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MASS SPECTROMETRY OF PYRIDINE COMPOUNDS—III*

REARRANGEMENTS IN THE MOLECULAR ION OF THE METHYL ESTER OF ISONICOTINIC ACID

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Abstract—In the molecular ion of the title compound some interesting rearrangements occur, as shown by diffuse peaks for the following reactions:

(a) Elimination of a molecule of water and of a hydroxycarbonyl radical from the molecular ion pointing to transformation of the ester group to a carboxylic acid function.

(b) Loss of a molecule of carbon monoxide and of a formyl radical from the molecular ion, indicating that the pyridine ring and the methoxyl group are linked together possibly by a 1,2-shift of the pyridine ring to the oxygen atom of the methoxyl group in the molecular ion.

INTRODUCTION

IN OUR PRECEDING PAPER[1] it has been shown, that in the molecular ion of isonicotinic acid an exchange between β- and hydroxylic hydrogens occurs prior to or during the expulsion of a hydroxyl radical and of a molecule of water. With the rationalization, given for this exchange, in mind we might expect that the methyl hydrogens are mixed with the β-hydrogens in the molecular ion of the methyl ester of isonicotinic acid.

The mass spectrum of the unlabeled methyl ester of isonicotinic acid appears to be very simple: the only important breakdown proceeds through the successive loss of a methoxyl radical, a molecule of carbon monoxide and of a molecule of hydrogen cyanide, when only peaks with a relative intensity $\geq 2\%$ are considered [$M^{+\cdot} = 137$ (90%); $(M—OMe)^+ = 106$ (100%); $(M—OMe—CO)^+ = 78$ (90%); $(M—OMe—CO—HCN)^+ = 51$ (58%)].

It is therefore quite possible to study the supposed exchange by examination of the mass spectra of site-specifically labeled methyl esters of isonicotinic acid.

The D-labeling has not given any indication for the suggested exchange prior to or during the loss of a methoxyl radical either in the ion source—or in the first—or in the second field free regions of the mass spectrometer.

Nevertheless, in the mass spectrum of the methyl ester of isonicotinic acid some diffuse peaks are observed pointing to rearrangements of the molecular ion, initiated by transfer of one of the β-hydrogens to the carbonyl oxygen atom, similar to that found in the molecular ion of isonicotinic acid.[1] Details of these rearrangements have been elucidated thanks to the D-labeling, as will be shown below.

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* For Part II, see ref. 1.

† This paper has been presented at the fifth meeting of the British Mass Spectroscopy Group, held at Bristol University on 13–15 July, 1971.

RESULTS AND DISCUSSION

1. *Successive loss of a molecule of water and of carbon monoxide from the molecular ion (Schemes 1 and 2)*

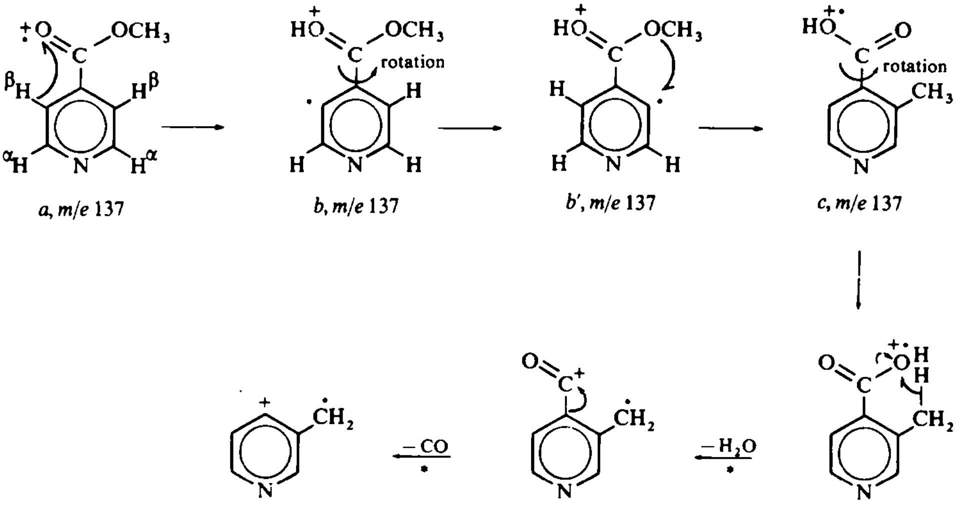
The molecular ion of the unlabeled methyl ester of isonicotinic acid appears to expel a molecule of water in the first- and in the second field free region of the mass spectrometer, as shown by the refocusing technique[3] and by an obvious diffuse peak in its mass spectrum (rel. int. 0·06%).

