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Two New Sterols from *Amoora yunnanensis*

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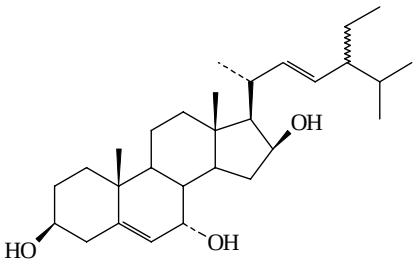
Abstract: Two new sterols, 3β,7α,16β-trihydroxy-stigmast-5,22-diene **1**, 3β,7α,16β-trihydroxy-stigmast-5-ene **2**, were isolated together with two known ergosterols, ergosta-5,24(28)-diene-3β,7α-diol, ergosta-5,24(28)-diene-3β,7β,16β-triol from the bark of *Amoora yunnanensis* (H. L. Li) C. Y. Wu. Their structures were deduced on the basis of spectral data.

Keywords: *Amoora yunnanensis* (H. L. Li) C. Y. Wu, Meliaceae, 3β,7α,16β-trihydroxy-stigmast-5,22-diene, 3β,7α,16β-trihydroxy-stigmast-5-ene.

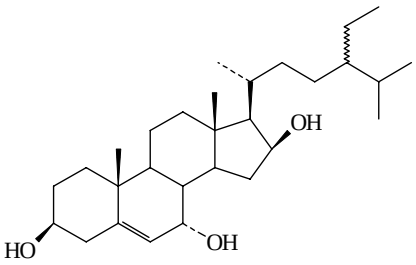
The genus *Amoora* comprising about 25-30 species is distributed in India and the Malay Peninsula. Six species are distributed in Yunnan province. *Amoora yunnanensis* (H. L. Li) C. Y. Wu, is mainly distributed in the South of Yunnan[1]. According to Pennington and Styles[2], *Amoora* cannot be considered as a valid genus. Up to now, chemical constituents for this genus have not been reported yet. In our chemical study on *Amoora yunnanensis*, tetranortriterpenoids or protolimonoids that were considered as chemotaxonomic markers of the family Meliaceae, were not isolated. In this paper, we report the isolation and structural elucidation of two new sterols, 3β,7α,16β-trihydroxy-stigmast-5,22-diene **1**, 3β,7α,16β-trihydroxy-stigmast-5-ene **2**. In addtion, known compounds ergosta-5,24(28)-diene-3β,7α-diol and ergosta-5,24(28)-diene-3β,7β,16β-triol were also obtained.

The air-dried and powdered bark (4.1 kg) of *A. yunnanensis* was extracted with EtOH three times under reflux (each process lasting three hours). After removal of the solvent by evaporation, the residues were suspended in H_2O and extracted with EtOAc, three times. The EtOAc fraction was concentrated *in vacuo* to get 56 g residues. The residues were separated repeatedly by chromatography on silica gel column, eluted with $CHCl_3$-Me_2CO to afford 3β,7α,16β-trihydroxy-stigmast-5,22-diene **1** (8 mg), 3β,7α,16β-trihydroxy-stigmast-5(6)-ene **2** (15 mg), ergosta-5,24(28)-diene-3β,7α-diol (14 mg) and ergosta-5,24(28)-diene-3β,7β,16β-triol (16 mg). The known compound ergosta-5,24(28)-diene-3β,7α-diol were identified by direct comparing its spectral data with those reported in the literature[3]. Ergosta-5,24(28)-diene-3β,7β,16β-triol which was published early[4] was determined by the detailed analysis of its spectral data.

