

The relation between natural and synthetical glycerylphosphoric acids. Pt. 2 / by Frank Tutin and A.C.O. Hann.

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No. 66

THE RELATION BETWEEN
NATURAL AND SYNTHETICAL
GLYCERYLPHOSPHORIC ACIDS

PART II.

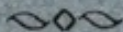
BY

FRANK TUTIN

AND

A. C. O. HANN

(From the Transactions of the Chemical Society, 1906)



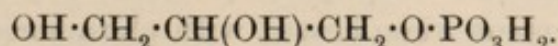
THE WELLCOME CHEMICAL RESEARCH LABORATORIES
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CLXX.—*The Relation between Natural and Synthetical Glycerolphosphoric Acids. Part II.*

By FRANK TUTIN and ARCHIE CECIL OSBORN HANN.

WILLSTÄTTER and Lüdecke (*Ber.*, 1904, 37, 3753) have described the preparation of some salts of a lævorotatory glycerylphosphoric acid obtained from egg-lecithin, from which they concluded that the natural glycerylphosphoric acid has the unsymmetrical configuration



They also prepared the synthetical acid by heating together glycerol and phosphoric acid, and claimed to have found essential differences between the barium and calcium salts of the product thus obtained and the corresponding salts of the natural acid, not only with respect to solubility, but especially in the composition of the preparations when dried by heat (*loc. cit.*).

In an account of an investigation by Dr. Power and one of us (*Trans.*, 1905, 87, 249), it was shown that the above-mentioned differences in composition observed by Willstätter and Lüdecke were due to the fact that their synthetical salts were not pure, but were contaminated with salts of the di-ester, $\text{C}_3\text{H}_5(\text{OH})\begin{smallmatrix} \text{O} \\ \diagup \quad \diagdown \\ \text{O} \end{smallmatrix}\text{PO}\cdot\text{OH}$. It was also stated that "the observation of Willstätter and Lüdecke that the glycerylphosphoric acid from lecithin possessed optical activity, although certainly proving the presence of the unsymmetrical acid in the preparation, does not necessarily exclude the possibility that some of the symmetrical acid was also present." Furthermore, that "the same possibility naturally exists with respect to the homogeneity of the synthetical acid."

With the endeavour definitely to ascertain the nature of the natural and synthetical glycerylphosphoric acids, the present authors have

synthesised the unsymmetrical, or α -acid, $\begin{smallmatrix} \text{CH}_2\cdot\text{O}\cdot\text{PO}_3\text{H}_2 \\ | \\ \text{CH}\cdot\text{OH} \\ | \\ \text{CH}_2\cdot\text{OH} \end{smallmatrix}$, and the sym-

metrical, or β -acid, $\begin{smallmatrix} \text{CH}_2\cdot\text{OH} \\ | \\ \text{CH}\cdot\text{O}\cdot\text{PO}_3\text{H}_2 \\ | \\ \text{CH}_2\cdot\text{OH} \end{smallmatrix}$, and compared the barium and brucine

salts of these compounds, on the one hand with the corresponding salts of the glycerylphosphoric acid prepared from glycerol and phosphoric acid* and, on the other, with those of the racemised acid from egg-lecithin.†

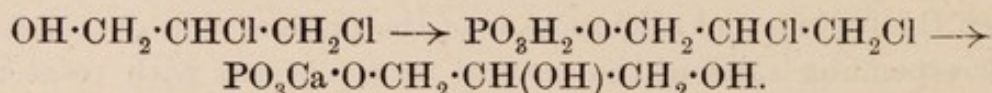
* Subsequently referred to as the "synthetical acid."

† Subsequently referred to as the "natural acid."

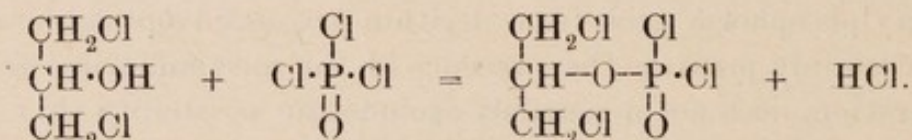
The synthetical glycerylphosphoric acid was prepared from glycerol and phosphoric acid under such conditions as are known to give only the mono-ester (compare Power and Tutin, *Trans.*, 1905, 87, 249).

