Available online at www.sciencedirect.com
ScienceDirect
Phytochemistry 68 (2007) 1327–1330
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Cyathenosin A, a spiropyranosyl derivative of protocatechuic acid from *Cyathea phalerata*

Moacir Geraldo Pizzolatti [a,*], Ines Maria Costa Brighente [a], Adailton João Bortoluzzi [a], Jan Schripsema [b], Luiz Gonzaga Verdi [a]

[a] *Laboratório de Produtos Naturais e Cristalografia, Departamento de Química, Universidade Federal de Santa Catarina, 88040-900 Florianópolis, Brazil*
[b] *Grupo Metabolômica, Laboratório de Ciências Químicas, Universidade Estadual do Norte Fluminense, 28015-620, Campos dos Goytacazes, RJ, Brazil*

Received 24 August 2006; received in revised form 11 January 2007
Available online 26 March 2007

Abstract

Cyathenosin A, a spiropyranosyl derivative of protocatechuic acid was isolated from the stem pith of *Cyathea phalerata* Mart. Its structure was determined by MS, 1D and 2D NMR spectroscopic analyses and confirmed by single crystal X-ray analysis. Cyathenosin A is the first example of a naturally occurring compound containing a spirocyclic orthoester pyranosidic structure.

Keywords: *Cyathea phalerata*; Cyatheacae; Cyathenosin A; Spiro-orthoester pyranosidic; Spirocyclic compound; Cyathenoside A

1. Introduction

Ferns (Pteridophyta) represent a division of the plant kingdom with over 12,000 species, of which many are tree ferns found in the tropical and subtropical regions of the world. *Cyathea phalerata* Mart. is a tree fern which grows up to 4 m tall in the tropical and subtropical areas of Brazil. Its stem pith is used for several inflammatory diseases. Lowland folk in southeastern Brazil use the alcohol extract of the pith for the treatment of varicose veins and hemorrhoids. In previous studies on the *Cyathea* genus, several classes of compounds were found including fernene, filicene and hopane triterpenes (Arai et al., 1994; Arai et al., 1995), phenolic acids such as coumaric, caffeic, and protocatechuic acids and flavonoids represented mainly by kaempferol glycosides (Hiraoaka and Hasegwa, 1975; Hiraoka and Maeda, 1979; Bringmann et al., 1999). In the search for the biologically active substances of this folk medicine, kaempferol-3-neohesperidoside was isolated in a previous study and it was shown to have antioxidant and hypoglycemic ativity (Cazarolli et al., 2006). We report here the isolation and structural elucidation of an orthoester spiropyranosyl derivative of protocatechuic acid with a new structural feature, along with two known glucosides: 4-*O*-β-D-glucopyranosyl caffeic acid (**2**) and 4-*O*-β-D-glucopyranosyl coumaric acid (**3**), from the aqueous residue.

2. Results and discussion

The EtOH extract dried from the stalk of *C. phalerata*, after extraction with $CHCl_3$ and acetone, was subjected to repeated silica gel column chromatography affording cyathenosin A (**1**), 4-*O*-β-D-glucopyranosyl caffeic acid (**2**) and 4-*O*-β-D-glucopyranosyl coumaric acid (**3**). The compounds (**2**) and (**3**) were identified on the basis of IR, MS, ^1H and ^{13}C NMR, and 2D NMR spectroscopic analyses and comparison with the literature data (Bringmann et al., 1999).

Cyathenosin A (**1**) was isolated as colorless prism-like crystals. With direct-inlet EI-MS a molecular ion peak $[M]^{+\cdot}$ was obtained at *m*/*z* 314. Together with the number

* Corresponding author. Tel./fax: +55 4837216844.
E-mail address: mgpizzo@qmc.ufsc.br (M.G. Pizzolatti).

0031-9422/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.phytochem.2007.02.009

Table 1
^{1}H NMR and ^{13}C NMR, and HMBC spectroscopic data for compound **1**[a]

C	δ_C	δ_H	HMBC
1	126.1 *s*		H-5
2	110.9 *d*	7.52 *d* (1.6)	(H-5)
3	147.9 *s*		H-2, H-5, H-6
4	151.8 *s*		H-2, H-5 (H-6)
5	109.0 *d*	6.96 *d* (8.3)	(H-2)
6	126.3 *d*	7.68 *dd* (1.6–8.3)	H-2
7	169.2 *s*		H-2, H-6
1′	127.3 *s*		H-2′, H-3′
2′	73.6 *d*	3.75 *m* (overlapped)	
3′	75.9 *d*	3.73 *m* (overlapped)	
4′	70.6 *d*	3.53 *m*	
5′	77.6 *d*	3.83 *m* (overlapped)	
6′	61.9 *t*	3.72 *m* (overlapped)	H-5′
		3.81 *m* (overlapped)	

