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SYNTHESIS OF (22*R*,23*R*)- AND (22*S*,23*S*)-[4-^{14}C]-24-EPIBRASSINOLIDE

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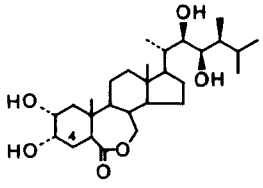
SUMMARY

The title compounds were synthesized for metabolic studies in plants and animals, with ^{14}C labelling being made at position 4 of the epibrassinolides 4 and 5 in an overall radiochemical yield of 3.22% and 4.46%, respectively, based on barium [^{14}C]carbonate.

Key words: (22*R*,23*R*)-[4-^{14}C]-24-Epibrassinolide, (22*S*,23*S*)-[4-^{14}C]-24-Epibrassinolide, Plant growth regulator, Carbon-14.

INTRODUCTION

Brassinosteroids are a new class of plant growth regulating substances. Since the first isolation of brassinolide (1) from rape pollen (1), a number of related brassinosteroids have been isolated and identified from various plants (2, 3). Many related compounds have been synthesized, and the structure-activity relationships of brassinosteroids have been investigated (4). Among these synthetic analogues, (22*R*,23*R*)-24-epibrassinolide (2) and (22*S*,23*S*)-24-epibrassinolide (3) are promising candidates for applications in agriculture (5). To facilitate metabolic and distribution studies of 2 and



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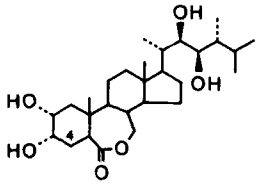

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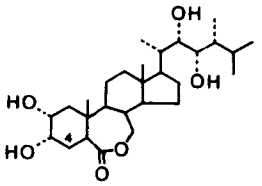
4 : [4-^{14}C]



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5 : [4-^{14}C]

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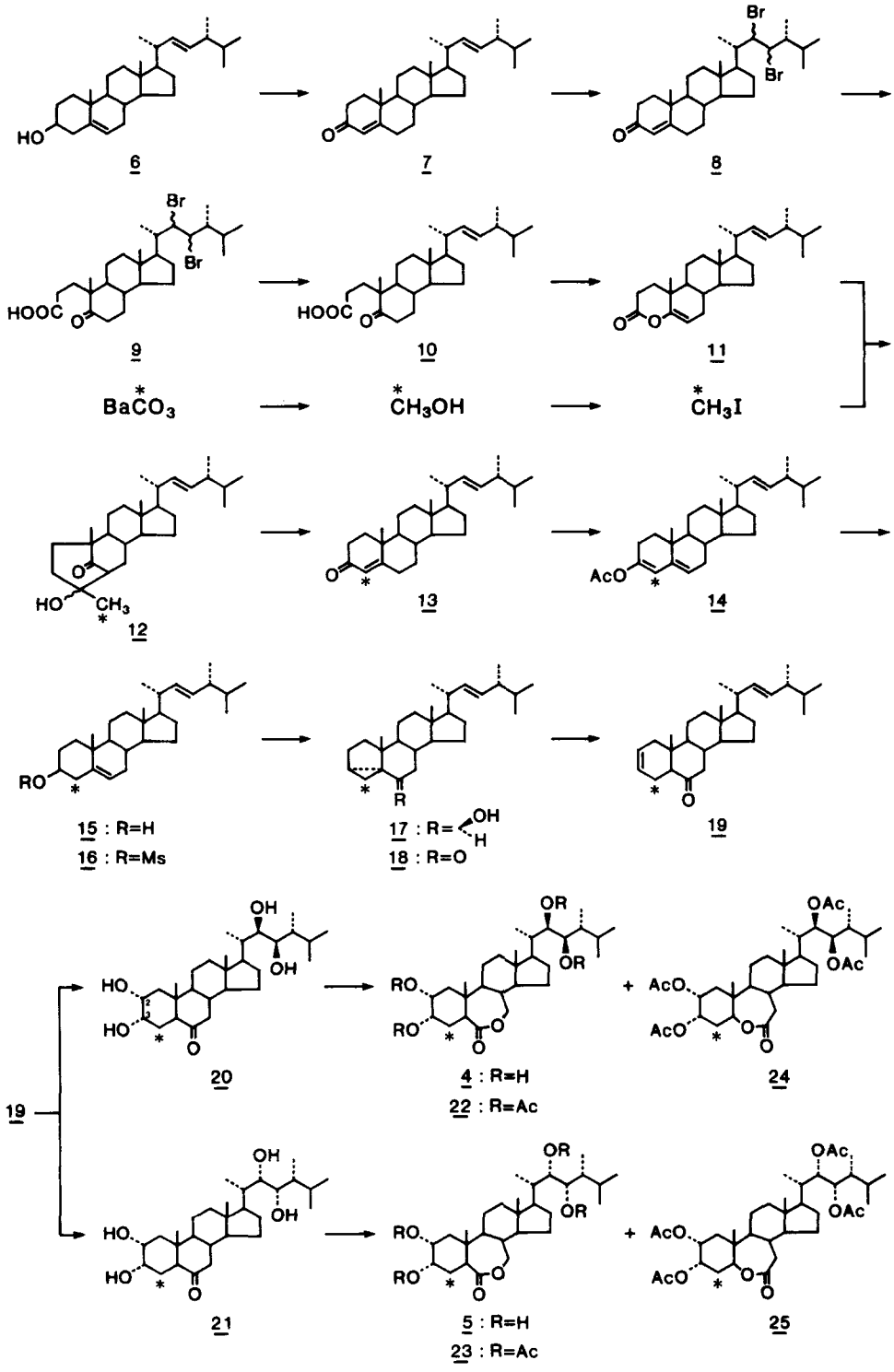
3 as well as to study their roles in the growth of grain and vegetables, we have synthesized (22*R*,23*R*)-[4-^{14}C]-24-epibrassinolide ((22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-[4-^{14}C]ergostan-6-one) (4) and (22*S*,23*S*)-[4-^{14}C]-24-epibrassinolide ((22*S*,23*S*,24*R*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-[4-^{14}C]-ergostan-6-one) (5).

