368. *Alkaloids of Calabash-curare and* Strychnos *Species. Part II.* Isolation of New Alkaloids.*

By A. R. Battersby, R. Binks, H. F. Hodson, and D. A. Yeowell.

The bark of *Strychnos toxifera* Schomb. has been found to contain mainly quaternary alkaloids, together with a small amount of non-quaternary bases. Fractionation of the quaternary material by adsorption and partition chromatography has yielded three new alkaloids named hemitoxiferine-I, macusine-A, and macusine-B, together with the known xanthocurine. The properties of the new alkaloids are described.

The series of toxiferines isolated by King [6] from *Strychnos toxifera* bark has been re-examined and several of the alkaloids have been identified.

The powerful physiological effect of the various types of plant extract known as " curare " has stimulated much interest in their chemical investigation. The studies described here and in other Parts of this series are concerned mainly with the alkaloids of calabash-curare and of *Strychnos* species.

The first important chemical investigations of the alkaloids of calabash-curare were made by Wieland and his collaborators.[1-4] The total quaternary alkaloid mixture obtained as the reineckate complex was subjected to a preliminary separation by repeated precipitation with water from acetone solution. The sparingly soluble reineckate fraction, which accounted for nearly all the curare-activity of the total quaternary bases, was further fractionated by chromatography on acid-washed alumina. C-Curarine chloride was the first crystalline calabash-curare alkaloid thus to be isolated.[1]

The barks of *Strychnos toxifera* and other *Strychnos* species have long been known to be important constituents of many South American calabash-curares and in 1947 Wieland, Bähr, and Witkop [4] described an investigation, essentially by the above method, of the quaternary bases of *S. toxifera* bark from British Guiana. They isolated in addition to toxiferine-II,† also obtained [4] from calabash-curare, a new crystalline alkaloid, toxiferine-I chloride with physiological activity surpassing that of any alkaloid then known. Subsequently toxiferine-I chloride, also designated C-toxiferine-I chloride, was isolated by Karrer and Schmid [5] from a calabash-curare of Venezuelan origin.

In 1949, King [6] reported an examination of the quaternary alkaloids of the bark of *S. toxifera* obtained from British Guiana; this is almost certainly the plant examined by Wieland. By chromatography of the quaternary reineckates, he isolated toxiferine-I chloride and toxiferine-II, together with a series of alkaloids designated toxiferines III—XII.

Partition chromatography on powdered cellulose [7,8] has proved the most successful method for fractionation of the complex quaternary alkaloid mixtures obtained from calabash-curare and *Strychnos* species; more than 60 alkaloids have been isolated so far in this way. The Zurich group obtained [9,10] mavacurine, fluorocurine, fedamazine, and the tertiary bases caracurines I—IX and nordihydrotoxiferine from *S. toxifera* of Venezuelan origin.

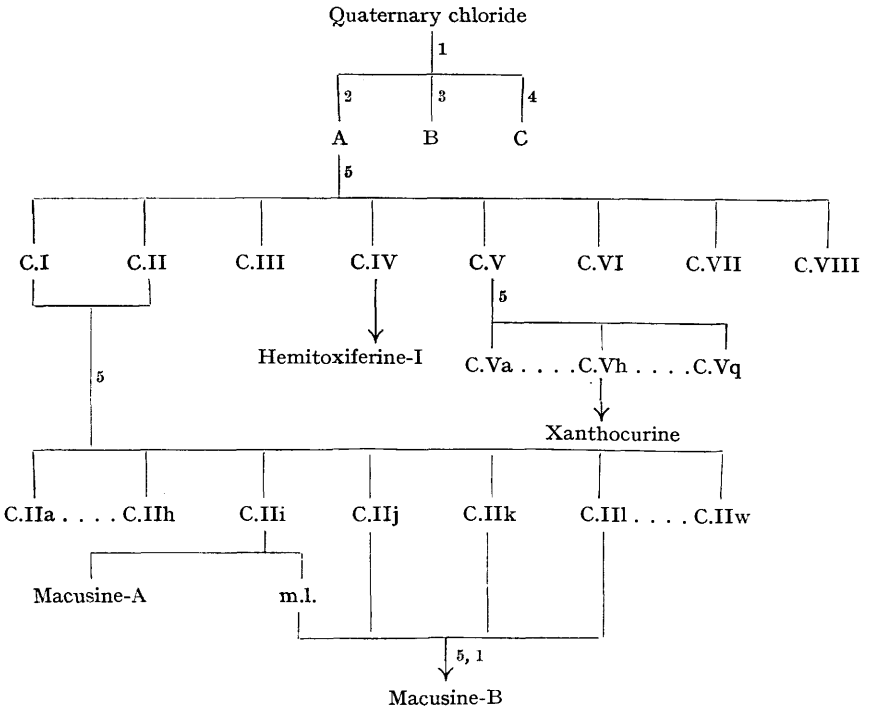
* Part I, *J.*, 1960, 736.

