Mass Spectrometry of Trimethylsilyl Derivatives of Disubstituted Pyridines, Quinolines, Pyrimidines, and Pteridines

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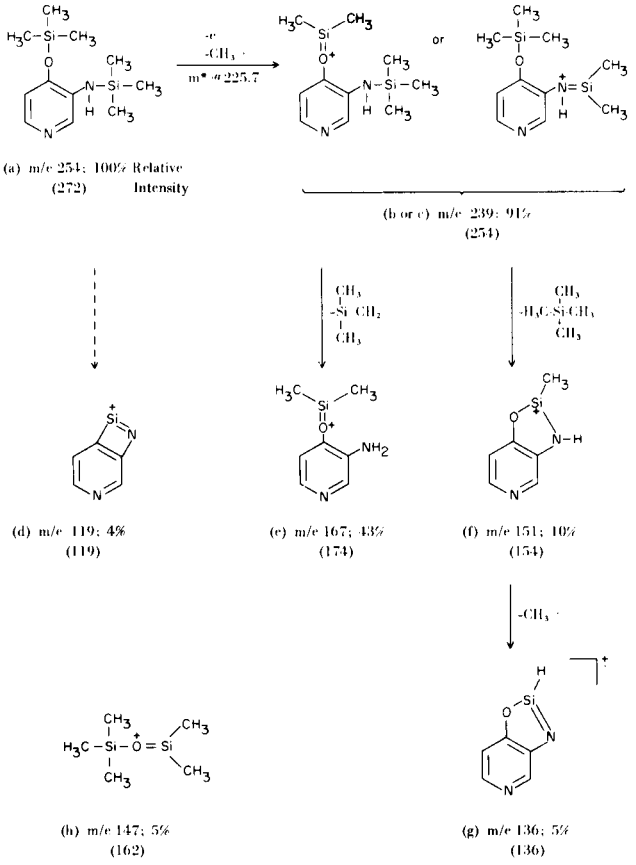
The mass spectra of substituted pyridines, quinolines, pyrimidines, and pteridines have received wide treatment in the literature. Brown and Moser (1) have recently reported on the mass spectra of pyridinecarboxylic acids and have found that M-44 is the preferred fragmentation for 2-substituted compounds whereas M-17 is predominant for those substituted at the 4 position. Brown, *et al.* (2) have studied aminoquinolines, which lose HCN from the M^+ yielding the common M-27 fragment. The mass spectra of these compounds have been reviewed by Porter and Baldas (3) and Budzikiewicz, Djerassi, and Williams (4). With the prevalent use of the combination of gas chromatography-mass spectrometry (GC/MS), it is commonplace to synthesize derivatives of compounds which are volatile and stable (to GC conditions) and, therefore, more amenable to GC/MS. The trimethylsilyl derivatives are among those compounds most frequently used for this purpose. Little has been published, however, on the mass spectra of the TMSi derivatives of these compounds although combined GC/MS of some pteridines as their TMSi derivatives has recently been reported by two groups of workers (5,6).

One unique property which certain TMSi derivatives have shown is an ability to form intense doubly-charged ions with little or none of the corresponding singly-charged species present. McCloskey and co-workers (7) have observed intense doubly-charged M-30 ions in the mass spectra of the di-TMSi derivatives of long chain diols, and have shown that these ions arise from the loss of two methyl radicals and two electrons. This observation has been extended to include the di-TMSi derivatives of aromatic amines (8) and aminohydroxy, dihydroxy, and dicarboxy compounds (9). Further, it has been demonstrated that the intensity of the doubly-charged M-30 ion is position-dependent in the TMSi derivatives of diamino, dihydroxy, and aminohydroxy benzenes and naphthalenes (10), whereas it is independent of the nature of the functional group. These results have encouraged us to examine the mass spectra of the TMSi derivatives

SCHEME I



of diverse functional group-substituted heterocyclic compounds in order to determine the extent of this phenomenon. We now wish to report on a study of the mass spectrometry of these substances, with special reference to the formation of the doubly-charged M-30 ion.

