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ORIGINAL ARTICLE

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Heartwood extractives from the Amazonian trees *Dipteryx odorata*, *Hymenaea courbaril*, and *Astronium lecointei* and their antioxidant activities

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Abstract Heartwood extracts from Amazonian trees cumaru-ferro (*Dipteryx odorata*), jatoba (*Hymenaea courbaril*), and guarita (*Astronium lecointei*) exhibit antioxidant activities comparable with that of α-tocopherol, a well-known antioxidant. This article reports the characterization of the antioxidant compounds in the extracts of the three heartwoods. Silica gel column chromatography of the cumaru-ferro EtOAc extract yielded (–)-(3*R*)-7,2′,3′-trihydroxy-4′-methoxyisoflavan and (+)-(3*R*)-8,2′,3′-trihydroxy-7,4′-dimethoxyisoflavan. Silica gel column chromatography followed by preparative high-performance liquid chromatography of the jatoba EtOAc extract yielded (–)-fisetinidol and (+)-*trans*-taxifolin. Chemical structures were assigned using electron-ionization mass spectrometry, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy including nuclear Overhauser effect spectroscopy (NOESY), as well as optical rotation and circular dichroism. Gas chromatography-mass spectrometry demonstrated that the isolated compounds were predominant in the EtOAc extracts. In the guarita EtOAc extract, catechin and gallic acid were identified by comparing their retention times and mass fragmentation patterns with those of authentic samples. Antioxidant activity determined by the 1,1-diphenyl-2-picrylhydrazyl assay demonstrated that all these compounds had activities comparable with that of α-tocopherol.

Key words Heartwood extractives · Antioxidant activity · *Astronium lecointei* · *Dipteryx odorata* · *Hymenaea courbaril*

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Introduction

As part of our studies aimed at identifying new applications for Amazonian wood, we have characterized 11 Amazonian tree species in terms of density, amount of Klason lignin and α-cellulose, chemical structure of lignin, and soluble components in methanol (MeOH) and alkali extracts.[1] It was found that the heartwood of Amazonian trees tends to contain large amounts of MeOH-soluble components, and that heartwood extracts of cumaru-ferro (*Dipteryx odorata*), jatoba (*Hymenaea courbaril*), and guarita (*Astronium lecointei*) exhibited high antioxidant activity comparable with α-tocopherol, a well-known antioxidant.

Various plant extractives exhibit bioactivities such as antioxidative, oestrogenic, antifungal, antimicrobial, and antibacterial activities. The extracts of some South American trees have been reported to have antiviral,[2] antioxidative,[3] antimicrobial,[4] antibacterial,[5] anticancer activity,[6] and antiplasmodial activity.[7] In these studies, however, bark, roots, or leaves were primarily investigated, rather than the wood itself. Furthermore, the extractives from some species have not been well studied, so it is unclear which compounds in the extracts contribute to the observed bioactivities.

The present article describes the isolation and structure elucidation of (–)-(3*R*)-7,2′,3′-trihydroxy-4′-methoxyisoflavan (**1**) and (+)-(3*R*)-8,2′,3′-trihydroxy-7,4′-dimethoxyisoflavan (**2**) from cumaru-ferro heartwood, and of (–)-fisetinidol (**3**) and (+)-*trans*-taxifolin (**4**) from jatoba heartwood (Fig. 1). The antioxidant activities of these isolated compounds, as well as of authentic gallic acid and catechin identified as

