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Antibiotic Action of *Solanum incanum* Linnaeus

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The fruits of *Solanum incanum* Linnaeus are extensively used in Kenya for the treatment of cutaneous mycotic infections and other pathological conditions. The therapeutic activity of the berries has been attributed to their content of solanine and related glycoalkaloids, which are saponins and cytostatic poisons. In the present study, however, a simpler more potent antimicrobial substance with a phosphorylated structure similar to the purine adenine was isolated from the berries. The crystals of this compound were effective inhibitors of the growth of gram-positive and -negative bacteria, yeasts, dermatophytes, and some pathogens of agricultural produce. High concentrations of the substance caused hemolysis of erythrocytes.

The bushy herb *Solanum incanum* Linnaeus (bitter apple) of *Solanaceae* is widely distributed throughout the highlands of Kenya and is regarded as a noxious weed in widespread areas of Nairobi. However, in the rural districts, it serves as a natural remedy for a variety of injuries (13, 14), and its topical use as an antiseptic for wounds and dermatomycosis is common. The presence of pharmacologically active glycosidal alkaloids in *Solanaceae* has been known for centuries and, until recently, antispasmodic drugs obtainable from this family were the only therapeutic agents alleviating Parkinsonism. *Solanum* constituents that act as anticholinergic or parasympatholytic agents include tropine and hyoscine, the tropane alkaloids that occur abundantly in *Datura fastuosa* and *Atropa belladonna* (3).

The glycosidal alkaloids in particular have been associated with antibiotic activity. Solanocapsine (of *S. pseudocapsicum*) is antibacterial and the drug solanine, found in great quantities in potatoes (*S. tuberosum*), is toxic to various species of fungi (10, 14). Both are representative of antimicrobial steroidal alkaloids that occur in only a few genera of plants.

In addition to antimicrobial activity, some glycoalkaloids display antitumor effects as well. Beta-solamarine (isolated from *S. dulcamara*) inhibits sarcoma 180 in mice, and subsequent investigations have revealed the cytostatic activity of other steroidal glycoalkaloids such as solanine, which has been characterized as a mitotic poison (7, 9). Most of these compounds have limited therapeutic value.

The current investigation of the antimicrobial compounds in *S. incanum* was initiated because Kenyans continue to utilize successfully the juice of the berries for dermal infections that are frequently resistant to the most common commercial preparations containing zinc or sulfur. Its therapeutic effect against a wide variety of pathogenic conditions (including snake bites) is probably due to components that have not so far been described.

MATERIALS AND METHODS

Preparation of extract. The ripe and unripe fruits of *S. incanum* were collected from several areas of Nairobi, Kenya, and the green viscous juice was obtained from these through incisions of the pericarp. Centrifugation of the juice for 30 min at 3,000 rpm resulted in a clear supernatant fluid that was subsequently applied to silica gel adsorption columns (Fig. 1).

Columns of silica gel (60 to 120 mesh, 20 by 1.5 cm) were first washed with 100 ml of the mixed solvent of *n*-hexane and acetone (90:10) before 20 ml of the fluid sample was applied. Pigments and phenolic derivatives were eluted from the column with the hexane-acetone mixture, followed by a solvent consisting of benzene-ethyl acetate-formic acid (50:49:1). The final effluent of ethyl acetate-formic acid-water-butanone (50:10:10:30) contained a compound that showed antibiotic activity. This last fraction was neutralized to pH 7 with NH_3 (30%), concentrated to half its volume under vacuum in a desiccator over $CaCl_2$ and H_2SO_4, and rechromatographed on Dowex-50 (hydrogen ion form) ion exchange columns. The Dowex column (20 by 1.5 cm) was washed successively with 100-ml aliquots of acetone, distilled water, 2 N NaOH, 2 N HCl, ethanolic (65%) NH_4OH, 2 N HCl, and repeated changes of water until the effluent was neutral before application of the sample. The toxicant was removed from

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the column with 0.1 N sodium acetate as the first fraction. This produced clear needle-like crystals, with marked bioactivity after its decolorization over activated charcoal and the evaporation of the solvent.

Test cultures. The test organisms employed for assaying antibiotic activity (Table 1) were obtained from a collection of stock cultures in the Department of Veterinary Pathology and Microbiology, University of Nairobi. These were identified by standard procedures previously described (1, 4, 5).

