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THE CONDENSATION OF AZIRIDINE WITH PHOSPHATIDIC ACID; SYNTHESIS OF 0-(1,2-DIACYL-*sn*-GLYCERO-3-PHOSPHORYL)-ETHANOLAMINE

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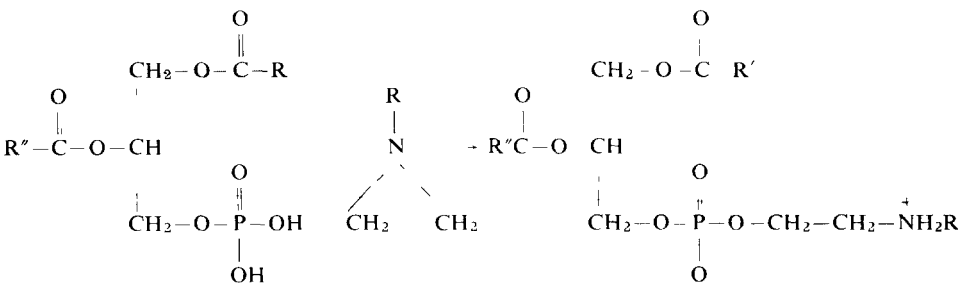
The condensation of N-tritylaziridine with phosphatidic acid is facile and gives a good yield of N-trityl phosphatidylethanolamine which is detritylated to a phosphatidylethanolamine This novel route is illustrated by the synthesis of 0-(1,2-dioctadecanoyl-*sn*-glycero-3-phosphoryl)-ethanolamine

Introduction

Several total syntheses of 0-(1,2-diacyl-*sn*-glycero-3-phosphoryl)-ethanolamine[1] (P E) have been published[2], and welcome improvements and modifications to the most successful of the traditional methods continue to appear[3–5] Attempts to develop new routes have also been made, but have met with partial success only[6] We describe now a short and practical synthesis by a novel route in which the key step is the condensation of an aziridine(II) with a phosphatidic acid (I)



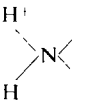
I II III

The condensation of aziridine and phosphoric acids

The ring-opening-addition of phosphoric acids to aziridine has received scant attention in the literature Two early studies of the reaction of (syrupy) phosphoric acid with aziridine variously describe the products as 2-amino-ethyldihydrogen phosphate[7] and as tris-(2-amino-ethyl)-phosphate[8] In

contrast, 2-aminoethyl-dibenzyl phosphate was not obtained by the action of aziridine on dibenzylhydrogen phosphate[9]. More recently, the successful condensation of aziridine with cytidine diphosphate[10a], and with monophenylphosphate[10b], gave moderate yields. However, early in the present study, the reaction of phosphatidic acid (I) with aziridine (II, R = H) gave P.E. (III, R = H) albeit in poor yield.

By analogy to the other ring-opening reactions of aziridine[11], the condensation probably proceeds via the initial formation of the strained protonated species



which then undergoes nucleophilic attack by the phosphate anion, nucleophilic attack by another aziridine molecule leads to di- (and ultimately) polymerisation and presumably is a major cause of the poor yield[12].

N-tritylaziridine [II, R = C$(C_6H_5)_3$]

It would appear, therefore, that an aziridine derivative, e.g., with an electron attracting substituent on the nitrogen atom[13] would be more suitable, since it would be less prone to polymerisation, whereas the ring carbon atoms would be more susceptible to nucleophilic attack. The resulting product would be an N-substituted P.E., and, therefore, the substituent chosen must be easily removable to yield P.E. (III, R = H).

Initially, however, the trityl group was selected as the N-substituent. N-tritylaziridine was readily prepared in good yield by the reaction of tritylbromide and aziridine in the presence of triethylamine. In this case the large bulk of the trityl group should prevent N-tritylaziridine from acting as an efficient nucleophile and preclude its polymerisation. It is not certain if the N-trityl group enhances the electrophilic character of the aziridine ring carbon atoms[11], and its choice was influenced by the fact that the recovery of P.E. from N-trityl P.E. is straightforward[5].

Phosphatidic acids

The phosphatidic acid, 1,2-dioctadecanoyl-*sn*-glycero-3-phosphoric acid (I, R′ = R″ = $C_{17}H_{35}$), used in this work was prepared by the method of Bird and Chadha[15].

