J. G. Liehr, C. L. Weise, P. F. Crain†, George H. Milne†,

Dean S. Wise**, Leroy B. Townsend** and James A. McCloskey†*

Institute for Lipid Research, Baylor College of Medicine, Houston, Texas 77025

Departments of Medicinal Chemistry† and Biochemistry*,
University of Utah, Salt Lake City, Utah 84112

Mass spectra of certain selenobases and selenonucleosides, and some of their trimethylsilyl and O,N-permethyl derivatives have been studied from the standpoint of structural characterization, and in order to ascertain the influence of selenium on normal fragmentation patterns. Molecular ion abundances of the selenouracils are intermediate between those of the corresponding oxygen and sulfur analogs. Fragmentation processes are similar to those of the corresponding normal bases and nucleosides but with additional ions resulting from expulsion of Se or SeH in most cases. Trimethylsilylation occurs at approximately the same rate as for normal bases and nucleosides but the products show decreasing stability with prolonged heating. A least squares procedure is demonstrated which generates monoisotopic mass patterns and assists in interpretation of the mass spectra.

Introduction.

Synthesis of selenium-containing analogs of nucleosides and their bases has been reported in relation to their potential as anti-cancer agents (2). An interesting series of experiments has been reported in which ^{75}Se was incorporated into growing E. coli, from which selenonucleosides were isolated (3), one of which was identified as 4-selenouridine based on comparison with the authentic compound (3,4). In view of the established utility of mass spectrometry for the characterization of nucleoside structure (5,6) we have studied the mass spectra of a number of selenium-containing analogs of purine and pyrimidine bases and their nucleosides. Results from selenium substitution at various sites in the heterocyclic moiety were examined to determine the influence on normal modes of base and nucleoside fragmentation, and in some cases to compare the behaviour of corresponding oxygen, sulfur and selenium analogs. The constraints of decreased volatility resulting from selenium substitution were also considered because of the requirement that some of the more polar nucleosides be converted to volatile derivatives for mass spectrometry, and because of the potential importance of gas chromatography-mass spectrometry as a technique for examination of mixtures of nucleosides and bases (7).

Discussion of Mass Spectra.

The mass spectrometry of organoselenium compounds has been reviewed by Agenas (8), and several detailed comparisons of oxygen, sulfur and selenium-containing molecules have been reported by Duffield, et al., (9).

Deutsch and co-workers have reported some data from the mass spectrum of 3,7-dimethyl-6-selenopurine with brief comment on its interpretation (10). From these and other reports on selenoheterocycles (11,12) several general characteristics of their mass spectra can be summarized: (a) molecular ion abundances tend to lie between those of the analogous oxygen and sulfur analogs, the latter being characteristically greater than the former (13); (b) direct expulsion of Se or SeH from molecular or fragment ions is commonly observed; (c) in the case of complex mass spectra in which peaks from different ion species may fall close together in the spectrum, the interpretation of the spectrum may be confused by the complex pattern resulting from the six stable isotopes of selenium; (d) in some cases thermal instability of organoselenium compounds leads to the presence of artifacts in the spectrum generated during sample vaporization (8). These characteristics have been generally observed in the present study, with the exception of that relating to thermal instability, which in the case of selenonucleosides was only slightly more noticeable than in work with normal nucleosides. In such cases, chemical conversion to more volatile derivatives appears to offer a generally satisfactory solution.

Bases and Nucleosides.

The two isomeric monoselenouracils 1 and 2 and 2,4-diselenouracil (3) offer an opportunity for comparison with uracil (14) and the corresponding thiated uracils (15), 4-6. A well-known general property of sulfur-containing compounds is their tendency to form more abundant

1, X = Se
4, X = S

2, X = Se
5, X = S

3, X = Se
6, X = S
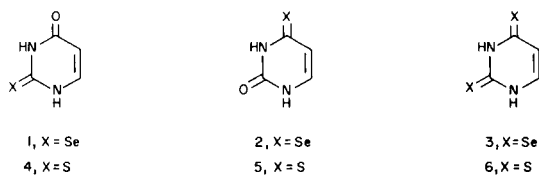
molecular ions than their oxygen-containing analogs (13). It is therefore of interest to examine the same property in relation to selenium, which is slightly more metallic in character than sulfur. A qualitative survey of the mass spectra of organoselenium compounds (8) shows that molecular ion abundances are enhanced by replacement of oxygen by selenium. In the relatively few studies in which data from all three analogs were reported, it appears that selenium is intermediate between oxygen and sulfur (9,11c). Data from 70 eV mass spectra from the present study and from the literature using an identical instrument (LKB 9000) are shown in Table 1 (16). These comparisons show that for the selenouracils, molecular ion abundances lie between those of the corresponding oxo- and thio-analogs, but that the positional effects (C-4 vs. C-2) differ. The positional order of abundances for **1-3** is therefore 2,4 > 2 > 4, while for the sulfur analogs **4-6** it is 2,4 > 4 > 2. The general observation as to the effects of selenium being intermediate between oxygen and sulfur supports the observations made for five-membered saturated heterocycles by Djerassi and co-workers (9) and 1,2,5-selenodiazole analogs by Pedersen and Moller (11c).

