Mass Spectrometry of Tautomeric Compounds. Part IV.[1] Structure of the Molecular Ions of 4-Hydroxycoumarins

By **Hisao Nakata,*** Department of Chemistry, Aichi Kyoiku University, Kariya, Aichi, Japan
Akira Tatematsu, Hideo Yoshizumi, and **Shinobu Naga,** Faculty of Pharmacy, Meijo University, Showa-ku, Nagoya, Japan

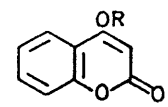
The structure of the mass spectral molecular ions of 4-hydroxycoumarins is discussed. From the fact that variation of inlet temperatures did not affect the spectral patterns, the molecular ion is considered not to be an equilibrium mixture of keto and enol forms. In contrast to earlier proposals, the enolic nature of the molecular ion is demonstrated by comparison of the spectra with those of acetylated derivatives and by deuterium-labelling experiments.

In order to rationalize the observed mass spectral fragmentation patterns, the molecular ions of heteroaromatic hydroxy-compounds such as 4-hydroxy-5-methyl-6-phenyl-2-pyrone,[2] 4-hydroxy-2-pyridones,[3] 4-hydroxybenzopyridones,[4] and several 4-hydroxycoumarins [5-7] have previously been assigned the less stable keto structures. Recent investigations, however, have shown that in most cases the molecular ions of tautomeric compounds have the structure of the most stable form that predominates in solution and in the solid state,[8,9] and no evidence has been obtained for keto–enol tautomeric changes in molecular ions or in fragment ions after electron impact in a mass spectrometer.[9-12] The structures of the molecular ions of these compounds should not be postulated arbitrarily for the purpose of interpreting the observed spectra; plausibility of a fragmentation scheme does not necessarily imply its validity.[9] It is, therefore, desirable to re-examine the spectra and fragmentations of these tautomeric compounds; the present paper provides evidence that the molecular ions of 4-hydroxycoumarins are enolic.

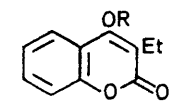
RESULTS AND DISCUSSION

In the mass spectrum of 4-hydroxycoumarin (1), two intense peaks appear at *m/e* 121 and 120. These have been ascribed [5-7] to cleavage of the oxygen-containing ring of the molecular ion in the keto-form. The spectrum of 3-ethyl-4-hydroxycoumarin (2) exhibits strong peaks at *m/e* 121 and 120, the relative intensities of which are similar to those from compound (1), but also shows an intense $M - 15$ peak at *m/e* 175. This peak probably arises from the corresponding enol molecular ion, because 3-ethyl-4-methoxycoumarin (4), which can only exist as an enolic structure, also affords a similarly intense $M - 15$ peak.†

If the peaks at *m/e* 121 and 120 from compound (2) are produced from the keto molecular ion, the foregoing observations indicate that both keto and enol structures must be present as the molecular ion of (2). In order to check for possible equilibration in the mass spectrometer, a technique due to Zamir *et al.*[13] was applied. As is seen from Table 1, the intensity ratios [*m/e* 121] : [*m/e* 175] and [*m/e* 120] : [*m/e* 175] are almost constant over a relatively large temperature variation of the inlet system. This implies that the molecular ions do not equilibrate, and that each of the three ions of *m/e* 175, 121, and 120 is generated from a molecular ion of the same structure.[13] Since the keto form of compound (2) is expected to afford an $M - 28$ peak (either $M - CO$ or $M - C_2H_4$) which is not observed, the molecular ion is presumably the enol form.



(1) R = H
(3) R = Me
(5) R = Ac



(2) R = H
(4) R = Me
(6) R = Ac

This was confirmed by examining the spectra of the 4-acetoxy-derivatives. In general, acetates of phenols [1,14,15] and enol acetates of aliphatic ketones [16,17] lose a keten molecule upon electron impact, and the resulting $M - 42$ ion has been assigned phenolic [1,14,15] and enolic [16,17] structures, respectively. In the spectra

† A possibility that the $M - 15$ peak of (4) might originate from the methoxy methyl group was excluded by the fact that 4-methoxycoumarin (3) itself did not show the corresponding $M - 15$ peak in any appreciable amount.

