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2″-*O*-Methylgyrophoric Acid, a New Lichen Tridepside

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Abstract

A new metabolite has been detected in the lichens *Diploschistes gyrophoricus* and *Rinodina alba*, and its chromatographic behaviour shown to be identical with that of 2″-*O*-methylgyrophoric acid (6), the total synthesis of which is described.

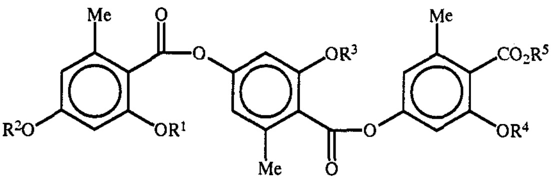
Introduction

The gyrophoric acid chemosyndrome comprises a cohort of lichen tridepsides based on gyrophoric acid (1). Biosynthetic elaboration of the parent tridepside (1) may proceed by decarboxylation, *O*-methylation, *C*-hydroxylation or a combination of these processes. Of the various possible mono-*O*-methyl derivatives of gyrophoric acid (1), four are known to occur naturally: methyl gyrophorate (2),[1] 4-*O*-methylgyrophoric acid (3),[1] umbilicaric acid (4)[1] and ovoic acid (5).[2]

In this report we describe the detection in lichen extracts and total synthesis of the remaining isomer, 2″-*O*-methylgyrophoric acid (6).

The New Tridepside

Lumbsch and Elix[3] recently described the new lichen *Diploschistes gyrophoricus* Lumbsch & Elix and found that this species contained a minor unknown in



	R¹	R²	R³	R⁴	R⁵
(1)	H	H	H	H	H
(2)	H	H	H	H	Me
(3)	H	Me	H	H	H
(4)	Me	H	H	H	H
(5)	H	H	Me	H	H
(6)	H	H	H	Me	H

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[1] Culberson, C. F., 'Chemical and Botanical Guide to Lichen Products' (University of North Carolina Press: Chapel Hill 1969).
[2] Huneck, S., Schreiber, K., and Sundholm, G., *Phytochemistry*, 1980, **19**, 885.
[3] Lumbsch, H. T., and Elix, J. A., *Plant Syst. Evol.*, 1989, **167**, 195.

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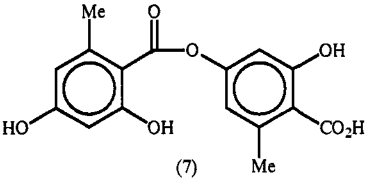
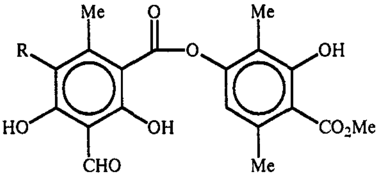
addition to gyrophoric acid (1). The chromatographic behaviour of this unknown was similar but not identical to that of umbilicaric acid (4). Subsequently Giralt and Barbero[4] encountered this unknown in *Rinodina alba* Metzler ex Arn. where it co-occurs with gyrophoric acid (1), umbilicaric acid (4), lecanoric acid (7) and the cortical depsides atranorin (8) and chloroatranorin (9). We have now undertaken the synthesis of 2″-*O*-methylgyrophoric acid (6) as outlined in Scheme 1.

