# STEROIDS XLI.[1)] PREPARATION OF 9α, 11ξ-TRITIATED ESTRONE-3-METHYL ETHER AND FOLLOWING SYNTHESES OF 17α-$CH_2X$-DERIVATIVES OF ESTRADIOL

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SUMMARY

*The preparation of 9α, 11ξ-tritiated estrone-3-methyl ether with high specific activity is described. The following syntheses gave 17α-$CH_2X$-derivatives of estradiol (X = CN and SCN) with high specific activity and radiochemical purity higher than 98%. Radiolysis and storage conditions are examined.*

## I N T R O D U C T I O N

During our work on the synthesis and the biological effects of steroid hormones, especially those with a 17α-$CH_2X$-substituent [1], we investigated the possibility of producing labelled compounds. Without such compounds pharmacokinetic research work is scarcely done at present.

Two requirements are to be considered with labelling:

1. The radioactive isotope must be in a position biochemically stable in order to allow the qualitative assay after metabolizing and

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1) 40.Mitt.: K.Ponsold u. B.Schönecker, Tetrahedron, to be published.

2. the specific activity of the labelled substance has to be at least 1 Ci/mM in order to allow the quantitative assay of the steroids which are used in very low quantities.

Generally the carbon atoms of the steroid nucleus represent stable positions. Therefore carbon-14 can be used for labelling. But the preparation of carbon-14 labelled compounds implies a complicated synthesis (ring opening and ring closure). Besides that, the expensive carbon-14 results in maximum specific activity of only 60 mCi/mM for one labelled position.

In view of this tritium is an alternative to carbon-14. It is essentially cheaper, the synthetic efforts are lower and the realizable specific activity (theoretically 29 Ci/mM) is three decimal powers higher. However, not all the hydrogen atoms of the steroid nucleus are equally stable, biochemically. This is taken into consideration by choosing the position of labelling.

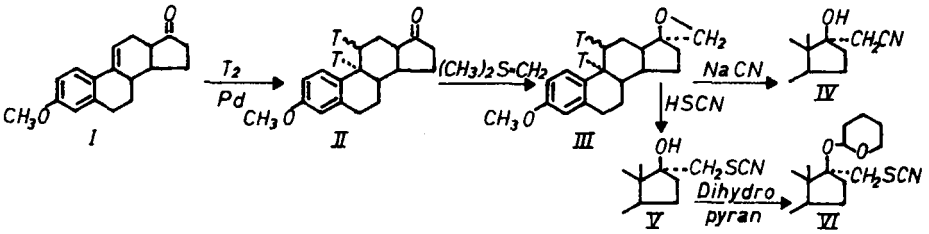
We decided therefore to incorporate tritium. As a method we used the reduction of an olefinic double bond with tritium gas which results in a specifically labelled product of a maximum of 58 Ci/mM. The frequently used method of WILZBACH [2] does not satisfy the requirements given above.

For our investigations we needed tritium labelled 17α-$CH_2X$-derivatives of estradiol-3-methyl ether using estrone-3-methyl ether as starting material. As this compound is easily accessible by total synthesis and usable also as a key-product for steroids with different basic structures, the labelling of estrone-3-methyl ether is of general importance. Estrogens labelled with tritium in 6,7-position [3-5] and 2,4-position [6] have been known for quite some time. However, labelling in these positions is unfavourable for biochemical investigations because metabolism is preferred in these positions. Based on other investigations of our department [7] 9,11-dehydro-estrone-3-methyl ether (I) is very easily accessible. As the 9,11-positions of estrogens are not metabolized we decided to prepare 9α,11ξ-tritiated estrone-3-methyl ether.

Recently a paper has been published also describing the tritium labelling of estrone-3-methyl ether in 9,11-position [8], but without experimental details.

For the preparation of 17α-$CH_2X$-substituted steroids we found a fundamental way in our department via 17β-spiro-epoxides [9] and

their cleavage with nucleophilic agents [17].



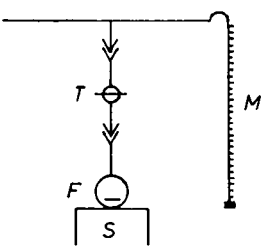

In this way we obtained 3-methoxy-estra-1,3,5(10)-trien-17β-spiro-1',2'-oxiran-9α,11ξ-$^3$H (III), which gave by opening with NaCN 3-methoxy-17α-cyanomethyl-estra-1,3,5(10)-trien-17β-ol-9α,11ξ-$^3$H (IV) and with HSCN 3-methoxy-17α-thiocyanomethyl-estra-1,3,5(10)-trien-17β-ol-9α,11ξ-$^3$H (V), from estrone-3-methyl ether-9α,11ξ-$^3$H (II). V was transferred to 17β-tetrahydropyranyl ether (VI) by reaction with dihydropyran.

