for abundant investigations tending to clarify the subject and enabling fairly definite procedures to be adopted for the qualitative and quantitative examination of these products.

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THE CHEMISTRY AND PROPERTIES OF GLYCERO-PHOSPHATES (GLYCERINOPHOSPHATES)

By Gaston DuBois

In an article on "Calcium Glycerophosphate," which appeared in two numbers of the *Journal de Pharmacie et de Chimie* of May 1 and 16, 1913, the authors, E. François and E. Boismenu, start their critical review of the literature on glycerophosphates by pointing to the great number of publications on the subject.
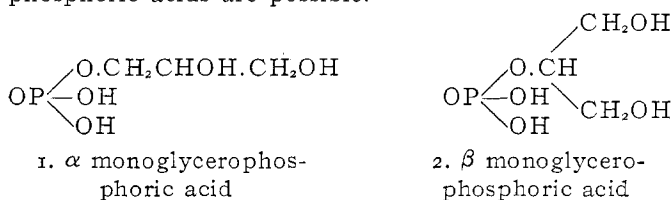
They believe that in spite of the comparatively voluminous literature few of those interested in this subject can draw exact conclusions from those publications as to the nature or composition of synthetic glycerophosphates and of the products found on the market.

In the literature including the leading authoritative publications on organic chemistry we find such contradictory statements as to make it impossible for any one unfamiliar with the subject to recognize which are correct.

The writer having had some experience in the manufacture of glycerophosphates, and having, therefore, spent some time studying the chemistry of these products, deemed it advisable to collect the most important known facts on the subject and to endeavor to draw conclusions by adding his experience to that gathered from the research work of others, and also to point out a few of the erroneous statements and inconsistencies found in some publications.
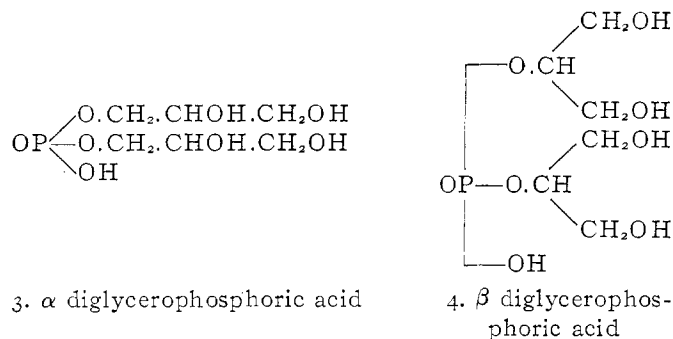
Before going into the details of the chemistry and properties of glycerophosphates, and the findings of the various chemists who have developed this field, let us briefly examine what compounds are theoretically possible as a result of the action of phosphoric acid or its salts, on glycerine.

By the interaction of 1 molecule phosphoric acid with one molecule glycerine, two isomeric monoglycerophosphoric acids are possible.

$$OP\begin{cases} O.CH_2.CHOH.CH_2OH \\ OH \\ OH \end{cases}$$

$$OP\begin{cases} O.CH \begin{cases} CH_2OH \\ CH_2OH \end{cases} \\ OH \\ OH \end{cases}$$

1. α monoglycerophosphoric acid
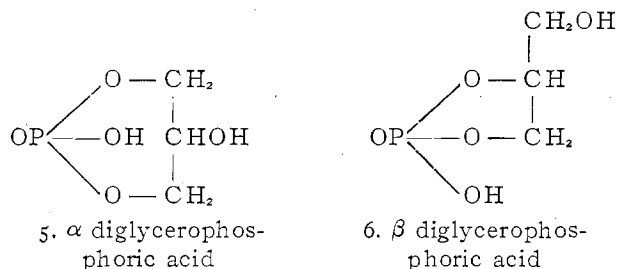
2. β monoglycerophosphoric acid

These acids can form mono- and dibasic salts. The dibasic calcium salts of above acids are the main components of calcium glycerophosphate of the market.

If one molecule phosphoric acid interacts with two molecules glycerine, again two isomeric diglycerophosphoric acids are possible:

$$OP\begin{cases} O.CH_2.CHOH.CH_2OH \\ O.CH_2.CHOH.CH_2OH \\ OH \end{cases}$$

$$\begin{cases} O.CH \begin{cases} CH_2OH \\ CH_2OH \end{cases} \\ OP-O.CH \begin{cases} CH_2OH \\ CH_2OH \end{cases} \\ OH \end{cases}$$

