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METHODS FOR THE STRUCTURE ELUCIDATION OF ALKALOIDS[1]

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ABSTRACT.—The strategy for the identification and structure elucidation of alkaloids is discussed. Some problems that may be encountered with the various spectral methods are briefly reported, e.g., the influence of the solvent in fdms and traces of acid in solvents in ^{1}H nmr. The use of trifluoroacetic acid as shift reagent in ^{1}H nmr is described. Examples of the use of the various spectrometric methods are given, i.e., the revision of the structure of kribine, the determination of the structure of two alkaloids isolated from *Strychnos dale* (10,10′-dimethoxy-3α,17α-*Z*-tetrahydrousambarensine and its N'_b-methyl derivative). Finally, the need for a reinvestigation of the structure of the alkaloid hayatine is discussed, and the isolation of an alkaloid with a structure similar to the original hayatine structure is reported.

Since the isolation of the first alkaloids in the early nineteenth century, the methods used in the identification and structure elucidation of such compounds have changed considerably. Originally, pure chemistry (e.g., the making of derivatives and performing degradative reactions) was the only tool available. However, over the past 40 years a number of nondegradative spectral methods have been introduced, which had a great impact on natural products research (Table 1). Application of such new methodology

TABLE 1. Approximate Dates of Introduction of Spectral Methods in Natural Product Research

Date	Method
1800	chemical methods
1950	uv and ir
1960	ms, ^{1}H nmr (60-100 MHz), tlc, ord, cd
1970	^{13}C nmr
1980	high-resolution ^{1}H nmr (300-500 MHz), 2D-nmr

resulted in a number of structural revisions. To better understand the importance of the various methods, it is probably preferable to describe how to proceed when a compound is isolated and how to determine its structure, whether it is known or unknown (Schemes 1 and 2).

In fact, the identification and structure elucidation is first of all a matter of classification. Based on the knowledge of the isolation method used, one knows whether the isolated compounds have an acidic, neutral, or basic character. The plant from which a compound has been isolated also adds important information, as chemotaxonomy can be used to determine which groups of compounds are likely to be found. For example, if one has isolated a basic compound from a plant belonging to one of the families Apocynaceae, Loganiaceae, or Rubiaceae, the compound is most likely to be an terpenoid-indole alkaloid, of which about 2000 are known, all isolated from these three families. Furthermore, the genus to which the plant belongs can be used to reduce even further the number of possibilities. In the three families mentioned, of the important alkaloid-containing genera, about 200-300 different alkaloids have been isolated so far, some of which are more or less ubiquitous, others being typical for the genus.

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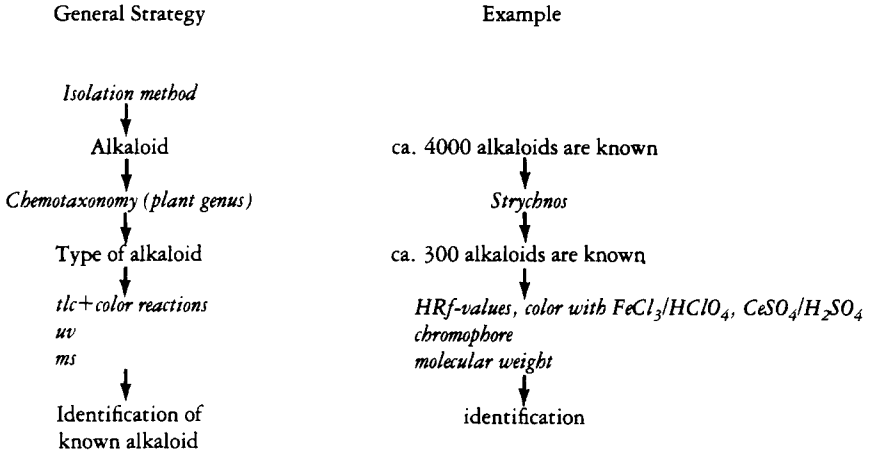


SCHEME 1. Strategy for the Identification of Alkaloids

Having reduced the problem to the identification within a limited number of compounds, one starts by studying the compound by means of tlc in combination with a specific color reagent, which often allows a preliminary identification. The recording of a uv spectrum usually allows a further reduction of the number of possible structures. Finally, a mass spectrum may result in a definite identification, as the molecular weight and the fragmentation pattern are quite specific features. In most cases, with these methods a known alkaloid can be identified. Eventually [α], ord, and cd may be necessary to confirm the stereochemistry. Ir and nmr spectra can be used to confirm the identity further. In the case of an unknown structure, other spectral methods such as ^{1}H nmr, ^{13}C nmr, and ir are usually needed for the structure elucidation (Scheme 2).

The first question with an unknown alkaloid is whether it is possible to relate it to a known one by comparing ms data. One can think of simple derivatives having, for example, extra hydroxy, methoxy, acetyl, or *N*-oxide substituents. If such an approach does not lead to any structural hypothesis, further spectral data will have to be gathered. First of all, ^{1}H nmr may give a lead by showing characteristic features already known from other alkaloids. For example, a dimeric alkaloid that was recently isolated from *Tabernaemontana* species, showed features in the ^{1}H nmr that were known from the spectra of vallesamine-apparicine type (**1**) [doublets for H-6a,b at 4.39 and 4.49 ppm (J=17.5 Hz), and for H-21a,b at 3.97 and 3.45 ppm (J=15.2 Hz)] and vobasine type (**2**) of alkaloids ($COOCH_3$ at 2.47 ppm and NCH_3 at 2.61 ppm) (Figure 1). Subsequently, the complete spectrum was assigned, and the structure **3** was proposed for this alkaloid (1,2). Where necessary, a ^{13}C nmr can be used to further confirm the structure. In the case of a completely new structure, the structural features that can be recog-

SCHEME 2. Strategy for Structure Determination of Alkaloids

Strategy for Unknown Alkaloid		
Chemotaxonomy	↦	type of alkaloid
uv	↦	chromophore
ms	↦	M^+, known fragments, Simple derivative of known alkaloid (fragments +14, +16, +30 m.u. etc.)
^{1}H nmr	↦	characteristic features, Eventually complete assignment
^{13}C nmr	↦	characteristic features, Functional groups
ir	↦	functional groups

The structural elements found are, based on biosynthetic reasoning, combined into possible structures. These structures are compared with all spectral data. This will lead to a final proposal for a structure.

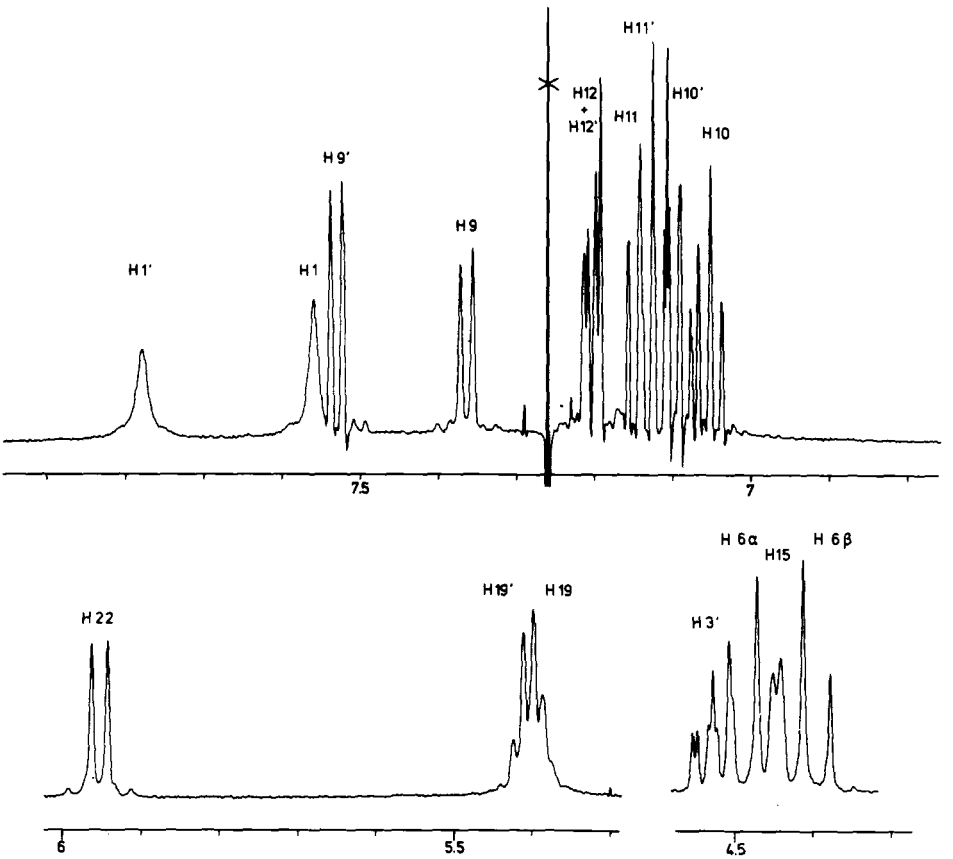


FIGURE 1. ^{1}H nmr vobparicine (**3**) (500 MHz, $CDCl_3$)

nized by the various spectral methods in combination with knowledge of the biosynthetic building blocks should be used to generate different possible structures. Subsequently, these structures can be fitted in the spectral data to see which match all the recorded data.

What can be predicted by means of biosynthetic reasoning can be seen in Scheme 3, which shows various alkaloids that can be thought of as the product of a reaction of one of the reactive groups in the dialdehyde (formed after enzymatic hydrolysis of strictosidine) with one of the N-H functions present in the molecule. In fact, all of these alkaloids have been found over the past few years. Similarly, dimeric alkaloids can be predicted. An example which I predict will be found in the near future in a *Strychnos* species is presented in Scheme 4. The compound results from the condensation of 18-desoxy Wieland-Gumlich aldehyde with akagerine, which also has an aldehyde function. Such a condensation is similar to that in the (bio-)-synthesis of caracurine V (**4**) from two molecules of Wieland Gumlich aldehyde (**5**).

This was a brief summary of the strategy we usually employ in our studies of alkaloids. What are the possibilities of the various methods mentioned? A brief survey of the different methods and, in some cases, their pitfalls will be given below.

