RESEARCH PAPERS

CARDIOTONIC SUBSTANCES FROM *BERSAMA ABYSSINICA* FRES. SUB. SPECIES *ABYSSINICA*

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Three crystalline materials have been isolated from the leaves of *Bersama abyssinica* sub. sp. *abyssinica*, N.O. Melianthaceae. Some chemical and pharmacological evidence is presented that these materials are bufodienolide aglycones. The material isolated in greatest quantity has a toxicity similar to that of scillaridin.

Bersama abyssinica, N. O. Melianthaceae, is a tree with a wide distribution in Africa occurring from S. Rhodesia to Ethiopia. Leaves from this tree have been shown to be toxic to cattle (Harker and Gourlay, 1959) and the clinical and pathological findings in cattle, rabbits and mice after poisoning by leaves and crude leaf extracts have been described (Gourlay and Harker, 1960). Investigation of the leaves of this tree have resulted in the isolation of several toxic materials: the most potent of these have produced in cattle the characteristic symptoms of poisoning by the leaves (Gourlay, Harker and Lock, 1962). The method of isolation of some of these materials and some of their chemical and pharmacological properties are described in this paper.

MATERIALS AND METHODS

Plant materials were air dried and reduced to moderately fine powder in a disintegrator.

Chromatographic purification and separation; materials. Alumina, chromatographic grade, was heated for 1 hr. at 110° and 14 mm. Hg, and then equilibrated with water vapour for a week over aqueous saturated sodium bromide solution. Chloroform was rendered low in ethanol by washing four times with water, drying over calcium chloride and distilling. Isopropanol was dried over anhydrous sodium sulphate and distilled.

Identification of active fractions. From 0·1 ml. to 1·0 ml. of eluate was evaporated to dryness on a staining tile and the residue covered with concentrated sulphuric acid; characteristic colours developed.

Chromatography on paper. Whatman No. 1 or No. 4 paper was soaked in 20 per cent formamide in acetone for 5 min. and the acetone blown off the paper. After applying the samples, the paper was run in 50 per cent xylene and methylethyl ketone by the ascending method at the ambient temperature of this laboratory (ca. 22°) (Kaiser, 1955). After drying at 110°, the paper was layered on to a thick film of concentrated sulphuric acid on a glass plate; characteristic colours developed.

Pharmacological solutions. Crystalline materials were dissolved at a concentration of 1 per cent in ethanol and the ethanol diluted with 9 parts of water. This solution was subsequently diluted with physiological solutions as required.

Toxicity determinations. Mice. White mice, 16–22 g., were dosed intraperitoneally, intravenously or orally. Observations for deaths were made for 24 hr. Approximate LD50 determinations were obtained graphically.

Cats and Vervet monkeys. Animals were anaesthetised with ether and infused intravenously with dilutions of the drugs in normal saline at the rate of 1 ml./min. until death (de Lind Van Wijgaarden, 1926). Electrocardiographic records were taken from some animals. Respiration was recorded by Gaddum's (1941) method.

Isolated hearts from rabbits and guinea-pigs were perfused with Ringer's solution by Langendorff's method.

RESULTS

Extraction. 5 kg. of powdered leaves were percolated with 100 litres of 75 per cent ethanol. After concentration to 10 litres extraction with chloroform yielded 35 g. of oily semi-solid material. Preliminary purification of this was achieved by chromatography on a column 8 cm. diameter by 18 cm. high containing 800 g. alumina. Some inert oily material was removed by the passage of 3 litres of chloroform. Active material was then eluted with 3 litres of 10 per cent isopropanol in chloroform. Removal of the solvent left 4·2 g. of slightly oily solid residue. Subsequent elution of the column with methanol yielded 2·8 g. of material containing only small amounts of toxic activity. In Table I is summarised the toxic activity of the various stages so far described.

TABLE I

THE TOTAL TOXIC ACTIVITIES OBTAINED FROM LEAVES OF *B. abyssinica* DURING PRELIMINARY EXTRACTIONS AND PARTIAL PURIFICATION BY CHROMATOGRAPHY

Stage	Total amount of sample	kg.-mouse units*
Concentrate of percolate from 5 kg. leaves	10 litres	2490
Total residue from chloroform extract of percolate ..	35 g.	2200
Partially purified material from 1st chromatography	4·2 g.	1240
Methanol eluate from 1st chromatography	2·3 g.	5

* A kg.-mouse unit is defined as ONE median lethal dose per kg. administered intraperitoneally to white mice.

Fractionation of active components. The partially purified material was dissolved in chloroform and applied to a column of alumina, 330 g., of dimensions 2·8 cm. diameter and 54 cm. length. Linear gradient elution with 0 to 10 per cent isopropanol in chloroform was then started. After the passage of 1·25 litres of eluent, 25 ml. samples were fractionally collected. Samples of individual fractions were tested with sulphuric acid and combined in groups according to the colour reaction. Solvent was removed and the residues weighed. Aliquotes of the individual samples were taken for paper chromatography and toxicity tests. In Table II is summarised the results obtained by the fractionation.

