Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 3 (1): 149-154
Received: 04 -04-2014
Accepted: 17-04-2014

Nisha Chaudhary
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110 062, India

Shahnaz S. Husain
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110 062, India

Mohammed Ali
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110 062, India

Correspondence:
Mohammed Ali
Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi – 110 062, India
Email: maliphyto@gmail.com
Tel: +91-11-22191082

New Phenolic, triterpenic and steroidal constituents from the fruits of *Cuminum cyminum* L.

Nisha Chaudhary, Shahnaz S. Husain, Mohammed Ali

ABSTRACT
Cuminum cyminum L. (Family: Apiaceae) is a small annual herb with slender, angular branched stem cultivated as a spice throughout the world. Its fruits are used to treat dyspepsia, insomnia, cold, fever, gastrointestinal, gynecological and respiratory disorders, toothache, diabetes, hypertension and epilepsy. Phytochemical analysis of the methanolic extract of the fruits of *C. cyminum* led to the isolation of a fatty acid ester identified as *n*- tricosanyl *n*- octadec-9- enoate (**1**) and five terpenic and steroidal constituents characterized as 1,4,5,8-tetrahydroxynaphthyl geranilan-10′-al 1′-oate (**2**), lanost-5,20 (22)-dien-3α-olyl *n*-docosanoate (**3**), labdan-6α,16,20-triol-16-(10′,11′- dihydroxy anthraquinone-2′-oate) (**4**), stigmast-5-en-3β-O-D-arabinopyranosyl-2′-benzoate (**5**) and lanost-5,24-dien-3β-ol 3β-O-D- arabinopyronosyl-2′- *n*-octadec-9″, 12″-dienoate (**6**). The presence of these phytoconstituents are reported for the first time in *C. cyminum* fruits and their structures have been established on the basis of spectral data analysis.

Keywords: *Cuminum cyminum*, fruits, methanolic extract, terpenic constituents, steroidal glycoside, aliphatic ester.

1. Introduction
Cuminum cyminum L. (Family Apiaceae), known as cumin, is a small annual herb with slender, angular branched stem. The plant is indigenous to Egypt and Syria and one of the most cultivated popular spices throughout the world. It thrives well from 9 to 26 ˚C and is intolerant to long periods of dry heat and frost. Cumin seeds resemble caraway seeds, but are slightly smaller having oblong shape, thicker in the middle, compressed laterally with nine ridges and yellow-brown in colour. Cumin is the second most popular spice in the world after black pepper. It is added in some Dutch cheeses, traditional bread in France, Brazilian cuisine, chili powder, sofrito, garam masala, curry powder, cuisines, baharat and other preparations of food of the Middle East, India, Cuba and Mexico. Cumin is spread on the meat along with other common seasonings. The cumin seeds are considered as carminative, analgesic, eupeptic, antispasmodic, astringent, used to treat digestive disorders, cough, diarrhea, dyspepsia, flatulence, morning sickness, colic, dyspeptic headache and bloating and to improve liver function [1-3]. A mixture of powdered cumin seeds, honey, salt and butter is applied to alleviate pain from scorpion bites [2, 4]. The seeds contained a volatile oil mainly composed of monoterpene hydrocarbons, oxygenated mono- and sesqui- terpenes, fatty acids, aldehydes, ketones and esters. The major compounds occurring in cumin are cuminaldehyde, limonene, α- and β-pinenes, 1,8-cineole, *o*- and *p-cymenes*, α- and γ-terpinenes, safranal and linalool [5-9]. The other components present in the cumin are monoterpenic, flavone, 2-C-methyl-D-erythritol, sesquiterpene lactone and alkyl glycosides, amino acids, fatty acids and aromatic compounds [10-17]. Several nutrients, e.g., vitamins, amino acids, protein, minerals, starch, sugars, tannins, phytic acid and dietary fiber components have also been reported in cumin seeds [18, 19]. The present paper describes the isolation and characterization of the chemical constituents from the seeds of *C. cyminum* of Delhi region.

2. Materials and Methods
2.1 General
Melting points were determined on a Perfit apparatus without correction. The IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on Bruker spectrospin spectrometer using TMS as an

internal standard. ESI MS analyses were performed on a JEOL SX 102/Da-600 instrument equipped with direct inlet probe system. Column chromatography separations were carried out on silica gel (Merck, 60–120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60 F254) were used for analytical thin layer Chromatography and visualized by exposure to iodine and UV radiations.

2.2. Plant materials

The dried seeds of *C. cyminum* were obtained from a local market of Khari Baoli, Delhi and authenticated by Dr. H.B. Singh, Scientist, Herbarium Section, National Institute of Science and Information Resources (CSIR), New Delhi. A voucher specimen (No. NISCAIR/ RHMD/2010-11/1465/63) is deposited in the herbarium.

2.3. Preparation of extract

The fruit powder of *C. cyminum* (1 kg) was extracted exhaustively in a Soxhlet apparatus with methanol for 72 hr. The methanolic extract was concentrated under reduced pressure to obtain dark brown viscous mass (11.3%). A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents.

