from 1 mole of I and 2 moles of 2-methylmercaptobenzothiazole etho-*p*-toluenesulfonate as described for III. The orange dye was recrystallized from methanol (1200 ml. per gram of dye); yield 35%, m.p. 280–282° dec.

Anal. Calcd. for $C_{29}H_{28}IN_3S_3$: N, 6.55; S, 15.00. Found: N, 6.49; S, 14.92.

3-Ethyl-8-(3-ethyl-2(3)-benzothiazolylidene)-8H-indeno-[1,2-d]thiazolium Iodide (X).—The condensation of IX (1.0 g., 1 mol) with 2-methylmercaptobenzothiazole etho-*p*-toluenesulfonate (1.10 g., 1 mol), as described for III, yielded X as orange crystals; 200 ml. of methanol per gram of dye was used for recrystallization: m.p. 261–262°, yield 22%.

Anal. Calcd. for $C_{21}H_{19}IN_2S_2$: N, 5.71; S, 13.08. Found: N, 5.46; S, 12.92.

3-Ethyl-8-(1-ethyl-4(1)-quinolylidene)-8H-indeno[1,2-d]-thiazolium Iodide (XI).—Compound IX (1.5 g., 1 mol), 4-phenylmercaptoquinoline etho-*p*-toluenesulfonate (2.3 g., 1 mol), triethylamine (0.5 g.) and 15 ml. of absolute ethanol were refluxed for 20 minutes. The dye was recrystallized from methanol (50 ml. per gram of dye) as tiny black needles; yield 10%, m.p. 218–220°.

Anal. Calcd. for $C_{28}H_{21}IN_2S$: I, 26.21; N, 5.79. Found: I, 26.20; N, 5.59.

3-Ethyl-2-methyl-8-(1-methyl-4(1)-quinolylidene)-8H-indeno[1,2-d]thiazolium Iodide (XII).—Compound I (1.0 g., 1 mol) was condensed with 4-phenylmercaptoquinoline metho-*p*-toluenesulfonate (1.2 g., 1 mol) as above; the reflux time was 5 minutes. After recrystallization from methanol (150 ml. per gram of dye), the dye was obtained as black crystals, m.p. 235°, yield 50%.

Anal. Calcd. for $C_{23}H_{21}IN_2S$: I, 26.21; N, 5.79. Found: I, 26.15; N, 5.53.

3,3′-Diethyl-8′-(1-methyl-4(1)-quinolylidene)-8H-indeno-[1,2-d]-thiazolothiacyanine Iodide (XIII).—Dye XII (0.26 g., 1 mol) was condensed with 2-methylmercaptobenzothiazole etho-*p*-toluenesulfonate (0.19 g., 1 mol) as described for III; the mixture was refluxed for 10 minutes. The dye was washed with water followed by cold ethanol and recrystallized from methanol (200 ml. per gram of dye) as felted black needles, m.p. 240–243°, yield 25%.

Anal. Calcd. for $C_{32}H_{28}IN_3S_2$: I, 19.66; N, 6.51. Found: I, 19.42; N, 6.55.

MIDDLETOWN, CONN.

[CONTRIBUTION FROM THE WM. H. NICHOLS CHEMICAL LABORATORY, NEW YORK UNIVERSITY]

