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A reinvestigation of the synthesis of arsonolipids (2,3-diacyloxypropylarsonic acids)

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Abstract

A reinvestigation of the reactions leading to arsonolipids (2,3-diacyloxypropylarsonic acids) has been carried out in order to understand why the yields of their preparation were only moderate, although they are better than those reported for 2,3-diacyloxypropylphosphonic acid (phosphotidic acid). Thus, the reaction of glycidol and of 3-chloro-1,2-propanediol with alkaline sodium arsenite, "Na_3AsO_3", gives the desired product, 2,3-dihydroxypropylarsonic acid, and ~10% of an arsenic-containing glycerol dimer which is removed during the preparation of these arsonolipids. The step which is mainly responsible for the diminished yields is due to the reaction of the $-As(SPh)_2$ or $-AsO_3H^-$ precursor with the activated acid chlorides or carboxylic acid anhydrides to give an intermediate which cyclizes with the primary hydroxy group of the 2,3-dihydroxypropyl moiety. This cyclization does not allow the primary hydroxy group to be acylated. Such cyclization could not be avoided with RCOCl/py, $(RCO)_2O$/DMAP, or RCOOH/DCC/DMAP acylating systems.

Keywords: Arsonolipids; 2,3-Diacyloxypropylarsonic acids; Glycidol (2,3-epoxypropanol); Trisodium arsenite

1. Introduction

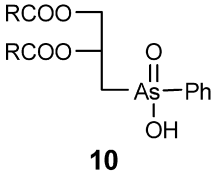
A new class of lipids **5** (see Scheme 1 for formulae), called arsonolipids (Tsivgoulis et al., 1991a) was prepared from *rac*-, *R*-, and *S*-2,3-dihydroxypropylarsonic acids **2** (Tsivgoulis et al., 1991b; Serves et al., 1992; Lacoste et al., 1992). Thus, acylation of the mono *n*-tetrabutylammonium **3** (Tsivgoulis et al., 1991b) or the di *n*-tetrabutylammonium **4** (Serves et al., 1992) salts of **2** with $(RCO)_2O$/py and RCOCl/py, respectively, gave the arsonolipids **5** in only 20–40% yields.

Based on the report (Kamai and Chadaeva, 1957) that reaction between acyl chlorides or carboxylic acid anhydrides and dithioarsonites (Eqs. (1) and (2), respectively) took place under drastic conditions (150–160 °C/5–18 h), we prepared the dithioarsonite **6**, acylated it (RCOCl/py) to **7** and after hydrogen peroxide oxidation of **7** the arsonolipids **5** were prepared with essentially the same yields (20–50%) (Serves et al., 1993). Better

$$R\text{–}As(SR')_2 + 2R''COCl \rightarrow R\text{–}AsCl_2 + 2R''COSR' \quad (1)$$

$$R\text{–}As(SR')_2 + 2(R''CO)_2O \rightarrow R\text{–}As(OCOR'')_2 + 2R''COSR' \quad (2)$$

yields (60–75%) were obtained for the preparation of arsinolipids **10** from **11** by a procedure analogous to that shown in Scheme 1 (**11** in place of **6**) using the RCOCl/py and RCOOH/DCC/DMAP acylating systems (Kordalis and Ioannou, 2000), implying that in the reaction of **6** with RCOCl/py some side-reactions took place and two probable by-products were reported (Serves et al., 1993).



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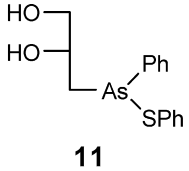

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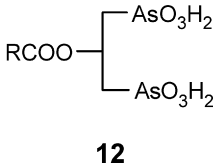

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Abbreviations: RCOOH, fatty acid; RCOCl, fatty acyl chloride; $(RCO)_2O$, fatty acid anhydride; RC(O)SH, thioalkanoic acid; py, pyridine; DMAP, 4-dimethylaminopyridine; DCC, *N*,*N*′-dicyclohexylcarbodiimide; DHU, 1,3-dicyclohexylurea; Phosphotidic acid, 2,3-diacyloxypropylphosphonic acid; Glycidol, 2,3-epoxypropanol; Capric acid, decanoic acid; Myristic acid, decatetranoic acid; Palmitic acid, hexadecanoic acid; Thiomyristic acid, thiotetradecanoic acid; Thiopalmitic acid, thiohexadecanoic acid.

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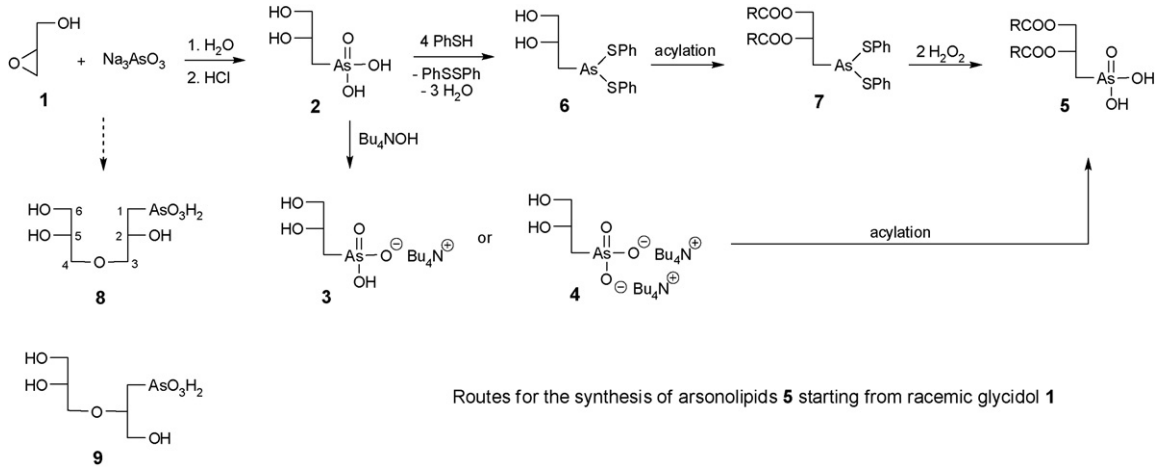


Scheme 1.