Fungal cultures were grown on Sabouraud dextrose agar (BBL) at 25 C and harvested in 0.15 M NaCl, and the spore number was estimated with a hemacytometer. The suspensions were adjusted in Welcome opacity tubes (no. 5) and stored at 4 C until required. The fungal plates were prepared by mixing 1.5 ml of the suspension with 13.5 ml of liquified Sabouraud dextrose agar that had been cooled to 50 C. These plates were used immediately for the bioassay and showed growth in 3 days at 25 C.

Bacterial cultures were grown on 1% bovine blood agar or Trypticase blood agar slants (Oxoid, London), and suspensions of these organisms were made in 0.15 M NaCl. Experimental plates were prepared by using cotton swabs to inoculate the blood agar plates heavily with suspensions of bacteria.

Bioassay procedure. Aqueous solutions of the

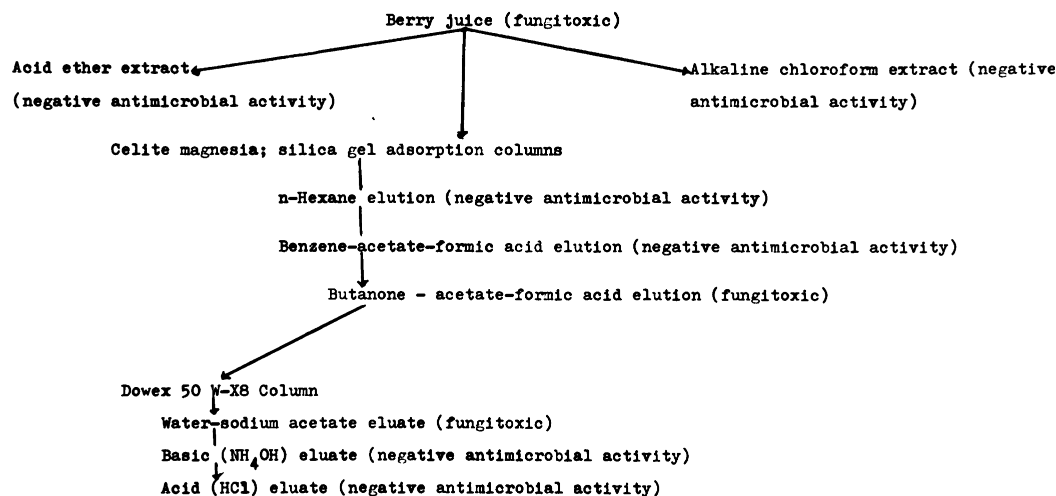

FIG. 1. *Extraction chart of S. incanum fractions and antibiotic activity. Test organisms: Microsporum gypseum and Keratinomyces ajelloi.*

TABLE 1. *Susceptibility of microorganisms to antibiotic of S. incanum (50 μg of solanum crystals per disk)*[a]

Bacteria	Zone of inhibition (mm)	Fungi	Zone of inhibition (mm)
Streptococcus pyogenes	26	*Microsporum nanum*	32
Staphylococcus aureus	20	*Microsporum gypseum*	21
Listeria monocytogenes	12	*Microsporum canis*	22
Clostridium perfringens	25	*Microsporum equinum*	22
Clostridium septicum	25	*Keratinomyces ajelloi*	24
Corynebacterium diphtheriae	20	*Microsporum distortum*	35
Corynebacterium pyogenes	15	*Trichophyton tonsurans*	23
Bacillus anthracis	18	*Crytococcus neoformans*	20
Salmonella gallinarum	20	*Geotrichum candidum*	19
Salmonella typhimurium	16	*Candida albicans*	17
Shigella sonnei	18	*Colletotrichum lindemuthianum*	35
Pseudomonas aeruginosa	18		
Proteus vulgaris	15		
Pasteurella haemolyticum	22		
Haemophilus gallinarum	22		
Klebsiella pneumoniae	9		
Escherichia coli	15		
Brucella abortus	20		
Actinbacillus lignieresii	20		
Bacillus subtilis	15		

[a] Diameter of disks, 6.35 mm.

crystalline antibiotic were applied to 6.35-mm sterile absorbent disks (Carl Schleicher and Schuell Co., Keene, N.H.) centrally placed on the agar surface. The fungal plates were sealed with Cellotape and incubated aerobically at 25 C under atmospheric conditions. The zones of inhibited growth were measured after 72 h (Fig. 2). Bacterial plates were incubated at 37 C aerobically for 24 and 48 h, except those of *Clostridium perfringens* and *C. septicum*, which were incubated anaerobically, and *Brucella abortus*, which was grown in an atmosphere of 5% O_2, 10% CO_2, and 85% N_2.

