Studies on the Constituents of Orchidaceous Plants. VII.1) The C-24 Stereochemistry of Cyclohomonervilol and 24-Isopropenylcholesterol, Non-conventional Side Chain Triterpene and Sterol, from Nervilia purpurea SCHLECHTER2)

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The C-24 stereochemistry of cyclohomonervilol, a non-conventional side chain triterpene isolated from Nervilia purpurea, was determined to be 24S by chemical transformation to 24S-dihydrocyclofuntumienol. Separation of a 24-epimeric mixture of chemically synthesized 24-isopropenylcholesterol was effectively achieved by reversed-phase high-performance liquid chromatography (HPLC), and the C-24 stereochemistry of each epimer was determined on the basis of chemical correlation with sitosterol or clionasterol. Then, 24S-isopropenylcholesterol, isolated from N. purpurea, was identified as 24S-isopropenylcholesterol by proton nuclear magnetic resonance and HPLC comparisons with the synthetic sample.

Keywords—Nervilia purpurea; Orchidaceae; cyclohomonervilol; 24S-isopropenylcholesterol; reversed-phase HPLC; stereochemistry; triterpene; sterol; 2D-NMR

In previous papers,3) we reported the isolation of cyclohomonervilol and 24ξ-isopropenylcholesterol, non-conventional side chain triterpene and sterol, from Nervilia purpurea SCHLECHTER (Orchidaceae) and proposed the structures 1a and 3a, respectively, but the stereochemistry at the C-24 position of these compounds remained uncertain. Isopropenylcholesterol has recently been obtained as a 24-epimeric mixture (4a) from Caribbean sponge, Verongia cauriformis, by a combination of silver nitrate-silica gel thin-layer chromatography (TLC), reversed-phase high-performance liquid chromatography (HPLC), and preparative gas chromatography (GC) by Djerassi and co-workers,4) who also synthesized 4a starting from fucosterol (5a) and isolated one of the C-24 epimers in an almost pure state. However, the stereochemistry at the C-24 position of that epimer was not determined. In this paper we wish to present full details of the stereochemical assignments of cyclohomonervilol (1a) and 24ξ-isopropenylcholesterol (3a) and also to describe the effective separation of an epimeric mixture of 24-isopropenylcholesterol.

Cyclohomonervilol (1a) was converted to the benzoate (1b) for practical convenience for TLC and HPLC analyses. Oxidation of 1b with osmium tetroxide gave a diol (6) as an amorphous material, which showed the molecular ion peak at m/z 592 (C_{39}H_{60}O_{4}) in the mass spectrum (MS). Brief treatment of 6 with hydrogen periodate in aqueous dioxane and subsequent reduction with sodium borohydride afforded a mixture of epimeric alcohols, which could be separated by preparative TLC to give 7a, mp 144.5—146.5 °C, MS m/z: 562 (M^+, C_{39}H_{58}O_{3}), and 8a, mp 150—152 °C, MS m/z: 562 (M^+, C_{39}H_{58}O_{3}). Then, we examined the transformation of the alcohols, 7a and 8a, into the 24-ethyl compound (9), which is distinguishable from its C-24 epimer by the chemical shift difference of the 29-methyl signals and the reversed-phase HPLC behavior.3a,5)

First, an attempt at the reductive desulfurization of 7a mesylate with sodium iodide–zinc
powder\textsuperscript{6}) did not give the desired compound, but only a mixture of elimination products. Then, the alcohol \(7a\) was derived to the phenoxythiocarbonate (\(7b\)) and subsequently reduced with tributyltin hydride\textsuperscript{7}) in toluene to yield a crystalline material, which showed four peaks (in an approximate ratio of 7:8:7:78) on HPLC with a reversed-phase column using chloroform–acetonitrile (2:8) as the eluting solvent (Fig. 1). Each of these peaks could be isolated by repeated preparative HPLC; the fourth peak (main product) was found to be identical with \(24S\)-dihydrocyclofuntumienol benzoate (\(9\)),\textsuperscript{3a}) prepared from cyclofuntumienol

\[ \text{Chart 1} \]

\[ \text{Chart 2} \]
beozate (10), by MS, proton nuclear magnetic resonance (1H-NMR), and HPLC comparisons. The other peaks were considered to be elimination products on the basis of their MS data (M⁺ peak at m/z 544). Among them, the third peak was assigned to 10 by MS and HPLC analyses.

