152. *Alkaloids of Calabash Curare and* Strychnos *Species. Part I. Chemistry and Structure of Hemitoxiferine-I and Toxiferine-I, including the Preparation of Toxiferine-I.*

By A. R. Battersby and H. F. Hodson.

Degradation of toxiferine-I with dilute acid yields two crystalline salts. One of these is named hemitoxiferine-I and has been shown to be identical with alkaloid A 8 from *Strychnos toxifera*; the chemistry of this alkaloid is studied, leading to the proof of its identity with caracurine-VII methochloride. Hemitoxiferine-I has been dimerised in the presence of hot pivalic acid to give toxiferine-I readily and in good yield. Since caracurine-VII is the Wieland–Gumlich aldehyde (IV), hemitoxiferine-I has structure (V); the structure of toxiferine-I can then be derived. The dimerisation of the Wieland–Gumlich aldehyde base by pivalic acid has been examined.

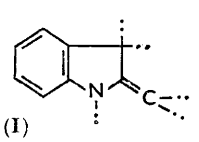
Toxiferine-I was one of the first crystalline alkaloids to be isolated from *Strychnos toxifera* [1a] and was subsequently separated from a sample of calabash curare [1b] of Venezuelan origin. This quaternary alkaloid is a powerful physiological agent and though extensive tests have not been possible in the past, owing to the minute amounts of material available, the pharmacological results obtained so far [2] show that toxiferine-I warrants careful examination as a neuromuscular blocking agent.

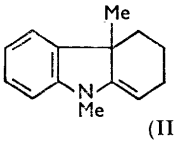
At the outset of the present study, little chemical work had been done on toxiferine-I and the then available knowledge can be summarised as follows.

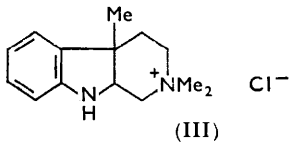
Toxiferine-I chloride was assigned the empirical formula $C_{20}H_{23}ON_2Cl$ by Wieland, Bähr, and Witkop; [1] King [3] gave $C_{20}H_{23}ON_2Cl,3H_2O$ for the air-dried salt. von Philipsborn, Schmid, and Karrer [4] suggested that the alkaloid has a C_{40} molecule, that is $C_{40}H_{46-48}O_2N_4^{2+}$ for the cation,[5] because of several strong similarities between the properties of toxiferine-I

[1] (*a*) Wieland, Bähr, and Witkop, *Annalen*, 1941, **547**, 156; (*b*) Schmid and Karrer, *Helv. Chim. Acta*, 1947, **30**, 1162.
[2] Herring and Marsh, *Fed. Proc.*, 1951, **10**, 309; Paton and Perry, *Brit. J. Pharmacol.*, 1951, **6**, 299.
[3] King, *J.*, 1949, 3263.
[4] von Philipsborn, Schmid, and Karrer, *Helv. Chim. Acta*, 1956, **39**, 913.
[5] Bernauer, Berlage, Schmid, and Karrer, *ibid.*, 1958, **41**, 1202.

and those of curare alkaloids with established C_{40} molecular formulæ. On the basis of colour reactions and ultraviolet spectra, toxiferine-I was assigned [6] the methyleneindoline chromophore (I) as also were *C*-dihydrotoxiferine, *C*-alkaloids H, and the tertiary alkaloid caracurine-Va. It was known [7] that toxiferine-I undergoes a change on treatment with dilute aqueous acid at room temperature, and the reaction product showed two spots when


(I)


(II)


(III)

chromatographed on paper. The two products were not isolated, but the faster-running material gave the characteristic colour reactions of orange with ceric sulphate and citron yellow with cinnamaldehyde–hydrochloric acid.

