Preparation of Phosphorus Esters of Long-Chain Hydroxy Fatty Acids[1]

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Abstract

Several procedures are evaluated for synthesis of phosphorus-containing derivatives of some long-chain hydroxy-fatty acids, including ricinoleic and lesquerolic acid and their hydrogenated products. The conventional method of preparing phosphate esters by reaction of an alcohol with a dialkyl phosphorochloridate is unsatisfactory, but introduction of the dialkyl phosphoryl group was accomplished by using dimethylformamide as catalyst. Phosphorus derivatives are obtained more conveniently by ester interchange between a long-chain hydroxy compound and a low molecular weight dialkyl phosphite. Purification of the phosphite esters was accomplished by partitioning between acetonitrile and petroleum ether and also by chromatography on a column of silicic acid. Attempts to prepare phosphorus derivatives of the allylic hydroxyl of methyl dimorphecolate resulted in extensive dehydration to the conjugated triene.

Introduction

An investigation of phosphorus-containing derivatives of long-chain hydroxy-fatty acids was undertaken as part of a program to find potential uses for the hydroxy unsaturated oils present in castor, *Dimorphotheca,* and *Lesquerella* seed. Phosphorus derivatives of several non-hydroxylic fatty acids have already been prepared and evaluated as plasticizers (1,2). However, ricinoleic acid, 12-hydroxy-*cis*-9-octadecenoic acid, the chief component of castor oil, is the only long-chain hydroxy fatty acid for which phosphorylated derivatives have been reported (3). Recently the presence of two new naturally occurring hydroxy fatty acids, dimorphecolic (9-hydroxy-*trans, trans*-10,12-octadecadienoic) and lesquerolic (14-hydroxy-*cis*-11-eicosenoic) have been reported (4,5) to occur in major proportions in the seed oils of *Dimorphotheca* and *Lesquerella,* respectively. It seemed worthwhile to undertake the preparation of phosphorus esters of these unsaturated hydroxy fatty acids and their hydrogenated derivatives since the products might be expected to find use as agricultural chemicals, plasticizers, surface coatings, flame retardants, and hydraulic fluids. Procedures are presented for the reaction between the hydroxyl group of the methyl esters of several long-chain fatty acids and the following phosphorus-containing compounds: phosphorus trichloride, phosphorus oxychloride, dialkylphosphorochloridates, and dimethyl phosphite.

Experimental

Spectrophotometric Techniques. Infrared spectra were obtained with a Perkin-Elmer Infracord, Model 137, on ca. 0.5% solutions with CCl_4 or CS_2 in 1-mm cells. Ultraviolet spectra were measured in methanol solution at 8λ per 10 ml using a Beckman spectrophotometer, Model DK-2. The absorptivities at 2.76μ (OH) were determined with the Beckman spectrophotometer. The settings used were scale A × 1; sensitivity 50; period 5 min; time constant 0.6. Measurements were made on solutions in CCl_4 at concentrations of about 5 g per liter, using 1-cm near-IR fused quartz cells.

Starting Materials

Methyl Ricinoleate. This ester was prepared by alcoholysis of castor oil followed by fractional distillation of the mixed methyl esters under reduced pressure (6). The ester had the following characteristics: $[\alpha]^{25}_{D} = 5.05$; $n^{25}_{D} = 1.4600$; a = 0.079 at 2.76μ.

Methyl Dimorphecolate. Dimorphotheca oil obtained from seed of *D. sinuata*[3] was converted to the mixed methyl esters containing about 65% methyl dimorphecolate by methanolysis using either sodium methoxide or anhydrous anion exchange resin catalyst (8). Methyl dimorphecolate could not be successfully distilled even at pressures below 100μ owing to excessive dehydration to the conjugated triene (4) but was concentrated effectively by partitioning the mixed *Dimorphotheca* methyl esters between acetonitrile and petroleum ether (8,9). Only those fractions containing at least 92% methyl dimorphecolate, as judged by characteristic absorption due to conjugated diene (a >99 at 231 mμ) and to hydroxyl (a >0.081 at 2.76μ), were used for syntheses.

