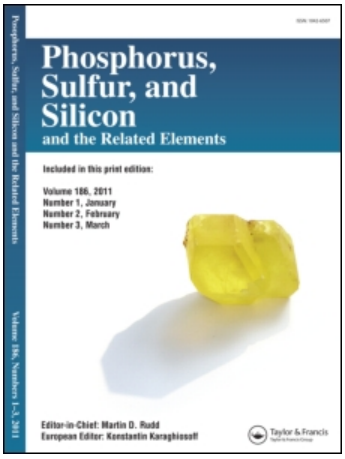


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# *rac*-1,2-DIACYLOXYPROPYL-3-ARSONIC ACIDS: ARSONOLIPID ANALOGUES OF PHOSPHONOLIPIDS

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# *rac*-1,2-DIACYLOXYPROPYL-3-ARSONIC ACIDS: ARSONOLIPID ANALOGUES OF PHOSPHONOLIPIDS

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A new class of lipids, arsonolipids or *rac*-1,2-diacyloxypropyl-3-arsonic acids, have been synthesized by acylating tetrabutylammonium hydrogen *rac*-1,2-dihydroxypropyl-3-arsonate with myristic, palmitic and stearic anhydrides in the presence of pyridine. The yields are moderate to low due to decomposition of *rac*-1,2-dihydroxypropyl-3-arsonic acid and its salts by acylating agents. Acid salts of arsonolipids were prepared by neutralization but neutral salts could not be made.

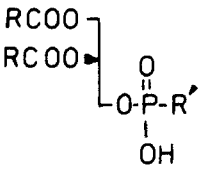
*Key words:* Arsonic acids; acylation; decomposition; arsonolipids; salts of arsonolipids.



## INTRODUCTION

Phosphonolipids of type (1) are known to exist in nature[1] but the synthetic ones[2] of type (2) are of greater interest for they can be used to regulate or perturbate metabolic pathways[2] or they can be used as analogues of phospholipids in biophysical and biochemical studies of model membranes.[3]

Although arsenic containing lipids have been isolated from certain marine organisms,[4] arsonolipids[5] (3), which are analogues to phosphonolipids (2), have not been detected. Arsonolipids are expected to have interesting properties because the nature and the polarity of their hydrophilic group can be changed easily for arsonic acids can be interconverted, As(V) $\rightleftharpoons$ As (III), by a variety of reagents.[6] Such property is not shown by P(V) in phospholipids and phosphonolipids.

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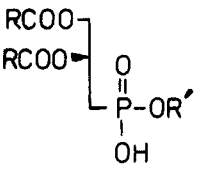


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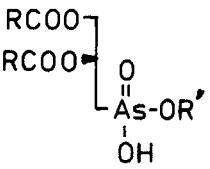


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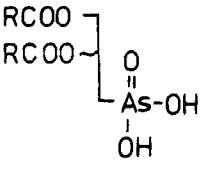


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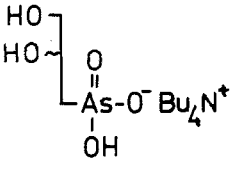


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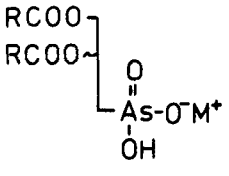


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We have initiated a program[5,7] on arsenic analogues of phosphonates and herein report on the synthesis of three members of arsonolipids (**4**), namely, *rac*-1,2-distearoyloxy, *rac*-1,2-dipalmitoyloxy, and *rac*-1,2-dimyristoyloxylpropyl-3-arsonic acids, as well as the synthesis of some hydrogen arsonate salts, (**6**).

