L-leucylglycinate.—Benzyl N-carbobenzoxy-L-prolyl-L-leucylglycinate (0.85 g.) was dissolved in 2 ml. of acetic acid and stirred with 4 ml. of HBr in acetic acid (31% w./w.) until the evolution of CO_2 had stopped (15 min.). This solution was poured into 100 ml. of stirred, dry ether, and the solid tripeptide ester hydrobromide which separated was washed several times by decantation of dry ether. It was kept under ether for 1 hr., filtered, washed thoroughly with ether, and dried *in vacuo* over $CaCl_2$. It was dissolved in 5 ml. of acetic acid, reprecipitated by pouring into ether, then washed, isolated, and dissolved in 5 ml. of anhydrous methanol. The precipitation and washing with ether were repeated to give a solid which was dissolved in 15 ml. of methanol and treated with Dowex IRA-410 resin (OH form), with stirring, until the pH of the solution was approximately 8. The resin was filtered and washed with methanol. The combined filtrate and washings were evaporated under reduced pressure to give a pale yellow oil (0.46 g.). It was dissolved with 0.46 g. of N-carbobenzoxy-S-benzyl-L-cysteine[10] in 2 ml. of freshly distilled tetrahydrofuran. The solution was cooled in ice–water and to it was added 0.29 g. of dicyclohexylcarbodiimide in 1 ml. of tetrahydrofuran. The mixture was kept at room temperature overnight and diluted with 15 ml. of ethyl acetate. The insoluble dicyclohexylurea was filtered and washed with 5 ml. of ethyl acetate. The combined filtrate and washings were evaporated to give a colorless oil, which was dissolved in 20 ml. of ethyl acetate and washed successively with 1 *N* HCl, water, 0.5 *N* $NaHCO_3$, water, and saturated saline. The solution was dried ($MgSO_4$), filtered, and evaporated to give 0.73 g. of a colorless oil. Of this, 0.6 g. was dissolved in 2 ml. of acetic acid and stirred with 3 ml. of HBr in acetic acid (31% w./w.) until the evolution of CO_2 had stopped (12 min.), when the solution was poured into 100 ml. of dry ether. The solid S-benzyl tetrapeptide benzyl ester hydrobromide which separated was purified and the free base was isolated by the method already described for the preparation of the tripeptide ester. The product was obtained as a pale yellow oil (0.39 g.). N-Carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparagine (0.57 g.)[6] was suspended in 4 ml. of dimethylformamide at 0° and to it was added with stirring, 0.44 g. of dicyclohexylcarbodiimide. After 5 min. at 0°, a solution of the S-benzyl tetrapeptide benzyl ester (0.39 g.) in 2 ml. of dimethylformamide was added to the mixture which was then stirred at room temperature for 2 hr. and kept at 3° for 48 hr. To it were then added 0.8 ml. of acetic acid and 70 ml. of water. This caused a white solid to separate, and after being stirred for 0.5 hr. it was filtered, washed thoroughly with water, and dried *in vacuo* over P_2O_5 to give 1.22 g. of product which was dissolved in 7 ml. of dimethylformamide. The solution was filtered from dicyclohexylurea, and water was added to the filtrate until precipitation was complete. The separated solid was filtered, washed with water, and dried *in vacuo* over P_2O_5 to give 0.76 g. of off-white amorphous material, which was dissolved in 5 ml. of dimethylformamide. The peptide material was reprecipitated by the addition of 10 ml. of 1-propanol followed by hexane to saturation. The separated solid was filtered, ground in a mortar under 30 ml. of methanol, filtered again, and washed with methanol, then ether. The white product was dried *in vacuo;* yield 0.3 g., m.p. 228–230°, $[\alpha]^{20}D$ −24.0° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{72}H_{91}N_{11}O_{15}S_2$: C, 61.1; H, 6.48; N, 10.9. Found: C, 60.7; H, 6.49; N, 10.8.

