Phytochemistry, 1968, Vol. 7, pp. 1691 to 1693. Pergamon Press. Printed in England

SHORT COMMUNICATION

ISOLATION AND PURIFICATION OF TREMATOXIN FROM *TREMA ASPERA*

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(*Received* 24 *January* 1968, *in revised form* 20 *March* 1968)

Abstract—A toxic compound, trematoxin, has been isolated from *Trema aspera* (Ulmaceae) and purified using fractional precipitation of lead salts, adsorption chromatography on polyamide, sephadex gel filtration and thin-layer chromatography on cellulose. The compound is highly fluorescent and has a LD 100 of 12–15 mg/kg when administered intraperitoneally to mice and guinea pigs. It is shown to be a glycoside and, when hydrolysed with 2 N HCl, two fluorescent aglycones can be extracted and purified.

INTRODUCTION

Trema aspera Blume (Ulmaceae) grows widely in the coastal districts of Australia, and is the cause of a disorder in cattle and sheep.[1] The plant is a large leafy shrub or small tree attaining a height of about 15 ft, and is commonly found in cleared rain forest country but is not confined to these areas. It occurs in New South Wales, Queensland, North-West Australia and New Guinea; also it is closely allied to *Trema ambionensis* and *T. orientalis* of tropical areas north of Australia. The plant growing in its natural state is readily eaten by cattle and sheep when they have access to it, and animals which eat the leaves usually die within a few days. As the number of animals affected by this disorder is often large, it represents a serious economic loss.

A toxic principle, trematoxin, has been isolated from *T. aspera* and its toxicity to laboratory animals has been determined. The present paper covers its isolation from the dried leaf material and some preliminary chemical work to determine its structure.

RESULTS

Toxicity of Trematoxin

A dilute aqueous solution (pH 7) of purified trematoxin was injected intraperitoneally to mice and guinea pigs and the results (Table 1) show that the LD 100 for mice and guinea pigs is 12–15 mg/kg and that all animals survived when a smaller dose was given. This could mean that the toxin has a specific mode of action.

Chemical Nature of the Toxin

200 mg of trematoxin after purification (see Experimental) were dissolved in 100 ml of water, and then run through a short column of Amberlite IR-120 (H^+) (10 g) to remove metals and ammonium ions. The eluate was concentrated to give granular crystals (150 mg) which were dried under vac. (0·01 mm) at 20°. Analysis of the crystals gave C = 50·38 per cent,

[1] C. R. MULHEARN, *Aust. Vet. J.* **18**, 68 (1942).

TABLE 1. TOXICITY OF TREMATOXIN

Weight of dissolved oil (in mg)	Number of animals tested	Average body weight of live animals, g	Result
Mice			
6	5	28	4/4
4	2	28	2/2
2	5	28	5/5
1	4	20	4/4
0·25	12	20	10/12
0·1875	4	20	0/4
0·1250	4	20	0/4
Guinea pigs			
20	1	415	1/1
6	1	220	1/1
4	4	334	4/4
2	2	287	1/2
1	2	355	0/2

H = 5·56 per cent, N = 0, and on heating they decomposed above 250°. Analyses for heavy metals, halogens, phosphorus and sulphur were negative. When 1 per cent $FeCl_3$ was added to a dilute solution (0·1 mg/ml) of trematoxin the solution turned dark blue instantaneously. Trematoxin gave a negative Liebermann–Burchard test, and i.r. spectrum (KBr disc) failed to show the four peaks characteristic of a steroidal sapogenin.[2] An aqueous 0·9 per cent sodium chloride solution of trematoxin, having a concentration of 10 mg/ml, did not haemolyse sheep's red blood cells in 10 hr. An alcoholic solution of trematoxin (0·1 mg/ml) shows very weak absorption in the u.v. at 320 nm. No change in this spectrum was seen when acid or alkali was added.

Trematoxin (10 mg) was heated for 3 hr with 2 N HCl and the solution evaporated to dryness under reduced pressure at 25°. Analysis of an aqueous aliquot of the residue using paper chromatography showed the presence of glucose and arabinose.[3] A 2 N HCl solution of trematoxin (200 mg) was heated at 100° for 3 hr. After cooling the solution was extracted with ethanol/benzene (1:4), the extract run through a florisil column (5 g) and evaporated to yield a dark yellow fluorescent oil (80 mg). This was purified on silica gel G plates 0·5 mm thick with chloroform/ethanol (99:1) as the solvent to give two fluorescent "aglycones", (i) R_f 0·9 (25 mg) and (ii) R_f 0·5 (15 mg).

