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TRI- AND TETRAOXYGENATED XANTHONES FROM *SWERTIA PETIOLATA**

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Key Word Index—*Swertia petiolata*: Gentianaceae: high altitude herbs; 1-glucosyloxy-3-hydroxy-5,8-dimethoxy xanthone; 1,8 dihydroxy-5,8-dimethoxyxanthone, 1,3-dihydroxy-7-methoxyxanthone; 1,7-dihydroxy-3-methoxy-xanthone.

Abstract—A new tetra-oxygenated xanthone glycoside: 1-glucosyloxy-3-hydroxy-5,8-dimethoxyxanthone has been isolated and identified from the methanolic extract of the aerial parts of *Swertia petiolata* alongwith 1,8-dihydroxy-3,5-dimethoxyxanthone; 1,3-dihydroxy-7-methoxyxanthone and 1,7-dihydroxy-3-methoxyxanthone.

INTRODUCTION

Xanthones have been regarded as mutagenetic [1, 2] as well as producing varying responses to the central nervous and cardiovascular systems [3–5]. Most of the xanthones and their glycosides and other derivatives which are known to possess such properties have so far been isolated from the plants of the family Gentianaceae. *Swertia petiolata* D. Don a native of the high altitude Himalayan region is a sub-species of a well known Ayurvedic herb *S. Chirata*. *Swertia petiolata* is bitter in taste and used for its laxative and antimalarial properties in the folk medicine of the region. In our earlier report [6] we isolated two compounds 2-hydroxydimethylterephthalate (**1**) and 1,3-dihydroxy-5,8-dimethoxyxanthone (**2**), **1** was found to be a major constituent. In this communication we report the presence of a new tetraoxygenated xanthone glycoside, 1-glucosyloxy-3-hydroxy-5,8-dimethoxyxanthone (**2a**), and three other known tri- and tetraoxygenated xanthones **3**–**5** from the methanolic extract of the plant.

*Part 4 in the series 'Constituents of High Altitude Himalayan Herbs'. For Part 3 see Khetwal, K. S. and Harbola, S. (1989) *J. Nat. Prod.* **52**, 837.

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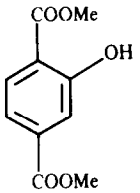
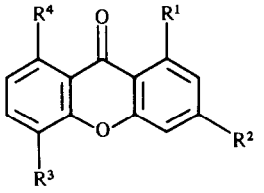
RESULTS AND DISCUSSION

The yellow coloured compound **2a**, $C_{24}H_{22}O_{12}$, mp 239–243°, isolated from the ethyl acetate fraction appeared brown on exposure to iodine vapour, UV light (365 nm) and with 15% H_2SO_4 spray. With iron(III) chloride it gave a green colour and showed positive Feigl [7] and Molisch tests. It did not leave the base line on TLC with the solvents methanol–chloroform (1:9, 1:4); methanol–benzene (1:19, 1:9, 1:4), methanol (100%), chloroform (100%) and acetone (100%). However, it was mobile on Whatman nos 1 and 3 chromatographic paper when developed in *n*-butanol–ethanol–water (4:1:5), *n*-butanol–acetic acid–water (4:1:5) and acetic acid–water (3:2). These data were sufficient to consider **2a** as a xanthone glycoside.

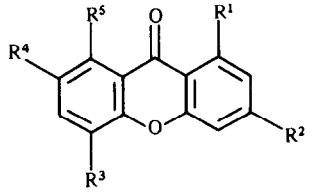
It was prone to hydrolysis and showed the ion of its aglucone (*m/z* 288) in its mass spectrum which clearly indicated it to be a xanthone-*O*-glucoside. After emulsin hydrolysis an aglucone, **2**, was isolated and glucose identified (PPC) in its hydrolysate.



1



2 $R^1 = R^2 = OH$, $R^3 = R^4 = OMe$
2a $R^1 = Oglc$, $R^2 = OH$, $R^3 = R^4 = OMe$
2b $R^1 = R^2 = OAc$, $R^3 = R^4 = OMe$
2c $R^2 = R^3 = R^4 = OMe$, $R^1 = OH$



3 $R^1 = R^5 = OH$, $R^2 = R^3 = OMe$, $R^4 = H$
4 $R^1 = R^2 = OH$, $R^3 = R^5 = H$, $R^4 = OMe$
5 $R^1 = R^4 = OH$, $R^2 = OMe$, $R^3 = R^5 = H$

The yellow crystalline aglycone (**2**), MS *m/z* 288 $[M]^+$ $C_{15}H_{12}O_6$ purified by repeated TLC and HPLC methods, visualized yellow brown in UV light (365 nm), brown with iodine vapour and 15% H_2SO_4 spray and green with iron(III) chloride. These colour reactions coupled with its IR 3360 (OH), 1650, 1615 (>C=O), 1160, 1100, 1060 cm^{-1} (C–O–C) and UV spectra λ_{max} 220, 224, 304 and 376 nm supported it to be a tetraoxygenated xanthone.

