SHORT COMMUNICATIONS

Although mercaptoethanol had essentially no effect in the spectrophotometric assay system containing indoleacetaldehyde, DPN and aldehyde dehydrogenase, the addition of mercaptoethanol to both systems, in which indoleacetaldehyde was generated enzymically from tryptamine, abolished the effect of the steroids. This was true with but one exception. Cortisone retained its inhibitory effect in the system in which indoleacetaldehyde was generated by partially purified monoamine oxidase.

The mechanism by which steroid hormones alter the rate of aldehyde oxidation is not known and the role of mercaptoethanol in altering the response to steroids in the various systems is not yet clear. However, the results here in vitro show that steroid hormones do, under certain conditions, affect the rate of acid formation from both serotonin and tryptamine. Whether or not steroid hormones affect the metabolism in vivo of aldehydes is not known and any possible physiological significance of the results reported here must await further study.

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Microbiological transformations of steroids. Aromatization of Ring A, cleavage of Ring D and oxidation of the side chain in certain 19-nor steroids

It is well known that a number of microorganisms are able to produce C-1,2 dehydrogenation in several Δ^{4} -3-keto steroids¹⁻⁴. KUSHINSKY⁵ and LEVY have demonstrated that when this Ring A unsaturation is introduced into 19-nortestosterone by means of Corynebacterium simplex or Pseudomona testosteroni, the compound is rapidly aromatized leading to the Ring A phenol. This aromatization proceeds with equal facility when 19-norprogesterone is incubated with Corynebacterium simplex as has been reported by BOWERS et al.⁷. Bio-oxidation involving both Ring D lactone formation and Ring A dehydrogenation has been observed^{8,9}. Streptomyces lavendulae has been shown⁹ to metabolize progesterone to 1,4-androstadiene-3,17-dione and I-dehydrotestosterone but in these experiments of PETERSON et al.⁹ the opening of Ring D to form the lactone was not observed. We now wish to report the aromatization of 19-nortestosterone (I) through its incubation with Bacillus sphaericus; and the side-chain degradation, Ring D cleavage and aromatization of Ring A, of 19norprogesterone (IV) by means of Streptomyces lavendulae.

Bacillus sphaericus (A.T.C.C. No. 7055) was grown in 250 ml of Fries medium,

at 30° (for details, see ref. 10). At the end of 24 h, 150 mg of I were added in 2.0 ml ethanol and the incubation continued for 48 h longer. The culture was extracted with ethyl acetate and the dried extracts were crystallized directly from ethyl acetate and recrystallized twice from acetone-methanol to give 90 mg of 3-hydroxy-1,3,5 (10)-estratrien-17-one (III), m.p.*, $262-264^{\circ}$; $[\alpha]_{D}^{22}$, $+162^{\circ}$ (dioxane); λ_{max} (in ethanol), 280 m μ (E 2,300); ν_{max} , 3400, 1715, 1615, 1575 cm⁻¹. The infrared spectrum of III was identical with that of an authentic sample of estrone. The mother liquors of III were chromatographed on Whatman paper No. 1 using the toluene-propylene glycol system of ZAFFARON1^{11,12} and the ligroin-propylene glycol system of SAVARD¹³. By these methods another 15 mg of III were obtained and identified by comparing its physical constants including infrared spectrum with an authentic sample. A second compound was eluted from the paper and crystallized from ethyl acetate to give minute amounts of 19-nor-4-androstene-3,17-dione (II), m.p., 162-166°; λ_{max} (in methanol), 240 m μ ; ν_{max} 1740, 1670 and 1617 cm⁻¹. The infrared spectra were identical to a known standard of II.

Streptomyces lavendulae (A.T.C.C. No. 8664) was grown in a nutrient broth at 30° as described above, 50 mg of IV were added, and incubation continued 24 h longer. The culture was extracted and chromatographed as mentioned, yielding several metabolites detected on paper with the Zimmermann reagent and the Turnbull's blue test for enols¹⁴. The major product gave after two crystallizations from acetonemethanol, 10 mg of 17 β -acetyl-1,3,5-(10)-estratrien-3-ol (V), m.p., 242–244°; λ_{max} (in ethanol), 280 m μ ; ν_{max} , 3400, 1685, 1624, 1585 and 1250 cm⁻¹. It was identified by comparison with an authentic sample of V. A second compound was eluted and crystallized from ethyl acetate to give 8 mg of (II), m.p., 163-166°; λ_{max} (in methanol), 250 m μ . Its infrared spectrum was identical to that of II. A compound located on a toluene-propylene glycol paper chromatogram with a polarity between standards of estrone and estradiol was eluted and crystallized from acetone to give 1.5 mg of 17α-0xa-17-0x0-D-homo-1,3,5 (10)-estratrien-3-ol (VII), m.p., 338-339°; λ_{max} (in methanol), 280 m μ ; ν_{max} , 3215, 1677, 1612, 1577 cm⁻¹ (cf. ref. 15, 16). The acetate of VII was formed in the usual manner with pyridine and acetic anhydride giving VIIa, m.p., 148–151°; v_{max}, 1763, 1730, 1610 and 1580 cm⁻¹. The compound was identified by comparing with authentic samples of estrololactone** and its acetate.

Minute amounts of III, IV and 1,3,5 (10)-estratriene- $3,17\beta$ -diol (VI) were detected and identified by comparison of spot tests, chromatographic behavior, u.v. absorption and infrared spectra with known standards.

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^{*} 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 were dispersed in KBr.

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Biosynthesis of [75Se]selenomethionine and [75Se]selenocystine

The chemical synthesis^{1,2} of the selenium analogs of the sulfur amino acids is difficult and not readily adaptable to the production of small quantities of material labelled with radioactive isotopes of high specific activity. Biosynthesis in yeast followed by hydrolysis and ion-exchange fractionation provides a convenient preparation for both [75Se]selenomethionine and [75Se]selenocystine. The physical properties of 75Se (gamma emitter, half life 127 days) are convenient for measurements in vivo and in vitro.

Baker's yeast (Saccharomyces cerevisiae) is grown on the low-sulfur medium of WILLIAMS AND DAWSON³, containing per liter: glucose, 20 g; (NH₄)₂HPO₄, 3.5 g; KH₂PO₄, 0.2 g; MgSO₄·7H₂O, 0.25 g; sodium citrate, 1.0 g; L-asparagine monohydrate, 2.5 g; biotin, 10 µg; calcium pantothenate, 0.5 mg; inositol, 10 mg; thiamine, 6 mg; pyridoxine, I mg; Zn^{2+} (as acetate), 400 μ g; Fe³⁺ (as chloride), 150 µg; Cu²⁺ (as chloride), 25 µg. H₂⁷⁵SeO₃ (ORNL Se-75-P-2 Processed, Enriched) is added and the medium adjusted to pH 5 with H_3PO_4 . Up to 10 mC/l of ⁷⁵Se has been used without noticeable decrease in the growth rate of the yeast. After inoculation with 50 mg of pressed yeast, growth is carried out in 3-l Fernbach flasks containing 500 ml of medium. Vigorous agitation provides aeration and the temperature is maintained at approx. 30°. Growth and subsequent chemical manipulations are carried out in a fume hood to prevent inhalation of volatile selenium compounds.

The yeast is harvested by centrifugation after 18-24 h. Incorporation of ⁷⁵Se into cells proceeds to approx. 50 % and then stops. This yield can be increased by adding glucose to the medium after the first harvest, readjusting to pH 5 and allowing growth to proceed for another 24 h. An additional 25 % yield is obtained.