PERGAMON

Phytochemistry 54 (2000) 215–220

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Normethyl pentacyclic and lanostane-type triterpenes from *Adiantum venustum*

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Received 5 August 1999; received in revised form 27 December 1999

Abstract

Phytochemical studies on the aerial parts of *Adiantum venustum* resulted in the isolation of normethyl lupane-type, a normethyl oleanane-type and a lanostane-type triterpene. Structures of these triterpenes have been established as 30-normethyl lupane-20-one, 30-normethyl olean-3-one-30β-ol and lanost-20(22)-ene-30-ol on the basis of spectral data analyses and chemical methods.

Keywords: *Adiantum venustum*; Adiantaceae; Aerial parts; Triterpenes; Adiantulupanone; Adiantuoleanone; Adiantulanosterol

1. Introduction

Adiantum venustum G. Don (Adiantaceae) is a small fern found commonly in the north-eastern Himalayas, Kashmir and Shimla. The plant is used in the treatment of biliousness, inflammatory diseases of chest, tumors, ophthalmia, hydrophobia, cold, headache (Chadha, 1985; Chopra et al., 1980) as antibacterial (Chopra et al., 1980; Kshirsagar and Mehta, 1972) and antiviral (Husson et al., 1986) drug. Earlier investigation on this fern led to the isolation of adiantone, 3-filicene, kaempferol, hakonanol ketone (Rangaswami and Thanu, 1967) fernane-type triterpene (Banerjee et al., 1991) and a tirucallane triterpene (Chopra et al., 1997). In this paper, we report the isolation and structural elucidation of two normethyl pentacyclic triterpenes and one lanostane-type triterpene from aerial parts of the plant.

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2. Results and discussion

Compound **1**, named adiantulupanone, had the composition of $C_{29}H_{48}O$ established on the basis of mass spectrum and ^{13}C-NMR data. It responded positively to the Liebermann–Burchard test, indicating a triterpenoid skeleton. Its IR spectrum exhibited a carbonyl group (1700 cm^{-1}) absorption band. Negative Zimmermann test ruled out the existence of 3-oxo group. Its ^{1}H-NMR spectrum was consistent with the proposed structure and clearly showed six quaternary methyl functionalities as three-proton each singlets at δ 0.815 (Me-23), 0.781 (Me-24), 0.931 (Me-25), 0.760 (Me-26), 0.548 (Me-27) and 0.902 (Me-28) and a three-proton singlet at δ 2.091 assigned to C-29 methyl group deshielded by C-20 carbonyl group (see Table 1). The remaining methylene and methine protons resonated between δ 2.928–0.665. Its broad band decoupled ^{13}C-NMR spectrum and DEPT spectra exhibited the presence of 29 carbon signals ($CH_3 \times 7$, $CH_2 \times 11$, -CH × 5, -C- × 5, CO × 1). The absence of carbon signals between δ 56.05–210.71 in the ^{13}C-

0031-9422/00/$ - see front matter
PII: S0031-9422(00)00063-7

Table 1
^{1}H-NMR spectral data of compound **1**, **2**, **3** (600 MHz, $CDCl_3$)[a]

