684. *Curare Alkaloids. Part X. Some Alkaloids of* Strychnos toxifera *Rob. Schomb.*

By Harold King.

The object of this investigation was the isolation of the alkaloids of *Strychnos toxifera* as this liane is the source of the renowned calabash-curare of British Guiana. By chromatography of the less soluble reineckates twelve crystalline quaternary salts, toxiferines-I to -XII have been obtained, two as the chlorides, and the remainder as picrates. Toxiferine-I chloride, an extremely potent curarising agent, was identical with the toxiferine-I chloride of Wieland, Bähr, and Witkop. Toxiferine-II picrate of these investigators was also encountered, the remaining ten toxiferines being apparently new. The curarising activity of some of these is comparable with that of toxiferine-I chloride. The neutral substance $C_{21}H_{25}O_3N_3$ found by the above-named investigators in calabash-curare from Venezuela has now been found in *St. toxifera*. The striking colour reactions of these alkaloids suggests that they are all derived biogenetically from tryptophan.

The pharmacologist Boehm (*Abhandl. Kgl. sächs. Ges. Wissensch.*, 1895, **22**, 203) showed that there were three kinds of curare: tube-curare, pot-curare, and calabash-curare, distinguishable by their containers and by their chemical characteristics. The chemical constituents of the two first-named curares have been elucidated in earlier parts of the present series of communications. The third type, calabash-curare, is dealt with indirectly in this communication and was formerly the one most frequently encountered in commerce, its main source of supply being the northernmost countries of S. America (British Guiana, Venezuela, and Colombia).

According to Richard Schomburgk (" Travels in British Guiana," 1840—1844), his brother Robert was the first to recognise a *Strychnos* species as furnishing the active ingredient of the renowned calabash-curare prepared by the Macusi Indians in British Guiana and he named it *St. toxifera*. Robert Schomburgk made an extract of this liane and showed that it alone was sufficient to confer the paralysing properties on calabash-curare. Richard Schomburgk collected a further supply of *St. toxifera*, and the German chemist Heintz examined it but failed to prepare any pure alkaloid.

Boehm (*loc. cit.*, 1897, **24**, 4) examined 17 specimens of calabash-curare and isolated a highly active amorphous quaternary alkaloid for which he proposed the formula $C_{19}H_{25}ON_2Cl$ and which he called curarine; the amount of non-quaternary alkaloid was very variable and in all cases minute. In a small sample of *St. toxifera* bark which he obtained from Holmes, from the museum of the Pharmaceutical Society in London, Boehm confirmed the presence of a curarising principle.

In 1935 (*Nature*, **135**, 469) I recorded some preliminary observations in this field; from

St. toxifera I isolated an amorphous quaternary iodide, to the extent of 0·2% of the bark, which chemically and physiologically closely resembled a curarine iodide isolated from calabash-curares from British Guiana. All attempts to crystallise either of these iodides or other salts by direct crystallisation failed.

In 1937 Wieland, Konz, and Sonderhoff (*Annalen*, **527**, 160), by use of the chromatographic adsorption of the reineckates of commercial calabash-curare on alumina, were able for the first time to crystallise the anthraquinone-2-sulphonate and picrate of an active principle. In a second communication Wieland and Pistor (*ibid.*, 1938, **536**, 68) described the preparation of the crystalline chloride and iodide of the above active principle which they now called C-curanine I and in addition they isolated a second weaker alkaloid, C-curarine chloride II and its iodide in crystalline form. They also stated, without details, that *St. toxifera* bark, supplied by the National Institute for Medical Research, Hampstead, contained, not C-curarine I, but a much more active principle which they named toxiferine.

In a later communication (*ibid.*, 1941, **547**, 140) Wieland, Pistor, and Bähr described some additional properties of C-curarines I and II from calabash-curare and of a third quaternary alkaloid C-curarine III. In a fourth contribution to this subject (*ibid.*, p. 156) Wieland, Bähr, and Witkop raised the number of crystalline quaternary alkaloids which they had isolated from various calabash-curares to seven, and from *St. toxifera* bark they isolated two, toxiferine-I chloride and toxiferine-II picrate both in the crystalline state. The last-named was identical with one of the seven alkaloids from calabash-curare and readily underwent isomerisation to the alkaloids toxiferine-IIa and toxiferine-IIb. The formula attributed to toxiferine-I chloride was $C_{20}H_{23}ON_2Cl$, whilst to toxiferine-II chloride was assigned the formula $C_{20}H_{25}ON_2Cl$. It is noteworthy that the formula proposed by Boehm for his amorphous preparation differed from the former by only one carbon atom.

Owing to the intervention of World War II, I was not able to return to a study of calabash-curare and the alkaloids of *St. toxifera* until 1948. As it was known that the Indians put the extracts of more than one plant into their curare preparations and even mix *Chondrodendron* species with *Strychnos* species, it was evident that the safest scientific approach to the problems presented by calabash-curare lay in an examination of botanically identified *Strychnos* species and in particular of *St. toxifera*. It was also clear from Wieland's and Karrer's results that the problem involved the separation of mixtures of quaternary alkaloids, avoiding acidic conditions, a comparatively new and difficult field of alkaloidal chemistry for which new methods were needed.