2.4. Isolation of phytoconstituents

The viscous dark brown mass was adsorbed on silica gel (60-120 mesh) for column chromatography, after being dissolved in a little quality of methanol for preparation of a slurry. The slurry (200 g) was air dried and subjected to chromatography over a silica gel column packed in petroleum ether. The column was eluted successively with petroleum ether, mixture of petroleum ether and chloroform (9:1, 3:1, 1:1, and 1:3), chloroform and the mixture of chloroform and methanol (99:1, 97:3, 95:5, 92:8, 9:1, 3:1, 1:1, 1:3). Various fractions were collected separately and matched by TLC to check the homogeneity. Similar fractions having the same Rf values were combined and crystallized. The isolated compounds were recrystallized to get the following compounds:

2.5. *n*-Tricosanyl oleate (1)

Elution of column with petroleum ether furnished colorless powder of **1**, recrystallized from acetone–methanol (1:1), 133.5 mg (0.154% yield), R_f 0.57 (petroleum ether); m.p. 151-155° C; UV λmax: 251 nm; IR ν_{max} (KBr): 2923, 2823, 2852, 1736, 1608, 1463, 1677, 1240, 1070, 840, 719 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.37 (1H, m, H-9), 5.30 (1H, m, H-10), 3.77 (2H, t, J=6.0 Hz, H2-1′), 2.31 (2H, t, J=7,2 Hz, H_2-2), 2.23 (2H, m, H2-8), 2.05 (2H, m, H_2-11), 1.60 (2H, m, CH_2), 1.37 (2H, m, CH_2), 1.35 (2H, m, CH_2), 1.32 (2H, m, CH_2), 1.30 (20 H, brs, 10 x CH_2), 1.28 (36H, brs, 18 x CH_2), 0.89 (3H, t, J=6.3 Hz, CH_3-18), 0.85 (3H, t, J= 6.1 Hz, CH_3-23′); ^{13}C NMR ($CDCl_3$): δ 173.45 (C-1), 129.83 (C-9), 121.05 (C-10), 63.04 (C-1′), 31.65 (CH_2), 29.34 (25 x CH_2), 29.27 (3 x CH_2), 29.03 (3 x CH_2), 22.78 (CH_2), 15.26 (CH_3-18), 13.01 (CH_3-23′); +ve TOF MS m/z (rel.int.): 604 $[M]^+$ ($C_{41}H_{80}O_2$) (1.3), 281 (43.8), 265 (11.6), 239 (8.7).

2.6. Tetrahydroxynaphthyl geranilan-10′-al 1′-oate (2)

Elution of the column with petroleum ether-chloroform (3:1) yielded pale yellow crystals of **2**, recrystallized from acetone-methanol (1:1), 6.8 mg (0.06 % yield); R_f 0.72 (petroleum ether-chloroform, 3:1); m.p. 89-92°C; UV- λmax (MeOH): 205, 249, 292 nm (log ε 1.3, 1.6, 5.7); IR ν_{max} (KBr): 3386, 3231, 2928, 2871, 1721, 1680, 1637, 1575, 1461, 1306, 1213, 1169, 1054, 839 cm^{-1}; ^1H NMR ($CDCl_3$): δ 9.92 (1H, d, J=6.8 Hz, H-10′), 7.83 (1H, d, J=8.0 Hz, H-2), 7.42 (1H, d, J=8.5 Hz, H-7), 7.37 (1H, d, J=8.0 Hz, H-3), 7.21 (1H, d, J=8.5 Hz, H-6), 2.87 (1H, m, H-3′), 2.30 (2H, d, J= 7.2 Hz, H_2-2′), 1.83 (1H, m, H-7′), 1.67 (2H, m, H_2-4′), 1.51 (2H, m, H_2-5′), 1.28 (2H, m, H_2-6), 1.27 (3H, d, J=7.0 Hz, Me-8′), 1.23 (3H, d, J=7.0 Hz, Me-9′); ^{13}C NMR ($CDCl_3$): δ 156.45 (C-1), 116.18 (C-2), 125.85 (C-3), 151.74 (C-4), 149.13 (C-5), 125.76 (C-6), 128.08 (C-7), 145.09 (C-8), 126.18 (C-9), 1334.58 (C-10), 169.28 (C-1′), 34.05 (C-2′), 33.91 (C-3′), 31.52 (C-4′), 29.67 (C-5′), 25.85 (C-6′), 33.68 (C-7′), 22.71 (C-8′), 22.86 (C-9′), 192.85 (C-10′); +ve FAB MS m/z (rel.int.): 360 $[M]^+$ ($C_{20}H_{24}O_6$) (6.2), 191 (11.8), 169 (16.7).

2.7. Lanostdienyl docasanoate (3)

Elution of the column with chloroform gave colorless crystals of **3**, recystallized from acetone- methanol (1:1), 7.5 mg (0.09% yield); R_f : 0.56 (chloroform); m.p. 111-114 °C; UV- λmax: 210 nm (log ε 4.8); IR ν_{max} (KBr): 2925, 2851, 1724, 1655, 1443, 1381, 1250, 1088, 722 cm^{-1}; ^1H NMR ($CDCl_3$): δ 5.37 (1H, m, H-6), 5.10 (1H, m, H-22), 4.50 (1H, dd, J = 4.5, 5.0 Hz, H-3β), 1.98 (3H, brs, Me-21), 2.20 (2H, m, H_2 – 2′), 1.31 (60H, m, 28 x CH_2, 4 x CH), 1.27 (3H, brs, Me-29), 1.25 (3H, brs, Me-28), 1.19 (3H, brs, Me-30), 1.17 (3H, brs, Me-19), 0.96 (3H, d, J = 6.2 Hz, Me-27), 0.93 (3H, brs, Me-18), 0.91 (3H, d, J = 6.3 Hz, Me-26), ^{13}C NMR ($CDCl_3$): δ 35.25 (C-1), 29.02 (C-2), 78.55 (C-3), 38.45 (C-4), 141.83 (C-5), 121.51 (C-6), 29.36 (C-7), 42.52 (C-8), 50.83 (C-9), 37.36 (C-10), 21.15 (C-11), 26.73 (C-12), 45.08 (C-13), 50.11 (C-14), 32.32 (C-15), 30.29 (C-16), 51.86 (C-17), 16.71 (C-18), 18.36 (C-19), 136.15 (C-20), 22.58 (C-21), 123.79 (C-22), 27.81 (C-23), 39.61 (C-24), 28.17 (C-25), 23.62 (C-26), 24.93 (C-27), 26.13 (C-28), 28.36 (C-29), 15.78 (C-30), 169.69 (C-1′), 34.50 (C-2′), 31.25 (C-3′), 29.62 to 24.81 (C-4′ to C-20′), 22.41 (C-21′), 14.18 (C-22′). +ve FAB MS m/z: 748 $[M]^+$ ($C_{52}H_{92}O_2$) (17.8), 425 (11.6), 339 (8.3), 323 (56.1).

