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BIOSYNTHESIS OF MORPHINE

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BIOSYNTHESIS OF MORPHINE

Don Robert Baker
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Biosynthesis of Morphine

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BIOSYNTHESIS OF MORPHINE

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July 1959

I. MORPHINONE

Introduction

In 1952 Schöpf[1] reported that morphinone could possibly be the precursor of the morphine type alkaloids found in the opium poppy, Papaver Somniferum. He postulated that methylation of the enol of morphinone and the phenolic hydroxyl would lead to thebaine. Morphinone itself could be reduced to morphine, which could then be methylated to codeine. Also, neopine might be formed through isomerization of morphinone's α,β-unsaturated ketone followed by reduction and methylation.

It has been shown[2] that silver carbonate oxidizes codeine to codeinone in relatively high yield. Therefore, it is reasonable to believe that morphinone may be prepared by utilization of this oxidizing agent. Since the phenolic hydroxyl group in morphine would have to be protected

before it could be exposed to oxidation and, since it would be desirable to have a molecule as closely related to codeine as possible, methoxymethylmorphine was prepared.

Methoxymethylmorphine could then be oxidized by silver carbonate to form methoxymethylmorphinone. Hydrolysis of the methoxymethylmorphinone would then lead to morphinone.

Unforeseen complications arose in the final step. Therefore, a series of various hydrolysis procedures and various isolation procedures were investigated. Previous work in this laboratory describes unsuccessful attempts to prepare morphinone by acid hydrolysis at elevated temperatures using various concentrations of acetic acid as well as equally unsuccessful attempts to prepare morphinone by the hydrolysis of methoxymethylmorphinone at room temperature using a one molar potassium acid sulfate solution. In the present work it was found that the instability of morphinone in aqueous alkali had been largely responsible for the difficulties encountered in the previous methods.

The carbonyl group of codeinone is very unreactive to Grignard reagents. However, treatment with methyl lithium[3] gives a high yield of one isomer of 6-methylcodeine. Since morphine derivatives having nuclear substituted methyl groups, i.e., 5-methyldihydromorphinone,[4] have been found to have increased analgesic effect, the possibility of preparing 6-methylmorphine from methoxymethylmorphinone and methyl li-

thium was investigated.

To complement this, the various acetyl derivatives of 6-methylmorphine were prepared.

Discussion

Preparation of Methoxymethylmorphine

Methoxymethylmorphine was prepared by Mannich's[5] procedure from sodium morphinate and chloromethyl ether in a chloroform solvent. The sodium morphinate was prepared from commercial morphine hydrate (Mallinckrodt U.S.P. IX). Three runs were necessary in the preparation of the sodium morphinate. The first run gave a very poor yield of a light brown powder and was later reconverted back to morphine. The last two runs went in good yield. The chloromethyl ether was prepared from methanol, formaldehyde, and hydrogen chloride according to the method found in Organic Syntheses.[6] The amount of chloromethyl ether in the preparation was determined first by hydrolysis of a small sample and then titrating that solution with standard silver nitrate solution to determine the chloride content.

The methoxymethylmorphine was prepared in two runs, giving yields of 62 and 80% (based on the sodium morphinate consumed). No difficulty was encountered in preparing a crystalline product as was reported by Mannich.[5]

Preparation of Methoxymethylmorphinone

Silver carbonate was prepared in a manner similar to that reported[2] for the oxidation of codeine. The product was stored in a vacuum desiccator over magnesium perchlorate in the dark. Its activity was tested by oxidizing codeine to codeinone. Methoxymethylmorphine was oxidized by silver carbonate to form methoxymethylmorphinone. Pure methoxymethylmorphinone was obtained by removing all phenolic material using sodium hydroxide washes and then removing the ketonic material from the unoxidized material with bisulfide extractions.

Saturated ketones are liberated by the addition of saturated potassium carbonate solution (pH 10) and are then removed by benzene extractions. The bisulfite addition product of a saturated ketone is rapidly decomposed under these conditions. The α, β-unsaturated ketones are liberated under much more basic conditions (pH 13.5).

Methoxymethylmorphinone is released slowly from its bisulfite addition product. Most of it is released in eight or ten hours. Methoxymethylmorphinone appears to be sensitive to heat, since the compound darkens considerably when heated to 80°C in a vacuum for eight hours.

Preparation of Morphinone

1. Ion exchange methods. A model compound on which to test hydrolysis conditions of methoxymethylmorphinone is methoxymethylmorphine, since it is readily hydrolyzed to morphine and has the same functional groups as methoxymethylmorphinone with the exception of the α, β-unsaturated ketone at carbon 6. Since the hydrolysis is acid

catalyzed, and the compound contains a basic nitrogen atom, it seems feasible that a strong sulfonic acid resin could be used for the hydrolysis. This could be followed by the addition of a basic solution for the elution of the alkaloid. The liberated formaldehyde that is formed during the hydrolysis could easily be removed by passing distilled water through the column during the period of hydrolysis. This would remove all formaldehyde and the possibility of its further reaction. To find a resin, which would hold the alkaloid and from which the alkaloid could be readily eluted, was the first problem.

The first resin tried was Dowex 50 x 7.5 (20-50 mesh). It was found that morphine was slowly eluted from the resin. Therefore, a resin of a smaller percentage cross linkage was tried. In this case, Dowex 50 x 2 (200-400 mesh) was tried. It was shown, with the use of the Model 14 Cary Spectrophotometer, that a nearly quantitative elution of morphine was possible using this resin.

Methoxymethylmorphine was added to the column prepared from Dowex 50 x 2 (200-400 mesh). Distilled water was allowed to flow through the column for five hours. At the end of this time 1 N sodium hydroxide was added to elute the hydrolyzed alkaloid. The first fractions contained mainly morphine. Later fractions indicated elution of non-phenolic materials and suggested a method of separating the unhydrolyzed material from the phenolic material.

These conditions were applied to the hydrolysis of methoxymethylmorphinone. The sodium hydroxide eluant rapidly turned dark, even while in an ice bath. Therefore, in later trials, the pH of the basic eluant was adjusted to 8.67 and extracted with chloroform. Even under these precautions only a very small amount of crude material was obtained. Also under these conditions the column tended to channelize and was eluted very poorly. Since the strong base rapidly darkened, more mild elution conditions were tried on methoxymethylmorphine.

Therefore, in trial 3 methoxymethylmorphine was added to a column containing Dowex 50 x 1 (50-100 mesh) and was eluted with a borate buffer of pH 9.6. These results indicated almost complete hydrolysis during the five-hour reaction time as well as a good elution. Similar conditions were applied to the hydrolysis of methoxymethylmorphinone. A typical run is given in the experimental section. The perchlorate was prepared and its infra-red spectrum shows the presence of a phenolic OH group (a medium band at 2.8 microns), an α, β-unsaturated ketone (very strong band at 5.98 microns), and a weak band at 5.80 microns, which may be some saturated ketone impurity.

It seemed that the formation of the saturated ketone was occurring while the morphinone was in basic solution. Therefore, elution from the resin was tried with a 10% solution of potassium chloride. In this method the morphinone was eluted as the ammonium ion.

Then the pH was adjusted and the morphinone extracted from the aqueous solution. In this case the time in basic solution could be reduced to a minimum. But still a very small amount of impurities were indicated by the infra red spectrum.

2. Acid hydrolysis methods. Previous methods using ion exchange resins indicated that the decomposition of morphinone was due to the basicity of the eluant, and not to reaction with liberated formaldehyde. Therefore, as a trial, methoxymethylmorphinone was hydrolyzed in 1 N hydrochloric acid instead of on the ion exchange column.

The perchlorate was prepared and showed a similar spectrum when compared to morphinone perchlorate. The ultraviolet spectrum was also similar to morphinone. Analysis and melting point on recrystallized material, however, indicated some unusual behavior that might be accounted for by the addition of formaldehyde to morphinone, or by hydration. Since phenols and α, β-unsaturated ketones are known to react with formaldehyde, morphine was chosen as a model upon which to examine the reaction of formaldehyde upon phenols. These reactions will be discussed later. In summary, however, the conclusion was reached that formaldehyde did not react with the phenol under conditions similar to those encountered in the hydrolysis.

Morphinone was finally prepared free of the saturated ketone by removal of the free base from the aqueous solution as soon as the free

base was formed. This was accomplished by thoroughly mixing the cold aqueous solution with chloroform while the pH of the solution was being adjusted to the isoelectric point.

Reaction of Morphinone with Sodium Borohydride

As a means of structure proof, a sample of morphinone was reduced to morphine with sodium borohydride. This indicated the position of the double bond in morphinone and the nuclear skeleton.

Morphinone, with a small amount of saturated ketone impurity, was reduced with sodium borohydride to morphine (80% yield). Melting point, mixed melting point, infrared and ultraviolet spectra were identical with morphine.

After the morphine had been removed from the reaction solution, a small amount of an impurity was indicated by the ultraviolet spectrum of the extracted mother liquors. It was thought that this small amount of material still left in the mother liquors could have arisen from the reduction of the saturated ketone impurity found in the original morphinone. For this reason some neomorphine was prepared using the procedure of Small.[7] The ultraviolet spectra of neomorphine and dihydromorphine in base were compared with that of the ultraviolet spectrum of the extracted mother liquors. The spectra are quite different.

Later experimental work indicated that the impurity found in the extracted mother liquors was actually a product of the decomposition of morphine. Morphine when heated in a basic solution presumably oxidizes to form a small amount of material which has a spectrum identical with that found in the extracted mother liquors of the sodium borohydride reduction.

Morphinone was again reduced with sodium borohydride under conditions which did not expose morphine to basic oxidation. In this case no impurity could be found in the extracted mother liquors as had been found in the previous trial.

Hydrogenation of Morphinone

The position of the double bond in morphinone is shown by morphinone's reduction to morphine. A further check on the position of the ketone is indicated by catalytic hydrogenation of the double bond of morphinone to form dihydromorphinone. Crystals of dihydromorphinone were isolated from the hydrogenation and found to have an identical infrared spectrum when compared with an authentic sample of dihydromorphinone. After sublimation it had a melting point of 256-258°C. Reported as 266-267°C.[8]

Reaction of Diazomethane and Morphinone

Several reactions with diazomethane and morphinone were tried. The first methods tried involved using N-nitrosomethylurea[9] for the pre-

paration of the diazomethane and in each case no codeinone could be prepared. Only an orange oil, that darkened on standing, could be isolated in each case, no matter what method was used in the isolation of the product. Typical procedures are given in the experimental section.

It was thought that some base possibly had reacted with the morphinone in the previous trials, so another method of preparing diazomethane was tried. The method was that of Organic Syntheses[10] involving distillation of the diazomethane. This procedure removed all possibility of base being present in the diazomethane solution. Again, as in the previous procedure, only an oil could be isolated. This would tend to indicate that reaction was also taking place at the ketone group.

The same reaction conditions were tested on morphinone, codeinone, and morphine. In the case of morphine, codeine was produced, but in the case of codeinone and morphinone only a dark oil was isolated. This would further indicate that reaction was also taking place at the ketone group, possibly to form a pyrazoline as has been noticed by others.[11] Even by shortening the reaction time to five minutes, a mixture of the phenol and a dark oil were produced.

Reactions with Morphine, Formaldehyde and Base

Since phenols and α, β-unsaturated ketones are known to react with formaldehyde, morphine was chosen as a model upon which to ex-

amine the reaction of formaldehyde upon phenols.

Morphine did not react with formaldehyde in a pH 10 buffer. However, it was found that morphine, when heated with aqueous base (pH 10), evidently oxidizes to some extent and forms a soluble material that is left in the aqueous mother liquors after the morphine has been removed. This material has the same spectrum as that found in the extracted mother liquors of the sodium borohydride reduction of morphinone.

Pseudomorphine, an oxidation product of morphine, was prepared so as to compare its ultraviolet spectrum with that of the extracted mother liquors. The spectra are very different; evidently some other oxidation product is involved. The method of Fulton[12] and Balls[13] was used in the preparation of pseudomorphine.

Preparation of 6-Methylmorphine and 6-Methylcodeine

6-Methylmorphine was easily prepared from methoxymethylmorphinone. Methoxymethylmorphinone was found not sufficiently soluble in ether to allow its addition as an ether solution, so it was added as a solid to the ether solution of the methyl lithium. The methyl lithium was prepared so as to be free of methyl iodide, which could react with the tertiary nitrogen.

The easiest way to prepare the methyl lithium free of methyl iodide is to prepare the methyl lithium in high yield from the methyl

iodide, thus leaving no methyl iodide in the solution. It should be noted that the methyl lithium concentration should not be greater than one molar concentration during its preparation, since coupling takes place as a side reaction, and the percent yield is lowered.

The 6-methyl-3-methoxymethylmorphine was not isolated but hydrolyzed to 6-methylmorphine. The hydrolysis was accomplished by allowing the 6-methyl-3-methoxymethylmorphine to stand in 1 N hydrochloric acid for five hours.

6-Methylcodeine, a known compound,[14] was prepared as a derivative of 6-methylmorphine by reaction with diazomethane.

6-Methyldihydromorphine was prepared by hydrogenation with platinum oxide catalyst.[15]

Acetyl Derivatives of 6-Methylmorphine

Treatment of 6-methylmorphine with sodium hydroxide solution, acetic anhydride, and chloroform readily gave the 3-acetyl-6-methylmorphine. The rate of hydrolysis of the acetic anhydride is slow compared with its reaction with the phenolate ion.

Treatment of the 6-methylmorphine with methyl lithium and acetic anhydride after the general method of Houben[16] for the preparation of acetyl derivatives of tertiary alcohols failed to yield the diacetyl derivative. Refluxing 6-methylmorphine with acetic anhydride and pyridine also failed to yield the diacetyl compound.

The 6-acetyl compound was prepared from methoxymethylmorphinone by treating it with methyl lithium, followed by acetic anhydride and selective hydrolysis. The 6-acetyl-6-methyl-3-methoxymethylmorphine was not isolated but hydrolyzed to 6-acetyl-6-methylmorphine. The hydrolysis was accomplished by refluxing the 6-acetyl-6-methyl-3-methoxymethylmorphine in a pH 3.0 buffer for six hours. The rate of hydrolysis was followed by observing the ultraviolet spectra of the hydrolysis solution in base. Since phenols show a bathochromic shift in base, the rate of production of the phenol is easily followed.

The diacetyl compound could now be prepared from the 6-acetyl derivative by refluxing with acetic anhydride and pyridine. The method of preparing the 3-acetyl derivative was tried on 6-acetyl-6-methylmorphine in an attempt to prepare the diacetyl compound, but only starting material and a light oil were obtained. Possibly the phenol is somewhat hindered by the 6-acetyl group.

The hydrolysis rates of the phenolic acetyl group can easily be followed by the appearance of phenolate ion absorption in the ultraviolet (300 mμ). An accurately weighed sample of about 1.0 mg. was dissolved in 10 ml. of 95% ethanol and to this was added a small amount of 1 N sodium hydroxide to make a solution of 0.01 N base. The results are tabulated as follows:

Compound	Weight mg	Time for Half Reaction seconds
3,6-diacetylmorphine	0.952	100
3,6-diacetyl-6-methylmorphine	1.403	120
3-acetyl-6-methylmorphine	1.010	40

This would tend to indicate that the phenolic acetyl is somewhat hindered by the 6-acetyl group.

Attempted Preparation of Thebaine from Codeinone

The method of preparing dihydrothebaine from dihydrocodeinone[17] was used in the hope that thebaine could be prepared from codeinone.

As soon as the methyl sulfate was added to the codeinone solution, an immediate dark coloration took place. Isolation of the product produced only a dark material that was only slightly soluble in benzene or chloroform. No attempt was made to recover any unreacted codeinone.

Experimental

All melting points are corrected and those above 200°C were taken in evacuated capillaries; microanalyses were performed in the Microchemical Laboratory, University of California.

Preparation of Sodium Morphinate

A 62.0 g portion of morphine hydrate (0.205 moles) was added to a solution prepared by reacting 5.27 g of sodium (0.229 moles) with a mixture of 93 g (115 ml) of 95% ethanol and 57.3 ml of water. The mixture was shaken for some time since the last portion of morphine went into the solution with some difficulty. Slowly 250 ml of ether was added to precipitate the sodium morphinate. The mixture was allowed to stand for one hour and was then filtered and washed with ether. The precipitate was redissolved in 500 ml of ethanol and reprecipitated with 1500 ml. of ether. The light tan precipitate was filtered and dried in a vacuum at room temperature for eight hours. It yielded 69.1 grams of sodium morphinate · C_2H_5OH (96% yield).

Preparation of Chloromethyl Ether

The purity of the formaldehyde solution was determined after the procedure by Walker.[18] The determination indicated a 36.2% solution of formaldehyde.

A 102 g (96 ml) portion of 36.2% formaldehyde solution (1.23 moles), containing approximately 12% methyl alcohol (0.38 moles) as a preservative was added to 35 g (43.8 ml) of methyl alcohol (1.09 moles) in a 300 ml, three-necked, pear-shaped flask. The flask was fitted with a gas bubbler and a reflux condenser. The flask was cooled with a stream of running water.

Hydrogen chloride was bubbled through the solution for one hour and thirty minutes, at which time another layer began to form. Hydrogen chloride was bubbled into the solution for an additional hour and forty minutes. The layers were separated and the ether layer was dried with calcium chloride. Calcium chloride (anhydrous) was added to the cooled aqueous solution. The resulting ether layer, light yellow in color, was decanted from the mixture and added to the previous ether fraction. The combined ether layers were fractionated and 55.3 g of a clear solution were collected. A few mls of a lower-boiling fraction were collected and discarded. About 10 ml of brown liquid remaind in the distilling flask.

Determination of Chloromethyl Ether

A sample of approximately 0.5 g of chloromethyl ether was accurately weighed, placed in a 100 ml volumetric flask, and diluted to a volume of 100 ml with distilled water. Two one-ml aliquots were taken and titrated to determine their chlorine content.

This was done by adding, to each sample, 10 ml water, 5 ml acetone, 3 drops dichlorofluoresein indicator, and just enough sodium acetate to turn the solution green. The resulting solution was then titrated to the pink end point with standardized 0.01 M silver nitrate solution. Calculation indicated that the chloromethyl ether solution was 93.3% chloromethyl ether. This would indicate a 52% yield in the preparation of chloromethyl ether.

Preparation of Methoxymethylmorphine

A 58.9 g portion of dried sodium morphinate · C_2H_5OH (0.167 mole) and 240 ml of dry chloroform was added to a three-necked 500 ml flask equipped with a stirrer, reflux condenser, nitrogen line and dropping funnel. With stirring and cooling, 14.5 g (13.9 ml) of 93.3% chloromethyl ether solution (0.167 mole) in 27 ml of dry chloroform was added to the mixture. Addition was complete after 40 minutes. The mixture was then stirred for an additional hour at room temperature.

The mixture was filtered and the insoluble portion was dissolved in 1 N hydrochloric acid. The pH of the acid solution was adjusted to 8.67 and 1.9 g of dried morphine were recovered. The filtrate was washed with twenty 50 ml portions of 1 N sodium hydroxide. The last five washings gave a negative Mayer reagent test. These sodium hydroxide washings were adjusted to pH of 8.67 and 5.1 g of recovered morphine was obtained.

The chloroform solution was dried for thirty minutes over magnesium sulfate and filtered. The filtered solution was then evaporated, under reduced pressure, to a thick paste and then seeded with two grains of a previously prepared sample. This was allowed to stand overnight in order to evaporate to dryness. The methoxymethylmorphine was then dried in a vacuum, at room temperature for eight hours and stored over magnesium perchlorate. Yield: 37.5 g; m.p. 92-94°C (80%). Reported[5]: m.p. 94-96°C.

Preparation of Silver Carbonate

A 447 g portion of silver nitrate (2.63 moles) was dissolved in 4,500 ml of water in a 10 liter battery jar. To this was added, with stirring, 239 g of sodium bicarbonate (2.85 moles) dissolved in 2,200 ml of water. The aqueous solution was decanted from the yellow precipitate and the precipitate divided into two equal parts. Each was placed in a 2 liter, glass stoppered shaking graduate. Each part was then washed with ten 1,200 ml portions of distilled water. The precipitates were then washed with five 1,400 ml portions of methanol. The silver carbonate was transferred into a Büchner funnel and washed with 1,500 ml of ether. The ether was removed by drying the silver carbonate in a vacuum oven at room temperature overnight and then storing it in a black evacuated desiccator over magnesium perchlorate. Yield: 343 g (95%).

Oxidation of Codeine to Codeinone

A 125 ml portion of benzene was added to a 500 ml three-necked flask fitted with a solvent stripper, reflux condenser, and stirrer. To this was added 6.0 g of codeine (0.05 mole, Mallinckrodt) and 25 ml of benzene was stripped off. The solution was allowed to cool and 27.5 g of green silver carbonate (0.1 mole) was added.

The mixture was stirred rapidly while refluxing for one hour and 10 ml of benzene stripped off. The mixture turned black almost immediately upon the addition of the silver carbonate. The mixture was filtered, while hot, through a sintered glass funnel, but a large amount of black material passed through. The resulting mixture was reheated and filtered through super cel. The black material was digested with two 50 ml portions of benzene and then passed through the super cel while still hot.

The solution was evaporated in vacuum until crystals started to form. The flask was placed on a steam bath. More benzene was evaporated off with the use of nitrogen until crystals again just began to form. The solution was cooled and the crystals centrifuged off and dried in a vacuum oven at room temperature. The benzene solution was further concentrated, and a second crop of crystals harvested. The first crop yielded 3.1 g of crude codeinone (m.p. 168-170°C). The second crop yielded 1.4 g (m.p. 159-162°C). These had a characteristic red melt.

Preparation of Methoxymethylmorphinone

A 16.45 g portion of methoxymethylmorphine (0.05 mole) was dissolved in 330 ml of benzene in a three-necked one-liter flask equipped with stirrer, nitrogen atmosphere, and solvent stripper with reflux condenser.

A 30 ml portion of benzene was distilled off and the solution cooled to allow the addition, with stirring, of 69 g of green silver carbonate (0.25 mole). The mixture was heated to reflux, whereupon the silver carbonate turned black.

After one hour of refluxing, during which time 20 ml of benzene was stripped from the reaction, the mixture was again cooled, and an additional 69 g of silver carbonate added. The mixture was again heated to reflux for an additional hour, then filtered while hot through super cel. Most of the precipitate was returned to the flask and digested three times with 70 ml portions of benzene. The combined benzene solutions were then evaporated in a vacuum with the aid of nitrogen to approximately 200 ml.

The combined benzene solutions (orange in color) were cooled and washed twice with 100 ml portions of 1 N sodium hydroxide. The reddish basic washes were washed three times with 25 ml portions of benzene. The pH of the basic washes was adjusted to 8.67 and the solution heated on a steam bath in order to remove the excess benzene.

The precipitated material turned black and was filtered off and dried to yield 0.5 g of black powder.

The combined benzene solutions were washed with 20 ml of water. The benzene solution was extracted with four 110 ml portions of buffered bisulfite solution. This solution is made by dissolving 11.4 g of $NaHSO_3$ in 110 ml of water and mixing this with a solution made by dissolving 41.6 g of Na_2SO_3 in water to form 330 ml of solution. This 440 ml of buffered solution had a pH of 7.00. Following the bisulfite extraction, the benzene solution was washed with a small amount of water, dried over magnesium sulfate, filtered, and evaporated in vacuum to yield 4.95 g of recovered crude yellow methoxymethylmorphine. The light yellow bisulfite extract was made basic (pH 10.0) with concentrated potassium carbonate solution and extracts were washed with a small amount of water, dried over magnesium sulfate, filtered and evaporated in vacuum to yield 1.12 g of recovered light yellow methoxymethylmorphine. (Total recovery of methoxymethylmorphine, 37%).

The bisulfite extracts were made basic by the addition of 15 ml of 15 N sodium hydroxide. To this was added 500 ml of chloroform, and the mixture was placed on the shaker. Four two-hour chloroform fractions were taken, and each time 500 ml of fresh chloroform was added to the basic bisulfite solution, after which the mixture was put back on the

shaker. These chloroform fractions were washed with a small amount of water, dried over magnesium sulfate, filtered, and evaporated to dryness. The chloroform from the first two-hour period yielded 2.50 g of methoxymethylmorphinone (m.p. 124-125°C), 1.33 g (m.p. 123-124°C) from the second, 0.82 g (m.p. 123-125°C) from the third, and 0.52 g from the fourth fraction. Fraction five, taken over a period of twelve hours, and fraction six, taken over the next succeeding ten-hour period, yielded 0.92 g of material (m.p. 118-120°C for fractions four, five and six). Total yield: 6.19 g, 38%. Decomposition takes place on melting.

An analytical sample was prepared by recrystallizing from benzene-hexane three times and drying in vacuum overnight at 80°C (H.N.R.)

Anal.	Calc'd for $C_{19}H_{21}NO_4$:	C, 69.70; H, 6.47
	Found:	C, 69.73; H, 6.43
	$[\alpha]_D^{25}$	-176° (c. 0.982, 95% ethanol)
		m.p. 125.6-129.8°C

Reduction of Methoxymethylmorphinone to Methoxymethylmorphine

Run 1 (H.N.R.)

A 0,12 g sample of methoxymethylmorphinone (0.367 mmole) was dissolved in 6 ml of methanol. To this solution was added a suspension of 0.28 g of sodium borohydride (7.34 mmoles) in 6 ml of methanol

and the mixture allowed to stand at room temperature for one and one-half hours. It was then concentrated to half its volume and 5 ml of 10% sodium hydroxide was added. After heating to boiling, the mixture was diluted with an equal volume of water, cooled, and extracted four times with equal volumes of chloroform. The combined chloroform solutions were washed with a quarter volume of water, dried over magnesium sulfate, filtered, and evaporated to dryness; the resulting 0.08 g of yellowish glass became a white solid when seeded with methoxymethylmorphine. After drying, this solid had m.p. 93-95°C, but a 50:50 mixture of it with known methoxymethylmorphine had a m.p. 90-94°C, while a 50:50 mixture with methoxymethylmorphinone had a m.p. 89-95°C.

Run 2 (H.N.R.)

Run 2 was carried out like Run 1 but on a larger scale (in order to have better purification), using 0.205 g of methoxymethylmorphinone (0.626 mmole). A glass which solidified with seeding with methoxymethylmorphine and weighed 0.16 g was obtained. This white solid was dissolved in 10 ml of benzene and extracted three times with equal volumes of a mixture consisting of one part of 1 N sodium bisulfite solution and three parts of 1 N sodium sulfite solution. The combined water solutions were made basic by the addition of saturated potassium carbonate solution and then washed three times with equal volumes of benzene. These benzene washes were combined with the original benzene solution,

washed with a quarter volume of water, dried over magnesium sulfate, filtered and evaporated to dryness. The glass formed was easily solidified (by scratching) to 0.10 g of white solid m.p. 94-96°C. Two 50:50 mixtures of this solid, with known methoxymethylmorphine, had m.p. of 93-95°C and 94-95°C.

Adsorption of Morphine on Dowex 50 x 7.5 (20-50 mesh).

A 5.0 g portion of Dowex 50 x 7.5 (20-50 mesh), about 6.5 ml, was added to a 10 ml buret. About 1,000 ml of distilled water was passed through this ion exchange column. A check of the last water passed through the column showed no ultraviolet absorption (λ, 250-310 mμ).

Next, 104 mg of morphine hydrate was dissolved in 10 ml of 1 N hydrochloric acid which was then added to the column. Following the morphine solution, 40 ml of distilled water was added. The first 50 ml of solution passed through the column and collected after thirty minutes showed no ultraviolet absorption.

A solution of 1 N sodium hydroxide was then added to the column and fractions taken every 15 mls. Each fraction was acidified with a small amount of concentrated hydrochloric acid. Ultraviolet absorption was run on each of the fractions. The results are as follows:

Fraction	Minutes of flow	Ml of soln. after acidification	Mg of morphine (hydrate)
1	5	16.1	0.8
2	5	16.2	27.2
3	6	15.4	7.9
4	7	15.8	7.6
5	8	17.0	7.5
6	10	16.0	7.7

The amount of morphine has been calculated from the acidified fraction's ultraviolet absorption as compared to that of morphine, $\lambda_{max}^{1\ \underline{N}\ HCl}$ 286 mμ, ϵ = 1515.

Adsorption of Morphine on Dowex 50 x 2 (200-400 mesh)

The ion exchange column was prepared in a similar fashion as the above but instead 5.0 g of Dowex 50 x 2 (200-400 mesh) (about 3 cm of resin in a 1.75 cm diameter column) was used since the rate of flow is much slower.

A solution of 136.6 mg of morphine hydrate dissolved in 10 ml of 1 N hydrochloric acid was added to the column. This solution was followed by 50 ml of distilled water which, after passing through the column, showed no ultraviolet absorption.

A solution of 1 N sodium hydroxide was now added to the column. Four fractions were collected and each was acidified with a small amount of conc. hydrochloric acid. The ultraviolet absorption spectra were run on each of the acidified fractions.

Fraction	Ml of soln after acidification	Color	Percent of original morphine
1	22.6	Lt. brown*	91.5
2	16.0	Clear	6.4
3	38.5	Clear	2.5
4	31.0	Clear	0.65
			101.0

* The color was later shown to be due to coloring matter found in the sand used in preparing the column.

Twenty ml of fraction one was adjusted to a pH of 8.67 using a pH meter. The mixture was cooled in the refrigerator and then centrifuged. A small dark layer was removed and the crystals were washed three times with 5 ml portions of cold water. The crystals were then dried in a vacuum oven for four hours at 80°C. The crystals showed a m.p. of 249.5°C, with decomposition starting at 244°C. The yield was 82 mg of dried white crystals.

Hydrolysis of Methoxymethylmorphine

Trial 1: The ion exchange column containing 5.0 g of Dowex 50 x 2 (200-400 mesh) was prepared as in the above case.

A solution of 93.2 mg of methoxymethylmorphine dissolved in 4 ml of 0.1 N hydrochloric acid was diluted to 40 ml with water. This solution was passed through the column followed by distilled water. The first 50 ml of solution were collected after 15 minutes (with the aid of one or two lbs of nitrogen pressure). The solution showed no ultraviolet absorption spectrum.

A total volume of 400 ml of distilled water was passed through the column over a period of five hours. This solution showed no ultraviolet absorption.

A solution of 1 N sodium hydroxide was then added to the column and six fractions of 15 ml were taken. Each fraction was first acidified with conc. hydrochloric acid and then the ultraviolet spectrum ran on the acidified solution. Each was then basified with 1 N sodium hydroxide and again the ultraviolet spectrum of each fraction was run. The first six fractions were collected within 20 minutes after the addition of the sodium hydroxide solution.

The first fraction (yellow in color) when in basic solution showed a λ_{max}^{NaOH} 298 mμ λ_{min}^{NaOH} 279 mμ. The minimum has a greater optical density than pure morphine in basic solution. Using the optical density at 298 mμ and ϵ=2490, 35.8 mg of morphine was in this fraction.

In acidic solution this fraction showed 35.4 mg of morphine to be present using the value of the optical density at λ_{max}^{HCl} 286 mμ and ϵ=1515.

The second fraction (slightly yellow in color) when in basic solution showed λ_{max}^{NaOH} 298 mμ; λ_{min}^{NaOH} 278 mμ. The minimum also had a greater absorption than that of pure morphine. Using the same calculation as in the first fraction, the basic solution indicated 21.0 mg of morphine present in the fraction while the acidic solution indicated 19.8 mg of morphine in the fraction.

The third fraction in basic solution indicated only a slight hump at 298 mμ and a λ_{max}^{NaOH} 284 mμ and λ_{min}^{NaOH} 276 mμ. This trend continued in later fractions until the last fraction (number 7, comprising 100 ml of basic eluant collected after one hour) showed a λ_{max}^{NaOH} 283 mμ and λ_{min}^{NaOH} 261 mμ.

Trial 2: The ion exchange column containing 5.0 g of Dowex 50 x 2 (200-400 mesh) was prepared as in previous runs.

A 101.5 mg portion of methoxymethylmorphine was dissolved in 4 ml of 0.1 N hydrochloric acid and diluted to 50 ml with water. This solution was passed through the column followed by distilled water. Water was passed through the column for 5.5 hours with a total volume of solution of about 200 ml.

A solution of 1 N sodium hydroxide was then added to the column and two fractions taken. The first fraction of 30 ml was collected after three minutes (with the use of nitrogen pressure). The second fraction of 50 ml was collected after five minutes. Chloroform was added to the

column after the sodium hydroxide and a fraction of 100 ml was collected. The chloroform was used to elute the unhydrolyzed material. This chloroform solution was extracted two times with 25 ml portions of 1 N sodium hydroxide.

The first sodium hydroxide fraction was then extracted four times with approximately 10 ml portions of chloroform (36 ml of chloroform, total volume). The second fraction was extracted three times with approximately 10 ml portions of chloroform (28 ml of chloroform, total volume). The ultraviolet absorption spectra were run on these chloroform extracts.

Part of each extracted basic fraction was saved and the ultraviolet absorption taken. The remainder of each fraction was acidified, and its ultraviolet absorption was taken also. The remainder of each acidified fraction was then adjusted to a pH of 8.67. A brown precipitate formed in the first fraction and was centrifuged off. The fraction was cooled and allowed to stand overnight in an ice bath. The crystals of morphine that formed were centrifuged off and washed three times with 5 ml portions of water. Next the crystals were dried in a vacuum oven at 80°C for five hours. They yielded 39.1 mg of crude morphine, m.p. 242-245°C with decomposition. (It began to darken at 228°C.)

The ultraviolet absorption is summarized in the following table:

Fraction	Extraction Solution		Aqueous Solution after Extraction			
	$\lambda_{max}^{CHCl_3}$	Yield, mg	λ_{max}^{NaOH}	Yield, mg	λ_{max}^{HCl}	Yield, mg
1	284 mμ	0.75	298 mμ	79.4	286 mμ	77.0
2	285 mμ	6.9	298 mμ	2.5	284 mμ	4.1
3*	284 mμ	14.2				

*In Fraction 3, the chloroform fraction, the values given are for the fraction itself; no material was indicated in the sodium hydroxide washes of this fraction.

The yields of the extraction solutions are calculated on the basis that the chloroform extract consisted of methoxymethylmorphine, $\lambda_{max}^{CHCl_3}$ 285 mμ, $\epsilon = 1320$; $\lambda_{min}^{CHCl_3}$ 263.5 mμ. The yields indicated in acid solution and basic solution columns were based on the ultraviolet absorption extinction coefficients in each of these solutions.

Trial 3: A 101.5 mg portion of methoxymethylmorphine (0.308 mmole) was dissolved in 5.0 ml of 0.1 N hydrochloric acid and diluted to 50 ml.

An ion exchange column (i.d. 17 mm) was prepared, using 10.0 g of Dowex 50 x 1 (50-100 mesh). The resin had been alternately washed with 1 N sodium hydroxide and 1 N hydrochloric acid and converted to the H form. This was then washed with a large amount of water.