In order to avoid the preparation of acyl chlorides, thus simplifying the synthesis of **5**, we used the acylating system RCOOH/DCC/DMAP (Eibl et al., 1983) which is equivalent to the $(RCO)_2O$/DMAP system (Gupta et al., 1977) which were used for the synthesis of lipids and phospholipids. The acylation of **6** with RCOOH/DCC/DMAP followed by oxidation gave **5** with yields 35–55% (this work) essentially similar to those obtained with the RCOCl/py system, but lower than those obtained for the preparation of arsinolipids **10** (Kordalis and Ioannou, 2000) and pseudo-arsonolipids **12** (∼80% from 2-hydroxy-1,3-bis(arsonic acid) following the sequence: reduction with thiophenol, acylation, and hydrogen peroxide oxidation) (Terzis and Ioannou, 2002). The yields of arsonolipids **5** are, however, much better than the yields (18–25%) reported for the acylation of L-dihydroxypropylphosphonate with carboxylic acid anhydrides in the presence of tetraethylammonium salt of the fatty acid to give the L-phosphotidic acid (Baer and Basu, 1970).

These results imply that the acylating systems RCOCl/py and RCOOH/DCC/DMAP react with the dithioarsonite **6** at the expected hydroxy groups and at As(III) as well, and understanding the nature of the various intermediates will probably improve the yields in the preparation of **5**, since these arsonolipids have quite interesting biophysical (Faturos et al., 2001; Gortzi et al., 2001; Fatouros et al., 2005a,b), biochemical (Supuran et al., 1996; Rogers et al., 1996), and biological (Gortzi et al., 2002, 2003; Antimisiaris et al., 2003, 2005; Fatouros et al., 2006) properties.

The complete route to **5** examined in this paper (Scheme 1) requires the synthesis of **2**, its reduction by thiophenol to **6**, acylation of **6** and oxidation of the acylated dithioarsonite **6** to **5**. In this paper we examined more closely the preparation of **2** and of **5** at large scale and compare the yields of **5** with the yields obtained for **10** and **12**. In order to understand the acylation of **6**, we did competitive experiments using cholesterol, Chol–OH, (having a secondary –OH group which is acylated slower than a primary –OH) in the presence of triphenyl trithioarsenite, $As(SPh)_3$, as a model for **6**.

2. Experimental

2.1. Materials

Cholesterol 99% and capric acid were from Sigma, while myristic acid was from Serva. *N*,*N*′-Dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) were from Aldrich. Racemic glycidol was prepared from 3-chloro-1,2-propanediol (Aldrich) (Rider and Hill, 1930). Palmitic anhydride (Lapidot et al., 1969) and triphenyl trithioarsenite, $As(SPh)_3$ (Serves et al., 1995b) were prepared by literature procedures. The cation exchange resin Dowex AG 50W-X8 (H^+) and the anion exchange resin Dowex AG 1-X8 (acetate) were from Bio-Rad, while silica gel 60 for column chromatography and silica gel 60 H for thin layer chromatography (TLC) were from Merck. Ethanol and dichlomethane were dried over A_4 molecular sieves.

2.2. Methods

TLCs were run on microslides. The spots were made visible by iodine vapors (especially for "As_2O_3", $As(SPh)_3$ and PhSSPh) followed by spraying with 35% sulfuric acid and charring. Cholesterol and cholesteryl palmitate gave a red color before being charred. IR spectra were taken in KBr discs on a PerkinElmer, model 16PC FT-IR spectrometer, while ^1H NMR (at 400 MHz) and ^{13}C NMR (at 100 MHz) spectra were obtained on a Bruker, model DPX Avance, spectrometer. The reference standard in D_2O was DSS (2,2-dimethyl-2-silapentane-5-sulfonate, sodium salt). Elemental analyses were done by the Centre of Instrumental Analyses, University of Patras, Patras, Greece, and C.N.R.S., Vernaison, France.

2.3. The reaction of rac-glycidol *1* with alkaline sodium arsenite, Na_3AsO_3: preparation of rac-2,3-dihydroxypropylarsonic acid, rac-*2*

To a solution of arsenic(III) oxide (3.960 g, 20 mmol As_2O_3) and sodium hydroxide (4.80 g, 120 mmol) in water (7.5 ml) in

a 25 ml round-bottomed flask having a short and thick magnetic follower, was added drop-wise during 3 h racemic glycidol (2.963 g, 40 mmol). The very viscous solution was stirred for another 2 h, cooled (ice-water) and concentrated hydrochloric acid (10.0 ml, 120 mmol) was added portion-wise, the pH at the end being ~2. The partly precipitated NaCl and As_2O_3, which did not react, were centrifuged and washed with cold water (2.5 ml). The combined supernatants contained by TLC (MeOH/conc. NH_3 4:1) the acid **2** (R_f 0.45) and As_2O_3 (R_f 0.50) while glycerol was not visible at R_f 0.80. The supernatants, diluted with water (20 ml) were applied onto the strongly cation exchange resin (Dowex AG 50W-X8, H^+ form, 2.2 cm × 26 cm, capacity 170 mmol H^+) and eluted with water (500 ml) (Lacoste et al., 1992). The eluate was evaporated (rotary, 60 °C) to an oil which was dried very well (for total removal of HCl) in vacuum to give a foam plus glass (~6.3 g). This mass was dissolved in water (15 ml), applied portion-wise onto the strongly anion exchange resin (Dowex AG 1-X8, acetate form, 3 cm × 32 cm, capacity 313 mmol AcO^-) and eluted with a) water (200 ml), and b) 1 M aqueous acetic acid (1000 ml) at a flow rate of 5 ml/min, collecting 50 ml fractions. Fractions 1–6 gave a solid plus oil (1.16 g) containing, by TLC, glycerol and As_2O_3, from which only 430 mg As_2O_3 could be isolated by adding water (3 ml) and then methanol (5 ml) and centrifuging. Fractions 9–10 gave 98 mg of a glass (compound **8**) and fractions 11–16 gave the product **2** (4.22 g, 53%) as a glass plus foam.

