[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY]

Reduction of 7α-Bromosteroids with Tritium[1]

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Received August 12, 1959

7α-Bromocholesterol acetate was reduced catalytically with tritium with the incorporation of the bulk of tritium in the 7α- position of the resulting cholesterol-H³. Details of the isotope distribution are shown and a possible mechanism to account for this distribution is indicated.

A growing interest in tritium as a radioactive tracer, caused by its low cost and the high specific activities made possible, stimulated this study on the specific labeling of steroids. It was of particular interest to study the stability of the isotopic label and also to investigate the position(s) of the introduced isotope.

The introduction of tritium in specific positions of the steroid molecule is carried out the most readily either by the tritiation of a multiple bond or by the reduction of an appropriate halosteroid with tritium.[2] An excellent study of the first method, including the distribution of isotope and also of the mechanism as applied to the deuteration of the double bond of cholesterol acetate, has already been published.[3] The present paper provides a study of the second method, namely the catalytic reduction of 3β-acetoxy-7α-bromo-Δ⁵-steroids with tritium.[4] Labeling in the 7- position has the advantage that the label would "stick" in most biological experiments; *e.g.*, in contradistinction to 16-tritiated steroids, where oxidation to the 17-ketone would render the label labile by enolization of its α-ketone.

7α-Bromocholesterol acetate[5] was reduced catalytically[4] with a hydrogen-tritium mixture, containing one Curie (1 C) per mmole. The reduced product was saponified with methanolic sodium hydroxide solution and the cholesterol obtained had, after chromatographic purification, an activity of 0.75 C per mmole (calculated 0.5 C per mmole).

That the introduction of significantly more than one atom of tritium for one atom of bromine was accomplished without exception was substantiated by the palladium catalyzed reduction of a series of steroids, *e.g.*, 7α-bromo-3β-acetoxypregn-5-en-20-one, 7α-bromo-3β,17α-dihydroxypregn-5-en-20-one 3β-acetate and 7α-bromoandrostenolone acetate.[6]

The mechanism by which this catalytic hydrogenation took place with the usual stereospecificity might possibly have been brought about by coordination of the activated hydrogen atoms with palladium atoms in a d^2sp^3 fashion, whereby the attack of such "palladium hydride" would be sterically controlled. The attack of "palladium tritide" on the polarized C_7-Br_α bond might proceed through a cyclic intermediate formed from the less hindered α-side of the steroid molecule, which then in turn collapses by the heterolysis of the carbon-bromine bond, assisted by tritide ion transfer to C_7 and tritium cation transfer to the negatively charged bromine.[7]

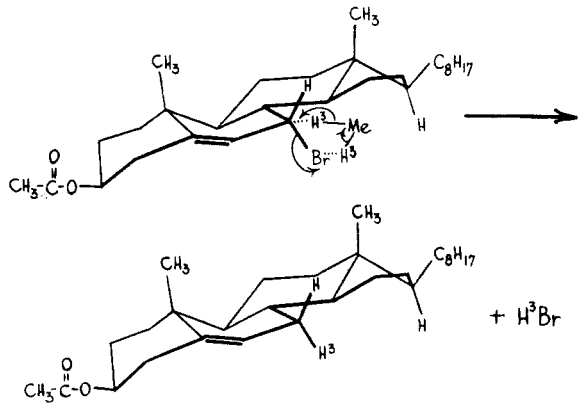

Figure 1

The axial orientation of the introduced isotope was established by reducing 7α-bromocholesterol acetate with pure deuterium.[4] Deuterium was chosen for this experiment because the use of tritium in a concentration sufficient for detection and interpretation of the conformation of the isotope by infrared analysis would have made the labeled product extremely unstable due to auto-

(1) Presented in part at the 135th Meeting of the American Chemical Society, Boston, Mass., April 1959. This investigation was supported in part by the AEC contract AT(30-1)-918.

(2) The reduction of ketones is omitted intentionally, since this would necessitate the rather lengthy and involved preparation of lithium aluminum tritide.

(3) D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, **77**, 139 (1955).

(4) Compare D. K. Fukushima, S. Lieberman, and B. Praetz, *J. Am. Chem. Soc.*, **72**, 5205 (1950).

