SOME OBSERVATIONS ON THE CURARISING ACTIVITY OF *GONIOMA KAMASSI*, E. MAY

PRELIMINARY OBSERVATIONS

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The total bases from *Gonioma kamassi* have been divided into tertiary and quaternary fractions and electrophoretic patterns of these are shown. The LD50 values for mice of the various fractions have been determined. The curariform action has been located in the quaternary fractions, and potencies estimated in comparison with tubocurarine. The curariform action has been shown to be truly neuromuscular, and strong evidence presented that the action is depolarising. Effects on respiration and duration of blocking action, compared with tubocurarine have been shown, and the presence of a prolonged parasympathetic ganglion blocking activity in the quaternary fraction is also demonstrated.

IN 1911 Dixon reported the pharmacology of an alkaloidal fraction of *Gonioma kamassi*, South African boxwood, and showed the presence of weak curarising activity. The description given of the preparation of Dixon's sample suggests that it may well have been a mixture of tertiary and quaternary alkaloids. In 1951, Schlittler and Gellért isolated from the bark of *G. kamassi* a tertiary alkaloid which they called Kamassin; Gellért and Witkop (1952) have since demonstrated the identity of this alkaloid with quebrachamine, and it is probable that Dixon in fact investigated a mixture of this alkaloid and curarising substances.

It seemed pertinent to reinvestigate the alkaloidal fractions from *G. kamassi* particularly with relation to the curarising activity and the possibility of the presence of quaternary alkaloids. Investigation has shown, as might be expected, that curarising activity occurs in the quaternary fraction. This fraction presents a complexity similar to those from the South American *Strychnos* species and gives rise to similar difficulties of isolating the individual alkaloids.

EXPERIMENTAL METHODS

Preparation of plant material. All materials were air dried and reduced to moderately fine powder in a disintegrator. The samples were defatted by continuous extraction with isohexane.

Extraction of Alkaloids

General. All evaporations and concentrations were made under reduced pressure. All representative samples were adjusted to contain the equivalent of 0·5 g. plant material per ml.

Method. The defatted material was continuously extracted with methanol, the methanol removed and the residue repeatedly extracted with hot water. The combined aqueous extracts were then filtered and concentrated.

Separation of total base chlorides. Basic compounds present in crude extracts were precipitated as reineckates from dilute acid solution. The reineckates were dried, dissolved in acetone, filtered and reconverted to chlorides by the Kapfhammer method using silver sulphate and barium chloride.

Separation of (*a*) *tertiary and* (*b*) *quaternary base chlorides.* (*a*) (i) The solution of total base chlorides was made alkaline with ammonia and repeatedly extracted with ether freed from peroxides. The ether fractions were combined and extracted with dilute hydrochloric acid to yield tertiary base chlorides.

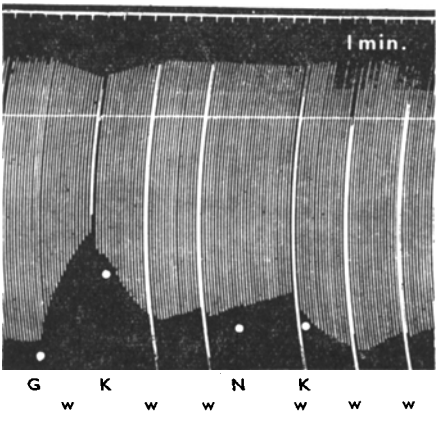

FIG.1. The effect of potassium chloride on the block induced by gonioma. Isolated rat diaphragm. 50 ml. bath. At G, 10 mg. gonioma, at w, wash, at K, 30 mg. KCl. N, normal dose position: no dose given; kymograph running continuously.

(ii) Material precipitated by ammonia and insoluble in ether was filtered off during the last ether extraction, washed with ether and water, dissolved in acid and the bases separated as chlorides by the reineckate process.

(*b*) The ammoniacal aqueous liquid was made acid and the quaternary bases separated as chlorides by the reineckate process.

Electrophoresis on paper. Whatman No. 1 paper was used with 15 per cent acetic acid as electrolyte. A potential gradient of 30 V./cm. was applied for one hr.

