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Neocandenatone, an isoflavan-cinnamylphenol quinone methide pigment from *Dalbergia congestiflora*

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Abstract

A purple pigment neocandenatone (vestitol[6→9″;7O→7″]obtusaquinone) was isolated from the heartwood of campincerán (*Dalbergia congestiflora*), an endemic Mexican tree. The isoflavan-cinnamyl phenol quinone methide structure of this compound was elucidated by HRMS, IR, and ^{1}H and ^{13}C NMR spectroscopic analysis, including 2D experiments (COSY, NOESY, HMQC and HSQC).
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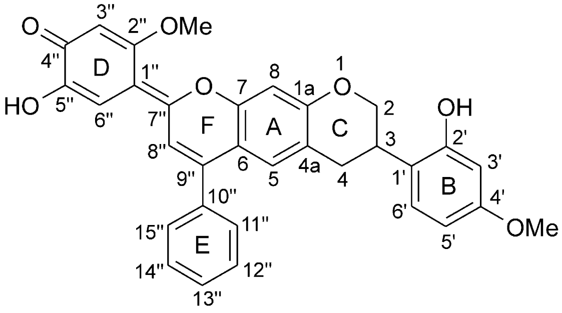
1. Introduction

Dalbergia species (Leguminosae) are known for their deeply pigmented heartwoods of varying colors. Often valued for their use in wooden crafts, the heartwoods are also used in traditional medicine and have antibiotic and cytotoxic activities (Yahara et al., 1989; Zhao et al., 2000; Goda et al., 1992; Hamburger et al., 1987; Ansari et al., 2002). Chemical investigations of *Dalbergia* heartwoods have shown the typical occurrence of monomeric isoflavonoids, neoflavonoids and cinnamylphenols (Goda et al., 1992; Dewick, 1994; Donnelly and Roland, 1994; Geiger, 1988; Gregson et al., 1978). A limited number of oligomers with mixed monomers of the isoflavonoid-neoflavonoid and isoflavonoid-cinnamylphenol type have also been reported (Bekker et al., 2002; Czakó and Márton, 2001; Hamburger et al., 1988).

Campincerán (*Dalbergia congestiflora*), also known as granadillo, is a tree endemic to Michoacán State, México. The wood is known for its hardness and resistance to insect attack and is used by native people to manufacture musical instruments. The purple pigment from its heartwood is also extracted to dye fabrics and when combined with resins, to paint hand crafted and wooden appliances. Recently, Barragán et al., (1995, 1998) have proposed using the pigment as a colorant for foods and cosmetics. Herein, we report the isolation and structural determination of this pigment as a new isoflavan cinnamylphenol quinone methide called neocandenatone **1**. Neocandenatone **1** (vestitol[6→9″;7O→7″]obtusaquinone) is an isomer of candenatone, isolated from *Dalbergia candenatensis* (Hamburger et al., 1988), a dimer of the isoflavan vestitol and the cinnamylphenol obtusaquinone. Both vestitol and obtusaquinone have been commonly found in *Dalbergia* heartwoods (Bekker et al., 2002; Gregson et al., 1978). A related isomer, retusapurpurine, extracted from *Dalbergia retusa* cell cultures has recently been reported (Czakó and Márton, 2001).



Neocandenatone **1**

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Table 1
^{1}H NMR (400 MHz) and ^{13}C (100 MHz) (296 K in methanol-d_4) spectroscopic assignments for neocandenatone **1**