[1] Part III, H. Nakata and A. Tatematsu, *Org. Mass Spectrometry*, 1971, **5**, 1343.
[2] G. Kersaint, C. Goetschel, and C. Mentzer, *Bull. Soc. chim. France*, 1963, 1966.
[3] A. M. Duffield, C. Djerassi, G. Schroll, and S.-O. Lawesson, *Acta Chem. Scand.*, 1966, **20**, 361.
[4] R. T. Coutts and K. W. Hindmarsh, *Org. Mass Spectrometry*, 1969, **2**, 681.
[5] A. P. Johnson, A. Pelter, and M. Barber, *Tetrahedron Letters*, 1964, 1267.
[6] A. Pelter, P. Stainton, A. P. Johnson, and M. Barber, *J. Heterocyclic Chem.*, 1965, **2**, 256.
[7] J. P. Kutney, G. Eigendorf, T. Inaba, and D. L. Dreyer, *Org. Mass Spectrometry*, 1971, **5**, 249.
[8] N. Schamp and M. Vandewalle, *Bull. Soc. chim. Belges*, 1966, **75**, 539.
[9] H. Nakata and A. Tatematsu, *Bull. Chem. Soc. Japan*, 1969, **42**, 1678.
[10] J. K. MacLeod, J. B. Thomson, and C. Djerassi, *Tetrahedron*, 1967, **23**, 2095.
[11] R. I. Reed and V. V. Takhistove, *Tetrahedron*, 1967, **23**, 2807.
[12] J. Diekman, J. K. MacLeod, C. Djerassi, and J. D. Baldeschwieler, *J. Amer. Chem. Soc.*, 1969, **91**, 2069.
[13] L. Zamir, B. S. Jensen, and E. Larsen, *Org. Mass Spectrometry*, 1969, **2**, 49; see also M. E. Rennekamp, J. V. Paukstelis, and R. G. Cooks, *Tetrahedron*, 1971, **27**, 4407.
[14] C. B. Thomas, *J. Chem. Soc.* (*B*), 1970, 430.
[15] A. A. Gamble, J. R. Gilbert, and J. G. Tillett, *Org. Mass Spectrometry*, 1971, **5**, 1093.
[16] H. Nakata and A. Tatematsu, *Org. Mass Spectrometry*, 1970, **4**, 211.
[17] D. G. Boocock and E. S. Waight, *J. Chem. Soc.* (*B*), 1968, 258.

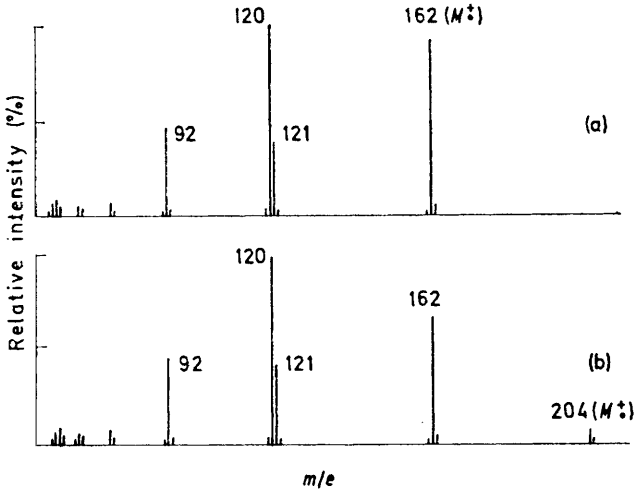
of 4-acetoxy-3-ethylcoumarin (6) and 3-ethyl-4-hydroxycoumarin (2), peak positions and relative intensities were essentially the same, except for the molecular ion peak of (6). This similarity reveals the structural identity of

TABLE 1

Effect of inlet temperature on peak intensity ratios

Temp. (°C)	175	200	225	250	275
[*m/e* 121] : [*m/e* 175]	0·46	0·49	0·47	0·49	0·49
[*m/e* 120] : [*m/e* 175]	0·43	0·48	0·46	0·49	0·49

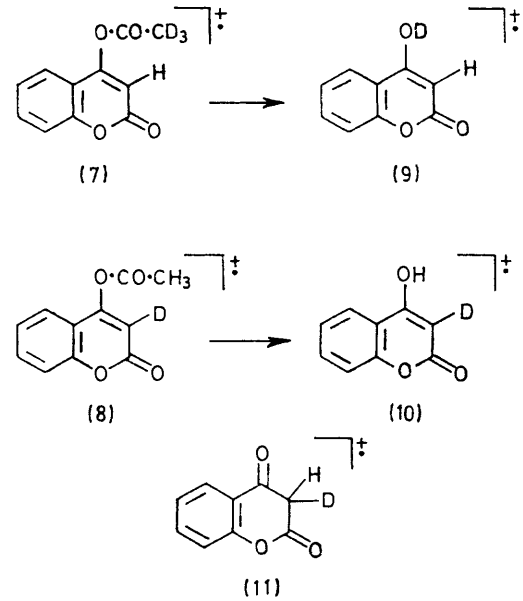
the M — 42 ion of (6) with the molecular ion of (2); the enolic nature of the latter ion is thus indicated. The same results were obtained in the case of 4-hydroxycoumarin (1) and 4-acetoxycoumarin (5) (see Figure).



