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Note

Chemical study on Garhwal Himalayan Lichen: Usnea emidotteries

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Usnea species are medicinally important, so we take unexplored species, *Usnea emidotteris*. Seven components 2-hydroxy-3-methoxy-4, 6-dimethyl ethyl benzoate, 2, 4-dihydroxy-3, 6-dimethyl ethyl benzoate, 2-hydroxy-4-methoxy-3, 6-dimethylbezoic acid, usnic acid, barbatic acid, diffractaic acid and evernic acid are isolated first time from *Usnea emidotteries* and identified from their physicochemical data.

Keywords: Lichen, Usnea emidotteris, usnic acid, barbatic acid, diffractaic acid, evernic acid

IPC Code: Int. Cl.^{8} C07C

Lichens are known as valuable plant resources and usnic acid, an antibiotic found in lichen species is reported to be antineoplastic[1]. *U. campestris* is known to be antifungal and antibacterial[2]. Lichen acids isolated from *U. longissima* are growth inhibitors[3]. Sterols have been isolated from *U. longissima*[4]. *U. diffracta* is known to be analgesic, antipyretic[5] and shows anti-inflammatory activity[6]. *U. misaminensis* is reported to have smooth muscle relaxant activity[7]. This is the first report of the chemical analysis of *Usnea emidotters* Mot.

Dried and powdered plant material (1 kg) was extracted with ethanol (95%). The concentrated mass was extracted by light petroleum (60-80)°C. Six (**1-6**) compounds were isolated from petroleum extract while compound **7** was isolated from petroleum free mass.

Results and Discussion

In compound **1**, the EI-mass spectrum revealed M$^+$ at *m/z* 224 corresponding to molecular formula $C_{12}H_{16}O_4$. DEPT spectrum showed the presence of six quaternary, one methine, one methylene and four methyl carbons. The ^{13}C spectrum shows signal at 172.1 ppm which corresponds to the carbonyl carbon of an ester group. ^{1}H NMR spectrum indicated the presence of an ethyl ester group, δ 4.38(2H, q, *J*=7 Hz, C-1′) and 1.40(3H, t, *J*=7 Hz, C-2′). The δ 3.80 (3H, s, 10-OCH_3) can be assigned to the protons of the methoxy group bound to an aromatic ring. In HMBC spectrum C-10 methoxyl hydrogen showed its long range correlation with C-3 affirming the attachment of methoxyl group at C-3 (^{13}C singlet at 116.2). Proton at C-5 showed its correlation with C-4, C-6 & 3J_{CH} correlations with C-9, C-8, C-3 & C-7. Compound **1** was identified as 2-hydroxy 3-methoxy-4, 6-dimethyl ethyl benzoate.

In compound **2**, the EI-mass spectrum revealed ion peak at *m/z* 210 corresponding to molecular formula $C_{11}H_{14}O_4$. IR KBr cm^{-1}: 1736(ester linkage), 1618, 1578, 1508, 1477(semicircle stretching of aromatic nucleus), 893. The combined ID ^{1}H NMR and HMQC indicated the presence of two phenolic protons, one upfield at δ 5.1 ppm and the second downfield proton at 12.12 ppm affirming its proximity with carbonyl oxygen of an ester group. The signal at δ 172.4 corresponds to the presence of a carbonyl carbon of an ester group. The downfield value of 4.38 (2H, q, *J*=7.1 Hz, C-1′) showed its direct linkage with carbonyl carbon, while *J* values for both, two protons quartet and three-protons triplet, 7 Hz, substantiate an ethyl ester group. The presence of one proton singlet at δ 6.2 indicates its attachment with C-5 carbon. The long range C-H correlation of C-5 aromatic proton were observed in HMBC spectrum ($^2J_{CH}$ and $^3J_{CH}$ correlations) with C-4; C-8; C-1; C-9. The structure was further confirmed by HMBC spectrum. Compound **2** on hydrolysis with dil acid gave a compound which revealed EI-MS ion peaks at *m/z* 182 corresponding to molecular formula $C_9H_{10}O_4$. The other ion peaks observed at *m/z* 164, 138, 136, 123 and 109. IR KBr cm^{-1}: 3400, 1635. The ^{1}H NMR spectrum showed signals at δ 2.65 (3H, s, 3-CH_3), 2.49 (3H, s, 6-CH_3) corresponding to two methyl groups. δ 6.34 (1H, s, 5-H) indicated penta substituted benzene ring. The hydrolyzed product was characterized as 2,4-dihydroxy-3,6-dimethyl benzoic acid[3].

