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Stereochemistry of Some Acetyl Coenzyme A Condensations

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The absolute configuration of four naturally occurring α-alkylmalic acids (ethyl, *n*-propyl, isopentyl, and benzyl) has been established by synthesis, using the addition of alkyl lithium cuprates to the (R)-(+)-epoxide of dimethyl itaconate. It is concluded that α-alkylmalic acids biosynthesized from acetyl-CoA and α-ketoacids are, with few exceptions, formed by attack at the *re* face of the carbonyl group to generate (R)-(−) enantiomers.

INTRODUCTION

A well-recognized reaction of acetyl coenzyme A in biological systems is nucleophilic addition to the carbonyl group of α-ketoacids to afford α-alkylmalic acids (*1*) (Eq. (1)). Table 1 lists the naturally occurring α-alkylmalic acids. Malic and citric acids are, of

$$CH_3COSCoA + R\text{-}CO\text{-}COOH \longrightarrow R\text{—}\underset{\displaystyle COOH}{\overset{\displaystyle OH}{\overset{|}{\underset{|}{C}}}}\text{—}CH_2COOH \qquad (1)$$

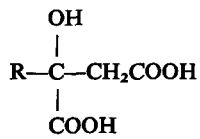
course, intermediates in basic metabolic cycles, while homocitric acid is an intermediate in lysine biosynthesis and α-isopropylmalic acid is the first intermediate in leucine biosynthesis. Some of these acids occur as esters of hydroxylated alkaloids: α-isobutylmalic and α-benzylmalic acids from the pyrrolizidine alkaloids cornucervine (*2*) and phalaenopsine (*3*) respectively, α-isopentylmalic acid from the antitumor alkaloid deoxyharringtonine (*4*), and the related acids X and XI from other *Cephalotaxus* alkaloids. 2-(4-Hydroxybenzyl)-malic acid (eucomic acid) XIII was isolated recently from both *Petalostemon gattingeri* and *Eucomis punctata L'Hérit.* (*5*).

For III–VII, clear evidence is available that the natural acids are constructed enzymatically according to Eq. (1), although alternate routes such as hydration or glyoxylate condensations (*6*) are also possible. Nothing is yet known about the biosynthesis of acids VIII–XIII, though it is logical to assume that they too are synthesized from acetyl coenzyme A since the requisite ketoacids for VIII, IX, XII, and XIII are known to occur in biological systems.

A new asymmetric center is created during the enzymatic reaction of Eq. (1), and most of the acids in Table 1 for which optical rotations have been measured appear to occur in nature as single enantiomers. Two steric courses possible for the condensation are shown below; acetyl coenzyme A may attack either the *si* or *re* face (*14*) of the ketoacid, leading to (S) or (R) products.

