RECENT RESEARCHES ON INDOLE ALKALOIDS

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Since this is a meeting of pharmaceutical chemists and biochemists, it seemed best to devote my lecture to a review of the general chemistry of two groups of alkaloids rather than select any one substance to cover in great chemical detail. And because part of my lecture will be on some of the calabash curare alkaloids[1], it is a great pleasure for me to have Professor Schmid acting as Chairman. You will see as I go along the brilliant work which Professor Karrer and Professor Schmid with their co-workers carried out on the three or four curare alkaloids we shall consider. In fact, this lecture ought to be regarded as a joint one with my colleagues in Zürich. Professor Wieland[1, 2], who is with us too, and Professor Boekelheide[1, 3] in the United States also made important discoveries by isolation and by chemical study in this field, but much of their chemical work was on alkaloids which I shall not be able to discuss today. Finally, Professor Marini-Bettolo[4] and his co-workers in Rome have been very active in isolating and characterizing alkaloids in this area of study.

The pharmacological interest of curarizing agents is due to the use of these substances in surgery. With their aid, it is possible to achieve the required degree of muscular relaxation for surgical operations when using lighter degrees of anaesthesia than would otherwise be necessary. The potential dangers of deep anaesthesia will be familiar to you. Tubocurarine and the synthetic curarizing agents are well known to you all and the quaternary alkaloids of calabash curare, some of which are highly active curarizing agents, had obvious medicinal interest. Perhaps I should explain that calabash curare is that type of South American arrow poison which is packed in dried calabashes.

The first major problem to be overcome in this field was the complexity of the mixture of quaternary alkaloids present in curare, and the initial successes came in the late 1930's and early 1940's to the school of Heinrich Wieland in Munich[5] using chromatographic methods on alumina. They isolated among other alkaloids two that I shall talk about later, namely, toxiferine-I and the so-called dihydrotoxiferine-I. Dr Harold King[6] in England worked on the bark from a single plant, *Strychnos toxifera*, which is known to be one of the important natural products used by the South American Indians in their preparation of calabash curare. By using Wieland's method, he isolated toxiferine-I and several other alkaloids. However, the large number of alkaloids present in calabash curare only became clear when partition chromatography on cellulose was developed by the Munich and Zürich schools[7, 8]. This showed, for example, the presence of at least forty different alkaloids in a sample of South American curare and we found[9]

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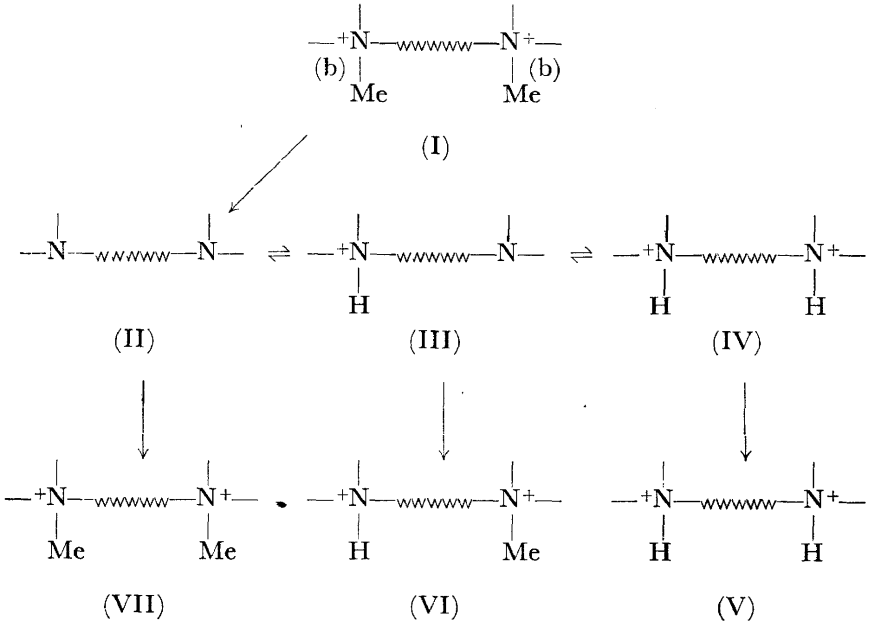
that even the pure plant material *Strychnos toxifera* contains at least thirty quaternary alkaloids. Chromatography on cellulose columns has led to the isolation of more than seventy crystalline curare alkaloids. Most of them are only available in very small amount and, as a result, much of the chemical work has been carried out on a semi-micro scale. Also, the work must be conducted with great care because of the high toxicities of these compounds. Thus the dose in the *head drop* test on mice for toxiferine-I is 9 γ/kg (lethal dose 23 γ/kg). Dihydrotoxiferine-I is somewhat less toxic—45 γ/kg in head drop and 100 γ/kg lethal dose.

