304 (22.0), 286 (99.0), 272 (39.7), 271 (24.4), 258 (35.2), 227 (89.0), 226 (100.0), 211 (31.6), 200 (66.7), 190 (26.2), 176 (61.3), 171 (23.4), 159 (17.2), 131 (61.3), 95 (10.4), 71 (15.3), 43 (51.4).

Anal. Calcd for $C_{25}H_{30}O_{12}$: C, 57.47; H, 5.79; mol wt, 522. Found: C, 57.51; H, 5.80; mol wt (MS), 522.

Leucanthinin (3a). *Melampodium leucanthum* was collected in Motley County, Texas, 18 miles north of Dickens on Farm road 3203 on June 25, 1974 (Stuessy-Stuessy 3560). Dried leaves (570 g) were extracted with cold $CHCl_3$ and worked up as described before.[4] From the combined $CHCl_3$ extracts 2.8 g of crude material was obtained. The crude syrup (1.0 g) was chromatographed over 100 g of silica gel (Merck 0.05–0.2 mesh) using *n*-propyl acetate as eluent and taking 15-ml fractions. The progress of the chromatographic run was monitored by TLC. Fraction 15–25 contained a material which was homogeneous by TLC. The fractions were combined and evaporated in vacuo providing a crystalline material (40 mg). Recrystallization from $CHCl_3$–Et_2O gave pure **3a:** mp 163–164 °C; uv λ_{max} (MeOH) 226 nm (ϵ 5.6 × 10^3); CD (*c* 2.1 × 10^{-4}, MeOH) $[\theta]_{222}$ −62 × 10^3, $[\theta]_{243}$ +3 × 10^3; ir ν_{max} (neat) 3480, 1760, 1740, 1720, 1670, 1630 cm^{-1}; ^{13}C NMR ($CDCl_3$) 169.4, 167.9, 167.5, 164.8 (>C=O); 145.0 d (-CH=); 140.5, 133.7, 130.9 (=C<); 122.7 d (=CH–); 121.0 t ($=CH_2$); 74.6 d, 73.6 d, 70.9 d, 70.6 d, 59.6 d (HCO); 59.1 (>CO); 52.0 d (>CH); 50.6 q ($-OCH_3$); 32.0 t ($-CH_2-$); 20.8 q, 19.1 q, 15.8 q, 13.6 q ($-CH_3$). The mass spectrum showed significant peaks at *m/e* 464.1687 (M^+), 404.1488 (M − CH_3COOH), 348.1193 (M − $C_5H_8O_3$), 288.1012 (M − $C_5H_8O_3$ − CH_3COOH), 229.0858 (M − $C_5H_8O_3$ − CH_3COOH − $C_2H_3O_2$), 183.0814 ($C_{13}H_{11}O$), 131.0491 (C_9H_7O), 116.0495 ($C_5H_8O_3$), 99.0463 ($C_5H_7O_2$), 71.0519 (C_4H_7O, base peak).

Anal. Calcd for $C_{23}H_{28}O_{10}$: mol wt, 464.1682. Found: mol wt (MS), 464.1687.

The acetate **3b** [uv λ_{max} (MeOH) 211 nm (ϵ 7.2 × 10^3); ir ν_{max} (neat) 1770, 1738, 1720, 1680, 1235, 1140 and 990 cm^{-1}] showed no parent peak but exhibited significant mass spectral peaks at *m/e* 446.1647 (M − CH_3COOH), 288.1002 (M − $C_5H_8O_3$ − CH_3COOH − CH_2CO), 270.0889 (M − $C_5H_8O_3$ − $2CH_3COOH$), 229.0866 ($C_{14}H_{13}O_3$), 183.0825 ($C_{13}H_{11}O$), 131.0466 (C_9H_7O), 116.0493 ($C_5H_8O_3$), 99.0422 ($C_5H_7O_2$), 81.0343 (C_5H_5O), 71.0486 (C_4H_7O, base peak).

Melampolidin (4). Collections of young shoots of *M. leucanthum* were made in Presidio County, Texas, 2.3 miles south of Marfa on Highway 67 on July 24, 1973 (Stuessy-Fischer No. 2044). Dried plant material (95 g) yielded 420 mg of crude syrup which was worked up and chromatographed as described above. Fraction 7–12 gave 95 mg of melampolidin (4) as a gum: uv λ_{max} (MeOH) 222 nm (ϵ 1.2 × 10^4); CD (*c* 8.4 × 10^{-5}, MeOH) $[\theta]_{218}$ −58 × 10^3, $[\theta]_{249}$ −3 × 10^3, $[\theta]_{265}$ −7 × 10^3; ir ν_{max} (neat) 3450, 1760, 1740, 1710, 1670, 1630 cm^{-1}; significant mass spectral peaks at *m/e* 432.1798 (M − H_2O), 274.1213 (M − $C_7H_{12}O_5$), 242.0935 ($C_{15}H_{14}O_3$), 215.1059 ($C_{14}H_{15}O_2$), 159.0663 ($C_7H_{11}O_4$), 131.0728 ($C_6H_{11}O_3$, base peak), 117.0553 ($C_5H_9O_3$), 99.0435 ($C_5H_7O_2$), 91.0550 (C_7H_7), 71.0513 (C_4H_7O). Since in the mass spectrum no parent peak was obtained for 4 the M − H_2O data were used for the determination of the empirical formula.

Anal. Calcd for $C_{23}H_{30}O_9$: mol wt, 432.1798. Found: mol wt (MS), 432.1784.

Acknowledgment is made to the National Science Foundation (Grant GB-42644) for support of this work. The authors thank Judy Abraham and Joseph Abraham for technical assistance, Professor N. S. Bhacca for NMR data, and Professor Tod Stuessy, The Ohio State University, for collecting and identifying the plant material.

Registry No.—**1,** 35878-52-5; **2,** 60295-53-6; **3a,** 60295-54-7; **3b,** 60295-55-8; **4,** 60295-56-9.

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Mass Spectrometry of Cytokinin Metabolites. Per(trimethylsilyl) and Permethyl Derivatives of Glucosides of Zeatin and 6-Benzylaminopurine

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The mass spectra of the Me_3Si and permethylated derivatives of a comprehensive series of synthetic isomeric glucosides of the cytokinins zeatin and 6-benzylaminopurine (6-BAP) have been recorded by combined gas chromatography–mass spectrometry. Comparison of these spectra with those obtained for a number of glucosyl metabolites of zeatin and 6-BAP allows unambiguous structural assignments to be made. Detailed analysis of the mass spectral fragmentation patterns indicate that, a priori, it should be possible to assign the sugar ring size (furanose vs. pyranose) in such compounds on the basis of characteristic fragment ion intensities. Mass spectra of the Me_3Si derivatives show more significant isomer differences than the corresponding permethylated compounds and their method of preparation appears less prone to multiple product formation. A thermal 1,3 migration of the sugar moiety from N_3 to N_9 was observed in the GC–MS of the derivatives of the 3-β-D-glucopyranoside of 6-BAP.

