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PHYTOCHEMICAL STUDIES ON *SWERTIA CORDATA*

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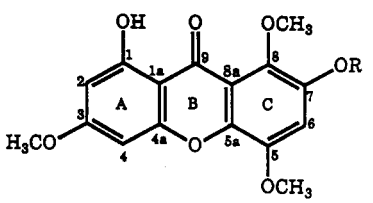
ABSTRACT.—Two new xanthones, 1-hydroxy-3,5,7,8-tetramethoxyxanthone [**1**] and 1,7-dihydroxy-3,5,8-trimethoxyxanthone [**2**], have been isolated from *Swertia cordata* and their structures were determined by using 2D-nmr techniques such as HMQC, HMBC, and HOHAHA experiments.

Swertia cordata (G. Done) Clark (Gentianaceae) is a perennial herb widely distributed in the northern areas of Pakistan. The plant finds extensive usage in folk medicine as an alterative, febrifuge, and anthelmintic as well as a bitter tonic. Previously a terpene and some xanthones were reported from this plant (1), but no attempt was made to characterize the xanthones. In view of the medicinal properties of *S. cordata* and the monoamine oxidase inhibiting activity of certain xanthones (2), it was considered worthwhile to undertake phytochemical investigations of this plant.

Our studies on the petroleum ether-soluble fraction of the EtOH extract of the aerial parts led to the isolation of two new xanthones, 1-hydroxy-3,5,7,8-tetramethoxyxanthone [**1**] and 1,7-dihydroxy-3,5,8-trimethoxyxanthone [**2**], along with two known xanthones, swertianolin and mangiferin, which are reported from this plant for the first time.

The eims of compound **1** showed the molecular ion peak *m/z* 332. The hreims (*m/z* 332.0897) of **1** afforded the molecular formula $C_{17}H_{16}O_7$ (calcd 332.0896), suggesting ten double bond equivalents in the molecule. The uv spectrum indicated the presence of a xanthone nucleus (3,4). No bathochromic shift was observed in the uv spectrum on addition of a few drops of NaOH in MeOH. This established that the OH group was not present at the C-3 or C-6 positions (3,5). The presence of a hydrogen-bonded OH signal at δ 2.17 in the ^{1}H-nmr spectrum further indicated that the OH group was peri to the carbonyl function, i.e., at C-1 or C-8 (6).



1 R=Me
2 R=H

The ^{1}H-nmr spectrum of **1** showed two sets of meta coupled doublets at δ 6.29 and 6.42 ($J_{2,4}$=2.3 Hz) assigned to H-2 and H-4, respectively. A sharp singlet at δ 6.95 was assigned to H-6. The four MeO singlets at δ 3.85, 3.88, 3.92, and 3.95 and an H-bonded OH at δ 12.17 indicated that the substance is a pentaoxygenated xanthone. A comparison of the ^{1}H-nmr spectrum of **1** with known pentaoxygenated xanthone derivatives (5,6) indicated that one of the aromatic rings was double substituted while the other was triply substituted.

The position of the MeO groups was established by nOe difference measurements. Separate irradiations of the doublets at δ 6.29 (H-2) and 6.42 (H-4) resulted in 8.3% and 7.9% nOe's, respectively, on the MeO at δ 3.85 at C-3 confirming their respective assignments.

[1]The authors regret the sad demise of Mr. M. Feroz.

These nOe results confirmed that ring A has OH and MeO substituents at C-1 and C-3, respectively. Similarly, when the singlet at δ 6.95 (H-6) was irradiated it caused 9.2% and 8.5% nOe's at δ 3.92 and 3.95, respectively, indicating that this aromatic methine (H-6) was in close proximity to the two MeO groups in ring C.

The ^{13}C-nmr spectrum of **1** exhibited 17 carbon resonances (Table 1). The multiplicities were determined by DEPT experiments (7,8). Comparison of the ^{13}C-nmr assignments of **1** with 1-hydroxy-3,5,6,7-tetramethoxyxanthone (9–11) showed that in the latter the aromatic methine carbon present in ring C resonated at δ 95.50 (C-8) whereas in compound **1** it appeared 10.7 ppm downfield (δ 106.22) and was assigned to C-6. This suggested that compound **1** does not belong to the 1,3,5,6,7-pentaoxygenated xanthone series. This was further substantiated by HMBC experiments (12–14).

