[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD CALIF., AND THE PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNA.]

Mass Spectrometry in Structural and Stereochemical Problems. XXXI.[1] Aporphines and Related Alkaloids[2]

BY M. OHASHI, J. M. WILSON, H. BUDZIKIEWICZ, M. SHAMMA, W. A. SLUSARCHYK, AND CARL DJERASSI

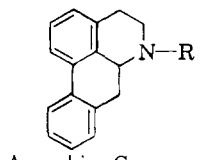
RECEIVED MAY 3, 1963

Mass spectra have been measured for typical members of the aporphine, cularine, berbine, and benzylisoquinoline groups of alkaloids and attention is called to a number of significant fragmentation features, which may be of assistance in structure studies.

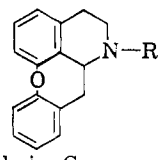
Although the use of mass spectrometry has been very fruitful in the elucidation of the structures of naturally occurring indoles,[3,4] it is only recently that this method has been applied to other classes of alkaloids.[5-7] The availability, from earlier optical rotatory dispersion studies,[8] of a diverse group of aporphine alkaloids[9] and related types, has prompted the presently described survey of their mass spectra in order to examine the utility of this physical method for diagnostic purposes of such ring systems.

The method of using compounds which differ only in the substitution pattern of an aromatic ring for comparison of mass spectra has proved to be useful in studies of indole alkaloids.[3,4] It is, however, of use only when there is attached to the aromatic nucleus an alicylic system which will fragment readily. When this is not present, the nature of the aromatic substituents will have a greater effect on the fragmentation pattern, as was found in the case of colchicine alkaloids.[6]

The alkaloids studied fall into four main groups, according to the ring system present. These are

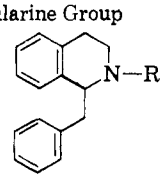


Aporphine Group



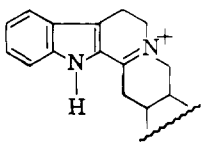
Cularine Group

Berbine Group

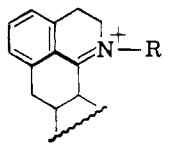


Benzylisoquinoline Group

Aporphine Group.—The simplest member of this series studied is N-nornuciferine (I).[10] In its mass spectrum (Fig. 1) the base peak is formed by loss of a hydrogen atom from the molecular ion. An intense M-1 peak has also been observed in the mass spectra of alkaloids of the yohimbine and ajmalicine type, where it was shown[11] that such a peak is produced by the loss of one of the hydrogen atoms next to nitrogen and that the ion formed is largely of the type a. By analogy, the M-1 ion in the aporphine spectra would have the structure a′. Peaks at *m/e* 266 and 250

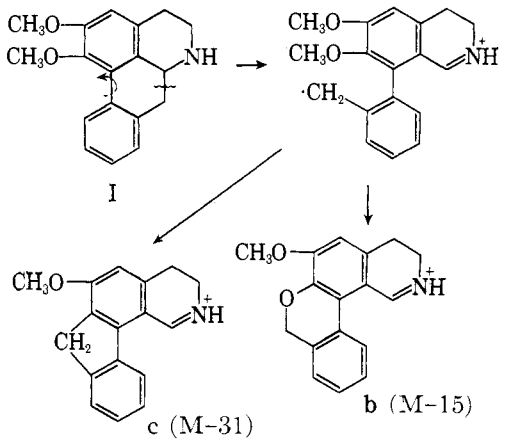


a



a′

(M−15 and M−31) are due to the loss of methyl and methoxyl from one of the aromatic methoxyl substituents. This is a typical behavior of aromatic methyl ethers, except that the usual fragmentation of the M-15 ion by loss of carbon monoxide[12] is not observed. Stabilization of the two observed ions may be possible by initial fission of the benzylic bond and formation of a new ring, as in the scheme



Some support of this scheme is found in the mass spectra of stephanine (IIa), crebanine (IIb), and bulbocapnine (III) (Fig. 3), where the M-31 peak is greatly reduced in intensity. The M-15 peak is reduced in intensity in II but not in III; although bulbocapnine (III) cannot form b, the M-15 ion can be stabilized as will be discussed later.

