Eudesmanolides from *Wedelia prostrata*

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Five *cis*- and *trans*-eudesmanolide sesquiterpenes were isolated from *Wedelia prostrata* (Compositae) collected in Egypt and their structures were elucidated by spectroscopic methods. The conformations of the *trans*-eudesmanolides are discussed on the basis of their coupling constants in the ¹H-NMR spectra.

Key words *Wedelia prostrata*; Compositae; eudesmanolide; sesquiterpene lactone; conformation; biological activity

Chemical and biological studies of *Wedelia* species (Compositae) have led to the isolation of many kaurenoid diterpenes^{2–4)} and eudesmanolide sesquiterpenes.^{5–8)} In the course of our phytochemical studies on Egyptian plants,⁹⁾ we have investigated the constituents of the aerial parts of *Wedelia prostrata* collected in Assiut, Egypt and isolated three *trans*-eudesmanolide sesquiterpenes **1**, **2** and **3**, and two *cis*-eudesmanolide sesquiterpenes **4** and **5**. In this paper, we wish to describe the isolation and structural characterization of the two new compounds **1** and **2**, and three known compounds **3**, **4** and **5**.

The chloroform-soluble fraction of the methanol extract of *W. prostrata* was repeatedly subjected to column chromatography on silica gel, followed by preparative thin layer chromatography (PTLC) on silica gel to give a mixture of compounds **1** and **2**, together with compounds **3** (0.23% yield), **4** (0.033% yield) and **5** (0.036% yield). The mixture was further subjected to high-performance liquid chromatography (HPLC) on reversed-phase silica gel to give **1** and **2** in 0.019% and 0.022% yields, respectively. Compounds **3**, **4** and **5** were identified by comparison of the ¹H-NMR data with published values.^{10,11)}

Compound **1** has the molecular formula C₂₄H₃₄O₈ as determined by high-resolution (HR) FAB-MS (*m/z*: 451.2308 (M + H⁺)). The ¹³C-NMR spectrum of **1** showed three ester carbonyl carbons, of which one was attributed to a lactone function (163.59 ppm), and the other two to ester groups (166.68, 176.29 ppm). Furthermore, the ¹³C-NMR spectrum revealed two carbon double bonds (133.21 (t), 131.96 (s) and 127.74 (d), 139.23 (s) ppm), so the molecule was tricyclic and contained two hydroxyl groups (3450 cm⁻¹). The ¹H-NMR showed two vinyl protons (5.80, 6.61 ppm) which were characteristic of the *exo*-methylene- γ -lactone function commonly encountered

in constituents of plants of this genus.^{5–8,10,11)} The two ester groups were assigned as an isobutyrate (1.21 (3H, d, *J*=6.96 Hz), 1.24 (3H, d, *J*=6.96 Hz), 2.61 (1H, septet, *J*=6.96 Hz)) and a tiglate (1.73 (3H, d, *J*=6.60 Hz), 1.74 (3H, d, *J*=6.60 Hz), 6.77 (1H, q, *J*=6.60 Hz)). Two additional methyl groups (1.27 (3H, s), 1.30 (3H, s)) were present in the molecule. The ¹H–¹H correlation spectroscopy (COSY) spectrum of **1** indicated the presence of the partial structures –C–CH(OH)–CH₂–CH₂–C(OH)–[I] and –C–CH–CH(OCOR₁)–CH–CH(OCOR₂)–CH(OCOR₃)–C–[II]. The secondary hydroxy group of the partial structure [I] has an equatorial configuration, as shown by the coupling constants (*J*=11.36, 4.40 Hz). These data suggested compound **1** to be a eudesmanolide sesquiterpene. The stereochemistry between H₅ and H₁₅ was determined by comparison of the chemical shift values of C₅ and C₁₅ of **1** with those of the known compounds **3**, **4** and **5**, as follows. The values (δ 43.51, 14.28 ppm) of **1** were very similar to those of a *trans*-eudesmanolide **3** (δ 43.43, 14.27 ppm),¹²⁾ and were very different from those of *cis*-eudesmanolides **4** and **5** (δ 58.68, 28.95 and 59.38, 28.58 ppm, respectively). Namely, these data showed compound **1** to be a *trans*-eudesmanolide. For the confirmation of the positions of the ester groups and the stereochemistry of compound **1**, the difference nuclear Overhauser effect (NOE) spectra were taken (Fig. 1). Irradiation of the bridgehead methyl (H-15) (δ 1.30 ppm) showed enhancement of the signals at H-8 (4.8%), H-9 (6.3%), and the methine proton of the isobutyrate (1.1%),