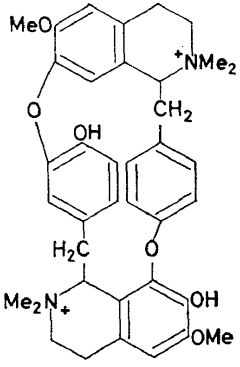


I want to turn now to the chemistry of toxiferine-I and c-dihydrotoxiferine-I. The account which follows is, of necessity, a simplified one and I cannot do full justice to the very skilful work of the men who carried out these researches on the laboratory bench. The many complexities and inter-relationships will be stripped away in the logical account which follows. It must be done this way for otherwise the principal facts would be lost in too much detail.

In the early days, there was some confusion about the true molecular formulae of these alkaloids and formulae involving twenty carbon atoms and two nitrogen atoms were commonly used, but the Zürich group showed[10] by a very neat partial quaternization method that these alkaloids contain forty carbon atoms and four nitrogen atoms, two of the nitrogen atoms being quaternary. The method involves pyrolysis of the quaternary alkaloid (I) to give the corresponding tertiary base (II) which is then treated with half an equivalent of mineral acid. This affords an equilibrium mixture of the species (II), (III), and (IV). Methylation then yields a mixture of the tertiary base [because the diprotonated species (IV) is unaffected (IV) = (V)], the

mono-metho derivative (VI), and the original diquaternary alkaloid (VII) [= (I)]. This preparation of a mono-metho derivative (VI) with one *N*(b)-methyl group in each C_{39} unit is conclusive evidence that the original alkaloid molecule has two *N*(b)-methyl groups in a C_{40} unit. I have outlined this because of its importance in the historical development of curare chemistry. The molecular weight of alkaloids is now more readily determined by mass spectrometry; I shall give an example later.

Toxiferine-I is very similar in its properties to dihydrotoxiferine-I and other alkaloids of established C_{40} molecular formulae, so it was suggested[10]



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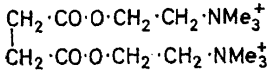
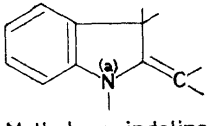


(IX)

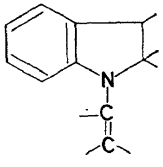
that toxiferine-I is also a C_{40} molecule and we shall see presently that this is so. This result makes clear the relationship of the calabash curare alkaloids to other quaternary curarizing agents. Thus tubocurarine (VIII) and the synthetic materials, such as succinylcholine (IX), have two quaternary nitrogen atoms set some distance apart. The calabash curare alkaloids now turn out to be in the same class.

