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Triterpenoids and flavonoids from *Inula viscosa* (L.) Aiton (Asteraceae)

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Riassunto. *Diversi studi sono stati condotti sulla estrazione degli steroli e dei terpeni di* Inula viscosa *(L.) Aiton (Asteraceae), un rappresentante erbaceo della flora pugliese, usato come erba medicinale nella medicina popolare. Con l'ausilio di diverse tecniche, incluse NMR e spettrometria combinata GC/MS, sono stati identificati lo stigmasterolo, come il principale sterolo, e un'amirina, come il principale terpene, nell'estratto di questa pianta. Per quanto riguarda i composti flavonoidi, sono identificati il flavanone sakuranetina e due flavonoli, iaceidina e 3,3' dimetil quercitina.*

Abstract. *Various studies have been carried out on the extraction of sterols and triterpenes in* Inula viscosa *(L.) Aiton (Asteraceae), a herbaceous member of the Apulian flora, used as a medicinal herb in folk medicine. Using various techniques, including NMR and combined GC/MS spectrometry, stigmasterol as the major sterol and an amyrin as the major triterpene were identified in extracts of this plant. As far as the flavonoid compounds are concerned, the flavanone sakuranetin and two flavonols, jaceidin and 3,3' dimethyl quercetin, were identified.*

Key words: *Flavonoids,* Inula viscosa *(L.) Aiton, Sterols, Triterpenoids*

Introduction

Inula viscosa (L.) Aiton (Tribe Inulae, Asteraceae) is a herbaceous perennial plant, widespread in the eastern regions of Italy, which has long been used as a medicinal herb in folk medicine of the Mediterranean countries (Font Quer, 1973; Rigual Magallón, 1972).

Some previous papers and various screening reports have described the sterols and triterpenes present in *Inula* genus (Sethi *et al.*, 1979; Topcu & Oksuz, 1990; Burdi *et al.*, 1992; Ahmad & Ismail, 1991; Okzsuz & Topcu, 1987; Saxena *et al.*, 1984; Singha, 1983; Oksuz, 1976).

Among the terpene compounds, some authors have previously attempted to determine the nature of taraxasterol, a triterpene present in *I. viscosa* plants (De Laurentis *et al.*, 2000).

Previous works on the flavonoid content of flowers (Wollenweber *et al.*, 1991) or of the whole plant (Benayache *et al.*, 1991; Ricca & Nicotra, 1978) have been carried out. A chloroform extract of flowers of *I. viscosa* was saponified with 5% KOH to obtain a unsaponifiable lipidic fraction, which was separated as acetyl derivatives into five main groups (A-E) by a combination of CC on silica gel with hexane-ethyl acetate (7:3), preparative argentation (silver-plate) TLC and further purification by HPLC fractionation. The sterols isolated from group A showed molecular ions EIMS at m/z 412, 400 and 414 respectively. Based on mass, ^{1}H- and ^{13}C-NMR spectral data, which were in close agreement with those previously reported, compounds 1-3 were identified

as **1** stigmasterol (24-α-ethylcholesta-5,22-dien-3β-ol) (DOMINGUEZ & ZAMUDIO, 1972), **2** campesterol (24-α-methylcholest-5-en-3β-ol) (FARAPUSI & BASSIR, 1972), and **3** β-sitosterol (24 α-ethylcholest-5en-3β-ol) (ITOH *et al.*, 1982). The two sterols isolated from group B showed by NMR and MS a Δ_7, 24Δ- ethyl sterol and a Δ5, 24-ethylidene sterol corresponding to **4** 22-dihydrospinasterol (GINER *et al.*, 1989) and to **5** fucosterol respectively (HUI & LI, 1976). Band C yielded **6** moretenol, a triterpene having the hopane structure (CHANDLER *et al.*, 1982). **7** α-amyrin (urs-12-en-3Δol) (LAVIE *et al.*, 1968), **8** β-amyrin (olean-12-en-3βol) (FARAPUSI & BASSIR, 1972; LAVIE *et al.*, 1968) and **9** taraxasterol (18Δ, 19Δ-urs-20 (30)-en-3βol) (ITOH *et al.*, 1982; LAVIE *et al.*, 1968) were isolated from band D. **10** Lupeol, having a lupane structure (BAKER *et al.*, 1999), was identified from band E. The MS and ^{1}HNMR, ^{13}CNMR data of the acetates of **1-10** were in close agreement with those of authentic compounds. Three further flavonoids were identified with spectral methods in a reexamination of the chloroform extract of the flowers of *I. viscosa* (WOLLENWEBER *et al.*, 1991): comparison with authentic samples or literature data identified them as **11** hispidulin, **12** 3,3'-Di-O methyl quercetin (5,7,4'-trihydroxy-3,3'-dimethoxyflavone) and **13** sakuranetin. The aim of this work is to identify sterol, triterpene and flavonoid compounds in plants of *I. viscosa* from Apulia, Italy.

