2,4-Di-*O*-methylgyrophoric Acid and 2,4,5-Tri-*O*-methylhiascic Acid. New Tridepsides from *Parmelia damaziana*

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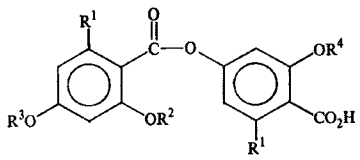
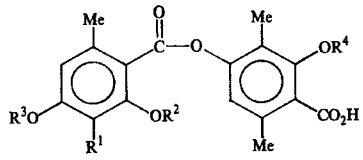
Abstract

The new tridepsides 2,4-di-*O*-methylgyrophoric acid (8) and 2,4,5-tri-*O*-methylhiascic acid (5) in addition to the known metabolites atranorin (9), chloroatranorin (10), ursolic acid (11), 5-*O*-methylhiascic acid (4) and gyrophoric acid (7) have been isolated from the lichen *Parmelia damaziana*. The structure of the new tridepsides has been established by total synthesis.

Minor biosynthetic variations observed within a lichen genus or group of related species can produce a large number of structurally related metabolites or a chemosyndrome.[1] The structural variations within a chemosyndrome take several forms; thus *para*-depsides such as (1) may vary in the length of the polyketide-derived side chain (R^1), the degree of oxidation of this side chain (i.e. CH_2COR or CH_2CH_2R) or the degree of methylation of the phenolic groups (i.e. R^2, R^3, R^4 = Me or H).[1,2] Depsides based on β-orcinol moieties (2) show greater variations in the degree of oxidation of the group R^1 (CO_2H, CHO, CH_2OH, Me) but similar variations in the methylation of the phenolic groups.[3,4]



(1)

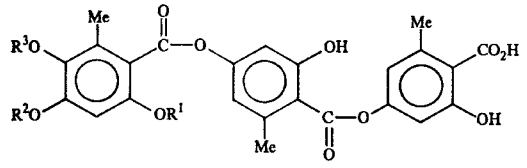


(2)

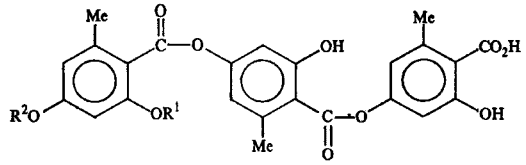
Recently we reported that *Parmelia horrescens* Nyl. and *P. pseudofatiscens* Elix nom. nud. (≡ *P. afrorevoluta* Krog & Swinsc.) produced 4,5-di-*O*-methylhiascic acid (3),[5] 5-*O*-methylhiascic acid (4)[6] and gyrophoric acid (7) in addition to the common cortical depsides atranorin (9) and chloroatranorin (10). The related species *Parmelia minarum* Nyl. (syn. *P. dissecta* Nyl.) produced a mixture of (4) and (7) in addition to atranorin (9) and chloroatranorin (10).[6]

[1] Culberson, C. F., and Culberson, W. L., *Syst. Bot.*, 1976, **1**, 325.
[2] Culberson, C. F., Culberson, W. L., and Esslinger, T. L., *Bryologist*, 1977, **80**, 125.
[3] Culberson, C. F., Nash, T. H., III, and Johnson, A., *Bryologist*, 1979, **82**, 154.
[4] Culberson, C. F., and Culberson, W. L., *Exp. Mycol.*, 1978, **2**, 245.
[5] Elix, J. A., and Engkaninan, U., *Aust. J. Chem.*, 1976, **29**, 2701.
[6] Elix, J. A., and Jayanthi, V. K., *Aust. J. Chem.*, 1977, **30**, 2695.