Phytohormones, and in particular the adenine derived cytokinins, evoke their biological responses at extremely low concentrations and occur free in plants in minute amounts. For example, the natural cytokinin zeatin (1) induces growth of carrot phloem tissue at concentrations of less than 0.1 μg/l. (5 × 10^{-10} M).[1] Zeatin-related compounds, e.g., N^6-(3-methyl-2-butenyl)adenosine, are ubiquitous, although minor, components of *t*-RNA hydrolysates from animals, plants, and

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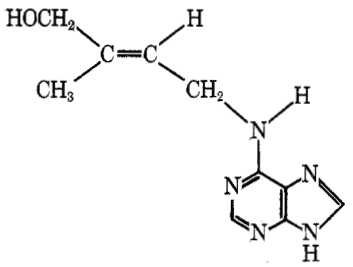
microorganisms. Because of the minute quantities of material involved, mass spectrometry has been and continues to be an essential means of providing information about the chemical nature of these compounds.[2,3]



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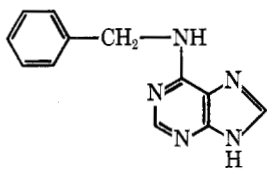


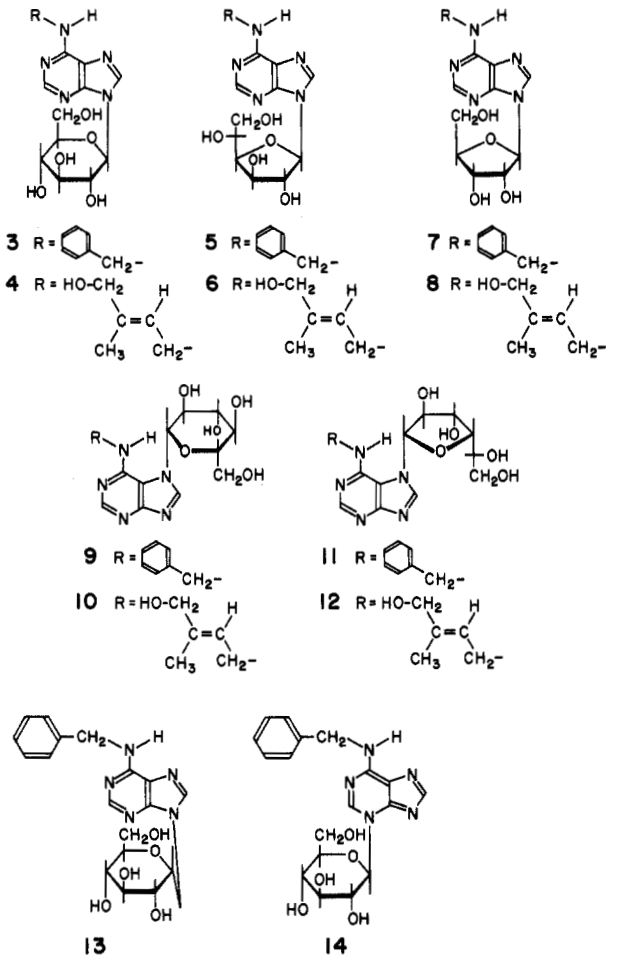
2

As part of a continuing study into the mechanism of action of cytokinins during plant growth, we have isolated a variety of metabolites from different plant systems following exogenous uptake of labeled zeatin and the synthetic cytokinin, 6-benzylaminopurine (6-BAP, **2**). As well as those metabolites of zeatin previously identified in plants such as its 9-riboside and 9-riboside 5′-phosphate plus adenosine and adenosine 5′-phosphate, several new stable metabolites of zeatin and 6-BAP have been isolated. In the main these have proved to be *N*-glucosides of the parent cytokinins,[3c-e] although an unusual amino acid conjugate of zeatin, lupinic acid, together with *O*-β-D-glucopyranosyl zeatin were isolated from lupin seedlings.[4] Preliminary identification and tentative assignments of structure have relied heavily on mass spectrometry, with unequivocal structural assignments being possible only after direct comparison with authentic synthetic samples. In the course of such comparisons, direct probe insertion of the underivatized compounds has often proved unreliable and on occasions given irreproducible mass spectra due to the instability of some of the more thermolabile glucosides. On the other hand, mass spectrometry of the volatile trimethylsilyl (Me_3Si), permethyl, acetyl, and trifluoroacetyl derivatives of nucleosides and related compounds has been utilized to obtain valuable structural information. A number of papers have been published analyzing the mass spectrometric fragmentation behavior of such derivatives using isotope labeling and high-resolution mass spectrometry.[5-11] As both the Me_3Si and permethyl derivatives of these compounds are amenable to gas chromatography, the GC–MS combination provides the additional comparison criteria of retention time and sample purity.

In this study the GC–MS spectra of Me_3Si and permethyl glucosyl metabolites of 6-BAP and zeatin have been compared with those recorded for the series of synthetic isomers **3, 5, 9, 11, 13, 14** and **4, 6, 10, 12,** respectively. It is also the purpose of this work to try to rationalize the characteristic fragmentations present in the mass spectra to determine whether structural assignments can be made from the GC–MS data without the necessity of reverting to direct comparisons with model compounds. That such an approach had previously been subject to uncertainty is illustrated by the incorrect assignment of a furanose structure to the sugar residue of the 7-glucosyl metabolite of 6-BAP based on a correlation of the mass spectral fragmentations of the Me_3Si derivative with previously published work on simple sugars (vide infra).

Results and Discussion

Derivatization and Gas Chromatography. Trimethylsilylation of the cytokinin glycosides **3–14** was carried out according to previously published methods for related compounds. Me_3Si ethers, as expected, were formed at all sugar hydroxyls and at the side-chain hydroxyl of the zeatin derivatives. No multiple derivatives were formed. Permethylation of the 9-glucosides by the Hakamori technique[11,12] gave good yields of homogeneous products, which eluted at slightly higher temperatures than the corresponding silylated compounds. N-Methylation at N_6 of the adenine moiety accounts for an extra site of derivatization in the permethylated derivatives. Poorer yields and multiple products were observed with the more labile 7-glucosides. If reaction times in excess of 5 or 10 min were used, only the products of hydrolysis of the glycosidic bond were obtained. With reaction times of 3 min or less, compounds of the expected molecular weight were mixed with the products of hydrolysis and partial methylation. In the particular case of the permethylation of 6-BAP-7-β-D-glucofuranoside (**11**) four products with the expected molecular ion at *m/e* 457 were formed in addition to those of hydrolysis and partial methylation. Of the components with molecular ion at *m/e* 457, one differed markedly from the rest in that the ion at *m/e* 210 was of very low intensity. This ion arises from loss of methylenimine from the b + H ion (see below) and is characteristic of a dialkylamino function at N_6. Its low intensity probably indicates methylation of an imino tautomer of **11**. Permethylation of the 7-β-D-glucopyranosides of 6-BAP (**9**) and zeatin (**10**) also gave low yields and mixtures of products, although only one derivative with molecular weight corresponding to the fully methylated, intact glucoside was formed. The fact that all cases of silylation of *N*-glucosides gave reproducible derivatization to a homogeneous product under the same reaction conditions suggests that this derivative has greater utility for the analysis of this group of compounds.



