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Gigantenol, a New Ergosteryl Triterpene from the Basidiocarps of *Macrocybe gigantea* (Massee) Pegler & Lodge.

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ABSTRACT

Phytochemical investigation of *Macrocybe gigantea* (Massee) Pegler & Lodge afforded a new ergosteryl triterpene. The chemical structure was established based on extensive spectroscopic data analyses, including IR, ^{1}H NMR, ^{13}C NMR, Mass spectroscopy.

Keywords: Mushroom, Phytochemical, Spectroscopy, Triterpene

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INTRODUCTION

Mushrooms have been valued as a delicacy preferred by people of different world cultures through all ages. There is a history of ethnomedicinal use of some of these species. Consequently, attention of the scientific community was drawn to the nutritional and medicinal benefits of mushrooms [1], leading to the exploration of nutritional and pharmacological benefits of several mushroom species, including their antioxidant properties [2], prevention of underlying oxidative stress related pathological conditions such as cancer [3], heart ailments [4], diabetes [5], inflammation [6], gastric ulcer [7], hepatic damage [8] etc. However, lack of proper documentation and seasonal nature of availability has severely limited the scope of research in this vast field, a large segment of which is lying unexplored and even uncharacterized till date [9].

Macrocybe gigantea (family: Tricholomataceae) is a wild edible mushroom growing in the tropical areas of India, Japan, Korea, China and parts of Africa. The fruiting body of *M. gigantea* finds many ethnopharmacological uses in Asian cultures [10]. Primary investigations in our laboratory involving organic extracts of *T. giganteum* have revealed its potent *in vitro* antioxidant and NOS activation properties [11]. Furthermore, the ethanolic extract of *T. giganteum* has been found to induce apoptosis in Ehrlich's ascites carcinoma cells [12] and forestomach cancer in Swiss albino mice [13]. However, reports of isolation and characterization of phytochemicals from *M. gigantea* are scanty. In this work the isolation and structure determination of a new ergostane type triterpene, *gigantenol* is described.

MATERIALS AND METHODS

Following collection of basidiocarps of *M. gigantea* from the Contai town of Coastal West Bengal, India, they were cleansed thoroughly and dried for 24 h in a forced air dehydrator at 35°C and the powdered material was exhaustively extracted by maceration with methylene chloride-methanol mixture (3×500ml) in cold. Evaporation of the solvent left a brownish residue that was dissolved in minimum volume of methylene chloride. The residue was then subjected to silica-gel column chromatographic separation. Elution with solvents on a petroleum ether-ethyl acetate gradient afforded one major fraction as a sticky mass at the 10% ethyl acetate in petroleum ether solvent polarity range.

RESULTS AND DISCUSSION

Crystallization of this mass from EtOAc-acetone yielded a pure compound I as colorless needles, melting point 162 ºC. The molecular mass and molecular formula of the compound was settled from the HR-ESI-MS (+) peak at 421.3443 $[M+Na]^+$ compatible with the molecular formula $C_{28}H_{46}ONa$ (calc. 421.3449) and having a molecular mass of 398 *amu*. The molecular formula revealed the existence of six double bond equivalents in it.

EIMS peak at *m/z* 398$[M]^+$ and *m/z* 380$[M\text{-}H_2O]^+$ were the two most prominent peaks at the higher mass region. This indicates that apart from a hydroxyl group, the structural moiety lacks any major functionality. Loss of the side chain from the molecular ion gave rise to the prominent peak at *m/z* 271. Again, loss of a methyl group followed by the

$OH^{\bullet}$ radical produced the EIMS peak at *m/z* 366 $[M\text{-}OH^{\bullet}\text{-}CH_3]^+$. Other part in the higher mass region of the EIMS spectrum was relatively non-structured indicating the relatively non functionalized nature of the molecule. The thin film IR spectrum (KBr disc) showed a broad band around 3435 cm^{-1} and a matching absorption at 1041 cm^{-1} indicating the presence of an O-H group. The C=C stretching vibrations of the molecule were detected as a moderately intense peak at 1656 cm^{-1}, indicating their non-conjugated nature. Presence of reasonable number of CH_2 and CH_3 groups was apparent from their characteristic intense bending absorptions at 1459 cm^{-1} and 1372 cm^{-1} respectively. It also exhibited sharp C – H stretching bands at 2941 and 2872 cm^{-1}.