Fig. 1. NOEs Observed in Difference NOE Experiments on Compound **1**

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Table 1. ^1H -NMR Spectral Data for **1**–**5** (in CDCl_3 , 600 MHz)

	1	2 ^{a)}	3	4	5
H-1	3.90, dd, $J=11.36$, 4.40	3.80, dd, $J=10.62$, 5.12	4.58, dd, $J=10.99$, 4.40	3.97, d, $J=4.76$	3.96, d, $J=4.76$
H-5	1.42, d, $J=2.56$	1.43, d, $J=2.57$	1.88, d, $J=2.93$	1.59, d, $J=10.99$	1.50, dd, $J=10.99$, 1.83
H-6	5.46, dd, $J=4.03$, 2.56	5.45, dd, $J=3.67$, 2.57	5.96, dd, $J=2.93$, 1.84	5.53, dd, $J=10.99$, 10.26	5.68, dd, $J=10.26$, 10.25
H-7	3.22, ddd, $J=4.03$, 3.66, 2.57	3.35, ddd, $J=3.67$, 3.66, 2.20	3.16, dddd, $J=8.06$, 4.03, 3.30, 1.84	3.25, ddd, $J=10.26$, 9.53, 2.57	3.17, dddd, $J=10.26$, 9.16, 2.93, 2.20
H-8	5.43, dd, $J=3.66$, 1.47	5.49, dd, $J=3.66$, 1.47	4.88, dd, $J=8.06$, 4.40	4.95, dd, $J=9.53$, 3.66	4.86, dd, $J=9.16$, 3.29
H-9	4.61, dd, $J=2.57$, 1.47	4.64, dd, $J=2.20$, 1.47	5.22, d, $J=4.40$	5.47, d, $J=3.66$	4.05, dd, $J=3.29$, 2.93 ^{b)}
H-13a	5.80, s	5.87, s	5.66, d, $J=3.30$	5.80, s	5.57, d, $J=2.20$
H-13b	6.61, s	6.60, s	6.24, d, $J=4.03$	6.25, d, $J=2.57$	6.22, d, $J=2.93$
H-14	1.27, s	1.30, s	1.34, s	1.35, s	1.31, s
H-15	1.30, s	1.35, s	1.36, s	1.28, s	1.16, s
1-Ester	—	—	1.99, 3H, s	—	—
6-Ester	1.21, 3H, d, $J=6.96$ 1.24, 3H, d, $J=6.96$ 2.61, 1H, septet, $J=6.96$	1.23, 3H, d, $J=6.96$ 1.28, 3H, d, $J=6.96$ 2.66, 1H, septet, $J=6.96$	1.20, 3H, d, $J=6.90$ 1.21, 3H, d, $J=6.90$ 2.58, 1H, septet, $J=6.96$	1.22, 6H, d, $J=6.96$ 2.59, 1H, septet, $J=6.96$ 2.06, s	1.185, 3H, d, $J=6.97$ 1.187, 3H, d, $J=6.97$ 2.55, 1H, septet, $J=6.97$
9-Ester	1.73, 3H, d, $J=6.60$ 1.74, 3H, s 6.77, 1H, q, $J=6.60$	1.81, 3H, d, $J=1.10$ 1.90, 3H, d, $J=7.32$ 6.18, 1H, dd, $J=7.32$, 1.10	1.93, 3H, s	—	—

a) Values in CD_3OD . b) Changed to d, $J=3.29$ and D_2O addition.

respectively. Irradiation of H-14 (δ 1.33 ppm) showed enhancement of the signal at the methine proton (2.4%) of the isobutyrate. Irradiation of H-8 (δ 5.43) showed enhancement of the signals at H-7 (4.2%) and H-9 (4.0%), respectively. Furthermore, NOEs (4.5, 13.2 and 10.2%) were observed between H-1 and H-5, between H-5 and H-6, and between H-6 and H-13a, respectively. These data indicated that the structure should be represented as **1**.