SPECTRAL METHODS

Uv.—As one of the oldest spectrometric methods, uv is still an important tool in the identification and structure elucidation of alkaloids. In the earlier mentioned classification system, uv spectra play an important role. Considering indole alkaloids, the uv spectra play an important role. Considering indole al-

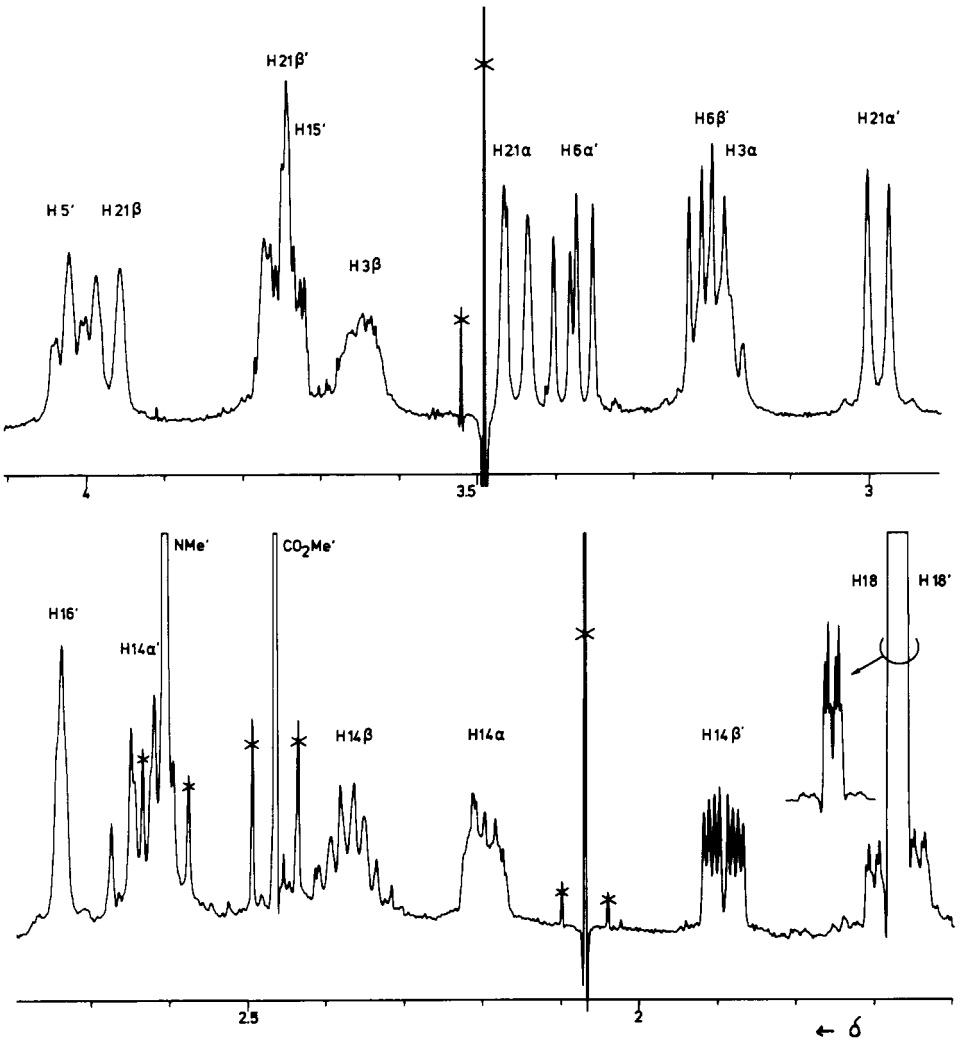
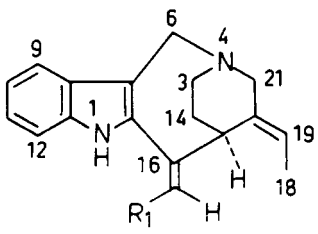


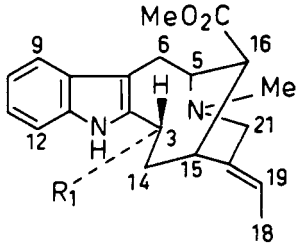
FIGURE 1. *Continued.*

kaloids, the uv spectrum gives information about the aromatic part of the molecule (3). In Figure 2, some of the characteristic spectra of the larger groups of indole alkaloids are presented. Also, substitution patterns in the aromatic part can be learned from the uv (Figure 3). Eventually, measuring uv spectra at various pH can give information about the presence of phenolic groups.

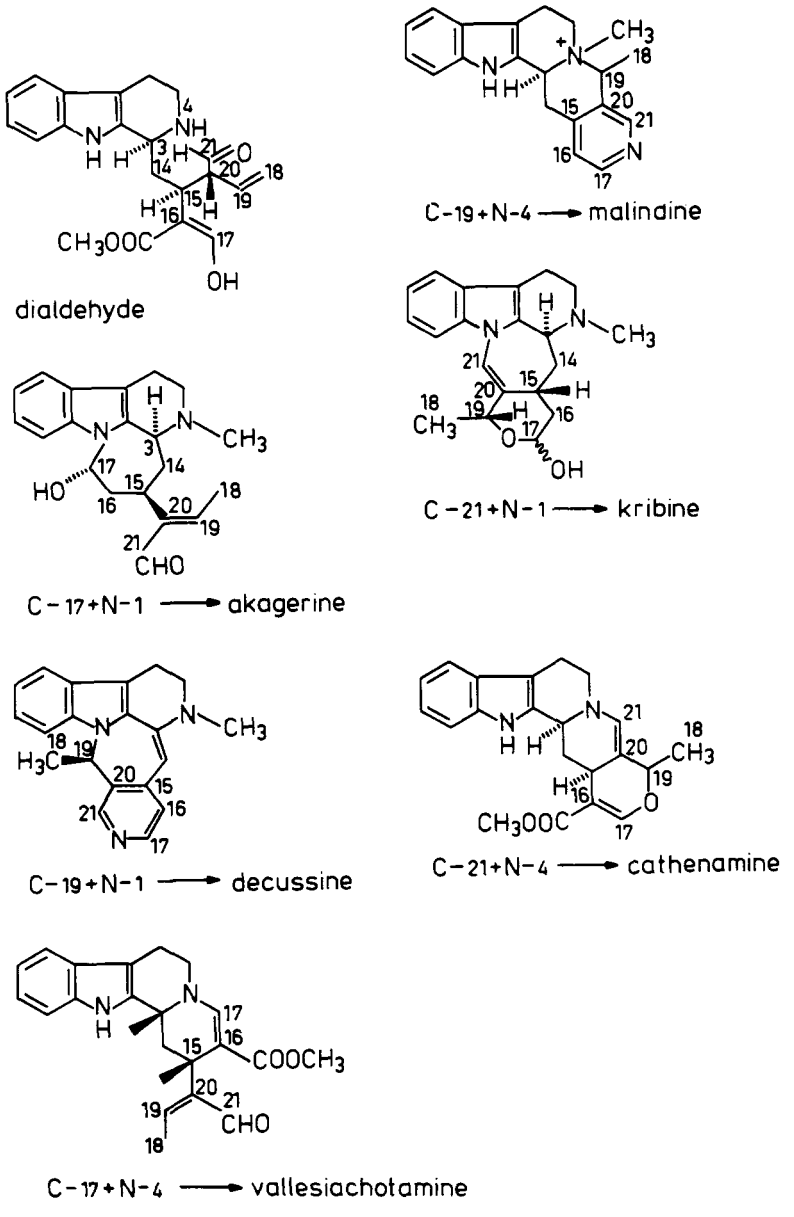
IR.—Information about functional groups can be obtained from ir spectra. Furthermore, because of the highly characteristic pattern of absorption, ir spectra are a useful tool in the identification of known



1 R_1=H apparicine
3 R_1='3-vobasinyl (**2**) vobparicine



2 R_1=OH vobasinol

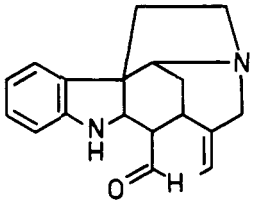


SCHEME 3. Some alkaloids derived from strictosidine

compounds by direct comparison of spectra. However, due to the advent of mass and nmr spectrometry, the role in the structure elucidation has been reduced.

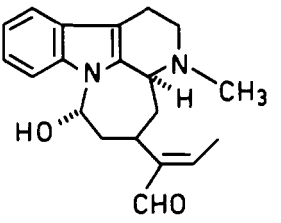
ORD, CD.—Over the past twenty-five years, the chiroptical methods have been developed into an indispensable method in the determination of the absolute stereochemistry in certain types of alkaloids. In the case of the indole alkaloids particularly, the configuration at the carbons C-2 and C-3 can be ascertained by means of these methods. An excellent review on the application of the chiroptical methods in natural product research has been given by Scopes (4). For indole alkaloids, data have been presented for heteroyohimbine (5), yohimbine (6-8), eburnane (6,7,9), sarpagan (10), iboga and voacanga (11), strychnine (12), and indolenine type (13) of alkaloids.

TLC.—Among the methods that are useful in the identification and structure elucidation of alkaloids, thin-layer chromatography should not be forgotten. Important information can be obtained from retention behavior of a compound in tlc, and color reactions can give information about the presence of certain chromophores.

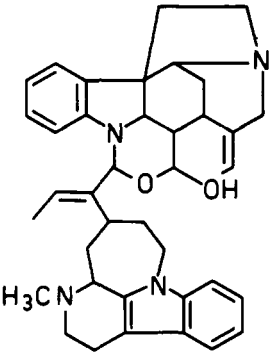


18-desoxy-Wieland Gumlich aldehyde

+

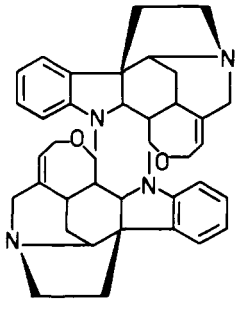


akagerine

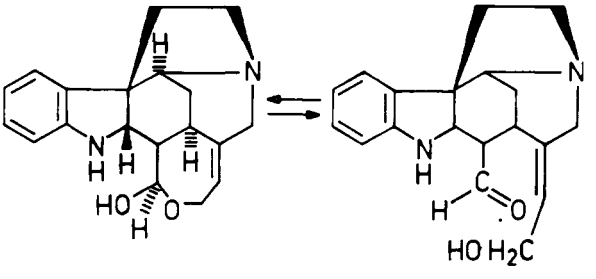


alkaloid which can be expected to be found in *Strychnos* species

SCHEME 4. Structure of as yet unknown alkaloid which may be formed in *Strychnos* by condensation of two known precursors



4 caracurine V



5 Wieland Gumlich aldehyde

Interesting work has been presented, for example, by Phillipson and Shellard (14,15) on the relationship of retention behavior in tlc and the configuration and aromatic substitution pattern of some heteroyohimbine and oxindole alkaloids. The stereochemistry of the D/E ring junction in the ajmalicine type of alkaloids was found to strongly influence retention. Alkaloids with a *cis* D/E junction (H-15α, H-20α) [reserpinine (**6**), tetrahydroalstonine (**7**)] have higher HRF-values than those with a *trans* D/E junction (H-15α, H-20β) [tetraphylline (**8**), ajmalicine (**9**)]. These observations were used to obtain information on the stereochemistry of some new *Mitragyna* alkaloids. Speciogynine and speciociliatine were known from uv, ir, ms, and ^{1}H nmr to be isomers of mitragynine (**10**). From the retention behavior of these alkaloids, compared to some alkaloids with known structures, the stereochemistry of these alkaloids was determined to be *trans* D/E for speciogynine (**11**) and *cis* D/E for speciociliatine (**12**).

