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Mass Spectrometric Analysis of Cytokinins in Plant Tissue VII. Quantification of Cytokinin Bases by Negative Ion Mass Spectrometry*

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Abstract. A study of derivatives of N^6-(isopent-2-enyl)adenine formed by substitution at N-9 indicated that sensitivity of detection by chemical ionization mass spectrometry was maximized by a pentafluorobenzyl substituent and negative ion monitoring. O-*t*-Butyldimethylsilyl-9-pentafluorobenzyl derivatives of zeatin (Z), *cis*-zeatin (*cis*-Z), and dihydrozeatin (DZ) were characterized by mass spectrometry. A procedure was based on these stable derivatives and negative ion chemical ionization mass spectrometry for quantification of zeatin and dihydrozeatin in plant tissue.

The purine plant hormones termed cytokinins occur in plants as free bases [e.g., zeatin, 6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purine], as ribosides (e.g., zeatin riboside), and as glucosides, nucleotides, and alanine conjugates (Letham and Palni 1983).

For gas chromatography–mass spectrometry (GC-MS) analysis of cytokinins, it is necessary to convert these polar compounds into volatile derivatives. Two types of derivatives have been used frequently, namely, trimethylsilyl (TMS) and permethyl. These have been chosen, not because they are uniquely suitable for cytokinin analysis, but simply because they are the derivatives which have been used most frequently in GC studies of many types of biochemicals. Very recently, *t*-butyldimethylsilyl (*t*BuDMS) derivatives have been found to be particularly suitable for GC-MS of cytokinin bases, and these derivatives are superior to TMS for this purpose (Hocart et al. 1986). Trifluoroacetyl (TFA) derivatives of cytokinins have been prepared (Ludewig et al. 1982) but have been employed for GC-MS analysis on only one occasion (Tsui et al. 1983).

There are problems associated with all of the four derivatives mentioned above. TMS and TFA derivatives are hydrolyzed instantly in aqueous solvents and cannot be subjected to thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC). Multiple derivative formation often occurs during preparation of TMS derivatives (Palni et al. 1986). While permethyl derivatives are stable in aqueous solutions and can be chromatographed, the preparation of the necessary derivatization reagents and the derivatization procedures are time-consuming and inconvenient. Furthermore, formation of multiple derivatives of cytokinins can occur during permethylation unless the procedure is carefully controlled (Palni et al. 1986). The *t*BuDMS derivatives of cytokinin bases are also subject to hydrolysis. While the *t*BuDMS moiety attached to the isoprenoid oxygen of cytokinin bases is stable in aqueous solvents, the group at N-9 is hydrolyzed very rapidly.

The sensitivity and reliability of GC-MS methods for cytokinin quantification would be improved by a more rational approach to derivatization methods. In developing improved derivatization procedures for cytokinins, consideration should be given to the desirability of the following: (1) use of chemical reactions which exhibit selectivity for cytokinins by recognizing their distinctive structural features (this would improve the specificity of the analyses); (2) preparation of derivatives which are stable to permit further purification by liquid chromatography; (3) preparation of derivatives with halogen (especially F), or other "electron-capturing" groups in the molecule, would probably greatly enhance sensitivity when used in conjunction with negative ion chemical ionization (CI) mass spectrometry.

* For part VI, see Letham and Singh (1989).

Quantification of cytokinin bases is particularly important, since these compounds are possibly the active forms of cytokinin (Letham and Palni 1983). Hence, an endeavor was made to find new derivatives of cytokinin bases which would meet the criteria mentioned above.

Experimental Procedures

Determination of Mass Spectra and GC-MS

All spectra were determined with a Finnigan 4530 mass spectrometer. Electron-impact mass spectra (EI-MS) were determined at 70 eV and a source temperature of 150°C. Samples were applied to a heated rhenium wire mounted at the tip of the direct inlet probe. Pulsed positive ion–negative ion chemical ionization (PPINICI) spectra were taken at 140 eV (source temperature of 120°C; reagent gas, NH_3 at 1 Torr) either by the desorption method using the probe fitted with a rhenium wire or during GC (Hewlett Packard BP1 capillary column, 25 m × 0.31 mm; carrier gas, helium; head pressure 60 kPa; 200–280°C at 15°/min).

