

DIRHAMNOSYL FLAVONOID AND OTHER CONSTITUENTS FROM *Brillantaisia palisatii*

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DIRHAMNOSYL FLAVONOID AND OTHER CONSTITUENTS FROM *Brillantaisia palisatii*. A mixture containing sitosterol and stigmasterol; a new triterpene 3-*epi*-ursolic acid; another triterpene mixture comprising α -amyrin, β -amyrin and lupeol; verbascoside, a phenylpropanoid glycoside; and lespedin, a glycosyl flavonoid, were isolated. The less polar compounds (steroids and triterpenoids) were isolated from the hexane partition of the crude ethanolic extract while the more polar ones (phenylpropanoid glycoside and glycosyl flavonoid) were isolated from the ethyl acetate partition of the same extract. The structures of all compounds were established using modern spectrometric methods of elucidation. The spectroscopic data of Lespedin, a rare dirhamnosylflavonol with hypotensor activity and of the triterpene, 3-*epi*-ursolic acid, are also reported.

Keywords: *Brillantaisia palisatii*; dirhamnosyl flavonol; triterpenes.

INTRODUCTION

Brillantaisia palisatii Lind. belongs to the family Acanthaceae, order Scrophulariales, superorder Lamiiflorae¹. Into this family *B. palisatii* is classified in the subfamily Acanthoideae, *tribus* Hygrophyllae². Acanthaceae is composed of about 250 genera with almost 2500 species spread over tropical regions, at the Mediterranean, United States of America and Australia³. The main four distribution centers of this family are Indomalay (*Strobilanthes* and *Andrographis*), Africa (*Barleria*), Brazil (*Ruellia*) and Central America (*Aphelandra* and *Odontonema*). These plants show a wide variety in habit and biological types. Most of them are shrubs or herbs, climbing plants, xerophytes and marshy plants are also very common. Trees, however, are rare in the family⁴. The chemistry of Acanthaceae shows a great diversity of different compounds such as alkaloids⁵, iridoids⁶, lignans⁷, flavonoids⁸, terpenoids⁹ and phenylpropanoid glycosides¹⁰. Some species of this family are used in folk medicine to treat several diseases, especially gastrointestinal ailments. In fact, several compounds isolated from species belonging to the family Acanthaceae have improved pharmacological activities.

METHODOLOGY

Aerial parts of *Brillantaisia palisatii* Lind. were collected near the Rio de Janeiro Botanical Garden, Rio de Janeiro State, Brazil, in April, 1997. Several herbarium samples (Voucher numbers = 289308, 251777, 173808, 267291, etc.) have been deposited at the Rio de Janeiro Botanical Garden herbarium. There are no reports about the traditional uses of this species as well as about its chemical composition.

Vegetative organs were extracted with ethanol and after that, extraction procedures using liquid-liquid partition were made in order to produce extracts with hexane, dichloromethane, ethyl acetate and

n-butanol. The purification step using chromatographic methods lead to several known molecules, including the rare favonol lespedin and also to a new one, a triterpene named 3-*epi*-ursolic acid.

RESULTS

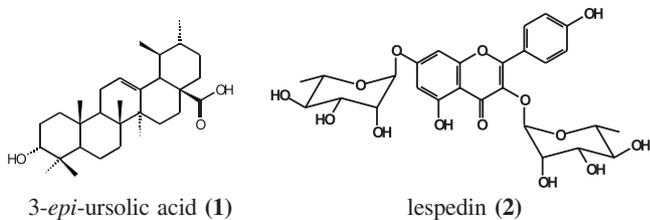
The hexanic partition of the *B. palisatii* crude ethanolic extract was chromatographed on a silica gel column to give two main fraction apparently pure on TLC. These fractions were analyzed by GC revealing that they were actually mixtures. The less polar mixture, eluted with hexane-EtOAc (95:5) consisted of three compounds (0.0229%). GC-MS analysis of this mixture showed that all of the three compounds have a mass spectra with $[M]^+ = m/z$ 426 in agreement with the following formula $C_{30}H_{50}O$. The characterization of the constituents of the less polar triterpene mixture as well as of the more polar constituents was simplified by the assignment of the carbon atoms in the ¹³C NMR. As the chemical shift of a sp² carbon atom is very characteristic for each triterpenoid skeleton, ¹³C NMR spectroscopy has been very frequently employed for the structural analysis of triterpene mixtures¹¹. To distinguish carbon types (multiplicity), the attached proton test (APT) was used. Thus, it appeared in the ¹³C NMR spectrum signals at δ 78.8 ppm relative to C₃ of the three constituents; δ 150.7 and 109.2 ppm corresponding to olefinic carbons C₂₀-C₂₉ of the lupane skeleton; δ 121.6 and 145.0 ppm corresponding to olefinic carbons C₁₂-C₁₃ of the oleanane skeleton and δ 124.3 and 139.4 ppm corresponding to olefinic carbons C₁₂-C₁₃ of the ursane skeleton. These data, together with those obtained by GC-MS and ¹H NMR, confirmed the presence of lupeol, α -amyrin and β -amyrin in this triterpene mixture in the following estimated proportions: 2:1:1, respectively.

