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Mass Spectrometry with Chemical Ionisation

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The fundamental processes occurring in the interaction of organic molecules with Brønsted and/or Lewis acids in the gas phase are discussed and the fragmentation of the principal classes of organic compounds initiated by chemical ionisation is examined. It is shown that the unique advantages of mass-spectrometry with chemical ionisation are manifested most strikingly in structural studies of complex natural and synthetic products with labile bonds.
The bibliography includes 107 references.

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I. INTRODUCTION

The origins of the development of chemical ionisation (CI) must be sought in experiments with molecular beams and the study of ion–molecule reactions in the gas phase†. The bibliography of the research on ion–molecule reactions during 1900–1966 includes more than 430 references and half of these cover the period since 1962.[4] In 1952 Tal'roze and Lyubimova detected for the first time the CH_5^+ ion (the methonium ion) in the ionisation chamber of a mass-spectrometer[5]. The first mass spectra with chemical ionisation[6,7] (CIM-spectra) were obtained in 1965 by Munson and Field on the Esso chemical physics mass-spectrometer[6,7]. These were the spectra of C_2H_6, n-C_3H_8, H_2O, and NH_3.[6,7] However, the application of CI in analytical chemistry began in 1966.[8] The extensive incorporation of CI in practical research is associated with the modification in 1969 of the two most common high-resolution mass-spectrometers—AEI MS-902 [9] and Du Pont/CEC 21-110C.[10] The development of a combined analytical method based on a gas–liquid chromatograph and a mass-spectrometer with chemical ionisation (GLC/MS-CI [11]) led to the intensive employment of CI in studies on complex biochemical mixtures. The application of CI in physicochemical research is associated with the development by Field of a method for the estimation of the kinetic parameters of reactions involving the molecular decomposition of protonated molecular ions and a method for the investigation of the kinetic parameters of reversible reactions[12] and also to some extent with the development of a procedure for the estimation of the proton affinities of the test compounds[13]. Finally the development of the CI-mass-spectrometry of negative ions made it possible to investigate S_N2 reactions in the gas phase[14-17].

The Essential Features of Chemical Ionisation‡. Problems of Nomenclature

At low pressures (10^{-5} mmHg and below) the ionisation of molecules and unimolecular decomposition of the ions formed take place in the ionisation chamber of the mass-spectrometer under electron impact. For example, the following reactions take place in methane:

$$CH_4 + e \rightarrow CH_4^{+\cdot}, CH_3^+, CH_2^{+\cdot}, CH^+, \ldots + 2e \quad . \tag{1}$$

The ions formed under these conditions are primary and the spectrum recorded is the primary spectrum of the reactant gas.

With increase of pressure, ion–molecule reactions between the primary ions and neutral molecules begin to occur together with reactions of type (1). For example, the following reactions take place in methane[5,21]:

$$CH_4^{+\cdot} + CH_4 \rightarrow CH_5^+ + CH_3^{\cdot}\,, \tag{2}$$

$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2\,, \tag{3}$$

$$CH_2^{+\cdot} + CH_4 \rightarrow C_2H_4^{+\cdot} + H_2\,, \tag{4}$$

$$CH_2^{+\cdot} + CH_4 \rightarrow C_2H_3^+ + H_2 + H^{\cdot}\,, \tag{5}$$

$$C_2H_3^+ + CH_4 \rightarrow C_3H_5^+ + H_2\,. \tag{6}$$

The ions formed under these conditions can be secondary or reagent ions and the spectrum recorded is the secondary spectrum of the reagent gas. Reactions (2)–(6) assume a significant importance at pressures above 0.01 mmHg and are characterised by extremely high rate constants (virtually each collision of a primary ion with a neutral molecule terminates in reaction). At 1 mmHg the CH_5^+, $C_2H_5^+$, and $C_3H_5^+$ ions constitute 95% of the overall ionic current (48, 51, and 6% respectively)[4]. It is significant that the secondary ions as a rule do not interact

† The term chemoionisation (chemiionisation) appeared in the literature in 1949 [1] and is used (in contrast to CI) to denote a wide variety of reactions between neutral atoms and molecules, leading to the formation of electrons and ions[2,3].

‡ In principle, chemical ionisation can be achieved in at least three ways[18]: (1) on double mass-spectrometers (tandems); (2) by ionic cyclotron resonance; (3) using the high-pressure mass-spectrometric technique. In the present review this problem is discussed only in relation to the use of the high-pressure mass-spectrometric technique. The experimental aspect of CI is examined in other reviews[19,20].

(or interact very slowly) with the neutral molecules of the reagent gas, i.e. the ratios of the intensities of the signals of the secondary ions in a specified range of reactant gas pressures do not vary to a first approximation (in the case of methane the $C_2H_3^+$ ion is an exception).

If approximately 0.1% of the test substance (the *substrate*) is added to the recent gas, then secondary ions interact with the substrate molecules, a proton or a hydride ion being transferred, i.e. they act like Brønsted and/or Lewis acids [reactions (7) and (8)]. The BH_2^+ ion will henceforth be referred to as the *protonated molecular ion* or the quasi-molecular ion $[M + H]^+$ and the ion B^+ will be called a *quasi-molecular ion* $[M - H]^+$. The BH_2^+ and B^+ ions can decompose under certain conditions [reactions (9)–(11)]. Reactions (12)–(14) illustrate the formation of cluster-ions§. The ions on the right-hand side of Eqn.(7)–(14) form the CH_4 spectrum of BH. The interaction with electrons and with the primary ions of the substrate molecules can be neglected in view of the low concentrations of the latter:

$$BH + CH_5^+ (C_2H_5^+) \rightarrow BH_2^+ + CH_4 (C_2H_4) \quad , \tag{7}$$

$$BH + C_2H_5^+ (CH_5^+) \rightarrow B^+ + C_2H_6 (CH_4 + H_2) \quad , \tag{8}$$

$$BH_2^+ \rightarrow B^+ + H_2 \quad , \tag{9}$$

$$BH_2^+ \rightarrow A_i^+ + B_i \quad , \tag{10}$$

$$B^+ \rightarrow A_{i'}^+ + B_{i'} \quad , \tag{11}$$

$$BH + C_2H_5^+ \rightarrow BH \cdot C_2H_5^+ \quad , \tag{12}$$

$$BH + C_3H_5^+ \hookrightarrow BH \cdot C_3H_5^+ \quad , \tag{13}$$

$$BH + BH_2^+ \rightarrow (BH)_2 H^+ \quad . \tag{14}$$

II. MAIN DEFINITIONS, PRINCIPLES, AND RELATIONS

One must distinguish two most common types of interactions occurring during CI: (1) acid–base interactions and (2) interactions of the oxidation-reduction type. The ions formed in the former and latter cases have even and odd numbers of electrons respectively. Examples of interactions of the first type are provided by reactions (7)–(14). Reactions of the second type are charge-transfer processes:

$$M + X^{+\cdot} \rightarrow M^{+\cdot} + X, \tag{15}$$

where M is a substrate molecule and X = He, Ne, Ar, Xe, NO, N_2, etc. The internal energy of the molecules ion formed (E_{in}) is equal in the given case to the difference between the recombination energy $RE(X^{\cdot})$ of the reagent ion in the gas phase and the ionisation potential IP(M) of the substrate molecule[23]: $E_{in} = RE(X^+) - IP(M)$. Exact knowledge of this quantity can prove extremely useful in theoretical studies. Charge transfer between the substrate molecule and reagent ion can be observed (and is indeed observed) for reagent gases of the first type (see Table) against the background of acid–base interactions if the recombination energy of reactant ions is fairly large. In the CH_4 spectra a phenomenon of similar kind has been observed for the reagent ions $C_2H_4^+$, $C_2H_5^+$ [29] and CH_5^+.[30]

Only processes leading to the formation of ions with an even number of electrons will be examined in detail in the present review. The following generalisations are applicable to them.

1. In contrast to photoionisation and ionisation by electrons, CI processes occur with an equilibrium of atomic nuclei and electrons (in the ground state).
2. The internal energy of the ions formed is much lower than in ionisation by electrons and approaches the energy of ions formed in ion–molecule reactions in the condensed phase.
3. The ions formed in CI interact with gas molecules, transferring the excess energy until the occurrence of thermal equilibrium; it is therefore assumed that the range of ionic energies is fairly narrow and corresponds approximately to the range of energies in the usual chemical reactions in solution[29,31]¶.

The reagent gases used for chemical ionisation [PA(X) is the proton affinity and $RE(X^+)$ (eV) the recombination energy].

