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A dimeric proanthocyanidin from *Stryphnodendron adstringens*

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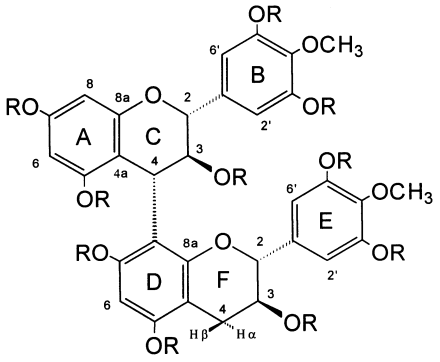
Abstract

A new proanthocyanidin, 4′-*O*-methylgallocatechin-(4α→8)-4′-*O*-methylgallocatechin of the rare 4′-methoxy derivatives of prodelphinidin, was isolated from the stem bark of *Stryphnodendron adstringens*. The structure was determined on the basis of spectroscopic data including 1-D (^{1}H, ^{13}C) and 2-D NMR (^{1}H–^{1}H-COSY, HSQC, HMBC) experiments of its peracetylated derivative.

Keywords: Stryphnodendron adstringens; Mimosaceae; Proanthocyanidins; 4′-*O*-methylgallocatechin-(4α→8)-4′-*O*-methylgallocatechin

1. Introduction

Stryphnodendron adstringens (Martius) Coville (Mimosaceae), popularly known as 'barbatimão', is a native Brazilian tree widely used in folk medicine as a wound healing agent (de Siqueira, 1982; Santos, Torres, & Leonart, 1987). Its stem bark is described in the Brazilian Pharmacopoeia, with a content of at least 20% tannins (Farmacopéia Brasileira, 1959). The antiinflammatory activity of an acetone soluble fraction from the stem bark was attributed to the presence of proanthocyanidins (Lima, Martins, & de Souza, 1998). In our previous reports, we described the isolation and identification of various flavan-3-ols, prodelphinidins and prorobinetinidins (de Mello, Petereit, & Nahrstedt, 1996a, 1996b). We report here on a new dimeric compound of the rare 4′-*O*-methylated proanthocyanidins.



1: R = H
1a: R = Ac

2. Results and discussion

The ^{1}H NMR spectrum ($CDCl_3$) of the peracetate **1a** showed close structural resemblance to that of the corresponding derivatives of gallocatechin-(4α→8)-gallocatechin (Petereit, Kolodziej, & Nahrstedt, 1991) concerning proton chemical shifts, absence of conformational isomerism and, in part, to gallocatechin-(4α→8)-catechin (Helsper, Kolodziej, Hoogendijk, & van Norel, 1993), except for two three-proton singlets at δ 3.79 and δ 3.77 indicating two methoxyls. All heterocyclic protons could readily be assigned from the ^{1}H–^{1}H-COSY spectrum. The long-range couplings between the H-2(C) and H-2(F) proton signals and the respective H-2′/6′ signals allowed to distinguish between pyrogallol type B- and E-ring proton signals. The large coupling constants of the C-ring protons ($J_{2,3}$ = 10 Hz and $J_{3,4}$ = 9.4 Hz) confirmed the 2,3-*trans*–3,4-*trans* relative configuration for ring C. The broadened H-2(F) and H-3(F) protons over-

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lap at δ 5.02 ppm and do not allow to determine the 2,3 relative stereochemistry with certainty. This observation has already been reported for the peracetylated gallocatechin-(4α→8)-catechin (Helsper et al., 1993) and can be explained by conformational changes of ring F (Balas, Vercauteren, & Laguerre, 1995). However, acid degradation of **1** (Thompson, Jacques, Haslam, & Tanner, 1972) yielded the lower unit as 4′-*O*-methylgallocatechin (TLC) clearly indicating the 2,3-*trans* position in the F ring system. The large coupling constants ($J_{3,4\alpha}=4.9$ Hz and $J_{3,4\beta}=7.1$ Hz) of the H-4 (F) protons (600 MHz) allow to locate the quasi-axial H-4β(F) being *trans* to H-3(F); these data indicate the stereochemistry shown for **1a** (Clark-Lewis, Jackman, & Spotswood, 1964; Davis, Cai, Davies, & Lewis, 1996). A 2,3-*trans*–3,4-*trans* relative configuration of ring C of the proanthocyanidin was also confirmed by the downfield position (Δ ca. 4-5 ppm) of the C-2 resonance in **1a** due to the equatorial substituent at C-4 ('γ gauche effect') in comparison to the 2,3-*cis* configurated analogue (Fletcher, Porter, Haslam, & Gupta, 1977; Porter, Newsman, Foo, Wong, & Hemingway, 1982). The methoxyl groups were set at the C-4′ positions of ring B and E based on the results of the HMBC experiment, which showed correlations between both C-4′ carbons (δ 144.2 and 144.1 ppm) with the respective H-2′/6′ (δ 6.81 ppm and δ 6.60 ppm) and methoxyl protons at δ 3.77 and 3.79 ppm. This arrangement was confirmed by weak NOE interactions to the B- and E-ring two proton singlets upon irradiation of the methoxyl protons and by the typical chemical shifts for *ortho–ortho* disubstituted methoxyl carbons at ca. 61 ppm (Nakano, Alonso, Grillet, & Martin, 1979) for both methoxyl groups.

