methane and the solution treated with 1 ml. of a 30% solution of hydrogen bromide in glacial acetic acid. The reaction mixture was kept at room temperature for 30 min., diluted with dichloromethane and washed successively with water, saturated aqueous sodium bicarbonate and water. Moisture was removed with granular sodium sulfate and the solution, after filtration through decolorizing carbon, concentrated *in vacuo*. Dissolved in a mixture of benzene and pentane (1:1), the residue crystallized as short needles: 57 mg. (61%), m.p. 160–161°. Recrystallization failed to change this value. The pure bromide showed $[\alpha]_D^{20}$ −40.0° in U.S P. chloroform (*c* 0.73) and could be recovered unchanged from this solvent.

Anal. Calcd. for $C_{26}H_{19}BrN_2O_{11}$ (615.35): C, 50.75; H, 3.11; N, 4.56; Br, 12.99. Found: C, 50.95; H, 3.05; N, 4.74; Br, 12.68.

1,5-Di-O-benzoyl-2,3-di-O-p-nitrobenzoyl-α-L-arabinose (IV) *from 5-O-benzoyl-2,3-di-O-p-nitrobenzoyl-α-L-arabinosyl bromide* (V). A solution of the bromide V (76 mg.) in 10 ml. of dry benzene was stirred with 150 mg. of silver benzoate for 30 min. at room temperature. The mixture was then filtered through decolorizing carbon, the filtrate evaporated *in vacuo* and the residue crystallized from benzene-pentane to give 51 mg. (62%) of 1,5-di-*O*-benzoyl-2,3-di-*O*-*p*-nitrobenzoyl-α-L-arabinose, melting at 169–170°; the substance failed to depress the melting point of a sample prepared through the *p*-nitrobenzoylation of 1,5-di-*O*-benzoyl-α-L-arabinose.

1-O-Acetyl-5-O-benzoyl-α-L-arabinose (VI) *from ethyl 5-O-benzoyl-1-thio-β-L-arabinoside* (I). To a stirred solution of 5.0 g. of ethyl 5-*O*-benzoyl-1-thio-β-L-arabinoside in 125 ml. of acetonitrile was added 5.90 g. (1.1 molar equivalents) of mercuric acetate. The salt dissolved within a few minutes and then a precipitate began to form. The mixture was stirred for 2 hr., filtered and the filtrate concentrated *in vacuo*. After solution in dichloromethane the residue was treated with hydrogen sulfide and the solution filtered through decolorizing carbon. Evaporation of the filtrate afforded a partly crystalline mass (5.53 g.) which was recrystallized from 50 ml. of ether to yield 2.12 g. of crude 1-*O*-acetyl-5-*O*-benzoyl-α-L-arabinose. After two recrystallizations from a mixture of ethyl acetate and pentane the pure ester (1.70 g., 34%) was obtained as plates melting at 117–118°, $[\alpha]_D^{20}$ −49.3° (*c* 0.53). The substance failed to mutarotate in 20% aqueous pyridine and could be recovered unchanged after 24 hr. in this solvent mixture. In aqueous solution it reduced 1.03 molar equivalents of sodium periodate in 24 hr. at room temperature.

Anal. Calcd. for $C_{14}H_{16}O_7$(296.27): C, 56.75; H, 5.44. Found: C, 56.77; H, 5.47.

1-O-Acetyl-5-O-benzoyl-2,3-di-O-p-nitrobenzoyl-α-L-arabinose (VII). To a solution of 1.25 g. of *p*-nitrobenzoyl chloride in 10 ml. of pyridine was added 500 mg. of 1-*O*-acetyl-5-*O*-benzoyl-α-L-arabinose. After standing at room temperature overnight the mixture was diluted with water and the product extracted with dichloromethane. The extract was washed successively with 3*N* sulfuric acid, saturated aqueous sodium bicarbonate and water. Moisture was removed with granular sodium sulfate and the solution filtered through decolorizing carbon. Removal of solvent left an amorphous residue; dissolved in a mixture of benzene (10 ml.) and pentane (10 ml.) this yielded 750 mg. (75%) of 1-*O*-acetyl-5-*O*-benzoyl-2,3-di-*O*-*p*-nitrobenzoyl-α-L-arabinose as very pale yellow prisms melting at 149–151°, $[\alpha]_D^{20}$ +38.6° in chloroform. A further recrystallization failed to change this melting point but raised the rotation to $[\alpha]_D^{20}$ +39.7° ($CHCl_3$, *c* 0.82).

