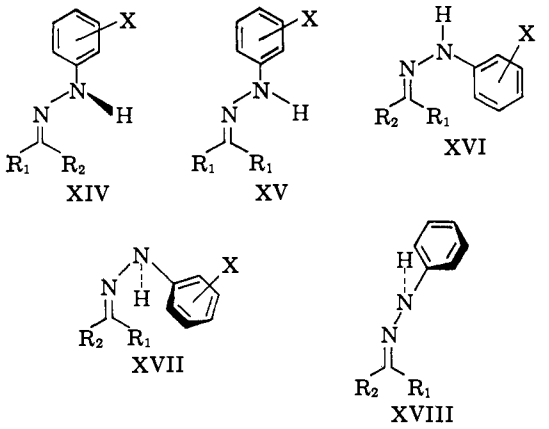
TABLE VI

CALCULATED EFFECTS OF RING ANISOTROPY ON *cis*- AND *trans*-HYDROGENS

	H_1 (*cis*)	H_1 (*trans*)	α-CH_3 (*cis*)[a]	α-CH_3 (*trans*)[a]
Conf. XV				
ρ	3.69	4.19	4.0	4.7
z	0	0	0	0
P.p.m.	−0.218	−0.318	−0.159	−0.087
$\nu_{cis} - \nu_{trans}$	−0.08		−0.072	
Conf. XVI				
ρ	2.04	3.12	1.53	3.84
z	0	0	0	0
P.p.m.	−1.159	−0.340	−1.744	−0.186
$\nu_{cis} - \nu_{trans}$	−0.819		−1.558	
Conf. XVII				
ρ	1.8	2.4	1.8	2.6
z	1.6	2.3	1.7	2.8
P.p.m.	+0.272	+0.127	+0.313	+0.114
$\nu_{cis} - \nu_{trans}$	+0.145		+0.199	
Conf. XVIII				
ρ	2.6	3.7	2.3	4.2
z	1.45	0.9	1.9	1.0
P.p.m.	−0.120	−0.154	−0.091	−0.110
$\nu_{cis} - \nu_{trans}$	+0.034		+0.201	
$\nu_{cis} - \nu_{trans}$ (exp)	−0.5 to −0.7		+0.05 to +0.2 (α-CH_3) −0.3 (α-methine)	

[a] The (averaged) position of the methyl hydrogens was taken to be the center of the triangle formed by the three hydrogens.

molecular hydrogen bonding—conformations XIV (pyramided nitrogen) and XV (planar nitrogen)[7] are the only ones that are consonant with the results; *e.g.*, solvent effects, and do not suffer from severe nonbonded interactions and loss of π–sp^3 overlap. Using Johnson and Bovey's nuclear shielding values[8] we have calculated for conformations XV–XVIII the effect of a benzene ring on various hydrogens. Table VI summarizes the results. It is clear from a comparison of the calculated and experimental values that the anisotropy of the ring is not the dominant contributor to the magnetic nonequivalence between *cis*- and *trans*-hydrogens. This conclusion is further supported by the fact that hydrazones and N-methylhydrazones show effects analogous to those observed with phenyl- and ring-substituted phenylhydrazones.



Experimental

Preparation of Carbonyl Derivatives.—All carbonyl derivatives are known compounds and were prepared by usual procedures.

N.m.r. Spectra.—All n.m.r. spectra were determined at 60 Mc. on a Model A-60 spectrometer (Varian Associates, Palo Alto, Calif.), at about 36°. Undegassed solutions were used with tetramethylsilane as internal reference.

Acknowledgment.—We thank the United States Atomic Energy Commission for financial support, Grant AT(11-1)-1189.

(7) Evidence favors a pyramidal configuration for the nitrogen of aniline and N-substituted anilines; *e.g.*, J. C. Evans, *Spectrochim. Acta*, **16**, 428 (1960); A. T. Bottini and C. P. Nash, *J. Am. Chem. Soc.*, **84**, 734 (1962).

(8) C. E. Johnson, Jr., and F. A. Bovey, *J. Chem. Phys.*, **29**, 1012 (1958).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF OREGON, EUGENE, OREGON]

Calabash Curare Alkaloids. Specific Deuterium Labeling and Nuclear Magnetic Resonance Studies[1-3]

BY MARCEL GRDINIC,[4a,b] DAVID A. NELSON, AND V. BOEKELHEIDE

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The important calabash curare alkaloids—dihydrotoxiferine, curarine-I, calebassine, toxiferine-I, C-alkaloid-A, and C-alkaloid-E—have been synthesized with specific deuterium labeling at the 17- and 17′-positions. Through comparative n.m.r. studies of these derivatives and by the use of spin decoupling experiments, confirming evidence has been obtained for the structural assignments and spectral interpretations of these alkaloids.

Of the various contributions made to the chemistry of calabash curare since the first isolation studies of Wieland,[5] undoubtedly the most significant of which was the linking of the important alkaloids of this group with Wieland–Gumlich aldehyde and thus, in turn, with the strychnine family.[6-10] The identity of Wieland–Gumlich aldehyde with caracurine VII and 18-desoxy Wieland–Gumlich aldehyde with hemidihydrotoxiferine made possible the direct syntheses of dihydrotoxiferine,[7] toxiferine-I,[7,8,10] curarine-I,[11] cale-

(1) This investigation was supported in part 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) For the preceding communication, see V. Boekelheide, O. Ceder, T. Crabb, Y. Kawazoe, and R. N. Knowles, *Tetrahedron Letters*, **26**, 1 (1960).

(3) Preliminary presentation of this work was made at the Second International Symposium on Natural Products at Prague, Aug. 29, 1962.

(4) (a) Research Associate, National Institutes of Health, 1960–1963; (b) Roche Anniversary Foundation Postdoctoral Fellow, 1963.

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

(6) K. Bernauer, S. K. Pavanaram, W. von Philipsborn, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **41**, 1405 (1958).

(7) K. Bernauer, F. Berlage, W. von Philipsborn, H. Schmid, and P. Karrer, *ibid.*, **41**, 2293 (1958).

(8) F. Berlage, K. Bernauer, W. von Philipsborn, P. Waser, H. Schmid, and P. Karrer, *ibid.*, **42**, 394 (1959).