Compound **2** has the same molecular formula $\text{C}_{24}\text{H}_{34}\text{O}_8$ (HR-FAB-MS m/z : 451.2352 ($M+1$)⁺) as that of compound **1**. The ^{13}C - and ^1H -NMR spectral data of **2** were very similar to those of **1** except for those of the ester at the C-9 position, as shown in Tables 1 and 2. The ^{13}C -NMR data showed the presence of a lactonic carbonyl carbon (165.48 ppm), two ester carbonyl carbons (167.75, 177.32 ppm), and two carbon double bonds (133.57 (t), 134.28 (s) and 128.29 (d), 140.82 (s) ppm). The ^1H - ^1H COSY spectrum also indicated the presence of two partial structures [I] and [II]. The secondary hydroxy group of the partial structure [I] has an equatorial configuration as shown by the coupling constants ($J=10.62$, 5.12 Hz). Furthermore, the ^{13}C -NMR data suggested **2** to be a *trans*-eudesmanolide (δ_{C} 15.10 (C-15), 44.73 ppm (C-5)), as discussed above for **1**. The main difference of compound **2** from compound **1** is an angeloyloxy group instead of a tigloyloxy group at the C-8 position. The NOE difference spectrum as well as the ^1H - ^1H COSY spectrum supported the structure **2**, as follows. Irradiation of H-14 (δ_{H} 1.30 ppm) and H-15 (δ_{H} 1.35 ppm) caused 3.3% and 3.9% enhancement of the methine proton of the isobutyrate (δ_{H} 2.66 ppm), respectively. Furthermore, irradiation of H-15 caused enhancement of the signals at H-8 (δ 5.49 ppm) (8.2%) and H-9 (δ 4.64 ppm) (7.0%), respectively. Irradiation of H-8 produced enhancement of the signals at H-7 (δ 3.35 ppm) (8.1%), H-9 (7.9%), and H-15 (5.0%), respectively. These results indicated that the structure should be represented as **2**.

Compounds **1** and **2** have the same configuration as

Table 2. ^{13}C -NMR Spectral Data for **1**–**5** (CDCl_3 , 150 MHz)

	1	2 ^{a)}	3	4	5
C-1	70.28	71.44	73.02	84.12	85.37
C-2	27.93	28.81	24.01	28.45	28.58
C-3	41.37	42.74	41.62	28.95	29.41
C-4	71.00	71.72	71.06	87.52	87.59
C-5	43.51	44.73	43.43	58.68	59.38
C-6	74.12	75.78	68.04	72.36	72.50
C-7	43.92	44.87	44.91	41.45	41.49
C-8	64.64	66.25	72.05	74.98	77.48
C-9	81.95	83.67	70.64	70.65	70.23
C-10	44.45	45.94	41.29	47.04	47.85
C-11	131.96	134.28	134.11	135.46	135.89
C-12	163.59	165.48	169.14	168.75	169.61
C-13	133.21	133.57	119.14	124.00	124.18
C-14	25.00	24.96	26.38	22.20	22.35
C-15	14.28	15.10	14.27	28.95	28.58
6-Ester	18.50	18.96	18.42	18.92	18.98
	19.01	19.56	18.94	18.96	19.03
	34.49	35.75	34.44	34.16	34.19
9-Ester	176.29	177.32	176.19	175.90	175.78
	11.81	16.07	21.01	21.11	—
	14.48	20.51	169.34	169.40	—
	127.74	128.29	—	—	—
	139.23	140.82	—	—	—
	166.68	167.75	—	—	—
1-Ester	—	—	20.24	—	—
	—	—	170.53	—	—

a) Value in CD_3OD .

that of compound **3**. However, the coupling constants (both $J=3.66$ Hz) between H-7 and H-8 of compounds **1** and **2** are very different from that ($J=8.06$ Hz) of compound **3**. The chemical shift values of the ^{13}C -NMR signals of carbons around the lactone group (C-6, 8, 9, 10 and 13) of **1** and **2** are also very different from those of **3**, as shown in Table 2. As Ragasa *et al.* reported in the case of *cis*-eudesmanolides, the conformation of the monoesters (**1**, **2**) seems to be different from that of the corresponding diester (**3**).⁵⁾ In Dreiding models, the dihedral angle between H-7 and H-8 corresponding to the

Table 3. Calculated and Observed ^1H - ^1H Coupling Constants of **1**–**3**¹³⁾

	Chair-chair conformation				Chair-boat conformation		
	Dihedral angle (°)	Calculated (Hz)	Observed (Hz)		Dihedral angle (°)	Calculated (Hz)	Observed (Hz)
			1	2			
H ₅ -H ₆	-55	2.5	2.56	2.57	-70	0.9	2.93
H ₆ -H ₇	-60	1.9	4.03	3.67	-75	1.9	1.84
H ₇ -H ₈	-40	3.9	3.66	3.66	+25	8.6	8.06
H ₈ -H ₉	+45	5.9	1.47	1.47	-50	2.5	4.40

