Synthesis of Teasterone and Typhasterol, Brassinolide-related Steroids with Plant-growth-promoting Activity

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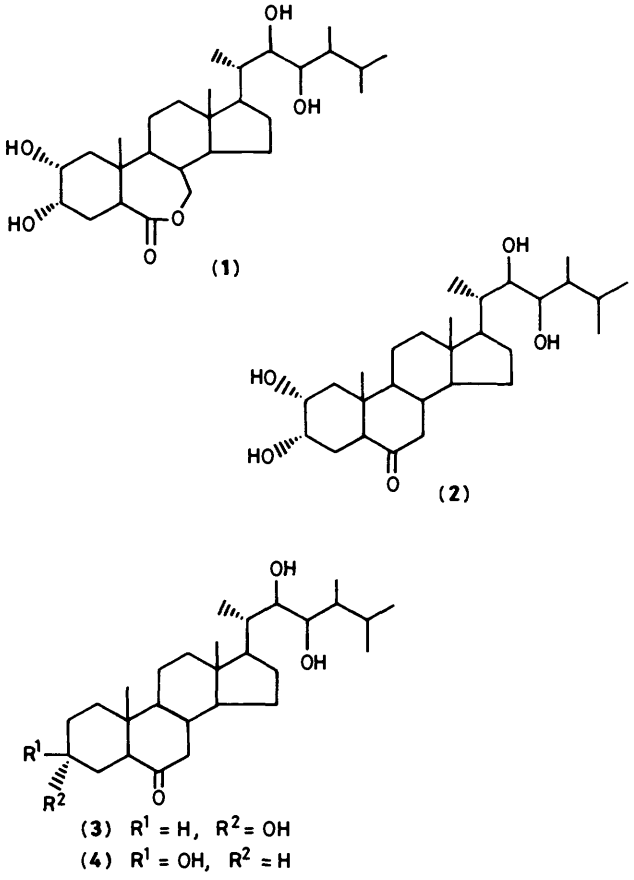
Brassinolide-related natural steroids, teasterone (**4**), (22*R*,23*R*,24*S*)-3β,22,23-trihydroxy-5α-ergostan-6-one, and typhasterol (**3**), 3α-isomer of (**4**), have been synthesized from crinosterol (**5**). The cyclopropyl ketone (**6**), obtained by solvolysis of crinosterol methanesulphonate followed by Jones oxidation, was transformed by treatment with acid and acetylation into (22*E*,24*S*)-6-oxo-5α-ergost-22-en-3β-yl acetate (**7**), which was epoxidized. Epoxide-ring opening of the separated (22*R*,23*R*)-epoxide (**9**) with 30% HBr–AcOH, followed by inversion reaction at the carbon bearing bromine, and acetylation, provided the (22*R*,23*R*,24*S*)-triacetate (**10**), which was saponified to yield teasterone (**4**). The sulphonate (**11**), derived from teasterone (**4**), was submitted to an improved inversion reaction with caesium acetate. Deprotection of the resulting 3α-acetate (**12**) provided typhasterol (**3**).

Since the discovery of brassinolide (**1**), (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-B-homo-7-oxa-5α-ergostan-6-one,[1a] a number of related steroids have been isolated from higher plants, and form a new class of plant-growth promoter. Among them are castasterone (**2**), (22*R*,23*R*,24*S*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one,[1b] typhasterol (2-deoxycastasterone) (**3**), (22*R*,23*R*,24*S*)-3α,22,23-trihydroxy-5α-ergostan-6-one,[1c,d] and teasterone (**4**), (22*R*,23*R*,24*S*)-3β,22,23-trihydroxy-5α-ergostan-6-one.[1e] These three steroids, in addition to brassinolide (**1**), have been found in the leaves of green tea (*Thea sinensis*).[1e,f] Furthermore, from the pollen of the pine tree (*Pinus thunbergii* Parl)[1d] and of corn (*Zea mays*),[1g] typhasterol (**3**) and/or teasterone (**4**) have recently been isolated along with castasterone (**2**).