Anal. Calcd. for $C_{28}H_{22}N_2O_{13}$ (594.48): C, 56.57; H, 3.73; N, 4.71. Found: C, 56.83; H, 3.65; N, 4.70.

5-O-Benzoyl-2,3-di-O-p-nitrobenzoyl-α-L-arabinosyl bromide (V) *from 1-O-acetyl-5-O-benzoyl-2,3-di-O-p-nitrobenzoyl-α-L-arabinose* (VII). 1-*O*-Acetyl-5-*O*-benzoyl-2,3-di-*O*-*p*-nitrobenzoyl-α-L-arabinose (295 mg.) was dissolved in 8 ml. of dichloromethane and 2 ml. of a 30% solution of hydrogen bromide in glacial acetic acid added. Mutarotation ceased after 3 min. at 20°; after 23 min. the solution was diluted with 15 ml. of dichloromethane and washed successively with water, saturated aqueous sodium bicarbonate and water. Moisture was removed with granular sodium sulfate, the solution filtered through decolorizing carbon and solvent removed *in vacuo*. From its solution in a mixture of 10 ml. of benzene and 10 ml. of pentane the residue afforded 230 mg. (75%) of 5-*O*-benzoyl-2,3-di-*O*-*p*-nitrobenzoyl-α-L-arabinosyl bromide melting at 160–161° either alone or in admixture with a sample prepared as described earlier from 1,5-di-*O*-benzoyl-2,3-di-*O*-*p*-nitrobenzoyl-α-L-arabinose.

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BETHESDA 14, MD.

[CONTRIBUTION NO. 1633 FROM THE STERLING CHEMISTRY LABORATORY, AND THE BINGHAM OCEANOGRAPHIC LABORATORY, YALE UNIVERSITY]

Contributions to the Study of Marine Products. L. Phospholipids of Sponges[1,2]

ROBERT A. LANDOWNE[3] AND WERNER BERGMANN[4]

Received July 11, 1960

The phospholipid fractions of two sponges, *Lissodendoryx isodyctialis* and *Speciospongia vesparia*, have been isolated and characterized. That from *L. isodyctialis* was found to consist of sphinegosine phosphate fatty acid esters, free of choline and sugars; the other, from *S. vesparia*, was a lecithin containing aldehyde in an enol ether linkage.

The study of the phospholipids of marine invertebrates has been continued as an extension of our comparative studies on the composition and evolution of the lipids of these organisms. In the previous report[5] the importance of isolation fol-

(1) This investigation was supported by a research grant, Nonr 253(00) from the Office of Naval Research.

(2) Taken in part from the dissertation submitted in 1958 by R. A. Landowne to the Graduate School of Yale University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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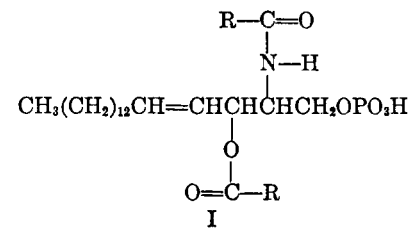
lowed by structural determination of the complex lipids was pointed out, as simple analysis for nitrogen and phosphorus can be misleading in the case of the atypical phospholipids of some marine organisms. The present work offers another example of the importance of characterization of phospholipids in this field, because both types of material studied here differ in structure from those previously described in ways not detectable by nitrogen and phosphorus analysis alone.

