compound reacted with alkali in a fashion similar to 5-(carboxy-ethylidene)-hydantoin and benzal-hydantoin and no evidence was obtained for conversion to a pyrimidine. This compound has been previously prepared by Johnson and Wrenshall.[11]

Discussion

The over-all yield of orotic acid obtained from aspartic acid was 39%. This yield is considerably better than by methods previously described in the literature. In addition, all of the reactions are technically simple and the yields are easily reproduced.

It appears most probable that the conversion of 5-(carboxy-methylidene)-hydantoin to orotic acid goes through an intermediate hydantoic acid which recyclizes rapidly in the presence of alkali to give a pyrimidine ring. Although the hydantoic acid has not been isolated the alternative mechanism involving direct ring enlargement is made improbable by the fact that three related hydantoins are not converted to pyrimidines on alkaline treatment.

(11) T. B. Johnson and R. Wrenshall, THIS JOURNAL, **37**, 2138 (1915).

It is of interest to note both the increase in $\epsilon_{max.}$ and wave length of maxima with increased conjugation in the four hydantoins described (Fig. 2).

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Summary

1. The total synthesis of orotic acid from aspartic acid has been described. The over-all yield obtained was 39%.

2. The rearrangement of 5-(carboxymethylidene)-hydantoin to orotic acid has been discussed.

3. Absorption spectra are given for 5-(carboxy-ethylidene)-hydantoin, 5-(carboxy-methylidene)-hydantoin, benzal-hydantoin and cinnamalhydantoin.

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[CONTRIBUTION FROM THE ENZYME RESEARCH LABORATORY, BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY, AGRICULTURAL RESEARCH ADMINISTRATION, USDA]

Synthesis of Cephalin[1]

BY W. GORDON ROSE

A supply of cephalin uncontaminated by other phospholipids is desired in this Laboratory as a substrate for certain enzyme investigations as well as for contemplating coupling experiments with purothionin.[1a] Since pure cephalin has never been obtained from natural sources, synthesis seemed to be the more promising alternative to obtain this material. Grün and Limpächer[2] described a synthesis of cephalin by heating distearin with phosphoric anhydride and adding ethanolamine carbonate to this reaction mixture. The product that was obtained sintered at 80°, melted with the formation of a meniscus at 177° and decomposed at 185°. Kabashima and Susuki[3a] and Kabashima[3b] also described a synthesis of cephalin. Their procedure consisted of heating together bromoethylamine picrate and the monosilver salt of dipalmitoglycerophosphoric acid. They obtained a yield of 0.5 g. of dipalmitocephalin that melted at 77° from 5 g. of the above silver salt. In spite of the poor yield, the Kabashima procedure appeared to be the more attractive because it seemed less likely that the groups attached to the glycerol would change places, and because of the probability that the unprotected amino group of ethanolamine also participated in the reaction with the phosphatidic acid. After several disappointing experiments on the acylation of glycerophosphoric acid with palmitoyl chloride by the Schotten–Baumann procedure, it appeared that dipalmitoglycerophosphoric acid was more readily accessible by the phosphorylation of dipalmitin.

α,γ-Dipalmitin was first prepared by the direct acylation of glycerol with palmitoyl chloride and dry pyridine. The reaction mixture was allowed to stand several days before adding ether and washing out the pyridine with dilute acid. The product so obtained melted at 68–69°. Averill, Roche and King[4a] reported 69.5°, and Jackson, Daubert, King and Longnecker[4b] reported 72.5°, obtained by Fischer's α-iodohydrin procedure. α,γ-Dipalmitin which we subsequently prepared through the trityl chloride procedure[5] melted at 73.5°. Grün[6] reported 72.6° for the melting point of α,γ-dipalmitin prepared from dihydroxyacetone. Dipalmitin prepared in this Laboratory through the iodohydrin procedure melted at 72.0–72.5° and hence was somewhat less pure

(1) Enzyme Research Laboratory Contribution No. 104.