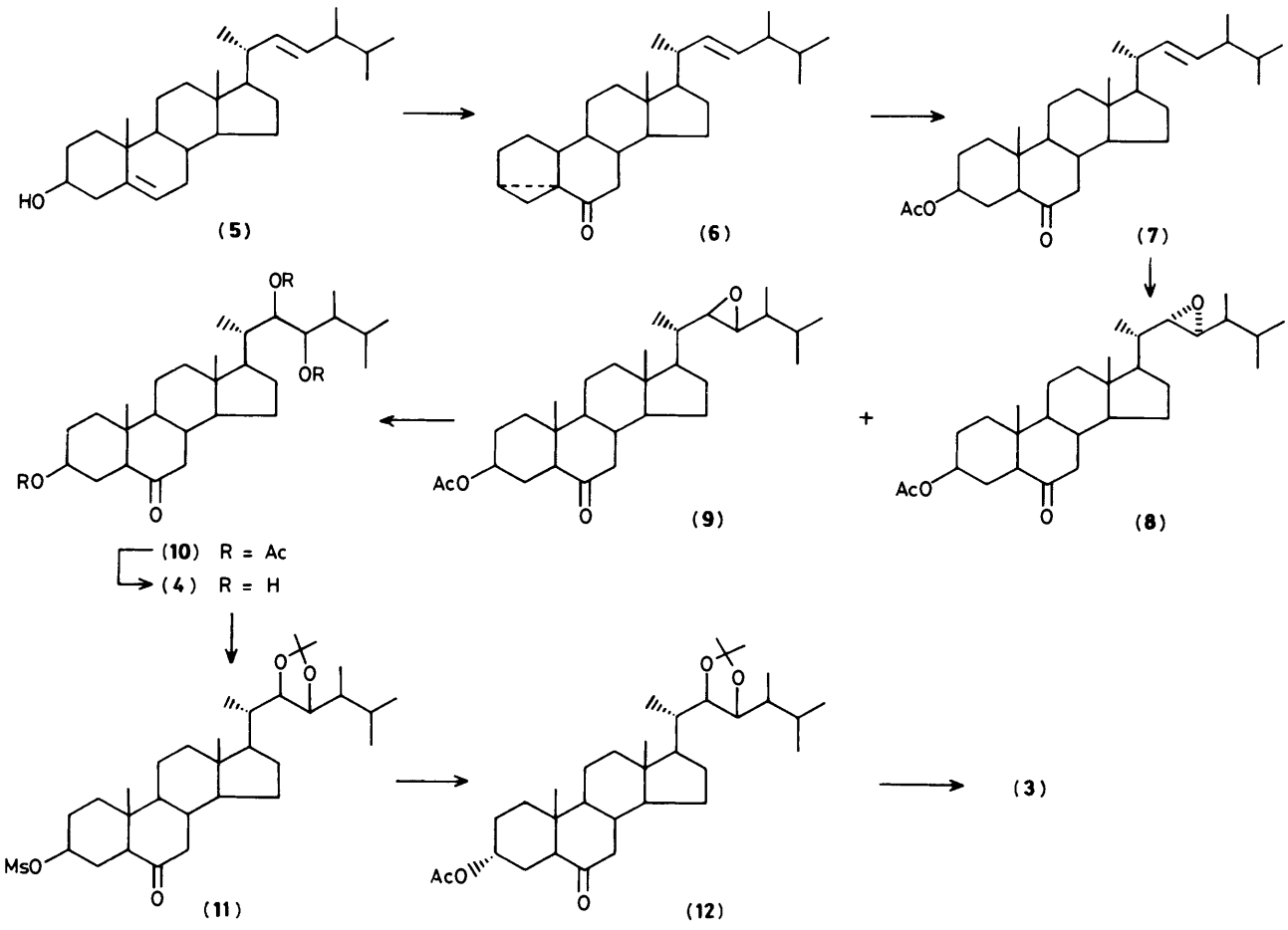
For a biosynthetic study of brassinolide (**1**), and a more detailed biological evaluation of typhasterol (**3**) and teasterone (**4**), we need sufficient amounts of each. In this paper, we report the first synthesis of teasterone (**4**) and our alternative synthesis[2] of typhasterol (**3**). For their synthesis we devised a route applicable for the synthesis of deuterio-labelled brassinolide, castasterone, typhasterol, and teasterone. These labelled compounds are necessary for our other programme, concerned with the microanalysis of natural brassinosteroids.[3]

