MARINE STEROLS. V.[1] ISOLATION AND STRUCTURE OF OCCELASTEROL, A NEW 27-NORERGOSTANE-TYPE STEROL, FROM AN ANNELIDA, PSEUDOPOTAMILLA OCCELATA

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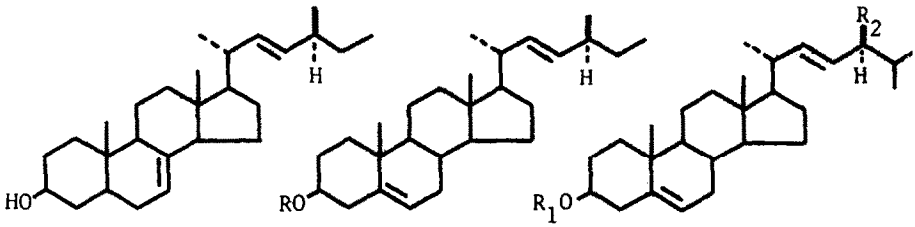
ABSTRACT

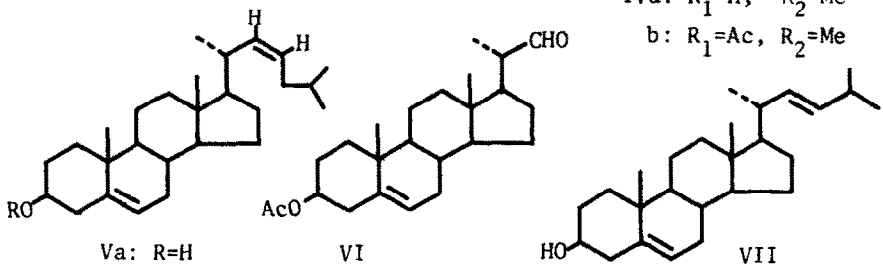
Occelasterol, a new marine C_{27} sterol, has been isolated from an annelida, Pseudopotamilla occelata, and its structure was confirmed as 22-trans-27-nor-(24S)-24-methylcholesta-5,22-dien-3β-ol (IIa) from the spectral data and by synthesis. This sterol, the second member of a class of sterols having 27-norergostane-type side chain, had been formerly regarded as 22-cis-cholesta-5,22-dien-3β-ol (Va). Gas-liquid chromatographic studies have shown that occelasterol is distributed in various amounts in most of marine invertebrates.

Our previous paper reported the occurrence of amuresterol (I), a novel sterol having a 27-norergostane-type side chain, in six species of asteroids in Hokkaido.[1] An unidentified peak, which corresponds to I, was also observed by gas-liquid chromatography (GLC) in the asteroids in southern part of Japan[2] so that it may be a fairly ubiquitous sterol, occurring in asteroids in Japanese coastal waters and, possibly, in other districts.

Since the asteroids biosynthesize sterols rather weakly and convert the ingested Δ^5-sterols into Δ^7 effectively,[3] it seems probable that at least a part of I was derived from the corresponding $\Delta^{5,22}$-sterol, namely, 22-trans-27-nor-(24S)-24-methylcholesta-5,22-dien-3β-ol (IIa). It also implies that this sterol must occur widely in marine invertebrates. The present paper will show the confirmation of the occurrence of this sterol in marine invertebrates.



I IIa: R=H b: R=Ac IIIa: R_1=H, R_2=H b: R_1=Ac, R_2=H IVa: R_1=H, R_2=Me b: R_1=Ac, R_2=Me



Va: R=H b: R=Ac VI VII

Peak	Component	Composition (%)
1	24-Norcholesta-5,22-dien-3β-ol (VII)	4.4
2	Occelasterol (IIa)	0.5
3	22-trans-Cholesta-5,22-dien-3β-ol (IIIa)	6.0
4	Cholesterol	50.1
5	22-trans-24-Methylcholesta-5,22-dien-3β-ol (IVa)	5.5
6	Desmosterol	17.9
7	24-Methylcholest-5-en-3β-ol	3.4
8	24-Methylenecholest-5-en-3β-ol	9.3
9	24-Ethylcholesta-5,22-dien-3β-ol	0.5
10	24-Ethylcholest-5-en-3β-ol	2.0

