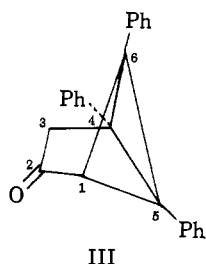155.5–156°. *Anal.* Calcd. for $C_{24}H_{18}O$: C, 89.42; H, 5.63; mol. wt., 322. Found: C, 89.24; H, 5.68; mol. wt., 318 (thermoelectric osmometer). Its in-

III

frared spectrum showed a five-membered ketone band at 1750 cm.$^{-1}$; the n.m.r. spectrum had a fifteen-proton multiplet centered at τ 2.8 (phenyl hydrogens), a one-proton singlet at τ 6.8 (bridgehead hydrogen), and a two-proton singlet at τ 7.4 (methylene hydrogens). The ultraviolet spectrum (dioxane) showed a shoulder at 243 mμ (log ϵ 4.23) and was thus similar to that reported by Masamune[1] for his diphenylbicyclobutane which has λ_{max} 242 mμ (log ϵ 4.17). Quantitative conversion of III to 3,4,5-triphenylphenol (identified by comparison with an authentic sample)[3] at 180° provided additional evidence for the proposed structure.

III
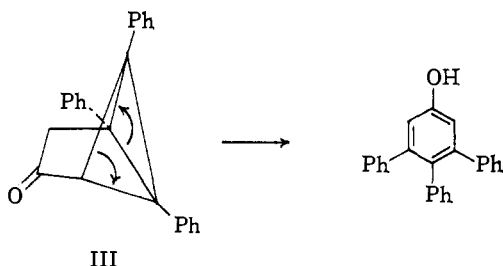
Treatment of I with silver oxide under conditions for a Wolff rearrangement[4] led to a 38% yield of crude, once-recrystallized acid, tentatively assigned structure IV, (1,2,4-triphenylcyclobut-2-enyl)acetic acid. The analytical sample had m.p. 178.5–180°. *Anal.* Calcd. for $C_{24}H_{20}O_2$: C, 84.68; H, 5.92. Found: C, 84.42; H, 5.78. The infrared spectrum showed a carbonyl band at 1710 cm.$^{-1}$. The ultraviolet spectrum (ethanol) had λ_{max} 257 mμ (log ϵ 4.12). In comparison, 1-phenylcyclobutene has λ_{max} 255 mμ (log ϵ 4.14).[5]

IV
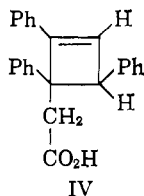
The n.m.r. spectrum showed a one-proton singlet at τ −0.1 (acid hydrogen), a fifteen-proton complex multiplet centered at τ 2.8 (phenyl hydrogens), a somewhat broad one-proton singlet at τ 3.7 (vinyl hydrogen), a somewhat broad one-proton singlet at τ 5.4 (tertiary hydrogen), and two one-proton doublets (J = 14 c.p.s.) centered at τ 6.5 and 7.4 (methylene hydrogens).

(3) Prepared by the method of A. Smith, *Chem. Ber.*, **26**, 65 (1893), as modified by J. B. Garner, *Am. Chem. J.*, **31**, 143 (1904); B. Prager, *et al.*, "Beilsteins Handbuch der Organischer Chemie," 4th Ed., Julius Springer, Berlin, Germany, 1923, Vol. VI, p. 721, Vol. VIII, p. 220.

(4) The reaction was run at 80° in dioxane–water in the presence of silver oxide, sodium thiosulfate, and sodium carbonate.

(5) J. W. Wilt and J. D. Roberts, *J. Org. Chem.*, **27**, 3430 (1962).

Typical of the spectra of other cyclobutenes,[6] the splitting of the vinyl hydrogen is very small, if not nonexistent, and was not resolved in our spectrum. The methylene hydrogens are nonequivalent because of their proximity to asymmetric carbon atoms[7a] and their splitting is typical of a *geminal* AB system.[7b] Thus the analysis and spectra are consistent with the proposed structure IV.

A reasonable mechanism for the formation of IV could involve prior formation of III which cleaves in the presence of base. However, reaction of III with mild base or under the Wolff rearrangement conditions led to *little or no acidic product*.[8] Similarly, I, in the absence of silver oxide, gave a low yield of acidic material. Therefore, IV is most likely formed *via* a route which involves intimate association with silver.[9]

Although a tricyclo[2.1.1.0^{5,6}]hexyl system has been prepared previously,[10] the unique position of the keto group in III allows an unusual opportunity for the investigation of several properties of this strained system. Such studies of III and its derivatives are in progress.

