PERGAMON

Phytochemistry 55 (2000) 359–362

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Eleganoside-A, B and C from *Pseudocalymma elegans*, a native of Brazil

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Received 15 December 1999; received in revised form 5 July 2000

Dedicated to Prof. Salimuzzaman Siddiqui, the founder of H.E.J. Research Institute of Chemistry

Abstract

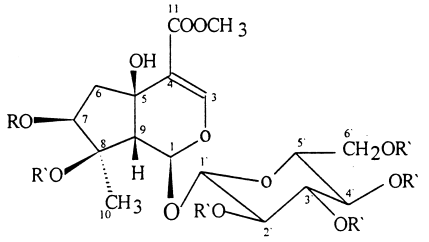
The toxic methanol-soluble part of *Pseudocalymma elegans* (leaves), a native of Brazil, yielded three new iridoidglucosides (**1a–3a**) as their acetate-derivatives (**1–3**) named eleganoside-A (**1a**), B (**2a**) and C (**3a**) which have been characterized with the aid of spectroscopic techniques, including 2D NMR.

Keywords: *Pseudocalymma elegans*; Brazil; Bignoniaceae; Structure elucidation; Iridoid-glucosides; Eleganoside-A, B and C; Acetate derivatives

1. Introduction

The family Bignoniaceae comprises 120 genera and 600 species found abundantly in all humid tropical countries except Australia. Many species of Bignoniaceae have been reported to have medicinal properties and in South America some are widely used as folk medicines (Jimenez et al., 1987). In our search for natural products from Bignoniaceous plants and their toxicology, we have investigated *Pseudocalymma elegans*, a native of Brazil. This plant is well known in Brazil due to its poisonous character against animals. Young leaves of *P. elegans* have shown a more poisonous property as compared to other parts of the plant (Tokarnia et al., 1969). The sign of toxicity starts by shaking of animal's muscles, then the animal feels very uncomfortable and ultimately dies within some minutes. In 1974, Tavares discovered the toxicological features of *P. elegans* (Tavares et al., 1974).

Iridoid glycosides (Damtoft et al., 1997), flavonoids (Subramanian et al., 1972), flavone glycosides (Okuda et al., 1975) and quinones (Thomson, 1968), for example, have been isolated and studied biologically. This communication describes the isolation from the Bignoniaceae and characterization of three new iridoid glucosides: eleganoside-A, B and C (**1a–3a**).



1	**R** = CH_2-$CH(OAc)$-CH_3	**R`** = Ac
1a	**R** = CH_2-$CH(OH)$-CH_3	**R`** = H
2	**R** = H_2C-$(CH_2)_6$-CH_3	**R`** = Ac
2a	**R** = H_2C-$(CH_2)_6$-CH_3	**R`** = H
3	**R** = CH_3	**R`** = Ac
3a	**R** = CH_3	**R`** = H

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0031-9422/00/$ - see front matter
PII: S0031-9422(00)00313-7

2. Results and discussion

2.1. Chemistry

The methanolic extract of the leaves of *Pseudocalymma elegans* yielded three new iridoidglucosides (**1a–3a**) which have been isolated as their acetate-derivatives (**1–3**) and characterized spectroscopically including 2D NMR.

Compound **1** showed a base peak at m/z 331.0989 in the HREIMS corresponding to the formula $C_{14}H_{19}O_9$ due to the acetylated glucose-moiety. Another peak at m/z 615.2001 was due to the M^+ excluding the side-chain ($C_5H_9O_3$). The molecular ion peak in the same spectrum was found at m/z 732.2389 with 2% intensity corresponding to the formula $C_{32}H_{44}O_{19}$ showing eleven degrees of unsaturation. Out of 11, six were due to the acetyl moieties, one (glucose-ring), one (double bond), and one (ester-carbonyl), respectively. The remaining two must be due to the rings in the aglycon-part of the molecule. These fragments were also counter checked in the EIMS of same compound. The IR spectrum of **1** showed the presence of two types of carbonyl functions due to unconjugated (1735 cm^{-1}) and conjugated (1715 cm^{-1}) esters. The hydroxyl and olefinic absorptions were observed at 3520 (OH) and 1610 (C=C) cm^{-1}.

