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A Preparative Method of DL-threo-3-Isopropylmalic Acid and DL-threo-[2-^{2}H]-3-Isopropylmalic Acid

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A practical preparative method of the titled compounds was exploited, during which unusually erythro-favored aldol condensation of an lower alkyl ester was observed.

One of the major prerequisites for enzyme research is availability of the substrate of an enzyme in question, and so is the case of 3-isopropylmalate dehydrogenase (IPMDH), which is an enzyme involved in the leucine biosynthesis and currently attracts wide attentions from genetic, biochemical as well as evolutionary standpoints. IPMDH is coded by leu B gene in procaryotes or by leu 2 gene in eucaryotes and those genes from various organisms have been cloned[1] and sequenced.[2] Since the substrate threo-D_S-3-isopropylmalic acid (IPM) has been obtained to date either by rather tedious isolation from the culture of a leucine auxotrophic mutant of Neurospora crassa[3] or by non-selective chemical synthesis,[4] an efficient preparative method has been highly desired. Naturally, optically active (2R,3S)-isomer of IPM is the most desirable, currently available approaches to obtain such chiral substrate requires, however, multistep chemical processes or are stereochemically disfavored; e.g. stereoselective syntheses by some chiral enolate methodologies might be a method of choice which would end up with undesired erythro products.[5] Furthermore, because no inhibitory side effects of the inactive enantiomer of the substrate upon the enzyme have been reported so far, racemic DL-threo-3-isopropylmalic acid (**1**) seems practically sufficient for studies on IPMDH. Hence, this communication is intended to describe a practical synthetic access to the racemate of **1**.

A simple aldol condensation of ethyl isovalerate **2** and veratraldehyde **3** was first attempted.[6] Veratraldehyde **3** was so chosen that an oxidative transformation of the aromatic ring into a carboxylic acid functionality at a later stage would be enhanced by the electron-donating methoxyl substituents.

The enolate of **2** generated by LDA in THF at -78 °C was allowed to react with **3** to give in 88% yield a diastereoisomeric mixture with significant selectivity (ca. 6:1), which was separated by flash chromatography.[7,8] The stereochemistry of the major product **4** was suggested by the ^{1}H-NMR spectrum to be erythro geometry,[9,10] which turned out to be undesirable. To confirm the stereochemistry, **4** was reduced

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with $LiAlH_4$ as shown in Fig. 1 to a diol, which was subsequently converted with 2,2-dimethoxypropane/p-TsOH into the 1,3-dioxane derivative **5** (88% yield).[9] The coupling constant (J = 3.5 Hz) of the oxygenated benzylic proton at 5.25 ppm supported the cis stereochemistry between the isopropyl and the aromatic groups (conformation undetermined). It may be emphasized here that this erythro-favored condensation is rather unusual because with lower alkyl esters essentially no stereoselectivity has been reportedly observed.[6] Typically, the Reformatsky reaction of methyl 2-bromoisovalerate with benzaldehyde showed no selectivity.[11]

Since hexamethylphosphoric triamide (HMPT) is known to promote a stereochemical reversal in ester enolization selectivity,[12] the condensation reaction was next conducted in the presence of HMPT. According to the amount of HMPT, the threo-selectivity was gradually improved and acceptable selectivity (ca. 1:4) was achieved in the presence of 2.30 molar equivalent of HMPT. The more polar threo-product **6** (63% yield after purification),[9] was again transformed into an acetonide **7** (84% yield) to confirm the trans-configuration of the substituents on the dioxane ring. Thus, the benzylic oxymethine proton signal was observed at 4.70 ppm as a doublet with J = 11 Hz.

Acetylation of **6** in a standard manner (Ac_2O/Pyridine/DMAP) gave **8** (87% yield).[9] Attempted ozonolysis to carboxylic acid in CH_2Cl_2 at -70 °C was unsuccessful. The dimethoxybenzene ring of **8** was oxidatively degraded with $RuCl_3$ (catalytic) and $NaIO_4$ to give **9** (85% yield).[9,13] Finally, alkaline hydrolysis of **9** gave in 92% yield the desired DL-threo-**1**. The 400 MHz ^{1}H-NMR spectrum was

