tograms which leave the base-line tangentially. Since this is a small effect in comparison to the total dye-uptake and may be an artifact, we prefer to regard this region of the riptograms with caution and base our interpretations rather on the prominent features of these curves. As to the general form of the riptograms and in the finding that the dye–protein precipitate has an essentially constant composition during the mutual precipitation, our results confirm those of Colvin.

The very interesting work of Lewin[27] in the preparation of crystalline dye–protein salts suggests that this class of compounds does not necessarily follow stoichiometric relations and may even be thought of as solid solutions. A solid solution should give a linear plot of $\bar{v}$ *vs.* C. Several such tests of the "plateau" data from different lysozyme–Orange II riptograms have given curvilinear plots, tending asymptotically to the maximum value, m. The results of this present work are not in contradiction to those of Lewin but rather show that under adequately defined conditions, the composition of dye–protein precipitates can be definite and constant throughout the whole course of the mutual precipitation.

We may enquire what effect soluble-complex formation between the dye and the protein would have on a riptogram. Since this would result in soluble bound dye passing through the filter and being included in the colorimetric analysis for unbound dye, the situation is analogous to that of incomplete filtration. Further, when sufficient dye has been added to solution to form an insoluble protein–dye precipitate, the *apparent* dye concentration decreases due to the conversion of the soluble complex into precipitate. The over-all effect on a riptogram is that it assumes an S-shape, having a negative slope as it leaves the base-line.[21] Since we observed no indications of an S-shape in our riptograms, we can exclude the existence of soluble lysozyme–Orange II complexes in the systems we have studied. At the highest concentration of lysozyme studied (2×10^{-5} m, pH 3.2, $\Gamma/2$ 0.10, 2.0°), we could have detected such an effect with certainty when a minimum of about 10% of the apparent absorbance was due to a soluble complex. This would mean a free Orange II concentration of 1.8×10^{-5} m and a concentration of bound (soluble) dye one-tenth as great. From this information we can calculate a free energy of association for this hypothetical soluble protein–dye complex of 4.5 kcal. mole^{-1}. Thus if soluble lysozyme–Orange II complexes do exist under the above conditions, their free energy of formation must be less than 4.5 kcal. mole^{-1}.

The system for riptographic analysis which we describe here appears to offer attractive possibilities for the analysis of simple protein mixtures. Preliminary experiments in the titration of insulin, protamine and lactoglobulin and certain mixtures of these proteins with Orange II give some promise of realizing such an analytical goal.

Acknowledgments.—The authors wish to thank Dr. H. Edelhoch and Dr. C. Tanford for helpful discussions.

MADISON, WISCONSIN

(27) L. Lewin, THIS JOURNAL, **73**, 3906 (1951).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF ROCHESTER]

Calabash Curare of the Piaroa Indians. Conversion of C-Curarine-I to C-Curarine-III[1,2]

BY A. ZÜRCHER, O. CEDER AND V. BOEKELHEIDE

RECEIVED OCTOBER 28, 1957

Calabash curare, obtained from the Piaroa Indians, has been studied to determine its chemical nature. Although the mixture is a very complex one, the principal alkaloids have been identified as C-curarine-I, C-toxiferine-II (C-calebassine), C-curarine-III, C-dihydrotoxiferine, caracurine-II and C-fluocurinine. A study of the halochrome reaction of C-curarine-I has led to the discovery that C-curarine-I is converted by acid to C-curarine-III.

Through the kindness of Dr. William J. Robbins of the New York Botanical Garden, a quantity of calabash curare from the Piaroa Indians living in the region of the upper Orinoco in Venzuela has been made available to us for chemical study.[3]

Since all of the curare was gathered at one time from one tribe of Indians, it was anticipated that the various calabashes would be reasonably uniform in alkaloidal content. Physiological tests of crude extracts from various calabashes chosen at random confirmed the fact that the crude extracts from different calabashes were of similar, and high, potency.[4] Likewise, paper chromatograms of random samples showed relatively little variation in content of the principal alkaloids.

Although methods of isolation have improved considerably since Heinrich Wieland's first studies on calabash curare,[5-8] the separation of these complex mixtures into their pure individual components in usable quantities still remains the chief stumbling block to chemical studies in this field. In recent years Karrer, Schmid and their group in

(1) This investigation was supported by a research grant (B-671) from The National Institute of Neurological Diseases and Blindness of the National Institutes of Health, Public Health Service.