Methyl Lesquerolate. Lesquerella oil obtained from seed of *Lesquerella lasiocarpa* was alcoholyzed with methanol using anhydrous anion exchange resin catalyst (8). The mixed methyl esters contained about 65% methyl lesquerolate, but partitioning between acetonitrile and petroleum ether was not as effective as with methyl dimorphecolate since only about half the methyl lesquerolate was recovered in a purity of 90% or more, compared with recovery of about 90% of methyl dimorphecolate of better than 90% purity. However, fractional vacuum distillation through a 30-in. Vigreux column could be used for concentrating the methyl lesquerolate, provided care was taken to keep the pressure low and the pot temperature below 230C. Above this temperature excessive dehydration occurs and diene is formed. The methyl lesquerolate, estimated at least 90% pure, distilled at 190–192C at 0.2 mm; $n^{25}_{D} = 1.4620$; a >0.076 at 2.76μ.

Methyl 9-hydroxystearate. This ester was prepared by hydrogenation of methyl dimorphecolate in acetic acid with 5% catalyst containing 10% rhodium on alumina (8). Crystallization from petroleum ether readily afforded the pure ester mp 52.5–53C (Unc.); $[\alpha]^{25}_{D} = -0.22$ (c = 17.55 in methanol, l = 1).

Methyl 14-hydroxyeicosanoate. Hydrogenation of methyl lesquerolate was readily accomplished in acetic acid using platinum oxide catalyst (8) and the product was purified by crystallization from commercial hexane. The purified ester had the following characteristics, mp 59–61C; a >0.109 at 2.76μ.

Methyl 12-Hydroxystearate. This ester, mp 53–

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[1] Presented at the AOCS meeting in Toronto, Canada, 1962.

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[3] The *Dimorphotheca aurantiaca* reported in the literature is the same species as *D. sinuata* which, according to Norlindh (7), is the proper designation.

55.5C and a >0.105 at 2.76μ, was prepared by hydrogenation of purified methyl ricinoleate using platinum oxide catalyst.

Phosphorus Compounds. Phosphorus trichloride, phosphorus oxychloride, dibutyl phosphorochloridate, diethylphosphorochloridate, and dimethyl phosphite were all redistilled before use.

Organic Nitrogen Compounds. N,N-Diethylaniline, triethylamine, 2,6-lutidine, and N-ethylmorpholine were dried over barium oxide and redistilled before use; acetonitrile and N,N-dimethylformamide were redistilled.

Reactions with PCl_3 (10)

Methyl Ricinoleate. A solution of 0.34 g (2.5 mM) phosphorus trichloride in 2 ml of petroleum ether was added dropwise to a stirred solution of 2.31 g (7.5 mM) of methyl ricinoleate and 1.12 g (7.5 mM) of N,N-diethylaniline in 10 ml petroleum ether cooled to 3C with an ice-water bath. After addition of the PCl_3 was completed, the bath was removed and the temperature rose spontaneously to the 42C refluxing temperature which was sustained for 0.75 hr by heating. The solid amine hydrochloride was filtered off. The filtrate was washed successively with dilute HCl, K_2CO_3, and water and dried with Na_2SO_4. Evaporation of solvent left 2.24 g of a liquid residue. A portion (500 mg) was fractionated by partition chromatography with methanol-wetted silicic acid (11), using benzene and 2 to 10% methanol as the eluting solvent. A fraction eluted by 4% and 6% methanol in benzene, comprising half the portion that was chromatographed, showed no characteristic absorption for hydroxyl (2.75μ) but characteristic maxima at 4.13μ (P—H), 8.02μ (P→O), and 10.35μ (P—O—C) (12,13,14,15). Analysis—found: C, 69.2; H, 10.9; P, 3.58. Calcd. for $(C_{19}H_{35}O_3)_3$ P: C, 70.9; H, 10.9; P, 3.22; for $(C_{19}H_{35}O_3)_2$ PH(O); C, 68.1; H, 10.6; P, 4.63. The product apparently is a mixture comprising approximately 3 parts phosphite triester and 1 part phosphite diester.

