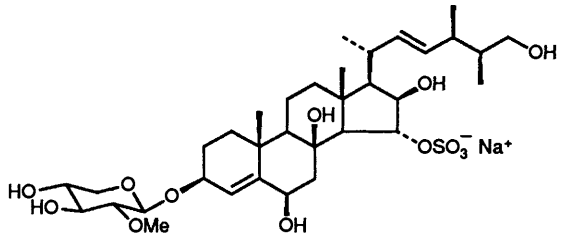
# Synthesis of 24-Methyl-26-hydroxysteroid Side-chains: Models for Stereochemical Assignments in Polyhydroxylated Marine Steroids

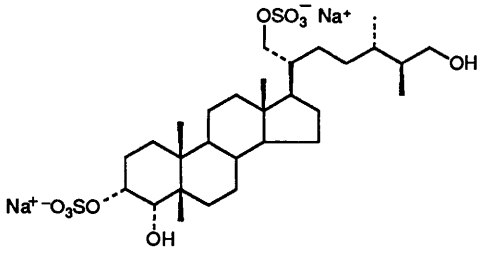
**Maria Valeria D'Auria, Francesco De Riccardis, Luigi Minale, and Raffaele Riccio***
*Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli 'Federico II,' Via Domenico Montesano, 49, 80131 Napoli, Italy*

Stereoisomers of $\Delta^{22E}$ and saturated 24-methyl-26-hydroxy steroids have been synthesized *via* a Claisen rearrangement as model compounds of established absolute configuration for stereochemical assignments at C-24 and C-25 of marine 24-methyl-26-hydroxy steroids. Analysis of NMR spectral data of synthetic compounds and of their MTPA esters proved the suitability of NMR spectroscopy for the assignment of configurations at C-24 and C-25 of unknown 24-methyl-26-hydroxy steroids. The absolute configurations at C-24 and C-25 of two naturally occurring marine steroids from a starfish and an ophiuroid have been established.

During our work on bioactive compounds from echinoderms[1] we have isolated and characterized several new polyhydroxylated steroids, all possessing a hydroxy function on the side-chain. Among these the recently isolated echinasteroside A (**1**) from the starfish *Echinaster sepositus*[2] and the disulphate




(1) echinasteroside A




(2)

steroid (**2**) from the ophiuroid *Ophiolepis superba*[3] present a 24-methyl-26-hydroxy substitution pattern. The structures of these compounds were determined by analysis of spectral data, but the stereochemistry at C-24 and C-25 remained undetermined. In order to solve such a problem and settle a method for future investigations, we have stereospecifically synthesized reference models with all possible configurations at C-24 and C-25 and we report here a set of spectral data allowing the unambiguous assignment of the target stereochemistry. Synthesis of model compounds (Scheme 1) was performed *via* a Claisen rearrangement reaction on a *cis*-allylic C-22 alcohol, a method developed by Sucrow and co-workers[4] which has grown into a general and effective methodology for the stereospecific functionalization of steroidal side-chains.[5]

