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Conhypoprotocetraric Acid, a New Lichen β-Orcinol Depsidone

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Abstract

The β-orcinol depsidone, conhypoprotocetraric acid (2), has been identified in the lichens *Relicina* cf. *incongrua* and *Lecanora myriocarpoides*. This new depsidone has been synthesized and characterized, and the biosynthetic implications are discussed.

Introduction

The biosynthetic interrelationship between the common lichen depsides and depsidones has been the subject of speculation for some time.[1–3] With simple orcinol derivatives, circumstantial evidence exists for such an interrelation in the form of the co-occurrence of isostructural depside–depsidone pairs in one organism.[1–4] Subsequently we have formulated a hypothetical biosynthetic scheme to account for these observations, and have employed a biomimetic-type approach for the synthesis of several orcinol depsidones.[5] Although this suggestion needs to be verified by *in vivo* labelling experiments, preliminary results indicate that this may be so.[6]

However, the more common β-orcinol depsidones, particularly those with more highly oxidized C 1 side chains, do not appear to have isostructural *para*-depside counterparts. This led Keogh[7] to suggest that they had a different mode of formation. Indeed it would appear likely that the majority of β-orcinol depsidones arise biosynthetically from a common precursor, hypoprotocetraric acid (1), by stepwise oxidation reactions. Secondary *O*-methylation, nuclear chlorination, decarboxylation or side chain esterification reactions can then be observed at various oxidation levels, ultimately leading to the vast array of naturally occurring derivatives. As a consequence, a series of parallel biosynthetic schemes of sequentially related β-orcinol depsidone derivatives can be constructed,

[1] Culberson, C. F., 'Chemical and Botanical Guide to Lichen Products' (University of North Carolina Press: Chapel Hill 1969).
[2] Mosbach, K., *Angew. Chem., Int. Ed. Engl.*, 1969, **8**, 240.
[3] Mosbach, K., in 'The Lichens' (Eds V. Amadjian and M. E. Hale, Jr) Ch. 16 (Academic Press: New York 1973).
[4] Culberson, C. F., *Bryologist*, 1965, **68**, 435.
[5] Elix, J. A., Jenie, U. A., and Parker, J. L., *Aust. J. Chem.*, 1987, **40**, 1451.
[6] Culberson, C. F., and Armaleo, D., *Exp. Mycol.*, 1992, **16**, 52.
[7] Keogh, M. F., *Phytochemistry*, 1978, **17**, 1192.

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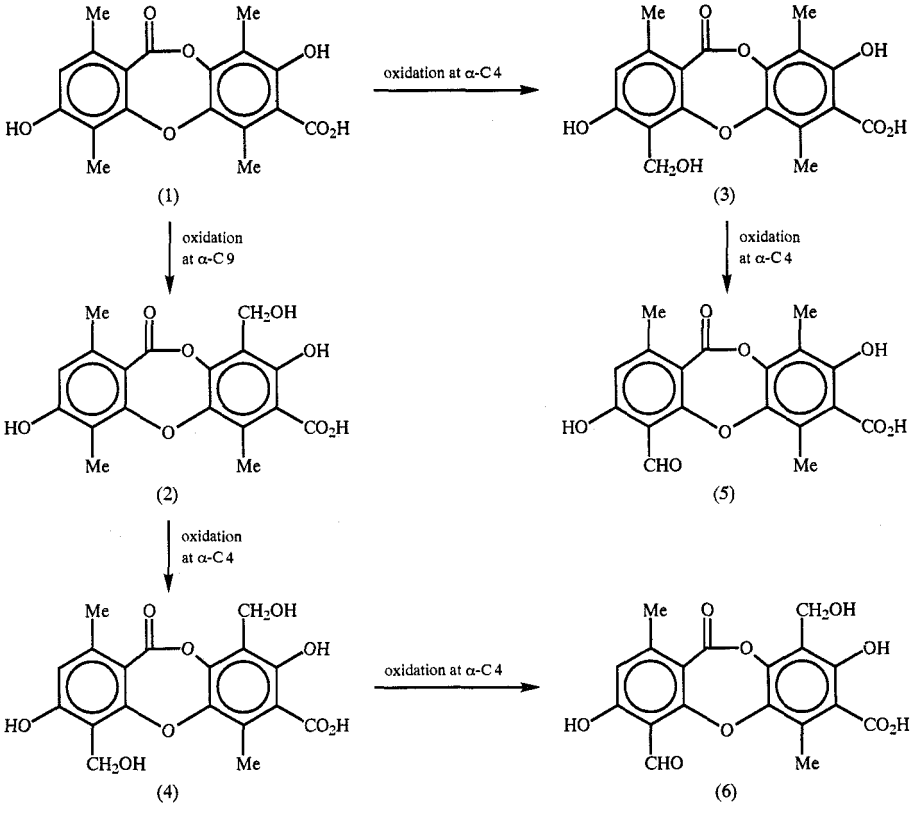
and Scheme 1 illustrates this relationship in the virensic acid–protocetraric acid chemosyndrome. Apart from depsidone (2), all of these compounds are known lichen derivatives.[8] The natural occurrence of the new compound, conhypoprotocetraric acid (2), which is a biosynthetic intermediate or 'missing link' according to Scheme 1, is described in the present paper.