Preparation of Derivatives for Mass Spectrometry

Benzyl and Allyl Derivatives. The following was added to 30 μg of N^6-(isopent-2-enyl)adenine (iP) in a derivatization vial: (1) 300 μl of a buffer (pH 10.9) containing 20 mg of tetrabutylammonium hydrogen sulfate and 25 mg of Na_2CO_3/ml of water; (2) 200 μl of $CHCl_3$; and (3) 20 μl of the appropriate halide (pentafluorobenzyl bromide, benzyl bromide, allyl bromide, 2,3-dichloro-1-propene). In the cases of the first three halides, the mixture was stirred at 23°C overnight, but with 2,3-dichloro-1-propene stirring was continued for 2 days. The aqueous solution was removed and the chloroform phase extracted by shaking with an equal volume of water three times. The chloroform solution was evaporated under vacuum. A few drops of chloroform were then added and also evaporated.

Cyanoethyl Derivatives. The following was added to 25 μg of iP in a derivatization vial: 150 μl of anhydrous *n*-butanol, 15 μl of acrylonitrile or 2-chloroacrylonitrile, and 5 μl of triethylamine. The mixture was kept at 80°C for 1 h and then evaporated under vacuum. A few drops of chloroform were then added and also evaporated.

Other Derivatives. Permethyl derivatives of iP and zeatin (Z) were prepared by a modification of the procedure of Eagles et al. (1974). Potassium *t*-butoxide (30 mg) was first heated at 70°C with dry redistilled dimethylsulfoxide (1 ml) under anhydrous conditions for 30 min. The resulting solution (50 μl) was added to the dried sample, followed by purified methyl iodide (10 μl). After 30 min, water (100 μl) was added and the mixture was then extracted with chloroform (three 100-μl volumes). The combined extracts were washed with water (100 μl) and evaporated for mass spectrometry.

O-Mono-*t*BuDMS derivatives of Z, dihydrozeatin (DZ), and *cis*-Z were prepared according to Hocart et al. (1986). Further derivatization with pentafluorobenzyl bromide was conducted as detailed above.

All derivatives of iP and also permethyl-Z were purified by TLC (Merck silica gel GF_{254}; solvent: chloroform–methanol, 9:1 vol/vol). The 9-pentafluorobenzyl (pfBz) derivatives of *O*-mono-*t*BuDMS Z, DZ, and *cis*-Z (henceforth termed *t*BuDMS-pfBz derivatives) were chromatographed with chloroform-ethyl acetate (5:1 vol/vol) as solvent. All derivatives were eluted with redistilled ethyl acetate, which was also used to wash the layers prior to sample application.

Quantification of Z and DZ in Tobacco Leaves

The laminae and midribs were excised from tobacco leaves and the resulting laminae tissue (20–30 g) was dropped into methanol–water–formic acid (15:4:1 by volume, 10 ml/g tissue) chilled to −20°C. After 48 h at this temperature [to inactivate phosphatase and other enzymes (see Bieleski 1964)], pentadeuterium-labeled Z ([$^2H_5$]Z), [$^2H_5$]Z riboside, [$^2H_5$]DZ riboside, and dideuterium-labeled DZ [1.0 μg of each; prepared according to Summons et al. (1979)] were added as internal standards and the tissue and solvent were homogenized. The homogenate was stirred at 4°C for 12 h and then centrifuged. An aqueous solution of the residue obtained by evaporation of the supernatant was chromatographed on a column of cellulose phosphate (Badenoch-Jones et al. 1984), and the fraction eluted with ammonia was further purified using a paraffin-impregnated silica gel column (Hall et al. 1987). The eluted fraction was subjected to TLC on Merck silica gel 60 PF_{254} (solvent: *n*-butanol–acetic acid–water, 12:3:5 by volume) using Meldola blue (D1), Fast green FCF (D2), Drimarene brilliant blue K-BL (D3), and Rhodamine B (D4) as marker dyes [for source, see Badenoch-Jones et al. (1984)]. These dyes exhibit R_f values of 0.27, 0.39, 0.44, and 0.55, respectively. The zone between dyes D3 and D4 (Z1) which contained Z, DZ, ZR, plus DZR was eluted with ethanol–water–acetic acid (25:25:2, by volume); elution of the zone between D1 and D2 (Z2) would yield an *O*-glucoside fraction (the *O*-glucosides of Z, DZ, ZR, and DZR), which could be used for quantification of these compounds by the procedure of Letham and Singh (1989). The cytokinin bases and ribosides in the evaporated eluate of Z1 were separated by use of a dihydroxyboryl polymer column (Jameson et al. 1987, Noodén et al. 1990). Both fractions were then further purified using Baker SPE C_{18} columns (Jameson et al. 1987). The ribosides were converted to per-trimethylsilyl derivatives and subjected to GC-MS analysis as described previously (Jameson et al. 1987), while the bases were converted to *t*BuDMS-pfBz derivatives as described above. The derivatized base fraction was chromatographed on a Merck precoated HPTLC silica gel $60F_{254}$ plate, which had been washed with redistilled ethyl acetate. The solvent was chloroform–ethyl acetate (5:1 vol/vol) and the plates were developed twice; a few drops of concentrated ammonia were added to the filter paper lining the tank. The UV-absorbing spot of deuterium-labeled Z plus DZ (R_f 0.11) was eluted with redistilled ethyl acetate (60 μl) by stirring in a small vial (100 μl). After centrifugation, the supernatant was concentrated to about 10 μl and an aliquot was injected into the GC for quantification by PPINICI mass spectrometry (selected ion monitoring). The remainder of the derivatized sample was subjected to HPLC [analytical μBondapak C_{18} column, Waters Assoc.; solvent, acetonitrile–water (3:2, vol/vol)]. The *t*BuDMS-pfBz derivatives of Z and DZ, which were separated during HPLC (retention times 8.76 and 11.03 min, respectively), were each resubjected to GC-MS anal-