Mass spectra of (a) 4-hydroxycoumarin (1) and (b) 4-acetoxycoumarin (5)

The present argument is similar to, but not identical with, that used in the ordinary external standard method [18] in organic mass spectrometry. Here, an ion of a known structure is produced by a well-defined fragmentation reaction in a mass spectrometer, and its further decomposition pathways are compared with those of unknown molecular ions. As the reliable application of the present method requires the exact structural identification of the fragment ion under consideration, the following labelling experiments were carried out to establish the structure of the M — 42 ion of acetoxycoumarins.

Acetylation of 4-hydroxycoumarin (1) with [$^2H_6$]acetic anhydride gave 4-[$^2H_3$]acetoxycoumarin (7). Refluxing compound (1) in deuterium oxide followed by rapid acetylation with unlabelled acetic anhydride gave 4-acetoxy[3-^{2}H]coumarin (8). In the elimination of keten from these deuteriated acetoxycoumarins upon electron impact, if the hydrogen or deuterium rearrangement of the acetyl methyl group proceeds through a four-membered cyclic transition state,[1,14-17] the resulting fragment ions must have enolic structures (9) and (10), respectively, which would be expected to show different breakdown patterns. This is actually the case. The acetate (7) showed intense peaks at *m/e* 122 and 120,



whereas the acetate (8) exhibited strong peaks at *m/e* 121 and 120. Alternatively, if the keten elimination were to take place through a six-membered cyclic transition state involving the double bond of the coumarin ring, both compounds (7) and (8) would produce a common fragment ion (11) of ketonic structure, in which the hydrogen and deuterium atoms are structurally and stereochemically equivalent. This is incompatible with the results.

It is concluded that the fragment ion from acetoxycoumarins after keten elimination has an enolic structure, which is identical with that of the molecular ion of 4-hydroxycoumarin itself. Consequently, fragmentation pathways of 4-hydroxycoumarins must be interpreted on the basis of the enol molecular ions. Several fragmentation schemes can be formulated, in which specific rearrangements of a hydroxy hydrogen atom are involved, as in the case of 4-hydroxy-2-pyrones.[19] Detailed discussion of this point will be included in a forthcoming paper.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. I.r. spectra were taken for solutions in chloroform or Nujol mulls with a JASCO IR-G spectrophotometer. N.m.r. spectra were measured on a Varian A-60 spectrometer (internal tetramethylsilane standard). Mass spectra were measured with a Hitachi RMU-7 spectrometer with a heated inlet system under the following conditions, unless otherwise stated: ionizing voltage 70—14 eV, ion accelerating voltage 1800 V, total emission current 80 μA, ion source temperature 250°.

The following coumarin derivatives were prepared and purified as described in the literature: 4-hydroxycoumarin (1), m.p. 214—216° (lit.,[20] 214—216°), $\nu_{max.}$ (Nujol) 1703

[18] P. Brown and M. M. Green, *J. Org. Chem.*, 1967, **32**, 1681.
[19] H. Nakata, Y. Hirata, and A. Tatematsu, *Tetrahedron Letters*, 1965, 123.
[20] M. A. Stahmann, I. Wolff, and K. P. Link, *J. Amer. Chem. Soc.*, 1943, **65**, 2285.

cm^{-1}; 3-ethyl-4-hydroxycoumarin (2), m.p. 154—156° (lit.,[20] 155—156°), $\nu_{max.}$ (Nujol) 1674 cm^{-1}; 4-methoxycoumarin (3), m.p. 124—125° (lit.,[21,22] 125—126°), $\nu_{max.}$ ($CHCl_3$) 1708 cm^{-1}; 3-ethyl-4-methoxycoumarin (4), m.p. 50—52° (lit.,[22] 52—53°), $\nu_{max.}$ ($CHCl_3$) 1710 cm^{-1}; 4-acetoxycoumarin (5), m.p. 109—111° (lit.,[23] 109—110°), $\nu_{max.}$ ($CHCl_3$) 1790 and 1726 cm^{-1}, δ ($CDCl_3$) 2·60 (3H, s), 6·85 (1H, s), and 7·56—8·20 p.p.m. (4H, m); 4-acetoxy-3-ethylcoumarin (6), m.p. 109—110° (lit.,[24] 110°), $\nu_{max.}$ ($CHCl_3$) 1774 and 1712 cm^{-1}.

