[1,2,3,4-^{13}C] TESTOSTERONE AND [1,2,3,4-^{13}C] ESTRADIOL

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ABSTRACT

The preparation of [1,2,3,4-^{13}C] testosterone and of [1,2,3,4-^{13}C] estradiol by total synthesis is described.
The ^{13}C labels are introduced by alkylating intermediate 1 with [1,2,3,4-^{13}C]1-iodo-3,3-ethylenedioxybutane (2) to obtain intermediate 10. Hydrolysis of the ketal function, cyclization, aromatization and removal of protective groups gave [1,2,3,4-^{13}C] estradiol. Labeled testosterone was prepared by methylating intermediate 10 and by subsequent treatment with acid. The labeled steroids can be used as tracers for in vivo metabolic studies and as internal standards for the development of definitive gc-ms quantitative methods.

INTRODUCTION

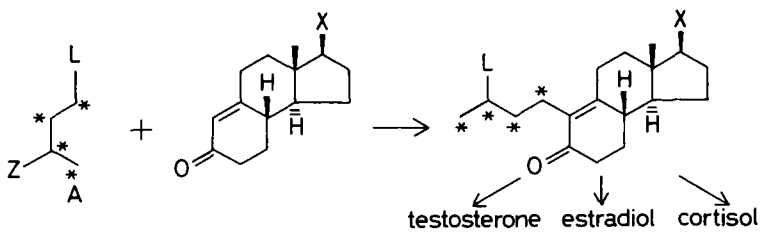
Although deuterium-labeled steroidal hormones are increasingly used to investigate endocrine disorders, there is a growing interest in using other stable isotopic tracers e.g. ^{13}C. ^{13}C atoms incorporated in the steroid nucleus cannot be exchanged. Furthermore, ^{13}C atoms alter the molecule much less than deuterium. The isotope effects caused by ^{13}C both in vitro (GC retention times, fragmentation in the mass spectrometer etc.) and in vivo are much smaller than deuterium isotope effects.

In this paper we report the syntheses of testosterone and estradiol both labeled at four positions in the A ring. The synthesis of

[1,2,3,4-^{13}C] cortisol acetate is the subject of the accompanying paper (8).

RESULTS AND DISCUSSION

The synthetic scheme used to incorporate four ^{13}C atoms in the A ring of a steroid nucleus is depicted in scheme 1.



SCHEME 1

"A", the isotope carrying fragment, must be capable of serving either as a Michael acceptor or as an alkylating agent: L denotes a leaving group and Z a (masked) carbonyl function. Furthermore, synthon "A" has to be built up from available ^{13}C chemicals. [1,2,3,4-^{13}C] 1-Iodo-3,3-ethylenedioxybutane was chosen as synthon "A". The non-isotopic compound has been described by Trost and Lee (1).

The somewhat modified synthesis is delineated in scheme 2.

$$CH_2{=}CH_2 + CH_3COCl \xrightarrow[CH_2Cl_2]{AlCl_3} CH_3COCH_2CH_2Cl$$

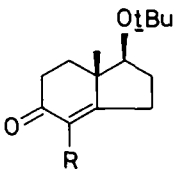
$$CH_3COCH_2CH_2Cl \xrightarrow[\text{acetone}]{NaI} CH_3COCH_2CH_2I$$

$$CH_3COCH_2CH_2I \xrightarrow[PPTS,\ \text{benzene}]{CH_2(OH)CH_2OH} CH_3C(OCH_2CH_2O)CH_2CH_2I$$

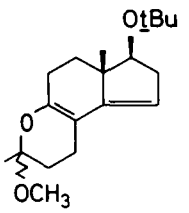
SCHEME 2

The last two steps have been modified slightly. Contrary to the published procedure, a reaction time of two hours in step 2 proved to be sufficient for complete substitution and in the last step pyridinium p-toluenesulphonate (PPTS) (2) was used as the catalyst. By the use of this catalyst the reaction time could be decreased. The product was obtained as a colourless liquid.

A good candidate for the "BCD" precursor would be des-A-17β-t-butoxy-estr-9-en-5-one (1). This compound has the right stereochemistry and an α,β-unsaturated ketone which can be alkylated at the right position. The synthesis of compound 1 is shown in scheme 3.