Treatment of individual fractions. Only those fractions showing major amounts of toxic activity have so far been investigated, namely, Nos. 4, 7 and 8 in Table II.

TABLE II

A SUMMARY OF THE RESULTS OF GRADIENT ELUTION, WITH ISOPROPANOL IN CHLOROFORM, FROM A COLUMN OF ALUMINA, OF 4·2 G. OF PARTIALLY PURIFIED TOXIC MATERIAL FROM BERSAMA LEAVES

Combined fraction No.	Gradient range per cent isopropanol	Tube numbers 25 ml. fractions	Colour* with sulphuric acid**	Total wt. residue of fraction g.	R_F and colour* of spot on paper with sulphuric acid†	kg.-mouse units	Designation of material crystallised from fraction
1	0·625–1·25	1–17	B V,	0·792	0·64 V, 0·73 Pc, 0·81 YB, 0·72 B, 0·91 BOr,	5	
2	1·25–2·5	18–28	P Or,				
3	1·25–2·5	29–38	B Or,				
4	1·25–2·5	39–53	Rd,	1·020	0·71 R	913	R
5	2·5–5	54–60	B V,	0·210	0·4 V, 0·51 R, 0·73 Pc,	3	
6	2·5–5	61–70	Rd,		0·29 V, 0·37 Y, 0·49 ROr, 0·61,		
7	2·5–5	71–84	Or Rd,	1·190	0·47 POr,	16	O
8	2·5–5	85–104	Or Rd,	0·986	0·43 Or,	49	Q
9	5·0–10	105–120	Or,	0·202	0·27 B, 0·36 Or, 0·75 Y, 0·69 V, 0·22 BOr,	5	
10	5·0–10	121–200	Y Or,				

* B, brown; Or, orange; Pc, peach; Rd, red; V, violet; Y, yellow.
** The colours attempt to express the maximal colour developing with H_2SO_4 from an aliquote of the fraction evaporated to dryness. Thus B V, indicates a brownish violet coloration.
† R_F values of entities separated on paper by Kaiser's method, the colour being that developed by immersion of the paper in sulphuric acid.

Fraction 4. The residue from this fraction tended to crystallise. Repeated washing with ether removed oil and green colouring matter. Almost white crystals were left. Yield: 350 mg. The crystals were dissolved in ethanol, and an equal quantity of water added and the solution treated with charcoal (BDH decolourising acid washed) and the recovered material recrystallised from methanol. The purified material is designated in this paper as substance "R".

Fraction 7. This residue which was oily was also treated with ether, in which the active material was found to be moderately soluble. Repeated crystallisation from this solvent removed much oily and green matter and the greenish white crystals (74 mg.) were treated with charcoal and recrystallised from methanol. The resulting crystals are designated as "O".

Fraction 8. This material was treated as for fraction 7 and designated as "Q".

A summary of some chemical and physical properties of R, O and Q is shown in Table III.

Pharmacological: Toxicity Determinations

Mice. Lethal and sublethal doses produced violent and prolonged clonic convulsions in this species. With lethal doses the mice frequently died in a tonic convulsion with the head drawn down onto the chest and the hind legs extended. With sublethal doses convulsions were prolonged

for as long as 24 hr. followed by recovery. Convulsions began within 5 min. after intraperitoneal injection, and within 10, orally.

TABLE III

SOME CHEMICAL AND PHYSICAL PROPERTIES OF MATERIALS R, O AND Q

Test	Material R	Material O	Material Q
Melting point ° (uncorrected) ..	237–241	224–226	224–226
Molecular wt.*	421	420	—
Optical rotation* $[\alpha]_D^{23°}$	+43	+43	—
Maximum ultra-violet absorption Å	300	300	300
Liebermann Immediate	pale yellow	red	bright yellow
Burchard After 30 sec.	pale green	olive green	blue green
Legal	negative	negative	negative
Raymond	negative	negative	negative

* This information is derived from results obtained by the Microanalytical Laboratories, Oxford

In Table I is shown the total toxic activity obtained during extraction of the leaves and preliminary purification of the extract. Table IV shows the approximate median lethal doses of the crystalline materials R, O and Q; the results for digitoxin are included for comparison.

Knee jerk. During the administration of toxic fractions in increasing amounts up to lethal doses, no increase in knee jerk reflex was observed.