Crinosterol (**5**), (22*E*,24*S*)-ergosta-5,22-dien-3β-ol, which was prepared according to the reported method,[4] is the starting material for the target molecules (**3**) and (**4**). Refluxing of the methanesulphonate of crinosterol (**5**) with aqueous acetone in the presence of potassium hydrogen carbonate was followed by Jones chromic acid oxidation to give the known (22*E*,24*S*)-3α,5-cyclo-5α-ergost-22-en-6-one (**6**)[5a] in 86% yield. Treatment of compound (**6**) with 5M-sulphuric acid in acetic acid under reflux and subsequent acetylation provided (22*E*,24*S*)-6-oxo-5α-ergost-22-en-3β-yl acetate (**7**) in 93% yield.

For the introduction of the desired (22*R*,23*R*)-diol function into the side-chain of compound (**7**), oxidation with osmium tetraoxide has already been found to be inadequate because of the low stereoselectivity for the (22*R*,23*R*)-diol isomer.[5] Thus, we adopted another method *via* the (22*R*,23*R*)-epoxide, which was developed by Mori *et al.*[6] Epoxidation of compound (**7**) with 1 equiv. of *m*-chloroperbenzoic acid (MCPBA) followed by separation by column chromatography afforded the less polar (22*R*,23*R*,24*S*)-22,23-epoxy-6-oxo-5α-ergostan-3β-yl acetate (**9**) and the more polar (22*S*,23*S*,24*S*)-isomer (**8**) in 63 and 31% yield, respectively. The stereochemical assignment of these epoxides (**8**) and (**9**) was tentatively made on the basis of the



reported observation that upon oxidation of (22*E*,24*S*)-5α-stigmast-22-en-3β-ol with MCPBA the corresponding (22*R*,23*R*)-epoxide was obtained as the major product.[7] This assignment for our epoxides was finally confirmed by transformation of the less polar epoxide (**9**) into typhasterol (**3**) (*vide infra*). The less polar epoxide (**9**) was converted into a mixture of the corresponding bromo acetates by treatment with 30% hydrobromic acid in acetic acid at room temperature. The mixture was heated at 100 °C with 80% aqueous acetic acid for 24 h and then acetylated to give (22*R*,23*R*,24*S*)-6-oxo-5α-



ergostane-3β,22,23-triol triacetate (**10**) in 63% yield. Saponification of triacetate (**10**) with 5% KOH–MeOH afforded teasterone (**4**), m.p. 200—201 °C, in almost quantitative yield. The (22*R*,23*R*,24*S*)-configuration of the synthetic teasterone (**4**) was confirmed by comparison of its ^{1}H n.m.r. data with those reported for brassinolide (**1**) and castasterone (**2**).[2,6]