ysis. Purification by HPLC did not alter the calculated ratios of endogenous cytokinins (Z, DZ) to the [^{2}H] internal standards.

When determination of cytokinin *O*-glucosides as well as cytokinin bases and ribosides is required, the above chromatographic sequence involving TLC is considered to be the purification procedure of choice. The TLC step provides a simple and complete separation of the two groups of cytokinins. However, when quantification of Z, DZ, ZR, and DZR only is required, the purification can be simplified. The four steps involving a paraffin-impregnated silica gel column, TLC, the dihydroxyboryl polymer, and Baker SPE C_{18} columns can be replaced by a bulk HPLC step using a Waters Bondapak C_{18}/Porasil B column (Badenoch-Jones et al. 1984) following by analytical HPLC (column: Waters μBondapak C_{18}, 7.8 × 300 mm; solvent: methanol–water–acetic acid, 25:74:1; flow rate, 3.5 ml min^{-1}) to give two cytokinin functions, Z plus DZ in one, and ZR plus DZR in the other (retention times 6.6 and 10.0 min, respectively).

The former procedure involving TLC was applied to all extracts mentioned in Results.

Results

iP was selected as a model base for initial studies and six new derivatives were prepared for assessment of suitability for quantification by mass spectrometry. These derivatives were 9-benzyl-iP (I), 9-pentafluorobenzyl-iP (II), 9-allyl-iP (III), 9-(2-cyanoethyl)-iP (IV), 9-(2-chloroallyl)-iP (V), and 9-(2-chloro-2-cyanoethyl)-iP (VI). Compounds IV and VI were prepared by Michael-type additions of acrylonitrile and 2-chloroacrylonitrile, respectively, to the N-9 position of iP. For comparison, the known derivative N^6,9-dimethyl-iP (VII) was also prepared. EI-MS of these derivatives are tabulated below (m/z values with relative intensities in parentheses; an asterisk denotes a chlorine isotope peak at +2 mass units):

I: 293 (M^+, 39), 292 (31), 278 (33), 250 (100), 238 (14), 225 (50), 224 (75), 202 (21).
II: 383 (M^+, 26), 368 (30), 340 (73), 328 (5), 315 (11), 296 (39), 202 (7), 181 (100).
III: 243 (M^+, 30), 228 (46), 200 (100), 188 (8), 174 (59), 160 (9).
IV: 256 (M^+, 30), 241 (53), 213 (100), 201 (9), 188 (15), 173 (6), 172 (6), 160 (4), 148 (6), 135 (49).
V: 277* (M^+, 25), 262* (44), 234* (100), 174 (66).
VI: 290* (M^+, 16), 275* (39), 247* (77), 222* (9), 160 (24), 148 (17), 135 (100).
VII: 231 (M^+, 39), 216 (41), 199 (12), 188 (100), 176 (11), 162 (31).