Synthesis of Pyrido[4,3-b]quinoline (2,10-Diazaanthracene) and Related Compounds

BY ANTHONY T. COSCIA AND S. CARLTON DICKERMAN

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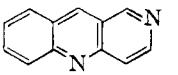
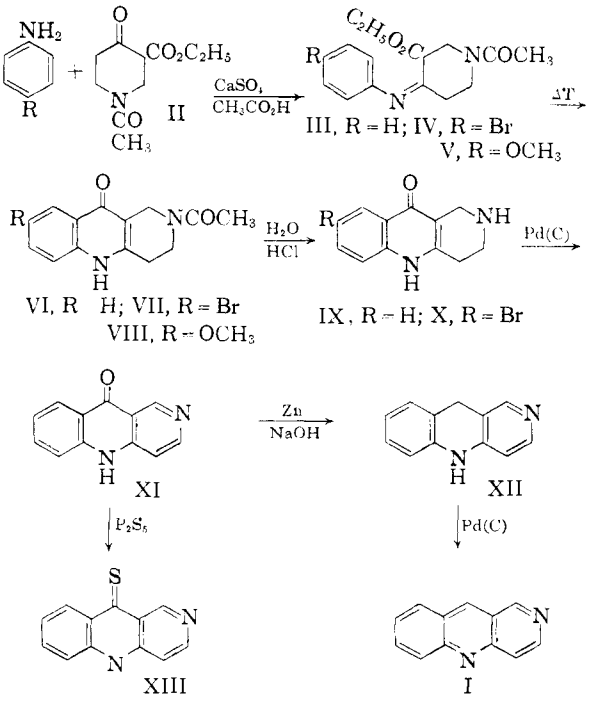
Pyrido[4,3-b]quinoline, previously unknown, has been synthesized from 1-acetyl-3-ethoxycarbonyl-4-piperidone and aniline. Various methods of removing oxygen from a pyridoquinolone have been investigated.

Although several derivatives of pyrido [4,3-b] quinoline (I) have been prepared[1–3], the parent heterocyclic compound has remained inaccessible. The failures of classical syntheses involving cyclization of acids derived from N-pyridylanilines[3–5] prompted us to investigate an approach which obviated such steps.[6]



I

1-Acetyl-3-ethoxycarbonyl-4-piperidone (II) was treated with aniline, *p*-bromoaniline and *p*-anisidine to yield the corresponding anils III, IV and V. The anil prepared from aniline was isolated as an intractable oil and the bromo and methoxy analogs were used to investigate experimental conditions for subsequent steps. The three anils were converted by heating to the corresponding tetrahydropyridoquinolones VI, VII and VIII. The possibility that the impure anil prepared from aniline contained an appreciable amount of anilide was excluded by quantitative estimation of the ethanol liberated during the cyclization reaction.[7] These precautions proved to be unnecessary since both the solid anil from *p*-bromoaniline and that prepared from aniline were converted eventually to pyrido[4,3-b]quinoline (I).



(1) St. von Niementowski and E. Sucharda, *J. prakt. Chem.*, **94**, 193 (1916); *Ber.*, **52B**, 484 (1919).

(2) B. Bobranski and E. Sucharda, *Rocz. Chem.*, **7**, 192 (1927); *C. A.*, **24**, 1381 (1930).

(3) G. B. Bachman and R. S. Barker, *J. Org. Chem.*, **14**, 97 (1949).

(4) W. O. Kermack and A. P. Weatherhead, *J. Chem. Soc.*, 726 (1942).

(5) V. A. Petrow, *ibid.*, 927 (1945).

(6) B. M. Ferrier and N. Campbell [*Chemistry & Industry*, 1089 (1958)] have recently reported the synthesis of pyrido[4,3-b]quinolin-10(5H)-one (XI) from N-4-pyridylanthranilic acid.

(7) We have been unable to prepare an anilide from the keto ester II and aniline although B. K. Blount, Wm. H. Perkin, Jr., and S. G. P. Plant [*J. Chem. Soc.*, 1975 (1929)] reported the syntheses of both anil and anilide from aniline and 2-ethoxycarbonylcyclohexanone. The anilide would be expected to cyclize to the non-linear isomer in a strongly acidic reaction medium.

The acetylated amines VI and VII were hydrolyzed to the bases IX and X which were dehydrogenated with palladium-on-charcoal. Both IX and X gave a bromine-free pyridoquinolone XI. Evidently, dehydrogenation of the bromo compound was accompanied by inverse substitution. Reduction of the pyridoquinolone XI with a mixture of zinc and sodium hydroxide produced 5,10-dihydro[4,3-b]quinoline (XII).[8]

The desired heterocyclic compound was prepared from XII by catalytic dehydrogenation. Pyrido-[4,3-b]quinoline (I) is a colorless solid of m.p. 138° having an ultraviolet spectrum similar to that of acridine.