(1a) Balls, Hale and Harris, *Cereal Chem.*, **19**, 279–288 (1942).

(2) Grün and Limpächer, *Ber.*, **60**, 151 (1927).

(3) (a) Kabashima and Susuki, *Proc. Imp. Acad. (Japan)*, **8**, 492–495 (1931); (b) Kabashima, *Ber.*, **71**, 76–80; 1071–1073 (1938).

(4) (a) Averill, Roche and King, THIS JOURNAL, **51**, 870 (1929); (b) Jackson, Daubert, King and Longenecker, *ibid.*, **66**, 289 (1944).

(5) Verkade, van der Lee and Meerburg, *Rec. Trav. Chim.*, **54**, 716–724 (1935).

(6) Schönfeld Hefter, "Fette und Fettprodukte," Vol. I, "Chemie und Gewinning der Fette," Julius Springer, Vienna, 1936, p. 251.

than that made using the trityl block. On further study of direct acylation of glycerol using palmitoyl chloride and pyridine or quinoline, it was found possible to obtain nearly pure dipalmitin melting at 73.0–73.5° in a yield of 67%, if the reaction mixture was not allowed to stand longer than two hours. Pure α,γ-dipalmitin can be easily obtained from this product by recrystallization from hexane.

We were unable to obtain the monosilver dipalmitoglycerophosphoric acid described by Kabashima. Attempts to purify the crude product resulting from the addition of one equivalent of alcoholic silver nitrate to the monosodium salt of dipalmitoglycerophosphoric acid in hot monomethyl ether of ethylene glycol invariably resulted in the production of the disilver salt. When the crude reaction product was heated with bromoethylamine picrate, the nitrogen content of the reaction product varied from 0.5 to 1.1%. The theoretical nitrogen value for dipalmitocephalin is 2.02%. Recrystallization from various solvents raised the melting point and the nitrogen content, but it was generally not possible to obtain a pure substance for analysis. In one experiment there was obtained 0.36 g. of an ether-insoluble substance that had 2.02% nitrogen but melted at 182–184°. Washing with ether and recrystallizing from alcohol gave a product having a m. p. of 191–193°. When cephalin was prepared by other procedures and the products compared with this one, it was apparent that this preparation was indeed dipalmitocephalin, despite the melting point of 77° reported for dipalmitocephalin by Kabashima. However, the small yield makes the method impractical in any case.

Carbobenzoxyethanolamine was condensed with crude dipalmitoglycerophosphoryl chloride resulting from the reaction of dipalmitin with phosphorus oxychloride in chloroform and pyridine. The carbobenzoxycephalin resulting from this reaction could not be cleaved with hydrogen[7] using platinum oxide or palladium black catalysts. Some cleavage was obtained with sodium in liquid ammonia,[8] but the product was difficult to purify. Cleavage of carbobenzoxycephalin with phosphonium iodide[9] gave a good yield of cephalin melting at 178–193°. Recrystallizing from alcohol raised this m. p. to 193–194°. β-Hydroxyethylphthalimide was also condensed with the crude dipalmitoglycerophosphoryl chloride reaction mixture with the formation of phthalylcephalin. This product was readily cleaved with hydrazine[10] giving an excellent yield of nearly pure cephalin that was readily purified by recrystallization from alcohol. Cephalin separates from this solvent in minute crystals which agglomerate to form spherulites. The crystalline nature of the product is apparent by microscopic examination in polarized light. Dr. F. T. Jones of the Western Regional Research Laboratory examined the optical and crystallographic properties of the product prepared through the silver salt synthesis, that prepared by the phosphonium iodide cleavage of carbobenzoxycephalin and the product obtained through the hydrazine cleavage of phthalylcephalin and said that there was no question as to their identity. X-Ray examination by Dr. K. J. Palmer and Merle Ballantyne of the Western Regional Research Laboratory showed the long spacings of all three specimens to be similar. The long spacings were also very similar to those of natural cephalins. Of the three methods investigated, it is believed that the synthesis through phthalylcephalin is the most satisfactory for practical purposes.

