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PREPARATION OF THE BOTH ENANTIOMERS OF *THREO*-2-AMINO-3-METHYLHEXANOIC ACID BY ENZYMATIC RESOLUTION AND THEIR CONVERSION TO OPTICALLY ACTIVE FORMS OF *THREO*-4-METHYLHEPTAN-3-OL, A PHEROMONE COMPONENT OF THE SMALLER EUROPEAN ELM BARK BEETLE†

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Abstract—The both enantiomers of *threo*-2-amino-3-methylhexanoic acid were prepared by resolving its racemic N-acetate with *Aspergillus* acylase. The amino acid enantiomers were converted to optically active forms of *threo*-4-methylheptan-3-ol, a pheromone component of *Scolytus multistriatus*.

(−)-4-Methylheptan-3-ol **1** and (−) - α - multistriatin[(1*S*, 2*R*, 4*S*, 5*R*) - 2, 4 - dimethyl - 5 - ethyl - 6, 8 - dioxabicyclo[3.2.1]octane] are beetle-produced pheromones responsible for the aggregation of the smaller European elm bark beetle, *Scolytus multistriatus* Marsham.[1] Some years ago one of us established the absolute configuration of the levorotatory alcohol to be 3*S*, 4*S* as depicted in **1** by synthesizing its antipode from (*R*)-(+)-citronellic acid.[2] The synthesis, however, was not stereoselective and afforded a mixture of (3*R*, 4*R*)-*threo*-**1** and its (3*S*, 4*R*)-*erythro*-isomer which was separated by preparative GLC. Since then we sought for a better synthetic route which would afford the natural (3*S*, 4*S*)-pheromone **1**.

The Fischer projectional formula of **1** made us to realize that an amino acid **A** might be an ideal starting material for the synthesis of **1**. The amino acid **A** is a homolog of alloisoleucine **B**, whose chemistry is thoroughly investigated.[3] Alloisoleucine **B** is known to be separable from its *erythro*-isomer **C** (isoleucine) by recrystallizing a mixture of their N-acetyl derivatives.[3] By analogy, it seemed possible to separate the N-acetate of the *threo*-acid **A** (homoalloisoleucine) from the undesired *erythro*-isomer. Then the well-known enzymatic enantio-selective hydrolysis of the racemic N-acetyl-α-amino acid would afford the optically active amino acid.[4] The conversion of **A** to the pheromone **1** seemed to offer no difficulty. The following is the successful account of our work along this line which emphasizes the importance of an enzymatic reaction in chiral syntheses.

(±)-*threo*-2-Amino-3-methylhexanoic acid **3** was prepared from 2-methylpentanal **2** by the Strecker synthesis. This new unnatural α-amino acid (±)-**3** was converted to its N-acetyl derivative (±)-**4** according to the procedure described for (±)-alloisoleucine.[5] This was recrystallized several times from acetone to give pure (±)-**4**. Its enzymatic resolution was attempted with a microbial acylase obtained from *Aspergillus*. The reaction was carried out at pH 6.7 ~ 6.8 in the presence of a trace amount of Co^{2+}.[6,7] After 4 days at 37°, the reaction mixture was concentrated *in vacuo* to give crystals of (2*S*, 3*R*)-(+)-**5**. Acidification of the mother liquor with dil HCl gave partially resolved N-acetate (2*R*, 3*S*)-(−)-**4**

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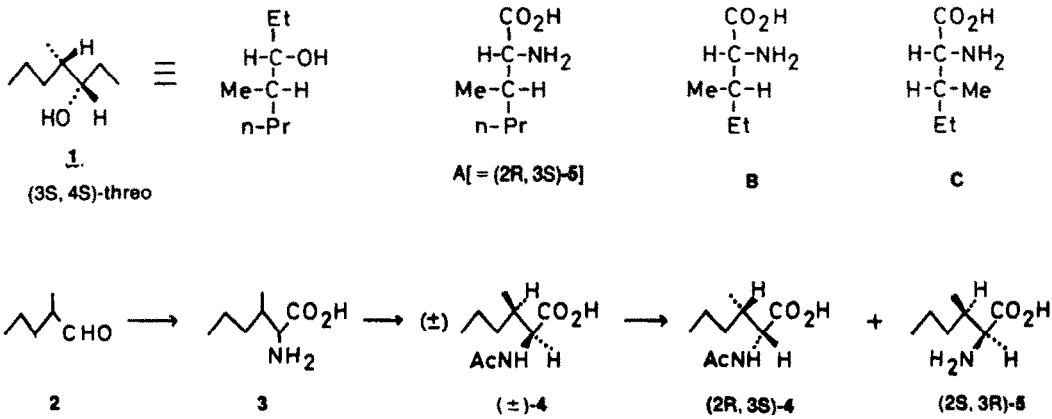


