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Original Research Article

Phytochemical investigation and isolation of new compounds from the stems of *Tinospora cordifolia* Miers

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ABSTRACT

Tinospora cordifolia (Willd.) Miers (Menispermaceae) is a glabrous, deciduous and trailing plant found in tropical India, China, Sri Lanka, Bangladesh and Myanmar. It is used to treat anemia, debility, diabetes, diarrhea, dysentery, dyspepsia, fever, jaundice, rheumatism, urinary and skin diseases and snake bites. Its air-dried stem powder was exhaustively extracted with methanol. The concentrated methanolic extract was adsorbed on silica gel (60-120 mesh) for the preparation of a slurry. The dried slurry was chromatographed over silica gel column packed in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol successively in order of increasing polarity to isolate a variety of phytoconstituents including cetyl alcohol (**1**) along with new chemical constituents characterized as *trans*-cinnamoyl-2-*n*-hexanyl-7-methoxynaphthyl amide (**2**), *trans*-cinnamoyl-2-*n*-pentanyl-6,7-dimethoxynaphthyl amide (**3**), *trans*-cinnamoyl-2-*n*-octanyl-7-methoxynaphthyl amide (**4**), β-D-arabinosyl-*O*-geranilan-10′-oate (**5**), 4,5,7-trimethoxy-2-naphthol-2-*O*-α-L-arabinofuranosyl-(2′→1′′)-*O*-α-L-arabinopyranosyl-2′′-*O*-pentane (**6**), 5,7-dimethoxy-2-naphthol-2-*O*-α-L-arabinopyranosyl-(2′→1′′)-α-L-arabinopyranosyl-2′′-*O*-decane (**7**) and 5-hydroxy-4'-methoxy-7-flavanoxy-(7→7'')-β-*O*-labdan-1-en-3''α,19''-olide-18''-oic acid (tinolabdenyl flavanone) (**8**). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

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1. Introduction

Tinospora cordifolia (Willd.) Miers, syn. *Menispermum cordifolium* Willd. (Menispermaceae), known as amrita, guduchi, giloe and heart-leaved moonseed, is a glabrous, deciduous, climbing shrub with creamy white, soft, porous and spiral stems; corky bark; simple, alternate, long petiolate, smooth, cordate leaves; unisexual, yellow or green flowers and red, glossy, ovoid berries. It is distributed throughout tropical India, China, Sri Lanka, Bangladesh and Myanmar up to an altitude of 300 m (Anonymous, 1976; Singh et al., 2003; Anonymous, 2007; Singh and Chaudhuri, 2017). Its stem possesses antiallergic, bactericidal, osteoprotective, antioxidant, genoprotective anti-inflammatory, uricosuric, antimalarial, antiperiodic, antispasmodic, antistress, hepatoprotective, antiviral, bitter, diuretic, febrifuge, immuno-stimulant, stomachic and tonic properties (Sachdeva et al., 2014; Biswas et al., 2015; Shah and Shah, 2015; Singh et al., 2015; Rong et al., 2016; Sharma and Dabur, 2016; Singh et al., 2016; Abiramasundari et al., 2017; Singh and Chaudhuri, 2017). It is used to treat anemia, debility, diabetes, diarrhea, dysentery, dyspepsia, fevers, jaundice, rheumatism, urinary and skin diseases, scorpion stings and snake bites. A stem paste is applied to fix bone fractures. It is taken to expel brain toxins. A leaf decoction is drunk to relieve gout (Kirtikar and Basu, 1975; Anonymous, 1976; Chopra, 1982; Sinha et al., 2004; Quattrocchi, 2012).

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The plant contained clerodane derived diterpenes (Bhatt et al., 1988; Bhatt and Sabata, 1989; Khan et al., 1989; Gangan et al., 1994, 1995, 1996,1997; Maurya et al., 1995, 1996, 1998, 2004; Wazir et al., 1995), giloin glycoside, giloinin, gilo-sterol, columbin, chasmanthin, palmarin, tinisporon, tinosporic acid and tinosporol, phenolic lignans (Hanuman et al., 1986, 1988), phenolic propane glycosides (Sipahimalani et al., 1994), steroids (Pathak et al., 1995), phytoecdysones, tinosporafuranol, tinosporafurandiol and β-sitosterol (Ahmad et al., 2010), alkaloids berberine, tinospporin, palmitine, tembetarine, choline, isocolumbin, and tetrahydropalmatine; octacosanol, heptacosanol, nonacosan-15-one, hydroxyecdysone, makisterone, giloinsterol, 18-nonderodane glycoside, furanoid diterpene glycosides, tinocordifoliside, tinocordiside, cordiside, cordifoliside, plamatosides and syringin (Swaminathan et al., 1989; Sarma et al., 1995; Ghosal and Vishwakarma, 1997; Pradhan et al., 1997; Sarma et al., 1998). Keeping in view the high reputation and wide application of *T. cordifolia* in the many indigenous systems, it has been aimed to carry out isolation and characterization of chemical constituents from the stems of this plant procured from Delhi.

2. Experimental

2.1. Materials and Methods

2.1.1. General procedures

All chemicals were acquired from Sigma-Aldrich unless otherwise stated. Melting points were determined on a thermoelectrically heated Perfit apparatus without correction. The IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. 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 DRX 400 MHz spectrometer with TMS as an internal standard. Mass spectra were scanned on a Jeol D-300 (EI/CI) system. Column chromatography was performed on silica gel (Qualigens, Mumbai, India), 60-120 mesh and solvents used were purchased from Merck Specialties (E. Merck, Pvt. Ltd. New Delhi, India). The purity of the isolated compounds was checked on precoated TLC plates with Silica gel 60 F_{254} (Merck, 0.25 mm) and the spots were visualized by exposure to iodine vapors or under UV radiations and spraying with ceric sulfate solution.

2.2. Plant material

The stem of *T. cordifolia* (Fig. 1) were procured from a local market, Khari Baoli (Fig. 2), New Delhi and identified by Prof. M. P. Sharma, Department of Botany, Jamia Hamdard. A specimen voucher of the drug was deposited in the herbarium of the Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard for future reference.

Fig. 1. The stem of *Tinospora cordifolia*.

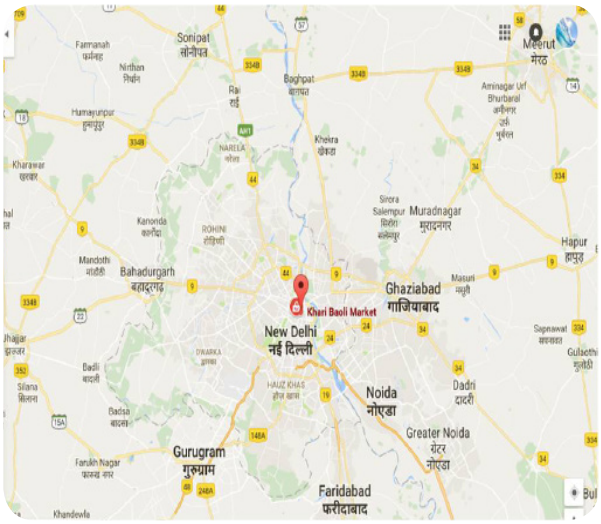

Fig. 2. The map of *T. cordifolia* area.