An example of the usefulness of color reactions in tlc are the characteristic colors that indole alkaloids

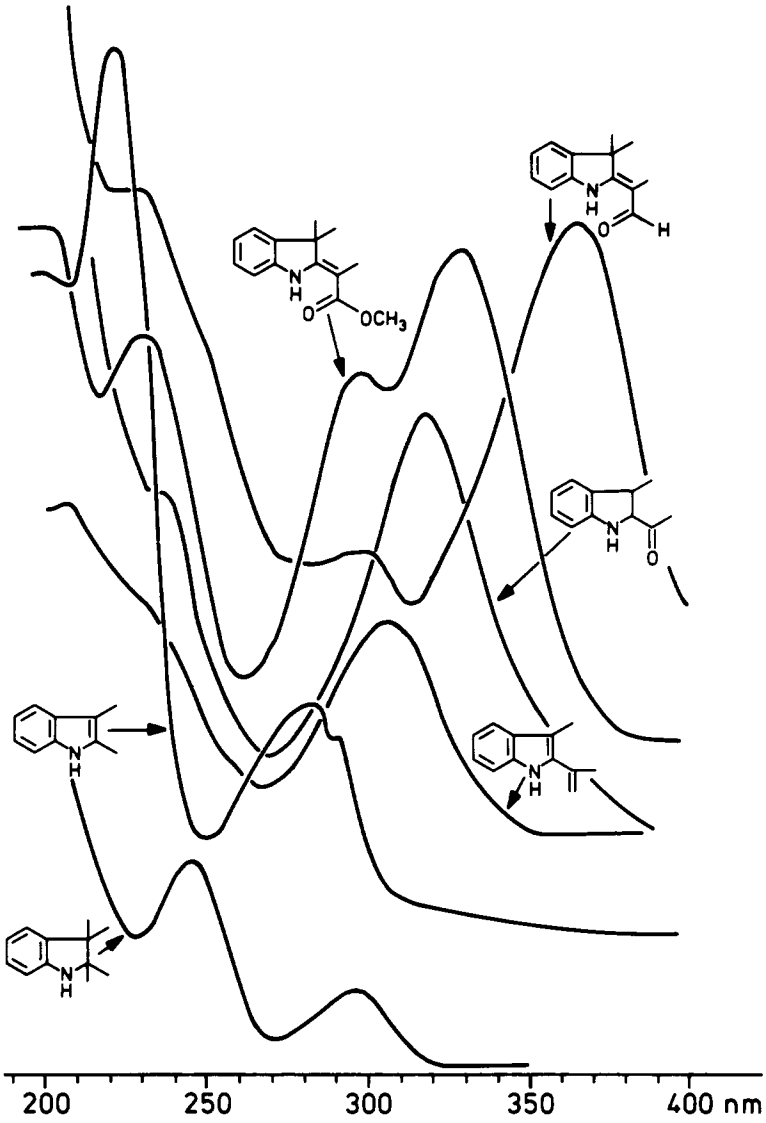


FIGURE 2. Uv spectra of some common indole chromophores (not normalized for concentrations)

give with ceric sulphate in H_2SO_4 acid or $FeCl_3$ in perchloric acid (16-19). A chromophore present in tubotaiwine (**13**) is readily recognized by its strong blue color; many other examples could be given. Observation of characteristic colors in a chromatogram immediately reduces the number of possible structures. Color reactions also allow the rapid recognition of degradation products such as *N*-oxides, as they usually give the same color reaction as the original compound, with, however, a more polar character on tlc.

It can thus be concluded that tlc, in combination with color reactions, is a powerful tool for: a) rapid identification of known compounds (e.g., References 16-18); b) classification of an unknown alkaloid into a certain group of compounds (e.g., References 16-18); and c) obtaining information about structural features such as stereochemistry, basicity, etc. For extensive review of tlc of alkaloids, see Svendsen and Verpoorte (19). Of course, with hplc and glc, similar information can be obtained; however, the unique combination with color reactions is missing (20).

X-RAY CRYSTALLOGRAPHY.—The importance, but also the limitations, of this method are well known. There is no doubt that in the case of crystalline compounds this method is very convenient. However, even in the case of structure elucidation with this technique, it will always be necessary to record the various spectral data in order to be able to identify the compound easily after future isolations.

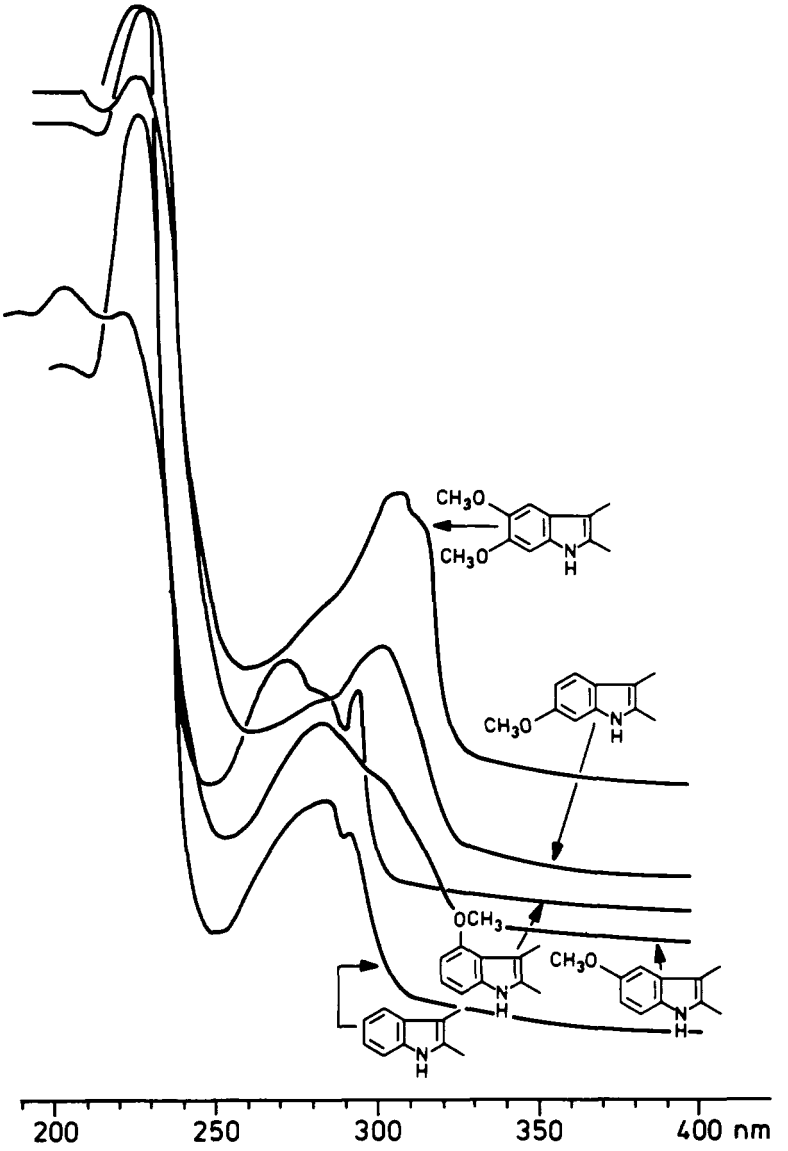
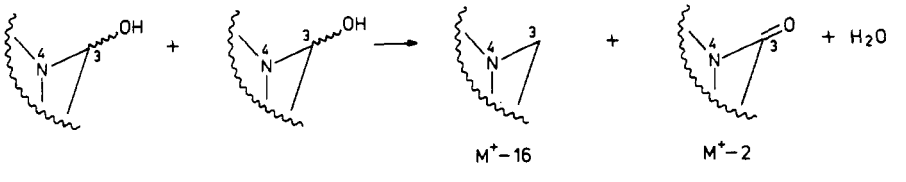


FIGURE 3. Uv spectra of some aromatic substituted indole alkaloids (not normalized for concentrations)

Ms.—In most cases, a molecular weight can be obtained by using ms. This, in combination with the fragmentation pattern and the uv spectrum, can result in the identification of an alkaloid in many cases. However, a molecular ion cannot always be obtained by means of eims. New methods such as cims, fdms, and fabms have proved to be very useful also, for alkaloids, in obtaining molecular ions from labile compounds. But even these methods may sometimes fail to give a clear molecular ion, as we have experienced, 3-Hydroxyiboga alkaloids (e.g., **14**) do not give a single clear molecular ion in any of the methods men-



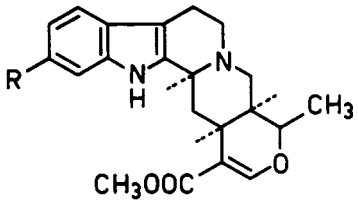
SCHEME 5. Ms fragmentation of 3-hydroxyiboga alkaloids

TABLE 2. Influence of the Solvent on the Fdms of 3*R*/*S*-hydroxyconopharyngine (**14**) (2)

Solvent	Emitter current (mA)	Fragments (*m*/*z*)												
		810	793	792	442	432	431	428	414[a]	413	412	398	397	396
CCl_4	9	+							50[b]		100		50	90
	12	+		+					60		40	40	80	100
	20		+	+					50		30	25	55	100
$CHCl_3$+1% EtOH . .	5				100				20	20	40	10	10	10
	10				40				40	40	100		45	85
	18				5				20	10	20	20	50	100
MeOH	10							100						
	12							10						
CD_3OD	10					100	35							
0.001 M HCl in H_2O . . .	8											40	100	
	10								1			25	100	
	12											10	100	

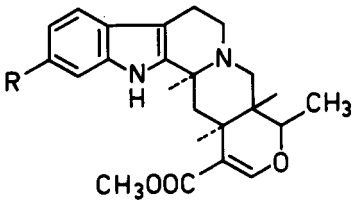
[a]M^+
[b]percent

cis D/E junction

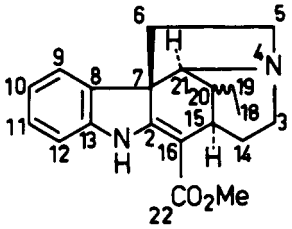


6 R=OCH_3 reserpinine
7 R=H tetrahydroalstonine

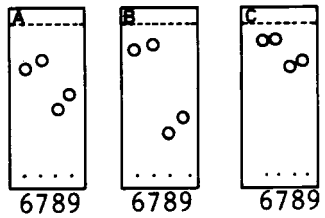
trans D/E junction



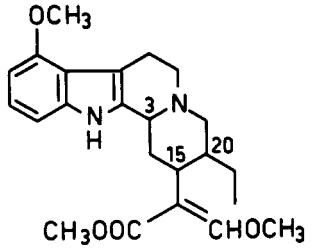
8 R=OCH_3 tetraphylline
9 R=H ajmalicine



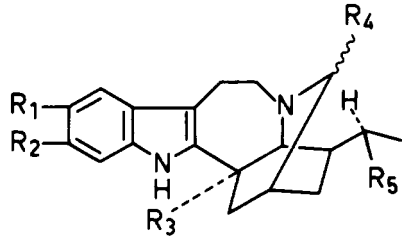
13 tubotaiwine



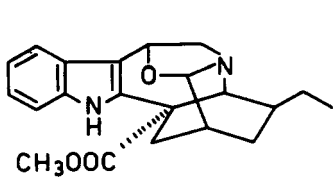
TLC-systems:
A:Al_2O_3, $CHCl_3$-C_6H_6 (1:1)
B:SiO_2, Et_2O
C:SiO_2, $CHCl_3$-Me_2CO (5:4)



