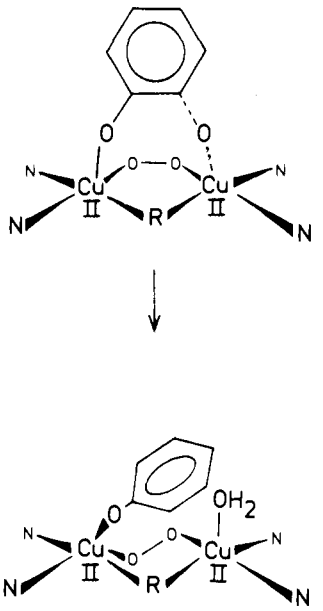

Figure 8. Proposed interaction of the oxy site effective structure with monophenolic (—) and diphenolic (---) substrates.

hemocyanins are consistent with this picture and certain hemocyanins exhibit low phenolase activity.[22] Finally, ligand displacement of peroxide by a substrate inhibitor supports the idea that a ternary complex is formed between the oxy "type 3" copper site and the substrate. Figure 8 demonstrates how the effective structure of the oxy "type 3" site is well-suited for reactions[23] with *o*-diphenol and monophenol under normal enzymatic turnover. In the case of catecholase activity, the "type 3" site is geometrically correct for coordination of both phenolic oxygens in ortho positions axially to both coppers with a M-M distance of ~3.6 Å. Oxidase activity is not observed with *m*- and *p*-phenols.[24,25] For cresolase activity, coordination of the monophenol to one copper would be followed by a rearrangement to a trigonal-bipyramidal intermediate as generally associated with square-planar and tetragonal associative substitution chemistry. This has the effect of labilizing the peroxide from one copper leaving a geometrically correct ortho-substrate-position-activated peroxide complex. Orientation of the monophenolic substrate by amino acid side chains constituting the active site is certainly another important factor for the observed specificity in hydroxylation. This deprotonated peroxide coordinated to only one copper should be extremely reactive and either directly hydroxylate the substrate or provide an oxene intermediate through the heterolytic polarization of the peroxide by coordination to one copper(II).

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Stereospecificity of Enzymatic Dehydrogenation during Tiglate Biosynthesis

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Abstract: (2*RS*,3*S*,4*S*)-[2-^{14}C,4-$^{3}H_1$]Isoleucine has been prepared by stereospecific synthesis, using LiAl[$^{3}H_4$] reduction of optically pure 2-butene oxide to introduce tritium stereospecifically at the prochiral methylene group of 2-butanol. The tritiated alcohol was carried forward in a malonic ester synthesis with ^{14}C-labeled dimethyl malonate, followed by Schmidt reaction of the *sec*-butyl malonic acid, to afford doubly labeled isoleucine. Feeding this substrate to *Datura innoxia* plants led to isolation of 3α,6β-ditigloyloxytropane, from which tiglic acid was obtained by hydrolysis and degraded to locate the labels. More than 95% of the tritium was retained in the tiglic acid, allowing the conclusion that the enzymatic dehydrogenation involves antiperiplanar elimination of the hydrogen at C-2 and the *pro-R* hydrogen at C-3 of (2*S*)-2-methylbutanoic acid.

Metabolism of isoleucine,[2] like that of other branched-chain amino acids, begins with transamination to the α-ketoacid followed by oxidative decarboxylation, yielding 2-methylbutanoyl coenzyme A (scheme I). The next metabolic step is enzymatic dehydrogenation to tigloyl coenzyme A. This dehydrogenation occurs in plants and microorganisms as well as in animals. It has been shown in the laboratories of Leete[3] and Wooley[4] that the tiglic acid component of the tropane ester alkaloids is derived specifically from isoleucine and 2-methylbutanoic acid in *Datura meteloides*, and Crout has shown[5] that isoleucine is the precursor of the geometric isomer angelic acid in *Cynoglossum officinale*. Neither the 2-hydroxy nor the 3-hydroxy derivatives of 2-methylbutanoic acid serve as precursors to tiglate,[4a,6] and angelate is not converted

