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Review - Synthesis of 6ß-Hydroxy-3-Keto-4-Ene Steroids. And Synthesis of 6ß-Hydroxy Metabolites of Anabolic Steroids.

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The misuse of anabolic steroids in sports is controlled by laboratories accredited by the Medical Comission (MC) of the International Olympic Committee (IOC). The confirmation of positive cases is based on a gas chromatographic (GC) - mass spectrometric (MS) identification of urinary excreted anabolic steroids and their metabolites **[1]**. For this purpose the synthesis of 6ß-hydroxy metabolites was necessary, which are used as reference compounds.
6ß-Hydroxylation of anabolic steroids in human was published for metandienone in 1963 by Rongone and Segaloff **[2]** and for boldenone in 1971 by Galetti and Gardi **[3]**. The confirmation of the metabolites was based on comparison with synthesized reference substances. In the metabolism of 4-chlorodehydromethyl-testosteroene Schubert et al. **[4, 5]** isolated a 6ß-hydroxy metabolite which was also prepared by microbiological oxidation with *Absidia glauca* **[6]**.

6ß-Hydroxylation of steroids was first described for cortisol in 1954 by Burstein et al.**[7]**, who isolated 6ß-hydroxycortisol from urine after intravenous administration of 270 mg of cortisol to a human subject with Cushing's syndrome and they compared it after acetylation with authentic 6ß-hydroxycortisol diacetate monohydrate. In 1956 Nadel et al. **[8]** published an improved method for isolation of 6ß-hydroxycortisol from urine and identified 6ß-hydroxycortisol also in urine of normal human subjects without prior cortisol application.

The first synthesis of a 6ß-hydroxy steroid with a 3-keto-4-ene structure was described in 1941 by Maximillian Ehrenstein **[9]** who synthesized the acetate of 6ß-hydroxyandrost-4-en-3,17-dione (Fig.1).

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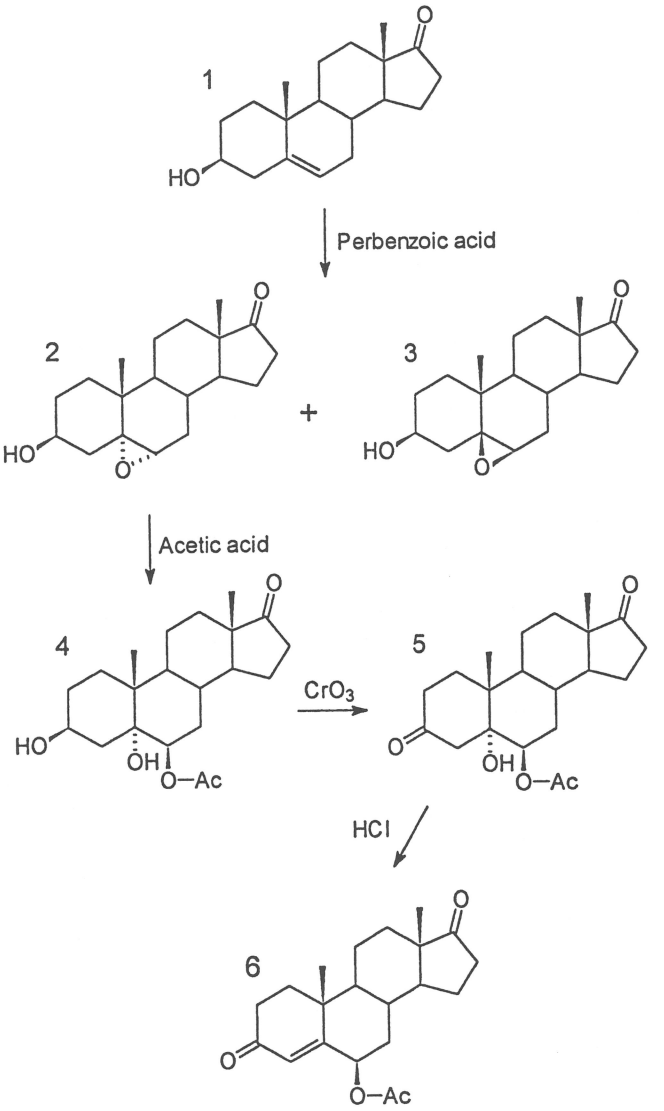