3. α diglycerophosphoric acid

4. β diglycerophosphoric acid

Some authors claim diglycerophosphoric acids to have the following constitution:[1]

$$OP\begin{cases} O-CH_2 \\ OH \quad CHOH \\ O-CH_2 \end{cases}$$

$$OP\begin{cases} O-CH \begin{cases} CH_2OH \\ O-CH_2 \\ OH \end{cases}$$

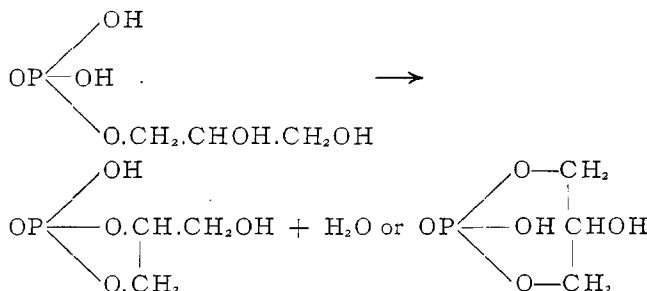5. α diglycerophosphoric acid

6. β diglycerophosphoric acid

The diglycerophosphoric acids, whatever their constitution may be, can form only monobasic salts. The salts of these acids are readily partly saponified by the action of alkali hydroxides yielding mainly monoglycerophosphates.

Diglycerophosphoric acids are formed when phosphoric acid is mixed with glycerine, preferably an excess of the latter, and the mixture heated to above 110° under reduced or atmospheric pressure. The formation of diglycerides at temperatures above 110° was demonstrated very conclusively by Adrian and Trillat,[2] by Power and Tutin,[3] and also by Carré.[4]

The question, which are the correct formulas for the α and β diglycerophosphoric acids, formulas 3 and 4 or 5 and 6 is not settled yet. Adrian and Trillat analyzed diglycerophosphoric acid and obtained figures for C and H corresponding about with the amount contained in formulas 3 and 4.

It is possible that both are correct as it is reasonable to expect that monoglycerophosphoric acid when heated could, by the elimination of water, form diglycerophosphoric acid:

$$OP\begin{cases} OH \\ OH \\ O.CH_2.CHOH.CH_2OH \end{cases} \longrightarrow$$

$$OP\begin{cases} OH \\ O.CH.CH_2OH \\ O.CH_2 \end{cases} + H_2O \text{ or } OP\begin{cases} O-CH_2 \\ OH \quad CHOH \\ O-CH_2 \end{cases}$$

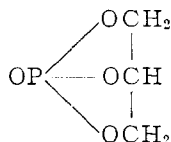We now come to the last class of esters formed

[1] Carré, *C. r. de l'Acad. des Sciences*, **137**, 1070–73, also **138**, 47–49.
[2] *J. Phar. et Ch.*, [6] **7**, 226–30.
[3] Power and Tutin, *J. Chem. Soc.*, **87**, 240–57.
[4] Carré, *C. r. de l'Acad. des Sciences*, **137**, 1070–73.

by the action of phosphoric acid on glycerine, the triglycerophosphoric ester. This compound has the following formula:

$$OP\begin{matrix} OCH_2 \\ | \\ OCH \\ | \\ OCH_2 \end{matrix}$$

It is, according to Carré, insoluble in water, alcohol and acetone, and forms a hard, spongy mass which can be powdered.

It is interesting to note from the following example the relative amounts of mono, di and tri esters formed under various conditions, which is shown in the following table taken from G. Prunier's investigations.

Equal amounts of 60 per cent phosphoric acid and glycerine 28° B. were heated together under atmospheric pressure.

Temp.	Hours heated	Monoether	Diether	Triether	Total
110°	72	39.3	37.4	0.6	77.3
110°	84	43.9	33.5	0.6	78.0
130°	12	36.8	29.0	0.6	66.4
130°	24	36.1	27.1	1.9	65.1

Equally interesting is the result of investigations by G. Prunier[1] on the rate and limit of esterification of phosphoric acid by glycerine under varying conditions of concentration, temperature and pressure.

The investigation shows that the best results are obtained if the water formed during the reaction is eliminated, which can best be attained, either by heating the reaction mixture under reduced pressure or by exposing a comparatively large evaporating surface. The limit of esterification under reduced pressure appears to be reached when 80 per cent of the acid is esterified and this point is arrived at more quickly at higher temperatures, 24 hours being required at 110°, 12 hours at 130° and 8 hours at 150°. Under atmospheric pressure the speed of esterification also increases with higher temperatures, but the limit of esterification decreases. For example with 85 per cent acid and glycerine 30° B. at 110° the limit of esterification is 75.8 per cent but drops to 60.9 per cent at 130° and to 49.4 per cent at 150°. Under atmospheric pressure the best results for the esterification are obtained at 110° and by using 60 per cent phosphoric acid and glycerine 30° B. whereby 78 per cent of the acid is esterified.

At higher temperatures and under reduced pressure the chief product is the diester, this being replaced, in part by the monoester when esterification is slow (namely, at higher pressures and lower temperatures) and by the triester when the operation is conducted at a still higher temperature.