2.3. Extraction and isolation

The air-dried powder of the stems (1.0 kg) was extracted with methanol exhaustively in a Soxhlet apparatus. The methanolic extract was concentrated under reduced pressure to obtain a reddish brown viscous mass (114 g). A small portion of the extract was analyzed chemically to determine the occurrence of different chemical constituents. It was dissolved in a small amount of methanol and adsorbed on silica gel (60-120 mesh) for column chromatography for preparation of a slurry. The slurry was dried in air and subjected to chromatography over 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, 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 homogeneity. Similar fractions having the same R_f values were combined and crystallized. The isolated compounds were purified to get the following compounds as explained in graphical abstract.

2.3.1. Cetyl alcohol (**1**)

Elution of the column with petroleum ether gave colorless flakes of **1**, 73.2 mg, m. p. 48-49 ºC; IR ν_{max} (KBr): 3365, 2927, 2841, 1415, 1362, 1260, 1042, 835, 728 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.45 (2H, t, *J*=6.6 Hz, H_2-1), 2.19 (2H, m, CH_2), 1.48 (2H, m, CH_2), 1.17 (24H, brs, 12 x CH_2), 0.87 (3H, t, *J*=7.5 Hz, Me-16). ^{13}C NMR ($CDCl_3$): δ 61.33 (C-1), 33.43 (CH_2), 32.09 (CH_2), 31.06 (CH_2), 28.82 (CH_2), 28.69 (CH_2), 28.49 (CH_2), 28.35 (CH_2), 25.10 (CH_2), 25.10 (CH_2), 24.15 (CH_2), 21.83 (CH_2), 13.34 (Me-16). EI-MS *m/z* (rel.int): 242 $[M]^+$ ($C_{16}H_{34}O$) (3.8).

2.3.2. *trans*-Cinnamoyl-2-*n*-hexanyl-7-methoxynaphthyl amide (**2**)

Elution of the column with chloroform afforded a yellowish semisolid mass of **2**, 81.5 mg, UV λ_{max} (MeOH); 275, 350 nm; IR ν_{max} (KBr): 3310, 2928, 2841, 1687, 1635, 1527, 1436, 1262, 1131, 1042, 935 cm^{-1}; ^1H NMR (MeOD): δ 7.43 (1H, d, *J*=10.6 Hz, H-5), 7.38 (1H, d, *J*=1.6 Hz, H-8), 7.18 (1H, d, *J*=1.6 Hz , H-1), 7.06 (1H, dd, *J*=1.6, 10.6 Hz, H-6), 7.03 (1H, m, H-2''), 7.01 (1H, m, H-3''), 6.94 (1H, m, H-5''), 6.83 (1H, d, *J*=8.4 Hz, H-4), 6.76 (1H, dd, *J*=1.6, 8.4 Hz, H-3), 6.72 (1H, m, H-4''), 6.65 (1H, m, H-6''), 6.40 (1H, d, *J*=15.6 Hz, H-7''), 5.85 (1H, d, *J*=15.6 Hz, H-8''), 3.49 (2H, m H_2-6'), 3.42 (2H, m H_2-5'), 2.78 (2H, m, H_2-1'), 2.71 (2H, m, H_2-2'), 1.36 (2H, m, H-3'), 1.31 (2H, m, H_2-4'), 3.91 (3H, s, OMe). ^{13}C NMR (MeOD): δ 110.19 (C-1), 148.44 (C-2), 144.44 (C-3), 115.07 (C-4), 140.61 (C-5), 121.81 (C-6), 155.54 (C-7), 112.58 (C-8), 147.90 (C-9), 126.91 (C-10), 34. 41 (C-1'), 34.17 (C-2'), 29.35 (C-3') 29.05 (C-4'), 40.95 (C-5'), 41.13 (C-6'), 129.28 (C-1''), 129.32 (C-2''), 129.91 (C-3''), 114.87 (C-4''), 123.42 (C-5''), 136.93 (C-6''), 117.38 (C-7''), 120.28 (C-8''), 167.78 (C-9''), 55.01 (OMe). EI-MS *m/z* (rel.int); 387 $[M]^+$ ($C_{26}H_{29}NO_2$) (1.7).

2.3.3. *trans*-Cinnamoyl-2-*n*-pentanyl-6,7-dimethoxynaphthyl amide (**3**)

Elution of the column with chloroform furnished a dark brown semisolid mass of **3**, 112.7 mg, UV λ_{max} (MeOH); 277, 348 nm; IR ν_{max} (KBr): 3315, 2922, 2837, 1685, 1638, 1531, 1442, 1259, 1130, 1038, 937 cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.37 (1H, s, H-8), 7.33 (1H, s, H-5), 7.04 (1H, d, *J*=1.6 Hz, H-1), 6.99 (1H, m, H-2''), 6.95 (1H, m, H-6''), 6.93 (1H, m, H-3''), 6.90 (1H, m, H-5''), 6.73 (1H, d, *J*=10.0 Hz, H-4), 6.65 (1H, dd, *J*=1.6, 10.0 Hz, Hz, H-3), 6.62 (1H, m, H-4''), 6.34 (1H, d, *J*=16.0 Hz, H-7''), 5.75 (1H, d, *J*=16.0, H-8''), 3.80 (3H, s, OMe), 3.74 (3H, s, OMe), 3.38 (2H, t, *J*=7.2 Hz, H_2-5'), 3.31 (2H, t, *J*=7.2 Hz, H_2-1'), 2.69 (2H, m H_2-4'), 2.61 (2H, m H_2-3'), 1.20 (2H, m, H_2-2'). ^{13}C NMR (DMSO-d_6): δ 123.21 (C-1), 148.51 (C-2), 124.74 (C-3), 118.77 (C-4), 111.61 (C-5), 156.80 (C-6), 159.81 (C-7), 142.02 (C-8), 149.75 (C-9), 149.27 (C-10), 48.35 (C-1'), 35.75 (C-2'), 35.50 (C-3'), 42.30 (C-4'), 42.50 (C-5'), 131.36 (C-1''), 130.72 (C-2''), 116.27 (C-3''), 115.85 (C-4''), 113.95 (C-5''), 130.67 (C-6''), 121.71 (C-7''), 116.47 (C-8''), 169.20 (C-9''), 56.43 (OMe), 56.41 (OMe). EI MS *m/z* (rel.int); 403 $[M]^+$ ($C_{26}H_{29}NO_3$) (1.6).

