Chemistry of Natural Compounds, Vol. 48, No. 4, September, 2012 [Russian original No. 4, July–August, 2012]

A NOVEL C29 STEROL FROM *Clerodendrum cyrtophyllum*[a]

Peng Wang,[1] Fujiang Guo,[1] Junjie Tan,[2] Cheng Huang,[1] Guangyin Wang,[1] and Yiming Li[1]*

UDC 547.918

*A novel C29 sterol, stigmasta-5, 22, 25-trien-7-on-3*β*-ol, has been isolated from the branches of* Clerodendrum cyrtophyllum *with four other known sterols. Their structures were elucidated by means of spectroscopic studies, including* ^{1}*H,* ^{13}*C NMR, and HR-ESI-MS. Bioassay tests show that they can inhibit Mcf-7 human breast cancer cell growth.*

Keywords: sterols, *Clerodendrum cyrtophyllum*, anticancer.

The genus *Clerodendrum* (Verbenaceae) contains more than 30 species distributed in China, some of which have been used as traditional chinese medicine (TCM), such as *Clerodendrum bungei* for treating boils, hemorrhoids, eczema, and hypertension [1], and *Clerodendrum serratum* for treating bacterial infection and rheumatism [2]. The characteristic chemical constituents of this genus are phenylpropanoid and phenylethanoid glycosides, flavonoids, and terpenoids [3]. *Clerodendrum cyrtophyllum* Turz. is a small shrub plant distributed mainly in southern China. Local inhabitants have used its branches as a folk medicine for the treatment of inflammation and cancer. Several types of constituents including, diterpenoids [4], phenylethanoid glycosides and terpenoids [5], have been identified from this plant. The present study reports on a new C29 sterol with four known sterols isolated from the branches of this plant. The primary bioassay showed they could inhibit the Mcf-7 human breast cancer cell growth.

Compound **1** was obtained as a white amorphous powder, easily soluble in $CHCl_3$. Its molecular formula was assigned as $C_{29}H_{44}O_2$ by HR-EI-MS, *m/z* 447.3241 $[M + Na]^+$. Its IR (KBr) spectrum showed the presence of a hydroxyl group (3465.5 cm^{-1}), a carbonyl group (1650.8 cm^{-1}), a double bond (1604.5 cm^{-1}), and C-H groups (2925.5, 2877.3 cm^{-1}). The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of **1** showed five olefinic protons at δ 5.67, 5.25, 5.17, and 4.68 (2H), and five methyl groups at δ 0.67, 0.83, 1.00, 1.17, and 1.62. The ^{13}C NMR (75 MHz, $CDCl_3$) and DEPT spectra showed that **1** had 29 carbon peaks. Six peaks at δ 165.2, 148.6, 137.0, 130.2, 126.1, and 109.5 suggested that **1** had three double bands, and one of them is a terminal double bond. The peak at δ 202.3 showed that **1** had a carbonyl group. Also the DEPT spectrum showed five methyl groups in **1**, which agreed with the ^{1}H NMR data. The above data showed that **1** is possibly a sterol. After checking the literature, we found that the ^{1}H and ^{13}C NMR data of **1** were very similar to those of stigmastanone (3*β*-hydroxystigmast-5-en-7-one) [6]. The only difference is that **1** has one more double bond, which causes the ^{1}H and ^{13}C NMR data of the side chain to be different from those of stigmastanone. After checking with 22-dehydroclerosterol [7], which has the C22-C23 double bond in the side chain, we show that **1** has one more double bond in C22-C23 compared with stigmastanone. So **1** is a new compound with the structure stigmasta-5,22, 25-trien-7-on-3*β*-ol.

The structures of **2–5** were elucidated mainly by their ^{1}H, ^{13}C NMR, and ESI-MS data as known compounds: 22-dehydroclerosterol-3-*O*-*β*-D-glucopyranoside (**2**), *β*-sitosterol (**3**), stigmasta-5,22-dien-3*β*-ol (**4**), and 22-dehydroclerosterol (**5**).

