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SWERTIFRANCHESIDE, AN HIV-REVERSE TRANSCRIPTASE INHIBITOR AND THE FIRST FLAVONE-XANTHONE DIMER, FROM *SWERTIA FRANCHETIANA*

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ABSTRACT.—The first flavone-xanthone *C*-glucoside, swertifrancheside, was isolated from *Swertia franchetiana*, and its structure was elucidated on the basis of spectroscopic analysis as 1,5,8-trihydroxy-3-methoxy-7-(5′,7′,3″,4″-tetrahydroxy-6′-*C*-β-D-glucopyranosyl-4′-oxy-8′-flavyl)-xanthone. This compound was a moderately potent inhibitor of HIV reverse transcriptase.

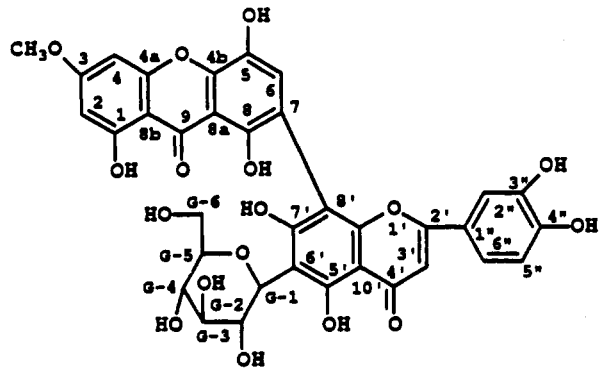
The genus *Swertia* (family Gentianaceae) comprises 170 species, of which 79 species are distributed in China (1). About 20 species of this genus have been used in Chinese traditional medicine for the treatment of hepatic, choleric, and inflammatory diseases (2,3). In previous phytochemical studies, xanthone derivatives, flavonoids, iridoid glycosides and triterpenoids have been reported as the main constituents, and more recently, dimeric xanthones, such as swertiaxanthone-I and swertipunicoside have been isolated from this genus (4,5).

Swertia franchetiana tastes very bitter, possesses the ability to reduce fever, and can be used as an antidote. It is employed in the southwestern part of China for the treatment of hepatogenous jaundice and cholecystitis. Xanthone glycosides and flavonoid glucosides have been isolated from this species (6). This paper deals with the isolation and structure determination of a new flavone-xanthone *C*-glucoside, swertifrancheside [**1**], from *Swertia franchetiana* through a series of one and two-dimensional nmr techniques, including COSY, phase-sensitive ROESY (6–9), reverse-detected HMQC (9) and HMBC (9,10).

RESULTS AND DISCUSSION

Swertifrancheside, a yellow powder, analyzing for $C_{35}H_{29}O_{17}$ (hrfabms), showed from its ^{1}H-, ^{13}C-, and APT nmr spectra the presence of one methoxy, seven aromatic methines, five aliphatic methines, one aliphatic methylene, two carbonyls, and 19 quaternary carbons. The reverse-detected HMQC spectrum of **1** revealed the direct attachment between protons and carbons, and the reverse-detected HMBC spectrum showed long-range correlations between ^{1}H and ^{13}C through three bonds and two bonds. Analysis permitted assignment of the quaternary carbons, and, thus, the elucidation of the structural skeleton.

The HMBC nmr spectrum of **1** (Table 1) showed correlation contours between 5′-OH (δ 13.65 and 13.64) and C-6′, 5′-OH and C-5′, 5′-OH and C-10′, H-6 (δ 7.25 and 7.24) and C-8, H-6 and C-5, H-6 and C-4b, and H-6 and C-8′. The latter correlation in particular indicated that C-8 of the flavone unit was connected to C-7 of the xanthone unit. The HMBC spectrum also showed the correlation contours between a methoxy (δ 3.88) and C-3, H-2 (δ 6.43, d, J=2 Hz) and C-1, H-2 and C-3, H-2 and C-4, and H-2 and C-8b. Due to the signal overlap of H-4 (δ 6.69, s) with the H-3′ (δ 6.70, 6.71, 2s) and H-5″ (δ 6.67, d) signals, it was very difficult to irradiate one of those protons to enhance carbon signals using the selective INEPT nmr technique. However, after