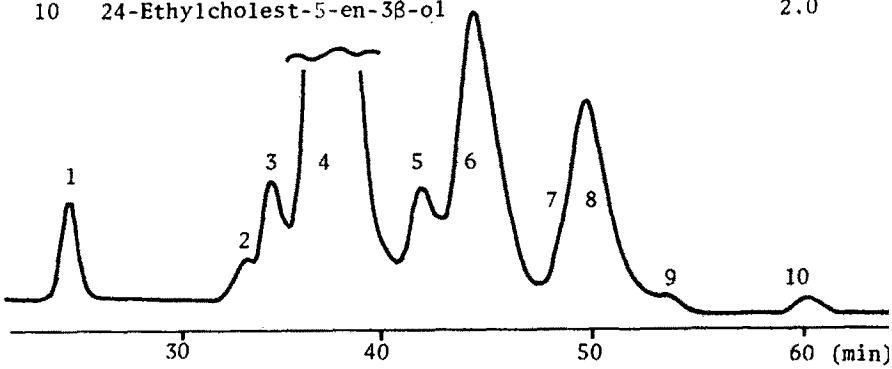


Figure 1. Gas chromatogram and composition of steryl methyl ether mixture from P. occelata (1.5% OV-17 at 252°)

From the knowledges[1] obtained from isolation and synthesis of I, the compound IIa was expected to show a slightly shorter retention time in GLC than 22-trans-cholesta-5,22-dien-3β-ol (IIIa) and intermediate polarity between IIIb and its 24S-methyl analog (IVb) on argentation chromatography of its acetate (IIb). We noticed the corresponding component, which we have designated occelasterol, in the sterol mixture of an annelida of the class Polychaeta, Pseudopotamilla occelata Moore (Fig. 1). It was formerly regarded as 22-cis-cholesta-5,22-dien-3β-ol[4] (Va) from its retention time in GLC (relative to cholesterol, 0.89 - 0.90 by a column of 1.5% OV-17 at 252°).

Extensive column and thin-layer chromatography (TLC)[5] of the acetate of diunsaturated sterol fraction from P. occelata over silver nitrate-impregnated silicic acid and silica gel HF(254 and 366) gave 3.2 mg of occelasteryl acetate, mp 138-141°, $[\alpha]_D$ -47±2°, containing ca. 5% of IIIb as a persisting impurity. As was expected, it eluted between IVb and IIIb, and was less polar than Vb though its retention time in GLC was identical with Vb. Hydrolysis of the acetate gave the free sterol, mp 128.5-129.5°, $[\alpha]_D$ -43±2°, shown to be pure by GLC.

The mass spectrum of occelasterol (Fig. 2) showed a molecular ion (M^+) at m/e 384 and other prominent ions at 369 (M^+-Me), 366 (M^+-H_2O), 351 (M^+-Me and H_2O), 273 (M^+-side chain), and 255 (M^+-side chain and H_2O) indicating that it is a diunsaturated C_{27} sterol ($C_{27}H_{44}O$) having an unsaturated side chain.[6] The strong ions at m/e 271 (M^+-side chain and 2H) and 300 (allylic cleavage of C-20 and C-22 with one hydrogen transfer) suggest the presence of a double bond at C-22 in the side chain.[7] This cracking pattern and its relative intensity were almost

