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TRITIUM LABELED TRANS-4-N-BUTYL-2,3-^{3}H-CYCLOHEXANECARBOXYLIC ACID. PREPARATION OF TESTOSTERONE 17β-TRANS-4-N-BUTYL-2,3-^{3}H-CYCLOHEXANECARBOXYLATE AND [4-^{14}C]-TESTOSTERONE 17β-TRANS-4-N-BUTYLCYCLOHEXANE CARBOXYLATE

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SUMMARY

The synthesis of *trans*-4-*n*-butyl-2,3-^{3}H-cyclohexanecarboxylic acid is described. The title compound was synthesized by catalytic tritiation of 4-*n*-butylcyclohex-2-enecarboxylic acid, which was obtained from a "double" Birch reduction of 4-*n*-butylbenzoic acid. The *trans*-acid was isolated from a mixture of *cis/trans* diastereomers by the selective formation of a thiourea inclusion complex. The title compound was used for the synthesis of the 17β-ester of testosterone. In addition, [4-^{14}C]-testosterone was esterfied using unlabelled *trans* acid. The three radiolabelled compounds will be used in metabolism studies.

Key words: *trans*-4-*n*-butylcyclohexanecarboxylic acid, labeling with trititum, thiourea inclusion complex, [4-^{14}C]-testosterone.

INTRODUCTION

It is mandatory to achieve azospermia in order to successfully develop methods for the regulation of male fertility with hormones. The low success rate of testosterone esters

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including testosterone enanthate, cypionate and undecanoate can be attributed to their short life *in vivo* and their ready conversion to testosterone as evidenced in the pharmakokinetic patterns resulting in supraphysiological serum testosterone peaks shortly after intramuscular injection of these esters. These pharmacokinetics of the testosterone esters are not considered favorable for male contraception [1].

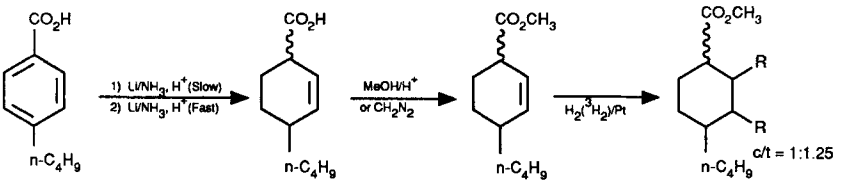
After a series of new long acting testosterone esters were synthesized and tested in rats as well as castrated male monkeys, it was found that compound **9** has the ability to maintain testosterone levels in the normal physiological range and exhibited the best physiological profile. A single intramuscular injection (40 mg) of compound **9** in aqueous suspension maintained serum testosterone as well as dihydrotestosterone levels in the normal range for about 3 months in monkeys weighing 2.8 to 4.6 kg [2].

In light of the results obtained with the new 17β-steroidal ester of *trans*-4-*n*-butylcyclohexanecarboxylic acid **1** it was desirable to synthesize radiolabeled *trans*-4-*n*-butylcyclohexanecarboxylic acid **1a** as well as the corresponding 17β-steroidal ester in order to study their metabolic fate *in vivo*. Using an intermediate of this synthetic methodology, we were able to obtain tritiated acid **1a** with high specific activity and using this material, the corresponding 17β-ester of testosterone was produced. This communication will describe our synthesis of **1** as well as the synthesis of tritiated acid **1a** and the corresponding testosterone ester **9a**.

DISCUSSION

4-*n*-Butylcyclohex-2-enecarboxylic acid **6** served as the immediate precursor to tritiated acid **1a** and was synthesized from 4-*n*-butylbenzoic acid **7**. Our route to **6** was based on the reported synthesis of various dihydro and tetrahydro derivatives of 4-alkylbenzoic acids via carefully controlled modified Birch reductions [3-6]. Thus, extended lithium-ammonia reduction of 4-*n*-butylbenzoic acid **7**, under conditions of protonation (thermodynamic control), gave the conjugated 3,4-dihydro derivative of **7**. Following isolation, the 3,4-dihydro derivative of **7** was subjected to an additional lithium-ammonia reduction under conditions of protonation (kinetic control) to afford a mixture of *cis/trans*-4-*n*-butylcyclohex-2-enecarboxylic acids **6**.