2.3.4. *trans*-Cinnamoyl-2-*n*-octanyl-7-methoxynaphthyl amide (**4**)

Elution of column with chloroform- methanol (3:1) yielded a brown sticky mass of **4**, 185.2 mg, UV λ_{max} (MeOH); 272, 345 nm; IR ν_{max} (KBr): 3318, 2927, 2845, 1689, 1641, 1525, 1435, 1265, 1130, 1047, 932 cm^{-1}; ^1H NMR (MeOD): δ 7.47 (1H, d, *J*=10.5 Hz, H-5), 7.38 (1H, d, *J*=2.0 Hz, H-8), 7.13 (1H, d, *J*=2.0 Hz, H-1), 7.05 (1H, dd, *J*=2.0, 10.5 Hz, H-6), 7.03 (1H, m, H-2''), 6.97 (1H, m, H-3''), 6.93 (1H, m, H-5''), 6.80 (1H, d, *J*=8.0 Hz , H-4), 6.76 (1H, dd, *J*=2.0, 8.0 Hz, H-3), 6.72 (1H, m, H-4''), 6.67 (1H, m, H-6''), 6.44 (1H, d, *J*=15.6 Hz, H-7''), 5.85 (1H, d, *J*=15.6 Hz, H-8''), 3.90 (3H, s, OMe), 3.50 (2H, t, *J*=7.2 Hz, H2-8'), 3.44 (2H, m, H_2-7'), 2.80 3(2H, t, *J*=7.2 Hz, H_2-1'), 2.73 (2H, m, H_2-2'), 1.41 (2H, m, H_2-3'), 1.37 (2H, m, H_2-4'), 1.31 (2H, m, H_2-5'), 1.29 (2H, m, H_2-6'); ^{13}C NMR (MeOD): δ 110.20 (C-1), 148.41 (C-2), 114.81 (C-3), 115.09 (C-4), 140.63 (C-5), 112.09 (C-6), 158.11 (C-7), 112.58 (C-8), 143.03 (C-9), 126.89 (C-10), 34.82 (C-1'), 34.60 (C-2'), 30.52 (C-3') 30.41 (C-4'), 29.54 (C-5'), 29.35 (C-6'), 40.91 (C-7'), 41.07 (C-8'), 130.65 (C-1''), 121.79 (C-2''), 124.08 (C-3''), 114.44 (C-4''), 123.44 (C-5''), 136.91 (C-6''), 117.39 (C-7''), 120.22 (C-8''), 167.80 (C-9''), 55.01 (OMe); EI MS *m/z* (rel.int); 415 $[M]^+$ ($C_{28}H_{33}O_2N$) (2.3).

2.3.5. β-D-Arabinosyl-*O*-geranilan-10′-oate (**5**)

Elution of column with chloroform-methanol (49:1) produced a yellow semisolid mass of **5**, 215.1 mg, IR ν_{max} (KBr): 3368, 3285, 2930, 2845, 1721, 1638, 1455, 1401, 1272, 1073, 921, 861 cm^{-1}: ^1H NMR (DMSO-d_6): δ 4.95 (2H, d, *J*=7.3 Hz, H-1), 4.21 (1H, m, H-2), 3.96 (1H, m, H-3), 3.85 (2H, d, *J*=8.1Hz, H_2-5), 3.53 (1H, m, H-4), 2.50 (1H, m, H-3'), 2.03 (1H, m, H-7'), 1.57 (2H, m, H_2-4'),1.37 (2H, m, H_2-5'), 1.33 (2H, m, H_2-6'), 1.25 (2H, m, H_2-2'), 1.21 (3H, d, *J*=6.3 Hz, Me-8'), 1.18 (3H, d, *J*=6.5 Hz, Me-9'), 0.87 (3H, t, *J*=6.5 Hz, Me-1'). ^{13}C NMR (DMSO-d_6): δ 107.16 (C-1), 85.07 (C-2), 72.96 (C-3), 76.11 (C-4), 63.55 (C-5), 14.28 (C-1′), 29.45 (C-

2′), 56.18 (C-3′), 29.47 (C-4'), 31.75 (C-5') 32.62 (C-6'), 48.13 (C-7'), 20.15 (C-8′), 17.86 (C-9'), 172.52 (C-10′); EI MS *m/z* (rel.int): 304 $[M]^+$ ($C_{15}H_{28}O_6$) (6.5), 149 (10.2), 133 (5.1).

2.3.6. 4,5,7-Trimethoxy-2-naphthol-2-*O*-α-L-arabinofuranosyl-(2′→1′′)-*O*-α-L-arabinopyranosyl-2′′-*O*-pentane (**6**)

Elution of column with chloroform- methanol (97:3 v/v) gave a colorless semisolid mass of **6**, 185.7 mg, UV λ_{max} (MeOH): 275 nm. IR ν_{max} (KBr): 3415, 3227, 2937, 2842, 1645, 1525, 1427, 1365, 1258, 1072, 938 cm^{-1}. $_1$H NMR (DMSO-d_6): δ 6.87 (1H, d, *J*=2.0 Hz, H-8), 6.73 (1H, d, *J*=1.6 Hz, H-1), 6.72 (1H, d, *J*=2.0 Hz, H-6), 6.69 (1H, d, *J*=1.6 Hz, H-3), 6.58 (1H, d, *J*=4.4 Hz, H-1'α), 4.69 (1H, d, *J*=4.0 Hz, H-1''α), 4.62 (1H, m, H-2'), 4.57 (1H, m, H-2''), 4.46 (1H, m, H-4'), 4.39 (1H, m, H-3'), 4.29 (1H, m, H-3''), 4.01 (1H, m, H-4''), 3.87 (2H, d, *J*=3.6 Hz, H_2-5'), 3.84 (2H, d, *J*=9.2 Hz, H_2-5''), 3.78 (3H, s, OMe), 3.77 (3H, s, OMe), 3.76 (3H, s, OMe), 3.66 (2H, s, H_2-1'''), 1.94 (2H, m, H_2-2'''), 1.23 (2H, m, H_2-3'''), 1.21 (2H, m, H_2-4'''), 0.83 (3H, t, *J*=6.3 Hz, Me-5'''); ^{13}C NMR (MeOD): δ 116.09 (C-1), 154.65 (C-2),116.16 (C-3), 149.12 (C-4), 147.29 (C-5), 120.17 (C-6), 149.13 (C-7), 116 .01 (C-8), 138.76 (C-9), 133.80 (C-10), 104.25 (C-1'), 87.28 (C-2'), 72.97 (C-3'), 87.45 (C-4′) 70.92 (C-5'), 97.75 (C-1''), 87.28 (C-2''), 72.59 (C-3''), 71.87 (C-4''), 70.90 (C-5''), 61.11 (C-1'''), 47.54 (C-2'''), 30.71 (C-3'''), 29.53 (C-4'''), 18.13 (C-5''''), 56.66 (OMe), 56.44 (OMe), 55.67 (OMe); EI MS *m/z* (rel.int.): 568 $[M]^+$ ($C_{28}H_{40}O_{12}$) (5.8), 335 (4.2), 233 (11.6), 203 (8.7).

