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KINETICS OF THE HYDROLYSIS AND ALCOHOLYSIS OF TETRAPOLYPHOSPHATE ESTERS

("ETHYL METAPHOSPHATE")

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"Ethyl metaphosphate" or tetrapolyphosphate esters are a potentially useful starting material for the preparation of polynucleotides. The kinetics of the reactions of the esters with excess water and ethanol have been measured by means of P^{31} n.m.r. and IR spectroscopy. Upon the addition of specific amounts of water or ethanol, substances could be prepared which consist mainly of linear tetrapoly-, tripoly- or pyrophosphate esters containing smaller amounts of other polyphosphates and orthophosphates in an equilibrium composition. Diethyl hydrogen orthophosphate reacts with cyclic polyphosphate esters to open the ring; with linear esters it reacts to form polyphosphates with a lesser degree of condensation. This latter reaction also proceeds to an equilibrium. No reactions between linear and cyclic polyphosphate esters were observed at room temperature, which implies that the rates of the disproportionation of the linear polyphosphate esters were low. Some organic solvents previously employed for the dehydrating polymerization of sugars, amino acids or nucleotides destroy the tetrapolyphosphate esters. The various substances now avail-

able from tetrapolyphosphate esters by the action of water or reactive solvents will differ in their capabilities of producing the dehydrating polymerization reaction. Thus, one may expect that very different products might result from very small differences in reaction conditions.

A considerable controversy exists about the preparation of polynucleotides by means of the so-called ethyl metaphosphate,[3-7] a compound which is obtained by refluxing phosphorus pentoxide with diethyl ether.[8-10] To clarify this problem investigations concerning the structure of "ethyl metaphosphate", which turned out to be a mixture of ring and chain tetrapolyphosphates, were obtained and the results published earlier.[10] For the preparation of polynucleotides from nucleotides or nucleosides and probably also for the preparation from a sugar and a purine or pyrimidine base by means of the mixture of tetrapolyphosphate esters, it may be expected that one of the initial steps of the reaction will be the attack of the tetrapolyphosphate esters on the hydroxyl groups of the compounds mentioned above, including the acid phosphate group of the nucleotide. For an understanding of these dehydrating polymerization reactions, some knowledge of the reaction of the tetrapolyphosphate esters with simple hydroxyl com-

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(3) G. Schramm, H. Grötsch and W. Pollmann, Angew. Chem., 74, 53 (1962).

(4) T. M. Jacob and H. G. Khorana, J. Am. Chem. Soc., 86, 1630 (1964).

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(8) K. Langheld, Ber., 43, 1857 (1910); 44, 2076 (1911).

(9) W. Steinkopf and I. Schubart, Ann., 424, 1 esp. 19 (1921).

(10) G. Burkhardt, M. P. Klein and M. Calvin, J. Am. Chem. Soc., 87, 591 (1965).

pounds such as water and ethanol is desirable but, unfortunately, is so far lacking.

It was known only that the final products of the hydrolysis and ethanolysis are all the three ethyl esters of orthophosphoric acid and orthophosphoric acid itself.[8,11,12] Sometimes esters of the pyrophosphoric acid were found.[8,11] Recently it was established by potentiometric titration of an aqueous solution of the mixture of tetrapolyphosphate esters that the esters easily add one mole of water.[13] In a paper chromatographic study,[14] esters of the tripolyphosphoric acid were discovered.

However, the course of the reaction and the nature of any other intermediates were quite unclear. The resolution of this problem should be possible by means of phosphorus n.m.r. measurements as first applied to the study of the hydrolysis and ethanolysis of the "ethyl metaphosphate" by Weill, Klein and Calvin.[15] In the experiments described in the present paper the kinetics of the reaction of the tetrapolyphosphate esters with an excess of water or ethanol and the reaction with specific amounts of water or ethanol as well as with diethyl hydrogen phosphate were investigated by means of ^{31}P and ^{1}H n.m.r., I.R. and molecular weight measurements.

In our samples of tetrapolyphosphate esters three different peaks are generally observed in the ^{31}P n.m.r. spectra in the presence of water or ethanol. They belong to the middle bonded (P_m), the terminal bonded (P_t) and the single or orthophosphate phosphorus (P_o),[16] (see Chart 1). Detectable amounts of branched bonded phosphorus (P_b) were found only in the absence of water or ethanol. When

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(11) K. Langheld, F. Oppmann and E. Meyer, Ber., 45, 3753 (1912).

(12) R. Rätz and E. Thilo, Ann., 572, 173 (1951).

(13) W. Pollmann and G. Schramm, Biochim. Biophys. Acta, 80, 1 (1964).

(14) W. Wieker, H.-P. Walter and E. Thilo, Ber., 97, 2385 (1964).

a sample of tetrapolyphosphate esters showing a ^{31}P n.m.r. spectrum of 20.5% P_t, 74.3% P_m, and 5.2% P_b (material D)[17] was allowed to react with an excess of water or ethanol at room temperature, the ratio of the ^{31}P n.m.r. peaks changed characteristically with the time of the reaction as can be seen from Fig. 2 and Table I. Fig. 1 shows some examples of the ^{31}P n.m.r. spectra of products of this reaction. There is no basic difference between the action of water and of ethanol, except that ethanol seems to react a little faster. A mixture of tetrapolyphosphate esters with another composition of cyclo (II), isocyclo(III) and linear tetrapolyphosphate esters (IV), which is indicated by its different ^{31}P n.m.r. spectrum (material B)[17], and also a mixture of bicyclo (I), cyclo and isocyclo tetrapolyphosphate esters (material A)[17] reacts with both water and ethanol in the same manner as material D (Table II), but differences in the rate can be observed. Only orthophosphate could be found in the phosphorus n.m.r. spectrum when the tetrapolyphosphate esters were refluxed with excess water for a period as short as 0.5 hr.

The composition of mixtures of tetrapolyphosphate esters was calculated from their ^{31}P n.m.r. spectra as described previously.[10] From that work, material D consists of 20.8% isocyclo, 48.6% cyclo (together, 69.4% cyclic esters) and 30.6% linear tetrapolyphosphate esters. For the destruction of the cyclic structures, one mole water for each mole cyclic ester should be necessary and the resulting linear tetrapolyphosphate ester should show a ^{31}P n.m.r. spectrum of 50% P_t and 50% P_m. Based on the composition given above, if 69.4% of one equiva-

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(15) G. Weill, M. P. Klein and M. Calvin, Nature, 200, 1005 (1963).

(16) J. R. Van Wazer, C. F. Callis, J. N. Shoolery and R. C. Jones, J. Am. Chem. Soc., 78, 5715 (1956).

