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Phytochemical investigation from wood residues of *Dalbergia spruceana* Benth

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Abstract: Dalbergia spruceana Benth (Fabaceae: Papilionoideae), known in the Brazilian Amazon as ¨jacarandá-do-pará" recognized for the natural resistance of its wood has little scientific information about its secondary metabolism. In this paper, we report a phytochemical study of the wood residues of *D. spruceana* using classical chromatographic techniques. Thus, chromatographic fractionation of methanolic extract resulted in the isolation of phenylpropanoid, isoflavonoids of different types (pterocarpan, isoflavonol, isoflavan, isoflavone) in addition to a neoflavonoid. A new isoflavonoid with an oxygenation pattern not previously reported was elucidated as 8,4,2'-trihydroxy-7,4'-dimethoxyisoflavonol. The structures of all the isolated compounds were determined by using 1D and 2D-NMR techniques, mass spectrometry ESI-MS and by comparison with literature data. Most of the compounds that were identified are isoflavonoids, which are types of flavonoids that are especially recognized for their contribution to the natural resistance of the wood.

Keywords: Fabaceae; flavonoid; pterocarpan; neoflavonoid; natural resistance.

INTRODUCTION

Dalbergia spruceana Benth [*Miscolobium spruceanum* Benth] (Fabaceae: Papilionoideae) is a species that is native to northern South America, and is known in the Brazilian Amazon as “jacarandá-do-pará". It has registered occurrence in the states of Amazonas, Amapá, Rondônia and Pará[1,2] and has great economic potential because its wood can be used for special purposes such as the manufacture of luxury furniture, decorative objects and musical instruments. It has

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medium to fast growth and easy propagation and is therefore recommended for reforestation and forest restoration programs.[3]

There is little scientific information about the secondary metabolism of *D. spruceana.* The chemical composition of the genus *Dalbergia* is represented mainly by phenolic compounds, which are found in different vegetative parts.[4] The planting of this species is very promising because it has propagation potential, economic potential because the wood has natural resistance, and it is also sought after for making stringed instruments, among other purposes. Woods with natural strength often have interesting secondary metabolites that have promising bioactive compounds.[5-10] In this paper, we evaluated the secundary metabolites extracted from the heartwood of *D. spruceana* using classical chromatographic techniques.

EXPERIMENTAL

General experimental procedures

NMR spectra were recorded on a Bruker Fourier 300 UltraShield (300 MHz for ^{1}H and 75 MHz for ^{13}C) and Bruker Avance III 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometers, at a temperature of 25 °C. The spectra were referenced to the residual solvent signal. HRESIMS data were obtained on an MicroTOF–Q–II, Bruker Daltonics, the ESI was operated in the positive and negative mode, and nitrogen was used as the drying gas (4.0 L/min) and nebulizing gas at 0.4-0.6 Bar. Mass-to-charge ratio was scanned in the 50-980 m/z range. For column chromatography (CC), silica gel (particle size 70-230 and 200–300 mesh, Sigma–Aldrich, Gallen Switzerland), hexane, ethyl acetate, acetone and methylene chloride were used. Analytical and preparative TLC was realized on silica gel 60 GF254 20 x 20 cm plates, with a layer thickness of 0.25 mm (Merck).

Acquisition and identification of wood residues

The *D. spruceana* from wood residues was supplied to the Wood Technology Laboratory of the National Institute for Amazonian Research (INPA) by a luthier. The largest residues had been previously evaluated for their technological properties and the smaller residues resulting from these procedures became available for phytochemical studies. The identification of the wood samples was done via macroscopic comparisons with standard samples from the xylotheque at INPA (INPA-Xil N0 4329).

Extraction and Isolation

The residues were chopped and ground (1.117 g) and submitted to maceration at room temperature with *n*-hexane and then methanol (seven days in each solvent), which provided yields of 0.05 and 3.67 %, respectively. The methanol extract (19 g) was purified on silica gel CC using gradient elution with Hexane/EtOAc (98:2 to 60:40) to obtain 90 fractions. Fraction 46 (20 mg) was purfied with preparative TLC using mixture of CH_2Cl_2/acetone (98:2) for isolation of compound **1** (10 mg). Additional purification of fraction 61 (70 mg) with silica gel column chromatography (230-400 mesh; 46 x 2.6 cm) and CH_2Cl_2 as eluent lead to further purification od subfractions 15 and 17 and final isolation of compounds **3** (3.5 mg) and **4** (5.5 mg). Fractions 71 (52.0 mg) and 73 (80.0 mg) were subjected to additional silica gel CC with mixture of CH_2Cl_2/acetone (98:2) as eluent for obtaining compunds **5** (10.0 mg) and **6** (3.5 mg).

Fractions 50 (970 mg) and 83 (18.1 mg) represent the solid compunds **2** (805 mg) and **7** (5.8 mg), respectively.

RESULTS AND DISCUSSION

From methanol extracts of *Dalbergia spruceana* Benth wood residues five known compounds as phenylpropanoid elemycin (**1**),[11] flavonoids maackiain (**2**),[12] 8-hydroxy-7-methoxy-3',4' methylenedioxypterocarpane (**3**),[13,14] dalbergine (**5**),[15] 3',4'-dihydroxy-7,8,2'-trimethoxy-isoflavan (**6**), pseudobaptigenin (**7**)[16] and one (**4**) previously undescribed isoflavonol were isolated. The isolated compounds were identified using 1D (^{1}H and ^{13}C) and 2D NMR (HSQC and HMBC), HRESIMS spectra and by comparison with literature data (Figure 1). HMBC correlations provide identification of flavonoids and reveal oxygenation patterns (Fig. 1). (see Supplementary material).