Let us look first at the chemistry of C-dihydrotoxiferine-I. Much effort was used[1] in the early chemical study of this alkaloid and the partial formulae below* summarize what was known just before the final breakthrough in

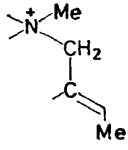


Methylene indoline

or

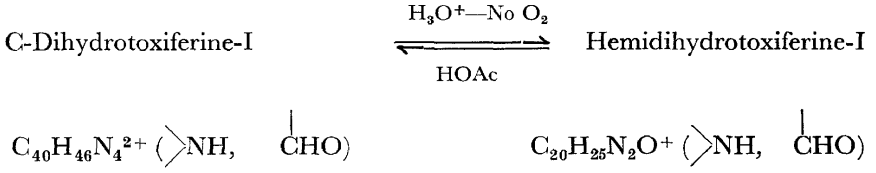


N-Vinylindoline



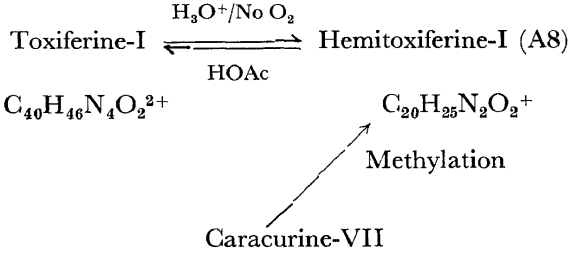
* The location of the enamine system will be considered later.

structural elucidation occurred. The first step came when the Zürich group showed[11, 12] that C-dihydrotoxiferine-I is converted by dilute mineral acid into a C_{20} alkaloid, hemidihydrotoxiferine-I which was found to be an indoline derivative by its ultra-violet absorption. Its infra-red spectrum showed the presence of an aldehyde group. Further, hemidihydrotoxiferine-I, a C_{20} molecule, was converted back into C-dihydrotoxiferine-I, a C_{40} molecule, by dilute acetic acid. Clearly, C-dihydrotoxiferine-I is formed by dimerization of hemidihydrotoxiferine-I as shown below. In deriving this relationship, many important results were obtained from researches on the corresponding tertiary base series.



Whilst this work on C-dihydrotoxiferine-I was proceeding, we were working independently on toxiferine-I and also on the isolation of new alkaloids from *S. toxifera*. I must say that we were extremely fortunate in this isolation work for the substance which crystallized most readily for us was a new alkaloid provisionally named A8. This was valuable because Hodson found that when toxiferine-I is treated with mineral acid in the absence of oxygen, it yields this same alkaloid, A8, and so a readily isolated alkaloid related to the rare toxiferine-I was available[13]. Very little was known at this stage about toxiferine-I but its ultra-violet spectrum was very similar to that of C-dihydrotoxiferine-I and corresponded to one or other of the methylene indoline or *N*-vinylindoline chromophores shown earlier.

It is one of the facets of scientific work that many useful discoveries are made by chance and this certainly occurred with alkaloid A8; for, as a result of trying to do something different, the conversion of A8 into toxiferine-I by acetic acid was carried out[13]. These results then allow the following relationships to be set down, ignoring for the moment the caracurine-VII.



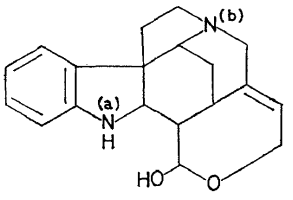
As for C-dihydrotoxiferine-I, there is a breakdown with mineral acid and a dimerization in the presence of an organic acid. Alkaloid A8 is now named hemitoxiferine-I because of its formation by fission of toxiferine-I.

Caracurine-VII is a tertiary base isolated[14] by the Zürich group from Venezualan *S. toxifera* and it is brought into the picture because it is the

only alkaloid in this large class of compounds which gives the highly characteristic colour reaction shown by hemitoxiferine-I (A8). Since caracurine-VII is a tertiary base and hemitoxiferine-I is a quaternary salt, the strong similarity of these two substances led to a comparison of the quaternary *N*(b)-metho-derivative of caracurine-VII with hemitoxiferine-I, and the two were identical[13]. All the interconversions set out above have now been established and I want to stress that the relationship of toxiferine-I and hemitoxiferine-I to caracurine-VII (and to other tertiary *S. toxifera* alkaloids) was discovered independently in Zürich[11, 12] in a way somewhat different from the one I have outlined. So, in total, there is ample evidence for the various relationships discussed above.

