1,4-Cyclohexadiene-1-alanine (2,5-Dihydrophenylalanine), a New Inhibitor of Phenylalanine for the Rat and *Leuconostoc dextranicum* 8086[1]

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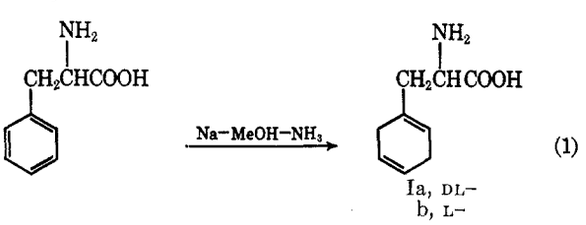
The loss of phenylalanine previously encountered as a side reaction on applying a dehydration–reduction procedure to certain peptides has been investigated. Free phenylalanine is found to undergo reductive conversion with sodium–methanol–ammonia into the new amino acid, 1,4-cyclohexadiene-1-alanine (2,5-dihydrophenylalanine). Structure was assigned on the basis of the amino acid's uptake of 2 molar equiv of hydrogen to yield β-cyclohexylalanine, its nmr spectrum, and its partial hydrogenation to 1-cyclohexene-1-alanine. This was identified mainly by nmr spectrum and conversion in acid into a γ-spirobutyrolactone hydrochloride, as indicated by infrared data. Convenient preparative syntheses are described of DL- and L-1,4-cyclohexadiene-1-alanine and their N-acetyl derivatives from commercial DL- and L-phenylalanine. Also isolated and characterized were DL-cyclohexene-1-alanine, γ-cyclohexanespiro-DL-aminobutyrolactone hydrochloride, and DL-β-(1-hydroxycyclohexyl)alanine. 1,4-Cyclohexadiene-1-alanine is an effective antagonist of phenylalanine for the growth of *Leuconostoc dextranicum* 8086 and of the young rat.

When evolidine and bacitracin were subjected to a dehydration–reduction procedure for identifying *endo*-asparagine and glutamine residues in peptides, partial loss of phenylalanine resulted.[2] This loss was attributed tentatively to reduction of the aromatic ring under the conditions of the Birch reduction used. We have now investigated this side reaction by treating phenylalanine itself with sodium–methanol–ammonia under similar conditions. The phenylalanine was partly converted into unidentified material with an unusual *brick-orange* ninhydrin color. When chromatographed on paper, the new material traveled with a slightly higher R_f than phenylalanine. On the amino acid analyzer it was eluted just after phenylalanine, with a characteristically high 440:570-mμ absorption ratio of 0.5, compared with 0.2 for phenylalanine. It differed in its chromatographic behavior on paper and on the analyzer also from known β-cyclohexylalanine and 3-cyclohexene-1-alanine. This communication reports the identification of the product as 1,4-cyclohexadiene-1-alanine (2,5-dihydrophenylalanine, I). With the aid of chromatographic observations convenient one-step procedures have been developed for syntheses from commercial DL- and L-phenylalanine of the DL and L forms of this new amino acid in crystalline state and in reasonable purity and yield.



(1)

Ia, DL–
b, L–

The ready synthesis of I and its properties were of special interest because of the structural relation this amino acid bears to certain biologically active tetrahydrophenylalanines, DL-1-cyclohexene-1-alanine and DL-3-cyclohexene-1-alanine, that have been accessible only *via* lengthy synthetic routes, *viz.*, condensations of ethyl acetamidocyanoacetate with appropriate cyclohexene-ω-methyl bromides. In the microorganism *Leuconostoc dextranicum* 8086, the tetrahydrophenylalanines are effective antagonists of phenylalanine and leucine, respectively.[3,4] Coplanarity of the ring and the bond attaching the side chain, considered to be a factor in the inhibitory activity toward phenylalanine, should be enhanced by the presence of a second double bond in the ring. Thus, I might be expected to be a more active inhibitor of phenylalanine than 1-cyclohexene-1-alanine. In the present study 1,4-cyclohexadiene-1-alanine has been tested as an antagonist of phenylalanine for *L. dextranicum* 8086 and compared with 1-cyclohexene-1-alanine. Its effect on the growth and survival of the young rat has also been investigated.

After treatment with excess sodium in liquid ammonia, either in anhydrous condition or in the presence of 0.5% water, phenylalanine was recovered quantitatively, and no reduction product was detected by amino acid analysis. Reductive conversion of phenylalanine to I is therefore not expected to be a serious side reaction of peptide synthesis under the conditions frequently used for the removal of the S-benzyl, N-carbobenzoxy, and N-tosyl protecting groups with the sodium–liquid ammonia reagent.

The products obtained on treatment of phenylalanine with sodium in liquid ammonia in the presence of methanol[5] consisted of mixtures of 2,5-dihydrophenylalanine and phenylalanine in proportions which varied with the conditions of the reaction. Since recrystallization did not significantly improve the purity of such products, attempts were made to bring reaction 1 close to completion. Microscale experiments examined by amino acid analysis indicated that this could be accomplished with large excesses of both sodium and methanol. With 20 equiv of sodium and 100 equiv of methanol, 1,4-cyclohexadiene-1-alanine was formed in 94% yield. From 10 g of DL-phenylalanine the crude DL-amino acid (Ia) was isolated in 55–60% yield directly from the dried reaction mixture by washing with water to remove salts. A single crystallization from 50% ethanol gave DL-1,4-cyclohexadiene-1-alanine in 43–48% yield and 92–95% purity. The product retained 0.5–2% phenylalanine and also had 3–5% of a

(1) This work was aided by Grant NB 04316 from the U. S. Public Health Service and by Muscular Dystrophy Associations of America.

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

(3) J. Edelson, P. R. Pal, C. G. Skinner, and W. Shive, *ibid.*, **79**, 5209 (1957).

(4) J. Edelson, C. G. Skinner, J. M. Ravel, and W. Shive, *Arch. Biochem. Biophys.*, **80**, 416 (1959).

