Phytochemistry, Vol. 28, No. 12, pp. 3569–3571, 1989.
Printed in Great Britain.

0031-9422/89 $3.00+0.00
© 1989 Pergamon Press plc

SWERTIABISXANTHONE-I FROM *SWERTIA MACROSPERMA*

HUI-MIN ZHOU, YONG-LONG LIU*, GÁBOR BLASKÓ†‡ and GEOFFREY A. CORDELL*†

Department of Phytochemistry, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Beijing 100094, People's Republic of China; † Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60680, U.S.A.

(*Received in revised form* 10 *May* 1989)

Key Word Index—*Swertia macrosperma*; Gentianaceae; xanthone derivatives; bisxanthone; swertiabisxanthone-I; selective INEPT.

Abstract—Swertiabisxanthone-I, a new bisxanthone, was isolated from *Swertia macrosperma*, and its structure elucidated as 1,3,5,8-tetrahydroxy-7-(1′,3′,5′,8′-tetrahydroxy-2′-xanthonyl)xanthone by ^{1}H, ^{13}C NMR and mass spectral evidence. In addition, two known xanthone derivatives were also identified.

INTRODUCTION

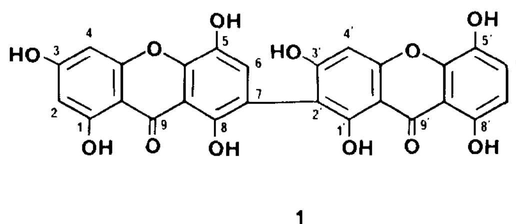
Plants of the genus *Swertia* (Gentianaceae) comprise about 70 species distributed throughout China, but especially in the south-western area [1]. About 20 species of *Swertia* have been used in traditional medicines for many years. Because these herbs taste extremely bitter and possess the ability to reduce fever, detoxify and act as choleretic and liver tonics, they have been mainly used for the treatment of hepatic and choleric and inflammatory diseases, such as hepatitis, cholecystitis, pneumonia, osteomyelitis, dysentery, scabies, spasm, pain and neurasthenia [2, 3]. *Swertia mileensis* and *S. mussoti* are especially efficacious for acute viral hepatitis and some preparations have been produced industrially in China [4]. Xanthone derivatives [5–8], flavonoids [9, 10], iridoid glycosides [11, 12] and triterpenoids [13, 14] have been reported as the main constituents of this genus.

Swertia macrosperma C. B. Clark is a medicinal plant used as a febrifuge, an antidote and a stomach tonic by the indigenous population in the south-western part of China. The chemical constituents of *S. macrosperma* have not been reported previously. Investigation of the whole plant of *S. macrosperma* led to the isolation and structure elucidation of swertiabisxanthone-I (**1**), a new dimeric xanthone with a C–C intermolecular linkage, and the identification of two known xanthone derivatives, 1,3,5,8-tetrahydroxyxanthone (**2**) and norswertianolin (**3**). This is the first discovery of a xanthone dimer of this skeletal type in Nature.

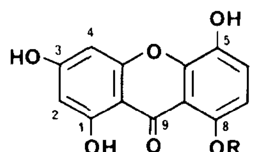
RESULTS AND DISCUSSION

From the ethyl acetate fraction obtained after partitioning the ethanol extract of the whole plants of *Swertia macrosperma* with different organic solvents, three compounds, swertiabisxanthone-I (**1**), together with the known 1,3,5,8-tetrahydroxyxanthone (**2**) and its 8-*O*-β-D-glucopyranoside (norswertianolin, **3**) were isolated by polyamide column chromatography.

Swertiabisxanthone-I (**1**) was obtained as an apricot coloured amorphous powder. Its UV spectrum in methanol and the spectra recorded in the presence of shift reagents were strikingly similar to those of 1,3,5,8-tetrahydroxyxanthone (**2**) (see Experimental). This indicated that **1** was a xanthone with the same oxygenation pattern as **2**. The field desorption mass spectrum (FDMS) of **1** afforded a molecular ion peak at m/z 518, and a fragment ion peak at m/z 259 $[M-259]^{+}$, suggesting that **1** was a dimeric xanthone with two equivalent monomeric xanthone units each bearing four hydroxy groups and linked by a C–C intermolecular bond. The ^{1}H NMR spectrum of **1** (Table 1) exhibited eight signals at δ9.66, 9.67, 11.13, 11.19, 11.24, 11.47, 11.92 and 12.23 (one proton each) due to the protons of the hydroxy groups of the aromatic rings, coupled signals for the *meta*-substituted aromatic protons at C-2 and C-4 (δ6.25 and 6.46, $J = 1.8$ Hz), two signals for the *ortho*-substituted aromatic protons at C-6′ and C-7′ (δ7.27 and 6.65, $J = 8.8$ Hz), and two singlets at



1



2 R = H

3 R = β-D-glucopyranosyl

*Authors to whom correspondence should be addressed.