Proton	**1**		**2**		**3**	
	α	β	α	β	α	β
1	0.976 *dddd* (10.6, 5.8, 10.6, 10. 6)	1.499 *dddd* (5.8, 4.0, 2.9, 13.5)	0.746 *dddd* (4.8, 9.4, 5.8, 8.4)	1.546 *dddd* (1.8, 3.6, 5.8, 9.4)	1.590 *dddd* (12.4, 12.4, 5.5, 6.6)	1.631 *dddd* (12.4, 12.4, 5.5, 5.0)
2	1.369 *m*	1.396 *m*	2.661 *dddd* (6.2, 9.4, 5.8, 8.4)	2.019 *dddd* (2.2, 3.6, 5.8, 8.4)	1.179 *m*	1.979 *m*
3	1.088 *dddd* (4.0, 9.1, 4.0, 13.1)	1.300 *m*	–	–	1.194 *dddd* (9.5, 13.5, 5.5, 11.8)	1.386 *dddd* (13.9, 5.5, 13.5, 9.5)
5	0.683 *dd* (10.1, 1.8)	–	0.673 *m*	–	1.354 *dd* (11.7, 5.5)	–
6	1.333 *m*	1.333 *m*	1.505 *m*	1.584 *m*	1.315 *m*	11.435 *m*
7	1.110 *dddd* (4.9, 4.0, 13.5, 4.0)	1.263 *dddd* (10.6, 6.7, 2.9, 2.8)	1.078 *dddd* (9.5, 4.0, 9.5, 8.2)	1.275 *m*	1.224 *m*	1.827 *m*
9	1.194 *dd* (12.5, 6.6)	–	1.116 *dd* (4.5, 5.5)	–	1.513 *m*	–
11	1.220 *m*	1.455 *m*	1.331 *m* (4.03)	1.505 *m* (13.76)	1.513 *m*	1.534 *m*
12	1.583 *m*	1.743 *m*	1.202 *m*	1.275 *m*	1.224 *dddd* (9.5, 5.0, 13.2, 8.5)	1.805 *dddd* (5.0, 8.5, 5.5, 13.9)
13	–	1.300 *m*	–	1.203 *m*	–	–
15	0.976 *dddd* (10.6, 5.8, 5.1, 10.6)	1.499 *dddd* (5.8, 5.1, 4.6, 13.3)	1.124 *m*	1.488 *m*	1.435 *m*	1.827 *m*
16	1.773 *dddd* (11.5, 6.6, 3.6, 10.6)	1.417 *dddd* (8.0, 4.76, 2.93, 6.60)	1.234 *m*	1.488 *m*	1.805 *m*	2.010 *m*
17	–	–	–	–	1.524 *dd*	–
18	1.455 *dd* (9.53, 10.25)	–	–	1.124 *m*	0.752 *s*	–
19	–	2.928 *m*	1.234 *m*	2.016 *d* (5.1)	0.897 *s*	–
21	2.240 *m*	1.602 *m*	1.203 *m*	1.331 *m*	1.564 *s*	–
22	0.732 *dddd* (7.3, 9.5, 3.6, 9.5)	1.535 *dddd* (4.3, 8.4, 8.8, 5.8)	1.124 *m*	1.353 *m*	1.165 *d* (5.3)	–
23	0.815 *s*	–	0.993 *s*	–	1.827 *m*	1.524 *m*
24	0.781 *s*	–	1.036 *s*	–	1.250 *m*	1.315 *m*
25	0.931 *s*	–	0.764 *s*	–	1.412 *m*	–
26	0.760 *s*	–	0.781 *s*	–	0.885 *d* (5.8)	–
27	0.548 *s*	–	0.822 *s*	–	0.806 *d* (6.5)	–
28	0.902 *s*	–	0.916 *s*	–	1.041 *s*	–
29	2.09 *s*	–	1.234 *s*	–	0.921 *s*	–
30	–	–	–	–	3.88 *d* (11.7)	3.80 *d* (11.7)
OH	–	–	3.901 *s*	–	–	–

[a] TMS; Coupling constants are provided in parentheses.

NMR and ^{1}H signals beyond δ 2.928 in the ^{1}H-NMR spectra suggested the saturated nature of the molecule without any carbinyl proton. The assignment of the carbon chemical shifts were made by comparison with the δ_c values of the corresponding carbon atoms in lupanes (Mahato and Kundu, 1994; Wenkert et al., 1978; Reynolds et al., 1986). The EIMS of **1** exhibited characteristic fragment ion peaks considered to arise from the cleavage of the lupane skeleton at *m/z* 397 $[M - Me]^+$, 369 $[M - COCH_3]^+$, 191 $[C_{8,14}–C_{9,11}$ fission$]^+$, 124 $[C_{16,17}–C_{13,18}$ fission$]^+$, 231, 176, 162, 148, 133, 109 $[124 - Me]^+$ and 81 $[124 - COCH_3]^+$ supporting the saturated skeleton of the molecule. The structure **1** was fully supported by extensive 2D-NMR experiments. A ^{1}H–^{13}C heteronuclear chemical shift correlation spectrum (HETERO-COSY) was recorded to locate the chemical shifts of various protons. The signals of C-5, C-9, C-13, C-18 and C-19 in ^{13}C-NMR spectrum could easily be correlated with the chemical shifts of their respective protons in the ^{1}H-NMR spectrum. In the 2D long range ^{1}H–^{13}C-NMR spectrum, compound **1** showed cross correlation for signals of Me-23 (with C-3, C-4, C-5 and C-24), Me-24 (with C-3, C-4, C-5, and C-23), Me-25 (with C-1, C-10 and C-9), Me-26 (with C-8, C-9, C-14 and C-27), Me-27 (with C-14, C-15, C-8 and C-26) and Me-28 (with C-17, C-16, C-18 and C-22) as well as CH-19 (with C-18 and C-21), CH-18 (with C-13, C-17 and C-19), CH-13 (with C-12, C-14 and C-18), CH-9 (with C-8, C-10 and C-11) and CH-5 (with C-4, C-6 and C-10). Compound **1** resisted to react with acetylating (Ac_2O–pyridine), hydrolysing (NaOH solution) and oxidising (CrO_3, $KMnO_4$) reagents. All these evidences indicated that the structure of **1** must be established as 30-normethyl lupane-20-one.