One bond ^{1}H/^{13}C connectivities were determined from the 2D HETCOR spectrum (7,8). The carbons at δ 97.41 (C-2) and 92.15 (C-4) were shown to be coupled to the protons at δ 6.29 (H-2) and 6.42 (H-4), respectively. Similarly the aromatic methine at δ 6.95 (H-6) showed cross peaks with the carbon at δ 106.22 (C-6), confirming the assignments. The four MeO carbons at δ 55.75, 57.94, 57.08, and 61.83 showed cross peaks with the protons at δ 3.85, 3.88, 3.95, and 3.92, respectively. The results obtained from nOe difference, COSY-45°, ^{13}C-nmr, and 2D heteroCOSY experiments indicated that ring A contained an OH group at C-1 and an MeO group at C-3, whereas ring C had three MeO groups located at C-5, C-7, and C-8. These assignments were furhter confirmed by the observation of long range proton-carbon connectivities by the HMBC experiment (12–14).

TABLE 1. ^{13}C-Nmr Assignments of Compounds **1** and **2**.

Carbon	Compound	
	1	**2**
C-1	166.65	166.53
C-1a	104.36	103.60
C-2	97.41	97.15
C-3	163.78	164.14
C-4	92.15	92.23
C-4a	157.06	156.57
C-5	141.56	141.06
C-5a	148.80	148.08
C-6	106.22	107.05
C-7	142.15[a]	154.05
C-8	144.59[a]	146.68
C-8a	116.25	114.25
C-9	181.10	180.23
Ar-OMe (at C-8)	61.83	61.90
Ar-OMe	57.94[b]	57.01[a]
Ar-OMe	57.08[b]	55.84[a]
Ar-OMe	55.75[b]	—

[a,b] The assignments may be interchanged.

The HMBC spectrum of **1** showed that the proton at δ 6.29 (H-2) was coupled to the carbons at δ 92.15 (C-4), 166.65 (C-1), 163.78 (C-3), and 104.36 (C-1a), whereas the proton at δ 6.42 (H-4) was coupled with the carbon at δ 97.41 (C-2), 163.78 (C-3), and 157.06 (C-4a), in agreement with the assignments in ring A. The methine proton at δ 6.95 (H-6) showed cross peak with the carbons at δ 144.59 (C-8), 142.15 (C-7), and 141.56 (C-5). If this aromatic methine had been located at C-7, then it would have been expected to show cross peaks with C-8a (δ 116.25). Similarly, if it were present at C-8 or C-5 then it would have been expected to show coupling with C-8a, C-9, and C-5a. No such cross peaks appeared in the HMBC spectrum of **1**, thereby confirming the assignment of the methine at C-6. These studies led to structure **1** for the new xanthone.

Compound **2** showed the molecular ion peak at *m/z* 318.0744, corresponding to the formula $C_{16}H_{14}O_7$ (calcd 318.0739). The molecular ion was further confirmed by fdms. The peak at *m/z* 303.0510 ($C_{15}H_{11}O_7$) arose due to the loss of an Me group. Compound **2** showed uv maxima at 238, 262, 314, and 385 nm and did not show any shift on addition of NaOH or

HCl solution, thus indicating the absence of a free OH group at C-3 or C-6 (3–5). The ir spectrum displayed peaks at 3320–3480 (OH), 1665 (C=O), and 1610 (C=C) cm^{-1}. The ^{1}H-nmr spectrum of **2** showed a distinct resemblance to that of **1**. The presence of only three MeO singlets in the ^{1}H-nmr spectrum indicated that compound **2** is a pentaoxygenated xanthone with three MeO and two OH functions.

