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Chemical constituents of *Evolvulus nummularius*

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Three new compounds, **1-3** together with known compounds, β-sitosterol and its glucoside, stigmasterol, *d*-mannitol, ursolic acid and oleanolic acid have been isolated from the aerial parts of *Evolvulus nummularius*. On the basis of their spectroscopic data and chemical study, the structures of the new compounds **1-3** have been established as 3-*O*-(4-stearoyl-*Z*-coumaroyl)-stigmast-5, *E*–22–dien-3β-ol; 16-(*E*-coumaroyloxy)-palmitic acid and 3β-hydroxy-urs-12-en-29β-oic acid, respectively. Possibly compound **3** is identical with plectranthoic acid, whose structure was wrongly assigned as 3α-hydroxy-urs-12-en-29β-oic acid.

Keywords: *Evolvulus nummularius*, Convolvulaceae, steroids, aliphatic ester, pentacyclic triterpenes

IPC: Int.Cl.[8] C07C

Evolvulus nummularius (L.) L. syn. *Convolvulus nummularius* L. of family Convolvulaceae, a perennial herb having small funnel shaped flowers grows in waste lands of the North Eastern states of India. The plant is used as febrifuge and vermifuge[1]. Previous study[2] revealed the presence of 7,3´,4´,5´-tetrahydroxyflavone and its 7-*O*-glycosides. The aerial parts of the plant are now phytochemically investigated. Herein is reported the isolation of three new compounds **1-3,** together with six known compounds. The known compounds were identified by comparison of their spectral data reported earlier. The antibacterial activities of the isolated compounds have also been screened and only compound **1** showed moderate activity against *Bacillus subtilis*.

A methanolic extract of the air-dried aerial parts of this plant was dissolved in distilled water and then successively partitioned with benzene, chloroform and *n*-butanol mixture. These fractions were column chromatographed using silica gel to isolate the phytochemicals. Compounds **1** and **2** were isolated from chloroform fraction and compound **3** from *n*-butanol fraction.

Compound **1** had the composition $C_{56}H_{88}O_4$ (M^+ 824), as determined by a combination of EI-MS, FAB-MS and elemental analysis. The IR spectrum in KBr showed the bands for ester (1735 cm^{-1}), conjugated ester (1725 cm^{-1}), olefinic double bonds (1685, 1665 and 1640 cm^{-1}) and phenyl (1605 cm^{-1}) functionalities. The ^{1}H NMR (**Table I**) exhibited two methyl singlets [δ 0.68 (3H), 1.00 (3H)], three methyl doublets [δ 1.02 (3H, *J* = 6.5 Hz), 0.81 and 0.84 (each 3H, *J* = 7.0 Hz)], one methyl triplet [δ 0.79 (3H, *J* = 7.0 Hz)], three olefinic protons [δ 5.35 (1 H, brd, *J* = 3.5 Hz), 5.15 (1H, dd, *J* =15.0 and 8.5 Hz), 5.01 (1H, dd, *J* = 15.0 and 7.5 Hz)] and one carbinol methine proton [δ 3.54 (1H, m)], assignable to stigmasterol unit of the molecule. The ^{1}H NMR spectrum also exhibited signals for a 1,4-disubstituted phenyl moiety [δ 7.61 (2H, d, *J* = 8.5 Hz) and 6.84 (2H, d, *J* = 8.5 Hz)], and two olefinic protons [δ 6.82 (1H, d, *J* = 12.5 Hz) and 5.81 (1H, d, *J* = 12.5 Hz) indicating the presence of a *Z*-coumaroyl moiety[3]. The ^{1}H NMR spectrum also showed a methyl triplet [δ 0.88 (3H, *J*= 7.0 Hz)], one methylene triplet [δ 2.34 (2H, *J* = 7.0 Hz)], one methylene quintet [δ 1.63 (2H, *J* = 7.0 Hz)] and fourteen methylene groups [δ 1.26 (brs)], assignable to stearoyl moiety. The ^{13}C NMR spectrum (**Table I**) indicated the presence of 56 carbons, 29 for the sterol moiety, 9 for the coumaroyl moiety and 18 for the stearoyl moiety. The attachment of the coumaroyl moiety at the C-3 position of the sterol moiety was confirmed by the HMBC correlation of C-3 proton (δ 3.54) with C-9´ (δ 166.55).

