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Calliphyllin, a New Diterpene from the Leaves of *Callicarpa macrophylla*#1

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A new diterpene designated calliphyllin, betulinic acid, 5,4′-dihydroxy-3,7,3′-trimethoxyflavone, 5,4′-dihydroxy-3,7-dimethoxyflavone and β-sitosterol have been isolated from the leaves of *Callicarpa macrophylla* Vahl. (Verbenaceae). Calliphyllin has been shown to possess 14α-hydroxy-isopimaric acid (1) structure on the basis of nmr and mass spectral and chemical evidence.

The genus *Callicarpa* (Verbenaceae)[2] comprises nearly 30 species, occurring mostly in East Asia, Malayasia and North Australia, a few in Polynesia, Colombia and West Indies. *Callicarpa macrophylla* Vahl. grows throughout North and East India, ascending to 6000 ft in the West Himalayas from Kashmir to Assam. It also grows abundantly in West Bengal and Bangladesh. It finds uses as folk medicine[3]. An aromatic oil extracted from its roots is used in the remedy of the disordered stomach. Hot extract of the leaves of *C. macrophylla* is known to be useful when applied to rheumatic joints[3].

Earlier studies on the leaves of *C. macrophylla* showed the presence of L(+)-α-amino-β-(*p*-methoxyphenyl)-propionic acid[4], calliterpenone and its monoacetate[5-7], β-sitosterol[7], β-sitosterol-β-D-glucoside[7], ursolic acid[7], 2α-hydroxyursolic acid[7], crategolic acid[7], 7-*O*-glucuronides of luteolin and apigenin[8], and 5,4′-dihydroxy-3,7,3′-trimethoxyflavone[9]. We now report the isolation, structure and stereochemistry of a new hydroxyditerpene acid, designated calliphyllin (**1**) and also the isolation of betulinic acid, 5,4′-dihydroxy-3,7,3′-trimethoxyflavone, 5,4′-dihydroxy-3,7-dimethoxyflavone and β-sitosterol from the leaves of *C. macrophylla*.

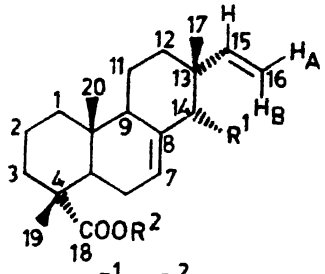
Results and Discussions

Chromatography of the crude petrol extract of the leaves of *C. macrophylla* on silica gel led to the isolation of β-sitosterol, betulinic acid, 5,4′-dihydroxy-3,7,3′-trimethoxyflavone, 5,4′-dihydroxy-3,7-dimethoxyflavone and calliphyllin arranged in order of increasing polarity.

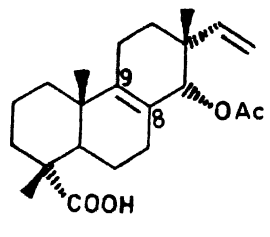
Calliphyllin (**1**) was crystallised from chloroform-petrol as colourless needles, m.p. 157°, $[\alpha]_D$ −19.8° ($CHCl_3$), $C_{20}H_{30}O_3$ (M^+ at *m/z* 318). To obtain structural information on this diterpene attention was forcussed on its spectral properties. Its ir spectrum showed the presence of a hydroxyl group (3 480 cm^{-1}) and a carbonyl group (1 710, 1 690 cm^{-1}). An interesting feature is that calliphyllin (**1**) showed its carboxylic carbonyl peak as a doublet (1 710 and 1 690 cm^{-1}) of equal intensity when taken in a KBr pellet, but as a singlet (1 685 cm^{-1}) in its chloroform solution. This is indicative of the presence of intermolecular hydrogen bonding in the crystal lattice of calliphyllin which is arrested in its solution. A number of monohydroxy acids are known in the literature with similar basic skeleton but occurrence of such doublet carbonyl peak in KBr pellet due to intermolecular hydrogen bonding is not reported so far.

#Part 32 of the series 'Terpenoids and Related Compounds; for Part 31 see Ref, 1. Presented in part at the 14th National Symposium on Organic Chemistry (NASOC-XIV), Calcutta, 1992, Abstract no. CP. 18.

