of glacial acetic acid added and finally precipitated with water. This product was combined with the solid product obtained above to yield 0.63 g. (54.3% yield). A sample was reprecipitated twice from 95% ethanol and dried in high vacuum at 78° for 4 hr. This amorphous product melted at 188.5–191°, $(\alpha)^{23}D$ −28.5 (*c* 1.0, in pyridine).

Anal. Calcd. for $C_{72}H_{87}O_{18}N_{15}\cdot 2H_2O$: C, 58.17: H, 6.17: N, 14.13. Found: C, 58.06: H, 5.90: N, 13.98.

Carbobenzoxy-nitro-L-arginyl-nitro-L-arginyl-L-valyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine *p*-Nitro-benzyl Ester (1+7).—To a cold solution of 0.557 g. (1.5 mmoles) of carbobenzoxy-nitro-L-arginine monohydrate dissolved in 5 ml. of tetrahydrofuran was added 0.357 ml. of tributylamine and 0.143 ml. of ethyl chloroformate. After 15 minutes at this temperature, it was combined with the solution of 1.27 g. (1.0 mmoles) of the heptapeptide *p*-nitrobenzyl ester dihydrobromide dissolved in 5 ml. of dimethylformamide containing 0.5 ml. of tributylamine. After stirring for 15 minutes at −5° and 2 hr. at room temperature, the reaction mixture was transferred to a separatory funnel with 200 ml. of tetrahydrofuran and 400 ml. of ethyl acetate. The organic layer was extracted once with water, twice with *N* hydrochloric acid, once with water, twice with saturated sodium bicarbonate and twice with water. The precipitate which formed during each extraction was redissolved by addition of dimethylformamide and tetrahydrofuran. Sufficient ethyl acetate was added to maintain the two layers. The organic layer was dried over anhydrous sodium sulfate and evaporated *in vacuo*. Precipitation from methanol–ether yielded 0.71 g. (49.1% yield) of an amorphous product which melted at 170–180°. Chromatography in two solvent systems indicated that this product was homogeneous: R_{fAW} 0.95, R_{fBAW} 0.90.

In preparation for analysis a sample was twice reprecipitated from 95% ethanol and once from methanol–dioxane–ethyl acetate. After drying *in vacuo* at 110° for 24 hr., the product melted at 173–180°, $(\alpha)^{22}D$ −34.45 (*c* 2.0 in pyridine).

Anal. Calcd. for $C_{67}H_{87}O_{18}N_{19}$: C, 55.63; H, 6.06; N, 18.40. Found: C, 55.38; H, 6.30; N, 18.15.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES AND THE NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE, BETHESDA, MARYLAND]

Oxidation of Kynuramine Derivatives by Monoamine Oxidase and the Enzymatic Conversion of Dihydronorkynuramine to Indigo

BY YUICHI KANAOKA,[1] HERBERT WEISSBACH, THOMAS E. SMITH AND BERNHARD WITKOP

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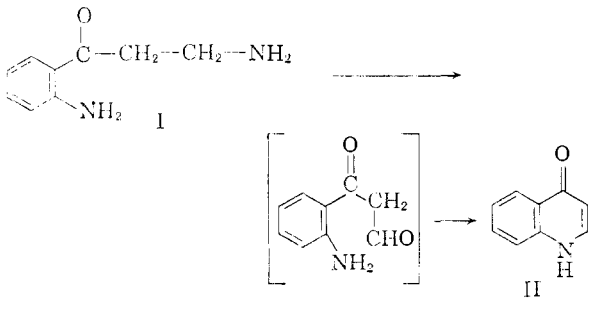
Norkynuramine (VI), dihydronorkynuramine (VIIa) and dihydrokynuramine, synthesized by standard methods, have been found to be oxidized by monoamine oxidase at rates of 20–40% compared with kynuramine, as determined manometrically. Only dihydronorkynuramine (VIIa) in this oxidation with monoamine oxidase gave rise to a colored product, namely indigo, which makes possible a simple qualitative visual test for the detection of that enzyme.

It has been shown in a preceding study[2] that kynuramine (I) is a good substrate for monoamine (but not diamine) oxidase and that its enzymatic oxidation and transformation to 4-hydroxyquinoline (γ-carbostyryl, II) may be conveniently followed spectrophotometrically. This method has been developed into a new rapid assay for monoamine oxidase. These enzymatic studies have now been extended to dihydrokynuramine, norkynuramine (VI), dihydronorkynuramine (VII) and its amino acid analog, *erythro*-*o*-amino-DL-phenylserine, substrates which were synthesized by standard methods (III-VIII).