(17) Various initial mixtures of tetrapolyphosphate esters having specific ratios of the ^{31}P n.m.r. peaks are designated in Table II as A, B, or D. If the ratio is 26.2% P_t, 69.8% P_m, and 4.0% P_b the ester is called material E according to reference (10).

TABLE I

Ratio of ^{31}P n.m.r. peaks of samples obtained by hydrolysis or alcoholysis of a mixture of tetraphosphate esters (material D)[17] at room temperature after a long reaction time.

Time, hours	Hydrolysis (10 g ester + 20 g water; 1 mole ester : 48 moles water)			Alcoholysis (10 g ester + 51.1 g ethanol; 1 mole ester : 48 moles ethanol)		
	P_o	P_t	P_m	P_o	P_t	P_m
10	30.6%	56.5%	12.9%	32.4%	64.3%	3.3%
15	32.0%	57.0%	11.0%	38.2%	61.8%	-
20	36.7%	55.3%	8.0%	38.8%	61.2%	-
24	42.4%	50.6%	7.0%	40.7%	59.3%	-
36	47.0%	49.8%	3.2%	44.3%	55.7%	-
48	54.8%	45.2%	-	45.1%	54.9%	-
60	59.5%	40.5%	-	46.6%	53.4%	-
72	63.2%	36.8%	-	47.8%	52.2%	-

Ratio of ^{31}P n.m.r. peaks of samples obtained from the reaction of different materials of tetraphosphate esters with an excess of water (a) or ethanol (b) at room temperature (3 g ester + 6 ml solvent).

a)

Time	Material A[17]				Material B[17]				Material D[17]			
	P_o	P_t	P_m	P_b	P_o	P_t	P_m	P_b	P_o	P_t	P_m	P_b
0# hr.	–	5.5%	77.6%	16.9%	–	12.5%	77.5%	10.0%	–	20.5%	74.3%	5.2%
0.25 hr.	4.6%	43.9%	51.5%	–	6.7%	50.1%	43.8%	–	7.2%	56.9%	35.9%	–
1 hr.	11.9%	48.7%	39.4%	–	13.3%	51.0%	35.7%	–	16.1%	54.8%	29.1%	–
5 hr.	22.0%	53.3%	24.7%	–	23.5%	53.2%	23.3%	–	27.3%	56.5%	16.2%	–
10 hr.	27.6%	52.8%	19.6%	–	28.2%	54.1%	17.7%	–	32.6%	55.2%	12.2%	–
24 hr.	37.2%	50.7%	12.1%	–	37.5%	51.3%	11.2%	–	39.4%	53.3%	7.3%	–

b)

Time	Material A				Material B				Material D			
	P_o	P_t	P_m	P_b	P_o	P_t	P_m	P_b	P_o	P_t	P_m	P_b
0# hr.	–	5.5%	77.6%	16.9%	–	12.5%	77.5%	10.0%	–	20.5%	74.3%	5.2%
0.25 hr.	8.9%	54.4%	36.7%	–	8.3%	55.9%	35.8%	–	6.0%	56.5%	37.5%	–
1 hr.	18.9%	61.3%	19.8%	–	20.6%	57.5%	21.9%	–	15.1%	61.6%	23.3%	–
5 hr.	31.9%	60.8%	7.3%	–	34.1%	58.6%	7.3%	–	29.0%	62.8%	8.2%	–
10 hr.	36.5%	58.6%	4.9%	–	36.4%	58.0%	5.6%	–	32.9%	62.4%	4.7%	–
24 hr.	49.1%	50.9%	–	–	53.2%	46.8%	–	–	46.2%	53.8%	–	–

\# The values at the time zero were obtained from the tetraphosphate esters dissolved in chloroform without water (a) or ethanol (b).

lent of water was added to material D, the resulting substance indeed showed a phosphorus n.m.r. spectrum with 49.9% P_t, 50.1% P_m, and a trace of P_o (substance V). When the equivalent amount of ethanol was used, 47.7% P_t, 51.3% P_m, and 1.0% P_o (substance VI) was found.

A calculation shows that for the preparation of a tripolyphosphate ester (66.7% P_t and 33.3% P_m) from the mixture of cyclic and linear tetrapolyphosphate esters, 4/3 mole water per mole cyclic esters and 1/3 mole water per mole linear ester is necessary. If, also based on the composition given above, the sum of 69.4% of 4/3 equivalents and 30.6% of 1/3 equivalents of water were added to material D, a substance showing a n.m.r. spectrum of 61.9% P_t, 34.4% P_m, and 3.7 P_o (substance VII) was obtained; if ethanol instead of water was used, the spectrum showed 62.5 P_t, 34.8% P_m, and 2.7% P_o (substance VIII).

For the preparation of the pyrophosphate ester (100% P_t) from material D, two moles water per mole of the cyclic compounds and one mole water per mole of the linear compound is necessary. If, again based on the composition given above, the sum of 69.4% of 2 equivalents and 30.6% of 1 equivalent of water were added to material D, the substance obtained showed a spectrum of 74.7 P_t, 11.3% P_m, and 14.0% P_o (substance IX); if ethanol was used instead of water, the spectrum showed 73.5% P_t, 13.4% P_m, and 13.1% P_o (substance X).

If, finally, the sum of 69.4% of 4 equivalents and 30.6% of 3 equivalents of water or ethanol was added, which should cause a complete conversion of the polyphosphate esters to orthophosphate, the values given in Table III were obtained.

It is expected that linear and cyclic polyphosphate esters will differ in their spectral properties, especially in the infrared region. According to Cramer and Hettler,[18] cyclic polyphosphate esters possess an IR absorption band at 1330 cm^{-1} not observed in linear esters. Pollmann and Schramm[13] published

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(18) F. Cramer and H. Hettler, Ber., 91, 1181 (1958).

IR spectra of tetrapolyphosphate esters claiming that a band at 1310 cm^{-1} disappeared upon the addition of small amounts of water. The spectra we recorded were very similar to those published by Pollmann and Schramm, but the band at 1330 (1310) cm^{-1} was not sufficiently characteristic to distinguish clearly between cyclic and linear polyphosphate esters.

TABLE III

Reaction of a mixture of tetraphosphate esters (material D)[17] with water or ethanol. 4 mole water (ethanol) per 1 mole cyclic, and 3 mole water (ethanol) per 1 mole linear esters were used.

