androsta-1,4-diene-3,17-dione by means of *Bacillus sphericus*.[4]

EXPERIMENTAL[9]

2α-Hydroxytestosterone (I) (100 mg.) was incubated with a two-day culture of *Bacillus sphericus* (A.T.C.C. No. 7055), in 250 ml. of nutrient broth in a Fernback Flask at 30°. The nutrient broth was prepared as follows: yeast extract 3 g., N-Z-Case (peptone) 5 g., and water 1000 ml. The steroid dissolved in 2 ml. ethanol was added aseptically and the incubation mixture rotated on a shaker for 48 hr. After incubation the broth was extracted with 3 × 100 ml. of redistilled ethyl acetate. The extracts were washed 2 × 50 ml. with $NaHCO_3$ and twice with distilled water. The ethyl acetate extracts were dried over anhydrous Na_2SO_4 and evaporated at 60° under vacuum. The residue was chromatographed on a silica gel adsorption column and the eluates from a 14:1 and 9:1 mixture of benzene–ethyl acetate were pooled. The dried residue was applied to No. 1 Whatman paper and run for 48 hr. in the ligroin propyleneglycol system of Savard.[10] A compound with a running rate of androsta-1,4-diene-3,17-dione was detected with the Zimmermann, Turnbull's blue (ferric chloride and potassium ferricyanide) and blue tetrazolium reagents. After eluting this zone, the dried material was crystallized twice from ether-hexane, giving 13 mg. of III, m.p. 148–150°, $[\alpha]_D^{22}$ +67° ($CHCl_3$). The ultraviolet spectrum in methanol showed λ_{max}^{MeOH} 253 mμ with a small shoulder at 284 mμ. The infrared spectra indicated maxima at 3425, 1740, 1675, 1620, and 1218 cm.$^{-1}$ The identity of III was established by comparison of its physical constants, including infrared spectra, with an authentic sample of 2-hydroxyandrosta-1,4-diene-3,17-dione[11] and its acetate. The acetate of III was prepared with acetic anhydride in pyridine and crystallized from ethyl acetate to give IV, m.p. 225–228°, λ_{max}^{MeOH} 246 mμ, ν_{max} 1768, 1730, 1670, 1645, 1610, and 1208 cm.$^{-1}$ In the 3:1 benzene–ethyl acetate eluate from the silica gel column, a minute amount of a compound more polar than III, was found. This product (II?) gave a negative test with potassium ferricyanide and ferric chloride, absorbed ultraviolet light at 238 mμ, and by infrared analysis was found to contain a pentacyclic ketone, a hexacyclic α,β-unsaturated ketone, and an absorption band indicating free hydroxy group.

The possibility of III being an artifact (since ketols can be oxidized with very mild oxidative agents) has been eliminated by incubating I with a denatured culture of *Bacillus sphericus*. After extraction and purification no enol could be detected with Turnbull's reagent and the recovery of the starting material was nearly quantitative.

Oxidation of 2-hydroxy-Δ⁴-3-keto steroids to their Δ¹-analogs could be brought about either by removal of the two hydrogens at carbon 1 and 2, or by oxidation of the alcoholic function at carbon 2 to the ketone, which would then enolize.[12] Axial hydroxyl functions are oxidized with greater ease, but little is known about similar oxidations in biological systems.[13]

Recently Kushinsky[14] suggested a 1- or 2-hydroxy intermediary in the 1,2-dehydrogenation. Since it is known that dehydration of hydroxy compounds proceeds most readily between an axial hydroxy function and an adjacent axial hydrogen, it is quite likely that a 1α-hydroxy derivative would be the intermediary in this reaction. The finding that the 2α-OH group of I, located in the equatorial position, does not interfere with the 1,2-dehydrogenation, is consistent with this mechanism. Steroids hydroxylated at position C_1 are being used to test this hypothesis although evidence by Levy and Talalay[15] suggests that the 1,2-dehydrogenation involves direct removal of hydrogen by an hydrogen acceptor.

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SHREWSBURY, MASS.

(4) A number of microorganisms[5–8] are able to produce dehydrogenation of steroids at the 1,2 position.

(5) E. Vischer, C. Meystre, and A. Wettstein, *Helv. Chim. Acta*, **38**, 835 (1955).