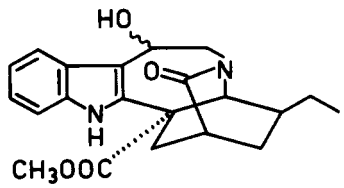
		H-3	H-15	H-20
10	mitragynine	α	α	α
11	speciogynine	α	α	β
12	speciociliatine	β	α	α



14 R_1=R_2=OCH_3, R_3=$COOCH_3$, R_4=OH, R_5=H 3*R/S*-hydroxyconopharyngine
15 R_1=R_2=R_5=H, R_3=$COOCH_3$, R_4=OEt 3-ethoxycoronaridine
18 R_1=R_2=R_5=H, R_3=$COOCH_3$, R_4=OH 3*R*-hydroxycoronaridine (eglandine revised)
19 R_1=R_2=R_5=H, R_3=$COOCH_3$, R_4=O 3-oxocoronaridine (eglandulosine revised)
21 R_1=OCH_3, R_2=R_5=H, R_3=$COOCH_3$, R_4=OH 3-hydroxyvoacangine



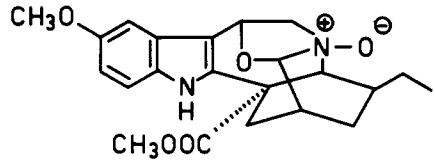
16 eglandine (old structure)



17 eglandulosine (old structure)

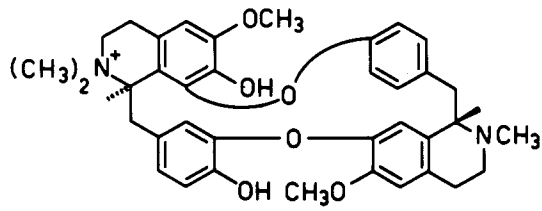
tioned (2). Strong fragments at M^+-2, M^+-16, M^+-17, and M^+-18 are always observed, which is due to reactions as shown in Scheme 5. It was also found that the solvent used to apply the alkaloid on the wire in fdms played an important role in the appearance of the spectrum. The results obtained under various conditions as summarized in Table 2 illustrate this nicely.

Such a variable behavior may lead to considerable confusion, as can be learned from literature. For example, the natural occurrence of the alkaloid 3-ethoxycoronaridine (**15**) is subject to some doubt (21). An even more interesting example is that of the alkaloids eglandine (**16**) and eglandulosine (**17**). In 1974, Le Men *et al.* (22) postulated structures **16** and **17** for these alkaloids. They were based among others upon ms data. With the abbreviated behavior of the 3-hydroxyiboga alkaloids in mind, a recent reinvestigation of these alkaloids resulted in revised structures for these alkaloids (**18,19**) (23). This also raises some questions about the alkaloid 10-*O*-methyleglandine-*N*-oxide (**20**) (24). In fact, this alkaloid might be 3-hydroxyvoacangine (**21**).



20 10-*O*-methyleglandine-*N*-oxide

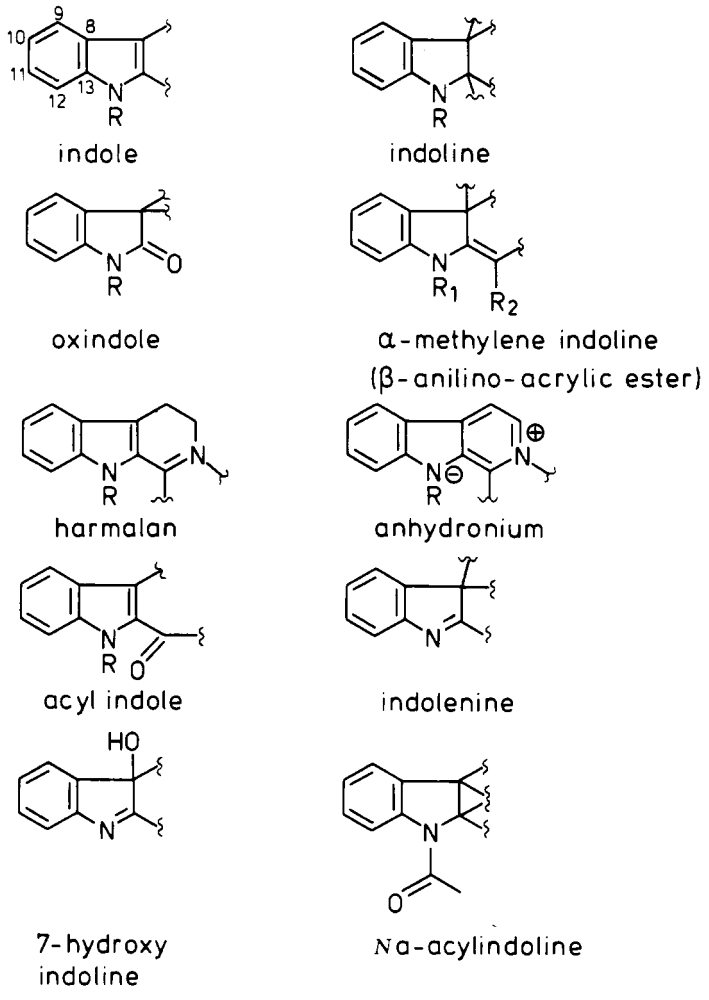
NMR.—When ^{1}H nmr was introduced in natural products research in the early 1960s, it proved immediately to be a very important tool in structure elucidation. It was the first nondestructive method that gave direct information on the presence of certain functional groups such as methyl, NH, hydroxyl, methoxyl, double bonds, aromatic protons, etc. For the more complex molecules, however, the aliphatic protons generally gave too complex patterns, allowing only its use as a fingerprint area for identification purposes. The revision of the structure of tubocurarine (**22**) (25) is in fact a classical example of the importance of ^{1}H nmr. Using this method, it became clear that only three *N*-methyl groups were present in the molecule and not four as previously thought.



22 tubocurarine

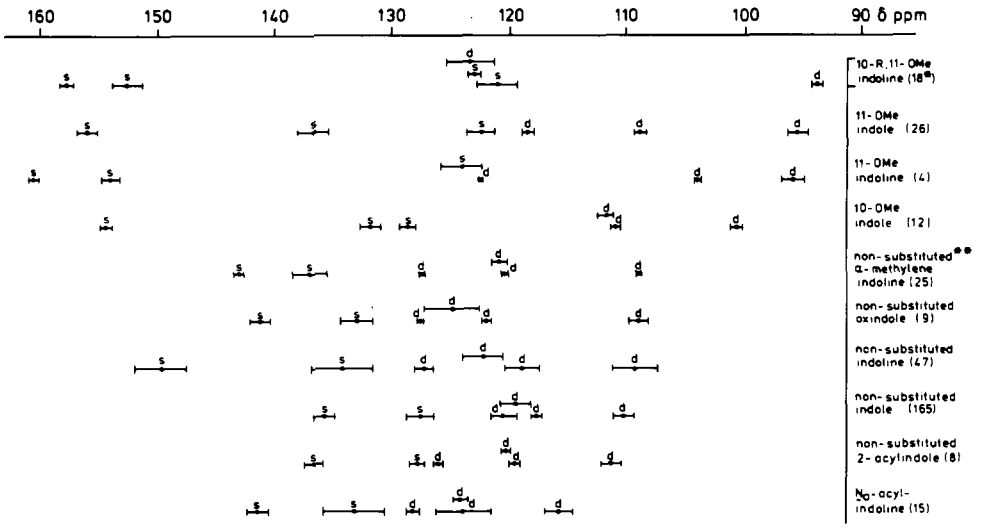
The introduction of ^{13}C nmr again was a major breakthrough as, finally, information could be obtained about all the features of a structure, e.g., the carbonskeleton, the backbone of a structure. Even the number of protons attached to each carbon could be determined. As quite a few spectra of different types of alkaloids have been published, one can draw some more general conclusions from these data. Such generalizations may be helpful in the structure elucidation of novel compounds, e.g., ^{13}C shifts have been calculated for the most common chromophoric groups among the indole alkaloids (Schemes 6,7) (26). From the shifts of the aromatic carbons, the structure of the indolic part of the molecule can be determined. With these data, collected from about 300 alkaloids, the substituent induced chemical shifts were calculated, allowing a prediction of the shifts in other related indole derivatives. In Table 3, the values as calculated for indole alkaloids with an hydroxy or methoxy group in position 11, the most often encountered site of substitution, are summarized. As can be seen, the shifts of the neighboring carbons are not symmetrical, and also the *ipso*-shift may vary quite a bit.

In the 1980s the highfield ^{1}H nmr, e.g., 300-500 MHz, became available as a routine method. Due to the increased resolution, the complete assignment of complex spectra became possible in many cases, particularly with the aid of decoupling methods. Again, this was a major breakthrough as now it was feasible to determine the relationship between all protons. This, in combination with nOe experiments, showed much about the stereochemistry of a compound, as coupling constants are dependent on the angle between the coupling protons. The introduction of two-dimensional nmr finally added an even more powerful method, allowing the rapid assignment of complex spectra and observation of all nOe effects within a molecule. Some examples of the use of 2D-nmr will be given below.



SCHEME 6. Some common chromophoric groups in indole alkaloids (nonsubstituted)

A problem that we recently encountered is worth mentioning. From a cell culture of a *Tabernaemontana* species, a series of alkaloids was isolated (27). One alkaloid was isolated in a minute amount. Its spectral data were determined. The ^{1}H nmr showed some of the characteristic features of the apparicine (**1**) nmr (Figure 4); however, all peaks were considerably shifted. A thorough analysis of the spectrum allowed its complete assignment, and it could be concluded that all signals observed in the spectrum of apparicine (28) were present; however, those signals due to protons close to N_b were shifted. Therefore, we first though of an *N*-oxide; however, in the ms, no $M^+ + 16$ could be observed, and, furthermore, the HRf-values in several tlc systems were identical to those of apparicine. Thus, we had to conclude that the alkaloid was apparicine (**1**), the major alkaloid in the cell culture. This left us with the problem of finding an explanation for the large changes in the ^{1}H nmr spectrum. Our first thought was the presence of traces of H_2O, but experiments showed that no changes in the shifts were obtained upon addition of small amounts of H_2O to a $CDCl_3$ solution. We then thought of the presence of traces of acid. Indeed, the addition of small amounts of trifluoroacetic acid (or DCl in D_2O) gave dramatic changes in the ^{1}H nmr of apparicine. We studied this in more detail. For several alkaloids, the influence of the addition of small amounts of trifluoroacetic acid on the shifts of the various protons was determined. An example is presented in Figure 5. It concerns the alkaloid quinidine (**23**). Several complex signals due to overlapping became easier to assign, whereas others became superimposed. The spectrum in the middle of Figure 5 corresponds to the addition of about one equivalent of acid. Further addition of acid resulted in changes of other protons, i.e., protons in the proximity of the less basic N_a. In Figure 6, the relationship of the shifts and the concentration of acid is shown. It is a clear linear relation until the first point of equivalence. Those protons closest to the protonated N_b do show the largest changes. The same applies for the protons close to N_a for the addition of the second equivalent of acid.



