

Eudesmanolides from *Wedelia prostrata*

Salwa F. FARAG,^{a,1)} Nasr A. EL-EMARY,^b and Masatake NIWA^{*,a}

Faculty of Pharmacy, Meijo University,^a Tempaku, Nagoya 468, Japan and Faculty of Pharmacy, Assiut University,^b Assiut 71526, Egypt. Received September 14, 1995; accepted December 8, 1995

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%),

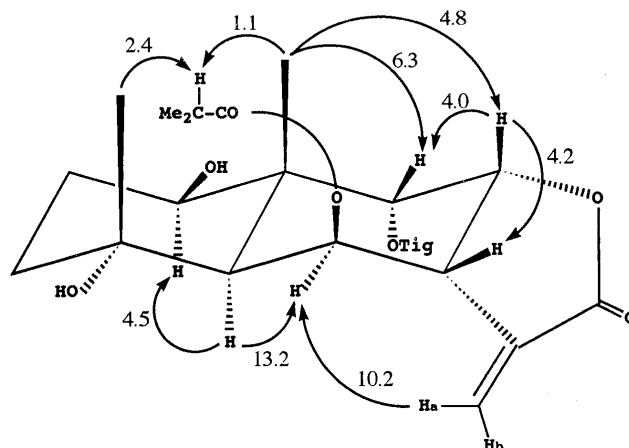
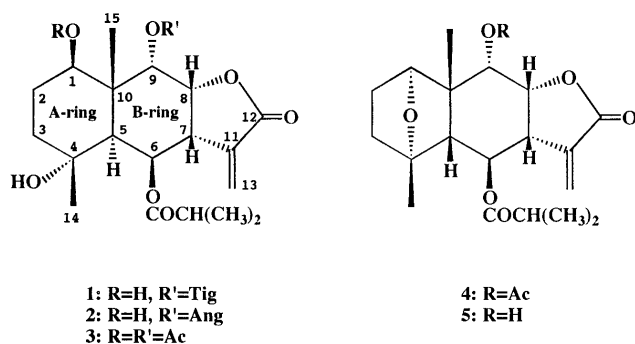


Fig. 1. NOEs Observed in Difference NOE Experiments on Compound **1**

* To whom correspondence should be addressed.

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, $J=6.96$ 1.21, 3H, d, $J=6.96$ 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 ($\text{M}+1$)⁺) 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)

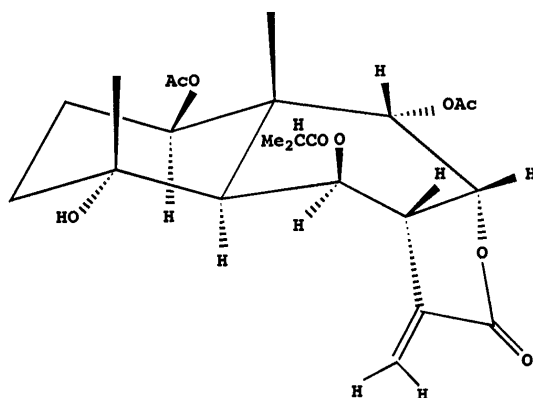
	1	2 ^{a)}	3	4	5
C-1	70.28	71.44	73.02	84.12	85.37
C-2	27.93	28.81	24.01	28.45	28.58
C-3	41.37	42.74	41.62	28.95	29.41
C-4	71.00	71.72	71.06	87.52	87.59
C-5	43.51	44.73	43.43	58.68	59.38
C-6	74.12	75.78	68.04	72.36	72.50
C-7	43.92	44.87	44.91	41.45	41.49
C-8	64.64	66.25	72.05	74.98	77.48
C-9	81.95	83.67	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, $[\alpha]_D -21^\circ$ ($c=2.0$, 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), $[\alpha]_D -16^\circ$ ($c=2.0$, 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), $[\alpha]_D +36^\circ$ ($c=1.0$, 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), $[\alpha]_D +69^\circ$ ($c=1.66$, 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), $[\alpha]_D +50^\circ$ ($c=1.0$, 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.

Acknowledgements The authors wish to thank Dr. K. Kato of Nippon Kayaku Co., Ltd. for the bioassay tests, Dr. Y. Terada and Mr. K. Terashima of Meijo University for the calculation of coupling constants, and Miss. J. Ito of Meijo University for the measurements of MS. One of the authors (S. F. Farag) is grateful to the Egyptian Government for the award of a scholarship.

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.