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Procyanidins from the bark of *Prunus africana*

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Abstract

From the aqueous acetone extract of the bark of *Prunus africana* (Hook.f.) Kalkman (Syn. *Pygeum africanum* Hook.f.) the flavanols catechin (1) and epicatechin (2), and the procyanidins B-1 (3), B-2 (4), B-3 (5), A-2 (6) and C-1 (7) were isolated and identified. Their structures were established as their peracetate derivatives, on the basis of chemical and spectral evidence. The ^{13}C-NMR spectrum of the higher molecular weight polymer fraction revealed a 3',4'-dihydroxylated B-ring oxidation pattern and the 2,3-*cis* relative stereochemistry of the constituent flavan-3-ol units. The mean average molecular size of the polymers was estimated to be about 8 to 9 flavan-3-ol-units.

Keywords: *Prunus africana*, Rosaceae, flavan-3-ols, procyanidins.

1. Introduction

Prunus africana (Hook.f.) Kalkman {(Syn. *Pygeum africanum* Hook.f. (Rosaceae)} is an evergreen tree, which is native to several African countries from Madagascar, Uganda, Ethiopia to sao Tome e Principe and Cameroon.

A chloroform extract of the bark exhibits good effects in the treatment of benign prostatic hyperplasia (BPH) (1). Phytochemical investigations aimed at isolating the active ingredients have been extensively undertaken (2, 3, 4, 5). Many compounds have been identified including fatty acids (C_{12}-C_{24}), sterols (β-sitosterol, β-sitosterol-3-O-glucoside, β-sitostenone and campesterol), pentacyclic triterpenoids and two linear alcohols, *n*-tetracosanol and *n*-docosanol (C_{22}) and their trans-ferulic esters. The efficacy of the extract in the treatment of benign prostate enlargement in man has been confirmed in clinical and pharmacological experiments (6, 7).

However, little is known about the structural design of the hydrophilic fraction of the title plant. This knowledge is important to prove whether phenolic compounds are involved in the treatment of BPH or not. The present investigation deals with the isolation and identification of polyphenols from the acetone-water (7:3) extract of *Prunus africana* bark.

2. *Experimental*

2.1. General.

^{1}H NMR spectra were recorded in $CDCl_3$, relative to $CHCl_3$, on a Varian Mercury 400 plus. CD spectra were measured in MeOH on a CD Spectrometer AVIV 62A DS. Acetylation was performed in Ac_2O-pyridine (1,2:1) at ambient temperature for 24 h. ESI mass spectrometer: Quattro LC-Z (Micromass). MALDI-TOF mass spectrometer: LAZARUS II (home built), N2-laser (LSI VSL337ND) 337 nm, 3 ns puls width, focus diameter 0.1 mm, 16 kV acceleration voltage, 1 m drift length, data logging with LeCroy9450A, 2.5 ns sampling time and expected mass accuracy +/- 0.1 %, sample preparation: acetylated compounds were deposited from a solution in $CHCl_3$ on a thin layer of 2,5-dihydroxybenzoic acid (DHB) crystals. Analytical TLC was done on silica gel GF_{254} plates (Merck) in solvent system EtOAc-HCO_2H-H_2O (18:1:1). Compounds were visualized as red spots by spraying with vanillin/HCl-reagent. Preparative TLC was performed on silica gel plates (Kieselgel 60 F254, 0.5 mm, Merck) using toluene-Me_2CO (7:3) for peracetate derivatives. Optical rotation ([α]) was measured using a Perkin-Elmer polarimeter 241.

