of the four separate isomers of a β-hydroxy-α-amino acid by this enzyme has not been reported. This is herein accomplished for β-phenylserine.[3] The cleavage of β-phenylserine is of additional interest due to the structural and possible metabolic relationships of the compound with epinephrine and chloroamphenicol.

When a purified rat liver preparation was employed as enzyme source, it was found (Table I) that *erythro*-β-phenyl-L-serine was rapidly cleaved at nine times the rate of *threo*-β-phenyl-L-serine. The respective D isomers were not measurably attacked under the conditions employed. This result regarding the *threo* isomers of β-phenylserine is analogous to the data obtained using racemic and L-threonine which indicated that L-threonine was selectively attacked.[4]

TABLE I

ENZYMATIC CLEAVAGE OF ISOMERS OF β-PHENYLSERINE

Enzymatic digests consisted of 2 cc. of 0.1 *M* borate buffer to which was added 1 cc. of substrate and 1 cc. of purified rat liver enzyme.[a] Incubated at 37° for 15 min. Enzymatic splitting of substrate was linear with respect to time over intervals reported.

Additions		Mg. N/cc.		μmoles formed/hr./mg. N	
μmoles	Substrate	enzyme	*p*H	Benzaldehyde[b]	Glycine[c]
5	*threo*-β-Phenyl-L-serine	0.27	8.3	2.4	
5	*threo*-β-Phenyl-D-serine	.62	8.3	<0.1	
5	*erythro*-β-Phenyl-L-serine	.04	8.4	21.6	
5	*erythro*-β-Phenyl-D-serine	.62	8.4	<0.1	
20	DL-Allothreonine[d]	1.03	8.1		3.1
20	*erythro*-β-Phenyl-DL-serine[d]	0.23[e]	8.0	27.0	28.6

[a] The enzyme was purified as described under Experimental. [b] Benzaldehyde determined directly on the enzymatic digest by measuring the increment in optical density at 250 mμ using a Model DU Beckman spectrophotometer.[5] [c] Glycine determined by ninhydrin decarboxylation and colorimetric determination of formaldehyde formed with chromatropic acid.[6] [d] Incubated 30 min. [e] Enzyme preparation more active.

erythro-β-Phenyl-L-serine was split at seven times the rate obtained with DL-allothreonine as substrate (Table I). The relative rate of cleavage was similar in a crude rat liver extract. Therefore, *erythro*-β-phenyl-L-serine is more rapidly cleaved by this enzyme than previously examined substrates.[1] This result also suggests that the β-phenyl-DL-serine employed in earlier enzymatic studies[2] was of the *threo* configuration. The rapid splitting of the *erythro* isomer coupled with the ease of determination of this cleavage[5] should facilitate the further purification of the enzyme. The *p*H optima for enzymatic cleavage of *erythro*-β-phenyl-DL-serine and DL-allothreonine were *p*H 8.3 and *p*H 8.0, respectively.

Benzaldehyde was identified as a product of the cleavage of *erythro*-β-phenyl-DL-serine, by steam distillation of the enzymatic digest and isolation as the 2,4-dinitrophenylhydrazone, m.p. 239.5° (cor.), mixed m.p. 239.5° (cor.). *Anal.* Calcd. for $C_{13}H_{10}N_4O_4$: N, 19.58. Found: N, 19.73. It was also demonstrated that glycine was formed in equimolar quantity with benzaldehyde (Table I).

As a preliminary step in ascertaining the effect of substitution of β-hydroxy-α-amino acids, α-methyl-DL-serine[3,7] was incubated with pigeon liver extract possessing activity with respect to other β-hydroxy-α-amino acids. No evidence of alanine formation was detected on paper chromatograms of the enzymatic digest.

Experimental

Enzyme Fractionation.—Rat livers were homogenized in a blendor with two volumes of cold water. The resulting extract was centrifuged at 42,000 times gravity for two hours. An equal volume of a saturated aqueous solution of ammonium sulfate was added to the supernatant. The resulting precipitate was taken up in a minimum volume of water and dialyzed against water at 5° until free of ammonia. The dialyzed solution was centrifuged and the precipitate discarded. The supernatant solution was diluted to a concentration of 1.5 mg. N/cc. To this solution were added, in order, an equal volume of 0.02 *M* sodium acetate solution and a half volume of an aged calcium phosphate gel[8] (dry weight: 35 mg./cc. of initial suspension). After standing for one hour the suspension was centrifuged and the enzyme eluted from the gel with 0.01 *M* phosphate buffer *p*H 7.3. This enzyme solution was dialyzed against running water at 5° for two hours and lyophilized. The activity of the purified rat liver enzyme was 20 μ moles of benzaldehyde formed/hr./mg. N when *erythro*-β-phenyl-DL-serine was employed as substrate. This preparation represented a five times purification of the original extract and 8% recovery of the original total activity.

