Nematicidal Activity of α-Chaconine: Effect of Hydrogen-ion Concentration

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Abstract: α-Chaconine, a steroid-glycoalkaloid from *Solanum tuberosum* L., was increasingly more toxic to a free-living nematode, *Panagrellus redivivus*, with decreasing acidity from about *p*H 5 to 7. A study of the toxicity to adult nematodes at three concentrations of α-chaconine in buffer from *p*H 4 to 7.5 indicated that the free base is the nematicidal form of the compound. The median effective doses (ED_{50}) of α-chaconine to inhibit the motility of *P. redivivus* were estimated as 85 μg/ml at *p*H 6.7, 170 μg/ml at *p*H 6.5, and 340 μg/ml at *p*H 6.2. *Key Words*: Nematicide, Steroid-glycoalkaloid, α-Chaconine, Toxicity, Hydrogen-ion concentration.

Plant parasitic nematodes cause disease in many important agricultural crops. Disease resistant varieties are not always available or suitable; hence, control of parasitic nematodes is usually dependent on the use of nematicides (17). Due to recent concern about pollution of the environment with persistent pesticides, we have undertaken a study of naturally occurring nematicides of plant origin. A better understanding of the structures and modes of action of natural pesticides may help in the preparation of safer bio-degradable pesticides for commercial use and in the selection of green manure crops for the control of plant pests (13).

We are studying a class of well-known plant products, the steroid-glycoalkaloids. In an early investigation of the toxicity of a steroid-glycoalkaloid preparation designated "solanine" (Merck), Fischer (8) showed that the "solanine"-induced hemolysis of red blood cells increased rapidly with decreasing hydrogen-ion concentration. Kuhn *et al.* (10, 11) reported that "solanine" (Merck) was a mixture of six steroid-glycoalkaloids. The two major alkaloids, α-solanine and α-chaconine, have been isolated from a large number of *Solanum* spp. (15). Our preliminary studies showed that α-chaconine had higher nematicidal activity than α-solanine. We report here the dependence of the nematicidal activity of α-chaconine [α-L-rhamnopyranosyl-(1–2 gluc.)-α-L-rhamnopyranosyl-(1–4 gluc.)-β-D-glucopyranosyl-Δ^5-solanidenol-(3β)] (Fig. 1) on hydrogen-ion concentration.

MATERIALS AND METHODS

We selected 0.05 M ββ′-dimethylglutaric acid—NaOH buffer (7) for its low nematode toxicity between *p*H 4 and 7.5. *Panagrellus redivivus* (Goodey), the test nematode, is a free-living saprophyte easily cultured on wet oatmeal (18). α-Chaconine was isolated from tuber sprouts (*Solanum tuberosum* L.) by a previously described method (3) and tested at 85, 170, and 340 μg/ml of buffer at about *p*H 4, 5, 6, 7, and 7.5. A population of *P. redivivus* was removed from oatmeal cultures, washed and held for 48 hr at 21 C in vials containing washed quartz sand (18) with α-chaconine in buffer or buffer alone and about 200 adult nematodes each. Nematodes were separated from the sand by dilution with tap water and decantation. The nematodes were isolated from the sand-free supernatants by filtration with reduced pressure in sintered-glass funnels. The nematodes were washed with tap water and held for an additional 24 hr in counting dishes containing tap water at 21 C. Total motile

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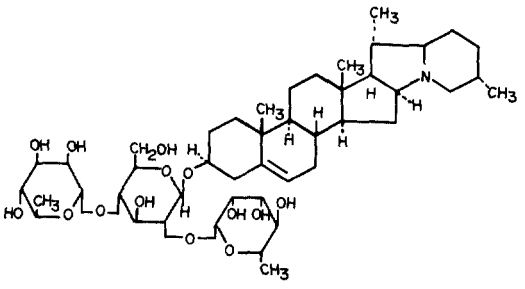

FIG. 1. Structure of α-chaconine.

and non-motile adults were counted for each sample with the aid of a microscope (10 to 60×).

The test of α-chaconine was conducted with duplicate samples at each *p*H and repeated three times.

RESULTS

The non-motile counts were progressively larger with increasing *p*H values at each level of α-chaconine (Fig. 2). After plotting the data on logarithmic probability paper, the median effective doses (ED_{50}) of α-chaconine to inhibit the motility of *P. redivivus* were estimated to be 85 μg/ml at *p*H 6.7, 170 μg/ml at *p*H 6.5, and 340 μg/ml at *p*H 6.2. However, the calculated free base concentrations (19) were nearly the same for each of the ED_{50} values: 1.0 μg/ml at *p*H 6.7, 1.3 μg/ml at *p*H 6.5, and 1.3 μg/ml at *p*H 6.2. A pK_b of 5.38 for the aglycone of α-chaconine, solanidine [Δ^5-solanidenol-(3β)], was determined by Bloom and Briggs (5). The free base concentrations for 170 μg α-chaconine/ml at several *p*H values were calculated (Fig. 2) by assuming that the pK_b of α-chaconine and solanidine are the same (5). The toxicity of α-chaconine to *P. redivivus* was proportional to the release of the free base. This is what one would expect if the free base were the toxic form of α-chaconine. The data suggest that the free base (Brönsted base) has high nematicidal activity and the conjugate acid has little or no nematicidal activity. Decreasing hydrogen-ion concentration above *p*H 7 had little or no effect on nematicidal activity since part of the free base precipitated (Fig. 2).