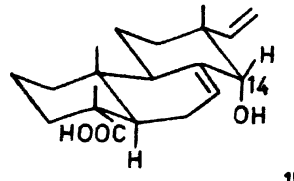
Its ^1H nmr spectrum (300 MHz; $CDCl_3$) indicated the presence of (i) three tertiary methyls on saturated carbons : singlets at δ 0.87, 0.88 and 1.25, (ii) a vinyl group attached to a quaternary carbon (δ 5.10, 1H, dd, *J* 17.6 and 0.6 Hz; 5.14, 1H, dd, *J* 10.4 and 0.6 Hz; 5.88, 1H, dd, *J* 17.6 and 10.4 Hz), (iii) an olefinic proton (δ 5.68, 1H, $W_{1/2}$ 11 Hz), (iv) and an oxymethine proton (δ 3.68, 1H, s). The foregoing evidence suggested that calliphyllin possessed pimaradiene[10] type structure.

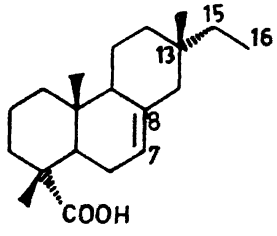


	R^1	R^2
1	OH	H
2	H	H
3	OH	Me

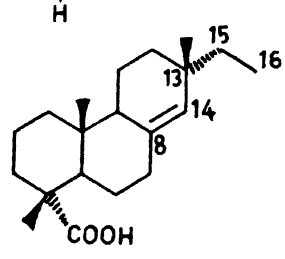


4





5



6

Calliphyllin formed an acetate (**4**) in which the secondary oxymethine proton suffered deshielding by 1.2 ppm (δ 4.93, 1H, s) and the acetate methyl appeared at δ 1.96 (3H, s). Interestingly, under the condition of acetylation the 7,8-double bond of **1** suffered isomerisation to 8,9-position as evident from the absence of the olefinic proton signal in its ^1H nmr spectrum, present initially in that of the parent compound (**1**). On treatment with diazomethane, **1** formed the corrresponding ester (**3**) which was well characterised on the basis of ^1H and ^{13}C nmr spectral studies (vide experimental).

The ^{13}C natural abundance spectrum (75 MHz; $CDCl_3$) of **1** showed essentially the carbon chemical shifts as those of isopimaric acid[11] (**2**) (Table 1), except for C-9, C-12 and C-14 The assignments of the carbon chemical shifts were based on ^{13}C APT and DEPT spectra, comparison with the ^{13}C nmr spectral data on compound **2** and chemical shift changes expected for a few carbons on introduction of the hydroxyl group in the cyclic hydrocarbon skeleton. The location of the only hydroxyl group was straight forward. Its secondary nature (evident both from the ^{13}C and the foregoing ^1H nmr spectral data) as well as appearance of the oxymethine proton as a sharp singlet allowed its placement at the only available position C-14 which is flanked by two adjacent tetrasubstituted carbons. Comparison of the ^{13}C nmr spectral data (Table 1) with isopimaric acid (**2**) showed the γ-gauche shielding effect of the OH at C-14 felt at C-12 and C-9, the magnitude of the upfield shift of these carbons was indicative of the α- and axial orientation of C-14 hydroxyl (conformation 1′). The ^{13}C shifts of the methyl ester (**3**) were also assigned by comparison (Table 1).

Further, in its mass spectrum, the base peak appeared at *m/z* 301 (M^+–OH) due to facile elimination of an allylic axial hydroxyl group present in the molecule. The mass fragmentation pattern (Chart 1) was fully consistent with the assigned structure **1** for calliphyllin.

Calliphyllin (**1**) upon hydrogenation (H_2, Pd–C) (10%)) followed by usual work-up and chromatography yielded a white uncrystallisable amorphous solid showing only one spot in tlc (R_f 0.5; $CHCl_3$–MeOH, 9 : 1) which was shown to be a mixture of dihydroisodextropimaric acid (**5**) and dihydrosandaracopimaric acid (**6**) in the ratio 3 : 1 as evident from the intensity ratio of the olefinic protons of these two products in the ^1H nmr spectrum of the mixture. Its ^{13}C nmr spectrum also showed it to be a mixture of **5** and **6**. The 15,16-double bond

of the vinylic group underwent reduction to form an ethyl group in each product. The product **5** was formed by the hydrogenolysis of the 14-OH group through the intermediacy of the allylic C-14 radical. The latter simultaneously underwent isomerisation to a small extent to form the C-7 allylic radical which collapsed by hydrogen capture to form **6**.