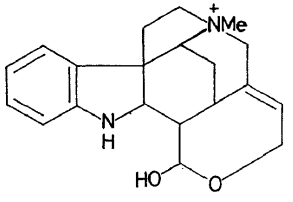
Just one piece of knowledge was now required and it came from the proof by the Zürich group[15] that caracurine-VII is in fact the Wieland–Gumlich aldehyde (X). This compound had been long known from Wieland's degradative work on strychnine. The possibility that caracurine-VII might be the Wieland–Gumlich aldehyde was considered by the Swiss chemists as the result of their extensive study of the chemistry of yet another alkaloid, C-fluorocurarine. However, another lecture would be required to review this. What I do want to make clear to you is the way in which many of the curare alkaloids have related structures and that knowledge from the study of one alkaloid greatly helped structural work on another.

The importance of knowing the structure of caracurine-VII cannot be over-emphasized since, in conjunction with all the relationships outlined above, it allowed structures to be written for C-dihydrotoxiferine-I and toxiferine-I and so opened the way to many other structures of alkaloids in this group.



(X)

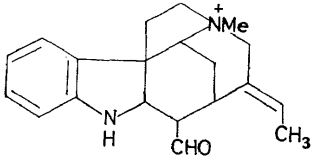
Caracurine-VII
(Wieland–Gumlich aldehyde)



(XI)

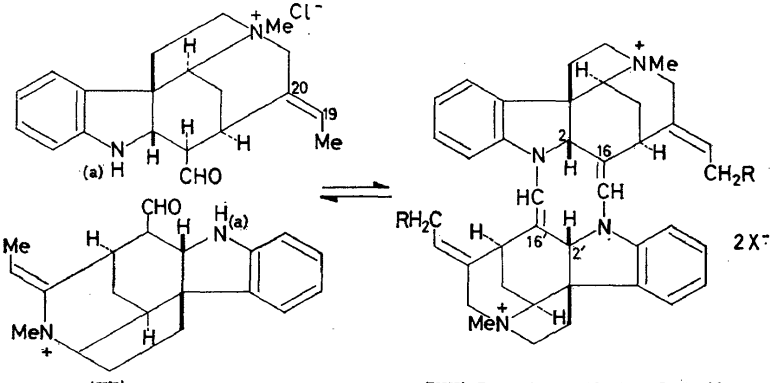
Hemitoxiferine-I (A8)

Knowing the structure of caracurine-VII, the Swiss workers suggested that hemidihydrotoxiferine-I has the structure (XII) and soon showed that



(XII)

this is so by unequivocal methods[11, 12]. Since hemitoxiferine-I is the *N*(b)-metho-derivative of caracurine-VII, it followed[12, 13] that it has the structure (XI). At this stage the structures of hemitoxiferine-I and hemidihydrotoxiferine-I were both known and it is necessary to consider first how C-dihydrotoxiferine-I is formed from two molecules of the aldehyde (XII). The >NH groups and CHO groups of the salt (XII) are lost in the dimerization and on this basis the structure (XIII) was proposed* by the Swiss workers[11, 12] for C-dihydrotoxiferine-I. It thus follows naturally from the Swiss interpretation of the dimerization reaction and from the relationships established in the foregoing paragraphs that toxiferine-I has the structure* (XIV) by dimerization of two molecules of hemitoxiferine-I[11–13].



(XII)
Hemidihydrotoxiferine - I

(XIII): Dihydrotoxiferine-I R=H
(XIV): Toxiferine-I R=OH
(XV): R=OAc

In this case it is the potential aldehyde group of the hemiacetal system in hemitoxiferine-I (XI) which condenses with *N*(a) of another molecule of the same substance. It can now be seen that C-dihydrotoxiferine-I should be called deoxytoxiferine-I, but the former name is established by long use.

