HIGH RESOLUTION MASS SPECTROMETRY OF NATURAL PRODUCTS

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During the past few years mass spectrometry has become an important technique for the organic chemist and was used with remarkable success for the solution of many structural problems. Alkaloids are one of the best examples in support of this statement. It was only four years ago that we first reported[1] on the use of mass spectrometry for the determination of the structure of indole alkaloids, and in the short period since at least fifty new structures in this area have been established in part, or entirely, on the basis of their mass spectra. A considerable part of these more recent examples stems from the industriousness of Djerassi's group at Stanford University, partly in collaboration with Janot's group in Gif (France). Additional examples appear almost constantly in the literature. In many other areas of natural products chemistry, such as amino-acids, fatty acids, steroids, carbohydrates, antibiotics, *etc.*, mass spectrometry has also been widely used[2]. The instruments employed in this work have been almost exclusively of the conventional single-focusing type, which has a mass range of up to mass 1000 or somewhat beyond and a resolving power permitting species differing by at least one full mass unit, which is generally sufficient.

Aside from this approach, which we now can almost call the conventional one, a new technique—high resolution mass spectrometry—is becoming more and more established. A number of years ago Beynon[3] showed that a suitably constructed mass spectrometer permits the determination of the mass spectra of organic molecules with such a resolution and precision that makes it possible to resolve two species of the same nominal mass differing, however, by a fraction of a mass unit due to the fact that they contain different elements. *Figure 1*, for example, shows the mass differences of various combinations of elements from the hydrocarbon of the same nominal mass. To make the table more comprehensible, combinations of elements are used which all add up to mass 352, but one has to keep in mind that the mass differences (extreme left and right columns) are, of course, the same regardless of actual mass as long as the differences in elemental composition are the same.

Thus, a species containing one nitrogen atom more and one carbon and two hydrogens less than the hydrocarbon (*i.e.* $C_{24}H_{50}N$ *v.* $C_{25}H_{52}$) differs by 12·6 mmu, while the species containing two nitrogen atoms is 25·1 mmu lighter than the saturated hydrocarbon. Consequently, if the necessary resolving power (in this case 1 part in 28,000) is available, one could resolve these three species and obtain three separate peaks in a

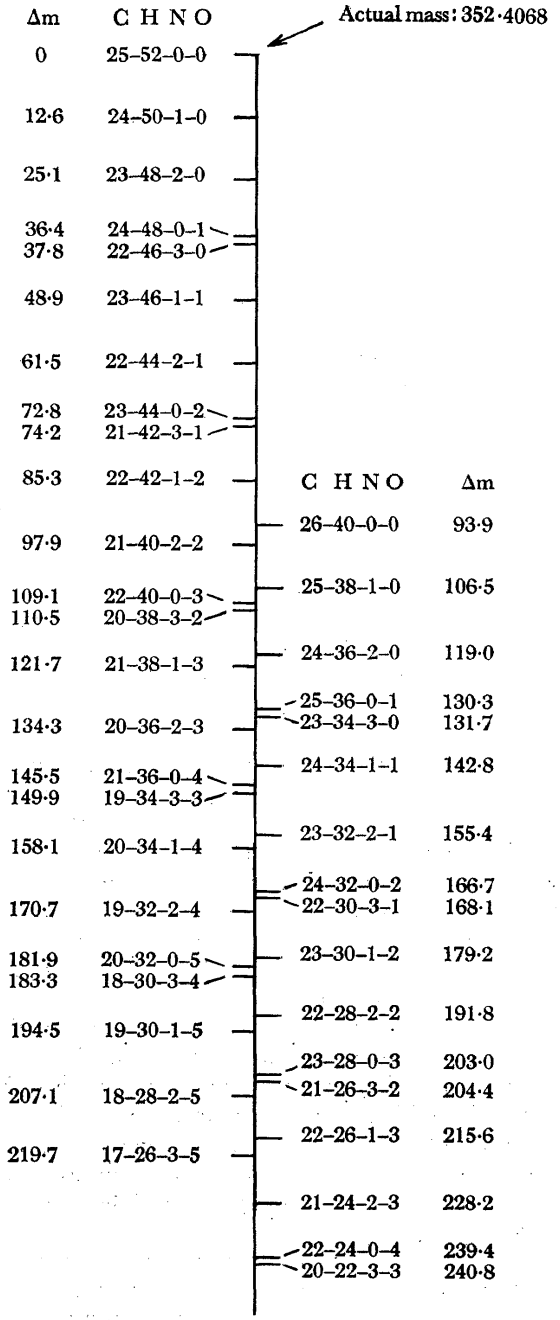


Figure 1. Graphic representation of the mass differences among various combinations of C, H, N and O all adding up to a nominal mass 352. Note that the right part differs by $+^{12}C$ and $-H_{12}$ from the left and thus represents a highly unsaturated set of combinations. The values at the extreme left and right indicate the difference (in millimass units, mmu) of the particular combination of elements from that of the hydrocarbon, $C_{25}H_{52}$

spectrum of a compound that can form all these three ions, or in a mixture if the components contribute these ions. Secondly, if the mass can be determined with an error much less than 12 mmu, one can assign the elemental composition to the species on the basis of its mass.

Some combinations of elements have, however, very similar masses and species differing by C_2H_2O *v.* N_3 (ΔM 1·5 mmμ) *e.g.* $C_{24}H_{48}O$ *v.* $C_{22}H_{46}N_3$, or C_3N *v.* H_2O_3 (ΔM 2·6 mmu) *e.g.* $C_{22}H_{40}O_3$ *v.* $C_{25}H_{38}N$ require a very accurate mass measurement at higher masses. Fortunately, these differ appreciably in heteroatom content as well as degree of saturation which makes it easier to select the more plausible combination and to reject those not compatible with the information obtained from the bulk of the remaining ions.

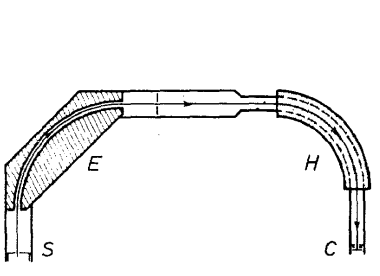
The major application of this technique has been the determination of the elemental composition of a certain compound by accurate measurement of the mass of its molecular weight, or the determination of the elemental composition of fragments formed on electron impact to confirm a proposed fragmentation mechanism. Beynon has presented quite a number of examples for both types. The most notable one of his earlier work was to show that anthraquinone eliminates in two consecutive steps one molecule of carbon monoxide each to form a specie that is best represented by biphenylene (dibenzcyclobutadiene)[3]. In most of these cases only the mass of the particular ion of interest has been measured, while the recording of an entire high resolution mass spectrum is very rare. The few published spectra of this type are those of rather small molecules, such as cyclic ethers[3] and ketones[4], or small esters[5], none of them exceeding molecular weight 150, for the simple reasons that it is tedious and time-consuming to record a spectrum in a fashion that mass differences in the order of millimass units are still measurable with reasonable accuracy.

For a long time Beynon possessed the only such instrument accessible to organic chemists, and even at the present time practically all the data published were obtained in three laboratories, namely, at ICI (Beynon)[3–6], at AEI[7] (the manufacturer of one of these instruments), and our own[8]. This situation will perhaps change very rapidly in the near future as high resolution mass spectrometers are now being produced commercially in great numbers.

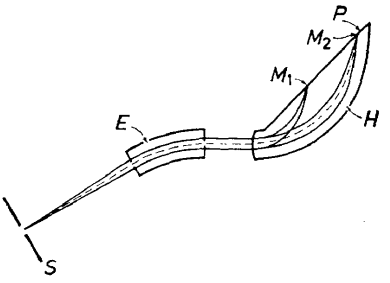
At this point it might be advisable to discuss briefly the two major types of high resolution mass spectrometers at present commercially available (*Figure 2*). In both systems an electrostatic field is used as an energy selector to take care of the energy spread within the ion beam due to initial kinetic energy which severely limits the resolving power of any single-focusing mass spectrometer, that is one using a magnetic field only for the deflection of the ion beam.

The two systems differ mainly in the manner in which the magnetic field refocuses the ion beam. In the Nier-Johnson type, which is the one utilized by the English manufacturer (AEI, Model MS-9) and also going to be used by Hitachi (Japan) the beam of ions of a particular mass is refocused at one point, at which the collector slit is placed, and the spectrum is scanned in a conventional way, for example, by changing the magnetic field. The Mattauch-Herzog system, however, focuses all the ions

simultaneously in one plane, which permits either placing a collector slit at any given point and obtaining a spectrum by scanning the magnetic field; or placing a photographic plate in the focal plane and obtain a sharp line for each mass. The latter method permits one to obtain, theoretically instantly, a complete high resolution spectrum, and the only time required is in fact the period in which ions have to bombard the photographic



Nier-Johnson System
AEI MS-9 (Engl.)
[Hitachi RMU6D (Jap.)]



