New Sesquiterpenoids from the Jordanian Medicinal Plant *Inula viscosa*

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Four new and 14 known compounds have been isolated from *Inula viscosa* of Jordanian origin. The new isolates are 11(13)-eudesmen-12-oic acids, 3*β*-hydroxyilicic acid (**1**), 3α-hydroxy-*epi*-ilicic acid (**2**), 2α-hydroxyilicic acid (**3**) and 9*β*-hydroxy-2-oxoisocostic acid (**4**).

Inula viscosa (L.) Ait. *(Dittrichia viscosa* (L.) Greuter) (Compositae) is known in folk medicine to possess antiinflammatory, antipyretic, and anthelmintic properties.[1,2] Previous studies on this plant led to the isolation and characterization of flavonoids[3−5] and sesquiterpenoids.[6−11] We report here the isolation and characterization of four new sesquiterpenoids of the eudesmane type from *I. viscosa*. These are 3*β*-hydroxyilicic acid (**1**), 3α-hydroxy-*epi*-ilicic acid (**2**), 2α-hydroxyilicic acid (**3**), and 9*β*-hydroxy-2-oxoisocostic acid (**4**). They were identified and characterized by their spectral data and chemical evidence. In addition, 14 known compounds have been isolated from the plant. These are 3,3′-di-*O*-methylquercetin,[3,4] 3-*O*-acetylpadmatin,[4] 3-*O*-methylquercetin,[4] hispidulin,[4] nepetin,[5] 2-desacetoxyxanthinin,[6] inuviscolide,[6,9] 2-oxoisocostic acid,[8] ilicic acid,[7,11−13] viscic acid,[7,11] *β*-sitosterol,[14] *β*-sitosteryl glucoside,[14] 3,7,4′-trimethoxy-5,3′-dihydroxyflavone,[15] and 11α,13-dihydroinuviscolide.[16] The last two compounds are reported for the first time from *I. viscosa*.

3*β*-Hydroxyilicic acid (**1**) was obtained as a white solid and had the molecular formula $C_{15}H_{24}O_4$ as established by HRMS. EIMS showed fragments at *m*/*z* 250 and 232 due to successive losses of two H_2O molecules from the molecular ion, indicating the presence of two hydroxyl groups. The IR spectrum confirmed the presence of the OH groups (3425 cm^{-1}), COOH (1710 cm^{-1}), and C=C (1615, 945 cm^{-1}).

The ^1H NMR spectrum exhibited two methyl singlets (*δ* 0.84 and 0.88), a pair of broad one-proton singlets (*δ* 6.02 and 5.57) for an exocyclic methylene group, and a one-proton double doublet at *δ* 3.23 (*J* = 11.5, 4.4 Hz) for an α-proton geminal to a hydroxyl group. The ^{13}C NMR spectrum showed 15 carbon signals. The multiplicity of each carbon was achieved by the DEPT experiment, which revealed the presence of three methines including a hydroxylated one (*δ* 78.9), thus confirming the presence of one secondary hydroxyl group, two methyls, six methylenes, and four quaternary carbons, which include a carboxyl carbon (*δ* 168.8), a trisubstituted olefinic carbon (*δ* 146.9), and a hydroxylated carbon (*δ* 74.4), indicating that the second hydroxyl group is tertiary.

2D NMR experiments (COSY, HMQC, and HMBC) showed different correlations that helped in the full assignment of hydrogens and carbons (Tables 1 and 2). Of particular significance were the long-range correlations between H-3α and each of C-2, C-4, and C-15, which confirms the location of the hydroxyl groups at C-3 and C-4. The stereochemistry of **1** was established by ROESY experiment. Thus, the correlations between H-3α and H-2α, between H-15 and H-14, and between H-1*β* and H-14 establish the configuration of the hydroxyl groups at C-3 and C-4. The presence of the secondary hydroxyl group in **1** was further confirmed by acetylation using Ac_2O/pyridine to afford a monoacetyl derivative **1a**. The ^1H NMR spectrum of **1a** showed the presence of a three-proton singlet at *δ* 2.00 corresponding to the acetyl group. The proton geminal to the acetyl group (H-3α) was downfield shifted and appeared at *δ* 4.51. It is noteworthy that the acetate **1a** was already reported as a natural product and characterized as its methyl ester. The NMR data obtained for the compound are compatible to those reported for its methyl ester derivative.[17]