Other synthetic routes from the quinolone XI to I were attempted. A classical method failed when neither phosphorus oxychloride nor pentachloride converted XI to the corresponding chloro compound. Bachman and Barker[3] reported identical results with a dimethyl derivative of XI. Phosphorus pentasulfide did convert XI to a thione XIII but in very poor yield. Desulfurization of the thione was accomplished with Raney nickel but was accompanied by extensive reduction of the ring system. Thioacridone behaved similarly.

Experimental[9]

1-Acetyl-3-ethoxycarbonyl-4-phenyliminopiperidine (III).—To a solution of 10.0 g. (0.047 mole) of 1-acetyl-3-ethoxycarbonyl-4-piperidone (II)[10] and 4.40 g. (0.047 mole) of aniline in 40 ml. of absolute ethanol were added 16.0 g. of Drierite and two to three drops of glacial acetic acid. This mixture was refluxed vigorously for 6 hr., filtered, and concentrated under reduced pressure. The sirupy residue failed to crystallize and was partially purified before subsequent use by solution in 30 ml. of phenyl ether and by distillation of 10 ml. of this solution at 100° and 1 mm. pressure; λ_{max} 308 mμ (log ε 4.21).

1-Acetyl-3-ethoxycarbonyl-4-(*p*-bromophenylimino)-piperidine (IV) was prepared in 63% yield from II and *p*-bromoaniline as described above. After recrystallization from 60% ethanol–water, IV was isolated as colorless needles of m.p. 149–150°, λ_{max} 315 mμ (log ε 4.37).

Anal. Calcd. for $C_{16}H_{19}BrNO_3$: Br, 21.8; N, 7.6. Found: Br, 21.6; N, 7.5.

1-Acetyl-3-ethoxycarbonyl-4-(*p*-methoxyphenylimino)-piperidine (V).—*p*-Anisidine and II gave V in 55% yield; m.p., after recrystallization from aqueous ethanol, 126–127°, λ_{max} 304 mμ (log ε 4.28).

Anal. Calcd. for $C_{17}H_{22}N_2O_4$: C, 64.2; H, 6.9; N, 8.8. Found: C, 64.6; H, 6.8; N, 8.9.

2-Acetyl-1,2,3,4-tetrahydropyrido[4,3-b]quinolin-10(5H)-one (VI).—A solution of the anil III in phenyl ether was heated at 245° under nitrogen for 40 min. On cooling, the solution deposited a yellow solid which was filtered and washed with hot benzene; yield 6.17 g. or 55% based on II. After two recrystallizations from dioxane VI was isolated as colorless microcrystals of m.p. 279–280°, λ_{max} 238 mμ (log ε 4.58).

Anal. Calcd. for $C_{14}H_{14}N_2O_2$: C, 69.4; H, 5.8. Found: C, 69.4; H, 6.0.

2-Acetyl-8-bromo-1,2,3,4-tetrahydropyrido[4,3-b]quinolin-10(5H)-one VII.—The anil IV was heated in phenyl ether at 275° under nitrogen; yield 83%. After recrystallization from methanol VII melted at 326–328°.

Anal. Calcd. for $C_{14}H_{13}BrN_2O_2$: N, 8.7. Found: N, 8.5.

2-Acetyl-8-methoxy-1,2,3,4-tetrahydropyrido[4,3-b]quinolin-10(5H)-one (VIII) was prepared in 82% yield from the anil V in the manner described above. After precipitation from dilute sodium hydroxide solution VIII melted at 320–321°.

Anal. Calcd. for $C_{15}H_{16}N_2O_3$: N, 10.3. Found: N, 10.1.

