



A bis-labdenic diterpene from *Moldenhawera nutans*

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Abstract

A new bis-diterpene, moldenin (**6**), derived from the esterification of 3-oxo-8(17)-15-labdenic acid with 3 β -hydroxy-8(17)-15-labdenol, four normal-labdene diterpenes **1–4**, and betulinic acid were isolated from the stems of *Moldenhawera nutans*. The structures were established on the basis of their spectral data. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: *Moldenhawera nutans*; Leguminosae; Labdene diterpenes; Bis-diterpene; Moldenin

1. Introduction

The genus *Moldenhawera* (Leguminosae) is represented in Brazil by approximately ten species most of which are dispersed in the northeastern region of the country. This genus is taxonomically and nomenclaturally confusing and it has been difficult to classify its species (Lewis, 1987). There are no previous phytochemical reports on the genus, and this work was initiated to investigate the compounds of *M. nutans* Queiroz & Allkin responsible for the activity exhibited by the CHCl₃ extract in the HIV-1 RT (p 66) Assay (72% at 200 μ g/ml).

The present paper describes the isolation and identification of four labdene diterpenes (**1–4**), a new bis-diterpene, moldenin (3 β -hydroxy-labd-8(17)-enyl 3'-oxo-labd-8'(17')-en-15'-oate) (**6**), and betulinic acid.

2. Results and discussion

Compounds **1–4** and **6** revealed a mauve colour with the vanillin-H₂SO₄ reagent. The labdene and

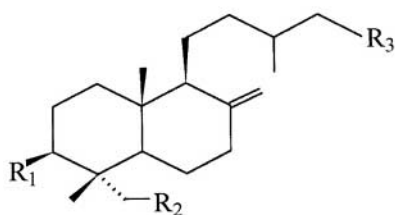
lupene nature of compounds **1–4** and betulinic acid, respectively, were suggested by ¹³C NMR, as well as EIMS, and their structures were established by comparison with the literature data (Jakupovic, Schuster & Wasshausen, 1991; Zdero, Bohlmann & King, 1992; Calderón, Quijano, Gómez-Garibay, Moran & Rios, 1987; Zdero, Bohlmann & King, 1991; Dentali, Hoffmann, Jolad & Timmermann, 1987; Mahato & Kundu, 1994). The diterpenes were recognized as members of normal-labdene series through their $[\alpha]_D$ values.

The ¹H NMR spectrum of the isolate **6** revealed the characteristic absorptions of eight methyl (δ 0.68, 0.77, 0.86, 0.91, 0.94, 1.02, 1.09), one oxy methine (δ 3.25), one oxy methylene (δ 4.07) and two vinyl (δ 4.49, 4.82 and 4.56, 4.89) groups. The ¹³C NMR spectrum showed the presence of 40 carbon resonances, in agreement with the M⁺ peak at m/z 611 displayed in the positive FABMS. In addition, the molecular formula of C₄₀H₆₄O₃ [M⁺–H₂O] (obs. 592.48419, calcd. 592.48555) observed in the HREIMS corroborated a bis-structure for **6**. The ¹³C NMR spectrum confirmed the previous data through the resonances displayed at δ 14.1, 14.5, 15.4, 19.4, 19.6, 21.7, 26.3, 28.3; δ 78.9; δ 62.8; δ 106.7, 147.3; and δ 107.5, 148.2, respectively. Inspection of the DEPT spectra also revealed the presence of fourteen methylene, six methine, four quaternary carbons, one ketone carbonyl (δ 218.0) and one ester carbonyl group (δ 173.3). The IR spectrum corroborated the presence of the latter groups through the

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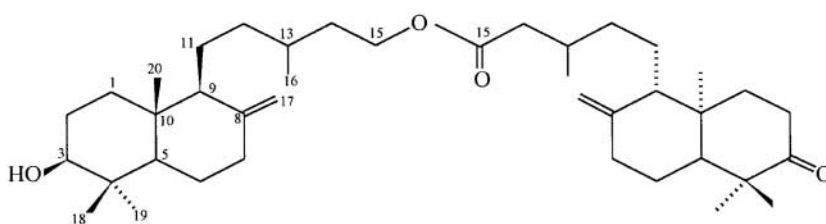
1: $R_1 = R_2 = H$; $R_3 = CO_2H$

