Tetrahedron, 1965, Vol. 21, pp. 1767 to 1775. Pergamon Press Ltd. Printed in Northern Ireland

HETEROCYCLIC STEROIDS—III [1]

MASS SPECTRA OF 6-AZASTEROIDS WITH TWO AROMATIC RINGS

U. K. PANDIT, W. N. SPECKAMP and H. O. HUISMAN
Laboratory for Organic Chemistry
University of Amsterdam, The Netherlands

(*Received 5 December* 1964)

Abstract—The mass spectra of the methyl ethers of 6-azaequilenin, 6-aza-14(β)-isoequilenin and their corresponding alcohols have been measured. The postulation of fragmentation pathways and the assignment of structures to the fragments has been based upon the study of suitably labeled deuterium derivatives and recognition of metastable ions. Significant differences in the spectra of stereoisomeric compounds have been related to different modes of decomposition which find their origin in the stereochemistry of the molecules. It is suggested that the mass spectra of these systems may be utilized in diagnosing the stereochemistry of the C/D ring junction.

THE application of mass spectrometry to problems of structural and stereochemical correlations in the field of natural products is fast assuming an increasingly important role.[2,3] The extensive investigations, notably of Djerassi and coworkers, have led to an understanding of the behaviour of steroids and complex alkaloid systems under conditions of electron-impact induced decomposition. Of particular utility, in recognizing the scope and limitations of the fragmentation patterns, has been the study of suitably labeled derivatives of the various alkaloids and steroids—a technique which has received much impetus from the Djerassi school.

In the course of our work pertaining to the programme on the total synthesis of azasteroids[1,4] it became of interest to examine the mass spectra of these steroids, both in order to derive corroboratory evidence for the structures of the synthesized compounds and to search for stereochemically controlled decomposition patterns which could be of potential analytical value. The latter objective was made particularly worthwhile in view of the availability of various stereoisomeric azasteroids via our synthetic schemes. Finally, it was expected that a study of this new class of novel steroids would be of fundamental interest in developing and extending our understanding of the relationship between mass spectrometric fragmentation patterns and molecular structure. In this communication we wish to describe the results on the study of the 6-azaestrogen analogues (I to VI) which incorporate rings A and B in the aromatized form.

The synthesis of compounds I, II, V and VI has been recently communicated.[1] The deuterated analogue (IV) was obtained by reduction of the alcohol (VII) with deuterium gas over Pd catalyst and followed by oxidative detosylation of the so

[1] H. O. Huisman, W. N. Speckamp, H. de Koning and U. K. Pandit, *Tetrahedron Letters* No. 20, 1275 (1964). This paper may be regarded as Part II of this series.
[2] K. Biemann, *Mass Spectrometry* Chap. 6-10. McGraw-Hill, New York (1962).
[3] H. Budzikiewicz, C. Djerassi and D. H. Williams, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vols. 1 and 2, Holden-Day, San Francisco (1964).
[4] H. O. Huisman, W. N. Speckamp and U. K. Pandit, *Rec. Trav. Chim.* **82**, 898 (1963).

Fig. 1
281 M+
266 (M−15)
238
225
212
210
Fig. 2
281 M+
266 (M−15)
225
210
Fig. 3
283 M+
265 (M−18)
250
239
224
212
210
Fig. 4
283 M+
265 (M−18)
250
236
224
223
210
100
60
20
150
200
250
300
m/e
MeO
I R = O
II R = OH, H
III R = O
IV R = OH, H
V R = O
VI R = OH, H
IX R = CH3
XI R = C2H5
VII
VIII
X
XII
C9H17
AcO
Ts
D2/Pd
NaH/THF
IV

formed dideuterated alcohol (VIII). Oppenauer oxidation of IV with aluminium isopropoxide and cyclohexanone yielded 14,15-d_2-6-aza-equilenin methyl ether (III).

Mass spectra of the methyl ethers of 6-aza-equilenin (I) *and 6-aza-14(β)-iso-equilenin* (V). The spectra of the corresponding equilenin and 14(β)-isoequilenin derivatives (IX and X) have been described and discussed in detail by Djerassi *et al.*[5] A comparison of the spectra of the latter compounds with those of their aza analogues (Figs. 1 and 2) reveals the striking correspondence between the overall spectral patterns of the stereochemically related pairs of ketones. Thus, for all the important peaks (except at *m/e* 165) observed in the spectra of IX and X, there are present peaks of almost comparable intensity, at one mass unit higher, in the spectra of ketones I and V respectively. The existence of such a regular shift by one mass unit in the position of the peaks, indicates that the electron-impact induced decomposition of the two systems proceeds by analogous pathways; a fact which is not surprising when one considers that the difference between the two series comprises only of the replacement of a CH unit by a nitrogen atom in the aromatic portion of the molecule.