The racemised natural acid was prepared in the form of its barium salt by the hydrolysis of lecithin with hot baryta solution. The latter substance was obtained from the yolk of egg by Bergell's method (*Ber.*, 1900, 33, 2584) and purified by the recrystallisation of its cadmium chloride compound (compare Willstätter and Lüdecke, *loc. cit.*).

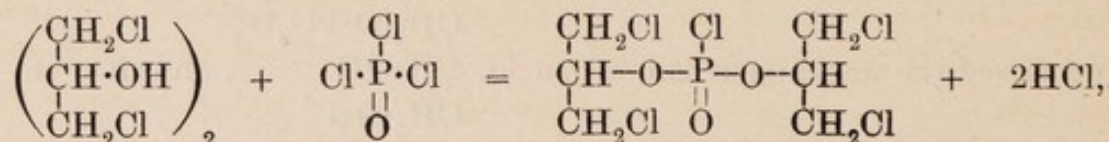
For the preparation of the pure α - and β -glycerylphosphoric acids, the two dichlorohydrins were employed. β -Dichlorohydrin was found to esterify to a small extent when heated with phosphoric acid, and when the resulting product was hydrolysed with milk of lime the calcium salt of α -glycerylphosphoric acid was obtained:



A similar method of procedure was found to be inapplicable in the case of α -dichlorohydrin, as this compound undergoes no esterification when heated with phosphoric acid. Since it had previously been observed that glycerylphosphoric acid could be obtained by the action of phosphoryl chloride on glycerol and subsequent hydrolysis of the product, it was anticipated that if α -dichlorohydrin were treated with this acid chloride a reaction would take place according to the equation:



This, however, is not the case, for when α -dichlorohydrin is treated with phosphoryl chloride at the ordinary temperature no action occurs, but at higher temperatures it was found that a reaction takes place according to the following equation:



and the resulting product, when hydrolysed with milk of lime, yielded calcium β -diglycerylphosphate, $([(\text{CH}_2\cdot\text{OH})_2\text{CH}\cdot\text{O}]_2\text{PO}_2)_2\text{Ca}$ (m. p. 249—250°). This compound crystallises from water with 13H₂O, and is soluble in all the usual organic solvents with the exception of light petroleum.*

* It may incidentally be noted that this fact affords conclusive proof that the 'di-ester' which is produced when glycerol and phosphoric acid are heated together at a somewhat high temperature (compare Power and Tutin, *loc. cit.*) consists of one or

When β -diglycerylphosphoric acid was boiled with water, it was found that hydrolysis slowly took place, giving the desired β -glycerylphosphoric acid, $(\text{CH}_2\cdot\text{OH})_2\cdot\text{CH}\cdot\text{O}\cdot\text{PO}_3\text{H}_2$.

The fact that the barium and brucine salts of these four preparations of glycerylphosphoric acid differ in composition and in many of their properties will be seen from the following table :

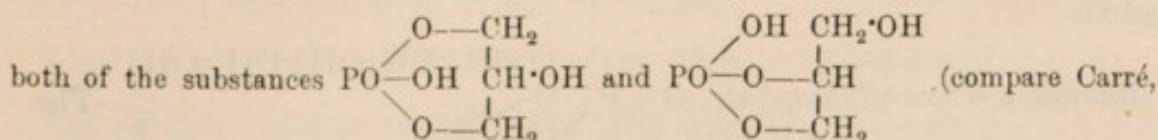
Barium Salts.

	α -Glycerylphosphate.	β -Glycerylphosphate.	Natural glycerylphosphate.	Synthetical glycerylphosphate.
Appearance	Well-defined leaflets	Quite amorphous	Leaflets, but not nearly so well defined as the α -salt	Granular powder
Percentage of H_2O in air-dried salt	1.0	5.1	2.4	2.5
Solubility in water at 17°	One part in 26.6	One part in 36.8	One part in 13.9	One part in 53.7

Brucine Salts.