2: $R_1 = OH$; $R_2 = H$; $R_3 = CH_2OH$

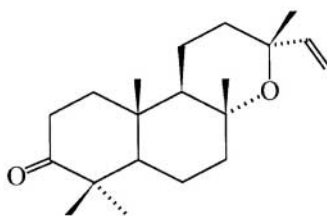
3: $R_1 = H$; $R_2 = OH$; $R_3 = CO_2H$

4: $R_1 = OH$; $R_2 = H$; $R_3 = CO_2H$

5: $R_1 = O$; $R_2 = H$; $R_3 = CO_2CH_3$



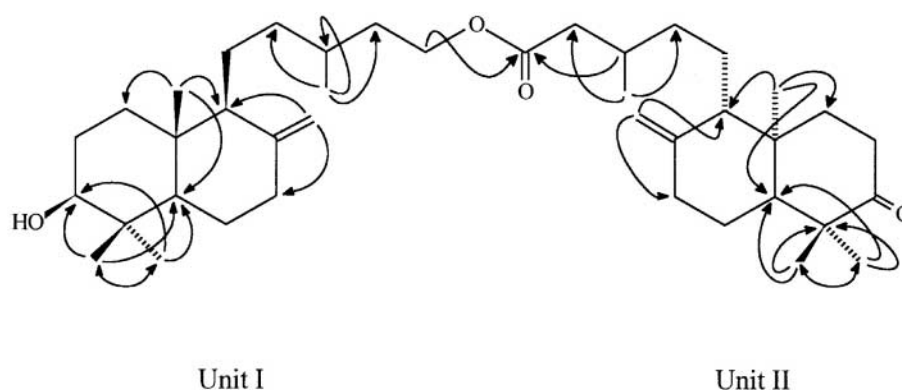
6



7

absorptions at 1712 and 1735 cm^{-1} . The correlation observed between H-15 of moiety I (δ 4.07) and the carboxyl carbon (C-15 of moiety II, δ 173.3) in the HMBC spectrum of **6** indicated the nature of the ester linkage connecting unit I to unit II (Fig. 1). Detailed analysis of the ^{13}C NMR spectrum showed the resemblance of the chemical shifts observed for the vinyl groups, methine and side chain carbons in both units, suggesting a close structural relationship between them. Unit I of compound **6** was recognized through the HMBC spectrum and by direct comparison with the ^{13}C NMR data of **2**. Thus, the correlations shown between C-9 (δ 56.8) with the H_2 -vinylic (δ 4.49, 4.82)

and Me-20 (δ 0.68), as well as between C-5 (δ 54.6) with Me-20, Me-18 (δ 0.77) and Me-19 (δ 0.98), besides the correlations observed between the resonance at δ 3.25 with the Me-18 (δ 15.4) and Me-19 (δ 28.3), indicated a 3β -hydroxy labdane skeleton for unit I. For unit II the ^{13}C NMR spectrum also showed absorption for two quaternary carbons (δ 47.8 and δ 218.0). Comparison of the chemical shifts observed for C-2 and C-4 of unit II with those of unit I demonstrated deshielding effects for both, respectively, C-2 ($\Delta\delta$ – 6.9 ppm) and C-4 ($\Delta\delta$ – 8.7 ppm). In addition, the Me-18 was shielded when compared with the signal in compound **1** (Experimental). These observations

Fig. 1. HMBC data of **6**.

suggested that the carbonyl group was at C-3 of unit II. Comparison with the ^{13}C NMR data (Table 1) of 3-cetomanoyl oxide **7** (Wehrli & Nishida, 1979) corroborated the previous assignment. The HMBC spectrum of **6** substantiated the previously mentioned structural inferences, showing correlations for unit II that pointed out the relationship between the side chain, vinyl and methyl groups. It was possible to observe correlations for the H_2 -vinylic (δ 4.56; 4.89) and Me-20 (δ 0.86) with the C-9 (δ 55.9), as well as

between the resonance at δ 55.2 (C-5) with Me-20, Me-18 (δ 1.02) and Me-19 (δ 1.09).