Fig.1 Synthesis of 6ß-acetoxyandrost-4-en-3,17-dione by Maximillian Ehrenstein [9] in 1941

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The synthesis was started with dehydroepiandrosterone **1** which was treated with perbenzoic acid yielding the 5α,6α- **2** and 5ß,6ß-epoxide **3** of dehydroepiandrosterone.
The 5α,6α-epoxide **2** was treated with glacial acetic acid yielding 6ß-acetoxy-3ß,5α-dihydroxyandrostan-17-one **4**, which was oxidized with chromic trioxide to 6ß-acetoxy-5α-hydroxyandrost-an-3,17-dione **5**. Dehydration of the tertiary 5α-hydroxy group to 6ß-acetoxyandrost-4-ene-3,17-dione **6** was achieved with hydrochloric acid.

The configuration of the 6-hydroxy position was first postulated with 6α, but Ehrenstein revised the exact nomenclature and configuration to be 6ß-hydroxy in 1947 **[10]**. The exact configuration could be established when in 1944 Plattner and Lang **[11]** confirmed the configuration of the 5α,6α- and 5ß,6ß-epoxide of cholesterol.
The different hydrolysis kinetic (Fig.2) of the 3ß,6ß-diacetoxy-5α-cholestane **13** and the 3ß,6α-diacetoxy-5α-cholestane **14** was the basis of this confirmation. The hydrolysis kinetic of the 6ß-acetoxy group in **13** was reduced compared to the 6α-acetoxy group in **14**. It was possible to obtain the mono-acetate 6ß-acetoxy-3ß-hydroxy-5α-cholestane **15** of 3ß,6ß-diacetoxy-5α-cholestane **13** using a partial hydrolysis with potassium hydroxide whereas for 3ß,6α-diacetoxy-5α-cholestane **14** both acetoxy groups were hydrolysed under the same conditions. This result is in agreement with the proposed 6α- and 6ß-configuration. The 6ß-acetoxy group has a axial configuration and the hydrolysis is sterically hindered by the neighboured 19-methyl group. The 19-methyl group is not effecting the hydrolysis of 6α-acetoxy group because its orientation is equatorial and the hydrolysis with potassium hydroxide is rapid and no mono acetate could be obtained.
The origin of the 3ß,6ß-diacetoxy-5α-cholestane **13** is shown in Fig.2. The acetate of cholesterol **7** was treated with perbenzoic acid yielding the 5α,6α-epoxide **9** and the 5ß,6ß-epoxide **10**. Hydrogenation of the 5ß,6ß-epoxide **9** with platinum in glacial acetic acid splitted the carbon 5 oxygen bond and 3ß-acetoxy-6ß-hydroxy-5α-cholestane **11** was obtained which then was acetylated to the 3ß,6ß-diacetoxy-5α-cholestane **13**.
On the other hand hydrogenation of the 5α,6α-epoxide **10** with platinum splitted the carbon 6 oxygen bond and 3ß-acetoxy-5α-hydroxy-cholestane was the reaction product.
The origin of the 3ß,6α-diacetoxy-5α-cholstane **14** is also shown in Fig.2. The starting substance was 3ß-hydroxy-5α-cholestane-6-one **8** which was reduced with sodium in ethanol to 3ß,6α-dihydroxy-5α-cholestane **12**. Acetylation with acetic anhydride yielded 3ß,6ß-diacetoxy-5α-cholestane **14**.

