ORIGINAL ARTICLE

Isolation of galangogalloside from rhizomes of *Alpinia galanga*

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Alpinia galanga commonly known as Kulingen. Plant is selected for isolation of newer constituents responsible various therapeutic activities like Antidiabetic and anti-inflammatory activities. The purpose of present study to isolate new phenolic glycoside from *Alpinia galanga* rhizomes. Methanolic extract of *Alpinia galanga* was column chromatographed and eluted with ethyl acetate-methanol (9:1) to isolate compound AG 13, Galangogalloside. New structure of Galangogalloside was interpreted by various spectral techniques (UV, IR, ^{1}HNMR, ^{13}CNMR, and MS). Chemical investigation of the methanolic extract of the rhizomes of *Alpinia galanga* furnished new gallic acid glycoside (Galangogalloside). New gallic acid glycoside isolated from medicinal plant or synthetic source for the first time.

Key words: AG 13, *Alpinia galanga,* galangogalloside, gallic acid glycoside, methanolic extract

INTRODUCTION

Alpinia galanga Willd is a perennial herbaceous plant with rhizomatous root stocks and tall leafy stems belonging Zingiberaceae family, commonly known as Kulingen, greater galangal.[1,2] This plant is well known for its richness in essential oils such as cineole, methyl cinnamate, myrecene, and methyl eugenol. This plant is also reported to contain various flavones like galangin, alpinin, kampferide and 3-dioxy-4-methoxy flavones.[3,4] *Alpinia galanga Willd* is known to possess antimicrobial activity, antioxidant activity, antifungal activity, anticancer activity, and gastroprotective activity.[5-7] Present paper reports the isolation of phenolic glycoside and structural determination evidences by means of various spectroscopic methods like UV, IR, NMR and Mass spectrometry.

MATERIALS AND METHODS

General

Melting point was determined in open capillary and is uncorrected. IR spectra were recorded using KBr pellets, recorded on Jasco FTIR-550 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DPX 300 Hz and Mass spectra on FAB-JEOL-MS 303 system. Purity of isolated compound was checked by TLC aluminium sheets –Silica gel 60 F254 (0.2 mm).

Plant

The dried rhizomes of *Alpinia galanga* (Zingiberaceae), collected in Pusad province of India were identified by Prof. Anjula Pandey, Taxonomist, National bureau of plant genetic resources, PUSA, New Delhi. A voucher specimen No. EP-542 is deposited in the Natural Medicine Research Center of this Institute.

Extraction and Isolation

Dried, ground rhizome of *Alpinia galanga* (3000 g) was defatted with petroleum ether, and successively extracted with methanol using Soxhlet apparatus. The methanolic extract was evaporated to yield a dark brown solid (35 g), which was subjected to Si-gel column chromatography (100-120mesh) eluted with, EtOAc–MeOH (9:1) to give compound AG 13 (670 mg).

Hydrolysis

Compound AG 13 (25 mg) was dissolved in 5 ml of HCl- MeOH (1:1) and refluxed for 1 hour. The solvent was evaporated under reduced pressure and the residue was dissolved in water (5 ml). It was extracted with petroleum ether to remove palmitic acid (TLC comparable) and then with solvent ether to separate gallic acid (TLC comparable). The sugars present in the aqueous extract were detected by TLC using standard samples of D-glucose (Rf 0.12) and L-arabinose (Rf 0.18) using n-BuOH-HOAc-H_2O, 4:1:5, top layer.

RESULTS

The methanolic extract was column chromatographed over silica gel using EtOAc–MeOH (9:1) as eluants to yield Compound AG 13 as galangogalloside [Figure 1], obtained as a pale yellow crystalline mass. It responded

