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MASS SPECTRA OF AMARYLLIDACEAE ALKALOIDS
THE LYCORENINE SERIES[1]

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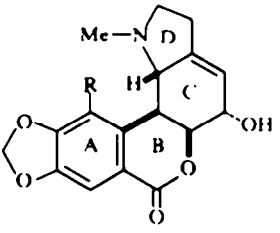
Abstract—The mass spectra of alkaloids belonging to the benzopyrano[3.4g]indole series are presented and discussed. It is shown that the fragmentation pattern for this particular carbon skeleton is quite characteristic and can be rationalized in terms of simple mechanisms. This permits the identification and location of various substituents within different parts of the molecule.

ALKALOIDS of the Amaryllidaceae family have enjoyed much active investigation,[3] resulting in the complete characterization of many bases of considerable structural variety. A mass spectrometric study at this time, based on a great number of different systems would appear desirable, since it should yield a set of independent data of advantage to future structural work.

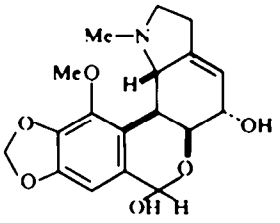
Our interest in the chemistry and biochemistry of these alkaloids led us to undertake a relatively broad and detailed study[4] of all structural types. We wish to present here the results obtained for one group of these bases—the benzopyrano[3.4g]indole series—represented by structures I–XV.

In an earlier paper,[4a] some characteristic features of the decomposition patterns of alkaloids of the crinine class were commented upon and Duffield *et al.*,[5] surveying the mass spectrometric behavior of several types of Amaryllidaceae alkaloids, have presented mechanistic interpretations of some of the dominant fragmentation modes. Very recently, a brief note[6] discussed, in part, the salient points of the mass spectra of several lactone alkaloids also included in the present report. A few applications to structural problems[4b, 7] indicate the potential utility of the technique. It would appear from this study that future chemical investigation of these lactone alkaloids should derive great benefit from the application of mass spectral methods, since the dominant modes of fragmentation of these compounds is readily interpretable in terms of skeletal type and substitution pattern. Furthermore, the generalizations derived from the mass spectra of such lactones appear equally applicable to relatively more complex structures, as has been demonstrated recently by confirmation of the structure of the alkaloid clivimine.[8]

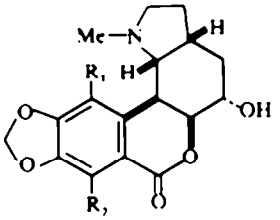
Both low and high resolution* mass spectra of the following substances have been obtained: the $\Delta^{3a,4}$-5-hydroxy compounds, hippeastrine (I), neronine (II) and krigeine (III) the corresponding 3a,4-dihydro derivatives, α-dihydrohippeastrine (IV), α-dihydroneronine (V), clivonine (VI), 0-acetylclivonine (VII) and α-dihydrocandimine (VIII), as well as the 5-desoxy derivatives, masonine (IX), dehydrokrigenamine (X), krigenamine (XI), homolycorine (XII), albomaculine (XIII), lycorenine (XIV) and deoxylycorenine (XV).



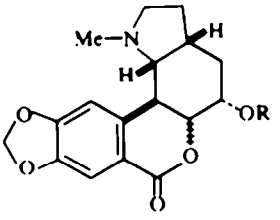
I R = H
II R = OMe



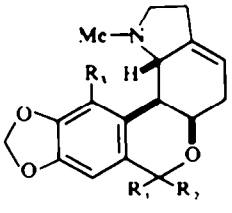
III



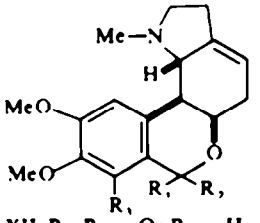
IV $R_1 = R_2 = H$
V R_1 = OMe, R_2 = H
VIII R_1 = H; R_2 = OMe



VI R = H
VII R = COMe



IX R_1, R_2 = O; R_3 = H
X R_1, R_2 = O; R_3 = OMe
XI R_1 = H; R_2 = OH; R_3 = OMe



XII R_1, R_2 = O; R_3 = H
XIII R_1, R_2 = O; R_3 = OMe
XIV R_1 = H; R_2 = OH; R_3 = H
XV $R_1 = R_2 = R_3 = H$

* In the high resolution mass spectra presented here, peaks are plotted in separate graphs according to their iso-heteroatomic content. The number of heteroatoms is indicated on each individual plot and the carbon-hydrogen ratio is given by the abscissa. The major divisions indicated on the abscissa correspond to the composition of saturated fragments. A fragment containing fewer hydrogens than the saturated ions thus appears *below* the major divisions and its composition is determined by counting down from the saturated position. For example, peak *b* in Fig. 10, drawn in the C_nH NO plot five units to the left of the division corresponding to the saturated mononitrogen-containing fragment, $C_nH_{2n+2}NO$, $C_7H_{16}NO$, has the composition $C_7H_{11}NO$. A peak marked by a short vertical line above it indicates more than seven degrees of unsaturation. Its true composition is determined by adding one carbon and substracting two hydrogens from the composition indicated by its position on the graph. A detailed description of this method is in preparation (D. H. Smith and A. L. Burlingame, *Tetrahedron Letters* in preparation).