2.2. Plant material.

Prunus africana *bark was collected from Nakuru, Kenya, in June 2002, air dried and ground into a fine powder. The plant identity was confirmed at the East African Herbarium (Nairobi) and a voucher specimen is deposited at the University of Petra, Amman, Jordan.*

2.3. Extraction, isolation and identification of compounds

The dried bark (500 g) was extracted successfully with acetone-water 7:3 (5 l) at room temperature. The water fraction was concentrated. Extraction of the residue with ethyl acetate (10 x 1000 ml) gave a 11.3 g brown solid fraction (ethyl acetate fraction) and 64 g red-brown solid fraction (remaining water fraction). The ethyl acetate fraction was subjected to CC on Sephadex LH-20 (3.8 x 50 cm) with EtOH 96% (3 l), MeOH (8 l) and acetone-water 7:3 to give 7 fractions (frs.). Frs. 3 (3700-4300 ml, 700 mg) was purified by MCI gel CHP-20P (2,5 x 25 cm, 75-100 µm; Mitsubishi Kasei corporation, Tokyo) chromatography using 20-80% MeOH (2 l) linear gradient to give compound 1 (subfrs. 66-79 , 18 mg) and compound 2 (subfrs. 33-46, 187 mg). Frs. 4 (4300-6200 ml, 610 mg) was purified by MCI system as above to give compound 4 (subfrs.90-95, 157 mg) and a mixture of compound 3 and compound 5 (subfrs. 101-111, 130 mg).

Final purification was achieved on preparative TLC after peracetylation of the mixture [*Rf* 0.56 (B-1-peracetate, 53 mg) and 0.50 (B-3-peracetate, 17 mg)]. Compound 6 (89 mg) was obtained from the Sephadex fraction 6 (6500-7100 ml; 734 mg) and MCI gel chromatography (subfrs. 56-66) as described above.

Epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (7): Fraction 5 (6200-6500 ml, 1.7 g) was separated on MCI-gel with the same gradient as above to afford compound 7 (procyanidin C-1; subfrs. 85-103, 67 mg). 20 mg were peracylated to give 7a: MALDI-TOF-MS: *m/z*: 1516 ([M + Na]$^+$). ^{13}C-NMR- ($CDCl_3$, 100 MHz, assignments waere based on HSQC and HMBC spectra): δ 27.6 [C-4 (I)], δ 31.7 [C-4 (F)], δ 34.1[C-4 (C)], δ 65.3 [C-3 (I)], 66.7 [C-3 (C)], δ 68.1 [C-3 (F)], δ 73.5 [C-2 (I)], δ 74.1 [C-2 (C)], δ 76.8 [C-2 (F)], δ 106.3 [C-8 (A)], δ 106.5 [C-6 (G)], δ 107.7 [C-6 (A)], δ 107.9 [C-4a (G)], δ 108.6 [C-6 (D)], δ 114.6 [C-8 (G)], δ 115.1 [C-4a (A)], δ 115.3 [C-4a (D)], δ 116.7 [C-8 (D)], δ 119-125 [C-2' (B), C-5' (B), C-6' (B), C-2' (E), C-5' (E), C-6' (E), C-2' (H), C-5' (H), C-6' (H)], δ 150.8 [C-8a (G)], δ 152.7 [C-8a (D)], δ 154.5 [C-8a (A)].

2.4. Isolation of the polymeric extract

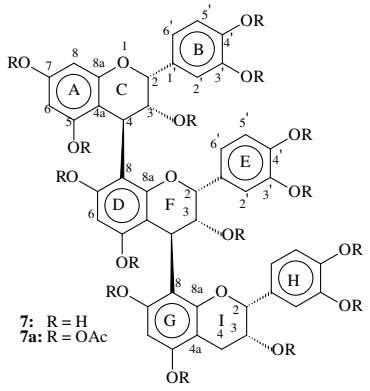
The remaining water fraction was subjected to column chromatography on Sephadex LH-20 (3.8 x 50 cm) with MeOH-H2O 1:1 (15 l) until the eluent material became colorless. The polymeric procyanidin extract was then eluted with acetone-water 7:3 (6 l) and freeze-dried (15 g).