Materials[3]

ω-Bromo-*o*-acetaminoacetophenone (III).—To a hot solution (70°) of 25 g. of *o*-acetaminoacetophenone (m.p. 76.5°)[4] in 170 ml. of glacial acetic acid was added dropwise and with stirring a solution of 22.6 g. of bromine in 140 ml. of glacial acetic acid. The reaction mixture was irradiated with light from a 125-watt ultraviolet lamp. The yellow crystalline addition product which formed initially dissolved after 10–20 min., during which the color of bromine was discharged. The temperature had to be kept at 65 ± 5°. In experiments where the temperature was raised above 75°, colorless crystals, m.p. 169–170° (dec.), presumably the dibromo compound, were formed. After 2 hr. the reaction mixture was evaporated *in vacuo*, the residue taken up in chloroform, washed with water and the dried chloroform extract was again evaporated to dryness. The residue was crystallized from benzene–hexane to yield pale brown needles, m.p. 124–125.5° (reported 126–127°).[5] The yield was 27.1 g. or 74% of theory.

ω-Phthalimido-*o*-acetaminoacetophenone V.—To a solution of 10.0 g. of ω-bromo-*o*-acetaminoacetophenone (III) in 30 ml. of dimethylformamide[6] was added 8.3 g. of potassium phthalimide over a period of 15 min. with stirring. A slightly exothermic reaction occurred. The mixture was then warmed to 70° and maintained there for 30 min. After cooling 200 ml. of chloroform was added to the reaction mixture, the organic phase was washed with 150 ml. of water, 100 ml. of 0.5 *N* sodium hydroxide and with three portions of 50 ml. of water. The chloroform extract was dried (Na_2SO_4) and evaporated *in vacuo*. The residue was triturated with ethyl acetate to give a crystalline powder, m.p. 212–216° (sintering at 205°). The yield was 5.36 g. or 43% of theory. Recrystallization from ethyl acetate gave almost colorless prisms, m.p. 222–224°.

(1) Visiting Scientist of the USPHS from the University of Hokkaido, Sapporo, Japan.

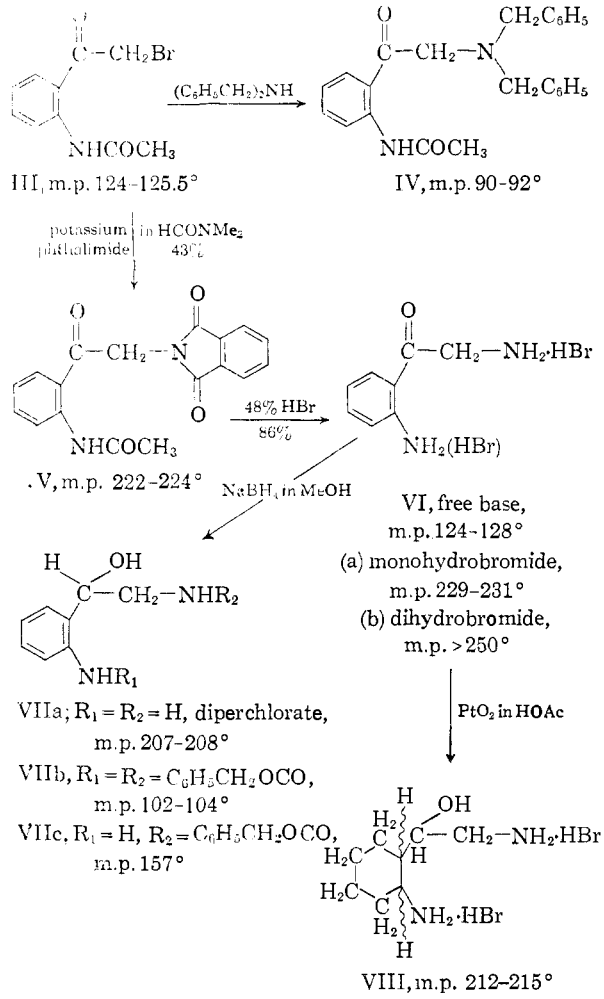
(2) H. Weissbach, T. E. Smith, J. W. Daly, B. Witkop and S. Udenfriend, *J. Biol. Chem.*, **235**, 1160 (1960).

(3) All melting points are uncorrected. We are indebted to Mr. H. G. McCann and associates of the Analytical Services Unit of this Laboratory for the analytical data.

(4) H. Gevekoht, *Ber.*, **15**, 2086 (1882); A. v. Baeyer and F. Bloem, *ibid.*, **15**, 2154 (1882).