Lissodendoryx isodyctialis. The sponge, *L. isodyctialis*, collected from the waters around Bermuda, was extracted for lipids by standard methods[6] and its phospholipid content isolated. When purified by precipitation from glacial acetic acid this fraction was found to have a typical nitrogen to phosphorus ratio of 1:1 but exhibited both ester (5.8 μ) and amide (6.1 μ) carbonyl bands in the infrared. The high melting point (181.5–183°), insolubility in ether, and its stability to air and moisture were indicative of a sphingoside, and the persistence of the ester infrared absorption through purification steps tended to rule out the presence of glycerides in the mixture. Hydrolysis yielded no detectable choline. If choline had been present in the original molecule, it was not lost during extraction of the organism, as normal choline-containing sphingomyelin was obtained from the anemone *A. elegantissima* by the same procedures used here.[5] Nor did hydrolysis yield carbohydrate (Molisch and Fehlings tests) even though the material resembled cerebrosides by its insolubility in acetic acid. Sphingosine, identified by infrared comparison with the known triacetyl derivative, was isolated. Two moles of fatty acid (calculated as C_{16} acids) were obtained per molecule of sphingoside, the molecular weight of the sphingoside being based on the assumption of one nitrogen and phosphorus atom per molecule. Analysis of the fatty acids, as their methyl esters, by gas chromatography[7] showed a mixture ranging from C_{10} to C_{22}. Many of the minor components appeared to be branched chain acids; the major components were palmitate (38%) and palmitoleate (17%).

Hydrogenation cleaved the fatty acid ester linkage to yield 66% of theoretical yield (based on C_{16}) of free fatty acid. This behavior is typical of the reactions of allylic esters[8] and indicates that most, if not all, of the fatty acid was esterified at the allylic hydroxyl group of sphingosine. Hydrogenation of sphingosine esters does not give quantitative yields of acids; Carter[9] *et al.* isolated only 80% of acetic acid from sphingosine triacetate and in our experience the yield of acetic acid was somewhat less than this.

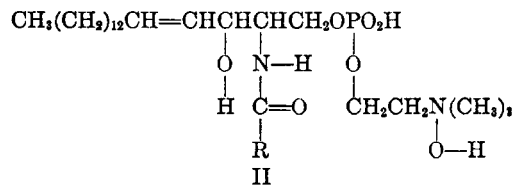
Evidence for a monophosphate ester grouping was obtained by study of the action of alkaline phosphatase.[10] This enzyme liberated 10.5% of the phosphate in thirty minutes, while simple hydrolysis at the same *p*H liberated 4.3%. Lack of material made more complete studies impossible, but it is felt that a monophosphate ester is the most likely structure present.

Consideration of the above evidence leads to the proposal of structure I for the phospholipid from *L. isodyctialis*.



I

This structure best accomodates the amide, allylic ester and phosphate linkages, taking into consideration also the normal sphingomyelin structure II.[11]



II

The name acylsphingomyelin phosphatidic acid is suggested for this new subclass of natural products.[12]

The only previous report of an acyl sphingomyelin known to us is that of Thannhauser and Reichel[13] who treated a beef brain sphingomyelin fraction with esterase and obtained free fatty acids. Their sphingomyelin fraction, however, was not purified by washing with acetic acid as in the present work, and more recent findings have indicated that hydrolecithins are ether insoluble and contaminate sphingomyelin fractions.[14] The possibility that the esterase-liberated fatty acids from their material originated from impurities certainly

(5) W. Bergmann and R. A. Landowne, *J. Org. Chem.*, **23**, 1241 (1958).

(6) W. R. Bloor, *J. Biol. Chem.*, **17**, 377 (1914); **22**, 133 (1915).

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(10) L. A. Heppel, *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds., Academic Press, New York, 1955, vol. II, p. 530.

(11) H. E. Carter, F. J. Glick, W. P. Norris, and G. E. Phillips, *J. Biol. Chem.*, **142**, 449 (1942).

(12) The term "phosphatidic acid" follows from the accepted nomenclature for the glycero-phosphatides that do not contain a nitrogen base in ester linkage with the phosphate and are therefore phosphate mono-esters.