9-Deamidooxytocin.—The preceding compound (200 mg.) was dissolved in 150 ml. of boiling, redistilled liquid ammonia. A freshly prepared sodium stick was dipped into the stirred solution and was removed whenever the whole solution became blue. When this color faded (12 sec.) the stick was momentarily reintroduced into the solution. After the color so produced had faded, the ammonia was removed by lyophilization (water pump) and the solid residue was dissolved in 200 ml. of 0.25% acetic acid. The pH of this solution was adjusted to 6.5 and a slow stream of air (CO_2-free) was bubbled through it for 5 hr. Potassium ferricyanide (0.02 *N*, 2 ml.) was then added and was not consumed. Any ferrocyanide and excess ferricyanide ions were removed by passage of the solution through a column of AG 3-X4 resin (4 × 4 cm.). The column was washed with water and the volume of the combined eluate and washings was made up to 250 ml. Of this, 1 ml. was diluted to 5 ml. and assayed for avian depressor activity. The volume of the solution was reduced to 50 ml. by evaporation in a rotary evaporator under reduced pressure, with the temperature not exceeding 25°, and it was then submitted to countercurrent distribution in the solvent system 1-butanol–1-propanol–water containing 0.5% acetic acid and 0.1% pyridine (6:1:8).[13] After 300 transfers the distribution was visualized by the development of the Folin–Lowry color[15] of samples of lower phase. Three peaks of partition coefficients 0.11, 0.67, and 1.4 were seen. On examination, the materials of K = 0.11 and K = 1.4 were obviously by-products. The contents of the tubes representing the peak of K = 0.67 were concentrated and lyophilized. The average yield of lyophilized powder in three preparations was 28 mg., $[\alpha]^{19}D$ −31.7° (*c* 0.6, 1 *N* acetic acid).

Anal. Calcd. for $C_{43}H_{65}N_{11}O_{13}S_2$: C, 51.2; H, 6.50; N, 15.3. Found: C, 51.4; H, 6.57; N, 15.2.

A sample was hydrolyzed in 6 *N* HCl at 110° for 22 hr. in an evacuated tube and analyzed for amino acids on a Beckman-Spinco analyzer.[23] The following molar ratios were obtained, with the value of aspartic acid being taken as 1: aspartic acid 1.0, proline 1.0, glycine 0.9, glutamic acid 1.0, cystine 1.0, isoleucine 1.0, leucine 1.0, tyrosine 0.9, and ammonia 2.2.

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(23) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

Synthesis of β-(4-Hydroxy-1-naphthyl)-DL-alanine[1]

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The synthesis of β-(4-hydroxy-1-naphthyl)-DL-alanine by hydrolysis of 5-(4-hydroxy-1-naphthyl)methylenehydantoin or -thiohydantoin has been carried out. This new synthetic amino acid, an analog of tyrosine, is useful for histochemical demonstration of tyrosinase activity.

