Xanthones from *Halenia corniculata*

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A new xanthone, a new xanthone glycoside and a known xanthone have been isolated from the chloroform and ethyl acetate fractions of *Halenia corniculata* (Gentianaceae). They were identified as 1,3-dihydroxy-2,4,5,7-tetramethoxyxanthone, 1-hydroxy-2,7-dimethoxy-3-*O*-β-D-glucopyranosylxanthone and 1,2,3-trihydroxy-5-methoxyxanthone. 1,3-Dihydroxy-2,4,5,7-tetramethoxyxanthone is a revised structure from 1,6-dihydroxy-2,3,4,8-tetramethoxyxanthone reported by our previous paper.

Key words *Halenia corniculata*; Gentianaceae; xanthone; xanthone glycoside

As a result of the investigation of 30 compounds belonging to the xanthones, iridoids and flavonoids were isolated and identified from *Halenia corniculata* and 27 of them were described in our previous publications.[1—5]

The structure elucidation of compound **1** which was described previously as 1,6-dihydroxy-2,3,4,8-tetramethoxyxanthone[3] was studied again, carefully revised and was corrected to 1,3-dihydroxy-2,4,5,7-tetramethoxyxanthone. Now we report here the isolation and structural elucidation of compound **1** with one new compound (**2**) and one known compound (**3**).

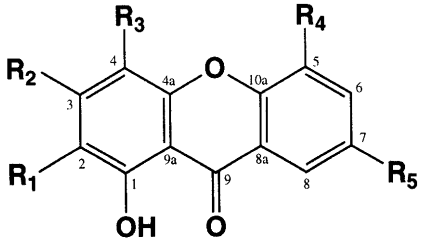
Compounds **1** and **3** were isolated from the chloroform extract and subjected to column chromatography on silica gel using a hexane–chloroform as an eluent; compound **2** was obtained from the mixture of ethyl acetate and n-buthanol extract using this method with a chloroformy–methanol solvent system. After isolation, compounds **1**—**3** were crystallized from the methanol and purification was achieved by recrystallization.

The UV spectra of these compounds exhibited three or four absorption bands of decreasing intensity at 235—380 nm which is characteristic of xanthones.[6] The indication of free hydroxyl groups in **1**—**3** was obtained from their UV spectra recorded with classical shift reagents. Strong bathochromic shifts were observed in all three compounds upon addition of 5% $AlCl_3$, indicating the existence of OH group at peri to the carbonyl function and/or ortho-dioxide groups in the xanthone skeleton. After addition of HCl, no change was observed in the spectrum of **1** and **2**, while the first maximum of **3** was shifted to a short wavelength by 8 nm, which confirmed that **1** and **2** have hydroxyl group at C-1 or C-8 positions and that **3** additionally has the ortho-dihydroxyl group.

The mass spectrum of **1** showed the following fragments at *m/z* (%): 348 $[M]^+$ (74), 333 $[M-CH_3]^+$ (100), 305 $[M-CH_3-CO]^+$ (35), 290 $[M-2CH_3-CO]^+$ (23), 275 (4), 260 (2.5), which indicated that the major fragmentation was initiated by loss of CH_3 and CH_3CO. This is characteristic for the xanthone substituted by OCH_3 at positions C-2, C-4, C-5 and C-7,[7—9] while the appearance of fragments at *m/z* (%): 274 $[M]^+$ (100), 259 $[M-CH_3]^+$ (75), 231 $[M-CH_3-CO]^+$ (84), 202 (6), and particularly that at *m/z* 259 in the massspectrum of **3** confirmed the presence of OCH_3 group at C-5.[10]

The ^{1}H-NMR spectrum of **1** exhibited one singlet signal at δ12.8 characteristic of the peri-OH group to the carbonyl group at C-9, two doublets at δ 7.16 and 6.85 (J=3 Hz), indicative of a pair of meta coupled aromatic protons; it also exhibited intense singlets at δ 3.92, 4.01, 4.04 and 4.09 indicating these four respective methoxyl groups. The ^{13}C-NMR spectra showed 13 carbon signals due to the xanthone nucleus, 4 signals due to the methoxyl groups at δ 55.9, 56.5, 61.1 and 61.7. Also, the appearance of signal C-9 at δ 181.2 indicated only one OH group substitution in peri-position to this carbonyl group.[11] Chemical shifts of OCH_3 groups showed that two of them (at δ 61.1, 61.7) existed at diortho-substituted and others (at δ 55.9, 56.5) in another ring at meta-positions.[11—13] All these signals appeared at the distortionless enhancement by polarization transfer (DEPT) spectrum, one ring fully substituted and the other with two OCH_3 groups. Full structure elucidation of this compound was made by the comparison of NMR data with known compound 1,7-dihydroxy-2,3,4,5-tetramethoxyxanthone, which was previously isolated from *Halenia corniculata*[3,4] and *Halenia elliptica*.[14] Thus, we revised the structure of **1** as 1,3-dihydroxy-2,4,5,7-tetramethoxyxanthone.