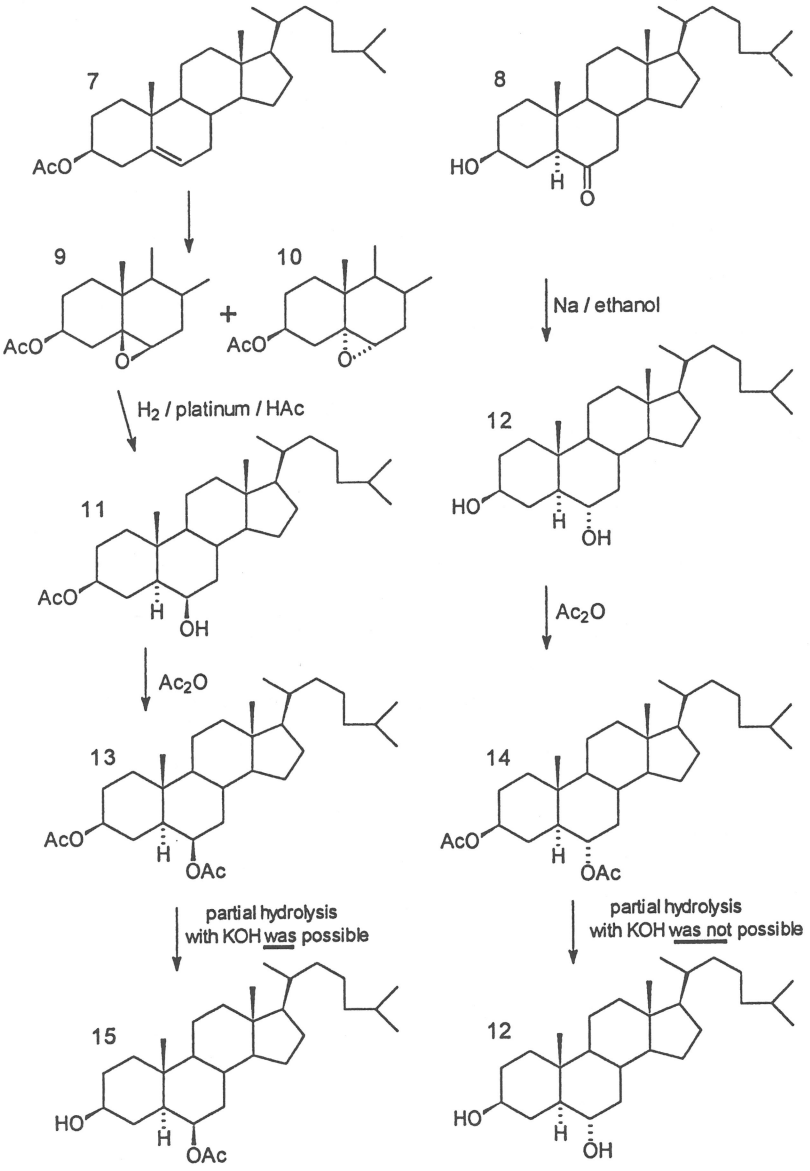

Fig.2 Confirmation of the 6α- and 6ß-acetoxy group in 3ß,6α-diacetoxy-5α-cholestane and 3ß,6ß-diacetoxy-5α-cholestane by Platter and Lang [11] in 1944.

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Reaction of the 6ß-acetoxy group.

The 6ß-acetoxy group of 3-keto-4-ene steroids can be converted to the more stable 6α-acetoxy configuration by treatment with hydrochloric acid in chloroform **[12, 13]**. This is shown in Fig.3 for 6ß-acetoxyandrost-4-ene-3,17-dione **6** which will rearrange by treatment with hydrochloric acid in chloroform at -10°C to the more stable 6α-acetoxyandrost-4-en-3,17-dione **16**.

The 6ß-acetoxy group can be hydrolyzed with diluted potassium hydroxide to 6ß-hydroxyandrost-4-ene-3,17-dione **18**, but is rearranged to 5α-androstane-3,6,17-dione **17** by excess treatment under alkaline conditions **[12, 13]**.