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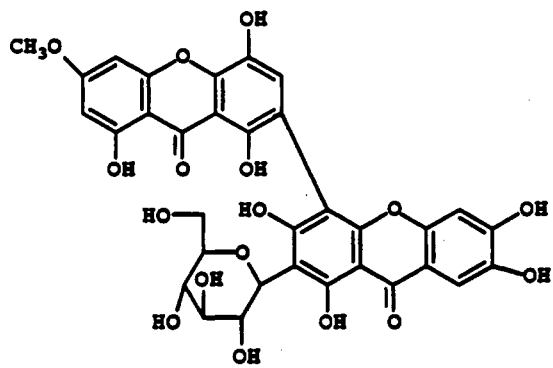

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expansion of the HMBC spectrum, it was possible to recognize the enhancements of these protons on irradiation of the carbons. Therefore, the contours between H-4 and C-3, H-4 and C-4a, H-4 and C-8b, H-4 and C-2, H-3′ (δ 6.70 and 6.71) and C-4′, H-3′ and C-2′, H-3′ and C-10′, H-3′ and C-1″, H-5″ and C-1″, H-5″ and C-3″, and H-5″ and C-4″ could be recognized. This spectrum also showed correlation contours between H-6″ (δ 7.09 and 7.11) and C-3″, H-6″ and C-2″, H-6″ and C-2′, H-2″ (δ 6.97 and 6.98) and C-2′, H-2″ and C-3″, H-2″ and C-4″, H-2″ and C-6″, H-G1 (δ 4.78) and C-G2, H-G1 and C-G3, H-G1 and C-6, H-G1 and C-5′, H-G1 and C-7′, which indicated that a glucose is connected to the C-6′ position of the flavone unit. According to the chemical shifts, two of the remaining four signals, at δ 185.18 and 153.47, which were more than three bonds away from their nearest protons and showed no correlation contours with any protons, should be assigned to C-9 and C-9′, respectively. Two other remaining signals, at δ 112.69 and 108.69, could be assigned to C-7 and C-8a, respectively, by comparison with the data of swertipunicoside [**2**]. Most of the other signals for similar carbons of **1** and **2** were very close, and, thus, the nmr data of **2** were assigned by analyzing the results of extensive selective INEPT and fully coupled ^{13}C-nmr spectra (4). In this HMBC spectrum, except for H-G1, which showed correlations with C-G2 and C-G3, neither H-G2, nor H-G3, H-G4, H-G5 and H-G6 showed correlations with any carbon; they were assigned from their correlations in the HMQC and COSY spectra.

The COSY nmr spectrum of **1** showed correlation contours between H-2 (δ 6.43) and H-4 (δ 6.69), H-6″ (δ 7.09, dt, 2, 8) and H-5″ (δ 6.68), H-6″ and H-2″ (δ 6.97 and 6.98), H-G1 (δ 4.78) and H-G2 (δ 3.63, m), H-G6a (δ 3.62, m) and H-G6b (δ 3.53, d, 12 Hz), H-G2 and H-G3 (δ 3.27, m), H-G6b and H-G5 (δ 3.29). The ROESY nmr spectrum showed clear correlation contours between the 3-methoxy and H-2, and the 3-

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of Swertifrancheside [**1**] from *Swertia franchetiana*.[a]

Compound	^{1}H	^{13}C	Correlated ^{13}C in HMBC[b]
1	—	161.93	—
2	6.43 (d, 2)	97.67	(1), (3), 4, 8b
3	—	166.88	—
4	6.69 (s)	93.12	2, (3), 4a, 8b
4a	—	157.49	—
4b	—	143.44	—
5	—	136.88	—
6	7.25 and 7.24 (2s)	126.72	(5), 8, 4b, 8′
7	—	112.69	—
8	—	150.29 and 150.00	—
8a	—	108.69	—
8b	—	102.22	—
9	—	184.18	—
3-OMe	3.88 (s)	56.36	3
2′	—	163.83 and 163.75	—
3′	6.71 and 6.70 (2s)	102.52	(2′), (4′), 10′, 1″
4′	—	182.18	—
5′	—	159.04 and 158.94	—
6′	—	107.57	—
7′	—	160.05	—
8′	—	103.16 and 103.01	—
9′	—	153.47	—
10′	—	103.49	—
1″	—	121.41	—
2″	6.98 and 6.97 (2d, 2)	113.46	2′, (3″), 4″, 6″
3″	—	149.74	—
4″	—	145.52	—
5″	6.68 (d, 8)	115.74	1″, 3″, (4″)
6″	7.11 and 7.09 (2dd, 8,2)	107.57	2′, 2″, 3″
5′-OH	13.65 and 13.64 (2s)	—	6′, (5′), 10′
G-1	4.78 (d, 9.5)	74.04	5′, (6′), 7′, (G-2), G-3
G-2	3.63 (m)	71.69	#
G-3	3.27 (m)	78.19 and 77.88	#
G-4	3.28 (m)	69.36	#
G-5	3.29 (m)	81.25 and 82.14	#
G-6a	3.62 (m)	60.33 and 60.11	#
G-6b	3.53 (d, 11.5)	—	#