(2) Preliminary disclosure of the present findings was made at the International Symposium on Curare, Rio de Janeiro, Brazil, August 4–11, 1957.

(3) Dr. Basset Maguire of the New York Botanical Garden, who has observed the Piaroa Indians during the gathering of plant material and the preparation of the curare, has identified the bark of *Strychnos toxifera*, R. Schomburgh, as being the only important botanical species used.

(4) We are indebted to Dr. Irwin Slater of Eli Lilly and Co. for the pharmacological testing.

(5) H. Wieland, W. Konz and R. Sonderhoff, *Ann.*, **527**, 160 (1937).

(6) H. Wieland and H. J. Pistor, *ibid.*, **536**, 68 (1938).

(7) H. Wieland, H. J. Pistor and K. Bähr, *ibid.*, **547**, 140 (1941).

(8) H. Wieland, K. Bähr and B. Witkop, *ibid.*, **547**, 156 (1941).

Zurich[9] and Theodor Wieland and his collaborators at Frankfort[10] have developed the use of partition chromatography over powdered cellulose as the most satisfactory method of isolation. For the separation and identification of small amounts of the alkaloids Marini-Bettolo has found paper electrophoresis to be quite valuable.[11] After some experimentation, we also settled upon partition chromatography over powdered cellulose as the most convenient procedure for isolation and, while our method owes much to the prior published work, some innovations have been made to suit our scale and method of operation that deserve mention.

First of all the powdered cellulose used in our experiments has been a commercial grade of Solka Floc that has been treated for a short period of time with concentrated hydrochloric acid.[12] Although Solka-Floc does not have the uniform particle size of the more expensive Whatman's powdered cellulose, the treatment with acid results in a product that in our experience has a higher efficiency for separation in partition chromatograms. Also, the acid treatment allows greater control over the water content of the powdered cellulose and, with increased water content, a higher ratio of the weight of alkaloids to the weight of powdered cellulose can be used without loss of efficiency in separation.

In the past when the mixture of quaternary chlorides has been freed of tertiary bases and other extraneous material, the first partition chromatogram to effect separation of the alkaloids has involved the use of a pyridine-containing solvent mixture—usually pyridine, ethyl acetate and water.[9,10,13] For large scale separations involving several hundred liters of solvent the use of pyridine is expensive and presents a health hazard. Investigation of other possible solvents revealed that a simple water–butanol mixture was equally as good, if not better, than the pyridine, ethyl acetate and water mixtures.

For the second partition chromatogram the most generally used solvent mixture has been methyl ethyl ketone, methanol and water.[9,10,13] The chief difficulty with this solvent mixture is the ease with which methyl ethyl ketone forms peroxides which then react readily with the sensitive indole alkaloids. When a search for a satisfactory substitute solvent mixture failed, attempts to control peroxide formation were made by converting the constant-temperature room used for all the partition chromatography into a semi-dark room. Finally, a further improvement was made which rendered the methyl ethyl ketone mixture satisfactory for routine use. This was to pass the solvent mixture through an ion exchange column having ferrous ion as the cation just prior to admitting it to the powdered cellulose column. Traces of peroxide were effectively eliminated in this way and the resulting chromatograms were noticeably improved.

The results of the isolation studies can best be described then by summarizing the outcome of a typical run. From 8 calabashes, whose total contents weighed about 650 g., there could be isolated about 10 g. of tertiary bases and 70 g. of a mixture of quaternary alkaloids as their chloride salts. Partition chromatography of the quaternary mixture using the *n*-butyl alcohol mixture gave a large number of eluate fractions which were divided into 32 groups, each of which contained anywhere from several to many components. When these groups were subjected individually to a second partition using the methyl ethyl ketone, methanol and water mixture, a large number of separate fractions were obtained of which a few could be crystallized directly or converted to crystalline derivatives. The crystalline alkaloids which have been identified are listed in Table I with an indication of the approximate amount obtained in a typical run.

TABLE I

CRYSTALLINE ALKALOIDS FROM PIAROA CURARE

1	C-Curarine-I chloride	2.8 g.
2	C-Toxiferine-II chloride (C-calebassine)	1.0 g.
3	C-Curarine-III chloride (C-fluorocurarine)	150.0 mg.
4	C-Dihydrotoxiferine chloride (C-alkaloid-K)	50.0 mg.
5	Caracurine-II picrate	45.0 mg.
6	C-Fluocurinine picrate	100.0 mg.

