Phytochemical studies on *Amberboa ramosa*

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Summary: Seven compounds have been isolated for the first time from *Amberboa ramosa* namely, octadecanoic acid (**1**), triacontanoic acid (**2**), (24*R*)-cycloartane-3β,30-diol (**3**), cycloart-5-ene-3β,25-diol (**4**), cycloartane-3α,24β,25-triol (**5**), saussureolide (**6**), 6,3′-dihydroxy-3,5,7,4′-tetramethoxyflavone (**7**), respectively. Their structures have been elucidated by EIMS, HREIMS, ^{1}H and ^{13}C NMR spectroscopic studies.

Introduction

The genus *Amberboa* belongs to the family Compositae and comprises six species. One of these is *Amberboa ramosa* Jafri that is an annual herbaceous plant found in India and Pakistan. The plant has tonic, aperient, febrifuge, deobstruent, cytotoxic and antibacterial activities [1]. Previously triterpenoids, flavanoids, steroids, and sesquiterpene lactones have been reported from *Amberboa* species [1,2,3]. The chloroform fraction of *A. ramosa* have now resulted in the isolation and structure elucidation of octadecanoic acid (**1**), triacontanoic acid (**2**), (24*R*)-cycloartane-3β,30-diol (**3**), cycloart-5-ene-3β,25-diol (**4**), cycloartane-3α,24β,25-triol (**5**), saussureolide (**6**), 6,3′-dihydroxy-3,5,7,4′-tetramethoxyflavone (**7**), respectively.

Results and Discussion

Column chromatography of the chloroform fraction of *Amberboa ramosa* resulted in the isolation and characterization of two fatty acids including octadecanoic acid (**1**), triacontanoic acid (**2**), three cycloartane type triterpenes namely cycloartane-3β, 30-diol (**3**), cycloart-5-ene-3β,25-diol (**4**), (24*R*)-cycloartane-3α,24β,25-triol (**5**), saussureolide, the sesquiterpene lactone (**6**) and 6,3′-dihydroxy-3,5,7,4′-tetramethoxyflavone (**7**), respectively. All of these have been reported for the first time from *Amberboa ramosa*.

Experimental

Column chromatography (CC): silica gel 70-230 mesh. and 230-400 mesh., respectively TLC: pre-coated silica gel 60 F_{254} plates. Optical rotations: Jasco-DIP-360 digital polarimeter. UV and IR spectra: Hitachi-UV-3200 and Jasco-302-A spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR, spectra: Bruker spectrometers operating at 500, 400, 125 and 100 MHz; chemical shifts δ in ppm relative to $SiMe_4$ as internal standard and coupling constants J in Hz. EI-, FAB-, HR-EI-, HR-FAB-MS: 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 of E-Merck.

Plant Material

Amberboa ramosa Jafri (Compositae), whole plant was collected in June 2002, from Karachi (Pakistan) and identified by Dr. Surraiya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, where a voucher specimen has been deposited.

Isolation

The whole plant (20 kg) was extracted with MeOH three times at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy mass (600 g). It was suspended in water and successively extracted with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The chloroform fraction (85g) was subjected to column chromatography over silica gel eluting with *n*-hexane-ethyl acetate, ethyl acetate, ethyl acetate-methanol and methanol in increasing

