New Triterpene and Antibacterial Labdenoic Acid Derivatives from *Moldenhawera nutans*

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Um novo triterpeno (22\(\beta\)-hidroxilupeol) foi isolado do extrato em metanol do caule de *Moldenhawera nutans* (Leguminosae) além de diterpenos derivados do ácido labdenôico de ocorrência comum nesta espécie. A partir do ácido labd-8(17)-en-15-óico foram preparados derivados com atividade in vitro discreta frente à *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella cholerasuis* e *Vibrio parahaemolyticus*.

A new triterpene derivative (22\(\beta\)-hydroxyxiluopel) was isolated from the MeOH extract of stems of *Moldenhawera nutans* (Leguminosae) together with labdenoic acid derivatives of common occurrence in this species. From the labd-8(17)-en-15-oic acid were prepared simple derivatives, which exhibited in vitro weak activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella cholerasuis*, and *Vibrio parahaemolyticus*.

**Keywords:** *Moldenhawera nutans*, Leguminosae, antibacterial labdene derivatives, 22\(\beta\)-hydroxyxiluopel

**Introduction**

Genus *Moldenhawera* Schrard. (Leguminosae: Caesalpinoideae), endemic to Northeast Brazil, is represented by approximately ten species.\(^1\) Previous phytochemical study of *M. nutans* resulted in the isolation of four known labdene diterpenes besides a new bis-diterpene named moldenin.\(^2\) The present work describes the results of the fractionation of the hexane phase obtained from the MeOH extract of *M. nutans*. Besides the known diterpenes previously isolated, it was also obtained the 3-oxo-labd-8(17)-en-15-oic acid (1) as a methyl derivative (1a), and three triterpenes, lupeol, betulin, and the new lupane derivative (2). The labd-8(17)-en-15-oic acid (3) was the predominant compound in this extract. From this compound, derivatives (4-8) were prepared and some of them were submitted to in vitro antibacterial assays.

**Results and Discussion**

The structural elucidation of 1a (Figure 1) was based on MS, IR, and NMR data analyses. Comparison of NMR data of the methyl ester derivative with moldenin,\(^2\) methyl ent-3-oxo-labd-8(17)-en-15-oate\(^3\) and literature data\(^4\) allowed establishing the labdanic structure. The normal series of this compound was confirmed by positive optical rotation.

The HREIMS of 2 exhibited a molecular ion signal at m/z 442.3829, indicating the molecular formula C\(_{30}\)H\(_{50}\)O\(_2\) (requires 442.3811). The \(^1\)H NMR data (Experimental section) showed characteristic signals of lupane triterpene, seven methyl groups, one isopropenyl (\(\delta\) 4.61, 4.71, and 1.70) and two signals of oxymethine hydrogens (\(\delta\) 3.68 and 3.20). The \(^13\)C NMR spectra (BB and DEPT 135\(^\circ\)) displayed 30 signals and confirmed the data above through the resonances displayed at \(\delta\) 19.3, 109.8, 150.3, as well as at \(\delta\) 78.0 and 79.7 for the isopropenyl and two oxymethine groups, respectively. The presence of an additional oxymethine signal indicated that 2 is a hydroxylated lupeol derivative. The localization of the hydroxyl group at C-22 of the lupane framework was proposed by comparison with \(^13\)C NMR data (Table 1) of 16-hydroxyluope\(^5\) and literature data\(^4\) allowed establishing the labdanic structure. The normal series of this compound was confirmed by positive optical rotation.

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insignificant effect observed at C-19 in the NMR spectra of 2a together with the coupling constants observed in the 1H NMR suggested that the hydroxyl group is in equatorial position. The 1HNMR nOe difference spectra of this compound permitted to corroborate the proposition. When the H-22 was irradiated it was possible to assign increments in Hα-21 (25%), H-29 (3%) and, H-19 (10%). These findings are indicative that all the affected protons were in the same plane. The fragmentation pattern observed in the MS of 2 was also indicative of a hydroxyl group in C-22 of the cyclopentyl ring, especially by mass fragments of 2 at m/z 374 and 2a at m/z 458 (Figure 2).