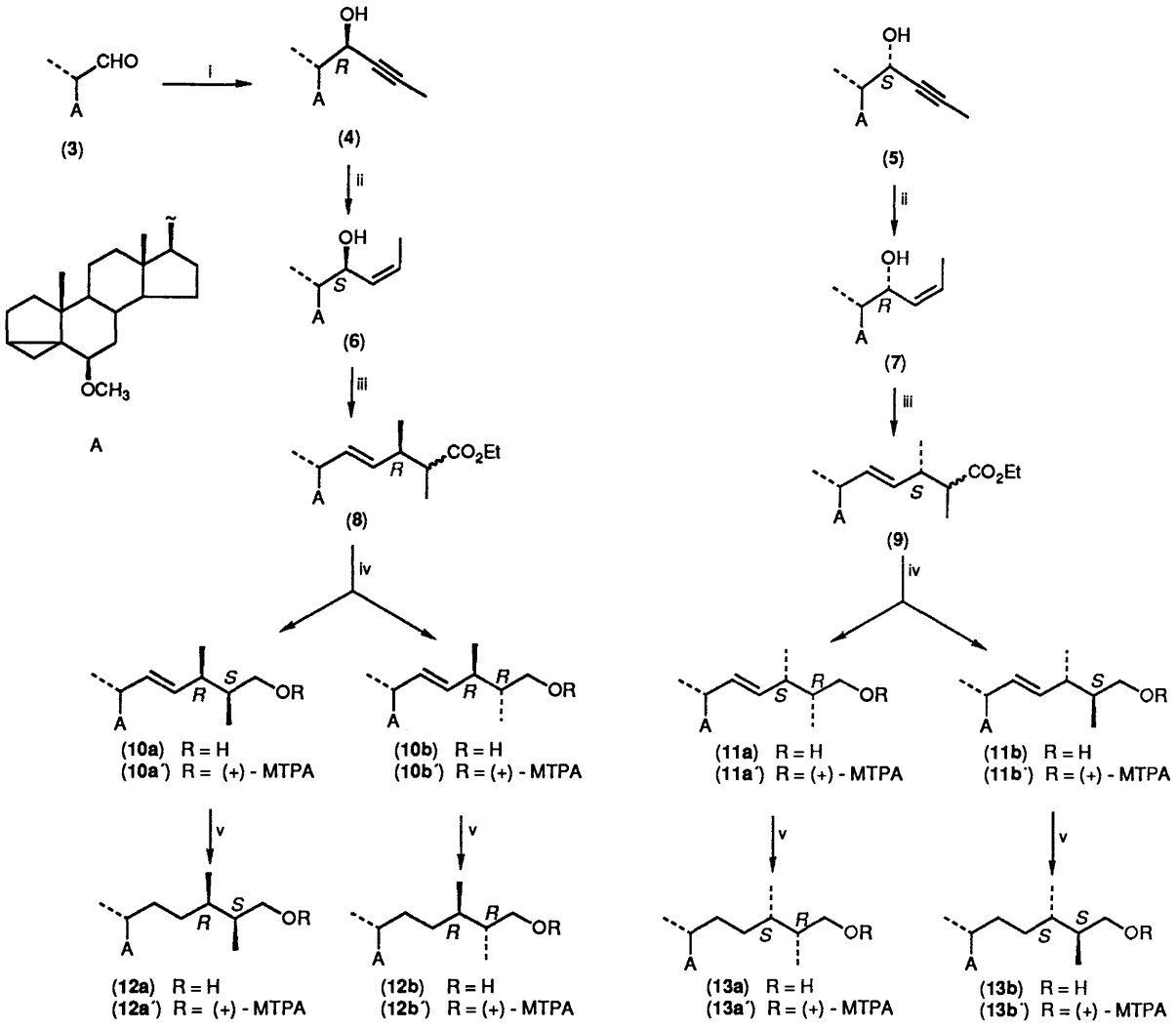
Allylic alcohols (**6**) and (**7**)[6] were converted into a mixture of two $\Delta^{22E}$ esters epimeric at C-25 [respectively (**8**) and (**9**)],[7] which, upon reduction by lithium aluminium hydride followed by HPLC separation, afforded the four possible $\Delta^{22E}$-24-methyl-26-hydroxy stereoisomers (**10a** and **b**) and (**11a** and **b**), which were eventually converted by catalytic hydrogenation into the saturated derivatives (**12a** and **b**) and (**13a** and **b**). While the configuration at C-24 of all synthetic models was established by the stereoselectivity of the Claisen rearrangement step, the stereochemistry at C-25 required to be determined.

Analysis of the $^1$H NMR spectra of the eight stereoisomers (**10a**)–(**13b**) revealed the presence of related pairs showing almost identical spectral data. Obviously these had to be pairs of compounds with the same relative configuration at C-24 and C-25, that is the *threo* pairs (**10a**)–(**11a**), (**12a**)–(**13a**) and the *erythro* pairs (**10b**)–(**11b**), (**12b**)–(**13b**). Stereochemistry at C-25 could be confirmed at this stage by application of the lanthanide-induced chemical shift (LIS) non-equivalence method described by Yasuhara and Yamaguchi[8] to the (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(*R*)-(+)-MTPA, Mosher's reagent[9]] esters of what was shown to be the *erythro* pair (**12b**)–(**13b**). According to Yasuhara and Yamaguchi[8] the magnitude of the shift induced by Eu(fod)$_3$ † for the OMe group ($LIS_{OMe}$) of the (*R*)-(+)-MTPA ester of a primary alcohol with an (*R*) stereogenic centre at C-2 is larger than that of the corresponding ester of the (*S*)-alcohol. When (*R*)-(+)-MTPA esters (**12b'**) and (**13b'**) were mixed in a 2:3 ratio a larger $LIS_{OMe}$ was clearly observed for the minor component upon addition of Eu(fod)$_3$ (0.3–0.5 and equiv.); thus 25*R* and 25*S* configurations could be assigned respectively to alcohols (**12b**) and (**13b**), and as a consequence the absolute configuration was established for all possible stereoisomers.

