Schisanwilsonenes A−C, Anti-HBV Carotane Sesquiterpenoids from the Fruits of *Schisandra wilsoniana*

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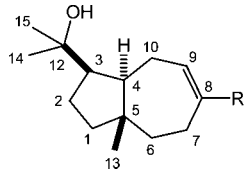
Three carotane-type sesquiterpenoids, schisanwilsonenes A (**1**), B (**2**), and C (**3**), were isolated from the fruits of *Schisandra wilsoniana.* Their structures and relative configurations were elucidated on the basis of spectroscopic methods including 2D-NMR techniques, and the structure of **1** was confirmed by a single-crystal X-ray diffraction experiment. Schisanwilsonene A, at 50 μg/mL, exhibited antiviral activity, inhibiting HBsAg and HBeAg secretion by 76.5% and 28.9%.

Plants belonging to the family Schisandraceae have proved to be a rich source of dibenzocyclooctane lignans, as well as lanostane and cycloartane triterpenes, some of which have been found to possess calcium antagonism, antilipid peroxidation, antitumor, or anti-HIV effects.[1–4] Several dibenzocyclooctane lignans isolated from *Kadsura induta*, *K. japonica*, *K. matsudai*, and *Schisandra arisanensis* were also reported to be active against hepatitis-B virus (HBV).[5–7] *Schisandra wilsoniana* A. C. Smith (Schisandraceae) is a medicinal plant indigenous to southern China. Its fruits are used in Chinese folk medicine as a substitute for "wu-wei-zi" to treat hepatitis, and the Et_2O extract was found to show anti-HBV activity, inhibiting HBsAg and HBeAg secretion by 7.6% and 19.5%, respectively. In our continuing search for antiviral natural products, three carotane-type sesquiterpenoids, **1**−**3**, were isolated from the fruits of *S. wilsoniana*. This is the first report of carotane-type sesquiterpenoids from the Schisandraceae. In this paper we describe the isolation and structure elucidation of **1**, **2**, and **3**, as well as their antiviral properties.



1. R = CH_2OH (11)

2. R = CH_2OCOCH_3 (11, 16, 17)

3. R = CHO (11)

Results and Discussion

An EtOH extract of the fruits of *S. wilsoniana* was suspended in H_2O and partitioned with Et_2O. Repeated column chromatography of the Et_2O portion on silica gel and on RP_{18} gel, followed by preparative TLC, yielded sesquiterpenoids **1**−**3**.

Compound **1**, colorless crystals, had the molecular formula $C_{15}H_{26}O_2$ on the basis of HRESIMS (*m*/*z* 261.1828 $[M + Na]^+$), which indicated three degrees of unsaturation. The IR absorption band at 3315 cm^{-1} and EIMS fragments at *m*/*z* 220 $[M - H_2O]^+$ and 202 $[M - 2H_2O]^+$ implied the presence of two OH groups in **1**. The ^{13}C NMR and DEPT spectra (see Experimental Section) revealed 15 signals due to three methyl, six methylene, three methine, and three quaternary carbons. The ^1H NMR signal at δ_H 5.78 (1H, t, J = 8.6 Hz) and ^{13}C NMR signals at δ_C 127.5 and 140.7 suggested a trisubstituted double bond. The other two degrees of unsaturation were due to two rings in the molecule. The double bond was assigned at C-8(9) by the HMBC correlations (Figure 1)

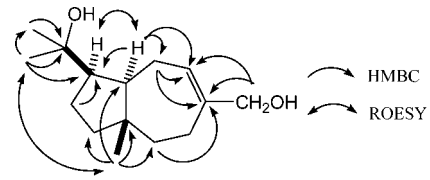


Figure 1. Key HMBC and ROESY correlations of **1**.

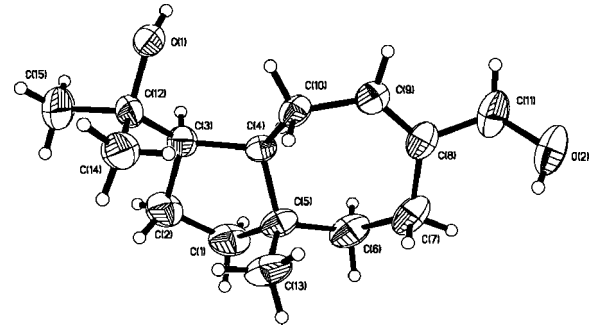


Figure 2. X-ray crystal structure of **1**.