SYNTHESIS

Position 4 in 24-epibrassinolide was selected for ^{14}C labelling because of its stability to metabolic loss and easy preparation of 4 and 5. In order to apply the well-established method (6) of ^{14}C-incorporation into position 4, the enol lactone 11 was synthesized from brassicasterol (6) in five steps in 24.4% overall yield. Thus, brassicasterone (7), prepared from 6 by Oppenauer oxidation, was selectively brominated at the 22E double bond for protection to give dibromide 8, which was converted into dibromo keto acid 9 by ozonolysis and oxidative cleavage. Debromination with zinc dust in acetic acid regenerated the 22E double bond to give keto acid 10, which was treated with acetic anhydride in the presence of sodium acetate to afford enol lactone 11.

Grignard reaction of 11 with [^{14}C]methyl iodide, prepared by the known method (7,8) from barium [^{14}C]carbonate via [^{14}C]methanol gave bridged ketone 12, which on alkaline treatment in methanol furnished [4-^{14}C]brassicasterone (13) in 69.9% yield from [^{14}C]methyl iodide. Enol acetylation of 13 with isopropenyl acetate and an acid catalyst gave a mixture of enol acetate 14 and its $\Delta^{2,4}$ isomer, which without separation was reduced with sodium borohydride in methanol to give [4-^{14}C]brassicasterol (15) in 32.5% yield together with a mixture of 3α-hydroxy isomer of 15, the 3β-hydroxy-4-ene derivative, and its 3α-hydroxy isomer. The mixture was reconverted by Jones' oxidation (9) to the enone 13. By three more repetitions of the above procedure, the desired compound 15 was prepared in 55.8% total yield from 13. 3,5-Cyclo-6-ol 17 was obtained from 15 in 91.7% yield by mesylation to give 16 followed by treatment with sodium carbonate in acetone. Jones' oxidation (9) of 17 gave 3,5-cyclo-6-one 18, which was treated with lithium bromide and camphorsulfonic acid in dimethylacetamide to afford 2,22-diene-6-one 19 in 81% yield. Oxidation of 19 with osmium tetroxide gave a stereoisomeric mixture of 2,3,22,23-tetraols. By repeated chromatography and recrystallization, (22*R*,23*R*)-tetraol 20 and (22*S*,23*S*)-tetraol 21 were separated from their 2β,3β-