2.3.1. Characterization of compound 8

By triturating the glassy compound **8** with MeOH/Et_2O and evaporation, it was transformed into foam, keeping tenaciously methanol and/or acetic acid, and having R_f 0.42, while **2** has R_f 0.31. It decomposed at ~213 °C. IR (KBr) (all bands are somewhat broad): 3412 vs, 2924 m, 1636 m, 1460 w, 1397 w, 1343 w, 1257 w, 1222 w, 1124 s, 1044 s, 902 s, 764 m. ^{1}H NMR (D_2O, DSS), δ = 2.85 (m, 2 H, H-1), 3.65 (m, 6 H, H-3, H-4, H-6), 3.90 (m, 1 H, H-5), 4.35 (m, 1 H, H-2). ^{13}C NMR (D_2O, DSS), δ = 40.96 C-1, 65.22 C-6, 67.15 C-2, 73.13 C-4, 74.82 C-5, 77.18 C-3. The ^{13}C peaks were assigned based on the spectra of polymerized glycidol (Sunder et al., 1999; Tokar et al., 1994; Dworak et al., 1995; Cassel et al., 2001). For analytical purposes, the acid **8** was converted into its barium salt as follows: To a solution of **8** (98 mg, 0.35 mmol) in methanol (2 ml) and water (0.5 ml) a clear solution of recrystallized barium hydroxide octahydrate (113 mg, 0.35 mmol) in methanol (5 ml) was added and the solution was evaporated and dried in vacuum. The solid was suspended in boiling methanol (3 ml), cooled to r.t. and centrifuged to give the barium salt of **8** (117 mg, 82%). It is soluble in water and insoluble in warm methanol and in warm acetone. M.p. ~205 °C dec. Calculated for $C_6H_{13}O_7AsBa$ (M_r 409.42): C 17.60, H 3.20%; found: C 17.64, H 3.15%. IR (KBr) (all bands are somewhat broad): 3412 s, 2920 m, 1460 m, 1452 mw, 1320 mw, 1111 ms, 1062 ms, 818 vs. ^{1}H NMR (D_2O, DSS), δ = 2.05 (m, 2 H, H-1, 3.57 and 3.65 (m, 6 H, H-3, H-4, H-6), 3.92 (m, 1 H, H-5), 4.26 (m, 1 H, H-2). ^{13}C NMR (D_2O, DSS), δ = 39.63 C-1, 65.30 C-6, 68.81 C-2, 73.04 and 73.14 C-4, 74.74 and 74.69 C-5, 77.72 and 77.75 C-3.

2.3.2. Characterization of compound 2

The glassy product **2** was converted into a foam by dissolving in warm methanol (7 ml), evaporating and drying in vacuum. Its m.p., IR, ^{1}H NMR, and ^{13}C NMR spectra were as published (Tsivgoulis et al., 1991a). It was 91% pure (impurities: acetic acid, methanol and traces of **8**). For analytical purposes a sample of it (110 mg, 0.55 mmol) in a centrifuge tube was dissolved in methanol (2 ml) and treated with a clear solution of recrystallized barium hydroxide octahydrate (173 mg, 0.55 mmol) in methanol (5 ml). After 3 h at r.t. centrifugation and drying in vacuum gave the barium salt of **2** (192 mg, 94%) as the dihydrate, insoluble in methanol and in water, and decomposing slowly at >195 °C (lit. Tsivgoulis et al., 1991a: 200 °C dec.). Calculated for $C_3H_7O_5AsBa{\cdot}2H_2O$ (M_r 371.38): C 9.70, H 2.99%; found C 9.89, H 2.72%. IR (KBr): 3380 s, broad, 2925 m, 1664 w, 1560 m, 1414 m, 1347 w, 1072 ms, 1035 ms, 814 vs, 646 w.

When 15 mol% excess glycidol was used then the crude reaction mixture contained by ^{1}H NMR 82% **2**, 9% **8**, and 9% glycerol. The isolated yield of **2** was ~60%.

When 3-chloro-1,2-propanediol was used as substrate (Tsivgoulis et al., 1991a) the crude reaction mixture contained, by ^{1}H NMR, 65% **2**, 8% **8**, and 27% glycerol.

2.4. Acylation of cholesterol in the presence of an equimolar quantity of triphenyl trithioarsenite, As(SPh)$_3$

2.4.1. General procedure

Triphenyl trithioarsenite (20 mg, 0.05 mmol), cholesterol (19.4 mg, 0.05 mmol), palmitic anhydride (x·0.05 mmol), and DMAP (x·0.05 mmol or x·0.005 mmol) (x = 1–6) were dissolved in dry dicholoromethane (2 ml) and stirred as shown in Table 1. In *all* cases TLC (petroleum ether, Et_2O/petroleum ether 1:10 and 1:3) showed (cholesterol which did not "react" even in the presence of excess palmitic anhydride), cholesteryl palmitate, phenyl thiopalmitate, and diphenyl disulfide. Evaporation and drying in vacuum gave white solids that were examined by IR: acid anhydride was detected only in the cases with large excess of palmitic anhydride (entries 4–7, 10,11, Table 1). Then, the solid of each run was dissolved in cholorform (1 ml) and chromatographed (silica gel 15 g in petroleum ether) eluting with (a) petroleum ether (25 ml) and (b) ether/petroleum ether 1:10 (100 ml). The fatty acid produced by hydrolysis of the anhydride was retained on the column, as was the free cholesterol. The first 80–90 ml gave ~40 mg of a solid which was analysed by IR and ^{1}H NMR. The IR spectra showed the absence of anhydride (1802 cm^{-1}), the presence of cholesteryl palmitate (1740 cm^{-1}), phenyl thiopalmitate (1704 cm^{-1}) and diphenyl disulfide (1576, 740, and 688 cm^{-1}). The ^{1}H NMR spectra permitted the identification (Tsivgoulis and Ioannou, submitted for publication) and quantitation of As(SPh)$_3$ (7.44–7.47 ppm for H-2 and H-6 of PhS–), cholesteryl palmitate (4.60 ppm RCOOC*H*), phenyl thiopalmitate (2.64 ppm, triplet, for CH_2CO– and 7.41, singlet, for PhS–), and PhSSPh (7.48–7.52 ppm, apparent doublet, for *ortho* protons of PhSSPh). Results are shown in Table 1.

