of the products precipitated in the reaction mixture. In the run with benzenethiol, the product was isolated as free base from the lower layer liberated from the reaction solution. In the other runs, the products were obtained by concentration of the reaction solution. Yields of the products are given in Table II. Melting points, analytical and NMR spectral data are recorded in Table IV.

As shown in the following examples, treatment of IV with aqueous $KHCO_3$ gave N,N-bis(alkylthiomethyl)hydroxylamines.

N,N-Bis(benzylthiomethyl)hydroxylamine: liquid; NMR (in DMSO-d_6) δ: 3.84 (4H, s, CCH_2S), 3.99 (4H, s, SCH_2N), 7.28 (10H, s, aromatic protons). *Anal.* Calcd. for $C_{16}H_{19}NOS_2$: C, 62.90; H, 6.28; N, 4.59. Found: C, 62.74; H, 6.22; N, 4.37.

Tetrahydro-3-hydroxy-2H-1,5,3-dithiazepine: prisms from $CHCl_3$, mp 94—95°. NMR (in DMSO-d_6) δ: 2.98 (4H, s, CH_2CH_2), 4.36 (4H, s, SCH_2N). *Anal.* Calcd. for $C_4H_9NOS_2$: C, 31.76; H, 6.01; N, 9.26. Found: C, 31.47; H, 6.00; N, 9.08.

As shown in the following examples, refluxing a chloroform solution of N,N-bis(alkylthiomethyl)hydroxylamine and acetic anhydride gave O-acetyl-N,N-bis(alkylthiomethyl)hydroxylamine.

O-Acetyl-N,N-bis(phenylthiomethyl)hydroxylamine: prisms from *n*-hexane, mp 40—42°. IR ν_{COO}^{KBr} cm^{-1}: 1754, 1210. NMR (in $CDCl_3$) δ: 1.68 (3H, s, CH_3CO), 4.61 (4H, s, SCH_2N), 7.15—7.58 (10H, m, aromatic protons). *Anal.* Calcd. for $C_{16}H_{17}NO_2S_2$: C, 60.16; H, 5.37; N, 4.39. Found: C, 60.10; H, 5.36; N, 4.38.

3-Acetoxytetrahydro-2H-1,5,3-dithiazepine: needles from AcOEt, mp 137—139°. IR ν_{COO}^{KBr} cm^{-1}: 1755, 1200. NMR (in DMSO-d_6) δ: 1.99 (3H, s, CH_3CO), 2.98 (4H, s, CH_2CH_2), 4.51 (4H, s, SCH_2N). *Anal.* Calcd. for $C_6H_{11}NO_2S_2$: C, 37.28; H, 5.74; N, 7.25. Found: C, 37.28; H, 5.76; N, 7.19.

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Determination of Optical Isomers of Clinofibrate by High-Performance Liquid Chromatography

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A method for separation and determination of optical isomers of 2,2′-(4,4′-cyclohexylidenediphenoxy)-2,2′-dimethyldibutyric acid (clinofibrate) by high-performance liquid chromatography was developed. *meso*, *d*- and *l*-isomers of clinofibrate were derivatized with D-(+)-α-methylbenzylamine to the corresponding diastereomer amides, which were separated from one another by high-performance liquid chromatography. Each isomer in clinofibrate was determined. The chromatographic conditions were as follows: column, stainless-steel two connecting columns (150 mm in length and 4 mm in i.d.) packed with LiChrosorb SI-60 (5 μm); mobile phase, *n*-hexane/isopropanol (500/3, v/v); flow rate, 1.6 ml/min; detector, UV photometer at 254 nm.