However, several other methods are available[2]. The DL-compounds are readily accessible by acylation of glycerol-3-phosphoric acid[16]. The action[17] of phospholipase D on natural[17a] or synthetic[17b] 1,2-diacyl-*sn*-glycero-3-phosphorylcholines (or other phospholipids) could be an attractive alternative in certain cases.

0-(1,2-dioctadecanoyl-sn-glycero-3-phosphoryl)-ethanolamine

The new procedure is illustrated by the synthesis of the title compound (III, $R'=R''=C_{17}H_{35}$, R=H)

The condensation of N-tritylaziridine [II $R=C(C_6H_5)_3$] and 1,2-dioctadecanoyl-*sn*-glycero-3-phosphoric acid (I $R'=R''=C_{17}H_{35}$) was effected in chloroform solution at room temperature during 40 hours The product N-trityl-0-(1,2-dioctadecanoyl-*sn*-glycero-3-phosphoryl)-ethanolamine [III, $R'=R''=C_{17}H_{35}$, $R=C(C_6H_{53})$] recovered in 66% yield, was identical (m p , $[\alpha]_D$, p m r) with an authentic sample prepared by the direct tritylation of 0-(1,2-dioctadecanoyl-*sn*-glycero-3-phosphoryl)-ethanolamine Detritylation was carried out by hydrogenation using a 10% Pd/C catalyst, alternatively, hot 90% acetic acid may be employed[5] The final product was identical with authentic 0-(1,2-dioctadecanoyl-*sn*-glycero-3-phosphoryl)-ethanolamine[4 18 19]

Experimental

General methods

Infrared spectra were determined on a Perkin-Elmer infracord spectrophotometer, model 257 Optical rotations were recorded for ethanol-free chloroform solutions on a Perkin-Elmer 141 Polarimeter P m r spectra were measured on a Perkin-Elmer R 12 spectrometer for deuterochloroform solutions, with tetramethylsilane as the internal standard Melting points were determined on a Kofler hot stage apparatus and are uncorrected Elemental analyses were carried out by Dr F Pascher, Bonn

1,2-Dioctadecanoyl-sn-glycero-3-phosphoric acid
(I, $R'=R''=C_{17}H_{35}$)

This compound was prepared by the method of Bird and Chadha[15], m p 72–73°, $[\alpha]_D+3\,5^\circ$ (C,12 5) (lit ,[20], m p 75 5–76 5° and $[\alpha]_D=+3\,7^\circ$)

Aziridine (II, R=H)

The compound was a generous gift from the Dow Chemical Company Limited

N-tritylaziridine [II, $R=C(C_6H_5)_3$]

A solution of aziridine (2 g, 0 046 mole) and triethylamine (6 g, 0 059 mole, *dried over KOH*) in dry benzene (150 ml) was cooled to 0° A solution of tritylbromide (20 g, 0 061 mole) in benzene was added with stirring over a 1hr period and further stirred for 1 hr at room temperature The precipitate

of triethylamine hydrobromide was removed, the solvent evaporated from the filtrate and the residue in hexane chromatographed on alumina (basic Woelm, grade I, 80 g) Initial fractions eluted with hexane contained trityl-bromide and some N-tritylaziridine, but subsequent fractions contained pure N-tritylaziridine, which crystallised from hexane as fine needles, (11 g, 83%), m p 125–126°, νmax (nujol mull), 3060, 695, 710 750 and 770 cm^{-1}, PMR, τ, 2 4–2 9 (15H, multiplet), 8 14 (2H, broad), 9 0 (2H, broad)

Found	C 88 13	H 6 61	N 4 84%
Calcd for $C_{21}H_{19}N$ (M = 285 3)	C 88 38	H 6 71	N 4 91%

N-trityl-O-(1,2-dioctadecanoyl-sn-glycero-3-phosphoryl)-ethanolamine [III $R' = R'' = C_{17}H_{35}$, $R = C(C_6H_5)_3$]

A mixture of N-tritylaziridine (99 mg, 0 35 mmole) and 1,2-dioctadecanoyl-*sn*-glycero-3-phosphoric acid (237 mg, 0 32 mmole) in ethanol-free dry chloroform (6 ml) was allowed to stand at room temperature for 40 hr, and then refluxed for 15 min (the formation of N-trityl P E was monitored by T L C on Silicagel G using chloroform methanol, 4 1 N-trityl P E, Rf ~ 0 8, appeared blue when sprayed with Zinzadze phosphate reagent, but became yellow on warming, the yellow colour is characteristic of trityl compounds)