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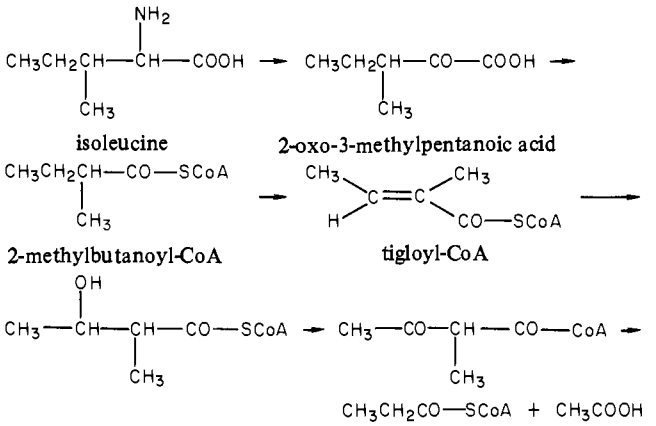
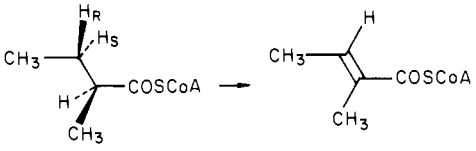
Scheme I. Metabolism of Isoleucine



to tiglate in *Datura* species,[7] so the plant apparently contains an enzyme capable of catalyzing the direct dehydrogenation of 2-methylbutanoyl coenzyme A. Several other natural products are derived by dehydrogenation of isoleucine.[8-10]

The known acyl-CoA dehydrogenases require flavin coenzymes. Hamilton has proposed an attractive mechanism in which both hydrogens are transferred as protons and the substrate forms a covalent compound with the flavin ring system,[11] while Walsh has argued that these desaturations proceed by proton-hydride mechanisms.[12] Regardless of detailed mechanism, an interesting stereochemical question arises when the proton removed from either the α- or β-carbon is initially one of the nonequivalent hydrogens of a prochiral methylene group (eq 1). Detachment

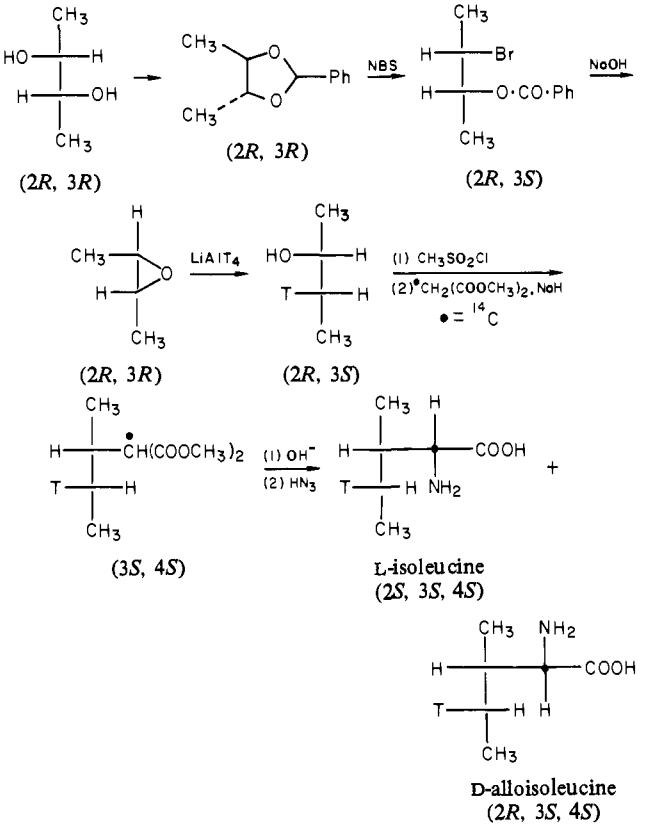


(1)

by a chiral enzyme should lead to stereospecificity in the selection of the proton. Indeed, several acyl-CoA dehydrogenases have been demonstrated to act in a totally stereospecific manner. Dehydrogenation of butanoyl-CoA by the butyric dehydrogenase of swine liver[13] specifically removes the *pro-R* hydrogens at both C-2 and C-3, while dehydrogenation of isobutyryl-CoA by *Pseudomonas putida*[14] or by the isobutyryl dehydrogenase of rats[15] removes the α-hydrogen along with a β-hydrogen specifically from the *pro-S* methyl group.

In this paper we report a study of the stereospecificity of tiglate formation in *Datura innoxia* from specifically labeled isoleucine.

