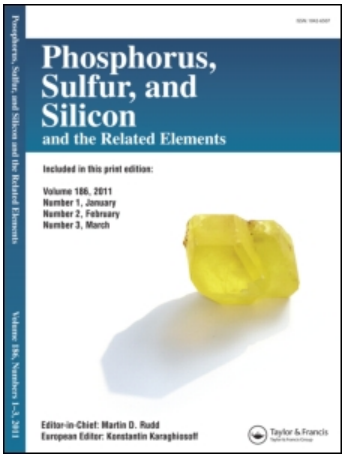

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SYNTHESIS OF (R)- AND (S)-1,2-DIACYLOXYPROPYL-3-ARSONIC ACIDS: OPTICALLY ACTIVE ARSONOLIPIDS

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SYNTHESIS OF (R)- AND (S)-1,2-DIACYLOXYPROPYL-3-ARSONIC ACIDS: OPTICALLY ACTIVE ARSONOLIPIDS

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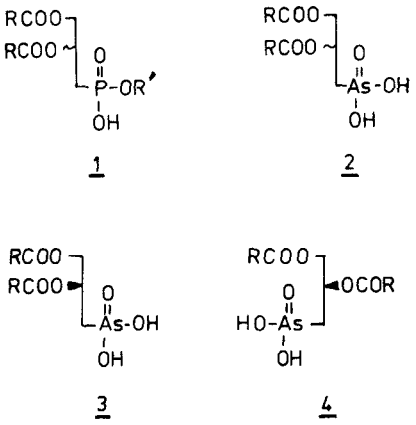
Reaction of trisodium arsenite with (R)- and (S)-glycidol affords in good yields the (R)- and (S)-1,2-dihydroxypropyl-3-arsonic acid, the tetrabutylammonium salt of which upon acylation with myristic, palmitic and stearic anhydrides in the presence of pyridine gives, in moderate to low yields, optically active arsonolipids, i.e., (R)- and (S)-1,2-diacyloxypropyl-3-arsonic acids. The thermotropic phase transitions of these arsonolipids are characterized. Results show that the transition temperatures and enthalpies of fusion of the racemates are lower than those of the enantiomers in the powder form presumably because of conglomerate formation. The thermotropic transition characteristics of the aqueous dispersions of the arsonolipids change with the acyl chain lengths, and the overall behaviour is similar to that of the aqueous dispersions of phospholipids.

Key words: Optically active arsonic acids; acylation; decomposition; optically active arsonolipids; thermotropic phase transitions; conglomerates.

INTRODUCTION

Racemic or optically active synthetic phosphonolipids (**1**) are of interest not only because they can be used to regulate or perturb metabolic pathways[1] but they can also be used as analogues of phospholipids in biophysical and biochemical studies to understand intermolecular interactions in model membranes.[2] Substituting As for P in phosphonolipids gives a new class of compounds, arsonolipids,[3] (**2**). The biophysical and biochemical properties of these molecules are of interest because the size, polarities and the hydrogen bonding properties of the —$AsO(OH)_2$ and the —$PO(OH)_2$ groups differ. Moreover, redox reactions on the arsenic center under mild[4] conditions offer potential for the study of such processes at the interfaces.

Following the synthesis of racemic arsonolipids (**2**)[5] we have synthesized the enantiomeric arsonolipids (**3**) and (**4**), namely (R)- and (S)-1,2-distearoyloxy, 1-2-dipalmitoyloxy-, and 1,2-dimyristoyloxypropyl-3-arsonic acids starting from (R)- and (S)-glycidol, respectively. We also report the thermotropic properties of homologous arsonolipids in aqueous dispersions as well as dry powders.



SCHEME I

RESULTS AND DISCUSSION

Investigating the Meyer reaction between sodium arsenite and 2-chloroethanol or 3-chloro-1,2-propanediol we found[3] that the reaction proceeds and *via* the epoxides which are formed *in situ*. Previously Chelintsev and Kuskov[6] noted that β-hydroxyethylarsonic acid can be prepared by bubbling ethylene oxide in aqueous potassium arsenite. Therefore the reaction of (R)- and (S)-glycidol, which are available commercially or through Sharpless' epoxidation,[7] should give (R)- and (S)-1,2-dihydroxypropyl-3-arsonic acids ($DPAH_2$) respectively which upon arylation[5] would give the desired optically active arsonolipids (**3**) and (**4**).

