PERGAMON

Phytochemistry 55 (2000) 779–782

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

A dimeric ArC_2 compound from *Peperomia pellucida*

Joaquim de C. Bayma *, Mara Silvia P. Arruda, Adolfo H. Müller, Alberto C. Arruda, Williams C. Canto

Departamento de Química, CCEN, Campus Universitário, Universidade Federal do Pará, 66075-900, Belém, PA, Brazil

Received 8 July 1998; received in revised form 1 December 1999

Abstract

Pellucidin A, a novel dimeric ArC_2 compound, together with dill-apiol have been isolated from the aerial parts of *Peperomia pellucida*. The structure of pellucidin A was established by 1D and 2D NMR spectroscopy (^1H–^1H COSY; ^1H–^{13}C COSY; DEPT; NOESY and double irradiation) and other spectroscopic techniques. The biogenesis of pellucidin A is also briefly discussed. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Peperomia pellucida*; Piperaceae; Dimeric ArC_2; Pellucidin A

1. Introduction

Peperomia pellucida (Piperaceae), whose popular name in the Amazon region is 'erva-de-jabuti', is a herb known for its medicinal properties. The whole herb is used as an emollient, diuretic and to control cough and cardiac arrhythmia (Berg, 1993; Pimentel, 1994). Previous chemical investigations on this plant established the occurrence of apiol, 2,4,5-trimethoxystyrene, flavones, flavonols and phytosterols (Manalo et al., 1983; Aqil et al., 1993, 1994). A chemical study of methanolic extract of the aerial parts has led to the isolation of a new dimeric ArC_2 compound which was named pellucidin A (**1**) along with the known phenylpropanoid dill-apiol (**2**) (Bernhard and Thiele, 1978). A plausible biosynthetic pathway for **1** is also proposed.

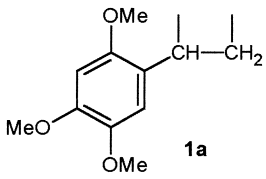
2. Results and discussion

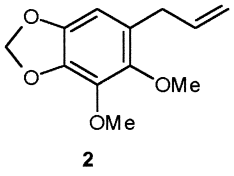
The methylene chloride soluble portion of the methanolic extract from aerial parts of *P. pellucida* was fractionated by column chromatography yielding compounds **1** and **2**.

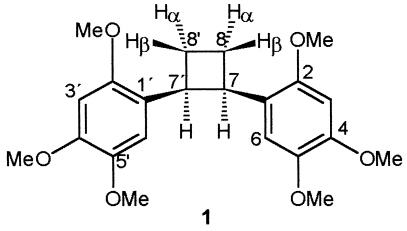
Pellucidin A (**1**), $C_{22}H_{28}O_6$ ($[M]^+$ *m*/*z* 388), showed absorptions at 1608, 1516 and 1481 cm^{-1} in its IR spectrum revealing its aromatic nature (Colthup et al., 1975). The ^1H NMR spectrum (Table 1) showed two signals (1H each, *s*) at δ 6.97 and 6.47 attributable to protons of a 1,2,4,5-tetrasubstituted aromatic ring (Patra and Mitra, 1979), a signal (3H, *s*) at δ 3.74 related to one aromatic methoxyl group, and two signals (1H each) at δ 2.31 and 1.95 suggesting the presence of one methylene group, and two singlets at δ 3.85 and 3.84 superimposed on a multiplet signal, all integrating 7H, characterizing two more aromatic methoxyl groups and a methine proton. The DEPT spectrum showed two carbons in the aliphatic carbon region due to methylene (δ_C 27.0) and methine (δ_C 40.6) groups. Furthermore, the HETCOR experiment correlated all the proton resonances with those of the corresponding one-bond coupled carbons. The ^1H–^1H COSY spectrum showed correlations of the methylene protons with each other (δ 2.31 and 1.95) and both with the methine proton (δ 3.85). These data indicated a partial structure **1a** for pellucidin A. The ^{13}C NMR spectrum (Table 1) is in agreement with structure **1a**. The ^{13}C NMR, DEPT and HETCOR spectra still revealed the presence of six signals in aromatic carbon region due to two methines (δ 112.2 and 98.2), three oxygenated (δ 151.3, 147.8 and 143.3) and one quaternary (δ 124.8), besides three signals (δ 56.7, 56.6 and 56.3) assigned to three methoxyl groups. After an evaluation of the ^1H, ^{13}C and mass spectral data, it was evident that **1** is a symmetric dimer of **1a**, resulting in four structural possibilities **1**, **1b**, **1c**,