Regarding the stability of glycerophosphoric acid or in other words its velocity of hydrolysis, F. Malengreau and G. Prigent[2] found that the decomposition of glycerophosphoric acid is a case of autocatalysis, caused by the action of the uncombined acid groups

[1] *Bull. de la Soc. Chim. de France,* **1907,** [4] 1, 1046–48.
[2] *Z. physiol. Chem.,* **1911,** 73, 68–84.

of the phosphoric acid, as when these are neutralized the decomposition at 100° is practically reduced to zero. With rising temperature the hydrolysis is notably accelerated. There is a certain acidity or concentration of H ions above and below which the stability of the compound increases. This explains why strong acids, such as HCl, H_2SO_4, acetic acid, etc., retard the decomposition owing to the increase in the concentration of the H ions in the solution. On the other hand weak acids such as oxalic and citric acids and their salts accelerate the hydrolysis of glycerophosphoric acid. The degree of acidity at which the hydrolysis is the greatest is represented by acid salts of glycerophosphoric acid.

The potassium salt decomposes more rapidly than the calcium and sodium salts. The salts of strong acids have mostly but a slightly accelerating effect but the salts of weak acids accelerate the hydrolysis reaction considerably.

Before leaving the subject of the possible compounds resulting from the esterification of phosphoric acid by glycerine, the investigations by A. Contardi, recently published should be briefly mentioned.[1]

By heating at 120–130° three molecules crystallized phosphoric acid under reduced pressure with one molecule glycerine, *glycerotriphosphoric* acid is obtained:

$$CH_2 — O — PO(OH)_2$$
$$CH — O — PO(OH)_2$$
$$CH_2 — O — PO(OH)_2$$
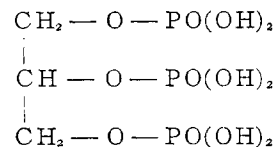
This triphosphoric ester heated with one molecule glycerine yields *diglycerotriphosphoric* ester:

$$\begin{matrix} CH_2.O & CH_2 — O — PO(OH) \\ CH.O & CH — O — PO(OH) \\ CH_2.O & CH_2 — O — PO(OH) \end{matrix}$$
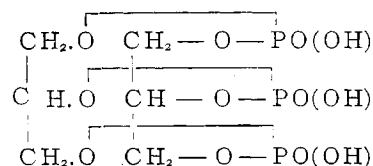
which compound yields when again heated with one more molecule glycerine the neutral triglycerotriphosphoric ester:

$$\begin{matrix} CH_2.O & CH_2 — O — PO — CH_2 — O \\ CH.O & CH — O — PO — CH — O \\ CH_2.O & CH_2 — O — PO — CH_2 — O \end{matrix}$$
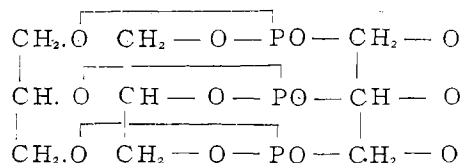
This compound is identical with Carré's triglycerophosphoric ester

$$CH_2 — O \atop CH — O {\Large >} PO \atop CH_2 — O$$

, the latter being just one-third of the formula proposed by Contardi.

The correctness of Contardi's formula for triglycerophosphoric ester has not yet been proved owing to

[1] *Gazz. Chim. Ital.,* **42,** II, 270–82.

the insolubility of this compound, which makes it impossible to determine the molecular weight.

P. Carré does not agree with Contardi's findings;[1] the question of the existence of above compounds is, therefore, not definitely settled.

In contrast to the methods used by all of the above mentioned authors for synthesizing glycerophosphoric esters, I find that the present manufacturing methods, judging from the patent literature, start not from phosphoric acid, but mostly from mono- and dibasic salts of ortho- or metaphosphoric acids. One patent describes the use of a mixture of metaphosphoric acid, disodium phosphate and glycerine; another patent covers the use of monosodium phosphate and glycerine, etc.

The purpose of deviating from the original method of Pelouze (phosphoric acid and glycerine) is partly in order to prevent the formation of triglyceroesters, by protecting one of the acid groups by a base, and partly for other practical reasons which do not enter into the subject of this paper.

We now come to the examination of the principal salts of glycerophosphoric acid found on the market, the calcium, sodium and potassium glycerophosphates.

CALCIUM GLYCEROPHOSPHATE.—An examination of various market products which I carried out in our laboratories showed how widely these products differ in composition.

Sample	H₂O of crysts. and moisture	Based on anhydrous product Ash	CaO	P₂O₅	Titration	Added org. acid fig. as citric acid
1	10.03	55.32	19.23	32.03	56.1%	–
2	9.43	50.93	22.70	28.15	80.7%	7%
3	8.79	54.66	24.45	29.58	85.3%	5%
4	10.39	56.35	22.2	33.68	71.0%	–
5	12.73	59.02	26.56	31.9	......	–
L. P. 6	1.25	60.32	26.96	33.77	99.29	–
Theory	7.89	60.47	26.68	33.80	100.0%	–

Sample No. 6 was a laboratory preparation almost free of water.