2.3.7. 5,7-Dimethoxy-2-naphthol-2-*O*-α-L-arabinopyranosyl-(2′→1′′)-α-L-arabinopyranosyl-2′′-*O*-decane (**7**)

Elution with column with chloroform-methanol (93:7) yielded a pale yellow semisolid mass of **7**, 103 mg, UV λ_{max} (MeOH): 277 nm; IR ν_{max} (KBr): 3421, 3230, 2941, 2845, 1645, 1532, 1428, 1368, 1261, 1073, 941 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 6.82 (1H, d, *J*=2.0 Hz, H-8), 6.70 (1H, d, *J*=2.0 Hz, H-1), 6.40 (1H, dd, *J*=2.0, 7.6 Hz, H-3), 6.24 (1H, d, *J*=2.0 Hz, H-6), 6.13 (1H, d, *J*=7.6 Hz, H-4), 5.28 (1H, d, *J*=5.2 Hz, H-1'), 5.25 (1H, d, *J*=4.4 Hz, H-1''), 4.98 (1H, m, H-2'') 4.42 (1H, m, H-2'), 4.30 (1H, m, H-3'), 4.22 (1H, m, H-3''), 3.78 (2H, d, *J*=4.0 Hz, H_2-5'), 3.75 (2H, d, *J*=6.0 Hz, H2-5''), 3.58 (1H, m, H-4'), 3.55 (1H, m, H-4''), 3.50 (2H, t, *J*=6.8 Hz, H-1'''), 3.67 (3H, s, OMe), 3.46 (3H, s, OMe), 2.32 (2H, m, H_2-2'''), 2.27 (2H, m, H_2-3'''), 1.99 (2H, m, CH_2), 1.54 (2H, m, CH_2), 1.24 (2H, m, CH_2), 1.20 (4H, s, 2 x CH_2), 1.16 (2H, m, CH_2), 0.83 (3H, t, *J*=7.2 Hz, Me-10'''); ^{13}C NMR (DMSO-d_6): δ 109.21 (C-1), 154.61 (C-2), 108.68 (C-3), 108.46 (C-4), 151.28 (C-5), 121.18 (C-6), 147.25 (C-7), 112.37 (C-8), 138.63 (C-9), 133.65 (C-10), 100.51 (C-1'), 78.19 (C-2'), 72.63 (C-3'), 70.83 (C-4'), 70.21 (C-5'), 92.28 (C-1''), 79.82 (C-2''), 72.78 (C-3''), 71.26 (C-4''), 69.53 (C-5''), 60.84 (C-1'''), 47.52 (C-2'''), 30.69 (C-3'''), 30.65 (C-4'''), 29.55 (C-5'''), 29.48 (C-6'''), 27.53 (C-7'''), 25.26 (C-8'''), 22.68 (C-9'''), 14.40 (C-10'''), 56.44 (OMe), 53.18 (OMe). EI MS *m/z* (rel.int.): 608 $[M]^+$ ($C_{32}H_{48}O_{11}$) (4.1).

2.3.8. Tinolabdenyl flavanone (**8**)

Elution of the column with chloroform-methanol (9:1) gave yellow colored solid mass of **8**, 93 mg, UV λ_{max} (MeOH): 293, 347 nm; IR ν_{max} (KBr): 3442, 3225, 2931, 2848, 1735, 1697, 1673, 1635, 1513, 1412, 1315, 1190, 1094, 970 cm^{-1}. ^{1}H NMR (MeOD): δ 7.29 (1H, d, *J*=8.4 Hz, H-3'), 6.83 (1H, d, *J*=8.4 Hz, H-2'), 6.78 (1H, d, *J*=8.4 Hz, H-6'), 6.52 (1H, d, *J*=8.4 Hz, H-5'), 6.15 (1H, d, *J*=8.4 Hz, H-6), 5.89 (1H, d, *J*=2.0 Hz, H-8), 5.34 (1H, dd, *J*=12.8, 2.8 Hz, H-2), 3.10 (1H, dd, *J*=12.8, 2.8 Hz, H-3a), 2.67 (1H, dd, *J*=12.8, 2.8 Hz, H-3b), 3.88 (3H, s, OMe), 7.54 (1H, dd, *J*=2.8, 7.6 Hz, H-2''), 6.27 (1H, d, *J*=7.6 Hz, H-1''), 5.31 (1H, d, *J*=2.8 Hz, H-3''β), 5.28 (1H, ddd, *J*=4.8, 10.9, 2.8 Hz, H-7''α), 2.53 (1H, m, H-8''), 2.48 (1H, m, H-9''), 2.26 (1H, t, *J*=7.2 Hz, H-5α), 2.03 (1H, m, H_2-6''a), 1.75 (1H, m, H-13''), 1.71 (2H, m, H_2-11''), 1.59 (2H, m, H_2-12''), 1.44 (1H, m, H_2-6''b), 1.42 (2H, m, H_2-14''), 1.27 (3H, s, Me-17''), 1.19 (3H, s, Me-16''), 0.97 (3H, d, *J*=7.6 Hz, Me-20''), 0.91 (3H, t, *J*=6.6 Hz, Me-15''). ^{13}C NMR (MeOD): δ 80.48 (C-2), 44.03 (C-3), 197.79 (C-4), 164.88 (C-5), 97.04 (C-6), 168.33 (C-7), 96.16 (C-8), 159.02 (C-9), 103.37 (C-10), 129.03 (C-1'), 119.25 (C-2'), 115.83 (C-3'), 152.67 (C-4'), 116.33 (C-5'), 113.83 (C-6'), 125.27 (C-1''), 137.12 (C-2''), 75.39 (C-3''), 39.93 (C-4''), 35.46 (C-5''), 28.45 (C-6''), 72.38 (C-7''), 34.90 (C-8''), 45.50 (C-9''), 36.22 (C-10''), 27.01 (C-11''), 30.13 (C-12''), 38.58 (C-13''), 30.74 (C-14''), 18.58 (C-15''), 28.01 (C-16''), 28.04 (C-17''), 177.16 (C-18''), 169.99 (C-19''), 24.60 (C-20''), 56.41 (OMe). EI MS *m/z* (rel.int.): 618 $[M]^+$ ($C_{36}H_{42}O_9$) (2.1).

