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BRANCHED-CHAIN FATTY ACIDS. XXIII. THE PRINCIPAL STRUCTURAL FEATURES OF C_{27}-PHTHIENOIC ACID[1]

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In the preceding paper (1) of this series it was shown that C_{27}-phthienoic acid is an α,β-unsaturated acid with three branching groups along the chain. The evidence was good that one of these branches is in the 2-position and another in the 4- or 5-position. In the present paper are reported deductions based on infrared spectra and chemical evidence which corroborate the structural features already proposed and extend the knowledge of the structure of this acid to include all aspects except the location of one methyl group and the geometrical isomerism at the double bond.

Infrared Spectra

In Fig. 1 are shown the infrared spectral transmission curves from 5 to 16 microns of stearic acid, C_{27}-phthienoic acid, C_{27}-phthianoic acid, and methyl C_{27}-phthienoate. The spectral region from 2 to 5 microns contained no features of interest to the present discussion. Stearic acid, included for comparison, has the characteristic absorption bands of long-chain normal fatty acids. Since our conclusions will be reached largely from empirical considerations we will not review the description of these characteristic bands and their interpretation as presented by Shreve and co-workers (2). More general background for structural interpretation of infrared spectra may be found in recent publications (3,4).

The most striking feature of the spectrum of phthienoic acid, in comparison with that of stearic acid or numerous other branched-chain acids[2] is its

relative complexity. Absorption bands of appreciable intensity occur at 6.08 μ, 8.56 μ, 10.06 μ, 12.50 μ, 13.26 μ, and 14.92 μ, and none of these are exhibited by saturated acids. The first of these bands, at 6.08 μ, corresponds to the well-known carbon-carbon double bond stretching frequency, and its presence gave the initial indication of unsaturation in this molecule. This has been confirmed (1) by hydrogenation, and ultra-violet spectra have shown the double bond to be in the α, β-position. The bands in the region beyond 10 μ are also consistent with the presence of a double bond, and all the bands listed above are absent from the hydrogenated acid, C_{27}-phthianoic acid. Although the curve for methyl phthienoate exhibits the C = C band at 6.05 μ, the only strong band in the long wavelength range that is coincident with any of the "unsaturation" bands of the acid is that at 13.28 μ. This difference cannot be attributed to any structural change of the skeleton in going from ester to acid, for a sample of acid was re-esterified with diazomethane and the spectrum of this sample of ester was the same as obtained with the original ester directly from distillation.

Although substitution of alkyl groups about a double bond has been correlated (3) with frequencies of the long wavelength bands in alkenes, this correlation is not applicable to an α, β-unsaturated acid. Rasmussen (3) has pointed out that substitution of a non-hydrocarbon group on an unsaturated carbon has a pronounced (and unpredictable) effect on the vibration frequencies responsible for the long wavelength bands. However, these bands are believed to result from deformation vibrations involving hydrogen, and a double bond about which all the hydrogen atoms have been substituted contributes none of the long wavelength bands. Thus, the presence of several such bands in phthienoic acid and of one in the ester strongly suggests the presence of at least one hydrogen on the unsaturated carbons. Although this can hardly be regarded as conclusive

it is consistent with chemical evidence to be presented below.

The infrared evidence appears entirely consistent with the view (1) that the double bond in phthienoic acid is in the α, β-position. Various workers (5,6) have reported that the carbonyl absorption found at 5.75 μ in saturated esters is shifted to longer wavelengths by 0.05 μ - 0.07 μ in conjugated esters. In order to determine whether such a shift is observable in phthienoic acid or its ester, their spectra in the region of 5.5 - 6.5 μ were carefully compared with the spectra of 3-methyl-3-nonenoic acid[3], 3-methyl-2-nonenoic[3], and the esters[3] of these acids. These compounds (0.2 M solutions in chloroform in cells of 0.1 mm. thickness) were run as consecutive series, in order to avoid slight calibration shifts which may result from ambient temperature changes. The bands were sharp and the peaks can be located to the nearest 0.01μ. From the data in Table I, it may be seen that the carbonyl absorption maxima for both phthienoic acid and its ester are shifted about 0.06 μ from the maxima recorded for the synthetic acid and ester known to be β, γ-unsaturated. The shift in absorption maxima between the 2- and 3-alkenoic acids is less than the shift between the corresponding esters, as expected from the known lack of homogeneity[3] of the acids. Although it has been reported (3) that the C = C stretching frequency is altered by conjugation with the carbonyl this is not observed in the present instances. The intensity of the C = C absorption, relative to the carbonyl band, is considerably enhanced in the synthetic pairs by conjugation, but less so in phthienoic acid and its ester. A possible explanation of this difference is the presence of a β-methyl in the former case and an α-methyl in the latter case. If this can be confirmed by study of additional examples it should be of considerable use in structural studies.

