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Note

Use of O-((—)-menthyl)-N,N′-diisopropylisourea for the preparation of diastereomeric menthyl esters for the chromatographic resolution of enantiomeric carboxylic acids

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One of the techniques commonly employed in the separation of enantiomers involves the preparation of diastereomeric derivatives using an optically pure chiral derivatizing reagent. The diastereomers are then separated by various chromatographic means, and the resulting elution patterns reflect the nature of the underivatized material. The purpose of the study presented here was to examine the usefulness of O-((—)-menthyl)-N,N′-diisopropylisourea as a reagent for preparing (—)-menthyl esters of enantiomeric carboxylic acids.

Previously used methods for the preparation of menthyl esters include acid catalyzed esterification using (—)-menthol [1]. This method requires the use of dry hydrogen chloride gas and a very large excess of (—)-menthol. Menthyl esters have also been prepared by first converting the acid to the acid chloride by refluxing with thionyl chloride [2] or oxalyl chloride [3], followed by treatment of the acid chloride with (—)-menthol. While all of these methods produce the desired end-product, namely diastereomeric (—)-menthyl esters of enantiomeric acids, the derivatization procedures themselves are somewhat involved and require considerable care in the maintenance of anhydrous conditions. Among the proposed advantages of O-((—)-menthyl-N,N′-diisopropylisourea ((—)-MDI) as a chiral derivatizing reagent are the simple techniques involved with its use, the completeness of its reaction with carboxylic acids, and the lack of potential racemization during derivatization.

(—)-MDI is an adduct of (—)-menthol and N,N′-diisopropylcarbodiimide:

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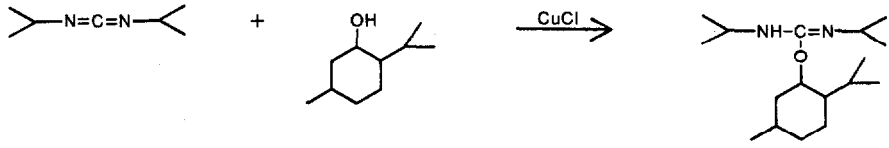
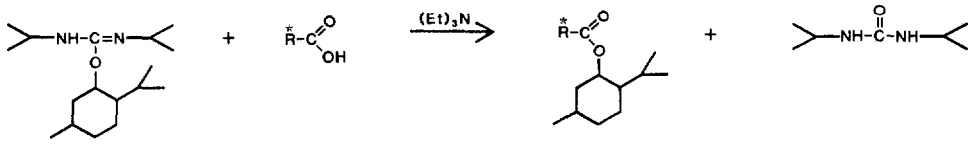


N,N'-diisopropylcarbodiimide (-)-menthol O-((-)-menthyl)-N,N'-diisopropylisourea ((-)-MDI)

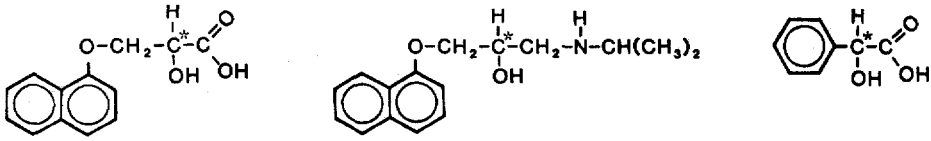
This reaction is catalyzed by monovalent copper. The derivatization reaction produces the two diastereomeric esters and N,N'-diisopropylurea:



(-)-MDI (±) Racemic acid Diastereomeric esters N,N'-diisopropylurea

Tertiary amines catalyze the derivatization; triethylamine was used as the catalyst in these studies.

Naphthoxylactic acid (2-hydroxy-3-(1-naphthoxy)propanoic acid; NLA), a major urinary metabolite of the β-adrenergic antagonist propranolol, and mandelic acid were used as test samples for the (—)-MDI derivatization reagent.



Naphthoxylactic acid (NLA) Propranolol Mandelic acid

Both of these acids have one chiral center. The diastereomeric derivatives were analyzed by capillary gas chromatography (GC) and capillary gas chromatography—mass spectrometry (GC—MS).

MATERIALS AND METHODS

Synthesis of O-((—)-menthyl)-N,N'-diisopropylisourea

(—)-Menthol (7.8 g, 0.050 mol; Mallinckrodt, New York, NY, U.S.A.) and N,N'-diisopropylcarbodiimide (6.3 g, 0.050 mol; Aldrich, Milwaukee, WI, U.S.A.) were dissolved in 25 ml tetrahydrofuran (Aldrich). Copper(I) chloride (10 mg; Fisher, Fair Lawn, NJ, U.S.A.) was added, and the mixture was stirred at room temperature overnight. The mixture was then evaporated in vacuo to 8 ml and chromatographed on a 250-g Al_2O_3 (W200 Basic; Woelm, Eschwege, G.F.R.) column eluting with 250 ml tetrahydrofuran. The tetrahy-

drofuran was removed by evaporation in vacuo, yielding 5.86 g of (−)-MDI (41.6% of theoretical) as a pale yellow liquid. No appreciable decomposition of (−)-MDI was detected four years after synthesis.