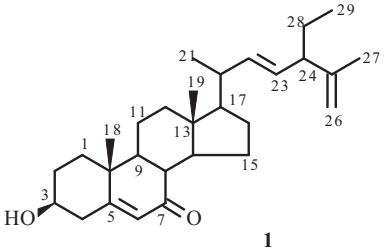
[a]Materials presented at the 9th International Symposium on the Chemistry of Natural Compounds (SCNC, People′s Republic of China, Urumqi, Oct. 16–19, 2011).

1) School of Pharmacy, Shanghai University of Traditional Chinese Medicine, 201203, Shanghai, P. R. China, e-mail: ymlius@163.com; 2) Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 201203, Shanghai, P. R. China.
Published in *Khimiya Prirodnykh Soedinenii*, No. 4, July–August, 2012, pp. 534–535. Original article submitted March 5, 2012.

0009-3130/12/4804-0594

TABLE 1. ^{1}H and ^{13}C NMR Spectra Data of Compound **1**, Stigmastanone (**6**), and 22-Dehydroclerosterol (**5**) ($CDCl_3$, δ, ppm, J/Hz)

C atom	**1**		**6**		**5**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	1.19 (s);1.94 (m)	36.4 (t)	1.19; 1.94	36.4 (t)		37.4 (d)
2	1.61 (m); 1.92 (m)	31.2 (t)	1.61; 1.92	31.2 (t)		30.2 (t)
3	3.65 (m)	70.5 (d)	3.67	70.5 (d)		71.8 (s)
4	2.40 (m); 2.50 (m)	41.8 (t)	2.41; 2.50	41.8 (t)		39.3 (d)
5	–	165.2 (s)	–	165.0 (s)		140.9 (s)
6	5.67 (d, J = 1.6)	126.1 (d)	5.69	126.1 (d)		121.9 (d)
7	–	202.3 (s)	–	202.3 (s)		32.1 (t)
8	2.22 (m)	45.4 (d)	2.24	45.4 (d)		32.0 (d)
9	1.48 (m)	49.9 (d)	1.50	50.0 (d)		50.3 (t)
10	–	38.3 (s)	–	38.7 (s)		36.9 (d)
11	1.54 (m);1.10 (m)	21.2 (t)	1.56; 1.10	21.2 (t)		21.2 (d)
12	2.02 (m)	38.6 (t)	2.03	38.3 (t)		39.8 (d)
13	–	43.0 (s)	–	43.1 (s)		42.3 (d)
14	1.28 (m)	50.0 (d)	1.28	50.0 (d)		56.9 (t)
15	1.29 (m); 2.40 (m)	26.4 (t)	1.29; 2.40	26.4 (t)		24.5 (d)
16	1.24 (m); 1.89 (m)	28.8 (t)	1.24; 1.89	28.5 (t)		29.1 (t)
17	1.08 (m)	54.6 (d)	1.09	54.7 (d)		55.9 (t)
18	0.67 (s)	12.1 (q)	0.68	12.0 (q)		12.1 (d)
19	1.17 (s)	17.3 (q)	1.20	17.3 (q)		19.4 (t)
20	1.33 (m)	40.0 d)	1.35	36.2 (d)		40.5 (d)
21	1.00 (d, J = 6.8)	21.0 (q)	0.93	19.0 (q)		21.0 (t)
22	5.17 (dd, J = 15.2, 7.2)	137.0 (d)	0.96; 1.36	33.9 (t)	5.33 (m)	137.6 (d)
23	5.25 (dd, J = 15.2, 8)	130.2 (d)	1.00; 1.22	26.3 (t)	5.38 (m)	130.3 (d)
24	1.43 (m)	52.0 (d)	0.95	46.1 (d)		52.3 (t)
25	–	148.6 (s)	1.66	29.0 (d)		148.6 (s)
26	4.68 (m)	109.5 (t)	0.83	19.6 (q)	4.88	110.2 (t)
27	1.62 (s)	20.2 (q)	0.81	19.0 (q)	1.73 (s)	20.3 (t)
28	2.40 (m)	25.7 (t)	1.14; 1.29	23.0 (t)		26.0 (d)
29	0.83 (t, J = 7.2)	12.2 (q)	0.85	12.3 (q)		12.4 (d)



1

Compounds **1–5** were evaluated against Mcf-7 human breast cancer cell growth using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) method [8]. The results showed that at a concentration of 25 μmol/L, the inhibition of Mcf-7 proliferation rate of **1** was 54.21%, that of **2** was 64.23%, and that of **5** was 56.10%. The inhibition rates were not much different when the concentration of the compounds increased to 50 μmol/L.