Mattauch Herzog System
CEC 21-110 (USA)
[Atlas SM I (Germ.)]

S = Ion source, E = Electrostatic field, H = Magnetic field
C = Focal point
P = Focal plane
(M_1 = Focal point of lower mass)
(M_2 = Focal point of higher mass)

Figure 2. The two principle designs of commercial high resolution mass spectrometers

emulsion to leave a measurable trace after development. This period is from a few seconds to a few minutes depending on the particular problem. The Mattauch-Herzog design is used in the American instrument (CEC 21-110) and will be available in the near future also from Germany (Atlas SM1).

A few examples from the most recent work in our laboratory will illustrate the results obtainable.

First, let us consider a problem concerning the fragmentation of some compounds of known structure. The conventional mass spectrum of ajmaline (I) is shown in *Figure 3* (top) to which were added the elemental composition of some peaks as determined from a high resolution spectrum. These data in general agree with the interpretation arrived at both in our laboratory[9] and by Spiteller[10], except that a few of them are multiplets, such as mass 158 and mass 182. The major component of the former is $C_{11}H_{12}N$ as expected, representing the *N*-methyldihydroindole system with the two carbons of the tryptamine bridge (C–5 and C–6). The oxygen-containing species must contain C–17 with the oxygen atom and is in fact

found with all compounds possessing a C–17, C–7 bond and oxygen at C–17 Obviously, the presence of such a doublet is structurally significant but impossible to detect without a high-resolution mass spectrometer. Similarly the peak at mass 182 consists of three components, one ($C_{12}H_{10}N_2$) representing the expected *N*-methyl-β-carboline fragment while the other major component contains only one nitrogen. Its genesis is discussed later.

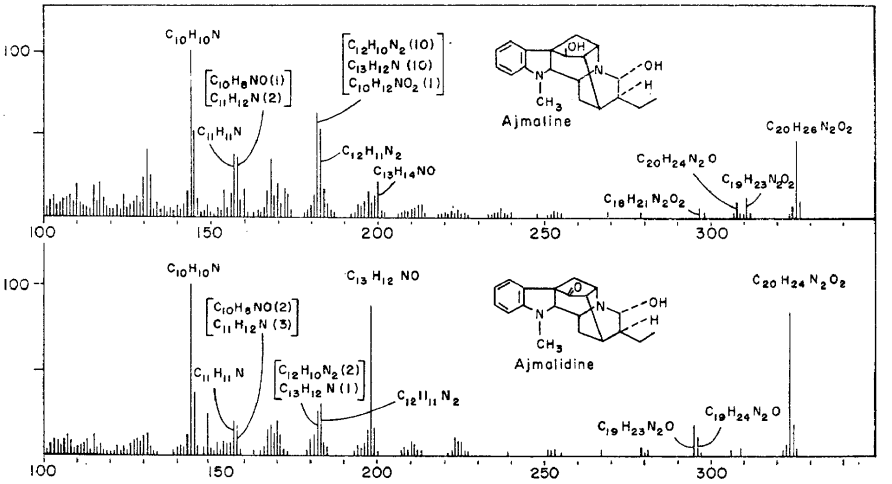
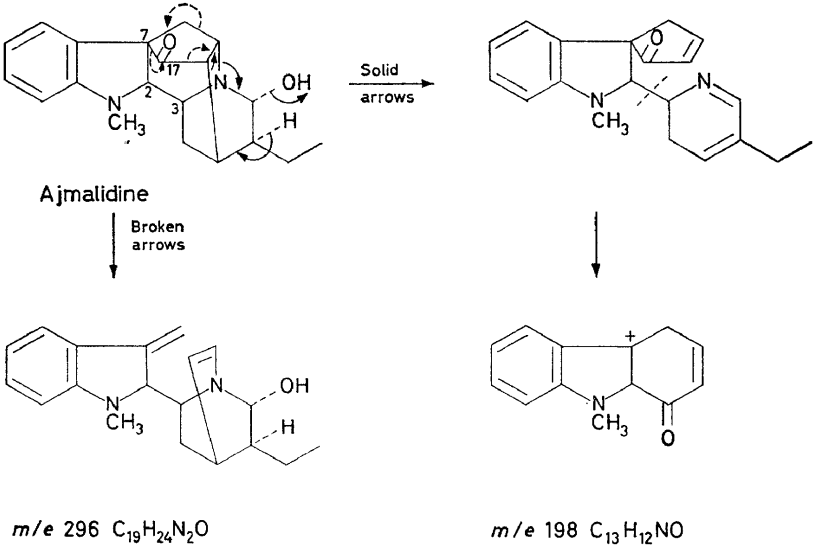


Figure 3. The conventional mass spectra of ajmaline (I, top) and ajmalidine (II, bottom)

The mass spectrum of ajmalidine (II) was somewhat puzzling to us as it seems that mere conversion of the secondary alcohol (I) to a ketone (II) considerably changed the appearance of the spectrum (*Figure 3*, bottom) in comparison with ajmaline. Particularly the intense peak at *m/e* 198 did



m/e 296 $C_{19}H_{24}N_2O$ *m/e* 198 $C_{13}H_{12}NO$

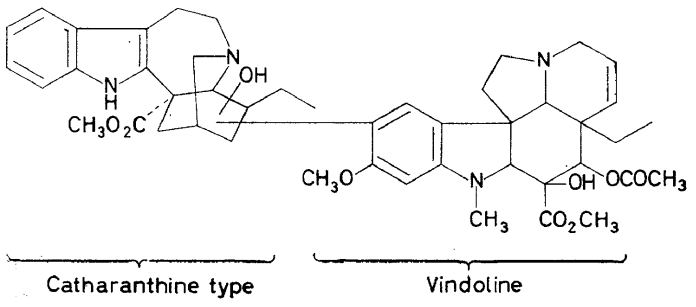
not fit into the picture as it has no equally significant counterpart in the spectrum above. Accurate mass measurement revealed however its elemental composition ($C_{13}H_{12}NO$) which requires the presence of the indole system plus the cyclopentanone ring. Scrutinizing the ajmaline spectrum one realizes that the peak at mass 200 has the analogous composition, except that there are two more hydrogens.

The nature of the peak at *m/e* 198 in ajmalidine can be interpreted as shown on page 99.

The elimination of the piperidine ring as shown, followed by cleavage of the C–2, C–3 bond leads to an ion of mass 198 ($C_{13}H_{12}NO$) which may rearrange to the carbazol-species shown. This is the final "product" in the case of ajmalidine. The same fragment from ajmaline (*m/e* 200) possesses however a hydroxyl group which can be eliminated as water leading to $C_{13}H_{12}N$ and resulting in a low peak at *m/e* 200 but a higher one at 182. Thus, merely determining the accurate mass of a few peaks in these two spectra resolved the earlier discrepancy[8b].

The second example concerns the structure of the "dimeric" indole alkaloid vinblastine (VLB). The previously proposed[11] structure (III) seemed to leave two questions unanswered: (1) the attachment of the vindoline portion to the other half of the molecule and (2) the position of the hydroxyl group. It was thought that a mass spectrum might shed some

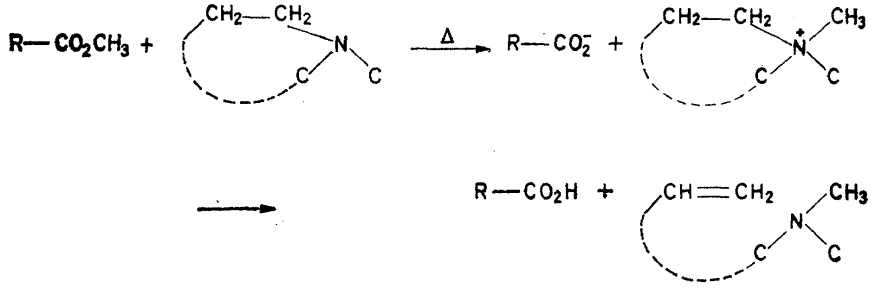
$C_{46}H_{56}N_4O_9$ (MW 808)



(III)

light on these questions and the first result, with a conventional, single focusing spectrometer led more to mystery rather than solution as the value obtained was 838, quite in disagreement with the proposed structure requiring 808. Shortly thereafter we realized that many compounds of this type are thermally unstable and show incorrect (higher) molecular weights, the first case noted being voacamine[12].

The reaction involved is a thermal one occurring during the heating of the sample in order to vaporize it. Molecules of very low vapour pressure (thus requiring considerable heat for vaporization even at very low pressure) and containing both an alkylating group and an alkylatable group seem to be prone to react with each other in the following manner:



The result is the production of a higher homologue as well as the free acid corresponding to the original methyl ester.

