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Isolation of β-Sitosterodiglucoside and β-Sitsteryl Arabinoside from Rhizomes *Alpinia galanga*

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This paper reports β-sitosterodiglucoside (**AG 7**) and β-sitsteryl arabinoside (**AG 8**), two constituents isolated from *Alpinia galanga* are characterized on the basis their spectral value.

Key Words: *Alpinia galnga*, Zingiberaceae, β-Sitosteryl arabinoside, β-Sitosterodiglucoside.

INTRODUCTION

Alpinia galanga (Zingiberaceae) is a perennial herbaceous plant growing 1-2 cm height[1]. The rhizomes of *Alpinia galanga* are commonly used for healing various diseases like gastralgia, diseases of heart, colic, diarrhoea and malaria fever, liver troubles, rheumatoid arthritis[2-5] and bronchial catarrh[6]. Various constituents isolated such as cineol, methyl cinnamate[7,8] and flavones galangin, alpinin[9,10], kampferide and 3-dioxy-4-methoxy flavone myrecene, (Z)-β-ocimene, α-pinene, borneol[7] phenylpropanoids[11], acetoxychavicol acetate[12], acetoxy-1,8-cineoles[13,14], methyl eugneol, caryophyllene[15], 2,3-dihydroxy-1,8-cineole glucosides[16]. The present investigation reports β-sitosterodiglucoside, β-sitosteryl arabinoside isolated form rhizomes of *Alpinia galanga*.

EXPERIMENTAL

The dried rhizomes of *Alpinia galanga* (Zingiberaceae), collected in Pusad, India were identified by Prof. Alka Chaturvedi, Department of Botany, Nagpur University, Nagpur, India. A voucher specimen is deposited in the Natural Medicine Research Center of this Institute.

Column chromatography[17] was carried out using silica gel 60-120 mesh (Hi media). Thin layer chromatography[18] (TLC) was carried out using silica gel G (Hi Media). IR spectra of the compounds were recorded in a Jasco IR spectrophotometer using KBr pellet. The FT-NMR spectra (Bruker) were recorded using $CDCl_3$ as solvent and TMS as internal standard.

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Preparation of extract and isolation of compounds from methanolic extract using column chromatography: Dried, ground rhizome of *Alpinia galanga* (3000 g) defatted with petroleum ether, successively extracted with MeOH using soxhlet apparatus. After evaporation, a dark brown solid (35 g) was obtained which is subjected to Si-Gel CC (100-120 mesh) and eluted with, EtOAc-MeOH (49:1) to give compound **AG 7** (17 mg, 0.4857 % yield), compound **AG 8** (56 mg, 0.16 %yield). The purity of eluted compound was tested using thin layer chromatrography developed in solvent system of ($CH_3COOC_2H_5$:$CHCl_3$, 19:1; $CH_3COOC_2H_5$:CH_3OH, 9.5:0.5).

Compound AG 1: Colourless crystals, m.p. 274-275 ºC (decom.), UV λ_{max} (MeOH): 257 nm. IR (KBr, cm^{-1}): 3510, 3452, 3360, 2923, 1636, 1516, 1450, 1382, FAB-MS m/z (Relative intensity): 738 $[M]^+$ ($C_{41}H_{70}O_{11}$) (2.1), 413 (13.6), 398 (12.3), 396 (14.1), 383 (21.2), 271 (14.1), 256 (24.6), 254 (22.5), 238 (18.9), 229 (24.6),214 (19.8), 199 (30.1). ^{1}H NMR (DMSO-d_6): presented in Table-1. ^{13}C NMR (DMSO-d_6): presented in Table-1.

Compound AG 2: Colourless crystals, m.p. 273-274 ºC (decomp.), UV λ_{max} (MeOH): 257 nm. IR (KBr, cm^{-1}): 3510, 3430, 2927, 1643, 1507, 1455, 1385, 1028. FAB-MS m/z (Relative intensity): 546 $[M]^+$ ($C_{34}H_{58}O_5$) (13.6), 413 (52.1), 398 (41.2), 396 (16.8), 383 (21.6), 271(11.9), 256 (18.7), 253 (17.9), 238 (14.8), 229 (18.7), 214 (23.5), 150 (78.9), 133 (36.1). ^{1}H NMR (DMSO-d_6): presented in Table-2. ^{13}C NMR (DMSO-d_6): presented in Table-2.

