pyridine, diluted with CH_2Cl_2 (20 ml), and cooled to −60° in a Dry Ice–acetone bath. While stirring at −60° a mixture of 12.5% phosgene in benzene (9.1 ml, 0.010 mol) and CH_2Cl_2 (25 ml) was added dropwise over 16 min. After slow equilibration to 25° the reaction mixture was evaporated to dryness under vacuum and the residue was broken up with water, filtered, and dried to give 3.48 g of crude **13**. Recrystallization from EtOH gave 3.24 g (85%) of pure **13** as colorless, thin, lathe-like crystals: mp 145–146°; ir ($CHCl_3$) 5.69 μ (C=O); nmr ($CDCl_3$) δ 1.32, 1.48 (s, 6, methyls), 4.88 (d, 1, J = 4 Hz, C-2 H), 5.17 (s, 2, benzylic CH_2), 5.93 (d, 1, J = 4 Hz, C-1 H), and 7.39 ppm (s, 5, benzene ring H); $[\alpha]^{30}D$ +59.8 (c 1, $CHCl_3$); mass spectrum molecular ion m/e 380 (calcd mol wt, 380).

Anal. Calcd for $C_{18}H_{20}O_9$: C, 56.84; H, 5.30. Found: C, 56.83; H, 5.11.

Conversion of 11 to 12.—A solution of **11** (0.532 g) in pyridine (10 ml) was treated with Ac_2O (0.5 ml) and stirred for 1 hr at 25° and 1 hr at 100–120°. Removal of pyridine and Ac_2O under vacuum gave **11** diacetate quantitatively: nmr ($CDCl_3$) δ 1.30, 1.50 (s, 6, isopropylidene methyls), 1.97, 2.05 (s, 6, acetyl methyls), 5.17 (s, 2, benzylic CH_2), 5.36 (d, 1, J = 4 Hz, C-2 H), 5.90 (d, 1, J = 4 Hz, C-1 H), and 7.35 ppm (s, 5, benzene ring H). The crude diacetate was hydrogenolyzed with 10% Pd/C catalyst under 50 psig of H_2 for 4 hr at 25° after which time tlc analysis (Et_2O, silica gel) indicated complete removal of the CBZ group (R_f change of 0.90 to 0.55). Concentration of the filtered solution gave 0.462 g of yellow oil which was dissolved in pyridine (15 ml), cooled to 0°, and treated with 0.2 ml of methanesulfonyl chloride in $CHCl_3$ (6 ml). The mixture was kept at 4° for 16 hr, and concentrated to dryness under vacuum. After water was added the product was extracted with EtOAc, and the EtOAc solution was washed with 2% H_2SO_4, saturated $NaHCO_3$ solution, and water and dried over anhydrous $MgSO_4$. Concentration of the filtered EtOAc solution followed by recrystallization of the residue from MeOH gave 0.299 g (52% overall from **11**) of **12**: mp 141–143° (lit.[21] mp 143°); nmr ($CDCl_3$) δ 1.30, 1.52 (s, 6, isopropylidene methyls), 2.07 (s, 6, acetyl methyls), 3.03 (s, 3, methanesulfonyl methyl), 5.37 (d, 1, J = 4 Hz, C-2 H), 5.91 (d, 1, J = 4 Hz, C-1 H).

Anal. Calcd for $C_{14}H_{22}O_{10}S$: C, 43.97; H, 5.80; S, 8.38. Found: C, 43.81; H, 5.68; S, 8.13.

Acid-Catalyzed Hydrolysis of 13.—Pure **13** (0.328 g) was refluxed in 5 ml of 1:1 v/v HOAc–water for 50 min. Cooling to 4° followed by filtration gave 0.064 g of recovered **13**, mp 144–145.5°. The filtrate was concentrated to dryness under vacuum and the residue was crystallized from water to give 0.026 g of **11**. Recrystallization from benzene gave prisms, mp 118–119.5°. The balance of **13** apparently underwent more extensive hydrolysis to water-soluble products.

Hydrogenolysis of 13.—Compound **13** (0.180 g) in 25 ml of THF was treated with 0.050 g of PtO_2 and hydrogenated under 50 psig H_2 at 25° for 2 hr. Filtration of catalyst followed by removal of THF under vacuum gave 0.098 g (84%) of **9**, mp 207–209° dec, whose ir (KBr) was identical with the ir of authentic **9**. One recrystallization from EtOH gave colorless needles, mp 229–231.5° dec. Similar reduction (1 hr) with EtOH in place of THF gave 82% of **9**, mp 204–205° dec, which on recrystallization from EtOH had mp 227.5–230° dec.