Of the remaining fractions many contain too little material to be useful. On the other hand, the gummy substances present in a number of these fractions undoubtedly can be obtained crystalline by further effort. A study of these substances by two dimensional paper chromatography as well as by the use of the many different color tests that have been developed for these alkaloids indicates rather clearly that the Piaroa curare is very similar in character to samples of calabash curare previously studied[9,10,13,14] and the likelihood of finding useful amounts of entirely new types of curare alkaloids here is rather remote. For that reason, we have been less concerned with the crystallization and identification of additional alkaloids from the Piaroa curare than with studying the chemistry of the alkaloids presently available to us.

Halochrome Reaction of C-Curarine-I.—The most abundant, and probably the most important, of the calabash curare alkaloids is C-curarine-I. Since its first isolation in 1938 by Heinrich Wieland,[6] much peripheral information regarding the molecule has been gained but there is still no clear insight into its structure. An important, but baffling, clue to the type of chromophore which is present is the brilliant violet color that develops when C-curarine-I is dissolved in strong acid. Wieland applied the term "Halochrome reaction" to this phenomenon and demonstrated that it was a reversible process, dependent only on *p*H and not on the type of acid employed.[7] The occurrence of the halochrome reaction is quite sensitive to changes in structure. Although C-curarine-I can be converted to the corresponding tertiary base, nor-C-curarine-I, without loss of the halochrome

(9) H. Schmid, J. Kebrle and P. Karrer, *Helv. Chim. Acta*, **35**, 1864 (1952).

(10) T. Wieland and H. Merz, *Chem. Ber.*, **85**, 731 (1952).

(11) G. B. Marini-Bettolo, M. Lederer, M. A. Jorio and A. Pimenta, *Gazz. chim. ital.*, **84**, 1155 (1954).

(12) The *Solka-Floc* (BW-40) was supplied by the Brown Co., Berlin, New Hampshire.

(13) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **33**, 512 (1950).

(14) For comparison by paper chromatography, paper electrophoresis and color tests of various species of strychnos, see G. B. Marini-Bettolo, Festschrift Arthur Stoll, p. 257, Birkhäuser, A. G., Basel, 1956; G. B. Marini-Bettolo and M. A. Iorio, *Gazz. chim. ital.*, **86**, 1305 (1956), and preceding papers in this series.

reaction, all of the other structural alterations so far investigated appear to destroy this phenomenon.

As a first approach to the chemistry of C-curarine-I, we took up a study of the halochrome reaction and observed that the violet solution had its main absorption at 550 mμ. Further, the intensity of absorption at this wave length was time-dependent; the extinction coefficient showed a steady decrease in value with time. It thus appeared that, even though the halochrome reaction might be reversible, a further slow reaction must be occurring to destroy the deep violet complex.

When a sample of C-curarine-I chloride was allowed to stand in concentrated hydrochloric acid the original violet solution gradually underwent a series of color changes giving various shades of reddish-brown and eventually became yellow. These changes were followed by making paper chromatograms of samples taken at regular intervals and it was found that, as the C-curarine-I disappeared, spots corresponding to six new substances could be detected. The substance having the highest R_f value showed a pale blue fluorescence, typical of C-curarine-III[7] (fluocurarine[9,15,16]). Further, its ultraviolet absorption spectrum and behavior in various color reactions corresponded to those of C-curarine-III.

To confirm the identity of the fluorescent substance a preparative experiment was carried out in which 104 mg. of C-curarine-I chloride was treated with acid and the product mixture was separated using the *n*-butyl alcohol mixture in a partition chromatogram over powdered cellulose. The first fluorescent fraction (6.9 mg.) was compared to C-curarine-III by paper chromatography and showed the same mobility in two different solvents as well as in paper electrophoresis using 5% acetic acid. Also, a mixture of the fluorescent fraction and C-curarine-III were not separated but gave a single spot in the paper chromatography and paper electrophoresis experiments. The ultraviolet absorption spectrum of the transformation product was identical to that of C-curarine-III and, similarly, showed the characteristic shift to longer wave lengths on the addition of base. Finally, it was possible to convert the remainder of our product to a crystalline β-anthraquinonesulfonate and the infrared spectrum of these crystals was identical in all respects to that of the β-anthraquinonesulfonate of C-curarine-III. Since the purity of the starting C-curarine-I was checked carefully, there can be no doubt that the treatment of C-curarine-I with acid yields C-curarine-III as one of its transformation products.