The above alkaloid solution was added to the column, and 820 ml of distilled water were passed through the column during the next five

hours. A buffer solution of pH 9.6 (0.5 M H_3BO_3, 0.5 M KCl, 320 ml of 1 N NaOH per liter) was added to the column and three fractions were collected. Then 100 ml of chloroform was passed through the column. The chloroform fraction was washed with 25 ml 1 N sodium hydroxide. The ultraviolet spectrum of the chloroform solution showed a shoulder at 270-280 mμ, ϵ=0.10.

From each of the aqueous fractions two 1 ml samples were taken. One sample was diluted with 1 N hydrochloric acid and the other with 1 N sodium hydroxide. The ultraviolet spectra were run on these samples. The data are shown on the following table:

Ultraviolet Spectra of Basicified Solution (1 N NaOH)

Fraction	Volume, ml	Dilution	λ_{max}, mμ	λ_{min}, mμ	E_{max}	Morphine (hydrate) mg
1	30	1/25	298	279	0.18	16.3
2	50	1/10	298	279	0.99	60.3
3	50	1/10	298	279	0.24	14.6
					Total:	91.2

Ultraviolet Spectra of Acidified Solution (1 N HCl)

Fraction	Volume, ml	Dilution	λ_{max}, mμ	λ_{min}, mμ	E_{max}	Morphine (hydrate) mg
1	30	1/25	285	261	0.11	16.5
2	50	1/10	286	261	0.59	59.5
3	50	1/10	285	261	0.144	14.4
					Total:	90.4

Theoretical yield of morphine (hydrate): 93.5 mg

The pH of each of the fractions was adjusted to 8.67 with a few drops of concentrated hydrochloric acid or sodium hydroxide. Crystals formed in fraction two and were centrifuged off and washed four times with 1 ml portions of water and then dried in a vacuum at room temperature for eight hours to yield 26.0 mg of dried crystals (m.p. 251-253°C). The solution was continuously extracted with chloroform for twenty-four hours and yielded only a dark material.

Hydrolysis of Methoxymethylmorphinone, Dowex 50 Hydrolysis

Trial 1: A solution of 104 mg of methoxymethylmorphinone (0.318 mmole) was added to the exchange column containing 5.0 g of Dowex 50 x 2 (200-400 mesh). The column had been previously washed with 1 N sodium hydroxide followed by 1 N hydrochloric acid and a large amount of distilled water.

After the alkaloidal solution was added, distilled water was passed through the column for five hours and a total of 600 ml of water was collected over this period. At the end of this period a 1 N sodium hydroxide solution was added to the ion exchange column, which was now cooled with an ice water jacket. Two fractions were collected (fraction one of 30 ml after two minutes and fraction two after five minutes).

Each fraction was washed in the cold with a 25 ml portion of chloroform to remove any unhydrolyzed material. The ultraviolet spectra

of these washes indicated that there was no unhydrolyzed material in the chloroform washes. The pH of each aqueous fraction was adjusted to 8.67 and extracted four times with 25 ml portions of chloroform. Samples were taken both before and after the extraction for the ultraviolet spectra.

The chloroform extracts of each fraction were combined and washed with a small amount of water, dried over calcium sulfate and evaporated to one or two ml in a vacuum, using a stream of nitrogen. A 10 ml portion of hexane was added to the chloroform solution. The resulting precipitate was centrifuged, washed with hexane and reprecipitated from chloroform.

The precipitate was dried at room temperature to yield 30.3 mg of dark yellow material.

Trial 2, pH 9.6 Elution: A 499.6 mg portion of methoxymethylmorphinone (1.57 mmoles) was dissolved in 10 ml of 0.1 N hydrochloric acid and added to an ion exchange column (i.d. 17 mm) containing 10 g of Dowex 50 x 1 (50-100 mesh). The resin had been previously washed with 1 N sodium hydroxide and 1 N hydrochloric acid followed by distilled water.

Water was passed through the column for five hours, after which time 760 ml had been collected. The ion exchange column was then surrounded by an ice bath and the pH 9.6 borate buffer was added to the column. Four fractions were collected (rate of flow: 25 ml/min) and each of these

fractions was worked up at 0°C as quickly as possible. Following the last buffered fraction, 100 ml of chloroform was passed through the column. The chloroform's ultraviolet spectrum was taken.

The pH of these four aqueous fractions was adjusted to 8.67 and each fraction extracted with four 25 ml portions of chloroform. Two 1 ml samples were taken from each fraction before and after the extraction. The ultraviolet spectra were run on these samples and also the chloroform extract to determine the completeness of the extraction.

Ultraviolet Spectra (Sample Basified with 1 N NaOH)

Fraction	Volume, ml	Dilution	λ_{max}, mμ	λ_{min}, mμ	E_{max}
Before Extraction:					
1	50	1/50	300	277	0.75
2	58				
3	50	1/50	300	277	0.56
4	50	1/10	302	277	0.93
After Extraction:					
1	50	1/10	295	276	0.18
2	58	1/10	295	277	0.18
3	50	1/10	295	277	0.14
4	50				
Chloroform Extracts (in chloroform):					
1	100	1/10	280	266	0.61
2	100	1/10	279	266	0.80
3	100	1/10	279	266	0.46
4	75	1/10	280	265	0.20

The chloroform extracts were combined and washed with a small amount of water, dried over sodium sulfate, filtered, and evaporated to dryness to yield a glass which, upon the addition of absolute ethanol, formed crystals that are not very soluble in chloroform (342.4 mg, 79%).

The perchlorate was made by dissolving 52 mg of morphinone in 3 ml of absolute methanol and adding 1.86 ml of 0.1 N ethanolic perchloric acid. The crystals were recrystallized three times from methanol-ether for analysis. Dried nine hours in a vacuum at 101°C. (m.p. 151-155°C hot stage). Material is hygroscopic.

Infrared Spectra: (KBr pellet)

2.8 microns; medium, broad band

5.80 microns; weak band

5.98 microns; strong, narrow band

Anal. Calc'd for $C_{17}H_{17}NO_3 \cdot HClO_4$: C, 53.20; H, 4.73

Found: C, 52.82; H, 5.01

$[\alpha]_D^{25}$ -125° (c, 0.75, methanol)

Trial 3, KCl Elution: A solution of 502 mg of methoxymethylmorphinone (1.53 mmoles) dissolved in 20 ml of 0.1 N hydrochloric acid was added to a Dowex 50 x 1 (50-100 mesh) ion exchange column that had been previously prepared as in Trial 2.

A total volume of 800 ml of distilled water was passed through the column during a five hour period. After this time a 10% potassium chloride solution was added to the column and over a period of one hour,

four 100 ml fractions were collected. The pH of each fraction was adjusted to 8.67 by first adding 20 ml of 1 M sodium bicarbonate and then adding a few drops of conc. ammonium hydroxide (in the cold). Each fraction was then quickly extracted three times with 50 ml portions of chloroform.

The chloroform extracts of fraction one and two were combined, washed with a little water, dried over sodium sulfate, and evaporated to a light yellow glass (120 mg, 28% yield). The chloroform extracts of fractions three and four were combined, washed, dried, and evaporated to 159 mg, (35%) of a darker glass.

The infrared spectra of the material from fractions one and two had a very weak band at 5.80 microns and a strong band at 5.95 microns.

Hydrochloric Acid Hydrolysis of Methoxymethylmorphinone

Trial 1: A 102 mg portion of methoxymethylmorphinone (0.312 mmole) was dissolved in 10 ml of 1 N hydrochloric acid and allowed to stand at room temperature for five hours. The solution was then diluted with 40 ml of water and 25 ml of 0.1 M disodium hydrogen phosphate solution and while being kept cold the pH was adjusted to 8.67. The adjusted solution was extracted quickly with four 25 ml portions of cold chloroform. The ultraviolet spectra was taken on the aqueous solution before and after extraction and on the chloroform extract to determine the completeness of the extraction.

The chloroform solution was washed with a small amount of water, dried over sodium sulfate, filtered, and evaporated in a vacuum to dryness to yield 68 mg (80%) of dried light yellow glass. The dried material was taken up in 5 ml of absolute ethanol. To this was added 2.19 ml of 0.1 N ethanolic perchloric acid. The crystals were recrystallized three times from methanol-ether for an analytical sample. It was dried for ten hours at 101°C and 0.02 mm of mercury to yield 27.6 mg of white crystals. The mother liquors were evaporated to dryness to yield 58.0 mg of light yellow crystals.

Anal. m.p. 140±2°C (hot stage)

Calc'd for $C_{17}H_{17}NO_3 \cdot HClO_4$: C, 53.20; H, 4.73

Calc'd for $C_{17}H_{17}NO_3 + CH_2O \cdot HClO_4$: C, 52.23; H, 4.87

Calc'd for $C_{17}H_{17}NO_3 \cdot HClO_4 \cdot CH_3OH$: C, 51.97; H, 5.33

Found: C, 52.0; H, 5.1

Trial 2: A solution of 1.04 g of methoxymethylmorphinone (3.18 mmoles) dissolved in 20 ml of 1 N hydrochloric acid was allowed to stand at room temperature for five hours. The solution was then cooled in an ice bath and to it was added 50 ml of chloroform. While stirring mechanically, the pH was adjusted to 8.7 with 20 M potassium carbonate (about 5 ml). The layers were quickly separated and the aqueous layer extracted three times with 50 ml portions of cold chloroform. The chloroform fractions were combined and washed with a small amount of

water. These were then dried over sodium sulfate and evaporated to dryness in a vacuum to yield 763 mg of dried light yellow glass (85% yield).

Part of the glass (500 mg) was dissolved in about 5 ml of absolute ethanol. Spontaneous crystallization took place. The crystals were separated, washed with ethanol, and air dried, to yield 450 mg of air-dried crystals. An analytical sample was prepared by drying the above crystals for twenty-four hours at room temperature, 0.01 mm. The infrared showed a medium broad band at 2.85 microns, no band at 5.80 microns, strong band at 5.98 microns (in KBr). It showed no m.p. but started to decompose at 148°C (evac.cap.).

Anal. Calc'd for $C_{17}H_{17}NO_3$: C, 72.06; H, 6.07

Found: C, 71.86; H, 6.37

$[\alpha]_D^{25}$ -194° (c, 0.87, methanol)

Reduction of Morphinone with Sodium Borohydride

Trial 1: A 98.7 mg portion of morphinone (0.249 mmole), prepared from the Dowex 50 hydrolysis using pH 9.6 eluant, was dissolved in 5 ml of methanol. This was added to a solution of 268 mg of sodium borohydride (7.1 mmoles). The sodium borohydride solution was prepared by dissolving the sodium borohydride in 2 ml of water first and then adding 5 ml of methanol.

The solution was allowed to stand for 1.5 hours and then evaporated to one-half its volume (on the steam bath, using nitrogen). A 10% solution of sodium hydroxide (5 ml) was added to the solution and the combined solutions brought just to the boil. Then 5 ml of water was added. The pH of the solution was adjusted to 8.67 and the methanol evaporated off in vacuum, using a stream of nitrogen. Crystals formed, were centrifuged off, washed three times with 1 ml portions of water, and dried five hours to yield 39.3 mg (40%) of morphine (m.p. 250-251.5°C with decomposition); mixed melting point with morphine (m.p. 253-254°C): 249.5-250.5°C.

The pH of the solution was readjusted to 8.67 and a second fraction of crystals collected, washed, and dried, to yield 22.4 mg (23%) of morphine (m.p. 249.5-250.5°C).

The ultraviolet spectrum was run on a sample of the mother liquors. The mother liquors were extracted six times with 25 ml portions of chloroform. Ultraviolet spectra were run on samples of the chloroform extract and the aqueous solution. The chloroform solution had a λ_{max} 286 mμ, λ_{min} 265 mμ, and indicated the presence of 17.4 mg (18%) of morphine in the chloroform extract. The aqueous solution had a λ_{max} 300 mμ, and a λ_{min} 283 mμ (about 4.5 mg of material) in a 1 N sodium hydroxide solution. The aqueous solution was evaporated to dryness in a vacuum and digested with two 10 ml portions of chloroform.

These chloroform digests were evaporated to yield only a small amount (less than 0.5 mg) of dried white powder.

Trial 2: A solution of 84 mg of morphinone (0.257 mmole), that had been prepared by hydrochloric acid hydrolysis and contained a small amount of saturated ketone impurity, dissolved in 7 ml of methanol was added to a solution containing 270 mg of sodium borohydride (7.1 mmoles). The sodium borohydride solution was made by dissolving the sodium borohydride in 2 ml of water and adding 3 ml of methanol.

The above reaction solution was allowed to stand at room temperature for one and one-half hours and then was evaporated to half the original volume on a hot water bath using a stream of nitrogen. An additional volume of 10 ml was added to the solution and again evaporated to half the original volume.

The pH was adjusted to 8.67 with a small amount of conc. hydrochloric acid. Bubbles of hydrogen (?) were formed upon the addition of the acid. The mixture was allowed to stand in an ice-salt bath for one hour before the crystals were filtered off, washed, and dried at 101°C, 0.01 mm, for fourteen hours to yield 60 mg (71%) of white crystals.

The ultraviolet spectrum of the mother liquors (in 1 N sodium hydroxide) was the same as that of morphine and indicated the presence of about 20 mg (24%) of additional morphine. Therefore, the mother liquors were extracted six times with 25 ml portions of chloroform which,

when dried and evaporated to dryness, yielded 14 mg of an oil which solidified into a white solid. Infrared spectrum of this material (in KBr) was identical with that of morphine.

Part of the above white crystals were sublimed at 150°C, 0.01 mm and dried for twelve hours at 101°C, 0.01 mm for an analytical sample. It had a m.p. of 247-250.5°C and a 50:50 mixed (m.p. 256-257°C) m.p. of 248.5-251.5°C.

Anal. Calc'd for $C_{17}H_{19}NO_3$: C, 71.55; H, 6.71

Found: C, 71.59; H, 6.63

$[\alpha]_D^{25}$ -118° (c, 0.91, methanol)

Reported m.p. 253-254°C;[19] $[\alpha]_D^{23}$ -130.9°[20]

Hydrogenation of Morphinone

A 92.2 mg sample of morphinone (0.326 mmole), prepared from the Dowex 50 hydrolysis using pH 9.6 eluant, was dissolved in 10 ml of absolute ethanol. Then, 150 mg of 5% Pd/C catalyst was added to the hydrogenation flask. An almost theoretical amount of hydrogen (0.96 mole of hydrogen per mole of compound) was taken up in the first thirty minutes. The solution was filtered through a sintered glass funnel and evaporated to approximately 0.5 ml and left to stand overnight. Crystals formed and were dried to yield 15 mg of dried material (m.p. 252-255°C). Comparison of its infrared spectrum with that of authentic dihydromor-

phinone (m.p. 259-260°C) revealed them to be identical. These crystals were sublimed at 190°C, 0.01 mm to give a powder; m.p. 256-258°C, 50:50, mixed with dihydromorphinone (m.p. 259-260°C), 260-261°C. Reported m.p. 266-267°C; $[\alpha]_D^{25}$ -194° (c, 0.98, dioxane).[8]

The mother liquors were evaporated to dryness to yield 63.4 mg of material having a phenolic and a saturated ketone band in the infrared. The spectrum was similar to dihydromorphinone but not identical (in KBr).

Reactions of Morphinone with Diazomethane Prepared from N-Nitrosomethylurea

Trial 1: The ethereal solution of diazomethane was prepared by adding 3.0 g of powdered N-nitrosomethylurea to a cooled 125 ml Erlenmeyer flask containing 20 ml of 40% potassium hydroxide and 30 ml of ether (in the hood). The solution was swirled, and as the ether layer became bright yellow, it was decanted off and fresh ether added. This was done until little yellow color formed in the ether layer.

The ether solution of the diazomethane was standardized by adding 2 ml of solution to an ethanolic solution of benzoic acid and titrating the excess benzoic acid with standardized 0.1 N sodium hydroxide.

A solution of 250 mg of morphinone (0.885 mmole), prepared from the hydrochloric acid hydrolysis and containing a small amount of saturated ketone impurity, dissolved in 20 ml of methanol, was cooled in an ice-salt

bath. Slowly, 70 ml of 0.05 M diazomethane solution was added to the solution of morphinone. The solution was allowed to stand in the cold for three hours and two hours at room temperature.

The solution was then evaporated in a vacuum to an orange oil, which partially crystallized on the addition of absolute ethanol. When more ethanol was added and the solution again evaporated, only a darker oil was obtained.

The dark oil was dissolved in chloroform, washed with 0.1 N sodium hydroxide, washed with water, dried over sodium sulfate, and evaporated to dryness to yield 210 mg of dark tar.

A 177 mg portion of the above dark tar was dissolved with heating in 10 ml of benzene. This was added to a column containing 10 g of alumina. No useful material was found from this chromatography.

Trial 2: The diazomethane solution was prepared as in Trial 1. A solution of 250 mg of morphinone (0.885 mmole) dissolved in 10 ml of methanol was cooled in an ice-salt bath, and to it was added 85 ml of 0.16 M diazomethane. The yellow solution was allowed to stand for fourteen hours in the refrigerator and at room temperature for three hours.

The yellow solution was added to 50 ml of 0.5 M acetic acid. The two layers were evaporated in a vacuum to remove the non-aqueous

solvents. The pH was adjusted to 9.0 and the solution extracted five times with equal portions of chloroform. These chloroform solutions were dried and evaporated to yield 0.23 g of yellow oil which darkened on standing. The infrared spectrum showed quite a decrease in the intensity of the band at 5.98 microns and was totally different when compared to codeinone.

Reaction of Morphinone with Diazomethane Prepared from p-Toylsulfonylmethylnitrosoamide

The diazomethane was prepared in the method given in Organic Syntheses[10].

A 25 ml sample of 0.125 M diazomethane was added in the cold to 104 mg of morphinone (0.368 mmole) dissolved in 10 ml of methanol. The morphinone that was used was that which had been prepared by hydrochloric acid hydrolysis and was free of saturated ketone. The yellow solution was allowed to stand in the refrigerator for one hour after which time excess 1 N acetic acid was added. The acidified solution had an orange color. The orange solution was diluted with 30 ml of 0.1 M phosphoric acid and the organic solvents removed with the aspirator at room temperature.

The pH was adjusted to 8.5 with potassium hydroxide solution and then extracted with four 20 ml portions of chloroform. These were dried over sodium sulfate and evaporated to yield 106 mg of dark oil.

Test of the Stability of Codeinone to Diazomethane

A 98 mg portion of codeinone (C.H.L.) was subjected to the same conditions as those listed above for morphinone and yielded an orange solution upon the addition of the acetic acid. Following the same extraction procedure, 123 mg of dark tar was produced.

Diazomethane Reaction with Morphine

Morphine hydrate (98 mg, 0.324 mmole) was dissolved in 25 ml of methanol. A 25 ml portion of the same diazomethane solution used in the previous two reactions was added to the alkaloidal solution. The reaction was almost instantaneous. The yellow solution was allowed to stand in the refrigerator for one hour and was then acidified with excess 1 N acetic acid to give a clear, colorless solution.

A 30 ml portion of 0.1 M phosphoric acid was added to the colorless solution, the organic solvents evaporated off, and the pH adjusted to 12.5 with potassium hydroxide. The adjusted solution was extracted with six 20 ml portions of chloroform. The chloroform extracts were dried over sodium sulfate and evaporated to dryness to yield 70 mg of white crystals which had a m.p. 151-152°C after drying for twenty-four hours at room temperature, 0.01 mm. Reported m.p. 156-157°C.[21]

Diazomethane Reaction with Morphinone (Short Reaction Time)

A 3.1 ml portion of 0.12 M diazomethane (0.372 mmole) (the same diazomethane as used in the previous three procedures) was added in the cold to a solution of 96 mg of morphinone (0.351 mmole) in 10 ml of methanol over a period of two minutes. After an additional three minutes, a few drops of 1 N acetic acid, followed by 30 ml of 0.1 M phosphoric acid, was added to the reaction solution. The organic solvents were evaporated off to yield a light yellow solution. The pH of this solution was adjusted to 12 with potassium hydroxide and extracted with four 20 ml portions of chloroform. The aqueous solution rapidly turned dark.

The chloroform extracts were washed, dried and evaporated to dryness to yield 20 mg of dark oil.

Stability of Morphine to pH 10 and Heat

A 102 mg portion of morphine hydrate was dissolved in 10 ml of 0.1 N hydrochloric acid and this solution was added to 70 ml of 0.1 M sodium borate solution. The pH was adjusted to 10.0 and the solution heated on the steam bath for 15 minutes. The solution was cooled and the pH adjusted to 8.67 and allowed to stand overnight in the refrigerator. No crystals formed.

The solution was extracted six times with 50 ml portions of chloroform and the ultraviolet spectra taken of the chloroform extracts

and the aqueous solution before and after extraction. The chlorofom extracts were evaporated to dryness to yield 70 mg of material.

The extracted aqueous solution showed λ_{max}^{NaOH} 301 mμ, λ_{min}^{NaOH} 283 mμ. Assuming $\epsilon = 2500$, this would indicate the presence of about 22 mg of material. There was only a slight indication of a point of inflection at 249 mμ, a point which is characteristic of morphine. This spectrum compares well with that of the extracted mother liquors of the sodium borohydride reduction of morphinone, Trial 1 (p.).

Stability of Morphine to Formaldehyde at pH 10

A 100 mg sample of morphine hydrate (0.33 mmole) was dissolved in 10 ml of 0.1 N hydrochloric acid. This acidic solution was then added to 70 ml of 0.1 M sodium borate solution and the pH adjusted to 10. Next, 1.0 ml of 1% formaldehyde solution (0.33 mmole) was added and the solution was allowed to stand at room temperature for 30 minutes. After that time the pH was readjusted to 8.67 and the solution was allowed to stand overnight in the refrigerator.

The crystals were filtered off, washed, and dried at 100°C, 0.01 mm, for four hours. This yielded 54.3 mg (58%) of morphine; m.p. 254.5-255.5°C, 50:50 mixed melting point with authentic morphine (m.p. 254.0-254.5°C) 254.0-254.5°C. All melted with decomposition.

The mother liquors were extracted four times with 50 ml portions of chloroform. The chloroform solution was then dried over sodium sulfate

and evaporated to dryness to yield 16.0 mg (17%) of dried yellow crystals (crystallized from methanol).

The ultraviolet spectrum was run on the extracted mother liquor (in 1 N sodium hydroxide) and gave a spectrum similar to that of morphine in 1 N sodium hydroxide. This indicated the presence of 12.9 mg (13%) of morphine hydrate.

Preparation of Pseudomorphine

A 5.0 g portion of morphine hydrate (16.5 mmoles) was dissolved in 37 ml of 0.5 N sodium hydroxide (18.5 mmoles). To this solution was added 4.5 g of mercurous chloride (19.0 meq) and the mixture boiled for one minute. The solution was cooled and basified with 1 N sodium hydroxide and then filtered, and the remaining material washed with additional base.

The filtrate and washes were combined and acidified with conc. hydrochloric acid, and then the pH was adjusted to 6.5 with conc. ammonium hydroxide.

The precipitate was filtered off and redissolved in 0.1 N hydrochloric acid, and again filtered. The acidic filtrate was again adjusted to a pH of 6.5. This precipitate was washed with a small amount of water followed by ethanol and ether. The washed precipitate was dried in a vacuum at room temperature for eight hours to yield 2.0 g (39%) of dried

white material plus 0.8 g of darker material. This white material starts to darken at 310°C and decomposes at approximately 319°C. Polstorff[22] reports decomposition at 327°C. Its ultraviolet spectra are shown in the graph. Its spectrum in 0.02 N hydrochloric acid compares well with that reported by Oestreicher, Farmilo and Levi.[23]

Preparation of Neomorphine

A suspension of 10 g of neopine hydrobromide (26.5 mmoles) in 60 ml of 15% hydrogen bromide in glacial acetic acid was heated slowly with stirring and refluxing on an oil bath. The bath temperature was 115°C at the end of one hour. Evolution of gas began at about 100°C. The bath temperature was slowly raised from 115°C to 145°C in the course of an hour. The solution was cooled and diluted with 60 ml of water. The pH of the solution was adjusted to 8.5 with conc. ammonium hydroxide. The dark gray solution was cooled in the refrigerator for eight hours and the crystals filtered, washed with a small amount of cold water, and dried in a vacuum at room temperature to yield 7.5 g of 6-acetylneomorphine. The crystals were recrystallized from 70 ml of ethanol to yield 5.0 g (56%) of dried 6-acetylneomorphine, m.p. 240-247°C, reported m.p. 243-251°C. $[\alpha]_D^{20}$ + 27.6° (c, 1.07, alcohol).[7]

A 4.0 g portion of recrystallized 6-acetylneomorphine (8.5 mmoles) was dissolved in 16 ml of 2 N sodium hydroxide and boiled for ten minutes. The solution darkened and a small amount of dark material was centrifuged off. Dry ice was added in small pieces to the light yellow solution. At a pH of about 8, a very gummy oil separated out. The solution was warmed slightly to prevent ice formation and, on standing a few minutes, the oil crystallized. Most of the crystals were centrifuged off and dried in a vacuum. The remaining light yellow solution was evaporated to dryness in a vacuum. The combined dried material was digested with two 40 ml portions of chloroform and filtered through super cel. The chloroform solution was allowed to stand for two days and the "coffin shaped" crystals filtered and dried in a vacuum to yield 1.15 g of transparent crystals, m.p. 142-145°C (hot stage). The crystals turned white on heating over 85°C.

These crystals were then dried in a high vacuum (0.2 mm) for eight hours at 101°C to yield a white powder, m.p. 238-239°C, with decomposition. Reported m.p. 240-241°C; $[\alpha]_D^{20}$ -9.2° (c, 1.04, 95% ethanol).[7]

The mother liquor was concentrated to 10 ml and the crystals that formed were filtered off and dried in a vacuum to yield 1.13 g of dried crystals. The mother liquors were further concentrated to yield a third crop of crystals (0.30 g).

Neomorphine's (m.p. 238-239°C) ultraviolet spectrum was run in 1 N sodium hydroxide solution, λ_{max} 297 mμ, ϵ=2180; λ_{min} 272 mμ, ϵ=1030.

Preparation of Methyl Lithium

The lithium that was used was in the form of wire. It had been stored in vaseline. The wire was cut in convenient lengths of about four inches long and the vaseline removed by rubbing with a piece of cloth (under pentane). The strips of cleaned lithium were then cut into smaller pieces (about 5 mm long) and placed under an inverted funnel containing pentane. The lithium pieces were washed with three alternate portions of pentane and dry ether, retaining the last ether wash in the funnel.

The cleaned and washed lithium (8.33 g, 1.2 moles) was placed in a one-liter three-necked flask equipped with stirrer, reflux condenser, dropping funnel, and nitrogen bubbler. Dry ether (300 ml) was placed in the flask. While the flask was cooled with an ice bath, 25 ml of methyl iodide (0.401 mole) was added during a 45 minute period. No evolution of gas was noted. After the addition of the methyl iodide, the flask was stirred an additional hour at room temperature.

The methyl lithium was filtered from the three-necked flask, using all glass connections, into a methyl lithium flask and the glass wool filter washed with ether. Excess lithium was carefully destroyed using methanol.

Two 3 ml aliquots were taken with hypodermic syringes and added to water and the liberated lithium hydroxide titrated with 0.1 N standardized hydrochloric acid, indicating 425 mls of 0.93 M methyl lithium (98.5%).

Preparation of 6-Methylmorphine

Dry ether (50 ml) was placed in a 250 ml round-bottom, three-necked flask equipped with stirrer, condenser, nitrogen line, and an adapter for the addition of solids.

The flask was cooled in an ice-salt bath, and 20 ml of 0.93 M methyl lithium was added by means of a syringe. Over a period of 15 minutes, 2.48 g of repurified, dried (m.p. 129-130°C) methoxymethylmorphinone (7.6 mmoles) was added as a solid. The light yellow solution was stirred in the cold for an additional hour.

The solution was tested for excess methyl lithium by adding a few drops to water and noting whether gas was evolved.

The solution was added to 60 ml of ice water and the layers separated. The aqueous layer was washed four times with 30 ml portions of ether. The combined ether solutions were then washed with a small amount of water and then extracted with four 40 ml portions of 1 N hydrochloric acid. The hydrochloric acid solution was allowed to stand for five hours at room temperature.

The pH of the acid solution was adjusted to 8.67 with conc. ammonium hydroxide. The adjusted solution was extracted four times with a total volume of 200 ml of chloroform. Almost immediately, 1.12 g (49%) of light crystals formed in the chloroform and were filtered off, washed with chloroform, and dried (m.p. 275-276°C, with decomposition).

The combined chloroform fractions were dried over sodium sulfate. (Ten ml of methanol had to be added to the chloroform solution while drying since additional material started to crystallize from the chloroform.) The organic solution was evaporated to yield 0.75 g (33%) of light tan solid.

Three additional 50 ml portions of chloroform were used to again extract the aqueous adjusted solution and a second fraction of 0.35 g (15%) was collected. Total yield: 2.22 g, 98%.

A sample was recrystallized once from acetone and twice from methylethyl ketone and analyzed as follows: (H.N.R.)

Anal. Calc'd for $C_{18}H_{21}NO_3$: C, 72.22; H, 7.07; C-CH_3, 5.03

Found: C, 71.95; H, 7.24; C-CH_3, 4.3

$[\alpha]_D^{25}$ -166° (c, 0.82, methanol)

m.p. 278.8-279.6°C

Preparation of 6-Methylcodeine (H.N.R.)

A 0.25 g portion of 6-methylmorphine (0,84 mmole) which had been recrystallized twice from methylethyl ketone was dissolved in methanol

and cooled in an ice bath.

After 1.75 g of finely powdered N-nitrosomethylurea (17 mmoles) had been added (in the hood) to a 125 ml Erlenmeyer, containing 12.5 ml of cooled 40% potassium hydroxide and 25 ml of ether, the mixture was stirred in an ice bath until the ether layer became bright yellow. The ether layer was carefully decanted into another cooled Erlenmeyer and kept cold in an ice bath. Further 10 ml portions of ether were added to the water phase, swirled, and decanted until very little or no color appeared in the ether layer.

The combined ether layers were added slowly with swirling to the methanol solution of 6-methylmorphine and the mixture clamped in an ice bath in the hood overnight, thus allowing it to return to room temperature slowly. The reaction mixture was then evaporated to dryness, leaving 0.255 g of a light oil which hardened upon scratching to a white powder (m.p. 95-100°C). To remove any phenolic material, the crude product was dissolved in 25 ml of ethyl acetate, extracted with 12 ml portions of 0.1 N sodium hydroxide, washed with 5 ml of water, dried over magnesium sulfate, filtered, and evaporated to dryness, giving 0.20 g (77%) of white powder now melting from 106-111°C. This white powder crystallized easily from 60-90° ligroin, giving 0.123 g (47%) of white needles which, after drying five hours in a vacuum oven at 80°C, had a melting point of 112.4-115°C.

6-Methylcodeine had been prepared previously by Findlay and Small[14] who reported a melting point of 114.5-116.5°C and an $[\alpha]_D^{20}$ -163° (c, 1.1 alcohol).

Preparation of 3-Acetyl-6-methylmorphine

A 0.35 g portion of crude 6-methylmorphine (1.17 mmoles) was dissolved in 25 ml of chloroform. Only about 75% of the material dissolved in the chloroform and the remainder was filtered off and dissolved in 1.75 ml of 1 N sodium hydroxide.

The sodium hydroxide solution and the chloroform solution were added together (in the cold). To this was added 0.138 ml (150 mg) of acetic anhydride (1.47 mmoles) and the mixture vigorously shaken for one minute.

The layers were separated and the chloroform solution washed with a small amount of water. The wash was combined with the aqueous solution and the pH was adjusted to 8.7 with 2 ml of sodium bicarbonate and a drop of conc. ammonium hydroxide. The adjusted solution was extracted three times with 10 ml portions of chloroform.

The combined chloroform layers were dried over sodium sulfate and evaporated to a light yellow oil which, on the addition of one drop of ethyl acetate, crystallized to a light tan powder (0.29 g, 75%).

Some of the light tan powder (0.25 g) was dissolved in about

10 ml of chloroform and then washed with three 25 ml portions of 0.5 M sodium carbonate and the sodium carbonate solutions back-washed with chloroform. The chloroform solutions were combined and washed twice with a small amount of water. The chloroform solutions were dried and evaporated to dryness to yield crystals. These were found to recrystallize nicely from ethyl acetate.

An analytical sample was prepared by recrystallizing once from ethyl acetate and then sublimed at 120° C, 0.01 mm, and dried for twelve hours at room temperature, 0.01 mm. It had a melting point of 166.5-167.5°C, with decomposition. The infrared spectrum showed a strong narrow band at 5.70 microns.

Anal. Calc'd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; Acetyl, 12.6

Found: C, 70.12; H, 6.60; Acetyl, 12.5

$[\alpha]_D^{25}$ -198° (c, 0.78, methanol)

In basic solution the acetyl group is readily hydrolyzed as is indicated by the gradual appearance of absorption at 300 mμ. This would be due to the formation of the phenolate ion.

Reaction of 6-Methylmorphine with Methyl Lithium and Acetic Anhydride

A 297 mg portion of 6-methylmorphine (0.99 mmole, m.p. 275-276°C) was dissolved in 50 ml of dry tetrahydrofuran in a dry 300 ml three-necked flask. A solution of 0.93 M methyl lithium (5.0 ml) was

added to the cooled flask and allowed to stir for 15 minutes. Then 0.50 ml of acetic anhydride (5.4 mmoles) was added and the solution heated to reflux on a water bath for 30 minutes The flask was then allowed to cool and was stirred for an additional eighteen hour at room temperature.

The red solution was added to 50 ml of 0.5 M acetic acid and evaporated to 50 ml on the aspirator. A few grains of sodium sulfite were added and the color changed to a light yellow. The yellow solution was extracted three times with 50 ml portions of chloroform. These chloroform extracts were then washed three times with 30 ml portions of 0.5 M sodium carbonate and two times with a small amount of water. The chloroform solution was then dried over sodium sulfate and evaporated to a yellow oil which crystallized on standing to yield 242 mg of material.

The pH of the aqueous acetic acid solution was adjusted to 8.67 with ammonium hydroxide and then extracted three times with 50 ml portions of chloroform. The chloroform extracts were washed and dried to yield 49 mg of an oil which crystallized on standing. It had a melting point of 131-135°C. The infrared spectrum of this material was very similar to that of 3-acetyl-6-methylmorphine.

A sample was sublimed at 120°C, .0.01 mm, and dried for fourteen hours at room temperature to yield a material that had a melting point of 156-160°C.

Anal. Calc'd for $C_{22}H_{25}NO_5$: Acetyl, 22.4

Found: Acetyl, 9.4

Reaction of 6-Methylmorphine with Acetic Anhydride and Pyridine

A 98 mg portion of 6-methylmorphine (0.328 mmole) was refluxed with 2.0 ml of acetic anhydride and 1.0 ml of dry pyridine for five hours (under nitrogen). The light yellow solution was evaporated to dryness in a vacuum with gentle heating. The oil was taken up in 1.0 ml of chloroform and washed three times with 10 ml portions of 0.1 M sodium carbonate. The sodium carbonate solution was back-washed with chloroform.