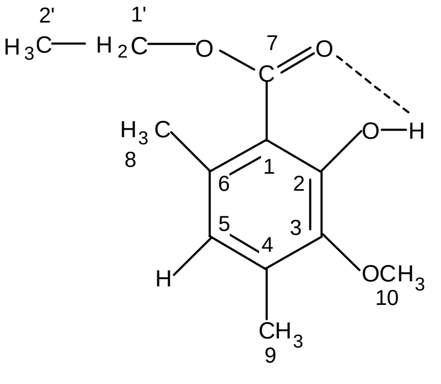
The EI-mass spectrum of compound **3** revealed ion peak at *m/z* 196 corresponding to molecular formula $C_{10}H_{12}O_4$. DEPT showed the presence of six quaternary, one methine and three methyl carbons. The protons having values of δ 2.6 (3H, s, 10-CH_3)

and 2.0 (3H, s, 8-CH_3) corresponded with methyl groups directly attached with benzene nucleus. ^{13}C value of 55.7 ppm relates with the presence of methoxy group. The ^{13}C NMR spectrum showed signal at 174.4 ppm corresponding to the carbon of the carbonyl group of a carboxylic acid. The above data were all consistent with the reported data[3] and it was identified as 2-hydroxy 4-methoxy-3, 6-dimethyl benzoic acid.

The EI-mass spectrum of compound **4** revealed the peak at *m/z* 344 corresponding to the molecular formula $C_{18}H_{16}O_7$ and ions at *m/z* 329, 260, 233 displayed the fragmentation pattern of the parent and fragment ions as reported[8]. ^1H NMR spectrum showed signal of an aromatic proton at δ 6.2 (1H, s). The three phenolic protons gave the downfield signal at δ 11.3 (1H, s, 9-OH), 13.30 (1H, s, 7-OH) 18.80 (1H, s, 3-OH) showing the intramolecular hydrogen bonding (chelation). The structure was finally established by the ^{13}C NMR spectrum using DEPT mode with flip angles at 45°, 90° and 135° to establish multiplicities. Compound **4** was identified as usnic acid by the study of its IR, MS, HMQC, HMBC and COSY. Circular dichroism of compound **4** showed a positive curve. Final identification of compound **4** was established as (+) usnic acid by comparison of m.p. 203-204°C [lit. 203-204°C] (ref. 9).

The EI-mass spectrum of compound **5** revealed ion peak at *m/z* 360 corresponding to molecular formula $C_{19}H_{20}O_7$. The ^1H NMR spectrum showed signals at δ 2.06 (3H, s, 10-CH_3), 2.09 (3H, s, 9′-CH_3), 2.62 (3H, s, 8′-CH_3), 2.71(3H, s, 8-CH_3) indicating the presence of four methyl groups. The presence of a peak at δ 3.95 (3H, s, 4-OCH_3) corresponds to the presence of methoxy group. The aromatic protons singlet occurred at δ 6.6 (1H, s, 5-H) and 6.7 (1H, s 6′-H) indicate the presence of pentasubstituted benzene rings. The downfield value of δ 11.5 (1H, s, 6-OH) displays the presence of chelated proton. ^{13}C NMR spectrum showed signal at δ 174.6 ppm corresponding to the carbon of the carboxylic acid while the signal at 174.4 corresponds to the presence of a carbonyl carbon of an ester group. On comparing mp 181-82°C [lit. m.p. 181-83°C] (ref. 3) and other physical data, the compound **5** was identified as 2-hydroxy-4-methoxy-3,6-dimethyl benzoic acid 4′-carboxy-3′-hydroxy-2′,5′-dimethylphenyl ester (barbatic acid).