(13) S. J. Thannhauser and M. Reichel, *J. Biol. Chem.*, **135**, 1 (1940).

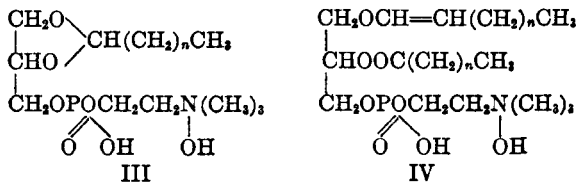
(14) E. Baer, D. Buchnea, and A. G. Newcombe, *J. Am. Chem. Soc.*, **78**, 232 (1956).

exists, since no confirmation by isolation of a purified product free of normal sphingomyelin and hydrolecithins has been reported.

Speciospongia vesparia. The major phospholipid component was an aldehydogenic lecithin.[15] The material was surprisingly insoluble in ethanol, and precipitation by ethanol from petroleum ether aided its purification and made it evident that the ester group as detected by its infrared absorption and later established by hydrolysis was not due to impurities. The purified phospholipid also gave a rapid fuchsin-aldehyde test. It was free of amino nitrogen but contained a mole of choline for each mole of nitrogen and of phosphorus. Hydrolysis gave aldehydes, acids, choline, and glycerol phosphate. The aldehyde was shown to arise from an enol ether by hydrogenation of the intact molecule followed by hydrolysis. Here, no free aldehyde could be found, but a stable glyceryl ether was isolated instead (as the salt of its phosphate ester). Whether the enol ether linkage was α or β (at C_1 or C_2 of the glycerol) was not determined.

Both the aldehydes and esters were isolated after hydrolysis, and from elementary analyses the average chain length of each moiety was eighteen carbon atoms. Gas chromatography of the methyl esters showed a mixture of components from C_{10} to C_{24} with no one particular ester predominating. The aldehydes were also a complex mixture. The 2,4-dinitrophenylhydrazones were not crystalline for this reason. Unsaturation was relatively high in that there was 0.77 double bond per mole of phospholipid in addition to the double bond of the enol ether.

An acetal phospholipid (III) isolated as a minor constituent from the sea anemone *A. elegantissima* was previously reported.[5] Aldehyde-containing compounds of this type were first noted by Feulgen and Bersin[16] and later confirmed by Thannhauser *et al.*[17] to be present in mammalian tissue. Other structures were proposed which included hemiacetal[18] and enol ether[19,20] attachment of the aldehyde moiety (IV). These investigators preferred to discount the existence of the acetal structure in nature. Our present finding in the sponge *S. vesparia* of an aldehyde-containing phospholipid (IV) which did not have the acetal linkage, even though similar isolation and identification procedures were used, makes it seem quite certain that both acetals and enol ethers, and perhaps even other types, exist in nature.



III IV

A comparison of the optical rotation for the several plasmalogens that have been reported is quite interesting although its interpretation is not particularly clear. While the sea anemone acetal plasmalogen[5] (III) had a negative rotation similar to Rapport's enol ether plasmalogen[19] and opposite to that of Thannhauser's acetal compound,[17] the sponge plasmalogen herein reported (IV) has a positive rotation even though it is structurally similar to Rapport's compound. It seems unwise at this point to attribute these rotational discrepancies to species differences. It should be pointed out that all natural lecithins have a positive rotation, and there is no apparent reason to expect the replacement of a fatty acid by an aldehyde (be it in the α- or β-position) to alter significantly the rotation of these compounds. Thus, a positive rotation for the aldehydogenic lecithins (IV) seems consistent with other data.

EXPERIMENTAL[21]

Isolation of L. isodyctialis phospholipid. The air-dried tissue (228 g.) was homogenized in a Waring blendor in eight portions with 200 ml. of 2:1 (v./v.) chloroform-methanol solvent mixture per portion. The residual tissue was centrifuged, divided into two parts, and homogenization was repeated with 200 ml. of chloroform for each part. The homogenate was centrifuged again and the supernatants were combined and washed with 10 volumes of water by the Folch method.[22] The chloroform extract (1.5 l.) was filtered and then added to 5.25 l. of acetone and refrigerated overnight.