Transformation of teasterone (**4**) into typhasterol (**3**) was carried out as follows. Teasterone (**4**) was submitted to acetonide formation and then methanesulphonation to give the sulphonate (**11**). In our previous paper,[2] the 3α-hydroxy group was introduced as a formate ester when the sulphonate (**11**) was heated with lithium carbonate and dimethylformamide. However, the yield of the inversion reaction was low (25%). Thus, we investigated a more efficient method. After trying several reagents for the inversion reaction at the C-3 position of 6-oxo-5α-cholestan-3β-yl methanesulphonate, we found that Ikegami's procedure[8] was satisfactory. The sulphonate (**11**) was refluxed in benzene with 5 equiv. of caesium acetate and 1 equiv. of 18-crown-6, and the desired (22*R*,23*R*,24*S*)-22,23-isopropylidenedioxy-6-oxo-5α-ergostan-3α-yl acetate (**12**) was obtained in 67% yield. Removal of the protecting groups with 80% aqueous acetic acid under reflux followed by saponification provided typhasterol (**3**), m.p. 226—229 °C (lit.,[2] 225—228.5 °C), in 94% yield, whose spectral data and chromatographic behaviour on t.l.c. were identical with those of an authentic sample.[2] Conversion of the sulphonate (**11**) into castasterone (**2**) and brassinolide (**1**) has already been described in our previous paper.[2]

Using the above described synthetic route, deuterio-labelled brassinolide, castasterone, typhasterol, and teasterone could be prepared from [26,26,26,28,28,28-$^2H_6$]crinosterol.[9] The results will be reported in due course.

In conclusion we were able to synthesize typhasterol (**3**) and teasterone (**4**) in quantities sufficient for physiological study by developing a more efficient route applicable for synthesis of deuterio-labelled brassinosteroids.

Experimental

M.p.s were determined on a Yazawa hot-stage microscope apparatus (BY-1) and are uncorrected. I.r. spectra were taken with a Hitachi Model 260-10 i.r. spectrometer. ^{1}H N.m.r. spectra were recorded with a Hitachi R-24B (60 MHz) or JEOL JNM-FX 200 (200 MHz) spectrometer, for $CDCl_3$ solutions with tetramethylsilane as internal standard unless otherwise noted. Mass spectra were taken with a Shimadzu GC-MS 9020 gas chromatograph-mass spectrometer with electron-impact source at 70 eV. Kiesel gel 60 F_{254} (Merck) was used for analytical t.l.c. Column chromatography was carried out with Kieselgel 60 (70—230 mesh, Merck). Work-up refers to dilution with water, extraction with an organic solvent indicated in parentheses, washing of the extract to neutrality, drying over anhydrous magnesium sulphate, and removal of the solvent under reduced pressure.

(22E,24S)-3α,5-*Cyclo*-5α-*ergost*-22-*en*-6-*one* (**6**).—(22*E*,24*S*)-Ergosta-5,22-dien-3β-ol (**5**) (3.5 g, 8.79 mmol) was dissolved in pyridine (30 ml) and treated with methanesulphonyl chloride (3 ml) at room temperature for 1 h. Work-up (ether) gave (22*E*,24*S*)-3β-methanesulphonyloxyergosta-5,22-diene (4.04 g).

A mixture of the sulphonate, potassium hydrogen carbonate (3 g), water (100 ml), and acetone (500 ml) was refluxed for 8 h. Removal of acetone by distillation, followed by work-up (ether), gave crude (22*E*,24*S*)-3α,5-cyclo-5α-ergost-22-en-6β-ol (3.5 g), which was dissolved in acetone (100 ml) and treated with Jones' chromic acid reagent (1.1 equiv.). The mixture was stirred at room temperature for 20 min. Work-up (ether) and chromatography on silica gel (3.5 cm i.d. × 25 cm) with benzene provided (22*E*,24*S*)-3α,5-cyclo-5α-ergost-22-en-6-one (**6**) (3.0 g, 86%), m.p. 105—107 °C (lit.,[5a] 105—108 °C) (from methanol). ^{1}H N.m.r., i.r., and mass spectra of the synthetic product (**6**) were identical with those reported.[5a]