The solids (343 mg) from several runs containing cholesteryl palmitate, PhSSPh, and RCOSPh, were pooled and recrystallized three times from acetone, to give pure, by TLC, cholesteryl

Table 1
Competitive acylation of cholesterol and of triphenyl trithioarsenite, $(PhS)_3As$, by 4-dimethylaminopyridine-activated palmitic anhydride

Entry	Reactants				Reaction conditions[a,b]	Recovered $As(SPh)_3$ [c] (%)	Yields of non-arsenic-containing products		
	Chol–OH[d] (mmol)	$(PhS)_3As$ (mmol)	$(RCO)_2O$ (mmol)	DMAP[e] (mmol)			RCOSPh[f,g] (%)	PhSSPh[g,h] (%)	RCOOChol (%)
1	0.05	0.05	0.05	0.005	r.t./24 h	28	25	20	23
2	0.05	0.05	0.10	0.01	$(RCO)_2O$ added slowly at 0 °C; then r.t./24 h	25	46	20	25
3	0.05	0.05	0.10	0.01	$(RCO)_2O$ added slowly to the reaction mixture having Et_3N at 0 °C; then r.t./24 h	35	43	14	10
4	0.05	0.05	0.10	0.01	r.t./24 h	18	23	21	52
5	0.05	0.05	0.20	0.02	r.t./24 h	20	19	24	54
6	0.05	0.05	0.25	0.025	r.t./24 h	0	0	81	66
7	0.05	0.05	0.30	0.03	r.t./24 h	0	4	94	48
8	0.05	0.05	0.05	0.05	r.t./24 h	30	24	21	28
9	0.05	0.05	0.10	0.10	r.t./24 h	24	23	23	61
10	0.05	0.05	0.20	0.20	r.t./24 h	19	10	46	70
11	0.05	0.05	0.25	0.25	r.t./24 h	13	3	63	60

[a] Cholesterol was detected by TLC at the end of all experiments.
[b] Palmitic anhydride was detected by IR only in entries 5–7 and 10,11.
[c] Some $(PhS)_3As$ decomposes on the silica gel column giving PhSSPh and As_2O_3.
[d] Chol–OH = cholesterol.
[e] DMAP = 4-dimethylaminopyridine.
[f] The yields have been calculated for the conversion $(PhS)_3As \rightarrow 3$ RCOSPh.
[h] The yields have been calculated for the conversion 2 $(PhS)_3As \rightarrow 3$ PhSSPh.
[g] The yields should be indicative when $(RCO)_2O$ is not in excess.

palmitate (65 mg), m.p. 74 °C sinters, 76–78 °C melts. Lit. (Swell and Treadwell, 1955): 75 °C cloudy, 80.5 °C melts. IR (KBr): 2916 vs, 2850 vs, 1742 s, 1464 m, 1380 mw, 1308 w, 1283 w, 1266 w, 1242 w, 1222 w, 1196 m, 1178 ms, 1012 w, 800 w, 726 w.

2.5. Preparation of rac-**5** ($R = C_{13}H_{27}$) via RCOOH/DCC/DMAP acylation of rac-**6**

2.5.1. Reduction of rac-**2** to rac-**6** with thiophenol

To a solution of *rac*-**2** (3.844 g, 19.2 mmol) (containing 3% methanol and 3% acetic acid) in methanol (100 ml), was added portion-wise thiophenol (8.21 ml, 76.8 mmol) and the solution with the precipitated diphenyl disulfide stirred overnight. Evaporation and drying in vacuo gave a solid which was extracted with boiling petroleum ether (2 × 50 ml and 2 × 25 ml) decanting while warm. The dried product *rac*-**6** contaminated by 5% diphenyl disulfide was a semi-opalescent oil (7.14 g, expected 7.07 g). IR (neat): 3354 br, s, 3056 w, 2922 w, 2870 w, 1580 m, 1476 s, 1438 s, 1398 w, 1082 s, 1060 s, 1024 s, 1002 w, 960 w, 868 w, 742 vs, 690 vs. ^{1}H NMR ($CDCl_3$), $\delta = 2.29$ (m, 2 H, CH_2As), 3.55 and 3.70 (both doublet of doublets, 2 H, CH_2OH), 4.22 (m, 1 H, C*H*OH), 7.22 (d, $J = 0.8$ Hz, 6 H, *meta* and *para* hydrogens), 7.40 (m, 4 H, *ortho* hydrogens) and protons of the phenyl disulfide impurity. ^{13}C NMR ($CDCl_3$), $\delta = 35.83$ *C*H_2As, 67.46 *C*H_2OH, 69.72 *C*HOH, 127.59 *para*-C, 129.03 *ortho*-C, 133.10 and 133.39 *ipso*-C, 133.93 *meta*-C. From the diphenyl disulfide impurity: the *ipso*-C was not visible at 136.97, the *ortho* and *meta*-C coincided with those of **3** and a very small peak at 127.14 (*para*-C) was visible.

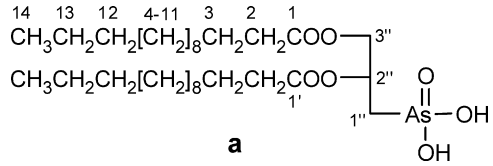
2.5.2. Acylation of rac-**6** to rac-**7**

To the oily *rac*-**6** (assuming to contain 19.2 mmol *rac*-**6**) was added myristic acid (10.51 g, 46.1 mmol), DMAP (560 mg, 4.6 mmol), and dissolved by dry dichloromethane (150 ml). Then, a solution of DCC (9.30 g, 45.1 mmol) in dry dichloromethane (50 ml) was added drop-wise during 3 h, and the heterogeneous (1,3-dicyclohexylurea, DHU) solution was stirred at r.t. for 2 days. TLC ($CHCl_3$/MeOH 20:1) showed DMAP (R_f 0.0), DHU (R_f 0.22), myristic acid (R_f 0.30, and a strong spot at $0.80 < R_f < 1.0$ containing the product and other non-polar by-products. The solution was evaporated and dried in vacuo (to remove dichlomethane which solubilizes some DHU) to give 27.3 g of a solid. This was suspended in ether (100 ml) filtered through celite and washed with ether (3 × 50 ml) to give a clear, faint yellowish solution.