The flocculent phospholipid precipitate was centrifuged and taken up in 150 ml. of chloroform. The solution was filtered and the phospholipids reprecipitated by adding the solution to 525 ml. of acetone. This process was repeated using 40 ml. of chloroform and 160 ml. of acetone. The precipitate was dried under a stream of nitrogen to yield 2.25 g. (1.0%) of light tan powder.

Purification. The phospholipid was stirred and ground in 100 mg. portions with 10 ml. of glacial acetic acid. The acetic acid insoluble fraction (50%) was filtered and washed twice with 10 ml. of acetone to remove acetic acid. The acetic acid filtrate was mixed with 14 volumes of acetone and let stand a few hours. Phospholipid, now acetic acid insoluble and identical with the original acetic acid insoluble fraction, precipitated and was filtered and washed with acetone. The

(15) A minor constituent (0.01%) was also isolated by precipitation with ethyl ether from petroleum ether solution. It was apparently a phospholipoprotein in that it had a N/P ratio of 12.6/1.

(16) R. Feulgen and T. Bersin, *Z. Physiol. Chem.*, **260**, 217 (1939).

(17) S. J. Thannhauser, N. F. Boncoddo, and G. Schmidt, *J. Biol. Chem.*, **188**, 417 (1951).

(18) E. Klenk and H. Debuch, *Z. Physiol. Chem.*, **299**, 66 (1955).

(19) M. M. Rapport, B. Lerner, N. Alonzo, and R. E. Franzl, *J. Biol. Chem.*, **225**, 859 (1957).

(20) G. V. Marinetti and J. Erbland, *Biochim. Biophys. Acta*, **26**, 429 (1957); G. V. Marinetti, J. Erbland, and E. Stotz, *J. Am. Chem. Soc.*, **80**, 1624 (1958).

(21) P, N, C, and H analyses by the Schwarzkopf Microanalytical Laboratory, Woodside 77, N. Y. Choline was determined in this laboratory gravimetrically as the reineckate.

(22) J. Folch, I. Ascoli, M. Lees, and J. A. Meath, *J. Biol. Chem.*, **191**, 833 (1951).

purification yield was 88%, m.p. 181.5–183° dec.; $[\alpha]_D^{27°}$ = +13.3° (1.25% in pyridine, α = +0.166°).

Anal. Calcd. for $C_{50}H_{98}NO_7P$: N, 1.64; P, 3.62. Found: N, 1.73; P, 3.73. N/P = 1.02.

Paper chromatography by the method of Bevan *et al.*[23] showed only one phosphorus positive spot which remained at the origin. The compound gave negative Molisch and Fehlings tests for sugar after acid hydrolysis. The ninhydrin test was negative but strongly positive after hydrolysis.

Acid hydrolysis and isolation of the esters. The sphingoside (101 mg.) was mixed with 90 ml. of 2*N* methanolic hydrochloric acid (anhydrous) and refluxing was begun. After 16 hr., 25 mg. of undissolved, unchanged starting material was filtered from the cooled solution, and the filtrate was extracted with three 50-ml. portions of petroleum ether (b.p. 30–60°). The petroleum ether was washed twice with 50 ml. of water and dried over anhydrous sodium sulfate overnight. Removal of the drying agent by filtration and the solvent by evaporation left 41 mg. of a mixture of methyl esters, m.p. 30–33°. The yield from the material that treated was 85% based on 2 moles of palmitate per mole of hydrolyzed sphingoside. Gas liquid chromatography of the mixture showed at least fourteen peaks from C_{19} to C_{22} with palmitate and palmitoleate being the two most abundant components, in concentrations of 38% and 17%, respectively.