	α -Glycerylphosphate.	β -Glycerylphosphate.	Natural glycerylphosphate.	Synthetical glycerylphosphate.
Appearance	Needles	Needles	Needles	Needles
Water of crystallisation	9 molecules	$11\frac{1}{2}$ molecules	$6\frac{1}{2}$ molecules	7 molecules
M. p. of dried salt ...	$157-158^\circ$	$157-158^\circ$	$158-159^\circ$	$158-159^\circ$
Optical rotation :				
In water	$[\alpha]_D - 25.3^\circ$	$[\alpha]_D - 23.9^\circ$	$[\alpha]_D - 23.9^\circ$	$[\alpha]_D - 24.6^\circ$
In alcohol	$[\alpha]_D - 32.5^\circ$	$[\alpha]_D - 28.1^\circ$	$[\alpha]_D - 27.9^\circ$	$[\alpha]_D - 28.2^\circ$

It will be seen from the above results that the natural and synthetical glycerylphosphoric acids are not identical. Moreover, neither of them consists of the pure α - or β -acid ; but, as has previously been mentioned, the natural acid must consist, in part at least, of the α - or unsymmetrical acid. Natural glycerylphosphoric acid is therefore a mixture of the α - and β -acids. As the synthetical acid is not identical with either of the pure isomerides, it may be concluded that this preparation is also a mixture of the α - and β -acids. It must, however, be admitted that the results obtained are somewhat anomalous, and do not permit of a ready explanation. For instance, if the barium salts of the natural and synthetical acids are both mixtures of the salts of



Compt. rend., 1903, **137**, 1070), and not the diglyceryl ester, $(\text{C}_3\text{H}_7\text{O}_3)_2\cdot\text{PO}_2\text{H}$ (compare Adrian and Trillat, *J. Pharm.*, 1898, **7**, 226), as the salts of the above-mentioned "di-ester" are insoluble in alcohol.

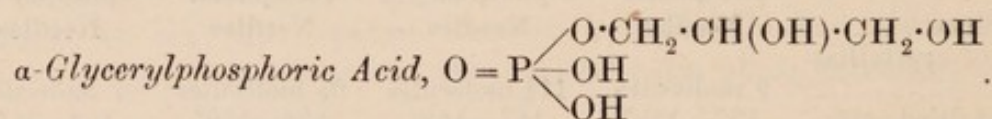
the α - and β -acids, it is difficult to see why one should be more readily, and the other more sparingly, soluble than the salts of either of the pure acids. Furthermore, with regard to the brucine salts, it would not be expected that the amount of water of crystallisation contained in those two which are mixtures would be less than that contained in either of the constituents of these mixtures when in their pure state.

The values obtained for the specific rotations of the brucine salts are recorded, for inasmuch as the structure of the acid radicle is different in the salts of the α - and β -acids, it was to be anticipated that they would exhibit a difference in rotatory power in alcoholic solution. The values obtained for the specific rotations in aqueous solution should, of course, only differ within the limits of experimental error.

Although both the natural and synthetical glycerylphosphoric acids appear to be mixtures, there was no indication that any separation could be effected by the crystallisation of their salts.

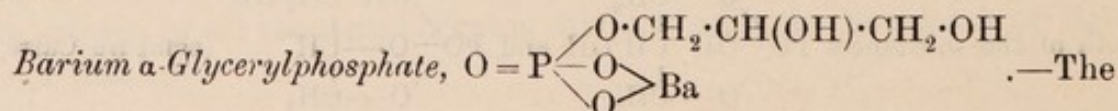
It may thus be concluded from the results of this investigation that the natural and the synthetical glycerylphosphoric acids are differently constituted mixtures of the α - and β -acids.

EXPERIMENTAL.



For this experiment, β -dichlorohydrin was prepared according to the method of Hübner and Müller (*Annalen*, 1871, 159, 179), by chlorinating dry allyl alcohol (b. p. 96–97°). The fractionated product boiled at 179–180°, and on analysis was found to be pure.