(5) P. Ruggli and H. Reichwein, *Helv. Chim. Acta*, **20**, 913 (1937).

(6) J. C. Sheehan and W. A. Bolhofer, THIS JOURNAL, **72**, 2786 (1950).



Anal. Calcd. for $C_{18}H_{14}N_2O_4$: C, 67.07; H, 4.38; N, 8.69. Found: C, 66.65; H, 4.33; N, 8.86.

ω-Dibenzylamino-*o*-acetaminoacetophenone (IV).—To the solution of 1.40 g. of the bromoketone (III) in 25 ml. of benzene was added 2.70 g. of dibenzylamine and the mixture was refluxed for 13 hr. The dibenzylamine hydrobromide which had separated was filtered off, washed once with benzene, and the combined filtrates were washed with water, dried (Na_2SO_4) and evaporated *in vacuo*. The residual brownish oil was dissolved in warm hexane and stored in a cold room to yield 0.8 g. of pale yellow needles, m.p. 90–92°.

Anal. Calcd. for $C_{24}H_{24}N_2O_2$: C, 77.39; H, 6.50; N, 7.52. Found: C, 77.51; H, 6.52; N, 7.45.

Norkynuramine. A. Dihydrobromide (VIb).—The phthalimido compound (V, 3.50 g.) was refluxed with 20 ml. of 48% hydrobromic acid for 2 hr. (bath temperature 135–140°). As the reaction progressed, the starting material dissolved to give a darkly colored clear solution which after completion of the reaction was allowed to cool. The precipitated phthalic acid was collected and washed with cold water. The combined water solution was evaporated *in vacuo* at 40–50°. The residue was dissolved in 40 ml. of cold water, filtered and again evaporated to dryness *in vacuo*. The residue (4.1 g.) was dried in a vacuum desiccator over sodium hydroxide and then dissolved in 25 ml. of warm methanol. When to this solution was added 15 ml. of glacial acetic acid contiaining 30% HBr, norkynuramine dihydrobromide crystallized as colorless needles on storage in the cold. The yield was 2.9 g. or 86% of theory. The analytical sample was recrystallized from hot ethanol from which it separated as colorless needles on addition of warm ethyl acetate. The compound sintered above 220° and showed progressive decomposition at 250°. The ultraviolet spectrum showed λ_{max}^{EtOH} 239, 257, 262, 376 mμ (log ε, 4.80, 4.07, 4.02, 3.97); $\lambda_{max}^{EtOH+1.0\ N\ NaOH}$ 255, 364 mμ (log ε, 4.10, 4.00); $\lambda_{max}^{KB'}$: 1693 cm.$^{-1}$ (C=O).

Anal. Calcd. for $C_8H_{10}N_2O \cdot 2HBr$: C, 30.80; H, 3.88; N, 8.98; Br, 50.23. Found: C, 30.91; H, 3.88; N, 8.94; Br, 50.22.

B. **Monohydrobromide (VIa).**—When 10.0 g. of the phthalimido compound V after hydrolysis with hydrobromic acid as above was recrystallized from ethanol, 6.0 g. (80%) of yellowish-brown needles, m.p. 229–231° (dec.) was obtained which showed λ_{max}^{EtOH}: 228, 257, 262 (shoulder), 371 mμ (log ε, 4.38, 3.86, 3.80, 3.79); $\lambda_{max}^{KB'}$ 1653 cm.$^{-1}$ (hydrogen bonded C=O).

Anal. Calcd. for $C_8H_{10}N_2O \cdot HBr$: C, 41.58; H, 4.79; N, 12.12; Br, 34.58. Found: C, 42.05; H, 4.83; N, 11.99; Br, 34.97.

Conversion of Monohydrobromide VIa to Dihydrobromide VIb.—When 0.2 g. of the monohydrobromide VIa was dissolved in 1 ml. of warm 48% hydrobromic acid containing 1 ml. of glacial acetic acid, colorless crystals separated on storage in the cold. After washing with acetic acid and ether the colorless needles were identified as the dihydrobromide VIb by m.m.p. and infrared spectra.

Free norkynuramine VI was obtained from the solution of 10 mg. of norkynuramine monohydrobromide in 2 ml. of cold water after the addition of excess 2.0 *N* aqueous ammonia. On storage in the cold the reddish crystals were collected, washed and dried; m.p. 124–128° (dec.) with sintering at *ca.* 110° (lit.[7] m.p. 123–128°, not sharp).