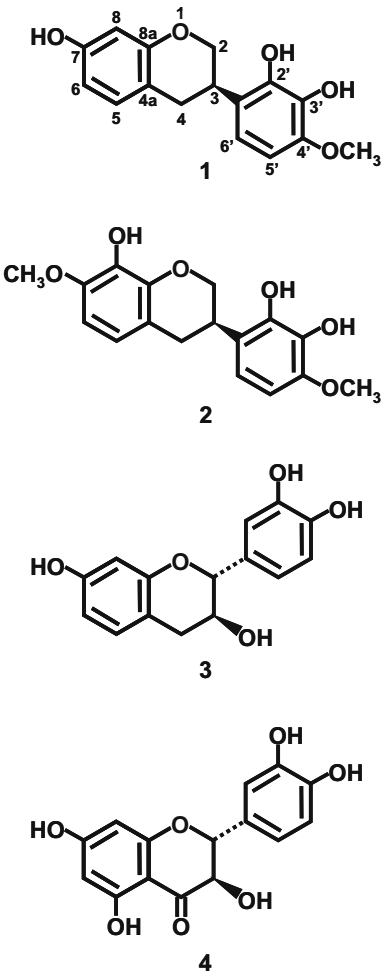

Fig. 1. Chemical structures of the compounds isolated in this study. ***1***, (–)-(3*R*)-7,2′,3′-Trihydroxy-4′-methoxyisoflavan; ***2***, (+)-(3*R*)-8,2′, 3′-trihydroxy-7,4′-dimethoxyisoflavan; ***3***, (–)-fisetinidol; ***4***, (+)-*trans*-taxifolin

guarita heartwood extractives by gas chromatography-mass spectrometry (GC-MS), were determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay.

Materials and methods

General

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Biospin AVANCE 400S instrument (400 MHz for ^{1}H and 100 MHz for ^{13}C NMR) in acetone-d_6 for compounds **1** and **2**, and in methanol-d_4 for compound **3**. Chemical shifts are given as δ values (ppm) relative to tetramethylsilane (TMS), which was used as an internal reference. Nuclear Overhauser effect spectroscopy (NOESY) spectra were recorded using the same NMR instrument. Electron-ionization mass spectrometry (EI-MS) analyses were carried using a JEOL MStation MS 700 instrument. Optical rotations and circular dichroism (CD) spectra were measured on a Jasco P-1020 polarimeter and a Jasco J-720 WI spectropolarimeter, respectively.

Plant materials

Samples of cumaru-ferro (*Dipteryx odorata*), jatoba (*Hymenaea courbaril*), and guarita (*Astronium lecointei*) wood were collected at Cotriguaçu in Mato Grosso, Brazil, in 2004.[1]

Extraction and isolation of antioxidant compounds

The three heartwood MeOH extracts previously prepared[1] were extracted with ethyl acetate (EtOAc), and the extracts were evaporated to give the EtOAc-soluble material. The EtOAc extract from cumaru (551 mg) was chromatographed on silica gel with chloroform/acetone (20:1) eluent, and fractions containing compound **1** (Rf value: 0.14) and compound **2** (Rf value: 0.23) were collected separately. The former and the latter fractions were rechromatographed on silica gel with benzene/MeOH (20:1) to provide purified compound **1** (Rf value: 0.1, 33.1 mg) and purified compound **2** (Rf value: 0.17, 17.1 mg). The EtOAc extract from jatoba (521 mg) was chromatographed on silica gel with chloroform/EtOAc (2:1), and fractions containing compound **3** (Rf value: 0.15) and compound **4** (Rf value: 0.3, 5 mg) were collected separately. The former fraction was subjected to preparative high-performance liquid chromatography (p-HPLC) on a Shimadzu LC-20AD equipped with a ultraviolet–visible (UV-VIS) detector and a reverse-phase Hypersil ODS column (10 mm i.d. × 150 mm, particle size 5 µm). The mobile phase was water/MeOH (91:1), the temperature was 40°C, the flow rate was 3.0 ml/min, and the separated compounds were detected at 280 nm. Compound **3** was collected at a retention time of 39 min (11.3 mg).