O = C = CH = CH = NH
m/e 69

of HCN and CO to form ions of m/e 149 and 121, respectively (17). These reactions are insignificant in the mass spectra of uracil (14), the isomeric thiouracils **4** and **5** (15), as well as 4-selenouracil (Figure 2). The characteristic retro-Diels-Alder reaction in which N-1, C-2 are expelled is evident in the case of **1** (Figure 1), but not **2** (m/e 133, Figure 2):

This process is useful in establishing sites of thiation (15), ^{18}O incorporation (18,19) and other structural features in the pyrimidine series. Its failure to operate in prominent fashion in the case of **2** may be due to differences in initial charge localization (the process is also less prevalent in **5** than **4**), or to increased contribution from enol-type tautomers in the gas phase which would block the cyclohexene-like structure which is required (20). The prominent m/e 68 in the spectrum of **2** is a doublet: $C_3H_4N_2$ and C_3H_2NO (2:1), the latter which corresponds to one hydrogen less than m/e 69 from **1**. Direct expulsion of CO from the molecular ion of **2** (m/e 148, Figure 2) mirrors the behavior of **5**, in which the same process is favored over the 2-thio isomer **4**. In both isomers **1** and **2** loss of SeH (m/e 95) and presence of Se$^+$ (m/e 80) are observed as in the case of many selenium-containing compounds (8).
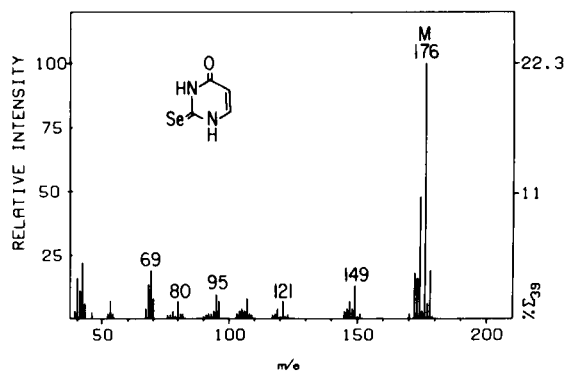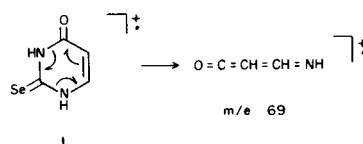

Figure 1. Mass Spectrum of 2-Selenouracil (1).

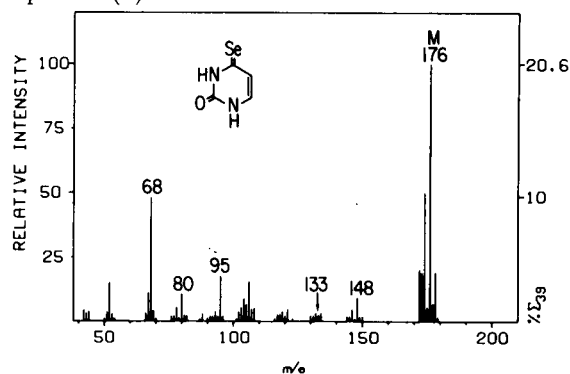As shown in Figure 1, the principal decomposition pathway in 2-selenouracil involves sequential expulsion
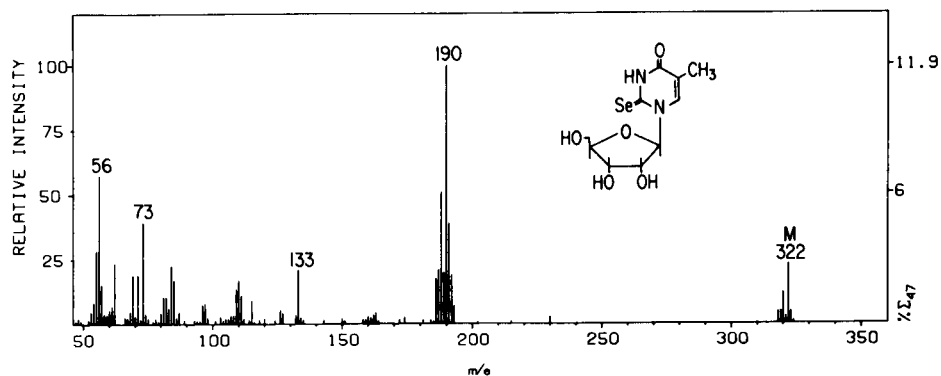

Figure 2. Mass Spectrum of 4-Selenouracil (2).

Behavior of the diseleno analog **3** was found to be qualitatively similar to that of 2,4-dithiouracil (6) recorded at 20 eV (15):

	(M-XH)$^+$	(M-XCN)$^+$	XCN$^+$
M$^+$ (3) 100% relative intensity	m/e 159 36%	m/e 134 20%	m/e 106 29%
M$^+$ (6) 100% relative intensity	m/e 111 88%	m/e 86 24%	m/e 58 24%

The Se$_2$ pattern is clearly recognizable in the abundant molecular ion region.

