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Chemical constituents from *Piptadenia rigida* "angico"

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Abstract: The phytochemical investigation of the roots of *Piptadenia rigida* Benth., Fabaceae, known as "angico", afforded sitosterol, lupeol, betuline, the chalcone isoliquiritigenin, the flavonoids, 7,4'-dihydroxyflavone, 7,3',4'-trihydroxyflavone, 7,8,3',4'-tetrahydroxyflavanone, 4-hydroxy-3,5-dimethoxybenzaldehyde and methyl-3,4-dihydroxy-benzoate. Both flavones were also isolated from the branches of this plant. Five derivatives of the aldehyde were obtained by diazomethane treatment. The structures of compounds were identified by IR, NMR and mass spectral data analysis of natural compounds and some derivatives, and by comparison with literature data.

Article

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Introduction

The *Piptadenia genus*, Fabaceae, comprise eighty tropical species frequently occurring in South America. In Brazil these species are popularly known as "angico" and have been found in the North, Northeast, and Central West. This genus had been considered as heterogenous that was divided in *Anadenanthera* Speg., *Pityrocarpa* Benth. and *Piptadenia* Benth. On the other hand the Brazilian botanists have considered as *Piptadenia* sensu Bentham. The *P. rigida* has been classified as belonging to Leguminosae-Mimosoideae (Correa, 1984; Lorenzi, 2002) and more recently as Fabaceae family (Morin, 2010). It has been described the inhalation of narcotic snuff from some *Piptadenia* species by the Caribbean and by South American native Indians (Correa, 1984; Lorenzi, 2002, Stromberg, 1954). The previous phytochemical study of *Piptadenia* species described the presence of indole alkaloids from the seeds of *P. peregrine, P. excelsa, P. macrocarpa,* and *P. leprostachy*a (Fish et al., 1955; Pachter et al., 1959; Legler & Tschesche, 1963, Yamasato et al., 1972). Steroids, heterosides and tannins have been identified in bark of *P. columbrina* (Bulhões et al., 1976) and the flavonoid anadantoside, 7,3',4'-trihydroxy-3-β-D-xylopiranosylflavane, was isolated from the bark of *P. macrocarp*a (Piacente et al. 1999). Besides the hallucinogenic properties of the seeds, the exudate from the wood has been used against respiratory infections (Shneider, 1937; Rangel, 1943). It is used in the popular medicine as anti leucorrhoea, anti diarrhea and as a cure for ulcer; the leaves have haemostatic function in cerebral disease and commotion (Bulhões et al., 1976; Piacente et al., 1999; Shneider, 1937; Rangel, 1943). This paper reports the first phytochemical study of *P. rigida* revealing for the first time the presence of a chalcone, three flavonoids, two benzoil derivatives, lupeol and betulin in *Piptadeni*a genus.

Materials and Methods

The roots and branches of *Piptadenia rigida* Benth., Fabaceae, were collected in the forest garden of the Instituto de Florestas (IF) of Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica-RJ, by Dr. Acácio G. de Carvalho, Departamento de Produtos Florestais, IF, UFRRJ. It was identified by Prof. Dr. José Aguiar Sobrinho, Departamento de Ciências Ambientais, IF, UFRRJ. Voucher specimen (No RBR-21438) is deposited at the Herbarium of Instituto de Biologia, UFRRJ.