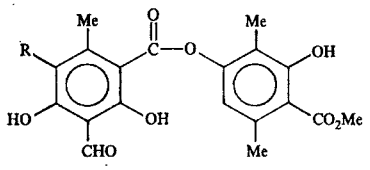
All these species belong to *Parmelia* subg. *Hypotrachyna* (Vain) Krog & Swinsc. and it is apparent that some members of this subgenus produce a chemosyndrome of tridepsides based on gyrophoric acid (7) and hiascic acid (6). We report here the examination of *Parmelia damaziana* Zahlbr. a further member of this subgenus.[7] In addition to atranorin (9), chloroatranorin (10), ursolic acid (11), gyrophoric acid (7) and 5-*O*-methylhiascic acid (4) we established that this lichen contained 2,4,5-tri-*O*-methylhiascic acid (5) and 2,4-di-*O*-methylgyrophoric acid (8). The latter metabolites were separated by vacuum liquid chromatography and the structure of these compounds has been established by unambiguous syntheses and by t.l.c. comparisons of the natural products with the corresponding synthetic derivatives in three independent solvent systems.[8]



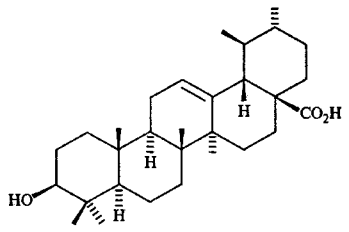
	R^1	R^2	R^3
(3)	H	Me	Me
(4)	H	H	Me
(5)	Me	Me	Me
(6)	H	H	H



	R^1	R^2
(7)	H	H
(8)	Me	Me



(9) R = H
(10) R = Cl



(11)

Synthesis of Tridepsides

The substituted benzoic acids, 2,4-dimethoxy-6-methylbenzoic acid (12) and 2,4,5-trimethoxy-6-methylbenzoic acid (13) were prepared by standard procedures[9] and condensed in turn with benzyl lecanorate (14) in the presence of trifluoroacetic anhydride.

Subsequent hydrogenolysis of the tridepside esters (15) and (16) so obtained yielded the corresponding tridepsides, 2,4-di-*O*-methylgyrophoric acid (8) and 2,4,5-tri-*O*-methylhiascic acid (5).

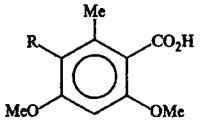
[7] Krog, H., and Swinscow, T. D. V., *Norw. J. Bot.*, 1979, **26**, 11.
[8] Culberson, C. F., *J. Chromatogr.*, 1972, **72**, 113.
[9] Bryan, A. J., Elix, J. A., and Norfolk, S., *Aust. J. Chem.*, 1976, **29**, 1079.

Experimental

The general experimental details have been published previously.[9]

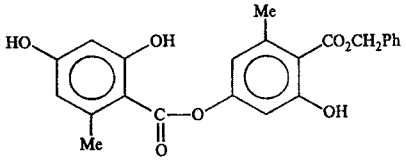
Ethyl 2,4,5-Trimethoxy-6-methylbenzoate

Ethyl 2,5-dihydroxy-4-methoxy-6-methylbenzoate[10] (2·26 g, 0·01 m), powdered anhydrous potassium carbonate (4·5 g) and dimethyl sulfate (1·0 ml) were stirred and heated under reflux in acetone (40 ml) for 22 h. The mixture was cooled and poured into cold dilute hydrochloric acid and extracted with ether. The ethereal solution was washed with water and dried ($MgSO_4$). The residue obtained on removal of the solvent was applied to a column of silica gel and eluted with 7% ethyl acetate/light petroleum. The major band yielded *ethyl 2,4,5-trimethoxy-6-methylbenzoate* (2·33 g, 91%) as a pale yellow oil (Found: C, 61·2; H, 7·3. $C_{13}H_{18}O_5$ requires C, 61·4; H, 7·2%). ^{1}H n.m.r. ($CDCl_3$) δ 1·36 t, J 7 Hz, CH_2CH_3; 2·20, s, $ArCH_3$; 3·72, 3·78, 3·86 each s, OMe; 4·36, q, J 7 Hz, CH_2CH_3; 6·38, s, ArH.