A study on the modified Meyer reaction between sodium arsenite and *rac*-glycidol was undertaken in order to find the conditions for higher yields of $DPAH_2$. The nucleophile in the Meyer reaction is the anion AsO_3^{3-},[6] which functions at high pH values in the presence of concentrated solutions of sodium hydroxide.[8] In our case sodium hydroxide is produced during the reaction and therefore we did not use it in excess. However, concentrated solutions of the reactants were used in order to suppress the hydrolysis of Na_3AsO_3. Slow addition of glycidol at room temperature followed by stirring at 50°C for 3 h completes the reaction. We did not see any dramatic improvement in the (titrimetric) yield when we used up to 30% mole excess of sodium arsenite over glycidol and vice versa. Under the conditions described in the experimental section the (R)- and (S)-$DPAH_2$ were isolated in good yields (70–80%) contaminated with 6–7% As_2O_3, 3–4% ethanol (by ^{1}H-NMR) and were essentially free of glycerol (overspotted TLC). Further removal of As_2O_3 was not carried out because the isolated yield drops to ~45%[3] and because As_2O_3 does not interfere in the acylation step which follows.

Nucleophilic additions to glycidol occur exclusively at the primary ring-carbon.[9] Therefore, the Meyer reaction with glycidol as substrate should give $DPAH_2$.[3] With α,β-epoxy alcohols in the strongly basic system of the Meyer reaction there is always a possibility of Payne rearrangement[9,10] (epoxide migration), before nucleophilic attack by the AsO_3^{3-} takes place, but in the case of (optically active)

glycidol the rearrangement is degenerate and does not lead to racemization.[9] The product (R)- or (S)-$DPAH_2$ is stable in alkaline environment,[3] and therefore optical purity of the product will depend on the optical purity of the starting glycidol.

Direct determination of the optical purity[11] of the (R)- and (S)-$DPAH_2$ via their Mosher esters or diesters[12] is not feasible because these acids or their salts upon reaction with acylating agents suffer decomposition[5,13] to various degrees. Because $DPAH_2$ is soluble only in water, DMSO and formic acid, Eu(III) based chiral shift reagents cannot be used[14] for the determination of their optical purity.

The very hygroscopic neutral salts *rac*-, (R)- and (S)-$DPA(Bu_4N)_2$ were prepared and acylated under the same conditions used for acylation of *rac*-$DPAH(Bu_4N)$.[5] The yields of arsonolipids (**3**) and (**4**), shown in the Table, are similar to those obtained by using the acid salt. A possible explanation is that after the acylation of the primary —OH group the following reactions take place:

$$RCOO^-pyH^+ \rightleftharpoons RCOOH + py$$

$$—As(O)(OBu_4N)_2 + RCOOH \rightarrow —As(O)(OH)(OBu_4N) + RCOOBu_4N$$

converting the neutral salt into the acid salt which then behaves as expected.[5,13] An unexplained observation was that the decomposition of the (S)-$DPA(Bu_4N)_2$ during the acylation (9 days) was 33–44% to As(III) while that of the (R)-DPA $(Bu_4N)_2$ under the same conditions was 10–15%.

$DPAH(Bu_4N)$ and $DPA(Bu_4N)_2$ could not be acylated in dichloromethane, 1,2-dichloroethane or DMF under various conditions using fatty acid anhydride and pyridine.

There is an increase by ~15°C in the capillary melting points in going from (optically active) phosphatidic acids[15] to phosphotidic acids[16] to arsonolipids (**3**) or (**4**) (Table) with the same acyl groups, implying stronger intermolecular hydrogen bonding of the head groups in arsonolipids. The melting points of the optically active arsonolipids (**3**) and (**4**) with the same acyl groups are the same but they are 5–10°C higher than the melting points of the corresponding racemic arsonolipids (**2**) (Table and Reference 5).