The present investigation started with alkaloid A 8, the isolation of which from *S. toxifera* bark has been briefly described; [8] our work on the isolation will be published in full in a subsequent paper. The striking colour reactions of alkaloid A 8 were identical with those described above for the product from toxiferine-I; accordingly the chemistry of toxiferine-I, particularly the effect of dilute acid upon it, was further investigated. This was made possible by the generosity of Dr. James Walker of the National Institute for Medical Research, who provided us with the collection of alkaloids isolated by the late Dr. H. King. The infrared spectrum of toxiferine-I chloride showed absorption bands indicating, in agreement with the ultraviolet evidence, the presence of C=C double bond(s) and, importantly, of hydroxyl group(s). The position of the C=C double-bond adsorption band in toxiferine-I corresponds exactly to that shown by the carbazolenine (II).

Chromatographic separation of the products of treatment of toxiferine-I with dilute acid gave two crystalline salts; that of lower mobility on the column, which is chromatographically indistinguishable from caracurine-II methochloride (see below), has been reserved for further study. The more mobile product has now been shown * to be identical with alkaloid A 8. Thus, in A 8 a readily available alkaloid related to the rare toxiferine-I was available.

Of the sixty or so alkaloids isolated from calabash curare and *Strychnos* species,[9] only the tertiary base caracurine-VII shows the same ultraviolet absorption and characteristic colour reactions as given by alkaloid A 8. This led to a direct comparison between alkaloid A 8 chloride and caracurine-VII methochloride, and the two were identical. Since caracurine-VII can be obtained in admixture with caracurine-II by treatment of caracurine-Va with dilute aqueous acid,[9] it follows that caracurine-Va is nortoxiferine-I.

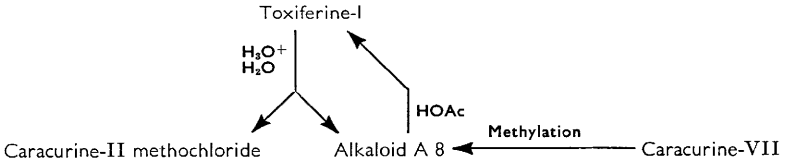
Alkaloid A 8 has ultraviolet absorption characteristic of an indoline which is replaced in 0·1N-hydrochloric acid by the benzenoid absorption of the indolinium cation. In known hexahydro-β-carbolines [10] and in the simple system (III) which we have synthesised,[11] the indoline nitrogen atom is not protonated under these conditions owing to the field effect of the closely placed, positively charged nitrogen atom. Alkaloid A 8 thus cannot contain the hexahydro-β-carboline system. Treatment of the alkaloid with acetic anhydride at 80° yielded a product with ultraviolet absorption characteristic of *N*-acylindolines; the indoline nitrogen atom is therefore secondary. The infrared spectrum of the alkaloid chloride shows absorption corresponding to >NH, OH, and alkene groups. Oxidation of

* The proof of identity in cases thus marked was by colour reactions, R_F values in two solvent systems, ultraviolet and infrared spectra, and where possible by m. p. and mixed m. p.

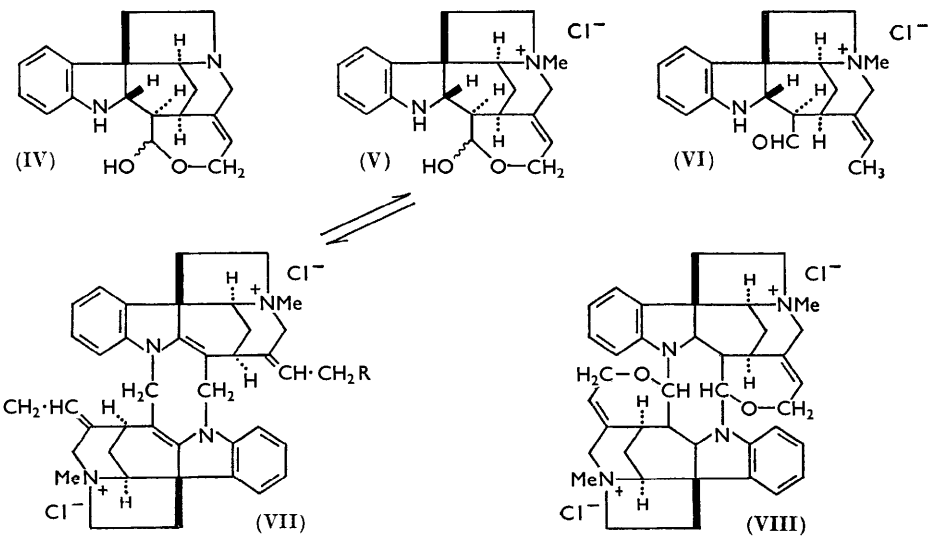
[6] Kebrle, Schmid, Waser, and Karrer, *Helv. Chim. Acta.*, 1953, **36**, 102.
[7] Asmis, Bächli, Schmid, and Karrer, *ibid.*, 1954, **37**, 1993.
[8] Battersby and Hodson, *Proc. Chem. Soc.*, 1958, 287.
[9] Karrer, *Bull. Soc. chim. France*, 1958, 99.
[10] Hodson and Smith, *J.*, 1957, 1877.
[11] Battersby and Hodson, unpublished work.

