The product was obtained in 87% yield, b.p. 97.5–98.5°/10 mm. (lit.,[13] b.p. 112–114°/18 mm.).

4-Phenylbutylamine was prepared by the reduction of 4-phenylbutyronitrile with lithium aluminum hydride-aluminum chloride according to the general procedure of Nystrom.[14] The product was obtained in 89% yield boiling at 114° at 12 mm. (lit.,[15] b.p. 123–124° at 17 mm.).

ω-Haloacid amides. N-(3,4-Dichlorophenyl)-3-chloropropionamide. β-Chloropropionyl chloride (13 g., 0.10 mole) in benzene (100 ml.) was added dropwise to a stirred benzene (150 ml.) solution of 3,4-dichloroaniline (32.5 g., 0.20 mole) at 20°. The precipitate of 3,4-dichloroaniline hydrochloride (19 g., 95% recovery) was filtered, and the filtrate was evaporated to dryness. Crystallization from ether-petroleum ether (b.p. 60–90°) gave the product melting at 108–110°, yield 22 g. (87%). Recrystallization from dilute ethanol solution raised the melting point to 110–111°.

The other compounds listed in Table II were prepared in the same manner.

Preparation of ω-aminoalkylcarboxamides. Method A. N,N'-Di(3,4-dichlorophenyl)-3-aminopropionamide. A mixture of *N*-(3,4-dichlorophenyl)-3-chloropropionamide (12.7 g., 0.05 mole) and 3,4-dichloroaniline (16.2 g., 0.10 mole) was stirred at 180° for 30 min. The reaction mixture was partitioned between 5% sodium carbonate solution (500 ml.) and ether (500 ml.). The ether solution was dried and evaporated, and the residue was steam-distilled until 3,4-dichloroaniline no longer appeared in the distillate. The nonvolatile residue was extracted with ether (300 ml.) and the ether extract was concentrated to a small volume and diluted with petroleum ether to give the product melting at 108–112°, yield 13.5 g. (71%). Crystallization from dilute ethanol raised the melting point to 114.5–115.5°. The compounds prepared by Methods A, B, C, D, and E are described in Table III.

Method B. N,N'-Di(3-phenylpropyl)aminoacetamide hydrochloride. Chloracetyl chloride (8.48 g., 0.075 mole) was added dropwise to a stirred mixture of 3-phenylpropylamine (20.3 g., 0.15 mole) and sodium carbonate (15.9 g., 0.15 mole) in toluene (75 ml.) at 10°. The mixture was then refluxed for 2 hr., while the water liberated in the reaction (1.4 ml.; theory, 1.4 ml.) was removed azeotropically in a Barrett trap. The suspension was filtered and the filtrate was shaken with 3*N* hydrochloric acid (200 ml.). The crude product (m.p. 128–145°) separated at the interface. Crystallization from ethanol-ether raised the melting point to 146.5–148°, yield 14.0 g. (53.8%).

Method C. N,N'-Di(2,4-dichlorobenzyl)aminoacetamide hydrochloride. A mixture of 2,4-dichlorobenzylamine (19 g., 0.108 mole) and glyoxal sodium bisulfite (14.4 g., 0.0504 mole) in 50% aqueous ethanol (160 ml.) was refluxed for 22 hr. The mixture was evaporated to dryness *in vacuo* and the residue was extracted with boiling ethanol (150 ml.). The ethanol extract was concentrated to about 50 ml., and ether (300 ml.) was added. On passing dry hydrogen chloride through the solution the crude product (m.p. 221–223°) precipitated, yield 19.5 g. (50%). Crystallization from ethanol raised the melting point to 224–225°.