It is interesting that the Claisen rearrangement of the individual allylic alcohols gave, in both cases, a major amount of the *erythro* product and this is in agreement with an expected predominance of the more stable (*E*) isomers of the intermediate ketene acetals formed during the reaction, when ethanol is lost from the orthoester intermediates.[10]

The 250 MHz $^1$H NMR and $^{13}$C NMR spectral data of all stereoisomers made straightforward the assignment of relative stereochemistry in the side-chain. Indeed, the most significative NMR data (Tables 1 and 2) depict a typical *threo* or *erythro* pattern for every pair in the $\Delta^{22E}$ or saturated side-chain series: the chemical shifts of the C-26, C-27, and C-28 protons and

† Europium tris-(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyloctane-3,5-dionate).




**Scheme 1.** *Reagents and conditions:* i, MeC≡CMgBr; ii, $H_2$, Lindlar catalyst; iii, $EtC(OEt)_3$, xylene, heat; iv, $LiAlH_4$; then HPLC on Partisil; v, $H_2$, Pt/C.

**Table 1.** Selected NMR data ($CDCl_3$) for side-chain signals in synthetic 24-methyl-26-hydroxy steroids.[a]

| | $^1H$ | | | $^{13}C$ | | | $^1H$ (*R*)-(+)-MTPA esters |
|---|---|---|---|---|---|---|---|
| Compound | 26-$H_2$ | 27-$H_3$ | 28-$H_3$ | C-24 | C-27 | C-28 | 26-$H_2$ |
| $\Delta^{22}$-Series | | | | | | | |
| (**10a**) (24*R*,25*S*) } *threo* | 3.44 dd, 3.60 dd | 0.91 d | 0.95 d | 39.2 | 14.1 | 17.5 | 4.13 dd, 4.28 dd |
| (**11a**) (24*S*,25*R*) } *threo* | 3.44 dd, 3.59 dd | 0.89 d | 0.95 d | 39.2 | 14.0 | 17.3 | 4.07 dd, 4.35 dd |
| (**11b**) (24*S*,25*S*) } *erythro* | 3.43 dd, 3.56 dd | 0.85 d | 0.99 d | 38.2 | 12.8 | 18.3 | 4.17 br d |
| (**10b**) (24*R*,25*R*) } *erythro* | 3.44 dd, 3.55 dd | 0.86 d | 0.99 d | 38.3 | 12.9 | 18.6 | 4.07 dd, 4.21 dd |
| Saturated side-chain series | | | | | | | |
| (**12a**) (24*R*,25*S*) } *threo* | 3.46 dd, 3.57 dd | 0.83 d | 0.78 d | 34.0 | 12.3 | 11.8 | 4.19 br d |
| (**13a**) (24*S*,25*R*) } *threo* | 3.46 dd, 3.56 dd | 0.80 d | 0.78 d | 34.1 | 14.8 | 11.3 | 4.10 dd, 4.28 dd |
| (**13b**) (24*S*,25*S*) } *erythro* | 3.44 dd, 3.65 dd | 0.92 d | 0.88 d | 35.7 | 17.2 | 14.0 | 4.15 dd, 4.29 dd |
| (**12b**) (24*R*,25*R*) } *erythro* | 3.45 dd, 3.63 dd | 0.91 d | 0.87 d | 35.0 | 16.9 | 13.7 | 4.10 dd, 4.35 dd |

[a] The chemical-shift values are given in ppm and were referred to $CHCl_3$ (δ 7.27) and central $CDCl_3$ ($\delta_C$ 77.0) signals.

those of the carbons 24, 27, and 28 are virtually identical in the $\Delta^{22}$ *threo* pair (**10a**)–(**11a**) and significantly different from those, likewise identical, of the alternative *erythro* pair (**10b**)–(**11b**); the same is true in the saturated side-chain series, with a more differentiated pattern exhibited by the carbon signals. Differentiation between the single stereoisomers of every *threo* or *erythro* pair could be achieved by analysis of $^1H$ NMR spectra of (*R*)-(+)-MTPA derivatives: in any pair the 26-methylene proton signals appear closer in the spectrum of the (25*S*)-isomer than in that of the (25*R*)-isomer. Such behaviour is reversed in the (*S*)-(−)-MTPA derivatives, the C-26 proton signals being now closer in the spectra of the (25*R*)-isomers.