Carbon	^{13}C δ	^{1}H δ, multiplicity, (J, Hz)	Carbon	^{13}C δ	^{1}H δ, multiplicity, (J, Hz)
2	69.70	H-2$_{ax}$ 3.83, *dd* (16, 8) H-2$_{eq}$ 3.90, *dd* (16, 2)	1″	104.3	
3	30.79	3.55, *dd* (8, 2)	2″	164.3	
4	29.90	H-4$_{ax}$ 2.65, *dd* (16, 8) H-4$_{eq}$ 2.70, *dd* (16, 8)	3″	128.6	7.80 *s*
5	98.8	6.95 *s*	4″	181.0	
6	116.4		5″	156.7	
7	149.9		6″	102.1	6.05 *s*
8	109.1	6.87 *s*	7″	163.7	
1a	159.1		8″	115.8	7.93 *s*
4a	116.4		9″	137.0	
1′	113.8		10″	154.6	
2′	155.4		11″, 15″	129.8	7.53 *s*
3′	103.0	6.26 *d* (4)	12″, 14″	129.0	7.53 *s*
4′	155.8		13″	130.1	7.53 *s*
5′	108.2	6.40 *dd* (8, 4)	4′ OCH_3	56.1	4.01 *s*
6′	130.3	6.95 *d* (8)	2″ OCH_3	54.9	3.81 *s*

2. Results and discussion

Strong IR absorption of neocandenatone **1** at 1618 cm^{-1} suggested an enone system (Hamburger et al., 1988). HR-FAB-MS revealed an $[M+H]^+$ ion peak at *m/z* 523.17568, (*m/z* expected 523.17422) in agreement with $C_{32}H_{27}O_7$. ^{1}H and ^{13}C NMR spectra together with COSY and HSQC spectral anaylsis permitted *a priori* assignation as an isoflavan-like compound.

The ^{1}H NMR spectrum (methanol-d_4) showed two proton singlets at δ 3.81 and 4.01 for the two methoxyl groups which were correlated by an HSQC spectrum to their carbons at δ_C 54.9 and 56.0 ppm, respectively. Five coupled protons, belonging to an ABMXZ, system were observed in the COSY spectrum. Those peaks were attributed to the aliphatic protons H-4$_{ax}$ (δ 2.65, *dd*, 16, 8 Hz), H-4$_{eq}$ (δ 2.70, *dd*, $J=16$, 8 Hz), H-3 (δ 3.55, *dd*, $J=8$, 2 Hz), H-2$_{ax}$ (3.83, *dd*, $J=16$, 8 Hz) and H-2$_{eq}$ (δ 3.90, *dd*, J =16, 2 Hz) in ring C of the presumed isoflavan unit. A COSY spectrum confirmed the substitution pattern in ring B as an ABX system with signals at δ 6.95 (*d*, $J=8$ Hz) for H-6′, δ 6.40 (*dd*, $J=8$, 4 Hz) for H-5′, and δ 6.26 (*d*, $J=4$ Hz) for H-3′. A sharp peak at δ 7.53 which integrated for five aromatic protons, was assigned to protons in ring E for H-11″/H-15″, H-12″/H-14″ and H-13″.

A ^{13}C-DEPT (90° and 135° sequence pulses) and a HSQC ^{1}H-^{13}C direct correlation showed that five signals at δ 6.95, 7.80, 7.93, 6.05 and 6.87 belonged to vinylic protons H-5, H-8″, H-8, H-3″ and H-6″, respectively. A strong correlation ^{3}J was observed in the HMBC spectrum for the proton signal at δ 3.81 (methoxyl on ring D) which was connected to the quaternary carbon at δ 164.3 (C-2″). The correlation from the proton signal at δ 7.8 (H-3″) to the carbonyl group at δ 181.0 (C-4″) confirmed its position on ring D. The methoxyl signal at δ 4.01 showed a ^{3}J correlation to the 4′ carbon (δ_C 155.8) in ring B. Proton signals at δ 6.95 (H-5) and 6.87 (H-8) showed a reciprocal correlation in the HMBC with the corresponding quaternary carbons at δ 149.9 (C-7a) and δ 116.4 (C-6a). H-5 also showed correlations to the carbon signals for C-4 (δ_C 29.90) and C-9″ (δ_C 137) which confirmed bridging of ring A with rings F and C. Complete ^{1}H and ^{13}C spectral assignments for neocandenatone **1** are given in Table 1.

The most crucial correlation in the HMBC spectrum was the ^{3}J correlation from the methylene protons on C-4 (δ_H 2.65 and 2.70) and C-2 (δ_H 3.83 and 3.90) to the *ipso* carbon C-1′ (δ_C 113.8) to confirm the connection from ring B to ring C. Other confirming long range correlations (filled arrows) observed by HMBC are shown in Fig. 1.

