C-3-Oxygenation of 17α-Methyl-5α-androstan-17β-ol by Rabbit Liver Homogenate†

Manfred E. Wolff* and Yasuji Kasuya

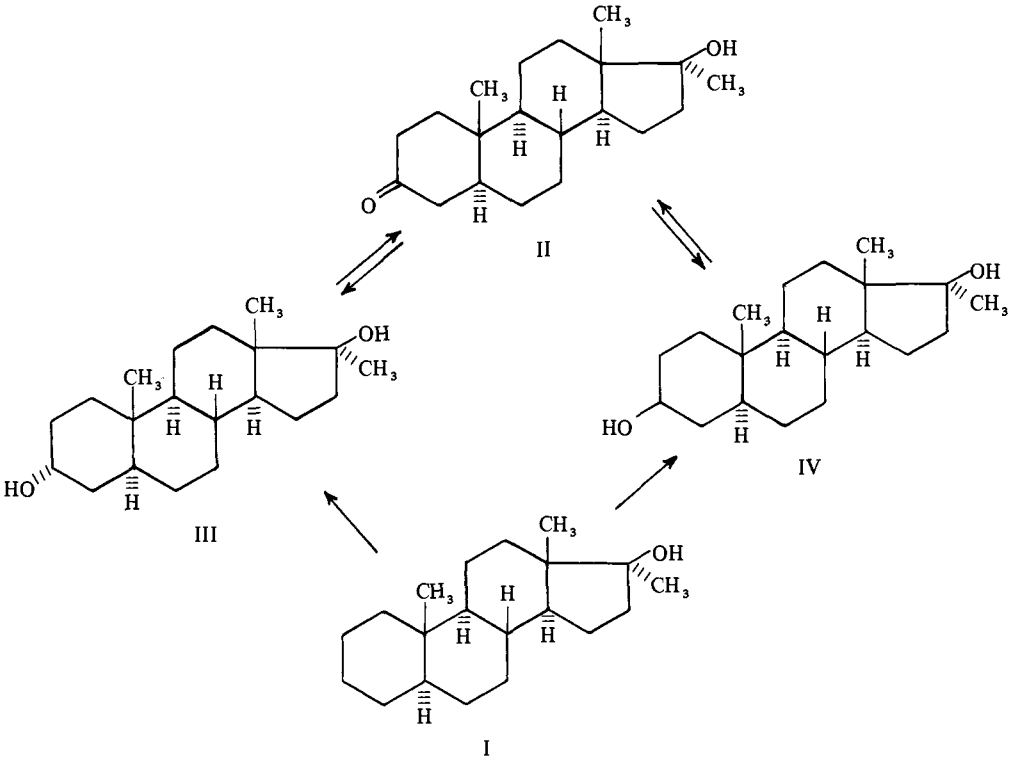
Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94122.
Received February 17, 1971

The possibility that 5α-androstane derivatives having a simple hydrocarbon A ring can be oxygenated metabolically to known C-3 oxygenated androgens was examined. The following 3-oxygenated androstanes were isolated and characterized by glc and mass spectrometry after incubation of 17α-methyl-5α-androstan-17β-ol (I) with rabbit liver homogenate: 17α-methyl-5α-androstan-3-on-17β-ol (II), 17α-methyl-5α-androstane-3α,17β-diol (III), and 17α-methyl-5α-androstane-3β,17β-diol (IV). A study of the metabolism of III and IV by rabbit liver homogenate was also carried out. The results show that III largely gives II and IV, whereas only a small conversion of IV to II and III was observed.

In previous papers, we have evaluated the characteristics of the 3-keto group which have importance in eliciting the biological response in androgenic steroids.[1a-k] We concluded[1i] that it is only the steric nature of the C-3 carbonyl group, rather than its electronic characteristics, which is important in this connection. It is known, however, that steroids having an unsubstituted A ring, such as androstan-17β-ol[2] and 5α-androstane,[3,4] are androgens. Again, as has been pointed out in a recent review of the area,[5] 17α-methyl-5α-androstan-17β-ol (I) has significant oral anabolic and androgenic activity[6] which has been confirmed in the clinic.[7] We considered it important, therefore, to examine the possibility that these ring A hydrocarbons owe their activity to their conversion *in vivo* to compounds oxygenated at C-3. This would be an alternative to the suggestion of Segaloff and Gabbard[3] that androgenicity is a property of the hydrocarbon skeleton 5α-androstane. To determine the occurrence of C-3 oxygenation, we investigated the metabolism of 17α-methyl-5α-androstan-17β-ol (I) by rabbit liver homogenate.

