stereomer. Recrystallization once again from acetonitrile (19 mL) gave 1.68 g of pure **5a** (>99%). An overall yield of 34% was obtained for the hydrogenation procedure, while the sodium cyanoborohydride procedure gave a 32% overall yield of **5a**: mp 143–144.5 °C; $[\alpha]^{25}_D$ −42.2° (*c* 1.0, CH_3OH); UV (0.1 N HCl in CH_3OH) λ_{max} 258 nm (% *A* 10.5), 263 (7.40), 267 (5.64); ^1H NMR (D_2O) δ 1.30 (t, *J* = 7 Hz, OCH_2CH_3), 1.54 (minor),[22] 1.59 (major, d, *J* = 6.5 Hz, Ala-CH_3), 1.75 (minor), 2.02 (major, m, Pro γ-CH_2), 2.02 (m, Pro β-H), 2.33 (m, Pro β-H, $PhCH_2CH_2$), 2.82 (m, $PhCH_2$), 3.48 (minor), 3.60 (major, m, Pro δ-CH_2), 3.96 (m, $PhCH_2CH_2CH$), 4.09 (minor), 4.33 (major, q, *J* = 6.5 Hz, Ala α-H), 4.27 (q, *J* = 7 Hz, OCH_2CH_3), 4.44 (m, Pro α-H), 6.37 (s, CH=CH), 7.39 (m, ArH_{2-6}); ^{13}C NMR (CD_3OD) 16.2, 17.4 (major), 18.1 (minor), 24.9 (minor), 27.7, 31.8, 33.7, 35.0, 35.2 (minor), 49.8, 57.9, 58.4 (minor), 61.7, 61.8, 62.3, 62.4, 65.6, 65.7, 129.3, 131.2, 131.4, 138.0, 142.9, 170.5, 171.2, 171.6, 172.0, 172.4, 176.5, 176.7 ppm. Anal. Calcd for $C_{24}H_{32}N_2O_9$: C, 58.53; H, 6.55; N, 5.69. Found: C, 58.49; H, 6.53; N, 5.61.

1-[*N*-[1(*S*)-Carboxy-3-phenylpropyl]-L-alanyl]-L-proline (Enalaprilat, 5b). Enalapril maleate, **5a** (50 g, 0.102 mol), and sodium hydroxide (20 g, 0.5 mol) were dissolved in 500 mL of water, and the mixture was permitted to stir overnight at room temperature. The reaction mixture was then added to a Dowex 50W-2X ion-exchange column (1.2 L, 50–100 mesh). The column was washed with water until the eluent was near neutrality. Then the product was removed from the column with 8 L of 2% pyridine in water. The appropriate fractions were concentrated, and the resulting solid was slurried in 300 mL of acetone. Filtration and drying gave 33.8 g of pure **5b**: R_f 0.55; mp 149–151 °C dec; $[\alpha]^{25}_D$ −67.0° (*c* 2.0, 0.1 N HCl); $[\alpha]^{25}_D$ −53.5° (*c* 1.0, CH_3OH); UV (0.1 N HCl) λ_{max} 257 (ε 179); ^1H NMR (D_2O) δ 1.55 (minor),[22] 1.59 (major, d, *J* = 6.5 Hz, Ala-CH_3), 1.82 (minor), 2.04 (major, m, Pro γ-CH_2), 2.04 (m, Pro β-H), 2.32 (m, Pro β-H), 2.18 (m, $PhCH_2CH_2$), 2.75 (m, $PhCH_2$), 3.54 (t, *J* = 6 Hz, $PhCH_2CH_2CH$), 3.63 (m, Pro δ-CH_2), 4.08 (minor), 4.29 (major, q, *J* = 6.5 Hz, Ala α-H), 4.51 (minor), 4.46 (major, dd, *J* = 5 Hz, *J* = 8 Hz, Pro α-H), 7.35 (m, ArH_{2-6}); ^{13}C NMR (D_2O, NaOD) 18.2, 22.9 (minor, Pro γ), 25.2 (major, Pro γ), 30.2 (major, Pro β), 32.3 (minor, Pro β), 32.6, 36.3, 48.3, 53.7 (major, Ala α), 55.2 (minor, Ala α), 62.3 (minor, Pro α), 62.6 (major, Pro α), 63.1, 126.9, 129.4, 129.5, 143.1, 175.3, 180.4, 182.2 ppm. Anal. Calcd for $C_{18}H_{24}N_2O_5 \cdot 0.5\ H_2O$: C, 60.49; H, 7.05; N, 7.84. Found: C, 60.60; H, 7.01; N, 7.79.