(9) A. R. Batters-By and H. F. Hodson, *Proc. Chem. Soc.*, 126 (1959).

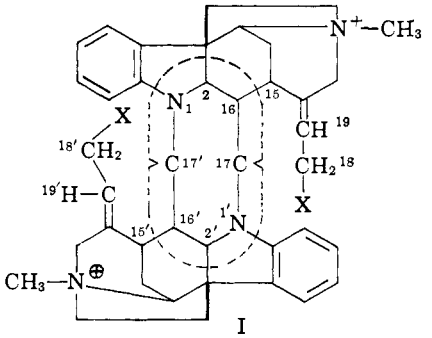
(10) A. R. Batters-By and H. F. Hodson, *J. Chem. Soc.*, 736 (1960).

(11) K. Bernauer, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **40**, 1999 (1957).

bassine,[11] C-alkaloid-A,[12] and C-alkaloid-E.[12] However, since the detailed mechanism of the dimerizations and photooxidations involved in these syntheses is not known, these transformations do not provide a basis for writing complete detailed structures for these alkaloids. The full assignment of structure has been deduced from spectral data, the n.m.r. evidence being by far the most useful.[2,13–15]

Since the structural assignments of these important calabash curare alkaloids are based on interpretations of n.m.r. which, although self-consistent, involve some unusual proton chemical shifts, it seemed of importance to test these interpretations by preparing these alkaloids with specific deuterium labeling and report on the results of such a study. For additional clarification, the n.m.r. spectra have been taken using a 100-Mc. instrument with suitable accessories to allow spin decoupling.[16]

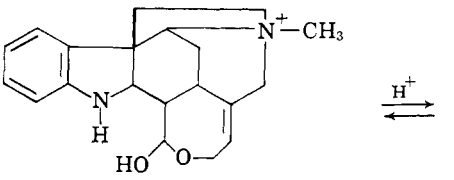
The alkaloids under consideration can be divided into two families: (1) the dihydrotoxiferine family including dihydrotoxiferine, curarine-I, and calebassine; and (2) the toxiferine-I family including toxiferine-I, C-alkaloid-E, and C-alkaloid-A. In the over-all structure I, the dihydrotoxiferine family corresponds to I, where X = –H, and the toxiferine-I family to I, where X = –OH; the nature of the central eight-membered ring (enclosed in the dotted circle) varies according to the member of the family under consideration. From various studies,[17] there is a wealth of evidence that the over-all carbon skeleton shown in I is correct for these alkaloids. However, assignment of the detailed structure within the central eight-membered ring is largely based on n.m.r. evidence and chemical analogy.



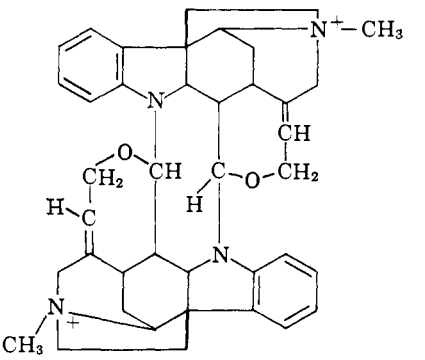
I

The dimerization of Wieland–Gumlich aldehyde methochloride (hemitoxiferine-I,II) may lead directly to toxiferine-I,[10] or may give caracurine-V dimethochloride (III)[7] which can subsequently be converted to toxiferine-I.

Similarly, 18-desoxy Wieland–Gumlich aldehyde methochloride (hemidihydrotoxiferine) can be dimerized by acid to dihydrotoxiferine and the spectral evidence

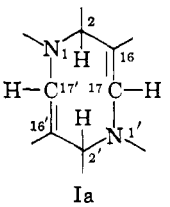


II

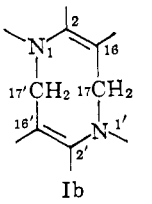


III

as well as the chemical evidence from direct conversion of caracurine-V to dihydrotoxiferine[7] clearly shows that dihydrotoxiferine differs from toxiferine-I only in having X = –H instead of –OH at the 19- and 19′-positions. Although the chemical evidence allows two possible arrangements of the central eight-membered ring, either Ia or Ib, for dihydrotoxiferine and toxiferine-I, structure Ib was favored.[7,17b] However, a study of simple strychnine derivatives by Knowles showed that in these model substances the stable arrangement was that shown by Ia.[18] Further, dihydrotoxiferine showed the presence of a vinyl proton in addition to those at 19 and 19′, which is required by Ia and not by Ib.[2,13,19]

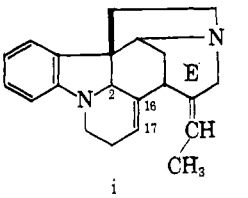


Ia



Ib

The spectra of dihydrotoxiferine and toxiferine-I in the region of 1.5–5.0 τ are shown in Fig. 1. As expected, the vinyl protons at 19 and 19′ appear as a quartet at 3.75 τ in dihydrotoxiferine and as a triplet at 3.62τ in toxiferine.[20] However, the assignment of



i

(12) K. Bernauer, F. Berlage, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **41**, 1202 (1958).

(13) W. Arnold, M. Hesse, H. Hiltebrand, A. Melera, W. von Philipsborn, H. Schmid, and P. Karrer, *ibid.*, **44**, 620 (1961).

(14) J. Nagyvary, W. Arnold, W. von Philipsborn, H. Schmid, and P. Karrer, *Tetrahedron*, **14**, 138 (1961).

(15) M. Hesse, H. Hiltebrand, Ch. Weissmann, W. von Philipsborn, K. Bernauer, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **44**, 2211 (1961).

(16) We are indebted to Mr. N. Bhacca of Varian Associates, Palo Alto, Calif., for determining these spectra using a 100-Mc. instrument. A description of the spin decoupling apparatus is available from Varian Associates.

(17) For reviews of the chemistry of the calabash curare alkaloids, see (a) K. Bernauer, *Fortschr. Chem. org. Naturstoffe*, **17**, 184 (1959), and (b) A. R. Battersby and H. F. Hodson, *Quart. Rev.* (London), **14**, 77 (1960).