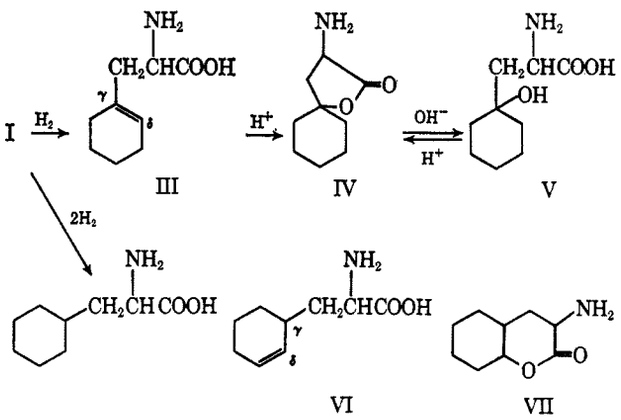
(5) Since these observations were first made, it was noted that lithium in methylamine converts certain phenylalanine peptides into mixtures of the cyclohexadiene and cyclohexene derivatives that can be cleaved with N-bromosuccinimide [M. Wilchek and A. Patchornik, *J. Amer. Chem. Soc.*, **84**, 4613 (1962)].

by-product, cyclohexene-1-alanine (III). The L isomer (Ib) was too soluble to be isolated directly in good yield from the aqueous solution of the reaction mixture. It was separated instead, in 83% yield or more, as a crystalline copper chelate that was washed free of reaction salts with water. Attempts to remove copper from the chelate in the conventional way with hydrogen sulfide led to 3–6% dehydrogenation to phenylalanine. A better product was obtained by treating the complex in concentrated ammonia with Chelex 100 chelating resin (NH_4^+ cycle). The liberated L-1,4-cyclohexadiene-1-alanine was isolated from 80% ethanol in 67% yield, then crystallized from the same solvent. Such products were over 92% homogeneous and contained less than 2% phenylalanine. Like the DL-amino acid, Ib gave satisfactory values on elemental analysis. By treating the crude aqueous mixtures with acetic anhydride in the presence of dilute base and extracting with ethyl acetate, Ia and b could also be isolated readily as highly crystalline, stable N-acetyl derivatives. The composition of the latter was confirmed by enzymatic deacylation with a crude preparation from *Aspergillus oryzae*, which, in each case, afforded I in close to the expected yield. Attempts to deacylate with hog kidney acylase were less successful. The mold acylase is generally more reactive toward derivatives of aromatic amino acids than toward those of aliphatic amino acids.[6]

Ib absorbed 2 molar equiv of hydrogen on catalytic hydrogenation over platinum to yield β-cyclohexylalanine, the identity and homogeneity of which were established by chromatography on paper and on the amino acid analyzer. Since the optical rotation of the hydrochloride after one recrystallization agreed in magnitude with that of a reference sample of D-β-cyclohexylalanine hydrochloride, no significant degree of racemization apparently accompanies the reduction of L-phenylalanine to the diene Ib. Ia was destroyed on heating in 6 *N* HCl at 110° for 18 hr, giving rise to numerous unidentified products. Such behavior is consistent with the loss of phenylalanine initially observed on dehydration–reduction–hydrolysis of evolidine and bacitracin.[2] Under certain mild conditions noted in the Experimental Section, Ib in the solid state tended to dehydrogenate spontaneously to phenylalanine. Ia and b were otherwise reasonably stable.

That the reduction product of phenylalanine had structure I was suggested by analogy with products of Birch reduction of certain dialkyl- and alkylalkoxybenzenes, considered to be 2,5-dihydrobenzene derivatives.[7] Moreover, of the five structures possible, the absence of appreciable absorption in the ultraviolet region at 225 mμ and at higher wavelengths eliminated the conjugated isomers (1,3-cyclohexadiene-1-, -2-, and -6-alanines). This left as possibilities only the two unconjugated isomers, 1,4-cyclohexadiene-1-alanine (I) and 1,4-cyclohexadiene-6-alanine (II).

Support for structure I was obtained from the results of the sequence of reactions I → III → IV. The cyclohexadienealanine was partially hydrogenated to a cyclohexenealanine, a reference sample of which was isolated by column chromatography on the amino acid analyzer, then crystallized. The crude hydrogenation product, which also contained phenylalanine and β-cyclohexylalanine, was refluxed overnight in 6 *N* HCl, and the basic lactone thus formed was purified by electrophoresis, then isolated as the crystalline hydrochloride which was examined in the infrared. It was expected that hydrogenation of I would take place preferentially at the less hindered double bond to yield 1-cyclohexene-1-alanine (III) and that lactonization of III would give γ-cyclohexanespiro-α-aminobutyrolactone (IV). Amino acids unsaturated in the γ–δ position, such as 2-cyclohexene-1-glycine,[3] β-methallylglycine, and allylglycine,[8,9] are known to form γ-lactones on heating in acid; ethyl 1-cyclohexene-1-propionate yields cyclohexane-γ-spirobutyrolactone on refluxing in HCl.[10] Such γ-lactones are generally characterized by an absorption band within the range 1781–1777 cm^{-1}.[11] Partial hydrogenation of 1,4-cyclohexadiene-6-alanine (II), in contrast, should yield 2-cyclohexene-1-alanine (VI). If lactonization of VI could be accomplished, the expected product would be the bicyclic 2-δ-lactone (VII) which should have a characteristic absorption band within the range 1750–1735 cm^{-1} like a number of steroid δ-lactones.[11,12] Since the relevant absorption band of the isolated lactone occurred at 1781 cm^{-1}, the lactone is thought to be IV; the cyclohexenealanine, III; and the reduction product of phenylalanine, 1,4-cyclohexadiene-1-alanine (I).



Nuclear magnetic resonance spectra were consistent with the structures assigned as I and III. The spectra of Ia, as the carboxylate anion in D_2O (Figure 1) and of N-acetyl Ib in dimethyl sulfoxide-d_6 showed the expected protons and, in particular, were clearly characterized by signals for three vinyl protons, a one-proton signal near 5.45 and a two-proton signal near 5.65. These are attributed to the vinyl proton on C-2 and to the two equivalent vinyl protons on C-4 and -5 of the ring, respectively. The spectrum of III had a one-proton signal near 5.5 assigned as the vinyl proton on C-2 of the ring. By contrast, the theoretical spectrum of II should indicate four vinyl protons, possibly as two separate two-proton signals; the spectrum of VI should show two vinyl protons.

(6) I. Chibata (see J. P. Greenstein, and M. Winitz, "Chemistry of the Amino Acids," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1961, pp 1771, 1772).

(7) A. J. Birch, *J. Chem. Soc.*, 430 (1944).

(8) J. Fillman and N. Albertson, *J. Amer. Chem. Soc.*, **70**, 171 (1948).

(9) H. L. Goering, S. J. Cristol, and K. Dittmer, *ibid.*, **70**, 3310 (1948).

(10) D. W. Mathieson, *J. Chem. Soc.*, 177 (1951).

(11) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y., 1958, pp 179–187.

(12) R. N. Jones, P. Humphries, and K. Dobriner, *J. Amer. Chem. Soc.*, **72**, 956 (1950).

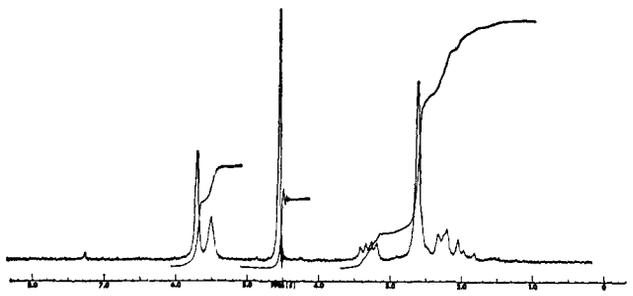


Figure 1.—Nmr spectrum of DL-1,4-cyclohexadiene-1-alanine as the carboxylate anion in D_2O.