DISCUSSION

The nematicidal test used in this study (18) is dependent on the fact that healthy specimens of *P. redivivus* are in continuous rapid motion under favorable conditions. When nematodes inactivated by the test compound or lack of oxygen are held in tap water for 24 hr, they usually become motile again (18). Dead nematodes start to disintegrate and are darker than living nematodes. Very few nematodes recovered from the effects of α-chaconine. On the contrary, after 48 hr of contact with α-chaconine, some of the nematodes moved slowly and stopped moving when held for an additional 24 hr in tap water.

Tarjan (16) found that the susceptibilities of *P. redivivus*, a saprophyte, and *Meloidogyne incognita* (Kofoid & White) Chit., a root-knot nematode, to several nematicides were nearly equal. The influence of *p*H on the nematicidal activity of α-chaconine, as tested against *P. redivivus*, may be an important phenomenon in assessing the role of alkaloids as disease and insect-resistance factors in plants. The toxicities of both natural and synthetic steroid-glycoalkaloids and related compounds to saprophytic and plant parasitic nematodes are being tested with respect to *p*H. Such compounds with high activity near neutrality may be effective in alkaline soils and tests to verify this are contemplated.

Many steroid-glycoalkaloids have been shown to be toxic to plant parasitic insects (6), tumors in mice (12), animal parasitic fungi (9), and plant parasitic fungi (9, 15). However, the toxicity and pharmacological

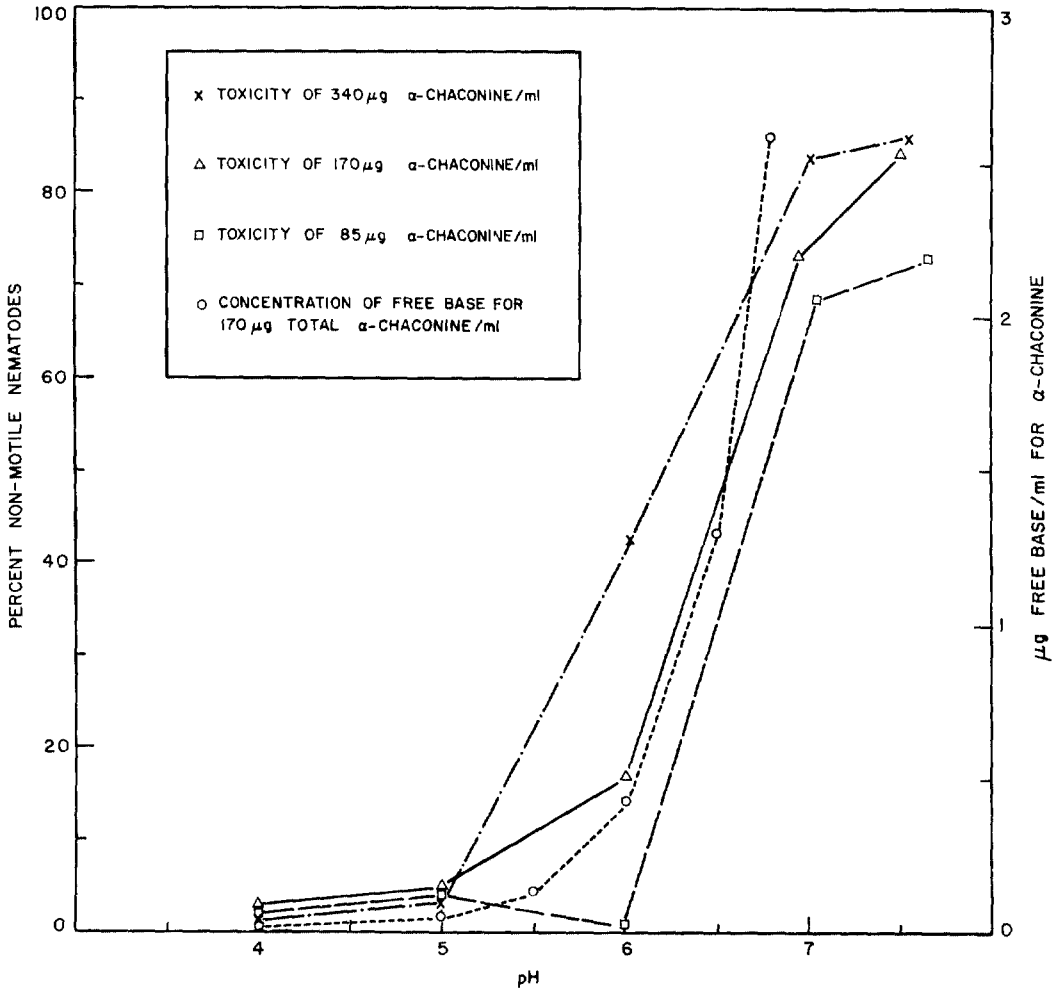

FIG. 2. Effect of hydrogen-ion concentration (*p*H) on the nematicidal activity of α-chaconine at three levels in 0.05 M ββ′-dimethylglutaric acid-NaOH buffer. The percentage of non-motile adult nematodes (*Panagrellus redivivus*) was used as an indicator of nematicidal activity at each *p*H tested. The toxicity percentages in this graph have been adjusted to compensate for the non-motile adult nematodes found in the buffer checks (4 to 9%). The concentration of the free base for 170 μg total α-chaconine/ml was plotted to show the correlation of the increase in free base concentration with the increase in nematicidal activity.

properties of α-chaconine are virtually unknown (15). The fungitoxicities of several steroid-glycoalkaloids have been shown to be *p*H-dependent (1, 4, 14), but apparently the effect of *p*H on the toxicity of steroid-glycoalkaloids to nematodes and other animals has received little attention (2, 8).

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