Compound **2**, designated adiantuoleanone, positive

in the Liebermann–Burchard and Zimmermann tests, was obtained as colourless crystalline mass. Its IR spectrum showed the presence of hydroxyl (3496 cm^{-1}) and carbonyl group (1710 cm^{-1}) absorption bands. The compound was assigned the molecular formula $C_{29}H_{48}O_2$ by ^{13}C-NMR and mass spectra. The ratio of carbons to hydrogens in the molecule indicated six degrees of cyclization and/or unsaturation. Since there is only one carbonyl group (δ 215.61) in the ^{13}C-NMR spectrum, the combined data strongly suggested it to be pentacyclic triterpene having a carbonyl group. In conjugation with DEPT edited spectral data, the normal ^{13}C-NMR spectrum (Table 2) confirmed the presence of 29 carbon atoms of the molecule to consist of one carbonyl, six quaternary (sp^3), four methine (sp^3), 11 methylene (sp^3) and seven methyl groups.

The ^{1}H-NMR data (Table -1) of **2** were based upon the analysis of the ^{1}H–^{1}H COSY spectrum. A solitary D_2O exchangeable hydroxyl signal at δ 3.901, a three-proton signal at δ 1.234 for C-29 methyl group attached to hydroxy substituted C-24 and six other tertiary methyl signals at δ 0.993 (Me-23), 1.036 (Me-24), 0.764 (Me-25), 0.781 (Me-28), 0.822 (Me-27) and 0.916 (Me-28) suggested a normethyl oleanone-type carbon framework of the molecule. Since only one set of CH_2 proton is strongly deshielded, the carbonyl group probably lies between a CH_2 group and a quaternary carbon, e.g., at C-3, the most common site for oxidation in triterpenes. The H-2 protons were correlated with the resonances at δ 0.746 and 1.546, hence these could be considered for the methylene at C-1. The non-equivalent protons of H-19 were observed at δ 1.234 and 2.016, exhibiting couplings with H-18 at δ 1.124 m. The most shielded methine resonance at δ 0.673 was assigned to H-5, which exhibited cross peak, at δ 1.505 and 1.584 corresponding to the H-6 methylene protons, which were further correlated with the H-7 methylene protons at δ 1.078 and 1.275. The assignments for the methyl resonances at δ 0.764 and 0.781 to H-25 and H-26 were straightforward as cross peaks were observed with the methylene protons of the 1 and 7 positions, respectively. Analogously, the methyl resonance at δ 0.822 was assigned to H-27 as it displayed cross peaks with methylene protons of the C-15 position. Based on the ^{1}H-NMR assignments, the ^{13}C-NMR chemical shifts for the protonated carbon resonances could readily be correlated by the one-bond ^{1}H–^{13}C COSY spectrum. The assignment of the resonance at δ 215.61 to C-3 was straightforward, whereas the assignments of other non-protonated carbon resonances were made on the basis of comparison with those for structurally similar oleanones (Mahato and Kundu, 1994; Knight, 1974; Marner et al., 1991) and by consideration of the empirical rules. In the 2D long range ^{1}H–^{13}C-NMR spectrum, compound **2** showed cross correlation for signals of angular methyls with the neighbouring carbons.

The most significant evidence for the structure elucidation of **2** was its electron impact mass spectrum. Apart from the molecular ion, the ion fragments of diagnostic importance appeared at m/z 413 $[M - Me]^+$, 410 $[M - H_2O]^+$ supporting the saturated nature of the molecule and carbonyl group at C-3. These data led to establish the structure of adiantuoleanone as 30-normethyl olean-3-one-30β-ol.