Ethanol Determinations.—The ethanol liberated during cyclization of two anils, III and IV, was qualitatively detected by passing the carrier gas, nitrogen, through a pyridine solution of 3,4-dinitrobenzoyl chloride. Ethyl 3,5-dinitrobenzoate of m.p. 89° was isolated.

In separate experiments the ethanol was collected in water and this solution was analyzed by the method of Shupe and Dubowski.[11] The amounts of ethanol detected corresponded to from 85 to 100% of pyridoquinolone isolated.

1,2,3,4-Tetrahydro[4,3-b]quinolin-10(5H)-one (IX).—A solution of 1.00 g. of VI in 15 ml. of 20% hydrochloric acid was refluxed for 7 hr. Upon cooling the solution deposited 0.84 g. (86%) of IX hydrochloride. The free base was isolated by dissolving the hydrochloride (3.6 g.) in a minimum amount of 10% sodium hydroxide solution, saturating this solution with solid sodium carbonate, evaporating the mixture to dryness, and extracting the residue with hot absolute ethanol. Evaporation of the ethanol gave 2.5 g. (83%) of colorless powder. After recrystallization from water IX melted at 255°.

Anal. Calcd. for $C_{12}H_{12}N_2O$: C, 72.0; H, 6.0. Found: C, 71.5; H, 5.9.

8-Bromo-1,2,3,4-tetrahydro[4,3-b]quinolin-10(5H)-one (X).—A solution of 1.00 g. of VII in 50 ml. of 20% hydrochloric acid was refluxed for 12 hr. and the free base (0.84 g., 95%) was prepared as described above. After recrystallization from 10% aqueous ethanol X was isolated as colorless needles of m.p. 300–303° with dec.

Anal. Calcd. for $C_{12}H_{11}BrN_2O$: N, 10.0. Found: N, 9.9.

Pyrido[4,3-b]quinolin-10(5H)-one (XI).—A mixture of 3.00 g. of IX, 1.0 g. of 5% palladium-on-charcoal and 25 ml. of phenyl ether was refluxed at a bath temperature of 235–245° under slightly diminished pressure for three hours. After cooling, the yellow crystals which precipitated were collected with the catalyst and this mixture was washed with ether. Crude XI (2.1 g., 70%) was isolated by extraction with 10% sodium hydroxide solution. After two recrystallizations from 50% ethanol or 50% methanol XI melted at 338–339° dec., reported[6] m.p. 339° dec., λ_{max} 249 mμ (log ε 4.61).

Anal. Calcd. for $C_{12}H_8N_2O$: C, 73.5; H, 4.1; N, 14.3. Found: C, 73.4; H, 4.41; N, 14.1.

Similar treatment of the bromo compound X gave a bromine-free product of m.p. 333–335° dec. The ultraviolet spectrum of this material was identical with that of authentic XI.

5,10-Dihydro[4,3-b]quinoline (XII).—A mixture of 1.0 g. of activated zinc dust, 0.50 g. of XI in 25 ml. of 10% sodium hydroxide solution, 4 ml. of ethanol and 25 ml. of toluene was heated at 125° for 3.5 hr. After allowing the mixture to cool, the aqueous phase was separated and extracted with toluene. The combined toluene extracts were washed with 10% sodium hydroxide solution, dried and the toluene removed under reduced pressure. The residue weighed 0.16 g. (34%) and melted at 205–209°. After two recrystallizations from 60% ethanol, XII melted at 219°; λ_{max} 303 mμ (log ε 4.12), λ_{max} of 9,10-dihydroacridine[12] 290 mμ (log ε 4.2).

Anal. Calcd. for $C_{12}H_{10}N_2$: C, 79.1; H, 5.5; N, 15.5. Found: C, 78.6; H, 5.3; N, 15.7.

Pyrido[4,3-b]quinoline (I).—A mixture of 0.070 g. of XII, 0.055 g. of palladium-on-charcoal and 15 ml. of toluene was refluxed vigorously for 9 hr. After filtering, the toluene was removed under reduced pressure and the residue was fractionally recrystallized from water to yield 0.040 g. (57%) of

(8) F. Ullmann and R. Maag [*Ber.*, **40**, 2521 (1907)] reported that 9,10-dihydroacridine is produced from acridone by reduction with sodium and ethanol.