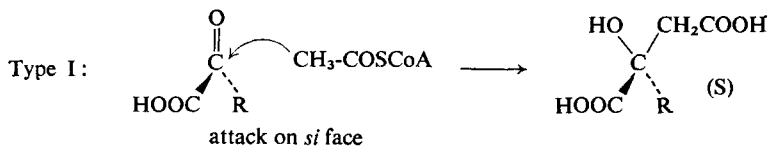
TABLE 1

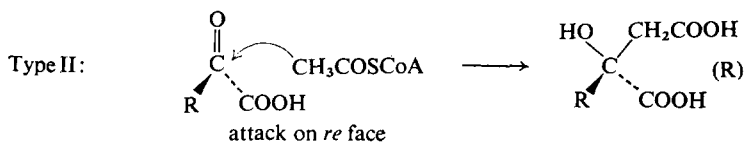
NATURALLY OCCURRING α-ALKYLMALIC ACIDS



I.	R = H	Malic	(*7*)
II.	R = CH_3	Citramalic	(*8*)
III.	R = C_2H_5	α-Ethylmalic	(*9*), (*10*)
IV.	R = CH_2COOH	Citiric	(*11*)
V.	R = *n*-C_3H_7	α-Propylmalic	(*10*)
VI.	R = CH_2CH_2COOH	Homocitric	(*12*)
VII.	R = $CH(CH_3)_2$	α-Isopropylmalic	(*13*)
VIII.	R = $CH_2CH(CH_3)_2$	α-Isobutylmalic	(*2*)
IX.	R = $CH_2CH_2CH(CH_3)_2$	α-Isopentylmalic	(*4*)
X.	R = $CH_2CH_2C(CH_3)_2OH$		(*4*)
XI.	R = $CH_2CH_2CH_2C(CH_3)_2OH$		(*4*)
XII.	R = $CH_2C_6H_5$	α-Benzylmalic	(*3*)
XIII.	R = CH_2-p-C_6H_4OH	Eucomic	(*5*)

Bentley (*15*) has classified the enzymes which catalyze attack of an acyl CoA unit on the *si* face of the ketoacid as Type I, and those which catalyze attack on the *re* face as Type II. Examples of Type I enzymes are those which catalyze the synthesis of (S)-(−) malic acid, (S)-(+) citramalic acid (*16*), and citric acid (in animals and most bacteria).





Type II enzymes are involved in the biosynthesis of citric acid in certain bacteria and (R)-(−) homocitric acid (*17*). Eggerer (*1*) has suggested that the replacement of magnesium ion in Type I systems by another Lewis acid constitutively present in the protein is responsible for inverting the direction of attack. The stereochemistry at the *methyl* group of acetyl CoA has been studied with the elegant use of chiral methyl groups (*18*); inversion of configuration takes place at methyl.

In order to ascertain whether the enzyme stereospecificity is related to the size of the

group R in the ketoacid or other factors, it would be helpful to know the absolute configurations of the remaining acids in Table 1. At the time we initiated this project, the configurations of III, V, and VII–XIII were all unknown; during the course of our work, Brandänge and his co-workers proved the configurations of III and XII as (S)-(+) by unambiguous chemical correlation (*19*) and assigned (S)-(+) configurations to VII–IX and XI as well by comparison of ORD curves of molybdate complexes (*19*, *20*). The opposite assignment, (S)-(−), has been made to natural VII on the basis of anomalous X-ray dispersion measurements (*21*). Heller and Tamm (*5*) have proven unambiguously that eucomic acid (XIII) is the (R)-(−)-enantiomer. This paper reports the determination of absolute configuration of acids III, V, IX, and XII by synthesis from a common intermediate of known configuration.