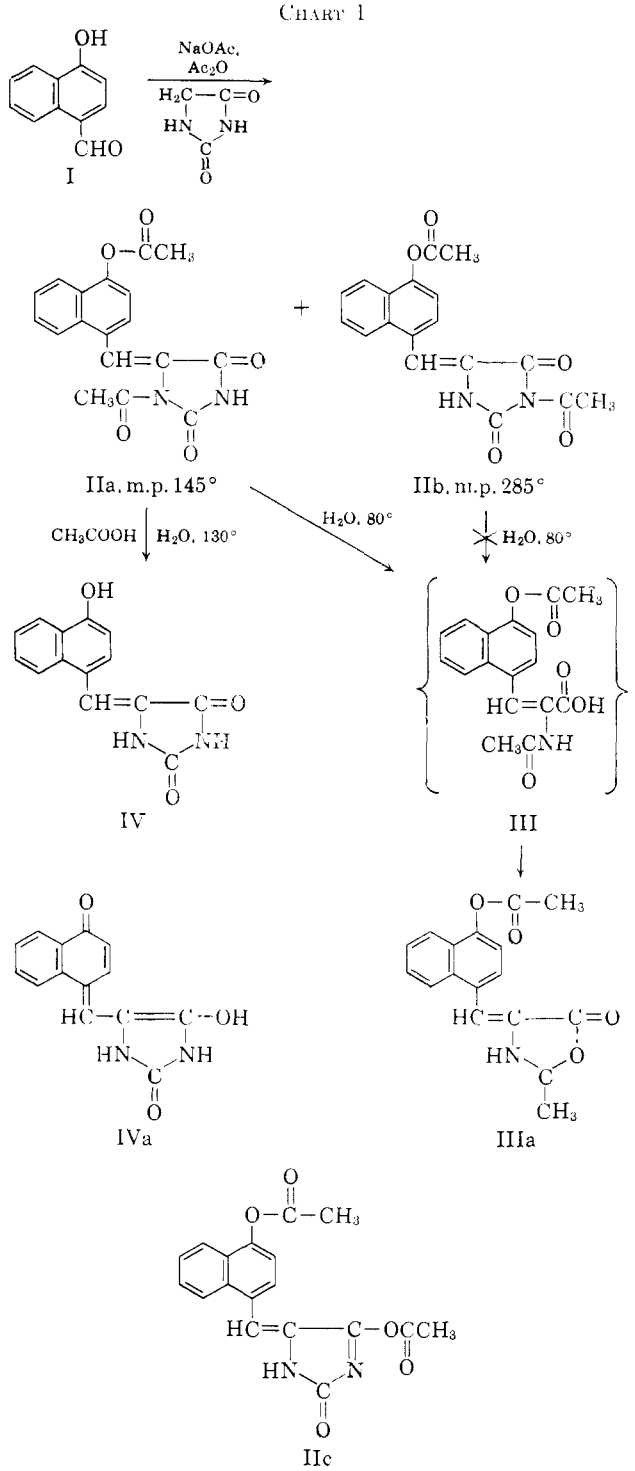
Our interest in the preparation of β-(4-hydroxy-1-naphthyl)-DL-alanine (XII) was initiated because of its possible use as a chromogenic substrate for histochemical demonstration of protein or enzyme synthesis. This amino acid, by virtue of its structural similarity to tyrosine, could be a substrate for tyrosinase or a good competitive amino acid to undergo peptide synthesis where tyrosine is involved.[2a] Histochemical visualiza-

(1) This investigation was supported by U. S. Public Health Service Grant CA-07339 and in part by Grants CY-2530 and AI-06261.

(2) (a) E. L. Smith and A. N. Dannenberg, *J. Biol. Chem.*, **215**, 45 (1955); (b) K. C. Tsou and A. M. Seligman, *J. Am. Chem. Soc.*, **74**, 3066, 5065 (1952); **76**, 3704, 6108 (1954); **77**, 4613 (1955).

tion could be made possible by coupling with a diazonium salt to an azo dye[2b] or by virtue of its fluorescence. The synthetic scheme is shown in Charts I–III.