3 : R = H
4 : R = $CH_2CH_2C(OCH_2CH_2O)CH_3$
5 : R = $CH_2CH_2COCH_3$
6 : R = $CH_2CH_2C(OCH_3)_2CH_3$



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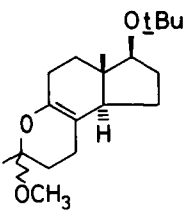

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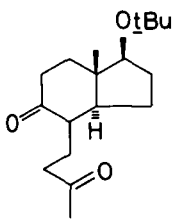

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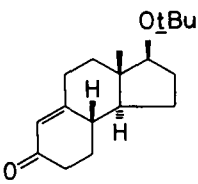
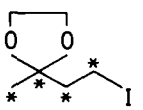
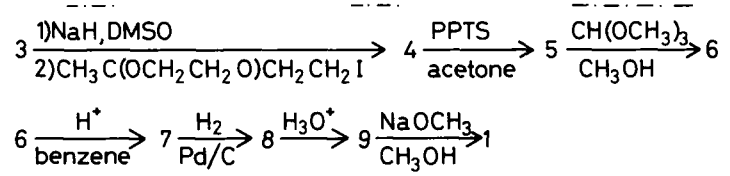


1



2 : * = ^{13}C

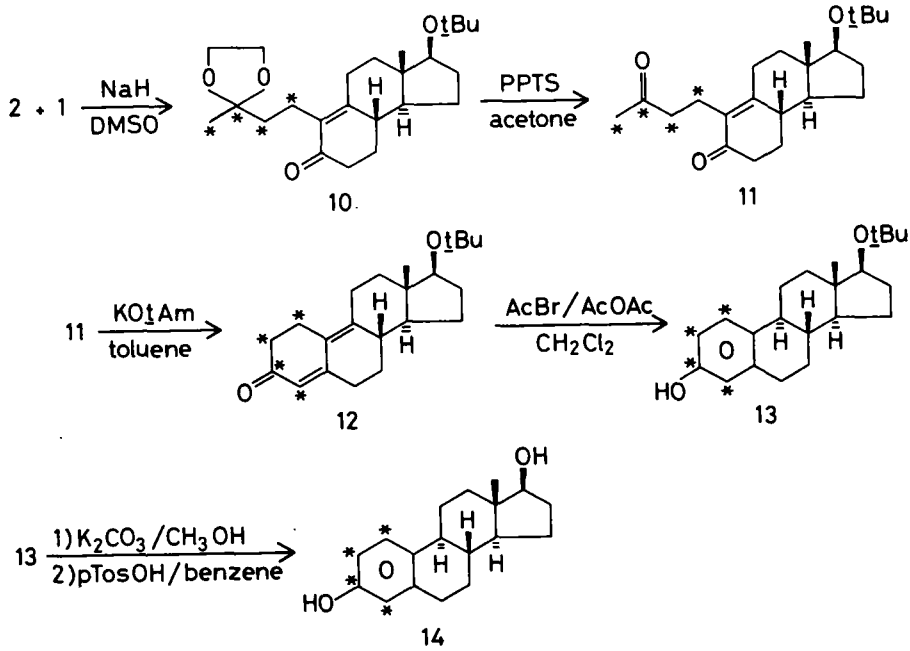


SCHEME 3

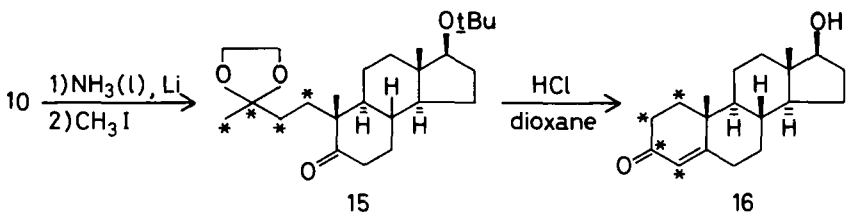
The starting material, the optically pure enone 3 was prepared according to (3). The dienolate anion of compound 3, prepared by treating a solution of compound 3 in DMSO with sodium hydride, was alkylated with 1-iodo-3,3-ethylenedioxybutane. The alkylated material 4 proved to be difficult to separate from the starting material. Therefore, the unpurified material was deketalized with PPTS in refluxing wet acetone to give compound 5. By crystallization compound 5 could be obtained pure (mp 92-93.5°C, $[\alpha]_D^{25}$ +72.3, c = 0.5, benzene) in 56% yield from compound 3. The direct hydrogenation of compound 5 yields a mixture of cis- and trans- fused ring systems. To circumvent this problem an alternative synthesis was set up (c.f. the work of Sauer and co-workers (4)). Ketalization of compound 5 with trimethoxymethane in methanol catalyzed by p-toluenesulphonic acid afforded compound 6. Without purification, compound 6 was treated with a trace of malonic acid in benzene. The resulting epimeric mixture 7 was hydrogenated using palladium on carbon as the catalyst. Acidic hydrolysis of the resulting epimeric mixture 8 yielded the diketone 9, which after treatment with sodium methoxide in methanol afforded des-A-17β- t-butoxy -10(9)-estren-5-one (1) in an overall yield of 50% from compound 5.

