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Note

Carbohydrates from *Detarium microcarpum* bark extract☆

Pedro Abreu,* Angela Relva

Departamento de Química, Centro de Química Fina e Biotecnologia, FCT-UNL, P-2829 516 Caparica, Portugal

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Abstract

The bark extract of the medicinal plant *Detarium microcarpum* was analysed for its carbohydrate content by GLC-CIMS. Preparative HPLC of the benzoylated carbohydrate fraction led to the isolation of L-quino-1,5-lactone, D-(−)-bornesitol, D-pinitol, *myo*-inositol, sucrose, D-glucose, and D-fructose benzoates, which were characterised by NMR spectroscopy experiments. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: *Detarium microcarpum*; L-Quino-1,5-lactone; D-(−)-Bornesitol; D-Pinitol; *myo*-Inositol

1. Introduction

The Leguminosae *Detarium microcarpum* Guill. (Perr) is catalogued as a major African medicinal plant.[1] The infusion of the bark is reported to possess diuretic, anti-inflammatory and anti-parasitic properties, whereas its fruits and leaves are used in the treatment of dysentery and syphilis.[1,2] The isolation of terpenoids and anti-HIV flavans from *D. microcarpum* extracts has been previously reported,[3,4] and the characteristics of oil recovered from its seeds were investigated.[5] A seed polysaccharide was evaluated as a stabiliser in processed fruit products.[6]

In this communication, we report the HPLC isolation of L-quino-1,5-lactone (L-quinide **1**), D-(−)-bornesitol (**2**), D-pinitol (**3**), *myo*-inositol (**4**), sucrose, D-glucose and D-fructose, from a *D. microcarpum* bark extract. These compounds were isolated in the form of their corresponding benzoylated derivatives, which were characterised by NMR experiments (^{1}H, ^{13}C, DEPT, COSY, HMQC, HMBC, NOESY).

2. Results and discussion

Chromatographic fractionation of the ethanolic bark extract of *D. microcarpum* afforded an aqueous fraction, whose ^{1}H and ^{13}C NMR spectra exhibited a sugar profile.[7] GLC-CIMS of this fraction after derivatization of the free sugars to alditol acetates,[8] indicated sucrose as the main constituent ($[M + NH_4]^+$ at m/z 696), the presence of mannitol and glucitol with $[M + NH_4]^+$ ions at m/z 452, and three unidentified compounds: two with $[M + NH_4]^+$ at m/z 422, and the third one at m/z 450.

The isolation of the carbohydrates was carried out by semi-preparative HPLC of the corresponding benzoylated mixture, monitored by diode-array detection. This method provides a faster alternative for separation of sugar mixtures, with better resolution when compared to the refractive-index detection, which precludes the use of gradient elution.[9,10] Prior to HPLC analysis, the benzoylated mixture was eluted on a RP-18 open column, yielding sucrose octabenzoate (73% of the total content), whose mono- and two-dimensional NMR spectra were in accordance with published data.[11] From the eight compounds further isolated by HPLC, α- and β-D-glucopyranose, and α- and β-D-fructofuranose perbenzoates, were readily identified by co-injection with standards and comparison of their corresponding NMR data.[12] The presence of these two sugars in the

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* Corresponding author. Tel.: +351-2-954464; fax: +351-2-954461

E-mail address: pma@dq.fct.unl.pt (P. Abreu).

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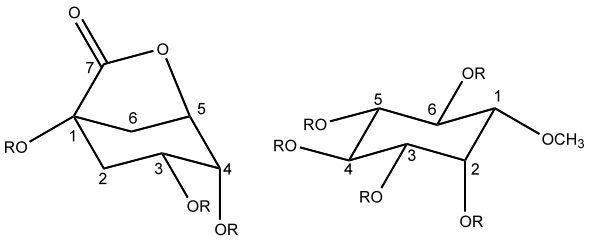
extract explains the CIMS detection of mannitol and glucitol, formed from the derivatization to alditol acetates. Although furanoid, pyranoid and acyclic derivatives could be obtained in various proportions from the benzoylation of fructose,[12,13] we have just detected the two anomeric fructofuranose pentabenzoates.