The significance of this result became apparent when, after our work was completed, we received the report of Volz and Theodor Wieland that C-toxiferine-II (calebassine[10]) is also converted by treatment with a formic acid–acetic anhydride mixture to C-curarine-III.[17] Thus, the fact that C-curarine-I and C-toxiferine-II, the two most abundant alkaloids of calabash curare, give a common degradation product establishes that these two important alkaloids have a close structural relationship.

It might be presumed that these two degradations involved essentially the same reaction and that the difference in the reagents used was not important. To test this we repeated the conversion of C-toxiferine-II to C-curarine-III as described by Volz and Wieland[17] and were able to confirm their findings. However, when we applied the same reaction conditions—formic acid and acetic anhydride—to C-curarine-I, there was no evident change and a paper chromatogram of the product indicated the presence of no C-curarine-III, but only the unchanged C-curarine-I. On the other hand, treatment of C-toxiferine-II with concentrated hydrochloric acid, under the conditions used for our degradation of C-curarine-I, gave a series of transformation products as evidenced by paper chromatography, but the presence of C-curarine-III could not be detected[18] nor was there any evident correspondence between these products and those obtained earlier from C-curarine-I. At present the explanation of why C-curarine-I and C-toxiferine-II yield the same degradation product, C-curarine-III, but under different conditions is not clear.

As a last point, it was possible to demonstrate, though, that the halochrome reaction of C-curarine-I is not directly related to its acid degradation to C-curarine-III. A series of experiments were carried out in which C-curarine-I was treated with acids of various strengths for various times with and without heating and the formation of C-curarine-III was observed by paper chromatography. The most efficient conversion appeared to occur when C-curarine-I in concd. hydrochloric acid was heated on the steam-bath for 5 minutes. However, even in acids as dilute as 1 *N*, where the violet color of the halochrome reaction is completely absent, the conversion of C-curarine-I to C-curarine-III proceeded at an appreciable rate.

It has been a common anticipation that the complexities of having so many different calabash curare alkaloids would be mitigated in part by a multiplicity of interrelationship between the alkaloids. If to the present observations we add the findings of Schmid and Karrer that C-dihydrotoxiferine[8] (C-alkaloid-K[9,15]) is transformed by dilute acid to C-alkaloid-D[19] and by acetic acid in pyridine to C-toxiferine-II,[20] it can be seen that five of the common alkaloids are closely related as shown below.[21] The recent experiments of Philipsborn, Schmid and Karrer[22] indicate that C-curarine-I, C-toxiferine-II, C-dihydrotoxiferine and C-alkaloid-D are all alkaloids having a molecular weight corresponding to C_{40} derivatives. Although sim-

(15) J. Kebrle, H. Schmid, P. Waser and P. Karrer, *Helv. Chim. Acta*, **36**, 102 (1953).

(16) J. Kebrle, H. Schmid, P. Waser and P. Karrer, *ibid.*, **36**, 345 (1953).

(17) H. Volz and Th. Wieland, *Ann.*, **604**, 1 (1957).

(18) Volz and Wieland, ref. 17, observed a similar behavior of C-toxiferine-II when it was treated with acid.

(19) H. Asmis, E. Bächli, H. Schmid and P. Karrer, *Helv. Chim. Acta*, **37**, 1993 (1954).

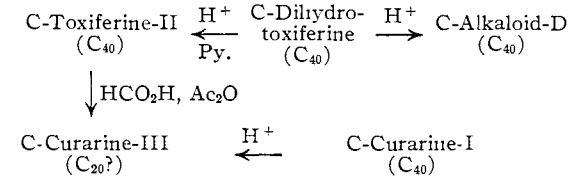
(20) H. Asmis, H. Schmid and P. Karrer, *ibid.*, **39**, 440 (1956).

(21) Whether all of these alkaloids occur in the plant or whether they are in part artifacts introduced in the preparation or work-up of the curare due to the sensitivity of these indole alkaloids is still questionable.