Acid–base reactions (type 1) (Refs.22–27)			Charge transfer (type 2) (Ref.28)		
gas (X)	reagent ions	PA(X), kcal mole^{-1}	gas	principal ion	$RE(X^+)$, eV
H_2	H_3^+	100	He	$He^{+\cdot}$	24.6
CH_4	CH_5^+	127	Ne	$Ne^{+\cdot}$	21.6; 21.7
CH_4	$C_2H_5^+$	159	Ar	$Ar^{+\cdot}$	15.8; 15.9
CH_4	$C_3H_5^+$	—	Ar	Ar^{2+}	~24
C_2H_4	$C_2H_5^+$	160	N_2	$N_2^{+\cdot}$	15.3
iso-C_4H_{10}	t-$C_4H_9^+$	195	N_2	N^+ or $(N_2)^{2+}$	
iso-C_5H_{12}	t-$C_5H_{11}^+$	—	N_2	$N_4^{+\cdot}$	
C_6H_{14}	$C_6H_{13}^+$	—	Kr	$Kr^{+\cdot}$	14.0; 14.7
C_8H_{18}	$C_8H_{17}^+$	—	Kr	Kr^{2+}	~21
C_8H_{18}	$C_6H_{13}^+$	—	CO	$CO^{+\cdot}$	14.0
C_8H_{18}	$C_5H_{11}^+$	—	CO	$(CO)_2^{+\cdot}$	11–12
H_2O	H_3O^+	165	CO_2	$CO_2^{+\cdot}$	13.8
H_2S	H_3S^+	170	CO_2	$(CO)_2^{2+}$	—
CH_3OH	$CH_3OH_2^+$	182	CO_2	$O_2^{+\cdot}$	9.7–17.0
NH_3	NH_4^+	207	Xe	$Xe^{+\cdot}$	12.1–13.4
CH_3NH_2	$CH_3NH_3^+$	216	Xe	Xe^{2+}	18–20
$(CH_3)_2NH$	$(CH_3)_2NH_2^+$	222	O_2	$O_2^{+\cdot}$	9.7–17.0
$(CH_3)_3N$	$(CH_3)_3NH^+$	227	CF_4	CF_3^+	IP=9.17
$(CH_3)_4Si$	$(CH_3)_3Si^+$	—	NO	NO^+	9.25 (8.3)

All three factors determine the specific nature of even-electron CIM-spectra, namely (a) the degree of fragmentation is smaller than in ionisation by electrons, (b) the peaks of quasi-molecular ions $[M + H]^+$ and /or $[M - H]^+$ are usually very intense, and (c) fragment ions giving rise to fairly intense peaks and having masses close to that of the quasi-molecular ions are as a rule identified in the CIM-spectrum.

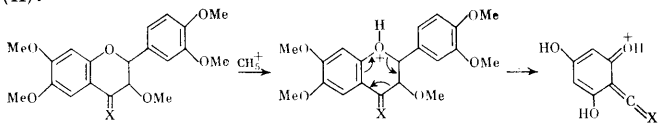
§ The presence of cluster ion peaks in the CIM-spectrum greatly simplifies the solution of the problem of the identification of the peak due to the quasi-molecular ion $[M + H]^+$.[22]

¶ However, one must bear in mind that, although comparison with behaviour in solution is attractive and fruitful, it suffers from certain limitations due to the following three factors: (1) the reaction time in CI is much shorter (approximately 10^{-6} s); (2) the energies of the ions are somewhat higher; (3) there is no solvation.

It is noteworthy that complex molecules, when the latter can be transferred to the gas phase without decomposition, can suffer less fragmentation than relatively simple ions, in view of the greater possibility of a rapid redistribution of the vibrational energy of the ions among the internal degrees of freedom under conditions where the local excess energy is still insufficient for bond dissociation[32].

It is assumed that in a non-polar or weakly polar molecule fragmentation occurs as a result of random attack by the reagent ion on a particular section of the molecule, but fragmentation is as a rule localised at the site of attack (see, however, Foltz and Fentiman[33]). It has been shown that the presence of a static dipole in the molecule appreciably increases the cross-section of ion–molecule reactions[34], so that it is reasonable to suppose that the greatest number of effective collisions of the reactant ions with substrate molecules involve functional groups[29]. When the molecule contains several functional groups, the attack occurs to some extent via all the functional groups and the CIM-spectrum can be usually regarded as a combination of ions formed as a result of attack via each functional group separately. Such behaviour can be accounted for by the fact that the ions formed in CI have a relatively low energy and the migration of the charge in an even-electron ion usually requires the simultaneous migration of atoms or a group of atoms in the ion.

It follows from the foregoing considerations that the fragmentation of a polyfunctional compound with an increased lability of the molecule can nevertheless lead to the appearance of an intense peak of the $[M + H]^+$ ion when the molecule contains at least one group which "leaves with difficulty". For example the presence of the carbonyl group, which is readily protonated with formation of a stable ion, in the molecule of the flavanoid 2,3-*trans*-3,5,7,3′,4′-pentamethoxyflavanone (I) leads to an appreciable increase of the stability of the ion $[M + H]^+$ compared with 2,3-*cis*-3,5,7,3′,4′-pentamethoxyflavan (II):[35,36]



(I): X=O, $[M + H]^+$(52%)*, *m/e* 181 (16%); (II): X=H_2, $[M + H]^+$(18%), *m/e* 167 (44%)*. (16)

An analogous example with fragmentation of 5α-androstan-17β-ol (III) and 17β-hydroxy-5α-androstan-3-one (IV), for which the $[M + H]^+$ peak intensities are 3.9 and 50.2% respectively, has been published[20]†. The low intensity of the peak due to the ion $[M + H]^+$ derived from compound (III) is due to the great ease of the elimination of the OH group as H_2O following its protonation. The sharp increase of the stability of the ion $[M + H]^+$ derived from compound (IV) is associated, as in the previous example, with the presence of the carbonyl group.

The tendency of various functional groups to be eliminated has not been subjected to a systematic comparative study, but it is known, for example, that NH_2 is eliminated in the form of NH_3 with difficulty, halogens are eliminated as HHal, and the acetoxy-group is eliminated as CH_3COOH (the last two processes take place relatively readily), etc. It has been suggested[29] that the ease of elimination of the functional group Y from the molecule RY for the same R is inversely related to the proton affinity of the HY molecules and the following sequence, in terms of increasing ease of elimination of the most common functional groups, has been proposed (based on the proton affinities taken from Haney and Franklin[37]):

$$NH_2 < CH_3S \simeq C_6H_5 \simeq CH_3O \simeq OC(:O)H < CN \simeq SH < OH < I \simeq Cl \simeq Br\ .$$

The influence of the nature of R on the ease of elimination of the functional group can be estimated from general considerations. Thus, if R is an alkyl group, then the ease of elimination of Y increases in the sequence[38]: $k_{prim} < k_{sec} < k_{tert}$.

The substrate molecule is protonated only if the proton transfer from the reagent ion is an exothermic reaction. The heat of proton transfer is determined by the proton affinity of the substrate molecule M and that of the conjugate base X of the reagent ion, which is a measure of the acid properties of the reagent ion as a Brønsted acid: $\Delta H = PA(X) - PA(M)$. Consequently, by varying the acid strength of the reagent ion, it is possible to vary the exothermic property of the proton transfer reaction and hence the degree of fragmentation[23].

It can now be regarded as established that thermally activated fragmentation can occur under CI conditions. Thus the extremely significant influence of the temperature of the ion source on the degree of fragmentation of t-pentyl acetate and benzyl acetate in the presence of iso-C_4H_{20} has been noted[12]. It was found that CH_3COOH is eliminated with absorption of heat. Furthermore, when account was taken of the insignificant exothermic effect of protonation in the presence of iso-C_4H_{10} (between −8 and −13 kcal mole^{-1}) and the large number of collisions (approximately 200) of the protonated ion with the surrounding gas molecules, it could be claimed with sufficient confidence that the weakly excited ions formed initially rapidly reach thermal equilibrium with the surrounding medium, i.e. their distribution with respect to energies becomes of the Boltzmann type or approaches the latter. Kinetic analysis of experimental data obtained from iso-C_4H_{10} spectra recorded over a fairly wide temperature range showed that the rate constants for the unimolecular decomposition of ions obey the Arrhenius equation[12]. The use of reagent ions with fairly high acid strengths leads to fragmentation in a vibrationally excited state before the attainment of thermal equilibrium with the surrounding medium. For example, the protonated molecular ion of N-valeryl-leucine, formed in a methane plasma, decomposes in the energy state equivalent to the energy state of the same ion in the isobutane plasma at a temperature higher by 167 ± 10 K than the temperature of the methane plasma[39]. Thus, when the CIM-spectra obtained with different reagent gases are compared, the temperature of the ion source must be taken into account.