Compound **3**, designated adiantulanosterol, was obtained as colourless crystalline mass from MeOH soluble alcoholic extract after removal of petroleum ether and EtOAc. It showed positive Liebermann–Burchard test and IR absorption's for a hydroxy group (3420 cm^{-1}) and unsaturation (1625, 1610 cm^{-1}). It had a molecular formula of $C_{30}H_{52}O$ on the basis of mass ($[M]^+$ 428) and ^{13}C-NMR spectra (Table 2).

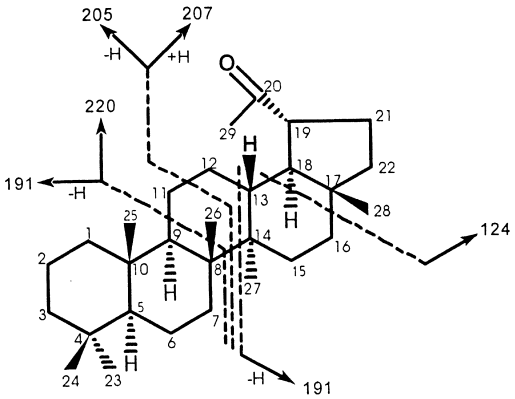
The mass spectrum exhibited diagnostically important ion fragments at m/z 138, 124 suggesting lanostane-type triterpene with saturated rings and the hydroxyl group at C-14 and C-8 unsaturated side chain.

Table 2
^{13}C-NMR data of **1**–**4** (150 MHz $CDCl_3$)

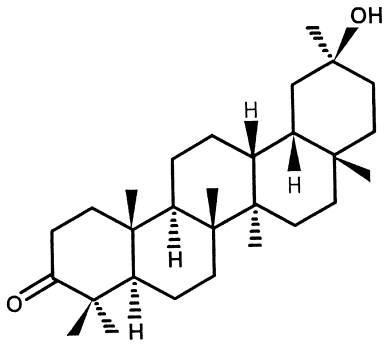
Carbon	1	2	3	4
1	41.24	40.27	35.82	35.66
2	24.06	33.73	28.32	28.35
3	42.05	215.61	29.24	28.98
4	33.39	37.30	39.17	38.96
5	56.05	56.09	58.32	58.37
6	18.65	18.24	17.91	18.06
7	33.35	42.06	29.55	29.63
8	41.87	41.80	60.05	59.89
9	50.28	50.23	50.20	49.89
10	37.36	37.03	38.66	38.77
11	20.82	18.62	18.65	18.71
12	22.15	18.65	39.14	39.43
13	48.90	58.80	42.70	43.85
14	44.76	42.00	42.46	43.06
15	41.24	20.90	29.55	28.67
16	33.22	21.64	28.37	29.41
17	41.87	33.22	51.60	52.10
18	53.93	49.68	16.30	16.33
19	53.62	41.13	15.73	15.76
20	210.71	77.35	143.98	144.10
21	22.91	32.97	17.99	18.15
22	40.27	32.17	120.68	121.23
23	33.36	16.43	20.36	19.96
24	21.56	14.74	19.89	20.39
25	16.58	21.55	30.74	30.76
26	15.84	15.89	21.92	21.96
27	14.58	33.37	22.88	23.21
28	16.63	16.47	20.13	21.96
29	30.92	22.03	15.45	15.64
30	–	–	64.87	64.91
31				171.82
32				21.06

The ^{1}H-NMR spectrum of **3** (Table 1) displayed a one-proton doublet at δ 5.165 assigned to H-22 and a three- proton deshielded singlet at 1.564 ascribed to C-21 methyl group attached to olefinic C-20 carbon atom. The presence of two doublets at δ 3.880 (J = 11.70 Hz) and 3.800 (J = 11.70 Hz) attributable to C-30 hydroxymethylene group and C-26 and C-27, secondary methyl doublets at δ 0.855 (J = 5.86 Hz) and 0.806 (J = 6.59 Hz) supported the C-20(22)-unsaturation instead of C-24(25)-unsaturation. The four tertiary methyl groups resonated as three-proton each singlets at δ 0.752 (Me-18), 0.897 (Me-19) 1.041 (Me-28) and 0.921 (Me-29). The ^{13}C-NMR spectrum of **3** exhibited signals due to seven methyl, 12 methylene, 6 methine ($5sp^3$, $1sp^1$) and 5 tertiary ($4sp^3$, $1sp^2$) carbon atoms. The chemical shifts of the carbon atoms of **3** were compared with the respective values of lanostanes (Knight, 1974; Tanaka and Matsunaga, 1990). Multiplicity of each carbon was determined by DEPT experiments and the ^{13}C-NMR values of important carbons were correlated with ^{1}H-NMR values in ^{1}H–^{13}C correlated 2D spectrum. The correlation of H-5, H-9 and H-17, Me-28, Me-30 and Me-18, Me-19 and Me-29 were established by ^{1}H–^{1}H 2D spectrum. The proton-relay coherence transfer experiment showed that the non-allylic alcohol was connected to a group located δ 3.88 and 3.80 and the vinylic proton showed a correlation with C-22 (δ_c 120.68).