2.8. Trihydroxy labdanyl anthraquinoate (4)

Elution of the column with chloroform-methanol (19:1) afforded brown colored mass of **4**, recystallized from chloroform-methanol (19:1), 6.9 mg (.05% yield); R_f value: 0.42 (chloroform- methanol, 19:1); m.p. 83-85° C; UV λmax (MeOH): 209, 258, 310 nm (log 5.3, 2.6, 1.3); IR ν_{max} (KBr): 3415, 3386, 3270, 2927, 2855, 1731, 1607, 1513, 1455, 1379, 1212, 1171, 1052 cm^{-1}; ^1H NMR (DMSO-d_6): 7.86 (1H, d, J=8.5 Hz, H-9′), 7.48 (1H, d, J=2.0 Hz, H-1′), 7.46 (1H, dd, J=2.0, 8.1 Hz, H-3′), 7.31 (1H, d, J=8.1 Hz, H-4′), 7.29 (1H, d, J=8.5 Hz, H-8′), 3.77 (2H, brs, H_2=16), 3.38 (1H, ddd, J=6.5, 4.3, 5.3 Hz, H-6β), 3.32 (2H, d, J=8.5 Hz, H_2-20), 2.85 (1H, d, J=5.3 Hz, H-5α), 2.68 (1H, m, H_2-1a), 2.60 (1H, m, H_2-1b), 2.01 (1H, m, H_2-2a), 1.57 (2H, m, H-8, H-13), 1.55 (1H, m, H-9), 1.53 (2H, m, H_2-2b, H_2-3a), 1.43 (1H, m, H_2-7a), 1.41 (2H, m, H_2-12a, H_2-14a), 1.38 (2H, m, H_2-11a, H_2-7b), 1.36 (1H, m, H_2-11b), 1.34 (2H, m, H_2-3b, H_2-14b), 1.31 (3H, brs, Me-19), 1.29 (1H, m, H_2-12b), 1.27 (3H, brs, Me-17), 1.25 (3H, d, J=7.0 Hz, Me-18), 0.95 (3H, t, J=7.0 Hz, Me-15); ^{13}C NMR (DMSO-d_6): δ 31.52 (C-1), 29.32 (C-2), 28.43 (C-3), 42.08 (C-4), 48.16 (C-5), 67.71 (C-6), 31.52 (C-7), 30.57 (C-8), 50.82 (C-9), 37.13 (C-10), 29.38 (C-11), 28.79 (C-12), 32.11 (C-13), 23.04 (C-14), 14.69 (C-15), 63.71 (C-16), 22.64 (C-17), 17.04 (C-18), 24.92 (C-19), 62.47 (C-20), 128.01 (C-1′), 143.51 (C-2′), 127.75 (C-3′), 126.89 (C-4′), 139.22 (C-5′), 174.61 (C-6′), 136.85 (C-7′), 126.40 (C-8′), 124.20 (C-9′), 154.20 (C-10′), 153.51 (C-11′), 136.68 (C-12′), 192.54 (C-13′), 129.65 (C-14′),173.36 (C-15′); +ve TOF MS *m/z* (rel.int.): 592 $[M]^+$ ($C_{35}H_{44}O_8$) (2.3), 325 (9.8), 283 (14.2), 267 (11.6).

2.9. β-Sitosterol 3-O-arabinobenzoate (5)

Elution of the column with petroleum ether – chloroform (1:3) gave colorless crystal of **5**; recystallized from acetone-methanol, 3.5mg 0.04 % yield); R_f value: 0.45 (petroleum ether- chloroform, 1:3); m.p. 131-133 °C; UV- λmax: 203, 251 nm (log ε 5.3, 1.8)**;** IR ν_{max} (KBr): 3384, 3260, 2921, 2851, 1734, 1638, 1510, 1458, 1379, 1037, 881, 717 cm^{-1}; ^1H NMR ($CDCl_3$): 7.28 (1H, dd, J=2.6, 8.0 Hz, H-2″), 7.26 (1H, J=2.2, 7.5 Hz, H-6″), 7.24 (1H, m, H-3″), 7.22 (1H, m, H-5″), 7.20 (1H, m, H-4″), 5.36 (1H, dd, J=6.0, 5.2 Hz, H-6), 5.30 (1H, d, J=7.2 Hz, H-1′), 4.60 (1H, brm, $w_{1/2=}$18.3 Hz, H-3α), 4.57 (2H, brs, H_2-5′), 4.17 (1H, dd, J=7.2, 6.3 Hz, H-2′), 3.96 (1H, m, H-3′), 3.42 (1H, m, H-4′), 1.01 (3H, brs, Me-19), 0.95 (3H, d, J=6.3 Hz, Me-21), 0.89 (3H, d, J=6.2 Hz, Me-26), 0.87 (3H, d, J=6.1 Hz, Me-27), 0.83 (3H, d, J=6.5 Hz, Me-29), 0.67 (3H, brs, Me-18), 2.91-1.09 (29H, m, 11 x CH_2, 7 x CH); ^{13}C NMR ($CDCl_3$): δ 37.16 (C-1), 31.68 (C-2), 71.05 (C-3), 42.11 (C-4), 141.87 (C-5), 121.02 (C-6), 30.91 (C-7), 31.28 (C-8), 51.26 (C-9), 36.30 (C-10), 21.09 (C-11), 39.73 (C-12), 42.66 (C-13), 56.80 (C-14), 24.61 (C-15), 28.73 (C-16), 56.17 (C-17), 11.84 (C-18), 19.42 (C-19), 36.06 (C-20), 18.67 (C-21), 33.49 (C-22), 26.80 (C-23), 45.91 (C-24), 29.75 (C-25), 19.25 (C-26), 19.72 (C-27), 28.76 (C-28), 11.05 (C-29), 104.81 (C-1′), 75.86 (C-2′), 68.58 (C-3′), 67.75 (C-4′), 65.92 (C-5′), 138.57 (C-1″), 129.57 (C-2″), 127.76 (C-3″), 126.35 (C-4″), 125.92 (C-5″), 129.46 (C-6″), 169.05(C-7″); 650 $[M]^+$ ($C_{41}H_{62}O_6$) (2.5), 413 (15.8).