Characterization of the nitrogen base. The sphingoside (292 mg.) was refluxed with 68 ml. of 2*N* methanolic sulfuric acid for 3.5 hr. The solution was filtered to remove 6 mg. of starting material and extraction with four 50-ml. portions of petroleum ether removed the esters. Methanolic potassium hydroxide (30%) was added to make the solution alkaline to phenolphthalein. Potassium sulfate was filtered and the solution was re-acidified with 1 drop of glacial acetic acid before being concentrated to a volume of 25 ml. at 50° in a rotary evaporator. An equal volume of water was added, and the solution was made alkaline again before extracting the free base three times with 50 ml. of ethyl ether. The ether was washed twice with 25 ml. of water, dried with anhydrous sodium sulfate, and evaporated to yield 49 mg. (50%) of crude base.

The base was acetylated overnight with 1 ml. of pyridine and 1 ml. of acetic anhydride. Two milliliters of water was then added to the mixture and the brown oil that floated to the top was removed and dried *in vacuo* at room temperature. Recrystallization from acetone was partially successful, m.p. 50–60°. The infrared spectrum was identical with both natural[24] and synthetic[25] triacetyl sphingosine.

Hydrogenolysis. A microhydrogenation apparatus[26] was used to hydrogenate 84.7 mg. (0.1 mmole) of sphinogoside, dissolved in 1 ml. of pyridine, and diluted with 5 ml. of benzene. The catalyst was 48.5 mg. of 5% palladium on charcoal. The reaction was stirred at room temperature under hydrogen at atmospheric pressure. The catalyst was then filtered off and the solvents removed in a rotary evaporator. The residue was taken up in 3 ml. of chloroform and 12 ml. of acetone was added. This precipitated 42 mg. of a mixture of hydrogenated starting material and the deacylated compound. The filtrate was taken to dryness and the residue extracted with acetone to leave an additional 10 mg. of insoluble product. The acetone solution was taken to dryness and the residue (30 mg.) extracted with ether. The ether extract contained 17 mg. of free fatty acid equivalent to 0.066 mmole of palmitic acid.

Enzymatic hydrolysis. A finely ground sample of sphingoside (52 mg.) was shaken for 10 min. in 10 ml. of water to obtain a nearly homogeneous mixture. Ten milliliters of 0.1*M* sodium borate solution ($Na_2B_4O_7{\cdot}10H_2O$) was added to bring the mixture to *p*H 9. A 15% magnesium chloride solution (0.5 ml.) and 0.1 ml. of enzyme solution containing 0.19 mg. (760 units) of alkaline phosphatase (Mann Laboratories, New York, N. Y.) was then added. The mixture was incubated at 36° in a water bath for 30 min., and then 3 ml. of 10% trichloroacetic acid was added to quench the reaction. The procedure was repeated with 32 mg. of sphingoside but without the enzyme. An enzyme blank was run by repeating the procedure without the phospholipid sample. Phosphorus was determined on all solutions by a modification of the Fiske-Subbarow procedure[27] using amidol[28] as the reducing agent. The optical density was determined at 550 mμ in a Bausch and Lomb colorimeter. Standards were obtained by adding magnesium phosphate in place of enzyme and/or lipid.

RESULTS

Solution	O.D.[a]	P Added, Calcd.	Free P, Found
Standard I	0.23	0.05 mg.	0.05 mg.
Standard II	0.46	0.10	0.10
Standard III	0.91	0.20	0.20
Enzyme blank	0.04	—	0.009
Lipid blank	0.76	3.7	0.16
Lipid sample	1.46[b]	6.0	0.63[c]

[a] After subtracting blank with water as zero. [b] Read at half-dilution. [c] After subtracting enzyme blank.

The yield of hydrolyzed phosphate was 4.3% without enzyme, and 10.5% with the enzyme.

Isolation and purification of S. vesparia phospholipids. The frozen tissue was defrosted for 24 hr. at room temperature and 2.3 kg. was shredded and homogenized in ten portions with a total of 2.1 l. of chloroform-methanol solvent mixture (2:1, v./v.). The tissue was filtered from the extract and rehomogenized with 1.2 l. more of chloroform. All filtrates were combined and 1.5 l. of methanol was added to obtain a homogeneous extract. The dried residual tissue was 0.31 kg.