The ^{1}H-NMR spectrum of **3** showed the presence of three OH signals at δ 12.95, 11.00, 10.45 and four signals of aromatic protons at δ 6.53 (1H, s), 7.31 (1H, dd, J=7, 2 Hz),



1: R_1=R_3=R_4=R_5=OMe, R_2=OH

2: R_1=R_5=OMe, R_2=OGlc, R_3=R_4=H

3: R_1=R_2=OH, R_3=R_5=H, R_4=OMe

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7.24 (1H, d, J=7 Hz), 7.56 (1H, dd, J=7, 2 Hz) and one OCH_3 group at δ 3.79, respectively. These results were very similar to 1,2,3,5-substituted xanthones isolated from this species earlier.[4] Consequently, **3** is 1,2,3-trihydroxy-5-methoxyxanthone, which was isolated and identified for the first time from *Centaurium erythrea* (Gentianaceae).[15,16]

For **2** there are two common fragments with *m/z* (%): 451 $[M+H]^+$ (36) and 289 $[M-glucose]^+$ (100) in the mass spectrum. To determine the structure of the sugar residue, **2** was hydrolysed and by the comparative paper chromatography with standard sugars it was determined as glucose. Furthermore, the appearance of doublet at δ 5.15 (J=7 Hz) in the ^{1}H-NMR spectrum was characteristic of the β-configuration of glucose.

Four signals of aromatic protons and two signals of OCH_3 groups were also observed at δ 7.63 (1H, d, J=9 Hz), 7.53 (1H, d, J=3 Hz), 7.50 (1H, dd, J=9, 3 Hz), 6.88 (1H, s) and 3.88, 3.77 (6H, s). By the comparison with known compounds this could then be substituted at 1, 2, 3, 7 positions. This conclusion was confirmed by ^{13}C-NMR spectroscopy, where two OCH_3 groups appeared at δ 60.4 and 55.8, one of them placed at the diortho-substituted position, C-2, and the other at C-7, as indicated by the nuclear Overhauser and exchange spectroscopy (NOESY). The structure of **2** was thus determined to be 1-hydroxy-2,7-dimethoxy-3-*O*-β-D-glucopyranosylxanthone. The aglycone of this compound was isolated and identified from *Veratrilla bailonii* (Gentianaceae).[17]

Experimental

General Procedures UV shift reagents were prepared according to ref.[6,18] and the UV spectra were recorded between 200 and 450 nm. The ^{1}H- and ^{13}C-NMR spectra were measured in DMSO-d_6 or $CDCl_3$ at 200 MHz, and the solvent signals were used as internal standard. TLC was carried out on pre-coated silica gel 60F 254 aluminium sheets.

Plant Material The aerial parts of *Halenia corniculata* L. (Cornaz) were collected in 1992 in the vicinity of Ulaanbaatar and identified by Dr. Ch. Sanchir (Herbarium of Botanical Institute, Ulaanbaatar, Mongolia). A voucher specimen (no. 92012) is on deposit at the Pharmacognosy and Phytochemistry (University of Lausanne, Switzerland).

Extraction and Isolation One kg of dry powdered whole plant was extracted at room temperature with 96% ethanol (3×5 l) and 70% ethanol (1×5 l). Then, 254 g of thick extract was fractionated successively with hexane (5×1 l), chloroform (5×1 l), ethylacetate (5×1 l) and *n*-buthanol (5×1 l) and afforded 15, 16, 24 and 67 g of extract, respectively. Sixteen g of chloroform extract was subjected to CC on silica gel using hexane–chloroform (1 : 1) as the eluent and 33 fractions were enriched in **1**; **3** was isolated from the above mentioned CC using hexane–chloroform (1 : 4) as the eluent and in 13 fractions this compound was dominant. **2** was isolated from the total ethylacetate and *n*-buthanol exracts. Ninety-one g of extract was subjected to CC and **2** was isolated from 23 fractions using chloroform–methanol (98 : 2) as the eluent. All compounds were purified by repeated crystallization from methanol and ethanol: **1** (53 mg), **2** (48 mg), **3** (80 mg).

Hydrolysis of Xanthone Glycoside Compound **2** was hydrolysed according to the standard procedure.[18] Sugar was analysed by paper TLC with *n*-buthanol–acetic acid–water (4 : 1 : 2) and visualized spraying with *p*-anisidine phtalate.