TABLE IV

THE APPROXIMATE MEDIAN LETHAL DOSES FOR MICE OF MATERIALS R, O, Q AND DIGITOXIN BY THE INTRAPERITONEAL AND ORAL ROUTES

Material	Median lethal dose mg./kg. Intraperitoneal	Oral
R	0·51	0·84
O	30·75	83·00
Q	9·30	16·20
Digitoxin	12·60	250·00

Blood pressure and respiration. Non-lethal doses of toxic materials in cats and monkeys produced a pressor response of up to 50 mm. Hg, recovery taking about 20 min. With lethal doses, heart irregularities supervened and the blood pressure fell abruptly to zero. Respiration continued after cessation of the heart beat (Fig. 1).

Isolated hearts. Non toxic doses produced on isolated hearts an increased contractility and tone with irregularities of rhythm due to extrasystoles. Large doses produced heart block, independent beating of auricles and ventricles and finally ventricular fibrillation (Fig. 2).

Lethal doses, Vervet monkeys and cats. Table V shows the lethal doses obtained by intravenous infusion of material R in cats and monkeys; material Q was not included owing to the small amount isolated. ECG recordings obtained simultaneously (Fig. 3) showed cardiac changes

sometimes beginning with slight bradycardia with an increased P—Q interval, and then, invariably, the development of ectopic beats, bundle branch block, gross irregularities and cardiac arrest following ventricular fibrillation. Results obtained with substance O and Q were similar.

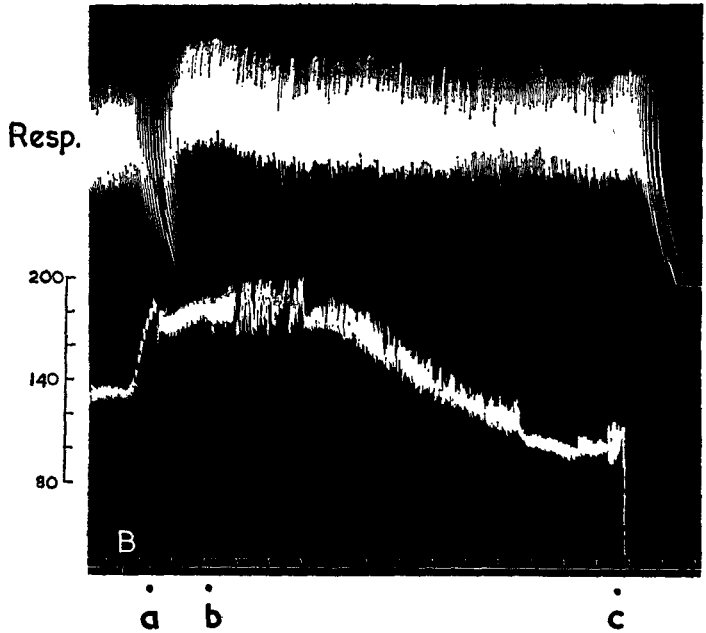

FIG. 1. Vervet monkey, 4·5 kg., anaesthetised with ether. At B, 0·5 mg. material R was given intravenously. At a, a pressor response of 50 mm. of mercury is seen; at b, irregularities of the heart's action occurred; at c, the blood pressure fell to zero, preceding the failure of respiration.

DISCUSSION

The preliminary investigations into the toxic activity of leaves of *B. abyssinica* lead to the suspicion that a strychnine-like substance was involved, mice, rabbits and cattle showing a convulsive response varying in intensity with the species. The absence, however, of any enhancement of the knee jerk reflex in cats indicated stimulation of the central nervous system at a level higher than the spinal cord. The results obtained on blood pressure and respiration in monkeys showed clearly a cardiotoxic effect, the heart invariably failing before the respiration. The cardiac effect was confirmed on isolated hearts; an initial increase of amplitude was seen first, followed by partial and then complete heart block, culminating, if the dose was large enough, in ventricular fibrillation. These responses, together with the initial rise of blood pressure shown in Fig. 1 and the convulsant effects, particularly prominent in mice, are all characteristic of a digitalis-like substance. Confirmation of this hypothesis is afforded by the ECG recordings (Fig. 3). The successive changes consisting of bradycardia with an increased P-Q interval, the development of

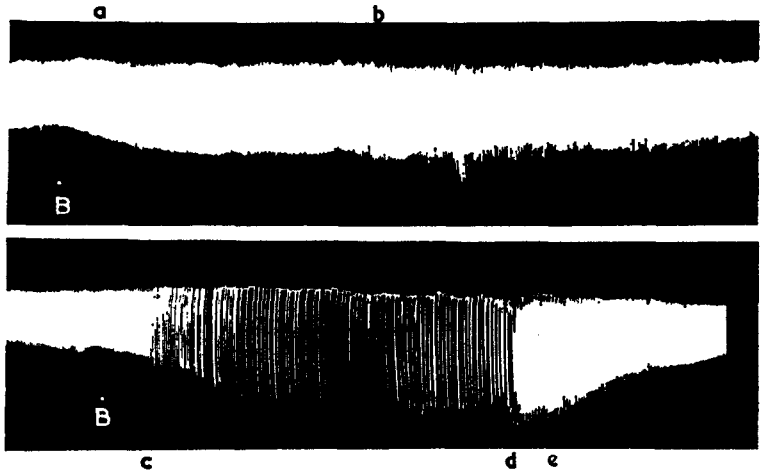

FIG. 2. The effect of material R on the isolated rabbit heart; Langendorff's preparation. Upper tracing: At B, 60 μg. of material R were injected into the perfusion fluid immediately above the heart. At a, an increase in amplitude occurred together with an increase in tone; there was no significant change in rate. At b, irregularities of rhythm occurred, due to extrasystoles.