The acid was converted to the methyl ester **5** by the usual methods (catalytic HCl in methanol at reflux or ethereal diazomethane) and the double bond was hydrogenated over Pt



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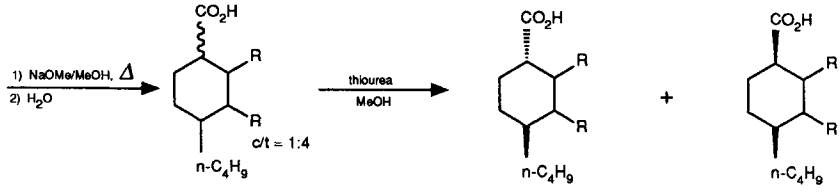
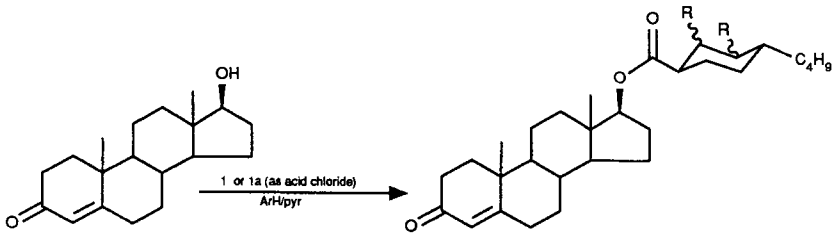
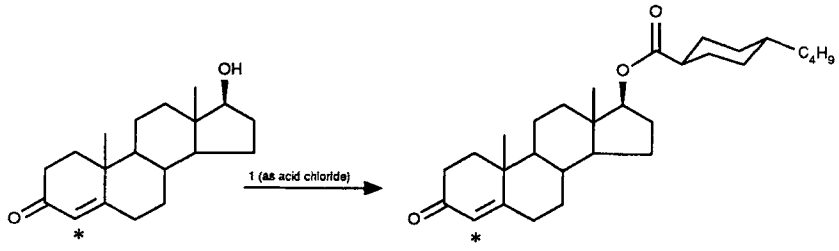


3 R = H; 3a R = ^{3}H — 1 R = H; 1a R = ^{3}H — 2 R = H; 2a R = ^{3}H



8 — 9 R = H; 9a R = ^{3}H



10 — 11

in ethyl acetate to give a mixture of *cis/trans*-4-*n*-butylcyclohexanecarboxylic acid methyl esters 4. For preparative work (10-70 g), the hydrogenation was done using a Parr shaker apparatus at 40-50 psi. In small scale reactions (10-250 mg), we found the reduction could be effected using a positive atmospheric pressure hydrogenation apparatus. By either method, 4 was obtained as a 1:1.25 mixture of *cis/trans* isomers. This *cis/trans* mixture was equilibrated to a 1:4 *cis/trans* mixture by refluxing 4 in methanol containing sodium methoxide. Hydrolysis of the methyl ester gave a mixture of *cis/trans*-4-*n*-butylcyclohexane-carboxylic acids 3.

The desired *trans* isomer was separated from the mixture using a novel procedure developed by van Bekkum *et al* [7,8]. They found that when a methanolic solution of a variety of *cis/trans*-4-alkylcyclohexanecarboxylic acids were treated with an excess of thiourea, the *trans* isomer was removed from the mixture in the form of an insoluble thiourea inclusion complex and the *cis* isomer remained in solution.

When a mixture of *cis/trans* diastereomers **3** was treated with thiourea, as described by van Bekkum *et al* [7,8], a thiourea inclusion complex was formed. The isolated thiourea inclusion complex was subsequently dissolved in aqueous base, followed by acidification, and extraction with ether gave *trans acid* **1** as a low melting solid. Analysis of the methyl ester of **1**, obtained by treatment with ethereal diazomethane, by HPLC showed this material to be greater than 96% *trans*. Extraction of the filtrate from the thiourea treatment gave a material that was greatly enriched with the *cis* isomer **2**.