*Compound **1** [(–)-(3R)-7,2′,3′-trihydroxy-4′-methoxyisoflavan].* Oil, $[\alpha]_D^{25}$ – 2.72° (MeOH; c1.71); CD: $[\theta]_{250}$ – 179, $[\theta]_{255}$ + 80, $[\theta]_{265}$ + 142, $[\theta]_{275}$ + 1023, $[\theta]_{285}$ + 1955, $[\theta]_{295}$ + 478, $[\theta]_{305}$ + 10, $[\theta]_{315}$ – 54, $[\theta]_{330}$ – 59 (MeOH; c0.05); ^{1}H NMR (acetone-d_6): δ 2.80 (1H, ddd, $J_{4eq,2eq}$ = 2.0, $J_{4eq,3ax}$ = 5.2, $J_{4eq,4ax}$ = 15.2 Hz, H-4$_{eq}$), 2.98 (1H, dd, $J_{4ax,3ax}$ = 7.6, $J_{4ax,4eq}$ = 15.0 Hz, H-4$_{ax}$), 3.49 (1H, m, H-3$_{ax}$), 3.70 (3H, s, OCH$_3$), 4.00 (1H, dd, $J_{2ax,2eq}$ = 10.0, $J_{2ax,3ax}$ = 10.0 Hz, H-2$_{ax}$), 4.26 (1H, ddd, $J_{2eq,4eq}$ = 2.2, $J_{2eq,3ax}$ = 3.2, $J_{2eq,2ax}$ = 10.0 Hz, H-2$_{eq}$), 6.29 (1H, d, $J_{8,6}$ = 3.3 Hz, H-8), 6.36 (1H, dd, $J_{6,8}$ = 3.3, $J_{6,5}$ = 8.3 Hz, H-6), 6.48 (1H, d, $J_{5',6'}$ = 8.3 Hz, H-5′), 6.62 (1H, d, $J_{6',5'}$ = 8.3, H-6′), 6.89 (1H, d, $J_{5,6}$ = 8.3 Hz, H-5); ^{13}C NMR (acetone-d_6): δ 30.1 (C-4), 32.2 (C-3), 55.5 (OCH$_3$), 69.9 (C-2), 102.8 (C-5′), 102.9 (C-8), 107.9 (C-6), 113.4 (C-4a), 116.9 (C-6′), 120.9 (C-1′), 130.1 (C-5), 133.4 (C-3′), 143.4 (C-2′), 146.8 (C-4′), 155.2 (C-8a), 156.6 (C-7); EI-MS: *m/z* 288 (M$^+$, 100), 256 (7), 228 (6), 166 (75), 154 (47), 123 (51).

*Compound **2** [(+)-(3R)-8,2′,3′-trihydroxy-7,4′-dimethoxyisoflavan].* Oil, $[\alpha]_D^{25}$ + 1.99° (MeOH; c0.28); CD: $[\theta]_{255}$ + 79, $[\theta]_{275}$ + 101, $[\theta]_{280}$ + 348, $[\theta]_{285}$ + 294, $[\theta]_{295}$ + 127, $[\theta]_{305}$ + 109, $[\theta]_{315}$ + 42, $[\theta]_{330}$ + 23 (MeOH; c0.05); ^{1}H NMR (acetone-d_6): δ 2.85 (1H, ddd, $J_{4eq,2eq}$ = 1.4, $J_{4eq,3ax}$ = 4.9, $J_{4eq,4ax}$ = 15.0 Hz, H-4$_{eq}$), 3.00 (1H, dd, $J_{4ax,3ax}$ = 11.3, $J_{4ax,4eq}$ = 15.0 Hz, H-4$_{ax}$), 3.51 (1H, m, H-3$_{ax}$), 3.77 (3H, s, 7-OCH$_3$), 3.80 (3H, s,