Some deductions may be made regarding the nature of the branching by correlating the spectrum of phthianoic acid with those of various types of branched-

chain acids[2] and with the known spectra of branched-chain aliphatic hydrocarbons (8). Firstly, there are indications that the molecule has nether the iso nor the neo configuration. The spectra of 2-methylalkanes, isobutyric, isocaproic, and 17-methloctadecanoic acids all exhibit two features which are absent from the spectra of phthianoic acid and most other acids. One is a band at 8.55 μ, prominent in hydrocarbons but relatively weak in acids. The other is the splitting of the band at 7.3 μ into a doublet. Although this splitting is obscured in the curve obtained in carbon disulfide solution by the proximity of solvent absorption it becomes clearly apparent in carbon tetrachloride solution. Other workers (9) have reported this splitting for isopalmitic and isostearic acids, as well as a similar but differentiable splitting for the corresponding neo acids. Hence the absence of any splitting in the case of phthianoic acid is evidence against both structures.

Of the various types of fatty acids examined[2], those containing a quaternary carbon atom more remote from carboxyl than the α-position have shown an absorption band at 8.8 μ. In phthianoic acid there are weak bands near this position, but their dissimilarity to those in the reference compounds indicates that a quaternary carbon is absent. This is consistent with the conclusion reached (1) from consideration of the index of refraction of phthianoic acid and the boiling point of methyl phthienoate.

From the spectra of 3- and 4-methylalkanes, the presence of ethyl and propyl groups has been associated with bands at 12.95 μ and 13.5 μ respectively. These bands are observed in 15- and 16-methyloctadecanoic acids and in 15-ethylheptadecanoic acid. In other instances where the ethyl is close to another branch or to carboxyl, a slight shift downward is observed. In a few instances, weaker bands in this region have resulted from other causes, but the absence of any absorption in the region at 12.5 - 13.8 μ seems valid evi-

dence that branching ethyl or propyl groups are not present in phthianoic acid. By analogy with other natural products, the presence of larger branching groups seems very unlikely.

The overlapping bands at 7.8 μ and 8.1 μ are characteristic of normal fatty acids (2), and study of numerous branched-chain acids[2] has shown that the positions and relative intensities of these bands remain unaffected unless there is a substituent no more remote from carboxyl than the δ-position. In phthianoic acid, the positions of these bands deviate from the normal in a manner most nearly resembling that of 2-methyloctadecanoic acid. Their intensities are about equal, whereas the shorter wavelength band is the stronger in nearly all acids except those branching at the α-position, in which instances this relationship is reversed. This suggests that in phthianoic acid there is a methyl in the α-position and is consistent with the hypothesis that there is another methyl no more remote than the δ-position. Again, this is in agreement with conclusions reached from independent evidence (1).

One further set of measurements was carried out in an attempt to assay the number of terminal methyls in the molecule. This is especially desirable since determination of this quantity by oxidation is not entirely reliable (1). Referring once again to hydrocarbons, it has been demonstrated (10) that their absorption at 7.3 μ is associated with methyl groups, and the intensity of this absorption is a function of the number of methyl groups. For example, Gore and Petersen (11) concluded, from this feature of the infrared spectrum, that the C_9 acid from polymyxin is a branched-chain acid, and this has been substantiated. Our correlations of the intensity of the 7.3 μ band with the number of methyl groups has proved definitive for saturated acids containing one, two, or three methyl groups, but extrapolation is hazardous. The two available samples (12) of acids with four methyl groups were 2, 3, 4-trimethylhexadecanoic acid and 4, 8, 12-trimethylocatadecanoic acid. When these

were run in the same cell at the same molar concentration the optical densities (against an arbitrary background) were 0.25 and 0.4 respectively. The value of 0.35 obtained for phthianoic acid is in good agreement for four methyl groups, with the branches not concentrated near carboxyl as in 2, 3, 4-trimethylhexadecanoic acid. Four is certainly a minimum figure, for the highest optical density obtained with any acid containing three methyl groups was 0.2. Without examples containing five methyl groups, there is no assurance that the range for this number does not overlap that for four methyls, but it is of interest that analysis by oxidation (1) has also indicated four methyl groups in phthianoic acid.