Using the above methodology, we were able to synthesize radiochemically and isomerically pure **1a** with a high specific activity (ca. 20 Ci/mmol). A portion of this material was used to synthesize the 17β-ester of testosterone **9a**. In addition, the 17β-ester of [4-^{14}C]testosterone **11** was synthesized using acid **1**. All the labeled compounds will be used for pharmakokinetic and metabolic studies.

EXPERIMENTAL PROCEDURES

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton (^{1}H) NMR spectra were recorded on a Varian EM-390 (90mHz) spectrometer as $CDCl_3$ solution, using TMS (Me_4Si) as an internal standard. UV spectra were recorded on a Varian-Cary model 210 UV/VIS spectrophotometer. Satisfactory microanalyses were performed by Midwest Microlabs, Indianapolis, Indiana. Catalytic reduction with tritium gas was performed by New England Nuclear (NEN) Research Products, Boston, Massachusetts. [4-^{14}C] Testosterone was obtained from New England Nuclear (NEN) Research Products, Boston, Massachusetts. Radio and isomeric purity was determined using HPLC (Waters Associates HPLC system, equipped with a M6000 solvent delivery system) with fractionation to obtain a radioactive profile. Tritium was counted using a Beckman LS-7500 Liquid Scintillation Counter. Preparative TLC were run on silica gel plates (20 x 20 cm, 1000μ, with 254 nm phosphor) obtained from Analtech, Newark, Delaware; 4-(*n*-Butyl)benzoic acid was purchased from Aldrich Chemical Company, Milwaukee, Wisconsin.

4-*n*-Butylcyclohex-2-enecarboxylic acid (6) via Double Birch Reduction of 4-*n*-butylbenzoic acid

To 4-*n*-butylbenzoic acid (7, 69.7 g, 0.39 mol) in a 5-L 3-necked flask equipped with a mechanical stirrer and a dry-ice/acetone condenser anhydrous ether (650 ml) was added. Anhydrous ammonia (ca. 2000 ml), redistilled from Na, was introduced into the flask. With slow stirring, lithium wire (10.85 g, 1.56 mol), cut into small pieces, was added over a 10 minute period. The dry-ice/acetone cooling bath was removed and the reaction was allowed to stir at reflux for 45 min. The cooling bath was reinstalled and absolute ethanol (70.0 ml, 1.17 mol) was added slowly over a 20 minute period to the blue solution. Within 5 minutes, the blue color disappeared and the solution turned yellow. The ammonia was allowed to evaporate under a stream of nitrogen and the reaction mixture was left standing overnight. Water (2000 ml) was added to the reaction mixture. The reaction flask was placed in an ice bath and the solution was acidified (pH = 1-2) with HCl. The aqueous solution was extracted with ether (3x1000 ml). The ether layers were washed with brine and then dried over sodium sulfate. Evaporation of the solvent *in vacuo* gave 68.6 g (97%) of 3,4-dihydro derivative of 7 as a waxy solid. The material was used with no further purification. UV (MeOH) λ_{max} = 273 nm; NMR ($CDCl_3$) δ 0.90(t, J = 4 Hz, -CH_3, 3 H), 2.33(br.d, J = 6 Hz, allylic-CH_2-, 2 H). 2.57(m, C-4 allylic methine, 1 H), 6.27(d, J = 10 Hz, C-2 vinylic, 1 H), 10.05(br.m, - CO_2 H, 1 H)ppm.

