Eudesmanolides from *Dimerostemma vestitum*

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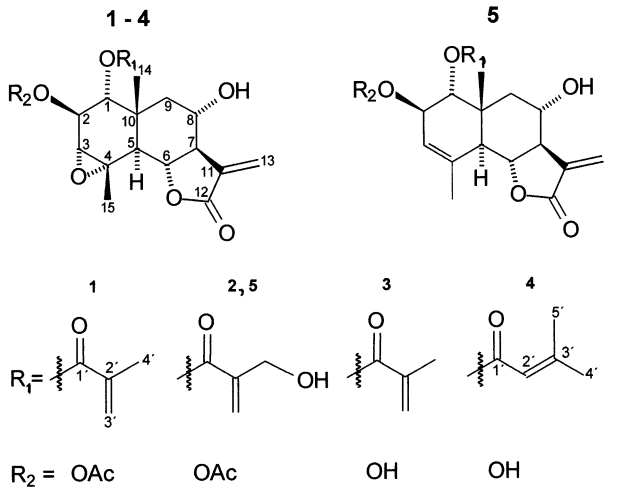
A leaf rinse extract with dichloromethane from *Dimerostemma vestitum* afforded five new eudesmanolides (**1**−**5**) along with the known flavone eupafolin. Compounds **1**−**5** were isolated by chromatographic methods, and their structures were elucidated by spectral analyses. Eudesmanolides **1**−**4** show a functional group substitution pattern so far unreported in the subtribe Verbesininae (tribe Heliantheae) of the family Asteraceae.

Dimerostemma Cass. (Asteraceae, tribe Heliantheae) is a small South American genus[1] that is placed in the subtribe Verbesininae,[2] and so far only four Brazilian species therein have been investigated chemically.[3−6] Among other terpenoids, these species contained mainly eudesmanolides with a substitution pattern not observed in any other genus.[3−6] Due to their unusual 4,15-epoxide rings, some of these eudesmanolides have been named dimerostemmolides.[3] An additional Brazilian member of this genus, *D. vestitum* (Baker) S. F. Blake,[1] has now been investigated. In addition to the known flavone eupafolin, the leaf rinse extract afforded five new eudesmanolides (**1**−**5**) with the general substitution pattern exhibited by isolates from other members of the genus. However, **1**−**4** showed a slightly changed substitution pattern so far undetected in the subtribe Verbesininae, which distinguish them from the dimerostemmolides. The structures of **1**−**5** were determined by means of spectral data interpretation. The structure of eupafolin was determined by means of UV and ^{1}H NMR spectroscopy, as well as comparison with data from the literature.[7]



A particular substitution pattern of the eudesmanolides isolated from the previously investigated *Dimerostemma* species includes a C-8α-OR group and a C-1α-ester residue. These features were also observed in **1**−**5** after comparison of their NMR spectral data with related compounds.[3−6] However, all of them showed a C-8α-OH group. The NMR spectral data of **1**−**4** indicated that these compounds differ among each other only in the nature of the ester group attached at C-1. In addition, **1**−**4** varied from one another by the presence of either a *β*-oriented hydroxyl or acetoxyl moieties at C-2. Besides other minor changes, the main feature that distinguished **1**−**4** from the dimerostemmolides is the position of the epoxide ring. While the former show a 4,15-epoxide ring (α- or *β*-oriented),[3−6] those from *D. vestitum* exhibit an α-oriented 3,4-epoxide ring.