Results and Discussion

The present study clearly shows the ability of rabbit liver homogenate to produce extensive oxygenation of I at C-3. The ratio of the total amount of 3-oxygenated products to unchanged parent compound is 2:1 after 60 min under the conditions of the experiment (Figure 1).



A study of the metabolism of III and IV by rabbit liver homogenate was also carried out. The results show that III largely gives II and IV (Figure 2) whereas only a small conversion of IV to II and III was observed (Figure 3).

Our results are compatible with the idea that the active androgens I and III exert their effect through conversion to the dihydrotestosterone derivative II. Moreover, the fact that the 3β-OH derivative IV is a less active androgen than either I or III[8] is in harmony with the finding that IV is not converted efficiently to II and III, and it is possible that the active form of all of these compounds is II.‡

Experimental Section

Steroids. 17α-Methyl-5α-androstan-17β-ol (I) was obtained by Wolff-Kishner reduction[10] of 17α-methyl-5α-androstan-3-on-17β-

†This investigation was supported in part by a Public Health Service Research Grant (AM-05016) from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

‡However, for evidence that IV has intrinsic activity, see Robel, *et al.*[9]

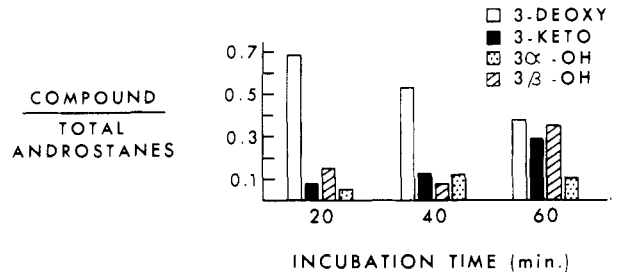


Figure 1. Formation of 3-oxygenated-17α-methyl-5α-androstan-17β-ols by rabbit liver homogenate.

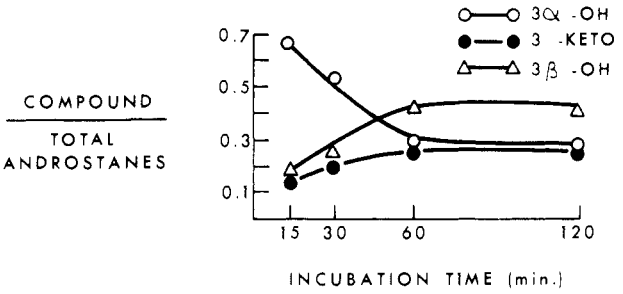


Figure 2. Incubation of 3α-hydroxy-17α-methyl-5α-androstan-17β-ol with rabbit liver homogenate.

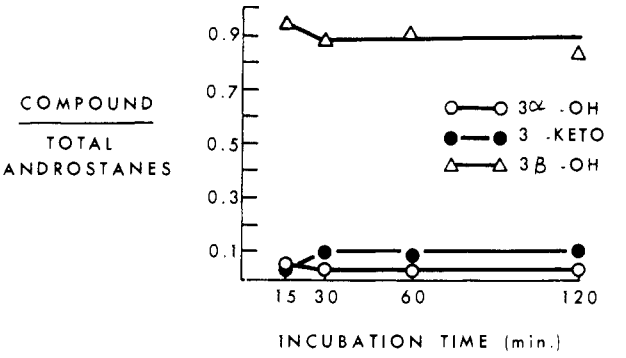


Figure 3. Incubation of 3β-hydroxy-17α-methyl-5α-androstan-17β-ol with rabbit liver homogenate.

ol; 17α-methyl-5α-androstan-3-on-17β-ol (II) was purchased from Searle Chemicals, Inc; Lot. No. V-31; 17α-methyl-5α-androstane-3β-17β-diol (IV) was made by reducing a soln of 17α-methyl-5α-androstan-3-on-17β-ol in MeOH with $NaBH_4$;[11] 17α-methyl-5α-androstane-3α,17β-diol (III) was prepd by treatment of androstan-3α-ol-17-one in anhyd PhH with MeMgBr.[12]

All steroids used in incubations were examined for purity by tlc and glc.