The combined chloroform solutions were washed with water, dried over sodium sulfate, and evaporated to 105 mg of a yellow oil. The infrared spectrum was also similar to that of 3-acetyl-6-methylmorphine.

Preparation of 6-Acetyl-6-methylmorphine

A 1.00 g portion of methoxymethylmorphinone (0.306 mmole, m.p. 129-130°C) was added over a period of 15 minutes to a cold solution composed of 100 ml of dry ether and 20 ml of 0.93 M methyl lithium. After stirring the solution for one hour in the cold, a test for excess methyl lithium was taken (using Michler's ketone).

This constitutes a method of determining the presence of Grignard or alkyl lithium compounds and is carried out as follows: A sample of 0.5-1.0 ml of the solution to be tested is placed in dry 1.2 x 10 cm test tube containing an equal volume of 1% solution of Michler's ketone in dry benzene. About 0.5 ml of water is carefully added and is followed by enough (a few drops) of a 0.2% solution of iodine in acetic acid to give a clear two phase solution. Finally, 2 ml of glacial acetic acid is added and the solution is well mixed. The mixture is allowed to settle and the color noted. A positive test will give a characteristic blue-green color; a negative test is a shade of pale yellow.

The test indicated the presence of excess methyl lithium.

A 2.00 ml portion of distilled acetic anhydride (21.3 mmoles, b.p. 137°C) was added with stirring over a period of 10 minutes. A white material precipitated from the solution upon the addition of the acetic anhydride. The mixture was refluxed on the water bath (with stirring) for two hours.

The mixture was added to 100 ml of 0.1 M phosphoric acid buffer (pH 3.0) and the ether removed on the rotary evaporator. Some gummy dark material precipitated from the solution. The aqueous solution now was at pH 5.0 and was adjusted back to 3.0 (some gummy material still remained). The gummy material was very soluble in chloro-

form. The chloroform solution was extracted with two equal portions of buffer. Evaporation of the washed and dried chloroform solution yielded 66 mg of red tar.

The buffer solutions were combined and refluxed in a nitrogen atmosphere for six hours. The rate of hydrolysis was followed using the ultraviolet spectra to indicate the amount of phenol formed.

The buffer solutions were washed with three 30 ml portions of chloroform. The pH of the aqueous solutions was adjusted to 8.7 with potassium hydroxide solution and then extracted with four 50 ml portions of chloroform.

The combined chloroform extracts were washed with water, dried over sodium sulfate, and evaporated to yield 1.00 g of dried white solid (88%, m.p. 220-221.5°C with decomposition).

A 100 mg sample of the above white solid was easily recrystallized (twice) from ethyl acetate to give long needles. An analytical sample was prepared by subliming the recrystallized material at 140°C, 0.01 mm to give a white solid having a m.p. 244-245°C.

The infrared spectra is very different when compared to 3-acetyl-6-methylmorphine.

Anal. Calc'd for $C_{20}H_{23}NO_4$: C, 70.36; H, 6.79; Acetyl, 12.6

Found: C, 70.32; H, 7.06; Acetyl, 13.0

$[\alpha]_D^{25}$ -212° (c, 0.65, methanol)

Preparation of 3,6-Diacetyl-6-methylmorphine

A 96 mg portion of recrystallized 6-acetyl-6-methylmorphine (0.281 mmole) was dissolved in a sdution of 1.0 ml of acetic anhydride and 2.0 ml of pyridine. This solution was refluxed in an atmosphere of nitrogen. Refluxing was discontinued after 1.5 hours since the solution has changed from colorless to dark orange.

The dark solution was evaporated in a vacuum using gentle warming. The thick oil was dissolved in 5 ml of chloroform and extracted with ten 5 ml portions of a pH 3.0 0.1 M phosphoric acid buffer.

The phosphate buffers were combined and the pH adjusted to 8.7 with potassium hydroxide solution. The adjusted solution was extracted with four 50 ml portions of chloroform which were then dried over sodium sulfate and evaporated to a light oil which crystallized on the addition of a drop of ethyl acetate. These were dried to yield 102 mg (94%). These crystals were much more soluble in ethyl acetate than the previous acetyl derivatives. The dried crystals were recrystallized from ethyl acetate-hexane. An analytical sample was prepared by subliming the recrystallized material at 125°, 0.01 mm, and drying for twenty-four hours at about 80°C, 0.01 mm. It had a melting point of 166-168°C, with decomposition.

The infrared spectrum had a doublet at 5.66 and 5.76 microns.

Anal. Calc'd for $C_{22}H_{25}NO_5$: C, 68.90; H, 6.57; C-CH_3, 12.1

Found: C, 69.34; H, 6.41; C-CH_3, 12.3

$[\alpha]_D^{25}$ -200° (c, 0.77, methanol)

Hydrolysis in basic solution shows the gradual formation of absorption at 300 mμ indicating the gradual formation of the phenolate ion.

Hydrogenation of 6-Methylmorphine

A 114 mg portion of 6-methylmorphine (0.38 mmole, m.p. 275-276°C) was dissolved in 10 ml of absolute ethanol. A 20 mg portion of platinum oxide was added to the hydrogenation flask. In 30 minutes 1.05 moles of hydrogen per mole of compound had been picked up.

The catalyst was filtered off and the solvent evaporated to dryness. This yielded 115 mg of dried white crystals. These were sublimed at 140°C 0.01 mm and dried at 101°C, 0.01 mm for fifteen hours, m.p. 204-206°C.

Anal. Calc'd for $C_{18}H_{23}NO_3$: C, 71.73; H, 7.69

Found: C, 71.5; H, 7.8

The last trace of moisture was hard to remove.

Reported for 6-methyldihydromorphine, m.p. 209-211°C, $[\alpha]_D^{20}$ -147° (c, 1.02, alcohol).[15]

Attempted Preparation of Thebaine from Codeinone

A 299 mg portion of codeinone (Chada) (1.00 mmoles) was dissolved in a solution of 5 ml of 3^{o} butanol, 0.01 ml of methanol, and 26 mg of sodium (nitrogen atmosphere).

At room temperature 0.095 ml of vacuum distilled methyl sulfate was added to the alkaloidal solution. At once dark coloration took place. The dark red mixture was left at room temperature for 45 minutes and refluxed on the steam bath for 20 minutes.

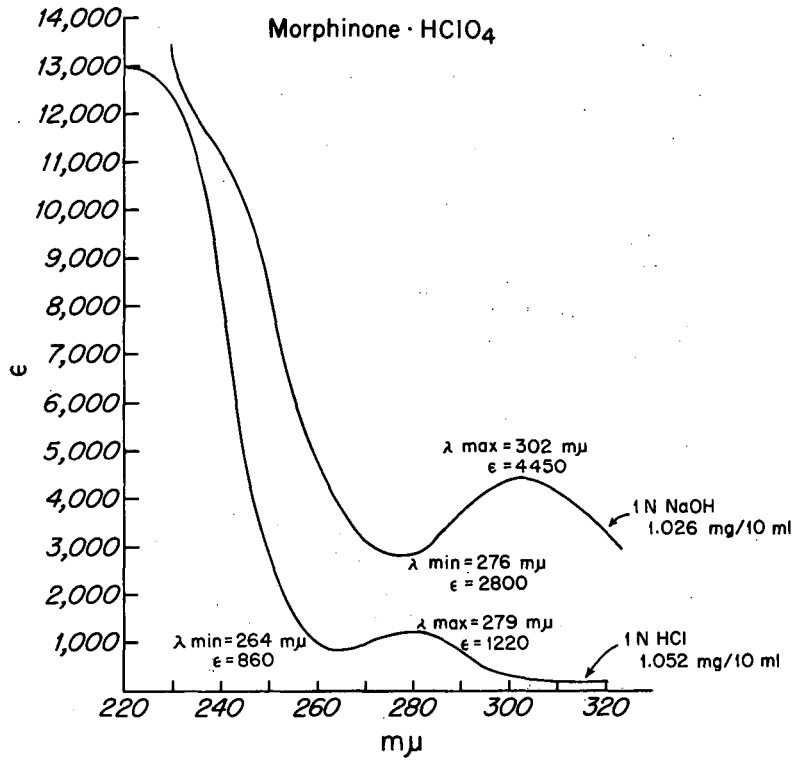
A 10 ml portion of water, containing two drops of ammonium hydroxide, was added to the cooled solution. The dark mixture was evaporated to half volume (205 mg of dark cinder-like material was filtered off). To the dark solution was added 30 ml of chloroform and 20 ml of bisulfite buffer. The organic phase was washed three additional times to remove all ketonic material. The bisulfite solutions were discarded.

The organic phase was washed, dried over sodium sulfate, and evaporated to yield 91 mg of dark material. The infrared spectrum of this material did not indicate any bands that are characteristic of thebaine. Not all of this material was soluble in benzene (10 ml, with heating). The mixture was added to an alumina column (10 g) but no useful material was recovered.

Attempted Preparation of 3,6-Diacetyl-6-methylmorphine by Two Phase Acetylation

A 54 mg portion of recrystallized 6-acetyl-6-methylmorphine (0.16 mmole) was dissolved in 0.5 ml of chloroform. In the cold, 0.020 ml of acetic anhydride (0.21 mmole) was added to the chloroform solution. Immediately 0.252 ml of 1 N sodium hydroxide was added and the mixture shaken for five minutes. One ml of water was added and the pH adjusted to 8.7 with potassium hydroxide solution. The layers were separated and the aqueous layer was washed with three 5 ml portions of chloroform.

The combined chloroform solutions were dried over sodium sulfate and evaporated to dryness to yield 57 mg of white powder. Addition of ethyl acetate yielded an oil and 17 mg of white crystals, m.p. 245-246°C.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12,000
11,000
10,000
9,000
8,000
7,000
6,000
5,000
4,000
3,000
2,000
1,000
ε
λ max = 302 mμ
ε = 4450
1 N NaOH
1.026 mg/10 ml
λ min = 276 mμ
ε = 2800
λ max = 279 mμ
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mμ
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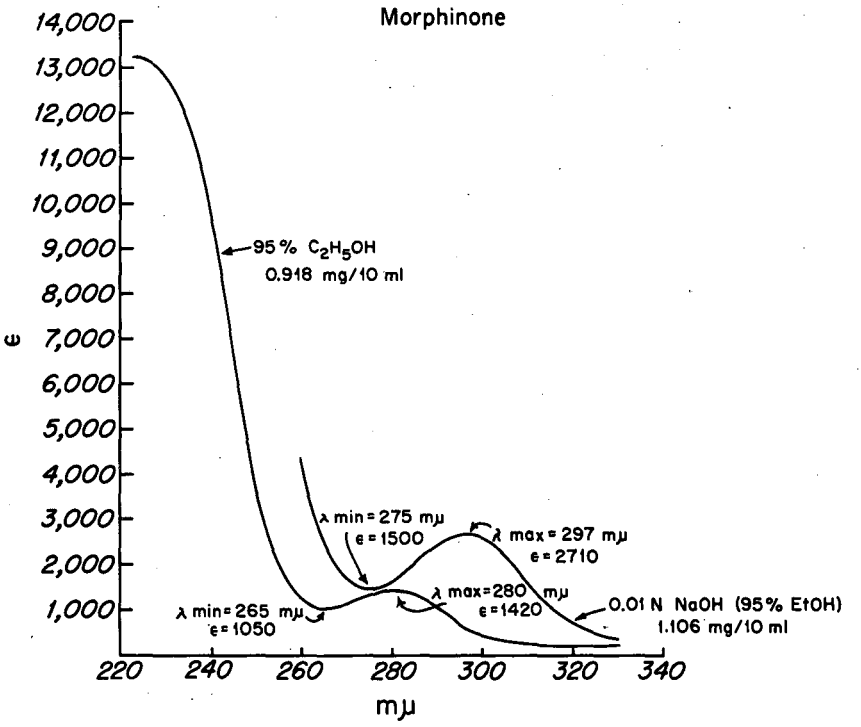
Morphinone
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13,000
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10,000
9,000
8,000
7,000
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5,000
4,000
3,000
2,000
1,000
ε
95% C2H5OH
0.918 mg/10 ml
λ min = 275 mμ
ε = 1500
λ max = 297 mμ
ε = 2710
λ max = 280 mμ
ε = 1420
λ min = 265 mμ
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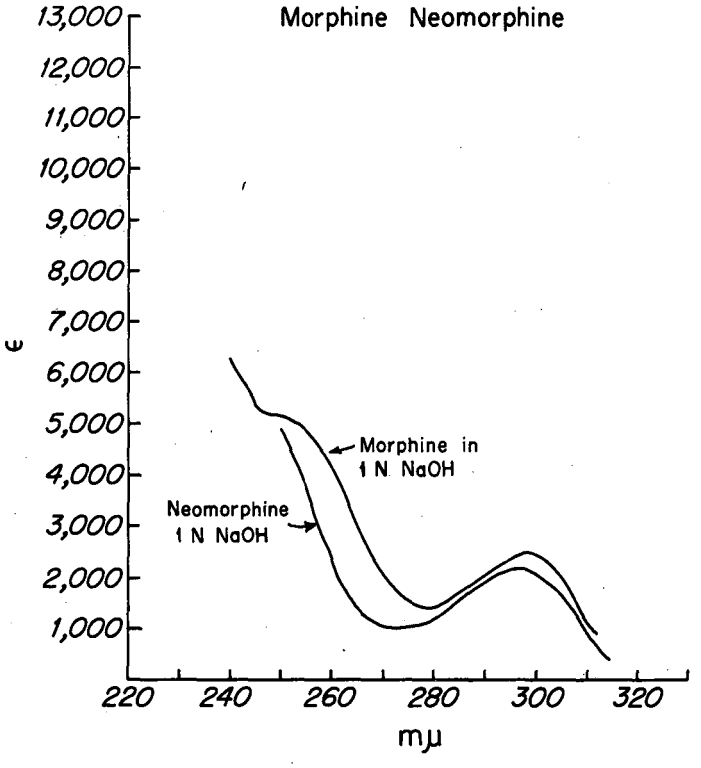
mμ

MU – 17034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2,000
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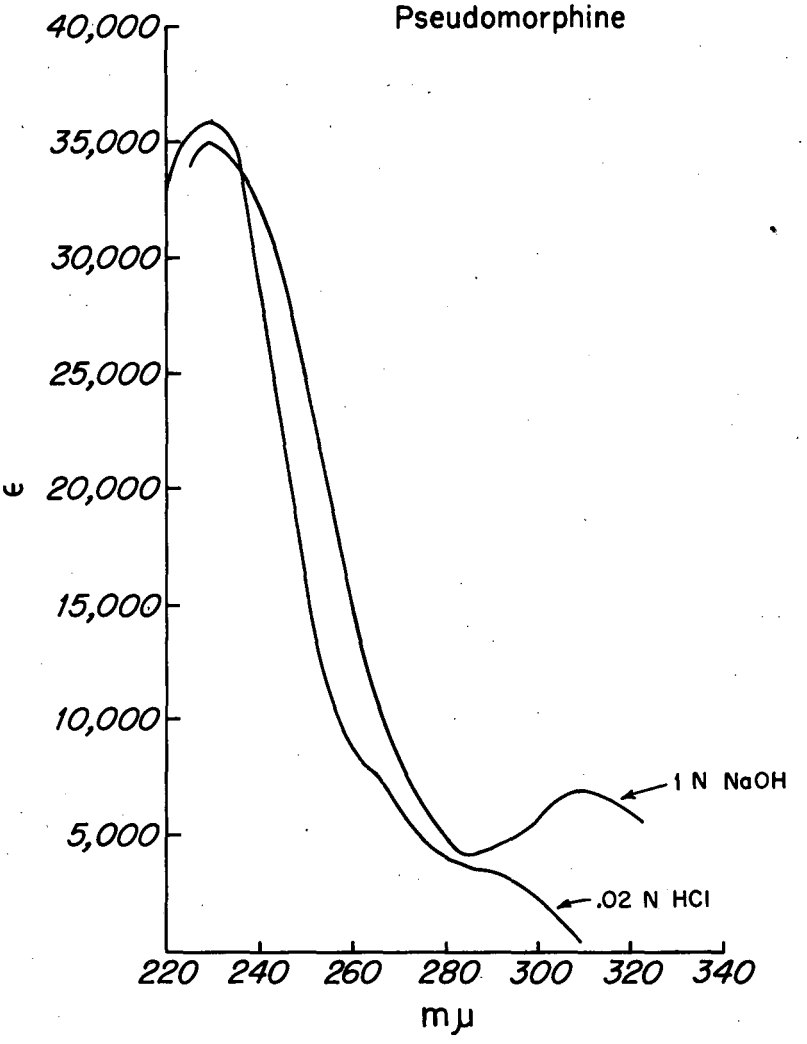
mμ

MU-17031


Pseudomorphine
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20,000
15,000
10,000
5,000
ε
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.02 N HCl
220
240
260
280
300
320
340
mμ

MU-17032

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II. PLANT STUDIES

INTRODUCTION

In 1805 Serturner[1] first isolated morphine from the opium poppy, Papaver Somniferum. Since then, this compound has been studied from many different aspects, particularly its isolation, production, chemistry, biochemistry, pharmacology, regulation, and control.

Since the time of the posulation of the correct structural formula of morphine by Gulland and Robinson[2, 3], many groups have attempted the synthesis of morphine by an unambiguous route. Early attempts at synthesis were based on a mode of biogenesis that was presented by Robinson[4]. These early postulates have been modified to meet the needs of the systems involved, but as yet very little work has been done to actually find the intermediate steps in the plants' synthesis of the opium alkaloids.

Prior to 1939, the separation and identification of intermediate compounds in photosynthetic plant processes was an almost impossible task using the classical methods of analysis then available. Despite the efforts of many able investigators, it was not until the advent of tracer-element methods of following the mechanism of carbon assimilation that any instrument showed promise of opening the door to this vast field of knowledge. The first work done in the field using this new tool was that of Ruben, Hassid, and

Kamen[5], who reported a method of tracer-element technique using $C^{11}O_2$ in following the mechanism of carbon assimilation in barley plants. This early work was still hampered by two major factors. One was the short half life of carbon-11 which precluded separation procedures requiring long times. The other difficulty was the lack of suitable methods for separating the complex mixture of materials found in the plant. The first difficulty was overcome by the discovery that the radioactive carbon-14 isotope could be made by an (n, p) reaction from N^{14} and it became possible to produce C^{14} in quantities which were adequate for biological tracer studies through the development of high neutron fluxes in nuclear reactors.

It was the rediscovery and development of an old process first reported by the Russian botanist Tswett[6] that helped in the development of methods of separating complex plant materials. Tswett was the first to be aware of the great possibilities of chromatography other than just a means of filtration through finely divided adsorbents. Partition chromatography on silica gel was introduced by Martin and Synge[7] in 1941. Paper chromatography was first described by Consden, Gordon, and Martin[8] in 1944. It was the combination of C^{14} isotope tracer techniques and chromatography that opened the door to the vast field of knowledge concerned with plant photosynthesis.

In 1950 McIntosh, Kelsey, and Geiling[9] reported their findings in growing the opium poppy under $C^{14}O_2$ in order to obtain radioactive morphine for metabolic studies. They were not interested in the mode of biosynthesis

of the morphine but only in obtaining radioactive morphine.

The intent of this present study was to grow the opium poppy under $C^{14}O_2$ and isolate the various opium alkaloids. In order to do this, a suitable chamber had to be constructed and a method developed for the separation and purification of the small amount of each alkaloid found in the poppy. Also, a means of degradation of the various alkaloids had to be developed in order to find the centers of highest radioactivity in the various alkaloids.

DISCUSSION

Description of the Opium Poppy

The opium poppy, Papaver somniferum L., belongs to the Papaveraceae family. The Papaveraceae family is dicotyledonous, dialypetalous, and superovaried. The family itself is of a simple classification. This is not true of its internal classification. The number of genera and the number of species within each genus vary according to botanists[10].

Much work has been done in order to find which plants contain alkaloids that come under national and international control. With the exception of the alkaloids, protopine, cryptopine and thebaine, none of the alkaloids of Papaver somniferum is found in any other plant. Farmilio, Rhodes, Hart and Taylor[11] analyzed 25 species (subspecies or varieties) of poppies closely related to Papaver somniferum by a method which they describe in their paper. They found that only Papaver somniferum and Papaver setigerum (a wild species of poppy of Western Europe) contain morphine.

Since Papaver somniferum is a cultivated plant and has been grown commercially over most of the world from latitude 56°N. to the equator, it is natural that many variations would occur, according to the varied climate and soil conditions. Also many hybrids have been developed by intervention of man through cross pollination which brings about many distinct varieties.

With this thought in mind, it should not to too startling to find that the opium poppy has varieties that are so different from one another that at first glance they would hardly be thought to belong to the same species. The flower may be single or double and the petals either plain or fringed. The petals may be white, pink, lavender, red, purple, or violet, or various combinations of these. The seeds may be black, brown, purple, pale or dark blue, gray or slate colored, red or pink, yellow, or white. There is very little connection between the color of the seeds and the general color of the flowers; possibly more between the seeds and the color of the spots at the base of the petals as to whether light or dark[10]. The capsules may be of different shapes and no relation can be detected between the shape and the alkaloid content. On the other hand, when the latex is being collected by means of incisions in the pod, some shapes are easier to incise than others. The capsules may be either open or closed and there are as many as two, three or more capsules on each plant. The plant may vary in height from 30 to 150 cm. or more when fully developed. The stems may be glabrous or hairy and the leaves may have various shapes.[12] But the one thing that you can say for all of them, no matter what the shape or color, they all contain morphine to a greater or less degree.

Since the poppy adapts itself very well to different types of soils and climatic conditions, horticulturists have succeeded after many years of trial in developing varieties that are well adapted to the soil and climate of

a particular region. Efforts have been made in developing closed capsule varieties in Turkey, for instance, since the morphine content is generally two or three percent higher in this variety than those with open capsules[13]. This may be due to leaching by water.

Cultivation of the Plant

The opium poppy will grow under varying conditions, but cannot endure extreme cold. In a cold climate the opium yield is greatly reduced. The humidity affects the yield to a great extent. In damp climates it is attacked by the pernospora and other plant diseases. For opium or extraction of alkaloids from the chaff, it is best to grow the poppies in a dry region where they can be irrigated, since the rain may leach the morphine out of the dry capsules. The poppy is sensitive to wind because the capsule is comparatively heavy and may be blown down by the force of the wind[10]. For the best results the soil should be a good loam, and fertilizing is advisable. In the early stages the ground should be kept well hoed to prevent the weeds from competing[14].

The poppy is an annual and may be sown in autumn or spring, the determining factor being the climate. The most important point is the alternation of the rainy and the dry period. In poppy growing areas the sowing time may therefore begin in September and continue until April; but the autumn poppy runs greater risks than the spring poppy if the winter is

severe[10]. The poppy was grown in California prior to 1944 for its seed[15].

The growth of the opium poppy at Hoxey on the Isle of Axholme[14] for the seed's oil is an example of its general cultivation. Seeds are sown at the end of February using a carrot drill, with about two pounds of seed per acre in rows about one foot apart. Later the young plants are thinned to about five inches apart. The plants flower about the beginning of July and the capsules are ripe enough six to eight weeks later for the oil to be extracted from the seeds.

Germination requires about two to three weeks. About a month later the first four leaves appear, and about two or three weeks later the stem begins to form. The plant reaches full development in about two months. The flowering season varies according to the climate, the sowing date, and other conditions. The plant flowers by day. The flowers last 30 to 40 hours. After the petals fall, the capsules continue to grow and are ripe in about two weeks[10]. It will be noted that the ripening date is different, depending on whether the plant is grown for its seeds' oil or for its alkaloids. Guillaume and Faure[16] state that the oil content of the seeds increases as the morphine content of the heads decreases.

Larvae of insects, such as the beetle, locust, spider, mite, etc., which destroy the roots, leaves, and capsules, are its natural enemies. A light dusting of the plants with California Spray Chemicals Ortho brand Rose Dust has been found to be effective in controlling powdery mildew and small insects.

When the capsules are ripe they may be incised, allowing the latex to flow from the husk of the capsule. This dried latex may later be collected. This is the method in use in countries where labor is cheap. Australia[17] uses a harvesting method whereby the tops are chopped off and the alkaloids are extracted from the crushed capsules and stems.

Geiling[9] and associates have grown the poppy in carbon-14 labeled CO_2 to produce radioactive morphine with an activity of 84×10^3 d.p.m./mg of morphine.

Prior to 1955 all of the investigations concerned with the growth of the opium poppy were those in which conditions were found for best growing the poppy outdoors in a certain climate. Mika[18] tried to resolve the difficulties encountered by those at the University of Chicago[19, 20], who were trying to prepare radioactive morphine. He made an attempt to correlate the growth and development and morphine content of plants grown under usual greenhouse conditions with those of plants grown under controlled temperatures and photoperiods. He found that the total morphine content was apparently increased by low night temperatures. His plants, grown at 65°F day temperature and 48° night temperature, contained the greatest total amount of morphine as a result of increased dry weight production.

He found that for his poppies the following culture conditions are indicated for optimal production of morphine in biosynthesis studies:

"After germination of the seeds at 70-80°F, seedlings are grown on an eight hour day at 60-70°F, utilizing natural sunlight. Induction of flowering occurs as a result of exposure to fourteen 18-hour days at 60°F when thirty to thirty-five leaves have formed. Floral induction and total leaf number are determined by microdisection. Plants are then transferred to the biosynthesis chamber, which is maintained on an 18-hour day at 60-65°F. Sunlight is supplemented by twelve 40-watt G.E. Deluxe Warm White lamps mounted inside the chamber. Radioactive morphine with a specific activity of 0.5 mc per gram of carbon has been obtained from Yuma poppies treated in this manner." For a more complete discussion of his work, his paper should be consulted.

Alkaloid Content of the Opium Poppy

Many different analyses have been made on the dried latex (or opium) of the poppy. Fulton[10] gives a summary of many findings up to 1944 on the alkaloid content of the plant and its opium. The Pharmacopeia of the United States XV[21] states that the dried opium should contain not less than 9.5% of anhydrous morphine, and gives a description of the material as it is received commercially. It also lists a method of analysis for morphine content in opium. Henry[22] gives a list of references to papers published in the twenty years prior to 1949 on the estimation of morphine, arranged under the following headings: "(a) opium, (b) galenical preparations, (c) poppy plants (poppy

straw and poppy heads), (d) preparations of morphine, (e) biological materials." In Table I are listed what Fulton[10] considers as the range of percentages of the various alkaloids found in opium. He states that the alkaloids vary so independently of one another that the averages are often meaningless.

He lists the other alkaloids that are present to a smaller degree as being porphyroxine, meconidine, cryptopine, protopine (or fumarine), pseudomorphine, codamine, lanthropine, laudanine, laudanidine (l-laudanine, tritopine), laudanosine, oxynarcotine, gnoscopine (dl-narcotine), hydrocotarnine, neopine, papaveramine, papaveraldine (xanthaline), and narcotoline.

The alkaloids of the plant are the same as those of opium, since the opium gum is the dried latex of the poppy plant, except that some of the minor alkaloids which occur only to a small extent may be formed from the major alkaloids by air oxidation.

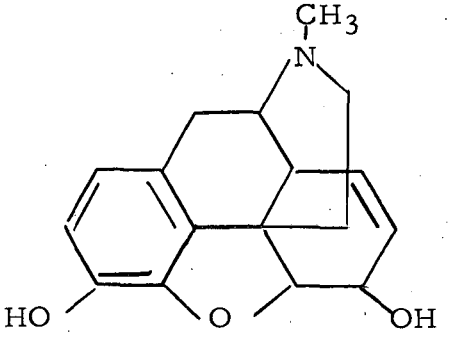
Schmid and Karrer[23] examined the water-soluble material of the plant after the alkaloids had been extracted. They found:

p-hydroxybenzaldehyde	p-hydroxybenzoic acid
vanillin	vanilic acid
p-hydroxystyrene	2-hydroxy-cinchoninic acid
fumaric acid	phthalic acid
dl-lactic acid	hemipinic acid

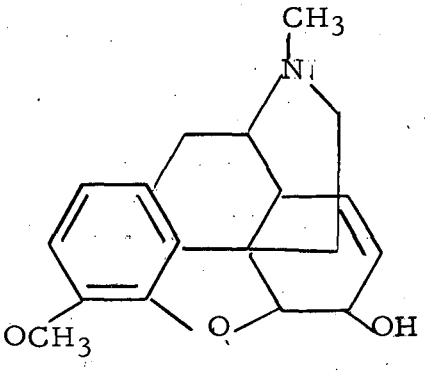
TABLE I

MAJOR ALKALOIDS FOUND IN OPIUM AND APPROXIMATE PERCENTAGES OF EACH

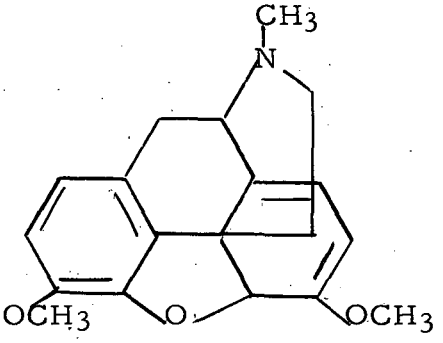
MORPHINE TYPES



Morphine
(3 - 23%)

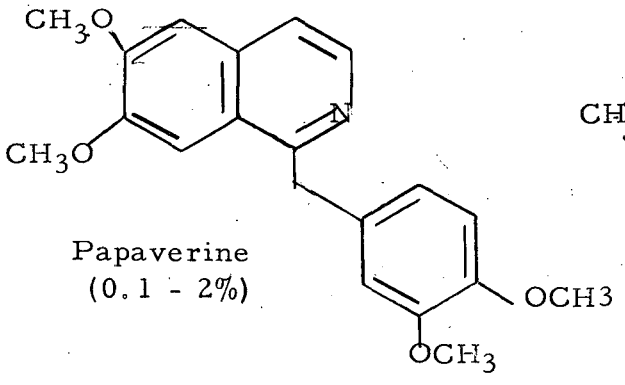


Codeine
(0.1 - 4%)

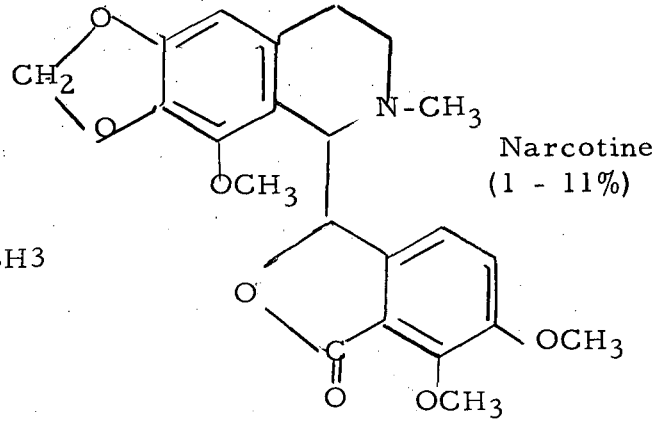


Thebaine
(0.1 - 4%)

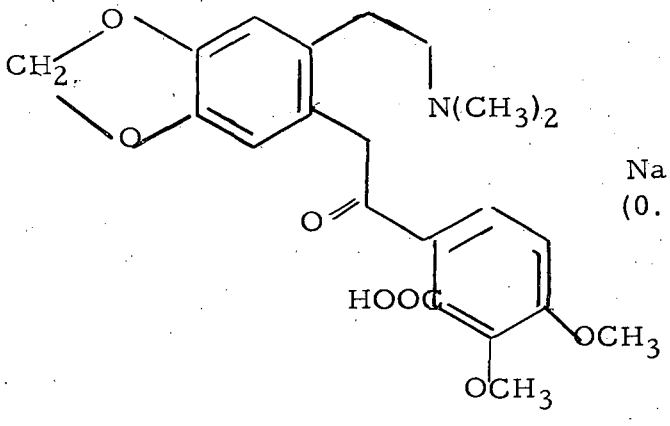
BENZYLISOQUINOLINE TYPES



Papaverine
(0.1 - 2%)



Narcotine
(1 - 11%)



Narceine
(0.1 - 1%)

benzoic acid m-hemipinic acid

p-hydroxycinnamic acid meconin

and a higher unsaturated carboxylic acid "J", and four unknown substances, "Fx", "Q", Wx", and an odorous material.

Morphine Content of the Plant during Growing Period

Poethke and Arnold[24] studied the morphine content of the plant during the growing period. The made observations over a period of years on various varieties. They found the following general percentage contents in various parts of the plant during the growth cycle (and provide their results by means of graphs that are readily understood):

Roots:

young plants -- 0.34 to 0.45% (0.39% average)

old plants -- 0.09 to 0.19% (0.13% average)

The morphine content decreased fairly regularly with growth.

Leaves:

The morphine content in the leaves increased with growth until the time the buds formed. At that time the leaves had a morphine content of 0.10 to 0.24% (0.20% average). The morphine content then decreased to 0.03 to 0.05% at the time of harvest.

Stalks:

At the time of bud formation the morphine content in the stalks was 0.14 to 0.34% (0.25% average). This content increased to 0.34 to 0.49% (0.42% average) at the time of the formation of the green unripe capsules. Content then rapidly decreased to 0.08 to 0.15% (0.11% average) at the time of harvesting.

Capsules:

The morphine content seemed to vary during the different years according to the weather. All plants in 1949 showed the highest morphine content in the fully ripened (brown) capsules, 0.38 to 0.40% (0.386% average). In 1948 the highest content was in the half-ripe (green) capsules, 0.39 to 0.60% (0.513% average).

Fuchs[25] reports the morphine content as being 0.25% for the unripe capsule; half-ripe capsule, 0.4%; and fully ripe, 0.3% (and no alkaloid content in the seeds).

Hills[17], of Australia, determined the percentages of morphine in the capsules of various varieties after the time the petals had fallen. He found that the percentages were higher just after the petals had fallen, but that the capsules had not yet reached their full weight. He found that the husks of the opium poppy increased in dry weight during the first two

weeks after petal fall, and then decreased 10% during the third and fourth weeks. The absolute weight of the morphine reached a maximum sometime after the 14th day after petal fall, the exact stage being dependent on seasonal conditions and upon the variety. Also, the morphine was concentrated in the upper half of the stem, and decreased approximately in a geometric progression in successive sections from the base of the capsule downwards.

Küssner is quoted in Chemical Abstracts[26] as saying that the "total alkaloid content seems to be a hereditary immutable property." He investigated and cultivated nine different varieties of Papaver somniferum. Two varieties, Prof. Freudl's Liebwerder blue poppy and Dauber silver poppy, had a reported yield of 116 and 114 g. of seed-free, dried capsules per sq. meter; and 0.471 and 0.440 g. of morphine per sq.meter, respectively. Henry[22] reporting on the same work of Küssner, reports that from seven commercial varieties grown in plots of 50 sq.meters, the yield in grams per sq. meter were

seeds:	141 to 200 g.
capsules:	65 to 116
morphine:	0.123 to 0.471 g.
nonphenolic bases (codeine, thebaine, papaverine, narcotine, etc.):	0.043 to 0.131 g.