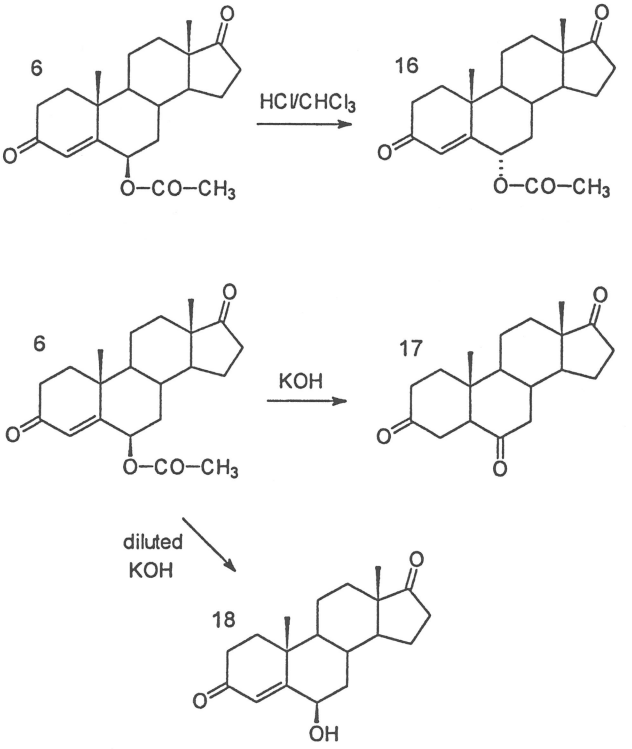

Fig.3 Reaction of 6ß-acetoxyandrost-4-ene-3,17-dione

In 1954 Sondheimer et al. **[14]** used a similar reaction way for the synthesis of 6ß-hydroxy-3-keto-4-ene steroids as Ehrenstein but started the synthesis with the 3-keto-4-ene steroid itself which he converted by ketalization to a 3-ethyleneketal with a shift of the double bond from C-4−5 to C-5−6.
The synthesis of 6ß-hydroxycortisone **25** by Sondheimer et al. **[14]** is illustrated in Fig. 4. Sondheimer started the synthesis with cortisone **19** which was treated with 2-methyl-2-ethyl-1,2-dioxalane to obtain the 3-ethyleneketal of cortisone **20** with a shift of the C-4-5 double bond to C-5-6. The reaction of the ketal with perbenzoic acid yielded a mixture of 5α,6α- and 5ß,6ß-epoxide **21** which both were opened with perchloric acid to 5α,6ß-dihydroxy-dihydrocortisone **22**. Acetylation of the 6ß-hydroxy group with acetic anhydride to the 5α-hydroxy-6ß-acetoxy-dihydrocortisone **23** followed by treatment with hydrochloric acid yielded 6ß-acetoxycorticone **24**. The 6ß-acetyl group was hydrolyzed with methanolic potassium hydroxide at 0°C to 6ß-hydroxycortisol **25**.

In the same year Romo et al. [15] published a two step preperative method. The reaction scheme is shown in Fig.5 for the synthesis of 6ß-hydroxytestosterone 17-acetate **28**. Romo started the synthesis with the 17-acetate of testosterone **26** which was converted to the enol acetate **27** with isopropenyl acetate catalyzed by para-toluenesulfonic acid. The resulting product was oxidized with monoperphthalic acid yielding the 6ß-hydroxy-testosterone 17-acetate **28** directly.

A similar two step preparative method was published by Dusza et al. in 1962 **[16]**. The reaction (Fig.6) started also with the 17-acetate of testosterone **26** which was converted by treatment with trimethyl orthoformate to the methyl-3,5-dien enol ether **29** which then was oxidized in same way as Romo oxidized the enol-acetate with monoperphthalic acid yielding the 6ß-hydroxytestosterone 17-acetate **28** as main product.

