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# MASS SPECTRAL DETERMINATION OF BENZAMIDE DERIVATIVES OF POLYAMINES SEPARATED BY HPLC

DANE R ROBERTS, MARK A WALKER and ERWIN B DUMBROFF

Department of Biology, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada

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**Key Word Index**—Polyamines, putrescine, spermidine, spermine, benzamide derivatives, MS, HPLC

**Abstract**—The identities of benzamide derivatives of putrescine, spermidine and spermine separated from plant tissue extracts by HPLC are confirmed by mass spectral analysis and recoveries are reported

## INTRODUCTION

Redmond and Tseng [1] used the classical Schotten–Baumann procedure to benzoylate polyamines for subsequent analysis by reversed-phase HPLC with UV detection Flores and Galston [2] applied the method to the analysis of polyamines from plant tissues using $HClO_4$ extraction and isocratic elution Efficiency of polyamine recovery from plant-tissue samples has not been reported, and to date, identification has relied upon retention times, co-chromatography and comparisons with TLC data We now report a modified separation procedure and recoveries of putrescine, spermidine and spermine through HPLC We also confirm the identities of the benzamide derivatives of the polyamines and identify the major ions in their mass spectra

## RESULTS AND DISCUSSION

Average recoveries and standard errors for $^{14}C$-putrescine, spermidine and spermine added to tissue slurries were $94 \pm 2$, $83 \pm 2$ and $70 \pm 2\%$, respectively Although the reasons for reduced recovery with increased chain length of the amines remain unclear, additional sample clean-up by inclusion of an ion-exchange chromatography step in the separation procedure [3] apparently does not reduce variation or yield higher recoveries than we obtained On the contrary, we found that standard errors remained acceptably low when the crude TCA extracts were derivatized without additional purification of the samples The recovery of unlabelled 1,6-hexanediamine, which is commonly used as an internal standard during polyamine analysis [1, 3], was $83 \pm 2 9\%$ It, therefore, accurately reflects recovery efficiency for spermidine but underestimates the values for putrescine and overestimates those for spermine To determine whether endogenous agmatine might be hydrolysed to putrescine during sample extraction and subsequent benzoylation, several HPLC runs were made with TCA-treated and benzoylated standards of agmatine, putrescine and mixtures of the two amines We found no evidence that agmatine contributed to the putrescine pool during these procedures

The low volatility and relatively high boiling points of the benzamide derivatives of the amines necessitated the use of solid probe analysis in lieu of combined GC/MS Probe temperatures of 140°, 180° and 390°, respectively, were required to detect the molecular ions of the benzamides of putrescine, spermidine and spermine (Table 1) The detection of several high $M_r$ ions is essential for positive identification of the amines since a number of low $M_r$ fragments, including the benzene carbonyl-ion base peaks at $m/z$ 105, are common to all three mass spectra A comparison of the spectra of derivatized plant samples and standards showed essentially identical fragmentation patterns and confirmed the reliability of the benzoylation procedure for the analysis of putrescine, spermidine and spermine derived from plant tissues

## EXPERIMENTAL

Polyamines were identified in cotyledons of recently germinated sugar maple seeds (*Acer saccharum* Marsh) and in carnation flower petals (*Dianthus caryophyllus* L) About 300 mg fr wt of plant tissue were extracted 3 × in 1 ml aliquots of cold 5% TCA in a Potter–Elvehjem homogenizer The samples were transferred to 16 × 125 mm screw-cap test tubes and, after addition of $^{14}C$ radioactive authentics and 1,6-hexanediamine [1] as int standards, the homogenates were centrifuged at 2600 *g* for 1 hr to remove suspended plant material (Extracts from some tissues may require partitioning with 2 ml $CH_2Cl_2$ prior to derivatization to avoid contaminant peaks that may overlap with 1,6-hexanediamine) A 500 μl aliquot of the supernatant from each sample was benzoylated following the procedure of ref [2] Derivatized extracts (10 μl) were injected into a Spectra-Physics 8000 liquid chromatograph and separated at 50° on a 150 × 4 6 mm column packed with 5 μm Ultrasphere-ODS (Beckman) using MeOH–$H_2O$–acetonitrile (8 11 1) programmed to 14 5 1 over 20 min for elution Peaks were detected by *A* at 254 nm Fractions corresponding to retention times of benzoylated standards were collected for recoveries, then dried and subsequently analysed by solid-probe MS without further purifi-

Table 1 Principal mass ion fragments of benzoylated putrescine and polyamine standards

| Compound | Ion peaks (*m/z*) | Normalized intensity (%) | Possible fragment structures |
|---|---|---|---|
| Putrescine | 105 | 100 | $[PhCO]^+$ |
| | 134 | 9 0 | $[PhCONHCH_2]^+$ |
| | 148 | 9 7 | $[PhCONH(CH_2)_2]^+$ |
| | 162 | 12 2 | $[PhCONH(CH_2)_3]^+$ |
| | 174 | 18 4 | $[PhCON\langle^{CH-CH_2}_{CH_2-CH_2}]^+$ |
| | 191 | 10 4 | $[PhCONH(CH_2)_4NH]^+$ |
| | 296 | 2 7 | $[PhCONH(CH_2)_4NHCOPh]^+$ molecular ion |
| Spermidine | 105 | 100 | As above |
| | 174 | 24 5 | As above |
| | 231 | 20 5 | $[PhCONH(CH_2)_3N\langle^{CH-CH_2}_{CH_2-CH_2}]^+$ |
| | 352 | 14 9 | $[PhCONH(CH_2)_3N(CH_2)_4NHCOPh]^+$ |
| | 457 | 1 0 | $[PhCONH(CH_2)_3N(COPh)(CH_2)_4NHCOPh]^+$ molecular ion |
| Spermine | 105 | 100 | As above |
| | 162 | 21 0 | As above |
| | 174 | 47 8 | As above |
| | 231 | 42 8 | As above |
| | 352 | 29 0 | As above |
| | 456 | 17 7 | $[PhCONH(CH_2)_3N(COPh)(CH_2)_4N(COPh)]^+$ |
| | 513 | 19 5 | $[PhCONH(CH_2)_3N(COPh)(CH_2)_4N(COPh)(CH_2)_3NH]^+$ |
| | 618 | 1 3 | $[PhCONH(CH_2)_3N(COPh)(CH_2)_4N(COPh)(CH_2)_3NHCOPh]^+$ molecular ion |

cation About 10 nmol of derivatized sample were redissolved in 50 μl of absolute MeOH and then dried on the solid probe of a VG Micromass 7070F mass spectrometer and analysed using an emission current of 100 μA, an accelerating voltage of 4 kV, an ion-source temp of 240° and ionizing voltages of 20 eV for spermidine and spermine and 70 eV for putrescine

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