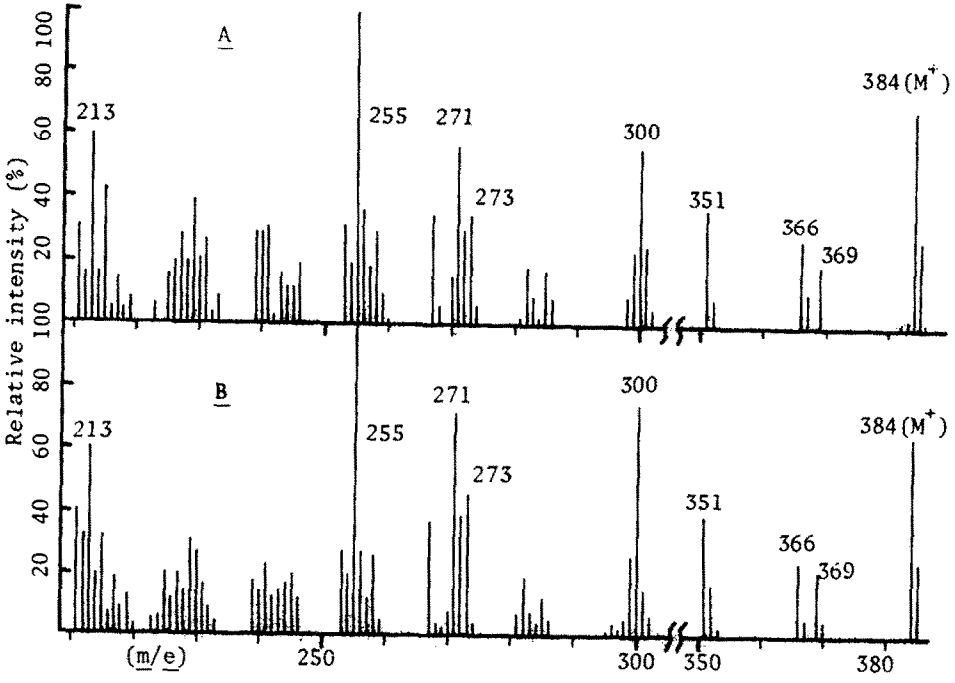


Figure 2. Mass spectra of occelasterol (A) and synthetic compound IIa (B)

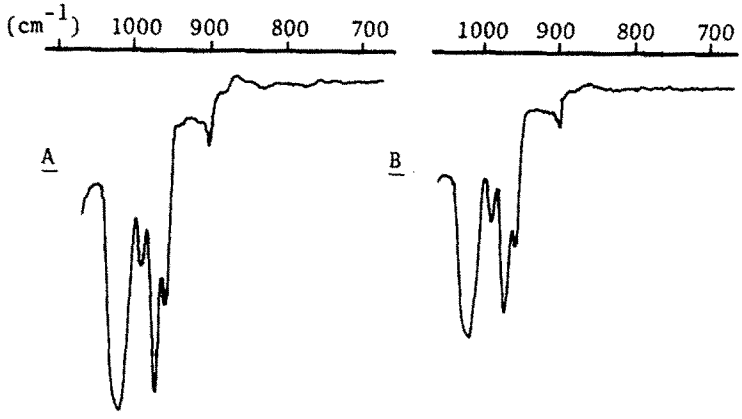


Figure 3. IR spectra (in $CHCl_3$) of occelasteryl acetate (A) and synthetic compound IIb (B)

indistinguishable from those of IIIa and Va.[8] The configuration at C-22 was found to be trans from the infrared (IR) spectrum which showed strong absorptions of trans-disubstituted double bond[9] at 958 and 972 cm^{-1} (Fig. 3). The nuclear magnetic resonance (NMR) spectrum of occelasteryl acetate (Fig. 4) showed signals of 18-Me (δ 0.69), 19-Me (1.02), acetoxy-Me (2.02), acetoxy-methine (4.3-4.8), and three olefinic protons at δ5.36 (1H, m, 6-H) and 5.12-5.22 (2H, unresolved m, 22, 23-H).