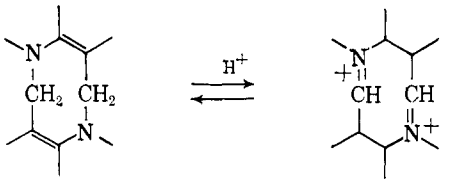
(18) R. N. Knowles, Ph.D. Dissertation, University of Rochester, 1960. In this work, for example, it was shown that structure i, when subjected to very strenuous conditions, showed no evidence for migration or equilibration of the double bond between carbons 16 and 17. However, when ring E was open, migration of the 16–17 double bond to the 2–16 position could not be prevented.

(19) In ref. 2 the deduction of structure from the n.m.r. evidence is correct, although the exact interpretation of the spectrum is incorrect due to an erroneous electronic integration of the spectrum supplied to us by L. F. Johnson of Varian Associates, Palo Alto, Calif.

(20) All of the n.m.r. spectra of these quaternary alkaloids were taken using deuterium oxide as solvent and the τ-values cited relate to tetramethylsilane as an external standard.

the protons at 17 and 17′ is not obvious. In the normal vinyl proton region, toxiferine shows a singlet at 3.92 τ and dihydrotoxiferine a singlet at 3.93 τ. These signals have been assigned to the 2- and 2′-protons, though, and the 17- and 17′-protons have been assigned the sharp signal at 2.93 τ. The shift of the vinyl protons downfield into the aromatic region has been attributed to the effect of the ring current of the adjacent aromatic ring.[13]

Because of the abnormal chemical shift of the signal assigned to the 17- and 17′-protons and its importance for the total structural assignments of the calabash curare alkaloids, we undertook the synthesis of toxiferine-I and dihydrotoxiferine having specific deuterium labeling at the 17- and 17′-positions, the absence of a signal in these deuterated derivatives making possible unequivocal assignments. As a first experiment, it was necessary to show that a deuterium label at the 17- and 17′-positions would not be lost readily through equilibration during normal handling of these compounds. In their review,[17b] Batters-By and Hodson have proposed that an easy equilibration occurs between Ib and Ic. Although this did not seem likely in view of Knowles' experiments,[18] the possibility of such an equilibration was tested by heating nordihydrotoxiferine in deuterioacetic acid (CH_3CO_2D). Within the limits of experimental error, there was no introduction of deuterium into the nordihydrotoxiferine molecule and thus, in fact, the proposed equilibration does not occur. Since the conditions employed in this test are essentially the same as those used for the dimerization of Wieland–Gumlich aldehyde methochloride,[7] this negative result opened the way to a synthesis of the desired 17,17′-deuterium labeled alkaloids. Knowing that equilibration and loss of deuterium would not occur during dimerization, our goal became the synthesis of 17-deuterio Wieland–Gumlich aldehyde.



Although a number of different routes to 17-deuterio Wieland–Gumlich aldehyde were investigated, the most successful path in our experience started with Wieland–Gumlich aldehyde itself. This was converted, following the procedure of Edwards and Smith,[21] through the corresponding oxime, nitrile, and acid to the N,N-dimethylamide IV. Reduction of IV using lithium diethoxyaluminodeuteride following the procedure of Brown and Tsukamoto[22a] gave 17-deuterio Wieland–Gumlich aldehyde V in 55% yield. Using the method of Batters-By and Hodson,[10] we were able to convert V to 17,17′-dideuteriotoxiferine-I in high yield.

For the preparation of 17,17′-dideuteriodihydrotoxiferine, our first approach involved the dimerization of 17-deuterio Wieland-Gumlich aldehyde to 17,17′-dideuteriocaracurine-V which, in turn, was

(21) P. N. Edwards and G. F. Smith, *J. Chem. Soc.*, 152 (1961).

(22) (a) H. C. Brown and A. Tsukamoto, *J. Am. Chem. Soc.*, **81**, 502 (1959); (b) K. Bernauer, F. Berlage, W. von Philipsborn, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **42**, 201 (1959).

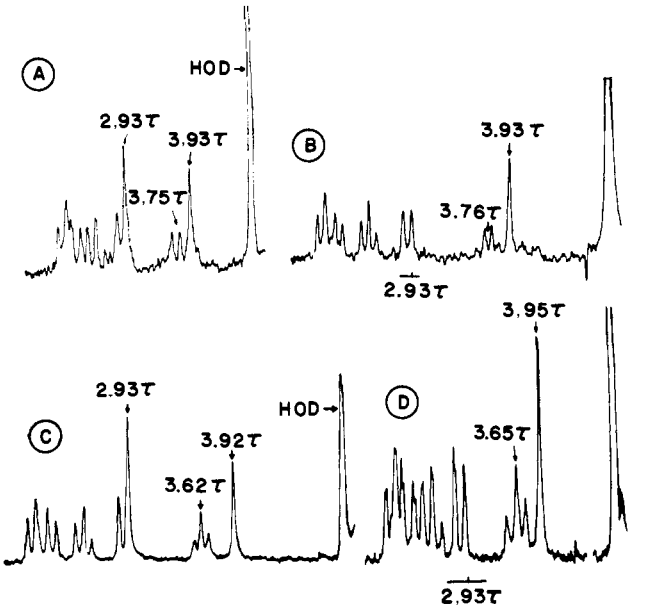
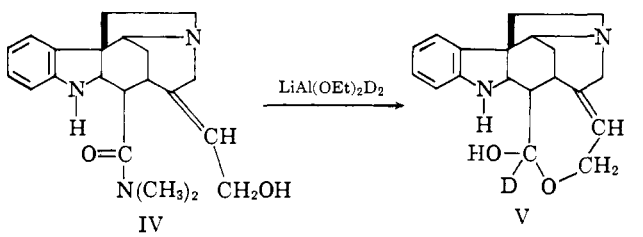


Fig. 1.—N.m.r. spectra of dihydrotoxiferine (A), 17,17′-dideuteriodihydrotoxiferine (B), C-toxiferine-I (C), and 17,17′-dideuterio-C-toxiferine (D) in the region of 1.5–5.0 τ, using D_2O as solvent (A and D, a Varian HR-60 B and C, a Varian HR-100).

carried on to 17,17′-dideuteriodihydrotoxiferine.[7] However, subsequently we found that the direct conversion of toxiferine to dihydrotoxiferine proceeds in much higher yield. This conversion has been reported previously by Schmid and Karrer,[22b] but the scale of their experiments limited their evidence for the identification of dihydrotoxiferine to comparative paper chromatography. We have carried out the conversion of toxiferine-I to dihydrotoxiferine on a preparative scale, both with and without deuterium labeling, and in our opinion it is the most convenient route to this alkaloid.