TABLE 1–^{13}C CHEMICAL SHIFTS* (δ, $CDCl_3$) OF THE DITERPENES CALLIPHYLLIN (**1**), ISOPIMARIC ACID (**2**) AND CALLIPHYLLIN METHYL ESTER (**3**)

Carbon	1[a]	2[b]	3[a]
1	38.6	39.2	38.6
2	17.9	18.3	17.9
3	36.8	37.2	36.8
4	46.1	46.4	46.4
5	44.4	45.4	44.8
6	25.1	25.7	25.1
7	127.1	121.5	126.6
8	136.9	136.0	137.6
9	46.9	52.4	46.9
10	34.7	35.5	34.8
11	19.2	20.5	19.1
12	27.3	36.0	27.6
13	40.9	37.5	40.8
14	79.3	46.5	79.5
15	146.3	150.7	146.5
16	113.7	108.7	113.0
17	22.0	21.9	21.8
18	183.9	183.5	178.7
19	17.1	17.5	17.3
20	15.0	15.7	14.9
OCH_3	–	–	51.7

*Values are in ppm downfield from TMS used as an internal standard in $CDCl_3$ solution; δ ($CDCl_3$) = 76.9 ppm.
[a]Measured in a Brucker AM 300L FT spectrometer at 75.0 MHz.
[b]Measured[11] at 15.077 MHz on a FT spectrometer.

The mixture upon repeated chromatography over silica gel followed by recrystallisation from chloroform–petrol afforded pure dihydroisodextropimaric acid (**5**) in a small amount, m.p. 171–73°, $[\alpha]_D$–4.5° (lit. 173–75°, $[\alpha]_D$–5°]. The ir spectrum of **5** indicated the presence of a carboxylic acid group (1 700 cm^{-1}) and a trisubstitued double bond (825 cm^{-1}). Its ^{1}H nmr spectrum revealed the presence of an olefinic proton (δ 5.27, 1H, bs), three tertiary methyls

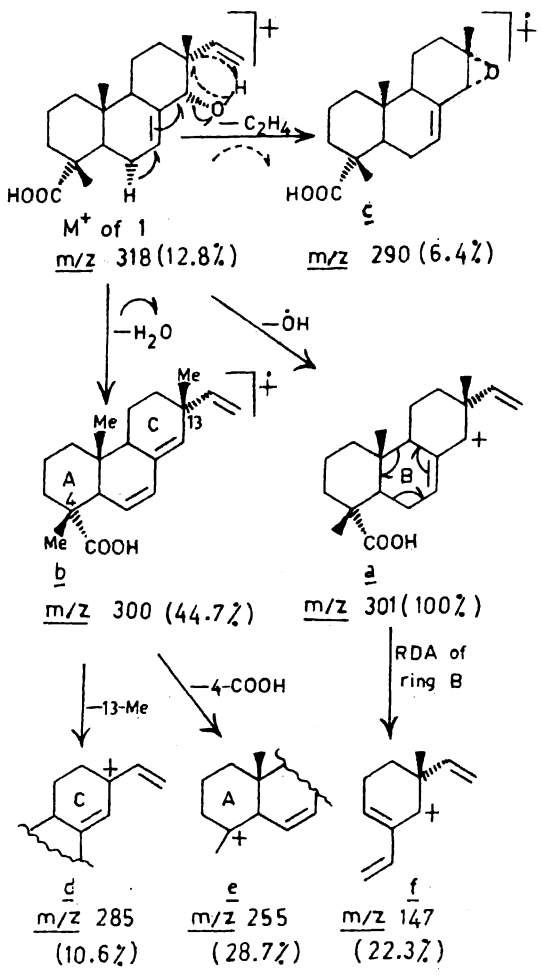


Chart 1

(δ 1.26, 0.89 and 0.70) and an ethyl group (δ 0.81, 3H, t; 1.18, 2H, m) and showed the absence of the isomer **6**.

Thus the formation of dihydroisodextropimaric acid (**5**) which was derived from isodextropimaric acid, a natural product of known absolute configuration established the structure as well as the stereochemistry of all the chiral centres excepting C-14 of calliphyllin (**1**).

