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IDENTIFICATION OF ASPARAGUSIC ACID AS A NEMATICIDE OCCURRING NATURALLY IN THE ROOTS OF ASPARAGUS

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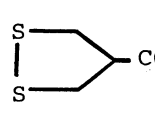
A nematicidal constituent, present at least at 35 ppm, was isolated from the roots of asparagus and identified as asparagusic acid, which has also been reported to exist in the edible part of the plant. The acid was toxic to several plant parasitic nematodes and would be a major factor in resistance of asparagus.

Several species of the genus Asparagus have been demonstrated to have chemicals in their roots which are antagonistic to plant parasitic nematodes.[1] For example, Rohde and Jenkins reported that the root diffusates of A. officinalis var. altilis L. (asparagus) were resistant to Trichodorus christiei and contained highly toxic substances, which were assumed to be glycosides with aglycone(s) of low molecular weight.[2] In connection with other studies,[3] we have searched the compounds in question in the roots of asparagus by the bioassay using Paratylenchus curvitatus and succeeded in the identification of one of the nematicides as asparagusic acid[4] (I). The present paper describes the identification and biological activity as a nematicide of the acid, which would probably be the major factor in resistance.

Initial extraction and purification of active substances were carried out by a modification of the Rohde procedure.[2] Fibrous and storage roots (30 kg) of 10-year-old asparagus were squeezed in water in a meat grinder and the extracted juice (35 l) was concentrated to about 15 l and diluted with ethanol to a 70% ethanol solution. The supernatant, obtained by centrifugation, was further concentrated to 3 l and extracted with ether repeatedly (7 l). The ether extract (fraction A, 5.0 g, active), after being evaporated, was reextracted with ethyl acetate and then separated by treatment with 10% aqueous sodium carbonate into neutral (2.85 g, inactive) and acidic fractions (B, 1.50 g, active). On the other hand, the afore-mentioned aqueous concentrate was extracted with butanol (9 l),



I R=H
II R=C_4H_9
III R=CH_3

and the butanol solution was evaporated below 50°C in vacuo to leave resinous material (86 g), which was triturated with acetone. The insoluble material (69 g) was removed and gave sarsasapogenin on acid hydrolysis. The acetone-soluble material (4.7 g) was

shaken with ethyl acetate (200 ml) and water (300 ml). Extraction of the acetate solution with alkali (0.2 N NaOH) afforded an active acidic mixture (C, 640 mg). The remaining acetate solution also gave active neutral substance (D, 400 mg), which was submitted to evaporation under reduced pressure (1 mmHg) below 50°C (bath-temperature), when the distillate was collected in a trap cooled in liquid nitrogen. The collected oil (24 mg), showing a single peak on GLC, was highly active and suggested to be butyl 1,2-dithiolane-4-carboxylate (II, asparagusic acid butyl ester) on the basis of the spectral data: Mass, m/e 206 (M^+), 150, 133, 132, 105, and 104; UV (EtOH), λ_{max} 328 nm (ε 250) and 204 (920); IR ($CHCl_3$), ν_{max} 2960, 2880, 1726, 1470, 1420, 1330, and 1200 cm^{-1}; NMR ($CDCl_3$), δ 0.93 (3H, t J = 7), 1.4 (4H, br m), 3.41 (5H, m), and 4.14 (2H, t J = 6). This was confirmed by comparison with an authentic specimen prepared from I (with BuOH and BF_3-ether). However, ester II was evidently an artifact formed during the isolation procedure, because fractions A and B showed no peak due to II on GLC, though II was readily soluble in ether. In fact, asparagusic acid (I) was isolated, only in low yield owing to the facile polymerization, by preparative TLC (SiO_2, toluene:HCOOEt:HCOOH = 5:4:1) in the same manner as reported,[4b] and identified by comparison with the authentic acid[4c] and derivation into the methyl ester[4b] (III) (Mass, IR, NMR, and GLC). This acid I was found to exist at least at 35 ppm concentration in the roots by GLC of samples obtained by esterification of fractions B and C with diazomethane, and appeared in the Rohde's "glycoside"[2] region on paper chromatogram (Whatman No. 1, BuOH:EtOH:H_2O = 10:1:2).

Asparagusic acid (I) inhibited completely the emergence of the 2nd-stage larvae of Heterodera rostochiensis and H. glycines from the cysts (emerged larvae:total larvae and eggs < 0.1%) at 50 ppm in water at 25°C even in the presence of their natural hatching stimulants, and possessed the nematicidal action against the 2nd-stage larvae of H. rostochiensis (mortality 99%) and Meloidogyne hapla (92%), and the larvae and adults of Pratylenchus penetrana (82%), and Paratylenchus curvitatus (82%) at 50 ppm in water at 25°C for 145 hr.

REFERENCES and FOOTNOTES

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