ISSN : 0974 - 7508 Volume 8 Issue 5

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 8(5), 2012 [194-197]

Isolation, characterization and crystal structure of natural β-sitosterol from the shell of *Celastrus orbiculatus thunb*

Chuan-Jin Wang*, Guang-Jun Zhu
Institute of Chemical Engineering, Nanjing University of Science and Technology, Nanjing, (CHINA)
E-mail: wangchuanjin302@sohu.com
Received: 25th March, 2012 ; Accepted: 25th April, 2012

ABSTRACT

The title compound, β-sitosterol is a phytosterol which was first isolated from the acetyl acetate fraction of Celastrus Orbiculatus Theunb shell and characterized by 1H-NMR and 13C-NMR. Besides, the crystal of the compound was acquired, and the relative configuration of β-sitosterol was determined by single-crystal X-ray diffraction analysis. It crystallizes in monoclinic system, space group P21, a=10.311(2), b=7.6050(15), c=35.257(7)Å, α=90°, β=94.66°, γ=90°, Z=4, C29H50O, Mr=414.0, Dc=1.043g/cm3, V=2755.5(10)Å3, μ(MoKa)=0.063mm-1, F(000)=968, the final R=0.0760 and wR=0.1894 for 5442 independent reflections (Rint=0.0988) and 2525 observed ones (I>2σ(I)). The bond lengths, bond angles, partial values of torsional angles are also given. Intramolecuar van der waals force contribute to the stability of the structure.

KEYWORDS

Celastrus orbiculatus thumb shell;
β-sitosterol;
Single-crystal x-ray structure;
Crystal structure.

INTRODUCTION

Celastrus Orbiculatus Thunb is one of the well-known medicinal plants belonging to the family celastraceae widely distributed in China[1]. The fruits of *Celastrus Orbiculatus* has been used as the traditional Chinese medicine in the treatment of insomnia[2]. In order to find new and bioactive compounds, the chemical constituents of the *Celastrus Orbiculatus* fruits shell were investigated and one compound, named β-sitosterol was isolated for the first time. It has been reported that β-sitosterol could treat increased serum and low-density lipoprotein cholesterol concentrations or as a protective agent against cancers[3]. The isolated compound was fully characterized by means of ^{1}H-NMR and ^{13}C-NMR as well as X-ray crystallographic studies. In this paper we report the X-ray crystallographic studies on the title compound.

RESULTS AND DISCUSSION

^{1}H-NMR and ^{13}C-NMR spectra showed the typical pattern of phytosterol. The ^{1}H-NMR spectrum exhibited two singlets at δ 1.02 and 0.69 that were assigned to the methyl group of C -18 and C-19, respectively. A single at δ 5.37 in ^{1}H-NMR can account for an olefinic proton at C-6. Other multplet at δ 3.52 equivalents to a

singlet proton was assigned for the proton of C-3. The low field signal may be due to the attachment of β-OH group at the C-3 carbon. Thus, assignment of hydroxyl group at C-3 and the double bond at C-5 were assigned accordingly. ^{13}C-NMR gave signal at 140.77 and 121.71 ppm for C5=C6 double bond, respectively, 71.8 for C3 β hydroxyl group, and 19.81 and 11.86 for the angular methyl carbon atoms for C18 and C19, respectively. On the basis of the above evidences, the structure of compound was suggested to β-sitosterol. The NMR data of compound was in good agreement with the previous data of β-sitosterol (Figure 1)[4].

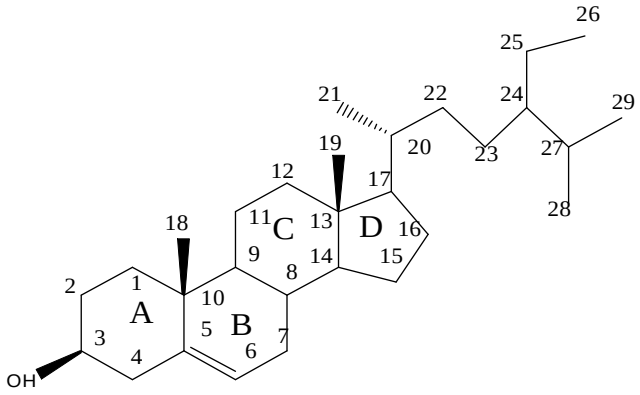

Figure 1: Chemical structure of β-sitosterol

The selected bond lengths, bond angles, partial values of torsional angles in TABLE 1 and 2, respectively. Figure 2 shows the molecular structure of the title compound.