III. MASS SPECTRA OF THE CHEMICAL IONISATION OF DIFFERENT CLASSES OF ORGANIC COMPOUNDS

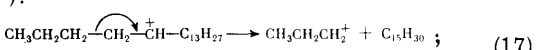
The most important factor in the study of the CI-fragmentation of organic compounds is that the reactions occurring in the ion source of the mass spectrometer obey the majority of the rules developed for ion–molecule reactions in the condensed phase. As one of the "mildest" ionisation methods, the CI procedure is most suitable in the study of the structures of labile molecules; it ensures

† As in the previous example, here the peak intensity is expressed as a percentage relative to the overall ionic current.

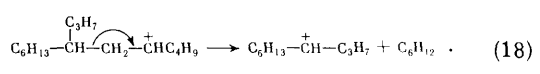
as a rule the presence of an intense peak due to the quasi-molecular ion for a fairly high degree of fragmentation. The nature of fragmentation in CI gives rise to the hope that this method will be most useful in the study of the content of functional groups in the substrate molecule and in the study of the nature and sequence of large blocks joined by labile bonds in the molecule. In ionisation by electrons [the electron impact (EI) method] the molecule is subjected to a more severe influence, as a result of which one can obtain data for the structure of its rigid skeleton. Thus the two methods (CI and EI) yield mutually supplementary information about the molecular structure.

1. Alkanes

The CH_4 spectra of n-alkanes consist of the peak due to the ion $[M - 1]^+$, which has the maximum intensity, and the peaks of the fragment alkyl ions, whose intensities are approximately equal but much lower than that of $[M - 1]^+$, located at intervals of 14 a.m.u.[8] The relative intensity of the ion $[M - 1]^+$ is independent of chain length, and the form of the CH_4 spectra, for example, for $n\text{-}C_{44}H_{90}$ and $n\text{-}C_8H_{18}$, is completely analogous. On the other hand, in the electron impact (EI) ultraviolet spectra of n-alkanes the maximum intensity corresponds to the $C_3H_7^+$, $C_4H_9^+$, and $C_5H_{11}^+$ ions, while the intensity of the peaks due to ions with large masses rapidly decreases with increase of m/e.[40] In the CH_4 spectra of branched alkanes the intensity of the ion $[M - 1]^+$ is somewhat lower than for n-alkanes, but the decrease is much smaller than in the case of electron impact, where the M^+ intensity becomes negligibly small[40]. The rate of dissociation of the C–C bond at the branching site increases appreciably, which can be attributed to exothermic β-cleavage (on the other hand, for unbranched alkanes the β-cleavage reaction is endothermic[8]):



(V) (endothermic process) (17)



(VI) (exothermic process) (18)

In the CH_4 spectrum of compound (VI) the peaks of the fragment alkyl ions $C_{13}H_{27}^+$ and $C_{10}H_{21}^+$ are appreciably more intense compared with those of other alkyl ions. In addition, the olefin ions $C_{13}H_{26}^+$ and $C_{10}H_{20}^+$, which have a considerable diagnostic importance, are formed in small but detectable amounts; the mechanism of the formation of these ions has not been elucidated. Until recently, it has been suggested that the fragment alkyl ions are formed in n-alkanes exclusively as a result of the electrophilic attack by the reagent ion on the C–C bond[8]. It has now been demonstrated experimentally that their formation via an alternative mechanism with abstraction of an olefin from the ion $[M - 1]^+$ also takes place[41].

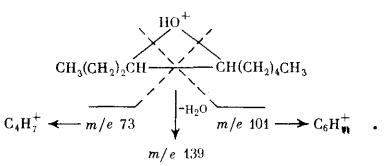
2. Cycloalkanes

Two series of ions have been detected in the CH_4 spectra of cycloalkanes: (1) $[M + 1]^+$ and $C_nH_{2n+1}^+$—the series of alkyl ions; (2) $[M - 1]^+$ and $C_nH_{2n-1}^+$—the series of alkenyl ions. The ion $[M + 1]^+$ is formed as a result of exothermic proton transfer from the reagent ion on ring opening. The ion $[M - 1]^+$ is formed when a hydride ion is split off from the cycloalkane molecule; it has the maximum intensity in the spectra. The study of the CH_4 spectra of cyclo-C_6D_{12} led to the conclusion that there is no hydrogen exchange between the substrate molecule and the reagent ions and that the $C_6D_{11}^+$ ion has the cyclohexyl and not the alkenyl structure. The presence of the $C_3D_7^+$ and $C_3D_6H^+$ ions indicates the occurrence of rearrangements with migration of hydrogen, because these ions originate from the hexyl ion $C_6D_{12}H^+$ and their peaks have approximately equal intensities[42].

3. Alkenes and Alkynes

The CH_4 spectra of monoalkenes resemble those of cycloalkanes, but the degree of fragmentation and the relative peak intensities corresponding to low m/e are greater for monoalkenes. Hydrogen is split off most readily from allyl positions. For polyenes, cycloalkenes, and alkynes, two series of ions are also observed, one of which originates from the quasi-molecular ion $[M + H]^+$ and the other from the quasi-molecular ion $[M - H]^+$.[43] A technique has been developed for the determination of the position of the double bond in the molecule of the initial olefin via its epoxy-derivative, using CH_4 or iso-C_4H_{10} as reagent gases (this method requires not more than 1 μg of the test substances):

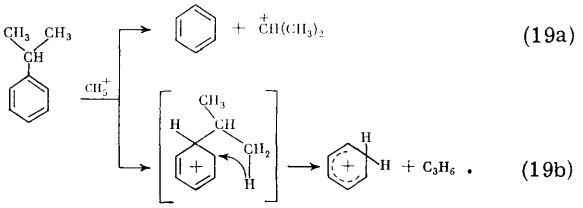
Scheme 1



The position of the epoxide ring and hence of the double bond can be readily established from the fragment peaks (see Scheme 1). Unfortunately no differences have been observed between the *cis*- and *trans*-isomers in any of the CIM-spectra of the epoxy-derivatives investigated[44].

4. Aromatic Hydrocarbons[45]

Aromatic rings are resistant to the action of reagent ions of the first type (see Table). For example, ions with a mass smaller than that of M^+ are not observed in the CH_4 spectrum of benzene. Together with the ions $[M + H]^+$, cluster ions having the composition $[C_6H_6 + C_2H_5]^+$, $[C_6H_6 + C_3H_5]^+$, etc. are formed. Alkyl groups are readily split off in the form of carbonium ions [reaction (19a)]. Furthermore, the alkyl side groups in aromatic hydrocarbons can undergo a fragmentation analogous to that of alkanes (see above). A hydride ion is split off from methyl groups with formation of a benzyl cation. In the presence of a side chain with three and more carbon atoms reactions involving hydrogen transfer and the elimination of an olefin [reaction (19b)] take place:



(19a)

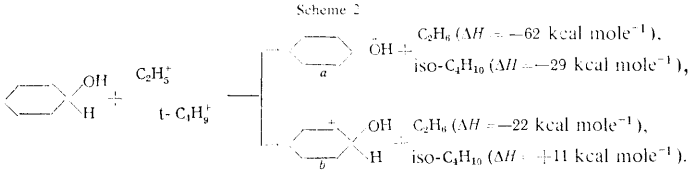
(19b)

5. Alcohols

The form of the iso-C_4H_{10} spectra of alkanols depends on the length and degree of branching of the hydrocarbon chain. For the first three members of the homologous series, the peak of the ion $[M + 1]^+$ has the maximum intensity in the spectrum. With increase of the length of the hydrocarbon chain, the intensity of the $[M + 1]^+$ peak falls sharply and the latter is altogether absent from the spectra of members of this series starting with C_8. The peak of the ion $[M - 1]^+$ is present with a fairly high intensity in the iso-C_4H_{10} spectra of all alcohols, with the exception of tertiary alcohols. The mechanisms of the formation of the ion $[M - 1]^+$ have been discussed in a number of studies[38,46,47]. One should note in the first place that initially the formation of this ion was considered exclusively in terms of hydride transfer from the alcohol molecule to the reagent ion[38]. However, the metastable transition $[M + 57]^+ \xrightarrow{*} [M - 1]^+$ has been observed[47]. Consequently at least some of the ions $[M - 1]^+$ should be formed when C_4H_{10} is split off from $[M + t\text{-}C_4H_9]^+$.

