Phytochemistry, Vol. 26, No. 8, pp. 2381–2384, 1987.
Printed in Great Britain.

0031–9422/87 $3.00 + 0.00

XANTHONES FROM FORMOSAN GENTIANACEOUS PLANTS*

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(*Received* 19 *August* 1986)

Key Word Index—*Tripterospermum lanceolatum; Swertia arisanensis; Gentianaceae*; xanthone; lanceolin; 1,4,8-trihydroxy-3,7-dimethoxyxanthone; methyllanceolin; 1,3-dihydroxy-4,7,8-trimethoxyxanthone; oleanolic acid; ursolic acid; sweroside; ^{13}C NMR spectroscopy.

Abstract—A reinvestigation of the whole plants of *Tripterospermum lanceolatum* gave, in addition to compounds isolated previously, two new xanthones, named lanceolin and methyllanceolin, respectively. The compound previously reported to be oleanolic acid was re-examined and identified as ursolic acid by a ^{13}C NMR study. An isomeric mixture, oleanolic acid and ursolic acid, and sweroside were isolated from the whole plants of *Swertia arisanensis*.

INTRODUCTION

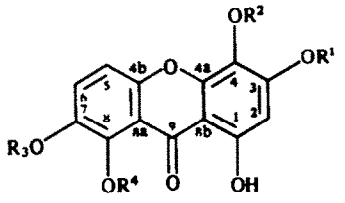
The *Gentianaceae* is known to produce xanthonoids, flavonoids and secoiridoids with potential therapeutic effects [1]. As part of a search for active constituents in various genera of Formosan *Gentianaceae*, the fresh whole plants of *Tripterospermum lanceolatum* (Hayata) Hara ex Satake and *Swertia arisanensis* Hayata were investigated.

Some time ago we isolated oleanolic acid **3**, mangiferin and two xanthone glycosides [2] from *Tripterospermum lanceolatum*. The new compounds show remarkable biological activities [3–5] and therefore we have reinvestigated the whole plant. The chloroform fraction afforded two kinds of new natural xanthones, named lanceolin (**1**) and methyllanceolin (**2**), respectively.

Swertia arisanensis is relatively common in south Taiwan. It is endemic under forests or along roadsides on mountains, and is frequently found in the middle and northern parts of the central mountain range from an altitude of 1400 to 3000 m.

We have now obtained an isomeric mixture (**5**) of oleanolic acid and ursolic acid, and a secoiridoid glucosides, sweroside (**6**), from *Swertia arisanensis* and the components were identified by spectral comparison. The ^{13}C NMR spectroscopy data of these compounds have not been described previously.

*Part IX in the series 'Studies on the Constituents of Formosan *Gentianaceous* Plants'. For part VIII see ref. [11].



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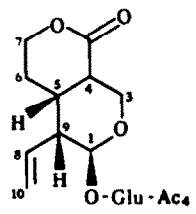

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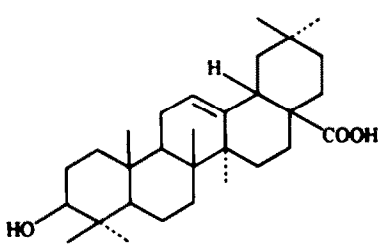

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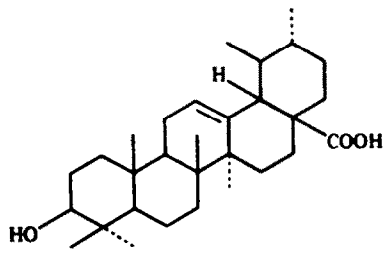