Both the Me_3Si and permethylated derivatives of 6-BAP-3-β-D-glucopyranoside (**14**) underwent thermal rearrangement during gas chromatography. The gas chromatographs and the GC–MS total ion current traces both showed two

peaks, a broad one followed by a very sharp one which coeluted with the corresponding derivative of 6-BAP-9-β-D-glucopyranoside (3). The mass spectra under both peaks were identical with each other and with those of the respective derivative of 3. This behavior would indicate a thermal rearrangement of the sugar moiety from N_3 to N_9 on the GC column, with rearrangement of any remaining N_3 derivative within the mass spectrometer ion source. The β configuration of the anomeric carbon of the sugar is retained during the rearrangement since the spectra of the products are easily distinguished from those of the corresponding derivatives of 6-BAP-9-α-D-glucopyranoside (13).

A similar migration has been reported for some 3-alkyladenine and 3-ribosyladenine derivatives and an intermolecular reaction mechanism has been proposed.[13] However, a necessary condition for those migrations was found to be the presence of an N_6 acyl substituent and a mercuric or hydrogen halide catalyst. A combination of inter- and intramolecular mechanisms, with the latter process predominating, has recently been found to be operating in the thermal rearrangement of 3-benzyladenine to 6-BAP.[14] The mechanism of the rearrangement in the 3-glucosyl derivatives of 6-BAP is under further investigation since 3-β-glucopyranosyl-6-BAP has been found as a metabolite of 6-BAP in radish seedlings and is itself highly active as a cytokinin.[3e]

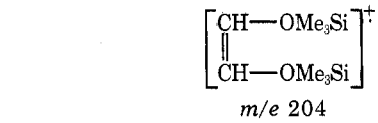
Mass Spectra. A. General. As expected, the fragmentation patterns of the derivatized zeatin and 6-BAP glucosides resembled those of previously published adenine ribosides except for those decompositions associated with the N_6 side chain or those characteristic of the sugar portion of the molecule. The assignments of structures to the major ions in the spectra (as in Table I) have been made on the basis of labeling studies carried out on other nucleosides[5,11] and derivatized carbohydrates[15] and have been supplemented by some high-resolution measurements. It must be emphasized that although structures have been assigned to certain ions throughout this paper, this does not imply that such structures are necessarily correct. Rather they are rationalizations based on available labeling data and chemical analogy.

Molecular ions were visible in all spectra, varying in intensity between 0.1% and 20% of the base peak. Spectra of Me_3Si derivatives were characterised by a base peak at *m/e* 73 and intense ions at *m/e* 75, 103, 129, 147, 169, 204, 217, 305, and 319 which are the well-documented ubiquitous species characteristic of Me_3Si carbohydrates and related compounds.[15] Permethylated derivatives showed a similar series of characteristic ions at *m/e* 71, 75, 88, 101, and 111.[18] The assignments in Table I have been grouped as far as possible according to the origin of the ionic species. The spectra of some of the silylated compounds are reproduced in full since our results indicate that this derivative has the most utility for structure assignment. The spectra of the successfully prepared permethylated compounds are in tabulated form, and available as supplementary material (see paragraph at end of paper).

Detailed examination of all spectra showed that apart from the obvious mass related assignments (e.g., hexose vs. pentose) the sugar ring size in glucosides could be established with certainty by the presence or absence of certain critical ions. Although a change of the position of substitution of the sugar moiety on the purine ring (e.g., 7 vs. 9) gave rise to markedly different spectra, it was not possible to assign the substitution pattern by inspection. The spectra of the derivatives of the 9-β- (3) and 9-α- (13) glucopyranosides of 6-BAP also showed major differences although there was no obvious structure–fragment ion relationship which could permit assignment of the configuration of the anomeric linkage with certainty.

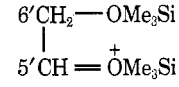
B. Me_3Si Derivatives of 6-BAP Glycosides. In this series we obtained spectra of the Me_3Si derivatives of 3, and of its 9-α anomer (13), the 9-β-glucofuranoside (5), the 7-β-glucopyranoside (9), the 7-β-glucofuranoside (11), and the 9-β-riboside (7). The spectra of 3, 5, 9, and 11 are shown in Figures 1–4, with the major ions of Table I signified, and exemplify the features which distinguish the glucopyranosides from glucofuranosides (see paragraph at end of paper regarding supplementary material).

In the spectra of the pyranosides (Figures 1 and 3) the ion at *m/e* 204 is more intense than *m/e* 205 while in the furanosides (Figures 2 and 4) the order is reversed. Our results indicate that this is the ion intensity data of greatest diagnostic value for assignment of sugar ring size. The ion at *m/e* 204 is a characteristic fragment ion of Me_3Si carbohydrates



m/e 204

and in hexose sugars has been shown to incorporate essentially the C2′–C3′ or C3′–C4′ pairs of sugar skeletal carbons.[16] The ion at *m/e* 205, apart from the isotope contribution of *m/e* 204,

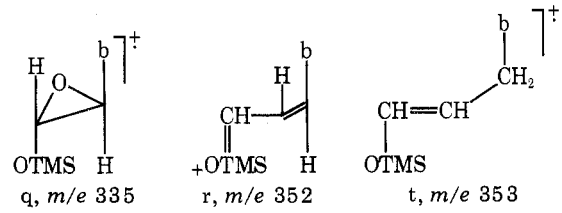


z, *m/e* 205

has been shown to arise in hexose sugars by cleavage of the C4′–C5′ bond with charge retention on the C5′–C6′ fragment to give ion z.[16] Analogous cleavages have been observed in the spectra of peracetyl[17] and permethyl[18] hexofuranosides. In the former derivative, however, ion z occurs at *m/e* 145 together with the ubiquitous triacetyloxonium species, thus limiting its use as a structural marker in this case.