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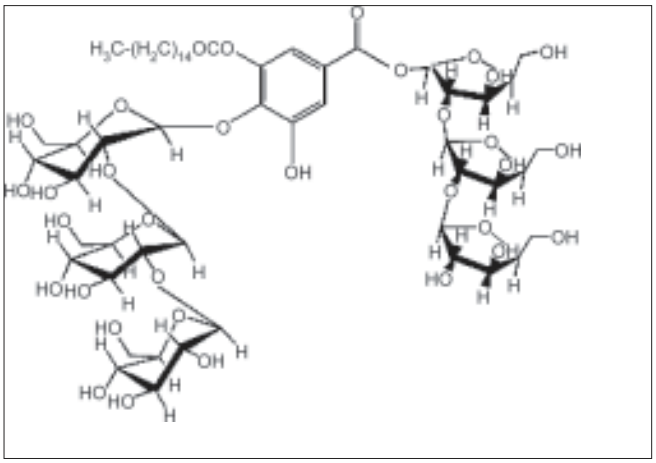

Figure 1: Structure of galangogalloside (Compound AG 13)

positively to the tests of phenolic glycosides. Compound AG 13 showed Rf value of 0.59 in EtOAc-acetone (99:1) solvent system. Its Melting point was determined by open capillary method and recorded as mp. 182°C-185°C, is uncorrected. The compound AG 13 showed UV λ_{max} at 275 nm; IR bands at 3402, 2931, 1722, 1637, 1509, 1399, 1232, 1054, 777/cm; Positive FAB-MS m/z at 1292 $[M]^+$ ($C_{56}H_{92}O_{33}$), (2.1), 565 (14.1), 539 (26.3) 413 (11.3), 397 (31.6), 391(30.7), 265 (18.9), 239 (12.3), 163 (35.1), 133 (52.3).

The ^{1}H-NMR and ^{13}C-NMR data of compound AG 13 are given in [Table 1].

DISCUSSION

Compound AG13, designated as Galangogalloside, the pale yellow crystalline mass obtained from ethyl acetate: Methanol (9:1) as eluants, exhibited its IR spectrum distinctive absorption bands for hydroxyl group (3402/cm), ester group (1722/cm) and methylene groups of long aliphatic chain (722/cm). Its positive ion FAB mass spectrum showed a molecular ion peak at m/z 1292 corresponding to the gallic acid glycoside esterified with a fatty acid, $C_{56}H_{92}O_{33}$ [Figure 2]

The ^{1}H NMR spectrum of AG 13 showed signal at δ 8.09 (2H, d, H-2) and δ 6.69 (2H, d, H-6) and the existence of one of the signal in the deshielded region at δ 8.09 indicated the location of the ester linkage at C-3. The ^{1}H NMR spectrum of AG 13 exibited signal for methylene protons at δ 2.50 (2H, brs, C-2′), primary methyl protons at δ 0.84 (3H, t, C-16′) and remaining methylene protons resonated between δ 2.26-1.22. The ^{1}H NMR spectrum of AG 13 put on view for signals of anomeric protons at δ 5.29 (H, d, G-1), δ 5.01 (2H, d, G-1′) δ 5.01 (H, d, G-1′′), anomeric protons of arabinose moieties of δ 4.93 (2H, br, A-1), δ 4.90 (1H, br, A-1′), δ 4.90 (1H, br A-1′′) and oxygenated methylene protons of the glucose and arabinose residues at δ 3.40-3.17