[a]Recorded in DMSO-d_6, chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz for ^{1}H and 90.8 MHz for ^{13}C; signal multiplicity and coupling constants (Hz) are shown in parentheses.

[b]The HMBC experiment was performed at 125.8/500.1 MHz with $J=6$ Hz; two-bond correlations are shown in parentheses; #indicates no clear correlations with this proton were observed on irradiation of the carbons.

methoxy and H-4. Integrated interpretation of this data suggested that this compound should have structure **1**, and the complete and unambiguous assignments of its ^{1}H- and ^{13}C-nmr data are shown in Table 1.

The ^{1}H signals for C-5′-OH (2s, δ 13.647 and 13.643) and H-6 (2s, δ 7.259, 7.249) appeared as two singlets without substantial change during the measurements taken with about 1 mg of **1** in about 0.4 ml of DMSO-d_6 at different temperatures (20°, 30°, 40°, 50° and finally 60°). In addition, the ^{13}C signals for C-8, C-2′, C-5′, C-8′, G-3, G-5, and G-6, appeared as two peaks with chemical shift differences of 0.05–0.3 ppm, indicating that there might be two isomers, instead of two conformers, in solution. These isomers might be derived by the different orientations of the major ring systems without

free rotation due to the existence of OH functions. This observation was noted previously for swertipunicoside [**2**] (4).

In order to explain the splitting observed in some of the ^{1}H- and ^{13}C-nmr signals of **1**, molecular mechanics calculations were performed on its structure (11), using the interactive program PCMODEL with the MMX force field. Initially, we supposed that such splitting in the nmr signals would be due to restriction in the rotation around bonds G-1, C-6′ and/or C-8′, C-7; but, according to the literature (12), restricted rotation of the *C*-glycosyl moiety at C-6 is observed in 6-*C*-β-D-glucosylflavones only if a MeO or an *O*-β-D-glucose group was attached to the 7-position, giving rise to split signals in the ^{13}C-nmr spectrum, run at 30° in DMSO-d_6. Coalescence of the split signal was observed as the temperature is increased (12). In our case, **1** contains an OH group at C-7 of the flavone nucleus and, thus, restricted rotation of the sugar moiety should not be expected, unless the bulky group at C-8 produced a significant effect.

Before attempting energy minimization of the whole structure of **1**, the xanthone, flavone, and sugar moieties were generated separately. In a first approach, the sugar was attached to the flavone. Rotation around bond G-1,C-6′, with and without the xanthone group linked to C-8′, did not show considerable differences in the level of the calculated rotational barriers, indicating that the xanthone moiety does not produce an important effect in the rotation of the *C*-glucosyl chain. This led us to focus on the rotation of the xanthone group around the bond C-7, C-8′.

After the global minimum of the *C*-6-β-glucosylflavone part was determined, the xanthone group was linked to the flavone, and when rigid rotor approximation calculations were performed around bond C-7, C-8′, four minima and four maxima were found. However, not all of the minima obtained from rigid rotation calculations are real. Hence, in order to determine if these minima are real or not, several energy minimization calculations have been performed freeing the structure from different torsion angles around bond C-7, C-8′. As a result, from these calculations, four rotamers (**1a**, **1b**, **1c**, and **1d**) were found with small relative energy between them (see Table 2). Two of the energy maxima were very high and were observed between rotamers (**1a** and **1d**, and **1b** and **1c**) (see Table 2). Interconversion from **1a** to **1b** and **1c** to **1d** on the other hand showed energy maxima of less than 1 kcal/mol. Such very high energy maxima were found to be due to strong nonbonding interactions of the van der Waals (VDW) type between O-8 and O-1′, and H-6 and O-7′ in one case, and between O-8 and O-7′, and H-6 and O-1′ in the other. It is noteworthy that when the dihedral angle C-9′, C-8′, C-7, C-8 is 0° or 180°, the interatomic distance O-8, O-1′ in the first case, and O-8, O-7 in the second, takes a value of less than the normal VDW radius for this oxygen atom type, which is about 1.74 Å (see Table 3). Considering that during the rigid rotation there is no minimization at all, the calculated energy of the maxima are higher than