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(7) *dextro*-α-Methylserine has been identified recently as a constituent of the antibiotic Amicetin; E. H. Flynn, J. W. Hinman, E. L. Caron and D. O. Woolf, THIS JOURNAL, **75**, 5867 (1953).

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LABORATORY OF BIOCHEMISTRY
NATIONAL CANCER INSTITUTE
NATIONAL INSTITUTES OF HEALTH
PUBLIC HEALTH SERVICE
BETHESDA, MARYLAND

4-Nitro- and 4-Amino-3-picoline

BY WERNER HERZ AND LIN TSAI

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In the course of other work in this Laboratory it was desirable to study the preparation of certain 4-substituted 3-picolines. It is well known that electrophilic substitution on the pyridine nucleus invariably introduces a group into the β-position, whereas similar reactions on pyridine 1-oxide have been shown by Ochiai and his school[1] and independently by den Hertog and co-workers[2] to proceed in a different manner. Thus nitration of pyridine 1-oxide leads primarily to 4-nitropyridine 1-oxide which may subsequently be reduced to 4-nitropyridine. By a similar method we have

(1) For a review of work by the Japanese investigators, see E. Ochiai, *J. Org. Chem.*, **18**, 534 (1953).

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now prepared 4-nitro-3-picoline which we required.[3] The position of the nitro group in the latter was established by reduction to the known 4-amino-3-picoline.[4] Since 4-nitro-3-picoline-oxide can also be hydrogenated directly to 4-amino-3-picoline in good yield, the method proves to be very convenient for the preparation of this amine.

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Experimental

3-Picoline 1-Oxide.—3-Picoline 1-oxide, which was extremely hygroscopic and was not isolated in pure form, melted at 33–36° (evacuated capillary) and was characterized by its picrate which after three recrystallizations from methanol furnished a sample of m.p. 141–143° (lit.[5] 138–139°).

Anal. Calcd. for $C_{12}H_{10}O_8N_4$: C, 42.61; H, 2.98; N, 16.57. Found: C, 42.80; H, 2.99; N, 16.2.

4-Nitro-3-picoline 1-Oxide.—Fifty grams of 3-picoline was mixed with 150 cc. of 40% peracetic acid and 9 g. of anhydrous sodium acetate. The mixture was allowed to stand for 20 hours at room temperature. Dilute hydrochloric acid (100 ml.) was added and the solvent was distilled under reduced pressure below 50°. The residue was dissolved in 505 g. of concentrated sulfuric acid and 84 g. of potassium nitrate was added slowly. The mixture was heated on a steam-bath for 14 hours, basified with concentrated potassium hydroxide solution and extracted several times with chloroform. The chloroform solution was dried over anhydrous sodium sulfate and evaporated to a small volume. Two crops of the nitro compound were collected as yellow crystals, m.p. 129–133°, total yield 29 g. (35% based on 3-picoline). Two recrystallizations from acetone followed by two more from methanol furnished pale yellow prisms, m.p. 136–138°.

Anal. Calcd. for $C_6H_6O_3N_2$: C, 46.75; H, 3.92; N, 18.18. Found: C, 47.24; H, 4.13; N, 18.1.

4-Amino-3-picoline.—One gram of 4-nitro-3-picoline 1-oxide was hydrogenated over 0.5 g. of palladium-black catalyst in 20 cc. of acetic acid and 1 cc. of acetic anhydride for 36 hours under a pressure of 54 pounds per sq. in. After filtering off the catalyst the solution was concentrated *in vacuo*, diluted with 20 ml. of water, made basic with 20% NaOH solution and extracted with five 25-ml. portions of ether. The combined ether extracts were dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The light brown solid residue was crystallized from benzene and gave colorless needles, m.p. 103–105°, yield 0.55 g. (78.5%). Two further recrystallizations from benzene–petroleum ether (b.p. 60–110°) afforded a sample with m.p. 107.4–108.6° (lit.[4] 108–109°).