(22E,24S)-6-*Oxo*-5α-*ergost*-22-*en*-3β-*yl Acetate* (**7**).—A solution of 22*E*,24*S*)-3α,5-cyclo-5α-ergost-22-en-6-one (**6**) (2.95 g, 7.45 mmol) in acetic acid was treated with 5M-sulphuric acid (4 ml) under reflux for 4 h. The reaction mixture was cooled and neutralized with 10% aqueous potassium hydroxide. Work-up (ethyl acetate) gave a crude product, which was acetylated with acetic anhydride (5 ml) and pyridine (10 ml) at room temperature overnight. Work-up (ethyl acetate) and chromatography on silica gel (2.5 cm i.d. × 25 cm) with benzene–ethyl acetate (50:1) provided (22E,24S)-6-*oxo*-5α-*ergost*-22-*en*-3β-*yl acetate* (**7**) (3.16 g, 93%), m.p. 140—141 °C (from methanol); δ ($CDCl_3$) 0.674 (3 H, s, 18-H_3), 0.770 (3 H, s, 19-H_3), 0.817 (3 H, d, *J* 6.6 Hz, 28-H_3), 0.835 (3 H, d, *J* 6.6 Hz, 26-H_3), 0.912 (3 H, d, *J* 6.6 Hz, 27-H_3), 1.009 (3 H, d, *J* 6.6 Hz, 21-H_3), 2.029 (3 H, s, acetyl), 4.67 (1 H, m, 3-H), and 5.16 (2 H, m, 22- and 23-H) (Found: C, 78.8; H, 10.6. $C_{30}H_{48}O_3$ requires C, 78.90; H, 10.59%).

(22R,23R,24S)- *and* (22S,23S,24S)-22,23-*Epoxy*-6-*oxo*-5α-*ergostan*-3β-*yl Acetate* (**9**) *and* (**8**).—To a solution of (22*E*,24*S*)-6-oxo-5α-ergost-22-en-3β-yl acetate (**7**) (1.44 g, 3.16 mmol) in dichloromethane (20 ml) was added MCPBA (550 mg, 3.18 mmol), and the mixture was stirred at room temperature for 15 h. Calcium hydroxide (2 g) was added and the mixture was stirred for 1 h. Filtration and removal of the solvent gave a crude product, which was applied to a column of silica gel (2.5 cm i.d. × 33 cm). Elution with benzene–ethyl acetate (25:1) gave the less polar (22*R*,23*R*,24*S*)-22,23-epoxy-6-oxo-5α-ergostan-3β-yl acetate (**9**) (942 mg, 63%) as an oil; δ ($CDCl_3$) 0.661 (3 H, s, 18-H_3), 0.773 (3 H, s, 19-H_3), 0.871 (3 H, d, *J* 7.1 Hz, 28-H_3), 0.912 (3 H, d, *J* 6.8 Hz, 26-H_3), 0.937 (3 H, d, *J* 6.8 Hz, 27-H_3), 1.054 (3 H, d, *J* 5.6 Hz, 21-H_3), 2.032 (3 H, s, acetyl), 2.55 (1 H, dd, *J* 6.6 and 2.2 Hz, 22-H), 2.71 (1 H, dd, *J* 6.3 and 2.2 Hz, 23-H), and 4.67 (1 H, m, 3-H); $\nu_{max.}$($CHCl_3$) 2 950s, 2 870s, 1 710s, 1 460m, 1 383m, 1 370m, 1 325w, 1 310w, 1 255s, 1 240s, 1 210s, 1 168w, 1 140w, 1 100w, 1 033s, 995w, 960w, and 905m cm^{-1}; *m/z* 412 (M^+ − 60), 401, 400, 372, 357, 341, 329, 313, 300, 297, 243, 229, 175, 161, 149, 147, 123, 121, and 107.

Further elution with the same solvent gave the more polar (22S,23S,24S)-22,23-*epoxy*-6-*oxo*-5α-*ergostan*-3β-*yl acetate* (**8**) (465 mg, 31%), m.p. 140—141 °C (from methanol); δ ($CDCl_3$) 0.665 (3 H, s, 18-H_3), 0.768 (3 H, s, 19-H_3), 2.032 (3 H, s, acetyl), 2.46 (2 H, m, 22- and 23-H), and 4.67 (1 H, m, 3-H); $\nu_{max.}$($CHCl_3$) 2 955s, 2 870s, 1 710s, 1 460m, 1 385m, 1 370m, 1 330w, 1 310w, 1 260s, 1 245s, 1 212s, 1 170w, 1 142w, 1 125w, 1 100w, 1 035s, 1 020m, 997w, 962w, and 910m (Found: C, 76.15; H, 10.2. $C_{30}H_{48}O_4$ requires C, 76.23; H, 10.24%). The m.s. of compound (**8**) was identical with that of its (22*R*,23*R*)-isomer (**9**).