An attempt was thus made to minimize the thermal reactions leading to this undesired result, and also to detect the formation of higher homologues by transmethylation. A high resolution spectrum obtained with a sample of VLB spread in a thin layer and recorded immediately after insertion of the sample into the ion source gave the results summarized in *Table 1.*

Table 1

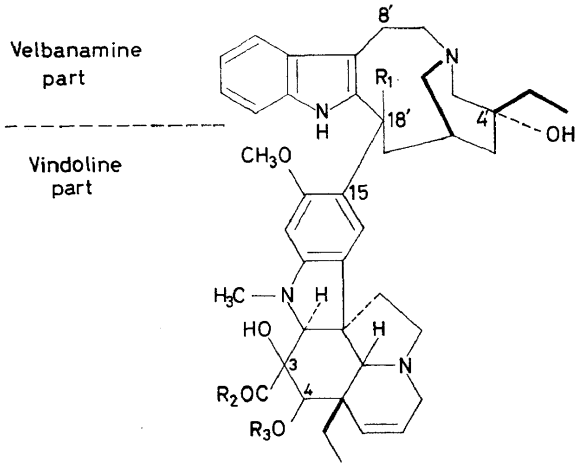
Mass found	*Error*[a]	*Composition*	*Elements lost*[b]
		Vinblastine (I)	
838·4538	+2·4	$C_{48}H_{62}N_4O_9$[c]	
824·4372	+1·2	$C_{47}H_{60}N_4O_9$[d]	
811·4234	−0·3	$C_{45}{}^{13}CH_{58}N_4O_9$	
810·4219	+1·5	$C_{46}H_{58}N_4O_9$	
752·4153	+0·4	$C_{44}H_{56}N_4O_7$	$C_2H_2O_2$
751·4080	+1·0	$C_{44}H_{55}N_4O_7$	$C_2H_3O_2$
750·4014	+2·2	$C_{44}H_{54}N_4O_7$	$C_2H_4O_2$
651·3906	−0·5	$C_{40}H_{51}N_4O_4$	$C_6H_7O_5$
650·3858	+2·6	$C_{40}H_{50}N_4O_4$	$C_6H_8O_5$
591·3723	+2·6	$C_{38}H_{47}N_4O_2$	$C_8H_9O_7$
154·1219	−1·3	$C_9H_{16}NO$	$C_{37}H_{42}N_3O_8$

[a] Difference in millimass units between mass found and value calculated for the elemental composition in the third column (^{12}C = 12·000000).
[b] From mass 810 in case of I.
[c] Dimethylation product.
[d] Monomethylation product.

It should be noted that the ions of mass 810, 824 and 838 differ, in fact, by CH_2 supporting the assumption that the former is the actual molecular weight of VLB and the two latter ones are formed by attachment of one or two methyl groups during pyrolysis. The accurate mass of the species 810 required an elemental composition $C_{46}H_{58}N_4O_9$ and this is further corroborated by the mass of the isotope peak at 811.

These data required now a revision of the earlier formula (III), namely a nonacyclic ring system instead of a decacyclic one. Omission of the C–5, C–18 bond is the best solution, since none of the chemical cleavage products of VLB contain such a bond. Of the other fragments listed the one of mass 752 is of interest as it corresponds to the decarboxylation product of the

methylating species. Furthermore, the loss of $C_6H_8O_5$ corresponds to the loss of the C–3, C–4 bridge in the vindoline part, the only area with such a high oxygen content. An indication of the point of attachment is provided by the presence of the $C_9H_{16}NO$ species present here as well as in velbanamine itself, where it is most certainly due to the piperidine ring with the



(IV) $R_1 = COOCH_3$

$R_2 = OCH_3; R_3 = COCH_3$

ethyl and hydroxyl group and the two adjacent carbon atoms (C–1 and C–7), *i.e.* leaving C–18 or C–8 as the only possibilities. This conclusion was supported by the mass spectrum of another derivative of VLB which contained a peak whose elemental composition required the presence of both aromatic systems (low H:C ratio), the alicyclic part of vindoline (high oxygen content) and a total number of carbon atoms requiring the loss of the $C_9H_{16}NO$-moiety mentioned above, again eliminating the possibility that the vindoline part were attached to this moiety.

Thus the earlier formula was revised to (IV) both on the basis of the above evidence[8d] and additional chemical data[13]. This structure presupposes the fact that the hydroxyl group of velbanamine is attached to C–4 which is based on mass spectrometric evidence to be discussed later.

These two examples outline the refined information one obtains with a double focusing mass spectrometer. The first example in particular followed the approach used by Beynon and his school, namely to obtain a low resolution mass spectrum of the conventional type, select a few of the more interesting peaks (such as the molecular weight and a few intense fragment peaks) and to check whether the accurate mass of these species is in agreement with the proposed elemental composition, or to get some clues regarding the identity of an otherwise unexplainable fragment. This technique is therefore more accurately described as low resolution mass spectrometry with occasional accurate mass measurement.

This seemed a rather uneconomical approach because one makes actual use of only a very small fraction of the available information. Let us consider a simple example. The low resolution mass spectrum of deoxydihydro-N_b-methylajmaline(V) (*Figure 4*) shows only very few significant peaks

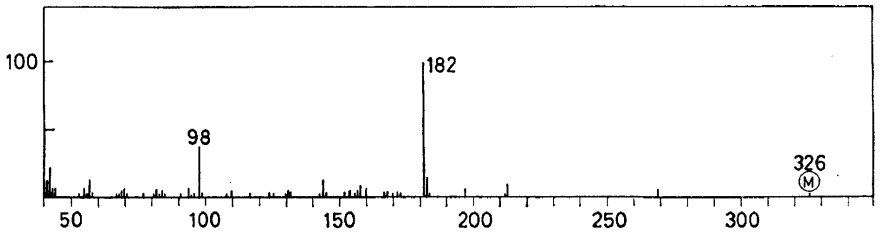


Figure 4. The conventional mass spectrum of deoxydihydro-N_b-methyl-ajmaline (V)

and no more could be said about it than that the compound has most probably a molecular weight of 326. Again the conventional approach, determining the elemental composition of the molecular weight and of the few conspicuous fragment ions does not lead much further, except that they are due to $C_{21}H_{30}N_2O$ (326), $C_{13}H_{12}N$ (minor component) and $C_{11}H_{20}N$ (major component) at 182 and C_5H_8NO at 98. All the small peaks are frequently neglected in conventional mass spectrometry as they are not very significant as long as only mass and intensity are the criteria. We always considered that it is a pity to discard all the information contained in this part of the spectrum and felt that their elemental composition might be the needed additional criterium to make them useful.

It is however a formidable problem to determine the accurate mass of all the ions in a spectrum as it requires a few hundred measurements with an accuracy of almost one part in a million (less than 1 in 10^5 is rarely useful). But when we realized the potentialities residing in this information we set out to elaborate a technique to achieve this.

One might think it could be done by simple extension of the conventional approach and measure one peak after another as done when using a Nier-Johnson instrument (such as the AEI MS-9). The time and number of manipulations required, however, make this approach very impractical. It is therefore desirable to separate the recording of the mass spectrum, which requires a mass spectrometer, from the measurement of the mass.

Having available a double-focusing mass spectrometer of the Mattauch-Herzog type, which actually records all these data instantly on the photographic plate, the problem is then a matter of retrieving this information. As the distances of the various focal points are directly related to the square root of the corresponding masses, it is possible to determine the accurate mass of any species by measuring the exact distance of this line to two other lines produced by ions of known mass. Thus, mass measurement is reduced to distance measurement, which requires only an optical system (comparator), and not the mass spectrometer itself, thus greatly facilitating the measurements. We therefore set out to develop a technique which permits semi-automatically measuring the distances of all lines and converting these data, using a computer, to the accurate mass of the ion in question,

and further to the elemental composition possible for such a mass. Once this was accomplished, we found ourselves in another dilemma, namely, the elemental composition of hundreds of ions. Ways had to be found for the condensation of these data to a comprehensible form, that can be looked over at a glance, and this resulted in what we call an "element map", for reasons that will become immediately obvious.