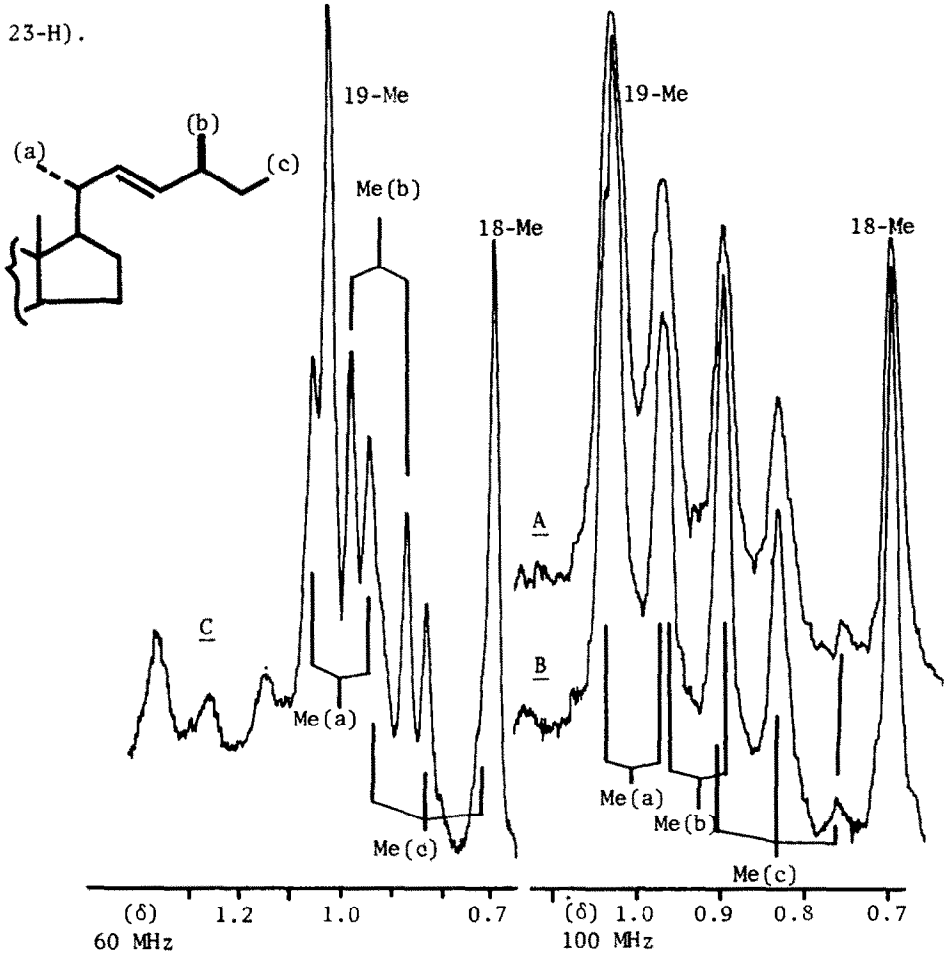


Figure 4. NMR spectra of occelasteryl acetate (A) and synthetic compound IIb (B and C)

The resolution of primary and secondary methyl signals was relatively low due to the minute amount isolated but signals of a primary methyl were present at δ 0.83 and 0.75 as a part of triplet enveloped by two doublets of secondary methyl bonded at C-20 and C-24. This pattern is quite similar to that observed in amuresterol[1] (I) and rules out other systems where normal or tertiary butyl group was attached at C-23. The position of 18- and 19-Me in NMR[10] and the specific rotations fall well within the boundary of 3β-hydroxy-Δ^5-sterols. From these results, it is evident that occelasterol is 22-trans-27-nor-24-methylcholesta-5,22-dien-3β-ol (IIa) as expected. Confirmatory evidence for this structure was provided by its partial synthesis by an unequivocal route.