Pharmacological

Toxicity determinations. White mice, 16–20 g. were injected intraperitoneally in groups of 10 per dose. Mortalities per cent were converted to probits and approximate LD50 determinations obtained graphically.

Isolated rat diaphragm—phrenic nerve preparation. This preparation was arranged according to the method described by Chou (1947). Doses of drug were added by an automatic syringe device, Lock (1961).

Gastrocnemius nerve-muscle preparation. Cats and grey monkeys were anaesthetised by chloralose, 80 mg./kg., and sodium phenobarbitone, 120 mg./kg. respectively, and arranged according to the method of Bülbring and Burn (1941). In these preparations doses of drug were given either into the external iliac artery or the jugular vein. Respiration was recorded by Gaddum's (1941) method. Some cats were arranged for recording contractions of the nictitating membrane after supra-maximal square-wave stimulation of the preganglionic fibres at a frequency of 10/sec.

Assay method. The isolated rat diaphragm was used for approximate potency determinations of the curarising activity. It was found that samples of gonioma, in amounts producing more than a 30 per cent inhibition, resulted in a very slow rate of recovery compared with tubocurarine.

The addition of KCl to the bath during the recovery period, as suggested by West (1947), resulted in a rapid expansion of the trace, but after removal of the KCl the inhibition increased and tended to return to its

TABLE I

THE TOXICITIES TO MICE, CURARISING POTENCIES AND TOTAL ACTIVITIES OF GONIOMA LEAF, BARK AND WOOD CRUDE EXTRACTS. SAMPLES WERE STANDARDISED TO CONTAIN 0·5 G. OF CRUDE DRUG PER ML.

Sample	Wt. residue mg./ml.	mg. residue equivalent to 1 mg. tubocurarine	LD50 to mice mg./kg. i.p.	Total activity per kg. orig. material in terms of tubocurarine
Leaves	47·0	490	135	660
Bark	80·4	142	71	1133
Wood	26·0	1270	557	39

expected position as if the KCl had not been added (Fig. 1). Two applications of KCl during the wash periods are shown after a dose of gonioma sufficient to produce a 48 per cent inhibition. On each occasion the trace expanded during the application of KCl and contracted to above the base line during the subsequent wash period. Full recovery was reached only after two complete cycles, that is, 26 min. Without the addition of KCl a similar dose of gonioma took much the same time for recovery.

In view of these observations, curarising potencies of various fractions were determined on the rat diaphragm by matching, doses being added in the order ABBA, and in amounts to produce not more than a 30 per cent inhibition. It is with these limitations that curarising potencies are presented in this paper.

RESULTS

The relative amounts of curarising activity in leaves, bark and wood is shown in Table I. Not unexpectedly, samples of bark showed the highest activity, and in view of the relatively low potency of wood, further work has been confined to leaves and bark.

Table II shows relative activities of tertiary and quaternary fractions and the ether and water insoluble fraction obtained during separation (see methods). The relatively low oral toxicity to mice of the quaternary fraction from bark is also included.

TABLE II

THE TOXICITIES TO MICE, CURARISING POTENCIES AND TOTAL ACTIVITIES OF TOTAL BASES, QUATERNARY AND ETHER: WATER INSOLUBLE FRACTIONS OF GONIOMA LEAF AND BARK

Sample		Wt. residue mg./ml.	mg. residue equivalent to 1 mg. tubocurarine	LD50 to mice mg./kg. i.p.	Equivalent mg. tubocurarine per kg.
Leaf	Total bases	8·0	34·5	12·8	490
	Quaternary	2·0	35·0	12·6	166
	Ether: water insoluble	2·5	30·0	10·6	166
Bark	Total bases	23	38·2	22·4	1200
	Quaternary	8·4	45·8	14·0 oral 840	366
	Ether: water insoluble	7·8	25·0	13·0	624

The tertiary bases contained no curarising activity, although difficulty was initially experienced in obtaining a fraction uncontaminated with quaternary substances. The ether and water insoluble complex, initially discarded during filtrations to prevent emulsions while extracting the tertiary components from ammoniacal bases with organic solvents was found to contain as much curarising activity as the quaternary fraction.