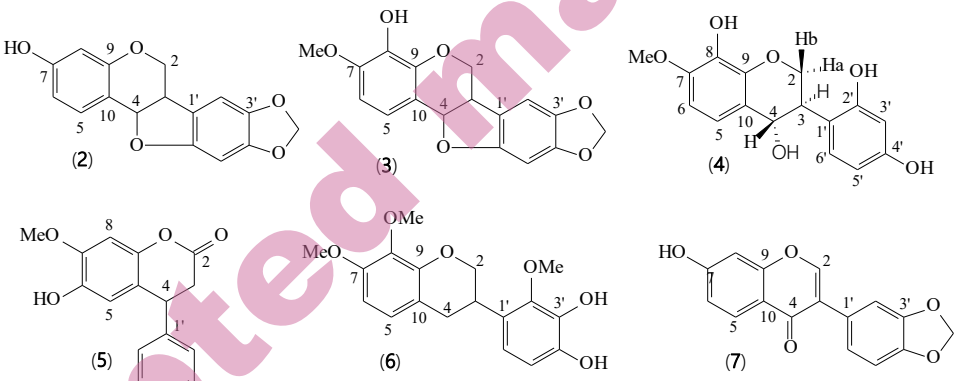

Fig. 1. Structures of the flavonoids from the wood residues of *Dalbergia spruceana*

The molecular formula of compound **4**, $C_{17}H_{16}O_7$, was determined based on the HRESIMS ion $[M+Na]^+$ at m/z 341.0994. The ^{1}H NMR spectrum revealed an aromatic system of ABX-type spins observed by the set of hydrogen signals in the region between δH 7.26-6.38, in addition to the *ortho*-coupled signals at δH 6.97 and 6.69 (Table 1). The signals of oxymethylene at δH 4.34 and 3.63, methine at δH 3.65 and oxymethine at δH 5.58 were indicative of 4-hydroxyisoflavonol. The ^{1}H and ^{13}C NMR data with significant NOESY and HMBC correlations are represent in Table I. The HMBC correlations H-5/C-4, C-7 and C-9 and H-6'/C-3, C-1' and C-2' revealed oxygenation patterns for A and B rings. The NOESY correlations H-2a/H-3 and H2b/H-4 indicated *trans* orientation of H-3 and H-4. Based on 1D and 2D NMR data, the structure of new isoflavonoid was elucidated as 8,4,2' trihydroxy-7,4'-dimethoxyisoflavonol.

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TABLE I. ^{1}H and ^{13}C-NMR spectroscopic data for compound **4** in $(CD_3)_2CO$.

H/C	δ H (*J* in Hz)	NOESY	δ C	HMBC
2	4.33 m (2a) 3.63 m (2b)	H-3, H4	66.5	C-3, C-4, C-9, C-1’
3	3.65 m	H-2a	39.6	C-2, C-4, C-1’
4	5.58 d (6.7)	H-2b	78.6	C-2, C-3, C-10, C-1’
5	6.97 d (8.5)		120.5	C-4, C-7, C-9
6	6.74 d (8.5)	H_3-7	105.8	C-5, C-7, C-8, C-9, C-10
7			148.1	
8			135.1	
9			144.2	
10			114.5	
1’			119.4	
2’			160.8	
3’	6.38 d (2.2)		96.2	C-1’, C-4’, C-5’
4’			161.2	
5’	6.47 dd (8.1 and 2.2)	H_3-4’	106.0	C-1, C-3’
6’	7.26 d (8.1)		125.0	C-3, C-1’, C-2’
OMe-7	3.84 s		55.7	C-7
OMe-4’	3.74 s		54.8	C-4’

^{1}H NMR (300 MHz, J/Hz); ^{13}C NMR (75 MHz)

In the Fabaceae family, the isoflavonoids are restricted almost entirely to Papilionoideae[17] and are especially recognized for their contribution to the natural resistance of its woods, such as is the case for the wood of *Dalbergia spruceana*.

CONCLUSION

A phytochemical analysis of woody residues of *Dalbergia spruceana* was conducted and resulted in the identification of mainly isoflavonoids of different types (pterocarpan, isoflavonol, isoflavan, isoflavone) in addition to a neoflavonoid. Among these, compound **4** was previously undiscribed. The woody residues of this species of Fabaceae gave us an opportunity to gain further knowledge regarding its secondary metabolism, and therefore increase the value of solid residues from *Dalbergia spruceana* discarded by the wood processing sector.

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ИЗВОД

ФИТОХЕМИЈСКО ИСПИТИВАЊЕ ДРВНИХ ОСТАТАКА *Dalbergia spruceana* Benth

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Биљка *Dalbergia spruceana* Benth (Fabaceae: Papilionoideae) позната у бразилском Амазону као „jacarandá-do-pará", иако је препозната по природној отпорности има мало научних информација о њеном секундарном метаболизму. У овом раду описано је фитокемијско испитивање дрвних остатака *D. spruceana* применом класичних хроматографских техника. Хроматографским фракционисањем метанолног екстракта изоловани су фенилпропаноиди, изофлавоноиди различитих типова (птерокарпан, изофлавонол, изофлаван, изофлавон) као и неофлавоноиди. Нови изофлавоноид, са јединственом структуром која је настала оксидацијом која раније није пријављена, дефинисан је као 8,4,2'-трихидрокси-7,4'-диметоксиизофлавонол. Структуре свих изолованих једињења одређене су коришћењем 1D и 2D- NMR техника, масене спектрометрије ESI-MS и поређењем са литературним подацима. Већина идентификованих једињења су изофлавоноиди, типови флавоноида за које је добро познато да доприносе природној отпорности дрвета.

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