‡On leave from the Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary.

Table 1. ^{1}H NMR data for compounds **1**–**3***

C	H-2	H-4	H-6	H-7	H-4′	H-6′	H-7′	Anomeric H
1	6.25 (*d*, *J* = 1.8 Hz)	6.46	7.15 (*s*)		6.62 (*s*)	7.27 (*d*, *J* = 8.8 Hz)	6.65	
2	6.19 (*d*, *J* = 2.0 Hz)	6.38	7.22 (*d*, *J* = 9.0 Hz)	6.58				
3	6.19 (*d*, *J* = 2.0 Hz)	6.39	7.26 (*d*, *J* = 9.0 Hz)	7.12				4.78 (*d*, *J* = 7.2 Hz)

*Recorded in $CDCl_3$. Chemical shift values are given in ppm (δ) using TMS as internal standard.

δ7.15 and 6.62 (one proton each), which were tentatively assigned to 6-H and 4′-H, respectively. The chemical shift values for 2-H, 4-H, 6′-H and 7′-H were essentially unchanged compared to the ^{1}H NMR data for 1,3,5,8-tetra-hydroxyxanthone (**2**). The singlet at δ7.15 was shifted *ca* 0.1 ppm upfield and the singlet at δ6.62 was shifted *ca* 0.2 ppm downfield relative to the corresponding chemical shift values of 6-H and 4-H in **2**. These data suggested the presence of a 2′-7 linkage between the two monomeric xanthone moieties and established the assignments of the signals at δ7.15 and 6.62 as 6-H and 4′-H, respectively. The location of the connection between the two monomeric xanthone units was further supported by ^{13}C NMR measurements. In the ^{13}C NMR spectrum of **1**, almost all of the carbon signals occurred in pairs (Table 2), with the exception of the C-2 (δ98.42) and C-7′ (δ109.30) resonances, where the corresponding signals occurred at δ113.31 and 124.95, and could be assigned to C-2′ and C-7, respectively. The presence of C-2 at δ98.42 as a protonated aromatic carbon, and the absence of the C-2′ signal as a methine carbon, supported the fact that one side of the linkage between the monomeric units was located at C-2′. Selective INEPT irradiation of 4′-H (δ6.62) resulted in resonance enhancements at δ101.09 and 113.31 permitting the assignment of C-8′b and C-2′, respectively. The chemical shift value and the quaternary character of the latter signal reaffirmed the linkage at the C-2′ position. Selective INEPT irradiation of 6-H (δ7.15) enhanced the signals at δ142.74 and 149.91 which could be assigned to C-4b and C-8, respectively. That the signal of C-7′ occurred at δ109.30, and that the corresponding methine carbon signal for C-7 was missing from the ^{13}C NMR spectrum of **1**, supported the second linkage location to involve C-7. Consequently, the quaternary carbon appearing at δ124.95 could be assigned to C-7 and the methine carbon at δ126.45 to C-6. It should be noted that when the APT spectrum of **1** was obtained on the Varian XL-300 spectrometer using standard Varian pulse programs the quaternary C-7 carbon could not be observed, probably due to its long relaxation time. The APT spectrum recorded on the Nicolet NMC-360 instrument with a modified pulse program applying a 2 sec delay time between acquisitions resulted in the detection of the C-7 quaternary carbon atom at δ124.95. Thus, the structure of **1** was established as 1,3,5,8-tetrahydroxy-7-(1′,3′,5′,8′-tetrahydroxy-2′-xanthonyl)-xanthone.

The ^{1}H and ^{13}C NMR data of compounds **2** and **3** were in good agreement with those described in the literature for 1,3,5,8-tetrahydroxyxanthone and 1,3,5,8-tetrahydroxyxanthone-8-*O*-β-D-glucopyranoside, i.e. norswertianolin, respectively [15].

