

Mass Spectra of C-Nucleosides II. An Unusual Fragmentation Reaction of the Heterocyclic Moiety of Pyrazomycin and Some Closely Related Compounds (I).

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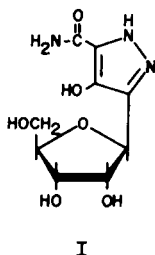
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The mass spectrum of pyrazomycin has been obtained and reveals a fragmentation pattern with several important peaks which are not normally found in the mass spectra of C-nucleosides. It has now been established, using model compounds, that these unusual fragment ions are a direct result of the juxtaposition of the exocyclic hydroxy and carboxamido groups of the aglycon. It appears that a facile elimination of ammonia and ethanol from *o*-hydroxycarboxamides and *o*-hydroxyethylesters, respectively, may be a general fragmentation reaction for aromatic heterocycles. A mass spectrum of the TMS derivative of pyrazomycin has also been obtained and factors which may result in exceptions to the empirical B+30 (M-103) rule for C-nucleosides are discussed.

The antibiotic pyrazomycin was isolated from a fermentation broth of *Streptomyces candidus* and assigned the structure 4-hydroxy-3(5)-(β -D-ribofuranosyl)pyrazole-5(3)-carboxamide (I) (2). This structural assignment was based on degradation studies and certain physicochemical data, excluding mass spectra. A previous mass spectral



study on C-nucleosides (3) had established certain empirical rules which could have been applied to the structural assignment of pyrazomycin and would have provided considerable support for the proposed structure (2a) prior to the total synthesis (4a).

This prompted our initial interest in obtaining a mass spectrum of pyrazomycin which is shown in Figure 1. The elemental compositions shown were from the corresponding high resolution mass spectrum. A recent report (4b) giving limited mass spectral data for pyrazomycin has indicated the presence of an ion of mass 180 without any mention of a molecular ion. The m/e 180 was attributed

to the loss of 2H₂O and the 5'-CH₂OH. Although if this were the case, then a peak at m/e 192 should have been observed rather than a peak at 180. Our spectrum (Figure 1) shows an abundant molecular ion and no peak at m/e 180 (or 192), raising the possibility that the previous observation was associated with artifacts, possibly from pyrolysis. The molecular ion (M) composition (Figure 1) when corrected for ribose yields a composition of C₄H₄N₃O₂ for the heterocyclic moiety. Exchange of active hydrogens by solution of I in deuterium oxide prior to a determination of the spectrum results in a molecular ion shift from m/e 259 to 266, indicating the presence of seven active hydrogens, corresponding to four in the aglycon. Cleavage of the glycosyl bond to furnish the BH⁺ or BH₂⁺ ions as the predominant peaks was not observed. The reduced intensity of these peaks and the carbohydrate fragment peak is a characteristic feature of C-nucleosides (3-6). In fact, the most abundant ion in the mass spectrum (Figure 1) was observed at m/e 156 which corresponds to B+30 (M-103) and this fragmentation, when it is a major process, has been suggested to be a definite diagnostic feature in the mass spectrum of a C-nucleoside. The peaks m/e 140 and 170 corresponding to normal nucleoside fragmentation reactions (7,8) were observed and the exact masses of these peaks confirmed a composition of C₄H₄N₃O₂ for the heterocyclic moiety.

With the exception of the minor ions of m/e 241 (M-H₂O) and m/e 223 (M-2H₂O), the important peaks

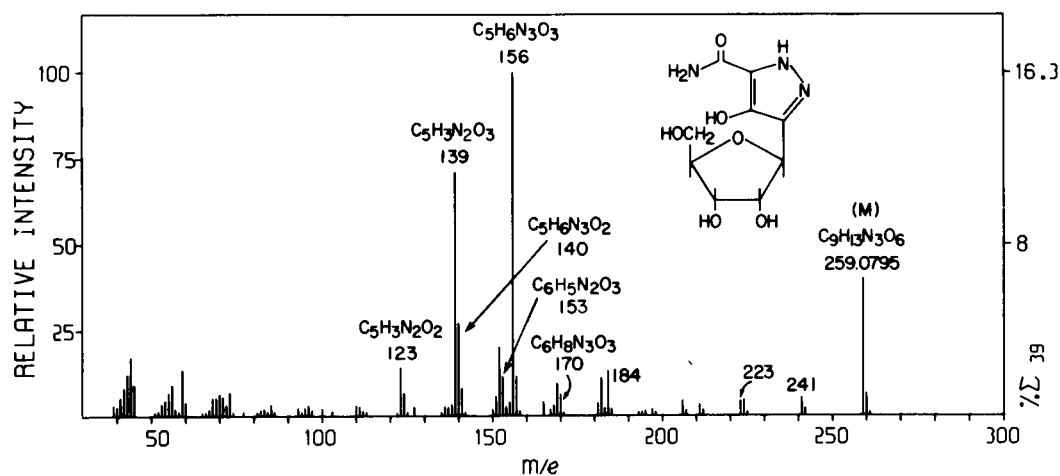
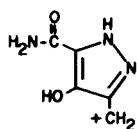
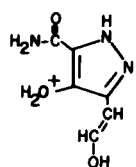