Time	Water		Ethanol	
	P_o	P_t	P_o	P_t
24 hr. at 20°	59.3%	40.7%	43.8%	56.2%
0.5 hr. at 75°	61.9%	38.1%	53.8%	46.2%
1.5 hr. at 75°	62.4%	37.6%	65.5%	34.5%
5 hr. at 75°	66.3%	33.7%	78.2%	21.8%

In very dilute solutions the broad infrared absorption between 900 and 1100 cm^{-1}, caused by P-O-P and P-O-C vibrations, could be resolved into three strong bands which occur at 940, 1005, and 1040 cm^{-1} (Fig. 3). The band at 1005 cm^{-1} is found in the spectra of all polyphosphate esters prepared by heating P_4O_{10} with diethyl ether (materials A to E).[17] The bands at 940 and 1040 cm^{-1}, however, do not appear in the spectra of materials A and B, but appear in those of materials D and E and increase from D to E. If a small amount of water or ethanol (0.75 equivalent) is added to material B, the band at 1005 cm^{-1} decreases; it disappears, except for a small shoulder, if 1 equivalent or more of water (ethanol) is added (substance V to X). Simultaneously, the band at 1040 cm^{-1}, and to a small extent that at 940 cm^{-1}, increases. The addition of 0.75 equivalent of water or ethanol to material B should destroy three quarters of the cyclic structures; the addition of 1 equivalent or more should completely destroy the cyclic structures, since they are expected to be the most sensitive ones against an attack of a nucleophilic substance (see P n.m.r. results and reference [10]).

In the spectrum of triethyl orthophosphate two strong bands are observed, one at 1040 and another at 980 cm^{-1}. The band at 940 cm^{-1} has completely disappeared. The very small absorption at 980 cm^{-1} in spectrum 8, Fig. 3, shows that already some orthophosphate has formed in this substance (see also P n.m.r. results). These results show that cyclic and linear polyphosphate esters can be distinguished by their IR spectra in the 1100 to 900 cm^{-1} region if the samples are very dilute. Cyclic esters show a characteristic absorption at 1005 cm^{-1}, linear esters at 1040 cm^{-1}, and a less characteristic feature at 940 cm^{-1}.

The U.V. spectra showed no special features down to 2400 Å.

The preparation of natural polynucleotides from nucleotides has been claimed[3] but could not be reproduced.[4-7] Two reaction centers at the nucleotide molecule are potentially available for the attack of the polyphosphate esters: the phosphate group and the hydroxyl groups of the sugar. To find out which is favored, it should be known, if the mixtures of tetrapolyphosphate esters or any of their reaction products with water or ethanol do react easily with simple orthophosphate esters. Therefore, diethyl hydrogen orthophosphate was mixed with cyclic and also with linear polyphosphate esters. In separate experiments the cyclic and linear esters were also mixed together. The ratio of the phosphorus n.m.r. peaks of the initial materials was determined. The ratio of the peaks of the mixtures obtained from equimolar amounts of them can be easily calculated as the arithmetic mean, if it is supposed that no reaction takes place. However, in those cases where strong deviations from the calculated ratios are found in the spectra of the mixtures, it can be concluded that a reaction occurred. As a cyclic ester, material B was used; material B consists of 95.0% cyclic tetrapolyphosphate esters (40.0% isocyclic and 55.0% cyclic esters) and 5.0% linear tetrapolyphosphate esters (according to reference [10]). As linear esters (see later) the reaction products of material D with water (substance V) or ethanol (VIII and X) were used. Table IV lists the n.m.r. results for the starting materials and also the calculated and measured values for the mixtures. The IR spectra of material B and of mixtures (1) to (3) show a strong absorption at 1005 cm^{-1}, typical of cyclic compounds (see above); it has disappeared in mixture (4).

To find out which solvents are useful for reactions with polyphosphate esters, solutions of material D in different carefully dried solvents were also examined by means of phosphorus n.m.r. measurements. Changes in the spectra must indicate a reaction between the polyphosphate esters and the solvent. No changes were found, even upon prolonged heating (80° or reflux, 5 hr), if the ester was dissolved in chloroform, dichloro methane and polychlorinated ethanes or propanes. Heating for a shorter time (10 min) is possible in nitrobenzene, acetonitrile, propionitrile, dimethyl formamide, dioxane, methyl acetate and ethyl acetate. Prolonged heating (5 hr) causes slight changes of the ratios of the peaks; the changes were very small if the solutions remained at room temperature (72 hr).

The simultaneous presence of P_o and P_b peaks was never observed in samples of "ethyl metaphosphate" dissolved in polychlorinated hydrocarbons. However, when material D[17] was heated for 5 hr at 157° in dimethyl formamide, for example, a spectrum with 6.1% P_b, 68.8% P_m, 22.2% P_t, and 2.9% P_o was observed. This may indicate that reorganization processes occur in highly polar solvents at an appreciable rate, especially at higher temperatures.

TABLE IV

Ratio of ^{31}P n.m.r. peaks of mixtures of cyclic and linear polyphosphate and orthophosphate esters

Initial Materials (in chloroform):

	P_o	P_t	P_m	P_b
Material B (cyclic tetrapolyphosphates)	-	12.5%	77.5%	10.0%
substance V (linear tetrapolyphosphates)	trace	49.9%	50.1%	-
substance VIII (linear tripolyphosphates)	2.7%	62.5%	34.8%	-
substance X (pyrophosphates)	13.1%	73.5%	13.4%	-

Equimolar Mixtures (in chloroform):

Components	n.m.r. calculated (%)				n.m.r. found (%)			
	P_o	P_t	P_m	P_b	P_o	P_t	P_m	P_b
(1) B + V	-	31.2	63.8	5.0	trace	33.4	62.6	4.0
(2) B + VIII	1.3	37.5	56.2	5.0	trace	38.2	59.2	2.6
(3) B + X	6.5	43.0	45.5	5.0	trace	40.8	56.8	2.4
(4) B + $(C_2H_5)_2HPO_4$	50.0	6.3	38.7	5.0	trace	37.9	62.1	trace
(5) V + $(C_2H_5)_2HPO_4$	50.0	25.0	25.0	-	8.8	64.5	26.7	-
(6) VIII + $(C_2H_5)_2HPO_4$	51.3	31.3	17.4	-	14.6	69.2	16.2	-

If solutions in formamide, dimethyl sulfoxide, acetone, ethyl formate, ethyl orthoformate, or tetrahydrofuran are heated for 5 hr (the first two solvents at 90°, the others at reflux temperature), the phosphorus spectra show significant changes: the P_b disappears, the P_m peak decreases, the P_t peak increases, and sometimes P_o is found. With the exception of dimethyl sulfoxide, similar changes were obtained if the solutions stood at room temperature for 72 hr. However, the changes in the phosphorus n.m.r. spectrum in dimethyl sulfoxide solution at room temperature were negligible. A reaction also takes place when the ester is treated at 20° or 80° with pyridine, acetic acid, or acetic anhydride. Spectra showing entirely different absorptions than those of pure material D arise. Absorptions occur at +12, +25, +28, and +36 p.p.m. in pyridine and at +0.5 (only in acetic acid), +10, +16, +19, +26, +33, +39 p.p.m. in acetic acid and acetic anhydride.

