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Three New Aliphatic Acids from Lichens of Genus *Parmelia* (Subgenus *Xanthoparmelia*)

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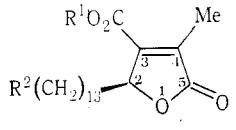
Abstract

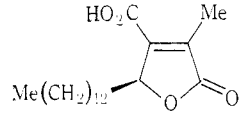
The aliphatic acids, constipatic acid [2-(14′-hydroxypentadecyl)-4-methyl-5-oxo-2,5-dihydrofuran-3-carboxylic acid], protoconstipatic acid [2-(14′-hydroxypentadecyl)-4-methylene-5-oxotetrahydrofuran-3-carboxylic acid] and dehydroconstipatic acid [4-methyl-5-oxo-2-(14′-oxopentadecyl)-2,5-dihydrofuran-3-carboxylic acid], have been identified as constituents of various *Xanthoparmelia* lichens from Australia.

Introduction

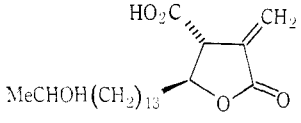
In 1975 Kurokawa and Filson[1] reported the detection of a number of unidentified fatty acids in lichens of genus *Parmelia* (subgen. *Xanthoparmelia*) by thin-layer chromatographic methods. We have now isolated and characterized three of these compounds, namely constipatic acid (1), protoconstipatic acid (5)* and dehydroconstipatic acid (2). The spectroscopic properties of these compounds indicated that they were closely related to the known lichen acids, lichesterinic acid (4) and protolichesterinic acid (6). Authentic samples of the latter two acids were obtained by extraction of *Cetraria australiensis* Weber ex Kärn.[3,4]



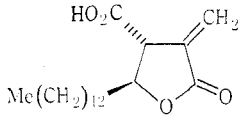
	R^1	R^2
(1)	H	MeCHOH
(2)	H	MeCO
(3)	Me	MeCHOH



(4)



(5)



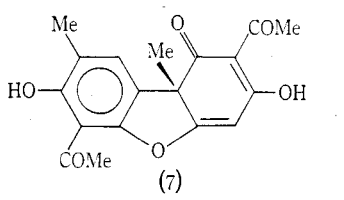
(6)

* Very recently Huneck and coworkers[2] have described the isolation of murolic acid, a stereoisomer of (5), from the lichen *Lecanora muralis* (Schreb.) Rabenh.

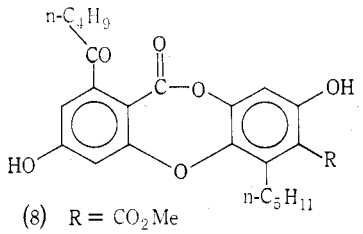
[1] Kurokawa, S., and Filson, R. B., *Bull. Nat. Sci. Mus., Tokyo, B*, 1975, **1**, 35.
[2] Huneck, S., Schreiber, K., Höfle, G., and Snatzke, G., *J. Hattori Bot. Lab.*, 1979, No. 45, 1.
[3] Kärnefelt, I., *Bot. Not.*, 1977, **130**, 125.
[4] Asano, M., and Kanematsu, T., *Ber. Dtsch. Chem. Ges.*, 1932, **65**, 1175.

Discussion

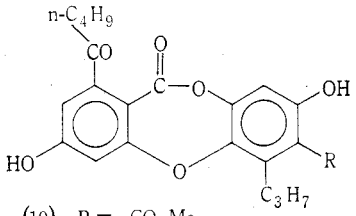
The extraction of *Parmelia constipata* Kurok. & Fils. led to the isolation of constipatic acid (1) in addition to the known lichen derivatives, usnic acid (7), loxodin (8)[5] and norlobaridone (9).[6] Constipatic acid (1) was also obtained by extraction of *Parmelia xanthosorediata* Elix where it co-occurs with usnic acid (7), loxodin (8), norlobaridone (9), conloxodin (10),[7] conorlobaridone (11)[7] and a second fatty acid, protoconstipatic acid (5). The third related acid, dehydroconstipatic acid (2), was isolated by preparative layer chromatography of the crude extract of *Parmelia barbatica* Elix. This lichen also produces usnic acid (7), constipatic acid (1) and the known depsides, barbatic acid (12) and 4-*O*-demethylbarbatic acid (13).[8]



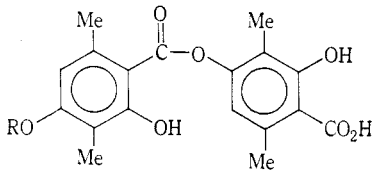
(7)



(8) R = CO_2Me
(9) R = H



(10) R = CO_2Me
(11) R = H



(12) R = Me
(13) R = H

The molecular formulae of the optically active acids (1), (2) and (5) were determined by elementary analysis and/or high-resolution mass measurements on the respective molecular ions. Infrared absorption in the carbonyl region indicated that all three compounds possessed carboxylic acid and unsaturated lactone moieties (see also[9]).