2.10. Lanostdienyl arabinolinoleiate (6)

Elution of the column with chloroform-methanol (99:1) yielded colorless crystals of **6**, recystallized from acetone-methanol (1:1), 4.9 mg (0.04% yield); R_f 0.49 (chloroform-methanol, 1:99); m.p. 77-81°C; UV λmax (MeOH): 205, 242 nm (log ε 4.7, 1.3); IR ν_{max} (KBr): 3517, 3385, 2926, 2855, 1731, 1646, 1456, 1381, 1229, 1149, 1037, 723 cm^{-1}; ^1H NMR (DMSO-d_6): δ 5.33 (1H, m, H-6), 5.24 (1H, m, H-24), 3.74 (1H, dd, J = 4.5, 9.3 Hz, H-3α), 1.82 (3H, brs, Me-26), 1.75 (3H, brs, Me-27), 1.26 (3H, brs, Me-29), 1.23 (3H, brs, Me-28), 1.18 (3H, brs, Me-30), 1.12 (3H, brs, Me-19), 0.98 (3H, d, J=6.8 Hz, Me-21), 0.91 (3H, brs, Me-18), 5.21 (1H, d, J=7.5 Hz, H-1′), 4.34 (1H, dd, J=7.5, 6.8 Hz, H-2′), 4.02 (2H, d, J=6.5 Hz, H_2-5′), 3.98 (1H, m, H-3′), 3.44 (1H, m, H-4′), 2.09 (2H, t, J=6.6 Hz, H_2-2″), 5.20 (1H, m, H-9″), 5.17 (1H, m, H-10″), 5.08 (1H, m, H-12″), 4.98 (1H, m, H-13″), 2.80 – 1.27 (44H, m, 20 x CH_2, 4 x CH), 0.89 (3H, t, J = 6.2 Hz, Me- 18″); ^{13}C NMR ($CDCl_3$): δ 35.15 (C-1), 28.76 (C-2), 76.02 (C-3), 38.30 (C-4), 142.81 (C-5), 118.78 (C-6), 29.24 (C-7), 42.47 (C-8), 50.65 (C-9), 37.23 (C-10), 21.11 (C-11), 26.75 (C-12), 44.58 (C-13), 49.81 (C-14), 31.28 (C-15), 31.03 (C-16), 51.75 (C-17), 16.67 (C-18), 18.34 (C-19), 36.52 (C-20), 18.77 (C-21), 36.49 (C-22), 24.26 (C-23), 125.43 (C-24), 139.86 (C-25), 23.61 (C-26), 24.89 (C-27), 26.04 (C-28), 28.34 (C-29), 15.73 (C-30), 103.78 (C-1′), 81.17 (C-2′), 72.58 (C-3′), 67.04 (C-4′), 62.80 (C-5′), 172.03 (C-1″), 34.81 (C-2″), 31.83 (C-3″), 29.16 (C-4″), 29.28 (C-5″), 29.26 (C-6″), 29.31 (C-7″), 32.56 (C-8″), 128.22 (C-9″), 120.05 (C-10″), 46.81 (C-11″), 122.85 (C-12″), 115.82 (C-13″), 33.16 (C-14″), 29.30 (C-15″), 29.28 C-16″), 22.89 (C-17″), 14.17 (C-18″); +ve FAB MS m/z (rel.int.): 821 $[M + H]^+$ ($C_{53}H_{89}O_6$) (1.3).

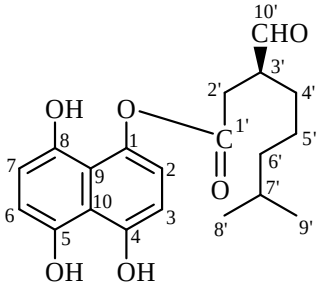
3. Results and discussion

Compound **1**, named *n*-tricosanyl oleate, was obtained as a colorless powder from petroleum ether eluants. It exhibited characteristic IR absorption bands for ester (1736 cm^{-1}), unstauration (1608 cm^{-1}) and long aliphatic chain (719 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 604 corresponding to the molecular formula of a fatty acid ester $C_{41}H_{80}O_2$. The ion peaks arising at *m/z* 265 $[CH_3\ (CH_2)_7CH=CH(CH_2)_7\text{-}CO]^+$, 239 [M-265] and 281 $[CH_3\ (CH_2)_7CH=CH(CH_2)_7\text{-}COO]^+$ indicated that oleic acid was esterified with a C_{23} aliphatic alcohol. The ^1H NMR spectrum of **1** showed two one-proton multiplets at δ 5.37 and 5.30 assigned to vinyl H-9 and H-10 proton, respectively. A two-proton triplet at δ 3.77 (J= 6.0 Hz) was ascribed to oxygenated methylene H2-1′ proton. The other methylene proton resonated from δ 2.31 to δ 1.28. Two three-proton triplets at δ 0.89 (J=6.3 Hz) and 0.85 (J=6.1 Hz) were accounted to the terminal C-18 and C-23′ primary methyl protons, respectively. The ^{13}C NMR spectrum of compound **1** displayed signals for ester carbon at δ 173.45 (C-1), vinylic carbons at δ 129.83 (C-9) and 121.05(C-10), oxygenated methylene carbon at δ 63.04 (C-1′), other methylene carbons in the range of δ 31.61-22.78, and methyl carbons at δ 15.26 (Me-18) and 13.01(Me-23′). On the basis of the above account the structure of 1 has been characterized as *n*- tricosanyl *n*- octadec-9-enoate.