Methyl Dimorphecolate. A solution of phosphorus trichloride in petroleum ether was added to a solution of methyl dimorphecolate and N,N-diethylaniline in petroleum ether and the mixture was worked up and fractionated as was methyl ricinoleate. However, the product obtained showed intense characteristic ultraviolet absorption at 260–280 mμ, indicating conjugated triene. Apparently dehydration was the predominating reaction.

Reactions with $POCl_3$ (10)

Methyl Ricinoleate. A solution of 0.31 g (2.0 mM) of $POCl_3$ in 2 ml commercial hexane was added to a solution of 3.74 g (1.2 mM) of methyl ricinoleate and 1.29 g (1.2 mM) of 2,6-lutidine in 20 ml commercial hexane and the mixture was refluxed at 71C for 2 hr. The amine hydrochloride was filtered off. The filtrate, after washing, drying, and evaporation of solvent, left 3.16 g of a liquid residue. Fractionation of a 500-mg sample by chromatography with a methanol-wetted silicic acid column using methanol-benzene as the eluting solvent afforded a major portion, which was mainly unreacted methyl ricinoleate. A minor fraction, which showed a strong characteristic infrared absorption at 8.10μ (P→O) and at 9.78μ (P—O—C), appeared to be chiefly a triester of phosphorus. Analysis—Calcd. for $(C_{19}H_{35}O_3)_3$ PO: C, 69.8; H, 10.71; P, 3.16. Found: C, 67.8; H, 10.6; P, 3.06.

Methyl Dimorphecolate. When methyl dimorphecolate was treated with $POCl_3$ under similar conditions the characteristic absorption due to hydroxyl disappeared after 2½ hr but the isolated reaction product showed intense absorption at 260–280 mμ characteristic of conjugated triene. Again dehydration rather than esterification was the predominant reaction. When the same reactants were refluxed in petroleum ether at 42C for 2½ hr, no esterification occurred as indicated by undiminished hydroxyl absorption at 2.75μ.

Reactions with Dialkyl Phosphorochloridates (10)

No esterification occurred, as judged from the sustained intensity of the absorption at 2.75μ, when methyl ricinoleate was treated under a variety of conditions with dibutyl phosphorochloridate using 2,6-lutidine as an HCl-acceptor. Conditions tried included reaction at room temperature without solvent (4 days), refluxing isooctane solution (100C), and refluxing toluene (113C). Similarly no esterification was observed when diethyl phosphorochloridate was used in refluxing tetrahydrofuran at 65C, with triethylamine as an HCl-acceptor, or with metallic copper as catalyst and potassium carbonate as HCl-acceptor in either refluxing benzene or methyl ethyl ketone.

