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Phenolic Compounds from *Frullania nisquallensis*

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Abstract—Five phenolic compounds were isolated from the MeCOEt extract of *Frullania nisquallensis* (Jubulaceae), namely methyl 2,4-dihydroxy-3,6-dimethylbenzoate, methyl 2,4-dihydroxy-6-methylbenzoate, acacetin, betuletol, and pectolinaringenin. Revised ^{13}C-NMR data of methyl 2,4-dihydroxy-6-methylbenzoate and betuletol are reported.

Keywords—*Frullania nisquallensis* · Jubulaceae · phenolic compounds · flavonoids · ^{13}C-NMR

Frullania species, epiphytic stem-leafy liverworts, belong to the Jungermanniales and are interesting from the viewpoint of medicinal chemistry since they are a rich source of sesquiterpene lactones which can produce intence allergic contact dermatitis.[1] From previous extensive chemotaxonomic investigations, sesquiterpene lactones and bibenzyls have been recognized as valuable chemosystematic markers for *Frullania* species.[2-4] Recently, flavonoids were also demonstrated to be significant in the chemotaxonomy of this species.[5] This paper describes the phenolic compounds, two orsellinic acid derivatives and three flavonoids, isolated from the MeCOEt extract of *Frullania nisquallensis* Sull.

Experimental

General Experimental Procedures - Mp's were determined on a Kofler hot-stage apparatus and are uncorrected. Uv spectra obtained in MeOH on a Beckman DU-50 spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively. ^{1}H-^{13}C HETCOR and HMBC experiments were performed on the same spectrometer, using standard Varian pulse sequences. Flash chromatography was performed using silica gel Merck G60 (230-400 mesh), and reversed-phase preparative TLC with Whatman PLKC 18F linear K reversed-phase (1000 mm, 20 x 20 cm) plates. Sephadex LH-20 (Sigma) was employed for gel permeation chromatography.

Plant Material - The plant material was collected in Oregon, U.S.A. in June, 1992 and authenticated by Dr. R.W. Spjut. A voucher specimen (WBA # 1774) has been deposited at the U. S. National Herbarium.

Extraction and Isolation - Dried pulverized *F. nisquallensis* (900 g) was extracted sequentially with hexane, MeCOEt and MeOH. The MeCOEt extract (18.1 g) was partitioned between hexane and 80% aqueous MeOH. H_2O was added to the aqueous MeOH fraction until a 60% aqueous MeOH mixture was achived, and this was extracted thoroughly with $CHCl_3$. The $CHCl_3$ fraction was dried under vacuum to yield 6 g of $CHCl_3$-soluble extract. This extract was subjected to gel permeation chromatography on Sephadex LH-20, eluting initially with

hexane-CH_2Cl_2 (1:4), followed by CH_2Cl_2, CH_2Cl_2-Me_2CO (1:1), Me_2CO, and finally MeOH. The CH_2Cl_2-Me_2CO (1:1) fraction was further subjected to flash chromatography on silica gel and eluted with hexane-EtOAc (4:1), followed by hexane-EtOAc (1:1), and finally EtOAc. Based on their TLC patterns, similar fractions were combined to obtain a total of 10 fractions.

Fraction 1 (12 mg) was purified by silica gel column chromatography (eluent: hexane-Me_2CO, 9:1) to yield methyl 2,4-dihydroxy-3,6-dimethylbenzoate (1, 5 mg). Fraction 2 (68 mg) was purified by reversed-phase preparative TLC (90% aqueous MeOH) to obtain methyl 2,4-dihydroxy-6-methylbenzoate (2, 10 mg). Fraction 7 (120 mg) on further purification by silica gel column chromatography (eluent: $CHCl_3$-Me_2CO, 19:1) afforded acacetin (3, 21 mg).

The CH_2Cl_2 fraction from the Sephadex LH-20 column was loaded onto a silica gel column, and eluted with hexane-EtOAc gradient to give a total of 14 fractions. Fraction 4 (70 mg) was purified by silica gel column chromatography (eluent : $CHCl_3$-Me_2CO, 19:1) to yield 3,5,7-trihydroxy-6,4′-dimethoxyflavone (betuletol) (4, 11 mg). Fraction 5 (126 mg) on further purification by silica gel column chromatography (eluent : hexane-Me_2CO, 7:3) afforded 5,7-dihydroxy-6,4′-dimethoxyflavone (pectolinaringenin) (5, 7 mg).

Methyl 2,4-dihydroxy-3,6-dimethylbenzoate (1) - Colorless needles ($CHCl_3$); mp 139° [Lit. 6) mp 139°] ; EIMS *m/z* (rel. int., %) 196 [M]+ (32), 164 (73), 136 (100), 107 (22), 79 (25), 77 (28) ; ^{1}H-NMR ($CDCl_3$) δ: 2.08 (3H, s, 3-Me), 2.44 (3H, s,6-Me), 3.90 (3H, s, OMe), 4.99 (1H, brs, 4-OH), 6.18 (1H, s, H-5), 12.01 (1H, s, 2-OH); ^{13}C-NMR ($CDCl_3$) δ: 172.6 (COOMe), 163.1 (C-2), 157.9 (C-4), 140.1 (C-6), 110.5 (C-5), 108.4 (C-3), 105.3 (C-1), 51.8 (OMe), 24.0 (6-Me), 7.6 (3-Me).

