The Mass Spectra of Formycin, Formycin B and Showdomycin Carbon Linked Nucleoside Antibiotics (1)

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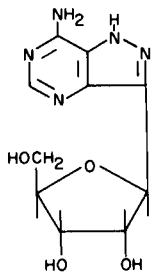
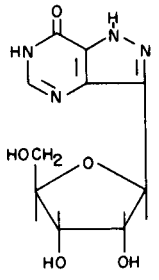
The mass spectra of the nucleoside antibiotics formycin (I), formycin B (II) and their heterocyclic aglycons (7-aminopyrazolo[4,3-*d*]pyrimidine and pyrazolo[4,3-*d*]-7-pyrimidone) have been studied utilizing high resolution mass spectroscopy. The major electron-impact fragmentation pattern exhibited by the heterocyclic aglycons can be explained by the initial expulsion of hydrogen cyanide followed by decarbonylation or by loss of a second mole of hydrogen cyanide. There is a striking difference in the fragmentation pattern of these nucleoside antibiotics where the glycoside linkage is a C-C bond as compared to nucleosides possessing a C-N bond linkage. The base peak observed for formycin and formycin B occurred at B + 30 in direct contrast to the usual nucleosides where the B + 1 or B + 2 normally occurs as the predominant peak. This pattern suggests that such a carbon linked nucleoside should be readily identified by this means. The mass spectrum of showdomycin (3-β-D-ribofuranosylmaleimide, III) was also determined and found to exhibit a similar parent peak at B + 30. This base + 30 peak has been assigned to the aglycon plus a protonated formyl group which results from fragmentation of the sugar. Mass spectrometry should prove very useful in the future structural elucidation of carbon linked nucleoside derivatives since these features (B + 30 and M-103) may be considered diagnostic.

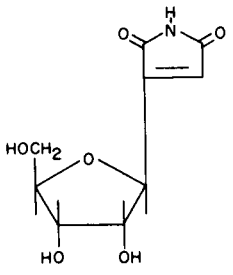
The isolation (2-4) from natural sources and subsequent characterization (5,6) of formycin (I) and formycin B (II) as carbon-linked nucleoside antibiotics created considerable interest in this class of nucleosides. The isolation (7) from *Streptomyces showdoensis* and characterization of the nucleoside antibiotic showdomycin (8) (III) as 3-β-D-ribofuranosylmaleimide, a structural relative of *pseudo*-uridine, illustrates the general occurrence of carbon linked nucleosides as natural products. The increasing importance which mass spectroscopy occupies in the area of structural elucidation of nucleic acid derivatives (9), carbohydrates (10), and certain nucleoside antibiotics (11) suggests the possible application of this tool to the area of carbon-linked nucleosides. It is of interest that the structural elucidation of the nucleoside antibiotics, formycin, formycin B, and showdomycin, was accomplished without the use of mass spectroscopy. We now wish to report the mass spectra of formycin, formycin B and showdomycin and show that the initial structural elucidation of these nucleoside antibiotics could have been facilitated by the use of mass spectroscopy.



