MASS SPECTRA OF URIDINE AND PSEUDOURIDINE: FRAGMENTATION PATTERNS CHARACTERISTIC OF A CARBON-CARBON NUCLEOSIDIC BOND

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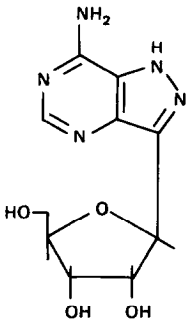
SUMMARY

The mass spectrum of uridine contains intense peaks at m/e 112, 113 and 133, generated by uracil, protonated uracil, and the ribose moiety respectively. This pattern is characteristic of all nucleosides containing a carbon-nitrogen nucleosidic bond. In contrast, the spectrum of pseudouridine lacks these peaks, but has an intense peak at m/e 141 due to protonated 5-formyluracil. The carbon-carbon nucleosidic bond is not cleaved in the mass spectrometer; neither the sugar nor the base moiety undergoes fragmentation independently. This feature is predicted to be general for nucleosides containing a carbon-carbon nucleosidic bond.

Pseudouridine (5-ribofuranosyluracil) is the only "unusual" nucleoside characterized by a carbon-carbon (C-C) nucleosidic bond which has thus far been found in transfer RNA. However, Townsend, Robins and their associates have recently demonstrated that the C-C nucleosidic bond is not unique to pseudouridine, but is also a structural feature of certain nucleoside antibiotics, among them formycin (7-amino-3-β-D-ribofuranosylpyrazolo[4,3-d]pyrimidine, 1), an isomer of adenosine, and laurusin (3-β-D-ribofuranosyl-pyrazolo[4,3-d]-7-pyrimidone, 2), an isomer of inosine (Robins et al, 1966), and showdomycin (3-β-D-ribofuranosylmaleimide, 3) (Darnell et al, 1967). It seems likely that more such structures will be encountered in the future.



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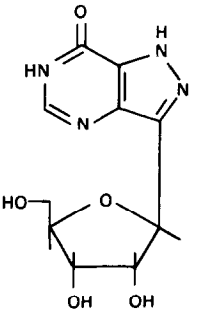

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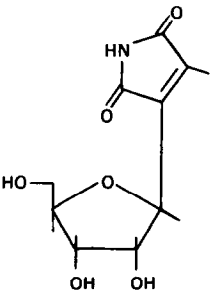

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The natural nucleoside components of DNA and RNA have very characteristic mass spectra, the most prominent features of which result from one fundamental reaction: scission of the carbon-nitrogen (C-N) nucleosidic bond in the molecular ion (Biemann and McCloskey, 1962). If one designates the mass of the sugar moiety as S and that of the base moiety as B, intense peaks are then observed in C-N nucleoside mass spectra at m/e values of B + 1, B + 2 and S (Fig. 1 and 2) (Biemann and McCloskey, 1962). This characteristic fragmentation has helped elucidate the structure of unusual C-N nucleosides, including substituted adenosine cytokinins (Biemann et al, 1966; Burrows et al, 1968) and the antibiotic cordycepin (Hanessian et al, 1966). In contrast, we have found that the C-C nucleosidic bond of pseudouridine is not cleaved in the mass spectrometer, and consequentially the mass spectrum of this compound differs strikingly from that of its isomer, uridine (Fig. 1).

EXPERIMENTAL

Uridine (Calbiochem A grade) and pseudouridine (Calbiochem A grade, a mixture of α and β isomers) were purchased from the California Corporation for Biochemical Research, and used without purification. Mass spectra were recorded on an AEI MS-9 mass spectrometer, using a direct-insertion probe. High-resolution mass measurements were performed by reference to fragment ions of perfluorotri-n-butylamine.

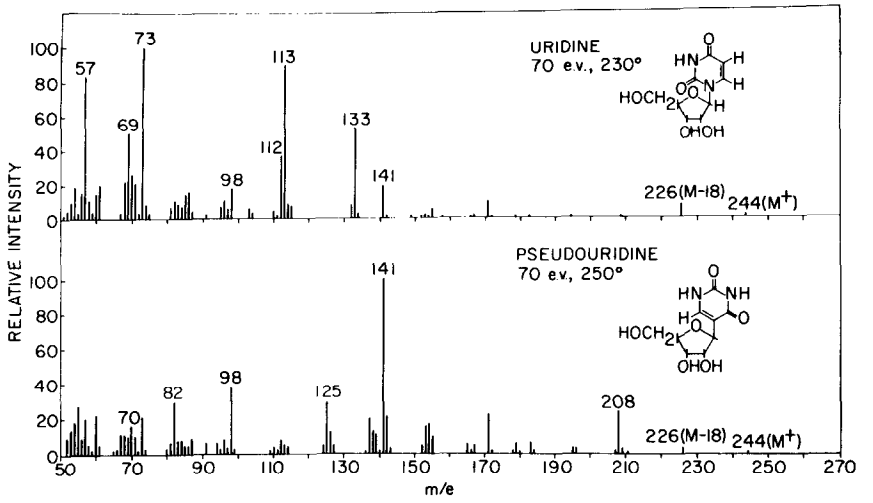

Figure 1. Mass spectra of uridine and pseudouridine at 70 e.v. Peaks characteristic of C-N nucleosides occur in the uridine spectrum at m/e 112 (B + 1), 113 (B + 2), 133 (S), and 141 (B + 30).