Mixed melting point determinations revealed that the racemic arsonolipids (**2**) form racemic mixtures (conglomerates) and, in principle, microcalorimetric measurements[11,17] should provide an estimate of the optical purity of arsonolipids (**3**) and (**4**). Since in the samples of (**3**) and (**4**) we did not detect any endotherm due to their respective conglomerates we conclude that they are >95% optically pure.

From the Table it is evident that the enthalpies of fusion of the racemic arsonolipids (**2**) are substantially lower than the enthalpies of fusion of the corresponding optically active arsonolipids (**3**) or (**4**). However, the enthalpies of fusion of the (S)-arsonolipids, (**4**), are lower than the enthalpies of fusion of the (R)-arsonolipids, (**3**), although their melting points are virtually the same. Since optical antipodes should differ only in their optical rotation,[18] the difference in the enthalpies of fusion must be due to a difference in the intermolecular interactions that may also be reflected in the packing of the head groups.

The arsonolipids are not easily dispersed in water by sonication but they do so in buffer solutions, pH ≥ 8, because they are converted into their acid salts. The

TABLE

Physical constants and elemental analyses of 1,2-dihydroxypropyl-3-arsonic acids and 1,2-diacyloxypropyl-3-arsonics acids.

Compound	Yield, %	Capillary m.p., °C	Thermal analys. m.p., °C	Thermal analys. ΔH, kcal/mol	Optical purity, %	$[\alpha]_D^{25}$	Molecular formula	Elemental analyses C Calcd	Elemental analyses C Found	Elemental analyses H Calcd	Elemental analyses H Found	Tm^e, °C	ΔH^e, kcal/mol
(R)-DPAH$_2$[a]	79	222 dec.	-	-	-	-17.6[d]	$C_{13}H_{19}O_5As$	-	-	-	-	-	-
(S)-DPAH$_2$[b]	75	220 dec.	-	-	-	+17.8[d]	$C_{13}H_{19}O_5As$	-	-	-	-	-	-
rac-2, R=$C_{17}H_{35}$	-	91-93[c]	91.1	35.0	-	-	$C_{31}H_{61}O_7As$	-	-	-	-	58.6	4.9
(R)-3, R=$C_{17}H_{35}$	19	100-101	102.0	69.2	> 95	+2.0[e]	$C_{31}H_{61}O_7As$	59.98	59.94	9.90	9.86	54.0	5.0
(S)-4, R=$C_{17}H_{35}$	25	101-102	101.6	64.0	> 95	-2.0[e]	$C_{31}H_{61}O_7As$	59.98	60.25	9.90	9.89	48.4	5.2
rac-2, R=$C_{15}H_{31}$	-	96-97[c]	96.0	35.0	-	-	$C_{29}H_{59}O_7As$	-	-	-	-	74.0	8.5
(R)-3, R=$C_{15}H_{31}$	19	103-105	104.1	59.9	> 95	+2.0[e]	$C_{29}H_{59}O_7As$	62.11	62.37	10.27	10.30	66.0	7.2
(S)-4, R=$C_{15}H_{31}$	30	103-104	104.0	49.3	> 95	-2.0[e]	$C_{29}H_{59}O_7As$	62.11	62.20	10.27	10.29	66.0	7.2
rac-2, R=$C_{21}H_{43}$	-	102-103[c]	100.6	32.0	-	-	$C_{35}H_{71}O_7As$	-	-	-	-	> 80	-[g]
(R)-3, R=$C_{21}H_{43}$	37	107-110	109.6	65.7	> 95	+2.0[e]	$C_{35}H_{71}O_7As$	63.91	64.17	10.59	10.31	> 80	-[g]
(S)-4, R=$C_{21}H_{43}$	28	107-110	109.2	43.1	> 95	-2.0[e]	$C_{35}H_{71}O_7As$	63.91	64.14	10.59	10.64	> 80	-[g]

a: contains 7.2% As_2O_3 ; **b**: contains 6,3% As_2O_3 ; **c**: ref. 5 ; **d**: (c, 5.0 ; H_2O) ; **e**: (c, 1.0 ; $CHCl_3$/MeOH 7:2 v/v) ; **f**: dispersed in Tris buffer pH 8.00 ; **g**: slow evaporation of water takes place above ~80 °C.