SCHEME 7. Chemical shifts of the aromatic carbons in the most common chromophoric groups in indole alkaloids [d=doublet(CH), s=singlet(C)] (26)

*number of alkaloids for which ^{13}C-nmr data have been reported in parentheses

**all alkaloids with a β-anilinoacrylic ester chromophore

From the experiments, it was concluded that trifluoroacetic acid can be a useful ^{1}H nmr shift reagent in the structure elucidation of alkaloids. It offers the following possibilities: a) identification of signals of protons close to the protonated nitrogen, b) obtaining information about the stereochemistry around the nitrogen, and c) improvement of spectral resolution. It also means that care has to be taken to avoid contamination with traces of acid, as it can cause considerable confusion in the assignment of ^{1}H nmr data.

APPLICATIONS

Finally, some examples will be given of the use of the various methods mentioned in the structure elucidation of some alkaloids.

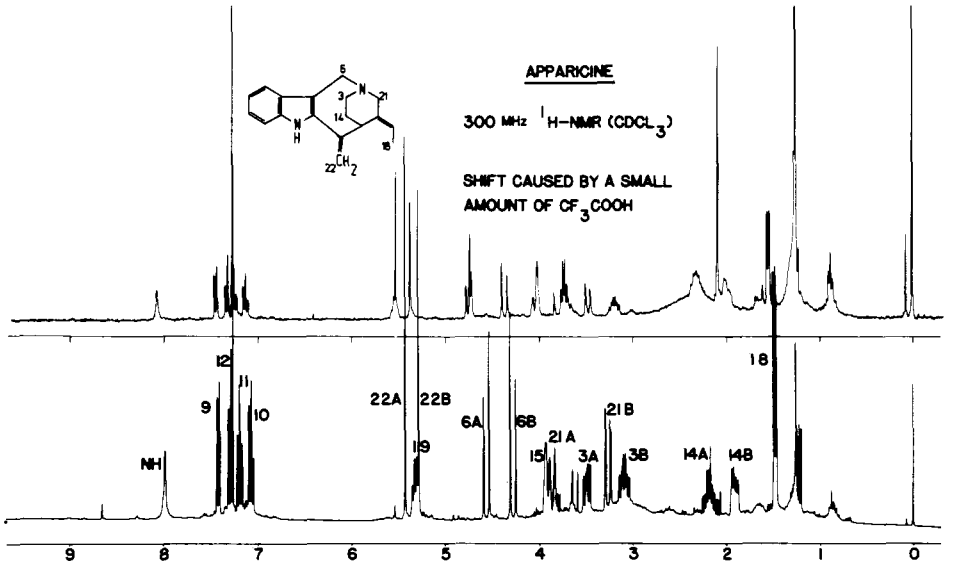


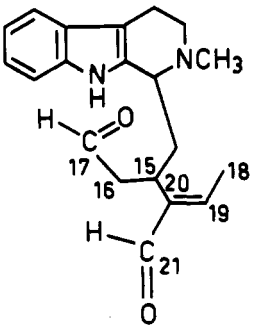
FIGURE 4. Influence of traces of acid on ^{1}H nmr of apparicine (**1**) (300 MHz, $CDCl_3$)

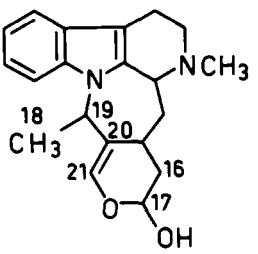
TABLE 3. Substituent Induced Chemical Shifts in 11-Hydroxy or 11-Methoxy Substituted Indole Alkaloids (26)

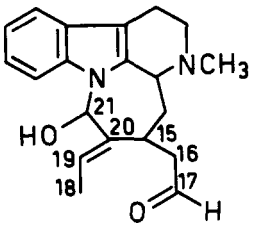
carbon	11-hydroxy			11-methoxy					
	indoles	harmalan	anhydronium	indoles	indolines	oxindoles	α-methylene-indolines[a]	harmalan	anhydronium
8 *p*	−5.6[b]	−3.7	−0.5	−5.4±0.9	−9.4	−9.2±0.2	−7.4	−2.1	+0.5
9 *m*	+0.9	+2.5	−0.1	+0.4±0.6	+0.3	−0.3±1.5	+0.3	+2.4	+0.5
10 *o*	−9.7	−6.4	−9.5	−11.0±1.1	−12.9	−14.4±0.9	−15.1	−10.0	−9.4
11 *ipso* . . .	+31.1	+27.3	+23.6	+35.0±0.9	+33.6	+31.8±0.6	+32.5	+33.9	+32.6
12 *o*	−13.0	−17.4	−15.6	−14.9±1.0	−11.1	−13.1±0.1	−12.4	−21.3	−16.1
13 *m*	−1.0	+2.4	−1.5	+0.8±0.9	+1.3	+0.9±0.7	+0.8	−0.3	+0.1

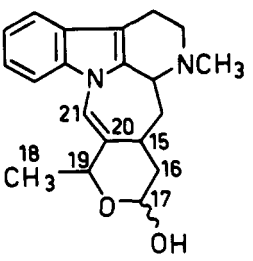
[a]all the alkaloids have a β-anilinoacrylic ester chromophore
[b]ppm (δ)

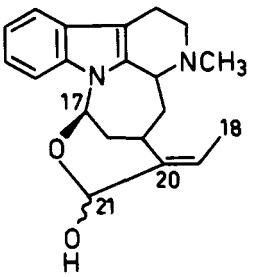
REVISED STRUCTURE KRIBINE.—The advantages of high resolution nmr and 2D-nmr compared with 60-100 MHz nmr, which were used until about ten years ago, can be demonstrated with the recent revision of the structure of kribine.











SCHEME 8. Some possible structures for kribine

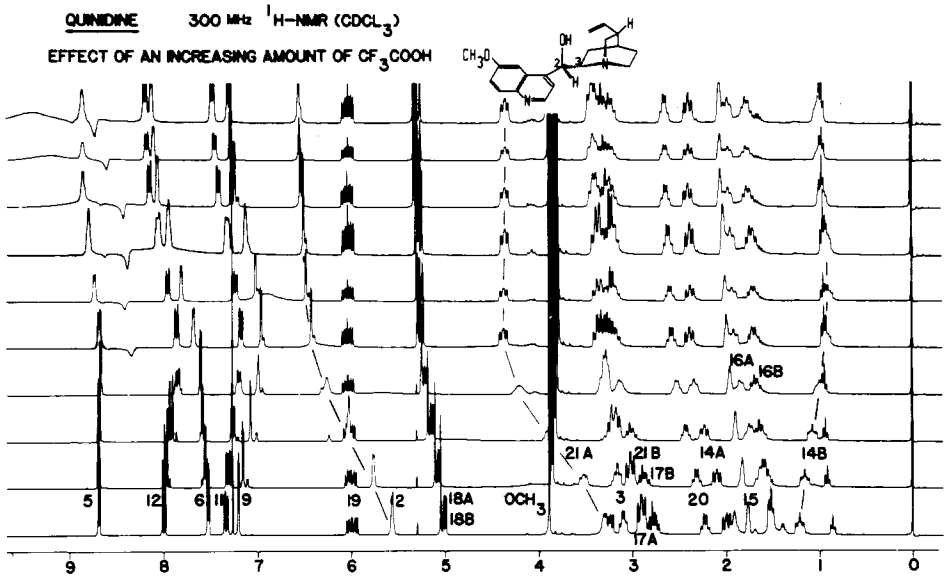
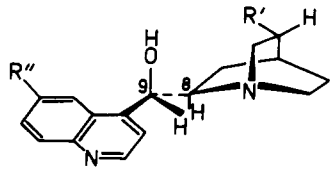


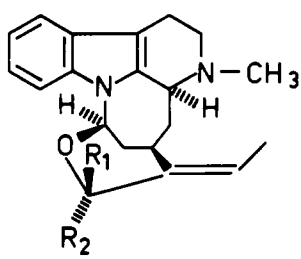
FIGURE 5. ^{1}H nmr of quinidine (**23**) with increasing amount CF_3COOH added (300 MHz, $CDCl_3$)

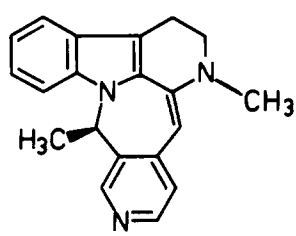


23 quinidine

Kribine was isolated about thirteen years ago (29), and based on the interconversion with the known alkaloid akagerine and its spectral data, structure (**24**) was proposed. Kribine is a mixture of two epimers, present in a 1:1 ratio. Also, the two methoxy compounds (**25,26**) corresponding to the two epimers were isolated. The isolation of the alkaloid decussine (**27**) at the end of the 1970s (30,31) raised the question of whether the akagerine or kribine-type of alkaloids could be the precursor for this alkaloid. This resulted in a reinvestigation of the structure of kribine, using more advanced nmr methods.

The first question to be answered was whether the quartets at ca. 5 ppm in the ^{1}H nmr were due to a strongly shielded vinylic proton or to a strongly deshielded aliphatic proton. With *O*-methylkribine, a heteronuclear decoupling experiment was performed





24 R_1=H, R_2=OH⇌ R_1=OH, R_2=H kribine
25 R_1=OCH_3, R_2=H 21-*O*-methylkribine
26 R_1=H, R_2=OCH_3 epi-21-*O*-methylkribine