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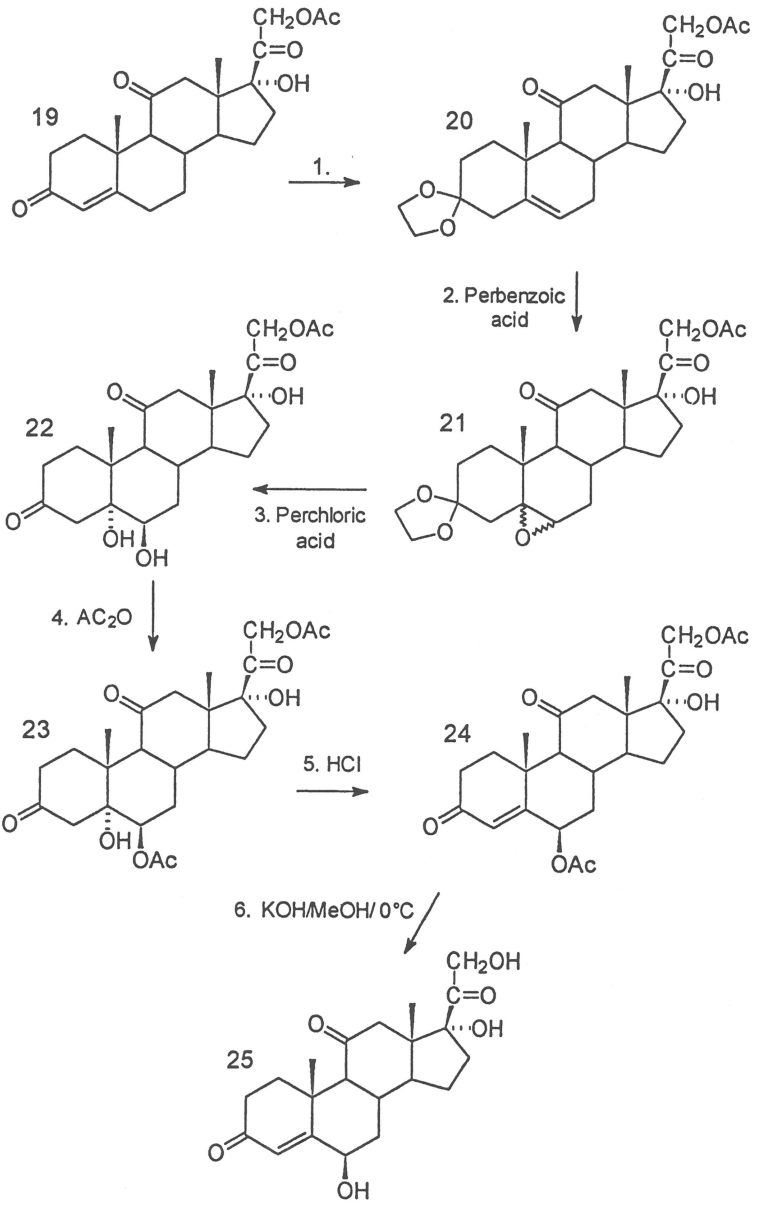

Fig. 4 Synthesis of 6ß-hydroxycortisone by Sondheimer et al. [14] in 1954.

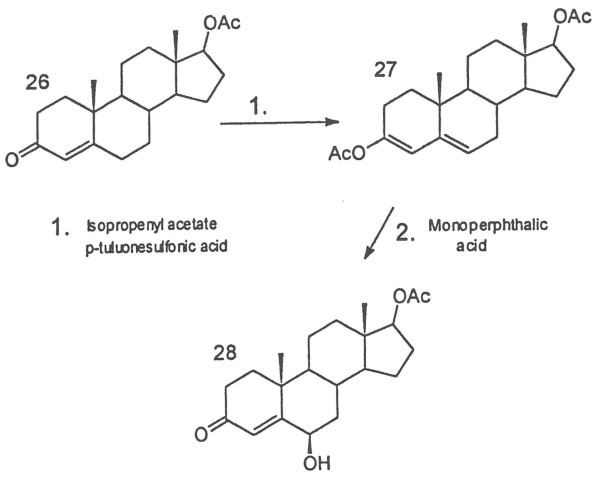

Fig. 5 Synthesis of 6ß-hydroxytestosterone 17-acetate by Romo et al. [15] in 1954.