Since labd-8(17)-en-15-oic acid (3) showed antibacterial activity (Table 2), some of its simple chemical derivatives were prepared and also evaluated. Derivative 3 was refluxed with MeOH/HCl to obtain the isomeric methyl esters 4a and 5. Next, 4a was submitted to allylic oxidation by t-butyl chromate6 and furnished 6a. It was also prepared the epoxy derivatives 7a and 8a from 4a by reaction with MCPBA. The derivatives 7a and 8a are new and they were characterized by spectrometric analyses data. Compounds 1a, 2, 4a, and 6a-8a were submitted to antibacterial evaluation and none of them was active; while the acid derivatives (1, 4, 6-8) obtained after saponification, together with 9, a labdane previously obtained from this plant, showed moderate antibacterial activity at 90 μg per disc (Table 2). These results indicate the need of the presence of an acid group for the antibacterial activity and also that structural features contribute to the inhibition area as well.

### Experimental

#### General procedures

The 1H and 13C NMR, DEPT, COSY, and HETEROCOSY (J 140 and 9 Hz) spectra were obtained
Table 2. Diameter of inhibition zones of compounds 1, 3, 4, 6-9 from M. nutans in agar diffusion test in mg per disk

<table>
<thead>
<tr>
<th>Sample</th>
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<th>SA</th>
<th>SC</th>
<th>VP</th>
<th>PA</th>
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<tr>
<td>6</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>7 + 8*</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>-</td>
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<tr>
<td>9</td>
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<td>-</td>
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<td>34</td>
<td>30</td>
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<tr>
<td>Chloramphenicol</td>
<td>22</td>
<td>-</td>
<td>26</td>
<td>34</td>
<td>23</td>
</tr>
</tbody>
</table>

EC = Escherichia coli; SA = Staphylococcus aureus; SC = Salmonella cholerasuis; VP = Vibrio parahaemolyticus; PA = Pseudomonas aeruginosa. *evaluation performed in mixture.

on a Varian Gemini 2000 instrument employing CDCl3 as both solvent and reference. The FTIR spectrum was recorded on a JASCO spectrophotometer Mod. Valor III. MS was recorded on a Micromass Autospec spectrometer (HRMS) and an HP model 5973 spectrometer (EIMS). Melting points were measured on a Microquímica MIAPF 301 apparatus and are uncorrected. Column chromatography was carried out on silica gel TLC plates and are uncorrected. Column chromatography was carried out on silica gel 60 and, silica gel TLC plates and are uncorrected. Chromatography was carried out on silica gel with mixtures of hexane/EtOAc as eluents.

Plant material

The plant material of M. nutans were collected at sandy soil of Reserva do Parque da Lagoa do Abaeté, Salvador, BA, Brazil in the spring of 1997 and identified by Prof. Maria L. S. Guedes of Herbarium Alexandre Leal Costa, where a voucher (#029057) is deposited.

Extraction and isolation

The powdered stem (4 kg) of Moldenhawera nutans was extracted with MeOH. The methanol extract (153 g) was partitioned with hexane, furnishing 73.2 g of extract. The hexane phase was purified through CC over silica gel with mixtures of hexane/EtOAc as eluents.

Preparation of derivatives

Acetylation of 22β-hydroxy lupel (2)

Compound 2 (15.0 mg) was added to a solution of pyridine (0.5 mL) and acetic anhydride (2.0 mL) and the mixture was left at room temperature for 24 h. Cold H2O was added and the diacetyl derivative (2a, 14.3 mg) was extracted with CHCl3.

3ß, 22ß-Diacetoxy lup-20(29)-ene (2a)

Oil, EIMS: 70 eV (rel. int.) m/z: 526 [M⁺] (35), 466 (42), 458 (50), 416 (46), 398 (10), 289 (21), 276 (39), 249

Methyl 3-oxo-labd-8(17)-en-15-oate (1a)