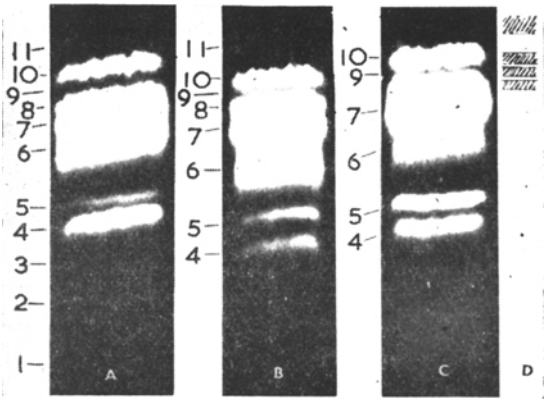

FIG. 2. The electrophoretic patterns of fractions from *G. kamassi* bark. U.V. light 360 Å; electrolyte, acetic acid 15 per cent; potential gradient 30 V/cm. for 1 hr.; paper Whatman No. 1. A, total base; B, ether : water insoluble; C, quaternary; D, Dragendorff positive zones. Fluorescent colours 1, 2 and 3, whitish; 4 blue; 5 yellow; 6 blue; 7 yellow; 8 green; 9 blue; 10 yellow; 11 dark purple. No. 11 does not show on the photograph; it is present in A and B but not in C.

Identification of components

Paper chromatography, with a large number of solvent mixtures including Karrer's A, B, D & E (Schmid and Karrer, 1950; Schmid, Kebrle and Karrer, 1952) have produced indifferent results with gonioma extracts and fractions. Paper electrophoresis has, however, made evident the presence of a large number of positively charged components most of

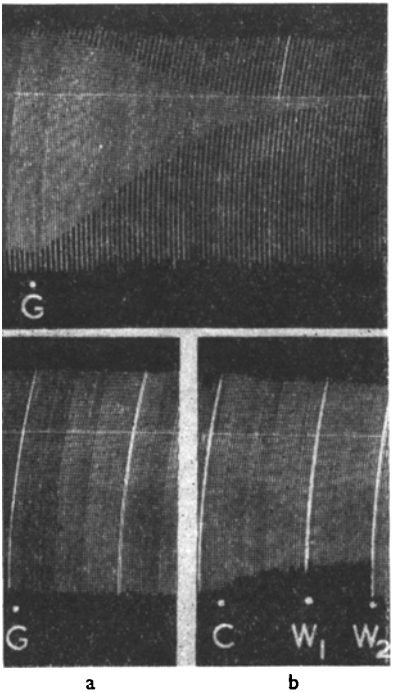

FIG. 3. The effect of direct muscular and neural stimulation of the isolated rat diaphragm exposed to gonioma, and also the application of gonioma to nerve alone, Upper Fig., at G, 0·16 mg./ml. gonioma in the bath. Ten stimulations per min. alternately to nerve and muscle. Lower Fig. a, at G, 0·8 mg./ml. gonioma applied to the nerve. The nerve from the diaphragm was passed through a rubber seal into a glass tube which contained the electrodes. Lower Fig. b, at C, 10 per cent cocaine applied to the nerve; W, wash in the main bath; W_2 replacement of the cocaine by Tyrode, kymograph off 15 min.

which show strong fluorescence and some a positive reaction with Dragendorff's reagent. Fig. 2A, B, and C, shows the electrophoretic patterns of total bases, ether: water insoluble, and quaternary fractions from bark under ultra-violet light of wavelength 360 Å, and at D the position of bands acting with Dragendorff's reagent.

A deep purple zone (at 11 in Fig. 2A and B), which had the greatest rate of migration, showed a transient violet coloration with ceric sulphate. This fluorescence had not sufficient actinic power to show in the photograph. This band was found to be identical with the tertiary component

identified by Gellért and Witkop (1952) as quebrachamine and the reaction with ceric sulphate (Kebrle, Schmid, Waser and Karrer, 1953) is in accord with the indol group in its structure. It also gave a positive reaction with Draggendorff's reagent. It is of interest that no other component showed a similar colour reaction, in marked contrast with the