2.5.3. Oxidation of rac-**7** to rac-**5** and PhSSPh with H_2O_2

To the ether solution of impure *rac*-**7** was added water (20 ml), stirred vigorously to give an emulsion, and then aqueous hydrogen peroxide 30% (5.23 ml, 46.1 mmol) was added drop-wise (10 min). The emulsion gave a suspension which after 3 h was centrifuged. The upper ether phase was decanted and worked-up (see below), and the water (lower phase, positive for hydrogen peroxide by the $TiOSO_4$ test) was syringed off. The middle solid phase was the product *rac*-**5** ($R = C_{13}H_{27}$), which was dried in vacuum to give 7.58 g (expected 11.90 g) of a white solid. It was recrystallized from boiling absolute ethanol (10 ml/g), cooling at r.t. overnight. The product *rac*-**5** ($R = C_{13}H_{27}$) (6.239 g, 52.4%), pure by TLC ($CHCl_3$/MeOH 20:1, R_f 0.08, $CHCl_3/CH_3COOH$ 10:1, R_f 0.24), shrinks at 85 °C and melts at 90–92 °C. (lit.

Tsivgoulis et al., 1991b: 91–93 °C). Its IR (KBr) spectrum is as published (Tsivgoulis et al., 1991b).



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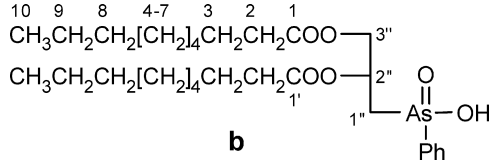
^{1}H NMR ($CDCl_3$), $\delta = 0.88$ (t, 6 H, H-14 and H-14′), 1.26 (m, 40 H, H-4 to H-13 and H-4′ to H-13′), 1.61 (m, 4 H, H-3 and H-3′), 2.33 (m, 4 H, H-2 and H-2′), 2.71 (d, $J = 6.8$ Hz, 2 H, H-1″), 4.19 and 4.37 (both doublet of doublets, 2 H, H-3″), 5.47 (m, 1 H, H-2″), 6.34 (broad, 2 H, $As(OH)_2$). ^{13}C NMR ($CDCl_3$), $\delta = 14.13$ C-14 and C-14′, 22.71 C-13 and C-13′, 24.66 C-3, 24.83, C-3′, 29.13–29.70 C-4 to C-11 and C-4′ to C-11′, 31.93 C-12 and C-12′, 33.19 C-1″, 33.97 C-2 and C-2′, 63.97 C-3″, 65.21 C-2″, 172.49 C-1′, 173.09 C-1. The assignments are based on the work of Amato et al. (1990).

The ether phase was evaporated to give a solid (10.50 g) in which diphenyl disulfide (4.19 g) and excess myristic acid (1.75 g) should be present. It was chromatographed (silica gel 70 g in ether) eluting with a) ether (350 ml), b) $CHCl_3$/MeOH 10:1 (200 ml), and c) $CHCl_3$/MeOH 5:1 (300 ml), collecting 50 ml fractions. Fractions 1–7 gave 6.41 g of a mixture (by ^{1}H NMR and TLC) of diphenyl disulfide (∼3 g), myristic acid (∼2.5 g), and phenyl thiomyristate (RCOSPh) (∼1 g). Fractions 8–9 gave an oil (96 mg) which could not be identified. Fractions 10–13 gave a foam (2.29 g) which, by IR and ^{1}H NMR, contained RCOO– and $–AsO_3H_2$ groups, melted at 48–50 °C but on recrystallization from methanol (8 ml) no chromatographically pure compound could be isolated. It is probably the lyso compound which underwent acyl group migration (Serdarevich, 1967) during the work-up. Fraction 14–17 gave a solid (1.15 g) containing, by IR and ^{1}H NMR, RCOO– and $–AsO_3H_2$ groups and DMAP.

2.6. Preparation of the arsinolipid rac-**10** ($R = C_9H_{19}$) via RCOOH/DCC/DMAP acylation of rac-**11**

This was prepared according to Kordalis and Ioannou (2000), in dry dichloromethane. After reduction, acylation and oxidation, the product did not precipitate and it was isolated by column chromatography (silica gel 50 g in ether for a scale of 2.2 mmol). Elution with a) Et_2O (400 ml) b) $CHCl_3$/MeOH 1:1 (10 ml, in order to push the product into the silica gel), and c) $CHCl_3$/MeOH 10:1 (800 ml) collecting 100 ml fractions gave the product *rac*-**10** ($R = C_9H_{19}$) in the fractions 10–11 as an oil (905 mg, 70%). The product absorbs moisture very easily (Kordalis and Ioannou, 2000). Calculated for $C_{29}H_{49}O_6As{\cdot}H_2O$ (M_r 586.62): C 59.37, H 8.76%; found 59.80, 8.83%. IR (neat): 1740 vs (C=O), 892 m (As=O), 756 s (As–OH).



b

^{1}H NMR ($CDCl_3$), $\delta = 0.88$ (t, 6 H, H-10 and H-10′), 1.26 (m, 24 H, H-4 to H-9 and H-4′ to H-9′;), 1.57 (m, 4 H) H-3 and H-3′, 2.26 (m, 4 H), H-2 and H-2′), 2.70 (m, 2 H), H-1″, 4.12 and 4.35 (both doublet of doublets, 2 H, H-3″), 5.41 (m, 1 H, H-2″), 7.49 (m, 2 H, *meta*-H), 7.56 (m, 1 H, *para*-H), 7.80 (d, $J = 7.2$ Hz, 2 H, *ortho*-H), 8.1 and 8.3 (broad, 2 H, H_2O). ^{13}C NMR ($CDCl_3$), $\delta = 14.11$ C-10 and C-10′, 22.70 C-9 and C-9′, 24.87 C-3, 24.94, C-3′, 29.08 – 29.47 C-4 to C-7 and C-4′ to C-7′, 31.91 C-8 and C-8′, 33.82 and 34.04 C-2 and C-2′, 34.90 C-1″, 64.50 C-3″, 66.35 C-2″, 129.37 *ortho*-C, 130.22 *meta*-C, 132.73 *para*-C, 134.29 *ipso*-C, 172.40 C-1′, 173.12 C-1.

