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ANTIVIRAL ELLAGITANNINS FROM *SPONDIAS MOMBIN**

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Key Word Index—*Spondias mombin*; Anacardiaceae; didehydroellagitannins; geraniin; galloylgeraniin; antiviral activity.

Abstract—Two ellagitannins with antiviral properties were isolated from the leaves and stems of *Spondias mombin* by means of a bioguided assay. Geraniin, the main component, and galloylgeraniin, a new didehydroellagitannin, showed pronounced antiviral activity against *Coxsackie* and *Herpes simplex* viruses.

INTRODUCTION

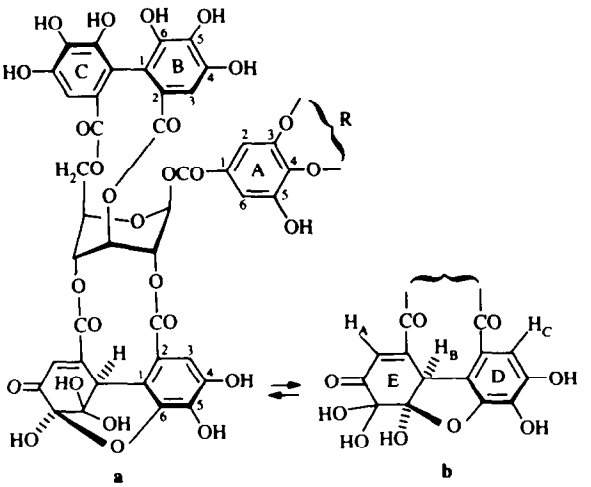
The results of a screening programme for antiviral properties of higher plants showed that an 80% EtOH extract from the leaves and stems of *Spondias mombin* (Anacardiaceae) exhibited pronounced antiviral activity against *Coxsackie* B_2 and *Herpes simplex* 1 viruses.

Spondias mombin is a deciduous tree *ca* 18 m in height and 1.5 m in girth, which originates from tropical Central America, but is now widespread in south east Asia as well as in east and west Africa. This tree is sometimes cultivated for its edible plum-like fruits and its leaves have a folk reputation for use as an oxytocic, an antidiarrheal and an antimicrobial agent, for the treatment of wounds and as an astringent [1, 2]. The ellagitannins geraniin (**1**) and galloylgeraniin (**2**) were isolated from the 80% ethanol extract of the leaves and stems in the present work.

RESULTS AND DISCUSSION

By a combination of different chromatographic techniques (droplet counter current chromatography (DCCC), column chromatography on silanized silica gel, Avicel cellulose and Sephadex LH-20) compounds **1** and **2** were isolated from the *n*-butanol–ethyl acetate (1:1) fraction.

Geraniin was identified by complete spectral analysis (UV, IR, ^{1}H NMR, ^{13}C NMR, mass) which coincided with the spectral data in the literature [3, 4]. The condensation of geraniin with *O*-phenylenediamine yielded phenazine A and B, which gave corilagin and phenazine C upon hydrolysis. The NMR and mass spectral data of these products were also in agreement with those of the literature [4, 5]. An interesting fact is that the presence of a didehydrohexahydroxydiphenoyl (DHHDP) group can easily be recognized in the negative FAB mass spectrum when adding methanol. The $[M-H]^-$ ion is then accompanied by an $[M-OH+OMe-H]^-$ ion due to the formation of acetals in alcoholic solutions.



a **b**

1 R=H,H
2 R=H, Galloyl

Galloylgeraniin, a yellowish powder, showed an $[M-H]^-$ ion peak at *m/z* 1103 and an [M-galloyl-H]$^-$ ion peak at *m/z* 951 in the negative FAB mass spectrum. This last peak was attributable to geraniin. Therefore this compound had to be geraniin containing an extra, depsidically linked, galloyl group. The location of the extra galloyl group was determined by inspection of the ^{1}H and ^{13}C NMR spectra, which showed signals arising from an equilibrium mixture of *m*- and *p*-digallates [6]. From these findings, the new ellagitannin was assigned as 1-*O*-digalloyl-2,4-*O*-(*R*)-didehydrohexahydroxydiphenoyl-3,6-*O*-(*R*)-hexahydroxydiphenoyl-β-D-glucopyranose ($^1C_4$).

The isolated compounds were tested in our chemotherapeutic battery [7–9]. Geraniin and galloylgeraniin possessed antiviral activity against *Coxsackie* B_2 and *Herpes simplex* type 1 at a concentration of 50 μg ml^{-1} (reduction factor of the viral titre = 10^3). These results confirm the antiherpes activity found for geraniin and related tannins by means of the assay of plaque formation by Fukuchi *et al.* [10]. For both compounds we found a

*Part 8 in the series 'Plant Antiviral Agents'. For Part 7, see ref. [11].

weak antibacterial activity against *Enterobacter aeruginosa* and *Proteus vulgaris* at 500 $\mu g\,ml^{-1}$. Both compounds were inactive against all other bacteria and fungi tested at concentrations up to 500 $\mu g\,ml^{-1}$.