6
7
8
9
10
11
$BaCO_3$
CH_3OH
CH_3I
12
13
14
15 : R=H
16 : R=Ms
17 : R=
18 : R=O
19
20
4 : R=H
22 : R=Ac
24
21
5 : R=H
23 : R=Ac
25

hydroxy isomers in yields of 26% and 35%, respectively. Bayer-Villiger oxidation of the (22*R*,23*R*)-tetraol 20 with trifluoroperacetic acid in dichloromethane gave the (22*R*,23*R*)-7-oxa-lactone 4 contaminated with a small amount of its 6-oxa isomer. (22*S*,23*S*)-Tetraol 21 also gave the (22*S*,23*S*)-7-oxa-lactone 5 contaminated with its 6-oxa isomer. The crude lactones 4 and 5 were purified by transformation into tetraacetate 22 and 24 followed by chromatography, recrystallization, and hydrolysis. The yields of pure 4 and 5 were 50% and 49.6%, respectively, from the tetraol 20 and 21. The overall radiochemical yields of 4 and 5 are 3.20% and 4.46%, respectively, based on barium [^{14}C]carbonate. Specific radioactivity of 4 and 5 was 56.78 mCi/mmol (118.1 μCi/mg).

EXPERIMENTAL

The usual work-up of extracts consists of washing to neutrality, drying with sodium sulfate, filtration, and evaporation of the solvent *in vacuo*. Column chromatography was carried out by method A: silica gel (Merck No 7734) or method B: prepacked silica gel (Merck Lobar B) with a solvent system for elution indicated in parentheses.

(24R)-Ergosta-4,22E-dien-3-one (brassicasterone)(7)

To a stirred solution of brassicasterol (6) (90 g, 226 mmol) in dry toluene (1.62 l) and cyclohexanone (432 ml) was added dropwise a solution of aluminum isopropoxide (23 g, 113 mmol) in toluene (520 ml) under nitrogen over 30 min, during which the mixture was distilled at 135°C to complete the reaction. The reaction mixture was concentrated further to about 2 l by distillation, cooled in an ice bath, and mixed with 25% aq. potassium sodium tartrate (360 ml). The organic layer was separated and the aqueous layer was extracted with toluene. The usual work-up of the combined organic layer gave a crystalline residue (162 g), which was recrystallized from dichloromethane-methanol to give 7 (79.9 g, 202 mmol), m.p. 129.5 - 131°C, in 89% yield.

(24R)-22,23-Dibromoergosta-4-en-3-one (8)

To a stirred solution of 7 (72.5 g, 183 mmol) and pyridine (36.2 ml) in dichloromethane (725 ml) was added dropwise a solution of bromine (30.7 g, 192 mmol) in acetic acid (260 ml) over 1.7 hr at -40°C. After stirring for 10 min at -40°C (the reaction was follwed by reverse phase HPLC (methanol-water 98:2)), the reaction mixture was poured into water (1 l) and extracted with dichloromethane (2 x 300 ml). The usual

work-up of the extracts followed by crystallization from acetone-methanol gave 8 (67.9 g, 122 mmol), m.p. 199 - 201°C, in 66.9% yield.

(24R)-22,23-Dibromo-5-oxo-3,5-seco-4-norergostan-3-carboxylic acid (9)

Into a stirred solution of 8 (31 g, 51 mmol) and acetic acid (263 ml) in ethyl acetate (775 ml) was introduced ozone (5.6 g, 116 mmol, 14.1 g/m^3 air), generated by an ozone generator (Nippon ozone IO-2-2A), for 1 hr at -15°C. After expelling excess ozone by bubbling nitrogen for 5 min, water (134 ml) and 30% aq. hydrogen peroxide (26.3 ml) were added to the mixture at -15°C. After being stirred for 6 hr at room temperature, the reaction mixture was poured into water (1 l) and extracted with ethyl acetate (2 x 1.5 l). The extracts were washed with 5% aq. sodium sulfite (1 l) and water (1 l), dried with sodium sulfate, and evaporated to dryness to give raw crystalline 9 (37.5 g), which was used for the next reaction without any purification.