β-Sitosterol[13], betulinic acid[14], 5,4′-dihydroxy-3,7,3′-trimethoxyflavone[9,15] and 5,4′-dihydroxy-3,7-dimethoxyflavone[15,16] were isolated from other fractions of the silica gel column chromatography of the petrol extract of the plant material.

Experimental

M.ps. are uncorrected. Ir spectra were measured in KBr pellets and uv spectra in 95% aldehyde-free ethanol. ^1H nmr spectra were run at 300 MHz and ^{13}C nmr at 75 MHz, both in $CDCl_3$ using TMS as the internal standard. Mass spectra were recorded in a mass spectrometer having a direct inlet system and operating at 70 eV; figures within parenthesis after *m/z* values represent relative intensities of peaks. For column chromatography, silica gel (B.D.H., 60–120 mesh) was used; tlc : microscopic slides coated with silica gel-G (Qualigens) fractions with similar tlc behaviour were combined; spots were visualised in uv light and on exposure to I_2 vapour. Petrol used had b.p. 60–80°. Analytical samples were routinely dried over P_2O_5 for 24 h under reduced pressure. Anhydrous Na_2SO_4 was used for drying organic solvents. The plant material was collected in the neighbourhood of Calcutta.

Extraction : Air-dried and milled leaves of *C. macrophylla* Vahl. (2.5 kg) were extracted in a Soxhlet apparatus with petrol and $CHCl_3$ successively for 40 h each. The extracts were concentrated under reduced pressure and separately chromatographed over silica gel, elution being carried out with solvents and solvent mixtures of increasing polarity.

Treatment of the petrol extract :

Isolation of β-sitosterol : The petrol extract concentrate (18 g) was subjected to column chromatography (cc) over silica gel (340 g) fractions of 400 ml each being collected. The residue obtained from the combined petrol-EtOAc (19 : 1) eluate fractions upon repeated cc over silica gel afforded β-sitosterol crystallising from petrol as colourless needles (0.0012%), m.p. 137°, $[\alpha]_D$–37° ($CHCl_3$); acetate (Ac_2O/Py, room temp., 20 h), m.p. 134°, $[\alpha]_D$–30.5° ($CHCl_3$). The identity was confirmed in each case by direct comparison (m.m.p. –no depression, superposable ir) with an authentic sample.

Isolation of betulinic acid : The petrol–EtOAc (9 : 1) eluate fractions from the main cc afforded a solid residue which upon repeated cc over silica gel followed by crystallisation from $CHCl_3$–MeOH furnished betulinic acid in needles (0.001%), m.p. 278–80°; R_f 0.55, $CHCl_3$–MeOH (9 : 1); $[\alpha]_D$ + 11° (MeOH, *c* 0.08); ν_{max} (KBr) 3 440 (OH), 1 690 (C=O), 1 590, 1 450, 1 235, 985, 885 cm^{-1}. The identity was confirmed by direct comparison with an authentic sample.

Isolation of 5,4′-dihydroxy-3,7,3′-trimethoxyflavone and 5,4′-dihydroxy-3,7-dimethoxyflavone : The yellowish gummy solid residue obtained from the earlier eight petrol–EtOAc (4 : 1) eluate fractions of the main cc showing identical spots in tlc were combined and subjected to further chromatography over silica gel. Petrol–ethyl acetate (17 : 3) eluate fractions of this chromatogram afforded a light yellow solid having two well-separated spots in tlc (R_f 0.6 and 0.5; silica gel-G; $CHCl_3$–MeOH, 19 : 1). The compounds were separated by preparative tlc and finally purified by crystillisation. The compound (R_f 0.6; silica gel-G; $CHCl_3$–MeOH, 19 : 1) crystallising from chloroform–petrol as a yellow microcrystalline solid, was identified as 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (0.0004%), m.p. 166–67°; λ_{max} (EtOH) 361 (log ε 4.40), 256 (4.40), 206 nm (4.69); λ_{max} (EtOH + $AlCl_3$) 361 (log ε 4.23), 268 (4.32), 209 nm (4.62); λ_{max} (EtOH + NaOAc) 358 (log ε 4.34), 256 (4.36), 206 nm (4.66); ν_{max} (KBr) 3 500, 3 450 (OH), 1 665 (chelated C=O), 1 605, 1 520, 1 500, 1 460, 975, 815, 720 cm^{-1}; ^1H nmr δ 12.64 (1H, s, OH-5), 7.71 (1H, d, *J* 1.8 Hz, H-2′), 7.68 (1H, dd, *J* 8.4 and 1.8 Hz, H-6′), 7.05 (1H, d, *J* 8.4 Hz, H-5′), 6.45 and 6.36 (each 1H, d, *J* 2.1 Hz, H-8 and H-6), 6.01 (1H, s, OH-4′), 3.86, 3.88 and 3.99 (each 3H, s, three aromatic methoxyl groups).