The compound **5** was hydrolyzed to give fragment A and B. The spectral data of fragment A was similar to that of compound **3** identified as 2-hydroxy-4-



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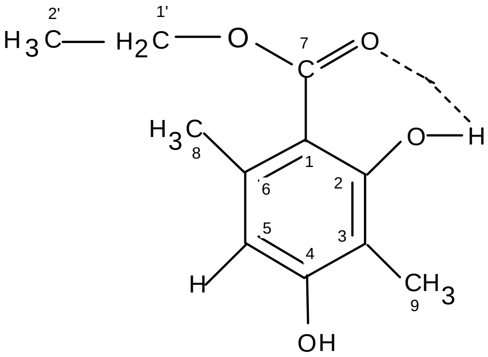

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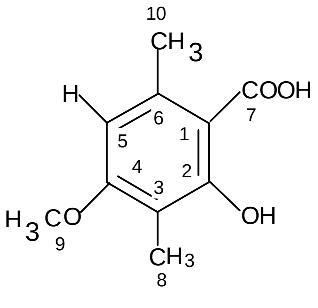

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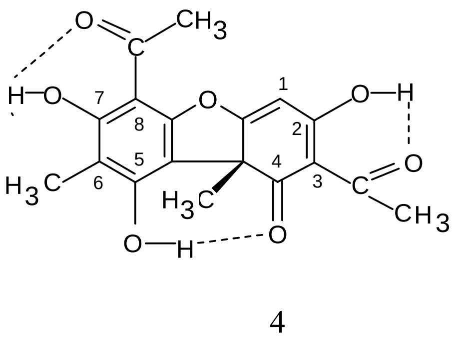

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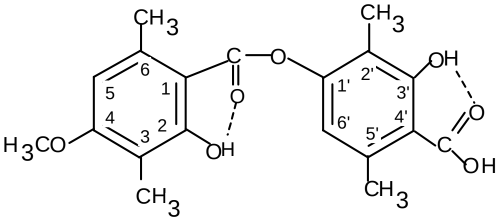

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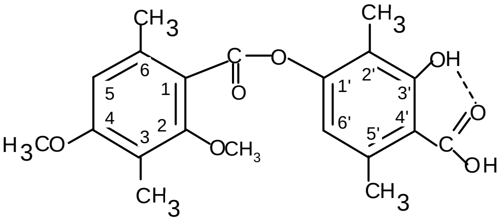

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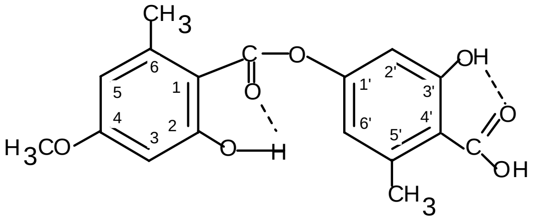

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methoxy-3,6-dimethyl benzoic acid and fragment B was characterized as 2,4-dihydroxy-3,6-dimethylbenzoic acid [3].

In compound **6**, the EI- mass spectrum revealed ion peak at *m/z* 374 corresponding to molecular formula $C_{20}H_{22}O_7$. The ^{1}H NMR spectrum showed signals at δ 2.13(3H, s, 3-CH$_3$), 2.14(3H, s, 2′-CH$_3$), 2.45 (3H, s, 6-CH$_3$), 2.62 (3H, s, 5′-CH$_3$) indicating the presence of four methyl groups in the molecule. The signal at δ 3.84 (3H, s) is due to the attachment of methoxy group with the aromatic ring. The δ value of 6.68 (1H, s, 5-H) and 6.76 (1H, s, 6′-H) indicated the presence of pentasubstituted benzene rings and the phenolic proton occurred at δ 11.69 (1H, s, 2′-OH). The compound was confirmed by m.p. (192-94)°C [lit. m.p. 190-94 °C] (ref. 3). Compound **6** was identified as 2,4-dimethoxy-3,6-dimethylbenzoic acid 4′-carboxy-3′-hydroxy-2′,5′-dimethylphenyl ester (diffractaic acid).