3. Results and Discussion

Compound **1** had IR absorption bands for hydroxyl group (3365 cm^{-1}) and long chain aliphatic hydrocarbon (728 cm^{-1}). Its mass spectrum showed a molecular ion peak at *m/z* 242 corresponding to a molecular formula of cetyl alcohol, $C_{16}H_{34}O$. The ^{1}H NMR spectrum of **1** exhibited a two-proton triplet at δ 3.45 (*J*=6.6 Hz) assigned to hydroxymethylene H_2-1 proton. The other methylene protons resonated between 2.19- 1.17. A three-proton triplet at δ 0.87 (*J*=7.5 Hz), was ascribed to primary C-16 methyl protons. The ^{13}C NMR of **1** displayed signals for hydroxylmethylene carbon at δ 61.33 (C-1), other methylene carbons between δ 33.43-21.83 and methyl carbon at δ 13.34 (C-16). The absence of any proton singlet beyond δ 3.45 and carbon signal after δ 61.33 in the down field region suggested saturated nature of the molecule. On the basis of above evidences the structure of **1** has been elucidated as 1-hexadecanol (Fig. 3).

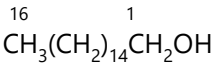

Fig. 3. Cetyl alcohol (**1**).

Compound **2** showed IR absorption bands for amide group (3310, 1687 cm^{-1}) and aromatic ring (1635, 1527, 1042 cm^{-1}). On the basis mass and ^{13}C NMR spectra the molecular ion peak of **2** was determined at *m/z* 387 consistent to a molecular formula of an aromatic alkyl amide, ($C_{26}H_{29}NO_2$). Its ^1H NMR spectrum exhibited four one-proton doublets at δ 7.43 (*J*=10.6 Hz), 6.83 (*J*=8.4 Hz), 7.38 (*J*=1.6 Hz) and 7.18 (*J*=1.6 Hz) assigned to aromatic ortho-coupled H-5 and H-4 and meta-coupled H-8 and H-1 protons, respectively. Two one-proton double doublets at δ 7.06 (*J*=1.6, 10.6 Hz) and 6.76 (*J*=1.6, 8.4 Hz) were ascribed to ortho-meta coupled H-6 and H-3 protons. Other aromatic protons appeared as one-proton multiplets at δ 7.03 (H-2''), 7.01 (H-3''), 6.94 (H-5''), 6.72 (H-4'') and 6.65 (H-6''). Two one-proton doublets at δ 6.40 and 5.85 with coupling interactions of 15.6 Hz each were accounted to *trans*-oriented vinylic H-7'' and H-8'' protons, respectively. The methylene protons appeared as two-proton multiplets between 3.49-1.31. A three-proton singlet at δ 3.91 was due to methoxy protons.

The ^{13}C NMR spectrum of **2** displayed signals for amide carbon at 167.78 (C-9''), aromatic and vinylic carbons from 155.54 to 110.19, methylene carbons between δ 41.13-29.05 and methoxy carbon at δ 55.01. The DEPT spectrum of **2** exhibited the presence of one methoxy, six methylene, thirteen methine and six quaternary carbons. The ^1H-^1H COSY spectrum of **2** showed interactions of H-1 with H-3, H-8 and H_2-6'; H-6 with H-5, H-8 and OMe; H_2-1' with H_2-2' and H_2-3'; and H-7'' with H-8'', H-2'' and H-6''. The HMBC spectrum of **2** exhibited corrections of H-1, H-3 and H_2-6' with C-2; H-6, H-8 and OMe with C-7; H_2-2' with C-1'; H-8'' with C-9''; and H-2'', H-6'' and H-7'' with C-1''. The HSQC spectrum of **2** displayed correlation of aromatic protons with their respective carbon signals; vinylic protons at δ 6.40 (H-7'') and 5.85 (H-8'') with the carbon signals at δ 117.38 (C-7'') and 120.28 (C-8'') respectively; and methylene proton signals with the corresponding carbon signals. These evidences led to establish the structure of **2** as *trans*-cinnamoyl-2-*n*-hexanyl-7-methoxy-naphthyl amide, a new aromatic amide (Fig. 4).

Compound **3**, $[M]^+$ at *m/z* 403 ($C_{26}H_{29}NO_3$), showed IR absorption bands for amide group (3315, 1685 cm^{-1}) and aromatic ring (1638, 1531, 1038 cm^{-1}). Its ^1H NMR spectrum exhibited aromatic protons from δ 7.37 to 6.62, *trans*-oriented vinylic protons as doublets at δ

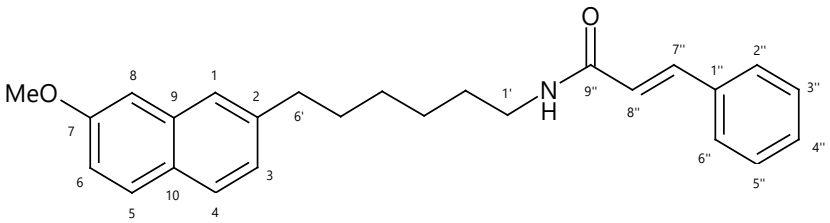

Fig. 4. *trans*-Cinnamoyl-2-*n*-hexanyl-7-methoxynaphthyl amide (**2**).

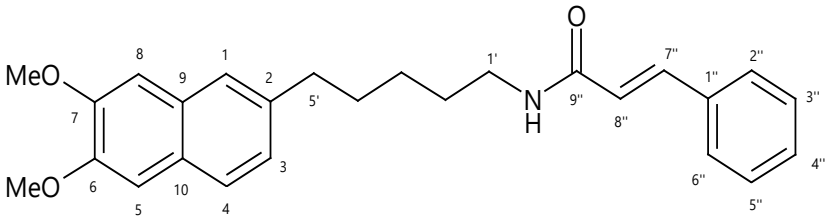

Fig. 5. *trans*-Cinnamoyl-2-*n*-pentanyl-6,7-dimethoxynaphthyl amide (**3**).

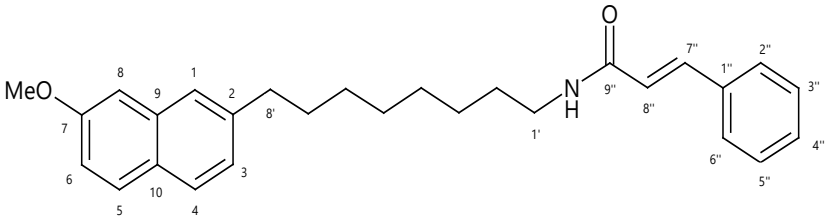

Fig. 6. *trans*-Cinnamoyl-2-*n*-octanyl-7-methoxynaphthyl amide (**4**).