To the crude dienic acid (68.6 g, 0.38 mol), dissolved in anhydrous ether (650 ml), in a 5-L 3-necked round bottomed flask equipped with a mechanical stirrer and a dry-ice/acetone condenser, was introduced approximately 1500 ml of anhydrous ammonia, redistilled from sodium. With slow stirring, lithium wire (7.9 g, 1.14 mol), cut into small pieces, was added over a 10 minute period. The cooling bath was removed and the reaction was allowed to stir at reflux for 1 hour. The cooling bath was reinstalled and absolute ethanol (70.0 ml, 1.18 mol) was added slowly over a 20 minute period. Just as the solution began to lose the blue color, ammonium chloride (142.7 g, 2.66 mol) was added in one portion. The ammonia was allowed to evaporate and water (2000 ml) was added. The reaction flask was placed in an ice bath and the reaction mixture was acidified (pH = 1-2) with HCl. The aqueous solution was extracted with ether (3x1000 ml) and the ether layers were washed with brine and then dried over sodium sulfate. Evaporation of the solvent *in vacuo* gave 68.3 g (98.7%) of the acid 6. NMR ($CDCl_3$) δ 0.90(t, J = 4 Hz, -CH_3, 3 H),

2.26(m, C-4 methine, 1 H), 3.10(m, C-1 methine, 1 H), 5.76(m, C-2 and C-3 vinylic methines, 2 H)ppm.

4-*n*-Butylcyclohex-2-enecarboxylic acid methyl ester (5)

Ethereal diazomethane (ca. 28.1 mmol from 8.6 g of Diazald) was distilled into an ice cold ether solution (25 ml) of 4-*n*-butylcyclohex-2-enecarboxylic acid (6, 3.56 g, 19.5 mmol). Most of the excess diazomethane was destroyed with the addition of acetic acid and the last traces of diazomethane were removed by bubbling nitrogen through the solution. The ether was evaporated to afford 3.80 g (99%) of methyl ester 5. NMR ($CDCl_3$) δ 0.90(t, J = 4 Hz, $-CH_3$, 3 H), 3.10(m, C-1 methine, 1 H), 3.67(s, $-CO_2CH_3$, 3 H), 5.67(br.s, C-2 and C-3 vinylic methines, 2 H)ppm.

4-*n*-Butylcyclohexanecarboxylic acid methyl ester (4)

The following procedure was done using a positive atmospheric pressure hydrogenation apparatus. Platinum oxide (81-84% PtO_2, Engelhard, 5.0 mg. 10 mole%) was suspended in ethyl acetate (2.0 ml). Once a hydrogen atmosphere had been established, the mixture was stirred until the uptake of hydrogen had ceased and free platinum metal was clearly visible. The methyl ester (5, 33.64 mg, 0.17 mmol), dissolved in ethyl acetate (250 µl), was injected with a gas-tight syringe through a rubber septum. The reaction mixture was stirred for 1.5 hrs. The mixture was filtered and the residue rinsed with additional ethyl acetate. The ethyl acetate was evaporated under a stream of nitrogen and finally *in vacuo* to give 23.8 mg (70%) of saturated methyl ester 4. Note: Methyl ester 4 is somewhat volatile, as evidenced by the low yield, and there was some loss while the material was under vacuum. HPLC analysis (Waters and Associates µPorasil column, 1.0% EtOAc/heptane, 1.0 ml/min, Waters and Associates R-401 differential refractometer) of methyl ester 4 showed the material to be a 1:1.25 *cis/trans* mixture. NMR ($CDCl_3$) δ 0.90(t, J = 4 Hz, $-CH_3$, 3 H), 2.27(t of t, J = 10 Hz, J′ = 3 Hz, C-1 methine of *trans* isomer), 2.51(br. quintet, J = 3 Hz, C-1 methine of *cis* isomer), 3.65(s, *trans*-CO_2CH_3), 3.67(s, *cis*-CO_2CH_3)ppm.

