Chemical Studies on the Lichen. I. The Structure of Isolecanoric Acid, a New *ortho*-Depside Isolated from *Parmelia tinctorum* Despr.

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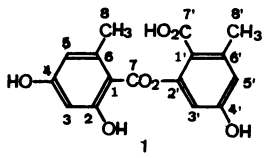
Synopsis. A new *ortho*-depside, isolecanoric acid was isolated from *Parmelia tinctorum* Despr. and 2-(2,4-dihydroxy-6-methylbenzoyloxy)-4-hydroxy-6-methylbenzoic acid was assigned to this substance from studies on the hydrolysis products and from analyses of the ^1H and ^{13}C NMR spectra. This substance is the first *ortho*-depside isolated from the genus Lichen.

Lichens produce many unusual secondary metabolites ("lichen substance") hardly found in other plants.[1] The major phenolic substances, among them are divided into classes of *meta*-depside, *para*-depside, depsidone, etc, but *ortho*-depside has not been isolated. From a lichen, *Parmelia tinctorum* Despr. (Umenoki-goke in Japanese) *para*-depsides, atranolin and lecanoric acid have been isolated.[2]

In our studies on the constituents of *Parmelicaeae* we found the presence of a new depside in *P. tinctorum* Despr. besides them and named it isolecanoric acid (**1**). In this paper we wish to report the isolation and structure of **1**.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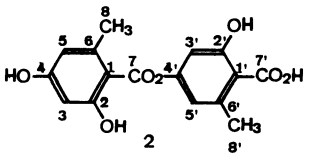
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The lichen which occurred on stone walls was collected and extracted with acetone followed by reextraction with methanol. The later extract was subjected to chromatography on a column of Sephadex LH-20 eluting with methanol. The last effluent gave **1** as colorless powder, mp >300 °C (colored at 220 °C) in 0.03% yield from wet plant. Compound **1** is optically inactive and from its elemental analyses was suggested the formula $C_{16}H_{14}O_7$. The UV spectrum of **1** in ethanol shows the absorption maxima at 211, 269, and 303 nm, which was similar to that of lecanoric acid (**2**) in the spectral pattern.[2]

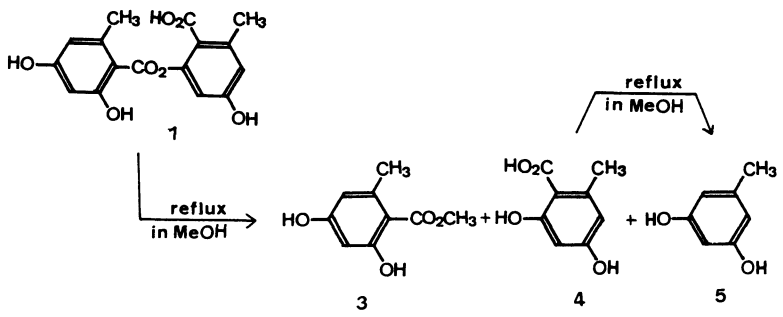
The ^1H NMR spectrum of **1** in a mixture of $CDCl_3$ and CD_3OD shows the small difference in the chemical shifts as compared with that of **2**.[3] And the spectrum of **1** shows the signals ascribed to two aromatic methyl protons at δ 2.56 (3H, s) and 2.62 (3H, s), and to the protons attached to two 1,2,3,5-tetrasubstituted benzene rings at δ 6.22 (1H, d, J=2 Hz) and 6.30 (1H, d, J=2 Hz), and 6.48 (1H, d, J=2.1 Hz) and 6.54 (1H, d, J=2.1 Hz).

Treatment of **1** with diazomethane in methanol gave a tetramethyl derivative of **1** as colorless needles, mp 154—155 °C [MS (20 eV): M+ 374], and its ^1H NMR spectrum in $CDCl_3$ shows the signals ascribed to three methoxyl groups and to one

Table 1. Carbon-13 NMR Chemical Shifts of **1**, **2**, **3**, and **4** in DMSO-d_6

Compound	1	2	3	4
1	107.6	107.7	107.0	—
2	160.7[a]	160.7[a]	161.2[a]	—
3	100.6	100.6	100.4	—
4	161.4[a]	161.4[a]	161.5[a]	—
5	110.1	110.2	110.3	—
6	140.3	140.0	141.1	—
7	167.6	167.4	170.3	—
8	21.7	21.3	22.2	—
1′	116.3	116.0	—	105.0
2′	151.1	159.3	—	161.8
3′	107.1	107.5	—	100.6
4′	165.6	152.6	—	164.5
5′	112.5	114.9	—	110.9
6′	142.2	140.7	—	142.9
7′	171.8	170.9	—	173.3
8′	23.0	21.7[b]	—	23.5
CH_3	—	—	51.6	—

In parts per million downfield from tetramethylsilane. Values bearing the same superscript may be interchanged.



1 3 4 5

Scheme 1.

methoxycarbonyl group at δ 3.82 (6H, s), 3.86 (3H, s), and 3.90 (3H, s).

After having been refluxed in methanol followed by separation with a column of Sephadex LH-20 in the same manner as described above, compound **1** gave methyl orsellinate (**3**) and orsellinic acid (**4**) in a molar ratio of ca. 1:1 and a trace amount of orcinol (**5**).[4] Since **4** gives **5** by further treatment with boiled methanol, compound **5** should be formed by decarboxylation of **4** after methanolysis of **1**.

