Stereoselective Saturation of a Carbon–Carbon Double Bond under Wilzbach Conditions[1]

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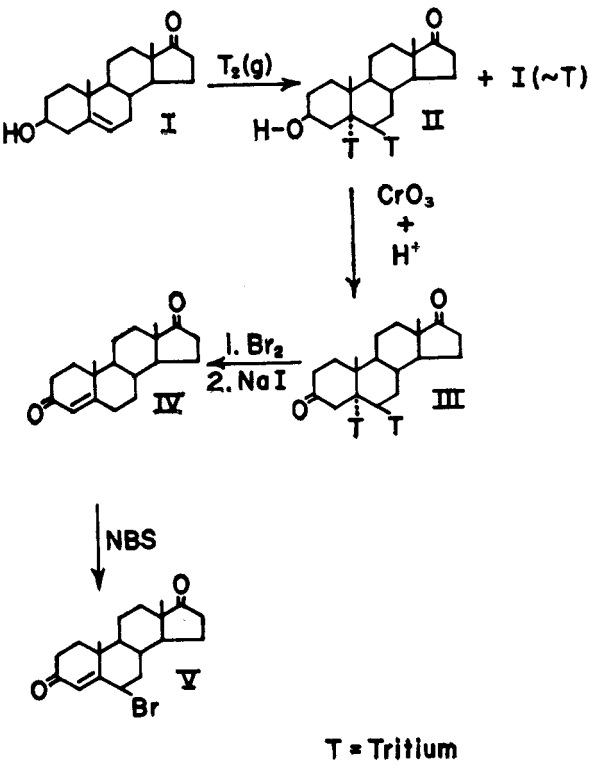
After submitting 3β-hydroxyandrost-5-en-17-one to the Wilzbach procedure, labeled starting material and 3β-hydroxy-5α-androstan-17-one were isolated. Proof is given that the double bond is saturated, predominantly by *trans* addition, yielding tritium in the 5α- and 6β-positions.

The Wilzbach tritium gas exposure method[5] has been used frequently for the labeling of organic compounds with this isotope. In recent years its application to unsaturated compounds has received critical attention,[6–9] and it has been shown that, in addition to substitution of tritium for hydrogen, the saturation of double bonds can take place. Thus, saturation accounts for the total radioactivity incorporated in some fatty esters and for the major part of that introduced in some steroids.[9]

The stereochemistry of the saturation process, however, has received little attention. It was of interest to determine this to help clarify the mechanism and to see if this reduction procedure could be useful in preparing stereospecifically labeled tritium compounds.

The compound chosen for investigation was 3β-hydroxyandrost-5-en-17-one (I). It had been shown[9] that, when I and 3β-acetoxypregn-5-en-20-one were exposed to tritium gas, saturated products having only the A–B *trans* ring juncture were isolated. Therefore, there was stereospecific addition of tritium at the 5α-position. This study presents evidence that the tritium at carbon 6 is mostly β-oriented, and therefore saturation of I by the Wilzbach procedure takes place predominantly by *trans* addition yielding 3β-hydroxy-5α-androstan-17-one-5α,6β-H[3] (II). Data are also presented for the distribution of tritium at other positions in II.

SCHEME I



Procedure and Results

Compound I was exposed to carrier-free tritium and the resultant mixture was partially purified[5] and treated as follows (also see Scheme I).

A. Chromatography in the paper systems ligroin–propylene glycol and *n*-heptane–dimethyl sulfoxide was used to separate I and 3β-hydroxy-5α-androstan-17-one (II). No 3β-hydroxy-5β-androstan-17-one was found.

B. Chromic acid oxidation of II to 5α-androstane-3,17-dione (III) with the reagent of Bowden, *et al.*,[10] was followed by treatment with base. Loss of tritium during the base treatment was 4%.

C. Conversion of III *via* the 2,4-dibromodione to androst-4-ene-3,17-dione (IV) was effected by the method of Rosenkranz, *et al.*[11] (loss of activity III → IV was 47%).

D. The androstenedione IV was divided in two portions. (1) One part was equilibrated with 2% potassium hydroxide in 80% aqueous methanol and suffered a loss of 91% of its radioactivity (the tritium at carbon 6). (2) The second part of the androstenedione was brominated with N-bromosuccinimide (NBS) to give 6β-bromoandrost-4-ene-3,17-dione (V) with a loss of 78% of the activity at carbon 6.

Discussion

The exposure of I to tritium gas yielded the 5α-product II and radioactive starting material. No 3β-hydroxy-5β-androstan-17-one could be detected. The distribution of tritium in androstanedione III obtained from II was as follows: 4% at carbons 2, 4, and 16 (procedure B); 45% at carbon 5 (procedure C); 46% at carbon 6; and 5% at other positions (procedure D-1).