***cis/trans*-4-*n*-Butylcyclohexanecarboxylic acid (3)**

To dry methanol (5.0 ml) in a dry 50 ml flask was added sodium (ca. 100 mg) and the mixture was stirred until the sodium was dissolved. The methyl ester (4, 47.0 mg, 0.237

mmol) was added to the above mixture as a methanolic solution. The mixture was refluxed for 24 hrs. The reaction mixture was cooled to room temperature and water (1.0 ml) was added. The reaction mixture was refluxed an additional 24 hrs. The methanol was evaporated and the residue was diluted with water (3.0 ml) and extracted with ether. The aqueous portion was cooled in an ice bath and made acidic (pH = 1-2) with aqueous HCl and extracted with ether (3x5.0 ml). The ether extracts were washed with brine, combined and dried over sodium sulfate. Evaporation of the solvent gave 46.52 mg of **3**. NMR ($CDCl_3$) δ 0.90(t, J = 4 Hz, -CH_3, 3 H), 1.10-2.25(br.m, 15 H), 2.27(t of t, J = 10 Hz, J′ = 3 Hz, C-1 methine for *trans* isomer), 2.51(br. quintet, J = 3 Hz, C-1 methine for *cis* isomer)ppm.

trans-4-_n_-Butylcyclohexanecarboxylic acid (1)

cis/trans Acid (**3**, 46.5 mg) was dissolved in a minimum amount of methanol (ca. 100 µl) and to this solution was added thiourea (115.4 mg). With heating, additional methanol was added until the mixture was homogeneous. The solution was allowed to cool to room temperature then cooled to 4°C and finally cooled to -20°C. The supernatant was pipetted from the solid inclusion complex and the solid was rinsed with cold methanol (3x). The inclusion complex was dissolved in 5% aqueous KOH and the solution was chilled in an ice bath. The solution was made acidic (pH = 1-2) with sulfuric acid and the mixture was extracted with ether (3x5.0 ml). The ether extracts were washed with water (4x) and brine (1x). The ether extracts were dried over sodium sulfate and evaporation of the solvent gave 11.81 mg of *trans* acid **1** mp =37-39°C. A small sample of the acid was re-esterified with diazomethane and HPLC analysis of the ester showed it to be greater than 96% *trans*. NMR ($CDCl_3$) δ 0.90(t, J = 4 Hz, -CH_3, 3 H), 1.10-2.25(br.m, 15 H), 2.27(t of t, J = 10 Hz, J′ = 3 Hz, C-1 methine, 1 H)ppm; Analysis calculated for $C_{11}H_{20}O_2$: C-71.70; H-10.94. Found: C-72.00; H-11.19.

Testosterone 17β-_trans_-4-_n_-butylcyclohexanecarboxylate (9)

trans-Acid (**1**, 10.0 mg, 0.051 mmol) was dissolved in anhydrous benzene (1.0 ml) and treated with an excess of oxalyl chloride (16 µl, 0.18 mmol). The mixture was stirred until gas evolution had ceased. The benzene and excess oxalyl chloride were removed under a stream of nitrogen. Additional benzene (500 µl) was added and was also evaporated. The acid chloride was finally dissolved in benzene (250 µl).

To testosterone (8, 21.8 mg, 0.075 mmol), dissolved in benzene (1000 µl) and pyridine (100 µl), the above acid chloride was added. The reaction was allowed to stir for 1.5 hr. The reaction was diluted with water (2.0 ml) and the aqueous mixture was extracted with ether (3x5.0 ml). The ether extracts were washed with brine, combined, and dried over sodium sulfate. The solvent was evaporated to give 30.2 mg. The material was chromatographed on a single preparative TLC plate (40% EtOAc/Hexane) to give ester (9, 19.6 mg). Recrystallization of the material from methanol afforded 15.3 mg, mp = 133-134°C; NMR ($CDCl_3$) δ 0.91(t, J = 4 Hz, $-(CH_2)_3\underline{CH_3}$, 3 H), 0.94(s, 18-$CH_3$, 3 H), 1.17(s, 19-$CH_3$, 3 H), 4.65(t, J = 9 Hz, 17α-H, 1 H), 5.76(br.s, C-4 H, 1 H)ppm; Analysis calculated for $C_{30}H_{46}O_3$: C-79.25; H-10.20. Found: C-79.33; H-10.20.