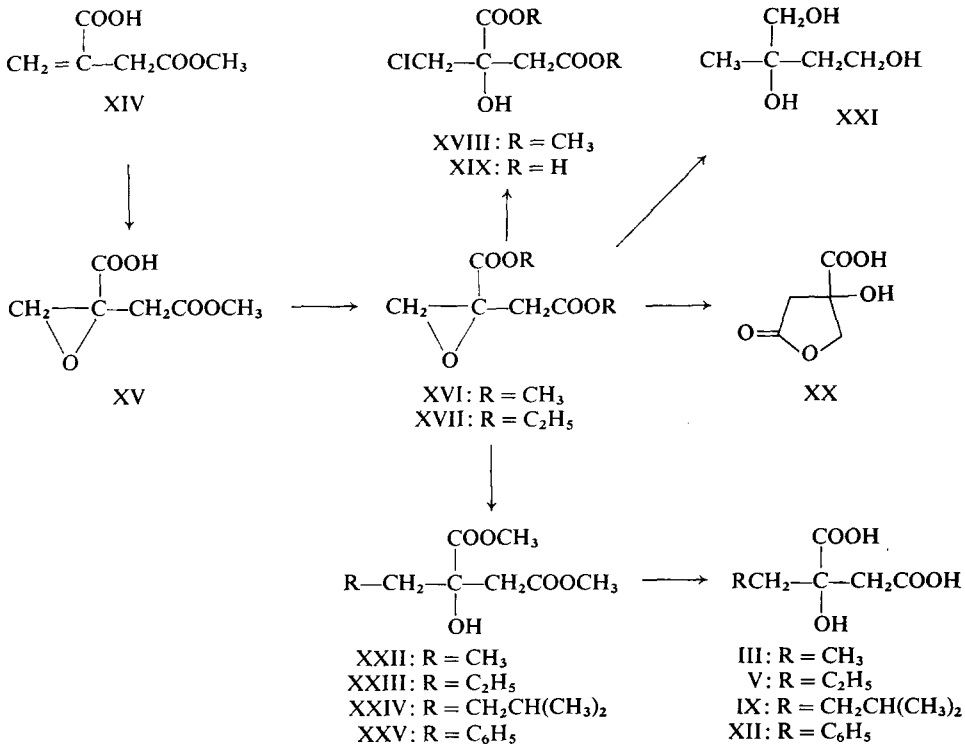
METHODS AND RESULTS

It was found that the optically active malic acids III, V, IX, and XII could easily be prepared from a common precursor, the epoxide XVI, using the reaction with dialkyl lithium cuprates. Weinreb had already reported the use of this method to synthesize racemic α-isopentylmalic acid (*22*) and Brandänge independently used optically active XVI to prepare several α-alkylmalic acids (*19*).

To prepare the optically active epoxide XVI, we first attempted an asymmetric peroxidation of itaconate esters with percamphoric acid, but like most epoxidations reported with chiral peracids (*23*) this gave very low optical yields. A satisfactory preparation of XVI was epoxidation of the half ester XIV with pertrifluoroacetic acid, resolution of epoxide XV with cinchonidine, and methylation with diazomethane. The absolute configuration of (+)-XVI was established unambiguously as (R) by two independent transformations: (a) although heating the epoxide with aqueous acid led to the known butyrolactone XX, treatment with gaseous HCl at room temperature gave (S)-(+)-dimethyl chlorocitramalate (XVIII), hydrolyzed to (S)-(+)-chlorocitramalic acid (*24*); (b) lithium aluminum hydride reduction of (+)-XVI gave (S)-(−)-2-methyl-1,2,4-butanetriol (XXI), characterized as its crystalline bis-*p*-nitrobenzoate (*16*). These results confirm the configuration of the epoxide (*19*) as (R)-(+). From the magnitude of the rotation of XIX the optical purity of XVI can be estimated as about 88%. In his studies Brandänge (*19*) had used an epoxide of 14% optical purity, obtained by asymmetric synthesis.

Reaction of epoxide XVI with the lithium cuprates prepared from methyl, ethyl, isobutyl, and phenyl lithium, respectively, led smoothly in 62–87% yields to the malate esters XXII–XXV, which were hydrolyzed to the crystalline α-alkylmalic acids by hydrochloric acid. When the (R)-(+)-epoxide was used, all four malic acids III, V, IX, and XII, as well as their dimethyl esters, were obtained as dextrorotatory isomers.

This synthesis from (R)-(+)-XVI, without affecting the configuration at the chiral center, establishes the configurations of all four α-alkylmalic acids as (S)-(+). The result agrees with the findings of Brandänge for III, IX, and XII, and with the (S)-(+) configuration assigned to the *Cephalotaxus* acid IX by Weinreb and Klyne on the basis of CD measurements (*25*).



Several conclusions may be drawn from these results and those of Brandänge (*19*, *20*):

1. Levorotatory α-alkylmalic acids have the (R) configuration XXVI when the alkyl group is methyl, ethyl, *n*-propyl, *n*-butyl, isobutyl, isopentyl, β-carboxyethyl, benzyl and *p*-hydroxybenzyl, and it seems safe to conclude that this will hold true for any primary alkyl group. Levorotatory α-isopropylmalic acid also has the absolute configuration represented by XXVI, though due to the strictures of the configurational

convention it is the (S) enantiomer; Brandänge's erroneous assignment of the (R) configuration to (−)-α-isopropylmalic acid based on CD comparison with other alkylmalic acids (*19*) is apparently due to this notational confusion rather than to a chemical error. Thus it is likely that all α-alkylmalic acids of the configurational series XXVI are levorotatory. It is of interest that the related series of simple levorotatory alkylsuccinic acids have the similar configuration XXVII (*28*), although for primary alkyl groups this is (S), again due to the configurational convention.