The other alcohol (8a) was also deoxygenated in the same way as above and the products were separated by preparative HPLC to give 9 as the main product and 10 as a minor product. Identification of these compounds was achieved by MS and HPLC comparisons with authentic samples.

From the above results, the stereochemistry at the C-24 position of cyclohomonervilol (1a) was determined to be S.

Next, we examined the effective separation of a C-24 epimeric mixture of 24-isopropenylcholesterol (4a) and the stereochemical assignment of each eimer.

24-Isopropenylcholesterol (4a) was prepared from fucosterol (5a) according to Djerassi's description. Benzoylation of 4a yielded the benzoate (4b), which showed two peaks in an approximate ratio of 51 : 49 on reversed-phase HPLC using a TSK-GEL ODS-120A column (developed with chloroform–acetonitrile 2 : 8) as illustrated in Fig. 2. Thus, preparative HPLC of this mixture gave each epimer; the more mobile epimer (2b) showed mp 130—131 °C and the less mobile one (3b), mp 150—151 °C.

Alkaline hydrolyses of these epimeric benzoates (2b and 3b) afforded the corresponding alcohols, 2a, 141—142 °C, [α]D — 38.8 ° (chloroform), and 3a, 135—135.5 °C, [α]D — 39.8 ° (chloroform), respectively, whose MS patterns were identical with each other. Further, the 1H-NMR spectra of 2a and 3a, and those of 2b and 3b closely resembled each other, except for
### Table I. 200 MHz $^1$H-NMR Spectral Data for Synthetic 24R- and 24S-Isopropylcholesters and Natural 24S-Isopropylcholesterol from Nervilia purpurea and Their Benzoates (δ Values in CDCl$_3$ and Coupling Constants in Hz)

<table>
<thead>
<tr>
<th>Compound (C-24 config.)</th>
<th>3-H</th>
<th>6-H</th>
<th>18-CH$_3$</th>
<th>19-H$_3$</th>
<th>21-CH$_3$</th>
<th>26-CH$_3$</th>
<th>27-CH$_3$</th>
<th>29-CH$_3$</th>
<th>30-H$_2$</th>
<th>Others</th>
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<tr>
<td>2a (synthetic)$^{b)}$</td>
<td>3.53</td>
<td>5.36</td>
<td>0.673</td>
<td>1.006</td>
<td>0.906</td>
<td>0.904</td>
<td>0.798</td>
<td>1.560</td>
<td>4.62,</td>
<td>4.74</td>
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<tr>
<td>(R/z)</td>
<td>m</td>
<td>br d</td>
<td>s</td>
<td>s</td>
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<td>d</td>
<td>d</td>
<td>br s</td>
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<td></td>
<td>(5.8)</td>
<td>(6.6)</td>
<td>(6.4)</td>
<td>(6.4)</td>
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<tr>
<td>3a (synthetic)</td>
<td>3.52</td>
<td>5.35</td>
<td>0.666</td>
<td>1.006</td>
<td>0.922</td>
<td>0.911</td>
<td>0.802</td>
<td>1.565</td>
<td>4.60,</td>
<td>4.74</td>
</tr>
<tr>
<td>(S/β)</td>
<td>m</td>
<td>br d</td>
<td>s</td>
<td>s</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>br s</td>
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<tr>
<td>3a (natural)</td>
<td>3.52</td>
<td>5.35</td>
<td>0.666</td>
<td>1.006</td>
<td>0.922</td>
<td>0.911</td>
<td>0.802</td>
<td>1.566</td>
<td>4.60,</td>
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<td>(S/β)</td>
<td>m</td>
<td>br d</td>
<td>s</td>
<td>s</td>
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<td>d</td>
<td>d</td>
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<tr>
<td>2b (synthetic)</td>
<td>4.89</td>
<td>5.44</td>
<td>0.684</td>
<td>1.067</td>
<td>0.911</td>
<td>0.91,</td>
<td>0.802</td>
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<tr>
<td>3b (synthetic)</td>
<td>4.88</td>
<td>5.44</td>
<td>0.676</td>
<td>1.067</td>
<td>0.929</td>
<td>0.914,</td>
<td>0.804</td>
<td>1.566</td>
<td>4.61,</td>
<td>4.75</td>
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<tr>
<td>(S/β)</td>
<td>m</td>
<td>br d</td>
<td>s</td>
<td>s</td>
<td>d</td>
<td>d</td>
<td>d</td>
<td>br s</td>
<td>br s</td>
<td>m</td>
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<tr>
<td>(4.3)</td>
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<td></td>
<td>(6.4)</td>
<td>(6.0)</td>
<td>(6.2)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>3b (natural)</td>
<td>4.88</td>
<td>5.44</td>
<td>0.678</td>
<td>1.068</td>
<td>0.930</td>
<td>0.915,</td>
<td>0.806</td>
<td>1.569</td>
<td>4.61,</td>
<td>4.75</td>
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<tr>
<td>(S/β)</td>
<td>m</td>
<td>br d</td>
<td>s</td>
<td>s</td>
<td>d</td>
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<td>br s</td>
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<td>(6.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>m</td>
</tr>
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</table>