Figure 1



m/e 140.0456

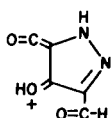


m/e 170.0555

remaining in the spectrum are not common to nucleoside fragmentation. In fact, the most interesting peaks were found to be associated with some unusual structural features of the heterocyclic moiety. The expulsion of NH_3 from m/e 156 to produce m/e 139 has now been established (*vide infra*) as a major process. This process would be energetically unlikely if the amino group were directly bound to an unsaturated ring carbon, as in adenosine or cytidine. The elimination of NH_3 from the *o*-hydroxycarboxamido grouping in structure I was assumed



m/e 156.0406

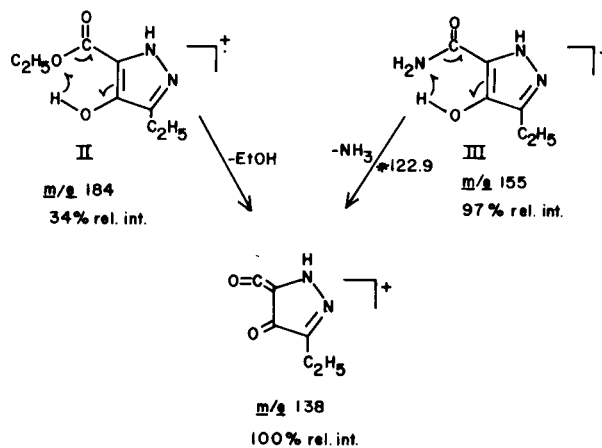


m/e 139.0139

to proceed *via* a favorable 6-membered transition state as depicted above. This assumption required corroboration and prompted us to synthesize model compounds in order to not only confirm that the *o*-hydroxycarboxamido grouping was actually responsible for this facile elimination of NH_3 but also to exclude the possibility that participation by the ribosyl moiety might be involved in this elimination. The initial approaches envisaged (9) were unsuccessful; however, an alternate approach (13) *via* treatment of ethyl 3-ethyl-4-hydroxypyrazole-5-carb-

oxylate (II) with liquid ammonia furnished III (14).

The low resolution mass spectra of these compounds (II and III) confirmed the assumption, *vide supra*, that the elimination was indeed a direct result of the juxtaposition for the substituents at C4 and C5 and independent of the ribosyl moiety. It was observed that II and III eliminate ethanol and ammonia, respectively, to give m/e 138. Additional corroboration for the assumption that ex-



pulsion of NH_3 was actually occurring rather than a simple loss of the 4-hydroxy group (both 17 mass units) was obtained from the mass spectrum of the *N,O*-*d*-4 derivative of III which showed the loss of ND_3 rather than OD .

The facile eliminations noted above for II and III and the m/e 156, m/e 139 fragments from pyrazomycin would tend to cast some doubt on the *absolute* validity of the M-103 (B+30) base peak (100% rel. int.) to function as a diagnostic tool for the unequivocal assignment of *C*-nucleoside structure. It would appear that under propitious circumstances the elimination of an exocyclic substituent or some portion of an exocyclic substituent may

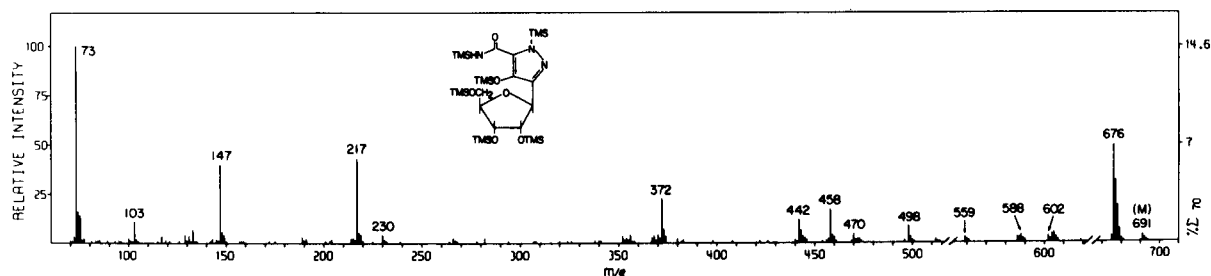
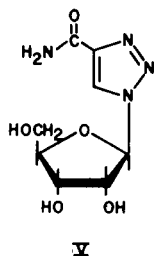
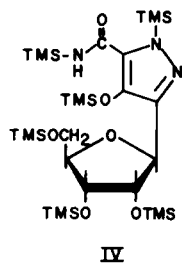


Figure 2

occur in preference to fragmentation of the sugar moiety of the molecular ion. In that case, a M-103 peak for the original nucleoside may not be observed as the major peak (100% rel. int.) since there would now be a B+30 peak for the daughter ion as the most intense peak.