(22) W. von Philipsborn, H. Schmid and P. Karrer, *Helv. Chim. Acta*, **39**, 913 (1956).

ilar evidence is not available for C-curarine-III, the indicative criteria of a high R_c value and low toxicity[15] would suggest that C-curarine-III is a C_{20} derivative. In this case the acid-catalyzed transformations of C-curarine-I and C-toxiferine-II to give C-curarine-III are best considered as cleavages in which roughly half of the molecule in each instance is converted to C-curarine-III. Thus, the importance of C-curarine-III to a solution of the over-all structural problem of these alkaloids is apparent.

Alkaloid interrelationships[23]



Acknowledgment.—We wish to express our indebtedness to Dr. Robbins and the New York Botanical Garden for their part in gathering the Piaroa curare and, in particular, to thank Dr. John Wurdack who made the difficult trip up the Orinoco River.

Experimental

Isolation Studies on Calabashes of Piaroa Curare.—The procedure eventually developed as most satisfactory for routine execution on a large scale is described below for a typical run. As indicated in the discussion, numerous experiments were made with other methods and other solvents before it was concluded that this scheme was best suited to our own situation.

In the usual run, 8 calabashes were taken and smashed in a heavy brass cylinder and the contents (total weight averaged about 650 g.) extracted by vigorously stirring them first for 20 hours in 5 l. of distilled water and then for another hour after addition of 5 l. of methanol. The resulting mixture was allowed to stand for 24 hours before the clear solution was removed by careful syphoning. The turbid residue, after dilution with 2.5 l. of methanol, was filtered and the filtrate was combined with the clear solution removed previously. The combined extracts then were concentrated to a volume of 4 l. by passage over a climbing film evaporator. The precipitate, which separated, was removed by decantation.

After the solution had been brought to a *p*H of 8 by addition of sodium hydroxide, it was extracted continuously with methylene chloride in a large percolator until the returning methylene chloride was colorless (3 days). Concentration of the methylene chloride extracts usually gave about 10 g. of tertiary bases per run. These have been combined and reserved for future investigation.

The aqueous solution then was concentrated again in the climbing film evaporator to a volume of about 2.5 l. and the resulting clear solution was separated from any amorphous precipitate that had formed. After the clear solution had been warmed to 35°, there was added over a period of 30 minutes with continuous stirring 5 l. of a prepared aqueous picric acid solution whose temperature was kept at about 60°. The picric acid solution was prepared by taking 2.5 l. of an aqueous picric acid solution saturated at 85° and diluting it with an equal volume of water. Under these conditions the mixture of alkaloid picrates readily could be separated by filtration from the still warm solution (but not above 50°). Examination of the mother liquor from the picrate precipitation showed the absence of any significant quantity of alkaloids. The picrate precipitate then was redissolved by taking it up in 3 l. of freshly-distilled acetone and adding to the mixture 2 l. of warm (70°) distilled water and the clear solution was passed immediately over an ion exchange resin (Dowex-II-X4, Cl^-) to recover the alkaloid mixture in the form of their quaternary chlorides. After concentration of the solution to a volume of 2 l., it was passed over acid-washed alumina to remove any extraneous material still remaining. Concentration of the eluate then gave the purified quaternary chlorides in yields ranging from 60 to 70 g.

A number of two-dimensional paper chromatograms of the chloride mixture were run at this stage—the solvent mixtures "C" and "D" of the Zurich laboratories[9] as well as other combinations were tried. In our hands the most effective separations were achieved using a water–*n*-butyl alcohol mixture in one direction and methyl ethyl ketone, methanol and water mixture in the second direction. The distribution of the alkaloids as indicated by fluorescence and by spraying with ceric sulfate and the other test reagents was quite similar to those described in the publications from the Zurich[9] and Frankfort[10] laboratories. It would seem that the Piaroa curare is very similar in the nature and number of alkaloids it contains to that reported on in these previous publications. However, to establish conclusively the presence or absence of a particular alkaloid in this way is not satisfactory in most cases.