From the above figures we find the moisture in the products varying from 8.79–12.73 per cent, the ash from 50.93–59.02 per cent, CaO from 19.23–26.56 per cent, P₂O₅ from 28.15–33.68 per cent. All of the figures for ash, CaO, P₂O₅ being based on dry substance.

If we compare their solubilities we find still greater differences and it is, therefore, evident that these products must differ widely from one another.

G. Prunier examined samples which showed solubilities in water ranging from 1 : 11 up to 1 : 60 parts of water.

The causes for these divergences are mainly:

1. The presence of organic acids (citric acid, etc.) which increase the solubility.

2. The presence of diglycerophosphates which also increase the solubility.

3. Different mixtures of α and β glycerophosphates the β glycerophosphate being less soluble than the α isomeride.

The two first causes will not be discussed in this paper as organic acids or diglycerides are impurities added for the purpose of apparently increasing the

[1] *Compt. rend.,* **1912,** 155, 1520–21.

solubility. That the solubility is mostly only apparently increased is explained by the fact that although such products will at first readily dissolve in 25–40 parts of water, on longer standing, however, calcium citrate will gradually be formed and precipitate out of the solution and in the case of diglycerides being present, they will slowly hydrolyze forming monoglycerophosphates and also some free phosphoric acid. Furthermore, as is shown in this paper, the presence of organic acids (weak acids) or their salts will increase the hydrolysis of monoglycerophosphates, thereby causing CaHPO₄ to be formed.

The mistake has been repeatedly made by various authors in determining the solubility of pure calcium glycerophosphate, not to take into consideration the fact that the products examined may have contained one or the other of the two possible isomerides or even a mixture of both of these, and the result, naturally has been that very widely differing statements were made concerning the solubility of these products.

Tutin and Hann in an endeavor to finally ascertain the nature of the natural and synthetical[1] glycerophosphoric acids, have prepared these products and their barium salts and also the α and β monoglycerophosphoric acids separately and their barium salts under such conditions as they hoped would exclude the formation of other products.

As the determination of the properties of the α and β isomerides is of the utmost importance as a basis for a thorough knowledge of the composition of commercial products and of the requirements to be established for such products, the writer will briefly discuss a part of Tutin and Hann's work and results. This appears all the more necessary in view of the importance of their researches and because their findings regarding the solubility of α barium glycerophosphate as well as that of the synthetical product do not coincide with the results of more recent investigations.

Tutin and Hann prepared α barium glycerophosphate by chlorinating dry allyl alcohol and thereby producing β-dichlorhydrin, which was heated with one and a half molecules of crystallized phosphoric acid at a temperature of 150–155°. The product obtained was boiled with milk of lime and the α-calcium glycerophosphate thereby obtained, converted into the barium salt.

It should be noted that the yield in the esterification of β-dichlorhydrin with phosphoric acid is small and that furthermore, the heating of the mixture at 155° for several hours does not exclude the possibility of HCl being driven off, whereby phosphoric acid would combine with glycerine allowing the formation of β glycero- or diglycerophosphates, or even glycerodiphosphoric esters.

In connection with the above method of preparing α calcium glycerophosphate, it is interesting to compare the Eng. Pat. 2883, whereby monochlorhydrin or monoacetin is heated with syrupy phosphoric acid until *no more hydrochloric acid* or *acetic acid is evolved.*

[1] By synthetical glycerophosphoric acid is meant the product obtained by heating glycerine and phosphoric acid for 24 hours at 105–110° C.

We find here an attempt to produce the esterification by introducing phosphoric acid in the position occupied by the chlorine in monochlorhydrin whereas Tutin and Hann did just the opposite; they intended to introduce phosphoric acid in the only remaining hydroxyl group of β dichlorhydrin leaving the chlorine groups intact.

Tutin and Hann

$$CH_2.Cl \qquad\qquad CH_2.Cl$$
$$|\qquad\qquad\qquad\quad |$$
$$CH.Cl \ + \ H_3PO_4 \ = \ CH.Cl \qquad + H_2O$$
$$|\qquad\qquad\qquad\quad |$$
$$CH_2.OH \qquad\qquad CH_2.OP(OH)_2$$
$$\qquad\qquad\qquad\qquad\qquad ||$$
$$\qquad\qquad\qquad\qquad\qquad O$$

β dichlorhydrin

Eng. Pat. 2883

$$\qquad\qquad\qquad\qquad\quad O$$
$$\qquad\qquad\qquad\qquad\quad ||$$
$$CH_2.Cl \qquad\qquad CH_2.OP(OH)_2$$
$$|\qquad\qquad\qquad\qquad |$$
$$CH.OH + H_3PO_4 = CH.OH \qquad + HCl$$
$$|\qquad\qquad\qquad\qquad |$$
$$CH_2.OH \qquad\qquad CH_2.OH$$

α monochlorhydrin

In view of the above, I am of the opinion that the figure obtained by Tutin and Hann for α barium glycerophosphate, soluble $1 : 26.6/17°$, cannot be accepted as final. This figure does not correspond with the solubility determined quite recently by Langheld, Oppmann and Meyer,[1] which they found to be $1 : 11.9$.