The extract was washed by the Folch procedure[22] in two 3 l. batches, with 20 l. of water for each portion. After 4.5 hr., fresh water was substituted and washing continued for 13.5 hr. more. The chloroform extract was concentrated to 0.70 l. at 60° and 20 mm. pressure, and then added to 2.45 l. of acetone. This mixture was kept at 5° for 2 days.

One milliliter of saturated ethanolic magnesium chloride ($MgCl_2.6H_2O$) was added to coagulate the phospholipids which were centrifuged and collected by washing the centrifuge bottles with 80 ml. of chloroform. Reprecipitation with 320 ml. of acetone then followed and the precipitate was dissolved in 35 ml. of petroleum ether. This solution was added to 175 ml. of ethyl ether to yield a small precipitate (10 mg.). The combined acetone-chloroform mother liquors were taken to dryness at 60° and 20 mm. to yield 6.5 g. of lipid material which was acetone soluble except for 20 mg. This acetone insoluble fraction was also ether insoluble and was therefore combined with the first ether insoluble fraction. The total yield was 0.01% of the dry weight.

The ether–petroleum ether solution was taken to dryness and the residue found to be completely soluble in 100% ethyl ether. The ether was removed, and the lipid reprecipitated from 75 ml. of chloroform with 525 ml. of acetone. The phospholipid was taken up in 100 ml. of petroleum ether

(23) T. H. Bevan, G. I. Gregory, T. Malkin, and A. G. Poole, *J. Chem. Soc.*, 841 (1951).

(24) K. Mislow, *J. Am. Chem. Soc.*, **74**, 5155 (1952).

(25) D. Shapiro and K. Segal, *J. Am. Chem. Soc.*, **76**, 5894 (1954).

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(27) C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, **66**, 375 (1925).

(28) R. J. L. Allen, *Biochem. J.*, **34**, 858 (1940).

and the solution dried over anhydrous sodium sulfate. The yield of a gummy amorphous material was 2.93 g. (0.92%). Quantitative precipitation from petroleum ether with an equal volume of ethanol gave a powdery, light brown material. Paper strip chromatography according to the method of Bevan *et al.*[23] gave only one phospholipid spot which was negative to ninhydrin but indicative of a lecithin, according to its R_f value of 0.92. It gave a positive fuchsin-aldehyde test with mercuric acetate. The test was negative without the mercury salt. The infrared showed a strong carbonyl peak at 5.78 μ and the melting behavior was also typical of the lecithins with softening occurring at 90°, and meniscus formation at 210–220°. $[\alpha]_D^{24°} = +12.6°$ (1.11% in chloroform, $\alpha = +0.14°$).

Anal. Calcd. for $C_{48}H_{98}NO_8P$: P, 3.65; N, 1.65; choline, 14.3. Found: P, 3.65; N, 1.60; choline, 14.9. N:P:choline = 0.97:1:1.04.

The ether insoluble fraction had an infrared spectrum similar to the major phospholipid fraction. Its melting behavior was also similar.

Anal. Found: P, 1.05; N, 6.01. N:P = 12.6:1.

No further identification was attempted.

Quantitative hydrogenation of the plasmal lecithin. An 18.9-mg. sample of the phospholipid (23.9 μM) was dissolved in 8 ml. of *n*-butyl ether after the platinum catalyst (19 mg.) was reduced in the microhydrogenation apparatus.[26] At 22° and 754 mm., the sample required 1.04 ml. of hydrogen or 0.946 ml. at STP (42.2 μM). The number of double bonds was 1.77 per mole of phospholipid.

Alkaline hydrolysis of hydrogenated phospholipid. The compound (500 mg.) was dissolved in 75 ml. of *n*-butyl ether and hydrogenated over platinum in a Parr shaker for 21 hr. The catalyst was then filtered off and the solvent removed at 2 mm. and room temperature. The residue was dissolved in 30 ml. of ethyl ether and 30 ml. of 0.5*N* methanolic potassium hydroxide was added. This cloudy mixture was shaken in a stoppered flask at room temperature for 21 hr. Water (20 ml.) was then added and the mixture extracted twice with 25 ml. of ether. The ether extract was washed twice with 10 ml. of water and dried over sodium sulfate. The solvent was evaporated to yield an oil containing a white solid. The oil was removed by dissolving it in 10 ml. of ether leaving 30 mg. (17%) of the solid whose infrared spectrum was typical of a fatty acid salt.