(6) E. Vischer, C. Meystre, and A. Wettstein, *Helv. Chim. Acta*, **38**, 1502 (1955).

(7) A. Nobile, N. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, N. E. Tully, M. A. Jernik, and E. B. Hershberg, *J. Am. Chem. Soc.*, **77**, 4184 (1955).

(8) T. H. Stoudt, W. J. McAleer, J. M. Chemerda, M. A. Kozlowski, R. F. Hirschmann, V. Marlott, and R. Miller, *Arch. Biochem.*, **59**, 304 (1955).

(9) The melting points were taken on a Fisher-Johns block and are uncorrected; the infrared spectra were recorded on a Perkin-Elmer Model 12C, all samples dispersed in potassium bromide.

(10) K. Savard, *Recent Progress in Hormone Research*, **9**, 185 (1954).

(11) We wish to express our thanks to Dr. J. S. Baran for sending us a sample of 2-hydroxyandrosta-1,4-diene-3,17-dione.

(12) Compare E. T. Stiller and O. Rosenheim, *J. Chem. Soc.*, 353 (1938); Ch. Meystre, H. Frey, W. Voser, and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956).

(13) Compare the oxidation of quinic acid to 5-dehydroquinic acid by mutants of *Escheria coli* and others: D. B. Davis, *J. Bact.*, **64**, 729 (1952).

(14) S. Kushinsky, *J. Biol. Chem.*, **230**, 31 (1958).

(15) H. R. Levy and P. Talalay, *J. Am. Chem. Soc.*, **79**, 2658 (1957).

Reactions of δ-Valerolactone with *ortho*- and *peri*-Naphthylenediamines

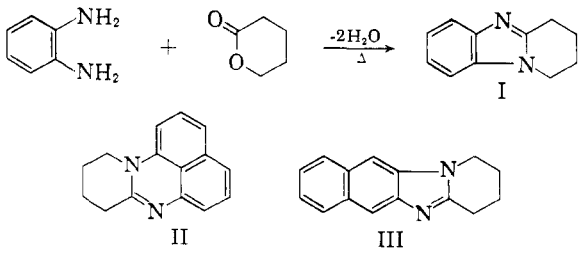
WILLIAM L. MOSBY

Received September 10, 1958

Although butyrolactone[1] and γ-valerolactone[2] have been condensed with *o*-phenylenediamine[1,2] and with 1,2-naphthylenediamine,[2] the use of δ-valerolactone in such reactions has not been reported. Insomuch as 1,2-, 2,3-, and 1,8-naphthylenediamines, as well as δ-valerolactone, are all available commercially, these reactions appeared of interest.

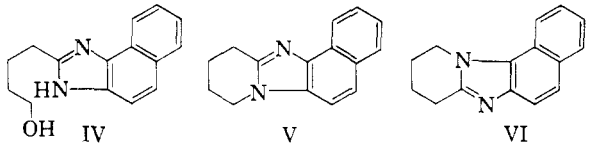
To evaluate the reaction, the known[3,4] 1,2,3,4-tetrahydropyrido[*a*]benzimidazole (I) was prepared. Although the yield was only 15%, the nature of the product was unquestionable, and the authenticity of the reaction was demonstrated. Further, despite the rather low yield, this is easily the most convenient preparation of I yet reported, and the yield could possibly be improved by minor modifications.

(1) A. Bistrzycki and W. Schmutz, *Ann.*, **415**, 1 (1917).
(2) W. Reppe *et al.*, *Ann.*, **596**, 209 (1955).
(3) R. Huisgen and R. Rist, *Ann.*, **594**, 159 (1955).
(4) K. H. Saunders, *J. Chem. Soc.*, 3275 (1955).

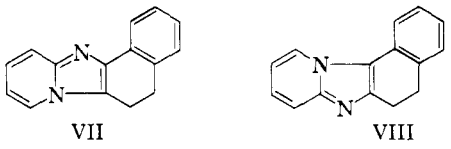


The reaction was then extended to 1,8- and 2,3-naphthylene diamines, which produced II and III in about 70 and 80% yields, respectively. The structures of these new products were supported by microanalyses and by ultraviolet and infrared spectra. Efforts to aromatize III by heating with palladium-charcoal or rhodium-charcoal at 195–200° in trichlorobenzene or in ethylene glycol, or by treatment with sulfur, chloranil, or lead tetraacetate, were unsuccessful. This is in accord with the difficulty reported[4] in the aromatization of I. Oxidation of III in acetic acid with chromic acid readily gave the 6,11-quinone, again without dehydrogenating the pyridine ring.

