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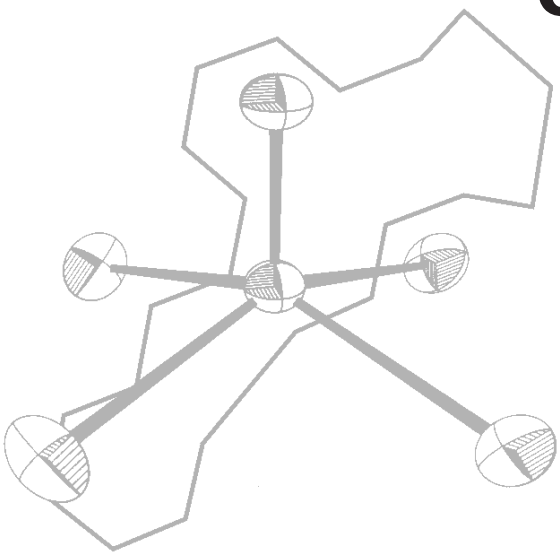

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The Structure of Chalybaeizanic Acid and Quaesitic Acid, Two New Lichen Depsidones Related to Salazinic Acid

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The depsidones chalybaeizanic acid (1,4,10-trihydroxy-8-methyl-3,7-dioxo-1,3-dihydro-7*H*-isobenzofuro-[4,5-*b*][1,4]benzodioxepin-5,11-dicarbaldehyde) (4) and quaesitic acid (11-formyl-1,4,10-trihydroxy-8-methyl-3,7-dioxo-1,3-dihydro-7*H*-isobenzofuro[4,5-*b*][1,4]benzodioxepin-5-methyl hydrogen fumarate) (5) have been isolated from the lichens *Xanthoparmelia amphixanthoides* and *Hypotrachyna quaesita* respectively, and their structures determined by a combination of spectroscopic evidence, partial synthesis, derivatization or degradation reactions.

The continuation of our phytochemical survey of lichens[1] has led to the identification of a further two depsidones from several species. These compounds have been characterized by the normal spectroscopic techniques from which their respective structures have been deduced.

Chalybaeizanic Acid (4)

In his monographic study of the lichen genus *Xanthoparmelia*,[2] Mason Hale observed that *X. chalybaeizans* produced five major phenolic metabolites. This included the common cortical dibenzofuran usnic acid, together with the β-orcinol depsidones norstictic acid (1), salazinic acid (2), and consalazinic acid (3) together with the 'chalybaeizans unknown', a compound we have called chalybaeizanic acid. Although Hale detected this unknown in a number of *Xanthoparmelia* species by microchemical methods he did not isolate it nor elucidate its structure.

Recently we have undertaken a larger scale extraction of *Xanthoparmelia amphixanthoides* (Stein. & Zahlbr.) Hale which contains the same five metabolites as *X. chalybaeizans*, and followed this by fractional crystallization of the extract. This led to the isolation of chalybaeizanic acid (4), the structure of which followed initially from the spectroscopic properties.

In particular, the ^{1}H n.m.r. spectrum showed a *C*-methyl resonance (δ 2·52), an aromatic proton signal overlapping a methine proton resonance (6·87), a methine proton (7·01), two aldehyde proton signals (10·43, 10·63) and two intramolecularly hydrogen-bonded hydroxy signals (12·31, 13·42). High-resolution mass measurement on the molecular ion established that the molecular formula of (4) was $C_{18}H_{10}O_{10}$, whereas that of the co-occurring salazinic acid is $C_{18}H_{12}O_{10}$. The similarities of the ^{1}H n.m.r. and mass spectra of (2) and (4) were thus not surprising.

The structure of chalybaeizanic acid (4) was ultimately established by synthesis and derivatization. Oxidation of salazinic acid (2) with pyridinium dichromate under mild conditions led to the formation of chalybaeizanic acid (4), albeit mixed with unreacted salazinic acid (2). The properties [thin-layer chromatography (t.l.c.), high-performance liquid chromatography (h.p.l.c.), ultraviolet absorption (u.v.)] of this synthetic sample of chalybaeizanic acid (4) were found to be identical to those of the natural material.