Alkaloids of the Opium Poppy other than Morphine

Korbosch[27], over 35 years ago, made one of the most thorough studies of the alkaloids in the plant during various times in its growth period. Most of his results were only qualitative, but in each case a positive and sure identification was made of each alkaloid. In the young plants of the variety "Smyrna dunkel" narcotine could be detected after 12, 16, 20, and 25 days growth. Plants older than 30 days (32 to 42 mm. long) also contained codeine. Those older than 36 days (5 to 7 cm. long) also contained papaverine and morphine. These four alkaloids were also found on the 38, 44, 48, and 51st days of growth. In an analysis on the 52nd day (15 to 25 cm. long), narcotine, codeine, and morphine were found in the roots; and narcotine, papaverine, codeine, and morphine were found in the leaves and stems. Plants, grown from seed from the firm of Hagge & Schmitt of Erfurt, contained narcotine, codeine, and morphine when they were only 21 days old and 4 cm. long. He summarizes his findings by stating that:

> Narcotine and a small amount of amorphous alkaloid was present as a mere trace in the ripe seed. After three days growth, narcotine content was found to increase in the small sprouting seeds.

The alkaloids appeared in the following order in the plant:

narcotine, codeine, morphine, papaverine, and thebaine.

The blooming plant contains narcotine, papaverine, codeine, and morphine in all its organs, except the stamens, up to the time of maturity.

The appearance of the milky juice was not the same in all parts of the plant.

The grown plant contained narcotine, codeine, and morphine in all its organs.

Seeds which were germinated in a nitrogen-free ground also form narcotine.

The narcotine which was developed by the sprouting seeds originated from the seed's protein reserve.

Narcotine was present in the very young plants in a fairly large amount. The amount was much larger in the flower bud than in the unripe seed capsule.

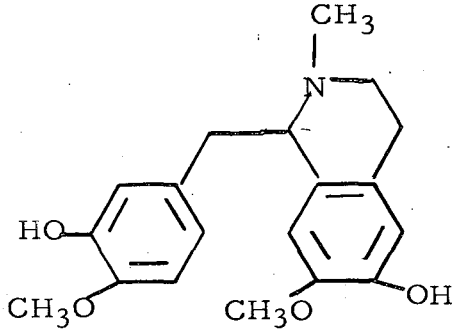
Italie[28] reported findings similar to Kerbosch, but he stated that the order of appearance in the plant is narcotine, codeine, morphine, papaverine, narceine, and thebaine. (Kerbosh didn't list narceine in his findings.) In one variety that he studied he detected an amorphous alkaloid, codeine, and morphine in plants 3 to 3.5 cm. long (15 days old). Twenty different samples of opium of different origins were tested for

narcotine, papaverine, narceine, thebaine, codeine, and morphine. Narcotine was absent from French "Bernard" and "Leyden black poppy" opiums. Papaverine was absent from two types of "Bengal" opium and was also not found in "Patna" and "Benares" opium. The other four alkaloids were detected in all samples. (Could this work by Italie be the same as that reported by an Italie[29] in 1910?)

The seeds of the poppy have been examined quite a number of times by U.S. Treasury chemists and no morphine could be found in them. They contained mere traces of basic material which could not be identified with the alkaloids known in opium.[10]

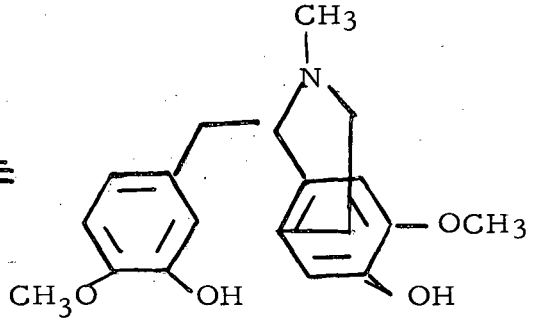
Biogenesis of Opium Alkaloids

The first attempt at a mode of biogenesis after the verification of the Gulland and Robinson structure[2, 30] was by Robinson and Sugasawa[4]. They suggested a mode of biogenesis involving the union of two aromatic nuclei of a base of the laudanosine type of benzyltetrahyd roisoquinoline structure (I).



(I-A)

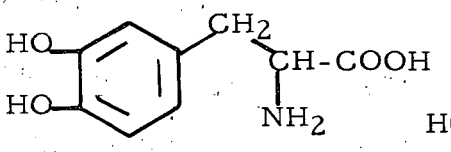
≡



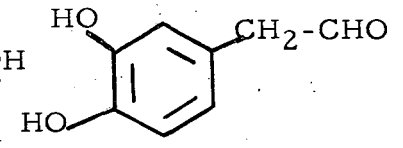
(I-B)

From (I) there are four different possible ways for the two aromatic nuclei to unite. From (I-A) there are two aporphine type bases and from (I-B) there is the possibility of sinomenine and an isomer of sinomenine. (The three previous types of compounds are known to occur in nature, but the isomer of sinomenine has not been isolated.)[31]

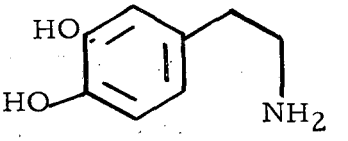
This structure (I) could arise from two moles of 3,4-dihydroxyphenylalanine (II) which could, in part, be converted to 3,4-dihydroxyphenylacetaldehyde (III) and also to β-(3,4-dihydroxyphenyl)-ethylamine (IV), and these two fragments later uniting to form (V).[31'] At what stages the N and O methylations might occur is not known[4, 32].



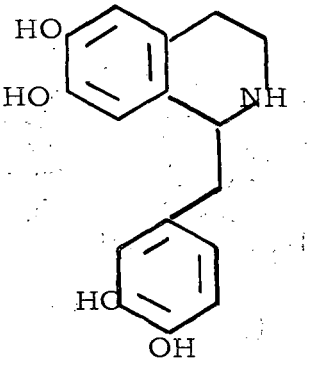
(II)



(III)

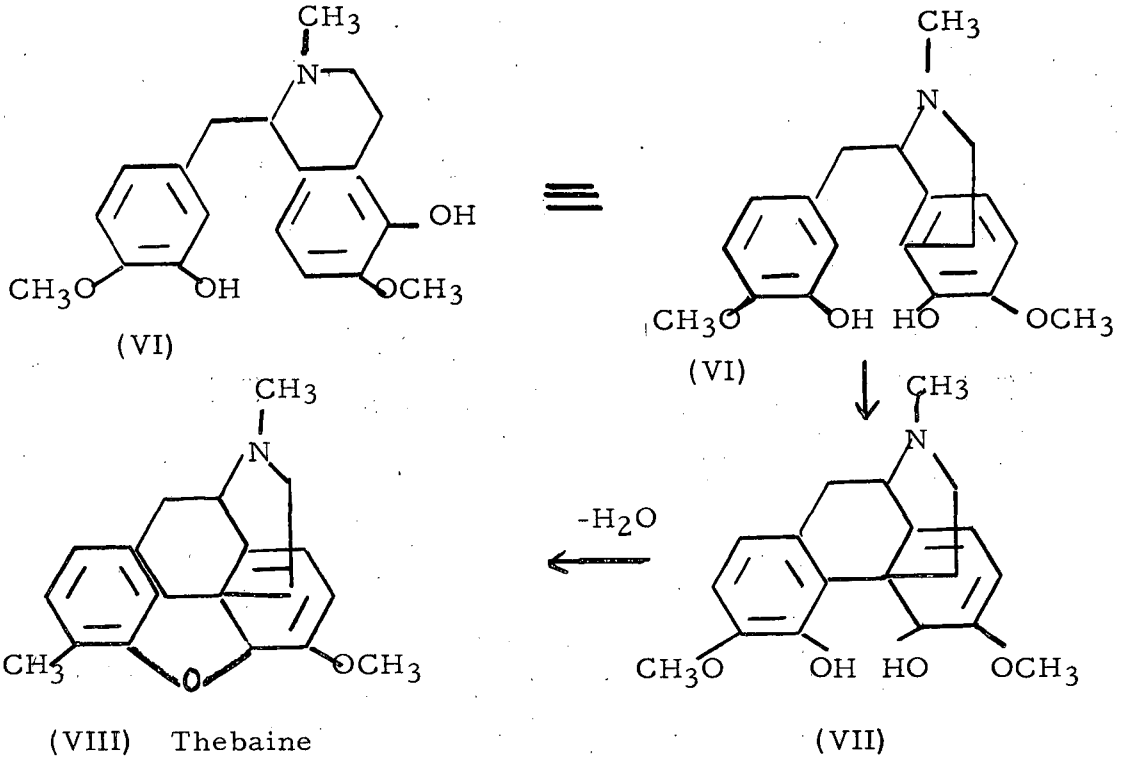


(IV)

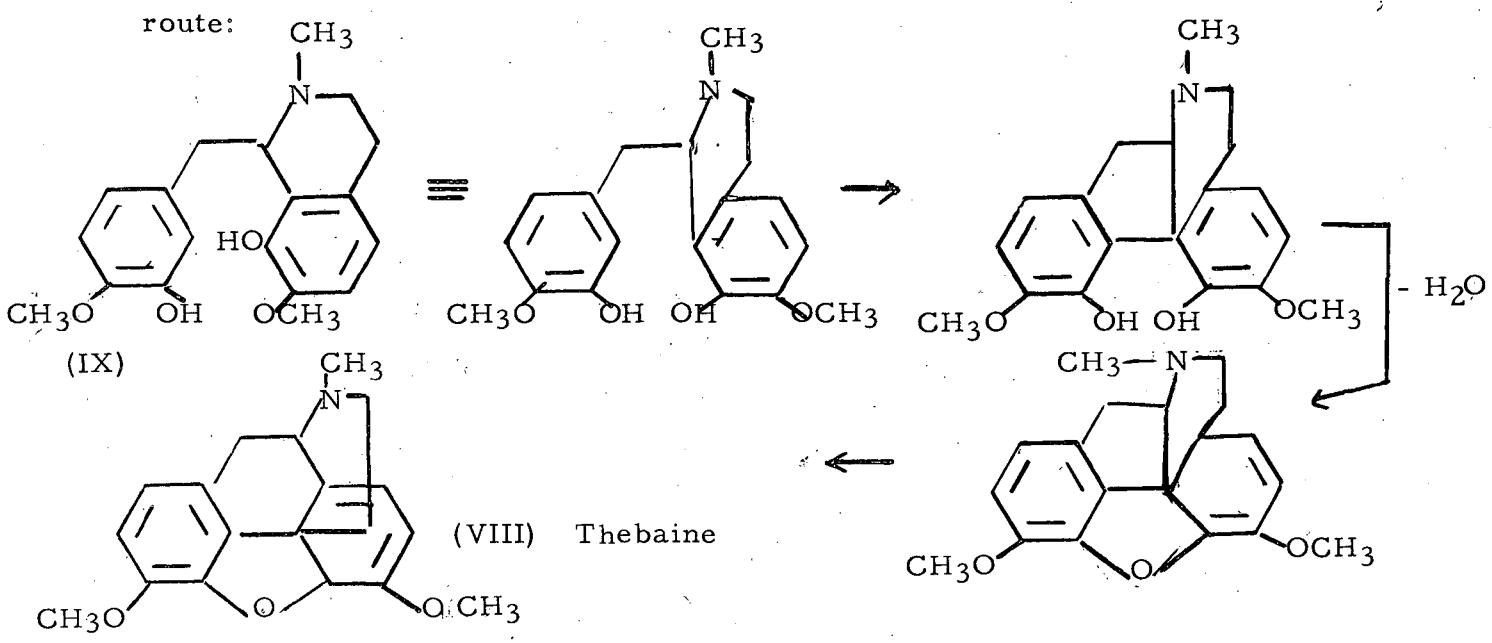


(V)

The biogenesis of thebaine may go by this same type of route as is shown in (VI) to (VIII), but the isoquinoline precursor (VI) has an arrangement of substituents not found in nature and would require two different fragments as compared to just one in structure (I).

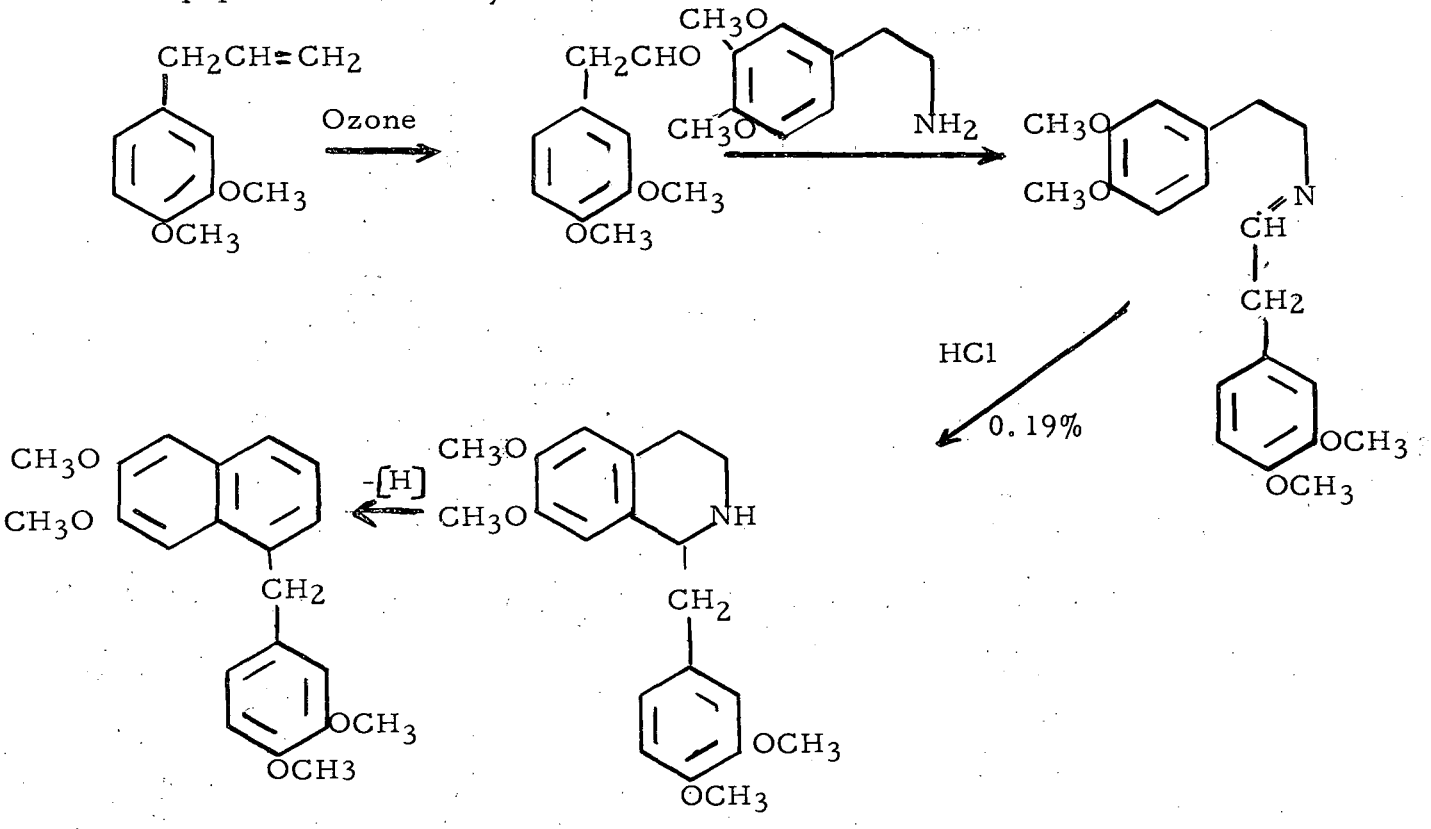


For this reason Robinson and Sugasawa[4] suggest the following possible route:



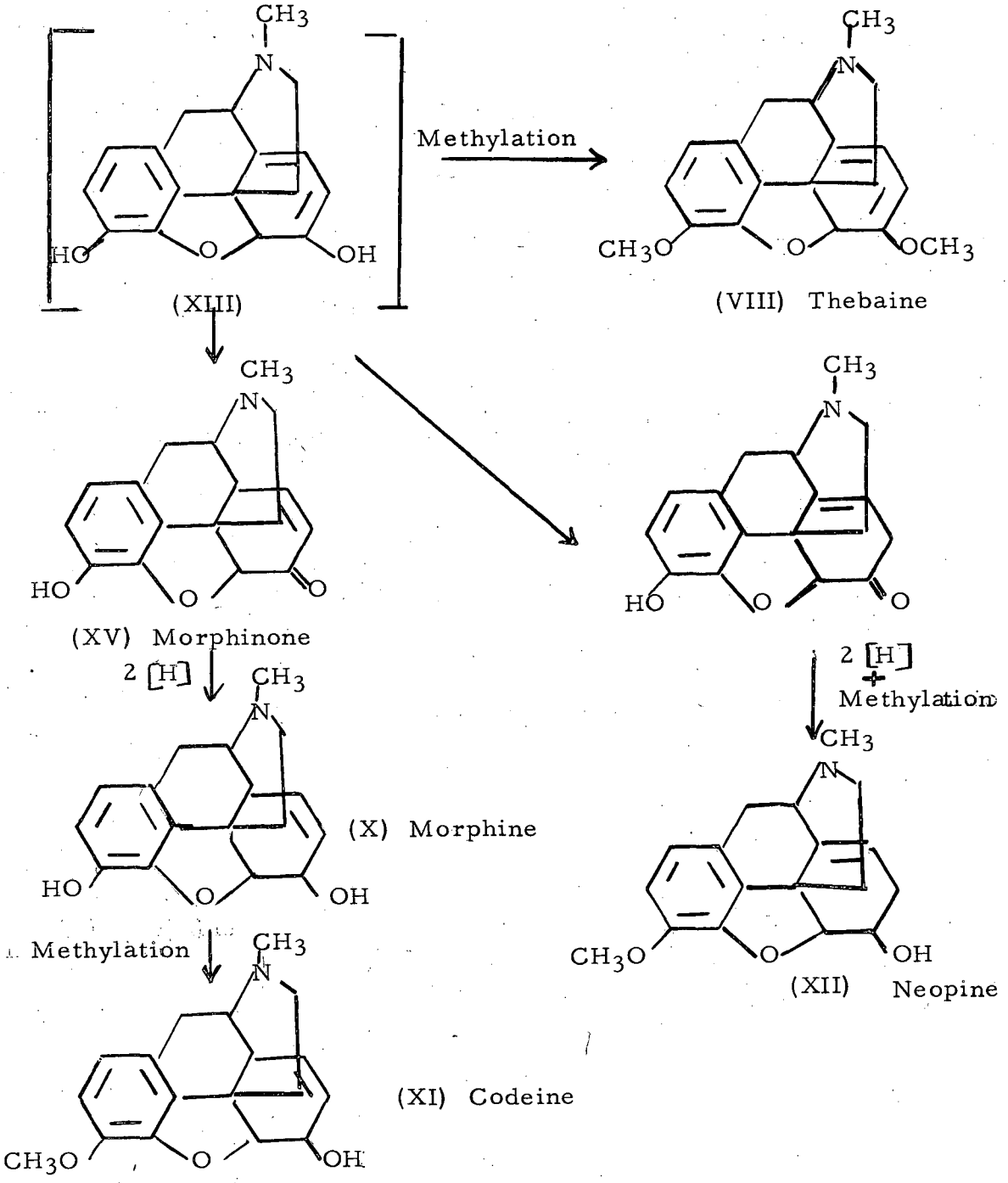
In this case (IX) might be made in a manner similar to (I) except that ring closure would have to take place ortho to the OH instead of para. Attempts to follow experimentally this method of conversion of laudanosine - type bases to bases of the aporphine and morphine series have met with failure.[33]

Spath and Berger[34] in 1930 presented a scheme for the "biogenesis" of papaverine. Briefly it is as follows:

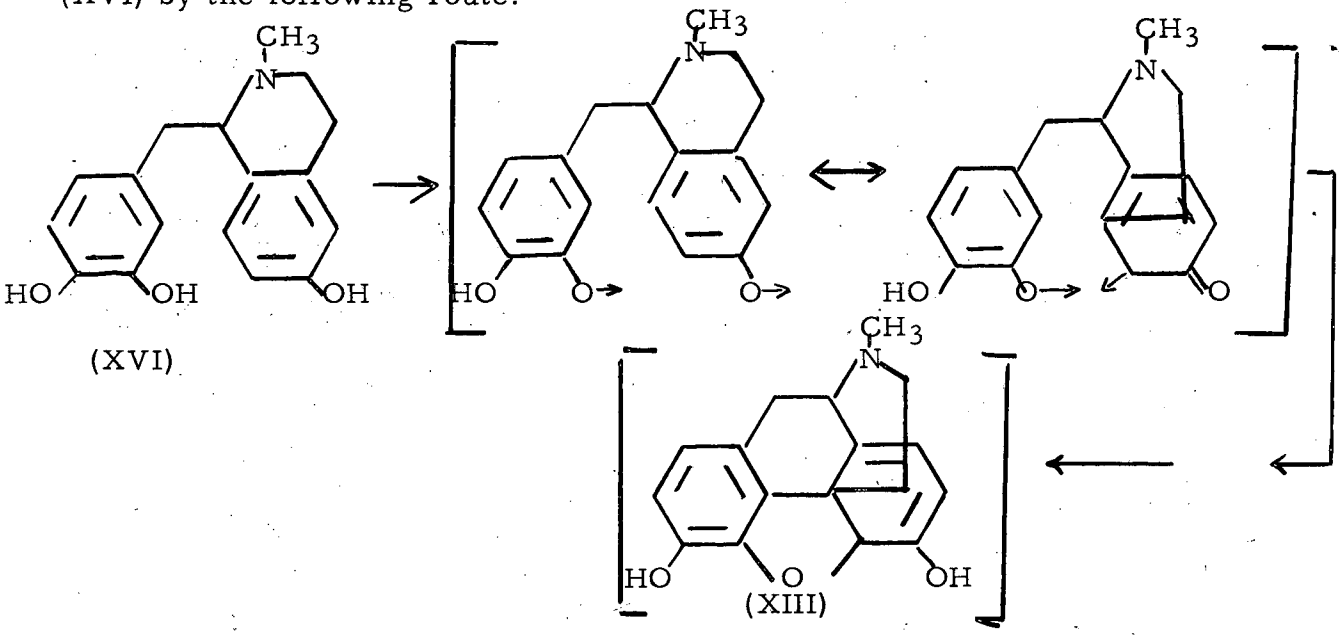


A more recent approach to the biogenesis of morphine has been presented by Schopf[35] who starts with the enol form of morphinone (XIII). Methylation of the enol would lead to thebaine (VIII). From the enol one

could get morphinone (XV), which could be reduced to morphine (X), which could be methylated to codeine (XI). Also neopine (XII) could be made by 1, 2-ketonization of the enol followed by reduction and methylation.



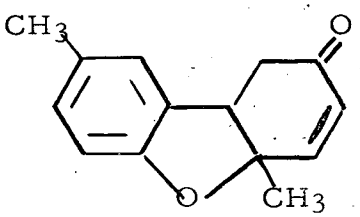
Schop believes that (XIII) could possibly result from the oxidation of 1-(3':4':-dihydroxybenzyl)-6-hydroxy-2-methyl-tetrahydroisoquinoline (XVI) by the following route:



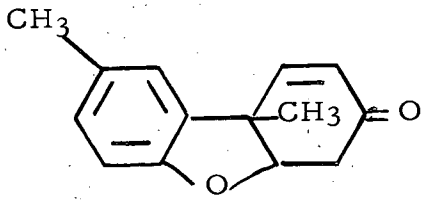
(XVI)

(XIII)

The basis of this ring closure was proposed on the basis of the presumed oxidation of p-cresol to the ketone (XVII). However, Barton and his co-workers[36] have since shown that the product of oxidation of p-cresol has the structure (XVIII) not (XVII).

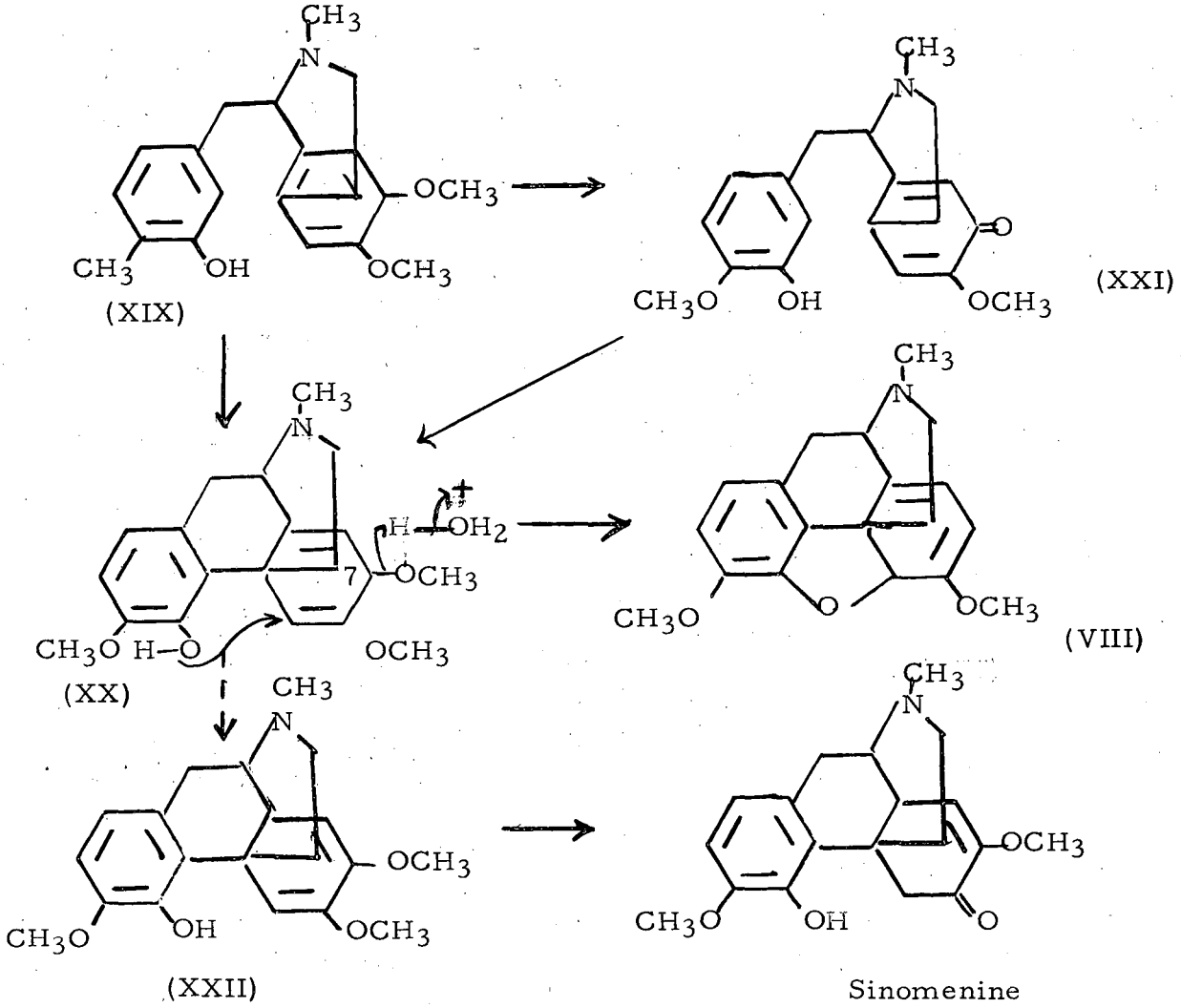


(XVII)

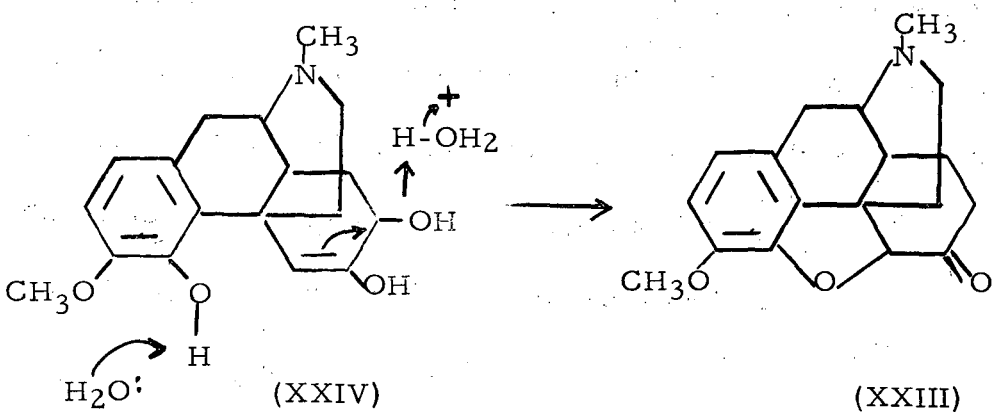


(XVIII)

Bentley[37,38], however, proposed a mode of biogenesis of thebaine (VIII) from laudanine (XIX) via (XX), which may be formed directly or via (XXI); closure of the oxide bridge is represented as the allylic expulsion of methoxyl from position 7 (XX). Migration of the 8:14 double bond of (XX) would give (XXII), in which this type of expulsion could not occur, and hydrolysis of (XXI) would then afford sinomenine. In this manner, concludes Bentley, thebaine (and from it the other morphine type alkaloids) and sinomenine could arise from the two enantiomorphic forms of laudanine.

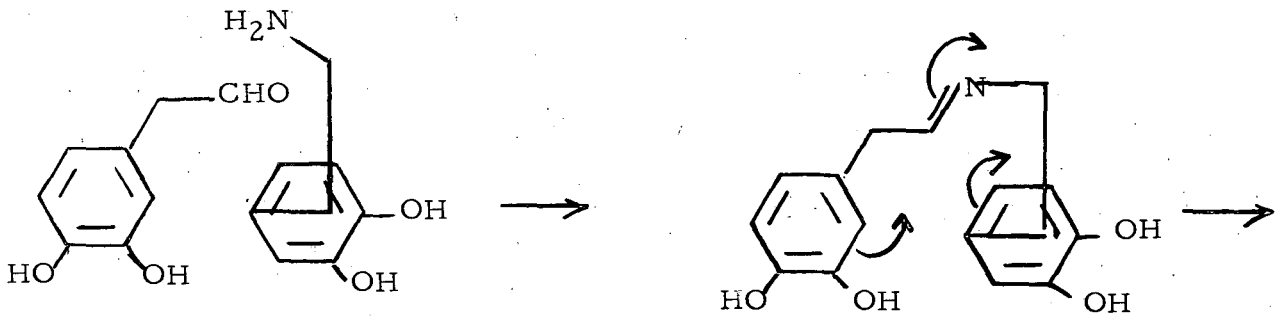


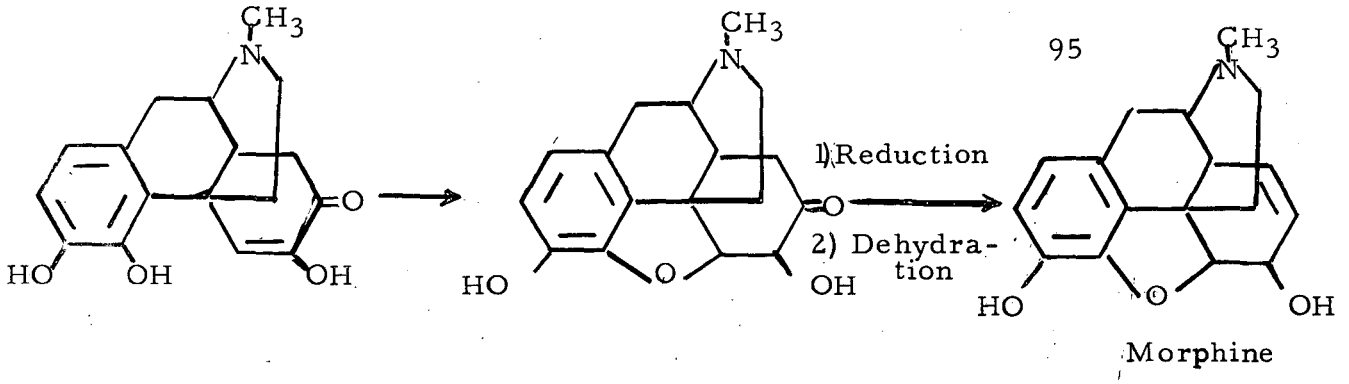
As an analogy for the expulsion of the group depicted in (XX), Bentley cites the formation of (+)-dihydrocodeinone (XXIII) from dihydrosinomeninone (XXIV) in acids.



(XXIV) (XXIII)

Cohen[39] describes a somewhat different approach to the biogenesis of the morphine type alkaloids. This is outlined in the following sequence of reactions:

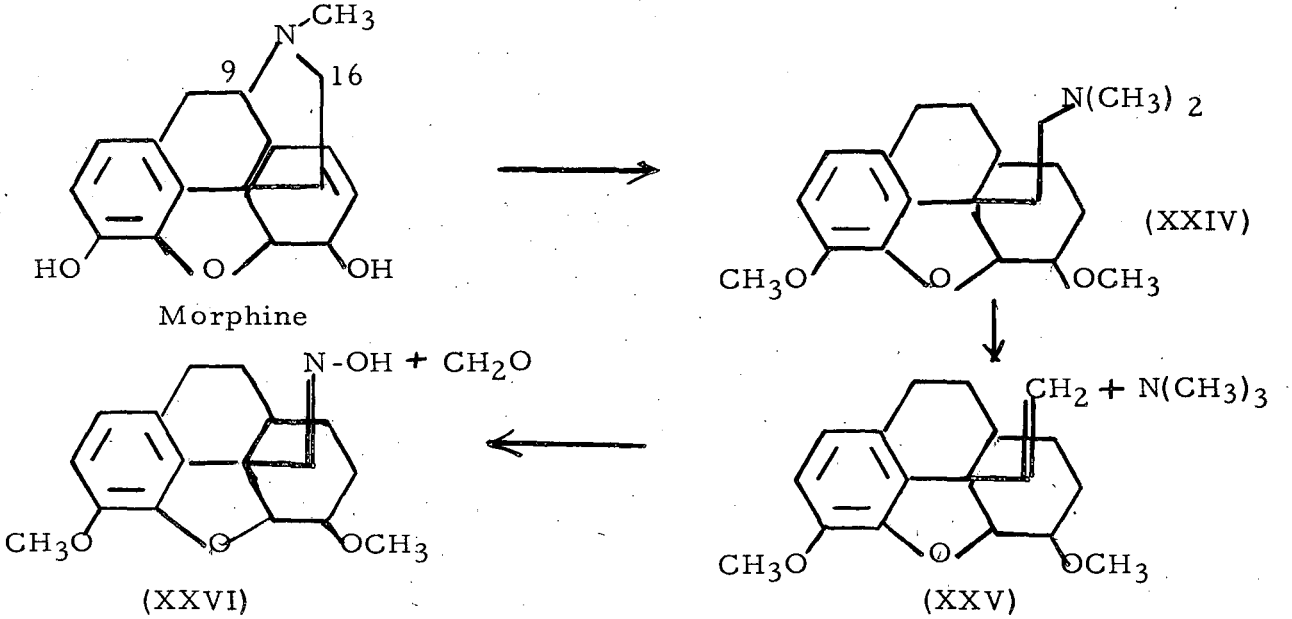




Morphine

Battersby and Harper[40], in trying to show a connection between the amino acids and morphine alkaloids, fed plants with α-C^{14}-DL-tyrosine and isolated the major alkaloids. They diluted the morphine with inactive morphine, which was further purified as the picrate, and finally had a constant activity of 429,000 disintegrations per minute per millimole.

