sion. The resulting blue mixture was stirred 4 hr. at room temperature and worked up as described for the metalations with *n*-butylsodium. Recrystallization of the crude product from hexane gave 20.0 g. (63%) of V, m.p. 105–107°. The solvent was evaporated from the filtrate and the residue distilled to give 2.8 g. (21%) of recovered amine I and 2.6 g. of viscous residue. The infrared spectrum of the latter was similar to that of V.

The other runs listed in Table III were effected similarly, allowing the metalations to proceed under the indicated conditions before adding the benzophenone. No evidences of the presence of *o*-isomer III could be found in any of the experiments.

Metalation with Phenylpotassium.—To a suspension of phenylpotassium prepared from 15.64 g. (0.40 mole) of potassium sand and 21.6 g. (0.20 mole) of anisole in 165 ml. of heptane[15] was added 13.5 g. (0.10 mole) of amine I in 30 ml. of heptane. The light-red suspension was stirred 18 hr. at room temperature, and a solution of 45.5 g. (0.25 mole) of benzophenone in 125 ml. of benzene was added. After stirring for 4 hr., the reaction mixture was decomposed with 40 ml. of *t*-butyl alcohol and then worked up in the usual manner. Recrystallization of the crude amine fraction from hexane gave 23.0 g. (73%) of amino alcohol V, m.p. 106–107°, and distillation of the filtrate afforded 1.9 g. (14%) of recovered amine I.

Metalation with Phenyllithium.—To a solution of phenyllithium prepared from 3.43 g. (0.49 mole) of lithium wire and 36.9 g. (0.235 mole) of bromobenzene in 150 ml. of ether[16] was added 13.5 g. (0.10 mole) of amine I in 60 ml. of ether. The brown solution was stirred for 18 hr. at room temperature, and a solution of 53.5 g. (0.29 mole) of benzophenone in 100 ml. of ether was added. The resulting purple suspension was stirred for 4 hr., then decomposed with methanol and worked up in the usual manner. Removal of solvent from the amine fraction followed by cooling gave 6.7 g. (21%) of amino alcohol III, m.p. 152–153°. The mixture m.p. with an authentic sample of III[10] was 152–153.5°. Distillation of the filtrate gave 9.6 g. (71%) of recovered amine I.

(15) A. A. Morton and E. J. Lanpher, *J. Org. Chem.*, **23**, 1638 (1958).

(16) R. G. Jones and H. Gilman, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 339.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY, STANFORD, CALIF.]

Mass Spectrometry in Structural and Stereochemical Problems. XXVII.[1] Mass Spectral Fragmentation Processes and Hydrogen Transfer Reactions of Amides and Amines[2]

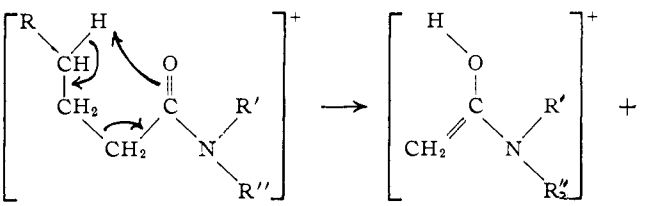
BY Z. PELAH,[3a] M. A. KIELCZEWSKI,[3b] J. M. WILSON, M. OHASHI, H. BUDZIKIEWICZ AND CARL DJERASSI

RECEIVED MARCH 1, 1963

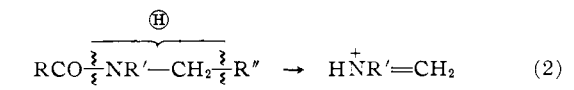
Numerous deuterated analogs of aliphatic, cycloalkyl and steroidal N-acetylamines have been synthesized and their mass spectra compared with those of the non-labeled parent substances. Such differential labeling has been shown to be of mechanistic significance, clarifying many hydrogen transfer reactions, as well as of analytical consequence by uncovering the composite nature of several mass spectral peaks. The empirical composition of these fragments was such that even high-resolution mass spectroscopy could not have achieved these results. Mechanistic generalizations are feasible and lead to the conclusion that fission of the carbon–carbon bond next to nitrogen is one of the key operations. In amides, if the acyl group can be lost by ketene elimination, such processes occur in order to give rise to the more stable amine ion. In cycloalkylamines and their amides, a single carbon–carbon bond fission cannot fragment the molecule, and subsequent rearrangement results in the formation of a stable, even-electron ion. In amides, this process is accompanied by ketene expulsion to provide the more favored amine ion. Attention is called to a number of competing fragmentation processes and to the differences encountered with the relatively stable nuclear system of the steroids, where rupture of the bond between the ring and the nitrogen takes precedence over ring fission.

The ultimate aim of this series of investigations is to reach that level of sophistication which will permit the prediction (and hence interpretation) of the principal modes of mass spectral fragmentation of fairly complicated organic molecules. For this purpose, it is necessary to study in detail the behavior, upon electron impact, of many common functional groups, often superimposed upon certain fundamental ring systems.[4] In the course of examining the desulfurization of thiazolidines,[5] a number of steroidal amides were obtained and their mass spectra measured. Examination of these spectra prompted us to undertake a detailed study —utilizing deuterated substrates for labeling purposes— of the mass spectral fragmentation behavior of secondary and tertiary amides as well as of certain precursor amines. The experimental results and conclusions constitute the subject matter of the present paper.

Aliphatic Secondary and Tertiary Amides.—The mass spectra of a wide variety of aliphatic amides have been discussed by Gilpin,[6] who found that two processes predominated.



RCH=CH$_2$ (1)



(2)

Process 1 occurs when the acyl group contains a hydrogen atom γ to the carbonyl group; it must be the same process which operates in the spectra of various carbonyl compounds[7] and which has been confirmed by labeling experiments in certain esters.[8] Process 2 is less well defined but appears to be analogous to the behavior of amines[9] and ethers.[10] It has not been possible, with the evidence so far published, to determine the origin of the hydrogen atom which is transferred to the ionized fragment.

(1) Paper XXVI: C. Djerassi, H. Budzikiewicz, R. J. Owellen, J. M. Wilson, W. G. Kump, D. J. Le Count, A. R. Battersby and H. Schmid, *Helv. Chim. Acta*, **46**, 742 (1963).

(2) Supported by grants No. A-4257 and CRTY-5061 from the National Institutes of Health, U. S. Public Health Service.

(3) (a) On leave from the Israel Institute for Biological Research, Ness-Ziona, Israel; (b) Rockefeller Fellow, 1961–1962, on leave from the University of Poznan, Poland.

(4) For examples of our approach toward studying the mass spectral behavior of the carbonyl group in bicyclic (decalin) or tetracyclic (steroid) systems, see E. Lund, H. Budzikiewicz, J. M. Wilson and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 1528 (1963), and D. H. Williams, J. M. Wilson, H. Budzikiewicz and C. Djerassi, *ibid.*, **85**, 2091 (1963), and references cited therein.

(5) C. Djerassi, N. Crossley and M. A. Kielczewski, *J. Org. Chem.*, **27**, 1112 (1962).

(6) J. A. Gilpin, *Anal. Chem.*, **31**, 935 (1959); see also F. W. McLafferty, *ibid.*, **28**, 306 (1956).

(7) F. W. McLafferty, *ibid.*, **31**, 82 (1959).

(8) Ng. Dinh-Nguyen, R. Ryhage, S. Ställberg-Stenhagen and E. Stenhagen, *Arkiv Kemi*, **18**, 393 (1961).

(9) R. S. Gohlke and F. W. McLafferty, *Anal. Chem.*, **34**, 1281 (1962).

(10) F. W. McLafferty, *ibid.*, **29**, 1782 (1957).

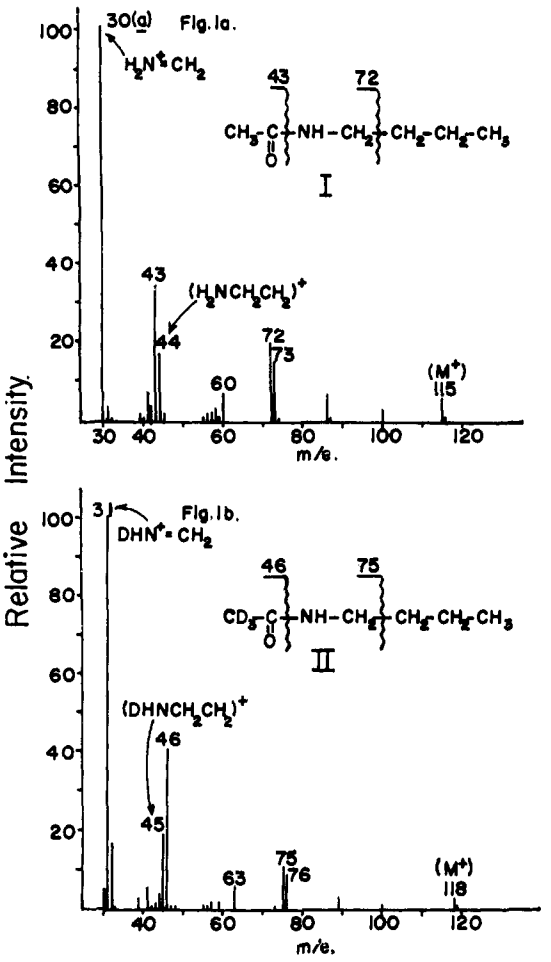


Fig. 1.—(a) Mass spectrum of *n*-butylacetamide (I); (b) mass spectrum of *n*-butyl-d_3-acetamide (II).

(a) **N-Butylacetamide** (Fig. 1).—In order to clarify the nature of this and other processes, we have prepared deuterated analogs of some simple secondary and tertiary amides and examined their mass spectra. In Fig. 1 are shown the mass spectra of *n*-butylacetamide (I) and *n*-butyl-d_3-acetamide (II). The base peak in the spectrum (Fig. 1a) of I appears at *m/e* 30 and can be ascribed to process 2. This peak shifts almost completely to *m/e* 31 in the spectrum (Fig. 1b) of II, showing that the hydrogen atom originates from the acetyl group. The following two processes can now be suggested for the genesis of this ion

$$\left[\begin{array}{l}\mathrm{CO{-}NHCH_2R}\\ \mathrm{CH_2{-}H}\end{array}\right]^+ \rightarrow [\mathrm{H_2NCH_2R}]^+ + \mathrm{CH_2{=}CO} \rightarrow$$

$$\mathrm{H_2\overset{+}{N}{=}CH_2} + \cdot\mathrm{R}$$
a, *m/e* 30

$$[\mathrm{CH_3CONHCH_2}\tfrac{\xi}{\xi}\mathrm{R}]^+ \rightarrow \left[\begin{array}{l}\mathrm{CO{-}\overset{+}{N}H{=}CH_2}\\ \mathrm{CH_2{-}H}\end{array}\right] + \mathrm{R}\cdot \rightarrow$$

$$\mathrm{H_2\overset{+}{N}{=}CH_2} + \mathrm{CH_2{=}CO}$$

Loss of ketene in this manner has been observed in the formation of some peaks in the mass spectra of N-acetyldihydroindole alkaloids.[11] The ion a which is produced has the same structure as the ion of *m/e* 30, which forms the base peak in all primary amine mass spectra.[9]

The peak at *m/e* 44 (Fig. 1a) appears to be formed by a similar process to that shown above. It moves to *m/e* 45 in the spectrum (Fig. 1b) of II, and so must involve the loss of ketene.[12] Its formation can be visualized as

$$[\mathrm{CH_3CONHCH_2CH_2CH_2CH_3}]^+ \rightarrow$$
$$\mathrm{CH_2{=}CO} + [\mathrm{H_2NCH_2CH_2}\tfrac{\xi}{\xi}\mathrm{CH_2CH_3}]^+ \rightarrow$$
$$[\mathrm{H_2NCH_2CH_2}]^+ \rightarrow \mathrm{H_2\overset{+}{N}{=}CHCH_3}$$
b, *m/e* 44

The ion formed (b) will not be as stable as a and this difference in stability is reflected in the difference in abundance which is found (Fig. 1).

The peak at *m/e* 43 moves to *m/e* 46 in the spectrum (Fig. 1b) of the deuterated analog. It is, therefore, the

(11) For a general discussion of ketene loss, see K. Biemann, "Mass Spectrometry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp. 112–113; and H. Budzikiewicz, J. M. Wilson, C. Djerassi, J. Levy, J. LeMen and M.-M. Janot, *Tetrahedron*, in press, and references cited therein.