NMR data of compound **1a** indicated the presence of an oxygenated quaternary carbon (δ 76.6) showing $^{2}J_{CH}$ cross-peaks with four methylenic protons in the HMBC spectrum, two secondary carbons bearing benzoate groups (δ 65.6 and 66.6), and another secondary oxygenated carbon (δ 65.6) whose geminal methine proton (δ 5.90) exhibited a three-bond correlation with a carbonyl ester at δ 171.2 ppm. These features, in addition to the observed proton coupling in the ^{1}H–^{1}H COSY spectrum, and corresponding coupling constants and NOE interactions, suggested the structure of L-quino-1,5-lactone tribenzoate, which was confirmed by chemical correlation with a standard prepared from L-quinic acid according to a described procedure.[14] The triacetyl derivative **1b** was also synthesised and characterised by NMR. Although stereoisomeric quinic acid lactones formed from L-quinic acid have been previously characterised,[15–18] to our knowledge, this is the second reported occurrence of lactone **1** as a natural product.[19] Complete NMR data of **1a** and **1b** are presented herein for the first time. The sequential $NaBH_4$ reduction and acetylation of **1** resulted in several unidentified products, thus explaining the non-detection of this metabolite by GLC-CIMS.

Compounds **2a** and **3a** displayed a similar *O*-methyl-inositol pattern of their ^{1}H and ^{13}C NMR spectra: five secondary carbons bearing benzoate groups; one methoxy group; one methine proton at δ 3.92 and 4.24 ppm, respectively; and five low-field methine protons in the range of δ 5.63–6.28 ppm. Detailed analysis of COSY, HMQC and HMBC spectra, allowed us to assign the structures of D-(−)-bornesitol and D-pinitol to compounds **2** and **3**, whose acetylated derivatives showed $[M+NH_4]^+$ ions at m/z 422 in CIMS. The conformations of benzoates **2a** and **3a** were assigned on the basis of proton coupling constants and NOE interactions in the NOESY spectra.

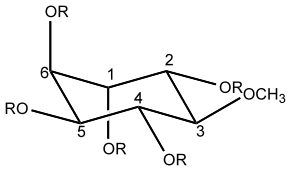
The ^{1}H and ^{13}C NMR spectra of **4a**, in comparison with those of **2a** and **3a**, lack the methoxy group and show six benzoate substituents. Analysis of two-dimensional spectra led to the structure of *myo*-inositol (**4**), whose hexacetate displays the $[M+NH_4]^+$ ion at m/z 450.

The identity of compounds **2**, **3** and **4**, was further confirmed by GLC co-injection of their acetylated derivatives with authentic samples. The NMR data of the benzoylated derivatives **2a**, **3a** and **4a** are reported herein for the first time.

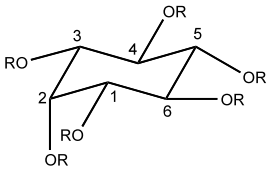


1 R = H
1a R = Bz
1b R = Ac

2 R = H
2a R = Bz



3 R = H
3a R = Bz



4 R = H
4a R = Bz

3. Experimental

General.—^{1}H (400 MHz) and ^{13}C (100.61 MHz) one- and two-dimensional NMR spectra were recorded on a Bruker ARX-400 spectrometer. Preparative HPLC of benzoylated carbohydrates was carried out on a D-7000 Merck instrument equipped with a DAD detector L7450A, in a range 200–450 nm, using Lichrospher Si60 and Lichrospher 100 RP18 columns (250 × 8 mm, 10 μm), Rheodyne injector 7725I, loop of 200 μL, flow rate 6.0 mL/min. Normal- and reversed-phase column chromatography were conducted on Silica Gel of 70–230 mesh and LiChroprep RP-18 of 40–63 μm, respectively. E. Merck Silica Gel, cellulose and HPTLC-NH_2 plates 0.25-mm thick were used for TLC. Gas–liquid chromatography of acetylated carbohydrates (Ac_2O–*N*-methylimidazole)[20] and alditol acetates[8] was performed on a DB-225 column (20 m × 0.104 mm, 0.1 μm; J&W) fitted to a Carlo–Erba GC 6000 Vega Series 2 chromatograph equipped with a flame-ionisation detector and a split–splitless injection-system used in the split mode. Hydrogen was used as carrier gas at a flow rate of 80 Kpa, with oven temperature of 200 °C raised at 5 °C/min to 220 °C, where it was kept for 20 min. The injection port and detector were heated to 250 and 300 °C, respectively. A Finnigan MAT 95 mass spectrometer was used in GC–MS with chemical ionisation with NH_3.

