Studies on Hypolipidemic Agents. IV.\textsuperscript{1}) Syntheses and Biological Activities of \textit{trans}- and \textit{cis}-2-(4-Alkylcyclohexyl)-2-oxoethyl Arenesulfonates

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\textit{trans}- and \textit{cis}-2-Diazo-1-(4-alkylcyclohexyl)-1-ethanones were reacted with arenesulfonic acids to afford the corresponding 2-(4-alkylcyclohexyl)-2-oxoethyl arenesulfonates. The esterase-inhibitory activity and hypolipidemic effect of the arenesulfonates were examined, and it was found that in most cases, the \textit{trans}-isomers were more active than the corresponding \textit{cis}-isomers.

Stereoselective syntheses of several biologically potent \textit{trans}-isomers (\textit{trans}-3) were also developed.

\textbf{Keywords} — diazoketone; \textit{trans}-arenesulfonate; \textit{cis}-arenesulfonate; 4-alkylcyclohexyl methylketone; \textit{\alpha}-bromoketone; \textit{\alpha}-hydroxyketone; esterase-inhibitory activity; chymotrypsin-inhibitory activity; hypolipidemic activity; structure–activity relationship

We have previously reported the synthesis and esterase-inhibitory activity as well as hypolipidemic effect of 2-oxoalkyl arenesulfonates.\textsuperscript{1}) In the preceding paper,\textsuperscript{1\textsuperscript{c}} we also found that stereoisomeric mixtures of several 2-(4-alkylcyclohexyl)-2-oxoethyl arenesulfonates possess considerable activities. In general, the pharmaceutical activities of stereoisomers are considerably different.\textsuperscript{2,3}) Thus, stereoselective synthesis or separation of the stereoisomers of arenesulfonates (3) is important for pharmaceutical evaluation. In this paper, we wish to report preparations and pharmaceutical evaluations of both stereoisomers of the arenesulfonates (3), as well as stereoselective synthesis of the \textit{trans}-arenesulfonates (\textit{trans}-3).

\textbf{Chemistry}

Catalytic hydrogenation of 4-isopropylbenzoic acid over platinum oxide catalyst afford-
ed a stereoisomeric mixture of trans- and cis-4-isopropylcyclohexanecarboxylic acid (1a), whose separation by distillation was difficult. However, the cis- and trans-isomers (ratio ca. 3:1) of 2-diazo-1-(4-isopropylcyclohexyl)-1-ethanone (2b), which were obtained by treating a stereoisomeric mixture of 4-isopropylcyclohexane-carbonyl chloride with diazomethane, could be separated into trans- (trans-2b) and cis-isomers (cis-2b) by column chromatography.

The stereochemistry of the separated trans-isomer (trans-2b) and cis-isomer (cis-2b) was confirmed as follows. The proton nuclear magnetic resonance (1H-NMR) spectra of trans-2b and cis-2b in CDCl₃ showed the signal of the methine proton (Ha and Hb) at the 1-position of the cyclohexane ring at δ 2.18 ppm (1H, t, J = 12 Hz) and δ 2.46 ppm (1H, m, W₁₂ = 13 Hz), respectively. The former signal can be assigned as the axial proton and the latter, the equatorial proton based on comparisons with the data reported by Jensen et al. Although the signal of Ha overlapped partially with the signals of protons at other positions on the cyclohexane ring in the 100 MHz 1H-NMR spectrum of trans-2b, it was clearly isolated in the 400 MHz 1H-NMR spectrum at δ 2.16 ppm (1H, tt, J = 3.5 and 12 Hz). Furthermore, there is no difference between the chemical shifts of the two methyis on the isopropyl group of trans-2b (δ 0.85 ppm, d, J = 6.5 Hz) and those of cis-2b (δ 0.85 ppm, d, J = 6.5 Hz). These results indicate that the isopropyl group in both trans-2b and cis-2b is equatorial. From the above spectral observations, trans-2b and cis-2b were clearly assigned as trans-isomer and cis-isomer, respectively. Stereoisomeric mixtures of other diazoketones (2a and 2c–e), which were obtained from the corresponding acyl halides of 1b–e by treatment with diazomethane, were also separated into the trans-isomer (trans-2a and trans-2c–e) and the cis-isomer (cis-2a and cis-2c–e) in the same manner as described for trans-2b and cis-2b. The structure of trans-2a, trans-2c–e, cis-2a and cis-2c–e were similarly confirmed. The physical data for trans-2 and cis-2 are listed in Table I.