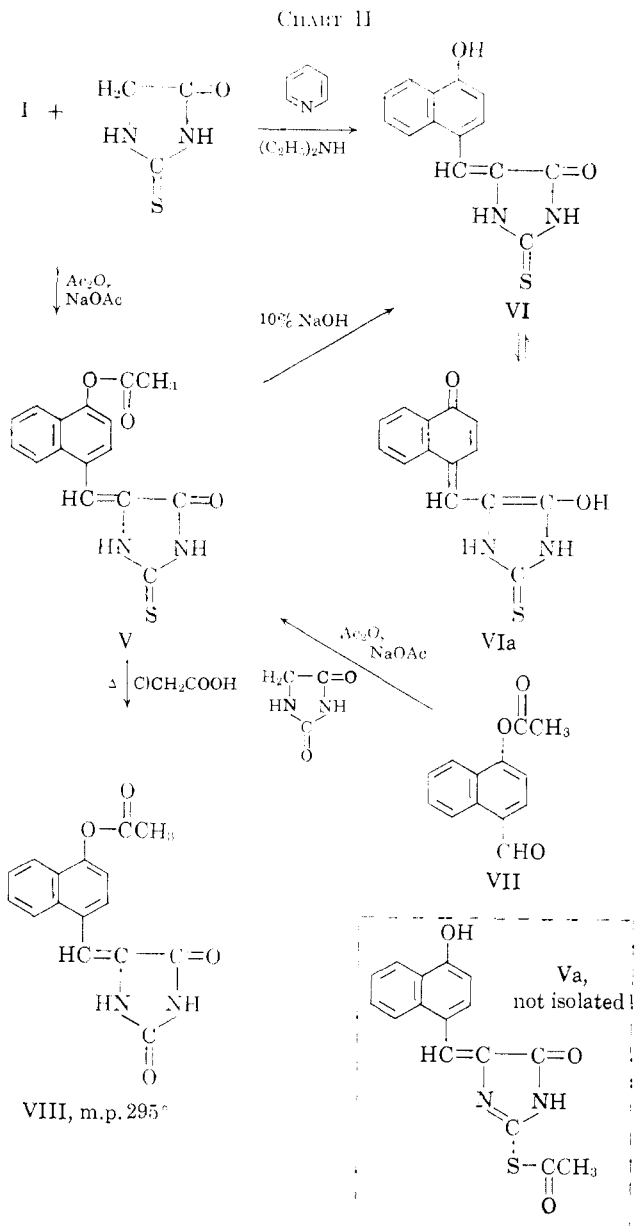
Chart I



4-Hydroxy-1-naphthaldehyde (I)[3,4] was condensed with hydantoin or 2-thiohydantoin. With hydantoin in acetic anhydride and sodium acetate, the isomeric 5-(4-acetoxy-1-naphthylidene)hydantoin monoacetyl derivatives IIa and IIb were obtained. The assignment of these structures was based on their infrared spectra. Indirect support for IIa was also provided by the isolation of an oxazolidone (IIIa) when IIa was

(3) R. Adams and I. Levine, *J. Am. Chem. Soc.*, **45**, 2373 (1923).
(4) L. Gatterman and Th. von Horlacher, *Ber.*, **32**, 284 (1899).

Chart II

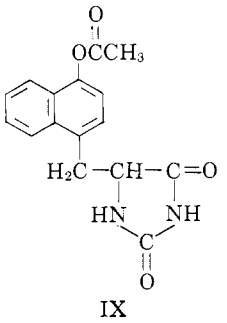


subjected to mild hydrolysis. Under the same conditions, IIb was stable, making its assignment preferable to IIc.

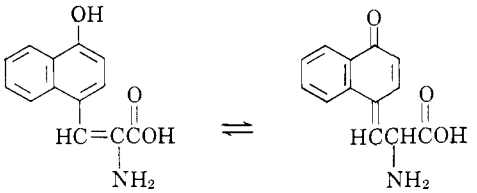
In order to prepare 5-(4-hydroxy-1-naphthylidene)-hydantoin (IV) directly, reaction of the aldehyde I with hydantoin was carried out in a basic solvent medium such as pyridine with diethylamine. While the loss of the aldehydic group during the reaction indicated that condensation did take place, no IV could be isolated. After numerous trials, IIa was hydrolyzed in boiling acetic acid–water; deacetylation occurred and a compound was obtained as fine orange crystals. Its color, infrared spectrum, and faint quinone-like odor lend support to the assignment of a quinonoid structure IVa rather than IV.

The condensation of I with 2-thiohydantoin in acetic anhydride and sodium acetate gave 5-(4-acetoxy-1-naphthylidene)-2-thiohydantoin (V) as the main product (Chart II). Since it was possible to have the S-acetyl derivative Va rather than the O-acetyl compound V under the reaction conditions, 4-acetoxy-1-naphthaldehyde (VII) was condensed with thiohydan-