From the results described above, the following conclusions may be drawn:

(1) Mixtures of tetrapolyphosphate esters (ethyl metaphosphate) are highly sensitive toward a nucleophilic attack by water or ethanol. Both solvents react with the esters in the same manner. When an excess of water or ethanol is used, the P_b structural unit disappears suddenly, the amount of the P_m unit drops sharply, and the amount of the P_t unit increases (in the first 0.25 hr of the reaction; see Figs. 1 and 2, and Tables I and II). This means the ring structures I, II and III are very sensitive and were opened first by the attack of water or ethanol to form linear tetrapolyphosphate ester, which is in accordance with results of Weill, Klein and Calvin.[15] The further reaction of the linear ester with water and ethanol occurs at a lower rate, giving an increasing amount of orthophosphate, whereas the amount of the P_m structural unit decreases and the P_t peak stays nearly constant (in the first 10 hr under the conditions given in Fig. 2 and Table II). This means that orthophosphate esters (or ortho-

phosphoric acid) is split off from the polyphosphate esters; tripoly- and pyrophosphate esters are formed successively. Formerly it was supposed that no tripolyphosphate esters were formed—i.e., the tetrapolyphosphate esters were split into two pyrophosphate esters.[15] The destruction of the pyrophosphate molecule occurs at a much lower rate, and is far from being complete even after 72 hr at 20°; at 100° after 0.5 hr, only orthophosphates are present.

(2) If, based on the composition of the mixture of tetrapolyphosphate esters as was given earlier,[10] those specific amounts of water or ethanol were added which were calculated to be necessary for the formation of linear tetrapoly-, tripoly- or pyrophosphate esters, substances were obtained whose ^{31}P n.m.r. spectra correspond to the calculated ones of the tetrapoly-, tripoly- and, to some extent, also with that of the pyrophosphate ester (Table V).

TABLE V

Ratio of ^{31}P n.m.r. peaks of the substances prepared from material D and water or ethanol. These products are assumed to be mainly tetrapoly-, tripoly- and pyrophosphate esters.

	P n.m.r. calculated (%)				P n.m.r. found (%)						
					with water				with ethanol		
	P_o	P_t	P_m		P_o	P_t	P_m		P_o	P_t	P_m
tetrapolyphosphate	-	50.0	50.0	V	trace	49.9	50.1	VI	1.0	47.7	51.3
tripolyphosphate	-	66.7	33.3	VII	3.7	61.9	34.4	VIII	2.7	62.5	34.8
pyrophosphate	-	100.0	-	IX	14.0	74.7	11.3	X	13.1	73.5	13.4

Molecular weight determinations showed in particular that substances V and VI have an average degree of condensation of that of the tetrapolyphosphate esters, VII and VIII of that of the tripolyphosphate esters, and X of that of the pyrophosphate esters. The IR spectra confirm that no significant amounts of cyclic compounds are present in the substances V to X.

There is a large increase of the viscosity in the series of the substances V, VII, and IX. The substances VI, VIII, and X have much lower viscosities which do not differ appreciably. As is expected, all the substances show a large peak of the OH protons in the ^{1}H n.m.r. spectra increasing from VI, VIII, to X and from V, VII to IX. This peak lies at 12.5 (VI), 12.7 (VIII), 12.8 (X), 12.9 (V), 13.2 (VII), and 13.3 p.p.m. (IX).

From all these measurements we conclude that the mixtures V and VI consist mainly of linear tetrapolyphosphate esters, VII and VIII of linear tripolyphosphate esters, and IX and X of pyrophosphate esters. However, the P n.m.r. spectra of the pyrophosphate esters clearly establish that these substances are actually equilibrium mixtures with only an average degree of condensation of that of the proposed structure. They contain a principal compound having the proposed structure together with other larger and shorter condensed chain phosphates.

Some conclusions about the amount of the principal compound in mixtures which contain mainly pyrophosphate esters can be drawn from their n.m.r. spectra. In mixture IX, for example, the amount of pyrophosphate will be lowest, 50.7%, if the middle bonded phosphorus exists only in the form of tripolyphosphate and will be highest, 61.7%, if P_m exists only in the form of tetrapolyphosphate. Higher polyphosphates should be expected to contribute insignificantly to the equilibrium composition of that mixture.

Similar equilibria of phosphate esters were also observed by Schwarzmann and Van Wazer[19] in mixtures of triethyl orthophosphate and P_4O_{10}.

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(19) E. Schwarzmann and J. R. Van Wazer, J. Am. Chem. Soc., 83, 365 (1961).

For the complete destruction of the pyrophosphate structure, an excess of water is necessary. Table III shows that the attack by a small amount of water proceeds only slowly. The reaction is far from being complete even at 75° after 5 hours.

(3) It was found that the attack by water (ethanol) on linear tetrapolyphosphate ester splits off orthophosphate successively. Thus, the mixtures of tripoly- and of pyrophosphate esters cannot be formed only by a direct attack by water (ethanol) on tetrapolyphosphate and tripolyphosphate, but must also originate from an attack of orthophosphate already formed on these substances. This reaction proceeds only to a certain equilibrium, as the spectra of substances IX and X show. If an excess of water (ethanol) is present, the attack by the orthophosphate is masked by the much faster attack by water (ethanol). The results obtained when orthophosphate itself is added to mixtures of polyphosphate esters, as summarized in Table IV, ascertain this assignment. In mixtures of diethyl hydrogen orthophosphate and tripoly- or tetrapolyphosphate as well, reactions occurred leading to the same equilibria as described above. Triethyl orthophosphate reacts in the same manner, but very much slower. If diethyl hydrogen orthophosphate was added to cyclic tetrapolyphosphates (material B),[10] a mixture with 37.9% P_t, and 62.1% P_m was obtained. The molecular weight and the IR spectrum suggest that it consists mainly of linear pentapolyphosphate ester. The P n.m.r. and IR spectra obtained from mixtures of cyclic tetrapolyphosphates (material B) and linear tetrapoly- (V), tripoly- (VIII), or pyrophosphate (X) do not indicate any reaction between the components. The most sensitive atom for an attack by the linear esters is doubtlessly the branched bonded phosphorus, but its amount does not markedly decrease in these mixtures. The small decrease observed must be caused

EXPERIMENTAL

When neither water nor ethanol was involved, all measurements were performed under conditions of extreme dryness.[10] The preparative and analytical procedures followed those of reference (10). Absolute ethanol was used.

No elemental analysis of substances V to X was carried out since the P, C, and H content is predetermined by the values of material D and the amount of water or ethanol added.