The synthesis of [1,2,3,4-^{13}C] estradiol (14) from compound (1) is depicted in scheme 4.

Alkylation of compound 1 with ^{13}C labeled 1-iodo-3,3-ethylenedioxybutane was accomplished using sodium hydride as the base in DMSO. The alkylated product (10) was isolated in 40% yield. Deketalization of compound 10 (PPTS, acetone, reflux) yielded compound 11. Ring closure of diketone 11 was effected using potassium t-amylate in toluene to give [1,2,3,4-^{13}C] 17β-t-butoxy-estra-4,9-dien-3-one (12).



SCHEME 4



SCHEME 5

Aromatization of compound 12 using a method published by Danishefsky (5) (acetylbromide and acetic anhydride in dichloromethane) gave compound 13. Hydrolysis of the acetate function with potassium carbonate in methanol/water and removal of the t-butyl protective group with p-toluenesulphonic acid in refluxing benzene afforded [1,2,3,4-^{13}C] estradiol in a yield of 25% from compound 10.

[1,2,3,4-^{13}C] testosterone was prepared according to scheme 5.

Treatment of compound 10 in liquid ammonia with lithium generated the enolate anion which could be alkylated with methyliodide to yield the β-methylated product (15). Hydrolysis of the ketal function of compound 15, aldol condensation and dehydration accompanied with the removal of the t-butyl protective group was accomplished by reaction of compound 15 with 50% hydrochloric acid in refluxing dioxane. ^{13}C labeled testosterone was obtained in 28% yield from compound 10.

EXPERIMENTAL

General remarks:

Melting points were determined on a Mettler FP 2 melting point apparatus. IR spectra were recorded on a Unicam SP 200 spectrophotometer. ^{1}H NMR spectra were recorded at 60 Mhz (Varian A-60 or Hitachi-Perkin Elmer R-24B or Jeol C 60 HL), at 100 Mhz (Varian XL 100) or at 200 Mhz (Nicolette 200). ^{1}H chemical shifts are reported in ∂-units (ppm) relative to $CHCl_3$ and converted to ∂-TMS values using ∂-$CHCl_3$ = 7.25 ppm. ^{13}C NMR spectra were recorded at 25.16 Mhz (Varian XL 100). ^{13}C chemical shifts are denoted in ∂-units (ppm) relative to the solvent $CDCl_3$ and converted to ∂-TMS values using ∂-$CDCl_3$ = 76.9 ppm. Mass spectra were recorded on a MAT 100 spectrometer. Optical rotations were measured on a Perkin Elmer 241 polarimeter. Elemental analyses were performed in the micro-analytical section of this department. All solvents used were purified according to standard procedures.

1β-t-butoxy-4-(3'-oxobutyl)-7aβ-methyl-7,7a-dihydro-5(6H)-indanone (5).

NaH (1.3 g of dispersion, washed free of oil with pentane) was dispersed in 40 ml of absolute DMSO. Whilst being dispersed, 5.5 g of t-butyl ether (3) in 40 ml of dry DMSO was added. The reaction mixture was stirred under a nitrogen atmosphere for 20 hours at room temperature. To the resulting reddish-brown solution 1-iodo-3,3-ethylenedioxybutane (6 g) dissolved in 10 ml of DMSO was added. After 4 hours at room temperature the reaction was quenched with 30 ml of a 20% NH_4Cl solution. The reaction mixture was thoroughly extracted with ether (4 x 100 ml). The organic layers were washed with water (4 x 50 ml) and brine (2 x 50 ml). After drying over $MgSO_4$ the solvent was removed, giving 6 g of an oil. This oil was dissolved in 70 ml of 95% acetone and after adding 2 g of PPTS refluxed for 2 hours. The acetone was removed and replaced with 100 ml of ether. The etheral phase was washed with 20 ml of brine, dried over $MgSO_4$ and evaporated. There was obtained 6 g of an oil which after crystallization from hexane at -20°C afforded 3.2 g of compound 5. An additional amount of compound 5 (0.5 g) was obtained by