A pilot experiment on the alkaloidal reineckates from 465 g. of *St. toxifera* bark, substantially by Wieland's methods, had shown the presence of four or more alkaloids by their isolation as crystalline picrates, one of which was probably identical with toxiferine-I picrate; in addition the neutral substance $C_{21}H_{25}O_3N_3$ which Wieland, Bähr, and Witkop had found in calabash-curare from Caracas in Venezuela was encountered.

A repetition of the chromatographic experiment on a 4·5-kg. scale was then undertaken. The alkaloidal reineckates, after preliminary purification by hot-water extractions as recommended by Wieland, Pistor, and Bähr, were submitted to chromatography on three successive columns of alumina, each 50 cm. long. By elution with acetone, the first column gave four clearly defined zones, A, B, C, and D, A at the top representing the most tenaciously-held components. The filtrate and eluate put on to the second column gave evidence, on development with acetone, of eleven bands numbered xi to i downwards, a yellow solution Y passing through without adsorption, followed by a rose-coloured solution R. These two coloured fractions were separated on a third column into a yellow filtrate, followed by a rose-coloured filtrate; negligible amounts of adsorbed material were left on the third column.

During the development of the first column it was noticed that the roseate reineckate solutions became bleached in the surface layers of the column nearest the glass walls by the action of daylight. The second and third columns were accordingly run shielded by a black paper collar, and the column was examined only in artificial light. There is apparently no mention of this phenomenon by other workers.

The zones A, B, C, and D were then sectioned and the recovered reineckates converted into chlorides in neutral solution by means of silver sulphate and barium chloride. The alkaloids in zones xi—i of the second column were converted into chlorides similarly, and the solutions R and Y from the third column were treated in the same way. Each fraction was then evaporated to dryness at $<50°$, warmed with absolute alcohol, separated from sodium chloride, concentrated to a small volume, and kept.

Under these conditions fraction D deposited a chloride, crystallising in needles, the properties of which on recrystallisation agreed in most respects with the toxiferine-I chloride of Wieland, Bähr, and Witkop. Fraction R also deposited a crystalline chloride which could be crystallised from water and as it appears to be new is called *toxiferine*-III *chloride*. These were the only fractions crystallised directly as chlorides.

Each of the remaining fractions was then converted into its picrate in aqueous solution, and the amorphous picrates dried and then warmed with a small volume of dry acetone and kept. Those fractions which did not crystallise were then treated with acetone and water in various, often laborious, ways described in the Experimental section under each zone.

Eventually twelve crystalline quaternary alkaloids were isolated, two as their chlorides and ten as their picrates; others have been encountered in traces. Their properties, their highly characteristic colour reactions, and the positions they occupied on the adsorptive columns are summarised in the following table.

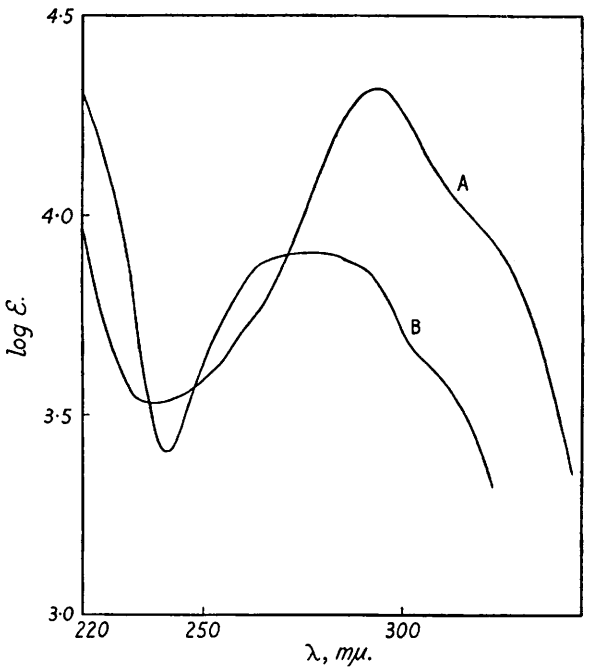
Positions on columns.	Identity.	M. p.	H_2SO_4.	HNO_3.	Cr_2O_7'' in 50% H_2SO_4
A	Toxiferine-IV picrate	230°	—	—	—
B	Toxiferine-IV picrate	238—239	Nil	Carmine	Bluish-purple ⟶ carmine
B	Toxiferine-V picrate	270	Blue	Deep green ⟶ pink	Deep blue ⟶ purple
C	Toxiferine-VI picrate	>300	Pale straw	Carmine	Blue ⟶ purple ⟶ red
C	Toxiferine-II picrate	about 200	Pale straw	Carmine	Bluish-purple ⟶ reddish-purple
D	Toxiferine-I chloride	—	Nil	Deep rose	Bluish-purple ⟶ reddish purple
D	Toxiferine-I picrate	278	Blue	Deep green ⟶ rose	Bluish-purple ⟶ reddish-purple
D	Toxiferine-VII picrate	>300	Pale straw	Deep rose	Reddish-purple ⟶ carmine
xi—ix	Toxiferine-VIII picrate	>300	Pale straw	Rose to carmine	Carmine
iv—i	Toxiferine-IX picrate	>300	Nil	Carmine	Bluish-purple
R	Toxiferine-III chloride	285	Yellow	Bluish-green ⟶ dirty colour	Deep blue
R	Toxiferine-X picrate	264 (explodes)	Blue	Orange	Carmine
R	Toxiferine-XI picrate	277	Blue	Deep green ⟶ pink	Bluish-purple ⟶ reddish-purple
Y	Toxiferine-XII picrate	>333	Blue	Orange	Carmine