Table 1: ^{1}H and ^{13}C NMR values of galangogalloside

Position	1H NMR		13CNMR
	Alpha	Beta	
1	-	-	134.46
2	8.09 d (1.90)	-	121.25
3	-	-	166.78
4	-	-	160.58
5	-	-	150.26
6	6.69 d (1.90)	-	115.39
7	-	-	169.67
1′	-	-	173.57
2′	2.50 brs	2.50 brs	59.34
3′	2.26 m	2.24 m	55.70
4′	1.84 m	1.84 m	51.38
5′	1.34 m	1.34 m	33.96
6′	1.22 brs	1.22 brs	31.26
7′	1.22 brs	1.22 brs	29.197
8′	1.22 brs	1.22 brs	29.07
9′	1.22 brs	1.22 brs	29.07
10′	1.22 brs	1.22 brs	28.43
11′	1.22 brs	1.22 brs	26.55
12′	1.22 brs	1.22 brs	26.55
13′	1.22 brs	1.22 brs	24.57
14′	1.22 brs	1.22 brs	22.43
15′	1.22 brs	1.22 brs	.....
16′	0.84 t (6.30)	-	17.83
G-1	5.29 d (7.20)	-	102.06
G-2	4.25 m	-	72.56
G-3	3.87 m	-	70.66
G-4	3.82 m	-	69.31
G-5	4.39 m	-	76.81
G-6	3.40 d (12.90)	3.35 d (12.50)	62.18
G-1′	5.01 brs	-	92.31
G-2′	4.25 m	-	71.57
G-3′	3.82 m	-	70.29
G-4′	3.57 m	-	67.90
G-5′	4.39 m	-	75.41
G-6′	3.40 d (12.90)	3.35 d (12.90)	61.20
G-1″	5.01 brs	-	88.35
G-2″	3.92 m	-	71.57
G-3″	3.82 m	-	70.01
G-4″	3.57 m	-	66.50
G-5″	4.37 m	-	76.81
G-6″	3.40 d (12.90)	3.35 d (12.90)	61.17
A-1	4.93 brs	-	104.24
A-2	4.25 m	-	77.64
A-3	3.57 m	-	73.96
A-4	4.53 m	-	83.01
A-5	3.32 d (12.30)	3.28 d (12.30)	64.46
A-1′	4.93 brs	-	98.12
A-2′	4.16 m	-	75.83
A-3′	3.57 m	-	73.19
A-4′	4.53 m	-	81.96
A-5′	3.32 d (12.30)	3.28 d (12.30)	63.81
A-1″	4.90 brs	-	96.97
A-2″	3.61 m	-	74.94
A-3″	3.57 m	-	72.56
A-4″	4.50 m	-	81.06
A-5″	3.25 d (10.50)	3.17 d (10.50)	83.15

Coupling constants in Hertz are provided in parenthesis.