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RESULTS AND DISCUSSION

Lanceolin (**1**), $C_{15}H_{12}O_7$, showed UV, IR and NMR spectra characteristic of a 1,3,4,7,8-pentaoxygenated xanthone [6,7]. A bathochromic shift with aluminium trichloride ($AlCl_3$) suggested the presence of a C-1 or C-8 hydroxy function. The ^{1}H NMR spectrum (DMSO-d_6) showed two singlets at δ3.82 (3H) and 3.92 (3H) assignable to the protons of two OMe groups. A singlet at δ6.53 (1H) was attributed to the proton located at the C-2 position and a pair of doublets (1H, J = 9 Hz) arose from protons at the C-5 and C-6 positions. Two singlets at δ11.43 (1H) and 11.87 (1H) were assignable to the protons of the C-1 and C-8 hydroxyl groups [8] (exchanged by D_2O) and indicated that **1** is a 1,8-dihydroxyxanthone. The UV spectrum of **1** showed a bathochromic shift with $AlCl_3$, but was unchanged on addition of sodium acetate (NaOAc) and (NaOAc)–boric acid (H_3BO_3). Based on the above data, compound **1** is 1,4,8-trihydroxy-3,7-dimethoxyxanthone and the identity was proved by comparison of its mp and spectral properties (MS, UV, IR and NMR) with those of the aglycone of lanceoside [2]. The ^{13}C NMR spectrum of **1** also confirmed the above assigned structure. According to our knowledge, lanceolin has not been reported as a natural product.

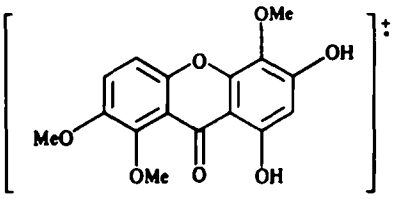
Methyllanceolin (**2**), $C_{16}H_{14}O_7$. $[M]^+$ at m/z 318, showed IR, UV and NMR spectra characteristic of a 1,3,4,7,8-pentaoxygenated xanthone [2,6]. A bathochromic shift with $AlCl_3$ and NaOAc, clearly indicated that compound **2** possessed a free C-1 or C-8 hydroxyl group and a C-3 or C-6 hydroxyl group. The ^{1}H NMR spectrum (DMSO-d_6) of **2** showed three singlets (3H each) centered at δ3.76, 3.80 and 3.90 assignable to the protons of three OMe groups. A singlet at δ6.50(1H) appeared at relatively high field and was attributed to the proton located at the C-2 position and a pair of doublets (1H, J = 9 Hz) centered at δ7.25 and 7.39 were assignable to the protons located at the C-5 and C-6 positions, respectively. The compound possessed a chelatable hydroxyl group, as indicated by an $AlCl_3$ induced UV shift and a strongly deshielded singlet at δ13.13, belonging to the C-1 or C-8 hydroxyl proton [8] and another broad singlet at δ9.36 was attributed to the proton located at C-3. The UV spectrum was unchanged on addition of NaOAc + H_3BO_3 and the mass spectral fragmentation pattern is shown in Scheme 1. Based upon the above results compound **2** is a 1,3-dihydroxy-4,7,8-trimethoxyxanthone or a 3,8-dihydroxy-1,4,7-trimethoxyxanthone.

The structure of **2** was confirmed by its ^{13}C NMR data (Table 1), in which all the carbon atoms were assigned by means of broad-band heterodecoupling and off-resonance decoupling spectra and comparison with those of **1** (Table 1). In the ^{13}C NMR spectrum of **2**, two methoxyl carbon signals shifted downfield to δ60.9 and 61.0, clearly indicating that **2** possesses an *ortho*-methoxyl group [9,10]. Therefore, according to the above results and a negative reaction of compound **2** with Gibb's reagent, the structure of **2** was determined as 1,3-dihydroxy-4,7,8-trimethoxyxanthone (**2**).

The isomeric mixture (**5**) was recrystallized from methanol as colourless needles, mp 223–224°. It showed a violet-red response in the Liebermann Burchard reaction and the IR spectrum exhibited absorption bands at 3400 (alcoholic OH) and 1690 cm^{-1} (carboxylic CO). The mass spectrum showed a molecular ion at m/z 456, and fragments at m/z 248 (base peak), 207, 203, 189 and 133 suggesting that **5** is oleanolic acid (**3**) or ursolic acid (**4**) [12].