cis/trans-4-_n_-Butyl-2,3-^{3}H-cyclohexanecarboxylic acid methyl ester (4a)

NEN Research Products was sent 55.36 mg of 5 and using the above experimental procedure, produced 3000 mCi of tritiated 4a with a specific activity of approximately 58 Ci/mmol. The material was received as a solution in anhydrous methanol (5.0 ml) and was used without characterization.

cis/trans-4-_n_-Butyl-2,3-^{3}H-cyclohexanecarboxylic acid (3a)

To dry methanol (5.0 ml) in a dry 50 ml flask, was added sodium (ca. 100 mg) and the mixture was stirred until the sodium was dissolved. The methanol solution of tritiated ester 4a (ca. 3000 mCi) was added to the reaction flask and the mixture was heated at reflux for 24 hrs. The reaction mixture was cooled to room temperature and water (3.0 ml) was added. The mixture was refluxed an additional 24 hrs. The methanol was evaporated and the aqueous mixture was diluted to 5.0 ml with water. The aqueous mixture was extracted with ether (3x5.0 ml). The ether extracts were washed with brine, combined, and dried over sodium sulfate. Evaporation of the solvent gave 100.9 mCi of the unhydrolyzed ester 4a. The aqueous portion was cooled in an ice bath and made acidic (pH = 1-2) with aqueous HCl and extracted with ether (3x5.0 ml). The ether extracts were washed with brine, combined, and dried over sodium sulfate. Evaporation of the solvent gave 2897.73 mCi of the *cis/trans*-acid 3a.

trans-4-_n_-Butyl-2,3-^{3}H-cyclohexanecarboxylic acid (1a)

The tritiated acid from above was diluted with cold *trans*-4-*n*-butylcyclohexane-

carboxylic acid (**1**, 21.0 mg) which adjusted the specific activity to approximately 20 Ci/mmol. The material was dissolved in a minimum amount of methanol and thiourea (75 mg) was added. The mixture was heated and additional methanol was added until the mixture was homogeneous. The solution was allowed to cool to room temperature then cooled to 4°C and finally cooled to -20°C. The supernatant was pipetted from the solid inclusion complex and the solid was rinsed with cold methanol (3x). The inclusion complex was dissolved in 5% KOH and the solution was chilled in an ice bath. The mixture was made acidic (pH = 1-2) with sulfuric acid and the mixture was extracted with ether (3x). The ether extracts were washed with water (4x) and brine (1x). The combined ether extracts were dried over sodium sulfate and evaporation of the solvent gave 1800 mCi of *trans* acid **1a**, which solidified when chilled. Treatment of the methanol supernatant from above with an additional portion of thiourea (37 mg), and processing as described above, gave an additional 1060 mCi of **1a**.

Purification of *trans*-4-*n*-butyl-2,3-^{3}H-cyclohexanecarboxylic acid (1a)

A small aliquot from approximately 1/2 of the acid (ca. 900 mCi) from the first thiourea treatment was converted to the methyl ester using ethereal diazomethane. This material was assayed by HPLC (Waters and Associates μPorasil column, 1% ethyl acetate/heptane, 1.0 ml/min) to ascertain its isomeric and radiochemical purity. By HPLC the material was predominantly *trans* **1a**, but there was approximately 9% of a less polar radiochemical impurity. The acid was recrystallized three times from cold (-78°C) acetone to afford 122.36 mCi of **1a**. Conversion of a small aliquot to the methyl ester and analysis by HPLC showed the material to be radiochemically pure and 98.7% *trans* isomer (Fig. 1). Tritiated acid **1a** was stored at 4°C as a solution in 10% ethanol/toluene. HPLC analysis of **1a**, after a storage period of one year, showed that no appreciable decomposition had occured.