Of the twelve picrates recorded in this table, five are unmelted at 300°; it is possible they are the picrates of alkaloids of a greater degree of complexity than the others, and there is some support for this in some of the analytical figures. As some of these picrates have been obtained pure only in 10—30-mg. quantities, the formulæ given in the Experimental section must be regarded as tentative; in no case have determinations of water of crystallisation been made as the effect of heat was unknown, so that it is very probable that water of crystallisation is incorporated in the formulæ. Many of these picrates explode on micro-analysis, even when mixed with copper oxide; this does not necessarily invalidate the analyses when micro-analytical methods are used.

Five of the picrates give a blue colour with strong sulphuric acid and, as toxiferine-I picrate but not toxiferine-I chloride gives this colour, it is probable that the nitro-groups act as oxidising agents.

Of the twelve alkaloids isolated from *St. toxifera*, only two—toxiferine-I chloride and toxiferine-II picrate—appear to have been isolated previously. None of the remaining ten alkaloids appears to be identical with any of the alkaloids isolated by Wieland and by Karrer (*Helv. Chim. Acta*, 1947, **30**, 2085) and their collaborators from calabash-curare. The colour reactions, however, of the alkaloids from *St. toxifera* and of the alkaloids from calabash-curare and the analytical figures suggest a close relationship between the two groups of alkaloids. There is no evidence that the calabash-curares examined by Wieland or Karrer came from British Guiana where *St. toxifera* is known to be used in the preparation of such curare; on the

contrary the evidence points to Venezuela and Colombia as being the source of their curares. It is, however, significant that toxiferine-II picrate has been found in *St. toxifera* from British Guiana and in calabash-curare from Venezuela, and that the neutral substance $C_{21}H_{25}O_3N_3$ has now been found in *St. toxifera* having first been found in calabash-curare from Venezuela. It is therefore certain that *Strychnos* species are used in the preparation of the calabash-curares from Venezuela and Colombia, for toxiferine-II and the neutral substance $C_{21}H_{25}O_3N_3$ might very well occur in *Strychnos* species other than *St. toxifera*. The alternative that the calabash-curare alkaloids so far isolated have come from *St. toxifera* and have undergone some change in the native method of preparation of the poison cannot lightly be dismissed as there is evidence that toxiferine-I chloride can undergo change when kept in solution. A further unknown factor is the strong alkalinity of Brockmann's alumina and its effect on these alkaloids. The early acetone percolates from such alumina columns were found to contain triacetone dialcohol, $HO{\cdot}CMe_2{\cdot}CH_2{\cdot}CO{\cdot}CH_2{\cdot}CMe_2{\cdot}OH$, m. p. 57°, produced by trimerisation of the acetone.



A = *Toxiferine-I chloride.*
B = *Toxiferine-III chloride.*

The absorption spectra of toxiferine-I chloride and toxiferine-III chloride have been determined in water (see figure). The curve for the former agrees very well with that given by Wieland, Bähr, and Witkop.

The present communication throws very little light on the chemical structure of these alkaloids. Their colour reactions suggest that they are all indole derivatives, possibly derived biogenetically from tryptophan and retaining the spatial distribution of the two nitrogen atoms of tryptamine. The non-quaternary nitrogen atom is non-basic and is probably the indole-nitrogen atom. This communication does, however, throw considerable light on the formidable problem involved in the isolation of this complex mixture of quaternary alkaloids, which only represent a less soluble fraction of the total alkaloids present, on a sufficient scale for their further study.

I am indebted to Dr. W. D. M. Paton for a preliminary determination of the approximate paralysing activities of 8 out of 12 of the quaternary alkaloids isolated from *Strychnos toxifera*. Toxiferines-I and -III were examined as chlorides, the remainder as picrates by dissolving 3 mg. of each in 10 c.c. of aqueous acetone (water : acetone = 2 : 8) and then diluting the solution with saline as required. No correction has been applied for the differing molecular weights of the anionic components.

	Frog test $E.D._{50}$,* μg./kg.	Rabbit head drop test, mg./kg.
Toxiferine-I chloride	7·5	0·011
,, -III ,,	4600	0·45
,, -IV picrate	100	0·18
,, -V ,,	250	0·40
,, -VI ,,	15	0·008
,, -IX ,,	400	>0·4
,, -XI ,,	15	0·008
,, -XII ,,	>1160	>0·4

* $E.D._{50}$ = Dose effective in paralysing the righting reflex in 50% of the animals.

It will be observed that toxiferines-I, -VI and -XI are of approximately equal potency; on the basis of the frog test these alkaloids are about 200 times as active as *dextro*tubocurarine chloride.

Experimental.

