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ISOPRENOID TRIETHER LIPIDS FROM *CALDARIELLA*

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Key Word Index—*Caldariella*; lipids; ether lipids; isoprenoids; tri-*O*-phytanylglycerol.

In the *Caldariella* group of extreme thermoacidophile bacteria [1], the major lipids [2,3] are macrocyclic sn-1,2-diethers of glycerol derived from a range of C_{40} diols with unique ω,ω'-biphytanyl structures. In the hope of shedding some light on their biosynthesis, we investigated minor lipid components, here shown to be triethers of glycerol with partly-or fully-saturated C_{20} (phytanyl) alcohols.

Separation of minor lipids

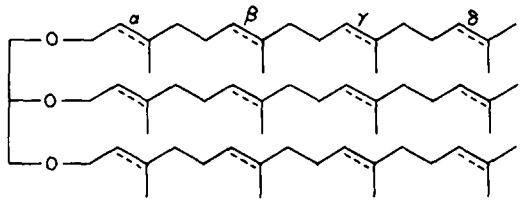
From *ca* 350 g of lyophilized cells of the MT-3 and MT-4 strains of *Caldariella acidophila*, some 25 g of total lipid was obtained, of which *ca* 0.5 g (accumulated over a series of experiments) was directly soluble in hexane and separated accordingly. Chromatography of this fraction afforded the thianaphthenequinone described elsewhere [4] as major component, a complex mixture of hydrocarbons, and some 30 mg of glycerol triethers. These on TLC (Si gel; hexane–Et_2O, 7:3) afforded two components, R_f 0.5 and 0.4; the former predominated in the triether fraction from MT-4.

Tri-O-phytanylglycerol (**1**)

The IR spectrum of the less polar triether showed only saturated alkyl and ether bands (ν_m 1110, 1375–1385, 1465, 2850–2950 cm^{-1}) and the ^1H NMR spectrum showed, per 11 CH_2–O and CH–O protons (δ 3.40, *br*) some 72 saturated CH_2 and CH protons (δ1.22–1.60, *br*) and 45 CH_3 protons (δ 0.86, *d*, J = 9Hz). Satisfactory MS were not obtained. Treatment with BCl_3 gave glycerol (TLC) and an alkyl chloride $C_{20}H_{41}Cl$ (by MS); similarly HI cleavage gave $C_{20}H_{41}I$. The ^1H NMR spectra of these halides showed, per 2 CH_2X protons (δ 3.15, *t*) 24 CH_2 and CH protons (δ 1.22–1.60) and 15 CH_3 protons (δ 0.86, *d*, J = 9 Hz) and their identification as phytanyl halides was confirmed by conversion into the acetate which was identical (MS, NMR, and GLC) with authentic phytanyl acetate. Thus structure (**1**) for the less polar triether is established.

The more polar component

The more polar triether component was very similar to (**1**) but contained some unsaturation (addition ν_m 1665 cm^{-1}; NMR data below). Hydrogenation gave a product identical with (**1**) which was similarly converted into



(**1**) (no unsaturation)
(**2**) (unsaturation as broken lines, see text)

authenticated phytanyl acetate. The extent and location of unsaturation in this component (which is probably a mixture) were in part extablished by ^1H NMR methods. If we regard (**1**) biogenetically as a tri-*O*-geranylgeranylglycerol which has been completely reduced, then in a partly-reduced analogue there are 12 possible double bond sites and these are of three distinguishable kinds: (i) in the α-residue, bearing allylic CH_2–O; (ii) in the β- and γ-residues; (iii) in the δ-residue, with terminal =CMe_2.

The resolved signals, assignments, and approximate integrals (calibrated from the total of the C$\underline{H}_2$–O and C$\underline{H}$O signals, δ 3.40 and 3.95; 11 protons) in the ^1H NMR spectrum of the more polar triether fraction are: δ 0.86 (21H, *d*, J = 9 Hz, C$\underline{H}_3$–CH sat); 1.10–1.40 (36H, *br*, C$\underline{H}_2$ and C$\underline{H}$, sat); 1.57 (6H, *s*, one C$\underline{H}_3$–CMe= in δ-residue); 1.66 (18H, *s*, C$\underline{H}_3$–C= in α, β, γ and one C$\underline{H}_3$–CMe= in δ-residue); 2.00 (18H, *br*, C$\underline{H}_2$–CH=); 3.40 (9H, *br*, sat C$\underline{H}_2$–O and C$\underline{H}$–O); 3.95 (2H,*d*, J = 8 Hz, =CH–C$\underline{H}_2$–O); 5.05 (5H, *br*, =C$\underline{H}$–CH_2); 5.35 (1H, *t*, J = 8 Hz, =C$\underline{H}$–CH_2O). The data are satisfied if in the more polar triethers there are (approximately) 6 double

bonds, viz. one out of the 3 possible in α-residues, three out of the 6 possible in β- and γ-residues, and two out of the 3 possible in δ-residues (cf. structure 2).

