Phorbic Acid, a New Acid from *Euphorbium*

ERLING BERNATEK, ARNOLD NORDAL and GUNNAR OGNER

*Universitetets Farmasøytiske Institutt, Blindern-Oslo, and
Universitetets Kjemiske Institutt, Blindern-Oslo, Norway*

From the crude drug *Euphorbium* a new acid, phorbic acid, has
been isolated in the form of a dilactone — dilactophorbic acid. The
structure of this acid has been elucidated and shown to contain two
γ-lactone rings which are combined in a spiro system.

The water-soluble organic acids from *Euphorbium* have been investigated by
Nordal and Bærheim Svendsen.[1] Esterification of the crude mixture of acids
with ethanol and sulphuric acid yielded a mixture of esters, which was partly
separated by fractionation *in vacuo*. The lower-boiling fractions contained
mainly ethyl L-malate together with some diethyl succinate and presumably a
small amount of triethyl citrate. The fraction 220—235°C (11—12 mm Hg)
could be brought to crystallization and the pure crystals had m.p. 90°C, and
$[\alpha]_D^{18} = + 33°$. Cryoscopic measurements gave a molecular weight of about
230. The substance was evidently an ester and contained at least one lactone
ring. No unsaturation could be detected. It was treated with hydrazine hydrate,
but the product was not crystalline and was therefore converted into the benzy-
lidene hydrazide. This product was amorphous and could not be identified as
a derivative of any known acid. The previous investigation was terminated
mainly because of lack of material.

This paper deals with some further developments in the structural investiga-
tion of the ester m.p. 90°C, and the corresponding acid. In its unlactonized form
this acid has been named phorbic acid by the present authors. Extraction of
Euphorbium with water and the working up of this extract to a crude mixture
of acids were performed as described previously.[1] This mixture contains impuri-
ties of several kinds, inorganic as well as organic. Some of these, especially
calcium salts, can be removed by dissolving the mixture in acetone and filter-
ing off the sludge which is formed. Esterification of the organic acids with
ethanol and sulphuric acid is a method which conceivably can give rise to
artefacts, *e.g.* through rearrangements or decomposition. A milder method,
viz. treatment with diazoethane was therefore tried and an identical ester was
produced. By this method and by distilling the ester mixture at lower pressure
$(10^{-2}$ mm Hg) the yield of pure ester, m.p. 90°C, can be increased to about
11 % based on the crude acid mixture.

By acid hydrolysis of the ester a free acid can be isolated, which has the composition $C_8H_8O_6$ and m.p. 154°C. Further the acid can be prepared by alkaline hydrolysis and subsequent passage of the basic solution through a cation-exchange resin column. Diazoethane converts this acid back to the original ester, which proves that no changes in the molecule apart from hydrolysis have taken place during the hydrochloric acid or alkali treatments. In aqueous solution the acid is titrated with 0.01 N sodium hydroxide as a dibasic acid in such a way that the first equivalent of alkali is consumed instantaneously and the second more slowly. By dissolving the acid in 0.5 N potassium hydroxide and back-titrating with standard hydrochloric acid the substance can be titrated as a tribasic acid. An attempt to determine C-methyl groups with the usual oxidative method showed that no such groups are present in the molecule. This is also confirmed by the infra-red spectrum where no methyl group absorption can be observed. A strong absorption occurs at 1780 cm^{-1} and is evidently caused by carbonyl stretching vibrations in a γ-lactone carbonyl group. Spectroscopic evidence does not indicate the presence of a δ-lactone ring. Evidently the acid $C_8H_8O_6$ contains two γ-lactone rings and it has therefore been named dilactophorbic acid. One of the lactone rings apparently must be opened more easily by alkali than the other. The unlactonized acid is not very stable as shown by chromatography. A freshly prepared solution of the free acid (prepared from the tripotassium salt by means of an ion-exchanger) gives three spots on the chromatogram. If this solution is concentrated and finally dried at 105° one of the spots gradually disappears.