In Fig. 1, the n.m.r. spectra of 17,17′-dideuterio-C-toxiferine and 17,17′-dideuteriodihydrotoxiferine are compared with the spectra of the corresponding protonated samples. In each case the only evident change due to deuteration is the diminution of the signal at 2.93 τ. The fact that this signal does not disappear completely is not because of incomplete deuterium labeling, for a comparison of the integrated area of



this region of the vinyl and aromatic protons shows a loss within experimental error of exactly two protons for the deuterated species. Rather, the fact that a signal remains at 2.93 τ in the deuterated compounds shows that the observed signal in the protonated species is actually a superposition of the signals from the 17- and 17′-protons with a signal from the aromatic protons. In any case, it is clear that the signal from the 17- and 17′-protons occurs at 2.93 τ, and thus the arrangement of the internal eight-membered ring in

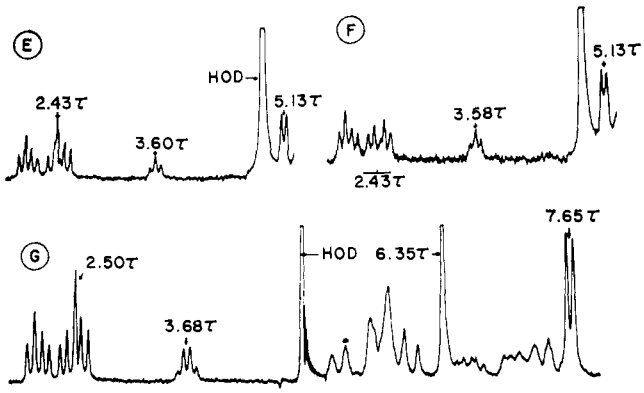
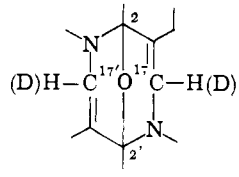


Fig. 2.—N.m.r. spectra of C-alkaloid-E (E), 17,17′-dideuterio-C-alkaloid-E (F), in the region of 1.7–5.3 τ, and curarine-I (G), using D_2O as solvent (Varian HR-100).

toxiferine-I and dihydrotoxiferine is established as being correctly represented by Ia.

As mentioned previously, dihydrotoxiferine on photooxidation gives a mixture of products of which curarine-I is one.[11] Similarly, toxiferine-I on photooxidation is converted to C-alkaloid-E.[12] There is good evidence that curarine-I and C-alkaloid-E have the same pattern of substitution in the internal eight-membered ring and differ only in the presence or absence of hydroxyl at the 18- and 18′-positions.[17] Schmid and Karrer and their collaborators have assigned structure Id to the internal eight-membered ring unit for curarine-I and C-alkaloid-E.[14] Again, this assignment was based largely on n.m.r. evidence. The absence of a signal in the 3.93-τ region which, in dihydrotoxiferine and toxiferine-I had been assigned to the 2- and 2′-protons, is in accord with an ether linkage having replaced the 2- and 2′-hydrogens. Further, curarine-I and C-alkaloid-E show signals at 2.50 and 2.43 τ, suggesting that the 17- and 17′-protons in these alkaloids have a similar environment to that present in dihydrotoxiferine and toxiferine-I.



Id

When 17,17′-dideuteriotoxiferine-I was subjected to this photooxidation experiment,[12] it was possible to obtain a pure sample of 17,17′-dideuterio-C-alkaloid-E. In Fig. 2, the n.m.r. spectrum of C-alkaloid-E is compared to that of the deuterated species. The introduction of deuterium at the 17- and 17′-positions causes the loss of the strong signal at 2.43 τ. This is in full accord with structure Id and provides good corroborative evidence that Id correctly represents curarine-I and C-alkaloid-E. Due to lack of material, it was not possible to prepare a sample of 17,17′-dideuteriocurarine-I. However, the spectrum of curarine-I shown in Fig. 2 is clearly analogous to that of C-alkaloid-E and the signal due to the 17- and 17′-protons is readily discernible.

Schmid and Karrer and their collaborators have also shown that dihydrotoxiferine when subjected to air oxidation in a pyridine solution containing an aliphatic

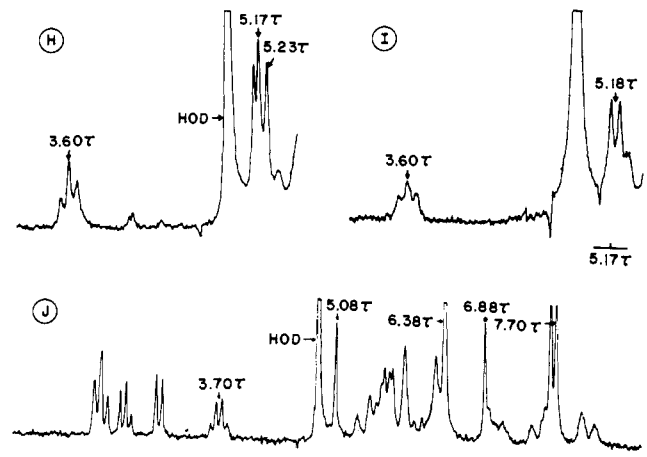
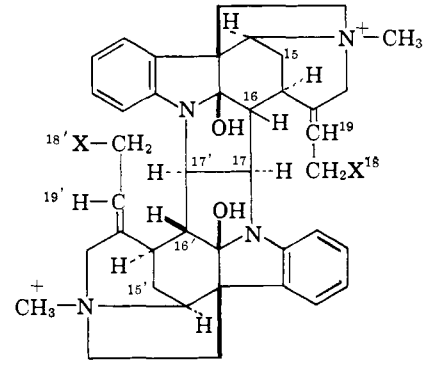