The same C4′–C5′ bond cleavage, but with charge retention on the larger portion of the molecule, would give rise to an ion at *m/e* 470. Fox and co-workers[19] used the presence of the ion at this mass, together with the relative intensities of *m/e* 204 and *m/e* 217, to assign a furanose structure to the sugar moiety in 6-BAP-7-glucoside, the major stable metabolite of 6-BAP in soybean callus tissue. Examination of Figures 1–4, however, reveals that *m/e* 470 is most intense in Figure 3, the spectrum of the 7-glucopyranoside (9). Comparison of their published spectrum with Figure 4, the spectrum of Me_3Si-6-BAP-7-β-D-glucofuranoside, shows a number of dissimilarities, while it is almost identical with Figure 3, thus indicating that their metabolite is almost certainly the 7-glucopyranoside 9. Compound 9 has also been isolated as the major metabolite of 6-BAP in radish seedlings and its identity recently confirmed by comparison with an authentic synthetic sample.[20]

Other differences between the furanosides and pyranosides include the relative intensities of the ions at *m/e* 355 and 353. The ion at *m/e* 355 (q) is especially prominent in the spectrum



q, *m/e* 335 r, *m/e* 352 t, *m/e* 353

of Me_3Si 11 and its origin has previously been established.[11] The ion at *m/e* 353 (t) has an accurate mass corresponding to $C_{18}H_{23}N_5OSi$ (b + C_3H_4OR, R = Me_3Si). It thus appears to be the hydrogen rearranged analogue of the "ion m" (b + C_3H_3OR, R = CD_3) in the spectra of perdeuteriomethylated ribonucleosides[11] and the ion *m/e* 352 (r) (b + C_3H_3OR, R = Me_3Si) which is present but mostly at low intensity in all the

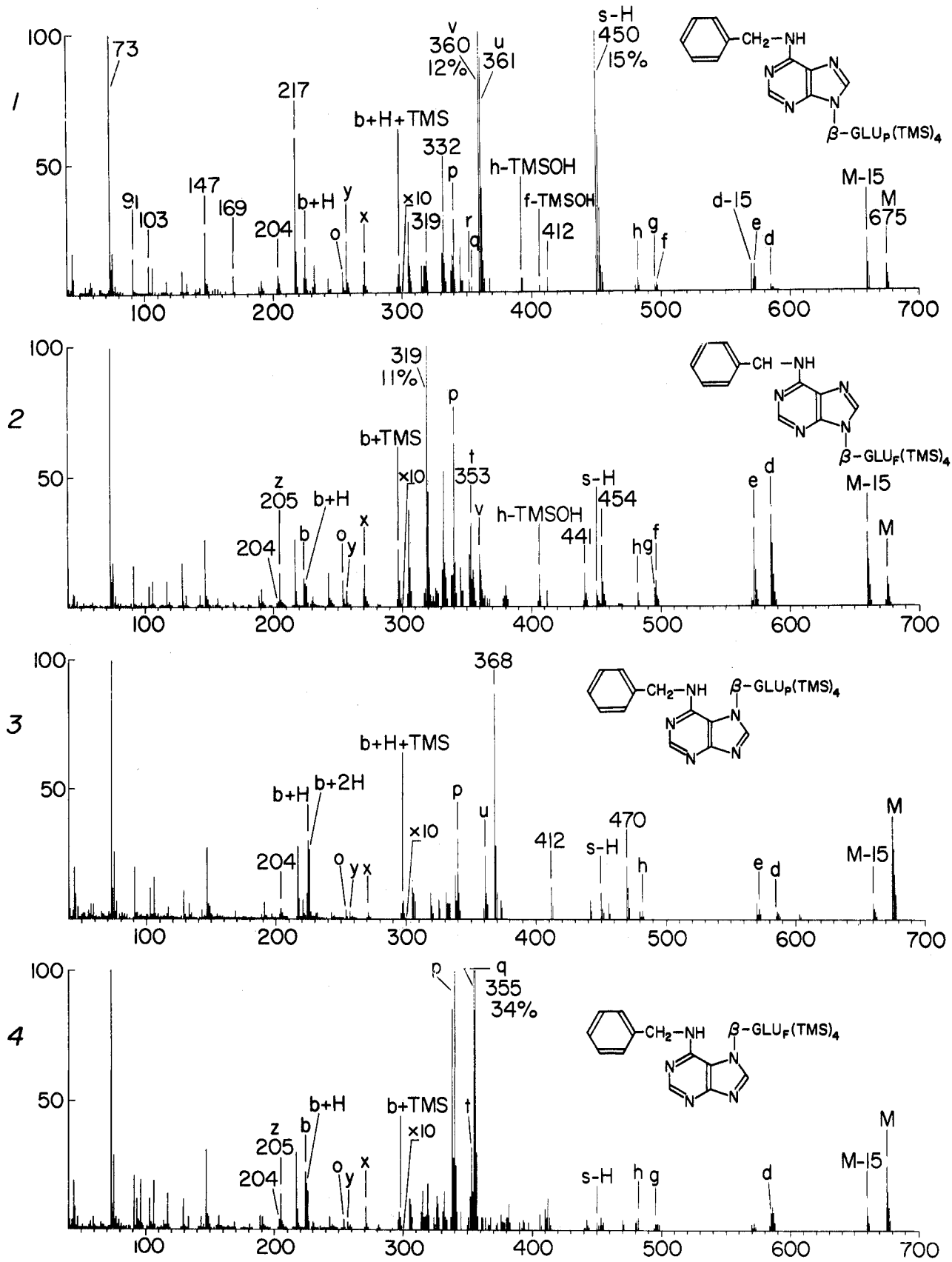
Table I. Summary of Major Decomposition Pathways of Permethyl and Pertrimethylsilyl Purine Glycosides

