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Unusual amount of (−)-mesquitol from the heartwood of *Prosopis juliflora*

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A large amount of flavonoid has been extracted and isolated from the heartwood of *Prosopis juliflora*, an exogenous wood species of Kenya. Structural and physicochemical elucidation based on FTIR, ^{1}H and ^{13}C NMR, GC-MS and HPLC analysis clearly demonstrated the presence of (−)-mesquitol as the sole compound without any noticeable impurities. The product was able to slow down oxidation of methyl linoleate induced by AIBN. The important amount and high purity of (−)-mesquitol present in the acetonic extract of *P. juliflora* could therefore be of valuable interest as a potential source of antioxidants from a renewable origin.

Keywords: antioxidant; heartwood; flavonoid; *Prosopis juliflora*; mesquitol

1. Introduction

Wood is a natural composite constituted mainly of cellulose, lignin and hemicelluloses. In addition to these polymeric materials it may contain low molecular weight compounds present in more or less important quantities called extractives. Wood extractives have been described to have a crucial effect on the natural durability of wood, explaining the resistance of some wood species to biodegraders (Celimene, Micales, Ferge, & Young, 1999; Haupt et al., 2003; Mori, Aoyama, & Hokkaido, 1997; Neya, Hakkou, Petrissans, & Gérardin, 2004; Reyes Chilpa, Gomez-Garibay, Moreno-Tores, Jimenez-Estrada, Quiroz-Vasquez, 1998; Windeisen, Wegener, Lesnino, Schumacher, 2002). The aim of the present article is to report the identification and isolation of (−)-mesquitol, a relatively unusual flavonoid, obtained in high yields from the heartwood of *Prosopis juliflora*, an exogenous wood species native to South and Central America, and introduced to Kenya in the early 1970s for rehabilitation of arid and semi arid lands.

2. Results

Extraction of *P. juliflora* heartwood with acetone leads to a large amount of light brown extractives ranging between 7 and 8%. Contrary to heartwood, sapwood contains a smaller amount of extractives of ~2%. ^{1}H NMR analysis of the crude extract indicates the

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presence of a main component showing typical flavanol signals. Indeed, NMR analysis indicated a characteristic ABX system corresponding to two hydrogen atoms at the C_4 position of the (C) ring of a flavanol structure at ~2.8 ppm, and two additional signals at approximately 4.0 and 4.5 ppm, characteristic of hydrogen at C_3 and C_2 positions, respectively. Indeed, signals at 2.71 (dd), 2.89 (dd) and 4.09 (m) ppm are characteristic of an ABX system corresponding to the two hydrogen atoms at the C_4 position and to the hydrogen at the C_3 position of the (C) ring of a flavanol structure. This attribution is corroborated by the presence of a doublet at 4.79 ppm, corresponding to the hydrogen atom at the C_2 position. Moreover, additional signals appearing in the NMR spectra of the crude extract concern aromatic signals, which could be attributed to (A) and (B) rings. Comparison with spectral data of reference flavanols like (+)-catechin and (−)-epicatechin, reported in the literature to be present in several wood species (Mämmelä, 2001; Mayer, Koch, & Puls, 2006; Pietarinen, Willför, Vikström, & Holmbom, 2006), indicates that the flavanol present in *P. juliflora* heartwood has a different structure.