as crystals. Further work-up of the mother liquor (see Experimental) slightly increased the yields of the products and the total yield of pure (2*S*,3*R*)-**5**, $[\alpha]_D^{21}$ + 44.9° (5N–HCl), was 55% of the theoretical amount. Since the amino acid (2*S*,3*R*)-**5** was hitherto unknown, we felt it necessary to check its optical purity by a modern direct method. This was later achieved after its conversion to (2*S*,3*R*)-**6** and found to be 98% (*vide infra*). It should be noted that the *Aspergillus* acylase showed very high enantioselectivity even with this unnatural substrate. This makes worth while to define the substrate specificity of the commercially available acylase more precisely so as to use it as a reagent for optical resolution.

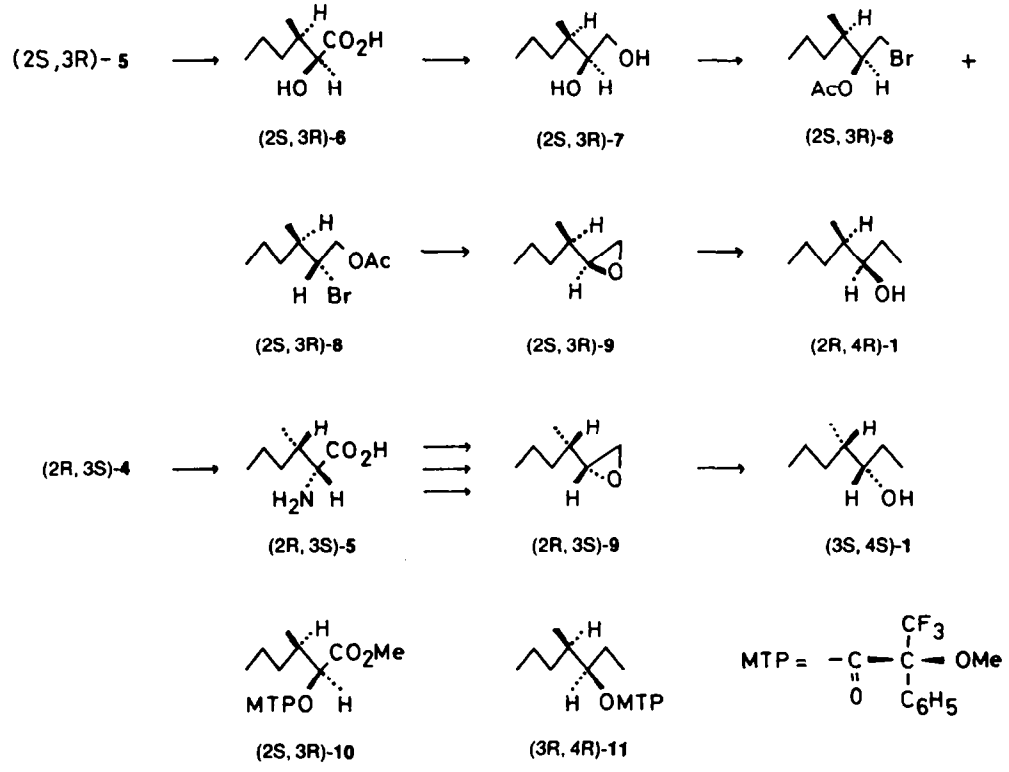
The conversion of (2*S*,3*R*)-**5** to the antipode [(3*R*,4*R*)-**1**] of the natural pheromone was carried out in the following manner. The (+)-amino acid **5** was treated with nitrous acid to give a hydroxy acid (2*S*,3*R*)-(+)-**6**. This deamination reaction is known to proceed with retention of configuration.[8] LAH reduction of (2*S*,3*R*)-**6** gave a glycol (2*S*,3*R*)-**7**. This was treated with HBr–AcOH to give a mixture of acetoxy bromides (2*S*,3*R*)-**8** and (2*S*,3*R*)-**8'**. Treatment of this mixture (**8**+**8'**) with NaOMe–MeOH gave an epoxide (2*S*,3*R*)-(−)-**9**. The S_N2 ring opening of the epoxide **9** with Me_2CuLi yielded (3*R*,4*R*)-(+)-*threo*-4-methylheptan-3-ol **1** with 95% chemical purity as determined by glc. This was further purified by preparative glc to give pure (3*R*,4*R*)-**1** with $[\alpha]_D^{23.5}$ + 22.7° (n-hexane).