It was seen from Figure 2 that the title compound has four rings, 3-hydroxy and 17, side-chain. The C–C bond distances in the four rings ranging from 1.345 (9) to 1.576 (10) Å and the C–C–C angles from 99.8 (4) to 125.9 (6)° are normal [5]. The distance between C (5) and C (6) is 1.345 (9) Å, within the normal range of double bond (C=C), revealing it is a normal C=C. Ring A is a strained chair, and ring C a symmetrical chair in both polymorphs. As expected however ring A is further from the ideal chair conformation than ring C. The influence of the double bond also imparts an approximate 7α/8β half-chair conformation on ring B. Ring D is a 13β/17α half-chair as frequently found in phytosterols.

EXPERIMENTAL

Instrument

Melting point was obtained in an X-6 digital

TABLE 1: Selected bond distances (Å) and bond angle(°)

Bond	Dist.	Bond	Dist.	Bond	Dist.
O(1)-C(3)	1.442(7)	C(1)-C(2)	1.526(8)	C(1)-C(10)	1.532 (7)
C(2)-C(3)	1.509(9)	C(3)-C(4)	1.514(9)	C(4)-C(5)	1.503(9)
C(5)-(6)	1.345(9)	C(5)-C(10)	1.544(9)	C(6)-C(7)	1.466(9)
C(7)-C(8)	1.508(7)	C(8)-C(14)	1.538(7)	C(8)-C(9)	1.565(6)
C(9)-C(11)	1.538 (7)	C(9)-C(10)	1.539(7)	C(10)-C(18)	1.541 (8)
C(11)-(12)	1.551 (8)	C(12)-C(13)	1.520 (7)	C(13)-C(14)	1.522(7)
C(13)-(19)	1.535(7)	C(13)-C(17)	1.562(7)	C(14)-C(15)	1.536(7)
C(15)-(16)	1.525(8)	C(16)-C(17)	1.548(8)	C(17)-C(20)	1.532(8)
C(20)-(22)	1.524(8)	C(20)-C(21)	1.565(9)	C(22)-C(23)	1.493(8)
C(23)-(24)	1.576(10)	C(24)-C(27)	1.480(12)	C(24)-C(25)	1.502(11)
C(2)-C(1)-(10)	113.5(5)	C(3)-C(2)-C(1)	111.4(5)	O(1)-C(3)-C(2)	110.7(6)
O(1)-C(3)-C(4)	108.4(6)	C(2)-C(3)-C(4)	112.5(6)	C(5)-C(4)-C(3)	109.2(6)
C(6)-C(5)-C(4)	120.9(7)	C(6)-C(5)-C(10)	119.7(5)	C(4)-C(5)-C(10)	117.1(6)
C(5)-C(6)-C(7)	125.9(6)	C(6)-C(7)-C(8)	110.8(5)	C(7)-C(8)-C(14)	112.7(5)
C(7)-C(8)-C(9)	109.7(4)	C(14)-C(8)-C(9)	107.9(4)	C(11)-C(9)-C(10)	113.3(5)
C(11)-(9)-C(8)	112.9(4)	C(10)-C(9)-C(8)	111.8(4)	C(1)-C(10)-C(9)	109.0(4)
C(1)-(10)-C(18)	107.5(5)	C(9)-C(10)-C(18)	112.3(5)	C (1)-C(10)-C(5)	108.6(5)
C(9)-(10)-C(5)	110.1(5)	C(18)-C(10)-C(5)	109.4(5)	C(9)-C(11)-C(12)	113.4(5)
C(13)-C(12)-C(11)	111.1(5)	C(12)-C(13)-C(14)	105.3(4)	C(12)-C(13)-C(19)	111.5(5)
C(14)-C(13)-C(19)	111.3(5)	C(12)-C(13)-C(17)	117.8(5)	C(14)-C(13)-C(17)	99.8(4)
C(19)-C(13)-C(17)	110.3(4)	C(13)-C(14)-C(15)	105.2(4)	C(13)-C(14)-C(8)	115.9(5)

Symmetry transformation: a: -*x*, *y*+1/2, -*z*

Full Paper

TABLE 2: Partial values of torsional angles (°)