Acknowledgment.—The author wishes to thank Professor Ernest Wenkert for the n.m.r. spectra and the National Science Foundation for a postdoctoral fellowship.

(6) See, for example, E. H. White and H. C. Dunathan, *J. Am. Chem. Soc.*, **86**, 453 (1964).

(7) (a) See L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, p. 99 ff for a detailed explanation of this phenomenon; (b) *ibid.*, p. 85.

(8) In fact under these conditions, a new ketone is formed whose structure is now being investigated.

(9) A referee has suggested an alternative structure to IV: (1,3,4-triphenylcyclobut-2-enyl)acetic acid (V). With the data now available it is

V
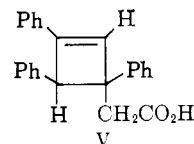
not possible to distinguish between structures IV and V. However, we prefer IV at this time for the following reason. A mechanism involving base cleavage of a silver complex of III (or a similar species) leads directly to IV whereas mechanisms leading to V involve several intermediates which we feel would collapse just as readily to 3,4,5-triphenylphenol. But in fact no phenol can be detected by infrared analysis of the crude product from the attempted Wolff rearrangement of I.

(10) J. Meinwald, C. Swithenbank, and A. Lewis, *J. Am. Chem. Soc.*, **85**, 1880 (1963).

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The Acetaldehyde–2-Deoxy-D-ribose 5-Phosphate Aldolase Complex: Comparison of the Product Obtained on Borohydride Reduction and Hydrolysis with Synthetic N^6-Ethyllysine

Sir:

Previous investigations have demonstrated that interaction of rabbit skeletal muscle aldolase and yeast transaldolase with their respective substrates, dihydroxyacetone phosphate and fructose 6-phosphate, involves Schiff base intermediates.[1–4] Moreover, it

(1) B. L. Horecker, S. Pontremoli, C. Ricci, and T. Cheng, *Proc. Natl. Acad. Sci. U. S.*, **47**, 1942 (1961).

(2) E. Grazi, T. Cheng, and B. L. Horecker, *Biochem. Biophys. Res. Commun.*, **7**, 250 (1962).

(3) E. Grazi, P. T. Rowley, T. Cheng, O. Tchola, and B. L. Horecker, *ibid.*, **9**, 38 (1962).

(4) J. C. Speck, Jr., P. T. Rowley, and B. L. Horecker, *J. Am. Chem. Soc.*, **85**, 1012 (1963).

has been shown that these intermediates are formed by reaction of the ε-amino group of a lysine residue of these enzymes with the carbonyl group of the C^{14}-labeled substrates, since borohydride reduction of each enzyme–substrate complex, followed by acid hydrolysis, gave a radioactive amino acid which was identified as N^6-β-glyceryllysine by periodate oxidation and by comparison with synthetic N^6-β-glyceryl-DL-lysine.[3,4] Recently, evidence has been presented which indicates that 2-deoxy-D-ribose 5-phosphate aldolase and 2-keto-3-deoxy-6-phospho-D-gluconate aldolase, purified from extracts of *Lactobacillus plantarum* and *Pseudomonas fluorescens*, respectively, also form Schiff base intermediates with their substrates.[5] An inactive, labeled protein was isolated from each enzyme following borohydride reduction of the enzyme in the presence of its radioactive substrate. Similar treatment of these enzymes in the absence of their substrates did not result in appreciable loss of enzymatic activity.