of δ_H 1.83 (1H, m, H-4) and 2.21, 2.88 (1H each, m, H-10) with δ_C 127.5 (C-9), and δ_H 2.21, 2.88 (H-10) and 1.22, 1.76 (1H each, m, H-6) with δ_C 140.7 (C-8). HMBC correlations of methyl protons at δ_H 0.88 (3H, s, Me-13) with the carbons at δ_C 42.0 (C-1), 50.3 (C-4), 44.6 (C-5), and 41.1(C-6) implied that C-1, C-4, and C-6 were connected to C-5. The EIMS fragments at *m*/*z* 59 ($C_3H_7O^+$) and NMR signals at δ_H 1.23 (s, Me-14), 1.21 (s, Me-15) and δ_C 74.4 (C-12) suggested the presence of an isopropanol group ($(CH_3)_2COH$), which was supported by HMBC correlations of Me-14 and Me-15 to C-12 (δ_C 74.4), and Me-14 to C-15 (δ_C 32.5). HMBC cross-peaks of Me-14, H-4, and H-2 to C-3 (δ_C 53.2) indicated that the isopropanol group was connected to C-3. The NMR signals at δ_H 3.93 (2H, s, H-11) and δ_C 70.6 (C-11) indicated the presence of a CH_2OH group. HMBC cross-peaks of H-11 to C-7 (δ_C 26.0), C-8, and C-9 suggested the connection between C-11 and C-8.

The ROESY cross-peak between Me-13 and Me-14 (Figure 1) indicated that the isopropanol group was β-oriented, while the ROESY correlation of H-3 with H-4 implied that H-3 and H-4 were α-oriented. The structure and relative stereochemistry of **1** was confirmed by single-crystal X-ray crystallography (Figure 2). Therefore, **1** was determined as 2-(1*R**,3a*S**,8a*R**,*E*)-6- (hydroxymethyl)-3a-methyl-1,2,3,3a,4,5,8,8a-octahydroazulen-1-yl)propan-2-ol, and it was named schisanwilsonene A.

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Table 1. Anti-HBsAg and Anti-HBeAg Effects of **1**−**3** in the HepG2 2.2.15 Cell Line

compound	conc (μg/mL)	HBsAg (inhibition %)	HBeAg (inhibition %)
1	200	[a]	[a]
	100	[a]	[a]
	50	76.5	28.9
	25	27.5	11.3
2	200	[a]	[a]
	100	58.6	0
	50	20.5	0
	25	5.6	0
3	200	28.3	8.8
	100	11.7	6.1
	50	5.5	1.4
	25	0	1.1
lamivudine(3TC)[b]	200	28.6	34.8

[a] These compounds were cytotoxic. Cell damage was assessed by means of the MTT assay; for cell growth inhibition, inhibition percentage (P) ≥ 25% was considered cytotoxic. [b] Positive control.

Compound **2** had the molecular formula $C_{17}H_{28}O_3$ (HREIMS). The IR spectrum indicated the presence of OH (3446 cm^{-1}). The ^{1}H and ^{13}C NMR data of **2** were quite similar to those of **1**; 17 carbon signals due to the olefinic carbons, a carbonyl, two sp^3 quaternary carbons, two sp^3 methines, six sp^3 methylenes, and four methyl groups were clearly indicated. Fragment ions at *m*/*z* 59 (CH_3COO^+) and 43 (CH_3CO^+) in the EIMS spectrum, as well as NMR signals at δ_H 2.06 (s, Me-17) and δ_C 171.1 (C-16) and 21.1 (C-17) suggested the presence of a CH_3CO group. The linkage of C-11 and C-16 via an oxygen atom was deduced from HMBC correlations of H-11 (δ_H 4.40) to C-16 (δ_C 171.1). ROESY correlations of H-3 with H-4, and Me-13 with Me-14, indicated that **2** had a configuration similar to that of **1**. Thus, the structure of **2** was determined to be ((1*R**,3a*S**,8a*R**,*E*)-1-(2-hydroxypropan-2-yl)-3a-methyl-1,2,3,3a,4,5,8,8a-octahydroazulen-6-yl)methyl acetate, and it was named schisanwilsonene B.