The ^{13}C NMR spectra of **3** and **4** were assigned as shown in Table 1. Except for the signals carbons 11–13, 17–22, 27, 29, and 30, all the other signals were superimposable on those in the spectrum of **4**, listed in Table 1. Since the ^{13}C NMR spectrum of **5** was found to exhibit a good coincidence with those of **3** and **4**, it was concluded that **5** is an isomeric mixture of oleanolic acid (**3**) and ursolic acid (**4**).

Sweroside was acetylated to yield sweroside tetraacetate (**6**) and recrystallized from methanol as colourless needles. The mp, UV, $[\alpha]_D$, IR, ^{1}H NMR and mass spectrum of **6** agreed with those of reported data [13]. It



m/z 318 $[M]^+$ (46.8)
↓ −Me
m/z 303 (100)
↓ −Me
m/z 288 (29.8) —(−CHO)→ m/z 259 (8.6) —(−CO)→ m/z 231 (8.4)
↓ −Me
m/z 273 (8.5)

Scheme 1. Diagnostic fragmentations in the mass spectrum of xanthone **2**.

Table 1. The ^{13}C NMR Chemical Shifts of compounds 1–6

Carbon number	1*,†	2†	Carbon number	5‡ 3‡	5‡ 4‡	Carbon number	6§
C-1	157.3	159.2	C-1	38.4	38.7	C-1	96.0
C-2	95.0	94.6	C-2	27.2	27.2	C-3	151.0
C-3	160.0	158.4	C-3	79.4	78.2	C-4	105.3
C-4	129.7	127.5	C-4	38.7	38.8	C-5	27.6
C-5	106.1	113.4	C-5	55.2	55.2	C-6	24.7
C-6	124.1	124.4	C-6	18.3	18.3	C-7	68.0
C-7	140.4	137.6	C-7	32.6	33.0	C-8	130.9
C-8	147.9	145.3	C-8	39.3	39.5	C-9	42.1
C-8‡	101.4	102.7	C-9	47.6	47.5	C-10	120.7
C-4†	147.0	145.3	C-10	37.0	36.9	>C=O	164.5
C-4‡	147.9	149.4	C-11	23.0	17.1	-OCOCH$_3$	20.5
C-8†	106.1	113.4	C-12	122.6	125.2	-OCOCH$_3$	168.7
C-9	184.0	180.6	C-13	143.6	138.3		168.9
OMe	56.7	56.4	C-14	41.6	42.0		169.5
	56.7	60.9	C-15	27.7	28.2		170.0
		61.0	C-16	23.4	24.2	C-1′	96.4
			C-17	46.5	47.5	C-2′	70.5
			C-18	41.0	52.7	C-3′	72.3
			C-19	45.9	39.1	C-4′	68.3
			C-20	30.6	38.8	C-5′	72.3
			C-21	33.8	30.7	C-6′	61.7
			C-22	32.4	36.7		
			C-23	28.1	28.0		
			C-24	15.6	15.7		
			C-25	15.3	15.4		
			C-26	16.9	17.0		
			C-27	26.0	23.5		
			C-28	179.9	179.9		
			C-29	33.1	23.2		
			C-30	23.6	21.2		

*See refs [9–11].
†In DMSO d_6, δ: ppm, TMS.
‡In pyridine, δ: ppm, TMS.
§In $CDCl_3$, δ: ppm, TMS.

was identified by mmp and IR comparison with authentic sample.

EXPERIMENTAL

All mps were uncorr. IR spectra were recorded on a Hitachi model 260–30 infrared spectrophotometer. Specific rotations were determined with a Jasco model dip-181 digital polarimeter. ^1H- and ^{13}C-NMR spectra [δ(ppm), J(Hz)] were run on a Bruker 100 MHz FT-NMR spectrometer. Mass spectra were determined on a Jeol JMS-D-100 mass spectrometer.