Methyl 2,4-dihydroxy-6-methylbenzoate (2) - Colorless needles (hexane/EtOAc); mp 136° [Lit.[6] mp 138°]; EIMS *m/z* (rel. int., %) 182 [M]+ (43), 150 (100), 122 (73), 94 (30), 69 (44), 66 (44); ^{1}H-NMR ($CDCl_3$) δ: 2.47 (3H, s, 6-Me), 3.90 (3H, s, OMe), 5.62 (1H, brs, 4-OH), 6.20 (1H, dd, *J*=0.8, 2.4 Hz, H-5), 6.25 (1H, d, *J*=2.4 Hz, H-3), 11.78 (1H, s, 2-OH) ; ^{13}C-NMR ($CDCl_3$) δ: 172.1 (COOMe), 165.3 (C-2), 160.3 (C-4), 144.0 (C-6), 111.3 (C-5), 105.6 (C-1), 101.2 (C-3), 51.8 (OMe), 24.2 (6-Me).

5,7-dihydroxy-4′-methoxyflavone (3; acacetin) - Yellow needles ($CHCl_3$/MeOH); mp 263° [Lit.[7] mp 261°] ; UV, λ_{max}^{MeOH} nm 267, 306 (sh), 325; EIMS *m/z* (rel. int., %) 284 $[M]^+$ (100), 256 (7), 241 (18), 152 (21), 135 (6), 132 (66), 124 (30), 117 (25), 89 (43), 69 (46) ; ^{1}H-NMR (DMSO-d_6) δ: 3.85 (3H, s, OMe), 6.18 (1H, d, *J*=2.0 Hz, H-6), 6.49 (1H, d, *J*=2.0 Hz, H-8), 6.87 (1H, s, H-3), 7.10 (2H, d, *J*=9.0 Hz, H-3′, 5′), 8.03 (2H, d, *J*=9.0 Hz, H-2′, 6′), 12.9 (1H, brs, 5-OH).

3,5,7-trihydroxy-6,4′-dimethoxyflavone (4; betuletol) - Yellow powder ; mp 225° [Lit.[8] mp 222-224°] ; UV, λ_{max}^{MeOH} nm 255, 270, 340 (sh), 364 ;EIMS *m/z* (rel. int., %) 330 $[M]^+$ (63), 312 (28), 287 (76), 165 (16), 144 (13), 135 (47), 92 (20), 77 (43), 69 (100); ^{1}H-NMR (DMSO-d_6) δ: 3.75 (3H, s, 6-OMe), 3.83 (3H, s, 4′-OMe), 6.55 (1H, s, H-8), 7.10 (2H, d, *J*=9.1 Hz, H-3′, 5′), 8.12 (2H, d, *J*=9.1 Hz, H-2′, 6′), 9.50 (1H, brs, 3-OH), 10.70 (1H, brs, 7-OH), 12.50 (1H, brs, 5-OH); ^{13}C-NMR (DMSO-d_6) δ: 176.2 (C-4), 160.5 (C-4′), 157.3 (C-7), 151.7 (C-5), 151.5 (C-9); 146.4 (C-2), 135.7 (C-3), 130.8 (C-6), 129.4 (C-2′, 6′), 123.3 (C-1′), 114.0 (C-3′, 5′), 103.5 (C-10), 93.8 (C-8), 60.0 (6-OMe), 55.4 (4′-OMe).

5,7-dihydroxy-6,4′-dimethoxyflavone (5; pectolinaringenin) - Yellow powder; mp 213-214° [Lit[9] mp 216-217°]; UV λ_{max}^{MeOH} nm 276, 331 ; EIMS *m/z* (rel. int., %) 314 $[M]^+$ (27), 299 (17),

296 (16), 271 (21), 167 (9), 139 (20), 89 (17), 69 (100); ^{1}H-NMR (DMSO-d_6) δ: 3.74 (3H, s, OMe), 3.85 (OMe), 6.60 (1H, s, H-3), 6.87 (1H, s, H-8), 7.10 (2H, d, J=9.0 Hz, H-3′, 5′), 8.03 (2H, d, J=9.0 Hz, H-2′, 6′), 10.8 (1H, brs, 7-OH), 13.0 (1H, brs, 5-OH); ^{13}C-NMR (DMSO-d_6) δ: 182.1 (C-4), 163.3 (C-2), 162.3 (C-4′), 157.4 (C-7), 152.7 (C-5), 152.4 (C-9), 131.4 (C-6), 128.3 (C-2′, 6′), 122.8 (C-1′), 114.6 (C-3′, 5′), 104.1 (C-10), 103.0 (C-3), 94.3 (C-8), 59.9 (6-OMe), 55.5 (4′-OMe).