I
Formycin



II
Formycin B



III
Showdomycin

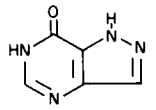
TABLE I

Mass Spectral Pattern of *C*-Nucleosides

Formycin (I) m/e	% Relative Intensity	Formycin (I) m/e	% Relative Intensity	Formycin (I) m/e	% Relative Intensity	Formycin B (II) m/e	% Relative Intensity
277	7.3	134	6.4	43	44.2	174	5.70
268	7.7	133	7.3	42	18.7	167	4.90
267	50.0	132	7.3	41	23.2	166	21.20
250	9.6	122	10.9	40	18.7	165	100.00
249	8.4	121	10.9	39	21.4	164	10.60
247	7.3	120	11.8	38	14.3	163	17.50
241	10.7	119	20.7	37	11.6	162	13.80
240	9.6	118	8.7	36	6.6	161	9.00
238	7.3	110	7.3	32	13.2	160	6.10
237	10.9	109	11.8	31	46.6	152	4.10
232	8.9	108	19.8	30	13.4	150	35.80
231	20.0	107	10.7	29	55.6	149	32.50
230	20.0	106	13.6	28	81.5	148	6.50
227	9.1	105	8.7	27	27.8	147	4.10
221	8.7	103	10.9	26	13.1	146	5.70
220	7.7	95	12.8	25	6.6	144	5.70
219	7.7	94	22.3	20	8.8	138	6.50
218	7.7	93	13.2	19	19.1	137	11.80
215	8.7	92	23.6	18	59.0	136	17.90
212	7.3	91	8.7	17	76.0	135	6.50
206	9.1	90	7.3	16	75.0	133	6.50
202	9.6	82	10.9	15	25.5	132	4.90
201	18.0	81	29.4	14	9.5	123	4.90
200	15.4	80	10.7	11	9.1	122	7.30
194	9.6	79	12.8			121	8.90
193	9.6	78	11.8	Formycin B (II)		119	12.6
192	29.6	77	7.3	269	3.66	118	6.5
191	8.0	76	12.0	268	9.35	117	4.9
188	7.3	73	11.1	238	5.05	111	4.1
186	18.3	72	8.0	237	4.70	110	6.5
185	13.2	70	9.8	232	6.50	109	8.9
179	8.4	69	9.6	221	4.20	108	13.8
178	34.8	68	26.8	220	10.60	107	10.6
177	17.7	67	18.7	219	4.90	106	9.8
176	18.0	66	13.6	216	4.90	105	11.4
174	8.7	65	24.6	207	4.10	104	6.5
173	8.2	64	17.6	206	4.10	103	7.3
166	8.2	63	9.1	204	4.10	97	4.1
165	34.2	62	7.5	203	10.60	96	9.0
164	100.0	61	13.2	202	16.70	95	9.0
163	14.3	60	30.5	201	4.10	94	21.5
162	25.0	59	12.6	195	6.50	93	11.4
161	10.9	58	7.3	193	4.10	92	14.2
160	9.6	57	13.0	192	6.10	91	8.1
150	11.4	56	9.1	191	18.70	90	4.9
149	45.5	55	18.6	187	13.00	84	6.1
148	29.6	54	44.0	186	4.10	83	6.5
147	10.9	53	27.3	180	10.60	82	11.4
146	8.2	52	17.0	179	59.30	81	19.1
145	14.8	51	7.7	178	15.50	80	10.6
144	42.8	50	7.7	177	8.10	79	12.2
137	10.2	46	15.2	176	7.30	78	11.4
136	19.1	45	81.0	175	4.50	77	11.0
135	25.0	44	84.0			76	13.0

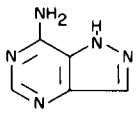
TABLE I (continued)