1,3-Dihydroxy-2,4,5,7-tetramethoxyxanthone (**1**): Yellow crystalline compound. mp 206—208 °C. Black green colour with 3% $FeCl_3$. UV lmax nm (MeOH): 236.0, 269.5, 320.0, 378.5; λ_{max} ($AlCl_3$): 229.5, 270.5, 322.5, 387.5. λ_{max} (CH_3COONa): 276.0, 361.0; λ_{max} (CH_3COONa/H_3BO_3): 270.5, 321.0, 384.5; λ_{max} (NaOH): 236.5, 278.0, 362.5. ^{1}H-NMR ($CDCl_3$) δ: 6.85 (1H, d, J=3 Hz, H-6), 7.16 (1H, d, J=3 Hz, H-8), 12.80 (1H, s, OH-1), 3.90, 3.92, 4.03, 4.08 (3H each, s, 4×OCH_3). ^{13}C-NMR (DMSO-d_6) δ: 149.7 (C-1), 129.8 (C-2), 149.8 (C-3), 129.1 (C-4), 148.4 (C-4a), 149.5 (C-5), 106.7 (C-6), 156.1 (C-7), 95.3 (C-8), 120.8 (C-8a), 181.2 (C-9), 102.8 (C-9a), 141.8 (C-10a), 61.7, 61.1, 56.5, 55.9 (4×OCH_3). EI-MS *m/z* (%): 348 $[M]^+$ (74), 333 $[M-CH_3]^+$ (100), 305 $[M-CH_3-CO]^+$ (35), 290 $[M-2CH_3-CO]^+$ (23), 275 (4), 260 (2.5).

1-Hydroxy-2,7-dimethoxy-3-*O*-β-D-glucopyranosylxanthone (**2**): Pale yellow crystalline compound. mp 263—265 °C. Black green colour with 3% $FeCl_3$. UV λ_{max} nm (MeOH): 235.0, 262.0, 297.0, 371.0; λ_{max} ($AlCl_3$): 275.0, 319.0, 425.0. Unchanged upon addition of other reagents. ^{1}H-NMR (DMSO-d_6) δ: 7.63 (1H, d, J=9 Hz, H-5), 7.53 (1H, d, J=3 Hz, H-8), 7.50 (1H, dd, J=3, 9 Hz, H-6), 6.88 (1H, s, H-4), 12.77 (1H, s, OH-1), 3.78, 3.88 (3H each, s, 2×OCH_3), 5.15 (1H, d, J=7 Hz, H-1′ anomer). ^{13}C-NMR (DMSO-d_6) δ: 153.5 (C-1), 131.7 (C-2), 157.9 (C-3), 93.9 (C-4), 152.4 (C-4a), 119.5 (C-5), 125.2 (C-6), 155.8 (C-7), 105.1 (C-8), 119.8 (C-8a), 180.5 (C-9), 103.8 (C-9a), 150.4 (C-10a), 55.8 (OCH_3-7), 60.4 (OCH_3-2), 100.0 (C-1′), 73.2 (C-2′), 77.3 (C-3′), 69.6 (C-4′), 76.6 (C-5′), 60.7 (C-6′). EI-MS *m/z* (%): 451 $[M+H]^+$ (36), 289 $[M-glucose]^+$ (100). On hydrolysis, **2** yielded the the aglycone, 1,3-dihydroxy-2,7-dimethoxyxanthone.[17]

1,2,3-Trihydroxy-5-methoxyxanthone (**3**): Colorless or pale yellow crystalline compound. mp 283—285 °C. Black green colour with 3% $FeCl_3$. UV λ_{max} nm (MeOH): 243.0, 312.0, 361.0; λ_{max} ($AlCl_3$): 244.0, 265.0, 280.0, 339.0, 415.5; λ_{max} ($AlCl_3$/HCl): 243.0, 264.0, 279.0 (sh), 330.0, 408.0; λ_{max} (CH_3COONa): 292.0, 353.0; λ_{max} (CH_3ONa): 244.0, 391.0, 350.0. λ_{max} (NaOH): 242.0, 314.0, 356.0. ^{1}H-NMR (DMSO-d_6) δ: 6.53 (1H, s, H-4), 7.31 (1H, dd, J=2, 7 Hz, H-6), 7.24 (1H, t, J=7 Hz, H-7), 7.56 (1H, dd, J=2, 7 Hz, H-8), 12.93 (1H, s, OH-1), 11.0, 10.45 (2H each, s, OH-2, OH-3), 3.79 (3H, s, 5-OCH_3). ^{13}C-NMR (DMSO-d_6) δ: 159.2 (C-1), 130.7 (C-2), 152.3 (C-3), 94.2 (C-4), 153.9 (C-4a), 146.0 (C-5), 114.3 (C-6), 123.8 (C-7), 120.3 (C-8), 120.4 (C-8a), 180.4 (C-9), 102.2 (C-9a), 144.8 (C-10a), 59.9 (OCH_3-5). EI-MS *m/z* (%): 274 $[M]^+$ (100), 259 $[M-CH_3]^+$ (75), 231 $[M-CH_3-CO]^+$ (84), 202 (6)

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