The Wittig reaction of (20S)-3β-acetoxybisnorchol-5-en-21-al[11] (VI), with the ylide generated from ((2S)-2-methyl)butyltriphenylphosphonium bromide and butyllithium in hexane at room temperature, gave a steryl acetate containing a small amount of IIIb due to the contaminated isoamyl alcohol in the starting (2S)-2-methylbutan-1-ol. This condition has been known to afford 22-trans isomer predominantly and leave the configuration at C-20 intact.[12] Purificataion of the steryl acetate gave the main product (IIb) shown to be pure by GLC, mp 142-144°, $[\alpha]_D$ -47°, mixed mp with occelasteryl acetate 138-143°. Hydrolysis of IIb gave the free sterol (IIa), mp and mixed mp with occelasterol, 128 - 129°, $[\alpha]_D$ -44°. IR, NMR, and mass spectra, and the retention time in GLC of the synthetic compound IIa and b were identical with those of occelasterol and its acetate, respectively. Some of the spectral comparisons are given in Figs. 2, 3, and 4. A better resolution of the methyl signals was obtained in the NMR operating at 60 MHz rather than

at 100 MHz (Fig. 4). The observed identity in the physicochemical properties of the synthetic and natural compounds supports that the configuration at C-24 of occelasterol is S (24α). Occelasterol, therefore, is 22-trans-27-nor-(24S)-24-methylcholesta-5,22-dien-3β-ol (IIa), the second member of a class of sterols having a 27-norergostane type side chain. It is significant to note that the disputed 24-norcholesta-5,22-dien-3β-ol[13] (VII), whose origin and biogenesis still remain unsettled, is the C-26 demethylated form of occelasterol.

Many workers, including ourselves, had noticed this component in marine invertebrates by GLC and regarded it as 22-cis-cholesta-5,22-dien-3β-ol (Va) from its retention time and mass spectrum.[4,14] However, there has been no firm proof for the identity of this sterol with Va. In only one example, Idler et al.[14a] have isolated it from the scallop, Placopecten magellanicus, by GLC and reported the presence of IR absorption due to cis-disubstituted double bond at 690 cm^{-1} (in KBr). There was a significant discrepancy between Idler's report and that of synthetic Va by Svoboda et al.[8] who recorded three absorptions between 700 and 750 cm^{-1} (in CS_2). We have examined the sample, supplied by Dr. Svoboda, in KBr and found it to show the same absorptions as in CS_2. In C-22 unsaturated sterols, the cis isomer is more polar than the trans isomer on argentation chromatography.[8, 12b, 15] In contrast, the compound from P. magellanicus showed it to be rather less polar than 22-trans-cholesta-5,22-dien-3β-ol (IIIa).[5] Such a disagreement can be explained if the compound from P. magellanicus was identical with occelasterol (IIa). It is also suggested that "22-cis-cholesta-5,22-dien-3β-ol" reported in other marine invertebrates

is, if not entirely, not Va but is IIa.

Our GLC studies indicated that occelasterol is widely distributed in marine invertebrates. For example, the mussel Mytillus coruscum, the sea cucumber Stichopus japonicus, and the tunicate Halocynthia aurantium contain a relatively high amount (~2%) of IIa. It was also observed in trace amounts in the Gastropoda Littorina brevicula, the sea anemone Epiactis japonica, the sea urchin Strongylocentrotus intermedius, and the crustacean Hemigrapsus sanquineus. It was not detected only in the sea hare Aplysia juliana[16] and the chiton Cryptochiton stelleri. Among these, the mussels,[17] the sea anemones,[17,18] and the crustaceans[17,19] are known to be unable to synthesize sterols. This irrelevance of the relative level of IIa to the presence or absence of ability of sterol biosynthesis indicates that IIa in these animals are derived by internal modification from ingested sterol and, at least partly, by accumulation via the food chain. The major biomass in marine water is the diatoms and the sterol of some diatoms was recently reported to be entirely composed of 22-trans-(24S)-24-methylcholesta-5,22-dien-3β-ol, the 24α isomer of brassicasterol.[20] The $\Delta^{5,22}$-C_{28} sterols in marine invertebrates, particularly the plankton feeders such as P. occelata, M. coruscum, and H. aurantium, seem to be accumulated from the diatom sterol as a primary source. Indeed, the compound IVa, mp 151°, $[\alpha]_D$ -45°, isolated from P. occelata, was identical with diatom sterol and different from brassicasterol, mp 151°, $[\alpha]_D$ -66°,[21] showing a small diamagnetic shift of 21- and 28-methyl signals in the NMR spectrum of its acetate (IVb) in accordance with the result reported by Rubinstein et al.[20a] Although the conclusive process can not be