When methyl ricinoleate was treated with diethyl phosphorochloridate using triethylamine as HCl-acceptor, dimethylformamide as catalyst and acetonitrile as solvent according to the recently reported procedure of Cramer and Winter (16), reaction occurred as indicated by distinctly decreased absorbance due to hydroxyl at 2.76μ. Diethyl phosphorochloridate, 2.14 g (12.4 mM), was added dropwise to a solution of 15.6 g (50.0 mM) of methyl ricinoleate, 1.26 g (12.5 mM) of triethylamine and 0.91 g (12.5 mM) of dimethylformamide in 7.0 ml of acetonitrile at −2C. After the mixture had been stirred for 17 hr at room temperature a copious precipitate of amine hydrochloride had formed, and the decreased intensity of absorption at 2.76μ indicated ca. 60% of the theoretical amount of phosphorylation had occurred. The solid amine hydrochloride was filtered off. The filtrate was dissolved in benzene, washed with water, and dried with $MgSO_4$. The solvent was removed by distillation under reduced pressure with a rotary evaporator. A 10-g portion of the resultant liquid residue was fractionated by crystallization from petroleum ether at −68C. After filtration, unreacted methyl ricinoleate was found in the solid phase and evaporation of the filtrate yielded a liquid residue of a crude triester which showed strong characteristic absorption at 9.60μ (P—O—C) and 7.85μ, (P→O) and weak absorption at 2.75μ (OH). Analysis—Calcd. for $(C_2H_5O)_2$ P(O) $(C_{19}H_{35}O_3)$: P, 6.92. Found: P, 4.97. Chromatography on a methanol-wetted silicic acid column and elution with methanolic benzene afforded a product which was free of hydroxyl (absence of a max at 2.75μ) and still showed characteristic absorption at 7.85 and 9.60μ, but the product was unstable and decomposed spontaneously on standing.

This reaction was also carried out with methyl dimorphecolate. A solution of 6.20 g (20.0 mM) of methyl dimorphecolate, 4.04 g (40.0 mM) of triethylamine, and 2.92 g (40.0 mM) of dimethylformamide in 30 ml of acetonitrile was cooled to 2C in an ice bath, and 6.88 g (40.0 mM) of diethyl phosphorochloridate was added dropwise while the cooled solution was stirred. After the reaction mixture at

1–8C had been stirred for 30 hr, the intensity of absorption at 2.75μ (OH) had decreased by more than 50% while absorption at 231 mμ (conjugated diene) had decreased by only 24%. Absorption at ca. 268 mμ indicated the presence of only a small amount of conjugated triene but higher temperature and longer reaction time increased triene formation. On filtering the reaction mixture, 2.70 g (20.0 mM) of solid triethylamine hydrochloride was obtained. Analysis—Calcd. for $C_6H_{16}NCl$: N, 10.2; Cl, 25.8. Found: N, 10.3; Cl, 25.6.

The filtrate was diluted with 35 ml of ethyl ether, washed with several portions of cold water and dried with $MgSO_4$. After removing the solvent at reduced pressure 5.5 g of an orange liquid residue was obtained. Attempted fractionation of a portion of this crude product by chromatography on silicic acid was unsuccessful since no discrete zones were obtained on eluting successively with petroleum ether, benzene, and methanolic benzene. Attempted partitioning between 10% aqueous methanol and petroleum ether resulted in hydrolysis. Diethyl phosphate was recovered from the methanol layer. The residue obtained by evaporation of solvent from the petroleum ether layer contained no phosphorus but showed characteristic absorption at 231 mμ (diene), 268 mμ (triene), and 2.77μ (OH), indicating the presence of a mixture of methyl dimorphecolate and methyl 8, 10,12-octadecatrienoate. Apparently dehydration and hydrolysis occurred in the aqueous medium. Systematic partitioning (17) of the product between petroleum ether and acetonitrile was more successful since the acetonitrile layer yielded a fraction containing 6.27% P. Theory for diethyl phosphorylated dimorphecolate $C_{23}H_{43}O_6P$; $(C_2H_5O)_2$ $(C_{19}H_{33}O_3)$ P(O); 6.95% P. However, the product was unstable and decomposed spontaneously on storage even at 0C.

Transesterification with Dimethyl Phosphite (18,19,20)

The transesterification reaction was carried out bebetween dimethyl phosphite and the methyl ester of each of the following hydroxy acids: ricinoleic, dimorphecolic, lesquerolic, 9-hydroxystearic, 12-hydroxystearic, and 14-hydroxyeicosanoic. The crude products obtained from the unsaturated fatty acid esters were liquid, whereas the products from the saturated esters were partially solid.