In each spectrum, an M-43 ion yielded the base peak or was a very prominent fragment ion. This ion was a cyclization ion analogous to that of m/z 340 shown in Fig. 8.

PPINICI mass spectra were then determined with 0.40 nmol of each derivative under identical conditions (Table 1). When the ion currents associated with the base peaks of the derivatives were compared, both in the positive and negative ion spectra, the current was greatest for the m/z 202 fragment ion in the negative ion spectrum of II. This ion was also prominent in the negative ion spectra of I, III, IV (the base peak), and V and is due to cleavage of the N-9 substituent with negative charge localization on the purinyl moiety. Only one derivative, namely VI, exhibited a negative ion spectrum in which the base peak was due to M^-; the chlorine atom present presumably facilitated associative resonance capture ionization.

Table 1. Principal ions observed in CI mass spectra of derivatives of iP.

No.	Derivative and MW	m/z values[a] Positive MS	Negative MS
I	Benzyl (293)	226 (294, 45%)	224 (202, 70%; 292, 85%)
II	Pentafluorobenzyl (383)	384 (204, 40%; 316, 15%)	202
III	Allyl (243)	244 (204, 7%)	242 (202, 31%; 218, 18%)
IV	Cyanoethyl (256)	257	202 (255, 60%)
V	Chloroallyl (277)	278[b]	162 (202, 40%; 277,[b] 25%)
VI	Chloro-cyanoethyl (290)	291[b] (257, 56%; 204, 58%)	290[b] (254, 22%)
VII	Permethyl (231)	232	230

PPINICI spectra (scanned over the range m/z 200–550) were taken with 0.40 nmol of each derivative (amount determined from UV spectrum).
[a] The base peak is listed first, and then, in parentheses, the less intense peaks are given when these occur.
[b] Chlorine isotope peak present at +2 mass units.

Since the pfBz derivative of iP yielded an intense negative ion at m/z 202 (essentially the only ion in the negative ion spectrum), this derivative may be ideal for quantification of cytokinin bases generally, especially in view of its stability in aqueous solvents. Hence, preparation of pfBz derivatives of Z, DZ, and *cis*-Z was investigated. Z, DZ, and *cis*-Z have a side chain OH group and this would need to be derivatized for GC, as well as the 9-position. Hence, the OH group was first converted to a *t*BuDMS ether and the 9-position was then derivatized with pentafluorobenzyl bromide under the conditions used for preparation of pfBz-iP. This involved an extractive alkylation procedure using tet-

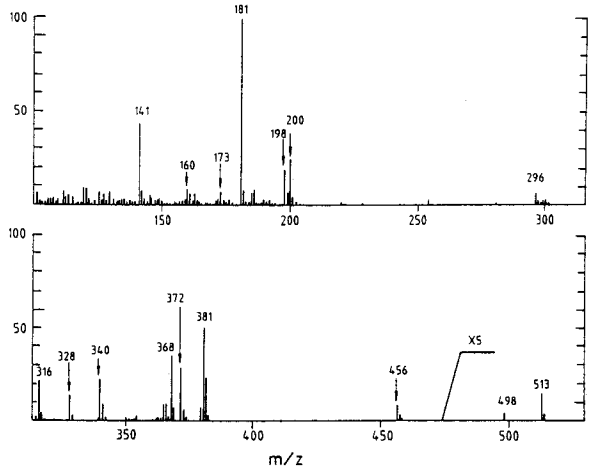

Fig. 1. EI-MS of *t*BuDMS-pfBz Z.

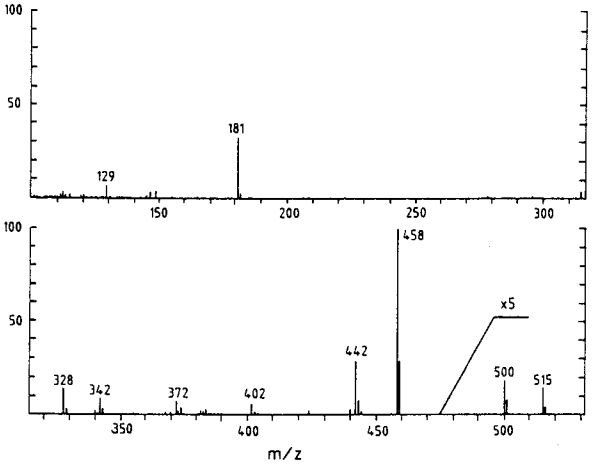

Fig. 2. EI-MS of *t*BuDMS-pfBz DZ.