Mass Spectral Pattern of *C*-Nucleosides

Formycin B (II)		Showdomycin (III)					
m/e	% Relative Intensity	m/e	% Relative Intensity	m/e	% Relative Intensity	m/e	% Relative Intensity
75	5.7	229	9.0	97	16.8	32	8.7
74	4.9	213	6.4	96	11.3	31	97.0
73	14.6	212	16.8	95	29.7	30	16.1
72	4.9	211	77.4	94	22.6	29	81.0
71	8.9	201	6.4	93	13.5	28	35.5
70	10.6	200	6.4	92	6.8	27	56.5
69	9.8	193	8.7	91	9.7	26	26.8
68	17.0	183	6.0	89	7.1	25	16.1
67	23.6	182	14.5	87	10.3	24	6.5
66	26.8	181	16.5	86	34.0		
65	19.5	180	40.4	85	53.2	Pyrazolo[4,3-*d*]-7-pyrimidone	
64	18.7	178	6.4	84	17.4		
63	9.8	171	9.1	83	35.5		
62	7.7	170	8.7	82	17.7		
61	15.5	169	17.1	81	29.0		
60	22.0	168	6.8	80	29.0		
58	4.9	165	7.4	79	11.9		
57	18.7	164	9.7	78	7.4	137	7.5
56	11.4	163	6.8	77	9.7	136	100.0
55	31.0	157	10.3	75	11.6	134	6.3
54	35.8	156	38.7	74	30.7	109	5.1
53	31.0	155	9.7	73	63.0	108	4.6
52	25.2	153	12.9	72	12.2	107	3.0
51	17.0	152	38.7	71	19.4	100	1.8
50	10.6	147	6.4	70	23.6	92	5.5
47	4.9	144	6.8	69	23.6	89	1.8
46	4.1	141	11.0	68	58.0	88	2.2
45	13.8	140	48.4	67	35.5	87	2.1
44	71.5	139	27.4	66	26.8	86	2.1
43	48.0	138	17.7	65	8.1	82	4.8
42	26.0	137	20.0	64	9.4	81	4.6
41	23.6	136	11.0	63	14.2	80	2.2
40	19.5	135	9.0	62	6.5	89	1.5
39	31.0	134	6.4	61	21.0	78	1.5
38	18.7	132	8.4	60	37.2	77	2.1
37	11.4	128	9.0	59	9.4	68	2.8
33	4.9	127	93.5	58	24.2	67	1.6
32	8.5	126	100.0	57	81.0	66	7.2
31	66.0	125	9.7	561	42.0	65	3.3
30	14.6	124	16.5	55	100.0	64	3.1
29	59.5	123	49.7	54	27.2	62	2.4
28	85.5	122	24.2	53	59.7	55	7.8
27	30.1	121	11.9	52	38.7	54	35.8
26	18.7	114	7.1	51	41.0	53	21.0
25	4.5	113	6.4	50	18.7	52	4.0
19	17.0	112	14.5	47	9.7	51	2.5
18	100.0	111	26.8	45	21.0	50	1.6
17	71.5	110	87.0	44	40.4	44	2.2
16	27.6	109	29.7	43	58.7	43	1.5
15	30.1	108	25.8	42	51.7	42	1.5
14	10.6	107	7.1	41	38.7	39	4.5
		106	6.4	40	38.7	38	32.0
Showdomycin (III)		104	8.1	39	90.4	37	4.5
		99	18.7	38	48.4	36	71.6
230	4.8	98	26.4	37	20.3	35	10.4

29	2.8
28	56.6
27	11.2
26	1.5
25	1.5
24	1.5

7-Aminopyrazolo[4,3-*d*]pyrimidine



m/e	% Relative Intensity
136	10.50
135	100.00
134	2.20
119	2.40
108	8.14
107	2.20
94	1.55
93	6.20
92	1.55
82	1.55
81	16.70
80	2.40
78	1.94
66	15.10
65	2.70
64	4.50
56	5.43
55	7.00
54	33.00
53	17.60
52	5.43
51	2.50
43	11.00
42	2.70
40	2.25
39	5.80
38	4.66
37	2.30
29	4.07
28	33.00
27	7.28

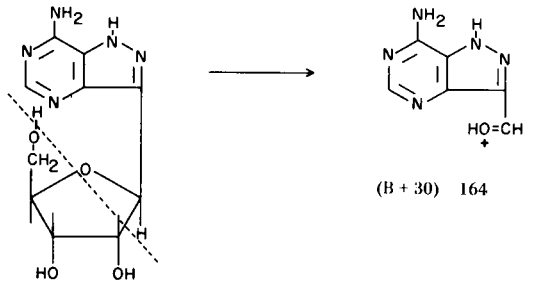
EXPERIMENTAL

Formycin and formycin B were purchased from Meiji Seika Kaisha Ltd., Yokahoma, Japan and were used without further purification. The heterocyclic bases, 7-aminopyrazolo[4,3-*d*]-pyrimidine and pyrazolo[4,3-*d*]-7-pyrimidone, were prepared *via* the reported procedures (12). Mass spectra were obtained with a consolidated Electrodynamics Corporation double focusing mass spectrometer *via* a direct insertion probe. The voltage of the electron beam was the standard 70 ev. The internal standard for high-resolution mass measurements was perfluorokerosene. A low gain was used to obtain a spectrum for use in the initial assignment of mass numbers for the peaks observed in the major fragmentation pattern. The gain was then increased to obtain peaks for use in corroborating the structural assignments. The various patterns obtained are recorded in Table I.