Symbol	Type	Description	Ref
		A. Ion Closely Related to the Molecular Ion	
M	$M^{\cdot+}$	Molecular ion	
M − 15	$M^{\cdot+} - \cdot CH_3$	Methyl loss from Me_3Si derivatives	
a	$M^{\cdot+} - \cdot R$	R = $Si(CH_3)_3$; R = CH_3 (permethyl)	
c	$M^{\cdot+} - \cdot OR$		
d	$M^{\cdot+} - ROH$		
e	$M^{\cdot+} - \cdot CH_2OR$	Lost from sugar and/or zeatin side chain	
f	$M^{\cdot+} - ROH - \cdot OR$		
g	$M^{\cdot+} - 2ROH$		
h	$M^{\cdot+} - \cdot CH_2OR - ROH$		
j	$d - CH_2NH$	Loss of methylenimine from ion d	
k	$M^{\cdot+} - C_3H_8OR$	Cyclization ion of zeatin derivatives (M − 131, Me_3Si; M − 73, permethyl)	23–25
l	$M^{\cdot+} - C_4H_6OR$	Cleavage β to N_6 in zeatin side chain (M − 143, Me_3Si; M − 85, permethyl)	
M − 29	$M^{\cdot+} - CH_2NH$	Loss of methlenimine from N_6 methyl derivatives	26
M − 91	$M^{\cdot+} - C_7H_7$	Loss of N_6 benzyl from 6-BAP derivatives	
		B. Ions Formed by Cleavage of Glycosidic Bond	
b	Base	Simple cleavage, charge retention on base	2, 7
b + H	Base + H	Cleavage with single hydrogen transfer	
b + 2H	Base + 2H	Cleavage with double hydrogen transfer	
b + Me_3Si	Base + Me_3Si	Cleavage with Me_3Si migration	
b + H + Me_3Si	Base + H + Me_3Si	Cleavage with Me_3Si and hydrogen migration	
s	Sugar	Simple cleavage, charge retention on sugar	
s − H	Sugar − H	Cleavage, H transfer to base, charge retention on sugar	
		C. Ions Derived from b or b + H	
n	$b + H - CH_2NH$	Loss of methylenimine from permethyl derivatives	
c′	$b - \cdot OR$	Loss of OR from zeatin side chain	
e′	$b + H - \cdot CH_2OR$	Loss of CH_2OR from zeatin side chain	
k′	$b + H - C_3H_8OR$	"Cyclization ion" in zeatin derivatives	
l′	$b + H - C_4H_6OR$	Cleavage β to N_6 in zeatin derivatives	
$C_6H_6N_5$	*m/e* 148	Cleavage of N_6 substituent from b + H in permethyl derivatives	11
		D. Ions Incorporating Base and Portion of Sugar	
o	$b + CH_2O$	b + H with C-1′ and ring oxygen of sugar (b + 30)	2, 28
p	$b + C_2H_3OR$	b + H with C-1′ and C-2′ − OR (b + 58 permethyl; b + 116, Me_3Si)[a]	7
q	$b + C_2H_2O_2R$	b with C-1′, C-2′ − OR, and ring oxygen (b + 131, Me_3Si)[a]	7
r	$b + C_3H_3OR$	b with C-1′, C-2′, and C-3′ − OR (b + 70, permethyl; b + 128, Me_3Si)[a]	11
t	$b + C_3H_4OR$	As above with one extra hydrogen (b + 129, Me_3Si)[a]	
		E. Ions Derived from s − H	
u	s − ROH		
v	s − H − ROH		
w	$s - \cdot CH_2OR$		
x	s − 2ROH		
y	$s - ROH - \cdot CH_2OR - H$		
z	$CH_2OR - CHOR\cdot$	Cleavage of C-4′–C-5′ bond of glucofuranosides with charge retention on "side chain"	16, 17
	m/e 144	Characteristic of adenosine derivatives	
		F. Ubiquitous Ions	
	m/e 73, 103, 129, 147, 169, 204, 217, 305, 319	Ions common to Me_3Si carbohydrates and related compounds	15, 16
	m/e 88, 75, 71, 101, 111	Ions common to permethyl sugar derivatives	18, 29
	m/e 91	Tropylium cation (6-BAP) derivatives	

[a] Formula established by high-resolution mass measurement.

spectra of Me_3Si 6-BAP-glycosides. The ion *m/e* 319, common to many Me_3Si carbohydrates, has been proposed as an indicator of furanosides.[16] It is an intense ion in the spectrum of Me_3Si **5** (Figure 2) but is as weak in the spectrum of Me_3Si 11 (Figure 4) as it is in the spectra of the pyranosides, indicating that use of such an indicator is incorrect in this series of compounds.

The cleavage of the glycosidic bond of nucleosides and the well-documented hydrogen transfers which accompany this process are also dependent on the nature of the sugar moiety. In Me_3Si derivatives the migrating hydrogens have been shown to arise from the sugar skeletal carbons[5] and while this is also true of permethyl derivatives, there is also some migration of the hydrogen atoms of the O-2′ methyl group.[11] In



Figures 1–4. Mass spectra of Me_3Si derivatives of 6-BAP glycosides.

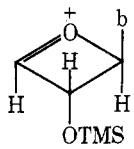
the Me_3Si 6-BAP glucosides, sugar ring size appears to influence this process quite significantly since in the pyranosides (Figures 1 and 3) b + H or b + 2H > b and in the furanosides (Figures 2 and 4) b > b + H > b + 2H. In the riboside (7) both b + H and b + 2H are larger than b. Migration of a Me_3Si group is also a favored process giving rise to intense ions at b + Me_3Si and b + H + Me_3Si at *m/e* 297 and 298 in all compounds.

Mass spectral features allowing assignment of the position of substitution of the sugar on the purine ring are more diffi-

cult to detect since differences present in the spectra of one pair of positional isomers are not always consistent for other pairs. In the case of the 3-β-glucosides, as mentioned above, the derivatized sugar undergoes migration from N_3 to N_9 and hence identical spectra result for these two positional isomers. The spectra of the 7- and 9-glucosides have obvious quantitative differences, for example in the ion abundance ratio M/(M − 15). However, these differences are probably the result of a number of interacting factors and any reliable decision as to the sugar position could only be made by direct comparison with reference spectra. A recent study has shown that, with reference compounds available, the differentiation between 7- and 9-substituted nucleosides by chemical ionization mass spectrometry appears feasible.[21]

The ions s − H, u, and v (as defined in Table I) at *m/e* 450, 361, and 360, respectively, are very intense in the Me_3Si 9-β-glucopyranosides of 6-BAP (**3**), zeatin (**4**), and adenine.[22] This indicates that particularly favorable conditions exist in this group of compounds for cleavage of the glycosidic bond, with charge retention on the sugar moiety. In all other spectra of the Me_3Si glucosides, s − H, u, and v are less than 5% rel intensity. In the spectrum of Me_3Si **5**, and in the corresponding zeatin derivative **6**, *m/e* 319 is a very intense ion (11% rel intensity), while it is only 1–2% rel intensity in the other spectra.

The ion at *m/e* 368 is present, but at low intensity in all spectra except that of Me_3Si **9**, where it is a characteristically intense peak. High-resolution mass measurement indicates

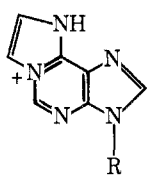


m/e 368

the formula for this ion of $C_{18}H_{22}N_5O_2Si$ or b + $C_3H_3O_2R$, R = Me_3Si. This ionic species most likely contains the first three skeletal carbons and ring oxygen of the sugar with a structure isomeric with that shown.

The ion at *m/e* 169, normally characteristic of Me_3Si ribonucleosides,[7] has 10% rel intensity in the spectrum of Me_3Si 6-BAP-9-β-D-ribofuranoside (**7**) and is also a reasonably significant ion in the spectra of the 9-glucopyranosides **3** and **13**. It is present but at lower abundance (2–3% rel intensity) in the remaining spectra.