### Table 1. Physical Data for trans-2 and cis-2

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>R¹</th>
<th>mp (°C)</th>
<th>MS (M⁺)</th>
<th>1H-NMR (CDCl₃) δ ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>trans-2a</td>
<td>-C₂H₅</td>
<td>Oil</td>
<td>180</td>
<td>0.60–2.40 (1H, m) 2.18 (1H, t, J = 12 Hz) 5.27 (1H, s)</td>
</tr>
<tr>
<td>cis-2a</td>
<td>-C₂H₅</td>
<td>Oil</td>
<td>180</td>
<td>0.60–2.04 (1H, m) 2.38 (1H, br m) 5.33 (1H, s)</td>
</tr>
<tr>
<td>trans-2b</td>
<td>-CH(CH₃)₂</td>
<td>34–35</td>
<td>194</td>
<td>0.85 (6H, d) 0.96–2.36 (10H, m) 2.18 (1H, t, J = 12 Hz) 5.24 (1H, s)</td>
</tr>
<tr>
<td>cis-2b</td>
<td>-CH(CH₃)₂</td>
<td>Oil</td>
<td>194</td>
<td>0.85 (6H, d) 0.95–2.15 (10H, m) 2.46 (1H, br m) 5.31 (1H, s)</td>
</tr>
<tr>
<td>trans-2c</td>
<td>-CH₂CH₂CH₃</td>
<td>Oil</td>
<td>208</td>
<td>0.60–2.35 (18H, m) 2.18 (1H, t, J = 12 Hz) 5.24 (1H, s)</td>
</tr>
<tr>
<td>cis-2c</td>
<td>-CH₂CH₂CH₃</td>
<td>Oil</td>
<td>208</td>
<td>0.65–2.14 (18H, m) 2.40 (1H, br m) 5.33 (1H, s)</td>
</tr>
<tr>
<td>trans-2d</td>
<td>-CH₂CH(CH₃)₂</td>
<td>36–37</td>
<td>208</td>
<td>0.89 (6H, d) 0.70–2.38 (12H, m) 2.20 (1H, t, J = 12 Hz) 5.27 (1H, s)</td>
</tr>
<tr>
<td>cis-2d</td>
<td>-CH₂CH(CH₃)₂</td>
<td>Oil</td>
<td>208</td>
<td>0.85 (6H, d) 1.00–2.04 (12H, m) 2.38 (1H, br m) 5.32 (1H, s)</td>
</tr>
<tr>
<td>trans-2e</td>
<td>-C(CH₃)₃</td>
<td>58–59</td>
<td>208</td>
<td>0.84 (9H, s) 0.60–2.32 (9H, m) 2.18 (1H, t, J = 12 Hz) 5.25 (1H, s)</td>
</tr>
<tr>
<td>cis-2e</td>
<td>-C(CH₃)₃</td>
<td>32–33</td>
<td>208</td>
<td>0.82 (9H, s) 0.65–2.28 (9H, m) 2.38 (1H, br m) 5.36 (1H, s)</td>
</tr>
</tbody>
</table>

a) All compounds were light yellowish oils. All IR spectra of these compounds in CHCl₃ showed the presence of a diazo group and a carbonyl group at 2100 and 1630 cm⁻¹, respectively. b) 1H-NMR (400 MHz) spectrum at 50 °C in CDCl₃: 0.86 (6H, d, J = 6.8 Hz) 0.92–1.15 (3H, m) 1.30–1.65 (3H, m) 1.80 (2H, br d, J = 11.2 Hz) 1.89 (2H, br d, J = 11.2 Hz) 2.16 (1H, tt, J = 3.5, 12 Hz) 5.20 (1H, s). c) Lit.: trans-isomer: mp 61.5–63 °C, cis-isomer: mp 34–36 °C.
Treatment of the trans- (trans-2) and cis-diazoketones (cis-2) with arenesulfonic acids afforded the corresponding trans- (trans-3) and cis-arenesulfonates (cis-3) in fairly good yields, respectively.