Scheme II. Preparation of Chirally Tritiated Isoleucine



Synthesis of Labeled Isoleucine

L-Isoleucine chirally deuterated at C-4 has recently been prepared biosynthetically from labeled threonine or α-aminobutyric acid.[16] For a chemical synthesis, the malonic ester[17] or acetamidocyanoacetate[18] synthesis of isoleucine from 2-butyl halides or sulfonates appeared to be especially suitable for the preparation of samples specifically labeled at C-4, since malonic ester displacements on secondary halides occur cleanly with inversion of configuration, and the requisite 2-butyl halides or sulfonates with a hydrogen isotope of fixed configuration at C-3 are accessible by several routes. One possibility is the hydroboration of *cis*-2-butene with the reagent derived from B_2D_6 and (+)-α-pinene to give (2*R*,3*S*)-3-deuterio-2-butanol,[19] but the product of this reaction was reported to be only 56% optically pure, too low for our purpose. An alternate method which also fixes the relative configuration at the two chiral centers is hydride reduction of 2-butene oxide. (−)-(2*R*,3*S*)-[3-$^2H_1$]- or [3-$^3H_1$]-2-butanol of essentially 100% optical purity can be obtained[20,21] by reduction of (+)-2-butene oxide with LiAl[$^2H_4$] or LiAl[$^3H_4$], and we chose this method to prepare the tritium-labeled alcohol.

The optically active epoxide was prepared from optically pure (−)-(2*R*,3*R*)-2,3-butanediol, using a sequence based on the work of Seeley and McElwee[22] which represents an improvement over previous methods.[20] In this sequence (Scheme II) the dioxolane prepared from the optically active diol and benzaldehyde is brominated with *N*-bromosuccinimide in CCl_4 to give (2*R*,3*S*)-2-(3-bromobutyl)benzoate by a single Walden inversion.

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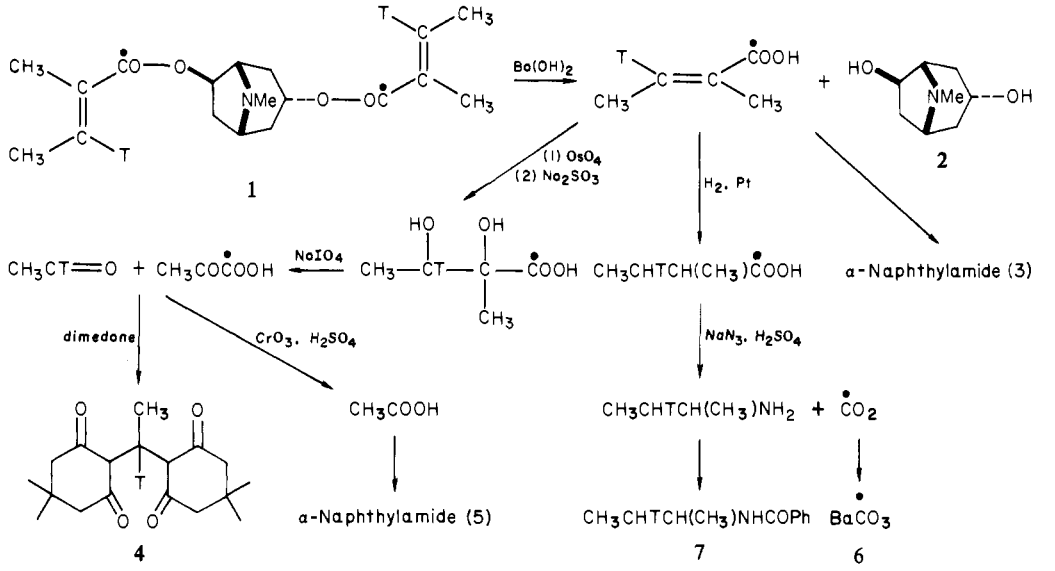
Scheme III. Degradation of 3α,6β-Ditigloyloxytropane



Treatment of the bromobenzoate with NaOH in ethylene glycol then gives the (+)-epoxide of 97% optical purity in 56% yield. This short sequence from a commercially available starting material is probably the best route for the preparation of this chiral epoxide.

The levorotatory alcohol obtained by reduction of the epoxide with LiAl[$^3H_4$] was converted to the methanesulfonate and subjected to nucleophilic displacement with the anion of dimethyl malonate. The inversion of configuration which accompanies this displacement generates the chiral center at C-3 in the (*S*) configuration present in L-isoleucine. For biosynthetic studies involving tritium it is customary to double label the substrate with ^{14}C as an internal standard for the measurement of tritium. For this purpose, [2-^{14}C]dimethyl malonate, prepared by treatment of [2-^{14}C]malonic acid with diazomethane, was used in the malonate displacement. The product was hydrolyzed to the crystalline diacid (+)-(3*S*,4*S*)-[2-^{14}C,4-$^3H_1$]-2-carboxy-3-methylvaleric acid.