Strychnos toxifera bark (5·6 kg.) was allowed to soak in methyl alcohol (6 l.) and then transferred to 4 large glass percolators, and methyl alcohol was allowed to percolate slowly through the mixture until the alkaloidal reaction of the percolate was very weak. The total percolate (10 l.) was concentrated under reduced pressure to about 1 l., whereafter water (1 l.) was added and the remaining methyl alcohol removed. Water (5 l.) was then added to extract the alkaloids from the fat and resins, which were precipitated, and after some hours the aqueous solution was decanted and the residual fat and resin were extracted with 2 further small portions of water. Finally ether and water were added to the fat, and the aqueous portion was separated. The total aqueous extract was concentrated at 50° to 3·7 l., treated with saturated ammonium reineckate solution (3·5 l.), and kept for 24 hours for the precipitate to become granular. The solid was collected, washed, and dried in a vacuum; yield, 131 g. This was treated with acetone (1310 c.c.) and after a few hours the insoluble reineckates (21 g.) were removed by filtration. The acetone solution (about 1800 c.c.) was transferred to a 12-l. flask fitted with a stirrer, and 8 l. of water at 70° were run in rapidly in a thin stream. When cold the precipitated amorphous solid (38 g.) was collected, dried in a vacuum, redissolved in acetone (380 c.c.), stirred, and treated in a similar way with water (2375 c.c.) warmed to 70°. The precipitated gum (28 g.) was collected after being kept overnight, and dried. The precipitation process was repeated once more, giving finally 23·5 g. of dried reineckates. The various mother-liquors were concentrated below 50°, and 102 g. of crude reineckates were recovered for future examination.

Chromatography.—A column (49 × 4 cm.) containing 700 g. of Brockmann's alumina wetted with 500 c.c. of acetone was prepared, the alkaloidal reineckates (23·5 g.) in acetone (1970 c.c.) were run through, and the column was developed with acetone (1250 c.c.) which gave four broad zones. The uppermost zone (A) was brownish, the second and longest (B) cream-coloured, the third (C) yellow, and the bottom (D) cream-coloured. These probably correspond to the zones 4, 3, 2b and 2a in the diagram of Wieland, Bähr, and Witkop (*Annalen*, 1941, **547**, 174). The cream colour of B and D is superficial and caused by daylight, the interior of these bands being rose-coloured as recorded by these workers. From this column the least adsorbed percolate was yellow and this was followed by a rose-coloured solution. The percolate was collected in a series of flasks in approx. 200-c.c. batches, the first percolate being deep yellow, the second orange-yellow, and the remainder rosy.

A second column of similar size was then prepared and shielded by a black paper collar from direct daylight to prevent superficial bleaching of the bands. The contents of the flasks containing the percolates from the first column were then put through in sequence and the column developed with 625 c.c. of acetone. As before, the percolate was collected in approx. 200-c.c. portions in a series of flasks, the first four being pure yellow in colour, and the fifth containing yellow and red components, whilst the sixth—ninth percolates were rose-coloured only. Inspection by artificial light showed that at this stage the lowest roseate band had completely passed through the column. The second column now showed a very complex appearance, eleven bands being visible, their position and colour from the top downwards being (xi) brown, (x) pink, (ix) deep cream, (viii) pink, (vii) deep cream, (vi) yellow, (v) pink, (iv) yellow, (iii) pink, (ii) deep yellow, and (i) yellow.

The fifth flask of percolate containing red and yellow components was separated into its yellow and its red components by percolation through a third, smaller column containing 250 g. of alumina. Only traces of material were left on the column after development. The yellow percolates, Y, were all combined, and the rosy percolates, R, similarly combined.

The columns were then sectioned; the first gave four fractions A—D, the second gave eleven fractions xi—i, and the non-adsorbed material was contained in fractions R and Y. Each band was then eluted first with acetone, then with methyl alcohol and finally with water, the solvents were removed and the residues converted in each case into chloride by treatment in succession by Kapfhammer's process with excess of silver sulphate solution and exactly with barium chloride solution to remove silver and sulphate ions. Each solution was then taken to dryness, weighed, and warmed with absolute ethyl alcohol and then filtered from inorganic chlorides, mainly sodium chloride which arises from the high alkali content of Brockmann's alumina. Each of the 15 fractions was then kept in a small volume of absolute alcohol in a sealed vessel, but only fractions D and R deposited crystalline alkaloidal chlorides. D corresponds to the band 2a from which Wieland, Bähr, and Witkop isolated toxiferine-I chloride.

After separation of these two crystalline alkaloidal chlorides, each alcoholic solution was evaporated to dryness, the residue dissolved in water, and excess of sodium picrate solution added. The amorphous picrates were dried in each case and weighed. The isolation of crystalline picrates from these fractions was a laborious process. The behaviour of each fraction was as follows.

Fraction A (1·74 g., as chloride) contained 0·27 g. of sodium chloride and gave an amorphous picrate (1·81 g.). This was boiled with acetone (15 c.c.), a gum remaining insoluble from which nothing crystalline could be isolated by a variety of procedures. The main acetone solution did not crystallise on dilution with water (5 c.c.) and was added with agitation to water (400 c.c.) warmed to 70°. When the solution had cooled nearly to room temperature, the amorphous precipitate was collected on a fluted paper, and the filtrate kept so that acetone could slowly evaporate. On prolonged storage a crystalline picrate grew on the walls of the flask in streaks (0·1992 g.). This was dissolved in boiling 90% aqueous acetone (8 c.c.) with difficulty, but when it was in solution a portion (4·5 c.c.) of the acetone was distilled off and the liquor straight-way deposited minute needles of *toxiferine*-IV *picrate*, m. p. 230° (60 mg.). The mother-liquors gave a second crop of the same salt which was incorporated in fraction B (see below, where the properties of toxiferine-IV chloride are given). No other crystalline picrate was obtained from fraction A.

