Phytochemical Studies on *Adiantum incisum*

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Summary: Four compounds were isolated for the first time from *Adiantum incisum*, along with one already reported compound, and their structures have been elucidated by EIMS, HREIMS, FAB, HRFABMS, ^{1}H and ^{13}C-NMR spectroscopic data.

Introduction

Adiantum incisum belongs to the genus *Adiantum* of family Adiantaceae. It is a fern used in Ayurvedic medicine and is well known for its antibacterial, antiviral, antifungal and other biological activities [1, 2]. *Adiantum incisum* Forssk. grows abundantly in the northern hilly areas of Pakistan [3]. Literature survey on this species shows that several classes of triterpenes have been reported [4,5]. The methanolic extract of *A incisum* showed strong phytotoxicity which prompted us to carry out phytochemical studies and four compounds were isolated for the first time from *Adiantum incisum*, their structures have been elucidated by EIMS, HREIMS, FAB, HRFABMS, ^{1}H- and ^{13}C-NMR spectroscopic data.

Results and Discussion

Repeated column chromatography of the ethanolic extract of *Adiantum incisum* resulted in the isolation of five compounds (**1-5**).

Compound (**1**) was isolated as colorless amorphous solid. The IR spectrum gave the characteristic peaks for carbonyl moiety in the molecule; the EIMS showed molecular ion peak at *m/z* 466.70, the HREIMS gave the molecular ion peak at 466.4956 consistent with the molecular formula $C_{31}H_{62}O_2$. The rest of the molecular ion peaks differed from one another by 14 mass units, indicating the presence of saturated hydrocarbon chain in the molecule. The ^{1}H-NMR showed the characteristic signal for a terminal methyl at δ 0.85. The methylene signals resonated between δ 1.15-1.25 as a broad singlet. The methylene protons adjacent to the carboxyl group resonated at δ 2.30. The ^{13}C-NMR spectrum showed 31 carbon signals. The BB and DEPT experiments showed the presence of one methyl and 29 methylene and one quaternary carbon signals. From the physical and spectroscopic data, the compound **1** was identified as hentriacontanoic acid [6].

Compound (**2**) was isolated as amorphous solid. The IR, mass and other spectral data indicated the presence of one additional double bond in the molecule. The IR bands at 1645 cm^{-1} and 1705 cm^{-1} were attributed to olefinic and carboxylic moieties respectively in the compound. The effervescence on treatment with sodium bicarbonate solution substantiated the presence of free carboxylic group. The peaks in mass spectrum showed difference of 14 mass units indicating saturated long chain in the molecule. The ^{1}H-NMR spectrum showed the olefinic signal at δ 5.36, the rest of the signals were similar to compound (**1**). The ^{13}C-NMR spectrum showed 35 carbons out of which one CH_3, 31 CH_2, two CH and one quaternary carbon signals were identified. The exact molecular mass was confirmed by HREIMS which showed peak at *m/z* 520.7343 consistent with the molecular formula $C_{35}H_{68}O_2$ and the compound was identified as 2-pentatriacontenoic acid (**2**) [6].

Compound (**3**) was isolated as colorless oil. The IR spectrum indicated the presence of ester (1740 cm^{-1}) and aromatic functionalities in the molecule. The EIMS showed the peak at *m/z* 390 while HREIMS gave the peak at 390.3798 matched with the molecular formula $C_{24}H_{38}O_4$. The ^{1}H-NMR showed signals at δ 0.87 (12H, t, J=7.0 Hz) for terminal methyls. The aromatic signals resonated at δ

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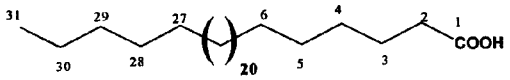
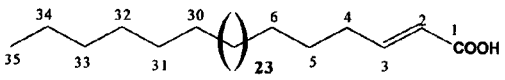
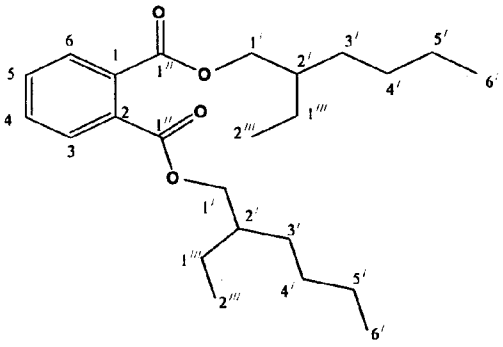