The ^{1}H n.m.r. spectrum of constipatic acid (1) exhibited a three-proton doublet at δ 1·18 (J 6 Hz) coupled to a one-proton multiplet at 3·88, characteristic of the terminal 1-hydroxyethyl group.[10] The presence of this group was further substantiated by the mass spectrum which showed a significant peak at m/e 45. In the ^{1}H n.m.r. spectrum of (1) the three-proton doublet at δ 2·10 (J 2 Hz) was assigned to the 4-methyl group. This group exhibited long-range coupling to H2 on the lactone ring, the latter proton signal appearing as a multiplet at δ 5·14. This assignment was confirmed by comparison with the ^{1}H n.m.r. spectrum of lichesterinic

[5] Komiya, T., and Kurokawa, S., *Phytochemistry*, 1970, **9**, 1139.
[6] Gream, G. E., and Riggs, N. V., *Aust. J. Chem.*, 1960, **13**, 285.
[7] Begg, W. R., Chester, D. O., and Elix, J. A., *Aust. J. Chem.*, 1979, **32**, 927.
[8] Elix, J. A., *Aust. J. Bot.*, 1976, **24**, 663.
[9] Van Tamelen, E. E., and Bach, S. R., *J. Am. Chem. Soc.*, 1958, **80**, 3079.
[10] Keogh, M. F., and Zurita, M. E., *Phytochemistry*, 1977, **16**, 134.

acid (4) since this showed a three-proton doublet at δ 2·17 (J 2 Hz) and a one-proton multiplet at 5·12 due to the methyl and lactone ring protons respectively.

Constipatic acid (1) was converted into methyl constipatate (3) by treatment with ethereal diazomethane at room temperature. The high-resolution mass spectrum of methyl constipatate (3) showed diagnostic peaks at m/e 367, 338, 279 and 169 resulting from cleavage of a methyl group, the 1-hydroxyethyl moiety (with hydrogen transfer), the methoxycarbonyl group and β-cleavage of the side chain respectively. The ^{1}H n.m.r. spectrum of methyl constipatate (3) further substantiated the proposed structure of (1) for it exhibited three-proton doublets at δ 1·08 (J 6 Hz) and 2·12 (J 2 Hz) and a three-proton singlet at 3·88 due to the terminal, 4-methyl and ester methyl groups respectively. The absence of any further methyl signals in the ^{1}H n.m.r. spectrum of (1) or (3) confirmed the linear nature of the aliphatic chain.

The structure of dehydroconstipatic acid (2) was established in a similar manner. Here the ^{1}H n.m.r. spectrum exhibited a three-proton singlet at δ 2·16 and a two-proton asymmetric triplet at 2·43, characteristic of the $MeCOCH_2$ functionality.[11] In this case the mass spectrum exhibited a significant peak at m/e 43. Further, the ^{1}H n.m.r. spectrum of (2) showed a three-proton doublet at δ 2·24 (J 2 Hz) and a one-proton multiplet at 5·12 due to the ring methyl and H 2 protons respectively. This confirmed that the substitution pattern of the lactone ring of (2) was the same as that for constipatic acid (1). The mass spectrum of (2) was consistent with this formulation and showed diagnostic peaks at m/e 348, 322 and 155 arising from cleavage of water, C_2H_4O and of the aliphatic side chain β to the lactone ring from the molecular ion.

The spectroscopic properties of protoconstipatic acid (5) showed similarities to both constipatic acid (1) and protolichesterinic acid (6). Thus the ^{1}H n.m.r. spectrum of (5) exhibited a three-proton doublet at δ 1·10 (J 6 Hz) and a one-proton multiplet at 3·77 indicative of the terminal 1-hydroxyethyl group. Two further one-proton multiplets at δ 6·00 and 6·29 were assigned to the 4-methylene protons, while the one-proton signals at 3·77 and 4·79 were assigned to H 2 and H 3 respectively.