The "element map" of deoxydihydro-N_b-methylajmaline is shown in *Figure 5.* In principle it lists the elemental composition and relative intensity

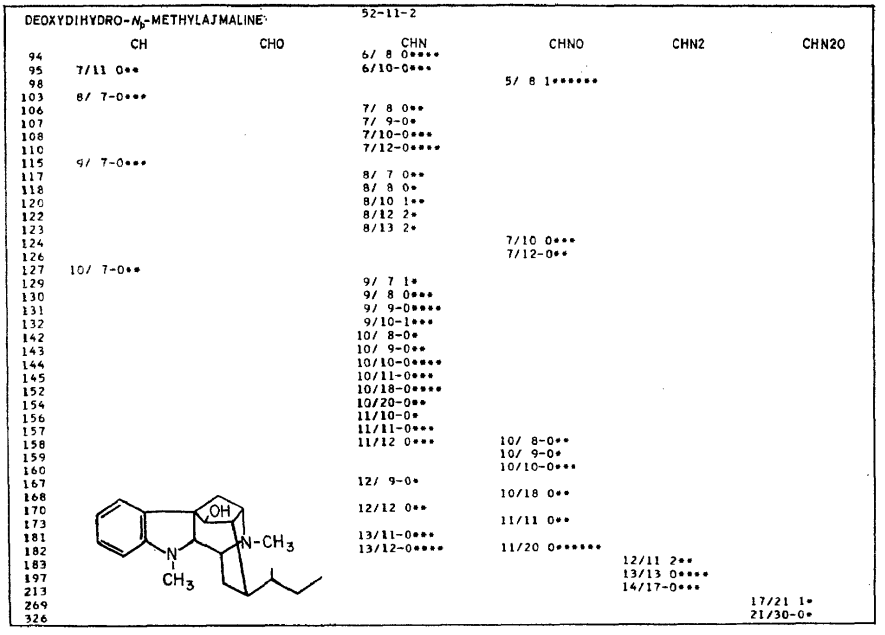


Figure 5. Element map of deoxydihydro-N_b-methylajmaline (V)

(represented by the number of asterisks, on a logarithmic scale to preserve space and to permit listing also of the species of low abundance) of all the ions in the spectrum. To obtain a meaningful picture, the ions are separated according to heteroatom-content representing parallel columns, starting with hydrocarbon ions at the left to CHN_2O ions on the right. The elemental composition of each ion is given by the number of C-atoms and H-atoms listed while the number and kind of heteroatoms is given by the heading of the column in which it is listed. Also stated is the deviation (in millimass units) of the found mass from the mass calculated for the elemental composition of the species stated.

The entire element map is assembled by a computer (IBM 7094) which is fed only all the line positions and a set of known masses of the calibration compound (perfluorokerosene) which had been added to the sample and whose lines are thus measured along with the lines of the spectrum in question. The computer time required for the entire process is about 10–30 seconds (!).

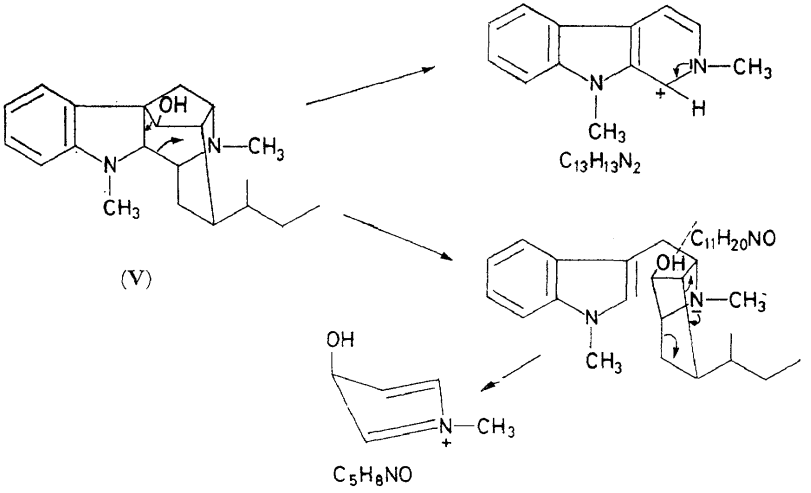
The lower right corner of the element map of deoxydihydro-N_b-methylajmaline thus indicates the elemental composition, $C_{21}H_{30}N_2O$. The entry 17/21 in the same column shows that a C_4H_9 fragment can be lost. It is the only ion not involving the loss of N or O and thus requires the presence of a saturated C_4-side chain. Formation of the next smaller fragment is already accompanied by the loss of oxygen and 7,8 or 9 carbon atoms (see CHN_2-column). Loss of 8 carbons leads to a β-carboline type of ion ($C_{13}H_{13}N_2$), the major fragment in this group.

In the CHNO column we find mainly ions of high hydrogen content which implies that the oxygen atom is located in the alicyclic area of the molecule rather than as a substituent at the aromatic ring (as in later examples). The most abundant species is $C_{11}H_{20}NO$ and it is also the largest one in this column which implies that the alicyclic part of the molecule can easily be split off involving 11 carbon atoms, leaving ten with the aromatic part containing the other nitrogen atom. The abundant species C_5H_8NO further suggests that the non-aromatic N and O are within at most 5 carbon atoms.

At mass 182 a doublet is found, one component being the above mentioned one ($C_{11}H_{20}NO$) while the second one is rather low in hydrogen content and contains only one nitrogen atom ($C_{13}H_{12}N$). From this one can again conclude that the aromatic system can retain up to 13 carbon atoms without involving the second nitrogen atom. Similarly the CHN column reveals that the alicyclic ions (high hydrogen content) of this type encompass at best 10 carbon atoms as shown by the entry 10/20.

The absence of any entries (above mass 94) in the column CHO indicates that the oxygen atom is present in a form which prevents the formation of reasonably large fragments containing oxygen but not nitrogen. The scarcity of C,H-ions requires that at least some of the heteroatoms are an integral part of the ring system.

All the conclusions outlined above are in line with the known structure[14] of deoxydihydro-N_b-methylajmaline and the major peaks can be interpreted along conventional lines[9]:



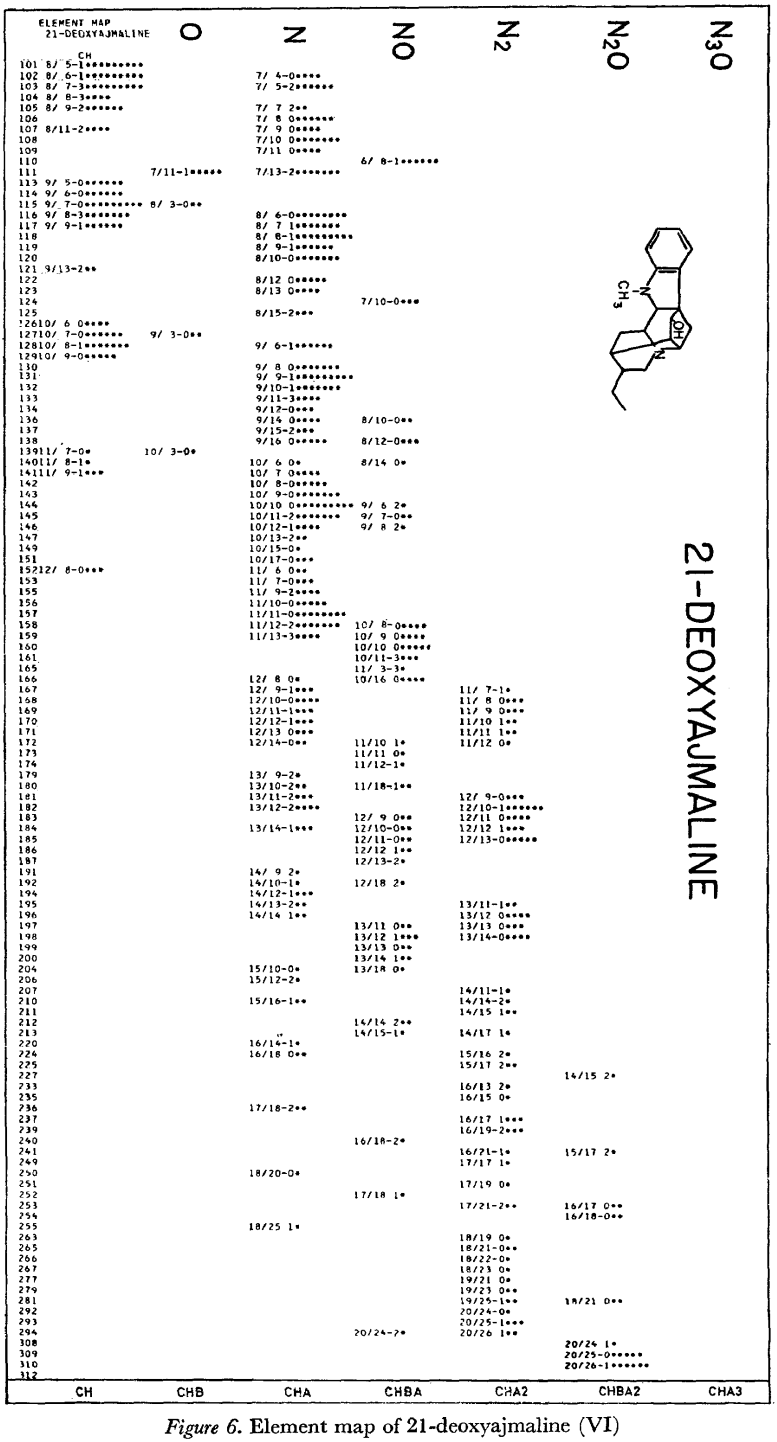


Figure 6. Element map of 21-deoxyajmaline (VI)

In *Figure 5* only 46 species were listed in the range from mass 93 to 326 because a weak spectrum was selected in order to allow clearly visible reproduction in the available space. As those lines due to the calibration compound, isotope peaks and doubly charged species are not listed, a total of about 100 lines were measured, and processed by the computer.