Finally, transesterification of **6** with sodium methoxide permitted recognition of each unit. The molecular ion peaks at m/z 308 and m/z 334 in the EIMS of the alcohol derivative **2** and of the methyl ester **5**, respectively, afforded in the reaction, established the alcohol $3\beta,15$ -dihydroxy-8(17)-labdene for unit I and, 3-oxo-8(17)-labden-15-oic acid for unit II. Thus, mordenin has the structure **6**.

Table 1
 ^1H NMR and ^{13}C NMR data of mordenin (**6**) and ^{13}C NMR data of **7**

Position	6		7 Wehrli & Nishida, 1979		
	Unit I		Unit II		
	^1H	$^{13}\text{C}^{\text{b,c}}$	^1H	$^{13}\text{C}^{\text{b,c}}$	^{13}C
1	1.30–1.62 ^d	37.1	1.45; 1.65 ^d	37.6	37.8
2	—	27.9	2.40; 2.60 ^d	34.8	33.8
3	3.25 (<i>dd</i> , 11.50; 4.47)	78.9	—	218.0	217.3
4	—	39.1	—	47.8	47.3
5	1.10 ^d	54.6	1.60 ^d	55.2	54.9
6	—	24.0	1.40–1.50 (<i>ax</i>) ^d ; 1.60–1.70 (<i>eq</i>) ^d	25.0	20.8
7	2.00–2.40 ^d	38.1	—	37.9	42.5
8	—	147.3	—	148.2	74.4
9	1.55 ^d	56.8	1.65 ^d	55.9	54.9
10	—	39.3	—	39.4	36.5
11	1.30–1.62 ^d	21.4	—	21.0	15.5
12	—	35.9	—	35.6	35.7
13	1.50 ^d	30.4	1.90 ^d	30.9	74.3
14	—	35.8	2.10 (<i>dd</i> , 11.90; 5.96); 2.25 (<i>dd</i> , 11.92; 5.90)	42.1	147.9
15	4.07 (<i>t</i> , 6.84)	62.8	—	173.3	110.3
16	0.91 (<i>d</i> , 6.24)	19.4	0.94 (<i>d</i> , 6.57)	19.6	28.5
17	4.49 (<i>br s</i>); 4.82 (<i>br s</i>)	106.7	4.56 (<i>br s</i>); 4.89 (<i>br s</i>)	107.5	24.8
18	0.77 (<i>s</i>)	15.4	1.02 (<i>s</i>)	21.7	20.8
19	0.98 (<i>s</i>)	28.3	1.09 (<i>s</i>)	26.0	26.6
20	0.68 (<i>s</i>)	14.5	0.86 (<i>s</i>)	14.1	15.0

^a Spectral data were recorded in CDCl_3 , $\delta_{\text{TMS}} = 0$ ppm, at either 300 MHz for ^1H or 75 MHz for ^{13}C . Parentheses indicate multiplicity and coupling constant (Hz).

^b Multiplicity in the ^{13}C were obtained by DEPT 135° and DEPT 90° experiments.

^c Chemical shifts assigned from HMBC.

^d Chemical shifts were assigned from HMQC.

3. Experimental

^1H (300 MHz), ^{13}C NMR (75 MHz), DEPT 135° and DEPT 90° (90.8 MHz), HMBC and HMQC (500 MHz): CDCl_3 as int. standard; CC: Si gel (70–230 mesh-Merck); LH-20 Sephadex (Pharmacia), $\text{CHCl}_3/\text{MeOH}$; TLC and PLC: precoated sheets and plates of Si gel 60 F₂₅₄ (Merck), respectively.

3.1. Plant material

Stems of *Moldenhawera nutans* were collected by Dr. Eudes da Silva Velozo, in the vicinity of Salvador, BA (Brazil) at the 'restinga' of Reserva do Parque da Lagoa do Abaeté. The plant material was identified by Professor Maria Lenise da Silva Guedes, and a voucher was deposited at the Herbarium Alexandre Leal Costa of the Universidade Federal da Bahia (HALC/IB/UFBA) under number 029057.