The ^{1}H NMR spectral data of **1** showed two doublets of doublets at δ 5.96 (J = 3.0, 0.5 Hz) and 6.20 (J = 3.3, 0.5 Hz), which are typical for the H-13 exocyclic methylene protons of an unsaturated *γ*-lactone containing an α-oriented hydroxyl group at C-8.[8] The presence of signals at δ 57.5 and 59.9 (with the latter correlated to a 1H doublet of doublets at δ 3.26 in the HMQC spectrum) in the ^{13}C NMR spectrum suggested that **1** contains an epoxide ring.[9] The position of the epoxide ring was determined by inspection of molecular models and by ^{1}H−^{1}H COSY and ^{1}H−^{13}C HMBC correlations. The doublet of doublets at δ 3.26 (J = 6.0, 1.0 Hz) was assigned to H-3, which was coupled with a doublet of doublets at δ 5.03 (J = 6.0, 1.3 Hz) assigned to H-2. A correlation between C-15 (δ 23.4) and H-3 was observed in the HMBC spectrum. The presence of an acetoxyl moiety at C-2 was proposed due to the deshielding effect of this group in the H-2 signal and the presence of a 3H singlet at δ 2.11. Two quartets of doublets at δ 6.12 (J = 2.8, 1.0 Hz) and 5.78 (J = 2.5, 1.0 Hz), as well as a 3H double doublet at δ 1.96 (J = 2.8, 2.5 Hz) indicated a methacrylate side chain at C-1, as observed in other eudesmanolides from the genus *Dimerostemma*.[3−6] The substitution pattern at C-1 and C-2 is also supported by the more downfield chemical shift exhibited by C-1 (δ 75.3), since the methacrylate group causes a more intense deshielding effect than the acetate group at C-2 (δ 70.5). Moreover, in the HMBC spectrum the carbonyl carbon of the methacrylate residue (δ 169.9) showed coupling to a doublet of doublets at δ 4.67, which was assigned to H-1, which was further coupled to the signals at δ 5.03 (H-2) and 3.26 (H-3) as could be observed in the ^{1}H−^{1}H COSY spectrum. The H-3 signal also showed a *W* coupling (J = 1.0 Hz) with the doublet of doublets at δ 4.67 (J = 1.3, 1.0 Hz) assigned to H-1, which in turn coupled with H-2

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Table 1. ^{1}H NMR Spectral Data of **1**−**5** (400 MHz, $CDCl_3$)

H	1	2	3	4	5
1	4.67 dd (1.3, 1.0)	4.73 dd (1.3, 1.0)	4.63 dd (1.3, 1.0)	4.59 dd (2.5, 1.3)	4.80 dd (1.3, 1.0)
2	5.03 dd (6.0, 1.3)	5.06 dd (4.8, 1.3)	4.06 dd (6.0, 1.3)	4.06 dd (5.0, 2.5)	5.00 (s) br
3	3.26 dd (6.0, 1.0)	3.22 dd (4.8, 1.0)	3.17 dd (6.0, 1.0)	3.12 dd (5.0, 1.3)	5.40 (s) br
5	2.38 d (11.7)	2.39 d (11.6)	2.39 d (11.6)	2.38 d (11.4)	2.55 d (11.6)
6	4.20 dd (11.7, 10.9)	4.21 t (11.6)	4.20 dd (11.6, 10.9)	4.20 dd (11.4, 10.6)	3.92 dd (11.6, 11.6)
7	2.58 dddd (10.9, 7.6, 3.3, 3.0)	2.59 dddd (11.6, 7.6, 3.3, 3.0)	2.60 dddd (10.9, 7.9, 3.3, 3.0)	2.62 dddd (11.4, 10.6, 3.3, 3.0)	2.50 dddd (11.6, 6.0, 3.3, 3.0)
8	4.21 ddd (11.4, 7.6, 4.3)	4.18 ddd (7.6, 6.1, 4.6)	4.22 ddd (9.6, 7.8, 4.3)	4.20 ddd (10.6, 8.3, 4.3)	4.18 ddd (6.0, 4.8, 2.3)
9a	1.65 dd (11.7, 4.3)	1.64 dd (12.4, 6.1)	1.65 dd (12.6, 4.3)	1.64 dd (12.6, 8.3)	1.64 dd (12.4, 4.8)
9b	1.48 dd (11.7, 11.4)	1.48 dd (12.4, 4.6)	1.48 dd (12.6, 9.6)	1.53 dd (12.6, 4.3)	1.48 dd (12.4, 2.3)
13a	6.20 dd (3.3, 0.5)	6.20 d (3.3, 0.7)	6.20 dd (3.3, 0.5)	6.19 dd (3.0, 0.5)	6.20 dd (3.3, 0.7)
13b	5.96 dd (3.0, 0.5)	5.96 d (3.0, 0.7)	5.96 dd (3.0, 0.5)	5.95 dd (3.3, 0.5)	5.94 dd (3.0, 0.7)
14	1.25 s (br)[a]	1.25 s (br)[a]	1.28 s (br)[a]	1.21 s (br)[a]	1.25 s (br)[a]
15	1.54 s[a]	1.58 s (br)[a]	1.60 s[a]	1.59 s (br)[a]	1.91 s (br)[a]
2′				5.71 q (1.3)	
3′	5.78 dq (2.5, 1.0)	5.94 s (br)	6.12 dq (2.8, 1.0)		5.83 s (br)[a]
4′	1.96 dd[a] (2.8, 2.5)	4.37 s (br)[b]	1.96 q[a] (2.8, 2.5)	2.19 d[a] (1.3)	4.37 s (br)[b]
5′				1.96 d[a] (1.3)	
3″	6.12 dq (2.8, 1.0)	6.27 s (br)	5.63 dq (2.5, 1.3)		6.27 s (br)
OAc	2.11 as	2.04 s[a]			2.01 s (br)[a]

[a] Integrated to 3H. [b] Integrated to 2H.

