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STENOSPORIC ACID, A NEW DEPSIDE IN *RAMALINA STENOSPORA**

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Abstract—Column chromatography of an extract of the lichen *Ramalina stenospora* Müll. Arg. yielded usnic acid (0·013%), a mixture of atranorin and chloroatranorin (0·007%), perlatolic acid (0·40%) and a new *para*-depside, stenosporic acid (0·37%). Stenosporic acid was proven to be 4-(2-hydroxy-6-propyl-*p*-anisate)-6-pentyl-β-resorcylic acid. A methyl ester synthesized by condensation of the appropriate phenolic units was found to be identical to the methyl ester prepared from stenosporic acid with diazomethane.

INTRODUCTION

LICHENS produce a large number of compounds,[1] of which depsides (and tridepsides), depsidones, dibenzofurans and a depsone are particularly characteristic of the lichen-forming fungi. Biogenetically, all of these compounds appear to arise in lichens by intermolecular coupling of acetate-polymalonate-derived phenolic units. The present report concerns the isolation and proof of structure of a new *para*-depside of the orcinol series.

RESULTS AND DISCUSSION

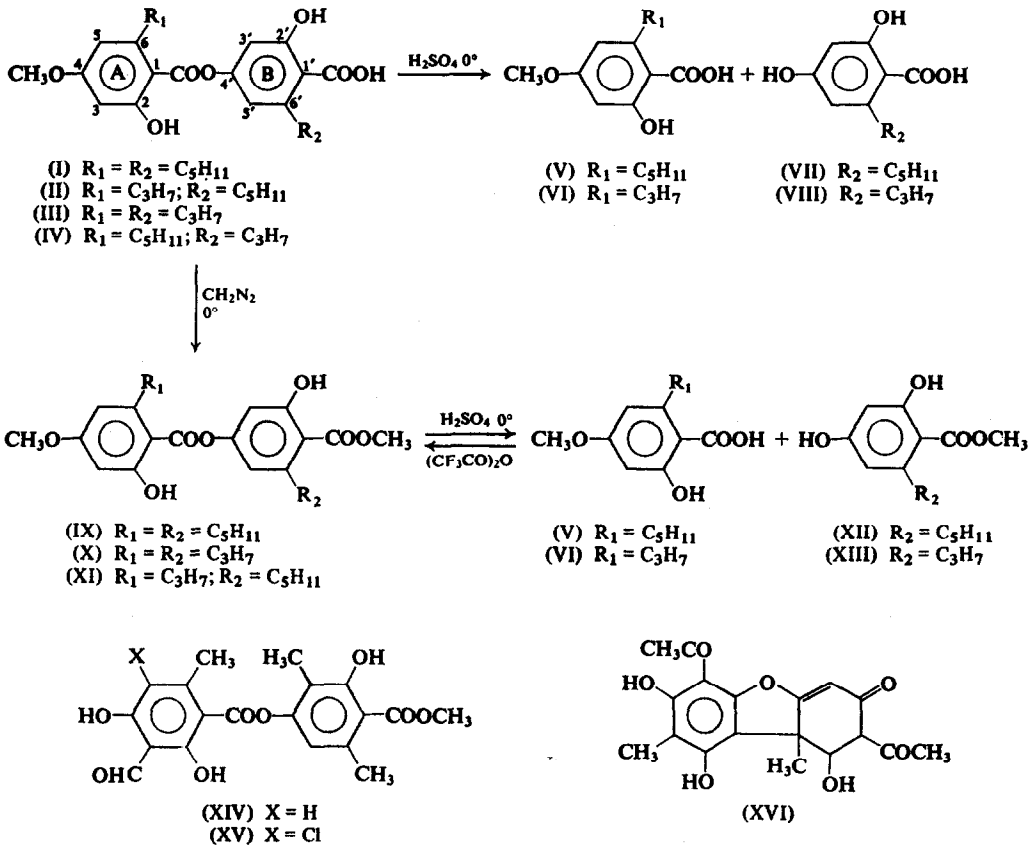
Preliminary microchemical tests on fragments of herbarium samples of *Ramalina stenospora* Müll. Arg. from Florida[2] showed usnic acid, perlatolic acid and an unidentified substance, which was difficult to resolve from perlatolic acid by TLC. Column chromatography of a larger extract separated two major products, one identified as perlatolic acid (I) by comparison with an authentic sample and the other, a new compound named stenosporic acid. The latter appeared to be closely related to perlatolic acid. The u.v., i.r. and NMR spectra and the results of microhydrolyses of stenosporic acid, perlatolic acid (I), divaricatic acid (III) and sphaerophorin agreed with the structure II for stenosporic acid.