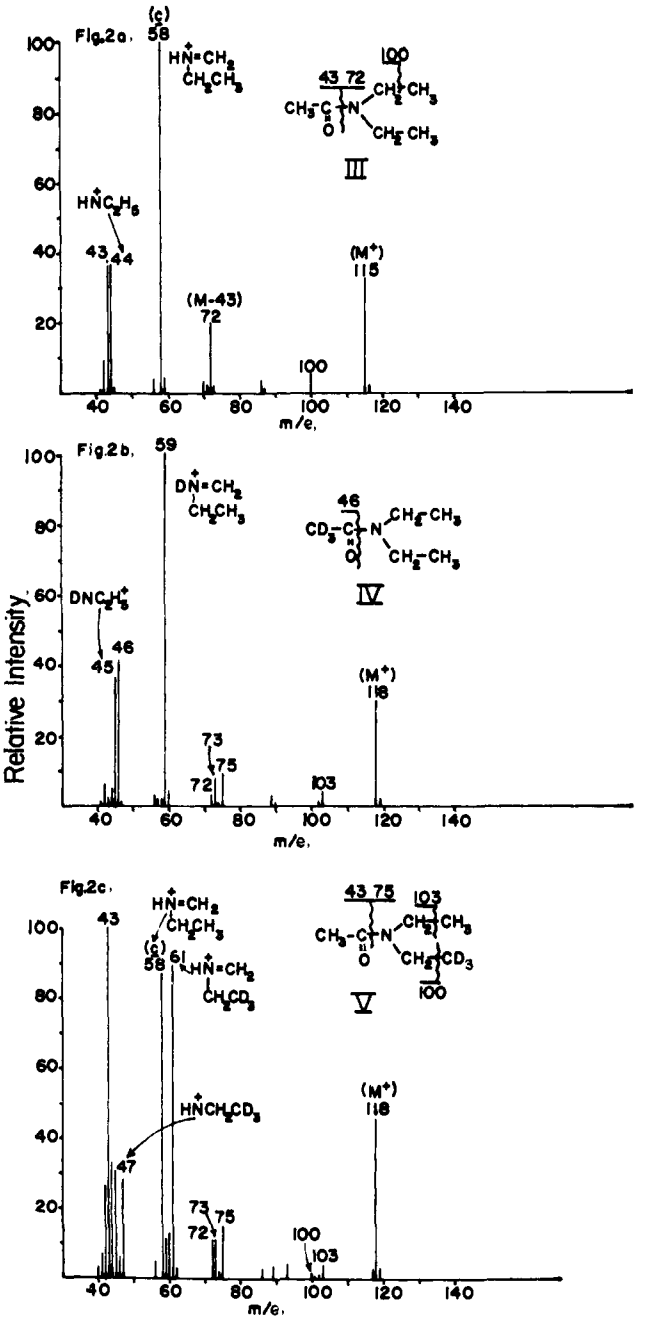
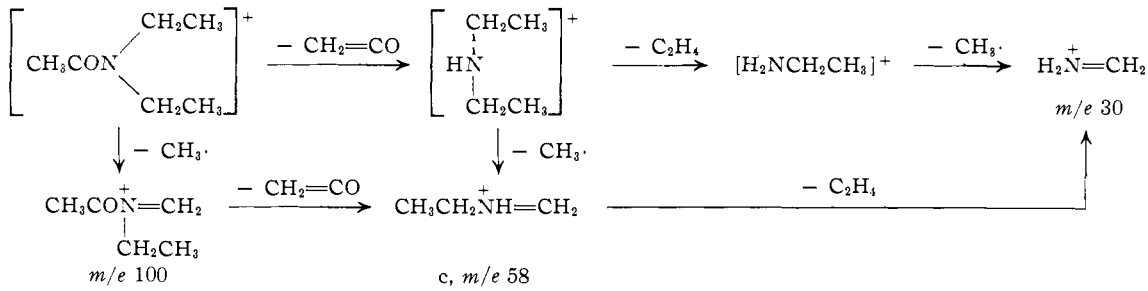


Fig. 2.—(a) Mass spectrum of N,N-diethylacetamide (III); (b) mass spectrum of N,N-diethyl-d_3-acetamide (IV); (c) mass spectrum of N-ethyl-N-β,β,β-d_3-ethylacetamide (V).

(12) In the spectrum of this compound (and of several others in this series), where there are neighboring peaks in the spectrum of the undeuterated compound, both of significant intensity, the shifts in the peaks of their polydeuterated analogs cannot be assigned unambiguously by sole consideration of arithmetic. However, in most cases we find that there is only one interpretation possible in terms of the behavior of analogous compounds.



$CH_3C{\equiv}O^+$ ion. The peaks at *m/e* 72 and 73 (M-43 and M-42) upon deuteration move largely to *m/e* 75 and 76. Consequently, they cannot involve loss of the acetyl function and must be ascribed to fission (see Fig. 1) of the alkyl group β to the nitrogen atom with and without hydrogen rearrangement. The ion of *m/e* 60 is probably a protonated acetamide (see shift to *m/e* 63 in Fig. 1b); its formation will be discussed in greater detail in the section dealing with amides of steroidal and other amines.

(b) **N,N-Diethylacetamide** (Fig. 2).—In the spectra of tertiary amides, Gilpin[6] reports that the predominant processes are those operative in the secondary amide spectra, especially process 2. In order to substantiate this postulate, we have examined the mass spectra of N,N-diethylacetamide (III) and of two deuterated analogs, N,N-diethyl-d_3-acetamide (IV) and N-ethyl-N-β,β,β-trideuterioethylacetamide (V). As found before,[6] the base peak in the spectrum (Fig. 2a) of III occurs at *m/e* 58. It shifts to *m/e* 59 in IV (Fig. 2b) and is split almost equally between *m/e* 58 and *m/e* 61 in V (Fig. 2c). This is indicative of the following processes which were already noted in the secondary amide spectra.

The two processes shown for the formation of c both operate as demonstrated by the presence of metastable ions at 33.8 ($100^+ \rightarrow 58^+ + 42$; calcd. 33.6) and 46.3 ($73^+ \rightarrow 58^+ + 15$; calcd. 46.1). The relation of *m/e* 30 to *m/e* 58 is borne out by the shifts observed upon deuterium labeling. It moves to *m/e* 31 in IV (Fig. 2b) and is split between *m/e* 30 and 31 in V (Fig. 2c).

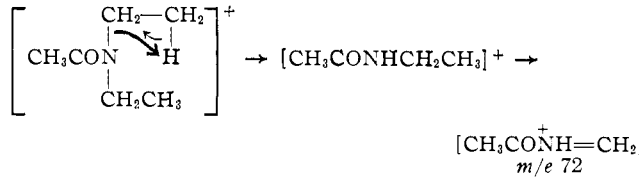
The peak at *m/e* 43 appears to consist only of the acetyl ion, as was found with I, but the peak at *m/e* 44 is more complex. It is shifted mostly to *m/e* 45 in IV (Fig. 2b), which implies that the acetyl group is lost as ketene and that the intermediate $[HN(C_2H_5)_2]^+$ may be invoked. Loss of ethyl from this ion will produce $[HNC_2H_5]^+$ which should result in a split between *m/e* 44 and 47 in the spectrum (Fig. 2c) of V. This is found, but accounts for only 70% of the peak, the remainder being at *m/e* 45. The most reasonable explanation for the formation of this peak would be

$$[CH_3CON(C_2H_5)_2]^+ \rightarrow [N(C_2H_5)_2]^+ \rightarrow [HNC_2H_5]^+ + C_2H_4$$

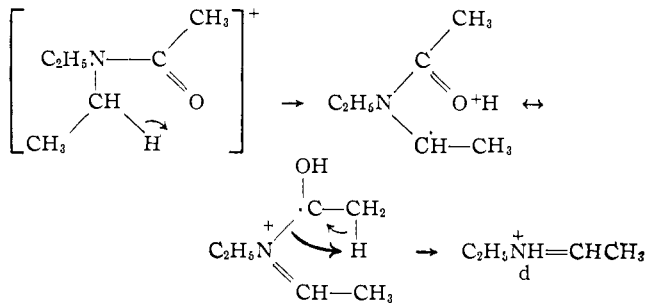
The main objection to this suggestion is that if this process is responsible for about 30% of the *m/e* 44 peak in the spectrum of III, then 30% of this peak should remain at *m/e* 44 in the spectrum (Fig. 2b) of the trideuterioacetyl derivative IV, while in fact no more than 10% of the *m/e* 44 peak remains unmoved. The discrepancy can be rationalized if the first step, loss of the acetyl group, takes place with a double rearrangement of hydrogen atoms. As will be seen in the discussions of other amide spectra, this is not an uncommon process. The formation of the *m/e* 45 peak in the spectrum of IV must then be partly due to the process

$$\underset{\text{IV}}{[CD_3CON(C_2H_5)_2]^+} \xrightarrow{-CHD_2CO\cdot} [ND(C_2H_4)C_2H_5]^+ \xrightarrow{-C_2H_4} \underset{m/e\ 45}{[NDC_2H_5]^+}$$

The peak (Fig. 2a) at *m/e* 72 (M-43) is split (Fig. 2b and 2c) into three peaks in both of the deuterated species. The largest part of it shifts to *m/e* 75 in the spectrum (Fig. 2b) of IV. The trideuterioacetyl group must, therefore, be retained in this ion. The loss of 43 mass units can also involve the other part of the molecule as the elements of $(C_2H_4 + CH_3)$ in the manner



This mechanism is confirmed by the presence of peaks *m/e* 72 and 73 in the spectrum (Fig. 2c) of the d_3-ethyl analog V. The trideuterioacetyl derivative IV also exhibits (Fig. 2c) peaks at *m/e* 72 and 73, which account for most of the remainder of the M-43 peak in the undeuterated compound. The existence of a substantial peak at *m/e* 75 in V requires that loss of the acetyl group must also have occurred. Since in the trideuterioacetyl species IV, the M-45 peak (*m/e* 73) is more intense than the M-46 peak (*m/e* 72), this cleavage must take place predominantly with a double rearrangement of hydrogen atoms. Simple fission will result in the formation of an ion with a divalent nitrogen atom, so hydrogen rearrangement may be more probable because it can give rise to a more stable species such as d.

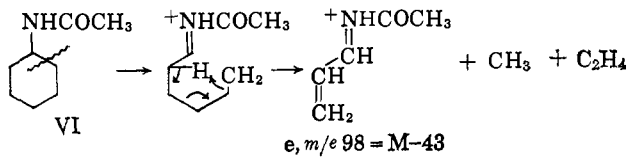


It is impossible to make an accurate determination of the origin of the hydrogen which is gained by the neutral fragment because of the composite nature of the peak, especially since the presence of $C_2H_5NHCO^+$ should also be taken into consideration. It would require several double labeling experiments to settle this subtle point.

Secondary Amides of Cycloalkylamines. (a) **N-Acetylcyclohexylamine** (Fig. 3 and Table I).—In the mass spectrum (Fig. 3) of N-acetylcyclohexylamine, aside from the molecular ion, there are found substantial peaks at *m/e* 43, 56, 60 and 98. The shifts of these peaks are shown in Table I as percentages where possible.[12] The M-43 peak (*m/e* 98) again appears to represent a mixture, but it becomes apparent from the shifts in the spectrum (see Table I) of the trideuterioacetyl derivative VII that the major constituent of this peak contains the acetyl group intact.[12a] The hydrogen

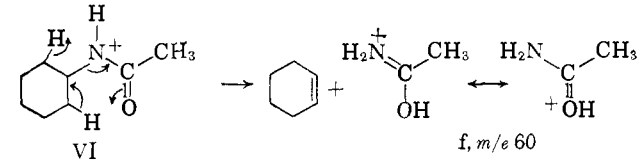
(12a) The same result is also inferred from the high-resolution mass spectrum of VI which was measured using an A.E.I. MS-9 double focusing mass

atom at C-1 is retained (see VIII, Table I) and so is one of the hydrogens at C-2 (see X, Table I). Since the fragment (or fragments) corresponding to 43 mass units, which is lost, must come from the ring, it must be C_3H_7 and can be lost in the following manner to give the ion e.