<u>Reaction with an Excess of Water and Ethanol.</u> <u>A. Data of Table I and Fig. 2, Spectra of Fig. 1.</u>— (1) 10.0 g (0.023 mole) material D[17] were quickly dissolved in 20.0 g (1.11 mole) water. An ice bath was used to compensate for the heat of the reaction during the first 5 min. The solution was then allowed to stand at room temperature (25°) and samples for the n.m.r. measurements were taken from the mixture after certain time intervals. The same concentrations were applied when the solution was refluxed. (2) 51.1 g (1.11 mole) ethanol were used instead of water. Procedure as described under (1).

<u>B. Data of Table II.</u>— 3.0 g (0.007 mole) of material A,[17] B or D were dissolved in 6 ml water or ethanol, respectively. Procedure as described under (1).

<u>Preparation of Linear Polyphosphate Esters.</u>— The composition of the initial mixture of tetrapolyphosphate esters (material D) was calculated from the P n.m.r. spectrum according to reference (10). Based on this composition, certain amounts of water or ethanol are necessary to produce linear tetrapoly-, tripoly- or pyrophosphates. Those amounts were calculated, as the sample calculation for substance V shows. All substances were weighed with an accuracy of 0.1 mg.

<u>A. Substance V:</u> (linear tetrapolyphosphate esters). Material D consists of 69.4 mole % or 65.9 weight % cyclic esters and 30.6 mole % or 34.1 weight % linear esters. 40.0590 g of material D contain, therefore, 26.3989 g (0.0611 mole) of cyclic esters and 13.6601 g (0.0270 mole) of linear esters. Necessary

amounts of water: for 0.0270 mole linear esters $\hat{=}$ 0 mole water; 0.0611 mole cyclic esters $\hat{=}$ 0.0611 mole water = 1.1006 g. 40.0590 g material D was dissolved in 20 ml $CHCl_3$. 1.006 g water was slowly added while cooling with ice. The final composition (49.9% P_t; 50.1% P_m) was reached after 2 hr as the P n.m.r. spectrum of a sample taken from the mixture showed. The solvent was evaporated under vacuum (at least at 0.5 mm Hg) and a viscous oil was obtained.

Molecular weight: $(C_2H_5)_6P_4O_{13}$ 506.3; $(C_2H_5)_4H_2P_4O_{13}$ 450.2; found 483,421.
$n_D 20°$ 1.436; density 20° 1.4139; viscosity 20° 35.11 poise.

B. Substance VI: (linear tetrapolyphosphate esters). 46.7978 g material D = 30.8398 g (0.0714) of cyclic esters and 15.9580 g (0.0315 mole) of linear esters were used. Necessary amount of ethanol: 0.0714 mole = 3.2894 g. Material D was dissolved in 20 ml $CHCl_3$ and the ethanol was slowly added under cooling. The final composition (1.0% P_o, 47.7% P_t, 51.3% P_m) was reached after 10 hr. After evaporation of the solvent (at least at 0.5 mm Hg) an oil was obtained.

Molecular weight: $(C_2H_5)_6P_4O_{13}$ 506.3; $(C_2H_5)_5HP_4O_{13}$ 478.2; found 467,449.
$n_D 20°$ 1.433; density 20° 1.3517; viscosity 20° 3.95 poise.

C. Substance VII: (linear tripolyphosphate esters). 44.4721 g material D = 29.3071 g (0.0678 mole) cyclic esters and 15.1650 g (0.030 mole) linear esters were used. Necessary amount of water: 1/3 of 0.030 mole and 4/3 of 0.0678 mole = 1.8090 g. Procedure as described under V. The final composition (3.7% P_o, 61.9% P_t, 34.4% P_m) was reached after 2 hr.

Molecular weight: $(C_2H_5)_4HP_3O_{10}$ 370.2; $(C_2H_5)_3H_2P_3O_{10}$ 342.1; found 394,405.
$n_D 20°$ 1.435; density 20° 1.4135; viscosity 20° 47.92 poise.

D. Substance VIII: (linear tripolyphosphate esters). 45.1864 g material D = 29.7778 g (0.0689 mole) cyclic esters and 15.4086 g (0.0304 mole) linear esters. Necessary amount of ethanol: 1/3 of 0.0304 mole and 4/3 of 0.0689 mole = 4.6991 g. Procedure as described under VI. Final composition (2.7% P_o, 62.5% P_t, 34.8% P_m)

reached after 24 hr.

Molecular weight: $(C_2H_5)_4HP_3O_{10}$ 370.2; $(C_2H_5)_5P_3O_{10}$ 398.2; found 362,407.

n_D20° 1.431; density 20° 1.3388; viscosity 20° 3.69 poise.

E. Substance IX: (pyrophosphate esters). 43.0554 g material D = 28.3735 g (0.0657 mole) cyclic esters and 14.6819 g (0.0290 mole) linear esters were used. Necessary amount of water: 0.0290 mole and twice 0.0657 mole = 2.8898 g. Procedure as described under V. Final composition (14.0% P_o, 74.7 P_t, 11.3% P_m) reached after 10 hr.

Molecular weight: $(C_2H_5)_3HP_2O_7$ 262.1; $(C_2H_5)_2H_2P_2O_7$ 234.1; no satisfactory molecular weights could be determined in aceto nitrile (found 377,425).

n_D20° 1.434; density 20° 1.3875; viscosity 131.12 poise.

F. Substance X: (pyrophosphate esters). 44.7761 g material D = 29.5074 g (0.0683 mole) cyclic esters and 15.2687 g (0.0302 mole) linear esters. Necessary amount of ethanol: 0.0320 mole and twice 0.0683 mole = 7.6845 g. Procedure as described under VI. Final composition (13.1% P_o, 73.5% P_t, 13.4% P_m) reached after 10 hr.

Molecular weight: $(C_2H_5)_3HP_2O_7$ 262.1; $(C_2H_5)_4P_2O_7$ 290.2; found 292,349 (in acetonitrile); 293,295 (in methyl acetate).

n_D20° 1.427; density 20° 1.3029; viscosity 20° 2.76 poise.

Data of Table III.— (attempt to produce orthophosphate).

(a) 5.3876 g material D = 3.5504 g (0.0082 mole) cyclic esters and 1.8372 g (0.0036 mole) linear esters. Necessary amount of water: 3 times 0.0036 mole and 4 times 0.0082 mole = 0.7855 g. To a solution of material D in 5.4 ml $CHCl_3$, the water was added under cooling with ice. The mixture stood at room temperature (25°) under vigorous stirring for 24 hr and was then refluxed.