This reaction demands a rather drastic rearrangement of the molecular ion and without labeling data it may be rationalized in the following way (Scheme 1):

As proposed earlier for isonicotinic acid,[1] first one of the β-hydrogens may migrate to the carbonyl oxygen atom in the molecular ion *a* to produce ion *b*, which is similar to its rotation isomer *b*′.

Then, the methyl group may be transferred to the vacant β-position of the pyridine nucleus giving ion *c* or its rotation isomer *c*′, which essentially has the structure of β-methyl isonicotinic acid. Expulsion of a molecule of water may then proceed through migration of one of the methyl hydrogens to the hydroxylic oxygen atom in a six-membered transition state as shown in sequence *c*′ → *d*, a well-known process in benzoic acids with *ortho* substituents containing α-hydrogens.[2]

Finally, carbon monoxide may be eliminated from ion *d* to give ion *e*.



SCHEME 1. Rationalization of the successive loss of water and carbon monoxide from the molecular ion of the methyl ester of isonicotinic acid.

This idea has been checked by studying the mass spectra of suitably deuterated* methyl esters of isonicotinic acid. Relevant data have been summarized in Table 1.

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* Labeled compounds have been prepared which give diffuse peaks for loss of water molecules which are separated as far as possible from the [M—methoxyl]$^+$-peaks.

The data from this Table show that the rationalization, presented in Scheme 1, can be partly true not only because a β- and an ω-hydrogen atoms participate in the expulsion of water, but also to some extent an α-hydrogen atom. Furthermore it must be noted that the *molecule of water* does contain *one α-hydrogen—and/or one β-hydrogen atom at the most*, as diffuse peaks for loss of D_2O from the α-d_2 and β-d_2 analogues have not been observed; this restriction is not applicable to the ω-hydrogens (*cf.* Table 1). The data of Table 1 further indicate that the three kinds of hydrogen atoms do not participate equally in the loss of water.

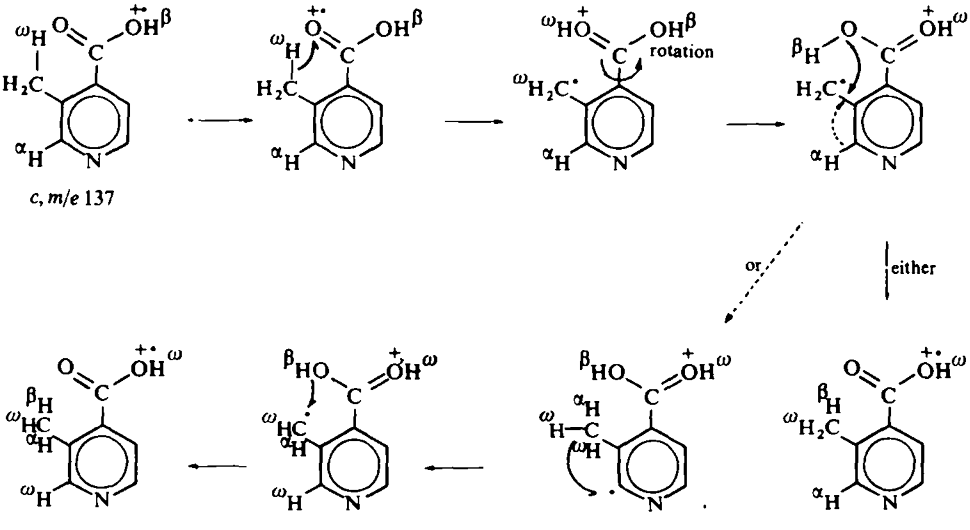
It is therefore not possible to interpret them quantitatively by use of a simple rationalization. However, inspection of the observed ratios for the β-d_2, ω-d_2 and ω-d_3 analogues points to a rather extensive mixing of β- and ω-hydrogen atoms before loss of a molecule of water.*

With this knowledge it is possible to predict roughly the ratios when the following assumptions are made:

(a) In a fraction of ions *c* or *c'* (Scheme 1) one β-hydrogen and three ω-hydrogen atoms are randomly distributed by exchange over the hydroxyl- and methyl group.

