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ISOLATION, CHARACTERIZATION AND THIOL EXCHANGE REACTION OF PENICILLAMINE SELENOTRISULFIDES

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Penicillamine selenotrisulfides formed by the reaction of selenite with D-, L-, and DL-penicillamines in the aqueous solution have been isolated and submitted to the spectroscopic and chromatographic analyses. FAB-MS spectra indicated the molecular ion peak $(M+1)^+$ at m/z 377, which confirmed the molecular formula($C_{10}H_{20}N_2O_4S_2Se$) for all these selenotrisulfides. The [^{77}Se]NMR and HPLC results showed that symmetric selenotrisulfides(DD and LL) were formed from D- and L-penicillamines, respectively, while asymmetric selenotrisulfide (DL) in addition to symmetric ones was formed from DL-penicillamine. The occurrence of thiol exchange reaction of selenotrisulfides was verified.

Selenium has been recognized as a biologically important element with both nutritious and toxic natures. As a possible intermediate in the bioconversion of dietary selenium into biologically active organoselenium compounds, selenotrisulfides have been presumed by Ganther(1). The occurrence of the following reaction has been presumed for the formation of selenotrisulfide in vivo, since the reaction in vitro was proposed by Painter(2).

$$4RSH + H_2SeO_3 \rightleftharpoons RSSeSR + RSSR + 3H_2O$$

Ganther showed a UV-absorption spectrum of selenotrisulfide formed from glutathione(1). However, selenotrisulfides are, in general, too labile to be isolated, so that they have not so far been well characterized by physico-

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chemical methods. On the other hand, we found that selenotrisulfide formed from penicillamine was exceptionally very stable and could be isolated, because it was less soluble in water than corresponding disulfide. This report deals with the properties of penicillamine selenotrisulfides, which may be regarded as a model compound in the metabolism of selenium in biological systems.

Materials and Methods

Reagents: D-, L-, and DL-penicillamines were the commercial products of reagent grade. They were used as supplied. Selenious acid and its sodium salt of analytical reagent grade were also obtained from commercial source. Glass-distilled deionized water and glass-distilled methanol were used to prepare the mobile phases for HPLC.

Isolation of penicillamine selenotrisulfides: D-, L-, and DL-penicillamines were dissolved separately in water until almost saturated, and each solution was added with aqueous saturated solution of sodium selenite at a molar ratio of 4:1. The mixed solution was allowed to stand for 30 min at ambient temperature. The precipitate formed was collected by filtration and rinsed with small volume of cold water and methanol. The selenotrisulfide crystals thus obtained from D- and L-penicillamines were the colorless needles with mp 192-3°C and 197°C, respectively, while DL-penicillamine selenotrisulfide crystallized in the colorless plate with mp 180°C.

Spectroscopic Measurements: Fast atom bombardment mass spectra were measured on a JMSDX-300 mass spectrometer(JEOL, Tokyo, Japan), [^{77}Se]NMR spectra on JNM-FX-200(JEOL), and UV-absorption spectra on UVIDEC-505(Jasco, Tokyo, Japan).

High-Performance Liquid Chromatography: The chromatograph used was LC-3A(Shimadzu, Kyoto, Japan) equipped with a variable wave length UV-detector SPD-2(Shimadzu). The stationary phase was Develosil ODS-10(Nomura Chemicals Co., Seto, Japan) packed in a stainless steel tubing(15 cm x 4.5 mm i.d.). The mobile phase conditions are given in the legend of Fig. 3.

Results and Discussion

Three different crystals of selenotrisulfides obtained from D-, L-, and DL-pencillamines gave almost identical FAB-MS spectra. Fig. 1 shows those for D- and L-penicillamine selenotrisulfides, where the molecular ion peak, $(M+1)^+$, was observed at m/z 377, which confirmed the molecular weight(M=376) consistent with the molecular formula of $C_{10}H_{20}N_2O_4S_2Se$ in each of these compounds. The elemental analyses of these crystals showed that they were isolated as monohydrate.

The [^{77}Se]NMR spectra(Fig. 2) indicated that D- and L-penicillamine selenotrisulfides in the pH 2.0 D_2O solution gave a signal at 583 ppm, where dimethylselenide was used as a primary standard for the chemical shift of [^{77}Se](0 ppm) and diphenyldiselenide as a secondary standard(461.7 ppm).