Pale yellow oil. [α]D25 33.4° (c 0.1, CHCl3), IR(film) νmax/cm⁻¹: 3080, 2950, 1737, 1706, 1643, 1455, 1385, 1160, 1008, 890; C21H34O3 (Found: C, 75.1; H, 10.5%. Requires: C, 75.4; H, 10.2%), EIMS: 70 eV (rel. int.) m/z: 334 [M⁺] (18), 319 (4), 303 (7), 233 (51), 220 (15), 205 (48), 177 (16), 163 (22), 137 (34), 135 (100), 123 (68), 109 (68); 1H NMR (300 MHz, CDCl3): δ 4.88 (s, 1H, H-17a), 4.55 (s, 1H, H-17b), 3.64 (s, 3H, OCH3), 1.08 (s, 3H, Me-19), 1.01 (s, 3H, Me-18), 0.93 (d, J 6.6 Hz, 3H, Me-16), 0.85 (s, 3H, Me-20); 13C NMR (75 MHz, CDCl3): δ 35.3 (C-1), 34.4 (C-2), 216.2 (C-3), 47.4 (C-4), 54.8 (C-5), 24.8 (C-6), 37.6 (C-7), 146.9 (C-8), 55.6 (C-9), 39.0 (C-10), 21.1 (C-11), 37.3 (C-12), 30.5 (C-13), 41.5 (C-14), 173.2 (C-15), 21.4 (C-16), 107.2 (C-17), 19.3 (C-18), 25.6 (C-19), 13.8 (C-20), 51.0 (OCH3).

β,22ß-Diacetoxy lup-20(29)-ene (2a)

White amorphous powder; mp 158-160 °C. [α]D25 71.2° (c 0.1, CHCl3); IR(film) νmax/cm⁻¹: 3401, 2934, 2868, 1643, 1455, 1385, 880; HREIMS: m/z 442.3829 (C20H29O2 requires 442.3811), EIMS: 70 eV (rel. int.) m/z: 442 [M⁺] (12), 427 (4), 374 (100), 291 (6), 273 (57), 247 (14), 234 (12), 219 (8), 207 (23), 189 (31), 175 (14), 161 (13), 147 (16), 135 (35), 121 (24), 107 (26); 1H NMR (300 MHz, CDCl3): δ 4.97 (m, 1H, H-17b), 4.56 (m, 1H, H-17a), 3.94 (s, 3H, OCH3), 0.95 (s, 3H, Me-19), 0.94 (s, 3H, Me-18), 0.85 (s, 3H, Me-20); 13C NMR (75 MHz, CDCl3): δ 35.4 (C-1), 34.5 (C-2), 216.2 (C-3), 47.4 (C-4), 54.8 (C-5), 24.8 (C-6), 37.6 (C-7), 146.9 (C-8), 55.6 (C-9), 39.0 (C-10), 21.1 (C-11), 37.3 (C-12), 30.5 (C-13), 41.5 (C-14), 173.2 (C-15), 21.4 (C-16), 107.2 (C-17), 19.3 (C-18), 25.6 (C-19), 13.8 (C-20), 51.0 (OCH3).

β,22ß-Hydroxy lupel (2)

White amorphous powder; mp 158-160 °C. [α]D25 71.2° (c 0.1, CHCl3); IR(film) νmax/cm⁻¹: 3401, 2934, 2868, 1643, 1455, 1385, 880; HREIMS: m/z 442.3829 (C20H29O2 requires 442.3811), EIMS: 70 eV (rel. int.) m/z: 442 [M⁺] (12), 427 (4), 374 (100), 291 (6), 273 (57), 247 (14), 234 (12), 219 (8), 207 (23), 189 (31), 175 (14), 161 (13), 147 (16), 135 (35), 121 (24), 107 (26); 1H NMR (300 MHz, CDCl3): δ 4.97 (m, 1H, H-17b), 4.56 (m, 1H, H-17a), 3.94 (s, 3H, OCH3), 0.95 (s, 3H, Me-19), 0.94 (s, 3H, Me-18), 0.85 (s, 3H, Me-20); 13C NMR (75 MHz, CDCl3): δ 35.4 (C-1), 34.5 (C-2), 216.2 (C-3), 47.4 (C-4), 54.8 (C-5), 24.8 (C-6), 37.6 (C-7), 146.9 (C-8), 55.6 (C-9), 39.0 (C-10), 21.1 (C-11), 37.3 (C-12), 30.5 (C-13), 41.5 (C-14), 173.2 (C-15), 21.4 (C-16), 107.2 (C-17), 19.3 (C-18), 25.6 (C-19), 13.8 (C-20), 51.0 (OCH3).
Preparation of derivatives of labd-8(17)-en-15-oic acid

