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Note

Determination of pentazirinocyclodiphosphathiazene in biological fluids by high-performance liquid chromatography

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Several cyclodiphosphathiazene derivatives have been synthesized and investigated by Labarre and co-workers [1, 2] to develop effective antitumor substances with fewer side-effects. Among these cyclodiphosphathiazene derivatives, pentaziridinocyclodiphosphathiazene (SOAz) (Fig. 1) has been found to have the strongest antitumor activity on experimental tumors — especially on murine L 1210 and P 388 leukemias and on B 16 melanoma — and the lowest toxicity [1, 2].

For elucidation of the metabolism, absorption and excretion of SOAz, it seems important to establish a quantitative method for the determination of the concentration of SOAz in biological fluids. There are no reports of a method for the determination of SOAz. Therefore, we examined various methods for assay of SOAz in biological fluids, and established a successful analytical method employing high-performance liquid chromatography (HPLC) with a refractive index (RI) detector.

Furthermore, it was found that no metabolites of the compound SOAz are observed in plasma and urine under the present method. However, it was also found that SOAz changes mainly into two kinds of compounds spontaneously (Fig. 1). This report describes these results.

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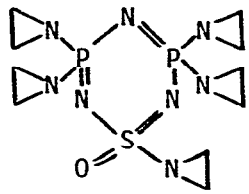
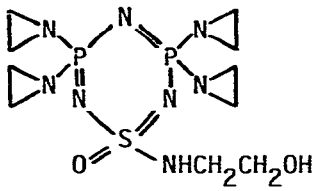


SOAz



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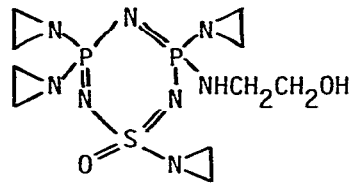

F-2

Fig. 1. Structures of SOAz and its decomposition products.

EXPERIMENTAL

Materials

SOAz was synthesized and purified by Otsuka Chemical Co. (Tokushima, Japan). The other chemicals used were obtained from Wako Pure Chemicals (Osaka, Japan). Dichloromethane and methanol were liquid chromatographic grade materials. The other chemicals used were analytical grade materials.

HPLC instrumentation

A Shimadzu LC-3 liquid chromatograph equipped with a Model SIL-1A high-pressure injector and a Model RID-2A detector (Kyoto, Japan) was used. A μBondapak C_{18}/Porasil chromatographic column (particle size, 8—10 μm; 30 cm × 3.9 mm I.D.) from Waters Assoc., Milford, MA, U.S.A., was used for the separation; the mobile phase was 5 m*M* KH_2PO_4—methanol (70:30, v/v) and the flow-rate 1.0 ml/min. The column was maintained at room temperature. Peak areas were determined with a Shimadzu Model C-R1A Chromatopac apparatus.

A JEOL Model JMS D 300 mass spectrometer with an electron impact (EI) and chemical ionization (CI) ion source (Tokyo, Japan) and a Varian FE-80 nuclear magnetic resonance (NMR) spectrometer were used for identification of the decomposition products of SOAz. The mass spectrometric analyses were carried out under the following conditions: ionization energy 190 eV, ionization current 300 μA, accelerating voltage 3.0 kV; isobutane was used as reagent gas for the measurement of CI mass spectra. ^{31}P-NMR spectra were measured

using deuterated chloroform as solvent and a deuterium oxide solution containing 85% phosphoric acid as external standard.

Analytical procedure

Samples of 1.0 ml of plasma, urine or other biological fluids were adjusted to pH 10.0 with 1 *N* sodium hydroxide, and extracted with 5 ml of dichloromethane for a few minutes. The organic layer containing SOAz was separated by centrifugation at 2000 *g* for 5 min. This extraction was repeated once using 2 ml of dichloromethane. The combined organic layer was dried under nitrogen at room temperature. The residue was dissolved in 100 μl of a solution of 5 m*M* KH_2PO_4—methanol (70:30, v/v; mobile phase for HPLC), and 40 μl of this solution were injected into the liquid chromatograph.

A calibration curve for the determination of SOAz by HPLC was prepared by plotting the peak area against the concentration. The calibration curve was linear at concentrations of 0.5—400 μg/ml.

RESULTS AND DISCUSSION

SOAz dissolves easily in chloroform, dichloromethane, methanol, acetone or water, but it is very unstable under strong acidic conditions. SOAz does not absorb or fluoresce in UV light, and it is hard to obtain any derivative of SOAz without decomposition. Also, it was found that SOAz could be extracted with chloroform, dichloromethane or 1,2-dichloroethane from biological fluids. Various conditions were examined for the extraction of SOAz from biological fluids following its administration and for its HPLC separation on the basis of its physicochemical properties described above. The following procedure was found to be the simplest and most rapid and to be the most reliable with highest recovery. The biological samples were adjusted to pH 10.0 with 1 *N* sodium hydroxide solution, then extracted with dichloromethane.

