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A new oxo-sterol derivative from the rhizomes of *Costus speciosus*

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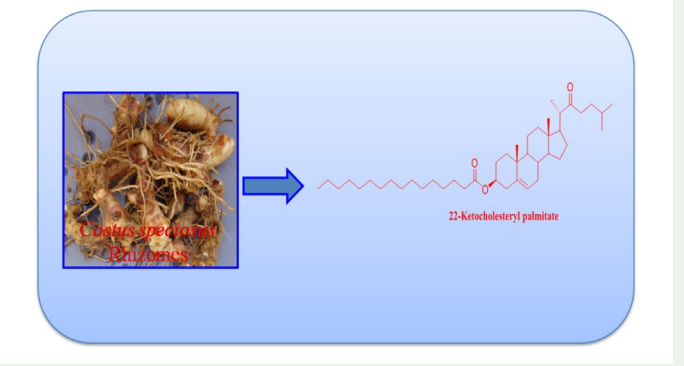
ABSTRACT

Chemical investigation of the rhizomes of *Costus speciosus* led to the isolation of a new compound, 22-ketocholesteryl palmitate (**1**) along with four known compounds, 24-methylenecycloartanol (**2**), cycloartanol (**3**), stigmasterol (**4**) and linoleic acid (**5**). The structure of new compound was characterised by extensive 1D-, 2D-NMR and mass spectrometry (GC-MS and HR-ESI-MS) techniques.



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1. Introduction

Costus speciosus Koen. (Family: Zingiberaceae), an esteemed Indian ornamental plant, has been extensively used in oriental systems of medicine. The rhizomes of this plant are attributed to be bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant, tonic, improves digestion and as stimulant herb that clears toxins (Deni 2008; Gupta 2010). *C. speciosus* is well known to exhibit diverse

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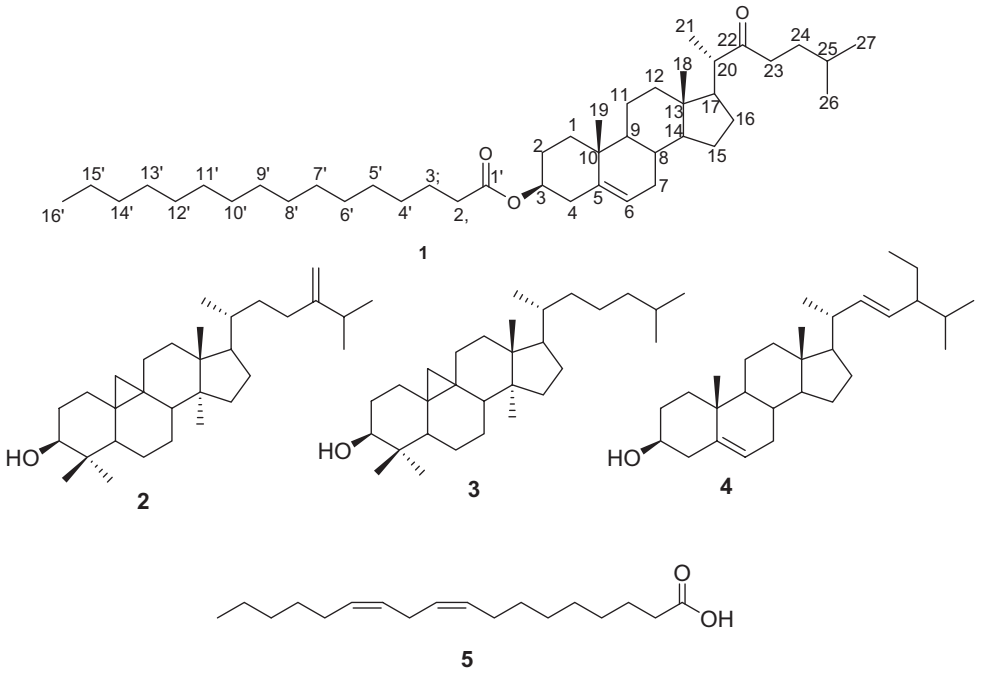

Figure 1. Structure of isolated compounds from *C. speciosus* rhizomes.

biological activities, including antidiabetic, hypolipidemic, anticholinesterase, hepatoprotective, antibacterial and antifungal (Srivastava et al. 2011). Isolation of oxo acids, branched fatty acid esters, sesquiterpene lactones, steroids, steroid glycosides, saponins such as diosgenin, dioscin and gracillin as major constituents was reported from the rhizomes of *C. speciosus* (Srivastava et al. 2011; Al-Attas et al. 2015). Diosgenin isolated from rhizomes of *C. speciosus* also displayed excellent anti-inflammatory, antioxidant and anti-angiogenic activities (Selim & Al Jaouni 2016). In continuation to our previous studies on isolation of compounds from western Himalayan regions (Agnihotri et al. 2014; Thakur & Agnihotri 2016; Walia et al. 2016), in present work, we had isolated one new compound, 22-keto-cholesteryl palmitate (**1**) along with four known compounds, 24-methylenecycloartanol (**2**), cycloartanol (**3**), stigmasterol (**4**) and linoleic acid (**5**) from rhizomes of *Costus speciosus* (Figure 1).