The ^1H NMR spectrum of **1** displayed the presence of nine methyl signals. Out of them, six were due to the acetyl-moieties, one to a methoxyl group, one to a tertiary methyl (H-10) and one to a primary methyl due to the side-chain (H-3″). The basic skeleton contained only one methylene (H-6) which appeared at δ 2.44 as a double doublet with coupling constants of 4.1 and 3.8 Hz. The signal due to the H-7 methine proton was observed in the spectrum at δ 5.24 as a double doublet having the coupling constants 4.3 and 3.5 Hz. In addition to four normal glucose methine signals containing acetate moieties, the signals of an anomeric proton (H-1′) at δ 4.81 (*d*, $J=8.08$ Hz), an olefinic methine at δ 7.32 (*s*) and a methine attached to two oxygens at δ 5.77 (*br,s*) were also observed.

The ^{13}C NMR spectrum of **1** exhibited 32 carbon signals in the broad-band spectrum which were resolved into nine methyls, three methylene, ten methine and ten quaternary-carbons with the help of DEPT experiments. Among the nine methyls, six were due to the acetate moieties and appeared in the carbon spectrum between the range of δ 20.6–21.6. The remaining three methyls appeared at δ 51.7, 18.4 and 17.5 due to the methoxyl group, C-3″ and C-10, respectively. The three methylenes in the molecule resonated at δ 61.9, 60.4 and 43.5 and were due to the C-6′ of sugar moiety, C-1″ and C-6, respectively. The olefinic methine resonated at δ 149.0. Another signal of a methine to which the side chain is attached, appeared at δ 76.5. The C-9 resonated at δ 56.3. An anomeric signal was observed at δ 95.9. The olefinic quaternary carbon resonated at δ 115.8 and the C-5 containing OH appeared at δ 68.2. A quaternary-carbon resonating at δ 166.0 attested for C-11. The six carbonyl functions due to the acetate moieties appeared between the range of δ 169.4–170.6.

The various connectivities in **1** were determined through selective HMBC experiments (Fig. 1). Some chemically acetylated natural iridoids from the same source have been isolated by one of us and their data were compared with **1** (Krebs, 1987; 1991a,b). Compound **1** was isolated as an acetate-derivative and thus the original metabolite would have the structure as **1a** and is named eleganoside-A.

Compounds **2** and **3**, isolated from the same source, contained the same skeleton with some minor changes compared to **1**. Due to this reason, only points of difference have been explained here. Compound **2** contains an β-oriented side-chain of eight carbon atoms at C-7. The position of side-chain in **2** at C-7 was determined by the HMBC experiment (Fig. 2). The $-H_2CO-$ (H-1″) attached to C-7 and terminal methyl (H-8″) of the chain appeared in the proton NMR spectrum at δ 3.70 (*t*, $J=7.4$ Hz) and 0.87 (*t*, $J=7.3$ Hz), respectively, Similarly, compound **3** contained no chain but a methoxyl group at the same position (C-7) with the same orienta-

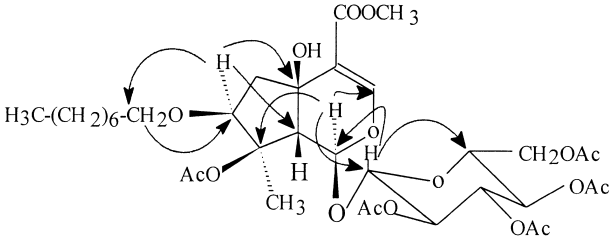

Fig. 2. Selective HMBC connectivities in **2**.

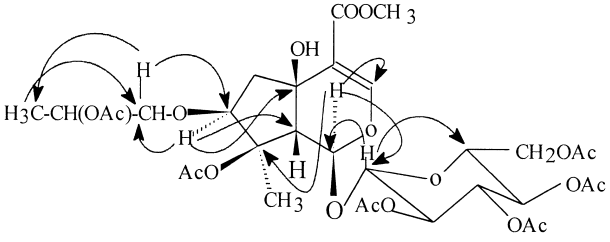

Fig. 1. Selective HMBC connectivities in **1**.