The M-29 peak (*m/e* 252 in Fig. 1) is found in all compounds of this type which have the NH grouping. Those with an N-methyl function, *e.g.*, nantenine (IV, *m/e* 296 in Fig. 2), stephanine (IIa), crebanine (IIb), and bulbocapnine (III) (*m/e* 282 in Fig. 3), exhibit an

(1) For paper XXX, see H. Budzikiewicz, C. Djerassi, A. H. Jackson, G. W. Kenner, D. J. Newman, and J. M. Wilson, *J. Chem. Soc.*, in press.

(2) The work at Stanford University was supported by Grant No. AM-04257 from the National Institutes of Health, U. S. Public Health Service, while that at The Pennsylvania State University was subsidized by the National Science Foundation Grant No. G-19876. We thank Profs. T. Takamoto, T. R. Govindachari, B. R. Pai, S. M. Kupchan, and Drs. J. Schmutz, and R. H. F. Manske for specimens.

(3) For introduction, see K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Chapter 8.

(4) For more recent references see C. Djerassi, H. Budzikiewicz, R. J. Dwellen, J. M. Wilson, W. G. Kump, D. J. Le Count, A. R. Battersby, and H. Schmid, *Helv. Chim. Acta*, **46**, 742 (1963); C. Djerassi, *Pure Appl. Chem.*, **6**, 575 (1963).

(5) Pyrrolizidine alkaloids: C. C. Culvenor, J. D. Morrison, A. J. C. Nicholson, and L. W. Smith, *Australian J. Chem.*, **16**, 131 (1963).

(6) Colchicine alkaloids: J. M. Wilson, M. Ohashi, H. Budzikiewicz, F. Šantavý, and C. Djerassi, *Tetrahedron*, in press.

(7) G. Spiteller and M. Spiteller-Friedmann, *Tetrahedron Letters*, 153 (1963), where a few members of the quinoline and isoquinoline alkaloid classes are discussed.

(8) C. Djerassi, K. Mislow, and M. Shamma, *Experientia*, **18**, 53 (1962).

(9) For a review of aporphine and related alkaloids see R. H. F. Manske (R. H. F. Manske and H. C. Holmes, Ed.), "The Alkaloids," Academic Press, Inc., New York, N. Y., 1954, Vol. IV.

(10) S. M. Kupchan, B. Dasgupta, E. Fujita, and M. L. King, *Tetrahedron*, **19**, 227 (1963).

(11) L. D. Antonaccio, N. A. Pereira, B. Gilbert, H. Vorbrüggen, H. Budzikiewicz, J. M. Wilson, L. J. Durham, and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 2161 (1962); see also G. Spiteller and M. Spiteller-Friedmann, *Monatsh.*, **93**, 795 (1962).

(12) Z. Pelah, J. M. Wilson, M. Ohashi, H. Budzikiewicz, and C. Djerassi, *Tetrahedron*, in press; see also C. S. Barnes and J. L. Occolowitz, *Australian J. Chem.*, **16**, 219 (1963).