**Enzyme-Inhibitory Activity (in Vitro Experiments)**

Methyl butyrate and N-acetyltyrosine ethyl ester (ATEE) were used as substrates for the determination of esterase and chymotrypsin activities, respectively (Table II).

**Pharmacological Examination (in Vivo Experiments)**

Male Wistar rats (7 weeks old) were used, with five animals in each experimental group. A test compound (0.3 mmol) was mixed with 5 ml of olive oil and the mixture was orally administered to rats at the dose of 0.3 mmol per kg. Blood for the determination of plasma triglyceride was taken from the orbital vein of the rats at 2 h after the administration. Plasma triglyceride was analyzed by using a commercially available analysis kit (Determiner TG-S Kyowa). Decreases of the triglyceride were expressed as percentage values with respect to the control value obtained for animals given olive oil containing no test compound.

**Results and Discussion**

The physical and biological data for the trans- (trans-3) and cis-arenesulfonates (cis-3) are listed in Tables II and III. As shown in Table II, in most cases, except for trans-3g and cis-3g, the trans-isomers (trans-3) exhibited 4 to 20 times more potent esterase-inhibitory activity.
than the cis-isomers (cis-3). On the other hand, alkyl substituents on the cyclohexane ring of trans-3 increased the esterase-inhibitory activity in the following order: ethyl > isopropyl > isobutyl > sec-butyl > tert-butyl. An ethoxy substituent on the benzene ring as in 3c decreased the activity. Chymotrypsin-inhibitory activity of the trans-isomers (trans-3) was also more potent than that of the cis-isomers (cis-3). Further, in the tests of plasma triglyceride-reducing effect in vivo, the trans-isomers (trans-3) showed a more potent hypolipidemic action than the cis-isomers (cis-3).

**Stereoselective Synthesis of trans-Isomers (trans-3)**

Biological tests of the trans- (trans-3) and the cis-arenesulfonates (cis-3) showed that trans-3 exhibited more potent esterase-inhibitory activity as well as greater hypolipidemic effect than cis-3. Thus, we devised a synthetic scheme for a facile synthesis of trans-3, which might be applicable to large-scale preparation (Chart 2).

Catalytic hydrogenation of 4-alkylacetophenones (4) over rhodium–platinum (3 : 1) was carried out at room temperature under a pressure of 50–60 atm in acetic acid to afford mixtures of the acetylcyclohexanes (5) and a small quantity of the cyclohexyl alcohols. The mixtures were subjected to Jones oxidation\(^9\) to afford stereoisomeric mixtures (5). Equilibration of the stereoisomeric mixtures (5) by refluxing in methanol in the presence of sodium methoxide\(^{10}\) proceeded successfully to give the trans-isomers (trans-5) in 74—78% yield. Bromination of the active methyl group of trans-5 was performed according to \(\ldots\)
procedure of Bettahar et al.\textsuperscript{11) to give the bromoacetylcyclohexanes (6) in 73–84\% yield. Hydrolysis of 2-bromo-1-(trans-4-isobutylcyclohexyl)-1-ethanone (6b) into 2-hydroxy-1-(trans-4-isobutylcyclohexyl)-1-ethanone (7b) under various basic conditions was examined as shown in Table IV in order to find optimum conditions. In this reaction, the yield was almost independent of the kind of bases (entries 1–6) and the reaction temperature (entries 1–5) except in the case of potassium formate as a base, which required a long reaction time at 40 °C (entry 6). Addition of a larger excess of sodium iodide than that used in entry 6 rather lowered the yield (entry 7). Thus, the conditions of entry 4 are recommended as a general method for the preparation of 7 starting from 6.