Acetylation of **3** with Ac_2O-pyridine at room temperature afforded a monoacetyl derivative (**4**). The C-30 acetoxymethylene protons shifted at δ 4.13 (*d*, J = 11.7 Hz) and 4.00 (*d*, J = 11.7 Hz) in the ^{1}H-NMR spectrum of **4**, the acetylated product. The presence of a three-protons singlet at δ 2.07 in its ^{1}H-NMR and two signals at δ 171.80 and 21.06 in the ^{13}C-NMR spectrum for one acetyl function confirmed the presence of one acetylable hydroxyl group in the molecule. On the basis of these evidences, the structure of **3** has been elucidated as lanost-20(22)-ene-30-ol.



1



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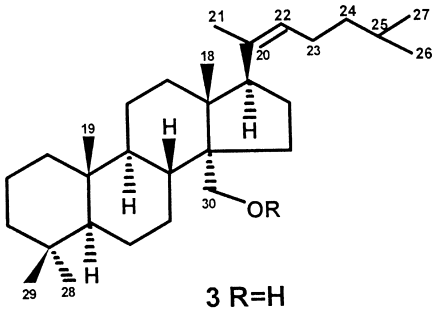

3 R=H

4 R=Ac

3. Experimental

3.1. General experimental procedures

Melting points were uncorrected. IR spectra were recorded as KBr pellets on Hitachi 270-30 spectrophotometer. UV spectra was measured on a Beckman DU-64 spectrophotometer in $CHCl_3$. MS was determined on a Hitachi M-80 spectrometer. ^{1}H-NMR (600 MHz) and ^{13}C- NMR (150 MHz) spectra were recorded on a JEOLGX spectrometer in $CDCl_3$. Chemical shifts are reported on δ (ppm) scale using TMS as an internal standard. Silica gel G (Merck, 60–120 mesh) was used for column chromatography (cc). Pre-coated Silica gel plates (Merck, Kieselgel 60 F-254, 0.20 mm) were used for analytical TLC and vanillin–H_2SO_4 was used as spraying reagent.

3.2. Plant material

The aerial parts of *A. venustum* were procured from Herba Indica, Chandigarh and identified in the Department of Botany, Hamdard University by Dr. M.P. Sharma. A voucher specimen is deposited in our laboratory.

3.3. Extraction and isolation

Air-dried aerial parts (3 kg) were extracted (Soxhlet) with 95% EtOH (6 l × 4, 2.5 h for each extraction) at 80°C. The combined extracts were evaporated in vacuo (below 45°C) to give a residue (300 g) which was sequentially refluxed (below 45°C) with solvents of increasing polarity, viz. petrol, C_6H_6, EtOAc and MeOH. The EtOAc soluble fraction (50 g) was subjected to Silica gel (600 g) column chromatography using gradient solvent system of petroleum ether and $CHCl_3$, increasing the amount of $CHCl_3$ from 0 to 40% to get compounds **1** and **2**. The MeOH soluble fraction (20 g) was chromatographed over 400 g Silica gel column and eluted with $CHCl_3$ to obtain compound **3**.

3.4. Adiantulupanone (*1*)

Elution of the column of EtOAc soluble fraction with petroleum ether–$CHCl_3$ (8:2) gave colourless rhombic crystals of **1**, recrystallised from $CHCl_3$–MeOH (1:1), 180 mg, m.p. 212–215°C, R_f 0.69 (toluene–EtOAc, 9:1), UV ($CHCl_3$) λ_{max} nm (log ε) 239 (1.8); IR (KBr) ν_{max} cm^{-1} 2945, 2865, 1700, 1625, 1460, 1435, 1375, 1155, 1040, 970; ^{1}H-NMR (Table 1), ^{13}C-NMR (Table 2). EIMS *m/z* (rel. int.) $[M]^+$412 $C_{29}H_{48}O$ (43.8), 397 (22.2), 369 (33.1), 231 (13.8), 220 (4.9), 219 (5.3), 207 (11.0), 205 (14.4), 193 (52.3), 191 (100), 176 (10.3), 173 (23.1), 162 (19.8), 148 (32.2), 137 (33.8), 133 (18.1), 124 (30.4), 109 (35.8), 95 (75.6), 83(17.3), 81(60.1), 69 (70.0), 66 (38.1), 55 (48.0). HRMS *m/z* 412.7315; found for $C_{29}H_{48}O$; required *m/z* 412.7005.