Methyl Ricinoleate. A solution of 31.25 g (0.10 M) methyl ricinoleate and 6.07 g dimethyl phosphite (0.055 M) was heated to 137–150C while nitrogen was bubbled through the reaction mixture. Methanol evolved was collected and the course of the reaction was followed by observing the quantity of methanol produced and the decrease in intensity of the band at 2.76μ (OH) due to the hydroxy ester. The reaction product was dissolved in petroleum ether; washed with cold, aqueous 1*N* K_2CO_3 and then with water till neutral; dried with $MgSO_4$; and partitioned between acetonitrile and petroleum ether. Unreacted methyl ricinoleate and the phosphorus diester dissolve preferentially in the acetonitrile whereas dehydration and polymeric products, with lower phosphorus content, dissolve preferentially in the petroleum ether. Although prolonged heating of the reaction mixtures resulted in decreased absorption at 2.76μ due to OH, in later stages the decrease was not proportional to the amount of methanol evolved, and prolonged heating resulted in increased solubility in the petroleum ether layer on partitioning. Thus, after 30 min of heating, the hydroxyl absorption had decreased 75% and only 11% of the product was found in the petroleum ether layers, and after 75 min the hydroxyl absorption had decreased by 82%, but 18% of the product was found in the petroleum ether. Five hr heating resulted in complete disappearance of characteristic hydroxyl absorption, but more than half (55%) of the product was extracted by the petroleum ether. Accordingly, for methyl ricinoleate a suitable reaction time is ca. 45 min.

The fractions obtained by partitioning between acetonitrile and petroleum ether were fractionated further by silicic acid chromatography, using ethyl ether-petroleum ether as the eluting solvent. A 1-g sample of the acetonitrile extract yielded a fraction of unreacted methyl ricinoleate and another fraction containing a long-chain diester of phosphorus. Analysis—Calcd. for $(C_{19}H_{35}O_3)_2$ PH(O): C, 68.00; H, 10.68; P, 4.61. Found: C, 68.0; H, 10.6; P, 4.38.

When a mixture of methyl ricinoleate and dimethyl phosphite in a 1:1 mole ratio was heated and then fractionated, the same long-chain diester of phosphorus was obtained, but in smaller yield.

Methyl Lesquerolate. When a mixture of methyl lesquerolate and dimethyl phosphite in a 2:1.1 mole ratio was heated 90 min at 140–159C, chromatographic fractionation of the product yielded a long-chain diester. Analysis—Calcd. for $(C_{21}H_{34}O_3)_2$ PH(O): C, 69.40; H, 10.94; P, 4.26. Found: C, 69.3; H, 10.8; P, 3.83.

Methyl Dimorphecolate. When a solution of methyl dimorphecolate and dimethyl phosphite was heated alone, or in the presence of N-ethylmorpholine, two phases formed. The lower layer was strongly acidic and soluble in water. The upper layer, insoluble in water, showed intense characteristic ultraviolet absorption at 260–280 mμ, indicating that triene was present, and that dehydration rather than transesterification had occurred.

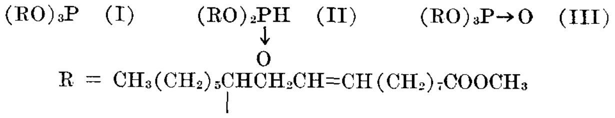
Methyl 9-Hydroxystearate. Heating a mixture of methyl 9-hydroxystearate and dimethyl phosphite in a 2:1.1 mole ratio at 130–150C for 2 hr resulted in evolution of methanol, a 17% decrease in hydroxyl content and, a partially solid reaction product. After the product was washed, most of the unreacted methyl 9-hydroxystearate was removed by crystallization from a petroleum ether solution at −10C. Removal of solvent then gave a liquid residue. Fractionation of a 500-mg portion of this liquid product on silicic acid using ethyl ether-petroleum ether as eluting solvent, afforded a liquid long-chain diester of phosphorus. Analysis—Calcd. for $(C_{19}H_{37}O_3)_2$ PH(O):C, 67.0; H, 11.21; P, 4.59. Found: C, 67.4; H, 11.2; P, 4.51.