The pharmacological activity of the calabash curare alkaloids was mentioned at the outset and so there was a clear interest in making toxiferine-I in quantity. The required intermediate, Wieland–Gumlich aldehyde, can be prepared from the readily available strychnine. Also, by using groups other than methyl to quaternize *N*(b), analogues of toxiferine-I can be made.

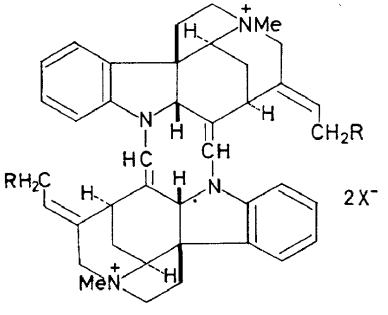
However, the dimerization of hemitoxiferine-I in acetic acid or in acetate buffer gave toxiferine-I in admixture with several other products and the process was not a simple one[13, 17]. One side product is the diacetyl derivative (XV) formed by ready esterification of the allylic hydroxyl groups. To overcome this we used[13] trimethyl acetic acid as the condensing catalyst because here there is strong steric hindrance of acylation reactions. This approach afforded toxiferine-I directly from hemitoxiferine-I in good yield with few by-products and this method and similar ones have been widely

* Initially the positions 2,16 and 2′,16′ were favoured for the central double bonds of dihydrotoxiferine-I and of toxiferine-I. More recent work involving nuclear magnetic resonance[3, 16] and chemical degradation[16] has proved that the illustrated positions are the correct ones.

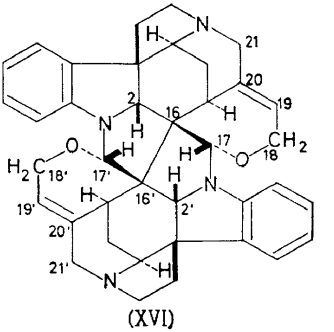
used. Analogues of toxiferine-I have been made and one such analogue (the diallyl analogue) is commercially available for use in medical practice. It has been prepared from *N*(b)-allyl Wieland–Gumlich aldehyde by Fürst, Boller and Els (F. Hoffmann-La Roche) and it has been found in many clinical trials to be an excellent agent for muscle relaxation, of short duration, specific and without side effects.

One of the remarkable features of the chemistry of the alkaloids we have discussed so far is the ease with which they are transformed into other substances. This is perhaps not surprising when one looks at the diazacyclo-octadiene system which forms the centre of toxiferine-I and dihydrotoxi-ferine-I. These changes lead to two groups of alkaloids and we can discuss one of these substances further. The very ready cleavage of toxiferine-I by dilute acid in the absence of oxygen has been mentioned already. However, quite a different reaction occurs in the presence of oxygen and, under the right conditions with very dilute acid, oxygen and a platinum catalyst, it is possible to convert toxiferine-I in high yield into a different substance called caracurine-II dimethochloride. This product has a lower physiological activity than toxiferine-I itself and so not only is there the chemical interest of working out its structure, but also the interest of structure–activity relationships. Much of the structural work was carried out by Swiss and British workers[18].

Pyrolysis of the quaternary alkaloid caracurine-II dimethochloride gives the tertiary base caracurine-II, which can also be made by a more direct route[18]. The molecular formula of this large molecule was shown to be $C_{38}H_{38}N_4O_2$ by many analyses and by mass spectrometry (kindly carried out by Dr R. I. Reed, Glasgow). This proof that there are thirty-eight hydrogen atoms is of considerable importance in the later argument. We need not consider all the details of the structural determination, but the constitution of caracurine-II was finally shown to be (XVI). The key steps in the argument were these: hydroxyl groups were absent by spectroscopic examination and catalytic hydrogenation showed the presence only of *two* double bonds in addition to the two benzene rings.