(b) In another fraction of ions *c* or *c'*, satisfying the first assumption, the α-hydrogen atom next to the methyl group exchanges with the equilibrated hydrogen atoms from the methyl group. This can not be distinguished from an exchange of an α-hydrogen atom with a mixture of one β- and three ω-hydrogen atoms so that the exchanged α-hydrogen atom will be randomly distributed over the hydroxyl- and methyl group.

Some ways, along which such an exchange could take place are shown in Scheme 2.



SCHEME 2. Rationalization of the participation of one α-, one β-, and three ω-hydrogen atoms in the loss of a molecule of water from ion c' (cf. scheme 1).

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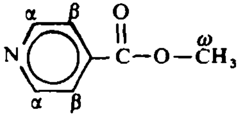
* α,β-scrambling in the unrearranged molecular ion is not considered because of the absence of such scrambling in the molecular ions of isonicotinic- and nicotinic acid.[1]

TABLE 1. OBSERVED[a] AND CALCULATED RATIOS FOR LOSS OF LABELED AND UNLABELED MOLECULES OF WATER FROM THE MOLECULAR IONS OF SITE SPECIFICALLY DEUTERATED METHYL ESTERS OF ISONICOTINIC ACID.



IN THE FIRST AND SECOND FIELD FREE REGION OF THE MASS SPECTROMETER AT 70 EV, DERIVED FROM THE AREAS OF THE CORRESPONDING DIFFUSE PEAKS

Labeled position and corresponding equation,[b] used for the calculated ratios $[H_2O]/[HDO]$ and/or $[HDO]/[D_2O]$	Ratio in the first field free region				Ratio in the second field free region			
	$[H_2O]/[HDO]$		$[HDO]/[D_2O]$		$[H_2O]/[HDO]$		$[HDO]/[D_2O]$	
	Obs[c]	Calc[d]	Obs[c]	Calc[d]	Obs	Calc[e]	Obs	Calc[e]
α-d_2 $:\dfrac{4i-2ix}{x+ix}=\dfrac{[H_2O]}{[HDO]}$	5·5	5·5	—	—	6·6	6·0	—	—
β-d_2 $:\dfrac{8i+2ix}{4-ix-x+4i}=\dfrac{[H_2O]}{[HDO]}$	1·4	1·5	—	—	1·9	1·6	—	—
ω-d_2 $:\dfrac{4i+4ix}{8-x-ix+8i}=\dfrac{[H_2O]}{[HDO]}$	0·5	0·5	—	—	0·6	0·5	—	—
ω-d_2 $:\dfrac{8-x-ix+8i}{4-2x}=\dfrac{[HDO]}{[D_2O]}$	—	—	5·8	6·3	—	—	7·0	7·2

ω-d_3[g]	$: \dfrac{4 + 4i + x + ix}{8 - 4x} = \dfrac{[HDO]}{[D_2O]}$	—	—	2·1	1·8	—	—	2·6	2·1
α,β-d_4	$: \dfrac{8i - 4ix}{4 + 4i + x + ix} = \dfrac{[H_2O]}{[HDO]}$	0·9	0·9	—	—	1·1	1·0	—	—
α,β-d_4	$: \dfrac{4 + 4i + x + ix}{2x} = \dfrac{[HDO]}{[D_2O]}$	—	—	—[f]	16	—	—	—[f]	18
α,ω-d_4[g]	$: \dfrac{8 + 8i - x - ix}{4 + 4x} = \dfrac{[HDO]}{[D_2O]}$	—	—	3·4	3·7	—	—	3·8	4·3
β,ω-d_4[g]	$: \dfrac{4i + 4 + ix + x}{8 - 4x} \quad \dfrac{[HDO]}{[D_2O]}$	—	—	1·5	1·8	—	—	1·8	2·1

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[a] Average of at least five runs; estimated accuracy ±10%

