Stereoselective Synthesis of Crinosterol [(22*E*,24*S*)-Ergosta-5,22-dien-3β-ol]

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A stereoselective synthesis of crinosterol, (22*E*,24*S*)-ergosta-5,22-dien-3β-ol, was developed from (20*S*)-6β-acetoxy-3α,5-cyclo-5α-pregnane-20-carbaldehyde using the Claisen rearrangement of an appropriate precursor of established absolute configuration.

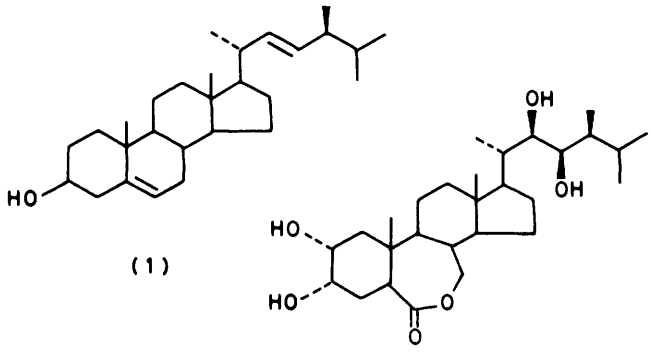
Crinosterol (1) [(22*E*,24*S*)-5α-ergosta-5,22-dien-3β-ol] is a marine sterol, and is an epimer at C-24 of the common phytosterol brassicasterol.[1] Compound (1), obtained as a mixture with the (24*R*)-isomer from the oyster *Crassostrea virginica*, has been used [2] as the starting material for the synthesis of brassinolide (2) an important plant-growth promoting steroid.[3]

Our interest in the synthesis of brassinolide [4a,b] prompts this report of the successful synthesis of compound (1) from (20*S*)-6β-acetoxy-3α,5-cyclo-5α-pregnane-20-carboxaldehyde (3) which is readily available from stigmasterol *via* its 3α,5-cyclo-6β-acetoxy derivative.[5]

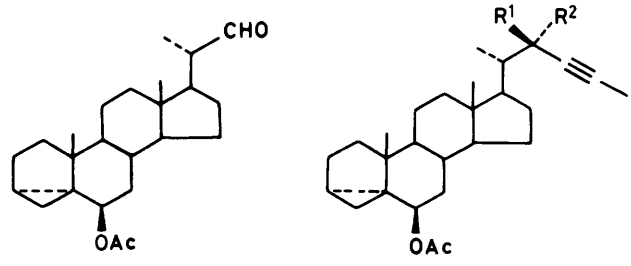
The stereospecific and regiospecific features of the Claisen rearrangement have already proved to be excellent for the stereospecific construction of modified side-chains [6] of steroids and we have now adapted this reaction to the synthesis of the sterol (1). Treatment of the aldehyde (3) with propyn-1-yl-magnesium bromide gave a 1 : 1 mixture of (22*R*)- and (22*S*)-6β-acetoxy-3α,5-cyclo-26,27-bisnor-5α-cholest-23-yn-22-ol (4a) and (4b). These epimeric alcohols were easily separated by column chromatography and their stereochemistry was established by the modified method of Horeau using g.l.c.[7] These results suggested the configuration at C-22 to be (22*R*) for the less polar and (22*S*) for the more polar isomer. The assignment was confirmed by the following transformation of the alcohol (4a). Half-hydrogenation of the (22*R*)-alcohol over Lindlar catalyst gave the allylic (22*S*)-alcohol (5) with (*Z*) stereochemistry, as established by ^{1}H n.m.r. analysis. Claisen rearrangement of compound (5) using triethyl orthopropionate gave a mixture (apparent from the ^{1}H n.m.r. spectrum) of the two olefinic esters (6), epimeric at C-25; in fact the (24*S*) stereochemistry follows that of the (22*S*)-precursor (5) which was previously established.

The mixed esters could not be separated by either thin layer or column chromatography and were reduced to the corresponding diols (7a) which were selectively esterified with toluene-*p*-sulphonyl chloride in pyridine to give the 26-mono-toluene-*p*-sulphonates (7b). This mixture on treatment with lithium aluminium hydride gave the single alcohol (7c). The ^{1}H n.m.r. spectrum of this compound (7c) is identical with that of the previously synthesized compound [4b] and shows a signal at δ 0.996 for the C-21 methyl group which is diagnostic for the stereochemistry at C-24. Thus the assignments of the stereochemistry at C-24 of the mixture of esters (6) and of the parent alcohol (4a) were confirmed.