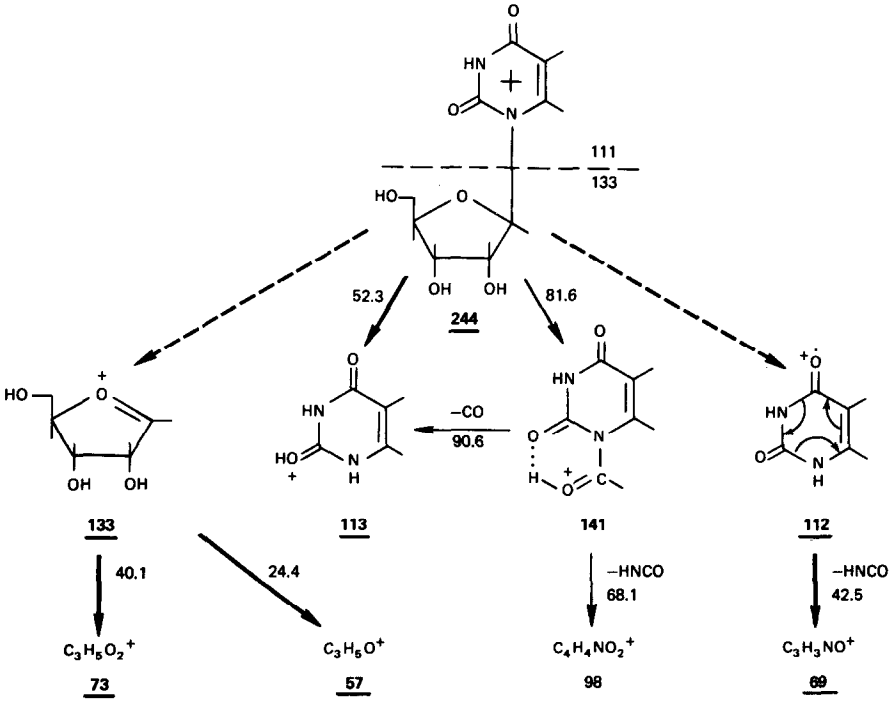

Figure 2. Fragmentation patterns of uridine (B=111, S=133). M/e values for observed metastable ions are given to 0.1 atomic mass unit, together with the transitions to which they are assigned. Heavy arrows and underlined mass numbers designate the principal reactions and fragment ions. Dashed lines indicate reactions for which no metastable ions were observed, but which account reasonably for the presence in the spectrum of the observed peaks.

RESULTS

The principal fragmentation sequences of uridine (Fig. 2) and pseudouridine (Fig. 3) need be traced only through the second stage in order to account for the major peaks in the spectra of these compounds. Uridine undergoes cleavage of the nucleosidic bond, and the charge either remains with the intact sugar moiety to generate daughter ions at m/e 133, or is transferred, together with one or two hydrogen atoms, to the uracil fragment, which gives rise to protonated uracil at m/e 113 (which does not undergo further fragmentation) and the uracil cation at m/e 112. The latter loses

