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Note

Gas chromatography and mass spectrometry of N-(phosphonacetyl)-L-aspartic acid

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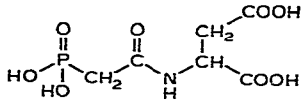
N-(Phosphonacetyl)-L-aspartic acid (PALA; NSC 224131), a transition-state inhibitor of the enzyme aspartate transcarbamylase, is currently under investigation (as the tetrasodium salt) by the National Cancer Institute as a potent antitumor agent[1]. During the course of preparing the drug radiolabeled with ^{14}C, a more sensitive and efficient method for the separation and quantitation of PALA (I) and its synthetic precursors, phosphonacetic acid and aspartic acid, was needed.



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A gas chromatographic method is described herein for the determination of the methyl ester derivatives of PALA, phosphonacetic acid, and aspartic acid. The proposed method is suitable for drug stability and metabolism studies.

EXPERIMENTAL

Materials and methods

PALA, as the tetrasodium salt, was synthesized by the method of Swyryd *et al.*[2]. Phosphonacetic acid (PA) was prepared from triethyl phosphonoacetate (Aldrich, Milwaukee, Wisc., U.S.A.) by the method of Balsiger *et al.*[3]. N,O-Bis(trimethylsilyl)-trifluoroacetamide (BSTFA), hexamethyldisilazane (HMDS), and trimethylchlorosilane (TMCS) were purchased from Pierce (Rockford, Ill., U.S.A.). Tris–hydrochloride and L-aspartic acid (>98% pure) were obtained from commercial suppliers. Diazomethane was generated from Diazald® (Aldrich). AG 50W-X8 and AG 1-X8 ion-exchange resins (200–400 mesh) were purchased from Bio-Rad Labs. (Rockville Centre, N.Y., U.S.A.).

Thin-layer chromatography

Thin-layer chromatography (TLC) was carried out on pre-coated polyethyleneimine (PEI) cellulose F plates, 20 × 20 cm × 0.10 mm (EM Labs., Elmsford,

N.Y., U.S.A.) with 1.2 *M* LiCl as the developing solvent[4]. Components were visualized by the detection system of Bandurski and Axelrod[5].

Gas-liquid chromatography

Gas–liquid chromatography (GLC) was performed at 100°–300° with 16°/min heating rate on a 6 ft. × 2 mm I.D. glass column containing Gas-Chrom Q (100–200 mesh) coated with 3% OV-17 (Analabs, North Haven, Conn., U.S.A.) using a Hewlett-Packard instrument (Model 5830) equipped with a flame ionization detector. The helium carrier gas flow-rate was 31 ml/min and injections were made on column at 250°.

Preparation of derivatives

Samples of tetrasodium PALA were desalted over AG 50W-X8 cation-exchange resin and the eluent was freeze-dried in a screw-cap test tube.

Per-methyl derivatives. Freshly prepared ethereal diazomethane was added to the PALA, PA, or aspartic acid samples and the mixture was allowed to react for 1 h at room temperature. PALA tetrasodium salt (2 mg) was added to either 2.0 ml of methanol–HCl or *n*-butanol–HCl, ultrasonicated for 5 min, then heated at 75° for 30 min. In both cases excess reagent and solvent were removed under a stream of nitrogen gas and the residue was dissolved in acetonitrile.

Per-trimethylsilyl (TMS) derivatives. PALA (2 mg) was treated with either 0.5 ml of BSTFA–acetonitrile (1:1) or pyridine–HMDS–TMCS (10:2:1) at 105° for 30 min.

Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) was performed using a Hewlett-Packard 5700A gas chromatograph interfaced with a DuPont 21-491 mass spectrometer. The ionization voltage was 70 eV. Accumulation of spectra and manipulation of data were performed using a DuPont 21-094 data system.

RESULTS AND DISCUSSION

TLC of PALA (free acid) and PA on PEI-impregnated cellulose plates developed in 1.2 *M* LiCl gave a single spot for PALA at R_F 0.50–0.64 and a single spot for phosphonacetic acid at R_F 0.59–0.73. Aspartic acid remained at the origin in this system. The minimum quantities of PALA and PA detected were 25 and 5 μg, respectively. Other TLC and visualization systems investigated did not result in either improved separation or lower limits of detection[6].

GLC was then investigated as a means of separating PALA from PA and aspartic acid. Fig. 1 shows a typical chromatogram. The ratio of tetra- to penta-methyl ester of PALA remained constant at 96.5:3.5 over derivatization periods of 5–120 min. In order to determine the percentage of PALA derivatized over these time periods, PALA-[^{14}C] derivatized samples were chromatographed over AG 1-X8 anion-exchange resin and eluted with 0.1 *M* Tris–hydrochloride buffer (pH 8). Any underivatized PALA would remain bound to the column. Measurement of the radioactivity recovered indicated complete conversion of PALA free acid to methylation products.