## RESULTS AND DISCUSSION

Since epoxides can be converted to vicinal diacyl derivatives[8,9] we tried to prepare 2,3-epoxypropyl-1-arsonic acid. Reaction of allylarsonic acid with various epoxidizing agents gave at best 10% (by $^1$H-NMR) of product, an unexpected result since arsonic acids act as catalysts for epoxidation of alkenes.[10]

Direct acylation of *rac*-1,2-dihydroxypropyl-3-arsonic acid, $DPAH_2$,[5] by fatty acyl chloride/pyridine lead to its decomposition.[7] $DPAH_2$ with fatty acid anhydrides in dichloromethane/pyridine or in melt gave no product, due to its insolubility, while in 85% aqueous phosphoric acid solvent[11] no arsonolipid was detected under conditions which glycerol gave 65% isolated tripalmitin.

Blocking of the $-AsO(OH)_2$ group by alcohols[12–14] or by dibutyltin oxide[15] was also unsuccessful in the case of $DPAH_2$ because polymerization and/or cyclization took place.

Next effort was to try to acylate a $DPAH_2$ salt despite of the probable decomposition.[7] The prepared $DPAH_2$ salts were all hydrated and insoluble in non polar solvents.[5] However, we found that the tetrabutylammonium hydrogen *rac*-1,2-dihydroxypropyl-3-arsonate (**5**) is soluble in dichloromethane and dry chloroform. The salt is obtained as a glass, it is very hygroscopic but it can be dried in vacuo at 60°C for 6 h, when in <3 mmol quantity, and used as such. Alternatively, it can be dried in vacuo over phosphorus pentoxide till constant weight (~10 days).

Acylation of (**5**) in dry chloroform with three equivalents of palmitoyl chloride for 24 h gave 20% decomposition to As(III), traces of the desired arsonolipid (**4**) and small amounts of lyso-arsonolipid i.e., 1-acyl-2-hydroxypropyl-3-arsonic acid and starting (**5**) (by TLC analysis).

Titration of As(III) in the presence of $Bu_4N^+$ by standard $I_2$ solution should be done very slowly (~6 h) in order for the insoluble $Bu_4N^+I_3^-$, which is formed, to be in very low concentrations and to react with As(III). Titration of As(III) with standard $KBrO_3$ in the absence of special redox indicators did not work in our case.

The acylation of (**5**) with fatty acid anhydrides in refluxing chloroform solution and in the presence of less than stoichiometric amount of pyridine proceeds smoothly and slowly. Usually 6–8 days are required for the anhydride to disappear (by IR) and the system usually develops a light brown color. The working up is remarkably simple compared to that of the corresponding phosphonolipids.[16] Thus, acidification with sulfuric acid removes almost all the tetrabutylammonium cation due to favourable partition of $Bu_4N^+HSO_4^-$ in the aqueous phase and by adding ether to the organic phase almost pure arsonolipid is precipitated. Absolute ethanol is a better recrystallization solvent than chloroform (both gave recoveries >80%) because it removes the lyso-arsonolipid more effectively from the arsonolipid. However, for the dimyristoyl arsonolipid chloroform is preferred in order to avoid

alcoholysis due to slow rate of crystallization in absolute ethanol. The yields (27–42%) of pure arsonolipids (**4**), although small, are better than those reported for the corresponding phosphonolipids.[16]

When stoichiometric amount of pyridine was used for the acylation of (**5**) the yields of (**4**) and the decomposition of (**5**) did not noticeably increase. The yields can be somewhat increased (by ~5%) by using five moles of anhydride, the decomposition of (**5**) now being ~30%.