† The nomenclature in this series is very confused. Toxiferine-II is not the same alkaloid as C-toxiferine-II; the latter is identical with C-calebassine.

[1] Wieland, Konz, and Sonderhoff, *Annalen*, 1937, **527**, 160.
[2] Wieland and Pistor, *Annalen*, 1938, **536**, 68.
[3] Wieland, Pistor, and Bähr, *Annalen*, 1941, **547**, 140.
[4] Wieland, Bähr, and Witkop, *Annalen*, 1941, **547**, 156.
[5] Schmid and Karrer, *Helv. Chim. Acta*, 1947, **30**, 1162.
[6] King, *J.*, 1949, 3263.
[7] Schmid, Kebrle, and Karrer, *Helv. Chim. Acta*, 1952, **35**, 1864.
[8] Wieland and Merz, *Chem. Ber.*, 1952, **85**, 731.
[9] Äsmis, Schmid, and Karrer, *Helv. Chim, Acta*, 1954, **37**, 1983.
[10] Äsmis, Waser, Schmid, and Karrer, *Helv. Chim. Acta*, 1955, **38**, 1661.

We now report an investigation of the quaternary alkaloids from the bark of *S. toxifera* Schomb. obtained from British Guiana. The alkaloid content of the bark was almost identical with that of the *S. toxifera* bark examined by King,[6] as shown by two-dimensional paper-chromatographic comparison of the total quaternary alkaloids from the two materials. King's examination of the bark included Wieland's separation into " easily soluble " and " sparingly soluble " reineckate fractions, of which only the latter was further fractionated: the present study was of the total alkaloid content of the bark.

The finely ground bark was exhaustively percolated with 1 : 4 aqueous methanol to give a solution from which the tertiary bases were extracted. This revealed a sharp difference in alkaloid content between the *S. toxifera* bark from British Guiana and the bark of the same species from Venezuela.[9] None of the alkaloids described later in this paper, or toxiferine-I obtained by Wieland and by King, was isolated from the Venezuelan material. Most of the crystalline alkaloids isolated from the latter bark are tertiary bases, whereas the tertiary base fraction from our material represents only about 1% of the total alkaloid content. These tertiary bases have as yet been examined only by paper chromatography which indicates that caracurine-VII is the major component.



1, Al_2O_3 column. 2, Elute with EtOH. 3, Elute with MeOH. 4, Elute with aq. MeOH. 5, Partition chromatography on cellulose.

Apart from the step to remove tertiary bases, our fractionation followed closely that used by earlier workers.[6,9,11] The quaternary bases were precipitated by aqueous ammonium reineckate at pH 2 and the acetone-soluble part of the reineckate complex was converted into quaternary chloride by Kapfhammer and Bischoff's method.[12] The chloride mixture in ethanol was adsorbed on to a column of acid-washed alumina from which three fractions, A, B, and C, were obtained by elution successively with ethanol, methanol, and 50% aqueous methanol; fractions B and C have so far not been examined.

A preliminary study by partition chromatography on powdered cellulose of fraction

[11] Bächli, Vamvacas, Schmid, and Karrer, *Helv. Chim. Acta*, 1957, **40**, 1167.
[12] Kapfhammer and Bischoff, *Z. physiol. Chem.*, 1930, **191**, 182.

