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The Structure of Echinocarpic Acid. A Benzyl Ester from the Lichen *Parmelia norcrambidiocarpa*

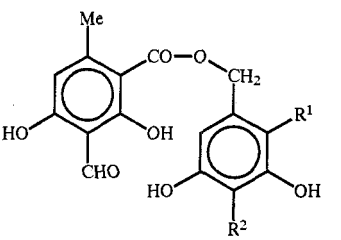
John A. Elix, Labunmi Lajide and Judith H. Wardlaw

Department of Chemistry, The Faculties, Australian National University, Canberra, A.C.T. 0200.

Abstract

The benzyl ester echinocarpic acid (1′,4′-dihydroxy-6′-methoxy-3′-oxo-1′,3′-dihydroisobenzofuran-5′-ylmethyl 2,4-dihydroxy-3,6-dimethylbenzoate) (7) has been isolated from the lichen *Parmelia norcrambidiocarpa* together with atranorin and chloroatranorin. The structure of the compound (7) followed from a combination of spectroscopic and chemical data.

Depsides and depsidones derived biosynthetically from β-orsellinic acid moieties are common lichen metabolites, but the related benzyl esters are much more restricted in number with only four known representatives; namely alectorialic acid (1), alectorialin (2), barbatolic acid (3) and barbatolin (4).[1]



	R^1	R^2
(1)	CO_2H	Me
(2)	H	Me
(3)	CO_2H	CHO
(4)	H	CHO

During a chemosystematic study of the lichen genus *Parmelia* in New Zealand,[2] Mason Hale observed that *P. norcrambidiocarpa* Hale produced three major metabolites. These included the common cortical depsides atranorin (5) and chloroatranorin (6) together with the unknown 'echinocarpic acid'. The last compound was first detected by Kurokawa in *P. echinocarpa* Kurok.[3] [= *Relicina echinocarpa* (Kurok.) Hale[4]], but he did not elucidate the structure of this compound. Subsequently we have undertaken a larger scale extraction of *P. norcrambidiocarpa* and followed this by fractional crystallization of the extract. This led to the isolation of echinocarpic acid (7), the structure of which followed,

[1] Elix, J. A., and Jayanthi, V. K., *Aust. J. Chem.*, 1987, **40**, 1841.
[2] Hale, M. E., *Smithsonian Contrib. Bot.*, 1987, **66**, 1.
[3] Kurokawa, S. *Jpn. J. Bot.*, 1965, **40**, 264.
[4] Hale, M. E., *Phytologia*, 1974, **28**, 479.

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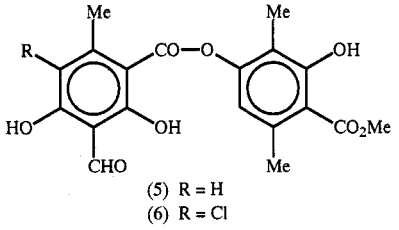
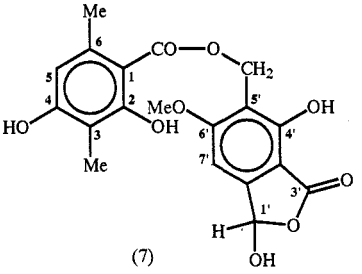
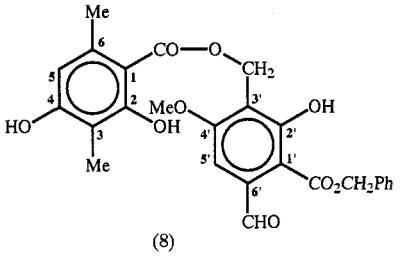
initially, from the spectroscopic properties. In particular, the ^{1}H n.m.r. [$(CD_3)_2SO$] spectrum showed two *C*-methyl resonances (δ 1·93, 2·19), an *O*-methyl signal (δ 3·88), an OCH_2Ar signal (δ 5·38), two aromatic proton signals (δ 6·22, 6·93), a methine proton (δ 6·63) coupled to a hydroxy proton (δ 7·88), another hydroxy proton signal (δ 10·12) and two intramolecularly hydrogen-bonded hydroxy signals (δ 11·56, 12·62). Furthermore, the broad one-proton signal at δ 6·63 was typical of a methine proton of a hydroxy lactone system,[5,6] so following biosynthetic considerations these data were compatible with the structure (7). Echinocarpic acid did not exhibit a molecular ion in the e.i. mass spectrum, but did show daughter ions at *m/z* 196, 182, 164 and 136 consistent with that expected for structure (7).