Plant material.—*D. microcarpum* was collected in January 1994 at Contuboel, Guinea-Bissau, and identified at the Herbarium of Botany Centre (LISC), Lisbon, where a voucher specimen is deposited.

Extraction and isolation.—Powdered bark of *D. microcarpum* (2.6 Kg) was extracted successively with

petroleum ether, $CHCl_3$ and EtOH. The EtOH extract (10 g) was submitted to reversed-phase flash chromatography using a step gradient of MeOH (0–100%) in water, and the corresponding fractions were analysed for their composition in silica gel, cellulose and HPTLC-NH_2 plates, using H_2SO_4, vanillin–HCl, $AlCl_3$, aniline–diphenylamine–phosphoric acid and 1-naphthol as spray reagents. The aqueous fraction, whose TLC and NMR analysis revealed a sugar composition, was evaporated to dryness (1.43 g) and derivatised with benzoyl chloride according to a described procedure.[9] The resulting benzoylated mixture (3.72 g) was eluted on a RP-18 open column with 1:1 to 0:1 water–MeCN, yielding sucrose perbenzoate (2.70 g) and a less polar fraction F_1 (1 g). Successive SiO_2 column chromatography (hexane–CH_2Cl_2 gradient) and normal-phase HPLC (4:1 to 1:4 hexane–EtOAc) of F_1, yielded, in order of increasing polarity, the benzoates of D-pinitol (**3a**) (323 mg), β-D-glucopyranose (8 mg), a mixture of D-(−)-bornesitol (**2a**) (157 mg) and *myo*-inositol (**4a**) (142 mg) further resolved by RP-18 HPLC (4:1 MeOH–water), α-D-fructofuranose (9 mg), quinic acid 1,5-lactone (**1a**) (4 mg), α-D-glucopyranose (8 mg) and β-D-fructofuranose (7 mg).

NMR data

Quinic acid 1,5-lactone tribenzoate (**1a**). ^{1}H NMR ($CDCl_3$): δ 7.29–8.08 (15 benzoyl protons), 5.90 (t, 1 H, $J_{4,3}$ 4.4, $J_{4,5}$ 5.6 Hz, H-4); 5.59 (oct, 1 H, $J_{3,2a}$ 11.2, $J_{3,2e}$ 7.2 Hz, H-3), 5.16 (t, 1 H, $J_{5,6ax}$ 0, $J_{5,6eq}$ 6.0 Hz, H-5), 3.32 (oct, 1 H, $J_{6eq,6ax}$ 11.6 Hz, H-6eq), 2.94 (d, 1 H, H-6ax), 2.74–2.80 (m, 2 H, H-2ax,2eq); ^{13}C NMR ($CDCl_3$): δ 171.2 (C-7), 164.8–165.0 (benzoate carbons), 128.4–133.9 (benzoyl carbons), 76.6 (C-1), 73.8 (C-5), 66.6 (C-3), 65.6 (C-4), 34.5 (C-6), 33.9 (C-2).

Quinic acid 1,5-lactone triacetate (**1b**). ^{1}H NMR ($CDCl_3$): δ 5.40 (t, 1 H, $J_{4,3}$ 4.4, $J_{4,5}$ 5.6 Hz, H-4), 5.08 (oct, 1 H, $J_{3,2a}$ 11.2, $J_{3,2e}$ 7.2 Hz, H-3), 4.80 (t, 1 H, $J_{5,6ax}$ 0, $J_{5,6eq}$ 6.0 Hz, H-5), 3.04 (oct, 1 H, $J_{6eq,6ax}$ 11.6 Hz, H-6eq), 2.48 (d, 1 H, H-6ax), 2.21–2.23 (m, 2 H, H-2ax,2eq), 2.08 (s, 3 H, OAc), 2.07 (s, 3 H, OAc), 1.94 (s, 3 H, OAc); ^{13}C NMR ($CDCl_3$): δ 171.2 (C-7), 169.3 (OAc), 169.1 (OAc), 169.0 (OAc), 75.9 (C-1), 73.5 (C-5), 65.4 (C-3), 64.5 (C-4), 33.5 (C-6), 33.2 (C-2).