The initial hypothesis that α-hydrogen is split off preferentially in the spectra with CH_4 also proved to be invalid, because it was soon found[46] that in the CH_4 spectrum of 1-decan-1,1-d_2-ol the observed degree of elimination of α-deuterium would be expected on statistical grounds (10%). A more detailed study of this problem was undertaken[47] and it was found that specific elimination of α-deuterium is not observed in the CH_4 spectrum of cyclohexan-1-d-ol, but the extent of this process is inconsistent with that expected on statistical grounds. On the other hand, in the presence of iso-C_4H_{10} α-deuterium is split off preferentially (93%). It has been suggested that the abstraction of hydrogen is determined by the thermochemistry of competing reactions (Scheme 2):

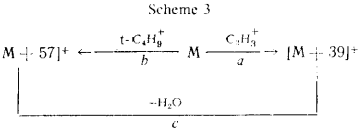
Scheme 2



If one takes into account the fact that endothermic reactions in CI are slow, it is not surprising that in the presence of iso-C_4H_{10} a specific exothermic reaction, involving the formation of the ion a with elimination of α-hydrogen, takes place, while in the presence of CH_4 the specific elimination of α-hydrogen is not observed (see Scheme 2).

The intensity of the peaks of the cluster ions observed in the iso-C_4H_{10} spectra of alcohols, namely $[M + 39]^+$, $[M + 57]^+$, and $[2M + 1]^+$, depends greatly on the conditions under which the spectrum is recorded[38]. Earlier studies of the CI-fragmentation of alcohols[38] led to the idea that the ions $[M + 39]^+$ and $[M + 57]^+$ are formed via pathways a and b:

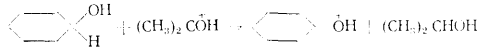
Scheme 3



However, in spectra with a high resolution only the peaks of ions having the composition $[M + C_4H_9 - H_2O]^+$ were found and not $[M + C_3H_3]^+$.[47] Consequently the ion $[M + 39]^+$ is formed when H_2O is split off from $[M + 57]^+$ (pathway c). It has been observed recently that the initial reaction between t-$C_5H_{11}OH$ and iso-C_4H_{10} involves the formation of the cluster ion $[C_9H_{20}OH]^+$, which rapidly dissociates with formation of $[C_9H_{19}]^+ + H_2O$ and $[C_5H_{11}]^+ +$ neutral products[48]. It is noteworthy that in the iso-C_4H_{10} spectra of alkanols, starting with C_5, the C_nH_{2n+1} peak has the maximum intensity in the spectrum. When CH_4 is used, the ion decomposes further with formation of a series of alkyl and alkenyl ions[38].

As regards the analytical applications of CI, it is of interest to examine the method whereby primary, secondary, and tertiary alcohols can be distinguished, and which is based on the significant differences between the ease of elimination of the hydride ion from the molecules of these compounds. When $(CH_3)_2CO$ or CH_3CHO is added to CH_4 in proportions of 20:1, methane reagent ions react almost completely with the carbonyl compound [reaction (20)]:

$$CH_5^+ (C_2H_5^+) + (CH_3)_2CO \rightarrow CH_4 (C_2H_4) + [(CH_3)_2COH]^+, \quad (20)$$

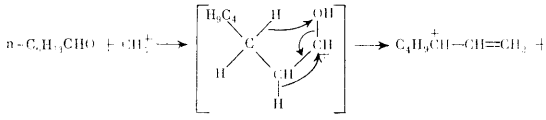


(21)

The oxonium ions formed in reaction (20) interact with secondary alcohols. This involves the elimination of hydrogen (in all probability from the α-position) and the formation of the ion $[M - 1]^+$ [reaction (21)]. An analogous reaction with primary alcohols takes place only in the presence of acetaldehyde. The ion $[M - 1]^+$ is not formed from tertiary alcohols[49].

6. Aldehydes and Ketones

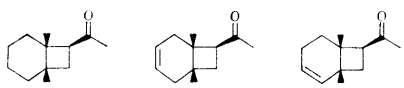
The CH_4 spectra of aliphatic aldehydes contain the peak due to the ion $[M + H - H_2O]^+$ in addition to that of the ion $[M + 1]^+$:



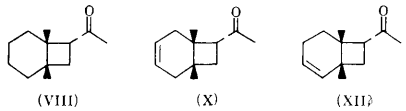
(22)

The driving force of reaction (22) is apparently the formation of a substituted allyl ion[50]. However, it must be emphasised that there is at present no independent evidence for the elimination of β-hydrogen from aldehyde molecules.

The CH_4 spectra of aliphatic ketones recorded under analogous conditions do not contain the $[M + H - H_2O]^+$ peak. Thus the spectrum of 2-octanone $C_{16}H_{13}COCH_3$ consists virtually of three peaks: $[M + 1]^+$, $[M + 29]^+$, and $[M + 41]^+$.[50] However, one should note that the formation of the ion $[M + H - H_2O]^+$ has been observed in the bicyclic ketones (VII)–(XII) and it has been found that the $[M + H - H_2O]^+$ peak for the *endo*-isomers (X) and (XII) is twelve times more intense than for the *exo*-isomers (IX) and (XI).[51] A test based on Dreiding models showed that the carbonyl oxygen in the *endo*-isomers (VIII), (X), and (XII) can approach closely to the hydrogen atoms of the six-membered ring, whereas in the *exo*-isomers it cannot. However, an increase of peak intensity is observed only for (X) and (XII) but not for (VIII), because in compounds (X) and (XII) hydrogen is transferred from the allyl position to the carbonyl group[51]:



(VII) (IX) (XI)

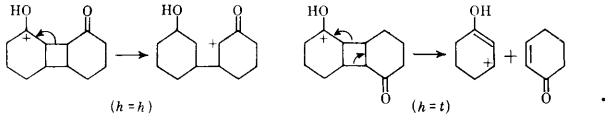


(VIII) (X) (XII)

7. Photodimers of Cyclic α,β-Unsaturated Ketones

The photodimerisation of α,β-unsaturated ketones results in the formation of isomeric compounds: $(h-h)$ and $(h-t)$. Comparison of the intensities of the peaks of the ions $[M+H]^+$ and $[M/2+H]^+$ in the CH_4 spectra of these compounds[52] showed that the intensity of the $[M/2+H]^+$ fragment peak for the $(h-h)$ isomers is appreciably lower than for the $(h-t)$ isomers. This fairly general phenomenon was explained by the fact that, in the β-cleavage of the C–C bond in the $(h-h)$ isomer, the positive charge must be localised in the vicinity of the carbonyl group, which inhibits the fragmentation[52]:

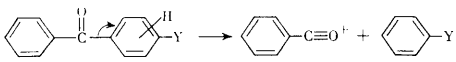
Scheme 4



$(h=h)$ $(h=t)$

8. Substituted Benzophenones[53]

The peaks of the ions $[M+H]^+$ and substituted and unsubstituted benzoyl ions were observed in the CIM-spectra of substituted benzophenones. It is believed that an increase of electron density at the substituted benzene ring leads to its preferential protonation. Since the fragmentation of the ion $[M+H]^+$ formed in this case leads to the formation of an unsubstituted benzoyl ion, its intensity in the CIM-spectrum increases:

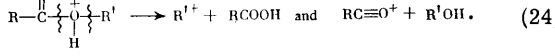


(23)

Protonation of the carbonyl oxygen apparently leads to the stable ion $Ar-\overset{+}{C}OH-Ar$, which exhibits no tendency towards fragmentation. Under electron impact, electron-donating substituents promote the formation of substituted benzoyl ions, probably in consequence of the stabilisation of the positive charge produced.

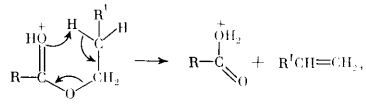
9. Esters

The peak of the ion $[M+H]^+$ in the CIM-spectra of esters is fairly intense (5% of the overall ionic current in the CH_4 spectrum of n-heptyl propionate) and exhibits no tendency towards a decrease with increase in the length of the hydrocarbon chain of the alcohol for a specified acid group. The ions R'^+ and $R-C\equiv O^+$, formed in heterolytic α-cleavage, give rise to moderately intense peaks:

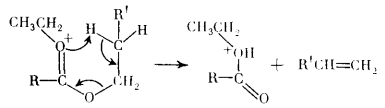


(24)

Alkenyl ions, formed when H_2 is split off from alkyl ions, are observed. As in the McLafferty rearrangement, processes responsible for β-cleavage lead either to the formation of the ion of a protonated carboxylic acid, which usually exhibits the maximum intensity in the spectrum [reaction (25)], or to transesterification if addition of the reagent ion takes place instead of protonation [reaction (26)]:



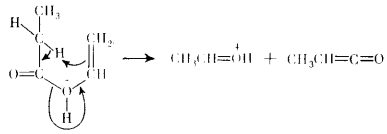
(25)



(26)

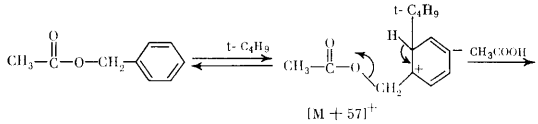
In the latter case ions with a moderate peak intensity are formed[54,55].