(b) 4.4147 g material D = 2.9093 g (0.0067 mole) cyclic esters and 1.5054 g (0.0030 mole) linear esters. Necessary amount of ethanol: 3 times 0.003 mole and 4 times 0.0067 mole = 1.6493 g. Procedure as described under (a). 4.4 ml $CHCl_3$ used. No stirring necessary after the first 5 min.

Data of Table IV.--- (mixtures of cyclic and linear polyphosphate and orthophosphate esters).

(1) Material B[17] and tetrapolyphosphate ester (substance V): 1.3538 g material B with 95% cyclic compounds (1.2861 g, 2.98 mmole) dissolved in 3 ml $CHCl_3$. To this was added 1.4572 g substance V (2.98 mmole, average mol. wt. calc. from the initial composition of material B[10] and amount of water added: 489) dissolved in 1 ml $CHCl_3$.

(2) Material B and tripolyphosphate ester (substance VIII): 1.8713 g material B (95% = 1.7777 g, 4.11 mmole) dissolved in 3 ml $CHCl_3$. To this was added 1.5495 g substance VIII (4.11 mmole, average mol. wt. calc. 377), dissolved in 1 ml $CHCl_3$.

(3) Material B and pyrophosphate ester (substance X): 2.1112 g material B (95% = 2.0056 g, 4.64 mmole) dissolved in 3 ml $CHCl_3$. To this was added 1.2422 g substance X (4.67 mmole, average mol. wt. calc. 266), dissolved in 1 ml $CHCl_3$.

(4) Material B and diethyl hydrogen phosphate: 6.0825 g material B (95% = 5.7784 g, 13.37 mmole) dissolved in 10 ml $CHCl_3$. To this was added 2.0605 g (13.37 mmole) $(C_2H_5)_2HPO_4$. Molecular weight determinations after 72 hr: $(C_2H_5)_6HP_5O_{16}$ calc. 586.3; found 535, 547 in ethyl acetate.

(5) Tetrapolyphosphate ester (substance V) and diethyl hydrogen phosphate: 3.4964 g substance V (7.15 mmole, average mol. wt. calc. 489), dissolved in 3 ml $CHCl_3$. To this was added 1.1019 g (7.15 mmole) $(C_2H_5)_2HPO_4$, dissolved in 1 ml $CHCl_3$.

(6) Tripolyphosphate ester (substance VIII) and diethyl hydrogen phosphate: 3.7285 g substance VIII (9.89 mmole, average mol. wt. calc. 377), dissolved in 3 ml $CHCl_3$. To this was added 1.5242 g (9.89 mmole) $(C_2H_5)_2HPO_4$, dissolved in 1 ml $CHCl_3$.

All ^{31}P n.m.r. and IR spectra were recorded after reaction times of 48 and 72 hr.

Reaction of Tetrapolyphosphate Esters (Material D) with Various Solvents.--- The solvents, given above, were dried directly by adding tetrapolyphosphate esters; the solutions were allowed to stand for 24 hr at 25° and the solvents then recovered by distillation under vacuum at 25° (nitrobenzene at 60°).

3 g esters (material D) were dissolved in 6 ml purified solvent, and the P n.m.r. spectrum recorded (after 72 hr at 25°, after 10 min and 5 hr at 80° or the reflux temperature).

Molecular weight determinations were carried out by an ebullioscopic method, using the apparatus of Suchardu and Bobranski.[20]

Acetonitrile and methyl acetate (only for substance IX) were used as solvents.[10] Substances VII and IX were completely soluble only on heating. No reaction between the solvents and the substances occurred during the determinations, as evidenced by ^{31}P n.m.r. measurements. The values obtained for substance X are inconsistent with the P n.m.r. results of this substance: the molecular weights found are too high, probably as a result of strong associations even in acetonitrile. All molecular weights obtained in chloroform, by this method and by means of an osmometer, gave values much higher than those obtained in the other two solvents. The results strongly depend on the content of the OH groups in the substances and on the concentrations: associations must occur in chloroform.[10]

N.M.R. Measurements.— All the ^{31}P n.m.r. spectra were recorded with a Varian V-4300-C spectrometer operating at 24.6 mc/s, in conjunction with an apparatus for "continuous averaging".[21] The curves were cut out and weighed for quantitative analysis. The signal to noise ratio for the measurements in water and ethanol was poorer than that of the spectra of the concentrated solutions of materials A to E in chloroform.[10] Therefore, the values in Tables

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(20) K. Rast in Houben-Weyl's "Methoden der Organischen Chemie," 3/1, Georg Thieme Verlag, Stuttgart, 1955, p. 327.

(21) M. P. Klein and G. W. Barton, Jr., Rev. Sci. Instr., 34, 754 (1963).

I, II, and III may have a limit of error of several percent. Amounts of P_b and P_o listed as "trace" are estimated to be about 1%. Smaller amounts may have escaped detection because of the relatively poor signal to noise ratio. The chemical shifts were determined using 85% H_3PO_4 as an external standard. No attempt was made to reach an accuracy of better than ± 2 p.p.m. Some of the samples were stored in tightly closed tubes for several days in dry ice before the measurements. It was ascertained by F n.m.r. measurements that a further reaction did not occur at this temperature.

The proton n.m.r. measurements were made with a Varian A-60 spectrometer using chloroform as an internal standard. The chemical shifts are given with respect to tetramethyl silane as reference.

IR Measurements.— The IR spectra were recorded with a Perkin-Elmer IR spectrophotometer 237. A 1.5% solution of the ester in $CHCl_3$ was used, and the absorption of the solvent compensated with a second cell with pure solvent. (Cell path 0.1 mm).

Acknowledgments.— We wish to thank the Deutsche Akademische Austausch-dienst, Bad Godesberg, Germany, for awarding a fellowship to one of us (G. B.).

LEGENDS FOR FIGURES

Chart 1: No legend

Fig. 1: Phosphorus n.m.r. spectra of samples obtained from the hydrolysis of a mixture of tetraphosphate esters (material D)[17] with an excess of water at room temperature.

a) Unhydrolysed sample dissolved in chloroform.

b) to e) Samples dissolved in water (conc. 10 g in 20 g water; 1 mole ester to 48 moles water); b) after 0.25 hr, c) after 1 hr, d) after 24 hr, e) after 48 hr.

The abscissa is the chemical shift in part per million from 85% H_3PO_4.

Fig. 2: Ratio of P_m, P_t and P_o peaks of the ^{31}P n.m.r. spectra of samples obtained by hydrolysis (a) or alcoholysis (b) of a mixture of tetraphosphate esters (material D)[17] at room temperature versus the time of reaction.

a) Hydrolysis with an excess of water (conc. 10 g ester in 20 g water; 1 mole ester to 48 moles water.

b) Alcoholysis with an excess of ethanol (conc. 10 g ester in 51.1 g ethanol; 1 mole ester to 48 moles ethanol.

