PERGAMON

Phytochemistry 50 (1998) 443–447

PHYTOCHEMISTRY

A bis-labdenic diterpene from *Moldenhawera nutans*

Juceni P. David [1], Jorge M. David [2], Shu-Wei Yang, Geoffrey A. Cordell *

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois, Chicago, Il 60612, U.S.A.

Received 17 December 1997; accepted 27 May 1998

Abstract

A new bis-diterpene, moldenin (**6**), derived from the esterification of 3-oxo-8(17)-15-labdenic acid with 3β-hydroxy-8(17)-15-labdenol, four normal-labdane 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.

Keywords: *Moldenhawera nutans*; Leguminosae; Labdane 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 $CHCl_3$ extract in the HIV-1 RT (p 66) Assay (72% at 200 μg/ml).

The present paper describes the isolation and identification of four labdene diterpenes (**1**–**4**), a new bis-diterpene, moldenin (3β-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-H_2SO_4 reagent. The labdene and lupene nature of compounds **1-4** and betulinic acid, respectively, were suggested by ^{13}C 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-labdane series through their $[\alpha]_D$ values.

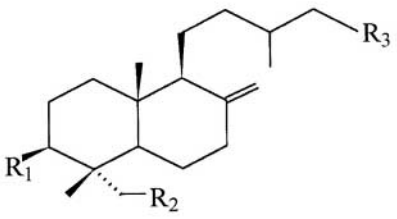
The ^1H NMR spectrum of the isolate **6** revealed the characteristic absorptions of eight methyl (δ 0.68, 0.77, 0.86, 0.91, 0.94, 1.02, 1.09), one oxy methine (δ 3.25), one oxy methylene (δ 4.07) and two vinyl (δ 4.49, 4.82 and 4.56, 4.89) groups. The ^{13}C NMR spectrum showed the presence of 40 carbon resonances, in agreement with the M^+ peak at m/z 611 displayed in the positive FABMS. In addition, the molecular formula of $C_{40}H_{64}O_3$ [M^+-H_2O] (obs. 592.48419, calcd. 592.48555) observed in the HREIMS corroborated a bis-structure for **6**. The ^{13}C 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

* Corresponding author. Tel.: +001-312996-7245; Fax: +001-312-996-3272.

[1] Permanent address. Faculdade de Farmácia, 40170-290, Salvador (BA), Brazil.

[2] Permanent address. Instituto de Química, Universidade Federal da Bahia, 40170-290, Salvador (BA), Brazil.

0031-9422/98 $ - see front matter
PII: S0031-9422(98)00590-1



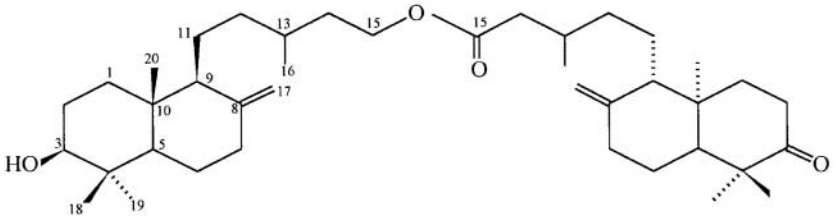
1: $R_1 = R_2 = H$; $R_3 = CO_2H$

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

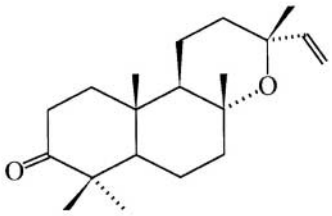
3: $R_1 = H$; $R_2 = OH$; $R_3 = CO_2H$

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

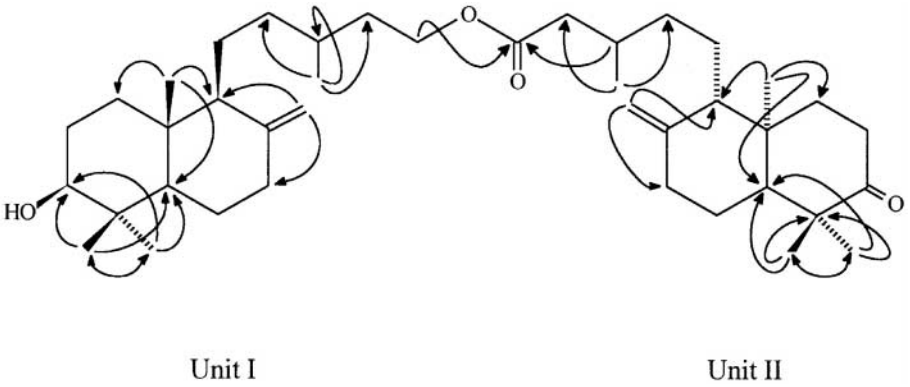


Fig. 1. HMBC data of **6**.