the alkaloid on a micro-scale [12] by the Kuhn–Roth procedure did not yield propionic acid or higher homologues of this acid, showing that the alkaloid does not contain a C–R group where R is ethyl or a larger alkyl group.

During our exploratory work on alkaloid A 8, the possibility that it might contain a carbinolamine function led to an investigation of its reaction with zinc and acetic acid. The product was a complex mixture which gave colour reactions totally different from those of the starting material. Accordingly, we examined the action of acetic acid alone on the alkaloid. Alkaloid A 8 heated at 120° with acetic acid in the absence of air yielded material with ultraviolet spectrum and colour reactions identical with those of toxiferine-I. Partition chromatography on cellulose of the reaction products allowed the isolation of toxiferine-I in 23% yield. By combining this result with those described above, the relations shown below are established.



At this stage in our investigations, Karrer, Schmid, and their co-workers [13] showed by direct comparison that caracurine-VII is identical with the Wieland–Gumlich aldehyde (IV). Alkaloid A 8 chloride is thus the Wieland–Gumlich aldehyde methochloride (V), which we have confirmed by rigorous comparison * with an authentic sample. The conversion of alkaloid A 8, $C_{20}H_{25}O_2N_2Cl$, into toxiferine-I having a C_{40} molecule is thus a dimerisation; accordingly alkaloid A 8 is now named hemitoxiferine-I.



Closely related studies were being carried out concurrently by Karrer, Schmid, and their co-workers [14] on *C*-dihydrotoxiferine.† Degradation of this alkaloid with acid gives the labile hemidihydrotoxiferine, with colour reactions and ultraviolet spectrum identical

† Though this name seems to be established by long use, it is a misnomer; *C*-dihydrotoxiferine is in fact [14,15] deoxytoxiferine-I.

[12] Garbers, Schmid, and Karrer, *Helv. Chim. Acta*, 1954, **37**, 1983.
[13] Bernauer, Pavanaram, von Philipsborn, Schmid, and Karrer, *ibid.*, 1958, **41**, 1405.
[14] Bernauer, Schmid, and Karrer, *ibid.*, 1958, **41**, 1408.
[15] Bernauer, Berlage, von Philipsborn, Schmid, and Karrer, *Helv. Chim. Acta*, 1958, **41**, 2293.

with those of hemitoxiferine-I. With 5% acetic acid, hemidihydrotoxiferine is reconverted into *C*-dihydrotoxiferine. Hemidihydrotoxiferine has infrared absorption indicative of >NH and aldehyde functions, whereas the *C*-dihydrotoxiferine cation, $C_{40}H_{46}N_4^{2+}$, does not have >NH absorption and does not contain oxygen. On the basis of this and other evidence, structures (VI) and (VII; R = H), respectively, were suggested [12] for hemidihydrotoxiferine and *C*-dihydrotoxiferine.