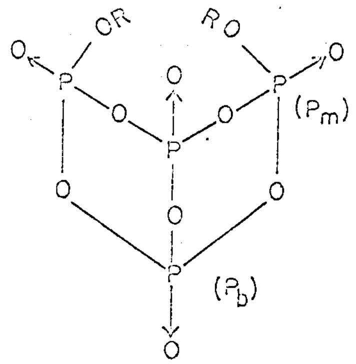
Fig. 3: IR spectra of samples of polyphosphate esters (dissolved in chloroform, absorption of the solvent compensated).

1) to 4) Tetrapolyphosphate esters: 1) material A, 2) material B, 3) material D, 4) material E.

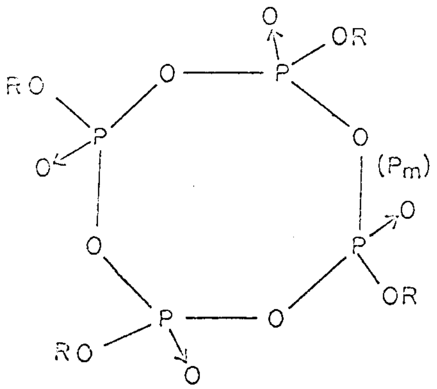
5) to 8) material B + different amounts of ethanol: 5) 3/4 equivalent, 6) 1 equivalent (substance VI), 7) 4/3 equivalent (substance VIII, 8) 2 equivalents (substance X).

9) triethyl orthophosphate.

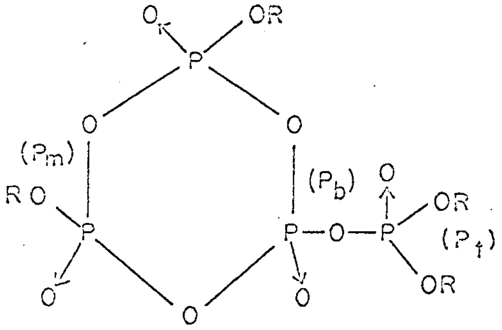
The ordinate, in all cases, measures the transmittance.



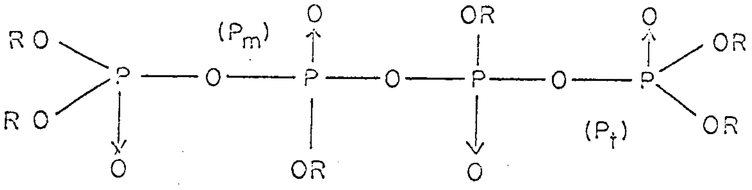
(I) BICYCLO TETRAPOLYPHOSPHATE ESTERS



(II) CYCLO TETRAPOLYPHOSPHATE ESTERS



(III) ISOCYCLO TETRAPOLYPHOSPHATE ESTERS



(IV) TETRAPOLYPHOSPHATE ESTERS

R = C_2H_5, TO A SMALL EXTENT ALSO H.