For the conversion of this malonic acid to isoleucine, the one-step Schmidt procedure[23] proved to be more satisfactory than the alternative bromination–decarboxylation–amination sequence. Treatment with hydrazoic acid in sulfuric acid and chloroform, followed by ion-exchange chromatography, gave the crystalline amino acid. Amino acid analysis showed the product to be a 48:52 mixture of isoleucine and alloisoleucine; as expected, the conversion of carboxyl to amino showed little stereospecificity.

This synthetic mixture of (2*S*,3*S*,4*S*)-[2-^{14}C,4-$^3H_1$]isoleucine (L-isoleucine) and (2*R*,3*S*,4*S*)-[2-^{14}C,4-$^3H_1$]alloisoleucine (D-alloisoleucine) is epimeric at the α-carbon. While it would be possible to separate the components by enzymatic resolution of the *N*-acetyl derivatives with hog kidney acylase,[24] for the purpose of this investigation separation is unnecessary, since the asymmetry at C-2 is destroyed by the initial metabolic step, transamination. Either the D-alloisoleucine will not be metabolized at all or, if it is, it will be converted into the same labeled α-methylbutyrate as derived from the L-isoleucine.

Incorporation and Analysis. An aqueous solution of the labeled isoleucine was fed to *Datura innoxia* plants by the wick method. After 14 days the plants were harvested and the roots separated and dried and then extracted in the usual way. The alkaloids were obtained from the extract by partition chromatography; hyoscyamine and hyoscine were nonradioactive, while the two tigloyl esters meteloidine and 3α,6β-ditigloyloxytropane were both radioactive.

Table I. Activities (dpm/mM × 10^{-6}) of 3α,6β-Ditigloyloxytropane and Its Degradation Products

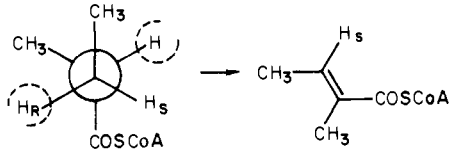
compd	^3H	^{14}C	$^3H/^{14}C$
3α,6β-ditigloyloxytropane (1) picrate	3.21	0.210	15.3
3α,6β-dihydroxytropane (2)	<0.01	<0.001	
tigloxy-α-naphthylamide (3)	1.56	0.105	14.9
acetaldehydedimedone (4)	1.53	<0.001	
acetyl-α-naphthylamide (5)	<0.01	<0.001	
barium carbonate (6)[a]		0.11	
N-benzoyl-2-aminobutane (7)	1.50	<0.01	
isoleucine fed			15.6

[a] Assayed as previously described.[25]

The latter alkaloid, present by far in major amount, was degraded as outlined in Scheme III. Alkaline hydrolysis of the alkaloid provided 3α,6β-dihydroxytropane and tiglic acid, which was crystallized from water. A portion of the tiglic acid was reduced to 2-methylbutanoic acid, which was degraded by the Schmidt reaction, and the carbon dioxide collected as barium carbonate. The remainder of the tiglic acid was hydroxylated with osmium tetroxide, and the resulting dihydroxyacid was cleaved with sodium metaperiodate. Half of the acetaldehyde from this cleavage was collected as its crystalline dimedone adduct, and half was oxidized to acetic acid and isolated as the crystalline α-naphthylamide. The activities of the various degradation products are listed in Table I.

Results and Discussion

The figures in Table I show that the dehydrogenation leading to tiglate is remarkably stereospecific; 95.5–98% of the tritium at C-4 of isoleucine is retained in the tiglic acid. Hydrogen is lost almost exclusively from the (4*R*) position of isoleucine. It is possible to conclude that tiglic acid is formed by an antiperiplanar elimination of the hydrogen at C-2 and the *pro-R* hydrogen at C-3 of (2*S*)-2-methylbutanoic acid, as represented in eq 2.



