Isolation of β-sitosterol diglucosyl caprate from *Alpinia galanga*

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ABSTRACT

Background: The purpose of present investigation to isolate β-sitosterol diglucosyl caprate from the rhizomes of *Alpinia galanga.* **Methods:** The methanolic extract of the rhizomes of plant *Alpinia galanga* was subjected to column chromatography and was eluted with ethyl acetate-methanol (99:1) to yield compound (AG5) β-sitosterol diglucosyl caprate. Various spectral techniques such as Ultraviolet (UV), Infrared (IR), Hydrogen Nuclear Magnetic Resonance (1HNMR), Carbon Nuclear Magnetic Resonance (13CNMR), and MASS spectrometry (MS), were employed to determine and elucidate. **Results:** Chemical and spectral investigation of extract furnished a new steroidal glycoside. **Conclusion:** The isolated compound β-sitosterol diglucosyl caprate could serve as a lead compound in synthesis of steroids.

Key words: *Alpinia galanga*, methanolic extract, rhizomes, β-sitosterol diglucosyl caprate

INTRODUCTION

Alpinia galanga is a perennial herb, which belongs to the Zingiberaceae family, commonly known as Kulingen.[1,2] Various essential oil constituents, such as, cineole, methyl cinnamate, myrecene, and methyl eugenol are obtained from this plant. This plant is also reported to contain various flavones like galangin, alpinin, kampferide, and 3-dioxy-4-methoxy flavone.[3,4] *Alpinia galanga* is attributed with various pharmacological activities such as, antimicrobial, antioxidant, antifungal, anti-cancer, and gastroprotective.[5-7] The present article gives an account of the isolation of steroidal glycone and structural determination grounds by means of various spectroscopic methods like UV, IR, NMR, and MS.

MATERIAL AND METHODS

General

The melting point was determined in open capillary and is uncorrected. IR spectra were recorded using KBR pellets, on a Jasco FTIR-550 spectrophotometer. IH NMR and 13C NMR spectra were recorded on Bruker DPX 300 Hz. The Mass spectra were recorded on the FAB-JEOL-MS 303 system. Purity of the isolated compound was checked by TLC aluminum sheets – silica gel 60 F254 (0.2 mm). All the reagents and solvents used in present study were of AR grade and procured from Rankem (Ranbaxy laboratory, Okhla, New Delhi).

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Plant

The dried rhizomes of *Alpinia galanga* (Zingiberaceae) were collected in the Pusad province of India and identified by Prof. Anjula Pandey, Taxonomist, National Bureau of Plant Genetic Resources, PUSA, New Delhi. A voucher specimen No. EP-542 has been deposited in the Natural Medicine Research Center of this Institute.

Extraction and isolation

The defatting of the dried, ground rhizomes of *Alpinia galanga* (3000 g) was performed with petroleum ether and successively extracted with methanol using the soxhlet apparatus. The methanolic extract was evaporated to yield a dark brown solid, which was subjected to Silica gel column chromatography (100 – 120 mesh), and eluted with EtOAc – MeOH (99:1) to give compound AG5 (78 mg).

RESULTS

The methanolic extract was column chromatographed over silica gel using EtOAc – MeOH (99:1) as an eluent to

yield colorless crystals of compound β-sitosterol diglucosyl caprate (AG5) [Figure 1], which were recrystallized from acetone. The compound AG5 responded positively to the tests of steroids and showed an Rf value of 0.62 in the EtOAc – MeOH (49:1) solvent system. The melting point for isolated compound AG5 was determined by the open capillary method and was recorded as 184°C – 187°C, which was uncorrected. The compound AG5 showed IR bands at 3510, 3432, 3360, 2923, 2850, 1721, 1641, 1504, 1453, 1389, 1263, and 1032 cm-1. The positive FAB-MS showed m/z at 892 [M]+ (C_{51} H_{88} O_{12}) (1.1), 479 (16.1), 464 (33.6), and 413 (92.3). The structure of compound AG5 was further supported by the 1H-NMR and 13 C-NMR data given in Table 1.