One molecular proportion of β -dichlorohydrin and one and a half molecular proportions of crystallised phosphoric acid were heated together in a paraffin bath for eight hours at 150–155°. The brown, oily product was then poured into an excess of milk of lime, and the mixture boiled for two hours. The liquid was cooled and filtered, the filtrate deprived of the excess of lime by means of carbon dioxide, and again filtered. On evaporation, *calcium α -glycerylphosphate* separated in colourless, glistening crystals; it was freed from calcium chloride by precipitating with alcohol, and collected on a filter. About 3 grams of crude calcium salt were obtained from 40 grams of β -dichlorohydrin.



crude calcium salt obtained as above was dissolved in water, the liquid rendered alkaline with ammonia, and the calcium precipitated and

removed as oxalate. An excess of barium hydroxide solution was added to the filtrate, and the liquid boiled until free from ammonia. After cooling, the excess of baryta was removed by means of carbon dioxide, and the filtered solution evaporated to a small bulk. The *barium α -glycerylphosphate* which separated was recrystallised from water, when it was obtained in glistening leaflets. This salt, like the barium salts of the other preparations of glycerylphosphoric acid, dissolves more sparingly in hot than in cold water.

0.4975 of air-dried salt, when heated at 125° , lost 0.0052 H_2O .
 $\text{H}_2\text{O} = 1.0$.

$\text{C}_3\text{H}_7\text{O}_6\text{P}\text{Ba}, \frac{1}{2}\text{H}_2\text{O}$ requires $\text{H}_2\text{O} = 2.8$ per cent.

0.4923, dried at 125° , gave 0.3722 BaSO_4 . $\text{Ba} = 44.5$.

$\text{C}_3\text{H}_7\text{O}_6\text{P}\text{Ba}$ requires $\text{Ba} = 44.6$ per cent.

The solubilities of this and succeeding barium salts were determined by parallel experiments, conducted at the same time, in the following manner. A quantity of the pure salt was placed in a small stoppered bottle and mechanically shaken for eleven hours with about 15 c.c. of water. The temperature of the liquid was 17° . A weighed quantity of the filtered solution was then evaporated to dryness on a water-bath, and the residue weighed. 6.6122 of the solution gave on evaporation 0.2484 of residue, therefore one part of the salt is contained in 26.6 parts of solution.

Brucine α -Glycerylphosphate, $\text{O} = \text{P} \begin{cases} \text{O} \cdot \text{CH}_2 \cdot \text{CH}(\text{OH}) \cdot \text{CH}_2 \cdot \text{OH} \\ \text{OH}, \text{C}_{23}\text{H}_{26}\text{O}_4\text{N}_2 \\ \text{OH}, \text{C}_{23}\text{H}_{26}\text{O}_4\text{N}_2 \end{cases}$ — The brucine

salt of α -glycerylphosphoric acid, as well as the brucine salts subsequently described, was prepared by precipitating exactly a solution of the respective barium salt with a solution of brucine sulphate, the barium sulphate being removed by filtration. *Brucine α -glycerylphosphate* readily crystallises from water in rosettes of needles, which, after drying at 100° , melt at $157-158^{\circ}$.

0.3074 of air-dried salt, when dried at 100° , lost 0.0443 H_2O .
 $\text{H}_2\text{O} = 14.4$.

In order to ascertain whether the amount of water of crystallisation was a constant quantity, the salt was recrystallised from a dilute solution, and again allowed to dry in the air.

0.3016, on drying at 100° , lost 0.0436 H_2O . $\text{H}_2\text{O} = 14.5$.

The air-dried and the dehydrated salts were then analysed :

0.2021 of air-dried salt gave 0.3860 CO_2 and 0.1270 H_2O .
 $\text{C} = 52.1$; $\text{H} = 7.0$.

0.2048 of salt, dried at 100° , gave 0.4591 CO_2 and 0.1143 H_2O .
 $\text{C} = 61.1$; $\text{H} = 6.2$.