3α-Hydroxy-*epi*-ilicic acid (**2**) ($C_{15}H_{24}O_4$) was isolated as an amorphous solid. Its MS and IR spectra were similar to those of **1**. Acetylation of **2** afforded a monoacetyl derivative **2a**, thus confirming that the two compounds contained the same functional groups. Comparison of their ^1H and ^{13}C NMR spectra also indicated that the two compounds were closely related and could be diastereomers. Significantly, the chemical shift of C-15 in **2** (*δ* 26.7) compared to that in **1** (*δ* 17.1) was indicative of an equatorial methyl in **2**. Furthermore, the signal for the proton geminal to the hydroxyl group appeared as a broadened singlet, indicating an axial OH group at C-3. Extensive 2D-NMR studies (COSY, HMQC, HMBC) and NOEDS confirmed the structure of compound **2**. The most useful NOE's were observed when protons H-3*β*, H-15, and H-7α were irradiated, which led to establishing correlations between the following sets of protons: H-3*β* and each of H-2α, H-2*β*, and H-15; H-15 and each of H-3*β* and H-5α; H-7α and H-5α. The unusual chemical shifts for the H-13 signals in DMSO-d_6 (*δ* 4.93 and 5.59) are worth noting. The upfield shift of these signals could be explained as a result of solvent effects. When the spectrum of compound **2** was recorded in CD_3OD, the H-13 signals were shifted downfield to *δ* 5.29 and 5.75, which is consistent with their normal values.

It is interesting to note that both compounds **1** and **2** possess a trans diol fragment. The two compounds

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**4**

H	1	2	3	4
lα	1.14 m	0.95 m	0.93 m	2.11 d, 16.1
1β	1.35 m	1.51 br d, 12.2	1.59 br d, 10.6	2.48 d, 16.1
2α	1.38 m	1.31 m		
2β	1.52 m	2.03 m	3.60 m	
3α	3.23 dd, 11.5, 4.4		1.26 m	5.77 br s
3β		3.26 br s	1.86 m	
5α	1.14 m	1.31 br d, 12.0	1.17 br d, 12.8	2.51 m
6α	1.87 m	1.51 m	1.86 m	1.88 m
6β	1.14 m	1.29 m	1.08 m	1.24 ddd, 12.5, 12.5, 12.5
7α	2.34 m	2.45 m	2.43 m	2.61 m
8α	1.36 m	1.29 m	1.31 m	1.39 m
8β	1.52 m	1.51 m	1.56 m	1.70 m
9α	1.14 m	1.13 m	1.17 br d, 12.8	3.47 dd, 11.0, 3.2
9β	1.36 m	1.13 m	1.37 br d, 12.8	
14-CH_3	0.84 s	0.98 s	0.85 s	0.74 s
15-CH_3	0.88 s	1.01 s	0.95 s	1.88 br s
H-13	6.02 br s	5.59 br s	5.87 br s	5.97 br s
H-13	5.57 br s	4.93 br s	5.26 br s	5.44 br s

[a] *J* values are given in Hz. The chemical shifts were determined in DMSO-d_6. The values are in ppm downfield from TMS.

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**4**

carbon	1	2	3	4
1	39.4	34.9	50.5	50.2
2	28.3	25.2	63.5	197.9
3	78.9	74.0	52.6	125.6
4	74.4	72.5	70.7	162.9
5	52.7	45.5	53.6	45.6
6	26.5	26.3	25.9	27.8
7	40.1	40.0	40.0	36.8
8	27.5	27.6	27.3	35.0
9	44.5	43.9	44.6	75.3
10	34.3	33.3	33.5	42.3
11	146.9	155.1	150.8	147.6
12	168.8	172.0	172.9	169.6
13	122.2	112.6	117.4	119.9
14	19.1	18.8	19.4	10.9
15	17.1	26.7	23.4	21.8

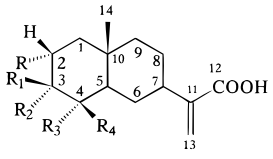
[a] The chemical shifts were determined in DMSO-d_6. The values are in ppm downfield from TMS.

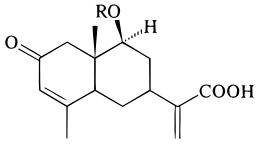
could, therefore, originate from a common precursor, the 3α,4α-epoxide, by two different openings. Such an epoxide had previously been isolated from *I. viscosa*.[7]