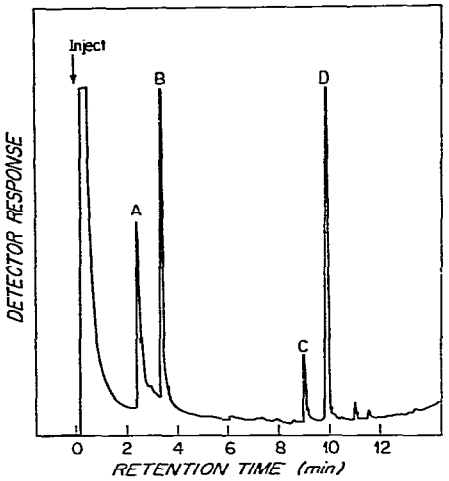

Fig. 1. Gas chromatogram of aspartic acid, phosphonacetic acid, and N-(phosphonacetyl)-L-aspartic acid derivatives. A = aspartic acid methylation products (t_R 2.45 min); B = phosphonacetic acid trimethyl ester (t_R 3.41 min); C = N-(phosphonacetyl)-L-aspartic acid pentamethyl ester (t_R 8.99 min); D = N-(phosphonacetyl)-L-aspartic acid tetramethyl ester (t_R 9.91 min).

Attempts to prepare methyl or *n*-butyl esters of PALA with methanol–HCl of *n*-butanol–HCl by standard procedures were unsuccessful[7]. The TMS derivative or PALA was prepared but was not sufficiently stable for reproducible GLC. The TMS derivative of PA is known[8].

The detector response was linear over the range of 1–25 μg of PALA and the minimum amount of drug which was easily detected and quantified was 1 μg. We are working on a more sensitive assay to determine drug and metabolite concentrations for plasma and tissue samples.

The mass spectrum of PALA tetramethyl ester after GC is shown in Fig. 2. Table I lists some of the major ions in this spectrum along with their proposed structures. By MS, the major component of the aspartic acid methylation products is the dimethyl ester. GC–MS, particularly selected ion monitoring, may be a way of determining drug/metabolite concentrations in biological fluids, in addition to aiding in the structural characterization of any metabolites.

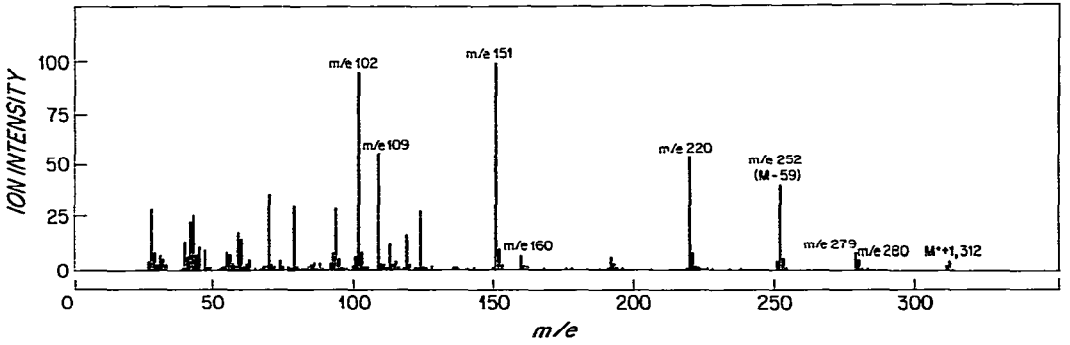

Fig. 2. Mass spectrum of N-(phosphonacetyl)-L-aspartic acid tetramethyl ester after GC.

TABLE I

SELECTED IONS AND PROBABLE STRUCTURES FROM THE MASS SPECTRUM OF PALA TETRAMETHYL ESTER

Ion	*m/e*	*Probable structure*
M + 1	312	
M − OCH_3 (M − 31)	280	
M − $COOCH_3$ (M − 59)	252	
M − $COOCH_3$ − CH_3OH (M − 91)	220	
M − 151	160	$[CH_3OOC–CH_2CH(COOCH_3)–NH]^+$
M − 160	151	$[O=C–CH_2–P(O)(OCH_3)_2]^+$
M − 202	109	$[O=P(OCH_3)_2]^+$
M − 209	102	$[CH_3OOC–CH_2CH=NH_2]^+$

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