Analysis showed the ester to have the composition $C_{10}H_{12}O_6$ and to contain one ethoxyl group. It gives a negative reaction on aldehydic and ketonic groups. It is insoluble in cold water but dissolves in hot water giving an acid solution. Upon cooling it separates unchanged from the aqueous solution. Direct titration with 0.01 N sodium hydroxide gives an equivalent weight of ca. 224 i.e. the ester behaves like a monobasic acid. If, on the other hand, it is dissolved in cold 0.5 N potassium hydroxide and back-titrated with standard hydrochloric acid, the equivalent weight is 76.8. Here the molecule has consumed three equivalents of alkali. The infra-red spectrum of the ester shows clearly that no free carboxyl or hydroxyl groups are present. There is a strong band at 1809 cm^{-1} which must be attributed to a γ-lactonic carbonyl group even if it occurs at a somewhat higher frequency than considered normal. There is also a fairly strong absorption at 1742 cm^{-1} due to an ester group.

The above mentioned facts all points towards $C_{10}H_{12}O_6$ being a monoethyl ester containing two γ-lactone rings. Due to the limited number of carbon atoms in the molecule, the two lactone rings can be combined to a cyclic skeleton in only three ways (hydrogen atoms not shown):

(I)   (II)   (III)
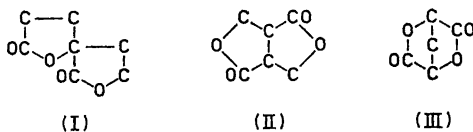
These skeletons must carry the following substituents:

(I): $-COOC_2H_5$
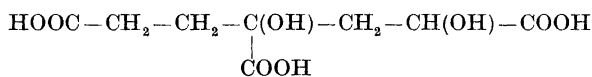(II): $-CH_2-COOC_2H_5$
(III): $-CH_2-CH_2-COOC_2H_5$
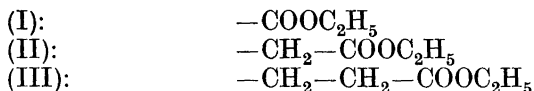
A large number of isomers can be conceived through: (*a*) different orientation or fusion of the rings relative to each other, (*b*) different points of substitution.
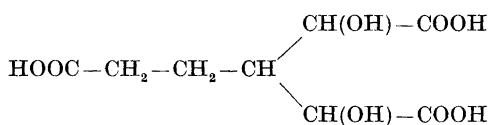
To unravel this very complicated set of possibilities it was considered whether pertinent information could be had from the periodate oxidation of phorbic acid. Alone this information would be meagre, but in combination with periodate oxidation of the reduced acid, *i.e.* a pentahydric alcohol, it might become a powerful tool. The numerous possibilities were analysed thoroughly and it was found that the method would at least reduce the probable structures to a small number.

The ester was not attacked by periodate in acid milieu or at pH *ca.* 7. With lithium aluminium hydride it was reduced smoothly to a pentahydric alcohol, which could be isolated as a tetrabenzoate, while the free alcohol was difficult to separate from the reaction mixture. In order to remove lithium ions from the syrupy alcohol an aqueous solution was passed through a mixed bed ion exchange resin. The purified compound was still a syrup but an infra-red spectrum showed that hydroxyl was the only functional group present in the molecule. Chromatographic analysis proved the purity of the substance which had the expected composition $C_8H_{18}O_5$. In the course of 15 min at room temperature the alcohol consumed two equivalents of periodic acid and after that point a very slow reaction continued. Formaldehyde could be detected in the reaction mixture and occurred in an amount of two moles per mole of starting material. Another carbonyl compound was present, but gave no defined derivatives *e.g.* with 2,4-dinitrophenylhydrazine. The oxidation mixture was therefore precipitated with barium hydroxide whereby periodate, iodate and sulphate ions were removed. Excess barium was precipitated with carbon dioxide. On evaporation a syrup remained which contained carbonyl groups and reduced Tollens' and Fehling's reagents. Its infra-red spectrum indicated a β-ketoaldehyde structure (abs. maxima at 1720 and 1680 cm^{-1}). Oxidation with potassium permanganate afforded succinic acid, which was identified chromatographically.