For the large scale partition chromatography the powdered cellulose (Solka-Floc, BW-40)[12] was prepared by first stirring it with 6 times its weight of a 33% hydrochloric acid solution for 8 minutes and then washing it free of acid with distilled water. The powdered cellulose then was collected by filtration and the resulting solid had a water content of about 70%. A homogeneous slurry was prepared by adding *n*-butyl alcohol to the wet cellulose such that the resultant mixture corresponded to 70% *n*-butyl alcohol, 24.5% water and 5.5% dry cellulose by weight. Using this slurry a column 15 cm. in diameter was packed to a height of 130 cm., the uniformity of the column being tested by passing through a dye (quinalizarin or azobenzene). The purified quaternary chlorides (60 g.) then were stirred vigorously with 200 ml. of the cellulose–*n*-butyl alcohol mixture to give a homogeneous slurry which was run onto the top of the column and elution of the column was begun using *n*-butyl alcohol saturated with water (the *n*-butyl alcohol employed in all of these experiments was purified by washing with sodium bisulfite and distilling prior to use). The flow rate of the column was set at 25 fractions of 450 ml. each per day with collection of fractions being done automatically. At the end of 10–12 days all of the bands except the very slow moving ones were eluted from the column. The column was then brought to a horizontal position and the cellulose was extruded by means of compressed air. Division of the extruded powdered cellulose now was made mechanically to give 9 sections which were extracted individually with methanol and water. Examination of the fractions from the *n*-butyl alcohol elution by paper chromatography using the methyl ethyl ketone mixture led to an arbitrary division of the 275 fractions into 23 groups. These in addition to the previous 9 from the extrusion made a total of 32 groups which were concentrated individually. In the case of 4 of these groups the residual gums crystallized spontaneously. Although for the most part these crystals were still mixtures, a surprising quantity (*ca.* 1.0 g.) of C-curarine-I chloride was obtained directly in the pure state.

At this stage residues from the 32 groups now were subjected individually to a second partition using powdered cellulose prepared as before but eluting with a water-saturated solution of methyl ethyl ketone containing 1–3% methanol (solvent "C" of the Zurich group[9]). To eliminate traces of peroxides which were present even though the methyl ethyl ketone was purified and distilled prior to use, the solvent mixture was passed over an ion exchange column (Dowex-50-X4) having ferrous ion as the cation.

As indicated in the discussion all of the partition chromatography was done in a semi-dark, constant-temperature room. In each case the size of the column and amount of solvent used varied according to the particular group. The various fractions from the elutions were examined for fluorescence, color reactions and ultraviolet spectra so that in combining fractions pure individual components would be kept separate wherever possible. After concentration then, a large number of separate gums were obtained and those which appeared to be relatively pure were subjected to attempts at crystallization, either directly or by conversion to some other derivative such as picrate, tetraphenylborate, etc. The alkaloids thus far obtained in crystalline form

(23) ADDED IN PROOF.—K. Bernauer, H. Schmid and P. Karrer, *Helv. Chim. Acta*, **40**, 1999 (1957), have just extended these interrelationships by converting C-dihydrotoxiferine to C-curarine-I and to C-toxiferine-II (*C. calebassine*).

together with their amount are given in Table I. Since these all have been described previously, their identification has been made in the usual way by comparison of melting points, R_f values, rotations and ultraviolet spectra. Undoubtedly, with further effort and time the number of crystalline alkaloids isolated from Piaroa curare can be increased to several times the present list.

The Halochrome Reaction of C-Curarine-I.—When a sample of C-curarine-I chloride was dissolved in concentrated hydrochloric acid, it gave a deep violet solution whose ultraviolet absorption spectrum showed maxima and extinction values (calculated on the basis that C-curarine-I chloride is $C_{40}H_{44}ON_4Cl_2$) as follows: 262 mμ (ε, 2.18×10^4), 295 (ε, 1.26×10^4) and 550 (ε, 3.77×10^3). By comparison the ultraviolet spectrum C-curarine-I chloride in ethanol has maxima at 260 mμ (ε, 2.58×10^4) and 296 (ε, 1.18×10^4). After short periods of time, dilution of the concentrated acid solution gave back a solution having a pure C-curarine-I spectrum. On the other hand, solutions of C-curarine-I in strong acid which had been allowed to stand at room temperature for an hour showed a decrease of intensity at the longer wave lengths (550 mμ) to about one-half the original value. A solution of C-curarine-I in concentrated hydrochloric acid which had been allowed to stand under nitrogen until the original violet color had undergone a series of changes through various reddish-browns to an eventual yellow was investigated by paper chromatography using the methyl ethyl ketone mixture. C-Curarine-I could no longer be detected but, instead, there were spots corresponding to six new compounds. The fastest moving of these, having an R_c value of approximately 2.2, showed a bluish fluorescence reminiscent of C-curarine-III (fluocurarine). Comparative paper chromatography using *n*-butyl alcohol as solvent as well as the ultraviolet spectrum of the substance strongly supported the conclusion that it was C-curarine-III.