The structure II assigned to stenosporic acid was confirmed by an independent synthesis of the methyl ester. For this synthesis, perlatolic acid (I) from *Cladonia evansii* was converted to its methyl ester (IX) with CH_2N_2. Hydrolysis of the ester with conc. H_2SO_4 yielded 2-hydroxy-6-pentyl-*p*-anisic acid (V) and methyl 6-pentyl-β-resorcylate (XII). Similarly, divaricatic acid (III) from *Evernia mesomorpha* gave a methyl ester (X) that was hydrolyzed to 2-hydroxy-6-propyl-*p*-anisic acid (VI) and methyl 6-propyl-β-resorcylate (XIII). Condensation of the acid fragment (VI) from methyl divaricatate with the ester fragment (XII) from

* The essence of this paper was presented at the meeting of the Phytochemical Society of North America, held at Banff, Canada, 19–22 August 1969.

[1] C. F. CULBERSON, *Chemical and Botanical Guide to Lichen Products*, University of North Carolina Press, Chapel Hill (1969); S. HUNECK, *Progress in Phytochemistry*, Vol. 1, p. 223, Interscience Publishers, New York (1968).

[2] B. J. MOORE, *Bryologist* 71, 161 (1968).



(I) $R_1 = R_2 = C_5H_{11}$
(II) $R_1 = C_3H_7$; $R_2 = C_5H_{11}$
(III) $R_1 = R_2 = C_3H_7$
(IV) $R_1 = C_5H_{11}$; $R_2 = C_3H_7$

(V) $R_1 = C_5H_{11}$
(VI) $R_1 = C_3H_7$

(VII) $R_2 = C_5H_{11}$
(VIII) $R_2 = C_3H_7$

(IX) $R_1 = R_2 = C_5H_{11}$
(X) $R_1 = R_2 = C_3H_7$
(XI) $R_1 = C_3H_7$; $R_2 = C_5H_{11}$

(V) $R_1 = C_5H_{11}$
(VI) $R_1 = C_3H_7$

(XII) $R_2 = C_5H_{11}$
(XIII) $R_2 = C_3H_7$

(XIV) X = H
(XV) X = Cl

(XVI)

methyl perlatolate proceeded smoothly in trifluoroacetic anhydride according to the method of Neelakantan *et al.*[3] The depside ester was identical to methyl stenosporate prepared from natural stenosporic acid (mixed m.p., i.r. spectrum).

Both of the orcinol-type depside constituents extracted from *R. stenospora* in the present study are new to the family Ramalinaceae.[1,4] These *para*-depsides are closely related to compounds previously reported for the family, although the genus *Ramalina* is best known for the production of orcinol-type *meta*-depsides, compounds in which the ester linkage to the B ring connects at the 3′-position. The 2-hydroxy-6-propyl-*p*-anisic acid unit, which occurs as the A ring in stenosporic acid, also occurs as the A ring in ramalinolic and sekikaic acids, *meta*-depsides in the genus *Ramalina*. The same unit, modified only in the *O*-methylation pattern of the phenolic hydroxyls, is found as the A ring in two additional *meta*-depsides from *Ramalina*, boninic acid and paludosic acid. The B rings of several *meta*-depsides already well known in the genus are biogenetically related to 6-pentyl-β-resorcylic acid, the B ring of perlatolic and stenosporic acids.

Few lichens have been reported to produce both a depside or depsidone and its lower homologue, probably because such mixtures were very difficult to detect before the advent of TLC. The joint occurrence of perlatolic acid and imbricaric acid, the lower homologue with a C_3 side-chain on the B ring, is reported in the older literature but not confirmed in a recent

[3] S. NEELAKANTAN, R. PADMASANI and T. R. SESHADRI, *Tetrahedron* **21**, 3531 (1965).
[4] G. FOLLMANN and S. HUNECK, *Willenowia* **5**, 181 (1969).

microchemical study.[5] On the other hand, the *meta*-depside, cryptochlorophaeic acid occurs with a lower homologue, paludosic acid, in *Ramalina paludosa*.[6]

EXPERIMENTAL

The elemental analysis was performed by the Schwarzkopf Microanalytical Laboratory, Woodside, N.Y. U.S.A. Merck SiO_2-F_{254} plates were used for TLC. M.p.s are corrected.