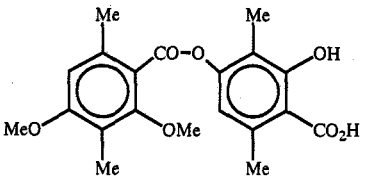


Scheme 1

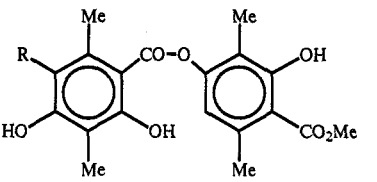
The New Depsidone

The neotropical lichen *Relicina* cf. *incongrua* (Hale) Hale was shown by h.p.l.c. and t.l.c. to produce four phenolic metabolites, three of which corresponded to known lichen substances.[9] These included the common pigment usnic acid, the depside diffractaic acid (7) and the β-orcinol depsidones hypoprotocetraric acid (1) and conhypoprotocetraric acid (2) (then of unknown structure). The latter compound was subsequently found to be the major constituent of a second species, *Lecanora myriocarpoides* Vainio from Brazil, which in addition contained minor quantities of hypoprotocetraric acid (1) and the common cortical depsides atranorin (8) and chloroatranorin (9).

[8] Elix, J. A., and Yu, J., *J. Hattori Bot. Lab.*, 1993, **74**, 317.
[9] Hale, M. E., *Smithson. Contrib. Bot.*, 1976, **26**, 1.



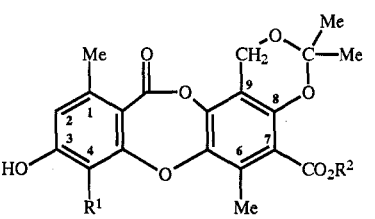
(7)



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

Subsequently we attempted the synthesis of conhypoprotocetraric acid (2) by the catalytic hydrogenolysis of conprotocetraric acid (4). This reaction gave convirensic acid (3) as the major product, with preferential reduction occurring at the sterically less hindered 9-hydroxymethyl group. However, the yields of convirensic acid were extremely poor and none of the expected isomeric, minor hydrogenolysis product (2) could be detected.

To inhibit reduction at the 9-hydroxymethyl group, protocetraric acid (6) was first derivatized by refluxing with acetone to form protocetraric acid 8,9α-acetonide (10). The 7-carboxy group of (10) was then protected by selective benzylation with benzyl bromide and potassium hydrogen carbonate to give benzyl protocetrarate 8,9α-acetonide (11). Treatment of (11) with sodium borohydride then effected reduction of the aldehyde group to give the corresponding alcohol, benzyl conprotocetrarate 8,9α-acetonide (12). Catalytic hydrogenolysis of (12) effected reduction of the 4-hydroxymethyl group as well as removal of the acetonide and benzyl protecting groups to give a mixture of the target molecule, conhypoprotocetraric acid (2), and convirensic acid (3).