3.2. Isolation

The powd. stem (4.0 kg) was repeatedly extd. with MeOH at rt. The conc. syrupy residue (153.5 g) was partitioned between hexane/MeOH- H_2O (9:1) followed by $\text{CHCl}_3/\text{MeOH}-\text{H}_2\text{O}$ (6:4). The CHCl_3 phase (61.2 g) was CC on Si gel eluting with mixtures of pet. ether/EtOAc in order of increasing polarity. Fractions (100 ml) were collected and combined on the basis of TLC. Four fractions were further purified: the two eluted with pet. ether/EtOAc 9:1 (3.5 g; 1.6 g) and the two eluted with 8:2 (1.7 g; 2.18 g). The first fraction (3.5 g) afforded the pure compound **1** (355.4 mg, 0.0089%) after CC on Si gel with $\text{CHCl}_3/\text{EtOAc}$ (8:2). CC on Si gel using $\text{CHCl}_3/\text{EtOAc}$ (8:2) of the second fraction (1.6 g) followed by further CC on Si gel eluting with pet. ether/EtOAc/HOAc (42:7:1) furnished **2** (39.8 mg, 0.001%), betulinic acid (29.4 mg, 0.00074%) and moldenin (**6**). The latter was obtained pure (40.9 mg, 0.001%) after gel permeation on Sephadex LH-20 using $\text{CHCl}_3/\text{MeOH}$ (1:4) as eluent. Compound **3** (107.3 mg, 0.0027%) was obtained pure from the third (1.7 g) main fraction after CC on Si gel eluting with $\text{CHCl}_3/\text{MeOH}$ (95:5) followed by gel permeation on Sephadex LH-20 using as eluent the mixture $\text{CHCl}_3/\text{MeOH}$ (1:4). The fourth fraction (2.18 g) afforded **4** (721.3 mg, 0.018%) after CC on Si gel eluting with $\text{CHCl}_3/\text{MeOH}$ (9:1).

3.3. Labd-8(17)-en-15-oic acid (**1**)

Oil; $[\alpha]_{\text{D}} + 26.8^\circ$ (c 0.8, MeOH); ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$) δ : 35.8 (C-1), 19.4 (C-2), 41.9 (C-3), 33.6 (C-4), 56.9 (C-6), 24.5 (C-6), 42.2 (C-7), 148.6 (C-8), 55.5 (C-9), 39.7 (C-10), 20.9 (C-11), 39.1 (C-12), 33.7 (C-13), 38.4 (C-14), 179.8 (C-15), 19.6 (C-16), 106.3 (C-17), 21.8

(C-18), 30.7 (C-19), 14.5 (C-20). Identification by direct comparison with spectral data of labdenes from the literature (Zdero et al., 1992).

3.4. 3 β ,15-Dihydroxy-labd-8(17)-ene (**2**)

Oil, $[\alpha]_{\text{D}} + 23.2^\circ$ (c 0.4, CHCl_3); EIMS m/z (rel. int.): 308 (4), 290 [$\text{M} - \text{H}_2\text{O}$] (17), 275 (8), 247 (4), 207 (10), 193 (9), 175 (21), 152 (26), 135 (100), 107 (27); ^{13}C NMR (CDCl_3) δ : 37.9 (C-1), 20.9 (C-2), 78.9 (C-3), 38.2 (C-4), 54.6 (C-6), 24.0 (C-6), 41.9 (C-7), 148.1 (C-8), 56.6 (C-9), 39.1 (C-10), 21.0 (C-11), 37.1 (C-12), 30.9 (C-13), 35.7 (C-14), 64.0 (C-15), 19.6 (C-16), 106.7 (C-17), 15.4 (C-18), 28.3 (C-19), 14.6 (C-20). Identification by direct comparison with spectral data from the literature (Zdero et al., 1991).

3.5. 19-Hydroxy-labd-8(17)-en-15-oic acid (**3**)

Crystals; $[\alpha]_{\text{D}} + 23.0^\circ$ (c 0.5, MeOH). Identification by direct comparison with spectral data from the literature (Zdero et al., 1991).