Methanolysis of 13.—Compound **13** (0.105 g) was refluxed in dry methanol (5 ml) for 19 hr and subsequently solvent was removed under vacuum. Nmr ($CDCl_3$) showed a new singlet at δ 3.80 ppm corresponding to 2.5 H (methyl carbonates from ring opening). Tlc indicated complete disappearance of **13** and formation of three reaction products. No evidence was found for methanolysis of the CBZ group in that peaks corresponding to benzyl alcohol were not observed.

Registry No.—**7**, 37056-03-4; **7** mono-*p*-nitrobenzyl derivative, 37056-04-5; **8**, 37056-05-6; **9**, 2875-90-3; **11**, 37056-07-8; **11** diacetate, 37056-08-9; **12**, 37056-09-0; **13**, 37056-10-3.

Acknowledgments.—The author is indebted to Dr. Richard S. Treptow for optical rotation measurements and to Mr. John D. Wendel for his able technical assistance with synthetic aspects of the work.

(21) K. Freudenberg and K. v. Oertzen, *Justus Liebigs Ann. Chem.*, **574**, 37 (1951).

Use of L-1,4-Cyclohexadiene-1-alanine in Peptide Synthesis as a Phenylalanine Analog

GOBICHETTIPALAYAM RAMAN NAGARAJAN,[1] LILLIAN DIAMOND, AND CHARLOTTE RESSLER*

Division of Protein Chemistry, Institute for Muscle Disease, and Department of Biochemistry, Cornell University Medical College, New York, New York 10021

Received June 21, 1972

In constructing analogs of biologically active peptides with potential inhibitory activity, a residue to replace phenylalanine has been needed. For this purpose, L-1,4-cyclohexadiene-1-alanine (L-2,5-dihydrophenylalanine, L-DiHPhe, **1**), a new and effective antagonist of phenylalanine,[2-5] appeared to be a likely candidate. It is readily available by Birch reduction of commercial phenylalanine and it also occurs naturally in several bacterial sources.[6] The present note examines attempts to incorporate L-DiHPhe into peptides and into a variety of derivatives suitable for peptide synthesis. Dehydrogenation to the phenylalanine compound and spirolactonization were considered to be the major likely side reactions.[2] When this study was essentially complete, incorporation of D-1,4-cyclohexadienylglycine into semisynthetic penicillins and cephalosporins came to our attention.[7] This diene was N-protected as an enamine or *t*-BOC derivative, and coupling was effected by a mixed anhydride procedure. No information, however, was given concerning dehydrogenation.

It was recently established that dehydrogenation of L-DiHPhe in the solid state is associated with a hydrated form of the amino acid which is unstable if stored and if an attempt is made to desiccate it.[2,8] Precautions were thus taken to store the solid as a stable salt or in aqueous solution and to avoid subjecting it to a high vacuum;[8] acylations were done under nitrogen. To confirm structure and determine the content of the corresponding phenylalanine compound all products were examined carefully by nmr, column chromatography, or uv absorption.

L-1,4-Cyclohexadiene-1-alanine methyl ester hydrochloride (**2**) was obtained in high yield by application of the Brenner–Huber method[9] to DiHPhe hydrate and was purified by crystallization.[10] Nmr evidence

(1) Visiting Research Fellow, 1967–1969.

(2) M. L. Snow, C. Lauinger, and C. Ressler, *J. Org. Chem.*, **33**, 1774 (1968).

(3) B. A. Shoulders, R. M. Gipson, R. J. Jandacek, S. H. Simonsen, and W. Shive, *J. Amer. Chem. Soc.*, **90**, 2992 (1968).

(4) C. Ressler, D. S. Genghof, C. Lauinger, and M. L. Snow, *Fed. Proc.*, **27**, 764 (1968).

(5) D. S. Genghof, *Can. J. Microbiol.*, **16**, 545 (1970).

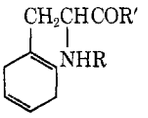
(6) Private communications: T. Yamashita, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan, 1968, and G. E. Mallett, Lilly Research Laboratories, 1968. See also T. Yamashita, N. Miyairi, K. Kunugita, K. Shimizu, and H. Sakai, *J. Antibiot.*, **23**, 537 (1970), and J. P. Scannell, D. L. Pruess, T. C. Demny, T. H. Williams and A. Stempel, Abstracts of Papers, 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970.