Anal. Calcd. for $C_{18}H_{35}O_2K$: C, 67.08; H, 10.86. Found: C, 67.13; H, 10.45.

The alcoholic solution was filtered, acidified with 1 ml. of concd. hydrochloric acid, and extracted again with three 25-ml. portions of ether. The extract was washed twice with 10 ml. of water and dried. This solution of fatty acids was esterified with diazomethane distilled with the ether from a solution of 1.2 g. of *N*-nitroso-*N*-methyl-*p*-toluenesulfonamide, slowly added to 30% ethanolic potassium hydroxide. The excess diazomethane was allowed to evaporate with the ether and the methyl esters remaining were combined with the ether soluble methyl esters first extracted from the alkaline hydrolysis mixture. The yield was 142 mg. (76%).

The material filtered from the alkaline hydrolysis mixture contained phosphorus. It was taken up in 2 ml. of chloroform and precipitated with 8 ml. of acetone. The precipitate was dissolved in 1 ml. each of pyridine and benzene and reprecipitated with 8 ml. of acetone. The dried powder had no carbonyl band in the infrared and had a strong hydroxyl peak typical of a glyceryl ether phosphate. The yield was 25 mg. (8%).

Anal. Calcd. for $C_{21}H_{43}O_6PK_2$: C, 50.40; H, 8.60. Found: C, 49.47; H, 8.83.

Acid hydrolysis. The lecithin (84 mg.) was dissolved in 1 ml. of chloroform, and 2 ml. of concd. hydrochloric acid and 8 ml. of ethanol were added. This mixture was allowed to stand over a few milligrams of mercuric acetate for 24 hr., after which time hydrolysis seemed complete, as the solution was almost clear. The solution was then filtered and extracted three times with 10 ml. of petroleum ether. The solvent was removed from the extract, the residual oil taken up in 2 ml. of ethanol and 2 ml. of 2,4-dinitrophenylhydrazine reagent added. A precipitate formed immediately but the mixture was refrigerated overnight. The 2,4-dinitrophenylhydrazones were centrifuged and dissolved in benzene. The benzene solution was passed through an alumina column to purify the 2,4-dinitrophenylhydrazones. The benzene was removed to yield a red oil which could not be successfully crystallized. The yield was 25 mg. (53%).

NEW HAVEN, CONN.

[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

A Unique Fatty Acid from *Limnanthes douglasii* Seed Oil: The C_{22} Diene

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The C_{22} dienoic fatty acid of *Limnanthes douglasii* seed oil (representing 10% of the total fatty acid) is shown to be the previously unknown *cis*-5-*cis*-13-docosadienoic acid.

An earlier paper from this laboratory[2] reports that the major components of seed oil from *Limnanthes douglasii* are *cis*-5-eicosenoic, *cis*-5-docosenoic, *cis*-13-docosenoic acids, and a C_{22} acid of undetermined structure. This paper reports the isolation and characterization of the remaining component as a previously unknown docosadienoic acid.

Concentration of the docosadienoic acid. A concentrate of the C_{22} diene acid (I) was obtained by low temperature crystallization of mixed free acids from heptane and subsequent countercurrent distribution (Fig. 1 and Table I).

(1) One of the laboratories of the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(2) C. R. Smith, Jr., M. O. Bagby, T. K. Miwa, R. L. Lohmar, and I. A. Wolff, presented before the Division of Organic Chemistry, 137th Meeting American Chemical Society, Cleveland, Ohio, April 5–14, 1960. C. R. Smith, Jr., M. O. Bagby, T. K. Miwa, R. L. Lohmar, and I. A. Wolff, *J. Org. Chem.*, **25**,1770 (1960).