Angle	(°)	Angle	(°)
C(10)-C(1)-C(2)-C(3)	54.6(8)	C(1)-C(2)-C(3)-O(1)	-178.3(5)
C(1)-C (2)-C(3)-C(4)	-56.9(8)	O(1)-C(3)-C(4)-C(5)	177.4(6)
C(2)-C(3)-C(4)-C(5)	54.6(8)	C(3)-C(4)-C(5)-C(6)	144.2(7)
C(3)-C(4)-C(5)-C(10)	-52.9(9)	C(4)-C(5)-C(6)-C(7)	-176.4 (7)
C(10)-C(5)-C(6)-C(7)	21.1(12)	C(5)-C(6)-C(7)-C(8)	-32.3(10)
C(6)-C(7)-C(8)-C(14)	169.7(5)	C(6)-C(7)-C(8)-C(9)	49.5(6)
C(7)-C(8)-C(9)-C(11)	170.1(5)	C(14)-C(8)-C(9)-C(11)	47.0(6)
C(7)-C(8)-C(9)-C(10)	-60.7(6)	C(14)-C(8)-C(9)-C(10)	176.3(4)
C(2)-C(1)-C(10)-C(9)	-169.0(5)	C(2)-C(1)-C(10)-C(18)	69.0(6)
C(2)-C(1)-C(10)-C(5)	-49.2(7)	C(11)-C(9)-C(10)-C(1)	-64.8(6)
C(8)-C(9)-C(10)-C(1)	166.2(4)	C(11)-C(9)-C(10)-C(18)	54.2(7)
C(8)-C(9)-C(10)-C(18)	-74.9(6)	C(11)-C(9)-C(10)-C(5)	176.2(5)
C(16)-C(17)-C(20)-C(21)	-177.7(6)	C(22)-C(23)-C(24)-C(25)	-177.5(3)
C(19)-C(13)-C(17)-C(20)	44.8(7)	C(23)-C(24)-C(27)-C(29)	-175.6(7)
C(21)-C(20)-C(22)-C(23)	-84.3(7)	C(19)-C(13)-C(17)-C(20)	44.8(7)

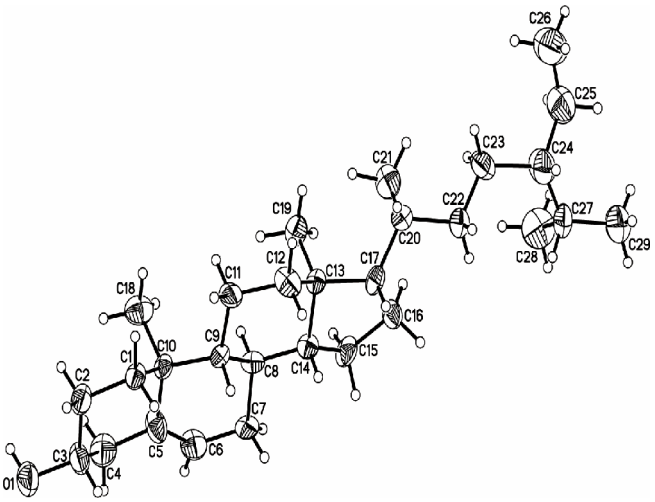

Figure 2: X-ray crystal structure of β- sitosterol

melting point instrument without correction. The elemental analysis was performed on a CHN-O-Rapid auto analyzer. The ^{1}HNMR (500MHz) and ^{13}CNMR (500MHz) spectra were recorded on a Bruker AvanceⅢ-500 spectrometer and tetramethylsilane (TMS) was used as an internal standard. Silica gel(200-300 mesh for column chromatography (CC) and GF_{254} for TLC) was obtained from Qingdao Marine Chemical Company(Qingdao, China). Cromasil C_{18} (40-60μm for column chromatography) was obtained from Boshi Company (Shanghai, China). Single-crystal structures of compound were measured on an Enraf-Nonius CAD4 diffractometer etc.

Extraction and isolation

The shell of *Celastrus Orbiculatus thumb* was collected in Chuzhou County, Anhui Province, China, in January 2011. The plant was identified by Prof. Jianwei Chen of Nanjing University of Chinese Medicine. A voucher specimen (No.PA110101) has been deposited at the Department of Pharmaceutical Engineering, Institute of Chemical Engineering, Nanjing University of Science and Technology, Nanjing, China.

The dried shell of *Celastrus Orbiculatus* (100g) was cut into small pieces and extracted with EtOAc (1liter×3). The solvent was removed by rotary evaporation and the yellow brown extract (2.0g) was obtained. The EtOAc extract was subjected to silica gel chromatography using stepwise elution with petroleum ether-CH_3COCH_3 (100:0, 100:1, 100:2, 100:4, 100:8, 100:16, 100:32, 100:100, and 0:100) to afford 90 fractions (A1-A90). A51-A60 were permeated through Cromasil C_{18} using stepwise elution with CH_3COCH_3- H_2O (50:50, 55:45, 60:40, 65:35, 70:30, 75:25, 80:20, 85:15, 90:10, 95:5, and 100:0) to give 11 subfractions F1-F11. Fraction of F8 was further purified with recystallization with CH_3Cl-CH_3COCH_3-MeOH (1:1:1) system to afford β-sitosterol (50mg) with a yield of about 0.05%.