Lactone IV, on treatment with dilute aqueous sodium hydroxide at 100°, was converted in good yield into a new neutral product, which was characterized by chromatographic behavior, melting point, infrared spectrum, and elemental analysis, and is considered to be β-(1-hydroxycyclohexyl)alanine (V). Heating in 6 *N* HCl at 110° for 18 hr converted V almost quantitatively back into lactone IV, as indicated by chromatography and electrophoresis.

L- and DL-2,5-dihydrophenylalanine effectively inhibited the growth of *L. dextranicum* 8086 in a medium free of phenylalanine and tyrosine. L-2,5-Dihydrophenylalanine was 85% inhibitory at a concentration of approximately 1 μg/ml. In the same experiment the DL-amino acid was 85% inhibitory at 12 μg/ml; DL-cyclohexane-1-alanine (III), at 10 μg/ml (approximate values derived from a steep portion of curve of growth response *vs.* inhibitor concentration). Inhibition of the growth of this organism by Ia or b was reversed in a competitive manner by DL-phenylalanine over a 20-fold range of increasing concentration (Table I).

TABLE I

REVERSAL OF INHIBITION OF L-1,4-CYCLOHEXADIENE-1-ALANINE BY DL-PHENYLALANINE IN *Leuconostoc dextranicum* 8086

	DL-Phenylalanine, μg/6 ml				
	0	6	12	60	120
L-1,4-Cyclohexadiene-1-alanine, μg/6 ml	% transmittance[a]				
0	48	25	22	21	22
7	85	41	22	22	24.5
14	90	47	24.5		22
35	89	57	26	22	21
70	88	78	32	22	22
140	86	82	64	22	21
350	88	86	83	65	25
700		88	87	75	39
1400			86	74	54

[a] An inverse measure of culture turbidity or growth.

Inhibition indices (ratios of concentration of inhibitor to concentration of phenylalanine) were 7, 25, and 45 at the levels of inhibition of 55, 80, and 85% and were the same for the L and DL forms of the inhibitor. Since corresponding inhibition indices for III were somewhat higher (20, 48, and 59), DL-2,5-dihydrophenylalanine appears to be somewhat more effective than DL-cyclohexene-1-alanine in inhibiting DL-phenylalanine. Probably it is closer to phenylalanine with respect to the average conformation of the alicyclic moiety (1,4-cyclohexadienyl *vs.* 1-cyclohexenyl). A more extensive study has been undertaken of the activity of I with other microorganisms and with amino acids other than phenylalanine.

Table II gives the effect of 2,5-dihydrophenylalanine at various dosages on the growth and survival of the young rat on a complete diet. The amino acid was injected after preliminary experiments showed that it markedly decreased food consumption when incorporated into the diet. Although it also had this effect when injected, results of paired feeding indicated that the toxicity probably was not associated primarily with the anorectic action (expt 1). The LD_{50} and 19/20 confidence limits of DL-2,5-dihydrophenylalanine equal 6.9 (5.1–9.3) mg/100 g of body weight, administered daily. Average survival at this dosage for an 18-day period is approximately 15 days.

The L, D, and DL forms of 2,5-dihydrophenylalanine were about equally toxic to the rat (expt 3 and 4).[13] N-Acetyl-L-2,5-dihydrophenylalanine in dosage equivalent to LD_{83} of DL-2,5-dihydrophenylalanine did not affect survival, although it depressed food consumption and growth somewhat (expt 2). This could suggest a low reactivity of mammalian acylase toward this substrate, and in agreement was the observation that hog kidney acylase acted only very slowly upon acetyl Ia and b.

The toxic effects of DL-2,5-dihydrophenylalanine at the LD_{83} dose level (9.7 mg/100 g) were significantly reversed by supplementing the diet with 3% phenylalanine. Anorexia was largely alleviated and growth was only a little below that of controls receiving the phenylalanine supplement. Of seven animals, all survived, and except for one, their condition appeared to be excellent (expt 2). However, such supplements afforded little or no protection at higher dosage levels of the inhibitor. At 14.5 mg/100 g, a supplement of 2% phenylalanine plus 2% tyrosine chiefly prolonged survival (expt 3). At 17.6 mg/100 g of Ib, the 3% phenylalanine diet did not even appreciably prolong survival (expt 4). 2,5-Dihydrophenylalanine apparently tends to act in an irreversible way when present in high concentration in mammalian tissue. The mechanism by which it exerts its inhibitory effect, perhaps in some relation to protein synthesis,[14] remains to be elucidated.

Experimental Section

Materials.—L-, D-, and DL-phenylalanine (CP), glucono-δ-lactone, and mevalo-δ-lactone were obtained from Mann Research, Inc., New York, N. Y. A reference sample of DL-3-cyclohexene-1-alanine was a gift of Dr. W. Shive; D-β-cyclohexylalanine·HCl and L-β-cyclohexylalanine, gifts of Dr. H. S. Anker and Dr. A. Meister. DL-Homoserine-γ-lactone·HCl, mp 199–200 (lit.[8] mp 198–199.5°), was prepared from DL-homoserine by the procedure described for the L-lactone·HCl.[15] Chelex 100 resin was purchased from Bio-Rad Laboratories, Richmond, Calif. The mold acylase was a crude preparation from *Aspergillus oryzae* designated "α-amylase, Type IV" and obtained from Sigma Chemical Co., St. Louis, Mo.

(13) With β-2-thienylalanine, another phenylalanine antagonist, stereoselective differences in toxicity were likewise noted for the rat and certain microorganisms. These could be correlated with the organism's ability to utilize D-phenylalanine and to synthesize phenylalanine for growth [M. F. Ferger and V. du Vigneaud, *J. Biol. Chem.*, **179**, 61 (1949)]. Thus, toxicity of D-2,5-dihydrophenylalanine could reflect an organism's ability to convert it into L-2,5-dihydrophenylalanine and its inability to synthesize L-phenylalanine at a significant rate.

(14) Several inhibitory analogs of phenylalanine are known to be able to be incorporated into protein or to serve *in vitro* as substrates or inhibitors of the phenylalanine-activating enzyme of *E. coli* 9723 [T. W. Conway, E. M. Lansford, Jr., and W. Shive, *Arch. Biochem. Biophys.*, **107**, 120 (1964)].

(15) S. M. Birnbaum and J. P. Greenstein, *ibid.*, **42**, 212 (1953).