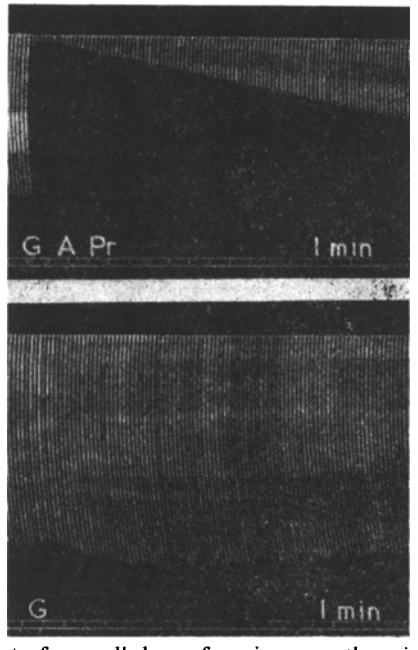

FIG. 4. The effect of a small dose of gonioma on the sciatic-gastrocnemius preparation, and also the effect of neostigmine on a gonomia produced block in the same preparation.

Vervet monkey, 6·2 kg., sodium phenobarbitone:

(a) At G, 28 mg. gonioma; at A, 1·5 mg. atropine; at Pr. 2 mg. neostigmine, all intra-arterially.

(b) At G, 11 mg. gonioma intra-arterially.

curarising strychnos alkaloids, most of which show colours with this reagent (Lederer, 1959).

A notable difference between leaf (not shown) and bark is the low intensity in the former of the two pronounced yellow bands at 4 and 6 in the bark. Electrophoretic patterns of the quaternary bases (Fig. 2C) show only five groups of components with three Dragendorff positive zones in contrast to eleven groups in the total bases. The ether: water insoluble fraction (Fig. 2B) is similar to the former except for the absence of band 2, tending to confirm the suggestion that quaternary bases are rendered insoluble by the presence of resinous substances.

From Table II it will be seen there is an appreciable apparent loss of activity during the division of the total bases into tertiary and quaternary components. This activity, however, was found to be present as a contaminant of the tertiary alkaloidal fraction. Repeated transference

between aqueous and non-aqueous solvents with acid and alkali did not achieve separation of these fractions, but chromatography of the crude tertiary fraction on alumina in isohexane allowed the passage through the column of the tertiary alkaloids; the curarising activity was retained at the top of the column and was elutable with ethanol.

Pharmacological Properties of the Quaternary Fraction from Bark

As the activities of quaternary fractions of leaves and bark appeared qualitatively similar, results reported below are confined to the quaternary fraction from bark. During the presentation of results this sample will be referred to as gonioma.

Site of blocking effect. While Dixon appreciated the similarity of the paralysis resulting from gonioma to that of "curare", he did not investigate the site of the blocking action. It was therefore important to ascertain whether gonioma is in fact a neuromuscular blocking agent.

In Fig. 3 will be seen the effect of the application of gonioma to the isolated rat diaphragm during alternate stimulations of the nerve and

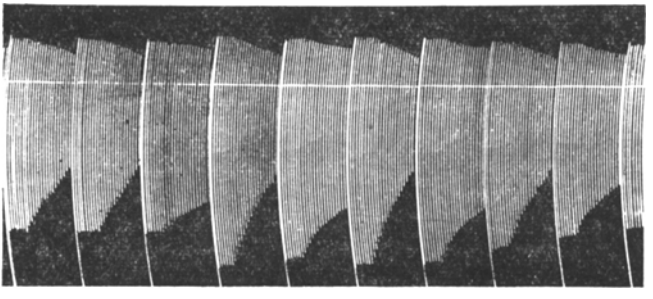

FIG. 5. The effect of neostigmine on alternate doses of tubocurarine and gonioma on the isolated rat diaphragm-phrenic nerve preparation. At T, 100 μg., tubocurarine; at G, 3 mg. gonioma; at Pr., 0·25 μg./ml. neostigmine in the Tyrode; at N, normal Tyrode resumed.

muscle. After sufficient time to produce a complete block to neural stimulation, there is no appreciable loss of excitability of the muscle to direct stimulation. The application of gonioma to the nerve alone, in a concentration five times greater than that necessary to produce the block shown in the first part of this experiment, produced no block in 3½ min. (Fig. 3a). The application of 10 per cent cocaine hydrochloride to the nerve showed an inhibition not removed by washing the main bath, but removed by replacing the cocaine solution by Tyrode, thus showing the validity of the method (Fig. 3b). It is concluded that the action of gonioma is that of a true neuromuscular blocking agent.