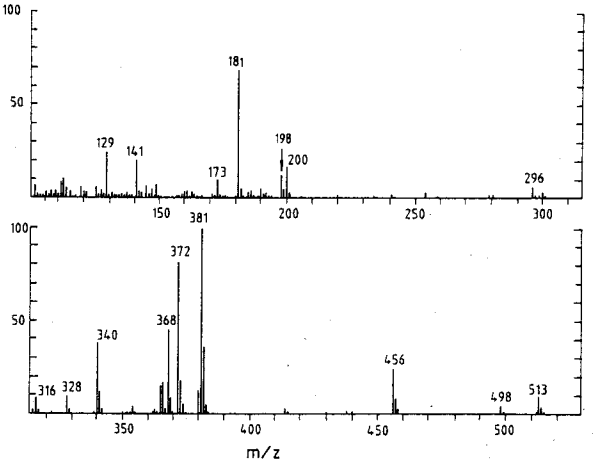

Fig. 3. EI-MS of *t*BuDMS-pfBz *cis*-Z.

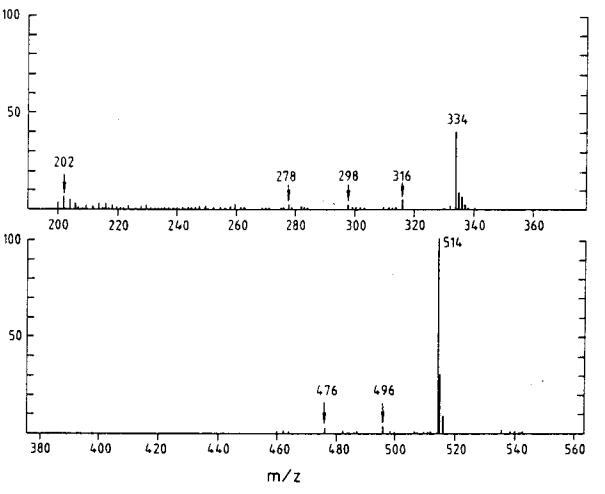

Fig. 4. Positive ion CI mass spectrum of *t*BuDMS-pfBz *cis*-Z. The spectrum was taken by the PPINICI method. The spectrum of the Z (*trans*) derivative was not significantly different from that of the *cis*-isomer.

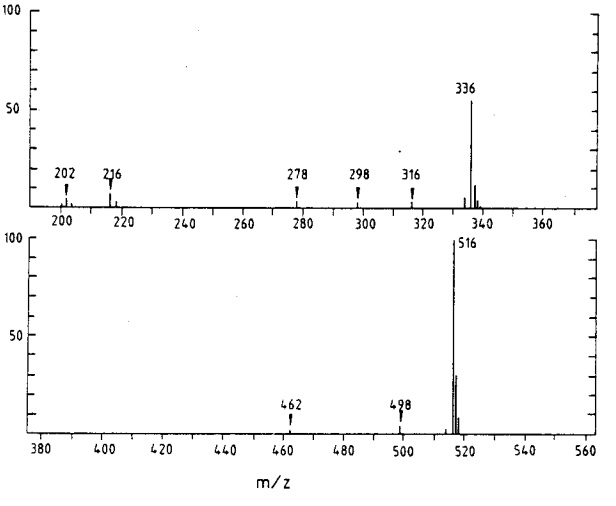

Fig. 5. Positive ion CI mass spectrum of *t*BuDMS-pfBz DZ. The spectrum was taken by the PPINICI method.

rabutylammonium hydrogen sulfate as an ion-pairing reagent. The resulting products are termed *t*BuDMS-pfBz derivatives.