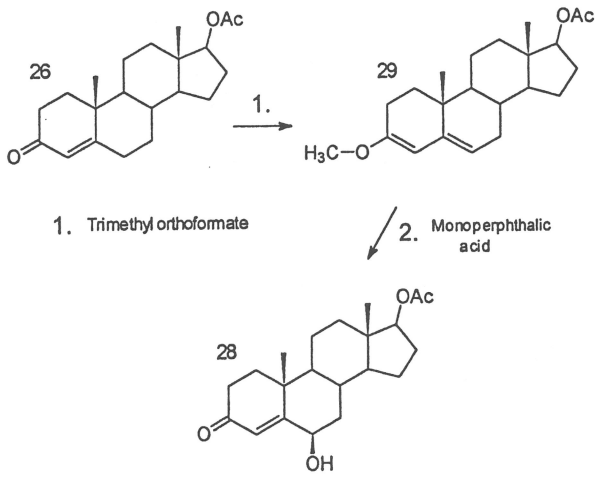

Fig. 6 Synthesis of 6ß-hydroxytestosterone 17-acetate by Dusza et al. [16] in 1962

In 1967 Gardi and Lusignani published a similar procedure to synthesize 6ß-hydroxy steroids [17]. They converted androst-4-en-3-ones to their corresponding n-alkyl-3,5-dien enol ethers especially ethyl enol ethers and expose them dissolved in ethanol to direct sunlight. The autooxidation yielded 6ß-hydroxyandrost-4-en-3-one steroids in high yield.

This reaction is shown for the synthesis of 6ß-hydroxytestosterone 17-acetate **28** in Fig.7. The 17-acetate of testosterone **26** was converted with triethyl orthoformate to the corresponding ethyl enol ether **30** which was autooxidized by direct sunlight yielding 6ß-hydroxytestosterone 17-acetate **28**.

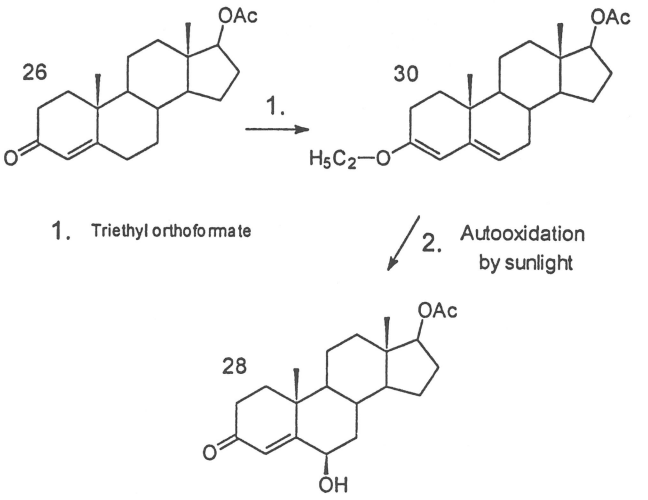

Fig. 7 Synthesis of 6ß-hydroxytestosterone 17-acetate by Gardi and Lusignani [17] in 1962 via autooxidation by sunlight

We tried this reaction to obtain 6ß-hydroxy steroids of the anabolic steroids 4-chlorodehydromethyltestosterone, fluoxymesterone and metandienone. As the formation of n-alkyl-3,5-dien enol ethers of 17α-methyl-17ß-hydroxy steroids by commonly described methods led to dehydration of the acidic lable 17ß-hydroxy group we used trimethylsilyl enol ethers which can be easily performed and exposed them to light.

6ß-Hydroxytestosterone

The 6α- and 6ß-hydroxy isomers of testosterone were synthesized as reference compound to establish the exact configuration of the 6-hydroxy group for the synthesized 6-hydroxy products of metandienone, 4-chlorodehydromethyltestosterone and fluoxymesterone.
The reaction scheme for the synthesis of 6ß-hydroxytestosterone **34** is presented in Fig.8. The reaction started with testosterone **31** which was trimethylsilylated with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA)/ trimethyliodosilane (TMIS) to testosterone TMS ether TMS enol ether **32**. The TMIS was generated by addition of ammonium iodide to MSTFA. This reaction mixture yielded with more than 98% the enol isomer with a 3,5-diene structure. The bis-TMS product **32** was isolated from the rection mixture, dried, suspended in isopropanol, and exposed to a 60 watt spotline lamp for 6 h while stirring. The reaction yielded with about 65% one main reaction product 6ß-hydroxytestosterone 17-TMS ether **33**. The TMS group was hydrolysed by addition of hydrochloric acid to the reaction mixture.