2. Results and discussion

Compound **1** was obtained as white solid. Its molecular formula was determined as $C_{43}H_{74}O_3$ on the basis of HR-ESI-MS which had also suggested seven degrees of unsaturation. The ^{13}C spectrum of this compound showed the presence of signals for one double bond, one carbonyl and one carboxyl group. Presence of peaks at 14.0, 22.6, 25.0, 31.9, 34.6, 173.2 along with group of peaks in between 29.0 and 29.6 are characteristic for the presence of a long fatty acid chain. Hence this molecule should be an ester of long fatty acid chain. Long fatty acid chain was characterised as palmitic acid by preparation of its methyl ester (Agnihotri et al. 2009) followed by comparison of fragmentation pattern in GC-MS (Adams 2007). After deletion of fatty acid

characteristics from ^{13}C spectra, the remaining peaks suggested the molecule should have another tetracyclic structure with five methyls, ten methylenes, eight methines and four quaternary carbons. The comparison of NMR data of compound (**1**) with previous literature strongly indicated another moiety of this molecule as cholesterol (Table S1) (Plouguerné et al. 2006; Augustin et al. 2015). The methyl signal at δ_H 0.70 (3H, s, Me-18) showed its HMBC correlations with C-12 (δ_C 39.5), C-13 (δ_C 42.4), C-14 (δ_C 55.9) and C-17 (δ_C 52.0), whereas, methyl at δ_H1.02 (3H, s, Me-19) confirmed by its HMBC correlation with C-5 (δ_C 139.6), C-9 (δ_C 49.9) and C-10 (δ_C 36.5) (Figure S7, S8). The presence of carbonyl moiety at C-22 (at δ_C 214.9), was confirmed by its HMBC correlations with methyl at δ_H 1.10 (3H, s, Me-21) and methylene protons at δ_H 2.36, 2.43 (m, H-23α, β). The esterification at C-3 has been confirmed from the correlations in HMBC between the C=O (at δ_C 173.2 ppm) and H-4 protons at δ_H 2.31(m, H-4α, β) (Figure S7, S8). The H-4 protons at δ_H 2.31 also showed HMBC correlations with C-3 (at δ_C 73.5) and C-5 (at δ_C 139.6). The linkage of fatty acid chain was fixed at C-3 position further supported by downfield shift of its proton at δ_H 4.60 (m) (Lee et al. 2014). From all the above evidences mentioned, the structure of compound (**1**) was established as a new sterol 22-ketocholesteryl palmitate.

The other known compounds were identified as, 24-methylenecycloartanol (**2**) (Barla et al. 2006), cycloartanol (**3**) (Khuong-Huu et al. 1975; Barla et al. 2006), stigmasterol (**4**) (Wu et al. 1998) and linoleic acid (**5**) (Kamurthy et al. 2015) using comparison of NMR data with previously published reports.

3. Experimental

3.1. General

The ^1H, ^{13}C-NMR, DEPT, HSQC, HMBC and COSY spectra were recorded on a Bruker Avance 600 NMR. The gas chromatography/mass spectrometry (GC/MS) analyses were conducted using a Shimadzu QP 2010 using a DB-5 (J&W Scientific, Folsom, CA, USA) capillary column (30 m × 0.25 mm i.d., 0.25 µm thickness).The mass spectrometry was performed on a Q-TOF triple-quadrupole mass spectrometer equipped with ESI source (Micromass, Manchester, UK) software Masslynx v4.1.The data acquisition was performed using negative ion mode over a mass range of *m/z* 100–1000.

3.2. Plant material

The rhizomes of *C. speciosus* were collected from CSIR-IHBT farm. The plant material was authenticated in-house by the taxonomist. A voucher specimen was deposited at CSIR-IHBT Herbarium, Palampur, India (Voucher # PLP-17601).

3.3. Extraction and isolation

Air-dried rhizomes of *C. speciosus* (4 kg) were crushed and extracted by using a percolator with 95% EtOH (7 times, 3 L × 24 h × 1, followed by 2.6 L × 24 h × 6) at room temperature. The ethanolic extracts were combined and dried in a rotavapor at 40 ± 5 °C to yield a semi-solid residue (179.2 g). Part of this extract (170.0 g) was suspended in H_2O (500 mL) and successively extracted with $CHCl_3$ (500 mL × 3, 50.2 g). Part of $CHCl_3$ fraction (36.0 g) was chromatographed over a dry silica gel (500 g, 230–400 mesh) column (4.5 × 48.2 cm) using