Esterification of 7 into the trans-arenesulfonates (trans-3) was performed by the same method as described in the preceding paper.\textsuperscript{12)} The properties of the products were identical with those of the trans-arenesulfonates (trans-3) obtained from the trans-diazoketones (trans-2). The synthetic route to trans-3 starting from 4 seems to be suitable for large-scale operation.

### Conclusion

We prepared the trans- (trans-3) and the cis-arenesulfonates (cis-3) from the pure trans-(trans-2) and the cis-diazoketones (cis-2), respectively, and their esterase-inhibitory activity and hypolipidemic effect were evaluated. The trans-isomers (trans-3) were synthesized in six steps starting from the acetophenone derivatives (4) and the synthetic route seems to be...
suitable for large-scale operation. The biological activities of the trans-isomers (trans-3) were found to be more potent than those of the cis-isomers (cis-3). Among the effective trans-isomers (trans-3), we consider that trans-3a, trans-3b and trans-3f may be favorable as hypolipidemic agents, and these compounds are now undergoing pre-clinical studies.

**Experimental**

All melting point were recorded with Yanagimoto micromelting point apparatus and are uncorrected. Spectral data were obtained as follows: infrared (IR) spectra with a Hitachi 260-50 spectrophotometer; mass spectra (MS) with a JEOL JMS-01G-2 spectrometer; 1H-NMR spectra with JEOL JMN-FX 100 and Bruker WH-400 spectrometers (using tetramethylsilane as an internal standard). Chemical shifts of 1H-NMR spectra are given in δ values (ppm).

**Starting Materials**——Stereoisomeric mixtures of the 4-alkylcyclohexanecarboxylic acids (1a–e) were prepared by catalytic hydrogenation of the corresponding 4-alkylbenzoic acids over platinum oxide according to the procedure described in the preceding paper.12 1a: bp 133—134 °C/1 mmHg. 1b: bp 128—130 °C/3 mmHg (lit.13) bp 118—119 °C/0.8 mmHg. 1e: bp 173 °C/20 mmHg. MS m/z: 184 (M+). 1H-NMR (CDCl₃): 0.75—1.90 (18H, m), 2.00—2.75 (1H, m), 11.00 (1H, br). 1d: mp 92—94 °C (lit.14) mp 111 °C). MS m/z: 184 (M+). 1H-NMR (CDCl₃): 0.75—2.15 (18H, m), 2.20—2.75 (1H, m), 11.70 (1H, br). 1e: bp 120 °C/1 mmHg. The 4-alkylacetophenones (4a–d) were prepared from alkylbenzene and acetyl chloride by means of the Friedel–Crafts reaction according to the procedure of Allen.15 4a: bp 94—96 °C/1 mmHg (lit.14) bp 118 °C/13 mmHg. 4b: bp 98—99 °C/2 mmHg (lit.15) bp 110 °C/3 mmHg. 4c: bp 98—101 °C/1 mmHg (lit.16) bp 133—136 °C/2 mmHg. 4d: bp 100—102 °C/2 mmHg (lit.17) bp 134—135 °C/11 mmHg.

**2-Diazo-1-(trans-4-ethylcyclohexyl)-1-ethanone (trans-2a) and 2-Diazo-1-(cis-4-ethylcyclohexyl)-1-ethanone (cis-2a)**——Typical procedure for the syntheses of the stereoisomeric mixtures (2a–e) and for the separation into the trans-isomers (trans-2a–e) and the cis-isomers (cis-2a–e). A mixture of thiouyl chloride (30 ml) and 4-ethylcyclohexanecarboxylic acid (1e) (1.7 g) was stirred for 2 h under reflux, and then the reaction mixture was evaporated under reduced pressure. The residue (4-ethylcyclohexylbenzyl chloride (100 ml) of diazomethane (obtained from 7.0 g of nitrosomethylurea) under stirring with ice-cooling. After being stirred for 1 h, the reaction mixture was evaporated under reduced pressure to give 2a as a crude oil (stereoisomeric mixture, cis:trans = ca. 3:1). The crude oil (2a) (2.0 g) was chromatographed on a long silica gel column with chloroform as an eluent. From the first eluate, the cis-isomer (cis-2a) was obtained as a yellowish oily product. Yield, 0.3 g (51%). IR ν cm⁻¹: 2100 (N=O), 1630 (CO). Similar procedures were used for the preparations of the other stereoisomeric mixtures (2b–e) and for the separations into the trans-isomers (trans-2b–e) and the cis-isomers (cis-2b–e).

