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.

(5) Birnbaum, S. M.; Levintow, L.; Kingsley, R. B.; Greenstein, J. P. *J. Biol. Chem.* **1952**, *194*, 455–470.
(6) Greenstein, J. P. "Methods in *Enzymology*", Academic Press: New York, 1957; Vol. 3, pp 554–570.
(7) Gillespie, H. B.; Snyder, H. R. "Organic Syntheses"; Blatt, A. H.; Ed.; Wiley: New York, 1943, Collect. Vol. 2, pp 491–494.
(8) Halpern, B.; Sargeson, A. M.; Turnbull, K. R. *Chem. Ind. (London)*, **1963**, *1399*.
(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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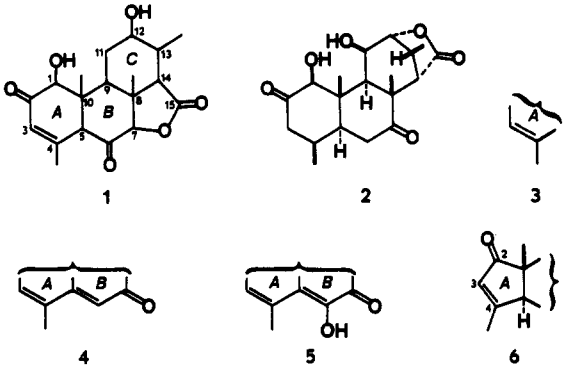
The terpenoids eurycomalactone and dihydroeurycomalactone were isolated from the Southeast Asian medicinal plant *Eurycoma longifola* Jack and structures 1 and 3,4-dihydro-1, respectively, were proposed for them.[2]



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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Table I. ^{1}H NMR Shifts (δ) and Coupling Constants (Hz) for 2–6 in $CDCl_3$

proton(s)	2	3	4	5	6
		δ			
1	4.07	4.04	4.20	4.15	
3α	2.77				
3β	2.51	6.11	6.29	6.09	5.90
4	2.26				
5	2.37	2.87			2.82
6α	2.31	2.81	6.24		2.70
6β	2.98	2.83			2.78
9	1.73	1.86	2.33	2.27	1.99
11	4.61	4.77	4.89	5.00	5.15
12	4.34	4.37	4.39	4.39	4.32
13	2.86	2.89	2.94	2.96	3.02
14	2.93	2.96	3.01	2.97	2.80
4-Me	0.97	1.94	2.16	2.40	2.10
8-Me	1.51	1.55	1.53	1.59	1.50
10-Me	1.21	1.25	1.45	1.46	1.47
13-Me	1.15	1.16	1.19	1.19	1.14
1-OH	4.35	4.35	4.50	4.47	
11-OH	3.21	3.15	2.73	2.72	1.85
		J			
1–10H	0	3.5	1.5	0	
1–3α	1.0	0	0	0	
3α–3β	13.7				
3α–4	7.6				
3β–4	1.6				
3–4Me	0	1.3	1.2	1.2	1.2
3–6	0	0	0.5		0
4–5	4.0				
4–4Me	7.6				
5–6α	3.7	3.3			5.1
5–6β	13.3	13.0			7.0
6α–6β	14.5	14.4			12.5
9–11	3.6	3.6	3.9	3.8	3.4
11–12	4.7	4.7	4.8	4.8	5.0
11–11OH	5.6	5.7	6.0	6.0	5.9
12–14	1.2	1.0	1.3	1.2	1.2
13–13Me	7.0	7.0	7.0	7.0	7.0

Table II. ^{13}C NMR Shifts (δ) for 2, 5, and 6 in $CDCl_3$

carbon	2	3[a]	5[a]	6
1	83.0 d	83.1	84.0	
2	206.8 s	196.7	*b*	212.0
3	38.8 t	124.1 d	126.4 d	127.0 d
4	47.4 d	161.6 s	*b*	177.6 s
5	52.7 d	53.1	*b*	53.9
6	45.5 t	*c*	*b*	41.8
7	209.3 s	204.4	*b*	209.8
8	47.8 s	*c*	45.7	47.7
9	50.2 d	49.5	52.9	51.9
10	50.9 s	51.3	48.1	48.8
11	69.9 d	69.9	68.8	67.6
12	81.5 d	81.3	81.3	83.7
13	36.0 d	36.4	45.4	37.0
14	32.3 d	32.6	31.7	31.4
15	176.3 s	175.4	*b*	176.5
4-Me	15.1 q	24.0	24.5	24.2
8-Me	15.5 q	12.5	16.6	17.5
10-Me	23.1 q	22.2	22.6	21.4
13-Me	16.6 q	17.1	16.4	16.4

[a] Some acetone-d_6 was added to dissolve this sample. [b] Singlets that could not be assigned due to weakness of signal. [c] Although 19 peaks were reported to be found for 3, only 18 chemical shifts were listed, and the one at δ 77.2 is almost certainly $HCCl_3$.

the molecular ion peak (m/e 346.1404; calcd for $C_{19}H_{22}O_6$, 346.1416) and longest wavelength maximum (λ_{max}^{MeOH} 288 nm, ϵ ~9200) for 4 in the mixture of 2–4.