6.34 (*J*=16.0 Hz, H-7'') and 5.75 (*J*=16.0 Hz, H-8''), two methoxy proton signals as three-proton singlets at δ 3.80 and 3.74 and methylene proton signals between δ 3.38-1.20. The ^{13}C NMR spectrum of **3** displayed the presence of twenty six carbons including amide carbon at δ 169.20 (C-9''), aromatic and vinylic carbons in the range of 159.81 to 111.61, methoxy carbons at δ 56.43 and 56.41 and methylene carbons from δ 48.30 to 42.50. The DEPT spectrum of **3** showed the existence of two methoxy, five methylene, and twelve methine carbons in the molecule. The ^{1}H-^{1}H COSY spectrum of **3** showed correlations of methoxy protons with H-5 and H-8; H-1 and H-3 with H_2-5'; and H-7'' with H-2'', H-6'' and H-8''. The HMBC spectrum of **3** exhibited interactions of H-1, H-3 and H_2-5' with C-2; H-5 and H-8 with C-6; H-2'', H-6'' and H-7'' with C-1''; and H-8'' with C-9''. The HSQC spectrum of **3** displayed correlations of aromatic and vinylic proton signals with their respective carbons. On the basis of this discussion the structure of **3** has been elucidated as *trans*-cinnamoyl 2-*n*-pentanyl-6,7-dimethoxynaphthyl amide (Fig. 5), a new aromatic amide derivative of **2**.

Compound **4**, a derivative of **2**, had $[M]^+$ at *m/z* 415 ($C_{28}H_{33}O_2N$) and showed IR absorption bands for amide group (3318, 1689 cm^{-1}), unsaturation (1641 cm^{-1}) and aromatic ring (1525, 1047 cm^{-1}). Its ^{1}H NMR spectrum exhibited aromatic proton signals from δ 7.47 to 6.67, *trans*-vinylic protons at δ 6.44 (*J*=15.6 Hz) and 5.85 (*J*=15.6 Hz), methylene protons between δ 3.50-1.29 and methoxy protons at δ 3.90. The ^{13}C NMR spectrum of **4** displayed aromatic and vinylic carbons in the range of 158.11 to 110.20, methylene carbons from δ 41.07-29.35, amide carbon at δ 167.80 (C-9'') and methoxy carbon at δ 55.01. The DEPT spectrum of **4** showed the presence of one methoxy, eight methylene and thirteen methine carbons in a molecule. The ^{1}H-^{1}H COSY spectrum of **4** exhibited interactions of H-8 with H-6 and OMe; H-1 with H-3 and H_2-8'; and H-7'' with H-8'', H-1'' and H-6''. The HMBC spectrum of **4** indicated that H-6, H-8 and OMe interacted with C-7; H-1, H-3 and H_2-8' interacted with C-2; and H-2'', H-6'' and H-7'' interacted with C-1''. The HSQC spectrum of **4** showed correlations of aromatic, vinylic and methylene protons signals with their respective carbons. These evidences led to establish the structure of **4** as *trans*-cinnamoyl-2-*n*-octanyl-7-methoxy-naphthyl amide (Fig. 6), a new naphthyl amide derivative.

Compound **5** gave positive tests for glycosides and showed IR absorption bands for hydroxyl groups (3368, 3285 cm^{-1}) and ester function (1721 cm^{-1}). Its molecular ion peak was established at *m/z* 304 on the basis of mass and ^{13}C NMR spectral data corresponding to a molecular formula of a monoterpenic glycoside, ($C_{15}H_{28}O_6$). The ion peaks generating at *m/z* 149 $[C_5H_9O_5]^+$ and 133 $[C_5H_9O_4]^+$ suggested that a pentose sugar was linked to an aglycone monoterpene. Its ^{1}H NMR spectrum exhibited a one-proton doublet at δ 4.95 (*J*=7.3 Hz) assigned to anomeric H-1 proton. The other sugar protons appeared as one-proton multiplets at δ 4.21 (H-2), 3.96 (H-3) and 3.53 (H-4) and as a two-proton doublet at δ 3.85 due to oxymethylene protons. Two three-proton doublets at δ 1.21 (*J*=6.3 Hz) and 1.18 (*J*=6.5 Hz) and a three-proton triplet at δ 0.87 (*J*=6.5 Hz) were ascribed to secondary C-8′ and C-9′ and primary C-1′ methyl protons, respectively. The remaining methine and methylene protons appeared from δ 2.50 to 1.25. The ^{13}C NMR spectrum of **5** displayed signals for the amide carbon at δ 172.52 (C-10′), anomeric carbon at δ 107.16 (C-1), other sugar carbons between δ 85.07-63.55 and methyl carbons at δ 20.15 (C-8′), 17.86 (C-9′) and 14.28 (C-1′). Acid hydrolysis of **5** yielded D-arabinose, R_f 0.18 (*n*-butanol-acetic-water, 4:1:5). The data led to formulate the structure of **5** as β-D-arabinosyl-*O*-geranilan-10′-oate, a new acyclic monoterpenic arabinoside (Fig. 7).

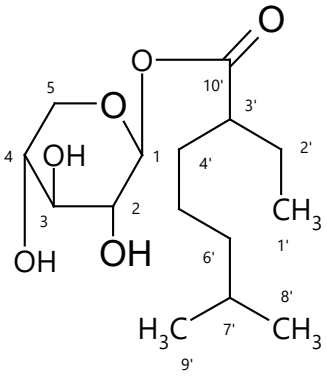

Fig. 7. β-D-Arabinosyl-*O*-geranilan-10′-oate (**5**).

Compound **6** gave positive tests for glycosides and showed UV absorption maxima for aromaticity at 275 nm and IR absorption bands for hydroxyl groups (3415, 3227 cm^{-1}) and aromatic ring (1645, 1525, 1072 cm^{-1}). Its molecular ion peak was established at *m/z* 568 on the basis of mass and ^{13}C NMR consistent with a molecular formula of a naphthyl diglucoside pentane, $C_{28}H_{40}O_{12}$. The ion fragments generating at *m/z* 203 $[C_5H_8O_4\text{-}(CH_2)_4\text{-}Me]^+$ and 335 $[C_5H_8O_4\text{-}C_5H_8O_4\text{-}(CH_2)_4\text{-}Me]^+$ and 233 [M-335, $C_{10}H_4(OMe)_3\text{-}O]^+$ indicated that trimethoxynaphthol was linked with an alkylated dipentose unit. The ^{1}H NMR spectrum of **6** exhibited four one-proton doublets at δ 6.87 and 6.72 with coupling interactions of 2.0 Hz each and at δ 6.83 (*J*=1.6 Hz) and 6.69 (*J*=1.6 Hz) assigned to *meta*-coupled aromatic H-8, H-6, H-1 and H-3 protons, respectively. Two one-proton at δ 6.58 (*J*=4.4 Hz) and 4.69 (*J*=4.0 Hz) were ascribed correspondingly to α-oriented anomeric H-1′ (furanic) and H-1′′ protons, other sugar protons appeared between δ 4.62-3.84, three singlets at 3.78, 3.77 and 3.76 integrating for three-protons each were due to methoxy protons, oxymethylene H_2-1′′′ protons at δ 3.66, other