Table 2. ^{13}C NMR data for compounds **1**–**3**

C	**1**		**2**	**3**
1	162.01	159.52 (1′)	162.07	162.74
2	98.42	113.31 (2′)	98.42	98.18
3	166.14	164.14 (3′)	166.20	165.29
4	93.80	94.23 (4′)	94.29	93.50
4a	157.27	156.24 (4′a)	157.27	156.29
4b	142.74	143.02 (4′b)	143.10	144.68
5	137.03	136.30 (5′)	137.08	140.79
6	126.45	123.47 (6′)	123.47	120.73
7	124.95	109.30 (7′)	109.24	112.46
8	149.91	151.62 (8′)	151.67	149.24
8a	106.99	107.24 (8′a)	107.24	111.74
8b	100.85	101.09 (8′b)	101.16	102.55
9	183.59	183.59 (9′)	183.47	180.55
G-1				103.28
2				73.50
3				75.99†
4				69.73
5				77.45†
6				60.85

*Recorded in $CDCl_3$.
†These values may be interchanged.
G: β-D-Glucopyranosyl.

EXPERIMENTAL

General. Mp uncorr. The ^{1}H NMR spectra were obtained on a FX-100 spectrometer operating at 100 MHz, on a Varian XL-300 spectrometer operating at 300 MHz, or on a JNM-GX 400 spectrometer operating at 400 MHz. The ^{13}C NMR measurements were recorded on a FX-100 spectrometer operating at 25.05 MHz, on a Varian XL-300 instrument operating at 75.4 MHz, or on a Nicolet NMC 360 spectrometer operating at 90.8 MHz. The selective INEPT experiments [16] were performed on a Nicolet NMC 360 spectrometer. Data sets of 16K covering a spectral width of 10 000 Hz were acquired. Proton pulse widths were calibrated by using a sample of HOAc in 10% C_6D_6 (^{1r}J = 6.7 Hz) in a 5 mm tube [17]. The radio frequency field strength for the soft proton pulse was on the order of 25 Hz in these experiments. For 6-H and 4′-H a value of 8 Hz was used for $^3J_{CH}$. Ten thousand acquisitions were accumulated in each irradiation. EIMS (at 70 eV) and FDMS (emitter current: 0–25 mA) were measured on a MAT-711 mass spectrometer.

Plant material. The whole plants of *S. macrosperma* were collected in Yunnan Province (China) in August 1986 and identified by Prof. Zhao-Yi Zhu (Department of Medicinal Plant

Resources, Institute of Medicinal Plant Development, People's Republic of China). A voucher specimen has been deposited at the Herbarium of IMPLAD.

Extraction and separation. The air-dried whole plants of *S. macrosperma* (19 kg) were cut into pieces and exhaustively extracted initially with 95% EtOH (twice), followed by 50% EtOH. The extracts were combined and concd under red. pres. until only H_2O remained. The filtrate was successively extracted with petrol, CH_2Cl_2 and EtOAc, respectively. The EtOAc extract (32.7 g), after evapn of the solvent, was repeatedly subjected to medium pressure chromatography (MPC) on Polyamide eluted with CH_2Cl_2 containing increasing amounts of MeOH. The fractions were collected according to the UV detected bands on the column. Each fraction was *ca* 250 ml. The ppt. (170 mg) from fraction 25 eluted with CH_2Cl_2–MeOH (20:1) was subjected to another Polyamide column (25 g) using $CHCl_3$ containing increasing amounts of MeOH as eluent. From the fractions eluted with $CHCl_3$–MeOH (10:1) 30 mg of compound **2** was obtained. From fractions 61–63 and 69 of the first separation 30 mg of compound **3** and 20 mg of compound **1** were isolated by repeated column chromatograpy on Polyamide using CH_2Cl_2–MeOH (8:1) as eluent.

Swertiabisxanthone-I (**1**). Apricot coloured amorphous powder, pptd from MeOH, mp >320°. R_f value: 0.29 on Polyamide plate developed by pyridine–MeOH (5:1) eluent. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1660, 1630 (C=O, conj.), 1620, 1590, 1505 (Ar); UV λ_{max}^{MeOH} nm: 224, 255, 280, 338,(MeOH + NaOMe) 230, 252, 306, 362, (MeOH + $AlCl_3$) 220 (sh), 255, 294, 332, 370, (MeOH + $AlCl_3$ + HCl) 220 (sh), 254, 292, 324, 370, (MeOH + NaOAc) 250 (sh), 270, 364, (MeOH + NaOAc + H_3BO_3) 254, 280, 338; ^{1}H NMR (DMSO-d_6, 400 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 75.4 MHz and 90.8 MHz), see Table 2; FDMS m/z: 518 $[M]^+$, 259 $[M-259]^+$.