Test for hemolytic activity. Serial dilutions of the bioactive compound were made in 0.15 M NaCl, and 0.5-ml amounts were mixed with equal volumes of a 2% suspension of washed bovine erythrocytes. These mixtures were incubated at 37 C in a water bath for 20 h.

Drug identification procedures. Although the total elucidation of the structure of the antibiotic has not been completed, the following procedures indicate the presence of a phosphorylated base similar in configuration to the purine adenine.

Ignition of the crystals with calcium oxide resulted in calcium phosphate, which (when reacted with ammonium molybdate in the presence of nitric acid) produced ammonium phosphomolybdate, a clear indication that the crystals contain phosphorus. This was further substantiated by the production of a white precipitate of magnesium ammonium phosphate with magnesia mixture (6).

The same crystals gave positive results with various chemical tests for purines, including the formation of an insoluble silver salt or a complex with mercury (8). The movement of these crystals on thin-layer chromatographic plates (Silica Gel GF_{254}, type 60) in solvent systems of butanol-acetone-35% NH_3-water (50:40:3:15) and *n*-propanol–methanol–35% NH_3-water (45:15:30:10) follow closely the movement of adenine, and both substances are visualized by the same staining procedures employing eosin-mercury or silver-nitrate-bromophenol blue spray reagents (11).

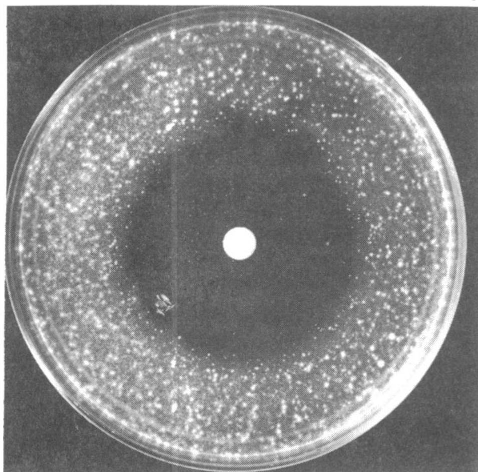

FIG. 2. *Zone of growth inhibition of Microsporum gypseum by 0.01 ml of active extract of S. incanum L.*

When injected into a Zipax SCX cation-exchange column (DuPont) and subjected to high-pressure liquid chromatographic procedures, the crystals are eluted from the column with 0.1 N HNO_3 at the same rate as adenosine-5'-monophosphate (AMP). However, the elution of the antimicrobial compound is greatly diminished relative to AMP with alkaline phosphate buffers (pH 8 to 9), perhaps due to a net positive charge in such media (Fig. 3).

RESULTS

Colorless, water-soluble, hygroscopic crystals obtained from the fruits of *S. incanum* were moderately soluble in ethanol and insoluble in ether, acetone, chloroform, hexane, and other hydrocarbon solvents. Those recrystallized from ethanol several times had a melting point of 113 to 115 C.

These crystals inhibited the growth of all organisms listed in Table 1. They also caused hemolysis of bovine erythrocytes within 1 h of incubation at concentrations approximating 50 mg/ml and partial hemolysis after 20 h when diluted to 2.5 mg/ml. No hemolysis was observed when the concentration was less than 1.5 mg/ml.

Data obtained with thin-layer chromatography and chemical procedures indicated that the crystals are of a phosphorylated purine structure that comes off the liquid chromatography cation-exchange column along with the nucleotide AMP in acidic media but exhibit more basic characteristics at higher pH values.

DISCUSSION

Hygroscopic crystals of an antibiotic constituent in *S. incanum* was isolated by us and displayed antibacterial activity against both gram-positive and gram-negative organisms. In addition, it showed anti-*Candida* properties, but its action against dermatophytes was the most prominent. The susceptibility of various organisms responsible for dermatomycosis to these solanum crystals was demonstrated not only on petri dish cultures but also in clinical trials (not reported herein) on persons infected with *Microsporum* organisms resistant to the topical antifungal agents frequently employed for such infections. In several instances severe cutaneous mycoses that persisted for weeks and that tended to spread cleared within 3 days subsequent to treatment with solanum crystals dissolved in glycerol.