Methyl 12-Hydroxystearate. Heating a mixture of methyl 12-hydroxystearate and dimethyl phosphite in a 2:1.1 mole ratio for 2½ hr at 140–160C resulted in an 82% decrease in intensity of absorption at 2.76μ (OH). The product was washed in the usual manner and partitioned between acetonitrile and petroleum ether. Silicic acid chromatography of a 1-g sample of the petroleum ether solubles afforded a liquid long-chain diester of phosphorus. Analysis—Calcd. for $(C_{19}H_{37}O_3)_2$ PH(O):C, 67.60; H, 11.21; P, 4.59. Found: C, 67.8; H, 11.2; P, 4.46. Silicic acid chromatography of a 1-g sample of the acetonitrile solubles afforded a liquid oil with analysis approximating a mixed monomethyl monocarbomethoxyheptadecyl diester of phosphorus. Analysis—Calcd. for (CH_3O) $(C_{19}H_{37}O_3)$ PH(O): C, 61.21; H, 10.53; P, 7.89. Found: C, 58.0; H, 10.3; P, 7.61.

Methyl 14-Hydroxyeicosanoate. Heating a mixture of methyl 14-hydroxyeicosanoate and dimethyl phosphite in a 2:1.1 mole ratio for 5 hr at 120–150C resulted in a 40% decrease in intensity of absorption at 2.76μ (OH). The product was washed in the usual manner with water and K_2CO_3, and unreacted methyl 14-hydroxyeicosanoate was removed from the washed product by crystallization from a petroleum ether solution. Silicic acid chromatography of the residue from the petroleum ether mother liquor afforded a long-chain diester of phosphorus. Analysis—Calcd. for $(C_{21}H_{41}O_3)_2$ PH(O): C, 68.99; H, 11.43; P, 4.24. Found: C, 69.0; H, 11.4; P, 4.06. When the same mixture was heated for 1 hr at 130–150C and the product washed up, silicic acid chromatography afforded a solid product, mp 46–50.5C (50–53C after crystallization from petroleum ether) which analyzed for a mixed methyl and long-chain fatty acid diester of phosphorus. Calcd. for (CH_3O) $(C_{21}H_{41}O_3)$ PH(O): C, 62.85; H, 10.77; P, 7.36. Found: C, 60.9; H, 10.6; P, 7.18.

Discussion

One of the oldest methods for preparation of phosphorus esters is reaction of a phosphorus halide with a hydroxylic compound using an organic base as an HCl-acceptor (10,21,22,23). Reaction of methyl ricinoleate with PCl_3 in this way followed by silicic acid chromatography afforded a product which comprised ca. 75% by weight of the triester(I) and 25% of the diester(II). Methyl ricinoleate treated with $POCl_3$ in the same type of reaction afforded a small yield of a phosphoric triester(III).



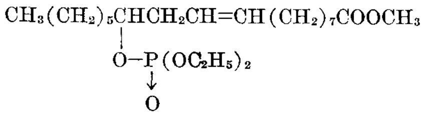
$(RO)_3P$ (I) $\quad (RO)_2\overset{}{\underset{\underset{O}{\downarrow}}{P}}H$ (II) $\quad (RO)_3P\rightarrow O$ (III)

$R = CH_3(CH_2)_5\underset{|}{C}HCH_2CH{=}CH(CH_2)_7COOCH_3$

All these phosphorus-containing products exhibited characteristic absorption maxima at 7.85–8.10μ and 9.60–10.35μ, within the regions commonly assigned to P⟶O and P–O–C respectively (15).

Methyl dimorphecolate is so susceptible to dehydration that when it was treated with either PCl_3 or $POCl_3$ under conditions usable with methyl ricinoleate the resultant product was a conjugated triene.