D-(−)-Bornesitol pentabenzoate (**2a**). ^{1}H NMR ($CDCl_3$): δ 7.26–8.20 (20 benzoyl protons), 6.28 (t, 1 H, $J_{1,2}$ 10.8, $J_{1,6}$ 10.4 Hz, H-1), 6.23 (q, 1 H, $J_{5,6}$ 2.7, $J_{5,4}$ 2.6 Hz, H-5), 6.10 (t, 1 H, $J_{3,2}$ 10.8, $J_{3,4}$ 10.0 Hz, H-3), 5.93 (t, 1 H, H-2), 5.63 (dd, 1 H, H-6), 3.92 (dd, 1 H, H-4), 3.46 (s, 3 H, OCH_3); ^{13}C NMR ($CDCl_3$): δ 165.2–165.6 (benzoate carbons), 128.0–133.3 (benzoyl carbons), 78.1 (C-4), 71.6 (C-3), 71.0 (C-2), 70.3 (C-6), 70.1 (C-1), 67.1 (C-5), 58.4 (OCH_3). Anal. Calcd for $C_{42}H_{34}O_{11}$: C, 70.58; H, 4.79. Found: C, 70.16; H, 4.84.

D-(+)-Pinitol pentabenzoate (**3a**). ^{1}H NMR ($CDCl_3$): δ 7.25–8.13 (20 benzoyl protons), 6.12 (brs, 3 H, $\Delta\nu_{1/2}$ 8 Hz, H-1, H-2, H-5), 6.00 (d, 1 H, $J_{6,5}$ 9.2, $J_{6,1}$ 0 Hz, H-6), 5.95 (d, 1 H, $J_{3,2}$ 0, $J_{3,4}$ 8.6 Hz, H-3), 4.24 (t, 1 H, $J_{4,5}$ 8.6 Hz, H-4), 3.57 (s, 3 H, OCH_3); ^{13}C NMR ($CDCl_3$): δ 164.7–165.5 (benzoate carbons), 128.4–133.7 (benzoyl carbons), 79.2 (C-4), 71.9 (C-3), 71.4 (C-5), 70.4 (C-6), 68.5 (C-2, C-1), 60.9 (OCH_3). Anal. Calcd for $C_{42}H_{34}O_{11}$: C, 70.58; H, 4.79. Found: C, 71.02; H, 4.83.

myo-*Inositol pentabenzoate* (**4a**). ^{1}H NMR ($CDCl_3$): δ 7.25–8.18 (30 benzoyl protons), 6.40 (t, 2 H, $J_{4,3}$ 10.3, $J_{4,5}$ 10.0, $J_{6,1}$ 10.3, $J_{6,5}$ 10.3 Hz, H-4, H-6), 6.39 (brs, 1 H, $\Delta\nu_{1/2}$ 7 Hz, H-2), 6.10 (t, 1 H, H-5), 5.89 (dd, 2 H, $J_{1,2}$ 2.4, $J_{3,2}$ 2.4 Hz, H-1, H-3); ^{13}C NMR ($CDCl_3$): δ 165.2–165.6 (benzoate carbons), 128.3–133.7 (benzoyl carbons), 70.9 (C-2), 70.2 (C-4, C-6), 69.8 (C-1, C-3), 69.2 (C-5). Anal. Calcd for $C_{48}H_{36}O_{12}$: C, 71.64; H, 4.51. Found: C, 72.06; H, 4.53.

NMR data of perbenzoates of sucrose, α- and β-D-glucopyranose, and α- and β-D-fructofuranose were previously reported,[11,12] as well as the 100 MHz ^{1}H NMR spectrum of **1b**.[16]

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