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Note

Gas chromatographic analyses of diastereomeric lactamides

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The enantiomers of lactic acid derivatives have been separated[1–3] by gas chromatography (GC) using chiral stationary phases. The diastereomers of lactic acid esters have also been separated by GC using achiral stationary phases (SE-30, SP-1000), by protecting the hydroxyl groups using trimethylsilyl (TMS)[4], trifluoroacetyl (TFA)[4] or acetyl groups[5]. However, no examples were reported of the separation of diastereomeric lactic acid derivatives (lactamides or lactic acid esters) whose hydroxyl groups are free by using a packed column with an achiral stationary phase.

In this paper, the GC separation of diastereomeric lactamides by the use of a packed column filled with a silica support coated with an achiral stationary phase (SE-52) is reported. The diastereomeric lactamides in which the hydroxyl groups were not protected have not been separated by the use of a packed column with SE-52 as a stationary phase. However, it was found that the diastereomeric lactamides were clearly separated when the samples were injected immediately after injection of a pyridine solution of N-trimethylsilylimidazole (TMS-Im).

EXPERIMENTAL

Gas chromatography

A Hitachi 163 gas chromatograph equipped with a flame-ionization detector was used. A stainless-steel column (4 m × 3 mm I.D.) was filled with Chromosorb W AW DMCS, which was coated with SE-52 silicone gum (5%, 80–100 mesh). The temperature of the injection port was 300°C. The oven temperature was increased at the rate of 2°C/min from 100 to 250°C. The flow-rate of the carrier gas (nitrogen) was 39 ml/min. A chloroform solution of the sample was applied 10 sec after the injection of 3 μl of a pyridine solution of TMS-Im (TMS-Im–pyridine = 2:1).

Materials

N-[(S)-Lactoyl]-(S)-α-methylbenzylamine (Ia). To a cooled solution of (*S*)-lactic acid (0.011 mol), (*S*)-α-methylbenzylamine (0.011 mol) and N-hydroxysuccinimide (0.013 mol) in ethyl acetate (20 ml), dicyclohexylcarbodiimide (0.012 mol) was added. After the usual work-up, an oily product was obtained. This crude oil was purified by the use of preparative silica gel thin-layer chromatography [developing solvent: chloroform–ethanol (19:1)]. Yield: 34% (oil). $[\alpha]_D^{25} = -116°$ ($c = 1.4$, chloro-

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form). Analysis: Calculated for $C_{11}N_{15}NO_2$, C68.36, H7.82, N7.24%; found, C67.47, H7.82, N7.53%.

The following five lactamides were prepared in a similar manner.

N-[(S)-Lactoyl]-(R)-α-methylbenzylamine (Ib). Yield: 18% (oil). $[\alpha]_D^{25} = +82°$ ($c = 1.4$, chloroform). Analysis: calculated for $C_{11}H_{15}NO_2$, C 68.36, H 7.82, N 7.24%; found, C 67.64, H 7.88, N 7.30%.

N-[(S)-Lactoyl]-(S)-α-ethylbenzylamine (IIa). Yield: 27% (oil). $[\alpha]_D^{16} = -121°$ ($c = 1.4$, chloroform). Analysis: calculated for $C_{12}H_{17}NO_2$, C 69.53, H 8.26, N 6.75%; found, C 68.81, H 8.24, N 6.90%.

N-[(S)-Lactoyl]-(R)-α-ethylbenzylamine (IIb). Yield: 43% (oil). $[\alpha]_D^{24} = +109°$ ($c = 1.4$, chloroform). Analysis: calculated for $C_{12}H_{17}NO_2$: C 69.53, H 8.26, N 6.75%; found, C 68.82, H 8.33, N 6.76%.

N-[(S)-Lactoyl]-(S)-α-naphthylethylamine (IIIa). Yield: 10%. M.p.: 104–105°C. $[\alpha]_D^{24} = -76°$ ($c = 1.4$, chloroform). Analysis: calculated for $C_{15}H_{15}NO_2$, C 74.04, H 7.04, N 5.75%; found, C 73.52, H 7.00, N 5.68%.

N-[(S)-Lactoyl]-(S)-alanine isobutyl ester (IVa). To a cooled mixture of (*S*)-$+32°$ ($c = 1.1$, chloroform). Analysis: calculated for $C_{15}H_{15}NO_2$, C 74.04, H 7.04, N 5.75%; found, C 73.13, H 7.02, N 5.63%.