Acetylation of a mixture of salazinic acid (2) and chalybaeizanic acid (4) by treatment with acetic anhydride and sulfuric acid led to the formation of the expected salazinic acid hexaacetate (8) and chalybaeizanic acid heptaacetate (9).

Chalybaeizanic acid (4) is a further representative of the β-orcinol depsidones, and may arise biosynthetically by selective oxidation of the co-occurring salazinic acid (2).[3,4]

Quaesitic Acid (5)

In 1986 Kurokawa[5] reported the new lichen species *Parmelia quaesita* Kurok. (*Hypotrachyna quaesita* (Kurok.) DePriest & B. Hale) which contained the common cortical depsides atranorin and chloroatranorin, the β-orcinol depsidone fumarprotocetraric acid (11), together with an unknown which he termed quaesitic acid. Although Kurokawa detected the compound by microchemical methods he did not attempt to isolate it nor elucidate its structure. Analytical h.p.l.c. indicated that the major metabolites detected by Kurokawa were accompanied by minor amounts of salazinic acid (2) and protocetraric acid (10). Recently, we have undertaken a larger scale extraction of *H. quaesita* and followed

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Table 1. ^{1}H n.m.r. data (δ) for β-orcinol depsidones

Spectra were recorded at 300 MHz

Proton(s)	(10)[A]	(11)[B]	(1)[B]	(2)[A]	(4)[C]	(5)[A]
1-Me	2·47	2·54	2·46	2·50	2·52	2·53
6-Me	2·67	2·66	—	—	—	—
HC(OH)O	—	—	6·90	7·01	7·01	6·78
CH_2O	4·81	5·37	—	4·92	—	5·40
ArH	6·75	6·77	6·86	6·83	6·87	6·78
CHO	10·72	10·78	10·48	10·64	10·63 10·43	10·23
OH	—	—	—	12·28	12·31 13·42	—
(*E*)-CH=CH	—	6·74	—	—	—	6·76
9-Me			2·22	—	—	—

[A] Spectrum run in $(CD_3)_2CO$.
[B] Spectrum run in $(CD_3)_2SO$.
[C] Spectrum run in $CDCl_3$.

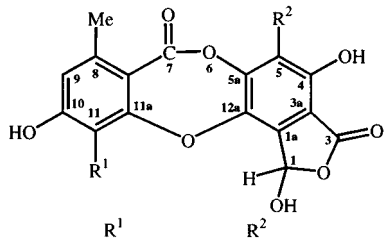
this by fractional crystallization and preparative t.l.c. of the extract. This led to the isolation of quaesitic acid (5), the structure of which followed initially from its spectroscopic properties.

In particular, the ^{1}H n.m.r. spectrum showed a *C*-methyl resonance (δ 2·54), an aromatic proton signal overlapping a methine proton resonance (6·78), an aldehyde proton (10·23), a two-proton singlet (5·40) due to the benzyloxy group and another two-proton singlet (6·76) assigned to alkene protons. This spectrum showed similarities with the ^{1}H n.m.r. spectra of fumarprotocetraric acid (11) and salazinic acid (2), both cometabolites of quaesitic acid (Table 1).

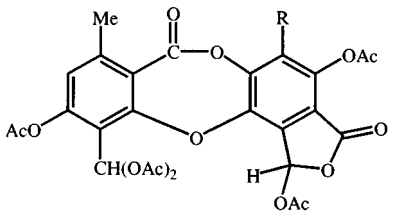
Although the mass spectrum of quaesitic acid did not show a molecular ion, the observed fragmentation pattern was very similar to that exhibited by galbinic acid (6).[6] This combination of spectroscopic data was consistent with structure (5) for quaesitic acid.

The structure of quaesitic acid (5) was ultimately established by degradation. Inadvertent methanolysis of quaesitic acid (5), by storing a methanolic solution of this compound at room temperature, efficiently converted (5) into salazinic acid α-methyl ether (7) and fumaric acid, the former establishing the core β-orcinol depsidone structure of quaesitic acid.