(7) J. E. Dolfini, H. E. Applegate, G. Bach, H. Basch, J. Bernstein, J. Schwartz, and F. L. Weisenborn, *J. Med. Chem.*, **14**, 117 (1971).

(8) C. Ressler, *J. Org. Chem.*, **37**, 2933 (1972).

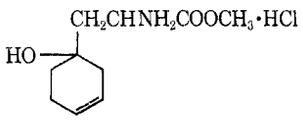
(9) M. Brenner and W. Huber, *Helv. Chim. Acta*, **36**, 1109 (1953).

(10) Compound **2** could also be prepared by starting from the Cu complex of L-DiHPhe. This was converted directly with $COCl_2$ to the *N*-carboanhydride by the general method of R. D. Hamilton and D. J. Lyman, *J. Org. Chem.*, **34**, 243 (1969). Without isolation the *N*-carboanhydride was then treated with MeOH–HCl.



	R	R′		R	R′
1	H	OH	6	Cbz	$OC_6H_4NO_2$
2	H·HCl	OMe	7	*t*-BOC	$OC_6H_4NO_2$
3	H	NH_2	8	Cbz	$NHCH_2COOBz$
4	Cbz	OH	9	*t*-BOC	$NHCH_2COOMe$
5	*t*-BOC	OH	10	*t*-BOC	$NHCH_2COOH$
			11	*t*-BOC	$NHCH_2CONH_2$

of three vinyl hydrogens supported its structure and excluded i, a possible product, under the acidic con-



i

ditions, of spirolactonization followed by methanolysis.[2] On storage, **2** tended to fall gradually in melting point and decompose with extensive dehydrogenation. However, freshly prepared **2** could be freed of HCl and treated with MeOH–NH_3 to give L-1,4-cyclohexadiene-1-alaninamide (**3**), which was isolated as the base in over 60% yield and which proved stable.

N-Benzyloxycarbonyl-L-1,4-cyclohexadiene-1-alanine (Cbz-L-DiHPhe, **4**) was obtained from the crude reduction mixture of L-Phe, *i.e.*, without first isolating L-DiHPhe, by treating it with benzyloxycarbonyl chloride (CbzCl). After purification by crystallization, the overall yield from Phe was 41%, which approached the yield obtainable from isolated L-DiHPhe. Compound **4** was stable under prolonged storage in the cold. An attempt to similarly prepare *N*-*tert*-butyloxycarbonyl-L-1,4-cyclohexadiene-1-alanine (*t*-BOC-L-DiHPhe, **5**) with *tert*-butyloxycarbonyl azide (*t*-BOC azide) was unsuccessful; the high concentration of salts in the crude reduction mixture of L-Phe seemed to retard acylation. L-DiHPhe was therefore first freed of salts by starting with the easily isolable copper complex and liberating L-DiHPhe from it with H_2S. This procedure was more convenient, especially on a large scale, than that described earlier,[2] involving Chelex resin in concentrated NH_3. Compound **5** is a low-melting solid (mp near 40°) that tended to decompose with dehydrogenation when stored at room temperature. Preferentially, it was used without isolation soon after it had been prepared or was isolated as the dicyclohexylammonium (DCHA) salt (**5a**), which was easily crystallized, was stable, and could be freed of DCHA before use.

Both **4** and **5**, when treated with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *p*-nitrophenol, gave in good yield their *p*-nitrophenyl ester Cbz-L-DiHPheNPE (**6**) and *t*-BOC-L-DiHPheNPE (**7**), which proved stable. Compounds **6** and **7** coupled smoothly with glycine benzyl ester and glycine methyl ester, respectively, to yield *N*-benzyloxycarbonyl-1,4-cyclohexadiene-1-alanylglycine benzyl ester (Cbz-L-DiHPheGlyBz, **8**) and *N*-*tert*-butyloxycarbonyl-1,4-cyclohexadiene-1-alanylglycine methyl ester (*t*-BOC-L-DiHPheGlyOCH$_3$, **9**). These dipeptides were also prepared by a DCC coupling of **4** and **5** with the glycine esters. In each case, the *p*-nitrophenyl ester procedure gave a somewhat better yield and product.

Treatment of ester **9** in aqueous Me_2CO with 1 equiv of NaOH converted it in good yield to *N*-*tert*-butyloxycarbonyl-L-1,4-cyclohexadiene-1-alanylglycine (*t*-BOC-L-DiHPheGly, **10**). Likewise, treatment of **9** with MeOH–NH_3 gave without difficulty *N*-*tert*-butyloxycarbonyl-L-1,4-cyclohexadiene-1-alanylglycinamide (*t*-BOC-L-DiHPheGlyNH$_2$, **11**).