Fig. 3.—N.m.r. spectrum of calebassine (J), and partial spectra (region of 3.5–5.5 τ) of C-alkaloid-A (H) and 17,17′-dideuterio-C-alkaloid-A (I). Spectra taken with D_2O as solvent and using a Varian HR-100.

acid gives calebassine.[15,23] Likewise, a similar treatment of toxiferine-I gives C-alkaloid-A.[15] As was true in the previous photooxidation, the mechanism of these air oxidations is not known. Through a combination of evidence, but relying heavily on an interpretation of the n.m.r. spectra, the Swiss workers have assigned structure Ie to represent calebassine and C-alkaloid-A.[15] We have now repeated these air oxidations to convert 17,17′-dideuteriodihydrotoxiferine to 17,17′-dideuteriocalebassine and 17,17′-dideuteriotoxiferine-I to 17,17′-dideuterio-C-alkaloid-A.



Ie

calebassine (X = -H)
C-alkaloid-A (X = -OH)

The n.m.r. spectrum of calebassine is shown in Fig. 3. In contrast to the previous alkaloids we have discussed, there is no signal in the aromatic or vinyl proton region which must be attributed to the 17- and 17′-protons. This led the Swiss workers to assign the signal at 5.08 τ to the 17- and 17′-protons. As explanation for the fact that this signal is a singlet even though there is a proton on the adjacent carbon (C-16), it was assumed that the dihedral angle is wrong for the coupling of the spins of these two protons. That this interpretation is truly correct is shown by the fact that the signal at 5.08 τ is completely absent in the n.m.r. spectrum of the corresponding 17,17′-dideuteriocalebassine.

In the case of C-alkaloid-A, as shown in Fig. 3, this region of the spectrum shows a combination of three signals. That the central one of these three

(23) H. Asmis, E. Bachli, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **38**, 1993 (1955).

signals is due to the 17- and 17′-protons is evident from the absence of this signal in the spectrum of the 17,17′-dideuterio-C-alkaloid-A.

The two neighboring signals in the region of 5.21 τ have been assigned to the 18- and 18′-protons which by coupling with the 19- and 19′-vinyl protons should appear as a doublet. In turn, the signal for the 19- and 19′-vinyl protons should appear as a triplet and the triplet occurring at 3.60 τ is readily identified with the 19- and 19′-protons. Since the coupling constant (J = 7 c.p.s.) is the same for both signals, these assignments are reasonable. However, it was of interest to prove this deduction by doing double irradiation experiments.[24] When the solution of C-alkaloid-A was scanned with simultaneous irradiation 155 c.p.s. higher than the scanning radiation, the triplet at 3.60 τ coalesced to a singlet. Similarly, when the experiment was repeated with 17,17′-dideuterio-C-alkaloid-A but with simultaneous irradiation 155 c.p.s. below the scanning radiation, the doublet coalesced to a singlet at 5.18 τ. This confirms the assignment of the 18-, 18′-, 19-, and 19′-protons. In addition, it strengthens the evidence that structure Ie correctly represents the calebassine and C-alkaloid-A molecules.

In the early efforts to prepare specifically labeled deuterio derivatives of the calabash curare alkaloids, a second method for preparing 17,17′-dideuteriodihydrotoxiferine was developed that deserves mention. Reduction of Wieland–Gumlich aldehyde with lithium aluminum deuteride gave the 17-deuterio derivative VI. This was treated with hydrobromic acid followed by zinc dust in the usual procedure for removing the hydroxyl group at the 18-position.[7] The resulting compound (VII) was then subjected to an Oppenauer oxidation. It was anticipated that the primary deuterium isotope effect in the Oppenauer oxidation would be nearly maximal. Thus, the resulting hemidihydrotoxiferine should be labeled with deuterium at the 17-position to the extent of about 80%. In fact, the Oppenauer oxidation did lead to marked enrichment of the deuterium isotope at the 17-position. However, the Oppenauer oxidation was complicated by the fact that it led to a mixture of products, VIII and IX.

The Oppenauer oxidation of the protonated species corresponding to VII was first reported by Fritz, Besch, and Wieland,[25a] who indicated that 18-desoxy Wieland–Gumlich aldehyde (VIII) was first formed and then underwent an easy air oxidation to give nor-C-curarine-III (IX). More recently, the German authors have described a modification in which nitrobenzene is used as solvent to promote the oxidation and increase the yield of nor-C-curarine-III.[25b] In our experiments with the Oppenauer oxidation, we employed potassium t-butoxide and benzophenone in boiling benzene with rigid exclusion of air. Nevertheless, the major product of the Oppenauer oxidation was IX. Although the accompanying 18-desoxy Wieland–Gumlich aldehyde could be isolated, it did not appear to be particularly susceptible to air oxidation. These results support the conclusion that the formation of nor-C-curarine-III is a direct result of the action of the Oppenauer reagent. Plausible mechanisms for this reaction are readily available.[26]

The products from the Oppenauer oxidation, VIII and IX, were combined, reduced with zinc and sulfuric acid, quaternized with methyl iodide, and then dimerized to give dihydrotoxiferine following the over-all procedure described previously by Schmid and Karrer.[27] The n.m.r. spectrum of this product, as judged from comparative electronic integrations in the aromatic region, indicated that deuterium labeling at the 17- and 17′-positions had occurred to the extent of about 80%, in full support of the predicted maximal primary isotope effect in the Oppenauer oxidation. However, the difficulties of this route combined with the disadvantage of partial labeling made this approach less desirable than the procedure described first.

Experimental[28]

Dimethylamide of 18-Hydroxy-2β,16α-cur-19-en-17-oic Acid[29] (IV).—The preparation of Wieland–Gumlich aldehyde from strychnine was carried out following the procedure of Anet and Robinson.[30] Several hundred grams of Wieland–Gumlich aldehyde were prepared and our yields were consistently about 36% of pure crystalline material. This is in contrast to the report of Edwards and Smith,[21] but is in agreement with the original claim of Anet and Robinson.[30] Conversion of Wieland–Gumlich aldehyde to the oxime was carried out according to the procedure of Wieland and Kaziro,[31] and this in turn was carried through the nitrile to methyl 18-hydroxy-2β,16α-cur-19-en-17-oate by the procedure of Edwards and Smith.[21] Then, a solution of 14.8 g. of methyl 18-hydroxy-2β,16α-cur-19-en-17-oate (m.p. 153–155°) in 200 g. of anhydrous dimethylamine was heated in a sealed vessel at 105° for 18 hr. The vessel was opened and the dimethylamine was allowed to evaporate. The crystalline residue weighed 14.7 g. and showed only one spot (R_f 1.9) on

(24) A. L. Bloom and J. N. Shoolery, *Phys. Rev.*, **97**, 1261 (9155).