If this is correct, then e should be split in the spectrum of the 3-deuterio analog IX, as there exist two possible ways in which C_3H_7 can be lost and these become non-equivalent in IX. Although the other constituent of the peak interferes, it can be seen (Table I) that IX does indeed exhibit peaks at *m/e* 98 and 99, and that the former, the less intense of the two, is of about half the intensity of the *m/e* 101 peak shown by VII. The other constituent of the peak at *m/e* 98 in Fig. 3 becomes M-45 and M-46 in the spectrum (Table I) of the trideuterioacetyl analog VII and again must be due to the loss of the acetyl group with and without double hydrogen rearrangement. As was found with N,N diethylacetamide (III) the rearranged product predominates. However, the mechanism suggested in that case (*vide supra*) can only be partly operative since the hydrogen atom at C-1 is only lost to an extent of 5% of the total peak (see VIII, Table I) (*i.e.*, 25% of the hydrogen is transferred from C-1 since the double rearrangement process constitutes 20% of the M-43 fragment).

The peak at *m/e* 60 in Fig. 3 is more easily explained. It moves completely to *m/e* 63 in the spectrum of VII (Table I) and so must contain the acetyl group. It shifts largely to *m/e* 61 and 62 in the d_4-derivative X, but does not appear to contain C-1 as judged from the retention of this peak at *m/e* 60 in the 1-*d*-labeled derivative VIII. It must, therefore, be due to a double rearrangement which produces the ion $CH_3\overset{+}{C}ONH_3$ or $CH_3C(OH)\overset{+}{N}H_2$, which would be analogous to the $RCO_2H_2^+$ ion found in the mass spectra of esters.[13] The hydrogen atoms involved in the transfers have the origins 3% C-1, 58% C-2, 28% C-3. The remainder, about 11%, must have come from C-4.[14] McLafferty and Hamming[15] found that in a similar rearrangement in the mass spectrum of *sec*-butyl acetate, most of the hydrogen atoms involved in the transfer came from carbon atoms γ to the carbonyl group. The ion formed will be the stabilized fragment f arising from a process such as

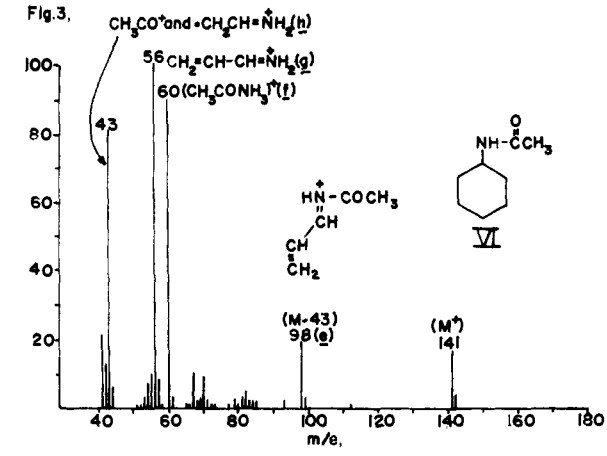


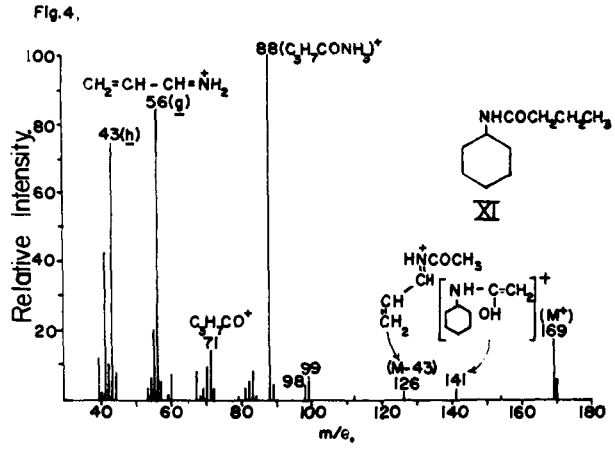
spectrometer. This spectrum exhibited a doublet at *m/e* 98.1282 (calcd. for $C_6H_{12}N$, 98.1284) and *m/e* 98.0917 (calcd. for C_5H_8NO, 98.0919) with relative intensities $C_5H_8NO:C_6H_{12}N$ = 3:1. We thank Dr. H. J. M. Fitches of A.E.I. Ltd., Manchester, England, for this and other high-resolution measurements reported in this article.

(13) A. G. Sharkey, Jr., J. L. Schultz and R. A. Friedel, *Anal. Chem.*, **31**, 87 (1959).

(14) The given values were calculated without accounting for the isotopic impurity (see Table I) present in 3-*d*-N-acetylcyclohexylamine (IX). Judging from its mode of synthesis (see Experimental), the extra deuterium atom is almost certainly at C-4 and so will make a contribution of only about 2% to the figure given for C-3. However, no correction was made for possible isotope effects.

(15) F. W. McLafferty and M. C. Hamming, *Chem. Ind.* (London), 1366 (1958).





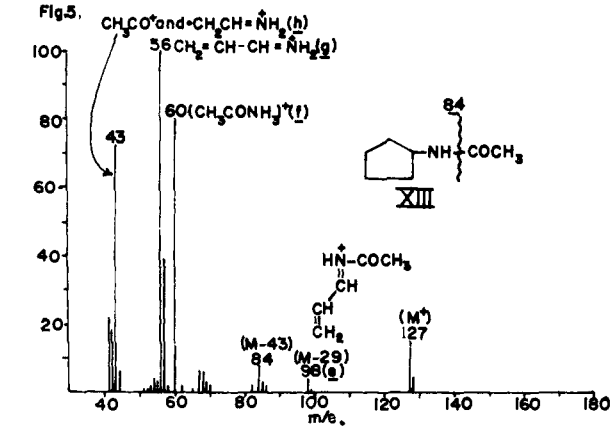


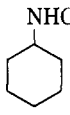
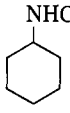
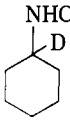
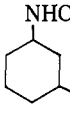
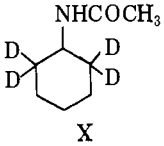
Fig. 3.—Mass spectrum of N-acetylcyclohexylamine (VI).
Fig. 4.—Mass spectrum of N-*n*-butyrylcyclohexylamine (XI).
Fig. 5.—Mass spectrum of N-acetylcyclopentylamine (XIII).

The base peak in Fig. 3 is located at *m/e* 56 and was shown by high-resolution mass spectrometry to consist of a single species $C_3H_6N_3^+$. It shifts to *m/e* 57 in the spectrum of the trideuterioacetyl analog VII and otherwise shows the same shifts (Table I) as the M-43 fragment e. It can, therefore, be visualized as being formed by loss of ketene from e (*m/e* 98) or initial loss of ketene (VI → *m/e* 99) can be invoked, giving the ion g.

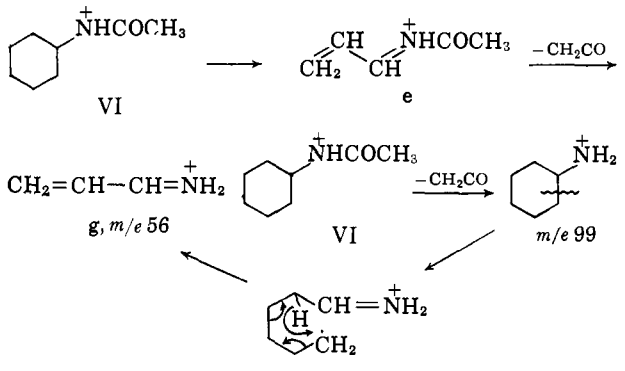
The peak (Fig. 3) at *m/e* 43 is made up of two species. As expected, the acetyl ion contributes, being present at *m/e* 46 in VII and at *m/e* 43 in all the other deuterated analogs. It accounts for about 60% of the intensity of the peak at *m/e* 43 in Fig. 3. The ion which constitutes the remainder of this peak retains the hydrogen atom at C-1 (VIII, Table I) and two hydrogen atoms at C-2 (X, Table I), but does not contain those at C-3 (IX, Table I) to a significant extent. In the spectrum of the

TABLE I[a]

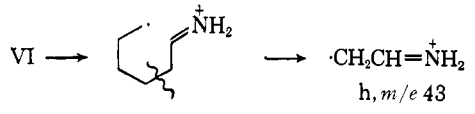
PRINCIPAL MASS SPECTRAL PEAKS OF N-ACETYLCYCLOHEXYLAMINE AND DEUTERATED ANALOGS

Compound	M^+	M-43	*m/e* 60	*m/e* 56	*m/e* 43
 VI	141	98	60	56	43
 VII	143(2%) 144(98%)	98(5%) 99(20%) 100(0–11%) 101(64–75%)	63	57	Mainly 44 and 46
 VIII	141(2%) 142(98%)	98(5%) 99(95%)	60(93%) 61(7%)	57	43(60%) 44(40%)
 IX	141(12%) 142(75%) 143(13%)	98(36%) 99(59%) 100(5%)	60(86%) 61(12%) 62(2%)	56(49%) 57(51%)	43(98%) 44(2%)
 X	143(1%) 144(13%) 145(86%)	99 (Over 50%)	60(15%) 61(54%) 62(31%)	57 (Over 90%)	43 (>50%) 45(~38%)

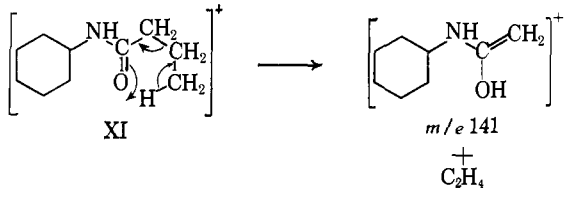
[a] Tables I–V show the shifts of the principal peaks of the compounds discussed when specifically labeled with deuterium. In cases where there are peaks of significant intensity adjacent to the peak in question, it is sometimes impossible to determine quantitatively the extent of the shift in the spectra of polydeuterated analogs. In most examples of this type, we have given whatever qualitative information could be derived; but in a few cases where the adjacent peaks were very intense and the compound was of low isotopic purity, we have found it impossible to make any assignment, so a few blank spaces will be found in the tables.



trideuterioacetyl compound VII, it keeps one of the deuterium atoms. It follows that structure h can be attributed to this ion, which can be formed from the same intermediate invoked in the genesis of g.



(b) **N-*n*-Butyrylcyclohexylamine** (Fig. 4).—The mass spectrum (Fig. 4) of N-*n*-butyrylcyclohexylamine (XI) can be interpreted easily in terms of the information we have obtained from the study of the acetyl derivatives VI–X. The ions which do not contain the acyl group appear at the same mass numbers in the acetyl and butyryl compounds, namely *m/e* 43 and 56 corresponding to h and g, the formation of the latter ion being clarified by a metastable peak at *m/e* 31.9 (calcd. 31.7 for *m/e* 99 → 56). There may also be a contribution to *m/e* 43 from $CH_3CH_2CH_2^+$. The *n*-butyryl ion $C_3H_7C{\equiv}O^+$ appears at *m/e* 71 and the protonated *n*-butyramide, analogous to f, at *m/e* 88. The fragment at *m/e* 126 corresponds to the loss of 43 mass units which can take place in a manner similar to the formation of e. The only peak of significant intensity which has no counterpart in the mass spectrum of the acetyl analog is the M-28 peak at *m/e* 141. It can be readily explained by invoking process 1 which Gilpin[6] finds to be operative whenever there is a γ-hydrogen atom in the acyl chain. Since the *m/e* 141 ion corresponds to the molecular ion of N-acetylcyclohexylamine (VI), it is not surprising that the butyryl homolog XI exhibits (Fig. 4) an *m/e* 98 peak, typical (M-43 = e in Fig. 3) of the N-acetyl series. An alternative possibility would be the direct loss of C_3H_7CO.

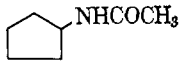
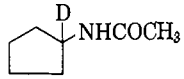
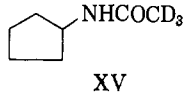
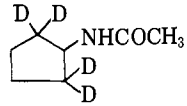


(c) **N-Acetylcyclopentylamine** (Fig. 5 and Table II).—In the mass spectrum (Fig. 5) of N-acetylcyclopentylamine (XIII), there are obvious resemblances to the spectrum of the cyclohexyl analog VI, especially the presence of the three most intense peaks at *m/e* 43, 60 and 56. The peak at *m/e* 60 must be due to the protonated acetamide f, its structure and the origin[16] (3% C-1, 72% C-2, 25% C-3) of the two transferred hydrogen atoms being established clearly by the spectra (Table II) of the deuterated analogs XIV–XVI.

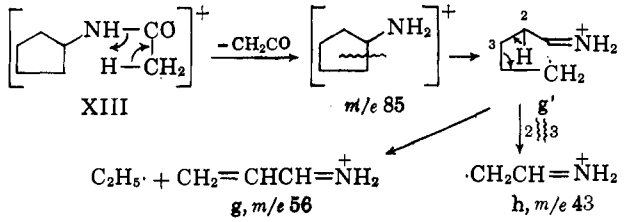
(16) These values have been corrected to allow for the incomplete deuteration; zero isotope effect has been assumed.