$$\overset{18}{CH_3}\,(CH_2)_7\,\overset{10}{CH}=\overset{9}{CH}\,(CH_2)_7\,\overset{1}{CO}\text{-}O\overset{1'}{CH_2}\,(CH_2)_{21}\,\overset{23'}{CH_3}$$

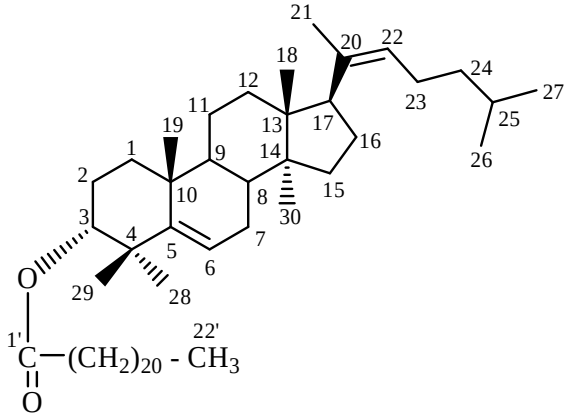
n-Tricosanyl oleate (**1**)

Compound **2**, designated as Tetrahydroxynaphthyl geranilan-10'-al-1'-oate, was obtained as pale yellow crystals from petroleum ether-chloroform (3:1) eluants. Its UV absorption maxima at 249 and 292 nm indicated aromatic nature of the molecule. It showed IR absorption bands for hydroxyl groups (3386, 3231 cm^{-1}), ester function (1702 cm^{-1}), aldehydic group (1680 cm^{-1}) and aromatic ring (1637, 1575, 1054 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **2** was determined at *m/z* 360 consistent with the molecular formula of a naphthyl ester, $C_{20}H_{24}O_6$. The ion peaks arising at m/z 191, 169 $[CO – O\ fission]^+$ suggested that a C_{10} unit was linked to a tetrahydroxy naphthyl ring. The ^1H NMR spectrum of **2** exhibited four one-proton doublets at δ 7.83 (J= 8.0 Hz), 7.42 (J = 8.5), 7.37 (J= 8.0 Hz) and 7.21 (J = 8.5 Hz) assigned correspondingly to ortho-coupled aromatic H-2, H-7, H-3 and H-6 protons. A two-proton doublet at δ 2.30 (J = 7.2 Hz) and two one-proton multiplets at δ 2.87 and 1.83 were ascribed to methylene H_2-2′ attached to the ester group and to methine H-3′ and H-7′ protons, respectively. The other methylene protons appeared as two-proton multiplets at δ 1.67, 1.51 and 1.28. Two three-proton doublets at δ 1.27 (5= 7.0 Hz) and 1.23 (J = 7.0 Hz) and a one-proton doublet at δ 9.92 (J=6.8 Hz) were due to secondary C-8′ and C-9′ methyl and C-10′ aldehydic protons, respectively. The ^{13}C NMR spectrum of **2** displayed signals for ester carbon at δ 169.28 (C-1′), aldehydic carbon at δ 192.85 (C-10′), aromatic carbons between δ 156.45 – 116.18, methyl carbons at δ 22.71 (C-8′) and 22.88 (C-9′) and methylene and methine carbons between δ 34.05 – 25.85. On the bans of the foregoing discussion, the structure of **2** has been elucidated as 1,4,5,8-tetrahydroxynaphthyl geranilan-10′-al 1′-oate. This is a new naphthyl ester.



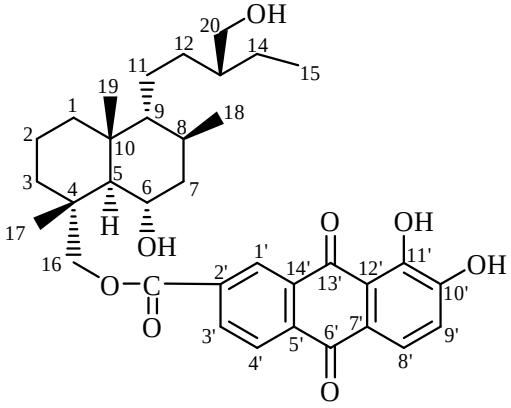
Tetrahydroxynaphthyl geranilan-10'-al-1'-oate (**2**)

Compound **3**, designated as lanostdienyl docosanoate, was obtained as a colourless crystalline mass from chloroform eluants. It responded positively to Liebermann-Burehardt test for triterpenoids and showed distinctive IR absorption bands for ester function (1724 cm^{-1}), unsaturation (1655 cm^{-1}) and long aliphatic chain (722 cm^{-1}). On the basis of mass and ^{13}C NMR spectral data, the molecular ion peak of **3** was determined at m/z 748 consistent with the molecular formula of a triterpenic ester $C_{52}H_{92}O_2$. The ion peaks arising at m/z 425 $[M - 323]^+$, 323 $[CH_3(CH_2)_{20}CO]^+$ and 339 $[CH_3(CH_2)_{20}COO]^+$ suggested that a C_{22} fatty acid was esterified with a triterpenol. The ^1H NMR spectrum of **3** showed two one-proton multiplets in the deshielded region at δ 5.37 and 5.10 assigned to vinylic H-6 and H-22 protons, respectively. A one-proton double doublet at δ 4.50 with coupling interactions of 4.5 and 5.0 Hz was ascribed to β-oriented oxygenated methine H-3 proton. A three-proton broad singlet at δ 1.98 was due to C-21 methyl protons located on the vinylic C-20 carbon. Five three-proton broad singlets at δ 1.27, 1.25, 1.19, 1.17 and 0.93 and two three-proton doublets at δ 0.96 (J = 6.2 Hz) and 0.91 (J = 6.3 Hz) were associated correspondingly to tertiary C-29, C-28, C-30, C-19, C-18 and secondary C-27 and C-26 methyl protons. A three-proton triplet at δ 0.76 (J = 6.5 Hz) was due to primary C-22′ methyl protons. The ^{13}C NMR spectrum **3** exhibited signals for ester carbon at δ 169.69 (C-1'), vinylic carbons at δ 141.83 (C-5), 121.51 (C-6), 136.15 (C-20) and 123.79 (C-22), oxygenated methine carbon at δ 78.55 (C-3) and methyl carbons from δ 28.36 to 14.18. The ^1H and ^{13}C NMR spectral data of the triterpenic skeleton of **3** were compared with the reported data of lanostenes [20-22]. On the basis of spectral data analysis, the structure of **3** has been established as lanost-5,20 (22)-dien-3α-olyl *n*-docosanoate. This is a new triterpenic ester.