RESULTS AND DISCUSSION

Compound **AG 7**, designated as β-sitosterol diglucoside was obtained as colourless crystal from ethyl acetate:methanol (49:1) as eluants. It responded positively to steroidal glycosides. Its infrared spectrum exhibited characteristics absorption bands at 3510, 3452, 3360 cm^{-1} and band for unsaturation is at 1636 cm^{-1}. The positive ion FAB mass spectrum of **AG7** showed a molecular ion peak at m/z 738 corresponding to steroidal diglycoside ($C_{41}H_{70}O_{11}$). It indicated 7 double bond equivalents; 4 of them were adjusted to the tetracyclic carbon skeleton of the steroidal nucleus, 1 in the vinylic linkage and the remaining 2 in the sugar moieties. The prominent ion fragments generated at m/z 413 $[M\text{-}C_{12}H_{21}O_{10}]^+$, 398 $[413\text{-}Me]^+$, 396 $[413\text{-}H_2O]^+$, 271 $[413\text{-}C_{12}H_{21}O_{10}]^+$, 256 $[271\text{-}Me]^+$, 214 [256-ring D fission]$^+$, 199 $[214\text{-}me]^+$ and 383 $[398\text{-}me]^+$ supported the presence of β-sitosterol as a glycone moiety.

The ^{1}H NMR spectrum of **AG 7** exhibited a 1 proton doublet at δ 5.52 d (J = 5.2) assigned to vinylic H-6 proton. One proton broad multiplet at δ 3.62 with w1/2 =18.5 Hz was ascribed to 3α′ methine proton (axial) interacting with C-2 equatorial, C-2 axial and C-4 equatorial, C-4 axial protons. Three doublets, integrating three protons, each at δ 0.90 (J = 6.1 Hz), δ 0.84 (J = 6.3 Hz) and δ 0.80 (J = 6.1 Hz) were accounted to C-21, C-26, C-27 secondary methyl protons, respectively. Two tertiary-18 and C-19 methyl signals appeared as 3 protons, each broad signal at δ 0.64 and δ 0.95, respectively. Three proton triplet at δ 0.82 (J = 6.1 Hz) was associated with C-29 primary methyl protons. Two 1 proton doublets at δ 5.33 (J = 7.1 Hz), δ 5.08 (J = 7.3 Hz) were attributed corresponding to anomeric protons H-1'and H-1" protons.

TABLE-1
^{1}H NMR AND ^{13}C NMR OF COMPOUND β-SITOSTERODIGLUCOSIDE

Position	^{1}H NMR		^{13}C NMR
	α	β	
1	1.42 m	2.89 m	36.83
2	1.94 m	1.81 m	29.01
3	3.62 brs (w 1/2=18.5)	–	73.46
4	2.53 d (12.1)	2.50 brs	40.32
5	–	–	141.49
6	5.52 d (5.7)	–	121.20
7	2.12 m	2.35 m	29.26
8	–	1.67 m	31.41
9	1.55 m	–	49.60
10	–	–	36.22
11	2.35 m	1.46 m	20.59
12	1.22 m	1.81 m	38.68
13	–	–	41.85
14	1.17 m	–	56.17
15	1.22 m	1.94 m	23.86
16	1.23 m	1.15 m	27.78
17	1.43 m	–	55.43
18	0.64 brs	–	11.70
19	0.95 brs	–	19.71
20	–	2.19 m	35.47
21	0.90 d (6.1)	–	18.61
22	1.50 m	1.17 m	33.35
23	2.25 m	2.19 m	25.45
24	1.21 m	–	45.14
25	1.47 m	–	28.71
26	0.84 d (6.3)	–	19.08
27	0.80 d (6.1)	–	19.06
28	1.22 m	1.81 m	22.61
29	0.82 t (6.1)	–	11.68
1’	5.33 dd (7.1)	–	100.77
2’	4.92 brs	–	81.28
3’	4.01 m	–	70.10
4’	3.48 m	–	73.84
5’	4.45 m	–	76.81
6’	3.12 d (9.0)	3.09 d (9.0)	61.09
1’’	5.08 d (7.3)	–	88.22
2’’	4.20 d (6.5)	–	72.38
3’’	4.03 m	–	67.74
4’’	3.46 m	–	72.29
5’’	4.25 m	–	76.77
6’’	3.06 dd (9.9)	3.04 d (9.9)	60.75

Coupling constants in Hertz are provided in parenthesis.