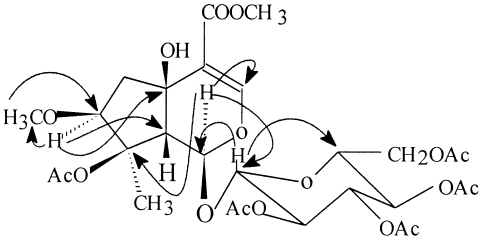

Fig. 3. Selective HMBC connectivities in **3**.

tion (β). The position of second methoxyl moiety in **3** at C-7 was also determined through the HMBC technique (Fig. 3) which appeared at δ 3.89. The detailed data of **1–3** are given in the experimental section. Due to insufficient amounts of **2** and **3**, the carbon spectra could not be taken and thus their structures were elucidated with the aid of proton NMR and HMBC techniques. Compounds **2** and **3** are also new glucoiridoids and were isolated as their acetate derivatives. Their corresponding original metabolites would have structures **2a** and **3a** and were named eleganoside-B and eleganoside-C, respectively.

3. Experimental

3.1. General experimental

The proton and carbon spectra were recorded in $CDCl_3$ at 400 and 100 MHz, respectively.

3.2. Collection and extraction

The leaves of *P. elegans* were collected from Rio de Janeiro (Brazil). Further details regarding the extraction procedures and the toxicology of crude samples can be obtained from the previously published results (Krebs, 1987; 1991a,b).

3.3. Isolation, purification and characterization

An amount of 50 g of the total MeOH extract (213 g) was chromatographed on Sephadex LH-20. As a result of this, five fractions were obtained. Fraction No. 3 showed toxicity and thus was subjected to silica gel column using $CHCl_3$, $CHCl_3$–MeOH, pure MeOH and finally, MeOH-H_2O as mobile phase. The fractions eluted with 25% MeOH in $CHCl_3$ were mixed on the bases of same TLC profiles. In order to separate the components of the mixed fractions the crude mixture was acetylated by dissolving the crude sample (1.0 g) in 3 ml pyridine and into it 4.5 ml Ac_2O was added. The reaction mixture was left overnight at room temperature and then refluxed for an hour, water was added and the organic material was recovered in $CHCl_3$ to afford a gum which on purification through silica gel column (elution with 10% $CHCl_3$ in hexane) and then with thin layer chromatography (developed with 30% $CHCl_3$ in hexane), yielded compounds **1–3** as white powders.

Eleganoside-A (**1**) Hexaacetate: Amount: 18 mg; Yield: 0.0047%; mp: 184.6°C; $[\alpha]_D$: −69.7° ($CHCl_3$: *c* 0.3); IR ν_{max} ($CHCl_3$) cm^{-1}: 3520 (OH), 1735 (CO, ester), 1715 (CO, conjugated ester), 1610 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.32 (1H, *s*, H-3), 5.77 (1H, *br. s*, H-1), 5.26 (1H, *t*, *J* = 9.8, 9.5 Hz, H-3′), 5.24 (1H, *dd*, *J* = 4.3, 3.5 Hz, H-7), 5.08 (1H, *t*, *J* = 9.8 Hz, H-4′), 4.97 (1H, *dd*, *J* = 9.8, 8.1 Hz, H-2′), 4.81 (1H, *d*, *J* = 8.08 Hz, H-1′), 4.37 (1H, *dd*, *J* = 12.4, 4.4 Hz, H-6′A), 4.15 (1H, *dd*, *J* = 12.39, 2.3 Hz, H-6′B), 4.70 (1H, *m*, H-2″), 3.75 (3H, *s*, OCH_3), 3.69 (1H, m, H-5′), 3.46 (2H, *d*, *J* = 6.3 Hz, H-1″), 3.17 (1H, *br. s*, H-9), 2.96 (1H, *br. s*, OH), 2.44 (2H, *dd*, *J* = 4.1, 3.8 Hz, H-6), 2.11, 2.04, 2.03, 2.02, 2.00, 1.94 (each 3H, OAc), 1.37 (3H, *s*, H-10) and 1.19 (3H, *d*, *J* = 6.1 Hz, H-3″); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 93.3 (*d*, C-1), 149.0 (*d*, C-3), 115.8 (*s*, C-4), 68.2 (*s*, C-5), 43.5 (*t*, C-6), 76.5 (*d*, C-7), 84.1 (*s*, C-8), 56.3 (*d*, C-9), 17.5 (*q*, C-10), 166.0 (*s*, C-11), 97.0 (*d*, C-1′), 71.1 (*d*, C-2′), 72.1 (*d*, C-3′, 68.6 (*d*, C-4′), 73.8 (d, C-5′), 61.9 (*t*, C-6′), 60.4 (*t*, C-1″), 70.3 (*d*, C-2″) 18.4 (*q*, C-3″), 51.7 (*q*, OCH_3), 20.6, 20.8, 20.4, 21.1, 21.6 (*q*, $-OCCH_3$), 170.6, 170.4, 170.1, 169.8, 169.5 and 169.4 (*s*, $-OCCH_3$); EIMS: *m*/*z* 732 [M^+, 2%], 615 [M-$C_5H_9O_3$, side chain]$^+$, 331 [100%, acetylated glucose moiety]$^+$, 271 [24%, 331-AcOH]$^+$; HRMS: *m*/*z* 732.2389 (calcd. *m*/*z* 732. 2476 for $C_{32}H_{44}O_{19}$), 615.2001 (calcd. *m*/*z* 615.1924 for $C_{27}H_{35}O_{16}$), 331.0989 (calcd. *m*/*z* 331.1028 for $C_{14}H_{19}O_9$), 271.0763 (calcd. *m*/*z* 271.0817 for $C_{12}H_{15}O_7$).