Method D. N,N'-Di(2-phenylethyl)aminoacetamide hydrochloride. Ethyl chloroacetate (163 g., 1.33 mole) was added dropwise with stirring to 2-phenylethylamine (485 g., 4.0 moles) without external cooling. During the addition period of 45 min. the temperature rose to 125°. The reaction mixture was heated at 135–140° for 1 hr. and the ethanol from the reaction was removed by distillation. Water (2 l.) and 2*N* hydrochloric acid (712 ml., 1.42 moles) were added at 75°, and the solution was allowed to cool overnight. The crude product (m.p. 220–222°) was recovered by filtration, yield 357 g. (84%). Extraction with acetone (1 l.) removed the colored impurities, 325 g. (76.2%). Crystallization from 1-propanol (5 ml./g.) raised the melting point from 230–232° to 233–234°. (lit.,[16] m.p. 231°).

Method E. N,N'-Di(4-ethoxyphenyl)-3-aminopropionamide. *p*-Phenetidine (137 g., 1.0 mole) was heated with acrylic acid (36.0 g., 0.5 mole) at 180–190° for 5 hr. Crystallization of the dark-brown reaction mixture from dilute ethanol gave the product melting at 140–141°, yield 82 g. (47.8%). Recrystallization from dilute ethanol raised the melting point to 141–142°.

LaSalle, Quebec

(13) J. von Braun, G. Blessing, and F. Zobel, *Ber.*, 56, 1988 (1923).

(14) R. F. Nystrom, *J. Am. Chem. Soc.*, 77, 2544 (1955).

(15) J. von Braun, *Ber.*, 43, 2837 (1910).

(16) J. von Braun and W. Munch, *Ber.*, 60, 345 (1927).

[Contribution from the Department of Biological Sciences, Stanford Research Institute]

Potential Anticancer Agents.[1] XLVII. Alkylating Agents Related to Phenylalanine Mustard. IV.[2] Transformation Products of Ethyl *o*-Amino-α-benzamidocinnamate

ABELARDO P. MARTINEZ, W. A. SKINNER, WILLIAM W. LEE, LEON GOODMAN, AND B. R. BAKER

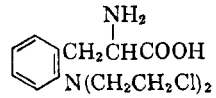
Received June 23, 1960

Ethyl *o*-amino-α-benzamidocinnamate (VI) readily rearranged to ethyl *o*-benzamidopyruvate (XIII) in aqueous acetic acid at room temperature, indicating that VI had a *cis*-relationship of the *o*-aminophenyl and α-benzamido groups. When VI was treated with hydrazine at room temperature, a hydrazide (VIII) was obtained with the reverse conformation, that is, the *o*-aminophenyl and benzamido groups were *trans*, as VIII was readily cyclized to 3-benzamidocarbostyril (XI).

The *p*-isomer of phenylalanine mustard has excellent anticancer properties in transplanted experimental tumors[2] and some utility in man. As the *m*-isomer of phenylalanine mustard appears to have a better chemotherapeutic index than the *p*-isomer against some tumors such as Sarcoma 180

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, Contract No. SA-43-ph-1892. The opinions expressed in this paper are those of the authors and are not necessarily those of the Cancer Chemotherapy National Service Center. For the preceding paper in this series, *cf.* W. A. Skinner, A. P. Martinez, and B. R. Baker, *J. Org. Chem.*, 26, 152 (1961).

and Melanoma S-91 in mice,[3] the synthesis and evaluation of the *o*-isomer of phenylalanine mustard (I) was considered to be logical. However, in view

