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.

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 *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 *Piptadenia* Benth. On the other hand the Brazilian botanists have considered as *Piptadenia* sensu Bentham. 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with CHCl₃ and MeOH·H₂O (9:1). The fraction obtained with chloroform (13.4 g) was chromatographed on silica gel column and eluted with CH₂Cl₂ 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 CHCl₃ 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·H₂O (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 1H 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), AlCl₃-ErOH (1%) or exposure to iodine vapor, and 1H spectral analysis.

The alcohol function in 1, 2 and 3 was confirmed by the IR and 1H and 13C 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 1H and 13C NMR spectra of 4 product let us to analyze it by CG-MS and a mixture of 4a, 4b, 4c, 4d and 4e was identified, Rt [%; m/z (%):] 4a: 6.38 [4.4; m/z: 196 (100, M+), 181 (57), 165 (4), 153 (10), 132 (9), 123 (6)]; 4b: 7.18 [2.87; m/z: 210 (25, M+), 181 (100), 167 (5), 151 (5), 148 (17)]; 4c: 7.65 [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+), 182 (12), 181 (100), 167 (5), 151 (2), 137 (4), 121 (2), 107 (3)]; 4e: 9.02 [8.81; m/z: 224 (100, M+), 209 (52), 195 (12), 179 (10), 181 (62), 151 (10)], (Scheme 1). The 1H 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'-tri hydroxyflavone (8), and 7,4'-dihydroxyflavone (9), besides 4-hydroxy-3,5-dimethoxybenzaldehyde (4) and methyl 3,4-dihydroxy-
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, 1H and 13C NMR spectra analysis of natural substances and of the products obtained by PCC oxidation, sitosterone, 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 (ν C=O in 4) and 1692 (ν C=O in 5). The signals at δ 9.79 (s, 1H, aldehyde), 7.12 (s, 2H, Ar-H), 3.94 (s, 6H, 2x OCH3) and 51.2 (OCH3 of ester). The 1H NMR spectra (C-4, C-3 and C-1, respectively), 122.0, 116.1, 115.0 signals at δ 6.33 (d, 2H, 2xOCH3) identified this proposed benzoyl methyl ester structure by the doublet at δ 6.38 (s), 7.19 (s), 3.82 (s), 3.89(s), and multiples signals at δ 3.7-4.0, 2.8-3.12, and 2.15-2.5. The 13C NMR spectrum 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 (2xOCH3). The same analysis of the 1H NMR spectra of 5 let to identify the signals of 3H of the ABC system in aromatic ring by δ 7.8 (H, H-5), 7.7 (H, H-5) and 7.6 (H, H-5)

The 1H NMR spectrum of chalcone 4 showed band at cm⁻¹:

7.12 (s, 2H, Ar-H), 3.94 (s, 6H, 2x OCH3) and 51.2 (OCH3 of ester). The same spectral analysis of 1H 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 (2xOCH3). The same analysis of the 1H NMR spectra of 4 let to identify the signals of 3H of the ABC system in aromatic ring by δ 7.8 (H, H-5), 7.7 (H, H-5) and 7.6 (H, H-5) besides the signal at δ 3.69 (s, 3H, OCH3).

The 13C NMR data confirmed this proposed benzyol methyl esther structure by the signals at δ 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 (OCH3 of ester). The IR spectra of the residue obtained from treatment of 4 with diazomethane did not show signals of aldehyde and showed signals at δ 6.38 (s), 7.19 (s), 3.82 (s), 3.89(s), and multiples signals at δ 3.7-4.0, 2.8-3.12, and 2.15-2.5. The 13C NMR spectrum of this residue showed a signals of 3H of the ABC system in aromatic ring by δ 7.8 (H, H-5), 7.7 (H, H-5) and 7.6 (H, H-5) besides the signal at δ 3.69 (s, 3H, OCH3).

The 1H and 13C NMR chemical shift data of 7,4'-dihydroxyflavone proposed for this product showed δ 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 6.8 were compatible with a trihydroxyflavone. The doublet at δ 8.1 agrees with H-5 chemical shift of the ABC system in the flavone. The 13C NMR spectrum of 8 showed signal of quaternary carbons at δ 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 CCH 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+, 1%, C31H20O8], 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 1H NMR spectra analysis of 9 allowed to identify an ABC system in the ring A of flavone [δ 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 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 CH 6.71 (s). The carbon chemical shifts at δ 103.5 (CH-8), 105.1 (CH-3), 116.2 (CH-6) 114.3 (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).

Acknowledgment

The authors thank to Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Fundação Carlos Chagas Filho de Amparo a Pesquisa do Estado do Rio de Janeiro for scholarship and financial support and to Universidade Estadual do Norte Fluminense, Campos-RJ, for the 400 MHz NMR spectra.

The IR spectra of 7 and 8 showed band at cm⁻¹:

3238, 3030, 1630 (ν C=O), 1600, 1510 and 1272. The analysis of 1H NMR spectra of 7 and comparison with literature data of 7,8,3’,4’-tetrahydroxy-flavonone, isolated from Acacia melanoxylon (Foo,1987), were used to confirm the proposed structure of 7. The unambiguous 1H and 13C 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 6.33 (d, J=2.0 Hz, H-3), 6.45 (dd, J=8.7, J=2.0 Hz, H-5), 8.01 (d, J=8.7 Hz, H-6), two doublets of J=15 Hz (δ 7.65 and 7.84) and two signals of AA’BB’ system at δ 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:
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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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