Other reagents

(±)-Naphthoxylactic acid was prepared in these laboratories by a procedure analogous to that reported by Nelson and Bartols [4]. Racemic mandelic acid was obtained from Chem Service (Westchester, PA, U.S.A.); (−)-mandelic acid and (+)-mandelic acid were obtained from Aldrich, triethylamine from Eastman Kodak (Rochester, NY, U.S.A.), and ethyl acetate from Fisher. All solvents were ACS reagent grade or better. Ethereal diazomethane was generated from Diazald® (Aldrich).

Equipment

Capillary GC analyses were performed using a Varian 3700 gas chromatograph equipped with a split injector and a flame ionization detector. The column was a Grade A 60 m × 0.25 mm I.D. SP-2100 wall-coated open tubular (WCOT) glass capillary obtained from J. & W. Scientific (Orangeville, CA, U.S.A.). The straightened capillary ends were deactivated with Carbowax 20M.

Capillary GC-MS analyses were performed using the same gas chromatograph and column interfaced through an open-split interface to a Finnigan MAT-212 mass spectrometer equipped with a Spectrosystem SS200 data system.

Derivatization of enantiomeric acids

A 1-mg amount of each sample acid was dissolved in 400 μl of a solution of tetrahydrofuran—triethylamine (9:1, v/v); 10 μl (−)-MDI were added, and the reaction mixture was capped tightly in a vial and heated at 100°C for 16 h. The completeness of reaction was monitored by forming the methyl ester of any unreacted acid by derivatization with diazomethane in diethyl ether followed by capillary GC analysis. No methyl ester peaks were observed after 16 h heating with triethylamine as a catalyst.

Capillary GC—MS resolution of diastereomers

The diastereomeric menthyl naphthoxylactates were resolved under the following conditions: injector temperature 260°C; split ratio 1:350; column temperature programmed from 225°C to 240°C at a rate of 0.2°C/min; average linear velocity 21—22 cm/sec; open-split interface temperature 260°C; line-of-sight temperature 250°C; ion source temperature 240°C; ionization energy 70 eV. The conditions for the resolution of the diastereomeric menthyl mandelates were identical except that the injector temperature was 200°C and the column temperature was programmed from 165°C to 180°C at 0.3°C/min. Both analyses were complete before the final column temperature was reached. During both analyses the mass spectrometer was scanned from 45 to 400 a.m.u. at a rate of 3 sec/decade with an interscan time of 1 sec.

Determination of elution order

Menthyl mandelates. *R*-(−)-Mandelic acid and *S*-(+)-mandelic acid were

separately derivatized*. Aliquots of the two solutions were mixed in a 4:1 (v/v) ratio (*S*:*R* and *R*:*S*) and analyzed by capillary GC. Elution order was determined based upon the retention times of the separately derivatized *R* and *S* standards and the relative peak intensities of the two mixtures.

Menthyl naphthoxylactates. Because of the unavailability of optically pure *R*- and *S*-naphthoxylactic acid, and because NLA is a major urinary metabolite of propranolol, *R*-naphthoxylactic acid (R-NLA) was extracted from the urine of a rat dosed with S-(−)-propranolol. The sample was a 24-h urine collection from a male Sprague—Dawley rat injected intraperitoneally with 10 mg/kg of *S*-(−)-propranolol·HCl (Ayerst Labs., New York, NY, U.S.A.). The total urine volume was 14 ml. A 2-ml aliquot was adjusted to pH 1.5 with 6 *N* hydrochloric acid and extracted with 2 × 5 ml ethyl acetate. The combined organic phases were evaporated to dryness under a stream of nitrogen; the residue was derivatized using 90 μl tetrahydrofuran—triethylamine (9:1) and 10 μl (−)-MDI. A 6-μl aliquot of this solution was mixed with 6 μl of previously derivatized racemic NLA of comparable concentration. The resulting mixture was analyzed by selected ion monitoring (*m/z* 370, the molecular ion) capillary GC—MS. The elution order was determined by comparing the relative peak intensities of derivatized racemic NLA with and without the added derivatized urinary R-NLA.

