EXPERIMENTAL[9]

Lithiumphenyl solutions (1*M*) were prepared and standardized according to the method described by Jones and Gilman.[10]

Sodiumphenyl. In a flame-dried flask under a nitrogen atmosphere was placed 0.5 mole of sodium as a 45% dispersion in toluene[11] or a 50% dispersion in *n*-heptane.[11] The dispersion was immediately covered with dry benzene, and about 5 ml. of a solution of 28.7 g. (0.255 mole) of chlorobenzene in 25 ml. of benzene was added. As soon as formation of the sodiumphenyl commenced, as evidenced by a rise in temperature and the formation of black granular particles in the grey mixture, the stirrer was started and the flask immersed in a Dry Ice–methanol bath. The remainder of the chlorobenzene solution was then added during 30 min. to 1 hr., while maintaining the temperature of the reaction between 25–40° by proper adjustment of the cooling bath. After conclusion of the addition, the reaction mixture was stirred an additional 30 min. at room temperature to insure completion of the reaction. At the end of this time, the reaction mixture consisted of a black suspension of sodiumphenyl granules. This material was used immediately for further reaction (see below) without storage.

Reaction of alkyl halides with metallo diethylamides. (*Table* I). (*A*) *With lithium diethylamide.* To 200 ml. (0.2 mole) of a *M* lithiumphenyl solution was added dropwise with stirring over 20 min. 16.1 g. (0.22 mole) of diethylamine in 25 ml. of dry ether. After stirring for an additional 10–20 min., a solution of an equivalent of the alkyl halide in an equal volume of ether was added during 0.5 hr., and the reaction completed by refluxing for the time indicated. The reaction mixture was cooled and decomposed by the addition of 50 ml. of water, and the ether layer, to which was added an ether extract of the aqueous layer, was extracted with several portions of 5*N* hydrochloric acid solution, washed with water and dried over $CaCl_2$. The acidic extracts were combined, made strongly basic with 5*N* sodium hydroxide solution, salted out with sodium chloride, and extracted with several portions of ether. The ether layer containing the basic fraction was then dried over potassium hydroxide. The solvents were removed from both fractions and the residues distilled to yield recovered halide and amine. Material from the neutral fraction boiling in the range 80–103° (760 mm.) was tested with potassium permanganate solution to determine the presence of olefin. Only traces of material giving a positive test for unsaturation were obtained.

(*B*) *With sodium diethylamide.* To 0.25 mole of sodiumphenyl, prepared as described above, was added during 30 min., 20.5 g. (0.28 mole) of diethylamine in 25 ml. of benzene and the black, sirupy mixture stirred an additional 30–60 min. to insure formation of the sodium diethylamide. To the stirred liquid was added during 30 min. an equivalent of the alkali halide in an equal volume of benzene, and the reaction then completed as indicated in Table I. The reaction mixture was cooled, and 20 ml. of ethanol added cautiously to decompose any unreacted sodium followed by water. The reaction mixture was then worked up as described above for lithium diethylamide. Since the olefins formed in these reactions boil close to that of the toluene or heptane used as dispersant for the sodium, the neutral material was carefully fractionated and the various fractions tested with potassium permanganate solution to determine the presence of olefin. If olefin was indicated present in the fraction, the amount present was estimated by the methods indicated in Table I.

The following new amines were prepared by these methods. From *n*-octyl bromide there was obtained *N,N*-diethyl-*n*-octylamine, b.p. 221–224° (760 mm.), 93–95° (10 mm.).

Anal. Calcd. for $C_{12}H_{27}N$: C, 77.75; H, 14.69. Found: C, 77.70; H, 14.67.

From 2-ethylhexyl bromide there was obtained *N,N*-diethyl-2-ethylhexylamine, b.p. 205–208° (760 mm.).

Anal. Calcd. for $C_{12}H_{27}N$: C, 77.75; H, 14.69; N, 7.56. Found: C, 77.75; H, 14.34; N, 7.56.

Reaction of n-octyl bromide with metallo phenyls. (*A*) *With lithiumphenyl.* To 250 ml. (0.25 mole) of a *M* solution of lithiumphenyl in ether was added dropwise during 2.5 hours, 48.3 g. (0.25 mole) of *n*-octyl bromide in 50 ml. of ether. The reaction mixture was stirred for 30 min. at room temperature, then stirred and refluxed for 1.5 hr., cooled and decomposed with water. The ether layer was washed with dilute hydrochloric acid, saturated sodium bicarbonate solution and water, and, after combining with an ether extract of the aqueous layers, dried over calcium chloride. The solvent was removed and the residue distilled *in vacuo* to give 5.7 g. (12%) of recovered *n*-octyl bromide, b.p. 77–81° (10 mm.), 32.8 g. (69%) of *n*-octyl benzene, b.p. 125–127° (10 mm.) (reported[12] b.p. 264–265° (760 mm.), and 2.8 g. of gummy residue. A small low-boiling forerun gave a negative test with potassium permanganate solution.

(*B*) *With sodiumphenyl.* To 0.25 mole of sodiumphenyl was added during 45 min., 48.3 g. (0.25 mole) of *n*-octyl bromide in 50 ml. of benzene. The reaction mixture was stirred at room temperature for 1 hr., then stirred and refluxed for 1 hr. After cooling and decomposing with methanol followed by water, the benzene layer was worked up as described above for lithium phenyl. There was obtained 15.3 g. (32%) of recovered *n*-octyl bromide, b.p. 79–82° (10 mm.), 16.3 g. (35%) of *n*-octyl benzene, b.p. 129–134° (12 mm.), and 14.9 g. of high boiling residue. A small low-boiling forerun gave only a weak positive test with potassium permanganate solution.

Department of Chemistry
Duke University
Durham, N. C.

(9) Analyses by Clark Microanalytical Laboratories, Urbana, Ill. Melting points and boiling points are uncorrected. Distillations described herein were performed on a 30 cm. Vigreux column.

(10) R. G. Jones and H. Gilman, *Org. Reactions*, Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 353.

(11) Generous samples of these materials were obtained from the National Distillers Corporation through the courtesy of Dr. V. L. Hansley.

(12) F. Eisenlohr and L. Schulz, *Ber.*, **57**, 1815 (1924).

Microbiological Synthesis of 2-Hydroxyandrosta-1,4-diene-3,17-dione[1]

C. Gual, S. R. Stitch,[2] M. Gut, and R. I. Dorfman

Received August 25, 1958

The recent publication on the synthesis of 2-hydroxy-$\Delta^{1,4}$-3-keto steroids by Baran[3] prompts us to report, as part of a study on possible precursors to the aromatic ring A, the microbiological conversion of 2α-hydroxytestosterone to 2-hydroxy-

(1) Supported in part by Research Grants No. C-2207 and CRTY-5001, Division of Research Grants, National Institutes of Health, U. S. Public Health Service.

(2) Present address: Radiobiological Research Unit, A.E.R.E., Harwell, Didcot, Berkshire, England.

(3) J. S. Baran, *J. Am. Chem. Soc.*, **80**, 1687 (1958).

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.

THE WORCESTER FOUNDATION FOR
EXPERIMENTAL BIOLOGY
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).