DISCUSSION

This appears to be the first report of the isolation of a toxic compound from a member of the Ulmaceae. Trematoxin appears to act principally as a liver toxin in guinea pigs and mice. These animals usually die within 2 or 3 days after dosing, and the autopsy findings resemble those reported by Mulhearn[1] for sheep.

The method of purification and isolation of trematoxin used in this study recovers most of the original toxicity in the plant but some loss takes place at the lead precipitation step. No satisfactory alternative to this step has been found. An attempt to determine the molecular

[2] M. C. WALL, C. R. EDDY, M. L. MCCLENNAN and M. E. KLUMP, *Anal. Chem.* **24**, 1337 (1952).
[3] D. H. NORTHCOTE, *Brit. Med. Bull.* **22**, 2, 180 (1966).

weight of trematoxin and its aglycones by mass spectrometry has so far been unsuccessful owing to the involatile nature of these compounds and the difficulty of obtaining sufficiently pure samples. However, the behaviour of trematoxin on dialysis indicates that the molecular weight is probably over 1000.

EXPERIMENTAL

Source of Plant Material

Leaves of *Trema aspera* were gathered from an area outside Brisbane, Queensland, and air dried in a room away from direct sunlight. The sample was identified by the Queensland Government Botanist and specimens were filed by him under the voucher number (BRI-058459).

The air-dried leaf was broken up in a hammer mill in order to facilitate extraction and storage. The moisture loss was found to be approximately half the weight of the green leaf. The presence of the toxic substance was followed during purification by concurrent toxicity tests on guinea pigs.

Isolation and Purification

100 g of milled air-dried leaf was extracted three times with 50 per cent aqueous ethanol (at 50°) and the extract concentrated under reduced pressure to a small volume. The extract was dialysed in a Visking dialysis tube for 20 hr and the non-diffusible fraction centrifuged to remove precipitated material. The supernatant was adjusted to pH 6, and sufficient 20 per cent lead acetate solution was added to give complete precipitation. The precipitate was separated by centrifuging, washed twice with distilled water, and the lead salts then decomposed by adding 20 per cent NaH_2PO_4 solution.[4] After centrifuging, the supernatant was adjusted to pH 4, run through a polyamide/celite column (20 g:40 g) made up with water[5] and the column washed with 2 l. of water. The toxin was displaced from the polyamide by washing the column with 1 l. of 0·1 N NH_4OH followed by water till the eluate was neutral. This was concentrated under reduced pressure, the dried residue dissolved in 50 per cent aqueous methanol (20 ml), and run through a column of Sephadex G 50 (50 g) made up with the same solvent.[6,7] A narrow yellow fluorescent band was seen under u.v. light when the solvent was run through the column. This band was collected and purified by a much longer column of Sephadex G 50 (150 g). A final purification of the fluorescent fraction was achieved using cellulose thin-layer plates 0·5 mm thick and ethanol/water (3:1) as a solvent. A yellow fluorescent band, $R_f = 0{\cdot}9$, was recovered from the cellulose by washing it with 50 per cent aqueous methanol. Concentration of these washings yielded a light yellow oil (200 mg) which solidified on drying under reduced pressure to give pure trematoxin.

Acknowledgements—The author is much indebted to Sir Rudolph Peters for his helpful advice and encouragement. The author wishes to thank Dr. D. B. Cater of the Pathology Department for the post-mortem and histological examinations, Dr. D. H. Williams of the chemistry Department for many NMR and mass spectra, Dr. E. C. Bate-Smith for his suggestions in preparing this manuscript, and Professor Young for use of apparatus and facilities at the Biochemistry Department. The author received a maintenance grant from the Queensland Department of Primary Industries during the course of this study.

[4] R. Hill, personal communication.
[5] B. S. Chandler and T. Swain, *Nature* **183**, 989 (1959).
[6] I. Forrest, Ph.D. Thesis, University Cambridge (1966).
[7] D. Gupta, *Nature* **200**, 574 (1963).