4′-OCH_3), 4.06 (1H, dd, $J_{2ax,2eq}$ = 10.0, $J_{2ax,3ax}$ = 10.0 Hz, H-2_{ax}), 4.37 (1H, ddd, $J_{2eq,4eq}$ = 1.6, $J_{2eq,3ax}$ = 4.9, $J_{2eq,2ax}$ = 10.0 Hz, H-2_{eq}), 6.39 (1H, d, $J_{6,5}$ = 8.4 Hz, H-6), 6.48 (1H, d, $J_{5',6'}$ = 8.6 Hz, H-5′), 6.63 (1H, d, $J_{6',5'}$ = 8.4 Hz, H-6′), 6.64 (1H, d, $J_{5,6}$ = 8.4 Hz, H-5); ^{13}C NMR (acetone-d_6): δ 30.3 (C-4), 32.2 (C-3), 55.4 (4′-OCH_3), 59.7 (7-OCH_3), 69.7 (C-2), 103.0 (C-5′), 107.7 (C-6), 114.7 (C-4a), 117.0 (C-6′), 120.8 (C-1′), 123.9 (C-5), 133.6 (C-3′), 135.6 (C-8), 143.5 (C-2′), 146.9 (C-4′), 147.7 (C-8a), 148.6 (C-7); EI-MS: *m/z* 318 (M^+, 100), 288 (7), 256 (6), 228 (5), 166 (53), 153 (88).

Compound ***3*** *[(−)-fisetinidol].* Oil, $[\alpha]_D^{25}$ − 24.0° (MeOH; c1.11); CD: $[\theta]_{230}$ − 992, $[\theta]_{235}$ − 24, $[\theta]_{240}$ + 587, $[\theta]_{245}$ + 455, $[\theta]_{255}$ + 287, $[\theta]_{260}$ + 227, $[\theta]_{270}$ + 408, $[\theta]_{275}$ − 291, $[\theta]_{280}$ − 353, $[\theta]_{285}$ − 1447, $[\theta]_{290}$ − 2098, $[\theta]_{295}$ − 627, $[\theta]_{300}$ + 302 (MeOH; c0.05); ^{1}H NMR (CD_3OD): δ 2.70 (1H, dd, $J_{4ax,3ax}$ = 7.6, $J_{4ax,4eq}$ = 16.0 Hz, H-4_{ax}), 2.89 (1H, dd, $J_{4eq,3ax}$ = 4.8, $J_{4eq,4ax}$ = 16.0 Hz, H-4_{eq}), 4.02 (1H, m, H-3_{ax}), 4.64 (1H, d, $J_{2ax,3ax}$ = 7.1 Hz, H-2_{ax}), 6.31 (1H, d, $J_{8,6}$ = 2.4 Hz, H-8), 6.36 (1H, dd, $J_{6,8}$ = 2.4, $J_{6,5}$ = 8.1 Hz, H-6), 6.88 (1H, d, $J_{5,6}$ = 7.9 Hz, H-5), 6.73 (1H, dd, $J_{6',2'}$ = 1.7, $J_{6',5'}$ = 9.4 Hz, H-6′), δ 6.78 (1H, d, $J_{5',6'}$ = 9.3 Hz, H-5′), 6.85 (1H, d, $J_{2',6'}$ = 1.5 Hz, H-6′); ^{13}C NMR (CD_3OD): δ 31.9 (C-4), 67.5 (C-3), 81.7 (C-2), 102.3 (C-8), 108.2 (C-6), 111.2 (C-4a), 113.8 (C-2′), 114.8 (C-5′), 118.3 (C-6′), 130.0 (C-5), 131.0 (C-1′), 145.0 (C-3′), 145.0 (C-4′), 154.9 (C-8a), 155.6 (C-7); EI-MS: *m/z* 274 (M^+, 46), 256 (12), 213 (5), 185 (8), 167 (18), 152 (43), 123 (100).