The mechanism of the chemotherapeutic action of solanum crystals has not been thoroughly investigated. Although the hemolysis experiments with erythrocytes indicated that

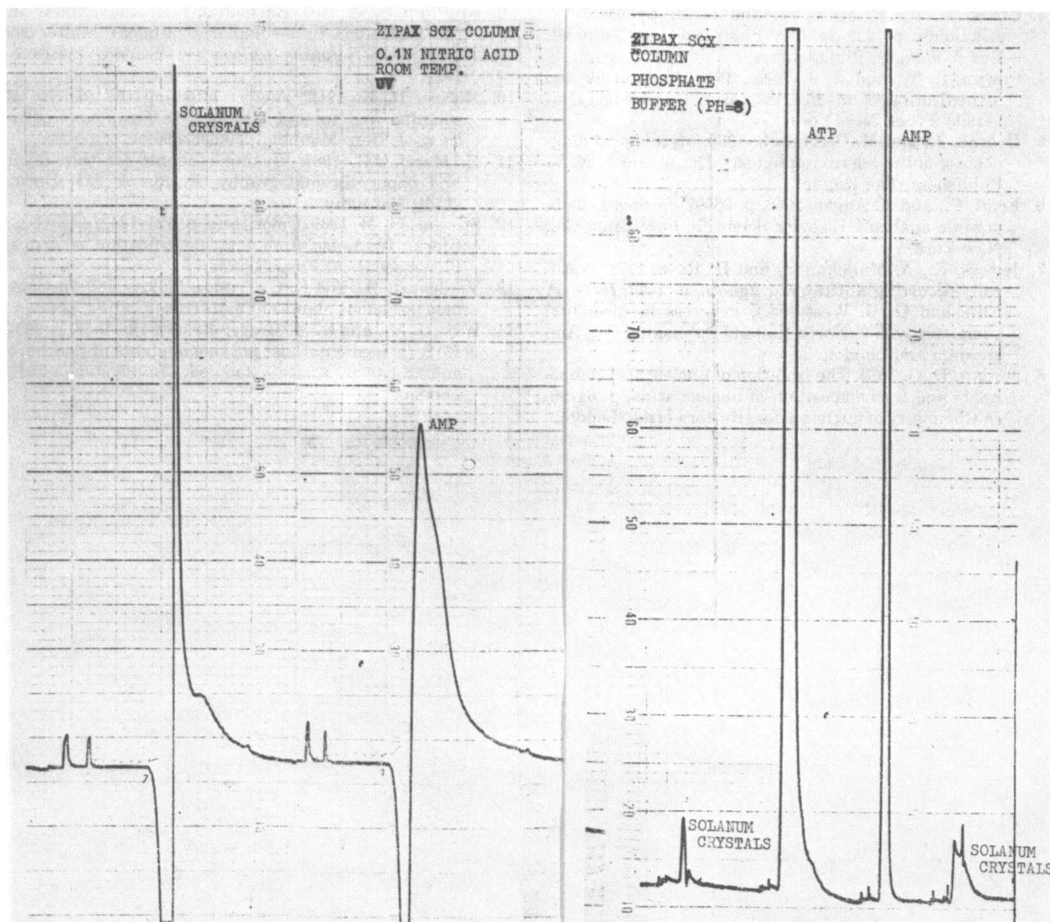

FIG. 3. *Elution pattern of 0.1 mg of solanum crystals, AMP, or adenosine-5'-triphosphate (ATP) per ml of solution from Zipax SCX cation-exchange column in high-pressure liquid chromatograph. Solanum crystals efficiently eluted from the column with 0.1 N nitric acid at the same rate as AMP show decreased movement in alkaline phosphate buffer.*

the crystals may act as a sapogenic surface active agent at high concentration, this seemed less important at very low, but biologically effective, dosages. Microscopic examination of thin, Araldite-embedded sections of fungal cultures inoculated with the toxicant showed distorted hyphae and almost total inhibition of sporulation, suggesting that biosynthesis of some essential cellular components may be inhibited or the toxicant may act as an antimetabolite.

Several constituents of *Solanum* have been associated with antibiotic activity, mainly the steroidal alkaloids (10). However, chromatographic techniques, microcrystal tests, and a series of chemical tests for drug identification (12) show that the pronounced bioactivity does not reside in the presence of solanine or related glycoalkaloids but rather that the crystals are of a simpler plant metabolite, purine-like in structure and probably phosphorylated.

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