EXPERIMENTAL

Plant material. Leaves and stems of *S. mombin* L. were collected in the National Botanical Garden of Belgium in Meise (Belgium).

Extraction and isolation of compounds **1** *and* **2**. Leaves and stems were cut into small pieces and macerated and percolated with 80% EtOH. After rotary evapn of EtOH, the H_2O residue was successively extracted with CCl_4, Et_2O and *n*-BuOH–MeCOEt (1:1). This last fr. was chromatographed × 3 over a Sephadex LH-20 column (120 × 2.5 cm) with a gradient from H_2O to MeOH and further with MeOH–Me_2CO (1:1) to give 9 frs. Fr. 8 was further purified by CC on silanized silica gel 60 (0.063–0.200 nm, Merck) (35 × 4 cm) eluted with MeOH–H_2O (1:9–10:0), by DCCC with *n*-BuOH–Me_2CO–H_2O (7:2:11) in the ascending mode and finally by CC on Sephadex LH-20 (80 × 2 cm) with MeOH to afford **1** (1.7 g).

Fr. 9 was first chromatographed over Sephadex LH-20 eluted with MeOH–H_2O (9:1–10:0) and then with MeOH–Me_2CO (1:1) to give 14 frs. Frs 9–11 was further purified by DCCC with *n*-BuOH–PrOH–H_2O (2:1:3) in the descending mode, by CC on Avicel micro-crystalline cellulose (20 × 2 cm) eluted with H_2O–HOAc–Me_2CO (18:1:1–6:1:3) and finally by CC on Sephadex LH-20 with MeOH to yield **2** (22 mg).

Chromatography. Reversed-phase HPLC was performed on a Merck Lichrosorb RP-18 column (7μ, 250 × 4 mm) with MeCN–MeOH (1:1, A) and H_2O–HCO_2H (19:1, B) using the following elution profile: 0–4 min 90% B, 4–16 min linear to 70% B, 16–24 min linear to 30% B. Flow rate: 2 ml min^{-1}. Detection: UV-absorption at 280 nm. R_t (**1**): 10.0 min, R_t (**2**): 12.1 min.

TLC was carried out using (a) silica gel F_{254} (Merck) with EtOAc–HOAc–HCO_2H–H_2O (100:11:11:27), (b) cellulose (Merck) with 15% HOAc and (c) RP-8 $F_{254}S$ (Merck) with H_2O–MeCN–HOAc (19:1:1, 6 cm and 17:0:3, 12 cm).

Ellagitannins were detected by their absorption or violet fluorescence under UV light and by a spray of satd aq KIO_4 or HNO_2. R_f values for **1**: 0.35 (a), 0.45 (b) and 0.70 (c); R_f values for **2**: 0.28 (a), 0.50 (b) and 0.55 (c).

Galloylgeraniin (**2**). $C_{48}H_{32}O_{31} \cdot xH_2O$. UV λ_{max} (nm): 259. FABMS (NOBA as matrix): *m/z* 1103 $[M-H]^-$, 951 $[M-galloyl\text{-}H]^-$, in MeOH 1117 $[M-OH+OMe-H]^-$. ^{1}H NMR (200 MHz, Me_2CO-d_6 + D_2O, δ): 6.46 (1H, *s*, H_A), 6.55 (1H, *d*, J = 2 Hz, Glc H-1), 6.82 (1/2 H, *S*, HHDP), 6.85 (1/2 H, *s*, HHDP), 7.13 and 7.15 [4.5 H, *s*, HHDP, H_C, terminal galloyl (2H), *p*-depside galloyl(1/2H)], 7.30 and 7.35 (each 3/4 H, *s*, *m*-depside galloyl). ^{13}C NMR (50 MHz, Me_2CO-d_6 + D_2O, δ): Glc (a/b form): 91.1/91.6 (C-1), 68.8/69.4 (C-2), 62.4/61.4 (C-3), 64.9/65.9 (C-4), 71.8/72.4 (C-5), 63.0/62.8 (C-6); HHDP: 114.7, 116.1 (1b, 1c), 124.5 (2b, 2c), 106.9, 109.4 (3b, 3c), 143.8, 144.4, 144.8 (4b, 4c, 6b, 6c), 135.3, 136.4, 136.6 (5b,5c); DHHDP: 114.2 (1d), 117.7 (2d), 112.7 (3d), 144.8 (4d), 138.2 (5d), 142.0/145.8 (6d), 44.7/50.8 (1e), 153.1/147.8 (2e), 128/124.5 (3e), 191.4/194.3 (4e), 95/91.1 (5e), 91.6/107.6 (6e); terminal galloyl: 110, 118.7, 138.9, 145.8; *p*-depside galloyl: 110, 126.3, 132.5, 150.1; *m*-depside galloyl: 116.1, 117.2, 119.0, 138.9, 144.0, 146.5.

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