MATERIAL AND METHODS

Plant Material

Flower samples (1 kg) of *I. viscosa* were collected in October 1999 during the flowering stage in an uncultivated field in the neighbourhood of Bari, Apulia, Italy. A voucher specimen was deposited in the herbarium of the Botanical Institute of the University of Bari.

Identification procedures

Recrystallization of acetated steroids and triterpenes was obtained from acetone-methanol.

Preparative TLC: silica gel, developed for 3 h with hexane-ethylacetate (6:1).

TLC argentation: silica gel-silver nitrate (4:1) developed for 3 h with chloroform-dichloromethane (3:2).

HPLC: Shimadzu (L-C 10 AD pump; SPD M6A Diode Array detector) with a Spherisorb ODS-2μ column (Sigma-Aldrich) 25 cm x 4,6 mm i.d.

The mobile phase was methanol (flow rate: 2 ml/min), with isocratic elution.

GC-MS: (Hewlett Packard HP 6890) spectrometry system operating in EI (70 eve) mode. A HP-5 MS fused silica capillary column of 30 m x 0,25 mm i.d. was used for analysis; column temperature: 280 °C. The MS results were based on comparison of their retention indices with those ones reported in literature.

^{1}H-NMR and ^{13}C-NMR spectra were determined with a Varian EM-390 at 300 MHz and with a Bruker AC at 50 MHz instruments respectively in deuterolchloroform

Acetylation: acetic anhydride-pyrinin anhydride at room temperature overnight.

Extraction and isolation

Plant material (1 kg) was placed in a stainless steel tank and macerated in chloroform-methanol (1:1) for 48 h; the extract was then drained from the tank. This procedure was repeated 3 times. The extracts were then combined and the solvent eva-

porated in vacuo, producing a dark green waxy mass (45 g).

This material was saponified by refluxing with 5% KOH in 90% methanol for 3 h, followed by isopropyl-ether extraction to yield unsaponificable lipid (1.950 g).

The unsaponificable fraction was subjected to CC over silica gel (150 g) by elution in order of increasing polarity with 0.5 lhexane, 1.0 lhexane-ethylacetate (9:1), 1.2 lhexane-ethylacetate (9:1), 1.2 lhexane-ethylacetate (7:3), 1.0 l ethylacetate and then 0.75 l MeOH.

The first fraction eluted with hexane-ethylacetate (7:3) yielded the sterol and triterpene mix, which was subsequently acetylated: the acetate fraction (860 mg) was subjected to argentation TLC.

The acetate mix gave five bands (referred to as bands A-E in order of polarity, beginning with the least polar). The fractions recovered were subjected to further fractionation by HPLC, giving the following sterols and terpenes as their acetyl derivatives.

· Band A (Rf 0.68-0.79) 150 mg, 65 mg of which was subjected to HPLC fractionation, yielding: **1** (25 mg), **2** (10 mg) and **3** (20 mg);

· Band B (Rf 0.48-0.68) 50 mg, 15 mg of which was subjected to HPLC fractionation, yielding a mix of **4** (3 mg) and **5** (6 mg);

· Band C (Rf 0.22-0.48) 13 mg, yielding **6** (3 mg);

· Band D (Rf 0.17-0.23) 135 mg, 45 mg of which was submitted to HPLC fractionation, yielding **7** (25 mg), **8** (15 mg) and **9** (3 mg);

· Band E (Rf 0.10-0.17) 10 mg, yielding **10** (4 mg).

The MS and ^{1}H-NMR results of the acetyl derivatives of **1-10** consisted with those of authentic compounds.

Isolation of flavonoids

The dried flowers of *I. viscosa* (0.8 Kg) were macerated in ethanol at 95° in a stainless tank for a week; the extract was then drained from the tank. This process was repeated 2 times; the extracts were then combined and the solvent evaporated in vacuo, producing a yellow mass (51 g), which was defatted twice with hexane. A first portion of residue was chromatographed with hexane-ethylacetate (8:2) and then chloroform-acetone (9:1) to yield 21 mg of **11** (hyspidulin). A second portion of residue was chromatographed with hexane-ethylacetate (7:3) and then chloroform-methanol (8,5:1,5) to yield 35 mg of **12** (3,3'-di-O-methyl quercetin). The last portion yielded 25 mg of **13** (sakuranetin), after the chromatographic procedure with hexane-ethylacetate (9:1) and ethylacetate-methanol (9:1).

Sterols and triterpenoids identification

1. sterols

Stigmasterol acetate (**1**) $C_{31}H_{50}O_2$ mp 142-44° (chloroform): MS m/z; 394(M-HOAc)$^+$ (base peak), 255(53), 81(51), 69(34), 55(54).