Extraction and Separation. Fresh material of *Tripterospermum lanceolatum* (0.65 kg) was collected at Alishan, Taiwan, in July 1979, chipped and extracted several times with hot MeOH. The MeOH extract was evaporated under red. pres. The H_2O soluble part of the MeOH extract was successively extracted with $CHCl_3$, EtOAc and *n*-BuOH. The $CHCl_3$ extracted fraction was chromatographed on a silica gel column. The column was eluted with benzene and benzene–EtOAc to afford lanceolin (1) in the former fraction and methyllanceolin (2) in the latter fraction eluted with bnzene–EtOAc (19:1), respectively, and ursolic acid (3) eluted with benzene–EtOAc (9:1). Fresh material of *Swertia arisanensis* (0.6 kg) was collected at Mt. Hohuan, Taiwan, in November, 1983, chipped and extracted as described above. The *n*-BuOH extract was chromatographed on a silica gel column. The column was eluted with benzene, benzene–EtOAc, $CHCl_3$, $CHCl_3$–MeOH and MeOH to afford an isomeric mixture (5) eluted with benzene–EtOAc (9:1) and sweroside eluted with $CHCl_3$–MeOH (4:1).

Lanceolin (1,4,8-*trihydroxy*-3,7-*dimethoxyxanthone*) (1). Yellow needles (MeOH), mp 233–234°, red colour under UV and negative reaction with Fehling's soln. (Found: C, 59.29; H, 3.84; $C_{15}H_{12}O_7$ requires: C, 59.21; H, 3.98%) UV λ_{max}^{MeOH} nm (log ε): 237 (4.26), 269 (4.51), 309 (3.81), 343 (4.04), 400 (3.48); + $AlCl_3$: 245, 280, 327, 383. IR ν_{max}^{KBr} cm^{-1}: 1640, 1620, 1600, 1580. ^1H NMR (DMSO-d_6): δ 3.82 (3H, *s*, OMe), 3.92 (3H, *s*, OMe), 6.53 (1H, *s*, H-2), 6.87 (1H, *d*, J = 9 Hz, H-5), 7.27 (1H, *d*, J = 9 Hz, H-6), 11.43 (1H, *s*, ex. D_2O, 1-OH or 8-OH), 11.87 (1H, *s*, ex. D_2O, 1-OH or 8-OH). ^{13}C NMR (DMSO-d_6): Table 1.

Methyllanceolin (1,3-*dihydroxy*-4,7,8-*trimethoxyxanthone* 2). Yellow needles (MeOH), mp 218–220°, red colour under UV and negative reaction with Fehling's soln. (Found: C, 59.98; H, 4.44; $C_{16}H_{14}O_7$ requires: C, 60.38; H, 4.43%) UV λ_{max}^{MeOH} nm (log ε): 235 (4.27), 264 (4.32), 322 (3.96), 387 (3.64), + $AlCl_3$: 230, 272, 330,

450, + NaOAc: 235, 280, 330, 390. MS *m/z*: 318 $[M]^+$ (46.8), 303 (100), 288 (29.8), 273 (8.5), 259 (8.6), 231 (8.4) (Scheme 1). IR ν_{max}^{KBr} cm^{-1}: 1650, 1620, 1570. ^1H NMR (DMSO-d_6): δ3.76 (*s*, OMe), 3.80 (*s*, OMe), 3.90 (*s*, OMe), 6.50 (1H, *s*, H-2), 7.25 (1H, *d*, J = 9 Hz H-5), 7.39 (1H,*d*, J = 9 Hz, H-6), 9.60 (1H, *br s*, ex. D_2O, 3-OH), 13.13 (1H,*s*,ex. D_2O, 1-OH). ^{13}C NMR (DMSO-d_6): Table 1.