The solubility $1 : 53.7$, found by Tutin and Hann for the synthetical barium glycerophosphate, obtained by heating glycerine and phosphoric acid for 24 hours at $105-110°$, is also so far off from the solubility of α barium glycerophosphate that their result would apparently require confirmation.

I compare in the preceding paragraph the synthetical barium glycerophosphate, obtained by Tutin and Hann, with the α barium glycerophosphate obtained by Langheld, Oppmann and Meyer. This comparison is justifiable as the two products should be almost identical. The main product of esterification of glycerine by phosphoric acid under the conditions as carried out by Tutin and Hann, is the α-isomeride. This corresponds to the fact that when glycerine is treated with an equivalent amount of hydrochloric acid, α chlorhydrin is the chief product obtained, only a small amount of β chlorhydrin being formed.

We find that in all cases in which mono esters of glycerine are formed at low temperatures, by the interaction of acids or their salts on glycerine, the α isomeride is the chief product of the reaction.

If the solubility of synthetical barium glycerophosphate of $1 : 53.7$ found by Tutin and Hann were correct, this barium compound would then be less soluble than the corresponding synthetical calcium glycerophosphate, the solubility of which was determined by Power and Tutin and also by Adrian and Trillat.

[1] *Ber. d. chem. Ges.*, **45**, 3757 (1912).

Synthetic

Ca glycerophosphate.......	1 : 22.4/16°	Power and Tutin[1]
Ca glycerophosphate.......	1 : 22.0/25°	Adrian and Trillat

I find, however, that the barium glycerophosphates are more soluble than their corresponding calcium salts. Let us for instance compare the solubilities of the barium and calcium salts prepared from crystallized sodium glycerophosphate, which latter product was prepared for the first time about seven years ago and has since been examined very carefully by various authors and recognized as pure β sodium monoglycerophosphate.

β Ca glycerophosphate.....	1 : 59.5/18°	Rogier and Fiore
β Ca glycerophosphate......	1 : 77 /15°	Paolini
β Ba glycerophosphate....	1 : 22.2/21°	Rogier and Fiore
β Ba glycerophosphate.....	1 : 36.8/17°	Tutin and Hann

If we admit that the synthetical calcium glycerophosphate prepared as above described, contains mostly the α isomeride, we can conclude that the solubility of α calcium glycerophosphate in water must be about $1 : 22/16°$, and that the solubility of the α barium glycerophosphate is greater than that of the calcium salt or as found by Langheld, Oppmann and Meyer, $1 : 11.9/22°$, which, as these authors remark, corresponds very closely to the figure obtained by Tutin and Hann for barium glycerophosphate produced from lecithin, the solubility of which they found to be $1 : 13.9$.

The foregoing leads forcibly to the conclusion that the synthetical glycerophosphates produced at low temperatures ($100-110°$), and the natural glycerophosphates obtained from lecithin are almost identical, and in all probability, a mixture of the α and β isomerides, in which the α isomeride predominates.

By eliminating the statements, which by their contradictory nature and inconsistency have tended so far to obscure the true knowledge of the chemistry and properties of glycerophosphates, and by condensing from the foregoing the actual facts, we come to the following description of the principal salts of glycerophosphoric acid:

CALCIUM MONOGLYCEROPHOSPHATE.—Both the α and β isomerides are known.

α CALCIUM GLYCEROPHOSPHATE is a white amorphous or crystalline powder of the following formula:

$$CH_2 — OP \overset{O}{\underset{O}{\diamondsuit}} Ca$$
$$|\qquad\quad ||$$
$$CHOH \quad O \qquad (+ H_2O)$$
$$|$$
$$CH_2OH$$

When precipitated from a cold, saturated, aqueous solution, by prolonged heating on the water bath, it separates as a fine crystalline powder, which after drying at 70° C. is practically anhydrous.

When precipitated out of a cold aqueous solution by the addition of alcohol, it forms an apparently amorphous precipitate which on drying below 70° C. retains one molecule of water; when, however, it is precipitated from the solution of its corresponding sodium salt, by the addition of $CaCl_2$, it forms a crys-

[1] *J. Chem. Soc. Transactions*, **89**, 249-57.

talline precipitate, containing one molecule of water of crystallization, which is not driven off when the product is dried below 70° C. Both products are hygroscopic.
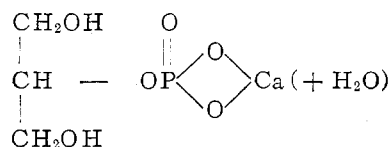
Anhydrous α calcium glycerophosphate is soluble in about 22 parts of water at 20° C., and precipitates out on heating, being soluble only in 108 parts of water at 100°.

It is insoluble in alcohol.