We wish now to report the synthesis of N^6-ethyl-DL-lysine (I) and the comparison of this substance with the isolated radioactive amino acid obtained after borohydride reduction of the C^{14}-acetaldehyde–2-deoxy-D-ribose 5-phosphate aldolase complex. The preparation of N^6-ethyl-DL-lysine was carried out by a procedure similar to that described for synthesis of N^6-β-glyceryl-DL-lysine.[4] 5-δ-Bromobutylhydantoin was allowed to react in a sealed tube at 90–100° for 50 hr. with a 15.5-fold excess of anhydrous ethylamine in absolute ethanol. Evaporating this reaction mixture gave crude 5-δ-(N-ethylamino)butylhydantoin as a light-brown sirup which was hydrolyzed without further purification to N^6-ethyl-DL-lysine by dissolving it in 2 M sodium hydroxide and heating this solution under nitrogen at 100° for 40 hr. This hydrolysis mixture then was acidified to pH 4 with concentrated hydrochloric acid and the resulting solution desalted on an Amberlite CG-120 column by the procedure of Dreze, *et al.*[6] Evaporating the portion of 4 N hydrochloric acid eluate containing N^6-ethyl-DL-lysine gave the dihydrochloride as a nearly colorless, very hygroscopic glass. This product was subjected to one more cycle of the desalting operation and dried *in vacuo* over barium oxide before analysis. *Anal.* Calcd. for $C_8H_{20}Cl_2N_2O_2$: C, 38.88; H, 8.16; N, 11.34. Found: C, 39.84, 40.00; H, 8.07, 8.09; N, 11.56, 11.66.[7] All attempts to recrystallize this dihydrochloride failed, and neither the free base nor the picrate could be obtained crystalline. N^6-Ethyllysine (extent of racemization unknown) was prepared also from poly-L-lysine. Poly-L-lysine (molecular weight, 210,000; degree of polymerization, 1640)[8] was boiled under reflux with a twofold excess of a water solution of acetaldehyde at pH 8.0 for 10 min. After cooling to 0°, this mixture was reduced with sodium borohydride at pH 6. The resulting mixture was hydrolyzed with 6 N hydrochloric acid at 110°. Isolation of N^6-ethyllysine from the solution of the hydrolysate by paper chromatography (phenol–water solvent system) gave this product in approximately 15% yield.

(5) E. Grazi, H. Meloche, G. Martinez, W. A. Wood, and B. L. Horecker, *Biochem. Biophys. Res. Commun.*, **10**, 4 (1963).

(6) A. Dreze, S. Moore, and E. J. Bigwood, *Anal. Chim. Acta*, **11**, 554 (1954).

(7) Microanalysis by Spang Microanalytical Laboratory, Ann Arbor, Mich.

(8) Purchased from Mann Research Laboratories, Inc., New York, N. Y.

2-Deoxy-D-ribose 5-phosphate aldolase was purified from extracts of *L. plantarum* by modification of a method previously described.[9] The reduced enzyme-substrate complex was prepared[5] and hydrolyzed in a sealed tube with 6 N hydrochloric acid at 110° for 24 hr. The hydrolysate was chromatographed and the single radioactive band cut out and eluted. As shown in Table I, this material was identical on cochromatography in two solvent systems with synthetic N^6-ethyllysine. Identical fingerprint patterns were obtained with ninhydrin and by autoradiography.

TABLE I

PAPER CHROMATOGRAPHY OF AMINO ACIDS[a]

Compound	R_f in solvent A[b]	R_f in solvent B[c]
Lysine	0.58	0.11
Synthetic N^6-ethyl-DL-lysine	0.82	0.18
Radioactive amino acid from 2-deoxy-D-ribose 5-phosphate aldolase–acetaldehyde[d] + synthetic N^6-ethyl-DL-lysine (cochromatogram)	0.85[e]	0.18[e]
Synthetic N^6-ethyl-DL-lysine + product from poly-L-lysine–acetaldehyde (cochromatogram)	0.83, 0.95[f]	

[a] All chromatograms were developed overnight at room temperature. [b] Solvent A: phenol–water (80%), descending chromatography. [c] Solvent B: butanol–pyridine–water (1:1:1), ascending chromatography. [d] A trace of this substance (insufficient to give a ninhydrin test) was used for cochromatography. [e] The values given by synthetic N^6-ethyl-DL lysine (detected by ninhydrin spray) and the radioactive spot (detected by autoradiography) were identical. [f] Unidentified spot.

These findings provide additional evidence for the generality of immonium ion catalysis in aldolase-catalyzed reactions and for the reaction scheme shown below.

2-deoxy-D-ribose 5-phosphate aldolase
+ → $CH_3CH=N-(CH_2)_4-CH-C=O$
acetaldehyde
                                          NH

                                          protein

             ↓ NaBH₄

       $CH_3CH_2-NH-(CH_2)_4-CH-C=O$
                                          NH

                                          protein

             ↓ H₂O (HCl)

       $CH_3CH_2-NH-(CH_2)_4-CH-COOH$
                                          NH_2

              I

(9) W. E. Pricer and B. L. Horecker, *J. Biol. Chem.*, **235**, 1292 (1960).

(10) To whom inquiries concerning this work should be addressed.

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Direct Iodination of the Sugar Moiety in Nucleosides

Sir:

Reagents such as methyltriphenoxyphosphonium iodide (triphenyl phosphate methiodide, I) and iodotriphenoxyphosphonium iodide (triphenyl phosphite diiodide, II) have been utilized by Rydon and