A more common situation is shown in *Figure 6*, which represents the element map of a closely related compound, 21-deoxyajmaline (VI), containing more than 170 ions, requiring measurement of 250–350 line positions, but spectra containing 500–1000 lines are not uncommon.

The conclusions that can be drawn from *Figure 6* are similar to those of *Figure 5*. The CH and especially the CHO columns are sparsely populated, the CHN-group comprises a major part of the ions, and are both aromatic and alicyclic, the latter ending with 10 C-atoms ($C_9H_{16}N$ however more intense, analogous to $C_{10}H_{17}N$ in *Figure 5*). One of the major differences is found in the CHN_2-column which goes up to C_{20} because of the more rigid carbon skeleton of (VI) and the very low abundance of the M—C_4H_9 species which requires in this case cleavage of two bonds in addition to loss or rearrangement of one hydrogen atom.

The element map of ibogaine (VII) an isomer of (VI) with different carbon skeleton and different arrangement of the heteroatoms is shown in *Figure 7*. The absence of CHO-ions and CHN_2 ions (except $C_{19}H_{24}N_2$ due to loss of the methoxyl group and the isolated entry $C_6H_{11}N_2$ of low intensity which is probably due to an impurity), as well as the high population of the CHNO-column in the high mass part entirely due to aromatic ions (low H:C ratio) requires that the oxygen atom is closely associated with the aromatic part which in turn contains one nitrogen atom, but is far removed from the alicyclic nitrogen atom.

It may be noted that the element map clearly shows the intensity relationship of the various peaks (except that they are shown on a logarithmic scale), the peaks at *m/e* 122, 124, 135, 136, 149, 186, 199, 225 and 310 being the most intense ones as previously found with a single focusing spectrometer[15]. Their elemental composition is in agreement with the earlier interpretation, except for *m/e* 225 which contains only one nitrogen atom and must thus be due to loss of the alicyclic nitrogen probably along with carbon atoms 3, 4, 5, 19 and 20.

It may be noted that there is an entry corresponding to $C_{20}H_{27}N_2O$, a species containing apparently one hydrogen atom more than ibogaine. This ion is in fact the ^{13}C-isotope peak of ibogaine but its mass was found about 2 mmu high which thus falls within our specified limit (± 3 mmu) of the species containing ^{12}CH instead of ^{13}C (mass difference 4·5 mmu). This feature which may repeat itself for fragments is quite irrelevant with respect to the purpose of the element map but could of course be corrected for.

As a final example of an indole alkaloid the element map of velbanamine (VIII) shall be discussed briefly (*Figure 8*). As mentioned earlier its complete structure was essential for the elucidation of the structure of vinblastine[8d, 13], of which velbanamine is a cleavage product. The main question, the position of the hydroxyl group can be deduced from the element map. Both the absence of aromatic and the abundance of alicyclic CHNO-species place the hydroxyl in the alicyclic region of the molecule. The

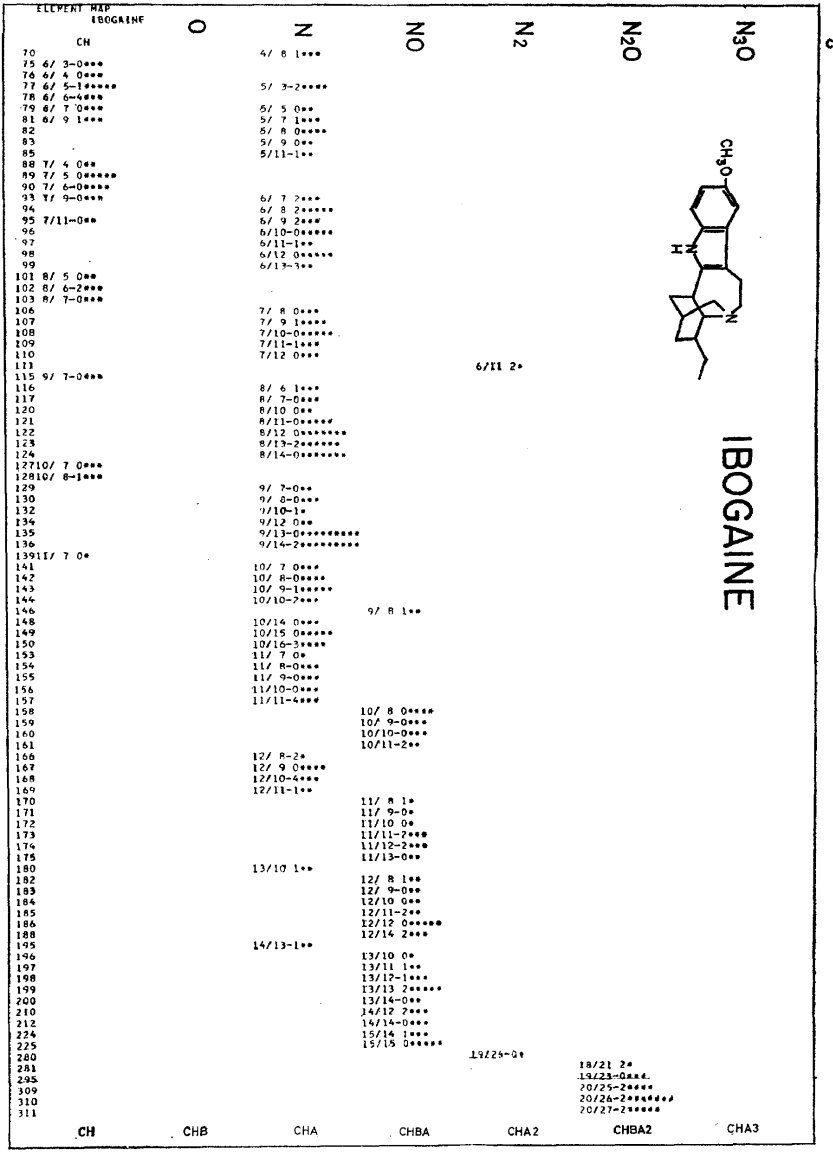


Figure 7. Element map of ibogaine (VII)

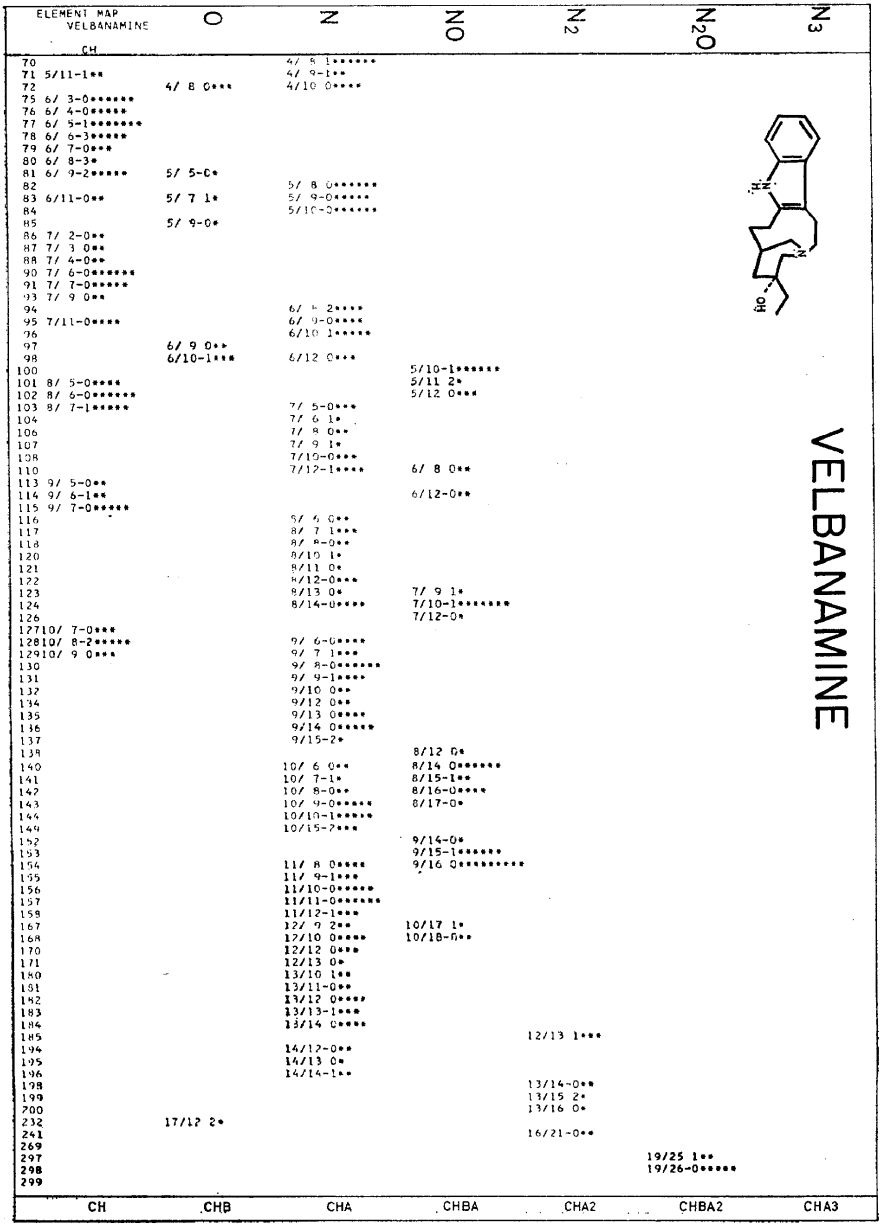
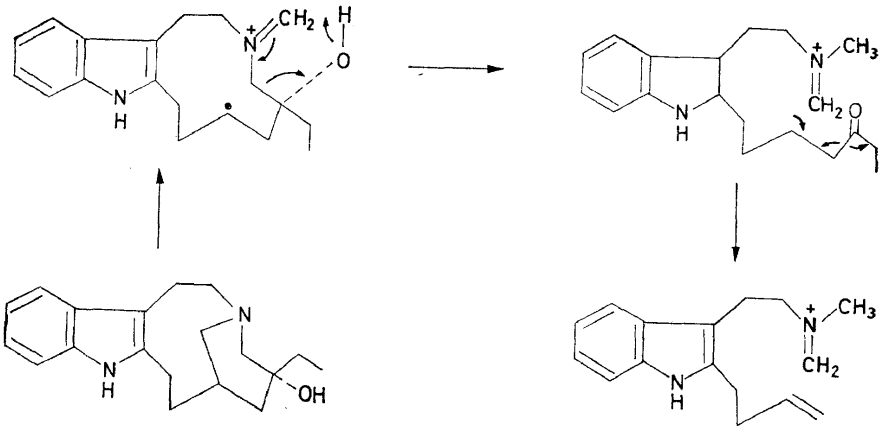