Lanostdienyl-*n*-docosanoate (**3**)

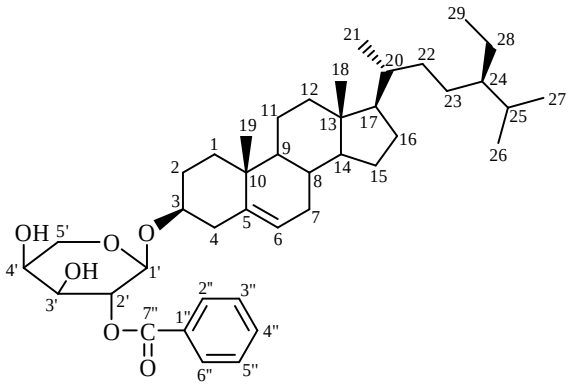
Compound **4**, named trihydroxy labdanyl anthraquinoate, was obtained as a brown coloured mass from chloroform-methanol (19:1) eluents. It gave positive tests for phenols and had UV absorption maxima at 258 and 310 nm for anthraquinones. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3415, 3386, 3270 cm^{-1}), ester function (1731 cm^{-1}) and aromatic ring (1607, 1513, 1052 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **4** has been established at m/z 592 consistent with the molecular formula of an anthraquinone unit linked to C_{20} compound, $C_{35}H_{44}O_8$. The ion peaks arising at m/z 267 $[C_{15'} - O \text{ fission}]^+$, 325 $[M - 267]^+$ and 283 $[C_{16} - O \text{ fission}]^+$ indicated that dihydroxy anthraquinonoate unit was linked with the diterpenoid. The ^1H NMR spectrum of **4** exhibited four one-proton doublets at δ 7.48 (J = 2.0 Hz), 7.31 (J = 8.1 Hz), 7.29 (J = 8.5 Hz) and 7.86 (J = 8.5 Hz), assigned to aromatic meta-coupled H-1′, and ortho-coupled H-4′, H-8′ and H-9′ protons, respectively. A one-proton double doublet at δ 7.46 with coupling interactions of 2.0 and 8.1 Hz was ascribed to ortho-, meta-coupled H-3′ proton. A one-proton triple doublet at at δ 3.38 (J=6.5, 4.3, 5.3 Hz), a broad singlet at δ 3.70 and a doublet at δ 3.32 integrating for two protons each were attributed correspondingly to β-oriented carbinol H- 6, oxygenated methylene H_2-16 and hydroxymethylene H_2-20 protons. Two three-proton broad singlets at δ 1.27 and 1.31, a three-proton doublet at δ 1.25 (J = 7.0 Hz) and a three-proton triplet at δ 0.95 (J = 7.0 Hz) were associated with the tertiary C-17 and C-19, secondary C-18 and primary C-15 methyl protons, respectively of labdane-type diterpenoid. The other methine and methylene protons resonated in the range of δ 2.85 - 1.29. The ^{13}C NMR spectrum of **4** exhibited signals for keto carbons at δ 192.54 (C-13′) 174.61 (C-6′), ester carbon at δ 173.36 (C-15′), aromatic carbons from δ 154.20 to 124.20 oxygenated methylene carbons at δ 63.71 (C-16) and 62.47 (C-20), carbinol carbon at δ 67.71 (C-6) and methyl carbons at δ 14.69 (C-15), 22.64 (C-17), 17.04 (C-18) and 24.92 (C- 19). The ^1H and ^{13}C NMR values of labdanyl unit of **4** were compared with those of the related diterpenes [23, 24]. On the basis of the foregoing account the structure of **4** has been established as labdan-6α,16,20-triol-16-(10′,11′- dihydroxy anthraquinone -2′-oate). This is a new labdane type diterpene linked with an anthraquinone moiety.



Trihydroxy labdanyl anthraquinoate (**4**)