(5) K. Ziegler, A. Späth, W. Schumann, and E. Winkelmann, *Ann.*, **551**, 80 (1942). For the assignment of the α-orientation for the bromine atom see A. E. Bide, H. B. Henbest, E. R. H. Jones, and P. A. Wilkinson, *J. Chem. Soc.*, **1948**, 1788; L. F. Fieser, *Exp.*, **6**, 312 (1950).

(6) Marcel Gut and Milan Uskoković, *J. Org. Chem.*, **24**, 673 (1959).

(7) Compare F. J. McQuillin, *Chem. and Ind.*, **1951**, 251.

radiation, and because a determination of the orientation of deuterium substituents in steroids had already been accomplished.[8] The deuterated compound showed two weak bonds at 2155 and 2130 cm.$^{-1}$, characteristic of the axial orientation of the deuterium and in excellent agreement with the data in the literature,[8] while no absorption around 2170 cm.$^{-1}$ (equatorial orientation) could be observed.

This result is in agreement with the proposed mechanism, and a series of well known controlled oxidations was applied to the tritiated molecule to establish the relative concentration of the isotope in different positions.

Tritiated cholesterol (500 mC/mmole) was converted to cholesterol acetate (same specific activity) and the acetate was treated with *N*-bromosuccinimide[5] to give 7α-bromocholesterol acetate at a specific activity of 115 mC/mmole, the 7α-position therefore accounting for 77% of the total activity.

The conclusion that the total activity lost is derived from the 7α- position is based on spectral evidence (no equatorial isotope was detected). This suggests that the Wohl-Ziegler bromination proceeds in this case by preferential removal of the 7α-hydrogen by the succinimide radical followed by preferential formation of the 7α-bromo compound in the reaction of the allylic radical with *N*-bromosuccinimide.

Since only one hydrogen is removed in one step it seems proved that the original 7β-hydrogen is retained in the end product and thus the relation between the configuration of the bromine in the product and the configuration of the removed hydrogen established.

Oxidation of cholesterol (500 mC/mmole) with aluminum *tert*-butoxide[9] gave Δ^4-cholestenone containing 410 mC/mmole, indicating that the positions 2, 3, 4, and 6 contained at least 18% of the isotope. Finally, oxidation of cholesterol acetate (500 mC/mmole) with *tert*-butyl chromate[10] yielded 7-ketocholesterol acetate, which, after hydrolysis and thorough equilibration, yielded 7-ketocholesterol with an activity of 70 mC/mmole, demonstrating for the positions 8, 7, 6, and 4 an isotope content of 86%.

Although the incorporation of more than the calculated amount of isotope had been observed and discussed[3] before, it is noteworthy that milder conditions, such as palladium *vs.* platinum catalyst, neutral solution (ethyl acetate *vs.* acetic acid) and a much shortened reaction time bring about the same effect. Since the positions which have been analyzed for isotope content account for 104% (4, 6, 7, and 8 = 86% and 2, 3, 4, and 6 = 18%), there is little likelihood that there would be any isotope in ring C or D. Possible introduction of isotope into position 4 might be explained by the fact that the 7α-bromocholesterol acetate, which was not chromatographed prior to the reduction, might have contained minute amounts of 4β,7α-dibromocholesterol acetate.[11] The small amount of isotope (9%) found in positions 4, 6, 7β- and 8β- might result either from an isomerization of the bromide, which is easily brought about by polar solvents[12] or by the substitution of bromine for tritium in a S_N^2 fashion.

The two oxidation experiments lead to the conclusion that roughly 80% of the label is at the 7-position. The spectral evidence indicates that the bulk of the label must be at 7α.

EXPERIMENTAL

Radioactivity measurements were made on small aliquots by liquid scintillation in a Packard Tri-Carb spectrometer (Packard Instrument Co., La Grange, Ill.) with an error of ±10%. The infrared spectra were recorded on a Beckman model IR4 infrared spectrophotometer. Melting points were determined on a Fisher-Johns hot stage and are uncorrected. The chromatographic separations were made either on Davison Silica Gel Mesh 100-200 or on Woelm alumina, neutral, activity grade 1.

7α-Bromocholesterol acetate from cholesterol acetate. This reaction was carried out as described by Bide *et al.*[13] and the product melted at 107–109°.