The compound **6** was hydrolyzed to give fragment C and D. Fragment C: The EI-mass spectrum revealed ion peak at *m/z* 210 corresponding to molecular formula $C_{11}H_{14}O_4$. IR KBr cm^{-1}: 3050, 1690. The ^{1}H NMR spectrum showed signals at δ 2.14 (3H, s, 2-OCH$_3$), 2.59 (3H, s, 6-CH$_3$), 3.84 (3H, s, 4-OCH$_3$), 3.87 (3H, s, 2-OCH$_3$) and 6.58 (1H, s, 5-H). The fragment C was identified as 2,4-dimethoxy-3,6-dimethylbenzoic acid [3]. The spectral data of fragment D was similar to that of fragment B was identified as 2, 4-dihydroxy-3, 6-dimethylbenzoic acid.

In compound **7**, the EI-mass spectrum revealed ion peak at *m/z* 334 corresponding to molecular formula $C_{17}H_{18}O_7$. The ^{1}H NMR spectrum showed signals at δ 2.64 (3H, s, 6-CH$_3$), 2.65 (3H, s, 5′-CH$_3$) corresponding to the two methyl groups. δ 3.87 (3H, s, 4-OCH$_3$) indicated the presence of methoxy group and δ value of 6.74 (1H, d, *J*=2.47Hz, 2′-II), 6.82 (1H, d, *J*=2.47 Hz, 6′-H) relates to the presence of tetra substituted benzene nucleus. The acidic proton occurred at δ 11.17 (1H, s, 3′-COOH). The structure was further confirmed by comparing its m.p. 169-71°C [lit. mp 169-71°C] (ref. 3). The structure of compound **7** was found to be 2-hydroxy-4-methoxy-6-methyl benzoic acid 4′-carboxy3′-hydroxy-5′-methylphenylester (evernic acid).

The compound **7** was hydrolyzed to give fragment E and F. Fragment E: The EI-mass spectrum revealed ion peak at *m/z* 182 corresponding to the molecular formula $C_9H_{10}O_4$. IR KBr cm^{-1}: 3600, 3300, 3000, and 1620. The ^{1}H NMR spectrum showed signals at δ 2.57 (3H, s, 4-OCH$_3$), 11.56 (1H, s, 2-OH). The δ value of 6.33 (1H, d, *J*=2.54 Hz, 3-H), 6.35 (1H, d, *J*=2.54 Hz, 5H) showed metacoupled protons. The fragment E was identified as 2-hydroxy-4-methoxy-6-methylbenzoic acid[3]. Fragment F: The EI-mass spectrum revealed ion peak at *m/z* 168 corresponding to molecular formula $C_8H_8O_4$. IR KBr cm^{-1}: 3530, 3440, and 1605. The ^{1}H NMR spectrum showed signals at δ 2.52 (3H, s, 6-CH$_3$), 6.46 (1H, d, *J*=2.56 Hz, 5-H). The fragment F was identified as 2,4-dihydroxy-6-methylbenzoic acid[3].

Experimental Section

Plant material (*Usnea emidotteris* Mot.) was collected from Pauri (Garhwal), Uttaranchal, India and identified by Dr D K Upreti, National Botanical

Research Institute, Lucknow, Uttar Pradesh, India. A voucher specimen is deposited in his herbarium.

EI-MS was recorded on JMS–SX102A 5890 series II spectrometer. Optical rotation was measured on a JASCO DIP 140 digital polarimeter. IR or FTIR 8100 Shimadzu spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-A600 spectrometer with TMS as an internal standard and chemical shifts were expressed on the δ (ppm) scale. TLC was performed on silica gel using $CHCl_3$-MeOH-H_2O (64:32:10, lower layer) and the spots were detected by spraying with 5% H_2SO_4 reagent followed by heating.

