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CHLOROSULPHOLIPIDS OF *TRIBONEMA AEQUALE*

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Key Word Index—*Tribonema aequale*, Xanthophyceae, Chrysophyta, chlorosulpholipids, docosane-1,14-disulphate

Chlorosulpholipids were first discovered in the alga *Ochromonas danica* (Division—Chrysophyta; Class—Chrysophyceae)[1,2] where they constitute 15% of the lipids and 3% of the dry weight of heterotrophically-grown stationary phase cells.[2] Two series of chlorosulpholipids were found to be present in *O. danica*, one based on *N*-docosane-1,14-diol disulphate and one, much less abundant, based on *N*-tetracosane-1,15-diol disulphate. The 13-chloro-, 11,15-dichloro-, 2,2,11,13,15-pentachloro- and 2,2,11,13,15,16-hexachloro derivatives of *N*-docosane-1,14-diol disulphate have been identified along with several partly characterized tri- and tetrachloro derivatives.[3] The 14-chloro-, 2,12,14,16,17-pentachloro- and 2,2,12,14,16,17-hexachloro derivatives of *N*-tetracosane-1,15-diol disulphate have also been detected.[3]

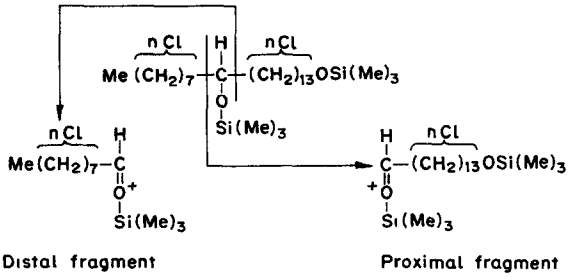

FIG 1. PRINCIPAL MS FRAGMENTATION OF CHLOROSULPHOLIPID DIOL-TMS ETHERS

In the present work we report the detection of *N*-docosane-1,14-diol disulphate and mono-, di-, tri-, tetra-, penta- and hexachloro derivatives of it in the filamentous alga *Tribonema aequale* (Xanthophyceae, Chrysophyta). The identification of these compounds was based upon the GC–MS analysis of the trimethylsilylethers of the mixture of diols produced by solvolysis of the extracted chlorosulpholipid fraction isolated from the alga (see Table 1). The chlorosulpholipids of *O. danica* were isolated and analysed in an identical manner for comparison purposes.

[1] HAINES, T H., POUSADA, M, STERN, B and MAYERS, G L (1969) *Biochem. J* **113,** 565
[2] ELOVSON, J and VAGELOS, P R (1969) *Proc. Nat. Acad. Sci. U.S.A.* **62,** 957
[3] HAINES, T. H (1971) *Progress in the Chemistry of Fats and Other Lipids* (HOLMAN, R. T M, ed), Vol XI, pp 299–345, Pergamon Press, New York

TABLE 1 GAS-CHROMATOGRAPHIC AND MS ANALYSIS OF THE CHLOROSULPHOLIPID DIOL-TMS ETHERS OF *Tribonema aequale*

GLC Peak	*A*	*B*	*C*		*D*		*E*		*F*	*G*
R_t (min–sec)	8–22	11–23	14–19		16–30		18–30		20–56	22–59
Temp of elution °C	227	242	252		265		265		265	265
Number of Cl atoms	0	1	2		3		4		5	6
Position of Cl atoms*		0–1	0–2	1–1	0–3	1–2	1–3	2–2	2–3	2–4
MS Fragmentation										
M^+	486	520	–	–	—	—	–	–	—	—
M-Me	471	505	539	539	573	573	607	607	641	675
M-HCl	–	484	518	518			–	—	620	654
M-[Me + HCl]	–	–	503	503	537	537	571	571	605	639
M-[TMSCl]	– –	–	– –	–	480	480	514	514	—	– –
M-[Me + TMSCl]	– –	–	431	431	– –		–	–	533	567
Proximal fragment (PF)†	373	407	441	407	—	—	–	441	475	509
PF-HCl	–		405	371	–	405	439	405	439	473
PF-2 HCl			369		403	369	403	369	403	437
PF-3 HCl	– – –		–	–	367		367		367	401
PF-4 HCl	–	—	–	–	–	—	—	—		365
Distal fragment (DF)‡	215	215	215	249	215	249	249	283	283	283
DF-HCl	– –	—	—	213	–	213	213	247	247	247
DF-2 HCl		– –	–		– –		–	211	211	211

* This refers to the position of the chlorine atoms relative to the secondary alcoholic oxygen atom. Of the pair of figures (e.g. 1–3), the first (i.e. 1) refers to the number of chlorine atoms distal to this oxygen, whilst the second (i.e. 3) refers to the number which are proximal.