The foregoing experiments demonstrate the stability of L-1,4-cyclohexadiene-1-alanine to the conditions of peptide coupling by the carbodiimide and nitrophenyl ester procedures, to esterification under acidic conditions and to deesterification under alkaline conditions, to ester amidation, and to acylation by *tert*-butyloxycarbonyl azide. Carbobenzyloxylation led to 4–10% dehydrogenation, but the product, Cbz-L-DiHPhe, could be improved by recrystallization. L-DiHPhe-OCH$_3$·HCl and *t*-BOC-L-DiHPhe dehydrogenated gradually and extensively upon storage; these products, however, can be used soon after preparation. Thus, with appropriate care, L-1,4-cyclohexadiene-1-alanine can be used in peptide synthesis. *t*-BOC-L-DiHPhe-NPE so far appears to be the reagent of choice for introducing this amino acid into peptides.

Experimental Section

L-PheGly and L-PheNH$_2$ acetate were purchased from Mann Research Laboratories, New York, N.Y. Elemental analyses were carried out by Micro-Tech Laboratories, Skokie, Ill. Nmr spectra were obtained on a Varian T-60-C spectrometer by Sadtler Research Laboratories, Philadelphia, Pa. Melting points were taken in open capillaries on a Thomas-Hoover apparatus and are corrected.[2] Optical rotations were determined and automatic amino acid analyses[11] were carried out as described elsewhere.[2] Phe and DiHPhe were analyzed in system 2[2] and L-PheGly and L-DiHPheGly, in system 1,[2,12] in which they eluted 29 and 57 ml, respectively, after γ-aminobutyric acid. Ascending paper chromatography was done on Whatman No. 1 paper in *n*-BuOH–PYR–AcOH–H_2O (30:20:6:24).

Determination of Contamination of DiHPhe Derivatives by Phe Compounds.—Compounds **4** and **8** were deprotected with Na(NH_3) and **5** and **10** with trifluoroacetic acid (0.8 ml for 50 μmol, 15 min at 25°). The products were determined on the amino acid analyzer. Compounds **2**, **3**, **5**, **5a**, and **9**–**11** were each examined by uv absorption; compounds **6** and **7** were examined indirectly as **8** and **9**. Occasionally, nmr spectra were obtained. In mixtures in CD_3OD, the $-C(CH_3)_3$ singlet of *t*-BOC-L-Phe, at δ 1.39, appeared separate from that of *t*-BOC-L-DiHPhe, at δ 1.45. Likewise, the $-C(CH_3)_3$ singlet of *t*-BOC-L-DiHPheGlyOCH$_3$ in acetone-d_6 was distinguishable from that of *t*-BOC-L-PheGlyOCH$_3$. Integration of the $-C(CH_3)_3$ protons gave the amount of Phe impurity, thus supplementing the use of the aromatic protons for this purpose.

L-1,4-Cyclohexadiene-1-alanine Methyl Ester Hydrochloride (2).—L-DiHPhe·0.75H_2O (2.6 g, 14.4 mmol) was added in portions over a 30-min period to a mixture of anhydrous MeOH (36 ml, 0.88 mol) and $SOCl_2$ (6 ml, 83 mmol) in a magnetically stirred bath at −10°. The mixture was allowed to come to room temperature, where it was kept for 18 hr. It was then concentrated to dryness. The oil was taken up in MeOH, which was then evaporated, and the process was repeated three times. The residue was crystallized from MeOH–Et_2O; the yield was 2.46 g (79%), mp 122–125°. Compound **2** was recrystallized (charcoal) three times from $CHCl_3$–Et_2O: mp 125–126°; $[\alpha]_D$ −30.4° (*c* 1, H_2O). It contained 2.8% Phe compound. **2** had R_f 0.85; L-Phe-OCH$_3$·HCl had R_f 0.79. Nmr for **2** in DMSO-d_6: δ 2.45–2.65 (allylic, 6 H), 3.7 (OCH$_3$, 3 H), 4.1 (α-CH, 1 H), 5.5–5.8 (vinyl, 3 H), 8.8 (NH_3^+, 2–3).

Anal. Calcd for $C_{10}H_{16}ClNO_2$: C, 55.2; H, 7.41; N, 6.44. Found: C, 55.7; H, 7.47; N, 6.62.

(11) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(12) C. Ressler and D. V. Kashelikar, *J. Amer. Chem. Soc.*, **88**, 2025 (1966).