(9) Melting points are uncorrected. Microanalysis by W. Manser, Zürich, and by Schwarzkopf Micro-analytical Laboratory, Woodside 77, N. Y. Ultraviolet spectra were measured in 95% ethanol solution with a Beckman model DU spectrophotometer.

(10) S. C. Dickerman and H. G. Lindwall, *J. Org. Chem.*, **14**, 534 (1949).

(11) L. M. Shupe and K. M. Dubowski, *Am. J. Clin. Pathol.*, **22**, 901 (1952). We are indebted to Dr. J. V. Fiore for bringing this excellent method to our attention.

(12) E. R. Blout and R. S. Corley, THIS JOURNAL, **69**, 765 (1947).

I and 0.012 g. of recovered XII. An additional recrystallization from water afforded an analytical sample of I as off-white needles of m.p. 138–139°; λ_{max} 239 mμ (log ε 4.60), λ_{max} of acridine[12] 250 mμ (log ε 5.02).

Anal. Calcd. for $C_{12}H_8N_2$: C, 80.0; H, 4.4; N, 15.5. Found: C, 80.1; H, 4.6; N, 15.5.

Pyrido[4,3-b]quinolin-10(5H)thione (XIII).—A solution of 0.250 g. (1.25 millimoles) of XI and 0.32 g. (1.44 millimoles) of phosphorus pentasulfide in 6 ml. of anhydrous pyridine was refluxed for 5 hr. The pyridine was removed under reduced pressure and the residue was extracted with hot dilute sodium hydroxide solution. The addition of acid to this extract precipitated crude XIII as an amorphous solid which contained phosphorus and which resisted purification. After extraction with carbon disulfide, followed by dilute sodium bicarbonate solution, the residue was partially soluble in ethanol. Evaporation of this ethanol extract gave a solid which was recrystallized from methanol; XIII was isolated in the form of red needles, 0.010 g. (4%), m.p. 298–301°.

Anal. Calcd. for $C_{12}H_8N_2S$: N, 13.2; S, 15.1. Found: N, 13.0; S, 14.9.

Attempted Synthesis of I from Pyrido[4,3-b]quinolin-10(5 H)-thione (XIII).—An ethanol solution of crude thione XIII and Raney nickel[13] was heated to reflux. The reaction was followed by withdrawing aliquots and measuring the optical density at 239 mμ. The spectral changes indicated rapid and extensive reduction of the ring system. Less active Raney nickel failed to remove the sulfur.

Model experiments with thioacridone indicated that a maximum of only 25% of acridine was formed after 5 min. and that after 1 hr., when desulfurization was complete, only 2–5% of acridine was present in the mixture.

Attempted Synthesis of 10-Chloro[4,3-b]quinoline.—A mixture of 0.050 g. of XI and 5 ml. of phosphorus oxychloride was refluxed for 3 hr. and then poured on crushed ice. Only unreacted XI (0.040 g.) could be isolated. A mixture of phosphorus pentachloride and oxychloride was also used but without success.

(13) R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers, THIS JOURNAL, **65**, 1013 (1943).

NEW YORK 53, N. Y.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE UNIVERSITY OF TEXAS M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE]

The Synthesis of *m*-[Di-(2-chloroethyl)-amino]-DL-phenylalanine

BY T. S. OSDENE, DARRELL N. WARD, WILLIAM H. CHAPMAN AND HENRY RAKOFF

RECEIVED NOVEMBER 26, 1958

The synthesis of *m*-[di-(2-chloroethyl)-amino]-DL-phenylalanine in five steps from *m*-nitrobenzyl bromide and acylamino malonic esters is reported. The compound was prepared as a potential tumor inhibitor.