(25) (a) H. Fritz, E. Besch, and Th. Wieland, *Angew. Chem.*, **71**, 126 (1959); (b) *Ann.*, **663**, 150 (1963).

(26) For example, the following sequence of equilibrium steps could plausibly lead to the final resonance-stabilized vinylamide structure ii.

ii

(27) W. von Philipsborn, K. Bernauer, H. Schmid, and P. Karrer, *Helv. Chim. Acta*, **42**, 461 (1959).

(28) Microanalysis by Micro-Tech, Skokie, Ill.

(29) The nomenclature used here follows that of Edwards and Smith, ref. 21.

(30) A. L. F. Anet and R. Robinson, *J. Chem Soc.*, 2253 (1955).

(31) H. Wieland and K. Kaziro, *Ann.*, **506**, 60 (1933).

paper chromatography using solvent "C" (methyl ethyl ketone, methanol, and water[32]). Recrystallization from methanol gave white crystals, m.p. 182–184°. In the infrared, the amide carbonyl appeared as a strong band at 1640 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{27}N_3O_2$: C, 71.36; H, 7.70; N, 11.89. Found: C, 71.20; H, 7.60; N, 11.68.

17-Deuterio Wieland–Gumlich Aldehyde (V).—Although the general procedure used was that of Brown and Tsukamoto,[22] several modifications were introduced. A mixture of 11.5 g. of lithium aluminum deuteride in 1760 ml. of anhydrous ether was stirred overnight. The solution was separated from inorganic residue by centrifugation and decantation. Analysis of the clear solution showed a content of 5.6 mg. of $LiAlD_4$/ml. To this clear solution (1420 ml.), held at 0° with stirring, there was added dropwise a solution of 17.4 g. of absolute ethanol in anhydrous ether. The resulting clear solution was then added dropwise with stirring over a period of 20 min. to a solution of 9.7 g. of the dimethylamide (IV) in 3.13 l. of anhydrous tetrahydrofuran held at 0° under a nitrogen atmosphere. After the addition was complete, the mixture was stirred for 2 hr. longer at 0°. Then, 2 *N* aqueous sulfuric acid was added with stirring until the mixture was acidic. The aqueous layer was separated and the organic layer was extracted again with aqueous sulfuric acid. The combined acid extracts were then made alkaline with concentrated ammonium hydroxide and the precipitated organic base was extracted with chloroform; after it had been dried over sodium sulfate, it was concentrated under reduced pressure to give 8.7 g. of crude product. This was purified by chromatography over powdered cellulose (Whatman No. 1) using a solvent mixture of 1-butanol–acetic acid–water (77:6:16). Fractions of 18 ml. each were taken and fractions 70–150 were combined to give 4.7 g. of chromatographically pure 17-deuterio Wieland–Gumlich aldehyde. Recrystallization of a sample from chloroform gave white needles, m.p. 211–213°, undepressed by admixture of authentic Wieland–Gumlich aldehyde. The corresponding picrate melted at 231–234° dec.[33] The presence of deuterium was evident from the infrared spectrum of V which showed a weak absorption band at 2060 cm.$^{-1}$. However, conclusive evidence of its identity was provided from comparison of the n.m.r. spectra of the deuterated sample with that of Wieland–Gumlich aldehyde itself. This in turn permits the assignment of the doublet at 4.48 τ in the Wieland–Gumlich aldehyde spectrum to the 17-proton.

The Methochloride of 17-Deuterio Wieland–Gumlich Aldehyde.—To 3.0 g. of 17-deuterio Wieland–Gumlich aldehyde in 130 ml. of chloroform there was added 10 ml. of methyl iodide and the mixture was allowed to stand at room temperature for 1 hr. After the crystalline precipitate had been collected by filtration, it was redissolved in 150 ml. of an acetone–water mixture (7:3) and passed over an ion-exchange column (Dowex-2, Cl^- form). The eluate was concentrated to give the crystalline methochloride (R_c 1.5 using solvent "C").[34] The corresponding methopicrate melted at 232–234°, undepressed by admixture of an authentic sample of Wieland–Gumlich aldehyde methopicrate. The infrared spectrum of the methochloride showed weak C–D absorption at 2065 cm.$^{-1}$. The n.m.r. spectrum of the methochloride of 17-deuterio Wieland–Gumlich aldehyde differed from that of the methochloride of Wieland–Gumlich aldehyde, itself only in the absence of the doublet at 4.48 τ (J = 7) which can thus be assigned to the 17-proton.

17,17′-Dideuteriotoxiferine-I.—A solution of 2.9 g. of the methochloride of 17-deuterio Wieland–Gumlich aldehyde in 26 ml. of pivalic acid was placed in an evacuated sealed tube and heated at 120° for 16 hr. The pivalic acid was then removed under reduced pressure, the residual solid was taken up in 10 ml. of water, and this, in turn, was concentrated to dryness. The crude product was then recrystallized from ethanol to give 2.2 g. of crystalline 17,17′-dideuteriotoxiferine-I dichloride. This was chromatographically pure, giving only one spot (R_c 0.42) on paper chromatography using solvent C. The corresponding picrate was prepared and found to melt at 273–278° dec. in agreement with an authentic sample of toxiferine picrate.[35] The n.m.r. spectrum of the 17,17′-dideuteriotoxiferine dichloride differed from that of toxiferine-I dichloride only in the change in signal at 2.93 τ (see Fig. 1). In the infrared, the –C–D bond gave rise to a band at 2232 cm.$^{-1}$.