Compound ***4*** *[(+)-trans-taxifolin].* Yellowish crystal, $[\alpha]_D^{30}$ + 4.08° (MeOH; c0.5); ^{1}H NMR (CD_3OD): δ 4.61 (1H, d, $J_{3,2}$ = 11.1 Hz, H-3), 5.02 (1H, d, $J_{2,3}$ = 11.4 Hz, H-2), 5.95 (1H, d, $J_{6,8}$ = 2.1 Hz, H-6), 5.99 (1H, d, $J_{8,6}$ = 2.0 Hz, H-8), 6.86 (1H, d, $J_{5',6'}$ = 8.2 Hz, H-5′), 6.92 (1H, dd, $J_{6',2'}$ = 2.2, $J_{6',5'}$ = 8.2 Hz, H-6′), 7.07 (1H, d, $J_{2',6'}$ = 2.1 Hz, H-2′); ^{13}C NMR (CD_3OD): δ 72.2 (C-3), 83.6 (C-2), 95.1 (C-8), 96.3 (C-6), 100.8 (C-4a), 114.9 (C-5′), 115.0 (C-2′), 120.0 (C-6′), 129.0 (C-1′), 145.0 (C-3′), 145.9 (C-4′), 163.4 (C-8a), 164.2 (C-5), 167.0 (C-7), 197.5 (C-4); EI-MS: *m/z* 304 (M^+, 46), 256 (12), 213 (5), 185 (8), 167 (18), 152 (43), 123 (100).

Gas chromatography-mass spectrometry

The EtOAc extracts from cumaru, jatoba, and guarita (0.1 mg) were dissolved in pyridine (20 µl) and subjected to trimethylsilylation using *N*,*O*-bis (trimethylsilyl)-trifluoroacetamide (Wako, Japan). The trimethylsilylated derivatives were analyzed on a Shimadzu GCMS QP 2010 equipped with a DB-1 capillary column (30 m × 0.32 mm i.d., film thickness 1 µm) using helium gas as the mobile phase. The oven temperature was programmed to increase from 150°C to 280°C at 7°C/min, and the ionization energy was 70 eV.

Evaluation of antioxidant activity by the DPPH assay

DPPH radical scavenging activity was measured according to the method of Inatani et al.[8] Ethanol solutions (0.5 ml) of compounds **1**–**4**, catechin, and gallic acid were each combined with 3 ml of 0.1 mM DPPH in ethanol, and the mixtures were shaken vigorously. After 20 min at room temperature, the absorbance of each mixture was measured at 517 nm on a Jasco V-530 spectrophotometer. Five concentrations (0.01, 0.05, 0.1, 0.5, and 1.0 mg/ml) were measured for each sample.

Results and discussion

Isolation and structure elucidation

We had found that EtOAc-soluble components of the heartwood MeOH extracts from cumaru-ferro, jatoba, and guarita exhibited antioxidant activities comparable with that of α-tocopherol.[1] The EtOAc extracts from these three species were analyzed by GC-MS (Fig. 2). The GC-MS profile of the guarita extract shows strong peaks at 11.8 min (compound **5**, Fig. 2c) and 24.8 min (compound **6** in Fig. 2c). One of these predominant compounds (compound **5**) in guarita was identified as gallic acid by comparing its retention time and mass fragmentation pattern with those of authentic compound as reported previously.[9] Compound **6** was identified as catechin by GC-MS using authentic sample.