In the usual mass spectral patterns of the naturally occurring nucleosides of DNA and RNA, intense peaks are usually found at B + 1, B + 2 and 133 (D-ribose) where B is the molecular weight of the heterocyclic base. Thus, rupture of the carbon nitrogen bond joining the sugar to the aglycon is characteristic of these spectra (11,13). This pattern has been helpful in the mass spectral study and structural elucidation of new nucleosides isolated in small quantities from such natural sources as transfer RNA (14-16).

Observation of the mass spectral patterns of formycin (I), formycin B (II) and showdomycin (III) as given in Table I show a decided absence of major peaks for B + 1 and B + 2 and D-ribose (133) which are characteristic for the initial cleavage of the glycosidic bond of *N*-ribosides. Indeed in each instance the major peak is found at B + 30 where B is the heterocyclic aglycon. The most intense peak for formycin is found at m/e 164 and for formycin B at m/e 165. In showdomycin the major peak is observed at m/e 126. This is strong evidence that the carbon-carbon bond of the glycosyl linkage is *not* ruptured to any major extent under these conditions. The B + 30 peak is strongly suggestive of a formyl type residue attached to the base. Thus the D-ribofuranose residue is probably fragmenting in formycin as shown in Scheme 1.

SCHEME I



267

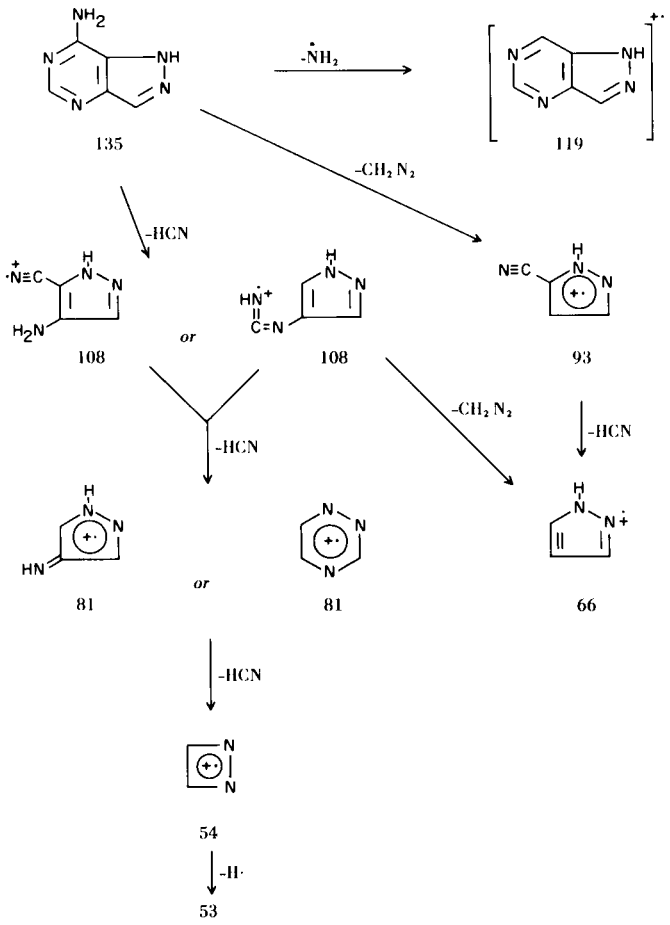
(B + 30) 164

Such a pattern would appear to be general for nucleosides which possess a sugar moiety attached to a heterocyclic base *via* a C-C bond. Recent studies of *pseudo*-uridine also exhibits a major peak at B + 30 (m/e 141) upon fragmentation in the spectrometer.