In comparing the spectra of the isomeric 6-azaequilenin ethers (Figs. 1 and 2) it is to be noted that while the patterns resemble each other in exhibiting strong peaks for the molecular ions (base peaks) and for ions at *m/e* values 266, 225 and 210, they are significantly different in the appearance of two prominent peaks at *m/e* 238 and *m/e* 212 in the spectrum of the 14α-isomer (Fig. 1). It may be recalled here that although the analogous ions at *m/e* 237 and *m/e* 211 appear in comparable intensity in the spectrum of IX, no structural assignments have been offered for these fragments.[6] The examination of the mass spectrum of 14,15-d_2 analogue (III) however, provides clues for plausible structures of the ions 212 and 238 and for possible fragmentation pathways leading to their formation. The important peaks in the spectra of ketones I and V are discussed in detail in the sequel.

Peak M-15 (*m/e* 266, Figs. 1 and 2). The origin of the M-15 ion in the spectra of ring A aromatic steroids has been ascribed to a combined loss of the CH_3 moiety from the tertiary C_{13} and from the ether oxygen at C_3.[5] By analogy, the peak at *m/e* 266 may be attributed to a similar loss from the methoxy ketones I and V. Further, it has been suggested that the formation of *m/e* 165 ion in the spectrum of IX involves the decomposition of a 2-methoxyphenanthrene species via successive losses of CH_3 and CO,[6] a manner of decomposition which has been observed for several aromatic methyl ethers.[7,8] The absence of *m/e* 166 peak in the spectra of I and V would appear to dispute the involvement of the latter type of fragmentation in the azasteroid series.

Peak M-56 (*m/e* 225; Figs. 1 and 2). The formation of this fragment, which appears as a strong peak in the spectra of both isomers, may be readily visualized as a consequence of the loss of ring D via process *a'*.* Evidence for the structure of ion *a* (*m/e* 225) is derived from the spectrum of the deuterated derivative (III) in which this

* For the sake of simplicity all bond cleavages are written as homolytic fission.

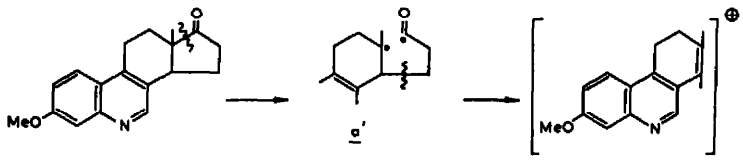
[5] C. Djerassi, J. M. Wilson, H. Budzikiewicz and J. W. Chamberlin, *J. Amer. Chem. Soc.* **84**, 4544 (1962).

[6] C. Djerassi, J. M. Wilson, H. Budzikiewicz and J. W. Chamberlin, *J. Amer. Chem. Soc.* **84**, 4549 (1962)

[7] Z. Pelah *et al. Tetrahedron* **19**, 2233 (1963).

[8] F. Meyers and A. G. Harrison, *Canad. J. Chem.* **42**, 2008 (1964).

peak is entirely shifted to *m/e* 226. A metastable ion at 181 in the spectrum of III further supports the implication of process *a'*, ($283^+ \rightarrow 226^+ + 57$; calc. 180·5).

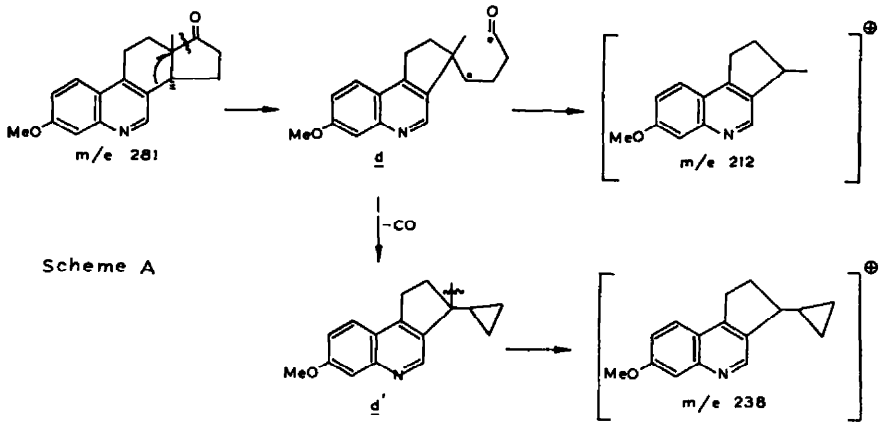


m/e 281 a (m/e 225)