Eleganoside-B (**2**) Pentaacetate: Amount: 8 mg; Yield: 0.0021%; mp: 183.5°C; $[\alpha]_D$: −47.6° ($CHCl_3$: *c* 0.1); IR ν_{max} ($CHCl_3$) cm^{-1}: 3535 (OH), 1720 (CO, ester), 1715 (CO, conjugated ester), 1615 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.30 (1H, *s*, H-3), 5.66 (1H, *br. s*, H-1), 5.25 (1H, *t*, *J* = 9.6, 7.9 Hz, H-3′), 5.06 (1H, *t*, *J* = 9.7, 7.9 Hz, H-4′), 4.96 (1H, *dd*, *J* = 9.6, 7.9 Hz, H-2′), 4.81 (1H, *d*, *J* = 7.9 Hz, H-1′), 4.67 (1H, *dd*, *J* = 4.4, 3.2 Hz, H-7), 3.75 (3H, *s*, OMe), 3.73 (1H, *m*, H-5′), 2.88 (1 H, *br. s*, H-9), 2.79 (1H, *br. s*, OH), 2.40 (2H, *dd*, *J* = 4.4, 3.6 Hz, H-6), 3.70 (2H, *t*, *J* = 7.4 Hz, H-1″), 2.13, 2.10, 2.02, 1.99, 1.94 (3H each, *s*, CH_3CO–), 1.24 (12H, *br. s*, H-2″ to H-7″), 1.15 (3H, *s*, H-10), 0.87 (3H, *t*, *J* = 7.3 Hz, H-8″); EIMS: *m*/*z* 744 [M^+, 2%], 615[M-$C_8H_{17}O$, side chain]$^+$, 413 [M-331]$^+$, 331 [40%, acetylated glucose moiety]$^+$, 271 [10%, 331-AcOH]$^+$, 169 [100%]$^+$; HRMS: *m*/*z* 744.3199 (calcd. *m*/*z* 744.3204 for $C_{35}H_{52}O_{17}$), 615.2012 (calcd. *m*/*z* 615.1925 for $C_{27}H_{35}O_{16}$), 331.1143 (calcd. *m*/*z* 331.1028 for $C_{14}H_{19}O_9$), 271.0763 (calcd. *m*/*z* 271.0817 for $C_{12}H_{15}O_7$).