TABLE II

PRINCIPAL MASS SPECTRAL PEAKS OF N-ACETYLCYCLOPENTYLAMINE AND DEUTERATED ANALOGS

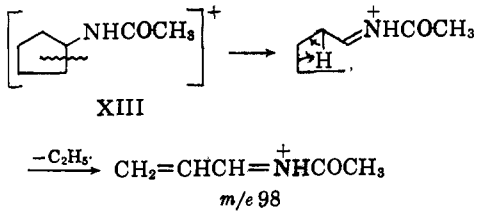
Compound	M^+	M-29	M-43	*m/e* 60	*m/e* 56	*m/e* 43
 XIII	127	98	84	60	56	43
 XIV	127(2%) 128(98%)	98(4%) 99(96%)	84(17%) 85(83%)	60(95%) 61(5%)	56(3%) 57(97%)	43(81%) 44(19%)
 XV	129(6%) 130(94%)	101	84(43%) 85(57%)	63	57	46(84%)
 XVI	129(6%) 130(29%) 131(65%)	98(7%) 99(57–68%)	Mostly 88 87(~20%)	60(16%) 61(57%) 62(27%)	56(10%) 57(80%) 58(10%)	43(60–90%) 44(?) 45(10–20%)

The peak shifts summarized in Table II require that the ion of *m/e* 56 contain C-1, C-2 (with one of the attached hydrogen atoms) and one hydrogen atom from the acetyl group. Its structure, therefore, can be represented also by g, which is formed as follows, the intermediacy of the *m/e* 85 species being established by the presence of a metastable peak at *m/e* 37.2 (calcd. 36.9 for *m/e* 85 → *m/e* 56). The empirical formula, $C_3H_6N^+$, of this ion was established by the mass measurement (found, 56.0686; calcd., 56.0680) of this peak in the high-resolution spectrum.



The ion h at *m/e* 43 can be produced by fission of the 2,3-bond in g′, the neutral product being cyclopropane or propene rather than the trimethylene biradical. As is the case with the cyclohexyl homolog VI (Fig. 3), the major constituent of the peak at *m/e* 43 is the acetyl ion.

In contrast to the behavior of the cyclohexyl series, the M-43 peak (*m/e* 84) in the mass spectrum (Fig. 5) of N-acetylcyclopentylamine (XIII) consists entirely of



ions formed by loss of the acetyl group. It is possible, therefore, to derive the origin of some of the hydrogen atoms back-transferred to the acetyl group in the double rearrangement by an examination of the peak shifts (Table II) incident to deuteration: C-1 contributes 30% and C-2 between 40 and 45%, these figures being obtained by considering that only about one-half of these peaks owes its genesis to such a process. There may be some degree of randomization in this reaction.

The loss of 29 mass units to give the ion of *m/e* 98 appears to be analogous to the formation of the ion e from VI. It involves the loss of an ethyl radical from the ring as shown (Table II) by the mass spectrum of the 2,2,5,5-d_4 analog XVI.

N-Ethylcycloalkylamines.—The availability of certain amines in the preparation of their N-acetyl amides, notably those labeled with deuterium, permitted a rationalization of their major fragmentation processes. Since in a number of respects the mass spectral behavior of these amines bears a distinct mechanistic resemblance to that of the corresponding amides, a discussion of these features seems appropriate at this stage.

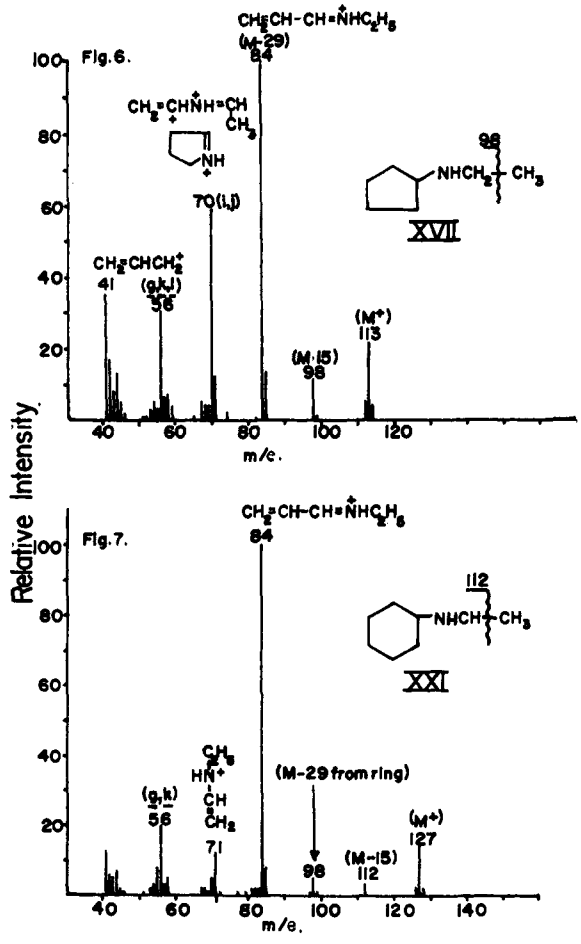
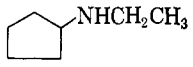
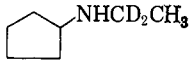
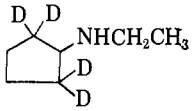
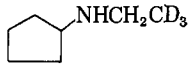
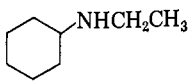
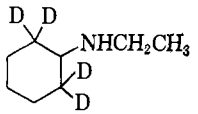


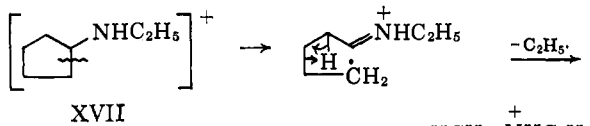
Fig. 6.—Mass spectrum of N-ethylcyclopentylamine (XVII).
Fig. 7.—Mass spectrum of N-ethylcyclohexylamine (XXI).

TABLE III

PRINCIPAL MASS SPECTRAL PEAKS OF N-ETHYLCYCLOPENTYLAMINE, N-ETHYLCYCLOHEXYLAMINE AND THEIR DEUTERATED ANALOGS

Compound	M+	M-15	M-29	M-43	*m/e* 56	*m/e* 41
 XVII	113	98	84	70	56	41
 XVIII	114(3%) 115(97%)	100	86(~90%)	72(~75%) 71(~25%)	56(37%) 58(40–60%)	41
 XIX	115(6%) 116(29%) 117(65%)	102(~65%)	85(75%)	72(~69%)	Mainly 57 and 58	Mainly 43
 XX	114(12%) 115(88%)	98	84(4%) 87(96%)	70(71%) 73(29%)	Mainly 56 and 57 59(7%)	41
	M+	M-15	M-29	M-43	*m/e* 71	*m/e* 56
 XXI	127	112	98	84	71	56
 XXII	129(2%) 130(13%) 131(85%)	116(85%)	99 100 101	85(~95%)	73(~90%)	57 and 58

(a) **N-Ethylcyclopentylamine** (Fig. 6 and Table III). —In the mass spectrum (Fig. 6) of N-ethylcyclopentylamine (XVII), there are found prominent fragment ions at *m/e* 98, 84, 70, 56 and 41. The peak at *m/e* 98 (M-15) is due to the loss of CH_3, which is shown to be the methyl of the ethyl group by the behavior of the β,β,β-trideuterioethyl analog XX (Table III). The M-29 (*m/e* 84) is only due to a small extent (~4%) to loss of the N-ethyl group. It moves to *m/e* 85 in the spectrum of the 2,2,5,5-d_4 analog XIX and is therefore analogous to e in the amide spectra (Fig. 3 and 5).

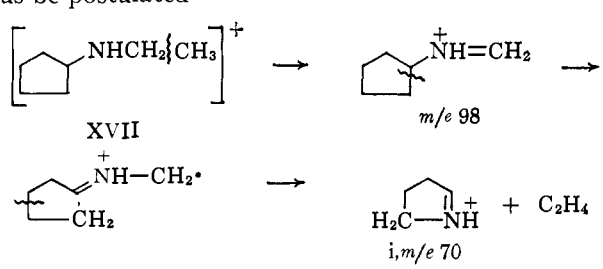


XVII

$CH_2{=}CHCH{=}\overset{+}{N}HC_2H_5$

m/e 84

The shifts (Table III) of the peak at *m/e* 70 in the spectrum of N-(β,β,β-d_3-ethyl)-cyclopentylamine (XX) show that there are two different processes operating. The predominant one involves loss of the methyl group, but retention of the α-carbon of the ethyl group (see XVIII in Table III) as well as of two C-2 hydrogen atoms (see XIX in Table III). The stable ion i can thus be postulated

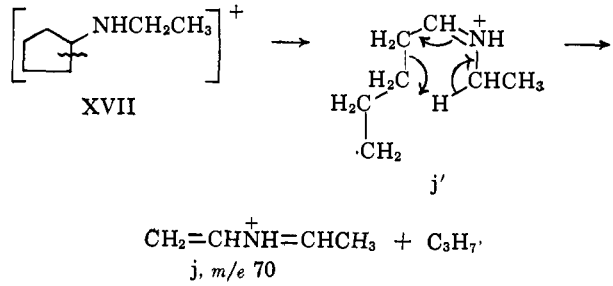


XVII

m/e 98

i, *m/e* 70

The second (minor) constituent of the peak at *m/e* 70 (25–30%) retains (see Table III) all three deuterium atoms of XX but only one of XVIII. Its formulation as j seems unambiguous and its formation is believed to involve first the expected[9] fission of a carbon-carbon bond next to nitrogen (see also postulated precursor to *m/e* 84 ion), followed by a hydrogen transfer *via* a six-membered intermediate j'.



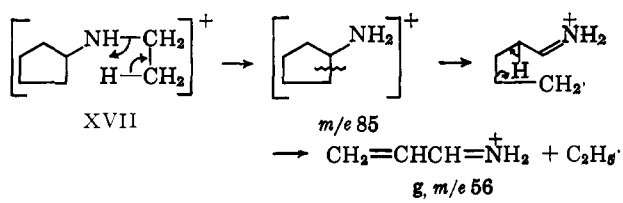
XVII

j'

$CH_2{=}CH\overset{+}{N}H{=}CHCH_3 + C_3H_7\cdot$

j, *m/e* 70

The peak at *m/e* 56 is also composite. The presence of peaks at adjacent masses causes some difficulty in assigning shifts of this peak, but the following proposals seem tenable. The formation of g is expected, since loss of ethylene should be possible in the same manner as the loss of ketene in the amide XIII. This is substantiated by the presence of a metastable peak at *m/e* 37.1 ($85^+ \rightarrow 56^+$, calcd. 36.9).



XVII

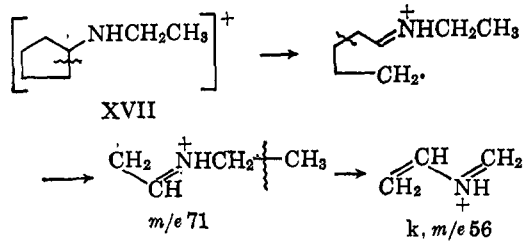
m/e 85

$\rightarrow CH_2{=}CHCH{=}\overset{+}{N}H_2 + C_2H_5\cdot$

g, *m/e* 56

It should appear at *m/e* 56 in XVIII, 57 in XIX and 57 in XX and such shifts are in fact noted in Table III. The results summarized in the table show that the other major component of the *m/e* 56 fragment must

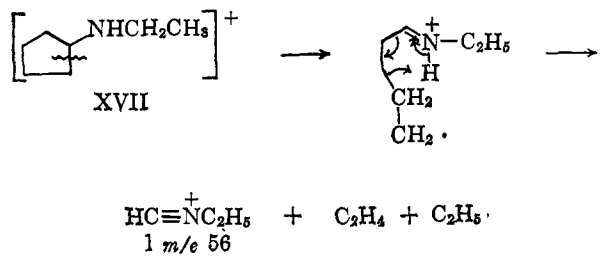
retain both deuterium atoms in XVIII but none in XX. Such an ion, k, could be formed as



XVII

m/e 71 k, *m/e* 56

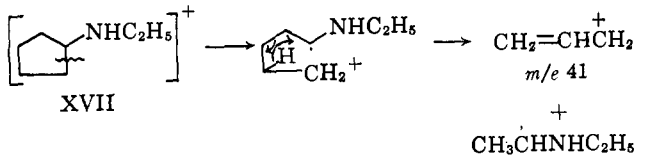
This proposal is supported by the presence of a metastable peak at *m/e* 44.2 (calcd. 44.2 for transition *m/e* 71 → *m/e* 56) and by the partial shift (Table III) to *m/e* 58 in XIX. Evidence of the presence of a third constituent is given by the shift (only to an extent of about 7%) to *m/e* 59 in the spectrum of XX. This could be explained by the formation of l.