**Table 2.** Selected NMR data ($CD_3OD$) for side-chain signals in synthetic 24-methyl-26-hydroxy steroids and in natural compounds (**1**) and (**2**).[a]

| Compound | $^1H$ | | | $^{13}C$ | | | $^1H$ (*R*)-(+)-MTPA esters |
|---|---|---|---|---|---|---|---|
| | 26-$H_2$ | 27-$H_3$ | 28-$H_3$ | C-24 | C-27 | C-28 | 26-$H_2$ |
| $\Delta^{22}$-Series | | | | | | | |
| (**10a**) (24*R*,25*S*) } *threo* | 3.28 dd, 3.60 dd | 0.90 d | 0.95 d | 39.7 | 13.8 | 17.1 | 4.19 dd, 4.31 dd |
| (**11a**) (24*S*,25*R*) } *threo* | 3.29 dd, 3.59 dd | 0.90 d | 0.97 d | 39.5 | 13.8 | 16.8 | 4.13 dd, 4.38 dd |
| (**11b**) (24*S*,25*S*) } *erythro* | 3.34 dd, 3.53 dd | 0.87 d | 1.02 d | 39.2 | 13.6 | 19.0 | 4.21 br d |
| (**10b**) (24*R*,25*R*) } *erythro* | 3.34 dd, 3.52 dd | 0.88 d | 1.02 d | 39.3 | 13.6 | 19.2 | 4.16 dd, 4.21 dd |
| Saturated side-chain series | | | | | | | |
| (**12a**) (24*R*,25*S*) } *threo* | 3.38 dd, 3.55 dd | 0.83 d | 0.81 d | 35.1 | 14.8 | 12.0 | 4.23 br d |
| (**13a**) (24*S*,25*R*) } *threo* | 3.38 dd, 3.49 dd | 0.81 d | 0.81 d | 35.1 | 15.1 | 11.6 | 4.14 dd, 4.34 dd |
| (**13b**) (24*S*,25*S*) } *erythro* | 3.36 dd, 3.58 dd | 0.93 d | 0.92 d | 36.8 | 17.5 | 14.4 | 4.22 dd, 4.32 dd |
| (**12b**) (24*R*,25*R*) } *erythro* | 3.37 dd, 3.57 dd | 0.91 d | 0.91 d | 36.1 | 17.4 | 14.1 | 4.16 dd, 4.38 dd |
| Natural compounds | | | | | | | |
| (**1**) (24*R*, 25*S*) | 3.46 dd, 3.58 dd | 0.91 d | 0.97 d | 40.4 | 14.5 | 17.5 | 4.17 dd, 4.33 dd (*R*)-(+)-MTPA ester 4.06 dd, 4.46 dd (*S*)-(−)-MTPA ester |
| (**2**) (24*S*,25*S*) | 3.34 dd, 3.62 dd | 0.92 d | | 36.7 | 17.4 | 14.3 | 4.24 dd, 4.32 dd (*R*)-(+)-MTPA ester 4.16 dd, 4.37 dd (*S*)-(−)-MTPA ester |

[a] The chemical-shift values are given in ppm and were referred to $CHD_2OD$ ($\delta_H$ 3.34) and central $CD_3OD$ ($\delta_C$ 49.0) signals.

In conclusion, NMR measurements resulted in a highly reliable method for stereochemical assignment in 24-methyl-26-hydroxy steroids and the following strategy for the analysis of an unknown natural product can be formulated: (a) identification of relative stereochemistry (*threo* or *erythro*) by comparison of NMR spectral data with those of reference models; (b) assignment of absolute configuration at C-25, and hence differentiation between the two *threo* or the two *erythro* isomers, by the shape of the C-26 methylene proton signals in the (*R*)-(+)- and (*S*)-(−)-MTPA derivatives.

$^1H$ and $^{13}C$ NMR spectral data of the natural echinasteroside A (**1**) exhibited a typical *threo* pattern (24*R*,25*S* or 24*S*,25*R*) (Table 2). Comparison of $^1H$ NMR spectra of the 26-MTPA derivatives revealed a closer C-26 proton signal in the spectrum of the (*R*)-(+)-MTPA ester, indicative of a 25*S* stereochemistry, and therefore the stereochemistry 24*R*,25*S* could be established in compound (**1**).