methylene protons resonated as multiplets at δ 1.92, 1.23 and 1.21 and a three-proton triplet at δ 0.83 (*J*=6.3 Hz) was accounted to primary C-5‴ methyl protons. The ^{13}C NMR spectrum of **6** displayed signals for aromatic carbons from δ 154.65 to 116.01, anomeric carbons at δ 104.25 (C-1′) and 97.75 (C-1″), other sugar carbons from δ 87.45 to 70.90, oxymethylene carbon at δ 61.11 (C-1‴), other methylene carbons in the range of δ 47.54 - 29.53 and methyl carbon at δ 18.13 (C-5‴). The ^{1}H-^{1}H COSY spectrum of **6** exhibited interactions of H-1′ with H-2′, H-1 and H-3; H-4′ with H-1′, H-3′ and H_2-5'; H-1″ with H-2′, H-2″ and H_2-5″; and H_2-1‴ with H-2'' and H_2-2‴. The presence of H-2′ (δ 4.62) and H-2″ (δ 4.52) in the deshielded region and carbon C-2′ and C-2″ signals at δ 87.28 suggested (2′→1″) linkage of sugar units and attachment of the alkyl group at C-2″. The HMBC spectrum of **6** showed interactions of H-1, H-3 and H-1′ with C-2′; H-2′, H-2″, H_2-5″ and H_2-1‴ with C-2″; and H-6 and H-8 with C-7. The HSQC spectrum of **6** indicated that aromatic protons from δ 6.87 to 6.69 interacted their respective carbon signals; anomeric proton signals at δ 6.58 (H-1′) and 4.69 (H-1″) interacted with the carbon signals at δ 104.25 (C-1′) and δ 97.75 (C-1″); and oxymethylene proton signals at δ 3.66 (H_2-1‴) interacted with the carbon signal at δ 61.11 (C-1‴). On the basis of these data the structure of **6** has been established as 4,5,7-trimethoxy-2-naphthol-2-*O*-α-L-arabinofuranosyl-(2′→1″)-*O*-α-L-arabinopyranosyl-2″-*O*-pentane, a new naphthyl diarabinosyl pentane (Fig. 8).

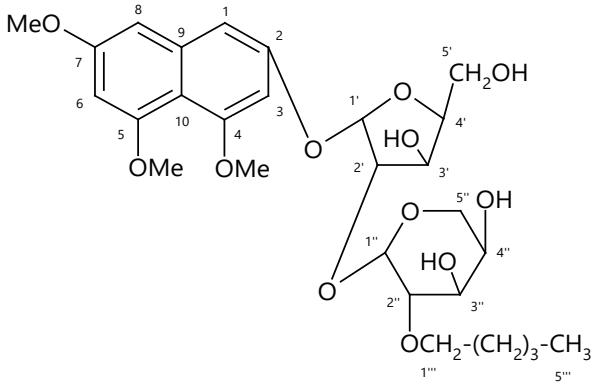

Fig. 8. 4,5,7-Trimethoxy-2-naphthol-2-*O*-α-L-arabinofuranosyl-(2′→1″)-*O*-α-L-arabinopyranosyl-2″-*O*-pentane (**6**).

Compound **7**, $[M]^+$ at *m/z* 608, was analyzed for ($C_{32}H_{48}O_{11}$). The structural feature of **7** showed resemblance to compound **6** except to existence of two methoxy groups in the naphthol and *n*-decanyl chain. The ^{1}H NMR spectrum of **7** displayed two anomeric signals as one-proton doublets at δ 5.28 (*J*=5.2 Hz, H-1') and 5.25 (*J*=4.4 Hz, H-1'') indicating pyranose forms of the both the sugars linked in α-orientation, other sugar protons between δ 4.98-3.55, four one-proton doublets at δ 6.82, 6.70 and 6.24 with coupling interactions of 2.0 Hz each and at δ 6.13 (*J*=7.7 Hz) ascribed to *meta*-coupled H-8, H-1 and H-6 and ortho-coupled H-4 protons, respectively, and a one-proton double doublet at δ 6.40 (*J*=2.0, 7.6 Hz) accounted to *meta*-, *ortho*-coupled H-3 proton, a two-proton triplet at δ 3.50 (*J*=6.8 Hz) ascribed to oxymethylene H_2-1‴ protons, other methylene protons between δ 2.32- 1.16, a three-proton triplet at δ 0.83 (*J*=7.2 Hz) due to primary C-10‴ methyl protons and methoxy protons as three-proton singlets at δ 3.67 and 3.46. The ^{13}C NMR spectrum of **7** exhibited three aromatic deshielded signals at δ 154.61 (C-2), 151.28 (C-5) and 147.25 (C-7) due to the presence of phenolic hydroxyl group, anomeric carbon signals at δ 100.51 (C-1') and 92.28 (C-1''), an oxymethylene carbon signal at δ 60.84 (C-1'''), other methylene carbons from δ 47.52 to 22 .68 and methyl carbon at δ 14.40 (C-10'''). The ^{1}H-^{1}H COSY spectrum of **7** exhibited correlations of H-3 with H-1, H-4, H-1'; H-2' with H-1', H-3' and H-1''; and H_2-1‴ with H-2'' and H_2-2‴. The HMBC spectrum of **7** showed interactions of H-1, H-3, and H-1' with C-2; H-2', H-2'' and H-3'' with C-1‴; and H_2-1‴ with C-2''. The HSQC spectrum of **7** exhibited correlations of aromatic proton with the respective carbon signals, anomeric protons at δ 5.28 (H-1') and 5.25 (H-1'') with the respective carbon signals at δ 100.51 (C-1') and 92.28 (C-1'') and oxymethylene proton signal at δ 3.50 (H_2-1‴) with the carbon signal at δ 60.84 (C-1‴). On the basis of above spectral studies the structure of **7** was elucidated as 5,7-dimethoxy-2-naphthol-2-*O*-α-L-arabinopyranosyl-(2′→1″)-α-L-arabinopyranosyl-2″-*O*-decane, a new naphthyl diarabinosyl decane (Fig. 9).