(24R)-5-Oxo-3,5-seco-4-norergosta-22E-en-3-carboxylic acid (10)

To a stirred solution of 9 (75 g) and acetic acid (103 ml) in ether (1.03 l) was added zinc powder (51.7 g, 795 mmol) in portions at room temperature. After stirring for 1.5 hr at 35°C and refluxing for 50 min, the mixture was filtered and the filtrate worked up in the usual way to give a crystalline residue (50 g), which on column chromatography (method A, 50 g, benzene-ethyl acetate 10:1 to 5:1) followed by crystallization from ether-*n*-hexane gave 10 (23 g, 55 mol), m.p. 136 - 138°C in 49.1% yield from 8. Anal, Calcd for $C_{27}H_{44}O_3$: C, 77.83; H, 10.64. Found: C, 77.55; H, 10.66. IR:($CHCl_3$) 2950, 2850, 1700 cm^{-1}.

(24R)-4-Oxa-ergosta-5,22E-dien-3-one (11)

To a stirred solution of 10 (20 g, 47.9 mmol) in acetic anhydride (100 ml) was added anhydrous sodium acetate (6 g, 73 mmol) under nitrogen. After stirring for 3 hr at 135°C, the reaction mixture was concentrated to leave a crystalline residue which was extracted with ether (2 x 100 ml). The usual work-up of the extracts gave a residue (20 g), which on column chromatography (method A, 50 g, benzene-ethyl acetate 9:1) followed by crystallization from ether afforded 11 (15.9 g, 40 mmol), m.p. 132.5 - 133.5°C, in 83.4% yield. Anal. Calcd for $C_{27}H_{42}O_2$: C, 81.3; H, 10.6. Found: C, 81,2; H, 10.6. MS; m/z 398 (M+). IR;($CHCl_3$) 1740, 1685 cm^{-1}.

[^{14}C]Methanol

According to a modification of the Cox and Turner method (7), [^{14}C]carbon dioxide

generated from barium [^{14}C]carbonate (1 Ci, 3.32 g, 16.66 mmol) was introduced into a suspension of lithium aluminum hydride (2.6 g, 68.4 mmol) in diethylene glycol diethyl ether (80 ml) and the mixture was stirred for 1 hr at room temperature and for 2.5 hr at 70°C. Addition of tetrahydrofurfuryl alcohol (35 ml) followed by vacuum distillation (70 mm Hg) at 110°C accompanied with bubbling a slow nitrogen stream gave [^{14}C] methanol (887 mCi, 15.6 mmol, 56.78 mCi/mmol) in 88.7% yield.

[^{14}C]Methyl iodide

[^{14}C]Methyl iodide was prepared by the Ronzio and Murray method (8). Thus, a solution of [^{14}C]methanol (887 mCi, 15.6 mmol) in 55% aq. hydrogen iodide (40 mL) was heated for 10 min at 40 to 60°C then for 20 min at 90°C. Distillation of the reaction mixture accompanied with a slow nitrogen stream and redistillation of the distillate in a vacuum line gave [^{14}C]methyl iodide (887 mCi, 15.6 mmol) in quantitatve yield.

(24R)-3-Hydroxy-3-[^{14}C]methyl-3(5→6βH)abeo-A-ergosta-22E-en-5-one (12)

A Grignard reagent, prepared from [^{14}C]methyl iodide (887 mCi, 15.6 mmol) and magnesium turnings (396 mg, 16.5 mmol) in ether (20 ml), was added dropwise into a stirred solution of the enol lactone 11 (6.68 g, 16.78 mmol) in anhydrous dichloromethane (100 ml) at 0°C over 5 min under nitrogen. After stirring for 2 hr at 0°C and for 2.5 hr at room temperature, 1N aq. sulfuric acid (40 ml) was added to the reaction mixture at 0°C. Then the organic layer was separated and the aqueous layer extracted with ether (3 x 150 ml). The combined organic layers were worked up in the usual way and the crude product was crystallized from ethyl acetate to give 12 (675 mCi, 4.95 g, 11.9 mmol) in 76.1% radiochemical yield based on [^{14}C]methyl iodide.

(24R)-[4-^{14}C]Ergosta-4,22E-dien-3-one ([4-^{14}C]brassicasterone) (13)

To a stirred solution of 12 (675 mCi, 4.95 g, 11.9 mmol) in tetrahydrofuran-methanol (1:1, 130 ml) was added 1N aq. sodium hydroxide (7.0 ml) at 0°C. After stirring for 1.5 hr at 55°C under nitrogen, the reaction mixture was concentrated *in vacuo* to about 20 ml below 20°C and extracted with ether (2 x 100 ml). The extracts were worked up in the usual way to leave a crystalline residue, which on column chromatography (method A, 20 g, ether) followed by crystallization from ether gave 13 (620 mCi, 4.35 g, 10.9 mmol), m.p. 129.5 - 131°C, in 91.8% yield.