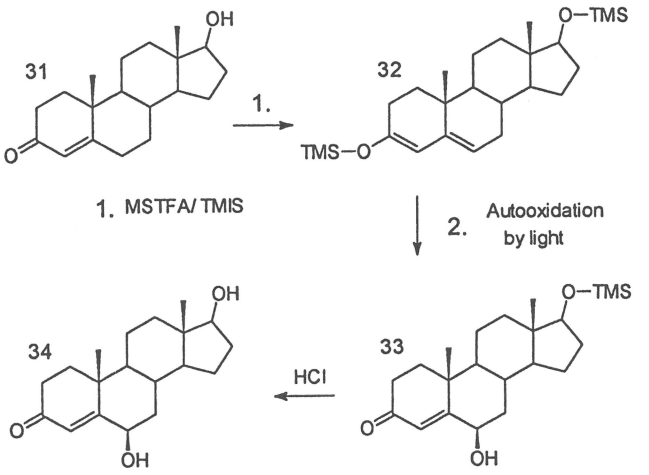

Fig.8 Synthesis of 6ß-hydroxytestosterone by Schänzer and Donike [18]

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6ß-Hydroxymetandienone

With the same reaction conditions as for the synthesis of 6ß-hydroxytestosterone 6ß-hydroxymetandienone **38** was synthesized. The reaction is shown in Fig.9. Metandienone **35** was trimethylsilylated with MSTFA/TMIS to metandienone 17-TMS ether 3-TMS enol ether **36** which was autooxidized in isopropanol by roomlight yielding with 65% 6ß-hydroxymetandienone 17-TMS ether **37** as main reaction product. The reaction mixture was stored for four weeks to darkness until the 17-TMS-ether was completely hydrolyzed to 6ß-hydroxymetandienone **38**. The hydrolysis with hydrochloric acid is also possible but was not performed in this experiment.

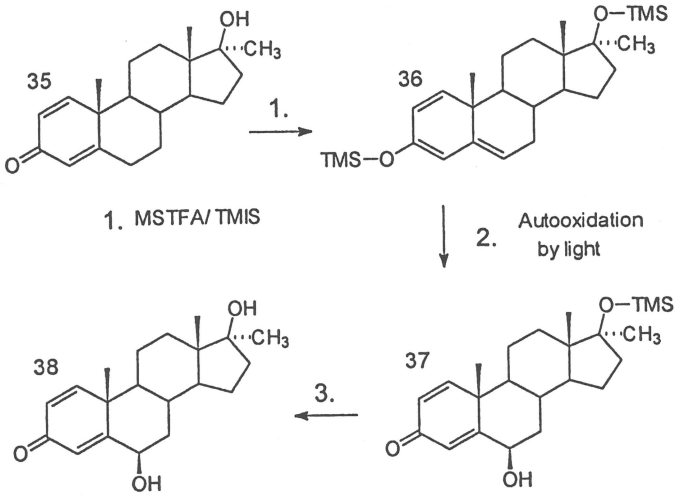

Fig.9 Synthesis of 6ß-hydroxymetandienone by Schänzer and Donike [18]
Metandienone **35**, metandienone 17-TMS ether 3-TMS enol ether **36**, 6ß-hydroxymetandineone 17-TMS ether **37**, 6ß-hydroxymetandineone **38**.

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6ß-Hydroxy-4-chlorodehydromethyltestosterone

The autooxidation of 4-chlorodehydromethyltestosterone in isopropanol was accompanied by generation of acidic side products (maybe hydrochloric acid) which hydrolyzed the bis-TMS enol ether to such an extend that the reaction was finished after 3.5 h yielding 42% of 4-chlorodehydromethyltestosterone, 29% of 6ß-hydroxy isomer, and 29% of further reaction products. The 17ß-O-TMS ether was completely hydrolyzed under this conditions. A further synthesis using alkaline reaction conditions was not performed.