*a) The higher-field signal was arbitrarily assigned to the 27-methyl group. b) The $^1$H-NMR signals of this compound are identical with those of Djerassi's synthetic 24$\zeta$-isopropylcholesterol obtained in an almost pure state; see ref. 4.
very slight differences between the chemical shifts of their methyl signals, as shown in Table I.
Assignments of three sec-methyl signals in these compounds were done with the aid of the
two-dimensional $^1$H--$^1$H shift correlation spectrum of 3a (Fig. 3), in which two doublets at
$\delta$ 0.911 and 0.802, correlated with a proton multiplet at $\delta$ 1.51, were assigned to the 26- and 27-
methyls, and the remaining doublet at $\delta$ 0.922 was assigned to the 21-methyl.

In order to determine the stereochemistry at the C-24 position in 2a and 3a, we then
examined the transformation of 2b and 3b into the corresponding 24-ethyl compounds,
sitosterol benzoate (17) and clionasterol benzoate (21), respectively. These sterols (17 and 21)
are distinguishable from each other by their relative HPLC mobilities, as reported in our
previous paper.8)

Osmium tetroxide oxidation of 2b under controlled conditions gave a diol (14), whose
MS showed the molecular ion peak at $m/z$ 564 ($C_{37}H_{56}O_4$). Treatment of 14 with hydrogen
periodate in aqueous dioxane for a short time, followed by reduction with sodium
borohydride, gave the 28-epimeric alcohols, 15a and 16a, which could be separated by preparative TLC.

The less polar alcohol (15a), mp 131–133 °C, was converted to the phenoxythiocarbonate (15b) and subsequently reduced with tributyltin hydride to yield a crystalline material, which showed three peaks (in an approximate ratio of 25 : 38 : 37) on HPLC (Fig. 4a). Separation of these products was successfully performed by preparative HPLC; the third peak was proved to be identical with sitosterol benzoate (17) by MS and HPLC comparisons. The other peaks must be elimination products, based on their MS data (M + - C₆H₅COOH peak at m/z 396). Of these, the second peak was assigned to fucosterol benzoate (5b) by HPLC analysis.

The more polar alcohol (16a) was also deoxygenated in the same manner and the crude product was separated by preparative HPLC to give 17 (the major product) and 5b. Identification of these compounds was done by MS and HPLC comparisons with authentic samples.

Similarly, treatment of the less mobile epimer (3b) with osmium tetroxide in pyridine
gave a diol (18), MS m/z: 564 (M⁺, C₃₇H₅₆O₄). Subsequent oxidation of 18 with hydrogen periodate, followed by sodium borohydride reduction, led to a mixture of 28-epimeric alcohols, which on preparative TLC gave 19a (less polar), mp 161-161.5°C, and 20a (more polar), mp 132-132.5°C.

When both of 19a and 20a were subjected to the deoxygenation reaction in the same manner as above and the crude products were separated by preparative HPLC (Fig. 4b), the same compound (21) was obtained as the major product; it was found to be identical with authentic clionasterol benzoate (21) by means of MS and HPLC. Also, one of the minor products in both cases was identified as 5b.