The cross peak correlation observed in a NOESY experiment between the proton at δ 6.05 (H-6″) and

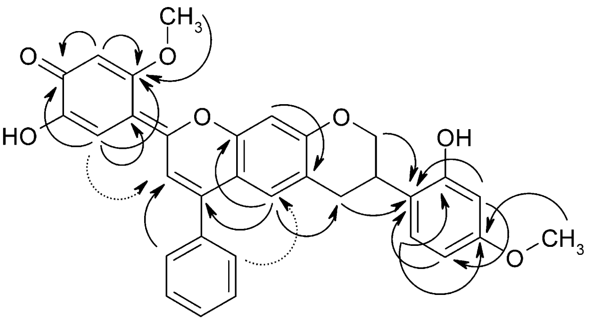

Fig. 1. Long range ^{1}H–^{13}C correlation observed by HMBC (filled arrows) and NOE interactions ^{1}H–^{1}H (dotted arrows) in neocandenatone **1**.

proton 8″ in ring F (δ_H 7.93) confirmed the close spatial relationship between rings D and F. Also, an NOE interaction observed between the large peak at δ 7.53, and the vinyl proton H-5 (δ_H 6.95) confirmed the spatial proximity of ring E to ring A.

3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded on a Brüker AM 400 MHz spectrometer with TMS as internal standard. Standard pulse sequences for a Brüker instrument were used for the gradient enhanced absolute value COSY, phase sensitive double quantum filtered DQF-COSY, phase sensitive NOESY and gradient enhanced HMQC and HMBC experiments. HR-MS (FAB) spectra were recorded using a VG-70E instrument in a matrix of 0.1 M oxalic acid/2:1 thioglycerol:glycerol. IR spectra were recorded on a Perkin-Elmer FT-IR Spectrum 2000 instrument. Whereas UV–vis spectra were obtained using a Perkin-Elmer Lambda 19 model. TLC was performed on precoated Merck aluminum sheets (silica gel 60 F_{254}, 0.20 mm). Preparative plates of silica gel (Merck 20×20 cm, Kieselgel G F_{254}, 2 mm) were activated in an oven at 110 °C for 5 h before sample application. CC was performed on a Sephadex LH-20 column (30×5 cm) with a flow rate of 20 ml/h.

3.2. Plant material

The heartwood of *Dalbergia congestiflora* was collected in Michoacán, México. A voucher specimen (EBUM-10194) was deposited in the herbarium of the University of San Nicolás de Hidalgo. Morelia, Michoacán, Mëxico.

3.3. Extraction and isolation

Dried and ground heartwood (100 g) of *D. congestiflora* was extracted with MeOH (4×200 ml). The resulting solution was filtered and combined extracts were concentrated on a rotatory evaporator to give an amorphous purple material (20 g). The dried extract was placed in a Soxhlet apparatus and extracted under reflux with diethyl ether (250 ml). The ethereal fraction contained (*S*)-dalbergione (Ollis et al., 1964). The residual fraction was applied onto Sephadex LH-20 and eluted with a gradient of 20, 40, 60, 80 and 100% MeOH–H_2O(V:V) mixtures (200 ml each) to give 150 mg of a solid product after evaporation. The solid was purified by prep. TLC (20×20 cm, 2 mm) using EtOAc–EtOH (5:1) as eluent to give neocandenatone **1** (50 mg).

3.3.1. Neocandenatone *1* (vestitol[6→9″;7→7″]obtusaquinone)

Purple amorphous powder, UV–vis(MeOH) λ_{max} nm (log ϵ) 580 (3.55), 547 (3.62), 322 (3.44); λ_{max} (+NaOMe) 601 nm; λ_{max} (+ HCl) 506 nm; IR (KBr) cm^{-1}, 3424, 2926, 2852, 1618, 1511, 1459, 1366, 1245, 1209, 1114, 958 and 878; HR-FABMS *m/z* 523.17568 ($C_{32}H_{27}O_7$ requires 523.17422); and ^{1}H and ^{13}C NMR spectroscopic data, see Table 1.

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