Figure 8. Element map of velbanamine (VIII)

largest CHN_2-species corresponds to the loss of C_3H_5O which is most compatible with the attachment of the hydroxyl group at C–4 permitting elimination of C_2H_5CO in the following manner:



Velbanamine
($C_{19}H_{26}N_2O$)

$C_{16}H_{21}N_2$ (*m/e* 241)

It now remains to demonstrate that element maps of other systems appear in fact different from those shown before. A few steroids may serve as examples. *Figure 9*, the map of a steroid (IX) oxygenated at positions 3, 11 and 17 already shows a strong preponderance of C,H-ions containing up to 17 C-atoms demonstrating the presence of a carbon skeleton of at least that many carbon atoms uninterrupted by heteroatoms. The elimination of CO from cyclic ketones is a well-known reaction as is the elimination of H_2O from hydroxyl compounds. The maximum number of carbon atoms lost without heteroatoms is two, requiring the absence of any long aliphatic side chain or oxygen-free rings at the terminus of the molecule. The preponderance of both CHO_2 and CHO ions indicates an even distribution of the oxygen atoms. The smallest CHO_2 species contains 5 carbon atoms, the shortest connection between 2 O-atoms (C–11, 12, 13, 17 and 18). The next larger ion of this type includes 7 C-atoms, probably ring *D* with C–11 and 12, but not C–18.

The element map of another steroid of about the same size and also containing three oxygen atoms (androsterone acetate, X) is shown in *Figure 10*. Comparison with *Figure 9* clearly indicates the difference in the oxygen distribution between (IX) and (X). First, there are 19 C-atoms retained in the largest CH-ion. Second, the CHO_2-column is almost empty while the CHO-column is highly populated requiring the close connection of two oxygen atoms which are easily lost together.

The detailed interpretation of an element map in terms of fragmentation processes is outlined in *Figure 11* on hand of the high mass parts of *Figures 9* and *10*. These maps contain all the information present in conventional

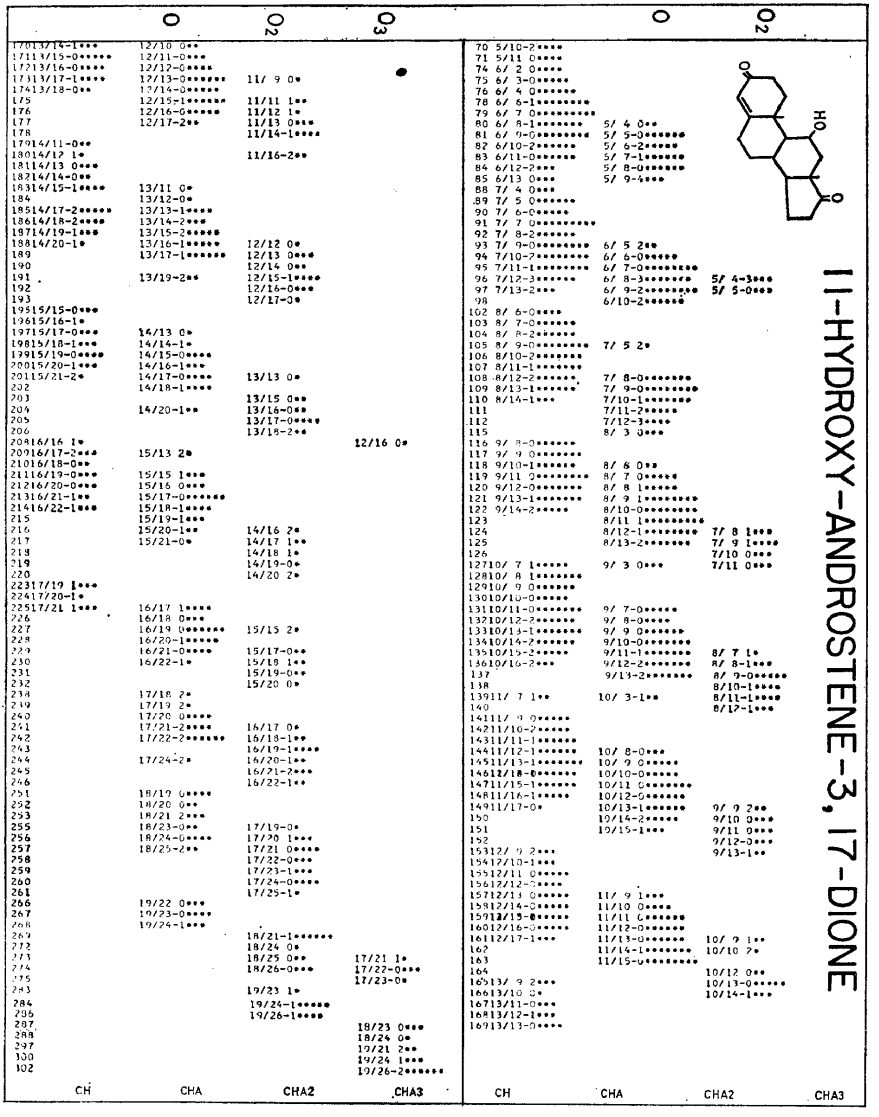


Figure 9. Element map of 11-hydroxy-Δ^4-androsten-3, 17-dione (IX)

spectra namely intensity and mass of the various ions, except that more importantly the elemental composition is shown as well.