L-1,4-Cyclohexadiene-1-alaninamide (3).—A suspension of **2** (1 g, 4.6 mmol, 2.8% Phe compound) in 50 ml of EtOAc was freed of HCl with 1.3 ml of Et_3N, as described for **8**. The oil was dissolved in 50 ml of dry MeOH and amidated for 3 days, essentially as described for **11**. The product was concentrated to dryness, and the solid residue was crystallized from EtOAc, yielding 0.48 g (63%), mp 98–100°. The needles of **3** were redissolved in EtOAc at 55–60° (charcoal) and allowed to crystallize at room temperature: mp 103–104°; $[\alpha]^{27}D$ −21.6° (*c* 0.8, 1 *N* AcOH), with 2% Phe compound. **3** had R_f 0.7; L-PheNH$_2$ had R_f 0.68.

Anal. Calcd for $C_9H_{14}N_2O$: C, 65.0; H, 8.49; N, 16.9. Found: C, 64.7; H, 8.55; N, 16.8.

***N*-Benzyloxycarbonyl-L-1,4-cyclohexadiene-1-alanine (4).**—L-Phe (10 g, 60.5 mmol) was reduced with Na–MeOH–NH_3.[2] The dry residue was suspended in 300 ml of H_2O, cooled in a water bath (15–20°), and placed under N_2. The solution was adjusted to pH 8.5 when most of the solid dissolved. A solution of CbzCl (12.3 g, 72 mmol) in 80 ml of Et_2O was added dropwise over a 70-min period, along with 69 ml of 2 *N* NaOH to maintain the pH. The mixture was stirred overnight.[13] The oily bottom phase was separated and diluted with 80 ml of H_2O. The solution was extracted with Et_2O, adjusted to pH 2 with 4 *N* HCl, and extracted with 120 ml of EtOAc. The extract was dried ($MgSO_4$) and concentrated to a syrup, which was taken up in CCl_4 and diluted with petroleum ether (bp 30–60°). Cooling this extract yielded 12.75 g of a white solid, mp 72–79°. Recrystallization of 6 g from CCl_4 (45–50° bath) yielded 3.48 g (41%), mp 77–81°. After three recrystallizations **4** melted at 82–83.5°: $[\alpha]^{20}_{546}$ −1.8°; $[\alpha]^{27}D$ +2.6° (*c* 1.1, MeOH). It contained 2.6% Phe compound and 3.3% Ene.

Anal. Calcd for $C_{17}H_{19}NO_4$: C, 67.8; H, 6.36; N, 4.65. Found: C, 68.0; H, 6.38; N, 4.68.

***N*-Benzyloxycarbonyl-L-1,4-cyclohexadiene-1-alanine *p*-Nitrophenyl Ester (6).**—DCC (2.06 g, 10 mmol) dissolved in 10 ml of EtOAc was added to a solution of **4** (3 g, 10 mmol, 2.6% Phe) and *p*-nitrophenol (1.67 g, 12 mmol) in 50 ml of EtOAc, which had been placed in an ice bath. The mixture was stirred under N_2 for 30 min at 5° and then for 90 min at room temperature. AcOH (0.1 ml) was added, and after 5 min the urea was filtered off. The filtrate was concentrated to a pale yellow, crystalline residue, which was then recrystallized from 70 ml of hot EtOH containing 60 μl of AcOH, yielding 3.57 g (85%), mp 114–118.5°. This material was redissolved in EtOH and recrystallized: mp 118.5–120°; $[\alpha]^{25}D$ −33.2° (*c* 1, DMF), with 2.8% or less of the Phe derivative. Nmr for **6** in $CDCl_3$: δ 2.65–2.8 (allylic, 6 H), 4.6–5.0 (α-CH, 1 H), 5.3 ($C_6H_5CH_2O$, 2 H), 5.4–5.6 (NH, 1 H), 5.75–5.95 (vinyl 3 H), 7.35–7.57 (aromatic, 7 H); 8.4–8.57 (aromatic adjacent to $-NO_2$, 2 H).

Anal. Calcd for $C_{23}H_{22}N_2O_6$: C, 65.4; H, 5.25; N, 6.63. Found: C, 65.6; H, 5.29; N, 6.61.