† and ‡ see Fig. 1.

The chlorosulpholipid diol-TMS ether mixture was separated into seven major peaks (*A*–*G*) by GLC. Peak *A* had a retention time very close to that of cholestane (8 min 34 sec) and a R_t and MS identical to that of the TMS ether of *N*-docosane-1,14-diol of *O. danica*. The highest *m/e* value in the MS was 486 (v. low intensity), accompanied by medium intensity peaks at *m/e* values of 471 (M-Me), 373 (proximal fragment) and a base peak at *m/e* 215 (distal fragment). The M^+ peak is frequently missing from the MS of the TMS ethers of the chlorosulpholipid diols[2] and the principal fragmentation is that shown in Fig. 1 giving the proximal (C-1-OTMS → C-14-OTMS) and distal (C-22 → C-14-OTMS) fragments.[2] The distal fragment is very abundant and is frequently the base peak. The proximal fragment is much less abundant and sometimes cannot be seen in the higher chlorinated species, its degree of chlorination may then be elucidated by the characteristic *m/e* values of the "Proximal Fragment minus nHCl" fragment ions.

Peak *B* had a retention time and mass spectrum identical to that of the TMS ether of the monochloro-*N*-docosane-1,14-diol of *O. danica*. The molecular ion cluster had peaks at *m/e* values of 520 and 522 in the ratio of 2:1 as had the M-Me cluster at *m/e* values of 505 and 507. The *m/e* values of the M^+ and M-Me ions were therefore 34 and 36 mass units greater than for the TMS ether of *N*-docosane-1,14-diol, indicating the presence of one chlorine atom. The present of fragment ions at *m/e* values of 215 and 407/409 (ht. ratio 2:1) indicated that the chlorine atom is located in the proximal fragment. However the precise position of the chlorine atom cannot be determined from the MS. The lack of a fragment ion at *m/e* 397 shows that the monochloro species of *T. aequale* may differ from that of *O. danica* where its presence has been interpreted[2] as being due to loss of TMSCl from the M-15 ion by interaction of the Cl and TMS groups, a situation which is most likely if they are bonded to adjacent carbons.

Peak *C* had a retention time identical to that of the TMS ether of the dichloro-*N*-docosane-1,14-diol of *O. danica*. The highest *m/e* values in the MS were a Cl_2 cluster at 539 which corresponds to the M-Me fragment ion of the TMS-ether of dichloro-*N*-docosane-1,14-diol. The non-chlorinated ion at *m/e* 215 and the Cl_1 cluster at *m/e* 249 correspond to non- and monochlorinated distal fragments and therefore suggest that peak *C* is a mixture of two dichlorinated diols, one having no Cl atoms distal and two Cl atoms proximal to the secondary alcoholic oxygen and the other having one Cl atom distal and one proximal. This is confirmed by the presence of a Cl_1 cluster at *m/e* 407 (mono Cl proximal fragment) and a Cl_2 cluster at *m/e* 441 (diCl proximal fragment). The presence of a Cl_1 cluster at *m/e* 371 corresponds to a M-[Me + TMSCl] ion and suggests that one or both of the dichloro species has a Cl atom adjacent to a TMS group.

Peak *D* had a retention time identical to that of the TMS-ether of the trichloro-*N*-docosane-1,14-diol peak of *O. danica*. The highest *m/e* values in the mass spectrum were a Cl_3 cluster at 573 corresponding to the M-Me ion Again there was evidence of two diol species in the peak. A non-chlorinated ion at *m/e* 215 (distal fragment) and peaks at *m/e* 403 (triCl proximal fragment—2 HCl) and *m/e* 367 (triCl proximal fragment—3 HCl) suggest the presence of a trichloro species with all three Cl atoms proximal to the secondary alcoholic oxygen. A Cl_1 cluster at *m/e* 249 (distal fragment) and peaks at *m/e* 405 (diCl proximal fragment—HCl) and *m/e* 369 (diCl proximal fragment—2 HCl) indicate the presence of a trichloro species with one Cl atom distal and two proximal to the secondary alcoholic oxygen.

Peak *E* had a retention time identical to that of the TMS ether of the tetrachloro-*N*-docosane-1,14-diol of *O. danica*. Again the MS was devoid of the molecular ion and the highest *m/e* value was at 607 (M-Me). A Cl_1 cluster at *m/e* 249 (distal fragment) and peaks at *m/e* values of 439, 403 and 367 (triCl proximal fragment—HCl, 2 and 3 HCl respectively) indicate the presence of a tetrachloro species with one Cl distal and three proximal to the secondary alcoholic oxygen. A Cl_2 cluster at *m/e* 283 (distal fragment) and peaks at *m/e* 441 (diCl proximal fragment), *m/e* 405 (diCl proximal fragment—HCl) and *m/e* 369 (diCl proximal fragment—2 HCl) indicate the presence of a tetrachloro species with Cl atoms distributed equally on either side of the secondary alcoholic oxygen.