Fraction B (3·625 g., as chloride) gave 1·026 g. of sodium chloride on dissolution in absolute alcohol; the yield of picrate was 1·52 g. This picrate was warmed with acetone (15 c.c.), leaving an insoluble picrate (90 mg.) which on dissolution in 90% acetone (6 c.c.) deposited minute needles of toxiferine-IV picrate, m. p. 225—235° (76 mg.). The main acetone mother-liquor was poured with agitation into water (200 c.c.) at 70°, and just before the temperature of the solution had reached room temperature it was filtered as described above and the solution kept. The filtered solid was redissolved in a small volume of acetone and again poured into water (200 c.c.) at 70°, and this process repeated twice more. In this way 4 filtrates containing a low concentration of acetone were obtained; on long storage, the first three deposited a crystalline picrate in nodules or streaks on the walls of the flasks similar in growth to a mould. The crystalline solid (0·366 g.) was collected and dissolved in 90% acetone (20 c.c.), and a portion of the acetone (7 c.c.) distilled off. Toxiferine-IV picrate (0·186 g.) separated in very small needles, m. p. 234°. This was mixed with the toxiferine-IV picrate (60 mg.) from fraction A and recrystallised from 90% acetone (20 c.c.), some acetone being distilled off after dissolution was effected. Toxiferine-IV picrate crystallised in chrome-yellow minute glistening flat needles (0·136 g.), m. p. 238—239° (Found: C, 54·5; H, 4·8; N, 11·7. $C_{21}H_{27}O_4N_2,C_6H_2O_7N_3$ requires C, 54·1; H, 4·9; N, 11·7%).

The acetone mother-liquors which had given the crop of picrate weighing 186 mg. deposited, on storage, a clearly-defined mixture of red needles (20 mg.) and yellow needles in balls (48 mg.). The latter were clearly toxiferine-IV picrate and on crystallisation from the mother-liquor of the purest material gave a further 100 mg. of toxiferine-IV picrate, m. p. 232°. The red needles (20 mg.) were crystallised from 90% acetone (2 c.c.) and separated in characteristic bronze-coloured needles or leaflets (11·1 mg.), m. p. 270° (decomp.). This is named *toxiferine*-V *picrate* (Found: C, 55·4; H, 4·9; N, 11·9. $C_{21}H_{27}O_3N_2,C_6H_2O_7N_3$ requires C, 55·5; H, 5·0; N, 12·0%).

Fraction C (1·186 g., as chloride) contained sodium chloride (0·29 g.) and gave an amorphous picrate (1·03 g.). This was dissolved in acetone (15 c.c.); on storage the solution deposited an anisotropic granular solid in small quantity, unmelted at 300°. It was boiled with water (25 c.c.) in which it was very sparingly soluble, and the insoluble solid (10 mg.) collected. The aqueous filtrate was concentrated and deposited microscopic plates, trapezoidal in shape. The colour reactions and melting-point behaviour of the two fractions were the same. There was insufficient of the plates for analysis. As this salt is different from the toxiferine-II picrate recorded by Wieland, Bähr, and Witkop which should occur in this fraction, it is called *toxiferine*-VI *picrate* (Found: C, 53·4; H, 4·5; N, 11·4. $C_{21}H_{25}O_5N_2,C_6H_2O_7N_3$ requires C, 52·9; H, 4·4; N, 11·4%). The high m. p. however suggests greater complexity than this formula indicates.

The original acetone mother-liquor was poured with agitation into 300 c.c. of water at 70°, and filtered from amorphous matter when the temperature had almost fallen to room temperature, and the filtrate kept. The amorphous solid was dissolved in acetone, the solution poured into 300 c.c. of water at 70°, the precipitated solid collected when the solution had cooled, and the whole process repeated once more. On long storage the first two filtrates deposited nodular crystals (88 mg.) which were collected and dissolved in 90% aqueous acetone, and some acetone was distilled off. A picrate separated in compact crystals (11 mg.) in nodular form, m. p. 200° (decomp.; after sintering) (Found: C, 53·8; H, 5·2; N, 12·3. Calc. for $C_{20}H_{29}O_3N_2,C_6H_2O_7N_3$: C, 54·4; H, 5·4; N, 12·2%). It is possible that this picrate is a slightly impure form of toxiferine-II picrate described by Wieland, Bähr, and Witkop. These authors found toxiferine-II in this fraction of the chromatogram and give a m. p. 216° and evidence in favour of a formula $C_{20}H_{25}ON_2,C_6H_2O_7N_3,2H_2O$. Both salts give a carmine colour with nitric acid.