Testosterone 17β-(*trans*-4-*n*-butyl-2,3-^{3}H-cyclohexane)carboxylate (9a)

The acid (ca. 900 mCi, 8.57 mg, 0.047 mmol), obtained from the first thiourea treatment, was dissolved in dry benzene (250 μl) and was treated with oxalyl chloride (10.0 μl, 0.115 mmol). When gas evolution had ceased (ca. 1 hr) the solvent was removed under a stream of nitrogen. Additional benzene (500 μl) was added and was also evaporated. The acid chloride was finally dissolved in benzene (250 μl).

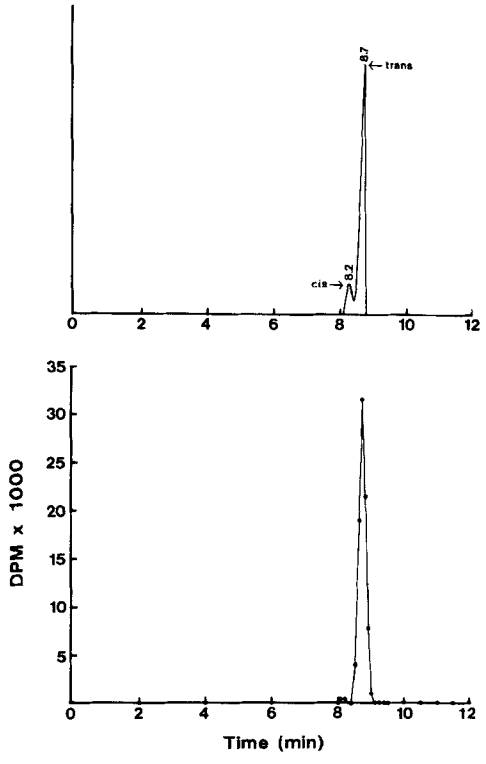


Fig. 1 HPLC analysis of unlabeled (1) and ^3H-labeled (1a) as the methyl ester on a normal phase column (Waters and Associates, μ-Porasil, 30 cm x 3.9 mm) using EtOAc:Heptane (1:99) solvent system at a flow rate of 0.9 mL/min, differential refractometer detector.

To testosterone (8, 27.3 mg, 0.095 mmol), dissolved in benzene (1000 µl) and pyridine (100 µl), the above acid chloride was added. The reaction was allowed to stir for 1.5 hr. The reaction was diluted with water (2.0 ml) and the aqueous mixture was extracted with ether (3x5.0 ml). The ether extracts were washed with brine, combined, and dried over sodium sulfate. The solvent was evaporated under a stream of nitrogen and the ester was isolated via preparative TLC using 40% ethyl acetate/hexanes. The ester was eluted from silica gel using ethyl acetate. Evaporation of the solvent gave 306.78 mCi of testosterone ester 9a. HPLC analysis (Waters and Associates Novapak C_{18} column, 100% acetonitrile, 0.5 mL/min) showed the material to be radiochemically pure *trans*.

A single recrystallization from methanol gave 224.37 mCi of 9a that was shown to be greater than 99% *trans* by HPLC (Fig. 2). The specific activity of ester 9a was determined by first caculating the mass by UV spectroscopy and the material obtained had a specific activity of 18.73 Ci/mmol. The tritiated testosterone ester 9a was stored at 4°C as a solution in 10% ethanol/toluene. HPLC analysis of 9a, after a storage period of one year, showed that no appreciable decomposition had occured.

