PN 1212

Aromatization *in vivo* of 3 β,17β-bisacetoxy-17α-ethynyl-19-nor-androst-4-ene (SC-11 800)

Clinical interest in the 19-nor-steroids began with the synthesis of 17α-ethynyl-19-nor-testosterone by DJERASSI *et al.*[1]. The ever-increasing widespread use of oral 19-nor-progestins prompts this preliminary report of an investigation concerning the metabolism of SC-11 800 which began in these laboratories two years ago. Reported metabolic alterations *in vivo* of certain 19-nor-steroids to compounds possessing androgenic and/or estrogenic activity, suggested the feasibility of investigating urinary metabolites of SC-11 800.

Following the oral administration of 60 mg of SC-11 800 to hospitalized women, 24-h urine specimens were collected, preserved by the addition of chloroform, and stored at −20°. The entire specimen was adjusted to pH 4.5 and acetate buffer was added. Incubation was carried out with 1000 units/ml of β-glucuronidase (EC 3.2.1.31) (Ketodase) for 72 h at 37°. The hydrolyzed urine was extracted for 24 h on a constant extractor utilizing ethyl acetate. The phenolic fraction was prepared according to the method described by BROWN[2]. Preparation and preliminary purification of the neutral fraction was done according to our method[3]. Spot tests, as described by AXELROD[4] were used to locate the phenolic compounds.

Pre-, during and post-drug urinary levels of etiocholanolone, androsterone, dehydroepiandrosterone, 11-oxo-etiocholanolone, 11-hydroxyetiocholanolone, 11-oxo-androsterone and 11-hydroxyandrosterone remained the same. Significant urinary estrogen alterations of estrone, estradiol and estriol were encountered. Also, a different phenolic metabolite appeared only in the urine collected during the ingestion period. The residue of the phenolic fraction was chromatographed in a toluene–propylene glycol system for 24 h. The new area was eluted and re-chromatographed in a methylcyclohexane–formamide system for 24 h, from which it was eluted and re-run in a light petroleum–toluene/methanol–water system for 4 h. This area gave positive reactions to 0.2 saturated $KMnO_4$, $FeCl_3$–$K_3Fe(CN)_6$, and urea–$SnCl_2$ reagent. The pink color formed with the latter reagent is positive only for 17α-ethynyl estradiol[4]. Re-chromatography of the phenolic compound in the above systems failed to separate from a standard of 17α-ethynyl estradiol, either on the side limbs or on admixed papergrams. Acetylation of the phenolic compounds failed to resolve it from the acetoxy-derivative of the standard in the above described systems. In methanol no maximal absorption was found, while the λ_{max} in H_2SO_4 was 275, 360, 460 and 520 mμ, the same as observed for the standard compound, as previously reported after chromatography[5]. Quantitation by means of the Kober color method[6] followed by an Allen correction, demonstrated the occurrence of 58 μg of 17α-ethynyl estradiol per 24-h urine. Repeated studies gave essentially the same results. The phenolic compound caused a bathochromic shift of 20 mμ in the maximal absorption when the estradiol reagent was used. This was also true of the standard.

As a control, 60 mg of the administered drug from the same lot number was worked up in the same manner as described above. All efforts failed to demonstrate the presence of any 17α-ethynyl estradiol as a contaminate in this sample.

These findings are in keeping with those reported for other 19-nor-steroids. ENGEL[7] studied 19-nor-testosterone, *in vivo*, and found a conversion of 0.03% to estrone. BROWN AND BLAIR[8] found that administering 17α-ethynyl-19-nor-testosterone

resulted in the formation of 17α-ethynyl estradiol and estrone. Likewise, BREUER[9] reported the same conversion in 0.1 and 0.05–0.1 % yield, respectively. KAISER[10] reported an absolute excess excretion of total estrogens to be only 7 μg following the administration of 200 mg of esterized nor-testosterone derivatives and emphasizes there is a shift in the relationship among the individual estrogen fractions depending on the compound given. A similar, but more detailed study has been reported by LANGECKER[11]. In the present study, it was felt that perhaps a greater conversion to a phenolic derivative might occur due to the presence of the 3β-hydroxy group. Apparently, the esterification of the 3β-alcohol prevented any accelerated aromatization, as we found SC-11800 to form in about 0.1 % yields the biologically potent compound, 17α-ethynyl estradiol.

ENGEL[7] reported the conversion of 19-nor-testosterone to two new androgens, 19-nor-androsterone and 19-nor-etiocholan-3α-ol-17-one. BREUER[12] reported no gross alteration in 17-ketosteroids or 17-ketogenic steroids following the use of 19-nor-compounds. Our results failed to demonstrate any alteration in the seven above mentioned androgens by our fractionation procedure.

Isolation and identification of the major urinary metabolites of SC-11800, as well as the clinical observations, will be published elsewhere. In conclusion, we have demonstrated the removal *in vivo* of the 3β,17β-bisacetoxy group with the concomitant aromatization of ring A of SC-11800 to form 17α-ethynyl estradiol.

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