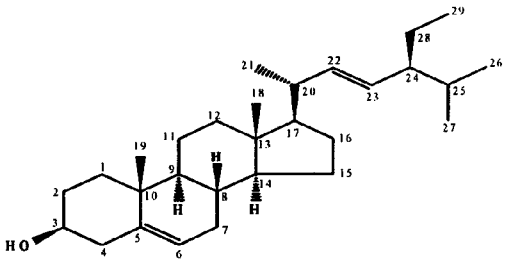
Hentriacontanoic acid (**1**)



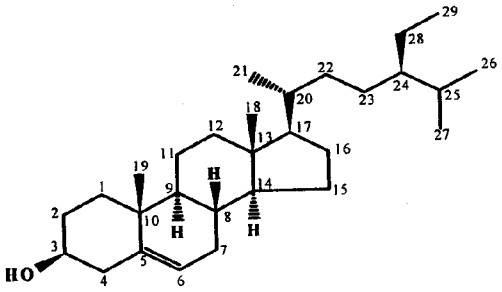
2-Pentatriacontenoic acid (**2**)



Octoil (**3**)



Stigmasterol (**4**)



β-Sitosterol (**5**)

7.72 (2H, d, *J*=7.1 and 1.2 Hz), and *δ* 7.54 (2H, dd, *J*=7.1 and 1.0 Hz), the ^{13}C-NMR 24 carbon signals in which four were CH_3, four quaternary, six methine and ten methylenes. The quaternary carbons of carbonyl group were observed at *δ* 168.2, methyl at *δ* 14.5 and *δ* 11.4 while aromatic signals were observed at *δ* 129.2 and *δ* 131.3 which confirms the dimeric nature of the molecule. The physical and spectroscopic data confirmed the compound (**3**) as Octoil [7].

Compound (**4**) was isolated as colorless needles from the ethyl acetate soluble fraction, subjected to CC over silica gel and eluted with *n*-hexane: $CHCl_3$, (8.5:1.5). The molecular formula $C_{29}H_{48}O$ was established through HREIMS showing molecular ion peak $[M]^+$ at *m/z* 412.3920 (calcd. for $C_{29}H_{48}O$, 412.3926). The nature of oxygen in **4** was shown to be hydroxyl as indicated by IR spectrum (3432 cm^{-1}). The mass spectrum showed characteristic fragmentation pattern of a Δ^5, Δ^{22} sterol [8]. The ^{1}H-NMR spectral data of **4** was consistent with that of stigmasterol [9]. It displayed signals for two tertiary methyl groups (*δ* 0.84, 0.65), two multiplets for three olefinic protons at *δ* 5.33 (1H) and 5.15 (2H) and a multiplet for the carbinylic proton at *δ* 3.28. The ^{13}C-NMR (BB and DEPT) spectrum of **4** disclosed the presence of 29 carbon signals for six methyl, nine methylene, eleven methine and three quaternary carbons. The above data confirmed **4** as stigmasterol [10].

The EIMS of compound (**5**) showed the $[M]^+$ peak at *m/z* 414, which was confirmed by FD-MS. The HREIMS showed the $[M]^+$ peak at *m/z* 414.3857 in agreement with the molecular formula

$C_{29}H_{50}O$ (calcd. for $C_{29}H_{50}O$; 414.3861) corresponding to five degrees of unsaturation. The IR spectrum showed the hydroxyl bands at 3450 cm^{-1} and bands at 3050, 1650 and 815 cm^{-1} were due to trisubstituted double bonds. In the EIMS spectrum of (**5**), characteristic fragment ions for β-sitosterol were observed at *m/z* 399, 396, 381, 329 and 303. The last two ions were diagnostic for sterols having Δ^5-unsaturation. Other important fragments were observed at *m/z* 273 and 255 for [M-side chain]$^+$ and [M-side chain-H_2O]$^+$, respectively. The ^{1}H-NMR spectrum of (**5**) was characteristic of a steroidal molecule. Two singlets integrating for 3H each appeared at δ 0.63 and 0.92 due to the quaternary CH_3-18 and CH_3-19, respectively. Three doublets integrating for 3H at δ 0.88 ($J_{21,\ 20}$ = 6.5 Hz), 0.83 ($J_{26,\ 25}$ = 6.5 Hz) and 0.81 ($J_{27,\ 25}$ = 6.5 Hz) were due to secondary CH_3-21, CH_3-26 and CH_3-27, respectively, while a triplet integrating for 3H at δ 0.77 ($J_{29,\ 28}$ = 7.0 Hz) was due to the primary CH_3-29. The olefinic signal integrating for 1H at δ 5.32 (*m*) was assigned to H-6 of the Δ^5-bond. The chemical shift and splitting pattern of the signal integrating for 1H at δ 3.36 was consistent with the H-3α and a 3β-hydroxyl function. The comparative study of its NMR spectroscopic data with reported data revealed its identity as β-sitosterol (**5**) [11,12].