chromatography of the residue (Al_2O_3, hexane/ether) together with 0.75 g of compound 3. The total yield amounted to 3.7 g (57%), mp 92-93.5°C, $[\alpha]_D^{25}$ +72.3 (c = 0.5, benzene). Calculated for $C_{18}H_{28}O_3$: C 73.9% H 9.65%, found: C 73.5%, 73.7%, H 9.58%, 9.41%.

^1H NMR: δ1.05 (s,3H), 1.15 (s,9H), 2.1 (s,3H), 3.5 ppm (bt,1H),

^{13}C NMR: δ79.5 (C1), 25.3 (C2), 29.5 (C3), 169.6 (C3a), 131 (C4), 198.4 (C5), 33.2 (C6), 33.7 (C7), 44.5 (C7a), 15.7 (C8), 19.9 (C9), 41.8 (C10), 208.3 (C11), 29.5 (C12), 72.7 (C13), 28.4 ppm (C14).

Des-A-17β-t-butoxy-9-estren-5-one (1).

To a solution of 3 g of compound 5 in 30 ml of methanol, 3 ml of trimethoxymethane and 0.4 ml of a 0.5% solution of p-toluenesulphonic acid in methanol were added. After 5 hours at room temperature tlc (Al_2O_3/ether) indicated that the reaction was complete. The reaction mixture was poured into 20 ml of a saturated $NaHCO_3$ solution and extracted with ether (3 x 50 ml). The organic layers were dried over $MgSO_4$ and evaporated to yield 2.9 g of an oil, which according to ^1H NMR consisted of the ketal (6) (^1H NMR: δ0.85 (s,3H), 0.95 (s,9H), 1.1 (s,3H), 3.0 ppm (s,6H)). This oil was refluxed in 50 ml of benzene containing 20 mg of malonic acid. During the reflux about 20 ml of distillate was collected in a water separator; this took approximately 90 minutes. After cooling, the reacton mixture was diluted with 50 ml of ether and washed with $NaHCO_3$ solution and brine. After drying over $MgSO_4$ and evaporation there remained 2.5 g of a slightly yellow oil which according to ^1H NMR consisted of two epimeric mixed ketals (7) in a 1:1 ratio. (^1H NMR: δ0.9 (s,3H), 1.1 (s,9H), 1.3 and 1.32 (2s, 3H), 3.1 and 3.15 (2s,3H), 4.9 ppm (bs, 1H)). The 2.5 g of compound 7 in 50 ml of ethylacetate was hydrogenated with 350 mg of 10% palladized carbon. After 3 hours the catalyst was filtered off. Evaporation of the solvent gave 2.4 g of compound 8 (^1H NMR: δ0.75 (s,3H), 1.1 (s,9H), 1.3 (s,3H), 3.15 and 3.17 ppm (2s,3H)). This material was directly treated with 0.5 ml of 1N HCl in 25 ml of methanol and after stirring for 2 hours at room temperature, according to tlc, compound 8 was completely converted to diketone 9. Solid $NaOCH_3$ (220 mg) was added and the reaction mixture was refluxed for one hour. It was cooled, acidified with 1N HCl and most of the methanol removed. Ether (50 ml) was added to the residue. The organic layer was washed with a saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$ and evaporated to yield 2.3 g of compound 7 as a semi-solid material. Recrystallization from hexane at -40°C gave 1.7 g (50% from compound 5) of crystalline des-A-17β-t-butoxy-9-estren-5-one, mp 122-123.5°C, $[\alpha]_D^{25}$ -11.3 (c= 1.0, $CHCl_3$). Reported mp 125-128°C, $[\alpha]_D^{25}$ -10.9 ($CHCl_3$) (7).

^1H NMR: δ0.9 (s,3H), 1.1 (s,9H), 3.5 (bt,1H), 5.75 ppm (bs,1H).

^{13}C NMR: δ199.5 (C5, steroid numbering), 36.1 (C6), 30.9 (C7), 50.3 (C1), 166.7 (C9), 124.9 (C10), 36.6 (C11), 31.1 (C12), 42.4 (C13), 38.1 (C14), 23.6 (C15), 27.1 (C16), 79.7 (C17), 72.9 and 28.4 ppm (C t-butyl).