CHART III

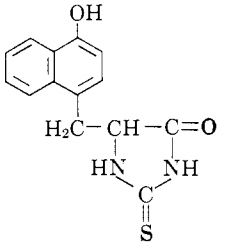


IX

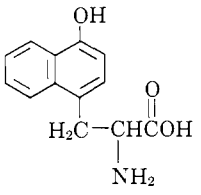


X, m.p. >300°
R_f 0.54

Xa.
R_f 0.65



XI



XII

toin directly to yield V. That no diacetyl compound was isolated could be taken as evidence that the hydrogen atoms on amide nitrogens 1 and 3 are less acidic in the thiohydantoin than those in the hydantoin. When the condensation was carried out in pyridine with diethylamine, 5-(4-hydroxy-1-naphthylidene)-2-thiohydantoin (VI) was obtained as a hydrate. The same product was obtained when V was deacetylated in 10% sodium hydroxide solution. Based on the infrared spectral data, it appears that VI, like its hydantoin analog IV, exists also in a tautomeric quinonoid form VIa, at least in the solid state. Desulfurization of the thiohydantoin V by prolonged boiling in aqueous chloroacetic acid gave 5-(4-acetoxy-1-naphthylidene)hydantoin (VIII), as shown by infrared and elemental analysis.

When the hydantoin IIa was refluxed with hydriodic acid in glacial acetic acid, a low yield of 5-(4-acetoxy-1-naphthyl)methylenehydantoin (IX) was isolated. Reduction of IIa by sodium amalgam in 2% sodium hydroxide yielded a mixture of the sodium salt of the hydantoic acid and β-(4-hydroxy-1-naphthyl)dehydroalanine (X). This amino acid (X) was later prepared directly by hydrolysis and was confirmed by paper chromatography. Reduction of VI with sodium amalgam and aqueous sodium hydroxide led to 5-(4-hydroxy-1-naphthyl)methylene-2-thiohydantoin (XI) in low yields. The formation of XI during the reaction was indicated by the appearance of a brilliant blue color in the solution, owing probably to the C-sodium salt of XI. Hydrolysis of 5-(4-acetoxy-1-naphthylidene)thiohydantoin (V) gave the dehydroamino acid (X), which could be shown by paper chromatography to consist of the expected two isomeric forms X and Xa (Chart III). Hydrolysis of XI to β-(4-hydroxy-1-naphthyl)-DL-alanine (XII) was effected in 5 days with barium hydroxide solution (reflux). The characterization of X and XII could not be done by elemental analysis alone, and supporting evidence was provided by paper-chromatographic analysis. Only the purified sample showed activity in a simulated tyrosinase experiment and the dehydroamino acid X appeared to be a competitive inhibitor. The details of the biological study will be reported elsewhere. A preliminary account is given in the Experimental Section.

Experimental Section

5-(4-Acetoxy-1-naphthylidene)hydantoin (IIa).—A mixture of 10.6 g. of I,[5] 5.3 g. of hydantoin, and 5 g. of freshly fused sodium acetate in 50 ml. of acetic anhydride was refluxed with stirring for 2 hr. The solution was evaporated *in vacuo* to a tarry mass which was triturated in benzene. The fine, bright yellow crystals of IIa thus formed melted at 145°; λ_{max}^{KBr} 3.25 (m) (NH), 3.40 (s), 5.70 (s), 5.80 (s), 6.02 (m) μ.

Anal. Calcd. for $C_{18}H_{14}N_2O_5$: C, 63.90; H, 4.17; N, 8.28. Found: C, 63.69; H, 4.37; N, 8.03.

When the heating period was decreased to 30 min. and the solution was decomposed with water, the tarry material obtained on trituration in benzene and recrystallization from methanol–water, gave a pale yellow solid, m.p. 283–286° dec., assigned the structure IIb; λ_{max}^{MeOH} 230 mμ (ε 26,600).

Anal. Calcd. for $C_{18}H_{14}N_2O_5$: C, 63.90; H, 4.17; N, 8.28. Found: C, 63.92; H, 4.28; N, 8.27.

This product and IIa have almost identical infrared spectra. The 8.70-μ band of IIa is split into a doublet (in IIb) at 8.60 and 8.85 μ.

Hydrolysis of IIa.—IIa (1.8 g.) was heated in a 50:50 mixture of benzene and water for 1 hr. The benzene layer was separated and evaporated to dryness. A methanolic solution of the residue was treated with charcoal, filtered, and treated with water to give 1.5 g. of slightly tan crystals: m.p. 75°; λ_{max}^{KBr} 5.65 (shoulder, lactone) 5.72 (s), 5.90 (s) μ.