The EI-MS (probe) of *t*BuDMS-pfBz derivatives of Z, DZ, and *cis*-Z are presented in Figs. 1–3, respectively, and PPINICI spectra (positive ion) are shown in Figs. 4 and 5. The negative CI spectra of *t*BuDMS-pfBz Z, DZ, and *cis*-Z (scanned m/z 200–500) exhibited only one ion, $[M-181]^-$, at m/z 332, 334, and 332, respectively. The above CI spectra were taken during GC, which readily separates the *t*BuDMS-pfBz derivatives of the three bases (retention times for DZ, *cis*-Z, and Z: 12.9, 13.5, and 14.6

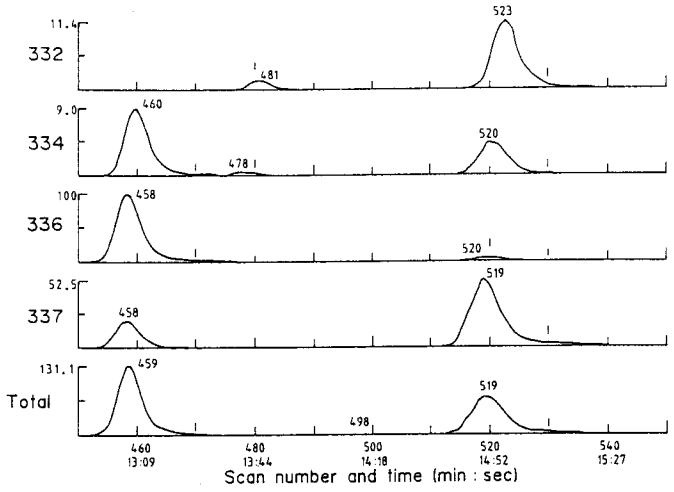

Fig. 6. Negative ion currents used to quantify Z and DZ in tobacco leaf extracts as *t*BuDMS-pfBz derivatives. The currents were recorded during GC-MS analysis with selected ion monitoring. DZ: m/z 334 [$^2H_0$] and 336 [$^2H_2$]; Z: m/z 332 [$^2H_0$] and 337 [$^2H_5$]. The extracts were purified by the extended procedure using TLC to permit quantification of glucosides in the same samples.

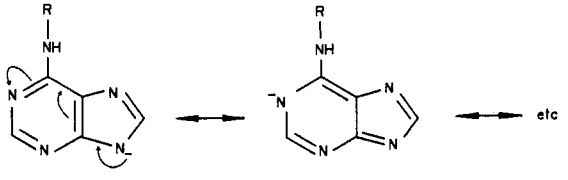

Fig. 7. Delocalization of negative charge in the purine ring system.

min, respectively). The positive ion CI spectra exhibited pronounced MH^+ ions (usually base peaks), while the $[M\text{-}181]^-$ ions noted above correspond to the ion at m/z 202 in the negative ion CI mass spectrum of pfBz-iP. The ratios of ion currents in PPINICI spectra, $[M\text{-}181]^-$ over MH^+, were calculated to be: Z, 83; DZ, 67; *cis*-Z, 80. Hence, for quantification of the *t*BuDMS-pfBz derivatives under the conditions of mass spectrometry used in this study, the negative ion CI mass spectra will give much greater sensitivity than the positive spectra.

The *t*BuDMS-pfBz derivative of Z was compared with the permethyl derivative by introducing 0.4 nmol of each into the mass spectrometer via the direct inlet probe. The MH^+ ion derived from permethyl Z (m/z 262) was much more intense than the base peak of the negative ion spectrum (m/z 260), but the ion current associated with MH^+ was only about 50% of that due to m/z 332 in the negative ion spectrum of *t*BuDMS-pfBz Z. When the two derivatives were introduced in equal amounts into the mass spectrometer via the GC, this difference in ion current was considerably greater (about fivefold). The results obtained indicate that quantification of iP and Z from the $[M\text{-}181]^-$ ion of the pfBz derivatives would be more sensitive than quantification using the permethyl derivatives, the only other known derivative of these compounds suitable for GC-MS which is stable in aqueous solvents.

As an example of the use of *t*BuDMS-pfBz derivatives for cytokinin quantification, the levels of Z and DZ in tobacco leaves were determined. [$^2H_5$]Z, [$^2H_2$]DZ, and [$^2H_5$]-labeled ribosides were added to the extracting solvent; the extract was purified and the fraction containing Z and DZ was derivatized to give *t*BuDMS-pfBz cytokinins for PPINICI mass spectrometry. Negative ion current traces from selected ion monitoring used for quantification are presented in Fig. 6. While clear peaks were evident in the negative ion currents, the cytokinins were not detected in the positive ion profiles for the molecular ions (the base peaks in the positive ion spectra). The values determined for Z in immature, expanded presenescent, and early senescent leaves were 2.90, 5.10, and 1.08 ng/g, respectively; the corresponding values for DZ were 0.75, 0.93, and 0.50 ng/g. More detailed data for cytokinin base and riboside levels in tobacco leaves in relation to induction of leaf senescence will be reported elsewhere.