All lactone alkaloids of the Amaryllidaceae conform to the gross structural pattern illustrated by formulae I–XV (except for macronine[9] which, although a lactone, exhibits the skeletal arrangement of the tazettine class of alkaloids) and the compounds thus differ only in substitution pattern, degree of oxygenation and unsaturation. The mass spectra reflect these differences only in the mass-shift of peaks and not in the fundamental modes of fragmentation, which appear notably indifferent to peripheral structural modifications. One mode of decomposition dominates: cleavage of the labile bonds in ring C with fragmentation of the molecule into two parts, one representing the pyrrolidine ring (plus substituents), the other (a less abundant fragment) encompassing the aromatic lactone moiety. A further general and noteworthy feature is the low abundance of the molecular ion in the spectra of all $\Delta^{3a,4}$-compounds. Ions corresponding to loss of one, two and three hydrogen atoms are usually more intense than the molecular ion. This somewhat unusual elimination of hydrogen (partially thermal in nature) would generate $\Delta^{3a,4}$-conjugation (or aromatization of ring C). In addition, the $\Delta^{3a,4}$-bond is expected to enhance the lability of ring C, also resulting in molecular ions of low abundance. Such conclusions are corroborated by the mass spectra of the 3a,4-dihydro derivatives, all of which show quite prominent molecular ions.

The dominant fragmentation sequence of the $\Delta^{3a,4}$-compounds is conveniently illustrated by the spectrum of hippeastrine (I, Figs. 1 and 10). It shows very intense peaks at *m/e* 125 (**b**, $C_7H_{11}NO$) and 96 (**b**$_1$, $C_6H_{10}N$). Such N-containing fragments, comprising the pyrrolidine nucleus, are the most characteristic feature of all alkaloids in the lycorenine series. All other ions are by comparison minor contributors, although the sequence of peaks **a** ($C_{10}H_6O_4$, *m/e* 190), **a**$_1$ ($C_9H_6O_3$, *m/e* 162), **a**$_2$ ($C_8H_6O_2$) and **a**$_3$ ($C_8H_5O_2$) are significant because they convey important structural information. The peaks at *m/e* 125 and 190 completely account for the two parts of the molecule (i.e. 125 + 190 = 315 = M^+), and an explanation for their genesis is reasonably apparent: a *retro*-Diels–Alder reaction would conveniently generate both fragment **a** ($C_8H_6O_4$, *m/e* 190) and **b** ($C_7H_{11}NO$, *m/e* 125). This fragmentation can be thought of as a concerted reaction of the *retro*-Diels–Alder type but, of course, a stepwise sequence, i.e. cleavage of the 11b,11c-bond following that of 5,5a, is equally feasible. In Scheme I, the fragmentation is illustrated as a concerted process, yielding either ion **a** or **b**.

Subsequent elimination of carbon monoxide from **a** is unexceptional and results in the benzofuranyl fragment **a**$_1$ ($C_9H_6O_3$). Elimination of neutral carbon monoxide* and the formyl radical from ion **a**$_1$ yields species **a**$_2$ ($C_8H_6O_2$, *m/e* 134) and **a**$_3$ ($C_8H_5O_2$, *m/e* 133), respectively. This process is best formulated as involving the ether oxygen of the benzofuranyl ion radical, since the methylenedioxy moiety does not appear to undergo this fragmentation† in analogous systems. The absence of metastable peaks does not permit a distinction between the possible pathways **a**-CO-H and **a**-CHO for the generation of ion **a**$_3$.

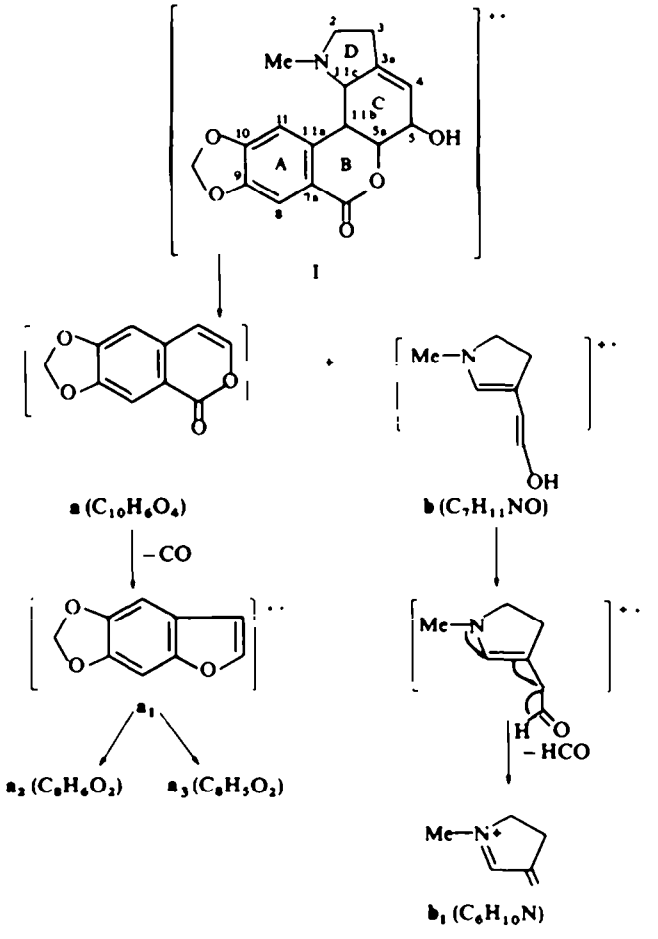
The elimination of a formyl radical (·CHO) from fragment **b** may proceed by the pathway outlined in Scheme I. Transfer of the hydroxyl hydrogen atom is established

* Data from the literature [W. H. Pirkle, *J. Am. Chem. Soc.* **87**, 3022 (1967)] concerning the elimination of CO from 2-pyrone would suggest other possibilities for the formulation of fragment **a**$_1$.