Compound **3** had the molecular formula $C_{15}H_{24}O_2$ (HRESIMS). Comparison of the NMR data of **3** with those of **1** indicated that the only difference was that the CH_2OH group in **1** was replaced by a CHO group in **3**. NMR signals at δ_H 9.30 (1H, s, H-11) and δ_C 196.1 (C-11) as well as an EIMS fragment ion at *m*/*z* 207 [M − CHO]$^+$ indicated the presence of a CHO group in **3**. HMBC cross-peaks of H-11 to C-7 (δ_C 20.2), C-8 (δ_C 144.7), and C-9 (δ_C 157.0) demonstrated that the CHO group was located at C-8. ROESY correlations of H-3 with H-4 and Me-13 with Me-14 indicated that **3** had a configuration similar to that of **1**. Thus, the structure and relative configuration of **3** was determined as (1*R**,3a*S**,8a*R**,*E*)-1-(2-hydroxypropan-2-yl)-3a-methyl-1,2,3,3a,4,5,8,8a-octahydroazulene-6-carbaldehyde, and it was named schisanwilsonene C.

Compounds **1**−**3** were tested for anti-HBV activity *in vitro*, and the results are summarized in Table 1. Compound **1** exhibited antiviral activity at 50 μg/mL, inhibiting HBsAg and HBeAg secretion by 76.5% and 28.9%. Compound **2** displayed an anti-HBsAg effect of 58.6%, at a concentration of 100 μg/mL. However, compound **3** showed a weak anti-HBsAg effect. On the basis of the structures of compounds **1**−**3**, it was concluded that the substituent at C-11 may be important to anti-HBV activity for carotane-type sesquiterpenoids.

Experimental Section

General Experimental Procedures. Melting points were measured on an XT-4 micromelting point apparatus and are uncorrected. Optical rotations were run on a JASCO P-1020 polarimeter at room temperature. IR spectra were recorded on an Avatar 360 FT-IR ESP spectrometer in CH_2Cl_2. Mass spectra were determined on a HP5989A mass spectrometer for EIMS, a Waters Micromass GCT mass spectrometer for HREIMS, and a Bruker APEX 7.0 TESLA FT-MS apparatus for HRESIMS. ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker DRX-400 spectrometer in $CDCl_3$. Analytical and preparative TLC were run on silica gel plates (GF_{254}, Yantai Institute of Chemical Technology, Yantai, China). Spots were observed under UV light and visualized by spraying with 10% H_2SO_4, followed by heating. Column chromatography (CC) was performed on silica gel (200−300 mesh and 300−400 mesh; Qingdao Marine Chemical Factory, Qingdao, China) and Lichroprep RP_{18} gel (40−60 μm, Merck, Darmstadt, Germany). X-ray crystallographic analysis was carried out on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods using the program SHELXS. Non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were located by geometry and rode on the related atoms during refinements with a temperature factor 1.5 times the latter.[8]

Plant Material. Fruits of *Schisandra wilsoniana* were collected in August of 2005 in Heqing, Yunnan, China. The identity of the plant material was verified by one of the authors (D.-F.C.), and a voucher specimen (DFC20050801) has been deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, People's Republic of China.

Extraction and Isolation. The dried and powdered materials (8 kg) were extracted with 95% EtOH at reflux, three times, and filtered. The filtrate was evaporated *in vacuo* to give a residue (1600 g), a portion of which (1500 g) was suspended in H_2O (5 L) and partitioned with Et_2O. The combined Et_2O solution was concentrated to yield a residue (400 g), 200 g of which was subjected to CC on silica gel (200−300 mesh, 2 kg, 10 × 120 cm) eluted successively with petroleum ether (PE)/acetone (50:1, 30:1, 20:1, 10:1, 4:1, 3:1, 3:2, 1:1) and acetone to yield fractions 1−9. A fraction eluted with PE/acetone (20:1) was chromatographed over silica gel with PE/acetone (20:1) repeatedly and PTLC with PE/EtOAc (10:1) to afford **2** (5 mg); a fraction eluted with PE/acetone (10:1) was chromatographed over silica gel with PE/acetone (10:1) and PTLC with PE/EtOAc (5:1) to afford **3** (10 mg); and a fraction eluted with PE/acetone (3:1) was chromatographed over RP_{18} gel with MeOH/H_2O (7:3) to afford **1** (30 mg).