Peak *F* had a retention time identical with that of the TMS-ether of a pentachloro-*N*-docosane-1,14-diol peak of *O. danica*. The mass spectrum had a highest *m/e* value at 641 (M-Me), a Cl_2 cluster at *m/e* 283 (distal fragment) and peaks at *m/e* values of 475, 439, 403 and 367 corresponding to a trichlorinated proximal fragment and its dehydrochloro derivatives. The pentachloro species therefore has two Cl atoms distal and three proximal to the secondary alcoholic oxygen.

Peaks *G* had a retention time and mass spectrum identical with that of the TMS-ether of the hexachloro-*N*-docosane-1,14-diol peak of *O. danica*. The MS had a highest *m/e* value at 675 (M-Me), a Cl_2 cluster at *m/e* 283 (distal fragment) and peaks at *m/e* values of 509, 473, 437, 401 and 365 corresponding to a tetrachlorinated fragment and its dehydrochloro derivatives. The hexachloro species therefore has two Cl atoms distal and four proximal to the secondary alcoholic oxygen.

EXPERIMENTAL

Tribonema aequale Pascher, obtained from the Culture Collection of Algae and Protozoa, The Botany School, Cambridge, was grown on Bold's Basal Medium[4] contained in Roux bottles standing upright six inches in front of a double bank of "warm white" fluorescent tubes (4750 lx) and continuously aerated, these conditions provided

[4] DEASON, T R and BOLD, H C (1960) *University of Texas Publication* No 6022, 1

a temp of 20–21°. The filaments were harvested by filtration and washed twice with H_2O. The resulting material (wet wt 8.74 g) was then extracted three times with $CHCl_3$–MeOH (2:1, v/v) after first disrupting the cells by cycles of freezing and thawing followed by grinding with acid-washed silver sand. The chlorosulpholipids were then obtained from this extract as described previously for *O. danica*[5] and converted to their diols by solvolysis.[6] The diol mixture was then silylated and subjected to GC–MS using a Pye 104 gas chromatograph linked via a single-stage silicone rubber membrane separator[7] to an AEI MS-30 mass spectrometer. The GLC separation was performed on a 5 ft × 4 mm i.d. glass column packed with 1% SE-30 on 80–100 mesh Gas Chrom Q programmed to 5 min isothermal at 210° followed by a linear increase of 5°/min up to 265° and then held. The He gas flow through the column was 50 ml/min. Low resolution MS were obtained with an electron energy of 24 eV, an emission current of 300 μA and a source temp of 250°.

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CRYSTALLINE CHEMICAL COMPONENTS OF *CHEILANTHES LONGISSIMA*

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Key Word Index—*Cheilanthes longissima*, Polypodiaceae, fern, cheilanosin, 7-*O*-methylapigenin, 3,7-di-*O*-methylkaempferol; 3,7,4′-tri-*O*-methylkaempferol, sitosterol, sitosterol D-glucoside.

Plant. Cheilanthes longissima. Geographical source. Darjeeling (Himalayas). *Previous work*: on this species nil; on other *Cheilanthes* species, *C. farinosa*,[1–4] *C. tenuifolia*[5] and *C. mysurensis*.[6]

Present work. Dried and coarsely powdered fern (aerial parts) was successively extracted with boiling petrol and C_6H_6. The extracts, being identical (TLC), were mixed. On concentration a solid was deposited. The filtered solid (**1**) and the evaporated mother liquor (**2**) were separately chromatographed on silica gel. From chromatography of (**1**): $CHCl_3$–MeOH (49:1) eluted cheilanosin; $CHCl_3$–MeOH (97:3) eluted 3,7-di-*O*-methylkaempferol and then a flavonoid mixture; $CHCl_3$–MeOH (19:1) eluted sitosterol D-glucoside. The flavonoid mixture was separated by preparative TLC using C_6H_6.dioxan:AcOH (90:25:4) into 3,7-di-*O*-methylkaempferol and 7-*O*-methylapigenin. From chromatography of (**2**): Petrol–C_6H_6 (9:1) eluted a triterpene compound in very low yields; C_6H_6 eluted sitosterol; C_6H_6–EtOAc (49:1) eluted 3,7,4′-tri-*O*-methylkaempferol. Appropriate

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