Monopicrate.—To the solution of 31 mg. of norkynuramine dihydrobromide VIb in 2 ml. of water was added 2 ml. of a saturated aqueous solution of picric acid. The yellow crystals which separated were collected and recrystallized from aqueous methanol giving 21 mg. of long yellow needles, m.p. 154–155.5°.

Anal. Calcd. for $C_8H_{10}N_2O \cdot C_6H_3N_3O_7$: C, 44.33; H, 3.45; N, 18.47. Found: C, 44.40; H, 3.44; N, 18.46.

The identical picrate was obtained from a solution of the monohydrobromide VIa in an analogous manner.

N,N'-Dicarbobenzyloxynorkynuramine.—To the solution of 1.90 g. of potassium carbonate in 20 ml. of water, covered with 30 ml. of ether, at 0°, 0.94 g. of norkynuramine dihydrobromide VIb in 20 ml. of water was added. The solution of 1.35 g. of carbobenzyloxy chloride in 10 ml. of ether was added to the mixture with cooling and stirring over a period of 10 minutes. Stirring was continued overnight at −5°. The ether layer was separated and the aqueous phase was extracted with ether. The combined ether extracts were washed with 1 *N* hydrochloric acid and water, dried (Na_2SO_4) and evaporated *in vacuo*. The residue was recrystallized from benzene–hexane, giving 0.85 g. of colorless rods, m.p. 108–110°(sintering at 104°).

Anal. Calcd. for $C_{24}H_{22}N_2O_5$: C, 68.89; H, 5.30; N, 6.70. Found: C, 68.61; H, 5.44; N, 6.80.

Octahydronorkynuramine (VIII).—Attempted catalytic hydrogenation of norkynuramine dihydrobromide with palladium on carbon as catalyst and ethanol or acetic acid as solvents was without success. When platinum oxide was used, the uptake of hydrogen would not decelerate after absorption of one mole. In the event, the solution of 1.75 g. of norkynuramine dihydrobromide VIb in 20 ml. of water and 30 ml. of acetic acid was hydrogenated with 0.50 g. of platinum oxide under atmospheric pressure and room temperature which led to the uptake of 3.8 moles of hydrogen. On evaporation of the filtrate *in vacuo* a crystalline residue was obtained which was dried in a vacuum desiccator over sodium hydroxide overnight, then crystallized from ethanol–ether, giving colorless prisms, m.p. 212–215° (dec., sintering at 175°).

Anal. Calcd. for $C_8H_{18}N_2O \cdot 2HBr$: C, 30.02; H, 6.29; N, 8.75. Found: C, 30.09; H, 6.49; N, 9.12. The compound showed no absorption in the ultraviolet.

Dihydronorkynuramine Diperchlorate (VIIa).—To the solution of 250 mg. of norkynuramine dihydrobromide VIb in 5 ml. of methanol was slowly added with cooling (ice–salt mixture) and stirring a solution of 300 mg. of sodium borohydride in 5 ml. of methanol. Stirring was continued for 30 min. at −10°, then for 1 hr. at room temperature. The reaction was checked by taking aliquots and examining them for the disappearance of λ_{max} 360 mμ. The mixture

(7) S. Gabriel and W. Gerhard, *Ber.*, **54**, 1067 (1921).

was taken to dryness *in vacuo*, 5.0 ml. of 1.0 *N* sodium hydroxide was added followed by extraction with ethyl acetate of the aqueous phase previously saturated with potassium carbonate. The organic extracts were washed once with saturated sodium chloride solution, dried (Na_2SO_4) and evaporated *in vacuo* to give 98 mg. of a sirup which withstood all attempts at crystallization and was, therefore, dissolved in 2 ml. of 40% perchloric acid, concentrated *in vacuo* at room temperature and stored in a cold room. The brown crystals which eventually separated were collected and recrystallized from ethanol–ether to give 73 mg. of colorless fine needles, m.p. 207–208° (dec.); λ_{max}^{EtOH}: 234, 289 mμ (log ε, 4.06, 3.50); λ_{max}^{KBr}, 3440 cm.$^{-1}$ (OH).

Anal. Calcd. for $C_8H_{12}N_2O \cdot 2HClO_4$: C, 27.21; H, 3.99; N, 7.93. Found: C, 27.38; H, 4.13; N, 8.03.