† A. L. Burlingame and B. R. Simoneit, unpublished results from this laboratory.

by the retention of a deuterium atom in fragment $\mathbf{b}_1$ ($C_6H_{10}N$) in the spectrum of hippeastrine-OD. A metastable ion at *m/e* 74·1 (calc. 73·7) confirms the transition from **b** to $\mathbf{b}_1$. The postulated sequences appear to be quite general and essentially invarient for all compounds of this type. The fragmentation pattern thus readily permits the location of substituents in both moieties of the molecule. For example, in the spectrum of neronine (II, Fig. 2) the peak corresponding to fragment **a** is shifted by 30 m.u. (*m/e* 220, $C_{11}H_8O_5$) due to the presence of an additional OMe substituent. Hydroxyl substitution at C-5 is immediately evident from the mass and composition of fragment **b**. In the case of the 5-desoxy derivatives (IX–XV), an ion

SCHEME I



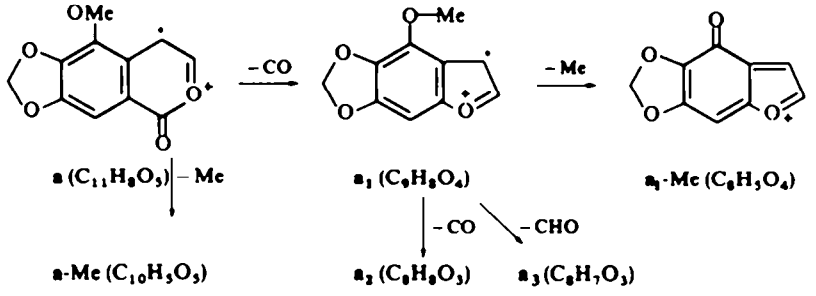
of type **b** gives rise to an intense peak at *m/e* 109, of the expected composition $C_7H_{11}N$ (cf. Figs 4, 5 and 6).

Substances with OMe substituents in the aromatic ring (cf. neronine, Fig. 2; albomaculine, Fig. 5) show some additional peaks, since now the loss of Me radicals is superimposed upon the general pathway. Such eliminations should, of course, be facile, since they contribute to the stability of the ions formed; for neronine (II, Fig. 2) one observes peaks corresponding to the sequence sketched in Scheme II.

It is to be noted that the loss of a Me radical generates another carbonyl oxygen function which might now partake in the expulsion of carbon monoxide.

While the significant features of the fragmentation pattern of the hemiacetyl members (cf. III, XI, XIV) of this series are readily ascertained in the light of the foregoing discussion, they are less suited to mass spectrometric analysis due to thermal lability toward elimination of water. The spectrum of krigeine (III, Fig. 3)

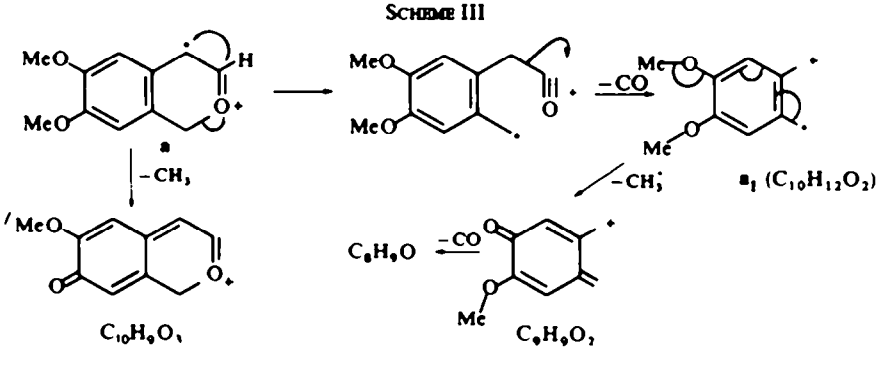
SCHEME II



may serve as an example. In this case, two OH functions are available for the elimination of water. Since the loss of water in the case of the 5-hydroxy lactones is a minor process, the facile elimination of water in the hemiacetals can be ascribed to the hemiacetal OH function. Species resulting from elimination of the elements of water either upon electron impact or thermally would give rise to quite different fragmentation patterns. Fig. 3 illustrates this point. Most notably, the M-H_2O peak (*m/e* 329) is very intense and, while the usual and expected fragments are easily recognizable (e.g. the intense peaks **b**, *m/e* 125; $\mathbf{b}_1$, *m/e* 96, as well as **a**, *m/e* 222; **a**-OH, *m/e* 205; $\mathbf{a}_1$, *m/e* 194), other ions arising probably from the M-H_2O species are now also quite abundant.

The mass spectrum of the ether, deoxylycorenine, XV, Figs. 6 and 11, exhibits the usual fragmentation pattern leading to ions **a** ($C_{11}H_{12}O_3$) and **b** ($C_7H_{11}N$). The further decomposition of **a** is interesting, because elimination of the elements of carbon monoxide occurs even though no carbonyl function is originally present. Scheme III depicts formulation of the prominent fragments of the **a** group (cf. Fig. 11):