(7) Bergmann and Zervas, *Ber.*, **65**, 1194 (1932).

(8) Loring and du Vigneaud, *J. Biol. Chem.*, **111**, 385–392 (1935).

(9) Harington and Mead, *Biochem. J.*, **29**, 1602–1611 (1935).

(10) Ing and Manske, *J. Chem. Soc.*, 2346 (1926). It is of interest to note that Barber and Wragg, *Nature*, **158**, 514 (1946), and Mosher, THIS JOURNAL, **68**, 1565 (1946) have found that the intermediate in the hydrazine cleavage of phthalimides is a salt of phthalhydrazide, instead of the intermediate postulated by Ing and Manske.

Experimental[11]

Palmitoyl Chloride.—Technical palmitic acid was esterified with methanol. The ester was fractionally distilled and recrystallized from acetone at −18°. The recrystallized product was again fractionally distilled, then saponified. The product so obtained melted at 63.5° and set at 61°. Commercially pure palmitic acid melted at 63.0–63.5° and set at 60°. In spite of the similarity of melting and setting points of these products, dipalmitin made from commercial pure palmitic acid melted at 72.0–72.5° and the melting point could not be raised by fractional crystallization, whereas dipalmitin made from laboratory-purified palmitic acid melted at 73.5°.

Purified palmitic acid (128 g.) was heated under reflux for two hours with 70 g. of thionyl chloride and 100 ml. of carbon tetrachloride. The solvent and excess thionyl chloride were then removed with the aid of a water pump vacuum and the residue distilled rapidly. This distillate was then carefully redistilled yielding 118 g. of palmitoyl chloride boiling at 152–154° at 1 mm.

Preparation of α,γ-Dipalmitin.—Glycerol was distilled in vacuum to obtain an anhydrous product. This (4.60 g.) was placed in a two-necked flask provided with a Hershberg stirrer and a dropping funnel. Anhydrous quinoline (25 ml.) and alcohol-free chloroform[12] (10 ml.) were then added and the reaction flask surrounded with a water-bath maintained at 10–15° by the occasional addition of ice. The stirrer was then started, and 27.5 g. of palmitoyl chloride in 50 ml. of purified chloroform were added during the course of forty-five minutes. A semi-solid mush formed in the reaction mixture during the latter stage of the addition. The reaction mixture was stirred two hours at 10–15°, then one hour at room temperature. Ether (800 ml.) and water were added and the mixture shaken in a separatory funnel. The ether layer was dried with anhydrous magnesium sulfate, filtered and left at 5° overnight. The crystalline dipalmitin (19.0 g.) so obtained melted at 73.0–73.5°. Recrystallization from 600 ml. of hexane gave 18.2 g. of a product that melted at 73.5° and set at 70.5–70.0°. Crystallization from hexane and filtering at 42, 25 and 0° gave three fractions that all melted at 73.5° and gave corrected theoretical active hydrogen values, as determined by the Zerewitinoff method.

Carbobenzoxyethanolamine.—Freshly distilled ethanolamine (24.4 g.) in 50 ml. of water was placed in a three-

(11) All melting points are uncorrected.