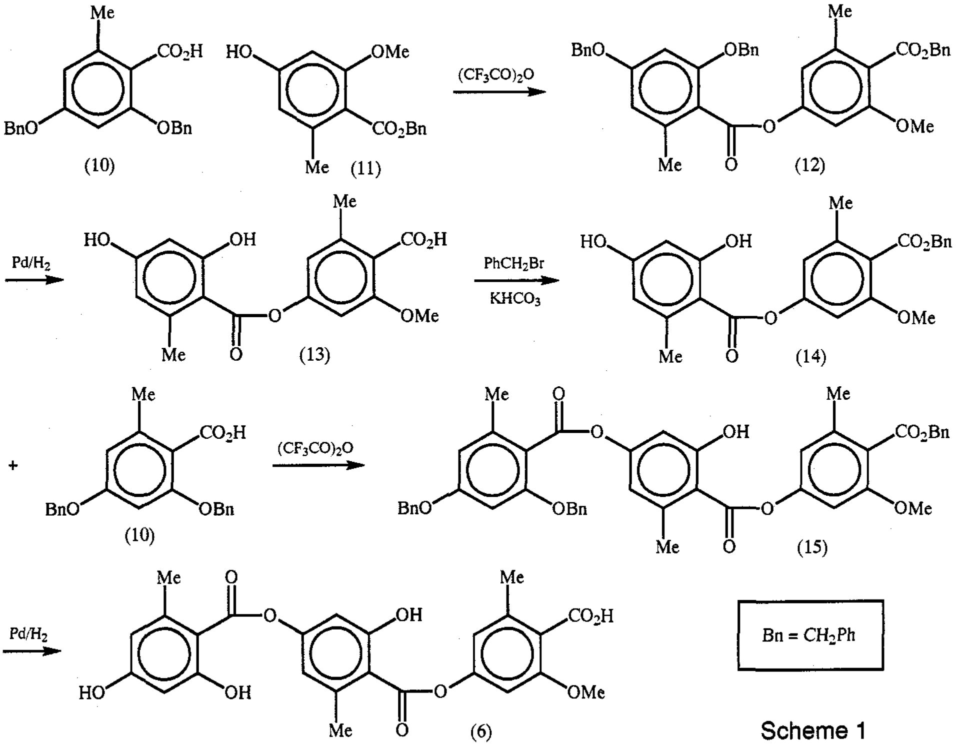


(7)



(8) R = H
(9) R = Cl

The synthetic strategy was to utilize suitable protected benzoic acids and phenols as the appropriate mononuclear precursors.[5] The overall procedures for such syntheses are now well established.[6] The preparations of 2,4-dibenzyloxy-6-methylbenzoic acid (10)[7] and benzyl 4-hydroxy-2-methoxy-6-methylbenzoate (11)[8]



Scheme 1

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[4] Giralt, M., and Barbero, M., *Mycotaxon*, in press.
[5] Elix, J. A., Yu, J., and Tønsberg, T., *Aust. J. Chem.*, 1991, **44**, 157.
[6] Manaktala, S. K., Neelakantan, S., and Seshadri, T. R., *Tetrahedron*, 1966, **22**, 2373.
[7] Bryan, A. J., Elix, J. A., and Norfolk, S., *Aust. J. Chem.*, 1976, **29**, 1079.
[8] Elix, J. A., and Lajide, L., *Aust. J. Chem.*, 1981, **34**, 2005.

have been described previously. The condensation of the benzoic acid (10) and the phenolic ester (11) was achieved by treatment with trifluoroacetic anhydride, and afforded the depside ester benzyl 2,4-di-*O*-benzyl-2′-*O*-methyllecanorate (12) in good yield. Catalytic hydrogenolysis of the benzyl ester (12) proceeded to give 2′-*O*-methyllecanoric acid (13). Selective benzylation of the carboxy group of (13) was achieved by treatment with benzyl bromide and potassium hydrogen carbonate, and gave benzyl 2′-*O*-methyllecanorate (14). The latter depside ester was then esterified by reaction with 2,4-dibenzyloxy-6-methylbenzoic acid (10) in the presence of trifluoroacetic anhydride to give benzyl 2,4-di-*O*-benzyl-2″-*O*-methylgyrophorate (15). Catalytic hydrogenolysis of (15) then gave the required tridepside, 2″-*O*-methylgyrophoric acid (6). The ^{1}H n.m.r. and mass spectral data recorded for this synthetic depside were consistent with those observed previously for related tridepsides.[2,5]

The thin-layer chromatographic behaviour of synthetic 2″-*O*-methylgyrophoric acid (6) in three independent solvent systems was identical with that of a minor metabolite present in *Diploschistes gyrophoricus* and *Rinodina alba*, and this was further confirmed by comparative h.p.l.c. and mass spectrometry.

Experimental

^{1}H n.m.r. spectra were recorded at 300 MHz on a Varian Gemini XL300 spectrometer. Chemical shifts are expressed in ppm (δ) relative to tetramethylsilane as internal standard. Mass spectra were recorded on a VG micromass 7070F mass spectrometer at 70 eV linked on line to a Finnigan Incos data system. Melting points are uncorrected. Microanalyses were carried out by the Australian National University Microanalytical Service Unit.

Chromatography

The lichen fragments were freed as far as possible from obvious organic substrate material, and extracted with warm acetone for thin-layer chromatography (t.l.c.) or with warm methanol for high-performance liquid chromatography (h.p.l.c.). Compounds were identified by t.l.c. according to the methods standardized for lichen products,[9–12] and by h.p.l.c. with retention index values (RI) calculated from benzoic acid and solorinic acid controls.[13] For t.l.c. standard R_F values were determined in three independent t.l.c. solvent systems: (A) toluene/dioxan/acetic acid (180:45:5); (B) hexane/t-butyl methyl ether/formic acid (140:72:18); (C) toluene/acetic acid (170:30). For h.p.l.c. a Spectra System, a Phenomenex Hypersil 5C18 column (250 by 4·6 mm) and a spectrometric detector operating at 254 nm with a flow rate of 1 ml/min were used. Two solvent systems were used: 1% aqueous orthophosphoric acid and methanol in the ratio 3:7 (*A*) and methanol (*B*). The run started with 100% *A* and was raised to 58% *B* within 15 min, then to 100% *B* within a further 15 min, followed by isocratic elution in 100% *B* for a further 10 min.