The arsonolipids (**4**) (R = $C_{17}H_{35}$; $C_{15}H_{31}$; $C_{13}H_{27}$) are white amorphous powders, stable in A.R. $CHCl_3$ for more than 50 days. They can be kept as solids at room temperature for more than 50 days and are stable at −20°C for more than 6 months. They are moderately soluble in warm $CHCl_3$, slightly soluble in $CCl_4$, DMF, DMSO, and absolute ethanol, and sparingly soluble in petroleum ether, ether, ethyl acetate and acetone. Their melting points decrease regularly with decreasing of the length of the fatty acyl chain and they are far lower than that of free $DPAH_2$.[5] Their IR spectra (KBr pellets) are qualitatively similar and differ from the corresponding phosphonolipids.[16] All bands are sharp. There is a weak band at 2600 $cm^{-1}$ and a strong band at 1730 $cm^{-1}$ characteristic of the stretching of As—OH and C=O groups, respectively. The combination vibration of the $AsO_2H$ which usually occurs at 1690 $cm^{-1}$,[6,17] is absent in our arsonolipids. The stretching vibration of the hydrogen bonded As=O (As=O . . . H—O—As) which occurs at 940 $cm^{-1}$ in simple arsonic acids[17] and at 900 $cm^{-1}$ in 1,2-dihydroxypropyl-3-arsonic acid[5] is split into two peaks, a weak at 900 $cm^{-1}$ and a strong one at 860 $cm^{-1}$ which signify a stronger hydrogen bonding as a result of the side-by-side packing of the arsonolipid molecules. This explanation is corroborated by the splitting at 2270 and 2200 $cm^{-1}$ of the deformation of the As—OH group. The bands at 790 and 760 $cm^{-1}$ are probably due to asymmetric and symmetric stretching, respectively, of the As—O group which in simple alkylarsonic acids is found at 780 $cm^{-1}$.

The 60 MHz $^1$H-NMR spectrum of the more soluble in $CDCl_3$ dimyristoyl arsonolipid is consistent with the structure ($\delta$: 5.4 —COOC<u>H</u>—; 4.2 —COOC<u>H</u>$_2$; 2.7 —C<u>H</u>$_2$—As; 2.2 —C<u>H</u>$_2$COO—; 1.1 (—C<u>H</u>$_2$—)$_{11}$; 0.9 C<u>H</u>$_3$—) but it is not informative. High resolution $^1$H- and $^{13}$C-NMR spectra of these arsonolipids in organic solvents and in water are under study.

The hydrogen arsonate salts (**6**) were prepared by neutralizing the acid (**4**) with equivalent amount of metal hydroxide in methanol. The neutral salts could not be prepared by the same method because rapid methanolysis to lyso-arsonolipid took place. Also neutral or acid salts could not be prepared with divalent cations, such as $Mg^{2+}$ and $Ba^{2+}$, in the biphasic system of Folch et al.[18] as phospholipids can.[19,20] In our case the free arsonolipids (**4**) were quantitatively recovered.

All of the prepared acid salts (**6**) were monohydrates except the barium salts of dimyristoyl and dipalmitoyl (**6**), which were dihydrates. The potassium salts of dimyristoyl and dipalmitoyl arsonolipids are somewhat hygroscopic while the other salts are not. Their solubilities do not strongly depend on their cations but rather on their fatty acyl chain length. Thus the dimyristoyl (**6**) are the most soluble and the distearoyl (**6**) are the least soluble in organic solvents ($CHCl_3$ > MeOH > $Et_2O$ ≃ petroleum ether). Their $R_f$ values in the acidic developing solvent, $CHCl_3/CH_3COOH$, depend only on the fatty acyl chain length (Table). The thermal

TABLE

Physical constants and elemental analyses of the arsonolipids (**4**) and their acid salts (**6**)