Table I — ^{1}H and ^{13}C NMR spectral data and HMBC correlation of **1** in $CDCl_3$ (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR)

Carbon No	δ_C	δ_H	HMBC (H→C)
1	37.21t		
2	28.21t		
3	71.90d	3.54m	C-1', C-2, C-4, C-5,
4	42.14t		
5	140.62s		
6	121.77d	5.35 brd (3.5)	C-4, C-5, C-7, C-8
7	31.89t		
8	31.88d		
9	50.11d		
10	36.47s		
11	21.05t		
12	37.74t		
13	42.29s		
14	56.74d		
15	24.27t		
16	28.73t		
17	56.03d		
18	12.21q	0.68 s	
19	19.35q	1.00 s	
20	40.46d		
21	21.18q	1.02 d (6.5)	
22	138.28d	5.15 dd (15.0,8.5)	C-20,C-21, C-24
23	129.25d	5.01dd (15.0,7.5)	C-20, C-24
24	51.21d		
25	31.88d		
26	19.01q	0.84 d (7.0)	
27	19.78q	0.81d (7.0)	
28	25.37t		
29	12.01q	0.79 t (7.0)	
1′	126.99s		
2′	129.88d	7.61d (8.5)	C-1', C-3', C-7'
3′	115.86d	6.84 d (8.5)	C-2', C-4', C-1″
4′	158.02s		
5′	115.6d	6.84 d (8.5)	C-4', C-6', C-1″
6′	129.88d	7.61 d (8.5)	C-1', C-5', C-7′
7′	143.30d	6.82 d (12.5)	
8′	117.04d	5.81 d (12.5)	
9′	166.55s		
1″	167.65s		
2″	33.96t	2.34 t (7.0)	C-1″, C-3″
3″	28.73t	1.63 quin (7.0)	
4″-17″	29.04-31.51t	1.26 brs	
18″	14.08q	0.88 t (7.0)	

J values(Hz) are given in parentheses

The HMBC interaction of C-3′/5′ proton (δ 6.84) with C-4′ (δ 158.02) and C-1″ (δ 167.65) also confirmed the attachment of the stearoyl moiety at C-4′ position of the coumaroyl moiety. The FAB-MS of compound **1** recorded mass ion at *m/z* 825 $(M+H)^+$ along with other mass ions at *m/z* 411, 397 (sterol moiety-Me + H)$^+$, 395 (sterol moiety - H_2O + H)$^+$, 329, 273, 271, 255, 213, which are characteristic of C_{29} $\Delta^{5,22}$ steroids[4]. The EI-MS showed mass ions at *m/z* 412, 284, 164 and 147 suggesting the presence of stigmasterol, stearic acid and coumaric acid moieties in the molecule. The compound on saponification with 1*N* aqueous methanolic KOH afforded stigmasterol, *Z*-coumaric acid and stearic acid. On the basis of the foregoing evidence, the compound **1** was defined as 3-*O*-(4-stearoyl-*Z*-coumaroyl)-stigmast-5, *E* 22-dien-3β-ol.