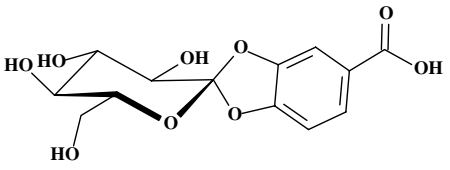
[a] *J* in Hz, in CD_3OD, ^{1}H and ^{13}C NMR at 400 and 100 MHz, respectively. Weak HMBC correlations are indicated between brackets.

of carbon atoms obtained from the ^{13}C NMR spectra (Table 1), this indicated that the molecule has the molecular formula $C_{13}H_{14}O_9$. The IR spectrum showed absorptions characteristic of hydroxyl (3352 cm^{-1}) and carbonyl (1690 and 1671 cm^{-1}) suggesting the presence of carboxylic acid and glycosyl groups. The ^{1}H NMR spectrum had three aromatic signals [δ_H 7.68 (1H, *dd*, $J = 1.6$ and 8.3 Hz), 7.52 (1H, *d*, $J = 1.6$ Hz) and 6.96 (1H, *d*, $J = 8.3$ Hz)] which were attributed to a 1,2,4-trisubstituted aromatic unit, and complex signals [δ_H 3.84–3.70 (5H) and 3.56–3.51 (1H)] for six protons attached to oxygenated carbons indicating a glycosyl moiety. However, the signal for the anomeric proton was absent. The ^{13}C NMR spectrum taken in CD_3OD showed 13 carbon signals, and the DEPT experiments indicated one oxymethylene, four oxymethynes, three aromatic methynes and five quaternary carbons including one carbonyl. Interpretation of the ^{13}C NMR (Table 1) chemical shifts showed the presence of protocatechuic acid and glucosyl units, but the anomeric carbon of the glucosyl unit yielded a signal at δ 127.3, much further downfield than expected. All the methynic and methylenic carbons were assigned through direct ^{1}H–^{13}C correlations in the HMQC (Table 1) spectrum. In the HMBC spectrum, the δ_C 127.3 (*s*, C-1′) resonance showed cross-peaks for δ_H 3.75 (1H, *m*, H-2′) and 3.73 (1H, *m*, H-3′), which confirmed that this carbon corresponds to C-1′ of the glucosyl moiety. The carbon-13 resonance in low field indicates that it is linked to both oxygens of the protocatechuic moiety. The carboxylic carbon δ_C 169.2 showed cross-peaks for δ_H 7.52 (1H, *d*, $J = 1.6$ Hz, H-2), 7.68 (1H, *dd*, $J = 1.6$–8.3 Hz, H-6) and 6.96 (1H, *d*, $J = 8.3$ Hz, H-5), and also other long range correlations observed as H-5/C-2, C-3, C-4, C-6; H-2/C-3, C-5, C-6, C-4; and H6/C-2, C-3, respectively, confirming the structure assigned. This compound thus possesses the unique structural feature of a glucopyranosyl C-1′ spiro fused to the catechol group of the protocatechuic acid which however, might have the *R* or *S* configuration at C-1′.

The compound was obtained as colorless crystals after several recrystallizations from methanol. Analysis of the single crystal X-ray diffraction of the compound not only confirmed the presence of the spirocyclic carbon at C-1′, but also the glucopyranosyl ring, indicating that the hydroxyl groups at C-2′ and C-4′ are in the β orientation and the hydroxyl and hydroxymethyl groups linked at C-3′ and C-5′ are in the α orientation. The X-ray analysis also showed the presence of one water molecule and two cyathenosin A molecules in the asymmetric unit. This indicates the molecular formula of $C_{26}H_{30}O_{19}$, which is in agreement with the elemental analysis. The presence of water in the crystal structure is associated with the extensive hydrogen bond network. The O1W atom is in an approximately tetrahedral environment and the water molecule forms an H-bond with four neighboring cyathenosin A molecules. Thus, the structure was determined as a protochatecuic acid derivative containing a glycopyranosyl unit fused to the catechol group through the C-1′ spiro with a *R* configuration as shown in Figs. 1 and 2, which was named cyathenosin A (**1**).

