

ISOLATION OF METHYL GALLATE AS THE ANTITUMOR PRINCIPLE  
OF *ACER SACCHARINUM*ARTHUR E. BAILEY,<sup>1</sup> R. OWEN ASPLUND\**Department of Chemistry, University of Wyoming, Laramie, Wyoming 82071*

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A report by Kupchan *et al.* (1) of antitumor activity in the leaves of *Acer negundo* L. prompted the testing of *Acer saccharinum* L. (Aceraceae) for antitumor activity. An EtOH/H<sub>2</sub>O extract of the leaves of this plant inhibited B16 melanoma in mice, and isolation of the active material was carried out. The separation yielded methyl gallate (methyl-3,4,5-trihydroxy benzoate) as the active compound. Methyl gallate was tested against B16 melanoma in mice following standard protocols (2) yielding T/C values of 188% and 172% at 25 mg/kg, and testing against tumor cells in culture, again following standard protocols (2), gave the following results: B16 melanoma ID<sub>50</sub>  $4.0 \times 10^{-5}$  M, L1210 ID<sub>50</sub>  $2.2 \times 10^{-5}$  M, and P388 ID<sub>50</sub>  $2.8 \times 10^{-5}$  M. Finally, the compound was tested for inhibition of reverse transcriptase using the assay detailed by Kacian and Spiegelman (4) and was found to be inhibitory with an I<sub>50</sub> of  $4.80 \times 10^{-3}$  M.

Methyl gallate occurs widely in the Aceraceae as well as other plant families. It has previously been reported to inhibit L1210 leukemia *in vivo* (3). Investigators should be aware that antitumor activity detected in random screening of plants may be due to this compound. The inhibition of reverse transcriptase by this compound is an observation that has not previously been reported. This is also the first report of its cytotoxicity.

## EXPERIMENTAL

**ISOLATION.**—Green leaves of *A. saccharinum* were collected in the area around Lakewood, Colorado. A voucher specimen is on file at the American Medical Center, Lakewood, Colorado (identified by date, 8/23/79, and collector, G. C. Sharma). Leaves (40 kg) were ground, extracted with MeOH-H<sub>2</sub>O (1:1), and filtered. The filtrate was dried, taken up in H<sub>2</sub>O, and extracted with CHCl<sub>3</sub> and BuOH. The BuOH phase was dried, taken up in H<sub>2</sub>O, adjusted to pH 8 with NH<sub>4</sub>OH, and extracted again with BuOH. The BuOH phase was dried and separated by two preparative tlc steps in succession (developed in EtOAc-MeCOEt-HOAc, 5:3:1 and toluene-EtOAc-HOAc, 5:4:1 both on Si gel GF). In each case the most rapidly moving brown band was isolated. The material thus obtained was crystallized three times from H<sub>2</sub>O and dried. The isolate was identified as methyl gallate by comparison of its physical (mp, co-tlc, combustion analysis) and spectral properties (ir, <sup>1</sup>H nmr, <sup>13</sup>C nmr) with those of an authentic sample.

**ASSAYS.**—The *in vivo* testing was performed on 6 mice per group using 5-fluorouracil as a positive control (T/C 145 and 141%).

The ID<sub>50</sub> values for the cytotoxicity determination in L1210, B16, and P-388, represent the concentrations of the compound required to reduce cell growth to one-half that of the control. The compound was tested on normal human fibroid cells at  $10^{-3}$  M and was found to have no effect.