[b] The equations have been derived using the assumptions, mentioned in the text

[c] Corrected for natural ^{13}C and ^{15}N contributions (7%) and for loss of hydroxyl (±10% with respect to the loss of water from the molecular ion of the unlabeled compound)

[d] Calculated with $x = 0{\cdot}37$ and $i = 1{\cdot}7$

[e] Calculated with $x = 0{\cdot}37$ and $i = 2{\cdot}1$

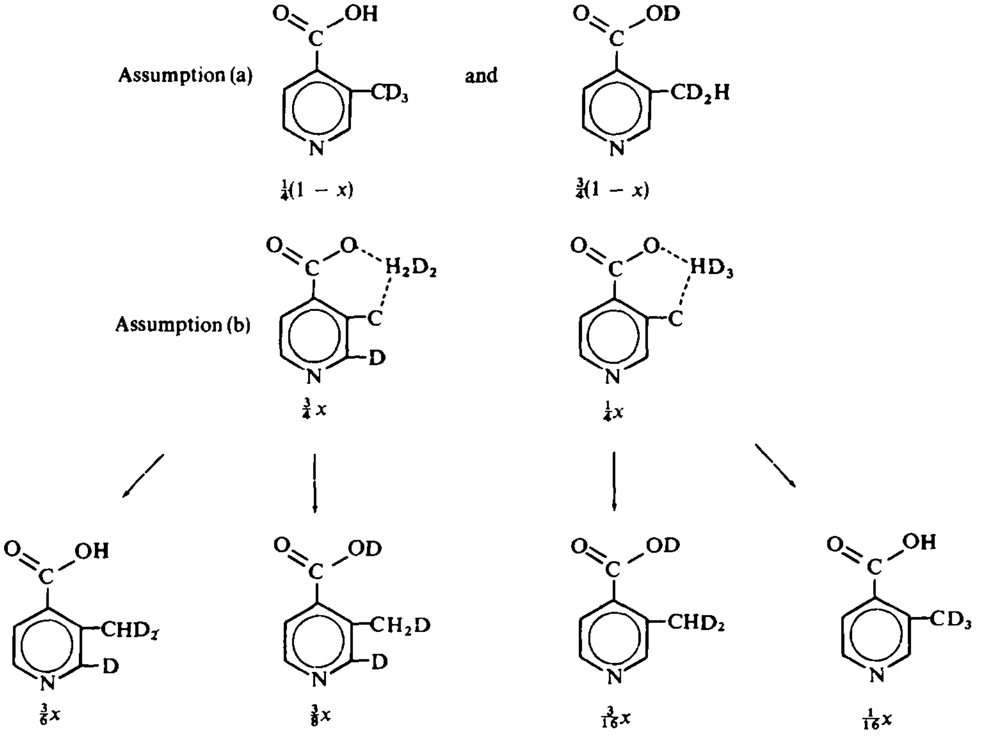
[f] These values have been omitted because of the *very low intensity* of the diffuse peak for loss of D_2O

[g] The ratio $[H_2O]/[HDO]$ has been omitted because of the unreliable intensity of the diffuse peak for the loss of H_2O (either very low intensity and/or interference with normal peaks)

Ions with a *gem-diol* structure (which resemble ion *b* in Scheme 1) do enter into this scheme. As no indication is found for the loss of a molecule of methanol from the molecular ion *a* or *b* (Scheme 1), we prefer a β-methyl isonicotinic acid structure (ion *c* or *c′*) as precursor for the expulsion of a molecule of water as assumed before in Scheme 1.

Using assumption (a) and (b) above, calculation of the various fractions of ions *c* (or *c′*) (*cf.* Scheme 1) with deuterium in a specific position, generated by mixing of one α-, one β- and three ω-hydrogen or deuterium atoms then proceeds as follows: Suppose that (1-*x*) ions *c* (or *c′*) of the ω-d_3 compound satisfy assumption (a) and that *x* ions *c* (or *c′*) satisfy assumption (b).

Then the following deuterated ions *c* (or *c′*) with the corresponding fractions are generated by the mixing:



All these ions may then eliminate a molecule of water as shown in Scheme 1, sequence $c' \rightarrow d$.