DISCUSSION

The compound AG5, named as β-sitosterol diglucosyl caprate, was obtained in a colorless crystalline form. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3510, 3432, 3360 cm^{-1}), ester group (1721 cm^{-1}), and unsaturation (1641 cm^{-1}). The positive ion FAB-Mass spectrum showed a molecular ion peak generated at 413, which indicated a β-sitosterol nucleus in the molecule. The ion peak arising at m/z 479 $[C_{22}H_{39}O_{11}]^+$ and 464 $[C_{21}H_{36}O_{11}]^+$ indicated the location of a diglycosidic moiety esterified with capric acid in β-sitosterol glycoside.

The ^{1}H NMR of compound AG5 exhibited signals at δ 5.32 (Vinylic, 1H, d, H-6), δ 0.91(3H, d, CH_3, C-21), δ 0.82 (3H, d, CH_3, C-26) and δ 0.80 (3H, d, CH_3, C-27), δ 0.65 (3H, br, C-18), 0.95 (3H, br, C-19). Anomeric C-1 proton and other protons of the glucose moiety were observed at δ 5.01 (H-1'), δ 4.38 (H-2'), δ 4.03 (H-3'), and δ 3.09 (H-6'). A broad signal integrating 12 protons at δ 1.23 was attributed to the methylene protons. ^{13}C NMR spectral data of AG5 showed the presence of 51 carbon atoms in the molecule. Signals at δ 140.38, δ 121.22, and δ 73.38 were assigned to C-5, C-6 unsaturated carbons, and C-3 carbinol carbon, respectively. The anomeric carbon appeared at δ 100.79 (C-1'). The remaining sugar carbons resonated in the range of δ 73.86 – 63.07. A signal at δ 18.03 was attributed to methyl position (C-10'''). The deshielding of the C-2' Carbon signal at δ 81.29 in the ^{13}C NMR and a one proton

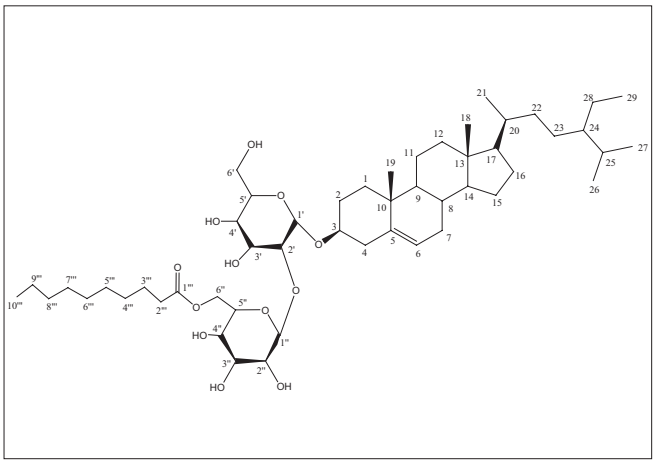

Figure 1: Structure of β-sitosterol diglucosyl caprate (compound AG 5)

Table 1: ^{1}H NMR and ^{13}C NMR values of β-sitosterol diglucosyl caprate (compound AG 5)