2. Of the naturally occurring α-alkylmalic acids, it can now be seen that those with

primary alkyl groups of three or more carbons are all levorotatory and thus belong to the (R) series XXVI; the natural (–)-α-isopropylmalic acid also has configuration XXVI.

At first sight citramalic acid (II) does not fit this generalization, since the common acid of nature is dextrorotatory. However, the (+) acid is that formed by the mesaconase-catalyzed hydration of mesaconic acid, not by acetyl coenzyme A condensation. However, Sai *et al.* (*29*) have subsequently shown that the acid formed from pyruvate and acetyl CoA by respiration-deficient mutants of brewers' yeasts is (–)citramalate. Moreover, the citramalate condensing enzyme was found to be identical with the α-isopropylmalate synthetase of leucine biosynthesis (*29*), and it has a broad substrate specificity which includes α-ketobutyrate (*29*) and α-ketovalerate (*9*, *10*), as well as pyruvate and α-ketoisovalerate. The majority of the α-alkylmalic acids formed by acetyl coenzyme A condensations thus appear to be constructed by identical or similar enzymes, and one can reasonably predict that the α-ethyl and α-*n*-propylmalic acids formed in cultures of bacteria and bakers' yeast (*9*, *10*), for which optical rotations were not measured, will prove to be levorotatory like their naturally-occurring homologs. Except for mammalian citrate synthase and citrate cleavage enzyme, in which acetyl CoA attacks the *si* face of the keto acid, α-alkylmalic acids biosynthesized from acetyl CoA and α-ketoacids are assembled by Type II enzymes with attack at the *re* face of the carbonyl group. The finding of Strassman and Ceci (*9*) that the condensing enzyme for most of the alkylmalic acid syntheses is different from the mammalian citrate synthase lends support to the conclusion that while the type II enzymes have broad substrate specificity, they lead to levorotatory α-alkylmalic acids of configuration XXVI, and a different enzyme is required to achieve the opposite configuration.

EXPERIMENTAL SECTION

Melting points were determined on a Thomas–Hoover apparatus and are uncorrected. Nmr spectra were recorded on a Varian HA-100 spectrometer; chemical shifts are reported in δ units, with tetramethylsilane as an internal standard. Infrared spectra were recorded on a Perkin–Elmer Model 257 spectrometer. Optical rotations were determined on a Perkin–Elmer Model 141 polarimeter; *c* is expressed as grams per 100 ml of solution. Elemental analyses were performed at Galbraith Laboratories, Knoxville, Tenn., Atlantic Microlab, Inc., Atlanta, Ga., and the University of Georgia.

Percamphoric acid oxidation of diethyl itaconate. Diethyl itaconate (4.40 g) was added to 0.034 mole of monopercamphoric acid (*26*) in 50 ml of chloroform. After stirring 4 days at room temperature, the solution was poured into 10% $NaHCO_3$ and extracted with ether. Distillation gave 4.34 g (92%) of ethyl β, γ-epoxy-β-carbethoxybutyrate (XVII), D_{25} 1.0870, $[\alpha]_D^{21}$ –0.088° (neat); the ir and nmr spectra were identical with those of an authentic sample prepared by pertrifluoroacetic acid oxidation (*24*).

β-Carboxy-β-hydroxy-γ-butyrolactone (*XX*). A solution of 2.50 g of epoxide XVII in 35 ml of water and 3 ml of 2 *M* H_2SO_4 was refluxed for 6 hr. The water was removed under reduced pressure and the residue refluxed with 50 ml of absolute ethanol for 10 hr. The solution was neutralized with aqueous $NaHCO_3$, concentrated to remove the ethanol, and continuously extracted with chloroform for 24 hr. Distillation of the

extracts gave 1.79 g (83%) of the *ethyl ester* of XX, bp 120–132°C (0.05 mm, Kugelrohr); nmr ($CDCl_3$): 4.35 (4H, two quartets), 4.0 (s, 1H, hydroxyl), 3.05 and 2.65 (AB quartet, 2H, $J = 17.6$ Hz), and 1.29 (t, 3H, $J = 6.5$ Hz); mass spec: $M^+ = 174$.