Fig. 2. Stereostructure of Compound **3**

coupling constant value ($J=3.66$ Hz) of **1** and **2** is about -40° . However, the dihedral angle corresponding to the coupling constant value ($J=8.06$ Hz) of **3** is about $+25^\circ$. If compound **3** exists in the chair-chair conformation, the B ring and the lactone ring would have big torsional strains. Consequently, the B ring of **3** may exist in the boat form. The ^1H - ^1H coupling constants in the chair-chair form and chair-boat form were calculated by using the Dreiding models and the extended Karplus equation, and are shown in Table 3.¹³⁾ These data support the view that compounds **1** and **2** exist in the chair-chair form and compound **3** in the chair-boat form, as shown in Figs. 1 and 2, respectively.

Finally, compounds **1** and **2** showed 80 and 87% inhibition, respectively, of the growth of HeLa S₃ cells at the concentration of 1.0 $\mu\text{g}/\text{ml}$. However, compounds **3**, **4** and **5** showed no inhibitory activity.¹⁴⁾

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus, and are uncorrected. Optical rotations were measured on a JASCO DIP-181 digital polarimeter at 25°C . UV spectra were taken in CH_3OH with a JASCO UVIDE-610 spectrometer, and IR spectra with a JASCO FT/IR-5000 spectrometer. MS and HR-MS were obtained under electron impact (EI) conditions with a Hitachi M-80 spectrometer and under FAB conditions with a JEOL DX-110 spectrometer. The ^1H -, ^{13}C -, two-dimensional (2D) NMR and difference NOE spectra were measured with JEOL α -400 and α -600 spectrometers.

Plant Material The plant was collected at Assiut, Egypt in March 1994.

Extraction and Isolation The air-dried material (3.8 kg) was powdered and extracted with MeOH at room temperature. The solvent was evaporated *in vacuo* to give a dark tar (458.7 g, 12.07% from the dried material). The MeOH extract was fractionated with hexane (11.8 g), CHCl_3 (60.4 g), EtOAc (41.0 g) and BuOH (45.0 g), successively. A part of the CHCl_3 -soluble fraction (30.0 g) was chromatographed on SiO_2 using a stepwise gradient solvent system of CHCl_3 and EtOAc (each 1 l) to give 5 fractions, 0.3 g (10:0), 5.1 g (9:1), 5.3 g (8:2), 3.5 g (5:5), and 0.3 g (0:10), followed by a mixture of CHCl_3 and MeOH (9:1) to give

a sixth fraction (15.7 g). The second fraction eluted with 10% EtOAc (3.0 g) was repeatedly chromatographed on SiO_2 with hexane-acetone followed by benzene-acetone to give crude compounds **4** and **5**. Each compound was purified by preparative TLC (SiO_2 , benzene-acetone (8:2)) to give pure **4** (92 mg) and **5** (100 mg). The fourth fraction eluted with 50% EtOAc (1.0 g) was subjected to column chromatography on SiO_2 using a gradient solvent system of hexane-acetone to give a mixture of compounds **1** and **2** (100 mg). The mixture was separated by reversed-phase HPLC (Develosil ODS-5, 2 i.d. $\times$ 25, MeOH - H_2O (80:20)) to give compounds **1** (25 mg) and **2** (30 mg), respectively. The third fraction eluted with 20% EtOAc (1.0 g) was chromatographed on SiO_2 using a mixture of benzene-acetone (20:1) and then rechromatographed on SiO_2 with benzene-ether (1:1) to give compound **3** (200 mg).

Compound 1 Colorless gum, $[\alpha]_D^{25} -21^\circ$ ($c=2.0$, CHCl_3). UV λ_{max} nm (ϵ): 213 (20400). IR (KBr): 3450, 1719 cm^{-1} . FAB-MS m/z : 451 ($\text{M}+\text{H}^+$). EI-MS m/z : 345, 262, 227, 191, 162. HR-FAB-MS: Calcd for $\text{C}_{24}\text{H}_{35}\text{O}_8$: 451.2332. Found: 451.2308. ^1H -NMR: Given in Table 1. ^{13}C -NMR: Given in Table 2.

