

A rapid, preparative separation of methionine sulfoximine from methionine sulfoxide

In studies to determine quantitative relationships regulating the inhibition^{1,2} and the inactivation³ of cerebral glutamine synthetase by the convulsant DL-methionine-DL-sulfoximine (MSO), it became necessary to prepare radioactive MSO free of traces of methionine sulfoxide (MSI). Since in the synthesis of MSO⁴, the final step consists in the reaction of the sulfoxide with sodium azide to yield the sulfoximine (35–45 % conversion), a major problem has been the purification of the final product. A procedure for the rapid, chromatographic separation of the two compounds on a preparative scale is described in the present note.

On the basis of the behavior of MSI and MSO on an analytical column⁵, use has been made of Dowex 50 in the ammonium form, the stepwise elution of MSI and MSO being effectively accomplished with ammonium formate buffers⁶.

Experimental

Separation of MSI and MSO. A glass column 20 × 1 cm with a teflon stopcock was loosely packed with a small amount of pyrex glass wool above the constriction to support the resin. A slurry of Dowex-50W-X8 (200–400 mesh) prepared in the ammonium form and equilibrated with 0.2 M ammonium formate, pH 3–4 in 40 % ethanol, was poured into the column to a height after compaction of 10 cm. A solution of 100 mg each of MSO and MSI (Nutritional Biochemicals, Cleveland, Ohio) in 2 ml of 2 M HCOOH (pH 1.9) was allowed to percolate into the resin. Elution was started with 0.2 M ammonium formate, pH 3.4 (40 % ethanol) in the reservoir at a level 25 cm above the resin to maintain a flow rate of 0.8 ml/min. Fractions (5 ml) were collected and starting at tube 9, the buffer in the reservoir was changed to 0.5 M ammonium formate, pH 7.0 (40 % ethanol). Separation was complete after a total of 13 fractions had been collected.

Isolation of MSO and MSI. The fractions containing MSI (tubes 3–7) and MSO (tubes 9–11) were reduced in volume to approximately 2 ml at 50° *in vacuo*. Each

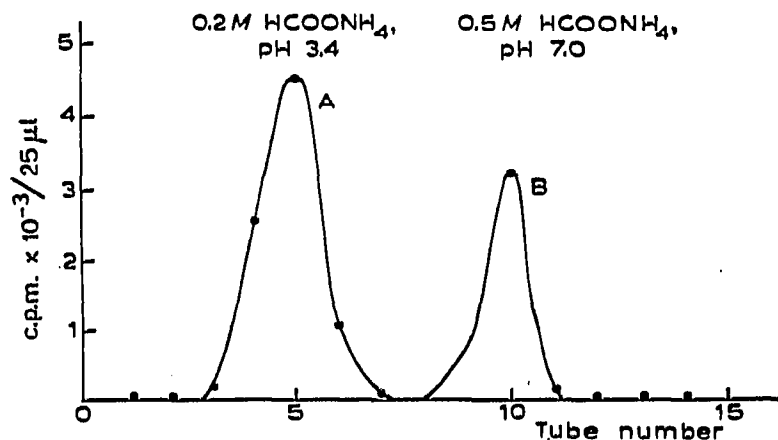


Fig. 1. Elution profile of a mixture of (A) MSI-¹⁴C and (B) MSO-¹⁴C after passage on Dowex 50W NH₄⁺ (200–400 mesh). Column: 10 × 1 cm; temperature: 25°; elution rate: 0.8 ml/min; fraction volume: 5 ml. The radioactivity was counted as described in the text.

fraction was then mixed with 40 ml absolute ethanol to precipitate the desired compound in 80 % yield for MSI and 70 % for MSO. The compounds were chromatographically pure compared to authentic samples of MSO and MSI run on Whatman No. 1 paper, descending, for 15 h in the following solvents: *tert.*-butanol-formic acid-water (70:15:15), R_F MSI = 0.45, R_F MSO = 0.35, R_F methionine = 0.51; *n*-propanol-acetic acid-water (60:15:25), R_F MSI = 0.51, R_F MSO = 0.44, R_F methionine = 0.70. Fig. 1 illustrates the separation of 1-¹⁴C-MSO from 1-¹⁴C-MSI in a typical experiment in which 200 mg (5.2×10^6 c.p.m.) of a mixture (53 % MSI, 47 % MSO) of these two compounds was applied to the column. The total weight recovery was 150 mg (75 %), 80 mg of which was in tubes 3-6 (MSI) and the remainder in tubes 8-11 (MSO). The radioactivity was determined in a Packard room temperature liquid scintillation counter (Model 2211) with counting fluid of the following composition: 300 ml ethyl cellosolve, 300 ml of dioxane, 100 ml of xylene, 7 g of 2,5-diphenyloxazole, 0.35 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene and 30 g of naphthalene.

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