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Isolation and Characterization of Water-soluble New Gingerlanosterol from *Zingiber officinale* Rhizomes

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The rhizomes of *Zingiber officinale* after hydro-distillation of the essential oil have yielded one new compound Gingerlanosterol (Lanostan-3β, 25-diol-3β-D-glucopyranosyl 1,4-β-D-glucopyranoside) from waste water of ginger along with known hexanoic phosphate. The structure of the new compound was elucidated by 500 MHz NMR aided by EI-MS, IR spectra and chemical methods.

Key Words: Gingerlanosterol, *Zingiber officinale* rhizomes.

INTRODUCTION

Ginger *(Zingiber officinale* Rosc.) is a perennial herb belonging to Zingiberaceae. It is commercially cultivated in India, Nepal, China, Taiwan, Philippines, Fiji, Jamaica, Nigeria, Japan and Australia. India is the major producer and exporter of ginger producing about 50% of the total world production. In India, ginger is cultivated on an area of about 65,000 ha with an annual production of 1.89 lakh tonnes of rhizomes. The rhizomes (ginger: commonly known as *adrak* in India) are frequently prescribed in Indian system of medicine for the treatment of cough, stomach, asthma, worms, leprosy, skin, gastrointestinal and respiratory diseases. Ginger is a well-known spice, widely used for flavoring a variety of foods and for the preparation of oleoresin and volatile oils. Gingerols are the constituents responsible for the taste of ginger, including the pungency of the oleoresin[1], antiulcer[2], antifungal[3], prostaglandin biosynthesis inhibiting[4], antirhinoviral[5], insecticidal[6] compounds have been reported from the rhizomes. A number of other constituents have been reported from ginger as shogaols and gingerdiols[7, 8], diarylheptanoids[9, 10], phenylbutenoids[11], sesquiterpenes[12] and some more reports from ginger constituents[13–17]. Firstly, the volatile oil of *Z. officinale* was examined and identified as major and minor constituents and reported[18]. The major constituents of volatile oil mainly

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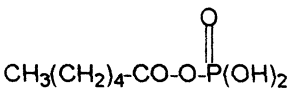
consist of borneol (5.2%), neral (9.9%), geraniol (3.6%), geranial (14.3%), zingiberene (17.4%), nerolidol (2.5%) and cadinol (3.0%). After hydrodistillation of volatile oil from rhizomes and from waste water of ginger was isolated and identified one new compound gingerlanosterol **(2)** along with known hexanoic phosphate **(1)** and other compounds mostly sugar molecules on the basis of NMR spectra. This paper deals with the isolation and structural elucidation of gingerlanolsterol.



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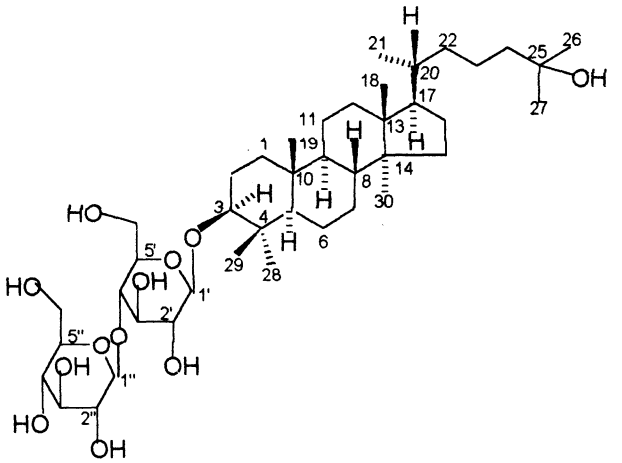

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Fig. 1. Structures of compounds **1** and **2**

EXPERIMENTAL

Melting points were determined on Electrochemical Eng. melting point apparatus and optical rotation was measured on an AA-10 model polarimeter. IR spectra were recorded on a Thermo Mattson 60-AR spectrophotometer. UV spectra were recorded using a UV-Vis spectrometer TU-180PC. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained on a Brucker Avance (DRX-500) using $CDCl_3$, CD_3COCD_3, C_5D_5N as solvents. EI-Mass spectra were recorded on a Jeol JMS-SX 102A spectrometer. Column chromatography was performed over silica gel 70-230 (Merck). TLC analyses were performed on precoated silica gel glass plates 60 F_{254} (Merck) and visualized under UV light and by spraying with (vanillin 1 g/sulfuric acid 5 mL/ethanol 94 mL) solution followed by heating (100-110°C).