Anal. Calcd. for $C_7H_{10}O_5$: C, 48.28; H, 5.79. Found: C, 47.99; H, 5.85.

The *free acid* (XX) was obtained by stirring 3.50 g of the ester with 40 ml of concentrated HCl at room temperature for 24 hr and then concentrating the solution to dryness. The oily acid (2.65 g, 90%), had infrared and nmr spectra identical with those reported (*24*).

The *methyl ester*, prepared with ethereal diazomethane in 96% yield, had bp 103–107°C (0.05 mm); nmr: 4.55 and 4.32 (AB quartet, 2H, $J = 10$ Hz), 3.82 (s, 3H), 3.65 (s, 1H, hydroxyl), 3.10 and 2.58 (AB quartet, 2H, $J = 18$ Hz); mass spec: $M^+ = 160$.

Anal. Calcd. for $C_6H_8O_5$: C, 45.01; H, 5.04. Found: C, 45.25; H, 5.30.

Methyl β,γ-epoxy-β-carboxybutyrate (XV). To 4.35 g of trifluoroacetic anhydride in 20 ml of methylene chloride was added 0.692 g of 98% hydrogen peroxide. After stirring for 1 hr, 2.0 g of methyl 3-carboxy-3-butenoate (XIV) (*27*) in 50 ml of methylene chloride was added and the solution refluxed for 4 hr. The solution was concentrated at reduced pressure and the residue taken up in ether, filtered, and concentrated to yield 2.27 g of acid XV; ir (neat) 3200 (br), 1735, 1720, and 1180 cm^{-1}; nmr ($CDCl_3$): 9.4 (s, 1H), 3.69 (s, 3H), and 2.95 (two overlapping quartets, 4H).

Resolution of acid XV. The acid (2.27 g) was heated with 4.54 g of cinchonidine in acetone until dissolution occurred; the salt which precipitated on cooling was collected and recrystallized four times from aqueous acetone. The *cinchonidine salt* had mp 169–170°C, $[\alpha]_D^{16}$ −54.47°C (H_2O, $c = 2.17$).

Anal. Calcd. for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65. Found: C, 65.98; H, 6.63.

An aqueous solution of 1.166 g of the cinchonidine salt was passed through 13 g of AG 50W-X4 Bio-Rad cation exchange resin, hydrogen form, 200–400 mesh. The eluant was concentrated to afford 0.415 g of the acid, $[\alpha]_D^{18}$ 24.44° ($CHCl_3$, $c = 4.61$).

The *methyl ester* (XVI), prepared with diazomethane in methanol, distilled at 80–90°C (3 mm, Kugelrohr); ir (neat): 2960, 1735, 1198, and 750 cm^{-1}; nmr ($CDCl_3$): 3.75 (s, 3H), 3.65 (s, 3H), 2.95 (two overlapping quartets, 4H); mass spec: $M^+ = 174$; $[\alpha]_D^{20}$ 12.75° ($CHCl_3$, $c = 1.2$).

Anal. Calcd. for $C_7H_{10}O_5$: C, 48.28; H, 5.79. Found: C, 48.21; H, 5.65.

Absolute Configuration of Methyl β, γ-Epoxy-β-carbomethoxybutyrate (XVI)

A. By conversion to chlorocitramalic acid. A benzene solution of 400 mg of epoxide XVI, $[\alpha]_D$ 12.75°, was saturated with gaseous HCl. The resulting solution was stirred 24 hr and distilled to give 440 mg of (S)-(+)-dimethyl chlorocitramalate (XVIII), bp 130–140°C (0.05 mm, Kugelrohr); ir (neat): 3505, 2960, 1740, and 1205 cm^{-1}; nmr (CCl_4): 3.91 (s, 3H), 3.75 (s, 2H), 3.70 (s, 3H), 2.85 and 2.26 (AB quartet, 2H, $J = 15.2$ Hz); $[\alpha]_D^{18}$ 4.0° ($CHCl_3$, $c = 7.87$); lit. (*24*) $[\alpha]_D^{25}$ 4.0° ($CHCl_3$).

