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An unusual caffeic acid derived bicyclic [2.2.2] octane lignan and other constituents from *Cordia rufescens*

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ABSTRACT

This work reports isolation of an unusual lignan with a bicyclic [2.2.2] octene skeleton, named rufescenolide (**1**), from stems of *Cordia rufescens*, along with β-sitosterol, stigmasterol, syringaldehyde, 3-β-*O*-D-glucopyranosyl-sitosterol, methyl caffeate, 4-methoxy-protocatechuic acid and methyl rosmarinate. Structural characterizations employed IR spectroscopic, ESIHRMS and mono and dimensional NMR spectroscopy.

1. Introduction

The genus *Cordia*, which belongs to the Boraginaceae family, encompasses approximately 250 species and has a wide range of uses in traditional medicine. For instance, members of this genus are used to treat rheumatism, painful menstruation, bladder diseases and gastric ulcers (Akhtar and Ahmad, 1995; Coelho et al., 2004; David et al., 2007). In addition to fatty acids which are recognized as compounds of chemotaxonomic significance for this genus (Velasco and Goffman, 1999), pharmacologically active quinones, chromones, terpenes, polyphenols and flavonoids which have analgesic, anti-inflammatory, anti-arthritic and larvicidal activities have been isolated from the genus *Cordia* (Kuroyanagi et al., 2003; Ioset et al., 2000; Bayeux et al., 2002; Mori et al., 2008; Menezes et al., 2005). *Cordia rufescens*, commonly known as "ramela de velho" or "pau-bombo," is a small shrub from northeastern Brazil that is used in folk medicine as an abortifacient, anti-inflammatory agent and a treatment for dysmenorrhea and dyspepsia (Barroso et al., 2002). Previous chemical studies of *C. rufescens* A. DC. (Synonymy of *Cordia piauhiensis* Fresen) identified the presence of both saponins and aryl naphthalene lignans (Santos et al., 2003; Silva et al., 2004). In a previous study regarding the pharmacological activities of ethyl acetate extract of *C. rufescens*, it was found to be active on lymphocyte proliferation (Costa et al., 2008). However, the chemical composition of this extract was not evaluated, and this is the purpose of this study.

2. Results and discussion

The structure of the new lignan (Fig. 1) was characterized by analyzing a variety of spectroscopic data. The quasi-molecular ion at *m*/*z* 359 of compound **1** was detected using an APCI-LC–MS operating in the positive ion mode, and the ^{1}H and ^{13}C NMR spectra (including DEPT) were consistent with a molecular formula of $C_{19}H_{18}O_7$. This formula was confirmed using an ESIHRMS operating in the negative ion mode, which had a peak corresponding to an [M−H] with an *m*/*z* of 357.0975 (calcd. for $C_{19}H_{17}O_7$, 357.0974). The signals at δ 51.9, δ 167.8 and δ 178.8 in the ^{13}C NMR spectrum were identified as a methoxyl group and two carboxyl groups at C-9′ and C-9, respectively. Resonances corresponding to the presence of aliphatic, aromatic and sp^2 carbons were also observed, but only methine signals were found in the aliphatic region. These resonances occurred at δ 44.4, while signals for benzyl and oxymethine carbons were at δ 44.1 (C-7), δ 73.5 (C-4′) and δ 82.6 (C-3′). The ^{13}C NMR spectra also showed four sp^2-unsaturated carbons at δ 116.5, δ 142.0, δ 133.6 and δ 139.7, which corresponded to an α, β, γ and δ-unsaturated ester. Six signals from a

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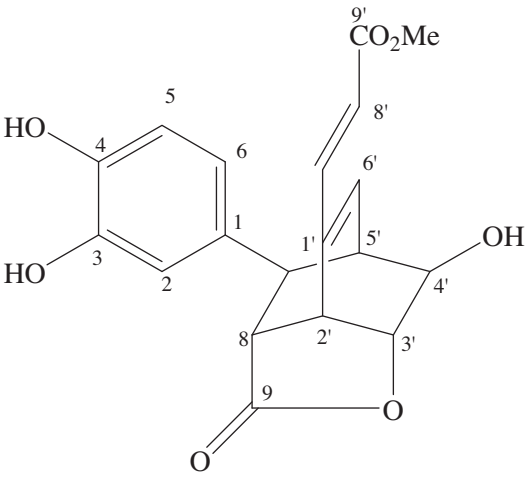

Fig. 1. Structure of compound **1**.