TABLE 2. Relative Energies of Rotamers **1a–1d** with Their Respective Values for the Dihedral Angle C-9′, C-8′, C-7, C-8.

Dihedral angle	Relative energy (kcal/mol)	Rotamer
0°	376[a]	
58.60°	0.57	**1d**
106.93°	1.33	**1c**
180°	409[a]	
−121.12°	1.29	**1b**
−63.77°	0.00	**1a**

[a]Maximum value observed by rigid rotation.

TABLE 3. Some Selected Distances (DIS) in Å When the Dihedral Angle C-9′, C-8′, C-7, C-8 Takes Values 0° and 180°.

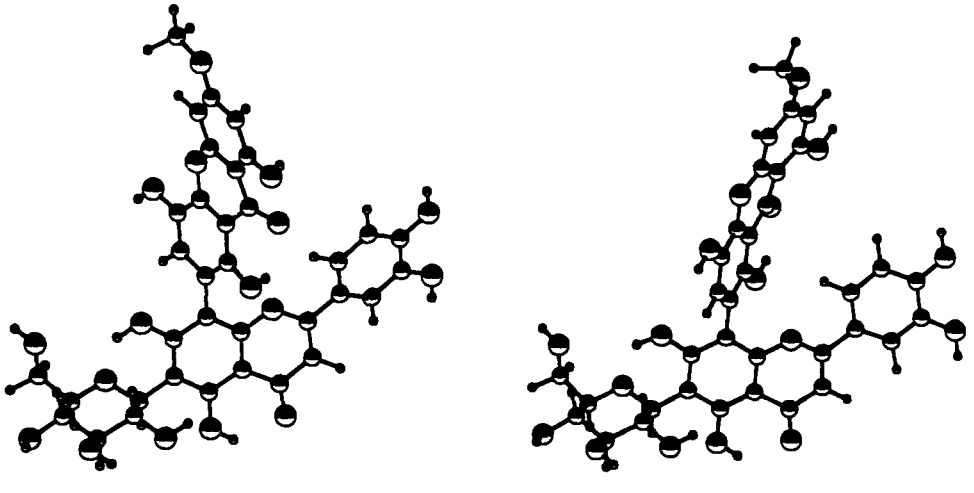
Dihedral angle	DIS O-7, O-8′	DIS H-6, O-7′	DIS H-6, O-8′	DIS O-7, O-7′
0°	1.588	1.583	—	—
180°	—	—	1.647	1.526

reality. Hence, in order to find more accurate values for the rotational barriers, we have performed rotation with full relaxation of all degrees of freedom, except for the torsion around bond C-7, C-8′ (dihedral driving). The rotation during the dihedral driving was made in steps of 2°, and the last structure was rotated to the next step. We have observed during the rotation that when the dihedral angle is approaching 0° or 180° (energy maxima in rigid rotation), the interacting oxygens bend apart to minimize the strong VDW repulsion. At 45° after this point, one oxygen crosses over the other, thereby reaching the maximum energy values (rotational barrier). We found barriers with a relative energy of 21 kcal/mol for the interconversion between **1a** and **1d**, and 22 kcal/mol for the interconversion between **1b** and **1c**. These values are quite high and explain the absence of coalescence of the signals in the nmr experiment run at 60°, and hence give rise to the existence of the two observed major atropoisomers. Figure 1 shows a stereoscopic view of the selected rotamers **1a** and **1d**.