3. Results and discussion

3.1. The reaction of alkaline arsenite with electrophiles

The reaction of alkaline sodium arsenite, "Na_3AsO_3", with *short* chain alkyl halides is the best method (the Meyer reaction; Meyer, 1883) for the preparation of aliphatic arsonic acids. With *medium* chain alkyl halides the reaction is very "temperamental" (Pietsch, 1965) and with *long* chain alkyl halides it does not work because the mixing of a lipophilic halide with the aqueous arsenite is very poor (Dixon, 1997). The lipophilicity of the medium and long chain alkyl halides can be overcome by using as substrates α-halo acids RCHXCOOH which in the Meyer reaction will give $RCH(AsO_3H_2)COOH$ that can be thermally decarboxylated to $RCH_2AsO_3H_2$ (Adams et al., 1984; Ioannou, 2002a). Alkaline arsenite reacts with epoxides (Chelintsev and Kuskov, 1946) giving α-hydroxy arsonic acids and we exploited this feature in order to prepare arsonolipids **5** (Serves et al., 1992) and arsinolipids like **10** and more complex ones (Kordalis and Ioannou, 2000; Ioannou, 2002) using glycidol as a substrate.

The nucleophile in the Meyer reaction is the trianion, AsO_3^{3-}, which is present in minute amounts in aqueous solutions of sodium arsenite (Serves et al., 1994). Its concentration can increase in the presence of excess sodium hydroxide (an undesirable strategy because the HO^- is also nucleophilic) or by using as concentrated solutions of sodium arsenite as possible. The reaction of glycidol with concentrated sodium arsenite gives a very viscous solution of disodium 2,3-dihydroxypropylarsonate (see **4**) and stirring is very difficult when the preparation is run at a large scale [e.g. 40 or 80 mmol As(III)].

For isolation of **2** the solution is "neutralized" by an equivalent amount of hydrochloric acid. When the preparation is run at a small scale [2–10 mmol As(III)], evaporation and drying in vacuum is relatively easy and extraction with absolute ethanol gives quite pure **2** (Tsivgoulis et al., 1991a; Serves et al., 1992, 1993) free of **8** but contaminated with As_2O_3, ethanol and, occasionally, with traces of glycerol. When the synthesis is run on a 40 or 80 mmol As(III) scale, the drying is not convenient and therefore the sodium chloride is removed by a strongly cation exchange resin (Lacoste et al., 1992) and the eluate, after evaporation, is dried very well in order to remove all hydrochloric acid because it diminishes the capacity of the strongly anion exchange resin which is used to purify **2**. From the latter resin, elution with water removes As_2O_3, glycerol (Serves et al., 1995a) and any

base catalysed polymerized glycidol (Sunder et al., 1999). From this fraction only 25–30% of the As_2O_3 that did not react was recovered because As_2O_3 is volatile in water when evaporated (rotary, 60 °C). Elution with 1 M aqueous acetic acid gave small amounts (~10%) of **8** as a pair of diastereoisomers, running just above **2** on TLC and its detection in the original mixture was not possible. Next, **2** was eluted either free of **8** or containing traces of it.

When a concentrated solution of aqueous alkaline sodium arsenite reacted with *rac*-3-chloro-1,2-propanediol, the relative molecular proportions of **2**, **8** and glycerol in the crude reaction mixture were 1:0.09:0.89 (Tsivgoulis et al., 1991a), when reacted with stoichometric amount of *rac*-glycidol the proportions were 1:0.11:0.27 (this work) and when reacted with 15 mol% excess *rac*-glycidol the proportions were 1:0.08:0.24 (this work). The yields of **2** from *rac*-glycidol (60–80%) (Serves et al., 1992; this work) are better than the yields from *rac*-3-chloro-1,2-propanediol (45–50%) (Tsivgoulis et al., 1991a; this work). The latter substrate gives more glycerol because of the concomitant glycidol formation (Serves et al., 1994) which consumes HO^-. Lowering the OH^- concentration results in a decrease of the concentration of the active nucleophile AsO_3^{3-} (Serves et al., 1994), and glycidol is hydrolysed to glycerol under HO^- catalysis.

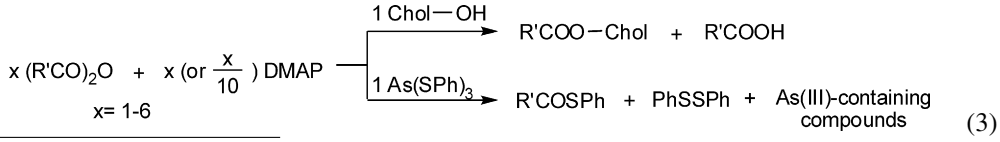
The arsonic acid **2** could not be obtained pure. When isolated by extraction and "crystallization", it contained small amounts of As_2O_3, ethanol, traces of glycerol, and, probably, water (Tsivgoulis et al., 1991a; Serves et al., 1992, 1993). Isolation by anion exchange chromatography, however, gives **2** contaminated by acetic acid, methanol, traces of **8**, and, probably, water (Section 2.3). The tenaciously held acetic acid and methanol are efficiently removed during the preparation of **6**. The other impurity **8**, is removed during "crystallization" of **2** (Tsivgoulis et al., 1991a; Serves et al., 1992) or during the preparation of the dilithium (Lacoste et al., 1992) or barium (this work) salts of **2**. If not removed during the preparation of **6** it can be removed from the arsonolipid **5** due to different solubility of acylated **8** (this work).

The assignment of the structure of the by-product **8** (instead of **9**) is based on the ^{13}C NMR spectra of polymerized glycidol in CD_3OD (Sunder et al., 1999) and in D_2O (Tokar et al., 1994; Dworak et al., 1995) and on the work of Cassel et al. (2001). The production of **8**, probably containing traces of **9**, comes from the primary alkoxide of the anion **4**. Our data indicate that reaction of the alkoxide of **8** with glycidol to give a trimer is unlikely. The dimeric **8** is obtained as a pair of diastereoisomers in equal proportions (by ^{13}C NMR of its barium salt). The production of **8** in essentially the same proportion compared to **2** starting from either glycidol or 3-chloropropane-1,2-diol, implies that the latter gives the epoxide much faster than the Meyer reaction on $-CH_2Cl$, a conclusion which has been reached earlier (Serves et al., 1994).