XVII

$$HC{\equiv}\overset{+}{N}C_2H_5 \quad (1\ m/e\ 56) \quad + \quad C_2H_4 + C_2H_5\cdot$$

The peak at *m/e* 41 in Fig. 6 does not contain any of the hydrogens of the ethyl group but retains two of the four deuterium atoms of the ring-labeled 2,2,5,5-d_4 analog XIX. It may possibly be represented as being formed by the process



XVII

m/e 41

The composite character of the *m/e* 56 and *m/e* 70 peaks and the elucidation of the nature of the individual constituents represent a striking illustration of the importance of differential deuterium labeling, which is required in an examination of detailed fragmentation processes. It should be noted that high-resolution mass spectrometry would have been of no assistance in this instance.

(b) **N-Ethylcyclohexylamine** (Fig. 7 and Table III).—The only labeled analog of N-ethylcyclohexylamine (XXI) available is the 2,2,6,6-d_4 derivative XXII. This is sufficient, however, for the interpretation of the mass spectrum (Fig. 7), since it is now possible to predict some of the cleavages on the basis of the above detailed analysis of the cyclopentyl lower homolog. The M-15 peak at *m/e* 112 should again be expected to be due to loss of the methyl from the ethyl group. According to the shifts of the M-29 peak in the spectrum (Table III) of XXII, the formation of the M-29 peak (*m/e* 98 in Fig. 7) does not involve loss of the N-ethyl group to a significant extent. Rather, it appears to be formed by loss of C_2H_5 from the ring in a random manner.

By far the most intense peak in the spectrum (Fig. 7) of N-ethylcyclohexylamine (XXI) is the M-43 peak at *m/e* 84. The loss of the elements of C_3H_7 from cyclohexylamine derivatives is by now a well-defined process and the ion formed in this case will have

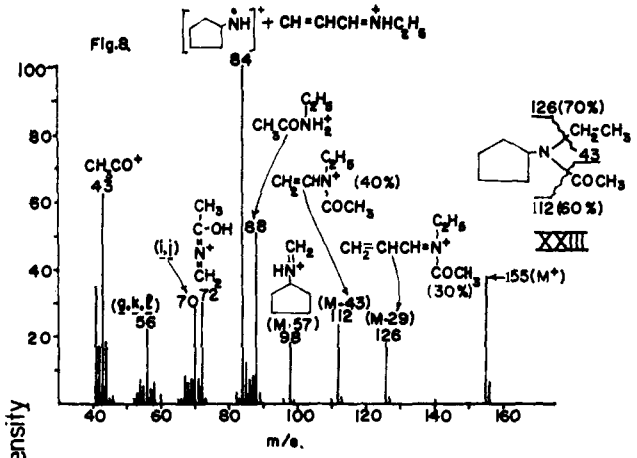


Fig. 8.—Mass spectrum of N-ethyl-N-acetylcyclopentylamine (XXIII).

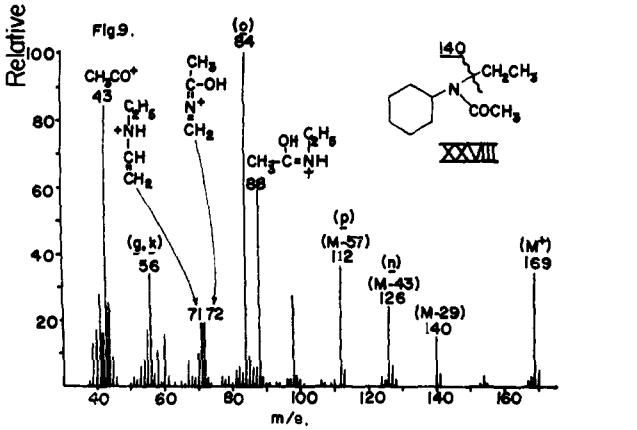
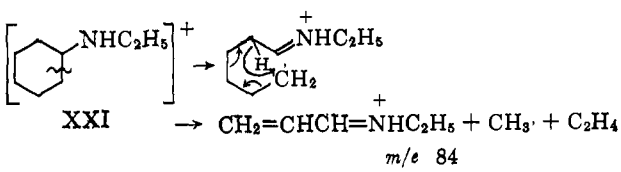


Fig. 9.—Mass spectrum of N-ethyl-N-acetylcyclohexylamine (XXVIII).

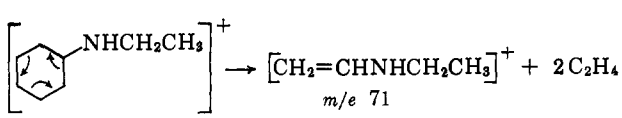
the same structure as the M-29 ion from N-ethylcyclopentylamine (XVII), a conclusion which is confirmed by the shift (Table III) to *m/e* 85 in the spectrum of the 2,2,6,6-d_4-labeled derivative XXII.



XXI

m/e 84

The peak at *m/e* 71 moves to *m/e* 73 in XXII and hence can be most easily explained by a simple fission of the ring.



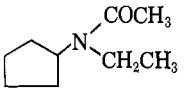
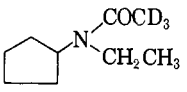
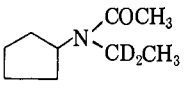
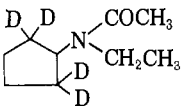
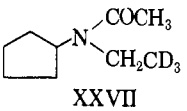
m/e 71

The peak at *m/e* 56 shifts to *m/e* 57 and 58 in XXII. It seems probable, therefore, that it consists of the same two ions, g and k, which were found in the spectrum (Fig. 6) of N-ethylcyclopentylamine (XVII); the precursor of k will have the same structure in both cases (*m/e* 71 above).

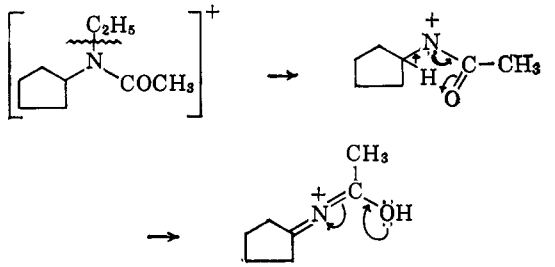
N-Ethyl-N-acetylcycloalkylamines. (a) **N-Ethyl-N-acetylcyclopentylamine** (Fig. 8 and Table IV).—N-Ethyl-N-acetylcyclopentylamine has a fairly complicated mass spectrum (Fig. 8) but it has been possible with suitable labeling to make assignments to all the peaks of significant intensity (Table IV).

TABLE IV

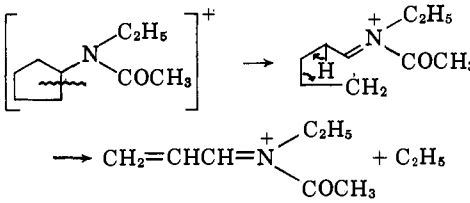
PRINCIPAL MASS SPECTRAL PEAKS OF N-ETHYL-N-ACETYLCYCLOPENTYLAMINE AND DEUTERATED ANALOGS

Compound	M+	M-29	M-43	M-57	*m/e* 88	*m/e* 84	*m/e* 72	*m/e* 70	*m/e* 56	*m/e* 43
 XXIII	155	126	112	98	88	84	72	70	56	43
 XXIV	157(3%) 158(97%)	129	112(37%) 113(22%) 115(41%)	99	91	84(15%) 85(85%)	75	71	56, 57	Mainly 46
 XXV	156(7%) 157(93%)	126(65%) 128(35%)	114	100	90	84(35%) 86(65%)	74	72	56, 57, 58	43
 XXVI	157(4%) 158(28%) 159(68%)	127(14%) 128(8%) 129(29%) 130(49%)	113(30%) 114(22%) 115(18%) 116(30%)	101(31%) 102(69%)	88, 89, 90	85(48%) 86, 87, (88?)	73 or 72	72 or 73	56, 57, 58	Mainly 43
 XXVII	157(8%) 158(92%)	126(75%) 129(25%)	115	98	91	84(40%) 87(60%)	72, (73?) 74 75	70, 73	56, 57, 59(~5%)	Mainly 43

In contrast to the behavior of the amines, the M-29 peak (*m/e* 126) is produced to a large extent by loss of the N-ethyl group. It may be possible for the resultant ion to stabilize itself by an internal hydrogen transfer

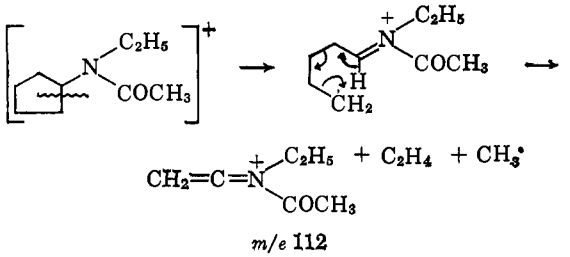


The remaining 20–30% of the peak is formed by loss of C_2H_5 from the ring, mainly by the process which was operative for the secondary amides.



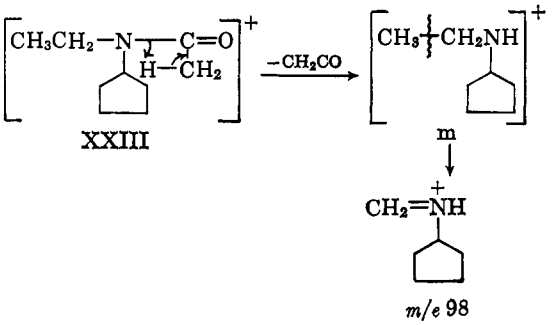
m/e 126

The loss of 43 mass units in Fig. 8 is also a complex process. Expulsion of acetyl with and without rearrangement accounts for about 60% of the peak. The remainder must involve the loss of the elements of C_3H_7 from the ring as can be seen from the shifts in Table IV. This could take place as



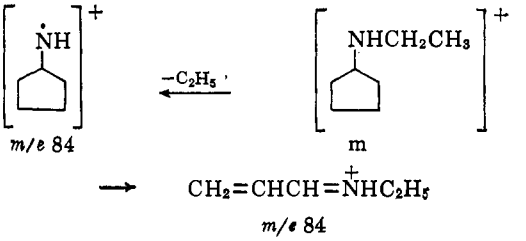
m/e 112

The M-57 peak (*m/e* 98 in Fig. 8) contains one hydrogen atom from the acetyl group as well as the α-carbon atom but not the methyl of the ethyl group (see Table IV). It would therefore be analogous to process 2 which was found in aliphatic amide spectra and can be formulated in the same manner.



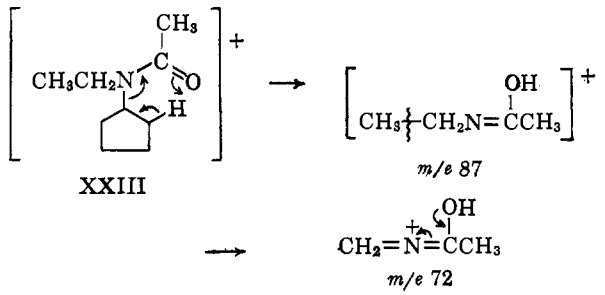
m/e 98

The ion analogous to f, in this case protonated N-ethylacetamide, appears at *m/e* 88. This assignment follows from the shifts in Table IV, where it can be seen that the ion contains the ethyl and acetyl groups and one (in some cases two) hydrogen atom from C-2. The most intense peak in the spectrum (Fig. 8) is at *m/e* 84 and appears to consist of two different species, one of which is the ion which one would expect by analogy to the behavior of N-ethylcyclopentylamine (XVII). This is present to an extent of about 65% of the *m/e* 84 peak, the remainder involving loss of the ethyl group from the intermediate species m. The *m/e* 84 fragment depicted with an electron-deficient nitrogen atom is probably stabilized further by migration of one of the adjacent hydrogens.



m/e 84

The peaks at *m/e* 70 and 72 both appear to be mixtures of ions of different origin, but the shifts (Table IV) are sufficiently clear to allow assignment of the major contributing fragment in each case. The peak at *m/e* 72 retains the acetyl group (see XXIV in Table IV) and the α-carbon of the ethyl group (see XXV in Table IV), but the larger part of it does not contain the methyl of the ethyl group (see XXVII in Table IV). The possibility of a shift of one mass unit in the 2,2,5,5-d_4 derivative XXVI makes the following process an attractive proposition, although the mass spectral shifts (see Table IV) are not unambiguous.