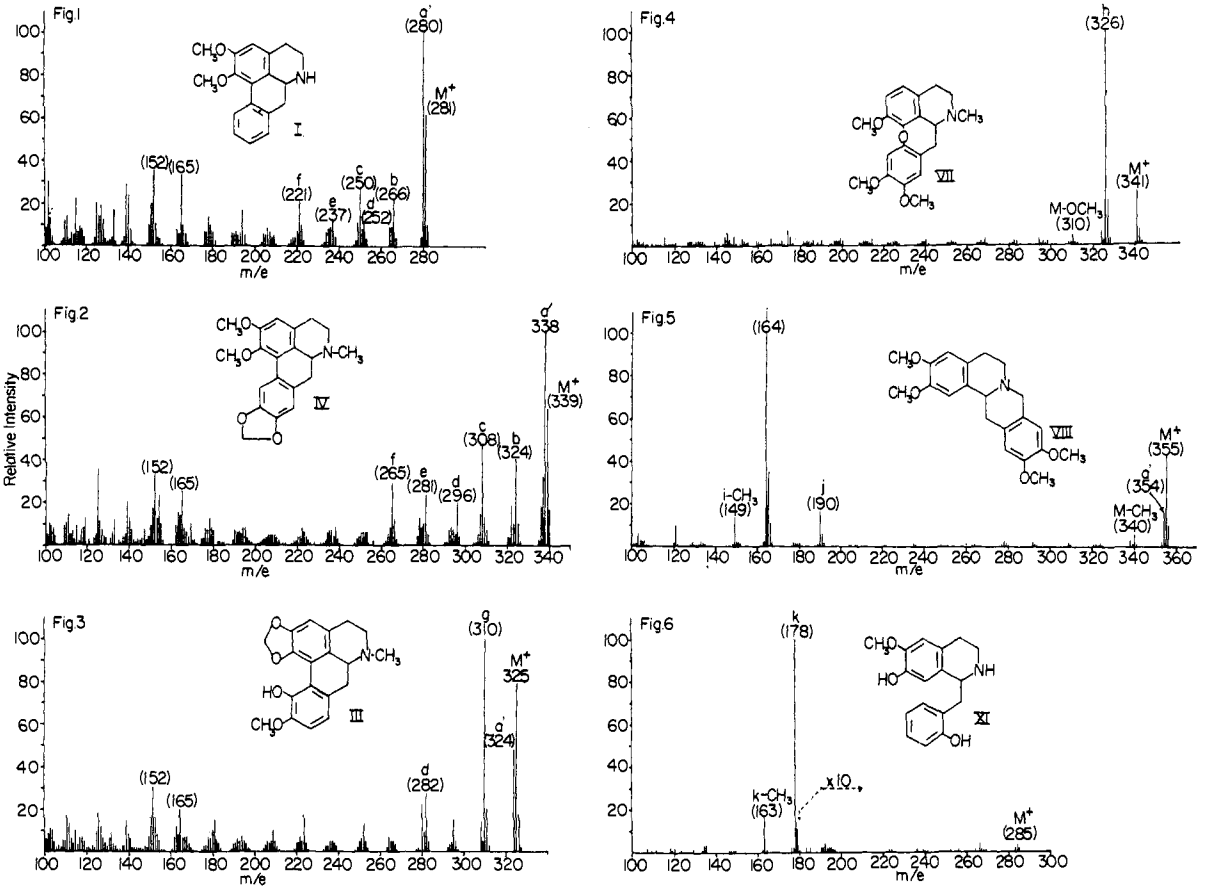
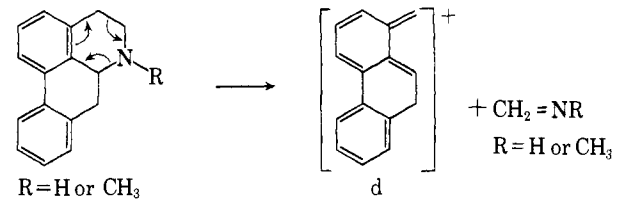
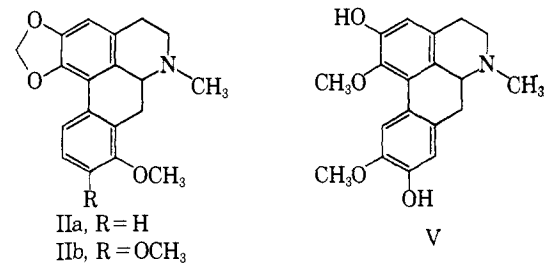


Fig. 1.—Mass spectrum of N-nornuciferine (I).
Fig. 2.—Mass spectrum of nantenine (IV).
Fig. 3.—Mass spectrum of bulbocapnine (III).
Fig. 4.—Mass spectrum of cularine (VII).
Fig. 5.—Mass spectrum of xylopinine (VIII).
Fig. 6.—Mass spectrum of 1-(2'-hydroxylbenzyl)-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (XI).