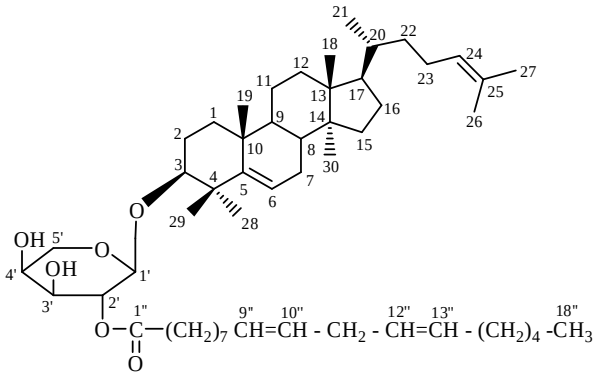
Compound **5**, named β-sitosterol 3-O-arabinobenzoate, was obtained as a colorless crystalline mass from petroleum ether-chloroform (1:3) eluents. It gave positive tests for glycosides and showed characteristic absorption bands for hydroxyl groups (3384, 3260 cm^{-1}), ester function (1734 cm^{-1}), unsaturation (1638 cm^{-1}) and aromatocity (1510, 1037 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular weight of **5** was established as *m/z* 650 consistent to the molecular formula of a steroidal glycosidic ester, $C_{41}H_{62}O_6$. The ion peak arrising at *m/z* 413 due to removal of the glycosidic chain indicated that β-sitosterol unit was linked as an aglycone to the sugar unit. The ^1H NMR spectrum of **5** displayed five deshielded signals as one-proton double doublets at δ 7.28 (J = 8.0, 2.6 Hz) and 7.26 (J = 7.5, 2.2 Hz) and three one-proton multiplets at δ 7.24, 7.22 and 7.20 assigned to five aromatic protons. A one- proton double doublet at δ 5.36 with coupling interactions of 6.0 and 5.2 Hz was ascribed to vinylic H- 6 proton. A one-proton broad multiplet at δ 4.60 with half width of 18.3 Hz was accounted to oxygenated methine H-3α proton. Two three-proton broad singlets at δ 0.67 and 1.01, four three-proton doublets at δ 0.95 (J=6.3 Hz), 0.89 (J=6.2 Hz), 0.87 (J=6.1 Hz) and 0.83

(J=6.5 Hz) were associated correspondingly to tertiary C-18 and C-19, secondary C-21, C-26 and C-27 and primary C-29 methyl protons, all attached to saturated carbons. A one-proton doublet at δ 5.30 (J = 7.2 Hz) was due to anomeric H-1′ proton. The other sugar protons appeared as a one-proton double doublet at δ 4.17 (J = 7.2, 6.3 Hz), two one-proton multiplets at δ 3.96 and 3.42 and a two-proton broad singlet at δ 4.57. The other methine and methylene protons resonated from δ 2.91 to 1.09. The ^{13}C NMR spectrum of **5** exhibited signals for aromatic carbons from δ 138.57 to 125.92, ester carbon at δ 169.05 (C-7"), vinylic carbons at δ 141.87 (C-5) and 121.02 (C-6), oxygenated methine carbon at δ 71.05 (C-3), anomeric carbon at δ 104.81 (C-1'), other sugar carbons from δ 73.86 to 65.92 and steroidal carbons between δ 56.80-11.05. The ^{1}H NMR and ^{13}C NMR spectral data of the steroidal nucleus were compared with other stigmastene- type molecules [25-27]. The presence of H-2 signal in the deshielded region at δ 4.17 and C-2′ signal at δ 75.86 suggested location of the phenyl ester at C – 2′. On the basis of these evidences the structure **5** has been formulated as stigmast-5-en-3β-O-D-arabinopyranosyl-2′-benzoate. This is a new steroidal glycosidic ester.



β-Sitosteryl-3-*Oβ-D*-arabino-2'-benzoate (**5**)

Compound **6**, named lanostdienyl arabinolinoleiate, was obtained as a colorless crystalline mass from chloroform- methanol (99:1) Eluants. It gave positive tests for glycosides and had IR absorption bands for hydroxyl groups (3517, 3385 cm^{-1}), ester function (1731 cm^{-1}), unsaturation (1646 cm^{-1}) and long aliphatic chain (723 cm^{-1}). On the basis of mass and ^{13}C NMR spectra, the molecular ion peak of **6** was determined at m/z 821 $[M + H]^+$ consistent with the molecular formula of a tetracyclic triterpenic glycosidic ester $C_{53}H_{89}O_6$. The ^{1}H NMR spectrum of **6** exhibited six one-proton deshielded multiplets at δ 5.33, 5.24, 5.20, 5.17, 5.08 and 4.98 assigned to vinylic H-6, H-24, H-9″, H-10″, H-12″ and H-13″ protons, respectively. A one-proton double doublet at δ 3.74 with coupling interactions of 4.5 and 9.3 Hz was ascribed to α-oriented oxygenated H-3 methine proton. Seven three-proton broad singlets at δ 1.82, 1.75, 1.26, 1.23, 1.18, 1.12 and 0.91 were ascribed to C-26 and C-27 methyl protons located on the vinylic C-25 carbon and to tertiary C-29, C-28, C-30, C-19 and C-18 methyl protons, respectively. A three- proton doublet at δ 0.98 (J=6.8 Hz) and a three-proton triplet at δ 0.89 (J=6.2 Hz) were associated with correspondingly to secondary C-21 and primary C-18″ methyl protons. A two-proton triplet at δ 2.09 (J = 6.6 Hz) was due to C-2″methylene protons adjacent to the ester group. The other methine and methylene protons appeared from δ 2.80 to 1.27. A one-proton doublet at δ 5.21 (J = 7.5 Hz) was accounted to anomeric H-1′ proton. The other sugar protons appeared in the range of δ 4.34 – 3.44. The ^{13}C NMR spectrum of **6** showed signals for vinylic carbons from δ 142.81 to 115.82, ester carbon at δ 172.03 (C – 1″), oxygenated methine carbon at δ 76.02 (C-3), anomeric carbon at δ 103.78 (C-1′), other sugar carbons between δ 81.17 - 62.8 and methyl carbons in the range of δ 28.34 -14.17. The shifting of H-2′ signal as a double doublet at δ 4.34 (J = 7.5, 6.8 Hz) in the deshielded region in the ^{1}H NMR spectrum and C-2′ at 81.17 in the ^{13}C NMR spectrum suggested the location of the ester linkage at C – 2'. The ^{1}H and ^{13}C NMR spectral data of the triterpenic skeleton of **6** were compared with the reported data of lanostenes [20-22]. On the basis of spectral data analyses, the structure of **6** has been elucidated as lanost-5, 24-dien-3β-ol 3β-O-D- arabinopyranosyl-2′-*n* – octadec -9″, 12″-dienoate. This is a new lanostenyl glycosyl ester.