If the alcoholic component of the ester molecule possesses basic properties, for example, contains a double bond, it can itself serve as a proton acceptor. In this case reactions of type (25) and (26) are suppressed and are replaced by a proton transfer reaction with formation of a protonated aldehyde and the elimination of a ketene-like molecule[54]:



(27)

The additional nucleophilic centre can also become an acceptor of the reagent ion[56]. This concept made it possible to identify the ions $[M-3]^+$ in the iso-C_4H_{10} spectra of acetates, which had not been previously assigned[56,57]:



(28)

For formates, these ions are manifested as $[M+11]^+$ species, for propionates as $[M-17]^+$ species, etc.

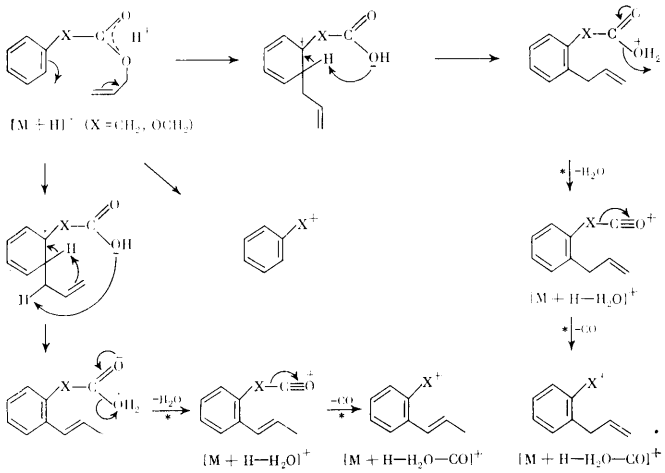
10. Fragmentation of Allyl Phenylacetates as an Example of Skeletal Rearrangements in Chemical Ionisation[58]

Under electron impact, the decomposition of the molecular ion is frequently accompanied by rearrangements[50,60]. In CI the rearrangements, particularly skeletal rearrangements, are encountered much more rarely. Field and Munson noted the occurrence of skeletal rearrangements in the bicyclo[2,2,2]octane[42], bicyclo[2,2,2]octene[43], and norbornadiene[61] systems using CH_4 as the reagent gas. The ions formed as a result of the rearrangements constitute approximately 18% of the overall ionic current. The formation of the rearranged ion $[CH_2OH]^+$ has been studied[62] in the protonation of $(CH_3)_2CO$ using H_2 as the reagent gas.

In the CH_4 spectra of allyl phenylacetate and allyl phenoxyacetate the ions formed in skeletal rearrangements constituted more than 40% of the overall ionic current[58]. The principal pathway to the fragmentation of the above compounds involves skeletal rearrangements and includes the successive elimination of elements of H_2O and CO from $[M+H]^+$ (Scheme 5). The *o*-allylation stage is analogous to the reaction in the condensed phase (the Claisen rearrangement)[63,64]. Allyl *ortho*-substitution is accompanied by the migration of H to the carbonyl group either from the *ortho*-position in the ring or from the allyl group. In

both cases the driving force of the reaction is the rearomatisation of the ring before the elimination of H_2O. If both *ortho*-positions in the ring are blocked, as, for example, in allyl 2',6'-dichlorophenylacetate, then a simple ester fragmentation is observed, i.e. the splitting off of allyl alcohol with subsequent elimination of CO. Electron-accepting groups in the *para*-position also suppress *o*-allylation. Electron donors in the *para*-position promote a sharp intensification of competing fragmentation reactions with formation of benzyl (tropylium) ions. There is no rearrangement on ionisation by electrons and only the formation of tropylium ions is observed. Thus the EI-sprectra of allyl phenylacetates are simpler than the CIM-spectra.

Scheme 5



11. Amines

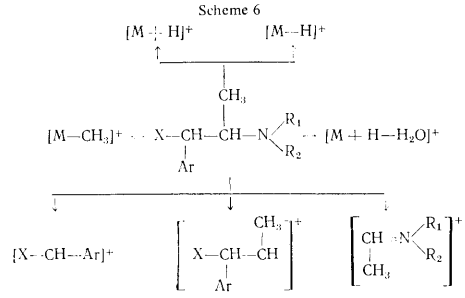
It has already been noted that the NH_2 group belongs to those which "leave with greatest difficulty" the protonated substrate molecule (Section II). Similarly the peak of the ion $[M + H]^+$ has the maximum intensity in the CIM-spectra of amines. The main fragmentation process on protonation of the amine molecule is due to the α-cleavage of the C–C bond relative to N. Thus the intensity of the peak with *m/e* 142 in the CH_4 spectrum of tributylamine is 19.9% of the overall ionic current, which is apparently due to the stabilising influence of the nitrogen atom[8,20]:

$$(n\text{-}C_4H_9)_3N + CH_5^+ \rightarrow \underset{m/e\ 142}{(n\text{-}C_4H_9)_2\overset{+}{N}{=}CH_2} + C_3H_8 + CH_4 \quad . \qquad (29)$$

The peak of the ion $[M - H]^+$ has a high intensity, probably for the same reason. Ions due to β- and γ-cleavage give rise to peaks with low and approximately equal intensities. The very intense peak due to the ion $[M - C_4H_9]^+$ was observed in the H_2 spectrum of tributylamine[65]. The postulate that the degree of fragmentation is independent of chain length, which is general for CI with transfer of even-electron species, has been confirmed in relation to polymeric tertiary amines[66].

The CH_4 spectra of four types of phenethylamine [α-amphetamine (XIII), *dl*-metamphetamine (XIV), *dl*-ephedrine (XV), and *dl*-methylephedrine (XVI)] have been investigated. There is no peak of the molecular ion in the EI-spectra of these compounds. The maximum peak of the ion $[CH_3{-}CH{=}N^{R_1}_{R_2}]^+$ is due to the α-cleavage of the C–C bond in relation to N and, with the exception of low-intensity ionic peaks with *m/e* 91 ($C_7H_7^+$) for (XIII) and *m/e* 77 ($C_6H_5^+$) for (XV), other characteristic peaks are absent from the EI-spectra of (XIII)–(XVI).

Scheme 6



(XIII): X = H, $R_1 = R_2 = H$;
(XIV): X = H, R_1 = H, $R_2 = CH_3$;
(XV): X = OH, R_1 = H, $R_2 = CH_3$;
(XVI): X = OH, $R_1 = R_2 = CH_3$.

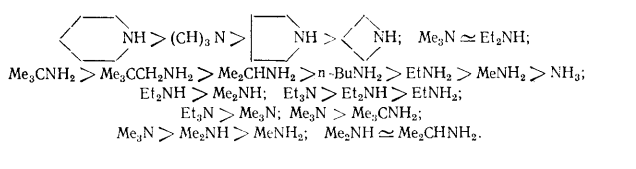
On the other hand, Scheme 6 shows that the CH_4 spectra of these compounds would alone be sufficient for the complete determination of their structure[65].

12. The Relative Basicity of Amines in the Gas Phase

The knowledge of relative basicities in the gas phase is of considerable interest for theoretical chemistry, since it makes it possible to infer the internal electronic effects in the molecule in the absence of a solvent. The determination of the relative basicities by the CI method is based on the fact that proton transfer is observed only in exothermic reactions with a negligible activation energy, i.e. the reaction

$$XH^+ + M \rightleftarrows X + MH^+ \qquad (30)$$

can proceed to the right only when $PA(M) > PA(X)$ (Section II). The relative basicities of amines determined in this way can be arranged in the following sequences[67]:



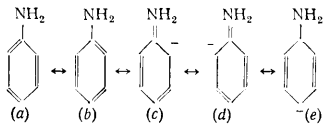
In conformity with the $+I$ effect, when the hydrogen in NH_3 is replaced by alkyl groups, the basicity of the amine should increase, which we observed in the gas phase. However, in solution there is a different order of basicities owing to the influence of solvation. For example, in aqueous solutions the basicity of methylamines falls in the following sequence:

$$Me_2NH > Me_3N > MeNH_2 > NH_3.$$

The explanation of this phenomenon is that the stabilisation of the system as a result of the formation of hydrogen bonds with H_2O diminishes with decrease of the number of H atoms linked to N and in the case of Me_3N the given effect predominates over the increase of the stability of the cation due to the $+I$ effect of the methyl groups[67,68].