Anal. Calcd. for $C_7H_{11}ClO_5$: C, 39.92; H, 5.26. Found: C, 40.15; H, 5.33.

Hydrolysis following the published procedure (*24*) gave (S)-(+)-chlorocitramalic acid (XIX), $[\alpha]_D^{22}$ 6.59° (H_2O, $c = 2.79$); lit. $[\alpha]_D^{25}$ 7.5° (H_2O, $c = 1.0$). The ir, nmr, and mass spectra were identical with those of an authentic sample prepared according to the procedure of Suh and Hite (*24*).

B. By conversion to 2-methyl-1,4,5-butanetriol (XXI). To a solution of 0.155 g of

$LiAlH_4$ in ether was added 193.6 mg of epoxide XVI, $[\alpha]_D$ 12.75°. The mixture was refluxed for 24 hr, cooled and hydrolyzed by the dropwise addition of 0.15 ml of water, 0.15 g of 15% NaOH, and 2 ml of water. The solid was filtered and washed with hot dioxane and the combined filtrate and washings distilled, affording 110 mg (82%) of the triol XXI, bp 120–130°C (0.03 mm, Kugelrohr); ir (neat): 3350, 2950, and 1055 cm^{-1}; nmr (D_2O): 3.79 (t, 2H), 3.45 (s, 2H), 1.8 (t, 2H), 1.25 (s, 3H); $[\alpha]_D^{19}$ −2.80° (H_2O, c = 5.24); lit. (*16*) $[\alpha]_D^{19}$ −1.7° (H_2O, $c = 3.3$).

The *bis-p-nitrobenzoate* was prepared following the procedure described by Weber (*16*), chromatographed with 9:1 benzene–ethyl acetate over silica gel, and crystallized from chloroform; mp 126–128°C, $[\alpha]_D^{23}$ 9.4° ($CHCl_3$, $c = 1.86$); Weber reports mp 128°C, $[\alpha]_D^{25}$ 12.5°.

General Procedure for Dialkyllithium Cuprate Additions.

All reactions were carried out under dry nitrogen with careful exclusion of oxygen and moisture. A solution of 2.0 mmoles of cuprous iodide (cuprous bromide was used in the reaction with phenyl lithium) in 20 ml of ether was cooled to −10 to −30°C and treated with 4.0 mmole of the alkyl or aryl lithium (normally the 1.4 to 1.8 *M* solutions from Foote Mineral Co., except for isobutyl lithium, which was freshly prepared). After stirring for 5 min, 1.0 mmole of epoxide XVI was added and the solution stirred for 30 min. The reaction mixture was hydrolyzed by stirring 30 min with 5–10 ml of saturated NH_4Cl solution and extracted with ether. The extracts were washed with saturated brine, dried over Na_2SO_4, concentrated and distilled.

α-*Ethylmalic acid* (*III*). Following the general procedure above with racemic epoxide XVII and methyl lithium, *diethyl α-ethylmalate*, bp 80–90°C (0.3 mm), was isolated in 85% yield; ir (neat): 3500, 2980, 1745, and 1190 cm^{-1}; nmr ($CDCl_3$): 4.2 (two quartets, 4H), 3.70 (s, 1H, hydroxyl), 2.91 and 2.62 (AB quartet, 2H, J = 16 Hz), 1.72 (q, 2H), 1.29 (two triplets, 6H), and 0.88 (t, 3H); mass spec: $M^+ = 218$.

Anal. Calcd. for $C_{10}H_{18}O_5$: C, 55.03; H, 8.31. Found: C, 54.89; H, 8.26.

