3.6 g. (0.02 mole) of β-naphthylamine hydrochloride in 50 ml. of 75% ethanol. After standing at room temperature for 48 hours, 10 ml. of water was added and the solution cooled. A yield of 2.65 g. (90%) of crude orange crystals was obtained which after recrystallization from ether–ethanol melted at 157°. The product was identified as 2-amino-α,β-azo-naphthalene (IV).

Anal. Calcd. for $C_{20}H_{15}N_3$: N, 14.13. Found: N, 14.10.

The filtrate was evaporated under vacuum in an attempt to recover methylurea. However, no residue was obtained.

Reaction of Nitrosoguanidine with β-Naphthylamine Hydrochloride.—To 1 g. (0.011 mole) of nitrosoguanidine[10] was added a solution of 4.0 g. (0.022 mole) of β-naphthylamine hydrochloride in 80 ml. of 50% ethanol. After standing at room temperature for 72 hours, the solution was diluted with 40 ml. of water and the orange-red precipitate filtered. A yield of 3 g. (90%) of 2-amino-α,β-azo-naphthalene was obtained.

Anal. Calcd. for $C_{20}H_{15}N_3$: N, 14.13. Found: N, 14.01.

To the filtrate was added 125 ml. of a 1% solution of ammonium picrate. The resulting precipitate was filtered off and weighed 1.5 g. (47%) and melted at 315° (dec.). This was identified as guanidine picrate by analysis.

Anal. Calcd. for $C_7H_8N_6O_7$: N, 29.11. Found: N, 28.93.

(10) E. Lieber and G. B. L. Smith, THIS JOURNAL, **57**, 2479 (1935).

DEPARTMENT OF CHEMISTRY
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Preparation of 6,7-d_2-Estrone Acetate

BY W. H. PEARLMAN AND M. R. J. PEARLMAN

Estrogens stably labeled with deuterium should prove very useful in metabolism experiments. A route for the preparation of such compounds is indicated in this report starting with Δ^6-dehydroestrone, a substance first prepared by Pearlman and Wintersteiner[1] from equilin and now obtainable in about 40% yield by aromatization of $\Delta^{1,4,6}$-androstatrienedione-3,17 by a procedure recently described by Rosenkranz, *et al.*[2] The conditions for the catalytic reduction of the equilin isomer to estrone were somewhat modified in the present study and deuterium gas was employed; the yield of 6,7-d_2-estrone acetate from Δ^6-dehydroestrone acetate was practically quantitative and the content of stably bound deuterium almost theoretical. Inasmuch as the partial synthesis of estradiol[3] and recently of estriol from estrone has been achieved, the preparation of these estrogens with deuterium in ring B seems feasible.

Experimental[4]

Ninety-eight milligrams of the acetyl derivative, m.p. 139–140°, of Δ^6-dehydroestrone, m.p. 260–262° (kindly furnished by Syntex, S. A., Mexico City, D. F., through the courtesy of Dr. G. Rosenkranz) was dissolved in 35 ml. of cyclohexane and shaken in deuterium at atmospheric pressure at 25° in the presence of 98 mg. of 5% palladium-on-charcoal catalyst (previously treated with deuterium); the uptake of gas ceased in about 20 minutes. The deuterated product was recovered and crystallized from alcohol to give 91 mg., m.p. 125–126°, which did not depress the melting point on admixture with estrone acetate, m.p. 125–126°. This product was refluxed for 1.5 hours with 5% potassium hydroxide in 90% methanol and then allowed to remain at room temperature for 48 hours. The estrogenic material was recovered, treated with acetic anhydride in pyridine for 24 hours and the acetate chromatographed over 2 g. of aluminum oxide (Harshaw Chemical Co.) and eluted with petroleum ether:ethyl ether (1:1) to yield 63.5 mg. of colorless material. It yielded, on crystallization from alcohol, a product, m.p. 125–126°, $[\alpha]^{29}D$ +152° ± 6° (abs. ethanol), ϵ 796, $\lambda_{max.}^{alc.}$ 270 mμ; ϵ 438, $\lambda_{min.}^{alc.}$ 250 mμ; *Anal.* Calcd. for $C_{20}H_{24}O_3$: C, 77.02; H, 7.70. Found: C, 76.55; H, 7.58. Isotope analysis[5]: found 8.23 atom % excess deuterium (theoretical value, 8.33, based on the introduction of 2 atoms of deuterium). This product did not depress the melting point on admixture with estrone acetate, m.p. 125–126°, $[\alpha]^{29}D$ +155 ± 6° (abs. ethanol), ϵ 798, $\lambda_{max.}^{alc.}$ 270 mμ; ϵ 441, $\lambda_{min.}^{alc.}$ 250 mμ.

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(1) Pearlman and Wintersteiner, *J. Biol. Chem.*, **132**, 605 (1940); Pearlman and Wintersteiner, *Nature*, **165**, 815 (1950).

(2) Rosenkranz, Djerassi, Kaufman, Pataki and Romo, *ibid.*, **165**, 815 (1950).

(3) Estradiol may likewise be obtained by aromatization of $\Delta^{1,4,6}$-androstatrienol-17-one-3,17 acetate.[2]

(4) All melting points are corrected. The carbon and hydrogen analyses were performed by Mr. James Rigas.

(5) The "falling-drop method" was employed as described by Keston, Rittenberg and Schoenheimer, *J. Biol. Chem.*, **122**, 227 (1937–1938), and modified by M. Cohn in "Preparation and Measurement of Isotopic Tracers," J. W. Edwards, Ann Arbor, Mich., 1947.

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The Use of Silver Nitrate and Sodium Dichromate in the Detection of Purines by Paper Partition Chromatography[1]

BY ROSE M. REGUERA AND ISAAC ASIMOV

The technique of paper partition chromatography has been frequently applied in the last few years to the determination of the nature and quantity of the purine and pyrimidine bases present in nucleic acids.[2–10] Vischer and Chargaff[3] have introduced a "sulfide-spot" technique to render the separated bases visible prior to quantitative photometric determination. This technique involves the precipitation of the bases as mercury salts, with subsequent conversion to mercuric sulfide.

(1) The work described in this paper was done with the aid of a grant from the United States Public Health Service.

(2) Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).

(3) Vischer and Chargaff, *ibid.*, **176**, 703 (1948).

(4) Vischer and Chargaff, *ibid.*, **176**, 715 (1948).

(5) Chargaff, Vischer, Doniger, Green and Misani, *ibid.*, **177**, 405 (1948).

(6) Vischer, Zamenhof and Chargaff, *ibid.*, **177**, 429 (1948).

(7) Chargaff, Magasanik, Doniger and Vischer, THIS JOURNAL, **71**, 1513 (1949).

(8) Holiday and Johnson, *Nature*, **163**, 216 (1949).

(9) Markham and Smith, *Biochem. J.*, **45**, 294 (1949).

(10) Chargaff, Zamenhof and Green, *Nature*, **165**, 756 (1950).