Article

J. Mex. Chem. Soc. **2023**, *67*(3)
Special Issue

ISSN-e 2594-0317

Pulcaffeate, a Trimeric Caffeic Acid Derivative from *Bourreria pulchra*

Gilda J. Erosa-Rejón[1,2], Alejandro Yam-Puc[2], Luis M. Peña-Rodríguez[2*], Olov Sterner[1*]

[1]Division of Organic Chemistry, Lund University, PO Box 124, SE-22100, Lund, Sweden.
[2]Laboratorio de Química Orgánica, Unidad de Biotecnología, Centro de Investigación Científica de Yucatán. Calle 43 # 130 Col. Chuburná de Hidalgo, Mérida, Yucatán, México 97205.

***Corresponding author:** Olov Sterner, email: Olov.Sterner@organic.lu.se; Tel.: +46 46 222821;
Luis M. Peña-Rodríguez, email: lmanuel@cicy.mx; Tel: +52-9998-990767.

Received Febraury 23rd, 2023; Accepted April 18th, 2023.

DOI: http://dx.doi.org/10.29356/jmcs.v67i3.1985

Dedicated to Dr. Víctor Manuel Loyola Vargas on the occasion of his 75th birthday.

Abstract. The trimeric caffeic acid derivative pulcaffeate (**1**) was isolated from the organic crude extract of the roots of *Bourreria pulchra* (Boraginaceae). The biosynthesis of **1** appears to involve an intramolecular Diels-Alder reaction between a cyclohexa-1,3-diene intermediate, generated by the oxidation of the aromatic ring of a cinnamic acid unit, and the conjugated double bond of a caffeic acid unit. The structure of the new metabolite was elucidated through a detailed analysis of its ^{1}H and ^{13}C NMR spectroscopic data. This is the first report of a trimeric metabolite occurring in *B. pulchra*.
Keywords: Boraginaceae; cinnamic acid; caffeic acid; intramolecular cycloaddition; Diels-Alder reaction.

Resumen. Pulcafeato (**1**) un derivado trimérico del ácido cafeico fue aislado del extracto orgánico crudo de las raíces de *Bourreria pulchra* (Boraginaceae). La biosíntesis de **1** parece llevarse a cabo mediante una reacción intramolecular de Diels-Alder entre un intermediario de cyclohexa-1,3-dieno, generado durante la oxidación del anillo aromático de una unidad de ácido cinámico, y el doble enlace conjugado de una unidad de ácido cafeico. La estructura del nuevo metabolito fue elucidada mediante el análisis detallado de sus datos espectroscópicos de RMN de ^{1}H y ^{13}C. Este es el primer reporte sobre la presencia de un metabolito trimérico en *B. pulchra*.
Palabras clave: Boraginaceae; ácido cinámico; ácido cafeico; cicloadición intramolecular; reacción de Diels-Alder.

Introduction

Bourreria pulchra Millsp. (Boraginaceae), a medicinal plant commonly known as "bakalche" and "azar del monte", is used in Yucatecan traditional medicine as an antiviral and antipyretic, as well as for the treatment of cutaneous diseases [1]. Recently, as part of a project directed towards the detection, isolation, and identification of bioactive metabolites from the native flora of the Yucatan peninsula, the root extract of *B. pulchra* showed DNA-interacting activity when tested using the DNA-methyl green assay [2]. As a result of a phytochemical investigation of the root extract of *B. pulchra*, we wish to report herein on the isolation and characterization of pulcaffeate (**1**), a novel trimeric caffeic acid derivative which appears to be formed via an intramolecular Diels-Alder cycloaddition between an intermediate diene derived from cinnamic acid and the conjugated double bond of a caffeic acid unit.