Fraction D (0·96 g., as chloride) gave 0·16 g. of sodium chloride on dissolution in absolute acohol. The alcoholic mother-liquor on concentration readily deposited needles (0·15 g.) which on recrystallisation from absolute alcohol (7 c.c.) gave toxiferine-I chloride (0·105 g.) (Found, on air-dried solid: C, 60·5; H, 8·0; N, 7·1. Calc. for $C_{20}H_{23}ON_2Cl,3H_2O$: C, 60·5; H, 7·4; N, 7·1%. Drying at 100° removed only a portion of the water. Found: loss, 5·8. Calc. for $C_{20}H_{23}ON_2Cl,3H_2O$, losing 1·5 H_2O: H_2O, 6·8%. Found, on solid dried at 100°: C, 65·4; H, 6·8. Calc. for $C_{20}H_{23}ON_2Cl,1{\cdot}5H_2O$: C, 64·9; H, 7·1%). Wieland and his collaborators describe this salt as a dihydrate, losing all its water of crystallisation at 80° in a vacuum. A portion of this salt (12 mg.) gave an amorphous picrate on precipitation with sodium picrate solution. This derivative was dried and crystallised from 90% aqueous acetone by boiling off some of the acetone, and separated in glistening elongated leaflets (14·3 mg.), m. p. 278—280° (decomp.). Wieland, Bähr, and Witkop record m. p. 270° and publish a photograph of the crystals with which the above preparation is in agreement. Toxiferine-I chloride gives a brownish-green colour with strong nitric acid and dissolves in concentrated sulphuric acid to a colourless solution, in agreement with the observations of Wieland. On the other hand, with dichromate in 50% sulphuric acid toxiferine-I chloride gives an intense bluish-purple colour, whereas Wieland and his collaborators find that it gives a carmine colour.

The final alcoholic recrystallisation mother-liquor of toxiferine-I chloride was evaporated to dryness at <40°, the residue dissolved in water, and sodium picrate solution added. The amorphous picrate

(80 mg.) was crystallised from aqueous acetone. It separated in microscopic triangular plates (11 mg.), unmelted at 308° (Found: C, 58·0; H, 4·6; N, 12·6. $C_{21}H_{25}O_2N_2,C_6H_2N_3O_7$ requires C, 57·3; H, 4·8; N, 12·4%). By careful working through of the mother-liquors another preparation, microscopic plates in clusters (14·8 mg.), unchanged at 300°, was obtained (Found: C, 57·3; H, 5·6; N, 12·5%). Both crops gave the same colour reactions—a rosy solution with strong nitric acid, a very pale straw-coloured solution in strong sulphuric acid, and an intense purple, changing to a redder shade, with dichromate in 50% sulphuric acid. The facts that the picrate is unmelted at 300° and that it came from a solution which should have given toxiferine-I picrate suggests that the toxiferine-I might have polymerised with loss of water and possibly formation of an ether link. The formula $C_{40}H_{44}ON_4,2C_6H_2O_7N_3,2H_2O$ (requiring C, 57·3; H, 4·8; and N, 12·9%) would also fit the analyses of both preparations. This picrate is named *toxiferine*-VII *picrate*.

That toxiferine-I chloride is changeable is supported by the following observation. The original alcoholic mother-liquor which gave toxiferine-I chloride was concentrated and when cold deposited a further crop of needles identical in appearance with the previous crop of toxiferine-I chloride. The flask was sealed with an air-tight glass stopper and, when kept for some weeks, the needles slowly dissolved. The alcohol was removed, the residue dissolved in water, and sodium picrate solution added. The amorphous picrate (0·5 g.) was dissolved in hot aqueous acetone, and eventually compact clusters of a crystalline picrate (19 mg.) were obtained, unmelted at 310° (Found: C, 55·6; H, 4·5; N, 13·6%). The colour reactions (rose to carmine with nitric acid, bluish-purple passing to carmine with dichromate in 50% sulphuric acid, and straw-coloured in strong sulphuric acid) resembled those of toxiferine-VII picrate, but the analytical figures show a considerable discrepancy. The amount of material available precluded further purification.

Column 2. *Fractions* xi—i. Each of these fractions was freed from alcohol-insoluble material, mainly sodium chloride, and the residues were converted into picrates, all of which were amorphous at first. Each fraction was separately dissolved in hot acetone and kept. Those which did not crystallise were treated with a little water, and the acetone was then allowed to evaporate off very slowly at room temperature. Fractions viii—v yielded no crystalline material.

Fractions xi—ix on slow evaporation deposited a crystalline picrate in pale yellow nodular forms. As they seemed to be identical they were combined (yield, 61 mg.). They were dissolved in boiling 90% aqueous acetone, and a portion of the acetone was boiled off. When the solution was then kept, *toxiferine*-VIII *picrate* separated in balls of needles (41 mg.), unmelted at 300° (Found: C, 57·3; H, 4·8; N, 11·8. $C_{22}H_{25}O_3N_2,C_6H_2O_7N_3$ requires C, 56·6; H, 4·6; N, 11·8%). The colour reactions are recorded in the table. The high melting point of the picrate suggests a more complex structure, possibly a double molecule. As fractions xi—ix are adjacent to fraction D of the first column, it is possible that this picrate is a purer form of the second high-melting picrate found in fraction D.