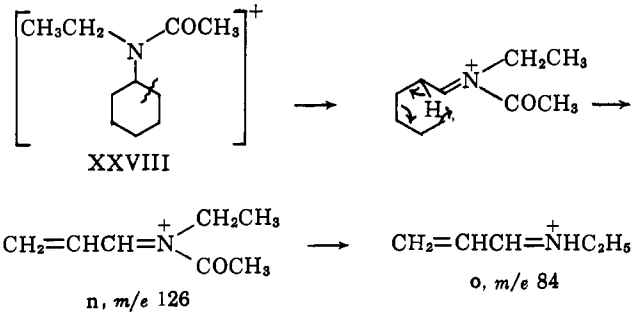


XXIII *m/e* 87 *m/e* 72

In view of the complete move of the peak at *m/e* 70 to *m/e* 71 in the trideuterioacetyl derivative XXIV, one can postulate the loss of ketene to give an N-ethylcyclopentylamine intermediate (XXIII → m = XVII), which decomposes in the manner outlined above for the amine XVII to form the ions i (*m/e* 70) and j (*m/e* 70). The peak shifts (Table IV) of the deuterated species XXV and XXVII are in accordance with these conclusions.

The movements of the peak at *m/e* 56 (Table IV) indicate that after loss of ketene, the resulting ion decomposes as does N-ethylcyclopentylamine (XVII) to form the ions g, k and l, the latter again being present only to a very small extent (see shift to *m/e* 59 exhibited by XXVII). The *m/e* 43 peak appears to consist mostly of the acetyl ion (see Table IV).

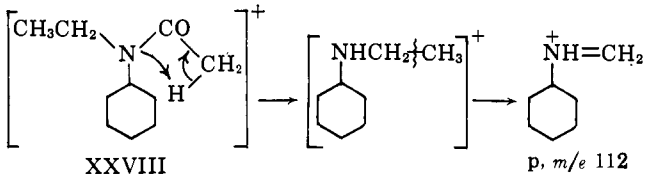
(b) **N-Ethyl-N-acetylcyclohexylamine** (Fig. 9).—No labeled derivatives of N-ethyl-N-acetylcyclohexylamine (XXVIII) have been prepared but the mass spectrum (Fig. 9) can be readily explained by analogy with those of N-ethyl-N-acetylcyclopentylamine (XXIII, Fig. 8) and N-ethylcyclohexylamine (XXI, Fig. 7). The M-29 peak will be formed by a similar mechanism as in the cyclopentyl analog, with loss of the ethyl group predominating, because six-membered rings do not lose C_2H_5 as readily as cyclopentane rings. The M-43 peak will be partly due to loss of acetyl and partly due to the process



XXVIII n, *m/e* 126 o, *m/e* 84

The ion formed, n, can further lose ketene to produce o (*m/e* 84), which forms the base peak of the spectrum (Fig. 9). Loss of ketene could also be the first step in the production of this ion and in the formation of the *m/e* 56 and 71 fragments, which probably have the same structure as the ions of the same mass in the spectrum (Fig. 7) of N-ethylcyclohexylamine (XXI).

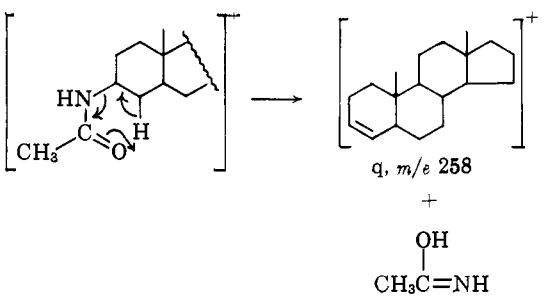
The production of the *m/e* 112, 88 and 72 ions can be rationalized by analogy to N-ethyl-N-acetylcyclopentylamine (XXIII, Fig. 8). Thus the M-57 ion p (at *m/e* 112 in this case) is formed by successive loss of ketene and methyl. The peaks (Fig. 9) at *m/e* 72 and 88 are almost certainly due to the same ions as in the cyclopentyl analog XXIII (Fig. 8), since the ring carbons are not involved. It is expected that the *m/e* 43 peak consists mostly of the acetyl ion.



XXVIII p, *m/e* 112

Amides of Steroidal Amines.—In the mass spectra (Fig. 10) of 3α- (XXIX) and 3β- (XXX) N-acetylaminoandrostane, it is possible with the labels available (Table V) to determine the origin of the methyl group lost in the formation of the M-15 peak. When the contribution of the amide methyl group is subtracted (using the trideuterioacetyl derivative), it is found that loss of C-19 accounts for about half of the remainder of the peak, *i.e.*, the two quaternary methyl groups are lost equally readily. This should be the case in any androstane derivative where there is no substituent sufficiently close to either methyl group to favor its preferential cleavage. It is interesting to note that, except for relatively minor quantitative differences (see Tables VI–VIII), the mass spectra (Fig. 10a and 10b) of the two isomeric amides XXIX and XXX are very similar.

The M-59 ion (*m/e* 258) does not appear to bear any analogy to any of the intense peaks in the spectra of the



q, *m/e* 258 +

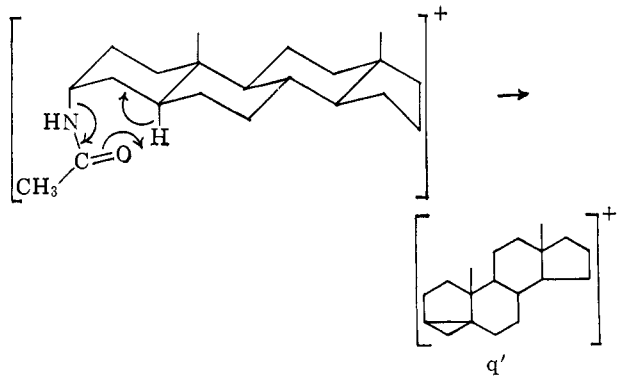
simpler amides (*e.g.*, VI in Fig. 3). It can, however, be explained as the loss of acetamide, for which the following mechanism can be proposed, involving the familiar six-membered ring transition state. A similar mechanism can be put forward involving the hydrogen atom on C-2, which will produce an ion q with a 2,3- rather than a 3,4-double bond. However, the distribution of deuterium in this peak in the spectrum of the 2,2,4,4-d_4 derivative (see Table V) shows that not more than 40% of the hydrogen transferred comes from the 2- and 4-positions in the α-series and not more than 54% in the β-series. More extensive labeling of ring A hydrogen atoms shows (Table V) that in over 80% of all cases this peak involves a hydrogen transfer from C-2, C-4 or C-5. Transfer from C-5 could be rationalized as illustrated on the next page. The ion q′ may rearrange to a less strained structure with a double bond. It is more difficult to visualize the above transfer when the amide group is β. Minor contributions to this peak involve transfer of hydrogen from C-1, C-3 and C-19 and are summarized in Table VI. The totals in both cases are greater than 100% which would indicate that there is an isotope effect which favors transfer of deuterium over hydrogen. Similar conclusions can be reached from Table

TABLE V

PRINCIPAL MASS SPECTRAL PEAKS OF 3α- (XXIX) AND 3β- (XXX) N-ACETYLAMINOANDROSTANE AND DEUTERATED ANALOGS

Compound	M^+	M-CH_3	M-59	M-74	*m/e* 204	*m/e* 189	*m/e* 60	*m/e* 56
XXIX and XXX	317	302	258	243	204	189	60	56
2,2,4,4-d_4- (3α)	320(7%)	305(8%)	261(39%)	245(4%)	204(87%)	189(87%)	60(9%)	56(33%)[a]
	321(93%)	306(92%)	262(61%)	246(53%)	205(13%)	190(13%	61(49%)	57(67%)
				247(43%)			62(42%)	
2,2,4,4-d_4- (3β)	320(9%)	305(9%)	261(53%)	245(6%)	204(92%)	189(87%)	60(9%)	56(30%)[a]
	321(91%)	306(91%)	262(47%)	246(68%)	205(8%)	190(13%)	61(45%)	57(70%)
				247(26%)			62(46%)	
5α-d_1- (3α)	318(87%)	303(88%)	258(49%)	243(40%)	204(21%)	189(31%)	60(77%)	56
	319(13%)	304(12%)	259(51%)	244(60%)	205(79%)	190(69%)	61(22%)	
							62(1%)	
5α-d_1- (3β)	318(87%)	303(87%)	258(34%)	243(23%)	204(26%)	189(38%)	60(86%)	56
	319(13%)	304(13%)	259(66%)	244(72%)	205(74%)	190(62%)	61(14%)	
				245(5%)				
3β-d_1	318	303	258(6%)	243(4%)	204(94%)	189(95%)	60(94%)	56(32%)
			259(94%)	244(96%)	205(6%)	190(5%)	61(6%)	57(68%)
3α-d_1	318	303	258(5%)	243(2%)	204(95%)	189(99%)	60(90%)	56(27%)
			259(95%)	244(98%)	205(5%)	190(1%)	61(10%)	57(73%)
1α-d_1 (3α)	318(89%)	303(92%)	258(5%)	243(4%)	204(90%)	189(84%)	60(82%)	56(61%)
	319(11%)	304(8%)	259(89%)	244(91%)	205(10%)	190(16%)	61(18%)	57(39%)
			260(6%)	245(5%)				
1α-d_1- (3β)	318(93%)	303(94%)	258(4%)	243(3%)	204(89%)	189(88%)	60(83%)	56(33%)
	319(7%)	304(6%)	259(90%)	244(91%)	205(11%)	190(12%)	61(17%)	57(67%)
			260(6%)	245(6%)				
2,2,3β,4,4,5α-d_6- (3α)	321(2%)		262(15%)	247(16%)	204(21%)	189(18%)	60(10%)	
	322(20%)	308(80%)	263(77%)	248(77%)	205(69%)	190(73%)	61(36%)	
	323(79%)		264(8%)	249(7%)	206(10%)	191(8%)	62(54%)	
2,2,3α,4,4,5α-d_6- (3β)	321(2%)		262(16%)	247(18%)	204(11%)	189(17%)	60(6%)	
	322(19%)	308(80%)	263(76%)	248(74%)	205(79%)	190(68%)	61(39%)	
	323(79%)		264(8%)	249(8%)	206(10%)	191(15%)	62(55%)	
19-d_1- (3α and 3β)	318(97%)	302(36%)	258(3%)	243(37%)	204(27%)	189(15%)	60(95%)	56(60%)
	319(3%)	303(64%)	259(97%)	244(63%)	205(73%)	190(85%)	61(5%)	57(40%)
d_3-Acetyl- (3α and 3β)	319(2%)	302(25%)	258	243	204	189	63	56(37%)[a]
	320(98%)	305(75%)						57(63%)

[a] The presence of intense peaks at *m/e* 55 and 57 raises difficulties in the calculation of shifts of the *m/e* 56 peak in the spectra of polydeuterated analogs. In these cases, we have assumed that no ion in this mass region contains more than one deuterium atom.



q'

V by making a comparison between the spectra of the 2,2,3,4,4,5-d_6-derivative and of the compounds individually deuterated in these positions (92% *vs.* 97% transfer, respectively).

The peak at *m/e* 243 (M-74) appears to be derived by loss of a methyl group from q or q'. The spectrum of the 19-deuterio analog indicates that it is predominantly (*ca.* 2:1) C-18 rather than C-19 which is lost. The hydrogen transfer for peak M-74 is summarized in Table VII. Again the total exceeds 100% and an isotope effect can be assumed to be operating. The differences in the isotope distributions in Tables VI and VII indicate that species q and q' do not undergo this methyl expulsion to the same extent.

The peak at *m/e* 204 is formed by fission of the 1,10- and 4,5-bonds; the resultant ion r loses a methyl group to form the ion s of *m/e* 189. The methyl group lost must be C-18, since C-19 is retained (Table V). This is predictable since the vinylic 10,19-bond would not be labile.

By far the most intense peak in the spectrum (Fig. 10) is at *m/e* 60. It shifts completely (see Table V) to *m/e* 63 in the spectrum of the trideuterioacetyl analog and so must be the protonated acetamide ion f. The source of the hydrogen for the double transfer involved is summarized in Table VIII.