The second compound (R_f 0.5; silica gel G; $CHCl_3$–MeOH, 19 : 1) was crystallised from $CHCl_3$–MeOH as yellow needles (0.0005%), m.p. 250°. It was identified as 5,4′-dihydroxy-3,7-dimethoxyflavone by spectral analysis and finally by comparing its physical and spectral data reported

earlier, λ_{max} (EtOH) 351 (log ε 4.27), 268 (4.27), 204 nm (4.51); λ_{max} (EtOH + $AlCl_3$) 350 (log ε 4.26), 270 (4.25), 270 nm (4.56); λ_{max} (EtOH + NaOAc) 352 (log ε 4.09), 268 (4.12), 207 nm (4.52); ν_{max} (KBr) 3 260 (OH), 1 670 (α,β-unsaturated C=O), 1 605, 1 590, 1 505, 945, 815, 720 cm^{-1}; ^1H nmr δ ($CDCl_3$ + CD_3OD) 7.92 (2H, d, *J* 8.1 Hz, H-2′ and H-6′), 6.81 (2H, d, *J* 8.1 Hz, H-3′ and H-5′), 6.35 (1H, d, *J* 2.7 Hz, H-8), 6.21 (1H, d, *J* 2.7 Hz, H-6), 3.78 and 3.71 (each 3H, s, two aromatic methoxyl groups); ^{13}C nmr 178.1 (C-4), 165.7 (C-7), 161.6 (C-5), 160.2 (C-4′), 157.2 (C-9), 157.0 (C-2), 138.9 (C-3), 130.8 (C-2′ and C-6′), 121.5 (C-1′), 116.0 (C-3′ and C-5′), 106.0 (C-10), 98.2 (C-6), 92.5 (C-8), 56.1 (OCH_3), 60.1 (OCH_3).

Isolation of calliphyllin : The white solid obtained from the later thirteen petrol–EtOAc (4 : 1) eluate fractions of the main cc showing one major and another minor spots in tlc, was subjected to repeated cc over silica gel, followed by crystallisation from $CHCl_3$–petrol to afford colourless needles-shaped crystals of calliphyllin (**1**; 0.016%), m.p. 157° (Found : C, 75.2; H, 9.28. $C_{20}H_{30}O_3$ requires : C, 75.07; H, 9.43%); $[\alpha]_D$–19.8° ($CHCl_3$, *c* 0.124); ν_{max} (KBr) 3 480 (OH), 2 910, 2 850, 1 710, 1 690 (C=O), 1 630, 1 450, 1 380, 1 230, 995, 835, 710 cm^{-1}; ν_{max} ($CHCl_3$) 3 675, 3 610 (OH), 3 010, 2 970, 1 685 (C=O), 1 630, 1 510, 1 460, 1 380, 920, 840, 730 cm^{-1}; ^1H nmr δ 5.88 (1H, dd, *J* 17.6 and 10.4 Hz, H-15), 5.68 (1H, bd, $W_{1/2}$ ≈ 11 Hz, H-7), 5.14 (1H, dd, *J* 10.4 and 0.6 Hz, H_A-16), 5.10 (1H, dd, *J* 17.6 Hz and 0.6 Hz, H_B-16), 3.68 (1H, s, H-14), 1.25 (3H, s, CH_3-17), 0.88 (3H, s, CH_3-19), 0.87 (3H, s, CH_3-20); Ms *m/z* (relative intensity) 318 (M^+) (12.8), 301 (ion *a*) (100), 300 (*b*) (44.7), 290 (*c*), (6.4), 285 (*d*) (10.6), 255 (*e*) (28.7), 233 (10.6), 173 (27.7), 161 (15.9), 159 (17.0), 147 (*f*) (22.3), 145 (18.1), 133 (27.7), 131 (25.5), 123 (46.8), 109 (42.6), 91 (46.8), 81 (51.1), 79 (44.7), 67 (71.3), 55 (74.5).