Table 1. T.l.c. data for lichen fatty acids

On silica gel; *A*, benzene/dioxan/acetic acid 180/45/5; *B*, n-hexane/diethyl ether/formic acid 130/80/20; *C*, toluene/acetic acid 200/30

Compound	$R_F(A)$	$R_F(B)$	$R_F(C)$
Atranorin (standard)	0·75	0·76	0·78
Norstictic acid (standard)	0·41	0·32	0·27
Constipatic acid (1)	0·31	0·35	0·29
Protoconstipatic acid (5)	0·26	0·34	0·26
Dehydroconstipatic acid (2)	0·40	0·41	0·42
Lichesterinic acid (4)	0·44	0·64	0·42
Protolichesterinic acid (6)	0·36	0·48	0·37

A comparison with the ^{1}H n.m.r. spectrum of protolichesterinic acid (6) confirmed the latter assignments. In (6) the signals arising from the lactonic 4-methylene protons occur at δ 5·99 and 6·38, and those due to H 2 and H 3 occur at 3·62 and 4·78 respectively. Understandably the mass spectrum of protoconstipatic acid (5)

[11] Duran, I., and Keogh, M. F., *Phytochemistry*, 1977, **16**, 1605.

showed no significant difference to that of constipatic acid (1), and the structure of this former acid followed. [Subsequent o.r.d. studies have confirmed that constipatic acid (1) and dehydroconstipatic acid (2) have the (2*S*) configuration comparable with (−)-lichesterinic acid (4), while protoconstipatic acid (5) has the (2*S*,3*R*) configuration comparable with (−)-protolichesterinic acid (see[12]).]

As thin-layer chromatography is now the most widely used method for the identification of lichen constituents,[13] the relevant data for constipatic acid (1), protoconstipatic acid (5) and dehydroconstipatic acid (2) are listed in Table 1.

Experimental

The general experimental details have been published previously.[14] O.r.d. measurements were made on a Jasco UV/5 ORD recorder fitted with a Sproul Scientific ss 20 CD modification.

Extraction of Parmelia constipata *Kurok. & Fils.*

The lichen material was collected on schist boulders along High Eden Road, 6·5 km west of Springton, S.A., *J. A. Elix 869* (MEL).

The dried thallus (136 g) was extracted with anhydrous ether in a Soxhlet extractor for 20 h. The solution was cooled and usnic acid (7) (0·96 g) filtered off. This was identified by comparison with authentic material (t.l.c., m.s., ^{1}H n.m.r.). The ethereal filtrate was evaporated and the residue extracted with hot benzene. The benzene-soluble fraction was concentrated to yield a mixture of loxodin (8) and norlobaridone (9) (5·2 g), identified by comparison with authentic samples (t.l.c., m.s.). The benzene-insoluble residue was recrystallized from acetic acid to give *constipatic acid* (1) (0·25 g, 0·2%) as white flakes, m.p. 108–109°, $[\alpha]_D^{25}$ −24° ($CHCl_3$) (Found: C, 68·4; H, 9·9. $C_{21}H_{36}O_5$ requires C, 68·4; H, 10·0%) (Found: mol. wt, 368·2559. $C_{21}H_{36}O_5$ requires mol. wt, 368·2563). ν_{max} (Nujol) 3400 (broad OH), 1740 (lactone C=O), 1702 cm^{-1} (acid C=O). ^{1}H n.m.r. δ ($CDCl_3$) 1·18, d, *J* 6 Hz, CH_3CHOH; 1·27, br s, $(CH_2)_{13}$; 2·10, d, *J* 2 Hz, 4-CH_3; 3·88, m, CH_3CHOH; 5·14, m, H 2. Mass spectrum *m/e* 368 (M, 3%), 353 (19), 350 (32), 332 (12), 325 (14), 324 (49), 306 (12), 305 (11), 304 (12), 280 (24), 279 (100), 261 (13), 179 (12), 168 (11), 165 (15), 155 (50), 151 (11), 142 (42), 141 (10), 139 (10), 137 (10), 123 (15), 111 (19), 109 (15), 97 (30), 95 (25), 83 (34), 81 (21), 71 (19), 69 (44), 67 (24), 57 (30), 55 (55), 45 (40), 43 (10), 41 (37).