TABLE II

EFFECT OF DL-, L-, D-, AND ACETYL-L-1,4-CYCLOHEXADIENE-1-ALANINE (2,5-DIHYDROPHENYLALANINE) ON THE GROWTH AND SURVIVAL OF THE YOUNG RAT

Expt no.	Supplement to basal diet[a]	Change in body wt,[b] g	Average food consumption,[c] g/day	Average survival,[d] days	Duration of expt, days	Mortality, %
1[e]	None (*ad libitum*)	46–138	9.3	18	18	0 (0/9)
	None (pair-fed)	47–77	5.1	18[f]	18	0 (0/9)
	DL-2,5-Dihydrophenylalanine, 3 mg (pair-fed)	46–64	5.1	15.3 ± 5.2	18	33 (3/9)
2	None	54–209	14.2	28[g]	28	0 (0/7)
	DL-2,5-Dihydrophenylalanine, 5 mg	53–112	5.5	1.53 ± 8.1	28	86 (6/7)
	Acetyl-L-2,5-dihydrophenylalanine, 6.3 mg	51–155	10.9	28	28	0 (0/7)
	3% L-Phe	53–184	11.9	28	28	0 (0/7)
	3% L-Phe + DL-2,5-dihydrophenylalanine, 5 mg	54–161[h]	10.5	28[g]	28	0 (0/7)
3	None	52–233	10.3	35	35	0 (0/9)
	DL-2,5-Dihydrophenylalanine, 8 mg	55	3.1	9.1 ± 2.2	13	100 (8/8)
	D-2,5-Dihydrophenylalanine, 8 mg	57	3.9	7.9 ± 2	9	100 (9/9)
	2% L-Phe + 2% L-Tyr	58–216	9.6	35	35	0 (0/9)
	2% L-Phe + 2% L-Tyr + DL-2,5-dihydrophenylalanine, 8 mg	56–151	3.8	16.6 ± 10.8[i]	35	89 (8/9)
4	None	76–129	10.6	10[g]	10	0 (0/8)
	DL-2,5-Dihydrophenylalanine, 13 mg	69	3.8	7.6 ± 1.8	10	100 (5/5)
	L-2,5-Dihydrophenylalanine, 13 mg	78	3.4	8.1 ± 1.4	10	100 (8/8)
	3% L-Phe	74–123	9.3	10	10	0 (0/7)
	3% L-Phe + L-2,5-dihydrophenylalanine, 13 mg	74–57	4.5	8.5 ± 1.2	10	88 (7/8)

[a] For mode of administration, see "Methods." [b] Starting and final weight of survivor(s) at end of experiment. [c] In expt 3, within first 2 weeks; otherwise, average for the duration of the experiment. [d] Standard deviation. [e] At end of experiment serum Phe/Tyr ratios, determined on the amino acid analyzer, were 0.72, 0.81, and 0.76, respectively. [f] $P < 0.20 > 0.10$, compared by the "t" test, with the group receiving DL-2,5-dihydrophenylalanine only. [g] $P < 0.01$; see footnote *f*. [h] One rat weighed 49 g, the other six averaged 180 g. [i] $P < 0.10 > 0.05$; see footnote *f*.

Methods.—Infrared spectra were taken on a Perkin-Elmer Model 137 spectrophotometer, on Nujol mulls or on pressed potassium bromide disks containing 0.3 wt % of sample, or both. Ultraviolet spectra were taken on a Beckman DU spectrophotometer. Nuclear magnetic resonance was determined on a Varian Associates A-60 spectrometer in deuterium oxide with sodium 3-(trimethylsilyl)-1-propanesulfonate as internal standard, or in dimethyl sulfoxide-d_6 with tetramethylsilane as internal standard. Chemical shifts are expressed in parts per million, δ, downfield from the standard. Optical rotations were taken in a 2-dm cell in a Rudolph polarimeter, Model 80. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill. Evaporations were carried out below 30° under reduced pressure on a rotary evaporator. Melting points were taken in capillaries and are uncorrected. They varied with the rate of heating. Samples of amino acids were placed in a bath 30° below melting point and, when the temperature reached 10° below melting point, were heated at a rate of 3–4°/min. Quantitative hydrogenations were carried out in Brown[2] Hydroanalyzers purchased from Delmar Scientific Laboratories, Inc., Maywood, Ill. Just before use the $NaBH_4$ solution (1 M in ethanol, 4 N) was standardized by hydrogenation of cyclohexene (99%, chromatoquality). Catalyst was platinum generated *in situ* on Darco K-B carbon.[16,17]

Amino acid analyses were performed on a Beckman-Spinco automatic amino acid analyzer, Model 120.[18] System 1 refers to the 50-cm column, 50°, pH 4.26; system 2, to the 15-cm column, 50°, pH 4.26; system 3, to the 15-cm column, 50°, pH 5.28. Ninhydrin color yield constants[18] are in system 2 unless indicated otherwise. Paper chromatography (ascending) was carried out on Whatman No. 3MM paper in 1-butanol–pyridine–acetic acid–water (30:20:6:24). Electrophoresis was carried out on strips of the same paper for 3 hr at 9 V/cm in pyridinium acetate buffer, pH 5.7, unless indicated otherwise. The papers were developed by spraying with 0.15% ninhydrin in acetone and heating briefly at 105°.

Preparations of Ia and b were checked spectrophotometrically for approximate content of phenylalanine by measuring the absorbance at 258 mμ of a 10^{-2} M solution. For determining the purity of samples containing less than 5% phenylalanine, amino acid analysis was used. Stabilities were examined at a concentration of 2 mg/ml, and products were determined by amino acid analysis. If not indicated, total recovery was 85% or more.

Enzymatic deacylations were carried out with a crude preparation from *A. oryzae*.[6] To a solution of 15 mg of acetyl Ia or b in 1 ml of 0.2 M potassium phosphate buffer, pH 7, were added 12 mg of the crude enzyme. The mixture was incubated 72 hr at 37°. Also incubated were controls containing Ib, enzyme, and enzyme plus Ib. Mixtures were then immersed for 3 min in a bath at 100° and centrifuged; a portion was analyzed on the amino acid analyzer. Correction was made for amino acids, particularly phenylalanine (*ca.* 1–4%), present in the enzyme preparation and formed during incubation.

Microbiological assays were carried out by Shankman Laboratories, Los Angeles, Calif. *Leuconostoc dextranicum* 8086 was used with a previously described amino acid medium free of phenylalanine and tyrosine.[3] The medium was modified by omitting pantethine. The organism was incubated at 30–32° for 16 hr. Inhibition of growth was determined photometrically with a Beckman Model C colorimeter in terms of transmittance readings of the turbid culture medium. An opaque object read 0 transmittance; distilled water, 100.