Figure 3. Mass Spectrum of 2-Seleno-1-(β-D-ribofuranosyl)thymine (7).
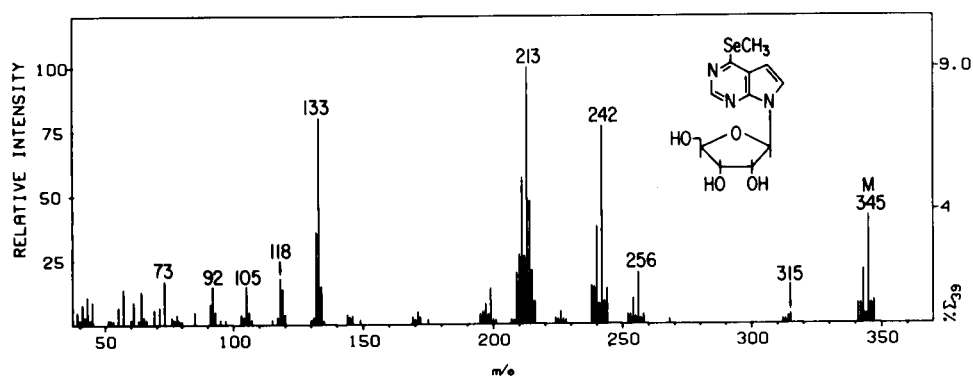
Figure 4. Mass Spectrum of 2-Methylseleno-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (9).

A group of nucleosides which were sufficiently volatile for direct vaporization was studied in order to determine the effects of selenium on the principal modes of nucleoside fragmentation, most of which involve cleavage of sugar bonds with the base remaining intact (21). Two models whose mass spectra were qualitatively very similar to normal nucleosides were 2-seleno-1-(β-D-ribofuranosyl)-thymine (7) and 8-methylselenoinosine (8). The spectrum of 7 (Figure 3) is dominated by the base + H species, with a modest but prominent peak (m/e 133) representing the sugar moiety. The spectrum is strikingly similar in


7
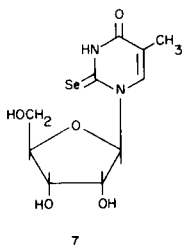
appearance to that of 1-(β-D-ribofuranosyl)thymine (22) with the obvious exception of higher mass of base-containing ions and the characteristic appearance of the selenium isotope cluster; also in parallel are lower mass peaks representing the sugar, such as m/e 73 ($C_3H_5O_2^+$).

Strong correlation can normally be made between the tendency for charge retention in the base and the balance of base vs. sugar-related ions (5). Therefore, in pyrimidine nucleoside spectra, the sugar ions are of moderate abundance while in the case of purine nucleosides the ion population is usually dominated by base-containing ions. In the spectrum of 1-(β-D-ribofuranosyl)thymine, in which sugar ions compete effectively with those containing the base, the distribution is largely undisturbed by the presence of selenium in the base, leading to the conclusion that the heteroatom has relatively little influence on the normal modes of fragmentation.

To some extent these observations can be extended to 8-methylselenoinosine (8), whose mass spectrum resembles that of the parent nucleoside, inosine (22-24): the molecular ion is of low abundance, and the base + H

8, m/e 362 (3.0 %)
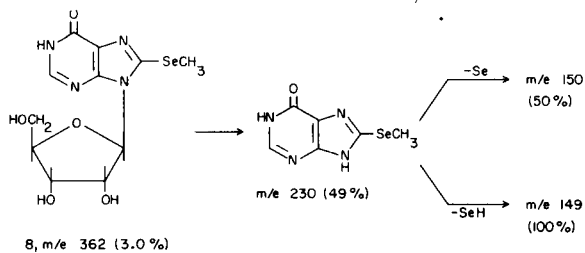
ion is prominent, to the exclusion of the base + CH_2O and other normal fragment ions. However, unlike inosine the presence of selenium is strongly reflected in the abundant ions of m/e 149, 150 due to loss of SeH and Se from m/e 230. Although the latter process is common in the mass spectra of many organoselenium compounds its prevalence in the case of **8** suggests the possibility of some thermal degradation, which cannot be regorously excluded. Similar loss of Se from the base + H ion was observed in the mass spectrum of 4-methylseleno-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine ((**9**) 6-methylthio-7-

9   10
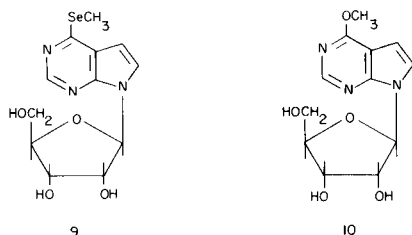
deazapurine riboside), Figure 4. The product ion, m/e 133, coincides in mass with the sugar fragment m/e 133, but the high resolution mass spectrum of **9** shows the absence of $C_5H_9O_4$, as is often the case for purine nucleosides (5). Other fragmentation products representing the base plus C-1', O-4'(m/e 242), base plus C-1', 2', O-2' (m/e 256), and M- 5' CH_2O (m/e 315) are seen at abundance levels that are normal to conventional nucleosides. In addition, less intense peaks due to the base + 2H species are superimposed on the isotopic cluster from the base + H ions (m/e 213). Further loss of the methyl group bound to selenium produces m/e 199 (m* = 185.1). The high resolution spectrum of **9** shows m/e 73 to be the common sugar fragment $C_3H_5O_2^+$ (25), while degradation products of the base are represented by m/e 92 ($C_5H_4N_2^+$), 105 ($C_6H_5N_2^+$) and 118 ($C_6H_4N_3^+$). The charactersitic sequential loss of three molecules of HCN from the 7-deazadenine moiety (26) is not observed as a consequence of methylseleno substitution. Otherwise

the overall features of the conventional nucleoside spectrum are observed. This conclusion is further supported by the mass spectrum of 4-methyl-7-(β-D-ribofuranosyl)pyrrolo-[2,3d]pyrimidine (**10**, 6-methoxy-7-deazapurine riboside), which is dominated by the base + H peak (m/e 149, 100%, 25%Σ) and base + CH_2O (m/e 178, 67%), with smaller contributions from m/e 192 (base + C_2H_4O, 14%) and the molecular ion (m/e 281, 4.5%).