The location of the interflavanoid linkage in **1a** was recognized by the ^{3}J long-range correlations (HMBC) of the H-4(C) proton (δ 4.48 ppm) with the C-8a(A) (δ 155.9 ppm) and C-8a(D) (δ 152.7 ppm) carbons as key correlations in the case of the 4→8 linked procyanidin B_3-peracetate (Balas & Vercauteren, 1994). The corresponding C-8a(A) signal was assigned by the ^{2}J H,C correlation from C-8a(A) to H-8(A) and the ^{3}J coupling to the H-4(C) proton signal. A negative argument for the 4,8-interflavonoid bond is the lacking cross peak in the HMBC from C8a(D) to any D ring proton.

Supporting evidence was obtained from MALDI-TOF mass spectroscopy ($[M+Na]^+$ m/z 1081) of the acetate **1a**. The negative Cotton effect in the 210–240 nm region of the CD-spectrum of **1a** indicated a 4α-flavanyl substituent with a 4*S* configuration (Barrett et al., 1979; Botha, Young, Ferreira, & Roux, 1981). On the basis of all arguments, the new proanthocyanidin **1** has been established as 4′-*O*-methylgallocatechin-(4α→8)-4′-*O*-methylgallocatechin, which represents a rare example of the 4′-methoxylated proanthocyanidins outside the *Ouratea*-proanthocyanidins (Monache, Pomponi, Martini-Bettolo, D'Albuquerque, & de Lima, 1976).

3. Experimental

3.1. General

^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian Gemini 200 (200 MHz) or a Bruker AM 600 (600 MHz), δ are given relative to $CHCl_3$ (7.26 ppm). ^{13}C NMR were recorded at 50 or 150 MHz. CD spectrum was recorded in MeOH on a Jasco J 600. Acetylation was in Ac_2O–pyridine (1,2:1) at ambient temperature for 24 h. MALDI-TOF mass spectrometer: LAZARUS II (home built), N_2-laser (LSI VSL337ND) 337 nm, 3 ns puls width, focus diameter 0.1 mm, 16 kV acceleration voltage, 1 m drift length, data logging with LeCroy9450A, 2.5 ns sampling time and expected mass accuracy ±0.1%. Sample preparation: the acetylated compounds is deposited from a solution in $CHCl_3$ on a thin layer of 2,5-dihydroxybenzoic acid (DHB) crystals. Analytical TLC was done on silica gel GF_{254} plates (Merck) in the solvent system EtOAc–HCO_2H–H_2O (18:1:1). Compounds were visualized as red spots by spraying with vanillin/HCl-reagent. Prep. TLC was performed on silica gel plates (Kieselgel 60 F_{254}, 0.5 mm, Merck) using toluene–Me_2CO (7:3).

3.2. Plant material

The stem bark of *S. adstringens* was collected in São Gerônimo da Serra (State of Paraná, Brazil; 22°S, 51°W) in October 1995. A voucher specimen (HUM 3800) is deposited at the Herbarium of the Botanical Department of State University of Maringá, Brazil.

3.3. Extraction and isolation

Air-dried stem bark (2750 g) was extracted with Me_2CO–H_2O (7:3, 29 L). The combined extracts were filtered, evapd under reduced pressure and lyophilized (1142 g). A portion (400 g) was redissolved in 4 l H_2O and extracted with EtOAc (40 l). After evapn of solvents, the EtOAc extract and the remaining H_2O phase gave dark brown solids of 98 and 302 g, respectively. A portion (18.5 g) of the EtOAc extract was subjected to CC on Sephadex LH-20 (800 × 56 mm; eluents: 50% EtOH (5 l), EtOH (5 l), 50% MeOH (3 l), MeOH (1,4 l) and 70% Me_2CO (2 l); 15 ml frs) to yield 21 main fractions (indicated below with roman numbers). The 'main fractions' were further separated by MLCCC, which was carried out with the solvent system EtOAc–*n*PrOH–H_2O (140:8:80) on PC: Inc. ITO multilayer coil separator–extractor, flow rate 1.0 ml min^{-1}, using the upper-phase as mobile phase (these frs are indicated below with asteriks).