The interest in aromatic nitrogen mustard derivatives as tumor inhibiting substances has led to the development of various new types. One of the most interesting is *p*-[di-(2-chloroethyl)-amino]-phenylalanine (I), which was synthesized independently by Bergel and his collaborators[1] who made the DL-, D- and L-forms, and also by Larionov, *et al.*,[2] who prepared the DL-form only. This compound has been reported to have pronounced carcinostatic activity against certain tumors.[3] Luck and his co-workers[4] have studied I and a number of related homologs. Most interesting is their finding that at least a temporary regression of the Cloudman S-91 melanoma in mice is obtained on treatment with I, since the melanomas are generally resistant to nitrogen mustards. The foregoing findings have led to the use of this drug in clinical trials at various centers as an anti-cancer drug.

Phenylalanine can be hydroxylated *in vivo* in the *para* position to give the amino acid tyrosine,[5] a possibility which has been eliminated in I by the presence of the nitrogen mustard group in that position. Since this hydroxylation is of importance in a number of metabolic functions of phenylalanine, it was reasoned that a derivative of I should be made in which hydroxylation in the para position could occur. Therefore, the synthesis of *m*-[di-(2-chloroethyl)-amino]-DL-phenylalanine (II) was undertaken.

(1) F. Bergel and J. A. Stock, *J. Chem. Soc.*, 2409 (1954); F. Bergel, V. C. Burnop and J. A. Stock, *ibid.*, 1223 (1955).

(2) L. F. Larionov, A. S. Khokhlov, E. N. Shkodinskaja, O. S. Vasina, V. I. Trooshetkina and M. A. Novikova, *Lancet, Lond.*, **2**, 169 (1955).

(3) See, for example: P. C. Koller and U. Veronesi, *Brit. J. Cancer*, **10**, 703 (1956); J. F. Holland and W. Regelson, *Ann. N. Y. Acad. Sci.*, **68**, 1122 (1958); R. Papac, D. A. G. Galton, M. Till and E. Wiltshaw, *ibid.*, **68**, 1126 (1958); N. Blokhin, L. Larionov, N. Perevodchikova, L. Chebotareva and N. Merkulova, *ibid.*, **68**, 1128 (1958).

(4) J. M. Luck, *Science*, **123**, 984 (1956); J. M. Luck, *Cancer Research*, **17**, 1071 (1957); H. E. Smith and J. M. Luck, **23**, 837 (1958).

(5) A. B. Lerner, *Adv. Enzymology*, **14**, 73 (1953).

Results and Discussion

The synthetic approach to II closely paralleled that employed by Bergel, *et al.*,[1] and the Russian workers[2] for the synthesis of I. Two syntheses were achieved, the only difference between them being in the utilization of different protecting groups on the α-amino nitrogen introduced in the first stage of the synthesis. In series "a" the acetyl group was used while in series "b" the benzoyl group was used. Ethyl acetamidomalonate or ethyl benzamidomalonate was condensed with *m*-nitrobenzyl bromide to give ethyl acetamido-(*m*-nitrobenzyl)-malonate (IIIa) or ethyl benzamido-(*m*-nitrobenzyl)-malonate (IIIb) in 75% yields. Compounds IIIa and IIIb were then reduced by catalytic hydrogenation to the corresponding amino derivatives, ethyl acetamido-(*m*-aminobenzyl)-malonate (IVa) or ethyl benzamido-(*m*-aminobenzyl)-malonate (IVb) in almost quantitative yields. The aromatic amino group was then hydroxyethylated with ethylene oxide in 50% acetic acid solution. The ethyl acetamido-[*m*-di-(2-hydroxyethyl)-aminobenzyl]-malonate (Va) and the ethyl benzamido-[*m*-di-(2-hydroxyethyl)-aminobenzyl]-malonate (Vb) were obtained initially as gums. A seed crystal of Va was obtained from Dr. B. R. Baker[6] which made it possible to isolate the crystal-

(6) During the course of this work we learned from Dr. Howard W. Bond of the Cancer Chemotherapy National Service Center that Dr.