We can now predict the ratio of $[HDO]/[D_2O]$, assuming that an isotope effect *i* is operative in the cleavage of a methyl C—H bond with respect to a methyl C—D bond during the elimination of a molecule of water.

This ratio becomes then:

$$\frac{[HDO]}{[D_2O]} = \frac{\frac{3}{4}(1-x) + i\cdot\frac{3}{4}(1-x) + 2\cdot\frac{3}{8}x + 2i\cdot\frac{3}{8}x + i\cdot\frac{3}{16}x + \frac{3}{16}x}{2\cdot\frac{3}{4}(1-x) + \frac{3}{8}x + 2\cdot\frac{3}{16}x} = \frac{4 + 4i + ix + x}{8 - 4x}$$

In the same way the other ratios, listed in Table 1, have been obtained.

The best fit of this redundant system of equations to the observed values is obtained for $x = 0{\cdot}37$ and $i = 1{\cdot}7$ in the first field free region and for $x = 0{\cdot}37$ and $i = 2{\cdot}1$ in the second field free region (Table 1). The agreement of the observed ratios with the calculated ratios is satisfactory and therefore supports the assumptions made before. Also the observation of a larger isotope effect i for ions, decomposing in the second field free region, with respect to the first field free region, is anticipated as the first-mentioned ions do have a relatively lower internal energy.

The calculated fraction x might suggest that in roughly one third of the ions c (or c') an α-hydrogen atom is incorporated either in the methyl group or in the hydroxyl group, i.e. α-, β- and ω-hydrogens could participate in the elimination of a molecule water in the ratio of $\simeq 1:3:9$, resp.

The loss of a molecule of water may be followed by the elimination of a molecule of carbon monoxide as shown by the refocusing techniques[3] and by the occurrence of very weak diffuse peaks in the spectra. This process can easily be rationalized from the acid structure (*cf.* sequence $c' \rightarrow d \rightarrow e$ in Scheme 1).

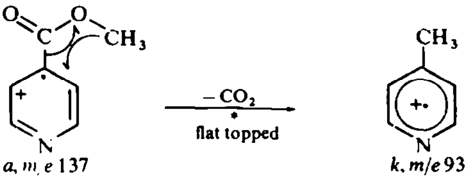
2. *Loss of a hydroxycarbonyl radical from the molecular ion*

Another decomposition of the molecular ion which supports the intermediacy of an acid structure is the loss of a hydroxycarbonyl radical from the molecular ion as shown by the refocusing technique.[3] This hydroxycarbonyl radical is probably (eliminated from the molecular ion in two successive steps *viz.* hydroxyl and carbon monoxide resp., as only loss of hydroxyl from the molecular ion has been observed, using the refocusing technique.[3] Similar observations have been reported earlier in the literature.[4] The D-labeling has shown that the hydroxylcarbonyl radical contains all kinds of hydrogen atoms. The shape of the peaks of refocused $(M{-}COOH(D))]^{\pm}$ ions suggests that they are contaminated with the peaks of refocused $\{[M{-}H(D)]{-}CO_2\}^{\pm}$-ions, thus preventing us from giving reliably calculated percentages of α-, β- and ω-hydrogens present in the expelled hydroxycarbonyl radical.

3. *Loss of carbon dioxide from the molecular ion*

In the spectra of all the investigated methyl esters of isonicotinic acid a flat-topped[5] diffuse peak is found for the loss of 44 a.m.u. from the molecular ion both in the first- and second field free region. This must correspond to the elimination of a molecule of carbon dioxide, which in principle might point to the formation of an acid structure c or c' (Scheme 1).

We prefer, however, the original methyl ester structure as precursor for the loss of carbon dioxide, as a diffuse peak for this reaction has not been observed in the mass spectrum of isonicotinic acid. Rationalization of this reaction from the ester structure has been presented in Scheme 3, sequence $a \rightarrow k$.



SCHEME 3. Rationalization of the loss of carbon dioxide from the molecular ion of the methyl ester of isonicotinic acid.