^{1}H NMR: δ, 0.68(3H, s, H-18), 0.78(3H, d, 7.0, H-27), 0.79(3H,t, 7.5, H-29), 0.84(3H, d, 6.5, H-26), 0.99(3H, s, H-19), 1.01(3H, d, 6.5, H-21), 1.99(s, OAc-3β), 2.29-2.31(1H, br s, each, H2-4), 4,60(1H,m, H-3), 4.99(1H, dd, 8.1 and 14.7, H-23), 5.01(1H, dd, 8.6 and 15.2, H-22), 5.36(1H, br d, 4.7, H-6). ^{13}CNMR: C_1 (37.3), C_2(27.6), C_3(73.8), C_4(38.2), C_5(139.4), C_6(122.4), C_7(31.7), C_8(31.7), C_9(50.4), C_{10}(36.3), C_{11}(20.8), C_{12}(39.6), C_{13}(42.6), C_{14}(56.5), C_{15}(24.2), C_{16}(28.1), C_{17}(56.3), C_{18}(11.7), C_{19}(19.2), C_{20}(36.3), C_{21}(18.7), C_{22}(139.2), C_{23}(121.8), C_{24}(75.6), C_{25}(34.2), C_{26}(16.5), C_{27}(16.5), C_{28}(29.6),

C_{29}(11.8), acetyl (171,2 and 21,4).

Campesterol acetate (2) $C_{30}H_{50}O_2$ mp:136-137° (chloroform) MS m/z: 442(M)$^+$ (rel. int.2%), 382 (M-HOAc)$^+$ (14), 367(10), 40(2.6), 147(17), 43(100). ^{1}H NMR:δ4.60(1H, m, H-3), 1,99(1H, s, OAc-3β), 2,29, 2,31(1H, br s, each, H2-4), 5,36(1H,br, d, 4.7, H-6), 0,66(3H, s, H-18), 0,99(3H, s, H-19), 0,90(3H, d, 6.2,H-21), 0,84(3H, d 6.2, H-26), 0,79(3H, d, 6,8, H-27), 0,76(3H, d, 6.5, H28). ^{13}CNMR: C_1(38.0), C_2 (23.4), C_3 (80.9), C_4(37.5), C_5(55.10), C_6(18.20), C_7(32.4), C_8(39.8), C_9(47.2), C_{10}(36.7), C_{11}(23.50), C_{12}(121.5), C_{13}(145.1), C_{14}(41.7), C_{15}(26.7), C_{16}(26.0), C_{17}(32.3), C_{18} (47.1), C_{19}(46.7), C_{20}(36.2), C_{21}(18.5), C_{22}(33.2), C_{23}(29.8), C_{24}(38.8), C_{25}(32.4), C_{26}(20.6), C_{27} (18.6), C_{28}(15.4), acetil, (171.2 and 21.2).

Sitosterol acetate (3) $C_{31}H_{52}O_2$ mp127-128 MS m/z: 456(M)$^+$ (rel. int.3%), 396(M-HOAc)$^+$ (57),147(16),145(19), 93(15), 81(19), 57(21), 55(23), 43(100). ^{1}H NMR:δ 4,5(1H, m, H-3α), 1,99(3H, s, OAc-3β), 2.2- 2.3(1H, br s, each, H2-4), 5,34(1H,br d, 4.7, H-6), 0,67(3H, s, H-18), 0,68(3H, s, H-19), 0,90(3H, d, 6.6, H-21), 0,82(3H, d, 6.7, H-26), 0,80(3H, d, 6.7, H-27), 0,83(3H, t, 7.4, H-29).
^{13}CNMR: C_1(37.1), C_2(31.8), C_3(72.0), C_4(42.4), C_5(140.2), C_6(121.4), C_7(31.8), C_8(31.8), C_9(49.8), C_{10} (36.8), C_{11}(21.3), C_{12}(39.6), C_{13}(42.4), C_{14}(42.4), C_{15}(24.0), C_{16}(27.8), C_{17}(55.9), C_{18}(12.2), C_{19}(19.1), C_{20}(35.8), C_{21}(18.7), C_{22}(33.5), C_{23}(26.1), C_{24}(45.6), C_{25}(29.1), C_{26}(19.7), C_{27}(18.7), C_{28}(23.2), C_{29} (11.6).