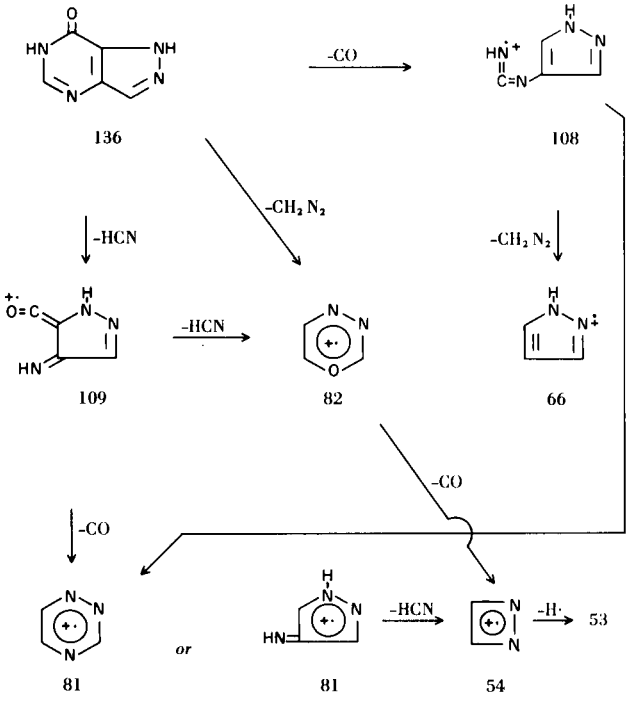
In order to better interpret the mass spectral pattern of formycin and formycin B the corresponding bases [7-aminopyrazolo[4,3-*d*]-pyrimidine (12) and pyrazolo[4,3-*d*]-7-pyrimidone (12]were similarly examined (see Table I). Examination of the peaks observed for 7-aminopyrazolo[4,3-*d*]pyrimidine reveals a fragmentation pattern strikingly similar to that observed for adenine (18). For example both compounds show major peaks at m/e values of 135, 108, 81, 66, 54, 53, 43 and 28. A possible fragmentation pattern is shown in Scheme II for 7-aminopyrazolo[4,3-*d*]pyrimidine. Such a pattern suggests rupture in the pyrimidine ring as a major early step. Thus, similar fragments would follow in the case of adenine (18) with the intact corresponding imidazole ring.

Comparison of the peaks obtained from pyrazolo[4,3-*d*]-7-pyrimidone with those of hypoxanthine (18) again reveal considerable similarity, with corresponding peaks at m/e values of 136, 54

SCHEME II (20)



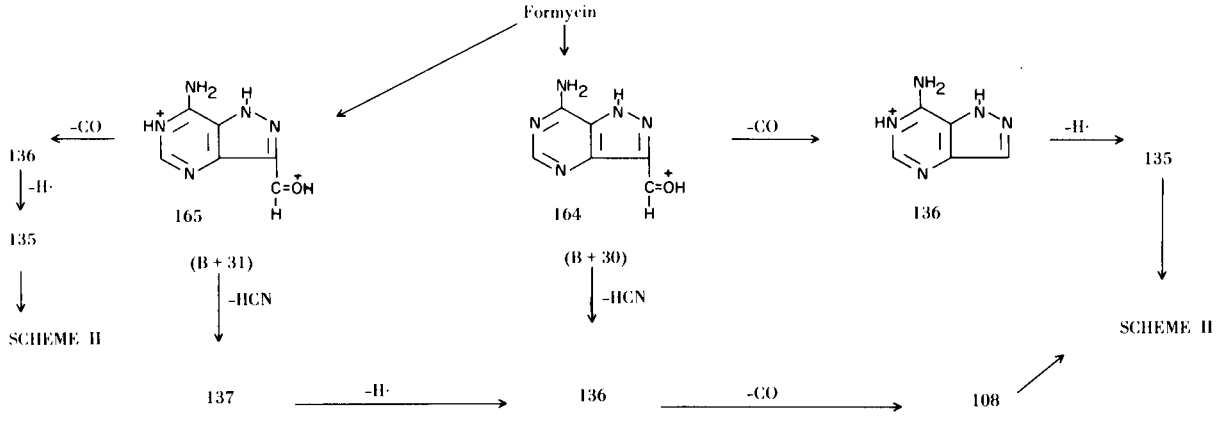
REACTION SCHEME III



and 28. Similar peaks of smaller intensity are also found at m/e 109, 108, 82, 81. A possible fragmentation pattern for pyrazolo-[4,3-*d*]-7-pyrimidone is illustrated in Scheme III. Thus loss of HCN from the pyrimidine ring would appear to be predominant in the patterns observed in both this compound and hypoxanthine. Of particular interest is the similarity of the species of m/e 81, 66, 54 and 53 which are common to both Scheme II and Scheme III and are also found in the mass spectral patterns of adenine and hypoxanthine (18). Similar structures can be drawn for the imidazole ring. The peaks (Table I) for the two pyrazolo[4,3-*d*]pyrimidines at m/e 27 may be ascribed to HCN. The peak of m/e at 28 may be a protonated species of HCN or possibly nitrogen gas, N_2.