Position	^{1}HNMR		^{13}C NMR
	Alpha	Beta	
1	1.41 m	2.40 m	36.87
2	1.94 m	1.86 m	36.26
3	3.25 brm (w1/2 = 18.2)	----	73.48
4	2.63 d (J = 11.2)	2.34 d (J = 6.1)	41.12
5	-------	----	140.38
6	5.32 d (J = 4.9)	----	121.22
7	2.50 m	2.48 m	29.01
8	----	1.78 m	31.41
9	1.62 m	----	51.64
10	----	-----	36.25
11	2.01 m	1.45 m	20.61
12	1.13 m	1.83 m	37.68
13	----	----	41.05
14	1.16 m	----	56.18
15	1.18 m	1.50 m	23.87
16	1.76 m	1.53 m	27.81
17	1.41 m	----	55.44
18	0.65 brs	---	11.70
19	0.95 brs	----	19.72
20	----	2.12 m	35.49
21	0.91 d J = 6.5)	---	18.62
22	1.50 m	1.20 m	33.36
23	1.18 m	1.65 m	25.46
24	1.20 m	---	45.15
25	1.55 m	---	29.16
26	0.82 d (J = 6.1)	---	19.10
27	0.80 d (J = 6.3)	---	18.94
28	1.16 m	1.55 m	22.62
29	0.78 d (J = 6.2)	-----	11.68
G-1'	5.01 d (J = 7.1)	----	100.78
G-2'	4.38 d (J = 4.8)	----	81.29
G-3'	4.03 m	----	71.77
G-4'	3.96 m	----	70.13
G-5'	4.50 m	----	76.77
G-6'	3.09 (J = 8.6)	3.04d (J = 8.6)	61.12
G-1"	4.89 d (J = 7.3)	---	100.79
G-2"	4.25 d (J = 6.3)	---	73.86
G-3"	4.01 m	----	71.81
G-4"	3.86 m	----	67.03
G-5"	4.43 m	----	76.79
G-6"	3.69 d (J = 11.3)	3.64 d (J = 11.3)	63.07
1'''	----	---	171.61
2'''	2.69 d (J = 7.3)	2.59 d (J = 7.3)	59.24
3'''	1.45 m	1.41 m	49.61
4'''	1.23 brs	1.23 brs	28.81
5'''	1.23 brs	1.23 brs	28.81
6'''	1.23 brs	1.23 brs	28.81
7'''	1.23 brs	1.23 brs	28.81
8'''	1.23 brs	1.23 brs	28.81
9'''	1.23 brs	1.23 brs	28.81
10'''	0.84 t (J = 6.1)	---	18.03

doublet at δ 4.38 (J = 4.8 Hz) indicated the attachment of the second glucose moiety at C-2'. The shifting of the oxygenated C-6" methylene proton signal at δ 3.69 (J = 11.3 Hz) and δ 3.64 (J = 11.3 Hz) and the methylene carbon at δ 63.07 (C-6") suggested the location of the ester group at C-6". A signal at δ 171.61 was assigned to C-1"' ester carbon. The 1H-1H COSY spectra showed the correlation of H-3(δ H, 3.25 ppm) with H_2-2/H_2-4, H-6 (δ H, 5.32 ppm) with H_2-7, and H-8 (δ H, 1.78 ppm) with H_2-7/H-9/H-14. The C-H HMBC spectra exhibited the correlation of carbon at δc 140.38 (C5) with H_2-1/H_2-4/H_3-19, carbon at δc 36.25 (C10) with H-9/H-6/H_3-19, and δc 41.05 (C13) with H-17/H_2-15/H_3-18. In addition to this, correlation of H-3 with G-1'/G-1" corresponded to the linkage between sitosterol and diglucopyranose. The HMBC and 1H-1H COSY spectra showed the correlation of carbon at δc 45.15 (C24) with H_2-28, whereas, δH 1.20 (H-24) with H_2-28. The acid hydrolysis of AG5 yielded capric acid, β-D–glucose, and β-Sitosterol. Thus the structure of compound AG5, β-sitosterol-3-β-D-glucopyranosyl (2 →1")-β-D-glucopyranosyl 6"-n-decanoate was determined.

CONCLUSION

The present study deals with isolation and structural elucidation of a newer steroidal glycoside compound AG5. The data obtained by UV, IR, ^{1}H-NMR, ^{13}CNMR, MASS spectrometry, and a chemical test, has resulted in compound AG5, and has confirmed that the compound is steroidal in nature and is elucidated as β-sitosterol-3-β-D-glucopyranosyl (2 →1")-β-D-glucopyranosyl 6"-n-decanoate for the first time.

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