Feeding experiments were carried out with male Sherman rats housed in metal cages with raised floors. Except for expt 1 of Table II, the rats were kept in groups and consumed food *ad libitum*. The basal diet was a commercial diet already described.[19] Phenylalanine (Phe) and tyrosine (Tyr) supplements were incorporated into the diet. Rats receiving these supplements were started on the diet 2 days before the experiment. 2,5-Dihydrophenylalanine isomers were dissolved in distilled water, acetyl Ib was dissolved in water by adjusting to pH 7 with dilute NaOH solution, and rats were injected subcutaneously daily with the solutions. Controls were injected with water. LD_{50} was calculated from a plot of daily dose *vs.* percentage of mortality and evaluated according to the method of Litchfield and Wilcoxon.[20]

DL-1,4-Cyclohexadiene-1-alanine (DL-2,5-Dihydrophenylalanine) (Ia).—In a 3-l. 5 three-necked flask, fitted with a gas

(16) C. A. Brown and H. C. Brown, *J. Amer. Chem. Soc.*, **84**, 2829 (1962).
(17) H. C. Brown, K. Sivasankaran, and C. A. Brown, *J. Org. Chem.*, **28**, 214 (1963).
(18) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).
(19) C. Ressler, J. Nelson, and M. Pfeffer, *Nature*, **203**, 1286 (1964).
(20) J. T. Litchfield, Jr., and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).

inlet tube and protected by drying tubes of Mallcosorb, 10 g (0.060 mol) of DL-phenylalanine were placed in 250 ml of dry methanol (stored over Linde 4A Molecular Sieves). The flask was immersed in a Dry Ice–Cellosolve bath, and 2 l. of liquid ammonia were collected. The temperature of the bath was maintained near −40° while sodium (36 g, 1.6 g-atoms) was added to the clear solution in portions with magnetic stirring over a period of 1.5 hr. (It was desirable to keep a volume of ammonia of at least 2 l. to avoid precipitation of a white solid.) The blue color was allowed to fade before each successive addition of sodium. When addition of sodium was complete, ammonium chloride (84 g, 1.6 mol) was added carefully in portions, and the ammonia was allowed to evaporate overnight. Methanol and residual ammonia were removed by evacuating the flask at the water pump *via* a Kjeldahl bulb for at least 2 hr.

The dried reaction residue was suspended in 50–100 ml of cold water, adjusted to pH 7 with 6 *N* HCl, and filtered, The solid was resuspended in 30 ml of water and filtered (five to ten times) until free of chloride ion. It was washed on the filter several times first with absolute ethanol, then with ether, and was dried by evacuation at the water pump: weight 6.0 g of product containing 95% I, 0.3% Phe and 4.5% III. The product was recrystallized as large clusters of fine needles from 50% ethanol (25 ml/g): weight 4.9 g (48%). It contained 95% I, 0.5% Phe, and 4.4% III; 97% recovery.

A portion was recrystallized twice, with little change in composition, mp 235–236° dec. The analytical sample had 96% I, 0.7% Phe, and 3.6% III. Medium to strong infrared bands were observed at ν_{max}^{KBr} 3080–2870 (b), 2580 (b), 1620–1575 (d), 1500, 1420, 1320, 960 and 858 cm^{-1}; uv spectrum (1.8×10^{-3} *M*, water), λ [mμ (ε)] 220 (654), 225 (302), 230 (104), 235 (22), 258 (6), 270 (11). The nmr spectrum of the sodium salt in D_2O (Figure 1) was as follows: 1.82–2.32 (2 H unsymmetrical multiplet, C*H*$_2$ of side chain); 2.61 (4 H, broad singlet C*H*$_2$CH=CH on C-3 and -6 of ring); 3.31 (1 H, multiplet, C*H*NH$_2$); 4.54 (singlet, exchangeable H); 5.50 (1 H, broad singlet, C*H*=C< on C-2 of ring); and 5.69 (2 H, broad singlet, C*H*=C*H* on C-4 and -5 of ring).

Anal. Calcd for $C_9H_{13}NO_2$: C, 64.7; H, 7.84; N, 8.38. Found: C, 64.6; H, 7.81; N, 8.33.

Paper chromatography showed a brick-orange spot, R_f 0.54, and a trace of a yellow spot, R_f 0.65 (III). Elution volumes in system 1 and 2 are 94 and 30 ml; constant 19.6. Corresponding chromatographic data for phenylalanine follow: purple spot, R_f 0.5; elution volumes 80 and 27 ml; constant 22.9.

Ia containing less than 0.5% Phe was stable in aqueous solution for 4 days at 25° and in 1 *N* ammonia for 18 hr. When heated in 1 *N* NaOH at 110° for 18 hr, 6–10% Phe and some insoluble material formed. Under the same conditions in 6 *N* HCl, less than 1% Ia was left and nine unidentified peaks formed, some of which behaved as basic material on electrophoresis. In water saturated with H_2S, 2.1% Phe formed within 18 hr at 25°; 18% Phe within 4 days. The solid heated for 5 hr at 100° formed 2.6% Phe. Another sample of solid Ia containing 3.6% Phe was unchanged after standing 6 months at room temperature.

Ia containing 5% Phe was made up as 0.1 *M* aqueous solution by addition of several drops of concentrated HCl and dilution. Catalytic hydrogenation[17] of 250-μl samples resulted in an uptake of 1.8 molar equiv of hydrogen (95%). Paper chromatography (purple spot, R_f 0.7) and amino acid analysis of the reduction mixture showed an 86% yield of β-cyclohexylalanine (elution volume in systems 1 and 2, 135 and 40 ml; constant 23.7).

N-Acetyl-DL-cyclohexadiene-1-alanine.—DL-Phenylalanine, 10 g, was reduced as described under Ia. After isolation of 6 g of crude Ia, the aqueous mother liquor was concentrated to a slurry of 150 ml and 9.75 ml of 2 *N* NaOH were added. The mixture was cooled to −5° with an ice–salt bath. Twelve portions each of precooled acetic anhydride, 1.6 ml, and 2 *N* NaOH, 8 ml, were added with vigorous stirring at 2-min intervals, essentially as described for acetylating phenylalanine.[21] Ten minutes after the last addition, the mixture was adjusted to pH 3 with 6 *N* H_2SO_4 and concentrated to 200 ml. It was then adjusted to pH 1 and extracted three times with equal portions of ethyl acetate. The extract was concentrated to dryness. The residue was re-evaporated several times with 8-ml portions of fresh ethyl acetate to remove traces of acetic anhydride. The light tan solid was collected on the filter with petroleum ether (bp 30–60°) and was air dried: weight, 2.93 g; mp 108–132°. Trituration with a small volume of warm ethyl acetate left 2.34 g (45%): mp 133–138°; over-all yield, including Ia, 77%. Recrystallization from ethyl acetate (Norit A) gave 2.2 g of hard clusters of small needles, mp 134–139°.

The same product was obtained when isolated Ia was used as the starting material. For analysis, the solid was recrystallized twice with no change in melting point: uv spectrum (7×10^{-4} *M*, methanol), λ [mμ (ε)] 220 (996), 225 (533), 230 (232), 240 (27), 250 (4), 258 (5), 270 (8).