Type of blocking action. Tubocurarine, the toxiferines and the ethroidine alkaloids have in common the property of being antagonised by neostigmine, and small doses do not cause an exaggerated response to nerve stimulation in the normal muscle. This is in contradistinction to

the depolarising agents such as decamethonium. With gonioma the onset of paralysis of the sciatic-gastrocnemius preparation is rapid and doses large enough to produce an appreciable degree of inhibition do not normally show an initial expansion of the trace. When, however, a suitable small dose is given an expansion is marked, as will be seen in Fig. 4b.

When a dose sufficiently large to produce a 95 per cent inhibition was given to the same animal, after atropine, the administration of neostigmine produced a slight but definite increase in the degree of inhibition. This is shown in Fig. 4a.

A similar enhancement by neostigmine of the inhibition by gonioma was also demonstrable on the isolated rat diaphragm (Fig. 5), tubocurarine being antagonised in the expected way. Percentage inhibitions are shown in Table III.

Further evidence in this direction was obtained using chicks according to the method of Buttle and Zaimis (1949).

After the intravenous injection of 2·4 mg./kg. of gonioma, spastic paralysis occurred, the characteristic opisthotonic attitude being assumed.

TABLE III

THE EFFECT OF NEOSTIGMINE ON THE INHIBITIONS PRODUCED BY TUBOCURARINE AND GONIOMA ON THE ISOLATED RAT DIAPHRAGM (TAKEN FROM FIG. 5)

Drug	Per cent inhibition									
			P	P	P	P				
Tubocurarine 100 μg. ..	41·5		20		36·2		33·3		34·9	
Gonioma 3·3 mg.		44·5		50		52·3		50		45·3

At P, neostigmine, 0·25 μg./ml. was added to the Tyrode solution.

These observations present strong presumptive indications that unlike the other natural alkaloids mentioned above, the blocking activity of gonioma is of a depolarising nature.

Duration of action of the blocking effect. Reference to the more prolonged effect of gonioma compared with tubocurarine has already been made in connection with the isolated rat diaphragm assay of gonioma fractions. On gastrocnemius-sciatic nerve preparations both in monkeys and cats, gonioma produced a more prolonged blocking effect than tubocurarine. Fig. 6 shows that an 83 per cent inhibition in the gastrocnemius of a vervet monkey is followed by recovery in 20 min., whereas recovery from a 70 per cent inhibition produced by gonioma required 32 min. Results on cats gave a similar slower rate of recovery from gonioma.

Effect on respiratory muscles. Also in Fig. 6 will be seen the effect of the two drugs on respiration. Gonioma produced a slight decrease in amplitude of the respiratory movements, but the amount of air passing through the lungs was practically unchanged. Gonioma appears to have little, if any, relatively greater effect on the respiratory muscles than tubocurarine.

Effect on vagus stimulation. Doses of gonioma, well below those required to produce neuromuscular block, cause a profound and long lasting inhibition of the response of the blood pressure to stimulation of the peripheral end of the cut vagus.

Fig. 7 shows that an intravenous dose of gonioma insufficient to produce either expansion or contraction of the gastrocnemius twitch produced a complete block to vagal stimulation, although the fall in blood

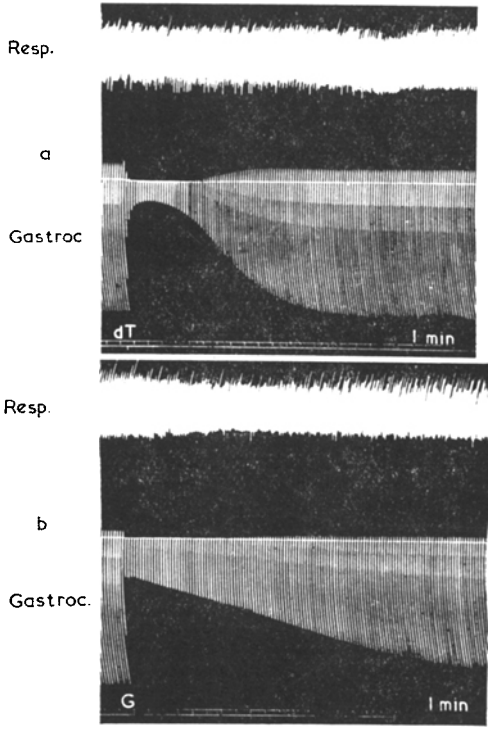

FIG. 6. The relative effects on respiration and gastrocnemius twitch of tubocurarine and gonioma.
Vervet monkey, 5·3 kg., sodium phenobarbitone.
(a) At dT, 400 μg. tubocurarine.
(b) At G, 14 mg. gonioma.