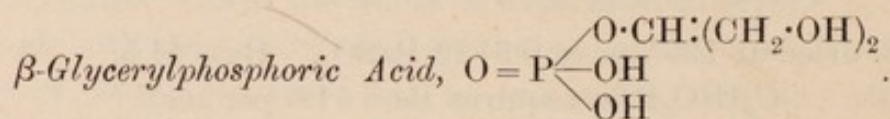
$\text{C}_{49}\text{H}_{61}\text{O}_{14}\text{N}_4\text{P}, 9\text{H}_2\text{O}$ requires $\text{C} = 52.4$; $\text{H} = 6.9$; $\text{H}_2\text{O} = 14.4$ per cent.

$\text{C}_{49}\text{H}_{61}\text{O}_{14}\text{N}_4\text{P}$ requires $\text{C} = 61.3$; $\text{H} = 6.3$ per cent.*

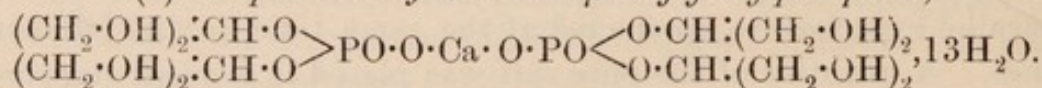
The specific rotation of the salt dried at 100° was determined in aqueous and in alcoholic solution.

0.2178, dissolved in 25 c.c. of absolute alcohol, gave $\alpha_D - 34'$ in a 2-dcm. tube, whence $[\alpha]_D - 32.5^{\circ}$.

0.1608, dissolved in 25 c.c. of water, gave $\alpha_D - 19.5'$ in a 2-dcm. tube, whence $[\alpha]_D - 25.3^{\circ}$.



(a) Preparation of Calcium β -Diglycerolphosphate,



The α -dichlorohydrin required for this preparation was obtained from Kahlbaum, and was submitted to a careful fractionation. Only the portion boiling from $173-176^{\circ}$ was employed, which was found to be pure. Equivalent molecular proportions of α -dichlorohydrin and phosphoryl chloride were boiled together in a round-bottomed flask attached to a reflux condenser, when a copious evolution of hydrogen chloride ensued. At the end of two and three-quarter hours the dark liquid was poured into a large excess of milk of lime, the mixture boiled for two hours in a flask attached to a reflux condenser, then filtered, and the excess of lime removed from the filtrate by means of carbon dioxide. On evaporating the filtrate, the crude calcium β -diglycerolphosphate, which separated as a scum on the surface of the liquid, was removed from time to time and dried on a porous tile. From 50 grams of the dichlorohydrin about 18 grams of crude calcium salt were obtained. When crystallised from water, calcium β -diglycerolphosphate was obtained in long, slender needles, which melted at $249-250^{\circ}$. It dissolves very readily in alcohol and in ethyl acetate, but less readily in water, chloroform, or benzene; it is insoluble in light petroleum. When the crystallised salt is heated, it suffers a decrease in weight, but it was found impracticable to estimate the water of crystallisation by this method, as the temperature necessary for complete dehydration also causes gradual decomposition.

0.3081 of air-dried salt gave 0.0230 CaO . $\text{Ca} = 5.3$.

The air-dried salt was then analysed in the usual manner.

* One atom of hydrogen becomes fixed as HPO_3 .

0.1804 gave 0.1243 CO_2 ; 0.1169 H_2O , and 0.466 of residue, $\text{Ca}(\text{PO}_3)_2$.

$\text{C} = 18.8$; $\text{H} = 7.2$; $\text{Ca}(\text{PO}_3)_2 = 25.8$.

$\text{C}_{12}\text{H}_{28}\text{O}_{16}\text{P}_2\text{Ca}, 13\text{H}_2\text{O}$ requires $\text{C} = 18.8$; $\text{H} = 7.1$; $\text{Ca} = 5.2$; $\text{Ca}(\text{PO}_3)_2 = 25.9$ per cent.