In aqueous solutions the basicity of anilines is much lower than that of ammonia and methylamines [$pK_b(C_6H_5NH_2) = 9.42$; $pK_b(NH_3) = 4.77$]. An attempt was made to account for this finding by the contribution of resonance structures (*c*)–(*e*) to the additional stabilisation of anilines, which

would vanish on protonation, and also by the electron-withdrawing influence of the phenyl group[69]:



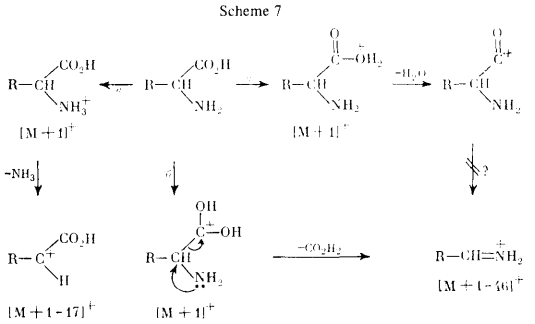
However, the difference between the resonance energies of benzene and aniline is not more than 2–4 kcal mole^{-1} and the contribution of structures (*c*)–(*e*) to the resonance stabilisation should be minimal, since they include charge separation. In the gas phase aniline is more basic than NH_3, which can apparently be accounted for by the high polarisability of the phenyl group, stabilising the positive charge. This hypothesis is confirmed by the fact that the basicity of *N*,*N*,*N*-triphenylamine is higher than that of aniline[70].

The low basicity of pyridine (lower than that of NH_3) has nowadays come to be explained by the sp^2 hybridisation of the N-atom, but, it has been shown[70] that in reality the basicity of pyridine in the gas phase exceeds that of dimethylamine and that the reason for the low basicity of pyridine in aqueous solution should therefore be sought in the first place in the effect of solvation and not hybridisation.

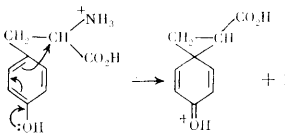
13. Aminoacids and Peptides

The presence of the ions $[M + 1]^+$, $[M + 1 - 18]^+$, $[M + 1 - 17]^+$, and $[M + 1 - 46]^+$ is characteristic of the CIM-spectra of the majority of aminoacids[71,72]:

Scheme 7

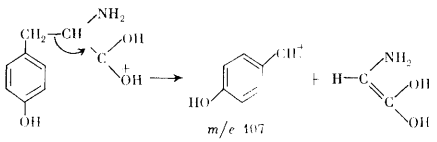


The fragmentation of glutamic acid, arginine, citrulline, and ornithine does not fit within the framework of this scheme[73]. The ion $[M + 1]^+$ has high-intensity peaks in all cases; the above aminoacids, where the peak due to the ion $[M + 1 - 18]^+$ has a high intensity and which are formed on pyrolytic elimination of H_2O, are an exception. The fragmentation of glycine, alanine, valine, leucine, isoleucine, and proline is confined to pathways *a* and *b*. A second H_2O molecule is abstracted from hydroxyaminoacids—serine, threonine, and 3-hydroxyproline. The peak due to the metastable ion, confirming the abstraction of CO, has not been observed in any of the CIM-spectra of aminoacids[72]. Fragmentation with elimination of elements of CO, which is extremely common under electron impact, is apparently encountered very rarely under CI conditions. The abstraction of NH_3 from $[M + 1]^+$ is of greatest theoretical interest. The fact that the peak of the ion $[M + 1 - 17]^+$ is not observed in the spectra of unsubstituted aliphatic aminoacids is evidence against a simple interpretation of the elimination, represented in Scheme 7 (pathway *b*). The elimination of elements of NH_3 is observed only for aminoacids containing a second nucleophilic group, which apparently indicates the involvement of this group in substitution and is accompanied by the formation of new bonds, for example:



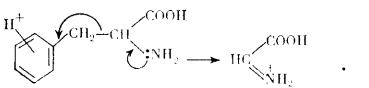
(31)

Such "co-operative" reactions are apparently of general importance in CI-fragmentation processes involving molecules of compounds with two and more nucleophilic centres (see, for example, Chizhov et al.[95]). The fairly intense peak of the ion with *m*/*e* 107, formed on cleavage of the α,β-bond, merits attention in the CIM-spectrum of tyrosine:



(32)

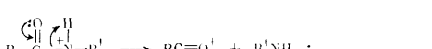
An analogous cleavage has been observed also for other aromatic aminoacids: phenylalanine, tryptophan, and histidine. In the absence of electron-donating substituents, the charge can be localised also on the amino-fragment:



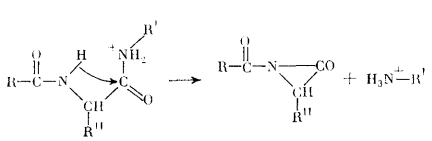
(33)

One of the most important methodological problems in bio-organic chemistry at the present time is the determination of the aminoacid sequence in peptides. The solution of this problem by mass spectrometry is based on the preferential cleavage of amide bonds in the ionisation of the peptide molecule[74]. To ensure their adequate volatility, peptides are modified by acylating the terminal amino-group and esterifying the terminal acid group. The EI-spectra usually contain only a complete set of amino-fragments (acylium ions). In addition, the interpretation of the EI-spectra of peptides is complicated as a result of the fragmentation of the molecules with elimination of side chains and requires the use of high-resolution mass spectrometers. The low peak intensities in the region of high masses lower the sensitivity of the method.

In CI there is usually a complete set of fragments of both the amino-type [reaction (34)] and the acyl type [reaction (35)]:



(34)



(35)

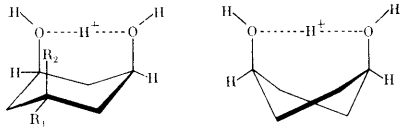
The presence of two series of ionic peaks in the CIM-spectra of peptides reduces the uncertainty in the determination of the aminoacid sequence and one can use for this purpose mass spectra with single focussing[75]. Owing to the lower degree of fragmentation and the relatively small difference between the densities of peaks due to ions with large and small masses, a much smaller amount of the specimen is required than in the recording of the EI spectrum (by a factor of approximately 100).[76] The CI method makes it

possible to record the spectra of peptides with a higher molecular weight than in the EI method.

The determination of satisfactory mass spectra of involatile substances and particularly peptides is one of the fundamental and most difficult problems in modern mass spectrometry. The most promising approaches in this field are those which do not require the modification of the initial substance in order to increase its volatility. Thus the application of the desorption method in an electric field apparently makes it possible to investigate unmodified peptides with a lower volatility and a greater variety of aminoacids than in the CI method[77]. However, the number of fragment ion peaks in the mass spectrum is frequently insufficient for the determination of the aminoacid sequence in the initial peptide. Considerable advances have been made recently also in the determination of the CIM-spectra of involatile substances by means of special methods[78-80]. Satisfactory spectra have been obtained for nonapeptides containing unprotected arginine and histidine, which made it possible to determine unambiguously the aminoacid sequences[78].

14. The Dependence of the Fragmentation in CI on the Stereochemistry and Conformation of Molecules in Relation to Cyclic Polyols and Steroid Aminoalcohols

As early as 1971, it was established[46] in relation to α,ω-diols, α,ω-dithiols, and α,ω-diamines with a long hydrocarbon chain that the formation of intramolecular hydrogen bonds stabilises the quasi-molecular ion $[M+H]^+$.[46] This phenomenon was used to investigate the stereochemistry of cyclic polyols[81]. The formation of proton bridges in the chair-conformation of *cis*-cyclohexane-1,3-diol (XVII) or the twist-form of *cis*-cyclohexane-1,4-diol (XVIII) lead to a marked suppression of the elimination of H_2O molecules from $[M + H]^+$. The addition of a third hydroxy-group to the molecule to give (XIX, R_2 = OH) results in a further stabilisation of the ion $[M + H]^+$ in consequence of the formation of two additional hydrogen bonds.



(XVII): $R_1 = R_2 = H$; (XVIII)

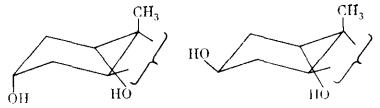
(XIX): $R_1 = H$, $R_2 = OH$;

(XX): $R_1 = H$, $R_2 = CH_3$.

The presence of a bulky substituent in the 5-position in (XX, $R_2 = CH_3$) destabilises the structure with a proton bridge and increases the degree of fragmentation. It is noteworthy that this increase is more pronounced for a high ionisation energy (CH_4 or iso-C_4H_{10} at high temperatures).

In contrast to their *cis*-isomers, the CIM-spectra of *trans*-cyclohexane-1,3-diol and *trans*-cyclohexane-1,4-diol contain intense peaks due to the ions $[M + H - H_2O]^+$ and $[M + H - 2H_2O]^+$, because stabilising proton bridges are not formed in the ions $[M + H]^+$ derived from these compounds. As in the spectra of monohydric alcohols, the spectra of the *trans*-isomers also contain an extremely intense peak due to the quasi-molecular ion $[M + H]^+$. It is believed[82] that a linear structure of the O...H⁺...O fragment, which cannot occur in *cis*- and *trans*-cyclohexane-1,2-diols, is necessary for the formation of a strong hydrogen bond; the flexibility of the cyclohexane ring leads to the final disappearance of the stereochemical advantage of the formation of the proton bridge in the *cis*-isomer, so that the CIM-spectra of *cis*- and *trans*-cyclohexane-1,2-diols are completely similar. However, the ion $[M + H]^+$ derived from cyclopentane-1,2-diols is appreciably more stable for the *cis*-isomer, which is probably associated with rigidity of the cyclopentane ring.