The ^1H NMR spectrum showed resonances of three ethylenic hydrogens, one as triplet at δ_H 5.20 and two as overlapping doublets at around δ_H 5.18. The low field complex multiplet type H-atom signal around δ_H 3.61 was ascribed to hydrogen atoms attached to the oxygen bearing carbon, i.e. H–C(3). The spectrum exhibited altogether five methyl H-atom signals out of which the high field singlet signal at δ_H 0.54 was indicative of a methyl group so disposed that it feels the shielding anisotropic effect of the cyclohexane core, and was attributed to H–C(19). Another singlet methyl group H-atom signal at δ_H 0.79 and was ascribed to H–C(18). A set of four methyl group H-atom signals appeared as doublets at δ_H 0.81 (3H, d, *J=6.7*), δ_H 0.83 (3H, d, *J=6.6*), δ_H 0.91 (3H, d, *J=6.8*) and δ_H 1.01 (3H, d, *J=6.5*) were assigned to H–C(25), H–C(26), H–C(27) and H–C(28) respectively, the latter two protons are homoallylic, hence show up as a little bit deshielded. H–C(5) resonated as a triplet signal at δ_H 1.84, indicating that it is in close proximity to the oxygen, confirming that C(3)-OH was α- to the cyclohexane ring. Other two protons in close proximity to the oxygen, i.e. the α-H–C(2) and α-H–C(4) showed up at δ_H 2.02.

Rest of the aliphatic region was of complex pattern and showed a surplus of overlapping H-atom signals, and the complete assignment has been done by analogy with NMR data of similar skeletons [14, 15]. The ^1H NMR data is easily conceivable considering the stereochemical representation of the molecule (Figure 1).

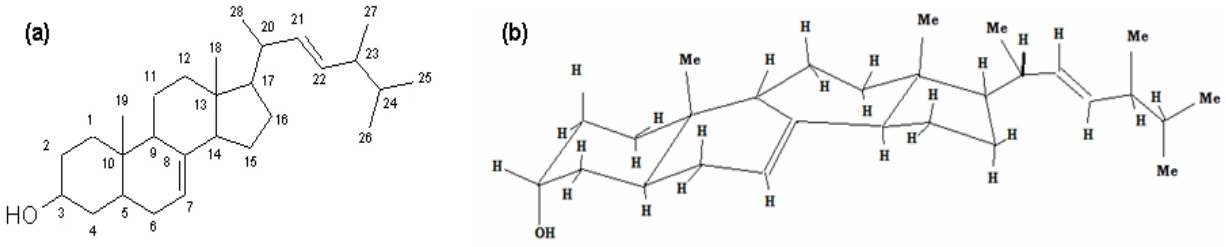

Figure 1: Structural formula of Gigantenol. (a) Standard representation; (b) Stereochemical representationl.

The ^{13}C NMR spectrum accounted for all the twenty eight carbon resonances, comprising six methyl, eight methylene, eleven methine and three quaternary carbon atoms. The presence of an endocyclic double bond was supported by the low field quaternary carbon signal at δ_C 139.5 and another low field methine C-atom signal at δ_C 135.6. The molecule showed another set of low field C-atom methine signals at δ_C values of 131.8 and 117.4 suggesting the presence of another double bond. Another low-field methine C-atom signal, directly bonded to oxygen atoms at δ_C value of 71.0 was ascribed to C(3). One of the carbons at AB ring junction, C(5) is pretty close to the oxygen atom in space,

and consequently, showed up as CH signal at δ_C 55.9. The allylic CH signals due to carbons at C(9), C(20) and C(23) appeared downfield at δ_C values of 55.1, 49.4 and 42.8 respectively. The CH2 carbons C(1), C(2) and C(4) are closer to the oxygen at C(3) and showed up as CH2 signals at δ_C values of 39.2, 37.9 and 37.1 respectively. The quaternary signals at δ_C values of 43.3 and 34.2 were ascribed to the carbons at ring junction AB and CD, i.e. C(10) and C(13) respectively. The relatively upfield CH signal at δ_C 33.1 was assigned to the aliphatic carbon, C(24). A complete assignment of C and H resonances is presented in Table 1. Based on all these observations, the triterpene structure I have been proposed for the compound (Figure 1). The compound was named *Gigantenol*, after the species from which it was isolated.