A showed that about two-thirds of the chloride mixture was rapidly eluted from the column and consisted of a mixture of alkaloids with R_C values greater than 1·5 [R_C gives movement-rate on chromatograms relative to C-curarine I (taken as 1·0)]. The components of this fast-running fraction, which almost certainly corresponds to the uninvestigated " easily-soluble " reineckate fraction of King, had ultraviolet spectra characteristic of 2,3-disubstituted indoles (*e.g.*, yohimbine types). Therefore a large quantity of fraction A chloride was examined by partition chromatography with a large alkaloid : cellulose ratio, to isolate the fast-running fraction and subsequently to separate the components of this fraction by chromatography with a much lower alkaloid : cellulose ratio. The diagram shows this approach together with an indication of the further columns which were necessary to obtain crystalline alkaloids. Many fractions have yet to be fully examined, but the work so far has yielded three new quaternary alkaloids. The isolation of one, hemitoxiferine-I, has been briefly recorded earlier,[13] and its structure and chemistry are discussed in Part I of this series.[14] The other two alkaloids are named macusine-A and macusine-B after the Macusi tribe of Indians who carried out the first investigations on *Strychnos toxifera*;[15] we intend to continue this system of nomenclature for other new alkaloids from *S. toxifera*.

Macusine-A, $C_{22}H_{27-29}O_3N_2^+$, has been characterised as the crystalline chloride, iodide, and picrate; macusine-B, $C_{20}H_{25-27}ON_2^+$, as the chloride, iodide, and perchlorate. Both alkaloids have ultraviolet spectra characteristic of 2,3-disubstituted indoles and almost identical with that of yohimbine methochloride. The infrared spectrum of macusine-A has bands at 3320 (OH), 3120 (⩾NH), and 1729 cm.$^{-1}$ (CO_2R), whereas that of macusine-B shows bands at 3310 (OH) and 3210 cm.$^{-1}$ (⩾NH) and no ester band.

In addition to the new alkaloids, a small quantity of a yellow quaternary alkaloid was isolated. This was shown with very high probability to be xanthocurine, an alkaloid of unknown structure, previously obtained[16] from a Columbian calabash. Our alkaloid corresponded closely with authentic xanthocurine in chromatographic behaviour in three solvent systems, in ultraviolet absorption including the marked bathochromic shift in alkaline solution, in rotation, and in colour reaction; the infrared spectrum of our material was very similar to that of the authentic sample, but a completely satisfactory spectral comparison was not possible owing to lack of material.

During preliminary work, a small quantity of a sparingly soluble alkaloid was isolated from the material which moved most rapidly down the partition column. This has proved to be macusine-B thiocyanate; undoubtedly the anion is derived from the ammonium reineckate used to precipitate the alkaloids. The properties of this thiocyanate called to mind those of a so-called " neutral product " isolated first by Wieland, Bähr, and Witkop[4] from Venezuelan calabash-curare and later by King[6] from *S. toxifera*. The infrared and ultraviolet spectra of King's sample of this material show conclusively that it is the thiocyanate of a quaternary 2,3-disubstituted indole. The analyses which King and the German workers gave in support of formula $C_{21}H_{25}O_3N_3$ for this substance also fit $C_{20}H_{25}ON_2^+CNS^-$ and cannot exclude $C_{20}H_{27}ON_2^+CNS^-$. Indeed, it is probable from the infrared spectrum of King's sample that it is identical with macusine-B thiocyanate; however, the minute amount of his material available to us prevented our getting a spectrum of the usual quality.

It is convenient at this stage to summarise the results of investigations made on the other alkaloid samples obtained by the late Dr. H. King in his isolation work on *S. toxifera* bark. Until now the relationship of toxiferines III—XII to the other sixty or so calabash-curare and *Strychnos* alkaloids isolated since 1949 was unknown. Toxiferine-I and toxiferine-III had been stored as quaternary chlorides; the remaining alkaloids were as

[13] Battersby and Hodson, *Proc. Chem. Soc.*, 1958, 287.
[14] Battersby and Hodson, *J.*, 1960, 736.
[15] McIntyre, " Curare, Its Natural History and Clinical Use," University of Chicago Press, Chicago, 1947.
[16] Giesbrecht, Meyer, Bächli, Schmid, and Karrer, *Helv. Chim. Acta*, 1954, **37**, 1974.

the quaternary picrates and for the work described below were converted into chlorides by ion-exchange. There was insufficient toxiferine-VI for examination even by the micromethods useful in this field; no sample of toxiferine-VII was available.

Toxiferine-I chloride had decomposed considerably; the picrate was homogeneous and apparently is quite stable.

Toxiferine-III chloride had decomposed completely to red material which gave many coloured and fluorescent spots on paper chromatography.

Toxiferine-IV was shown by colour reactions, ultraviolet spectrum and paper chromatography to be largely C-alkaloid A; [7] it contains two impurities with similar colour reactions and ultraviolet spectrum but with different R_C values.

