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).

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RECEIVED JUNE 26, 1950

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, $\lambda^{alc.}_{max.}$ 270 mμ; ε 438, $\lambda^{alc.}_{min.}$ 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, $\lambda^{alc.}_{max.}$ 270 mμ; ε 441, $\lambda^{alc.}_{min.}$ 250 mμ.

Acknowledgment.—This investigation was supported by a grant-in-aid from the United States Public Health Service, under the National Cancer Institute Act.

(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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RECEIVED JUNE 30, 1950

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).

In connection with investigations in these laboratories into the nucleic acids of normal and cancerous tissues, it has been found desirable to modify the technique to avoid the following disadvantages of the sulfide-spot method: (1) The method will not detect less than 5 micrograms of any given base. (2) It will not distinguish between one base and another, so that a careful consideration of RF values is necessary. (3) Spot-visibility is low and becomes worse with time. Even the darkest spots are barely visible after 24 hours. (4) The use of ammonium sulfide reagent is, understandably, unpleasant.

Silver nitrate has already been used to precipitate purines in the separation of these compounds from purine nucleosides.[11] We have now developed a visualization procedure utilizing silver nitrate as follows: (a) The mixture of purine and pyrimidine bases[12] in dilute acid solution (*e. g.*, 5% trichloroacetic acid) is resolved in the usual chromatographic manner, using water-saturated butyl alcohol as the moving solvent.[3] The chromatogram is allowed to dry. (b) A 2% solution of silver nitrate is allowed to trickle from a pipet over the suspended chromatogram. (c) After draining about 5 minutes, the chromatogram is passed through a bath of 0.5% sodium dichromate. The entire sheet is thus covered with a red silver chromate precipitate. (d) The chromatogram is then transferred immediately to a 0.5 N nitric acid bath, in which the silver chromate slowly dissolves. Left behind are less soluble red deposits (presumably a purine/silver chromate complex) at the positions occupied by adenine and guanine. (e) The chromatogram is removed from the nitric acid bath *while the background is still slightly pink*, and then washed with water. It should be noted that over-exposure of the chromatogram to acid in step (d) will lead to the leaching out of the purine complex as well. The guanine chromate-spot is the more sensitive to the nitric acid, so that it is usually smaller than the adenine chromate-spot and shows a greater tendency to fade with time.

If the characteristics of the chromate-spots and the sulfide-spots are compared, the following points may be noted: (1) The chromate-spot is detectable when as little as 0.5 microgram of purine is used. It is thus a full order of magnitude more sensitive than is the sulfide-spot. (2) The chromate-spot is specific for purines and does not form with cytosine,[13] thymine or uracil. Since the RF values for adenine and guanine are quite different, identification of the spots is thus possible at a glance. (3) The chromate-spots are brilliantly red, sharply defined, and highly visible. The adenine spot, particularly, is only slightly subject to fading and can be kept as a semi-permanent record. (4) In preparing the chromate-spot, the use of ammonium sulfide is avoided.

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RECEIVED JULY 24, 1950

(11) Kerr and Seraidarian, *J. Biol. Chem.*, **159**, 211 (1945).

(12) Only those bases occurring naturally in nucleic acids, *i. e.*, adenine, guanine, cytosine, thymine, and uracil, are here considered.

(13) A sample of cytosine was made available to us through the courtesy of Dr. Elkan R. Blout of Polaroid Corporation, Cambridge, Massachusetts.

Paper Partition Chromatography of Some Simple Phenols[1]

BY RICHARD F. RILEY[2]

It was desirable to apply the technique of partition chromatography to the identification of degradation products of a phenyl ether derived metabolically from Myanesin (3-(*o*-toloxy)-1,2-propanediol).[3] The application of this technique to the separation of common phenols has very recently been reported.[4] Our work, carried out prior to the appearance of the earlier publication, is presented as our methods differ considerably and likewise gave satisfactory results.

Experimental

The one dimensional descending boundary technique was employed throughout.[5] Solutions of phenols for examination were applied to Whatman no. 1 filter paper as micro drops to give ~ 0.5 cm. spots containing ~ 20 μg. of compound.

The various phenols were visualized by spraying the dried developed chromatograms with a 2% aqueous solution of phosphomolybdic acid and then exposing the moist strips to ammonia vapor. Di- and trihydroxy phenols containing —OH groups *ortho* or *para* to each other appear immediately as well-defined dark blue spots which darken on exposure to ammonia vapor. Simple phenols and polyhydroxy phenols in which —OH groups are in *meta* positions appear as spots shading from blue to green after exposing the sprayed chromatogram to ammonia vapor. Other reagents were also tested: ammoniacal silver reagent[6] will make visible *ortho* and *para* dihydroxy phenols; resorcinol and derivatives give red spots after spraying with 2% fructose and heating carefully in the presence of hydrochloric acid. Sprays of 1 to 2% aqueous or alcoholic ferric chloride may be useful in some instances; however, for the phenols listed, the phosphomolybdic acid reagent appeared to be more sensitive and general than the other reagents mentioned.

Results.—A summary of R_f values determined under our operating conditions for several phenols in several solvent combinations is given in Table I.

Amyl alcohol–water achieves a separation of mono, di- and tri-hydroxyl phenols although resolution of members within a group is not particularly satisfactory. Addition of benzene to alcohol–water systems was, in general, found to increase the dispersion, though decreasing the R_f values at the same time so as to give rather incomplete resolution. However, by allowing the

(1) Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

(2) University of California Atomic Energy Project, Post Office Box 4164, Los Angeles 24, California.

(3) R. F. Riley, THIS JOURNAL, **72**, 5712 (1950).

(4) R. A. Evans, W. H. Parr and W. C. Evans, *Nature*, **164**, 574 (1949).

(5) R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

(6) To 0.1 N $AgNO_3$ solution add concentrated ammonium hydroxide dropwise until the precipitate formed just redissolves.