2,2-Dihydrospinasterol acetate (4) $C_{31}H_{52}O_2$: mp 142-143°, MSm/z (rel. int.): 456(M)$^+$(100), 399(32), 273(31), 255(66), 231(25), 213(20); ^{1}H-NMR: δ 0,52 and 0,78 (6H, s, each, 3H, H-18 and 19), 0.89(3H, d, 6.2 , H-21), 0,80 and 0,82 (6H, s, each 3H, d, J=6,2 ,H-26 or 27), 0,83 (3H, t, 7,1, H-29). ^{13}C-NMR: C_1(37.3), C_2(27.6), C_3(73.8), C_4(38.2), C_5(29.4), C_6(122.4), C_7(141.7), C_8(32.1), C_9(50.4), C_{10}(36.3), C_{11}(20.8), C_{12}(39.6), C_{13}(42.6), C_{14}(56.5), C_{15}(24.2), C_{16}(28.1), C_{17}(56.3), C_{18}(11.7), C_{19}(19.2), C_{20}(36.3), C_{21}(18.7), C_{22}(33.8), C_{23}(27.8), C_{24}(45.6), C_{25}(29.2), C_{26}(19.5), C_{27}(18.5), C_{28}(23.6), C_{29}(11.8), acetyl (170.8 and 21.4).

Fucosterol acetate (5) $C_{31}H_{50}O_2$ mp:121-22°. MS m/z: 394(M-HOAc)$^+$ (base peak), 297(26), 296(98), 147(21), 145(37), 95(28), 80(99).
^{1}H NMR: δ 4.60(1H, m, H-3Δ), 2.00 (3H, s, OAc-3Δ), 2.10, 2.15 (2H, br s, each 1H, H-4), 5.35(1H,br d,5.0, H-6), 0.87(3H,s, H-18), 0,99(3H,s, H-19), 0,98(3H,d, 6.0, H-21), 2.10(1H, sept, 6.5,H-25), 0,96(6H, d, each 3H, 6.5, H-26 and H-27), 5.10(1H, q, 6.8, H-28), 1,57(3H, d, 7.1, H-29). ^{13}CNMR: C_1(37.0), C_2(27.6), C_3(73.6), C_4(38.3), C_5(139.4), C_6(122.4), C_7(31.8), C_8(31.8), C_9(49.8), C_{10}(36.2), C_{11}(21.02), C_{12}(39.5), C_{13}(42.4), C_{14}(56.5), C_{15}(24.2), C_{16}(28.0), C_{17}(55.2), C_{18}(11.7), C_{19}(19.2), C_{20}(36.5), C_{21}(18.9), C_{22}(35.1), C_{23}(25.8), C_{24}(46.8), C_{25}(34.7), C_{26}(22.1), C_{27} (22.1), C_{28}(15.3), C_{29}(13.2).

2. *triterpenes*

Moretenol(Hop-22(29)en-3βol) acetate (6) $C_{32}H_{52}O_2$ mp=238-39° MS m/z :468 (M)$^+$, 408(15), 202(31), 189(100), 148(11), 136(22), 122(21), 107(22), 95(28), 81(30). ^{1}H-NMR δ 0.67-1.14(18H,s, Me x 6), 1.66(3H,s, C=C-Me), 2.04(3H, s, CH3-CO), 4.50(1H, m, H3), 4.65(2H, br s, 5.2, C=CH2).^{13}CNMR: C_1 (38.5), C_2(27.5), C_3(78.6), C_4(38.7), C_5(55.1), C_6(18.1), C_7(33.9), C_8(40.9), C9(50.4), C_{10}(37.1), C_{11}(20.8), C_{12}(25.6), C_{13}(38.4), C_{14}(42.5), C_{15}(30.6), C_{16}(29.1), C_{17}(48.0), C_{18}(48.0),

C_{19}(49.2), C_{20}(38.8), C_{21}(29.8), C_{22}(140.1), C_{23}(27.8), C_{24}(16.1), C_{25}(15.9), C_{26}(16.2), C_{27}(15.2), C_{28}(28.1), C_{29}(11.2), acetyl (170.8 and 17.2).

α Amyrin acetate (7) $C_{32}H_{52}O_2$ mp 130-133° (methanol-ether) MS m/z 468(M)+, 219(35), 218(99), 189(27), 135(24), 120(24), 109(18), 107(23), 69(24), 43(28). ^{1}H-NMR δ 0.81-1.12(24H, s, Me x 8), 1.90(2H,m, CH2-CH=C), 2.00(3H, s, CH3CO), 4.45(1H, m, 15, H3), 5.10(1H, t, 3.1, CH=C). ^{13}C-NMR: C_1(38.0), C_2(23.4), C_3(80.9), C_4(37.5), C_5(55.10), C_6(18.20), C_7(32.4), C_8(39.8), C_9(47.2), C_{10}(36.7), C_{11}(23.50), C_{12}(21.5), C_{13}(45.1), C_{14}(41.7), C_{15}(26.7), C_{16}(26.0), C_{17}(32.3), C_{18} (47.1), C_{19}(46.7), C_{20}(31.2), C_{21}(34.5), C_{22}(37.2), C_{23}(27.8), C_{24}(16.8), C_{25}(15.4), C_{26}(16.6), C_{27}(25.6), C_{28}(28.4), C_{29}(33.2), C_{30}(23.6), acetil, (171.2 and 21.2).