Anal. Calcd. for $C_{17}H_{13}NO_4$: C, 69.15; H, 4.44; N, 4.73. Found: C, 68.86; H, 4.30; N, 4.50.

We have assigned the azlactone structure, IIIa, to this product.

5-(4-Hydroxy-1-naphthylidene)hydantoin (IV).—IIa (3 g.) was dissolved in boiling acetic acid. Some resinous material formed and was filtered. To the hot filtrate, water was added until an orange precipitate appeared; yield 2 g.; m.p. 213°; λ_{max}^{KBr} 2.90 (vs), 5.68 (s), 5.90 (s), 11.20 μ (no C–CH_3 at 3.5).

Anal. Calcd. for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02. Found: C, 65.91; H, 4.30; N, 10.78.

On the basis of infrared data and the characteristic quinone odor of the compound, we have assigned the structure IVa instead of IV, at least in solid state.

Condensation of 4-Hydroxy-1-naphthaldehyde with 2-Thiohydantoin. A. 5-(4-Acetoxy-1-naphthylidene)-2-thiohydantoin (V).—I (8.6 g.), 2-thiohydantoin (5.8 g.), 80 ml. of acetic anhydride, and 5 g. of freshly fused sodium acetate were heated slowly to 120° for 1 hr., cooled, and filtered. The solid was washed with acetic acid and then water. The yellow residue was recrystallized from methanol–water to give bright yellow, fine crystals: m.p. 275–278°; yield 4.7 g.; λ_{max}^{KBr} 3.20 (m), 3.42 (s), 3.50 (sh), 5.56 (s), 5.78 (vs), 6.02 (s) μ.

Anal. Calcd. for $C_{16}H_{12}N_2O_3S$: C, 61.52; H, 3.87; N, 8.97. Found: C, 61.54; H, 3.67; N, 9.18.

To confirm the O-acetyl substitution of the product, the condensation was carried out with 4-acetoxy-1-naphthaldehyde (VII, 1.8 g.), 2-thiohydantoin (0.97 g.), 20 ml. of acetic anhydride, and 0.6 g. of sodium acetate. The mixture was heated at 130° for 40 min. Water was added to decompose the excess acetic anhydride, giving a solid which was recrystallized from ethanol (charcoal): yield 0.46 g. of bright yellow crystals, m.p. 276°, alone or in mixture with the product obtained as described above.

Anal. Found: C, 61.89; H, 3.93; N, 8.74.

B. 5-(4-Hydroxy-1-naphthylidene)-2-thiohydantoin Hydrate (VI).—4-Hydroxy-1-naphthaldehyde (10.3 g.) and 2-thiohydan-

toin (7.0 g.) in 11 ml. of pyridine and 5 ml. of diethylamine were heated under reflux for 8 hr. After standing overnight, the soft mass was extracted with hot, dilute acetic acid (1:1) then with ethanol. On cooling, the extracts gave fine orange-red crystals, m.p. 215 and 210°, in a sealed, evacuated tube. The solids were combined (8 g.) and recrystallized twice from ethanol-water (charcoal) to give orange crystals, m.p. 215°. If the melting point was taken very slowly, it was about 300°; λ_{max}^{KBr} 2.95 (sh), 3.05 (s), 5.75 (s), 5.88 (s), 6.10 (s) μ.

Anal. Calcd. for $C_{13}H_{10}N_2SO_2 \cdot H_2O$: C, 58.33; H, 4.17; N, 9.72. Found: C, 58.71; H, 4.01; N, 9.90.

The VI can also be obtained from V by deacetylation in 10% NaOH.

Desulfurization of V.—A suspension of V (0.75 g.) in 10 ml. of water and 3 g. of acetic acid was refluxed for 2 hr. Chloroacetic acid (2 g.) in 6 ml. of water was added, and the reflux was continued for 6 hr. Again, 3 g. of chloroacetic acid in 6 ml. of water was added, and the suspension was refluxed for 5 hr. more. Ethanol (10–15 ml.) was added until the solid dissolved, and the solution was filtered while hot. After cooling, an orange-yellow, fluffy solid was collected and suspended in boiling water. Ethanol was added to obtain a clear solution, and insoluble material was filtered. The filtrate was concentrated (boiling) to its original water volume. When cool, golden yellow short needles of VIII were collected: yield 0.43 g. (60%), m.p. 290–295° dec.