Keywords——hypolipidemic agent; clinofibrate; high-performance liquid chromatography; determination of optical isomers; diastereomer amide

2,2′-(4,4′-Cyclohexylidenediphenoxy)-2,2′-dimethyldibutyric acid, clinofibrate, is a new hypolipidemic agent[2] having the chemical formula shown in Fig. 1. This new aryloxy compound has three isomers, namely the *d*-form, *l*-form and *meso*-form, which differ in the configurations around the two asymmetric carbons in the molecule. Usually, synthesized

1) Location: *4-2-1, Takatsukasa, Takarazuka-shi, Hyogo 665, Japan.*

2) K. Toki, Y. Nakamura, K. Agatsuma, H. Nakatani, and S. Aono, *Atherosclerosis*, **18**, 101 (1973); D. Kritchevsky and S.A. Tepper, *Atherosclerosis*, **18**, 93 (1973); S. Sakamoto, K. Yamada, T. Anzai, and T. Wada, *Atherosclerosis*, **18**, 109 (1973); E. Schacht, "Topics in Current Chemistry," Springer-Verlag, Inc., New York, 1977, pp. 99—123.

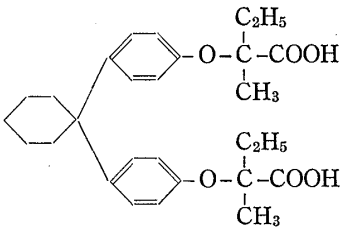

Fig. 1. Chemical Formula of 2,2′-(4,4′-Cyclohexylidenediphenoxy)-2,2′-dimethyldibutyric Acid (Clinofibrate)

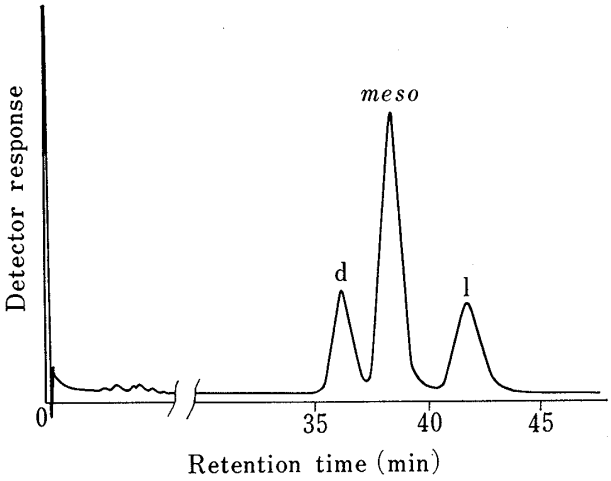

Fig. 2. Chromatogram of the Diastereomer Amide of Clinofibrate

Conditions: column LiChrosorb SI-60 (5 μm) (300 mm×4 mm i.d.); mobile phase, *n*-hexane/isopropanol (500/3 v/v); flow rate, 1.6 ml/min; detection, UV (254 nm).

clinofibrate is a mixture of the *meso* and the racemic isomers and is not optically active. The physicochemical properties of the meso and the racemic isomers are different from each other. Thus, it is important to know the *meso*-racemic isomer ratio of this compound for quality control of clinofibrate. Analysis of the meso and the racemic isomers in clinofibrate has been attempted by phase solubility analysis[3] and infrared spectroscopy,[4] but these methods are not entirely satisfactory as regards accuracy and precision, and moreover the latter method requires the pure isomers, which are difficult to obtain, as analytical standards. Recently, high-performance liquid chromatographic methods[5] were reported for the separation of the optical isomers in optically active carboxylic acids as their diastereomeric amides or esters. Therefore, high-performance liquid chromatography (HPLC) was studied in this work as a method to separate and determine the optical isomers in clinofibrate.

The isomers of clinofibrate were derived to the diastereomer amides with D-(+)-α-methylbenzylamine *via* acid chlorides, using thionyl chloride. The diastereomer amides of the three isomers were completely separated from one another by HPLC on a LiChrosorb SI-60 or μ-Porasil column using a solvent system consisting of *n*-hexane/isopropanol (500/3, v/v) as a mobile phase. Fig. 2 shows the chromatogram obtained on a LiChrosorb SI-60 column.