Beacuse the mass spectrum of **9** shows a balance of features representing normal nucleoside fragmentation as well as processes associated with presence of selenium, the same compound was subjected to chemical ionization (CI) to examine the extent to which selenium-specific processes survive. Methane reagent gas generally produces extensive fragmentation with nucleosides (27), and the methane CI spectrum shown in Figure 5 is no exception. The molecular weight region is emphasized by the protonated molecular ion (m/e 346) and characteristic adduct ions M + C_2H_5 (m/e 374) and M + C_3H_5 (m/e 386). The usual products (27) base + 2H (m/e 214) and base + CH_2O (m/e 242) are present but a surprising number of ions associated with the presence of selenium are observed which have no analogy in the electron ionization spectrum. Expulsion of Se from MH^+ (m/e 266), from base + 2H (m/e 134) and from base + CH_2O (m/e 148) and from the base + 2H species (m/e 120) are without counterpart in Figure 4. Since conditions of chemical ionization are milder than those associated with electron impact, these reactions are evidently a consequence of the even-electron character of the precursor (MH) in which the base is protonated. The abundant ion of m/e 97 is assigned the composition $CH_3SeH_2^+$, and presumably bears the proton transferred during initial ionization by CH_5^+.

For the final compound of the free nucleoside series a model was chosen, 6-γ,γ-dimethylallyseleno-9-(β-D-ribofuranosyl)purine (**11**), which contains a functional group that is normally influential in directing the course of fragmentation and thus generates a number of side chain-related ions. The mass spectrum of the nitrogen-contain-
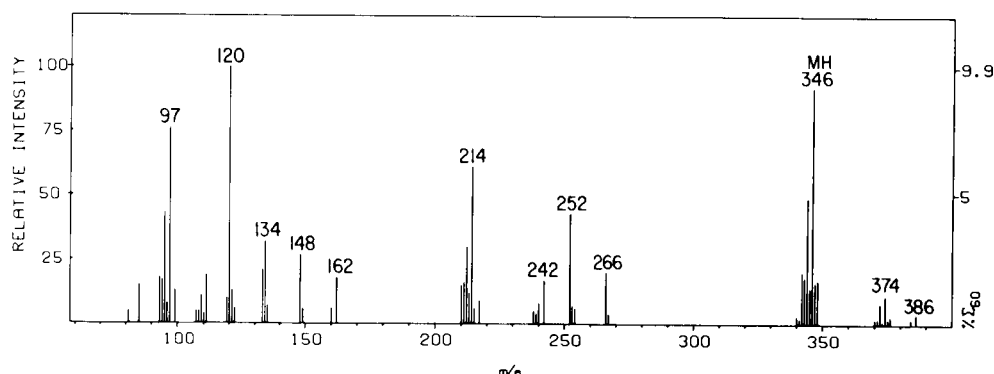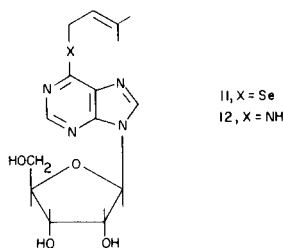

Figure 5. Chemical Ionization (Methane) Mass Spectrum of 4-methylseleno-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]-pyrimidine (**9**).

II, X = Se
12, X = NH

ing counterpart N⁶-(3-methyl-2-butenyl)adenosine (12) has been studied extensively (28) and has been a valuable model in the characterization of a number of natural analogs of 12 that exhibit cytokinin activity. The primary fragmentation process from 12 is formation of the tricyclic ion a by loss of C_3H_7 from both the molecular ion and base + H species (29,30). In the mass spectrum of 11 these ions (structure a) are present but are sharply reduced in abundance by the replacement of Se for NH:

R = H or ribosyl

a
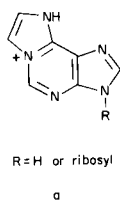
M - C_3H_7 is not observed and base + H - C_3H_7 (m/e 225) is reduced from the base peak in the spectrum of 12 (m/e 160) to only 7% in 11. The most prominent ions are associated with fragmentation of the base + H species:

m/e 188 (55%)   -Se
m/e 187 (86%)   -SeH

m/e 268 (17%)   (arrows)   m/e 200 (79%)
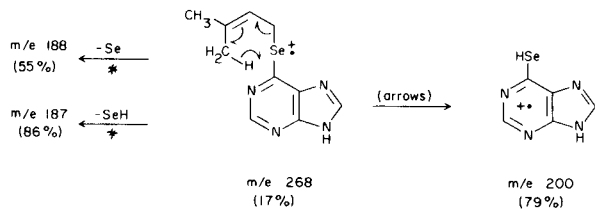
The mechanism shown for formation of m/e 200 follows the analogous major pathway proposed in the case of 12 (31), supported by deuterium labeling in the terminal

methyl groups (30). The base peak of the spectrum was observed to be m/e 69 ($C_5H_9{}^+$) while the related ion m/e 41 ($C_3H_5{}^+$), also derived from the isopentenyl moiety, is likewise abundant (94.5%). Both are formed from 11 but in lower abundance.