27 decussine

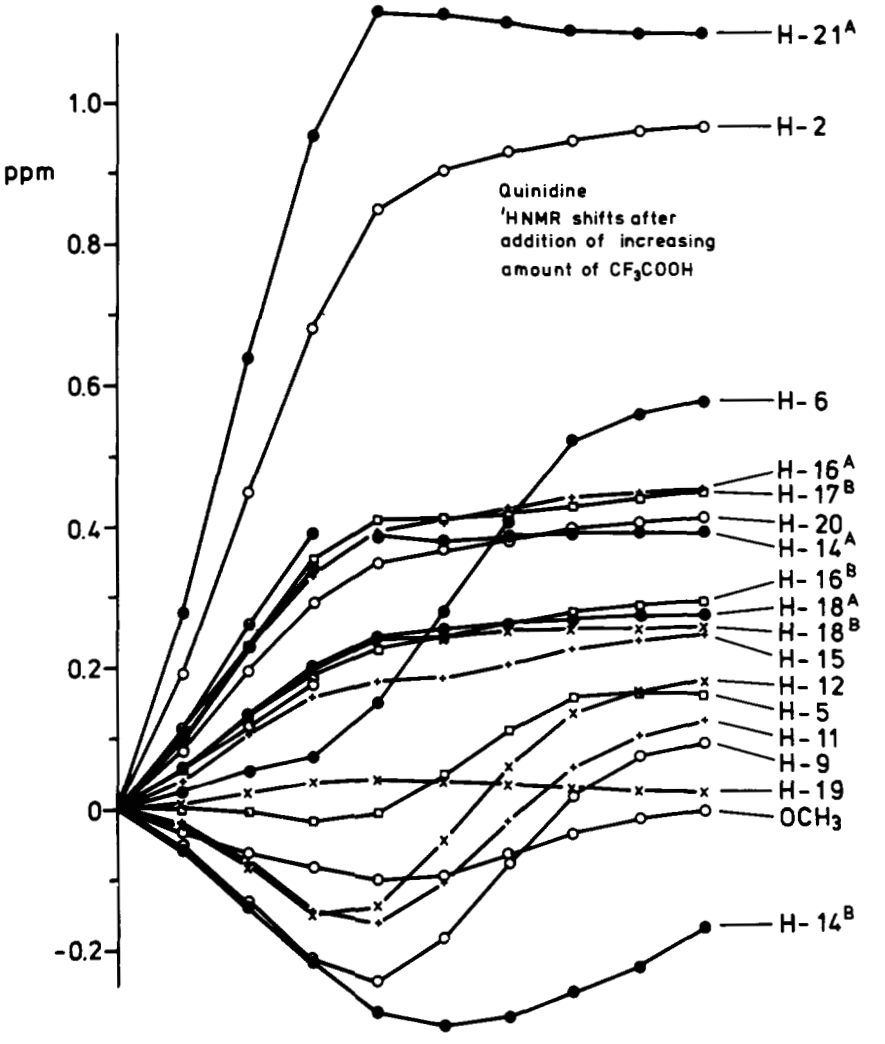


FIGURE 6. Relation between changes in chemical shifts of the protons in quinidine (**23**) and the amount of added CF_3COOH

to see if this proton coupled with an aliphatic or a vinylic carbon in the ^{13}C nmr. From the selective proton-carbon decoupling experiment, it was learned that the proton was aliphatic. Thus, the structure originally proposed was incorrect.

The next question was, of course, which is the correct structure? Based on the interconversion with akagerine, the number of possible structures was limited; in Scheme 8 some of the possibilities are shown. High resolution ^1H nmr was then used to learn more about the structure of the *O*-methylkribine. A 300 MHz ^1H nmr was recorded in $CDCl_3$ (Figure 8). In this spectrum, quite a few signals overlapped, hampering a complete assignment. Even a 500 MHz spectrum did not improve the resolution sufficiently (Figure 9). A simple change of the solvent, C_6D_6 instead of $CDCl_3$, resulted in a considerable improvement (Figure 7). This in combination with a COSY spectrum (Figure 10) resulted in the complete assignment of the ^1H nmr spectrum. From the coupling between the various protons it could be learned that structure (**28**) was the correct structure. The coupling constants also gave information about the stereochemistry of the D-ring (couplings between H-3, H-14a, H-14b, and H-15). For the stereochemistry of the *E*-ring, a chair form could be concluded from the coupling

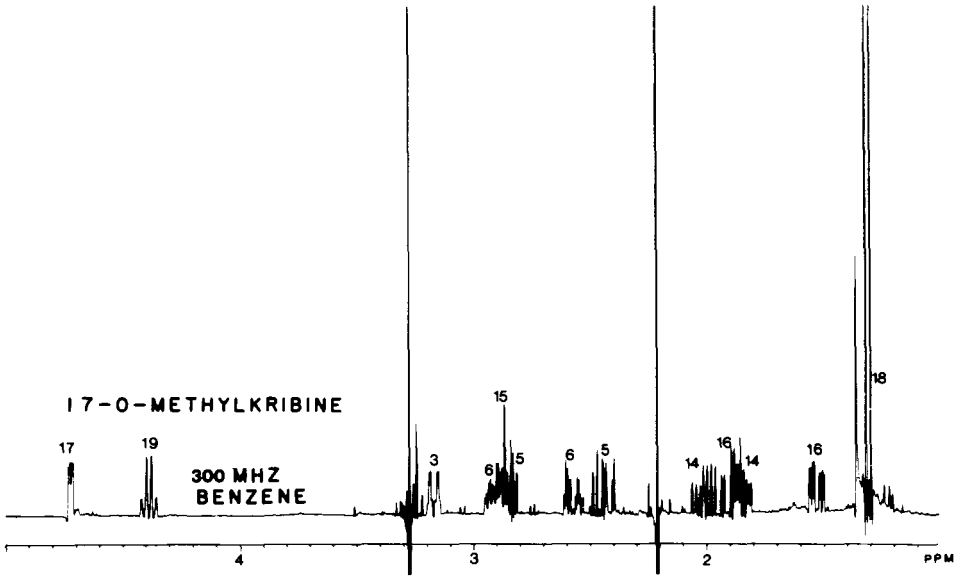


FIGURE 7. ^{1}H nmr of *O*-methylkribine in C_6D_6 (300 MHz)

between H-16a and H-15, H-17 and H-16a and H-16b, the *O*-methyl group thus being in an axial position. The configuration of C-19 was finally determined by means of some nOe difference experiments (Figure 11, 12). This experiment suggested a strong interaction between H-21 and H-18. The stereochemistry must thus be as presented in Figure 13. Comparison of the ^{13}C nmr data of the two *O*-methylderivatives further confirmed this structure. Structures (**28-32**) represent kribine and its derivatives (32).

ALKALOIDS *STRYCHNOS DALE*.—From the leaves of *S. dale*, some semidimeric alkaloids have recently been isolated (33). From the ms, it was concluded that the alkaloids were related to the cinchophyllines (34). The uv also showed the presence of a 10-*O*-methylindole chromophore. From the ^{1}H nmr, the conclusion was that instead of

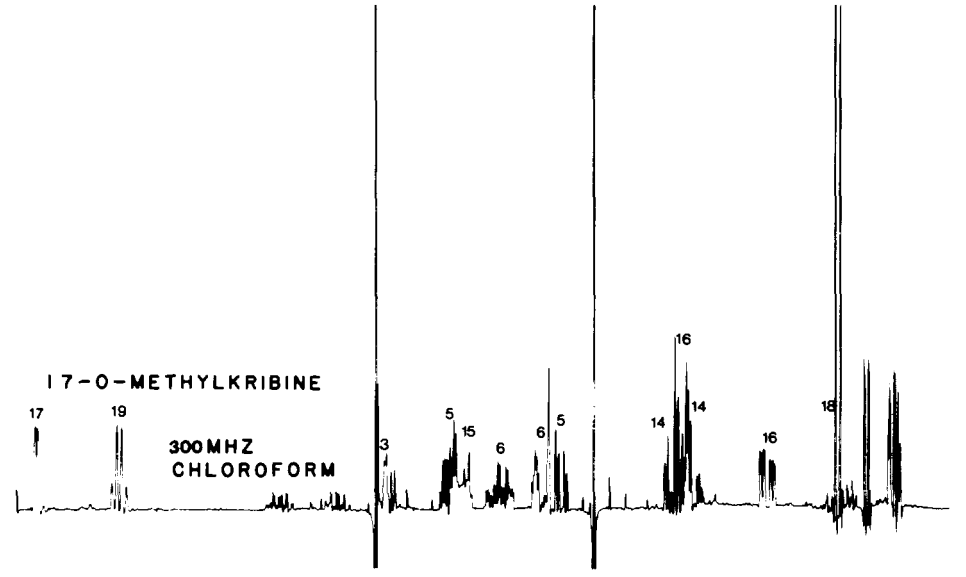


FIGURE 8. ^{1}H nmr of O-methylkribine in $CDCl_3$ (300 MHz)

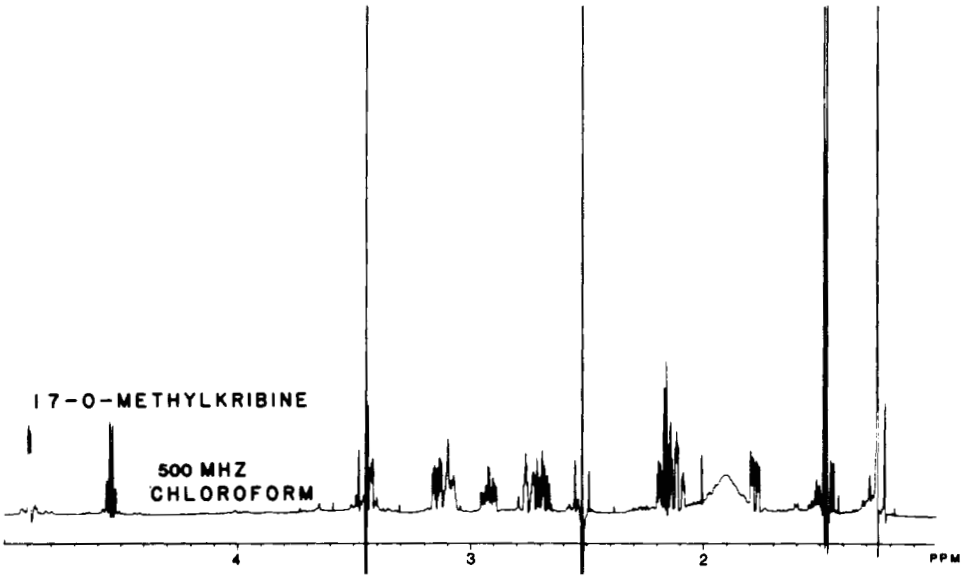
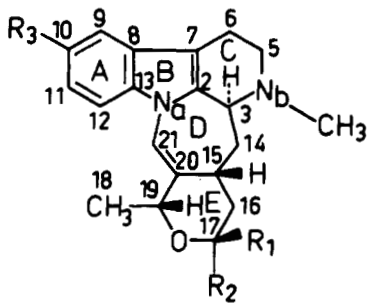
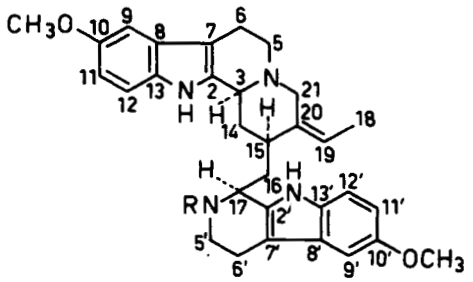


FIGURE 9. ^{1}H nmr of *O*-methylkribine in $CDCl_3$ (500 MHz)



28 R_1=OH, R_2=H⇌R_1=H, R_2=OH, R_3=H kribine
29 R_1=OCH_3, R_2=R_3=H 17-*O*-methylkribine
30 R_1=R_3=H, R_2=OCH_3, epi-17-*O*-methylkribine
31 R_1=OCH_3, R_2=H, R_3=OH 10-hydroxy-17-*O*-methylkribine
32 R_1=H, R_2=OCH_3, R_3=OH 10-hydroxy-epi-17-*O*-methylkribine

the 18-19 double bond as present in the cinchophyllines, these alkaloids had a 19-20 double bond. They thus belonged to the usambarensine type of alkaloids (**33**,**34**) (35-37). This left only the problem of the stereochemistry to be solved, i.e., the configuration at C-3 and C-17. The stereochemistry at C-3 can be determined in several ways