4-[$^2H_3$]*Acetoxycoumarin* (7).—4-Hydroxycoumarin (1) (1 g), [$^2H_6$]acetic anhydride (1 ml), and pyridine (7 drops) were warmed on a water-bath (50—70°) for 5 min. The resulting solution was poured into cold water (30 ml) with stirring and the mixture was set aside at room temperature for *ca.* 1 h. The precipitate was collected, washed with cold water, dried *in vacuo* ($CaCl_2$) and recrystallized from light petroleum–benzene to give 4-[$^2H_3$]acetoxycoumarin (7) as pale yellow crystals, m.p. 109—110°, $\nu_{max.}$ ($CHCl_3$) 1780 and 1727 cm^{-1}, δ ($CDCl_3$) 6·50 (1H, s), and 7·10—7·80 p.p.m. (4H, m) (no OAc signal).

4-*Acetoxy*[3-^{2}H]*coumarin* (8).—4-Hydroxycoumarin (1) (1 g), 99·75% deuterium oxide (5 ml), and dry pyridine (6 drops) were refluxed for 2 h. The deuterium oxide was removed *in vacuo*, freshly distilled acetic anhydride (1 ml) was added, and the mixture was warmed for a few minutes in the absence of atmospheric moisture. The mixture was poured into cold water (30 ml); the precipitate was collected and purified as in the preceding experiment to yield 4-acetoxy[3-^{2}H]coumarin (8), m.p. 107—109°, δ ($CDCl_3$) 2·45 (3H, s) and 7·10—7·80 p.p.m. (4H, m). The olefinic proton signal, δ *ca.* 6·5 p.p.m., was very small.

Mass Spectra of 3-Ethyl-4-hydroxycoumarin (2) *at Various Temperatures*.—The sample (2) was placed in the reservoir *ca.* 1 h before the spectra were recorded. The temperatures of the inlet system were maintained constant to $\pm 1°$ as checked by thermocouples placed on the reservoir. The measurement was carried out at 20 eV to suppress consecutive fragmentations. The ratios of the intensities (Table 1) are mean values of ten determinations, obtained by pen-recording. In this case, the collector slit was opened in order to allow all ions to pass through quantitatively, so that each peak had a nearly rectangular shape. The relative standard deviations of peak intensity determinations were *ca.* 1%. The isotope abundance from the *m/e* 120 peak was corrected for calculating the *m/e* 121 peak height. At 70 eV, the data were rather scattered, probably owing to further fragmentation reactions, but essentially the same trend was found.

Mass Spectra of Deuteriated Acetoxycoumarins.—When a heated inlet system was used, hydrogen scrambling took place to a considerable extent and the results were not straightforward. Even with a direct inlet system, some scrambling occurred; moreover, the spectra were complicated by the presence of small amounts of non-deuteriated isomers. Therefore, the isotopic purity was corrected from measurements of peak heights at *m/e* 162 and 163. For calculating the ratio of the intensities of peaks at *m/e* 121 and 122, isotope contributions from adjacent peaks were also considered. After these corrections, the specificity of the fragmentation shown in Table 2 was obtained. 4-[$^2H_3$]Acetoxycoumarin (7) predominantly gave a fragment ion at *m/e* 122, whereas 4-acetoxy[3-^{2}H]coumarin (8) afforded an ion at *m/e* 121. An intense peak at *m/e* 120 was observed in both spectra.

TABLE 2

Specificity of fragmentation of compounds (7) and (8)

Deuteriated acetoxycoumarin	Intensity ratio (%) [*m/e* 121] / [*m/e* 121] + [*m/e* 122]	Intensity ratio (%) [*m/e* 122] / [*m/e* 121] + [*m/e* 122]
(7)	17·1	82·9
(8)	81·3	18·7

[2/136 *Received*, 24*th January*, 1972]

[21] F. Arndt, L. Loewe, R. Un, and E. Ayca, *Chem. Ber.*, 1951, **84**, 319.

[22] J. Cieslak, S. Lewak, and I. Chmielewska, *Roczniki Chem.*, 1959, **33**, 349 (*Chem. Abs.*, 1960, **54**, 3404).

[23] R. Eisenhauer and K. P. Link, *J. Amer. Chem. Soc.*, 1953, **75**, 2044.

[24] C. Mentzer and P. Meunier, *Bull. Soc. chim. France*, 1943, 356.