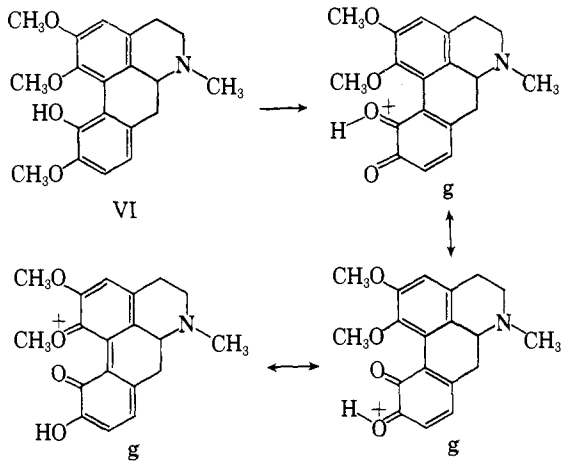
M-43 peak. Thus, the fragment lost must be methylene imine, expelled by a cyclic process as





Since d is an odd-electron ion, it can further lose methyl or methoxyl radicals to produce the even-electron ions e (= d − CH_3) and f (= d − OCH_3) by expulsion of part or all of the extranuclear methoxyl substituent.

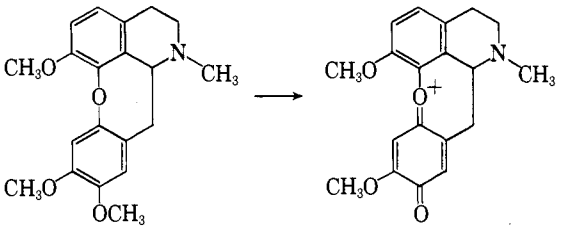
In the spectra of three of these alkaloids, bulbocapnine (III), boldine (V), and isocorydine (VI), the M-15 peak is much more intense than exhibited by I; in III (Fig. 3) and VI it is the base peak. All these compounds have four oxygen functions in the aromatic rings, so it is possible to stabilize the positive charge by distribution among several oxygen atoms



Peaks at *m/e* 152 and 165 are present in all of the above considered spectra. The existence of peaks at the same mass in the spectra of compounds with very different oxygenation patterns cannot be explained easily, but such peaks may serve as empirical sign posts in the consideration of spectra of unknown alkaloids.

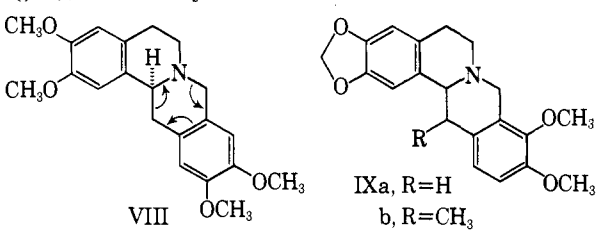
Cularine Group.—Only one example of this class, cularine itself (VII), was available. Its spectrum (Fig. 4) is strikingly different from those of the aporphine group. The base peak and the only significant fragment is the M-15 ion. The methyl group lost is

probably from the *p*-methoxy function; the resulting ion h will possess the very high stability of the *p*-quinoid system which is analogous to the behavior of *p*-anisidine.[13]

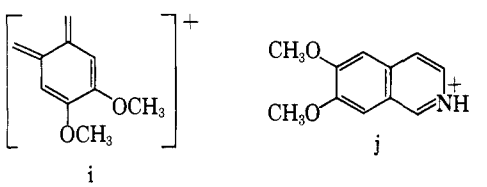


VII h (M-15)