Anal. Calcd for $C_{11}H_{15}NO_3$: C, 63.1; H, 7.23; N, 6.69. Found: C, 63.3; H, 7.20; N, 6.58.

Enzymatic deacylation gave approximately 50% of a product consisting of 93% I, 4% Phe, and 3% III.

L-1,4-Cyclohexadiene-1-alanine (L-2,5-Dihydrophenylalanine) (Ib).—L-Phenylalanine, 10 g, was reduced in the presence of 250 ml of methanol as described for DL-phenylalanine. The dry residue left after removal of the ammonia and methanol was dissolved in 700 ml of water. The solution was adjusted to pH 5 with concentrated HCl and clarified by filtration. A portion examined on the amino acid analyzer showed 95% I, 1% Phe, and 4% III.

To the solution was added a hot solution of 12 g of cupric acetate hydrate in 100 ml of boiling water. It was chilled overnight, and the crystalline copper chelate was collected by filtration, washed first with cold water until free of chloride ion (about 1 l.), then with cold absolute ethanol and ether, after which it was evacuated at the water pump for several hours in the absence of desiccant: weight, 10–12 g (83–100%). For analysis, a sample was recrystallized twice from 50% ethanol. The soft blue needles melted at 235–236°.

Anal. Calcd for $C_{18}H_{24}N_2O_4Cu$: C, 54.6; H, 6.11; N, 7.08. Found: C, 54.8; H, 6.38; N, 6.82.

For amino acid analysis the chelate was dissolved in several drops of 6 *N* HCl, and the solution was diluted with water. It contained 97% I, 0.2% Phe, and 3% III.

Crude and recrystallized material were stored at 5° over silica gel desiccant in an unevacuated container for 3 and 7 months, respectively, with no change in composition.

Liberation of L-1,4-Cyclohexadiene-1-alanine (Ib) from Copper Chelate.—The copper chelate (3.0 g containing 94% I, 0.3% Phe, and 5.6% III) was powdered finely and dissolved in concentrated ammonia (90 ml). Moist Chelex resin (NH_4^+ cycle, 200 ml) was added, and the mixture was stirred magnetically for 10 min. The resin was filtered off, and the filtrate was concentrated immediately and rapidly to dryness. Meanwhile, the resin was washed by resuspending it five times in water (100 ml), stirring it for 10 min, and filtering it until the final washing was ninhydrin-negative. The combined washings were quickly concentrated to dryness, and the residues were triturated with water (20 ml) and taken to dryness three times. The residue was suspended in 20 ml of water and adjusted from pH 7.5 to 5.5 with several drops of 6 *N* HCl. The mixture was concentrated to dryness and taken up in a minimum of hot 80% ethanol (water bath). After standing overnight in the cold, the solid, which tended to occlude solvent, was collected on the filter and pressed dry with a rubber filter mat. It was washed first with 80% ethanol, then with absolute ethanol, followed by ether. It was dried by evacuation at the water pump for 1 hr: weight, 1.7 g (67%); $[\alpha]^{25}D$ −61.6° (*c* 0.74, water). It contained 96% I, 0.2% Phe, and 3.8% III; 96% recovery. The nmr spectrum in D_2O was as follows: 2.42–2.62 (6 H, unsymmetrical multiplet, C*H*$_2$ of side chain and C*H*$_2$ on C-3 and -6 of ring); 3.83 (1 H, multiplet, C*H*NH$_2$); 4.67 (singlet, exchangeable H); 5.72 (1 H, broad singlet, C*H*=C< on C-2 of ring); and 5.81 (2 H, broad singlet, C*H*=C*H* on C-4 and -5 of ring).

Solid Ib thus prepared tends to undergo gradual, partial conversion to phenylalanine. It is particularly labile to high vacuum. Samples are stored at −15° under nitrogen and before use purity is rechecked chromatographically. Preferably, Ib is stored as the stable copper salt and liberated as needed, or converted into a stable form in the following manner.

The product, 1.3 g, was dissolved in 65 ml of hot 80% ethanol (water bath). After cooling to room temperature, the solution was seeded and allowed to stand. Crystallization started overnight. After 2 days the mixture was placed in the cold for a week. The dense aggregates of plates were then collected by filtration and were air dried: weight, 800 mg which contained 94.6% I, 2.6% Phe, and 2.8% III; $[\alpha]^{26}D$ −62.1° (*c* 0.5, 1 *N* acetic acid). The mother liquor was concentrated to dryness, and the residue was allowed to crystallize from 10 ml of solvent:

(21) V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **98**, 295 (1932).

weight, 189 mg of comparable purity. For analysis a similar sample was recrystallized: $[\alpha]^{28}D$ −60.2° (*c* 0.5, 1 *N* acetic acid). It contained 96.6% I, 1.7% Phe, and 1.7% III. Medium to strong infrared bands were observed at ν_{max}^{KBr} 3030–2850 (b), 2620, 1590–1560 (d), 1480, 1390, 1320, 1220, 1135, 965, 895, 855 cm^{-1}.

Anal. Calcd for $C_9H_{13}NO_2$: C, 64.7; H, 7.84; N, 8.38. Found: C, 65.0; H, 7.86; N, 8.53.

N-Acetyl-L-1,4-cyclohexadiene-1-alanine.—L-Phenylalanine, 10 g, was reduced as described for Ib. After removal of the ammonia and methanol, 250 ml of water were added to the dry residue. The slurry was adjusted to pH 7 with 6 *N* HCl, and 24.5 ml of 2 *N* NaOH were added. The mixture was cooled to −5° with an ice–salt bath and treated with 15 portions each of acetic anhydride (4 ml), and 2 *N* NaOH (20 ml) essentially as described for acetylating Ia. The mixture was adjusted to pH 3, concentrated to 450 ml, readjusted to pH 1, and extracted with three 450-ml portions of ethyl acetate. This yielded 10.8 g, mp 133–154°. Two recrystallizations from ethyl acetate gave 7.3 g (58%) of sheaves of large lustrous plates, mp 162–169°. For analysis the solid was again recrystallized: mp 163–169°; $[\alpha]^{26}D$ +10.7° (*c* 1, methanol); uv spectrum (7×10^{-4} *M*, methanol), λ [$m\mu$ (ϵ)] 220 (1063), 225 (551), 230 (253), 240 (28), 250 (17), 258 (19), 270 (18). The nmr spectrum in dimethyl sulfoxide-d_6 was as follows: 1.75 (3 H, singlet, CH_3CO); 2.21, 2.28 (2 H unsymmetrical doublet, CH_2 of side chain); 2.51 (4 H, broad singlet, $CH_2CH{=}CH$, on C-3 and -6 of ring); 4.31 (1 H, multiplet, probably sextet, CHNH); 5.40 (1 H, broad singlet, CH=C< on C-2 of ring); 5.61 (2 H, broad singlet, CH=CH, on C-4 and -5 of ring); 7.93 (1 H, doublet, J = 9 cps, CONH); and 12.4 (1 H, broad singlet, COOH).