The results so far leave us with the choice between two dihydroxytricarboxylic acids which can give dilactones:
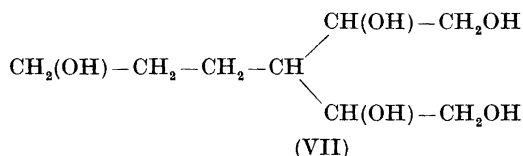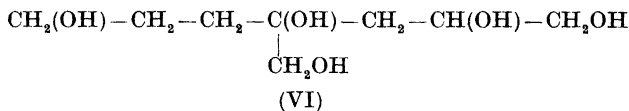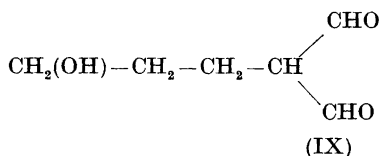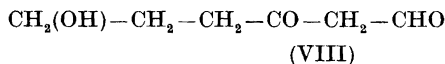
$$HOOC-CH_2-CH_2-\underset{\underset{\displaystyle COOH}{|}}{C(OH)}-CH_2-CH(OH)-COOH$$

(IV)

$$HOOC-CH_2-CH_2-CH\overset{\displaystyle CH(OH)-COOH}{\underset{\displaystyle CH(OH)-COOH}{<}}$$

(V)

None of these entities will react rapidly with periodate as the α-hydroxycarboxylic moiety only tardily is attacked by this reagent.[2] On reduction with lithium aluminum hydride the acids (IV) and (V) will give the pentahydric alcohols (VI) and (VII) respectively:

$$CH_2(OH)-CH_2-CH_2-C(OH)-CH_2-CH(OH)-CH_2OH$$
$$CH_2OH$$
$$(VI)$$

$$CH_2(OH)-CH_2-CH_2-CH\begin{array}{c}CH(OH)-CH_2OH\\ \\ CH(OH)-CH_2OH\end{array}$$
$$(VII)$$

Both these substances will consume two equivalents of periodate and produce two moles of formaldehyde. The main oxidation products will in the two cases be (VIII) and (IX):

$$CH_2(OH)-CH_2-CH_2-CO-CH_2-CHO$$
$$(VIII)$$

$$CH_2(OH)-CH_2-CH_2-CH\begin{array}{c}CHO\\ \\ CHO\end{array}$$
$$(IX)$$

Only structure (VIII), however, is compatible with the infrared spectrum of the oxidation product and with the ready formation of succinic acid with potassium permanganate. Also the consumption of periodate in excess of two moles is rationalised by this formula as β-dicarbonyl compounds are known to react slowly with that reagent.

In order to confirm this finding a high resolution proton magnetic resonance spectrum of the ester $C_{10}H_{12}O_6$ was recorded. The spectrum revealed the presence of three uncoupled sets of protons: (1) A triplet at $\tau = 1.27$ and a quartet at $\tau = 4.18$ show that the compound contains an ethyl ester group: CH_3CH_2OCO-. (2) A triplet at $\tau = 4.87$ must be due to a single proton α to a methylene group. The methylene group contributes a doublet at $\tau = 2.72$. From the chemical shift it is reasonable to assume that this $-CH_2CH-$ group is bound as shown in formula IV. (3) At slightly higher field than the doublet at $\tau = 2.72$ the spectrum contains a broad band with partially resolved fine structure. Measurements of the area under this band compared to other peaks in the spectrum tell that the band is due to four protons. A likely proton configuration is $-CH_2CH_2-$, somewhat unsymmetrically substituted so that the two methylene groups are slightly nonequivalent. The two last mentioned groupings are located in the skeleton of the ester and must therefore also be found in the unlactonized phorbic acid.

The interpretation of the spectrum supports the formula (IV) for phorbic acid while it definitely rules out formula (V).