Table 1: 1H(300 MHz)- and 13C(75.5 MHz)- NMR Spectral Data for Gigantenol in CDCl3

Position	δ_H in ppm	δ_C in ppm
CH2(1)	2.00, *m* and 1.62, m	39.2
CH2(2)	2.02, *d,* 5 and 1.75, m	37.9
CH(3)	3.61, *m*	71.0
CH2(4)	2.02, *d,* 5 and 1.66, m	37.1
CH(5)	1.84, *t,* 6	55.9
CH2(6)	1.80, *m*	39.2
CH(7)	5.20, *t,* 5	135.6
C(8)		139.5
CH(9)	1.75, *m*	55.1
C(10)		43.3
CH2(11)	1.60, *m* and 1.42, *m*	31.4
CH2(12)	1.64, *m* and 1.40, *m*	29.6
C(13)		34.2
CH(14)	1.78, *t,* 5	49.4
CH2(15)	1.26, *m*	21.5
CH2(16)	1.33, *m*	22.9
CH(17)	1.46, *m* and 1.38, *m*	40.2
Me(18)	0.79, *s*	13.0
Me(19)	0.54, *s*	12.1
CH(20)	1.70, *m*	49.4
CH(21)	5.18, *d,* 6	131.8
CH(22)	5.18, *d,* 6	117.4
CH(23)	1.30, *m*	42.8
CH(24)	1.28, *m*	33.1
Me(25)	0.81, *d,* 6.7	19.6
Me(26)	0.83, *d,* 6.6	17.6
Me(27)	0.91, *d,* 6.8	19.9
Me(28)	1.01, *d,* 6.5	21.1

REFERENCES

[1] Mallikarjuna SE, Ranjini A, Haware DJ, Vijayalashmi MR, Sashirekha MN. J Chem 2013; 1-5 (Article ID 805284).

[2] Khatua S, Palu S, Acharya K. Res J Pharm Technol 2013; 6: 496-505.

[3] Biswas G, Chatterjee S, Acharya K. Digest J Nanomaterials Biostruct 2012; 16: 1543-1560.

[4] Biswas G, Rana S, Sarkar S, Acharya K. Pharmacologyonline 2011; 2: 808-817.

[5] Biswas G, Acharya K. Int J Pharm Pharm Sci 2013; 5: 391-394.

[6] Biswas G., Sarkar S. and Acharya K. Lat Am J Pharm 2010; 29: 549-553.
[7] Chatterjee A, Khatua S, Chatterjee S, Mukherjee S, Mukherjee A, Paloi S, Acharya K, Bandyopadhyay SK. Glycoconjugate J 2013; 30(8): 759-768.
[8] Chatterjee S, Dey A, Datta R, Dey S, Acharya K. Int J Pharm Tech Res 2011; 3: 2162-2168.
[9] Hawksworth DL, Rossman AY. Phytopathology 1997; 87: 888-891.
[10] Lee DH, Kim JH, Park JS, Choi YJ, Lee JS. Peptides 2004; 25: 621-627.
[11] Chatterjee S, Saha GK, Acharya K. Pharmacologyonline 2011; 3: 88-97.
[12] Chatterjee S, Biswas G, Chandra S, Saha GK, Acharya K. Bioproc Biosyst Eng 2013; 36: 101-107.
[13] Chatterjee S, Biswas G, Chandra S, Saha GK, Acharya K. Int J Pherm Sci Rev Res 2014; 26: 189-196.
[14] Lai TK, Biswas G, Chatterjee S, Dutta A, Pal C, Banerji J, Bhuvanesh M, Reibenspies J H, Acharya K. Chem Biodivers 2012; 9: 1517-1524
[15] Bandyopadhyay D, Basak B, Chatterjee A, Lai TK, Banerji A, Banerji J, Neuman A, Prangé T. Nat Prod Res 2006; 20(10): 961 - 965