The more polar mixture (0.00487%), eluted with hexane-EtOAc (9:1), presented characteristic signals of olefinic carbons for sitosterol and stigmasterol¹² in an estimated proportion of 1:1. Another compound (0.00299%), isolated with hexane-EtOAc (8:2), showed the ¹³C NMR spectrum with a group of signals at δ 178.02 (C₂₈), 140.50 (C₁₃), 121.48 (C₁₂) and 74.53 (C₃), consistent with the signals of the 3-*epi*-ursolic acid (**1**)¹¹, which was supported by ¹H NMR data

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showing a multiplet centered at δ 3.5 ppm (H_3). The confirmation of this proposal was made by GC-MS analysis of the methylated molecule, which showed just one signal ($R_1 = 76.02$ min.) corresponding to 3-methyl-*epi*-ursolate, with mass spectra having $[M]^+ = m/z$ 470.

The ethyl acetate partition of *B. palisatii* crude ethanolic extract was chromatographed on a silica gel column to give a mixture of two compounds apparently pure on TLC. This mixture was eluted from the column with $CHCl_3$:MeOH (7:3). After 1H NMR analysis it was clear that this compound actually was a mixture of two substances: verbascoside (the major constituent, 0,00868%) and lespedin (the minor constituent, 0,00967%). The separation was performed by Sephadex LH-20 chromatography. Analysis by 1H NMR and ^{13}C NMR could identify these two compounds unequivocally, when compared with literature data^{13,14}.



3-*epi*-ursolic acid (1)

It was obtained as colorless amorphous powder ($CHCl_3$): mp 250-252 °C; 1H NMR (200 MHz, $CDCl_3$, TMS): δ (ppm): 0.73-1.11 (H methyl signals), 3.5 (*m*, H_3), 5.1 (*t*, J 3.2 Hz, H_{12}); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ (ppm): 15.5 (C_{24}), 15.7 (C_{25}), 16.5 (C_{29}), 16.6 (C_{26}), 18.4 (C_6), 21.4 (C_{30}), 23.3 (C_{11}), 23.6 (C_{27}), 24.2 (C_{16}), 27.4 (C_2), 27.9 (C_{15}), 28.4 (C_{23}), 30.7 (C_{21}), 32.9 (C_7), 36.7 (C_{22}), 37.0 (C_{10}), 38.8 (C_1 , C_4 , C_{20}), 39.1 (C_{17}), 39.5 (C_8), 42.0 (C_{14}), 47.2 (C_{17}), 47.4 (C_9), 52.8 (C_{18}), 55.3 (C_5), 74.5 (C_3), 121.5 (C_{12}), 140.5 (C_{13}), 178.0 (C_{28}).

GC (DB-1, Temp. Prog. 60°-240° / 3° min.) – 76.02 min.

EIMS m/z (rel. int.): 470 [M]⁺ (3), 411 (2), 262 (100), 249 (6), 233 (7), 207 (33), 203 (84), 189 (22), 133 (81), 119 (33), 95 (28), 69 (41), 55 (59), 43 (79).

Lespedin (2)

It was obtained as an yellow needles (MeOH): mp 219-221 °C; U.V. λ_{max} (log ϵ) MeOH: 341.4 (0.958), 265.6 (1.807), 204.6

(1.985).U.V. λ_{max} (log ϵ) MeOH + $AlCl_3$: 348.6 (0.850), 273.6 (1.355), 207.6 (2.036).U.V. λ_{max} (log ϵ) MeOH + $AlCl_3$ + HCl: 341.0 (0.803), 275.0 (1.565), 206.6 (1.861). U.V. λ_{max} (log ϵ) MeOH + AcONa: 343.4 (0.493), 265.4 (0.680), 203.0 (1.746); 1H NMR (200 MHz, MeOD, TMS): δ (ppm): 0.95 (*d*, J 5.6 Hz, H_6 Rha3), 1.28 (*d*, J 5.9 Hz, H_6 Rha7) 3.30-4.30 (signals relative to protons 2-5 belonging to the two rhamnose residues), 5.40 (*d*, J 1.6 Hz, H_1 Rha3), 5.57 (*d*, J 1.6 Hz, H_1 Rha7), 6.44 (*d*, J 2.1 Hz, H_8), 6.70 (*d*, J 2.1 Hz, H_6), 6.94 (*d*, J 8.8 Hz, 2H, $H_{3',5'}$), 7.78 (*d*, J 8.8 Hz, 2H, $H_{2',6'}$); ^{13}C NMR (50 MHz, MeOD, TMS): δ (ppm): 17.7 (C_6 Rha7), 18.1 (C_6 Rha3), 71.2 (C_5 Rha7), 71.6 (C_5 Rha3), 71.8 (C_2 Rha3), 72.1 ($C_{2,3}$ Rha7 and C_3 Rha3), 73.1 (C_4 Rha7), 73.5 (C_4 Rha3), 95.5 (C_8), 99.8 (C_6), 100.5 (C_1 Rha7), 103.5 (C_1 Rha3), 107.5 (C_{8b}), 116.6 ($C_{3',5'}$), 122.3 (C_1), 131.9 ($C_{2',6'}$), 136.4 (C_3), 157.9 (C_{8a}), 159.7 (C_2), 161.7 (C_4), 162.9 (C_5), 163.5 (C_7), 179.7 (C_4).

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