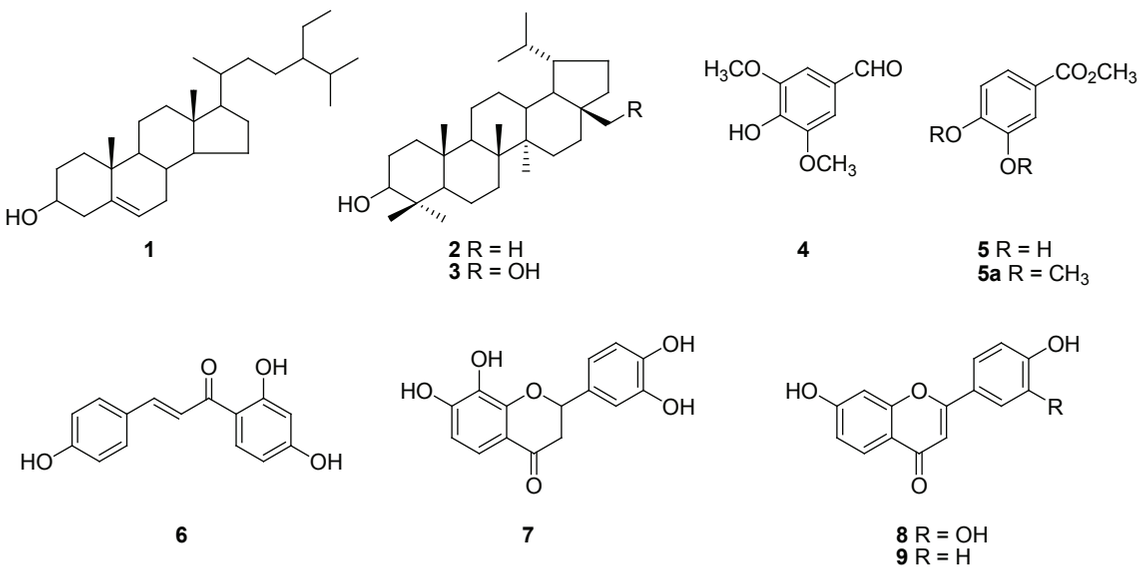
The powdered dried root (6.3 kg) and branches (2.0 kg) were extracted exhaustively with CH_2Cl_2 and MeOH at room temperature. The solvents were removed under vacuum to yield the residues PRBD (*P. rigida* branches dichloromethane, 1.0 g) and PRBM (*P. rigida* branches methanol, 83.0 g) from the branches, and PRRD (*P. rigida* root dichloromethane, 19.2 g) and PRRM (*P. rigida* root methanol, 310.5 g) from the root. The methanol extract from the branches PRBM (60.0 g) was partitioned

with $CHCl_3$ and MeOH:H_2O (9:1). The fraction obtained with chloroform (13.4 g) was chromatographed on silica gel column and eluted with CH_2Cl_2 and mixture of this solvent with EtOAc and MeOH in increasing polarity. 140 fractions were collected. Fractions 64-73 and 75-79 yielded the flavonoids **9** (90.0 mg, mp 289-290 °C) and **8** (80.0 mg, mp 212-213 °C), respectively. The residue PRRD was dissolved in methanol and filtered to obtain two fractions, a solid (PRRD-1, 6.9 g) and solution (PRRD-2, 12.0 g). The solid was chromatographed on silica gel column using $CHCl_3$ increasing the polarity with methanol to methanol 100%. Twenty fractions were collected, and fractions 8-15 were reunited and crystallized from methanol yielding the solid **1** (440.0 mg, mp 132-133 °C). The fraction PRRD-2 was fractionated on silica gel column using the same systems of solvent and 27 fractions were collected and analyzed by TLC plate. Fractions 5-12 afforded **1** (522.0 mg). Preparative TLC of the fractions 13-25 with hexane:ethyl acetate (8:2) yielded **3** (53.6 mg, mp 258-259 °C) and **4** (26.4 mg, gum). The extract PRRM was dissolved in MeOH:H_2O (8:2) and partitioned with hexane (PRRMH, 2.24 g), chloroform (PRRMC, 11.9 g) and methanol. Chromatography fractionation and TLC analysis of PRRMH were used to isolate **2** (138.0 mg, PF 194-195 °C) from fractions 4-16, and **1** (210.0 mg) from the fractions 19-23. PRRMC was filtered on silica gel column with dichlorometane, ethyl acetate and methanol to yield PRRMCD (270.0 mg), PRRMCE (6.3 g), PRRMCM (5.3 g). The IR and ^{1}H NMR spectra of fraction PRRMCD let to identify mixture of aliphatic acids. Fraction PRRMCE was fractionated on silica gel CC using chloroform as initial solvent and increasing the polarity with methanol and fifty fractions were collected. Chromatography fractionation and TLC analysis of fractions 8-13 (294.0 mg) afforded **5** (20.0 mg, oil) and **6** (15.0 mg, mp 158-159 °C). Fractions 27-33 afforded the flavanone **7** (22.0 mg, oil); fractions 45-53 were fractionated by preparative TLC using chloroform:ethyl acetate (1:3) to obtain the crystalline material identified as the flavone **8** (20.0 mg, mp 213-214 °C). The purity of the compounds was checked by silica gel TLC plate, revealed by visualization with UV (254 and 366 nm), $AlCl_3$-ETOH (1%) or exposure to iodine vapor, and ^{1}H spectral analysis.