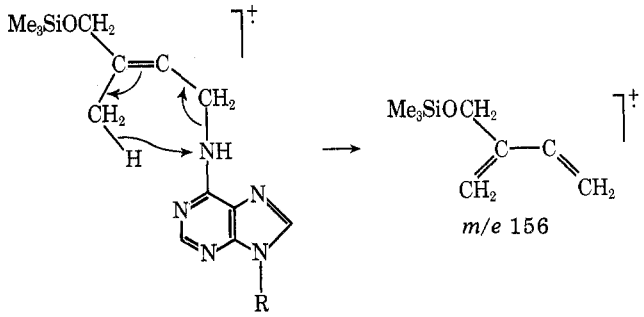
C. Me_3Si Derivatives of Zeatin Glycosides. In this series Figures 5–8 show the spectra of Me_3Si **4** and **10**, the 9-β- and 7-β-D-glucopyranosides, and Me_3Si **6** and **12**, the 9-β- and 7-β-D-glucofuranosides of zeatin. The spectrum of Me_3Si **8**, the 9-β-D-riboside of zeatin, is available as supplementary material. Some of the major ions are similar to those in the spectra of the Me_3Si derivatives of 6-BAP, but with additional peaks resulting from fragmentations of the zeatin side chain. The ions at *m/e* 610 (*m/e* 508 in the riboside), "cyclization ion" k, and at *m/e* 160 (k′) are analogous to the ion at *m/e* 160 in



k, R = glucosyl (Me_3Si)	*m/e* 610
R = ribosyl (Me_3Si)	*m/e* 508
k′, R = H	*m/e* 160

zeatin itself[23] and 6-*N*-(3-methyl-2-butenylamino)-9-β-ribofuranosylpurine.[24,25] In some cases this is accompanied by an intense hydrogen rearrangement ion at *m/e* 611 (e.g., Figure 7).

The intense ions at *m/e* 638, 201, and 202 result from loss of the Me_3SiOCH_2· radical from the side chain in $M^{\cdot+}$, b, and b + H, respectively, although there could be some contribution to *m/e* 638 from the same loss of the C-6′ portion of the sugar moiety. The ion at *m/e* 156 ($C_8H_{16}OSi$), which is prominent in all spectra, probably arises by the rearrangement process shown, with charge retention on the zeatin side chain.



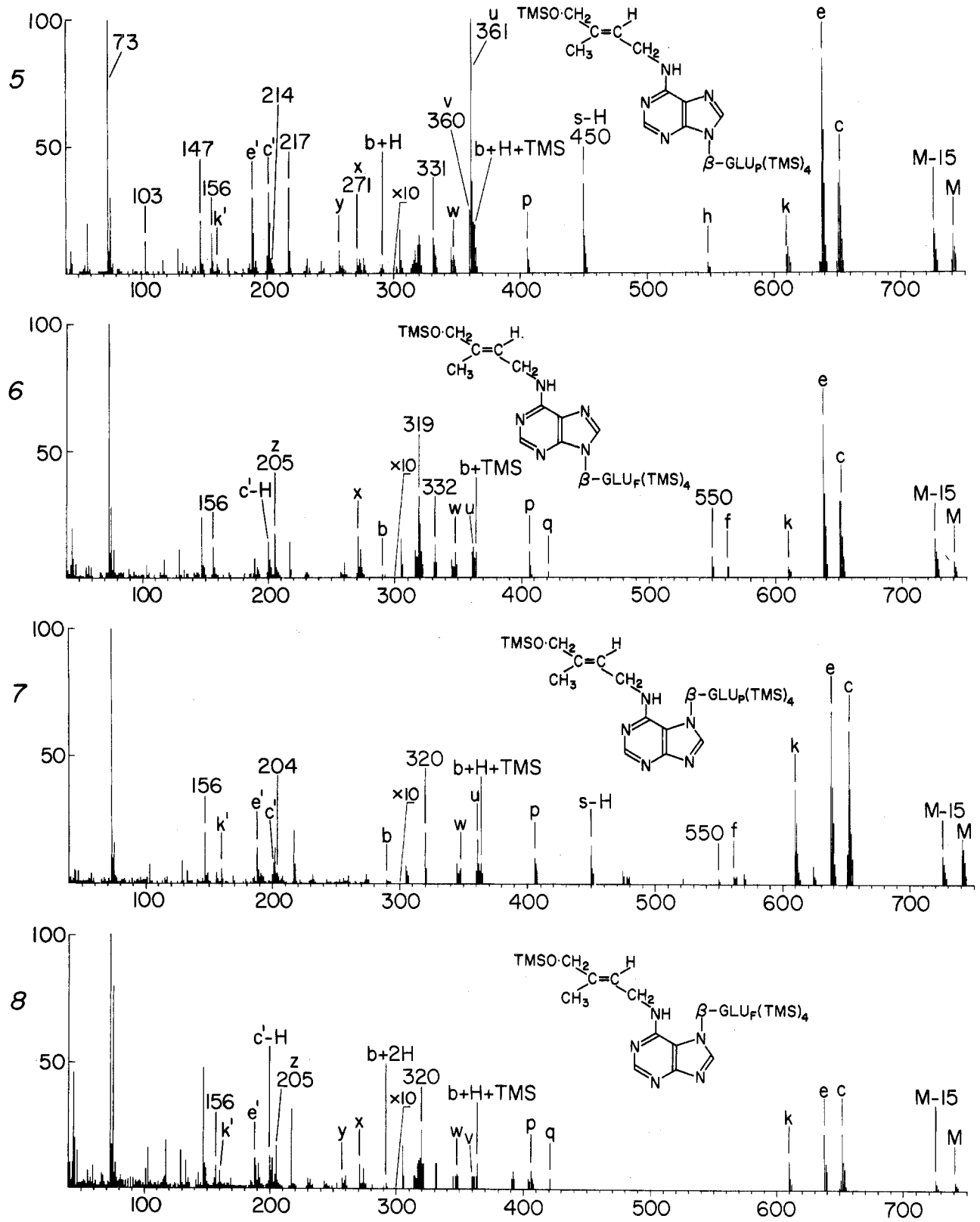
The indicators of sugar ring size evident in the 6-BAP spectra are also shown by the corresponding zeatin derivatives and include the relative intensities of the *m/e* 204 and 205 peaks, the latter being more intense in the spectra of Me_3Si **6** and **12** (Figures 6 and 8). Ion q (*m/e* 421) is also present in these spectra (0.2 and 0.4%, respectively), in accordance with the situation in the 6-BAP derivatives and the low intensity of this species is undoubtedly a reflection of how the decomposition pathways in zeatin are highly modified by the isopentenyl side chain.

The ion at *m/e* 319 is again of significant intensity in the spectrum of the 9-β-furanoside **6**, while the intense ions at *m/e* 450, 361 (u), and 360 (v) are characteristic of the 9-β-pyranoside **4** (Figure 5).