Anal. Calcd. for $C_{16}H_{12}N_2O_4$: C, 64.86; H, 4.08; N, 9.46. Found: C, 65.01; H, 4.26; N, 9.79.

Reduction of IIa. A. With HI and Glacial Acetic Acid.—The hydantoin IIa (0.46 g.), 5 ml. of glacial acetic acid, and 5 ml. of hydriodic acid (*d* 1.7) were refluxed for 5 hr. The dark solution was concentrated *in vacuo* to dryness. Water was added. The solution was again evaporated to dryness, and the residue was dissolved in ethanol and filtered. The filtrate was concentrated to half its original volume and left for 2 days to give a dark red solid, which was recrystallized from methanol–water (charcoal): yield 55 mg. of **5-(4-acetoxy-1-naphthyl)methylenehydantoin (IX)**, light brown, fine crystals, m.p. 252–255°.

Anal. Calcd. for $C_{16}H_{14}N_2O_4$: C, 64.43; H, 4.70; N, 9.40. Found: C, 64.35; H, 4.60; N, 9.35.

B. With Sodium Amalgam and Aqueous NaOH.—One gram of IIa in 100 ml. of 5% aqueous NaOH was added to 100 g. of 2.5% Na–Hg. When the vigorous reaction had subsided, the solution was refluxed with stirring for 8–10 hr. cooled to room temperature, acidified with HCl, and evaporated to dryness. The residue was extracted with methanol (Soxhlet). The methanol was removed, and the solid was extracted with ether and then dissolved in a minimum of water. After standing at 4° overnight, 0.3 g. of *impure* **β-(4-hydroxy-1-naphthyl)-DL-alanine (XII)** was collected as a solid, m.p. 300°. It gave a positive coupling test with tetrazotized di-*o*-anisidine and a positive ninhydrin test. A small amount of **β-(4-hydroxy-1-naphthyl)dehydroalanine (X)** was also formed. This acid also gave a positive ninhydrin test and was confirmed later by its R_f value on paper chromatography.

Reduction of V.—The thiohydantoin V (5 g.), dissolved in 50 ml. of 10% NaOH and 60 ml. of water, was added to 200 g. of Na–Hg (2.5%). The mixture was stirred under nitrogen at room temperature for 50 hr. and filtered. The solution changed from a light yellow color to blue. It was then acidified to pH 2.0 with 6 *N* HCl. A pink precipitate formed while the solution changed color again from blue to violet and red. The pink crystals were collected and recrystallized from ethanol-water to give 1.0 g. of fine pink crystals (XL), m.p. 173–175° dec.

Anal. Calcd. for $C_{14}H_{12}N_2O_2S$: C, 62.10; H, 4.41; N, 10.32. Found C, 62.47; H, 4.37; N, 9.93.

It was found to couple with tetrazotized diorthoanisidine in Na_2CO_3 solution.

β-(4-Hydroxy-1-naphthyl)alanine (XII).—Product XI (1.0 g.) and 20 ml. of saturated $Ba(OH)_2$ were refluxed (bath temperature 120–130°) under nitrogen for 5 days. After the solution was cooled to room temperature, it was neutralized to pH 7.0 with 2 *N* H_2SO_4 with occasional cooling and stirring. The $BaSO_4$ precipitate was filtered, and the filtrate was freeze dried to give light brown fine crystals. This residue was resuspended in water, acidified to pH 5, boiled for a few minutes, and filtered while hot. This process was repeated twice, and the combined filtrates were extracted with ether. The aqueous solution was then decolorized with charcoal, and the colorless solution was again freeze dried to give 120 mg. of XII. This amino acid melts above 300°, gives a positive ninhydrin test, and couples with tetrazotized di-*o*-anisidine. This material still contained a trace of hydantoic acid which could be removed by gentle warming with Amberlite IRC-50. Ultimately, the purity of the amino acid was confirmed by paper chromatography; λ_{max}^{KBr} 2.9–3.0 (broad strong band), 5.90–6.10 (broad diffused band) μ (resembling tyrosine). The purified amino acid melted at 360° dec.