(equatorial−equatorial coupling, $J = 1.3$ Hz). The equatorial coupling constants exhibited by H-1, H-2, and H-3, together with correlations observed in the ^{1}H−^{1}H COSY spectrum as well as data from analogous compounds,[5,6] led to the proposal of the orientation of these hydrogen atoms as β, α, and β, respectively. The NOEs observed between H-2/H-1 and H-2/H-3 after irradiation of the H-2 signal confirmed their relative configurations. The chemical shifts, coupling constants, and data from the ^{1}H−^{1}H COSY, and NOE spectra enabled data to be assigned for the H-5 to H-9 positions, whose NMR signals were in accordance with those shown by analogous compounds in the literature.[3−6] HMQC and HMBC correlations permitted the complete assignments of that ^{1}H and ^{13}C NMR spectra of **1**. The ESIMS data showed a $[M + Na]^+$ ion at *m*/*z* 429, which is consistent with the assignment of **1** as the new eudesmanolide 1α-methacryloyloxy-2β-acetoxy-3α,4α-epoxy-8α-hydroxyeudesm-11(13)-en-6α,12-olide. The HRMS showed a molecular ion corresponding to the proposed molecular formula $C_{21}H_{26}O_8$.

Compound **2** differs from **1** only in the nature of an ester residue, and its ^{1}H NMR data showed typical signals of a 4-hydroxymethacrylate side chain attached at C-1 instead of those from a methacrylate ester unit. The chemical shifts, coupling constants, and the ^{1}H−^{1}H COSY, HMQC, and HMBC data were also almost identical to those of **1**. The ESIMS data showed a $[M + Na]^+$ ion at *m*/*z* 445, thus confirming **2** as the 4-hydroxymethacrylate analogue of **1**. The HRMS showed a molecular ion corresponding to the proposed molecular formula $C_{21}H_{26}O_9$.

Compound **3** lacked an acetate singlet at δ 2.11 in the ^{1}H NMR spectrum and showed the typical signals of a methacrylate ester residue attached at C-1. The presence of the β-oriented hydroxyl group attached to C-2 was deduced on the basis of the chemical shift of H-2 (δ 4.06) instead of the downfield signal at δ 5.03 (**1**) or 5.06 (**2**). The coupling constants ($J_{2,3} = 6.0$ Hz and $J_{1,2} = 1.3$ Hz) confirmed the β-orientation of the hydroxyl group at C-2. The ESIMS data showed a $[M + Na]^+$ ion at *m*/*z* 387.11, thus confirming **3** as an analogue of **2**. The HRMS showed a molecular ion corresponding to the proposed molecular formula $C_{19}H_{24}O_7$.

Compound **4** differs from compound **3** only in the nature of the ester residue. The ^{1}H NMR spectrum showed two 3H doublets at δ 1.96 ($J = 1.3$ Hz) and 2.19 ($J = 1.3$ Hz), which were typical of the two olefinic methyl groups of the senecionate ester residue.[9] The signal at δ 5.71 was suggested by the olefinic hydrogen of this ester residue. The ESIMS data showed a $[M + Na]^+$ ion at *m*/*z* 401 and confirmed **4** as the senecionate analogue of **3**. The HRMS showed a molecular ion corresponding to the proposed molecular formula $C_{20}H_{26}O_7$.

Compound **5** lacked a 3,4-epoxide ring, as could be observed in the ^{1}H NMR spectrum. The presence of a 3,4-double bond was supported by the presence of a broad singlet at δ 5.40 typical of an olefinic hydrogen, which was assigned to H-3. This assignment was also supported by the ^{1}H−^{1}H COSY and HMQC data. The ^{1}H NMR spectrum of **5** showed a large 3H singlet at δ 1.91, which was attributed to an olefinic methyl hydrogen and assigned to H-15. The ^{1}H NMR spectrum of this compound also showed another 3H singlet at δ 2.01, which was assigned to an acetate residue attached to C-2. The position of the acetate unit was confirmed by the H-2 signal (broad singlet), which was shifted downfield to δ 5.00. The ^{1}H NMR spectrum also showed typical signals of a 4-hydroxymetacrylate ester residue attached at C-1. The ESIMS data showed a $[M + Na]^+$ ion at *m*/*z* 429 and confirmed the proposed structure for **5**. The HRMS showed a molecular ion corresponding to the proposed molecular formula $C_{21}H_{26}O_8$. This compound is the OAc-2 derivative of a known eudesmanolide previously reported from *D. brasilianum*.[5]