The compounds studied and the relative intensities of their M-30 doubly-charged ions are listed in Table I. Inspection of the data reveals that of the di-functional group-substituted compounds investigated, only the 2,4-

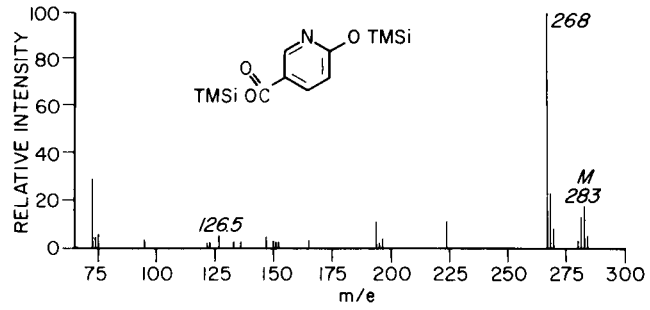


Figure 1. Mass spectrum (70 eV) of the di-TMSi derivative of 2-hydroxy-5-carboxypyridine.

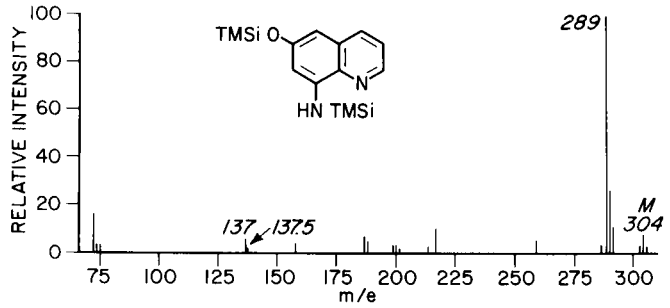


Figure 2. Mass spectrum (70 eV) of the di-TMSi derivative of 6-hydroxy-8-aminoquinoline.

and 4,6-substituted pyrimidines and the 2,4-substituted pteridines show the intense doubly-charged ions previously observed with other di-TMSi derivatives (8-10). In particular, the disubstituted pyridines show doubly-charged M-30 ions of much lower intensity than *meta* and *para* disubstituted benzenes (10). Qualitatively the same behavior is noted, however, in that the 2,5-isomers (analogous to the *para* isomers of benzene) show more intense M-30^{++} than do the 2,3- and 3,4-isomers (analogous to the *ortho* benzenes and naphthalenes). The mass spectrum of the di-TMSi derivative of 2-hydroxy-5-carboxypyridine (Figure 1) is representative of the 2,5-isomers. The spectrum is simple, dominated by fragmentations involving the TMSi groups. Peaks are observed at M^{+}, m/e 283, M-H^{+}, m/e 282; M-$CH_3$$^{+}$, m/e 268; M-($CO_2$+$CH_3$)$^{+}$, m/e 224; M-OTMSi^{+}, m/e 194; and the M-30^{++} at m/e 126.5 (5%). As this compound contains a single nitrogen atom and, therefore, has an odd molecular weight, the doubly-charged M-30 ion appears at a half-mass unit and is readily recognized. 2,6-Diaminopyridine is the only pyridine studied which can be compared to the analogous *meta*-substituted benzenes and it shows an M-30^{++} ion of $< 1\%$ relative intensity. The di-TMSi of *m*-aminophenol, however, exhibits an M-30^{++} almost as intense as that of the *para* isomer (17% *vs* 18% relative intensity) (10). No 2,4- or 3,5-disubstituted pyridines were available, and thus it was not possible to determine whether these isomers behaved more like *m*-aminophenol.