From the foregoing results, the stereochemistry at the C-24 position of 2a and 3a was proved to be 24 (R/δ) and 24 (S/δ), respectively. It is noteworthy that the 24δ-epimer (2b) is eluted faster than the corresponding 24β-isomer (3b), as in the case of typical C-24 epimers of sterol benzoate.8)

Turning now to the stereochemistry of 24-α-isopropenylcholesterol (3a) isolated from Nervilia purpurea, the MS and ¹H-NMR spectra of the free sterol and the benzoate (3b) were found to be identical with those of the synthetic 3a and 3b, respectively (see Table I). This was further substantiated by comparison of the HPLC behaviors of both benzoates. Therefore, the natural sterol was proved to be 24(S/β)-isopropenylcholesterol (3a). On the other hand, Djerassi’s synthetic specimen,4) obtained in an almost pure state, must be the 24 (R/α)-epimer (2a), since the reported ¹H-NMR data are substantially identical with those of our synthetic 2a.

It is of interest from the biosynthetic viewpoint that no trace of the epimeric counterparts of 1a and 3a could be found in the extracts of Nervilia species, while both of the 24-epimers occur4) in the marine sterol.
Experimental

Melting points were determined with a Kofler-type apparatus and are uncorrected. Optical rotations were measured in chloroform solutions on a JASCO DIP-4 automatic polarimeter at 22 °C. NMR spectra were taken on Varian Associates XL-200 and JEOL JNM-GX 400 spectrometers in CDCl₃ solutions with tetramethylsilane as an internal standard; chemical shifts are recorded in δ values. MS measurements were done on a JEOL D-300 mass spectrometer using a direct inlet system; ionization energy, 70 eV; accelerating voltage, 3 kV. HPLC and preparative HPLC were performed on a Waters Associates ALC/GPC 201 D compact-type liquid chromatograph using a TSK-GEL ODS-120A column (column size 25 cm × 4.6 mm i.d.; detector setting, UV 240 nm) with CHCl₃–CH₂CN (2:8) as the eluent (flow rate 0.6 ml/min). Preparative TLC was carried out on Merck Kieselgel GF₂₅₄ with CHCl₃ or CHCl₃–hexane (1:1 and 2:8) and plates were examined under ultraviolet (UV) light (for UV-absorbing material). Extraction of substances from silica gel was done with MeOH–CH₂Cl₂ (5:95) prepared in vacuo. Mallinckrodt silica gel was used for ordinary column chromatography. For drying of organic solutions, anhydrous MgSO₄ was used.

Benzylation of Cyclohomonervilol (1a)—Cyclohomonervilol (1a) (4 mg) was treated with benzyloxyl chloride (0.01 ml) and pyridine (0.2 ml) overnight at room temperature and the reaction mixture was worked up as usual. Recrystallization of the product from MeOH gave cyclohomonervilol benzoate (1b)(5 mg), colorless needles, mp 156–158 °C. MS m/z: 558 (M+, C₃₉H₆₀O₄), 436 (M–C₆H₅COOH), 421, 281, 211. 1H-NMR δ: 0.20, 0.45 (each 1H, d, J=4.0 Hz, 19-H₂), 0.86 (3H, d, J=6.0 Hz, 26– or 27-CH₃), 0.86 (3H, d, J=6.0 Hz, 21-CH₃), 0.92 (3H, d, J=6.0 Hz, 26– or 27-CH₃), 0.90 (3H, s, 32-CH₃), 0.96 (3H, s, 18-CH₃), 1.57 (3H, brs, 29-CH₃), 4.62, 4.75 (each 1H, m, C=CH₂), 4.76 (1H, m, 3-H), 7.50 (3H, m, phenyl), 8.01 (2H, m, phenyl). Osmium Tetroxide Oxidation of Cyclohomonervilol Benzoate (1b)—Osmium tetroxide (10 mg) was added to a solution of 1b (5 mg) in pyridine (0.5 ml) and the mixture was allowed to stand overnight with stirring at room temperature. Then, a solution of sodium bisulfite (0.2 g) in water (1 ml) was added and the whole was stirred for 1 h, poured into ice water, basified with Na₂CO₃, and extracted with CH₂Cl₂. The CH₂Cl₂ solution was washed successively with 2% HCl and dil. Na₂CO₃, dried, and concentrated in vacuo. Silica gel column chromatography of the residue with MeOH–CH₂Cl₂ (5:95) gave a diol (6) (5.5 mg), an amorphous material. MS m/z: 592 (M+, C₃₈H₅₈O₃), 470 (M–C₆H₅COOH), 452 (M–C₆H₅COOH–CH₂O)(base peak), 437, 410, 344, 283.