When 1,2-naphthylenediamine was condensed with δ-valerolactone, two products were isolated, each in rather low yield. The less soluble of the two is probably IV, a structure supported by the microanalyses and by infrared and ultraviolet spectra. The second product was shown by analysis to have the formula $C_{15}H_{14}N_2$, and could be either V or VI. Compound V has been prepared previously,[5,6] and comparison of the infrared spectrum of an authentic sample[6] with that of the $C_{15}H_{14}N_2$ product demonstrated their identity. No evidence was found for the formation of VI under the conditions studied, and efforts to convert IV into either V or VI by the action of heat alone, left it unaffected.



The structure of a homolog of compound V also was examined. Reitmann[7] condensed 2-aminopyridine with 2-bromo-1-tetralone, and assigned the product structure VII, although the possibility of structure VIII was not disproven. We have repeated the preparation of Reitmann's product, and oxidized it with chromic acid to the known[6] 5,6-dihydrobenzo[*e*]pyrido[*a*]benzimidazole-5,6-dione. The accuracy of structure VII is, therefore, confirmed.



EXPERIMENTAL[8]

1,2,3,4-Tetrahydropyrido[a]benzimidazole (I). A mixture of 5.00 g. of polymeric δ-valerolactone and 5.40 g. of *o*-phenylenediamine was heated for 16 hr. in an oil bath at 230°. The cooled melt was boiled several times with water (discarding the aqueous extracts) then extracted several times with (a total of 200 ml. of) hot benzene. The benzene solution was passed through a column of alumina and stripped of solvent *in vacuo*. The resulting pale yellow solid (2.40 g.), upon crystallization from cyclohexane, afforded 1.30 g. (15.1% yield) of white prisms, m.p. 100–102.5° (lit.[3,4] 100–101°; 101–102°); λ_{max} 248.5–251,* 254, 276.5, and 283 mμ. (ε 5770, 5970, 5560, and 6425.)

8,9,10,11-Pyrido[a]perimidine (II). A mixture of 5.00 g. of polymeric δ-valerolactone, 7.90 g. of 1,8-diaminonaphthalene, and 5 ml. of chlorobenzene was heated for 3 hr. at 230–250°, allowing the solvent and water to distill out. The residual oil was dissolved in hot acetic acid and drowned into 300 ml. of water. Ammonium hydroxide was added until a slight permanent precipitate was produced, and the mixture was clarified with charcoal and filtered. Basification of the filtrate with ammonium hydroxide yielded an oil which crystallized when triturated with benzene and ligroin (yield: 7.85 g., 70.6%, m.p. 133–136.4°). Two recrystallizations from methyl cyclohexane gave yellow prisms, m.p. 147.8–149.5°, λ_{max} 235 and 330 mμ. (ε 34,500 and 16,630.)

Anal. Calcd. for $C_{15}H_{14}N_2$: C, 81.08; H, 6.31; N, 12.61. Found: C, 80.85; H, 6.45; N, 12.84.

The *picrate* formed yellow felted needles from methanol, m.p. 234.3–235.0° dec.

Anal. Calcd. for $C_{21}H_{17}N_5O_7$: C, 55.87; H, 3.77; N, 15.52; O, 24.83. Found: C, 56.14; H, 3.45; N, 15.30; O, 24.65.

1,2,3,4-Tetrahydronaphtho[2,3-d]pyrido[a]imidazole (III). The product was obtained crude (m.p. 180–184°) in 80.5% yield from 2,3-naphthalenediamine by the same technique used to prepare II. Crystallization from benzene or nitromethane gave yellow needles, m.p. 191–193°, λ_{max} 241, 259,* 310,* 320, 323, and 338.5 mμ. (ε 37,700, 3035, 6990, 9030, 9100, and 6910.)

Anal. Calcd. for $C_{15}H_{14}N_2$: C, 81.08; H, 6.31; N, 12.61. Found: C, 80.88; H, 6.20; N, 12.81.

The *picrate* was prepared in methanol and crystallized from methyl Cellosolve; m.p. 271.5–272.0°.