Spectral data of steroid (**2**) were instead indicative of an *erythro* (24*S*,25*S* or 24*R*,25*R*) structure. $^1H$ NMR spectra of the 26-MTPA derivatives showed again a closer signal for C-26 methylene proton in the spectrum of the (*R*)-(+)-MTPA ester, therefore the 24*S*,25*S* stereochemistry could be established in compound (**2**).

## Experimental

Spectra were obtained on the following instruments: Bruker WM-250 with Fourier Transform ($^1H$ and $^{13}C$ NMR); Kratos MS 50 (mass spectra); Perkin-Elmer Polarimeter Mod. 141 (optical rotations). 'The usual work-up' refers to dilution with water, extraction with diethyl ether, washing to neutrality, drying over $MgSO_4$ or $Na_2SO_4$, filtration, and evaporation under reduced pressure.

(22*S*,23*Z*)- and (22*R*,23*Z*)-6β-Methoxy-3α,5-cyclo-26,27-dinor-5α-cholest-23-en-22-ol (**6**) and (**7**) were obtained from the aldehyde (**3**)[11] as described in ref. 6; their spectral data as well those of their synthetic precursors (**4**) and (**5**) were in full agreement with reported values.

*Ethyl* (22E,24R,25S)- *and* (22E,24R,25R)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*oate* (**8**)[7].—The (22*S*,23*Z*)-allylic alcohol (**6**) (1g) was heated under reflux in dry xylene (30 ml) with triethyl orthopropionate (10 ml) and propionic acid (0.2 ml); solvent (7 ml) was removed by distillation after 1.5 h and reflux was continued for an additional 1.5 h. Usual work-up gave an epimeric mixture (**8**) (1.1 g); *m/z* 470 ($M^+$); $\delta_H(CDCl_3)$ 0.44 (1 H, m, 4-H), 0.65 (1 H, m, 4-H), 0.73 (3 H, s, 18-$H_3$), 0.97 (ds, *J* 7 Hz, 28-$H_3$), 1.00 (3 H, d, *J* 6.3 Hz, 21-$H_3$), 1.03 (3 H, s, 19-$H_3$), 1.08 (ds, *J* 7 Hz, 27-$H_3$), 1.25–1.27 (3 H, ts, $OCH_2Me$), 2.77 (1 H, br t, *J* 3 Hz, 6-H), 3.33 (3 H, s, OMe), 4.13–4.14 (2 H, qs, *J* 7 Hz, $OCH_2Me$), 5.09 (dd, *J* 13 and 7 Hz, 23-H), and 5.22 (dd, *J* 13 and 7 Hz, 22-H) overlapping with a m at δ 5.25 for 22- and 23-H of the other C-25 epimer.

*Ethyl* (22E,24S,25R)- *and* (22E,24S,25S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*oate* (**9**)[7].—The (22*R*,23*Z*) allylic alcohol (**7**) (500 mg) was heated under reflux in dry xylene (20 ml) with triethyl orthopropionate (5 ml) and propionic acid (0.1 ml); solvent (5 ml) was removed after 1.5 h and the reflux was continued for an additional 1.5 h. Usual work-up gave an epimeric mixture (**9**) (540 mg); *m/z* 470 ($M^+$); $\delta_H(CDCl_3)$ 0.44 (1 H, m, 4-H), 0.66 (1 H, m, 4-H), 0.73 (3 H, s, 18-$H_3$), 0.98 (ds, 28-$H_3$), 1.01 (3 H, d, *J* 6.3 Hz, 21-$H_3$), 1.03 (3 H, s, 19-$H_3$), 1.07–1.09 (ds, 27-$H_3$), 1.25–1.26 (3 H, ts, $OCH_2Me$), 2.78 (1 H, br t, *J* 3 Hz, 6-H), 3.33 (3 H, s, OMe), 4.11–4.12 (qs, $OCH_2Me$), 5.11 (dd, *J* 13 and 7 Hz, 23-H), 5.27 (dd, *J* 13 and 7 Hz, 22-H) overlapping with a m at δ 5.24 of the other C-25 epimer.

(22E,24R,25S)- *and* (22E,24R,25R)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*ol* (**10a**) *and* (**10b**).—Lithium aluminium hydride (*ca.* 300 mg) was slowly added to a stirred solution of ester mixture (**8**) (500 mg) in dry diethyl ether (50 ml); the mixture was kept at room temperature for 4 h before being quenched with methanol. Usual work-up afforded the alcohol mixture (**10a**) and (**10b**) (350 mg). This was fractionated by HPLC on a Whatman Partisil M9 10/25 column, with a mixture of hexane–ethyl acetate (95:5) as eluant (flow rate 10 ml $min^{-1}$) to give pure alcohol (**10a**) ($t_R$ 33 min) (46 mg) and alcohol (**10b**) ($t_R$ 26 min) (147 mg).