	R^1	R^2
(10)	CHO	H
(11)	CHO	CH_2Ph
(12)	CH_2OH	CH_2Ph

The structure of convirensic acid (3) has been previously established by unambiguous synthesis,[8] so the structure of the isomeric, minor hydrogenolysis product must be (2). The natural occurrence of conhypoprotocetraric acid (2) has been tentatively reported previously[10,11] (as unknown Q-1) as has its synthesis by partial reduction of protocetraric acid (6), but (2) was not isolated nor was the structure of this compound established rigorously. The synthetic sample of conhypoprotocetraric acid (2) prepared above was found to be identical (t.l.c., h.p.l.c., m.s.) with the unknown substance present in *Relicina* cf. *incongrua* and *Lecanora myriocarpoides*.

Experimental

The general experimental details have been described previously.[8]

[10] Culberson, C. F., Culberson, W. L., and Johnson, A., *Mycologia*, 1992, **84**, 705.
[11] Culberson, C. F., Culberson, W. L., and Johnson, A., *Bryologist*, 1981, **84**, 16.

Detection of the New Depsidones by Comparative Chromatography

(*a*) The lichen *Relicina* cf. *incongrua* Hale was collected on canopy branches in mature secondary rainforest, elevation 150 m, about 9 km NW of Gamboa along Naval Pipeline Road, Canal Zone, Panama, M. E. Hale 43448, 11 February 1974 (US). Comparative h.p.l.c. and t.l.c. indicated the presence of usnic acid, hypoprotocetraric acid (1) (major), diffractaic acid (7) (minor), conhypoprotocetraric acid (2) (minor) [standard t.l.c. R_F values:[12] R_F(A) 0·04; R_F(B) 0·12; R_F(C) 0·03; R_F(G) 0·21; standard h.p.l.c. RI value:[13] 12]. The chromatographic behaviour of the last compound was identical with that of a synthetic sample prepared below.

(*b*) The lichen *Lecanora myriocarpoides* Vainio was collected on the bark of a tree near Sitio, Minas Geraes, Brazil, E. Vainio 5594a (TUR-5121, lectotype). Comparative h.p.l.c. and t.l.c. indicated the presence of atranorin (8), chloroatranorin (9), conhypoprotocetraric acid (2) (major), hypoprotocetraric acid (1) (minor) and physodalic acid (trace) and an unknown (minor). The chromatographic behaviour of the major compound was identical with that of a synthetic sample prepared below.

Protocetraric Acid 8,9α-Acetonide (10)

Protocetraric acid (6) (1·51 g) was heated under reflux in acetone (270 ml) and dimethyl sulfoxide (16 ml) with magnesium sulfate (1·6 g) for 90 h. After removal of most of the acetone, water (300 ml) was added and the mixture was extracted with ethyl acetate, minimal agitation being used. The organic extract was washed repeatedly with water and brine and dried ($MgSO_4$). The volume was reduced to approx. 50 ml, and the precipitate of unreacted protocetraric acid was removed by filtration. The filtrate afforded protocetraric acid 8,9α-acetonide (10) (0·271 g, 16%) as a colourless solid, m.p. >330°. ^{1}H n.m.r. [300 MHz, $(CD_3)_2CO$] δ 1·50, s, $OCMe_2$; 2·43, 2·51, 2s, ArMe; 4·95, s, $ArCH_2$; 6·82, s, H2; 10·76, s, CHO; 12·75, s, 3-OH.

Benzyl Protocetrarate 8,9α-Acetonide (11)