Compound (7c) was converted into crinosterol (1) by treatment with acidic aqueous dioxane. The physico-chemical properties of compound (1) agree well with those reported for the natural compound.[8] However, since the m.p.s, i.r. and mass spectra, and g.l.c. retention times of (1) and its 24-epimer brassicasterol are identical, the ^{1}H n.m.r. spectra appear to be the only feature which differ. In fact, comparison of the ^{1}H n.m.r. spectra of crinosterol (1) and brassicasterol clearly shows that the 21-methyl doublet occurs at lower field for the former than for the latter.[8]



(1) (2)



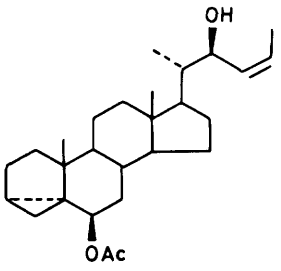
(3) (4)

a; R^1 = OH, R^2 = H
b; R^1 = H, R^2 = OH

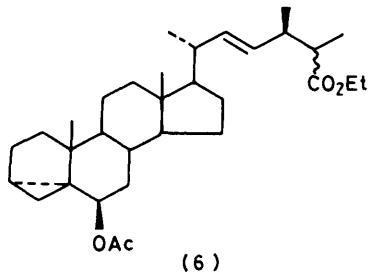
Experimental

All m.p.s are uncorrected. I.r. spectra were recorded for solutions in chloroform or for Nujol mulls. ^{1}H N.m.r. spectra were recorded on Varian XL-60 or XL-200 spectrometers in [^{2}H]chloroform solutions with $SiMe_4$ as internal standard. Routine optical rotations were recorded with a Perkin-Elmer 141 spectropolarimeter for 1% solutions in chloroform. Mass spectra were determined on a Varian 112 S mass spectrometer by direct inlet. The progress of each reaction and column chromatography was monitored by t.l.c. on E. Merck silica gel HF_{254} microplates or by g.l.c. using a 2-m silanized glass column of 3% SE30 on Gaschrom Q support, operating at 220—240 °C.

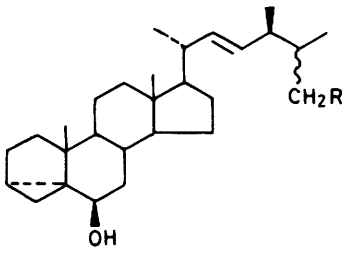
(22R)- *and* (22S)-6β-*Acetoxy*-3α,5-*cyclo*-26,27-*bisnor*-5α-*cholest*-23-*yn*-22-*ol* (4a) *and* (4b).—A solution of ethylmagnesium bromide was prepared from bromoethane (6.8 ml)



(5)



(6)



(7)

a; R = OH
b; R = $OSO_2C_6H_4Me$
c; R = H

and Mg (2.81 g) in diethyl ether. The solution was added to propyne (17.5 ml) in tetrahydrofuran (25 ml) at −70 °C (argon atmosphere). The resulting mixture was stirred at −15 °C for 30 min and then allowed to warm to room temperature. After 60 min benzene (15 ml) was added and the mixture was stirred at 0 °C during the addition (15 min) of the aldehyde (3) (3.6 g) in freshly dried tetrahydrofuran (50 ml). The mixture was stirred for 30 min and then it was treated with aqueous ammonium chloride before being extracted with diethyl ether. The dried organic layer was evaporated under reduced pressure to give the crude adduct which was chromatographed on silica gel G-Celite (1 : 1 v/v; 200 ml) with 5% ethyl acetate–hexane to give the less polar *epimer* (4a) [(22*R*)] (1.4 g), m.p. 148—150 °C (from heptane), $[\alpha]_D^{20}$ +44°; $\nu_{max.}$ 1 730, 2 220, and 3 480 cm^{-1}; δ 0.3—0.6 (3 H, m), 0.73 (3 H, s), 1.00 (3 H, s), 1.86 (3 H, m, 25-H), 2.01 (3 H, s, OAc), 4.45 (1 H, m, 22-H), and 4.55 (1 H, m, 6α-H); *m/z* 412 (Found: C, 78.5; H, 10.0. $C_{27}H_{40}O_3$ requires C, 78.60; H, 9.77%).