(2)

After this work was completed, Crout and his collaborators reported an efficient chemical synthesis of (2*S*,4*S*)-[3,4-$^3H_2$]-isoleucine, as well as the biological conversion of [3-^3H]threonine

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into (2*S*,4*R*)-[4-$^3H_1$]isoleucine.[26] Professor Crout very kindly supplied us with a sample of this latter (4*R*)-tritiated isoleucine, which was fed to *Datura meteloides*. The meteloidine isolated had lost 98% of the tritium, confirming the conclusion that the *pro-R* hydrogen at C-4 of isoleucine is lost and the *pro-S* hydrogen retained on the way to tiglate. This confirmation is important, since it proves that the enzymic dehydrogenation is genuinely stereospecific and does not remove the *pro-R* hydrogen of the (4*S*)-tritiated isoleucine simply because of a kinetic isotope effect.

The antiperiplanar elimination of hydrogen observed here finds a close parallel in the antiperiplanar elimination of H_{2R} and H_{3R} in the dehydrogenation of butyryl coenzyme A to *trans*-crotonate.[13] The interesting result that the dehydrogenation of butyryl-CoA and α-methylbutyryl-CoA follow identical stereochemical pathways is not unexpected, since a single swine liver acyl-CoA dehydrogenase is able to dehydrogenate both of these acids, as well as isobutyryl-CoA.[27] At least in humans, a separate enzyme system exists for the dehydrogenation of isovaleryl-CoA.[28]

This result provides another striking example of the stereospecificity of enzymatic selection at prochiral centers. The stereochemistry of dehydrogenation of α-methylbutyryl-CoA to angelic acid, the geometrical isomer of tiglate, is unknown, but Crout has recently demonstrated that in the biosynthesis of necic acids from isoleucine, the double bond with the angelate (*E*) geometry is formed by loss of the 4-*pro-S* hydrogen of isoleucine.[26] Thus the antiperiplanar alignment appears to be maintained in enzymic dehydrogenation of isoleucine to either (*E*) or (*Z*) products.

Experimental Section

Melting points were taken in open capillaries on a Hoover apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Model 257 grating spectrophotometer. NMR spectra were recorded on Varian HA-100 and T-60 instruments; chemical shifts are reported as δ units calibrated against tetramethylsilane. Optical rotations were measured in a 1-dm cell in a Perkin-Elmer Model 141 polarimeter; *c* is expressed as g/100 mL of solution. Radioactivity measurements on synthetic compounds were carried out on a Beckman LS-355 Liquid Scintillation System, using toluene/PPO cocktails for polar compounds and Trinex-toluene/PPO for amino acids; we thank Mrs. Cheryl Mothershed for her assistance. Radioactivity of degradation products was counted on a Nuclear Chicago Mark II liquid scintillation counter using dioxane–ethanol as solvent with the usual scintillators.[29] Samples were counted in duplicate and activities were reproducible to 5%.

Tritium-Labeled Isoleucine. (+)-(2*R*,3*R*)-2,3-Epoxybutane. A mixture of 34.1 g (0.230 mol) of redistilled triethyl orthoformate, 24.3 g (0.229 mol) of redistilled benzaldehyde, 1 g of *p*-toluenesulfonic acid, and 19.8 g (0.220 mol) of redistilled (2*R*,3*R*)-2,3-butanediol,[30] $[\alpha]^{24}_D$ −12.69° (neat, 1 dm) was slowly heated in an oil bath to 140 °C with stirring during 2 h while volatile products were distilled through a short-path still. The residual solution was diluted with 60 mL of ether, washed with two 30-mL portions of sodium bicarbonate solution, dried over potassium carbonate, and concentrated at reduced pressure. Vacuum distillation gave 33.3 g (0.187 mol, 85%) of (4*R*,5*R*)-4,5-dimethyl-2-phenyl-1,3-dioxolane: bp 123–126 °C (21mmHg); $[\alpha]^{24}_D$ −28.06° (neat, 1 dm).

A solution of 32.3 g (0.181 mol) of the levorotatory dioxolane in 50 mL of carbon tetrachloride was added dropwise over 1 h to a slurry of 32.3 g (0.186 mol) of *N*-bromosuccinimide in 350 mL of carbon tetrachloride, cooled in an ice bath. The mixture was stirred in the dark for 1 week at room temperature, cooled in ice, and filtered. The filtrate was washed with three 60-mL portions of saturated sodium bicarbonate, dried ($MgSO_4$), and concentrated at reduced pressure. Distillation of the residue afforded 34.6 g (0.135 mol, 74.5%) of (2*R*,3*S*)-2-(3-bromobutyl)benzoate: bp 80–90 °C (0.15mmHg); $[\alpha]^{25}_D$ −5.51° (neat, 1 dm).