***N*-Benzyloxycarbonyl-L-1,4-cyclohexadiene-1-alanylglycine Benzyl Ester (8a). Nitrophenyl Ester Coupling.**—A suspension of GlyBz·HCl (1.53 g, 7.6 mmol) in 70 ml of EtOAc and 1.05 ml of Et_3N was stirred for 3.5 hr. The solid was filtered off and the filtrate was concentrated. The oil was taken up in 10 ml of EtOAc, **6** (2.2 g, 5.2 mmol) was added, and the solution was allowed to stand at 25° overnight. It was then shaken successively with 0.5 *N* NH_3, H_2O, 0.5 *N* HCl, and H_2O. The solution was dried ($MgSO_4$) and concentrated to a white solid, 2.28 g (98%), mp 112–116°. Compound **8a** was recrystallized twice from EtOAc–petroleum ether: mp 118.5–119.5°; $[\alpha]^{26}_{546}$ −18.6° (*c* 1, MeOH); $[\alpha]^{27}D$ − 14.9° (*c* 1.25, MeOH). Crude and analytical materials contained 2.8 and 2.6% PheGly.

Anal. Calcd for $C_{26}H_{28}N_2O_5$: C, 69.6; H, 6.29; N, 6.25. Found: C, 69.7; H, 6.34; N, 6.15.

8b. Carbodiimide Coupling.—A suspension of GlyBz·HCl (0.20 g, 0.99) mmol) in 4 ml of THF was freed of HCl with 175 μl of Et_3N, as for **8a**. The oil was taken up in 4 ml of THF, and to this was added **4** (0.25 g, 0.83 mmol) containing 6% Phe and 5.5% ene compounds. The solution was cooled in a bath at 5° under a stream of N_2. A solution of DCC (0.17 g, 0.83 mmol) in 1.5 ml of THF was then added. The mixture was stirred for 2 hr and then filtered. The solvent was removed, and the residue was taken up in EtOAc. The solution was shaken with 5% $NaHCO_3$, H_2O, 0.2 *N* AcOH, and H_2O. The extract was dried ($MgSO_4$) and concentrated, and the residue was crystallized from EtOAc–petroleum ether, yielding 0.24 g (65%), mp 115–117°, $[\alpha]^{26}_{546}$ −19.7° (*c* 1, MeOH), with 5.8% Phe compound. A mixture of **8b** with **8a** had mp 116–118°.

***N*-*tert*-Butyloxycarbonyl-L-1,4-cyclohexadiene-1-alanine Dicyclohexylammonium Salt (5a).**—An aqueous solution (110 ml) of L-DiHPhe liberated by H_2S from the Cu complex (3.3 g, 16.7 mequiv) was stirred under a stream of N_2 as a solution of *t*-BOC azide (4.77 g) in 110 ml of dioxane and MgO (1.34 g) was added. Stirring was continued at room temperature and in an N_2 atmosphere for 60 hr. The mixture was filtered, and the filtrate, after being extracted twice with 150 ml of EtOAc, was cooled, adjusted to pH 7 with 20% citric acid, concentrated to 60 ml, and then adjusted to pH 3. The pasty mixture was extracted with 100 ml of EtOAc. The aqueous phase was saturated with NaCl and reextracted twice with 75 ml of EtOAc. The combined organic extract of **5** was washed with saturated NaCl and then dried ($MgSO_4$). It was concentrated to 15 ml and diluted with 75 ml of Et_2O. DCHA (6.05 g) diluted with several milliliters of Et_2O was then added. The crystals were collected after 1 hr in the cold, 5.53 g (74%), mp 208–209°. Recrystallization from approximately 100 ml of EtOH gave 4.63 g, mp 209–210°, with 1.7% Phe, 4.4% ene, and 93.9% DiHPhe compounds. For analysis, material was recrystallized three times from MeOH, mp 210–210.5° dec, $[\alpha]^{24}D$ +6.3° (*c* 1.2, MeOH), with 2.5% Phe compound.

Anal. Calcd for $C_{26}H_{44}N_2O_4$: C, 69.6; H, 9.89; N, 6.24. Found: C, 69.5; H, 9.97; N, 6.42.

***N*-*tert*-Butyloxycarbonyl-L-1,4-cyclohexadiene-1-alanine (5).**—To a suspension of *t*-BOC-L-DiHPheDCHA (0.8 g, 1.78 mmol) in 25 ml of EtOAc at 5° were added 8 ml of 0.5 *N* H_2SO_4, and the mixture was shaken quickly. The organic phase was separated, washed twice with H_2O, and dried ($MgSO_4$). The extract was then concentrated almost to dryness, and 10 ml of petroleum ether (bp 37–51°) were added. The clear solution was set aside at −35°. The crystals of **5** were collected by filtration in the cold, mp 39–42°. These contained 1–3% Phe compound and were suitable for further work; yields ranged from 80 to 96%. Nmr for **5** in CD_3OD: δ 2.45 [$C(CH_3)_3$, 9 H], 2.28–2.67 (allylic, 6 H), 4.3 (α-CH, 1 H), 5.57–5.72 (vinyl, 3 H).