Extraction of Ramalina stenospora *Müll. Arg.*

An air-dried sample (29·5 g) of the lichen, collected by B. J. Moore in 1967 from branches of Azalea bushes at Mt. Plymouth, Lake County, Florida, U.S.A., was extracted in a soxhlet with anhydrous, peroxide-free ethyl ether. The residue from evaporation of the filtered extract was chromatographed through a column of SiO_2, eluting first with benzene and then with increasing concentrations of ether in benzene. The compounds separated are described below in the order in which they were eluted from the column.

A small sample (2·2 mg, 0·0076%) of a crude mixture, m.p. 193·5–194·5°, of atranorin (XIV) and chloroatranorin (XV) (reported[7] for atranorin, m.p. 196° and for chloroatranorin, m.p. 208°) was tentatively identified by TLC (acetone–$CHCl_3$, 1:1 v/v) and microcrystal tests. This mixture was not studied further.

Usnic acid (XVI, 4·0 mg, 0·013%) was recrystallized once from $CHCl_3$–ethanol and identified by TLC (benzene–dioxane–HOAc, 90:25:4 v/v and hexane–Et_2O–HCO_2H, 5:4:1 v/v) and by an i.r. spectrum. The product, m.p. 199–200° (reported[7] m.p. 203–204°) was not purified further.

Perlatolic acid (I) was recrystallized from hexane, yielding colourless crystals (128 mg, 0·40%), m.p. 107·5–108·0°. A portion of the sample was recrystallized from ethanol–water, m.p. 108·5–109·0° (reported[8] 107–108°). Mass spectrum (130°): no molecular ion detected, principal fragments 238, 224, 220, 206, 182, 168, 164, 150 and 124 *m/e*. U.v. (95% ethanol): λ_{max} (log ϵ) 216 (4·64), 270 (4·26), and 307·5 (4·04) nm; λ_{min} (log ϵ) 242 (3·93) and 290 (3·95) nm. I.r. (nujol): ν 3060 (OH), 2500–2800 (acid OH), 1670 and 1645 (C=O), 1620 and 1575 (benzenoid), 1240 (s), 1215 (s), 1165 (s) and 1148 (s) cm^{-1}. NMR ($CDCl_3$): δ 0·92 (terminal methyls of alkyl side-chains, 6 H), 1·3 (intermediate methylenes of alkyl side-chains, 12 H), 3·0 (aryl-substituted methylenes of alkyl side-chains, 4 H), 3·7 (—OCH_3, singlet, 3 H), 6·36 (aryl H of ring B, 2 H), 6·66–6·75 (aryl H of ring A, 2 H), and 11·2 (—O*H*, 2 H) ppm. The i.r. spectrum was identical to one of perlatolic acid extracted from *Cladonia evansii* and a mixed m.p. was not depressed.

A small intermediate fraction that contained both perlatolic acid and stenosporic acid was collected from the column. This fraction was not purified.

Stenosporic acid was recrystallized from hexane and from ethanol–water, yielding colorless needles (110 mg, 0·37%), m.p. 112–113°. (Found: C, 66·38; H, 6·61. $C_{23}H_{28}O_7$ required: C, 66·33; H, 6·78%.) Mass spectrum (130°): M^+ 416 and principal fragments at 224, 210, 206, 192, 178, 168, 164, 150, and 135 *m/e*. U.v. (95% ethanol): λ_{max} (log ϵ) 215·5 (4·64), 270·5 (4·27), and 307·5 (4·06) nm; λ_{min} (log ϵ) 242 (3·96) and 290·5 (3·98) nm. I.r. (nujol): ν 3060 (OH), 2500–2800 (acid OH), 1675 and 1650 (C=O), 1620 and 1573 (benzenoid), 1245 (s), 1215 (s), 1195 (s), 1165 (s), and 1145 (s) cm^{-1}. NMR ($CDCl_3$): δ 0·95 (terminal methyls of alkyl side-chains, 6 H), 1·45 (intermediate methylenes of alkyl side-chains, 8 H), 3·0 (aryl-substituted methylenes of alkyl side-chains, 4 H), 3·85 (—OCH_3, 3 H), 6·43 (aryl H of ring B, 2 H), 6·66–6·87 (aryl H of ring A, 2 H), and 11·2 (O*H*, 2 H) ppm. A small sample of stenosporic acid (24 mg) was converted to the methyl ester, m.p. 35–36° (from hexane). Mass spectrum (110°): M^+ 430·1994 (Calc. for $C_{24}H_{30}O_7$, 430·1983) and principal fragments at 238, 206, 193, 182, 178, 163, and 150 *m/e*. U.v. (95% ethanol): λ_{max} 215, 271 and 307 nm; λ_{min} 241 and 293·5 nm. I.r. (nujol): ν 1670 (C=O), 1620 and 1580 (benzenoid), 1245 (s), 1213 (s), 1163 (s), and 1148 (s) cm^{-1}.