Berbine Group.—A typical example of this group is xylopinine (VIII).[14] Its mass spectrum (Fig. 5) shows that the cleavage of the ring system is predominant. The base peak (*m/e* 164) corresponds to fission of two benzylic bonds in the molecular ion with ionization of the nonnitrogenous fragment i. This ion can lose a methyl group from one of the methoxyl functions, furnishing the ion of *m/e* 149. The other fragment from the cyclic process can lose one hydrogen atom to provide species j (*m/e* 190 in Fig. 5). In tetrahydroberberine (IXa), fragment i and i − CH_3 occur in the same place (*m/e* 164 and 149) as in the xylopinine spectrum (Fig. 5), while ion j is now found at *m/e* 174.



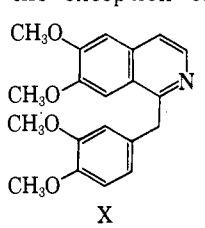
VIII IXa, R=H b, R=CH_3



i j

In the upper mass range (Fig. 5), the molecular ion is abundant and there is a significant M-1 peak, but other peaks in this range are of low intensity. Thalictricarvine (IXb) has a similar spectrum, an important difference being that the molecular ion is somewhat smaller, presumably due to the presence of an additional tertiary center at one of the bonds involved in the main fission. The ion i shifts to *m/e* 178 (14 mass units for addition of a methyl group); j moves to *m/e* 174 and is less abundant than in Fig. 5 because the extra methyl group will help stabilize the positive charge on i.

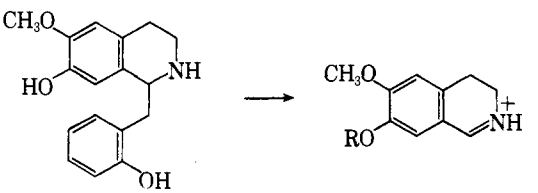
Benzylisoquinoline Group.—Most of the compounds which were examined in this class were tetrahydroisoquinolines with the exception of papaverine (X).



X

In the spectrum of the latter, the molecular ion, the M-1, M-15 (base peak), and M-31 peaks are the ions of significant abundance. Further fragmentation results in a large number of peaks of very low intensity, none of which are of value to the natural products chemist. This lack of significant fragmentation is typical of aromatic compounds where no obvious site for further cleavage is available.

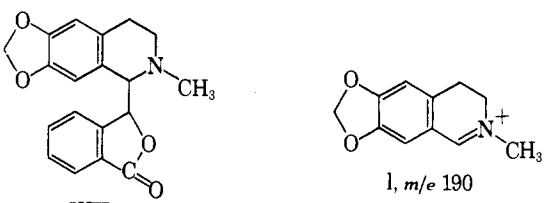
The benzyltetrahydroisoquinoline alkaloids, however, have very characteristic mass spectra. In the spectrum of 1-(2′-hydroxybenzyl)-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (XI) (Fig. 6), the molecular ion peak has an intensity of 0.2% of the base peak (k = *m/e* 178 in Fig. 6). The latter is formed by fission of a bond which is doubly benzylic and *β* to a nitrogen atom. Similar behavior was observed in the mass spectra of some derivatives of cactus alkaloids which contained the 1-isobutyltetrahydroisoquinoline group.[15]



XI, R = H k, *m/e* 178 (R = H)
XII, R = CH_3 *m/e* 192 (R = CH_3)

The only other ion of significant abundance is that (*m/e* 163 in Fig. 6) formed by loss of a methyl group from k. In the spectrum of 1-(2′-hydroxybenzyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (XII), the base peak k shifts to *m/e* 192, the molecular ion moves to *m/e* 299 and otherwise there exists a great similarity to that of XI.

Another relevant member of this group which was examined was hydrastine (XIII). The base peak occurs at *m/e* 190 (l) and loses a methyl group to only a small extent. There is no observable molecular ion since the presence of the additional oxygen function makes the doubly benzylic bond weaker than in XI or XII.