Compound **1**, white powder, mp. 132-134 °C, $[\alpha]_D^{26}$ -35.3 (*c* 0.15, $CHCl_3$), showed in its EI-MS spectrum a molecular ion peak at *m/z* 444 in accordance with the formula $C_{29}H_{48}O_3$ and the presence of 29 carbons was confirmed by its ^{13}C NMR spectrum. HRFAB-MS: *m/z* $[M-1]^-$ found: 443.3573, required: 443.3525. The IR spectrum revealed absorption bonds for -OH at 3405 cm^{-1} and C=C at 1665 cm^{-1}. The ^1H and ^{13}C NMR spectra of **1** exhibited the presence of six methyls (two of which were tertiary methyls), seven methylenes, ten methines (three of which were oxygenated), two characteristics quaternary carbons at δ_C 42.0 and 37.1, and four olefinic carbons with corresponding proton signals at δ_H 5.58 (d, *J* = 3.9 Hz), 5.38 (dd, *J* = 15.4, 9.0 Hz), 5.29 (dd, *J* = 15.4, 8.6 Hz). These data proposed that **1** possessed a stigmast skeleton having two double bonds and three hydroxyls substitution. Two double bonds were assigned to be located between C-5 and C-6, as well as C-22 and C-23, respectively, by comparison of chemical shifts and coupling constants of three olefinic proton signals with those of relative compounds[3, 5]. The assignment was further confirmed by an HMBC experiment, in which two olefinic protons [δ_H 5.39 (H-22), 5.29 (H-23)] showing cross peaks to δ_C 51.1 (d, C-24) and 35.3 (d, C-20), respectively, unambiguously indicated an olefinic linkage between C-22 and C-23. Long range coupling for the olefinic proton δ_H 5.58 (H-6) to δ_C 42.0 (t, C-4), H-6 to 37.1 (s, C-10), and H-6 to 65.2 (d, C-7) in the HMBC spectrum, not only confirmed the position of another olefinic linkage between C-5 and C-6 but also indicated a hydroxyl substitution at C-7. Small coupling constant (*J* = 4.0 Hz) for H-7 attributed to *ea* coupling between H-7 and H-8 suggested that the 7-OH occupied an α configuration[3]. The inference was further supported by NOESY spectrum, in which a NOE correlation between H-7 and H-8 (β-H) was observed. The other two hydroxyls were placed at C-3 and C-16 position, respectively, based on cross peaks between δ_H 2.32 (2H, H-4) to δ_C 71.4 (d, C-3), and δ_H 4.27 (H-16) to δ_C 42.0 (C-13) in HMBC spectrum of **1**. The correlation between δ_H 4.27 (H-16) and 1.14 (H-17) in ^1H-^1H COSY spectrum also supported the assignment. The stereochemistry at C-16 was determined from NOESY spectrum of **1**, with a NOE interaction between H-16 and H-17 (α-H). Thus, 16-OH was determined as having a β configuration. In ^{13}C NMR spectrum, the signals for C-26, C-27 and C-29 were not in pairs, indicating only one C-24 epimer (24S or 24R) rather than a mixture[5] for compound **1**. The chemical shift difference between the epimers published in previous literature [5-7] is too small to identify the configuration at C-24 in compound **1**. Compound **1** was deduced to be 3β,7α,16β-trihydroxy-stigmast-5,22-diene.



1



2

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (400 MHz).*

C	**1**	**2**	H	**1**	**2**
1	37.0 t	37.0 t		1.56, 1.85 m	1.55, 1.85 m
2	31.4 t	31.4 t		1.68, 1.86 m	1.70, 1.86 m
3	71.4 d	71.4 d		3.55 m	3.56 m
4	42.0 t	42.0 t		2.32 m	2.28, 2.36 m
5	146.3 s	146.4 s			
6	123.8 d	123.9 d		5.58 d (3.9)	5.59 d (5.0)
7	65.2 d	65.3 d		3.85 t (4.0)	3.83 t (4.0)
8	37.5 d	37.5 d		1.55 m	1.55 m
9	42.4 d	42.4 d		1.22 m	1.22 m
10	37.1 s	37.2 s			
11	20.4 t	20.4 t		1.52, 1.80 m	1.52, 1.78 m
12	39.5 t	39.3 t		1.98 m	2.0 m
13	42.0 s	42.0 s			
14	46.9 d	47.5 d		1.30 m	1.32 m
15	34.9 t	36.5 t		2.32, 1.20 m	2.37, 1.22 m
16	72.9 d	72.7 d		4.27 m	4.39 m
17	61.0 d	61.0 d		1.14 m	1.10 m
18	13.0 q	12.8 q		0.89 s	0.87 s
19	18.2 q	18.3 q		0.98 s	0.99 s
20	35.3 d	30.3 d		2.30 m	1.27 m
21	21.0 q	18.3 q		1.06 d (6.8)	1.00 d (6.8)
22	139.0 d	34.0 t		5.39 dd (15.4, 9.0)	1.32 m
23	131.1 d	26.5 t		5.29 dd (15.4, 8.6)	1.23 m
24	51.1 d	46.0 d		1.62 m	0.97 m
25	31.7 d	29.2 d		1.68 m	1.70 m
26	21.6 q	19.8 q		0.82 d (6.4)	0.81 d (7.0)
27	18.7 q	19.0 q		0.78 d (6.4)	0.79 d (7.0)
28	25.1 t	23.2 t		1.22 m	1.18 m
29	12.2 q	12.0 q		0.82 t (7.2)	0.83 t (7.6)