drawn at this stage, the coincidance of the configuration at C-24 of IIa and diatom sterol (IVa) seems to suggest that IIa was derived by demethylation from diatom sterol through biogenetic and/or non-biogenetic process in marine circumstances, and later distributed and accumulated in marine invertebrates via the food chain.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage and are uncorrected. Optical ratations were measured in $CHCl_3$ soln. NMR spectra were determined on a JEOL PS100 spectrometer operating at 100 MHz in $CDCl_3$ soln. with TMS as internal standard. Mass spectra were determined on a Hitachi RMU7 mass spectrometer. IR spectra were taken on a Hitachi 215 spectrometer. GLC was carried out on a Shimadzu GC4BPF gas chromatograph using a glass column (3 m x 3 mm i.d.) packed with 1.5% OV-17[22] on 80-100 mesh Shimalite W at 252°, with N_2 carrier gas flow-rate of 60 ml/min. Hydrolysis of steryl acetate was carried out by refluxing in 3% KOH-MeOH for 20 min followed by the usual work-up throughout.

Isolation of crude sterol Fresh material of commercial P. occelata (5 kg) was dried at 80° in vacuo and extracted exhaustively with ether. The evaporation residue (150 g) of the extract was dissolved in 10% KOH in 80% EtOH (0.9 l) and refluxed for 3 hr. The mixture was concentrated to 0.5 l in vacuo, diluted with 2 l of H_2O, and extracted with ether (0.5 l x 3). The combined extract was concentrated to 300 ml and washed thoroughly with H_2O and sat. NaCl soln. The suspended waxy substance was removed by filtration. Evaporation of the solvent gave 10 g of an oily residue which was dissolved in 40 ml of MeOH with heating, left to stand overnight, and the precipitate (2.4 g) was collected. The mother liquor was evaporated and the residue was chromatographed over a column of 200 g of silica gel and eluted with benzene. The fractions showing the same polarity with cholesterol in TLC were combined (1.3 g). The combined crude sterol (3.7 g) was acetylated in a usual manner with Ac_2O in pyridine and recrystallized twice from MeOH -$CHCl_3$ giving the crude acetate (3.4 g) which was found to be pure by TLC on silica gel (benzene). Gas chromatogram: See Fig. 1.

Isolation of occelasterol (IIa) The crude steryl acetate was chromatographed over a column of 20% (w/w) $AgNO_3$-silicic acid (500 g, 100 mesh, Mallinckrodt) and eluted with a mixture of benzene and hexane (3:10). The fractions (500 ml) were monitored by GLC and combined accordingly as follows: Fractions 1-5 gave no substances; fractions 6-10 (1.67 g), cholesteryl acetate associated with a trace of its C-24 alkylated analogs; fractions 11-14 (316 mg), a mixture of cholesteryl acetate (14%), desmosteryl acetate (7%), IVb (33%), IIb (8%), IIIb (35%), and