The bulk crystal of β-sitosterol was recrystallized in the mixture solution of CH_3Cl-CH_3COCH_3-

MeOH (1:1:1), and single crystal was obtained in constant temperature (25°C) on the basis of this. m. p. :138~139°C. Elemental Anal. Calcd. (%) for $C_{29}H_{50}O$: C, 84.06; H, 12.08; O, 3.86. Found (%): C, 84.01 H, 12.05; O, 3.94. ^{1}H-NMR(500 MHz, $CDCl_3$) δ_H=5.37 (1H, dd, H-6), 3.52 (1H, dddd, H-3), 2.31 (2H, d, H-4), 1.97 (1H, m, H-20), 1.85 (1H, m, H-8), 1.66 (2H, m, H-15), 1.62 (2H, t, H-12), 1.52 (2H, dd, H-7), 1.51 (2H, m, H-11), 1.48 (1H, m, H-14), 1.40 (1H, m, H-9), 1.31 (2H, m, H-16), 1.29 (1H, m, H-27), 1.27 (2H, m, H-25), 1.20 (2H, m, H-23), 1.17 (1H, m, H-17), 1.15 (2H, m, H-2), 1.13 (2H, m, H-1), 1.02 (3H, s, H-18), 0.98 (2H, m, H-22), 0.94 (3H, d, H-21), 0.92 (1H, m, H-24), 0.89 (3H, t, H-26), 0.85 (3H, d, H-29), 0.83 (3H, d, H-28), 0.69 (3H, s, H-18); ^{13}C-NMR (500 MHz, $CDCl_3$) δ_C = 140.77 (C-5), 121.71 (C-6), 71.81 (C-3), 56.78 (C-14), 56.08 (C-17), 50.15 (C-9), 45.86 (C-4), 42.32 (C-13), 39.79 (C-12), 37.26 (C-24), 36.51 (C-10), 36.15 (C-1), 33.96 (C-22), 31.92 (C-8), 31.68 (C-7), 29.69 (C-2), 29.18 (C-16), 28.24 (C-11), 26.12 (C-15), 24.30 (C-23), 23.08 (C-29), 21.09 (C-25), 19.81 (C-18), 19.39 (C-21), 19.04 (C-27), 18.78 (C-26), 18.25 (C-20), 11.98 (C-28), 11.86 (C-19).

Physical and spectra data of the title compound were almost identical with those reported in the literatures[4]. Its relative configuration was further established by X-ray cry-stallographic analysis.

Crystallographic data collection and structure determination

A colorless transparent crystal with dimensions 0.2mm×0.20mm×0.10mm was used for data collection. Unit cell parameters and 5442 intensities were measured on an Enraf-Nonius CAD-4 diffractometer with graphite-monochromated MoKα radiation (λ= 0.71073Å) in the range of 1.98°<θ<25.37° by $\omega/2\theta$ scanning. Corrections for LP and absorption with empirical ψ scanning technigue were applied and 2525 independent reflections with [$I>2^{\sigma}(I)$] were used in the structure determination. The structure was solved by the direct method using the program SHELXS-97[6] and refined on F^2 by full-matrix least-squares procedure with Bruker SHELXL-97 packing[7]. All non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms refined with riding model position parameters isotropically were located from difference Fourier map and added theoretically. The final full-matrix least-squares refinement gave R=0.0760, ωR=0.1894(ω=1/[$\sigma^2(F_0^2)$+(0.0700P)^2+0.0000P], where P=(F_0^2+2F_c^2)/3. S=1.001, $(\Delta 10)_{max}$=0.000, $(\Delta\rho)_{max}$=0.210 and $(\Delta\rho)_{min}$=0.142e/Å^3.

ACKNOWLEDGEMENTS

The authors are grateful to Associate Prof. Hua-Qin Wang of Nanjing University for single-crystal X-ray diffraction analysis. The project was supported by Independent Research Program (cultivation for National Natural Science Foundation of China) of Nanjing University of Science and Technology (No. 2010GJPY009).

REFERENCES

[1] Y.Q.Guo, X.Li, J.Xu, N.Li, D.L.Meng, J.H.Wang; Chem.Pharm.Bull., **52**, 1134-1136 **(2004)**.

[2] Jiangsu New Medicinal College; 'Dictionary of Chinese Herbal Medicine,' Shanghai People's Publishing House, Shanghai, **2**, 1563 **(1977)**.

[3] N.Petteri, P.Ilpo, I.Katja, M.Maija, M.Anne; Chemosphere, **71**, 493-499 **(2008)**.

[4] J.L.Wang, X.P.Tian, H.L.Guo, W.F.Zhang, S.J.Zhang; J.Qiqihar.University., **20**, 7-9 **(2004)**.

[5] H.Sun, T.Liu, Y.J.Shen; Chinese J.Struct.Chem., **29**, 1798-1801 **(2010)**.

[6] G.M.Sheldrick; SHELXS97, A Program for Automatic Solution of Crystal Structure, University of Göttingen, Germany **(1997a)**.

[7] G.M.Sheldrick; SHELXL97, A Program for Crystal Structure Refinement, University of Göttingen, Germany **(1997b)**.