veloped into a quantitative determination of coenzyme Q_{10} in urine.[8]

EXPERIMENTAL

About 1 mg. of a substituted benzoquinone was dissolved in 2 ml. of absolute ethanol, if necessary, with warming. The solution was cooled to room temperature, 2 drops of ethyl cyanoacetate was added, and then anhydrous ammonia was absorbed in the solution for 1–2 min. The colors formed by various substituted benzoquinones are listed in Table I.

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The Preparation of C^{14}-Labeled Spermine and C^{14}-Labeled Spermidine

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The reaction of acrylonitrile with putrescine to produce *N,N'*-bis(2-cyanoethyl)putrescine (I) and catalytic reduction of this nitrile to yield spermine (II) has been reported by Schultz.[1] Spermidine (III) was not obtained in this process by Schultz. The method has been adapted by us to the small-scale preparation, in one operation, of both C^{14}-labeled spermine and C^{14}-labeled spermidine. The cyanoethylation of putrescine-1-C^{14} with the use of nonisotopic acrylonitrile was carried out in ethanol solution. Reduction of the products, without isolation, by Raney nickel and hydrogen at 136–142° and 4700 p.s.i. yielded spermine and spermidine labeled with C^{14} in the putrescine moiety. The spermine and spermidine were separated chromatographically. Application of the procedure to the products of the cyanoethylation of nonisotopic putrescine by acrylonitrile-1-C^{14} afforded spermine and spermidine labeled with C^{14} in the propylamine moiety. The production of spermidine in this way may be due to the presence of its parent nitrile[2] (IV) among the products of the cyanoethylation of putrescine. As the reaction of acrylonitrile with amines is reversible,[3] the spermidine also could owe its origin to the partial dissociation of *N,N'*-bis-(2-cyanoethyl)putrescine to form IV; such a dissociation would be promoted by the elevated reduction temperature.

$$NC(CH_2)_2NH(CH_2)_4NH(CH_2)_2CN \quad (I)$$

$$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2 \quad (II)$$

$$H_2N(CH_2)_4NH(CH_2)_3NH_2 \quad (III)$$

$$H_2N(CH_2)_4NH(CH_2)_2CN \quad (IV)$$

EXPERIMENTAL

Spermine and spermidine from putrescine-1-C^{14} and acrylonitrile. To a suspension of 223 mg. of putrescine-1-C^{14} dihydrochloride[4] (1.38 mmoles) in 2 ml. of absolute ethanol was added 1.44 ml. of 1.92*N* sodium hydroxide which gave a solution of putrescine-1-C^{14} base. A solution of 164 mg. of nonisotopic acrylonitrile (3.09 mmoles) in 2 ml. of absolute ethanol was mixed with the putrescine-1-C^{14} solution. After being shaken for 5 min., the solution was kept at room temperature for 18 hr. It was diluted with 1 ml. of absolute ethanol, then refluxed for 1 hr. and kept at room temperature for 2 hr. The solution was transferred to a hydrogenation bomb and mixed with *ca.* 0.3 g. of Raney nickel catalyst and 20 ml. of absolute ethanol which had been saturated with ammonia at 20–23°. The mixture was shaken with hydrogen at 136–142° and 4700 p.s.i. for 30 min. The catalyst was filtered and washed thoroughly with absolute ethanol. Nearly all of the solvent was evaporated, with the use of a column, on the steam bath and the residue was neutralized to *p*H 7.0 with hydrochloric acid. Chromatographic separation of the spermidine and spermine was carried out upon Dowex 50 resin (2% cross linked, 100–200 mesh) in the hydrogen form. A column 24 × 1.5 cm. inside diameter was employed with gradient chromatography[4] using 300 ml. of water in the mixing flask and 2.5*N* hydrochloric acid in the reservoir. Spermidine appeared in the eluate between 344 and 468 ml. Spermine was eluted between 484 and 650 ml. These fractions were evaporated to dryness *in vacuo* over potassium hydroxide, and each was subjected to a second chromatography. The yields were 115 mg. (0.45 mmole) of spermidine trihydrochloride (0.034 μc per μ mole) and 114 mg. (0.33 mmole) of spermine tetrahydrochloride (0.034 μc per μ mole). The specific activity of the starting putrescine-1-C^{14} dihydrochloride was 0.034 μc per μ mole.

The above radioactive spermidine trihydrochloride and spermine tetrahydrochloride were found to be contaminated with small amounts of unknown material, and required further purification. An aliquot of the spermidine hydrochloride was recrystallized by dissolving it in a minimum quantity of absolute methanol, acidifying the solution with ethanolic hydrochloric acid, adding an equal volume of ethanol, and then adding ethyl acetate dropwise until precipitation occurred. After standing overnight at 5°, the precipitate was collected by centrifugation. The spermine was recrystallized by dissolving the hydrochloride in 1 ml. of water and adding a slight excess of 1*M* sodium phosphate solution of *p*H 7.2. On standing overnight at 5° spermine phosphate crystallized.

For identification of spermidine and spermine, the compounds were prepared as described with the use of nonisotopic putrescine dihydrochloride and acrylonitrile. An aqueous solution of the spermidine trihydrochloride from the column was treated with a few drops of 37% hydrochloric acid, then concentrated to a small volume and diluted with ethanol to start crystallization; m.p. 257–258° (uncorr.). The crystals showed an infrared spectrum identical with that of authentic spermidine trihydrochloride.

Anal. Calcd. for $C_7H_{22}Cl_3N_3$: C, 33.01; H, 8.71; Cl, 41.77; N, 16.50. Found (dried at 100° *in vacuo*): C, 33.30; H, 8.60; Cl, 41.85; N, 16.78.