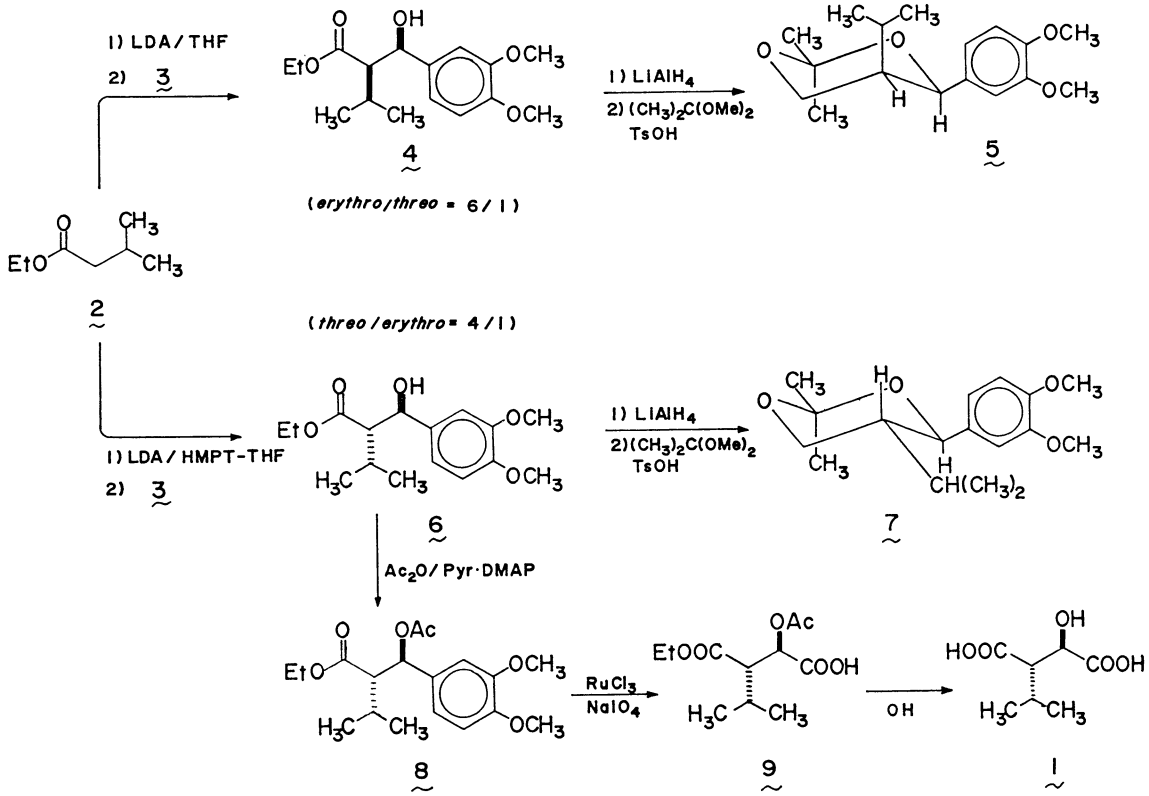

FIG. 1.

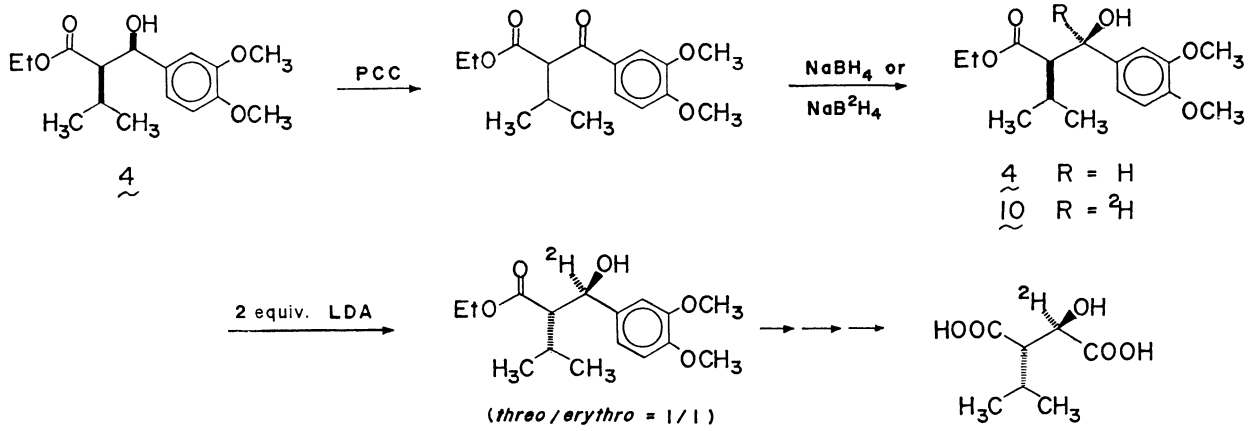

FIG. 2. [^{2}H]-6 11

completely superimposable with a natural compound obtained by the microbiological method.

Conversion of **4** to **6** via oxidation (PCC/CH_2Cl_2, 87%) and reduction ($NaBH_4$/i-PrOH, 92%) gave a mixture in much lower ratio of **6** to **4**. This precluded another route to **1** through alkylation of commercially available benzoylacetate ester.[14] Nevertheless, this oxidation-reduction sequence was undertaken to prepare a deuterated derivative **10** using NaB^2H_4 instead of $NaBH_4$ (Fig. 2). Thermodynamic equilibration through a dianion (LDA in THF) and regeneration afforded a mixture (ca. 1:1) of [3-^{2}H]-**4** and [3-^{2}H]-**6**. Subsequent purification of [3-^{2}H]-**6** followed by transformation described above yielded DL-threo-[2-^{2}H]-3-isopropylmalic acid (**11**), whose ^{1}H-NMR spectrum was identical with that of **1**, except that the C-2 methine signal disappeared and the C-3 methine signal appeared as a doublet. As described in the accompanying paper, **11** was used for the determination of the stereochemistry of the hydride transfer reaction to NAD catalysed by IPMDH.[15]

The present method provides a much simpler way to deal with **1** in quantities, which, we hope, will facilitate further biological studies in this area.