Condensation by-products, analogous to **8**, have also been encountered in the reaction of alkaline arsenite with DL-1,3-butadiene diepoxide (Tsivgoulis et al., 2007).

3.2. The competitive reaction of DMAP-activated carboxylic acid anhydride with cholesterol and triphenyl trithioarsenite $(PhS)_3As$

In order to understand the origin of the moderate yields of **5** obtained by RCOOH/DCC/DMAP acylation of **6** in dry, non de-aerated dichloromethane, we run competitive experiments using equimolar quantities of cholesterol and $(PhS)_3As$ according to Eq. (3) under various acylating conditions, Table 1.



(3)

Dioxygen in air has no effect on the acylation of cholesterol, but pure $As(SPh)_3$ in chloroform is air oxidized to As_2O_3 and PhSSPh at an extent of 5–10% in 3 days (Haikou and Ioannou, 2006). Therefore, a small amount of PhSSPh can come from the air oxidation of $(PhS)_3As$.

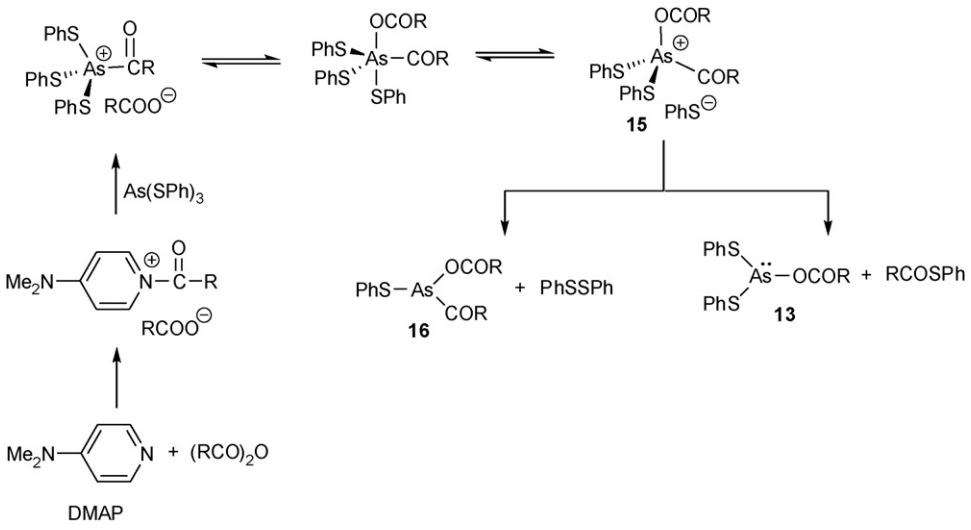
The reactions of Eq. (3) can conveniently be followed by TLC. In *all* cases *free* cholesterol was detected. Because the anhydride hydrolyses on TLC, its presence was confirmed by IR. The reaction mixture was chromatographed on silica gel. During this step, any anhydride that remained was hydrolysed to free acid which did not elute, as did cholesterol (which had not reacted or produced by hydrolysis of an intermediate; see below). Also, pure $As(SPh)_3$ was mainly air oxidized by ~65% during the chromatography (Tsivgoulis and Ioannou, submitted for publication). Therefore, the yields of $As(SPh)_3$, reported in Table 1, are underestimated and those of PhSSPh are overestimated, but these do not affect the conclusions presented below.

The detection of *free* cholesterol on TLC even when large excess of anhydride is present (entries 5–7, 10, 11; Table 1) means that, in solution, it was somehow protected from acylation as a compound which was hydrolytically labile. We hypothesize that cholesterol is "protected" as **14** by reacting with an arsenic(III) compound, e.g. $(PhS)_2As–OCOR$ **13** (see below), Eq. (4), because it is known (Gattow and Schwank, 1971) that $(AcO)_3As$ reacts with alcohols giving $(RO)_3As$ and AcOH.

$$\underset{\mathbf{13}}{(PhS)_2As–OCOR} + Chol–OH \rightarrow RCOOH + \underset{\mathbf{14}}{(PhS)_2As–OChol} \xrightarrow{H_2O} (PhS)_2As–OH + Chol–OH \quad (4)$$

Then, **14** hydrolyses on the silica gel giving free cholesterol, Eq. (4), because alkoxy esters of As(III) are not hydrolytically stable (Brill and Campbell, 1973; Baer et al., 1983). Therefore, the intermediate such as **13** must have been formed quite fast; in other words the reaction of $As(SPh)_3$ with activated acid anhydride competes with the acylation of cholesterol.

The yields of the wanted product, cholesteryl palmitate, reaches a plateau (60–70%) when $x = \sim 3$, Eq. (3), irrespective of the amount of DMAP (entries 4–7 and 9–11, Table 1).



Scheme 2.

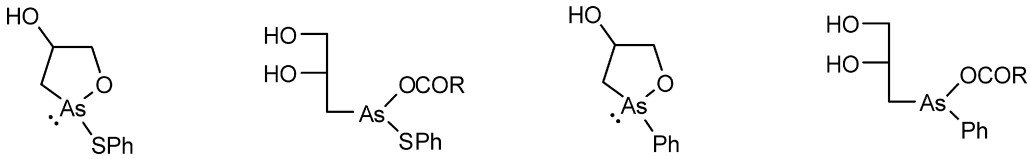
The remaining cholesterol is "blocked", as described above. Therefore, a slight excess of anhydride with catalytic amount of DMAP gives the best yields of cholesteryl ester without wasting anhydride. The data of Table 1 (entries 2 and 3) show that at low temperatures the $As(SPh)_3$ is more reactive towards DMAP-activated anhydride than the secondary –OH group, giving ~45% thiol ester.

The nature of the arsenic-containing compounds formed in these competitive experiments is not certain because of their lability and reactivity, but we can offer an approach to them. Previous results showed that when 1 mol $As(SPh)_3$ reacts with 3 mol $(RCO)_2O$ in the presence of 0.3 mol DMAP then ~60% RCOSPh is produced, according to Scheme 2, by attack of PhS^- on the activated carbonyl carbon of As^+–COR **15**, in a manner analogous to $DMAP^+$–COR, while attack at PhS– of **15** to give PhSSPh and **16** is not favored (Tsivgoulis and Ioannou, submitted for publication).