Chemical Studies

It has been shown in an earlier paper (13) that a methyl substituent is an α- or β-position in an acid may be conclusively located by study of the rate of alkaline hydrolysis of the amide. Application of this method to C_{27}-phthianamide has definitely established that there is a methyl group in the α-position and no substituent in the β-position. The apparent second order rate constant for hydrolysis of this amide was 0.063 liters moles^{-1} hours^{-1}. For 2-methyloctadecanamide, under the same conditions the constant was 0.058 for the average of four runs in which the spread was 0.054 to 0.061. The agreement between the values for these amides is considered within the limit of precision of the method; but, in any case, the rate for the phthianamide is not less than that of the 2-methyl amide, as would be the case for a 3-methyl amide. The possibility of an additional substituent in the 3-position is positively eliminated by the fact that the constant for 2, 3-dimethyloctadecanamide is only 0.0076. For 2, 3, 4-trimethyloctadecanamide, the constant is further reduced to 0.0040, and this is of considerable interest for the constant for a 4-methyl amide is the same as that for a normal amide (13).

This means that the presence of methyls in the 2- and 3-positions makes it possible for a 4-methyl substituent to hinder the carbonyl, whereas the 4-methyl substituent alone does not appreciably hinder the carbonyl. This is further support for the quasi ring structure of fatty acids, as hypothesized in an earlier paper (14).

The location of the second methyl at the 5-position, rather than the 4-position, has been accomplished by a study of the behavior of pthienoic acid on heating. It is known (7) that heating of an α, β-unsaturated acid to 200° causes relatively rapid equilibration with the β,γ-unsaturated acid, so if such an acid contains an asymmetric center in the γ-position heating must racemize this asymmetric center. It is further known (15) that heating of a γ-alkyl- β,γ-unsaturated acid above 200° causes equilibration with the γ-lactone, with the equilibrium shifted far towards the lactone. In absence of the γ-substituent, equilibrium is far towards the acid, with insignificant amounts of lactone being formed. When C_{27}-phthienoic acid was heated at 225-235° for forty hours the specific rotation was lowered from 17.7° to 13.7°, thus there was only partial racemization after adequate heating to effect complete racemization if the substituent were in the γ-position. Furthermore, there was very little lactone formation, for the material recovered after heating had an equivalent weight of 447, and the extinction coefficient in the ultra violet spectrum was 10,300. Thus, the recovered material was principally α, β-unsaturated acid. In addition to the other evidence cited above, which seems conclusive for the absence of a γ-substituent, it is also the case that a γ-substituent would cause the acid recovered after heat-equilibration to contain considerable β,γ-unsaturated acid.

Although it has been claimed (16) that there is no equilibration between β,γ-unsaturated acid and the γ,δ-unsaturated acid, the above evidence strongly

indicates that there was a very slow equilibration of this sort which caused the small observed lowering of the rotation. The properties of the recovered acid show that there was not enough conversion to lactone or general decomposition to explain the lowering of the rotation. This matter is being further investigated in this laboratory.

Since the ultraviolet spectra and optical rotations indicate that the mixture from which C_{27}-phthienoic acid was separated (1) contains other similar acids of different molecular weight, certain structural features of the levoratatory C_{29}-phthianoic acid (1) have been examined. The infrared spectrum of C_{29}-phthianoic acid is entirely similar to that of the dextrorotatory C_{27}-phthianoic acid, thus a similar skeletal structure is indicated. Furthermore, hydrolysis of C_{29}-phthianamide (1) gave the same rate constant as obtained for C_{27}-phthianamide, thus a α-methyl is also present in the higher molecular weight, levorotatory acid.

The geometrical isomerism around the double bond in the phthienoic acids has not been investigated, for the presence of the α-methyl places this study in an entirely unexplored field, and the synthesis of model compounds seems required.

Experimental

The infrared spectral transmission curves were recorded on a Baird infrared spectrophotometer. Except as otherwise indicated in the discussion, the acids were run in carbon disulfide solutions (about 50 g. per liter) in a cell of 0.9 mm. thickness. In these curves, the region from 6.2 to 7.2 μ is masked by solvent absorption. The methyl C_{27}-phthienoate was run as a liquid film of approximately 0.025 mm. thickness.