Anal. Calcd. for $C_{21}H_{17}N_5O_7$: C, 55.87; H, 3.77; N, 15.52; O, 24.83. Found: C, 55.53; H, 3.67; N, 15.79; O, 25.08.

Oxidation of III with chromic acid in acetic acid gave the *6,11-quinone* in good yield. Crystallization from acetonitrile gave fine yellow needles, m.p. 250–251°; λ_{max} 225,* 248, 275.5,* 282, and 332 mμ. (ε = 9310, 41,750, 14,750, 15,300, and 3400.)

Anal. Calcd. for $C_{15}H_{12}N_2O_2$: C, 71.43; H, 4.76; N, 11.10. Found: C, 71.25; H, 4.71; N, 11.23.

2-(4-Hydroxybutyl)-naphth[1,2]imidazole (IV). The reaction of 1,2-diaminonaphthalene with δ-valerolactone was run on 0.05 molar scale and worked up in the same manner used for the preparation of II and III. The oily product was

(5) G. Morgan and J. Stewart, *J. Chem. Soc.*, 1057 (1939).

(6) W. L. Mosby and R. J. Boyle, *J. Org. Chem.*, in press.

(7) J. Reitmann, U. S. Patent **2,057,978**, Oct. 20, 1936.

(8) All melting points were taken in Pyrex capillaries in a Hershberg melting-point apparatus using Anschütz thermometers. The ultraviolet spectra were all measured in ethanol solution using a Cary recording spectrophotometer, and points of inflection are indicated by asterisks. The infrared spectra were determined upon a Perkin-Elmer Model 321 recording spectrophotometer. The polymeric δ-valerolactone was obtained from the Union Carbide Chemicals Co. and the naphthylenediamines from the Aldrich Chemical Co.

extracted with hot benzene, leaving 2.05 g. (16.7% yield) of insoluble white solid, m.p. 170.8–173.6°. Recrystallization from acetonitrile improved the melting point only slightly (m.p. 172–174°); λ_{max} 222–227,* 243, 248.5, 274–277,* 283, 315, 321–325, and 328 mμ. (ϵ = 32,300, 48,600, 52,600, 4,600, 4,790, 4,175, 3,640, and 5,150.) The infrared spectrum in acetonitrile solution shows absorption at 2.85μ and 3.05μ attributed, respectively, to the OH and NH functions.

Anal. Calcd. for $C_{15}H_{16}N_2O$: C, 75.00; H, 6.66; N, 11.66; O, 6.66. Found: C, 74.46; H, 6.51; N, 11.94; O, 6.99.

The *picrate* was prepared in methanol and crystallized from methyl Cellosolve, m.p. 233.5–235.2°.

Anal. Calcd. for $C_{21}H_{19}N_5O_8$: C, 53.73; H, 4.05; N, 14.91; O, 27.31. Found: C, 53.93; H, 4.18; N, 15.15; O, 27.21.

8,9,10,11-Tetrahydrobenzo[e]pyrido[a]benzimidazole (V). Evaporation of the benzene extracts of the crude product IV and crystallization of the solid from benzene gave 0.98 g. (8.8% yield of white clusters, m.p. 160.2–162.2°. Recrystallization from benzene gave pure V, m.p. 161.5–162.5°.

5,6-Dihydrobenzo[e]pyrido[a]benzimidazole (VII), was prepared by Reitmann's method.[7] Crystallization from methylcyclohexane gave short, white needles, m.p. 159.4–160.6° (lit.[7] 157°); λ_{max} 253, 283–288, 298, 327–333, 341, and 356 mμ. (ϵ = 34,200, 5210, 4560, 10,980, 12,800, and 8290.)

Solution in hydrochloric acid gave the hydrochloride, which was purified by crystallization from methanol–ethyl acetate or from butanol. The fluffy white crystals had an *instantaneous* melting point of 307–308°.

Anal. Calcd. for $C_{15}H_{12}N_2 \cdot HCl$: C, 70.15; H, 5.06; N, 13.85; O, 10.91. Found: C, 69.96; H, 5.08; N, 13.97; O, 10.54.

The *picrate* was prepared in methanol and crystallized from methyl Cellosolve, m.p. 249–250°.