Experimental

General

Column chromatography was carried out on silica gel 70-230 mesh while flash chromatography was performed on silica gel 230-400 mesh. TLC was carried out on pre-coated silica gel 60 F_{254} plates visualized under uv light or by spraying with vanillin reagent. Optical rotations were measured on a Jasco-DIP-360 digital polarimeter. UV and IR spectra were recorded on Hitachi-UV-3200 and Jasco-302-A spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR, spectra were recorded on Bruker spectrometers operating at 500, 400, 100 MHz; chemical shifts have been reported in δ ppm relative to $SiMe_4$ as an internal standard. The coupling constants are reported in Hz. The EI-, FAB-, HR-EI-, HR-FAB-MS were performed on JEOL JMS-HX-110 and JMS-DA-500 mass spectrometers, *m/z* (rel. int). The purity of the isolated compounds was checked on pre-coated high performance thin layer chromatography (HPTLC) plates (E-Merck).

Plant Material

The stem and rhizome of the plant were collected from Murree hills of Pakistan in September, 2003 and identified at the NARC (National Agriculture Research Council), the voucher specimen number is 369-B.

Isolation

The dried stem and rhizomes of plant (2.9 kg) were extracted thrice with EtOH (10 L) at room temperature. The combined ethanolic extract was evaporated under reduced pressure to obtain a thick gummy mass (51.33 g). The ethanolic extract was subjected to column chromatography over silica gel eluting with *n*-hexane, chloroform, chloroform-methanol and methanol in increasing order of polarity. The fractions obtained from *n*-hexane-chloroform (7.5:2.5) were combined and again chromatographed over silica gel using *n*-hexane-chloroform gradiently to obtain (**1**) and (**2**), respectively. The fractions obtained from *n*-hexane-chloroform (3:2) were combined and re-chromatographed over silica gel to obtain (**3**), The fractions obtained from *n*-hexane-chloroform (3.2:6.8) were combined and re-chromatographed over silica gel using *n*-hexane-chloroform gradiently as eluent to obtain (**4**) and (**5**).

Hentriacontanoic acid (**1**):

Colorless amorphous solid, (25 mg); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 2.30 (2H, *t*, H-2), 1.15-1.25 (brs, H-3-H-30), 0.85 (3H, *t*, H-31). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 180.2 (C-1), 34.31 (C-2), 29.5-31.7 (C-3- C28), 25.1(C-29), 14.5(C-31), HREIMS *m/z* 466.4956 (Calcd. for $C_{31}H_{62}O_2$, 466.8321).

2-Pentatriacontenoic acid (**2**):

Colorless amorphous solid, (22 mg); IR (KBr) ν_{max} cm^{-1}: 1705 (acid, C=O), 1645 (C=C) ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.05 (1H, *dt*, H-2), 5.36 (1H, *d*, H-3), 2.12 (2H, d*t*, H-4), 1.25-1.37 (brs, H-5-H-34), 0.88 (3H, *t*, H-35). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 172.3 (C-1), 150.5 (C-3), 124.3 (C-2), 29.4-32.4 (C-4- C33), 23.4 (C-34), 13.8 (C-35),

HREIMS *m/z* 520.7343 (Calcd. for $C_{35}H_{68}O_2$, 520.9237).