33 R=H 10,10′-dimethoxy-3α,17α-*Z*-tetrahydrousambarensine
34 R=CH_3 10,10′-dimethoxy-N_b′-methyl-3α,17α-*Z*-tetrahydrousambarensine

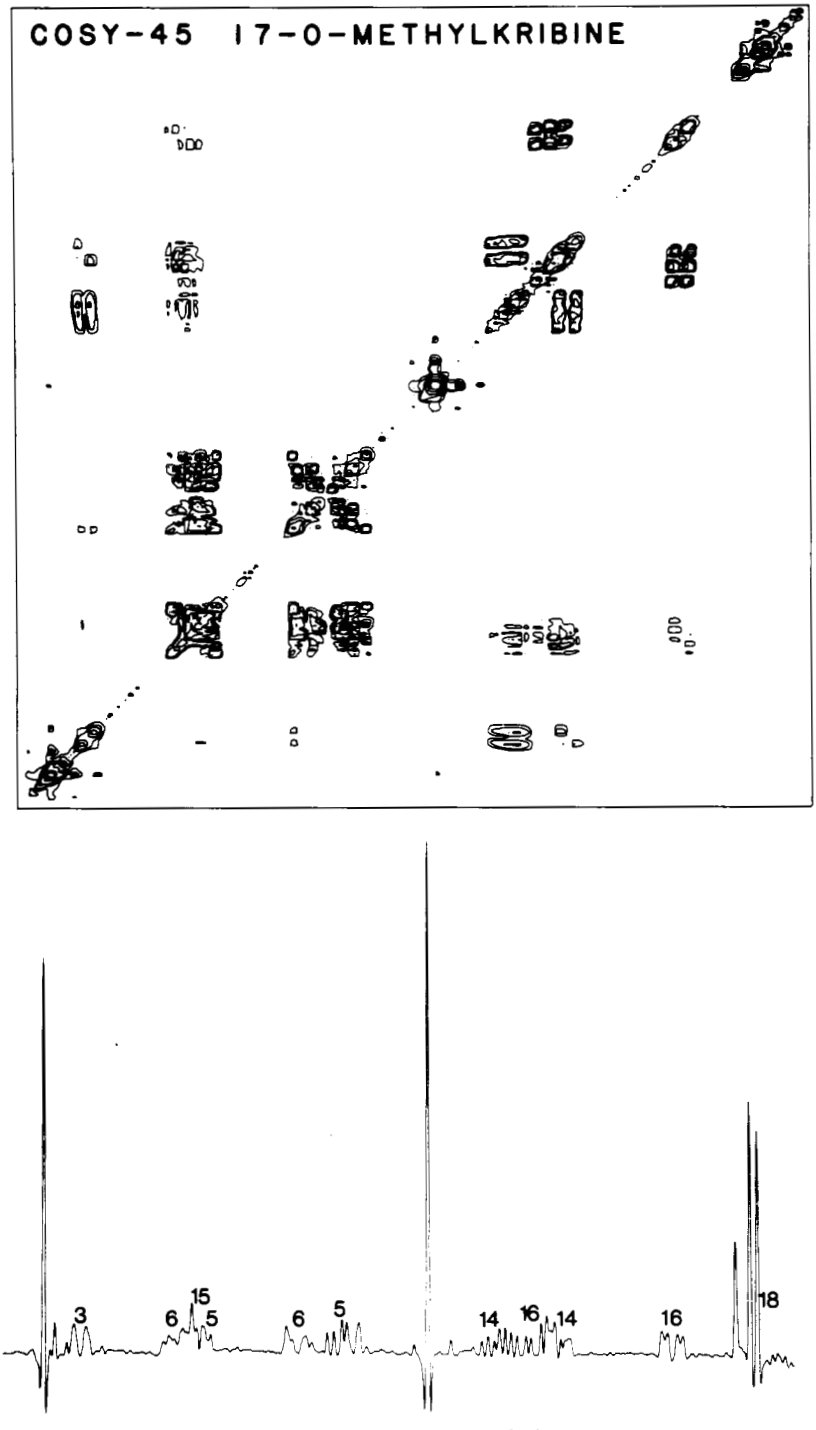


FIGURE 10. COSY-45 spectrum of *O*-methylkribine (300 MHz, $CDCl_3$)

(Table 4). The absolute stereochemistry can be determined by means of ord and cd (5-8). The conformation of the C and D rings (Figure 14) can be determined by means of ir (Bohlmann bands) (38-40), ^1H nmr (shifts of H-3) (38,40-43) and ^{13}C nmr (shifts of C-3, C-5, and C-6) (44,45). In Table 5, the characteristic spectrometric features for these four possibilities are summarized.

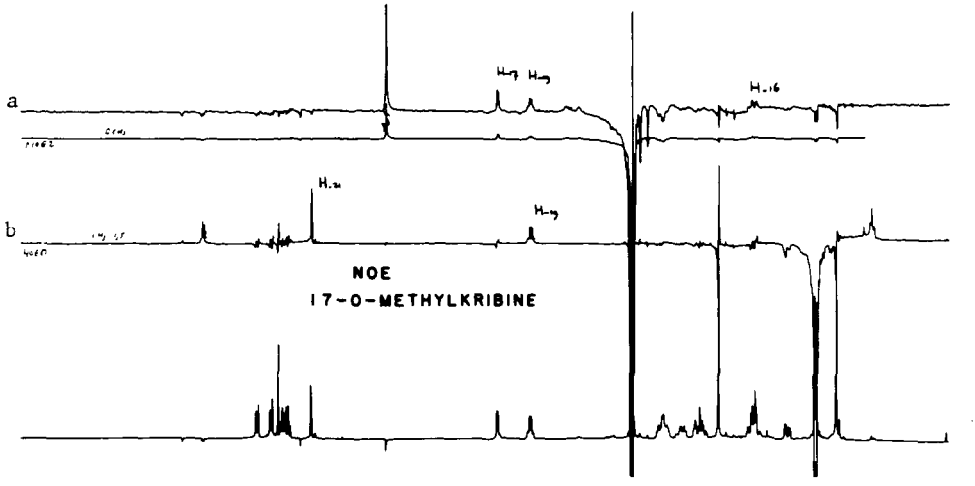


FIGURE 11. NOE-difference spectra of *O*-methylkribine (300 MHz, $CDCl_3$), *a* irradiated at OMe-signal, *b* irradiated at H-18

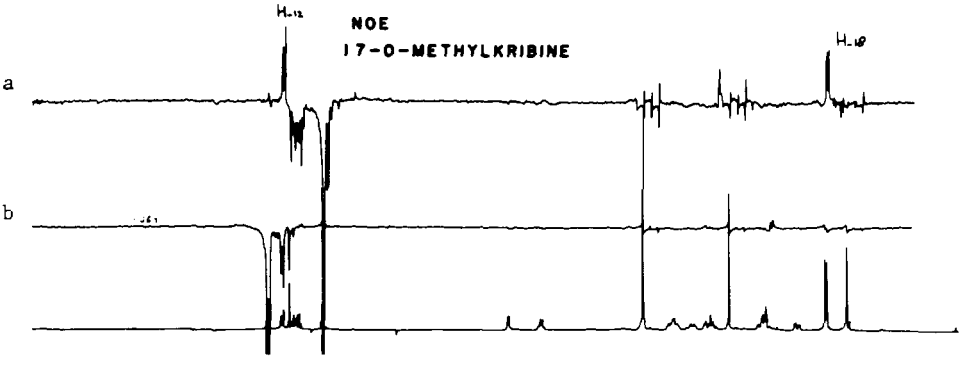


FIGURE 12. NOE-difference spectra of *O*-methylkribine (300 MHz, $CDCl_3$), *a* irradiated at H-21, *b* irradiated at H-9.

The ir and nmr data of the *S. dale* alkaloids corresponded with a 3α *trans*- or 3α *cis*-stereochemistry. The cd showed that the alkaloids had a 3α, 17α stereochemistry.

This was quite surprising as none of the so-far-reported usambarensine type of alkaloids had a 3α *trans* stereochemistry, they always had 3α *cis*. On the other hand, such a stereochemistry had been found for 3α,17α-cinchophylline (34). The only reasonable explanation for the difference in the conformation of the C and D rings for these alkaloids and the usambarensine type of alkaloids is a difference in the stereochemistry of the double bond. In the usambarensine type of alkaloids, this is known to be an *E*-configuration from X-ray crystallography (36). To establish the stereochemistry in the *S.*

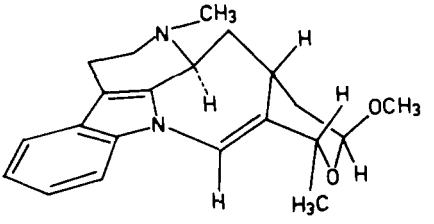


FIGURE 13. Stereochemistry of 17-*O*-methylkribine

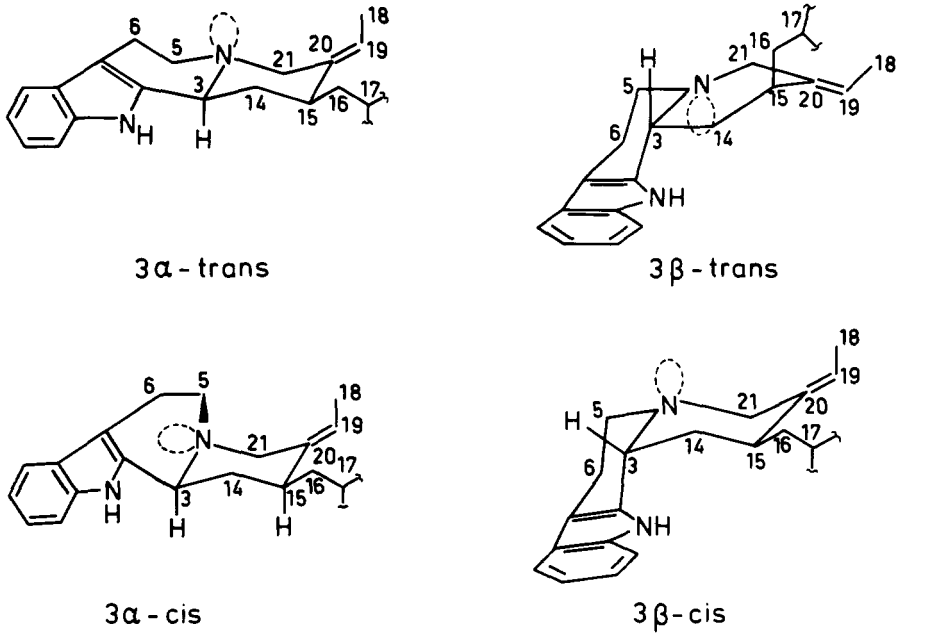


FIGURE 14. Some of the possible stereochemistries of the C/D-ring junction in the usambarensine-type of alkaloids

dale alkaloids, two-dimensional nmr was used. First, a COSY spectrum was obtained in order to be able to assign the complete spectra of the alkaloids. Subsequently, a NOESY (Figure 15) was recorded to determine the interaction between the various protons. In particular, the H-18 and H-19 protons with the surrounding protons had our interest. In the case of (**33**), a clear nOe was observed for H-18 and H-21, as well as for H-19 with H-16 and H-17. In (**34**) only clear cross peaks were observed for H-18 and H-21. An nOe-difference experiment for (**33**) further confirmed the interaction between H-19 and H-16 and H-17 (Figure 16), thereby firmly establishing a *Z*-configuration of the double bond (33). The two alkaloids are thus represented by the structures (**33,34**).