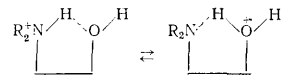
The results described above can be extended with reasonable confidence to more complex systems, although a significant difference between the stabilities of the ions $[M + H]^+$ derived from 5α-androstane-1α,3α-diol (XXI) and 5α-androstane-1α,3β-diol (XXII) was not observed[83].



(XXI) (XXII)

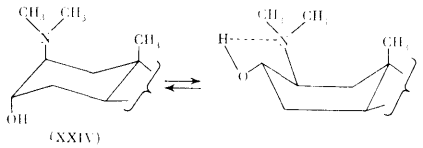
However, the use of milder ionisation conditions (95°C, direct injection of the specimen into the isobutane plasma[79]) made it possible to discover the differences between the spectra of (XXI) and (XXII) corresponding to the CIM-spectroscopic data for cyclohexane-1,3-diols[31].

When one of the hydroxy-groups is replaced by an amino-group, the main factor stabilising the quasi-molecular ion $[M + H]^+$ becomes proton transfer from oxygen to nitrogen in the bridge structure[83]:



(36)

This is why the *cis*-1,2- and *cis*-1,3-isomers are more stable than their *trans*-analogues, in which there is no proton transfer. It has been suggested[83] that the molecular conformation can play a very significant role in CI-fragmentation. The iso-C_4H_{10} spectra of 3α-dimethylamino-2β-hydroxypregnane (XXIII) and 2β-dimethylamino-3α-hydroxypregnane (XXIV) were interpreted from this standpoint. It has been shown by infrared spectroscopy[84] that an intramolecular hydrogen bond is formed in the corresponding *trans*-isomers of the cholestane series (evidently when the A ring of the steroid skeleton assumes the boat conformation):



(XXIV)

(37)

i.r.	30%	70%
CI	26.5%	73.5%

Using model compounds for which a transition to the boat conformation is known not to occur, the authors[83] estimated the conformational equilibrium for (XXIII) and (XXIV) on the basis of their iso-C_4H_{10} spectra. The results proved to be in excellent agreement with infrared spectroscopic data, which is extremely surprising bearing in mind the differences between the experimental conditions. Evidently further studies on these lines are extremely promising.

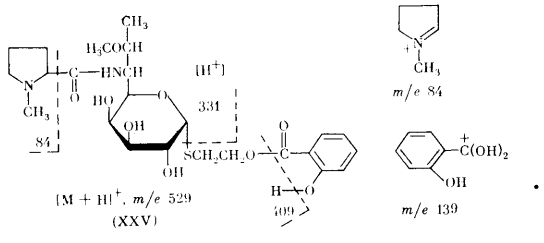
15. Carbohydrates, Nucleosides, and Macrolide Antibiotics

Mass spectrometry with electron impact is used nowadays to investigate carbohydrates and their derivatives[85]. When very small amounts of the specimen are available (sometimes $<10^{-6}$ g), valuable information about the molecular weight of the carbohydrate molecule, the nature and sequence of monosaccharide units, and in certain cases the position of the glycosidic linkages between the individual units can be obtained. The most suitable volatile derivatives for the study of the structures of oligosaccharides by the EI method are their acetates and methyl and trimethylsilyl ethers. However, the extremely low intensity and in certain cases the absence of the peak due to the molecular ion and the low intensities of the peaks due to fragment ions with high molecular weights impose very significant limitations on the analysis.

The most promising approach used to overcome these limitations involves the application of "mild" ionisation methods, for example, ionisation in a field[86], desorption in a field[86], and CI. It has been found that the degree of fragmentation under the conditions of ionisation in a field and desorption in a field is insufficient for the investigation of the structure. In studies involving the CI- fragmentation of carbohydrates and related compounds, the spectra of pentoses, hexoses, and their simple derivatives[32,87-92], oligosaccharide acetate[93,94], the methyl ethers of glycosyl- and biosyl-polyols[95], the antibiotics celesticetin and clindamycin, aminoacylthioglycosides[96,97], nucleosides[28,98,99], and macrolide antibiotics[24,100,101] were examined.

The results obtained fit within the framework of the generalisations developed in the study of the CIM-spectra of other classes of organic compounds. Thus the peak of the adduct ion $[M + NH_4]^+$ has been observed in the NH_3 spectra of oligosaccharide acetates (up to tetrasaccharides inclusive). The formation of this ion in the case of higher oligosaccharide acetates is apparently prevented by the thermal instability of these compounds when they are vaporised[93,94]. However, when the molecule contains a highly basic nitrogen atom, the peak of the ion $[M + NH_4]^+$ is absent from the NH_3 spectrum, since, instead of simple association, there is proton transfer from NH_4^+ to the nitrogen in the substrate molecule with formation of the ion $[M + H]^+$.[32] Thus the iso-C_4H_{10} and NH_3 spectra of the antibiotic celesticetin (XXV) differ markedly, because protonation in the presence of NH_3 can occur exclusively at nitrogen atoms remote from the aromatic part of the molecule and the formation of the ion with m/e 139 is strongly suppressed. On the other hand, in the iso-C_4H_{10} spectrum the ion has the maximum intensity, because protonation proceeds readily at the oxygen atom at the ester group of the salicylate. The peak of the methylpyrrolidinium cation with m/e 84 in the iso-C_4H_{10} spectrum has an extremely low intensity, while in the NH_3 spectrum its intensity is high[96]:

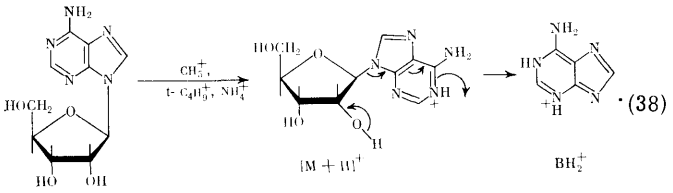
Scheme 8



The fragmentation of derivatives of pentoses and hexoses is extremely simple and in essence consists in successive and/or simultaneous elimination of functional groups, the degree of fragmentation being determined, as usual, by the acid strength of the reagent ions. For example, a detailed study of the fragmentation pathways of β-methyl-2,3,4,6-tetra-O-methyl-D-galactopyranoside using its deuterated analogues showed that the dominant process in the formation of ions with m/e 187 and 155 is the abstraction of methoxy-groups (in the form of methanol molecules) from the 3- and 4-positions and in the formation of ions with m/e 187 the most preferred process is abstraction from the 3-position. The first stage in the decomposition of the quasi-molecular ion $[M + H]^+$ with abstraction of methanol from the glycosidic centre was regarded as analogous to the rate-limiting stage in the acid hydrolysis of methylglycosides[90]. It has been shown[91] that the anomeric effect has a significant influence on the rate of decomposition of quasi-molecular ions and on the ratio of the intensities of the peaks due to the ions $[M+H]^+$ and $[M + H - CH_3OH]^+$ (m/e 251 and 219 respectively) and in the spectra of α- and β-methyl-2,3,4,6-tetra-O-methyl-D-galactopyranosides.

Examination of the iso-C_4H_{10} spectra of the methyl ethers of methyl deoxyhexosides led to the conclusion that the position of the deoxy-unit in 2,3-, 4-, and 6-deoxysaccharides can be determined from the ratio of the intensities of the peaks with m/e 125, 157, and 189 in spectra recorded at the same temperature[92].

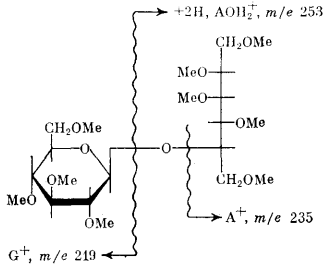
A complex aglycone, particularly when it contains powerful nucleophilic centres, tends to be split off in the form of a protonated species[27,88]. This decomposition pathway predominates in the CI-fragmentation of nucleosides:



(38)

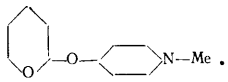
The formation of glycosyl ions in the given instance constitutes a much less probable fragmentation pathway[27,98]. The decomposition of the methyl ethers of glycosyl- and biosyl-polyols also proceeds with the preferential formation of the protonated aglycone (in this case the initial protonation apparently involves one of the five oxygen atoms in the methoxy-groups of the aglycone); however, also formed are the ions A^+ (probably on dissociation of the C–O bond on the side of the aglycone) and the glycosyl ion G^+ (with a much lower intensity) and it has been shown that the ratio of the intensities of the A^+ and AOH_2^+ peaks depends greatly on the position of the glycosidic linkage (1 → 2, 1 → 3, 1 → 4, and 1 → 6) in glycosylsorbitols[95]:

Scheme 9



The fragment ion peaks make it possible to determine directly the nature and sequence of the monomeric units of both glycosyl- and biosyl-polyols[95]. This is also true of the CIM-spectra of oligosaccharide acetates[93,94].