D. Permethylated Derivatives of 6-BAP Glycosides. The four 9-substituted glycosides (**3**, **5**, **7**, and **13**) methylated to afford homogeneous derivatives. The spectra of these compounds are available (see paragraph at end of paper regarding supplementary material). The 7-β-D-glucopyranoside (**9**), as mentioned before, methylated in low yield and this spectrum is included also. Results for the 7-β-D-glucofuranoside were ambiguous with respect to the spectrum of the desired product and are not included. A common feature of all the spectra is the strong molecular ion (10–20%). Taking into account the necessary mass shifts the major decomposition pathways are quite similar to those of the corresponding Me_3Si derivatives.

A striking feature of the spectrum of the glucofuranoside **5** is that *m/e* 89, the peak analogous to *m/e* 205 in the Me_3Si spectra, and corresponding to the glucose "side chain" cleavage, is in fact the base peak while there is an accompanying reduction in the intensity of *m/e* 88. Other features of the spectra similar to those seen in Me_3Si derivatives, are the presence of intense ions at *m/e* 218 (s − H) and 187 (u) in permethyl **3** (also seen in the spectrum of permethyl 9-β-D-glucopyranosyladenine)[22] and the relative intensity of *m/e* 155 (x) in permethyl **5**. The ion at *m/e* 111, analogous to *m/e* 169 in the Me_3Si spectra, mirrors the situation with those derivatives, being more intense in the 9-glucopyranosides and 9-β-riboside than in the glucofuranoside. The ion at *m/e* 114 appears to be characteristic of permethylated ribosides, having 24% rel intensity in the spectrum of permethyl **7**. McCloskey and co-workers have noted the characteristic presence of this ion in the spectrum of permethyladenosine.[11] In the specrum of permethyl **7** we have confirmed the formula $C_6H_{10}O_2$ for *m/e* 114 by a high-resolution mass measurement and the fact that it contains two methoxyl groups by its shift to *m/e* 120 in the MS of the perdeuteriomethylated analogue.

The permethylated 6-BAP derivatives also show similar behavior to the Me_3Si analogues with regard to the relative



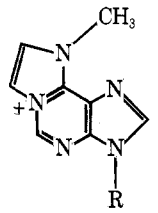
Figures 5–8. Mass spectra of Me_3Si derivatives of zeatin glycosides.

intensities of b, b + H, and b + 2H. The permethyl glucopyranosides **3**, **13**, and **9** have b + H > b, with b + H being the base peak. In the glucofuranoside **5**, the order of intensities is b > b + H > b + 2H.

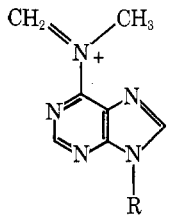
Expulsion of a methylenimine molecule, a process characteristic of methyl- and dimethylamino aromatics[26] and recently the subject of an isotope labeling study,[27] is a major process in the spectra of permethyl 6-BAP and zeatin glycosides. In the spectra of these derivatives of 6-BAP this process gives rise to an intense ion at *m/e* 210 where the expulsion is from the b + H ion at *m/e* 239, while in the 9-β-riboside the process also occurs from the molecular ion to give *m/e* 384. In the glucosides **5**, **9**, and **13** but not in **3**, methylenimine is expelled from the M − 15 ion to give an ion at *m/e* 413.

E. Permethylated Zeatin Glycosides. The spectra of permethylated zeatins **4**, **6**, **8**, and **10** are also available (see paragraph at end of paper regarding supplementary material).

The double bond of the isopentenyl side chain has a major directing influence on the fragmentation pattern, as it does in the corresponding Me_3Si derivatives. This factor gives rise to such characteristic features as the weak molecular ion, the intense ion at *m/e* 216 (base peak) corresponding to loss of methoxyl from b + H, and the "cyclization" and "side-chain cleavage" ions k and l.



k, R = glucosyl $(Me)_4$, *m/e* 392
R = ribosyl $(Me)_3$, *m/e* 348
k', R = H, *m/e* 194



l, R = glucosyl $(Me)_4$, *m/e* 380
R = ribosyl $(Me)_3$, *m/e* 336
l', R = H, *m/e* 162

As in the other methylated derivatives so far mentioned the distinction between glucofuranosides and glucopyranosides can be made on the basis of the relative intensities of *m/e* 88 and 89. In the spectrum of permethyl **6** the ions at *m/e* 434 ($M^{\cdot+} - OCH_3$) and 155 ($s - 2CH_3OH$) are also characteristically intense.

To summarize, the spectra of Me_3Si and permethyl derivatives of the individual isomeric glucosides of 6-BAP and zeatin are sufficiently diagnostic to allow structural assignments to be made. Metabolites of 6-BAP and zeatin extracted from various plant systems[3c-e] which have been unambiguously characterized by the GC–MS of their Me_3Si and permethyl derivatives are the 7- and 9-β-D-glucopyranosides **3**, **4**, **9**, and **10** and the 3-β-D-glucopyranoside of 6-BAP, i.e., **14**.

Experimental Section

Sources of Nucleosides. Zeatin-9-β-riboside was obtained from a commercial source. All other glycosides were synthesised as part of separate investigations into the structures of unusual cytokinin metabolites. For preliminary details of these syntheses see ref 3c–e and 20.

Derivatization Pertrimethylsilyl derivatives were prepared by dissolving a sample of the nucleoside (10–100 μg) in pyridine (10–20 μl), treating the mixture with BSTFA–TMCS, 99:1 (Regisil RC-2) (100 μl), and warming the solution at 60 °C for 30–60 min. Using this method all derivatizations were successful and gave essentially homogeneous products.

Permethyl derivatives were prepared according to the previously reported procedures for peptides[29] and nucleosides.[11] The nucleoside was treated with a 10-equiv excess of dimsyl anion solution for approximately 30 min, followed by the addition of a 10-equiv excess of methyl iodide. After a further 90 min the reaction was terminated by addition of water and the product was extracted, washed, and dried in the usual way. This procedure was not successful with the 7-substituted nucleosides, the only products being the permethylated fragments of hydrolysis of the glycosidic bond. In the case of the 7-glucopyranosides of zeatin and 6-BAP, using a 1-min contact with the anion solution and a 2-min contact with methyl iodide, the permethylated derivatives were prepared in slightly lower yield than for the corresponding 9-substituted derivatives. There were also some permethylated hydrolysis products present in the product. Multiple derivatives of the expected molecular weight and products of hydrolysis and incomplete methylation were formed in the attempted permethylation of the 7-glucofuranosides using the fast reaction sequence. The small quantity of material available precluded further experimentation.