Extraction and isolation

Dried and powdered plant material (1 kg) was extracted with ethanol (95%). The concentrated mass was further partitioned in light petroleum (60-80)°C. The petroleum extract (200 g) was column chromatographed over Si-gel using gradient elution with light petroleum (60-80)°C and ethyl acetate (99.5-0.5-90:10) afforded **1-6** compounds. The petroleum free mass concentrated and dried. The dry mass was chromatographed over silica gel (60-120 mesh) as adsorbent and elution was carried out with chloroform and methanol (86-14), to give compound **7**.

Hydrolysis. Depside (**5-7**) were dissolved in 1 mL conc.H_2SO_4 at 0°C and allowed to stand for 10 min. The solution changed to yellow in colour. Ice-cold water (5 mL) was poured into the solution and the white amorphous precipitate was extracted with 10 mL Et_2O. The Et_2O layer was washed with brine and dried over $MgSO_4$. The hydrolyzed products were separated by preparative TLC using hexane:iso-PrOH:AcOH (8:1:0.9), giving A-F.

Compound **1**: It was crystallized as white crystals in ethyl acetate m.p. (82-84)°C; IR (KBr, cm^{-1}): 1618, 1578, 1469; EI- mass: *m/z* 224,195, 178, 150, 223, 107, 77 and 65; ^{1}H NMR (500 MHz): δ 6.25 (1H, s, H-5), 2.50 (3H, s, H-8), 2.08 (3H, s, H-9), 3.80 (3H, s, H-10), 4.38 (2H, q, *J*=7.00 Hz, 1′-CH), 1.40 (3H, t, *J*= 7.00 Hz, 2′-CH_3) and 11.90 (1H, s, 2-OH); ^{13}C NMR (125 MHz): δ 140.0 (C-1), 162.0 (C-2), 161.2 (C-3), 110 (C-4), 105.4 (C-5) 105.7 (C-6), 172.4 (C=O, C-7), 24.6 (C-8), 7.7 (C-9), 55.3 (C-10), 61.1 (C-1′), 14.1 (C-2′).

Compound **2**: It was crystallized as white crystals in ethyl acetate m.p. (123-25)°C. $C_{11}H_{14}O_4$; IR (KBr, cm^{-1}): 893, 1618, 1578, 1508, 1477, 1736 (ester), 3590 (free hydroxyl group); EI-mass: *m/z* 210, 181, 136, 107, 77; ^{1}H NMR (500 MHz): δ 6.2 (1H, s, H-5), 5.1 (1H, s, 4-OH), 2.4 (3H, s, H-8), 2.1 (3H, s, H-9), 4.2 (2H, q, *J*= 7.1 Hz, H-1′) and 1.4 (3H, t, *J*= 7.1 Hz, H-2′), 12.12 (1H, s, 2-OH); ^{13}C NMR: 105.3 (C-1), 163.2 (C-2), 108.5 (C-3), 157.9 (C-4), 110.5 (C-5), 140.2 (C-6), 172.4 (C=O, C-7), 28.2 (C-8), 61.2 (C-1′), 14.2 (C-2′).

Compound **3**: It was crystallized as orange yellow crystals in ethyl acetate m.p. 186-88°C. $C_{10}H_{12}O_4$; IR (KBr, cm^{-1}): 3568, 1670, 1635, 1576, 1508, 1466, 897; EI-mass: *m/z* 196, 178, 150, 107, 77 and 57; ^{1}H NMR (500 MHz): δ 6.45 (1H, s, H-5), 2.00 (3H, s, H-8), 3.80 (3H, s, H-9), 2.60 (3H, s, H-10), 5.10 (1H, s, 2-OH); ^{13}C NMR: 162.4 (C-1), 163.4 (C-2), 110.7 (C-3), 105.6 (C-4), 106.6 (C-5), 141.7 (C-6), 174.4 (COOH), 7.9 (C-8), 55.9 (C-9), 24.5 (C-10).