N,N'-Dicarbobenzyloxydihydronorkynuramine (VIIb).—Amorphous dihydronorkynuramine, obtained as described above by $NaBH_4$ reduction of 0.32 mmole of norkynuramine dihydrobromide, was reacted in 10 ml. of ice-water containing 140 mg. (2 mmole) of potassium carbonate under 30 ml. of ether with a solution of 170 mg. (1 mmole) of carbobenzyloxy chloride in 5 ml. of ether under cooling. After stirring overnight at −5° the ether layer was separated and the aqueous phase was once more extracted with ether. The combined ether extracts were washed with 1.0 *N* hydrochloric acid and water, dried (Na_2SO_4) and evaporated *in vacuo*. The residue after recrystallization from benzene–hexane yielded 58 mg. of colorless needles, m.p. 102–104°.

Anal. Calcd. for $C_{24}H_{24}N_2O_5$: C, 68.56; H, 5.75. Found: C, 68.57; H, 6.06.

ω-N-Carbobenzyloxydihydronorkynuramine (VIIc).—When one equivalent of carbobenzyloxy chloride was used, the monocarbobenzyloxy compound, m.p. 157°, was obtained from the acid extract.

Anal. Calcd. for $C_{16}H_{18}N_2O_3$: C, 67.11; H, 6.34; N, 9.78. Found: C, 67.14; H, 6.45; N, 9.64.

Dihydrokynuramine.—When 170 mg. of kynuramine dihydrobromide was reduced with 300 mg. of sodium borohydride, as described in the case of norkynuramine, dihydrokynuramine was obtained as a colorless sirup (65 mg.); λ_{max}^{EtOH}, 238, 289 mμ (log ε, 3.76, 3.19). No crystalline derivative was obtained.

As in the case of norkynuramine, catalytic reduction of kynuramine dihydrobromide with platinum oxide proceeded with hydrogenation of the aromatic ring.

***o*-Nitrobenzylidene-*erythro*[8]-β-*o*-nitro-DL-phenylserine Ethyl Ester.**—A solution of 2.20 g. of glycine ethyl ester hydrochloride in 15 ml. of hot absolute ethanol was added to a solution of 0.37 g. of sodium in 10 ml. of absolute ethanol. After 1 hr. at room temperature the sodium chloride which had separated was removed by filtration. To the combined filtrate was added 4.70 g. of *o*-nitrobenzaldehyde and 0.3 ml. of triethylamine. The pale yellow mixture was occasionally agitated and then left overnight in the cold room. The crystals which separated were collected, washed with ethanol and then with ether. There was obtained 1.55 g. of colorless fine needles, m.p. 125–127°. From the mother liquor an additional crop of 0.48 g. was obtained on concentration, adding up to a total yield of 34%. Recrystallization from benzene and a small amount of hexane gave colorless needles, m.p. 126–127°.

Anal. Calcd. for $C_{18}H_{17}N_3O_7$: C, 55.81; H, 4.42; N, 10.85. Found: C, 56.12; H, 4.66; N, 10.83.

***erythro*-β-*o*-Nitro-DL-phenylserine.**—The solution of 1.20 g. of the benzylidene compound in 30 ml. of absolute ethanol containing 3.5 ml. (1.5 eq.) of 1.37 *N* ethanolic hydrochloric acid was refluxed for 20 mins., concentrated *in vacuo* and stored in a cold room overnight. The residual solvent was then removed *in vacuo*, the residue was washed with ether. By trituration with ethyl acetate in the cold it solidified gradually. Recrystallization from ethanol–ethyl acetate–ether gave 0.52 g. (56%) of colorless fine rods which sintered at 140° and gave a turbid melt at 155–160°. The crude hydrochloride (300 mg.) was stirred for 3 hr. at room temperature with 2.5 ml. of 1.0 *N* sodium hydroxide and 2.5 ml. of ethanol. The solution was adjusted with 1.0 *N* hydrochloric acid to a *p*H 6–7. The pale cream-colored crystalline powder (190 mg.) was collected and dried; m.p. 178–185° (dec.). It was recrystallized from water, giving colorless needles, m.p. 185–186.5° (dec.).

Anal. Calcd. for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.46; N, 12.39. Found: C, 48.01; H, 4.55; N, 12.12.

***erythro-o*-Amino-DL-phenylserine.**—A solution of 157 mg. of the *o*-nitro compound in 20 ml. of absolute ethanol and 2 ml. of 1.0 *N* hydrochloric acid was hydrogenated with 40 mg. of platinum oxide at atmospheric pressure and room temperature. The theoretical amount of hydrogen was absorbed within 3 hr. The colorless solution was filtered from the catalyst, evaporated to dryness at room temperature and the residue dissolved in 3 ml. of water, neutralized to *p*H 7 with 5% ammonia and concentrated to a small volume in a vacuum desiccator over sodium hydroxide. The crystals which had separated were collected and washed with cold water. There was obtained 91 mg. of a light brown powder which melted at 240–250° (dec.) with preliminary sintering at 230°. Purification was achieved by dissolving it in a small amount of water, addition of ethanol and storage in a cold room. The colorless fine rods thus obtained showed m.p. 255–260° (dec.).