17,17′-Dideuterio-C-alkaloid-E.—A solution of 100 mg. of 17,17′-dideuteriotoxiferine dichloride in a small amount of methanol was spread as a thin film on the bottom of a 20-cm. crystallization dish. The dish was wrapped with aluminum foil and cooled externally by circulating cold water. A 200-watt clear Mazda lamp was placed 20 cm. from the bottom of the crystallization dish and a stream of air was blown continuously over the surface of the film. After irradiation was begun, the reaction was stopped every 5 hr., the film was dissolved in methanol, and the progress of oxidation was checked by paper chromatography. The film was then reapplied and irradiation continued. After 20 hr. the methanol solution was subjected to preparative paper chromatography over Whatman No. 3 MM filter paper with solvent C for elution. The C-alkaloid-E fraction was cut out and eluted with methanol. Concentration of the eluate gave 25 mg. of crystalline 17,17′-dideuterio-C-alkaloid-E, which was chromatographically pure (R_c 0.36, ceric sulfate spray giving a fast-fading blue color). For spectra, the sample was purified further by dissolving it in an acetone–methanol mixture (9:1) and passing it over 0.5 g. of alumina (activity 1, Woelm). In the infrared, the C–D bond showed an absorption band at 2250 cm.$^{-1}$. The n.m.r. spectrum of 17,17′-dideuterio-C-alkaloid-E differed from that of an authentic sample of C-alkaloid-E only in the change in signal at 2.43 τ (see Fig. 2).

17,17′-Dideuterio-C-alkaloid-A.—A solution of 198 mg. of 17,17′-dideuteriotoxiferine-I dichloride in a mixture of 8.5 ml. of pyridine, 6.3 ml. of isobutyric acid, and 1.5 ml. of water was heated in a sealed tube at 125–130° for 7 hr. Then, the solvent was removed under reduced pressure, the residue was dissolved in methanol, and the methanol solution was passed over an ion-exchange column (Dowex-2, Cl^- form). The eluate was concentrated and subjected to preparative paper chromatography using Whatman No. 3 MM paper and solvent C. From the fluorescence under ultraviolet light, both C-alkaloid-A and C-alkaloid-E fractions were evident. These were removed separately and eluted with methanol. Concentration of the eluate from the minor fraction gave 14 mg. of 17,17′-dideuterio-C-alkaloid-E, whereas the major fraction yielded 63 mg. of 17,17′-dideuterio-C-alkaloid-A. For spectral purposes, the latter sample was purified further by dissolving it in an acetone–methanol mixture (9:1) and passing it over alumina (activity 1, Woelm). In the infrared, 17,17′-dideuterio-C-alkaloid-A shows a weak C–D band at 2100 cm.$^{-1}$. The n.m.r. spectrum of 17,17′-dideuterio-C-alkaloid-A differed from that of an authentic sample of C-alkaloid-A only in the signal at 5.17 τ as shown by Fig. 3.

17,17′-Dideuteriodihydrotoxiferine *via* 17,17′-Dideuteriocaracurine-V.—The conversion of 1.774 g. of 17-deuterio Wieland–Gumlich aldehyde to 17,17′-dideuteriocaracurine-V was carried out following the procedure described by Schmid and Karrer[7] and gave 950 mg. of pure product. This was then dissolved in 10 ml. of glacial acetic acid and the resulting solution was added gradually to 300 ml. of a hydrobromic–acetic acid mixture (prepared by saturating 10 ml. of acetic acid with hydrogen bromide and then diluting it with 290 ml. of acetic acid). The resulting solution was allowed to stand in the dark at room temperature for 68 hr. After the solvent had been removed under reduced pressure at room temperature, the crude 18,18′-dibromo derivative was redissolved in 450 ml. of glacial acetic acid and 50 g. of powdered zinc was added. The mixture was stirred at room temperature for 90 min.; then 100 ml. of methanol was added and the mixture was stirred for another hour. The mixture was filtered to remove excess zinc dust which was washed on the filter with methanol. The combined filtrates were concentrated under reduced pressure at room temperature. The residue was dissolved in 100 ml. of water and, after the solution had been made basic with ammonia, it was extracted with chloroform. Concentration of the chloroform gave 842 mg. of solid which was taken up in benzene and chromatographed over alumina (Woelm, activity 1 to which 18.5% water had been added). The fraction of the eluate containing the 17,17′-dideuterionordihydrotoxiferine was determined by paper chroma-

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

(33) The melting point reported for Wieland-Gumlich aldehyde is 211–213°, and that of the corresponding picrate is 233–234° dec.; see ref. 6.

(34) In ref. 10, Batters-By and Hodson report that Wieland-Gumlich aldehyde methochloride (alkaloid A-8) has an R_c of 1.5 and that the corresponding methopicrate melts at 233–235°. Their earlier report (*Proc. Chem. Soc.*, 287 (1958)) that the methopicrate of alkaloid A-8 melts at 134–136° is obviously a typographical error.

(35) H. Schmid and P. Karrer, *Helv. Chim. Acta*, **30**, 1162 (1947).

TABLE I[a]

Alkaloid	Position 2,2′ H-17 (τ)	2,2′ D-17 (τ)	16,16′ H-17 (τ)	16,16′ D-17 (τ)	17,17′ H-17 (τ)	17,17′ D-17 (τ)	18,18′ H-17 (τ)	18,18′ D-17 (τ)	19,19′ H-17 (τ)	19,19′ D-17 (τ)	$N^{\oplus}$-CH_3 H-17 (τ)	$N^{\oplus}$-CH_3 D-17 (τ)
Toxiferine-I	3.92 s	3.95 s			2.93 s	ϕ	5.18 d (J = 6)	5.22 d (J = 6)	3.62 t (J = 6)	3.65	6.33 s	6.33 s
Dihydrotoxiferine	3.93 s	3.93 s			2.93 s	ϕ	7.65 d (J = 6)	7.68 d (J = 6)	3.75 q (J = 6)	3.76 q (J = 6)	6.37 s	6.37 s
C-Alkaloid-E					2.43 s	ϕ	5.13 d (J = 6.5)	5.13 d (J = 6.5)	3.60 t (J = 6.5)	3.58 t (J = 6.5)	6.32 s	6.33 s
Curarine-I					2.50 s		7.65 d (J = 7)		3.68 q (J = 7)		6.35 s	
C-Alkaloid-A			6.88 s	6.83 s	5.17 s	ϕ	5.23 d (J = 7)	5.18 d (J = 7)	3.60 t (J = 7)	3.60 t (J = 7)	6.37 s	6.37 s
C-Calebassine			6.88 s	6.84 s	5.08 s	ϕ	7.70 d (J = 7)	7.73 d (J = 7)	3.70 q (J = 7)	3.67 q	6.38 s	6.38 s