***N*-*tert*-Butyloxycarbonyl-L-1,4-cyclohexadiene-1-alanine *p*-Nitrophenyl Ester (7).**—The concentrated extract of **5** before treatment with DCHA (see **5a**) was taken to dryness, and the syrupy residue of **5** (2.77 g, 10.4 mmol) was converted to the *p*-nitrophenyl ester, as described for **6**. Concentration of the reaction mixture left **7** as a residue, which solidified in the cold. This was triturated with petroleum ether and collected, wt 3.2 g (80%), mp 77–95°. Two recrystallizations from EtOH left 1.8 g (45% based on **5**), mp 102–104°. For analysis, **7** was recrystallized three times from EtOH: mp 104–105°; $[\alpha]^{28}D$ −44.3° (*c* 1, MeOH), with 2.5% or less of the Phe compound; nmr ($CDCl_3$) δ 1.47 [$C(CH_3)_3$, 9 H], 2.51–2.71 (allylic, 6 H), 4.4–4.8 (α-CH, 1 H), 4.98–5.09 (NH, 1 H), 5.61–5.71 (vinyl, 3 H), 7.2–7.35 (aromatic, 2 H), 8.2–8.35 (aromatic adjacent to $-NO_2$, 2 H).

Anal. Calcd for $C_{29}H_{24}N_2O_6$: C, 61.8; H, 6.23; N, 7.21. Found: C, 61.8; H, 6.17; N, 7.07.

***N*-*tert*-Butyloxycarbonyl-L-1,4-cyclohexadiene-1-alanylglycine Methyl Ester (9a). Nitrophenyl Ester Method.**—Distilled GlyOMe (0.356 g, 3.9 mmol) and **7** (0.712 g, 1.8 mmol) were coupled as described for **8a**, except that CH_2Cl_2 (10 ml) was the solvent. Compound **9** was crystallized from EtOAc–petroleum ether and recrystallized from Et_2O–petroleum ether, yielding 0.43 g (69%), mp 85–87°, $[\alpha]^{24}D$ −18.7° (*c* 0.6 AcOH), with 2.5% Phe compound.

Anal. Calcd for $C_{17}H_{26}N_2O_5$: C, 60.3; H, 7.74; N, 8.28. Found: C, 60.8; H, 7.87; N, 8.25.

9b. Carbodiimide Method.—GlyOMe (0.47 g, 5.3 mmol) and **5** (1.1 g, 4.1 mmol, with 4.4% Phe compound) obtained from the DCHA salt were coupled in CH_2Cl_2 (25 ml) with DCC (0.9 g, 4.4 mmol) as described for **8b** and isolated as described for **9a**. The yield was 0.89 g, mp 73–76°. Recrystallization from Et_2O–petroleum ether followed by MeOH–H_2O left 0.64 g (46%) of material, mp 76–81°, containing 5.3% Phe compound.

***N*-*tert*-Butyloxycarbonyl-L-1,4-cyclohexadiene-1-alanylglycine (10).**—To a solution of **9** (172 mg, 0.51 mmol, 5% Phe compound) in 1 ml of 66% Me_2CO was added dropwise 0.5 ml of 1 *N* NaOH. When base no longer was consumed, the solution was allowed to stand for 30 min; it was then adjusted to pH 7 with 5% citric acid and concentrated to a small volume. This was then extracted with wet EtOAc, cooled, acidified to pH 3, and again ex-

(13) This period can probably be shortened. Carbobenzyloxylation for 2 hr of L-DiHPhe liberated from the Cu complex with H_2S afforded **4** of similar purity in 57% yield (S. N. Banerjee, 1972, unpublished work).

tracted. The extract was washed with H_2O and dried ($MgSO_4$). On concentration, crystallization started and was complete after several hours in the cold; the yield was 118 mg (71%), mp 136.5–140.5°. For analysis, the material was recrystallized twice from EtOAc, mp 138.5–139.5°, $[\alpha]^{24}D$ −11.8° (c 0.7, MeOH), with 6% PheGly and 94% DiHPheGly derivatives.

