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CHEMICAL AND PHARMACEUTICAL STUDIES ON MEDICINAL PLANTS IN PARAGUAY.[1] GERANIIN, AN ANGIOTENSIN-CONVERTING ENZYME INHIBITOR FROM "PARAPARAI MI," *PHYLLANTHUS NIRURI*

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"Paraparai mi," *Phyllanthus niruri* L. (Euphorbiaceae), has traditionally been used for the treatment of urolitic disease and as a diuretic in Paraguay. In the screening tests for biological activity of about 60 Paraguayan medicinal plants, however, the 70% EtOH extract of "paraparai mi" was found to have inhibitory activity against angiotensin-converting enzyme (ACE). In continuation of our studies, the *n*-BuOH extract of the medicinal plant was found to have higher activity than the EtOH extract. ACE is a dipeptidyl carboxypeptidase that plays an important role in blood pressure regulation by catalyzing an important reaction. Several inhibitors of ACE from plant sources have been reported. Previously, Inokuchi and coworkers investigated ACE inhibitors extracted from Chinese crude drugs (2,3) and reported the inhibitors from the seeds of *Areca catechu* (4). We also reported inhibitory activity of crude extracts from natural products on ACE (5). Recently, Uchida *et al.* isolated some condensed tannins from "rhei rhizoma" as ACE inhibitors (6), and Kameda *et al.* reported (+)-catechin as the inhibitor from *Quercus stenophylla* (7). *P. niruri* has already afforded alkaloids (8), lignans (9–12), flavonoids (13–16), lup-20(29)-en-3β-ol (17), phthalic acid ester (18), fatty acid (19), and vitamin C (20). In pharmacological studies of the plant, diuretic action (21) and inhibition of induced liver injury (22) have been reported.

In this paper we report the substances that inhibit ACE activity from this medicinal plant. The *n*-BuOH extract, which was the active extract, was applied to a column of Sephadex LH-20, which was eluted with H_2O followed by 50% MeOH, MeOH, and Me_2CO. Based on the results of the activities of the extracts and eluates, it seemed that the active component in the EtOH extract was extracted with *n*-BuOH and then concentrated in the 50% MeOH eluate (Table 1). The 50% MeOH eluate was further chromatographed over a Sephadex LH-20 column and was eluted with 70% MeOH to afford three compounds. They were identified as ellagic acid, geraniin, and gallic acid by direct comparison with authentic samples. The isolated compounds and corilagin, a hydrolysis product from geraniin, were tested for inhibitory activity against ACE together with captopril. The 50% inhibitory concentration (IC_{50}) values of the test compounds are shown in Table 1. It was found that the IC_{50} for ACE activity of geraniin, the most active of the isolated compounds, was 4.0×10^{-4} mol/liter. In order to determine the type

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TABLE 1. Inhibitory Activity of Extracts, Elutions, and Isolated Compounds from *Phyllanthus niruri* on Angiotensin-Converting Enzyme (ACE).

Extracts	Inhibition % (100 μg/ml)	Eluates	Inhibition % (100 μg/ml)	Compounds	IC_{50} (mol/liter)
70% EtOH	34	H_2O	22	ellagic acid	5.0×10^{-3}
n-hexane	14	50% MeOH	60	geraniin	4.0×10^{-4}
precipitate	40	MeOH	40	gallic acid	7.7×10^{-3}
$CHCl_3$	27	Me_2CO	17	corilagin	3.7×10^{-3}
n-BuOH	50			captopril	5.0×10^{-9}
H_2O	30				

of inhibition, the kinetics of inhibition of ACE by geraniin were plotted according to Lineweaver and Burk. Geraniin produced a noncompetitive inhibition pattern at the concentration of IC_{50}. Ondetti *et al.* found that ACE was a zinc-containing metalloprotein (23). On the other hand, Okuda *et al.* reported that geraniin interacted with the heavy metal ions (24). The present study found that geraniin was a noncompetitive inhibitor for the enzyme. This result suggested that geraniin might interact with the zinc atom in ACE.

EXPERIMENTAL

MATERIALS.—Plant material, "paraparai mi," was purchased from local dealers in Asuncion, Paraguay. It was identified as *P. niruri*, and its voucher specimens are on deposit at both the institute of Toyama Medical and Pharmaceutical University and Asuncion University. Rabbit lung ACE was purchased from Sigma Chemical Co. and hippuryl-L-histidyl-L-leucine was from the Protein Research Foundation. All other chemicals were of analytical grade.

ACE ASSAY.—Test substances were dissolved in DMSO, and the fluorometric assay was carried out by the method of Friedland and Silverstein (25) as described previously (5). The inhibitory activities are shown in Table 1.