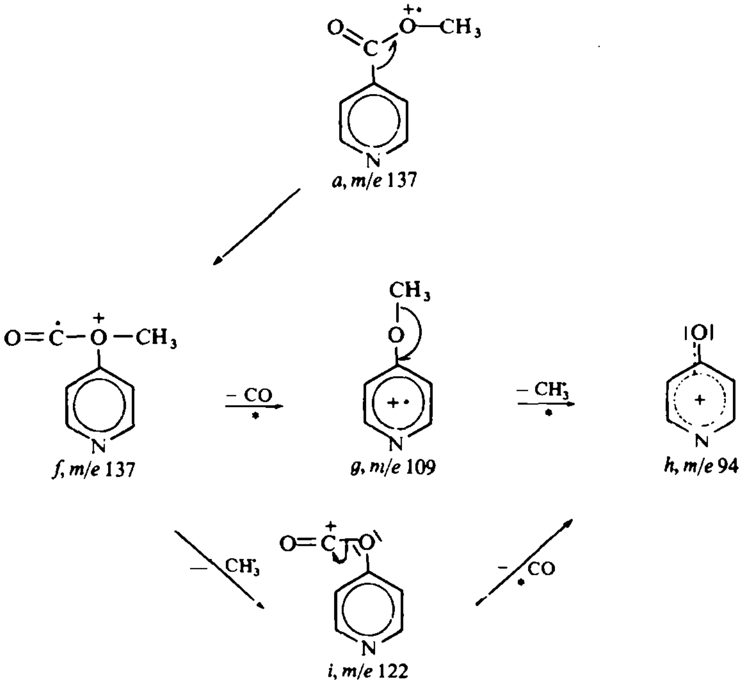
4. *Successive loss of carbon monoxide and of a methyl radical from the molecular ion and vice versa* (*Scheme* 4)

In all spectra of the various deuterated methyl esters of isonicotinic acid a conspicuous diffuse peak is found for the loss of 28 a.m.u. from the molecular ion both in the first- and in the second field free region

This must correspond with the elimination of a molecule of carbon monoxide, an unusual breakdown of esters upon electron impact (apart from methyl formate,[6, 7] which shows a similar decomposition).

In the latter compound this reaction has been explained by an initial shift of the formyl hydrogen atom to the oxygen atom of the methoxyl group, followed by expulsion of carbon monoxide.[8]

The present reaction can be likewise rationalized by an initial shift of the pyridine nucleus to the oxygen atom of the methoxyl group, followed by loss of carbon monoxide, as shown in Scheme 4, sequence *a* → *f* → *g*.



SCHEME 4. Rationalization of the loss of carbon monoxide and of methyl from the molecular ion of the methyl ester of isonicotinic acid and further degradations.

Ion *g*, represented by the structure of 4-methoxypyridine, decomposes further to ion *h* by expulsion of a methyl group, containing either the original methyl hydrogens or two original methyl hydrogens + one β-hydrogen as far as could be shown by the refocusing technique[3] applied to the peaks of $(M—CO—CH_xD_{3-x})^+$-ions, generated from the various labeled compounds.

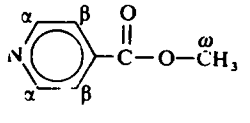
Unfortunately, the percentage of β-hydrogen, incorporated in the methyl group, cannot be calculated because of the very low intensities of the peaks of these refocused ions. Nevertheless, these observations may point to an exchange between the original methyl and β-hydrogens in ion *g* prior to or during its decomposition; exchange in an earlier stage e.g. in ions *a* and/or *f* is rather improbable as will be shown in section 5.

The loss of carbon monoxide and of a methyl radical from the molecular ion may also occur in the reverse order as shown by the refocusing technique[3] (*cf.* Scheme 4, sequence $f \rightarrow i \rightarrow h$); the intensities of the corresponding diffuse peaks, however, appear to be too low to permit conclusions about the nature of hydrogen atoms, present in the eliminated methyl radical.

5. *Loss of a formyl radical from the molecular ion* (*Scheme* 5)

The mass spectrum of the unlabeled methyl ester of isonicotinic acid shows a diffuse peak for loss of 29 a.m.u., i.e. a formyl radical, from the molecular ion (rel. int. 0·01%). In the spectra of the various labeled analogues diffuse peaks are found for expulsion of 29 a.m.u. and/or 30 a.m.u. as compiled in Table 2.

