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The first gas chromatographic resolution of carnitine enantiomers

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The enantiomers of carnitine are converted on-line in the injection port of a gas chromatograph into β-hydroxy-γ-butyrolactones and are separated on a derivatized β-cyclodextrin chiral stationary phase.

Carnitine (γ-trimethylammonium-β-hydroxybutyrate) is a zwitterionic compound formed *in vivo* from lysine and present in various animal and vegetable tissues, where it plays a fundamental role in the utilization of lipids.[1] The (*R*)-enantiomer **1** is considered a vitamin-like nutrient related to vitamins of the B-group. The (*S*)-enantiomer has considerable toxic effects due to competitive inhibition of carnitine acetyltransferase, leading to depletion of the carnitine storage.[2] Primary and secondary carnitine deficiencies are currently treated with single-enantiomer pharmaceutical preparations, and enantioselective assays are highly desirable for both bulk drug controls and biological fluid analysis. Resolution of carnitine enantiomers on a chiral stationary phase would be the method of choice in view of the high precision, accuracy and speed of analysis of modern chromatographic methodologies. However, the permanently charged trimethylammonium group and the low UV detectability[3] of carnitine pose severe obstacles to the development of simple procedures that are capable of detecting small enantiomeric impurities and that require only a minimum of sample manipulation.

Here we report on the first resolution of carnitine enantiomers by enantioselective gas chromatography on a β-cyclodextrin chiral stationary phase. Key to the new procedure is the known[4] stereoconservative conversion of **1** into (*R*)-β-hydroxy-γ-butyrolactone **2**, occurring *via* thermal intramolecular nucleophilic displacement of the trimethylammonium fragment by the carboxylate oxygen (Scheme 1). A related reaction has been exploited in the non-enantioselective GC analysis of acylcarnitines: their off-line heating in the presence of *N*,*N*-diisopropylethylamine yields the corresponding acyloxylactones that are amenable to GC-MS analysis.[5]

We have found that such reaction takes place promptly when solutions of carnitine in polar aprotic solvents are introduced in the hot GC injection port.[6] Moreover, preliminary experiments showed that preformed (*R*,*S*)-β-hydroxy-γ-butyrolactone could be baseline resolved by enantioselective GC[7] on a capillary column coated with heptakis(2,6-di-*O*-n-pentyl-3-*O*-trifluoroacetyl)-β-cyclodextrin.‡ Thus, combining the on-line derivatization procedure with enantioselective GC on a chiral phase, the polar zwitterionic, non volatile carnitine enantiomers can be separated by gas chromatography. Detection problems are easily solved since the on-line produced γ-butyrolactones have enhanced detector responses with either flame ionization (FID) or mass spectrometry (MS) compared to the poor UV detectability of underivatized samples (Fig. 1). MS detection in selected ion monitoring (SIM) mode has the added advantage of positive peak identification when trace amounts of the enantiomers are present in complex mixtures (Fig. 2).

The order of elution of the enantiomers from the derivatized β-cyclodextrin column is (*S*) before (*R*), as determined by

Scheme 1 *Solvents and conditions*. Off-line: DMSO or DMF, 150 °C, 2 h. On-line: CH_3NO_2, GC injection port at 200 °C.

† Deceased on 24 June 2001.

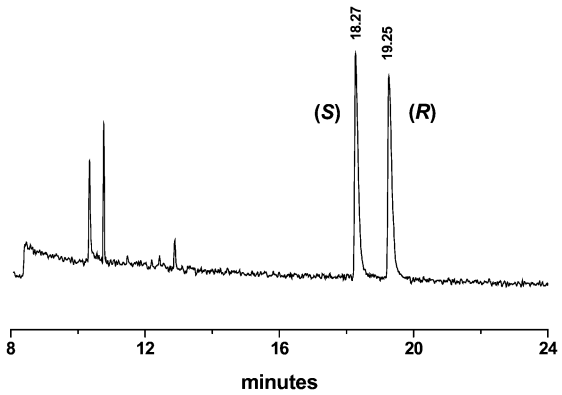

Fig. 1 Enantioselective GC of (*R*,*S*)-carnitine. Sample injected as 1 mg mL^{-1} solution in CH_3NO_2. Detection by MS (total ion current).