[1,2,3,4-^{13}C] 1-Iodo-3,3-ethylenedioxybutane (2).

In a 25 ml two-necked round-bottomed flask, provided with a gas inlet tube and connected to a mercury trap, 12 ml of dry CH_2Cl_2 was cooled in an ice bath. $AlCl_3$ (3.5 g) and [1,2-^{13}C] acetylchloride (1.9 g) were added successively. After stirring for 30 minutes at 0°C, [1,2-^{13}C] ethylene was passed through the solution. The stream of ethylene gas was adjusted with the aid of the stirring speed so that there

remained a constant reduced pressure in the reaction vessel. After 3 hours no more ethylene was consumed. The mixture was stirred for another 30 minutes and subsequently hydrolyzed by carefully adding it to a stirred, cooled (0°C) solution of 7 ml of concentrated HCl in 20 ml of ice water. The organic layer was separated. The aqueous phase was extracted with CH_2Cl_2 (3 x 50 ml). After washing with water (10 ml) and a saturated $NaHCO_3$ solution (2 x 10 ml) the organic layers were dried over $MgSO_4$ and evaporated (the temperature of the water bath used should not exceed 20°C because the product is rather volatile). To the residue a solution of 5 g of NaI in acetone (50 ml) was added. The mixture was refluxed and stirred for 2 hours in the dark, during which time a copious precipitate of NaCl had formed. The acetone was removed and replaced by a benzene (50 ml)/water(30 ml) mixture. The aqueous phase was extracted with benzene (2 x 50 ml). The benzene layers were washed with 20 ml of a 10% $Na_2S_2O_6$ solution and with brine (20 ml), dried over $MgSO_4$ and partly evaporated. To the resulting solution (about 100 ml) ethylene glycol (3 ml) and pyridinium paratoluenesulphonate (PPTS) was added. The mixture was refluxed, water being collected in a water separator. After 1.5 hours the benzene was evaporated; to the residue ether (100 ml) was added, the etheral solution washed with brine (30 ml), dried over $MgSO_4$ and evaporated to give 2.7 g of slightly yellow iodoketal. It was purified by passing it through 30 g of Al_2O_3 (elution CH_2Cl_2). There was obtained 2.4 g (41%) of colourless [1,2,3,4-^{13}C]-1-iodo-3,3-ethylenedioxybutane. (It is preferable, because of the unstability of the intermediate halo ketones, to perform this synthesis in one day. The iodoketal can be stored without decomposition. It solidifies if stored at -40°C).

[1,2,3,4-^{13}C] Estradiol (14).

Des -A-17β-t-butoxy-9-estren-5-one (1.1 g, 4 mmoles) dissolved in 6 ml of dry DMSO was treated with 4.5 mmoles of NaH dispersed in 6 ml of dry DMSO. After stirring the reaction mixture for 14 hours at room temperature under a nitrogen atmosphere [1,2,3,4-^{13}C] 1-iodo-3,3,-ethylendioxybutane (1 g, 4 mmoles) in 4 ml of dry DMSO was added in one portion. The brown reaction mixture was stirred for another 20 hours at room temperature. The reaction mixture was quenched with 5 ml of a 10% NH_4Cl solution. Addition of ether (10 ml) gave two clear layers. The aqueous phase was extracted with ether (4 x 50 ml). The ether layers were washed with water (2 x 20 ml) and brine (20 ml), dried over $MgSO_4$ and evaporated to afford a yellow oil (1.2 g). This oil was adsorbed on 100 g of Al_2O_3. Elution with CH_2Cl_2 gave 100 mg of compound 1 followed by 600 mg of the alkylated product 10 as a semi-solid.

^{1}H NMR: δ0.85 (s,3H), 1.12 (s,9H), 3.25 (t,1H), 3.8 ppm (d,4H).

^{13}C NMR: Enhanced signals due to C-13 labeling at about δ110, 37, 23 and 19 ppm; the natural abundance spectrum shows signals at δ197.8 (C5), 158.8 (C9), 134.0 and 133.1 (C10), 79.7 (C17), 71.8 and 28.3 (t-butyl), 64.1 (ketal), 50.4 (C8), 41.6 (C13), 10.5 ppm (C19).