This at least suggests that reduction of the postulated precursor tri-*O*-geranylgeranylglycerol occurs progressively from the oxygenated end of the isoprenoid chains. Unfortunately the structures of these very minor triethers do very little to clarify the biogenesis of the ω,ω'-biphytanyl skeleton in the major cyclic diether lipids of *Caldariella*.

EXPERIMENTAL

Culture methods and lipid extraction procedures are described elsewhere [1,3]. Direct extraction of the total lipid (25 g) with hexane gave a soluble fraction (540 mg) which was subjected to chromatography on Merck Kieselgel (70–230 mesh) in hexane with increasing proportions of Et_2O. The triethers eluted with 20% Et_2O and were further separated into (**1**) and (**2**) by TLC (Merck Kieselgel 60-F254) in hexane–ether (7:3) (R_f 0.5 and 0.4 respectively).

Hydrogenation of (**2**). The triether (10 mg) was treated with H_2 on Pd–C (5%; 15 mg) in MeOH (3 ml) for 24 hr; chromatography of the product, as above, gave (**1**) (NMR, IR) which was subsequently converted as for the natural saturated triether.

Ether cleavage etc. Treatment of (**1**) (15 mg) with 57% HI (1 ml; 24 h reflux) and work-up (extraction into hexane, washing with H_2O, aq. K_2CO_3, aq. $NA_2S_2O_3$, and H_2O, partition with hexane and 90% aq. MeOH, and TLC in hexane) gave phytanyl iodide, $C_{20}H_{41}I$ (MS and NMR). The product (10 mg) was refluxed with AgOAc (20 mg) in AcOH (2 ml) for 24 hr. The reaction mixture was taken up in Et_2O, centrifuged, washed (H_2O, aq. NaCl, aq. $NaHCO_3$, aq. $Na_2S_2O_3$, H_2O) and purified by TLC in hexane–ether (17:3). The product R_f 0.75, was identified as phytanyl acetate by comparison with authentic material [MS, NMR, and GLC (on a 2m × 3mm glass column packed with 1% OV-1 on Gas-Chrom Q 100/120 with 40 ml/min N_2 at 200°; retention time 6.24 min)]. Alternatively, the triether (10 mg) was treated with BCl_3 (1 ml with 1 ml $CHCl_3$; 14 hr at R.T.). Excess reagent was removed at reduced pressure and the alkyl chloride taken up in hexane for purification by TLC as above (phytanyl chloride, $C_{20}H_{41}Cl$ by MS and NMR). The undissolved residue was taken up in methanol and identified as glycerol by TLC (in $CHCl_3$–MeOH, 4:1 on Si gel, R_f 0.40, visualised with Ag–NH_3).

Spectra. All IR spectra were measured in CCl_4; NMR spectra at 100 MHz in CCl_4 with TMS standard; MS on the AEI MS-30.

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6β,22-DIHYDROXYHOPANE, A NEW TRITERPENE FROM THE FERN *CHEILANTHES MARANTAE*

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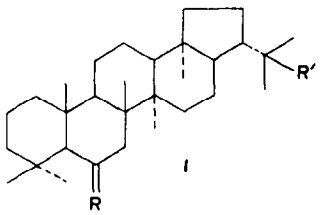
Key Word Index—*Cheilanthes marantae*; Sinopteridaceae; doradilla acanalada; fern; triterpenes, steroids; diplopterol; fernenol; 6β,22-dihydroxyhopane; sitosterol; sitosterol-*o*-β-D-glucoside.

Plant. Cheilanthes marantae L. local name doradilla acanalada. Specimen no. 322 deposited in the Herbarium of the Botany Department, University of La Laguna. *Source.* Monte de las Mercedes, Tenerife, Canary Islands. Collected in June. *Previous work.* None.

Present work. In addition to *saccharose, diplopterol, fernenol, sitosterol* and *sitosterol-*O-β-D-glucoside, the new natural triperpene *6β,22-dihydroxyhopane* (**1a**), characterized by its IR, PMR and MS spectra, was isolated from the stems and leaves of *Cheilanthes marantae*. The hopane framework of (**1a**) was deduced from its MS which showed the same fragmentation pattern as that of zeorin (**1d**) and related compounds [1]. The large downfield shifts of the methyl groups at C-4β, C-8β and C-10β observed in the PMR spectrum (*ca* 0.35 ppm) compared with 22-hydroxyhopane are compatible only with the presence of a C-6β hydroxyl group [2]. These assumptions were chemically confirmed by partial synthesis of (**1a**) from zeorin.



1

1a R = H; β-OH R' = OH
1b R = H; β-OAc R' = OAc
1c R = O R' = OH
1d R = H; α-OH R' = OH

EXPERIMENTAL

Mp's determined on a Koffler hot-stage apparatus, are uncorr. Optical rotations were measured in $CHCl_3$ and PMR spectra in $CDCl_3$ with TMS as internal reference.