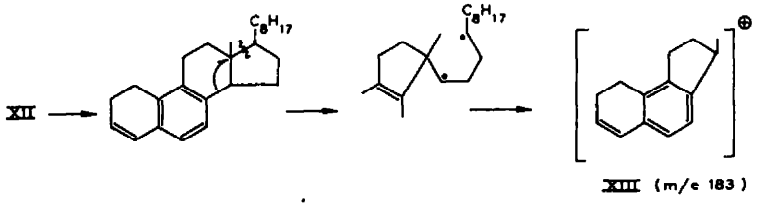
Peaks M-43 and M-69 (*m/e* 238 and *m/e* 212, Fig. 1). The overwhelming abundance of these two peaks in the 14α-isomer, indicates, that their formation is connected with stereochemically dependent processes. Information on the composition of ion *m/e* 238 comes from two sources. Firstly, deuterium labeling of IX has previously revealed that the fragment 237 retains C-16 and its associated hydrogen atoms;[6] the corresponding 238 ion, therefore, may be anticipated to possess a related structure. Secondly, it is observed that the peak *m/e* 238 moves to *m/e* 240 in the spectrum of 14,15-d_2-6-aza-equilenin methyl ether (III), implying the retention of the labeled hydrogens. On the other hand, the position of the ion 212 remains unaffected in the mass spectrum of the deuterated ketone (III) thereby indicating that the formation of the latter fragment involves either a loss, amongst others, of C_{14} and C_{15} with their attached hydrogens, or, what seems less likely, the operation of a complex rearrangement of the deuterium atoms prior to decomposition. In considering the possible mechanisms of fragmentation leading to ions 238 and 212 it must be borne in mind that such pathways should incorporate a step which would discriminate in favour of a *trans* stereochemistry for the C/D ring junction. A mechanism which provides a rationalization for the isotope labeling data and also permits the assignment of plausible structures to ions 238 and 212 is described in Scheme A. Moreover, if the two processes of C_8–C_{13} bond formation and ring D fission at C_{13}–C_{17} are visualized as concerted steps, then stereochemical consequences become implicit in Scheme A. An inspection of Dreiding molecular models indeed shows that such a synchronous rearrangement process would be more in keeping with the geometry of the *trans* C/D ring system than with its *cis* isomer. The breakdown of the rearranged species *d* (Scheme A), presumably via loss of a cyclobutanone radical or its mol. equiv., results in the formation of fragment 212 in which all the deuterium has been lost. A diffused peak at 159 (in the spectrum of III) supports the involvement of the fragmentation $283^+ \rightarrow 212^+ + 71$; calc. 158·8.

Expulsion of a carbon monoxide molecule from *d*, which is supported by a significant peak at *m/e* 253 in Fig. 1, may constitute a second mode of its decomposition. The resulting cyclopropane system *d'* can arise in either a concerted step, or via the intervention of a diradical species. A loss of the C_{18} methyl from *d'* gives rise to the formation of ion 238 in which the labeled hydrogens at C_{14} and C_{15} maintain their integrity. The conjugation of the C_{13} radical centre, with the cyclopropane ring and the aromatic nucleus, in ion 238, may provide the necessary driving force for the latter fragmentation. Breakdown of *d'* by loss of the cyclopropane ring may contribute in part to the population of the *m/e* 212 peak.