Detection of the New Depsides by Comparative Chromatography and Mass Spectrometry

(i) The lichen *Diploschistes gyrophoricus* Lumbsch & Elix was collected on rocks, El Cordobés, Estancia Las Pitangas, deto. Durazno, Uruguay, H. S. Osorio 2820, 4.ii.1953 (ANUC). Comparative h.p.l.c. and t.l.c. indicated the presence of gyrophoric acid (1) (major),

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[9] Culberson, C. F., *J. Chromatogr.*, 1972, **72**, 113.

[10] Culberson, C. F., and Ammann, K., *Herzogia*, 1979, **5**, 1.

[11] Culberson, C. F., and Johnson, A., *J. Chromatogr.*, 1982, **238**, 483.

[12] Elix, J. A., and Ernst-Russell, K. D., 'A Catalogue of Standardized Thin Layer Chromatographic Data and Biosynthetic Relationships for Lichen Substances' 2nd Edn (Australian National University: Canberra 1993).

[13] Feige, G. B., Lumbsch, H. T., Huneck, S., and Elix, J. A., *J. Chromatogr.*, 1993, **646**, 417.

crustinic acid (minor), 2″-*O*-methylgyrophoric acid (6) (trace), lecanoric acid (7) (trace) and unknown (minor).

(ii) The lichen *Rinodina alba* Metzler ex Arn. was collected on rocks, Alt Empordà, Cadaqués, Cala El Jonquet, 10 m alt., Catalonia, Spain, X. Llimona, 4.ix.1973 (ANUC). Comparative h.p.l.c. and t.l.c. indicated the presence of atranorin (8) (major), chloroatranorin (9) (trace), gyrophoric acid (1) (major), umbilicaric acid (4) (trace), 2″-*O*-methylgyrophoric acid (6) (minor) and lecanoric acid (7) (trace). Mass spectrum of crude acetone extract: (negative f.a.b.) *m/z* 481 (7%), 467 (35), 409 (6), 407 (19), 373 (65).

Benzyl 2,4-Di-O-benzyl-2′-O-methyllecanorate (12)

A solution of 2,4-dibenzyloxy-6-methylbenzoic acid (10)[7] (1·4 g, 4 mmol) and benzyl 4-hydroxy-2-methoxy-6-methylbenzoate (11)[8] (1·1 g, 4 mmol) in toluene (20 ml) and trifluoroacetic anhydride (2 ml) was stirred at room temperature for 24 h. The solvent was then evaporated and the residue purified by column chromatography over silica gel with 5% ethyl acetate/light petroleum as eluent. The major band afforded *benzyl 2,4-di-*O*-benzyl-2′-*O*-methyllecanorate* (12) (44%) which crystallized from ethyl acetate/light petroleum to form colourless needles, m.p. 116–120° (Found: C, 75·9; H, 5·7. $C_{38}H_{34}O_7$ requires C, 75·7; H, 5·6%). ^{1}H n.m.r. ($CDCl_3$) δ 2·19, s, 6′-Me; 2·43, s, 6-Me; 3·63, s, OMe; 5·07, 5·10, 2s, $ArOCH_2$; 5·36, s, CO_2CH_2; 6·43, s, H 3,5; 6·49, 6·53, 2d, *J* 2 Hz, H 3′, 5′; 7·40, m, Ph. Mass spectrum *m/z* 602 (M, 3%), 91 (100).

2′-O-Methyllecanoric Acid (13)

A solution of benzyl 2,4-di-*O*-benzyl-2′-*O*-methyllecanorate (12) (0·2 g, 0·3 mmol) in ethyl acetate (20 ml) and palladium on charcoal (10 mg) was stirred under an atmosphere of hydrogen for 24 h. The solution was then filtered and the solvent evaporated. The residue was crystallized from dichloromethane/light petroleum to give *2′-*O*-methyllecanoric acid* (13) (90%) as colourless needles, m.p. 162–164° (Found: C, 61·2; H, 4·7. $C_{17}H_{16}O_7$ requires C, 61·4; H, 4·85%). ^{1}H n.m.r. (CD_3COCD_3) δ 2·32, s, 6′-Me; 2·58, s, 6-Me; 3·82, s, OMe; 6·27, 6·36, 2d, *J* 2·4 Hz, H 3,5; 6·77, 6·92, 2d, *J* 2·0 Hz, H 3′,5′; 9·30, br s, 4-OH, CO_2H; 11·21, s, 2-OH. Mass spectrum *m/z* 331 (M − 1, 19%), 210 (20), 182 (87), 165 (67), 164 (100), 151 (34), 122 (34).