In order to confirm the spatial proximity of the MeO and aromatic protons in **2**, nOe difference measurements were performed. Irradiation of the doublet at δ 6.33 (H-2) caused 8.1% nOe at δ 3.85 (3-MeO). Similarly when the doublet at δ 6.36 (H-4) was irradiated, it caused 11.2% nOe at the 3-MeO protons. The reverse enhancements were also obtained when the 3-MeO was irradiated. These nOe effects indicated the close proximity of the two aromatic protons at C-2 and C-4 with the signal at δ 3.85. Therefore, this singlet was unambiguously assigned to the 3-OMe protons. When H-6 (δ 7.03) was irradiated, 14.4% nOe was observed for the 3H singlet at δ 3.87 (5-MeO). This established that only one MeO group was adjacent to the C-6 proton so that the other MeO group in ring C must be present at the C-8 position.

The ^{1}H-nmr and nOe difference spectra indicated that ring A of **2** bore an OH group at C-1 and an MeO group at C-3. Therefore, ring C must contain the remaining two MeO groups and an OH group. Because the ^{1}H-nmr spectrum showed only one downfield singlet at δ 10.30 due to a hydrogen-bonded hydroxylic proton (C-1) with the C-9 carbonyl group, this ruled out the position of the second OH at C-8 and indicated that it could either be at C-5 or C-7. Similarly the aromatic methine in ring C could be present either at C-6 or C-7 but not at C-5 or C-8 since ^{1}H as well as ^{13}C chemical shifts for the C-6 methine did not correlate with those of other known pentaoxygenated xanthone derivatives (5,6). The nOe between H-6 (δ 7.03) with the 5-MeO protons at δ 3.87 therefore established the position of the OH at C-7, the methine being at C-6.

The ^{13}C-nmr spectrum of **2** (Table 1) showed the presence of sixteen carbon signals. DEPT experiments showed the presence of three methine and three Me carbon signals. The three aromatic methines at δ 97.15, 92.23, and 107.05 were assigned to the C-2, C-4, and C-6 carbons, respectively. The C-9 carbon was found to resonate at δ 180.23 while the C-6 methine carbon resonated at δ 107.05, being approximately 10.90 ppm downfield in comparison to the known 1,3,5,6,7-pentaoxygenated xanthone derivatives (11,13). Comparison of these spectral data of **2** with those of **1** showed that **2** was the 7-*O*-demethyl derivative of **1** (1,7-dihydroxy-3,5,8-trimethoxyxanthone).

Two known xanthone glycosides, 1,5-dihydroxy-3-methoxy-8-β-D-*O*-glucoside xanthone and 1,6,7-trihydroxy-3-methoxy-2-β-C-glucoside xanthone were also isolated for the first time from the EtOH extracts of *S. cordata*. On the basis of ms, uv, ir, and nmr, these compounds were identified as swertianolin and mangiferin, respectively. Previously they were isolated from *Swertia japonica* (15) and *Mangifera indica* (16–18).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The uv spectra were recorded in MeOH, MeOH with 0.05 ml of 0.01 N NaOH, and MeOH with 0.01 N HCl on a Shimadzu UV-240 spectrophotometer. The ir spectra were recorded in $CDCl_3$ or as KBr discs on a Jasco 1RA-1 spectrophotometer. The ^{1}H- and ^{13}C-nmr spectra were recorded in $CDCl_3$ solution on a Bruker AM-300 or AM-400 nmr spectrometer with TMS as internal reference. Hreims were measured on Jeol HX 110 mass spectrometer, and fdms were recorded on Varian MAT 312 double focusing mass spectrometer connected to DEC PDP 11/34 computer system. Optical rotations were measured on a Polatronic Universal Australian standard C-157 polarimeter.

PLANT MATERIAL.—The aerial parts of *S. cordata* were collected in August 1988 from Dooge

Gali, Peshawar, Pakistan. The air-dried plant (17 kg) was soaked in EtOH (15 liters) for 144 h. The EtOH extract was filtered and evaporated under reduced pressure at 50° to afford a dark green semi-solid mass (300 g). This EtOH residue was repeatedly extracted with petroleum ether (40°–60°) (3 liters).