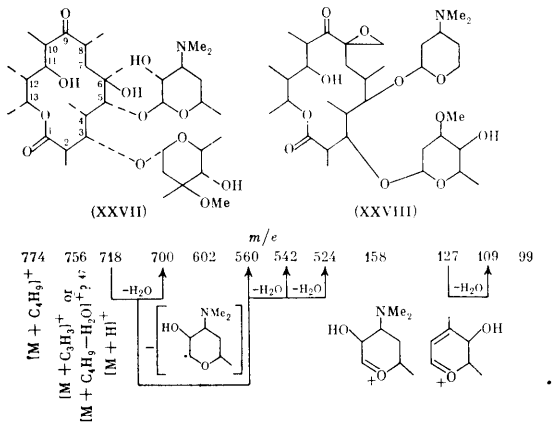
The ideas about the mechanism with the excitation centre remote from the site of bond dissociation were first formulated in the discussion of the fragmentation of macrolide antibiotics[100]. The possibility of fragmentation via this mechanism has been demonstrated rigorously and experimentally for compound (XXVI), which serves as a model of a glycoside with a complex aglycone[33]:



(XXVI)

The study of the CIM-spectra of macrolide antibiotics was stimulated by the fact that the EI peak of the molecular ion M^+ has an extremely low intensity and cannot always be detected. All the high-intensity peaks are located in the region of low mass numbers and the majority of them correspond to aminosugar fragments. The fragment ions corresponding to portions of the macrolide ring and neutral sugars are suppressed, so that it is extremely difficult to obtain structural information about these components of the molecule. On the other hand, in the iso-C_4H_{10} spectra of these compounds the peak of the ion $[M + H]^+$ has a very high intensity. The fragment ions in the middle part of the spectrum correspond to the successive elimination of sugar residues from $[M + H]^+$. Ions with low masses arise from sugar fragments when glyosidic linkages are broken with subsequent loss of one and more oxygen substituents—see Scheme 10 for the decomposition of the ion $[M + H]^+$ derived from erythromycin B (XXVII):

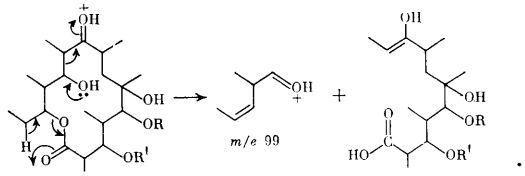
Scheme 10



(XXVII) (XXVIII)

Only the formation of fragment ions with *m/e* 99 and 602, giving rise to intense peaks, requires the dissociation of the C–C bond in the macrolide ring. The formation of an ion with *m/e* 99 can be regarded as the result of the protonation at the 9-oxo-group with subsequent opening of the ring at the lactone linkage as a result of the McLafferty rearrangement and the cleavage of the $C_{(10)}$–$C_{(11)}$ bond via a retroaldol type of fragmentation process (Scheme 11). The ion with *m/e* 602 corresponds to the protonated fragment (XXIX) after the elimination of one water molecule.

Scheme 11



(XXIX)

The structure of oleandomycin (XXVIII) resembles that of erythromycin B, but there are distinctive differences, the majority of which are clearly seen from the inspection of the iso-C_4H_{10} spectrum. Thus the peak of the ion arising in the retroaldol cleavage is very intense, but is displaced towards *m/e* 85, since the 13-substituent in (XXVIII) is a methyl group and not an ethyl group as in (XXVII). There is no peak due to the ion $[M + H - H_2O]^+$ in the spectrum of (XXVIII). This ion arises in acid-catalysed reactions involving macrolides which have a hydroxy-group in the 6-position, which is conformationally suitable for attack by the carbonyl group in the 9-position[102].

Information about the nature of the sugar units can be obtained by examining the ions arising in the cleavage of glycosidic linkages[24,100,101]. The iso-C_4H_{10} spectra of macrolide antibiotics with 16-membered rings have been examined[101]. They contain an extremely intense peak due to the $[M + H]^+$ ion and the peaks of fragment ions corresponding to the successive elimination of sugars and other smaller molecules—water, acetic acid, and methanol. The cleavage of C–C bonds is not a characteristic feature of the 16-membered macrolide ring.

The combination of CI, infrared, ultraviolet, and NMR spectroscopic methods and the mass-spectrometric method with electron impact together with a few rationally selected degradation reactions ensures a rapid and reliable determination of the structures of new compounds in this and related series.

IV. MASS SPECTROMETRY OF NEGATIVE IONS WITH CHEMICAL IONISATION. THE S_N2 REACTION IN THE GAS PHASE

Chemical ionisation by anions is significantly "milder" than ionisation by cations, which is understandable when one compares the proton (150–250 kcal $mole^{-1}$) and anion (5–25 kcal $mole^{-1}$)[17] affinities of molecules. Yet another cause of the "mildness" of CI by negative ions is associated with the fact that in the gas phase an odd-electron anion has sufficient energy for autoneutralisation, i.e. for the ejection of an electron, and the activation energy for the reaction must be very much smaller than the electron affinity of the neutral molecule. In the association of the molecule with an even-electron anion in the presence of an excess of internal energy there is a possibility of the reverse decomposition into a neutral species and an anion[17]. The CI-mass-spectrometry of negative ions has become readily available after the development of an ion source with a Townsend discharge, which makes it possible to obtain beams of positive and negative ions of comparable intensity[103,104]. The range of its analytical applications is limited apparently by the need to determine traces of substances, especially halogen-containing compounds (in particular, chlorine-containing pesticides[105] and insecticides[106]). Its application in general structural analysis is probably limited by inadequate fragmentation (however, see Hunt et al.[104]).

An interesting application of gas-phase reactions of anions as a guide to carrying out the corresponding reactions in solutions has been outlined[107].

The most fruitful application of the CI-mass-spectrometry of negative ions was found in physicochemical research. Having examined the changes in the heats and entropies of association of Cl^- in the series CH_3Cl, CH_2Cl_2, $CHCl_3$, and CCl_4 and having taken into account the fact that 1-bromoadamantane and 1-bromo[2,2,2]bicyclooctane do not combine with Cl^-, Br^-, and I^-, Dougherty and coworkers concluded that the ionic clusters RXY^- (where X and Y are halogens) are identical with the S_N2 transition state in Finkelstein's reactions[14]. Thus the data obtained for these ions can be used to estimate the influence of the solvent on the reactivity in S_N2 reactions. Both the influence of the nucleophilic properties of the attacking base[15] and the structural influence of the alkyl groups[16] have been examined. It has been found that the nucleophilic properties of Cl^-, Br^-, and I^- in the gas phase are approximately the same and that the differences between the nucleophilic properties of the same bases in Finkelstein's substitution reactions are caused mainly by the changes in the relative degrees of solvation of the reactants and the transition states in exchange processes[15].

It is believed that a branched hydrocarbon group interferes with S_N2 reactions. Thus the exchange of the halogen in neopentyl bromide via an S_N2 mechanism is to a large extent suppressed[108]. However, this classical model is apparently applicable solely to S_N2 reactions in the condensed phase, because it has been found[16] that the heats of association of Br^- increase in the sequence MeBr < EtBr < iso-PrBr < t-BuBr (α-branching) and EtBr < iso-BuBr < neopentyl bromide (β-branching). The entropies of association of these compounds in the gas phase vary approximately in parallel with the corresponding changes in solution. The conclusion that the structural influences of the alkyl groups on the reactivity in S_N2 reactions in solution are controlled exclusively by solvation is therefore valid.

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The foregoing review shows that the CI method has been finding increasingly wide applications in the identification and determination of the structures of organic compounds. Its advantages when used for these purposes compared with the electron impact method consist in the presence of stable MH^+ ions even in those cases where the M^+ ion is unstable. This makes it possible to find the molecular weight directly from the CIM-spectra. The differences between the CIM-spectra of structural isomers and stereoisomers are frequently greater than in the EI method, which makes the CI method particularly valuable for the solution of structural and stereochemical problems, which cannot be solved by EI-mass-spectrometry. Furthermore, the CI method has proved extremely promising for the investigation of the mechanisms of various gas-phase reactions and the determination of the intrinsic values of fundamental characteristics of molecules and other species, such as basicity and nucleophilicity. All these factors give rise to the hope that new valuable results will be obtained in the immediate future with the aid of the CI method.

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