The correct structures 2–6 were derived largely from ^{1}H–^{1}H NMR decoupling experiments and spectral comparisons among the various molecules. All five have the same C and D ring substitution pattern, as shown by the constancy of their 9–11 through 13–13 Me coupling constants (Table I, bottom). The coupling pattern observed for the protons on C-1 through C-6 of 2 can only be accommodated if the ketone carbonyls are at C-2 and C-7; many quassinoids share this oxygenation pattern.[7]

The stereochemistry shown for 2 is analogous to that of other quassinoids;[7] the relative configurations are strongly supported by ^{1}H–^{1}H coupling constants and by nuclear Overhauser effects.[8] The equatorial nature of the hydroxyl at C-1 was shown by the large NOE observed between H–C-1 and H–C-3α; the coupling constant of 1.0 Hz between these two protons is typical for axial α protons on different sides of the carbonyl group in a cyclohexanone, and the failure to observe coupling between H–C-1 and H–C-3 in 3–5 also supports this assignment.[9] The $J_{4,5}$ value of only 4 Hz and the finding that the sum of $J_{3\alpha,4}$ and $J_{3\beta,4}$ is only 9 Hz show the C-4-Me to be β and axial,[10] in accordance with its preparation by reduction of 3 with hydrogen/Pd.[2] H–C-5 must be axial from its 13.3 Hz coupling to H–C-6β.[10] The 8-Me show a very strong NOE as befits their 1,3-diaxial relationship. This puts H–C-11 α and axial, and its small coupling to H–C-12 requires the latter to be α and equatorial. The H–C-12 to H–C-14 coupling constant of 1.2 Hz requires a W arrangement,[9] and the configuration at C-13 must be as shown for $J_{12,13}$ and $J_{13,14}$ to be too small to observe (dihedral angles ~90°).

Given that dihydroeurycomalactone is 2, eurycomalactone is clearly 3, i.e., like samaderine B without the C-8-Me to C-13 ether bridge.[11] The additional double bond in 4 must be positioned as shown from its ^{1}H NMR parameters; its $J_{3,6}$ of 0.5 Hz is typical for such systems.[12] The spectral parameters of 5 are quite similar to those of 4 except for the absence of a vinyl hydrogen; its extra oxygen (MS) must be in a hydroxyl group replacing the vinyl hydrogen at C-6, since the C-3 vinyl hydrogen (coupled to the vinyl methyl protons) remains. From its molecular formula (MS), 6 has one less carbon and one less oxygen than any of the other compounds; spectral comparisons show it to be like 3 without the C-1 CHOH grouping. Samaderine A has a similar A ring.[13] Biogenetically, eurycomalactone 3 and its oxidation products 4–6 and reduction product 2 are closely related to the more highly oxidized samaderines[7,11,13] and less oxidized quassinoids such as klaineanone.[7] 3 may be biosynthesized from the latter compound by the sequence: oxidation α to the lactone carbonyl to give, with loss of CO_2, a 15-carboxyl group; lactonization toward C-12; and oxidation of the C-7 hydroxyl to a carbonyl group.

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Registry No. 2, 90584-30-8; 3, 23062-24-0; 4, 90605-25-7; 5, 90584-31-9; 6, 85643-76-1.

(7) Devon, T. K.; Scott, A. I. "Handbook of Naturally Occurring Compounds, Volume II"; Academic Press: New York, 1972; pp 362–364, 381.
(8) NOESY spectra were run on 2 on a Nicolet instrument at 300 MHz.
(9) Jackman, L. M.; Sternhell, S. "Applications of NMR Spectroscopy in Organic Chemistry", 2nd ed.; Pergamon Press: New York, 1969; pp 334–338.
(10) Bhacca, N. S.; Williams, D. H. "Applications of NMR Spectroscopy in Organic Chemistry", Holden-Day: San Francisco, 1964; pp 135–150.
(11) Zylber, J.; Polonsky, J. *Bull. Soc. Chim. Fr.* **1963**, 2016.
(12) Hobgood, R. T.; Goldstein, J. H., *J. Mol. Spectrosc.* **1964**, *12*, 76.
(13) Wani, M. C.; Taylor, H. L.; Wall, M. E.; McPhail, A. T.; Onan, K. D. *J. Chem. Soc., Chem. Commun.* **1977**, 295; 6 and samaderine A may have lost C-1 (or C-2) by the biological equivalent of a benzilic acid rearrangement followed by oxidation. These workers found antileukemic activity in samaderine E, which resembles 3 in many respects.