**2-(trans-4-Ethylcyclohexyl)-2-oxoethyl Benzenesulfonate (trans-3a) and 2-(cis-4-Ethylcyclohexyl)-2-oxoethyl Benzenesulfonate (cis-3a)**——Typical procedure for the syntheses of the trans-isomers (trans-3a–g) and the cis-isomers (cis-3a–g). Benzenesulfonic acid monohydrate (1.5 g) was added to an ethereal solution (50 ml) of the trans-diazoketone (2a) (0.5 g) under ice-cooling. After being stirred for 1 h at room temperature, the reaction mixture was washed with water and dried over sodium sulfate. The ethereal layer was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with chloroform as an eluent. From the second eluate, the trans-isomers (trans-2a) was obtained as a yellowish oily product. Yield, 0.3 g (15%). IR ν cm⁻¹: 2100 (N=O), 1630 (CO). Similar procedures were used for the syntheses of the trans-3a–g and cis-arenesulfonates (cis-3a–g). Other data are listed in Table I.

**2-(trans-4-Ethylcyclohexyl)-2-oxoethyl Benzenesulfonate (trans-3a)**——Typical procedure for the syntheses of the trans-isomers (trans-3a–g) and the cis-isomers (cis-3a–g). Benzenesulfonic acid monohydrate (1.5 g) was added to an ethereal solution (50 ml) of the trans-diazoketone (trans-2a) (0.5 g) under ice-cooling. After being stirred for 1 h at room temperature, the reaction mixture was washed with water and dried over sodium sulfate. The ethereal layer was evaporated under reduced pressure. The crude product was purified by column chromatography on silica gel with chloroform as an eluent. From the second eluate, the trans-isomers (trans-2a) was obtained as a yellowish oily product. Yield, 0.3 g (15%). IR ν cm⁻¹: 2100 (N=O), 1630 (CO). Similar procedures were used for the syntheses of the trans-3a–g and cis-arenesulfonates (cis-3a–g). Other data are listed in Tables II and III.

**1-(4-Isobutylcyclohexyl)-1-ethanone (5b)**——Typical procedure for the syntheses of 5a–d. Hydrogenation of 4-isobutylacetophenone (4b) (40.0 g) in AcOH (70 ml) was carried out in the presence of a catalytic amount of rhodium–platinum (1.5 g) under a pressure of 50 atm at room temperature (about 4 h). The reaction mixture was evaporated under reduced pressure to give a crude mixture of the acetylcyclohexane (5b) and the cyclohexyl alcohol. Jones reagent (120 ml) (consisting of CrO₃; 31 g, conc. H₂SO₄; 27 ml and water) was added dropwise to a solution of the mixture in aceton (100 ml) under ice-cooling. The whole was stirred for 5 h at room temperature, then water (200 ml) was added and the separated material was extracted with ether (200 ml × 2). The ethereal layer was washed with water (50 ml), dried over sodium sulfate, and evaporated under reduced pressure to give 5b as a crude oily product (stereoisomeric mixture, cis:trans = 1:1). The crude product was purified by column chromatography on silica gel with chloroform as an eluent, followed by recrystallization from ethanol to give the trans-arenesulfonate (trans-3a). Yield, 0.65 g (76%). A similar procedure was used for the preparations of the trans-3a–g and cis-arenesulfonates (cis-3a–g). Other data are listed in Tables II and III.
solution of sodium methoxide (9.9 g) in MeOH (350 ml) was added to the ketone (5b) (32.0 g). After being stirred for 4 h under refluxing, the reaction mixture was evaporated under reduced pressure to give trans-5b as a crude oily product, which was purified by distillation. Yield, 25.0 g (78%). bp 125—128 °C/22 mmHg. 1H-NMR (CDCl3): 0.86 (6H, d, J = 6.5 Hz), 0.78—2.45 (13H, m), 2.11 (3H, s). A similar procedure was used for the syntheses of trans-5a, trans-5c and trans-5d. trans-5a: Yield, 75%; bp 109—113 °C/19 mmHg. 1H-NMR (CDCl3): 0.86 (6H, d, J = 6.5 Hz), 0.60—2.50 (11H, m), 2.13 (3H, s). trans-5c: Yield, 76%; bp 127—130 °C/18 mmHg. 1H-NMR (CDCl3): 0.60—2.10 (18H, m), 2.10—2.50 (1H, br), 2.13 (3H, s). trans-5d: Yield, 74%; bp 127—131 °C/22 mmHg. 1H-NMR (CDCl3): 0.85 (9H, s), 0.70—2.45 (10H, m), 2.11 (3H, s).