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(5) K. E. Wilzbach, *J. Am. Chem. Soc.*, **79**, 1013 (1957).

(6) H. J. Dutton, E. P. Jones, L. H. Mason, and R. F. Nystrom, *Chem. Ind.* (London), 1176 (1958).

(7) R. F. Nystrom and D. E. Sunko, *Atomlight*, 1 (Jan., 1959).

(8) H. J. Dutton and R. F. Nystrom, "Advances in Tracer Methodology," Vol. 1, S. Rothchild, Ed., Plenum Press, New York, N. Y., 1963, p. 18.

(9) H. L. Bradlow, D. K. Fukushima, and T. F. Gallagher, *Atomlight*, 1 (Sept., 1959).

(10) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, **39** (1946).

(11) G. Rosenkranz, O. Mancera, J. Gatica, and C. Djerassi, *J. Am. Chem. Soc.*, **72**, 4077 (1950).

These values are easily calculated from the results of the selective removal of tritium (T) mentioned above and summarized in the following scheme.

$$\text{III} \xrightarrow[(-4\%)]{B} \text{III} \xrightarrow[(-47\%)]{C} \text{IV} \xrightarrow[(-91\%)]{D-1} \text{IV}$$
$$100\text{T} \qquad 96\text{T} \qquad 51\text{T} \qquad 5\text{T}$$

Hydrogen–tritium exchange as well as reduction was demonstrated, because III contained 9% tritium at positions other than 5 and 6 and recovered I also had tritium associated with it. The loss in activity (determined semiquantitatively) in oxidizing II to III appeared to be negligible indicating that there was little tritium at carbon 3 (see Experimental).

Androstenedione, with 91% tritium at carbon 6 (procedure D-1), lost 78% of the label at that position when brominated at 6β with NBS (procedure D-2). Since androstenedione-6α,7α-H^3 loses less than 5% of the label when converted to the bromide under the same reaction conditions,[12] the label at carbon 6 in IV was at least 78% β.

When one considers the way in which the primary isotope effect operates in the bromination, the data indicate that there may be a much higher orientation of tritium in IV. The first step in the bromination is probably an abstraction of a C-6 hydrogen atom either as a proton or free radical. The 6β-hydrogen is lost preferentially to the 6α because of better overlap of the vacated orbital with the C-4 double bond. With tritium at 6β, the ease of loss of the 6α-hydrogen approaches that of the 6β-tritium because of the isotope effect.[13] When the 6α-hydrogen is lost, the tritiated intermediate is then brominated to give a product with retention of tritium at 6. The net result is some inversion of tritium from 6β to 6α during the bromination.

If the isotope effect is thus operating in the 6β-bromination to cause all of the tritium retention, it follows that all the tritium originally at carbon 6 was in the β-position and the most likely mechanism is a *trans*-diaxial addition of tritium, either ionically as previously proposed[9] or by a free-radical mechanism.[14] If, as is less likely, the distribution of tritium at carbon 6 does approximate 22% α and 78% β, then there is probably an initial attack of $^{\cdot}H^3$ or $^{+}H^3$ at the 6-position *predominantly* from the β (axial) side, yielding an intermediate which assumes the more stable A–B *trans* conformation. The uptake of another tritium atom yields the 5α-structure exclusively. Attack at carbon 6 is favored because the intermediate is the more stable tertiary free radical or carbonium ion.

Experimental

Exposure to Tritium Gas.—To 500 mg. of 3β-hydroxyandrost-5-en-17-one (I) was added 3 c. of carrier-free tritium and the tube was sealed. After 10-day exposure the gas was "toeplered" out. Some of the tritium was removed by repeatedly dissolving the solids in methanol and evaporating to dryness until the specific activity of the residue remained constant.

Isolation of 3β-Hydroxy-5α-androstan-17-one (II).—An 800-γ aliquot of the methanol-equilibrated Wilzbach product was chromatographed on a ligroin–propylene glycol paper system for 4 hr. The chromatogram was scanned for radioactivity and showed four peaks of the following relative activities as determined by elution and scintillation counting of the eluates (Table I).

TABLE I

Peak no.	Compound	% activity	Distance run, cm.
1	Not identified[a]	20	Origin
2	I + II	70	2.5
3	Not identified[a]	4	13
4	Not identified[a]	6	35

[a] None of these peaks corresponded to 3β-hydroxy-5β-androstan-17-one.