EXTRACTION, SEPARATION, AND ISOLATION.—Dried powder of the material (185 g) was extracted with hot 70% EtOH (1 h × 3). The EtOH solution was concentrated in vacuo to give a 70% EtOH extract (40 g). The EtOH extract was partitioned between H_2O and *n*-hexane to afford *n*-hexane and aqueous layer and precipitate. The aqueous layer was successively extracted with $CHCl_3$ and *n*-BuOH to give $CHCl_3$ extract (1.5 g), *n*-BuOH extract (11 g), and H_2O extract (17 g), respectively. The *n*-BuOH extract was chromatographed on a Sephadex LH-20 column by stepwise elution with H_2O, 50% MeOH, MeOH, and Me_2CO. The 50% MeOH eluate was further chromatographed on a Sephadex LH-20 eluting with 70% MeOH to afford ellagic acid (20 mg), geraniin (430 mg), and gallic acid (5 mg). Ellagic acid and gallic acid are considered to be artifacts from geraniin.

IDENTIFICATION.—The isolated compounds were identified by direct comparison (uv, ir, ^1H and ^{13}C nmr, $[\alpha]D$) with the respective authentic samples.

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LITERATURE CITED

1. M. Arisawa, T. Hayashi, K. Ohmura, K. Nagayama, M. Shimizu, N. Morita, and L.H. Berganza, *J. Nat. Prod.*, **50**, 1164 (1987).
2. J. Inokuchi, H. Okabe, T. Yamauchi, and A. Nagamatsu, *Chem. Pharm. Bull.*, **32**, 3615 (1984).
3. J. Inokuchi, H. Okabe, T. Yamauchi, A. Nagamatsu, G. Nonaka, and I Nishioka, *Chem. Pharm. Bull.*, **33**, 264 (1985).
4. J. Inokuchi, H. Okabe, T. Yamauchi, A. Nagamatsu, G. Nonaka, and I. Nishioka, *Life Sci.*, **38**, 1375 (1986).
5. M. Arisawa, M. Nimura, A. Ikeda, H. Ueno, T. Hayashi, and N. Morita, *Shoyakugaku Zasshi*, **39**, 246 (1985).
6. S. Uchida, N. Ikari, H. Ohta, M. Niwa, G. Nonaka, I. Nishioka, and M. Ozaki, *Jpn. J. Pharmacol.*, **43**, 242 (1987).
7. K. Kameda, T. Takaku, H. Okuda, Y. Kimura, T. Okuda, and S. Arichi, *Wakan Iyaku Gakkaishi*, **4**, 43 (1987).
8. N.B. Mulchandani, and S.A. Hassarajani, *Planta Med.*, **50**, 104 (1984).
9. G.V. Krishnamurti and T.R. Seshadri, *Proc. Indian Acad. Sci.*, **24**, 357 (1946).
10. L.R. Row, C. Srinivasulu, M. Smith, and G.S.R.S. Rao, *Tetrahedron Lett.*, **17**, 1557 (1964).

11. L.R. Row, C. Srinivasulu, M. Smith, and G.S.R.S. Rao, *Tetrahedron*, **22**, 2899 (1966).
12. R.S. Ward, P. Satyanarayana, L.R. Row, and B.V.G. Rao, *Tetrahedron Lett.*, **32**, 3043 (1979).
13. J.S. Chauhan, M. Sultan, and S.K. Srivastava, *Planta Med.*, **32**, 217 (1977).
14. T. Nara, J. Gleye, C.E. Lavergnede, and E. Stanilas, *Plant. Med. Phytother.*, **11**, 82 (1977).
15. D.R. Gupta and B. Ahmed, *Shoyakugaku Zasshi*, **38**, 213 (1984).
16. D.R. Gupta and B. Ahmed, *J. Nat. Prod.*, **47**, 958 (1984).
17. J.S. Chauhan, M. Sultan, and S.K. Srivastava, *J. Indian Chem. Soc.*, **56**, 326 (1979).
18. S.B. Singh, P.K. Agrawal, and R.S. Thakur, *Indian J. Chem. Sect. B.*, **25**, 600 (1986).
19. M.U. Ahmad, S.K. Husain, and S.M. Osman, *J. Am. Oil Chem. Soc.*, **58**, 673 (1981).
20. S.K. Sinha and J.V.V. Dogra, *Natl. Acad. Sci. Lett. (India)*, **4**, 467 (1981).
21. L.A. van der Woerd, *Geneeskund. Tijdschr. Ned.-Indie*, **81**, 1963 (1941).
22. K.V. Syamasundar, S.B. Singh, R.S. Thakur, A. Hussain, Y. Kiso, and H. Hikino, *J. Ethnopharmacol.*, **14**, 41 (1985).
23. M.A. Ondetti, B. Rubin, and D.W. Cushman, *Science*, **196**, 441 (1977).
24. T. Okuda, K. Mori, M. Shiota, and K. Ida, *Yakugaku Zasshi*, **102**, 735 (1982).
25. J. Friedland and E. Silverstein, *Am. J. Clin. Pathol.*, **66**, 416 (1976).

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