N-trityl P E was isolated from the reaction mixture by chromatography on silica impregnated with triethylamine A silica column was prepared by stirring silica (SilicAR, CC-7, Mallinkrodt, 40 g) with chloroform benzene (1 1, v/v containing 20% triethylamine, 150 ml) and then pouring the slurry into the column The column was washed with benzene chloroform (1 1, v/v containing 1% triethylamine, 100 ml), the reaction mixture applied and elution continued with benzene chloroform (1 1, v/v containing 1% triethylamine, 150 ml) followed by chloroform benzene (4 1, v/v containing 1% triethylamine 250 ml) with which N-trityl P E was eluted After evaporation to dryness the last traces of the solvent and all triethylamine were removed by evacuation under high vacuum (0 01 mm Hg) at room temperature The residue (210 mg, 66%), m p 113–114°, $[\alpha]_D^{20} + 6\,45°$ (C,2 5) was identical with N-trityl P E (III $R' = R'' = C_{17}H_{35}$, $R = C(C_6H_5)_3$) prepared as follows

Tritylation of O-(1,2-dioctadecanoyl-sn-glycero-3-phosphoryl)-ethanolamine

The title compound[4]) (100 mg, 0 13 mmole, dried by continuous evacuation over P_2O_5) and tritylbromide (108 mg, 0 33 mmole) in dry chloroform (10 ml, ethanol free, freshly distilled over P_2O_5) were treated with anhydrous triethylamine (0 1 ml dried over KOH and distilled) The mixture was stirred at room temperature for 30 min The solvent was removed on a rotary evaporator and the compound purified by chromatography on SilicAR CC-7 (17 g) as described in the foregoing experiment The product obtained, after

drying on the pump over P_2O_5 for 30 hr (yield 128 mg, 97%) had m p 114–15°, $[\alpha]_D^{22}+6.6°$ (C, 2)

Found	C 72.29	H 9.56	N 1.49%
Calcd for $C_{60}H_{96}O_8NP\frac{1}{2}H_2O$ (M = 999.35)	C 72.10	H 9.78	N 1.40%

0-(*1,2-Dioctadecanoyl-sn-glycero-3-phosphoryl)-ethanolamine* (III, R′ = R″ = $C_{17}H_{35}$, R = H)

A suspension of N-trityl P E (III, R′ = R″ = $C_{17}H_{35}$, R = C $(C_6H_5)_3$) (120 mg, 0.12 mmole) in glacial acetic acid (25 ml) together with 10% Pd/C catalyst (150 mg) was hydrogenated (Parr hydrogenation apparatus) at 25 p s i for 20 hr The mixture was diluted with methanol (60 ml) and chloroform (60 ml), and filtered through a bed of hyflo supercell The solvent was removed, the residue triturated with anhydrous ether to give P E as a white insoluble powder, which crystallised from dioxan (80 mg 87%) m p 187 $[\alpha]_D = +6.15°$ (C, 1, chloroform-acetic acid 9:1), lit m p 188–89°, $[\alpha]_D = +6.2$ (ref 4), m p 180–182 (ref 20), $[\gamma]_D^{24}+6.0$ (C, 4.4) (ref 19)

Acknowledgments

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14) In N-tritylaziridine the averaged chemical shift of the aziridine ring protons is τ 8.57 (c f the experimental section) For comparison, a chemical shift value may be computed by adding the observed CH chemical shift in aziridine (τ 8.47) to the long range positive shielding contribution of the benzene rings in tritylaziridine, for a benzene ring rigidly positioned over the aziridine CH the latter contribution is estimated to be

ca τ 0 5 [using Dreiding models and the shielding field contour maps of the benzene molecule, given by C E Johnson and F A Bovery, J Chem Phys **29** (1958) 1012] The computed chemical shift is thus τ 8 47 $\pm$ 0 5 — τ 8 97 The observed lower chemical shift would indicate a lower electron density and enhanced electrophilicity of the aziridine ring carbons Since, in reality, the benzene rings are in motion, the calculations and arguments are less certain

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