Table I. Analyses of Optical Isomers of Clinofibrate

Lot No.	Isomer ratio (%)					
	meso			Racemic		
	1	2	$\bar{x}$	1	2	$\bar{x}$
S-1	58.1	58.3	58.2	41.9	41.7	41.8
S-2	58.6	58.6	58.6	41.4	41.4	41.4
S-3	62.5	62.3	62.4	37.5	37.7	37.6
S-7	54.2	54.4	54.3	45.8	45.6	45.7
S-9	49.1	49.5	49.3	50.9	50.5	50.7

3) M. Miyamoto and Y. Sakamaki, Abstracts of Papers, 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1977, p. 220.
4) H. Nakazawa and T. Doi, unpublished.
5) H. Furukawa, Y. Mori, Y. Takeuchi, and K. Ito, *J. Chromatogr.*, **136**, 428 (1977); J. Goto, M. Hasegawa, S. Nakamura, K. Shimada, and T. Nambara, *Chem. Pham. Bull.* (Tokyo), **25**, 847 (1977); Jr. D. Valentine, K.K. Chan, C.G. Scott, K.K. Johnson, K. Toth, and G. Saucy, *J. Org. Chem.*, **41**, 62 (1976); C.G. Scott, M.J. Petrin, and T. Mccorkle, *J. Chromatogr.*, **125**, 157 (1976).

Each peak was identified by mass spectrometry as well as from the HPLC retention time using appropriate standard. The ratio of the meso and the racemic isomers in clinofibrate was determined by their peak area ratios. Table I shows isomer analyses of some samples of clinofibrate.

Experimental

Apparatus——A Jasco Trirotar liquid chromatograph coupled with a Jasco UVIDEC-100 ultraviolet detector for monitoring at 254 nm (Japan Spectroscopic Co., Ltd.), a Shimadzu E1A computing integrator and a Shimadzu LKB-9000 mass spectrometer equipped with the GC-MS PAC-300 data processing system (Shimadzu Seisakusho Co., Ltd.) were used.

Column——LiChrosorb SI-60 (5 μm) silica packing (E. Merck, Darmstadt) was suspended in tetrabromoethane–carbon tetrachloride–dioxane (1 : 2 : 2, v/v) and packed into a stainless-steel column 150 mm in length and 4 mm i.d. under a pressure of 400—500 kg/cm^2 by the balanced-density slurry method.[6]

Reagents——Toluene (JIS guaranteed reagent) was dried on Molecular Sieve 4A (Union Carbide). D-(+)-α-Methylbenzylamine toluene solution was prepared by dissolving 3.0 g of D-(+)-α-methylbenzylamine (Aldrich, $[\alpha]_D^{20}$: +39°, neat) in 100 ml of dried toluene. Other reagents used were of analytical reagent grade.

Procedure——About 50 mg of clinofibrate was weighed into a 10 ml round-bottomed flask and 0.4 ml of thionyl chloride was added. The flask was stoppered tightly, the mixture was warmed for 5 minutes in a water bath at 60° with occasional shaking and then the excess thionyl chloride was removed *in vacuo* below 60°. Dried toluene and D-(+)-α-methylbenzylamine toluene solution (2 ml each) were added successively, and mixed well. The mixture was allowed to stand at room temperature for 10 minutes, and then the solvent was removed *in vacuo*. Chloroform (5 ml) was added to the residue and mixed well to make a sample solution. 5.0 μl of the sample solution was chromatographed under the following HPLC conditions: column, stainless-steel two connecting columns (150 mm in length and 4 mm in i.d.) packed with LiChrosorb SI-60 (5 μm); column temperature, ambient; mobile phase, *n*-hexane/isopropanol (500/3, v/v); flow rate, 1.6 ml/min; detector, UV photometer at 254 nm. The isomer composition of the sample was determined according to the following equation;

$$\textit{meso}, d\text{- or } l\text{-isomer ratio } (\%) = \frac{A_1, A_2 \text{ or } A_3}{A_1+A_2+A_3} \times 100$$

where A_1, A_2 and A_3 are the peak areas of *meso*, *d*- and *l*-isomers of clinofibrate, respectively.

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