The alcohol function in **1, 2** and **3** was confirmed by the IR and ^{1}H and ^{13}C NMR spectral analysis of the products with PCC oxidations. An ethereal solution of diazomethane was added to the methanol solution of **4** (13.0 mg) and **5** (15.0 mg) to confirm the phenol groups. The unexpected additional signals in the ^{1}H and ^{13}C NMR spectra of **4** product let us to analyze it by CG-MS and a mixture of **4a, 4b, 4c, 4**d and **4e** was identified, Rt [%; *m/z*: (%)]: **4a**: 6.38 [4.4; *m/z*: 196 (100, $M^{+\cdot}$), 181 (57), 165 (4), 153 (10), 123 (5), 93 (26)]; **4b**: 7.18 [2.87; *m/z*: 210 (25, $M^{+\cdot}$), 181 (100), 167 (5), 151 (5), 148 (17)]; **4c**: 7.56 [19.44; *m/z*: 210 (60), 195 (100), 167 (11), 153 (2), 139 (15), 137 (8), 107 (5)]; **4d**: 8.10 [64.44; *m/z*: 224 (37, $M^{+\cdot}$), 182 (12), 181 (100), 167 (5), 151 (2), 137 (4), 121 (2), 107 (3)]; **4e**: 9.02 [8.81; *m/z*: 224 (100, $M^{+\cdot}$), 209 (52), 195 (12), 179 (10), 181 (62), 151 (10)], (Scheme 1). The ^{1}H NMR spectral analysis confirmed **5a** as expected.

Results and Discussion

Chromatographic fractionation of the extracts from the roots and branches of *Piptadenia rigida* and analysis of the fractions allowed to identify sitosterol (**1**), lupeol (**2**), betulin (**3**), the chalcone isoliquiritigenin (**6**), the flavonoids 7,8,3',4'-tetrahydroxyflavanone (**7**), 7,3',4'-trihydroxyflavone (**8**), and 7,4'-dihydroxyflavone (**9**), besides 4-hydroxi-3,5-dimethoxybenzaldehyde (**4**) and methyl 3,4-dihydroxy-



1

2 R = H
3 R = OH

4

5 R = H
5a R = CH_3

6

7

8 R = OH
9 R = H

benzoate (**5**). The structures were established by IR, NMR and mass spectral data analysis of the natural compounds and some derivatives. The steroid **1** and the triterpenes **2** and **3** were identified by mp, IR, ^{1}H and ^{13}C NMR spectra analysis of natural substances and of the products obtained by PCC oxidation, sitostenone, lupenone and 3-oxo betulinic acid, respectively, and by comparison with values described in the literature (Chaurasia & Wichtl, 1987; Reynolds et al. 1986; Mahato & Kundu, 1994; Siddiqui et al., 1988).

The IR spectra of **4** and **5** showed band at cm^{-1}: 3377, 3076, 1605, 1512, 1462, 1251, 1680 ($\nu_{C=O}$ in **4**) and 1692 (($\nu_{C=O}$ in **5**). The signals at δ_H 9.79 (s, 1H, aldehyde), 7.12 (s, 2H, Ar-H), 3.94 (s, 6H, 2x OCH_3) were identified in the ^{1}H NMR spectrum of **4**. The ^{13}C NMR spectra (BBD and DEPT) of this aldehyde showed signals at 190.7 (HC=O, C-7), 147.8, 147.3x2 (C-4 and C-3,5), 128.3 (C-1), 106.6 (C-2,6) and 56.4 (2xOCH_3). The same analysis of the ^{1}H NMR spectra of **5** let to identify the signals of 3H of the ABC system in aromatic ring by δ_H 7.38 (brs, H-2), 7.32 (brd, *J*=8 Hz, H-6) and 6.79 (d, *J*=8 Hz, H-5) besides the signal at δ_H 3.69 (s, 3H, OCH_3). The ^{13}C NMR data confirmed this proposed benzoyl methyl esther structure by the signals at δ_C 166.5 (C=O of ester), 150.2, 145.0, 122.4 (C-4, C-3 and C-1, respectively), 122.0, 116.1, 115.0 (CH-6, 2 and 5) and 51.2 (OCH_3 of ester). The ^{1}H NMR spectrum of the residue obtained from treatment of **4** with diazomethane did not shows signals of aldehyde and showed signals at δ_H 6.38 (s), 7.19 (s), 3.82 (s), 3.89(s), and multiples signals at δ_H 3.7-4.0, 2.8-3.12, and 2.15-2.5. The ^{13}C NMR spectrum (BBD and DEPT) of this product showed δ_{CH} at 52.6, 60.6, 77.0, 102.1, 105.8, 105.9, 106.3, δ_{CH2} at 51.3, 34.0, 32.0, 27.4, and of OCH_3 at 56.11, 60.9. These data let us to analyze this product by the GC-MS whose chromatogram showed five peaks and corresponding mass spectrum of each one [**4a-4e**, Rt, % and *m/z* (%), see experimental] was detected. Those NMR spectral data besides the proposed ions structures (Scheme 1) for: *m/z* 181 (**4a**$_1$) and 153 (**4a**$_2$) from **4a**, *m/z* 181 (**4b**$_1$), 167 (**4b**$_2$), 148 (**4b**$_3$) from **4b**; *m/z* 195 (**4c**$_1$), 167 (**4c**$_2$), 153 (**4c**$_3$) from **4c**; *m/z* 181 (**4d**$_1$), 167 (**4d**$_2$), 148 (**4d**$_3$) from **4d**, and *m/z* 181 (**4e**$_1$), 153 (**4e**$_2$) from **4e**, were compatible with the proposed structures in the mixture of **4** derivatives (Scheme 1).