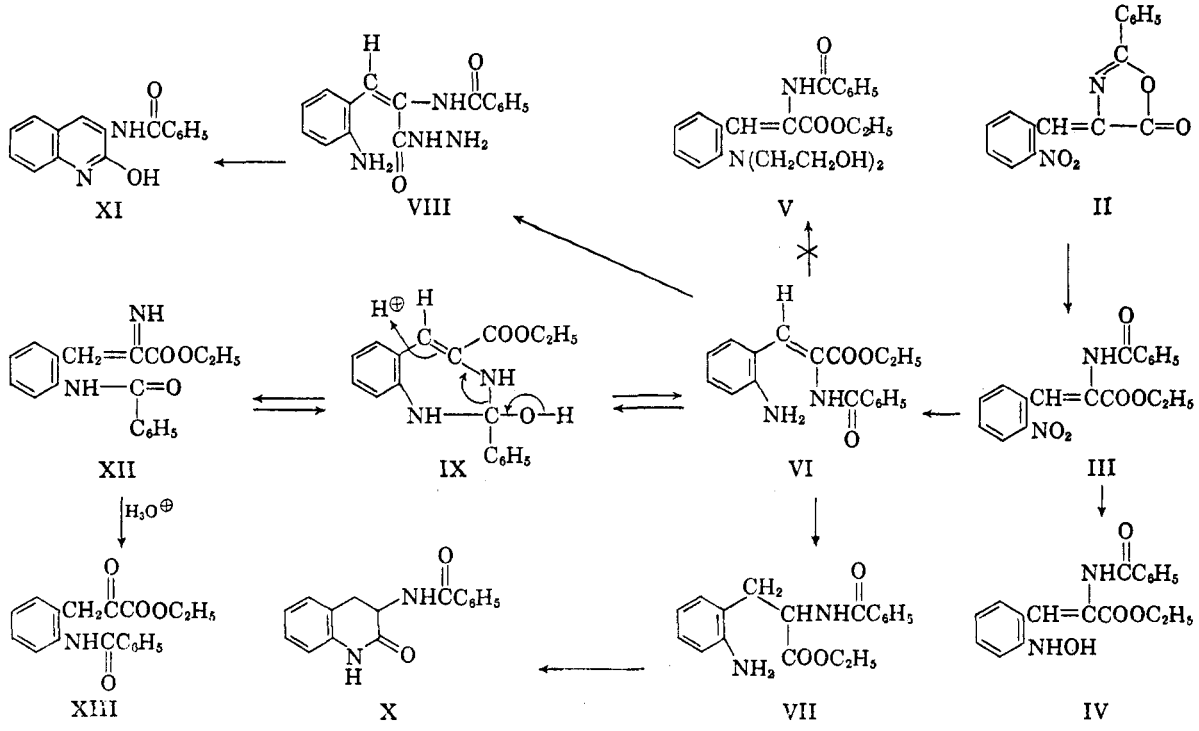


I

of the recent report of an elegant synthesis of *o*-phenylalanine mustard (I) by Connors and Ross,[4] we have discontinued endeavors towards synthesis of I and wish to present some unusual observations on the reactions of ethyl *o*-amino-α-benzamidocinnamate (VI), a projected intermediate to I.

Treatment of the azolactone (II), prepared in 85% yield from hippuric acid and *o*-nitrobenzaldehyde,[5] with ethanol containing sodium ethoxide afforded crystalline ethyl α-benzamido-*o*-nitrocinnamate (III) (R_f 0.64)[6] in 81% yield. Selective reduction of the nitro group of III proceeded smoothly in boiling aqueous ethanol with zinc and ammonium chloride to give a 70% yield of crystalline ethyl *o*-amino-α-benzamidocinnamate (VI) (R_f 0.76),[6] m.p. 113–115°, along with 5–10% of the highly insoluble carbostyril (XI). If this reduction of III with zinc was carried out at room temperature, the intermediate hydroxylamine (IV) was obtained as a crystalline solid, m.p. 145–146°, in 75% yield. Although IV had the same R_f at III on acetylated paper,[6] it was readily distinguished from III by its characteristic green fluorescence under ultraviolet light and by its positive reaction with Tollens reagent.

The unique reduction method of Balcon and Furst,[7] namely, hydrazine and Raney nickel in boiling ethanol, proceeded in a different course when applied to III; the high melting (280–281°) 3-benzamidocarbostyril (XI) was readily isolated in 26% yield. The mother liquors, as shown by paper chromatography,[6] contained the hydroxylamine (IV) and probably some of the amine VI. The ease of formation of this carbostyril (XI) appeared at the time to be explained on the basis that the intermediate aminocinnamate (VI) had a *cis*-relationship between the *o*-aminophenyl and carbethoxy groups, which could readily cyclize to the lactam (XI). However, subsequent work showed that these groups in VI were most probably *trans*.