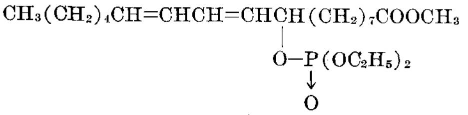
Many mixed phosphate esters are commonly prepared by reaction between a hydroxylic compound and a dialkyl phosphorochloridate in the presence of a basic HCl-acceptor (10,24,25,26,27). When methyl ricinoleate was treated with either diethyl phosphorochloridate or dibutyl phosphorochloridate, using various reaction conditions commonly employed, no evidence of esterification was observed. However, when the reaction was carried out with dimethylformamide as catalyst and acetonitrile as solvent, formation of a mixed phosphate triester (IV) was indicated.



(IV)

$$CH_3(CH_2)_5\underset{\underset{\underset{\underset{O}{\downarrow}}{O{-}P(OC_2H_5)_2}}{|}}{C}HCH_2CH{=}CH(CH_2)_7COOCH_3$$

When methyl dimorphecolate was treated with diethyl phosphorochloridate by this new procedure, a mixed phosphate triester (V) was formed along with some conjugated triene.

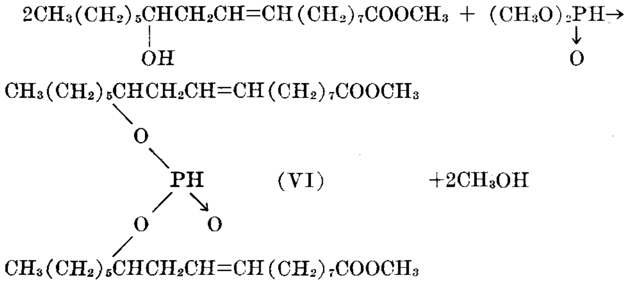


(V)

$$CH_3(CH_2)_4CH{=}CHCH{=}CH\underset{\underset{\underset{\underset{O}{\downarrow}}{O{-}P(OC_2H_5)_2}}{|}}{C}H(CH_2)_7COOCH_3$$

For purification of the triester (V), partitioning between acetonitrile and petroleum ether was the most suitable method. The conjugated triene enters the petroleum ether layer and the triester dissolves preferentially in the acetonitrile but it is unstable and decomposes spontaneously on standing.

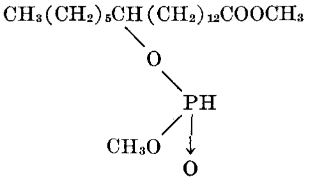
Another approach to the preparation of phosphorus-containing esters is by transesterification (18, 19,20,28) between a dialkyl phosphite and the methyl ester of a hydroxy fatty acid. The reaction may be represented as follows:



$$2CH_3(CH_2)_5\underset{\underset{OH}{|}}{C}HCH_2CH{=}CH(CH_2)_7COOCH_3 + (CH_3O)_2\underset{\underset{O}{\downarrow}}{P}H \rightarrow [CH_3(CH_2)_5CHCH_2CH{=}CH(CH_2)_7COOCH_3{-}O]_2\underset{\underset{O}{\downarrow}}{P}H \text{ (VI)} + 2CH_3OH$$

Phosphite diesters analogous to (VI) are formed when dimethyl phosphite is heated at a suitable temperature and time with any of the following methyl esters of long-chain hydroxy fatty acids: 9-hydroxystearic, 12-hydroxystearic, lesquerolic, and 14-hydroxyeicosanoic. Under the conditions used, methyl dimorphecolate dehydrated to the conjugated triene. Optimum reaction conditions for the various hydroxy acid esters differ, but, in general, prolonged reaction time and higher temperature increase the formation of less-polar products preferentially soluble in petroleum ether at the expense of the more polar products preferentially soluble in acetronitrile. The less polar products are, presumably, self-condensation products of the hydroxy esters, such as estolides. The more polar products include the long-chain phosphorus diesters. These can be fractionated to give analytical samples by elution from columns of silicic acid using ethyl ether-petroleum ether as eluting solvent.