1,3,4-substituted aromatic ring were observed at δ 114.6, δ 114.9, δ 119.3, δ 133.0, δ 143.5 and δ 144.2; these resonances are consistent with a caffeic acid moiety. All of the carbon atoms were unequivocally assigned based on *g*HMQC and *g*HMBC analyses. The *g*HMBC gave correlations between both signals at δ 3.15 (H-7) and δ 4.24 (H-3′) and the resonances at δ 178.8, which corresponds to an acyl group of an ester. It also showed correlations between both signals at δ 3.35 (H-5′) and δ 2.67 (H-8) and the resonance at δ 133.0 (C-1), which allowed assignment of the aromatic ring to C-7. The bicyclic [2.2.2.] octene system was established by the presence of a bridge connecting C-2′ to C-5′ through C-1′ and C-6′, respectively, and was corroborated by the observed correlations between the peaks at δ 4.11 (H-2′) and δ 139.8 (C-6′), as well as those at δ 3.35 (H-5′) and δ 133.6 (C-1′), respectively. In addition, the presence of a lactone group was confirmed by correlation in the *g*HMBC between peaks at δ 4.2 (H-3′) and δ 178.8 (C-9). The positions of the lactone linkage and the hydroxyl group on C-3′ (δ 82.6) and C-4′ (δ 73.5), respectively, were established by correlations between the resonances at δ 4.24 (H-3′) and δ 44.4 (C-8) and δ 133.6 (C-1′) and also between δ 4.01 (H-4′) and δ 139.8 (C-6′) and δ 44.1 (C-7).

The ^{1}H NMR spectrum (CD_3OD) of compound **1** showed signals corresponding to one isolated δ-hydrogen at δ 6.52 (d, J = 6.3, H-6′) as well as α and β hydrogens from an unsaturated ester at δ 6.27 (d, J = 15.9, H-8′) and δ 7.45 (d, J = 15.9, H-7′), respectively. In addition, the three hydrogens on the 1,3,4-substituted aromatic ring were observed at δ 6.32 (dd, J = 8.4 and 2.4 Hz, H-6), δ 6.42 (d, J = 2.4, H-2) and δ 6.63 (d, J = 8.4, H-5). Four methine hydrogens were observed at δ 2.67 (dd, J = 4.5 and 1.0 Hz, H-8), δ 3.15 (dd, J = 4.5, indt., H-7), δ 3.35 (ddd, J = 6.3, 3.5, indt., H-5′) and δ 4.11 (dd, J = 5.0, 1.0, H-2′), while two oxymethine hydrogens were found at δ 4.24 (d, J = 5.0, H-3′) and δ 4.01 (d, J = 3.5, H-4′). Resonance for a methoxyl hydrogen was also observed at δ 3.76.

Correlations observed in the ^{1}H–^{1}H COSY spectrum confirmed the bicyclic system, and suggests that a biosynthetic pathway to form this lignan occurs through cyclization of two caffeic acid molecules. The follower correlations were noted between: δ 4.11 (H-2′) to the signals δ 4.24 (H-3′) and δ 2.67 (H-8); resonance δ 3.15 (H-7) to δ 2.67 (H-8) and δ 3.35 (H-5′), δ 3.35 (H-5′) to H-6′ (δ 6.52), and, finally, between H-7 and H-4′. These data agreed with the previous evidence above that suggested a bicyclic system.