The ^{1}H NMR spectrum of chalcone **6** showed signals of ABC system [δ_H 6.33 (d, *J*=2.0 Hz, H-3), 6.45 (dd, J_1=8.7, J_2=2.0 Hz, H-5), 8.01 (d, *J*=8.7 Hz, H-6)], two doublets of *J*=15 Hz (δ_H 7.65 and 7.84) and two signals of AA'BB' system at δ_H 6.90 (d, *J*=8.7 Hz, H-3',5') and 7.67 (*J*=8.7 Hz, H-2'.6'). These data and the carbons chemical shift of **6** were identical to those of literature for isoliquiritigenin (Almtorp et al., 1991).

The IR spectra of **7** and **8** showed band at cm^{-1}: 3238, 3030, 1630 ($\nu_{C=O}$), 1600, 1510 and 1272. The analysis of ^{1}H NMR spectra of **7** and comparison with literature data of 7,8,3',4'-tetrahydroxy-flavanone, isolated from *Acacia melanoxylon* (Foo,1987), were used to confirm the proposed structure of **7**. The unambiguous ^{1}H and ^{13}C NMR chemical shift assignment, including the 1D and 2D spectra of **7**, are reported in the literature by Nascimento et al. (2003). The same spectral analysis of **8** allowed to identify two ABC system of hydrogen in an aromatic ring by the signals at δ_H 6.96 (brd, *J*=8.6 Hz, 1H), 6.98 (brs, 2H), 7,48 (brs, 1H), 7.45 (d, *J*=8.0 Hz, 1H), 8.01 (d, *J*=8.6 Hz, 1H). These six hydrogens and the singlet at δ_H 6.8 were compatible with a trihydroxyflavone. The doublet at δ_H 8.1 agrees with H-5 chemical shift of the ABC system in the flavone. The ^{13}C NMR spectrum of **8** showed signal of quaternary carbons at δ_C 180.4 (C=O), 165.7 (C-2), 165.3 (C-7), 157.8 (C-9), 151.0 (C-4'), 147.2 (C-3'), 124.1 (C-1'), 116.6 (C-10), and of δ_{C-H} at 127.8 (C-5), 114.2 (C-6), 116.5/116.9 (C-2'/5"), 120.2 (C-6'), 103.8/105.2 (C-3/8). The peak at *m/z* (%): 272 [$M^{+\cdot}$, 1%, $C_{15}H_{10}O_5$), 161(43), 178(100), and 133(14), confirmed the proposed structure as 7,3',4'-trihydroxyflavone for **8**, which carbon-13 data are registered in the literature (Agrawal, 1989).

The ^{1}H NMR spectra analysis of **9** allowed to identify an ABC system in the ring A of flavone [δ_H 6.92 (brd, *J*=8.0 Hz, H-6), 7.97 (d, *J*=8.0 Hz, H-5), 6.97 (brs, H-8)] and two doublets of para substituted aromatic ring [δ_H 6.94(d, *J*=8.7 Hz, H-3'.5') and 7.88(d, *J*=8.7 Hz, H-2',6') of a flavone with δ_{H-3} 6.71 (s). The carbon chemical shifts at δ_C 103.5 (CH-8), 105.1 (CH-3), 116.2 (CH-6) 116.4 (CH-3',5'), 117.0 (C-10), 122.9 (C-1'), 127.8 (C-5), 129.4 (CH-2',6'), 159.0 (C-9), 162.6 (C-7), 165.2 (C-2), 166.0 (C-4'), 180.0 (C-4) were compatible with 7,4'-dihydroxyflavone proposed for **9**. This compound was isolated from *Medicago sativa* and from *Vicia faba* (Fabaceae) (Wollehweber, 1994).