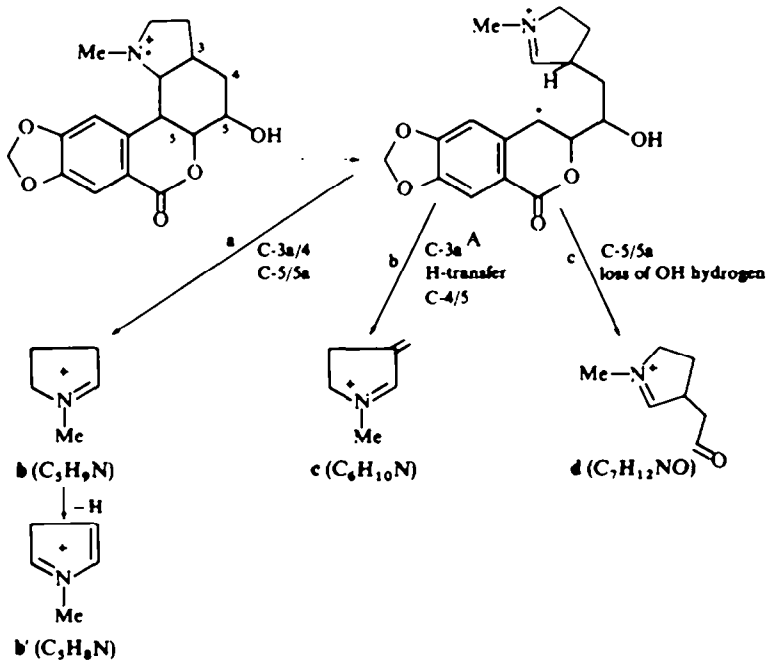
SCHEME III



In the spectra of the saturated lactones, α-dihydrohippeastrine (IV, Fig. 7), α-dihydroneronine (V, Fig. 8), α-dihydrocandimine (VIII), clivonine (VI, Fig. 9) and O-acetylclivonine (VII), the more intense molecular ion peaks are notable. This feature, as suggested above, may reflect a greater stability of the molecular ion, both with respect to hydrogen atom loss and the tendency towards ring C cleavage reactions. The latter still predominate, however, as evidenced by the appearance of fragments of type **a** and **b**, which are readily accounted for by concerted or stepwise cleavages of ring C. The expected ionic products derived from decomposition of α-dihydrohippeastrine (IV, Fig. 7) are shown in Scheme IV. The depicted sequence results in the elimination of the 4,5-bridge and yields the abundant ion **b** (*m/e* 83, C_5H_9N), typical for all dihydro derivatives. Loss of a hydrogen atom leads to **b'**.

The spectra of the dihydro compounds furnish some evidence that a stepwise

SCHEME IV



rather than concerted process should be considered for the decomposition of ring C. Peak **a**, ($C_{10}H_6O_4$), for example, is accompanied by a peak one mass unit higher (**a'**, $C_{10}H_7O_4$), indicating that fragmentation with hydrogen atom transfer does occur. In Scheme IV, intermediate **A** would provide the opportunity for such transfer reactions, and it is suggested that a hydrogen atom from C-3a is transferred to the radical site. Subsequent cleavage of the 4,5-bond would yield the nitrogen containing fragment **c** (*m/e* 96, $C_6H_{10}N$), a very abundant ion. Cleavage of the 5,5a-bond with subsequent loss of the hydroxyl hydrogen (path c) would yield peak **d** (*m/e* 126, $C_7H_{12}NO$), a sequence corroborated by the fact that in the spectrum of dihydrohippeastrine-OD, ion **d** does not retain the deuterium atom. The alternative decomposition, i.e. rupture of the 5,5a-bond followed by 3a,4-cleavage, results in the base peak **b** (C_5H_9N) already mentioned (path a). Of course, loss of an acetaldehyde radical from **d** would also account for the genesis of **b** and, likewise, a

"McLafferty" rearrangement involving the carbonyl function of d could be thought of as the source of b' (C_5H_8N). Unfortunately, metastable ions are not observed in these spectra and, therefore, a more precise delineation of pathways is not possible.

Only the alkaloid clivonine (VI, Fig. 9) differs in stereochemistry (*trans* B/C ring juncture) from the other compounds of this series, such that possible stereochemical effects on the fragmentation pattern could not be investigated extensively. The spectrum of clivonine is almost indistinguishable from that of its isomer dihydrohippeastrine (IV, Fig. 7), suggesting the absence of steric influence on the course of the decomposition. Such a result might be expected since all proposed pathways involve simple homolytic cleavages of labile bonds—reactions which should be unaffected by stereochemical detail.

The compounds appear, however, quite sensitive to instrument operating conditions. While the gross fragmentation pattern is unaffected, differences in detail can be noted between successive runs, implying that some of the fragmentation sequences discussed above (e.g. loss of hydrogen, H_2O) may represent partial thermal degradation.

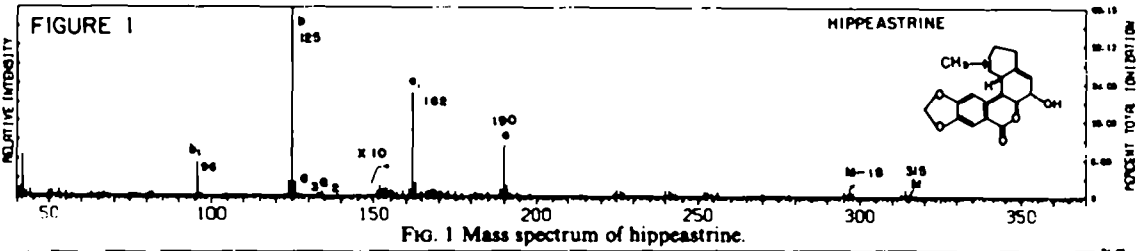


FIG. 1 Mass spectrum of hippeastrine.

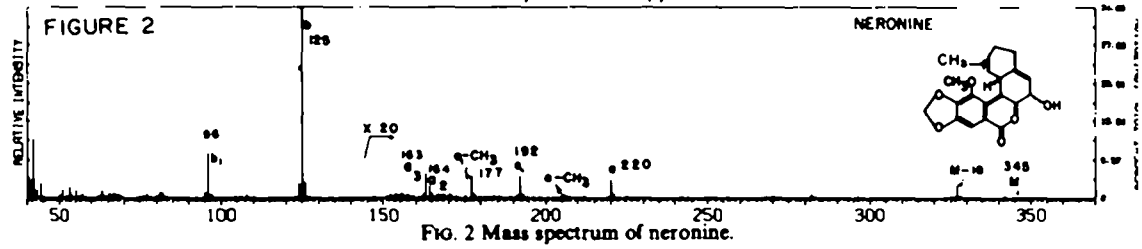


FIG. 2 Mass spectrum of neronine.