The morphine was converted to codeine methyl ether methiodide which yielded α-codemethine methyl ether by Hofmann's method. Reduction of this base to tetrahydrocodeimethine methyl ether (XXIV), followed by further Hofmann degradation, gave completely inactive trimethylamine.



Morphine

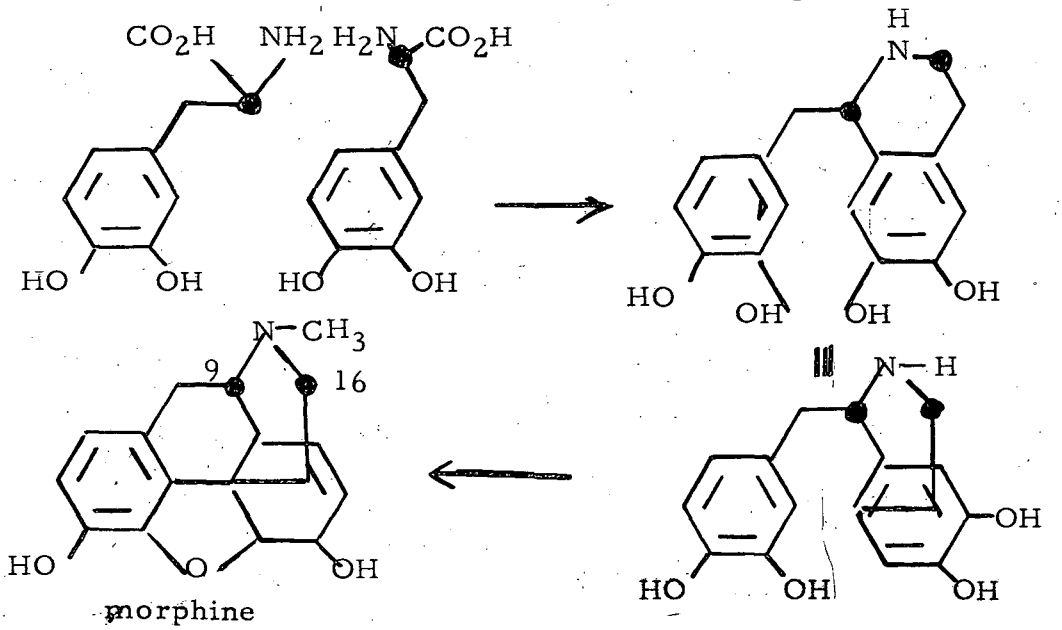
(XXIV)

(XXV)

(XXVI)

together with the morphenol derivative (XXV). This was hydroxylated (osmium tetroxide) and cleaved (periodate) to yield formaldehyde isolated as the dimedon derivative and the main bulk of the molecule as the oxime (XXVI). The dimedon derivative had a specific activity of 200,000 disintegrations per minute per millimole, and the activity of the oxime was 202,000 disintegrations per minute per millimole. These results would tend to dhow that half of the activity of the original morphine is located in position 16.

This would be consistent with such a general scheme as follows:



● Position of labeled atoms

Since half of the activity is found at positon 16, you might expect the other half to be found at position 9.

Independent experiments by Leete[41] confirmed the results found by Battersby and Harper. Leete fed α-C^{14}-DL-phenylalanine (0.12 mc, 44.7 mg.) and α-C^{14}-DL tyrosine (0.10 mc, 46.5 mg.) to poppy plants growing in an inorganic nutrient solution. The plants were three months old and the flowers were just opening. The plants were harvested after two weeks when the capsules had formed. The morphine was isolated without dilution and had a specific activity of 91,000 and 595,000 disintegrations per minute per millimole after feeding the phenylalanine and tyrosine, respectively. Half of the radioactivity of the morphine derived from the plants fed the tyrosine was removed when atoms 15 and 16 were taken off.

The most interesting aspect of the work by Leete is the fact that he reports how much radioactive tyrosine and phenylalanine he used in his experiments. But unfortunately he does not mention how many plants he used or how much morphine he isolated. The phenylalanine he used had a specific activity of 975,000,000 disintegrations per minute per millimole and the tyrosine had one of 860,000,000 disintegrations per minute per millimole. This would indicate that less than one part of tyrosine in 2,900 was converted to morphine and less than one part of phenylalanine in 21,000 was converted to morphine.

If tyrosine or phenylalanine are to be considered as precursors of morphine in the plant, this method of operation does not show them to be very efficient precursors of morphine. This may only represent a detoxification mechanism that is being used by the plant to rid itself of a small part of the tremendous excess of these amino acids. It would be interesting to see just to what the majority of the tyrosine and phenylalanine was converted.

Battersby and Harper[42] also fed methyl-C^{14}-L-methionine (0.223 mc) and C^{14}-labeled sodium formate (0.1 mc) to growing poppy plants. The alkaloids were isolated and the N-methyl groups were cleaved separately from each alkaloid with hydriodic acid and the liberated methyl iodide was collected as tetramethylammonium Reineckate. They show the following activities for the methionine experiment:

		Activities in disintegrations per minute per millimole x 10^{-5}		
	Alkaloid	N-me	O-me	Total N-me and O-me
Morphine	6.5	3.6	-	3.6
Codeine	11.9	4.4	5.0	9.4
Thebaine	9.3	2.1	2.3	6.7

The alkaloids from the experiment using formate were of a low specific activity, indicating the plant used formate less efficiently.

Even the experiments using the methionine indicate that only a small percentage of the radioactivity from the methionine actually reaches the alkaloids. Also, some of it reaches it in such a way that it is incorporated into other portions of the molecule rather than just the methyl groups.

It is possible, however, that a small portion of the radioactivity from the methionine found its way to the site of the methylation of the alkaloids.

Literature Survey of the Paper Chromatography of Opium Alkaloids

The paper chromatography of various alkaloids has been reviewed by Munier[43]. This review covers most of the work that had been published concerning the chromatography of the opium alkaloids prior to 1952. Other reviews concerning chromatography of alkaloids in general are those of Lederer and Lederer[44], Vega[45] and Brauniger[46]. The use of paper chromatography, as applied to drugs and pharmaceuticals, has been reviewed by Jung and Jacek[47] and Nauman[48]. A recent review on paper chromatography of alkaloids is that of Bettschart and Flück[49].

Munier found that the methods usually employed for the chromatography of amino acids were rarely applicable to alkaloids. He found that alkaloids with a pKa between four and eleven give elongated trailing spots in neutral solvents. The use of basic solvents, generally, gives good round spots, but the R_F values are usually near one. By using a suitable acid solvent, these difficulties can be overcome.

This technique, however, did not solve all the problems associated with the chromatography of certain alkaloids. The R_F values of morphine, codeine, and thebaine, when chromatographed on Whatman No. 1 paper with n-butanol:acetic acid (70:30 v/v, saturated with water), were 0.66, 0.75, and 0.86, respectively. Under these conditions, the spots are very close and in most cases overlapping. Munier[50] found that by impregnating particular salts in the paper, the R_F values could be greatly changed. The use of paper impregnated with a salt having the same anion as the acid in the solvent phase improved the separation.[51]

R_F Values

Paper: Durieux 122

System	1.	2.	3.	4.	5.	6.	7.	8.
Morphine	0.43	0.13	0.18	0.49	0.58	0.63	0.48	0.54
Codeine	0.58	0.22	0.28	0.61	0.70	0.74	0.60	0.61
Thebaine	0.78	0.52	0.52	0.86	0.85	0.87	0.77	0.80
Narcotine	0.96	0.91	0.89	0.94	0.93	0.93	0.88	0.89
Papaverine	0.95	0.91	0.88	0.94	0.92	0.92	0.88	0.90

1. Paper dipped in 1 M potassium dihydrogen phosphate and dried. n-Propanol:water (75:25 v/v) was employed as the mobile phase.

2. Paper: 0.2 M potassium dihydrogen phosphate.
Mobile phase: n-propanol:water (75:25 v/v).

3. Paper: 0.2 M potassium dihydrogen phosphate.

   Mobile phase: 2% butanol saturated with water.

4. Paper: 0.5 M potassium chloride.

   Mobile phase: 2% hydrochloric acid in n-butanol (saturated with water).

5. Paper: 0.2 M sodium acetate.

   Mobile phase: 2% acetic acid in n-butanol saturated with water.

6. Paper: 0.2 M sodium acetate.

   Mobile phase: 4% acetic acid in n-butanol saturated with water.

7. Paper: 0.1 M sodium acetate.

   Mobile phase: 2% acetic acid in n-butanol saturated with water.

8. Paper: 0.1 M sodium acetate.

   Mobile phase: 4% acetic acid in n-butanol saturated with water.

Since Munier's work using paper impregnated with various salts, others have tried various other systems. Bettschart and Flück[49] have separated morphine, thebaine, narceine, and codeine on a phosphate-citric acid buffer paper of pH 6.8; papaverine and narcotine on a pH 3.8-4.1 buffer paper, using ether.

Büchi and Schumacher[52] use Whatman No. 1 paper buffered at pH 3.5 and isobutanol:toluene (1:1 v/v) saturated with water as the mobile phase. They report the following R_F values for morphine, codeine, cryptopine, thebaine, narceine, papaverine, and narcotine: 0.03, 0.09, 0.15, 0.39, 0.47, 0.76, and 0.86, respectively.

Curry and Powell[53] used Whatman No. 1 paper dipped in 5% sodium dihydrogen citrate and dried. As the mobile phase they use the top layer of a n-butanol:water:citric acid (50 ml.:50 ml.:1 g.) mixture. Their main interest was in separating a large number of bases for toxicological examinations. Their results for various opium alkaloids do not look too good as a method of separation. The R_F values of morphine, codeine, narcotine, and papaverine are reported as 0.14, 0.16, 0.47, and 0.48, respectively.

Sun[54] reports using 0.5 M phosphate buffered paper with butanol or isopropanol solvents with fairly good separations achieved. Unfortunately, the abstracts fail to list the pH of the phosphate buffer.

A method that shows considerable promise is that of Krogerus and Tuderman[55]. They devised a two-dimensional paper chromatography using 1/7 M KH_2PO_4 paper, and first chromatographing with amyl alcohol, formic acid, water (100:18:4 v/v) mixture, followed by isopropanol-water solution (75:25 v/v). They report that negative results were obtained with several alcohols when the paper was treated with potassium chloride instead of with potassium dihydrogen phosphate. Likewise, inadequate results were found with treated paper when other acids were substituted for formic acid. The same was true with a number of other water:acid (acetic, hydrochloric, formic):alcohol (butanol, isobutanol, sec. butanol, isopropanol, isoamyl alcohol and amyl alcohol) mixtures. Only R_F values are reported for

morphine hydrochloride, codeine phosphate, and papaverine hydrochloride of the opium alkaloids.

Goldbaum and Kazyak[56] have used the method of buffered papers as a means of identification of alkaloids and other basic drugs. Their method involves chromatography on paper buffered at pH 3.0, 5.0, 6.5, and 7.5. Comparing the R_F values of one alkaloid at these pH's with that of codeine, run at the same time as a reference, they are able to identify a large number of alkaloids.

Reichelt[57] and Macek, et al.[58] have made use of paper impregnated with formamide to separate opium alkaloids and other alkaloids. This is done using a variety of solvent mixtures containing chloroform and benzene. Consistent R_F values are not obtainable, however, since they change greatly with the pH of the formamide, the temperature, and the method of impregnating the paper. From the results of Macek it appears that this method is one of the few methods that can separate narcotine and papaverine. One drawback of the method is that Dragendorf's reagent must be used. On this type of impregnated paper nearly 100 μg. of each alkaloid must be used to be detected. The more sensitive iodoplatinic acid reagent fades very rapidly as compared to Dragendorf's reagent. Perhaps a method of ultraviolet photography would be more suitable.

Numerous reports have been published concerning chromatography of various opium alkaloids using various solvent mixtures and untreated

paper. Thies and Reuther[59; 60] point out that the usual solvent mixtures like butanol-acetic acid-water are subject to esterification when stored for any length of time and separation of phases which results in irregular R_F values. Addition of an ester stabilizes the mixture and allows separations which the acid-alcohol solutions do not permit. For the system, butyl acetate:butanol:acetic acid (85:15:30 v/v), papaverine and narcotine have R_F values of 0.57 and 0.70, respectively.

Asahina and Ono[61, 62, 63] report using the top layer of a n-butanol:ammonium hydroxide:water (50:9:15 v/v) mixture to separate morphine, codeine, and narcotine with the following R_F values: 0.68, 0.86, and 0.93, respectively.

For detection of various opium alkaloids in urine and tissues, Mannering, Dixon, Carroll, and Cope[24] employed isoamyl alcohol:acetic acid:water (10:1:5), butanol:acetic acid:water (10:1:5), isoamyl alcohol: ammonium hydroxide:water (10:1:5) as solvents. Others who have used butanol-acetic acid mixtures to separate various opium alkaloids are Dobro and Kusafuk[65], Szymanska and Wasilewska[66], and Gore and Adshead[67].

Krogerus, et al.[68, 69] have used dioxane:formic acid:water solutions to separate morphine, codeine, papaverine, and narcotine.

Salversen and Paulsen[70] have separated seven analgesics related to morphine (morphine included) using ethyl acetate:toluene:acetic acid:

water (100:100:50:50) and chloroform:acetic acid:water (100:40:50).

Surface chromatography has been used by Borke and Kirch[71] for the separation of the major opium alkaloids.

Graf and List[72] and Burma[73] have reported applying paper electrophoresis methods to separate opium alkaloids but that the mobilities of the various compounds are nearly the same.

Methods of Chromatographic Separation (One-Dimensional)

Two systems have been found that work very well. One is similar to Munier's[50], using a molar potassium dihydrogen phosphate impregnated paper and a n-propanol:water (3:1 v/v) solution as the mobile phase. Very good separation is achieved for morphine, codeine, thebaine, and papaverine or narcotine. Papaverine and narcotine are not separated. Small, round, compact, nondiffused spots are achieved by this method.

The other method tried that was found to give good results was one similar to that of Büchi and Schumacher[52]. Unfortunately, in their work they neglected to publish the buffer that they used to impregnate the paper to a pH of 3.5, but a paper buffered by 0.1 M potassium citrate was found to work very well. The chief advantage of this paper is that substances that have R_F values higher than morphine or codeine are easily separated.

Besides the above general methods that have been tried there are those using the top layer of a n-butanol:ammonium hydroxide:water (50:9:15

v/v) mixture[19-21]. These have not been too successful. There was poor separation and the formation of "tails".

Employing dioxane:water:formic acid mixture on molar monopotassium phosphate paper appears to show promise. Several difficulties, however, were encountered. Unless extremely pure dioxane containing no peroxides is used, the Dragendorf reagent used in spraying the paper quickly fades. If iodo-platinic acid reagent is used to develop the spots, the paper turns very dark and the spots are not visible. Also, the spots tend to become diffused and are elongated, if this system is used.

Preliminary work was started using formamide-dipped paper before the details of Reichelt[57] or Macek, et al.[58] procedures had been received. The results that were obtained were very poor. It is nearly impossible to achieve reproducible chromatograms. With Reichelt and Macek's results available, this system should be be reinvestigated. Acetamide-impregnated paper was also tried. This has added advantages in that the paper is more standard when compared to the formamide paper.

Paper Chromatography of the Opium Alkaloids (Two-Dimensional)

Prior work has shown that good one direction chromatographic separation of the opium alkaloids can be achieved. One method involved using a Whatman No. 4 paper that had been buffered by molar potassium dihydrogen phosphate and n-propanol:water (3:1 v/v) solution as the mobile

phase.

The other method used Whatman No. 1 paper that had been buffered at pH 3.5 with 0.1 molar potassium citrate and toluene:isobutanol:water (1:1 saturated) as the mobile phase.

Numerous attempts to combine these two methods to form a two-dimensional method of chromatography have been tried. From these attempts it was found that Whatman No. 4 paper that had been buffered at pH 4.5 by a 0.2 molar potassium dihydrogen phosphate solution would give good separations when toluene:isobutanol:water (1:1 saturated) or n-propanol: ether:water (60:50:25) were used as mobile phases.

The systems involving ether solvents as the mobile phase develop very irregular and poorly separated spots if long equilibration times are used. Six to nine hours is sufficient for equilibration.

Other systems that were tried with varying amounts of success are outlined in the experimental section. It should be noted, however, that there is still no good system to separate papaverine and narcotine.

Description of Plant Chamber (Large Size)

The study of the biosynthesis of morphine by Papaver somniferum, using radioactive carbon dioxide, makes it necessary to grow the plants in a well sealed atmosphere so as to eliminate the danger of the gas escaping.

In order to grow the plants in a sealed atmosphere, it was necessary to construct a chamber (Figs. 1 and 2) in which the plants could grow. The plant chamber was constructed of Lucite in order to permit artificial lighting from outside the chamber. The chamber is approximately 30x30x30 inches. This size will accommodate nine pots of plants at a time.

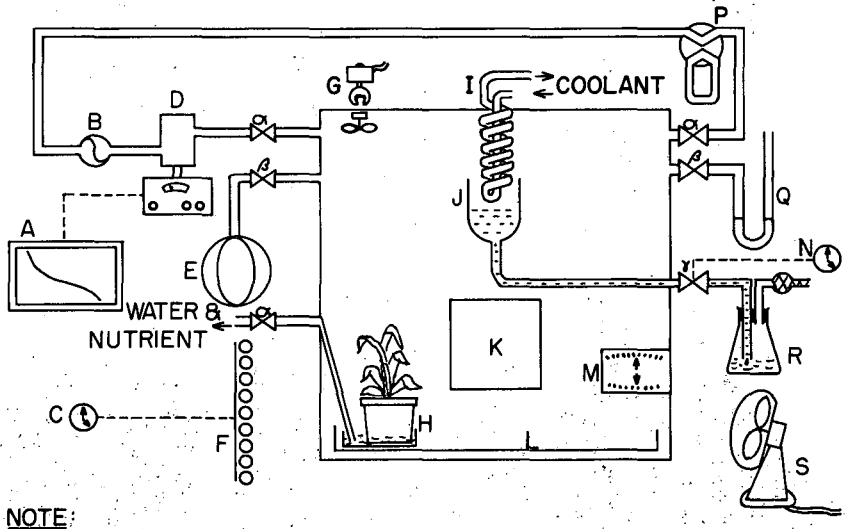
Lighting is furnished by two banks of nine, 48 inch, 40 watt fluorescent lights placed on opposite sides of the chamber. A bank of four fluorescent lights is suspended over the chamber. These lights furnish a light intensity of approximately 600 foot candles at the center of the chamber. The day length is controlled by a clock relay.

The humidity is controlled in the chamber by means of a stainless steel coil through which cold water is circulated. The water is cooled in a cold temperature bath by refrigeration. Temperatures as low as 4°C may be obtained with this cold water bath.

The room in which the plant chamber is located has been insulated and is cooled by a one horsepower, two-compressor air conditioner. Both of these compressors are regulated by thermostats. A heating fan, in series with a thermostat, is also used so that the temperature does not fall below a predetermined value. Circulation of the air around the chamber, during hours while the lights are running, is maintained by a 12 inch electric fan. The temperature in the room is continuously recorded on a three-day circular recorder.

ZN-1897

Fig. 1. Chamber for exposing plants to $C^{14}O_2$.



NOTE:

1) VALVES:

α-NORMALLY CLOSED NEEDLE VALVES

β-NORMALLY OPEN NEEDLE VALVES

γ-NORMALLY CLOSED SOLENOID VALVES

2) ONLY ONE PLANT AND FEED SYSTEM ILLUSTRATED

3) LIGHT BANKS ON RIGHT AND ON TOP NOT ILLUSTRATED

MU-17035

Fig. 2.

Fig. 2 key

A	Brown recorder
B	Circulation pump
C	Time clock for lights
D	Ionization chamber and vibrating reed electrometer
E	Polyvinyl balloon
F	Light bank
G	Magnetic powered fan
H	Plant, pot, watering tray, and feeding system
I	Stainless steel condenser
J	Condensate catch
K	Access port
L	Stainless steel floor tray
M	Humidity and temperature gauges
N	Time clock for solenoid valve
P	Carbon dioxide feed loop (shown closed to loop)
Q	Water filled manometer
R	Condensate collector, barium hydroxide solution and sodalime tube
S	Fan

Pressure changes in the chamber, due to temperature and barometric pressure, are minimized by an expansion bag prepared from polyvinyl chloride.

The plants can receive water and nutrient solution by means of a system of watering lines inside the chamber connected to the source of the solution outside the chamber. Also, it is possible to allow the condensate from the dehumidifying coils inside the chamber to flow back into the pots.

The concentration of the carbon dioxide in the chamber may be determined spectrophotometrically by means of the carbon dioxide's strong absorption at 4.3 microns.

Trial of Plant Chamber

Before growing the poppy in radioactive carbon dioxide, the poppy was allowed to grow in the plant chamber in order to determine the conditions for its growth.

On July 16, 1957, seeds of Papaver somniferum, variety Alba, were sown in a pot and placed in the greenhouse. The seeds germinated on July 21st and were allowed to grow in the greenhouse until the seventh of August, when they were sealed in the plant chamber. At this time there were two plants about one inch tall.

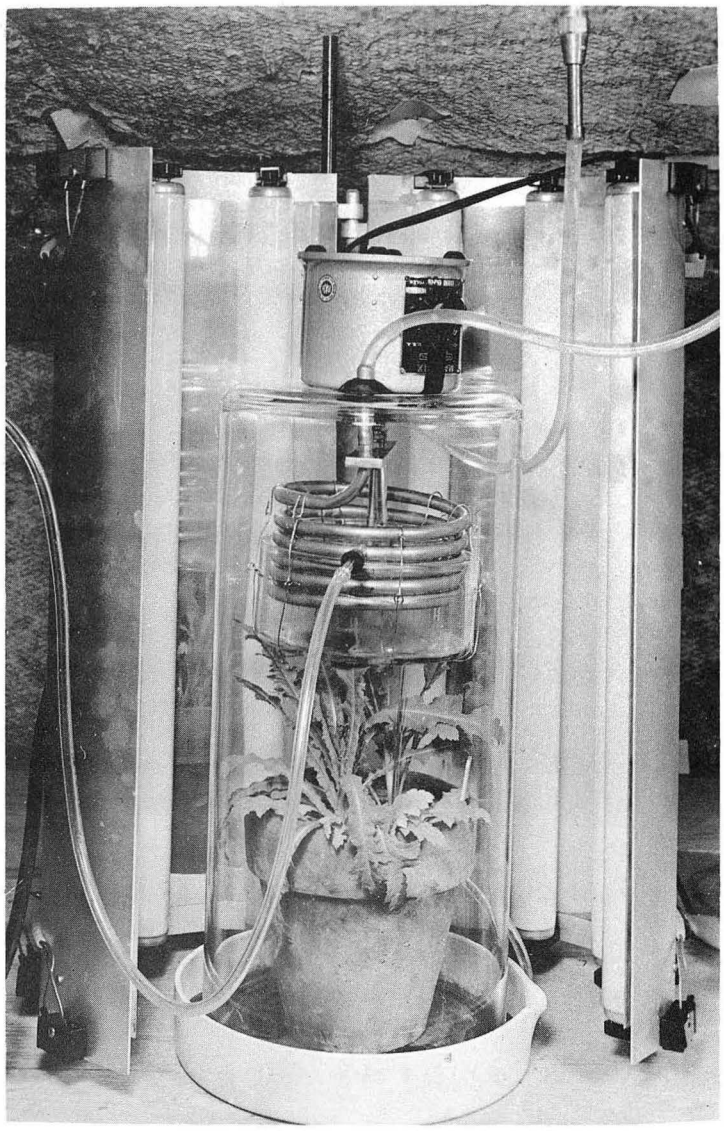
Initially the day length was twelve hours and the CO_2 concentration was 4%. Water (4-6°C) was circulated continuously through the dehumidifying coils. The temperature was regulated in the room at 20°±1°C, but the temperature in the chamber reaches approximately 24°C during the period when the lights are on around the chamber.

On August 13-14 the CO_2 concentration was reduced to approximately 1% by flushing the chamber with a stream of air. On August 15 it was noted that the plants had started to wilt and lose color; therefore, the day length was increased to fourteen hours and by August 20 they had regained much of their original color.

On August 26 the plants were fed 50 ml of a 1% fish emulsion which appeared to make the plants more healthy. On October 1 the plants were approximately four to five inches tall and appeared to be doing very well, except that their rate of growth was rather slow.

Description of Plant Chamber (Small Size)

Previously a plant chamber was described which was capable of holding nine pots at a time. The size of the chamber, however, made it impractical for small-scale, one-pot growth studies. For this reason a smaller chamber was constructed (Figs. 3 and 4).



ZN-1898

Fig. 3. Single plant chamber for exposure to $C^{14}O_2$.

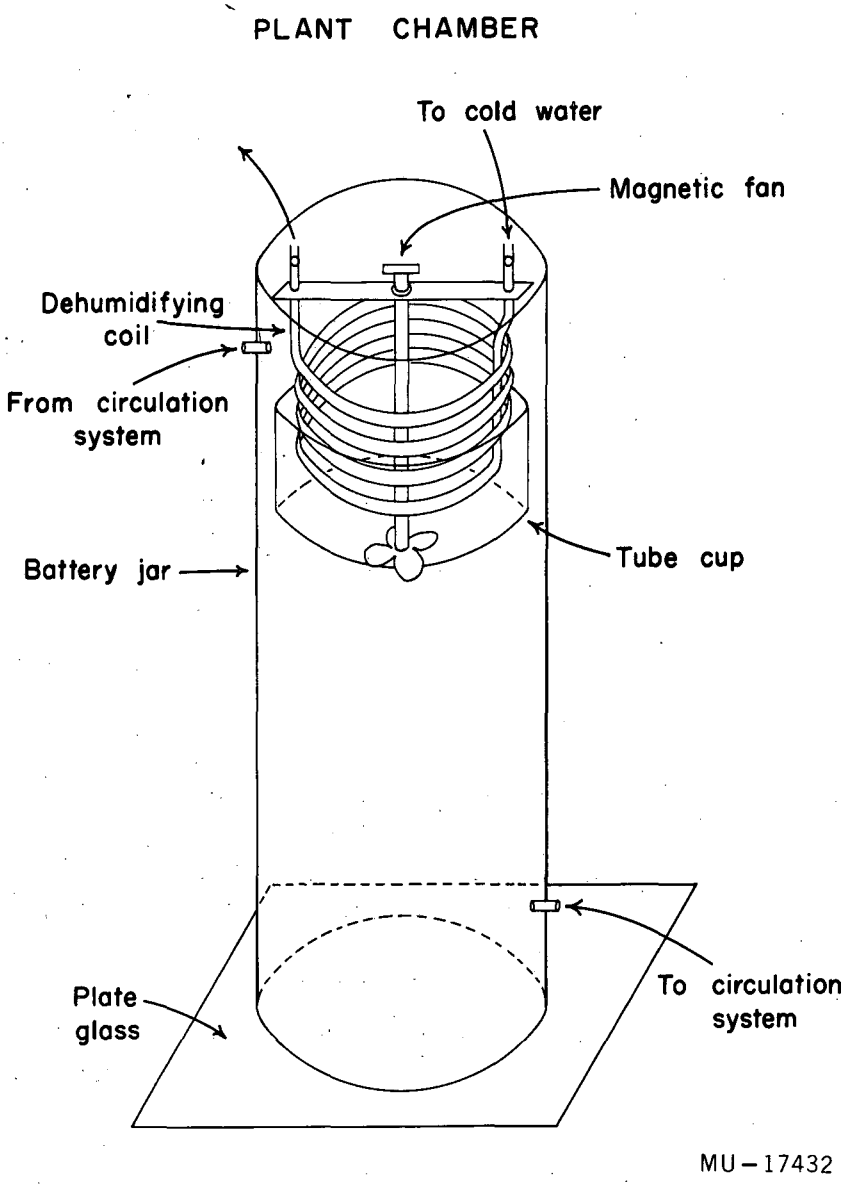

PLANT CHAMBER
To cold water
Magnetic fan
Dehumidifying coil
From circulation system
Battery jar
Tube cup
Plate glass
To circulation system
MU-17432

Fig. 4.

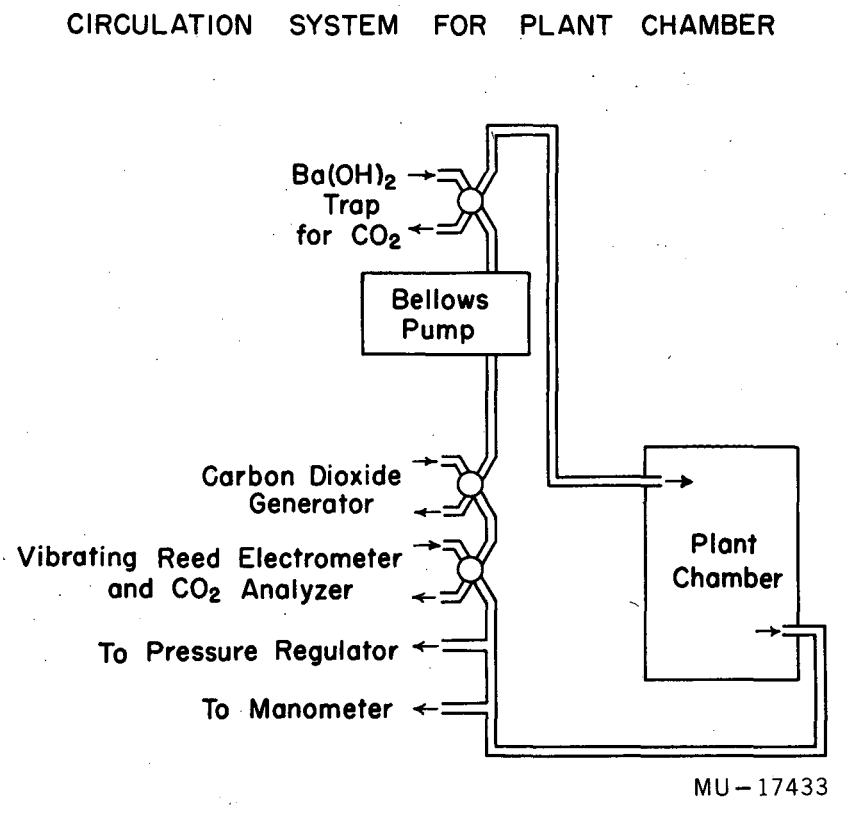


Fig. 5. Circulation system.

The chamber proper was an 18-inch by 8-3/4 inch diameter Pyrex battery jar. The jar was inverted over a 10-inch piece of 3/8 inch plate glass. A seal was achieved by using a liberal amount of stopcock grease between the battery jar and the plate glass. Two 1/2 inch holes were drilled in the top of the battery jar for the stainless steel dehumidifying coil. Two holes, the same size, were drilled on opposite sides of the jar so that the air in the chamber could be circulated through a system composed of a vibrating reed electrometer, carbon dioxide analyzer, carbon dioxide generator, and absorption traps.

The humidity in the chamber is controlled by means of a stainless steel coil through which cold water is circulated. The coil is 6-3/4 inches in diameter and was constructed from a 10-foot length of 3/8 inch stainless steel tubing. The condensate from the coil is collected in a tube cup suspended beneath the coils. The cup may contain sulfuric acid so that any carbon dioxide absorbed by the condensate is liberated. In the center of the coil is a magnetic fan used to circulate the air in the chamber.

The circulation system (Fig. 5) provides a means for controlling and recording the conditions inside the chamber. Air from the chamber may first be cycled through a vibrating reed electrometer which records the activity in the air from the chamber. The air can then be run through the carbon dioxide generator, the bellows pump, and the absorption tower.

The gas is then returned to the chamber.

Lighting is provided by eight 20-watt, 24-inch white fluorescent lights surrounding the chamber. A light intensity of 1200 foot candles can be achieved in the chamber.

The chamber has an air space of approximately 15 liters when it contains a seven inch flower pot.

Preliminary Chromatography of Poppy Extract

There are many methods of separating the various alkaloids from opium or dried poppy straw. None could be found that employed extraction of fresh plants. Two plants were extracted and the extracted combined alkaloids were chromatographed on paper impregnated with molar potassium dihydrogen phosphate. The alkaloids were easily separated and clearly visible when sprayed with iodoplatinic acid reagent.

Preliminary Runs Using Carbon-14

Four preliminary runs were tried with the opium poppy using a growing period of six hours in radioactive carbon dioxide. Runs 1, 2, 3, and 4 employed 2, 14, 50, and 191 microcuries of radioactive carbon dioxide, respectively. Runs 1, 3, and 4 were carried out in a 0.1% carbon dioxide atmosphere. Run 2 was carried out in a 1% carbon dioxide atmosphere and in this case the carbon dioxide uptake was fairly slow during the six-hour period.

Since all four runs were carried out in about the same manner, only run 4 is reported in the experimental section. Very little was learned from the first three runs because of the small amounts of activity used. It was found that it would also be advisable to use even higher amounts of activity in future work since the exposure times for the radioautographs are quite long when small amounts of activity are used. Also, smaller amounts of material could then be chromatographed and the tendency to overload the paper would be eliminated. This way better separations could be achieved.

From the chromatography results of run 4 it appears that more activity is found in thebaine than in codeine or morphine at the end of the six-hour period. The following order was found: papaverine and narcotine > thebaine > codeine > morphine. The amount of activity found in the papaverine and narcotine spot, however, must be discounted since other nonalkaloidal material is also found in the same area. Other solvent systems would have to be employed to give a different separation before any value can be taken concerning the amount of activity found for papaverine and narcotine.

The amount of activity in the thebaine appears to decrease after the six-hour period while the amount of activity found in the morphine and codeine steadily increases. However, at the end of nine days, the amount of

activity found in the codeine is still about twice that found in the morphine.

Purification Systems for Determination of the Specific Activities of the Morphine Type Alkaloids in Photosynthesis Studies

In order to determine the relative roles of the various alkaloids in the biosynthesis of morphine, it is imperative that some method be used to accurately determine the specific activities of the various alkaloids in the opium poppy after it has been exposed to radioactive carbon for various periods of time.