X-ray Crystal Analysis of 5b. Small rods of **5b** formed from water at room temperature. Preliminary X-ray experiments revealed cell constants of *a* = 9.798 (2) Å, *b* = 10.452 (2) Å, and *c* = 19.819 (5) Å and a space group of $P2_12_12_1$ for *Z* = 4. Of the 1619 unique reflections measured, 1360 were observed ($I \geq 3\sigma(I)$) by using Cu Kα radiation and an automatic four-circle diffractometer. The structure was solved by using direct method procedures[23] and refined by using full-matrix least-squares techniques. Anisotropic temperature parameters were refined for all non-hydrogen atoms while isotropic parameters were added but not refined for the hydrogen atoms. The function minimized was $\sum\omega(|F_o| - |F_c|)^2$ with $\omega = 1/(\sigma(F_o))^2$ to give an unweighted residual index of 0.046. Three molecules of water were found in the crystal lattice. Figure 1 is a perspective drawing of **5b** showing its absolute configuration and conformation in the solid state.

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Registry No. **1**, 64920-29-2; **2**, 13485-59-1; **3**, 90414-32-7; (*SSS*)-**4**, 75847-73-3; (*RSS*)-**4**, 76420-74-1; **5a**, 76095-16-4; **5b**, 76420-72-9.

Supplementary Material Available: X-ray structural analysis for **5b**, Table I, fractional coordinates and temperature factors, Table II, bond distances, Table III, bond angles, and Figure 2, X-ray numbering diagram (5 pages). Ordering information is given on any current masthead page.

(22) The rotameric distribution is indicated by *minor/major* terminology.

(23) The following library of crystallographic programs were used: MULTAN 80, University of York, York, England (1980); XRAY-72, University of Maryland, College Park, MD (1972); Structure Determination Package 17.0, Enraf-Nonius Corporation, Delft, Holland (1981); ORTEP-II, Oak Ridge National Laboratory, Oak Ridge, TN (1970).

Facile, Economical Synthesis of L-[α-^{2}H]-α-Amino Acids[1]

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Three methods have been suggested for preparation of L-[α-^{2}H]-α-amino acids. The method of Wong and Whitesides[2] employs NAD^2H to introduce the label in an enzymic reductive amination reaction. Chiral and isotopic purities are high, but an enzyme specific for the production of a particular amino acid must be used. Exchange without loss of chirality has been effected with transaminases such as aspartate aminotransferase,[3] but with amino acids which are poor substrates, large amounts of enzyme may be required or the reaction may fail. The potential also exists in this system for exchange of hydrogen at the β-carbon. A different approach is to prepare the DL-[α-^{2}H]amino acid and then resolve, most easily by an enzymic method. For example, Yamamoto, Upson, Linn, and Hruby[4] prepared DL-[α-^{2}H]phenylalanine from diethyl 2-benzyl-2-acetamidomalonate by simultaneous deacetylation and ester hydrolysis, followed by decarboxylation in D_2O. The product was then reacetylated and porcine kidney acylase was used for resolution by selective deacetylation of the L derivative.

We present here a two-step procedure which begins with the commercially available *N*-acetyl α-amino acids and produces high levels of deuteration in a first step under mild conditions. The product of this step is the *N*-acetyl DL-[α-^{2}H]amino acid, which is then resolved by porcine-kidney acylase I in the second step. In other experiments, deuteration of DL-phenylalanine was achieved quickly and simply with pyridoxal phosphate or pyridoxal chloride in basic D_2O.

Results

In a typical experiment, *N*-acetyl-DL-phenylalanine was dissolved at room temperature in deuterium oxide containing sodium hydroxide. Then acetic anhydride was added, producing a heterogeneous mixture; stirred and heated gently, the mixture became homogeneous and was allowed to stand for several hours. *N*-Acetyl-[α-^{2}H]-phenylalanine was precipitated with hydrochloric acid (yield 92%; deuteration >97% by NMR, no deuterium exchange was observed at other than α-position). Resolution with porcine kidney acylase I in protium oxide then produced L-[α-^{2}H]phenylalanine with >97% deuteration.