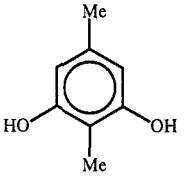
(5) R = H
(6) R = Cl



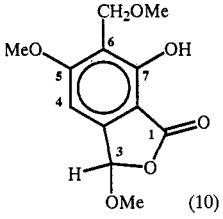
(7)



(8)



(9)



(10)

The structure of echinocarpic acid (7) was ultimately established by derivatization and subsequent degradation. Thus benzylation of echinocarpic acid (7) with phenyldiazomethane afforded the normal ester, benzyl echinocarpate (8), thereby confirming the presence of a hydroxy lactone moiety in (7).

Methanolysis of benzyl echinocarpate (8) by treatment with sodium methoxide in methanol afforded β-orcinol (9) (via the intermediacy of sodium 2,4-dihydroxy-3,6-dimethylbenzoate which was expected to decarboxylate under the basic reaction conditions) and the methyl pseudo-ester (10). High-resolution mass spectrometry established the molecular formula of (10) as $C_{12}H_{14}O_6$ and the structure followed from the ^{1}H n.m.r. spectrum and the mass spectral fragmentation pattern. In particular the ^{1}H n.m.r. spectrum exhibited two methoxy signals (δ 3·53, 3·62) where the respective methoxy groups were bonded to an aliphatic carbon atom, an aromatic methoxy resonance (δ 3·85), an OCH_2Ar signal (δ 4·85), an aromatic proton resonance (δ 6·33), an uncoupled methine proton signal (δ 6·87) and a hydroxy signal (δ 9·09). The structure of these cleavage products having been established, that of the precursor benzyl echinocarpate (8) and echinocarpic acid (7) followed.

[5] Elix, J. A., Gaul, K. L., and James, P. W., *Aust. J. Chem.*, 1985, **38**, 1735.
[6] Elix, J. A., Gaul, K. L., James, P. W., and Purvis, D. W., *Aust. J. Chem.*, 1987, **40**, 417.

Echinocarpic acid (7) differs from the other known lichen benzyl esters (1)–(4) since ester formation involves *ortho*- rather than a *meta*-hydroxymethyl substitution with respect to the position of dioxygenation of the B-ring.

Experimental

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

Extraction of Parmelia norcrambidiocarpa *Hale*

The lichen *P. norcrambidiocarpa* was collected on a fallen *Nothofagus* at the roadside in *Nothofagus* forest, Otago, *c.* 1 km west of Found Creek, Mt Aspiring National Park, South Island, New Zealand, J. Johnston, 1985 (CBG).

The lichen material (13·4 g) was dried and extracted with anhydrous ether in a Soxhlet extractor for 26 h. After evaporation of the solvent the residue (0·35 g) was shown by t.l.c. to consist of a mixture of atranorin (and chloroatranorin), echinocarpic acid and a smaller amount of a more polar compound. After warming with cyclohexane/ethyl acetate the residue yielded *echinocarpic acid* (7) (40 mg, 0·3%) as colourless crystals, m.p. 146–148° (Found: C, 55·4; H, 4·7. $C_{19}H_{18}O_9.H_2O$ requires C, 55·9; H, 4·9%). ^{1}H n.m.r. [$(CD_3)_2CO$] δ 2·02, 2·14, 2s, ArMe; 4·06, s, OMe; 5·43, s, OCH_2Ar, 6·26, 6·93, 2s, H5,7′; 6·71, s, H1′; 8·92, 10·50, 2s, OH; 11·91, 12·94, 2s, 2,4′-OH. ^{1} H n.m.r. [$(CD_3)_2SO$] δ 1·93, 2·19, 2s, ArMe; 3·88, s, OMe; 5·38, s, OCH_2Ar, 6·22, 6·93, 2s, H5,7′; 6·63, d, J 8·0 Hz, H1′; 7·88, d, J 8·8 Hz, 1′-OH; 10·12, s, 4-OH; 11·58, 12·62, 2s, 2,4′-OH. Mass spectrum *m/z* 299 (12%), 257 (17), 215 (19), 196 (19), 182 (34), 178 (19), 170 (15), 167 (21), 165 (14), 164 (62), 163 (12), 158 (14), 155 (10), 153 (11), 152 (16), 151 (19), 150 (36), 139 (17), 138 (29), 137 (25), 136 (81), 135 (19), 129 (13), 127 (12), 125 (13), 124 (12), 123 (21), 122 (11), 121 (26), 120 (21), 116 (13), 113 (14), 112 (12), 111 (20), 109 (23), 107 (32), 105 (15), 102 (18), 100 (14), 57 (100).