Eleganoside-C (**3**) Pentaacetate: Amount: 7.9 mg; Yield: 0.0021%; mp: 181.4°C; $[\alpha]_D$: −52.63° ($CHCl_3$: *c* 0.2); IR ν_{max} ($CHCl_3$) cm^{-1}: 3525 (OH), 1725 (CO, ester), 1720 (CO, conjugated ester), 1615 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.32 (1H, *s*, H-3), 5.68 (1H, *d*, *J* = 1.1 Hz, H-1), 5.25 (1H, *t*, *J* = 9.6, 9.5 Hz, H-3′), 5.08 (1H, *t*, *J* = 9.9, 9.5 Hz, H-4′), 4.93 (1H, *dd*, *J* = 9.6, 8.0 Hz, H-2′), 4.80 (1H, *d*, *J* = 8.0 Hz, H-1′), 4.69 (1H, *dd*, *J* = 4.0, 2.8 Hz, H-7), 4.30 (1H, *dd*, *J* = 12.4, 4.3 Hz, H-6′A), 4.13 (1H, *dd*, *J* = 12.4, 2.3 Hz, H-6′B) 3.75 (3H, *s*, OCH_3), 3.89 (3H, *s*, OMe), 3.73 (1H, *m*, H-5′), 2.94 (1H, *br. s*, OH), 2.88 (1H, *J* = 1.1 Hz, H-9), 2.39 (2H, *dd*, *J* = 4.1, 2.5 Hz, H-6), 2.13, 2.10, 2.02, 2.00, 1.94 (each 3H, *s*, CH_3CO-), 1.15 (3H, *s*, H-10); EIMS: *m*/*z* 646 [1%, M]$^+$, 331 [75%, acetylated glucose moiety]$^+$, 315 [M-

331]$^+$, 271 [16%, 331-AcOH]$^+$, 169 [100%]$^+$; HRMS: m/z 646.1999 (calcd. m/z 646.2108 for $C_{28}H_{38}O_{17}$), 331.1016 (calcd. m/z 331.1028 for $C_{14}H_{19}O_9$), 271.0763 (calcd. m/z 271.0817 for $C_{12}H_{15}O_7$).

Acknowledgements

Part of this research was carried out at Chemisches Institut der Tierärztlichen Hochschule, Hannover (Germany), under the DAAD program for which M.S.A. is very thankful to the German Govt.

References

Damtoft, S., Franzyk, H., Jensen, S.R., 1997. Iridoid glucosides from *Picconia excelsa*. Phytochemistry 45, 743–750.

Jimenez, C., Villaverde, M.C., Riguera, R., Castedo, L., Stermitz, F.R., 1987. Three phenylpropanoid glycosides from *Mussatia hyacinthina*. Phytochemistry 26, 1805–1810.

Krebs, H.C., 1991a. Ingredients from *Pseudocalymma elegans* (Vell.) Kuhlm.2. Isolation of a new iridoid glucoside and of durantoside II. Z. Naturforsch 46b, 1258–1260.

Krebs, H.C., 1991b. Chemische Untersuchungen an medizinisch relevanten Pflanzen und Marinen Invertibraten. PhD thesis. Chemisches Institut der Tierärztlichen Hochschule, Hannover, Germany, pp. 16–48.

Krebs, H.C., 1987. Ingredients from *Pseudocalymma elegans* (Vell.) Kuhlm.1. Isolation of allantion and sorbarin. Z. Naturforsch 42b, 1361–1363.

Okuda, T., Yoshida, T., Ono, I., 1975. Two new flavone glycosides from *Catalpa quata*. Phytochemistry 14, 1654–1656.

Subramanian, S.S., Nagarajan, S., Sulochana, N., 1972. Flavonoids of eight Bignoneaceous plants. Phytochemistry 11, 1499.

Tavares, M.I., de Rezende, A.M.L., Dobereiner, J., 1974. Intoxicacao experimental por *Pseudocalymma elegans* em co elhos e cobaios. Pesq. Agropec. Bras 9, 91–94.

Thomson, R.H., 1968. Naturally occurring quinones. Part XII. Extractives from *Tabebuia chrysanthe* Nichols from other Bignoniaceae. J. Chem. Soc. C, 850–853.

Tokarnia, C.H., Dobereiner, J., Canellla, C.F.C., Cuimaraes, D.J., 1969. Intoxicacas experimental por *Pseudocalymma elegans* (Vell.) Kuhlm. em bovinos. Pesq. Agropec. Bras. 4, 195–204.