TABLE 4. Determination of Stereochemistry C-3 and C/D-ring in Heteroyohimbine Type of Alkaloids

C-3 *R* or *S*	ord, cd
cis/*trans* quinolizidine	ir (Bohlmann bands)
	^{1}H nmr (shift H-3)
	^{13}C nmr (shifts C-3, C-5, and C-6)

REVISED STRUCTURE HAYATINE.—The last example concerns the alkaloid hayatine, which is believed to be due for a revision of its structure. This bisbenzyl-

TABLE 5. Characteristic Spectral Features for the Determination of the Stereochemistry at C-3 and of the C/D Ring Junction in Heteroyohimbine Type of Alkaloids

	3α-*cis*	3α-*trans*	3β-*cis*	3β-*trans*
Ir (Bohlmann bands)	−	+	−	+
^{1}H nmr: δ H-3	4.2	<4.0	4.2	<4.0
^{13}C nmr: δ C-3	53	60	55	54
C-5	51	53	53	52
C-6	18	22	17	22

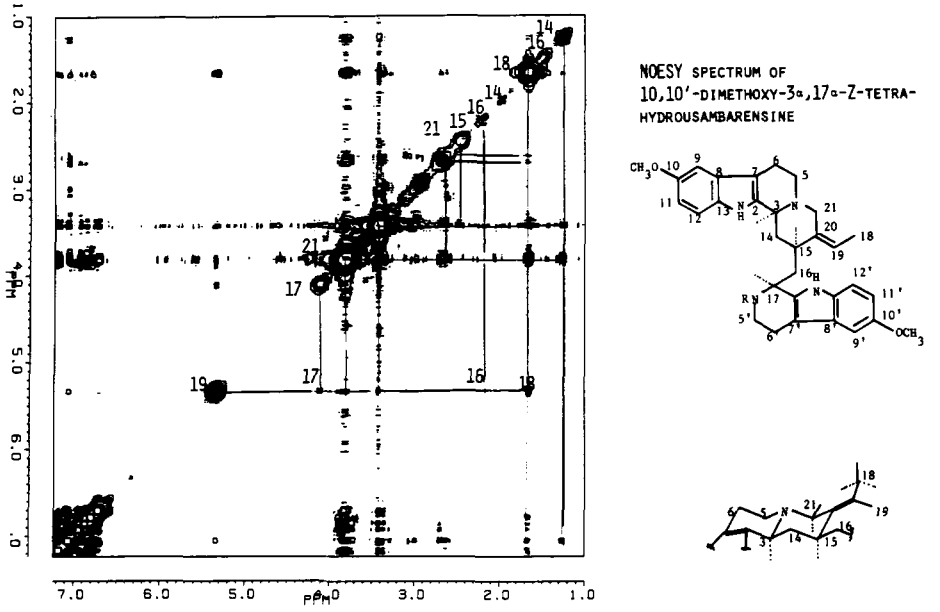


FIGURE 15. NOESY-spectrum of 10,10'-dimethoxy-3α-17α-*Z*-tetrahydrousambarensine (**33**) (300 MHz, $CDCl_3$)

isoquinoline alkaloid was first isolated by Bhattacharji and co-workers from *Cissampelos pareira* (46), together with the alkaloid (−)-bebeerine, which is identical with (−)-curine. The alkaloid was found to be only sparingly soluble in the common organic solvents. Only the hydrochloride could be dissolved in EtOH or H_2O. The alkaloid had [α]=0. It gave a positive Millon's reaction, and it was shown to possess two methoxy, two hydroxy, and two *N*-methyl groups. The alkaloid was later reisolated from the same source by Kupchan *et al.* (47), Srivastava and Klare (48) and Boissier *et al.* (49). In all those cases, l-curine was isolated from the same plant as a major component and isochondrodendrine as a minor component. In all cases, the alkaloids were characterized by their uv, ir, [α], mp, and comparison with authentic samples.

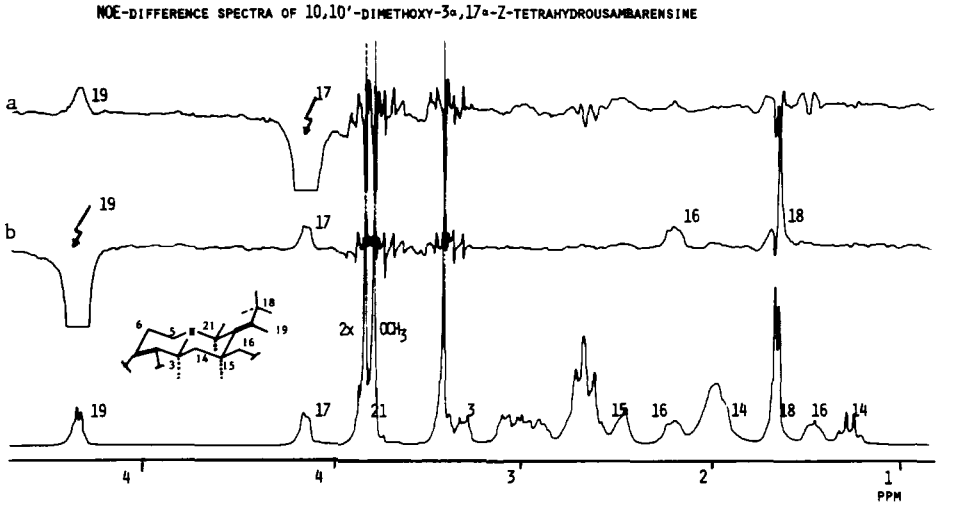
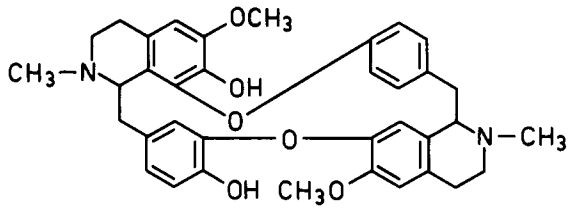


FIGURE 16. NOE-difference spectra of 10,10'-dimethoxy-3α17α-*Z*-tetrahydrousambarensine (300 MHz, $CDCl_3$), *a* irradiation at H-17, *b* irradiation at H-19

The first attempt to solve the structure of hayatine was made by Agarwal *et al.* (50). The fact that the color obtained with Millon's reagent was twice as intense as observed for (−)-curine lead to the conclusion that hayatine should have two reactive groups, as curine only has one. Therefore, structure (**36**) was proposed. An attempt to synthesize this alkaloid resulted in a small amount of a compound which did not show depression of mmp with hayatine methylethermethoiodide. This further supported the proposed structure.

In 1965, Milne and Plimmer (51) reported on the hrms of some bisbenzylisoquinoline alkaloids. They reported that the ms of hayatine was virtually the same as of curine. Also, the ^{1}H nmr data of the two alkaloids were found to be similar. It was therefore concluded that hayatine was the racemic mixture of curine. The studies of Bhatnagar *et al.* (52), who performed the Na/NH_3 fission of hayatine, led to the same conclusion. Ever since, hayatine has been accepted as being (±)-curine (**35**).



35 (±)-curine

However, a critical study of this history leads to the conclusion that somewhere a mistake has been made. The first time that hayatine was isolated, it was by means of column chromatography in which it was well separated from (−)-curine. It seems also rather unlikely that from one plant both the racemic mixture and the optically pure compound can be isolated by a simple silica gel column. Furthermore, the poor solubility as mentioned by Bhattacharji (46) was not mentioned at all by the later investigators who apparently had no problems in recording a ^{1}Hnmr spectrum in $CDCl_3$ solution. Also, the different HRf-values reported for (−)-curine and hayatine (53) point to the nonequivalence of these two alkaloids. Summarizing these arguments (Table 6) leads to the conclusion that the structure of hayatine should be revised.

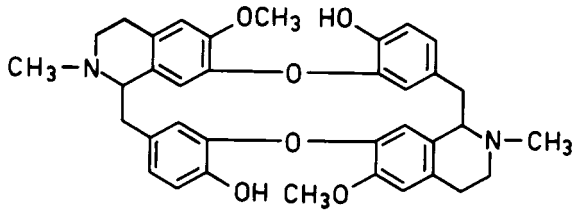
TABLE 6. Reasons That Hayatine is not Identical with (±)-Curine

Isolated from the same plant extract as (−)-curine
different HRf-values
separated by means of column chromatography
different solubilities
different intensity of coloration with Millon's reagent
different melting points

In fact we have recently isolated a small amount of a bisbenzylisoquinoline alkaloid from *Synclisia scabrida* (54) which did not dissolve in any of the common organic solvents, hampering the recording of proper nmr spectra. The few data we have been able to obtain all point to a structure as proposed for hayatine by Agarwal *et al.* (50). However, as authentic hayatine is no longer available, a direct comparison is not possible.

The evidence we have is founded first of all on the ms. The molecular weight is 594, and fragments are present which are typical for a head-tail coupled bisbenzylisoquinoline alkaloid (fragment at *m/z* 298). No fragments are observed at *m/z* 89 or 90, pointing to the absence of a nonsubstituted benzylic moiety (55). Similarly, the lack of a fragment at *m/z* 191 or 204 points to the absence of a tri-substituted isoquinoline

moiety (56). From the ^{1}H nmr and the ^{13}C nmr spectra, it was learned that the molecule was symmetrical. The structure (**36**) (50) fits into these structural requirements. The alkaloid we isolated is thus concluded to have structure (**36**). A reinvestigation of *C. pareira* seems necessary to clarify this matter further.



36 hayatine [original structure according to Agarwal *et al.* (50)]

FUTURE.—What will the future bring us? Observing the tremendous development in nmr techniques, it seems possible that in the future (1990s) by using homonuclear and heteronuclear shift correlated methods, a completely computerized structure elucidation will become feasible. Of course, we will miss the intellectual challenge of solving a structural problem; on the other hand, as pharmacognosists, we will obtain more time for the research we ought to be doing: the isolation of (new) biological active compounds from plants. Maybe twenty years from now, one will look back on the present period with all its emphasis on spectrometry in the same way in which we look back on the period in which microscopy governed pharmacognostical research, once an important tool, although it nearly meant the end of a discipline.

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