Toxiferine-V shows colour reactions, ultraviolet spectrum, and R_C values identical with those of toxiferine-I. It behaves exactly as does the latter alkaloid on treatment with dilute sulphuric acid; [13,14] this reaction was investigated by paper chromatography. King records toxiferine-V picrate, m. p. 270°, and toxiferine-I picrate, m. p. 278°, but our results leave no doubt that toxiferine-V is identical with toxiferine-I although King [6] reports its physiological activity to be considerably lower than that of toxiferine-I. Since the physiological activity reported for toxiferine-VI corresponds exactly with the unusually high activity of toxiferine-I, it may well be that in some way the test samples of toxiferine-V and toxiferine-VI were interchanged.

Toxiferine-VIII is homogeneous, with R_C 0·63 in solvent system " C "; its ultraviolet spectrum is identical with that of curacurine-IX methochloride [10] (R_C 1·1), but the two alkaloids differ so much in R_C values that they cannot be identical.

Toxiferine-IX has been shown to be identical with caracurine-II methochloride [14] by colour reactions, ultraviolet spectrum, and R_C value in solvent system " C "; the picrates of both alkaloids are unmelted at 300° and they have identical infrared spectra.

Toxiferine-X and -XII both have colour reactions and ultraviolet spectra characteristic of the toxiferine group of alkaloids, *i.e.*, they are methylene indolines. Toxiferine-XII has R_C 2·3 in solvent system " C "; toxiferine-X is inhomogeneous.

Toxiferine-XI is identical with toxiferine-I in all its properties, including infrared spectrum (Nujol and KBr disc) and degradation with dilute mineral acid.[13,14]

Early in our work a re-investigation was made of the separation of the quaternary alkaloids by adsorption chromatography of the alkaloid reineckates on alumina. A chromatographically homogeneous fraction, after conversion into the chloride, was shown by paper chromatography to consist of five major components. This result and the remarkable fact that in King's original separation it is now established that toxiferine-I was isolated from three widely spaced positions on the adsorption column show clearly that this method of fractionation is inferior to partition chromatography.

Experimental

Cellulose columns were packed dry and were washed and eluted with solvent which had been freed from peroxide by passage through "Amberlite " IR-120 resin in the ferrous phase [17] before entering the cellulose column. Solvent system " C " is water-saturated ethyl methyl ketone [7] containing stated amounts of methanol. Alumina was washed with 2N-hydrochloric acid, then with water until neutral, and dried at 110°. Solutions were evaporated at $\not>50°$ under a reduced pressure of nitrogen. Samples for analysis, determination of rotation, and ultraviolet and infrared absorption were dried at 100° over phosphoric oxide *in vacuo* unless otherwise stated.

Isolation of Alkaloids as Reineckate Complex.—Finely ground *S. toxifera* bark (15 kg.) was percolated with 80% aqueous methanol until the percolate gave only a weak colour with the ceric sulphate reagent; [7] the bark was then exhaustively percolated with 2% aqueous acetic acid. The total aqueous-methanolic percolate, in five portions (i)—(v), was evaporated as far as possible to dryness. The resinous residues (303 g., 221 g., 385 g., 393 g., and 307 g.) were treated separately with methanol (600—700 ml. per 100 g. of solid) at 35° with vigorous

[17] Zürcher, Ceder, and Boekelheide, *J. Amer. Chem. Soc.*, 1958, **80**, 1500.

swirling until they were broken down into solutions containing flocculent insoluble material. Each mixture was filtered through " Filtercel," and the filter-pad washed exhaustively with methanol. When kept overnight the filtrate deposited some resin; the solution was decanted and is here described as the " methanol-soluble fraction." The filter-pad and deposited resin were extracted exhaustively with water, and this solution is described as the " water-soluble fraction."

The " methanol-soluble fraction " from portions (i) and (ii) was reduced to *ca.* 1·5 l. and treated with water (2 l.). Concentrated hydrochloric acid (30 ml.) was added to the aqueous-methanolic solution until acid to Congo Red, and the methanol was then removed. The clear aqueous solution was extracted with 4 : 1 ether–chloroform (4 × 550 ml.) to remove neutral material, adjusted to pH 9 with an excess of ammonia, and again extracted with 4 : 1 ether–chloroform (4 × 550 ml.). Much material which was precipitated on basification was insoluble in ether–chloroform and, after the first extraction, the whole mixture was filtered through a sintered-glass funnel; the solid so removed is described as " solid D." Evaporation of the combined organic extracts left the non-quaternary bases as a dark resin (2 g.).