Compound 3 (1.0 g) was refluxed with 36 mL of an acidic methanolic solution (HCl 0.048 N) under stirring for 4.5 h. Sequentially, water was added and the solution was extracted with CHCl_3. The product was submitted to Si gel with AgNO_3 CC and eluted with hexane:EtOAc (C-15), 19.0 (C-16), 20.1 (C-17), 21.7 (C-18), 33.3 (C-19, 0.79 (s, 3H, Me-20); 13C NMR (75 MHz, CDCl_3): δ 33.6 (C-1), 19.1 (C-2), 39.5 (C-3), 33.3 (C-4), 51.8 (C-5), 25.4 (C-6), 36.9 (C-7), 125.4 (C-8), 140.4 (C-9), 38.9 (C-10), 19.0 (C-11), 41.4 (C-12), 31.5 (C-13), 37.3 (C-14), 173.6 (C-15), 19.0 (C-16), 20.1 (C-17), 21.7 (C-18), 33.3 (C-19), 19.6 (C-20), 51.9 (OCH_3).

Methyl labd-8-en-15-oate (5a)

Oil. [α]_D^25 30.2° (c 0.2, CHCl_3), C_{21}H_{36}O_2, EIMS: 70 eV (rel. int.) m/z: 320 [M]^+ (9), 305 (15), 196 (12), 191 (52), 177 (6), 163 (10), 149 (15), 135 (20), 122 (100), 109 (84); 1H NMR (300 MHz, CDCl_3): δ 5.79 (s, 1H, H-7), 3.66 (s, 3H, OCH_3), 1.68 (s, 3H, Me-17), 0.97 (d, J 6.5 Hz, 3H, Me-16), 0.84 (s, 3H, Me-19), 0.81 (s, 3H, Me-18) and 0.78 (s, 3H, Me-20); 13C NMR (75 MHz, CDCl_3): δ 39.2 (C-1), 19.0 (C-2), 42.2 (C-3), 32.7 (C-4), 54.7 (C-5), 23.8 (C-6), 122.1 (C-7), 135.1 (C-8), 49.8 (C-9), 36.6 (C-10), 25.3 (C-11), 37.1 (C-12), 30.8 (C-13), 41.7 (C-14), 172.9 (C-15), 18.5 (C-16), 22.2 (C-17), 21.7 (C-18), 33.1 (C-19), 13.5 (C-20), 51.7 (OCH_3).

Methyl 7-oxo-labd-8-en-15-oate (6a)

Oil. [α]_D^25 41.1° (c 1.0, CHCl_3); IR(film) ν_{max}/cm⁻¹: 2953, 1736, 1661, 1605, 1437, 1156, 1080, 1007; C_{21}H_{34}O_3, EIMS: 70 eV (rel. int.) m/z: 334 [M]^+ (30), 303 (7), 233 (52), 220 (15), 205 (44), 177 (10), 163 (10), 149 (13), 135 (100), 123 (48), 109 (56); 1H NMR (300 MHz, CDCl_3): δ 3.56 (s, 3H, OCH_3), 1.62 (s, 3H, Me-17), 0.90 (d, J 6.4 Hz, 3H, Me-16), 0.96 (s, 3H, Me-19), 0.80 (s, 3H, Me-18) and 0.76 (s, 3H, Me-20); 13C NMR (75 MHz, CDCl_3): δ 35.7° (C-1), 18.5 (C-2), 41.2° (C-3), 33.0 (C-4), 50.1 (C-5), 35.2° (C-6), 200.0 (C-7), 129.7 (C-8), 168.0 (C-9), 40.8 (C-10), 26.9 (C-11), 35.1 (C-12), 31.3 (C-13), 41.0° (C-14), 173.1 (C-15), 19.4 (C-16), 11.2 (C-17), 21.2 (C-18), 32.4 (C-19), 18.1 (C-20), 51.3 (OCH_3), \[^{\text{a}}\] values may be interchangeable.