Anal. Calcd. for $C_9H_{12}N_2O_5 \cdot 1\text{-}1/2H_2O$: C, 48.43; H, 6.77; N, 12.55. Found: C, 48.43; H, 7.36; N, 12.32.

Methods and Results

Enzymatic Oxidation of (Nor)kynuramine Derivatives by Monoamine Oxidase. A. Manometric Method.—One ml. samples of aqueous solutions of norkynuramine dihydrobromide, dihydronorkynuramine diperchlorate and of pure amorphous dihydrokynuramine (containing 5 mg. each) were each incubated at 37° with 0.3 ml. of a soluble, partially purified preparation of monoamine oxidase (containing 0.32 mg. of protein) from guinea pig liver (specific activity: 4.2 μmoles of kynuramine oxidized per mg. of protein per hour), 0.5 ml. of 0.5 *M* phosphate buffer (*p*H 8.0), 0.08 ml. of 0.1*N* potassium cyanide and 1.2 ml. of water in a Warburg apparatus. Oxygen uptake was determined and compared with that of kynuramine under the same conditions. The results are shown in Fig. 1.

B. Formation of Indigo from Dihydronorkynuramine as Measured Spectrophotometrically.—Dihydronorkynuramine diperchlorate (0.1 mg. in 0.1 ml. of water) was incubated with 0.5 ml. of a monoamine oxidase preparation, 0.3 ml. of 0.5 *M* phosphate buffer (*p*H 8.0) and 2.1 ml. of water in a 3-ml. silica cuvette. The incubation was run at room temperature; readings were taken at 600 mμ with a Beckman spectrophotometer (Model DU) and corrected for a corresponding blank containing no substrate. The results are shown in Fig. 2. The enzymatically formed material was identified as indigo in the following way. After a 2 hr. incubation the reaction product was extracted from the reaction mixture by three portions of 5-ml. of chloroform. The chloroform extracts were combined, filtered, concentrated and made up to a total volume of 4 ml. The spectrum was measured and compared with authentic indigo as shown in Fig. 3. No indigo was formed when norkynuramine was tested as substrate. In the case of dihydronorkynuramine half of the uptake of oxygen is due to enzymatic, the second half to spontaneous oxidation, assuming two discrete steps, *i.e.*, indoxyl and indigo.

Attempted Enzymatic Oxidation of *erythro-o*-Amino-DL-phenylserine to Indigo by Snake Venom

(8) The *erythro* configuration has been assumed to apply to this compound for reasons of analogy; it has been shown previously that this type of reaction leads into the *erythro* series: D. O. Holland, P. A. Jenkins and J. H. C. Naylor, *J. Chem. Soc.*, 273 (1953).

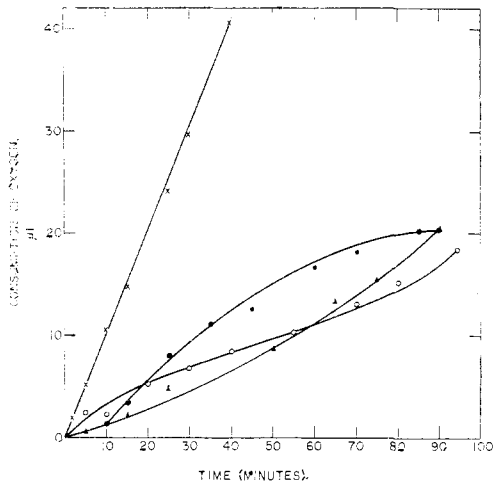


Fig. 1.—Comparative rates of oxygen uptake for kynuramine dihydrobromide (X–X–X), dihydrokynuramine (●–●–●), norkynuramine dihydrobromide (▲–▲–▲) and dihydronorkynuramine diperchlorate (O–O–O) on incubation with monoamine oxidase.