The aqueous, alkaline solution was adjusted to pH 2 with concentrated hydrochloric acid and stirred while an excess of ammonium reineckate solution (125 g. of ammonium reineckate in 2·5 l. of 2 : 1 water–acetic acid) was added. The precipitated complex was collected after 18 hr. at room temperature, washed thoroughly with water, then with ether, and dried at 35° *in vacuo* to give the quaternary reineckate complex (101 g.). Portions (iii), (iv), and (v), treated in the same manner, gave a further amount (180 g.) of reineckate complex.

The water-soluble fractions from portions (i)—(v) were combined, adjusted to pH 2 with concentrated hydrochloric acid, and treated with ammonium reineckate as above to give reineckate complex (20 g.).

The 2% acetic acid bark extract was separated as above into a " methanol-soluble fraction," a " water-soluble fraction," and " solid D." The " methanol-soluble fraction " gave some reineckate complex (70 g.) whereas the " water-soluble fraction " yielded a negligible amount.

" Solid D " from both aqueous-methanol and aqueous-acetic acid bark extracts was treated with 0·1N-hydrochloric acid, and the resulting solution decanted from insoluble matter and treated in the usual way with ammonium reineckate solution. The residue insoluble in hydrochloric acid was dissolved in 5% aqueous acetic acid, and then also treated with ammonium reineckate solution. The combined precipitates were collected and dried to give the reineckate complex (70 g.).

Fractionation of Alkaloids.—(a) *Isolation of hemitoxiferine-I.* The reineckate (200 g.) from the " methanol-soluble fraction " and the " water-soluble fraction " from the original 80% aqueous methanol bark extract were dissolved in acetone, the insoluble portion (21 g.) was rejected, and the soluble fraction converted into the chloride by Kapfhammer and Bischoff's method [12] as described by Äsmis, Schmid, and Karrer.[9] The total chloride (133 g.) was treated with ethanol (3 l.) and after removal of an insoluble residue (27 g.) was adsorbed on a column of acid-washed alumina (900 g.). Continued elution with ethanol (5·9 l.) gave fraction A (58 g.), and elution successively with methanol (2·8 l.) and 50% aqueous methanol (2·4 l.) gave fractions B (22·7 g.) and C (18·3 g.), all as brown resins.

Fraction A was dissolved in the minimum amount of absolute ethanol, and the solution filtered to remove ammonium chloride (6 g.). The filtrate was evaporated to dryness and a solution of the residue (52 g.) in solvent system " C " containing 6% of methanol (2·3 l.) was divided into two portions each of which was run on to a column of cellulose powder (600 g.; 66 × 5·5 cm.). Elution was continued with solvent system " C " containing 3% of methanol. Based on the results from a trial experiment seven fractions C.I—C.VII were collected from each column. The corresponding fractions from each column were combined, the total volumes and alkaloid contents of the various fractions being as follows:

	C.I	C.II	C.III	C.IV	C.V	C.VI	C.VII
Vol. (l.)	5	2·9	1·2	1·2	1·6	1	0·8
Alkaloid content (g.)	1·7	28·2	5·5	3·8	1·8	0·7	1·7

A final fraction C.VIII (10·2 g.) was obtained by washing the column with methanol (6 l.).

Fraction C.IV as a syrup in methanol readily gave crystals which were collected and recrystallised from ethanol (to give 0·8 g.) and from ethanol–ether to give pure hemitoxiferine-I chloride (alkaloid A8) (0·55 g.), m. p. >300°, $[\alpha]_D^{22}$ −41·0° (*c* 1·0 in H_2O). This alkaloid has

R_G 1·5 in solvent system " C " (for R_G values see p. 1850); it gives a persistent orange colour with the ceric sulphate reagent and a lemon-yellow colour with cinnamaldehyde and hydrogen chloride. The chloride is hygroscopic and satisfactory analyses could not be obtained; partially synthetic [14] hemitoxiferine-I chloride gave similar analytical results. The ultraviolet absorption of the natural alkaloid in water was: $\lambda_{max.}$ 241, 293, $\lambda_{min.}$ 225, 269 mμ (log ε 3·74, 3·33, 3·53, 2·86 respectively).

Hemitoxiferine-I picrate crystallised from aqueous acetone as yellow needles or elongated prisms, m. p. 234—236° (Found: C, 56·4; H, 5·1. $C_{20}H_{25}O_2N_2,C_6H_2O_7N_3$ requires C, 56·5; H, 4·9%).