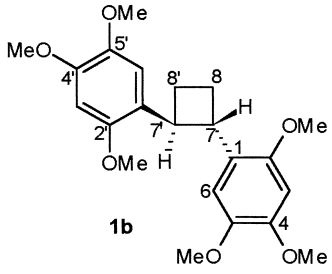
* Corresponding author.

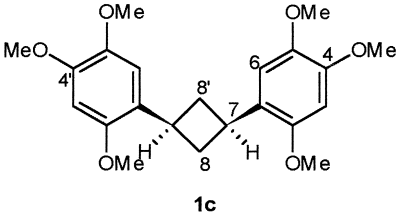
PII: S0031-9422(00)00224-7

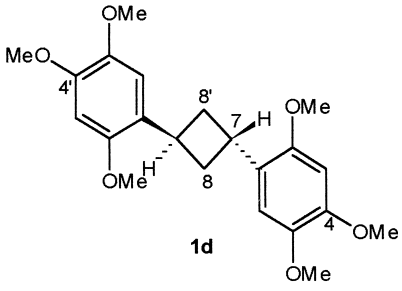












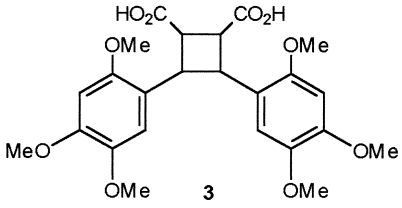


Table 1
^{1}H and ^{13}C NMR spectral data for compound **1** in $CDCl_3$ at 300 MHz

Position	δ_C	δ_H (mult., *J* Hz)	COLOC		NOESY
			$^{3}J_{CH}$	$^{2}J_{CH}$	
1/1′	124.9		H-3/3′		
2/2′	147.8		H-6/6′, OMe-2/2′	H-3/3′	
3/3′	98.2	6.47 (*s*)			OMe-2/2′ and 4/4′
4/4′	151.3		H-6/6′	H-3/3′	
5/5′	143.3		H-3/3′	H-6/6′	
6/6′	112.2	6.97 (*s*)	H-7/7′		8/8′ β, OMe-5/5′
7/7′	40.6	3.85 (*m*)			
		α-2.31 (*ddd*, 11.4,6.8 and 2.1)			8/8′ β, OMe-2/2′
8/8′	27.0				
		β-1.95 (*ddd*, 11.4, 4.3 and 2.2)			8/8′ α, OMe-2/2′
OMe-4/4′	56.3	3.74 (*s*)			3/3′
OMe-2/2′ and OMe-5/5′	56.6 and 56.1	3.84 and 3.85 (*s*, each)			

1d (all possessing a symmetric plane or symmetry operation).

The signals assigned to H-8/8′ α and β (δ 2.31 and 1.95) exhibited coupling patterns (*ddd*) consistent with **1** and **1b** but excluding **1c** and **1d**, which would show *dt* signals. These conclusions were confirmed by the presence of a fragment ion at *m/z* 360 (2.0%) in the mass spectrum (Scheme 1) and by double irradiation experiments. Irradiation of H-7/7′ (δ 3.85) collapsed the H-8/8′ α and β (δ 2.31 and 1.95) to a doublet of doublets, and irradiation of H-8/8′ α or β collapsed the other methylene proton to a doublet of doublets. The structure of pellucidin A was determined to be **1** by NOESY experiment which showed NOE's only between the protons H-8/8′ β (δ 1.95) and the aromatic protons H-6/6′ (δ 6.97), establishing a *cis* relationship between the two aromatic rings. In the case of **1b** NOE's would be expected between H-8′ β and H-6′, and between H-8α and H-6, indicating a *trans* relationship between the aromatic rings.