β Amyrin acetate (8) $C_{32}H_{52}O_2$ Ms m/z 468 (M)+, 409(38), 408(31), 218(43), 205(47), 191(69), 149(63), 137(51), 123(66), 121(41), 109(100), 107(35). ^{1}H NMR Δ 0.81-1.02(21H, s, Me x 7), 1.88(2H, m, CH2-CH=C), 1.99(3H, s, CH_3 acetyl), 4.50(1H, dd, 8.6, 8.6, H-3), 5.16(1H, br s, H-12). ^{13}C-NMR: C_1(38.0), C_2(23.4), C_3(80.9), C_4(37.5), C_5(55.10), C_6(18.20), C_7(32.4), C_8(39.8), C_9 (47.2), C_{10}(36.7), C_{11}(23.50), C_{12}(121.5), C_{13}(145.1), C_{14}(41.7), C_{15}(26.7), C_{16}(26.0), C_{17}(32.3) C_{18}(47.1), C_{19} (21.2), C_{20}(31.2), C_{21}(34.5), C_{22}(37.2), C_{23}(27.8), C_{24}(16.6), C_{25}(15.4), C_{26}(16.6), C_{27}(25.6), C_{28}(28.4), C_{29}(33.2), C_{30}(33.2), acetyl(171.2 and 21.2).

Taraxasterol acetate (9), $C_{32}H_{52}O_2$ mp 250-252° (ethyl-acetate-alcohol) MS m/z 468(M)+, 409(58), 408(28), 218(9), 205(73), 191(99), 189(47), 149(47), 137(35), 123(65), 121(41), 109(100), 107(21). ^{1}H-NMR: Δ 0.83-1.02 (21H, s, Me x 7), 1.04(3H, d, 6.2, CH_3-C), 2.05(3H, s, CH_3-CO), 2.12(1H, m, CH_3-CH), 2.41(1H, m, CH_2=C-CH), 4.48(1H, t, H-3). ^{13}CNMR: C_1(38.0), C_2(23.4), C_3(80.9), C_4(37.5), C_5(55.10), C_6(18.20), C_7(32.4), C_8(39.8), C_9(47.2), C_{10}(36.7), C_{11}(23.50), C_{12}(25.5), C_{13}(38.8), C_{14}(41.7), C_{15}(26.7), C_{16}(26.0), C_{17}(32.3), C_{18}(47.1), C_{19}(46.7), C_{20}(150.2), C_{21}(34.5), C_{22}(37.2), C_{23}(27.8), C_{24}(16.8), C_{25}(15.4), C_{26}(16.6), C_{27}(25.6), C_{28}(28.4), C_{29}(108.3), C_{30}(23.6), acetyl (171.2 and 21.2).

Lupeol acetate (10), $C_{32}H_{52}O_2$ mp 163° (methanol-ether) MS m/z 468(M)+, 218(44), 189(78), 135(55), 121(60), 109(70), 95(76), 43(100). ^{1}H NMR: Δ 0.84-1.06 (21H, s, Me x 7), 1.7(3H, br, CH_3-CH=CH), 2.1(3H, s, CH_3CO), 4.61(3H, m, H-3 overlapping with C=CH_2). ^{13}CNMR: C_1(38.0), C_2(23.4), C_3(80.9), C_4(37.5), C_5(55.10), C_6(18.20), C_7(32.4), C_8(39.8), C_9(47.2), C_{10}(36.7), C_{11} (23.5), C_{12}(25.4), C_{13}(38.7), C_{14}(41.7), C_{15}(26.7), C_{16}(26.0), C_{17}(32.3), C_{18}(47.1), C_{19}(46.7), C_{20}(150.2), C_{21}(34.5), C_{22}(37.2), C_{23}(27.8), C_{24}(16.8), C_{25}(15.4), C_{26}(16.6), C_{27}(25.6), C_{28}(28.4), C_{29} (109.4), C_{30}(19.2), acetil, (171.2 and 21.2).

Hispidulin (11) was firstly eluted with chloroform-ethyl-acetate (6:4) and then chloroform-methanol (8.5:1.5), mp 290-292° C (ethanol). IR (KBr) :3300, 3085 , 1660, 1600, 1550, 1470, 1450, 990, 820 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 3.76 (3H , s, OMe), 6.58(1H, s, H-8), 6.74 (1H ,s ,H-3), 6.91(2H, d ,J=8.8 Hz, H-3'and H -5'), 7.91(2H, d, J=8.8 Hz, H-2' and H-6'), 12.85 (1H, s, 5-OH). MS, m / z (%): 300(51, (M)+), 285(33), 257(44), 139(32), 69(100).