While examples of hydrogen transfers during molecular fragmentation in the mass



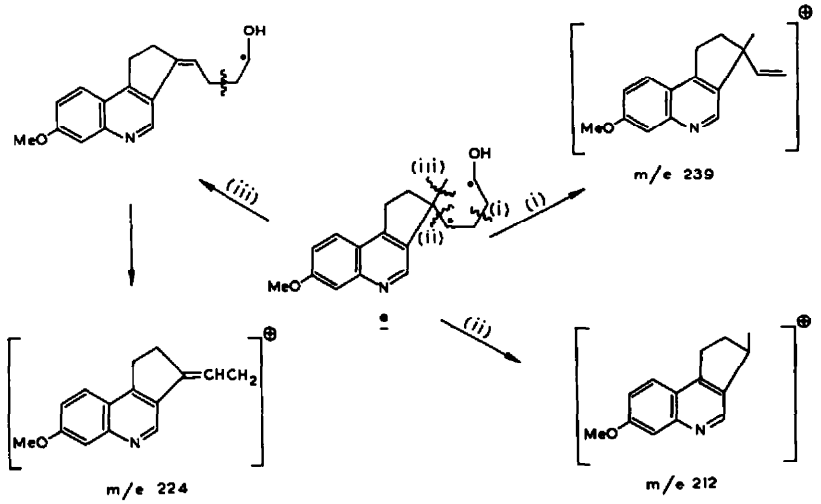
spectrometer are abundant in the literature,[9] the skeletal rearrangement suggested in Scheme A deserves some comment. The transformation in which the C_8–C_{14} bond is lost and a new bond between C_8–C_{13} is formed, corresponds to an overall $1 \rightarrow 2$ shift of an aromatic moiety. Such shifts are a well known phenomenon in carbonium ion intermediates[10] and are frequently observed in reactions involving radical species.[11] More recently, molecular rearrangements of aryl and alkyl substituents have also been suggested in the mass spectral studies of certain amides and alcohols,[12] and though no reference has been made in regard to the electronic nature of the latter reactions, their description, significantly, involves steps which proceed in a concerted manner. In connection with the scope of the rearrangement leading to ion *d* it is interesting to note that a related ring C contracted fragment has been suggested in the mass spectrum of the cardenolide digitoxigenin.[13] More pertinent to the present discussion, however, are the mass spectra of compounds IX, XI and 3β-acetoxy-19-norcholesta-5,7,9-triene (XII).[14] The origin of the fragments 211, 225 and 183 (XIII) from IX, XI and XII respectively, can be most conveniently explained on the basis of molecular rearrangement processes analogous to that described in Scheme A. It should be pointed out that all of the aforementioned compounds possess two structural features in common, namely, an aromatic ring B and a *trans* C/D configuration. These results strongly suggest the implication of a stereochemically controlled $1 \rightarrow 2$ aryl shift in ring B aromatic steroids.



[9] K. Biemann, *Mass Spectrometry* p. 107. McGraw-Hill, New York (1962).
[10] For example see: P. de Mayo, *Molecular Rearrangements* Part I. p. 1. Interscience, New York (1963).
[11] A. Fish, *Quart. Rev.* Vol. XVIII, p. 243 (1964).
[12] P. Funke, K. G. Das and A. K. Bose, *J. Amer. Chem. Soc.* **86**, 2527 (1964).
[13] M. v. Ardenne, R. T. Tümmler, E. K. Weiss and T. Reichstein, *Helv. Chim. Acta* **47**, 1038 (1964).
[14] A. v. d. Gen, "*Enige Substitutie- en Additiereacties in de Cholestaanreeks*" Thesis, University of Amsterdam (1964).

Mass spectra of methyl ethers of 6-aza-equilenin and 6-aza-14(β)-isoequilenin alcohols (II and VI). Directing our attention to the spectra of the aza-equilenin alcohols (Figs. 3 and 4) it is to be noted that both the compounds display strong molecular ion peaks as could be expected from partially aromatic systems. Stereochemical considerations again appear to play a significant role in characterizing the fragmentation patterns of the two isomers. Thus, whereas the C/D *cis* alcohol exhibits very pronounced peaks at *m/e* values 265, 250, 236, 224 and 210 (Fig. 4) the spectrum of the *trans* compound displays its characteristic ions at 239, 224 and 212.

The mass spectrum of alcohol II (Fig. 3) may be discussed in terms of the genesis and structures of ions 239, 224 and 212. Of these three, ions 239 and 224 move up by two mass units in the spectrum of the 14,15-d_2 alcohol IV while the peak at *m/e* 212 remains unaffected. A fragmentation pathway involving ring C contraction, in a manner similar to that described for the corresponding ketone, can lead to ion *e* which may serve as a precursor for the fragments 239, 224 and 212. The suggestion that fragment 224 is formed from ion *e* which itself arises through the intervention of a stereospecific process, implies, that though the same peak appears in the spectrum of alcohol VI its origin in the latter system must occur via a different pathway. The mode of formation of the peak at *m/e* 250 is not clear at present since a simple mechanism involving a loss of HOH and CH_3 units appears unlikely in view of the extremely small M-18 peak observed in the spectrum of alcohol II.