Volatile Derivatives of Bases and Nucleosides.

While many structurally simple nucleosides are sufficiently volatile to be directly sublimed into the ionizing region of the mass spectrometer, the more polar members of this class, e.g., guanosine, require blocking of the hydroxyl and amino groups in order to permit vaporization. Replacement of oxygen by selenium qualitatively appears to decrease volatility as expected, but the effect is not severe so that the mass spectra of most free seleno-nucleosides can be determined if those of the oxygen analogs can. Derivatization can therefore be applied to the more polar molecules, and in cases in which corroboration of certain structural features is required. In the present study, both trimethylsilylation and permethylation have been employed for a number of seleno bases and nucleosides. Representative results from their mass spectra are reported.

Trimethylsilylation was observed to proceed more slowly compared with the corresponding oxygen analogs, occasionally leading to incomplete reaction. The difference appears to be more pronounced for bases than for nucleosides. Figure 6a shows relative reaction yields for uracil and 2-selenouracil, based on gas chromatographic peak heights of the resulting derivatives. Uracil reaches a plateau after 30 minutes and is then stable toward further heating, while an equal weight of 2-selenouracil reacts more slowly and then degrades after 50 minutes of heating. By comparison, guanosine and 6-selenoguanosine produce the pentasilyl derivatives at approximately the same rate (Figure 6b), but at longer periods of time than shown in Figure 6b (several hours) the yield of selenoguanosine slowly decreases relative
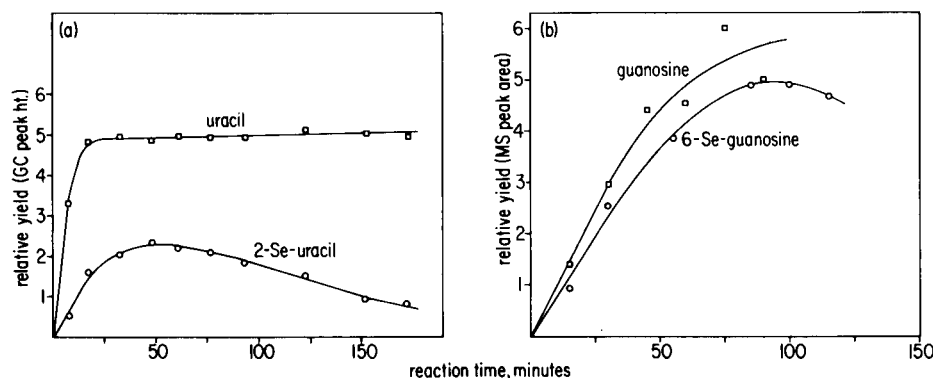

Figure 6. Relative Reaction Rates for Trimethylsilylation of (a) Uracil and 2-Selenouracil, (b) Guanosine and 6-Selenoguanosine.
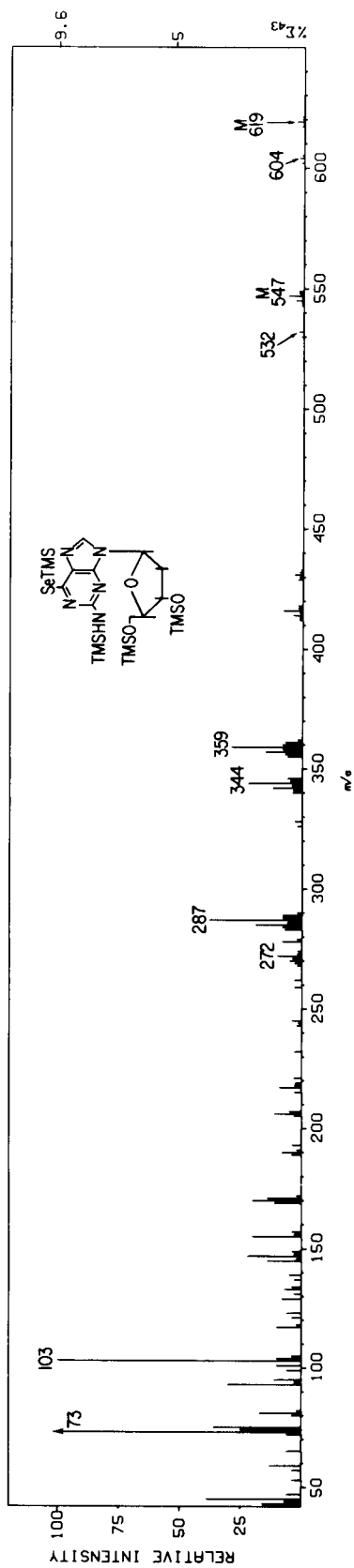
Figure 7. Mass Spectrum of the Trimethylsilyl Derivative of 6-Selenoguanosine (15).
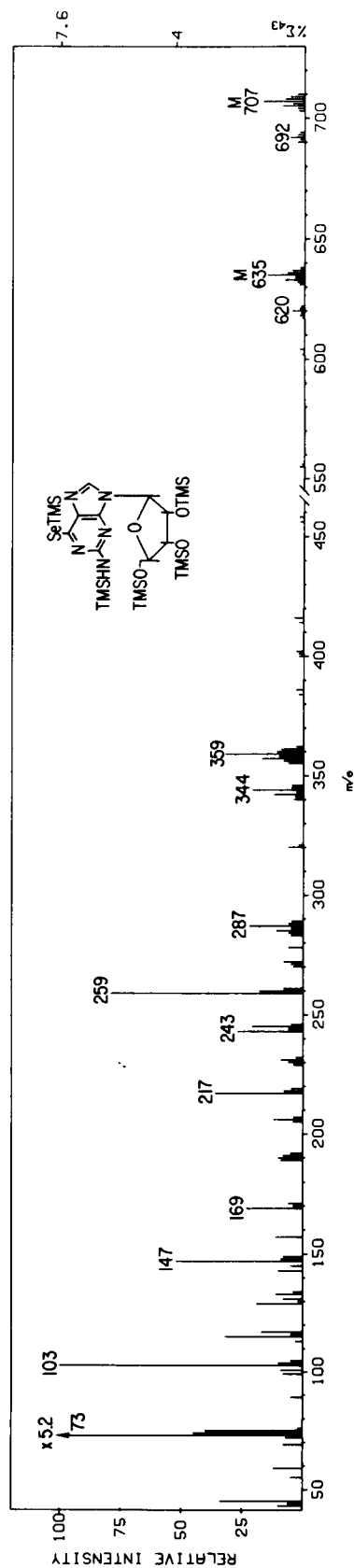
Figure 8. Mass Spectrum of the Trimethylsilyl Derivative of 6-Seleno-2'-deoxyguanosine (16).