(b) *Isolation of macusine-A.* Fraction C.II was combined with fraction C.I and further examined by partition chromatography on a second cellulose column (3 kg.; 160 × 7·6 cm.) with ethyl methyl ketone saturated with water as the developing solvent; 23 fractions C.IIa—C.IIw were obtained, the cuts being made on the basis of examination of the column in ultraviolet light. Fraction C.IIi (1·9 g.) readily crystallised from an ethanol syrup, to give *macusine-A chloride* (450 mg.); evaporation of the mother-liquor to dryness and crystallisation of the residue from water yielded a further crop (450 mg.) of the same material. Two recrystallisations from ethanol–ether gave the pure alkaloid as colourless prisms, m. p. 252° after previous darkening at 240° and sintering at 248°, $[\alpha]_D^{25}$ −57·5° ± 1·5° (*c* 1·455 in H_2O). The colour reactions of macusine-A are: ceric sulphate, pale grey; concentrated nitric acid, bright green rapidly changing to yellow; concentrated sulphuric acid plus one crystal of anhydrous ferric chloride, deep blue changing to green; cinnamaldehyde–hydrogen chloride, slow development of yellow. This chloride had R_G 3·6 in solvent system " C." A completely anhydrous sample was not obtained (Found: C, 64·4; H, 7·0; N, 6·6. Calc. for $C_{22}H_{29}O_3N_2Cl$: C, 65·25; H, 7·2; N, 6·9%. Found, in material dried at 100° and then kept in contact with the atmosphere for 3 days: C, 60·4; H, 7·15; N, 7·05. $C_{22}H_{29}O_3N_2Cl,2H_2O$ requires C, 59·9; H, 7·5; N, 6·35%).

A solution of the above chloride (22 mg.) in water (1 ml.) was treated with an excess of concentrated aqueous potassium iodide. The precipitate was collected and recrystallised from aqueous methanol, to give *macusine-A iodide* (25 mg.), m. p. 274° (decomp.) after previous darkening and sintering at 263° (Found: C, 53·5; H, 5·6; N, 5·6. $C_{22}H_{29}O_3N_2I$ requires C, 53·2; H, 5·9; N, 5·65. $C_{22}H_{27}O_3N_2I$ requires C, 53·45; H, 5·5; N, 5·65%), $\lambda_{max.}$ 222, 273, 277, 288, $\lambda_{min.}$ 251, 275, 286 mμ (log ε 4·73, 3·84, 3·84, 3·71, 3·58, 3·83, 3·70 respectively) in water.

Macusine-A picrate was prepared by precipitation in aqueous solution as usual with picric acid. Recrystallisation from dichloromethane–ether yielded the pure salt, m. p. 255° (decomp.) after extensive sintering and charring at 242° [Found: C, 56·2, 56·1, 56·3; H, 4·9, 4·8, 5·35; N, 11·9, 11·9%; equiv. (ultraviolet absorption [18]), 581, 574. $C_{22}H_{29}O_3N_2,C_6H_2O_7N_3$ requires C, 56·3; H, 5·2; N, 11·7%; equiv., 597·5. $C_{22}H_{27}O_3N_2,C_6H_2O_7N_3$ requires C, 56·5; H, 4·9; N, 11·8%; equiv., 595·5].

(c) *Isolation of macusine-B.* The mother-liquor from crystallisation of macusine-A above and fractions C.IIj, C.IIk, and C.III all contained the same mixture of alkaloids as shown by paper chromatography. They were combined (9·9 g.) and fractionated on a column of cellulose (2·5 kg., 190 × 8 cm.) with water-saturated butan-1-ol as the eluting solvent. The many fractions obtained were suitably combined on the basis of paper chromatographic checks to give seven major fractions (0·11 g., 1·2 g., 2·0 g., 1·8 g., 2·5 g., 1·8 g., 0·5 g.) which all failed to crystallise. Each fraction in ethanol was therefore percolated separately through separate columns of acid-washed alumina (45 × 0·8 cm.); much coloured material was removed and the second, third, fourth, and fifth fractions then yielded crystals from ethanol. They were collected, combined, and recrystallised from ethanol–ether to give *macusine-B chloride* (1·3 g.) as prisms, m. p. 248—249° (decomp.) after darkening from 230° (Found: C, 69·2; H, 7·5; N, 8·2. $C_{20}H_{27}ON_2Cl$ requires C, 69·25; H, 7·9; N, 8·1. $C_{20}H_{25}ON_2Cl$ requires C, 69·65; H, 7·3; N, 8·1%) [a second preparation, although dried under the same conditions, contained water of crystallisation and is probably a different crystalline *form* (Found: C, 65·8, 65·7; H, 7·4, 7·5. $C_{20}H_{27}ON_2Cl,H_2O$ requires C, 65·7; H, 7·45%)], $[\alpha]_D^{22}$ +15·6° ± 1·7° (*c* 1·21 in H_2O), $\lambda_{max.}$ 222, 273, 280, 291, $\lambda_{min.}$ 241, 278, 287 mμ (log ε 4·61, 3·84, 3·82, 3·74, 3·22, 3·81, 3·60 respectively) in water. The colour reactions of macusine-B duplicate those of macusine-A, save that with concentrated nitric acid a green-yellow colour is the final one. The chloride had R_G 3·0 in solvent system " C."