The spectrum of the C/D *cis* alcohol IV (Fig. 4) exhibits a strong M-18 peak corresponding to the loss of elements of water from the molecular ion. That this dehydration is an electronic and not a thermal process is shown from a study of the M-18 peak in the spectrum (of IV) run at varied electron energies and after heating a sample of the compound for different lengths of time. The results obtained are presented in Table 1. It may be observed that while the ratio M-18/M^+ remains almost unchanged with the time for which the alcohol is heated (compare runs 1 and 12, Table 1) it is, however, significantly dependent upon the energy of the bombarding electrons.

The elimination of water from cyclic and acyclic alcohols has been the subject of

considerable scrutiny recently. Labeling experiments argue strongly against the involvement of 1,2-elimination and in fact indicate that the hydrogen accompanying the hydroxyl group is derived from carbons at positions 3, 4 and 5 in relation to the

TABLE 1

Run*	Time (mts)	Voltage (ev)	$(M-18)^+/M^+$ (%)
1.	0	70	45·5
2.	12	60	45·0
3.	24	50	46·0
4.	36	40	4·5
5.	48	30	4·3
6.	60	70	45·0
7.	72	25	4·2
8.	84	22	4·1
9.	96	20	4·0
10.	108	18	3·7
11.	120	70	45·0
12.	180	70	44·5

* Inlet temp 185°.

latter function.[15–17] Further, a *cis* configuration of the eliminating groups has been regarded to facilitate the dehydration process.[18] The M-18 peak is therefore in complete accord with the expected behaviour of VI in which the C_{17}–OH and the C_{14}-tertiary hydrogen are in *cis* relationship to one another.

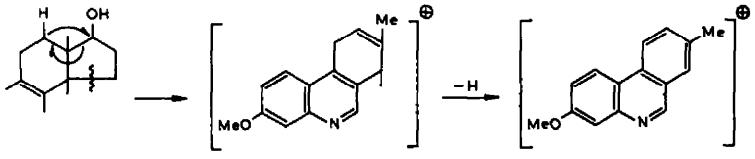
Although no data bearing upon the structure of fragment 250 is available at present, its origin from the *cis* alcohol (IV) can be best rationalized in terms of the loss of a methyl moiety and a water molecule. It is noteworthy in this connection that the *cis* alcohol, which readily loses water, also exhibits a correspondingly intense peak for ion 250. The *m/e* 224 ion (Fig. 4) corresponds to the familiar M-59 fragment observed in the mass spectrum of estradiol methyl ether and presumably arises via an analogous C_{13}–C_{17} cleavage of ring D. Consistent with the latter description of ion 224 a loss of hydrogen from the fragment can result in the formation of an aromatic ion *f* which is compatible with the prominent peak at *m/e* 223.

The peak at *m/e* 210 is present to a significant extent in the spectra of both pairs of ketones and alcohols (Figs. 1–4), and may represent a common genesis. In the deuterated systems, ketone III and alcohol IV, this peak is moved to *m/e* 211, thereby implying the retention of one of the C_{14} and C_{15} hydrogens. Owing to the lack of supporting evidence no structural assignment is suggested for ion 210, however, in view of the results of the labeling experiment it would appear that its formation may represent a loss of a C_4H_9O unit (M-73) incorporating the elements of cleavage of ring D and the C_{18} methyl group.

Stereochemical considerations. The possibility of distinguishing between stereochemical isomers via mass spectroscopy is of great interest in view of the potential

[15] H. Budzikiewicz, C. Djerassi and D. H. Williams, *Interpretation of Mass Spectra of Organic Compounds* p. 42. Holden-Day, San Francisco (1964).
[16] W. H. McFadden, D. R. Black and J. W. Corse, *J. Phys. Chem.* **67**, 1517 (1963).
[17] W. Benz and K. Biemann, *J. Amer. Chem. Soc.* **86**, 2375 (1964).
[18] C. G. MacDonald, J. S. Shannon and G. Sugowdz, *Tetrahedron Letters* No. 13, 807 (1963).

applicability of the method to minute quantities of materials. While the differences between the spectra of the C/D *trans* (I) and C/D *cis* (V) ketones are sufficiently characteristic in themselves, it is of significance that the prominent peaks at *m/e* 212 and *m/e* 238 in the spectrum of I can be related to fragments which have their origin in a stereospecific decomposition pathway. From the limited number of examples available at present it would appear that the molecular rearrangement invoked for the formation of ion 212 may be of general implication in aromatic ring B steroids with *trans* C/D fusion. The 14α-6-aza-equilenin alcohol (II) also shows the characteristic 212 ion which is absent in the spectrum of the corresponding 14β-isomer. The latter alcohol, on the other hand, is characterized by a strong M-18 peak in its spectrum which has been shown to arise from an electronic dehydration process. Characteristic M-18 ions have been similarly observed in the spectra of certain 17β-hydroxy-14β-D-homo-estrogens.[19] However, no evidence has been presented in the reported experiment[19] to distinguish between a thermal or an electronic loss of water.