EXPERIMENTAL

Instruments and Materials. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. The IR spectrum was recorded on a Nicolet-Magna-750-FTIR spectrometer (KBr pellets). ^{1}H and ^{13}C NMR spectra were obtained using a Bruker AV-400 instrument (^{1}H: 400 MHz and ^{13}C: 100 MHz). The EI-MS and HR-EI-MS spectral data were obtained on Bruker Esquire 3000 plus and Finnigan LCQ DECA mass spectrometers. Silica gel (200–300 mesh; Qingdao Haiyang, Co.,

China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), and ODS (Fuji silica chemical company, Tokyo, Japan) were used for column chromatography.

Extraction and Isolation. The branches of *Clerodendron cyrtophyllum* (9.2 kg), were reflux–extracted three times with 95% EtOH. The combined EtOH extracts were concentrated *in vacuo* to give a residue. The residue was suspended in water and partitioned successively against petroleum ether (3 × 3 L), methylene chloride (3 × 3 L) and *n*-butanol (3 × 3 L). The methylene-chloride soluble portion (152 g) was subjected to silica gel column chromatography eluting with petroleum ether–EtOAc mixture, and the fractions were further subjected to RP silica gel column chromatography and Sephadex LH-20 with MeOH–CH_2Cl_2 1:1 to give compounds **1** (11 mg), **2** (90 mg), **3** (45 mg), **4** (120 mg), and **5** (255 mg).

Stigmasta-5,22,25-trien-7-on-3β-ol (1). White amorphous powder. $[\alpha]_D^{20}$ –67.0° (*c* 0.085, $CHCl_3$). IR (KBr, cm^{-1}): 3465.4, 2925.5, 2877.3, 1650.8, 1604.5. HR-ESI-MS $[M + Na]^+$ 447.3241. For ^1H NMR and ^{13}C NMR, see Table 1.

ACKNOWLEDGMENT

This research program was supported by the Program for Professors of Special Appointment (Eastern Scholar) in Shanghai Institutions of Higher Learning "Xinglin" scholars and outstanding team training plan of SHUTCM and the National Natural Science Foundation of China (No. 81173518) and Shanghai Municipal Education Commission (No. 2010JW13).

REFERENCES

1. S. S. Liu, T. Zhou, S. W. Zhang, and L. J. Xuan, *Helv. Chim. Acta*, **92**, 1070 (2009).
2. J. C. Chen, Q. X. Zhu, and D. L. Cheng, *Pharmazie*, **54**, 145 (1999).
3. H. H. Nan, S. Zhang, and J. Wu, *Chin. Trad. Herb. Drugs*, **36**, 492 (2005).
4. X. D. Tian, Z. D. Min, N. Xie, Y. Lei, Z. Y. Tian, Q. T. Zheng, R. N. Xu, T. Tanaka, M. Iinuma, and M. Mizuno, *Chem. Pharm. Bull*., **41**, 1415 (1993).
5. Q. C. Zhao, Y. Li, H. M. Cai, T. Guo, and L. J. Wu, *Chin. J. Med. Chem*., **19**, 241 (2009).
6. A. Guerriero, M. D. Ambrosio, F. Pietra, C. Debitus, and O. Ribes, *J. Nat. Prod.*, **56**, 1962 (1993).
7. S. G. Leitao, M. A. C. Kaplan, F. D. Monache, T. Akihisa, and T. Tamura, *Phytochemistry*, **31**, 2813 (1992).
8. J. Carmichael, W. G. DeGraff, A. F. Gazdar, J. D. Minna, and J. B. Mitchell, *Cancer Res*., **47**, 936 (1987).