gel-to-fluid thermotropic phase transition profiles obtained at pH 8.00 by D.S.C. were, in all cases, symmetrical but broad presumably because of the equilibria:

$$—As(O)\ (OH)_2 \rightleftharpoons —As(O)\ (OH)O^- \rightleftharpoons —As(O)O_2^{2-}$$

From these profiles the temperature at the midpoint of the endothermic phase transition, T_m, and the enthalpy of the transition, ΔH, were obtained.[19] The size of the cooperative unit, n, is about 50 but because n is very sensitive to the presence of different ionic species and to traces of impurities[20] the number obtained should be viewed with caution. We did not observe such thermotropic transitions with the anhydrous arsonolipids (**3**) and (**4**) which suggests that the arsonolipids do form multilamellar liposomes in alkaline aqueous environments, and that water penetrates between the head groups of the arsonolipids. T_m and ΔH values reflect changes in the packing of the fatty chains and it is known that the packing is affected by changes in the chain length and the size of the polar head group[21] as well as in the state of ionization of the acid group, e.g. —O—P(O) $(OH)_2$.[22] The ~18°C change in T_m per —CH_2CH_2— and the ~2 Kcal/mol change in ΔH per —CH_2— group in arsonolipids (**3**) and (**4**) (Table) are approximately the same as the values observed in diacyl and tetraacyl acidic phospholipids.[23]

Comparing the T_m and ΔH values of (**3**) and (**4**) with those of the homologous phosphatidic acids[22,24] we see that the arsonolipids have slightly lower T_m and equal or higher ΔH values, implying larger separation among the neighboring acyl chains and somewhat stronger intermolecular polar head interactions respectively in arsonolipids. Therefore, the phase behaviour of the aqueous dispersions of arsonolipids (**3**) and (**4**) is similar to phosphatidic acids.

The *rac*-$DPAH_2$ and the racemic arsonolipids (**2**) (R = $C_{13}H_{27}$, $C_{15}H_{31}$ and $C_{17}H_{35}$) were found to be inactive as anti-AIDS and as anti-cancer agents in the National Cancer Institute screening tests.

EXPERIMENTAL

rac-Glycidol was prepared from 1-chloro-2,3-propanediol and methanolic sodium hydroxide[25] and used without completely removing the methanol by distillations. The optically active glycidols were from Aldrich. Their purities were checked by TLC (R_f 0.60 in $CHCl_3/Me_2CO$ 3:1 v/v) and determining the epoxide content by the alcoholic magnesium chloride hydrochlorination method.[26] The optical rotations of glycidols were +12.01° and −11.08° (neat) which were lower than the value of +15° reported by Sowden and Fisher[27] for (R)-glycidol prepared from D-mannitol indicating an optical purity of 0.80 or 90% enantiomeric excess. Methanolic tetrabutylammonium hydroxide (Eastman) was titrated with standard hydrochloric acid. The stearic, palmitic and myristic anhydrides were prepared from the corresponding fatty acids (Sigma and Ferak; 99% purity; recrystallized[28] before use) using dicyclohexylcarbodiimide in carbon tetrachloride.[2] Solvents and pyridine were dried over activated A4 molecular sieves and ethanol-free chloroform was prepared just before use by distillation from phosphorus pentoxide.

Optical rotations were measured on a Schmidt and Haensch Polatronic Universal polarimeter using a 5 cm cell. Differential scanning calorimetric studies were done using a Mettler TA 2000 instrument with scanning rate of 2.5°C/min. C, H elemental analyses were done by the Centre National de la Recherche Scienfique, Vernaison, France. The other techniques used were described previously.[3,13]