Gas Chromatography. All samples were run under approximately the same conditions, using a Perkin-Elmer 900 gas chromatograph fitted with a 6 ft × 0.125 in. (i.d.) glass column packed with 2% OV-17 on 80–100 Gas-Chrom Q, using nitrogen carrier gas flowing at 20 ml/min and programmed from 200 to 300 °C at 4 °C/min. Elution temperatures ranged from 240 °C for the Me_3Si ribosides to 290 °C for the permethylglucosides.

Gas Chromatography–Mass Spectrometry. Mass spectra were recorded using a Varian MAT 111 instrument equipped with a slit separator using a similar GC column to that above but with helium as carrier gas. The separator and line were isothermal at 300 °C and the column programmed from 240 to 300 °C at 6 °C/min. The mass spectrometer was operated at 80 eV, source temperature ca. 250 °C, and spectra were recorded on an oscillograph chart.

Registry No.—**3** permethyl derivative, 60282-18-0; **3** Me_3Si derivative, 60282-19-1; **4** permethyl derivative, 60282-20-4; **4** Me_3Si derivative, 60282-21-5; **5** permethyl derivative, 60282-22-6; **5** Me_3Si derivative, 60282-23-7; **6** permethyl derivative, 60282-24-8; **6** Me_3Si derivative, 60282-25-9; **7** permethyl derivative, 60282-26-0; **7** Me_3Si derivative, 60282-27-1; **8** permethyl derivative, 60282-28-2; **8** Me_3Si derivative, 60282-29-3; **9** permethyl derivative, 60282-30-6; **9** Me_3Si derivative, 60282-31-7; **10** permethyl derivative, 60282-32-8; **10** Me_3Si derivative, 60282-33-9; **11** Me_3Si derivative, 60282-34-0; **12** Me_3Si derivative, 60282-35-1; **13** permethyl derivative, 60282-36-2; **13** Me_3Si derivative, 60282-37-3.

Supplementary Material Available. Plotted mass spectra of Me_3Si derivatives of compounds **7**, **8**, and **13**, and mass spectra of permethylated 6-BAP glycosides **3**, **5**, **7**, **9**, and **13** (Table II) and permethylated zeatin glycosides **4**, **6**, **8**, and **10** (Table III) (3 pages). Ordering information is given on any current masthead page.

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Electrochemical Reduction of 3-Cyano-1-methylpyridinium Iodide, a Nicotinamide Adenine Dinucleotide Model Compound

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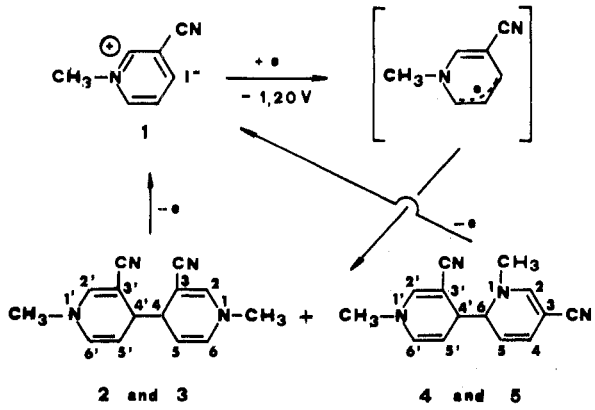
3-Cyano-1-methylpyridinium iodide (**1**) exhibits one polarographic wave at pH values below 11 ($E_{1/2}$ = −0.87 V vs. SCE). Electrolysis at the plateau potential of this wave, involving a one-electron uptake, leads to the formation of a mixture of four dimeric products, unambiguously identified as two diastereoisomer pairs: 3,3′-dicyano-1,1′-dimethyl-1,1′,4,4′-tetrahydro-4,4′-bipyridines **2** and **3**, and 3,3′-dicyano-1,1′-dimethyl-1,1′,6,4′-tetrahydro-6,4′-bipyridines **4** and **5**.

Dimeric products are known to arise from the one-electron reduction of nicotinamide adenine dinucleotide (NAD^+), and related compounds, by chemical,[1] electrochemical,[2] and photochemical[3] methods. While in a few cases these products could be isolated, their formation has been generally postulated on the ground of process stoichiometry and spectroscopic evidence. However, the literature on this subject lacks detailed evidence regarding the structure to be assigned to these dimeric compounds, and further research to obtain a deeper knowledge on the subject appears highly desirable. Accordingly, the electrochemical reduction of 3-cyano-1-methylpyridinium iodide (**1**), a NAD^+ model compound, was performed obtaining a mixture of four reduction products (Scheme I), that were isolated and unambiguously identified.

Scheme I



Results and Discussion

A. Polarographic Behavior. The reduction polarogram of **1**, recorded in the Britton–Robinson buffer solutions, exhibits one wave (A) from pH 2 up to pH 11. Another wave (B) appears at more negative potentials, at pH values greater than 11. Wave A is diffusion controlled (as ascertained from the limiting current variations with the mercury head height and temperature) over the investigated pH range. Its height is proportional to the concentration of **1**. The diffusion current constant, I, is 2.10 ± 0.05 $\mu A\ s^{1/2}\ (mM)^{-1}\ mg^{-2/3}$ for concentrations of **1** ranging from 0.05 to 5.0 mM, and this value corresponds to a one-electron Faradaic process. The half-wave potential, $E_{1/2}$, is pH independent for wave A (average value −0.87 V). The addition of surface active tetraethylammonium ions Et_4N^+ to a pH 9.5 buffered solution shifts $E_{1/2}$ toward more negative potentials (e.g., 25 mV when the concentration of Et_4N^+ was 0.1 M).

For the same pH 9.5 buffer, an increase of the ionic strength from 0.1 to 2.0 M, obtained by addition of KCl, causes $E_{1/2}$ to shift 40 mV toward more negative potentials. $E_{1/2}$ shifts also with the concentration of **1**: it is −0.86 V at 0.1 mM and -0.87_5 V at 1 mM.

From its slope over the pH range investigated, wave A seems related with a totally irreversible process ($E_{1/4} - E_{3/4}$ falls between 77 and 86 mV). The $\log i/(i_d - i)$ vs. E plot is not always strictly linear over the whole rising portion of wave A: such deviations are probably due to the chemical reaction which follows the one-electron uptake (see subsequent discussion) and to the adsorption of the depolarizer and/or the one-electron reduction products.

The second wave (B) has a partially kinetic character, as ascertained from the limiting current variations with the mercury head height and the temperature. Its $E_{1/2}$ is virtually pH independent and has a value of −1.60 V in a pH 11.5 buffer with a 1.0 mM concentration of **1**, and its height is about $\frac{3}{5}$ of the value of wave A. In the same buffer, addition of KCl, producing an increase in the ionic strength from 0.1 to 2.0 M, causes $E_{1/2}$ to shift 70 mV toward less negative potentials. The