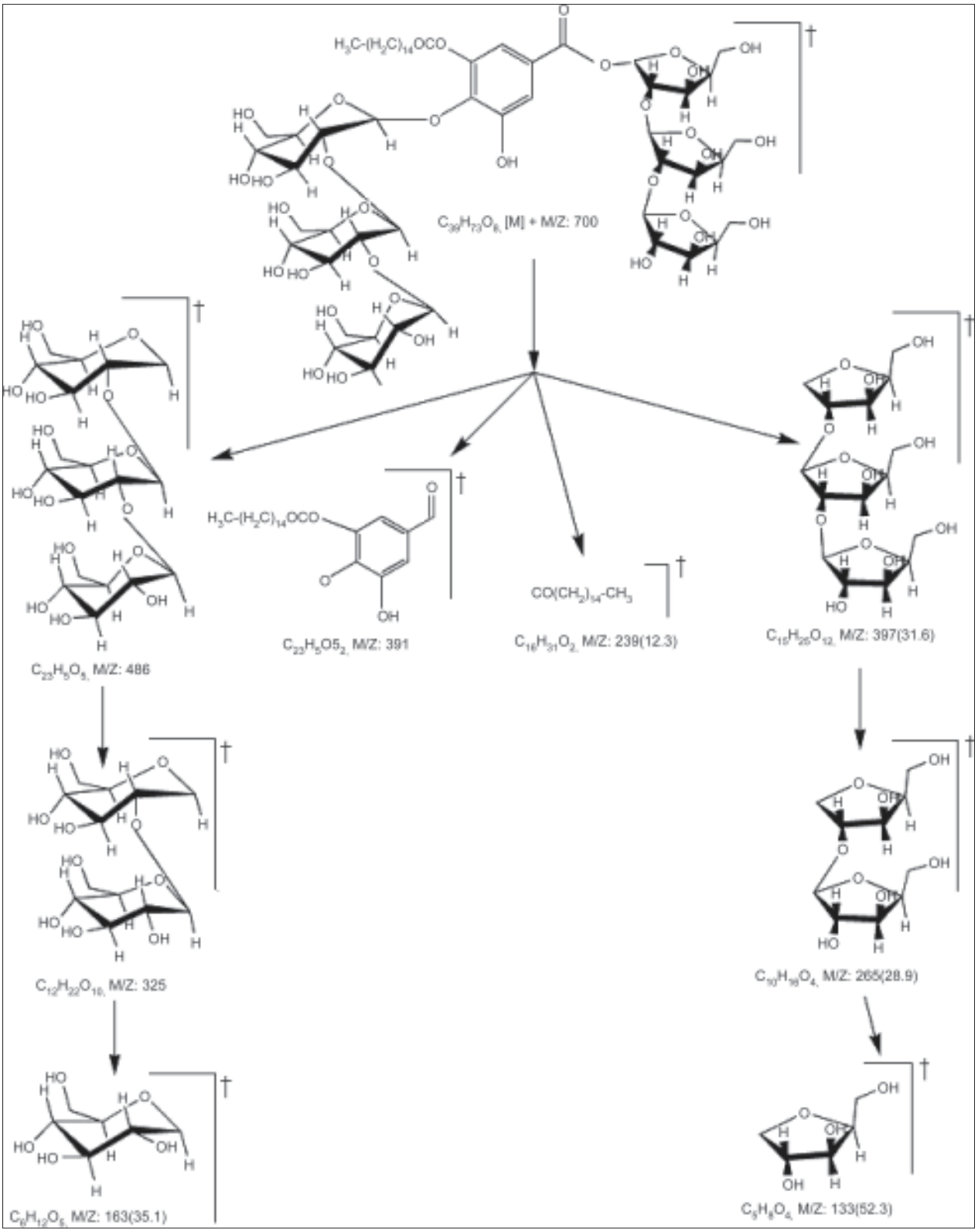

Figure 2: Mass fragmentation pattern of galangogalloside (Compound AG13)

The ^{13}C NMR spectrum of AG13 exhibited signals for ester carbons at δ 173.7 (C-1′) and δ 169.67 (C-7) methyl carbon at δ 17.83 (C-16′), and anomeric carbons at δ 102.06 (G-1), δ 92.31 (G -1′), δ 88.35 (G-1′′), δ 104.24 (A-1), δ 98.12 (A-1′) and δ 96.97 (A-1′′). The remaining sugar carbons appeared in the range δ 4.53-3.17. ^{13}C NMR signals for G-2 and G-2′ in the downfield region at δ 72.56 and δ 71.57 indicated the location of the glucose moieties at G-2 and G-2′.The ^{1}H NMR

signal for A-2 and A-2′ at δ 4.25 and δ 4.16 and ^{13}C NMR signals δ 77.64 and δ 75.83 suggested the existence of the arabinose units at A-2 and A-2′.

The ^{1}H-^{1}H COSY spectrum of AG 13 exhibited correlations of H-2 with H-6, H-G-1 with H-G-2, H-G-2 with H-G-1′/ H-G-1′′, H-A-1 with H-A-2, H-A-2 with H-A-1′, H-A-2′ with H-A-1′′, and H_3-16 with H_2-15. The HMBC spectra of AG 13 showed that C-3 interacted with H-2, C-4 interacted with H-G-1, C-G-2 interacted with H-G-1′/H-G-1′′, C-5 interacted with H-6, C-7 interacted with H-A-1, C-A-2 interacted with H-A-2′, C-A-2′ interacted with H-A-2′′ and C-16 interacted with H_2-15/H_2-14. The Appearance of the H-6 in the up field region, correlation of C-4 with H-G and C-5 with H-6 suggested the existence of glucosydic unit at C-4 position.

Acid hydrolysis of AG13 yielded palmitic acid, gallic acid, D-glucose and L-arabinose. On the basis of spectral data analysis and chemical reactions the structure of AG 13 has been established as 3,4,5-trihydroxybenzoic acid- 3-palmitoyloxy-4β-D-glucopyranosyl(G-2→G-1′)- β-D-glucopyranosyl (G-2′→G-1′′)-β-D-glucopyranosyl-7-β-L-arabinofuranosyl (A-2→A-1′)- β-L-arabinofuranosyl (A-2′ → A-1′′)- β-L-arabinofuranoside. This is new gallic acid glycoside isolated from medicinal plant or synthetic source for the first time.

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