Compound **2** of the molecular formula $C_{25}H_{38}O_5$, as determined by a combination of EI-MS and elemental analysis, showed UV-Vis absorption at 228 and 310 nm. Its IR spectrum exhibited the absorption bands for hydroxyl (3390 cm^{-1}), conjugated ester (1720 cm^{-1}), carboxyl (1710 cm^{-1}), conjugated double bond (1630 cm^{-1}) and phenyl (1600 cm^{-1}) functionalities. The ^{1}H NMR spectrum recorded signals for a *E*-coumaroyl moiety [δ 7.42 (2H, d, *J* = 8.5 Hz), 6.80 (2H, d, *J* = 8.5 Hz), 7.62 (1H, d, *J* = 16.0 Hz), and 6.28 (1H, d, *J* = 16.0 Hz)] and a hydroxypalmitic acid moiety [δ 4.18 (2H, t, *J* = 7.0 Hz), 2.28 (2H, t, *J* = 7.0 Hz), 1.52 (2H, quintet, *J* = 7.0 Hz), 1.26 (24H, brs) and 10.95 (1H, brs)]. The ^{13}C NMR spectrum with DEPT experiments showed the presence of 25 carbon resonances with 15 methylene, 6 methine and 4 quaternary carbons. The carbon resonance values (see Experimental section) of 6 methine and 3 quaternary carbons are in good agreement for a coumaroyl unit and of 15 methylene and 1 quaternary carbons for a hydroxypalmitic acid unit. The HMBC interaction of C-16 proton (δ 4.18) with C-9′ (δ 166.56), C-15 (δ 28.73) and C-14 (δ 25.96) indicated the attachment of palmitic acid unit to coumaroyloxy unit at C-16 position. The EI-MS recorded molecular ion peak at *m/z* 418 and other mass ions at *m/z* 256 (palmitic acid), 255, 164 (base peak, coumaric acid), 147 (coumaroyl unit), 119 and 60 also corroborating 16-coumaroyloxy-palmitic acid structure **2** for it. The compound on saponification with 1*N* aqueous methanolic KOH afforded *E*-coumaric acid and 16-hydroxypalmitic acid. From the above evidence the structure of compound **2** was elucidated as 16-(*E*-coumaroyloxy)-palmitic acid. To the best of the

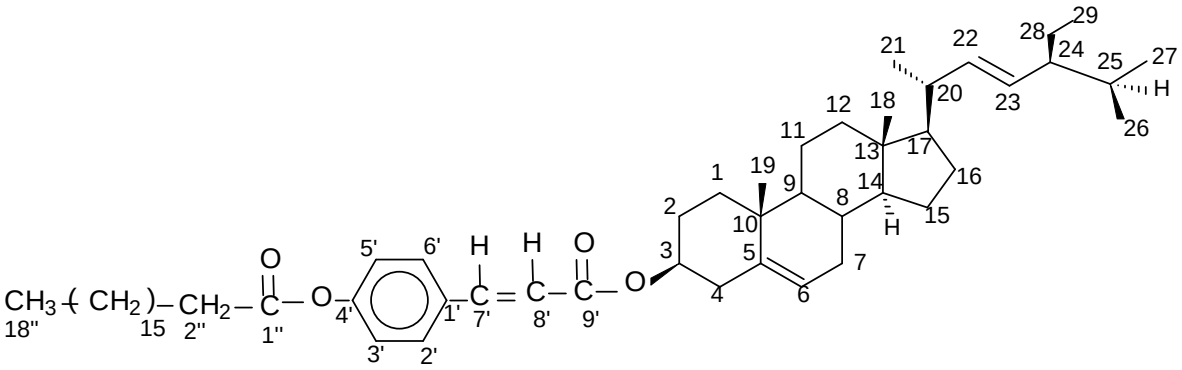
current knowledge, it is a new natural product. 16-Hydroxypalmitic acid (juniperic acid) was reported earlier from *Juniperus sabina* (Cupressaceae)[5].