A reversed-phase chromatographic column, μBondapak C_{18}, and an RI detector were used for separation and monitoring. A mobile phase consisting of water—methanol or 5 m*M* KH_2PO_4—methanol was found to be suitable for the separation of SOAz from biological fluid components extracted with dichloromethane. In this study, a solution of 5 m*M* KH_2PO_4—methanol (70:30, v/v) was used as the mobile phase since it resulted in the highest detection sensitivity and the best chromatographic separation of SOAz.

Known amounts of SOAz were added to plasma of rats. Chromatograms of HPLC with RI detection showing the separation of SOAz extracted from rat plasma and the extract of rat plasma control are given in Fig. 2. The retention time of SOAz was 7.0 min. As summarized in Table I, the recovery of SOAz from plasma was ca. 85%, and the detection limit for SOAz under this HPLC method was 0.5 μg/ml plasma. The reproducibility was ± 1.5—3.1%.

The results obtained for the chromatographic separation, recovery and detection limit were in good agreement with those obtained with urine, bile and other biological fluids.

Then, we tested the stability of SOAz at various temperatures in aqueous solution, rat plasma and urine. As shown in Table II, no decomposition of SOAz was observed at various temperatures for plasma, at 5°C or —25°C for

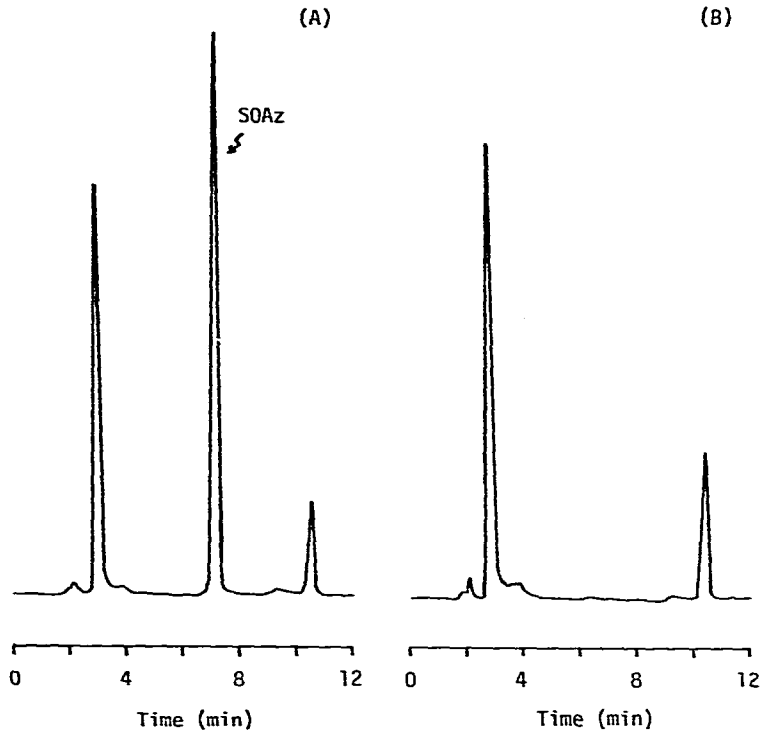

Fig. 2. HPLC chromatograms showing the separation of SOAz extracted from rat plasma (A) and the extract of rat plasma control (B).

TABLE I

RECOVERY ON EXTRACTION OF SOAz FROM PLASMA

Each value is the mean of three determinations.

Added (μg/ml)	Recovery from plasma (%)
1.0	75.4
5.0	81.7
10.0	81.9
40.0	89.4
200.0	97.1
Mean ± S.D. (%)	85.1 ± 7.8

its aqueous solution, or at −25°C for urine. However, SOAz decomposed to give small amounts of two compounds (F-1 and F-2, Fig. 1) at room temperature or at 5°C for aqueous solution and urine. The HPLC separation of these decomposition products and SOAz in urine is shown in Fig. 3. Each fraction eluting at retention times of 4.7 and 5.2 min from the HPLC column was collected separately and identified by the EI and CI mass spectra and ^{31}P-NMR spectra. SOAz: *m/z* 362 ($M^{+\cdot}$) and *m/z* 320 (M—aziridine) in EI mass spectrum;

TABLE II

STABILITY OF SOAz IN PLASMA, URINE AND AQUEOUS SOLUTION AT VARIOUS TEMPERATURES AFTER ADDITION OF SOAz AT A CONCENTRATION OF 500 μg/ml

Conditions	Compound*	Proportion found (%)		
		Plasma	Urine	Aqueous solution
Room temp., 24 h	SOAz	100.00	99.53	99.71
	F-1	—	0.36	0.26
	F-2	—	0.11	0.03
Room temp., 48 h	SOAz	100.00	98.47	99.37
	F-1	—	1.32	0.56
	F-2	—	0.21	0.07
5°C, 48 h	SOAz	100.00	99.88	100.00
	F-1	—	0.12	—
	F-2	—	—	—
—25°C, 48 h	SOAz	100.00	100.00	100.00
	F-1	—	—	—
	F-2	—	—	—

*The structures are indicated in Fig. 1.