Schisanwilsonene A (1): colorless crystals (acetone); mp 165−168 °C; $[\alpha]^{25}_D$ +52.3 (*c* 0.02, CH_3OH); IR ν_{max} (CH_2Cl_2) 3315, 2941, 2915, 2360, 2341, 1456, 1377, 1152, 1057, 1002, 945, 868 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.47, 1.32 (each 1H, m, H-1), 1.47, 1.73 (each 1H, m, H-2), 2.31 (1H, m, H-3), 1.83 (1H, m, H-4), 1.22, 1.76 (each 1H, m, H-6), 2.05, 2.25 (each 1H, m, H-7), 5.78 (1H, d, J = 8.6 Hz, H-9), 2.21, 2.88 (1H, m, H-10), 3.93 (2H, s, H-11), 0.88 (3H, s, H-13), 1.23 (3H, s, H-14), 1.21 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 42.0 (CH_2, C-1), 27.4 (CH_2, C-2), 53.2 (CH,C-3), 50.3 (CH, C-4), 44.6 (C, C-5), 41.1 (CH_2,C-6), 26.0 (CH_2, C-7), 140.7 (C, C-8), 127.5 (CH, C-9), 26.5 (CH_2, C-10), 70.6 (CH_2, C-11), 74.4 (C, C-12), 17.7 (CH_3, C-13), 27.1 (CH_3, C-14), 32.5 (CH_3, C-15); EIMS *m*/*z* 220 (36), 202 (12), 177 (65), 147 (50), 105 (58), 95 (56), 93 (60), 91 (74), 79 (55), 59 (100); HRESIMS *m*/*z* 261.1828 ([M + Na]$^+$, calcd for $C_{15}H_{26}O_2Na$, 261.1825).

Crystal data:[9] $C_{15}H_{26}O_2$, M_r = 238.36, orthorhombic, space group $P2_12_12_1$, a = 11.605(7) Å, b = 12.335(7) Å, c = 20.051(11) Å, α = β = γ = 90°, V = 2870(3) Å^3, Z = 8, D_{calc} = 1.103 Mg/m^3, approximate crystal dimension of 0.15 × 0.10 × 0.04 mm^3. Mo Kα (λ = 0.71073Å), F(000) = 1056, T = 293(2) K. The final R values were R_1 = 0.0917 and wR_2 = 0.1832 for 5899 observed reflections [$I > (2\sigma(I)$].

Schisanwilsonene B (2): white powder; mp 216−219 °C; $[\alpha]^{25}_D$ +30.4 (*c* 0.01, MeOH)); IR ν_{max} (CH_2Cl_2) 3446, 2925, 2853, 2359, 2340, 1734, 1717, 1375, 1233, and 1045 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.45, 1.32 (each 1H, m, H-1), 1.42, 1.71 (each 1H, m, H-2), 2.31 (1H, m, H-3), 1.86 (1H, m, H-4), 1.26, 1.74 (each 1H, m, H-6), 1.98, 2.22 (each 1H, m, H-7), 5.83 (1H, d, J = 8.6 Hz, H-9), 2.20, 2.89 (1H, m, H-10), 4.40 (2H, s, H-11), 0.89 (3H, s, H-13), 1.23 (3H, s, H-14), 1.21 (3H, s, H-15), 2.06 (3H, s, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 42.0 (CH_2, C-1), 27.4 (CH_2, C-2), 53.2 (CH, C-3), 50.1 (CH, C-4), 44.5 (C, C-5), 41.0 (CH_2, C-6), 26.3 (CH_2, C-7), 135.8 (C, C-8), 131.0 (CH, C-9), 26.7 (CH_2, C-10), 72.0 (CH_2, C-11), 74.3 (C, C-12), 17.7 (CH_3, C-13), 27.1 (CH_3, C-14), 32.5 (CH_3, C-15), 171.1 (C, C-16), 21.1 (CH_3, C-17); EIMS *m*/*z* 280 [M]$^+$ (0.5), 159 (84), 119 (41), 105 (57), 91 (69), 79 (42), 59 (67), 43 (100), and 41 (43); HREIMS *m*/*z* 280.2043 (calcd for $C_{17}H_{28}O_3$, 280.2038).