The acetonide obtained above was dried and used without further purification. Protocetraric acid 8,9α-acetonide (10) (0·303 g, 0·73 mmol) was dissolved in *N,N*-dimethylformamide (4 ml) and stirred with anhydrous sodium hydrogen carbonate (0·245 g, 2·92 mmol) and benzyl bromide (0·125 g, 87 ml, 0·73 mmol) at room temperature for 28·5 h. The mixture was diluted with ethyl acetate and poured into ice-cold water, and the organic layer was separated. The aqueous layer was extracted further with ethyl acetate and the combined organic layers were washed with water, with saturated sodium hydrogen carbonate, and dried ($MgSO_4$). Solvent was removed and the residue (183 mg) was purified by radial chromatography, 5–20% ethyl acetate/light petroleum being used as eluent. *Benzyl protocetrarate 8,9α-acetonide* (11) (0·057 g, 15%) crystallized from ethyl acetate/light petroleum as colourless hair-like crystals, m.p. 182–185° (Found: C, 66·6; H, 4·8. $C_{28}H_{24}O_9$ requires C, 66·7; H, 4·8%). ^{1}H n.m.r. (300 MHz, $CDCl_3$) δ 1·46, s, $OCMe_2$; 2·28, 2·50, 2s, ArMe; 4·89, s, $ArCH_2$; 5·34, s, OCH_2Ph; 6·71, s, H2; 7·30–7·45, m, Ph; 10·65, s, CHO; 12·17, s, 3-OH. Mass spectrum *m/z* 505 (1·4%), 504 (M, 1·9), 91 (100).

Benzyl Conprotocetrarate 8,9α-Acetonide (12)

A solution of the aldehyde benzyl protocetrarate 8,9α-acetonide (11) (0·177 g, 0·351 mmol) in anhydrous 1,2-dimethoxyethane (3 ml) was stirred with sodium borohydride (0·066 g, 1·75 mmol) and anhydrous ethanol (1 drop) at room temperature for 2·5 h. The mixture was poured into dilute aqueous tartaric acid (200 ml) and extracted repeatedly with ethyl acetate. The organic extract was washed with water and brine and dried ($MgSO_4$). The solvent was evaporated to yield a residue (0·187 g), a portion of which (0·025 g) was separated by preparative t.l.c. over silica gel (20 by 20 by 0·1 cm), 50% ethyl acetate/light petroleum being used as eluent. The first major band yielded *benzyl conprotocetrarate 8,9α-acetonide*

[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 (Aust. Natl Univ.: Canberra 1993).

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

(12) (13·5 mg, 57%) as a colourless solid, m.p. 154–155° (Found: C, 65·0; H, 5·0. $C_{28}H_{26}O_9.0{\cdot}5H_2O$ requires C, 65·2; H, 5·3%). ^{1}H n.m.r. (300 MHz, $CDCl_3$) δ 1·45, s, $OCMe_2$; 2·29, 2·41, 2s, ArMe; 4·87, s, $ArCH_2OCMe_2$; 5·21, d, $ArCH_2OH$; 5·34, s, OCH_2Ph; 6·61, s, H2; 7·34–7·42, m, Ph; 8·44, s, OH. Mass spectrum *m/z* 506 (M, 0·4%), 91 (100).

Conhypoprotocetraric Acid (2)

A solution of benzyl conprotocetrarate 8,9α-acetonide (12) (0·110 g) in glacial acetic acid (15 ml) was cooled to room temperature. Palladium-on-charcoal (0·1 g, 10%) was added and the mixture was stirred in an atmosphere of hydrogen at room temperature for 18 h. The catalyst was removed by filtration, and the filtrate was diluted with water (300 ml) and extracted repeatedly with ethyl acetate. The organic extract was washed with water and brine and dried ($MgSO_4$). The residue obtained on evaporation of the solvent was applied to three silica gel plates and eluted with toluene/ethyl acetate/formic acid (139 : 83 : 8). The major band contained convirensic acid (3), while the minor slow-moving band afforded *conhypoprotocetraric acid* (2 mg, 2·6%) as colourless microcrystals, m.p. >330° (Found: C, 60·1; H, 4·5. $C_{18}H_{16}O_8$ requires C, 60·6; H, 4·5%). ^{1}H n.m.r. (300 MHz, CD_3OD) δ 2·31, 2·36, 2·73, 3s, ArMe; 4·76, s, $ArCH_2OH$; 6·55, s, H2. Mass spectrum *m/z* 165 (5%), 149 (17), 139 (10), 125 (18), 111 (34), 97 (56), 83 (60), 71 (67), 57 (100). Standard t.l.c. R_F values: R_F(A) 0·04; R_F(B) 0·12; R_F(C) 0·03; R_F(G) 0·21. Standard h.p.l.c. RI 12.

Acknowledgment

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