Compound **3,** named nummularic acid had the composition $C_{30}H_{48}O_3$ (M^+456), which was determined by a combination of EI-MS, FAB-MS, ^1H and ^{13}C NMR spectra and elemental analysis. The IR spectrum of **3** in KBr indicated the existence of hydroxyl (3422 cm^{-1}), carboxyl (1695 cm^{-1}) and olefinic double bond (1652 cm^{-1}) functionalities. The ^{13}C NMR spectra with DEPT experiments (**Table II**) displayed 30 carbon signals. The carbonyl carbon of a carboxyl group was located at δ 180.08(s).The signals for a carbon bearing oxygen at δ 78.26(d) and olefinic carbons at δ139.42(s) and 125.76(d) were observed. Judging from the DEPT and HMQC spectra, the remaining carbon resonances were for 5 quaternary, 5 methine, 9 methylene and 7 methyl carbons. The deshielded olefinic methine carbon resonance at δ 125.76 and shielded olefinic quaternary carbon resonance at δ 139.42, suggested the location of olefinic unsaturation at C-12 position of the ursane skeleton[6]. The ^1H NMR spectrum (**Table II**) showed six methyl singlets [δ 0.90, 0.97, 1.04, 1.07, 1.24 and 1.26], one methyl doublet [δ 1.01 (J = 6.5 Hz)], one carbinol methine [δ 3.47 (dd, J = 10.5 and 5.5 Hz)] and one olefinic proton [δ 5.51 (t, J = 3.5 Hz)] characteristic of hydroxyursane skeleton[6]. The HMBC correlation of H-3 (δ 3.47) with C-2 (δ 28.26), C-4 (δ 39.51). C-23 (δ 28.93) and C-24 (δ 16.70) suggested the position of hydroxyl group at C-3. The coupling constants of H-3 indicated that the orientation of the hydroxyl group at C-3 was β and equatorial[7]. The HMBC interaction of H-18 (δ 2.66) with C-13 (δ 139.42), C-17 (δ 33.42), C-19 (δ 48.18) and C-29 (δ 180.08) and of H-19 (δ 1.84) with C-18 (δ 53.69), C-20 (δ 39.53), C-21 (δ 31.21), C-29 (δ 180.08) and C-30 (δ 21.53) also indicated the position of carboxyl group at C-19 position. The observed NOE effect between H-3 and H-23, H-5 and H-9, H-24 and H-25, H-12 and H-18, H-18 and H-28, H-19 and H-27, and H-19 and H-20 (**Figure 1**) confirmed the position of hydroxyl group (*β* and equatorial) at C-3, olefinic double bond at C-12 and carboxyl group (*β* and equatorial) at C-29 carbons. This NOE correlation also suggested the boat conformation of ring C and chair conformation of ring D in nummularic acid. The EI-MS of nummularic acid **3** showed mass ions at *m/z* 456 (M^{+}), 441 $(M\text{-}Me)^+$, 438 $(M\text{-}H_2O)^+$, 410 $(M\text{-}46)^+$, 248 (base peak), 207, 203, 189 and 133, characteristic of Δ^{12} ursane skeleton[8]. The compound on acetylation with acetic anhydride in pyridine afforded monoacetate **3a**, $C_{32}H_{50}O_4$ (M^+ 498). Based on these results, nummularic acid **3** was assigned as 3β-

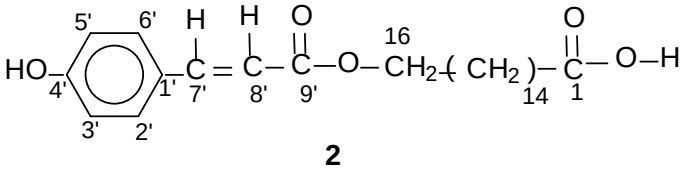
Table II — ^1H and ^{13}C NMR spectral data and HMBC correlation of nummularic acid **3** in C_5D_5N (400 MHz for ^1H and 100 MHz for ^{13}C NMR)

Carbon No	δ_C	δ_H	HMBC (H→C)
1	39.20 t		
2	28.26 t		
3	78.26 d	3.47 dd (10.5, 5.5)	C-2,C-4,C-23,C-24
4	39.51 s		
5	55.95 d	0.78 dd (11.0, 1.5)	
6	18.91 t		
7	33.70 t		
8	40.10 s		
9	48.18 d	1.56 m	C-5, C-8, C-10, C-11
10	37.41 s		
11	23.76 t	1.98 dd (11.0, 3.81)	C-12, C-13
12	125.76d	5.51 t (3.5)	C-9, C-11,C-13
13	139.42s		
14	42.63 s		
15	28.83 t		
16	25.05 t		
17	33.42 s		
18	53.69 d	2.66 d (11.0)	C-12, C-13,C-17,C-19,C-29
19	48.18 d	1.84 dd (11.0, 11.0)	C-18, C-20,C-21,C-29,C-30
20	39.53 d		
21	31.21 t		
22	37.59 t	1.96 m	
23	28.93 q	1.24 s	
24	16.70 q	0.97 s	C-3, C-4,C-5
25	15.80 q	0.90 s	C-5, C-9
26	17.65 q	1.04 s	
27	24.03 q	1.26 s	
28	17.60 q	1.07 s	
29	180.08s		
30	21.53 q	1.01 d	