The material corresponding to peak 2 was rechromatographed for 16 hr. in the same system. One radioactive peak was obtained which had the same running rate as the compounds in peak 2 above. The radioactive area was eluted with methanol, and the residue was chromatographed on a *n*-heptane–dimethyl sulfoxide paper system[15] for 15 hr., whereby the mixture was resolved into three zones: (1) unidentified material at the origin; (2) at 12.5 cm., a peak corresponding to 3β-hydroxyandrost-5-en-17-one (I); and (3) at 18.5 cm., a peak corresponding to 3β-hydroxy-5α-androstan-17-one (II). The peak corresponding to I contained twice the radioactivity of the peak corresponding to II. The peak containing the radioactive 3β-hydroxy-5α-androstan-17-one was eluted from the paper and diluted with authentic material to a total weight of 1.6 g. containing 3.7×10^7 c.p.m. The homogeneity of the material was established when repeated recrystallizations from ethyl acetate–ether and from methanol–ether did not alter the specific activity.

5α-Androstane-3,17-dione (III) from 3β-Hydroxy-5α-androstan-17-one (II).—A solution of 1.5 g. of 3β-hydroxy-5α-androstan-17-one (6700 c.p.m./μmole) in 50 ml. of acetone was cooled to 0–5° and a solution of chromic acid[10] was added until the brown color persisted. The mixture was stirred for 5 min. at 0–5° and then for another 5 min. at room temperature. Excess reagent was decomposed by adding 2 ml. of methanol and then the mixture was poured into water. The precipitate was extracted with methylene chloride and the organic layer was washed with a solution of sodium bicarbonate and with water and was finally dried over anhydrous sodium sulfate. The solvent was evaporated and the residue was recrystallized twice from dilute acetone to give 1.1 g. of leaflets, m.p. 130–132°, 6740 c.p.m./μmole (no apparent loss[16]). Equilibration of this material by the method described below for IV decreased the specific activity by 4%.

Androst-4-ene-3,17-dione (IV) from 5α-Androstane-3,17-dione (III).—5α-Androstane-3,17-dione (1.02 g., specific activity 48,800 d.p.m./μmole) was converted to the 2,4-dibromo saturated dione by the method of Rosenkranz, *et al.*[11] The crude dibromo product was dissolved in 50 ml. of acetone, 1.41 g. of sodium iodide was added, and the solution was refluxed under nitrogen with stirring for 20 hr. After that time the iodine color was discharged by the addition of a dilute sodium thiosulfate solution. The precipitate was filtered off, dried, and chromatographed on a silica gel absorption column. The benzene–ethyl acetate eluates gave first 2-iodoandrost-4-ene-3,17-dione[11] (8:2), m.p. 120–125° from benzene–hexane, specific activity 26,760 d.p.m./μmole, 24% yield; followed by androst-4-ene-3,17-dione (7:3), m.p. 172–174° from benzene–hexane, 27% yield, specific activity 26,000 d.p.m./μmole, 47% loss in activity. The latter had an identical infrared spectrum and the same running rate on a ligroin–propylene glycol system as an authentic sample.

Equilibration of Androst-4-ene-3,17-dione (IV).—Androst-4-ene-3,17-dione (32 mg., specific activity 7200 d.p.m./μmole) was treated under reflux with 100 ml. of 2% potassium hydroxide in 80% methanol for 2 hr. The mixture was neutralized with 6 *N* hydrochloric acid (pH 5–7). The methanol was removed by evaporation and the aqueous residue was extracted with benzene. After removal of solvent, the residue was purified to constant specific activity using thin layer chromatography on silica gel G (20% ethyl acetate in benzene) and recrystallization (benzene–hexane). Two equilibrations were necessary to obtain constant specific activity (620 d.p.m./μmole; % loss of tritium at C-6, 91%).

(12) H. J. Brodie, S. Baba, M. Gut, and M. Hayano, data to be published.

(13) For a more detailed discussion of a similar reaction, see E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, **78**, 6269 (1963).

(14) R. F. Nystrom and N. S. Ragen, *Chem. Ind.* (London), 1165 (1961); R. F. Nystrom, "Advances in Tracer Methodology," Vol. 1, S. Rothchild, Ed., Plenum Press, New York, N. Y., 1963, p. 46.

(15) S. Katz and J. R. Broich, *J. Chromatog.*, **6**, 514 (1961).

(16) The efficiency of the counter was not recorded when the final specific activity of II was determined. It usually varied by ±5%.