The firmly established relations and structures shown on page 738 being used in conjunction with this interpretation of the dimerisation, structure (VII; R = OH) must be written for toxiferine-I chloride as we briefly reported earlier; [8] (cf. ref. 15) this structure accommodates the pure methyleneindoline chromophore of the alkaloid and the presence of hydroxyl group(s) noted above. Soon afterwards the structure of hemidihydrotoxiferine was proved [15] to be (VI) by chemical correlation with the Wieland–Gumlich aldehyde (IV).

Further investigation of the product from the reaction of hemitoxiferine-I (alkaloid A 8) with acetic acid led to the isolation in about 50% yield of a crystalline chloride showing ultraviolet absorption and colour reactions identical with those of toxiferine-I. However, its R_F value on paper was greater than that of toxiferine-I (VII; R = OH) and its infrared spectrum showed a strong ester-carbonyl absorption at 1730 cm.$^{-1}$. This product is almost certainly di-*O*-acetyltoxiferine-I chloride (VII; R = OAc), and it therefore seemed probable that a higher yield of toxiferine-I might be obtained from hemitoxiferine-I with an acid such as pivalic acid in which there is strong steric hindrance of acylation. Hemitoxiferine-I chloride was treated at 120° with pivalic acid in the absence of air to give, in one step, 72% of pure toxiferine-I chloride by direct crystallisation. This is much the simplest method available for the preparation of toxiferine-I and since the Wieland–Gumlich aldehyde is obtainable in quantity from stychnine,[16] toxiferine-I can readily be prepared for full pharmacological study.

After our preliminary publication [8] of the preparation of toxiferine-I from hemitoxiferine-I, Karrer, Schmid, and their co-workers described [17] their study of this dimerisation. From hemitoxiferine-I with acetic acid and sodium acetate, a mixture of toxiferine-I, di-*O*-acetyltoxiferine-I, and caracurine-V methochloride (VIII) is formed. A second stage converts the last product into the first, and a third stage involves the hydrolysis of the diacetyl derivative. When the three stages are carried out without the isolation of intermediates, toxiferine-I is obtained in 62% yield. They also dimerised the Wieland–Gumlich aldehyde base (IV) under the same conditions to give caracurine-V, which is the tertiary base corresponding to structure (VIII).

The pivalic acid method for the preparation of toxiferine-I (VII) not only eliminates the side reaction of acylation, but also avoids the formation of more than traces of caracurine-V methochloride (VIII). However, when the Wieland–Gumlich aldehyde free base is dimerised by treatment with hot pivalic acid, the product is mainly caracurine-V, together with a smaller amount of nortoxiferine-I, the tertiary base corresponding to structure (VII). The factors controlling the various possible ring closures are thus delicately balanced.

Experimental

Degradation of Toxiferine-I by Aqueous Acid.—A solution of toxiferine-I dichloride (7 mg.) in water (1·5 ml.) and 0·1N-sulphuric acid (0·6 ml.) was kept at room temperature in a stoppered tube for 7 days. The colour of the solution gradually became strong violet, being most intense after 3 days and later fading to a very weak brown. Percolation of the solution through a small column of " Amberlite " IRA-400 resin in the chloride phase removed sulphate anions, and the percolate and washings were evaporated to dryness at room temperature in a desiccator. Paper chromatography showed the presence of two components in the residue, the faster running material giving an orange reaction with ceric sulphate and the slower-moving product a violet reaction with this reagent. The total residue was dissolved in water-saturated ethyl

[16] Wieland and Gumlich, *Annalen*, 1932, **494**, 191; Wieland and Kaziro, *ibid.*, 1933, **506**, 60; Anet and Robinson, *J.*, 1955, 2253.

[17] Berlage, Bernauer, von Philipsborn, Waser, Schmid, and Karrer, *Helv. Chim. Acta*, 1959, **42**, 394.

methyl ketone containing 10% (v/v) of methanol (total 2 ml.) and transferred to a column of powdered cellulose (2 g.; Whatman Standard grade). Elution with water-saturated ethyl methyl ketone containing 3% (v/v) of methanol and colour tests on the fractions obtained showed that the faster- and slower-moving components had been completely separated. Evaporation of the fractions containing the former gave crystalline material (3·3 mg.) which was shown to be identical with alkaloid A 8 (hemitoxiferine-I) by colour reactions (ceric sulphate and cinnamaldehyde–hydrochloric acid), ultraviolet spectrum, R_F values in two solvent systems, and infrared spectrum (Nujol).