A mixture of 33.4 g (0.13 mol) of the bromobenzoate and 12 g of sodium hydroxide in 100 mL of ethylene glycol was gradually heated to 140 °C while the epoxide was distilled, affording 5.9 g of crude product. Redistillation through a 40-cm Vigreux column gave 5.3 g (0.074 mol, 56%) of (2*R*,3*R*)-2,3-epoxybutane: bp 54–55 °C (760mmHg); lit.[31] bp 53.5 °C (746mmHg); $[\alpha]^{25}_D$ +45.78° (neat, 1 dm); IR (neat) 1250, 900, 800 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.2 (6 H, d, *J* = 5 Hz), 2.9 (2 H, dq). The observed rotation corresponds to an optical purity of about 97%, based on the maximum rotation of 47.23° (neat) reported by Lucas.[32]

(−)-(2*R*,3*S*)-[3-$^3H_1$]-2-Butanol. To a stirred mixture of 17.2 mg (0.45 mmol) of 95% lithium aluminum hydride and 5.0 mL of anhydrous ether was slowly added, via syringe, 360 mg (5.0 mmol) of the above dextrorotatory epoxide, followed by a rinse of 0.5 mL of ether. After the solution was warmed for 10 min on a water bath, 5.53 mg of $LiAl[^3H_4]$ (25 mCi, New England Nuclear, specific activity = 171.3 mCi/mmol) was added with cooling. The mixture was refluxed for 1.5 h on a water bath and cooled to room temperature, and 48 mg (1.26 mmol) of $LiAlH_4$ was added. After 1 h of additional reflux, the mixture was cooled in ice and treated with 0.15 mL of saturated aqueous Na_2SO_4 solution, followed by 400 mg (5.40 mmol) of (−)-2-butanol, $[\alpha]^{24}_D$ −10.75° (neat, 1 dm). A small lump of $MgSO_4$ was added to facilitate coagulation of the solids, the mixture was stirred for 3 h and filtered through Celite, and the solids were washed with three 2-mL portions of ether. The combined filtrate and washings were dried over anhydrous K_2CO_3 and filtered through sodium sulfate, and the ether removed by distillation through a Vigreux column below 55 °C. VPC analysis (0.25 in. × 10 ft, Carbowax 20M on Chromosorb W, 62 °C, retention time = 4.2 min) of the residue (773 mg) showed 96% 2-butanol along with unreacted epoxide (2%) and a little ether. A small sample of the pure 2-butanol, collected by preparative GLC on the same column, showed $[\alpha]^{28}_D$ −13.82° (ethanol, *c* = 1.15), specific activity = 3.15 × 10^9 dpm/mmol = 1.4 mCi/mmol.

(−)-(2*R*,3*S*)-[3-$^3H_1$]-2-Butyl Methanesulfonate. Methanesulfonyl chloride (0.4 mL) was added dropwise over 2.5 h, with stirring, to a solution of 500 mg of the above tritiated 2-butanol in 2 mL of dry pyridine, cooled to −10 to −20 °C. After the solution was stirred at 3° for 1 h, 1 mL of water was added and stirring continued for 20 min. The solution was diluted with 12 mL of ether and washed successively with 3 mL of cold 10% HCl, 3 mL of water, 3 mL of saturated aqueous $NaHCO_3$, and 3 mL of saturated brine. After being dried over $MgSO_4$, the solution was concentrated and distilled to afford 533 mg of mesylate: bp 70 °C (2.5mmHg); lit.[33] bp 66.8–67 °C (1.35mmHg); $[\alpha]^{25}_D$ −16.8° (chloroform, *c* = 1.01), lit.[33] $[\alpha]^{25}_D$ −17.07°; ^1H NMR ($CDCl_3$) 1.0 (t, 3 H, *J* = 8 Hz), 1.2 (d, 3 H, *J* = 8 Hz), 1.6 (m, 2 H), 3.0 (s, 3 H), 4.8 (sextex, 1 H, *J* = 8 Hz); specific activity = 3.52 × 10^9 dpm/mmol = 1.56 mCi/mmol.