The first method that was tried was that of elution of the alkaloid spots followed by the determination of the alkaloid's concentration in the eluant by its ultraviolet absorption. Unfortunately, elution of the spots with either methanol or water also eluted a strong ultraviolet-absorbing constituent of the paper. Prior washing of the paper failed to remove it. Another disadvantage of this procedure is that only relatively small amounts of material can be placed on the paper and successfully chromatographed. This seriously limits the amount of purification that can be done after elution of the alkaloidal spots.

Other methods that looked more promising were those of cellulose powder and alumina chromatography. Using a cellulose powder that had been previously buffered with 0.2 M potassium dihydrogen phosphate solution and n-propanol:ether:water (2:1:1 by volume) developing solution, it was possible

to separate a synthetic mixture of papaverine, narcotine, thebaine, codeine, and morphine. Only papaverine and narcotine were not separated from each other. Some difficulty was at first experienced in finding a suitable way of adding the alkaloidal mixture to the cellulose column. Since some of the alkaloids are fairly insoluble in the developing solvent and, since the addition of the alkaloids in some other solvent changes the absorbing character of the cellulose, it was necessary to spot the alkaloidal mixture on a small piece of buffered adsorbent paper which could then be added to the column. This was the procedure which was found to be the most satisfactory.

However, when a plant extract (run 5) was used with the above procedure, it was found that other materials having an ultraviolet fluorescence and absorption in the fractions containing the codeine were obtained. These impurities could not be separated from codeine by acid-base extraction. People at the Mallinckrodt Chemical Works[74] found that codeine could be separated from thebaine and neopine and other foreign materials by chromatography of the alkaloidal mixture on alumina. An adaptation of this method was found to work very well on the natural alkaloidal extract. In this way, codeine could easily be obtained in a state free from interfering materials.

Using a combination of these two methods it was possible to isolate the various alkaloids in fractions that contained no interfering ultraviolet absorbing material.

TABLE II

SYSTEM FOR SEPARATION OF MORPHINE, CODEINE AND THEBAINE

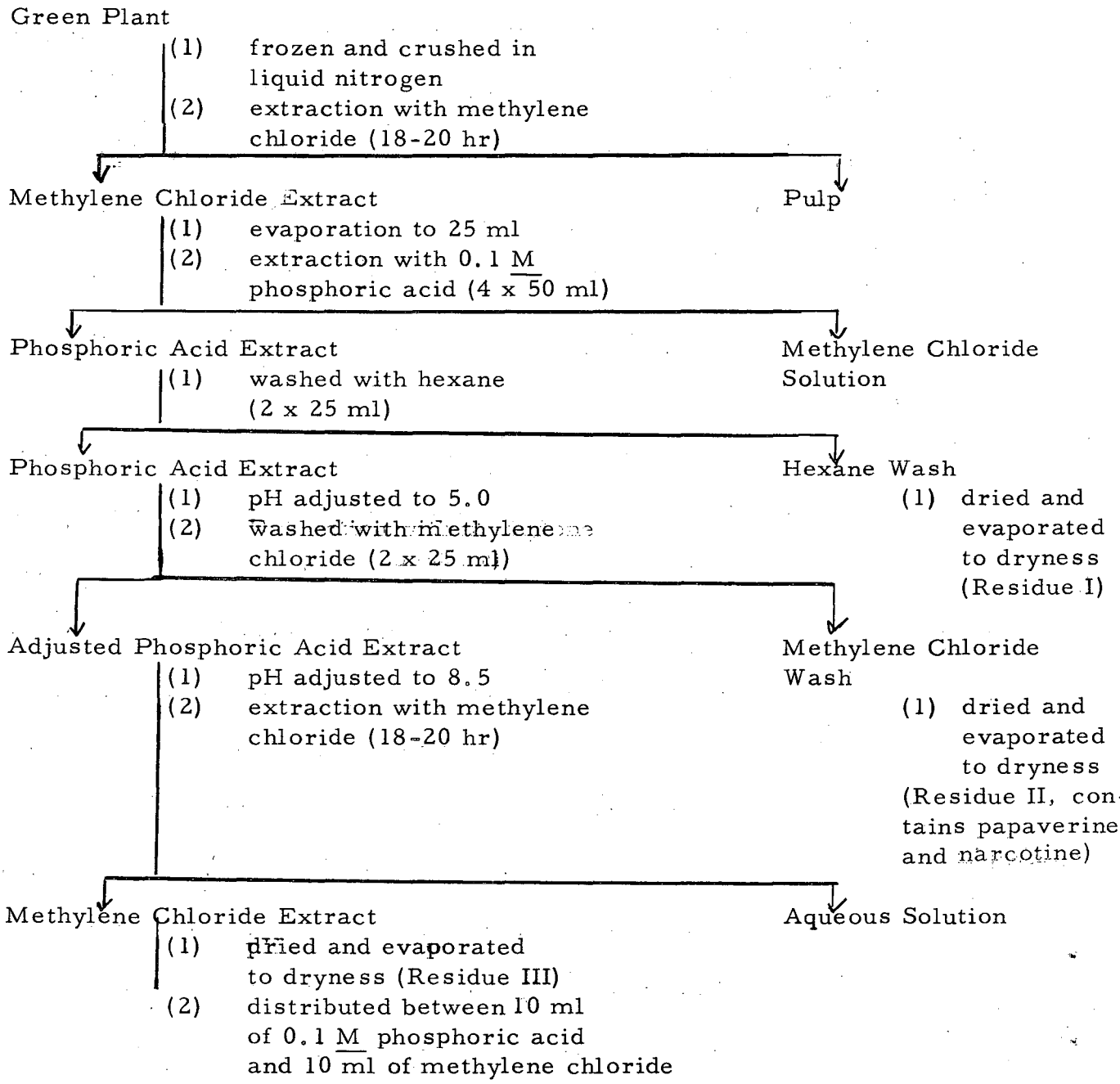


Table II (continued)

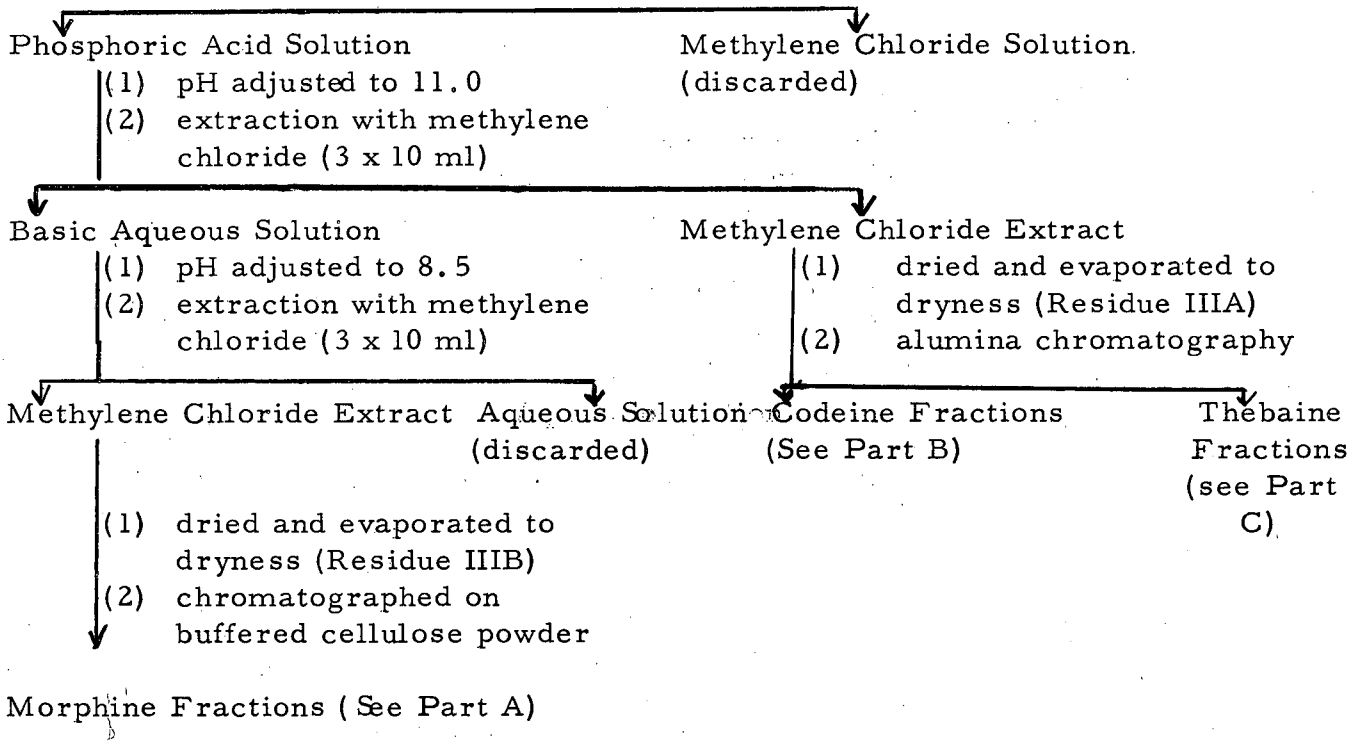


Table II (continued)

Part A Morphine Fractions

(1) combined and evaporated to dryness
(2) taken up in 0.1 M phosphoric acid (1 ml)
(3) washed with methylene chloride (2 x 1 ml) (discarded)
(4) pH adjusted to 11.0
(5) washed with methylene chloride (2 x 1 ml) (discarded)
(6) pH adjusted to 8.5
(7) extraction with methylene chloride (3 x 1 ml)

Methylene Chloride Extract
(1) dried and evaporated to dryness

Aqueous Solution (discarded)

Morphine (amount determined by spectra)

Part B Codeine Fractions

(1) combined and evaporated to dryness
(2) taken up in 0.1 M phosphoric acid (1 ml)
(3) washed with methylene chloride (3 x 1 ml) (washes discarded)
(4) pH adjusted to 11.0
(5) extraction with methylene chloride (4 x 1 ml)

Methylene Chloride Extract
(1) dried and evaporated to dryness

Aqueous Solution (discarded)

Codeine (amount determined by spectra)

Part C Thebaine Fractions

(1) combined and evaporated to dryness
(2) chromatographed on buffered cellulose powder

Thebaine Fractions

(1) work-up same as Part B above for codeine

Thebaine (amount determined by spectra)

Determination of the Specific Activities of the Morphine Type Alkaloids in Photosynthesis Studies

In run 6 a general system is described for the separation of small amounts of morphine, codeine, and thebaine from the opium poppy by which the specific activities of the alkaloids could be determined. An improved general system is outlined in Table II. The system described in run 6 was used on plants that were exposed to radioactive carbon dioxide and then placed in the greenhouse, in a special chamber for radioactive plants, for various periods of time. The length of time in this chamber varied from two to ten days, during which time the plants were allowed to grow under normal greenhouse conditions with no radioactive carbon dioxide in the chamber.

In run 6 the plant was exposed to radioactive carbon dioxide for six hours and then harvested and the alkaloids separated.

In run 7 the plants were exposed to radioactive carbon dioxide for two hours and then harvested. Because such a small amount of radioactive carbon dioxide was used, the run was not worked up beyond the initlal methylene chloride extraction. It was then discarded.

In run 8 the plants were grown in radioactive carbon dioxide for six hours and grown in the greenhouse, in the special chamber, for two days prior to harvesting.

In run 9 the plants were grown in radioactive carbon dioxide for two hours and then harvested.

In run 10 the plants were grown in radioactive carbon dioxide for six hours and then placed in the special chamber in the greenhouse for ten days prior to harvesting.

In run 11 the plants were grown in radioactive carbon dioxide for six hours and then placed in the greenhouse in the special chamber for five days before harvesting. A summary of the exposure data is found in Table III.

Morphine, codeine, and thebaine were each isolated from the above runs and the specific activities of each alkaloid were determined. This is summarized in Table IV.

Degradation of Morphine, Codeine and Thebaine

From the specific activity determinations it became apparent that the distribution of the radioactivity in the morphine, codeine, and thebaine was due, possibly, to the various methyl groups since each bears the same general carbon skeleton. The O^3-methyl of codeine could be removed by pyridine hydrochloride-ether cleavage[75]. The N-methyl groups of codeine and morphine could be removed by a von Braun degradation[76, 78].

The conversion of the thebaine to demethylated materials is somewhat more difficult. Neither of the above systems could be applied to the-

TABLE III

Summary of Exposure Data

	Run 6	Run 8	Run 9	Run 10	Run 11
Length of exposure to $C^{14}O_2$ (hours)	6	6	2	6	6
Length of time in special chamber (days)	0	2	0	10	5
Amount of $C^{14}O_2$ (microcuries)	400	390	1060	1070	1000
Wet weight of plants (grams)	43.9	34.1	36.9	34.7	34.1

TABLE IV

Specific Activities of Isolated Morphine Alkaloids
(dis.per min. per mg.)

	Run 6	Run 8	Run 9	Run 10	Run 11
Morphine	1,350	50,100	9,200[1]	380,000	450,000
Codeine	11,200		21,000	424,000	705,000
Thebaine	88,000	390,000	75,000	600,000[2]	670,000

(1) Most of this morphine sample was accidentally destroyed. The specific activity is probably much lower.

(2) Only a small amount of thebaine was isolated in this run. The specific activity is probably between 600,000 and 800,000 dis.per min. per mg.

baine itself. Thebaine is reported by Conroy[77] to be easily brominated by N-bromosuccinimide to the 14-bromocodeinone in very good yield. He also reports the conversion of 14-bromocodeinone to neopinone by catalytic hydrogenation, which he reported could be isomerized over activated charcoal to codeinone which is easily reduced by sodium borohydride to codeine. This codeine could then be used for demethylation reactions.

However, the attempted conversion of 14-bromocodeinone to neopinone by hydrogenation was very difficult. Conroy's hydrogenation could not be repeated on a moderate scale (100 mg). Various catalysts and hydrogenation procedures gave only mixtures of materials and no single major product was isolated in any of the methods tried.

Another method that was tried was that of treating 14-bromocodeinone with sodium borohydride to form neopine. However, the neopine was contaminated with two other alkaloidal materials. When 14-bromocodeinone (111 mg) was treated with lithium aluminum hydride in tetrahydrofuran, neopine was isolated in a nearly pure state. Neopine that was then formed is easily hydrogenated to dihydrocodeine which could be used in the demethylation reactions.

The series of reactions of thebaine to 14-bromocodeinone to neopine to dihydrocodeine which worked very well on a moderate scale (about 100 mg) was found to be almost entirely useless when applied on a scale of

less than 1 mg. The conversion of thebaine to 14-bromocodeinone is evidently seriously affected by a large concentration of N-bromosuccinimide since only under conditions of very good mixing and slow addition of the N-bromosuccinimide could any 14-bromocodeinone be isolated. Apparently, for this reason, carbon tetrachloride is a good solvent for the reaction, since the N-bromosuccinimide is only very slightly soluble in carbon tetrachloride.

The conversion of 14-bromocodeinone to neopine on a scale of less than 1 mg using lithium aluminum hydride and tetrahydrofuran as the solvent was in marked contrast to the moderate scale reaction. In the small-scale reaction, under identical conditions, no neopine could be isolated. Seven small-scale reactions of 15-bromocodeinone and lithium aluminum hydride in tetrahydrofuran were attempted. In each case various reaction conditions were tried and no neopine could be isolated from any of them.

The conversion of 14-bromocodeinone to neopine on a moderate scale using lithium aluminum hydride in ether solution instead of tetrahydrofuran was tried. The 14-bromocodeinone is only sparingly soluble in ether and thus is not a single phase reaction as it is when tetrahydrofuran is the solvent. As in the previous moderate scale reduction in tetrahydrofuran, neopine was isolated in good yield from the ether mixture. Preliminary small-scale reductions in ether with lithium aluminum hydride were encouraging since neopine was isolated. However, the yields have not been too good on material from the plant for some reason.

The degradation of dihydrocodeine is achieved in a manner similar to codeine. The pyridine hydrochloride cleavage of dihydrocodeine to remove the O^3-methyl to form dihydromorphine goes in better yield than that of the codeine cleavage. The von Braun degradation of dihydrocodeine to remove the N-methyl group by forming dihydronorcodeine, however, went in very poor yield. More work should be done in trying to improve the procedure.

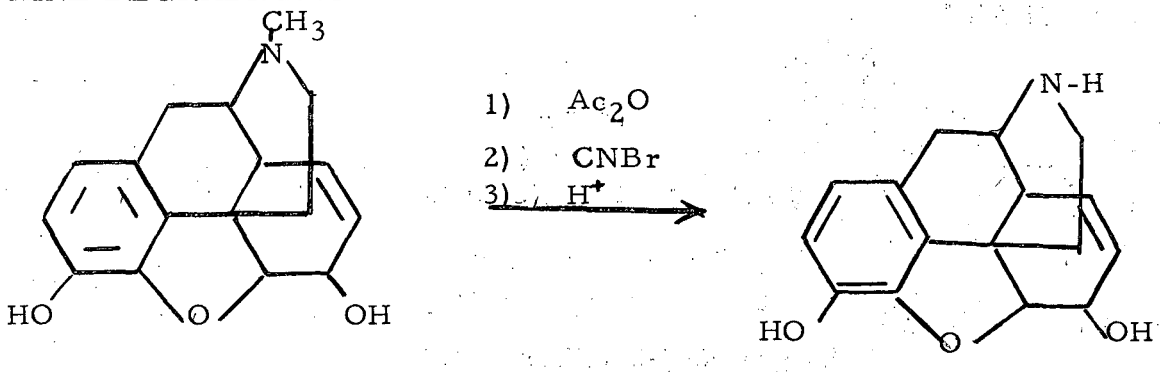
The methods of degradation of the alkaloids are outlined in Figs. 6 and 7.

Specific Activity Measurements of the Degradation Products of the Alkaloids from Run 11

The morphine and codeine from run 11 were diluted with additional material to give approximately one mg of each alkaloid. The codeine sample was divided into two portions -- one for the pyridine hydrochloride cleavage to morphine, the other for the von Braun degradation to norcodeine. The morphine sample was also divided into two portions. One was used in the von Braun degradation to normorphine. The other was saved in case something unforeseen happened to the first part.

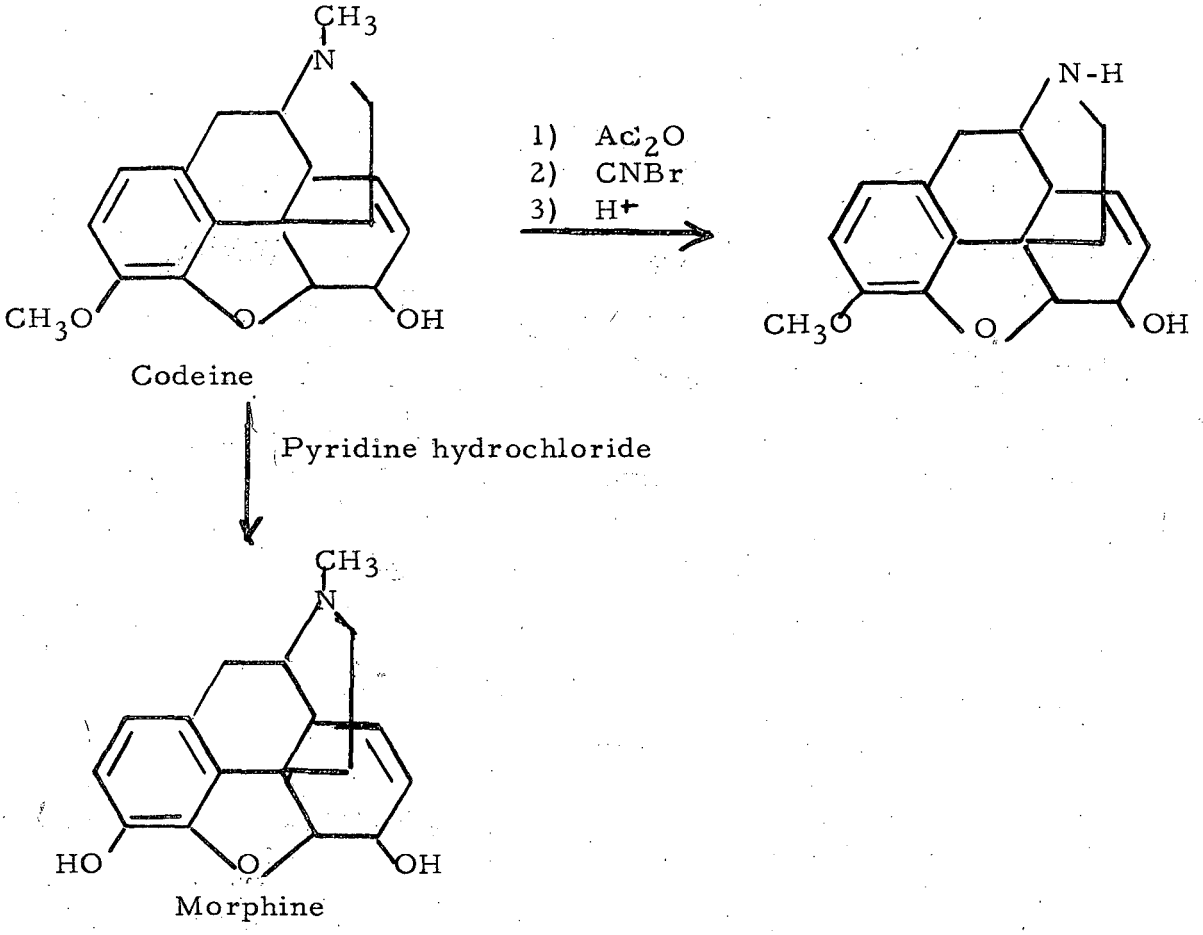
The specific activity measurements of the diluted alkaloids have been adjusted to the original specific activity as shown in Table V. A sample calculation is shown below.

MORPHINE DEGRADATION



Morphine Normorphine

CODEINE DEGRADATION



Codeine

Morphine

Fig. 6 Degradations of morphine and codeine

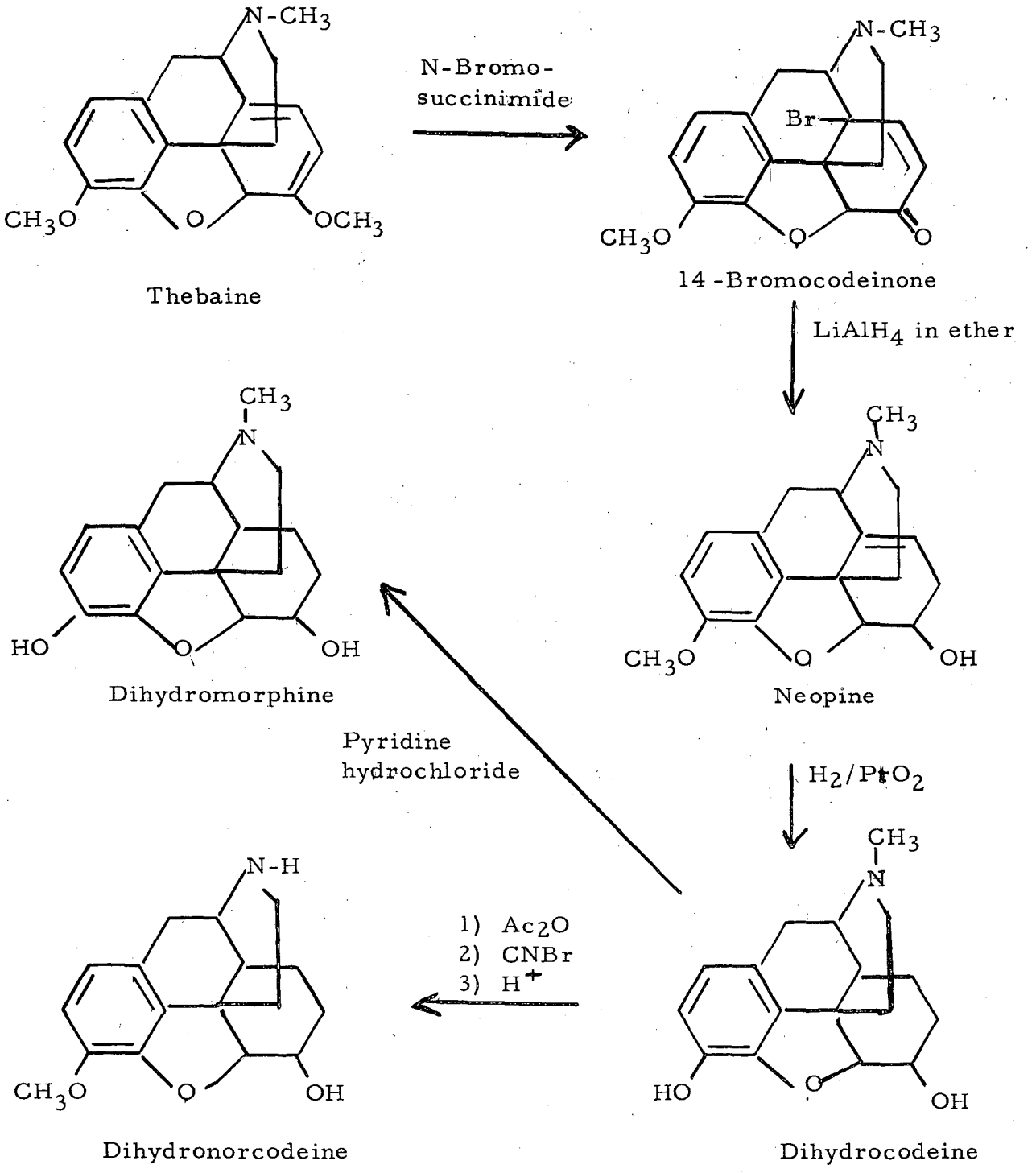


Figure 7. Degradation of Thebaine

$$\frac{\text{activity of diluted codeine}}{\text{activity of original codeine}} = \frac{\text{activity of diluted morphine}^{*}}{\text{activity of "original" morphine}^{*}}$$

$$\frac{89,000}{705,000} = \frac{71,000}{x}$$

$$x = 562,000 \text{ dis. per min. per mg.}$$

* This refers to morphine formed by ether cleavage.

From Table V it can be seen that even in plants which have been allowed to grow for five days after exposure to radioactive carbon dioxide, there still remains nonrandom labeling in the alkaloidal molecules. As an example of this, the N-methyl group of codeine contains 18% of the radioactive carbon and its O^3-methyl contains 24% where, if the molecules were randomly labeled, one would expect only about 5-6% of the radioactivity to be found in each of the methyl groups. However, it should also be noted that for some reason there is very little radioactivity in the N-methyl group of morphine.

Specific Activity Measurements of Degradation Products from Run 12

Since the specific activities of each of the alkaloids are beginning to level off and decrease at the end of five days after exposure, it would seem advisable to do degradation work on material when there is the greatest difference in specific activities. This difference is found in the time be-

TABLE V

Specific Activity Measurements of Degradation Products from Run 11

a) Disintegrations per minute per milligram

	Original value	Minus N-methyl	Minus O^3-methyl	Minus O^6-methyl	Ring Skeleton
Morphine	450,000	516,000	---	---	516,000
Codeine	705,000	607,000	562,000	---	464,000
Thebaine	670,000				

b) Disintegrations per minute per micromole

Morphine	137,000	140,000	---	---	140,000
Codeine	211,000	173,000	160,000	---	122,000
Thebaine	208,000				

tween two and six hours after introduction of the radioactive carbon dioxide to the plants. An example of this is found in runs 6 and 9. However, the amount of radioactivity found in the alkaloids of these runs was not great enough to allow degradation work. The only way around this problem is to use larger amounts of radioactive carbon dioxide. In run 12 a small plant (6.10 g) took up 20 mc of radioactive carbon dioxide during a six-hour period. The carbon dioxide was generated from barium carbonate having the highest specific activity available.

The alkaloids were each isolated and their specific activities determined. The alkaloids were degraded in the manner illustrated in Figs. 6 and 7, and the results are tabulated in Tables VI and VII.

The following tables show that 47% of the radioactive carbon of the codeine is located in its N-methyl group and 43% is found in the O^3-methyl. Also, 59% of the radioactivity is lost when the N-methyl group of morphine is removed. In the case of the thebaine, the O^6-methyl contains 55%, O^3-methyl contains no activity (-7%), and the N-methyl contains about 36% of the radioactivity.

These figures indicate that one of the last processes in the plant's synthesis of morphine, codeine or thebaine is the addition of methyl groups to the nucleus of the molecule. This was borne out in all cases except that of the O^3-methyl of thebaine.

TABLE VI

Specific Activity Measurements of Run 12 Alkaloids

	Isolated Alkaloids		Alkaloids after Dilution	
	A dis/min/mg	B dis/min/μmole B = A x mol. wt. x 10^{-6}	C dis/min/mg	D dilution factor D = A/C
Morphine	165,000	50,000	26,800	6.15
Codeine	1,600,000	478,000	69,500	23.0
Thebaine	22,500,000	7,000,000	216,000	104

TABLE VII

Specific Activity Measurements of Degradation Products of Run 12 Alkaloids

	Specific Activity of Diluted Products	Multiplication by Dilution Factors	
	A dis/min/mg	B dis/min/mg B = A x D (of Table VI)	C dis/min/μ mole C = B x mol. wt. x 10^{-6}
Morphine			
Normorphine (minus N-methyl)	12,200	75,000	20,300
Codeine			
Norcodeine (minus N-methyl)	38,200	880,000	251,000
Morphine (minus O^3-methyl)	39,500	910,000	274,000
Thebaine			
Dihydrocodeine (minus O^6-methyl)	101,000 (119,000)*	10,500,000	3,160,000
Dihydromorphine (minus O^6- and O^3-methyl)	117,000	12,200,000	3,370,000
Dihydronorcodeine (minus O^6- and N-methyls)	65,000	6,750,000	1,930,000

* From duplicate work-up procedure

SUMMARY OF TABLES VI AND VII

(All activities as dis/min/μmole)

	Original Value	Minus N-methyl	Minus O^3-methyl	Minus O^6-methyl	Ring - Skeleton
Morphine	50,000	20,300	--	--	20,300
Codeine	478,000	251,000	274,000	---	47,000
Thebaine	7,000,000	1,930,000 (minus N-methyl and O^6-methyl)	3,370,000	3,160,000	1,930,000

In this case the data would indicate that the methyl group that became the O^3-methyl of thebaine had been placed in position sometime much earlier in the plant's synthetic route to thebaine, Or it might also be possible that thebaine is receiving its O^3-methyl group from some source which has not yet had this group incorporate C^{14}. This last choice would seem most unlikely.

Tables VI and VII show that the nucleus of morphine (the molecule minus the methyl groups) has a specific activity of 20,300 disintegrations per minute per micromole (of normorphine). The nucleus of codeine would have a calculated specific activity of 47,000 disintegrations per minute per micromole (of normorphine). Since this last value was obtained by

subtracting one large experimental value from another large experimental value, the remainder is open to considerable doubt. Therefore, the value of 47,000 is within experimental error of being the same order of magnitude as that of the 20,300 found experimentally from the morphine degradation.

There are three general methods that might be employed in the plant's synthesis of the morphine-type alkaloids. One method involves the use of a precursor from which the alkaloids are produced by the plant. In the second method one of the alkaloids acts as the precursor for one or more of the other alkaloids. The third method is a combination of the two.

If it is assumed that during the time that the plant was allowed to grow in the presence of $C^{14}O_2$ it produced each of the alkaloids at the same rate as it did under natural conditions, the specific activities of each of the alkaloids (and their constituent parts) would be an adequate representation of their relative rates of incorporation of C^{14}, during which time the living plant was exposed to C^{14}. If a substance is to be a precursor for another substance or if they are to have a common precursor, the ratio of the specific activities of those parts of the molecule that are found in both substances must be the same. For example, alkaloid 1 has groups A, B and C, which are common to alkaloid 2. The specific activities of A:B:C are 5:2:1 in both alkaloid 1 as in alkaloid 2. Therefore, alkaloid 1 is related to alkaloid 2. Nothing is yet known as to whether alkaloid 1 is

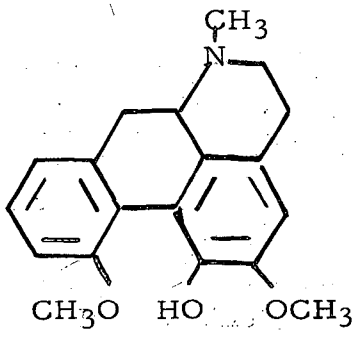
the precursor of alkaloid 2, or vice versa, or whether even a third substance is the precursor of alkaloid 1 or 2.

The only difference between morphine and codeine is the presence of the O^3-methyl in codeine. All other structural features are the same. The specific activities of the two portions of the molecule were determined. Morphine's N-methyl and nucleus had a specific activity of 29,700 and 20,300 disintegrations per minute per micromole, respectively, or a ratio of 1.46:1. The N-methyl and nucleus of codeine had a specific activity of 227,000 and 47,000 disintegrations per minute per micromole respectively, or a ratio of 4.8:1. Therefore, morphine is not a precursor of codeine unless during the methylation at O^3 the N-methyl group of the morphine is transmethylated. Similarly, codeine is not a precursor for morphine unless during the O^3-demethylation the codeine N-methyl is transmethylated. Using this same line of reasoning, thebaine could not be a precursor of codeine or vice versa, since thebaine's O^3-methyl is not active and codeine's O^3-methyl is active.

Since the specific activities of the nucleus of morphine and codeine are about the same order of magnitude, it is possible that there is a common precursor for these two alkaloids. One method of testing this hypothesis would be to remove the basic side-chain of both morphine and codeine and compare the ratios of the specific activities of the two carbon atoms that were

removed. This could be done using a method worked out by Batcho[79] on a large scale that would have to be adapted to small-scale procedures. It would also be interesting to find out how the ratios of the specific activities of carbon atoms comprising the side chain of morphine and codeine compare with those corresponding atoms found in the isoquinoline portion of the molecules of papaverine and narcotine as well as in the α and β positions of tyrosine, phenylalanine, etc. that had been isolated from the plant. A detailed study of the specific activities of the amino acids found in the poppy and their relationship with the alkaloids would be most helpful.

Since there is no radioactivity in the O^3-methyl of thebaine, in contrast to that of codeine, and since its nucleus had a much higher specific activity than that of codeine or morphine, it seems likely that it would also have a different precursor than morphine or codeine. The fact that thebaine, along with isothebaine, is also found in Papaver orientale and that neither morphine or codeine are found in that plant would add credulity to this hypothesis.



Isothebaine

In order to determine the radioactive purity of the various alkaloids, each of the purified alkaloids is rechromatographed on buffered paper and the developed chromatogram run through the paper strip counter to see if all the activity coincides with that of the alkaloid spot. A better way would be to convert, by chemical means, each of the alkaloids to some derivative and then purify that derivative. For example, morphine could be converted to codeine; codeine could be hydrogenated to dihydrocodeine; and thebaine hydrogenated to tetrahydrothebaine, to mention just a few of the possibilities.