(12) The chloroform was freed of alcohol by shaking with concentrated sulfuric acid, washing with water, partially drying with a small amount of calcium chloride, completely drying with phosphorus pentoxide, and finally distilling. This purified chloroform must be stored at low temperature to minimize the formation of phosgene.

necked flask provided with a Hershberg stirrer, the flask was surrounded with ice and water, and 68 g. of carbobenzoxy chloride[7] and 100 ml. of 4 *N* sodium hydroxide were added during the course of forty minutes. Stirring was continued for thirty minutes at ice temperature and for thirty minutes at room temperature. The reaction mixture was then made just acid to congo red paper, and extracted with ether. The ether layer was washed twice with 0.5 *N* hydrochloric acid and with water, dried with magnesium sulfate and left overnight at −30°. Filtration gave 35.2 g. of crystalline product melting at 62.0–62.5° and setting at 61°. Recrystallization from ether at −30° did not alter the melting point. The first ether filtrate was concentrated and added to the filtrate from the second crystallization. Cooling this to −30° gave 12.3 g., which also melted at 62.0–62.5°.

Anal. Calcd. for $C_{10}H_{13}O_3N$: C, 61.52; H, 6.71; N, 7.18. Found: C, 61.41; H, 6.35; N(Dumas), 7.18.

Carbobenzoxycephalin.—Phosphorus oxychloride (3.06 g.) was placed in a two-necked flask with a Hershberg stirrer and a dropping funnel and surrounded by a water-bath maintained at 10–15°. Dry pyridine (25 ml.) and purified chloroform (25 ml.) were added. The stirrer was started and 11.4 g. of dipalmitin in 50 ml. of chloroform was added during thirty minutes.

Although we did not isolate and purify the intermediate dipalmitoglycerophosphoryl chloride, we did obtain the acid analytically pure, as follows: Most of the chloroform was removed from the phosphorus oxychloride–dipalmitin reaction mixture, and it was stirred in a Waring Blendor with ice and water. This mixture was filtered and again stirred with ice and water in the Blendor. The mixture was then stored at 5° for several hours, filtered and allowed to dry in air and finally over phosphorus pentoxide. The crude product from 28.4 g. of dipalmitin, 7.67 g. of phosphorus oxychloride and 8 ml. of quinoline consisted of a quinoline salt of dipalmitoglycerophosphoric acid and several by-products; it weighed 35.3 g., sintered at 75° and melted at 88–90°. It was purified by dissolving in 170 ml. of chloroform and precipitating by slow addition of 600 ml. of Skellysolve F. Scratching, when about 200 ml. Skellysolve F had been added, induced crystallization. The purified product weighed 15.3 g., sintered at 85° and melted at 102–104°.[13] To obtain free dipalmitoglycerophosphoric acid, 5.2 g. of this purified quinoline salt was dissolved in 100 ml. of warm ethanol, the hot solution was clarified by filtration through cotton, 10 ml. of concentrated hydrochloric acid was added and the solution was cooled rapidly to room temperature. It was then cooled to 0° and filtered. This treatment with acid was repeated and the product was then recrystallized from absolute ethanol. It is necessary to work rapidly to minimize cleavage of the fatty acids from the phosphatidic acid. After drying in vacuum with solid potassium hydroxide, the product weighed 4.05 g. and melted at 62–63°.

Anal. Calcd. for $C_{35}H_{69}O_8P$: C, 64.78; H, 10.72; P, 4.77; equiv. wt., 324.46. Found: C, 64.81, 64.85; H, 11.05, 10.95; P, 4.77, 4.95; equiv. wt. (titration in neutral alcohol, thymolphthalein indicator), 330, 331.

After stirring the phosphorus oxychloride–dipalmitin reaction mixture thirty minutes at 30–35°, 3.90 g. carbobenzoxyethanolamine in 10 ml. of chloroform was added at 10–15° during twenty minutes. This reaction mixture was stirred one hour at room temperature and thirty minutes at 35–40°, cooled to 25° and 0.4 ml. of water in 4 ml. of pyridine was added. After stirring fifteen minutes at 25° and fifteen minutes at 35–40°, most of the chloroform was removed in vacuum. Ice, water and ether were added and the mixture agitated in a separatory funnel. The aqueous layer was badly emulsified and was drawn off and acidified with 2 *N* hydrochloric acid, which broke the emulsion. The ether layer that separated was returned to the main ether solution which was then washed with 4 portions of 0.5 *N* hydrochloric acid and with water. Sometimes emulsions formed which were separated by centrifuging in capped centrifuge bottles. The ether solution was filtered through cotton and left at 5° overnight. Filtration gave 1.5 g. of a by-product that melted at 69–77°. This was not further investigated. The ether filtrate was left at −18° for six hours, then filtered at this temperature giving 11.4 g. of material that sintered at 38° and melted at 40–46°. It was purified by warming with 250 ml. of methanol, decanting the solution from a small amount of molten insoluble material, chilling the solution to 0° and filtering. The resulting pasty product was recrystallized from 100 ml. of ether at −18°. Yield was 7.2 g. of carbobenzoxycephalin, m. p. 39.5–40°.