Natural compounds containing a carbon spiro-orthoester as a structural feature were first identified by Ganguly and co-workers in everninomycins, a group of oligosaccharide antibiotics isolated from the fermentation broth of *Micromonospora carbonaceae* (Ganguly et al., 1975). Cyathenosin A (**1**) thus represents the first reported natural occurrence of a spiro-orthoester glucoside in the plant kingdom.

Furthermore, it is interesting to note that several similar phenolic acid derivatives have been reported in ferns: Protocatechuic acid – 4-*O*-β-D-glucopyranoside was reported in the fern *Angiopteris lygodiifolia* (Hseu, 1981).



1

Fig. 1. Structure of cyathenosin A (**1**).

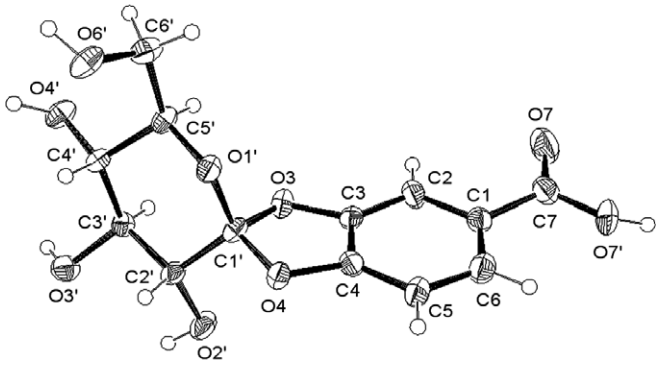


Fig. 2. X-ray crystallographic structure of cyathenosin A (**1**).

3. Experimental

3.1. General

^{1}H NMR (400 MHz, CD_3OD), ^{13}C NMR (100 MHz, CD_3OD) and 2D spectra were recorded on a Jeol Eclipse + 400 spectrometer. Residual solvent signals were used as internal standard, at 3.30 and 49.00 ppm for ^{1}H and ^{13}C NMR, respectively. Optical rotations were determined with a Schmidt and Haenschi polarimeter. EI mass spectra were measured on a Shimadzu QP5050A spectrometer. TLC: precoated silica gel type 60; CC: silica gel (230–400 mesh or 70–230 mesh) type 60.

3.2. Plant material

C. phalerata Mart was collected in March 2002 at lowland Atlantic Rain Forest in Plalhoça, Santa Catarina State, Southeastern Brazil. It was identified by Prof[a]. Dr[a]. Lana Silvestry and a voucher specimen (No. RBR 4287) was deposited in the herbarium of Departamento de Botânica da Universidade Federal Rural do Rio de Janeiro.

3.3. Extraction and isolation

A fresh stalk of *C. phalerata* Mart was peeled, cut into small pieces and extracted (9.0 kg) with EtOH–H_2O (9:1, v/v × 2) for 15 days at room temperature to afford following filtration and evaporation in vacuo, to get crude extract (189.0 g). An aliquot of the dried and powdered crude extract (30.0 g) was degreased with hexane and subsequently extracted with $CHCl_3$ and acetone, three times under stirring at room temperature. Each extract was filtered and concentrated in vacuo to afford the $CHCl_3$ (1.5 g) and acetone (4.2 g) extracts. A polar water soluble residue remained (24.3 g). Evaporation of the acetone extract led to precipitation of the main flavonoid glycoside kaempferol-3-neoesperidoside. From the $CHCl_3$ extract β-sitosterol was isolated. The aqueous residue (20.0 g) was prefractionated by column chromatography (5.5 cm × 25 cm) on silica gel (70–230 mesh) eluting with a EtOAc–MeOH gradient to give 47 fractions (200 mL each). Fractions 23–28 (EtOAc–MeOH 7:3) were subjected to flash CC (silica gel 60, 230–400 mesh) eluted with EtOAc–EtOH (4:1 + 3% AcOH) to yield cyathenosin A (**1**) (164.0 mg). Further flash CC of fractions 30–34 (EtOAc–MeOH 3:2) led to isolation of the 4-*O*-β-D-glucopyranosyl caffeic acid (**2**) (32 mg) and 4-*O*-β-D-glucopyranosyl coumaric acid (**3**) (8 mg).