It is neutral or very slightly alkaline towards phenolphthalein; on addition of one equivalent of acid, the acid salt is obtained which is neutral towards methyl orange.

The dry salt contains 26.68 per cent CaO and 33.80 per cent P_2O_5 and yields on incineration 60.47 per cent ash.

β CALCIUM GLYCEROPHOSPHATE is much less soluble than the α isomeride; it dissolves in about 60 parts of water at 20° C. It is obtainable as a crystalline or amorphous precipitate under the same conditions as were enumerated for the α isomeride. It has the following formula:

$$CH_2OH$$
$$CH \quad - \quad OP \diagdown^{O}_{O} Ca (+ H_2O)$$
$$CH_2OH$$

COMMERCIAL CALCIUM GLYCEROPHOSPHATE is a mixture of the two above described isomerides; therefore, no specific solubility can be given for this mixture. It is evident that very different results will be obtained if the solubility of such a mixture is determined by shaking an excess of the product with water and determining the amount of substance dissolved in a weighed amount of water, or whether the solubility is determined by adding to a weighed amount of the product barely as much water as is required to produce a clear solution. The first method is naturally worthless in this case, whereas the second method gives us the actual solubility.

Commercial products should dissolve in 40 to 50 parts of water at 20° C. For the rapid estimation and comparison of market products I would recommend the following tests:

Moisture Determination.—Dry the product at 120–130° C. or better still at 100° C., but under reduced pressure, to constant weight.

Titration.—If the product is acid towards phenolphthalein, 2 grams of the dried substance in 100 cc. of water are titrated with $N/2$ NaOH (determination of organic acids).

If the product is neutral or very slightly alkaline towards phenolphthalein, 2 grams in 100 cc. of water are titrated with $N/2$ HCl using methyl orange as indicator; 1 cc. $N/2$ HCl equals 0.105 gram calcium glycerophosphate. Although the titration is not an exact determination, still it is a convenient method for the rough estimation of calcium monoglycerophosphate.

If diglycerides are present, the titration will yield low results indicating, in some cases, the presence of 10 per cent or more diglycerides.

Ash Determination is made by very slow and careful incineration of 0.5 gram dried substance, until a white ash is obtained.

A low ash percentage indicates the presence of organic acids and other organic impurities including diglycerides. A high ash percentage indicates the presence of inorganic salts, such as chlorides, sulfates, phosphates.

Further qualitative and, if necessary, quantitative determinations of such impurities as chlorides, sulfates and phosphates complete the preliminary examination of the product.

SODIUM MONOGLYCEROPHOSPHATE.—Up to a few years ago, sodium glycerophosphate was known only in form of a syrupy liquid, or as a very hygroscopic white powder. The crystalline sodium glycerophosphate was discovered by a chemist of the Soc. Anon. des Etabl. Poulenc Frères, and the formation of this product was evidently due to a change made at the time in their method of manufacture.

V. Paolini, who was the first to publish results of investigations of this crystalline product,[1] found that it was pure disodium monoglycerophosphate and concluded that it was the β isomeride. This explains why it could be obtained in a crystalline form, whereas the products obtained by the interaction of glycerine and phosphoric acid at temperatures below 110° C., after conversion into the sodium salt, had never been known to crystallize, which can be explained by the fact that in this case, principally the α isomeride is formed.

In the early part of 1912, I analyzed sodium glycerophosphate, which, for purposes of purification, I recrystallized several times, and found

	Per cent	
Water	31.2	
Sod. monoglycerophosphate	68.9	by titration
Sod. monoglycerophosphate	68.5	by gravimetric P_2O_5 determination

Conflicting statements are found throughout the literature concerning the water of crystallization in sodium glycerophosphate; some authors give it as 1, some as 3, and again others as 7 molecules.

I found that the crystalline product contained $5^1/_2$ molecules of water, for which the following theoretical figures would correspond:

H_2O	31.41%
Sod. glycerophosphate	68.59%

these correspond very closely to our analysis figures (given above).

My views in this matter were confirmed by a publication by V. Paolini,[2] which appeared a few months later, in which the author having examined samples of four different sources, found them also to contain $5^1/_2$ molecules of water of crystallization.

α SODIUM MONOGLYCEROPHOSPHATE is obtainable as a colorless or slightly yellow syrupy liquid which,

[1] V. Paolini, *Atti R. Accad. dei Lincei, Roma*, **1911**, 20, I, 807–12 and also from same publication, **1912**, 21, II, 330–52.

[2] *Atti R. Accad. dei Lincei, Roma*, **1912**, 21, II, 350–52.

when dried completely under reduced pressure, forms a white porous mass which can be powdered but is very hygroscopic.

It has the following formula:

$$CH_2\ O{-}P\ \begin{matrix} O \\ \diagup ONa \\ \diagdown ONa \end{matrix}$$
$$CHOH$$
$$CH_2OH$$
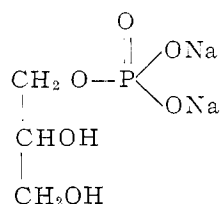
It mixes with water in all proportions but is practically insoluble in alcohol.