(22E,24R,25S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*ol*

**Table 3.** $^{13}C$ NMR ($CD_3OD$) side-chain signals in synthetic 24-methyl-26-hydroxy steroids[a]

| Compound | C-20 | C-21 | C-22 | C-23 | C-24 | C-25 | C-26 | C-27 | C-28 |
|---|---|---|---|---|---|---|---|---|---|
| $\Delta^{22}$-Series | | | | | | | | | |
| (**10a**) (24*R*,25*S*) | 41.2 | 21.4 | 137.2 | 133.5 | 39.7 | 42.1 | 66.8 | 13.8 | 17.1 |
| (**11a**) (24*S*,25*R*) | 41.2 | 21.4 | 137.1 | 133.5 | 39.5 | 42.0 | 66.7 | 13.8 | 16.8 |
| (**11b**) (24*S*,25*S*) | 4.13 | 21.6 | 137.8 | 131.7 | 39.2 | 42.1 | 66.8 | 13.6 | 19.0 |
| (**10b**) (24*R*,25*R*) | 41.3 | 21.5 | 138.0 | 131.9 | 39.3 | 42.7 | 67.0 | 13.6 | 19.2 |
| Saturated side-chain series | | | | | | | | | |
| (**12a**) (24*R*,25*S*) | 37.1 | 19.3 | 34.9 | 32.6 | 35.1 | 41.1 | 67.0 | 14.8 | 12.0 |
| (**13a**) (24*S*,25*R*) | 37.4 | 19.4 | 34.9 | 32.6 | 35.1 | 40.1 | 67.1 | 15.1 | 11.6 |
| (**13b**) (24*S*,25*S*) | 37.5 | 19.5 | 35.0 | 30.7 | 36.8 | 41.6 | 66.2 | 17.6 | 14.4 |
| (**12b**) (24*R*,25*R*) | 37.1 | 19.7 | 34.9 | 30.3 | 36.1 | 42.1 | 66.4 | 17.4 | 14.1 |

[a] At 62.9 MHz, values relative to $CD_3OD$ ($\delta_C$ 49.00) (central peak).

(**10a**). (Found: $M^+$, 428.3663. $C_{29}H_{48}O_2$ requires $M$, 428.3654); $\delta_H(CD_3OD)$ 0.48 (1 H, m, 4-H), 0.68 (1 H, m, 4-H), 0.78 (3 H, s, 18-$H_3$), 1.03 (3 H, d, $J$ 6.5 Hz, 21-$H_3$), 1.04 (3 H, s, 19-$H_3$), 2.84 (1 H br t, 6-H), 3.35 (3 H, s, OMe), and 5.25 (2 H, m, 22- and 23-H); remaining side-chain signals in Tables 1–2; $\delta_C(CD_3OD)$ C-1 34.5, C-2 25.8, C-3 22.8, C-4 13.9, C-5 36.1, C-6 84.1, C-7 36.6, C-8 31.7, C-9 49.5, C-10 44.5, C-11 23.7, C-12 41.5, C-13 43.9, C-14 57.7, C-15 25.2, C-16 29.7, C-17 56.7, C-18 12.8, C-19 19.7; side-chain carbon signals in Table 3.

(22E,24R,25R)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*ol* (**10b**). $m/z$ 428 ($M^+$); $\delta_H$ significative side-chain signals in Tables 1–2; remaining signals virtually identical with those of (**10a**); $\delta_C$ C-1 to C-19 as in (**10a**) ±0.1 ppm; side-chain signals in Table 3.

(22E,24S,25R)- *and* (22E,24S,25S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*ol* (**11a**) *and* (**11b**).—The C-25 epimeric mixture (**9**) (460 mg) was converted into the alcohol mixture (**11a**) and (**11b**) by using the same procedure described above. The alcohol mixture (330 mg) was fractionated by HPLC on a Whatman Partisil M9 10/25 column, with a mixture of hexane–ethyl acetate (92:8) as eluant (flow rate 10 ml $min^{-1}$) to give pure compound (**11a**) ($t_R$ 9 min) (33 mg) and compound (**11b**) ($t_R$ 12 min) (111 mg).