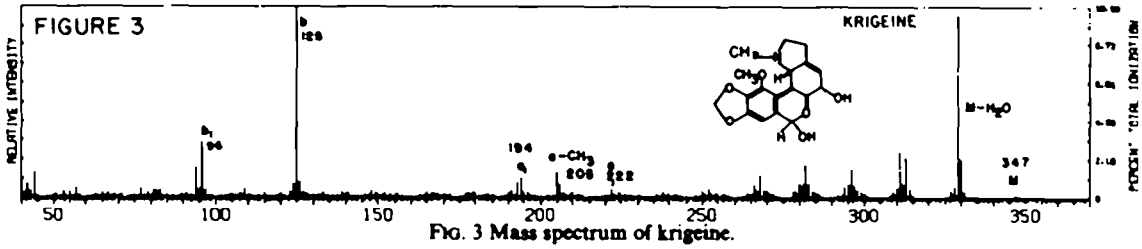


FIG. 3 Mass spectrum of krigeine.

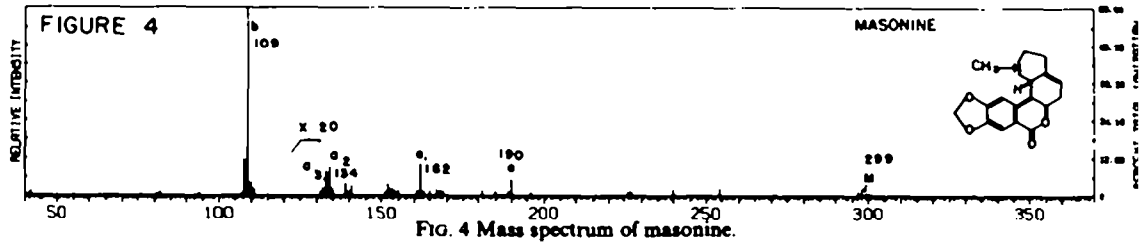


FIG. 4 Mass spectrum of masonine.

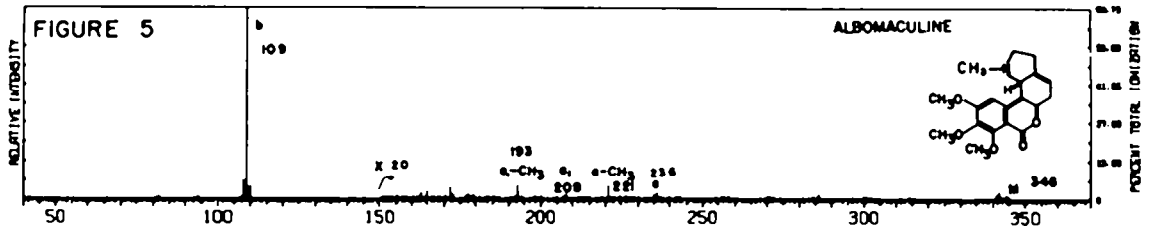


FIG. 5 Mass spectrum of albomaculine.

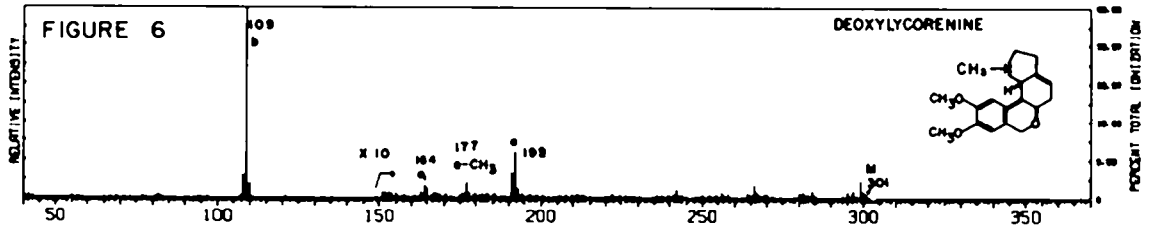


FIG. 6 Mass spectrum of deoxylycorenine.

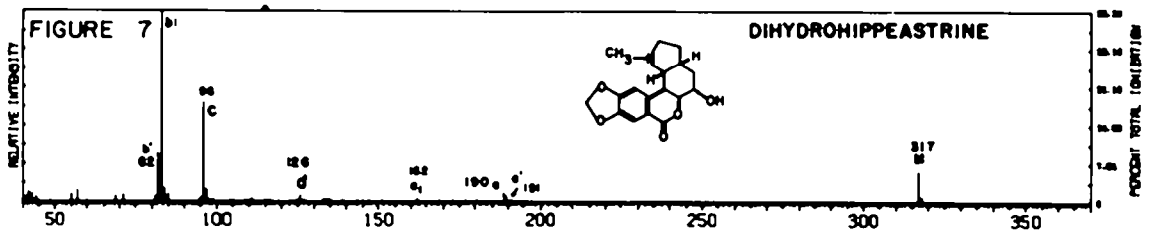


FIG. 7 Mass spectrum of α-dihydrohippeastrine.

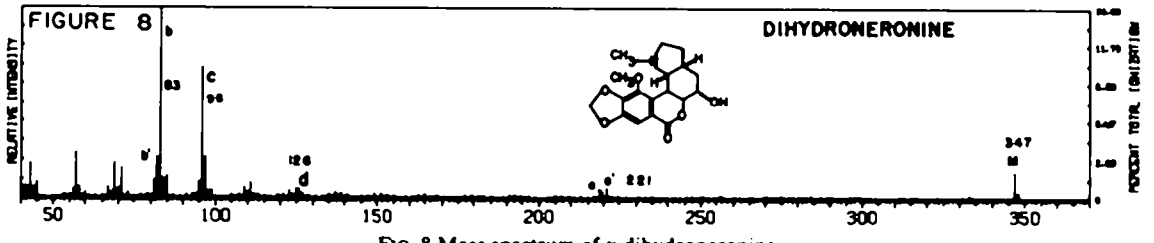


FIG. 8 Mass spectrum of α-dihydroneronine.