The ester (0.159 g) was heated under reflux with 20 ml of concentrated HCl for 4 hr, the reaction mixture concentrated at reduced pressure, and the solid residue crystallized from ethyl acetate to afford 0.112 g (95%) of *α-ethylmalic acid* (III), mp 118–119°C (lit. (*10*) mp 117°); ir (KBr): 3100 (br), 1695 cm^{-1}; nmr (D_2O): 3.03 and 2.69 (AB quartet, 2H, J = 16 Hz), 1.70 (q, 2H), and 0.88 (t, 3H).

Repeating the synthesis with (R)-(+)-epoxide XVI, $[\alpha]_D^{20}$ 12.75°, gave (*S*)-(+)-*dimethyl α-ethylmalate* (*XXII*), bp 90–100°C (0.3 mm, Kugelrohr) in 75% yield; ir (neat): 3540, 2990, 1740, and 1200 cm^{-1}; nmr ($CDCl_3$): 3.80 (s, 1H, hydroxyl), 3.79 (s, 3H), 3.68 (s, 3H), 2.95 and 2.65 (AB quartet, 2H, J = 15 Hz), 1.69 (q, 2H), and 0.88 (t, 3H) $[\alpha]_D^{25}$ 17.7° ($CHCl_3$, $c = 3.68$).

Anal. Calcd. for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.35; H, 7.40.

Hydrolysis as described above gave (S)-(+)-α-*ethylmalic acid* (III) in 81% yield. The acid was purified by chromatography over silica gel using benzene:ethyl acetate (85:15) and recrystallized from ethyl acetate–hexane. The pure acid had mp 145–146°C, $[\alpha]_D^{23}$ 15.2° (H_2O, $c = 1.33$).

Anal. Calcd. for $C_6H_{10}O_5$: C, 44.45; H, 6.22. Found: C, 44.19; H, 6.12.

α-*Benzylmalic acid* (*XII*). Following the general procedure above on twice the scale with racemic epoxide XVII and phenyl lithium, the crude product was chromatographed

in hexane over silica gel. Biphenyl was eluted in the first 100 ml, and the product was then eluted with ethyl acetate. Distillation gave 362 mg (65%) of *diethyl α-benzylmalate*, bp 130–140°C (0.05 mm); ir (neat): 3510, 1740, and 1205 cm^{-1}; nmr ($CDCl_3$): 7.18 (s, 5H), 4.15 (two quartets, 4H), 3.70 (s, 1H, hydroxyl), 2.98 and 2.60 (AB quartet, 2H, $J = 15.5$ Hz), 2.90 (s, 2H), and 1.15 (two triplets, 6H); mass spec: $M^+ = 262$.

Anal. Calcd. for $C_{15}H_{20}O_5$: C, 64.27; H, 7.19. Found: C, 64.15; H, 7.28.

The ester (146 mg) was hydrolyzed as described above and the crude acid recrystallized from ethyl acetate–hexane to afford 95.6 mg (82%) of *α-benzylmalic* acid (XII), mp 174–176°C; ir (KBr): 3500, 3000, (br), 1720, 1240; nmr (D_2O): 7.29 (s, 5H), 3.10 and 2.68 (AB quartet, 2H, $J = 17$ Hz), and 3.0 (d, 2H).

Repeating the synthesis with the (R)-(+)-epoxide XVI, $[\alpha]_D^{20}$ 12.75°, gave (S)-(+)-dimethyl α-benzylmalate (XXV), bp 130–135°C (0.05 mm), in 62% yield; ir (neat): 3530, 1745, and 1210 cm^{-1}; nmr ($CDCl_3$): 7.19 (s, 5H), 3.68 (s, 3H), 3.58 (s, 3H), 2.98 and 2.62 (AB quartet, 2H, $J = 15.5$ Hz), and 2.92 (s, 2H); mass spec: $M^+ = 234$; $[\alpha]_D^{24}$ 9.81° (ethanol, $c = 7.84$).

Hydrolysis of 200 mg of the ester gave 138 mg (78%) of *(S)-(+)-benzylmalic acid* (XII), mp 156–157°C (ethyl acetate-hexane), $[\alpha]_D^{23}$ 15.55° (H_2O, $c = 2.9$).