to guanosine. Different ordinate values are represented in Figure 6b, therefore only rates rather than yields can be compared.

The mass spectrum of the trimethylsilyl derivative **13** shows fragmentation products directly analogous and similar in abundance to those from silylated uracil and the isomeric thiouracils. The position of selenization is clearly indicated by prominent fragment ion m/e 99

OTMS

$OSi(CH_3)_2$

TMSSe   N

TMSSe   N ---- 99 (59%)

13   m/e 305 (96%)

SeTMS

TMSO   N

14
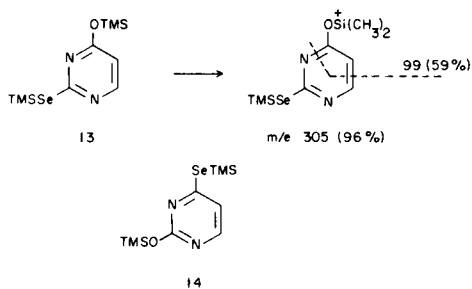
containing C-4,5 derived from the $M-CH_3$ ion precursor (32). The 4-seleno isomer **14** however fails to exhibit the analogous ion (m/e 163), suggesting the difference to be associated with the role of selenium in relation to loss of CH_3 from the C-4 substituent.

Trimethylsilylation of 6-selenoguanosine results in the mass spectrum shown in Figure 7, which exhibits molecular ions from the pentasilyl (**15**, m/e 707) and tetrasilyl (m/e 635) derivatives. Trimethylsilylation of guanosine under the same conditions leads to essentially complete conversion to the higher derivative. In Figure 7, the spectrum shows the site of incomplete silylation to be the base rather than the sugar as judged by the parallel appearance of base + H ions 72 amu apart: m/e 359 and 287.
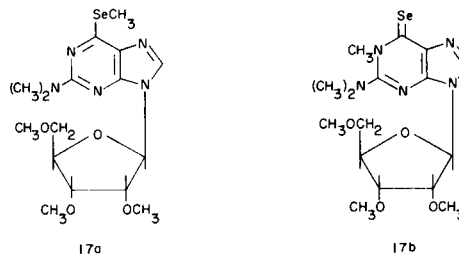
It is notable that in the present study the ion intensity ratio $M > M-CH_3$ was found to occur to an unusual extent in the mass spectra of silylated nucleosides. In spectra of conventional nucleosides the reverse is usually true, the most common exception being the nucleosides of guanine. Only the spectrum of the silyl derivative of 6-seleno-7-deazapurine riboside exhibited the ratio $M-CH_3 > M$, while the following showed the opposite behavior: derivatives of **7-9**, **11**, 2-selenocytidine, 2-selenouridine, and 7-selenoformycin.

Compared with pentasilyl guanosine (33) the number and abundance of base-containing ions is decreased, and a substantial fraction of total ion current is carried by sugar ions: m/e 259, 245, 243, 217, 169 and 103 (33). A similar effect is seen in the case of 2'-deoxyselenoguanosine

SeTMS

TMSHN   N   N

TMSOCH_2   O

TMSO   R

15, R = OTMS
16, R = H
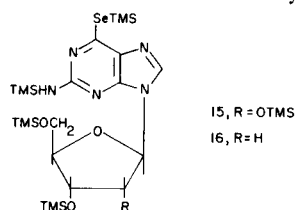
(**16**, Figure 8) in which the lower (trisilyl) derivative predominates. Both **15** and **16** exhibit relatively few of the normal nucleoside ions which contain the intact base and fragments of the sugar. The absence or lower abundance of base + CH_2O ions in Figures 7 and 8 (m/e 316 or 244) parallels the low abundance of the base + 2H species, which is mechanistically derived from loss of CO from the base + CH_2O ion (5). By contrast, the trimethylsilyl derivatives of guanosine and 2'-deoxyguanosine lead to significant populations of base-containing ions (5,33). However, unlike the free nucleosides which exhibit loss of Se and SeH, no significant new processes in Figures 7 and 8 are induced by the presence of selenium, so that the interpretation of the spectra can be made on the basis of the oxygen analogs in spite of the presence of two derivatives in each case. From the spectra of other silylated selenonucleosides it was determined that a normal series of base-containing ions is often present, so that their low abundance in Figures 7 and 8 does not appear to represent a general characteristic.