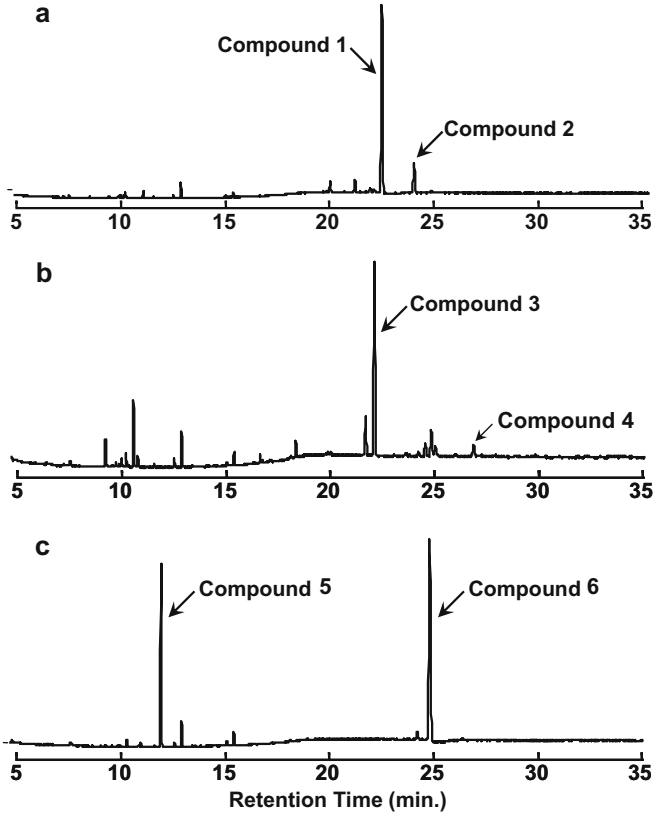

Fig. 2. Gas chromatography-mass spectrometry total ion current chromatograms of ethyl acetate extracts of **a** cumaru-ferro, **b** jatoba, and **c** guarita heartwood

GC-MS profiles of the EtOAc extracts from cumaru-ferro and jatoba show strong peaks at 22.4 min (compound **1**, Fig. 2a) and 22.2 min (compound **3**, Fig. 2b), respectively. These predominant compounds were isolated and their chemical structures were determined.

The ^{13}C NMR spectrum of compound **1** displays 16 signals: 3 signals in the aliphatic region at δ 30.1 (C-4), 32.2 (C-3), and 69.9 (C-2); 1 methoxyl carbon signal at δ 55.5; and 12 signals in the aromatic region (δ 100–160); suggesting that compound **1** has a C_{15} flavonoid skeleton with one methoxyl group. The ^1H NMR spectrum in the aromatic region of this compound displays signals at δ 6.29 (1H, d, J = 3.3 Hz, H-8), 6.36 (1H, dd, J = 3.3 and 8.3 Hz, H-6), and 6.89 (1H, d, J = 8.3 Hz, H-5), ascribed to a C7-substituted A ring, and signals at δ 6.48 (1H, d, J = 8.3 Hz, H-5′) and 6.62 (1H, d, J = 8.3 Hz, H-6′) ppm, ascribed to a 2′,3′,4′-trisubstituted B ring. The substitution pattern of the B ring was supported by the NOESY correlation between the H-4 and H-6′ protons. The presence of a methoxyl group was confirmed by a strong signal at δ 3.70. The remaining features in the aliphatic region are almost identical to those reported for the aliphatic protons of 7,4′-dihydroxy-3′-methoxylisoflavan.[10] Compound **1** is trimethylsilylated, as indicated by a fragment ion at *m/z* 73 [$(CH_3)_3Si^+$] on GC-MS, suggesting the presence of a hydroxyl group. The $[M]^+$ ion was found at *m/z* 288 by EI-MS, consistent with the molecular formula $C_{16}H_{16}O_5$. Therefore, it was concluded that compound **1** has a trihydroxymethoxyisoflavan structure. NOESY analysis showed correlations between the methoxyl group protons and both H-5′ and 6′ protons, indicating that the methoxyl group is attached at C-4′. The absolute stereochemistry at C-3 was determined as the *R*-configuration, based on the positive Cotton effects in the region 270–300 nm because it is demonstrated that (3*R*)-isoflavans have positive Cotton effects in this region.[11,12] The optical rotation was negative in MeOH at 25°C. Consequently, compound **1** was identified as (–)-(3*R*)-7,2′,3′-trihydroxy-4′-methoxy-isoflavan (**1**, Fig. 1). This compound has been isolated from the root of *Astragalus membranaceus*,[13] and was also found in the roots of *Smirnowia iranica*[14] and *Sophora arizonica*,[15] although the stereochemistry at the C-3 position was not determined.