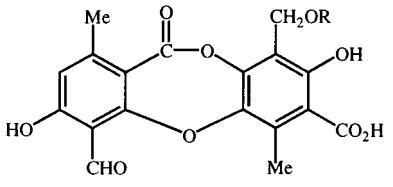
Quaesitic acid (5) is the second β-orcinol depsidone known in which biosynthesis has involved secondary side-chain esterification by fumaric acid.



	R^1	R^2
(1)	CHO	Me
(2)	CHO	CH_2OH
(3)	CH_2OH	CH_2OH
(4)	CHO	CHO
(5)	CHO	$CH_2OCOCH\overset{E}{=}CHCO_2H$
(6)	CHO	CH_2OAc
(7)	CHO	CH_2OMe



(8) R = CH_2OAc
(9) R = $CH(OAc)_2$



(10) R = H
(11) R = $OCOCH\overset{E}{=}CHCO_2H$

Experimental

The general experimental details have been described previously.[7]

Extraction of Xanthoparmelia amphixanthoides *(Stein. & Zahlbr.) Hale*

The lichen material was collected on sandstone, Rooiberg Pass, 19 km from Vanwyksdorp towards Calitzorp, Cape Province, Republic of South Africa, F. Brusse 4900 (CANB).

The lichen material (4·3 g) was dried and extracted with anhydrous ether in a Soxhlet extractor for 24 h. This procedure was then repeated with anhydrous acetone. After concentration, the extract from the latter yielded a colourless solid (39 mg), which t.l.c., h.p.l.c. and ^{1}H n.m.r. analyses revealed was approximately a 1 : 1 mixture of salazinic acid (2) and chalybaeizanic acid (4). Fractional crystallization of this mixture from aqueous acetone afforded salazinic acid (2) (5 mg, 1·2%) as colourless needles, m.p. 265° (dec.) (lit.[8] 260–280° (dec.)), identical with authentic material (t.l.c., h.p.l.c., ^{1}H n.m.r., u.v., mass spectrum). The mother liquor so obtained was enriched in *chalybaeizanic acid* (4) (2·6 mg, 0·6%), which on concentration crystallized in colourless microcrystals, m.p.

>300° (dec.) (Found: $M^{+\bullet}$, 386·0274. $C_{18}H_{10}O_{10}$ requires $M^{+\bullet}$, 386·0274). The homogeneity of this compound was confirmed by h.p.l.c. and ^{1}H n.m.r. spectroscopy. Mass spectrum: *m/z* 386 (M, 24%), 341 (30), 340 (28), 312 (34), 199 (24), 179 (56), 177 (16), 152 (48), 151 (62), 149 (34), 57 (100). Standard t.l.c. R_F values:[9,10] R_F (A) 0·11; R_F (B) 0·05; R_F (C) 0·10. Standard h.p.l.c. values:[6,11] R_t 17·90 min; R_I 0·10.

Oxidation of Salazinic Acid (2)

A solution of salazinic acid (2) (100 mg, 0·26 mmol) in *N,N*-dimethylformamide (10 ml) was cooled to 0° and stirred while pyridinium dichromate (121 mg, 0·32 mmol) was added. The mixture was stirred for a further 1 h at 0°, then poured into water (200 ml) and extracted with ethyl acetate. The combined organic extract was washed with water, brine and dried ($MgSO_4$). The residue (22 mg) obtained on evaporation of the solvent was analysed by t.l.c., h.p.l.c. and ultraviolet spectroscopy. This confirmed that the residue comprised a mixture of salazinic acid (*c.* 85%) and chalybaeizanic acid (4) (*c.* 15%) with identical R_F and R_t values and an identical ultraviolet absorption spectrum with the natural material isolated above.