Periodate Oxidation of the Diol (6) Followed by Sodium Borohydride Reduction—The above diol (6)(5.5 mg) was dissolved in dioxane (0.5 ml) and the solution was cooled in an ice-water bath. To this solution was added a solution of HIO₄ (0.01 ml) and pyridine (0.2 ml) overnight at room temperature, and the stirring was continued for 8 min. Then, MeOH (0.2 ml) and NaBH₄ (7 mg) were added and the stirring was continued for a further 30 min. Acetic acid (30 μl) was added to the above reaction mixture and the solution was concentrated in vacuo. The residue was extracted with CH₂Cl₂ and the CH₂Cl₂ extract was washed with brine, dried, and concentrated to give a crystalline residue (9 mg), which was separated by preparative TLC with hexane–CH₂Cl₂ (1:1) into two fractions. The less polar fraction (1.8 mg) was recrystallized from MeOH to give colorless needles (7a), mp 144.5–146.5 °C. MS m/z: 562 (M+, C₃₉H₅₈O₃), 544, 529, 422 (base peak), 407, 283. 1H-NMR δ: 0.19, 0.45 (each 1H, d, J=4.2 Hz, 19-H₂), 0.89–0.96 (CH₃ × 5), 0.983 (3H, s, 18-CH₃), 1.193 (3H, d, J=6.3 Hz, 29-CH₃), 3.97 (1H, m, 28-H), 4.78 (1H, m, 3-H), 7.50 (3H, m, phenyl), 8.08 (2H, m, phenyl).

Conversion of the Alcohols, 7a and 8a, to 24S-Dihydrocyclofuntumienol Benzoate (9)—The alcohol 7a (1.0 mg) was treated with phenyl chlororhodinocarbonate (5 μl) in pyridine (0.1 ml) with stirring for 12 h at room temperature. The reaction mixture was separated by preparative TLC to give the thiocarbonate (7b)(3.5 mg). MS m/z: 560 (M–C₆H₅OCS–H), 544 (M–C₆H₅OCSOH), 529, 440, 422, 407, 283. 1H-NMR δ: 0.19, 0.45 (each 1H, d, J=4.0 Hz, 19-H₂), 0.889–0.900 (CH₃ × 5), 0.96 (3H, s, 18-CH₃), 1.193 (3H, d, J=6.3 Hz, 29-CH₃), 3.97 (1H, m, 28-H), 4.78 (1H, m, 3-H), 7.50 (3H, m, phenyl), 8.08 (2H, m, phenyl).

The more polar fraction (2.0 mg) was recrystallized from MeOH to give colorless needles (8a), mp 150–152 °C. MS m/z: 562 (M+, C₃₉H₅₈O₃), 544, 529, 422 (base peak), 407, 283. 1H-NMR δ: 0.19, 0.45 (each 1H, d, J=4.0 Hz, 19-H₂), 0.890–0.924 (CH₃ × 5), 0.979 (3H, s, 18-CH₃), 3.84 (1H, m, 28-H), 4.78 (1H, m, 3-H), 7.50 (3H, m, phenyl), 8.08 (2H, m, phenyl).

The alcohol (8a)(1.0 mg) was also deoxygenated in the same manner as above and the product was separated by preparative HPLC to give 10 (0.01 mg) and 9 (0.2 mg). Identification of these compounds was confirmed.
by MS and HPLC comparisons with authentic samples.

**Hydroboration of i-Fucosterol Methyl Ether (11) Followed by Collins Oxidation**—Fucosterol (5a) was derived to the i-methyl ether (11) in the usual way. To a solution of 11 (198 mg) in tetrahydrofuran (THF) (13 ml), 1 M borane—THF complex (4 ml) was added slowly under stirring. Stirring was continued for 10 h at room temperature, and water was added to destroy the excess reagent. Then, 6 N aq. NaOH and 30% H2O2 (3 ml) were added dropwise over a period of 10 min with stirring under ice-cooling, and the mixture was stirred for a further 1.5 h at room temperature. The mixture was saturated with K2CO3 (10 g) and the organic layer was separated from the aqueous layer, which was washed with ether. The combined organic layer was dried over MgSO4 and concentrated. The residue (256 mg) was chromatographed on silica gel (4 g) with hexane and CHCl3—hexane (4 : 6) to give the alcohol (180 mg) as a colorless oil.