Synthesis of Methyl Stenosporate by Condensation

Divaricatic acid (III) was extracted from *Evernia mesomorpha* collected from dead trees in a bog at Big Arbor, Vitae Lake, Vilas County, Wisconsin, by W. L. Culberson in July 1953. The lichen (38 g) yielded usnic acid (1·1%) and divaricatic acid (6·7%), which was recrystallized from dilute ethanol, m.p. 129·0–129·5° (reported[9] m.p. 137° from dilute ethanol). Repeated recrystallizations did not raise the m.p. to the reported value although all tests indicated the product to be divaricatic acid (III). Mass spectrum (130°): M^+ 388 and principal fragments at 210, 196, 193, 192, 178, 164, 150 and 135 *m/e*. U.v. (95% ethanol): λ_{max} (log ϵ) 215

[5] W. L. Culberson and C. F. Culberson, *Contrib. U.S. Nat. Herb.* **34**, 449 (1968).
[6] C. F. Culberson, *Bryologist* **70**, 397 (1967).
[7] Y. Asahina and S. Shibata, *Chemistry of Lichen Substances*, Japan Society for the Promotion of Science, Tokyo (1954).
[8] Y. Asahina and I. Yosioka, *Chem. Ber.* **70**, 1823 (1937).
[9] Y. Asahina and M. Hiraiwa, *Chem. Ber.* **70**, 1826 (1937).

(4·61), 270·5 (4·28), and 307·5 (4·06) nm; λ_{min} (log ϵ) 241·5 (3·96) and 290·5 (3·97) nm. I.r. (nujol): ν 2500–2800 (acid OH), 1680 and 1635 (C=O), 1620 and 1590 (benzenoid), 1245 (s), 1215 (s), 1160 (s) and 1095 (s) cm^{-1}. The methyl ester X, prepared with CH_2N_2 in ether at 0°, was recrystallized from hexane, m.p. 74·0–75·5° (reported[7] m.p. 76°). Mass spectrum (100°): M^+ 402 and principal fragments at 210, 193, 178 and 150 *m/e*. U.v. (95% ethanol): λ_{max} 215, 271, and 307 nm; λ_{min} 241·5 and 293·5 nm. I.r. (nujol): ν 1670 (C=O), 1620 and 1590 (benzenoid), 1260 (s), 1215 (s), and 1148 (s) cm^{-1}.

Methyl divaricatate (231 mg) was hydrolyzed with conc. H_2SO_4 at 0° to give 2-hydroxy-6-propyl-*p*-anisic acid (VI) and methyl-6-propyl-β-resorcylate (XIII). The acid (VI), which was separated by its solubility in 5% aq. $NaHCO_3$, was further purified by preparative TLC and then recrystallized from methanol–water yielding colorless crystals (39 mg), m.p. 144–150° d (reported[9,10] m.p. 151° from ethanol–water and m.p. 157° from benzene). Mass spectrum (130°): M^+ 210 and principal fragments at 192, 164 and 135 *m/e*. U.v. (95% ethanol): λ_{max} 219, 267, 301·5 nm; λ_{min} 237·5 and 281·5 nm. I.r. (nujol): ν 2500–2800 (acid OH), 1575–1660 (strong unresolved C=O and benzenoid bands), 1268 (s), 1215 (m) and 1170 (m) cm^{-1}. The fraction insoluble in 5% aq. $NaHCO_3$, was recrystallized from methanol–water yielding a mixture (20 mg) of acid (VI) and the methyl ester (XIII), which was separated by preparative TLC (benzene–dioxane–HOAc, 90:25:4 v/v). The ester (XIII) was recrystallized from methanol–water, m.p. 78–80° (reported[8] m.p. 78°). U.v. (95% ethanol): λ_{max} 218, 266·5, and 303 nm; λ_{min} 240·5 and 286 nm. I.r. (nujol): ν 3300 (OH), 1640 (C=O), 1580 and 1550 (benzenoid), 1330 (s), 1270 (s), 1265 (s), and 1248 (s) cm^{-1}. Mass spectrum: M^+ 210 and principal fragments at 178, 150 and 122 *m/e*.