J. Mex. Chem. Soc. **2023**, *67*(3)
Special Issue

ISSN-e 2594-0317

Experimental

General experimental procedures

Optical rotation was measured using a polarimeter Perkin Elmer Model 341. ESI-HRMS spectra were recorded in a Waters Q-TOF Micro system spectrometer, using H_3PO_4 for calibration and as an internal standard. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were run in a Bruker DRX 500 spectrometer; the spectra were recorded in acetone-d_6, at 27 °C, and the solvent residual signals (2.05 and 29.9 ppm, for ^{1}H and ^{13}C NMR, respectively) were used as reference. Vacuum liquid chromatography (VLC) separations were carried out using TLC-grade silica gel (Merck), while column chromatography separations were run using silica gel 60 (230-400 mesh, Merck). TLC analyses were carried out using aluminum-backed silica gel 60 F_{254} (0.20 mm thickness) plates (Merck); chromatograms were first visualized by observing under a UV lamp (254 nm) and then spraying with 10 % sulfuric acid, followed by heating at 100 °C.

Plant material

The roots of *Bourreria pulchra* Millsp. were collected in Libre Unión (Cenote Xtojil), Yucatán, México in July 2003. The sample was identified by the taxonomist Paulino Simá, and a voucher specimen (PSimá 2661) has been deposited at the herbarium of the Unidad de Recursos Naturales of the Centro de Investigación Científica de Yucatán.

Extraction and isolation

Dried-ground roots (2 kg) were extracted with ethanol, three times at room temperature for one week. After filtration, the extracts were combined, and the solvent was evaporated under reduced pressure to give 63.10 g of organic extract. The extract was suspended in a mixture of water:methanol (9:1, v/v, 500 mL) and the resulting aqueous suspension was successively partitioned between n-heptane (three times, 2:1, v/v), dichloromethane (three times, 2:1, v/v), ethyl acetate (three times, 2:1, v/v) and butanol (three times, 1:1, v/v), to yield the corresponding low (4.35 g), medium-low (3.39 g), medium-high (7.28 g), and high polarity (24.08 g) fractions, respectively. The ethyl acetate (7.28 g) fraction was purified by VLC using gradient elution with mixtures of n-heptane, dichloromethane, and methanol to produce seven major fractions (A-G). Fraction C (3.70 g) was subjected to silica gel column chromatography and eluted with n-heptane/dichloromethane/methanol (2:7:1) to give 10 fractions (A2-J2). Column chromatography purification over silica gel of fraction G2 (400 mg), eluting with n-heptane/dichloromethane/methanol (2:7:1, v/v), yielded pulcaffeate (**1**) (106.1 mg) in pure form.

Pulcaffeate (1): brown amorphous powder; $[\alpha]^{20}_D$ +86.4 (c 2.0, $CHCl_3$/MeOH); ^{1}H (500 MHz) and ^{13}C (125 MHz), see Table 1. ESI-HRMS (positive ion) *m/z* 539.1553 (calcd. for $C_{28}H_{27}O_{11}$ $[M + H]^+$, 539.1553).

Results and discussion

The organic crude extract of the roots of *B. pulchra* was fractionated using a liquid-liquid partition procedure with solvents of increasing polarity. Successive silica gel column chromatography purifications of the medium-polarity (ethyl acetate) fraction resulted in the isolation of **1** in pure form. The ESI-HRMS of **1** (Fig. S1) showed a protonated parent ion peak at *m/z* 539.1394, indicating a molecular formula of $C_{28}H_{26}O_{11}$ and a structure with 16 unsaturation sites. The ^{1}H (Fig. S2) and ^{13}C NMR spectra of **1** (Table 1), together with data from the HSQC experiment (Fig. S3), revealed the presence of two trisubstituted aromatic rings, three carboxylic acid or ester carbonyls, and two additional carbon–carbon double bonds, which accounted for 13 unsaturation sites and suggested that the remaining three unsaturations corresponded to carbocyclic rings. A careful analysis of the coupling patterns between the various aromatic protons [δ 6.78 (dd, J = 2.1 Hz, H5), 6.75 (d, J = 8.1 Hz, H8), 6.61 (dd, J = 2.1, 8.1 Hz, H9), 6.56 (d, J = 2.2 Hz, H5''), 6.70 (d, J = 8.1 Hz, H8''), 6.40 (dd, J = 2.2, 8.1 Hz, H9'')] showed the typical *ortho* and *meta* patterns of two isolated ABC systems (Table 1), indicating that both aromatic rings were 1,3,4-trisubstituted. Similarly, the analysis of the coupling patterns of

J. Mex. Chem. Soc. **2023**, *67*(3)
Special Issue

ISSN-e 2594-0317

the vinylic protons [δ 6.28 (d, J = 15.8 Hz, H2'), 7.42 (d, J = 15.8 Hz, H3'), 6.61 (d, J = 6.1 Hz, H9')] indicated that one double bond was trisubstituted, and the other one was trans-disubstituted.