2-Bromo-1-(trans-4-isobutylcyclohexyl)-1-ethanone (6b) — Typical procedure for the syntheses of 6a—d. Bromine (14.0 g) was added in one portion to a solution of trans-5b (15.0 g) in MeOH (250 ml) at room temperature. After being stirred for 4 h, the reaction mixture was evaporated under reduced pressure. Water (20 ml) and benzene (100 ml) were added to the residue. The benzene layer was washed with water, dried over sodium sulfate and evaporated under reduced pressure to give 6b as a crude oily product, which was purified by distillation. Yield, 18.0 g (84%). bp 124—127 °C/1.5 mmHg. 1H-NMR (CDCl3): 0.70—2.10 (18H, m), 2.46—2.86 (1H, br), 3.97 (2H, s). Anal. Calcd for C11H20O2: C, 72.54; H, 11.45. Found: C, 72.47; H, 11.30. Compound 7b was also obtained by hydrolysis of 6b under various basic conditions as shown in Table IV (entries 1—7). Reaction conditions and data are listed in Table IV. A similar procedure was used for the syntheses of 6a, 6c and 6d. 6a: Yield, 73%. by 113—115 °C/2 mmHg. 1H-NMR (CDCl3): 0.86 (6H, d, J = 6.5 Hz), 0.60—2.20 (18H, m), 2.44—2.84 (1H, br), 3.96 (2H, s). Anal. Calcd for C12H22O2: C, 72.68; H, 11.18. Found: C, 72.47; H, 11.30. Compound 7b was also obtained by hydrolysis of 6b under various basic conditions as shown in Table IV (entries 1—7). Reaction conditions and data are listed in Table IV. A similar procedure was used for the syntheses of 7a—d. A solution of KOH (5.0 g) in MeOH (50 ml) was added dropwise to a solution of 6b (18.0 g) in MeOH (180 ml) below 10 °C. After being stirred for 1 h at the same temperature, the reaction mixture was concentrated to about 50 ml under reduced pressure. Water (30 ml) was added and the mixture was extracted with ether (100 ml × 2). The ethereal layer was washed with water, dried over sodium sulfate and evaporated under reduced pressure to give 7b as a crude oily product, which was recrystallized from hexane–ether. Yield, 9.3 g (68%). mp 69—70 °C. 1H-NMR (CDCl3): 0.86 (6H, d, J = 6.6 Hz), 0.80—2.10 (12H, m), 2.34 (1H, tt, J = 4, 12 Hz), 3.15 (1H, t, J = 5 Hz), 4.30 (2H, d, J = 5 Hz). Anal. Calcd for C11H19BrO: C, 53.45; H, 7.46. Found: C, 53.47; H, 7.75.

Enzyme-Inhibitory Activities — The esterase- and chymotrypsin-inhibitory activities were determined by the method described in the previous paper.1)

Pharmacology — The triglyceride level in plasma was measured by the same method as described in the preceding paper.1)

References and Notes


4) These compounds were reported in the preceding paper (ref. 1c).
13) Detailed physical data for the stereoisomeric mixture of 1e were not given.
14) The reason why the melting point of 1f was somewhat lower than the literature value [K. Alder, K. Heimback, and E. Kühle, Chem. Ber., 86, 1364 (1953)] might be that it is a stereoisomeric mixture.