EXPERIMENTAL

All m.ps. are uncorrected. IR spectra were taken on a Unicam S.P. 200 spectrometer by Mr. C. Kruk and associates. Mass spectral measurements were carried out on a A.E.I. MS2H spectrometer with the technical assistance of Messrs. W. J. Roozelaar, J. A. M. Spitteler and J. D. van Wageningen. Spectra of the deuterated derivatives III and IV were in addition taken on a Atlas CH_4 spectrometer. The ionizing potential employed in all measurements was 70 eV.

(±)-3-*Methoxy*-6-*aza*-17β-*hydroxy*-14,15-d_2-*estra*-1,3,5(10),6,8-*pentaene* (IV). A solution of VII (1·0 g) in dry tetrahydrofuran (20 ml) was reduced over 2 g of Pd–$CaCO_3$ (2%) with D_2 gas under atm. press. The catalyst was filtered and washed with tetrahydrofuran. Evaporation of the solvent left a residue which upon trituration with MeOH yielded 740 mg of crude VIII, mp 187–197°. The detosylation of VIII was achieved by refluxing 250 mg of the alcohol (VIII) with 2·0 g NaH (oil suspension, 50%) in 50 ml THF under an atm of N_2. After 18 hr, 50 ml of water was slowly added and the mixture diluted with ether and acidified with HCl to pH = 1. The organic layer was separated and extracted a second time with HCl. The combined aqueous-acidic layers were made basic with KOH (10%) following which the alcohol was extracted with ether. Drying of the ether solution and evaporation of the solvent gave 153 mg (95%) of crude IV. Crystallization from MeOH gave the pure alcohol, 95 mg, mp 191–193·5°, $\gamma_{cm^{-1}}^{KBr}$ 3370 (O—H), 2195 (C—D). Mass spectrum of the compound exhibited the molecular ion peak at *m/e* 285.

(±)-3-*Methoxy*-6-*aza*-14,15-d_2-*estra*-1,3,5(10),6,8-*pentaene*-17-*one* (III). A mixture of IV (379 mg), aluminium isopropoxide (2·3 g) and cyclohexanone (3·3 g) in 30 ml of xylene was refluxed with stirring for 4 hr. After cooling, 110 ml of ether–THF (2:1) was added, followed by 100 ml of KOH (7%). The organic layer was separated and extracted twice with dil. HCl (40 ml-10%, 40 ml-3%). The combined acidic extracts were made basic with 50 ml of KOH (20%) and extracted with ether (2 × 70 ml). The ether solution was dried over $MgSO_4$ and the solvent evaporated to leave an oily residue which was chromatographed over a florisil column (27 g of florisil, 60/100 mesh). Elution with cyclohexane–ethyl acetate (4:1) yielded the following products in order of their appearance from the column. (i) 6-aza-14,15-d_2-estrone methyl ether, 31 mg (8%), $\gamma_{cm^{-1}}^{KBr}$ 3390 (N—H), 1720 (C=O)

[19] S. N. Ananchenko, V. N. Leonov, V. I. Zarekskii, N. S. Wulfson and I. V. Torgov, *Tetrahedron* **20**, 1279 (1964).

and 2190 (C—D). (ii) 66 mg (17·5%) of 6-aza-14,15-d_2-equilenin methyl ether (III), mp 180°, $\gamma_{cm^{-1}}^{KBr}$ 1732 (C=O), 2200 (C—D), absence of an OH or NH band. The mass spectrum of the ketone exhibited the molecular ion peak at *m/e* 283. (iii) Small amounts of the starting alcohol.

Acknowledgements—We are grateful to Mr. H. de Koning for valuable assistance in the preparation of the labeled compounds III and IV. We also wish to acknowledge our indebtedness to Drs. H. J. Hofman of the Spectroscopic Division of this Laboratory for helpful discussions and to Dr. R. J. C. Kleipool of the Central Institute for Nutrition and Food Research T.N.O., Utrecht, for the spectra on the Atlas CH_4 Mass Spectrometer.