N-[(S)-Lactoyl]-(S)-alanine isobutyl ester (IVa). To a cooled mixture of (*S*)-lactic acid (0.011 mol), (*S*)-alanine isobutyl ester (0.011 mol) and N-hydroxysuccinimide (0.013 mol) in ethyl acetate (20 ml), dicyclohexylcarbodiimide (0.012 mol) was added. After the usual work-up, an oily product was obtained. This crude oil was purified by silica gel column chromatography [developing solvent: ethyl acetate–benzene (1:1)]. Yield: 19% (oil). $[\alpha]_D^{26} = -21°$ ($c = 1.4$, chloroform). Analysis: calculated for $C_{10}H_{19}NO_4$, C 55.28, H 8.81, N 6.44%; found, C 55.18, H 8.82, N 6.71%.

The following five lactamides were prepared in a similar method.

N-[(S)-Lactoyl]-(R)-alanine isobutyl ester (IVb). Yield: 13%. M.p.: 63–64°C. $[\alpha]_D^{26} = -0.75°$ ($c = 1.4$, chloroform). Analysis: calculated for $C_{10}H_{19}NO_4$: C 55.28, H 8.81, N 6.44%; found, C 55.72, H 8.80, N 6.36%.

N-[(S)-Lactoyl]-(S)-valine isobutyl ester (Va). Yield: 64% (oil). $[\alpha]_D^{26} = -1.8°$ ($c = 1.4$, chloroform). Analysis: calculated for $C_{12}H_{23}NO_4$, C 58.75, H 9.45, N 5.70%; found, C 58.50, H 9.40, N 5.77%.

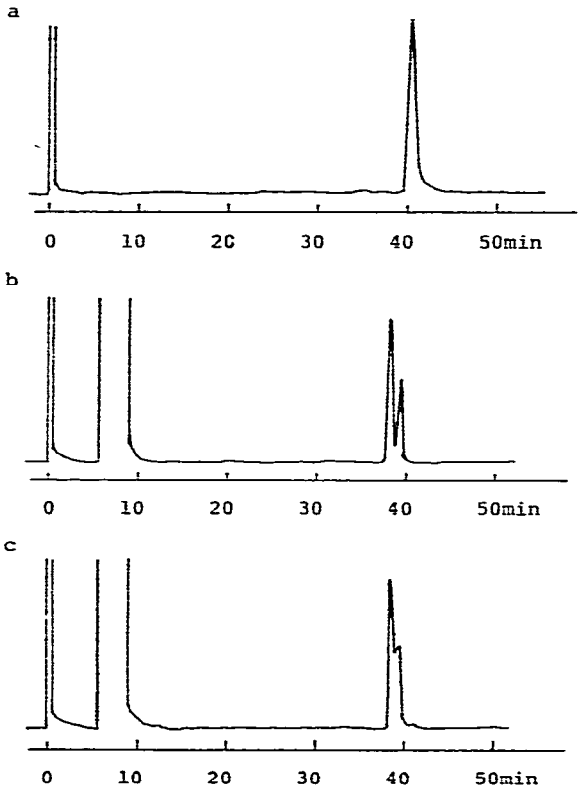
N-[(S)-Lactoyl]-(R)-valine isobutyl ester (Vb). Yield: 49% (oil). $[\alpha]_D^{26} = -10°$ ($c = 1.6$, chloroform). Analysis: calculated for $C_{12}H_{23}NO_4$, C 58.75, H 9.45, N 5.70%; found, C 58.50, H 9.46, N 5.87%.

N-[(S)-Lactoyl]-(S)-leucine isobutyl ester (VIa). Yield: 61% (oil). $[\alpha]_D^{26} = -14°$ ($c = 1.4$, chloroform). Analysis: calculated for $C_{13}H_{25}NO_4$, C 60.20, H 9.70, N 5.40; found, C 59.82, H 9.51, N 5.69%.

N-[(S)-Lactoyl]-(R)-leucine isobutyl ester (VIb). Yield: 65% (oil). $[\alpha]_D^{26} = +4.5°$ ($c = 1.4$, chloroform). Analysis: calculated for $C_{13}H_{25}NO_4$, C 60.20, H 9.70, N 5.40%; found, C 60.04, H 9.69, N 5.56%.

RESULTS AND DISCUSSION

The usual GC analysis of all diastereomeric pairs of the above prepared lactamides by using an SE-52 packed column gave a single peak on the chromatogram (Fig. 1a). However, when the application of the samples was performed immediately