Benzyl 2′-O-Methyllecanorate (14)

2′-*O*-Methyllecanoric acid (13) (0·1 g, 0·3 mmol), benzyl bromide (0·04 ml) and potassium hydrogen carbonate (0·04 g) were stirred in anhydrous *N,N*-dimethylformamide (10 ml) for 48 h. The reaction mixture was then poured into water and extracted with ethyl acetate. The organic extracts were washed well with water, dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by chromatography over silica gel with 5% ethyl acetate/light petroleum as eluent. The major band afforded *benzyl 2′-*O*-methyllecanorate* (14) (86%) which crystallized from ethyl acetate/light petroleum as colourless needles, m.p. 132–136° (Found: C, 67·9; H, 5·3. $C_{24}H_{22}O_7$ requires C, 68·2; H, 5·2%). ^{1}H n.m.r. ($CDCl_3$) δ 2·28, s, 6′-Me; 2·59, s, 6-Me; 3·81, s, OMe; 5·40, s, CH_2; 6·32, 6·34, 2d, *J* 2·2 Hz, H 3,5; 6·61, 6·63, *J* 2·0 Hz, H 3′,5′; 7·40, m, Ph; 11·40, br s, 2-OH. Mass spectrum *m/z* 422 (M, 7%), 165 (100), 91 (90).

Benzyl 2,4-Di-O-benzyl-2″-O-methylgyrophorate (15)

A solution of benzyl 2′-*O*-methyllecanorate (14) (40 mg) and 2,4-dibenzyloxy-6-methylbenzoic acid (10) (50 mg) in toluene (10 ml) and trifluoroacetic anhydride (1 ml) was stirred at room temperature, and the reaction monitored by thin-layer chromatography (15% acetic acid/toluene). After 4 h the solvent was evaporated and the residue chromatographed over silica gel with 10% ethyl acetate/light petroleum as eluent. Two major bands developed. The major faster moving band afforded *benzyl 2,4-di-*O*-benzyl-2″-*O*-methylgyrophorate* (15) (56%) as a colourless gum (Found: C, 73·2; H, 5·3. $C_{45}H_{40}O_{10}$ requires C, 73·0; H, 5·4%). ^{1}H n.m.r. ($CDCl_3$) δ 2·30, 2·45, 2·61, 3s, ArMe; 3·84, s, OMe; 5·09, 5·11, 2s, $ArOCH_2$; 5·40, s, CO_2CH_2; 6·50, 6·52, 6·53, 6·62, 6·65, 6·71, 6d, *J* 2·0–2·4 Hz, ArH; 7·40, m, Ph; 11·15, s, OH. Mass spectrum *m/z* 272 (40%), 91 (100).

2″-O-Methylgyrophoric Acid (6)

A solution of benzyl 2,4-di-*O*-benzyl-2″-*O*-methylgyrophorate (15) (50 mg) in ethyl acetate (10 ml) and palladium on charcoal (10 mg) was stirred under an atmosphere of hydrogen for 24 h. The solution was then filtered, the catalyst washed with ethyl acetate, and the solvent evaporated. The residue was crystallized from acetone/light petroleum to give *2″-O-methylgyrophoric acid* (6) (90%) as colourless crystals, m.p. 176–178° (dec.) (Found: C, 62·3; H, 4·8. $C_{25}H_{22}O_{10}$ requires C, 62·2; H, 4·6%). ^1H n.m.r. ($CDCl_3/CD_3SOCD_3$) δ 2·41, 2·62, 2·66, 3s, ArMe; 3·89, s, OMe; 6·33, 6·35, 2d, J 2·2 Hz, H3,5; 6·69, 6·78, 2d, J 2·0 Hz, H3″,5″; 6·72, s, H3′,5′; 10·02, br s, 2″-OH, CO_2H; 10·90, s, 4-OH; 11·21, s, 2-OH. ^{13}C n.m.r. (CD_3COCD_3/CD_3SOCD_3) δ 17·03, 18·70, 21·43 (Ar**C**H_3); 54·15 (OCH_3); 99·36, 101·69, 106·40, 110·11, 113·54, 113·70 (Ar CH); 103·21, 114·97, 122·0; 135·00, 138·08, 141·23 (Ar **C**C); 150·04, 151·28, 155·48 (Ar**C**O); 165·39, 166·80, 167·57 (C=O). Mass spectrum: (negative f.a.b.) m/z 481 (M − H, 50%), 339 (42), 325 (60), 311 (43), 283 (100), 255 (72); (e.i.) m/z 348 (50%), 182 (87), 165 (70), 164 (100), 150 (68).

Acknowledgments

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