The generality of the procedure was demonstrated by deuteration of four other *N*-acetyl α-amino acids: leucine, glutamic acid, methionine, and tyrosine. The α-deuteration of these amino acids was carried out by the procedure given above for *N*-acetylphenylalanine. α-Deuterated leucine and glutamic acid were obtained in 86% and 82% yield, respectively. The deuterium incorporation in these compounds was >97% by NMR. Similarly, the deuterium exchange reaction of methionine and tyrosine was followed

(1) This research was supported by the National Institute of General Medical Sciences under Grant No. GM-20198.

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(4) Yamamoto, D. M.; Upson, D. A.; Linn, D. K.; Hruby, V. J. *J. Am. Chem. Soc.* **1977**, *99*, 1564–1570.

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by NMR without product isolation. The final deuterium content was >97%. Resolution by porcine-kidney acylase has previously been achieved[5,6] for the methionine, leucine, and glutamic acid derivatives, while carboxypeptidase has been used for tyrosine.[6]

DL-[α-^{2}H]Phenylalanine was also prepared by the mild deuterium exchange reaction of DL-phenylalanine in the presence of catalytic amounts of pyridoxal phosphate or pyridoxal hydrochloride in KOD–D_2O. No β-deuteration was observed.

Experimental Section

Materials. Phenylalanine, *N*-acetylphenylalanine, *N*-acetylleucine, *N*-acetylmethionine, *N*-acetylglutamic acid, O,N-diacetyltyrosine, pyridoxal phosphate, and pyridoxal hydrochloride were obtained from Sigma Chemical Co. Porcine kidney acylase I (EC 3.5.1.14) was obtained from Sigma Chemical Co. Deuterium oxide (99.8% D) and acetic anhydride were supplied by Aldrich Chemical Co. All other materials were reagent grade.

***N*-Acetyl-DL-[α-^{2}H]phenylalanine.** Eleven grams of *N*-acetyl-DL-phenylalanine was dissolved at room temperature in 55 mL of deuterium oxide containing 2.2 g of sodium hydroxide. Then 21 mL of acetic anhydride was added, resulting in a heterogeneous mixture. The mixture was stirred and heated gently to about 50 °C during 10 min; during this period, the mixture became homogeneous. It was allowed to stand for 5 h at 40 °C. Then the mixture was placed in an ice bath and acidified to pH 2 with cold concentrated hydrochloric acid. The *N*-acetyl[α-^{2}H]phenylalanine precipitated, was removed by filtration, washed with cold water, recrystallized from water and dried (yield 92%): mp 147 °C [lit.[7] mp 150–151 °C for the protiated compound].

The ^{1}H NMR spectrum of the compound in CF_3CO_2H, compared with that of protiated *N*-acetyl-DL-phenylalanine, showed that the α-H (δ 5.0) was missing, indicating that the deuterium content at the α-position was >97%. No deuterium exchange was observed at other than α-position.

L-[α-^{2}H]Phenylalanine. In accordance with the method of Greenstein,[5,6] porcine kidney acylase I (500 mg) was added to a solution of *N*-acetyl-DL-[α-^{2}H]phenylalanine (43 mmol) in 700 mL of water that had been neutralized to pH 7.5 with 2 N LiOH. After being allowed to stand at 37 °C for 1 day, the mixture was brought to pH 5 with acetic acid, the protein was filtered off with the aid of charcoal, and the filtrate was concentrated in vacuo. At this point the L isomer began to crystallize, and the mixture was chilled for several hours. The crystals of L-[α-^{2}H]phenylalanine were filtered and washed with ethanol and finally recrystallized from water with the aid of a little charcoal (yield 51%). A single spot identical with that for authentic L-phenylalanine was observed by TLC: $[\alpha]^{23}_D$ −33.2° (*c* 1.51, H_2O) [lit. $[\alpha]^{20}_D$ −32.5° for L-[^{2}H]phenylalanine obtained by the aspartate–aminotransferase method;[3] $[\alpha]_D$ −33° (*c* 1.5, H_2O) for the protiated compound[8]]. Deuterium content was estimated at more than 97% by NMR.

DL-[α-^{2}H]Phenylalanine. A mixture of the potassium salt of phenylalanine (12 mmol), pyridoxal phosphate or pyridoxal hydrochloride (1.2 mmol) as catalyst, and KOD (24 mmol)–D_2O (15 mL) was refluxed for 2 h. After the mixture was cooled on ice and neutralized (pH ~5) with concentrated HCl while in an ice bath, crystals precipitated. The mixture was filtered and the precipitate was washed with cold water and methanol. The product was dissolved in water with a small amount of Norit and filtered. Methanol was added and the solution cooled in an ice chest until the product crystallized. The crystals were filtered and dried under vacuum (yield 80%): mp 270 °C [lit.[9] mp 271–273 °C for the protiated compound]. Deuterium incorporation was estimated at more than 97% by NMR.