Chart 1

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art 1

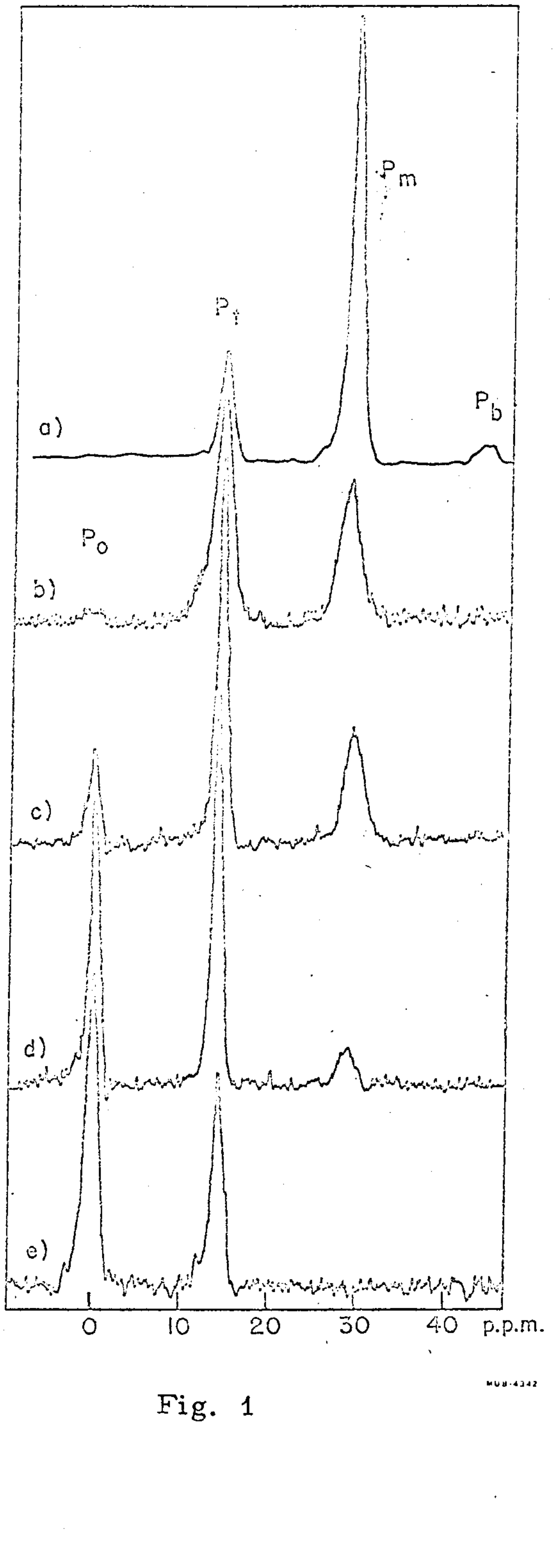

Fig. 1

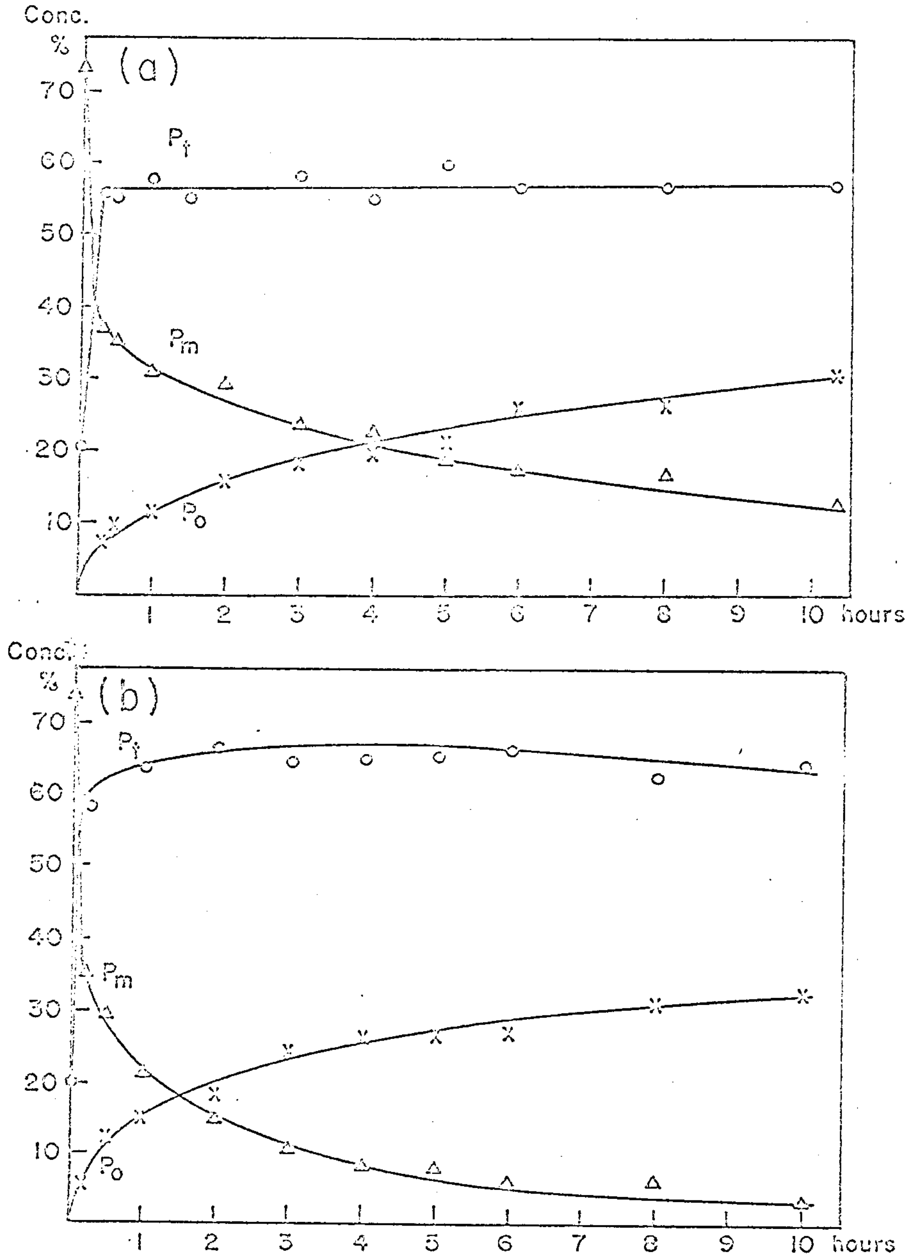

Fig. 2

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.2

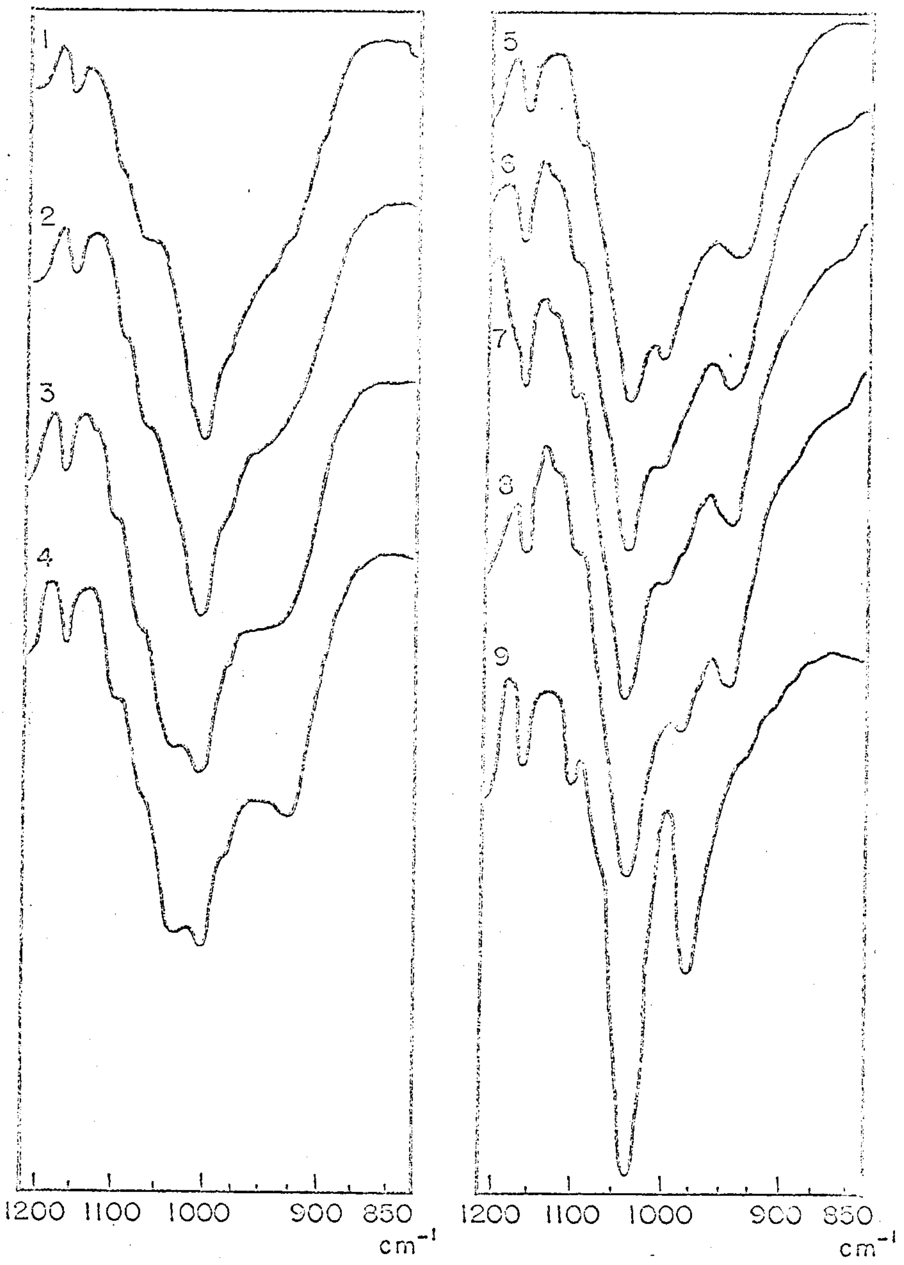

Fig. 3

MUB-4688