The mass spectrum of the di-TMSi derivative of 3-amino-4-hydroxypyridine as well as the spectrum of the di-TMSi-d_9 derivative were obtained. Proposed structures for the major ions observed in the spectra may be found in Scheme 1. The m/e values found in parenthesis are those observed with the TMSi-d_9 derivative and support the structures assigned. Ions similar to (f) and (g) have previously been observed in benzene and naphthalene derivatives, and fragmentation pathways involving loss of tetramethylsilane *via* a cyclic mechanism have been postulated (10). The peak at m/e 147 is frequently observed in the mass spectra of di-TMSi derivatives and is attributed to the rearranged species (h) (7,11,12).

The mass spectrum of the di-TMSi derivative of 6-hydroxy-8-aminoquinoline (IIe) is presented in Figure 2. The doubly-charged M-30 ion is apparent at m/e 137

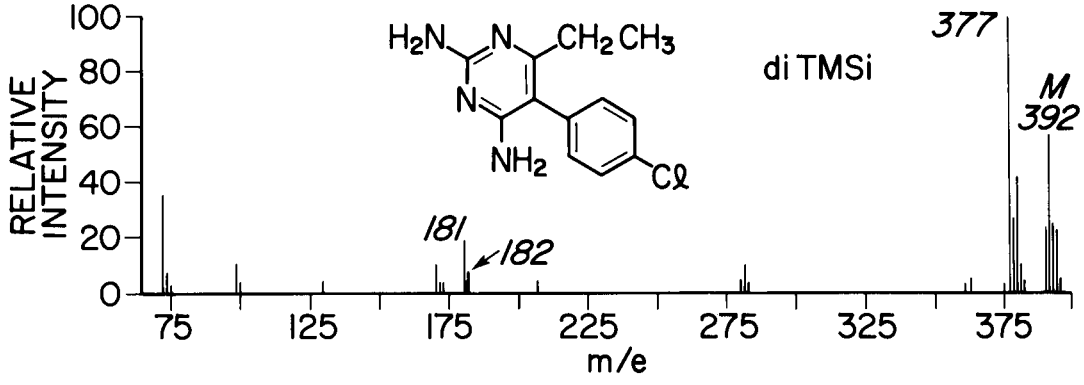


Figure 3. Mass spectrum (70 eV) of the di-TMSi derivative of pyrimethamine (2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine).

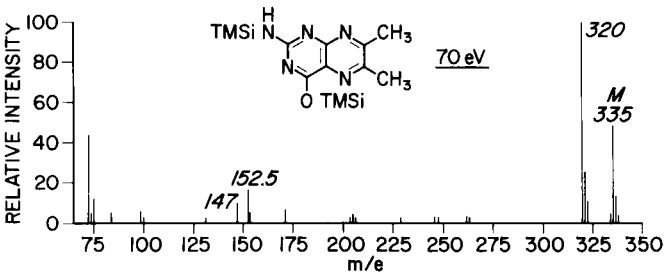


Figure 4. Mass spectrum (70 eV) of the di-TMSi derivative of 2-amino-4-hydroxy-6,7-dimethylpteridine.

(its ^{13}C isotope peak can be noted at m/e 137.5). Substitution in the 3,4-positions in the quinolines, as in the pyridines, appears to preclude the presence of intense doubly-charged M-30 ions (see compound IIc). Substitutions adjacent to the heteroatom (compound IIb) or the ring junction (compound IIe) evidently exert similar effects upon M-30^{++} intensity. When both substituents are thus positioned (compounds IIa and IId), doubly-charged M-30 ions of intermediate intensities are observed (see Table I).