Collins reagent (1 g) was added to a solution of the above alcohol (180 mg) in dry CH2Cl2 (5 ml) and the mixture was stirred for 16 h at room temperature. After removal of the reagent, the product was chromatographed on silica gel (5 g) with CH2Cl2 to afford the ketone (12) (160 mg) as an oil. MS m/z: 442 (M+, C30H50O2), 427, 410, 387, 255. 1H-NMR δ: 0.7-1.05 (CH3 ~ 5), 2.10 (3H, s, CH3-CO-), 2.75 (1H, m, 6-H), 3.33 (3H, s, OCH3).

**Witting Reaction of the Ketone (12)**—A solution of methyltriphenylphosphonium bromide (5 g) in dimethylsulfoxide (DMSO) (5 ml) was added to a solution of oil-free KH (570 mg) in DMSO (5 ml) under argon gas and the mixture was stirred for 1 h. Then, a solution of 12 (130 mg) in ether—DMSO (1 : 1) (1 ml) was added slowly under vigorous stirring, and the whole was stirred for 3 h at room temperature. Silica gel column chromatography of the reaction mixture with hexane and ether—hexane (5 : 95) gave the olefin (13) (97 mg) as an oil. MS m/z: 440 (M+, C31H52O), 425, 408, 385, 253.

**Acid Hydrolysis of the Olefin (13)**—A solution of 13 (97 mg) and p-toluenesulfonylic acid (3 mg) in aqueous dioxane (10 ml) was refluxed for 30 min. After cooling, the mixture was neutralized with aq. Na2CO3 and extracted with CHCl3. The extract was dried and concentrated. The residue was purified by preparative TLC [developed with CHCl3—MeOH—CH3CN (2 : 8)] to give 24-isopropenylcholesterol (4a) (56 mg), colorless needles (from MeOH), mp 119-122 C. MS m/z: 426 (M+, C31H52O), 408, 393, 385, 314, 299, 271, 255.

**Benzoate (4b):** colorless needles (from MeOH—isopropyl ether), mp 125—126 C. MS m/z: 530 (M+, C33H56O2), 408, 368, 253.

**Separation of the Epimeric Mixture of 24-Isopropenylcholesterol Benzoate (4b) by HPLC**—The benzoate mixture (4b) (43 mg) was subjected repeatedly to preparative HPLC on a TSK-GEL ODS-120A column with CHCl3—CH3CN (2.8 : 2) as the eluent at 20 C. The more mobile fraction afforded 24 (R/a)-isopropenylcholesterol benzoate (2b) (19 mg), colorless needles (from MeOH—isopropyl ether), mp 130—131 C, [α]D 19.4° (c = 0.82). 1H-NMR δ: see Table I. The less mobile fraction gave the 24 (S/β)-epimer (3b) (18 mg), colorless needles (from MeOH—isopropyl ether), mp 150—151 C, [α]D 19.2° (c = 0.78). 1H-NMR δ: see Table I.

**24(R/a)-Isopropenylcholesterol (2a)**—Compound 2b (7.5 mg) was refluxed with 5% KOH—MeOH (0.5 ml) for 1.5 h and the reaction mixture was worked up as usual. Recrystallization of the product from MeOH gave 2a (6.5 mg), colorless needles, mp 141—142 C, [α]D 38.8° (c = 0.40). MS m/z: 426 (M+, 408, 393, 385, 314, 299, 271, 255. High-resolution MS m/z: Found 426.3910, Calcd for C31H52O (M+) 426.3861. 1H-NMR δ: see Table I.

**24(S/β)-Isopropenylcholesterol (3a)**—The benzoate 3b (7.5 mg) was hydrolyzed in the same manner as above and the product was recrystallized from MeOH to give 3a (5.4 mg), colorless plates, mp 135—135.3 C, [α]D 19.2° (c = 0.78). MS m/z: 426 (M+, 408, 393, 385, 314, 299, 271, 255. High-resolution MS m/z: Found 426.3910, Calcd for C30H50O (M+) 426.3861. 1H-NMR δ: see Table I.