It is slightly alkaline to phenolphthalein and on addition of one equivalent amount of acid to its aqueous solution, the acid salt is obtained which is neutral to methyl orange.

Its solution can be heated up to 120° without decomposing.

β SODIUM MONOGLYCEROPHOSPHATE is obtainable in form of colorless or white monoclinic crystals, containing $5^1/_2$ molecules of water of crystallization.

It has the following formula:

$$CH_2OH$$
$$CH{-}O{-}P \begin{matrix} \diagup ONa \\ \diagdown ONa \end{matrix} + 5^1/_2\ aq.$$
$$CH_2OH\quad O$$
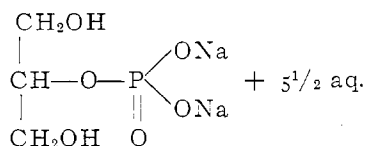
It is easily soluble in water; a saturated water solution at 20° C. contains about 40 per cent of the salt.

In the form of large crystals, this salt is, at 15°, stable when exposed to the air, whereas when finely powdered and exposed to the air it effloresces. This explains why the fine sodium glycerophosphate crystals found on the market contain usually more anhydrous sodium glycerophosphate than corresponds to a salt containing $5^1/_2$ molecules of water.

COMMERCIAL SODIUM GLYCEROPHOSPHATE.—The products that have been sold under the name of "sodium glycerophosphate 50 per cent" or "75 per cent" and also "about 100 per cent" which I have had the opportunity to examine, showed a surprising lack of uniformity in strength as well as purity.

Most of these products were in form of a syrupy liquid. It is of course impossible to produce a 75 per cent or even 50 per cent solution of β sodium glycerophosphate, as we have just seen that a saturated solution contains only about 40 per cent β sodium glycerophosphate crystals.

The liquid market products are composed of mixtures in which therefore mostly the α sodium glycerophosphate predominates.

Some manufacturers in determining the strength of their products have been guided merely by the specific gravity, others evidently have determined the strength on the basis of a variable amount of water of crystallization which, of course, accounts partly for the lack of uniformity in strength.

A 75 per cent solution, containing 75 per cent sodium glycerophosphate $+ 5^1/_2$ aq. is a syrupy liquid, which

mixes readily with water and is, therefore, quite convenient for use.

A preparation containing 75 per cent *anhydrous* sodium glycerophosphate, however, although it mixes in all proportions with water, has the great inconvenience of dissolving very slowly in cold water, and requiring many hours' stirring before complete solution is obtained. Even when heated the product does not dissolve readily.

I am, therefore, of the opinion that, for the sake of uniformity and convenience in handling, it should be required that the strength of the product be figured on the basis of its contents in sodium glycerophosphate $+ 5^1/_2$ aq. and this basis being admitted, a 75 per cent solution would unquestionably be the most desirable form for the consumer, if he must have a liquid preparation.

The last but not least interesting form in which sodium glycerophosphate is found on the market is the crystal form, which in itself is the best guarantee of its purity and, therefore, is preferable to all other forms wherever purity is of paramount importance.

The strength of the various sodium glycerophosphates can readily be determined by titration. This method yields for pure products checking results with the gravimetric method (P_2O_5 determination). It is carried out as follows:

Titration.—2–4 grams of the product accurately weighed are dissolved in 100 cc. of water and titrated with $N/2$ HCl, using methyl orange as indicator.

One cc. $N/2$ HCl equals 0.1576 gram monosodium glycerophosphate $+ 5^1/_2$ aq.

The product should also be tested for sulfates, chlorides and carbonates, which are almost invariably present in the liquid preparations but should not be present in the crystals.

The products should not contain any phosphates.

POTASSIUM GLYCEROPHOSPHATE.—Both α and β isomerides are obtainable as a colorless or slightly yellow syrupy liquid. When dried completely under reduced pressure a porous and very hygroscopic mass is obtained.

Potassium glycerophosphate mixes readily in all proportions with water. The product has never been obtained in crystal form in either of its modifications (α and β).

COMMERCIAL POTASSIUM GLYCEROPHOSPHATE.—This product is known mostly in form of 50 per cent and 75 per cent solutions.

Some manufacturers figure the contents on the basis of potassium glycerophosphate plus a certain amount of water of crystallization. I can see no reason for doing this as the product has never been obtained in crystal form. The only logical way of figuring the contents is on the basis of anhydrous potassium glycerophosphate.

These market products are best tested exactly as described for the sodium salts, whereby in the titration, 1 cc. $N/2$ HCl equals 0.124 gram potassium glycerophosphate.