6β-Bromoandrost-4-ene-3,17-dione (IV).—A solution of 105 mg. of androst-4-ene-3,17-dione (specific activity 7200 d.p.m./μ mole) in 20 ml. of carbon tetrachloride was refluxed with 181 mg. of NBS for 5 hr. in the dark. After filtration of succinimide, the solvent was removed by evaporation, and the residue was chromatographed on thin layer (silica, 10% acetone in benzene). Four zones were noted on development with iodine vapor; one contained the 6β-bromo compound and another slightly more polar zone contained starting material. The product was rechromatographed three times in the same system until constant specific activity and extinction coefficient were obtained. The pure product, after recrystallization from ethyl acetate had m.p. 168–170° dec.; ultraviolet absorption, λ_{max}^{MeOH} 246 mμ (ε 11,120)[17]; specific activity 2047 d.p.m./μmole; % loss at C-6, 78% (corrected for 9% stable tritium).

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(17) L. Dorfman, *Chem. Rev.*, **53**, 47 (1953); L. Ruzicka, W. Bosshard, W. H. Fischer, and H. Wirg, *Helv. Chim. Acta*, **19**, 1147 (1936).

Notes

Synthesis and Structures of α-Tolyl- and α-Xylylisobutyric Acids

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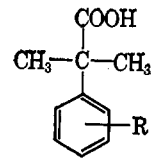
α-Arylisobutyric acids were required for a study of α,α-dimethylhomoaromatic acids. Previous workers prepared mixed α-tolylisobutyric acids by alkylation of toluene with ethyl α-bromoisobutyrate and aluminum bromide[1] or with methacrylic acid and aluminum chloride,[2] and claimed to have isolated the *ortho* and *para* isomers.[1,2] α-(*o*-Tolyl)isobutyric acid was oxidized by alkaline permanganate to an α,α-dimethylhomophthalic acid IV that readily formed an anhydride.[3] The



I, R = *o*-CH_3
II, R = *m*-CH_3
III, R = *p*-CH_3
IV, R = *o*-COOH
V, R = *m*-COOH
VI, R = *p*-COOH

structure of IV was further substantiated by two independent syntheses from *ortho*-substituted compounds.[3,4]

The α-(*m*-tolyl)isobutyric acid (II), m.p. 71–73°, reported in the present work is probably the isomer previously isolated and claimed to have the *para* orientation. The large increase in the proportion of this isomer with reaction temperature is more characteristic of a *meta* isomer (34% at −10–0° and 58% at 30–40°). The infrared absorption exhibited by this acid at 12.7 μ and the 13.2-μ band observed for the *ortho* isomer are consistent with the bands reported for other *meta*- and *ortho*-disubstituted benzenes.[5]

Alkaline permanganate oxidation of II resulted in an α,α-dimethylhomophthalic acid V that gave a broad aromatic signal in the n.m.r. spectrum. Fractionation of the acids obtained from oxidation of the mixture of α-tolylisobutyric acids led to the isolation of a less soluble, higher melting α,α-dimethylhomophthalic acid VI that showed the symmetrical splitting of aromatic bands in the n.m.r. spectrum expected for the *para* isomer.

The methyl proton portion of the n.m.r. spectrum of the mixture of α-tolylisobutyric acids in benzene or carbon tetrachloride exhibits a single *gem*-dimethyl proton peak and partially resolved aromatic methyl proton bands. It was shown, using samples of the *meta* and *ortho* isomers obtained by fractional crystallization, that the *meta* isomer is resolved in carbon tetrachloride solution and the *ortho* isomer is resolved in benzene solution (Fig. 1). Estimation of the isomer distribution using these curves gave values of the order obtained by gas chromatographic analysis of the methyl esters. In the study of the solvent effects for the aromatic protons of *para*-substituted benzenes, Schaefer and Schneider[6] suggested that the changes in relative chemical shifts are due to weak hydrogen bonding. The *ortho* effect, observed for the α-tolylisobutyric acids in benzene solution, occurs for the xylenes and a series of substituted toluenes. The utility of benzene as a solvent for n.m.r. spectroscopy is complicated by the presence of "ring current" effects and solvent–solute interactions. However, a study of the methyl protons of substituted toluenes in preparation in these laboratories indicates benzene is a solvent of choice for identification or estimation of *ortho* isomers.

Four of the five possible α- (*o*- and *m*-xylyl-) isobutyric acids were isolated from the condensations of *o*- or *m*-xylene with methacrylic acid. Only one, the α-(*p*-xylyl)isobutyric acid, had been previously reported.[2] *o*-Xylene recovered from a reaction mixture did not

(1) H. Rupe and J. Burgen, *Ber.*, **44**, 1222 (1911).
(2) J. Colonge and G. Weinstein, *Bull. soc. chim. France*, 820 (1951).
(3) S. Gabriel, *Ber.*, **20**, 1198 (1887).
(4) M. Anchel and A. H. Blatt, *J. Am. Chem. Soc.*, **63**, 1948 (1941).
(5) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1954, p. 64.
(6) T. Schaefer and W. G. Schneider, *J. Chem. Phys.*, **32**, 1218 (1960).