TABLE 2. RELATIVE PEAK AREAS[a] FOR DIFFUSE PEAKS AT 70 EV, REFERRING TO THE LOSS OF A FORMYL RADICAL FROM THE M^{+}-ION OF SITE-SPECIFICALLY DEUTERATED METHYL ESTERS OF ISONICOTINIC ACID.



IN THE SECOND FIELD FREE REGION.

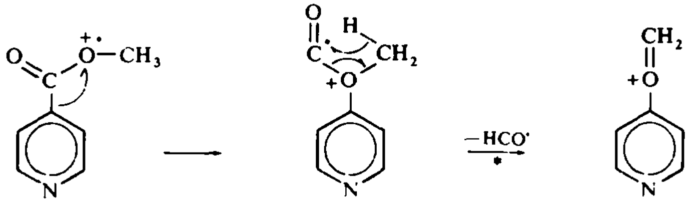
Compositon of the eliminated formyl radical	Labeled position							
	d_0	α-d_2	β-d_2	ω-d_2	ω-d_3	α,β-d_4	α,ω-d_4	β,ω-d_4
HCO	100	100	100	40	—	100	38	39
DCO	—	—	—	60	100	—	62	61

[a] Average of at least five runs: estimated accuracy ±5%

The data of this Table show:

1. The eliminated formyl radical contains exclusively one of the original methyl hydrogens.
2. An isotope effect of $(2 \times 39/61) = 1{\cdot}3$ operates in the cleavage of a carbon–hydrogen bond with respect to a carbon–deuterium bond of the methyl group.

These observations can be interpreted again assuming that the molecular ion *a* first rearranges to ion *f* without any mixing of original methyl hydrogens and ring hydrogens, thus supporting that the exchange, mentioned in the preceding section, starts after the formation of ion *g* (*cf.* Scheme 4). Then, transfer of a methyl hydrogen to the carbonyl carbon atom may occur in a four-membered transition state, resulting in the expulsion of a formyl radical to give ion *j* (Scheme 5). A similar rationalization has been given earlier for the loss of a formyl radical from the molecular ion of methyl formate.[8]



a, *m/e* 137 *f*, *m/e* 137 *i*, *m/e* 108

SCHEME 5. Rationalization of the loss of a formyl radical from the molecular ion of the methyl ester of isonicotinic acid.

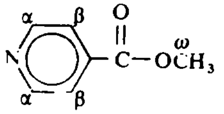
EXPERIMENTAL

Mass spectra were obtained with an AEI MS-902 mass spectrometer at 70 eV and at a constant ion repeller potential setting.

Ion source temperature: 150°; inlet temperature of the all-glass heated inlet system: 130°; trap current: 98 μA; filament current: 1·1 A; accelerating voltage: 8 kV; pressure in the ion source: 0·6 × 10^{-6} Torr; pressure in the analyser region: 0·2 × 10^{-7} Torr.

The methyl ester of isonicotinic acid and its deuterated analogues were purified by gas chromatography (30% SE-52). Label contents of all samples were measured at 70 eV and have been compiled in Table 3. The positions of deuterium incorporation were checked by NMR.

TABLE 3. D CONTENT[a] (IN %) OF DEUTERATED METHYL ESTERS OF ISONICOTINIC ACID



Ionization energy	Labeled position	d_1	d_2	d_3	d_4
70 eV	α-d_2	2·4	97·6	—	—
	β-d_2	2·3	97·7	-	—
	ω-d_2	1·4	98·6	—	—
	ω-d_3	—	1·6	98·4	—
	α,β-d_4	—	0·4	3·6	96·0
	α,ω-d_4	—	0·4	4·0	95·6
	β,ω-d_4	—	0·5	3·8	95·7

[a] Average of at least five measurements.

The methyl ester of isonicotinic acid and its deuterated analogues were prepared by interaction of the corresponding acids, described earlier,[1] with $SOCl_2$, followed by reaction of the intermediate isonicotinyl chloride hydrochloride with MeOH (or dideuteriomethyl- or trideuteriomethyl alcohol) and subsequent neutralization, as described in the literature.[9]

Dideuteriomethylalcohol was obtained by reduction of n-butylformate with LAD, analogous to the method of Nystrom *et al.*[10]

Trideuteriomethyl alcohol was a trade product of Merck.

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