The alkylation product (300 mg) was dissolved in 40 ml of acetone. After the addition of 1 ml of water and 330 mg of PPTS the mixture was refluxed during 3 hours. The acetone was removed and replaced with 100 ml of ether. The etheral solution was washed with brine (20 ml), dried over $MgSO_4$ and evaporated to yield the deketalized product (11) as an oil. This oil was dissolved in toluene (15 ml). 1N KOt-Am solution (1 ml) was added and the resulting reddish-brown solution was refluxed

under a nitrogen atmosphere for 30 minutes. After cooling, the reaction mixture was acidified with dilute acetic acid, diluted with ether (50 ml), washed with water (10 ml), $NaHCO_3$ solution (2 x 10 ml) and brine (10 ml). Drying over $MgSO_4$ and evaporation yielded the dienone (12) as a semi-solid. The dienone (12) was dissolved in CH_2Cl_2 (2 ml). To this stirring solution 0.2 ml of freshly distilled acetic anhydride was added followed by 0.1 ml of freshly distilled acetylbromide. After stirring the reaction mixture for one hour under a nitrogen atmosphere, it was carefully poured on 10 ml of a cooled (0°C) 5% $NaHCO_3$ solution. After stirring for 30 minutes at room temperature, the mixture was extracted with chloroform (4 x 10 ml). The organic layers were dried over $MgSO_4$ and evaporated. The residue (13), dissolved in methanol (50 ml) was stirred overnight with 0.8 g of K_2CO_3 and water (10 ml). The reaction mixture was neutralized with 1N HCl, the methanol was removed by distillation and the aqueous phase extracted with chloroform (4 x 10 ml). The organic layers were dried over $MgSO_4$ and evaporated. The residue was dissolved in benzene (15 ml), p-toluenesulphonic acid (0.13 g) was added and the mixture was refluxed during one hour under a nitrogen atmosphere. After cooling, 50 ml of a saturated $NaHCO_3$ solution was added, the layers separated and the aqueous layer extracted with ether (3 x 100 ml). The organic layers were extracted with 1N NaOH (3 x 30 ml). After filtration of the aqueous phase it was cooled to 0°C and acidified with concentrated HCl. Extraction with ether (3 x 50 ml), drying over $MgSO_4$ and evaporation yielded [1,2,3,4-^{13}C] estradiol (60 mg, 25% from compound 10). It was purified by HPLC on a Chemisorb SI 60.5 column. Elution with chloroform gave pure [1,2,3,4-^{13}C] estradiol, mp = 172-3°C.

^{1}H NMR (CD_3OD): ∂0.7 (s,3H), 3.7 (t,1H), ca 6.5 (d,2H, J = 155 Hz), ca 7.1 ppm (d,1H, J = 155 Hz). The UV and CD spectra of labeled and natural estradiol were superimposable.

[1,2,3,4-^{13}C] Testosterone (16).

Lithium metal (200 mg) was dissolved in 60 ml of liquid anhydrous ammonia. After stirring for 15 minutes at -50°C a solution of 300 mg of enone 10 in 8 ml of dry ether was added to the blue solution. After stirring the reaction mixture for 20 minutes, 3.2 ml of methyliodide in 10 ml of dry ether was added below the surface of the liquid. The resulting mixture was stirred at -50°C for 3 hours. The ammonia was evaporated using a water bath at 10°C. Water (10 ml) was added. The aqueous phase was extracted with chloroform (4 x 30 ml). The organic phase was washed with water (30 ml). After drying ($MgSO_4$) and evaporation there remained a light yellow oil. This oil (15) was dissolved in 10 ml of dioxane containing 2 ml of 6N HCl and refluxed under a nitrogen atmosphere during 5 hours. After cooling it was neutralized with saturated $NaHCO_3$ solution. Extraction with CH_2Cl_2 (4 x 30 ml), washing with water (20 ml), drying over $MgSO_4$ and evaporation yielded [1,2,3,4-^{13}C] testosterone (70 mg, 28% from compound 10). It was purified by HPLC using a LiChrosorb SI 60.5 column. Elution with ether gave pure [1,2,3,4,-^{13}C] testosterone (recovery 55%, mp = 152-3°C).

^{1}H NMR ($CDCl_3$): ∂0.7 (s,3H), 1.3 (d,3H, J = 4 Hz), 3.6 (t,1H), 5.7 ppm (d,1H, J = 158 Hz). The UV and CD spectra of the labeled and natural testosterones were superimposable.

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