3,3'-di-O-methyl quercetin (12) was eluted with chloroform-ethyl-acetate (3:2),

mp. 255-57°C (ethanol). IR(KBr), 3500, 1640, 1500, 1470, 1285, 1175, 810 cm^{-1}. ^{1}H-NMR (DMSO-d_6): Δ 3,83 (3H, s, OMe), 6.18(1H, d, 2.1, H-6), 6.50(1H, d, 2.1, H-8), 6.95(1H, d, 8.8, H-5'), 7.55(2H, m, H-2'and H-6'), 12.50(1H, s, 5-OH). MS, m/z(%): 330(100,$(M)^+$), 329(53), 315(45), 301(13), 287(56), 153(33), 151(43), 135(27), 108(30), 69(51).

Sakuranetin (13). ((2S)-5,4'-dihydroxy-7-methoxy flavanone) was eluted with chloroform-ethyl-acetate(6:4), m.p. 86-8°C (benzene). IR (KBr):366-2400, 1640, 1580, 1200, 835, 810 cm^{-1}. ^{1}H-NMR (CDC_{13}): δ 2.82(2H, m, 17.8, 10.9 and 4.2 H-2), 3.72(3H, s, OMe), 5.28(1H, dd, 10.8 and 4.2, H-2), 6.10(2H, s, H-6 and H-8), 6.82(2H, d, 8.8). MS, m/z(%); 286((M^+))(8.6), 193(34); 167(100); 120(58); 119(18).

Results and Conclusions

Sterols **1-5** were isolated from *I. viscosa* as acetyl derivatives using the described procedure: the red-brown colour with R1 reagent suggested the presence of a steroidal structure.

Sterol **1** (stigmasterol acetate) showed ^{1}H-NMR and mass spectra data consistent with an identification as stigmasterol (24Δ) acetate. Its proton spectrum emitted weak but distinctive signals: a triplet at δ 0.79 (H_3-29), and two double doublets due to olefinic protons at δ 5.01 (1H, J=8.6 and 15.2, H-22) and at δ 4.99 (1H, J=8.1 and 14.7, H-23). The olefinic proton (H-6) was a doublet at δ 5.36 (J=4.7) confirmed also by resonance of the ^{13}C NMR at 122.1 and 144.0 ppm.

The protonic spectrum of compound **2** (campesterol) showed six methylic signals: two singlets (H_3-18 an H_3-19) and four doublets (H_3-21, H_3-26, H_3-27, H_3-28) and a olefinic proton (H-6) at δ 5.36, confirmed by ^{13}C NMR, in accordance with a Δ_5 sterol.

The chemical shifts of ^{1}HNMR and ^{13}C NMR spectra were in accordance with the ones of an authentic sample of campesterol acetate (**2**). The protonic spectrum showed six methylic signals, two singlets (H_3-18 and H_3-19), three doublets (H_3-21, H_3-26 and H_3-27) and one triplet (H_3-29), whose chemical shifts obtained were in accordance with a Δ_5 sterol. The olefinic proton (H-6) was a multiplets at δ 5.34, confirmed by ^{13}CNMR at 122,1 and 144.1 ppm.

The sterol **4** (22-dihydrospinasterol) showed a protonic spectrum with six methylic signals, two singlets (H_3-18 and H_3-19), three doublets (H_3-21, H_3-26, and H_3-27), a triplet at δ 0.83 (H_3-29) and a olefinic proton at δ 5.12(m, H-7), whose chemical shifts were in accordance with a Δ_7 sterol. The olefinic proton (H-7) was confirmed by the resonance of the ^{13}C NMR at 122.4 (C-7)) and at 141.7 (C-8) ppm.

The compound **5** (fucosterol) showed a protonic spectrum with six methylic signals, two singlets (H_3-18 and H_3-19) and four doublets (H_3-21, H_3-26, H_3-27). The olefinic proton (H-6) was a doublet at δ 5.35 (J=5.0) and the olefinic proton (H-28) was a quartet (J=6.8) at δ 5.10, confirmed by the ^{13}C NMR at 122.4 (C-6), 139.4 (C-5) ppm and at 146.8 (C-24), 115.3 (C-28).

The compound **5** is a Δ_5 -Δ_{24} (28) sterol.

Compounds **6-10** had Rf values and coloured reactions (pink or violet) with the spray reagent R1, that suggested the presence of a triterpenoid structure. The measured values in ^{1}H and ^{13}C NMR were in accordance with those of authentic samples of moretenol, α amyrin, β amyrin, taraxasterol and lupeol acetate, respecti-

vely.