Because of the difficulties encountered in the degradation of thebaine by way of dihydrocodeine, it should be possible to degrade thebaine by other means in order to achieve the same goal. The small-scale method of preparation of 14-bromocodeinone is adequate enough to determine the specific activity of the product. However, some method of chromatographic purification of the crude reaction mixture would have to be worked out. In this manner, the amount of activity in the O^6-methyl could be determined. Thebaine could be hydrogenated to tetrahydrothebaine whose N-methyl group could be removed by a von Braun degradation and the O-methyls by pyridine hydrochloride-ether cleavage. Bentley[31] in Chapter XII of his book gives a review of the methods of preparation of tetrahydrothebaine.

EXPERIMENTAL

Methods of Chromatographic Separation

The chromatographic chamber that was used was fashioned from a two-liter graduated cylinder (with no pouring lip). At the top of the cylinder was placed a rubber stopper containing a glass trough and a separatory funnel to allow the addition of the mobile phase to the trough. Using this chamber, the mobile phase could be placed at the top of the paper and a descending technique used in developing the chromatogram.

Samples to be chromatographed are spotted approximately three inches from the top end of the paper. A solution of morphine, narcotine, codeine, thebaine, and papaverine was used in the trials of various papers and solvent systems. Approximately 1 mg of these compounds was dissolved in 1 ml of methanol and from this solution 0.050 ml was applied to the paper to form a spot no larger than 5 mm in diameter.

The chamber with the chromatogram was allowed to equilibrate with the solvent used in developing the chromatogram for a period of 12 to 24 hours. For this, a portion of the mobile phase is placed in a beaker at the bottom of the chamber.

The length of time needed for developing the chromatogram varied with the solvent, but the time usually was from three to four hours for Whatman No. 4 paper and about 12 hours for Whatman No. 1 paper. For

many volatile solvents it is essential that the temperature be nearly constant during this time since the air in the chamber may become unsaturated with respect to the mobile phase and cause evaporation from the developing chromatogram. The chamber was allowed to equilibrate from 12 to 24 hours in all cases, except those involving ether solvents as the mobile phase. The systems involving ether solvents as the mobile phase develop very irregular and poorly separated spots if long equilibration times are used. Six to eight hours is more than sufficient. This phenomenon has been noticed repeatedly using various papers and combinations of ether-containing solvents.

Iodoplatinic acid reagent was used to spray the paper to reveal the alkaloids.

The R_F values are taken as the ratio of the distance traveled by the center of the spot to the distance traveled by the solvent front (usually about 30 cm).

Whatman No. 4 Buffered Papers

1. 1 M Potassium Dihydrogen Phosphate Paper: Whatman No. 4 paper, that had been previously washed in oxalic acid, was dipped in 1 M potassium dihydrogen phosphate solution and allowed to dry. Using this paper and spotting with a solution containing a mixture of 25 to 50 micrograms of morphine, codeine, thebaine, narcotine and papaverine, the following R_F values were obtained:

System	Morphine	Codeine	Thebaine	Narcotine and Papaverine
(a)	0.50	0.59	0.71	0.87
(b)	0.51	0.61	0.78	0.89
(c)	0.53	0.61	0.75	0.83
(d)	0.07	0.16	0.43	0.88
(e)	0.08	0.13	0.38-0.47	0.93
(f)	0.21	0.32	0.46	0.87

Mobile phase and description of separation:

(a) n-propanol:water (3:1 v/v)

Very good separation with compact round spots.

(b) n-propanol:1 M citric acid (3:1)

Good separation with compact round spots

(c) N-propanol:1M citric acid:form ic acid (30:10:0.4)

Good separation, but there is a tendency to form beards on the spots.

(d) toluene:isobutanol:water (1:1 sat.)

Good separation, but thebaine has an enlarged spot.

(e) toluene:isobutanol:water (1:1 sat.) and molar in citric acid.

Good separation, but the spot of thebaine was elongated.

(f) dioxane:water:formic acid (90:9.5:0.5)

Good separation, but there is a tail on codeine and a beard on morphine

Also, toluene:isobutanol:tetrahydrofuran:water (1.:1:1:saturated) and n-butanol:28% ammonium hydroxide:water (50:9:15 v/v, top layer) were tried but only very poor separations could be achieved.

2. 0.5 M Potassium Dihydrogen Phosphate Paper: The Whatman No. 4 paper was dipped in 0.5 M potassium dihydrogen phosphate solution as in previous trials. The following R_F values were obtained:

System	Morphine	Codeine	Thebaine	Narcotine and Papaverine
(a)	0.45	0.54	0.63	0.82
(b)	0.06	0.14	0.45	0.70
(c)	0.27	0.35	0.53	0.89

Mobile phase and description of separation:

(a) n-propanol:water (3:1)

Good separation, but some tail on narcotine and papaverine.

(b) amyl alcohol:formic acid:water (50:9:2)

Good separation; very sharp spots for morphine and codeine, but thebaine has a beard.

(c) n-propanol:ether:water (60:50:25)

Very good separation and nice looking spots.

Also, isopropanol:water (3:1) was tried, but there was no separation.

3. 0.2 M Potassium Dihydrogen Phosphate Paper: The Whatman No. 4 paper was dipped in 0.2 M potassium dihydrogen phosphate (pH 4.5) as in the previous trials. The following R_F values were obtained:

System	Morphine	Codeine	Thebaine	Narcotine and Papaverine
(a)	0.47	0.55	0.66	0.80
(b)	0.04	0.09	0.30	0.94
(c)	0.08	0.15	0.43	0.69
(d)	0.33	0.41	0.65	0.88
(e)	0.06	0.10	0.23	0.83
(f)	0.33	0.39	0.58	0.91
(g)	0.35	0.45	0.68	0.89
(h)	0.50	0.59	0.74	0.88
(i)	0.61	0.72	0.88	0.96

Mobile phase and description of separation:

(a) n-propanol:water (3:1)

Good separation, but there is a tail on papaverine and narcotine.

(b) toluene:isobutanol:water (1:1 sat.)

Very good separation with nice compact spots.

(c) amyl alcohol:formic acid:water (50:9:2)

Good separation with the spot of thebaine somewhat elongated.

(d) n-propanol:ether:water (60:50:25)

Very good separation with nice compact spots.

(e) n-propanol:n-butyl ether:water (25:25:4)

Good separation with some elongation of the spots.

(f) n-propanol:tetrahydrofuran:ether:water (2:1:1:1)

Very good separation with good round spots.

(g) n-propanol:tetrahydrofuran:hexane:water (3:1:1:1)

Good separation, but thebaine's spot is somewhat elongated.

(h) n-propanol:ether:water (2:1:1)

Very good separation, with nice compact spots.

(i) n-propanol:ether:water (3:1:1)

Poor separation between thebaine, papaverine and narcotine.

Also, isopropanol:water (3:1), methyl cellosolve:water (1:1), tetrahydrofuran:water (3:1) were tried with little success.

Whatman No. 1 Paper

1. 1 M Potassium Dihydrogen Phosphate Paper: Whatman No. 1 paper was dipped in 1 M potassium dihydrogen phosphate solution and the chromatogram developed as in previous trials. The following R_F values were obtained:

System	Morphine	Codeine	Thebaine	Narcotine and Papaverine
(a)	0.42	0.50	0.61	0.82
(b)	0.08	0.15	0.52	0.70
(c)	0.10	0.19	0.38	0.79
(d)	0.04	0.16	0.27	very elongated

Mobile phase and description of separation:

(a) n-propanol:water (3:1)

Poor separation between morphine and codeine.

(b) amyl alcohol:formic acid:water (50:9:2)

Good separation, but the spots of thebaine, narcotine, and papaverine are elongated.

(c) tetrahydrofuran:ether:water (1:1:1 v/v, top layer)

Good separation, but the spots of thebaine and narcotine and papaverine are very elongated.

(d) ether:water (1:1 sat.)

Very small compact spots for morphine, codeine, and thebaine, but a very enlarged spot for narcotine and papaverine.

Also, n-propanol:0.2 M KH_2PO_4 plus citric acid to pH 3.5 (3:1) was tried and gave very poor separation and a large amount of tail formation.

2. 0.2 M Potassium Dihydrogen Phosphate Paper: Whatman No. 1 paper was dipped in 0.2 M potassium dihydrogen phosphate solution and the chromatograms developed as in the previous trials. The following R_F values were obtained:

System	Morphine	Codeine	Thebaine	Narcotine and Papaverine
(a)	0.38	0.47	0.61	0.73
(b)	0.11	0.20	0.34	0.89

Mobile phase and description of separation:

(a) n-propanol:0.2 M KH_2PO_4 plus citric acid to pH 3.5 (3:1)

Poor separation; spots had beards.

(b) tetrahydrofuran:ether:water (1:1:1 v/v, top layer)

Good separation, but the spots were quite elongated.

Also other papers were used with various solvents in which the pH of the paper was lowered to 3.5, but no improvement was noticed in the separations.

3. 1/7 M Potassium Dihydrogen Phosphate Paper: Whatman No. 1 paper was dipped in 1/7 M potassium dihydrogen phosphate solution and the chromatograms developed as in the previous trials. The following R_F values were obtained:

System	Morphine	Codeine	Thebaine	Narcotine and Papaverine
(a)	0.42	0.50	0.65	0.80
(b)	0.12	0.20	0.69	0.80
(c)	0.51	0.61	0.75	0.85
(d)	0.22	0.36	0.73	0.94

Mobile phase and description of separation:

(a) n-propanol:water (3:1)

Fair separation; all the spots were fairly enlarged.

(b) amyl alcohol:formic acid:water (50:9:2)

Good separation; some beard on thebaine;

(c) n-propanol:water:citric acid (3:1;molar)

Poor separation; very elongated spot for thebaine.

(d) toluene:isobutanol:citric acid (1:1;molar)

Very good separation and well shaped spots.

Also other papers were tried with various solvents in which the pH of the paper was lowered to 3.5 but no improvement was noticed in the separations.

Preliminary Chromatography of Poppy Extract

Two poppy plants (22 g), 60 days old, each having a green seed pod, were removed from their pots and immediately frozen in liquid nitrogen. The plants were pulverized while cold and extracted in a

Soxhlet extractor with 150 ml of methylene chloride for 24 hours (20 dumps per hour).

The green solution was then extracted six times with 25 ml portions of 0.1 M phosphoric acid. The aqueous solution was washed with methylene chloride and then the pH adjusted to 8.5 and continuously extracted with 150 ml of methylene chloride for 16 hours. The layers were separated and the methylene chloride solution dried over sodium sulfate and then evaporated to 20 ml.

From this solution, 0.5 ml was then chromatographed on Whatman No. 4, 1 M potassium dihydrogen phosphate paper using n-propanol: water (3:1 v/v) and later sprayed with iodoplatinic acid reagent. Spots having the following R_F values were developed: 0.06, 0.54, 0.64, 0.77, 0.94. The spots had the following colors and intensities: pink, faint; blue, strong; purple, medium; violet, faint; violet, faint, respectively.

Run 4

The six plants that were used were of the Alba variety, Papaver somniferum, and were planted September 5, 1957. They were allowed to grown in the greenhouse, under natural conditions, until October 7, 1957 (31 days) when they were transferred to the "silver box" to grow on a controlled twelve-hour day (6 A.M. to 6 P.M.). The plants appeared to be very healthy and showed no signs of disease.

When the plants were 96 days old, they were placed in the small plant chamber after having been placed in the light for three hours. The following is a summary of growth conditions during the plants' exposure to the radioactive carbon dioxide.

6:00 A.M.: Lights on

9:00 A.M.: Plants transferred to plant chamber

9:15 A.M.: Plant chamber sealed

9:30 A.M.: Carbon dioxide (15 ml) injected into chamber

9:33 A.M.: Carbon-14 dioxide (43 μc) injected into chamber

10:09 A.M.: Carbon-14 dioxide (2 μc) remained in the chamber and an additional 48 μc were injected into the chamber

11:00 A.M.: Less than 1 μc of carbon-14 dioxide remained in the chamber, and an additional 15 ml of carbon dioxide was injected into the chamber

11:03 A.M. An additional 100 μc of carbon-14 dioxide were added to the chamber

12:15 P.M.: The activity had dropped to less than 1 μc in the chamber

12:25 P.M.: Another 15 ml of carbon dioxide was injected into the chamber

3:30 P.M.: The plants were removed from the chamber with less than 0.5 μc of carbon-14 dioxide remaining in the chamber (lights off).

Total activity taken up: 191 μc

Total carbon dioxide taken up: 50 ml

Three plants were harvested immediately after being removed from the chamber and were frozen in liquid nitrogen and then pulverized. The remaining three plants were placed in the "hot box" in the greenhouse and harvested one at a time at the end of two days, five days and nine days.

Each group of plants was separated into three fractions for further treatment after they were harvested and frozen. One fraction was extracted with 95% ethanol in a Soxhlet extractor for 18 to 24 hours. The second fraction was extracted with methylene chloride in a Soxhlet extractor for 18 to 24 hours. The third fraction was placed in a vacuum desiccator and dried.

The alcohol extract from fraction one was used to prepare pheno :butanol:propionic acid two-dimensional chromatograms. A small amount of the solution was evaporated on an aluminum plate for counting. A sample from the pulp remaining after the alcohol extraction was burned and the liberated carbon dioxide trapped as barium carbonate which could then be counted.

The methylene chloride extract from fraction two was used mainly for the isolation of the alkaloids from the plant. Also, a small amount of it was evaporated to dryness and counted. The amount of activity for these three fractions is tabulated below.

Microcuries per gram of Whole Plant

	Ethanol Fraction		CH_2Cl_2 Fraction		Dried Fraction	Whole Plant
	Pulp	Soln.	Pulp	Soln.		
Plant 1 (8.46 g, 0 days)	2.34	6.23	4.18	0.22	8.30	70.2
Plant 2 (10.42 g, 2 days)	2.33	3.54	2.47	0.38	6.74	70.2
Plant 3 (3.88 g, 5 days)	2.22	1.28	2.48	0.39	3.08	11.9
Plant 4 (4.34 g, 9 days)	1.33	1.06	1.68	0.37	3.60	15.6

Total activity accounted for: 168 μc

It should be noted that a large amount of activity was found remaining in the Soxhlet extraction cups of the methylene chloride extractions.

The volume of the methylene chloride extract was concentrated to approximately 15 ml in vacuo. The concentrated extract was extracted with 0.1 M phosphoric acid (5 x 25 ml). The resulting phosphoric acid extract was adjusted to pH 8.5 with potassium hydroxide and extracted continuously at a moderate rate for 18 hours with methylene chloride. The

resulting methylene chloride extract was then evaporated to dryness *in vacuo* and the small amount of material resulting was taken up and spotted on a paper chromatogram. A one-dimensional descending system was used -- Whatman No. 4 paper buffered with 0.2 *M* potassium dihydrogen phosphate as the stationary phase and n-propanol:ether:water (2:1:1 v/v) as the mobile phase. These are tabulated below.

R_F Values

	Morphine	Codeine	Thebaine	Papaverine and Narcotine
Plant 1	0.36	0.41	0.51	0.82
Plant 2	0.37	0.42	0.52	0.78
Plant 3	0.41	0.51	0.67	0.88
Plant 4	0.38	0.46	0.67	0.82

Counts per Minute for the Alkaloidal Spots

	Morphine	Codeine	Thebaine	Papaverine and Narcotine	Origin (at start)
Plant 1	36	83	139	391	1100
Plant 2	579	1300	443	2990	6250
Plant 3	300	457	158	582	2185
Plant 4	187	388	58	292	1250

Counts per Minute per 1000 Counts at the Origin

	Morphine	Codeine	Thebaine	Papaverine and Narcotine
Plant 1	33	75	126	355
Plant 2	92	208	71	478
Plant 3	138	210	73	268
Plant 4	150	310	46	234

Cellulose Powder Chromatography of a Mixture of Opium Alkaloids

The buffered cellulose powder used was prepared by mising 0.2 M potassium dihydrogen phosphate (pH 4.6) solution and Whatman Standard Grade Ashless Cellulose Powder. The mixture was filtered and press-dried in a Buchner funnel using a rubber sheet diaphragm. The press-dried cellulose was then dried over phosphorus pentoxide in a vacuum desiccator.

The dried buffered cellulose was then added to a solution of n-propanol:ether:water (2:1:1 by volume) in a blender and the resulting slurry was added to the chromatography column (0.8 by 30 cm), prepared under laboratory air pressure (10 p.s.i.).

Approximately 0.5 mg of papaverine, narcotine, thebaine, codeine, and morphine was dissolved in 0.5 ml of chloroform:methanol (1:1) and spotted on two thick filter paper circles (0.8 cm in diameter). These were then added to the top of the cellulose column. Using an n-propanol:ether:water (2:1:1 by volume) solution, an initial fraction of 6.5 ml was collected. Then fractions of approximately 0.5 ml were collected every 12 minutes. The ultraviolet spectra of each fraction was taken and 0.1 ml of each fraction was used for rechromatographing on

0.2 M potassium dihydrogen phosphate-buffered Whatman No. 4 paper using n-propanol:ether:water (2:1:1 by volume) solution as the developing solvent.

Fractions 2 through 4 inclusive showed the presence of papaverine and narcotine. Fractions 4 through 10 showed the presence of thebaine. Fractions 13 through 17 indicated codeine. Fractions 17 through 22 indicated morphine.

Run 5

A local "non-alba" variety that had been planted October 4, 1957 was grown in the "silver box" controlled growth chamber (12 hour light period day) until the day of the experiment, January 22, 1958. A total volume of 60 ml of carbon dioxide was taken up over a six-hour period. The radioactive carbon dioxide was generated by adding 2.5 ml of 0.0265 M sodium bicarbonate (400 microcuries per ml) to sulfuric acid. However, the vibrating reed electrometer indicated only 610 microcuries of radioactive carbon dioxide had been generated.

At the end of the six-hour period in the growth chamber the plant was harvested and then frozen in liquid nitrogen. The pulverized plant material was divided into two fractions of 29.1 g and 15.2 g for extraction with methylene chloride and 95% ethanol, respectively. Each fraction was extracted for 20 hours in a Soxhlet extractor. The ethanol extract was used

in the phenol, butanol:propionic acid two-dimensional paper chromatography.

The methylene chloride extract (280 ml) was concentrated *in vacuo* to 25 ml and extracted with 0.1 M phosphoric acid (4 x 50 ml). This phosphoric acid extract's pH was then adjusted to pH 8.5 and continuously extracted with methylene chloride for a period of 24 hours.

The resulting methylene chloride extract was evaporated to dryness and later spotted on two filter paper circles that were then added to the buffered cellulose powder column (1 by 45 cm) An *n*-propanol:ether:water (2:1:1 by volume) solution was used as the developing solvent. After the first 15 ml of solution was collected, fractions of 0.55 ml were taken. From each fraction, 0.100 ml was used for running the ultraviolet spectra, 0.150 ml for plate counting, and 0.150 ml for rechromatographing on buffered paper. Radioactive papaverine and narcotine were found in Fractions 4 through 7, thebaine in Fractions 5 through 8, a green fluorescent material in Fractions 8 through 10, a blue fluorescent material in Fractions 12 through 19, a bright violet fluorescent material in Fractions 12 through 21, codeine in Fractions 15 through 18, morphine in fractions 20 through 26, and a yellow fluorescent material in Fractions 30 through 40. All of the alkaloids were radioactive.

The ultraviolet spectrum characteristic of thebaine was found in Fraction 8. Also, the ultraviolet spectra of Fraction 23 in both dilute acid and dilute base was similar to that of morphine. The spectrum of codeine was completely covered by those of the fluorescent materials also found in Fractions 15 through 18.

The small amount of solution remaining from Fractions 15 through 18 was evaporated to dryness and taken up in 1 ml of 0.1 M phosphoric acid and washed with methylene chloride. The pH of the aqueous solution was adjusted to pH 11.0 and the basic solution extracted with methylene chloride. Evaporation of the methylene chloride, followed by paper chromatography of the residue, revealed codeine and the same fluorescent materials.

Alumina Chromatography of a Mixture of Opium Alkaloids

Approximately 0.5 mg of thebaine, codeine, and neopine was dissolved in about 1 ml of benzene and added to the alumina chromatography column (0.8 by 30 cm). The column was prepared by adding 10 g of Merck basic aluminum oxide to the chromatography column containing benzene. After the sample had been added to the column, a solution containing 88.5% benzene, 10% chloroform, and 1.5% isopropyl alcohol, by volume, was added as the developing solvent. After collecting the initial 25 ml of solution, fractions of 2.3 ml were collected. Samples of

each fraction were rechromatographed on buffered paper. Thebaine was found in Fractions 4 and 5 and neopine in Fractions 5 and 6. Codeine was found in Fractions 15 through 24.

Run 6

A local "non-alba" variety that had been planted October 4, 1957 was grown in the "silver box" controlled growth chamber (12 hour light day) until the day of the experiment, February 4, 1958. The radioactive carbon dioxide was taken up by the plant over a six-hour period in the small growth chamber. The radioactive carbon dioxide was generated by adding 2.5 ml of 0.0264 M sodium bicarbonate (400 microcuries per ml) to sulfuric acid. The vibrating reed electrometer indicated 400 microcuries of radioactive carbon dioxide had been generated.

The plant (43.9 g) was harvested in the usual manner after the six-hour period and then extracted in a Soxhlet extractor for 20 hours with methylene chloride. The methylene chloride extract was evaporated from 200 ml to 25 ml in vacuo. Total radioactivity in the methylene chloride extract was 12.7 microcuries.

The concentrated methylene chloride extract was then extracted with 0.1 M phosphoric acid (4 x 50 ml). The resulting phosphoric acid extract was then washed with methylene chloride (2 x 25 ml). These washes were then dried and evaporated to dryness to yield a small amount of oil (Residue I). Chromatography on buffered paper indicated the pre-

sence of papaverine or narcotine, or both.

The pH of the washed phosphoric acid solution was adjusted to pH 5.0 with concentrated potassium hydroxide. The adjusted solution was then extracted with methylene chloride (2 x 25 ml). Evaporation of the methylene chloride extract yielded a small amount of material (Residue II) from which a sample was chromatographed. This showed that the methylene chloride removed most of the papaverine and narcotine from the aqueous solution. Also a small amount of thebaine was found. An unknown material having an R_F value between those of thebaine and papaverine and narcotine was also found. It was not fluorescent but gave an alkaloidal test with the iodoplatinic acid spray.

The pH of the adjusted aqueous solution was adjusted to pH 8.5. This adjusted solution was extracted continuously for 18 hours with methylene chloride. The resulting methylene chloride extract was dried over sodium sulfate and evaporated to dryness in vacuo to yield a dark residue (Residue III). Paper chromatography of a small portion of this methylene chloride residue indicated the presence of morphine, codeine, and thebaine, plus various fluorescent materials.

The alkaloidal regions of these chromatograms were cut from the papers and eluted with methanol. However, materials having an ultraviolet absorption were also eluted from the paper and greatly masked the spectra of the alkaloids.

The remainder of the methylene chloride residue (Residue III) from the pH 8.5 extraction was distributed between a mixture of 10 ml of 0.1 M phosphoric acid and 10 ml of methylene chloride. The methylene chloride was separated off and evaporated to yield a small amount of non-alkaloidal material. The pH of the aqueous solution was adjusted to 11.0 with potassium hydroxide. This basic solution was extracted with methylene choride (3 x 10 ml) and this methylene chloride extract was evaporated to dryness to yield a slight residue (Residue IIIA) containing codeine and thebaine.

The pH of the basic aqueous solution was adjusted to pH 8.6 and extracted with methylene chloride (3 x 10 ml). The extract was dried and evaporated to dryness to yield the crude morphine fraction (Residue IIIB).

The residue (Residue IIIA) from the basic extraction was dissolved in a small amount of benzene and added to an alumina column (0.8 by 30 cm). A solution of 88.5% benzene, 10% chloroform, and 1.5% isopropyl alcohol was used as the eluting solvent. After the first 24 ml of solvent was collected, 3 ml fractions were taken. A small portion of each fraction (0.100 ml) was chromatographed on buffered paper. Fractions 3 through 5 contained the thebaine. Fractions 11 through 16 contained the codeine.

The remainder of Fractions 11 through 16 was combined and evaporated to dryness. The resulting residue was taken up in 1 ml of chloroform. The chloroform solution was then extracted with 0.1 M phosphoric acid (3 x 1 ml). The phosphoric acid solution was washed with chloroform and then its pH was adjusted to 11.0 and it was extracted with methylene chloride (3 x 3 ml). The methylene chloride extract was taken up in 2 ml of 95% ethanol. The ultraviolet spectra was taken, and two fractions of 0.500 ml were plated for counting. The ethanol solution had a concentration of 0.079 mg per ml. λ_{max}^{EtOH} 287 mμ, λ_{min}^{EtOH} 265 mμ. The specific activity was 11,200 disintegrations per minute per mg of codeine.

Fractions 3 through 5 containing the thebaine from the alumina chromatography were evaporated to dryness on small filter paper circles. These were added to the buffered cellulose column (0.8 by 30 cm) using n-propanol:ether:water (2:1:1 by volume) as the eluting solvent. After the first fraction of 3.2 ml, fractions of 0.7 ml were collected, and from each of these 0.15 ml portions were rechromatographed on buffered paper. Fractions 7 and 8 contained the thebaine. These two fractions were evaporated to dryness and taken up with 1 ml of 0.1 M phosphoric acid. The acid solution was washed with methylene chloride and the aqueous solution's pH was adjusted to 11.0 with potassium hy-

droxide. The resulting basic solution was extracted with methylene chloride (3 x 1 ml). The methylene chloride extract was dried and then evaporated to dryness. The dry residue was taken up in 2 ml of 95% ethanol and its ultraviolet spectra taken. Two 0.500 ml portions were plated on aluminum discs for counting in the plate counter. Ultraviolet spectra indicated a concentration of 0.028 mg of thebaine per ml of solution. λ_{max}^{EtOH} 285 mμ, λ_{min}^{EtOH} 265 mμ. The specific activity was 88,000 disintegrations per minute per mg of thebaine.

The crude morphine fraction (ResidueIIIB) was spotted on small filter paper circles and added to the 0.2 M potassium dihydrogen phosphate cellulose powder column (0.8 by 30 cm). n-Propanol:ether:water (2:1:1 by volume) was used as the eluting solvent. After the first fraction of 10 ml, fractions of 0.5 ml were collected, and from each of these 0.10 ml portions were rechromatographed on buffered paper. Fractions 9 through 12 contained the morphine. Fractions 11 and 12 were evaporated to dryness and taken up in 1 ml of 0.1 M phosphoric acid. The acid solution was washed with methylene chloride and its pH adjusted to pH 11.0 and washed again with methylene chloride.

The resulting basic solution's pH was adjusted to 8.5 and the solution extracted with methylene chloride (3 x 1 ml). The methylene chloride extract was dried and evaporated to dryness and later taken up

in 2 ml of 95% ethanol. The ultraviolet spectra was taken in acid and basic solution and was similar to that characteristic of morphine. Also, 0.500 ml fractions were taken for plate counting. The specific activity was 1350 disintegrations per minute per mg of morphine.

Run 8

A local "non-alba" variety of Papaver somniferum (opium poppy) that had been planted October 18, 1957 was grown in the greenhouse under natural conditions until the day of the experiment, March 19, 1958. The plant showed no sign of flower formation. The radioactive carbon dioxide was generated from sodium bicarbonate (400 microcuries per ml). The vibrating reed electrometer indicated that 390 microcuries of radioactive carbon dioxide had been generated.

The plant (34.1 g) was removed from the small growth chamber after six hours' exposure to radioactive carbon dioxide and placed in the greenhouse, in the special chamber for radioactive plants, for two days. After this two-day period of natural growth, the plants were harvested in the usual manner. The general scheme used in Run 6 was followed for the isolation of the morphine, codeine, and thebaine. This is also outlined in Table I of the Discussion. However, through carelessness, the codeine fraction was lost. The morphine had a specific activity of 50,100 disintegrations per minute per mg of morphine. The thebaine had a specific activity of 390,000 disintegrations per minute per mg.

Run 9

Another of the local "non-alba" variety that had been planted October 18, 1957 was grown in the greenhouse under natural conditions until the day of the experiment, March 24, 1958. As in all the previous runs, the plant showed no flower formation.

In this run the radioactive carbon dioxide was generated from 29.5 mg of radioactive barium carbonate (36 microcuries per mg) with 200 mg of anhydrous barium carbonate to act as carrier for the gas during its generation. Concentrated sulfuric acid was used to generate the carbon dioxide from the barium carbonate. The reaction was done on a vacuum line and the carbon dioxide was trapped in a U-tube cooled by liquid nitrogen.

All of the radioactivity (1.060 millicuries) was taken up in two hours, at which time the plants (36.9 g) were removed from the chamber and harvested as before. The general scheme used in Run 6 was followed for the isolation of the morphine, codeine, and thebaine. Morphine had a specific activity of 9200 disintegrations per minute per mg. However, most of the morphine fraction was accidentally destroyed. The sample which was counted had an activity of only 14 counts per minute above background and part of this could have arisen from other sources. Codeine had a specific activity of 21,000 disintegrations per minute per mg and thebaine 75,000 disintegrations per minute per mg.

Run 10

The same type of local "non-alba" variety of Papaver somniferum that had been used in the past four runs was used in this run. The plants had been grown in the greenhouse since the day of their planting, October 22, 1957, until the day of the experiment, March 28, 1958. The plants took up 1070 microcuries of radioactive carbon dioxide in a period of six hours while in the growth chamber. The carbon dioxide had been generated from 29.7 mg of radioactive barium carbonate (36 microcuries per mg) and 218 mg of anhydrous barium carbonate as prepared in Run 9.

After the six-hour period in the growth chamber, the plants were removed and placed in the special chamber in the greenhouse and allowed to grow under natural conditions for 10 days before harvesting. The plants were harvested and the alkaloids separated as in the previous three runs that have been described.

The alkaloids had the following specific activities: morphine, 380,000; codeine, 424,000; thebaine, 600,000 disintegrations per minute per mg. Only a small amount of thebaine was isolated in this run. The specific activity is probably between 600,000 and 800,000 disintegrations per minute per mg.

Run 11

Plants from the same group as those used in Run 10 were used in this run. They had been grown in the greenhouse until the day of the experiment, April 10, 1958. The plants took up 1000 microcuries of radioactive carbon dioxide in the six-hour period they were in the plant chamber. The carbon dioxide was generated from 27.8 mg of radioactive barium carbonate (36 microcuries per mg) and 215 mg of anhydrous barium carbonate.

After the six-hour period, the plants were removed from the plant chamber and placed in the greenhouse, in the special chamber for radioactive plants, and allowed to grow under natural conditions for a period of five days, at which time they were harvested and each of the alkaloid's specific activity was determined.

The following specific activities were found: morphine, 450,000 disintegrations per minute per mg; codeine, 705,000 disintegrations per minute per mg; thebaine, 670,000 disintegrations per minute per mg.

Sufficient material remained so that preliminary degradation work could be done.

Ultraviolet Spectra of Alkaloids and Degradation Products

For the determination of the amounts of material for the various alkaloids and their degradation products, the following extinction coeffi-

cients were determined on pure analytical samples of the following compounds in 95% ethanol:

	95% ethanol	
	$\lambda_{max}(m\mu)$	ϵ_{max}
morphine hydrate	287	1515
codeine	286	1550
thebaine	285	7330
normorphine	287	1510
norcodeine	286	1490
dihydrocodeine	285	1600
dihydromorphine	285	1440
dihydronorcodeine	285	1590

Small Scale Conversion of Codeine to Morphine using Pyridine Hydrochloride

A 0.81 mg portion of codeine was sealed in a 5 mm Pyrex tube under nitrogen with 12 mg of dry pyridine hydrochloride. The sealed tube was heated in an oil bath melting point apparatus at a constant temperature of 220°C for 6 minutes.

The cold melt was dissolved in 3 ml of 0.1 M phosphoric acid which was then washed with methylene chloride (3 x 3 ml). The pH of the aqueous solution was adjusted to 5.0 with potassium hydroxide and

again washed with methylene chloride (5 x 3 ml). After each wash the pH was readjusted to 5.0.

The aqueous pH 5.0 solution's pH was adjusted to 8.5 and the adjusted solution extracted with methylene chloride (4 x 3 ml). This methylene chloride extract was dried and evaporated to dryness and later spotted on filter paper circles and chromatographed on a 0.2 M potassium dihydrogen phosphate buffered cellulose powder column (0.8 x 30 cm) using n-propanol:ether:water: (2:1:1 by volume) as the developing solvent. After the first 4.8 ml were collected, fractions of 0.95 ml were taken. Fractions 8 and 9 contained the morphine as determined by paper chromatography of 0.100 ml of each fraction. Fraction 8 also contained some other light blue fluorescent material so was not used.

Fraction 9 was evaporated to dryness and the morphine taken up from the residue with 95% ethanol. The ultraviolet spectra of this solution indicate the presence of 0.072 mg of morphine (9% yield).

Conversion of Codeine to Morphine using Codeine from Run 11

The codeine remaining from Run 11 was diluted with 1.22 mg of anhydrous codeine (m.p. 154-155°C) in 10 ml of 95% ethanol. Ultraviolet spectra indicated the presence of 1.39 mg of codeine. From this

solution two 0.250 ml portions were deposited on planchets for plate counting. This indicated a specific activity of 89,000 disintegrations per minute per mg. From the remaining solution, 5.0 ml were used in this experiment and 4.5 ml in the conversion of codeine to norcodeine.

The former was evaporated to dryness in a 5 mm Pyrex tube. A 20 mg portion of pyridine hydrochloride was added to the tube and it was sealed under a nitrogen atmosphere. The reaction and work-up was carried out as in the previous small scale run.

From the chromatography, after the first 4.5 ml had been collected, fractions of 0.90 ml were taken. After paper chromatography of 0.100 ml of each fraction, the morphine was found in Fraction 10. The remainder of Fraction 10 was evaporated to dryness and the morphine taken up in 95% ethanol. The ultraviolet spectra indicated the presence of 0.076 mg of morphine in 2.0 ml of ethanol (11% yield). The morphine had a specific activity of 71,000 disintegrations per minute per mg. This figure compared with the original codeine activity would be 562,000 disintegrations per minute per mg.

Small Scale Conversion of Morphine to Normorphine

A 0.91 mg portion of morphine was refluxed under nitrogen in 0.100 ml of acetic anhydride for 16 hours. (Bath temperature was 150°C). After the refluxing period, the acetic anhydride was removed

in vacuo at room temperature to yield crystalline diacetylmorphine. The crystalline solid was dissolved in 5 ml of benzene and the solution was washed with two 2 ml portions of 0.5 *M* sodium carbonate and two 2 ml portions of 0.5 *M* sodium carbonate and two 2 ml portions of water. The benzene solution was dried over sodium sulfate and then evaporated to dryness.