Anal. Calcd for $C_{11}H_{15}NO_3$: C, 63.1; H, 7.23; N, 6.69. Found: C, 63.3; H, 7.16; N, 6.76.

Enzymatic deacylation gave a theoretical yield of a product consisting of 95% I, 1% Phe, and 4% III.

Hydrogenation of Ib to L-β-Cyclohexylalanine.—Ib (1.0 g) was hydrogenated in the manner described for the preparation of III, except that reduction was allowed to continue overnight with a resultant uptake of 2 molar equiv of hydrogen. The catalyst was filtered off and washed, and the combined filtrates were concentrated to dryness. The residue was taken up in a minimum volume of boiling water and allowed to crystallize. The solid was collected on the filter and washed with cold water until the washings no longer gave a cloudy precipitate with acidic silver nitrate: weight, 0.45 g. Amino acid analysis indicated homogeneous β-cyclohexylalanine. The material (200 mg) was dissolved in a minimum volume of hot 3 *N* HCl from which it crystallized as long white needles: weight, 180 mg; $[\alpha]^{24}D$ +12.7° (*c* 2, 4% HCl). A reference sample of D-β-cyclohexylalanine·HCl had $[\alpha]^{24}D$ −12.9° (*c* 1.65) (lit.[22] $[\alpha]D$ +12.9° for L-β-cyclohexylalanine·HCl). Observed rotations are expressed in terms of weight of the free amino acid to allow comparison with the reported rotation expressed in this way (H. S. Anker, private communication).

DL-1-Cyclohexene-1-alanine (III).—Ia (1.0 g containing 8% Phe) dissolved in 10 ml of 70% acetic acid was hydrogenated until 1 molar equiv of hydrogen had been absorbed which required 45 min. The catalyst was filtered off directly and washed with hot water. Filtrate and washings were concentrated to dryness. The dry residue was suspended in cold water, and the solid was collected on the filter and washed until washings no longer formed a cloudy precipitate with silver nitrate. The product, 0.75 g, consisted of 70% III, 10% Phe, and 15% β-cyclohexylalanine. (In a run in which 10 ml of 6 *N* HCl were used in place of acetic acid, hydrogenation required 3 hr, and the product, 0.59 g, contained larger amounts of β-cyclohexylalanine.)

Crude III (272 mg) was purified by preparative chromatography on the amino acid analyzer with system 1 at 30°. The material was chromatographed in eleven 15- to 30-mg batches. Fractions were collected with a fraction collector and analyzed by the manual ninhydrin method.[23] Those containing the desired compound were combined, adjusted to pH 2, and applied to a column of Dowex 50 W-X8 (H^+ cycle, 100–200 mesh, 1.7-cm diameter, 100 ml of wet resin). The column was first washed with water and then eluted with 3 *N* ammonia. The basic eluate was concentrated to dryness, and the residue was taken up in water. The solution was adjusted with 6 *N* HCl to pH 5 and then concentrated to a low volume. The crystals which separated were filtered off after chilling and recrystallized from 50% ethanol: weight, 55 mg. For analysis, the material was recrystallized twice. The sheaves of needles melted at 219–221° dec (4°/min); 226–228° dec (6°/min) (lit.[3] mp 225–227°). Medium to strong infrared bands were observed at ν_{max}^{KBr} 3000–2700 (b), 1640–1585 (d), 1530, 1420, 1330–1315 (d), 1145–1040 (b), 875, 806 cm^{-1}. The nmr spectrum of the sodium salt in D_2O was as follows: 1.61 (4 H) and 1.98 (4 H, CH_2 on C-4 and -5 and CH_2 on C-3 and -6 of ring); 2.28 (2 H, CH_2 of side chain); 3.4 (1 H, $CHNH_2$); 4.5 (exchangeable H); and 5.50 (1 H, CH=C< on C-2 of ring). Insolubility prevented obtaining further detail.

Anal. Calcd for $C_9H_{15}NO_2$: C, 63.8; H, 8.94; N, 8.28. Found: C, 63.5; H, 8.92; N, 8.27.

The material was homogeneous when chromatographed on paper (brownish yellow spot, R_f 0.65) and on the analyzer (elution volumes in systems 1 and 2, 108 and 35 ml; constant 21.1). Corresponding chromatographic data for DL-3-cyclohexene-1-alanine: purple spot, R_f 0.65; elution volume in system 1, 110 ml; constant 19.5.

Heating III in 6 *N* HCl at 110° gave 17% IV after 1 hr, 75% IV and 1.5% III after 18 hr. Under the latter conditions DL-3-cyclohexene-1-alanine was 97% decomposed and gave six unidentified peaks.

γ-Cyclohexanespiro-DL-α-aminobutyrolactone Hydrochloride (IV).—A solution of 1.8 g of crude III in 45 ml of 6 *N* HCl was refluxed overnight. On cooling, crystals, shown chromatographically to be mainly β-cyclohexylalanine, separated and were filtered off. Concentration of the solution gave three similar additional crops which were removed: weight, 1.5 g. (A similar run with purer III yielded no solid at this point.) The final filtrate was concentrated to dryness. The residue was taken up in a minimum volume of ethanol and crystallized by addition of ether: weight, 225 mg; mp 176–187°. Crude lactone so obtained (525 mg) was electrophoresed for 18 hr on a Solka floc block in pyridinium acetate buffer, pH 3.5, 9 V/cm at 5°. A basic band with a yellow ninhydrin color was pressed out, and the extract was concentrated to dryness. The oily residue was taken up in a few milliliters of 6 *N* HCl, and the solution was taken to dryness. The residue was then dissolved in a minimum volume of ethanol at room temperature. On standing, crystals soon were deposited and were filtered off after cooling: weight, 188 mg; mp 198–200°. Ether added to the mother liquor gave a further crop of 80 mg melting at 185–189°. For analysis, the higher melting material was recrystallized from ethanol-ether (total volume, 50 ml) at 25°. After chilling overnight, the solid was collected: weight, 155 mg; mp 199–200°.

Anal. Calcd for $C_9H_{17}ClNO_2$: C, 52.6; H, 7.86; N, 6.81; Cl, 17.2. Found: C, 52.4; H, 7.84; N, 6.67; Cl, 17.1.

The material, R_f 0.8, was homogeneous on amino acid analysis in system 3 in which it was eluted at 477 ml as a wide skewed peak; constant 19.3. Electrophoresis showed a single basic product with a yellow ninhydrin color. Its infrared spectrum on a Nujol mull had a strong, sharp band at 1781 cm^{-1}. DL-Homoserine lactone hydrochloride served as reference γ-lactone and had a band at 1786 cm^{-1}; glucono-δ-lactone and mevalo-δ-lactone each had a band at 1724 cm^{-1}.