Plant material: The rhizomes were purchased from the local market, Lucknow, India in August 2002 and were identified by the taxonomist of the institute.

Extraction and isolation: 10 kg fresh rhizomes after crushing, hydrodistillation in a Clavengers type apparatus yielded an essential oil with an yield of 0.30%. After hydrodistillation of essential oil, the water in the crude material was filtered through a filter paper and concentrated *in vacuo*. After concentration and drying, 20 g dry extract was obtained . The details of extract preparation are shown in Fig. 2. The concentrated water extract on being subjected to normal phase

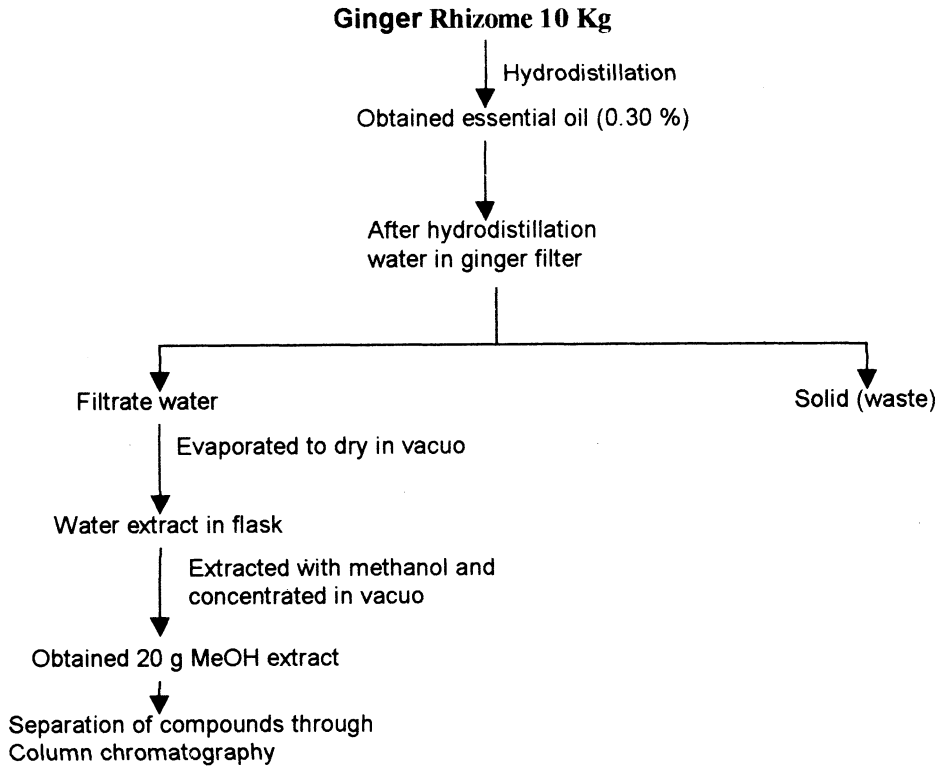

Fig. 2. Flow chart for separation of volatile oil and preparation of water extract

column chromatography over silica gel yielded 10 fractions (500 mL): Fraction Nos. 1 and 2 in EtOAc, 3 in EtOAc : MeOH (9 : 1), 4 in EtOAc : MeOH (8 : 2), 5 in EtOAc : MeOH (7 : 3), 6 in EtOAc : MeOH (6 : 4), 7 in EtOAc : MeOH (1 : 1), 8 in EtOAc : MeOH (3 : 7), 9 in EtOAc : MeOH (2 : 8), and 10 in MeOH. Fractions 1 and 2 were crystallized in a tube and the crystals were separated; the crystalline compound was further purified by column chromatography (CC) over silica gel with chloroform and methanol yielding a pure known compound which was identified as hexanoic phosphate (**1**, 5 mg), m.p. 157–159°C, R_f 0.34 on silica gel TLC plates developed by a solvent system of EtOAc/MeOH (9.5 : 0.5, v/v). Fraction 5 was crystallized in a tube and a solid compound insoluble in ethyl acetate, after filtration through a sintered funnel, was obtained as white powder (50 mg). Due to insolubility in $CHCl_3$, MeOH, pyridine and DMSO, acetylated product of this compound was synthesizes for NMR analysis. After acetylation, it was

further purified by CC over silica gel column with methylene dichloride/MeOH and a new compound gingerlansterol was obtained (**2**, 20 mg), R_f 0.46 on silica gel TLC plates developed by a solvent system of $CHCl_3$/MeOH (9.5 : 0.5, v/v). The other polar fraction contains only sugar molecules on the basis of NMR. The ^1H and ^{13}C-NMR, IR and mass spectral data of the new compound (**2**) are reported.