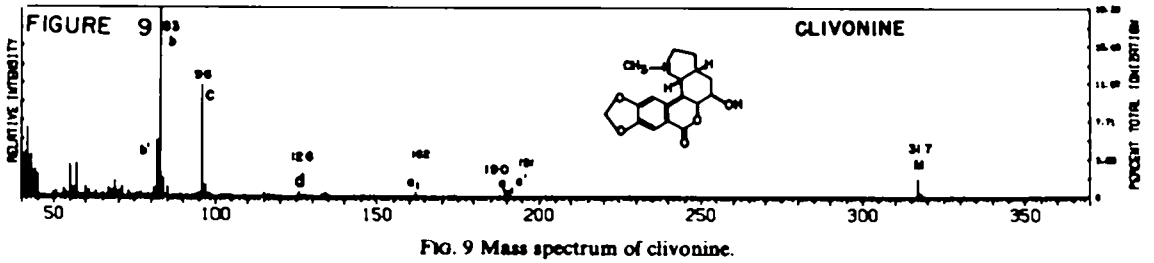


FIG. 9 Mass spectrum of clivonine.

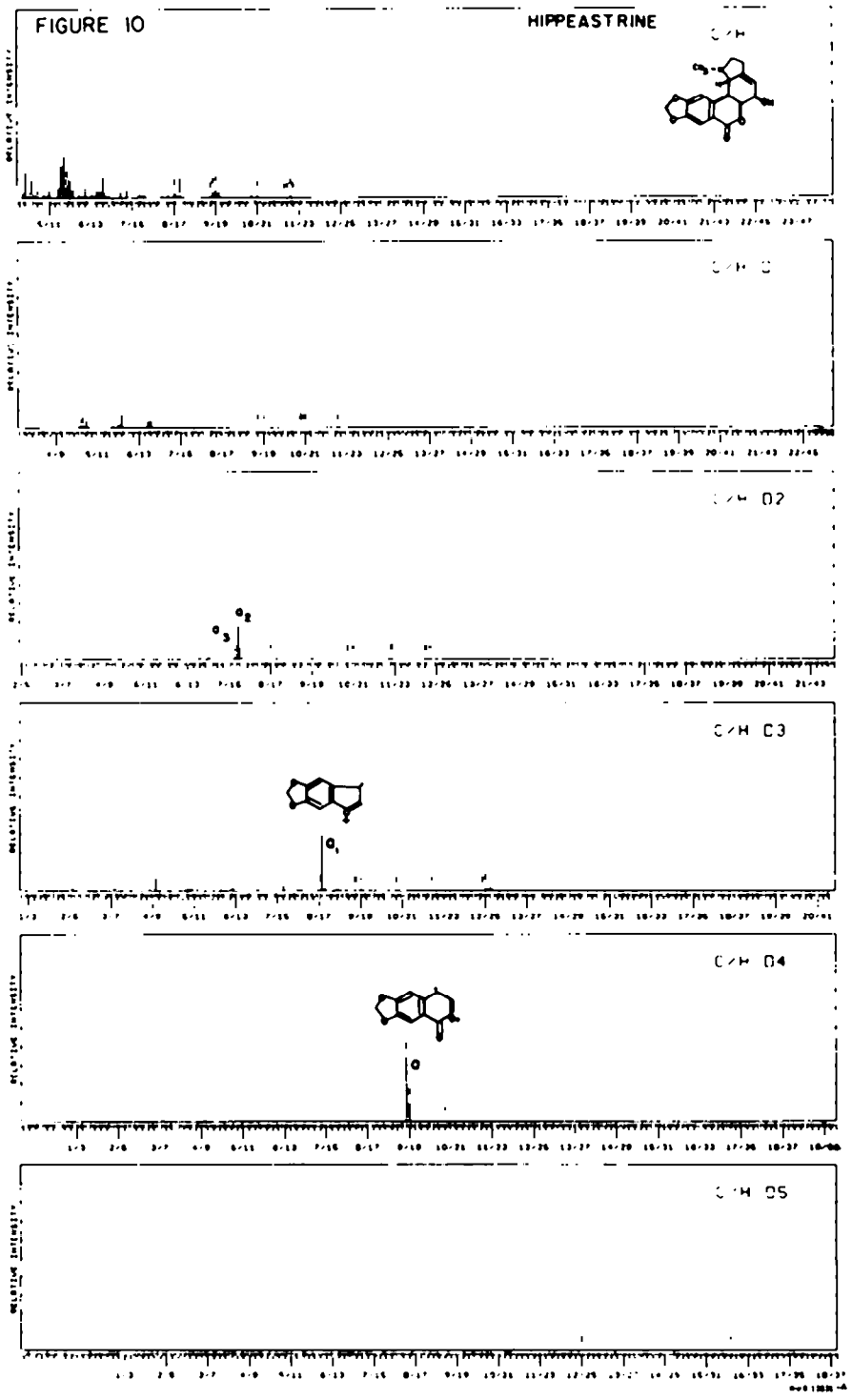


FIG. 10 High resolution mass spectrum of hippeastrine: Hydrocarbon and O-containing fragments.