In order to prepare optically pure (3*S*,4*S*)-**1**, the natural pheromone, we had to secure optically pure (2*R*,3*S*)-N-acetyl-α-amino acid **4**. For this purpose the partially resolved (−)-**4** was submitted again to enzymatic resolution with *Aspergillus* acylase. As expected, the recovered N-acetate was more enriched in the (−)-isomer. This was recrystallized several times from acetone to give pure (2*R*,3*S*)-(−)-**4**. Acid hydrolysis of (−)-**4** yielded (2*R*,3*S*)-(−)-*threo*-2-amino-3-methylhexanoic acid **5**, $[\alpha]_D^{22}$-43.8° (5N–HCl). This was converted to the natural pheromone (3*S*,4*S*)-**1** *via* the chiral epoxide (2*R*,3*S*)-**9** in entirely the same manner as described for its antipode. The synthetic pheromone was of 94% chemical purity as determined by glc. Further purification by preparative glc gave pure (3*S*,4*S*)-**1**, $[\alpha]_D^{22}$-21.7° (n-hexane).

The optical purities of our synthetic products were determined by the MTPA†-ester method.[9] In order to prove the high enantioselectivity of the acylase hydrolysis, a portion of the α-hydroxy acid (2*S*,3*R*)-**6** or (2*R*,3*S*)-**6** was esterified with CH_2N_2 to give the corresponding Me ester. This was treated with (*S*)-(−)-MTPA-Cl to give an MTPA-ester (2*S*,3*R*)-**10** or (2*R*,3*S*)-**10**. These two were analyzed by NMR spectroscopy. In both cases, even in the presence of the Sievers' shift reagent Eu(fod)$_3$‡, the OMe protons appeared as a sharp singlet. No definite sign of the presence of the other diastereomer was observable. The optical purities were therefore thought to be ≥96% considering the S/N ratio of the spectrometer. Upon glc analysis, the two diastereomers were separable and both (2*S*,3*R*)-and (2*R*,3*S*)-**10** were shown to be 98% diastereomerically pure. The enzymatic resolution was therefore highly successful. The possibility of racemization in the course of synthesis was excluded by proving the high optical purities of the final products. The alcohols (3*R*,4*R*)-**1** and (3*S*,4*S*)-**1** were converted to their respective MTPA-esters (3*R*,4*R*)-**11** and (3*S*,4*S*)-**11**. They were analyzed by NMR. No definite sign of the presence of the other diastereomer was observable within the limit of experi-

†α-Methoxy-α-trifluoromethylphenylacetic acid.

‡Tris (6, 6, 7, 7, 8, 8, 8 - heptaflouro - 2, 2 - dimethyl - 3, 5 - octanedionato) europium.



mental error. The optical purities were therefore in the same range as those of (2*S*, 3*R*)-6(~ 98%).§

In conclusion we synthesized the both enantiomers of *threo*-4-methylheptan-3-ol in sufficient quantities for biological evaluation. The use of an enzyme in the key resolution step was so successful that we were encouraged to continue our work on the application of biochemical systems in preparative organic chemistry.†

EXPERIMENTAL

All b.ps and m.ps were uncorrected. IR spectra refer to films for oils or Nujol mulls for solids and were determined on a Jasco IRA-1 spectrometer. NMR spectra were recorded as CCl_4 solns at 60 MHz with TMS as an internal standard on a Hitachi R-24A spectrometer. Optical rotations were measured on a Jasco DIP-4 polarimeter. Glc analyses were performed on Yanaco GCG-550F or G180 gas chromatographs.