Lanostan-3β,25-diol-3β-D-glucopyranosyl-1,4-β-D-glucopyranoside (2): White powder; m.p. 240-243°C; $[\alpha]_D^{23}$ + 32.8 (C 1.0, $CHCl_3$); UV λ_{max} ($CHCl_3$): 236 nm; IR (KBr, cm^{-1}) ν_{max}: 3565, 3368, 3250, 2989, 1607, 1477, 1427, 1375, 1310, 1123, 1075, 810, 776; EI-MS m/z (rel. int.) 446 $[M\text{-}C_{12}H_{21}O_{10}]^+$ (3.6), 428 (3.1), 410 (8.9), 408 (7.1), 337 (44.8), 319 (13.1), 284 (21.6), 257 (42.3), 242 (7.8), 212 (16.7), 185 (15.6), 163 (14.8), 141 (13.5), 129 (28.3), 127 (8.1), 123 (12.2), 113 (12.8), 109 (6.7), 97 (18.5), 95 (9.3), 83 (30.7), 72 (49.1), 69 (50.3), 55 (100); ^1H and ^{13}C NMR (Table-1).

TABLE-1
^1H AND ^{13}C-NMR SPECTRAL DATA OF 2 (ACETYLATED)

Position	^1H NMR		^{13}C NMR	Position	^1H NMR		^{13}C NMR
	α	β			α	β	
1	1.28 m	2.26 m	33.19	23	1.97 m	2.02 m	30.03
2	2.69 m	2.80 m	27.13	24	1.80 m	2.35	30.69
3	3.38 dd (4.5, 8.5)	–	80.02	25	–	–	64.08
4	–	–	39.91	26	1.26 br s	–	22.89
5	1.97 m	–	54.21	27	1.26 br s	–	21.59
6	1.76 dd (1.1,1.5)	1.65 m	18.45	28	1.34 br s	–	24.85
7	1.80 m	2.13 m	26.64	29	1.25 br s	–	29.56
8	–	1.37 br s	42.33	30	1.28 br s	–	14.30
9	1.43 br s	–	47.19	1′	5.34 d (5.5)	–	104.23
10	–	–	38.38	2′	4.30 m	–	75.24
11	1.31 m	1.49 m	21.12	3′	4.26 m	–	69.89
12	1.50 m	1.98 m	26.40	4′	4.20 m	–	68.47
13	–	–	49.29	5′	4.88 m	–	79.34
14	–	–	59.99	6′	3.25 d (5.5)	3.24 d(5.5)	62.15
15	2.13 m	1.98 m	31.85	1″	5.06 d (9.5)	–	90.18
16	2.17 m	1.83 m	32.13	2″	4.29 m	–	70.52
17	1.45 m	–	49.20	3″	4.22 m	–	70.54
18	0.88 br s	–	17.32	4″	4.13 m	–	68.74
19	1.11	–	19.21	5″	4.34 m	–	75.95
20	–	1.42 br s	31.93	6″	3.62 d (3.5)	3.16 d (3.5)	63.13
21	0.92 d (6.2)	–	19.30	OAc	2.18 to 2.02	–	20.75 to 21.59
22	2.27 m	2.04 m	19.96				

Coupling constants in Hertz are provided in parentheses.

Acetylation of 2: Compound **2** (25 mg) was refluxed with acetic anhydride (2.5 mL) and pyridine (1.0 mL) over a water bath for 4 h. After the usual work-up, it yielded the acetylated product.