RESULTS

(−)-MDI was found to react to completion with carboxylic acids to form menthyl esters after 16 h heating in tetrahydrofuran with triethylamine as a catalyst.

The retention behavior of the diastereomeric menthyl mandelates and menthyl naphthoxylactates from capillary GC—MS analysis is presented in

TABLE I

RETENTION BEHAVIOR OF THE DIASTEREOMERIC MENTHYL NAPHTHOXYLACTATES AND MENTHYL MANDELATES ON AN SP-2100 WCOT CAPILLARY COLUMN

Analysis	Column temperature	Adjusted retention time*, t'_R (min, ± 0.1)	Carboxylic acid enantiomer
Menthyl naphthoxylactates	225—240°C at 0.2°C/min	51.2	*R*-(−)
		53.7	*S*-(+)**
Menthyl mandelates	165—180°C at 0.3°C/min	40.5	*R*-(−)
		42.6	*S*-(+)***

*The elution time of a non-retained compound (butane) was subtracted from the absolute retention times to obtain the adjusted retention times.
**Determined from the data presented in Table II.
***Determined from chromatograms of derivatized optically pure mandelic acid standards.

*In the Cahn—Ingold—Prelog system of chiral notation, used here for the purposes of consistency, D-(−)-mandelic acid has the *R* configuration.

Table I. The retention times presented are adjusted for the void volume of the system. Exemplary chromatograms from the two analyses are presented in Figs. 1 and 2. Fig. 1 is a retrospective single ion plot of the molecular ion (*m/z* 370) of menthyl naphthoxylactate. No molecular ion was observed for the menthyl mandelates; the plot presented in Fig. 2 is a retrospective plot of *m/z* 107, corresponding to the $[(C_6H_5)C(OH)H]+$ fragment of menthyl mandelate. Both sets of diastereomers were resolvable on an SP-2100 WCOT capillary column with slight peak overlap. Baseline separation was obtainable under isothermal column conditions (225°C for the menthyl naphthoxylactates and 170°C for the menthyl mandelates) with some sacrifice in analysis time and peak broadening.

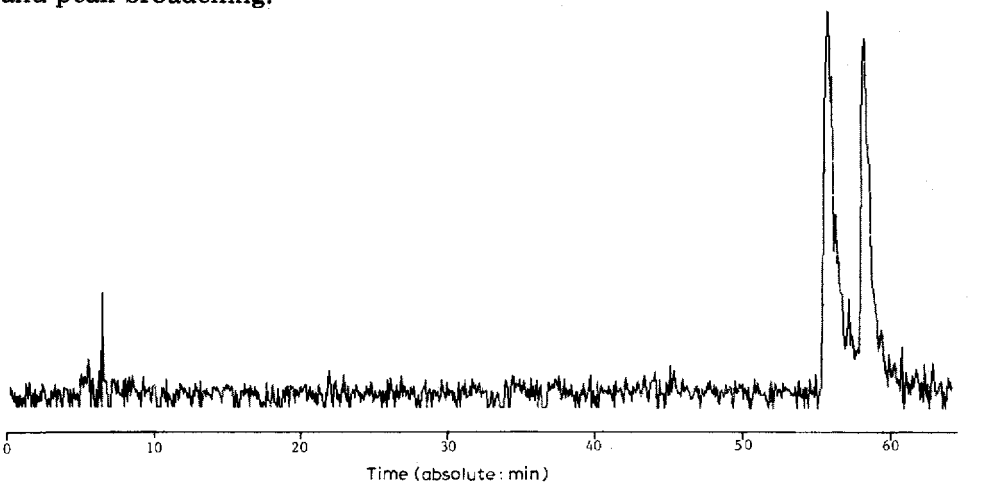


Fig. 1. Capillary GC—MS chromatogram of the diastereomeric menthyl naphthoxylactates. A 3-μl sample was injected (7—8 μg). The chromatogram is a restrospective single-ion plot of the molecular ion (*m/z* 370). See text for GC—MS parameters.