Anal. Calcd. for $C_{13}H_{13}NO_3$ (231.29): C, 67.50; H, 5.66; N, 6.05. Found: C, 67.58; H, 5.71; N, 6.10.

Paper Chromatography of XII and Related Compounds.—Ascending paper chromatography was carried out at 20–23° using 1-butanol–acetic acid–water (5:1.2:5 v./v.) as a solvent system, on Whatman No. 1 paper. The chromatogram was developed by spraying with 0.25% ninhydrin in acetone (w./v.) for amino acid, after warming over water vapor at 70°. The α-naphthol moiety was revealed by spraying with 0.1% aqueous tetrazotized di-*o*-anisidine (diazo blue B) and air drying; time 16–17 hr. The R_f values (±0.01) are listed in Table I.

TABLE I

R_f VALUES

Compound	By ninhydrin	By α-naphthol
4-Hydroxy-1-naphthaldehyde (I)		0.98
Glycine	0.24	Negative
Tyrosine	0.40	Negative
β-(4-Hydroxy-1-naphthyl)-DL-alanine (XII)	0.45	0.46
β-(4-Hydroxy-1-naphthyl)-dehydroalanine (X)	0.57, 0.64	0.57, 0.65

Hydrolysis of V.—A saturated solution of $Ba(OH)_2$ [prepared by boiling 50 ml. of distilled water with $Ba(OH)_2$ and filtering while hot] and V were refluxed under a slow stream of nitrogen for 5 days, after which no more ammonia was evolved. Upon cooling, the solution was acidified (H_2SO_4) at 0° to pH 6–6.5. The $BaSO_4$ was filtered and the filtrate was extracted three times with 50-ml. portions of ether. The aqueous solution was left at 4° overnight. If more precipitate formed and the solution remained basic, more 2 *N* H_2SO_4 was added until the pH was on the acid side, and the process was repeated. The filtrate was freeze dried to a brown powder. It was redissolved in hot water and decolorized with charcoal, and the aqueous solution was freeze dried again to dryness to yield light tan crystals: m.p. 300° dec.; λ_{max}^{KBr} 5.68 (weak shoulder), 5.80 (s), 6.10–6.32 (broad band) μ. It gave a positive ninhydrin test and coupled with tetrazotized di-*o*-anisidine.

Anal. Calcd. for $C_{13}H_{11}NO_3$: C, 68.60; H, 4.83; N, 6.11. Found: C, 68.68; H, 4.60; N, 6.40.

Paper chromatography of the dehydroamino acid showed two spots which have been assigned the tautomeric forms X and Xa. No further efforts have been made to separate these two forms.

Histochemical Demonstration of Tyrosinase Activity.[4]—Fresh-frozen sections (8–12 μ) of unfixed skin freshly excised from the rat, were incubated in 0.1 *M* phosphate buffer. After 2–3 hr. in the oven at 37°, the sections were examined quickly for reddish brown color and fixed in 10% formalin in alcohol for 20 min., cleared in xylene, and mounted in Permount[8]. The sections were examined under a fluorescence microscope; the dermal-epidermal junction showed brilliant blue fluorescence as evidence of activity. Attempts were also made to incubate the sections with 0.2 mg. of quinoxaline, and there was a qualitative increase of fluorescence and color change to greenish yellow.

Control sections were made with L-tyrosine[5] which showed only light brown and very weak blue fluorescence at the same sites. When 2 mg. of the dehydro amino acid X were added to both the L-tyrosine and XII solution, no brown or reddish brown sites could be seen under the microscope.[6]

In a preliminary way, this may suggest a competitive inhibition. Further study is in progress.

(5) T. B. Fitzpatrik, S. W. Becker, Jr., A. B. Lerner, and H. Montgomery, *Science*, **112**, 223 (1950).

(6) The present case may indicate a difference between phenol oxidase and tyrosinase, an amino acid phenol oxidase. For lack of proper evidence, we have retained the name "tyrosinase" in this paper.