Carrying two hydroxyl and three carboxyl groups phorbic acid *a priori* should be able to form a dilactone in two ways:

$$
\begin{array}{cc}
\text{(X)} & \text{(XI)}
\end{array}
$$
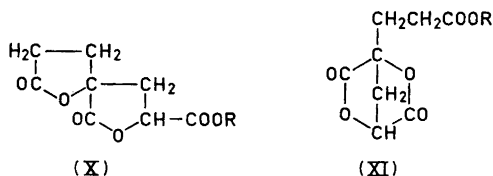
Chemical methods alone probably cannot decide between these two structures, especially as the free acids (R = H) may be interconvertible under more drastic conditions. The infra-red spectra of the ester and the free acid need therefore some closer attention. Steroidal spiro-γ-lactones are reported to absorb at a normal frequency,[3] and it may be expected that (X) (R = H or C_2H_5) will show no abnormal absorption *per se*. Likewise fused cyclic systems with γ-lactones are found to have a normal 5-ring lactone frequency.[4] Therefore also (XI) (R = H or C_2H_5) should have an absorption band around 1770 cm^{-1}. Now there is substitution effect in γ-lactones the nature of which is not well understood. It has been observed in several cases that a negative substituent in the γ-position raises the frequency of absorption considerably.[5] *E.g.*, in 1,4-hexanolide (γ-ethylbutyrolactone) a —COOCH$_3$ group in γ-position raises the carbonyl frequency to 1787 cm^{-1}. This, however, is measured in the pure liquid, dissolved in a nonpolar solvent the frequency will increase another 10—15 cm^{-1}. $C_{10}H_{12}O_6$ absorbs at 1809 cm^{-1} and from formula (X) (R = C_2H_5) it will be seen that we here have a —COOC$_2H_5$ group in γ-position in one of the rings. There is not found a separate frequency for the unsubstituted ring, which may be indicative of a certain interaction between the rings even as they are at right angles. In formula (XI) (R = C_2H_5) on the other hand, the carbethoxyl group is insulated from the ring system by two methylene groups and should be expected to have only a very small influence on the ring carbonyl frequency. The fact that substitution of —C_2H_5 with —CH$_3$ in the ester group lowers the carbonyl frequency about 16 cm^{-1}, also points in the same direction. Construction of the molecule (XI) with Courtauld's atom models is not possible if normal valency angles are to be retained. Such a molecule would contain considerable strain in its ring system and accordingly be unstable. It is therefore improbable that phorbic acid should lactonize to (XI) when there is a possibility to lactonize to a strain-free system as given by formula (X).

We shall therefore give a preference to formula (X) (R = C_2H_5) as a structural representation of $C_{10}H_{12}O_6$. The lower frequency of the acid (1780 cm^{-1}) may also be due to intermolecular (and perhaps intramolecular) hydrogen bonding.

EXPERIMENTAL

Isolation of the crude mixture of acids. 6.9 kg of powdered *Euphorbium* (quality according to *Pharmacopoea Norvegica*, Ed. V) was macerated for two days with 30 l of water and the unsoluble part filtered off. The filtrate was precipitated with 3 l of saturated,

aqueous lead acetate and the yellowish, voluminous precipitate (A) was filtered off. To remove water-soluble substances from the precipitate, this was twice suspended in 3 l of water and filtered.

The filtrate from A was precipitated with a slight excess of basic lead acetate solution and the precipitate (B) treated with water as A.

The two precipitates (A and B) were separately suspended in 3 l of water, decomposed with H_2S, filtered, and the two filtrates concentrated under reduced pressure to thick syrups. These were each treated with 900 ml of anhydrous ethanol, which in both cases produced yellowish precipitates that were filtered off. To remove the last traces of water from the mixtures of the acids, the alcohol was distilled *in vacuo*, and the residues again treated with 900 ml of anhydrous ethanol, which was removed under reduced pressure. This process was repeated three times. The yield of acids from precipitate A was 259 g and from precipitate B 145 g. Both were thick syrups. The yield of substances insoluble in alcohol from the two mixtures of acids were 100 and 30 g, respectively.