VIIb (3%); fraction 15 (112 mg), a mixture of desmosteryl acetate (20%), IIIb (67%), IIb (trace), and VIIb (13%); fraction 16(110 mg), a mixture of 24-ethylidenecholest-5-en-3β-yl acetate (6%), desmosteryl acetate (27%), IIIb (7%), and VIIb (60%); fraction 17 (650 mg), a mixture of desmosteryl acetate (93%), VIIb (3%), 24-ethylidenecholest-5-en-3β-yl acetate (2%), and 24-methylenecholest-5-en-3β-yl acetate (2%); fraction 18 (400 mg), a mixture of 24-methylenecholest-5-en-3β-yl acetate (92%) and desmosteryl acetate (8%). Fractions 11-14 were combined and purified twice using a column of 250 and 500 g of $AgNO_3$-silicic acid, giving a fraction (35 mg) containing IIb, IIIb, and IVb (1:2:2). This fraction was submitted to preparative TLC over $AgNO_3$-impregnated silica gel HF (254 and 366)(20% w/w) developing three times with hexane-benzene (3:1). The upper band (Rf: 0.6) gave IVb, mp 155°, $[\alpha]_D$ -52° (c, 1.18). Hydrolysis of IVb gave IVa, mp 151°, $[\alpha]_D$ -45° (c, 1.0). The lower broad band (Rf: 0.5) was further devided into upper (1/3) and lower(2/3) zones. The lower zone gave IIIb, mp 128°. Spectral data of IIIb were identical as reported previously.[4] The upper zone was composed of IIIb (58%), IIb (36%), and IVb (6%). Three separation of the upper zone in the same manner gave 3.2 mg of IIb, containing 5% of IIIb as estimated by GLC, as plates from MeOH, mp 138-141°, $[\alpha]_D$ -47±2° (c, 0.32). IR and NMR spectra: See Fig. 3 and 4. Hydrolysis of IIb gave occelasterol (IIa) as long needles from MeOH, mp 128.5-129.5°, $[\alpha]_D$ -43±2° (c, 0.26). ν_{max} ($CHCl_3$): 3600, 958, 972 cm^{-1}. Mass spectrum: See Fig. 2.

Synthesis of occelasterol (IIa) To a suspension of powdered ((2S)-2-methyl)butyltriphenylphosphonium bromide (636 mg) in 10 ml of dry hexane, 1.4 ml of 20% butyllithium in hexane dispersion was added and the mixture was refluxed in N_2 stream for 5 min and cooled to 40°. A suspension of the aldehyde[2] (VI, 450 mg) in 15 ml of dry hexane was added and the mixture was stirred at room temperature for 25 min. The mixture was diluted with hexane and H_2O, and the organic layer was washed with H_2O and sat. NaCl soln.,[2] and the solvent was evaporated. Acetylation[2] of the residue in a usual way and recrystallization from MeOH gave 130 mg of crude acetate. Most of the starting aldehyde remained in the mother liquor. The crude acetate was found by GLC to be composed of IIb (72%), cis isomer of IIb (10%), VI (5%), and the cis and trans isomers of IIIb and 27-norcholesta-5,22-dien-3β-yl acetate (13% in total) due to the butyl and isoamyl alcohol impurities in the starting (2S)-2-methylbutan-1-ol. The crude acetate was purified over a column of 125 g of $AgNO_3$-silicic acid eluting with hexane-benzene (10:3). The first eluate (100 mg) was found to be composed of IIb (80%) and impurities. It was purified again by $AgNO_3$-impregnated TLC to 75 mg of pure IIb, mp 142-144°, mixed mp with occelasteryl acetate 138-143°, $[\alpha]_D$ -47° (c, 0.87) from MeOH. ν_{max} ($CHCl_3$): See Fig. 3. ν_{max} (Nujol): 1730, 1660, 965, 959, 795 cm^{-1}. Mass spectrum (m/e): 366 (M^+-AcOH), 351 (M^+-AcOH and Me), 255 (M^+-AcOH and side chain). NMR: See Fig. 4. Anal. Calcd. for $C_{29}H_{46}O_2$: C, 81.63; H, 10.87. Found: C, 81.72; H, 11.02. Hydrolysis of the acetate gave free sterol (IIa) as needles from MeOH, mp and mixed mp with occelasterol 128-129°, $[\alpha]_D$ -44° (c, 0.73). ν_{max} ($CHCl_3$): 3600, 972, 958 cm^{-1}. NMR (δ): 0.69 (18-Me),

0.755 and 0.825 (part of triplet signal of a primary-Me), 0.887 and 0.955 (doublet of a secondary-Me at C-24), 1.01 (19-Me), 0.96 and 1.03 (21-Me), 5.34 (1H, m, 6-H), 5.08-5.20 (2H, m, 22, 23-H).
Mass spectrum: See Fig. 2.

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