(24R)-3-Acetoxy-[4-^{14}C]ergosta-3,5,22E-triene (14)

A mixture of 13 (620 mCi, 4.35 g, 10.9 mmol), *p*-toluene sulfonic acid (146 mg), and

isopropenyl acetate (230 ml) was refluxed for 4 hr under nitrogen. The mixture was concentrated to about 80 ml by distillation at atmospheric pressure and further to about 30 ml *in vacuo*. The concentrate was diluted with anhydrous ether (200 ml) and worked up in the usual way to give a crystalline residue (619 mCi, 4.81 g) as a mixture of 14 and its isomer ((24*R*)-3-acetoxy-[4-^{14}C]ergosta-2,4,22E-triene), which was used for the next reaction without separation.

(24R)-3β-Hydroxy-[4-^{14}C]ergosta-5,22E-diene ([4-^{14}C]brassicasterol) (15)

To a stirred solution of the mixture of 14 and its $\Delta^{2,4}$ isomer (619 mCi, 4.81 g, 10.9 mmol) in methanol-tetrahydrofuran (2:1, 360 ml) was added sodium borohydride (3.9 g, 103 mmol) in portions over 4 hr at room temperature. After addition of acetic acid (7 ml), the reaction mixture was concentrated *in vacuo*, suspended in water (100 ml), and extracted with ether (2 x 200 ml). The extracts were worked up in the usual way to leave a crystalline residue (4.3 g), which was chromatographed (method B, chloroform-acetone 49:1) to give 15 (202 mCi, 1.42 g, 3.55 mmol) in 32.5% yield and a mixture of 3α-hydroxy-5-ene, 3α-hydroxy-4-ene, and 3β-hydroxy-4-ene derivatives (417 mCi, 2.94 g, 7.36 mmol).

The mixture was reconverted to brassicasterone (13) by Jones' oxidation (9), which underwent enol-acetylation and sodium borohydride reduction as described above to give 15 (110 mCi, 711 mg, 1.78 mmol) and a by-product mixture. These procedures were repeated two more times to afford additional amounts (43 mCi, 302 mg, 0.757 mmol) of 15. The total amount of 15 is 2.44 g (346 mCi, 6.10 mmol) and its total yield is 55.8% from 13.

(24R)-3β-Methanesulfonyloxy-[4-^{14}C]ergosta-5,22E-diene (16)

To a stirred solution of 15 (346 mCi, 2.44 g, 6.10 mmol) and triethylamine (3.9 ml, 28 mmol) in anhydrous toluene-ether (1:1, 48 ml) was added methanesulfonyl chloride (0.84 ml, 10.9 mmol) at 0°C. After stirring for 50 min at 0°C and for 15 min at room temperature, cold water (2 ml) and cold 10% phosphoric acid (60 ml) were added to the reaction mixture. Extraction with ether (2 x 100 ml) and the usual work-up of the extracts followed by crystallization from ether gave 16 in quantitative yield.

(24R)-6β-Hydroxy-3α,5-cyclo-5α-[4-^{14}C]ergosta-22E-ene (17)

To a stirred solution of 16 (346 mCi, 2.92g, 6.10 mmol) in acetone (70 ml) and toluene (7 ml) was added a solution of sodium carbonate (815 mg, 7.65 mmol) in water

(18 ml) at room temperature. After gentle refluxing for 12.5 hr under nitrogen, the reaction mixture was concentrated *in vacuo* to about 30 ml below 40°C, mixed with water (50 mL), and extracted with ether (2 x 70 ml). The extracts were worked up in the usual way to give a crystalline residue (2.40 g), which was chromatographed (method A, 50 g, *n*-hexane-ethyl acetate 10:1) to give 17 (283 mCi, 1.99 g, 4.97 mmol) in 81.4% yield and [4-^{14}C]brassicasterol (15) (40.5 mCi, 0.285 g, 0.71 mmol) in 11.7% yield. Recovered 15 was converted to 17 (35.8 mCi, 252 mg, 0.63 mmol) *via* 16. The total yield of 17 is 92.1% from 16.