2α-Hydroxyilicic acid (**3**) was obtained as a white crystalline solid. The FDMS gave a molecular ion at *m/z* 268, which is consistent with a molecular formula of $C_{15}H_{24}O_4$. The EIMS did not show the molecular ion at 268 but showed instead ions that are compatible with loss of CH_3 (*m/z* 253) and H_2O (*m/z* 250) from the molecular ion. Other important peaks were *m/z* 235 $[M - H_2O - CH_3]^+$ and 232 $[M - 2H_2O]^+$. The IR spectrum of **3** showed absorption bands for hydroxyl (3455 cm^{-1}) and carboxyl (1695 cm^{-1}) groups. The ^{1}H NMR spectrum of **3** indicated the presence of an exocyclic methylene group as two broad singlets at δ 5.87 and 5.26 and a hydroxyl-bearing methine group as a multiplet at δ 3.60. This signal was shifted downfield to δ 4.83 in the monoacetyl derivative **3a**. The ^{13}C NMR spectrum exhibited 15 signals. The DEPT experiment confirmed the presence of two hydroxylated carbons at δ 63.5 and 70.7, which were assigned to C-2 and C-4, respectively, based on a combination of COSY, HMQC, and HMBC experiments. The location of the hydroxyl group at C-2 followed from the HMBC correlations between the C-14 methyl and C-1, the C-15 methyl and C-3, and each of H-lα and H-3α with C-2. The relative stereochemistry was confirmed by the ROESY spectrum in which significant correlations were observed between H-2β and each of H-1β, H-3β, the C-15 methyl and the C-14 methyl, thus establishing the α-configuration of the hydroxyl group at C-2.

9β-Hydroxy-2-oxoisocostic acid (**4**) was obtained as a gum having a molecular formula $C_{15}H_{20}O_4$ as indicated by HRMS (*m/z* 264.1363, calcd for 264.1362). It showed IR absorption bands for hydroxyl, carboxyl, and conjugated carbonyl groups at 3000, 1710, and 1685 cm^{-1}, respectively. The presence of the alcohol and carbonyl groups was also inferred from the EIMS, which showed $[M]^+$ at *m/z* 264 and prominent peaks at *m/z* 246 $[M - H_2O]^+$, 231 $[M - H_2O - CH_3]^+$, 218 $[M - H_2O - CO]^+$, and 203 $[M - H_2O - CO - CH_3]^+$. The ^{1}H NMR spectrum of **4** was consistent with the proposed structure. Besides the signals for an olefinic exomethylene (δ 5.44 and 5.97), it showed a broad singlet at δ 5.77 attributed to the olefinic proton (H-3) and a three-proton singlet at δ 1.88 for a methyl group attached to a double bond. The broadening of the H-3 signal could be accounted for by long-range coupling with the adjacent olefinic methyl group as indicated by the COSY spectrum; such coupling was indicative of the presence of the enone system.[18] The two doublets at δ 2.11 (J = 16.1 Hz) and 2.48 (J = 16.1 Hz) could be assigned to the geminal protons α to the carbonyl group in ring A, thus establishing the location of the hydroxyl group at C-9 in ring B. H-9 resonates as a double doublet (J = 11.0, 3.2 Hz) at δ 3.47. The large coupling constant is consistent with the β-orientation of the hydroxyl group. The stereochemistry at C-9 was also confirmed by the ROESY experiment. Significant is the correlation between H-1α (δ 2.11) and H-9α (δ 3.47), indicating the β-disposition of the hydroxyl group.

The ^{13}C-NMR assignments confirmed by DEPT, HMQC, and HMBC experiments are shown in Table 2 and are in agreement with the proposed structure. Finally, acetylation of **4** afforded a monoacetyl derivative **4a**, which confirmed the presence of the alcohol function in **4**.





1 R=R$_2$=H; R$_1$=R$_3$=OH; R$_4$=CH$_3$
1a R=R$_2$=H; R$_1$=OAc; R$_3$=OH; R$_4$=CH$_3$
2 R=R$_1$=H; R$_2$=R$_4$=OH; R$_3$=CH$_3$
2a R=R$_1$=H; R$_2$=OAc; R$_4$=OH; R$_3$=CH$_3$
3 R$_1$=R$_2$=H; R=R$_3$=OH; R$_4$=CH$_3$
3a R$_1$=R$_2$=H; R=OAc; R$_3$=OH; R$_4$=CH$_3$

4 R=H
4a R=Ac

Experimental Section

General Experimental Procedures. Melting points were determined using an Electrothermal Mel-Temp apparatus and were uncorrected. LRMS were obtained at 70 eV on a Finnigan MAT TSQ 70-triple quadruple instrument. HRMS (at 70 eV) and FDMS were obtained on a Finnigan MAT 711 A sector field instrument modified by AMD Intectra. IR spectra were recorded using KBr disks on an Impact 400-Nicolet IR spectrophotometer. NMR spectra were carried out on a Bruker

DPX-300 MHz (at 300 MHz for ^{1}H and 75 MHz for ^{13}C) in $CDCl_3$ and DMSO-d_6 using TMS as internal standard. Optical rotation measurements were made with a Perkin-Elmer 141 polarimeter. UV spectra were taken on a Camspec M 350 double beam UV-vis spectrophotometer. TLC was performed on silica gel 60 F254 (Merck) precoated glass plates (0.25 or 0.50 mm in thickness). Compounds were visualized under UV light or spraying with sulfuric acid−anisaldehyde spraying reagent.