As an alternative to trimethylsilylation, N,O-permethylation has been shown to produce nucleoside derivatives of lower molecular weight, slightly lower volatility, greater chemical stability, and whose mass spectra represent considerable structural detail (34-37). For comparison with the trimethylsilyl derivative **15**, the permethyl derivative of 6-selenoguanosine was prepared and its mass spectrum examined (Figure 9). The spectrum shows incorporation of six methyl groups (mw 431), but from

$SeCH_3$

$(CH_3)_2N$ N   N

CH_3OCH_2 O

CH_3O OCH_3

17a

Se

CH_3N N   N

$(CH_3)_2N$ N   N

CH_3OCH_2 O

CH_3O OCH_3

17b

the available evidence the assignment of structure from among the several possible tautomers cannot be made. Guanosine upon permethylation by the same method forms approximately equal amounts of enol and keto derivatives (35), corresponding to **17a** and **17b** in the present case. The mass spectrum of **17** shows a more stable molecular ion than **15** with no evidence of a lower pentamethyl derivative. Fragmentation produces a relatively even balance of base- and sugar-related ions, the mass of the base being clearly indicated by the intense b + H peak (m/e 257) and the characteristic b + CH_2O (m/e 286) and b + C_2H_4O (m/e 314) peaks. Loss of a methyl radical from b + H generates m/e 242, while the characteristic expulsion of CH_2NH from the dimethylamino function (38) yields m/e 228 which retains the characteristic selenium pattern. Of interest is the intense peak (m/e
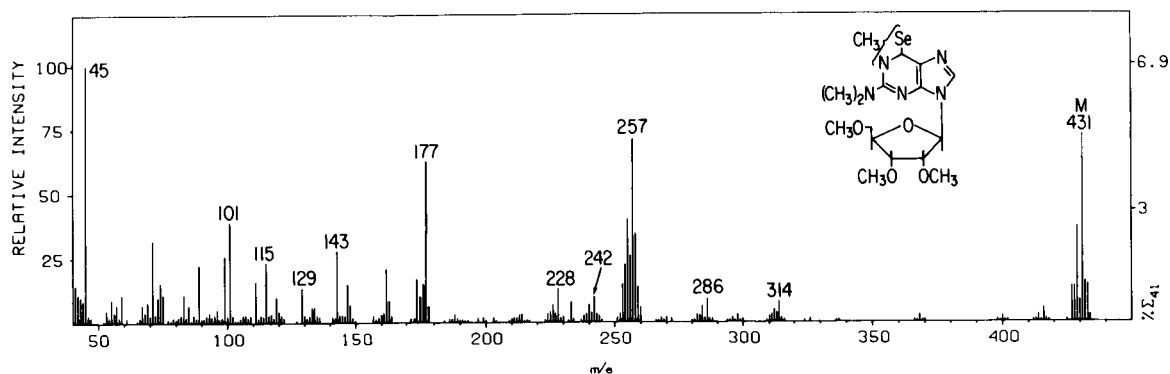
Figure 9. Mass Spectrum of the Hexamethyl Derivative of 6-Selenoguanosine (17).
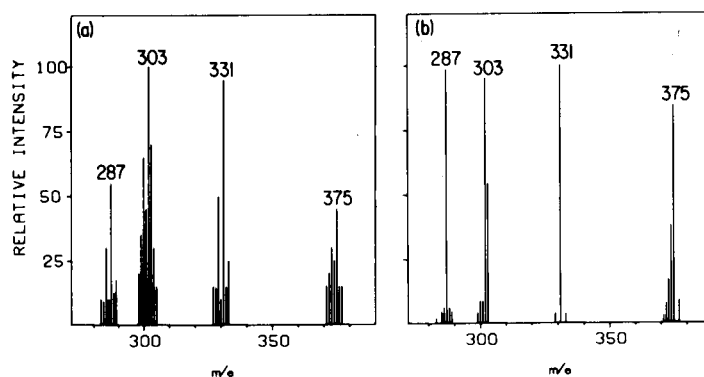

Figure 10. Partial Mass Spectra of the Trimethylsilyl Derivative of 8-Methylselenoinosine (18); (a) All Isotopic Species, (b) Monoisotopic Species.

177) corresponding to loss of Se from b + H, which has direct analogy in the behavior of 9 (Figure 4) which has the same 6-methylselenopurine structure. The likelihood of thermal decomposition as a route to m/e 177 cannot be rigorously excluded, but is judged as unlikely because the principal ion ratios (m/e 431, 257, 177) do not change during sample vaporization and because no loss of Se from the molecular ion is observed. Numerous low mass peaks shown in Figure 9 represent previously established fragmentation products of the 2',3',5'-O-trimethylribose moiety: m/e 174 (sugar - H), 143, 129, 115, 111, 101, 99 and 45 (35). As pointed out previously (35) the presence of O-methyl groups in the sugar prior to permethylation is readily differentiated and located by the introduction of CD_3 rather than CH_3 groups during derivatization.

Simplification of Isotopic Patterns.