(b) *Hydrolysis of β -Diglycerolphosphoric Acid*.—Calcium β -diglycerolphosphate was dissolved in water, two-thirds of the amount of sulphuric acid theoretically required to combine with the calcium added, and the mixture boiled for four hours. Excess of calcium hydroxide was then introduced, the mixture cooled, filtered, and the filtrate freed from the excess of lime by means of carbon dioxide. The filtrate was then evaporated to a small bulk, and the calcium β -glycerolphosphate, together with the calcium sulphate, precipitated by the addition of alcohol, and collected on a filter. The alcoholic filtrate contained some unchanged calcium β -diglycerolphosphate, from which a further quantity of the β -glycerolphosphate was obtained.

Barium β -Glycerolphosphate, $\text{O} = \text{P} \begin{array}{l} \text{O} \cdot \text{CH} : (\text{CH}_2 \cdot \text{OH})_2 \\ \text{O} \\ \text{O} \end{array} \text{Ba}$.—The crude

mixture of calcium β -glycerolphosphate and calcium sulphate was converted into the barium salts, and the barium sulphate removed by filtration. On evaporating the filtrate, barium β -glycerolphosphate separated as a white, amorphous powder, and was removed by filtration. It was then redissolved in cold water, the solution concentrated on a water-bath, and the barium salt which separated was collected on a filter. This operation was repeated several times, but the salt showed no indication of possessing a crystalline structure. From 45 grams of crude calcium β -diglycerolphosphate about 3 grams of barium β -glycerolphosphate were obtained.

0.5108 of air-dried salt, on heating at 125° , lost 0.0259 H_2O .
 $\text{H}_2\text{O} = 5.1$.

$\text{C}_3\text{H}_7\text{O}_6\text{PBa}, \text{H}_2\text{O}$ requires $\text{H}_2\text{O} = 5.5$ per cent.

0.4849 dried at 125° , gave 0.3643 BaSO_4 . $\text{Ba} = 44.2$.

$\text{C}_3\text{H}_7\text{O}_6\text{PBa}$ requires $\text{Ba} = 44.6$ per cent.

9.9378 of an aqueous solution, saturated at 17° , gave on evaporation 0.2702 of residue, therefore one part of the salt is contained in 36.8 parts of solution.

Brucine β -Glycerolphosphate, $\text{O} = \text{P} \begin{array}{l} \text{O} \cdot \text{CH} : (\text{CH}_2 \cdot \text{OH})_2 \\ \text{OH}, \text{C}_{23}\text{H}_{26}\text{O}_4\text{N}_2 \\ \text{OH}, \text{C}_{23}\text{H}_{26}\text{O}_4\text{N}_2 \end{array}$, $11\frac{1}{2}\text{H}_2\text{O}$.—

This salt, prepared in the manner previously described, crystallises from water in stout needles, and, after drying at 100° , melted at 157 — 158° .

0.4283, on drying at 100° , lost 0.0759 H_2O . $\text{H}_2\text{O} = 17.7$.

0.1981 of air-dried salt gave 0.3652 CO_2 and 0.1324 H_2O . $\text{C} = 50.3$;
 $\text{H} = 7.4$.

0.1893 of air-dried salt gave 0.3484 CO_2 and 0.1256 H_2O . $\text{C} = 50.2$;
 $\text{H} = 7.4$.

0.1787, dried at 100° , gave 0.4007 CO_2 and 0.1052 H_2O . $\text{C} = 61.2$;
 $\text{H} = 6.5$.

$\text{C}_{49}\text{H}_{61}\text{O}_{14}\text{N}_4\text{P}, 11\frac{1}{2}\text{H}_2\text{O}$ requires $\text{C} = 50.4$; $\text{H} = 7.1$; $\text{H}_2\text{O} = 17.7$
 per cent.

$\text{C}_{49}\text{H}_{61}\text{O}_{14}\text{N}_4\text{P}$ requires $\text{C} = 61.3$; $\text{H} = 6.3$ per cent.

0.2071, dissolved in 25 c.c. of absolute alcohol, gave $\alpha_D - 28'$ in a
 2-dcm. tube, whence $[\alpha]_D - 28.1^\circ$.