When methyl 14-hydroxyeicosanoate was reacted with dimethyl phosphite at 2:1 mole ratio for 1 hr at 150C, a product having the composition corresponding to the mixed diester of phosphorus, VII, was obtained although when the reaction time was extended to 5 hr a phosphite diester analogous to (VI) was formed.



(VII)

$$CH_3(CH_2)_5CH(CH_2)_{12}COOCH_3{-}O{-}\underset{\underset{O}{\downarrow}}{P}H{-}OCH_3$$

Conclusion

Although the long-chain hydroxy fatty acids are closely related homologs or analogs, the reactivity of the hydroxyl group towards phosphorus-containing reagents differs considerably. In general, except for esters containing an allylic hydroxyl (e.g., dimorphecolate), phosphorus triesters can be formed by reaction with PCl_3, mixed triesters by reaction with dialkyl phosphorochloridate, and symmetrical diesters and some mixed diesters by transesterification with dialkyl phosphite. The last is probably the most convenient method for preparation of phosphorus-containing esters of long-chain hydroxy fatty acids. In all cases the products are difficult to purify, but purification can be accomplished by partitioning between suitable pairs of immiscible solvents and chromatography on silicic acid.

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A Study of Detergent Biodegradability as Shown by Various Analytical Techniques[1]

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Abstract

The data discussed in this paper indicate the importance of the application of a number of different analytical procedures to a detergent biodegradation study, since no single technique is an adequate means of measuring the various aspects of detergent biodegradability.

This paper describes surface tension, colorimetric, manometric, radioactive, and GLC analytical methods applicable to detergent biodegradation analyses, with an interpretation of the results given by each method.

The biological system used in the studies is also described.

Introduction

DURING THE PAST few years, a number of investigators have studied detergent biodegradation using established chemical and biological methods.

In these studies, workers have generally used one or more of the following analytical techniques: direct colorimetric measurements using a dye capable of forming a detergent-dye salt, Warburg manometry, radioactive tracer techniques, and BOD tests. Although these methods can provide important biodegradation data, each method has distinct limitations. The application of several different analytical methods to a study will frequently provide data to support conclusions which could not be established from a single method of analysis.

The present study reports detergent biodegradability studies in which three of the above analytical methods and two additional methods—surface tension measurements and vapor chromatography—were used to evaluate the results. BOD tests were not used.

Procedure and Data

Preparation of Samples

The microbial culture employed in our studies was a stable mixed culture obtained from an activated sludge type waste treatment plant (Enid, Okla.). The culture was adapted to growth in the presence of detergent by three successive transfers in a medium containing 30 mg/liter of a sodium alkylbenzene sulfonate.

The composition of the medium used for this purpose, and also for all experimental detergent biodegradation studies, is shown in Table I.

One-liter quantities of the medium plus 30 mg of the detergent under study were added to 2-liter Erlenmeyer flasks and sterilized by autoclaving for 20 min at 20 pounds pressure. After cooling, the flasks were inoculated with 10 ml of a 72-hr bacterial culture and incubated at room temperature (25C) on a gyrorotary shaker. It was found that this apparatus provided adequate agitation and aeration for bacterial growth.

At desired intervals during the incubation period, aliquots were withdrawn from the flasks for analysis; or entire flasks were removed, depending upon the nature of the analysis desired.

Analytical Methods

Colorimetric. The principal colorimetric method used was the methyl green dye technique of Moore and Kolbeson (1). The method depends upon the formation of a dye-anionic detergent salt in aqueous buffered solution. The organic soluble dye-salt is extracted with benzene, and the concentration is determined from the absorbance at 610 mμ, using a suitable spectrophotometer.

Warburg Manometry. The manometric technique

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