Heating of C_{27}-Phthienoic Acid - 202 mg. of C_{27}-phthienoic acid,

$[\alpha]_D^{25}$ = +17.72±0.2°, were heated in a sealed tube for 6 hours at 225°. On molecular distillation of the product at 180° (0.4 mm.), there were obtained 180.3 mg., $[\alpha]_D^{25}$ = +16.23±0.45° (chloroform). Since the air in the tube had apparently caused some decomposition of the unsaturated acid, heating of the same sample was continued in a nitrogen-filled sealed tube for an additional 34 hours at 235°. After this heating period, there was recovered 160.5 mg. of acid, $[\alpha]_D^{25}$ = +13.73±0.50°, $\lambda_{max.}$ 216 m μ, ε 10,300, equivalent wt., 447.

C_{27}-Phthianamide - 250 mg. of C_{27}-phthianoic acid[4] were allowed to stand overnight in a solution of 1 ml. of purified thionyl chloride in 5 ml. of dry benzene. After excess thionyl chloride and benzene had been removed at reduced pressure, the residual acid chloride was dissolved in 10 ml. of pure, dry dioxane and added dropwise with swirling to 25 ml. of ice-cold concentrated ammonium hydroxide. After the precipitated amide had been crystallized twice from acetone there was obtained 103 mg. of m.p. 40-46.5°.

$C_{27}H_{55}NO$ (409.7) Calculated, N 3.42; found, N 2.74, 3.07. The average value for nitrogen indicates a purity of 84.8 percent for the amide. After the rate determination on this sample of amide the saponification was taken to completion. The total ammonia titrated corresponded to 84 percent purity for the amide, in excellent agreement with the above values obtained by Kjeldahl analysis. Also, the acid obtained after this hydrolysis was purified by use of Amberlite IRA-400 ion exchange resin and molecular distillation, and a pure sample of C_{27}-phthianoic acid was obtained.

Rate Constants - The method used has been described in detail (13). The previous work was not directed towards establishing absolute rate constants but apparent constants under a given set of conditions for use in structure determination. The principle uncontrolled variable which would be expected

to cause minor variations was the exact amount of water in the 1-propanol used as solvent. In order to establish a point of reference for the present determinations, and to evaluate the precision for runs using only about 0.008 mole of amide, several runs were made on 2-methyloctadecanamide and one run was made on 2, 3-dimethyloctadecanamide. The agreement in these instances with the previously published data (13) was reasonably good, but sufficiently different to emphasize the necessity for establishing a point of reference on known compounds.

The data was handled as previously described, using the equation

$$t = \frac{2.303}{k(a - b)} \left(\log \frac{b}{a} + \log \frac{a - x}{b - x} \right)$$

where a is the initial molarity of alkali, b is the initial molarity of amide, and x is the moles reacted in time, t. By plotting $\log \frac{a - x}{b - x}$ against t in hours, the equation was reduced to the form

$$k = \frac{2.303}{a - b} \frac{1}{\text{slope}}$$

In Table II are assembled the rate constants determined, expressed in liters moles^{-1} hours^{-1}. For C_{27}-phthianamide, complete data are given in Table III, and the plot for slope is given in Fig. 2.

Summary

The structure of C_{27}-phthienoic acid has been further investigated by use of infrared spectra, study of the behavior of the acid on heating, and study of the rate of hydrolysis of C_{27}-phthianamide. The known structural features of this acid are shown in the following formula:

$$C_4H_9\text{-}(CH_2)_{15}\text{-}\underset{CH_3}{CH}\text{-}CH_2\text{-}\underset{H}{\underset{|}{C}}\text{=}\underset{CH_3}{\underset{|}{C}}\text{-}CO_2H$$

C_{29}-Phthianoic acid, which is levorotatory, also has an α-methyl substituent, and has the same infrared spectrum as C_{27}-phthianoic acid. Thus, the mixture of acids of the phthioic type appears to consist primarily of acids of structure similar to the formula shown above.

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Footnotes

[1] This investigation was supported in part by a research grant from the National Institute of Health, Public Health Service, and by the Atomic Energy Commission.

[2] A comprehensive investigation has been made of the infrared spectra of branched-chain saturated acids, and this will be reported by one of us (N.K.F.) in another journal.

[3] The preparation of these compounds has been reported earlier (7), and as described in that paper each of these unsaturated acids contains appreciable amounts of the other unsaturated acid, but the esters are regarded as entirely homogeneous.