Acetylation of Mixture of Salazinic Acid (2) and Chalybaeizanic Acid (4)

The mixture of depsidones (2) and (4) (50 mg) was stirred in a solution of acetic anhydride (1·5 ml) and concentrated sulfuric acid (1 drop) at room temperature for 19·5 h. Water (15 ml) was then added and the solution stirred at room temperature for a further 3·5 h. The precipitate which formed was filtered, washed with water, and dried, to give the crude product (60 mg). The crude material was purified by preparative layer chromatography over silica gel with 35–40% ethyl acetate–light petroleum as eluent. Two major bands developed.

The faster moving band afforded hexaacetylsalazinic acid (20 mg), which crystallized from ethanol in colourless prisms, m.p. 173–174° (lit.[12] 178°). This material was identical with authentic[12] hexaacetylsalazinic acid (8) (t.l.c., h.p.l.c., ^{1}H n.m.r.).

The slower moving band yielded *heptaacetylchalybaeizanic acid* (9) (11 mg) as a colourless solid, m.p. >340° (Found: C, 54·0; H, 4·0. $C_{32}H_{28}O_{19}$ requires C, 53·6; H, 3·9%). ^{1}H n.m.r. ($CDCl_3$) δ 2·08, 2·14, 2·15, 2·17, 2·22, 2·36, 2·45, 2·58, 8s, Me; 6·95, s, ArH; 7·88, s, H 1; 7·97, 7·98, 2s, ArCH. Mass spectrum: *m/z* 674 ($M-CH_2CO$, 0·1%), 614 (8), 572 (30), 512 (28), 470 (74), 454 (16), 453 (16), 428 (50), 412 (30), 411 (49), 410 (76), 386 (40), 370 (34), 369 (44), 368 (100), 341 (21), 340 (55), 339 (20), 312 (12), 300 (28), 299 (30), 221 (15), 179 (20), 177 (14).

Extraction of Hypotrachyna quaesita *(Kurok.) DePriest & B. Hale*

The lichen material was collected on a fallen tree branch in disturbed *Nothofagus* forest, Mount Kaindi, Morobe Province, Papua New Guinea, *H. Streimann 33165* (CANB).

The lichen material (0·45 g) was dried and extracted with anhydrous ether for 42 h, and then extracted with acetone for 48 h. Evaporation of the combined solvent afforded a residue (71 mg) shown by t.l.c. to comprise a mixture of atranorin, chloroatranorin, fumarprotocetraric acid (11), quaesitic acid (5) and traces of salazinic acid (2) and protocetraric acid (10). The residue was purified by repeated preparative layer chromatography over silica gel with toluene–acetic acid (85 : 15) as eluent. Three major bands developed. The faster moving band containing atranorin and chloroatranorin was discarded. The second band afforded fumarprotocetraric acid (11) (2·0 mg, 0·4%), identical (t.l.c., h.p.l.c., u.v., ^{1}H n.m.r.) with authentic material. The third band yielded *quaesitic acid* (5) (6·4 mg, 1·4%), which crystallized from acetone in colourless microcrystals, m.p. >350° (dec.) (Found: C, 54·0; H, 3·0. $C_{22}H_{14}O_{13}$ requires C, 54·3; H, 2·9%). Mass spectrum: *m/z* 305 (14%), 205 (23), 149 (19), 139 (11), 137 (13), 126 (21), 124 (18), 113 (15), 112 (14), 111 (34), 110 (13), 109 (27), 99 (15), 98 (14), 97 (52), 96 (19), 95 (38), 57 (100). Standard t.l.c. R_F values:[9,10] R_F (A) 0·06; R_F (B) 0·09; R_F (C) 0·06. Standard h.p.l.c. values:[6,11] R_t 18·47 min; R_I 0·10.

Methanolysis of Quaesitic Acid (5)

A solution of quaesitic acid (5) (1 mg) in anhydrous methanol was permitted to stand at room temperature for 48 h. After this time no quaesitic acid could be detected by h.p.l.c. analysis—instead the presence of salazinic acid 5-α-methyl ether (6)[13] and fumaric acid was confirmed by comparison with authentic samples (h.p.l.c., u.v.). No maleic acid was detected. On evaporation of the solvent this product composition was confirmed by comparative t.l.c. in three independent solvent systems.

Acknowledgment

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