The structure of moretenol (**6**) was determined by using the data of ^{1}H NMR and spectrum seven singlet methyls at δ 0.67¸1.02, 18H, 3H each and a singlet at δ 1.66 of H-30, the presence of ddd at δ 2.88 of H-21 whose values of coupling constants (J=5.6; 11.0; 11.0) were in accordance with two equatorial/axial and axial/axial interactions. The presence of 3β-OH substitution derived by dd at δ 4.5 of H-3 whose values of c.c. (J=10.2, 4.6 Hz) were in accordance with one axial/axial and one equatorial/axial interaction. This substitution was confirmed by the resonance at 78.6 ppm of C-3 in ^{13}C NMR. The α amyrin (**7**), an urs-12-ene derivate, was determined by using the ^{1}H NMR data and spectrum: eight methyl, six singlets and two doublets (δ 0.96-1.12, 3H each, d, 6.5Hz, H-29 and H-30), a triplet at δ 5.1(J=3.1 Hz) due to H-12 and chemical shifts of C-12 at 121,5 ppm and C-13 at 138.1 ppm in the ^{13}C NMR. The presence of 3 βOH substitution was the same as for compound **6**. The compound **8** (β amyrin) resulted an olean-12-ene derivative by the analysis of chemical shifts of C-12 at 121,5 ppm and C-13 at 145,1 ppm. The protonic spectrum showed eight signals of tertiary methylic groups at δ 0.81-1.12 and a triplet at δ 5.16 (J=3,1 Hz) due to H-12. The presence of 3-OH substitution was the same as for compound **6**. The compound **9** (taraxasterol) resulted an urs-20 (30)-en derivative by the analysis of chemical shifts of C-20 at 150.2 ppm and C-29 at 108.5 ppm. The protonic specrum showed seven signals of tertiary methylic groups: six singlets (δ 0.83-1.02) and a doublet at δ 1.14(6.2 Hz) of H-29 and a singlet of methylene group at δ 2.41, a multiplet at δ 2.12 of H-19.

The compound **10** showed a lup-20 (29)-ene structure. The identification as lupeol was due to the characteristic olefinic signal of methylenic protons at δ 4.61 and 4.75 (br,s) overlapping H-3. The other signals were assigned by comparison of the data of the previous triterpenoid compounds with the ones reported in literature for lupeol.

The U.V. spectra of **11** was typical of a flavonoid compound; the shifts induced by addition of different reagents (**7-9**) and comparison of its physical and spectral data with the ones of authentic samples, allow us identify it as hispidulin.

Compound **12** was identified as a flavonol quercetin derivative; the induced shifts in the U.V. spectra allow us to conclude that **12** had two hydroxyl groups at C-4' and C-7 (**5, 14, 15**).

Compound **13** is a flavanone and it was identified as sakuranetin (M+ = 286, in agreement with the formula $C_{16}H_{14}O_5$). The ^{1}H-NMR spectra of this flavanone showed the AA'BB' system, characteristic of a 1,4-disubstituted aromatic ring (B ring) and also showed one signal due to a methoxyl group; after acetylation, **13** yielded a diacetate. Location of the OMe group at C-7 was easily deduced from the bathcromic shift of band II of the UV spectrum induced by sodium ethylate, characteristic of flavanones with one hydroxyl group at C-4' and one OMe group at C-7, as confirmed by the MS fragment.

There is a wide variety of phytosterol compounds, but the most frequent phytosterols found in nature are Δ sitosterol, campesterol and stigmasterol, which occur in free form or esterified to free fatty acids, sugars or phenolic acids.

A number of reports in literature have suggested that phytosterols may have some effects on reproductive systems and, in particular, that they possess estrogenic

activity (HANNA *et al.*, 1996), a moderate relaxant effect on isolated rabbit jejunum, an antimicrobial activity against *Bacillus subtilis* and a higher activity against *Escherichia coli* and *Staphylococcus aureus* (HARRAZ & AYAD, 1994). On the other hand, pentacyclic triterpenes showed significant anti-infiammatory and analgesic activity, a moderate antipyretic activity and an antimicrobial activity against *Fitobacteria* spp. and *Enterobacteria* spp. (DOS SANTOS *et al.*, 1999).

Therefore, further investigations should be carried out to determine the potential estrogenic and antimicrobial activity of phytosterols of *I. viscosa*.

LITERATURE CITED

AHMAD V. U. & ISMAIL N. 1991. 5-Hydroxy-3,6,7,2' 5'-pentamethoxyflavone from *Inula grantioides*. Phytochemistry, 30 (3): 1040-1041.

BAKER V. A., HEPBURN P. A., KENNEDY S. J. & JONES P. A. 1999. Safety of phytosterol esters. Part I. Assessment of oestrogenicity using a combination of in vivo and in vitro essay. Food and Chemical Toxicology, 37: 13-22.