The slow component was also recovered crystalline by evaporation of the appropriate fractions and was indistinguishable chromatographically from caracurine-II methochloride; the latter was prepared from an authentic sample of caracurine-II hydrochloride. An aqueous solution of this salt (*ca.* 1 mg.) was basified with sodium hydroxide, then saturated with potassium carbonate, and the caracurine-II base was extracted into chloroform. Evaporation of the dried extract left a gum which, in methanol, was treated with an excess of methyl iodide at room temperature. After 30 min., the solvents were removed and an aqueous solution of the residue was passed through " Amberlite " IRA-400 resin, chloride phase. After evaporation of the total percolate and washings, the residue was chromatographed on paper, giving only one spot (R_C 0·42 in solvent system " C " [18]). Caracurine-II methochloride gave a violet colour with ceric sulphate and no colour with cinnamaldehyde–hydrochloric acid.

By treatment of a sample of caracurine-II methochloride, derived from toxiferine-I, with picric acid in aqueous solution, *caracurine*-II *methopicrate* was precipitated; this crystallised from aqueous acetone as needles, m. p. >300° (Found: C, 58·1, 57·95; H, 4·6, 4·6. $C_{52}H_{50}O_{16}N_{10}$ requires C, 58·3; H, 4·7%; $C_{52}H_{50}O_{17}N_{10}$ requires C, 57·45; H, 4·65%).

Preparation of Hemitoxiferine-I Chloride (*Alkaloid A* 8 *Chloride*).—The Wieland–Gumlich aldehyde was prepared from strychnine by the method of Anet and Robinson.[16] It was found essential to prevent the temperature of the nitrosation reaction from rising above 70°. Moreover, the crude Wieland–Gumlich aldehyde base was best purified by percolating it in chloroform over alumina (Peter Spence, Type " H," containing 6% of water). The base recovered by evaporation of the total percolate then crystallised readily from benzene.

The pure base (1·1 g.) was dissolved in the least amount of methanol at 60°, and the solution was cooled to room temperature. After addition of methyl iodide (6 ml.), the mixture was kept for 10 min. at room temperature, then 30 min. at 0°, and the crystalline product was collected and dried to give the Wieland–Gumlich aldehyde methiodide (1·26 g.). Chromatographic examination of the mother-liquor showed that it contained more of the same salt. A solution of the methiodide (0·2 g.) in water (100 ml.) was percolated through " Amberlite " IRA-400, chloride phase; evaporation of the solution and washings gave a quantitative yield of the Wieland–Gumlich aldehyde methochloride, $[\alpha]_D^{24}$ −43° (*c* 1·0, in water). This product was shown (as above) to be identical with alkaloid A 8 chloride.

The Wieland–Gumlich aldehyde methopicrate crystallised from aqueous acetone as prisms, m. p. and mixed m. p. with alkaloid A 8 picrate, 233—235° (Found: C, 56·7; H, 5·0. $C_{26}H_{27}O_9N_5$ requires C, 56·5; H, 4·9%).

Action of Acetic Acid on Alkaloid A 8.—Alkaloid A 8 chloride (14 mg.) was heated at 90° for 16 hr. with acetic acid (1 ml.) in an evacuated sealed tube. The acetic acid was removed under reduced pressure and the residue (13·7 mg.) was fractionated on cellulose powder (6 g.) in water-saturated ethyl methyl ketone containing 3% of methyl alcohol. The eluted fractions were examined by paper chromatography and on this basis were combined to give three main fractions.

The slowest-moving fraction (4·3 mg.) by further chromatography under the same conditions as above gave crystalline toxiferine-I chloride identified (as above) with an authentic specimen. A portion was converted into the corresponding picrate which was recrystallised from aqueous acetone. The product had m. p. 276—278° (decomp.), unchanged on admixture with authentic toxiferine-I picrate having m. p. 278—280° (decomp.) in the same bath.