Lanostdienyl arabinolinoleiate (**6**)

4. Conclusion

Phytochemical investigation of a methanolic extract of the fruits of *Cuminum cyminum* on subjection to silica gel column chromatography led to the isolation of a variety of chemical constituents including acyl and phenolic esters, triterpenoids and a steroidal glycosidic ester. All the compounds are isolated from the fruits for the first time.

5. Acknowledgement

The authors are thankful to the Head, SAIF, Central Drug Research Institute, Lucknow for recording mass spectral data of the compounds.

6. References

1. Joshi SG. Medicinal plants: Family Apiaceae. Oxford and IBH Publishing Co. Pvt. Ltd, Delhi, 2000.
2. Mhaskar KS, Blatter E, Caius JF. editors. In Kiritikar and Basu's Illustrated Indian Medicinal Plants. New Delhi: Satguru Publications 2000, 5.
3. Perry LM. Medicinal Plants of East and Southeast Asia. The MIT Press, Massachusetts and London, 1980.
4. Al–Yahya M, Collpharm A. Phytochemical studies of plant used in traditional medicine in Saudi Arabia. Fitoterapia 1986; 57(3):179-82.
5. Agrawal S. Volatile oil constituents and wilt resistance in cumin (*Cuminum cyminum* L.). Curr Sci Ind 1996; 71:177–178.
6. Bettaieb I, Bourgou S, Wannes WA, Hamrouni I, Limam F, Marzouk B. Essential oils, phenolics and antioxidant activities of different parts of cumin (*Cuminum cyminum* L.). J Agric Food Chem 2010; 58:10410–10418.
7. Eikani MH, Goodarznia I, Mirza M. Supercritical carbon dioxide extraction of cumin seeds (*Cuminum cyminum* L.). Flav Frag J 1999; 14:29–31.

8. Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. Chemical and biological characteristics of *Cuminum cyminum* and *Rosemarinus officinalis* essential oils. Food Chem 2007; 102: 898–904.
9. Li R, Jiang ZY. Chemical composition of the essential oil of *Cuminum cyminum* L. from China. Flav Frag J 2004; 19:311–4.
10. Ishikawa T, Takayanagi T, Kitazima J. Water-soluble constituents of cumin: Monoterpenoid glucosides. Chem Pharm Bull 2002; 50:1471–1478.
11. El-Negoumy SI, Mansour RMA. Flavone glycosides of *Cuminum cyminum* seeds. Grasa Y Aceites 1989; 40:87–89.
12. Khafagy SM, Sarg TM, Abdel SNA, Gabr O. Isolation of two flavone glycosides from the fruits of *Cuminum cyminum* L. grown in Egypt. Pharmazie 1978; 33:296-297.
13. Kitazima J, Ishikawa T, Fujimatu E, Kondho K, Takayanagi T. Glycosides of 2-C-methyl-D-erythritol from the fruits of anise, coriander and cumin. Phyto-chemistry 2003; 62:115–120.
14. Takayanagi T, Ishikawa T, Kitazima J. Sesquiterpene lactone glucosides and alkyl glycosides from the fruit of cumin. Phytochemistry 2003; 63:479–484.
15. Toghrol F, Daneshpejouh H. Estimation of free amino acids, protein and amino acid compositions of cumin seed (*Cuminum cyminum*) of Iran. J Trop Pediatr Environ Child Health 1974; 20:109–111.
16. Laribi B, Kouki K, Mougou A, Marzouk B. Fatty acid and essential oil composition of three Tunisian caraway (*Carum carvi* L.) seed ecotypes. J Sci Food Agric 2010; 90:391–396.
17. Yetim H, Sagdic O, Ozturk I. Fatty acid composition of cold press oils of seven edible plant seeds grown in Turkey. Chem Nat Compd 2008; 44:634–636.
18. Uma PK, Geervani P, Eggum BO. Common Indian spices: Nutrient composition, consumption and contribution to dietary value. Plant Foods Hum Nutr 1993; 44:137–48.
19. Johri RK. *Cuminum cyminum* and *Carum carvi*: An update. Pharmacogn Rev 2011; 5(9):63-72.
20. Ali M. Techniques in terpenoid Identification. Birla Publications, Delhi, 2001, 211-228, 352-361.
21. Chung IM, Ali M, Ahmad A, Lim, JD, Yu CY, Kim JS. Chemical constituents of rice (*Oriza sativa*) hulls and their herbicidal activity against duckweed (*Lemna paucicostata* Hegelm 381). Phytochemical Analysis 2006; 17:36-46.
22. Khan MA, Ali M, Alam P. Phytochemical investigation of the fruit peels of *Citrus reticulata* Blanco. Nat Prod Res 2010; 24:610-620.
23. Mittal A, Ali M. Diterpenic labdane galactofuranosides from the roots of *Calotropis procera* (Ait.) R.Br. Indian J Chem 2013; 52B: 641-645.
24. Aslam M, Ali M, Dayal R, Javed K. Coumarins and a naphthyl labdanoate diarabinoside from the fruits of *Peucedanum grande* C.B, Clarke. Zeitsch. Naturforsch 2012; 67c:580-586.
25. Akhtar N, Ali M, Alam MS. New steroidal glycosides from the stem bark of *Mimusops elengi*. Chem Nat Compds 2010; 46(4):549-553.
26. Jung WS, Chung IM, Ali M, Ahmad A. New steroidal glycoside ester and aliphatic acid from the fruits of *Lycium chinensis*. J Asian Nat Prod Res 2012; 14(4):301-307.
27. Mustafa A, Ali M. New steroidal lactones and homomonoterpenic glucoside from fruits of *Malva sylvestris* L. Acta Poloniac Pharmac Drug Res 2011; 68(3):393-401.