Extraction of Parmelia xanthosorediata *Elix*

The lichen material was collected on rocks, Kowen Forest, 16 km east of Canberra, A.C.T., *J. A. Elix 1830* (MEL).

The dried thallus (8·2 g) was extracted and chromatographed as described previously.[7] Usnic acid (7), R_F 0·83 (210 mg), loxodin (8), R_F 0·41 (90 mg), conloxodin (10), R_F 0·36 (21 mg), norlobaridone (9), R_F 0·24 (110 mg), and conorlobaridone (11), R_F 0·20 (29 mg), were identified by comparison with authentic material (t.l.c., m.s., ^{1}H n.m.r.). Constipatic acid (1), R_F 0·29 (6 mg), was identified by comparison with that obtained from *P. constipata* (t.l.c., m.s., ^{1}H n.m.r., m.p.). The band at R_F 0·26 was recrystallized from benzene to give *protoconstipatic acid* (5) (12 mg, 0·15%) as white flakes, m.p. 102–103°, $[\alpha]_D^{25}$ −12° ($CHCl_3$) (Found: mol. wt, 368·2557. $C_{21}H_{36}O_5$ requires mol. wt, 368·2563). The homogeneity of this compound was confirmed by ^{1}H n.m.r. spectroscopy and the fact that it exhibited a single spot on t.l.c. in three independent solvent systems. ν_{max} (Nujol) 1743 (lactone C=O), 1710 cm^{-1} (acid C=O). ^{1}H n.m.r. δ (CD_3COCD_3) 1·10, d, *J* 6 Hz, CH_3; 1·31, br s, $(CH_2)_{13}$; 3·77, m, CH_3CHOH, H 2; 4·79 m, H 3; 6·00, 6·29, each m, 4-CH_2. Mass spectrum *m/e* 368 (M, 3%), 353 (24), 351 (11), 350 (41), 332 (10), 325 (18), 324 (63), 306 (16), 305 (11), 280 (26), 279 (100), 261 (11), 179 (12), 167 (12), 165 (14), 155 (45), 142 (43), 141 (12), 139 (12), 137 (10), 122 (14), 121 (20), 111 (19), 109 (16), 97 (32), 96 (14), 95 (32), 85 (13), 83 (43), 82 (14), 81 (29), 71 (21), 70 (15), 69 (69), 68 (16), 67 (39), 57 (41), 56 (20), 55 (97), 45 (78), 43 (39), 41 (61).

[12] Boll, P. M., *Acta Chem. Scand.*, 1968, **22**, 3245.
[13] Culberson, C. F., *J. Chromatogr.*, 1972, **72**, 113.
[14] Elix, J. A., *Aust. J. Chem.*, 1974, **27**, 1767.

Extraction of Parmelia barbatica *Elix*

The lichen material was collected on rocks, Kowen Forest, 16 km east of Canberra, A.C.T., *J. A. Elix 1445* (CANB).

The dried thallus (10·2 g) was extracted with anhydrous ether in a Soxhlet extractor for 20 h. The solution was cooled and filtered to yield usnic acid (7) (75 mg) which was identified by comparison with authentic material (t.l.c., ^{1}H n.m.r.). The ethereal filtrate was evaporated and the residue (0·79 g) adsorbed on five silica gel plates (100 by 20 by 0·1 cm) and eluted with 15% acetic acid/toluene. The fastest moving band yielded barbatic acid (12) (410 mg) which was identified by comparison with authentic material (t.l.c., ^{1}H n.m.r.). The second band was recrystallized from benzene to give *dehydroconstipatic acid* (2) (11·2 mg, 0·1%) as colourless needles, m.p. 91–92°, $[\alpha]_D^{25}$ −73·5° ($CHCl_3$) (Found: mol. wt, 366·2407. $C_{21}H_{34}O_5$ requires mol. wt, 366·2406). The homogeneity of this compound was confirmed by ^{1}H n.m.r. spectroscopy and the fact that it exhibited a single spot on t.l.c. in three independent solvent systems. ν_{max} (Nujol) 1735 (lactone C=O), 1706 cm^{-1} (acid C=O, ketone C=O). ^{1}H n.m.r. δ ($CDCl_3$) 1·27, br s, $(CH_2)_{13}$; 2·16, s, CH_3CO; 2·24, d, J 2 Hz, 4-CH_3; 2·43, t, J 7 Hz, CH_3COCH_2; 5·12, m, H2. Mass spectrum *m/e* 367 (11%), 366 (M, 46), 348 (24), 322 (10), 309 (19), 308 (15), 290 (10), 263 (11), 253 (12), 251 (13), 239 (11), 225 (20), 211 (10), 155 (28), 43 (100).