The relative stereochemistry of C-3′, C-4′, C-7 and C-8, as well as the location of the phenyl, hydroxyl and methoxy-acrylate groups, was established by 1D NOESY experiments. The most significant interactions for establishing the relative stereochemistry of the bicyclic [2.2.2.] octene ring were observed between the peaks at δ 4.01 (H-4′) and δ 3.15 (H-7), which indicated that the *exo* position of the 3,4-dihydroxyphenyl group was at C-7 (Fig. 2). Thus, the interaction between the resonances at δ 6.42 (H-2) and δ 2.67 (H-8) established that the C-8/C-3′ lactone was in an *endo* position. Finally, the interaction between the peaks at δ 4.11 (H-2′) and δ 6.27 (H-8′) established the relative stereochemistry of C-2′ and corroborated the *endo* configuration of the lactone group. Thus, it was possible to establish the stereochemistry of compound **1** as *rel* (2′*R*, 3′*R*, 4′*R*, 5′*S*, 7*R*, 8*R*).

Identification of β-sitosterol, stigmasterol, 3-β-*O*-D-glucopyranosyl-sitosterol (Correia et al., 2003), methyl caffeate (Islam et al., 2002), syringaldehyde (Chen et al., 2008), 4-methoxy-protocatechuic acid (Termentzi et al., 2009) and methyl rosmarinate (Lu and Foo, 1999) was accomplished via comparison to literature data.

3. Concluding remarks

While lignans in Boraginaceae were previously isolated from *Cordia* spp. (Silva et al., 2004) and *Ehretia ovafolia* (Yoshikawa et al., 1995), rufescenolide is the first reported lignan from this family to possess a bicyclic [2.2.2] octene skeleton to date. As rosmarinic and caffeic acid methyl ester derivatives were isolated along with compound **1**, it can be hypothesized that it may be a derivative of these acids, probably caffeic acid or caffeate derived. Yunnaneic acids C and D, which also possesses a bicyclic [2.2.2] octane, were also isolated from *Salvia yunnanensis* (Labiatae) (Tanaka et al., 1996). A better explanation for the biogenesis of these compounds involves a Diels–Alder reaction between the catechol ring of rosmarinic acid and the conjugated double bond of caffeic acid. Efforts developed in the syntheses of helicterins, bicyclic [2.2.2] octene neolignan dimers found in *Helicteres isora* (Sterculariaceae), appear to confirm the proposed biogenesis (Tezuka et al., 2000; Snyder and Kontes, 2009). In this way, the biosynthesis of rufescenolide (**1**) could be envisaged of result from coupling of two caffeic acid derivatives.

4. Experimental section

4.1. General experimental procedures

One-dimensional (^{1}H, ^{13}C and DEPT) and two-dimensional (^{1}H–^{1}H *g*COSY, *g*HMQC and *g*HMBC) NMR experiments were performed on Varian Gemini 2000 and Bruker AMX500 spectrometers operating at either 300 and 500 MHz (^{1}H), respectively, or 75 and 125 MHz (^{13}C), respectively. CD_3OD was used as the solvent with TMS as an internal standard. The 1D gNOESY experiments were performed using an Inova 500 spectrometer. The MS and the LC–MS analyses were conducted on a Shimadzu chromatographic system (mod. LCMS-2310). The detection of rufescenolide (**1**) was

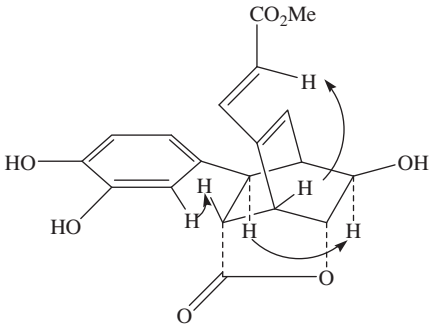

Fig. 2. Key correlations observed in the 1D NOESY.