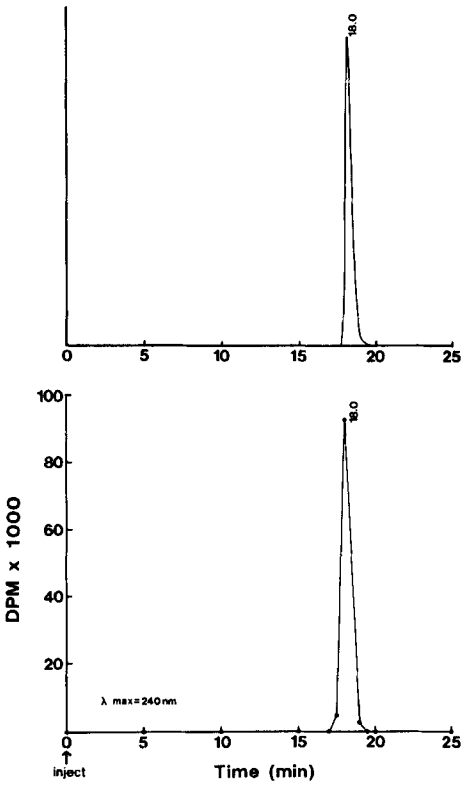


Fig. 2 HPLC analysis of unlabeled (**9**) and ^{3}H-labeled (**9a**) on a reverse phase C_{18} column (Waters and Associates, Novapak™) using MeCN (100%) at a flow rate of 0.5 mL/min, UV detector (λ_{max} = 240 nm).

[4-^{14}C]-Testosterone-17β-(*trans*-4-*n*-butylcyclohexane)carboxylat e (11)

Under dry nitrogen, a solution of *trans*-4-*n*-butylcyclohexanecarboxylic acid (**1**, 0.332 g, 1.9 mmol) in dry benzene (5 ml) was treated dropwise with oxalyl chloride (0.24 ml, 0.349 g, 2.75 mmol). The reaction was then stirred at room temperature until cessation of gas evolution (ca. 30 min). The solvent and excess oxalyl chloride were removed *in vacuo* under a stream of dry nitrogen. Benzene (3 ml) was then added to the residue and the solution was concentrated as before. The resulting oil was used in the subsequent reaction without purification.

[4-^{14}C]-Testosterone (**10**, 0.18 mmol, 9 mCi) was dissolved in dry benzene (2 ml) containing pyridine (0.2 ml). Under dry nitrogen a solution of *trans*-4-*n*-butylcyclohexanoyl chloride (0.36 mmol) in dry benzene (1 ml) was added via syringe. After stirring for 1 hr at room temperature, TLC (EtOAc/hexanes, 2:3) indicated completion of reaction. A dilute sodium hydroxide solution (0.05 N, 2 ml) was added and the mixture was stirred at room temperature for ca. 15 min. The mixture was taken up in ethyl acetate (ca. 40 ml) and diluted

with water (ca. 40 ml). The mixture was extracted with ethyl acetate (3x) and the organic fractions were washed successively with saturated sodium bicarbonate (1x), water (1x) and brine (1x). The organic fractions were combined and dried over anhydrous sodium sulfate. A radiochromatogram (EtOAc/hexanes, 2:3) taken of the organic fraction indicated a single radioactive product identical in R_f to that of the corresponding unlabeled ester. Scintillation counting of a 10 µl sample taken from a 250 ml dilution gave a yield of 7.76 mCi. Ester **11** was stored at 4°C as a solution in 10% ethanol/toluene. HPLC analysis of **11**, after a storage period of one year, showed that no appreciable deocmposition had occured.

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REFERENCES

1. E. Nieschlag, H.M. Behre, and G.F. Weinbauer (1989): In: Perspective in Andrology, edited by M. Serio. Serono Symposium Publications from Ravens Press. 53:517.

2. G.F. Weinbauer, G.R. Marshall, and E. Nieschlag, Acta Endocrinol (Copen.): 113:128, (1986).

3. F. Camps, J. Coll, and J. Pascual, J. Org. Chem., 32:2563 (1967)

4. R.L. Cargill, A.B. Sears, J. Boehm, and M.R. Willcott, J. Am. Chem. Soc., 95:4346 (1973).

5. H.D. Banks and W. Jacobs, J. Org. Chem. 40:790 (1975).

6. B.A. Pearlman, J. Am. Chem. Soc., 101:6404 (1979).

7. H. van Bekkum, P.E. Verkade, and B.M. Wepster, Proc. Koninkl. Ned. Akad. Wetenschap, Series B, 62:147 (1959).

8. H. van Bekkum, A.A.B. Kleis, D. Medema, P.E. Perkade, and B.M. Wepster, Rec. Trav. Chim. 81:833 (1962).