Other less diagnostic fragment ions in the mass spectrum of pyrazomycin (Figure 1) are m/e 182 and 184, which correspond in composition to $B + C_3H_4O$ and $B + C_2H_2O_2$, respectively. Compositions of m/e 123 and 152 indicate that they may be related to m/e 140 and 169 by loss of NH_3 , although no metastable peaks were observed. Also, the ion of m/e 153 differs in exact mass from m/e 170 by the value corresponding to NH_3 .

Results derived from Figure 1 can be correlated with the mass spectrum of the trimethylsilyl (TMS) derivative of pyrazomycin, Figure 2, and the structural assignments presented in Table I. The exact molecular mass corresponds to a composition in which 3 TMS groups have been incorporated into the aglycon, structure IV. An alternative structure for IV where the trimethylsilyl group residing at N-1 would be placed on an enolized carboxamide function at C5 has been excluded because the carboxamide group of the closely related model 1-(β -D-ribofuranosyl)-1,2,3-triazole-4-carboxamide (16) (V) fails to incorporate two TMS groups on the amide function under the same conditions of silylation. The presence of an amino group



in the aglycon of pyrazomycin is further supported by the presence of only three TMS groups in the aglycon, since the aglycon was shown to have four active hydrogens (*vide supra*), and under the usual conditions of silylation, amino groups incorporate only one silyl function (17).

The importance of these findings [the base bears an amino group not directly bound to it and the base incorporates three TMS groups] is that the ring system must have five rather than six members.

The principal fragment ions shown in Figure 2 do not include products in which cleavage of the glycosyl bond had occurred, such as B+H, B+2H, and B+HTMS (17). Similar to free pyrazomycin, this behavior is indicative of the C-C glycosidic bond structure analogous to that exhibited by the trimethylsilyl derivative of pseudouridine (18).

In Table I, ions of m/e 676, 588, 458, 442, and 372 correspond to the principal diagnostic fragment ions of trimethylsilylated nucleosides (17), with appropriate exact mass values in support of structure IV. The prominent upper mass ions m/e 603, 559, and 470 are not common to most nucleoside spectra, and appear to involve fragmentation of the sugar moiety. In Figure 2, the lower mass ions m/e 73, 103, 147, 217, and 230 are derived principally from the sugar moiety (17, 19-22). These

TABLE I

Structural Correlations for Diagnostic Ions from the Mass Spectrum of Trimethylsilylated Pyrazomycin

m/e (error (a), mmu)	Identity
691.3188 (1.2)	molecular ion $(TMS)_6$
676.2943 (0.2)	M- CH_3
602.2774 (2.1)	M-OTMS
588.2597 (1.3)	M - (5') CH_2OTMS
559.2566 (-0.3)	M- $C_2H_3O_2TMS$
498.2126 (3.0)	588 - TMSOH
470.2168 (-2.0)	$C_7H_4N_3O_3(TMS)_4$
458.2171 (2.4)	bH- $\overset{1'}{C}H = \overset{2'}{C}H-OTMS$
444.1969 (-2.1)	b- $\overset{1'}{C}HOTMS$
442.1850 (1.6)	458 - CH_4
372.1615 (2.0)	bH- $\overset{1'}{C}HO$

(a) Found minus theoretical exact mass values.

data show that the sugar is an unmodified pentafuranose moiety, but do not permit steric assignment of hydroxyl groups (*e.g.*, ribose *vs* arabinose). The mass spectrum of the TMS derivative of pyrazomycin is anomalous in that it shows the B+30 peak as a major fragment but with a significant reduction in relative intensity. Whether the decrease in intensity of this peak is characteristic for TMS derivatives or is actually a general occurrence due to derivatization (trifluoromethyl, acetyl, benzyl, *etc.*) is not known at the present time.