Reverse transcriptase activity was assayed in a 100  $\mu$ l total volume solution containing 50 mM Tris buffer (pH 8.1), 9 mM MgCl<sub>2</sub>, 40 mM KCl, 2 mM DTT, and 0.01% BSA. Enzyme (0.9 units per assay, Seikagaku America, Inc.), primer template [poly (rA)<sup>P(dT)</sup> 12-18, PL Biochemicals, 0.01 A<sub>260</sub>/assay], and inhibitor (as needed) were added to the assay tube, and it was incubated for 30 min at 37°. At that time 20 nmol (Methyl <sup>3</sup>H) TTP (125 cpm-pmol) was added, and the reaction was incubated for an additional 30 min at 37°. Contents of the tube were placed on a DEAE cellulose pad. The pad was washed thoroughly with H<sub>2</sub>O and 0.5 M Na<sub>2</sub>HPO<sub>4</sub>, dried, and counted by liquid scintillation. The number of counts is proportional to the enzyme activity and the strength of the inhibitor is related to the amount of reduction as compared to the control. The inhibitor effect is expressed as I<sub>50</sub> which is the concentration of the inhibitor that will reduce control activity by 50 per cent.

Full details of experimental procedures are available upon request from the senior author.

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CONSTITUENTS OF *GUETTARDA PLATYPODA*

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Chemical examination of the roots of *Guettarda platypoda* DC. (Rubiaceae), a plant used in traditional medicine as a febrifuge, gave quinovic acid, 3-(*O*-fucosyl)-quinovic acid, morroniside, sweroside, and 5  $\alpha$ -carboxystrictosidine. From the same plant Bhattacharyya *et al.* (1) isolated quinovic acid, rotundic acid,  $\beta$ -sitosterol, and a saponin.

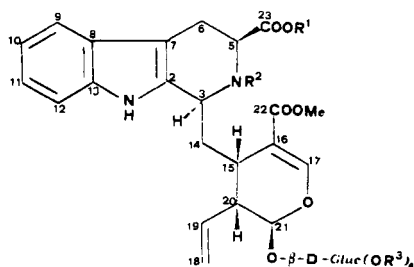
## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—<sup>1</sup>H-nmr spectra were recorded on a Varian EM 360 and <sup>13</sup>C-nmr spectra on a Bruker AM 400 spectrometers (TMS as internal reference); ms on an AEI MS 902 instrument, 70 eV; tlc and cc: Kieselgel 60 (Merck).

PLANT MATERIAL.—Roots of *G. platypoda* were collected near Itamaraca (Recife, Brazil) in March 1982. A voucher specimen of the plant (no. 5341), identified by Alda Chiappeta, is deposited at the Herbarium of the Instituto dos Antibioticos, Universidade Federal de Pernambuco, Recife, Brazil.

ISOLATION AND IDENTIFICATION.—The roots of *G. platypoda* (0.5 kg) were extracted three times with MeOH at room temperature (25 g). By means of cc of a fraction of the residue (14 g) eluting with CHCl<sub>3</sub>-MeOH (95:5), we obtained quinovic acid (150 mg) and 3-(*O*-fucosyl)-quinovic acid (350 mg) which were identified by direct comparison (<sup>1</sup>H nmr, ms, mp) with authentic samples and literature data (2). Elution with CHCl<sub>3</sub>-MeOH (90:10) yielded a mixture of two iridoids, which were re-chromatographed using H<sub>2</sub>O-saturated *n*-BuOH to give morroniside (190 mg) and sweroside (130 mg). The <sup>13</sup>C- and <sup>1</sup>H-nmr spectra of morroniside and sweroside were identical with those previously reported (3,4).

Eluting with CHCl<sub>3</sub>-MeOH (80:20) a basic fraction was obtained (300 mg), which was further purified using reversed-phase chromatography (Lichroprep RP-8, H<sub>2</sub>O-MeOH, 1:1) to give compound **1** [mp 225°, with dec; [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -261 (c 1.1, MeOH)]. <sup>1</sup>H- and <sup>13</sup>C-nmr data, in comparison with those reported for analogous compounds (5), suggested for **1** the structure of a tetrahydrodesoxycordifoline.



	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>
<b>1</b>	H	H	H
<b>2</b>	Me	H	H
<b>3</b>	Me	Me	H
<b>4</b>	Me	Ac	Ac