Fractions iv—i. These fractions, as picrates, when dissolved in pure acetone deposited microscopic crystals, the amount from fractions iv—ii being very small, whilst that from i amounted to 37·7 mg. This was boiled with acetone but remained insoluble and, as it is clearly distinct from any of the previously described salts, is named *toxiferine*-IX *picrate*. It was unmelted at 300° and showed the colour reactions recorded in the table (Found: C, 56·9; H, 4·8; N, 11·5. $C_{23}H_{27}O_3N_2,C_6H_2O_7N_3$ requires C, 57·3; H, 4·8; N, 11·5%). The high m. p. and insolubility in boiling acetone again suggest a more complex structure.

The filtrates of fractions iv—i were each treated with a little water and allowed to evaporate very slowly at room temperature. Each deposited a picrate in nodular form. As they were similar in appearance they were combined, dissolved in boiling 90% acetone in which they were slow to dissolve, and on long storage the solution deposited a picrate (3 mg.) as clusters of plates each shaped like an isosceles triangle. There was insufficient for analysis. Nitric acid gave a carmine solution, sulphuric acid no colour, whilst the Otto–Wieland reagent gave an intense bluish-purple tending to become redder.

Fraction R. This roseate solution, obtained from the development of column 3, on removal of the acetone and conversion into chloride, left a syrup (6·85 g.). This was dissolved in absolute alcohol and deposited inorganic salts (60 mg.). The alcohol was removed and the syrupy residue extracted with dry ether which removed a liquid (2·2 g.) which, when kept, gradually crystallised in large plates. These were recrystallised from low-boiling light petroleum and separated in long needles, m. p. 59° (Found: C, 61·5; 61·9; H, 9·6, 9·8. Calc. for $C_9H_{18}O_3$: C, 62·0; H, 10·4%). It was identified as triacetone dialcohol. It had evidently been formed by the catalytic effect of the alkali in Brockmann alumina on the acetone. D.R.-P. 481,290 describes triacetone dialcohol, m. p. 57°, as being produced by the action of solid alkali hydroxides on a mixture of acetone and diacetone alcohol.

Hesse, Reicheneder, and Eysenbach (*Annalen*, 1938, **537**, 67) describe the formation of diacetone alcohol when using acetone as the solvent with alumina columns, and this was confirmed by Karrer and Schmid (*Helv. Chim. Acta*, 1946, **29**, 1862), but the above seems to be the first recorded production of the symmetrical condensation product. Possibly a catalytic process for production of triacetone dialcohol could be based on these observations.

The residue insoluble in dry ether was dissolved in a small volume of absolute alcohol, and on storage the solution slowly deposited a crust of minute plates or needles. These were collected (0·118 g.) and crystallised from a small volume of water from which *toxiferine*-III *chloride* separated in clear tablets (88 mg.), m. p. 285° (decomp.) (Found, on air-dry solid: C, 60·5; H, 8·2; N, 7·1%; loss at 100°, 11·2. $C_{20}H_{37}ON_2Cl,3H_2O$ requires C, 59·9; H, 8·3; N, 7·0; $3H_2O$, 11·7%). The analytical figures correspond to a tetrahydrotoxiferine-I chloride but there is no other supporting evidence. A portion of pure toxiferine-III chloride in water was precipitated with sodium picrate solution to yield an amorphous picrate; all attempts to crystallise this failed. However, the aqueous mother-liquor from the final recrystallisation of toxiferine-III chloride on precipitation with sodium picrate solution gave an amorphous picrate (26 mg.) which on dissolution in aqueous acetone deposited needles, but insufficient for characterisation.

The original alcoholic mother-liquor from toxiferine-III chloride was evaporated to dryness, and the residue dissolved in water and converted into the picrate (3·95 g.). This failed to crystallise from aqueous acetone in the usual way but two new picrates were eventually obtained by the following process. The acetone solution was poured into 50 c.c. of water, heated to 70° with swirling of the contents, and when nearly cool was filtered on a fluted paper, and the filtrate R_1 kept. The contents of the fluted paper were dissolved in 20 c.c. of acetone and added to 200 c.c. of water at 70° with swirling. When nearly cool the solution was filtered as before and the filtrate R_2 kept. This process was repeated six more times, the volume of water for the last three treatments being raised to 400 c.c. each. In this way the solid filtered off on the final fluted paper was a fine powder from which all readily soluble picrates had been removed. It was dried (0·54 g.) and dissolved in aqueous acetone; crystals were gradually deposited and after two more crystallisations from aqueous acetone separated in fine needles growing in tufts (16 mg.). When heated it charred from 255° upwards and exploded at about 268°. *Toxiferine*-X *picrate* has distinctive properties (Found: C, 59·2; H, 5·0; N, 13·9. $C_{19}H_{23}N_2,C_6H_2O_7N_3$ requires C, 59·2; H, 5·0; N, 13·8%).

The eight aqueous acetone solutions, on storage in conical flasks exposed to the air, slowly lost acetone and solutions R_1 to R_7 all deposited orange needles together with some amorphous matter. These deposits were crystallised twice from aqueous acetone and gave *toxiferine*-XI *picrate* (53 mg.), m. p. 277°, which crystallised in orange-coloured thin pointed blades (Found: C, 58·3; H, 5·3; N, 12·7. $C_{21}H_{27}ON_2,C_6H_2O_7N_3$ requires C, 58·8; H, 5·3; N, 12·7%). The colour reactions of this salt are highly characteristic except that they resemble those of toxiferine-V picrate. On admixture of the two there was no definite lowering of m. p. As these two salts originate from widely different bands and analytical figures show a wide divergence they are regarded as different substances, and this view is supported by their relative curarising activities. Toxiferine-XI picrate also resembles toxiferine-I picrate in its colour reactions, biological activity, melting point, and appearance. As they are concentrated in widely differing parts of the chromatographic columns, toxiferine-XI reineckate having very little affinity for the alumina, they must for the present be regarded as different substances.