Preparation of Toxiferine-I Chloride (VII; R = OH).—The Wieland–Gumlich aldehyde methochloride (0·5 g.) was heated with pivalic acid (4 ml.) at 120° for 16 hr. in an evacuated sealed tube. After evaporation of the pivalic acid under reduced pressure, water (2 ml.) was added and the resultant solution was again evaporated to dryness. The total residue from two such experiments was dissolved in the smallest volume of hot ethanol, and the solution was

[18] Schmid, Kebrle, and Karrer, *Helv. Chim. Acta.*, 1952, **35**, 1864.

evaporated under nitrogen until crystallisation started. After the solution had been kept for 1 hr. at 0°, the crystals were collected and dried, to give pure toxiferine-I chloride (0·72 g.; 72%), shown to be identical (as above) with the natural alkaloid (Found: C, 62·55; H, 7·15; Cl, 9·25. Calc. for $C_{40}H_{46}O_2N_4Cl_2,4H_2O$: C, 63·4; H, 7·2; Cl, 9·35%). The partially synthetic toxiferine-I chloride had $[\alpha]_D^{23}$ −529° (*c* 1·0, in water); $\lambda_{max.}$ 293 mμ, $\lambda_{min.}$ 237 mμ (log ε, 4·61, 3·75, respectively, in ethanol).

Toxiferine-I picrate, prepared from the foregoing chloride, crystallised from aqueous acetone as orange plates, m. p. 278—280° (decomp.) (Found: C, 58·3; H, 5·0. Calc. for $C_{52}H_{50}O_{16}N_{10}$: C, 58·3; H, 4·7%).

Berlage *et al.*[17] record the m. p. of toxiferine-I picrate as 257—260° (decomp.). We find that decomposition occurs at 250—260°, dependent upon the rate of heating, and continues slowly as the temperature is raised; the constant recorded here, which was determined in an evacuated, sealed, soft-glass capillary, is that of the sudden flowing and frothing of the sample. This constant which is reproducible and is a good criterion of the purity of the material is clearly that recorded by Wieland *et al.*[16] (270°) and by King [3] (278°).

Action of Pivalic Acid on the Wieland–Gumlich Aldehyde.—A solution of the Wieland–Gumlich aldehyde base (0·3 g.) was heated at 120° for 18 hr. with pivalic acid (4 ml.) in an evacuated sealed tube. The pivalic acid was removed under reduced pressure, water (50 ml.) was added to the residue, and the solution was made basic with ammonia. Exhaustive extraction with chloroform and evaporation of the extract under reduced pressure gave a brown gum which was dissolved in benzene–chloroform (9 : 1 by vol.) and adsorbed on alumina (Peter Spence, Type " H," containing 12% of water; 25 g.). Elution with the same benzene–chloroform mixture gave pure caracurine-V (153 mg.), identified (as above) with authentic material. Chloroform and chloroform containing 2% of methanol eluted a number of unidentified substances. Finally, elution with methanol gave a brown resin (31 mg.) which was treated with methanol (2 drops) and methyl iodide (0·5 ml.). After 15 min., the methanol and methyl iodide were evaporated under reduced pressure and a solution of the residue in water was passed through " Amberlite " IRA-400 resin, chloride phase. The eluate, reduced to dryness *in vacuo*, gave a semicrystalline residue shown by ultraviolet spectrum, paper chromatography, and colour reactions to be mainly toxiferine-I chloride.

Grateful acknowledgment is made to the Curator of Forests, British Guiana, and to Dr. R. A. E. Galley and Dr. P. C. Spensley of the Tropical Products Institute for supplies of *Strychnos toxifera* bark, to Dr. James Walker for generous gifts of alkaloids, to Professor P. Karrer for samples of caracurines VII and II, and to the Medical Research Council for financial support.

THE UNIVERSITY, BRISTOL. [*Received, July 9th,* 1959.]