The pyrimidines behave more like the *meta* benzenes previously studied (10), in that the peaks resulting from the M-30^{++} ions are intense (13-18.5% relative intensity) in the 2,4- and 4,6-disubstituted compounds (see IIIa, IIIb and IIId, Table I). The mass spectrum (Figure 3) of the di-TMSi derivative of the antimalarial and coccidiostat, pyrimethamine (IIIb) is of interest and is also representative of this class of compounds. The molecular ion at m/e 392 and the M-15 ion at m/e 377 are the most intense ions in the spectrum, and both exhibit the mono-chloro isotope pattern. The pattern of the M-15 ion is that expected for a compound containing a single chlorine atom and is readily apparent. The pattern in the region of the molecular ion is somewhat obscured because of the presence of a M-1 peak whose ^{37}Cl isotope peak at m/e 393 also contains the ^{13}C isotope peak of the M^+ (^{35}Cl) thus distorting the usual pattern. However, the relationship

TABLE I

	Compound	Relative Intensity M-30^{++}	M-103	M^+	Base Peak (a)
	Di-TMSi derivative of:				
Ia	2,3-diaminopyridine	<1	15	15	M-15
Ib	2,5-diaminopyridine	4.2	3	52	M-15
Ic	2,6-diaminopyridine	<1	3	39	M-15
Id	3,4-diaminopyridine	<1	25	100	M
Ie	2-carboxy-3-hydroxypyridine	<1	<1	<1	M-15
If	2-hydroxy-5-carboxypyridine	5.0	<1	19	M-15
Ig	3-amino-4-hydroxypyridine	<1	10	100	M
IIa	2-carboxy-4-hydroxyquinoline	1.8	<1	13	M-15
IIb	2,6-dihydroxy-4-methylquinoline	7.5	1.3	36	M-15
IIc	3-carboxy-4-hydroxyquinoline	<1	<1	<1	M-15
IId	4-carboxy-8-hydroxyquinoline	2.8	<1	1.8	M-15
IIe	6-hydroxy-8-aminoquinoline	6.1	1.9	7.7	M-15
IIIa	2,4-dihydroxypyrimidine	13	<1	47	M-15
IIIb	2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine	18.5	<1	57	M-15
IIIc	4,5-diaminopyrimidine	2.4	6.1	18	M-15
IIId	4,6-dihydroxypyrimidine	13	<1	36	M-15
IVa	2-amino-4-hydroxy-6-methylpteridine	15	<1	53	M-15
IVb	2-amino-4-hydroxy-7-methylpteridine	13	<1	62	M-15
IVc	2-amino-4-hydroxy-6,7-dimethylpteridine	16	<1	48	M-15

(a) As is frequently done with TMSi compounds, the ion of m/e 73, $Si(CH_3)_3^+$, is excluded from consideration as the base peak.

of the M^{+} and the M+2 peaks is clearly due to the presence of one chlorine atom in the molecule. The ion at m/e 281 results from cleavage of the bond joining the rings, *i.e.*, loss of the *p*-chlorobenzene moiety. The $M\text{-}30^{++}$ appearing at m/e 181 is the fourth most intense peak in the spectrum. The remainder of the spectrum is characterized primarily by its paucity of signals.

Pteridines may be considered to be substituted pyrimidines, and thus TMSi derivatives of 2,4-disubstituted pteridines might be expected to yield intense M-30 doubly-charged ions. Although several papers have been published on the mass spectra of TMSi derivatives of pteridines (5,6) there has been no mention in the literature on the occurrence of these ions in the mass spectra of such compounds. The 70 eV spectrum of the di-TMSi derivative of 2-amino-4-hydroxy-6,7-dimethylpteridine is shown in Figure 4. Again, the dominant ions are the M^{+}, the $M\text{-}15^{+}$ and the $M\text{-}30^{++}$. Pteridines are widely distributed in nature (algae, fish scales, butterfly wings, *etc.*) and occur in very low concentrations. The sensitivity and selectivity of combined gas chromatography-mass spectrometry make this an attractive analytical tool for their determination. The most valuable information contained in the relatively simple spectra of their TMSi derivatives is the molecular ion and the number of active hydrogens as reflected by the number of TMSi groups. As has been pointed out by McCloskey, *et al.* (7) the number of reactive functional groups in a molecule can be readily determined by comparing the m/e values of the molecular ions of the TMSi derivative and the TMSi-d_9 derivative (formed by derivatization with BSA-d_{18}). For example, the molecular ion of the TMSi derivative of compound IVa is 321, whereas that for the TMSi-d_9 derivative is 339, a difference of 18 amu. This is the difference between 2 $Si(CH_3)_3$ and 2 $Si(CD_3)_3$ - compound IVa must contain two reactive functional groups. The presence or absence of intense doubly-charged M-30 ions might also be helpful in assigning substituent position.