Perlatolic acid (II) was isolated from *C. evansii* collected on Atlantic Beach, Carteret County, North Carolina, July 1960, by W. L. Culberson (No. 10,119). The lichen (340 g) yielded atranorin (0·43%), m.p. 197–198·5° from $CHCl_3$–petroleum ether (reported[7] m.p. 196°) and perlatolic acid (0·28%), m.p. 108·0–108·5° from ethanol–water. Mass spectrum (130°): M^+ 444 (very small) and principal fragments at 238, 224, 220, 206, 182, 168, 150 and 124 *m/e*. U.v. (95% ethanol): λ_{max} (log ϵ) 216 (4·64), 270 (4·26), and 307·5 (4·04) nm; λ_{min} (log ϵ) 242 (3·93) and 290 (3·95) nm. I.r. (nujol): ν 3060 (OH), 2500–2800 (acid OH), 1670 and 1645 (C=O), and 1620 and 1575 (benzenoid) cm^{-1}.

Methyl perlatolic (IX) was prepared with CH_2N_2 and recrystallized from methanol–water, m.p. 50–51° (reported[8] m.p. 48–49°). Mass spectrum (110°): M^+ 458 and principal fragments at 238, 221, 206, 182, 178, and 150 *m/e*. U.v. (95% ethanol): λ_{max} 216, 271, and 307 nm; λ_{min} 242 and 293·5 nm. I.r. (nujol): ν 1670 (C=O), 1620 and 1575 (benzenoid), 1248 (s), 1215 (s), 1165 (s), and 1145 (s) cm^{-1}.

Methyl perlatolate was hydrolyzed as described for methyl divaricatate above. The acid fraction, 2-hydroxy-6-pentyl-*p*-anisic acid (V), gave colorless crystals from methanol–water, m.p. 126–128·5° (reported[8] m.p. 126°). Mass spectrum (120°): M^+ 238 and principal fragments at 220, 192, 182, 164, and 135 *m/e*. U.v. (95% ethanol): λ_{max} 219, 262, and 302 nm; λ_{min} 237·5 and 281·5 nm. I.r. (nujol): ν 2500–2800 (acid OH), 1645 (C=O), 1620 (benzenoid), 1265 (s), 1210 (m), and 1160 (s) cm^{-1}. The ester fraction, methyl 6-pentyl-β-resorcylate (XII), was purified by preparative TLC and recrystallization from hexane to give a colorless solid, m.p. 77–78·5° (reported[11] m.p. 78°). Mass spectrum (100°): M^+ 238 and principal fragments at 206, 182, 178, 150, and 122 *m/e*. U.v. (95% ethanol): λ_{max} 218, 266, and 302·5 nm; λ_{min} 240·5 and 286 nm. I.r (nujol): ν 3330 (OH), 1650 (C=O), 1625 and 1585 (benzenoid), 1270 (s), 1223 (m) and 1175 (m) cm^{-1}.

A mixture of 2-hydroxy-6-propyl-*p*-anisic acid (VI, 25 mg, 0·11 millimoles) and methyl 6-pentyl-β-resorcylate (XII, 22 mg, 0·11 millimoles) in trifloroacetic anhydride (1·5 ml) was maintained at 50° for 1 hr. The reaction mixture was cooled, poured into ice water and extracted with ether. The ether solution was washed with 5% aq. $NaHCO_3$ and water and then dried. The principal product was separated by two successive preparative-layer separations (hexane–Et_2O–HCO_2H, 5:4:1 v/v) and finally by recrystallization from hexane. The compound (m.p. 36·5–37·5°) was identical to methyl stenosporate (XI) prepared from stenosporic acid (mixed m.p., i.r. spectra, and TLC).

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[10] Y. ASAHINA and H. AKAGI, *Chem. Ber.* **68**, 1130 (1935).
[11] Y. ASAHINA and F. FUZIKAWA, *Chem. Ber.* **68**, 634 (1935).