(22R,23R,24S)-6-*Oxo*-5α-*ergostane*-3β,22,23-*triol Triacetate* (**10**).—(22*R*,23*R*,24*S*)-22,23-epoxy-6-oxo-5α-ergostan-3β-yl acetate (**9**) (900 mg, 1.91 mmol) was treated with 30% HBr–AcOH (4 ml) at room temperature for 4 h. The reaction mixture was diluted with water (60 ml) and neutralized with solid sodium hydrogen carbonate. Work-up (ether) gave a mixture of the bromo-acetates (960 mg), which was heated at 100 °C with acetic acid (80 ml) and water (20 ml) for 24 h. The reaction mixture was poured into chilled aqueous sodium hydrogen carbonate. Work-up (ethyl acetate) gave a crude product, which was further treated with acetic anhydride (6 ml) and pyridine (10 ml) at 60 °C overnight. Work-up (ethyl acetate), followed by chromatography on silica gel (2.0 cm i.d. × 25 cm) with benzene–ethyl acetate (10:1), provided (22R,23R,24S)-6-*oxo*-5α-*ergostane*-3β,22,23-*triol triacetate* (**10**) (690 mg, 63%), m.p. 216—218 °C (from methanol); δ ($CHCl_3$) 0.692 (3 H, s, 18-H_3), 0.769 (3 H, s, 19-H_3), 0.902 (3 H, d, *J* 6.3 Hz, 26-H_3), 0.942 (3 H, d, *J* 6.3 Hz, 27-H_3), 0.954 (3 H, d, *J* 6.6 Hz, 28-H_3), 1.015 (3 H, d, *J* 6.8 Hz, 21-H_3), 1.994 (3 H, s, acetyl), 2.022 (3 H, s, acetyl), 2.028 (3 H, s, acetyl), 4.67 (1 H, m, 3-H), 5.16 (1 H, d, *J* 8.8 Hz, 22-H), and 5.33 (1 H, d, *J* 8.8 Hz, 23-H); *m/z* 454 (M^+ − 2 × 60), 388, 383, 371, 329, 311, 300, 271, and 111 (Found: C, 70.9; H, 9.5. $C_{34}H_{54}O_7$ requires C, 71.05; H, 9.47%).