| Compound | Yield % | Softening point, °C | M.p.*, °C | $R_f^+$ | Molecular formula | %As Calcd | %As Found |
|---|---|---|---|---|---|---|---|
| **4**, R=$C_{13}H_{27}$ | 33-40 | - | 91-3 | 0.36 | $C_{31}H_{61}O_7As$ | 12.1 | 11.8 |
| **4**, R=$C_{15}H_{31}$ | 27-31 | - | 96-7 | 0.38 | $C_{35}H_{69}O_7As$ | 11.1 | 10.9 |
| **4**, R=$C_{17}H_{35}$ | 29-42 | - | 102-3 | 0.40 | $C_{39}H_{77}O_7As$ | 10.2 | 10.2 |
| **6**, R=$C_{13}H_{27}$, M=Li | 100 | 170 | 200-2 | 0.36 | $C_{31}H_{60}O_7AsLi.H_2O$ | 11.6 | 11.7 |
| **6**, " M=Na | 100 | - | 167-170 | " | $C_{31}H_{60}O_7AsNa\cdot H_2O$ | 11.1 | 11.2 |
| **6**, " M=K | 100 | 165 | 173-5 | " | $C_{31}H_{60}O_7AsK\cdot H_2O$ | 10.8 | 11.2 |
| **6**, " M=$Ba_{\frac{1}{2}}$ | 100 | 170 | 175-8 | " | $C_{31}H_{60}O_7AsBa_{\frac{1}{2}}\cdot 2H_2O$ | 10.6 | 10.6 |
| **6**, R=$C_{15}H_{31}$, M=Li | 100 | 170 | 200-2 | 0.38 | $C_{35}H_{68}O_7AsLi\cdot H_2O$ | 10.7 | 11.1 |
| **6**, " , M=Na | 100 | - | 170-3 | " | $C_{35}H_{68}O_7AsNa\cdot H_2O$ | 10.5 | 10.2 |
| **6**, " , M=K | 100 | 163 | 170-2 | " | $C_{35}H_{68}O_7AsK\cdot H_2O$ | 10.2 | 10.3 |
| **6**, " , M=$Ba_{\frac{1}{2}}$ | 100 | 158 | 164-9 | " | $C_{35}H_{68}O_7AsBa_{\frac{1}{2}}\cdot 2H_2O$ | 9.8 | 9.8 |
| **6**, R=$C_{17}H_{35}$, M=Li | 100 | 170 | 200-2 | 0.40 | $C_{39}H_{76}O_7AsLi\cdot H_2O$ | 9.9 | 10.3 |
| **6**, " , M=Na | 100 | - | 170-3 | " | $C_{39}H_{76}O_7AsNa\cdot H_2O$ | 9.7 | 9.6 |
| **6**, " M=K | 100 | 160 | 169-171 | " | $C_{39}H_{76}O_7AsK\cdot H_2O$ | 9.5 | 9.5 |
| **6**, " , M=$Ba_{\frac{1}{2}}$ | 100 | 178 | 183-6 | " | $C_{39}H_{76}O_7AsBa_{\frac{1}{2}}\cdot H_2O$ | 9.3 | 9.3 |

*All compounds decomposed without melting

+ In $CHCl_3/Ch_3COOH$ 12:1 v/v

behaviour of (6) in capillary tubes is interesting. All but the sodium salts showed a softening point before decomposing. The decomposition temperatures are highest for the lithium salts while for the other salts are ~30°C lower. There is not a marked differentiation in the decomposition points of arsonolipids with the same cation, except the irregular trend of the barium salts which may be due to different degrees of hydration (Table).

The solid state (KBr pellets) IR spectra of arsonolipid salts (6) are all similar irrespective of the fatty acyl chain or cation. There is a broad, medium intensity band at 3600–3200 $cm^{-1}$ due to water of hydration. It is much stronger in the hygroscopic salts, in which weak bands appear at 2400 and 2250 $cm^{-1}$. The band at 2600 $cm^{-1}$ is absent as are the bands at ~2250 $cm^{-1}$. In the fingerprint region, the $\nu$(As=O) of the $As(OH)O_2^-$ group gives rise to a medium, relatively broad band at 890 $cm^{-1}$ which is characteristic of the spectra of all salts. The bands at 790 and 760 $cm^{-1}$ are absent.

## EXPERIMENTAL

The fatty acids (Sigma and Ferak) were of 99% purity and were recrystallized[21] before use. Carbon tetrachloride, dichloromethane and pyridine were dried over activated A4 molecular sieves. Ethanol-free chloroform was prepared just before use by distillation from phosphorus pentoxide. Methanolic tetrabutylammonium hydroxide (Eastman) was titrated with standard hydrochloric acid. $DPAH_2$ was prepared as described.[5] The stearic, palmitic and myristic anhydrides were prepared from the corresponding fatty acids using dicyclohexylcarbodiimide in carbon tetrachloride.[3] Palmitoyl chloride (b.p. 147–150°C/1 mmHg) was prepared from palmitic acid and redistilled thionyl chloride. The techniques used were described previously.[5,7]