4′-*O*-methylgallocatechin-(4α→8)-4′-*O*-methylgallocatechin (**1**): a portion (1170 mg) of the 'main fraction' VI (frs 171–214 of the Sephadex column, 2.7 g) was

subjected to MLCCC (10 ml frs^{-1}) to give 7 subfrs. Subfr.*5 (frs 59–90, 124 mg) contained two compounds, which were visualized as red spots by spraying with vanillin/HCl reagent. A portion (60 mg) of subfr.*5 was acetylated and purified by prep. TLC to give **1a** (33 mg) and epigallocatechin-peracetate. Treatment of 2 mg of subfr.*5 with 0.1 M ethanolic HCl (2 ml) at 60°C for 15 min. according to (Thompson et al., 1972) yielded epigallocatechin and 4′-*O*-methylgallocatechin as 'terminal' flavan-3-ol unit, which was detected by TLC on silica gel using reference substances. Compound **1a**: ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.7–2.4 ppm (3H, *all* s, -OAc), 2.65 [1H, dd, $J=7.1$, 16.6, H-4β(F)], 2.83 [1H, dd, $J=4.9$, 16.6, H-4α(F)], 3.77 [3H, s, -OCH_3(B)], 3.79 [3H, s, -OCH_3(E)], 4.48 [1H, d, $J=9.4$, H-4(C)], 4.73 [1H, d, $J=10.0$, H-2(C)], 5.02 [2H, m, H-2(F) and H-3(F)], 5.61 [1H, pseudo t, $J=9.4$, 10.0, H-3(C)], 6.49 [1H, d, $J=2.3$, H-6(A)], 6.52 [1H, d, $J=2.3$, H-8(A)], 6.60 [2H, s, H-2′/6′(E)], 6.65 [1H, s, H-6(D)], 6.81 [2H, s, H-2′/6′(B)]. ^{13}C NMR ($CDCl_3$, 50 MHz): δ 20.3-21.1 (-CO–CH_3), 25.1 [C-4(F)], 36.7 [C-4(C)], 60.9 [-OCH_3(E)], 61.0 [-OCH_3(B)], 68.4 [C-3(F)], 70.4 [C-3(C)], 77.8 [C-2(F)], 78.9 [C-2(C)], 108.1 [C-8(A)], 110.1 [C-6(A)], 111.5 [C-4a(D)], 115.2 [C-4a(A)], 117.0 [C-8(D)], 118.9 [C-2′/6′(E)], 119.9 [C-2′/6′(B)], 132.3 [C-1′(B)], 132.4 [C-1′(E)], 144.1 [C-4′(E)], 144.2 [C-4′(B)], 147.7 [C-7(D)], 148.0 [C-5(D)], 149.0 [C-5(A)], 149.7 [C-7(A)], 152.7 [C-8a(D)], 155.9 [C-8a(A)], 168.2-170.2 [-CO–CH_3]. CD: $[\Theta]_{235}$ −44500, $[\Theta]_{275}$ −15100.

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References

Balas, L., Vercauteren, J., & Laguerre, M. (1995). *Magnetic Resonance in Chemistry*, *33*, 85.

Balas, L., & Vercauteren, J. (1994). *Magnetic Resonance in Chemistry*, *32*, 386.

Barrett, M. W., Klyne, W., Scopes, P. M., Fletcher, A. C., Porter, L. J., & Haslam, E. (1979). *Journal of Chemical Society Perkin Transaction*, *I*, 2375.

Botha, J. J., Young, D. A., Ferreira, D., & Roux, D. G. (1981). *Journal of Chemical Society Perkin Transaction*, *I*, 1213.

Clark-Lewis, J. W., Jackman, L. M., & Spotswood, T. M. (1964). *Australian Journal of Chemistry*, *17*, 632.

Davis, A. L., Cai, Y., Davies, A. P., & Lewis, J. R. (1996). *Magnetic Resonance in Chemistry*, *34*, 887.

de Mello, J. C. P., Petereit, F., & Nahrstedt, A. (1996). *Phytochemistry*, *42*, 857.

de Mello, J. C. P., Petereit, F., & Nahrstedt, A. (1996). *Phytochemistry*, *41*, 807.

de Siqueira, J. C. (1982). *Spectrum J. Bras. Ci.*, *2*, 41.

(1959). *Farmacopéia Brasileira* (pp. 126). São Paulo.

Fletcher, A. C., Porter, L. J., Haslam, E., & Gupta, R. K. (1977). *Journal of Chemical Society Perkin Transaction*, *I*, 1628.

Helsper, J. P. F. G., Kolodziej, H., Hoogendijk, J. M., & van Norel, A. (1993). *Phytochemistry*, *34*, 1255.

Lima, J. C. S., Martins, D. T. O., & de Souza Jr., P. T. (1998). *Phytotherapy Research*, *12*, 218.

Monache, F. D., Pomponi, M., Martini-Bettolo, G. B., D'Albuquerque, I. L., & de Lima, O. G. (1976). *Phytochemistry*, *15*, 573.

Nakano, T., Alonso, J., Grillet, R., & Martin, A. (1979). *Journal of Chemical Society Perkin Transaction*, *I*, 2107.

Petereit, F., Kolodziej, H., & Nahrstedt, A. (1991). *Phytochemistry*, *30*, 981.

Porter, L. J., Newsman, R. H., Foo, L. Y., Wong, H., & Hemingway, R. W. (1982). *Journal of Chemical Society Perkin Transaction*, *I*, 1217.

Santos, C.A., Torres, K.R., & Leonart, R. (1987). *Plantas medicinais* (p. 39). Curitiba.

Thompson, R. S., Jacques, D., Haslam, E., & Tanner, R. J. N. (1972). *Journal of Chemical Society Perkin Transaction*, *I*, 1387.