TABLE VI

SOURCE OF TRANSFERRED HYDROGEN IN *m/e* 258 PEAK (FIG. 10) OF 3-N-ACETYLAMINOANDROSTANES

	Location of H atom	H transfer,[a] %
3α-Series	C-19	8
(XXIX)	C-1	9
	C-2,4	40
	C-3	6
	C-5	49
		Total 112
3β-Series	C-19	8
(XXX)	C-1	7
	C-2,4	54
	C-3	5
	C-5	34
		Total 108

[a] The values (taken from Table V) for the 2,2,4,4-d_4 derivatives are corrected for incomplete deuteration. The species at *m/e* 319 in the spectra of the 5-α-d_1 compounds are assumed to contain one deuterium atom in ring B and so values have not been corrected. The entry for the 1-*d* derivative was obtained by assuming that the ion at *m/e* 319 contains one deuterium atom at C-2 and that the 1-α- and 1-β-hydrogen atoms are transferred equally readily.

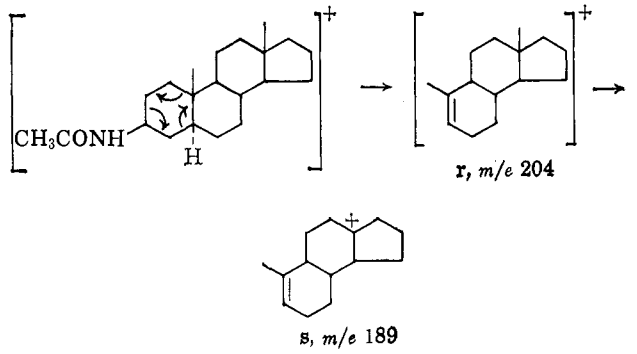


TABLE VII

SOURCE OF TRANSFERRED HYDROGEN IN *m/e* 243 PEAK (FIG. 10) OF 3-N-ACETYLAMINOANDROSTANES

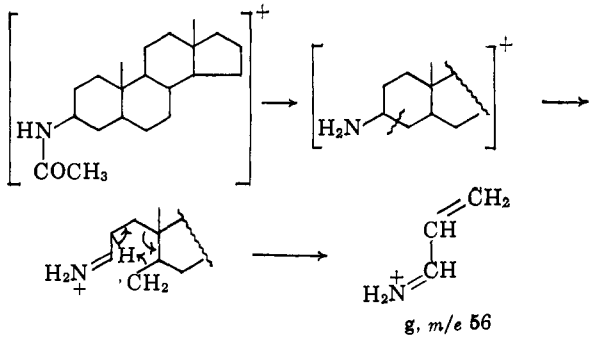
	Location of H atom	H transfer, %
3α-Series	C-19	...
(XXIX)	C-1	7
	C-2,4	54
	C-5	40
	C-3	4
	Total	105
3β-Series	C-19	...
(XXX)	C-1	5
	C-2,4	71
	C-5	23
	C-3	5
	Total	104

TABLE VIII

SOURCE OF DOUBLE HYDROGEN TRANSFER IN *m/e* 60 ($CH_3CONH_2^+$) PEAK (FIG. 10) OF 3-N-ACETYLAMINOANDROSTANES

	Location of H atom	H transfer, %
3α-Series	C-19	7
(XXIX)	C-1	18
	C-2,4	68
	C-3	3
	C-5	11
3β-Series	C-19	7
(XXX)	C-1	17
	C-2,4	70
	C-3	5
	C-5	7

The peak at *m/e* 56 shows (Table V) the correct shifts (to the extent of *ca.* 60%) for the presence of ion g which can be formed in the same manner as from N-acetylcyclohexylamine (VI), provided the 3,4- rather than the 2,4-bond is broken.



NOTE ADDED IN PROOF (July 1, 1963).—Recently[16a] the mass spectra of a large group of steroidal *Holarrhena*

(16a) V. Cerny, Colloquium at Stanford Unviersity, May 7, 1963; see also L. Dolejs, V. Hanus, V. Cerny and F. Sorm, *Collection Czech. Chem. Commun.*, **28**, 1584 (1963). We are indebted to these authors for an advance copy of their manuscript.

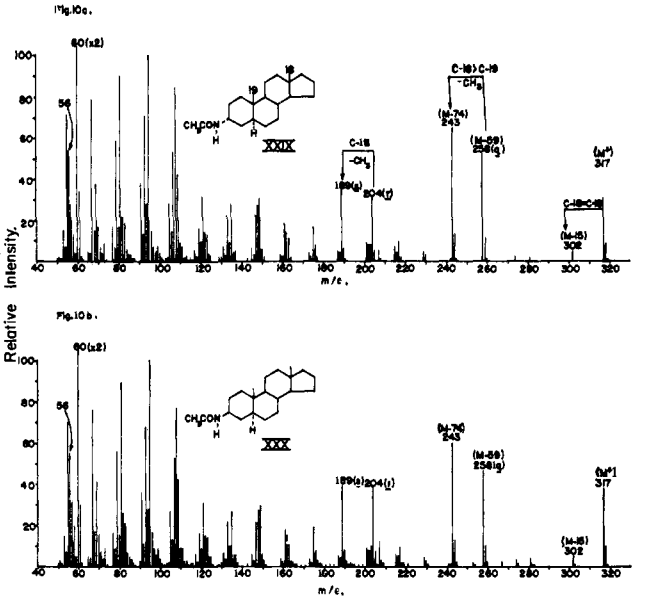
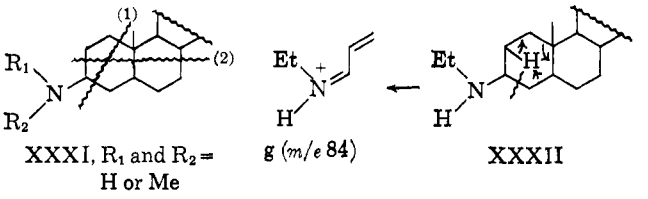


Fig. 10.—(a) Mass spectrum of 3α-N-acetylaminoandrostane (XXIX); (b) mass spectrum of 3β-N-acetylaminoandrostane (XXX).

alkaloids have been examined, all of which contain the partial structure XXXI. The most important peaks corresponded to fission (1), accompanied by rearrangement of one hydrogen, and the ion was represented[16a] analogous to species g. Another important fragment was supposed[16a] to arise from the rupture of three bonds, as indicated by a wavy line (2), and thus had to involve the loss of two unspecified hydrogen atoms.[16b] The availability in our laboratory of the various deuterated N-acetylaminoandrostanes made possible a very rapid confirmation of these assumptions as well as the detection of the hydrogens transferred.



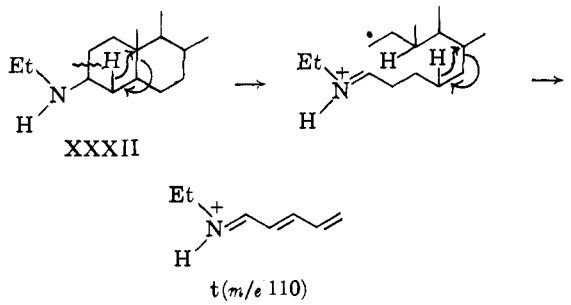
Lithium aluminum hydride reduction of 3β-N-acetylaminoandrostane (XXX) produced 3β-N-ethylaminoandrostane (XXXII, m.p. 56–58°, $[\alpha]^{25}D$ −5°) in which fragment g now corresponds to *m/e* 84. In agreement with this mechanism is the observation that the peak remained at *m/e* 84 in 5-d_1-XXXII, but was shifted to *m/e* 85 in 1-d_1-XXXII and 2,2,4,4-d_4-XXXII, thus demonstrating the transfer of the C-2 hydrogen atom.

The fragmentation corresponding to the wavy line (2) in XXXI was attributed[16a] to species t (*m/e* 110 in XXXI (R_1 = R_2 = Me) and XXXII). In agreement with this view, we find that t remains at *m/e* 110 in 1-d_1-XXXII, but appears at *m/e* 111 in 2,2,4,4-d_4-XXXII and in 5-d_1-XXXII. These shifts establish the rearrangement of the C-4 hydrogen atom and make it very likely that the second one is transferred from C-6 as indicated at the top of the next page.

Conclusions

It can be generally stated that cleavage of a bond β to a nitrogen will occur readily. This is facilitated by

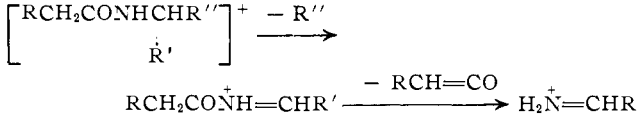
(16b) W. Vetter, P. Longevialle, F. Khuong-Huu-Laine, Q. Khuong-Huu and R. Goutarel, *Bull. Soc. Chim. France*, 1324 (1963), have studied the mass spectra of the same amines and have proposed migration of the 2-, as well as of the 4- and 6-, hydrogen atoms.



XXXII

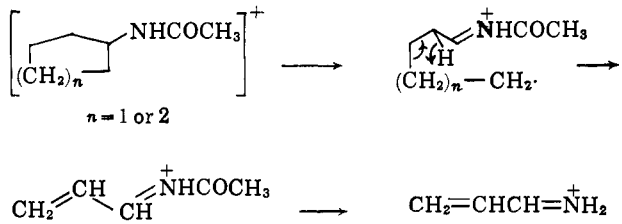
t(*m/e* 110)

stabilization of the resultant ion in the manner shown. The presence of the electron-withdrawing carbonyl group will not stabilize the ion, so if the acyl group can be lost by ketene elimination, a much more favored ion will result. This is reflected in the high intensity of the peaks of *m/e* 30 and 58 in the spectra (Fig. 1a and 2a) of N-*n*-butylacetamide (I) and N,N-diethylacetamide (III), respectively.



Cleavages of the same nature are to be found in the mass spectra of N-acetyl-α-amino acids[17] (R = H, R′ = alkyl and R″ = CO_2Me). The ion of *m/e* 43 is present, just as we find in all acetamides, and is always at least in part CH_3CO^+.

In the case of cycloalkylamines and their amides, a single cleavage β to nitrogen cannot fragment the molecule, but it is usually the initial step in the formation of the most stable ion. In both cyclopentyl and cyclohexyl compounds, fission β to the nitrogen is followed by a rearrangement which can form a stable even-electron ion.



n = 1 or 2

In amides, this is followed by loss of ketene to give the more stable amine-type ion. A competing process is noted in cyclic compounds, caused by the tendency of such cyclic systems to lose their side chains.[18] The predominant process of this type is the formation of the ion f, which has been discussed in detail. The steroid amides possess a stable polycyclic nucleus and give predominantly ions produced by fission of the bond between the ring and the nitrogen atom rather than fission of the ring.

Experimental[19]

3α-(XXIX) and 3β-(XXX) N-Acetylaminoandrostane.—A solution of 1.0 g. of androstan-3-one in 18 cc. of dry pyridine was warmed on the steam-bath for a few minutes with 0.5 g. of hydroxylamine hydrochloride. Most of the pyridine was removed *in vacuo*, water was added and the quantitatively precipitated androstan-3-one oxime was filtered, m.p. 186–187°. Crystallization from 94% aqueous ethanol provided rod-like, colorless crystals, m.p. 189–189.5°, $[\alpha]^{24}D$ +3.6° (*c* 0.8, acetone).

Anal. Calcd. for $C_{19}H_{31}NO$: C, 78.84; H, 10.80; N, 4.84. Found: C, 78.79; H, 10.85; N, 4.60.

A suspension of 1.6 g. of lithium aluminum hydride in 80 cc. of dry ether was heated under reflux for 30 min., followed by the dropwise addition of a solution of 0.6 g. of the oxime in 40 cc. of ether and continued heating for another 30 min. The reaction mixture was worked up by addition of a saturated, aqueous solution of sodium sulfate, separation of the ether phase and extraction of the remaining solid with ether. The combined organic extracts were washed with water, dried over magnesium sulfate and the ether removed. The resulting semisolid mixture of 3α- and 3β-aminoandrostane was mixed, without further purification, with 1 cc. of acetic anhydride and after 10 min. at room temperature, water was added, followed by dilute sodium carbonate solution. The crystalline mixture of amides XXIX and XXX was isolated with ether; yield 83%, m.p. 190–240°.

Slow crystallization from ethyl acetate provided in the first crop *ca.* 30% of colorless plates, m.p. 244–245°, which upon further crystallization provided the pure **3β-N-acetylaminoandrostane (XXX)**, m.p. 248–248.5°, $[\alpha]^{25}D$ −14° (*c* 0.7, chloroform), λ_{max}^{KBr} 6.13 μ.