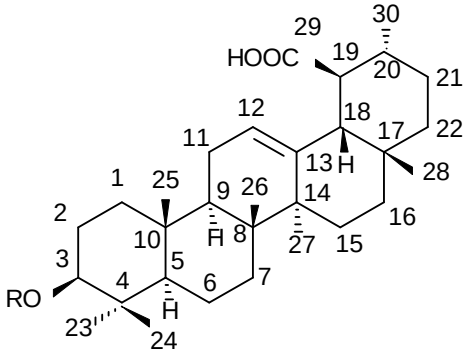
J values(Hz) are given in parentheses



1



2



3 R=H

3a R=Ac

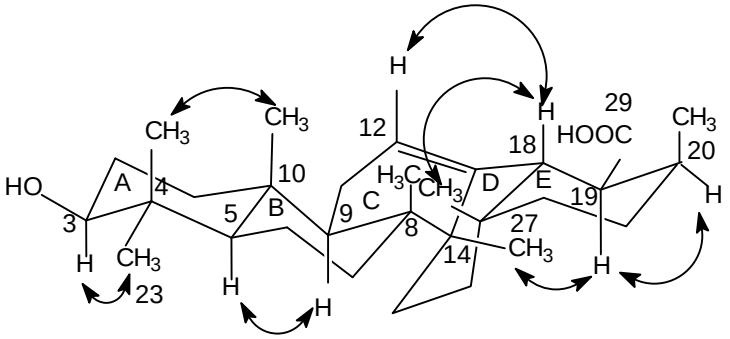


Figure 1— Observed NOE for compound **3**

hydroxyurs-12-en-29β-oic acid. Earlier Razdan *et al.*[9] reported plectranthoic acid [(19*S*)-3α-hydroxy-18α-urs-12-en-29β-oic acid], m.p. 296°C, $[\alpha]_D^{25}$ +42° (c 1.0, MeOH) from *Plectranthus rugosus*, but the physical and spectral observations on compound **3** differs much from those of that compound.However, the positive sign of specific rotation for both the compounds indicated that possibly both the compounds, plectranthoic acid and nummularic acid are the same and possibly plectranthoic acid was not isolated in pure state and its hydroxyl group at C-3 should have β-configuration.

In addition, six known compounds have been isolated namely, β-sitosterol and stigmasterol[10,11] from benzene fraction and β-sitosterol glucoside[12], ursolic acid[13,14], oleanolic acid[14,15] and *d*-mannitol from *n*-butanol fraction.

All the isolated compounds were assayed for antimicrobial activity. Only compound **1** showed moderate activity against spore forming bacterium, *Bacillus subtilis* at a concentration of 250 μg/disc. The other compounds were inactive (at a concentration of 250 μg/disc) against *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Klebsiella aerogens*, *Pseudomonas aeruginosa* and *Escherichia coli*.

The aqueous solution of the original extract of *E. nummularius* showed vermifuge activity against earthworm. Earthworms did not survive in the aqueous plant extract more than 15 min. Crude methanolic extract of the plant also showed moderate vermifuge activity against earthworms. The earthworms survived about 45 min. However, the isolated compounds did not show vermifuge activity against earthworms in aqueous solution/suspension. Possibly the vermifuge compounds eluded from the remaining aqueous part of the methanolic extract after extraction with benzene, chloroform and *n*-butanol. Further studies will be continued with the original aqueous extract of the plant to find out the vermifuge principle.

Experimental Section

General procedure. Melting points are uncorrected. IR spectra were recorded on a Shimadzu FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian XL400 spectrometer with usual pulse sequences operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. EI-MS and FAB-MS were taken on JEOL JMS-AX505 HA and JMS-700 M station mass spectrometers. Column chromatography (CC) was carried out on silica gel (Merck, 60-120 mesh) and thin layer chromatography (TLC) on silica gel G (Merck).

Plant material. Fresh aerial parts of *Evolvulus nummularius* were collected from the campus area of Tripura University (TU) at Suryamaninagar during August 2002 and was identified by Dr. B. K. Datta, Taxonomist, Department of Life Science, TU. A Voucher specimen (#BD-05/05) has been deposited in the Central National Herbarium, Botanical Survey of India, Shibpur, Howrah.

Extraction and isolation. Air-dried aerial parts (2.0 kg) were extracted thrice with MeOH (15 L) at RT. The combined extract was concentrated to dryness under reduced pressure. The major part of the residue (20 g) was dissolved in cold distilled water (200 mL) and partitioned between benzene, $CHCl_3$ and *n*-BuOH, successively. All the benzene, $CHCl_3$ and *n*-BuOH fractions were concentrated separately and subjected to silica gel column chromatography.