Anal. Calcd. for $C_{45}H_{80}O_{10}PN$: C, 65.42; H, 9.76; P, 3.75; N, 1.70. Found: C, 64.88, 64.88; H, 9.86, 9.93; P, 3.77; N (Dumas), 1.82, 1.82.

Phthalylcephalin.—β-Hydroxyethylphthalimide was prepared by adding 28.0 g. of ethanolamine to 59.2 g. of phthalic anhydride, and, after the initial heat of reaction had subsided, heating at 150° for thirty minutes. The mixture was allowed to cool to about 90°, and then poured into 800 ml. water. The crystals so obtained were chilled in ice and filtered, giving 46.7 g. of β-hydroxyethylphthalimide, m. p. 126–127°. Recrystallization from chloroform did not raise the melting point.[14]

Anal. Calcd. for $C_{10}H_9O_3N$: C, 62.82; H, 4.74; N, 7.33. Found: C, 62.81; H, 4.65; N, 7.40.

Phosphorus oxychloride (9.18 g.) was placed in a two-necked flask with 30 ml. of dry pyridine and 40 ml. of chloroform. The flask was provided with a Hershberg stirrer and surrounded by a water-bath at 10–15° and the stirrer was started. Then 34.2 g. of dipalmitin in 200 ml. of purified chloroform was added during the course of one hour. The solution was stirred at 25° for thirty minutes, then at 30–35° for thirty minutes, then cooled to 10–15° again and 11.45 g. of β-hydroxyethylphthalimide in 200 ml. of chloroform added during the course of one hour. The solution was then stirred at 25° for thirty minutes and at 30–35° for thirty minutes, cooled to 25° and 1.15 ml. of water in 4 ml. of pyridine added. Most of the chloroform was then removed with the aid of a water-pump vacuum, and ice and water were thereafter added to the residue. The mixture was transferred to a separatory funnel with the aid of ether, and was shaken moderately. The emulsified aqueous layer was withdrawn, acidified and the ether that separated added to the main ether solution. The ether layer was washed with dilute hydrochloric acid and with water and was then filtered through a thick soft paper or through cotton and left overnight at 5°. Filtration removed 3.4 g. of material that melted at 50–60°, but did not become clear until 160°. To obtain the principal reaction product the filtrate was next left for five hours at −18° and filtered at this temperature. The precipitate retained much ether, and was air-dried overnight as it melted in the retained solvent when it was dried in a vacuum desiccator without preliminary air drying. The dried product weighed 39.5 g. and melted at 43–46°. It was found later to be largely phthalylcephalin, but contained several other substances.

The crude material was purified by dissolving in 600 ml. of hot hexane, filtered hot and allowed to stand eighteen hours at room temperature. Filtration gave 4.8 g. of impure phthalylcephalin that sintered at 63° and melted at 70–76°. This was dissolved in 50 ml. of methanol, filtered hot and allowed to stand at room temperature overnight. Filtration gave 3.0 g. of crystals of phthalylcephalin that sintered at 64° and melted at 67–68°. The hexane filtrate (above) contained still more phthalylcephalin. It was concentrated to 200 ml., seeded with some of the crystals from the methanol crystallization and thereupon deposited

(13) In a private communication, E. B. Kester of the Western Regional Research Laboratory stated that the quinoline salt of dimyristophosphatidic acid contains one mole of quinoline to two moles of phosphatidic acid. It is probable that our dipalmitophosphatidic acid is of the same type.