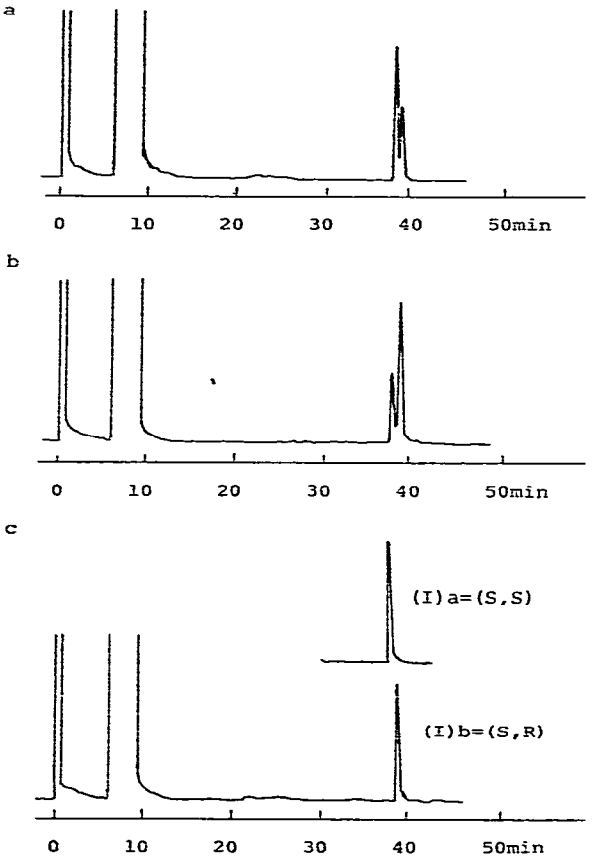


Fig. 1. Chromatograms of a mixture of diastereomeric lactamides Ia and Ib. (a) Without TMS-Im; (b) sample applied 10 sec after injection of TMS-Im; (c) sample applied 30 sec after injection of TMS-Im.

Fig. 2. Chromatograms of mixtures of diastereomeric lactamides Ia and Ib, and authentic diastereomers Ia and Ib. (a) Ia and Ib [a-rich, a = (*S*,*S*)]; (b) Ia and Ib [b-rich, b = (*S*,*R*)]; (c) authentic diastereomers.

after the injection of the pyridine solution of TMS-Im, the two diastereomers of each lactamide were well separated on the chromatogram and the tailing of the peaks was minimized. However, when the sample was injected more than 30 sec after injection of the TMS-Im solution, the two diastereomers were not separated (Fig. 1c). In this study all samples were injected 10 sec after the injection of TMS-Im solution (Fig. 1b).

In the GC analyses with TMS-Im, the identification of the peaks was carried out by using an authentic diastereomeric mixture in which the amount of one diastereomer was greater than the other. Fig. 2a shows a chromatogram of an (*S*,*S*)-lactamide-rich mixture and Fig. 2b shows a chromatogram of an (*S*,*R*)-lactamide-rich mixture. The chromatograms of the two authentic diastereomers in the analyses with TMS-Im are shown in Fig. 2c. Each of the two diastereomers emerged as a single peak. The retention times of the chromatographic peaks and the analytical conditions are given in Table I. It was found that the first peak was due to (*S*,*S*)-lactamide and

TABLE I

SEPARATION OF DIASTEREOMERIC LACTAMIDES BY GC WITH TMS-Im

Conditions: stainless-steel column (4 m × 3 mm I.D.) with 80–100 mesh Chromosorb W AW DMCS coated with 5% SE-52; carrier gas, nitrogen at a flow-rate of 39 ml/min; oven temperature, programmed from 100 to 250°C at 2°C/min.

Lactamide	*Retention time (min)*			*Separation factor**
	Without TMS-Im	*With TMS-Im*		
		First peak	*Second peak*	
Ia, Ib	40.5	39.0(*S,S*)**	39.9(*S,R*)***	1.023
IIa, IIb	43.6	42.0(*S,S*)	42.9(*S,R*)	1.021
IIIa, IIIb	68.0	64.4(*S,S*)	66.0(*S,R*)	1.024
IVa, IVb	38.9	37.5(*S,S*)	38.3(*S,R*)	1.021
Va, Vb	46.0	43.7(*S,S*)	45.2(*S,R*)	1.034
VIa, VIb	48.4	46.7(*S,S*)	47.5(*S,R*)	1.017

* Separation factor $= \frac{\text{retention time of second peak}}{\text{retention time of first peak}}$.

** a = (*S,S*): N-[(*S*)-lactoyl]-(*S*)-amine or -amino acid ester.

*** b = (*S,R*): N-[(*S*)-lactoyl]-(*R*)-amine or -amino acid ester.

the second peak to (*S,R*)-lactamide for all of the lactamides studied. The retention time of the peak analysed with TMS-Im was slightly higher than that of the peak without TMS-Im for all of the lactamides.

It seems that TMS-Im makes the stationary phase temporarily inert, and the adsorption of the hydroxyl and amide groups on the stationary phase weak. Thus it could be considered that the TMS-Im works as a temporary column conditioner, and the individual lactamides were separated clearly without tailing on the chromatogram. This method is simple and should be applicable to many other diastereomeric samples that are difficult to separate by the usual GC techniques.

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