Preparation of Liver Homogenate and Incubation. Livers of male New Zealand white rabbits (4 kg) were weighed and homogenized in a Waring blendor in Krebs-Ringer phosphate buffer (pH 7.4) at 4° (100 ml/10 g of tissue). The steroids were incubated with the liver homogenate for 15 min to 2 hr at 37° in a shaking bath, bubbling O_2 through the incubation mixture. Each incubation contd 100 ml of the liver homogenate (0.1 g of liver/ml) and 4 mg of steroid in 1 ml of EtOH. After the incubation was completed, the enzymatic reaction was stopped by the addn of 100 ml of MeOH-$CHCl_3$ (2:1, v/v) and, after shaking, the mixt was left in a refrigerator overnight.

Extraction. Coagulated proteins and ppt were removed by filtration and washed with MeOH-$CHCl_3$ (2:1). The filtrate and washings were combined, concd under vacuum, and subjected to repeated extn with petr ether (30–60°).

The petr ether ext was evapd to dryness under vacuum, and the dried ext was acetylated with Ac_2O and C_5H_5N at 65° for 2 hr. The mixt was evapd, and the residue was dissolved in a small amount of $CHCl_3$ and analyzed by gas chromatog.

Gas Chromatography and Mass Spectrometric Analysis. A Barber-Coleman Series 5000 gas chromatograph equipped with a flame ionization detector and a 1.8-m glass column was used. The glass column was packed with 3% OV-1 on 100–200 mesh Gas Chrom Q. Condns employed were: column temp 219°; injector 240°; detector 260°; He 2.1 kg/cm², 2.8 kg/cm²; and H_2 1.4 kg/cm².

The vapors eluted from the column were condensed in capillary tubes. Compds trapped from the effluents were subjected to mass spectrometry for final confirmation of identify.

Mass spectrometry was carried with an AEI MS-902 high-resolution mass spectrometer (70 eV).

References

(1) (a) M. E. Wolff, W. Ho, and R. Kwok, *J. Med. Chem.*, **7**, 577 (1964); (b) M. E. Wolff, W. Ho, and M. Honjoh, *ibid.*, **9**, 682 (1966); (c) M. E. Wolff and R. C. Boguslaski, *ibid.*, 11, 285 (1968); (d) M. E. Wolff, S-Y. Cheng, and W. Ho, *ibid.*, 11, 864 (1968); (e) M. E. Wolff and G. Zanati, *ibid.*, **12**, 629 (1969); (f) M. E. Wolff and G. Zanati, *ibid.*, **13**, 563 (1970); (g) M. E. Wolff, G. Zanati, G. Shanmugasundaram, S. Gupte, and G. Aadahl, *ibid.*, **13**, 531 (1970); (h) M. E. Wolff, G. Zanati, G. Shanmugasundaram, S. Gupte, and G. Aadahl, *Intra-Sci. Chem. Rep.*, **4**, 277 (1970); (i) M. E. Wolff and G. Zanati, *Experientia*, **26**, 1115 (1970); (j) G. Zanati and M. E. Wolff, *J. Med. Chem.*, **14**, 958 (1971); (k) G. Zanati and M. E. Wolff, *ibid.*, 14, 961 (1971).

(2) C. Huggins and E. V. Jensen, *J. Exp. Med.*, **100**, 241 (1954); K. L. Syndor, *Endrocrinology*, **62**, 322 (1958); R. I. Dorfman and F. A. Kincl, *Steroids*, **3**, 173 (1964).

(3) A. Segaloff and R. B. Gabbard, *Endrocrinology*, **67**, 887 (1960).

(4) R. I. Dorfman, W. H. Rooks, II, J. B. Jones, and J. D. Leman, *J. Med. Chem.*, **9**, 930 (1960).

(5) R. E. Counsell and P. D. Klimstra, in "Medicinal Chemistry," 3rd ed, Part II, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, pp 933–934, 941.

(6) E. F. Nutting, P. D. Klimstra, and R. E. Counsell, *Acta Endocrinol. (Copenhagen)*, **53**, 627, 635 (1966).

(7) I. A. Anderson, *Acta Endocrinol. (Copenhagen) Suppl.*, **63**, 54 (1962).

(8) R. E. Counsell and P. D. Klimstra, *ibid.*, **63**, 928 (1962).

(9) P. Robel, I. Lasnitzki, and E. E. Baulieu, *Biochemie*, **53**, 81 (1971).

(10) Huang-Minlon, *J. Amer. Chem. Soc.*, 71, 3301 (1949).

(11) E. Elisberg, H. Vanderhaeghe, and T. F. Gallagher, *J. Amer. Chem. Soc.*, 74, 2814 (1952).

(12) R. E. Counsell, P. D. Klimstra, and F. B. Colton, *J. Org. Chem.*, 27, 248 (1962).