0.1600, dissolved in 25 c.c. of water, gave $\alpha_D - 18.4'$ in a 2-dcm.
 tube, whence $[\alpha]_D - 23.9^\circ$.

Natural Glycerolphosphoric Acid.

The lecithin from which the racemised natural glycerolphosphoric acid was prepared was obtained in the form of its cadmium chloride compound according to Bergell's method (*loc. cit.*), and for this purpose the yolks of 144 eggs were employed. This cadmium chloride compound was purified by recrystallisation from a mixture of ethyl acetate and alcohol (compare Willstätter and Lüdecke, *loc. cit.*).

Barium Salt of Natural Glycerolphosphoric Acid.—The purified cadmium chloride compound of lecithin was boiled for one hour with a solution of barium hydroxide, the mixture then cooled, filtered, and the filtrate deprived of the excess of baryta by means of carbon dioxide. On concentrating the clear liquid, the barium salt of the natural glycerolphosphoric acid was deposited in the form of caseous flakes. This salt was optically inactive, having been racemised by the use of boiling liquids (Willstätter and Lüdecke, *loc. cit.*). It was recrystallised from water, when it was obtained in nodular aggregates of leaflets.

0.3950 of air-dried salt, on heating at 125° , lost 0.0093 H_2O .
 $\text{H}_2\text{O} = 2.4$.

$\text{C}_3\text{H}_7\text{O}_6\text{PBa}, \frac{1}{2}\text{H}_2\text{O}$ requires $\text{H}_2\text{O} = 2.8$ per cent.

0.3857, dried at 125° , gave 0.2912 BaSO_4 . $\text{Ba} = 44.4$.

$\text{C}_3\text{H}_7\text{O}_6\text{PBa}$ requires $\text{Ba} = 44.6$ per cent.

5.5110 of an aqueous solution, saturated at 17° , gave on evaporation 0.3968 of residue, therefore one part of the salt is contained in 13.9 parts of solution.

Brucine Salt of Natural Glycerolphosphoric Acid.—This salt was prepared in the same manner as the previously described brucine salts.

When crystallised from water, it was obtained in stout needles, which, after drying at 100° , melted at $158-159^{\circ}$.

0.4972, on drying at 100° , lost $0.0526 \text{ H}_2\text{O}$. $\text{H}_2\text{O} = 10.6$.

The estimation of the water of crystallisation was then repeated, using another sample of salt.

0.2980, on drying at 100° , lost $0.0314 \text{ H}_2\text{O}$. $\text{H}_2\text{O} = 10.5$.

The air-dried and the anhydrous salt were subsequently analysed, with the following results:

0.1946 of air-dried salt gave 0.3876 CO_2 and $0.1240 \text{ H}_2\text{O}$. $\text{C} = 54.3$; $\text{H} = 7.1$.

0.2332 of salt dried at 100° gave 0.5196 CO_2 and $0.1356 \text{ H}_2\text{O}$. $\text{C} = 60.8$; $\text{H} = 6.5$.

$\text{C}_{49}\text{H}_{61}\text{O}_{14}\text{N}_4\text{P}, 6\frac{1}{2}\text{H}_2\text{O}$ requires $\text{C} = 54.6$; $\text{H} = 6.8$; $\text{H}_2\text{O} = 10.9$ per cent.

$\text{C}_{49}\text{H}_{61}\text{O}_{14}\text{N}_4\text{P}$ requires $\text{C} = 61.3$; $\text{H} = 6.3$ per cent.

0.2464, dissolved in 25 c.c. of absolute alcohol, gave $\alpha_D - 33'$ in a 2-dcm. tube, whence $[\alpha]_D - 27.9^{\circ}$.

0.1593, dissolved in 25 c.c. of water, gave $\alpha_D - 18.3'$ in a 2-dcm. tube, whence $[\alpha]_D - 23.9^{\circ}$.

Synthetical Glycerolphosphoric Acid.