**Osmium Tetroxide Oxidation of 24(R/a)-Isopropenylcholesterol Benzoate (2b)**—Osmium tetroxide (20 mg) was added to a solution of 2b (10 mg) in anhydrous ether (200 ml) and anhydrous benzene (100 ml) and the mixture was allowed to stand overnight with stirring at room temperature. After removal of the solvent by evaporation, a solution of sodium bisulfite (0.2 g) in water (1 ml) and ethyl alcohol (1 ml) was added to the reaction mixture and the whole was stirred for 1 h. Then, the mixture was concentrated in vacuo, and the product was taken up in CH2Cl2. The CH2Cl2 solution was concentrated and the residue was purified by preparative TLC (developed with CHCl3) to give a diol (14) (10 mg) as an amorphous material. MS m/z: 564 (M+, C37H56O4), 442 (M+—C6H5COOH), 440 (M+—C30H50O), 425, 410, 395, 255.

**Periodate Oxidation of the Diol (14) Followed by Sodium Borohydride Reduction**—A solution of HIO4 (4.5 mg) in H2O (45 ml) was added to a stirred solution of the diol (14) (10 mg) in dioxane (0.5 ml) and the mixture was stirred for 5 min under ice-cooling. Then, MeOH (0.2 ml) and NaBH4 (10 mg) were added to the mixture and stirring was continued for 30 min. After decomposition of the excess reagent by addition of acetic acid (30 µl) under stirring, the reaction mixture was concentrated in vacuo. The product was taken up in CH2Cl2, and the CH2Cl2 solution was washed with brine, dried, and concentrated. The residue (3.2 mg) was separated by preparative TLC with CHCl3 into two fractions. The less polar fraction (0.8 mg) was recrystallized from MeOH to give the alcohol (15a), colorless needles, mp 131—133 C. 1H-NMR δ: 0.692 (3H, s, 18-CH3), 0.890 (3H, d, J = 6.7 Hz, 27-CH3), 0.943 (3H, d, J = 6.8 Hz, 26-CH3), 0.965 (3H, d, J = 6.3 Hz, 21-CH3), 1.070 (3H, s, 19-CH3), 1.188 (3H, d, J = 6.4 Hz, 29-CH3), 3.95 (1H, m, 28-H), 4.89 (1H, m, 3-H), 5.45 (1H, br d, J = 5.1 Hz, 6-H), 7.47 (3H, m, phenyl), 8.08 (2H, m, phenyl).

The more polar fraction (1.2 mg) was recrystallized from MeOH to give colorless needles (16a), mp 136—138 C.
The alcohol 15a (0.8 mg) was dissolved in pyridine (40 µl) and treated with phenyl chlorothioacetate (20 µl) for 14 h under stirring at room temperature. The reaction mixture was separated by preparative TLC to give the thiocarbonate (15b) (2.9 mg). MS m/z: 548 (M+ - C6H5COOH), 516 (M+ - C6H5OCSOH), 394, 296, 283, 255. This was reduced with tributyltin hydride (60 µl) in toluene (0.5 ml) at 130 °C in an oil bath for 5 h. Evaporation of the solvent in vacuo gave an oily residue, which was purified by preparative TLC with hexane–CHCl3 (1:1) to give a crystalline material (1 mg). This product was subjected to preparative HPLC on a TSK-GEL ODS-120A column with CHCl3–CH3CN (2:8) to give three components. The first eluate (ca. 0.08 mg) was considered to be an elimination product on the basis of the MS data, MS m/z: 394 (M+ - C6H5COOH), but was not identified. The second eluate (ca. 0.1 mg), MS m/z: 394 (M+ - C6H5COOH), was identified as fucosterol benzoate (5b) by MS and HPLC analyses. The third eluate gave sitosterol benzoate (17) (0.1 mg), MS m/z: 518 (M+), 396 (M+ - C6H5COOH). Its identity was confirmed by MS and HPLC comparisons with authentic samples.

The alcohol 16a (1.2 mg) was also treated with phenyl chlorothioacetate (20 µl) in pyridine (40 µl) and the resulting thiocarbonate (16b) (2.5 mg) was deoxygenated with tributyltin hydride (60 µl) in the same manner as above. Separation of the product by HPLC gave fucosterol benzoate (5b) (0.2 mg) and sitosterol benzoate (17) (0.3 mg). Their identities were confirmed by MS and HPLC comparisons with authentic samples.