2.5. Degradation with phloroglucinol

The polymeric extract of *Prunus africana* obtained as described above (5 g) was treated for 30 min. at room temperature with phloroglucinol (3 g) in 1% HCl in EtOH (50 ml) under continuous shaking (17, 18). The solution was then evaporated to dryness (7.9 g). A portion (7 g) was fractionated on Sephadex LH-20 (5.5x 68 cm) using EtOH (5 l), EtOH-MeOH 1:1 (5l) and acetone-water 7:3 (3 l) to give 5 fractions. Frs.2 (3400-4700 ml, 530 mg) was subjected to chromatography on MCI-gel CHP 20 P (25 x 250 mm) with a 10-80 % MeOH linear gradient (17 ml/frs.) to afford compound 1 (subfrs.68-83, 9 mg) and compound 2 (subfrs. 37-47, 80 mg). Frs. 3 (4700-6000 ml, 532 mg) was separated on MCI-gel with the same gradient as above to afford (subfrs. 76-89, 40 mg) compound 4. Compound 8 was achieved from Frs. 4 (6000-6200 ml, 1.2 g) followed by MCI-gel chromatography as described above (subfrs. 23-31, 872 mg).

3. Results and Discussion

Catechin (1), epicatechin (2), epicatechin-(4β→8)-catechin (procyanidin B-1, 3), epicatechin-(4β→8)-epicatechin (procyanidin B-2, 4), catechin-(4α→8)-catechin (procyanidin B-3, 5), epicatechin-(4β→8, 2β→O→7)-epicatechin (procyanidin A-2, 6) and epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (procyanidin C-1, 7) were isolated from the ethyl acetate fraction obtained from the aqueous acetone extract of *Prunus africana* bark. All compounds were isolated using CC on Sephadex LH-20 followed by MCI-gel. The identity of 1-6 was established by comparison of their optical rotation ([α]) and spectroscopic data [ESI- or MALDI-TOF-MS, ^{1}H-NMR] of the corresponding derivatives obtained after peracetylation with published values (8, 9, 10, 11, 12). Compound 7 was identified as epicatechin-(4β→8)-epicatechin-(4β→8)-epicatechin (procyanidin C-1). The ^{1}H-NMR data of its peracetate (7a) agreed with previously published data (12). The ^{13}C-NMR data of its peracetate are here reported for the first time.



The polymer fraction (obtained s. Exp) showed an optical rotation of $[\alpha]^{20}_{578}$ +81° (*c* 0.1; MeOH) which corresponds to a molar proportion of subunits with 2,3-*cis* stereochemistry of 84% (13). By integration of the signals close to δ 77 ppm and δ 84 ppm by the ^{13}C NMR spectroscopy (solvent: MeOH-d_4, 99 MHz) of the purified polymer fraction, a ca. 4:1 ratio was obtained for *cis* : *trans* isomers (14). The Integration of the signals at δ 114-118 revealed that the flavan monomers not only exhibited a 3',4'-dihydroxylated B-ring oxidation pattern but also showed 2,3-*cis* relative stereochemistry (15). The mean average molecular size of the polymers was estimated to be 8-9 flavan-3-ol units by integration of the corresponding signal of the C-3 signals of the extender units at 73 ppm and the corresponding signal of the lower flavan-3-ols at 68 ppm (16). To elucidate the structure in more details, the polymer was subjected to mild acid catalysed scission, and the generated extender flavan carbocations were captured with phloroglucinol (17, 18). The reaction resulted in the cleavage of the terminal units catechin (1) and epicatecin (2) in the ratio of ca. 1:9, respectively. A captured product was epicatechin-(4β→2)-phloroglucinol (8). The resulting products were identified by ^{1}H-NMR and MALD-TOF-MS of their peracetylated derivatives compared to the literature

data (8, 9, 10, 11, 12, 19). In conclusion, besides 5 procyanidins and 2 flavan-3-ols as starter units, a polymeric procyanidin mixture was isolated and characterized. The polymer showed exclusively the 3',4'-dihydroxylation and the dominance of 2,3-*cis*-configurated constituent flavan-3-ol units as well. The presence of epicatechin, and to a lesser extent catechin, in *Prunus africana* bark was consistent with this observation, where they were found to be disttributed in generally similar pattern as terminal units in the polymers.

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