Elution of the column of benzene fraction with benzene-$CHCl_3$ (9:1) afforded a residue which on rechromatography gave stigmasterol (40 mg), m.p. 170°C and β-sitosterol (7 mg), m.p. 138°C. Elution of the column of the $CHCl_3$ fraction with benzene-$CHCl_3$ (1:1) furnished a residue of two compounds. This residue on repeated column chromatography afforded compound **1** (55 mg) and compound **2** (22 mg).

Elution of the column of *n*-BuOH fraction with $CHCl_3$-EtOAc (9:1) afforded a residue of two compounds, which on repeated column chromatography gave ursolic acid (50 mg), m.p. 288°C and oleanolic acid (8 mg), m.p. 310°C. Elution of the column with $CHCl_3$-EtOAc (6:4) afforded nummularic acid **3** (36 mg), m.p. 275°C and with EtOAc gave β-sitosterol glucoside (25 mg), m.p. 287°C (dec). The column on elution with EtOAc-MeOH (7.5:2.5) furnished *d*-mannitol (35 mg), m.p. 167°C.

Compound **1**: Pale brown amorphous solid, m.p. 83-86°C; IR (KBr): 1735, 1725, 1685, 1665, 1640, 1605, 1460, 1026, 885 cm^{-1}; ^{1}H and ^{13}C NMR ($CDCl_3$): **Table I**; FAB-MS: *m/z* (%) 825 $[M+H]^+$ (3), 411(22), 397(23), 395(14), 329(4), 273(6), 271(4), 255 (11), 213(10), 43(100); EI-MS: *m/z* (%) 412(4), 396(5), 329(2), 284(4), 273(31) 271(2), 255 (5), 213 (7), 164 (100),147 (76). Anal. Found: C, 81.43; H, 10.82. Calcd. for $C_{56}H_{88}O_4$: C, 81.50; H, 10.75 %.

Compound **2**: Pale amorphous solid, m.p. 113-15°C; UV-Vis (MeOH) λ_{max} nm (log ε): 228 (4.13), 310(4.46); IR (KBr): 3390, 1720, 1710, 1630, 1600, 1595, 1515, 970, 880 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.26 (24H, brs, 12×CH_2), 1.52 (2H, quin., H_2-3), 2.28 (2H, t, J = 7.0 Hz, H_2-2), 4.18 (2H, t, J = 7.0 Hz, H_2-16), 6.28 (1H, d, J = 16.0 Hz, H-8′), 6.80 (2H, d, J = 8.5 Hz, H-3′,5′), 7.42 (2H, brd, J = 8.5 Hz, H-2′,6′), 7.62 (1H, d, J = 16.0 Hz, H-7′), 10.95 (1H, brs, -COOH); ^{13}C NMR ($CDCl_3$): δ 179.35 (s, C-1), 36.12 (t, C-2), 24.68 (t, C-3), 29.21-29.66 (t, C×10, C-4-13), 25.96 (t, C-14), 28.73 (t, C-15), 64.64 (t, C-16), 127.87 (s, C-1′), 129.25 (d, C-2′), 115.46 (d, C-3′),156.96 (s, C-4′),115.46 (d, C-5′), 129.25 (d, C-6′), 144.40 (d, C-

7′), 114.97 (d, C-8′), 166.56 (s, C-9′); EI-MS: *m/z* (%) 418 (M^+, 20), 256 (9), 255 (4), 164 (100), 147 (75), 119(25), 60 (10), 57 (27), 55 (31). Anal. Found: C, 71.65; H, 9.08. Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15%.

Nummularic Acid **3**: Colourless crystals, m.p. 275°C; $[\alpha]_D^{22}$ +70° (c 0.5, MeOH); IR (KBr): 3422, 1695, 1652,1456, 1387,1377, 1030, 997, 669 cm^{-1}; EI-MS: *m/z* (%) 456 (M^+,6), 438 (M-H_2O, 5), 423 (3), 410 (M-46, 4), 395 (3), 300 (9), 249 (94). 248 (100), 220 (23), 207 (74), 203 (98), 189 (34), 175 (21), 133 (85); ^1H and ^{13}C NMR (C_5D_5N): **Table II**. Anal. Found: C, 78.84, H,10.52. Calcd. for $C_{30}H_{48}O_3$: C, 78.90, H, 10.58%.