1-Hydroxy-3,5,7,8-tetramethoxyxanthone [**1**].—The petroleum ether-soluble extract (50 g) was cooled and left overnight at 0°, resulting in a yellow material being deposited which was filtered and recrystallized from MeOH as yellow needles: mp 198–200° (60 mg, % yield 3.5×10^{-4}); $[\alpha]_D^{24}$ −226° (*c*=0.44, $CHCl_3$); uv λ max (MeOH) (log ε) nm 240 (3.65), 263 (3.45), 313 (3.09), 381 (2.95); λ min (MeOH) (log ε) nm 245 (3.55), 289 (3.03), 356 (2.88); uv λ max (MeOH+NaOH) 293, 238, 263, 312, 356 nm; ir λ max (KBr) cm^{-1} 3445 (OH), 1665 (C=O), 1610, 1586 (C=C); fdms *m/z* 332; eims *m/z* (rel. int.%) 332 (100), 317 (56) $[M-Me]^+$ 314 (64), 289 (80), 255 (24), 274 (26), 167 (27), 149 (37), 122 (22), 69 (62); hreims *m/z* 332.0897 ($C_{17}H_{16}O_7$, calcd 332.0896), 317.0646 ($C_{16}H_{13}O_7$, calcd 317.0661), 314.0764 ($C_{17}H_{14}O_6$, calcd 314.0790), 289.0688 ($C_{15}H_{13}O_6$, calcd 289.0712); ^{1}H-nmr ($CDCl_3$, 300 MHz) δ 3.85 (3H, s, OCH_3 at C-3), 3.88 (3H, s, Ar-OCH_3), 3.92 (3H, s, OCH_3 at C-5 or C-7), 3.95 (3H, s, Ar-OCH_3 at C-5 or C-7), 6.29 (1H, d, $J_{2,4}$=2.3 Hz, H-2), 6.42 (1H, d, $J_{4,2}$=2.3 Hz, H-4), 6.95 (1H, s, H-6), 12.17 (1H, s, OH at C-1); ^{13}C-nmr ($CDCl_3$, 75 MHz) see Table 1.

1,7-Dihydroxy-3,5,8-trimethoxyxanthone [**2**].—The petroleum ether-insoluble portion of the EtOH extract (250 g) was extracted with $CHCl_3$ and filtered. The $CHCl_3$-insoluble portion (190 g) was dissolved in MeOH (2 liters). The residue obtained from the MeOH extract was acidified with 10% HCl and extracted with $CHCl_3$. The concentrated $CHCl_3$ residue was basified with 2% NaOH and filtered. The filtrate was extracted with $CHCl_3$ and the concentrated $CHCl_3$ residue was dissolved in EtOH. The EtOH-soluble portion was filtered, concentrated (5 g), and chromatographed over Si gel. Elution was carried out with $CHCl_3$-EtOH (4:1) to yield the new xanthone. Compound **2** was isolated as yellow crystals: mp 240–242° (15 mg, % yield 8.8×10^{-5}); $[\alpha]_D^{24}$ −207 (*c*=0.2, MeOH); uv λ max (MeOH) (log ε) 238 (4.10), 262 (4.24), 314 (3.96), 385 (3.37) nm, λ min 243 (4.06), 287 (3.71), 361 (3.27); uv (MeOH+NaOH) 236, 260, 314, 386 nm; ir λ max ($CHCl_3$) cm^{-1} 3480, 3320 (OH), 1665 (C=O), 1610 (C=C); *m/z* (rel. int. %) 318 (100), 303 (84), 289 (47), 275 (46), 205 (40), 149 (56), 122 (20), 69 (56); hreims *m/z* 318.0739 ($C_{16}H_{14}O_7$, calcd 318.0739), 303.0510 ($C_{15}H_{11}O_7$ calcd 303.0504); ^{1}H nmr ($CDCl_3$ 400 MHz) δ 3.85 (3H, s, OCH_3 at C-3), 3.87 (3H, s, OCH_3 at C-5), 3.92 (3H, s, OCH_3 at C-8), 6.33 (1H, d, $J_{2,4}$=2,3 Hz, H-2), 6.36 (1H, d, $J_{2,3}$=2.3 Hz, H-4), 7.03 (1H, s, H-6), 10.30 (1H, brs, OH at C-1); ^{13}C nmr ($CDCl_3$, 100 MHz) see Table 1.

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