Identification of the Aldehydic Metabolites

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THE METABOLISM of cyclosporine (CsA) has been previously investigated both in animals and in humans. Investigations conducted to date have revealed the presence of hydroxy, dihydroxy-*N*-demethyl, and *N*-demethyl metabolites of CsA in the bile of various animal species.[1,2] Modification of the terminal methyl group of the nine-carbon amino acid to a carboxylic acid has also been observed.[3] The isolation of the acid metabolite suggests the possible presence of an aldehyde as an intermediate in the metabolic pathway. The formation of such a metabolite could be potentially important due to the known reactivity of aldehydes toward proteins. The objective of this study is the isolation and identification of the aldehydic metabolite of CsA from human bile.

MATERIALS AND METHODS

Sample Preparation

Bile samples were obtained from adult liver transplant recipients who had a T tube in their common bile duct as part of their transplant procedure. The bile was extracted with diethyl ether, and the ether layer was evaporated under nitrogen at 50°C. The residue was purified by taking it up in aqueous methanol and washing with hexane. The extract was then dissolved in 50% acetonitrile in water for chromatography.

High-Performance Liquid Chromatography

HPLC was performed by using a 25-cm-by-10-mm, semipreparative, 5-μm C18 column (Supelco, Bellefonte, PA) that was heated to 70°C. The mobile phase consisted of a linear gradient program starting at 35% and increasing to 67% acetonitrile in water over 120 minutes with a constant flow rate of 4.0 mL/min. UV detection was performed at a 214-nm wavelength. After injection of the reconstituted bile extract, fractions of the eluent corresponding to the individual metabolite peaks were collected, and the organic solvent was removed under nitrogen at 50°C. The remaining aqueous solution was lyophilized. The residue was then subjected to fast atom bombardment (FAB) mass spectrometric analysis.

RESULTS AND DISCUSSION

An HPLC chromatogram of the metabolite mixture is depicted in Fig 1. Analysis of the metabolite peaks ALD1 and ALD2 by FAB mass spectrometry revealed two isomeric compounds with the molecular ion (M^+) at a mass-to-charge ratio (m/z) of 1,216, which corresponds to an increase of 14 atomic mass units (AMU) from CsA. This mass increase is consistent with the modification of a methyl group to an aldehyde. The M^+ ion loses 29 AMU to give a fragment ion at an m/z of 1,187. This loss can be attributed to an aldehydic CHO fragment. Furthermore, the appearance of an ion at m/z 1,090, which corresponds to a loss of 126 AMU from the M^+ ion, suggests that the aldehyde group is on the side chain of the nine-carbon amino acid no. 1 of CsA. The mass spectrum for the aldehyde metabolite of CsA is shown in Fig 2. For further confirmation of the aldehydic group, the HPLC-purified metabolites were reacted with hydroxylamine hydrochloride to form the corresponding oximes. As expected, the reaction products showed an M^+ sodium at M^+ sodium m/z of 1,254.

The presence of two isomers of this aldehydic metabolite is probably due to the open and cyclized forms of the nine-carbon amino acid (ie, CsA metabolites M17 and M18). Their mass spectra are identical, but the confirmation of these two isomers can be achieved by NMR spectroscopy. Because the aldehydic metabolites exist only in low concentrations in the bile of a patient receiving CsA therapy,

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Metabolites

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4-nm wavelength. After injection
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TS AND DISCUSSION

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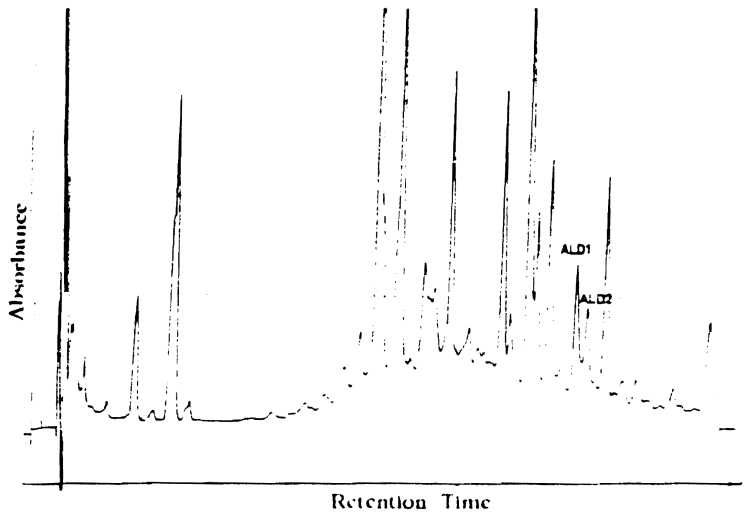

1. HPLC chromatogram of an injection of bile in which the aldehydic of CsA are desig-

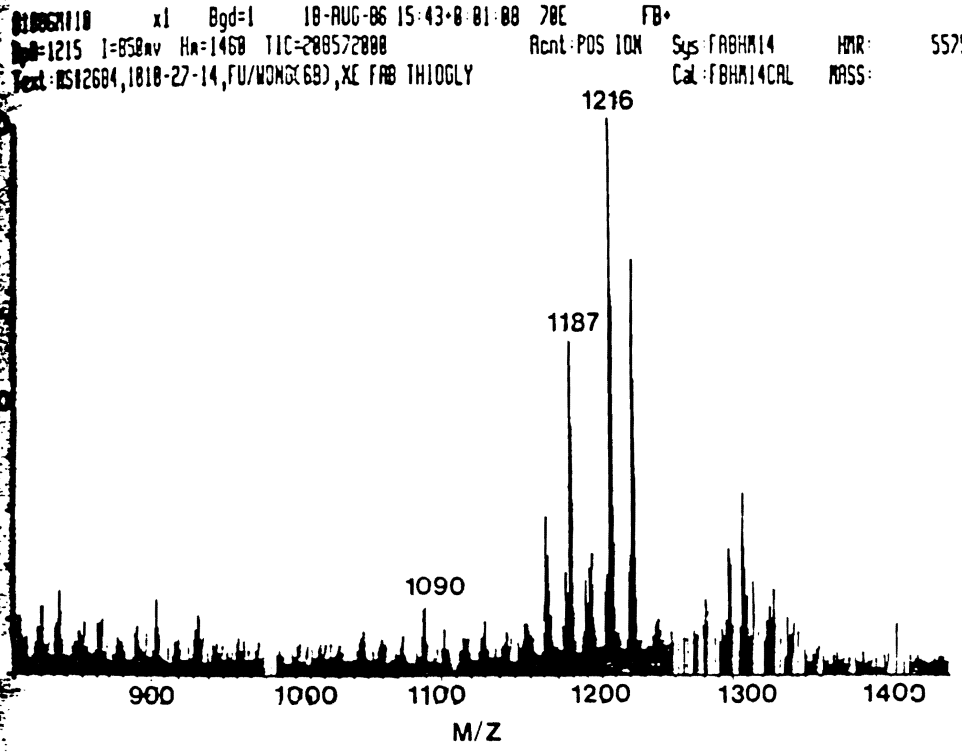

Fig. 2. Mass spectrum of an aldehydic metabolite of CsA. Both isomers have very similar patterns.

sufficient material for NMR spectroscopy has not been obtained. Synthetic samples of these aldehydic metabolites can be produced by mild oxidation of CsA and will allow both further chemical analysis and pharmacological testing for the immunosuppressive and toxic properties of these novel compounds.

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