A diastereomeric mixture of 2-*amino*-3-*methylhexanoic acid* 3

Aqueous NH_4Cl (60g in 200 ml water) was added to a soln of KCN (66g) in water (200 ml) and conc NH_3 aq (155 ml, 28%). The aldehyde 2(*ca* 90g, 100 ml) was added dropwise to the stirred and ice-cooled KCN–NH_4Cl soln at 0–20°. After stirring for 2 hr, ether (100 ml) was added to the mixture and the stirring was continued overnight at room temp. The ether layer was separated and the aq layer was extracted with ether. The combined ether soln was concentrated *in vacuo*. The residual oil, ν_{max} ~ 3350 (s), 2940 (s), 2900 (s), 2850 (s), 2220 (w), 1620 (m), 1450 (m), 1380 (m), 1065 (m), 945 (m), 740 (w) cm^{-1}, was mixed with conc HCl (500 ml) and heated under reflux for 15 hr. The dark soln was concentrated *in vacuo* and the residual solid was dissolved in hot water (300 ml). This was decolorized with activated charcoal while hot, filtered and dried up *in vacuo*. The residue was heated with 95% EtOH (500 ml) and filtered to remove NH_4Cl. The filtrate was concentrated *in vacuo* after the addition of C_5H_5N (100 ml), diluted with water and cooled to give 40g (31%) of crystalline 3. This was recrtstallized from water to give an analytical sample, m.p. 201–206°. ν_{max} ~ 3400 (m), ~ 2550 (m), 2120 (w), 1585 (s), 1495 (s), 1405 (m), 1350 (m), 1320 (m), 1295 (w), 1280 (w), 1260 (w), 1240 (w), 1175 (w), 1120 (w), 1060 (w), 1030 (w), 1000 (w), 930 (w), 890 (w), 850 (w), 830 (w), 810 (w), 785 (w), 735 (w) cm^{-1}. (Found: C, 57.18; H, 10.25; N, 9.88. Calc for $C_7H_{15}O_2N$: C, 57.90; H, 10.41; N, 9.65%).

(±)-threo-2-*Acetamino*-3-*methylhexanoic acid* (±)-4.

A suspension of 3 (120g) in Ac_2O (200 ml)–AcOH (200 ml) was heated under reflux for 5 min during which it became homogeneous. After cooling, the soln was concentrated *in vacuo*. The residue was mixed with water and concentrated *in vacuo*. The residue was dissolved in Me_2CO–C_6H_6, concentrated *in vacuo*, diluted with ether and set aside. The separated crystals were collected on a filter and recrystallized from Me_2CO. The first (60g, m.p. 130–131°), second (5.5g, m.p. 128–130°) and third (12.5g, m.p. 125–127°) crops were combined to give 78.5g (50%) of (±)-4. An analytical sample was recrystallized from Me_2CO to give prisms, m.p. 145–146°, ν_{max} 3320 (s), ~ 2500 (m), 1705 (s), 1620 (s), 1540 (s), 1340 (m), 1320 (m), 1275 (m), 1230 (m), 1150 (m), 1120 (w), 1080 (w), 1020 (w), 980 (m), 690 (m) cm^{-1}. (Found: C, 57.72; H, 9.01; N, 7.41%. Calc for $C_9H_{17}O_3N$: C, 57.73; H, 9.15; N, 7.48%). The yield of (±)-4 was 29–50% and dark brown oily N-acetate was obtained from the mother liquor after removal of crystalline (±)-4.

Enzymatic resolution of (±)-4.

(*i*) *Preparation of* (2S, 3R)-5. The N-acetate (±)-4 (43g) was dissolved in water (2.05 l) by adding NaOH aq (*ca* 8.6g in 100 ml water) to pH 6.7–6.8. Acylase (Acylase "Amano", 15,000 unit/g, 3g) and 7×10^{-4} M $CoCl_2$ were added to the soln and the mixture was kept at 37°. The acylase (2g) was added after 1 day. Another g of the acylase was added after 2 days. After 4 days at 37° the dark soln was concentrated *in vacuo* to *ca* 100 ml. The separated crystals of (2*S*, 3*R*)-5 (8.1g) were collected on a filter and washed with cold water. The combined filtrate and washings were concentrated *in vacuo* and acidified with 6N HCl (32.5 ml). The partially resolved (2*R*, 3*S*)-4 (28.3g) immediately separated from the soln and was collected on a filter. The filtrate was extracted with EtOAc. The EtOAc extract was concentrated *in vacuo* to give impure (2*R*, 3*S*)-4 (5.0g). The aq. layer was concentrated *in vacuo* to dryness. The residue was triturated with 99% EtOH and filtered to remove insoluble materials (17.4g). The filtrate was concentrated *in vacuo*. Pyridine (1 ml) and water (5 ml) were added to the residue and the precipitated (2*S*, 3*R*)-5 (1g) was collected on a filter. The total yield of (2*S*, 3*R*)-5 was 9.1g (55%). An analytical sample was obtained as prisms by recrystallization from water, m. p. 230–238° (dec.), $[\alpha]_D^{21}$ + 44.9 (*c* = 0.275, 5N HCl); ν_{max} ~ 3100 (m), ~ 2590 (m), 2100 (w), 1600 (s, sh), 1575 (s), 1505 (s), 1410 (m), 1350 (m), 1320 (m), 1295 (w), 1265 (w), 1240 (w), 1185 (w), 1120 (w), 1070 (w), 1020 (w), 945 (w), 900 (w), 860 (w), 815 (w), 790 (w), 760 (w), 740 (w), 715 (m) cm^{-1}. (Found: C, 57.68; H, 10.29; N, 9.67. Calc. for $C_7H_{15}O_2N$: C, 57.90; H, 10.41; N, 9.65%). The crude amino acid (7.3g) was recrystallized from a large volume of water to give 4.5g of pure (2*S*, 3*R*)-5.