## E X P E R I M E N T A L

The labelling experiments were made in a special apparatus at the Zentralinstitut für Kernforschung Rossendorf [10]. For measuring the specific activity we used a Beckman liquid scintillation spectrometer LS-233. A Berthold-Frieseke thin-layer scanner was employed for monitoring the chromatograms. The chemicals used were of p.a. grade. The solvent system for thin-layer chromatograms on Kieselgel (Merck) was benzene/methanol (9:1). After development the chromatogramms were sprayed with vanillin-sulfuric acid and heated to 180°C for making the spots visible. In order to minimize the radiolytic effects the labelled substances were stored at low temperatures.

### Estrone-3-methyl ether-9α,11ξ-$^3$H (II)

0.2 mM I (56.4 mg), 50 mg palladium black [11] and 10 ml ethyl acetate were put into a flask F with a magnetic stirrer. Flask F




was connected to the labelling apparatus via a vacuum tap T. The contents of the flask was frozen in with liquid nitrogen and the apparatus evacuated. Then the apparatus was filled with tritium; the manometer M showing 116 torr. With that 70 Ci tritium were in the flask. Tap T was closed, the liquid nitrogen removed, the flask warmed up to room temperature and agitated with stirrer S for one hour. During the reaction the catalyst assumed a fungeous appearance. The tritium pressure in flask F was 442 torr at room temperature. After reaction the content of the flask was again frozen in and tap T opened. The manometer M showed 70 torr. The remaining tritium (45 Ci) was reabsorbed and after aeration **the content of the flask was thawed. 1 ml of the highly active solution was removed and stored at -20°C for making radiolytic investigations. From the main part of the solution the** catalyst was filtered off and the filter washed with 15 ml of ethanol, which contained 230 mg of inert II. In this way the active II was diluted 5.5 fold. The solution was warmed up to 75°C under stirring in order to remove labile tritium. This was followed by freeze-drying and determination of the specific activity. The result was 10.45 ± 0.45 Ci/mM while the condensate had a total activity of about 6 Ci. The labelled product thus obtained was used without further purification because of being radiochemically pure according to t.l.c. The yield was quantitative (m.p. 169 - 171°C).

3-Methoxy-estra-1,3,5(10)-trien-17β-spiro-1',2'-oxiran-9α,11ξ-$^3$H (III)

450 mg of II (specific activity 2.6 Ci/mM) were dissolved in 8 ml of dimethylformamide. After adding 1 g of K-tert.butylate and 1 g of trimethylsulfonium-iodide the mixture was shaken for 20 minutes at room temperature. Then it was poured into ice-water and the precipitate formed was extracted with ethyl acetate. By freeze-drying 438 mg (yield 90%) of oxiran III (m.p. 103 - 105°C) were obtained. After determination of the specific activity (2.5 Ci/mM) the substance was used without further puri-

fication being radiochemically pure according to t.l.c.

3-Methoxy-17α-cyanomethyl-estra-1,3,5(10)-trien-17β-ol-9α,11§-$^{3}$H (IV)

438 mg of III (specific activity 2.5 Ci/mM) were dissolved in 50 ml of methanol and 1 g of NaCN was added. The reaction mixture was kept at 60°C for two hours. Then it was poured into ice-water and the precipitate extracted with ethyl acetate. After freeze-drying the specific activity and radiochemical purity were determined. On account of two impurities detected by t.l.c. the product was recrystallized twice from methanol. Thus 380 mg (yield 80%) of IV with a radiochemical purity higher than 98% and a specific activity of 2.4 Ci/mM were obtained (m.p. 144 - 147°C).