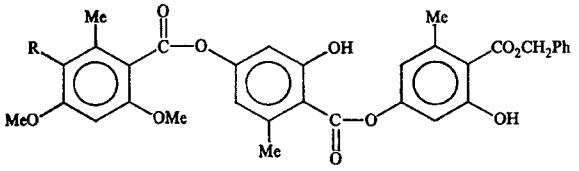


(12) R = H

(13) R = OMe



(14)



(15) R = H

(16) R = OMe

2,4,5-Trimethoxy-6-methylbenzoic Acid (13)

A mixture of ethyl 2,4,5-trimethoxy-6-methylbenzoate (2·54 g), potassium hydroxide (2·0 g), water (5 ml) and dimethyl sulfoxide (25 ml) was stirred and heated at 90° for 3 h. The solution was then cooled and poured into cold dilute hydrochloric acid and extracted with ether. The ethereal solution was washed with water and dried ($MgSO_4$). Evaporation of this solution gave the crude acid which was recrystallized from cyclohexane/toluene to give *2,4,5-trimethoxy-6-methylbenzoic acid* (13) (1·85 g, 82%) as colourless needles, m.p. 144–145° (Found: $M^{+\bullet}$, 226·0836. $C_{11}H_{14}O_5$ requires $M^{+\bullet}$, 226·0841). ^{1}H n.m.r. ($CDCl_3$) δ 2·40, s, $ArCH_3$; 3·76, s, OCH_3, 3·92, s, $2OCH_3$; 6·46, s, ArH; 11·60, s, CO_2H.

Synthesis of Depside Esters (15), (16)

The appropriate carboxylic acid [(12),[11] (13)] (1 mmol) and benzyl lecanorate[9] (14) (1 mmol) were dissolved in a solution of anhydrous toluene (4 ml) and trifluoroacetic anhydride (1 ml) and allowed to stand at room temperature for 12 h. The solvent was then removed under reduced pressure and the residue applied to two silica gel plates (100 by 20 by 0·1 cm) and eluted with 20% ethyl acetate/light petroleum. The faster-moving band was removed and extracted with ethyl acetate. Removal of the solvent gave the respective depside esters.

Benzyl 2,4-di-O-methylgyrophorate (15)* (55%) was recrystallized from n-hexane/acetone to form colourless prisms, m.p. 113–115° (Found: C, 67·8; H, 5·2. $C_{33}H_{30}O_{10}$ requires C, 67·6;

* Benzyl 4-[4-(2,4-dimethoxy-6-methylbenzoyloxy)-2-hydroxy-6-methylbenzoyloxy]-2-hydroxy-6-methylbenzoate.

[10] Aghoramurthy, K., and Seshadri, T. R., *Proc. Indian Acad. Sci., Sect. A*, 1952, **35**, 334.

[11] Robertson, A., and Robinson, R., *J. Chem. Soc.*, 1927, 2200.

H, 5·2%). ^{1}H n.m.r. ($CDCl_3$) δ 2·42, 2·54, 2·66, each s, $ArCH_3$; 3·78, 3·82 each s, OCH_3; 5·38, s, OCH_2; 6·34, s, H3, H5; 6·58–6·82, m, H3′,3″,5′,5″; 7·44, s, C_6H_5; 11·27, 11·75, each s, OH.

*Benzyl 2,4,5-tri-*O*-methylhiascate* (16)* (22%) was recrystallized from cyclohexane to form colourless prisms, m.p. 121° (with softening at 82°) (Found: C, 66·7; H, 5·1. $C_{34}H_{32}O_{11}$ requires C, 66·2; H, 5·2%). ^{1}H n.m.r. ($CDCl_3$) δ 2·36, 2·56, 2·74 each s, $ArCH_3$; 3·76, 3·88, 3·92, each s, OCH_3; 5·44, s, OCH_2; 6·48, s, H3; 6·62–6·86, m, H3′,3″,5′,5″; 7·44, s, C_6H_5; 11·16, 11·64, each s, OH.

Synthesis of Depsides (5), (8)

The appropriate depside ester (12), (13) (0·5 mmol) was dissolved in ethyl acetate (5 ml) containing 10% palladium-on-charcoal (25 mg) and the suspension was stirred in an atmosphere of hydrogen for 6 h. The catalyst was than filtered off and the solvent evaporated.