Compound **2** and isoflavan **1** were isolated by column chromatography of the cumaru EtOAc extract (Fig. 2a). ^{13}C NMR analysis indicated that compound **2** had 3 aliphatic carbons [at δ 30.3 (C-4), 32.2 (C-3), and 69.7 (C-2)], 2 methoxyl groups (at δ 55.4 and 59.7), and 12 aromatic carbons (δ 100–160), suggesting that compound **2** was a dimethoxyflavonoid. ^1H NMR analysis showed two pairs of AB coupling [at δ 6.39 (1H, d, J = 8.4 Hz, H-6) and 6.48 (1H, d, J = 8.6 Hz, H-5′), and at δ 6.63 (1H, d, J = 8.4 Hz, H-6′) and 6.64 (1H, d, J = 8.4 Hz, H-5)], indicating the presence of a 7,8-disubstituted A ring and a 2′,3′,4′-trisubstituted B ring. The substitution pattern of the B ring was supported by NOESY correlation between the H-4 and H-6′ protons. The presence of two methoxyl groups was confirmed by strong signals at δ 3.77 and 3.80. ^1H NMR analysis indicated that compound **2** also has an isoflavan structure because the coupling patterns of aliphatic protons were nearly identical to those for isoflavan **1**, described above. GC-MS analysis (*m/z* 73) suggested the presence of a hydroxyl group, and EI-MS ($[M]^+$ at *m/z* 318) was consistent with the molecular formula $C_{17}H_{18}O_6$. From these spectral data, compound **2** was concluded to be a trihydroxydimethoxyisoflavan. NOESY analysis indicated that the methoxyl groups are present at C-7 and C-4′ because of the correlation between the protons of one methoxyl group and both the H-5 and H-6 signals, and that between the other methoxyl group protons and both the H-5′ and H-6′ protons, respectively. The positive circular dichroism (CD) spectrum in the region 270–300 nm revealed that the C-3 is in the *R*-configuration.[11,12] The optical rotation was positive in MeOH at 25°C. Consequently, compound **2** was identified as (+)-(3*R*)-8,2′,3′-trihydroxy-7,4′-dimethoxylisoflavan (**2**, Fig. 1). Isoflavones, triterpenes, and methyl esters of fatty acids have been isolated from cumaru-ferro heartwood.[16] To the best our knowledge, however, the present report is the first to describe the isolation and structure elucidation of isoflavans **1** and **2** from cumaru wood.

^{13}C NMR analysis indicated that compound **3** has 3 aliphatic carbons [at δ 31.9 (C-4), 67.5 (C-3), and 81.7 (C-2)] and 12 aromatic carbons (δ 100–160), suggesting that compound **3** is a flavonoid (two signals were completely overlapped[17] at δ 145.0). ^1H NMR analysis indicated substitution at C-7, C-3′, and C-4′, because of the coupling patterns of aromatic protons (see data in Materials and methods for δ 6.31–6.85), and indicated a flavan structure because the coupling patterns of aliphatic protons (δ 2.70–4.64) were almost identical to those for the aliphatic protons of an authentic catechin. GC-MS analysis (*m/z* 73) suggested the presence of a hydroxyl group. The $[M]^+$ ion was found at *m/z* 274 in the EI-MS spectrum, consistent with the molecular formula $C_{15}H_{14}O_4$. Therefore, compound **3** was concluded to be fisetinidol (3,7,3′4′-tetrahydroxyflavan).[18–20] The negative and positive regions of the CD spectrum of flavan **3** were the complete reverse of (+)-fisetinidol[21] (the CD spectrum was shown in the report), which has the absolute stereochemistry [2*S*,3*R*] (Fig. 3). The optical rotation was negative in MeOH at 25°C. Consequently, compound **3** was identified as (–)-fisetinidol, (2*R*,3*S*)-3,7,3′,4′-tetrahydroxyflavan (**3**, Fig. 1). This compound has been isolated from *Rhoicissus tridentata*,[20] and is also found in the bark of *Castela tortuosa* Liebm[18] and *Burkea afficana*,[19] although the stereochemistries at C-2 and C-3 were not determined. Sesquiterpenes have been isolated as extractives from jatoba leaf resin,[22] and diterpenes have been isolated from jatoba seed resin[23–26] and trunk resin.[27] To date, however, heartwood extractives have not been well studied: the present report is the first describing the isolation and structural elucidation of (–)-fisetinidol from jatoba heartwood.