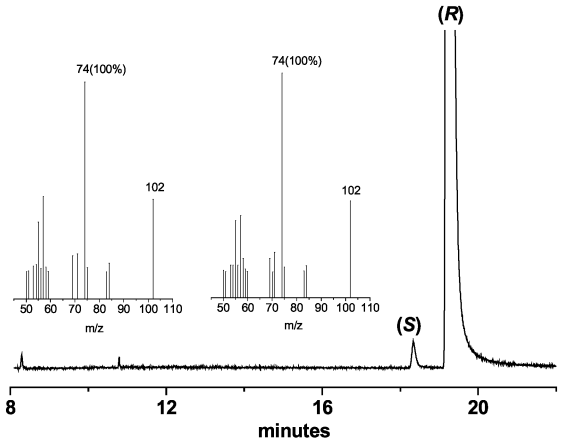

Fig. 2 Enantiomeric trace analysis of **1** containing 1% *ent*-**1** by GC-MS with SIM (*m*/*z* 74 + 102). EI-MS spectra at 18.27 and 19.25 min.

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chromatography of preformed γ-lactones of known configuration.[8,9] We have examined the occurrence of potential loss of stereochemical integrity during the ring closure reaction, by injecting samples of either **1** or *ent*-**1** of known§ enantiomeric excess (e.e.) and measuring the e.e.s of the corresponding γ-lactones by digital integration of peak area. With samples dissolved in CH_3NO_2 and the injection port kept at 200 °C we found a linear correlation with unitary slope between the two e.e. values, indicating that the on-line derivatization occurs with less than 0.05% inversion at the stereogenic center. Moreover, potential competitive side reactions in the hot injection port (*e.g.* β-elimination leading to the α,β-unsaturated 2(5*H*)furanone) should take place at the same rate for the two enantiomers and thus should leave the original e.e. unaffected. Both flame ionization (FID) and mass spectrometry (MS) detectors respond linearly over at least four-orders of magnitude, thus enabling the precise determination of enantiomeric excess up to e.e. = 99.95%. However, with solvents other than CH_3NO_2 or with higher injection port temperatures the stereochemical outcome reveals a sizeable inversion (up to 1% in DMSO and Tinj = 350 °C).

Carnitine samples that are present in forms other than the inner salt, like carnitine tartrate or chloride can be analyzed in the same way with a minimum of sample pretreatment. Thus, shaking a CH_3NO_2 solution of a carnitine salt with an anion-exchanging liquid resin[11] converts the carnitine into its inner salt that is ready for GC processing.

In conclusion we have demonstrated that carnitine samples can be enantioselectively analyzed by GC on a chiral stationary phase. The stereoconservative thermal cyclization of carnitine and the favourable gas chromatographic properties of the obtained γ-lactones form the basis of a simple GC method for enantiomeric profiling of carnitine samples. The new method drastically abbreviates sample preparation time and is highly precise, sensitive and specific.

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Notes and references

‡ Chiraldex B-TA, Astec Whippany, N.J., USA. 30 m × 0.25 mm I.D. fused-silica capillary column. Carrier gas helium at 2 mL min^{-1}, split injection with 1 : 5 and 1 : 20 ratios for FID and positive ion EI-MS detectors, respectively; injection port T = 200 °C, FID T = 350 °C. Temperature program: 100 °C isotherm for 5 min, to 160 °C at 10 °C min^{-1}, 160 °C isotherm for 15 min.

§ Enantiomeric compositions of enriched samples of carnitine were independently measured by enantioselective HPLC.[10]

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11 Amberlite LA-2 type resin (Fluka catalog) is a secondary amine liquid resin with an ion exchange capacity of 2.2-2.3 meq mL^{-1}. It is soluble in hexane and the resulting solution is immiscible with CH_3NO_2. In a typical procedure, a 1 mg mL^{-1} solution of carnitine salt is shaken with 0.5 mL of LA-2 resin diluted with 0.5 mL of n-hexane. After 5 minutes, the lower phase is directly sampled and introduced in the GC port.