*measured in $CDCl_3$, all values are in ppm, coupling constants in Hz, with TMS as internal standard.

Compound **2**, white powder, mp. 151-153 °C, $[\alpha]_D^{26}$ -60.0 (*c* 0.20, $CHCl_3$), its HRFAB-MS spectrum exhibited the molecular formula as $C_{29}H_{50}O_3$ ($[M]^+$ *m/z* found: 446.3710, required: 446.3760), which was supported by ^{13}C and DEPT spectra data. The IR spectrum also showed the presence of hydroxyl (3420 cm^{-1}) and olefinic (1668 cm^{-1}) absorption bands. The ^{1}H and ^{13}C NMR spectrum of **2** exhibited signals due to six methyls (two of which were tertiary methyls), nine methylenes, ten methines (three of which were oxygenated), and two olefinic carbons [corresponding carbon δ_C 123.9 (d), 146.4 (s), and proton δ_H 5.59 (d, J = 5.0 Hz)]. These data were similar to those of **1**, suggesting that **2** belonged to a stigmast with one double bond and three hydroxyls substitution. The molecular formula ($C_{29}H_{50}O_3$) was consistent with signals for only one double bond in ^{13}C NMR spectrum of **2**. Comparing the ^{13}C NMR spectra of the two compounds revealed that two more methylene groups (δ_C 34.0 and 26.5) were present in ^{13}C NMR spectrum of **2**, instead of δ_C 139.0 (d, C-22) and 131.1 (d, C-23) in ^{13}C NMR spectrum of **1**. The above data assumed that **2** was 22,23-dihydro-derivative

of **1**. In an HMBC experiment, the observation of cross signals between δ_H 1.00 (d, J = 6.8 Hz, H-21) to δ_C 34.0 (C-22), δ_H 1.10 (m, H-17) to 30.3 (d, C-20), and H-17 to C-22 confirmed the assumption. The stereochemistry at the other chiral centers in **2** were identical to those of **1**, as supported by its ^{1}H, ^{1}H-^{1}H COSY, and NOESY NMR spectra. Compound **2** also possessed one C-24 epimer (24S or 24R), the configuration at C-24 was also not determined. So compound **2** was elucidated as 3β,7α,16β-trihydroxy-stigmast-5-ene. All signals were assigned in **Table 1** based on the HMBC, HMQC, ^{1}H-^{1}H COSY and NOESY spectra of compounds **1** and **2**.

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References

1. Yunnan Institute of Botany, *Flora Yunnanica*, Science Press, Beijing, China, **1977,** Tomus 1, p. 231.
2. P. D. Pennington, B. T. Styles, A Generic Monograph of the Meliaceae, *Blumea*, **1975**, 22, p. 419.
3. J. A. Findlay, A. D. Patil, *Can. J. Chem.*, **1985**, *63*, 2406.
4. K. Masaru, K. M. C. A. Rao, A. J. Vallarupall, *Chem. Res. Synop.*, **1994**, *4*, 140, *Chem. Abstr.* **1994**, *121*, 31383y.
5. A. Madaio, V. Piccialli, D. Sica, G. Corriero, *J. Nat. Prod.*, **1989**, *52*, 952.
6. S. Thakur, P. Ghosh, T. Akihisa, N. Shimizu, T. Tamura, T. Matsumoto, *Indian J. Chem.*, **1988**, *27*B, 17.
7. T. Akihisa, T. Tamura, T. Matsumoto, W. C. M. C. Kokke, P. Ghosh, S. Thakur, *J. Chem. Soc. Perkin Trans. 1*, **1990**, 2213.

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