Toxiferine-I (R = OH)

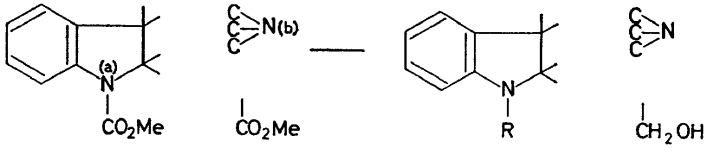


(XVI)

From this knowledge and knowing the molecular formula, it follows that there are fourteen rings in caracurine-II. Toxiferine-I contains eleven rings and so *three new rings* have been produced in the change. Chemical reduction

and many other studies established the presence of two cyclic carbinolamine ether residues which thus account for two of the new rings, and the final new ring is due to the formation of a new C—C bond somewhere in the molecule. The location of this new bond was determined by nuclear magnetic resonance. This depended on the fact that the protons at the positions 17 and 17′ give a well separated signal at 5·14τ due to their being attached to carbons which carry two electronegative groups and are, therefore, deshielded. Moreover, this signal is unsplit which showed in this case* that the adjacent carbon atoms are fully substituted. Hence, the new bond which has been formed joins position 16 to 16′ and, bearing in mind the structure of toxiferine-I from which caracurine-II dimethochloride is formed, structure (XVI) is established for caracurine-II. The main steps in the argument presented above were amply supported by other evidence. This structure has also been worked out independently by McPhail and Sim[19] at Glasgow, who used the X-ray method, and it is interesting that their results show that the separation of the two quaternary centres in caracurine-II methiodide is reduced to 8·6Å. Scale models indicate that this distance in toxiferine-I is about 14Å. The new central bond of caracurine-II alters the shape of the molecule and draws the two *N*(b)-nitrogen atoms together. This change probably accounts for the fall in physiological activity in the conversion from toxiferine-I to caracurine-II dimethochloride.

Let us turn now to the genus *Pleiocarpa* which has attracted interest because of the report by Raymond-Hamet[20] that crude extracts of the roots show a long-lasting hypotensive activity. These roots are rich in alkaloids and though many different bases are present[21] one could be isolated very readily. Several groups crystallized it[21–23] and it was named pleiocarpine. I want to outline briefly some of the main features of the structural work much of which was carried out in a collaborative way[21, 23, 24]. Pleiocarpine is a tertiary base with the molecular formula $C_{23}H_{28}N_2O_4$, and since it was shown to contain two carbonyl groups but no olefinic residues, it must be *hexacyclic*. The chromophore of pleiocarpine was established[21, *cf.* 23] as that shown in partial formula (XVII) by ultra-violet and infra-red spectroscopy and by the results obtained from the reduction of pleiocarpine by lithium aluminium hydride. These are shown in the partial formulae (XVII)→(XVIII).



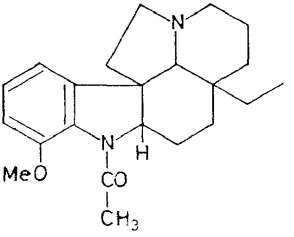
(XVII) (XVIII)

It was found that the reduction product contained[21, *cf.* 23] a mixture of the *N*(a)-methyl compound (XVIII; R = Me) and the >NH analogue (XVIII; R = H). The production of the former is straightforward and the latter is formed by fission of the intermediate carbinolamine[21] (XVIII;

* A suitable reference compound was available in which splitting by an adjacent proton was detected[18].