Table 1. ^{1}H (500 MHz) (δ; multiplicity; J) and ^{13}C (125 MHz) (ppm; multiplicity) NMR data for **1**. The coupling constants J are given in Hz.

Position	^{1}H	^{13}C	COSY	HMBC
1	-	170.8; s		
2	5.21; dd; 4.8, 8.0	74.2; d	H-3	C-1, C-3, C-4, C-1'
3	3.07; dd; 4.8, 14.3 3.00; dd; 8.0, 14.3	37.6; t	H-2	C-1, C-2, C-4, C-5, C-9
4	-	128.7; s		
5	6.78; d; 2.1	117.3; d	H-9	C-3, C-6, C-7, C-9
6	-	145.8; s		
7	-	144.9; s		
8	6.75; d; 8.1	116.1; d	H-9	C-4, C-6, C-7
9	6.61; dd; 2.1, 8.1	121.7; d	H-5, H-8	C-3, C-5, C-7
1'	-	166.8; s		
2'	6.28; d; 15.8	116.7; d	H-3'	C-1', C-4'
3'	7.42; d; 15.8	144.1; d	H-2'	C-1', C-4', C-5', C-9'
4'	-	135.1; s		
5'	4.18; ddd; 1.0, 3.9, 4.9	39.8; d	H-2'', H-6'	C-2''
6'	4.28; ddd; 1.0, 1.0, 4.9	83.4; d	H-5'	C-1''
7'	4.04; m	74.9; d	H-8'	
8'	3.36; ddd; 3, 3, 6.1	48.3; d	H-9'	
9'	6.61; d; 6.1	142.0; d	H-8'	
1''	-	178.8; s		
2''	2.69; m	45.2; d	H-5'	C-5', C-1''
3''	3.20; dd; 2.1, 2.7	45.0; d	H-8', H-2''	C-8', C-4'', C-5'', C-9'', C-2''
4''	-	134.9; s		
5''	6.56; d; 2.2	116.0; d	H-9''	C-9''
6''	-	145.7; s		
7''	-	144.8; s		
8''	6.70; d; 8.1	115.9; d	H-9''	C-9''
9''	6.40; dd; 2.2, 8.1	120.2; d	H-5'', H-8''	C-5'', C-8''
OMe	3.69; s	52.4; q		C-1

The ^{13}C NMR spectrum of **1** showed, in addition to the three carbonyl carbon signals, four aromatics (144.9, 144.8, 145.8, 145.7 ppm), three methine (83.4, 74.9, 74.2) and a methyl group (52.4) carbon signals, all having the characteristic chemical shift of oxygen-bearing carbons. The fact that the number of oxygen-bearing carbons was the same as the number of oxygen atoms in the molecular formula of **1**, suggested that the four oxygenated substituents in the aromatic rings were in the form of hydroxyl groups. Alternatively, the correlations observed in the HMBC experiment of **1** (Fig. S4 and S5) between the protons of the C-3 methylene (δ 3.00/3.07) and the two carbons of the aromatic methines C-5 (117.3) and C-9 (121.7), as well as between the proton of the C-3'' methine (δ 3.20) and the carbons of the C-5'' (116.0) and C-9'' (120.2) aromatic methines, indicated that one aromatic ring had a methylene group as a substituent, while the other was attached to a methine group. This allowed us to establish the nature of the three substituents in each of the aromatic rings as two hydroxyls and an alkyl group.