Compound **13**, being extremely reactive (Gattow and Schwank, 1971), reacts with cholesterol as per Eq. (4), giving $(PhS)_2$As–OChol **14**, thus protecting cholesterol from acylation. It should be noted that **14** could further react with the activated anhydride and cholesterol as per Scheme 2 and Eq. (4).

3.3. The preparation of rac-5 ($R = C_{13}H_{27}$) and rac-10 ($R = C_9H_{19}$) via RCOOH/DCC/DMAP acylation of rac-6 and rac-11, respectively, and spectral properties of the products

The above arguments can explain the high yields (~80%) in the preparation of **12** (Terzis and Ioannou, 2002) because inactivation of the secondary –OH does not take place intramolecularly because of the strain of a 4-membered ring would have possessed if it had been formed.



The moderate yields (~55%) in the preparation of **5** by RCOOH/DCC/DMAP acylation of **6** can be understood by the formation of a 5-membered ring, **17** from **18**. Evidence for the formation of **17** is the detection of phenyl thiomyristate, RC(O)SPh, arising *via* reactions analogous to those shown in Scheme 2. Acylation of **17** and hydrogen peroxide oxidation should give a lyso-arsonolipid which is removed during the isolation of **5**. The peroxide oxidation can convert any >As–SPh and > As–COR into As(V) because it is known that compounds of As(III), e.g. $Ar–As(SR)_2$ are oxidized by I_2/H_2O to arsonic acids $Ar–AsO_3H_2$ (Barber, 1929) and compounds such as Ph_2As-COMe (Steinkopf et al., 1928) or Me_2As-COMe (Albers et al., 1952) are oxidized to arsinic acids Ph_2AsO_2H or Me_2AsO_2H by O_2/H_2O and warming or spontaneously, respectively. From the preparation of **5** ($R = C_{13}H_{27}$) we isolated a significant amount of a low melting foam which most likely is lyso-arsonolipid. However, we could not isolate a chromatographically pure sample probably because acyl migration (Serdarevich, 1967) took place during the attempted purification.

The better yields (~70%) obtained for arsinolipids **10** are probably due to a slower nucleophilic attack of As(III) of **11** on the C=O carbon of the activated carboxylic acid anhydride compared with As(III) of **6**, and/or to a smaller extent of cyclization of **20** to **19** thereby increasing the amount of the primary –OH acylation.

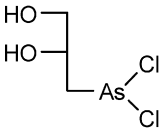
Analogous cyclization may have taken place when we (Tsivgoulis et al., 1991a; Serves et al., 1992) acylated salts of **2**,

e.g. **3** (an As(V) compound), with $(RCO)_2O$/py and RCOCl/py, respectively. In these cases, the mixed anhydride which should have been formed (see Mangroo and Gerber, 1988) suffered cyclization by attack of the primary –OH on As(V) giving the cyclic monoester of As(V), thus "protecting" the primary –OH from acylation.

The solid state IR spectra of the arsonolipids **5** (Tsivgoulis et al., 1991b) and arsinolipids **10** (Kordalis and Ioannou, 2000) have been discussed. The arsinolipid **10** ($R = C_9H_{19}$), however, showed only one band at 756 cm^{-1} for ν(As–OH) implying that there is only one type of hydrogen-bonding. The high-resolution ^1H NMR spectra of **5** ($R = C_{13}H_{27}$) resembles that of the arsenic-containing phosphonate studied in much detail by Amato et al. (1990). In **5** the RCOOC*H* proton resonates 0.25 ppm downfield compared with the RCOOC*H* proton of the phosphonate and it may be attributed to the different polar groups: $–CH_2AsO_3H_2$ and $–CH_2OP(O)(O^-)CH_2CH_2AsMe_3^+$. The high-resolution ^{13}C NMR spectrum of **10** ($R = C_9H_{19}$) resembles that of **5** ($R = C_{13}H_{27}$). The ^1H NMR spectrum showed the presence of water, also found by elemental chemical analysis, and this water absorption is a feature of arsinolipids (Kordalis and Ioannou, 2000).

3.4. Conclusion

The experiments reported herein threw light on the step that most affects the preparation of arsonolipids **5**. This is the reaction of an activated acyl group with the As(III) atom of **6**. The product obtained, **18**, intramolecularly gives a cyclic compound, **17**, thus blocking the primary –OH group of the substrate from being acylated.



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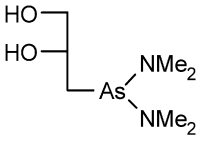

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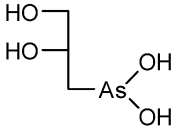

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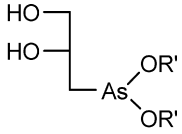

24

Searching for As(III) substrates other than **6** for the preparation of **5**, the dichloroarsine **21** and the diamide **22** deemed unsuitable because they were difficult to prepare (Serves et al., 1993). Compound **23** (Ioannou, 2000) should not be inert towards a catalysed acylation because acylation at the As(III) can take place, since it is known that α-furylarsine oxide reacts with acetic anhydride at 80–90 °C to give α-furyl diacetoxyarsine (Étiene, 1947), Eq. (5). Finally, esters like **24** with anhydrides can react at As(III) as well because it is known that, e.g. glycol ester of phenylarsonous acid and acetic anhydride at reflux for 6 h gave phenyl diacetoxyarsine (Kamai and Chadaeva, 1956), Eq. (6).

$$R–As(OH)_2 \underset{H_2O}{\overset{-H_2O}{\rightleftharpoons}} R–As=O \xrightarrow{(CH_3CO)_2O} R–As(OCOCH_3)_2 \quad (5)$$

$$Ph–As(OR)_2 + 2(CH_3CO)_2O \rightarrow 2CH_3COOR + Ph–As(OCOCH_3)_2 \quad (6)$$

Therefore, with **23** and **24**, intermediates having the >As–OCOR group can be formed and cyclization most likely cannot be avoided in their *catalysed* acylation.

Acknowledgment

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