To our knowledge, and from a detailed literature search, swertifrancheside is the first flavone-xanthone dimer to be isolated (4,5). This compound was subjected to anticancer (14 cell lines), antimalarial (2 strains) (13,14) and HIV-1 RT inhibitory tests (15,16). No cytotoxicity or antimalarial activity was observed, but this isolate showed inhibitory activity of 99.8% at 200 μg/ml (ED_{50}=30.9 μg/ml) in the HIV-1 reverse transcriptase inhibitory test (15,16). Swertipunicoside [**2**] displayed an ED_{50} of 3.0 μg/ml in this assay.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Cc was carried out with polyamide (Nylon 66, particle size 80–100 mesh, Lixian Chemical Co., Hunan Province, People's Republic of China). Mplc was performed



1a **1d**

FIGURE 1. Three-dimensional View of the Selected Rotamers **1a** and **1d**.

over a polyamide column (particle size 160–180 mesh). The uv spectrum was measured on a Phillips Pye Unicam PU8800 spectrophotometer, and the ir spectrum was taken on a Perkin-Elmer 983G instrument and recorded in a KBr pellet. The optical rotation was taken with a Perkin-Elmer 241 polarimeter, and ms were determined on DX-300 and Finnigan MAT-90 instruments in the positive-fab mode using glycerin and glycerin-NOBA as matrices. ^{13}C- and APT nmr spectra were taken on a Nicolet NT-360 instrument operating at 90.8 MHz, with a solution of 10 mg of **1** in 0.3 ml of DMSO-d_6. ^{1}H-Nmr, COSY, ROESY, HMQC and HMBC spectra were taken on a GE OMEGA 500 instrument operating at 500.1 MHz for ^{1}H- and homonuclear 2D nmr spectra, and 125.8 MHz/500.1 MHz for HMQC and HMBC spectra, using standard GE programs with the above solution.

PLANT MATERIAL.—The whole plant of *S. franchetiana* was collected in the suburbs of Lanzhou City, Gansu Province, People's Republic of China, in 1985. A voucher specimen is deposited in the Herbarium of the Institute of Medicinal Plant Development, Beijing, People's Republic of China.

ISOLATION OF SWERTIFRANCHESIDE [**1**].—The air-dried whole plant (5 kg) of *S. franchetiana* was cut into pieces and the extraction initiated with 95% EtOH (twice at 50° for two days), followed by 70% EtOH (once for 1 day). The extracts were combined and concentrated *in vacuo*, and the residue was suspended in H_2O. The suspension was successively extracted with petroleum ether, CH_2Cl_2, EtOAc, and *n*-BuOH, and the extracts were evaporated *in vacuo*, separately. The *n*-BuOH extract (480 g) was separated by cc on polyamide and eluted with H_2O containing increasing amounts of EtOH to yield 100 fractions (0.5 liter each), which were collected and monitored by tlc. Fractions 77–80 were subjected mplc on polyamide and eluted with $CHCl_3$ containing increasing amounts of MeOH. From fraction 40, eluted with $CHCl_3$-MeOH (8:2), a precipitate was obtained which was further purified over a Sephadex LH-20 column using MeOH as eluent to yield **1** (30 mg, 0.00006%); yellow powder after precipitation from MeOH; mp >320°; orange color with HCl-Mg, $[\alpha]_D$ +12.3° (*c*=0.17, MeOH); uv (MeOH) λ max (log ε) 256 (4.56), 280 (4.48), 340 (4.39) nm; [MeOH+NaOMe] 270 (4.48), 286 (4.51), 390 (4.34) nm; [MeOH+NaOAc] 254 (4.62), 282 (4.56), 328 (4.35), 396 (4.22) nm; [MeOH+NaOAc+H_3BO_3] 254 (4.69), 278 (4.61), 330 (4.35), 396 (4.18) nm; [MeOH+$AlCl_3$] 234 (4.60), 284 (4.56), 342 (4.21), 414 (4.27) nm; [MeOH+$AlCl_3$+HCl] 234 (4.63), 258 (4.54), 284 (4.57), 350 (4.33) nm; ir (KBr) ν max 3440, 1660, 1640, 1625, 1585 and 1480 cm^{-1}; ^{1}H- and ^{13}C-nmr data, see Table 1; positive fabms *m/z* 721 $[M+1]^+$, hrfabms: obsd. 721.1413 for $C_{36}H_{28}O_{17}$+H, calcd 721.1405.

CYTOTOXICITY, ANTIMALARIAL, AND HIV-1 RT INHIBITORY ASSAYS.—The biological evaluations for cytotoxic, antimalarial, and HIV-1 RT inhibitory activities of this compound were carried out according to established protocols (13–16).

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