Table 1
^1H, ^{13}C NMR and 2D NMR spectroscopic data for compound **1** (500 and 125 MHz, *J* = Hz) in CD_3OD.

Position	δ C	δ H	COSY (^1H–^1H)	HMBC (H → C)	1D NOESY
1	133.0				
2	114.6	6.42 (1H, d, *J* = 2.4)		C-7, C-6, C-3	H-8 (δ 2.67)
3	143.5				
4	144.2				
5	114.9	6.63 (1H, d, *J* = 8.4)	H-6	C-1, C-3	
6	119.3	6.32 (1H, dd, *J* = 8.4, 2.4)	H-5	C-7, C-5, C-4	
7	44.1	3.15 (1H, dd, *J* = 4.5, indt.)	H-5′, H-8	C-5′, C-4′, C-6′, C-1, C-6, C-2	H-4′ (δ 4.01)
8	44.4	2.67 (1H, dd, *J* = 4.5, 1.0)	H-2′, H-7	C-7, C-3′, C-1, C-9	H-2 (δ 6.42)
9	178.8				
1′	133.6				
2′	39.0	4.11 (1H, dd, *J* = 5.0, 1.0)	H-8, H-3′	C-5′, C-4′, C-3′, C-1, C-6′, C-7′	H-8′ (δ 6.27)
3′	82.6	4.24 (1H, d, *J* = 5.0)	H-2′	C-2, C-7, C-4′, C-9	
4′	73.5	4.01 (1H, d, *J* = 3.5)	H-5′	C-3′, C-6′	H-7 (δ 3.15), H-3′ (δ 4.24) H-5′ (δ 3.35)
5′	46.3	3.35 (1H, ddd, *J* = 6.3, 3.5, indt.)	H-7, H-4′, H-6′	C-8	
6′	139.8	6.52 (1H, d, *J* = 6.3)	H-5′	C-2′, C-7, C-4′, C-3′, C-7′	
7′	142.0	7.45 (1H, d, *J* = 15.9)	H-8′	C-2′, C-8′, C-1′, C-6′, C-9′	
8′	116.5	6.27 (1H, d; *J* = 15.9)	H-7′	C-1′, C-9	H-2′ (δ 4.11)
9′	167.8			51.87	
CH_3	51.9	3.77 (3H, s)		C-9′	

achieved in both positive and negative APCI modes. ESIHRMS was acquired using a micrOTOF system from Bruker. Optical rotations were measured on a Perkin-Elmer mod. 343 digital polarimeter. Conventional chromatographic methods were used for column chromatography (CC) (silica gel 60 (Acros, 63–200 and 40–63 μm)). Silica gel TLC plates (Merck) stained with iodine and viewed under UV light (254/366 nm) were used to monitor chromatographic purification procedures.

4.2. Plant material

Plant material was collected from two specimens in the semi-arid neighborhood of Morro do Chapéu, Bahia State, Brazil, in an Instituto Brasileiro do Meio Ambiente (IBAMA) authorized area. A voucher specimen is deposited at the Herbarium of the Universidade Estadual de Feira de Santana (HUEFS) under number 59087.