In instances in which the primary ion species is accompanied by another species one mass unit higher or lower due to differences in hydrogen content, the complexity of the isotopic pattern may lead to difficulty of interpretation. This problem is more prevalent in the

case of trimethylsilylated selenonucleosides which contain multiple isotope peaks representing six isotope of selenium, three of silcon, two of carbon, and additional minor isotopes. Resolution of the clusters into their mono-isotopic components is therefore a helpful means of distinguishing contributions from adjacent but different ion species. We have adopted the computer-based method of Crawford (39) for analysis of these patterns. The technique involves least squares calculations which utilize experimental ion abundance data and known or assumed elemental compositions. The closest monoisotopic fit is generated which shows the residual, i.e., unaccounted for, ion intensities, and the data are presented as summed intensities representing the principal isotopic species
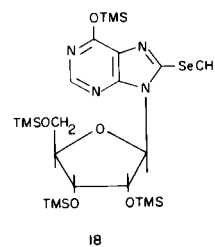
(^{12}C, ^{28}Si, ^{80}Se, ^{16}O, *etc.*). An example of the approach is demonstrated by the partial spectrum of base-containing ions from silylated 8-methylselenoinosine (18), Figure 10a. Inspection of the clusters centered around m/e 287 and 331 shows that only one ionic species is represented, but the m/e 303, 375 groups contain more than one. Reference to the general fragmentation scheme of silylated nucleosides (33) shows the probability that the ions shown are: m/e 375, base + HTMS; m/e 331, base + CH_2O; m/e 302, base + H; m/e 278, base + H - CH_3. Based on the corresponding elemental compositions the mono-isotopic spectrum shown in Figure 10b was generated. Purity of the m/e 287, 331 clusters is confirmed while the ratio of base + H to base + 2H ions, each of which arises by different fragmentation paths (33), was found to be 92:52. Similarly, the base + HTMS species is seen to be accompanied by a second ion one mass unit lower (base + TMS), plus several smaller ones of unknown origin.

In spite of the advantages to be gained in simplifying the isotopic patterns for detailed interpretation of the spectra, it is believed that characterization of selenium-containing compounds should in most cases be done using the raw mass spectrum. From the standpoint of the synthetic chemist the unique selenium isotopic pattern provides a simple means of verifying the presence and exact number of selenium atoms in final products, intermediates and unexpected by-products.

Table 1

Molecular Ion Abundances from the Mass Spectra
of Seleno and Thio Analogs of Uracil

Compound No.	% Σ_{40} (a)	Reference
uracil	43	5
1	46	(b)
2	43	(b)
3	33	(b)
4	45	5
5	50	5
6	43	(b)

(a) Calculated using the sum of all isotopic species of the molecular ion. (b) Present work.

EXPERIMENTAL

Sources of compounds are as follows: 1, 2-selenouracil (40), 2, 4-selenouracil (41); 3, 2,4-diselenouracil (40); 7, 2-seleno-1-(β-D-ribofuranosyl)thymine (41); 8, 8-methylselenoinosine (42); 9, 4-methylseleno-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (43); 10, 4-methoxy-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (44); 11, 6-γ,γ-dimethylallylseleno-9-(β-D-ribofuranosyl)-purine (45); 6-selenoguanosine (46); and 6-seleno-2'-deoxyguanosine (47).

Mass spectra were recorded using LKB 9000 and 9000S instruments; ionizing energy 70 eV, ion source temperature 250°. Compounds 13 and 14 were introduced by gas chromatograph (150°, 6 ft 1% OV-17) and the remainder by direct probe

over the approximate temperature range 70-130°.

The chemical ionization mass spectrum of 9 (Figure 5) was obtained using methane reagent gas at 0.5 torr using a CEC 21-110B instrument that was modified for high pressure operation (48). Exact mass measurements were made at R = 13,000-18,000 by peak matching and photographic recording using CEC 21-110B and Varian MAT 731 mass spectrometers.

Trimethylsilyl derivatives were made by the general procedure of heating the dried nucleoside or base in N,O-bis(trimethylsilyl)-trifluoroacetamide, trimethylchlorosilane, pyridine (100:13:1) for one hour at 78° (bases) or 100° (nucleosides). Temperatures over 120° for the selenouracils resulted in a notable decrease in reaction yield. The N,O-permethyl derivative 17 was prepared by a standard procedure using methyl iodide and methylsulfinyl carbanion (35).

Data in Figure 6a were acquired from gas chromatographic peak areas (128°, 1 ft 1% OV-17); the sample consisted of a mixture of equal weights (200 μg each) of uracil and 2-selenouracil which were silylated by the above procedure. Data for guanosine and 6-selenoguanosine (Figure 6b) were obtained from separate experiments in which guanosine points were derived from gas chromatographic peak heights (240°, 1 ft 1% OV-17), and 6-selenoguanosine from selected ion recording areas from the molecular ion (m/e 707) after direct probe introduction.

Isotopic pattern least squares calculations were carried out with an IBM 360-50 computer, using a program written in FORTRAN and PL/1, based on the work of Crawford (39). Core allocation was 200K bytes. Input data consisted of experimental mass-abundance data, a list of elements to be considered and suspected or potential elemental compositions of the principal ion species. Typical computation times, such as for the data shown in Figure 10, were three to five minutes.

REFERENCES AND NOTES

**Present address: Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.

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