(*ii*) *Preparation of* (2R, 3S)-4. The partially resolved N-acetate (61.4g) was fractionally recrystallized from acetone. Fractions with $[\alpha]_D$ − 13°to − 30° (EtOH) were combined (39g). This was suspended in water (2l) and dissolved by adding NaOH aq to pH 6.5. The acylase (3g) and $CoCl_2$-$6H_2O$ (44.0 mg) were added and the mixture was set aside at 37° for 5 days with the addition of further amount of the acylase (1g × 3) at intervals. Subsequent work-up as described above gave 25.5g of crude (2*R*, 3*S*)-4. This was fractionally recrystallized from acetone to give the first [17.5g, $[\alpha]_D^{22}$ − 28.7° (*c* = 1.286, EtOH)], second [5.5g, $[\alpha]_D^{22}$ − 15.6° (*c* = 0.973, EtOH)] and third [2.5g, $[\alpha]_D^{22}$ − 17.0° (*c* = 1.260, EtOH)] crops. The first crop was further purified by recrystallization from acetone to give 12.2g of pure (2*R*, 3*S*)-4 as prisms, m.p. 146–147°, $[\alpha]_D^{22}$ − 34.3° (*c* = 0.936, EtOH); ν_{max} 3320 (s), ~ 2480 (m), ~ 2320 (m), ~ 1880 (w), 1695 (s), 1620 (s), 1540 (s), 1425 (m), 1315 (m), 1305 (m), 1275 (s), 1230 (m), 1150 (m), 1115 (w), 1085 (w), 1025 (w), 955 (m), 865 (w), 835 (w), 745 (w), 700 (m) cm^{-1}. (Found: C, 57.81; H, 9.12; N, 7.46. Calc for $C_9H_{17}O_3N$: C, 57.73; H, 9.15; N, 7.48%).

(2R, 3S)-2-*Amino*-3-*methylhexanoic acid* (2R, 3S)-5

A suspension of (2*R*, 3*S*)-4 (12.2g) in 2N HCl (120 ml) was stirred and heated under reflux for 2 hr. The homogeneous soln was concentrated *in vacuo*. The residue was dissolved in water (50 ml) and concentrated *in vacuo* to remove HCl. The residual solid was dissolved in water (100 ml) and C_5H_5N (20 ml) was added to the soln. The precipitated (2*R*, 3*S*)-5 (10.0g, quantitative) was recrystallized from water to give 6.0g (63%) of pure (2*R*, 3*S*)-5, m.p. 230–240° (dec), $[\alpha]_D^{22}$ − 43.8° (*c* = 0.560, 5N HCl). The IR spectrum was identical with that of (2*S*, 3*R*)-5. (Found: C, 57.76; H, 10.48; N, 9.63. Calc for $C_7H_{15}O_2N$: C, 57.90; H, 10.41; N, 9.65%).

2-*Hydroxy*-3-*methylhexanoic acid* 6.