pressure in response to acetylcholine was unchanged. Little recovery from this blocking action took place until $3\frac{1}{2}$ hr. had elapsed. Larger doses did not influence the response to acetylcholine. Gonioma has therefore a prolonged blocking action on cardiac parasympathetic ganglia, but no appreciable atropine-like effect on the circulatory mechanism.

Effect on the blood pressure. Small doses of gonioma produce a fall in blood pressure (Fig. 7), and the fall is proportional to the dose until a level of about 40 mm. Hg is reached. Further doses produce no further fall. Recovery is rather prolonged; doses sufficient to produce maximal fall require an hour for recovery. The fall was not modified by previously

administered large doses of antihistamines. Experiments on the cat nictitating membrane showed that no block of the sympathetic ganglion occurred with doses of gonioma up to those producing complete block of the gastrocnemius. No further investigation of the mechanism of this effect was made during this series of experiments.

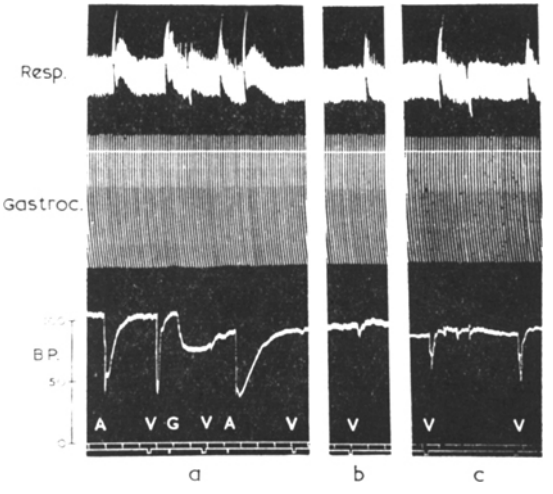

FIG. 7. The effect of gonioma on the response of blood pressure to acetylcholine and vagal stimulation. Vervet monkey, 1·6 kg., sodium phenobarbitone; respiration, gastrocnemius twitch, blood pressure in mm. Hg. At A, 30 μg. acetylcholine intravenously; at V, stimulation of the peripheral end of the cut vagus, at G, 3 mg. gonioma intravenously, b, after 40 min., c, after 210 min.

DISCUSSION

The paucity of curarising alkaloids so far reported from Africa is in marked contrast to the large number of different species yielding these from South America. *Gonioma kamassi* is, however, a notable example, but appears to have attracted little attention from either makers of arrow poisons or pharmacologists. There appear to be no reports of its use as an arrow poison in spite of the reasonably high toxicity of crude extracts by injection and negligible toxicity by mouth.

Of marked interest is that gonioma, unlike the other naturally occurring curarising alkaloids such as tubocurarine, the toxiferines and the erythrine-alkaloids, shows many properties of a depolarising blocking drug. While absolute proof must await the chemical separation of the curarising principle or principles, the combination of enhancement of the block by neostigmine, the increased response to nervous stimulation of the gastrocnemius muscle, and the characteristic opisthotonic attitude of the young chick after intravenous injection, present strong presumptive evidence that this is so.

The blocking effect resembles the toxiferines in its relatively prolonged action compared with tubocurarine; it does not show the relatively powerful action on the respiratory muscles, Paton and Perry (1951).

Of note also is the long acting block of the cardiac parasympathetic ganglia, and the less prolonged but appreciable hypotensive effect. These two properties cannot as yet be ascribed with certainty to the curarising activity, and it is felt that further pharmacological analysis should await separation of the chemical entities.

The potency of the curarising activity is also unknown. The most active fraction so far obtained during this series of experiments has shown a potency of one third that of tubocurarine; at least six components were shown to be present by electrophoresis.

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