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.

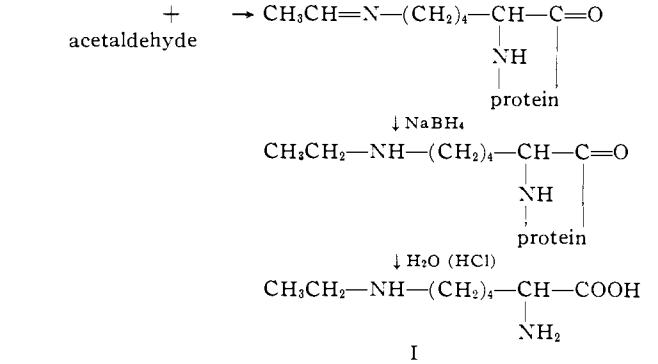
TABLE I

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.



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

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Direct Iodination of the Sugar Moiety in Nucleosides

Sir:

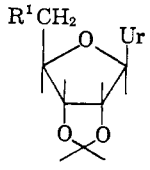
Reagents such as methyltriphenoxyphosphonium iodide (triphenyl phosphate methiodide, I) and iodotriphenoxyphosphonium iodide (triphenyl phosphite diiodide, II) have been utilized by Rydon and

co-workers[1] for the conversion of a variety of alcohols into the inverted iodides. More recently, Kochetkov, *et al.*,[2] and Lee[3] have extended this work to the direct halogenation of carbohydrate derivatives. As an extension of recent work in this laboratory on the synthesis of unusual deoxy- and polydeoxynucleosides,[4] we have now investigated the use of these reagents for the direct iodination of the sugar residue in nucleosides. The resulting products can then be converted into deoxynucleosides by hydrogenolysis of the iodine atom.

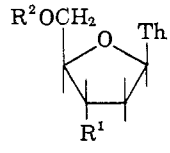
Our initial experiments involved the reaction of 2′,3′-O-isopropylideneuridine (IIIa, 2 mmoles) with I (2.3 mmoles)[1a] in anhydrous dimethylformamide at room temperature for 18 hr. Following evaporation of the solvent *in vacuo*, the residue was dissolved in chloroform, extracted with 5% sodium thiosulfate, washed with water, and dried. Direct crystallization from chloroform–hexane gave 5′-deoxy-5′-iodo-2′,3′-O-isopropylideneuridine (IIIb) in 77% yield as needles of m.p. 164–166°, indistinguishable from a sample of the same compound prepared by a different route.[5] Following hydrolysis of the isopropylidene group with acetic acid 5′-deoxy-5′-iodouridine (m.p. 184–186°) was obtained in 82% yield and was identical with a sample prepared according to Brown, *et al.*[6]

$(C_6H_5O)_3-P^+-CH_3\ I^-$ (I)

$(C_6H_5O)_3-P^+-I\ I^-$ (II)



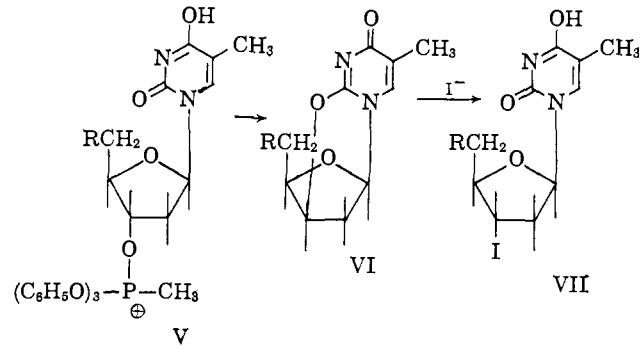
IIIa, R^1 = OH
b, R^1 = I



IVa, R^1 = OH, R^2 = acetyl
b, R^1 = I, R^2 = acetyl
c, R^1 = I, R^2 = H
d, R^1 = OH, R^2 = *p*-nitrobenzoyl
e, R^1 = I, R^2 = *p*-nitrobenzoyl

In a similar way 5′-O-acetylthymidine (IVa) was converted in 50% yield into 5′-O-acetyl-3′deoxy-3′-iodothymidine (IVb, m.p. 125–126°. *Anal.* Calcd.: C, 36.56; H, 3.84. Found: C, 36.81; H, 4.06). This yield could doubtless be raised by using a larger excess of I. Mild alkaline hydrolysis of IVb gave 3′-deoxy-3′-iodothymidine (IVc, m.p. 165.5–166° dec. *Anal.* Calcd.: C, 34.11; H, 3.72. Found: C, 34.37; H, 3.81) in 71% yield. Also, 5′-O-*p*-nitrobenzoylthymidine (IVd) with 2 equiv. of I gave a 70% yield of 3′-deoxy-3′-iodo-5′-O-*p*-nitrobenzoylthymidine (IVe, m.p. 154–156°. *Anal.* Calcd.: C, 40.73; H, 3.22; N, 8.38. Found: C, 40.88; H, 3.14; N, 8.21) which was isolated by chromatography on silicic acid.

