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A NOVEL DIMERIC STEROID, CALVASTERONE FROM THE FUNGUS *CALVATIA CYATHIFORMIS*

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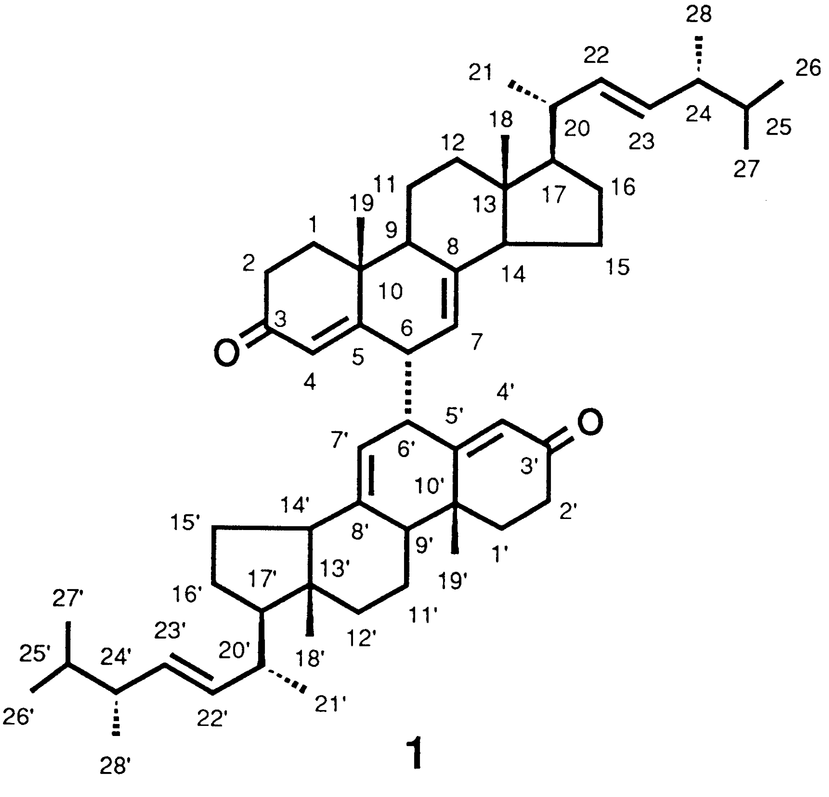
The structure and stereochemistry of a novel dimeric steroid, calvasterone (**1**), isolated from the fruiting body of *Calvatia cyathiformis*, has been established by spectroscopic data and chemical evidence.

KEYWORDS *Calvatia cyathiformis*; Lycoperdaceae; calvasterone; dimeric steroid

Calvatia cyathiformis (Bosc.) Morg. (Lycoperdaceae), a Chinese crude drug (Ma Bo in Chinese; Mabotsu in Japanese), has been used in China as hemostatic and pharyngodynia.[1] In the course of screening biologically active metabolites from the above fungus, we have isolated a novel dimeric steroid designated as calvasterone (**1**) from the dichloromethane extract of the fruiting body along with other related monomeric steroids. The structural elucidation of the above compound (**1**) is reported in this communication.

The dichloromethane extract of the fruiting body was chromatographed on silica gel with benzene-acetone (30:1) followed by LPLC using $CHCl_3$-hexane (1:1) to yield calvasterone (**1**).



1

Calvasterone (**1**), mp 202-204°C from acetone : $CHCl_3$ (5 : 1), $[\alpha]_D$ +58.2° (c=1.2, $CHCl_3$), gave a quasi-molecular ion at m/z 785 $(M-H)^-$ in the negative FAB-MS spectrum, and its elemental analysis confirmed the molecular formula $C_{56}H_{82}O_2$. The IR (1680 cm^{-1}) and UV (238nm sh, log ε 4.27) spectra indicated the presence of an α, β–unsaturated carbonyl group. The ^1H-NMR [2)] and ^{13}C-NMR [3)] spectra of **1** showed the presence of 41 protons and 28 carbons, which were exactly half number of the protons and carbons, respectively, as those confirmed from the molecular formula. Therefore **1** should have a dimeric structure. The ^1H-NMR spectrum of **1** exhibited signals for four tertiary (δ 0.57 and 1.23) and eight secondary (δ 0.81, 0.83, 0.90 and 1.02) methyl groups, thus suggesting a dimeric ergostane skeleton.[4)] This was also supported by the fact that the chemical shift values of the above secondary methyl groups and four coupled olefinic protons (δ 5.15, 5.22) were completely consistent with those of ergosterol. On the other hand, the ^1H-^1H COSY spectrum of **1** showed that four olefinic protons (δ 4.74, 5.85) coupled with two methine protons (δ 3.56), respectively. Also four tertiary carbons (δ 117.7, 121.3), four quarternary carbons (δ 141.4, 170.6), and two carbonyl carbons (δ 200.3) were observed in the ^{13}C-NMR spectrum of **1**. The above results indicated that **1** possessed four trisubstituted olefins and two α, β–unsaturated carbonyl groups in the dimeric ergostane skeleton.