BENAYACHE S., BENAYACHE F., DENDOUGUY H. & JAY M. 1991. Les flavonoides d'*Inula viscosa* L. Plantes Med Phytother, XXV (4): 170-176.

BURDI D. K.; HASAN M.; AHMAD V. U., QURESHI M. A. & MAHESAR M. A. 1992. Triterpene from flowers of *Inula grantioides* Boiss. J Sci Ind Res, 35 (9): 352-354.

CHANDLER R. F., HOOPER S. N., HOOPER D. L. & JAMIESON W. D. 1982. Herbal remedies of the maritime Indians:sterols and triterpenes of *Achillea millefolium* L. J Pharm Sci, 71 (6): 690-93.

DE LAURENTIS N., LOSACCO V., CORRENTE M. L., MILILLO M. A. & STEFANIZZI L. 2000. Attività antibatterica dei flavonoidi di *Inula viscosa* Ait. Acta Phytotherapeutica, III (3): 129-132.

DOMINGUEZ X. A., ZAMUDIO A. 1972. ß-Amyrin acetate and campesterol from *Pluchea odorata*. Phytochemistry, 11: 1179-1182.

DOS SANTOS M. H., DE OLIVEIRA T .T., NAGEM T. J. & OLIVEIRA J. R. 1999. Efeito de constituintes qui micos extraidos do fruto de *Rheedia gardneriana* sobre bactèrias patogenicas. Rivista Brasileira de Ciências Farmacêuticas, 35 (2): 297-301.

FARAPUSI S. I. & BASSIR O. 1972. Triterpenes from *Anstonia boonei*. Phytochemistry, 11: 3083-3084.

FONT QUER P. 1973. Plantas Medicinales. El Dioscòrides renovado. Pag. 785-790. Labor, Barcelona.

GINER J. L., MARGOT C. & DJERASSI C. 1989.Scope and regio chemical control of the allyl potassium reaction in the synthesis of sterols whit unsaturated side chains. J. Org. Chem., 54: 2117-2125.

HANNA A. G., YASSIN F. Y. S., ALLAM R. I.,YASSIN N. & EL-KASSABI I. 1996. A phytochemical screening of some Solanaceeous plants. Phytosterol and fatty acid constituents of *Solanum elaeagnifolium* and *Solanum nigrum* in addition to preliminary pharmacological and antimicrobial investigation. Egypt J. Pharm. Sci., 37 (1-6): 211-231.

HARRAZ F. M. & AYAD A. R. 1994. Phytochemical and biological investigation of *Cleome ambliocarpa* Barr.et Murb. Zagazig J. Pharm. Sci., 3 (A): 64-71.

HUI W. A. & LI M. M. 1976.Two new triterpenoids from *Rhodomyrtus tomento-*

sa. Phytochemistry, 15: 1741-1743.

Itoh T., Yoshida K., Tamura T. & Matsumoto T. 1982. Co-occurrence of c-24 epimeric 24-ethyl δ 7sterols in the roots of *Trichosanthes japonica*. Phytochemistry, 21 (3): 727-730.

Lavie D., Jain M. K. & Orebano T. O. 1968.Terpenoids VII. Constituents of *Euphorbia lateriflora* Schum. and Thonn. Phytochemistry, 7: 657-660.

Oksuz S. 1976. Psi-taraxasterol acetate from *Inula viscosa*. Planta Medica, 29 (4): 343-345.

Oksuz S. & Topcu. G. 1987.Triterpene fatty acid esters and flavonoids from *Inula Britannica*. Phytochemistry, 26 (11): 3082-3084.

Ricca G. S. & Nicotra F. 1978: Carbon-13 nuclear magnetic resonance spectra of some phytosterols. Gazz. Chim. It. 108: 713-715.

Rigual Magallòn A. 1972. Flora e vegetaciòn de la Provincia de Alicante, I.E.A. Excma. Pag. 220-224. Disputaciòn Provincial de Alicante.

Saxena V. K., Chaturvedi S. K., & Saxena G. K. 1984.Chemical constituents of bark of *Inula kappa* Acta Cienc. Indica [Ser.] Chem., 10 (1): 110-111

Sethi V. K., Gupta R. K. & Jain M. P. 1979. Chemical constituents of *Inula obtusifolia* Kerner. Indian. J. Pharm. Sci., 41 (5): 208-209.

Singha J. 1983. A study of bark of *Inula kappa*. Acta Cienc. Indica [Ser.] Chem., 9 (1-4): 24-25.

Topcu G. & Oksuz S. 1990. Guaianolides from *Inula anatolica*. Phytochemistry, 29 (11): 3666-3667.

Wollenweber E., Mayer K. & Roitman J. N. 1991 Exudate flavonoids of *Inula viscosa* Phytochemisty, 30 (7): 2445-2446.

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