The more polar *epimer* (4b) had m.p. 150—152 °C (from hexane), $[\alpha]_D^{20}$ +55°; $\nu_{max.}$ 1 730, 2 220, and 3 480 cm^{-1}; δ 0.3—0.6 (3 H, m), 0.73 (3 H, s), 1.00 (3 H, s), 1.86 (3 H, m, 25-H), 2.01 (3 H, s, OAc), 4.45 (1 H, m, 22-H), and 4.55 (1 H, m, 6α-H); *m/z* 412 (Found: C, 78.4; H, 9.8. $C_{27}H_{40}O_3$ requires C, 78.60; H, 9.77%).

(22S,23Z)-6β-*Acetoxy*-3α,5-*cyclo*-26,27-*bisnor*-5α-*cholest*-23-*en*-22*ol* (5).—A solution of the acetylenic alcohol (4a) (1.2 g) in ethyl acetate (150 ml) was hydrogenated at normal pressure and room temperature over Lindlar catalyst (600 mg). The reaction was complete after 60 min. The suspension was filtered over Celite, and the solvent was evaporated at reduced pressure. The residue was chromatographed on silica gel–Celite–$AgNO_3$ (1 : 1 : 0.3, v/v/w). Elution with 10% ethyl acetate–hexane afforded the pure (23Z)-*alcohol* (5) (1.06 g); $\nu_{max.}$ 1 730 and 3 480 cm^{-1}; δ 0.3—0.6 (3 H, m), 0.73 (3 H, s), 1.00 (3 H, s), 1.64 (3 H, m, 25-H), 2.01 (3 H, s, OAc), 4.46—4.68 (2 H, overlapping, 6α-H and 22-H), and 5.45—5.65 (2 H, m, 23- and 24-H); *m/z* 414 (Found: C, 78.3; H, 10.4. $C_{27}H_{42}O_3$ requires C, 78.20; H, 10.20%).

Ethyl (22E,24S,25R)- *and* (22E,24S,25S)-6β-*Acetoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*oate* (6).—The (23*Z*)-allylic alcohol (5) (500 mg) was heated under reflux in xylene (30 ml) with ethyl orthopropionate (4 ml) and propionic acid (100 μl) with continuous removal of ethanol. After 3 h the solution was washed with saturated sodium hydrogen carbonate solution and once with water and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was chromatographed to give the mixed esters (6) (390 mg, 65%), m.p. 109—111 °C (from methanol), $\nu_{max.}$ 1 720 cm^{-1}; δ 0.30—0.60 (3 H, m), 0.73 (3 H, s), 1.07 (3 H, s), 2.02 (3 H, s, OAc), 4.15 (2 H, m, CH_2OCO), 4.50 (1 H, m, 6α-H), and 5.10—5.30 (2 H, m, 22- and 23-H); *m/z* 498 (Found: C, 77.1; H, 9.8. $C_{32}H_{50}O_4$ requires C, 77.06; H, 10.10%).

(22E,24S,25R)- *and* (22E,24S,25S)-3α,5-*Cyclo*-5α-*ergost*-22-*ene*-6β,26-*diol* (7a).—The ester mixture (6) (500 mg) in absolute toluene (15 ml) was cooled to −35 °C and di-isobutylaluminium hydride (400 mg in 2 ml of toluene) was added under dry nitrogen. The solution was kept at −35 °C for 2 h before ethyl acetate was added. The solution was allowed to warm to room temperature and then was poured into saturated ammonium chloride solution. Usual work-up followed by rapid chromatography (on silica 230—400 mesh with 30% ethyl acetate–hexane) afforded the diol mixture (7a)(300 mg, 72%), m.p. 112—114 °C (from moist methanol); $\nu_{max.}$ 3 330 cm^{-1}; δ 0.30—0.60 (3 H, m), 0.74 (3 H, s), 1.04 (3 H, s), 3.30 (1 H, m, 6α-H), 3.50 (2 H, m, 26-H), and 5.10—5.30 (2 H, m, 22-H and 23-H); *m/z* 399 (M^+ − 15) (Found: C, 80.9; H, 11.2. $C_{28}H_{46}O_2$ requires C, 81.10; H, 11.18%).