Plant Material. *I. viscosa* was collected near Al-Hashimeia, west of Amman, Jordan, in October 1992, and identified by Prof. Dawud Al-Eisawi (Department of Biological Sciences, Faculty of Science, University of Jordan). A voucher specimen was deposited at the Herbarium of the University of Jordan, Amman, Jordan.

Extraction and Isolation. The dried, ground whole plant material (25 kg) was defatted with petroleum ether and then extracted extensively with ethanol (four times, 7 days each) at room temperature. The concentrated ethanolic extract (1300 g) was partitioned between $CHCl_3$ and H_2O. The dried chloroform extract was further partitioned between *n*-hexane and MeOH−H_2O (9:1). The aqueous methanol-soluble material (900 g) was chromatographed on a silica gel S (70−230 mesh, Merck) column eluted with a gradient of MeOH/$CHCl_3$ of increasing polarity to give six fractions (I−VI). Each fraction was further purified by a combination of column chromatography (silica gel, 400 mesh, Merck) and TLC using suitable solvent systems. Fraction I (80.0 g) afforded 2-desacetoxyxanthinin (400 mg), β-sitosterol (150 mg), 3,7,4′-trimethoxy-5,3′-dihydroxyflavone (15 mg), and ilicic acid (300 mg). Fraction II (40.0 g) gave inuviscolide (180 mg), 11α,13-dihydroinuviscolide (60 mg), 2-oxoisocostic acid (240 mg), and 3,3′-di-*O*-methylquercetin (100 mg). Fraction III (80.0 g) afforded 3-*O*-acetylpadmatin (100 mg), viscic acid (20 mg), and hispidulin (135 mg). Fraction IV (65.0 g) gave nepetin (200 mg), 3α-hydroxy-*epi*-ilicic acid (**2**) (15 mg), 9β-hydroxy-2-oxoisocostic acid (**4**) (17 mg), 3-*O*-methylquercetin (400 mg), 3β-hydroxyilicic acid (**1**) (60 mg), and β-sitosteryl glucoside (310 mg). Fraction V (44.0 g) afforded 2α-hydroxyilicic acid (**3**) (800 mg).

3β-Hydroxyilicic acid (1): white solid; mp 181−183 °C; $[\alpha]_D$ −36° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3425, 2925, 2350, 1710, 1615, 945 cm^{-1}; EIMS (70 eV) *m/z* $[M]^+$ 268 (17), 253 (20), 250 (7), 235 (13), 232 (10), 217 (9), 209 (100), 192 (34), 191 (98); HRMS *m/z* 268.1674 (calculated for $C_{15}H_{24}O_4$, 268.1673). Compound **1** was acetylated using Ac_2O/pyridine at room temperature for 24 h. The final product was purified to give **1a**: ^{1}H NMR (DMSO-d_6) δ 2.00 (3H, s, CH_3COO), 0.87 (3H, s, H-14), 0.99 (3H, s, H-15), 4.51 (1H, dd, *J* = 11.7, 4.8 Hz, H-3α), 5.87 (1H, br s, H-13), 5.31 (1H, br s, H-13′).

3α-Hydroxy-*epi*-ilicic acid (2) was obtained as an amorphous substance: $[\alpha]_D$ −20° (*c* 0.6, MeOH); IR (KBr) ν_{max} 3430, 2930, 2400, 1710, 1620, 950 cm^{-1}; EIMS (70 eV) *m/z* $[M]^+$ 268 (3), 253 (14), 250 (6), 235 (11), 232 (7), 217 (9), 209 (100), 192 (13), 191 (94). Compound **2** was acetylated at room temperature, and TLC purification of the residue afforded **2a**. ^{1}H NMR (DMSO-d_6) δ 2.02 (3H, s, CH_3COO) 0.98 (3H, s, H-14), 1.03 (3H, s, H-15), 4.58 (1H, broad signal, H-3β), 5.92 (1H, br s, H-13), 5.39 (1H, br s, H-13′).