(22E,24S,25R)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*ol* (**11a**). $m/z$ 428 ($M^+$); $\delta_H$ significative side-chain signals in Tables 1–2; remaining signals virtually identical with those of (**10a**); $\delta_C$ C-1 to C-19 as in (**10a**) ±0.1 ppm; side-chain signals in Table 3.

(22E,24S,25S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergost*-22-*en*-26-*ol* (**11b**). $m/z$ 428 ($M^+$); $\delta_H$ significative side-chain signals in Tables 1–2; remaining signals virtually identical with those of (**10a**); $\delta_C$ C-1 to C-19 as in (**10a**) ±0.1 ppm; side-chain signals in Table 3.

*General Procedure for Reduction of* $\Delta^{22}$ *Alcohols* (**10a**)–(**11b**) *to Corresponding Saturated Alcohols* (**12a**)–(**13b**).—Each $\Delta^{22E}$ alcohol (**10a**)–(**11b**) (*ca.* 10 mg) was hydrogenated at atmospheric pressure over 10% Pt/C in ethanol (8 ml) for 5 h. Removal of the catalyst by filtration, and evaporation of solvent, gave pure saturated alcohols (**12a**)–(**13b**).

*Physical data for compounds* (**12a**)–(**13b**):

(24R,25S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergostan*-26-*ol* (**12a**). (Found: $M^+$, 430.3793. $C_{29}H_{50}O_2$ requires $M$, 430.3806); $\delta_H(CD_3OD)$ 0.48 (1 H, m, 4-H), 0.69 (1 H, m, 4-H), 0.78 (3 H, s, 18-$H_3$), 0.98 (3 H, d, $J$ 6.5 Hz, 21-$H_3$), 1.04 (3 H, s, 19-$H_3$), 2.85 (1 H, br t, $J$ 3 Hz, 6-H), 3.35 (3 H, s, OMe); remaining side-chain signals in Tables 1–2; $\delta_C(CD_3OD)$ C-1 34.5, C-2 25.8, C-3 22.8, C-4 13.8, C-5 36.5, C-6 84.2, C-7 36.1, C-8 31.8, C-9 49.5, C-10 44.5, C-11 23.8, C-12 41.7, C-13 44.0, C-14 57.8, C-15 25.2, C-16 29.3, C-17 56.8, C-18 12.6, C-19 19.7; side-chain carbon signals in Table 3.

(24R,25R)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergostan*-26-*ol* (**12b**). $m/z$ 430; $\delta_H$ signals superimposable with those of isomer (**12a**) except for those reported in Tables 1–2; $\delta_C$ C-1 to C-19 as in (**12a**) ±0.1 ppm; side-chain signals in Table 3.

(24S,25R)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergostan*-26-*ol* (**13a**). $m/z$ 430; $\delta_H$ signals superimposable with those of isomer (**12a**) except for those reported in Tables 1–2; $\delta_C$ C-1 to C-19 as in (**12a**) ±0.1 ppm; side-chain signals in Table 3.

(24S,25S)-6β-*Methoxy*-3α,5-*cyclo*-5α-*ergostan*-26-*ol* (**13b**). $m/z$ 430; $\delta_H$ signals superimposable with those of isomer (**12a**) except for those reported in Tables 1–2; $\delta_C$ C-1 to C-19 as in (**12a**) ±0.1 ppm; side-chain signals in Table 3.

*General Procedure for Preparation of* (R)-(+)-*MTPA Derivatives* (**10a'**)–(**11b'**), (**12a'**)–(**13b'**) *for NMR Measurements*.—The required alcohol (**10a**)–(**11b**), (**12a**)–(**13b**) (3 mg) was treated with freshly distilled (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride (1–3 μl) in dry pyridine (0.1 ml) for 1 h at room temperature. The reaction was monitored by TLC [hexane–ethyl acetate (9:1)] and stopped when the starting-product spot disappeared. After removal of solvent, the product was eluted with $CH_2Cl_2$ through a Pasteur pipette filled (*ca.* 2 cm) with Silica gel. Significant $^1H$ NMR spectral data are reported in Tables 1 and 2.