Although methyl *p*-amino-α-benzamidocinnamate had been previously hydroxyethylated smoothly to methyl α-benzamido-*p*-[bis(2-hydroxyethyl)amino]cinnamate with ethylene oxide in aqueous acetic acid,[2] these conditions with the

(2) For the third paper on phenylalanine mustard, see A. P. Martinez, W. A. Skinner, W. W. Lee, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **83**, in press (1961), Paper XLV of this series. This paper contains the pertinent background references.

(3) M. O. Greene, B. R. Baker, and J. Greenberg, *Cancer Research*, **20**, 1160 (1960).

(4) T. A. Connors and W. C. J. Ross, *Chem. and Ind.*, 492 (1960).

(5) K. S. Norong and J. N. Ray, *J. Chem. Soc.*, 9761 (1931).

(6) Paper chromatograms were run by the descending technique on Schleicher and Schuell No. 2495 acetylated paper in benzene-methanol-water (2:6:1), unless otherwise indicated. Spots were detected by their ultraviolet absorption.

(7) D. Balcon and A. Furst, *J. Am. Chem. Soc.*, **75**, 4334 (1953).

o-aminocinnamate (VI) followed a completely different course. The crystalline product, isolated in 84% yield, proved to be ethyl *o*-benzamidophenylpyruvate (XIII), formed by migration of the α-benzoyl group to the *o*-amine. Merely stirring VI in aqueous acetic acid at room temperature gave the same rearrangement to XIII as could then be anticipated. The mildness of the conditions for conversion of VI to XIII strongly suggests that VI exists predominantly as the isomer with the α-benzamido and *o*-aminophenyl groups in a *cis*-relationship.[8]

The N→N migration of the N-benzoyl group in the conversion of VI to XIII may be analogous to the N→O and O→N acyl migrations which have been so thoroughly studied with acylated 2-aminoethanols[9-11] and for which cyclic intermediates have been proposed to explain the experimental facts. Thus, a cyclic intermediate, IX, could be proposed to explain the experimental facts. Thus, a cyclic intermediate, IX, could be proposed to explain the transamidation reaction (VI→XIII), but suffers from the objection that a seven-membered ring must be formed which would not favor the formation of XIII. As there was no evidence that VI was converted to XIII in *glacial acetic acid*, it can be suggested that an equilibrium between VI, IX and possibly XII exists, but that the equilibrium is far in favor of VI. However, the presence of water causes hydrolysis of the intermediate imine (XII) to the phenylpyruvate (XIII); as this latter step is irreversible under the conditions of the reaction, the reaction towards XIII would be driven to completion.

The apparent anomaly that the transamidation experiment indicated VI to have a *cis*-relationship between the *o*-aminophenyl and carbethoxy groups, while the hydrazine-Raney nickel reduction of III indicated a *trans*-relationship of these groups, was resolved by further study of the latter reaction.

That the *o*-aminophenylcinnamate (VI) was a probable intermediate in the conversion of III to the carbostyril (XI) with Raney nickel and hydrazine in boiling ethanol was shown by treatment of VI with hydrazine in boiling ethanol; a 50% yield of the carbostyril (XI) was obtained. However, when VI was stirred with hydrazine in ethanol at room temperature, a 70% yield of the hydrazide (VIII) was obtained, indicative that VIII was an intermediate in the direct conversion of VI to XI. When VIII was heated to 260°, cyclization to the carbostyril (XI) occurred. These results strongly indicate that the *o*-aminophenyl and α-benzamido groups of VIII have a *trans*-relationship, in contrast to VI where there is a *cis*-relationship of these groups. That this isomerization was not just a simple base catalyzed isomerization about the double bond was shown by the recovery of VI unchanged when boiled in ethanol containing diethylamine; a small amount of the carbostyril (XI), however, was again obtained.[8] The most logical explanation for these experimental results is that the *cis*-relationship of the *o*-aminophenyl and α-benzamido groups is the thermodynamically stable conformation in the ester (VI), whereas a *trans*-relationship of these groups is the more thermodynamically stable conformation in the hydrazide (VIII).