The correlation observed in the ^{1}H-^{1}H COSY experiment of **1** (Fig. S6) between the C-3 benzylic methylene protons and the proton of the oxygenated C-2 methine allowed the elongation of the side chain

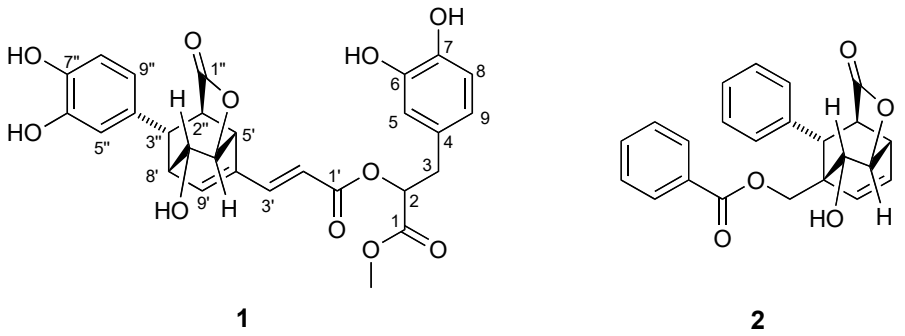
J. Mex. Chem. Soc. **2023**, *67*(3)
Special Issue

ISSN-e 2594-0317

attached to one of the aromatic rings. Furthermore, the correlations observed in the HMBC experiment of **1** between the C-2 methine proton and the C-1 (170.8 ppm) and C-1' (166.8 ppm) carbonyl carbons, together with that between C-1 and the protons of the methoxyl methyl group, allowed the identification of one of the substituents at C-2 as a methyl ester. Finally, the HMBC correlation observed between the C-1' carbonyl carbon and the H-2'/H3' vinylic protons of the trans-substituted double bond indicated C-1' being part of an α, β-unsaturated ester group and allowed the identification of the first part of the structure of the new metabolite.

A detailed analysis of the ^{1}H-^{1}H COSY experiment of **1** showed a clear correlation between the C-3''-benzyllic methine proton (3.20) and the protons at 3.36 (H8') and 2.69 (H2''), indicating it's being coupled to an allylic and α-keto methines, respectively. Similarly, the COSY correlations observed between the H-2'' (2.69) proton and the bridgehead C-5' methine proton (4.18), and between the C-5' proton and that of the oxygenated C-6' methine (4.28 ppm), together with the HMBC correlations observed between the H-2'' (2.69) and H-6' (4.28) methine protons and the C-1'' carbonyl carbon (178.8 ppm), allowed the construction of a *γ*-lactone ring. Finally, the COSY correlations observed between a second bridgehead methine proton (H-8', 3.36) and both the vinyllic proton of a trisubstituted double bond (H-9', 6.61) and the proton of a third oxygenated methine (H- 7', 4.04 ppm), together with the HMBC correlations between the vinylic H-3' proton and the C-5' (39.8 ppm) and C-9' (142.0 ppm) methine carbons, allowed the construction of the tricyclic central moiety. The data discussed supports the proposed structure **1** for the new metabolite, which we have designated with the common name pulcaffeate (Fig. 1).

The relative configuration of the tricyclic system was established on the basis of both the NOESY correlations between the H-7' and H-3'' protons and the value of the ^{1}H–^{1}H coupling constants between the different protons (Table 1), which are in agreement with those expected taking into account the dihedral angles observed in a Dreiding model of the strained system. Unfortunately, with the data at hand, it was not possible to establish the stereochemistry of C-2.



1 **2**

Fig. 1. Structures of pulcaffeate (**1**) and zeylena (**2**).

Trimeric metabolites that are structurally related to **1** are uncommon in the literature, although zeylena (**2**), a constituent of the roots of *Uvaria zeylanica* (Annonaceae), contains the same tricyclic moiety as **1** (Fig. 1) [3]. A detailed analysis of metabolites structurally related to **2**, led to the proposal that the biosynthesis of these unusual metabolites begins with the oxidation of a cinnamic acid molecule to produce an arene-oxide type intermediate which, as a result of the addition of a caffeic acid molecule, generates an ester intermediate that can undergo an intramolecular Diels-Alder reaction to form zeylena (**2**) [3]. Several synthetic preparations of **2** have exploited this biomimetic approach, showing the cycloaddition reaction to be efficient, even at room temperature [4,5]. A similar biosynthetic route can be expected for the formation of **1**, i.e., an initial oxidation of cinnamic acid to produce the corresponding arene-oxide which, as a result of the addition of a caffeic acid molecule, leads to the formation of the ester intermediate. The dimeric ester intermediate can then react with a second molecule of caffeic acid and undergo an intramolecular Diels-Alder cycloaddition to produce **1** (Fig. 2). The proposed biosynthesis could also explain the relative configuration established for the tricyclic system. To the best of our knowledge, this is the first report of a trimeric metabolite occurring in *B. pulchra*.