(24R)-3α,5-Cyclo-5α-[4-^{14}C]ergosta-22E-en-6-one (18)

To a stirred solution of 17 (319 mCi, 2.24 g, 5.62 mmol) in acetone-tetrahydrofuran (5:1, 60 ml) was added Jones' reagent (9) (2.8 ml) at 0°C. After stirring for 10 min at 0°C, excess reagent was quenched by adding isopropyl alcohol (2.0 ml). The reaction mixture was diluted with ether (100 ml) and passed through a column of silica gel (method A, 20 g, ether). The eluate was evaporated and the residue crystallized from acetone to give 18 (306 mCi, 2.14 g, 5.39 mmol), m.p. 110 - 111°C, in 96.0% yield.

(24R)-5α-[4-^{14}C]Ergosta-2,22E-dien-6-one (19)

To a stirred solution of 18 (306 mCi, 2.14 g, 5.39 mmol) in dry dimethylacetamide (21 ml) were added lithium bromide (1.05 g, 12.07 mmol) and D-10-camphorsulfonic acid (107 mg, 0.46 mmol) at room temperature. The mixture was stirred for 1.5 hr at 160°C under nitrogen, cooled, mixed with cold 1% aq. sodium bicarbonate (35 ml), and extracted with ether (3 x 50 ml). The extracts were worked up in the usual way to give a crystalline residue, which on chromatography (method B, *n*-hexane-ethyl acetate 100:1.5) followed by crystallization from acetone-ether gave 19 (248 mCi, 1.74 g, 4.36 mmol), m.p. 123 - 124°C, in 81% yield.

(22R,23R,24R)-2α,3α,22,23-Tetrahydroxy-5α-[4-^{14}C]ergostan-6-one (20) and (22S,23S,24R)-2α,3α,22,23-Tetrahydroxy-5α-[4-^{14}C]ergostan-6-one (21)

To a stirred solution of 19 (248 mCi, 1.74 g, 4.36 mmol) in *tert*-butyl alcohol-tetrahydrofuran (3:1, 36 ml) was added a solution of osmium tetroxide (2.33 g, 9.16 mmol) in carbon tetrachloride (13 ml) at 0°C under nitrogen. The mixture was stirred for 1.5 hr at 0°C and for 24 hr at room temperature. After dilution with tetrahydrofuran (100 ml), hydrogen sulfide was introduced into the reaction mixture, which was filtered. The residue was washed with hot methanol-tetrahydrfuran (1:4, 150 ml) containing

0.1% of water and filtered. The combined filtrates and washings were evaporated to give a residue (2.2 g), which was chromatographed (method B, chloroform-acetone 1:1) to give crude 21 (1.16 g) containing a small amount of its 2β,3β-hydroxy isomer and crude 20 (765 mg) containing a small amount of its 2β, 3β-hydroxy isomer. The crude 21 was separated by chromatography (method B, ethyl acetate) and recrystallization from ethyl acetate to give 21 (87.4 mCi, 717 mg, 1.54 mmol), m.p. 184 - 185°C, in 35.2% yield and the 2β,3β-hydroxy isomer (16.0 mCi, 131 mg, 0.28 mmol).

The crude 20 was also separated by chromatography (method B, dichloromethane-methanol 50:3) and crystallization from ethyl acetate to give 20 (66.8 mCi, 548 mg, 1.18 mmol), m.p. 241 - 242°C, in 26.9% yield and the 2β,3β-hydroxy isomer (9.71 mCi, 79.7 mg, 0.17 mmol).

(22R,23R,24R)-2α,3α,22,23-Tetrahydroxy-B-homo-7-oxa-5α-[4-^{14}C]ergostan-6-one ((22R,23R)-[4-^{14}C]-24-epibrassinolide) (4)

Trifluoroperacetic acid was prepared by addition of trifluoroacetic anhydride (4.20 g, 20 mmol) and powdered sodium sulfate (1.4 g, 9.9 mmol) to 30% aq. hydrogen peroxide (0.535 ml, 4.72 mmol) in dichloromethane (4.80 ml) at 0°C.