3.5. Adiantuoleanone (*2*)

Fractions eluted with petroleum ether–$CHCl_3$ (6:4) furnished colourless compound **2**, recrystallized from petroleum ether–$CHCl_3$ (1:1), 45 mg, R_f 0.71 (toluene–EtOAc–HCOOH, 5:4:1), m.p. 268–269°C; UV ($CHCl_3$) λ_{max} nm (log ε) 240 (1.5); IR (KBr) ν_{max} cm^{-1} 3.496, 3116, 2948, 1710, 1624, 1458, 1382, 1310, 1274, 1142, 1130, 1070, 1046, 974; ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2); EIMS *m/z* (ret. int.) 428 $[M]^+$ ($C_{29}H_{48}O_2$) (64.3), 413 (7.9), 410 (8.9), 234 (6.8), 222(4.2), 220 (4.6), 208(6.1), 206 (16.2), 193 (25.3), 190 (100), 189 (13.8), 178 (11.7), 166(8.9), 163 (14.2), 152 (8.1), 150 (14.3), 138 (27.4), 136 (10.2), 126 (60.3), 124 (28.4), 111(13.2), 109 (36.5), 97(10.1), 95 (56.3), 93 (24.4), 83 (58.7), 69 (68.1), 55 (55.3). HRMS *m/z* 428.5132, found for $C_{29}H_{48}O_2$; required 428.6999.

3.6. Adiantulanosterol (*3*)

Elution of the MeOH-soluble fraction with $CHCl_3$ furnished colourless crystals of 3, R_f 0.58 (toluene–EtOAc, 95.5), (125 mg, 0.004% dry wt.) m.p. 170–172°C; $[\alpha]_D^{20}$ −29.4 (*c* 0.07, $CHCl_3$); IR (KBr) ν_{max} cm^{-1} 3420 (OH), 2905 (CH, aliphatic), 1625 (C=C), 1610, 1445, 1425, 1360, 1240, 1005, 930 cm^{-1}; ^{1}H-NMR (Table 1); ^{13}C-NMR (Table 2); EIMS *m/z* 428 $[M]^+$ ($C_{30}H_{52}O$) (5.3), 220 (10.1), 208 (5.9), 192 (68.1), 178 (30.2), 164 (30.1), 152 (16.3), 149 (56.1), 138 (96.7), 124 (6.7), 121 (26.2), 111(17.1), 109 (15.6), 97 (24.1), 95 (23.6), 91 (47.3), 84 (52.8), 83 (27.5), 69 (62.2), 55 (100). Anal. calcd. for $C_{30}H_{52}O$: C 84.11, 12.15; found C 84.78, H 12.50. HRMS *m/z* 428.7617, found for $C_{30}H_{52}O$; required 428.7432.

3.7. Adiantulanosterol monoacetate (*4*)

Compound **3** (10 mg), dissolved in 1:1 mixture of pyridine–Ac_2O, was warmed slightly. On standing overnight and on usual work-up afforded a monoacetyl derivative **4** as white crystals m.p. 155–156°C, IR (KBr) ν_{max} cm^{-1} 1725 (C=0), 1610; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ 4.13 (1H, *d*, *J* = 11.7 Hz, H30a), 4.00 (1H, *d*, *J* = 11.7 Hz, H30b), 2.07 (3H, *s*, $COCH_3$), 1.63 (3H, *s*, Me-21), 1.06 (3H, *s*, Me-29); 0.96 (3H, *s*, Me-19), 0.90 (3H, *s*, Me-28), 0.87 (3H, *d*, *J* = 6.0 Hz, Me-26), 0.83 (3H, *d*, *J* = 6.5 Hz, Me-27), 0.77 (3H, *s*, Me-18); ^{13}C-NMR (Table 2).

Acknowledgements

The authors thank Professor Allauddin Ahmad, vice chancellor, Jamia Hamadard, New Delhi for providing necessary facilities.

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