[18] Cunningham, Dawson, and Spring, *J.*, 1951, 2305.

Macusine-B iodide was precipitated from an aqueous solution of the alkaloid chloride by potassium iodide and recrystallised from methanol–ether as prisms, m. p. 280—281° (decomp.) after darkening from 250° (Found: C, 55·3, 55·05; H, 5·9, 5·9; N, 6·2. $C_{20}H_{27}ON_2I$ requires C, 54·9; H, 6·2; N, 6·4. $C_{20}H_{25}O_5N_2I$ requires C, 55·05; H, 5·8; N, 6·4%).

Addition of perchloric acid to an aqueous solution of macusine-B chloride precipitated a solid which recrystallised from aqueous acetone to give *macusine-B perchlorate* as plates, m. p. 272—273° after darkening at *ca.* 250° (Found: C, 58·2; H, 6·3. $C_{20}H_{27}O_5N_2Cl$ requires C, 58·5; H, 6·6. $C_{20}H_{25}O_5N_2Cl$ requires C, 58·75; N, 6·2%).

Macusine-B thiocyanate was precipitated from an aqueous solution of the chloride by the addition of ammonium thiocyanate and, recrystallised from water or aqueous acetone, had m. p. 302° after sintering and darkening at 286°. The infrared spectrum of this material was identical with that of the quaternary thiocyanate obtained from trial fractionations of the total alkaloids from *S. toxifera* (see p. 1850).

(d) *Isolation of xanthocurine.* Fraction C.V above was deep yellow and was further fractionated on a cellulose column (600 g., 66 × 5·5 cm.) with solvent system " C " containing 1·5% of methanol. The eluate was divided into seventeen fractions (C.Va to C.Vq) and a further fraction (C.Vr) was obtained by washing the column with solvent system " C " containing 10% of methanol. A solution of fraction C.Vh in water deposited yellow needles (132 mg.) which were inhomogeneous and it was necessary to use a combination of recrystallisation from methanol and hand-picking to obtain xanthocurine chloride (6 mg.), m. p. >300°. The sample for determination of rotation was prepared as described by Giesbrecht *et al.*[16] and had $[\alpha]_D^{22}$ +757° ± 30° (*c* 0·0934 in MeOH). The authentic sample had $[\alpha]_D^{22}$ +717° ± 20° (*c* 0·181 in MeOH), $[\alpha]_D^{22}$ +706° ± 15° (*c* 0·260 in MeOH). Giesbrecht *et al.*[16] record $[\alpha]_D^{20}$ +813° ± 4° (*c* 0·566 in MeOH). The two samples of xanthocurine were indistinguishable in solvent system " C," solvent system " D," and water-saturated butanol, and showed the same blue-green colour with ceric sulphate.

A portion of our alkaloid was converted into the quaternary nitrate; this salt darkened at 280° and gradually decomposed. Giesbrecht *et al.*[16] report decomposition above 275° for xanthocurine nitrate.

We thank the Curator of Forests, British Guiana, Dr. R. A. E. Galley, and Dr. P. C. Spensley for supplies of *Strychnos toxifera* bark, Dr. James Walker for providing us with Dr. H. King's collection of alkaloids, Professor P. Karrer and Professor H. Schmid for a valuable gift of xanthocurine, and the Medical Research Council and Colonial Products Council for their financial support.

THE UNIVERSITY, BRISTOL. [*Received, November 25th,* 1959.]