Connectivities obtained from a COLOC experiment (Table 1) optimized for $^{2,3}J_{CH}$, enabled the correct assignment of non-hydrogenated aromatic carbons, and also confirmed the linking between the cyclobutane ring and the two 2,4,5-trimethoxyphenyl groups due to a three-bond connectivity from the methine protons H7/7′ (δ 3.85) with the protonated aromatic carbons C6/6′ (δ 112.2).

A biosynthetic pathway proposed for **1** might involve loss of two CO_2 from lignan **3**, but the occurrence of 2,4,5-trimethoxystyrene in *P. pellucida* (Manalo et al., 1983) suggests another possibility derived from an intermediate type 2,4,5-trihydroxystyrene. Thus, we believe that pellucidin A (**1**) is formed by stereospecific enzyme-mediated reductive coupling of the two 2,4,5-trihydroxy styrene units depicted in Scheme 2. A [2 + 2] cycloaddition reaction between two 2,4,5-trimethoxystyrene molecules is not plausible because of steric hindrance.

3. Experimental

3.1. General

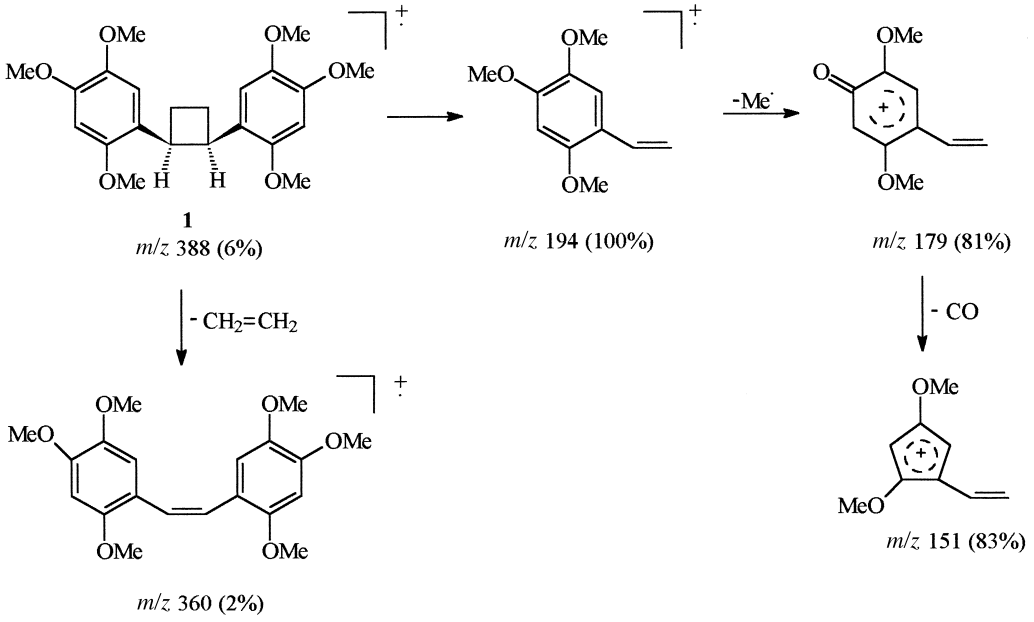
IR were recorded in KBr discs. ^{1}H and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, in $CDCl_3$ on a Varian GEMINI 300. EIMS were obtained by direct probe insertion at 70 eV. Column chromatography: silica gel (Merck 7734) TLC: precoated Kieselgel 60 F_{254} (Merck).