Schisanwilsonene C (3): white powder; mp 148−151 °C; $[\alpha]^{25}_D$ +56.2 (*c* 0.02, MeOH); IR ν_{max} (CH_2Cl_2) 3441, 2942, 2853, 2696, 2369, 1684, 1635, 1379, 1134, and 950 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50, 1.35 (each 1H, m, H-1), 1.41, 1.72 (each 1H, m, H-2), 2.36 (1H, m, H-3), 1.88 (1H, m, H-4), 1.16, 1.81 (each 1H, m, H-6), 2.01,

2.75 (each 1H, m, H-7), 6.80 (1H, d, $J = 8.6$ Hz, H-9), 2.50, 2.36 (1H, m, H-10), 9.30 (1H, s, H-11), 0.89 (3H, s, H-13), 1.26 (3H, s, H-14), 1.24 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 40.1 (CH_2, C-1), 27.2 (CH_2, C-2), 53.9 (CH, C-3), 49.3 (CH, C-4), 44.5 (C, C-5), 41.9 (CH_2, C-6), 20.2 (CH_2, C-7), 144.7 (C, C-8), 157.0 (CH, C-9), 28.3 (CH_2, C-10), 196.1 (CH, C-11), 74.3 (C, C-12), 17.7 (CH_3, C-13), 26.9 (CH_3, C-14), 33.0 (CH_3, C-15); EIMS *m/z* 236 $[M]^+$ (4), 207 (13), 95 (52), 91 (63), 81 (48), 79 (54), 59 (100), 55 (57), 43 (95), and 41 (64); HRESIMS *m/z* 259.1667 ($[M + Na]^+$, calcd for $C_{17}H_{28}O_2Na$, 259.1669).

Anti-HBV Assay. Drug stock solutions were prepared in DMSO and stored at −70 °C. Upon dilution with DMEM culture medium, the final DMSO concentration was ≤1%, a concentration having no effect on cell replication. Cell culture and other procedures were the same as those reported previously.[5,10] A HepG2-derived human hepatablastoma cell line (HepG2 2.2.15) was transfected with cloned HBV DNA to produce viral particles. All stock cultures were grown at 37 °C in a humidified atmosphere containing 5% CO_2, using T-25 flasks containing DMEM medium supplemented with 10% (v/v) fetal bovine serum, 0.03% (v/v) L-glutamine, 100 μg/mL penicillin, 100 μg/mL streptomycin, and 380 μg/mL G418. The HepG2 2.2.15 cell suspensions were seeded in 24-well microtiter plates and cultured for 48 h. Then, they were incubated at 37 °C for 9 days in the presence of test compounds (200, 100, 50, and 25 μg/mL) from the DMSO-diluted stock solution. The medium was refreshed every 3 days. Then, the culture supernatants were harvested to detect the HBsAg and HBeAg secretions using appropriate diagnostic ELISA kits (Shanghai SIIC KEHUA Biotech Co. Ltd.) as described in triplicate, and the SEM (standard error of the mean) of inhibition values varied no more than 5%. Cell damage was assessed by means of the MTT assay.

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Supporting Information Available: NMR spectra of the compounds **1**−**3**, as well as crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Zhang, X. M.; Chen, D. F.; He, X. J.; Yang, S.; Zheng, P.; Jiang, M. H. *Acta Pharmacol. Sin.* **2000**, *21*, 373–376.
(2) Yang, X. W.; Hattori, M.; Namba, T.; Chen, D. F.; Xu, G. J. *Chem. Pharm. Bull.* **1992**, *40*, 406–409.
(3) Kuo, Y. H.; Huang, H. C.; Yang Kuo, L. M.; Chen, C. F. *J. Org. Chem.* **1999**, *64*, 7023–7027.
(4) Chen, D. F.; Zhang, S. X.; Wang, H. K.; Zhang, S. Y.; Sun, Q. Z.; Cosentino, L. M.; Lee, K. H. *J. Nat. Prod.* **1999**, *62*, 94–97.
(5) Ma, W. H.; Ma, X. L.; Huang, H.; Zhou, P.; Chen, D. F. *Chem. Biodiversity* **2007**, *4*, 966–972.
(6) Kuo, Y. H.; Huang, H. C.; Kuo Yang, L. M.; Chen, C. F. *J. Org. Chem.* **1999**, *64*, 7023.
(7) Wu, M. D.; Huang, R. L.; Kuo Yang, L. M.; Huang, C. C.; Ong, C. W.; Kuo, Y. H. *Chem. Pharm. Bull.* **2003**, *51*, 1233.
(8) *SHELXL-97, Program for X-ray Crystal Structure Refinement*; University of Göttingen: Göttingen, 1997.
(9) Crystallographic data for the structures reported in this paper have been deposited at the Cambridge Crystallographic Data Center as supplementary publication number CCDC701112. Copies of the data can be obtained, free of charge, on application to the Director, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(10) Wu, T.; Huang, H.; Zhou, P. *Zhongguo Bing Du Xue* **1998**, *13*, 45–49.

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