While the mechanism of the iodination reaction normally results in an inversion of configuration,[1,2] it appears that in the present cases the resulting iodide has the same configuration as the alcohol. Thus IVb, IVc, and IVe all are identical in their melting points, mixture melting points, and infrared and nuclear magnetic resonance spectra with those of the appropriate compounds prepared from 3′-deoxy-3′-iodothymidine obtained by a different route and convincingly considered to have the iodine in the *ribo* (down) configuration.[7] These results lead to the conclusion that the first intermediate in the reaction, V, rapidly collapses to the O^2-3′-cyclonucleoside, VI, which is subsequently attacked by iodide ion to give a product with the iodine in the *ribo* configuration.



V VI VII

Such a mechanism is supported by the fact that 2′,3′-O-isopropylideneadenosine and 2′,3′-O-isopropylideneguanosine both react with I at room temperature to form quantitatively the respective 2′,3′-O-isopropylidene-N^3-5′ cyclonucleosides[8,9] as their iodide salts which are sufficiently stable to resist attack by iodide ion. Also O^2-3′-cyclothymidine (VI, R = H) on reaction with I at room temperature gave 3′,5′-dideoxy-3′,5′-diiodothymidine (VII, R = I, m.p. transition at 73–76°, melting at 119–121°. *Anal.* Calcd.: C, 25.99; H, 2.62; N, 6.06. Found: C, 25.88; H, 2.55; N, 5.96) in 58% yield. VII (R = I) was also obtained in 83% yield by direct treatment of thymidine with 3.0 equiv. of I. 2-Deoxyuridine was likewise converted into 2′,3′,5′-trideoxy-3′,5′-diiodouridine (m.p. transition at 77–80°, melting at 136–139°. *Anal.* Calcd.: C, 24.13; H, 2.25; N, 6.25. Found: C, 24.37; H, 2.43; N, 6.17) in 83% yield.

The reaction of I with nucleosides bearing *cis-vicinal* hydroxyl groups such as 5′-O-acetyluridine failed to produce iodinated derivatives. The starting material was, however, converted in high yield into two water-soluble compounds identified as 5′-O-acetyluridine-2′(3′) methyl phosphonate and uridine-2′(3′) methyl phosphonate by comparison with the same compounds prepared in quantitative yield by the condensation of 5′-O-acetyluridine and methylphosphonic acid in the presence of dicyclohexylcarbodiimide[10] followed by alkaline hydrolysis. A similar formation of uncharacterized acidic products during reaction of I with *cis-vicinal* diols has been previously observed.[3] The formation of such methyl phosphonate esters may be explained through a series of intramolecular attacks by the adjacent hydroxyl group upon the initial positively charged intermediate to give unstable cyclic structures with expulsion of phenol. Details of such a mechanism will be described later.

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(2) (a) N. K. Kochetkov and A. I. Usov, *Izv. Akad. Nauk SSSR*, 1042 (1962); (b) N. K. Kochetkov and A. I. Usov, *Tetrahedron*, **19**, 973 (1963).

(3) J. B. Lee and M. M. El Sawi, *Chem. Ind.* (London), 839 (1960).

(4) K. E. Pfitzner and J. G. Moffatt, *J. Org. Chem.*, in press.

(5) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **106**, 113 (1934).

(6) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 868 (1957).

(7) (a) A. M. Michelson and A. R. Todd, *ibid.*, 816 (1955); (b) J. J. Fox and N. C. Miller, *J. Org. Chem.*, **28**, 936 (1963).

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(10) The synthesis of monoesters of phosphonic acids by the carbodiimide route has recently been described by J. A. Maynard and J. M. Swan, *Australian J. Chem.*, **16**, 609 (1963).

We have also studied the iodination of several nucleoside derivatives with "diiodotriphenylphosphorane" prepared *in situ* by the reaction of triphenylphosphine and iodine.[11] While this reagent appears to be somewhat less reactive than I it was, however, used successfully for the preparation of IIb in 59% yield and of IVe in 50% yield.

Details of these and other studies on the iodination of nucleosides will be presented at a later date.

(11) G. A. Wiley, R. L. Hershkowitz, B. M. Rein, and B. C. Chung, *J. Am. Chem. Soc.*, **86**, 964 (1964).

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RECEIVED APRIL 3, 1964

Organic Syntheses By Means of Noble Metal Compounds. VI. Synthesis of Muconic Acid[1]

Sir:

We wish to report another new carbonylation[2] reaction of acetylene, namely, the formation of muconyl chloride by a reaction carried out in benzene in the presence of palladium chloride or its complex.[3] The formation of muconic acid from acetylene has been reported before,[4] but only in trace amounts.