XIII l, *m/e* 190

General Conclusions

The results discussed above show that mass spectrometry can be useful in distinguishing between the berbine group, the benzyltetrahydroisoquinolines, and the other classes. Difficulties may be encountered with the aporphine, cularine, and benzylisoquinoline groups where the nature of the fragmentation pattern is very much dependent on the substituents present rather than on the basic ring system.

In the berbine group, the main fragmentation processes are not affected to the same extent by differences in substitution, so it is possible from the spectrum to determine how the substituents are distributed between rings A and D. The same is true of the benzyltetrahydroisoquinolines, but with the following reservation. Since, in some cases, it is not possible to recognize the molecular ion, information may be obtained about rings A and B only. However, in most cases, a small molecular ion can be found and, if this is not so, as in the case of hydrastine, its absence will be of structural significance.

(13) G. Spiteller, *Monatsh.*, **93**, 1395 (1962).
(14) J. Schmutz, *Helv. Chim. Acta*, **42**, 335 (1959).
(15) See C. Djerassi, H. W. Brewer, C. Clarke, and L. J. Durham, *J. Am. Chem. Soc.*, **84**, 3210 (1962).

Experimental

All mass spectra were determined with a CEC mass spectrometer, Model 21-103 C, using a recently described[16] inlet system for the direct insertion of samples near the ion source. The ionization energy was maintained at 70 e.v. and the ionization current at 50 μa.

(16) J. F. Lynch, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *Experientia*, **19**, 211 (1963).

After the preparation of this manuscript, a communication appeared by Martell, *et al.*,[17] in which it is mentioned briefly that attempted measurement of the mass spectra of the aporphines bulbocapnine (III) and glaucine, using a heated metal inlet system, resulted in dehydrogenation to afford fully aromatic structures. Our results thus represent another instance where the superiority of the direct inlet procedure is demonstrated.

(17) M. J. Martell, T. O. Soine, and L. B. Kier, *J. Am. Chem. Soc.*, **85**, 1022 (1963).

[JOINT CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD, CALIF., AND VARIAN ASSOCIATES, PALO ALTO, CALIF.]

Unusual Chemical Shifts in the Nuclear Magnetic Resonance Spectra of 7- and 11-Keto Steroids

BY D. H. WILLIAMS, N. S. BHACCA, AND CARL DJERASSI

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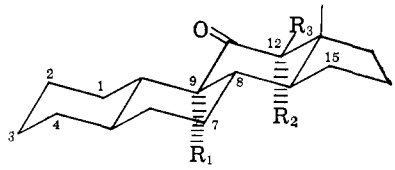
By a study of the n.m.r. spectra of 5α-androstan-11-one (Ia) and several deuterated analogs it has been established that the carbonyl group induces a considerable diamagnetic shift on the equatorial proton at C-1. A similar effect is observed in the deshielding of the equatorial proton at C-15 in a 7-keto steroid.

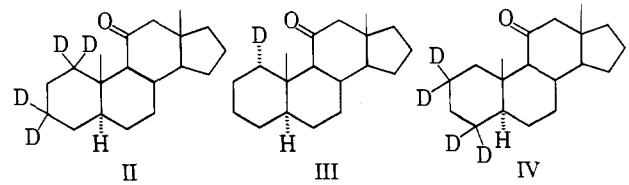
During the course of our investigation of the mass spectrometric fragmentation of 5α-androstan-11-one (Ia) *via* a study of its deuterated analogs,[1] we established that two enolizable hydrogens could readily be replaced by deuterium on treatment of the 11-ketone with base to give 9α,12α-d_2-5α-androstan-11-one (Ib), while a third deuterium atom could only be introduced with difficulty to give 9α,12,12-d_3-5α-androstan-11-one (Ic). The 9α,12α-d_2-structure was assigned to Ib on the basis of the known preference for axial as opposed to equatorial ketonization of enols[2] and mass spectrometric evidence.[1] In order to confirm our assignment we obtained the 100-Mc. n.m.r. spectra of Ia, Ib, and Ic, since the 60-Mc. spectra were not clearly resolved in the relevant downfield region. The spectrum (Fig. 1) of Ia showed downfield signals representing three protons, the tall peak at δ = 2.27 p.p.m. corresponding to two protons and a pair of smeared triplets at 2.45 p.p.m. accounting for the other proton. Initially they were assigned, very reasonably, to the protons at C-12 and C-9, respectively. The large splitting in the resonance pattern was assumed to arise



