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CRUSTINIC ACID, A TRIDEPSIDE FROM *UMBILICARIA CRUSTULOSA**

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Key Word Index—*Umbilicaria crustulosa*; Umbilicariaceae; crustinic acid; tridepside; lichen substance.

Abstract—The structure of crustinic acid extracted from *Umbilicaria crustulosa* has been elucidated as 4,6-dihydroxy-3-carboxy-2-methylphenyl lecanorate by spectroscopic and chemical means.

INTRODUCTION

In a study of the lichen genus *Umbilicaria* Hoffm. Posner *et al.* [1] detected the presence of a new lichen substance, crustinic acid, in the species *Umbilicaria cinereorufescens* (Schaer.) Frey, *U. crustulosa* (Ach.) Frey. *U. krempelhuberi* Müll. Arg., and *U. spodochroa* (Ach.) Frey and found that this acid was probably a tridepside. We have now established the structure of crustinic acid as 4,6-dihydroxy-3-carboxy-2-methylphenyl lecanorate (**1**) in the following manner.

RESULTS AND DISCUSSION

The UV spectrum of crustinic acid exhibited $\lambda_{\max}^{\mathrm{MeOH}}$ (log ε) 226 (4.19), 263 (4.26), 300 (4.08), and 313 nm (3.90), typical of a depside. The FAB mass spectrum of **1** showed a $[M+H]^+$ peak at m/z 485 (100%), corresponding to the formula $C_{24}H_{20}O_{11}$ (484), as well as fragment peaks at *m/z* 301 (72%, a) and *m/z* 151 (36%, b) confirming the presence of two orsellinic acid moieties in the molecule.

The singlets at δ2.51 (3H), 2.62 (3H), and 2.71 (3H) in the ^{1}H NMR spectrum (250 MHz, acetone-d_6) of **1** can be assigned to the three methyl groups, the two pairs of doublets at δ6.31, 6.40 (2H, $J=2.4$ Hz) and δ6.88, 6.90 (2H, $J=2.2$ Hz) to the aromatic protons of the S- and Z-rings and the singlet at δ6.47 (1H) to H-3″-of the A-part of the molecule.

Brief treatment of crustinic acid with ethereal diazomethane at 0° yielded methyl 4″-*O*-methylcrustinate (**2**). The FAB mass spectrum of **2** showed a $[M+H]^+$ peak at *m/z* 513, corresponding to the formula $C_{26}H_{24}O_{11}$ (512). The ^{1}H NMR spectrum (200 MHz, $CDCl_3$) of the compound exhibited the following signals: δ2.39, 2.62, 2.72 (3 × 3H, 3 × *s*, 3 × Me), 3.84 (3H, *s*, OMe-4″), 3.96 (3H, *s*, CO_2Me), 6.33 (2H, *s*, H-3′, H-5′), 6.48 (1H, *s*, H-3″), 6.70 (1H, *d*, $J=2.1$ Hz), 6.77 (1H, *d*, $J=2.3$ Hz) (H-3, H-5), 11.1, 11.3, 11.8 (3 × 1H, 3 × *s*, 3 × OH).

The final proof for the structure of crustinic acid came from hydrolysis of **2** with conc sulphuric acid. This yielded orsellinic acid (**3**) and a phenol identical with synthetic methyl 5-hydroxyeverninate (**4**) [2]: ^{1}H NMR (200 MHz, $CDCl_3$): δ2.45 (3H, *s*, Me-6), 3.91 (3H, *s*, OMe-4), 3.93 (3H, *s*, CO_2Me-1), 5.35 (1H, *s*, OH-5), 6.37 (1H, *s*, H-3), and 12.2 (1H, *s*, OH-2). The 4-position of the methoxyl group followed from the strong NOE between the methoxyl group and the proton at C-3. The isomeric structure methyl 3-hydroxyeverninate could be excluded because there was no NOE between the 6-methyl group and a proton in an ortho-position. Crustinic acid is the first representative of a mixed *para*- and *meta*-depside.

EXPERIMENTAL

Crustinic acid (**1**). *Umbilicaria crustulosa* (Ach.) Frey (11.8 g, Switzerland, Ticino, Maggia Valley, near Fusio on granite rocks, 1300 m a.s.; leg. S. Huneck, 27.10.1991, det. B. Posner, 1991) was pulverized and extracted with Et_2O (100 ml) for 10 hr. The extract was concd to a vol. of 5 ml, the resulting ppt (mainly gyrophoric acid) removed by filtration, the filtrate evapd to dryness (under vacuum) and the residue recrystallized twice from MeOH–H_2O and finally from Et_2O to yield crustinic acid (0.158 g, 1.34%) as needles, mp 178–180° (dec.). NaOCl + red. $C_{24}H_{20}O_{11}$ (484.40). $R_f=0.18$ (Merck silica gel, *n*-hexane–Et_2O–HCO_2H, 15:10:3). IR $\nu_{\max}^{\mathrm{KBr}}$ 490, 520, 555, 608, 680, 740, 800, 838, 880, 965, 980, 1050, 1075, 1140, 1180, 1250, 1310, 1375, 1420, 1440, 1500, 1580, 1620, 1640, 1660, 2930, 2980, 3180, 3380 cm^{-1}. ^{13}C NMR (62.76 MHz, acetone-d_6): δ15.68, 23.11, 24.31 (3 × Me), 101.93, 102.57, 109.61, 113.06, 117.86 (5 × =CH–), 129.35, 134.25, 134.52, 134.8, 136.2, 140.6, 144.0, 145.0, 155.4, 163.2, 164.4 (13 × =C=), 166.8, 170.8, 174 (3 × –CO–).