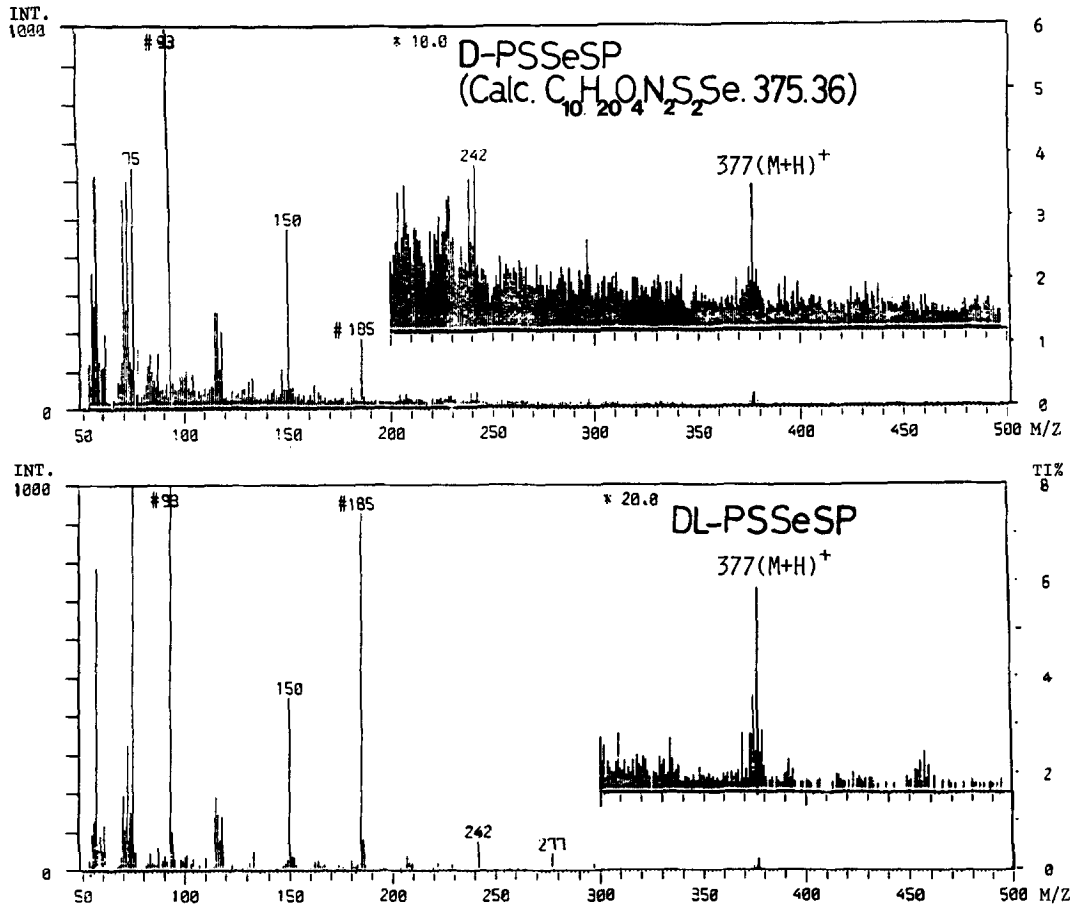

Fig. 1: Positive ion fast atom bombardment mass spectra of D-penicillamine selenotrisulfide(upper) and DL-penicillamine selenotrisulfide(lower).

However, selenotrisulfide obtained from DL-penicillamine gave two distinct signals at 580 ppm and 583 ppm. We already found that the reaction mixture of selenious acid and various thiols gave the [^{77}Se] signal in the region between 580 ppm and 730 ppm(3), whereas inorganic selenium compounds such as sodium selenate and sodium selenite and organic selenium compounds such as selenide, diselenide, and selenol gave the signals in the region above 1000 ppm and below 500 ppm, respectively(4,5). Accordingly, the signals observed at 580 ppm and 583 ppm may be assignalble to those of selenotrisulfides.

These isolated selenotrisulfides exhibited the identical UV-absorption spectra in 1N-HCl solution, and the molar extinction coefficient of an absorption maximum at 268 nm was calculated to be approximately 1800. This observation