For identification purposes, the crude extract was subjected to purification. Column chromatography over silica gel using an EtOAc/hexane(3/1v/v) mixture leads to a pale yellow solid in 60% yield (Rf 0.45, silica gel, EtOAc), melting at 81–83°C. ^{1}H NMR analysis of the purified product presented quite similar signals to those of the crude acetone extract. According to the large coupling constant observed between H_2 and H_3 ($J = 6.75$ Hz), the 3′,4′-dihydroxyphenyl group at the C_2 position is in trans position of the hydroxyl group at the C_3 position. This value is in good agreement with that observed for (+)-catechin. The main difference between spectra of reference flavanols and that of purified product from the extract of *P. juliflora* concerns the signals of the hydrogen atoms present on the (A) aromatic cycle. These two protons appear as 2 singlets just under 6 ppm in reference compounds, while they appear as 2 doublets with a typical benzenic ortho coupling constant ($J = 8.2$ Hz) at 6.38 and 6.42 ppm in the isolated product from the heartwood of *P. juliflora*. Further detailed analysis of ^{1}H–^{13}C HMQC and ^{1}H–^{13}C HMBC NMR data (Table 1) allowed unambiguous assignments of the structure of the compound present in *P. juliflora* acetonic extracts as 2,3-*trans*-3′,4′,7,8-tetrahydroxyflavan-3-ol (Figure 1). Such a compound has been previously isolated from the bark of *Dichrostachys cinerea* and described as (−)-mesquitol (Madhusudana, Jagadeeshwar, Ashok, Jhillu, & Kondapuram, 2004; Mämmelä, 2001). The specific rotation of the isolated product is similar to that of (−)-mesquitol reported in the literature (Madhusudana et al., 2003, 2004), allowing assignment of product configuration (compare $[\alpha]_D = -39$ (c 1.0, CH_3OH) to a value of –36 in the literature). The FTIR spectrum indicated characteristic hydroxyl group absorption at 3350 cm^{-1} and aromatic C = C skeletal vibrations at 1611, 1518 and 1470 cm^{-1}. Found microanalysis is in good agreement with that calculated for $C_{15}H_{14}O_6$. GC-MS analyses of the TMS derivatives of the purified product and of reference flavanols confirm the existence of three isomers appearing at distinct retention times. MS spectra of the different products present quite similar peaks, with a molecular ion peak for the penta-TMS derivative at m/z 650 and characteristic peaks at 355 and 368, similar to those reported in the literature (Soleas, Diamandis, Karumanchiri, & Goldberg, 1997). High performance liquid chromatography analyses corroborate preceding results, allowing identification of three different compounds presenting similar UV absorptions. In both cases, liquid and gas chromatographic analyses of *P. juliflora* extract confirmed high purity of the crude extract.

To confirm and generalise the presence of (−)-mesquitol in the heartwood of *P. juliflora*, analysis of extractives was investigated on six different trees selected randomly.

Table 1. NMR data for (−)-mesquitol.

Carbon no.	δ_C (ppm), multiplicity	$^1H - ^{13}C$ HMQC	$^1H - ^{13}C$ HMBC
2	83.4, CH	4.79 (d, $J = 6.75$ Hz, 2H)	2H-4, H-2′, H-6′
3	69.2, CH	4.09 (m, 1H)	2H-4, H-2
4	33.3, CH_2	2.71 (dd, $J = 15.7$, 7.5 Hz, 1H)	H-2, H-5
		2.89 (dd, $J = 15.8$, 5.0 Hz, 1H)	H-2, H-5
5	120.7, CH	6.42 (d, $J = 8.2$ Hz, 1H)	2H-4
6	109.8, CH	6.38 (d, $J = 8.2$ Hz, 1H)	H-5
7	145.5, qC	–	H-6, H-5
8	134.3, qC	–	H-6, H-5
9	151.1, qC	–	–
10	113.7, qC	–	2H-4, H-5
1′	129.2, qC	–	H-2, H-6′, H-5′
2′	115.4, CH	6.87 (d, $J = 1.6$ Hz, 1H)	H-2
3′	146.7, qC	–	H-2′, H-6′, H-5′
4′	146.7, qC	–	H-2′, H-6′, H-5′
5′	120.2, CH	6.78 (d, $J = 8.0$ Hz, 1H)	H-2, H-2′
6′	116.5, CH	6.75 (dd, $J = 8.0$, 1.6 Hz, 1H)	H-2

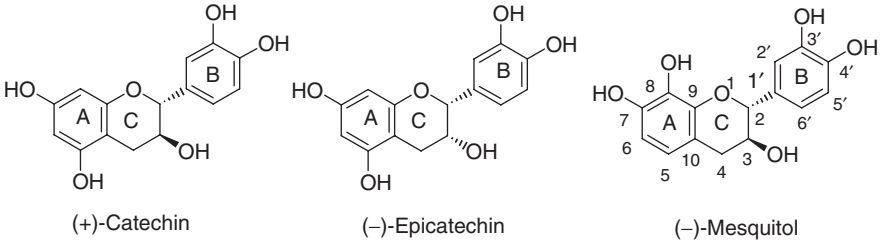

Figure 1. Structure of the different flavanols.