Methyl 4″-O-methylcrustinate (**2**). Prepd by treating crustinic acid (0.096 g) in Et_2O (15 ml) with the corresponding amount of CH_2N_2 in Et_2O at 0° for 30 sec. After recrystallization from $CHCl_3$–MeOH, **2** was obtained as

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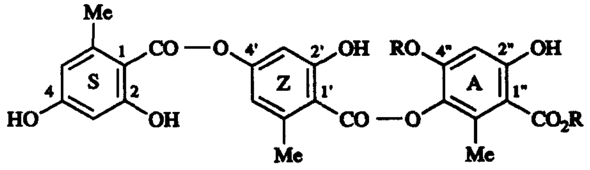
*In memory of the late Dr Ove Almborn.



R
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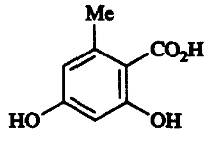
2 Me



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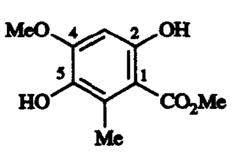

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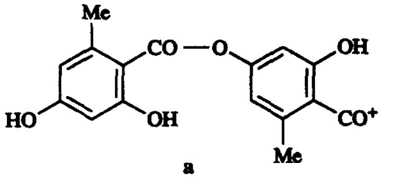

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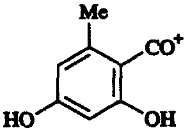

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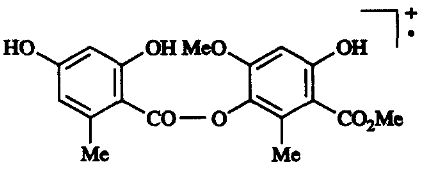

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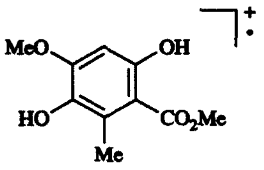

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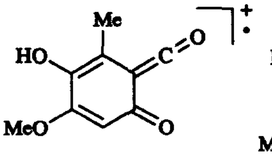

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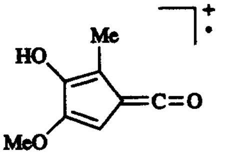

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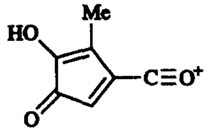

g

needles, mp 125–126°. $FeCl_3$ (EtOH)+wine red. $C_{26}H_{24}O_{11}$ (512.45). $R_f = 0.29$ silica gel, *n*-hexane–Et_2O–HCO_2H, 15:10:3). IR $\nu_{\max}^{\text{KBr}}$ 490, 520, 550, 608, 690, 785, 820, 880, 950, 980, 1030, 1070, 1130, 1190, 1240, 1302, 1325, 1375, 1440, 1575, 1610, 1655, 1675, 2850, 2890, 3410 cm^{-1}. FAB-MS, *m/z* 513 (8%, $[M+H]^+$), 241 (22), 215 (17), 151 (100); EI-MS, *m/z* 362 (6%, c), 212 (23, d), 180 (41, e), 151 (100, b).

Hydrolysis of methyl 4″-O-methylcrustinate (**2**). A soln of **2** (0.05 g) in conc H_2SO_4 was kept at 0° for 15 min and then poured into crushed ice (5 g). The ppt. was extracted with Et_2O (3 × 10 ml) and the extract sepd into an acid and a phenolic fr. by shaking with a soln of $NaHCO_3$ in H_2O (10%). After acidification with H_2SO_4 (10%) and recrystallization from MeOH–H_2O, the acid fr. gave orsellinic acid (**3**) (0.0038 g) as needles, mp 192–194° (dec.). NaOCl+red. R_f 0.40 (silica gel, C_6H_6–dioxane–HOAc, 90:25:4). After recrystallization from MeOH–H_2O, the phenolic fr. yielded rhombics and plates of mp 141–143°, identical in all respects with synthetic methyl 5-hydroxyeverninate (**4**). The crystals showed a characteristic green colour with NaOCl. $C_{10}H_{12}O_5$ (212.20). R_f 0.56 (silica gel, C_6H_6–dioxane–HOAc 22.5:6.25:1). IR $\nu_{\max}^{\text{KBr}}$ 450, 470, 530, 565, 605, 655, 720, 780, 820, 870, 940, 1025, 1052, 1100, 1160, 1195, 1220, 1280, 1330, 1375, 1420, 1440, 1475, 1485, 1620, 1640, 2950, 3465 cm^{-1}. EI-MS, *m/z* 212 (33%, $[M]^+$), 180 (100, e), 152 (8, f), 137 (18, g), 109 (8, g − CO), 81 (4, g − 2 CO), 69 (11).

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