DL-β-(1-Hydroxycyclohexyl)alanine (V).—A solution of IV (140 mg, 0.68 mmol) in 1.4 ml of 1 *N* NaOH was heated for 5 hr at 100°. It was then clarified by filtration, and the filtrate was concentrated to dryness. The thick residue was dissolved in several drops of water and adjusted to pH 5–6 with 0.9 ml of 1 *N* HCl. The mixture was cooled for 1 hr, and the white crystals were collected on the filter and washed with a few drops of water until free of chloride: weight, 70 mg; mp 178–179° dec. The mother liquor after concentration yielded 17 mg (68%). On a microscale the yield of V without isolation, as determined chromatographically, was 84%.

For analysis, the material was recrystallized twice from 50% ethanol. The dense lustrous prisms melted at 188–189° dec. Medium to strong infrared bands were observed at ν_{max}^{KBr} 3130–3000, 1635–1580 (d), 1510, 1450, 1410, 1330, 1290, 1170, 988, 924, 800–775 (b) cm^{-1}. The crystals required drying at 100° to reach constant weight for analysis.

Anal. Calcd for $C_9H_{17}NO_3$: C, 57.7; H, 9.15; N, 7.48. Found: C, 57.2; H, 8.91; N, 7.31.

The material was homogeneous when chromatographed on paper (purple spot, R_f 0.54) and on the analyzer (eluted in system 1 at 64 ml; constant 22.4). It behaved as a neutral substance

(22) M. B. Phillips and H. S. Anker, *J. Biol. Chem.*, **227**, 465 (1957).
(23) S. Moore and W. H. Stein, *ibid.*, **211**, 907 (1954).

on paper electrophoresis at pH 5.7. Heating V in 6 *N* HCl at 110° for 18 hr gave 1% III and 99% IV, the identity of which was confirmed by paper chromatography and electrophoresis at pH 5.7.

Registry No.—DL-Phenylalanine, 150-30-1; L-phenylalanine, 63-91-2; Ia, 16055-11-1; N-acetyl derivative of Ia, 16055-13-3; Ib, 16055-12-2; copper chelate of Ib, 16165-21-2; N-acetyl derivative of Ib, 16055-14-4; III, 16055-15-5; hydrochloride of IV, 16055-16-6; V, 16109-66-3.

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Preparation of Nucleosides *via* Isopropylidene Sugar Derivatives. III. Synthesis of 9-β-D-Gulofuranosyladenine and 9-α-L-Lyxofuranosyladenine[1]

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2,3:5,6-Di-*O*-isopropylidenegulonolactone was reduced to the corresponding gulofuranose derivative with sodium borohydride. A halogenose was prepared with thionyl chloride and condensed with 6-benzamidochloromercuripurine. Removal of all blocking groups yielded 9-β-D-gulofuranosyladenine. An intermediate in the reaction sequence, 9-(2,3-*O*-isopropylidene-β-D-gulofuranosyl)adenine was treated with sodium periodate and sodium borohydride to give, after removal of the isopropylidene group, 9-α-L-lyxofuranosyladenine. Assignment of anomeric configuration to this compound was made on the basis that it was shown to be the enantiomorph of 9-α-D-lyxofuranosyladenine, the structure of which had been demonstrated previously. Knowledge of the anomeric configuration of the α-L- and α-D-pentofuranosyl nucleosides enabled assignment of anomeric configuration to the hexofuranosyl nucleosides from which they were derived, 9-β-D-gulofuranosyladenine and 9-α-D-mannofuranosyladenine, respectively.

Because of potentially interesting biological properties of the products the preparation of hexofuranosyl nucleosides has received some attention in the last few years.[2] One such nucleoside, 9-β-D-gulofuranosyladenine (**6**), was first synthesized by the reduction of tetra-*O*-benzoyl-D-gulono-γ-lactone to tetra-*O*-benzoyl-D-gulofuranose with disiamylborane[3] followed by preparation of the halogenose, which was coupled with 6-benzamidochloromercuripurine.[4] Removal of the blocking groups gave the nucleoside in small yield (6%).

In a recent communication[5] the feasibility of using 2,3:5,6-di-*O*-isopropylidene hexofuranosyl halides for the preparation of hexofuranosyl nucleosides was demonstrated. In that case, 2,3:5,6-di-*O*-isopropylidene mannofuranosyl chloride was condensed with 6-benzamidochloromercuripurine, and the blocking groups were removed to give 9-α-D-mannofuranosyladenine in good yield. Application of this pathway with other hexoses, though possible, is not readily achieved because the acid-catalyzed acetonation of hexoses gives mixtures of isopropylidene derivatives, except in the case of mannose. This would lead to a great deal of waste of some difficulty obtainable rare sugars. However, in a recent report[6] it was demonstrated that 2,3:5,6-di-*O*-isopropylidene-D-gulono-γ-lactone could be reduced to the corresponding aldose. That report led to the application of the pathway for the preparation of the title compounds.

2,3:5,6-Di-*O*-isopropylidene-D-gulono-γ-lactone (**1**) was subjected to the sodium borohydride–ethyl ether–acetic acid medium described by Hulyalkar,[6] but it was found that a longer reaction time than that reported gave better results. When a reaction time of 20–24 hr was used, a yield of about 70% of 2,3:5,6-di-*O*-isopropylidene-D-gulofuranose (**2**) was consistently obtained. The anomeric hydroxyl group was exchanged for a chloro by reaction with thionyl chloride in pyridine.[7] A new halogenose, 2,3:5,6-di-*O*-isopropylidene-D-gulofuranosyl chloride (**3**), was obtained as an analytically pure oil by vacuum distillation. This oil (**3**) was coupled with 6-benzamidochloromercuripurine in refluxing xylene.[8] The isopropylidene group blocking positions 5′ and 6′ was removed with 70% aqueous acetic acid at 50°, and the *N*-benzoyl group was removed with sodium methoxide. The product, 9-(2,3-*O*-isopropylidene-β-D-gulofuranosyl)adenine (**5**), was obtained as irregular white crystals in good yield. The structure of **5** was concluded from the elementary analysis; the presence of the isopropylidene group was confirmed by the infrared peak at 1375 cm^{-1} (*gem*-dimethyl). The position of the isopropylidene group was verified by the formation of formaldehyde after periodate cleavage.

(1) (a) For parts I and II of this series, see ref 5 and 11. (b) Supported in part by Grant T-442 from the American Cancer Society and by Grant CA-07960 from the U. S. Public Health Service.

(2) See ref 4 and 5 for references to papers dealing with this subject.

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(5) L. M. Lerner and P. Kohn, *ibid.*, **31**, 339 (1966).

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