In the ^{13}C NMR spectra of **1**, **2**, **3**, and **4** in DMSO-d_6 as shown in Table 1, the signals ascribed to the carbons at the left hand of **1** (C-1—C-8) show the good agreement with those of **2**, whereas the signals at the right hand (C-1′—C-8′) show a little difference between **1** and **2**. The 2′-carbon bearing a benzoyloxy group in **1** appears at a field higher by 10.7 ppm than that of **4**, and 1′-, 3′-, and 5′-carbons in **1** (*ortho*- and *para*-potitions to C-2′) appear at fields lower by 11.3, 6.5, and 1.6 ppm respectively than those of **4**. The 4′-carbon in **2** appears at a field higher by 11.9 ppm than that of **4**, and 1′-, 3′-, and 5′-carbons in **2** appear at fields lower by 11.0, 6.9, and 4.0 ppm respectively than those of **4**. These results indicate that the ester bond in **1** should be linked at the 2′-position.

These ^1H NMR and ^{13}C NMR spectral data and all experimental results described above support the structure of isolecanoric acid (**1**) to be 2-(2,4-dihydroxy-6-methylbenzoyloxy)-4-hydroxy-6-methylbenzoic acid.

Compound **1** is the first *ortho*-depside isolated from the genus Lichen. Moreover we found the presence of **1** in *P. clavurifera* Räs. (Matsuge-goke in Japanese)[5] as a minor constituents with **2**.

Experimental

All melting points are uncorrected. The ^1H NMR spectra were measured with a Varian EM-390 90 MHz NMR spectrometer. The ^{13}C NMR spectra were measured with a JEOL JNM-PFT-60 NMR spectrometer at 15.04 MHz. Chemical shifts were expressed by δ value (ppm) from tetramethylsilane as internal standard. The IR spectra were measured with a Hitachi EPI-G3 spectrophotometer and the UV spectra were measured with a JASCO UVIDEC-510 spectrophotometer.

Isolation of Isolecanoric Acid (1). The lichen (300 g) which occurred on stone walls was collected and extracted with acetone (1500 cm³) followed by reextraction with methanol (1500 cm³) at room temperature for a week. The later extract was condensed to a syrup under reduced pressure and the syrup was subjected to chromatography on a column of Sephadex LH-20 (3.5×120 cm) eluting with methanol. The last effluent gave **1** as colorless powder after recrystallization from methanol: Mp >300 °C (colored at 220 °C); yield, 90 mg (0.03% from wet plant); UV (EtOH) 211 (log ε 4.56), 269 (4.21), and 303 nm (3.99); ^1H NMR (CD_3OD–$CDCl_3$) δ=2.56 (3H, s, Ar–CH_3), 2.62 (3H, s, Ar–CH_3), 6.22 (1H, d, *J*=2 Hz, Ar–H), 6.30 (1H, d, *J*=2 Hz, Ar–H), 6.48 (1H, d, *J*=2.1 Hz, Ar–H), and 6.54 (1H, d, *J*=2.1 Hz, Ar–H); ^{13}C NMR (DMSO-d_6) δ=21.7 (q), 23.0 (q), 107.6 (s), 110.1 (d), 112.5 (d), 116.3 (s), 140.3 (s), 142.2 (s), 151.1 (s), 160.7 (s), 161.4 (s), 165.6 (s), 167.6 (s), and 171.8 (s). Found: C, 58.93; H, 4.78%. Calcd for $C_{16}H_{14}O_7 \cdot 0.5H_2O$: C, 58.72; H, 4.63%.

Treatment of **1** (10 mg) in methanol (10 cm³) with an ether solution containing diazomethane followed by chromatography through silica gel with hexane–ethyl acetate (4:1) afforded a tetramethyl derivative of **1** as colorless needles after recrystallization from acetone–hexane: Mp 154—155 °C; yield, 4 mg; MS (20 eV): M^+ 374; ^1H NMR ($CDCl_3$) δ=2.30 (3H, s, Ar–CH_3), 2.42 (3H, s, Ar–CH_3), 3.82 (6H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 6.37 (2H, s, Ar–H), and 6.67 (2H, brs, Ar–H). Found: *m/z* 374.1374. Calcd for $C_{20}H_{22}O_7$: M, 374.1365.

Methanolysis of 1. Compound **1** (44 mg) was dissolved in methanol (50 cm³) and the solution was refluxed for 20 h. The mixture was concentrated and was subjected to chromatography on a column of Sephadex LH-20 in the same manner as described above, and afforded methyl orsellinate (**3**) (23 mg; mp 142—144 °C), orsellinic acid (**4**) (22 mg; mp 180—183 °C), and orcinol (**5**) (*ca.* 2 mg; mp 104—106 °C). Further treatment of **4** with boiled methanol gave **5**. These substances **3**, **4**, and **5** were proved to be identical with authentic samples which were prepared by methanolysis of lecanoric acid (**2**) in the same manner.

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References

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2) Y. Asahina, *Yakugaku Zasshi*, **47**, 533 (1926); G. B. Ahmann and A. Mathey, *Bryologist*, **70**, 93 (1967).

3) **2**: ^1H NMR ($CDCl_3$–CD_3OD) δ=2.57 (6H, s, Ar–CH_3), 6.21 (1H, d, *J*=3 Hz, Ar–H), 6.28 (1H, d, *J*=3 Hz, Ar–H), 6.60 (1H, d, *J*=3 Hz, Ar–H), and 6.67 (1H, d, *J*=3 Hz, Ar–H).

4) Y. Asahina and M. Yanagita, *Ber.*, **66**, 36 (1933); Y. Asahina and H. Akagi, *ibid*, **68**, 1130 (1935); G. Koller and J. Hamburg, *Monatsh*, **65**, 373 (1935).

5) From this lichen only salazinic acid was identified by means of microcrystal test. See, C. F. Culberson, "Chemical and Botanical Guide to Lichen Products," University of N. Carolina Press, Chapel Hill (1969), p. 413 and the references cited therein.