(14) Gabriel and Ohle, *Ber.*, **50**, 820 (1917), prepared β-hydroxyethylphthalimide, melting at 126.5 to 127.5°, by heating ethylene oxide and phthalimide in a sealed tube at 170°.

more crystals when allowed to stand at room temperature for three hours with occasional stirring. Filtration gave 14.7 g. that sintered at 60° and melted at 64–73°. This product was dissolved in 150 ml. of hot methanol, the hot solution filtered through cotton and allowed to stand sixteen hours at room temperature. Filtration gave 12.5 g., that sintered at 69° and melted at 70–72°. The two products that had been recrystallized from ethanol were combined and recrystallized from 200 ml. of hexane; the solution was filtered after standing one hour at room temperature. The yield was 13.25 g. of product that melted at 71–72°.

Anal. Calcd. for $C_{45}H_{76}O_{10}NP$: N, 1.70; P, 3.77; C, 65.74; H, 9.32; equiv. wt., 822.1. Found: N, 1.66; P, 3.85; C, 66.09; H, 9.47, equiv. wt., 825.

Cleavage of Phthalylcephalin.—Phthalylcephalin (4.11 g.) was dissolved in 100 ml. of hot neutral monomethyl ether of ethylene glycol and neutralized by the addition of 10 ml. of 0.5 *N* sodium hydroxide. Hydrazine hydrate in monomethyl ether of ethylene glycol (7.3 ml. of 1.45 *M* solution) was added, and the solution heated on a steam-bath, under reflux for thirty minutes. The solution was cooled, 10 ml. of 6 *N* hydrochloric acid was added, then allowed to stand at room temperature for fifteen minutes. The reaction mixture was then poured into 1 liter of cold water and allowed to stand for two hours. The cephalin separated out as a finely divided solid.

Removal of the cephalin was accomplished by taking advantage of the unexpected property that it is insoluble in ether but is very readily suspended therein. The water suspension was transferred to a separatory funnel with 300 ml. of ether, shaken and the aqueous layer removed. The ether layer and the solid that collected at the ether-water interface were washed with water until the washings were neutral. The ether containing the suspended solid was then filtered on a Hirsch funnel, washed with ether and dried. The dry residue amounted to 2.9 g. and melted at 178–200°. Recrystallization from 190 ml. of absolute alcohol gave 2.47 g. that sintered at 187° and melted with decomposition at 192–193° or 195–198° depending on the rate of heating. Further recrystallization from alcohol did not alter the melting point.

Anal. Calcd. for dipalmitocephalin, $C_{37}H_{74}O_8NP$: N, 2.02; P, 4.48; C, 64.22; H, 10.78; equiv. wt., 692.0. Found: N (Dumas), 2.07; amino N (Van Slyke in acetic acid), 1.86; P, 4.50; C, 63.75; H, 10.52; equiv. wt. (titration in neutral alcohol), 689.

It was necessary to add vanadium pentoxide to the substance, as described for phosphoric acid esters by Wagner-Jauregg and Griesshaber,[15] to obtain correct carbon analyses. Combustion of the substance alone, or with copper oxide gave carbon values that were 2 to 3% low.

Cleavage of Carbobenzoxycephalin.—Carbobenzoxycephalin (4.8 g.) was dissolved in 75 ml. of glacial acetic acid and 5.0 g. of phosphonium iodide added in small portions, with frequent stirring. The reaction mixture was left at 40° overnight, protected from atmospheric moisture with a drying tube. Alcohol (5 ml.) was then added to decompose the excess phosphonium iodide, then 200 ml. of water was added. Ether was added and the mixture was agitated in a separatory funnel. The water layer was removed and the interface layer was transferred to a Hirsch funnel and washed with ether. This product weighed 1.75 g., sintered at 135°, and melted at 178–193°. Recrystallization from absolute ethanol gave a product that melted at 193–194°.