Anal. Calcd. for $C_{11}H_{12}O_5$: C, 58.93; H, 5.39. Found: C, 58.82; H, 5.37.

α-Propylmalic acid (V). The general procedure above was followed with racemic epoxide XVII and ethyl lithium, giving *diethyl α-propylmalate*; ir (CCl_4) 3335, 1740, and 1185 cm^{-1}; nmr (CCl_4): 4.17 (two quartets, 4H), 3.43 (s, 1H, hydroxyl), 2.86 and 2.51 (AB quartet, 2H, $J = 14$ Hz), and 1.9–0.73 (m, 13H). Hydrolysis was effected by stirring overnight with concentrated HCl, then diluting with half the volume of water and refluxing 12 hr. The solvents were removed at reduced pressure and the solid slowly crystallized from chloroform–hexane, yielding α-propylmalic acid (V), mp 122–124°C; ir (CCl_4): 3500–2500 (br), 1730, 1260–1170 (br) cm^{-1}; nmr (D_2O): 3.12 and 2.78 (AB quartet, 2H, $J = 16$ Hz), 1.94–0.87 (m, 7H).

Repeating the synthesis with 0.5 g of (R)-(+)-epoxide XVI, $[\alpha]_D^{24}$ 6.51°, afforded 0.51 g (87%) of *(S)-(+)-dimethyl α-propylmalate* (XXIII), bp 95–100°C (0.5 mm, Kugelrohr); ir (CCl_4): 3540, 2960, 2880, 1740, and 1170 cm^{-1}; nmr (CCl_4): 3.74 (s, 3H), 3.62 (s, 3H), 3.40 (s, 1H), 2.82 and 2.54 (AB quartet, 2H, $J = 16$ Hz), 1.74–0.87 (m, 7H); $[\alpha]_D^{27}$ 13.54° ($CHCl_3$, $c = 3.44$). Hydrolysis with HCl as described above gave (S)-(+)-α-propylmalic acid (V), mp 133–135°C (hexane–chloroform); $[\alpha]_D^{31}$ 10.3° (H_2O, $c = 1.48$).

Anal. Calcd. for $C_7H_{12}O_5$: C, 47.73; H, 6.82. Found: C, 47.70; H, 6.83.

α-Isopentylmalic acid (IX). The general procedure above was followed using the racemic epoxide XVII and freshly prepared isobutyl lithium, affording *diethyl α-isopentylmalate (22)*, bp 110–118°C (0.3 mm, Kugelrohr), in 62% yield; ir (CCl_4): 3510, 2969, 1735, and 1180 cm^{-1}; nmr (CCl_4): 4.21 (m, 6H), 3.50 (s, 1H), 2.85 and 2.55 (AB quartet, 2H, $J = 14$ Hz), 1.47 (m, 11H), and 0.88 (d, 6H). The ester was hydrolyzed with HCl as described for the α-propylmalic acid, and the solid acid (IX) recrystallized slowly from hexane–chloroform, mp 122–124°C; ir (KBr): 3480, 3400–2700 (br), 1710 cm^{-1}; nmr (D_2O): 3.50 and 3.17 (AB quartet, 2H, $J = 16$ Hz), 2.10 (m, 5H), and 1.32 (d, 6H).

Repeating the synthesis with (R)-(+)-epoxide XVI, $[\alpha]_D$ 6.51°, gave a 74% yield of (S)-(+)-dimethyl α-isopentylmalate, bp 95–110°C (1.2 mm, Kugelrohr); ir (neat):

3510, 2960, 1745, 1440, 1220, and 1175 cm^{-1}. Acid hydrolysis gave 89% of (S)-(+)-α-isopentylmalic acid (IX), mp 136–138°C (hexane–chloroform); $[\alpha]_D^{34}$ 13.9° (H_2O, $c = 0.72$).

Anal. Calcd. for $C_9H_{16}O_5$: C, 52.94; H, 7.84. Found: C, 52.89; H, 7.87.

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