Esterification of the acids from precipitate A, using sulphuric acid as a catalyst. 100 g of the acids was dissolved in 500 ml of anhydrous ethanol. 10 ml of concentrated sulphuric acid was then added and the mixture refluxed for 4 h, whereafter the ethanol was distilled off *in vacuo*. The remaining mixture of esters was dissolved in ether, neutralized with a dilute solution of sodium carbonate, washed with a few ml of water, and finally dried over dehydrated sodium sulphate. After filtration the ether was distilled off.

Fractional distillation of the ester mixture. The ester mixture was fractionated at 10— 12 mm Hg whereby the following fractions were obtained (Table 1).

Table 1.

Fraction No.	B.p. interval in C°	Yield in g	Appearance of fraction
1	80 — 140	2.0	Yellowish liquid
2	140 — 175	3.2	Yellowish brown oily liquid
3	175 — 245	10.8	Brown syrup

A remarkably large amount of a black, thick fluid remained in the distillation flask. Fraction No. 3 was diluted with 5 ml of ethanol whereupon crystallization started almost immediately. The solid ester was filtered off and recrystallized twice from ethanol. The substance then appeared as white, scaly crystals, m.p. 91.5 — 92.0°C (Kofler micro hot stage). The yield of purified ester was 1.6 g or 1.5 % as calculated on the basis of the crude mixture of acids taken into work.

Esterification of the acids from precipitate A with diazoethane. 24 g of the acids was esterified with excess of diazoethane at 0°C. The reaction mixture was left at room temperature for 5 h whereupon it was left in a refrigerator. After two weeks a crystalline product had formed, which was filtered off and recrystallized twice from ethanol. Yield about 0.1 g. M.p. and mixed m.p. with the abovementioned ester: 91.5 — 92.0°C.

The rest of the ester mixture was fractionated *in vacuo* in the same way as the first prepared ester mixture. The result is shown in Table 2.

Table 2.

Fraction No.	B.p. interval in C°	Yield in g	Appearance of fractions
1	80 — 180	1.5	Yellowish liquid
2	180 — 200	0.5	Dark yellow oily liquid
3	200 — 220	3.0	Yellowish brown oily liquid
4	220 — 240	5.0	Brown syrup

Table 3.

Fraction No.	B.p. interval in C°	Yield in g	Appearance of fractions
1	40 – 120	2.4	Yellowish liquid
2	120 – 140	1.8	Yellowish liquid
3	140 – 170	3.0	Yellow liquid
4	170 – 190	5.8	Yellowish brown syrup
5	190 – 200	18.0	Brown syrup
6	200 – 220	3.8	Dark brown syrup

Upon dilution with ethanol the fractions 3 and 4 yielded a crystalline substance. Recrystallized from ethanol. M.p. and mixed m.p. with the previous esters: 91.5 – 92.0°C. The yield of solid ester from fractions 3 and 4 were 0.8 g and 0.1 g, respectively, or about 4 % of the acids taken into work.

The solid ester of m.p. 91.5 – 92.0°C. (Found: C 52.63; H 5.37; OC_2H_5 20.9. Calc. for $C_{10}H_{12}O_6$: C 52.62; H 5.30; OC_2H_5 19.7.) Direct titration in hot water; found: $E = 224$. Dissolved in cold potassium hydroxide and backtitrated; found: $E = 76.7$. After refluxing in potassium hydroxide: $E = 76.0$. Calc. E (monobasic): 228.2; E (tribasic): 76.1.

The ester $C_{10}H_{12}O_6$ gave no reaction with semicarbazide, p-nitrophenylhydrazine or with 2,4-dinitrophenylhydrazine, nor did it reduce Fehling's solution or Schiff's reagent. The ester gave no colour with ferric chloride and did not evolve hydrogen with metallic sodium in anhydrous ether.