Saponification of compound 1. A solution of compound **1** (15 mg) in 1*N* aqueous methanolic KOH (10 mL) was refluxed for 2 hr in an atmosphere of N_2 and the solvent was evaporated to a residue. The residue was dissolved in 20 mL of water and extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated and purified through silica-gel column to get stigmasterol (3.5 mg). The aqueous solution of the reaction mixture after $CHCl_3$ extraction was acidified with 2*N* HCl and extracted with Et_2O. The ethereal extract was concentrated and purified by column chromatography to yield *Z*-coumaric acid [*Z*-3(4-hydroxyphenyl)-2-propanoic acid] (2.0 mg), m.p. 133°C [lit[15]. 134-135.5°C (dec.)] and stearic acid (2.5 mg), m.p.70-72°C (lit.[16] 72°C). The products of saponification were identified by direct comparison (co-TLC and mixed m.p.) with the authentic samples.

Saponification of compound 2. Compound **2** (10 mg) in 1*N* aqueous methanolic KOH (7 mL) was refluxed for 2 hr in an atmosphere of N_2 and the solvent was removed by evaporation. The residue was then quenched with 15 mL of H_2O and acidified with 2*N* HCl and extracted with Et_2O. The ethereal extract was concentrated and purified by column chromatography to afford *E*-coumaric acid (3mg), m.p. 214°C [lit[17]. 215°C (dec.)] and 16-hydroxypalmitic acid (2.5 mg), m.p. 96°C (lit[5]. 95°C). Both the products were identified by direct comparison (co-TLC and mixed m.p.) with the authentic samples.

Acetylation of nummularic acid 3. To a solution of nummularic acid **3** (10 mg) in pyridine (1 mL), acetic anhydride (2 mL) was added and the solution was kept at RT (25°C) for 24 hr. The reaction mixture was poured in ice water and extracted thrice with $CHCl_3$. The combined $CHCl_3$ extract was washed with an aqueous $CuSO_4$ solution to remove pyridine, concentrated and subjected to column chromatography to get monoacetate **3a** in colourless needles (5 mg), m.p. 250°C, $C_{32}H_{50}O_4$ (M^+498); IR(KBr): 1735, 1700, 1650, 1460,1365, 1360, 1025, 820 cm^{-1} ; EI-MS: *m/z* (%) 498 (M^+,6), 438 (M-HOAc, 10), 300 (15), 248 (100), 203 (65), 189 (30).

Antimicrobial assay. The agar cup diffusion method[18] was used for antibacterial assay. Six bacteria namely, *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Klebsiella aerogens*, *Escherichia coli* and *Pseudomonas aeruginosa* were used as test microorganisms. All the bacteria mentioned above were incubated at 37°C for 24 hr by inoculating into Nutrient Broth (Hi-media). An inoculum containing about 10^6 bacterial cells/mL was spread by a glass spreader on each nutrient agar plate (100 µL inoculum/plate). The plates were dried at RT. The solution of each compound was made in DMSO at a concentration of 10 mg/mL. On each dry bacterium seeded plate wells of 5 mm diameter were made by a sterilized glass tip and solutions (25 µL) of different compounds were added. On one well of the plate only solvent was used as control. On each plate an appropriate reference antibiotic disc [amikacin disc (30 µg/disc) for the bacterium, *B. subtilis / K. aerogens*/ *E.coli* / *P. aeruginosa*.; *ceftriaxone disc* (30 µg/disc) for bacterium, *M. luteus* and penicillin G disc (10 units/disc) for bacterium, *S. aureus*; 5mm diameter, BEACON) was placed by pressing slightly and the plates were then incubated at 37°C for 24 hr. After this period, the positive antibacterial activity was ascertained by measuring the growth inhibition zone (mm).The reference antibiotic disc was used for comparison of antibacterial activity of the tested compounds under identical condition. Only compound **1** showed an inhibition zone of 13 mm against *B. subtilis* compared to 18 mm of the antibiotic, amikacin. All other compounds were inactive (inhibition zone below 7 mm) against the tested microorganisms.

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