26-(R)-(+)-*MTPA Ester and* 26-(S)-(−)-*MTPA Ester of the Natural Echinasteroside A* (**1**). Compound (**1**) (2 mg) was treated with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (2 μl) in dry pyridine (0.1 ml) at room temperature for 3 h. After removal of solvent, the product was purified by reverse-phase HPLC on a $C_{18}$ μ-Bondapak column (3.8 mm × 30 cm) with MeOH–water (65:35); FAB-MS (negative-ion mode) $m/z$ 919 ($M^-$). The (*S*)-(−)-MTPA ester was similarly prepared from compound (**1**) (1 mg) and freshly distilled (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (1 μl) and was purified as above; FAB-MS (negative-ion mode) $m/z$ 919 ($M^-$). Significant $^1H$ NMR spectral data are reported in Table 2.

26-(R)-(+)-*MTPA Ester and* 26-(S)-(−)-*MTPA Ester of the Natural Compound* (**2**).—Compound (**2**) (1 mg) was treated with (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (2 μl) in dry pyridine (0.1) at room temperature for 3 h. After removal of solvent the product was purified by reverse-phase HPLC on a $C_{18}$ μ-Bondapak column (3.8 mm × 30 cm) with MeOH–water (55:45); FAB-MS (negative-ion mode) $m/z$ 825 [$M(SO_3H)SO_3^-$] and 847 [$M(SO_3Na)SO_3^-$]; $\delta_H(CD_3OD)$ 0.73 (3 H, s, 18-$H_3$), 0.89 and 0.93 (each 3 H, ds, $J$ 7 Hz, 27- and 28-$H_3$), 0.95 (3 H, s, 19-$H_3$), 3.94 (1 H, m, 21-H) and 4.22 (m) (21-$H_2$) [partially overlapping with δ 4.19 (m, 3β- and 4β-H) and 4.24 (dd, $J$ 11.5 and 4.5 Hz, 26-H)], and 4.32 (1 H, dd, $J$ 11.5 and 6.5 Hz, 26-H). The (*S*)-(−)-MTPA ester of alcohol (**2**) was similarly prepared from compound (**2**) (1 mg) and (−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride; the $^1H$ NMR

($CD_3OD$) spectrum was very similar to that of the above (*R*)-(+)-MTPA ester except that the signal for the 26-methylene protons, which resonated as two dd at δ 4.16 (overlap with 21-, 3β-, and 4β-H signals) and δ 4.37.

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## References

1 L. Minale, R. Riccio, C. Pizza, and F. Zollo, in 'Natural Products and Biological Activities,' a NAITO Foundation International Symposium, ed. H. Imura, T. Goto, T. Murachi, and T. Nakajima, University of Tokyo Press, Elsevier Publisher Science, 1986, p. 59; R. Riccio, M. Iorizzi, and L. Minale in 'Biological Active Natural Products,' Annual Proceedings of the Phytochemical Society of Europe, and K. Hostettmann and P. J. Lea, Clarendon Press, Oxford, 1987, p. 153.
2 F. Zollo, E. Finamore, and L. Minale, *Gass. Chim. Ital.*, 1985, **115**, 303.
3 M. V. D'Auria, R. Riccio, E. Uriarte, L. Minale, J. Tanaka, and T. Higa, *J. Org. Chem.*, 1989, **54**, 234.
4 W. Sucrow, B. Shubert, W. Richter, and M. Slopianka, *Chem. Ber.*, 1971, **104**, 3689.
5 See, for example, J. R. Wiersig, N. Waespe-Sarcevic, and C. Djerassi, *J. Org. Chem.*, 1979, **44**, 3374; M. W. Preus and T. C. McMorris, *J. Am. Chem. Soc.*, 1979, **101**, 3066; M. Anastasia, A. Fiecchi, and A. Scala, *J. Chem. Soc., Chem. Commun.*, 1979, 858.
6 Y. Hirano and C. Djerassi, *J. Org. Chem.*, 1982, **47**, 2420.
7 M. Anastasia, P. Allevi, P. Ciuffreda, and A. Fiecchi, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2365.
8 T. Yasuhara and S. Yamaguchi, *Tetrahedron Lett.*, 1977, 4085.
9 J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.*, 1973, **95**, 512.
10 R. E. Ireland, R. H. Mueller, and A. K. Willard, *J. Am. Chem. Soc.*, 1976, **98**, 2868; R. E. Ireland and A. K. Willard, *Tetrahedron Lett.*, 1975, 3975.
11 W. G. Salmond and M. C. Sobala, *Tetrahedron Lett.*, 1977, 1695; R. F. N. Hutchins, J. J. Thompson, and J. A. Svoboda, *Steroids*, 1970, **15**, 113.

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