(*i*) (2S, 3R)-*Isomer*. A soln of $NaNO_2$ (5.0g) in water (10 ml) was added dropwise during 1 hr to a stirred and ice-cooled soln of (2*S*, 3*R*)-5 (6.0g) in 1.5 N H_2SO_4 (70 ml) at 0–5°. The stirring was continued for 2 hr at 0–30°. Subsequently the soln was concentrated *in vacuo* to 10 ml. Then it was thoroughly washed with ether to extract 6. The ether soln was dried ($MgSO_4$) and concentrated *in vacuo* to give 4.0g (66%) of (2*S*, 3*R*)-6 as an oil. This was used for the next step without further purification. A purer sample was obtained by micro-distillation, b.p. 87–91° (bath temp)/0.1 mm, n_D^{22} 1.4396; $[\alpha]_D^{22.5}$ + 15.8° (*c* = 0.621, EtOH); ν_{max} ~ 3360 (s), 2950 (s), 2860 (s), ~ 2500 (m), ~ 2300 (w), 1710 (s), 1450 (m), 1370 (m), 1260 (m), 1230 (m), 1140 (s), 1110 (w), 1065 (m), 1040 (m), 990 (m), 950 (w), 920 (w), 880 (w), 750 (w) cm^{-1}; δ 0.87 (3H, d, J = 6Hz), 0.93 (3H, t, J = 6Hz), 1.2–1.5 (5H, m), ~ 2.0 (1H, –OH), 4.22 (1H, d, J = 3Hz), 6.13 (1H, $-CO_2H$).

†At the EUCHEM Conference on Chemistry of Insects (14 August 1979), Dr. M. M. Blight of the University of Sussex reported her successful preparation of both (3*R*, 4*R*)-1 and (3*S*, 4*S*)-1 by HPLC separation of their MTPA esters.

(*ii*) (2R, 3S)-*Isomer*. (2*R*, 3*S*)-**5** (6.0g) was similarly treated as above to give 5.0g (82%) of (2*R*, 3*S*)-**6**. This was employed for the next step without further purification. A purer sample was obtained by micro-distillation, b.p. 91–95° (bath temp)/0.1 mm, n_D^{23} 1.4394; $[\alpha]_D^{23}$-15.8° (*c* = 0.378, EtOH). The IR and NMR spectra were identical with those of (2*S*, 3*R*)-**6**.

3-*Methylhexane*-1, 2-*diol* **7**.

(*i*) (2S, 3R)-*Isomer*. A soln of (2*S*, 3*R*)-**6** (4.0g) in dry ether (30 ml) was added dropwise to a stirred and ice-cooled suspension of LAH (1.6g) in dry ether (80 ml) at 0–5°. The mixture was stirred for 1 hr at 0–5° and for another hr at room temp. The excess LAH was carefully destroyed at 0–10° by the addition of water (1.6 ml), 10%NaOH aq (1.6 ml), and water (4.8 ml). The mixture was filtered and the solid was thoroughly washed with acetone. The combined filtrate and washings was concentrated *in vacuo*. The residue was distilled to give 2.7g (75%) of (2*S*, 3*R*)-**7**, b.p. 105–108°/5 mm, n_D^{23} 1.4435; $[\alpha]_D^{23}$ +11.8° (*c* = 0.999, EtOH); ν_{max}(film) 3340 (s), 2950 (s), 2910 (s), 2860 (s), 1460 (m), 1380 (m), 1220 (w), 1140 (w), 1060 (m), 1020 (m), 980 (w), 950 (w), 900 (w), 865 (w), 820 (w), 745 (w) cm^{-1}; δ ($CDCl_3$) ~0.8–~1.1 (6H, m, 0.84, 0.88, 0.94), ~1.1–~1.8 (5H, br), 3.54 (5H, br). (Found: C, 63.24; H, 11.97. Calc for $C_7H_{16}O_2$: C, 63.59; H, 12.20%).

(*ii*) (2R, 3S)-*Isomer*. This was prepared in the same manner as above from 5.0g of (2*R*, 3*S*)-**6** in 56% yield (2.5g), b.p. 101–102°/5mm, n_D^{22} 1.4439; $[\alpha]_D^{22}$-13.6° (*c* = 0.973, EtOH). (Found: C, 63.33; H, 12.10. $C_7H_{16}O_2$ requires: C, 63.59; H, 12.20%). The IR and NMR spectra were identical with those of (2*S*, 3*R*)-**7**.