Octoil (**3**):

Colorless oil, (26 mg),. IR (KBr) ν_{max} cm^{-1}: 1740 (ester, C=O), 1155 (C-O).
^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.72 (2H, *d*, H-3,6), 7.54 (2H, d*d*, H-4,5), 4.28 (4H, *t*, H-1$^{/}$), 1.29-1.67 (18H, brs, H-2$^{/}$,3$^{/}$,4$^{/}$,7$^{/}$,1$^{///}$), 0.87 (12H, *t*, H-6$^{/}$,2$^{///}$).
^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 168.2 (C-1$^{//}$), 132.8 (C-1,2), 131.3 (C-4,5), 129.2 (C-3,6), 68.5 (C-1$^{/}$), 39.1 (C-2$^{/}$), 30.7 (C-3$^{/}$), 29.3 (C-4$^{/}$), 24.1 (C-1$^{///}$), 23.4 (C-5$^{/}$), 14.5 (C-6$^{/}$), 11.4 (C-2$^{///}$).
HREIMS *m/z* 390.3798 (Calcd. for $C_{24}H_{38}O_4$, 390.5633).

Stigmasterol (**4**):

Colorless crystalline solid from $(CH_3)_2CO$ (41 mg), MP: 170-171 °C; IR ($CHCl_3$) ν_{max} cm^{-1}: 3432 (OH), 1648 (C=C).
^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.33 (1H, m, H-6), 5.15 (1H, dd, *J* = 15.2, 8.4 Hz, H-22), 5.02 (1H, dd, *J* = 15.2, 8.6 Hz, H-23), 3.28 (1H, m, H-3), 0.90 (3H, d, *J* = 6.5 Hz, Me-21), 0.83 (3H, d, *J* = 6.6 Hz, Me-26), 0.84 (3H, t, *J* = 7.0 Hz, Me-29), 0.81 (3H, d, *J* = 6.5 Hz, Me-27), 0.80 (3H, s, Me-19), 0.65 (3H, s, Me-18).
^{13}C-NMR ($CDCl_3$, 100 MHz): δ 140.9 (C-5), 138.4 (C-22), 129.4 (C-23), 121.7 (C-6), 71.9 (C-3), 57.0 (C-14), 56.0 (C-17), 51.3 (C-24), 50.3 (C-9), 42.5 (C-13), 42.2 (C-4), 40.5 (C-20), 39.7 (C-12), 37.5 (C-1), 36.6 (C-10), 32.2 (C-8), 32.0 (C-25), 31.9 (C-7), 31.8 (C-2), 28.9 (C-16), 25.4 (C-28), 24.4 (C-15), 21.2 (C-27), 21.1 (C-21), 21.0 (C-11), 19.4 (C-19), 19.0 (C-26), 12.4 (C-18) and 12.0 (C-29).
HREIMS *m/z*: 412.3920 (Calcd. for $C_{29}H_{48}O$, 412.3926).

β-Sitosterol (**5**):

Crystallized from methanol as colorless needles (43 mg), M.P.: 144° C
IR (KBr) ν_{max} cm^{-1}: 3450 (OH), 3050 (CH), 1650 (C=C)
^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.32 (1H, *m*, H-6), 3.36 (1H, *m*, H-3α), 0.92 (3H, *s*, CH_3-19), 0.88 (3H, *d*, $J_{21,\ 20}$ = 6.5 Hz, CH_3-21), 0.83 (3H, *d*, $J_{26,\ 25}$ = 6.5 Hz, CH_3-26), 0.81 (3H, *d*, $J_{27,\ 25}$ = 6.5 Hz, CH_3-27), 0.77 (3H, *t*, $J_{29,\ 28}$ = 7.0 Hz, CH_3-29),0.63 (3H, *s*, CH_3-18).
^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 141.0 (C-5), 121.1 (C-6), 71.9 (C-3), 56.9 (C-14), 56.6 (C-17), 50.5 (C-24), 50.4 (C-9), 43.8 (C-4), 43.0 (C-13), 40.8 (C-12), 39.5 (C-22), 38.7 (C-1), 37.2 (C-20), 37.1 (C-10), 33.0 (C-7), 32.9 (C-8), 29.9 (C-2), 29.7 (C-16), 29.5 (C-23), 25.9 (C-25), 25.8 (C-15), 23.7 (C-28), 21.5 (C-11), 19.7 (C-21), 20.1 (C-27), 19.5 (C-19), 18.2 (C-26), 12.0 (C-18), 11.9 (C-29).
HREIMS *m/z*: 414.3857 (Calcd. for $C_{29}H_{50}O$; 414.3861).

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