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7) W.C.Still, M.Kahn, and A.Mitra, J. Org. Chem., 43, 2923 (1978).
8) Preparative HPLC (Waters LC-500) was used for large scale synthesis.
9) Physicochemical properties of relevant compounds are as follow; **4**: mp 67.5-68.0 °C; IR(KBr) 3500, 1720, 1605, and 1600 cm^{-1}, EI-MS m/z 296 (M^+), 167, 139, 115, and 31, ^{1}H-NMR ($CDCl_3$) δ^{TMS} 1.00 (3H d, J=7 Hz), 1.04 (3H d, J=7 Hz), 1.10 (3H t, J=7 Hz), 2.30 (1H, m), 2.40 (1H d, J=2.5 Hz), 2.71 (1H dd, J=4.5 and 8 Hz), 3.86 (3H,s), 3.88 (3H,s), 3.96 (2H q, J=7 Hz), 4.90 (1H dd, J=8 and 2.5 Hz) and 6.84 ppm (3H,m); **5**: colorless oil, EI-MS m/z 279 (M^+), ^{1}H-NMR ($CDCl_3$) δ^{TMS} 0.68 (3H d, J=7 Hz), 1.06 (3H d, J=7 Hz), 1.39 (1H dq, J=1.5 and 3.5 Hz), 1.50 (3H,s), 1.55 (3H,s), 3.86 (3H,s), 3.88 (3H,s), 4.04 (1H dd, J=1.5 and 12 Hz), 4.16 (1H dd, J=3.5 and 12 Hz), 5.25 (1H d, J=3.5 Hz) 6.86 (2H, br.s) and 6.90 ppm (1H,s); **6**: colorless oil, EI-MS m/z 296, 167, 139, 115, and 31, IR(neat) 3520, 1730, 1605, 1597, 1520, 1460, 1260, 1240, 1160, 1030, 860, 810 and 760 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ^{TMS} 0.87 (3H d,J=7 Hz), 0.97 (3H d, J=7 Hz), 1.08 (3H t, J=7 Hz), 1.86 (1H sextet, J=7 Hz), 2.46 (1H dd, J=6 and 7 Hz), 3.20 (1H, OH), 3.80 (3H,s), 3.82 (3H,s), 4.83 (1H d, J=6 Hz), 6.76 (2H, br.s) and 6.80 (1H,s); **8**: colorless oil, EI-MS m/z 338 (M^+), 233, 209, 167, and 31, IR(NaCl) 2970, 1740, 1605, 1600, 1522, 1470, 1380, 1240, 1180, 1150, 1035, 865, 815, and 770 cm^{-1}, ^{1}H-NMR ($CDCl_3$) δ^{TMS} 0.92 (6H,br.d), 1.24 (3H t, J=7 Hz), 1.58 (1H,m), 1.92 (3H,s), 2.88 (1H dd, J=4 and 11 Hz), 3.88 (3H,s), 3.90 (3H,s), 4.20 (2H q, J= 7 Hz), 5.96 (1H d, J= 11 Hz), and 6.88 (3H,m); **9**: colorless oil, CI-MS(i-Butane) m/z 247 $(M+H)^+$, 205, 201, and 159, IR(NaCl) 3500, 3200, 3000, 1760, 1740, 1720, 1380, 1230, and 1190 cm^{-1}; and **1**: mp 117-117.5 °C, CI-MS(i-Butane) m/z 177 $(M+H)^+$, 159, 131, and 113, ^{1}H-NMR (D_2O) $\delta^{HDO=4.8\ ppm}$ 0.95 (3H d, J= 6.7 Hz), 0.98 (3H d, J= 6.7 Hz), 2.10 (1H,m), 2.52 (1H dd, J= 4.3 and 8.9 Hz) and 4.36 (1H d, J= 4.2 Hz), ^{13}C-NMR (D_2O) $\delta^{Dioxane=67.4\ ppm}$ 20.4, 20.8, 27.5, 56.9, 70.9, 178.3 and 178.8 ppm.
10) The conventional erythro/threo notation is coincident with the Heathcock's in this case. See W.A.Kleschick, C.T.Buse, and C.H.Heathcock, J. Am. Chem. Soc., 99, 247 (1977).
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14) $NaBH_4$ reduction of methyl 2-benzoylvalerate was reported to give a mixture (erythro/threo 66:34). See J.Canceill and J.Jacques, Bull. Soc. Chim. Fr., 1970, 2180.
15) NMR spectra were taken by a JEOL PS-100, FX-200, or GX-400 spectrometer. Mass spectra were obtained by a Hitachi M-80 or a Shimadzu LKB-9020DF spectrometer.

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