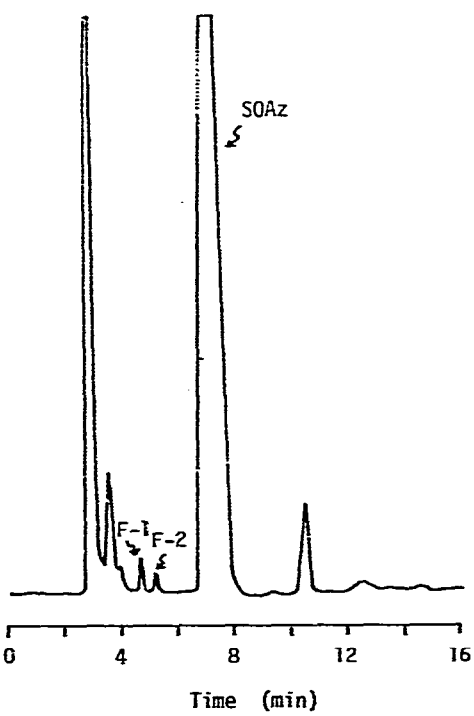

Fig. 3. HPLC chromatogram showing the separation of SOAz and its decomposition products in urine.

m/z 363 (M+1) and *m/z* 337 in CI mass spectrum; and δ 35.91 (P, singlet) in ^{31}P-NMR spectrum. F-1: *m/z* 362 (M—H_2O), *m/z* 350 (M—CH_2O), *m/z* 338 (M—C_2H_6NO) and *m/z* 320 in EI mass spectrum; *m/z* 381 (M+1), *m/z* 363 (M+1—H_2O) and *m/z* 338 (M—C_2H_6NO) in CI mass spectrum; and δ 35.35 (P, singlet) in ^{31}P-NMR. F-2: *m/z* 350 (M—CH_2O), *m/z* 338 (M—C_2H_6NO) and *m/z* 320 in EI mass spectrum; and *m/z* 381 (M+1) and *m/z* 320 in CI mass spectrum. (The ^{31}P-NMR spectrum of F-2 could not be measured because of small amounts collected.) It was also observed that F-2 decomposed further to another, unknown, compound.

Finally, SOAz (100 mg/kg) was administered intravenously to rats, and the time course of change in the concentration of SOAz in plasma was measured by the present method. The results obtained are shown in Fig. 4.

This method employing HPLC with an RI detector will be helpful for basic and clinical pharmacological studies on the compound SOAz.

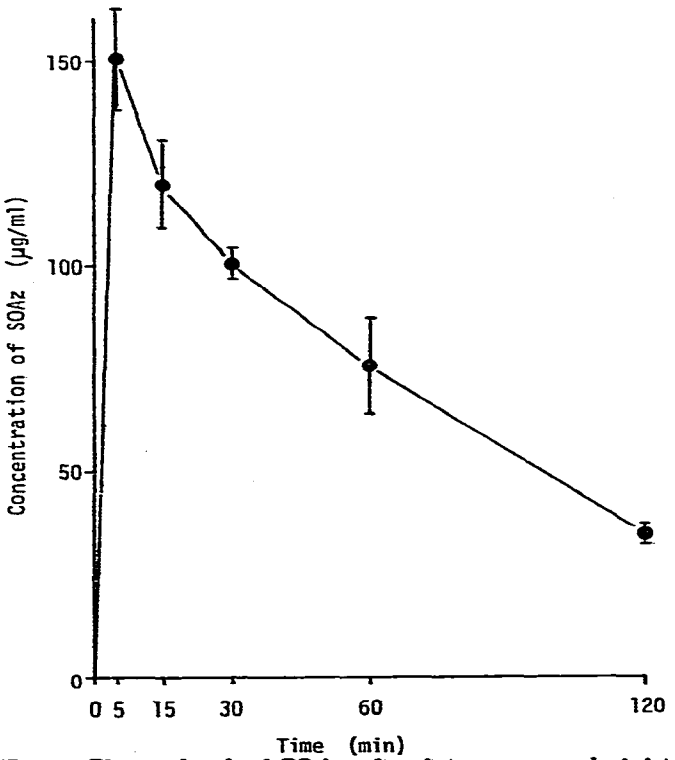

Fig. 4. Plasma level of SOAz after intravenous administration of SOAz (100 mg/kg) to rats. Results are the average for three rats.

REFERENCES

1 J.F. Labarre, F. Sournies, J.C. van de Grampel and A.A. van der Huizen, ANVAR French Patent No. 79-17336, July 4, 1979.

2 J.F. Labarre, F. Sournies, S. Cros, G. Francois, J.C. van de Grampel and A.A. van der Huizen, Cancer Lett., 12 (1981) 245.