Finally, the element map of a nitrogen-containing steroid, samandaridine (XI)[16] is shown in *Figure 12* and shall be discussed briefly. The upper right corner gives the elemental composition, $C_{21}H_{31}NO_3$ and shows that only one or two carbon atoms can be lost without heteroatoms. The single entry in the CHO_3 column ($C_{19}H_{25}O_3$) requires the location of the nitrogen in a

fashion permitting its loss only with two carbon atoms unless other hetero-atoms are lost also, *i.e.* at least one oxygen must be closely related to the nitrogen. Similarly, the loss of one oxygen alone is not frequent (only M—H_2O, M—CO, M—CO and CH_3). On the other hand the loss of either two oxygens or nitrogen and oxygen predominates, suggesting that

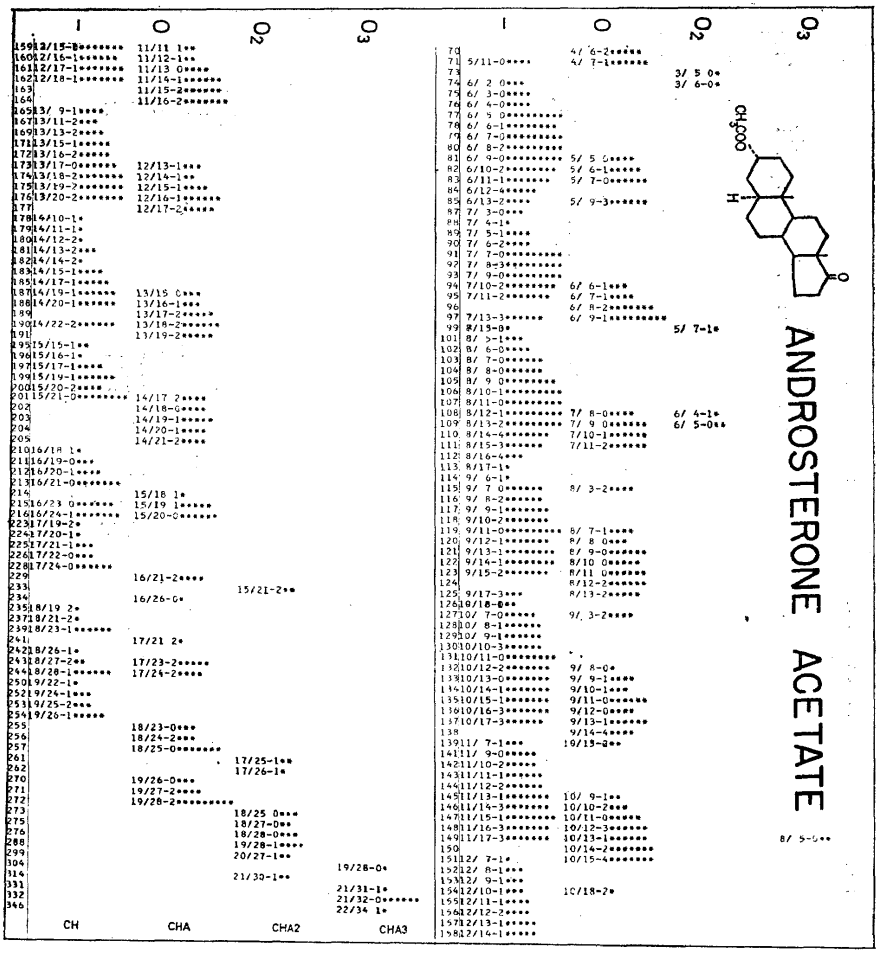


Figure 10. Element map of androsterone acetate (X)

the four heteroatoms are grouped together in that way but in different areas of the molecule.

The smallest members of the CHO_2 and CHNO series represent the lactone ring ($C_4H_{4-5}O_2$) and ring *A* ($C_4H_{7-8}NO$), respectively.

Although almost all the foregoing element maps, with the exception of velbanamine, for purposes of demonstration, dealt with compounds of well-known structure, it seems that element mapping is particularly valuable

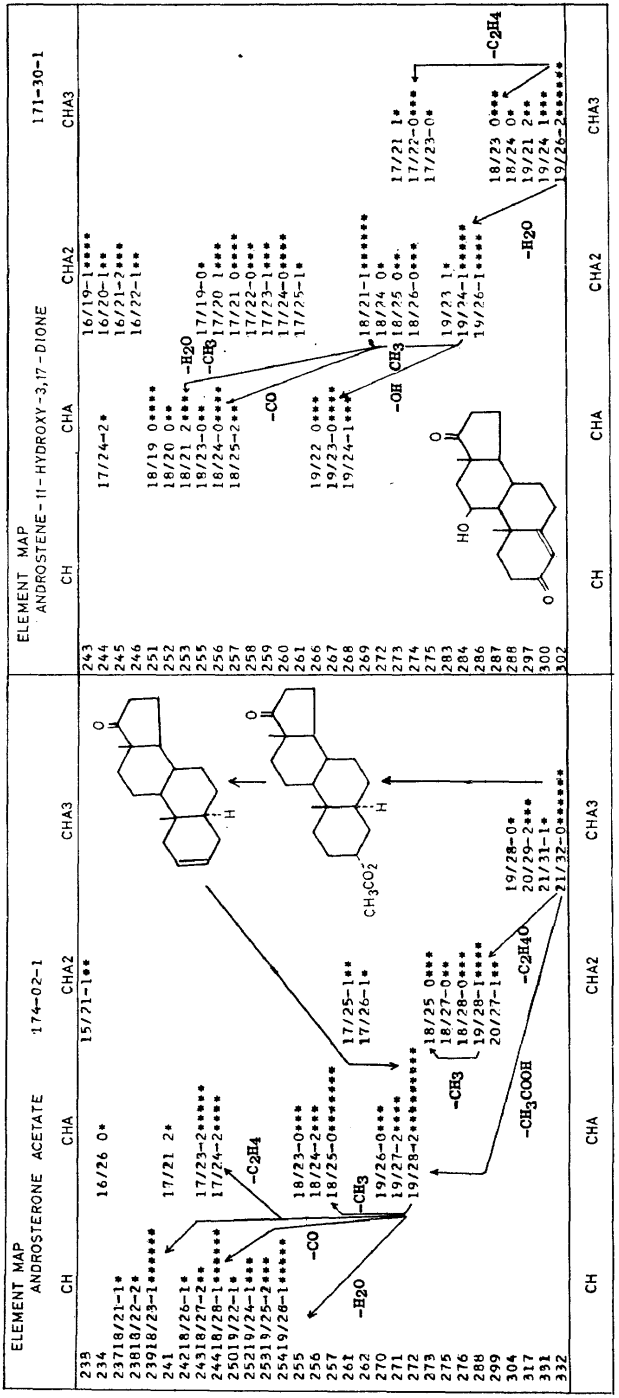


Figure 11. Comparison of the high-mass region of the element maps of (IX) and (X)

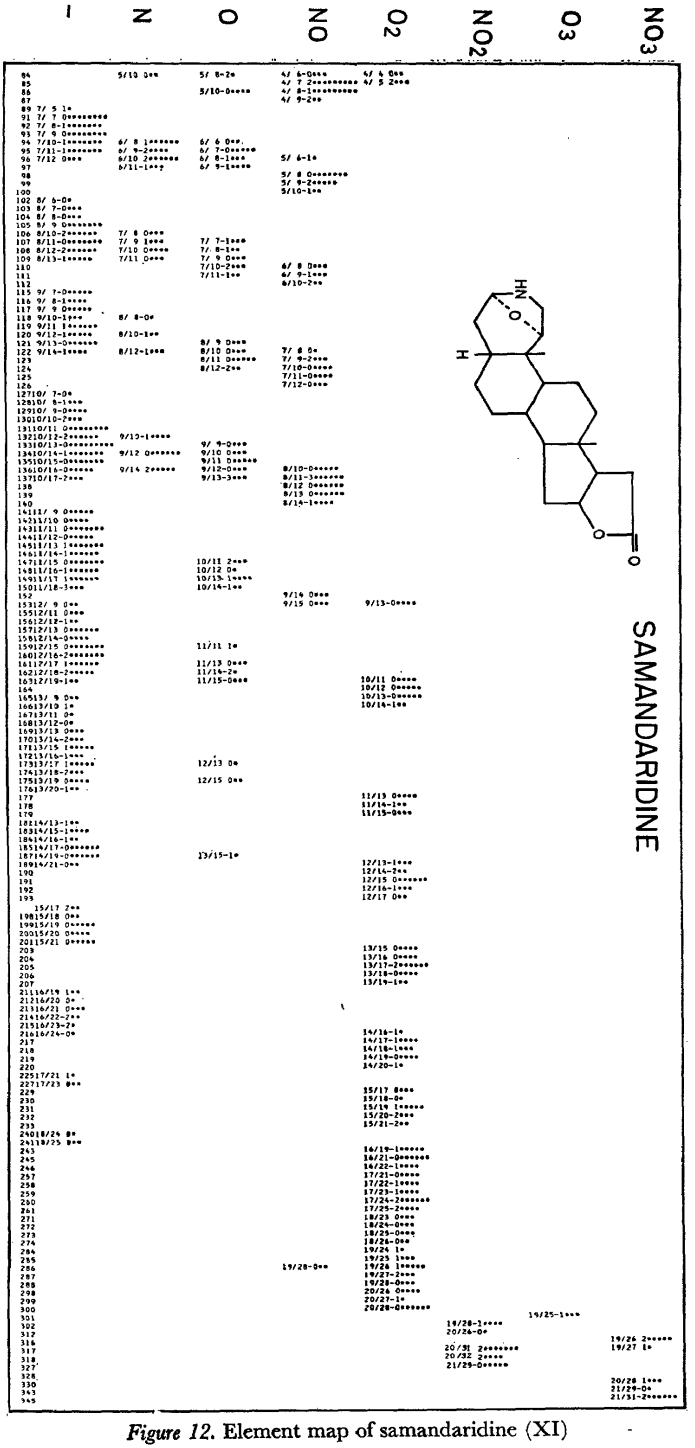


Figure 12. Element map of samandaridine (XI)

if little or nothing is known about the compound even not the general type of natural products to which it belongs.