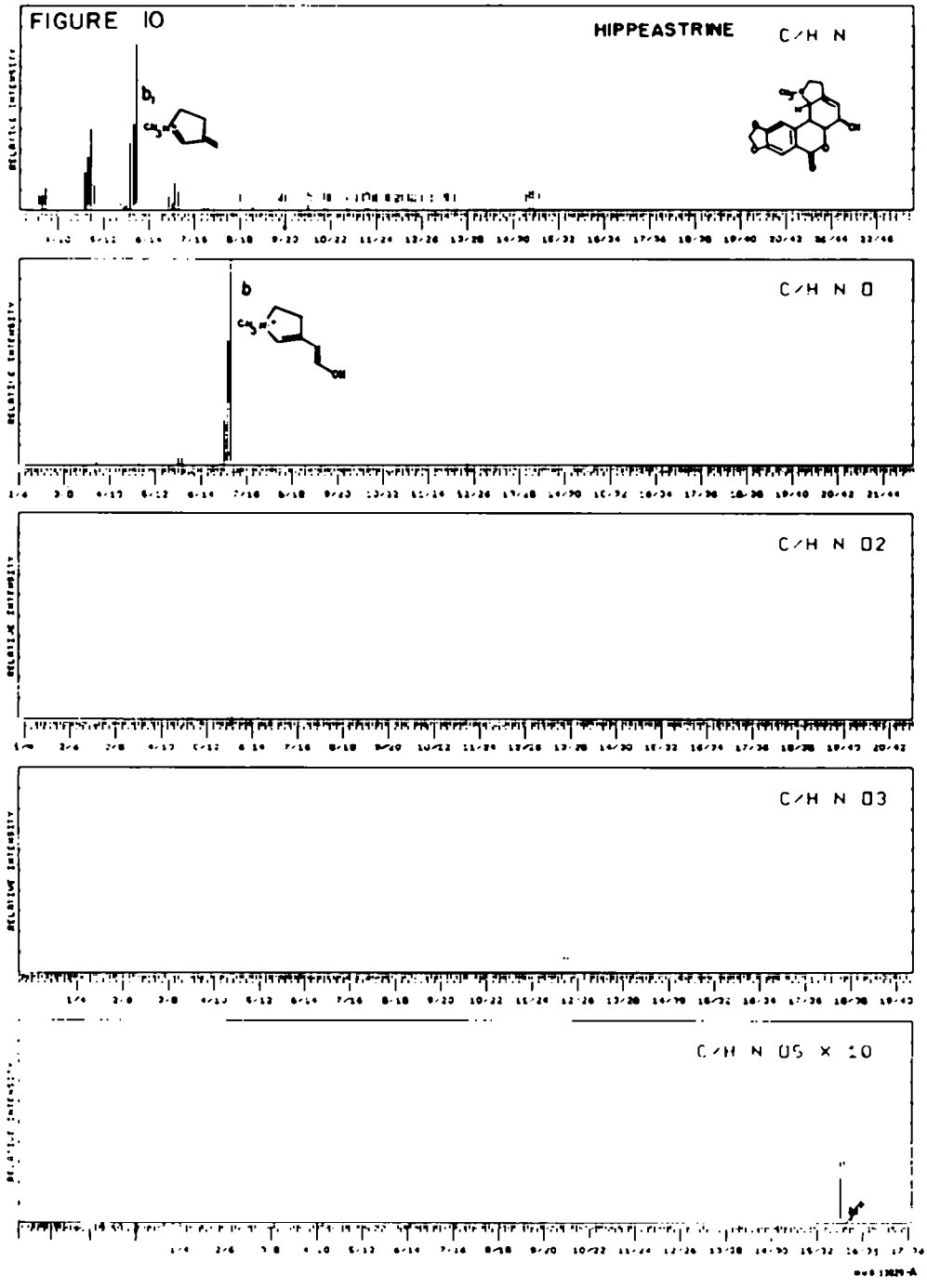


FIG. 10 High resolution mass spectrum of hippeastrine: N-containing fragments.