Dimethyl [2-^{14}C]Malonate. A mixture of 313.9 mg of sublimed malonic acid and 0.2 mg of [2-^{14}C]malonic acid (0.1 mCi, New England Nuclear, 45.9 mCi/mmol) was dissolved in 8 mL of ether and esterified with ethereal diazomethane. Concentration gave 382 mg of diester, specific activity = 2.26 × 10^8 dpm/mmol = 0.101 mCi/mmol.

(+)-(3*S*,4*S*)-2-Carboxy-3-methyl[2-^{14}C, 4-$^3H_1$]valeric Acid. To a mixture of 70 mg of 99% sodium hyride and 2 mL of tetrahydrofuran (distilled from calcium hydride) was added, with cooling and under a nitrogen atmosphere, 380 mg of the dimethyl [2-^{14}C]malonate over a period of 10 min. The cooling bath was removed and stirring continued for 30 min. The mixture was heated to 80 °C to bring the contents into solution, 0.39 mL of (2*R*,3*S*)-[3-$^3H_1$]-2-butyl methanesulfonate was added, and the solution was stirred at reflux for 44 h. The cooled mixture was distributed between 4 mL of water and 15 mL of ether, and the ether layer washed with 4-mL portions of water until neutral. The solution was dried over $MgSO_4$ and concentrated at reduced pressure to leave 560 mg of crude diester.

A mixture of the diester, 581 mg of KOH, and 4 mL of water was stirred at 70 °C for 4 h and then at room temperature overnight. The solution was concentrated to dryness at reduced pressure below 40° and the residue washed with 5 mL of ether and then acidified to Congo red with ice-cold 6 N HCl, keeping the temperature below 10 °C. The solution was extracted with six 6-mL portions of ether, and the extracts were dried ($MgSO_4$) and concentrated at room temperature. On being kept overnight in a vacuum desiccator over concentrated H_2SO_4, the residue solidified to colorless crystals: 331 mg (74%), mp 93 °C.

(+)-(3*S*)-2-Carboxy-3-methylvaleric acid, prepared from (−)-(2*R*)-2-butyl methanesulfonate in the same manner, had a melting point of 85 °C, $[\alpha]^{26}_D$ +3.41° (acetone, *c* = 1.99).

(2*RS*,3*S*,4*S*)-[2-^{14}C, 4-$^3H_1$]Isoleucine. A solution of the doubly labeled malonic acid (331 mg) in 1.5 mL of 100% sulfuric acid and 2 mL of $CHCl_3$ was vigorously stirred while 1.6 mL of a 5% solution of hydrazoic acid in $CHCl_3$ was added dropwise over 1 h. The mixture was stirred at 55 °C for 2 h, at which time gas evolution had nearly ceased, and then for another 4 h at 25 °C. The $CHCl_3$ layer was decanted and

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the sulfuric acid layer poured into 15 mL of ice water and washed with three 5-mL portions of ether. Solid $Ba(OH)_2 \cdot 8H_2O$ (6 g) was added, bringing the pH to 2–3, the mixture was filtered through Celite, and the precipitate was washed with 40 mL of water. The combined filtrate and washings were concentrated to 10 mL at reduced pressure and applied to the top of a Dowex 50W-X8 ion-exchange column (1 × 20 cm, hydrogen form, 14 g wet weight, previously washed with distilled water to neutrality). The column was washed with distilled water until no more sulfate ion was detected in the eluate by $Ba(OH)_2$. Elution with 200 mL of 0.5 N NH_4OH gave the amino acid, isolated as a colorless solid after concentration in vacuo. Recrystallization from a mixture of 10 mL of water and 15 mL of ethanol gave 63 mg plus a second crop of 27 mg (total 90 mg, 34%), mp 225 °C.

Analysis on a Beckman Model 119CL amino acid analyzer[34] showed that the amino acid was a mixture of 48.1% isoleucine and 51.9% alloisoleucine.

A protion of the labeled isoleucine (0.39 mg) was diluted with 86 mg of nonradioactive L-isoleucine (free of allosioleucine) and recrystallized from water. The $^3H/^{14}C$ ratio remained constant at 15.7, and the specific activity confirmed that the synthetic sample consisted of 50 ± 5% of L-isoleucine.