The picrate melted at 219–220° (lit.[4] 224–225°).

The N-acetyl derivative prepared according to Clemo and Swan's procedure[4] had a m.p. of 151–152° (lit.[4] 152–154°).

4-Nitro-3-picoline.—A solution of 5 g. of 4-nitro-3-picoline 1-oxide in 100 ml. of dry chloroform was cooled in an ice-bath to 3°. Twenty ml. of phosphorus trichloride was added dropwise under stirring. The rate of addition was regulated so as to keep the temperature of the reacting mixture below 10°. After the addition was completed, the mixture was stirred for 40 minutes below 10°, poured into about 100 g. of ice and basified cautiously with 20% sodium hydroxide solution. The aqueous and organic layers were separated and the aqueous solution was extracted with three 20-ml. portions of chloroform. The extracts were combined with the chloroform layer and dried over anhydrous sodium sulfate and the solvent was removed by distillation under reduced pressure. The yellow oil obtained was distilled *in vacuo* and 2.9 g. (65%) of 4-nitro-3-picoline was collected at 57–59° (0.4 mm.). Upon cooling in ice the liquid solidified into colorless crystals. An analytical sample was obtained by redistillation of the liquid, b.p. 67–69° (1.5 mm.), m.p. 27–29°.

Anal. Calcd. for $C_6H_6O_2N_2$: C, 52.17; H, 4.37; N, 20.28. Found: C, 51.49; H, 4.89; N, 20.0.

The picrate crystallized in bright yellow plates, m.p. 128–129°.

Anal. Calcd. for $C_{12}H_9O_9N_5$: C, 39.24; H, 2.47; N, 19.07. Found: C, 39.52; H, 2.97; N, 19.1.

Hydrogenation of 4-Nitro-3-picoline.—Four hundred and ninety milligrams of the nitro compound was hydrogenated over 100 mg. of palladium catalyst in ethanol. Three hundred milligrams (77%) of 4-amino-3-picoline (m.p. 107–109°) was isolated in the manner described previously.

TALLAHASSEE, FLORIDA

(3) We wish to thank the referee for pointing out that E. V. Brown, Abstracts of Papers, Los Angeles, Calif., Meeting of the A. C. S., March 15–19, 1953, p. 11M, has apparently prepared this compound by the persulfuric acid oxidation of 4-amino-3-picoline.

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The Formation of 9-Phenylfluorene and Triphenylmethane by Disproportionation of Triphenylmethyl

BY R. L. LETSINGER, R. COLLAT AND M. MAGNUSSON

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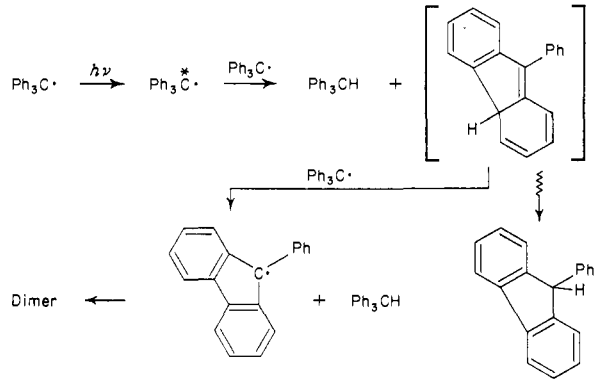
Triphenylmethyl undergoes a disproportionation when irradiated in benzene or a variety of other organic solvents[1] to yield 9-phenylfluoryl (isolated as the dimer or peroxide) and triphenylmethane. We have observed that the reaction takes a somewhat different course when the solvent is dioxane which contains potassium hydroxide. In this case the products are 9-phenylfluorene and triphenylmethane, isolated in about equimolar amounts. As in benzene, however, the triphenylmethyl does not undergo disproportionation in the alkaline dioxane solution in the absence of light.

Apparently this is the first time that 9-phenylfluorene has been isolated from a reaction of the triphenylmethyl radical. The data for both the reaction in alkaline dioxane and in benzene are compatible with the paths indicated.



By this scheme the initial step would be the abstraction of a hydrogen atom from one triphenylmethyl radical by another radical which has been activated by the absorption of light. This reaction would give triphenylmethane and a diradical which could cyclize to give the reactive intermediate II. In the presence of alkaline dioxane, II could undergo aromatization by an allylic shift of a hydrogen ion to give 9-phenylfluorene (III). On

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