As part of the study of the effect of Raney nickel and hydrazine on III, the effect of this large ratio of Raney nickel alone in boiling alcohol was investigated. A fair yield of the dihydrocarbostyril (X) was isolated.[12] This reaction most probably proceeds by reduction of the double bond of the cinnamate ester (VI) to give the hydrocinnamate (VII) followed by cyclization to X. As none of X could be detected when the nitro compound was reduced with hydrazine and Raney nickel, it is improbable that the carbostyril (XI) is an intermediate to X; it appears that reduction of the double bond to VII must occur prior to formation of X.

EXPERIMENTAL[13]

Ethyl α-benzamido-o-nitrocinnamate (III). A solution of 24.0 g. (0.082 mole) of 4-(*o*-nitrobenzylidene)-2-phenyl-2-oxazolin-5-one[4] (II) in 250 ml. of absolute ethanol and 0.3 g. of sodium ethoxide was refluxed for 1 hr., then the red-colored solution was refrigerated overnight. The crystalline product was collected on a filter, washed with cold water, and dried *in vacuo*; yield, 22.6 g. (81%), m.p. 167–168°; $\lambda_{max(\mu)}^{Nujol}$ 3.10 (NH); 5.78 (ester C=O); 6.10 (amide C=O); 6.20, 6.31, 6.70 (aryl and C=C); 6.55 (NO_2 and amide NH); 7.40 (NO_2); 7.97 (ester C—O—C); 13.7 (*o*-disubstituted benzene); 14.4 (benzoyl). The compound traveled as a single spot (R_f 0.64) on paper.[8]

Anal. Calcd. for $C_{18}H_{16}N_2O_5$: C, 63.5; H, 4.74; N, 8.23. Found: C, 63.8; H, 4.83; N, 8.56.

Ethyl o-amino-α-benzamidocinnamate (VI). A mixture of 1.56 g. (4.6 mmoles) of ethyl α-benzamido-*o*-nitrocinnamate (III), 4.04 g. of zinc dust, and 0.70 g. of ammonium chloride in 60 ml. of 95% ethanol and 6 ml. of water was refluxed for 10 min. on a steam bath. The mixture was filtered hot and the filtrate reduced to a volume of about 25 ml. *in vacuo*. Water was added until turbidity occurred; chilling of the solution overnight at 5° gave 1.0 g. (70%) of crystalline precipitate, m.p. 113–115°; $\lambda_{max(\mu)}^{Nujol}$ 2.85, 2.99, 3.09 (NH); 5.77, 5.83 (ester C=O); 6.02 (amide C=O); 6.21, 6.31 (aryl, C=C); 6.57 (amide NH); 7.95 (ester C—O—C); 13.3 (*o*-disubstituted benzene); 14.0 (benzoyl); no NO_2

(8) Small amounts of the carbostyril (XI) were isolated when the ester (VI) was heated in absolute or aqueous ethanol. Since the amount of XI did not increase on extending the reaction time, these results indicate that a proportionately small amount of isomer with the α-benzamido and *o*-aminophenyl groups having the *trans*-relationship may have been present in VI.

(9) M. Bergmann, E. Brand, and F. Weinmann, *Z. Physiol. Chem.*, **131**, 1 (1923).

(10) G. E. McCasland, R. K. Clark, Jr., and H. E. Carter, *J. Am. Chem. Soc.*, **71**, 637 (1949).

(11) S. Winstein and R. Boschan, *J. Am. Chem. Soc.*, **72**, 4669 (1950).

(12) H. Ueda, *Ber.*, **61B**, 146 (1928) has prepared this compound by a different route.

(13) Melting points were taken on a Fisher-Johns block and are uncorrected.

near 7.4. The compound traveled as a single spot (R_f 0.76) on paper.[6]

Anal. Calcd. for $C_{18}H_{18}N_2O_3$: C, 69.7; H, 5.85; N, 9.03. Found: C, 69.8; H, 5.73; N, 9.11.

Another crystal modification, m.p. 121–122°, which had the same infrared spectrum as the 113–115° material, was isolated in other experiments.