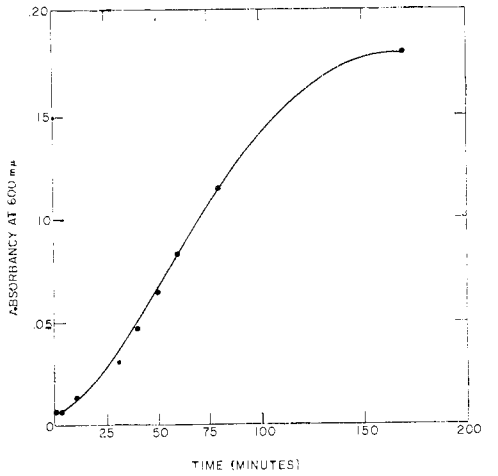


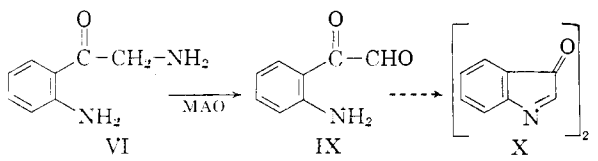
Fig. 2.—Enzymatic production of indigo, λ_{max} 600 mμ, from dihydronorkynuramine diperchlorate on incubation with monoamine oxidase at 25° as observed *in situ* in a spectrophotometer.

L-Amino Acid Oxidase.—A solution of 1.3 mg. (5 μmoles) of *erythro-o*-amino-DL-phenylserine in 1 ml. of water was incubated with 0.3 ml. of enzyme solution containing 0.3 mg. of crude rattlesnake (Crotalus adamanteus) enzyme, 0.5 ml. of 0.5 *M* Tris buffer (*p*H 8.0) and 1.2 ml. of water. On prolonged incubation no change was observed at 600 mμ, the region for visible absorption of indigo.

Although no indigo was formed in these experiments, it was of interest to test whether this compound was a substrate of L-amino acid oxidase as measured by oxygen uptake. Enzyme activity for these experiments was assayed manometrically in a Warburg apparatus. The incubation, total volume 3.0 ml., contained 2 mg. of snake venom, 22 μmoles substrate (side arm), 20 μmoles of Tris buffer (*p*H 7.2) and 0.2 ml. of 5 *N* NaOH in the center well. No activity was observed after a 3-minute incubation with *erythro-o*-amino-DL-phenylserine, although under the same conditions 0.6 μmole of L-kynurenine and 1.96 μmoles of L-leucine were oxidized.

Discussion

The enzymatic oxidation of norkynuramine, if it were to follow the same course as that of kynuramine, would be expected to lead, *via o*-aminophenylglyoxal (IX), to indolone (X), which is only stable as the dimeric indigo. The latter was not



VI IX X

formed on incubation with monoamine oxidase, for which norkynuramine is a much poorer substrate (Fig. 1) than the homologous amine in agreement with related observations on the retarding influence of *o*-substituents and β-hydroxyl groups in phenethylamines on the rate of doxiation by certain monoamine oxidases.[9] A

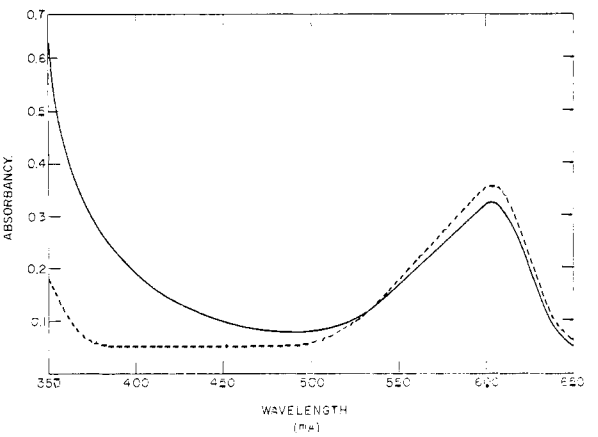
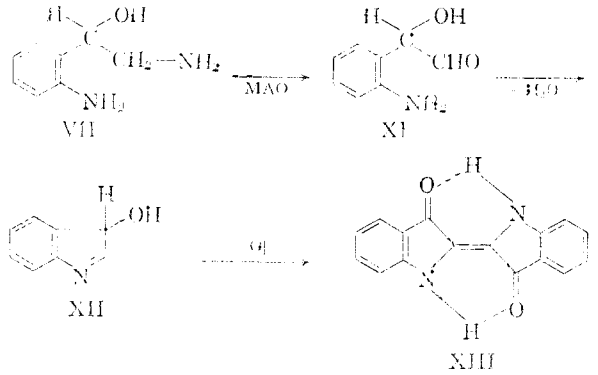


Fig. 3.—Ultraviolet absorption curve (solid line) of product obtained from incubation of 0.1 mg. of dihydronorkynuramine diperchlorate with monoamine oxidase at 37° for 2 hr. and subsequent extraction with chloroform. The dotted curve represents the absorption of indigo in chloroform, $c = 1.1 \times 10^{-5}$ *M*.

number of preliminary chromatographic and enzymatic studies, such as coupling the MAO oxidation with dehydrogenation by pure aldehyde dehydrogenase and DPN to form the corresponding glyoxylic acid or the attempt to arrest the intermediate glyoxal by addition of 2,4-dinitrophenylhydrazine failed to clarify the nature of the intermediate.