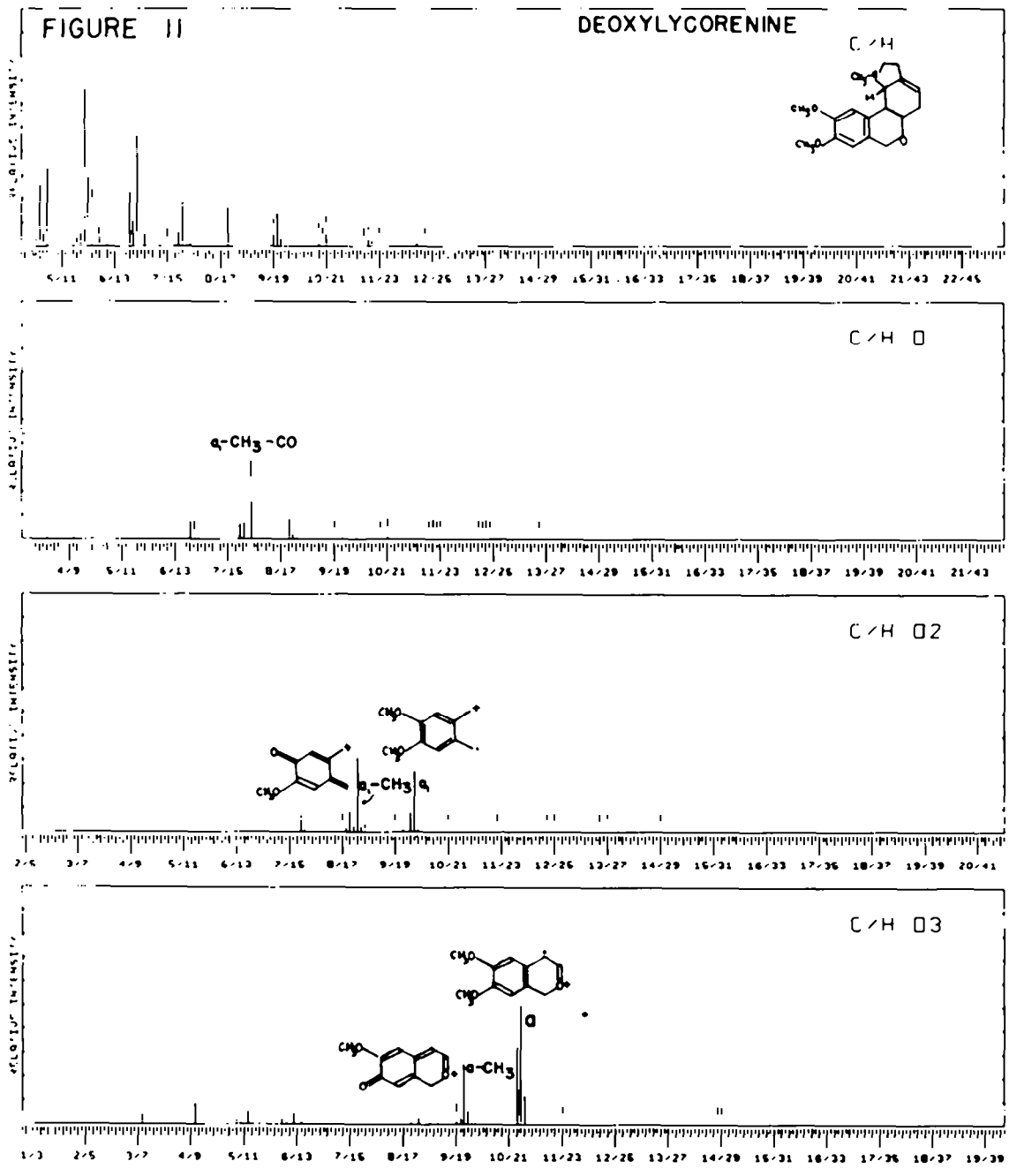


FIG. 11 High resolution mass spectrum of deoxylycorenine: Hydrocarbon and O-containing fragments

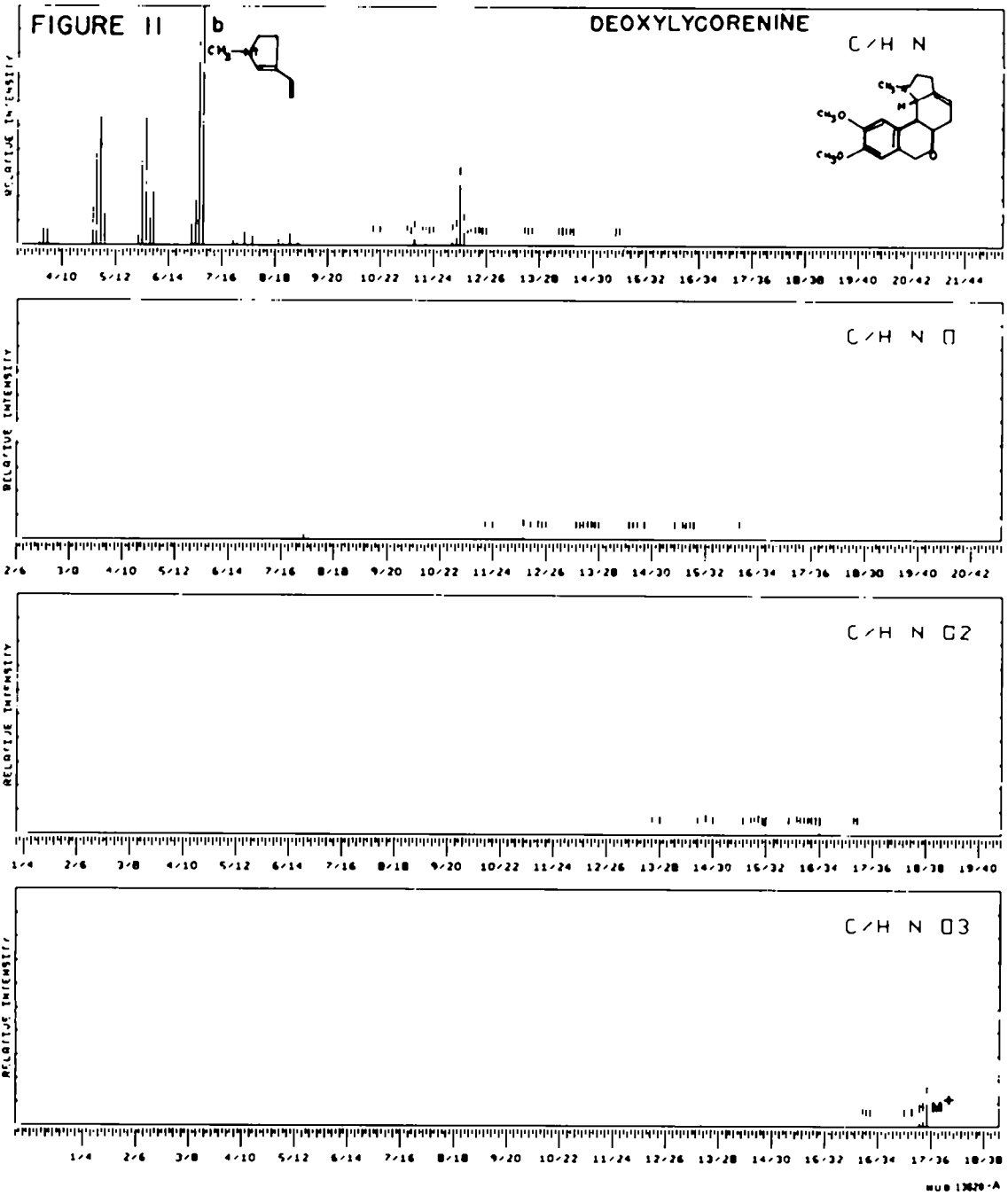


FIGURE II
b
DEOXYLYCORENINE
C/H N
C/H N O
C/H N O2
C/H N O3
RELATIVE INTENSITY
M+

EXPERIMENTAL

Low resolution spectra were determined on a modified C.E.C. 21-103C mass spectrometer [ionizing voltage: 70 eV; ionizing current: 20 μa; ion source temp: 250°]. High resolution mass spectra were obtained with a double focussing mass spectrograph (C.E.C. 21-110B) [ionizing voltage: 70 eV; ionizing current: 150 μa; temp at minimum value necessary to obtain ion beam]. In both instruments samples were introduced directly into the ion source chamber.

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[2] NASA predoctoral trainee, 1965–67.

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