In our reaction, muconyl chloride was a major product, accompanied by a considerable amount of fumaryl and maleyl chloride.

$$x\mathrm{CH}{\equiv}\mathrm{CH} + y\mathrm{CO} + z\mathrm{PdCl_2} \longrightarrow \mathrm{ClOC{-}CH{=}CH{-}CH{=}CH{-}COCl} + \mathrm{ClOC{-}CH{=}CH{-}CH{=}CH{-}COCl} + \mathrm{ClOC{-}CH{=}CH{-}CO{-}CH{=}CH{-}COCl}$$

In one of the typical examples, a benzene solution of dibenzonitrile–dichloropalladium was placed in a pressure reactor and an acetylene–carbon monoxide mixture (1:1) was introduced. The mixture was stirred at room temperature for several hours, and then carbon monoxide was charged up to a pressure of 100 kg./cm.^{2}. The mixture was heated to 100°. After the reaction, the formation of acid chloride was confirmed by observing an infrared absorption band at 1800 cm.$^{-1}$. The reaction mixture was refluxed with methanol in order to convert the acid chlorides to the corresponding esters. When the solvent was removed, the residue solidified on standing. Solid products were separated by filtration from the oily fraction, from which methyl maleate was obtained by distillation. The solid material was subjected to fractional sublimation and methyl fumarate was first collected at 80° (100 mm.). Further sublimation at 100° (10 mm.) afforded methyl muconate, identified with an authentic sample of a *trans-trans* isomer of methyl muconate (m.p. 152°; λ_{max}^{MeOH} 262.5 mμ (ϵ 34,400)).[5] *Anal.* Calcd. for $C_8H_{10}O_4$: C, 56.46; H, 5.92. Found: C, 56.49; H 5.84. The yield of methyl muconate was 38.5% and that of both the C_4 esters was 31.7%, based on palladium chloride.

In this reaction, the first step is presumed to be the coordination of 2 moles of acetylene to palladium as was assumed by Blomquist and Maitlis[6] in the case of diphenylacetylene. From this postulation it is reasonable to assume that the muconyl chloride, initially formed, should have a *cis-cis* form. By careful investigation of the reaction mixture using gas chromatography, the presence of a small amount of the *cis-cis* isomer was confirmed. We have found by using an authentic sample that the isomerization from the *cis-cis* to the *trans-trans* form in a benzene solution is particularly rapid in the presence of palladium chloride.

A mechanistic account of the reaction will be given in a forthcoming paper.

(1) Part V: J. Tsuji, J. Kiji, and S. Hosaka, *Tetrahedron Letters*, **No. 12**, 605 (1964).

(2) For recent review, see: C. W. Bird, *Chem. Rev.*, **62**, 283 (1962).

(3) Carbonylation of acetylene by the catalytic action of iodide and palladium compounds carried out in alcohol forming esters of acrylic, propionic, maleic, fumaric, and succinic acids was reported: G. Jacobsen and H. Spathe, German Patent 1,138,760 (1962); *Chem. Abstr.*, **58**, 6699 (1963).

(4) P. Pino, A. Miglierina, and E. Pietra, *Gazz. Chim. Ital.*, **84**, 443 (1954).

(5) J. A. Elvidge, R. P. Linstead, P. Sims, and B. A. Orkin, *J. Chem. Soc.*, 2235 (1950).

(6) A. T. Blomquist and P. M. Maitlis, *J. Am. Chem. Soc.*, **84**, 2329 (1962).

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RECEIVED MARCH 28, 1964

BOOK REVIEWS

Organic Reactions in Liquid Ammonia. Chemistry in Nonaqueous Ionizing Solvents. Volume 1, Part 2. By HERCHEL SMITH. John Wiley and Sons, Inc., 605 Third Ave., New York 16, N. Y. 1963. 363 pp. 17 × 25 cm. Price, $14.00.

Numerous reviews exist of limited aspects of organic reactions in liquid ammonia. This is the first book published which covers the subject as a whole. It is concerned with the properties of ammonia as a solvent for organic compounds, as a reagent with organic molecules, as a medium for the production of organic anions, and as a solvent for oxidation and reduction reactions. The most interesting section is that on reduction reactions involving alkali and alkaline earth metals. Dr. Smith has extensive research experience in this field, and presents an authoritative and balanced picture.

Literature surveys in a subject such as this are often very difficult, since the reactions are often incidental to some other aim and are usually not appropriately indexed. Dr. Smith has performed a signal service in collecting 1388 references with coverage up to part of 1962; very few references of which the reviewer is aware are missing.

The treatment is on the whole a practical one, clearly aimed to provide information of use to the research chemist. The theoretical aspects are not neglected, however, since at least a working knowledge of theory is required to direct experiment in this field. Theoretical chemists may, in fact, be stimulated by the information presented to delve deeper into some interesting problems.

The book is well presented, divided clearly by numerous subheadings, and adequately indexed with the references in alphabetical order of the initial author.

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