4.3. Extraction and isolation

Dried and ground stems of *C. rufescens* (1.5 kg) were extracted with MeOH (3 × 3 l) at room temp. for 72 h each. The resulting combined extract was suspended in H_2O and partitioned among hexane, $CHCl_3$ and EtOAc (200 ml each) to obtain *n*-hexane (6.8 g), $CHCl_3$ (3.9 g) and EtOAc (8.9 g) solubles. The $CHCl_3$ extract was separated by CC over silica gel 60 (63–200 μm) eluting with *n*-hexane–EtOAc (85:15 → 3:7). The fraction eluted with a *n*-hexane–EtOAc ratio (8:2) gave a mixture (126.4 mg) of β-sitosterol and stigmasterol. The fraction eluted with a *n*-hexane–EtOAc (3:7) was further fractionated by CC over silica gel (40–63 μm) eluted with $CHCl_3$–MeOH (85:15 and 7:3) yielding syringaldehyde (391.3 mg) and 3-β-*O*-D-glucopyranosyl-sitosterol (396.3 mg), respectively. The EtOAc phase was purified by CC over silica gel (40–63 μm) using $CHCl_3$–MeOH (100:0 → 7:3). The fractions eluted with a $CHCl_3$–MeOH (85:15) were subjected to further CC over silica gel (40–63 μm) using $CHCl_3$–MeOH (100:0 → 7:3) and MeOH as eluents, and the fraction eluted with a $CHCl_3$:MeOH (98:2) was followed by prep. TLC using a $CHCl_3$:MeOH (9:1) to yield methyl caffeate (26.1 mg) and 4-methoxy-protocatechuic acid (40.6 mg). The fraction that had been eluted with MeOH was purified by further CC over silica gel (40–63 μm) using $CHCl_3$:MeOH with the ratios of 96:4 and 9:1 as eluents to furnish compound **1** (49.6 mg) and methyl rosmarinate (62.7 mg), respectively.

4.3.1. Rufescenolide (**1**)

Amorphous solid; m.p. 176 °C (dec.); $[\alpha]^{25}_D$ + 15 (MeOH; c 0.5 mg/mL); IR (KBr) ν_{max} 3449.8, 1763.9, 1711.9, 1637.6, 1526.7, 1442.8, 1288.5, 1173.7, 1116.8, 1072.4, 986.6 cm^{-1}; positive-ion APCIMS *m/z* 359 $[M+H]^+$ and *m/z* 340 $[M+H-H_2O]^+$; ESIHRMS at *m/z* 357.0975 [M−H], calcd. for $C_{19}H_{17}O_7$, 357.0974; the ^1H NMR spectroscopic and ^{13}C NMR spectroscopic data, see Table 1.

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References

Akhtar, A.H., Ahmad, K.U., 1995. Anti-ulcerogenic evaluation of the methanolic extracts of some indigenous medicinal plants of Pakistan in aspirin-ulcerated rats. J. Ethnopharmacol. 46, 1–6.
Barroso, I.C.E., Oliveira, F., Branco, L.H.Z., 2002. O gênero *Cordia* L.: botânica, química e farmacologia. Rev. Lecta 20, 15–34.
Bayeux, M.C., Fernandes, A.T., Foglio, M.A., Carvalho, J.E., 2002. Evaluation of the antiedematogenic activity of artemetin isolated from *Cordia curassavica* DC. Braz. J. Med. Biol. Res. 35, 1229–1232.
Chen, Y., Xu, J., Yu, H., Qing, C., Zhang, Y., Wang, L., Liu, Y., Wang, J., 2008. Cytotoxic phenolics from *Bulbophyllum odoratissimum*. Food Chem. 107, 169–173.
Coelho, S.G., Haas, A.P.S., Von Poser, G.L., Schapoval, E.E.S., Elisabetsky, E., 2004. Ethnopharmacological studies of antimicrobial remedies in the south of Brazil. J. Ethnopharmacol. 90, 135–143.
Correia, S.J., David, J.P., David, J.M., 2003. Constituents of the bark of *Tapirira guianensis* (Anacardiaceae). Quim. Nova 26, 36–38.
Costa, J.F.O., David, J.P., David, J.M., Giulietti, A.M., Queiroz, L.P., Santos, R.R., Soares, M.B.P., 2008. Immunomodulatory activity of extracts from *Cordia superba* Cham and *Cordia rufescens* A. DC. (Boraginaceae), plant species native from Brazilian Semi-arid. Rev. Bras. Farmacogn. 18, 11–15.
David, J.P., Meira, M., David, J.M., Brandão, H.N., Branco, A., de Agra, M., Barbosa, M.R.V., de Queiroz, L.P., Giulietti, A.M., 2007. Radical scavenging, antioxidant and cytotoxic activity of Brazilian *Caatinga* plants. Fitoterapia 78, 215–218.
Ioset, J.R., Marston, A., Gupta, M.P., Hostettmann, K., 2000. Antifungal and larvicidal cordiaquinones from the roots of *Cordia curassavica*. Phytochemistry 53, 613–617.
Islam, M.S., Yoshimoto, M., Yahara, S., Okuno, S., Ishiguro, K., Yamakawa, O., 2002. Identification and characterization of foliar polyphenolic composition in sweet potato (*Ipomoea batatas* L.) genotypes. J. Agric. Food Chem 50, 3718–3722.
Kuroyanagi, M., Kawahara, N., Sekita, S., Satake, M., Hayashi, T., 2003. Dammarane-type triterpenes from the Brazilian medicinal plant *Cordia multispicata*. J. Nat. Prod. 66, 1307–1312.
Lu, Y.R., Foo, L.Y., 1999. Rosmarinic acid derivatives from *Salvia officinalis*. Phytochemistry 51, 91–94.
Menezes, J.E.S.A., Lemos, T.L.G., Pessoa, O.D.L., Braz-Filho, R., Montenegro, R.C., Wilke, D.V., Costa-Lotufo, L.V., Pessoa, C., Moraes, M.O., Silveira, E.R., 2005. A