Feeding Experiments. A solution of 10.87 mg of the synthetic isoleucine (^3H specific activity = 3.30×10^9 dpm/mM, $^3H/^{14}C$ = 15.6; total ^3H activity fed = 2.74×10^8 dpm) in 10 mL of water was fed by the wick method to nine *Datura innoxia* plants (2–3 months old) growing in soil in a greenhouse. After 14 days, the plants were harvested and the roots and aerial parts separated and dried at 60 °C for 48 h. The roots (60 g) were finely powdered in a Wiley mill, mixed with calcium hydroxide (15 g), moistened with water (30 mL), and extracted with five 150-mL portions of ether. The filtered extract was concentrated to a volume of about 2 mL. The alkaloids were separated from this extract by partition chromatography as previously described,[4a,35] affording the following compounds as their picrates: meteloidine (3.5 mg, ^3H activity = 1.17×10^6 dpm/mM, $^3H/^{14}C$ = 15.5, 99% retention of ^3H), 3α,6β-ditigloyloxytropane (63 mg, ^3H activity = 3.21×10^6 dpm/mM, $^3H/^{14}C$ = 15.3, 98% retention of ^3H), hyoscyamine (35 mg), and hyoscine (20 mg), the latter two being nonradioactive.

Degradation of the 3α,6β-Ditigloyloxytropane. Degradation products and their activities are recorded in Table I. Dilutions were carried out as necessary to facilitate manipulation.

The 3α,6β-ditigloyloxytropane picrate (62 mg) was dissolved in dilute sodium carbonate solution and extracted with ether. The residue obtained on evaporation of the solvent was refluxed with saturated barium hydroxide solution (10 mL) for 6 h, cooled, acidified with sulfuric acid, and continuously extracted with ether for 18 h. The solid residue obtained on evaporation of the ether extract was crystallized from water to afford 17 mg of tiglic acid. The aqueous solution remaining from extraction of the tiglic acid was made basic with sodium carbonate, concentrated to dryness, and extracted with hot ethanol. Concentration of the ethanol extracts left a solid residue which was sublimed at 180 °C and 0.001 mmHg to afford 8 mg of 3α,6β-dihydroxytropane, mp 212–213 °C, reported[36] mp 212 °C.

A portion of the tiglic acid was reduced to 2-methylbutanoic acid, which was subjected to a Schmidt reaction, affording 2-aminobutane (assayed as its *N*-benzoyl derivative) and carbon dioxide (collected as barium carbonate) by a previously described procedure.[3a]

Osmium tetroxide (130 mg) was added to a solution of tiglic acid (50 mg) in 100 mL of ether containing a drop of pyridine. After 18 h at room temperature the reaction mixture had deposited a black precipitate. The mixture was concentrated to dryness, and the residue was refluxed for 2 h with a solution of 0.5 g of sodium sulfite in 50 mL of 50% aqueous methanol. The filtered solution was concentrated to dryness and the residue extracted with hot methanol. The pale yellow residue remaining from evaporation of the methanol was dissolved in 20 mL of water, and 110 mg of sodium metaperiodate was added. The solution was kept for 2 h and then distilled into an ice-cooled receiver, water being added to the distillation flask at intervals until 60 mL of distillate had been collected. Half of this distillate was added to an aqueous solution of 140 mg of dimedone; after 10 h the crystalline precipitate (52 mg) was collected and recrystallized from aqueous methanol, affording colorless plates, mp 145–146 °C, identical with an authentic sample of the dimedone derivative of acetaldehyde.

The other half of the aqueous distillate was acidified with sulfuric acid, 1 g of chromium trioxide added, and the mixture distilled. The distillate was neutralized with sodium hydroxide and evaporated to dryness, and the residue of sodium acetate converted[37] to acetyl α-naphthylamide (8 mg).

Feeding of (2*S*,4*R*)-[4-^{14}C,4-$^3H_1$]isoleucine to *Datura meteloides* and Isolation of Meteloidine. (2*S*,4*R*)-[4-^{14}C,4-$^3H_1$]Isoleucine (10.65 mg, total ^{14}C activity = 2.58×10^6 dpm, $^3H/^{14}C$ = 2.0) was fed by the wick method to 6 four-month-old *D. meteloides* plants growing in soil. After 14 days the alkaloids were isolated and separated as previously described,[38] affording radioactive meteloidine (0.3% incorporation of ^{14}C, $^3H/^{14}C$ = 0.05, representing 98% loss of tritium). Hydrolysis yielded labeled tiglic acid and inactive teloidine. We thank Professor D. H. G. Crout for the generous sample of the labeled isoleucine.

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