Photooxidation of **1** under fluorescent lighting [5)] gave monomeric steroid, which was identical with ergosta-4,6,8(14),22-tetraen-3-one including the optical rotation. Furthermore, the presence of ergosta-4,7,22-trien-3-one moiety in **1** was confirmed using a combination of heteronuclear two-dimensional NMR techniques (^1H-^{13}C COSY and HMBC spectra). The methylene protons, which were assigned at C-6 of ergosta-4,7,22-trien-3-one, were not observed in the ^1H-NMR spectrum of **1**, whereas two methine protons (δ 3.56) were observed correlating with four olefinic protons [δ 4.74, H-7 (7'); 5.85, H-4 (4')], four tertiary carbons [δ 117.7, C-7 (7'); 121.3, C-4 (4')], and four quarternary carbons [δ 141.4, C-8 (8'); 170.6, C-5 (5')], respectively, in the ^1H-^1H COSY and HMBC spectra of **1**. Thus these two methine protons were assigned at C-6 (6'). Therefore each ergosta-4,7,22-trien-3-one moiety should be connected at C-6 (6').

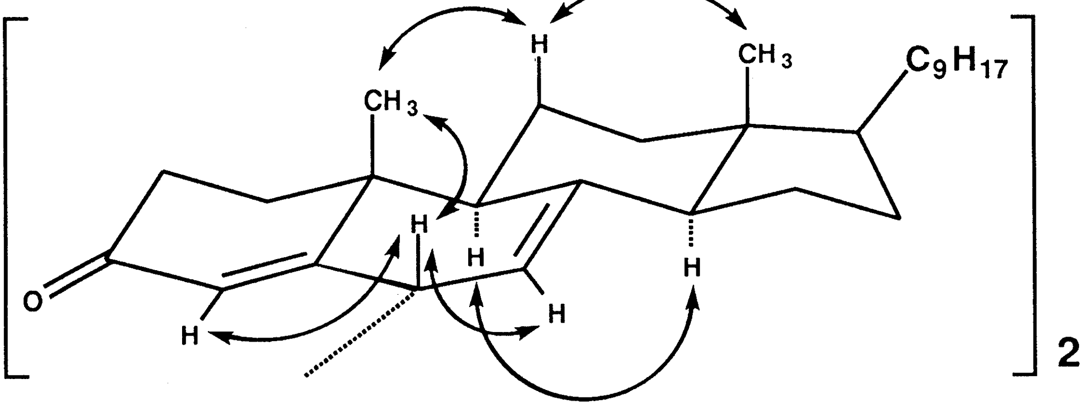


Fig. 1. NOESY Correlations of Calvasterone (**1**)

The stereostructure of **1** was corroborated by the NOE relationships observed in phase-sensitive NOESY spectrum (Fig. 1). The configuration at C-6 (6') can be explained to be *R* by the NOE's between H-6 (6') and H-19 (19'). From the above results, the structure of calvasterone (**1**) was established as (6,6'*R*)-biergosta-4,4',7,7',22,22'-hexaene-3,3'-dione.

Recently, a few dimeric triterpene derivatives have been found in natural sources.[6] Also dimeric steroidal alkaloids, cephalostatins,[7] and a Diels-Alder type dimeric steroid, bistheonellasterone [8] have been isolated from marine sources. Calvasterone (**1**) is the first example of a ergostane type dimeric steroid. Biological activities of calvasterone are now under investigation.

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2) ^{1}H-NMR (300 MHz, CDCl$_3$) δ: 0.57 (s, 18, 18'-H$_3$), 0.81 (d, J=6.7 Hz, 26, 26'-H$_3$), 0.83 (d, J=6.8 Hz, 27, 27'-H$_3$), 0.90 (d, J=6.8 Hz, 28, 28'-H$_3$), 1.02 (d, J=6.6 Hz, 21, 21'-H$_3$), 1.23 (s, 19, 19'-H$_3$), 1.79 (m, 14α, 14'α-H), 2.26 (m, 9α, 9'α-H), 2.36 (ddd, J=4.8, 6.5, 16.5 Hz, 2α, 2'α-H), 2.43 (ddd, J=5.0, 11.3, 16.5 Hz, 2β, 2'β-H), 3.56 (brs, 6, 6'-H), 4.74 (brs, 7, 7'-H), 5.15 (dd, J=8.2, 15.3 Hz, 22, 22'-H), 5.22 (dd, J=7.6, 15.3 Hz, 23, 23'-H), 5.85 (brs, 4, 4'-H).

3) ^{13}C-NMR (75 MHz, CDCl$_3$) δ: 12.4 (C-18, 18'), 17.5 (C-28, 28'), 19.7 (C-26, 26'), 20.0 (C-27, 27'), 21.1 (C-21, 21'), 22.3 (C-11, 11'), 22.9 (C-19, 19'), 23.0 (C-15, 15'), 28.1 (C-16, 16'), 33.1 (C-1, 1'), 33.2 (C-25, 25'), 33.8 (C-2, 2'), 38.1 (C-6, 6'), 39.0 (C-10, 10'), 39.2 (C-12, 12'), 40.5 (C-20, 20'), 42.9 (C-24, 24'), 43.8 (C-13, 13'), 46.1 (C-9, 9'), 55.3 (C-14, 14'), 56.1 (C-17, 17'), 117.7 (C-7, 7'), 121.3 (C-4, 4'), 132.6 (C-23, 23'), 135.6 (C-22, 22'), 141.4 (C-8, 8'), 170.6 (C-5, 5'), 200.3 (C-3, 3').

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