Benzyl Echinocarpate (8)

Echinocarpic acid (40 mg) was stirred with an excess of ethereal phenyldiazomethane[8] at room temperature for 15 min. Excess phenyldiazomethane was then destroyed by dropwise addition of acetic acid dissolved in ether until the reddish colour had disappeared. The solvent was evaporated and the residue separated into fractions by repeated radial chromatography with 2·5–30% ethyl acetate/light petroleum as eluent. The major band yielded *benzyl echinocarpate* (8) (34 mg, 69%) as a colourless oil. ^{1}H n.m.r. (300 MHz, $CDCl_3$) δ 2·09, 2·78, 2s, ArMe; 3·96, s, OMe; 5·42, 5·45, 2s, $ArCH_2$, OCH_2Ph; 6·14, 7·07, 2s, H5,5′; 7·40–7·48, m, Ph; 10·43, s, CHO; 11·92, 12·86, 2s, 2,2′-OH. Mass spectrum *m/z* 436 (27%), 345 (13), 327 (45), 280 (11), 207 (26), 151 (11), 91 (100). As this ester was unstable in deuterated chloroform, the degradation reaction was carried out immediately.

Methanolysis of Benzyl Echinocarpate (8)

A solution of benzyl echinocarpate (8) (13 mg, 0·027 mmol) in methanol (6 ml) was added to a solution of sodium (24 mg) in methanol (8·4 ml) and heated under reflux in a nitrogen atmosphere for 30 min. After cooling, the mixture was poured into ice-cold aqueous tartaric acid, and extracted with ethyl acetate, and the organic extract was washed with water and brine and dried ($MgSO_4$). After filtration and removal of solvent, the residue was separated by preparative t.l.c. over silica gel (20 by 20 by 0·1 cm) with 25% ethyl acetate/light petroleum as eluent. Two major bands developed. The first band afforded 2,5-dimethylbenzene-1,3-diol (9) (2·6 mg, 70%). ^{1}H n.m.r. (300 MHz, $CDCl_3$) δ 2·09, 2·20, 2s, ArMe; 6·22, s, 2OH, identical with authentic material (t.l.c., ^{1}H n.m.r.).[9]

The second band yielded *7-hydroxy-3,5-dimethoxy-6-methoxymethylphthalide* (10) (1 mg, 15%), as colourless crystals, m.p. >314° (Found: mol. wt, 254·0790. $C_{12}H_{14}O_6$ requires mol. wt, 254·0790). ^{1}H n.m.r. (300 MHz, $CDCl_3$) δ 3·53, 3·62, 3·85, 3s, OMe; 4·80, 4·91, 2d, J

[7] Elix, J. A., Venables, D. A., Lumbsch, H. T., and Brako, L., *Aust. J. Chem.*, 1994, **47**, 1619.
[8] Overberger, C. G., and Anselme, J. P., *J. Org. Chem.*, 1963, **28**, 592.
[9] Hesse, O., *J. Prakt. Chem.*, 1903, **68**, 14.

14·5 Hz, $ArCH_2$; 6·33, s, H 4; 6·87, s, OCHO; 9·09, s, 7-OH. Mass spectrum *m/z* 254 (M, 33%), 223 (17), 222 (45), 207 (15), 194 (29), 193 (22), 192 (100), 191 (78), 177 (18), 164 (21), 163 (17), 161 (15), 150 (20), 148 (13), 147 (11), 135 (21), 121 (10), 113 (21), 107 (14), 105 (17), 104 (11).

Acknowledgments

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