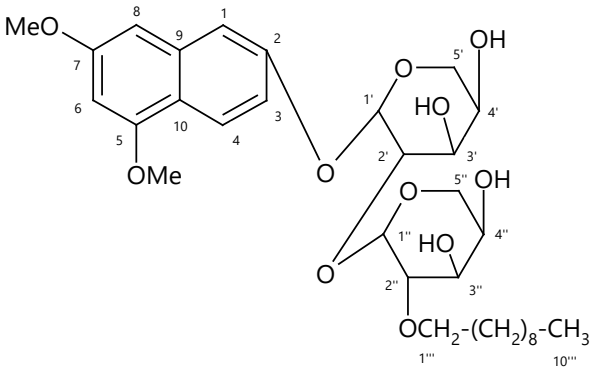

Fig. 9. 5,7-Dimethoxy-2-naphthol-2-*O*-α-L-arabinopyranosyl-(2′→1″)-α-L-arabinopyranosyl-2″-*O*-decane (**7**).

Compound **8**, named tinolabdenyl flavanone, had UV absorption maxima at 293, 347 nm for flavanone and showed IR absorption bands for lactone ring (1735 cm^{-1}), hydroxyl group (3442 cm^{-1}), carboxylic function (3225, 1697 cm^{-1}) and carbonyl group (1673 cm^{-1}). The absence of a shift with sodium acetate suggested bound nature of the C-7 hydroxyl bond. There was a shift of 25 nm of band II on addition of aluminum chloride solution indicating free C-5 hydroxyl group (Markham, 1982). Its molecular ion

peak was determined at *m/z* 618 on the basis of mass and ^{13}C NMR spectra consistent with the molecular formula of a diterpenic substituted with flavanone, $C_{36}H_{42}O_9$. The ^1H NMR spectrum of **8** exhibited three signals in non aromatic region placed as a one-proton double doublet at δ 5.34 (*J*=12.8 Hz, 2.8 Hz) due to vicinal coupling at H-2 proton separately with the axial and equatorial protons at position H_2-3, two one-proton double doublets at δ 3.10 (*J*=12.8 Hz, 2.8 Hz) and 2.67 (*J*=12.8 Hz, 2.8 Hz) characteristic of H_2-3 axial and H_2-3 equatorial, respectively, of flavanone moiety. Four one-proton doublets at δ 7.29 and 6.83 with coupling interactions of 8.4 Hz each and at δ 6.78 and 6.52 with *J* value of 6.8 Hz were ascribed to ortho-coupled H-3', H-2', H-6' and H-5' respectively. The meta-coupled H-6 and H-8 appeared as one proton doublets at δ 6.15 (*J*=2.0 Hz) and 5.89 (*J*=2.0 Hz), respectively. A one-proton double doublet at δ 7.54 (*J*=2.8, 7.6 Hz) and a doublet at δ 6.27 (1H, *J*=7.6 Hz) were accounted to *cis*-oriented vinylic H-2'' and H-1'' protons, respectively. A one-proton doublet at δ 5.31 (*J*=2.8 Hz) interacting with the vinylic H-2'' and a triple doublet at δ 5.28 (*J*=4.8, 10.9, 2.8 Hz) interacting with H_2-6 ax and H_2-6 eq and H-8 eq were accounted to H-3 ax and H-7 eq oxymethine protons, respectively. Four three-proton signals as singlets at δ 1.27 and 1.19, as a doublet at δ 0.97 (*J*=7.6 Hz) and as a triplet at δ 0.91 (*J*=6.6 Hz) were associated correspondingly with the tertiary C-17'' and C-16'', secondary C-20'' and primary C-15'' methyl protons. The remaining methine and methylene resonated from δ 2.53 to 1.42. The ^{13}C NMR spectrum of **8** displayed signals for lactone carbon at δ 169.99 (C-19''), carboxylic carbon at δ 177.16 (C-18''), carbonyl carbon at δ 197.79 (C-4), oxymethine carbons at δ 80.48 (C-2), 75.39 (C-3'') and 72.38 (C-7''), flavanonyl methylene at δ 44.03 (C-3) and methyl carbon at δ 18.58 (C-15''), 28.01 (C-16''), 28.04 (C-17'') and 24.60 (C-20''). The DEPT spectrum of **8** displayed the presence of five each of methyl and methylene, fifteen methine and eleven quaternary carbons. In ^1H-^1H COSY spectrum of **8** indicated that H-2 correlated with H_2-3, H-2' and H-6'; H-6 correlated with H-8; H-3'' correlated with H-1'', H-2'' and, Me-16 ''; and H-7'' correlated with H_2-6'' and H-8''. The HMBC spectrum of **8** exhibited correlations of H-2', H-3' and H-2 with C-1'; H-6, H-8 and H-7'' with C-7; and H-1'', H-2'' and Me-16'' with C-3''. The HSQC spectrum of **8** showed interaction of vinylic proton at δ 7.54 (H-2'') and 6.27 (C-1'') with their respective carbon signals at δ 137.72 (C-2'') and 125.27 (C-1''), aromatic proton signals with their corresponding carbons and oxymethine proton signals at δ 5.34 (H-2), 5.31 (H-3'') and 5.28 (H-7'') with the carbon signals 80.48 (C-2), 75.39 (C-3'') and 72.38 (C-7'') respectively. On the basis of these data, compound **8** was characterized as 5-hydroxy-4'-methoxy-7-flavanoxy-(7→7'')-β-*O*-labdan-1-en-3''α,19''-olide-18''-oic acid. It is a new diterpenic flavanone (Fig. 10).

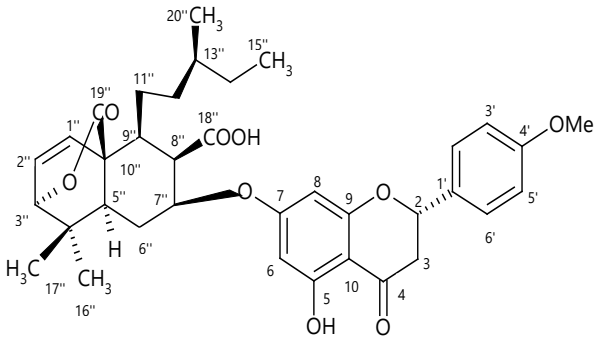

Fig. 10. 5-Hydroxy-4'-methoxy-7-flavanoxy-(7→7'')-β-*O*-labdan-1-en-3''α,19''-olide-18''-oic acid (**8**).

4. Concluding remarks

Phytochemical investigation of a methanolic extracts of the stem of *Tinospora cordifolia* led to the isolation of assorted nature of secondary metabolites as cetyl alcohol, three cinnamoyl naphthyl amides, geranilanoate arabinoside, naphthol diarabinosyl alkanes and a labdenyl flavanone. The presence of these crucially unmatched phytoconstituents and previously studied ones showed much enriched nature of this plant which enhances the understanding of therapeutic and traditional utility since ancient times. These secondary metabolites can also be used as analytical markers for quality control of the stem bark of this plant.

Conflict of interest

The authors declare that there is no conflict of interest.

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