Compound **4** was also isolated by column chromatography of the jatoba EtOAc extract (Fig. 2b) and subsequent GC-MS analysis showed the retention time and mass fragmentation pattern to be identical to those of authentic taxifolin. The ^1H NMR data were almost identical to those reported for taxifolin.[28] In particular, the signals for H-2 and H-3 appeared at δ 4.61 and 5.02 with a coupling constant (J value) of 11.4 Hz, which is well in accord with that for

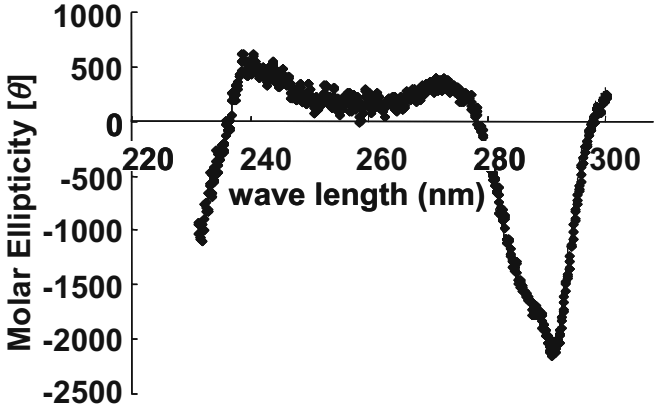

Fig. 3. Circular dichroism spectrum of compound **3**

Table 1. Antioxidant activities of reference compounds and compounds isolated from Amazonian trees

Compound	EC_{50} (μg/ml)	EC_{50} (μM)
Isoflavan **1**	50	174
Isoflavan **2**	70	220
Fisetinidol **3**	28	102
Taxifolin **4**	48	158
Gallic acid (authentic)	10	59
Catechin (authentic)	31	106
α-Tocopherol	51	118

EC_{50}, Oxidant concentration required for 50% consumption of the initial 1,1-diphenyl-2-picrylhydrazyl radical concentration

the *trans*-form (in the previous report[28] a much smaller *J* value of 2.8 Hz was demonstrated for the *cis*-form). The optical rotation was positive in MeOH at 30°C, indicating the absolute stereochemistry [2*R*,3*R*].[28] Consequently, compound **4** was identified as (+)-*trans*-taxifolin (**4**, Fig. 1).

Antioxidant activity

Although the extracts of some South American trees have been reported to have various bioactivities, the extractives from some species have not been well studied. Therefore, it is unclear which compounds in the extracts contribute to the observed bioactivities.

The antioxidant activities of isoflavans **1** and **2** from cumaru-ferro heartwood, (−)-fisetinidol and (+)-*trans*-taxifolin from jatoba heartwood, and authentic gallic acid and catechin, were evaluated (Table 1). Activity was expressed as the amount of oxidant required for 50% consumption of the initial DPPH radical concentration (EC_{50}). The activity of α-tocopherol was measured as a control. The EC_{50} values for isoflavans **1**, **2**, (−)-fisetinidol, (+)-*trans*-taxifolin, gallic acid, and catechin were 50 (174), 70 (220), 28 (102), 48 (158), 10 (59), and 31 (106) μg/ml (μM), respectively. These values are similar to that for α-tocopherol (51 μg/ml, 118 μM), indicating that these heartwood extractives have antioxidant activities comparable with that of α-tocopherol. These compounds would therefore contribute to the high antioxidant activities of cumaru, jatoba, and guarita heartwood methanol extracts reported previously.[1]

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