Ursolic acid (**4**). Colourless needles (EtOH), mp 240–242°, $[\alpha]_D^{23}$+49.2° (c = 0.5, pyridine), violet-red in Liebermann Burchard reaction. IR ν_{max}^{KBr} cm^{-1}: 3400 (alcoholic OH), 1690 (carboxylic CO). MS *m/z* (rel. int.): 456 $[M]^+$ (8), 248 (100), 207, (31), 203 (70), 189 (53), 133 (36). ^{13}C NMR ($CDCl_3$): Table 1.

Isomeric mixture (**5**). Colourless needles (MeOH), mp 223–224°, IR ν_{max}^{KBr} cm^{-1}: 3400 (alcoholic OH), 1690 (carboxylic CO). MS *m/z* (rel. int.): 456 $[M]^+$ (6.5), 248 (100.0), 207 (33.4), 203(56.7), 189 (20.0), 133 (39.4). ^{13}C NMR ($CDCl_3$): Table 1.

Sweroside tetraacetate (**6**). Colourless needles (MeOH), mp 167–168°, $[\alpha]_D^{24}$-177° (c = 1, $CHCl_3$). UV λ_{max}^{MeOH} nm: 243. IR ν_{max}^{KBr} cm^{-1}: 1750, 1710, 1620. ^{13}C NMR ($CDCl_3$):Table 1.

Acknowledgements. The authors are deeply indebted to the National Science Council of the Republic of China for financial support. Thanks are due to Prof. Dr H.-C. Shieh, President of Kaohsiung Medical College and Prof. Dr N. Morita, Dr M. Shimizu and Dr M. Arisawa, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Japan, for their encouragement. We also thank Prof. Dr Hiroyuki Inouye, Faculty of Pharmaceutical Sciences, Kyoto University, Japan, for his kind gift of authentic sweroside tetraacetate, Mr B.-G. Liu, Department of Chemistry, National Tsiung Hua University, for mass spectra and Miss B.-J. Luh and Miss. Y.-H. Tsai, Department of Chemistry, National Cheng Kung University, for NMR spectra and elemental analysis, respectively.

REFERENCES

1. Hostettmann, K. and Wagner, H. (1977) *Phytochemistry* **16**, 821.
2. Lin, C.-N., Chang, C.-H., Arisawa, M., Shimizu, M. and Morita, N. (1982) *Phytochemistry* **21**, 205.
3. Lin, C. -N., Chung, M.- I., Arisawa, M., Shimizu, M. and Morita, N. (1984) *Shoyakugaku Zasshi*, **38**, 80.
4. Lin, C. -N., Chiang, J.- R., Arisawa, M., Shimizu, M. and Morita, N. (1984) *Shoyakugaku Zasshi*, **32**, 155.
5. Hikino, H., Ohsawa, T., Kiso, Y. and Oshima, Y. (1984) *Planta Med.* **50**, 353.
6. Markham, K. R. (1965) *Tetrahedron* **21**, 3688.
7. Komatsu, M., Tomimori, T. and Mikuriya, N. (1969) *Chem. Pharm. Bull.* **17**, 156.
8. Ghosal, S., Shama, P. V. and Chaudhuri, R. K. (1974) *J. Pharm. Sci.* **63**, 1288.
9. Chaudhuri, R. K., Zymalkowski, F. and Frahm, A. W. (1978) *Tetrahedron* **34**, 1837.
10. Frahm, A. W. and Chaudhuri, R. K. (1979) *Tetrahedron* **35**, 2035.
11. Lin, C. -N., Chung, M.- I., Arisawa, M., Shimizu, M., Morita, N. *Phytochemistry* (submitted for publication).
12. Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
13. Inouye, H., Ueda, S. and Nakamura, Y. (1970) *Chem. Pharm. Bull.* **18**, 1856.