Ia, $R_1 = R_2 = R_3 = H$
b, $R_1 = R_2$ = D, R_3 = H
c, $R_1 = R_2 = R_3$ = D
d, $R_1 = R_2$ = H, R_3 = D
e, R_1 = D, $R_2 = R_3$ = H

via coupling of the C-9 proton with the single axial hydrogen at C-8 and the small splitting attributed to the hydrogens at C-7. However, very surprisingly, the spectrum (Fig. 2) of the 9α,12α-d_2-derivative Ib showed signals due to two downfield protons, the singlet at δ = 2.27 p.p.m. now containing one proton and the resonance at δ = 2.45 p.p.m. remaining unchanged. In the spectrum (Fig. 3) of the 9α,12,12-d_3-analog Ic the peak at δ = 2.27 p.p.m. had completely disappeared, but the signals at δ = 2.45 p.p.m. remained. Consistent with the assignment of the two-proton signal as being due to the protons at C-12, the singlet was reduced to half intensity in the spectrum (Fig. 4) of the 12β-d_1-ketone Id. *It must therefore be concluded that some proton in the molecule which is not adjacent to the carbonyl function must be giving rise to the most downfield signals at δ = 2.45 p.p.m., i.e.*, some proton is being deshielded much more than would normally be anticipated, and furthermore that the resonance due to the proton at C-9 does not occur in the expected downfield region. These deductions were confirmed by the spectrum (Fig. 5) of 9α-d_1-5α-androstan-11-one (Ie), which still showed three downfield protons.

In an effort to rationalize the above observations, we obtained the spectra of several other deuterated 5α-androstan-11-ones.[1] The spectrum of 8β-d_1-5α-androstan-11-one still exhibited the same downfield pattern as the undeuterated steroid, thus excluding the C-8 proton as giving rise to the resonance at δ = 2.45 p.p.m., despite the fact that it lies roughly in the plane of the carbonyl group and could therefore theoretically be deshielded. However, in the spectrum (Fig. 6) of 1,1,3,3-d_4-5α-androstan-11-one (II), the signals at δ = 2.45 p.p.m. did not occur. This result strongly suggested that the proton whose resonance occurred at δ = 2.45 p.p.m. was that oriented equatorially at C-1, in view of its proximity to the carbonyl function. That this was indeed the case was confirmed by the spectrum (Fig. 7) of 1α-d_1-5α-androstan-11-one (III)



which exhibited a broad single resonance at δ = 2.45 p.p.m.; the large coupling ($J \sim 12$ c.p.s.) in the spectrum (Fig. 1) of the parent ketone therefore arises due to geminal coupling of the two C-1 protons. The smeared triplet pattern (see Fig. 1) is due to further coupling with protons at C-2, a conclusion which is confirmed by the spectrum (Fig. 8) of 2,2,4,4-d_4-5α-androstan-11-one (IV). Now the resonance at δ = 2.45 p.p.m. appears as a broad doublet, since spin coupling with the C-2 protons has now been eradicated; the broadening of the doublet is due to the small coupling of the 1β-proton with deuterium nuclei at C-2. Even more interesting is the sharpening of the one proton

(1) D. H. Williams, J. M. Wilson, H. Budzikiewicz, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 2091 (1963).

(2) E. J. Corey and R. A. Sneen, *ibid.*, **78**, 6269 (1956).