3.3.1. Cyathenosin A (*1*)

Colorless crystals (MeOH); m.p. 169–171 °C; $[\alpha]_D^{25}$ +64.1 (MeOH–H_2O 30%), IR (KBr) ν_{max} cm^{-1} 3352, 2923, 2915, 1690, 1671, 1456, 1263, 1098, 1030, 978, 855,760,646, 536; EI MS (70 eV) *m/z* (%): 314 $[M]^+$ (13), 194 (15), 181 (10), 154 (100), 137 (46), 109 (14), 73 (39, 60 (53), 43 (50); for ^{1}H NMR and ^{13}C NMR spectroscopic data, see Table 1; CHN Anal. Calc. for $C_{13}H_{14}O_9$: C 49.69, H 4.49, O 45.82; for $C_{13}H_{14}O_9 \cdot 1/2H_2O$: C 48.30, H 4.68, O 47.02, found C 48.30, H 4.68, O 47.03. Crystal data: $C_{13}H_{15}O_{9.5}$, MW = 323.25, orthorhombic, space group $P2_12_12_1$, $a = 7.947(1)$ Å, $b = 18.060(1)$ Å, $c = 19.523(3)$ Å, $V = 2801.9(7)$ Å^3, $T = 293(2)$ K, $Z = 8$, μ (Mo Kα) = 0.134 mm^{-1}, $D_{calc} = 1.533$ g cm^{-3}, 2949 reflections collected, 414 parameters, GooF = 1.074, final indices: $R[I > 2\sigma(I)] = 0.0516$ and $wR(F^2) = 0.1484$ (all data). A colorless prismatic crystal was selected from a crystalline sample and isolated and mounted on a CAD-4 diffractometer. Cell parameters were determined from 25 carefully centered reflections in the θ range 6.71–17.00°. All data were corrected for Lorentz and polarization effects. The structure was solved with the SIR-97 program and refined by full-matrix least-squares methods using the SHELXL-97 program. All non-hydrogen atoms were refined with anisotropic displacement parameters. H atoms attached to C atoms were placed at their idealized positions, with C–H distances and U_{eq} values taken from the default settings of the refinement program. The H atoms of the acid and alcohol groups and the water molecule were found from the Fourier difference map and treated with the riding model. A full list of crystallographic data have been deposited with the Cambridge Crystallographic Data Centre, CCDC 283447. It is available by mail at CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK; by fax: +44 1223 336 033 or by internet facilities: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk.

Acknowledgements

The authors are grateful to CNPq for financial support and to Prof[a]. Maria da Graça Nascimento for helping with the optical rotation measurements.

References

Arai, Y., Koide, N., Ohki, F., Ageta, H., Yang, L.-L., Yen, K.-Y., 1994. Ferns constituents: triterpenoids isolated from leaflets of *Cyathea spinulosa*. Chem. Phar. Bull. 42 (2), 228–232.

Arai, Y., Hirohara, M., Mtsuhira, M., Tyoasaki, K., Ageta, H., 1995. Ferns constituents: triterpenoids isolated from leaflets of *Cyathea lepifera*. Chem. Phar. Bull. 43 (11), 1849–1852.

Bringmann, G., Günther, C., Jumbam, D.N., 1999. Isolation of 4-*O*-b-D-glucopyranosylcaffeic acid and galic acid from *Cyathea dregei* Kunze (Cyatheaceae). Pharm. Pharmacol. Lett. 9 (2), 41–43.

Cazarolli, L.H., Jorge, A.P., Zanatta, Leila, Sousa, E., Verdi, L.G., Horst, H., Pizzolatti, M.G., Szpoganicz, B., Silva, F.R.M.B., 2006. Follow-up studies on flavonoids and their complexes with vanadium: their anti-hyperglycemic potential roles in diabetes. Chem. Biol. Interact. 162 (3). Available online 12 August 2006.

Ganguly, A.K., Sarre, O.Z., Greeves, D., Morton, J., 1975. Structure of everninomicin D. J. Am. Chem. Soc. 97, 1982–1985.

Hiraoaka, A., Hasegwa, M., 1975. Flavonoide glycosides from five *Cyathea* species. Bot. Mag. Tokyo 88, 127–130.

Hiraoka, A., Maeda, M., 1979. A new acylated flavonol glycoside from *Cyathea contaminans* Copel, and its distribution in the Pterophyta. Chem. Phar. Bull. 27 (12), 3130–3136.

Hseu, T.H., 1981. Structure of angiopteroside (4-*O*-b-D-glucopyranosyl-L-*threo*-2-hexen-5-olide) monohydrate, a fern glycoside from *Angiopteris lygodiifolla* Ros. Acta Crystalogr., Sect. B 37, 2095–2098.