3.6. 3 β -Hydroxy-labd-8(17)-en-15-oic acid (**4**)

Crystals; $[\alpha]_{\text{D}} + 25.2^\circ$ (c 0.5, CHCl_3) of the methyl ester. Identification by direct comparison with spectral data of labdenes from the literature (Calderón et al., 1987; Dentali et al., 1987).

3.7. Methyl 3-oxo-labd-8(17)-en-15-oate (**5**)

EIMS m/z (rel. int.): 334 (32), 319 (20), 291 (9), 275 (10), 249 (41), 205 (76), 191 (23), 190 (25), 189 (83), 163 (37), 147 (36), 135 (61), 125 (75), 119 (81), 109 (76), 107 (100).

3.8. Moldenin (**6**). 3 β -hydroxy-labd-8(17)-enyl 3'oxo-labd-8'(17')-en-15'-oate

Oil; $[\alpha]_{\text{D}} + 15.8^\circ$ (c 0.90, CHCl_3); IR ν_{max} cm^{-1} : 3480, 2944, 2853, 1712, 1735, 1655, 1634, 1449, 1397, 1262, 1167, 1117, 1036, 888, 758; POSFABMS m/z (rel. int.): 611 (3), 593 (37), 317 (10), 288 (17), 225 (100), 209 (21), 171 (36), 155 (58), 135 (32), 121 (36), 119 (71), 117 (54), 109 (40), 105 (45), 69 (51); HREIMS: 592.48419 [$\text{C}_{40}\text{H}_{64}\text{O}_3$ ($\text{M}^+ - \text{H}_2\text{O}$)] EIMS m/z (rel. int.): 610 (6), 593 (26), 592 (100), 577 (23), 549 (25), 303 (12), 291 (8), 205 (37), 191 (16), 190 (15), 189 (41), 175 (33), 153 (16), 152 (35), 151 (15), 139 (32), 136 (38), 135 (100), 134 (40), 123 (52), 122 (30), 121 (14), 119(30), 109 (49), 107 (50); ^1H NMR and ^{13}C NMR: Table 1.

3.9. Transesterification reaction

Moldenin (**6**, 5.0 mg) was stirred with MeONa in MeOH (20 ml) for 12 hours. The methyl ester **5** was extracted from the reaction mixture with CHCl₃. After acidification (1% HCl), the methanolic phase was extracted with CHCl₃ to furnish the alcohol moiety **2**.

3.10. HIV-1 RT inhibition assays

The extract and the fractions from CC were monitored by the HIV-1 RT (p 66) assay according to established protocols (Tan, Pezzuto, Kinghorn & Hughes, 1991). Even though activity was observed for the CHCl₃ extract (72% at 200 µg/ml), none of the fractions or pure diterpenes showed activities.

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References

- Calderón, J. S., Quijano, L., Gómez-Garibay, F., Moran, M., & Rios, T. (1987). *Phytochemistry*, 26, 2639.
- Dentali, S. J., Hoffmann, J. J., Jolad, S. D., & Timmermann, B. N. (1987). *Phytochemistry*, 26, 3025.
- Jakupovic, J., Schuster, A., & Wasshausen, D. C. (1991). *Phytochemistry*, 30, 2785.
- Lewis, G. P. (1987). *Legumes of Bahia* (p. 33). Royal Botanic Gardens, Kew.
- Mahato, S. B., & Kundu, A. P. (1994). *Phytochemistry*, 37, 1517.
- Tan, G. T., Pezzuto, J. M., Kinghorn, A. D., & Hughes, S. H. (1991). *Journal of Natural Products*, 54, 143.
- Wehrli, F. W., & Nishida, T. (1979). In: W. Herz, H. Grisebach, & G. W. Kirby, *Fortschritte der Chemie Organischer Naturstoffe* (p. 56). New York: Springer-Verlag.
- Zdero, C., Bohlmann, F., & King, R. M. (1991). *Phytochemistry*, 30, 2991.
- Zdero, C., Bohlmann, F., & King, R. M. (1992). *Phytochemistry*, 31, 1631.