Lower tracing: At B, 400 μg. of material R were injected. At c, heart block and loss of tone developed; at d, auricles and ventricles began to beat independently; at e, ventricular fibrillation occured.

ectopic beats, increasing degrees of block and finally ventricular fibrillation are typical of digitaloid substances.

The chemical evidence indicates that the isolated materials are bufodienolides. The negative responses to the Legal and Raymond tests point to the absence of the five membered lactone ring, whereas ultraviolet absorption at 300 Å is characteristic of the six membered bufodienolide ring. The small molecular weights found for R and O indicate

TABLE V

THE LETHAL DOSES OF MATERIAL R BY CONTINUOUS INTRAVENOUS INFUSION INTO ETHERISED VERVET MONKEYS AND CATS

Species. Doses in μg./kg.	
Vervet monkey	Cat
93·0	110
78·0	127
93·5	130
90·5	110
98·8	
91·5	
94·2	
81·7	
Mean 90·1	119
S.E. ± 2·43	± 5·38

that the substances are genins and evidence has not been found of the occurrence of glycosides during the isolation of these materials. The similarity of the intraperitoneal and oral median lethal doses for mice,

particularly for material R, and the rapid onset of convulsions by the latter route, indicate a ready absorption by the gastrointestinal tract.

The vervet monkey was found to be more susceptible to the toxic action of material R than the cat and in this laboratory, on a small number of animals, the mean lethal dose for the cat (Table V) is similar to that reported by Chen (1950) for scillarenin viz. 110 μg./kg.; the elucidation of the structure of R would be of interest. It is of note that unlike most cardenolides and bufodienolides, the materials isolated from *B. abyssinica* produce a prolonged burning sensation on mucous membranes, but no bitter taste.

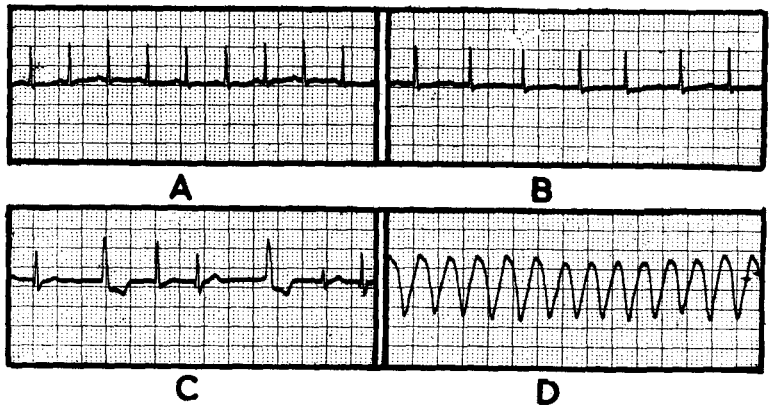

FIG. 3. Vervet monkey, 3·5 kg. anaesthetised with ether. Electrocardiographic records taken during continuous intravenous infusion with material R. At A, after 12·5; at B, 25; at C, 75; and at D, 87·5 per cent of the lethal dose.

Toxic activity has not been found in the fruit, seeds or bark of *B. abyssinica* sub. sp. *abyssinica*; the leaves of *B. abyssinica* sub. sp. *paullinioides* have been found to contain a toxic factor producing typical convulsions in mice. This and allied varieties of *Bersama* are being investigated.

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REFERENCES

Chen, K. K., Henderson, F. G. and Anderson, R. C. (1950). *J. Pharmacol., R.*, **99**, 395–400.
Gaddum, J. H. (1941). *J. Physiol.*, **99**, 257–264.
Gourlay, R. N. and Harker, K. W. (1960). *J. comp. Path. Therap.*, **70**, 464–474.
Gourlay, R. N., Harker, K. W. and Lock, J. A. (1962). In the press.
Harker, K. W. and Gourlay, R. N. (1959). *E. Afric. agric. J.*, **25**, 63–65.
Kaiser, F. (1955). *Chem. Ber.*, **88**, 556.
de Lind van Wijngaarden (1926). *Arch. exp. Path. Pharmak.*, **113**, 40.