*2,4-Di-*O*-methylgyrophoric acid* (8)† (70%) recrystallized from benzene/acetone to form colourless needles, m.p. 169–173° (Found: C, 62·6; H, 4·9. $C_{26}H_{24}O_{10}$ requires C, 62·9; H, 4·9%). ν_{max} (Nujol) 1680 (C=O) cm^{-1}; λ_{max} (95% ethanol) 225 (ε 19500), 255 (23000), 295 (10800) nm. ^{1}H n.m.r. (CD_3COCD_3) δ 2·42, 2·64, 2·70 each s, $ArCH_3$; 3·82, 3·88, each s, OCH_3; 6·44, s, H3, H5; 6·64–6·88, m, H3′,3″,5′,5″. Mass spectrum *m/e* 346 (3%), 213 (9), 196 (94), 180 (42), 179 (92), 178 (100), 167 (48), 152 (42), 150 (87), 122 (50). T.l.c.[8] R_F(A) 0·44, R_F(B) 0·37, R_F(C) 0·48.

*2,4,5-Tri-*O*-methylhiascic acid* (5)‡ (84%) was recrystallized from cyclohexane/toluene/acetone to form small colourless prisms, m.p. 173–176° (Found: C, 61·2; H, 5·2. $C_{27}H_{26}O_{11}$ requires C, 61·6; H, 5·0%). ν_{max} (Nujol) 1660 (C=O) cm^{-1}; λ_{max} (95% EtOH) 223 (ε 21400), 253 (19100), 300 (9500) nm. ^{1}H n.m.r. (CD_3COCD_3) δ 2·32, s, $ArCH_3$; 2·68, s, $2ArCH_3$; 3·68, 3·90, 3·92 each s, OCH_3; 6·72–6·86, m, ArH. Mass spectrum *m/e* 376 (8%), 226 (36), 211 (31), 209 (100), 193 (21), 168 (30), 150 (46), 122 (28). T.l.c.[8] R_F(A) 0·40, R_F(B) 0·35, R_F(C) 0·38.

Extraction of Parmelia damaziana *Zahlbr.*

The lichen material was collected on sandstone rocks 8 km north-east of Nerriga, Morton National Park, N.S.W., J. A. Elix—5093 (CBG).

The dried lichen thallus (66 g) was extracted with anhydrous ether in a Soxhlet extractor for 24 h. Evaporation of the ethereal solution yielded a grey solid (1·3 g). The residue was digested in acetone (20 ml) and the undissolved solid removed by filtration. This solid (0·174 g) was shown by comparative t.l.c. to comprise a mixture of atranorin (9) and chloroatranorin (10). The filtrate was then evaporated and the residue taken up in ether (3 ml). Some material dissolved and the solid (0·487 g) was removed by filtration. Analytical t.l.c. indicated that this solid consisted of a mixture of gyrophoric acid (7) and 5-*O*-methylhiascic acid (4) while the filtrate contained two additional phenolic metabolites. Evaporation of this filtrate yielded 0·65 g of solid which was subjected to vacuum liquid chromatography[12] in the following manner. The above residue was preadsorbed on silica gel (6 g) and added to a silica gel column (50 g, Merck G) according to the described technique.[12] Celite (5 g) was then added. The column was manually compressed and eluted with 1–3% acetic acid/toluene under vacuum; 100 ml fractions were collected. Fraction 13 yielded a colourless solid which after washing with benzene was shown to consist of ursolic acid (11) (10 mg), the identity of which was confirmed by comparison with authentic material (m.p., t.l.c., m.s.). The benzene-soluble portion of this fraction was applied to a silica gel plate (20 by 20 by 0·1 cm) and eluted with 15% acetic acid/toluene. The major band yielded 2,4-di-*O*-methylgyrophoric acid (8) (1·0 mg), identical with the synthetic material prepared above (^{1}H n.m.r., t.l.c. in three independent solvent systems). Further elution of the column with 5% acetic acid toluene yielded pure crystalline

* Benzyl 2-hydroxy-4-[2-hydroxy-6-methyl-4-(3,4,6-trimethoxy-2-methylbenzoyloxy)benzoyloxy]-6-methylbenzoate.

† 4-[4-(2,4-Dimethoxy-6-methylbenzoyloxy)-2-hydroxy-6-methylbenzoyloxy]-2-hydroxy-6-methylbenzoic acid.

‡ 2-Hydroxy-4-[2-hydroxy-6-methyl-4-(3,4,6-trimethoxy-2-methylbenzoyloxy) benzoyloxy]-6-methylbenzoic acid.

[12] Targett, N. M., Kilcoyne, J. P., and Green, B., *J. Org. Chem.*, 1979, **44**, 4962.