2α-Hydroxyilicic acid (3) was obtained as white solid: mp 155−157 °C; $[\alpha]_D$ −18° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3455, 2920, 1695, 1680, 1335, 1295 cm^{-1}; FDMS *m/z* 268 $[M]^+$; EIMS (70 eV) *m/z* 253 (3) $[M - CH_3]^+$, 250 (12), 235 (39), 232 (100), 217 (52), 209 (5), 204 (68), 192 (5), 121 (23), 87 (100), 84 (29). Compound **3** was acetylated by using Ac_2O/pyridine for 24 h at room temperature. The final dried product was purified by TLC to give **3a**: ^{1}H NMR (DMSO-d_6) δ 1.97 (3H, s, CH_3-COO), 0.91 (3H, s, H-14), 1.01 (3H, s, H-15), 4.83 (1H, br tt, *J* = 11.7, 4.0 Hz, H-2β), 5.96 (1H, br s, H-13), 5.46 (IH, br s, H-13′).

9β-Hydroxy-2-oxoisocostic acid (4): amorphous substance; $[\alpha]_D$ +29° (*c* 0.8, MeOH); IR (KBr) ν_{max} 3000, 2600, 1710, 1685, 1640, 1625, 945 cm^{-1}; EIMS (70 eV) *m/z* $[M]^+$ 264 (11), 246 (66), 231 (14), 218 (18), 203 (23), 137 (100), 123 (84), 151 (4), 107 (28), 95 (64); HRMS *m/z* 264.1363 (calculated for $C_{15}H_{20}O_4$, 264.1362). Compound **4** was acetylated as above, and the dried residue was purified to give **4a**: ^{1}H NMR (DMSO-d_6) δ 2.02 (3H, s, CH_3COO), 0.89 (3H, s, H-14), 1.89 (3H, br s, H-15), 4.79 (1H, br d, *J* = 11.0 Hz, H-9α), 5.81 (1H, br s, H-3), 5.95 (1H, br s, H-13), 5.40 (1H, br s, H-13′) .

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References and Notes

(1) Shtacher, G.; Kashman, Y. *J. Med. Chem.* **1970**, *13*, 1221−1223.
(2) Azoulay, P.; Reynier, J. P.; Balansard, G.; Gasquet, M.; Timon-David, P. *Pharm. Acta Helv.* **1986**, *61*, 345−352.
(3) Chiappini, I.; Fardella, G.; Menghini, A.; Rossi, C. *Planta Med.* **1982**, *44*, 159−161.
(4) Grande, M.; Piera, F.; Guenca, A.; Torres, P.; Bellido, I. S. *Planta Med.* **1985**, *51*, 414− 419.
(5) Wollenweber, E.; Mayer, K.; Roitman, J. N. *Phytochemistry* **1991**, *30*, 2445−2446.
(6) Bohlmann, F.; Czerson, H.; Schönewei*β*, S. *Chem. Ber.* **1977**, *110*, 1330−1334.
(7) Ulubelen, A.; Öksüz, S.; Gören, N. *Phytochemistry* **1987**, *26*, 1223−1224.
(8) Rustaiyan, A.; Jakupovic, J.; Chau-Thi, T. V.; Bohlmann, F.; Sadjadi, *A.Phytochemistry* **1987**, *26*, 2603−2606.
(9) Sanz, J. F.; Ferrando, C.; Marco, J. A. *Phytochemistry* **1991**, *30*, 3653−3655.
(10) Bohlmann, F.; Gupta, R. K. *Phytochemistry* **1982**, *21*, 1443−1445.
(11) Ceccherelli, P.; Curini, M.; Marcotullio, M. C.; Menghini, A. *Phytochemistry* **1985**, *24*, 2987−2989.
(12) Öksüz, S.; Topcu, G. *Phytochemistry* **1992**, *31*, 195−197.
(13) Fardella, G. *Fitoterapia* **1979**, *50*, 3−4.
(14) Al-Khalil, S.; Al-Eisawi, D.; Fisher, N. *Alex. J. Pharm. Sci.* **1992**, *6*, 307−309.
(15) Kumari, G. N.; Rao, L. J .M.; Rao, N. S. P. *Proc. Indian Acad. Sci.* **1986**, *97*, 171.
(16) Jakupovic, J.; Schuster, A.; Bohlmann, F.; King, R. M.; Lander, N. S. *Phytochemistry* **1988**, *27*, 3181−3185.
(17) Zdero, C.; Bohlmann, F.; Anderberg, A.; King, R. M. *Phytochemistry* **1991**, *30*, 2643−2650.
(18) Bohlmann, F.; Jakupovic, J.; Ahmed, M.; Schuster, A. *Phytochemistry* **1983**, *22*, 1623−1636.

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