The spermine tetrahydrochloride was thrice recrystallized from a mixture of 12% hydrochloric acid and ethanol. The infrared spectrum of these crystals was identical with that of authentic spermine tetrahydrochloride.

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(2) See O. Bayer, *Angew. Chem.*, **61**, 235 (1949).
(3) F. C. Whitmore, *et al.*, *J. Am. Chem. Soc.*, **66**, 725 (1944).
(4) H. Tabor, S. M. Rosenthal, and C. W. Tabor, *J. Biol. Chem.*, **233**, 907 (1958).

Anal. Calcd. for $C_{10}H_{30}Cl_4N_4$: C, 34.49; H, 8.68; Cl, 40.73; N, 16.09. Found (dried at 100° *in vacuo*): C, 34.50; H, 8.68; Cl, 40.56; N, 16.11.

Spermine and spermidine from putrescine and acrylonitrile-1-C^{14}. In a 25-ml. flask were mixed 278 mg. of nonisotopic putrescine dihydrochloride (1.7 mmoles), 5 ml. of absolute ethanol, and 1.59 ml. of 2.17*N* sodium hydroxide solution. Into this mixture was distilled, under high vacuum at room temperature, 204 mg. (3.8 mmoles) of acrylonitrile-1-C^{14}; the flask then was sealed under vacuum.[5] After *ca.* 44 hr. the solution was refluxed and treated by the above described procedure for the preparation of radioactive spermidine and spermine from putrescine-1-C^{14}. The yields were 116 mg. (0.46 mmole) of spermidine trihydrochloride showing 0.30 μc per μ mole and 221 mg. (0.64 mmole) of spermine tetrahydrochloride showing 0.60 μc per μ mole. These compounds contained small amounts of unknown material and were purified further as described for the products obtained from putrescine-1-C^{14}.

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(5) The acrylonitrile-1-C^{14} was prepared and mixed immediately after synthesis with the putrescine solution by Dr. S. Rothschild of Tracerlab, Inc., Boston, Massachusetts. The specific activity was approximately 0.26 μc per mmole.

Steroids. CXLIV.[1] Synthesis of Some 6α,17α-Dihaloprogesterones[2]

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Recent work in our laboratory[3] has demonstrated the potentiating effect upon progestational activity when a 6α-halogen atom is introduced into the progesterone molecule, notably of 17α-acetoxyprogesterone. As Engel and Jahnke[4] had observed that 17α-bromoprogesterone has about double the progestational activity of progesterone, it appeared of interest to synthesize certain progesterone analogs possessing halogen atoms at C-6 as well as C-17 and to subject them to biological assay.

For the synthesis of 6α-fluoro-17α-bromoprogesterone (VI) we selected a route patterned closely after the earlier described[5] preparation of 6α-fluoroprogesterone. 17α-Bromo-Δ^5-pregnen-3β-ol-20-one (II)[4,6] was transformed into its 5α,6α-epoxide III, which was opened to the fluorohydrin IV through the intervention of boron trifluoride.[5,7] Oxidation at C-3 by means of chromium trioxide in acetone solution[8] afforded the ketone V, whose dehydration with inversion at C-6 to 6α-fluoro-17α-bromoprogesterone (VI) was accomplished with hydrogen chloride in acetic acid.

The synthesis of 6α-chloro-17α-bromoprogesterone (XI) commenced with 17α-bromo-Δ^5-pregnen-3β-ol-20-one acetate (I),[6] which was chlorinated in carbon tetrachloride solution to the 3β-acetoxy-5α,6β-dichloride, VII. The acetoxy group was removed by exposure to hydrochloric acid and the 3β-hydroxy-5α,6β-dichloride (VIII) was oxidized[8] to 5α,6β-dichloro-17α-bromopregnane-3,20-dione (IX). Heating with ethanolic sodium acetate solution caused dehydrochlorination without inversion at C-6 to yield 6β-chloro-17α-bromoprogesterone (X), while the desired 6α-chloro-17α-bromoprogesterone (XI) was obtained by treatment of IX with hydrogen chloride in acetic acid solution.

A closely related reaction sequence was employed for 6α,17α-dichloroprogesterone (XVII). The enol diacetate XII[9] of Δ^5-pregnen-3β-ol-20-one was chlorinated to 5α,6β,17α-trichloropregnan-3β-ol-20-one acetate (XIII),[10] the 3-acetoxy group removed and the 3β-ol XIV oxidized with chromium trioxide.[8] Treatment of the resulting 5α,6β,17α-trichloropregnane-3,20-dione (XV) with sodium acetate led to 6β,17α-dichloroprogesterone (XVI), while exposure of XV to hydrogen chloride in acetic acid furnished directly the required 6α,17α-dichloroprogesterone (XVII).

Bioassays[11] in the rabbit showed that 6α-chloro-17α-bromoprogesterone (XI) and 6α,17α-dichloroprogesterone (XVII) possessed approximately 20% the oral progestational activity of Norlutin[12] (19-nor-17α-ethynyltestosterone).

(1) Paper CXLIII, A. Bowers and E. Denot, *J. Am. Chem. Soc.*, in press.

(2) Taken from the professional thesis of O. Candiani, submitted to the Instituto Politécnico Nacional, Mexico, D.F.

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(11) Assays by Endocrine Laboratories, Madison, Wisconsin.

(12) See D. A. McGinty and C. Djerassi, *Ann. N. Y. Acad. Sci.*, **71**, 500 (1958).