Acetylation of calliphyllin : A solution of calliphyllin (30 mg) in dry pyridine (1 ml) was treated with dry acetic anhydride (1 ml) and kept for over-night as room temperature. After usual work-up of the reaction mixture followed by chromatography of the product over silica gel, petrol–ethyl acetate (97 : 3) eluate fractions furnished a low melting compound (**4**) which could not be induced to crystallisation after rechromatography : ν_{max} (KBr) 3 360–3 440 (OH), 2 940, 1 725 (acetate C=O), 1 690 (C=O), 1 450, 1 235, 970, 825, 750 cm^{-1}; ^1H nmr δ 5.74 (1H, dd, *J* 17.6 and 10.0 Hz, H-15), 5.02 (1H, dd, *J* 17.6 and 0.9 Hz, H_B-16), 4.98 (1H, dd, *J* 9.9 and 0.9 Hz, H_A-16), 4.93 (1H, s, H-14), 1.96 (3H, s, -O-CO-CH_3), 1.27 (3H, s, CH_3-17), 0.89 (3H, s, CH_3-19), 0.88 (3H, s, CH_3-20).

Esterification of calliphyllin : Calliphyllin (20 mg) was dissolved in ether and cooled, and to it a cold solution of diazomethane in ether was added. The reaction mixture was kept over-night at room temperature. Then the solvent was removed and the residue chromatographed over silica gel (60–120 mesh). The petrol–ethyl acetate (9 : 1) eluate fractions afforded calliphyllin methyl ester (**3**; 10 mg), m.p. 67°; ν_{max} (KBr) 3 520–3 420 (OH), 2 940, 2 860, 1 725 (ester C=O), 1 640, 1 460, 1 245, 910, 830, 720 cm^{-1}; ^1H nmr δ 5.88 (1H, bd, *J* 17.6 and 10.9 Hz, H-15), 5.63 (1H, bd, $W_{1/2}$ ≈ 11 Hz, H-7), 5.12 (1H, dd, *J* 10.9 and 0.9 Hz, H_A-16), 5.07 (1H, dd, *J* 17.6 and 0.9 Hz, H_B-16), 3.61 (3H, s, OCH_3), 3.59, (1H, s, H-14), 1.24 (3H, s, CH_3-17), 0.87 (3H, s, CH_3-19), 0.85 (3H, s, CH_3-20)

Hydrogenation of calliphyllin : A solution of calliphyllin (50 mg) in absolute ethanol (3 ml) was hydrogenated in presence of Pd–C (10%) catalyst for 8 h at room temperature. The reaction was monitored by micro-tlc. The completion of the reaction was evident from the disappearance of the substrate spot (R_f 0.45; $CHCl_3$–MeOH, 9 : 1) and appearance of only one new less polar spot (R_f 0.50; $CHCl_3$–MeOH, 9 : 1).

Pd–C was filtered and the filtrate on evaporation followed by chromatography over silica gel furnished from petrol–ethyl acetate (3 : 17) eluate fractions a white amorphous solid, 25 mg; R_f 0.50, $CHCl_3$–MeOH (9 : 1). The latter was characterised as a mixture of dihydroisodextropimaric acid (**5**) and dihydrosandaracopimaric acid (**6**) in the ratio 3 : 1. The mixture was further subjected to repeated chromatography over silica gel to afford from petrol–ethyl acetate (3 : 22) eluate fractions a solid which upon repeated crystallisations from $CHCl_3$–petrol gave pure dihydroisodextropimaric acid (**5**), m.p. 171–73°, $[\alpha]_D$–4.5° ($CHCl_3$); ν_{max} (KBr) 3 420 (OH), 2 920, 2 860, 1 700 (C=O), 1 460, 965, 825, 755 cm^{-1}; ^1H nmr δ 5.27 (1H, bs, $W_{1/2} \approx 10$ Hz, H-7), 1.18 (2H, m, CH_2-15), 1.26 (3H, s, CH_3-17), 0.89 (3H, s, CH_3-19), 0.81 (3H, t, J 7.2 Hz, CH_3-16), 0.70 (3H, s, CH_3-20).

Treatment of the chloroform extract : The chloroform extract concentrate (green gummy residue, ~ 10 g) did not show any distinct spot in tlc and did not give any pure constituent upon chromatography.

Acknowledgement

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