1, 2-*Oxido*-3-*methylhexane* **9**

(*i*) (2S, 3R)-*Isomer*. 30% HBr in AcOH (15 ml) was added dropwise to the stirred and ice-cooled (2*S*, 3*R*)-**7** (2.7g) at 0–5°. The mixture was stirred for 10 min at 0–5° and for 1 hr at room temp. Subsequently it was poured into ice-water, neutralized with Na_2CO_3 with careful cooling and extracted with ether. The ether soln was dried ($MgSO_4$) and concentrated *in vacuo* to give 4.5g of crude **8** + **8'**, ν_{max} 2900 (s), 2860 (m), 1735 (s), 1450 (m), 1420 (m), 1370 (m), 1240 (s), 1100 (w), 1020 (s), 970 (m), 925 (w), 800 (w), 740 (w) cm^{-1}. This crude **8** + **8'** (4.5g) was added dropwise to a stirred and ice-cooled soln of NaOMe (from 0.8g of Na) in MeOH (10 ml) using 2 ml of MeOH to rinse the container of **8**. The mixture was stirred for 1 hr at room temp, poured into water, and extracted with n-penthane. The n-pentane soln was washed with water and sat NaCl aq, dried (K_2CO_3) and concentrated under atm press with a Vigreaux column. The residue was distilled to give 1.1g (47%) of (2*S*, 3*R*)-**9**, b.p. 66–67°/69 mm, n_D^{23} 1.4095; $[\alpha]_D^{23}$-4.2° (*c* = 0.867, ether); ν_{max} 3020 (m), 2900 (s), 2850 (s), 1450 (m), 1405 (w), 1370 (m), 1300 (w), 1255 (m), 1145 (w), 1120 (w), 1060 (w), 1005 (w), 950 (w), 930 (m), 895 (m), 860 (m), 850 (m), 815 (m), 770 (w), 735 (w) cm^{-1}; δ ~0.7–~1.10 (6H, m, 0.90, 0.99), ~1.30 (5H, br), 2.30–2.70 (3H, m).

(*ii*) (2R, 3S)-*Isomer*. In the same manner as described above, 918 mg (42%) if (2*R*, 3*S*)-**9** was obtained from 2.5g of (2*R*, 3*S*)-**7**: b.p. 62–65°/70 mm, n_D^{22} 1.4086; $[\alpha]_D^{22}$ +4.2° (*c* = 1.122, ether). The IR and NMR spectra were identical with those of (2*S*, 3*R*)-**9**.

threo-4-*Methylheptan*-3-*ol* **1**

(*i*) (3R, 4R)-*Isomer*. A soln of Me_2CuLi in ether was prepared by adding MeLi (an 85-ml-portion of a soln prepared from 5.14g of Li and 41g of MeBr in 240 ml of dry ether) to a suspension of CuI (5.71g) in ether (25 ml) at −30 to −20°. A soln of (2*S*, 3*R*)-**9** (1.14g) in ether (25 ml) was added dropwise to the stirred and cooled Me_2CuLi soln at −28 to −18°. After the addition, the reaction temp was lowered to −40° and then raised to 0° during 4 hr. The reaction flask was left to stand overnight in a refrigerator. Then sat NH_4Cl soln was added with cooling and the mixture was extracted with ether. The ether soln was washed with sat NH_4Cl soln and sat NaCl soln, dried (K_2CO_3) and concentrated under atm press. The residue was distilled to give 0.97g (75%) of (3*R*, 4*R*)-**1**, b.p. 99–101°/98 mm, n_D^{23} 1.4244; $[\alpha]_D^{22}$ + 18.4° (*c* = 0.522, n-hexane); ν_{max} ~3360 (s), 2930 (s), 2900 (s), 2840 (s), 1450 (s), 1370 (m), 1140 (w), 1095 (m), 1060 (m), 1030 (w), 960 (s), 940 (sh), 900 (w), 875 (w), 850 (w), 760 (w), 740 (w) cm^{-1}; δ(CCl_4, 100 MHz) 0.8–1.0 (9H, m, 0.80, 0.86, 0.92, 0.99), 1.28–1.48 (7H, m), 1.80 (1H, s, –OH), 3.28 (1H, m). The IR and NMR spectra of this product were identical with those of our previous sample.[2] (Found: C, 73.34; H, 13.91. Calc. for $C_8H_{18}O$: C, 73.78; H, 13.92%). Glc (Column, 5% PEG 20M, 2m × 4mm at 100° (+ 5°/min); Carrier gas, N_2, 20 ml/min): R_t 144 sec (2.7%), 355 sec (94.9%). This sample was therefore of 94.9% chemical purity. Further purification was carried out by preparative glc (Column, 15% PEG 20M 2m × 6mm at 110°; Carrier gas, N_2, 1.2 kg/cm^2). The purified sample showed $[\alpha]_D^{23.5}$ + 22.7° (*c* = 0.264, n-hexane); glc (Column, PEG 20M, 50m × 0.25mm at 70°(+ 3°/min); Carrier gas, N_2, 1.0 kg/cm^2): R_t 956–972 sec (100.00%).