Anal. Calcd. for $C_{21}H_{35}NO$: C, 79.44; H, 11.11; N, 4.41. Found: C, 79.68; H, 11.12; N, 4.37.

Concentration of the mother liquors led in 40% yield to **3α-N-acetylaminoandrostane (XXIX)**, m.p. 217–230°, raised to m.p. 241–242.5° upon further recrystallization from ethyl acetate, $[\alpha]^{26}D$ +13° (*c* 0.9, chloroform). The infrared spectra and especially the mass spectra (Fig. 10a and 10b) of the two isomers were very similar, but a mixture of the two amides melted at 187–220°.

Anal. Calcd. for $C_{21}H_{35}NO$: N, 4.41. Found: N, 4.25.

The deuterated analogs, whose relevant mass spectral information is collected in Table V, were prepared by identical procedures except that the following[20] labeled androstan-3-ones were used as starting materials: 1α-d_1, 19-d_1, 5α-d_1, 2,2,4,4-d_4. The amides (Table V) labeled solely at C-3 were obtained by substituting lithium aluminum deuteride for lithium aluminum hydride in the reduction of the oxime, while the 2,2,3,4,4,5α-d_6′-amides (Table 5) were prepared from 5α-d_2-androstan-3-one,[20] which was equilibrated with sodium methoxide in deuteriomethanol–deuterium oxide to the 2,2,4,4,5α-d_5-androstan-3-one, followed by conversion to the oxime, reduction with lithium aluminum deuteride and acetylation. The unseparated (see Table V) mixture of 3α- and 3β-N-trideuterioacetylaminoandrostane was prepared by using d_6-acetic anhydride (Merck, Ltd., Montreal) in the acetylation step, without attempting separation of the isomers, since this reaction was conducted on a very small scale and the mass spectra (Fig. 10a and 10b) of the parent compounds are in any event very similar.

1-d_1-N-Acetylcyclohexylamine (VIII).—The reduction of cyclohexanone oxime (0.5 g.) was effected in ether solution by heating under reflux for 30 min. with 1.2 g. of lithium aluminum deuteride and decomposing by the sodium sulfate technique (*vide supra*). The ether was removed carefully and the residual 1-d_1-cyclohexylamine treated immediately with 2 cc. of acetic anhydride. After the exothermic reaction had subsided, solid sodium carbonate was added followed by 50 cc. of water. The product was isolated with ether and crystallized from petroleum ether (b.p. 60–80°), whereupon there was obtained 384 mg. of 1-d_1-N-acetylcyclohexylamine (VIII) of 98% isotopic purity (see Table I), m.p. 105.5–106°, lit.[21] (for non-deuterated material) m.p. 104°.

3-d_1-N-Acetylcyclohexylamine (IX).—A solution of 1.0 g. of cyclohex-2-en-1-one (Aldrich Chemical Co., Milwaukee, Wis.) in 100 cc. of ether was shaken with 50 mg. of 10% palladium-on-charcoal catalyst in an atmosphere of deuterium until the gas uptake ceased. The catalyst was filtered, the ether was removed and the residue was heated under reflux for 30 min. with 20 cc. of 2.5% sodium hydroxide solution to exchange deuterium in any equilibratable position. The ketone was then removed by steam distillation and 1.2 g. of hydroxylamine hydrochloride added to the distillate. After making alkaline with solid sodium carbonate, the solution was cooled, the oxime filtered and recrystallized from petroleum ether (b.p. 60–80°); yield 1.0 g., m.p. 89–90°. This oxime was transformed into the desired amide (see Table I) by reduction with lithium aluminum hydride and acetylation.

2,2,6,6-d_4-N-Acetylcyclohexylamine (X).—Cyclohexanone (1.0 g.) was heated under reflux for 4 hr. with 0.2 g. of sodium methoxide and 10 g. of deuterium oxide, followed by the addition of 1.0 g. of hydroxylamine hydrochloride and 3 cc. of pyridine. After warming to 70° for 5 min., the solution was cooled, the

(17) C.-O. Andersson, R. Ryhage and E. Stenhagen, *Arkiv Kemi*, **19**, 417 (1962).

(18) J. H. Beynon, *Mikrochim. Acta*, 446 (1956).

(19) Melting points are corrected and were determined on the Kofler block. We are indebted to Messrs. R. Meier and J. Consul for the microanalyses. All mass spectra were obtained with a Consolidated Electrodynamics Corp. mass spectrometer model No. 21-103C using an all-glass inlet system heated to 200°. The ionizing energy was kept at 70 e.v. and the ionizing current at 50 μa.

(20) These ketones were prepared in our laboratory by R. H. Shapiro and M. A. Kielczewski in connection with a detailed study of the mass spectral fragmentation behavior of 3-keto steroids. Their syntheses will be covered in a future article.

(21) A. Baeyer, *Ann.*, **278**, 88 (1894).

oxime filtered (1.1 g., m.p. 88–89°) and recrystallized once from petroleum ether, whereupon it melted at 89.5–90°. Subsequent conversion to the amide X proceeded in the above-described manner.

2,2,6,6-d_4-N-Ethylcyclohexylamine (XXII).—The labeled amide X (200 mg.) in 20 cc. of dry ether was heated under reflux for 4 hr. with 0.5 g. of lithium aluminum hydride and 50 cc. of ether. After decomposing by the sodium sulfate technique, the ether was evaporated and the labeled cyclohexylamine (see Table III) distilled at a bath temperature of 164°.

N-Trideuterioacetylcyclohexylamine (VII).—Cyclohexylamine (50 mg.) was kept at room temperature for 2 min. in ether solution with 100 mg. of d_6-acetic anhydride. Solid potassium carbonate was added, followed by 3 cc. of water and 50 cc. of ether. The ether phase was dried and evaporated and the amide was recrystallized from petroleum ether; m.p. 105.5–106°.

Preparation of Deuterated N-Acetylcyclopentylamines (see Table II).—Cyclopentanone (3.3 g.) was dissolved in 20 g. of deuterium oxide and left at room temperature for 2 weeks with 1.5 g. of potassium carbonate, since the usual equilibration conditions with stronger base led to self-condensation. Solid potassium carbonate (4.0 g.) was added followed by 3.5 g. of hydroxylamine hydrochloride. After 5 min., the oxime was extracted with ether and the latter washed with water, dried and evaporated, leaving 3.5 g. of **2,2,5,5-d_4-cyclopentanone oxime**, m.p. 56–57°. The oxime (0.4 g.) was heated under reflux for 30 min. with excess ethereal lithium aluminum hydride and after decomposing with aqueous sodium sulfate solution, the ether phase was dried and 1 cc. of acetic anhydride was added. The ether was now removed, and the residue treated with 5 cc. of water and then mixed with solid potassium carbonate. The required **2,2,5,5-d_4-N-acetylcyclopentylamine (XVI)**[22] was extracted with ether and distilled at 0.1 mm.

1-d_1-Acetylcyclopentylamine (XIV) was prepared in an analogous fashion from unlabeled cyclopentanone oxime and substituting lithium aluminum deuteride for lithium aluminum hydride. **N-Trideuterioacetylcyclopentylamine (XV)** was obtained in the above-described manner by acetylation of cyclopentylamine with d_6-acetic anhydride in ether solution.

(22) The unlabeled amide has been described by E. K. Harvill, R. M Herbst, E. C. Schreiner and C. W. Roberts, *J. Org. Chem.*, **15**, 662 (1950).

Preparation of Deuterated N-Ethylcyclopentylamines (see Table III).—**2,2,5,5-d_4-N-Ethylcyclopentylamine (XIX)** and **N-β,β,β-d_3-ethylcyclopentylamine (XX)** were prepared by reducing the appropriate labeled amide (XV, XVI) with excess lithium aluminum hydride in ether solution (4 hr. reflux), while in the synthesis of **N-α,α-d_2-ethylcyclopentylamine (XVIII)**, lithium aluminum deuteride was employed for the reduction of N-acetylcyclopentylamine (XIII). In each instance, the amine[23] was purified by distillation (b.p. 119–120°) prior to mass spectra analysis.

Preparation of Deuterated N-Ethyl-N-acetylcyclopentylamines (Table IV).—For the preparation of the unlabeled N-ethyl-N-acetylcyclopentylamine (XXIII), the 2,2,5,5-d_4 analog XXVI, the N-α,α-d_2-ethyl-N-acetylcyclopentylamine (XXV) and the N-β,β,β-d_3-ethyl derivative XXVII, approximately 100 mg. of the appropriate amine (XVII–XX in Table III) was dissolved in 10 cc. of ether and kept at room temperature for 2 min. with 0.5 cc. of acetic anhydride. The ether was removed, 2 cc. of water was added, followed by 1.0 g. of sodium carbonate and then anhydrous magnesium sulfate. The solid mass was extracted thoroughly with ether, the solvent removed and the residual amide distilled under reduced pressure before mass spectral analysis. The trideuterioacetyl derivative XXIV was prepared in the same manner, except that d_6-acetic anhydride was employed in the last step. The unlabeled amide XXIII exhibited b.p. 241°, $n^{25}D$ 1.4721, λ_{max}^{capill} 6.06 μ and proved to be quite hygroscopic.

N-Ethyl-N-β,β,β-d_3-ethylacetamide (V).—Ethylamine was acetylated with d_6-acetic anhydride in ether solution as described above for N-ethylcyclopentylamine and the resulting N-ethyl-d_3-acetamide, in ether, was heated under reflux for 4 hr. with an excess of lithium aluminum hydride. The excess reagent was destroyed at 0° by the addition of aqueous sodium sulfate solution and the ether containing the labeled diethylamine was distilled directly from the reaction vessel into a cooled flask containing excess acetic anhydride dissolved in ether. The ether was then removed at room temperature by distillation under reduced pressure, solid sodium carbonate and water were added and the product was extracted with ether. After drying with anhydrous magnesium sulfate, the ether was removed and the amide V distilled at 24 mm. before mass spectral determination.

(23) The unlabeled amine has been reported by H. A. Shonle and J. W. Corse, U. S. Patent 2,424,063 (*Chem. Abstr.*, **41**, 7420 (1947)).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, POLYTECHNIC INSTITUTE OF BROOKLYN, BROOKLYN 1, N. Y.]

Conformational Aspects of Polypeptides. IX.[1] Synthesis of Oligomeric Peptides Derived from β-Methyl L-Aspartate

BY MURRAY GOODMAN AND FRANKLIN BOARDMAN[2]

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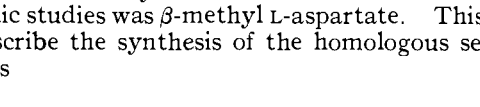
The synthesis of optically pure oligomeric peptides and polymers derived from β-methyl L-aspartate is described. The oligomers contain between 2 and 14 aspartate residues. Three general peptide synthetic methods were employed, utilizing the mixed anhydride, active ester and azide reaction sequences.

Introduction

Recent work by Karlson, Norland, Fasman and Blout,[3] and Bradbury, Downie, Elliott and Hanby[4] on the conformation of poly-β-benzyl L-aspartate has shown that a polypeptide derived from this amino acid in the L-configuration may form a left-handed helix. Past investigations of the polymers of a wide range of amino acids (*e.g.*, L-alanine, ε-benzyloxycarbonyl-L-lysine, γ-benzyl and γ-methyl L-glutamate[5,6] and L-methionine[7,8]) have shown that the L-configuration generally adopts a right-handed helix. Consequently, it was of interest to synthesize oligomers of an aspartate ester in order to determine the rotatory properties of peptides which have marginal helical stability. In order to avoid the possibility of acid solvolysis of a benzyl ester,[9] the amino acid chosen for synthetic studies was β-methyl L-aspartate. This paper will describe the synthesis of the homologous series of peptides



which may be abbreviated by the Brand–Edsall scheme as[10]

(1) This investigation was generously supported by a grant from the National Science Foundation (G8614). Previous paper in this series: M. Goodman, I. Listowsky, Y. Masuda and F. Boardman, *J. Am. Chem. Soc.*, in press.

(2) Submitted by Franklin Boardman to the faculty of the Polytechnic Institute of Brooklyn, 1962, in partial fulfillment of the requirements for the Ph.D. Degree.

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(4) E. M. Bradbury, A. R. Downie, A. Elliott and W. E. Hanby, *Proc. Roy. Soc.* (London), **A259**, 110 (1960).

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(8) G. E. Perlman and E. Katchalski, *ibid.*, **84**, 452 (1962).

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(10) E. Brand and J. T. Edsall, *Ann. Rev. Biochem.*, **16**, 223 (1947).