Discussion

In this contribution, improved derivatives for GC-MS quantification of cytokinin bases (Z, DZ, iP) have been developed. The *t*BuDMS-pfBz derivatives of Z and DZ, and the pfBz derivative of iP, possess the three desirable attributes mentioned in the introductory paragraphs. First, for formation of a pfBz derivative, the compound would need to possess an acidic group; in the present case this is the NH at position 9 of the purine ring (pK_a 9.8). In this respect, the derivatization possesses selectivity. Second, the derivatives are stable in aqueous solvents and can be chromatographed. Third, they

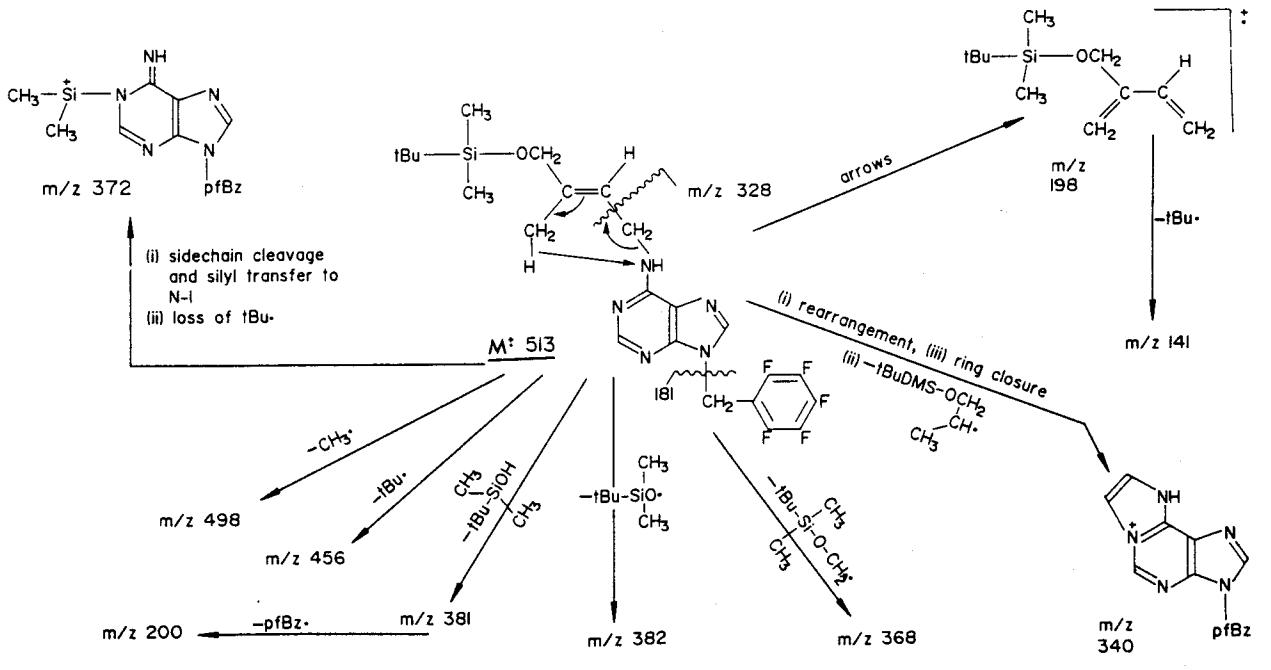

Fig. 8. Rationalization of the EI-MS of *t*BuDMS-pfBz Z. pfBz denotes a pentafluorobenzyl moiety.

contain five fluorine atoms per molecule. Compounds containing fluorine or chlorine atoms often exhibit intense anionic molecular ions due to resonance electron capture under CI conditions. Unfortunately, this did not occur with pfBz cytokinins because the pfBz moiety was cleaved under CI conditions and the negative ion current was concentrated in the M-181 fragment ion while an M^- ion was not detected. However, the intensity of the $[M-181]^-$ ion made it ideal for quantification, and its stability can be explained by the ability of the purine ring system to delocalize the negative charge as represented in Fig. 7.