The ^1H NMR spectrum of **2** showed the presence of four aromatic protons belonging to two *ortho*-coupled protons at δ7.38 and 7.14 (*d*, $J = 9$ Hz), and two *meta*-coupled ones at δ6.36 and 6.32 (*d*, $J = 1.5$ Hz). Two signals at δ13.17 and 5.7 due to hydroxyl protons and two at δ4.04 and 3.97 due to methoxyl protons in the ^1H NMR spectrum led us to consider the aglycone to be a dihydroxy dimethoxy xanthone. The absence of C-1 and C-8 carbonyl deshielded proton signals which should have occurred in the region δ8–8.5 indicated the aglycone to be oxygenated at C-1 and C-8. The low field signals at δ13.17 (chelated OH with carbonyl function) and 4.04 (OMe) led us to assume C-1 and C-8 are substituted with hydroxy and methoxy groups, respectively. Because **2** was negative to Tollen's reagent the other hydroxy group cannot be *ortho* to C-1 or C-8 and must be placed either at C-3 or C-6. In view of the strong acidic character of the compound **2** as the UV maxima undergoes a bathochromic shift with sodium acetate and superimposable [8] UV spectra in (MeOH + NaOAc) and (MeOH + NaOMe) indicate the attachment of hydroxyl at C-3. This was supported by a positive Gibbs test which requires a free *para*-position to the hydroxy group. Further, the position of the hydroxyl at C-3 was confirmed by the low field shifts (δ6.74 and 6.55) of *meta*-coupled protons in its acetate derivative **2b**. The position of the other methoxy group could be either C-5 or C-7. The melting point (217°) of 1,3-dihydroxy-7,8-dimethoxyxanthone was higher than that of **2** (192.5–194.5°). The ^{13}C NMR spectrum showed 15 signals belonging to carbonyl (*s*, δ180.57), two methoxyl groups (55.80 and 62.82) and twelve aromatic carbons (8 *s* and 4 *d*). These data showed the aglycone **2** as 1,3-dihydroxy-5,8-dimethoxyxanthone.

The glycoside **2a** on complete methylation with diazomethane followed by emulsin hydrolysis afforded 1-hydroxy-3,5,8-trimethoxyxanthone **2c** [2] indicating the linkage of the sugar moiety as C-1 of the aglycone unit. These data suggest 1-glucosyloxy-3-hydroxy-5,8-dimethoxyxanthone as the structure of **2a**.

The petrol concentrate afforded **3** and a mixture of **4** and **5**. Compound **3** $C_{15}H_{12}O_6$, mp 188°, belonged to 1:3,5,8-tetraoxygenated series of xanthones on the basis of its UV spectrum which showed λ_{max} 240, 250, 315 and 337 nm. The ^1H NMR spectrum showed the presence of two chelated hydroxyl groups at δ14.5 and 15.0, two methoxyls at δ3.85 and 3.90 and four aromatic protons, one pair each *meta*-coupled at δ6.37 and 6.48 (2H, *dd*, $J = 1.2$ Hz) and the other pair of *ortho*-coupled protons at δ6.60 and 7.18 (2H, *dd*, $J = 9$ Hz). Compound **3** was thus identified [9] as 1,8-dihydroxy-3,5-dimethoxyxanthone.

Compounds **4** and **5** which were isolated as a mixture from the silica gel G column chromatography were later separated by prep. TLC and identified as 1,3-dihydroxy-7-methoxyxanthone and 1,7-dihydroxy-3-methoxyxanthone by comparing their UV, IR and ^1H NMR spectra [10].

EXPERIMENTAL

Mps: uncorr. UV spectra: MeOH. IR as KBr pellets. ^1H NMR (90 MHz and 400 MHz and ^{13}C NMR (25 MHz.) in $CDCl_3$ using TMS as int. standard. MS at 70 eV.

Separation. Silica gel G (Glaxo 60–120 mesh) was used for CC and TLC. Whatman no 1 and 3 chromatographic papers were used for analytical and preparative purposes. Water Associates HPLC with variable wavelength (190–750 nm) UV detector, 6000 psi pump and Z-module μBondapak C_{18} cartridge was used for checking the purity of compounds. PC and TLC spots were visualized either by exposure to UV light (365 nm), I_2 vapour or $FeCl_3$ or with 15% H_2SO_4 spray. Sugar was analysed by PC using *p*-anisidine and benzidine as visualizing reagents.