Anal. Found: C, 69.7; H, 5.81, N, 9.07.

Ethyl α-benzamido-o-hydroxylaminocinnamate (IV). A mixture of 5.0 g. (15 mmoles) of ethyl α-benzamido-o-nitrocinnamate (III), 1.76 g. of ammonium, chloride and 10.2 g. of zinc dust in 140 ml. of ethanol and 10 ml. of water was stirred at room temperature for 30 min. The mixture was filtered through a Celite pad, then evaporated to dryness *in vacuo*. The white residue was stirred with water, filtered, and dried; yield, 4.7 g. Recrystallization from benzene gave 3.4 g. (75%) of analytically pure crystals, m.p. 145–146°; $\lambda_{max(\mu)}^{Nujol}$ 3.05, 3.10 (NH, NOH); 5.79, 5.87 (ester C=O); 6.05 (amide C=O); 6.23, 6.32, 6.70 (aryl C=C); 6.62 (amide NH); 8.05 (ester C—O—C); 13.4 (o-disubstituted benzene); 14.0 (benzoyl). The compound traveled as a single green fluorescent spot (R_f 0.76) on paper and gave a positive Tollens test.

Anal. Calcd. for $C_{18}H_{18}N_2O_4$: C, 66.2; H, 5.56; N, 8.58. Found: C, 66.4; H, 5.63; N, 8.36.

3-Benzamidocarbostyril (XI). *A. From the nitro ester* (III). A solution of 2.0 g. (5.9 mmoles) of ethyl α-benzamido-o-nitrocinnamate (III) and 1.0 g. of hydrazine hydrate (99%) in 20 ml. of absolute ethanol was warmed on a steam bath and a small amount of Raney nickel[14] added. The reaction mixture was warmed on the steam bath gently for 1 hr. while hydrogen was being evolved. The excess hydrazine was then destroyed by the addition of 1 g. of Raney nickel and the gases expelled by vigorous heating. The mixture was filtered hot through Celite, the filtrate concentrated to half its volume *in vacuo* and chilled in ice; yield, 0.40 g. (26%), m.p. 280–281°; $\lambda_{max(\mu)}^{Nujol}$ 2.99 (NH); 3.02 (enolic OH); 6.02 (amide C=O); 6.22, 6.32, 6.71 (aryl); 6.49 (amide NH); 11.1 (=CH); 13.2 (o-disubstituted benzene); 14.2 (benzoyl). The compound traveled as a single blue fluorescent spot (R_f 0.56) on paper.[6]

Anal. Calcd. for $C_{16}H_{12}N_2O_2$: C, 72.7; H, 4.58; N, 10.6. Found: C, 72.3; H, 4.79; N, 10.5.

B. From the amino ester (VI). A solution of 0.50 g. (1.47 mmoles) of the o-aminocinnamate (VI), 0.50 g. (10 mmoles) of 99% hydrazine hydrate and 10 ml. of ethanol was heated under reflux for 1.5 hr., then chilled at 0°. The white needles which formed were collected by filtration, washed, and dried, to yield 0.20 g. (51%) of hydroxyquinoline (XI), which was identical with the analytical sample in its paper chromatographic behavior and in its infrared spectrum.

Ethyl 3-(o-benzamidophenyl)pyruvate (XIII). A solution of 0.20 g. (0.64 mmole) of ethyl o-amino-α-benzamidocinnamate (VI) in 5 ml. of water and 1.3 ml. of glacial acetic acid was stirred at room temperature for 18 hr. After a few minutes of stirring, a solid began to separate from the solution and continued to do so during the time of the reaction. The solid was collected on a filter, washed with water, and dried; yield, 0.15 g. (78%), m.p. 112–114°. An analytical sample, m.p. 113–114°, was prepared by crystallization from methylene chloride–petroleum ether (b.p. 30–60°); $\lambda_{max(\mu)}^{Nujol}$ 2.99 (NH); 5.69 (α-ketoester C=O); 6.10 (amide C=O); 6.25, 6.70 (aryl); 6.48 (amide NH); 8.19, 8.34 (ester C—O—C); 13.1 (o-disubstituted benzene); 14.1 (benzoyl). The compound traveled as a single spot (R_f 0.74) on paper.[6]