To a stirred solution of 20 (66.8 mCi, 548 mg, 1.18 mmol) in dichloromethane (15 ml) was added at 0°C trifluoroperacetic acid prepared above. After stirring for 4.3 hr at O°C, a saturated aq. solution of sodium bisulfite (9 ml) was added to the reaction mixture. The mixture was poured into ice water (100 ml) and extracted with chloroform-methanol (5:1, 3 x 100 ml). The extracts were worked up in the usual way to leave a residue (491 mg), which was acetylated with acetic anhydride and pyridine in the presence of 4-dimethylaminopyridine for easy separation of 4 and its 6-oxa isomer. The crude acetate (661 mg) underwent chromatography (method B, benzene-ethyl acetate 4:1 to 2:1) followed by recrystallization from ether-*n*-pentane to give 7-oxa-lactone acetate 22 (35.3 mCi, 404 mg, 0.62 mmol), m.p. 159 - 161°C, in 52.8% yield from 20 and 6-oxa-lactone acetate 24 (5.55 mCi, 63.5 mg, 0.98 mmol), m.p. 193.5 - 195°C.

The 7-oxa compound 22 was dissolved in a solution of potassium hydroxide (141 mg, 3.2 mmol) in methanol (9 ml). The mixture was stirred for 1.7 hr at 82°C, mixed with 0.1N aq. sulfuric acid (35 ml), and extracted with chloroform-methanol (5:1, 2 x 15 ml). The extracts were worked up in the usual way and the residue crystallized from ethyl acetate to give 4 (32.2 mCi, 273 mg, 0.566 mmol), m.p. 265 - 266°C, in 48.2% yield

from 20. The overall yield of 4 is 3.22% based on barium [^{14}C]carbonate. The radiochemical purity of 4 is 98.8% as determined by HPLC and 98.4% by TLC (chloroform-methanol-acetic acid 12:1:0.1). A reverse dilution analysis of 4 (4.57 mCi, 38.7 mg, 0.08 mmol) diluted with unlabelled compound 2 (400 mg, 0.832 mmol) showed a constant specific activity (10.68 μCi/mg, 5.13 mCi/mmol).

(22S,23S,24R)-2α,3α,22,23-Tetrahydroxy-B-homo-7-oxa-5α-[4-^{14}C] ergostan-6-one ((22S,23S)-[4-^{14}C]-24-epibrassinolide) (5)

The tetraol 21 (87.4 mCi, 717 mg, 1.54 mmol) was oxidized with trifluoroperacetic acid prepared from trifluoroacetic anhydride (5.50 g, 26.1 mmol) and 30% aq. hydrogen peroxide (0.70 ml, 6.17 mmol) as described for 20 to give a residue (754 mg), which on acetylation followed by chromatography (method B, benzene-ethyl acetate 4:1) gave 7-oxa-lactone acetate 23 (49.8 mCi, 569 mg, 0.87 mmol) and 6-oxa-lactone acetate 25 (9.00 mCi, 103 mg, 0.158 mmol).

Alkaline hydrolysis of 23 with a solution of potassium hydroxide (260 mg, 4.64 mmol) in methanol (13 ml) as described for 22 followed by recrystallization from ethyl acetate gave 5 (44.6 mCi, 379 mg, 0.786 mmol), m.p. 198.5 - 200°C, in 51.0% yield from 21. The overall yield of 5 is 4.46% based on barium [^{14}C]carbonate. The radiochemical purity of 5 is 98.0% as determined by HPLC and 99% by TLC as described for 4. A reverse dilution analysis of 23 (34 mg, 4.0 mCi) diluted with unlabelled compound 3 (340 mg, 0.707 mmol) showed a constant specific activity (12.09 μCi/mg, 5.8 mCi/mmol).

Radioactivity was determined by an Aloka liquid scintillation countor LSC-672. Radiochemical purities were determined by HPLC which was run on a Shimazu LC-3A (column: Nucleosil $_5$C18 4.6 mm i.d. x 150 mm, mobile phase: acetonitrile-water 4:6 (1 ml/min), detector: Shimazu SPD-2A (208 nm), radioactive flow monitor: Packard Trace II 7150 (Monophase 40 plus (1 ml/min) as a scintilator) and TLC (precoated Silica gel plate Merck No 5715) with scintillation counting. [^{14}C]-Labeled compounds were identified with the corresponding authentic samples by comparison of TLC, mp, IR and/or NMR spectra.

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