The present study has corroborated the structure assigned to pyrazomycin and has also provided another example where a *C*-nucleoside exhibits the M-103 (B+30) fragment as the major peak (100% rel. int.) in the electron impact mass spectrum. However, of considerable interest was the observed expulsion of labile exocyclic groups from the aglycon as a major process. This would suggest that exceptions to the proposed empirical M-103 (B+30) rule may be encountered in the future due to the occurrence of specific exocyclic groups in an appropriate juxtaposition for rearrangement and fragmentation or merely due to the presence of a labile ring system or exocyclic group. Although all of the underivatized *C*-nucleosides studied thus far have exhibited the M-103 (B+30) peak as the major peak, exceptions may occur as other *C*-nucleosides are isolated and characterized. The present findings would indicate that further studies are in order to ascertain if the 100% relative intensity of the M-103 (B+30) peak of *C*-nucleosides is unique only to electron impact mass spectrometry and the free nucleoside, *per se*.

EXPERIMENTAL

Uv spectra were obtained from a Beckman DK-2 spectrophotometer, ir spectra from a Beckman IR-8 spectrophotometer, as nujol mulls and pmr spectra from a Varian A56/60 with TMS as an internal standard. Melting points were determined on a Thomas-Hoover Unimelt and are uncorrected. Elemental compositions (C,H,N) were determined by Heterocyclic Chem. Co., Harrisonville, Missouri.

Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was purchased from Regis Chemical Co., Chicago, Illinois.

Trimethylsilyl Derivatives of I and VI.

The nucleoside (10-40 μ g) was added to 50 μ l of BSTFA-TMCS reagent mixture and heated at 60° in a closed tube for 1 hour.

Mass Spectrometry.

Low resolution mass spectra were recorded using an LKB 9000 instrument. Samples were introduced by direct probe (I, III) or by a gas chromatograph (IV, 3 ft., 1% OV-17) under the following conditions: ionizing electron energy 70 eV, ion source and carrier gas separation temperatures 250°. The method for determining the spectra of *N,O*-perdeuterio-I and III was similar to that of Shaw *et al.*, (8). High resolution mass spectra were photographically recorded with a CEC 21-110B instrument, with all samples introduced by direct probe, after removal of reagents

by the direct inlet vacuum system in the case of IV. All experimental mass values for I agreed with ± 2 mmu of the theoretical values for the assigned compositions.

Ethyl 3(5)-Ethyl-4-hydroxypyrazole-5(3)-carboxylate (II).

An ethoxide solution, prepared by adding 1 g. (43.5 mmoles) of metallic sodium to ethanol (25 ml.), was cooled (5-10°) and with continuous stirring, a mixture of ethyl diazoacetate (2.28 g., 20 mmoles) and diethyl malonate (3.76 g., 20 mmoles) was then added slowly (20-30 minutes) while maintaining the reaction temperature at 5-10°. The mixture was then allowed to stand at 5° for 2 days. The solid which had formed was collected by filtration, washed with petroleum ether (30-60°) (10-20 ml.) and air dried. The solid (1.15 g., m.p. 250° dec.) was added to rapidly stirred aqueous 1*N* hydrochloric acid (20 ml.) and the pH of the mixture adjusted to 3-4 with concentrated ammonium hydroxide. The solid was collected and recrystallized from water, with charcoal treatment, giving 600 mg. (3.26 mmoles, 16%) of a white solid, m.p. 109-111°. The product was recrystallized again from water and dried *in vacuo* at room temperature to yield 430 mg., m.p. 109-111°; uv λ max (pH 1) 272 nm (ϵ , 5,800), λ max (methanol) 275 nm (ϵ , 5,620), 227.5 nm (ϵ , 9,650), λ max (pH 11) 317 nm (ϵ , 8,750), 237 nm (ϵ , 7,900).

Anal. Calcd. for C₈H₁₂N₂O₃: C, 52.16; H, 6.56; N, 15.20. Found: C, 52.12; H, 6.60; N, 14.92.

3(5)-Ethyl-4-hydroxypyrazole-5(3)-carboxamide (III).

Ethyl 3(5)-ethyl-4-hydroxypyrazole-5(3)-carboxylate (II, 0.5 g.) and 50 ml. of liquid ammonia were heated in a sealed reaction vessel at 75-80° for 12 hours. The ammonia was allowed to evaporate and the residue was recrystallized from a methanol-benzene mixture, with charcoal treatment, to give 350 mg. of crude product. Two recrystallizations from an ethyl acetate-benzene mixture gave, after drying *in vacuo* at 110°, 180 mg. of pure III, m.p. 168-170°; uv λ max (pH 1) 267 nm (ϵ , 5,500), λ max (pH 11) 310 nm (ϵ , 7,590), 235 nm (ϵ , 7,430), λ max (pH 12) 311 nm (ϵ , 7,350), 233 nm (ϵ , 6,730).

Anal. Calcd. for C₆H₉N₃O₂: C, 46.44; H, 5.82; N, 27.08. Found: C, 46.67; H, 5.82; N, 27.03.

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