Anal. Calcd. for $C_{18}H_{17}NO_4$: C, 69.4; H, 5.50; N, 4.50. Found: C, 69.5; H, 5.68; N, 4.64.

o-Amino-α-benzamidocinnamoylhydrazide (VIII). A solution of 0.50 g. (1.47 mmoles) of ethyl o-amino-α-benzamido cinnamate (VI), 0.50 g. (10 mmoles) of 99% hydrazine hydrate, and 20 ml. of 95% ethanol was stirred at room temperature for 18 hr. during which time a white solid precipitated. The crystalline solid was separated by filtration and washed several times with warm dichloromethane; yield, 0.30 g. (70%), m.p. 225° dec.; $\lambda_{max(\mu)}^{Nujol}$ 2.95, 3.01 and 3.12 (NH); 5.90 (hydrazide C=O); 6.05 (amide C=O); 6.55 (amide NH); 13.00 (o-disubstituted benzene); 14.01–14.12 (benzoyl). The compound did not give a satisfactory paper chromatogram in several solvent systems.

Anal. Calcd. for $C_{16}H_{16}N_4O_2$: C, 64.9; H, 5.44; N, 18.9. Found: C, 64.7; H, 5.37; N, 18.8.

When the hydrazide (VIII) was heated at 260° until gas evolution ceased, a white solid, m.p. 277°, was left as the residue. The infrared spectrum of this solid showed it to be the hydroxyquinoline (XI).

3-Benzamido-3,4-dihydrocarbostyril (X). A mixture of 0.50 g. (1.47 mmoles) of the ester (VI), 20 ml. of absolute ethanol, and 1.5 g. of Raney nickel was heated at reflux for 2.25 hr., then chilled to 0°, and filtered with the aid of Celite. The filtrate was evaporated to dryness *in vacuo* and the semisolid residue was dissolved in boiling dichloromethane. Addition of petroleum ether (b.p. 30–60°) to the point of turbidity and chilling gave 0.29 g. (57%) of a white, crystalline solid, m.p. 206–207°. The solid was recrystallized from dichloromethane–petroleum ether (b.p. 30–60°) to give the analytical sample, m.p. 207.5–208.5°, (Ueda[12] gave m.p. 205°); $\lambda_{max(\mu)}^{Nujol}$ 3.07, 3.15, 3.30 (NH); 5.89 (lactam C=O); 6.11 (benzamide C=O); 6.50 (amide NH); 13.36 (o-disubstituted benzene); 13.88 (benzoyl). The compound moved as a single spot on paper chromatography on Whatman No. 1 paper in 1-butanol-water with R_f 0.91 and in 1-butanol-methyl ethyl ketone–water (5:3:2) with R_f 0.91. The compound gave a purple absorbing spot under ultraviolet light, compared to XI which gave a blue fluorescent spot. This purple color, as well as the lactam C=O absorption at 5.89 μ, was compatible with the dihydrocarbostyril structure (X).[15]

Anal. Calcd. for $C_{16}H_{14}N_2O_2$: C, 72.2; H, 5.30; N, 10.5. Found: C, 72.2, 72.3; H, 5.57, 5.61; N, 10.3.

Acknowledgment. The authors wish to thank Dr. Peter Lim for the interpretations of infrared spectra, particularly for first suggesting the nature of XIII, and his staff for the spectrophotometry and the paper chromatograms. The authors are also indebted to Mr. O. P. Crews, Jr., and his staff for large-scale preparation of some intermediates.

MENLO PARK, CALIF.

(14) Sponge nickel catalyst, Davison Chemical Co., Cincinnati 29, Ohio.

(15) E. J. Reist, H. P. Hamlow, I. G. Junga, R. M. Silverstein, and B. R. Baker, paper XXXIV of this series, *J. Org. Chem.*, **25**, 1368 (1960).