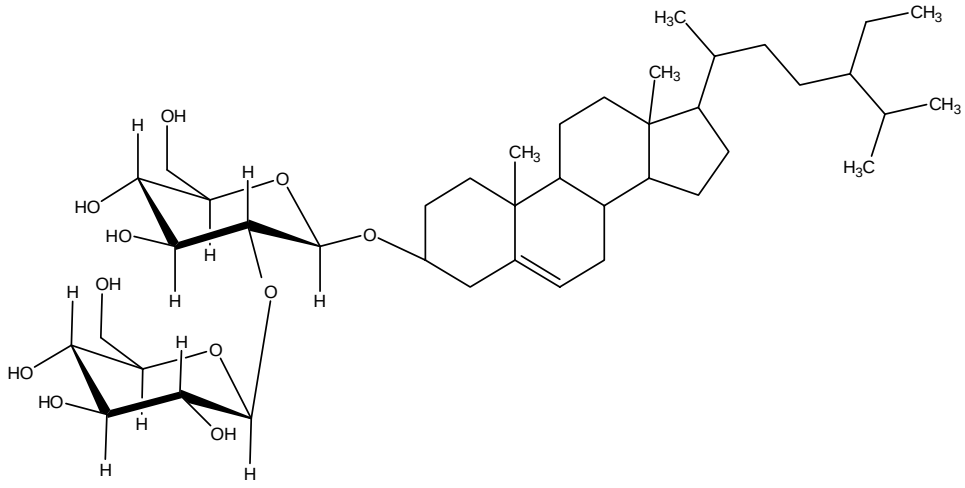
TABLE-2
^{1}H NMR AND ^{13}C NMR OF COMPOUND β-SITSTERYL ARABINOSIDE

Position	^{1}H NMR		^{13}C NMR
	α	β	
1	1.34 m	2.50 m	–
2	1.94 m	1.82 m	36.82
3	3.13 brs (w ½ 18.1)	–	29.26
4	2.54 d (12.0)	2.50 m	40.32
5	–	–	140.46
6	5.32 d (5.8)	–	121.19
7	2.12 m	2.09 m	31.39
8	–	1.63 m	31.41
9	1.55 m	–	49.61
10	–	–	36.21
11	2.35 m	1.46 m	20.59
12	1.12 m	1.83 m	38.31
13	–	–	41.85
14	1.23 m	–	56.17
15	1.23 m	1.78 m	23.85
16	1.23 m	1.25 m	27.77
17	1.43 m	–	55.43
18	0.65 brs	–	11.69
19	0.95 brs	–	19.70
20	–	2.15 m	35.46
21	0.91 d (6.5)	–	18.61
22	1.50 m	1.07 m	33.35
23	2.35 m	2.31 m	25.47
24	1.21 m	–	45.15
25	1.52 m	–	28.72
26	0.82 d (6.1)	–	19.09
27	0.80 d (6.3)	–	18.93
28	1.20 m	1.81 m	28.62
29	0.78 d (6.2)	–	11.67
1’	4.88 d (6.9)		100.78
2’	4.62 d (7.2)		76.98
3’	4.23 dd (7.2, 7.2)		76.74
4’	3.62 dd (7.2, 8.4)		70.13
5’	3.10 d (8.4)	3.05 d (8.4)	61.11

Coupling constants in Hertz are provided in parenthesis.

Four 1 proton doublets at δ 3.12 (J = 9.0 Hz), δ 3.09 (J = 9.0 Hz) and at δ 3.06 (J = 9.9Hz), 3.04 (J = 9.9 Hz) were associated with oxygenated methylene H_2-6' and H_2-6" protons, respectively. The other sugar protons appeared in the range of δ 4.92-3.46. The existence of the methyl signals between 0.64-0.95 supported the location of the methyl functionalities on the saturated carbons. The ^{13}C NMR spectra

of **AG 7** displayed important signals for vinyl carbons at δ 141.49 (C-5) and δ 121.20 (C-6), carbinol carbon at 73.46 (C-3), anomeric carbons at δ 100.27 (C-1') and d 88.22 (C-1") and other sugar carbons between δ 81.28-60.75. The appearance of the C-2' ^{1}H NMR signal in the deshielded range of δ 4.92 and ^{13}C NMR signal at δ 81.28 supported the linkage of another sugar moiety at this carbon. The ^{1}H NMR and ^{13}CNMR values were compared with the related molecules, *viz.* β-sitosterol, stigmasterol and lawsaritol. Alkaline hydrolysis of **AG 1** yielded β-sitosterol and D-glucose. On the basis of spectral data analysis and chemical reactions the structure of compound **AG 7** has been characterized as β-sitosterol-3β-D-glucopyranosyl (2'→1")-β-D-glucopyranoside. This is a new steroidal glycoside isolated from a natural or synthetic source for the first time.