Methyl 8β,9β-epoxy-labd-8-en-15-oate (7a)

Oil. [α]_D^25 38.2° (c 0.8, CHCl_3), C_{21}H_{36}O_3, EIMS: 70 eV (rel. int.) m/z: 336 [M]^+ (14), 321 (10), 305 (7), 278 (15), 264 (20), 253 (23), 251 (20), 207 (100), 177 (18), 163 (25), 149 (58), 125 (57), 121 (35), 109 (47); 1H NMR (300 MHz, CDCl_3): δ 3.53 (s, 3H, OCH_3), 1.13 (s, 3H, Me-17), 0.89 (d, J 6.4 Hz, 3H, Me-16), 0.96, 0.78 (s, 3H, Me-19 and Me-18) and 0.76 (s, 3H, Me-20); 13C NMR (75 MHz, CDCl_3): δ 33.2 (C-1), 17.2 (C-2), 41.5 (C-3), 32.8 (C-4), 42.3 (C-5), 23.9 (C-6), 43.5 (C-7), 62.2 (C-8), 72.2 (C-9), 38.5 (C-10), 18.4 (C-11), 41.4 (C-12), 31.0 (C-13), 35.1 (C-14), 173.5 (C-15), 19.6 (C-16), 21.2 (C-17), 21.9 (C-18), 33.5 (C-19), 17.2 (C-20), 51.3 (OCH_3).

Methyl 8α,9α-epoxy-labd-8-en-15-oate (8a)

Oil. C_{21}H_{36}O_3, [α]_D^25 42.2° (c 0.6, CHCl_3), EIMS: 70 eV (rel. int.) m/z: 336 [M]^+ (15), 321 (13), 305 (6), 278 (15), 264 (23), 253 (26), 251 (26), 235 (18), 207 (100), 177 (18), 163 (22), 149 (62), 125 (59), 121 (36), 109 (52);
\(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 3.53 (s, 3H, OCH\(_3\)), 1.24 (s, 3H, Me-17), 0.90 (d, \(J = 6.4\) Hz, 3H, Me-16), 1.00, 0.80 (s, 3H, Me-19 and Me-18) and 0.74 (s, 3H, Me-20); \(^{13}\)C NMR (75MHz, CDCl\(_3\)): \(\delta\) 33.4 (C-1), 16.7 (C-2), 41.4 (C-3), 33.6 (C-4), 53.8 (C-5), 29.2 (C-6), 29.0 (C-7), 64.7 (C-8), 72.5 (C-9), 38.7 (C-10), 19.7 (C-11), 41.4 (C-12), 31.4 (C-13), 37.0 (C-14), 173.5 (C-15), 19.5 (C-16), 21.4 (C-17), 21.9 (C-18), 33.2 (C-19), 16.5 (C-20), 51.3 (OCH\(_3\)).

Hydrolyses of the ester derivatives

All the methyl ester derivatives were submitted to hydrolysis by adding 1.5 mL of a solution of 10 mg NaOH in H\(_2\)O/EtOH (1:1) to 10 mg of each compound for 30 minutes. After this time, the reaction medium was saturated with 3 mL of brine and the produced ppted was acidified with 4 mL of an aqueous solution of H\(_2\)SO\(_4\) and extracted with CHCl\(_3\). The organic phase was washed and dried over Na\(_2\)SO\(_4\), yielding acid compounds.

Antibacterial assays

This assay was performed by disc diffusion method using the established protocol.\(^{11}\) The antibacterial activity using paper disk with \(\phi 9\) mm was determined through the microorganism growth inhibition halo for Staphylococcus aureus (ATCC 10708), Pseudomonas aeruginosa (ATCC 15442), Escherichia coli (ATCC 11229), Salmonella cholerasuis (ATCC 10708), and Vibrio parahaemolyticus (ATCC 17802) under the action of test substances (90 mg). Penicillin (10 mg), tetracycline (30 mg) and chloramphenicol (30 mg) were used as positive controls.

Supplementary Information

Supplementary data of 1a, 2a, 4a and 6a as \(^{13}\)C and \(^1\)H NMR spectra are available free of charge at http://jbcs.sbq.org.br, as PDF file.

Acknowledgments

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (Brazil), FAPESB and Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for fellowship support and grants. We are in debt to Dr. Ignacio Lopez of Universidad de Extremadura, Badajoz, Spain for HREIMS.

References