Results and Discussion

The $CHCl_3$-soluble part of the MeCOEt extract from *F. nisquallensis* was subjected to column chromatography on Sephadex LH-20, silica gel and reversed-phase C-18 PTLC to afford five compounds, which gave positive reactions when sprayed with the ferricyanide-ferric chloride reagent.

Compound **1** and **2** were identified by comparison of spectral data with reported values[6] as methyl 2,4-dihydroxy-3,6-dimethylbenzoate and methyl-2,4-dihydroxy-6-methylbenzoate, respectively. Compound **1** has been reported as a nematocidal principle of *Evernia prunastri*[10]. However, comparison of ^{13}C-NMR data of **1** and **2** in the literature[6] revealed disagreement on the assignments of C-2 and C-4. In compound **1** C-2 (163.03 ppm) appeared at lower field than C-4 (158.04 ppm), but in compound **2** C-2 (160.28 ppm) appeared at higher field than C-4 (165.19 ppm). With the aid of HETCOR and HMBC spectra, the ^{13}C-NMR spectral assignments of **2** have been confirmed. Thus, the protons at 2.47, 3.90, 6.20, and 6.25 ppm were found to have cross peaks with the carbon signals at 24.2, 51.8, 111.3, and 101.2 ppm, respectively, in the HETCOR spectrum. In the HMBC spectrum, the protons at 2.46 ppm attributed to the methyl group at C-6 showed 2- or 3-bond correlation with carbon atoms resonating at 105.6 (C-1), 111.3 (C-5), and 143.9 ppm (C-6). The protons at 6.25 (H-3) and 6.20 ppm (H-5) exhibited correlation peaks with 105.6 (C-1), 165.3 (C-2), 160.3 (C-4), 111.3 ppm (C-5) and 105.6 (C-1), 101.2 (C-3), 24.2 ppm (6-Me), respectively. The chelated hydrogen at 11.78 ppm also exhibited a 3-bond correlation with 101.2 ppm (C-3) and a 2-bond correlation with 165.3 ppm (C-2). Based on these results, the chemical shifts of C-2 and C-4 of compound **2** are unambiguously affirmed as 165.3 and 160.3 ppm, respectively.

Compounds **3** and **5** were identified as acacetin and pectolinaringenin, respectively, by comparison of physical and spectral data with literature values.[9,11,12] Anti-Herpes activity has been reported for compound **5**.[13]

MS, ^{1}H- and ^{13}C-NMR spectra of **4** suggested a 5,6,7,4′-tetra-substituted flavonol having a 6-methoxyl group.[14,15] Comparison of ^{1}H-NMR data of **4** with those of betuletol[8], which was synthesized by Wagner, gave good agreement. However, our data revealed a number of significantly different chemical shifts from the ^{1}H- and ^{13}C-NMR spectral data of **4** and those of betuletol[16] isolated from *Eupatorium glandulosum*. For this reason, ^{1}H- and ^{13}C-NMR spectral assignments of **4** were carried out. The methoxy protons at 3.83 ppm showed a cross peak with the carbon peak at 55.4 ppm in the HETCOR spectrum, and a 3-bond correlation peak at 160.5 ppm in the HMBC spectrum. The protons at 7.10 and 8.12 ppm, which revealed the cross peak with 114.0 and 129.4 ppm in the HETCOR spectrum, respectively, showed long range correlation peaks at 114.0, 123.3, 160.5 and 129.4, 146.4, 160.5 ppm, respectively in the HMBC spectrum. Based on these results, the presence of a methoxyl group at C-4′ was confirmed and the C-2 and B-ring carbons of **4** could be assigned. The chelated hydrogen at

12.50 ppm showed correlation peaks with resonances at 103.5, 130.8, and 151.7 ppm in the HMBC spectrum. The singlet proton at 6.55 ppm exhibited 2- or 3-bond correlation peaks with resonances at 103.5, 130.8, 151.5, and 157.3 ppm in the HMBC spectrum. One of the hydroxyl protons at 10.70 ppm also showed long range correlation peaks with resonances at 93.8, 130.8, and 157.3 ppm. Furthermore, the methoxyl protons at 3.75 ppm exhibited a cross peak with the carbon at 60.0 ppm in the HETCOR spectrum, and revealed a 3-bond correlation peak with the carbon at 130.8 ppm in the HMBC spectrum. From these results, the second methoxyl group was assigned to the C-6 position, and all of the A-ring carbons were unambigously assigned. Although, the proton of the 3-OH group showed only one 3-bond correlation peak with C-2 (146.3 ppm), C-3 and C-4 could be assigned unambiguously as 135.7 and 176.2 ppm, respectively.

Based on these results, compound **4** was characterized as betuletol, and this clearly indicates that the structure of the compound isolated from *E. glandulosum*[16] requires revision. This is the first report of ^{13}C-NMR data for **4**, and the first report of the isolation of compounds **1-5** from a *Frullania* species.

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