[4] In order to conserve material for biological testing, this phthianoic acid was obtained in part by hydrogenation of the heated sample of C_{27}-phthienoic acid, which contained some neutral material. This is no disadvantage for making amide for rate studies, however, since the purity of amide can be assessed by nitrogen analysis more precisely than the rate constant is determined. Also, the m.p. of the amide is of limited use for characterization, anyway, because it is a mixture of diastereoisomers.

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Table I

Wave Lengths of Double Bond Absorption Maxima

Compound	$\lambda_{(C=O)}$, μ	$\lambda_{(C=C)}$, μ
3-Methyl-3-nonenoic Acid	5.85	6.09
3-Methyl-2-nonenoic Acid	5.88	6.09
C_{27}-Phthienoic Acid	5.91	6.09
Ethyl 3-Methyl-3-nonenoate	5.79	6.08
Ethyl 3-Methyl-2-nonenoate	5.87	6.08
Methyl C_{27}-Phthienoate	5.85	6.08

Table II

Apparent Second Order Rate Constants for Saponification of Amides

The initial molarity of the potassium hydroxide in 1-propanol was 0.498.

Amide	Initial Molarity of Amide	k
2-Methyloctadecanamide	0.0336	0.057
	0.0134	0.054, 0.061 0.059 0.059
2,3-Dimethyloctadecanamide	0.0137	0.0076
2,3,4-Trimethylhexadecanamide	0.0340	0.0040
C_{27}-Phthianamide	0.00847	0.063
C_{29}-Phthianamide	0.0259	0.0635

Table III

Calculations for Rate Constant for Hydrolysis of C_{27}-Phthianamide

t (Hrs.)	x	a - x	b - x	$\log \frac{a - x}{b - x}$
4.1	0.00133	0.4970	0.00714	1.843
7.9	0.00220	0.4961	0.00627	1.898
11.3	0.00282	0.4955	0.00565	1.943
22.45	0.00445	0.4938	0.00402	2.089

$a = 0.4983$

$b = 0.00847$

$$\text{slope} = \frac{22.45 - 6.00}{2.089 - 1.870} = 75.1$$

$$k = \frac{2.303}{(0.4885)\ (75.1)} = 0.063$$

Figures

Figure 1 Infrared spectral transmission curves. Dotted lines indicate regions masked by absorption of solvent (carbon disulfide).

Figure 2 Determination of slope for rate constant for hydrolysis of C_{27}-phthianamide.

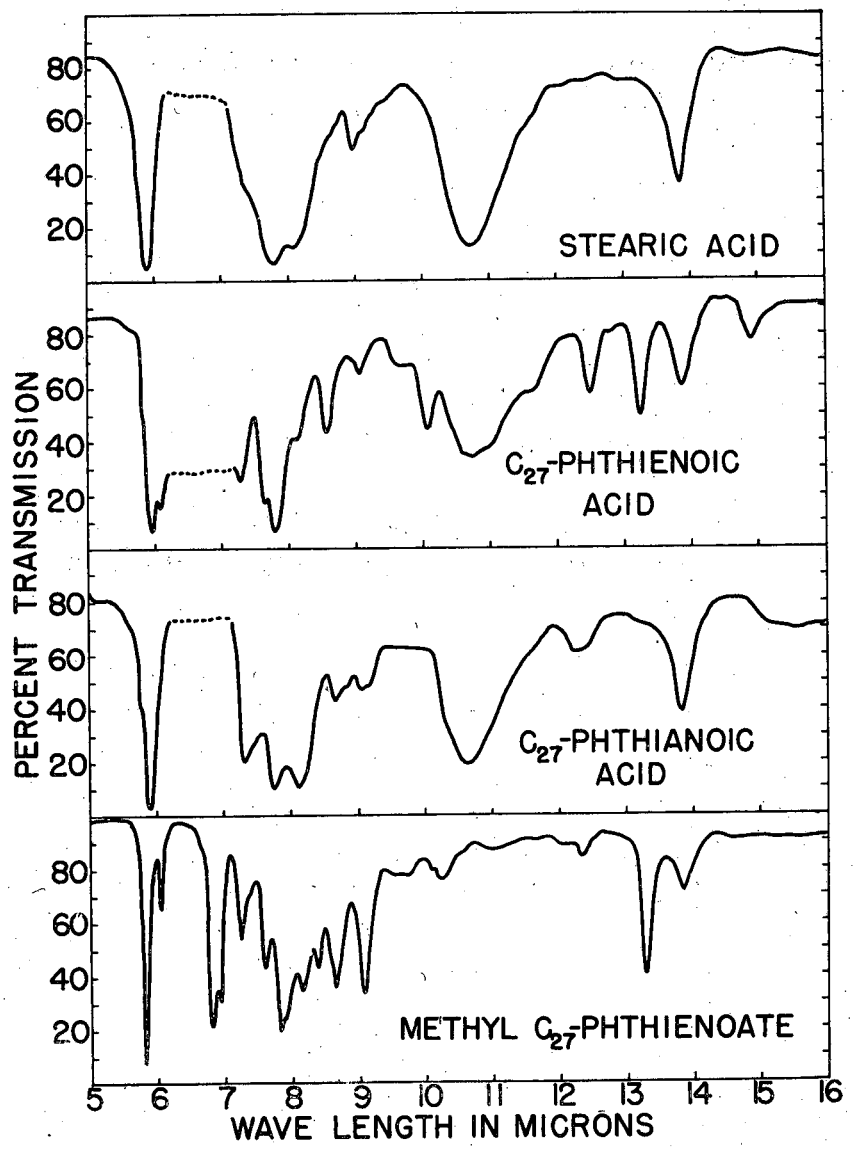
PERCENT TRANSMISSION
80
60
40
20
STEARIC ACID
C27-PHTHIENOIC ACID
C27-PHTHIANOIC ACID
METHYL C27-PHTHIENOATE
5 6 7 8 9 10 11 12 13 14 15 16
WAVE LENGTH IN MICRONS