Compound **4**: It was crystallized as yellow crystals in ethyl acetate m.p., 203-04°C. $C_{18}H_{16}O7$; IR (KBr, cm^{-1}): 2930 (intramoleculer bonded hydroxyl group), 1655, 1560, 1508, 1630, 1458, 1419; EI-mass: *m/z* 344, 329, 260, 233; ^{1}H NMR (500 MHz): δ 6.2 (1H, s, H-4), 2.65 (3H, s, H-2′), 2.69 (3H, s, H-2″), 1.70 (3H, s, C-1a-CH_3), 2.10 (3H, s, C-8-CH_3), 18.80 (1H, s, 3-OH), 13.30 (1H, s, 7-OH), 11.03 (1H, s, 9-OH); ^{13}C NMR: 198.05 (C-1), 105.22 (C-2), 191.15 (C-3), 98.32 (C-4), 101.53 (C-6), 163.55 (C-7), 109.34 (C-), 157.20 (C-9), 59.07 (C-1a), 179.38 (C-4a) 155.20 (C-6a), 103.95 (C-9a), 201.77 (C-1′), 27.89 (C-2′), 200.10 (C-1″), 31.27 (C-2″), 32.34 (C-1a-CH_3), 7.50 (C-8-CH_3).

Compound **5**: It was crystallized in ethyl acetate m.p. 183-84°C. $C_{18}H_{16}O_7$; IR (KBr, cm^{-1}): 2853, 1560, 1718, 1655, 1508, 1458; EI-mass: *m/z* 360, 179, 181; ^{1}H NMR (500 MHz): δ 6.60 (1H, s, H-5), δ 2.71 (3H, s, H-8), δ 3.90 (3H, s, H-9), δ 2.07 (3H, s, H-10), 6.70 (1H, s, H-6′), δ 2.62 (3H, s, H-8′), 2.09 (3H, s, H-9′), δ 11.50 (1H, s, 6-OH); ^{13}C NMR: 170.9 (C-1), 163.2 (C-2), 111.3 (C-3), 164.4 (C-4), 107.5 (C-5), 105.0 (C-6), 174.4 (C=O, C-7), 24.9 (C-8), 56.2 (C-9), 8.2 (C-10) 141.0 (C-1′), 153.4 (C-2′), 117.1 (C-3′), 164.0 (C-4′), 110.09 (C-5′), 117.0 (C-6′), 174.6 (C-7′), 23.8 (C-8′), 9.3 (C-9′) .

Compound **6**: It was crystallized in ethyl acetate m.p. 190-94°C (uncorrected). $C_{20}H_{22}O_7$; IR (KBr, cm^{-1}): 3450, 3050, 1740, 1640; EI-mass: *m/z* 374 $[M]^+$ 210, 194, 193, 182, 178, 165, 164, 136; ^{1}H NMR (500 MHz): δ 2.13 (3H, s, H-3), 2.14 (3H, s, H-2′), 2.45 (3H, s, H-6), 2.62 (3H, s, H-5′), 3.84 (1H, s, 4-OMe), 6.68 (1H, s, H-5) , 6.76 (1H, s, H-6′),11.69 (1H s, 2′-OH) .

Compound **7**: It was crystallized in ethyl acetate m.p. 169-71°C (uncorrected). $C_{20}H_{22}O_7$; IR (KBr, cm^{-1}): 3450, 3050, 1660, 1610; EI-mass: *m/z* 332 $[M]^+$ 168, 165, 164, 136; ^1H NMR (500 MHz): δ 2.64 (3H, s, H-6), δ 2.65 (3H, s, H-5′), 2.14 (3H, s, H-2′), 3.87 (3H, s, 4-OMe), 6.41 (1H, d, *J*=2.47 Hz, H-5), 6.47 (1H, d, *J*=2.44 Hz, H-3), 6.74 (1H, d, *J*=2.47 Hz, H-2′), 6.82 (1H, d, *J*=2.47 Hz, H-6′), 11.17 (1H s, 3′-COOH); ^{13}C NMR: δ 106.2 (C-1), 166.0 (C-2), 100.3 (C-3), 167.3 (C-4), 112.6 (C-5), 144.8 (C-6), 170.7 (C-7), 56.4 (C-8), 24.4 (C-9), 166.3 (C-1′), 110.0 (C-2′), 155.7 (C-3′), 111.8 (C-4′), 145.2 (C-5′), 117.8 (C-6′) 174.0 (C-7′), 24.8 (C-8′).

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