The crystalline residue was dissolved in 1 ml of chloroform. The solution was added to a cold solution (0°C) of 5 mg of cyanogen bromide in 1 ml of chloroform. The solution was allowed to stand 30 minutes in the ice bath and then 30 minutes at room temperature. The solution was then heated to reflux for two and one-half hours. The chloroform was evaporated to dryness *in vacuo* at room temperature. Two 1 ml portions of chloroform were added to the residue and each evaporated to dryness *in vacuo*. The residue was dissolved in 3 ml of chloroform and washed with two 1 ml portions of 0.1 *M* phosphoric acid. The acid washes were back-washed with chloroform. The combined chloroform solutions were dried over sodium sulfate and the solvent evaporated to dryness.

The crystalline residue was heated for five minutes with 0.5 ml of concentrated hydrochloric acid at 100°C. Four ml of water was added to the solution which was then heated to reflux for 3 hours. The solution was then evaporated to dryness *in vacuo*.

The crystalline normorphine hydrochloride was taken up in methanol-water solution and plated on filter paper circles. The filter paper circles were added to a 0.2 M potassium dihydrogen phosphate buffered cellulose powder column (0.8 x 30 cm). An n-propanol:ether: water (2:1:1 by volume) solution was used as the developing solvent. After 4.5 ml of the solution had been collected, 0.72 ml fractions were taken.

A 0.085 ml portion of each fraction was rechromatographed on Whatman No. 4 paper, buffered with 0.2 M potassium dihydrogen phosphate, using n-propanol:ether:water (2:1:1 by volume) as the developing solvent. Pure normorphine was detected in Fractions 9 and 10. Fractions 9 and 10 were each evaporated to dryness and the normorphine taken up from the residue with 95% ethanol. Ultraviolet spectra indicated the presence of 0.284 mg of normorphine (35% yield).

Conversion of Morphine to Normorphine using Morphine from Run 11

Morphine, remaining after counting, from Run 11 was diluted with 1.08 mg of morphine (monohydrate) in 10 ml of 95% ethanol. Ultraviolet spectra indicated the presence of 1.20 mg of morphine. From this, 10 ml of solution, two 0.500 ml portions, were plated on planchets for plate counting. This indicated a specific activity of 42,700 disintegrations per minute per mg of morphine. From the remaining solution, 5.0 ml

was used in this conversion of morphine to normorphine. The remaining 4.0 ml was evaporated to dryness and saved.

The 5.0 ml of morphine solution was evaporated to dryness in the small reaction flask used for the acetylation. A 0.100 ml portion of acetic anhydride was added to the flask and the reaction mixture refluxed under nitrogen for 16 hours as before. The conversion of the morphine to normorphine was then carried out as in the small scale conversion of morphine to normorphine (p. 173).

Ultraviolet spectra of the normorphine indicated the presence of 0.294 mg of normorphine (55% yield).

The normorphine had a specific activity of 49,000 disintegrations per minute per mg of normorphine. This figure compared with the original morphine activity would be 516,000 disintegrations per minute per mg of normorphine, or 150,000 disintegrations per minute per micromole of normorphine.

Conversion of Codeine to Norcodeine using Codeine from Run 11

The 4.5 ml of ethanol containing the "diluted" codeine (0.625 mg) that is described in the conversion of codeine to morphine using codeine from Run 11 was evaporated to dryness in the reaction flask used for the acetylation of the codeine. A 0.100 ml portion of acetic anhydride was added to the flask and the reaction mixture refluxed under nitrogen

for 8 hours. There was noticeable darkening of the solution. (This did not occur in the acetylation of morphine.) After the refluxing period, the acetic anhydride was removed *in vacuo* at room temperature to yield monoacetylcodeine. The solid was dissolved in 5 ml of benzene and the solution was washed with two 2 ml portions of 0.5 *M* sodium carbonate and two 2 ml portions of water. The benzene solution was dried over sodium sulfate and then evaporated to dryness.

The residue was dissolved in 1 ml of chloroform. The solution was added to a cold solution (0°C) of 5 ml of cyanogen bromide in 1 ml of chloroform. The solution was allowed to stand for 30 minutes in the ice bath and then for 30 minutes at room temperature. The solution was then heated to reflux for two and one-half hours. The chloroform was then evaporated to dryness *in vacuo* at room temperature. Two 1 ml portions of chloroform were added to the residue and each evaporated to dryness *in vacuo*. The residue was dissolved in 3 ml of chloroform and washed with two 1 ml portions of 0.1 *M* phosphoric acid. The acid washes were back-washed with chloroform. The combined chloroform solutions were dried over sodium sulfate and the solvent evaporated to dryness.

The residue was heated for 5 minutes with 0.5 ml of concentrated hydrochloric acid at 100°C. Four ml of water was added to the solution which was then heated to reflux for 3 hours. The solution was then evaporated to dryness *in vacuo*.

The norcodeine hydrochloride was taken up in methanol and plated on filter paper circles. The filter paper circles were added to a 0.2 M potassium dihydrogen phosphate buffered cellulose powder column (0.8 x 30 cm). An n-propanol:ether:water (2:1:1 by volume) solution was used as the developing solvent. After the first 4.0 ml of solvent, fractions of 0.85 ml were collected. A portion of each fraction (0.100 ml) was chromatographed upon Whatman No. 4, 0.2 M potassium dihydrogen phosphate buffered paper using n-propanol:ether:water (2:1:1 by volume) as the developing solvent. Norcodeine was indicated in Fractions 8, 9 and 10.

Fraction 9 was evaporated to dryness and the norcodeine taken up in 95% ethanol. The ultraviolet spectra indicated the presence of 0.125 mg of norcodeine (21% yield).

The norcodeine had a specific activity of 76,600 disintegrations per minute per mg of norcodeine. This figure compared with the original codeine activity would be 607,000 disintegrations per minute per mg of norcodeine.

Moderate Scale Preparation of 14-Bromocodeinone

Thebaine (6.22 g, 20 mmole) was suspended in 20 ml of 2:1 (by volume) acetone-water mixture. A solution of 3.7 g (20.8 mmole) of N-bromosuccinimide in 40 ml of 2:1 acetone-water was run in with

stirring over a period of 15 minutes. The temperature was maintained at 15-18°C by external cooling during the addition, and thereafter for 10 minutes. Water (100 ml) was added over a period of 30 minutes with stirring, when the bromocodeinone began to crystallize. Stirring was continued for one hour at 20°C and then for another 2 hours at 0°C. The product was sucked dry and washed with 50 ml of water. The yield, after drying in vacuum overnight was 5.6 g (75%). The material exhibited the same melting behavior as reported by Conroy[77]. When the sample is inserted in the melting point apparatus heated to 157°C, it melts to a deep red liquid immediately, but if inserted at 154°C or below, it does not melt, even if the temperature is raised above 157°C. Instead decomposition takes place in the solid state, leading to brown amorphous material.

Moderate Scale Sodium Borohydride Reduction of 14-Bromocodeinone

A solution, prepared by dissolving 300 mg of sodium borohydride in 2 ml of water and adding 5 ml of methanol, was added to a suspension of 100 mg of 14-bromocodeinone in 5 ml of methanol.

This solution was allowed to stand 90 minutes at room temperature and then placed on a steam bath under a stream of nitrogen. About half of the solution was evaporated off. Water was added and the solution concentrated. This was repeated once again.

The solution which was now basic was extracted with three equal volumes of methylene chloride. The methylene chloride extract was dried over sodium sulfate and evaporated to dryness to yield 100 mg of a light yellow oil. No attempt was made to obtain a crystalline product.

The infrared spectrum of the oil was similar to that of neopine. However, chromatography of a small sample on Whatman No. 4 potassium dihydrogen phosphate buffered paper revealed the presence of two other materials in addition to neopine. About half of the material was neopine, judging from the spots.

Half of the residue was hydrogenated in 5 ml of absolute ethanol using 4.6 mg of platinum oxide catalyst. The hydrogen uptake corresponded to approximately one mole, and was completed after 90 minutes. The catalyst was filtered off and the solution evaporated to dryness to yield a light yellow oil. About 1 mg of this oil was chromatographed on 10 g of basic alumina using benzene:chloroform:isopropanol (88.5:10:1.5 by volume) as the developing solvent. After the first 11.0 ml of solution collected, fractions of 4.7 ml were taken. A portion of each fraction was rechromatographed on buffered paper. An unidentified alkaloid was found in Fractions 4 and 5 and another in Fractions 5 through 7. Dihydrocodeine was found in Fractions 7 through 10.

Moderate Scale Lithium Aluminum Hydride Reduction of 14-Bromocodeinone in Tetrahydrofuran

A 111 mg portion of 14-bromocodeinone was dissolved in 5 ml of freshly distilled and purified tetrahydrofuran. To the solution was added 2 ml of a 0.96 M ether solution of lithium aluminum hydride. The solution was stirred under nitrogen for 90 minutes at room temperature. Ethyl acetate was added to neutralize the excess hydride. This was followed by the addition of 5.0 ml of 1.0 M hydrochloric acid solution. The acid solution was washed with three 5 ml portions of ether. The pH of the aqueous solution was adjusted to 10.0 and the mixture was extracted with methylene chloride (4 x 5 ml).

The methylene chloride extract was dried over sodium sulfate and evaporated to dryness to yield 81 mg of an oil whose infrared spectra was identical with that of neopine. Chromatography of a small portion of the oil on buffered paper indicated the presence of neopine as the major constituent with two minor alkaloidal contaminants.

Moderate Scale Hydrogenation of Neopine

Neopine (103 mg) was dissolved in 10 ml of absolute ethanol and 5.0 mg of platinum oxide catalyst was added. The solution was hydrogenated at atmospheric pressure and room temperature. One mole of hydrogen was taken up smoothly over a period of 90 minutes.

The catalyst was filtered off and the ethanol solution evaporated to dryness in vacuo to yield 100 mg of a colorless oil. The infrared spectrum of the oil was identical with that of an authentic crystalline sample of dihydrocodeine. Paper chromatography revealed the presence of only one material, dihydrocodeine. A small amount (about 1-2 mg) was chromatographed in 10 g of basic alumina using a benzene:chloroform: isopropanol (88.5:10:1.5 by volume) solution as the developing solvent. Fractions of 6 ml in volume were collected and dihydrocodeine was found in Fractions 6 through 10. This chromatography was the same as a trial chromatography of authentic dihydrocodeine.

Small Scale Bromination of Thebaine in Chloroform-Acetone

A small sample of thebaine (1.074 mg) was dissolved in 0.025 ml of chloroform. To this was added, with stirring, 0.0060 ml of a freshly prepared 10% solution of N-bromosuccinimide in acetone. The addition was done over a 15-minute period. Methanol was added to the colorless solution and then evaporated to dryness. Paper chromatography of a portion of the residue indicated the presence of 14-bromocodeinone and unreacted thebaine.

Small Scale Bromination of Thebaine in Carbon Tetrachloride

Thebaine (1.19 mg) was dissolved in 0.150 ml of carbon tetrachloride, and to this was added 0.92 mg of N-bromosuccinimide. The mixture was stirred under nitrogen for 17 hours. The mixture was taken up in methanol and evaporated to dryness. Paper chromatography of a portion of the residue indicated the presence of only 14-bromocodeinone.

Small Scale Lithium Aluminum Hydride Reduction of 14-Bromocodeinone in Tetrahydrofuran

A small sample of 14-bromocodeinone (1.021 mg) was dissolved in 0.050 ml of purified tetrahydrofuran. To this was added 0.020 ml of 0.96 M ether solution of lithium aluminum hydride. The solution was stirred under nitrogen for 90 minutes, after which time the excess hydride was neutralized with ethyl acetate. One ml of 1 N hydrochloric acid was added and the mixture washed with ether (3 x 1 ml). The aqueous solution was basified with a solution of potassium hydroxide and Rochelle salt. This basified solution was extracted with chloroform (3 x 3 ml). This chloroform extract was dried over sodium sulfate and then evaporated to dryness. Chromatography of a fraction of the residue revealed two alkaloidal spots, neither of which was neopine or starting material.

Moderate Scale Lithium Aluminum Hydride Reduction of 14-Bromocodeinone in Ether

14-Bromocodeinone (99 mg) was suspended in ether. To this was added 2.0 ml of a 0.96 M ether solution of lithium aluminum hydride. A white precipitate formed and the mixture was stirred for 30 minutes in a nitrogen atmosphere. Ethyl acetate was added to remove the excess hydride. A 1 N hydrochloric acid solution (10 ml) was added to the mixture. The precipitate dissolved and the phases were separated. The acid phase was washed with ether (3 x 25 ml). The washed acid solution was basified with a solution of potassium hydroxide and Rochelle salt. The resulting basic solution was extracted with methylene chloride (4 x 20 ml). This extract was dried over sodium sulfate and evaporated to dryness in vacuo to yield 66 mg of an oil.

The infrared spectra of the oil was similar to that of neopine. Paper chromatography indicated a small amount of some unidentified alkaloidal contaminant in addition to the neopine.

Approximately 1-2 mg of the oil was chromatographed on basic alumina using benzene:chloroform:isopropanol (88.5:10:1.5 by volume) as the developing solvent. Neopine was the only material eluted from the column with this solvent.

The remainder of the oil was hydrogenated in absolute ethanol (10 ml) using 9.9 mg of platinum oxide catalyst. After 140 minutes, no more hydrogen was being taken up, so the catalyst was filtered off and the solution evaporated to dryness. Chromatography, on basic alumina, of a small sample of the

resulting oil indicated that unhydrogenated neopine was a minor contaminant of the dihydrocodeine. Possibly the catalyst had been poisoned during the course of the hydrogenation.

Small Scale Lithium Aluminum Hydride Reduction in Ether

A small amount of 14-bromocodeinone (1.022 mg) was dissolved in chloroform and then evaporated to dryness in the reaction flask. Dry ether (0.250 ml) was added to the flask, followed by 0.025 ml of 0.81 M ether solution of lithium aluminum hydride. The mxture was stirred under nitrogen for 30 minutes, after which time the excess hydride was destroyed with ethyl acetate. One ml of 1 N hydrochloric acid was added to the mixture and the resulting two phases separated. The acid phase was washed with ether (3 x 1 ml) and then made basic with a solution containing potassium hydroxide and Rochelle salt. The resulting basic solution was extracted with chloroform (3 x 3 ml). This chloroform extract was dried over sodium sulfate and then evaporated to dryness to yield a small amount of oil.

Paper chromatography of a small fraction of the oil revealed only the presence of neopine.

The remainder of the oil was dissolved in 1.0 ml of absolute ethanol. Platinum oxide catalyst (about 1 mg) was added to the solution

and the mixture hydrogenated for 90 minutes at room temperature and atmospheric pressure. After this time the solvent was evaporated to dryness and then a small amount of benzene was added. This benzene solution containing the alkaloidal material and a small portion of the catalyst was added to the top of an alkaline alumina (10 g) chromatography column (0.8 x 25 cm). A benzene:chloroform:isopropanol (88.5:10:1.5 by volume) solution was used as the developing solvent. An aliquot from each fraction was rechromatographed on buffered paper. Dihydrocodeine was the only material eluted from the alumina column.

Small Scale Pyridine Hydrochloride Cleavage of Dihydrocodeine

Crystalline dihydrocodeine (0.572 mg) was dissolved in methanol and added to the bottom of a 5 mm Pyrex tube. The solvent was evaporated off under a stream of nitrogen and 20 mg of pyridine hydrochloride was added to the dry tube and the tube sealed under nitrogen.

The sealed tube was heated at 220°C for 6 minutes in a silicone oil melting point apparatus and then immediately cooled.

The cooled melt was taken up in 3 ml of 0.1 M phosphoric acid. The pH of the acid solution was adjusted to 5.0 with a 5% solution of potassium hydroxide. The adjusted solution was washed five times with equal parts of chloroform. After each wash the pH was again adjusted to 5.0. The pH of the washed solution was adjusted to 8.5

and this solution was extracted with chloroform (5 x 4 ml).

The chloroform extract was dried over sodium sulfate and evaporated to dryness. The residue from the chloroform extract was plated on filter paper circles which were added to the top of a 0.2 M potassium dihydrogen phosphate buffered cellulose powder column (0.8 x 30 cm). An n-propanol:ether:water (2:1:1 by volume) solution was used as the developing solvent.

After the 5.0 ml of solution had been collected, fractions of 0.62 ml were taken. From each fraction 0.10 ml was rechromatographed on buffered paper using the same developing solvent as in the column chromatography. Dihydromorphine was found in Fractions 9, 10 and 11. These fractions were combined and evaporated to dryness. The residue, containing some inorganic material, was washed with ethanol and its ultraviolet spectrum run. The ultraviolet spectrum indicated the presence of 0.160 mg of dihydromorphine.

Preparation of Dihydronorcodeine by Hydrogenation of Norcodeine

Recrystallized norcodeine (1.0 g) was dissolved in 25 ml of absolute ethanol. To this was added 57 mg of platinum oxide catalyst, and the mixture was hydrogenated at room temperature and atmospheric pressure for 90 minutes. One mole of hydrogen was taken up smoothly in this time. The catalyst was filtered off and the solution evaporated to

dryness to yield a crystalline product. The crystalline residue was recrystallized from ethanol, and the first crop of needles was sublimed at 140°C, 0.01 mm of mercury pressure, to yield 700 mg of white powder (m.p. 199-200°C). Reported[80] m.p. 194°C. $\lambda_{max}^{95\%\ EtOH}$ 285 mμ ϵ = 1590. $\lambda_{min}^{95\%\ EtOH}$ 258 mμ ϵ = 320.

Small Scale Conversion of Dihydrocodeine to Dihydronorcodeine

A methanol solution of dihydrocodeine (0.46 mg) was evaporated to dryness in the reaction flask used for the acetylation. The same conditions as those employed for the conversion of codeine to norcodeine using codeine from Run 11 (p. 176) were employed.

In the chromatography of the crude dihydronorcodeine, an initial fraction of 5.0 ml was taken and then fractions of 0.62 ml. Dihydronorcodeine was indicated in Fractions 8 and 9, Each fraction was evaporated to dryness and the dihydronorcodeine taken up in 95% ethanol. The ultraviolet spectra indicated the presence of only 0.027 mg of dihydronorcodeine.

Trial Thebaine Degradation to Dihydrocodeine

In a typical thebaine degradation 2.498 mg of thebaine was taken up in chloroform and evaporated to dryness in the flask used for the bromination. The residue was dissolved in 0.250 ml of carbon tetrachloride. To this solution 1.59 mg of N-bromosuccinimide was

added and the mixture stirred under nitrogen for 18 hours.

The mixture was evaporated to dryness, and to it was added 1 ml of dry ether. A 0.81 M ether solution of lithium aluminum hydride (0.100 ml) was added and the mixture stirred under nitrogen for 2 hours. Ethyl acetate was added to the mixture to remove the excess hydride. This was followed by 2 ml of 1 N hydrochloric acid. The phases were separated and the aqueous phase washed with ether (3 x 2 ml). The washed acid solution was made basic by the addition of a solution containing Rochelle salt and potassium hydroxide. This basic solution was extracted with chloroform (4 x 3 ml). The chloroform extract was dried over sodium sulfate and evaporated to dryness. Paper chromatography of a small fraction of the residue indicated neopine as the only product.

The residue from the hydride extract was dissolved in 1.0 ml of absolute ethanol. Platinum oxide catalyst (1 mg) was added and the mixture hydrogenated for 2 hours. A small sample (0.025 ml) was taken from the solution and chromatographed on buffered paper. Dihydrocodeine was the only alkaloidal material.

The remainder of the hydrogenation solution was evaporated to dryness and then taken up in benzene. Only about half of the dihydrocodeine went into solution; possibly the dihydrocodeine that did not go

into solution was in the form of a salt. This benzene solution was added to a chromatography column (0.8 x 25 cm) containing 10 g of basic alumina. A benzene:chloroform:isopropanol (88.5:10:1.5 by volume) solution was the developing solvent. After the first 4.5 ml, 2.0 ml fractions were collected and from each of them 0.200 ml was rechromatographed on buffered paper. Fractions 14 through 21 contained dihydrocodeine. These fractions were combined and evaporated to dryness. The ultraviolet spectra in ethanol indicated the presence of 0.575 mg of dihydrocodeine.

Run 12 -- Isolation of Morphine, Codeine, and Thebaine

The plants used in this experiment were a local "non-alba" variety that had been planted March 14, 1958 and allowed to grow in the "silver box" plant growth chamber from May 7, 1958 until the day of the experiment, July 29, 1958. The plant (6.10 g) took up a total of 20 millicuries of radioactive carbon dioxide during the six-hour period that it was in the chamber. The carbon dioxide was generated from 207.7 mg of barium carbonate (0.116 millicuries per mg).

After the six-hour period, the remaining radioactive carbon dioxide was flushed from the chamber and the plant harvested. The plant was frozen in liquid nitrogen and crushed in the usual manner. The crushed material was extracted in a Soxhlet extractor for 20 hours

with methylene chloride. The methylene chloride extract was evaporated from 150 ml to 25 ml *in vacuo*.

The concentrated methylene chloride extract was then extracted with 0.1 *M* phosphoric acid (4 x 45 ml). The resulting phosphoric acid extract was washed with hexane (2 x 25 ml). These washes were discarded since they contained no alkaloidal material.

The pH of the washed phosphoric acid solution was adjusted to 5.0 with a concentrated potassium hydroxide solution. The adjusted solution was extracted with methylene chloride (2 x 25 ml). This methylene chloride extract was dried over sodium sulfate and evaporated to dryness. This extract (II) contains papaverine and narcotine and other unknown plant material.

The pH of the adjusted aqueous solution was adjusted to 8.5 and the solution extracted continuously for 18 hours with methylene chloride. A good dispersion tube was used in the extraction. This methylene chloride extract was dried over sodium sulfate and then evaporated to dryness to yield a dark residue (III).

The residue from the methylene chloride extract (III) was distributed between a mixture of 10 ml of 0.1 *M* phosphoric acid and 10 ml of methylene chloride. The methylene chloride phase was discarded. The pH of the aqueous solution was adjusted to 11.5 with

potassium hydroxide. This basic solution was extracted with methylene chloride (3 x 10 ml). The methylene chloride extract was dried over sodium sulfate and then evaporated to dryness to yield a slight residue (IIIA). A small fraction (1/20) of the residue (IIIA) was chromatographed on Whatman No. 4 potassium dihydrogen phosphate buffered paper using n-propanol:ether:water (2:1:1 by volume) as the developing solvent. Codeine and thebaine were found.

The pH of the basic aqueous solution was adjusted to 8.6 and extracted with methylene chloride (4 x 10 ml). The extract was dried over sodium sulfate and evaporated to dryness to yield a slight brown residue (IIIB). Chromatography as in (IIIA) indicated the presence of morphine in (IIIB).

The residue (IIIA) from basic extraction was dissolved in a small amount of benzene and added to an alumina (10 g) column (0.8 x 25 cm). A benzene:chloroform:isopropanol (88.5:10:1.5 by volume) solution was used as the developing solvent. Fractions were collected and from each an aliquot was taken and chromatographed on buffered paper using the same system as was used on the crude extracts. Thebaine was found in the early fractions and codeine in the later fractions.

The fractions containing the codeine but not fluorescent contaminants were combined and evaporated to dryness. The residue was taken up in 1 ml of chloroform and extracted with 0.1 M phosphoric

acid (3 x 1 ml). The phosphoric acid solution was washed with chloroform and then its pH adjusted to 11.0. This basic solution was extracted with methylene chloride (3 x 3 ml). The methylene chloride extract was dried and evaporated to dryness. The residue was washed with 2.0 ml of water and its ultraviolet spectra indicated the presence of 0.050 mg of codeine. From this solution two 0.050 ml fractions were plated on planchets for counting and 0.200 ml was spotted on buffered paper and chromatographed as usual. The paper strip counter indicated that the only radioactive material was the codeine. The specific activity of the codeine from the plate counting was 1,600,000 disintegrations per minute per mg of codeine, or 478,000 disintegraions per minute per micromole of codeine.

The thebaine fractions were combined and evaporated to dryness on filter paper circles. These were added to the buffered cellulose powder column. An n-propanol:ether:water (2:1:1 by volume) solution was used as the developing solvent. After the first 5 ml of solution was collected, fractions of 1.4 ml were taken. From these, 0.100 ml aliquots were taken and rechromatographed on buffered paper. These indicated the presence of thebaine in Fraction 5 and 6. However, other nonalkaloidal fluorescent material was found in Fraction5 which contained most of the thebaine. In later runs, smaller fractions

should be collected from the chromatography. Fractions 6 and 7 were combined and then purified in the same manner as the codeine fractions. The paper chromatograms of the purified fractions 6 and 7 were run through the paper strip counter. The thebaine spot was the only location of radioactivity. Counting of the purified Fractions 6 and 7 revealed 22,500,000 disintegrations per minute per mg of thebaine, or 7,000,000 disintegrations per minute per micromole of thebaine.

The original Fraction 5, containing most of the thebaine, was diluted with 1.131 mg of thebaine and rechromatographed on cellulose powder as before. After collection of 6.2 ml, fractions of 0.54 ml were taken. Thebaine was found in Fractions 6, 7 and 8. Fractions 7 and 8 were evaporated to dryness and then taken up in ethanol and their ultraviolet spectra determined. From Fraction 8 samples were taken for counting and for rechromatographing on buffered paper. The thebaine spot was the only radioactive material found using the paper strip counter. The plate counting revealed that this diluted thebaine had a specific activity of 1,990,000 disintegrations per minute per mg of thebaine.

The crude morphine fraction (IIIB) was spotted on small filter paper circles and added to a 0.2 M potassium dihydrogen phosphate cellulose powder column (0.8 x 30 cm). An n-propanol:ether:water

(2:1:1 by volume) solution was used as the developing solvent. After the first 9.8 ml had been collected, fractions of 0.60 ml were taken. From these, 0.050 ml was rechromatographed on buffered paper. Morphine was found in Fractions 6 through 8. Fractions 7 and 8 were each evaporated to dryness and taken up in 1 ml of 0.1 M phosphoric acid. The acid solution was washed with methylene chloride and the pH of the aqueous solution adjusted to 11.0 and washed again with methylene chloride. The washed basic solution's pH was adjusted back to 8.5 and the solution extracted with methylene chloride (4 x 1 ml). The methylene chloride extract was dried and evaporated to dryness and later taken up in 95% ethanol. The ultraviolet spectra of the ethanol solution was determined. Also samples were taken for plate counting and paper chromatography. The counting indicated that the morphine had a specific activity of 165,000 disintegrations per minute per mg of morphine, or 50,000 disintegrations per minute per micromole of morphine.

Run 12 -- Conversion of Morphine to Normorphine

The remainder of purified Fraction 7 from the cellulose powder chromatography of the crude morphine was diluted with 0.844 mg of morphine to give morphine with a specific activity of 26,800 dis-

integrations per minute per mg of morphine, or a dilution factor of 6.15. About half of the morphine (0.47 mg) was evaporated to dryness in the flask used for the acetylation. The method of degradation was the same as that for the small scale conversion of morphine to normorphine, (p. 173).

Ultraviolet spectra indicated 0.056 mg of pure normorphine in the normorphine fractions. The normorphine had a specific activity of 12,200 disintegrations per minute per mg of normorphine. This figure multiplied by the dilution factor, 6.15, gives a value of 75,000 disintegrations per minute per mg of normorphine (undiluted) or 20,300 disintegrations per minute per micromole of normorphine.

Run 12 -- Conversion of Codeine to Morphine

The remainder of the purified codeine from Run 12 was diluted with codeine to give 1.13 mg of diluted codeine. The diluted codeine had a specific activity of 69,500 disintegrations per minute per mg of codeine or a dilution factor of 23.0. Fifty percent of the codeine was used in this conversion of the codeine to morphine. It was dissolved in methanol and evaporated to dryness in a 5 mm Pyrex glass tube. Approximately 20 mg of pyridine hydrochloride was added to the tube containing the codeine and the tube sealed under nitrogen. The reaction and its work-up was the same as in the pre-

liminary small scale conversion of codeine to morphine (p. 171).

In the cellulose powder chromatography after the first 9.0 ml had been taken, fractions of 0.75 ml were collected. From each fraction 0.100 ml aliquots were taken and chromatographed on buffered paper. Fractions 6 and 7 contained pure morphine as indicated from the paper chromatogram. Fractions 6 and 7 were combined and evaporated to dryness and the morphine taken up in 1 ml of 95% ethanol. The ultraviolet spectra of this solution indicated the presence of 0.041 mg of morphine. The morphine had a specific activity of 39,500 disintegrations per minute per mg of morphine, or 11,900 disintegrations, per minute per micromole of morphine. These values multiplied by the dilution factor, 23.0, give values of 910,000 disintegrations per minute per mg of morphine, or 274,000 disintegrations per minute per micromole of morphine. These last values then would be a comparison of the results that might have been obtained had undiluted codeine been converted to morphine.

Run 12 -- Conversion of Codeine to Norcodeine

Part of the remaining diluted codeine from Run 12, described in the conversion of codeine to morphine (p.196), was used in this conversion of codeine to norcodeine. This amounted to 48% of the

original diluted codeine. (Two percent was used for counting.) This diluted codeine was taken up in methanol and evaporated to dryness in the acetylation flask. The experimental procedure is the same as that employed in the conversion of codeine to norcodeine using material from Run 11 (p.176).

The norcodeine fractions were combined and evaporated to dryness, and the norcodeine taken up in 95% ethanol. The ultraviolet spectra indicated the presence of 0.124 mg of norcodeine. The norcodeine had a specific activity of 38,200 disintegrations per minute per mg of norcodeine, or 10,900 disintegrations per minute per micromole. These values multiplied by the dilution factor, 23.0, give the following values: 880,000 disintegrations per minute per mg of norcodeine and 251,000 disintegrations per minute per micromole of norcodeine.

Run 12 -- Conversion of Thebaine to Dihydrocodeine

Part of the diluted thebaine (0.541 mg) described in Run 12 was diluted with 5.000 mg of pure thebaine and divided into two equal fractions. One fraction was used in this series of reactions. It was taken up in chloroform and evaporated to dryness in the flask used for the bromination. The residue did not entirely dissolve when 0.250 ml of carbon tetrachloride was added. To the mixture 1.71 mg of

N-bromosuccinimide was added and the mixture stirred under nitrogen for 17 hours. After the 17-hour period, a small fraction was chromatographed on buffered paper. 14-Bromocodeinone was the only alkaloidal material found.

The mixture was evaporated to dryness and to it was added 1 ml of dry ether. Under a nitrogen atmospher, a 0.81 M ether solution of lithium aluminum hydride (0.100 ml) was added and the mixture stirred for 2 hours. Ethyl acetate was next added to the mixture to neutralize the excess hydride. This was followed by 2 ml of 1 N hydrochloric acid. The phases were separated, and the aqueous phase washed with ether (3 x 2 ml). The washed acid solution was made basic by the addition of a solution containing Rochelle salt and potassium hydroxide. The resulting basic solution was extracted with chloroform (4 x 3 ml). The chloroform extract was dried over sodium sulfate and evaporated to dryness. Paper chromatography of a small fraction of the residue indicated some other alkaloidal material in addition to the neopine.

The residue from the hydride extract was dissolved in 1.0 ml of absolute ethanol. Platinum oxide catalyst (1 mg) was added to the mixture and hydrogenated for 2hours. A small sample was taken and the solution chromatographed on buffered paper. Some unknown alkaloidal material was found in addition to the dihydrocodeine.

The remainder of the hydrogenation solution was evaporated to dryness. Only a fraction of the dihydrocodeine went into solution when benzene was added. The benzene solution was, however, added to the chromatography column containing alumina. A benzene:chloroform: isopropanol (88.5:10:1.5 by volume) solution was used as the developing solvent. After the first 8.5 ml, 3.2 ml fractions were collected. The unknown alkaloidal material was found in Fractions 5 and 6. Dihydrocodeine was found in Fractions 11 through 17. These fractions were combined and evaporated to dryness. The residue was washed with ethanol and its ultraviolet spectrum indicated the presence of 0.432 mg of dihydrocodeine. The dihydrocodeine had a specific activity of 101,000 disintegrations per minute per mg of dihydrocodeine, or 30,400 disintegrations per minute per micromole of dihydrocodeine. These values multiplied by the dilution factor, 104, give the following values: 10,500,000 disintegrations per minute per mg of dihydromorphine; and 3,160,000 disintegrations per minute per micromole of dihydrocodeine.

The remaining fraction of diluted thebaine was treated in the same manner as the method described above. This dihydrocodeine had a specific activity of 119,000 disintegrations per minute per mg of dihydrocodeine.

Run 12 -- Conversion of Dihydrocodeine to Dihydromorphine

Half of the dihydrocodeine (0.216 mg) remaining from the conversion of thebaine from Run 12 to dihydrocodeine was dissolved in methanol and evaporated to dryness in a 5mm Pyrex tube. Approximately 10 mg of pyridine hydrochloride was added to the tube which then was sealed under nitrogen. The work-up was the same as that of the small scale conversion of dihydrocodeine to dihydromorphine (p.186).

From the chromatography after the first 8.0 ml, fractions of 0.70 ml were collected. Dihydromorphine was found in Fractions 7 and 8. Each fraction was evaporated to dryness and taken up in 95% ethanol. The ultraviolet spectra of Fraction 8 indicated the presence of 0.028 mg of dihydromorphine. A small amount of potassium hydroxide was added to Fraction 7 and its ultraviolet spectrum underwent the characteristic base shift of phenols. The dihydromorphine had a specific activity of 117,000 disintegrations per minute per mg of dihydromorphine or 32,400 disintegrations per minute per micromole of dihydromorphine. These values multiplied by the dilution factor, 104, give the following values: 12,200,000 disintegrations per minute per mg of dihydromorphine and 3,370,000 disintegrations per minute per micromole of dihydromorphine.

Run 12 -- Conversion of Dihydrocodeine to Dihydronorcodeine

Half of the dihydrocodeine (0.216 mg) remaining from the conversion of thebaine from Run 12 to dihydrocodeine was dissolved in methanol and evaporated to dryness in the flask used for the acetylation. The same conditions as those employed for the conversion of codeine to norcodeine from Run 11 (p.176) and of the small scale conversion of dihydrocodeine to dihydronorcodeine (p.188) were employed.

In the chromatography of the crude dihydronorcodeine, an initial fraction of 2.5 ml was taken and then fractions of 0.55 ml. Dihydrocodeine was indicated in Fraction 14 which was evaporated to dryness and the dihydrocodeine taken up in 95% ethanol. The ultraviolet spectra indicated only 0.0072 mg of dihydronorcodeine being present. The dihydronorcodeine had a specific activity of 65,000 disintegrations per minute per mg of dihydronorcodeine or 18,600 disintegrations per minute per micromole of dihydronorcodeine. These values multiplied by the dilution factor of thebaine, 104, are as follows: 6,750,000 disintegrations per minute per mg of dihydronorcodeine and 1,930,000 disintegrations per minute per micromole of dihydronorcodeine. Because of the small amount of material isolated, the above figures are only accurate to within about ±20%.

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