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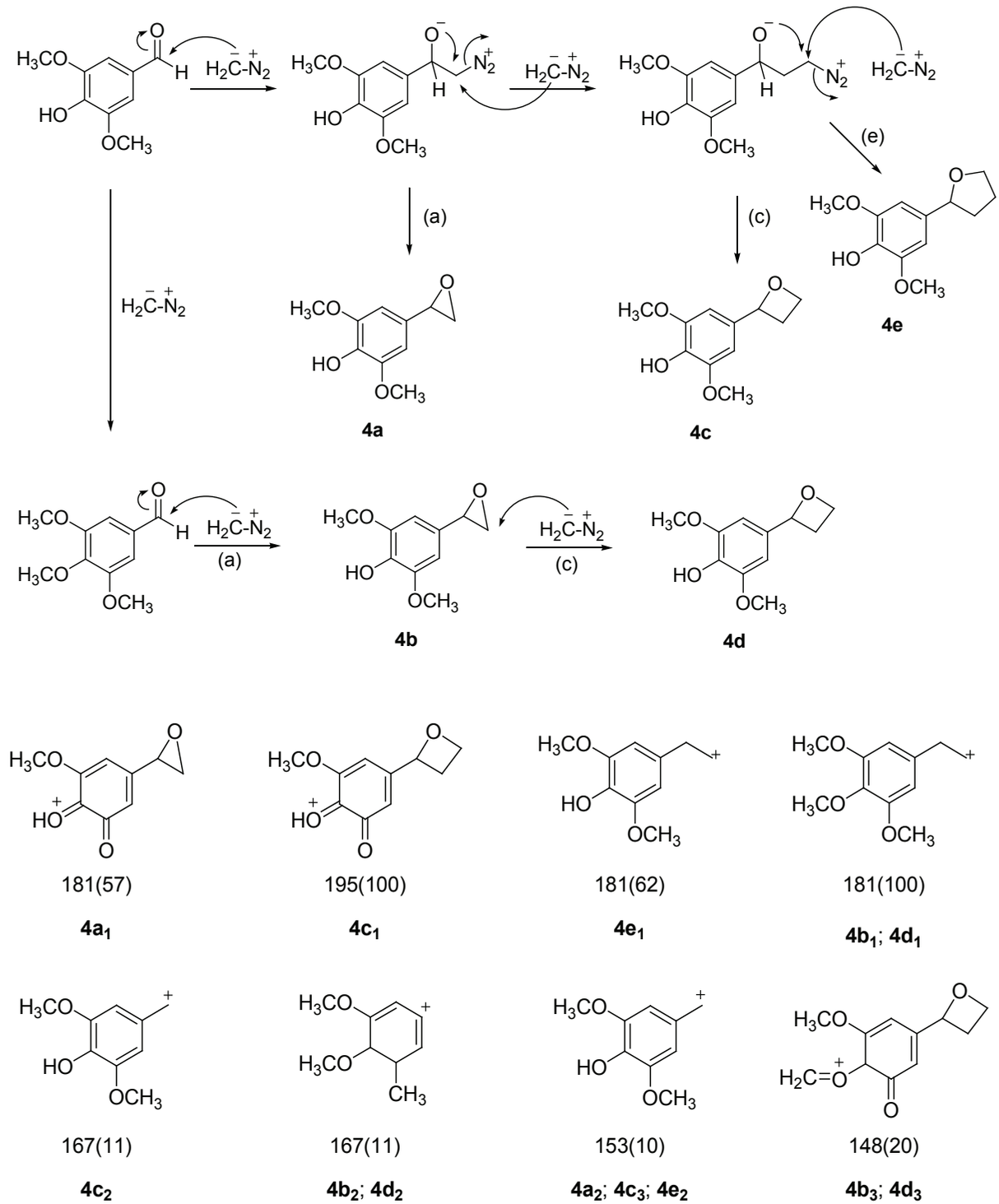

Scheme 1. Proposed mechanism for the formation of the **4** derivatives and for structures of ions peaks considered as principals in the mass spectra

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