Cholesterol-7α-H^3 from 7α-bromocholesterol acetate. This reaction was carried out as indicated by Fukushima *et al.*[4]

To a suspension of 3 g. of prereduced 5% palladium on calcium carbonate in 5 ml. of dry ethyl acetate was added 400 mg. of 7α-bromocholesterol acetate. Then the mixture was shaken for 35 min. together with a tritium-hydrogen mixture, containing 1 C per mmole. After removal of the residual gas the catalyst was filtered off, the solvent removed and the residue hydrolyzed by refluxing with 5% methanolic potassium hydroxide solution for 1 hr. After the usual workup and chromatography on alumina there was obtained 260 mg. cholesterol-7α-H^3, which, after recrystallization from ethanol, gave 241 mg. pure cholesterol-7α-H^3, m.p. 148–149°. This material had an activity of 75 × 10^6 dps/mg. or 0.75 C/mmole. On subsequent equilibrations the specific activity remained the same.

7α-Bromocholesterol acetate from cholesterol-7α-H^3. Cholesterol-7α-H^3 (0.5 C/mmole) was acetylated with acetic anhydride-pyridine and the resulting crude acetate chromatographed on alumina. The pure material had the same specific activity as the free alcohol. The acetate was then treated with *N*-bromosuccinimide as indicated above and the bromo compound obtained was recrystallized twice from petroleum ether. The product melted 106–109° and had a specific activity of 115 mC/mmole.

Cholestenone-7α-H^3 from cholesterol-7α-H^3. Cholesterol-7α-H^3 (0.5 C/mmole) was oxidized with acetone and aluminum *tert*-butoxide.[10] The crude cholestenone was

(8) E. J. Corey, M. G. Howell, A. Boston, R. L. Young, and R. A. Sneen, *J. Am. Chem. Soc.*, **78**, 5036 (1956).

(9) R. V. Oppenauer, *Rec. trav. chim.*, **56**, 137 (1937).

(10) R. V. Oppenauer and H. Oberrauch, *Anal. assoc. quim. Argentina*, **37**, 246 (1949).

(11) Another reduction of very carefully purified 7α-bromocholesterol acetate (m.p. 110–111°) with tritium was shown to contain this time 9% of the radioactivity in position 3 + 4. Compare S. Lieberman and D. K. Fukushima, *J. Am. Chem. Soc.*, **72**, 5211 (1952).

(12) H. Schaltegger and F. X. Müllner, *Helv. Chim. Acta*, **34**, 1096 (1951).

(13) A. E. Bide, H. B. Henbest, E. R. H. Jones, R. W. Peevers, and P. A. Wilkinson, *J. Chem. Soc.*, **1948**, 1783.

equilibrated with alkali and then chromatographed on alumina and had a specific activity of 410 mC/mmole.

7-Ketocholesterol from cholesterol acetate-7α-H³. Cholesterol acetate-7α-H³ was oxidized with *tert*-butyl chromate[10] and the crude 7-ketocholesterol acetate obtained was directly hydrolyzed by heating under reflux for 1 hr. in 1% methanolic potassium hydroxide solution. After the usual workup of the hydrolyzate, the product was chromatographed on silica gel and the pure material, obtained from the ethyl acetate-benzene eluates and recrystallized from methanol, had a specific activity of 70 mC/mmole.

Cholesterol-7α-D from 7α-bromocholesterol acetate. This reduction was carried out as described above, except that the palladium-on-calcium carbonate was prereduced with carrier free deuterium and that carrier free deuterium was used for the reduction proper. The deutero compound had two absorption bands in the C-D stretching region at 2155 and 2130 cm.$^{-1}$

3β-Hydroxypregn-5-en-20-one-7α-H³ from 3β-acetoxy-7α-bromopregn-5-en-20-one. The 3β-acetoxy-7α-bromopregn-5-en-20-one was prepared as indicated by Antonucci *et al.*[14] and the bromo compound was then reduced with a tritium-hydrogen mixture containing 0.5 C/mmole as described for the 7α-bromocholesterol acetate. The crude reaction product was hydrolyzed, worked up in the usual manner and finally chromatographed on silica gel. The pure pregnenolone had a specific activity of 325 mC/mmole.

3β,17β-Dihydroxypregn-5-en-20-one-7α-H³ from 3β, 17α-dihydroxypregn-5-en-20-one 3β-acetate. The bromination with *N*-bromosuccinimide followed by the reduction with tritium was carried out as described above. In this case the specific radioactivity amounted to 135% of the calculated amount.