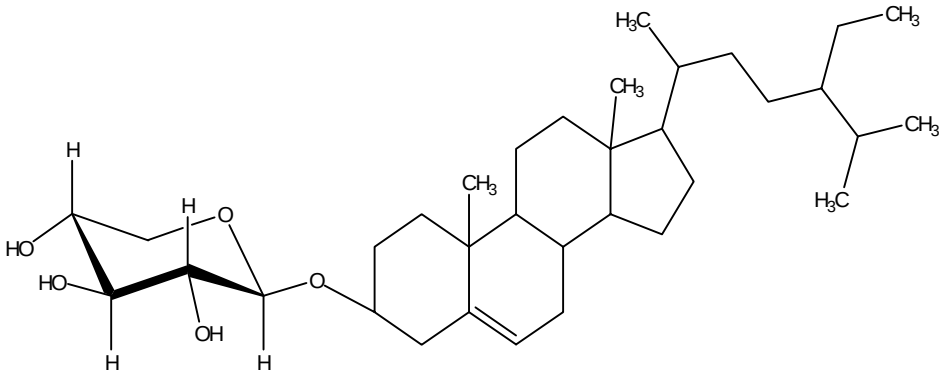
β-Sitosterol-3β-D-glucopyranosyl (2'→1")-β-D-glucopyranoside

Compound **AG 8**, named as β-sitosteryl arabinoside was obtained as colourless crystals from ethyl acetate:methanol (49:1) as eluants. It responded positively to steroidal glycosides. Its IR spectrum exhibited characteristics absorption bands for at 3510, 3430 cm^{-1} due to hydroxyl group and at 1643 cm^{-1} dye to unsaturation group.

The positive ion FAB-Mass spectrum of compound **AG 8** showed a molecular ion peak at 546 corresponding to molecular formula of a sterol glycoside, $C_{35}H_{58}O_5$. It indicated 6 double bond equivalents. Four of them were adjusted in the tetracyclic carbon framework of the sterol and 1 each in the vinylic linkage and sugar moiety. The prominent ion fragments arising at m/z 150 $[C_5H_{10}O_5]$, 133 $[C_5H_9O_4]^+$ and 413 $[M^+ - C_5H_9O_4]^+$, 133 $[C_5H_9O_4]^+$ and 413 $[M-C_5H_9O_4]^+$ indicated that a C_5 sugar was attached to the sterol. The ion fragments arising at m/z 398 $[414\text{-me}]^+$, 396 $[414\text{-}H_2O]^+$, 272 $[414\text{-}C_{10}H_{21}$, side chain$]^+$, 256 $[271\text{-me}]^+$, 253 $[271\text{-}H_2O]^+$, 221 [271-ring D]$^+$, 238 $[253\text{-me}]^+$ and 214 $[224\text{-me}]^+$ supported the presence of β-sitosterol moiety in the glycoside.

The ^{1}H NMR of compound **AG 8** exhibited a 1 proton doublet at δ 5.32 (*J* = 5.8 Hz) assigned to vinylic H-6 proton. One proton broad multiplet at δ 3.13 with w1/2 of 18.1 Hz was ascribed to 3α methine proton (axial) interacting with C-2 equatorial, C-2 axial and C-4 equatorial C-4 axial protons. Four doublets integrating three protons each, at δ 0.91 (*J* = 6.5Hz), δ 0.82 (*J* = 6.1 Hz) δ 0.80 (*J* = 6.3 Hz) and δ 0.78 (*J* = 6.2 Hz), were accounted to C-21, C-26 and C-27 secondary C-29 primary methyl protons, respectively. Two 3 proton broad signals at 0.65 and 0.95 were attributed C-18 and C-19 tertiary methyl protons. The appearance of all methyl signals in the range of δ 0.65-0.95 suggested that these functionalities were attached to saturated carbons.

Four 1 proton doublets at δ 4.38 (*J* = 6.9 Hz), δ 4.62 (*J* = 7.2 Hz), δ 3.10 (*J* = 8.4 Hz) were associated with the anomeric H-1', carbinol H-2' and oxygenated methylene H_2-5' protons, respectively. Two one proton double doublets at δ 4.23 (*J* = 7.2 Hz) and δ 3.62 (*J* = 7.2, 8.4) were accommodated to the carbinol H-3' and H-4', respectively. The ^{13}CNMR spectrum of **AG 8** exhibited important signals for vinylic carbons at δ 140.46 (C-5) and δ 121.19 (C-6), carbinol carbon at δ 73.47(C-3), anomeric carbon at δ 100.78 (C-1') and sugar carbons in the range δ 76.98-61.11. The ^{1}H and ^{13}C NMR chemical shifts of AG 2 were compared with related sterols. Acid hydrolysis of **AG 8**, yielded β-sitosterol and D-arabinose, on the basis of the forgoing discussion the structure of **AG 8** has been elucidated as β-sitosterol-3-β-D-arabinopyranoside. This is a new steroidal glycoside isolated from a natural or synthetic source for the first time.



β-Sitosterol-3β-D-arabinopyranoside

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