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Short Communication

Microbial Sulpholipids: (*R*)-13-Chloro-1-(*R*)-14-docosanediol Disulphate and Polychlorosulpholipids in *Ochromonas danica*

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During an investigation of the sulpholipids of the phytoflagellate *Ochromonas danica* we have described the sulphatide *S*-14-docosyl sulphate (Mayers & Haines, 1967; Mayers, Pousada & Haines, 1969). This sulpholipid was the first alkyl sulphate to be identified in a complex mixture of sulphatides. These sulpholipids are unique as polar lipids, since they have ionic groups at both ends of the molecule. Equally novel is the fact that chloride is a constituent part of the natural sulphatide. The present communication describes the second sulphatide in this group of sulphatides. It is *threo*-(*R*)-13-chloro-1-(*R*)-14-docosanediol disulphate.

Methods. Ochromonas danica was grown as described by Haines & Block (1962) and the sulpholipid mixture was isolated (Haines, 1965). The procedure was upgraded so that 7·5g. of sulpholipids was isolated. Although the mixture is separable into several spots on t.l.c., a preparation of the mixture analysed as: C, 42·56; S, 10·51%; or C/S ratio 11:1. Thus the mixture consists of C_{22}-alkyl disulphates. The diols (5·1g.) were obtained by solvolysis in moist dioxan with retention of configuration, and separated by column chromatography on silica gel (Mayers *et al.* 1969). Methods for g.l.c. and t.l.c. and laboratory techniques have been described by Mayers *et al.* (1969) and Gershengorn *et al.* (1968). At least seven different diols were demonstrated by t.l.c. Although we initially assumed these compounds were hydrolysis artifacts, solvolysis excluded this possibility. Analysis of spot I was not possible owing to insufficient quantity, but the chloride analyses of the other spots showed: spot II, Cl, 26·28% (3); spot III, Cl, 36·19% (5–6); spot IV, Cl, 22·45% (2–3); spot V, Cl, 17·91% (2); spot VI, Cl, 10·10% (1). Spot VII is the 1-(*R*)-14-docosanediol previously described together with tetracosanediol (Mayers *et al.* 1969). The dominant diols in the mixture were: hexachlorodocosanediol (spot III), 34%; chlorodocosanediol (spot VI), 24%; docosanediol (spot VII), 20%. Other substances constituted less than 10% each of the total diols. The substance characterized here is spot VI.

The compound was acetylated (Gershengorn *et al.* 1968) as shown in Fig. 1. This verified the analysis and t.l.c. properties of the substance and identified it as a diol. It also demonstrates its thermal decomposition to 14-oxodocosanol. 14-Oxodocosanol (X) was identified by comparison of its mass spectrum at 160° with that of the authentic material synthesized from 14-oxodocosanoic acid (Mayers *et al.* 1969). The mass spectrum of the substance at 240° is nearly identical with that of 14-oxodocosanol, although it exhibits some evidence for epoxide formation. Confirmation of the structure 13-chloro-1,14-docosanediol was obtained by mass spectrometry, in particular by close examination of the isotope peaks for those ions that contain chloride. For example the peak at 263 *m/e*, which is accompanied by isotopic peaks at 264, 265 and 266 *m/e* appropriate for 14 carbon atoms, 1

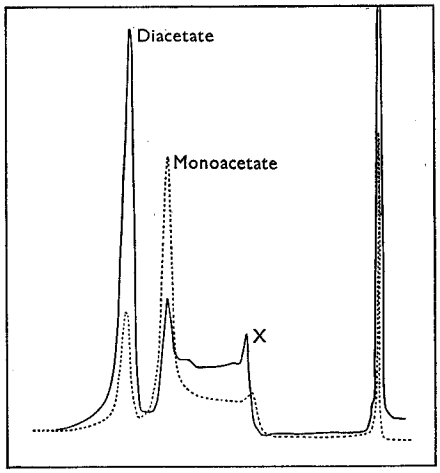

Fig. 1. G.l.c. of chlorodocosanediol after acetylation for 30min. (·····) and 1hr. (——) with acetic anhydride in pyridine. Samples were injected directly into the Perkin–Elmer model 881 chromatograph containing a 6 ft. × $\frac{1}{8}$ in. column (3% JXR on Chromosorb Q) at 230°.

chlorine atom and 2 oxygen atoms, is also accompanied by peaks at 245 ($263-H_2O$), 227 ($263-HCl$ or $263-2H_2O$), 209 ($263-H_2O-HCl$) and 191 *m/e* ($263-2H_2O-HCl$). The base peaks of the spectra were 142 and 143 *m/e*. Two particularly large metastable peaks at 108·3 and 109·3 *m/e* confirmed the decomposition of 142 and 143 *m/e* to 124 and 125 *m/e* respectively.

The n.m.r. spectrum in deuterochloroform showed peaks at τ 8·68 (CH_2), 9·10 (CH_3 terminal), 6·32 (CH_2.OH terminal) and a series of small peaks including the region of the triplet (τ 5·9–6·6). Integration of this area showed it contained two protons in addition to the protons of the primary hydroxyl. The i.r. spectrum of the chlorodiol is similar to that of 1,14-docosanediol (Mayers *et al.* 1969). Secondary chloride is identified by weak bands at 763, 681 and 613 cm.$^{-1}$ and also two medium bands at 1238 and 1305 cm.$^{-1}$. The optical rotation of the 13-chloro-1,14-docosanediol was found to be $[\alpha]_D^{27}$ +14·7° (*c* 0·0336 in chloroform). The magnitude of the rotation and its direction (Morris & Wharry, 1966) demonstrate that the substance is *threo*-(*R*)-13-chloro-1-(*R*)-14-docosanol (L-13, D-14). To confirm that the substance is *threo* it was converted into the *cis*-epoxide by standing at room temperature overnight in 0·2M-KOH. The corresponding *cis*-epoxide was synthesized from erucinol (*cis*-13-docosenol) by using *m*-chloroperbenzoic acid. Comparison of the t.l.c. properties of these substances (Roomi, Subbaram & Achaya, 1966) confirmed the assignment of *threo* to the natural chlorodiol. Further confirmation was obtained from the comparison of the t.l.c. properties (Roomi *et al.* 1966) of the natural chlorodiol to the *threo*- and *erythro*-chlorodiols obtained from erucic acid and brassidic acid respectively (Bharucha & Gunstone, 1956). Since the disulphate of 1-(*S*)-14-docosanediol is of the L-configuration and the secondary hydroxyl group of this material is inverted (D-configuration) then the presence of the L-13-chloro group in the molecule is accompanied by inversion of the orientation of the 14-hydroxyl group.

J. Elovson & P. R. Vagelos (personal communication) have obtained mass-spectral evidence for the existence of chlorodiols in *Ochromonas* sulphatides, including the one described in the present communication.

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