3-Methoxy-17α-thiocyanomethyl-estra-1,3,5(10)-trien-17β-ol-9α,11§-$^{3}$H (V)

To 560 mg of III (specific activity 1.6 Ci/mM), an ether solution of thiocyanic acid was added which was obtained from 1 g of $NH_4SCN$, dissolved in 3 ml of water, by addition of 1 g of phosphoric acid (85%) and following extraction with 15 ml of ether. The oxiran cleavage proceeded under dissolution of III, followed by the precipitation of V. After standing for two hours the reddish ether layer was removed and the crystals were washed with ether several times. After freeze-drying 570 mg of V (yield 85%) with a specific activity of 1.5 Ci/mM were obtained. The product was used without further purification having a radiochemical purity higher than 95% according to t.l.c. (m.p. 158 - 160°C).

3-Methoxy-17α-thiocyanomethyl-estra-1,3,5(10)-trien-17β-ol-tetrahydropyranyl ether-9α,11§-$^{3}$H (VI)

To a solution of 570 mg of V (specific activity 1.5 Ci/mM) in a mixture of 10.5 ml of benzene, 2.9 ml of methylen chloride and 1.2 ml of dihydropyran 0.5 ml of a solution of 22.6 mg of p-toluenesulfonic acid in 2.6 ml of benzene were added every 30 minutes. After 4 hours the reaction mixture was extracted with water to remove p-toluenesulfonic acid. After freeze-drying of

the organic layer an oily residue of 460 mg of VI (yield 64%) was obtained, which crystallized after dissolution in a mixture of 1.6 ml of diisopropyl ether and 9.2 ml of methanol and cooling. After a twofold recrystallisation from methanol 134 mg of VI with a specific activity of 1.4 Ci/mM and a radiochemical purity higher than 99% were obtained (m.p. 118 - 125°C).

The radiolytic decomposition of labelled compounds during storage is of great importance for long-range pharmacokinetic investigations. Therefore we studied the influence of storage temperature (+5 to -20°C) and state of aggregation (crystalline or dissolved in benzene with c = 20 mg/ml) upon the rate of radiolysis. For this purpose thin-layer chromatograms were evaluated numerically in order to calculate the percentage of radiolysis.

## RESULTS AND DISCUSSION

By presupposition the catalytic reduction of Δ9,11-estrone-3-methyl ether should proceed quantitatively. This was demonstrated by the specific activity of 10.45 ± 0,54 Ci/mM measured with the 5.5 fold diluted II. From that the specific activity of the undiluted II is 57.5 Ci/mM, a value agreeing within the margin of error with the theoretical value of 58 Ci/mM.

The tritium balance of the reaction shows rather high losses. 25 Ci of tritium disappeared during the reaction (70 Ci at the beginning against 45 Ci at the end; see sub II). According to the 0.2 mM of I we needed only 11.6 Ci for the quantitative reduction of the olefinic double bond, that means a loss of about 50% !

About 6 Ci were found in the condensate of freeze-drying, probably as the result of a catalytic isotope-exchange-labelling of the ethyl acetate (the 6 Ci found correspond with a specific activity of 60 mCi/mM), while the remaining 7.4 Ci are adsorbed on the catalyst (7.4 Ci correspond with 2.86 ml tritium).

This unfavourable balance can only be improved by reducing the amount of ethyl acetate used, because reducing both the tritium pressure and the amount of catalyst results, according to our investigations, in an incomplete reduction of the olefinic double bond

The investigation of radiolytic effects showed that the radiolytic decomposition must be taken into account primarily during

storage of crystalline substances. Decomposition cannot be excluded even by storage temperatures of -20°C (35 - 40% of decomposition products after 5 weeks). Storage in benzene solution is more favourable (2 - 3% of decomposition products after 5 weeks at -20°C) although the lower temperatures (below +5°C) are not, in any case, the better conditions. Precipitation of substance and solidification of the benzene under these conditions increase radiolysis. Warming up above room temperature accelerates the decomposition of both solid and dissolved substances considerably (warming up of a benzene solution to 65°C for 4 days results in 15 - 20% of decomposition products). Ethyl acetate must be completely avoided as storage solvent.

Besides that we found the interesting fact that estrone-3-methyl ether (II) showed, under the same storage conditions, a smaller decomposition rate than the $CH_2X$-derivatives. The normal chemical stability of labelled compounds is therefore not to be neglected if considerations about radiolytic effects are made.

As also the level of specific activity has an influence on the rate of radiolysis, the key-product for our radiochemical syntheses, the estrone-3-methyl ether-9$\alpha$,11$\xi$-$^3$H, was reduced in its original activity (10.45 Ci/mM) before storage by inert dilution. Of course the activity was maintained above the minimum of 1 Ci/mM required at the beginning.

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