Anal. Calcd. for $C_{21}H_{15}N_5O_7$: C, 56.12; H, 3.34; N, 15.59; O, 24.94. Found: C, 56.05; H, 3.03; N, 15.31; O, 25.04.

Acknowledgments. The author wishes to thank Dr. Jessie Gove for the infrared spectra, Mr. F. C. Dexter for the ultraviolet spectra, Mr. O. E. Sundberg and his associates for the microanalyses, and Mrs. Regina Moynihan for technical assistance.

RESEARCH DIVISION
AMERICAN CYANAMID CO.
BOUND BROOK, N. J.

Some 9,10-Disubstituted Phenanthrenes

WILLIAM L. MOSBY

Received October 13, 1958

For the synthesis of some new vat dye systems, a quantity of 9-bromo-10-nitrophenanthrene was required. The best procedure for the nitration of 9-bromophenanthrene to produce the 9-bromo-10-nitro compound, was described by Callow and Gulland.[1] Although the yield of pure material is only about 15%, this compound is potentially interesting as the starting point for a number of syntheses.

In an effort to prepare 10,10′-dinitro-9,9′-biphenanthryl, treatment of 9-bromo-10-nitrophenanthrene with copper powder in refluxing triethylbenzene gave no reaction, whereas the use of nitrobenzene as a solvent produced a black tar. The bromine atom also appeared surprisingly inert to nucleophilic displacement by several reagents. The bromonitro compound was recovered unchanged from treatment with boiling methanolic sodium methoxide or *p*-toluenesulfonamide in methyl Cellosolve, although boiling with piperidine readily gave 9-nitro-10-piperidinophenanthrene.

Several attempts were made to replace the halogen by cyanide before the proper conditions were found. Refluxing the bromonitro compound in pyridine with cuprous and potassium cyanides and a little acetonitrile afforded a fairly good yield of 10-nitro-9-phenanthrenecarbonitrile. However, the use of neither potassium cyanide in ethanol, nor cuprous cyanide in pyridine or in benzyl cyanide gave the desired reaction. When the nitrobromo compound was treated with cuprous and potassium cyanides in dimethyl sulfoxide, the sole product isolated (in 7% yield) was 9,10-phenanthrenedicarbonitrile. Presumably hydroxyphenanthrenes constituted the major products in this experiment.

Attempts to hydrolyze the nitronitrile by the action of 85% phosphoric or 96% sulfuric acids in boiling acetic acid, or by the action of hot polyphosphoric acid, left it unaffected. Under alkaline conditions, hydrolysis of the nitro group occurred, and 10-hydroxy-9-phenanthrenecarbonitrile was formed. Catalytic reduction of the nitronitrile produced 10-amino-9-phenanthrenecarbonitrile.

Reduction[2,3] of 9-bromo-10-nitrophenanthrene with stannous chloride, zinc and acid or ammonium sulfide, was known to be accompanied by debromination to yield 9-aminophenanthrene. This is, in fact, the major evidence for the constitution of the nitrobromo compound. Reduction by means of hydrazine and a palladium catalyst[4,5] also was accompanied by debromination. The lability of the halogen is not a specific characteristic of the 9,10-bromonitro compound, however, since an isomeric bromonitrophenanthrene (*v.i.*) and even 9-bromophenanthrene itself suffer hydrogenolysis of the bromine when treated with hydrogen in the presence of palladium. By employing a "neutral iron reduction" it was possible to isolate 10-bromo-9-phenanthrylamine in 35% yield.

Nitration of 9-acetamidophenanthrene[6] gave, in very low yield, an acetamidonitrophenanthrene, almost certainly the 9,10-isomer.

During one run of the nitration of 9-bromophenanthrene a product isomeric with the 9,10-bromo-

(1) R. K. Callow and J. M. Gulland, *J. Chem. Soc.*, 2424 (1929).

(2) J. Schmidt and G. Ladner, *Ber.*, **37**, 3573 (1904).

(3) P. C. Austin, *J. Chem. Soc.*, 1760 (1908).

(4) P. M. G. Bavin, *Can. J. Chem.*, **36**, 238 (1958).

(5) M. J. S. Dewar and T. Mole, *J. Chem. Soc.*, 2556 (1956).

(6) G. H. Keyes and L. G. S. Brooker, *J. Am. Chem. Soc.*, **59**, 74 (1937).