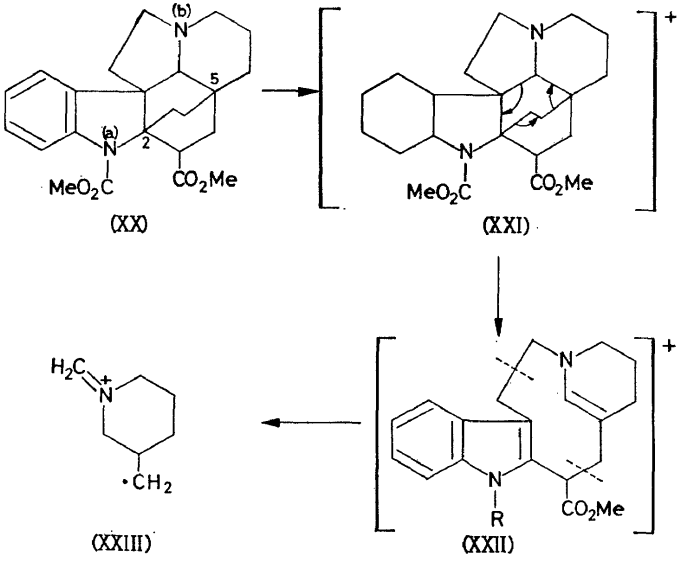
R = CH_2OH). As expected, the original ester group was reduced to the corresponding primary alcohol. The presence of this urethane group was the first unusual feature in the molecule.

Insight into the skeleton of pleiocarpine came from dehydrogenation experiments[24] which gave β-ethylindole, 3,5-diethylpyridine, and carbazoles, and these are the products normally obtained from such alkaloids as aspidospermine (XIX). Strong support for this type of skeleton for pleiocarpine



(XIX)

came from mass spectrometry and we are indebted to Dr R. I. Reed of Glasgow for these determinations. Pleiocarpine showed, in addition to the parent ion at *m/e* 396, a strong peak at *m/e* 368, that is 28 units less. This loss of 28 units of mass corresponds to the loss of ethylene and was first discovered in the *Aspidosperma* group of alkaloids by Biemann and his coworkers[25]. Many other examples of this process have come from the studies of Djerassi and his group[26, 27] The elimination is a cyclic process[25] and here I have anticipated what follows by illustrating the extrusion of ethylene on the pleiocarpine structure (XX). Loss of an electron to give the parent ion (XXI) is followed by the elimination for which the driving force[25–27] is the formation of the aromatic indole system and the relief of steric compression. The ion (XXII) can then fragment further to give many particles, one



of which has $m/e = 109$ and is assigned[25, 26] the structure (XXIII). This again is characteristic of the aspidospermine type of skeleton and we were pleased to see the $m/e = 109$ peak appearing strongly in the mass spectrum of pleiocarpine. So the combined evidence was very strong in favour of a skeleton similar to structure (XIX) for pleiocarpine.

A proton at position 2 of an *N*-acylindoline can be detected by nuclear magnetic resonance[28] and in the spectrum of pleiocarpine this signal was absent; position 2 (see structure XX) is, therefore, fully substituted. Taking the above results, the fact that pleiocarpine contains no *C*-methyl group and using biogenetic argument, structure (XX) seemed a plausible one for pleiocarpine. Extensive chemical work and N.M.R. spectroscopy[24] all gave results in agreement with this structure. For example, there was clear evidence for the ring sizes around *N*(b), for the full substitution at position 5 and for the position and environment of the ester carbomethoxyl group. You can see how closely related pleiocarpine is to aspidospermine; the two carbon unit which appears as an ethyl group in aspidospermine forms the basis of a new ring in pleiocarpine. It is interesting that there are several *Aspidosperma* alkaloids, for example, refractine and aspidofractine[27], which Djerassi and his co-workers proved independently to have the same hexacyclic system as is present in pleiocarpine.

We have looked at two types of indole alkaloid, but new ones are appearing steadily. This field is at present an extremely active one and is expanding at a remarkable pace. There can be little doubt that many interesting alkaloids will be found in this area, who knows, even one with valuable pharmacological properties. Certainly many vigorous groups are looking for such a substance and enjoying a rich harvest of chemistry and pharmacology in doing so. I hope I have been able to pass on to you some of the thrills of working in this field.

In conclusion, I wish to acknowledge the excellent work of my colleagues, Drs Binks, Hodson, Rao and Yeowell.

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[1] For complete reviews of the literature to the 1959–60 period see K. Bernauer. *Fortschr. Chem. org. Naturstoffe* **17**, 184 (1959);
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