Anal. Calcd. for $C_{37}H_{74}O_8NP$: C, 64.22; H, 10.78; N, 2.02; P, 4.48. Found: C, 63.98; H, 10.78; N, 2.06; P, 4.43.

Preparation of Derivatives.—In view of the wide differences in the melting point (195–198° for this dipalmitocephalin) and those of the cephalin preparations of Grün and Limpächer (177° for distearocephalin) and of Kabashima (77° for dipalmitocephalin) it seemed desirable to characterize further the product obtained in this work. This was done by the preparation of a 3,5-dinitrobenzamide and of the ethylene glycol ester of dipalmitoglycerophosphoric acid from the dipalmitocephalin obtained through phthalylcephalin.

Cephalin (173 mg.) was dissolved in 5 ml. of warm chloroform, 0.5 ml. of dry pyridine was added, followed by 62 mg. of 3,5-dinitrobenzoyl chloride, that had been purified by recrystallization from hexane. The reaction mixture was allowed to stand two hours, then most of the chloroform was removed with the aid of a water-pump and the residue was shaken with ether and 0.5 *N* hydrochloric acid. The ether layer was washed with dilute hydrochloric acid and water, cooled to −20° and filtered at this temperature. The product so obtained weighed 110 mg., sintered at 74° and melted at 77–78°. Recrystallization from hexane gave 63 mg. that melted at 82–84°. Further recrystallization did not raise the melting point.

Anal. Calcd. for dipalmitocephalin dinitrobenzamide, $C_{44}H_{76}O_{13}N_3P$: C, 59.64; H, 8.65; N, 4.74; P, 3.50. Found: C, 59.50; H, 8.55; N, 4.88; P, 3.45.

For the replacement of the $-NH_2$ group by the —OH group, 510 mg. of the cephalin was dissolved in 25 ml. of warm acetic acid and 10 ml. of 30% sodium nitrite was added during the course of thirty minutes. The first addition of sodium nitrite was characterized by the formation of much foam, but no foaming resulted from the final nitrite addition. Water (100 ml.) was added to the reaction mixture which was then agitated with ether. Two hundred and fifty mg. of unreacted cephalin was recovered. The ether layer was washed five times with water, chilled in ice and the slightly turbid solution was filtered. This filtrate was left twelve hours at −20° and filtered at this temperature. The product weighed 180 mg. and melted at 45–46°, with the formation of many minute bubbles in the melt. Recrystallization from hexane gave 156 mg. that melted at 49–51°, without the formation of bubbles. Further recrystallization from ether, methanol and ether did not alter the melting point.

Anal. Calcd. for the ethylene glycol ester of dipalmitoglycerophosphoric acid, $C_{37}H_{73}O_8P$: C, 64.13; H, 10.62; P, 4.48. Found: C, 62.81, 62.61; H, 10.31, 10.16; P, 4.47, 4.52; nitrogen was absent.

The low carbon values were obtained even when vanadium pentoxide was used, and identical values were obtained when no vanadium pentoxide was used and when a moderate amount was employed.

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Summary

Two new syntheses of dipalmitocephalin from dipalmitin are described that give over-all yields of about 25% of the dipalmitin used. A minute quantity of the same substance was also obtained by the published method of Kabashima. The product is microcrystalline, and melts at 195–198° (instead of 77° as reported by Kabashima). The constitution of the product follows from its synthesis by three independent methods, its quantitative reaction with nitrous acid, and the composition of a 3,5-dinitrobenzoyl derivative.

(15) Wagner-Jauregg and Griesshaber, *Ber.*, **70**, 1458 (1937).

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