The amount of extractives are similar to those obtained previously (7.5% ± 0.3). ^1H NMR and HPLC analyses clearly demonstrated the presence of (−)-mesquitol as the sole compound without any noticeable impurity. The important amount and high purity of the crude acetonic extract of *P. juliflora* could therefore be of valuable interest as a potential source of flavanols, which have been described as powerful antioxidants (Bors, Heller, Michel, & Saran, 1990; Valcic, Burr, Timmerman, & Liebler, 2000; Raza & John, 2007; Whiteside, Heimburger, & Johanning, 2004; Nijveldt et al., 2001; Sanchez-Moreno, Jimenez-Escrig, & Saura-Calixto, 2000; Sang et al., 2003). Antioxidant properties, estimated using a methyl linoleate oxidation inhibition test (Figure 2), showed that (−)-mesquitol, like (+)-catechin, is able to slow down oxidation of methyl linoleate induced by AIBN. In both cases, flavanols present higher antioxidant properties compared to BHT, chosen as the reference antioxidant.

3. Experimental section

3.1. *General experimental procedures*

Melting points were measured on Buchi Melting Point B-540 apparatus. Micro-analysis was carried out on Thermofinnigan Flash EA 1112 apparatus. ^1H and ^{13}C NMR spectra

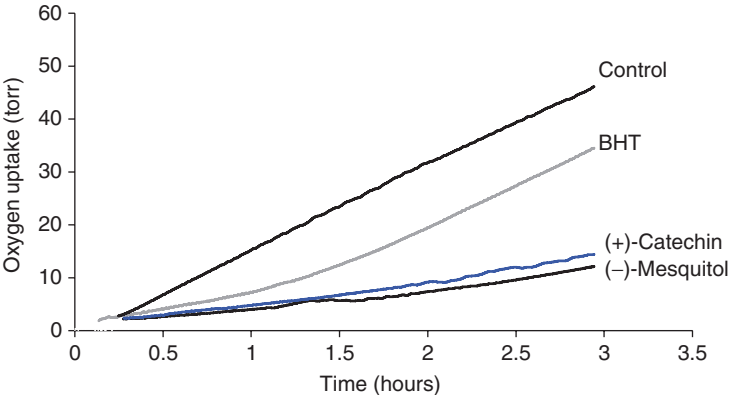

Figure 2. Antioxidant properties estimated using methyl linoleate oxidation inhibition.

were recorded in methanol D_4 on a Bruker AM 400 spectrometer. Chemical shifts were expressed in ppm and was calculated relative to TMS. FTIR spectra were recorded as KBr disks on a Perkin-Elmer FTIR spectrometer SPECTRUM 2000, between wave number range of 4000–500 cm^{-1}. Specific rotations were determined on a Perkin-Elmer 141 polarimeter (10 cm cell) at room temperature. Analytical thin-layer chromatography was performed on Merck 60 F254 pre-coated silica gel plates. Compounds were visualised with UV light.

3.2. *Extraction and isolation*

Prosopis juliflora heartwood was collected from the Baringo Forest (latitude 0°, 20′ N, longitude 35°, 57′E), Kenya. The voucher specimen is kept at the Department of Forestry and Wood Science, Moi University. Air dried heartwood was ground to fine powder, passed through a 115-mesh sieve and dried at 60°C before extraction with acetone using an Accelerated Solvent Extractor (Dionex ASE 200, Voisins Le Bretonneux, France). Extraction was performed in 33 mL cell size on 8 g of sawdust at 100°C under a pressure of 100 bars (3 static cycles of 5 minutes each). After extraction, the solvent was evaporated under reduced pressure and the crude extract dried under vacuum in a desiccator over P_2O_5. To check reproducibility of the nature and the amount of extractives, six trees about 27 years old growing at different sites were selected, randomly felled, debarked and transported while fresh to Moi University laboratories. Heartwood was differentiated from sapwood by colour, separated and extracted as above.

3.3. *GC-MS analysis*

(+)-Catechin (98% assay) and (−)-epicatechin (90% assay) were used as reference controls and purchased from Fluka-Sigma-Aldrich Chimie SARL (St Quentin Fallavier, France). Samples were analysed as trimethyl derivatives using the following procedure. In a screw-capped vial, a sample of ~1 mg of dry extract, (+)-catechin or (−)-epicatechin, was dissolved in 0.5 mL of anhydrous acetonitrile (Acros Organics), and 0.4 mL of *N*,*O*-*bis*-(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane

(BSTFA/1% TMCS) (Acros Organics) was added. The solution was sonicated for about 1 min and heated at 60°C for 60 min. After evaporation of the solvent in a stream of dry nitrogen, the residue was diluted in 1 mL of anhydrous acetonitrile. The GC-MS analysis was performed on a Clarus® 500 GC gas chromatograph (Perkin-Elmer Inc., USA) coupled to a Clarus® 500 MS quadrupole mass spectrometer (Perkin-Elmer Inc., USA). Gas chromatography was carried out on a 5% diphenyl / 95% dimethyl polysiloxane fused-silica capillary column (Elite-5ms, 60 m × 0.25 mm, 0.25 mm film thickness, Perkin-Elmer Inc, USA). The gas chromatograph was equipped with an electronically controlled split/splitless injection port. The injection (injection volume of 1 μL) was performed at 250°C in the split mode (split flow of 20 mL min^{-1}). Helium was used as carrier gas, with a constant flow of 1.2 mL min^{-1}. The oven temperature program was as follows: 200°C constant for 4 min, 200–330°C at a rate of 5°C min^{-1} and then constant for 330°C. Ionisation was achieved under the electron impact mode (ionisation energy of 70 eV). The source and transfer line temperatures were 250°C and 330°C, respectively. Detection was carried out in scan mode: m/z 35 to m/z 700 a.m.u. The detector was switched off in the initial 10 min (solvent delay). The three derivatised samples were analysed separately and in a mix (ratio 1 : 1 : 1).

The GC/MS spectrum of the penta-TMS derivative of (−)-epicatechin, retention time = 22.4 min, m/z (%): 650 (M^+, 1.7), 383 (2.5), 370 (6.9), 369 (14.3), 368 (47.5), 357 (2.9), 356 (6.3), 355 (20.1), 281 (1.9), 280 (3.1), 268 (1.6), 267 (7.2), 249 (2.0), 179 (9.5), 147 (4.1), 133 (1.7), 75 (5.7), 74 (7.3), 73 (100), 45 (7.1).

The GC/MS spectrum of the penta-TMS derivative of (+)-catechin, retention time = 23.4 min, m/z (%): 650 (M^+, 1.6), 383 (2.0), 370 (6.2), 369 (15.3), 368 (47.8), 357 (2.6), 356 (5.5), 355 (17.8), 281 (2.0), 280 (3.3), 268 (1.4), 267 (6.1), 249 (2.1), 179 (9.1), 147 (4.2), 133 (1.6), 75 (5.1), 74 (7.5), 73 (100), 45 (6.1).

The GC/MS spectrum of the penta-TMS derivative of (−)-mesquitol, retention time = 23.7 min, m/z (%): 650 (M^+, 1.6), 383 (5.8), 370 (6.1), 369 (12.9), 368 (39.7), 357 (1.3), 356 (2.8), 355 (8.7), 281 (1.3), 280 (2.2), 268 (4.5), 267 (19.5), 249 (1.9), 179 (4.7), 147 (3.8), 133 (2.2), 75 (5.3), 74 (7.4), 45 (6.7), 73 (100).

3.4. *HPLC analysis*

HPLC analysis was performed using a Supercosil™ LC-18 column (250 mm × 4.6 mm i.d.) at 35°C on a Waters liquid chromatograph (Waters SAS, Saint Quentin-en Yvelines, France) equipped with a system controller 600E, a manual injector system with a 20 μL loop and a Waters 2996 photo diode array (PDA) detector. The data were recorded on a 210–400 nm range with Empower software. Solvents used for elution were solvent A (water containing 0.05% of trifluroacetic acid) and solvent B (methanol (HPLC grade) containing 0.05% of TFA) at a flow rate of 1 mL min^{-1}. Elution was achieved with a binary gradient starting at 95% A and 5% B for 1 min, followed by a linear ramp to 50% A and 50% B at 10 min to finish at 100% B after 20 min. (+)-Catechin, retention time = 11.35 min, (−)-mesquitol, retention time = 12.40 min, (−)-epicatechin, retention time = 13.05 min.

3.5. *Antioxidant activity*

Oxidation of methyl linoleate (2 mL of a 0.4 M solution in 1-butanol) was performed in a closed borosilicate glass reactor containing 1 mL of a 9×10^{-3} M solution of AIBN in

1-butanol as initiator. The double shell reactor was thermostated at 60°C by an external heating bath. Oxygen (150 Torr) was bubbled by a gas-tight oscillating pump. A small condenser was inserted on the reactor in the gas circulation to ensure condensation of the solvent. Oxygen uptake was monitored continuously with a pressure transducer (Viatron model 104) in the presence of 1 mL of a 10^{-4} M solution in butan-1-ol of the supposed antioxidant compound to evaluate antioxidant properties. The volumes of the liquid and the gas phases were, respectively, 4 and 100 mL.

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