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$CH_3—(CH_2)_nCOOH$

1 n=16

2 n=28

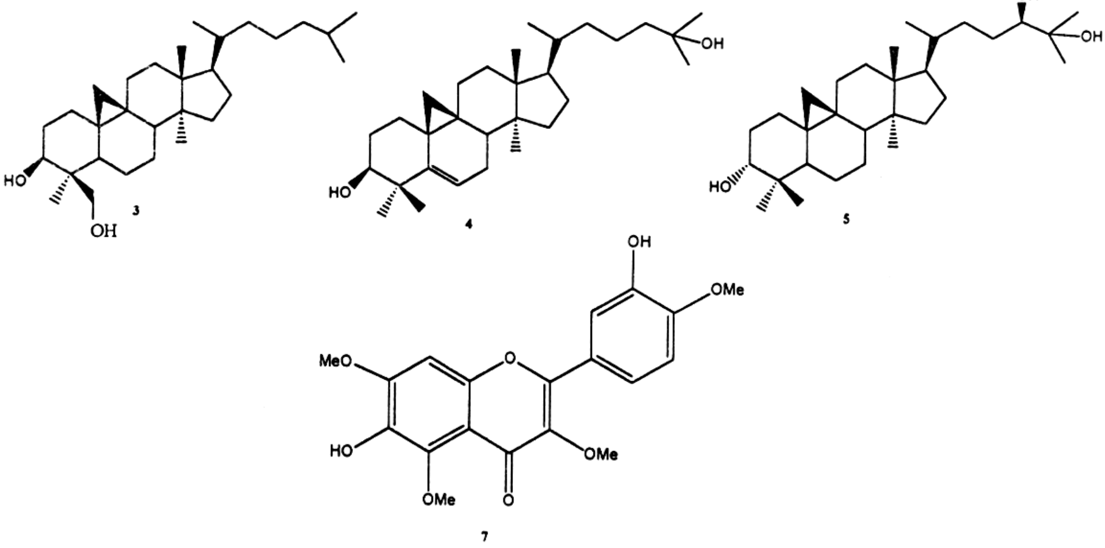

Fig. 1: Structures of compounds 1-7

order of polarity. The fractions obtained from *n*-hexane-ethyl acetate (8.6:1.4; eluted solvent = 3L) were combined and again chromatographed over silica gel using *n*-hexane-ethyl acetate (8.8:1.2) to obtain octadecanoic acid (**1**) and triacontanoic acid (**2**) from the head and tail fractions, respectively. The fractions obtained from *n*-hexane-ethyl acetate (8.0:2.0; eluted solvent = 2L) gave two major spots on TLC, were combined, and rechromatographed over silica gel using *n*-hexane-ethyl acetate (8.5:1.5) to afford cycloartane-3β,30-diol (**3**) from the head fractions and cycloart-5-ene-3β,25-diol (**4**) from the tail fractions, respectively. The fractions obtained from *n*-hexane-ethyl acetate (7.5:2.5; eluted solvent = 2L) were combined and rechromatographed over silica gel using n-hexane-ethyl acetate (7.8:2.2) as eluent to afford (24*R*)-cycloartane-3α, 24β,25-triol (**5**). The fractions obtained from *n*-hexane-ethyl acetate (6:4; eluted solvent = 3.5L) were combined and chromatographed over silica gel using *n*-hexane-ethyl acetate (6.5:3.5) to obtain saussureolide (**6**) from the head fractions and 6,3′-dihydroxy-3,5,7,4′-tetramethoxyflavone (**7**) from the tail fractions , respectively.

*Octadecanoic acid (**1**)*

It crystallized from chloroform, m.p 69-70°C; HREIMS: *m/z* 284.2715 $[M]^+$ (calcd. for $C_{18}H_{36}O_2$, 284.2709). The physical and spectral data showed complete agreement to those of octadecanoic acid (stearic acid) [4].

*Triacontanoic acid (**2**):*

It crystallized from benzene/acetone, m.p 93-94°C; HREIMS showed $[M]^+$ at *m/z* 452.4593 (calcd. for $C_{30}H_{60}O_2$, 452.4585). The physical and spectral data coincided with those of triacontanoic acid [5, 6].

Cycloartane-3β,30-diol (3):

It formed colorless crystals, m.p 195-197°C, $[\alpha]_D^{24}$ +78° ($CHCl_3$); HREIMS: *m/z* 444.3928 $[M]^+$ (calcd for $C_{30}H_{50}O_2$, 444.3954). The physical and spectral data showed complete agreement to those reported in the literature for cycloartane-3β,30-diol [7].

*Cycloart-5-ene-3β,25-diol (**4**):*

It was obtained as colorless crystals, m.p 198-199°C, $[\alpha]_D^{24}$ +69° ($CHCl_3$); HREIMS showed $[M]^+$ at *m/z* 442.3812 (calcd for $C_{30}H_{50}O_2$, 442.3808). The physical and spectral data coincided to those of cycloart-5-ene-3*β*,25-diol [8].

*(24R)-Cycloartane-3α,24β,25-triol (**5**):*

It crystallized from chloroform, m.p 201-203°C, $[\alpha]_D^{24}$ +73.7° ($CHCl_3$); HREIMS showed $[M]^+$ at *m/z* 460.3910 (calcd for $C_{30}H_{52}O_3$, 460.3916). The physical and spectral data were in complete agreement to those reported in the literature for (24*R*)-cycloartane-3*α*,24*β*,25-triol [9].

*Saussureolide (**6**):*

It was obtained as colorless gummy solid, $[\alpha]_D^{24}$ +19° (CH_3OH); HREIMS: *m/z* 298.1424 (calcd. for $C_{15}H_{22}O_6$, 298.1419). The physical and spectral data showed complete resemblance to those of saussureolide[10].

6,3′-Dihydroxy-3,5,7,4′-methoxyflavone (7):

It formed yellow crystals, m.p. 234-235°C; HREIMS: *m/z* 374.1019 (calcd. for $C_{19}H_{18}O_8$, 374.1002). The physical and spectral data coincided with those of 6,3′-dihydroxy-3,5,7,4′-tetramethoxyflavone [11].

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