Esterification of the acids isolated from precipitate A (cf. p. 2379). 70 g of the acids was esterified with diazoethane. Upon fractional distillation (10^{-2} mm Hg) the following fractions were obtained (Table 3):

The ester $C_{10}H_{12}O_6$ was isolated from the fractions Nos. 5 and 6. In minor quantities also from fraction No. 4. Total yield: 8 g or 11 %.

Acid mixture B gave upon esterification approximately 4 % of the ester $C_{10}H_{12}O_6$.

Isolation of the free acid

Saponification with potassium hydroxide. 200 mg of the ester $C_{10}H_{12}O_6$ was dissolved in 10 ml of 0.5 N potassium hydroxide. The solution was acidified with 1 N sulphuric acid to pH 2 and extracted 8 times with 20 ml of ether. The ether solution was dried over dehydrated sodium sulphate and the ether distilled off. The residue, a yellow oil amounting to approximately 30 mg, could not be brought to crystallization.

Saponification with hydrochloric acid. 1.1 g $C_{10}H_{12}O_6$ was refluxed for half an hour with 20 ml of 0.5 N hydrochloric acid, and the solution evaporated to about 4 ml. Upon drying *in vacuo* over potassium hydroxide pellets for three days, the solution was converted into a crystalline mass, which after recrystallization from anhydrous ethanol gave white, irregular crystals of m.p. 152 – 154°C. Yield: 0.6 g.

Isolation from the potassium salt with Dowex-50. 0.20 g $C_{10}H_{12}O_6$ was dissolved in 10 ml of 0.5 N potassium hydroxide and the solution was passed through a column of Dowex-50. 50 ml of the acid eluate was concentrated *in vacuo*, and the residue, 3 ml of a viscous clear liquid, was dried at 105°C for 24 h. The crystalline substance formed showed m.p. and mixed m.p. with the abovementioned acid 151 – 154°C.

Investigation of the free acid

(Found: C 47.89; H 4.01. Calc. for $C_8H_8O_6$: C 48.01; H 4.01.) Direct titration; found: $E = 100.0$. Backtitration after dissolution in potassium hydroxide at room temperature: found: $E = 68.9$. Backtitration after heating with potassium hydroxide for one hour; found: $E = 68.0$. Calc. E (dibasic) = 100; E (tribasic) = 66.6.

Esterification with diazoethane. A clear solution of 50 mg $C_8H_8O_6$ in 1 ml anhydrous ethanol was, under cooling with ice, treated with a slight excess of diazoethane. The

reaction mixture which had a yellowish colour, was left for 2 h at room temperature, concentrated to about 0.5 ml and put in the ice box. The crystalline product that soon appeared had m.p. 91.0 − 92.0°C alone and in mixture with the originally prepared ester, $C_{10}H_{12}O_6$. Yield: 40 mg. 50 mg of the yellow oil that was formed in an attempt of recrystallizing the free acid $C_9H_8O_6$, was esterified in the same way as mentioned above. Yield: 35 mg of $C_{10}H_{12}O_6$.

Solubility. $C_8H_8O_6$ is readily soluble in water and hot ethanol, but nearly insoluble in ether, carbon disulphide and carbon tetrachloride.

C-methyl determination of $C_8H_8O_6$ gave a negative result.

Reduction of the ester $C_{10}H_{12}O_6$ with lithium aluminum hydride

For the reduction was used a closed system consisting of a threenecked flask (250 ml), with a separatory funnel, a stirrer and a Soxhlet extraction unit. To the boiling suspension of 1.7 g of lithium aluminum hydride in 120 ml of anhydrous ether 1.6 g of $C_{10}H_{12}O_6$ was gradually added with stirring during approximately 4 h. Reflux and stirring of the reaction mixture was continued for another hour. Excess of the reagent was decomposed with ethyl acetate, whereafter water was added till a coarse, white mass had formed at the bottom of the flask. The ether was decanted off, dried over sodium sulphate and removed by distillation. Practically nothing was left in the distillation flask. The remainder of the reaction mixture was therefore suspended in 50 ml of water and filtered. This process was repeated twice with 25 ml of water.