The intensities of the $M\text{-}30^{++}$ produced by the TMSi derivatives of disubstituted heterocyclics are, as is the case with the analogous carbocyclic systems, usually dependent upon substituent positions, rather than the nature of the substituents. When the substituents are *ortho*- or *peri*-substituted in the latter compounds, intense M-103 ions are observed (10); these ions are also found in the spectra of similarly substituted heterocycles. They arise *via* the elimination of a methyl radical and the elements of tetramethylsilane (see Scheme 1 and ref. 10) only from those compounds in which the TMSi-substituted functional groups are on adjacent carbons or the *peri* positions of bicyclic compounds. When these ions are present, production of the M-30 doubly-charged ions is suppressed. An exception to this generalization occurs when one of the substituted functional groups is a carboxy group (*e.g.*, Ie and IIc). In this case, although the intensity of the $M\text{-}30^{++}$ ions is very low (< 1%), the M-103 pathway is also suppressed (< 1%). The di-TMSi-d_9 derivatives of 2,3-diaminopyridine (Ia-d_{18}) and 2-hydroxy-5-carboxypyridine (If-d_{18}), in addition to the previously mentioned 3-amino-4-hydroxypyridine (Ig-d_{18}), were prepared and the mass spectra of these compounds were obtained. As expected, the intense M-103 peaks observed in Ia and Ig shift to M-118 in Ia-d_{18} and Ig-d_{18}, and the intense $M\text{-}30^{++}$ observed in If shifts to $M\text{-}36^{++}$ in If-d_{18}.

The presence in the ring of a single heteroatom clearly exerts a negative influence on the production and/or stability of the doubly-charged M-30 ions, as the highest intensity values for the pyridines and quinolines are less than half those found for the benzenes and naphthalenes. For a yet unexplained reason with two heteroatoms, as in the pyrimidines and pteridines, the intensities of the doubly-charged M-30 ions approach those of the carbocyclic systems.

Preliminary evidence from our laboratories suggests that a TMSi-substituted nitrogen atom need not be a ring substituent to participate in the formation of a $M\text{-}30^{++}$, but may actually be the heteroatom in a cyclic system. Thus, the mass spectrum of *O,N*-di-TMSi-2-(4-thiazolyl)-5-hydroxy-benzimidazole exhibits a doubly-charged M-30 ion of 4.5% intensity for which the corresponding singly-charged ion is virtually absent (< 1%).

EXPERIMENTAL

Trimethylsilyl derivatives were prepared by treating < 0.1 mg. of the compound of interest with 30 μl. of *bis*-trimethylsilylacetamide (10 μl. of pyridine was added to increase the solubility of the sample). The reaction was carried out for 30 minutes at 50°. *Bis*-trimethylsilylacetamide-d_{18} (Merck Sharp & Dohme of Canada) was employed to prepare the TMSi-d_9 derivatives. Spectra were obtained by use of an LKB Model 9000 gas chromatograph-mass spectrometer. Column conditions: 1.5 ft. x 2 mm ID glass spiral column; 7.5% SE-30 on 80-100 mesh Chromosorb W; column temperatures, 140° to 180°; 30 ml./min. (helium). Spectrometer conditions: electron energy, 70 eV; source temperature 290°; accelerating voltage, 3.5 kV; trap current, 60 μA.

Acknowledgment.

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