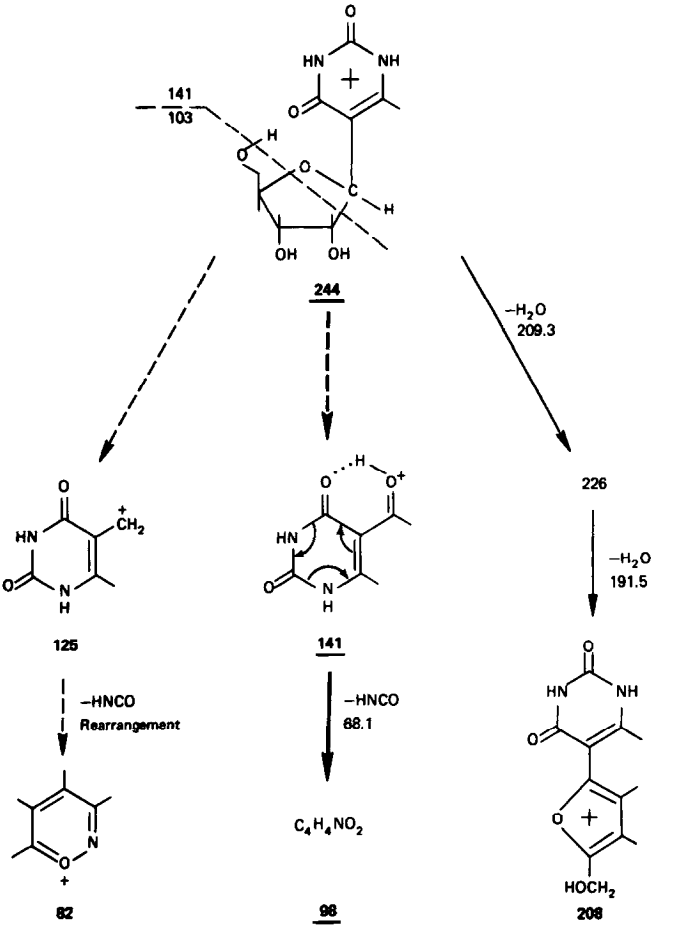

Figure 3. Fragmentation patterns of pseudouridine. Abundant fragment ions are invariably those for which resonance-stabilized structures can be written.

HNCO (43 mass units), in accordance with the established fragmentation pattern of uracil (Rice et al, 1965), generating a secondary fragment ion at m/e 69. The ribose ions at m/e 133 likewise undergo fragmentation, giving rise to secondary fragments chiefly at m/e 73 and 57. This major pattern accounts for the peaks at m/e 133, 113, 112, 73, 69, and 57, which dominate the mass spectrum of uridine.

A minor fragmentation sequence involves cleavage of the ribofuranose ring in the molecular ion, and generates the B + 30 fragment at m/e 141 (Biemann and McCloskey, 1962). This ion contains the uracil moiety and a CH_2O group derived from ribose, and undergoes at least two subsequent reactions: it either expells CO, contributing to the population of ions at m/e 113, or it ejects HNCO, generating a daughter ion of m/e 98.

The spectrum of pseudouridine, on the other hand, is dominated by an intense peak at m/e 141. This B + 30 ion has the same atomic composition as the corresponding ion derived from uridine, and likewise undergoes further fragmentation, losing HNCO and CO in succession (141 → 98 → 70). Another fragment ion, at m/e 125, consists of the uracil moiety plus a methylene group derived from ribose; it in turn probably gives rise to the fragment at m/e 82 through the expulsion of HNCO, although no metastable ion was detected for this transition. The pseudouridine molecular ion also has a much more pronounced tendency than its isomer to expell one or two molecules of water, generating peaks at m/e 226 and 208, but further fragmentation along this pathway is not significant. The ions produced in the principal fragmentation sequence of uridine are absent: there are no significant peaks at m/e 133, 113 or 112.

DISCUSSION

Two factors appear to contribute to the pronounced tendency of the uridine C-N nucleosidic bond to undergo fragmentation in the mass spectrometer, and to the complete stability of the pseudouridine C-C nucleosidic bond under the same conditions. The average C-N bond energy is 62 kcal/mole,

while the C-C bond averages approximately 80 kcal/mole (Gould, 1959); this difference should be reflected by a higher activation energy for cleavage of the stronger C-C bond. More important than this, however, is the fact that cleavage of a C-N nucleosidic bond generates a pyrimidine fragment in which the unpaired electron and/or positive charge is delocalized over the entire ring, while the corresponding C-C cleavage would generate a vinyl radical or cation. The latter structure is incapable of resonance stabilization, and consequently much more difficult to generate. The facile generation of a B + 30 fragment from both uridine and pseudouridine supports this argument, since these ions are formed by extension of the conjugated π-electron system of the pyrimidine ring. Resonance stabilization also accounts reasonably for the formation of the pseudouridine fragment ions at m/e 208 and 125 (Fig. 3).

These considerations should apply to the mass spectra of C-C nucleosides in general, and strongly suggest that the mass spectra of such compounds can provide compelling evidence for the existence of a suspected C-C nucleosidic bond.

REFERENCES

Biemann, K. and J. A. McCloskey, J. Am. Chem. Soc. 84, 2005 (1962).
Biemann, K., S. Tsunakawa, J. Sonnenbichler, H. Feldmann, D. Dütting, and H. G. Zachau, Angew. Chem. 78, 600 (1966).
Burrows, W. J., D. J. Armstrong, F. Skoog, S. M. Hecht, J. T. A. Boyle, N. J. Leonard, and J. Occolowitz, Science 161, 691 (1968).
Darnell, K. R., L. B. Townsend, and R. K. Robins, Proc. Natl. Acad. Sci. 57, 548 (1967).
Gould, E. S., "Mechanism and Structure in Organic Chemistry," p. 36 (Holt, Rinehart and Winston, New York, 1959).
Hanessian, S., D. C. DeJongh, and J. A. McCloskey, Biochim. Biophys. Acta 117, 480 (1966).
Rice, J. M., G. O. Dudek, and M. Barber, J. Am. Chem. Soc. 87, 4569 (1965).
Robins, R. K., L. B. Townsend, F. Cassidy, J. F. Gerster, A. F. Lewis, and R. L. Miller, J. Heterocyclic Chem. 3, 110 (1966).