Registry No. *N*-acetyl-DL-phenylalanine, 2901-75-9; *N*-acetyl-DL-[α-^{2}H]phenylalanine, 63570-52-5; L-[α-^{2}H]phenylalanine, 55836-70-9; DL-phenylalanine potassium salt, 55184-83-3; DL-[α-^{2}H]phenylalanine, 14246-24-3.

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(9) Marvel, C. S. "Organic Syntheses"; Horning, E. C., Ed.; Wiley: New York, 1955; Collect. Vol. 3, pp 705–708.

Structures of Eurycomalactone and Related Terpenoids

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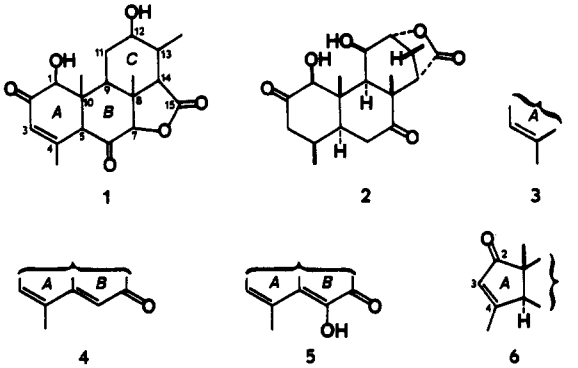
The terpenoids eurycomalactone and dihydroeurycomalactone were isolated from the Southeast Asian medicinal plant *Eurycoma longifola* Jack and structures 1 and 3,4-dihydro-1, respectively, were proposed for them.[2]



More recently, ^{13}C and 80-MHz ^{1}H NMR spectra were run on eurycomalactone and interpreted as supporting structure 1.[3] We wish to correct these structures to **3** and **2**, respectively, and to present evidence that related compounds **4**, **5**, and **6** also occur in the same plant.

In 1964, we ran 60-MHz ^{1}H NMR spectra on samples of eurycomalactone, dihydroeurycomalactone, and a "second bitter principle" of unknown structure (now **6**) for Le-Van-Thoi. We recently ran 250-MHz ^{1}H (Table I) and 62.9-MHz ^{13}C (Table II) NMR and UV and mass spectra on these samples.[4] It was apparent, e.g., from the 5-Hz coupling constant between two >CH–O– protons, that structures 1 and 3,4-dihydro-1 were incorrect; also, while **2** and **6**[5] were essentially pure, the eurycomalactone sample was actually a mixture of **2** (20%), **3** (30%), and two other compounds, which we have found to be 5,6-dehydroeurycomalactone (**4**, 30%) and 6-hydroxy-5,6-dehydroeurycomalactone (**5**, 20%). **5**[6] was readily separated from the others by virtue of its shorter retention time on a silica gel LC column, but the other compounds were only partially separated. It was still possible to obtain the ^{1}H NMR parameters for each compound (Table I) and to observe

(1) (a) University of Arizona. (b) University of Missouri.
(2) Le-Van-Thoi; Nguyen-Ngoc-Suong. *J. Org. Chem.* **1970**, *35*, 1104. The local name of this plant in Viet Nam is "tree which cures hundreds of diseases".
(3) Oei-Koch, A.; Kraus, L. *Sci. Pharm.* **1980**, *48*, 110.
(4) Comparison of the 250-MHz spectra (Bruker WM-250) with the 60-MHz spectra taken on thse samples in 1964 indicated no change in sample composition. The ^{13}C NMR spectrum of **2** was correlated with its ^{1}H NMR spectrum by heteronuclear decoupling.
(5) **6**: mp 263–264 °C; MS, *m/e* (M^+) calcd for $C_{18}H_{22}O_5$ 318.1467, obsd 318.1464; IR, 1779, 1718, 1700, 1628 cm^{-1}; UV, λ_{max}^{MeOH} 273 nm (ε 1800), 226 nm (ε 12100).
(6) **5**: mp 173 °C dec; MS, *m/e* (M^+) calcd for $C_{19}H_{22}O_7$, 362.1366, obsd 362.1359; UV, λ_{max}^{MeOH} 333 nm (ε 9700), 242 nm (ε 6100).

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