FIGURE 1

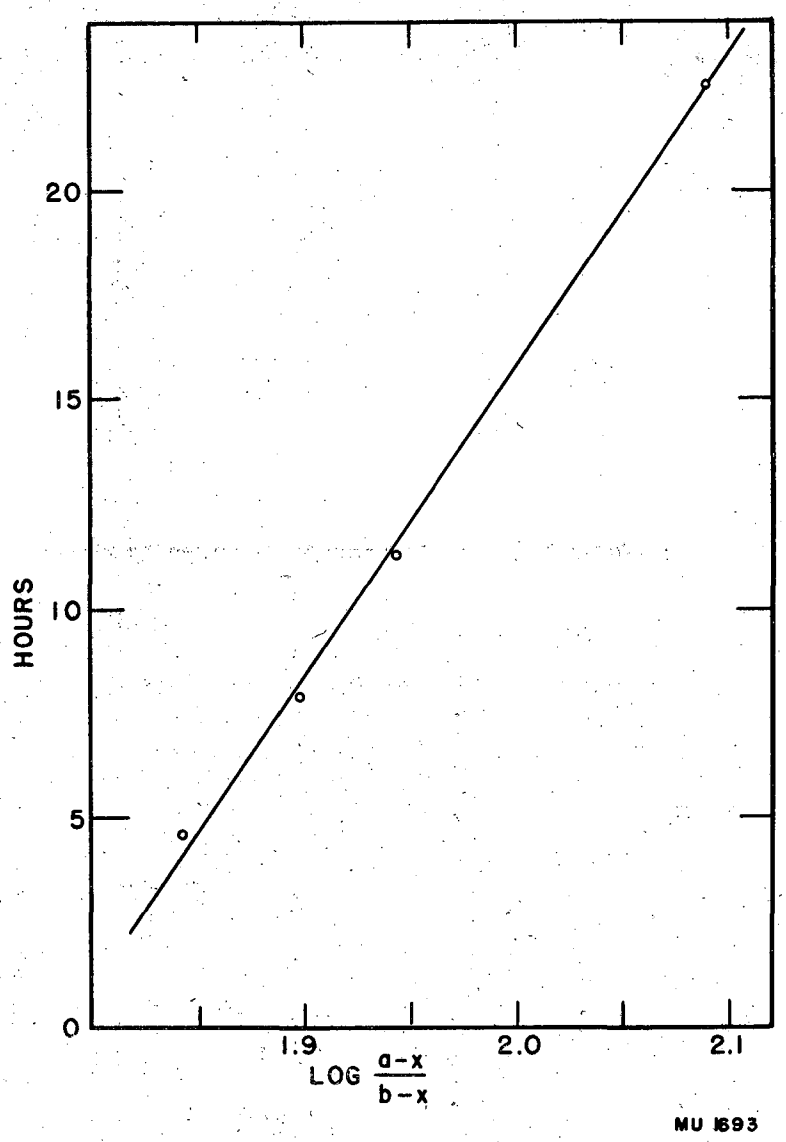

FIGURE 2