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Schmidt-Haensch Polartronic HH8 polarimeter, using 1.0 dm cells. IR spectra were recorded on a Nicolet FT-IR Protégé 520 instrument. NMR spectra were recorded on a Bruker ARX 400 spectrometer. Samples were dissolved in $CDCl_3$, and the spectra were calibrated at the solvent signals at δ 7.26 (^{1}H) or δ 77.0 (^{13}C). The optimizations in the HMBC spectra were as follows: $J_{CH} = 147$ Hz and $J^3_{CH} = 8.2$ Hz. ESIMS data were obtained using a Micromass Quattro LC system. HRMS data were obtained in a Micromass QTOF hybrid quadrupole orthogonal time-of-flight mass spectrometer operating at 7.000 mass resolution at UNICAMP, Campinas, SP. Spectra were taken using positive-ion electrospray ionization from 1:1 H_2O−MeOH solutions with the addition of a few microliters of formic acid. Vacuum-liquid chromatography (VLC) was carried out with Si gel 100−200 mesh ASTM (Merck), in a stepwise gradient system (100% hexane to 100% EtOAc), in glass columns with 5−10 cm i.d.

Flash chromatography was carried out with Si gel 230−400 mesh (Merck) in a 450 × 25 mm glass column, using an isocratic system, hexane−EtOAc (1:1) at 5 mL/min. Semi-preparative HPLC separations were carried out on a Shimadzu SCL-10 AVP liquid chromatograph system equipped with a SPD-M10AVP Shimadzu UV-DAD detector (the channels were set at 225 and/or 265 nm) and a Shimadzu column (ODS, 250 × 4.6 mm, 5 μm).

Plant Material. The leaves of *Dimerostemma vestitum* were collected by Prof. Dr. N. P. Lopes and F.B.D.C. in February 1998 at Tangará da Serra, MT, Brazil, and were identified by the taxonomist Prof. Dr. J. N. Nakajima, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil. A voucher specimen is on deposit at the Herbarium SPRF of Departamento de Biologia da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil, under the code SPRF 4614 (=N.P.L. collector's book # N.P.L. 121).

Extraction and Isolation. An aliquot (50 g) of dried leaves was rinsed with CH_2Cl_2 at room temperature for 5 min to obtain 4.1 g of a crude extract after solvent evaporation. This material was dissolved in MeOH−H_2O (4:1) to give 2.1 g of an organic-soluble residue after wax precipitation and solvent evaporation. This cleaned-up residue was then separated by VLC to give nine fractions of 300 mL each. Fractions 6 (306 mg) and 7 (830 mg) were found to contain sesquiterpene lactones via IR spectral analyses. These fractions were further separated by repeated flash column chromatography. The resulting subfractions were finally purified by reversed-phase HPLC by repeated injections of the selected fractions (ODS column, gradient elution, flow rate 1.0 mL/min; t = 0, 50% MeOH/H_2O; t = 30 min, 100% MeOH, hold at 100% for 5 min) to give the pure sesquiterpene lactones **1** (3 mg), **2** (5 mg), **3** (3 mg), **4** (5 mg), and **5** (9 mg), as well as 6 mg of the flavone eupafolin.

1α-Methacryloyloxy-2*β*-acetoxy-3α,4α-epoxy-8α-hydroxyeudesm-11(13)-en-6α,12-olide (1): amorphous powder; $[\alpha]^{25}_D$ +7.14° (*c* 5.6, MeOH); IR ($CHCl_3$) ν_{max} 3410, 2928, 1756, 1719, 1670, 1637, 1573, 1390, 1237, 1158, 1070, 970, 956, 756 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* [M + Na]$^+$ 429.19; HRMS *m*/*z* [M + Na]$^+$ 429.1526 (calcd for $C_{21}H_{26}O_8Na$, 429.1525); retention time (t_R) relative to 2,5-dimethylphenol (DMP) on HPLC (MeOH−H_2O, 55:45, 1 mL/min): 0.84.