*Tetrabutylammonium hydrogen rac-1,2-dihydroxypropyl-3-arsonate* (5). To a solution of $DPAH_2$ (2.23 mmol) in warm (70°C) absolute ethanol (22 ml) is added 2.23 mmol of methanolic tetrabutylammonium hydroxide. The solution is immediately cooled to 25°C and evaporated (rotary, ≤35°C) to give an oil which on drying 60°C/1 mmHg for 6 h, affords quantitatively the product, (5), as a glassy colorless solid. Found 16.52% As, calculated for $C_{19}H_{44}NO_5As$ 17,00% As. IR (in dry $CHCl_3$) 2420 w, 1640 m, 870 s, 740 w. $^1$H-NMR ($D_2O$), $\delta$: 0.9 (m, 12H, $C\underline{H}_3$), 1.4 (m, 16H, $C\underline{H}_2C\underline{H}_2CH_3$), 2.3 (d, $J$ = 6 Hz, $C\underline{H}_2$—As), 3.2 (broad, 8H, $C\underline{H}_2N^+$), 3.6 (m, 2H, $C\underline{H}_2OH$), 4.1 (m, 1H, $C\underline{H}OH$). The salt is insoluble in ether, soluble in acetone, methanol, ethanol, dichloromethane (1 g/7.0 ml) and dry chloroform (1 g/5.5 ml).

*Preparation of arsonolipids* (4) ($R$ = $C_{17}H_{35}$, $C_{15}H_{31}$ *and* $C_{13}H_{27}$). *General procedure.* To a solution of (5) (2.23 mmol) in dry chloroform (25 ml) is added 4.46 mmol dry pyridine and 6.69 mmol acid anhydride and the solution is refluxed for 8 days. TLC ($CHCl_3/CH_3COOH$ 12:1 v/v) shows esters ($R_f$ 0.92), free fatty acid ($R_f$ 0.76), the product ($R_f$ 0.36–0.40), and lyso-arsonolipid ($R_f$ ~ 0.15). Water (20 ml) is added with stirring, the pH of the aqueous layer is adjusted between 1.5–2.0 with 1M sulfuric acid and after vigorous stirring the phases are separated. The washing is repeated three more times, the sequence of reagent addition being important for the best extraction of $Bu_4NHSO_4$. The aqueous phases contain small amounts of unreacted $DPAH_2$ (by TLC), most of the $Bu_4N^+$, and by very slow titration with $I_2$ 8–15% decomposition of $DPAH_2$ to As(III) is found. The organic phase is warmed to dissolve any precipitated arsonolipid, equal volume of ether is added with stirring and the system is left at room temperature for >24 h to crystallize. The precipitated arsonolipid is separated by centrifugation and washed with $CHCl_3/Et_2O$ 1:1 v/v (1 × 2 ml) and $Et_2O$ (3 × 1 ml). The supernatants contain all the fatty acid, traces of product and most of the lyso-arsonolipid. The product is contaminated with traces of lyso-arsonolipid which can be removed by recrystallization from absolute ethanol (~15 ml/g) or chloroform (~8 ml/g) to give the pure arsonolipid as white amorphous solid. Yields and physical data are shown in the Table.

*Preparation of hydrogen arsonate salts,* (6). *General procedure.* To a warm solution of 0.15 mmol of arsonolipid (4) in 10 ml absolute ethanol a solution of 0.15 mmol of metal hydroxide in methanol is added, the resulting solution is cooled and evaporated (rotary, <30°C). The white amorphous precipitate is dried in a vacuum desiccator over phosphorus pentoxide till constant weight (~5 days). From

the weight and %As the water of crystallization has been determined. TLC ($CHCl_3/CH_3COOH$ 12:1 v/v) shows that no decomposition took place during the neutralization. Physical and analytical data are shown in the Table.

## ACKNOWLEDGEMENTS

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