Extraction and isolation. Aerial parts of *S. petiolata* were collected at an altitude of 14000 ft from Pindari Glaciers of Kumaon Himalaya, Uttar Pradesh, India. The material was identified at the Department of Botany, Kumaun University, Nainital where a voucher specimen is deposited. Shade dried aerial parts were pulverized and Soxhlet extracted with 80% MeOH, concd *in vacuo* and partitioned between $CHCl_3$ and H_2O (1:1). Both layers were separated and concd. The $CHCl_3$ layer was re-extracted with petrol (60–80°) while the H_2O concentrate was extracted with EtOAc. The insoluble deposit at the bottom of the EtOAc container afforded **2a** whereas the petrol extract on silica gel G CC followed by TLC afforded **3–5**.

1-*Glucosyloxy*-3-*hydroxy*-5,8-*dimethoxyxanthone* (**2a**). Yellow coloured; mp 239–243° (decomp); UV λ_{max}^{MeOH} nm: 254, 290, 302, 308, 330, 370; IR ν_{max}^{KBr} cm^{-1}: 3450, 1660, 1615, 1580, 1450, 1260, 1200, 1105, 1085, 1040, 950. Emulsin hydrolysis afforded glucose and an aglycone **2**. Glucose was identified by direct comparison with an authentic sample.

1,3-*Dihydroxy*-5,8-*dimethoxyxanthone* (**2**). Yellow crystalline, $C_{15}H_{12}O_6$, mp 192.5–193.5°. EIMS *m/z* 288 $[M]^+$; UV λ_{max}^{MeOH} nm: 220, 224, 305, 376; $\lambda_{max}^{MeOH+NaOAc}$ nm: 231, 254, 320, 390; these maxima are superimposable in (MeOH + NaOMe). IR ν_{max}^{KBr} cm^{-1}: 3360, 2950, 2860, 1650, 1610, 1470, 1435, 1320, 1300, 1210, 1160, 1100, 1060, 825. ^1H NMR ($CDCl_3$, TMS int. standard, 90 and 400 MHz): δ13.12 (1H, *s*, OH at C-1), 7.38 and 7.14 (each 1H, *d*, $J = 9.2$ Hz, H-6 and H-7), 6.36 and 6.32 (each 1H, *d*, $J = 1.5$ Hz, H-2 and H-4), 5.97 (1H, *br*, OH at C-3), 4.04 and 3.90 (each 3H, *s*; OMe at C-5 and C-8). ^{13}C NMR ($CDCl_3$, TMS int. standard, 25 MHz): δ180.59 (*s*, C-9), 166.48 (*s*, C-8), 163.38 (*s*, C-5a), 157.24 (*s*, C-1), 150.68 (*s*, C-3), 145.54 (*s*, C-4a), 144.38 (*s*, C-5a), 122.52 (*s*, C-8a), 113.87 (*s*, C-9a), 104.05 (*d*, C-2), 94.33 (*d*, C-4), 96.96 (*d*, C-6), 92.08 (*d*, C-7) and 2-OMe at 62.82 and 55.80.

1,3-*Acetoxy*-5,8-*dimethoxyxanthone* (**2b**). On acetylation (Ac_2O in pyridine) **2a** yielded **2b**. ^1H NMR ($CDCl_3$): δ7.34 and 7.14 (each 1H, *d*, $J = 9.2$ Hz), 6.74 and 6.55 (each 1H, *d*, $J = 2.2$ Hz), 3.90 (6H, *s*, OMe), 2.47 and 2.35 (each, 3H, Ac).

1-*Hydroxy*-3,5-*trimethoxyxanthone* (**2c**). Methylation of **2a** with diazomethane afforded a methylated derivative which on emulsin hydrolysis yielded **2c** as yellow crystals mp 213–214° (lit. 215°) MS *m/z*: 302 $[M]^+$; $C_{16}H_{14}O_6$ UV λ_{max}^{MeOH} nm: 204, 252, 272, 327; IR ν_{max}^{KBr} cm^{-1}: 3450, 1660, 1615, 1585. Formation of **2c** established the linkage of glucose to **2** at C-1.

Isolation of compound **3**. The silica G column on elution with petrol: C_6H_6 (50:50) yielded **3** $C_{15}H_{12}O_6$, mp 188° MS *m/z* 288 $[M]^+$, IR ν_{max}^{KBr} cm^{-1}: 3450, 1665, 1640, 1605, 1580. The other spectral data are given in the text.

Isolation of **4** and **5**. By using C_6H_6–EtOAc (3:1) a mixture of **4** and **5** was collected from the silica gel G column. Resolved by using EtOAc and MeOH–C_6H_6 (1:9) and identified [10] by direct comparing mp, MS, UV, IR and NMR.

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