The reaction is shown in Fig.10. 4-Chlorodehydromethyltestosterone **39** is trimethylsilylated with MSTFA/TMIS to 4-chlorodehydromethyltestosterone 17-TMS ether 3-TMS enol ether **40**. The reaction product is isolated by liquid/liquid extraction with n-pentane against water/potassium carbonate, dried, suspended in isopropanol, and autooxidized by room light (60 watt,spotline lamp). The 6ß-hydroxy 17-TMS ether **41** as intermediate was directly hydrolyzed in the acidic reaction mixture to 6ß-hydroxy-4-chlorodehydromethyltestosterone **42**.

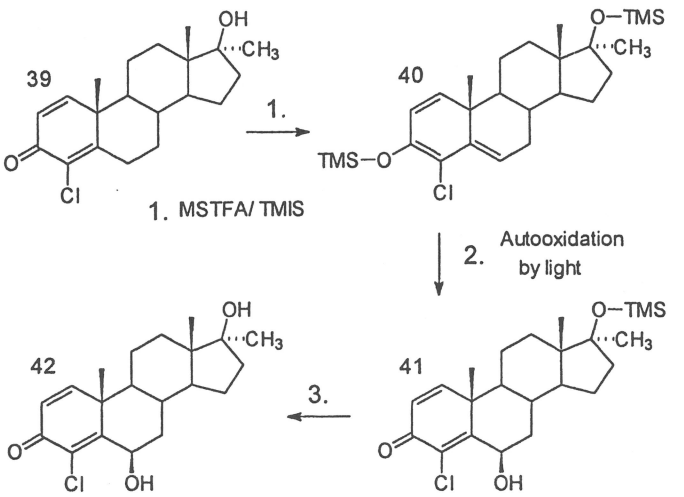

Fig.10 Synthesis of 6ß-hydroxy-4-chlorodehydromethyltestosterone by Schänzer and Donike **[18]**

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6ß-Hydroxyfluoxymesterone

The reaction scheme for the synthesis of 6ß-hydroxyfluoxymesterone **46** is presented in Fig. 11. The synthesis started with fluoxymesterone **43** which was trimethylsilylated with MSTFA/TMIS. The reaction was stopped after 10 min refluxing. Under these conditions fluoxymesterone 17-TMS ether 3-TMS enol ether **44** was obtained. The 11ß-hydroxy group reacts slowly and will not be trimethylsilyted in this short time. This derivative **44** was preferred as the 11-TMS group is difficult to hydrolyse. Even the hydrolysis with acetic acid yielded many decomposition products. The bis-TMS fluoxymesterone **44** was isolated, dried, suspended in isopropanol and exposed to a 60 watt lamp. The reaction mixture was stored after 6h at darkness for four weeks until the 17-TMS ether was completely hydrolzed and 6ß-hydroxyfluoxymesterone **46** was obtained.

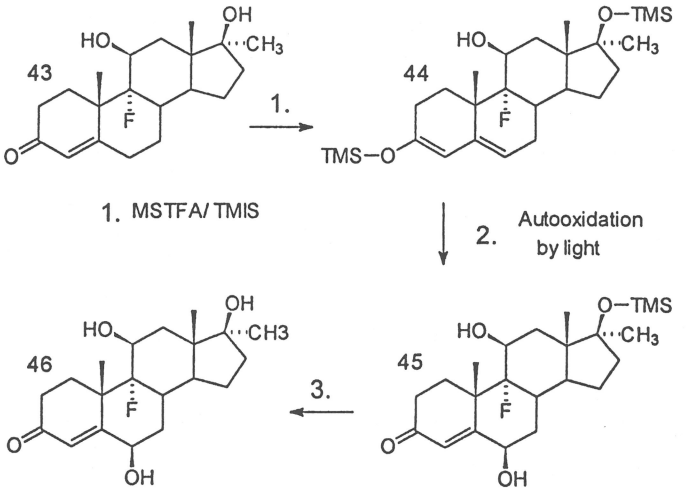

Fig. 11 Synthesis of 6ß-hydroxyfluoxymesterone by Schänzer and Donike [18].

Acknowledgments

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