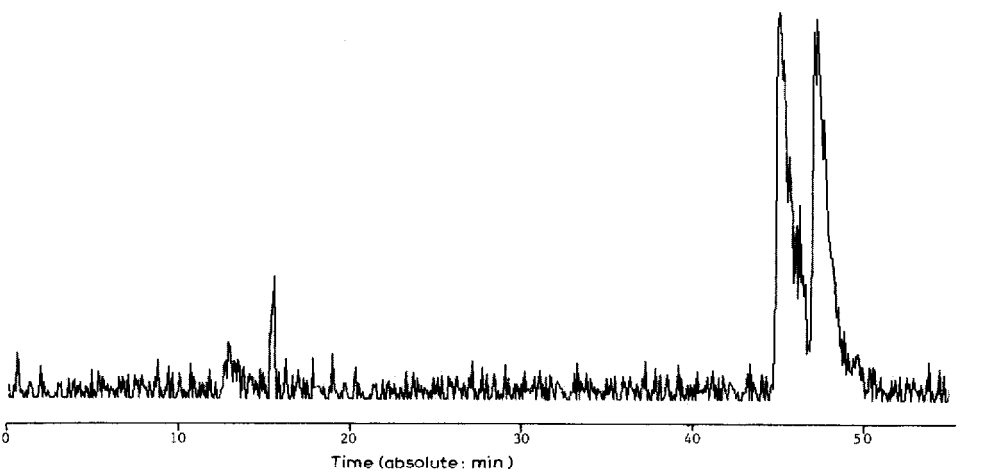


Fig. 2. Capillary GC—MS chromatogram of the diastereomeric menthyl mandelates, as a retrospective single-ion plot of the $[(C_6H_5)C(OH)H]^+$ fragment (*m/z* 107) of menthyl mandelate. The sample size was 3 μl (7—8 μg). For GC—MS conditions, see text.

The elution order of the menthyl mandelates was determined by comparing the retention times of the separately derivatized optically pure mandelic acid enantiomers, and by comparing the relative peak intensities of 4:1 (v/v) mixtures of the separately derivatized enantiomers. Under the conditions of the analysis, (—)-menthyl-*R*-(—)-mandelate eluted with a peak maximum at 40.5 min (adjusted retention time), and (—)-menthyl-*S*-(+)-mandelate eluted at 42.6 min. These retention times were reproducible to within ± 0.1 min. The relative peak intensities of the two 4:1 mixtures were consistent with the above elution order.

In order to determine the order of elution of the menthyl naphthoxylactates, *R*-naphthoxylactic acid (R-NLA) was extracted from the urine of a rat dosed with *S*-(—)-propranolol. The shift in the notation of the absolute configuration derives from the shift in priorities in the Cahn—Ingold—Prelog system of notation. It is assumed that the chiral center of the β-blocking side chain of propranolol remains unaltered during metabolism to NLA. The extracted R-NLA was derivatized and mixed with derivatized racemic NLA. Table II presents the relative peak intensities of this mixture and those of derivatized racemic NLA alone [from selected ion monitoring (*m/z* 370) capillary GC—MS]. From the results of the work of Nelson and Bartols [4], R-NLA is the negative rotating isomer. (—)-Menthyl-*R*-(—)-naphthoxylactate was found to elute before (—)-menthyl-*S*-(+)-naphthoxylactate on an SP-2100 column.

TABLE II

DETERMINATION OF THE ORDER OF ELUTION OF THE DIASTEREOMERIC MENTHYL NAPHTHOXYLACTATES FROM SELECTED-ION MONITORING (*m/z* 370) CAPILLARY GC—MS

Derivatized sample	Adjusted retention time, t'_R (min, ± 0.1)	Relative peak intensity
Racemic NLA	51.2	0.95
	53.7	1.00
Urinary R-NLA and racemic NLA	51.2	1.50
	53.7	1.00

DISCUSSION

O-((—)-Menthyl)-N,N′-diisopropylisourea has been synthesized and found to be useful for the preparation of diastereomeric (—)-menthyl esters of enantiomeric acids. (—)-Menthyl esters (from (—)-menthol) have been used in the past in the diastereomeric separation of lactic and glyceric acids [1], acyclic isoprenoid acids [2], phytanic [5] and pristanic [6] acids, and chrysanthemic acids [7]. The techniques involved with the use of (—)-MDI are relatively simple, with the minor disadvantage of the requirement of overnight heating in order to effect completeness of reaction. While this study has

demonstrated the applicability of (−)-MDI to analytical samples, it could be useful on a preparative scale as well. For instance, a large quantity of a racemic acid could be treated with (−)-MDI, and the resulting diastereomers resolved by preparative high-performance liquid chromatography. The separate esters could then be hydrolyzed and the optically pure enantiomers isolated. Such a procedure may offer advantages over repeated fractional crystallization in terms of yield, purity, or both.

The determination of the order of elution of the menthyl mandelates was straightforward due to the availability of optically pure standards and the extremely reproducible retention times obtainable from capillary GC techniques. While the determination of the elution order of the menthyl naphthoxylactates was indirect, it afforded a further demonstration of the usefulness of (−)-MDI in its application to the concentration levels of samples obtained from biological fluids. (−)-MDI thus has a wide range of potential applications as an analytical tool, and could be useful for preparative work as well.

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