J. Mex. Chem. Soc. **2023**, *67*(3)
Special Issue

ISSN-e 2594-0317

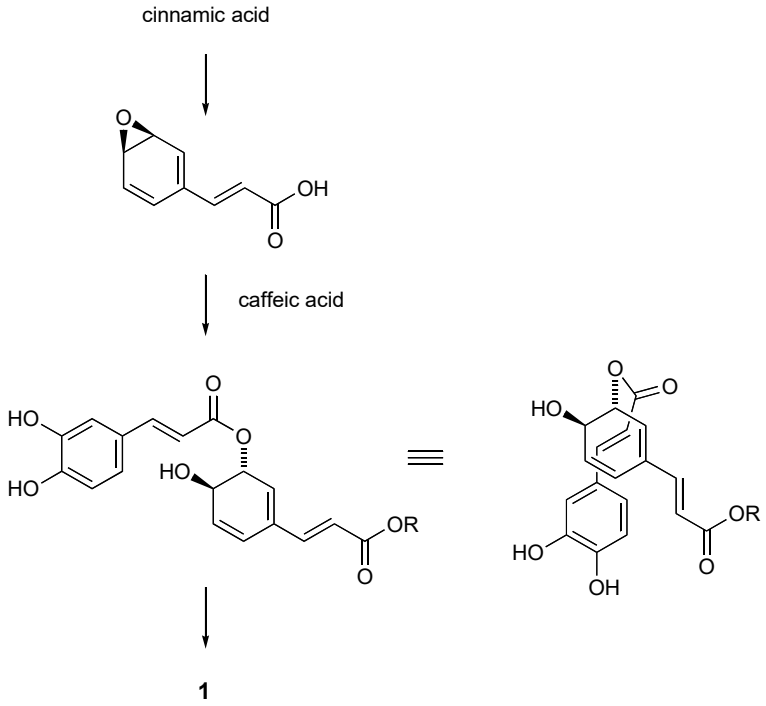


Fig. 2. Proposed biosynthesis of **1**.

Acknowledgements

The authors wish to thank Paulino Simá-Polanco and Lourdes Morales-Dzul for collecting, identifying, and preparing the plant material, as well as Fabiola Escalante-Erosa and Karlina García-Sosa, for technical assistance. G. E.-R. wishes to thank the EULADIV Alfa Project for supporting her research stays at CICY. The authors also gratefully acknowledge financial support from the Swedish Natural Science Research Council, the KAW Foundation, the Swedish Foundation for International Cooperation in Research and Higher Education, and FOMIX-Yucatán Project No. 66262.

References

1. Argueta, V. A.; Cano, L. M.; Rodarte, M. E. *Atlas de las plantas de la Medicina Tradicional Mexicana*. Instituto Nacional Indigenista; Mexico City, **1994**, Vol. 1, 483-485.
2. Fuentes-García, A.G., *Detección de Actividad Biológica en Extractos de Plantas Nativas de la Península de Yucatán*, B.Sc. Thesis. **2003**. Universidad Autónoma de Yucatán, Mexico.
3. Jolad, S. D.; Hoffmann, J. J.; Schram, K. H.; Cole, J. R.; Tempesta, M. S.; Bates, R. B. *J. Org. Chem.* **1981**, *46*, 4267-4272. https://doi.org/10.1021/jo00334a033.
4. Stipanovic, R. D.; Bell, A. A.; O'Brien, D. H.; Lukefahr, M. J. *Tet. Lett.* **1977**, *6*, 567-570. https://doi.org/10.1016/S0040-4039(01)92695-4
5. Bazan, A. C.; Edwards, J. M.; Weiss, U. *Tetrahedron.* **1978**, *34*, 3005-3015. https://doi.org/10.1016/0040-4020(78)88024-7.