cytotoxic meroterpenoid benzoquinone from roots of *Cordia globosa*. Planta Med. 71, 54–58.

Mori, K., Kawano, M., Fuchino, H., Ooi, T., Satake, M., Agatsuma, Y., Takenori, K., 2008. Antileishmanial compounds from *Cordia fragrantissima* collected in Burma (Myanmar). J. Nat. Prod. 71, 18–21.

Santos, R.P., Viana, F.A., Lemos, T.L.G., Silveira, E.R., Braz-Filho, R., Pessoa, O.D.L., 2003. Structure elucidation and total assignment of 1H and 13C NMR data for a new bisdesmoside saponin from *Cordia piauhiensis*. Magn. Reson. Chem. 41, 735–738.

Silva, S.A.S., Souto, A.L., Agra, M.F., Cunha, E.V.L., Barbosa-Filho, J.M., Silva, M.S., Braz-Filho, R., 2004. A new arylnaphthalene type lignan from *Cordia rufescens* A. DC. (Boraginaceae). Arkivoc 6, 54–58.

Snyder, S.A., Kontes, F., 2009. Explorations into neolignan biossyntheses: consise total syntheses of Helicterin B, Helisorin, and Helisterculin A from a common intermediate. J. Am. Chem. Soc. 131, 1745–1752.

Tanaka, T., Nishimura, A., Kuono, I., Nonaka, G., Young, T.-J., 1996. Isolation and characterization of yunnaneic acids A–D, four novel caffeic acid metabolites from *Salvia yunnanensis*. J. Nat. Prod. 59, 843–849.

Termentzi, A., Zervou, M., Kokkalou, E., 2009. Isolation and structure elucidation of novel phenolic constituents from *Sorbus domestica* fruits. Food Chem. 116, 371–381.

Tezuka, Y., Terazono, M., Kusumoto, T.I., Hanataka, Y., Kadota, S., Hattori, M., Namba, T., Kikuchi, T., Tanaka, K., Supriayatna, S., 2000. Helicterins A-F, six new dimeric (7.5, 8.2′)-neolignans from the Indonesian medicinal plant *Helicteres isora*. Helv. Chim. Acta 83, 2908–2919.

Velasco, L., Goffman, F.D., 1999. Chemotaxonomic significance of fatty acids and tocopherols in Boraginaceae. Phytochemistry 52, 423–426.

Yoshikawa, K., Kageyama, H., Arihara, S., 1995. Phenolic glucosides and lignans from *Ehretia ovalifolia*. Phytochemistry 39, 659–664.