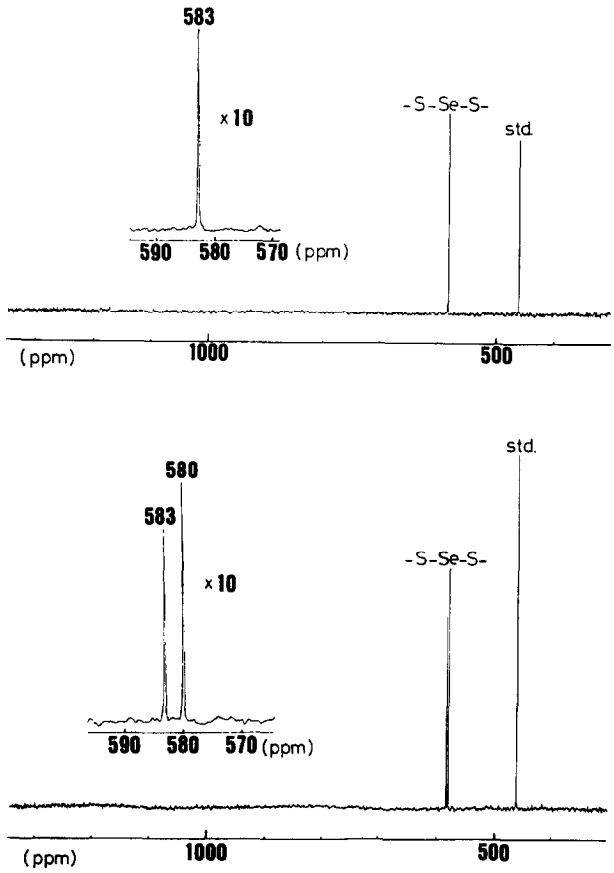

Fig. 2: [^{77}Se]NMR spectra of D-penicillamine selenotrisulfide(upper) and DL-penicillamine selenotrisulfide(lower) in D_2O at pH 1.2 by HCl.

is consistent with that in glutathione selenotrisulfide reported by Ganther (1). The absorption maximum at 268 nm, which is 10 to 20 nm higher than that of disulfides, is regarded as one of the characteristics of selenotrisulfides.

The high-performance liquid chromatographic analyses indicated that the isolated D- and L-penicillamine selenotrisulfides gave single peaks with the same retention time 12.3 min(Fig. 3), respectively, while DL-penicillamine selenotrisulfide exhibited two peaks with retention times 10.4 min and 12.3 min(Fig. 3b). The FAB-MS spectra of the fractionated eluents corresponding to these peaks were found to be consistent with that given in Fig. 1, indicating that all these peaks were attributable to penicillamine seleno-trisulfides. When the fraction of the peak at 12.3 min obtained from D-peni-cillamine selenotrisulfide was mixed with that from L-penicillamine seleno-

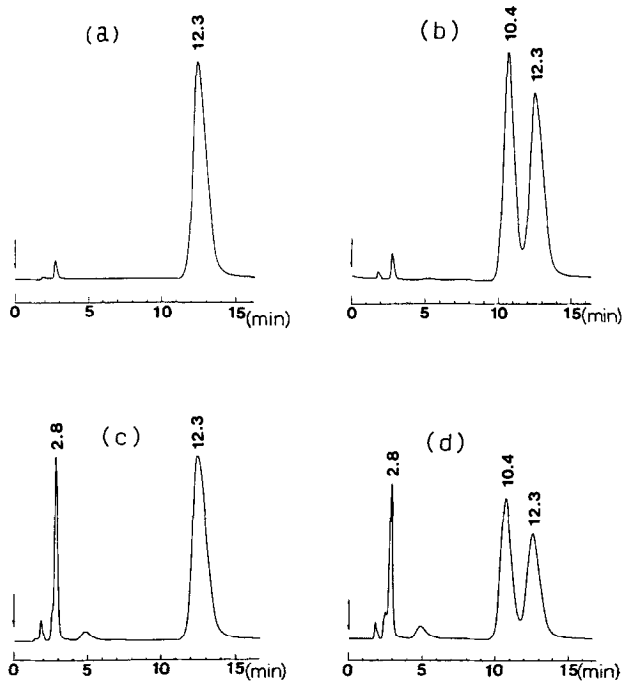

Fig. 3: High-performance liquid chromatograms of (a) D-penicillamine selenotrisulfide isolated, (b) DL-penicillamine selenotrisulfide isolated, (c) reaction mixture of D-penicillamine and selenite, and (d) reaction mixture of DL-penicillamine and selenite. Mobile phase; MeOH/H_2O=9/1(pH 4.0 by HCl), flow rate 1.0 ml/min, detection UV-210 nm.

trisulfide and rechromatographed under the same condition as in Fig. 3, we obtained two peaks at 10.4 min and 12.3 min. Furthermore, the same result was obtained when the fraction of the peak at 12.3 min from DL-penicillamine selenotrisulfide was rechromatographed, whereas the rechromatography of the fraction of the peak at 10.4 min exhibited only a single peak at 10.4 min. Fig. 3c and 3d show, respectively, the chromatograms of the reaction mixture of selenite with D- and DL-penicillamines, where the peaks corresponding to those in Fig. 3a and 3b emerged with an additional peak at 2.8 min. This peak was due to penicillamine disulfide overlapping with unreacted penicillamine.

These results, in combination with the above mentioned [^{77}Se]NMR observations, show that the HPLC peak with retention time at 12.3 min and the [^{77}Se]NMR signal at 583 ppm could be ascribed to symmetric penicillamine selenotrisulfides(DD and LL), and the peak at 10.4 min and the signal at

580 ppm to asymmetric one. Thus, it follows that the thiol exchange reaction could occur between respective selenotrisulfides formed from isomers of penicillamines. The results presented here suggest the occurrence of the thiol exchange reaction of selenotrisulfides formed from various thiols in vivo.

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