(S)-1,2-dihydroxypropyl-3-arsonic acid. To a stirred solution of arsenic trioxide (1.443 g; 7.31 mmol) in 13.0 M NaOH (3.4 ml; 43.6 mmol) was added dropwise during 30 min (S)-glycidol (epoxide content 96%) (1.021 g; 13.2 mmol) and the clear solution was stirred at 50°C for 3 h. TLC[3] showed only the product and traces of glycerol. After cooling to room temperature, the pH was adjusted to 4 with conc. hydrochloric acid and the heterogeneous system was left at +4°C for 24 h. Centrifugation gave 1.45 mmol As_2O_3 and a supernatant which, after adjusting the pH to 1.8 with 1:1 v/v HCl, was evaporated (rotary, <30°C) and dried in vacuo over P_2O_5 for 24 h. Extracting with boiling absolute ethanol (6 ×

4 ml), evaporating the extracts and drying in vacuo over P_2O_5 2.329 g of product contaminated with 11% As_2O_3, 8% ethanol and ~2% glycerol was obtained as a white amorphous mass. The As_2O_3 can be dropped to ~6% by dissolving the crude product in boiling ethanol (5 ml), adding equal volume of water and cooling at 4°C overnight. Filtration, evaporation and drying in vacuo over P_2O_5 gave 2.153 g white hygroscopic solid which contains 1.972 g of product (75% yield), 0.128 g As_2O_3, ~0.08 g ethanol and traces of glycerol.

From (R)-glycidol (epoxide content 92%) the (R)-1,2-dihydroxypropyl-3-arsonic acid was similarly prepared contaminated with ~7% As_2O_3, ~4% ethanol and traces of glycerol.

From *rac*-glycidol (epoxide content 83%) the *rac*-1,2-dihydroxypropyl-3-arsonic acid was similarly prepared (86% yield) contaminated with ~6% As_2O_3, ~5% ethanol and traces of glycerol.

Tetrabutylammonium rac-1,2-dihydroxypropyl-3-arsonate. To a solution of *rac*-$DPAH_2$ (2.38 mmol) (containing ~6% As_2O_3) in warm (70°C) absolute ethanol (20 ml) is added 4.76 mmol methanolic tetrabutylammonium hydroxide. The solution is cooled to 25°C and evaporated (rotary, <35°C) to give an oil which on drying at 60°C/1 mm Hg for 2 h, affords quantitatively the product as a hygroscopic glass. Found (subtracting the As_2O_3 present) 10.78% As, calculated for $C_{35}H_{79}N_2O_5As$ 10.97% As. IR (in $CHCl_3$): 1680 mw (combination band of AsO_2H), 880 m [ν(As = O)], 720 vs [rocking of $(CH_2)_3$]. ^{1}H-NMR (D_2O): 0.9 (d, J = 6 Hz, 24H, $C\underline{H}_3$), 1.4 (m, 32H, $C\underline{H}_2C\underline{H}_2$), 2.0 (d, J = 8 Hz, 2H, $C\underline{H}_2$—As), 3.1 (m, 16H, $C\underline{H}_2N$), 3.6 (m, 2H, $C\underline{H}_2OH$), 4.1 (m, 1H, $C\underline{H}OH$).

The salt is insoluble in ether, soluble in dichloromethane, chloroform, 1,2-dichloroethane and DMF.

From (R)- and (S)-$DPAH_2$ their tetrabutylammonium salts were prepared, using the same procedure, and acylated without delay.

*Preparation of (R)- and (S)-arsonolipids (**3**) and (**4**) (R = $C_{13}H_{27}$, $C_{15}H_{31}$ and $C_{17}H_{35}$). General procedure.* The acylations of (R)- and (S)-$DPA(Bu_4N)_2$ with fatty acid anhydrides in the presence of pyridine in dry chloroform were carried out using exactly the same conditions described for the acylations of *rac*-$DPAH(Bu_4N)$.[5] Yields and physical data are shown in the Table.

Differential scanning calorimetric studies of arsonolipid dispersions. To a known amount (~1 mg) of arsonolipid, 30 μl of 50 mM Tris buffer pH = 8.00 was added and the aluminum pan was sealed. The pan was then heated to 90°C and allowed to equilibrate for 10 min in the sample compartment of the instrument. The samples were cooled to below 10°C, equilibrated for 10 min, and then scanned on heating and cooling cycles at a rate of 2.5°C/min. The transition profiles obtained by repeated scans were indistinguishable.

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