The combined filtrates were concentrated *in vacuo* to 20 ml and a small precipitate that had formed was filtered off. To the filtrate was added 7 ml of 5 N sodium hydroxide and the solution was treated with 5 g of benzoyl chloride according to the Schotten-Baumann procedure. The reaction mixture was extracted three times with 15 ml of ether. The combined ether extract were dried over sodium sulphate, filtered and the ether distilled off. The residue, a yellow oil, was dissolved in 100 ml of methanol, mixed with 3 ml of conc. hydrochloric acid and refluxed for 2 ½ h in order to achieve transesterification. After concentration of the solution *in vacuo* to 15 ml, a white substance precipitated which after recrystallization from ethanol melted at 110 − 111°C. Yield: 0.7 g. The substance corresponds to a tetrabenzoate of the expected pentol. (Found: C 70.89; H 5.39. Calc. for $C_{36}H_{34}O_9$: C 70.81; H 5.61).

As the yield of benzoate was small by the above-mentioned method, a second sample of the ester was worked up in the following way: 3 g of $C_{10}H_{12}O_6$ was reduced with 2.5 g of lithium aluminum hydride in 100 ml of ether in the same way as the first sample. After completion of the reaction excess lithium aluminum hydride was decomposed with ethyl acetate whereupon 150 ml of water was added to the reaction mixture. The ether layer was removed by decantation, whereafter the mass of aluminum hydroxide was filtered off and washed three times with 25 ml of water. The combined filtrates were evaporated *in vacuo* to 25 ml and then adjusted to pH = 7 by means of sulphuric acid (25 %) and a pH paper. The precipitate that was produced, was filtered off and the filtrate evaporated *in vacuo* to a yellow syrup. After drying this was first extracted with cold acetone and ethanol, but as only minor amounts were dissolved, the residue was boiled with anhydrous ethanol and filtered hot. As no crystallization took place in the course of two weeks at − 15°C, the alcohol was distilled off whereby a yellow syrup remained which gave a flame test for lithium. The syrup was dissolved in 10 ml of water, and purified by means of a mixed bed ion exchange resin, MB-1, in order to remove the last traces of inorganic substances. After concentration of the eluate (100 ml) from the ion exchange column *in vacuo*, the pentol appeared as a glass-like mass, which could not be brought to crystallization. Yield: 1.6 g or 51 %. (Found: C 48.11; H 9.15. Calc. for $C_8H_{18}O_5$: C 49.49; H 9.34).

The fact that the pentol was not entirely dry may account for the rather low C content. The pentol was dried over potassium hydroxide *in vacuo*, but the mass thus formed still contained traces of water. However, the percentage of hydrogen will not be much altered even if the product contains small amounts of water. It is therefore concluded that the isolated substance is $C_8H_{18}O_5$.

Paper chromatography of $C_8H_{18}O_5$. Whatman No. 1 paper and descending chromatography were used, and the chromatograms were developed with butanol: ethanol:water

(4:2:1). To make the spots visible Tollens' reagent and a 1 % solution of lead tetraacetate in dry benzene were used.

When sprayed with Tollens' reagent, the chromatograms of the alcohol gave immediately a single spot. By steaming of the paper no more spots were detected. The lead tetraacetate spray gave a white spot on a yellowish brown background. For comparison erythritol and adonitol were used. The R_F values of the three substances were as follows: erythritol 0.47, adonitol 0.38 and $C_8H_{18}O_5$ 0.51.

Oxidation with periodic acid

The following solutions were used: (1) sodium periodate, 0.1030 N, (2) arsenic trioxide, 0.0981 N, (3) iodine, 0.1003 N, (4) starch, 0.2 %, (5) sodium bicarbonate, saturated solution, (6) potassium iodide 10 % (7) solutions of the substance to be tested (8) solutions in tartaric acid, used for comparison.