1,3,5,8-*Tetrahydroxyxanthone* (**2**). Yellow needles, mp 293° (from MeOH); IR ν_{max}^{KBr} cm^{-1}: 3420, 3380 (OH), 1670, 1645 (C=O, conj.), 1620, 1595, 1505 (Ar); UV λ_{max}^{MeOH} nm: 226, 252, 276, 334, (MeOH + NaOMe) 230, 256, 302, 356, (MeOH + $AlCl_3$) 220 (sh), 258, 286, 326, 372, (MeOH + $AlCl_3$ + HCl) 220 (sh), 260, 288, 328, 372, (MeOH + NaOAc) 246 (sh), 268, 358, (MeOH + NaOAc + H_3BO_3) 250, 276, 334; ^{1}H NMR (DMSO-d_6, 100 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 25.05 MHz), see Table 2; EIMS m/z (rel. int.): 260 $[M^+]$ (100), 232 (7), 231 (9), 203 (8), 152 (5), 130 (5), 116 (5), 108 (4).

Norswertianolin (1,3,5,8-*tetrahydroxyxanthone*-8-O-β-D-*glucopyranoside*) (**3**). Pale yellow needles, mp 263–264° (from MeOH); IR ν_{max}^{KBr} cm^{-1}: 3490 (OH), 1650 (C=O, conj.), 1620, 1585, 1505 (Ar); UV λ_{max}^{MeOH} nm: 220, 248, 271, 327, (MeOH + NaOMe) 232, 256, 295, 356, (MeOH + $AlCl_3$) 261, 278, 324, 358, (MeOH + $AlCl_3$ + HCl) 261, 278, 324, 358, (MeOH + NaOAc) 262, 352, (MeOH + NaOAc + H_3BO_3) 248, 271, 330; ^{1}H NMR (DMSO-d_6, 300 MHz), see Table 1; ^{13}C NMR (DMSO-d_6, 25.05 MHz), see Table 2; EIMS m/z (rel. int.): 260 $[M]^+$ aglycone (100), 232 (6), 231 (6), 203 (6), 152 (4), 130 (4), 116 (5).

Acknowledgements—The authors are grateful to Prof. Zhao-Yi Zho for identification of the plant material. This work was supported, in part, by a grant from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. We thank the Research Resources Center, University of Illinois at Chicago, the NMR Service of the Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing and the NMR Service of Beijing Medical University, for the provision of spectroscopic facilities.

REFERENCES

1. Hou, K.-Z. (1984) *A Dictionary of the Families and Genera of Chinese Seed Plants*, p. 473. Science Press, Beijing.
2. Jiangshu New Medical College (1977) *A Dictionary of Chinese Traditional Medicines*. Shanghai People's Press, Shanghai.
3. Song, W.-Z. (1986) *Zhong Yao Tong Bao* **11**, 643.
4. The 59th Hospital of People's Liberation Army (1972) *Zhong Cao Yao Tong Xun* **5**, 38.
5. Verma, D. L. and Khetwal, K. S. (1985) *Sci. Cult.* **51**, 305.
6. Chung, M. I., Gan, K. H., Lin, C. N. and Chen, I. J. (1986) *Kao-hsiung I Hsueh K'o Hsueh Tsa Chih* **2**, 131; (1986) *Chem. Abstr.* **105**, 91124u.
7. Nozaka, T., Morimoto, I., Watanabe, F. and Okitsu, T. (1984) *Shoyakugaku Zasshi* **38**, 96; (1984) *Chem. Abstr.* **101**, 197991g.
8. Bhan, S. and Kalla, A. K. (1981) *Res. J. Fac. Sci., Kashmir Univ.* **1**, 10; (1984) *Chem. Abstr.* **100**, 64973k.
9. Khetwal, K. S. and Verma, D. L. (1984) *Indian J. Pharm. Sci.* **46**, 25.
10. Khetwal, K. S. and Verma, D. L. (1982) *Nat. Appl. Sci. Bull.* **34**, 337.
11. Ikeshiro, Y. and Tomita, Y. (1984) *Planta Med.* **50**, 485.
12. Ikeshiro, Y. and Tomita, Y. (1984) *Planta Med.* **51**, 390.
13. Prakash, A., Basumatory, P. C., Ghosal, S. and Handa, S. S. (1982) *Planta Med.* **45**, 61.
14. Zhang, J. and Mao, Q. (1984) *Yaoxue Xuebao* **19**, 819.
15. Sakamoto, I., Tanaka, T., Tanaka, O. and Tomimori, T. (1982) *Chem. Pharm. Bull.* **30**, 4088.
16. Sarkar, S. K. and Bax, A. (1985) *J. Magn. Reson.* **62**, 109.
17. Bax, A. (1983) *J. Magn. Reson.* **52**, 76.