The third band contained 4-*O*-demethylbarbatic acid (13) (92 mg), identified by comparison with an authentic sample (t.l.c., ^{1}H n.m.r.), and the slowest band yielded constipatic acid (1), identified by comparison with the authentic material.

Methylation of Constipatic Acid (1)

Constipatic acid (1) (20 mg) in ethyl acetate (4 ml) was treated with an excess of ethereal diazomethane. After 20 min at room temperature the solvents were removed and the residue recrystallized from cyclohexane/toluene to give *methyl constipatate* (3) in quantitative yield as white flakes, m.p. 52–53° (Found: mol. wt of M−CH_3, 367·2536. $C_{21}H_{35}O_5$ requires mol. wt, 367·2535). The homogeneity of this compound was confirmed by ^{1}H n.m.r. spectroscopy and the fact that it exhibited a single spot on t.l.c. in three independent solvent systems. ν_{max} (Nujol) 1765 (lactone C=O), 1726 cm^{-1} (ester C=O). ^{1}H n.m.r. δ (CD_3COCD_3) 1·08, d, J 6 Hz, CH_3CHOH; 1·29, br s, $(CH_2)_{13}$; 2·12, d, J 2 Hz, 4-CH_3; 3·68, m, CH_3CHOH; 3·88, s, OCH_3; 5·16, m, H2; 7·31, br s, OH. Mass spectrum *m/e* 382 (M, <1%), 381 (4), 367 ($C_{21}H_{35}O_5$, 19), 364 (7), 350 (10), 339 (11), 338 ($C_{20}H_{34}O_4$, 42), 332 (10), 305 (16), 280 (25), 279 ($C_{18}H_{31}O_2$, 100), 169 ($C_8H_9O_4$, 41), 156 (28), 45 (52).

Extraction of Cetraria australiensis *Weber ex Kärn*

The lichen material was collected on dead *Baeckia* twigs, near Snowy River Bridge, 2·8 km east of Mt. Kosciusko, N.S.W., *J. A. Elix 4304* (CANB).

The dried thallus (4·32 g) was extracted with anhydrous ether in a Soxhlet extractor for 24 h. The residue (0·04 g) obtained on removal of the solvent was adsorbed onto two thick-layer plates (20 by 20 by 0·1 cm) and eluted with 15% acetic acid/toluene. Two major bands were removed. The faster band recrystallized from ethanol to give lichesterinic acid (4) (8 mg) as colourless plates, m.p. 123–123·5° (lit.[4] 123–124°), $[\alpha]_D^{25}$ −25·5° ($CHCl_3$) [lit.[15] $[\alpha]_D^{18}$ −31·4° ($CHCl_3$)]. ^{1}H n.m.r. δ ($CDCl_3$) 0·87, m, $(CH_2)_{12}CH_3$; 1·28, br s, $(CH_2)_{12}$; 2·17, d, J 2 Hz, 4-CH_3; 5·12, m, H2. Mass spectrum *m/e* 324 (M, 88%), 306 (15), 280 (62), 279 (100), 261 (37), 155 (52).

The second band recrystallized from benzene to give protolichesterinic acid (6) (9 mg) as white flakes, m.p. 105–106° (lit.[4] 106°), $[\alpha]_D^{25}$ −14° ($CHCl_3$) [lit.[16] $[\alpha]_D^{27}$ −12·7° ($CHCl_3$)]. ^{1}H n.m.r. δ ($CDCl_3$) 0·87, m, $(CH_2)_{12}CH_3$; 1·28, br s, $(CH_2)_{12}$; 3·62, m, H2; 4·78, m, H3; 5·99, 6·38, each m, 4-CH_2. Mass spectrum *m/e* 324 (M, 26%), 306 (7), 280 (24), 279 (100), 261 (11), 233 (7), 155 (19).

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[15] Asahina, Y., and Yasue, M., *Ber. Dtsch. Chem. Ges.*, 1937, **60**, 1053.
[16] Asano, M., and Azumi, T., *Ber. Dtsch. Chem. Ges.*, 1935, **68**, 995.