a step gradient consisting of hexane: EtOAc (9: 1 to 10: 0, each 1 L) to afford five subfractions, A (0.8 g), B (2.4 g), C (0.5 g), D (0.2 g), E (0.32 g), respectively. Sub fraction B (2.4 g) was chromatographed over dry silica gel (100.2 g, 230–400 mesh) column (3 × 32.6 cm) using an isocratic solvent system consisting of EtOAc: hexane (5: 95, 3L, each fraction 50 mL) to afford five sub fractions (B_1-B_5). Sub fraction B_1 (Fr No. 24–31, 40.2 mg) was chromatographed over dry silica gel (20.2 g, 230–400 mesh) column (1.8 × 18 cm) using isocratic solvent system consisting of EtOAc: hexane (2: 98, 500 mL, each fraction 25 mL) to afford a total 30 fractions. Fr·No.16–24, yielded compound **1** (4.2 mg). Sub fraction B_3 (Fr No. 32–38, 98.2 mg) was chromatographed over RP-18 column (1.8 × 18 cm) using isocratic solvent system consisting H_2O: methanol (20: 80 each fraction 25 mL) and total 20 fractions were collected. Fraction No. 6–8 after washing with acetone afforded compound **2** (12.2 mg). Fr. No. 15–20 on crystallisation yielded compound **3** (5.0 mg). The subfraction B_5 (510.0 mg) was chromatographed over RP-18 column (2.3 × 25 cm) using isocratic solvent system consisting H_2O: methanol (30: 70 each fraction 50 mL), total 45 fractions were collected. Fraction No.1–12 dried in a rotavapor and after washing with acetone afforded compound **4** (18.6 mg). Fraction No. 35–41 on crystallisation gave compound **5** (15.3 mg) Figure 1.

3.4. Hydrolysis of Compound 1 and GC-MS analysis

Compound **1** (2 mg) was heated under reflux with 5% methanolic KOH (1 mL) for 3 h. The reaction product was diluted with water (10 mL) and extracted with chloroform (10 mL × 2). The chloroform layer was dried over anhydrous Na_2SO_4, and the residue after evaporation was subjected to GC-MS analysis and was identified as methyl palmitate by comparison of RI and mass fragmentation with literature (Adams 2007; Agnihotri et al. 2009).

22-Ketocholesteryl palmitate (**1**): white solid.^{1}H NMR (600 MHz, $CDCl_3$) δ_H:1.13 (m, H-1α), 1.84^c (H-1β), 1.85^c (H-2α), 1.56^c (H-2β), 4.60 (m, H-3), 2.31 (m, H-4α, β), 5.36 (m,H-6), 1.52^c (H-7α), 1.95^c (H-7β), 1.43^c (H-8β), 0.97 (m, H-9α), 1.54^c (H-11α), 1.45^c (H-11β), 1.27^c (H-12α), 1.94^c (H-12β), 1.04^c (H-14α), 1.56^c (H-15α), 1.07^c (H-15β), 1.67 (m, H-16α), 1.18^c (H-16β), 1.60^c (H-17α), 0.70 (s, Me-18), 1.02 (s, Me-19), 2.51 (m,H-20), 1.10 (d, J = 6.8, Me-21), 2.36, 2.43 (m, H-23α, β), 1.43^c (H-24 α, β),1.57^c (H-25), 0.88^c (m, Me-26), 0.89^c (m, Me-27). Fatty acid chain: 2.26 (*t*, J = 3.0, H-2′α, β), 1.60^c (H-3′α, β), 1.22–1.32^c (H-4′-13′,CH_2 chain),1.25^c (H-14′α, β), 1.23^c (H-15′α, β), 0.87^c (m, Me-16′).^{13}C NMR (150 MHz, $CDCl_3$) δ_C: 36.9 (C-1), 27.7 (C-2), 73.5 (C-3), 38.0 (C-4), 139.6 (C-5), 122.3 (C-6), 31.7 (C-7), 31.8 (C-8), 49.9 (C-9), 36.5 (C-10), 20.9 (C-11), 39.5 (C-12), 42.4 (C-13), 55.9 (C-14), 24.4 (C-15), 27.4 (C-16), 52.0 (C-17), 12.0 (C-18), 19.2 (C-19), 49.4 (C-20) 16.5 (C-21), 214.9 (C-22), 39.5 (C-23), 32.3 (C-24), 27.6 (C-25), 22.3 (C-26), 22.3 (C-27). Fatty acid chain: 173.2 (C-1′), 34.6 (C-2′), 25.0 (C-3′), 29.0–29.6 (C-4′-13′), 31.9 (C-14′), 22.6 (C-15′), 14.0 (C-16′). HRESIMS: 637.5552 ([M − H]$^-$(Calcd for $C_{43}H_{73}O_3$,637.5560). (Note: ^{c} represents overlapped signal, assignments were done by 2D experiments.)

4. Conclusion

Western Himalayan flora is full with novel skeletons. Recently our group had introduced two new skeletons termioic acid A (Agnihotri et al. 2014), 14*R*, 17*S*, 20*R*-lupan-3-one (Thakur & Agnihotri 2016) and new rare molecule (Walia et al. 2016) from this region. In this study, the isolation of 22-ketocholesteryl palmitate (**1**) from *C. speciosus* is the first report of a 22-oxosterol derivative from plant source.

Supporting material

All spectral data associated with this article are provided as supplementary file.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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