This experiment was then repeated on preparative scale using 104 mg. of C-curarine-I chloride in 10 ml. of concentrated hydrochloric acid. After standing under nitrogen at room temperature for 94 hours, the solution was evaporated and the residue was subjected to partition chromatography over powdered cellulose using the *n*-butyl alcohol solvent. The first fluorescent band was collected and on concentration gave 6.9 mg. of a gum. The identity of this product (fraction 1) with C-curarine-III chloride was established by the following comparison. Comparative paper chromatography using either the *n*-butyl alcohol or the methyl ethyl ketone solvent mixtures showed identical behavior for both fraction 1 and C-curarine-III. Paper electrophoresis of a mixture of the two using 5% acetic acid gave only one spot. The fluorescence and color reactions of both were identical. As shown in the table below, the ultraviolet absorption spectra of fraction 1 in water both with and without added base, were identical to the corresponding spectra of C-curarine-III.

ULTRAVIOLET SPECTRA

	Maxima, mμ
Neutral solution	
Fraction 1	242, 299, 361
C-Curarine-III	242, 299, 361
Basic solution	
Fraction 1	250, 315, 388
C-Curarine-III	250, 315, 388

Finally, the remainder of the gum was converted to the corresponding crystalline β-anthraquinonesulfonate, m.p. 308–310° dec.[24] The infrared spectrum of the crystalline β-anthraquinonesulfonate of fraction 1 using a potassium bromide wafer corresponded in all respects to that obtained from an authentic sample of the β-anthraquinonesulfonate of C-curarine-III.

That the C-curarine-III could not have risen from impurities present in the sample of C-curarine-I was demonstrated by very careful check of the purity of C-curarine-I. In tests using paper chromatography with various solvents and in paper electrophoresis experiments no evidence of impurity could be found.

As yet it has not been possible to identify the other products formed during the acid treatment of C-curarine-I, but these are still under investigation.

(24) Reference 3 gives 308–310° dec. as the m.p. of the β-anthraquinonesulfonate of C-curarine-III.

ROCHESTER, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MASSACHUSETTS INSTITUTE OF TECHNOLOGY]

Fungichromin. Determination of the Structure of the Pentaene Chromophore

BY ARTHUR C. COPE AND HERBERT E. JOHNSON[1]

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Sodium periodate oxidation of fungichromin has been shown to yield, in addition to other products, 2-methyl-2,4,6,8,10-dodecapentaenedial. The structure of the dialdehyde was determined by reduction to 2-methyl-2,4,6,8,10-dodecapentaenediol, hydrogenation to 2-methyldodecane-1,10-diol and oxidation to 2-methyl-2,4,6,8,10-dodecapentaenedioic acid which was reduced to 2-methyldodecanedioic acid. Sodium periodate oxidation of hydrogenated fungichromin followed by oxidation of the aldehydes formed also yielded 2-methyldodecanedioic acid.

Fungichromin[2,3] is one of a number of crystalline antibiotic substances produced by members of the genus Streptomyces that have been isolated recently. Fungichromatin,[2] Fumagillin,[4-6] and Filipin[7,8] which are closely related to Fungichromin in molecular formula also have been produced, as have a large number of other similar antibiotic substances.[9] All of these substances are polyenic in character and are markedly similar in their antifungal activity.

Fungichromin was found to contain no methoxyl or acetoxyl groups, nitrogen, halogen or sulfur. A large active hydrogen value (lithium aluminum hydride in tetrahydrofuran) indicated the presence of 10–11 hydroxyl groups and C-methyl determinations gave a minimum value of 3–4 such groups. Absorption maxima in the ultraviolet spectrum occur at 311, 323.5, 339 and 357 mμ which upon correlation with other polyene compounds[9a] indicated a conjugated pentaene system. The molecular weight of

(1) National Institutes of Health Postdoctoral Fellow, 1955–1956.

(2) A. A. Tytell, F. J. McCarthy, W. P. Fisher, W. A. Bolhofer and J. Charney, "Antibiotics Annual, 1954-1955," Medical Encyclopedia, Inc., New York, N. Y., p. 716.

(3) F. J. McCarthy, W. P. Fisher, J. Charney and A. A. Tytell, *ibid.*, p. 719.

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