1α-(4-Hydroxymethacryloyloxy)-2*β*-acetoxy-3α,4α-epoxy-8α-hydroxyeudesm-11(13)-en-6α,12-olide (2): amorphous powder; $[\alpha]^{25}_D$ −43.33° (*c* 0.6, MeOH); IR ($CHCl_3$) ν_{max} 3408, 2939, 1757, 1719, 1668, 1637, 1573, 1381, 1238, 1152, 1056, 968, 756 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* [M + Na]$^+$ 445.27; HRMS *m*/*z* [M + Na]$^+$ 445.1469 (calcd for $C_{21}H_{26}O_9Na$, 445.1475); t_R relative to DMP on HPLC (mobile phase MeOH−H_2O, 55:45, flow 1 mL/min): 0.77.

1α-Methacryloyloxy-2*β*,8α-dihydroxy-3α,4α-epoxyeudesm-11(13)-en-6α,12-olide (3): amorphous powder; $[\alpha]^{25}_D$ +26.84° (*c* 3.8, MeOH); IR ($CHCl_3$) ν_{max} 3443, 2930, 2860, 1767, 1715, 1635, 1404, 1387, 1285, 1245, 1153, 1069, 961, 754 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* [M + Na]$^+$ 387.11; HRMS *m*/*z* [M + Na]$^+$ 387.1437 (calcd for $C_{19}H_{24}O_7Na$, 387.1420); t_R relative to DMP on HPLC (MeOH−H_2O, 55:45, 1 mL/min): 0.40.

1α-Senecioyloxy-2*β*,8α-dihydroxy-3α,4α-epoxyeudesm-11(13)-en-6α,12-olide (4): amorphous powder; $[\alpha]^{25}_D$ +29.37° (*c* 3.2, MeOH); IR ($CHCl_3$) ν_{max} 3408, 2980, 1758, 1721, 1631, 1440, 1392, 1297, 1269, 1155, 1145, 1123, 1070, 1011, 968, 757 cm^{-1}; ^{1}H and ^{13}C data, see Tables 1 and 2; ESIMS *m*/*z* [M + Na]$^+$ 401.28; HRMS *m*/*z* [M + Na]$^+$ 401.1612 (calcd for $C_{20}H_{26}O_7Na$, 401.1576); t_R relative to DMP on HPLC (MeOH−H_2O, 55:45, 1 mL/min): 0.45.

Table 2. ^{13}C NMR Spectral Data of **1**−**5** (100 MHz, $CDCl_3$)

C	1	2	3	4	5
1	75.3	75.5	77.7	76.5	75.5
2	70.5	70.5	69.0	69.4	70.5
3	59.9	59.9	61.3	61.3	120.0
4	57.5	57.5	59.9	60.1	136.0
5	44.4	44.4	44.6	46.9	44.4
6	77.9	77.9	78.0	78.2	77.9
7	57.2	57.2	57.6	57.4	57.2
8	66.9	66.9	66.9	67.0	66.9
9	47.1	47.0	47.0	44.2	47.0
10	40.0	40.0	39.8	39.7	40.0
11	136.0	136.8	136.2	136.9	136.8
12	*a*	*a*	170.1	170.2	*a*
13	120.7	120.7	120.7	120.6	120.7
14	18.7	19.6	18.8	19.9	19.6
15	23.4	23.4	23.7	23.7	23.4
1′	169.9	169.9	166.3	165.5	169.9
2′	136.7	139.3	136.7	115.5	139.3
3′	127.3	126.9	127.0	159.9	126.9
4′	19.6	62.8	19.9	20.9	62.8
5′				28.0	
OAc (CO)	165.9	164.9			164.9
OAc (Me)	21.2	21.2			21.2

[a] Not observed.

1α-(4-Hydroxymethacryloyloxy)-2*β*-acetoxy-8α-hydroxyeudesm-3,11(13)-dien-6α,12-olide (5): yellow gum; $[\alpha]^{25}_D$ +41.53° (*c* 7.8, MeOH); IR ($CHCl_3$) ν_{max} 3432, 2927, 2863, 1763, 1723, 1668, 1441, 1378, 1237, 1150, 1053, 1017, 960, 758 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m*/*z* [M + Na]$^+$ 429.14; HRMS *m*/*z* [M + Na]$^+$ 429.1588 (calcd for $C_{21}H_{26}O_8Na$, 429.1525); t_R relative to DMP on HPLC (MeOH−H_2O, 55:45, 1 mL/min): 0.59.

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