Dihydrokynuramine and dihydronorkynuramine (VII) are both enzymatically oxidized at a much slower rate than kynuramine (I, Fig. 1). While the oxidation of the former must be followed manometrically, dihydronorkynuramine (VII) on incubation with monoamine oxidase gives rise to indigo (XIII, Fig. 2) whose presence was proven by comparison of the characteristic visible absorption of the spectrum of the product with that of authentic indigo (Fig. 3). The initial relation between the optical density of λ_{max} of indigo at

(9) *Cf.* P. Hagen and N. Weiner, *Federation Proc.*, **18**, 1005 (1959); H. Blaschko and T. L. Chrusciel, *Brit. J. Pharmacol.*, **14**, 364 (1959).



600 mμ and time was a linear function (Fig. 2). The convertibility of dihydronorkynuramine to indigo did not exceed ~30% in a given experiment. For quantitative assays of monoamine oxidase kynuramine is superior although the enzymatic formation of indigo may serve as a qualitative *visual* test for the presence of monoamine oxidase.

It was of interest to test dihydronorkynuramine as a histochemical agent for staining tissue *in situ* and thus localize the areas containing monoamine oxidase. Microscopic inspection of liver and kidney slices incubated with dihydronorkynuramine showed the formation of the unmistakable dark blue indigo crystals. However these crystals formed only in solution and did not combine with the protein fibre. It may be possible that a useful histochemical stain may be developed along these lines, if suitable binding groups are introduced into the benzene part of dihydronorkynuramine. For example, halogens in positions 4 and 5 of indoxyl derivatives are known to increase the substantivity of the resulting indigo dyes.[10]

The oxidative degradation of L-kynurenine by snake venom L-amino acid oxidase to kynurenic acid has been developed into a convenient spectrophotometric assay of that enzyme.[11] The analogous reaction of *o*-amino-*erythro*-DL-phenylserine with snake venom L-amino acid oxidase did not lead to any uptake of oxygen and therefore not to a colored product, arising possibly from the expected indoxyl-2-carboxylic acid.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY, STANFORD, CALIF.]

Optical Rotatory Dispersion Studies. XLI.[1] α-Haloketones (Part 9).[2] Bromination of Optically Active *cis*-1-Decalone. Demonstration of Conformational Mobility by Rotatory Dispersion[3]

BY CARL DJERASSI AND J. STAUNTON[4]

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cis,cis-1-Decalol (IV) has been resolved for the first time through its 3β-acetoxy-Δ⁵-etienate (III) and a convenient preparation of the resolving agent, 3β-acetoxy-Δ⁵-etienic acid (II), is reported. The absolute configurations of the derived (−)-*cis*-1-decalone (V) and (−)-*trans*-1-decalone (VI) were established by comparing their rotatory dispersion curves with those of their 10-methyl analogs of known relative and absolute stereochemistry. Bromination of (−)-*cis*-1-decalone afforded in pure form all six possible monobromo-*cis*- and *trans*-1-decalones, whose rotatory dispersion curves were fully consistent with the assigned structures (VII–XII). Measurement of the rotatory dispersion curve of the axial (−)-2α-bromo-*cis*-1-decalone (XI) in solvents of different polarity demonstrated the existence of a 70–30 equilibrium between the "steroid" (XIa) and "non-steroid" (XIb) conformations of this ketone.

Recently, Zimmerman and Mais,[5] in connection with their studies on the stereochemistry of the ketonization process, investigated the bromination of *cis*-1-decalone. Of the six possible monobromo ketones (VII–XII),[6] they were able to isolate four (VII–X) in pure form and probably a fifth one (XII) in an impure state. Their structural assignments were based on infrared measurements (thus differentiating axial from equatorial isomers[7]), thermodynamic considerations and finally debromination as well as dehydrobromination experiments. While these elegant experiments[5] were quite self-consistent, it nevertheless seemed worth while to reinvestigate this bromination with optically active *cis*-1-decalone in order to apply rotatory dispersion measurements[8] to the various bromo ketones. Extensive rotatory dispersion studies with a variety of α-halocyclohexanones of known stereochemistry led to the enunciation of the "axial haloketone rule,"[9] which states that introduction of equatorial halogen does not change the sign of the Cotton effect of a cyclohexanone, while an axial bromine

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