While negative ion CI mass spectrometry is preferable for quantification of cytokinin bases as pfBz derivatives, EI-MS may be useful for quantification when sensitivity is less critical and for rigorous identification from the characteristic fragmentation patterns. Hence, the EI-MS of *t*BuDMS-pfBz Z has been rationalized in Fig. 8. The unusual ion m/z 372 is the counterpart of the ions at m/z 192 and 306 in the EI-MS of mono-*t*BuDMS-Z and di-*t*BuDMS-Z, respectively (see Hocart et al. 1986) and hence has been assigned the structure shown in Fig. 8. This unusual ion appears to be formed by transfer of the *t*BuDMS moiety to N-1 with cleavage of the N^6-substituent and subsequent loss of a *t*Bu radical. It is noteworthy that the intensity of this ion in the EI-MS or derivatized *cis*-Z (Fig. 3) greatly exceeds that in the spectrum of Z. Compared with the EI-MS of *t*BuDMS-pfBz Z and *cis*-Z, that of DZ is relatively simple and exhibits only three prominent ions: m/z 458, M^+-*t*Bu radical (−57); m/z 442, attributable to loss of *t*Bu radical plus CH_4 from M^+; and m/z 181, pentafluorobenzyl or pentafluorotrophylium ion.

Attempts to quantify cytokinin bases by gas chromatography using an electron capture detector have been restricted by lack of stable halogen-containing derivatives (Ludewig et al. 1982). The pfBz derivatives described herein would appear to be ideal for such work.

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References

Badenoch-Jones J, Letham DS, Parker CW, Rolfe BG (1984) Quantitation of cytokinins in biological samples using antibodies against zeatin riboside. Plant Physiol 75:1117–1125

Badenoch-Jones J, Rolfe BG, Letham DS (1984) Phytohormones, *Rhizobium* mutants, and nodulation in legumes V. Cytokinin metabolism in effective and ineffective pea root nodules. Plant Physiol 74:239–246

Bieleski RL (1964) The problem of halting enzyme action when extracting plant tissues. Anal Biochem 9:431–442

Eagles J, Laird WM, Selford R, Synge RLM (1974) Permethylation for mass spectrometry: Rearrangements for ester linkages and use of potassium *t*-butoxide. Biomed Mass Spectrom 1:43–48

Hall PJ, Badenoch-Jones J, Parker CW, Letham DS, Barlow BA (1987) Identification and quantification of cytokinins in the xylem sap of mistletoes and their hosts in relation to leaf mimicry. Aust J Plant Physiol 14:429–438

Hocart CH, Wong OC, Letham DS, Tay SAB, MacLeod JK (1986) Mass spectrometry and chromatography of *t*-butyldimethylsilyl derivatives of cytokinin bases. Anal Biochem 153:85–96

Jameson PE, Letham DS, Zhang R, Parker CW, Badenoch-Jones J (1987) Cytokinin translocation and metabolism in lupin species I. Zeatin riboside introduced into the xylem at the base of *Lupinus angustifolius* stems. Aust J Plant Physiol 14:695–718

Letham DS, Singh S (1989) Quantification of cytokinin *O*-glucosides by negative ion mass spectrometry. Plant Physiol 89:74–77

Letham DS, Palni LMS (1983) The biosynthesis and metabolism of cytokinins. Annu Rev Plant Physiol 34:163–197

Ludewig M, Dorffling K, Konig WA (1982) Electron capture capillary gas chromatography and mass spectrometry of trifluoroacetylated cytokinins. J Chromatogr 243:93–98

Noodén LD, Singh S, Letham DS (1990) Correlation of xylem sap cytokinin levels with monocarpic senescence in soybean. Plant Physiol 93:33–39

Palni LMS, Tay SAB, MacLeod JK (1986) GC-MS methods for cytokinins and metabolites. In: Linskens HF, Jackson JK (eds) Modern methods of plant analysis, Vol. 3. Springer-Verlag, Berlin, pp 214–253

Summons RE, Duke CC, Eichholzer JV, Entsch B, Letham DS, MacLeod JK, Parker CW (1979) Mass spectrometric analyses of cytokinins in plant tissues II. Quantitation of cytokinins in *Zea mays* kernels using deuterium labelled standards. Biomed Mass Spectrom 6:407–413

Tsui C, Shao LM, Wang CM, Tao GQ, Letham DS, Parker CW, Summons RE, Hocart CH (1983) Identification of a cytokinin in water chestnuts (corms of *Eleocharis tuberosa*). Plant Science Lett 32:225–231