(22E,24S)-3α,5-*Cyclo*-5α-*ergost*-22-*en*-6β-*ol* (7c).—To a stirred solution of the diol mixture (7a) (250 mg) in pyridine (10 ml) at 0 °C was added toluene-*p*-sulphonyl chloride (300 mg); the mixture was kept at 4 °C for 16 h. After usual work-up the monotoluene-*p*-sulphonate (7b) (300 mg) was obtained as an oil; δ 0.30—0.60 (3 H, m), 0.68 (3 H, s), 1.04 (3 H, s), 2.45 (3 H, s), 3.30 (1 H, m, 6α-H), 3.80 (2 H, m, 26-H_2), 4.95—5.15 (2 H, m, 22- and 23-H), and 7.25—7.85 (4 H, m, aromatic H) (Found: C, 73.6; H, 9.0. $C_{35}H_{52}SO_4$ requires C, 73.9; H, 9.2%). The monotoluene-*p*-sulphonate (7b) and lithium aluminium hydride (250 mg) were stirred together at 25 °C in diethyl ether (25 ml) for 5 h. Usual work-up afforded the oily alcohol (7c) (200 mg); $\nu_{max.}$ 3 015 and 3 060 cm^{-1}; δ 0.30—0.60 (3 H, m), 0.72 (3 H, s), 0.996 (3 H, d, 21-H, *J* 6 Hz), 1.02 (3 H, s), 3.24 (1 H, m, 6α-H), and 5.10—5.20 (2 H, m, 22- and 23-H); *m/z* 398 (Found: C, 84.4; H, 11.6. Calc. for $C_{28}H_{46}O$: C, 84.35; H, 11.63%). The compound showed physico-chemical properties identical with those of a sample prepared by a different route.[4b]

(22E,24S)-5α-*Ergosta*-5,22-*dien*-3β-*ol* (1).—Compound (7c) (150 mg) dissolved in dioxane (12 ml) was stirred for 7 h at 75 °C with water (12 ml) and toluene-*p*-sulphonic acid (12 mg). After being cooled, the usual work-up afforded the crude product (140 mg) which was purified by chromatography on silica gel G–Celite (1 : 1, v/v); 20% hexane–ethyl acetate eluted crystalline crinosterol (1) (130 mg), m.p. 152—154 °C (from methanol); δ 0.69 (3 H, s), 1.001 (3 H, d, 21-H, *J* 6 Hz), 1.010 (3 H, s), 3.54 (1 H, m, 3-H), 5.10—5.20 (2 H, 22-

and 23-H), and 5.35 (1 H, m, 6-H); *m/z* 398 (Found: C, 84.3; H, 11.7. Calc. for $C_{28}H_{46}O$: C, 84.35; H, 11.63%). All physicochemical properties are identical with those previously reported.[8]

Acknowledgements

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References

1 F. J. Schmititz, in ' Marine Natural Products,' ed. P. J. Scheuer, Academic Press, New York, 1978, vol. 1, p. 261. For a recent synthesis of brassicasterol from ergosterol see: M. Anastasia, P. Ciuffreda, and A. Fiecchi, *J. Chem. Soc., Chem. Commun.*, 1982, 1169.
2 M. J. Thompson, N. B. Mandava, W. J. Meudt, W. R. Lusby, and D. W. Spaulding, *Steroids*, 1981, **38**, 567.
3 M. D. Grove, G. F. Spencer, W. K. Rohwedder, N. B. Mandava, J. F. Worley, J. D. Warthen, G. L. Steffens, J. L. Flippen-Anderson, and J. C. Cook, *Nature (London)*, 1979, **281**, 216.
4 (*a*) M. Anastasia, P. Ciuffreda, and A. Fiecchi, *J. Chem. Soc., Perkin Trans. 1*, 1983, 379; (*b*) M. Anastasia, P. Ciuffreda, M. Del Puppo, and A. Fiecchi, *ibid.*, 1983, 383.
5 J. A. Steele and E. Mosettig, *J. Org. Chem.*, 1963, **28**, 571.
6 W. Sucrow and M. Slopianka, *Chem. Ber.*, 1975, **108**, 3721, and earlier papers; M. Anastasia, A. Fiecchi, and A. Scala, *J. Chem. Soc., Chem. Commun.*, 1979, 858.
7 C. J. W. Brooks and J. D. Gilbert, *J. Chem. Soc., Chem. Commun.*, 1973, 194.
8 R. W. Lang and C. Djerassi, *Helv. Chim. Acta*, 1982, **65**, 407.

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