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# PENTAGALLOYLGLUCOSE, A XANTHINE OXIDASE INHIBITOR FROM A PARAGUAYAN CRUDE DRUG, "MOLLE-I" (*SCHINUS TEREBINTHIFOLIUS*)

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As a part of our continuing search for biologically active substances from medicinal plants in Paraguay, we have examined inhibitory activities of 70% EtOH extracts of about 60 native crude drugs against xanthine oxidase (XO, EC 1.2.3.2). We report here that the extract of the aerial parts of Paraguayan "Molle-i" (*Schinus terebinthifolius* Raddi, Anacardiaceae) showed significant inhibitory activity (79.8% at 50 μg/ml) against cow's milk xanthine oxidase. Flavonoids (1–5), hydroxychalcones (1), coumarins (1), 2,8-dihydroxyadenine (6), and xanthones (7) have previously been reported as naturally occurring inhibitors of XO. An inhibitor of XO could potentially be useful as a therapeutic agent for hyperuricemia that causes gout (8), renal stones (8,9), or ischemic myocardium (10).

Bioassay-directed fractionation of the extract resulted in the isolation of an active substance as a colorless, amorphous powder. The compound was positive in the $FeCl_3$ reaction and showed absorption bands at 280 nm and 3400, 1720, and 1605 $cm^{-1}$ in the uv and ir spectra, respectively. The $^1$H-nmr spectrum indicated the presence of oxymethylene protons (δ 4.42, dd, $J$ = 4.4, 11.8 Hz; 4.54, d, $J$ = 11.8 Hz), of five oxygenated methine protons (δ 4.54, m; 5.61, dd, $J$ = 9.8, 8.3 Hz; 5.66, t, $J$ = 9.8 Hz; 6.00, t, $J$ = 9.8 Hz; 6.33, d, $J$ = 8.3 Hz), and of ten aromatic protons (δ 6.97, 7.01, 7.05, 7.11, 7.18, each 2H, s), which were characteristic of pentagalloylglucose (11). The compound was identified as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose by direct comparison (tlc, ir, $^1$H nmr, [α]D) with an authentic sample.

The inhibitory activity against XO of pentagalloylglucose was then compared with that of allopurinol, a well known XO inhibitor (12). As shown in Table 1, pentagalloylglucose exhibited the same level of activity as allopurinol. Kinetic analysis of the reaction of XO with pentagalloylglucose by Lineweaver-Burk plots revealed that it inhibited XO noncompetitively at the concentration of $2.12 \times 10^{-6}$ M. Inasmuch as pentagalloylglucose was reported to inhibit sialidase, hyaluronidase, alkaline phosphatase, and cholesterol oxidase (13), the inhibitory activity might be due to its tannic character. On the other hand, pentagalloylglucose was found to exert an antiherpetic (antiviral) effect (14). This is the first reported isolation of 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose from *S. terebinthifolius*.

TABLE 1. Inhibitory Activity of Pentagalloylglucose and Allopurinol Against Cow's Milk Xanthine Oxidase.

| Compound | $IC_{50}$ (M) |
|---|---|
| Pentagalloylglucose | $3.2 \times 10^{-6}$ |
| Allopurinol | $1.6 \times 10^{-6}$ |

## EXPERIMENTAL

PLANT MATERIAL.—Aerial parts of *S. terebinthifolius* were collected at Itapua, Paraguay in Oc-

tober 1985. The voucher specimens are deposited in the Herbal Garden of the Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University and Seccion Botanica, Facultad de Ciencias Químicas, Universidad Nacional de Asunción.

EXTRACTION AND ISOLATION OF PENTAGALLOYLGLUCOSE.—The aerial parts of air-dried *S. terebinthifolius* (100 g) were ground to a fine powder and extracted with 70% EtOH at boiling temperature (1h, 3 times) to give a brownish powder (36 g). A portion of this extract (8.4 g) was partitioned between $H_2O$ and *n*-hexane, and the aqueous layer was successively extracted with $CHCl_3$ and *n*-BuOH. The *n*-BuOH layer, the most active fraction (84.0% at 50 μg/ml), was chromatographed on a column of Sephadex LH-20. Elution of the column with MeOH afforded a mixture of pentagalloylglucose and an unknown compound which were separated by preparative layer chromatography [Si gel, $C_6H_6$-$HCO_2H$-ethyl formate (2:2:7)]. A colorless, amorphous powder (7 mg) was obtained and identified as 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose by direct comparison with an authentic sample.

ASSAY OF XANTHINE OXIDASE ACTIVITY.—Cow's milk xanthine oxidase was purchased from Boehringer Mannheim. Xanthine was obtained from ICN Pharmaceutical. All other chemicals were of analytical grade. Xanthine oxidase activity was measured by the method reported by Noro *et al.* (2). The inhibitory activity (%) was calculated by the formula $(1 - B)/A \times 100$, where A is the activity of the enzyme without test material and B is the activity of the enzyme with test material.

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