A comparison of the mass spectra of these two bases with the corresponding nucleoside antibiotics I and II revealed several interesting correlations. Essentially all major peaks found for the bases themselves may also be found in the spectra for the corresponding nucleoside. It thus appears that the B + 30 derivative undergoes decarbonylation to yield CO and the corresponding pyrazolo[4,3-*d*]pyrimidine according to Scheme IV. The aglycon

SCHEME IV



then undergoes further fragmentation according to Scheme II in the case of formycin and according to Scheme III in the case of formycin B. A significant peak in the mass spectra of I and II is also found at m/e B + 31 (165 in the case of I and 166 in the case of II). This is probably a protonated form of the B + 30 species.

It is interesting that a better molecular ion (parent peak m/e 267) was obtained for formycin than for formycin B. The problems of obtaining a good molecular ion in the mass spectrometry of nucleosides have recently been discussed (19).

Several of the peaks observed in the spectra of I and II may be accounted for by the loss of HCN from the B + 30 specie to give in the case of I, m/e 137 which then loses a hydrogen to give 136. This specie is comparable to m/e 108 in Scheme II with a protonated formyl group.

The mass m/e 136 may now decarbonylate to m/e 108 which may now fragment further to m/e 81 as indicated in Scheme II.

Because of the additional hydrogen present in the nucleosides more protonated species are found in the mass spectra of I and II than in the spectra of the corresponding pyrazolo[4,3-*d*]pyrimidine bases.

Formycin also appears to be undergoing fragmentation *via* dehydration. The peaks m/e 249, 230 and 231 are due to the loss of one and two moles of water, respectively, probably from the carbohydrate portion. It is interesting that this can occur without cleavage of the glycoside bond.

It is difficult to pick out further fragmentation patterns of the D-ribofuranose ring since the possibilities are considerable. It does appear, however, that many of the characteristics of the spectra of I and II may be seen in the mass spectra of showdomycin (III, Table I). There is a major peak at m/e 211 showing the loss of one mole of water from the nucleoside antibiotic. The familiar B + 30 and B + 31 peaks appear at m/e 126 and 127, respectively. The numerous peaks observed in III can be attributed to the breakdown of the maleimide ring which does not have the resonance stability of the pyrazolo[4,3-*d*]pyrimidine system. It is quite likely that the B + 30 fragment gives rise to very little absorption due to maleimide itself which is observed at low intensity at m/e 96.

It should be pointed out that although regular *N*-nucleosides often exhibit (11,12,15) a relatively weak peak at B + 30 the usual strong peaks at B + 1 and/or B + 2 which arise by the loss of ribose are essentially absent in the case of the *C*-nucleosides.

The B + 30 peak in the present study may also be considered as a M-103 peak where M is the molecular ion. (Formycin 267-103 = 164; formycin B 268-103 = 165; and showdomycin 229-103 = 126). Therefore, this would suggest that if an antibiotic of unknown structure displayed a major M-103 peak in the mass spectrum this would suggest the probability of a *C*-linked pentoside structure. This information would be especially useful prior to the actual determination of the structure of the heterocyclic base.

It does appear that the mass spectra of carbon linked nucleosides do lend themselves to distinctive patterns which may be interpreted and utilized for future structural problems, both in heterocyclic and nucleoside chemistry.

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(20) The fragment ions proposed in these reaction schemes have the potential of accomodating the positive charge by delocalization on a number of atoms, especially the heteroatoms. Therefore, although we have depicted the charge residing on a specific atom in certain structures, this has been done primarily to facilitate a better understanding of the subsequent fragmentation patterns proposed.

Received June 23, 1969 Salt Lake City, Utah 84112