3β-Hydroxyandrost-5-en-17-one-7α-H³ from 3β-acetoxy-7α-bromoandrost-5-en-17-one. This preparation had already been described.[6]

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(14) R. Antonucci, S. Bernstein, D. Giancola, and K. J. Sax, *J. Org. Chem.*, 16, 1126 (1951).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FACULTY OF SCIENCE, CAIRO UNIVERSITY, AND THE LABORATORIES OF THE MEMPHIS CHEMICAL COMPANY, CAIRO]

Experiments with Furochromones. A Color Test for Hydroxyfurochromones and Related Substances with Uranyl Acetate

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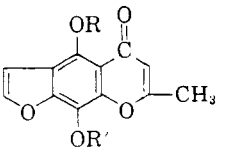
Received August 18, 1959

Syntheses for 8-(ω)-carboxymethoxy)-5-methoxy- (Ik) and 5-(ω-carboxymethoxy)-2-methylfuro-4′,5′,6,7-chromone (IId) are described. Whereas oxidation of IId with chromic acid effects the destruction of the furan ring with the formation of 5-(ω-carboxymethoxy)-6-formyl-7-hydroxy-2-methylchromone (IIIb), controlled oxidation with hydrogen peroxide in alkaline medium gives the corresponding furanosalicylic acid (Vc). The furanosalicylic acids Va–b and Vd are similarly obtained. Alkaline hydrolysis of Ik and IId results in the formation of the corresponding benzofuran derivatives (IVa–b) respectively. Hydroxyfurochromones and related substances (*cf.* Table I) give a color reaction with uranyl acetate solution. The importance of the free hydroxyl group in the *peri*- position to the carbonyl group is stressed (*cf.* Table II).

The recent publication of the preparation of a number of active water soluble derivatives of 2-methyl-5-hydroxy-8-methoxy-6,7-furochromone by the introduction of solubilizing groups, e.g., amino-,[1] or carboxyl group[2] prompts us to report some related work which we carried out some time ago.



Ia. R = R′ = CH_3
b. R = CH_2COOH; R′ = CH_3
c. R = H; R′ = CH_3
d. R = $CH_2COOC_2H_5$; R′ = CH_3
e. R = R′ = H
f. R = R′ = $CH_2COOC_2H_5$
g. R = R′ = CH_2COOH
h. R = H; R′ = CH_2COOH
i. R = H; R′ = $CH_2COOC_2H_5$
j. R = CH_3; R′ = $CH_2COOC_2H_5$
k. R = CH_3; R′ = CH_2COOH
l. R(R′) = $CH_2COOC_2H_5$; R′(R) = CH_2COOH.

(1) J. P. Fourneau, *Ann. Pharm. Franç.*, 11, 685 (1953).
(2) C. Musante and S. Fattuta, *Ann. Chim.* (Rome), 45, 918 (1955); L. Ritter and H. Kunsch, German Patent, 952,899, Nov. 22, 1956; *Chem. Abstr.*, 53, 2258 (1959).

The synthesis of 5-(ω-carboxymethoxy)-8-methoxy-2-methylfuro-4′,5′,6,7-chromone (Ib) by Mukerjee and Seshadri[3] now has been confirmed in our laboratories. The isomeric khellin derivative, namely, 8-(ω-carboxymethoxy)-5-methoxy-2-methylfuro-4′,5′,6,7-chromone (Ik) is now, similarly, prepared by allowing khellin-hydroquinone (Ie) to react with ethyl chloroacetate to yield Ii which, upon hydrolysis, produces Ik in an overall yield *ca.* 50% based on Ia used.

Refluxing 5,8-di(ω-carboxymethoxy)-2-methylfuro-4′,5′,6,7-chromone (Ig)[4] with 20% hydrochloric acid and/or 60% hydrobromic acid[5] does not accomplish the preparation of 8-(ω-carboxy-

(3) S. K. Mukerjee and T. R. Seshadri, *Proc. Indian Acad. Sci.*, 35A, 323 (1952).
(4) A. Schönberg and A. Sina, *J. Am. Chem. Soc.*, 72, 3396 (1950).
(5) These are common reagents to effect selective demethylation of chromones in position 5, without causing an undesired rearrangement (*cf.* H. Abu-Shady and T. O. Soine, *J. Am. Pharm. Assoc.*, 41, 325 (1952); S. K. Mukerjee and T. R. Seshadri (ref. 3).