Concluding, I wish to emphasize that while I believe the above data embodies the most important

investigations on the chemistry of glycerophosphates, and resulting conclusions, yet I fully realize that much remains to be done in order to define, without a doubt, the characteristics of the various isomeric glycerophosphoric esters, which naturally can be achieved only after the various compounds shall have been synthesized in such a way as to exclude the possibility of other compounds being formed.

Until such time the figures given for the solubilities, for instance, must be regarded only as tentative.

LABORATORIES OF THE MONSANTO CHEMICAL WORKS
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TOXICITY OF VARIOUS WOOD PRESERVATIVES[1]

By C. J. HUMPHREY AND RUTH M. FLEMING

During the past three years the writers have been conducting toxicity tests at the Forest Products Laboratory, Madison, Wisconsin, on various wood preservatives. These tests have been limited to substances in actual use in the industry or to those compounds submitted by coöperators as having a possible preservative value.

The toxicity of a given substance is not necessarily an ultimate criterion of its service value, but when correlated with other suitable properties, such as cost, ease of injection, permanence, non-corrosive action on metals and wood, fire resistance, and ease and safety in handling it assumes high importance in influencing our judgment as to what may normally be expected from the substance.

The writers have confined their attention to toxicity only, and with the view of bringing the essential data to the attention of the industry the more important results of our investigations to date are here presented.

While we are attacking the problem from two angles, namely, by the quick method of mixing the preservative with agar culture media and inoculating with fungi, and by the slower and probably more conclusive method of testing small injected wood blocks, the present paper will consider only the former, as the latter work is not yet far enough along to report upon.

Our method is essentially this: Agar culture media of the following formula is prepared:

Extract of 1 lb. lean beef in 1000 cc. distilled water.
25 grams Löfflund's malt extract.
20 grams agar-agar.
(Carefully filtered but reaction not adjusted; slightly acid.)

Seventeen, cc. of this medium are measured out into 50 cc. glass-stoppered bottles, using a specially graduated 17 cc. pipette, the bottles then being placed in clamp-frames and sterilized with steam at 100° C. for 25, 20 and 20 minutes, respectively, on three successive days.

The handling of the preservatives involved slight modifications for individual cases, but in all instances the concentrations reported are based on the actual weight of preservative in 20 cc. agar-preservative mixture. The character of the substance determined the method of introducing it into the agar medium.

With inorganic salts soluble in water, from 3 to 10 per cent solutions (grams per 100 cc. solution) were prepared and *measured* out into 50 cc. glass-

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stoppered bottles from either a 10 or 25 cc. standardized burette graduated in $^{1}/_{20}$ or $^{1}/_{10}$ cc., respectively. To each bottle sufficient distilled water was added to make 3 cc.

All other preservatives were *weighed* out into similar bottles on an analytical balance, and distilled water likewise added to make 3 cc. In the case of certain thick viscous oils, namely, wood tar, wood creosote, coal tar creosote and its fifth fraction, which do not readily emulsify with water, 5 to $33^{1}/_{3}$ per cent stock emulsions were prepared, using equal amounts of gum arabic and preservative and diluting with water to the desired concentration; these emulsions were then used in place of the crude preservative.

In a few instances where the preservatives were very low in toxic properties, more than the specified 3 cc. were necessary in order to secure the higher concentrations, and in these cases it became necessary to take into consideration the excess of preservative and to reduce the amount of agar by just this amount in order that the combined volume might not exceed 20 cc.

After the preservative had been introduced into the bottles as indicated, the stoppers were sealed in with a rubber-glycerine burette cock grease and the bottles were then sterilized along with the agar containers.

After sterilization both agar and preservative were heated on the water bath and the former was poured into the preservative bottle in a sterile culture box, thoroughly shaken, and then poured into sterile petri dishes 100 mm. in diameter and 10 mm. deep. After cooling, the surface of the medium was inoculated at the center with a weft of fungus mycelium 5 to 6 mm. square cut from a petri dish culture 2 to 3 weeks old. The test dishes thus prepared were then placed in an incubator and held at approximately 25° C. for from 4 to 10 weeks, usually 4 to 6. For each set of concentrations a check culture, using 17 cc. agar media plus 3 cc. of distilled water, was prepared.

The method as above outlined is not, by any means, intended to furnish the last word on the toxicity of a preservative, but it has the advantage of being quickly and easily applied and gives us at least valuable indications. The principal objection is the possibility in some instances that the preservative may combine with certain constituents of the culture media, thus rendering part of it inert. This is known to be the case with such compounds as zinc chloride, copper sulfate and mercuric chloride. With the oils this objection probably does not enter so seriously, but even here it would be difficult to say what might occur in such heterogeneous compounds as the various tar distillates and similar substances of highly complex constitution.

Another variable which only a large number of tests can correlate is the difference in susceptibility among different fungi. Since molds are, as a rule, much more resistant to toxic agents than true wood-destroyers, the writers have avoided their use, as well as that of bacteria and yeasts. Even among the true wood-destroying fungi considerable variation exists,