The actual oxidation was carried out in the following way: 5 ml aliquots of the test solution were mixed with 20 ml of the sodium periodate solution and allowed to react for certain periods of time (Table 4). Then the oxidation process was stopped by adding 25 ml of arsenic trioxide solution, 10 ml of the saturated sodium bicarbonate solution and 1 ml of the 10 % potassium iodide solution. The reaction mixture was put aside for 15 min. Excess of arsenite was then titrated with 0.1 N iodine using 3 ml of the starch solution as an indicator.

If the number of ml of 0.1 N iodine which is consumed by the titration is called a and the number of ml consumed by the blind test is called b, then

$$\text{Mole consumed periodic acid} = \frac{(a-b) \times 0.1003}{1000 \times 2}$$

A blind test corresponded to the theoretical value calculated on basis of the normality of the solutions.

Oxidation of the acid $C_8H_{12}O_8$. 0.3015 g of the ethyl ester $C_{10}H_{12}O_6$ was dissolved in 5 ml of 10 % potassium hydroxide. The solution was adjusted to about pH = 10 with sulphuric acid (10 %) and then diluted to 25 ml. (This corresponds to a 0.05 M solution of $C_8H_{12}O_8$.) The result of the oxidation is shown in Table 4.

Table 4.

Substance	Time of oxidation in hours	$a-b$	Mole HIO$_4$ consumed	Mole substance taken into work	Mole HIO$_4$ per mole substance
$C_8H_{12}O_8$	1/4	0.01	0	2.50×10^{-4}	0
»	1/2	0.01	0	»	0
»	1	0.06	0	»	0
»	2 1/2	0.05	0	»	0

Table 5.

Substance	Time of oxidation in hours	$a-b$	Mole HIO$_4$ consumed	Mole substance taken into work	Mole HIO$_4$ per mole substance
$C_8H_{18}O_5$	1/4	6.26	3.13×10^{-4}	1.50×10^{-4}	2.08
»	1/2	6.34	3.17×10^{-4}	»	2.11
»	3/4	6.61	3.30×10^{-4}	»	2.20
»	1 1/2	6.06	3.53×10^{-4}	»	2.36
»	2 1/2	7.28	3.64×10^{-4}	»	2.42

Temperature during the experiments: 20−21°C.

In another experiment the oxidation was performed at pH = 5−6. Nor in this case did the acid $C_8H_{12}O_8$ consume periodic acid.

Oxidation of the pentol $C_8H_{12}O_8$. The same oxidation procedure as described above was applied. This time erythritol and adonitol were used for comparison. The results of the oxidation are shown in Table 5.

Investigation of the oxidation products

The pentol $C_8H_{18}O_5$ was oxidized for 15 min according to the process mentioned above whereafter the reaction was stopped. 20 ml of the reaction mixture was distilled off through a 20 cm Vigreux column directly into a solution of 2,4-dinitrophenylhydrazine in dilute sulphuric acid. The derivative thus formed, melted at 165−166°C without further purification. The substance corresponds to the dinitrophenylhydrazone of form-aldehyde. A dimedone derivative melted at 189−190°C.

To obtain an estimate of the amount of formaldehyde formed during the oxidation of the pentol this was again oxidized with periodic acid the formaldehyde formed was treated with chromotropic acid, and the amount of colored complex formed was deter-mined spectrophotometrically. The results thus obtained indicate that each mole of $C_8H_{18}O_5$ splits off two mole formaldehyde when oxidized in the way mentioned above.

Infra-red spectra. The spectrometer was a Perkin-Elmer double beam apparatus (Model 21) in most cases, but some spectra were recorded on the Perkin-Elmer Infracord. The compounds were investigated in Nujol mulls, as liquid films, or in potassium bromide pellets.

N.M.R. spectrum. The compound ($C_{10}H_{12}O_6$) was dissolved in chloroform and a little TMS (tetramethylsilane) was added to serve as an internal standard. The high resolu-tion proton magnetic resonance spectrum was recorded on a Varian Associate Dual Purpose Spectrometer operating at 60 Ms/sec. The positions of the peaks relative to the TMS peak have been measured by standard side-band technique with an accuracy of about ± 0.01 p.p.m.

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