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 3β,15-dihydroxy-8(17)-labdene for unit I and, 3-oxo-8(17)-labden-15-oic acid for unit II. Thus, moldenin has the structure **6**.

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

Position	**6**				**7** Wehrli & Nishida, 1979
	Unit I		Unit II		
	^{1}H	$^{13}C^{b,c}$	^{1}H	$^{13}C^{b,c}$	^{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 (*dd*, 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 (*ax*)[d]; 1.60–1.70 (*eq*)[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 (*dd*, 11.90; 5.96);2.25 (*dd*,11.92; 5.90)	42.1	147.9
15	4.07 (*t*, 6.84)	62.8	—	173.3	110.3
16	0.91 (*d*, 6.24)	19.4	0.94 (*d*, 6.57)	19.6	28.5
17	4.49 (br *s*); 4.82 (br *s*)	106.7	4.56 (br *s*); 4.89 (br *s*)	107.5	24.8
18	0.77 (*s*)	15.4	1.02 (*s*)	21.7	20.8
19	0.98 (*s*)	28.3	1.09 (*s*)	26.0	26.6
20	0.68 (*s*)	14.5	0.86 (*s*)	14.1	15.0

[a] Spectral data were recorded in $CDCl_3$, $\delta_{TMS} = 0$ ppm, at either 300 MHz for ^{1}H or 75 MHz for ^{13}C. Parentheses indicate multiplicity and couping 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

^{1}H (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), $CHCl_3$/MeOH; TLC and PLC: precoated sheets and plates of Si gel 60 F_{254} (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 $CHCl_3$/MeOH-H_2O (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 $CHCl_3$/EtOAc (8:2). CC on Si gel using $CHCl_3$/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 $CHCl_3$/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 $CHCl_3$/MeOH (95:5) followed by gel permeation on Sephadex LH-20 using as eluent the mixture $CHCl_3$/MeOH (1:4). The fourth fraction (2.18 g) afforded **4** (721.3 mg, 0.018%) after CC on Si gel eluting with $CHCl_3$/MeOH (9:1).

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

Oil; $[\alpha]_D$ +26.8° (*c* 0.8, MeOH); ^{13}C NMR (C_5D_5N) 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. 3β,15-Dihydroxy-labd-8(17)-ene (*2*)

Oil, $[\alpha]_D$ +23.2° (*c* 0.4, $CHCl_3$); EIMS *m/z* (rel. int.): 308 (4), 290 [M − H_2O] (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; $[\alpha]_D$ +23.0° (*c* 0.5, MeOH). Identification by direct comparison with spectral data from the literature (Zdero et al., 1991).

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

Crystals; $[\alpha]_D$ +25.2° (*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*). 3β-hydroxy-labd-8(17)-enyl 3′oxo-labd-8′(17′)-en-15′-oate

Oil; $[\alpha]_D$ +15.8° (*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 [$C_{40}H_{64}O_3$ (M^+−H_2O]; 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); ^{1}H 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 $CHCl_3$. After acidification (1% HCl), the methanolic phase was extracted with $CHCl_3$ 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 $CHCl_3$ extract (72% at 200 μg/ml), none of the fractions or pure diterpenes showed activities.

Acknowledgements

The authors are indebted to Professor Maria Lenise da Silva Guedes (Curator of Herbarium Alexandre Leal Costae, Instituto de Biologia, UFBA, Salvador, BA, Brazil) for the identification of botanical material. The authors also are grateful to Ms. E. Mata and Dr. J.M. Pezzuto for the biological tests, and to Mr. R. Dvorak for the mass spectra. J.P.C. and J.M.D. thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for fellowships.

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.