Anal. Calcd for $C_{16}H_{24}N_2O_5$: C, 59.2; H, 7.46; N, 8.64. Found: C, 59.3; H, 7.41; N, 8.49.

N-tert-**Butyloxycarbonyl-L-1,4-cyclohexadiene-1-alanylglycinamide** (11).—Anhydrous MeOH (10 ml) was saturated with NH_3 distilled over Na. Ester 9 (275 mg, 0.81 mmol, with 5% Phe compound) was then added. The solution was allowed to stand at room temperature and, after 24 hr, was resaturated with NH_3. After 48 hr, the mixture was concentrated. The oily residue was taken up three times in MeOH and then twice in EtOAc, the solvent being evaporated off each time. Trituration with Et_2O gave a white solid, 230 mg (87%), mp 99–108°. Recrystallization from EtOAc–Et_2O left 166 mg (63%), mp 106–108°, $[\alpha]^{24}D$ +0.2° (c 0.9, MeOH), with 6% Phe compound.

Anal. Calcd for $C_{16}H_{25}N_3O_4$: C, 59.4; H, 7.79; N, 13.0. Found: C, 59.6; H, 7.85; N, 12.9.

Registry No.—**1**, 16055-12-2; **2**, 33423-61-9; **3**, 36959-88-3; **4**, 36959-89-4; **5**, 36959-90-7; **5a**, 36959-91-8; **6**, 36959-92-9; **7**, 36959-93-0; **8**, 36959-94-1; **9**, 36959-95-2; **10**, 36959-96-3; **11**, 36959-97-4.

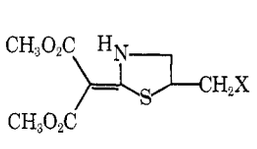
Acknowledgment.—This work was aided by Grant NS 04316 from the U. S. Public Health Service and by Muscular Dystrophy Associations of America. Preliminary experiments in acylating DL-1,4-cyclohexadiene-1-alanine were carried out by Dr. A. F. Wu.

Additions and Corrections

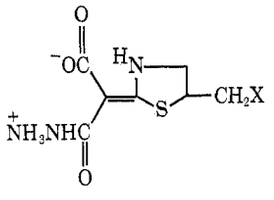
Vol. 37, 1972

George Just* and Phillip Rossy: The Action of Hydrazine and Its Derivatives on the Addition Products of Allyl Isothiocyanate and Dimethyl Malonate. A Correction.

Page 318. Column 2. The structures for Va–d and VIII should be given as



Va, X = Br
b, X = I
c, X = OH
d, X = ONO_2



VIII, X = I

Page 319. Column 1, paragraph 4, line 23. After "could be duplicated" the following sentences should be added. "However Worrall's assignment of the structure was wrong.[5] Compounds Va–d should be the thiazolidine and not the dihydrothiazine. Worrall suggested an intermediate dibromo compound. It has been shown[6] in analogous cases that the intermediate is a bromonium ion and the mechanism involves an ionic intermediate. The product of this type of cyclization usually has a five-membered and not a six-membered ring,[7] even if the carbonium ion leading to the six-membered ring is more favored.[6a,b] Reexamination of the nmr spectrum of Vd shows that there were two low-field protons at 4.7 ppm rather than one. Reduction of Vb with palladium on charcoal gives a compound which shows the presence of a methyl group (doublet at 1.4 ppm). Finally, treatment of Va with diethylamine or sodium hydroxide leads to a compound having a terminal methylene group (3030, 1645, and 870 cm^{-1} in ir spectrum). (Compound VIII has similarly been shown to have the five-membered structure.)"

(5) We wish to thank Dr. I. Monkovic and Dr. K. S. Dhami for having drawn our attention to this error, and for helpful discussions.

(6) (a) E. Demole and P. Enggist, *Helv. Chim. Acta*, **54**, 456 (1971); (b) O. Tanaka, *et al.*, *Tetrahedron Lett.*, 4235 (1968); (c) D. L. H. Williams, E. Bienvenue-Goetz, and J. E. Dubois, *J. Chem. Soc. B*, 517 (1969); (d) V. I. Staninets and E. A. Shilov, *Russ. Chem. Rev.*, **40**, (3), 272 (1971).

(7) H. S. Sachdev, K. S. Dhami, and M. S. Atwal, *Tetrahedron*, **14**, 304 (1961); (b) H. Singh, K. S. Bhandari, and K. S. Narang, *J. Indian Chem. Soc.*, **41**, 715 (1964); (c) T. Ajello and A. Miraglia, *Gazz. Chim. Ital.*, **78**, 921 (1948).