RESULTS AND DISCUSSION

Compound **2** named gingerlanosterol was obtained as a colourless amorphous solid from ethyl acetate and methanol (70 : 30) eluants. It responded positively to Liebermann-Burchard test[19] for triterpenes and tests for glycosides. Its IR spectrum showed absorption bands for hydroxyl groups at 3565, 3368, and 3250 cm^{-1}. It had an ion peak at m/z 446 generated due to expulsion of a $C_{12}H_{21}O_{10}$ moiety from the molecular ion peak. The prominent ion peaks are at m/z 55 $[C_{3,4}–C_{5,6}$ fission$]^+$, 69 $[C_{3,4}–C_{6,7}$ fission$]^+$, 83 $[C_{3,4}–C_{7,8}$ fission$]^+$, 113 $[C_{1,10}–C_{5,6}$ fission$]^+$, 319, 127 $[C_{1,10}–C_{6,7}$ fission$]^+$, 141 $[C_{1,10}–C_{7,8}$ fission$]^+$, suggesting the saturated nature of the rings A and B and the location of the hydroxyl group in ring A which was placed at C-3 on the basis of biogenetic consideration. The ion peaks appearing at m/z 446 $[M\text{-}C_{12}H_{21}O_{10}$, sucrose$]^+$, 410 $[428\text{-}H_2O]$, 284 $[428\text{-}C_8H_{17}O$ (side chain)-Me$]^+$, 257 $[428\text{-}C_8H_{17}O$-ring D-fission$]^+$, 242 $[257\text{-Me}]^+$, 227 $[242\text{-Me}]^+$, 212 $[227\text{-Me}]^+$, 320 $[C_{13,17}\text{-}C_{16,17}$ fission$]^+$, 129 $[C_8H_{17}O$, side chain$]^+$, 123 $[C_8H_{14}\text{-}C_9H_{11}$ fission-side chain$]^+$, 109 $[C_8H_{14}\text{-}C_{11,12}$ fission-side chain$]^+$ and 95 $[C_8H_{14}\text{-}C_{12,13}$ fission-side chain$]^+$ indicate the presence of $C_8H_{17}O$ side chain of a lanostane-type triterpene and saturated nature rings C and D.

The ^{1}H-NMR spectrum of **2** displayed a one-proton double doublet at δ 3.38 with coupling interactions of 4.5 and 8.5 Hz assigned to carbonol 3α-H. A three-proton doublet at δ 0.92 (J = 6.2 Hz) was accounted to C-21 secondary methyl protons. A six-proton broad signal at δ 1.26 was ascribed to C-26 and C-27 tertiary methyl functionalities attached to a oxygenated carbon. Five broad signals at δ 0.88, 1.11, 1.34, 1.25 and 1.28, all integrated for three protons each, were associated with C-18, C-19, C-28, C-29 and C-30 tertiary methyl protons. The presence of methyl proton signals in the range δ 1.34 0.88 supported the location of these groups on the saturated carbons. The C-1′ and C-1″ anomeric protons resonated as one-proton doublets at δ 5.34 (J = 5.5 Hz) and 5.06 (J = 9.5 Hz), respectively. Four one-proton doublets at δ 3.25 (J = 5.5 Hz), 3.24 (J = 5.5 Hz) and at δ 3.62 (J = 3.5 Hz) and 3.61 (J = 3.5 Hz) were attributed to oxygenated methylene protons of the sugar moieties at H_2-1′ and H_2-1″. The remaining carbinol protons of the glycone units appeared as multiplets between δ 4.88 and 4.13. The methine and methylene proton signals for triterpene moiety resonated in the range δ 2.80–1.28.

The ^{13}C-NMR spectrum of **2** showed the presence of 42 carbon signals in the molecule. The signals at δ 80.02 and 64.08 were assigned to carbional C-3 and C-25 carbons, respectively. The methyl carbon signals appeared at δ 17.32 (Me-18), 19.21 (Me-19), 19.30 (Me-21), 22.89 (Me-26), 21.59 (Me-27), 24.85 (Me-28), 29.56 (Me-29) and 14.30 (Me-30). Two deshielded signals at δ 104.23 and 90.18 were ascribed to anomeric C-1′ and C-1″ carbons. The remaining hydroxy methine carbons of the glycone moieties resonated in the range δ

62.15–79.34. The existence of all the carbon signals up to δ 104.23 indicated saturated nature of the molecule. The ^{1}H and ^{13}C-NMR values were compared with related triterpenes[20]. The ^{1}H-NMR and ^{13}C-NMR of acetylated compound show that the chemical shift and coupling pattern are consistent with the NMR data for gingerlanosterol. Treatment of **2** with acetic anhydride and pyridine yielded a hepta-acetate. On the basis of these spectral data analysis and chemical reactions, the structure of **2** has been established as lanostan-3β,25-diol-3β-D-glucopyranosyl-1,4-β-D-glucopyranoside.

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