Compound 2 Colorless needles, mp 165 – 167°C (from isopropyl ether), $[\alpha]_D^{25} -16^\circ$ ($c=2.0$, CHCl_3). UV λ_{max} nm (ϵ): 213 (14940). IR (KBr): 3400, 1717 cm^{-1} . FAB-MS m/z : 451 ($\text{M}+\text{H}^+$). EI-MS m/z : 432, 345, 262, 227, 191. HR-FAB-MS: Calcd for $\text{C}_{24}\text{H}_{35}\text{O}_8$: 451.2332. Found: 451.2352. ^1H -NMR: Given in Table 1. ^{13}C -NMR: Given in Table 2.

Compound 3 Colorless needles, mp 234 – 236°C (from ethyl ether), $[\alpha]_D^{25} +36^\circ$ ($c=1.0$, CHCl_3). UV λ_{max} nm (ϵ): 210 (9940). IR (KBr): 3504, 1769, 1727 cm^{-1} . EI-MS m/z : 392 ($\text{M}^+ - \text{CH}_3\text{COOH}$), 364, 304, 262, 244. HR-EI-MS: Calcd for $\text{C}_{21}\text{H}_{28}\text{O}_7$ ($\text{M}^+ - \text{CH}_3\text{COOH}$): 392.1834. Found: 392.1864. ^1H -NMR: Given in Table 1. ^{13}C -NMR: Given in Table 2.

Compound 4 Colorless needles, mp 116 – 117°C (from isopropyl ether), $[\alpha]_D^{25} +69^\circ$ ($c=1.66$, CHCl_3). UV λ_{max} nm (ϵ): 210 (9250). IR (KBr): 1783, 1756, 1731 cm^{-1} . EI-MS m/z : 392 (M^+), 333, 305, 244, 204. HR-EI-MS: Calcd for $\text{C}_{21}\text{H}_{28}\text{O}_7$: 392.1833. Found: 392.1812. ^1H -NMR: Given in Table 1. ^{13}C -NMR: Given in Table 2.

Compound 5 Colorless needles, mp 220 – 222°C (from isopropyl ether), $[\alpha]_D^{25} +50^\circ$ ($c=1.0$, CHCl_3). UV λ_{max} nm (ϵ): 212 (8330). IR (Nujol): 3518, 1765, 1707 cm^{-1} . EI-MS m/z : 350 (M^+), 293, 262, 234, 204. HR-EI-MS: Calcd for $\text{C}_{19}\text{H}_{26}\text{O}_6$: 350.1728. Found: 350.1742. ^1H -NMR: Given in Table 1. ^{13}C -NMR: Given in Table 2.

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References and Notes

- On leave from the Faculty of Pharmacy, Assiut University, Assiut, Egypt.
- MacLeod J. K., Lewis I. A. S., Moeller P. D. R., Oelrichs P. B., *J. Nat. Prod.*, **53**, 1256 (1990).
- Yang L.-L., Yen K.-Y., Konno C., Oshima Y., Kiso Y., Hikino H., *Planta Medica*, **52**, 499 (1986).
- Schteingart C. D., Pomilio A. B., *J. Nat. Prod.*, **47**, 1046 (1984).
- Ragasa C. Y., Padolina W. G., Bowden B. F., Li S., Tapiolas D. M., Coll J. C., *J. Nat. Prod.*, **56**, 386 (1993).
- Herz W., Sosa V. E., *Phytochemistry*, **25**, 1481 (1986).
- Bohlmann F., Ang W., King R. M., Robinson H., *Phytochemistry*, **23**, 2069 (1984).
- Bohlmann F., Zdero C., King R. M., Robinson H., *Phytochemistry*,

- 21**, 2329 (1982).
- 9) El-Emary N. A., Makboul M. A., Abdel-Hafiz M. A., *Bull. Pharm. Sci. Assiut University*, **13**, 51 (1990).
- 10) Bohlmann F., Ziesche J., King R. M., Robinson H., *Phytochemistry*, **20**, 751 (1981).
- 11) Bohlmann F., Zdero C., Jakupovic J., Ates N., King R. M., Robinson H., *Justus Liebigs Ann. Chem.*, **1983**, 1257.
- 12) Ober A. G., Fisher N. H., *Phytochemistry*, **26**, 848 (1987).
- 13) Haasnoot C. A. G., de Leeuw F. A. A. M., Altona C., *Tetrahedron*, **36**, 2783 (1980).
- 14) The details will be reported elsewhere.