[a] s = singlet, t = triplet, d = doublet, q = quartet.

tography and then converted directly to the corresponding methochloride as described for the methochloride of 17-deuterio Wieland–Gumlich aldehyde. The methochloride was then purified further by preparative paper chromatography over Whatman No. 3 MM paper using solvent C to give 149 mg. of pure crystalline 17,17′-dideuteriodihydrotoxiferine dichloride, whose mobility on paper chromatography and behavior in color tests was identical with that of authentic dihydrotoxiferine. Further, it was converted to the corresponding picrate, m.p. 182–185°, undepressed by admixture of authentic dihydrotoxiferine picrate.[7] The infrared spectrum of 17,17′-dideuteriodihydrotoxiferine dichloride showed a weak C–D band at 2220 cm.$^{-1}$. The n.m.r. spectrum differed from that of an authentic specimen of dihydrotoxiferine dichloride in the signal at 2.93 τ (see Fig. 1).

17,17′-Dideuteriodihydrotoxiferine from 17,17′-Dideuteriotoxiferine-I.—A solution of 200 mg. of 17,17′-dideuteriotoxiferine-I in 2 ml. of glacial acetic acid was added to 100 ml. of a hydrobromic–acetic acid mixture (prepared by saturating 5 ml. of glacial acetic with hydrogen bromide and then diluting with 95 ml. of glacial acetic acid). The mixture was allowed to stand in the dark at room temperature for 60 hr. after which it was concentrated at room temperature under reduced pressure. The residue was then redissolved in 160 ml. of acetic acid and treated with 6 g. of zinc dust. After the resulting solution had been stirred at room temperature for 90 min., 40 ml. of methanol was added and the solution was stirred for another 30 min. Removal of the excess zinc dust was followed by a brief washing of the zinc dust with methanol before the combined filtrates were concentrated to dryness. The residue was dissolved in water and treated with a saturated aqueous solution of sodium picrate. The precipitate of alkaloid picrate was collected, washed with water, then redissolved in methanol and passed over an ion-exchange column (Dowex-2, Cl^- ion). The eluate was concentrated to dryness; the residue was then taken up in an acetone–methanol mixture (80:20) and chromatographed over alumina (Woelm, activity 1). The main fraction of the eluate was concentrated to about 10 ml. and cooled to −80°. The crystals which separated were removed by centrifugation, redissolved in methanol, and then precipitated by dropwise addition of ether. This gave 90 mg. of 17,17′-dideuteriodihydrotoxiferine as white needles whose identity was established as in the previous experiment.

17,17′-Dideuteriocalebassine.—This preparation was carried out in the same manner described for the conversion of toxiferine to C-alkaloid-A. From 140 mg. of 17,17′-dideuteriodihydrotoxiferine dichloride there was obtained 25 mg. of 17,17′-dideuteriocalebassine dichloride whose behavior on paper chromatography and in color tests was identical with that of authentic calebassine. The n.m.r. spectrum of 17,17′-dideuteriocalebassine dichloride differed from that of an authentic specimen of calebassine dichloride in the signal at 5.08 τ.

17,17′-Dideuteriodihydrotoxiferine *via* the Oppenauer Oxidation.—The reduction of Wieland–Gumlich aldehyde to the 17-deuterio diol VI was accomplished using lithium aluminum deuteride following the procedure described for the corresponding reduction with lithium aluminum hydride.[30] The conversion of the 17-deuterio diol VI to the corresponding 18-desoxy derivative VII was accomplished following the procedure of Schmid and Karrer.[7] For the Oppenauer oxidation solvent-free potassium *t*-butoxide was carefully prepared and isolated as a fluffy, white, benzene-soluble powder (failure to remove all of the *t*-butyl alcohol results in the failure of the Oppenauer oxidation!). In a typical experiment, 10 ml. of benzene containing 500 mg. of potassium *t*-butoxide was added to a solution of 300 mg. of VII and 900 mg. of benzophenone in 60 ml. of dry benzene under a nitrogen atmosphere. The mixture was boiled under reflux for 2 hr. at which time paper chromatography under nitrogen of a probe sample using 1-butanol, acetic acid, and water (77:6:16) showed the absence of VI, the presence of a small amount of the 17-deuterio-18-desoxy Wieland–Gumlich aldehyde, but mainly, 17-deuterionor-C-curarine-III. The benzene solution was then shaken with three 25-ml. portions of water to remove potassium *t*-butoxide and dried over potassium carbonate. After concentration of the benzene solution, the residue was chromatographed over alumina (Woelm, activity 1 to which 15% water was added) using benzene for elution. The main fraction of the eluate was converted to the corresponding methochloride (as described for the preparation of Wieland–Gumlich aldehyde methochloride) and purified by chromatography over powdered cellulose using solvent C. There was isolated 87 mg. of crystalline 17-deuterio-C-curarine-III chloride. The ultraviolet absorption spectrum of this sample showed the characteristic absorption at 360 mμ which underwent a bathochromic shift of 20 mμ in the presence of base. The infrared spectrum showed a strong C–D band at 2100 cm.$^{-1}$, and supported the assignment of the 17-deuterio-C-curarine-III structure. The further conversion of 17-deuterio-C-curarine-III to 17-deuteriohemidihydrotoxiferine and hence to 17,17′-dideuteriodihydrotoxiferine was carried out following the previously reported procedure of Schmid and Karrer.[27]

N.m.r. Spectra.—The spectra of all of the quaternary alkaloids were observed using deuterium oxide as solvent and the spectra of the tertiary bases were taken using deuteriochloroform. Although portions of the n.m.r. spectra are presented in Fig. 1–3, the important signals are summarized in Table I.