Fraction Y. This yellow acetone solution represents the least adsorbable components of the columns. After conversion of the salts into chloride and removal of sodium chloride, a syrup (0·98 g.) was left from which dry ether removed a liquid which gradually crystallised almost completely, to give triacetone dialcohol. The ether-insoluble residue (0·43 g.) was converted eventually into picrate (0·4 g.). It dissolved immediately in acetone and straightway crystallised (0·11 g.). It was recrystallised from boiling 90% aqueous acetone and separated in orange needles or flattened prisms with striations (70 mg.). As this salt differs from the previous fractions it is called *toxiferine*-XII *picrate*. When heated it darkens at about 230° but is unmelted at 333°. The mother-liquors gave a further 17 mg. which gave similar analytical figures to those given by the first crop (Found, on first crop dried at 100°: C, 59·0, 58·5; H, 4·7, 4·9; N, 13·4. On second crop: C, 59·1, 58·8; H, 5·3, 5·0; N, 13·6. $C_{39}H_{46}ON_4,2C_6H_2O_7N_3$ requires C, 58·7; H, 4·8; N, 13·4%). It is difficult to fit the analytical figures to a molecular size comparable with toxiferine-I, *i.e.*, a C_{20} or closely similar unit; the high m. p. and insolubility in pure acetone suggest a more complex or double structure.

Chromatography of Reineckates from 465 *g. of* St. toxifera *Bark.*—A preliminary experiment on 6·2 g. of alkaloid reineckates from 465 g. of *St. toxifera* bark using a 48 × 3-cm. column of Brockmann's alumina (320 g.) gave a series of bands and a yellow percolate, very similar to the distribution shown in the diagram of Wieland, Bähr, and Witkop (*Annalen*, 1941, **547**, 174). In the light of the findings of the larger-scale experiment described above, toxiferine-IV picrate was isolated from the second band from the top of the column, toxiferine-VI and toxiferine-I picrate from the third band, and toxiferine-XI from the percolate. None was obtained sufficiently pure for analysis. However, from the fifth band, a substance sparingly soluble in alcohol was obtained which was then crystallised from water and separated in needles (34·9 mg.), m. p. 292° (decomp.) (Found: C, 68·2; H, 6·4; N, 11·3. Calc. for $C_{21}H_{25}O_3N_3$: C, 68·7; H, 6·8; N, 11·4%). With nitric acid it gave a bright yellow solution. The substance seems to be identical with the by-product of the same formula isolated by Wieland, Bähr, and Witkop (*loc. cit.*) from a similar position on an alumina column used for examining the alkaloids of a calabash-curare from Caracas, Venezuela.

Fractional Precipitation of the Alkaloids of St. toxifera *with Sodium Picrate Solution.*—The alkaloidal chlorides (12·56 g.) from 2·8 kg. of *St. toxifera* bark were prepared through the reineckates (19·9 g.) in the usual way and dissolved in 20 volumes of water and fractionally precipitated, with vigorous stirring, by adding 10 successive portions each of 28 c.c. of saturated sodium picrate solution. The initial starting material had an activity on the rat's diaphragm two-sevenths that of *dextro*tubocurarine chloride, according to Dr. B. D. Burns of this Institute. Each picrate fraction when dry was treated with pure acetone, the earlier fractions containing picrates, to the extent of 20—40%, insoluble in acetone. These have not been examined further. Each picrate solution was then added to about 10 volumes of water at 80°, the precipitated solid in each case being collected on a fluted filter paper before the solution was cold and then redissolved in a little acetone, and the process repeated until very little precipitable material was left. On long storage the filtrates slowly lost acetone, and crystalline material in the form of either reddish needles or very pale nodules separated from many of the fractions. A number of these fractions containing crystalline material of similar appearance, *viz.*, needles with a certain amount of amorphous matter, were collected (yield 0·73 g.). This material was dissolved in acetone (30 c.c.) and poured into water (300 c.c.) at 80°. After removal of a little amorphous precipitate when the temperature of the solution had fallen to 25°, the filtrate was kept and slowly deposited reddish-orange leaflets (0·1 g.). These were crystallised from 1 c.c. of 90% acetone with removal of some of the acetone by boiling, and gave crude toxiferine-V picrate, decomposing about 250°. Its identity was confirmed by direct comparison with toxiferine-V picrate described earlier. Characteristic was the reaction with strong nitric acid—a deep-green flash becoming a brownish-orange—and the pure blue colour produced by solution in strong sulphuric acid.

As chromatography revealed the presence of more than 12 alkaloids the fractionation of the picrates was not continued, but there is no doubt that other toxiferines could be isolated in a similar way and

that the unknown effect of the alkali contained in Brockmann's alumina on these substances could be thus avoided.

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