(22R,23R,24S)-3β,22,23-*Trihydroxy*-5α-*ergostan*-6-*one*, *Teasterone* (**4**).—(22*R*,23*R*,24*S*)-6-Oxo-5α-ergostane-3β,22,23-triol triacetate (**10**) (650 mg, 1.13 mmol) was treated with 5% KOH–MeOH (30 ml) under reflux for 1 h. Work-up (ethyl acetate) gave teasterone (**4**) (497 mg, 98%), m.p. 200—201 °C (from ethyl acetate); δ (C_5D_5N) 0.751 (3 H, s, 18-H_3), 0.785 (3 H, s, 19-H_3), 1.042 (3 H, d, *J* 6.8 Hz, 28-H_3), 1.113 (3 H, d, *J* 6.8 Hz, 26-H_3), 1.152 (3 H, d, *J* 6.8 Hz, 27-H_3), 1.271 (3 H, d, *J* 6.4 Hz, 21-H_3), 3.85 (1 H, m, 3-H), 3.99 (1 H, d, *J* 8.6 Hz, 22-H), and 4.16 (1 H, d, *J* 8.6 Hz, 23-H); *m/z* (as methylboronate–trimethylsilyl derivative[3a]) 544 (M^+), 529, 515, 488, 454, 439, 319, 305, 300, 229, 211, 189, 177, 171, 159, and 155 (Found: C, 74.8; H, 10.7. $C_{28}H_{28}O_4$ requires C, 74.95; H, 10.78%).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-6-*oxo*-5α-*ergostan*-3β-*yl Methanesulphonate* (**11**).—Teasterone (**4**) (170 mg, 0.379 mmol) was dissolved in acetone (10 ml) and treated with toluene-*p*-sulphonic acid (20 mg) at room temperature for 2 h. Work-up (ether) gave the corresponding acetonide, which was further treated with methanesulphonyl chloride (0.3 ml) and pyridine (4 ml) at room temperature for 1 h. Work-up (ethyl acetate) gave (22*R*,23*R*,24*S*)-22,23-isopropylidenedioxy-6-oxo-5α-ergostan-3β-yl methanesulphonate (**11**) (215 mg), δ ($CDCl_3$) 0.66 (3 H, s, 18-H_3), 0.78 (3 H, s, 19-H_3), 1.35 and 1.36 (6 H, 2 × 5, acetonide), 3.00 (3 H, s, mesyl), 3.70 (1 H, dd, *J* 8 and 4 Hz, 22-H), 3.94 (1 H, d, *J* 8 Hz, 23-H), and 4.60 (1 H, m, 3-H).

(22R,23R,24S)-22,23-*Isopropylidenedioxy*-6-*oxo*-5α-*ergostan*-3α-*yl Acetate* (**12**).—A solution of (22*R*,23*R*,24*S*)-22,23-isopropylidenedioxy-6-oxo-5α-ergostan-3β-yl methanesulphonate (**11**) (215 mg) in dry benzene (7 ml) was treated with caesium acetate (365 mg, 1.90 mmol) in the presence of 18-crown-6 (100 mg, 0.379 mmol) under reflux for 21 h. Work-up (ethyl acetate), followed by chromatography on silica gel (1.5 cm i.d. × 20 cm), with benzene–ethyl acetate (30:1) provided (22R,23R,24S)-22,23-*isopropylidenedioxy*-6-*oxo*-5α-*ergostan*-3α-*yl acetate* (**12**) (134 mg, 67%), m.p. 187—188 °C (from methanol); δ ($CDCl_3$) 0.68 (3 H, s, 18-H_3), 0.75 (3 H, s, 19-H_3), 1.35 and 1.36 (6 H, 2 × s, acetonide), 2.04 (3 H, s, acetyl), 2.54 (1 H, dd, *J* 11 and 5 Hz, 5α-H), 3.70 (1 H, dd, *J* 8 and 4 Hz, 22-H), 3.94 (1 H, d, *J* 8 Hz, 23-H), and 5.09 (1 H, m, $w_{\frac{1}{2}}$ 7 Hz, 3β-H) (Found: C, 74.6; H, 10.3. $C_{33}H_{54}O_5$ requires C, 74.67; H, 10.25%).

(22R,23R,24S)-3α,22,23-*Trihydroxy*-5α-*ergostan*-6-*one*, *Typhasterol* (**3**).—(22*R*,23*R*,24*S*)-22,23-Isopropylidenedioxy-6-oxo-5α-ergostan-3α-yl acetate (**12**) (120 mg, 0.226 mmol) was refluxed with 80% aqueous acetic acid (10 ml) for 4 h. Removal of the solvent under reduced pressure gave a residue, which was

then treated with 5% KOH–MeOH (5 ml) at room temperature for 1 h. Work-up (dichloromethane), followed by purification by chromatography on silica gel (1.5 cm i.d. × 20 cm) with chloroform–methanol (15:1), afforded typhasterol (**3**) (95 mg, 94%), m.p. 226—229 °C (lit.,[2] 225—228.5 °C) (from aqueous acetonitrile). Its physicochemical properties were identical with those of an authentic sample.[2]

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