The barium salt of the synthetical glycerolphosphoric acid was prepared under such conditions as are known to yield only the mono-ester (compare Carré, *Compt. rend.*, 1903, 137, 1070; Power and Tutin, *Trans.*, 1905, 87, 249). It was recrystallised from water, when it was obtained as a granular powder.

0.3036 of air-dried salt, on heating at 125° , lost $0.0077 \text{ H}_2\text{O}$. $\text{H}_2\text{O} = 2.5$.

$\text{C}_3\text{H}_7\text{O}_6\text{P}\text{Ba}, \frac{1}{2}\text{H}_2\text{O}$ requires $\text{H}_2\text{O} = 2.8$ per cent.

5.9158 of an aqueous solution, saturated at 17° , gave on evaporation 0.1102 of residue, therefore one part of the salt is contained in 53.7 parts of solution.

Brucine Salt of Synthetical Glycerolphosphoric Acid.—This salt was prepared in the same manner as the previously described brucine salts, and possessed the same appearance and melting point as the brucine salt of the natural acid.

0.4987, on drying at 100° , lost $0.0570 \text{ H}_2\text{O}$. $\text{H}_2\text{O} = 11.4$.

0.4948, „ „ 100° , „ $0.0588 \text{ H}_2\text{O}$. $\text{H}_2\text{O} = 11.9$.

0.3750, „ „ 100° , „ $0.0448 \text{ H}_2\text{O}$. $\text{H}_2\text{O} = 11.9$.

0.2064 of air-dried salt gave 0.4070 CO_2 and 0.1324 H_2O . $\text{C} = 53.8$;
 $\text{H} = 7.0$.

0.1968, dried at 100° , gave 0.4392 CO_2 and 0.1156 H_2O . $\text{C} = 60.9$;
 $\text{H} = 6.5$.

$\text{C}_{49}\text{H}_{61}\text{O}_{14}\text{N}_4\text{P}, 7\text{H}_2\text{O}$ requires $\text{C} = 54.1$; $\text{H} = 6.8$; $\text{H}_2\text{O} = 11.6$ per cent.
 $\text{C}_{49}\text{H}_{61}\text{O}_{14}\text{N}_4\text{P}$ requires $\text{C} = 61.3$; $\text{H} = 6.3$ per cent.

0.2366, dissolved in 25 c.c. of absolute alcohol, gave $\alpha_D - 32'$ in a 2-dcm. tube, whence $[\alpha]_D - 28.2^\circ$.

0.1607, dissolved in 25 c.c. of water, gave $\alpha_D - 19'$ in a 2-dcm. tube, whence $[\alpha]_D - 24.6^\circ$.

The brucine salt of the synthetical glycerylphosphoric acid was also prepared by Carré (*loc. cit.*), who assigned to it a formula containing 9 molecules of water, whereas the above results indicate the presence of only 7 molecules. In addition to this, Carré stated the melting point of the salt to be 181° , whereas it has been observed by the present authors to be $158-159^\circ$. It was considered possible that these discrepancies were due to the fact that Carré prepared his brucine salt from the free acid, while the brucine salts described in the present communication were all prepared from the corresponding barium salts by double decomposition. In other words, as the synthetical glycerylphosphoric acid appears to be a mixture, and undergoes slow hydrolysis when heated with water, it was thought possible that the free acid used by Carré might have become altered in composition on account of its constituents differing in their relative velocities of hydrolysis. The acid was therefore liberated from a quantity of the synthetical barium salt, and boiled with water for two and a half hours. It was then converted into the brucine salt, and the latter crystallised, when it was found to be identical with the preceding preparation.

0.3032, when heated at 100° , lost 0.0354 H_2O . $\text{H}_2\text{O} = 11.7$.

$\text{C}_{49}\text{H}_{61}\text{O}_{14}\text{N}_4\text{P}, 7\text{H}_2\text{O}$ requires $\text{H}_2\text{O} = 11.6$ per cent.

The melting point of the dried salt was the same as previously observed. The authors are therefore unable to confirm the figures given by Carré for the melting point and water of crystallisation of the brucine salt of synthetical glycerylphosphoric acid.

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