3.2. Plant material

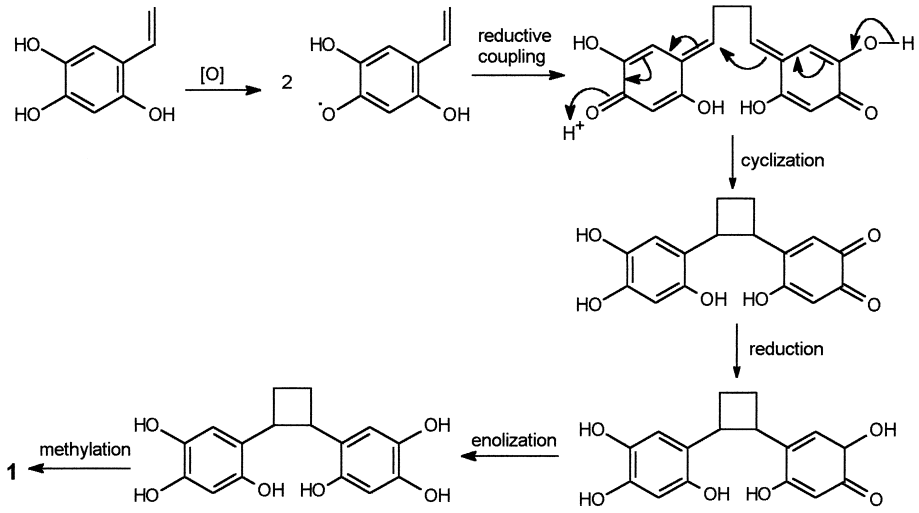
Five kilograms of aerial parts of *P. pellucida* resulted from a cultivation period of 6 weeks. A specimen is on file in Museum Paraense Emílio Goeldi (Belém, Pará) and was identified by Dra. Maria Elisabeth van den Berg.

3.3. Extraction and isolation

Dried and ground aerial parts (418 g) were extracted with hot EtOH (2 L). The EtOH extract was concentrated under reduced pressure and the viscous concentrate (59 g) partitioned between 5% HCl and methylene chloride. Column chromatography of the methylene chloride soluble-phase (12 g) on silica gel and elution with a gradient mixture of *n*-hexane/EtOAc (92:8) gave fractions 180–199 (148.7 mg) and 219–229



Scheme 1. Main fragments in the EI mass spectrum of **1**.



Scheme 2. Suggested biosynthetic pathway for **1**.

(143.1 mg). Further purification of both fractions was performed on preparative TLC (*n*-hexane/EtOAc 95:5) followed by crystallization from *n*-hexane/EtOAc to give **1** (18 mg) and **2** (35 mg).

3.4. Pellucidin A (*1*)

Pale colourless amorphous, mp 109.6–110.5°C (*n*-hexane/EtOAc); IR ν^{film} cm^{-1}: 2916, 2850, 1608, 1516, 1451, 1395, 1207, 1042, 815; EI/MS *m*/*z* (rel. int.): 388 $[M]^+$ (6), 360 (2), 194 (100), 179 (81), 151 (83), 136 (19), 121 (13), 108 (11), 91 (19), 77 (18), 69 (22); ^1H and ^{13}C NMR spectral data: see Table 1.

Acknowledgements

The authors are grateful to Financiadora de Estudos e Projetos (FINEP) for financial support, and to Departamento de Química da Universidade Federal de São Carlos for the NOESY and mass spectra. One of us (W.C. Canto) also thanks to Conselho Nacional de Desenvolvimento Científico e Tecnológico/CNPq for scholarship.

References

Berg, M.E. Van den, 1993. Plantas Medicinais da Amazônia. Contribuíção ao Conhecimento Sistemático, 2nd Edition. CNPQ/MPEG, Belém, p. 56.

Pimentel, A.A.M.P., 1994. Cultivo de Plantas Medicinais na Amazônia, FCAP, Serviço de Documentação e Informação, Belém (p. 51).

Manalo, J.B., Han, B.H., Han, Y.N., Park, M.H., Anzaldo, F.E., 1983. Arch. Pharmacol. Res. 62, 133.

Aqil, M., Khan, I.Z., Ahmad, M.B., 1993. Sci. Phys. Sci. 5, 213.

Aqil, M., Rahman, F.A., Ahmad, M.B., 1994. Sci. Phys. Sci. 61, 141.

Bernhard, H.O., Thiele, K., 1978. Helv. Chim. Acta 61, 2273.

Colthup, N.B., Daly, L.H., Wiberley, S.E., 1975. Introduction to Infrared and Raman Spectroscopy, 2nd Edition. Academic Press, London, p. 260.

Patra, A., Mitra, A.K., 1979. Indian J. Chem. 17 B, 412.