(*ii*) (3S, 4S)-*Isomer*. In the same manner as described above 780 mg (76%) of (3*S*, 4*S*)-**1** was prepared from 900 mg of (2*R*, 3*S*)-**9**, b.p. 105–108°/102 mm, n_D^{22} 1.4244; $[\alpha]_D^{22}$ − 20.0° (*c* = 0.769, n-hexane). The IR and NMR spectra were identical with those of (3*R*, 4*R*)-**1**. (Found: C, 73.83; H, 14.06. Calc. for $C_8H_{18}O$: C, 73.78; H, 13.92%). Glc (Column, 5% PEG 20M, 2m × 4mm at 100° (5°/min); Carrier gas, N_2, 20 ml/min): R_t 177 sec (3.4%), 408 sec (94.0%). This sample was therefore of 94.0% chemical purity. Further purification was carried out by preparative glc (Column, 15% PEG 20 M, 2 m × 6 mm at 110°; Carrier gas, N_2, 1.2 kg/cm^2). The purified sample showed $[\alpha]_D^{22}$ − 21.7° (*c* = 0.572, n-hexane); glc (Column, PEG 20M, 50 m × 0.25 mm at 70° (+ 3°/min); Carrier gas, N_2 1.0 kg/cm^2): R_t 1451 sec (99.83%): $n_D^{22.5}$ 1.4262.

Determination of the optical purities of (2S, 3R)-**6** *and* (2R, 3S)-**6**

The both enantiomers of **6** were converted to the corresponding (*S*)-(−)-MTPA esters in the conventional manner. They were analysed by glc and NMR. Glc (Column, 5% FFAP, 1.5 m × 2 mm at 130° (after 26 min + 2°/min to 170°); Carrier gas, N_2, 1.0 kg/cm^2): our (2*S*, 3*R*)-**10**. R_t 44.0 min (99%), 45.8 min (1%); our (2*R*, 3*S*)-**10**. R_t 43.0 min (1%), 46.2 min (99%). Therefore the optical purity of our (3*R*, 4*R*)-**1** and (3*S*, 4*S*)-**1** was 99% by glc. NMR of (2*R*, 3*S*)-**10** (25.3 mg **10** in 0.35 ml CCl_4): δ 3.45 (3H, s, OMe), 3.65 (3H, s, CO_2Me), 5.01 (1H, d, J = 3Hz, CHOMTP). NMR of (2*R*, 3*S*)-**10** in the presence of Eu(fod)$_3$ (67.8 mg **10** + 69.4 mg Eu(fod)$_3$ in 0.35 ml CCl_4): δ 4.02 (3H, s, CO_2Me), 4.66 (3H, s, OMe), 5.88 (1 H, d, J = 3Hz, CHOMTP). NMR of (2*S*, 3*R*)-**10** (55.8 mg **10** in 0.35 ml CCl_4): δ 3.58 (3H, s, OMe), 3.68 (3H, s, CO_2Me), 4.99 (1H, d, J = 3Hz, CHOMTP). NMR of (2*S*, 3*R*)-**10** in the presence of Eu(fod)$_3$ (74.2 mg **10** + 43.2 mg Eu(fod)$_3$ in 0.35 ml CCl_4): δ 3.96 (3H, s, CO_2Me), 4.35 (3H, s, OMe), 5.66 (1H, d, J = 3Hz, CHOMTP). No definite sign of the presence of the other diastereomer was observable in these NMR spectra.

Determination of the optical purities of (3R, 4R)-**1** *and* (3S, 4S)-**1**

The both enantiomers of **1** were converted to the corresponding (*S*)-(−)-MTPA esters in the conventional manner. They were analyzed by NMR. NMR of (3*R*, 4*R*)-**11** in the presence of Eu(fod)$_3$ (66.0 mg **11** + 31.9 mg Eu(fod)$_3$ in 0.35 ml CCl_4): δ 4.80 (3H, s, OMe). NMR of (3*S*, 4*S*)-**11** in the presence of Eu(fod)$_3$ (76.2 mg **11** + 28.4 mg Eu(fod)$_3$ in 0.36 ml CCl_4): δ 4.27 (3H, s, OMe). NMR of (*S*)-(−)-MTPA ester of (±)-**1** in the presence of Eu(fod)$_3$ (67.6 mg sample + 27.0 mg Eu(fod)$_3$ in 0.35 ml CCl_4): δ 4.25 (1.5H, s, OMe), 4.75 (1.5H, s, OMe). No cross-contamination of the other diastereomer was observable by this NMR analysis.

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