As such an example we shall discuss the element map of an extremely rare and poisonous substance produced by the Columbian frog *Phyllohates bicolor* and tentatively named "kokoi venom". It had been isolated and described very recently and its characteristics were found to be the following[17]:

Basic material p*K* 7–8 (carbinolamine?)
UV: End absorption with shoulders at 220, 230, and 260 mμ
IR: Band at 5·9 μ

Toxicity: LD_{50} 2·7 μg/kg mouse.

Dr Witkop kindly made about 400 micrograms of the venom available to us in order to obtain some more information about this molecule by mass spectrometry. A high resolution mass spectrum taken with about 50 micrograms led to the element map shown in *Figure 13.*

Evaluation of the characteristics of the map along the lines of the previous examples reveals some facts that permit drawing significant conclusions† about this molecule:

50 μg Kokoi venom

Facts	*Conclusions*
MW 399·2413	$C_{24}H_{33}NO_4$ 9 rings or double bonds
CH 17/17	Carbonskeleton C_{17} (potentially) highly unsaturated
	OH groups in ring system
No intense C_4H_7NO	Not ring *A* of Samandarine
No intense $C_6H_{12}NO$	Not ring *F* of Tomatidine
$C_4H_{10}NO$	NO within 4 C
$C_7H_{6-8}NO$	NO within 4 C
$C_7H_8NO_2$	NO_2 within 7 C.

† *Figure 13* illustrates the occasional appearance of "imaginary" entries in an element map due to the closeness in mass of two sets of combinations of elements, in this case the aforementioned C_3N *v.* H_3O_3 combinations which lead to duplication of some of the entries of the CHN-column in the CHO_3 column, as indicated by their identical intensity. These ions are definitely of the CHN-type because (1) the general features of this element map excludes the presence of three oxygens closely bunched together and (2) most of the CHO_3 entries are of relatively high hydrogen content and would, in fact, correspond to completely saturated trihydroxy species, requiring a polyhydroxy sidechain retaining the charge, which is also relatively unlikely. Considerations of this type permit a decision between two entries due to the same ion and thus imply that in this case all the CHO_3-ions, at least those of low carbon number should be eliminated from consideration as they are correctly listed in the CHN-group.

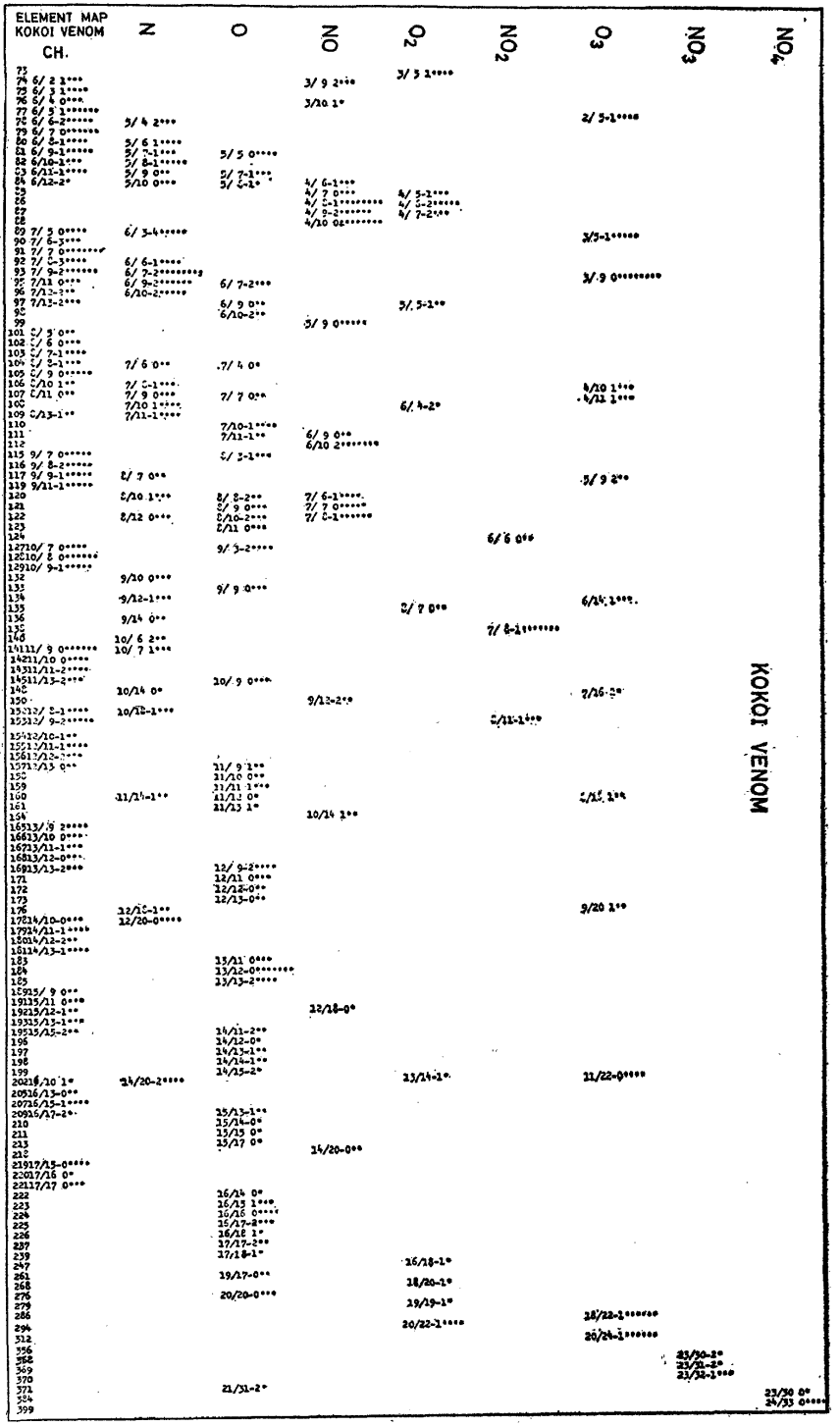
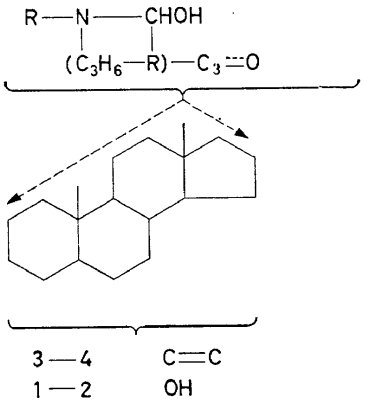


Figure 13. Element map of the kokoi venom: (See Footnote on p. 115 for explanation of the CHO_3 column)

These conclusions may now be moulded into a tentative working hypothesis for the structure of the venom:



(XII)

The $C_{17}H_{17}$ ion would suggest a steroid-type carbon skeleton, to one terminus of which (ring *A* or *D*) is attached the $C_4H_{8-10}NO$ grouping which in turn should have another oxygen atom within three additional carbon atoms. The carbinolamine grouping as shown is supported by the ion $C_{23}H_{32}NO_3$ due to loss of CHO, which would result from the open amino-aldehyde. It should be noted that expression (XII) shows a total of 26 carbon atoms, *i.e.* two of them are common and must be involved in the connection of the carbinolamine ring with the basic carbon skeleton (ring *A* or *D*).

Obviously further work in progress has to establish the validity of the above working hypothesis which is based on this single high resolution mass spectrum.

In conclusion it seems that in "element mapping" we have found a new and entirely different way of interpreting mass spectra. Making use of the elemental composition of *all* the ions formed in the spectrometer rather than a selected few as hitherto done and representation in the form of an element map makes it possible to exploit the tremendous amount of information that can be obtained with a double focusing mass spectrometer. In addition, the element map displays the distribution of the heteroelements within the molecule and adds a new dimension to mass spectrometric information which may be likened to the comparison of a black-and-white picture with colour photography. In the former one we used to associate various tones of grey with colour, which may or may not have been the correct one. The colour picture relays this information directly. Analogously, we were used to associating mass numbers with various combinations of elements but as was illustrated above, the element map shows this directly and we have hardly even used mass numbers in the second half of this paper nor was it necessary to draw detailed mechanisms to support conclusions.

Finally, it is a pleasant duty to mention two important factors which have made this work possible: First, the enthusiasm and devotion of all my associates, particularly Drs P. Bommer, W. McMurray and Mr D. Desiderio and, second, the support of this somewhat expensive work by the National Science Foundation (G-21037) and the National Institutes of Health, Public Health Service (GM-09352) as well as the use of the facilities of the M.I.T. Computation Center.

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