Osmium Tetroxide Oxidation of 24(S/-)-Isopropenylcholesterol Benzoate (3b)——The benzoate 3b (10 mg) was allowed to react with osmium tetroxide (20 mg) in anhydrous ether (200 µl) and anhydrous benzene (100 µl) overnight at room temperature and the reaction mixture was treated in the same manner as described for 2b. Purification of the product by preparative TLC (developed with CHCl3) afforded a diol (18) (7 mg) as an amorphous material. MS m/z: 564 (M+, C37H56O4), 442 (M+ - C6H5COOH), 440, 424, 410, 395, 255.

Periodate Oxidation of the Diol (18) Followed by Sodium Borohydride Reduction—The above diol (18) (7 mg) was dissolved in dioxane (0.4 ml) and a solution of HIO4 (4.0 mg) in H2O (40 µl) was added under vigorous stirring. Stirring was continued for 5 min under ice-cooling, then MeOH (0.16 ml) and NaBH4 (7 mg) were added and the reaction mixture was concentrated in vacuo. The product was extracted with CH2Cl2 and the CH2Cl2 extract was washed with brine, dried, and concentrated. The residue (5.1 mg) was separated by preparative TLC with CHCl3 into two fractions. The less polar fraction (0.9 mg) was recrystallized from MeOH to give the alcohol 19a, colorless needles, mp 161-161.5 °C. 1H-NMR δ: 0.689 (3H, s, 18-CH3), 0.905 (3H, d, J=6.8 Hz, 27-CH3), 0.913 (3H, d, J=6.9 Hz, 26-CH3), 0.954 (3H, d, J=6.5 Hz, 29-CH3), 3.95 (1H, m, 28-H), 4.87 (1H, m, 3-H), 5.43 (1H, br d, J=5.0 Hz, 6-H), 7.47 (3H, m, phenyl), 8.08 (2H, m, phenyl).

The more polar fraction (1.0 mg) was recrystallized from MeOH to give the alcohol 20a, colorless needles, mp 132-132.5 °C. 1H-NMR δ: 0.688 (3H, s, 18-CH3), 0.903 (3H, d, J=6.8 Hz, 27-CH3), 0.913 (3H, d, J=6.9 Hz, 26-CH3), 0.955 (3H, d, J=6.4 Hz, 21-CH3), 1.069 (3H, s, 19-CH3), 1.183 (3H, d, J=6.6 Hz, 29-CH3), 3.84 (1H, m, 28-H), 4.89 (1H, m, 3-H), 5.44 (1H, br d, J=5.0 Hz, 6-H), 7.47 (3H, m, phenyl), 8.08 (2H, m, phenyl).

Conversion of the Alcohols, 19a and 20a, to Clionasterol Benzoate (21)——Phenyl chlorothioacetate (20 µl) was added to a solution of the alcohol 19a (0.9 mg) in pyridine (40 µl) and the mixture was stirred for 18 h at room temperature. The reaction mixture was separated by preparative TLC to give the thiocarbonate (19b) (3.0 mg). This was dissolved in toluene (0.5 ml), then tributyltin hydride (50 µl) was added and the mixture was heated at 130 °C in an oil bath for 5 h. Evaporation of the solvent in vacuo gave an oily residue, which was purified by preparative TLC with hexane–CHCl3 (1:1) to give a crystalline material (0.6 mg). This was subjected to preparative HPLC on a TSK-GEL ODS-120A column with CHCl3–CH3CN (2:8) as the eluent to give four components. The first and second eluates (ca. 0.1 mg each) were considered to be elimination products on the basis of the MS data, MS m/z: 394 (M+ - C6H5COOH), but were not identified. The third eluate (ca. 0.05 mg), MS m/z: 394 (M+ - C6H5COOH), was identified as fucosterol benzoate (5b) by MS and HPLC analyses. The fourth eluate gave clionasterol benzoate (21) (0.2 mg), MS m/z: 518 (M+), 396 (M+ - C6H5COOH), which was identified by MS and HPLC comparisons with an authentic sample.

The more polar alcohol (20a) (0.8 mg) was also treated with phenyl chlorothioacetate (20 µl) in pyridine (40 µl) and the resulting thiocarbonate (20b) (2.5 mg) was deoxygenated with tributyltin hydride (30 µl) in the same manner as above. Separation of the products by HPLC gave fucosterol benzoate (5b) (0.1 mg) and clionasterol benzoate (21) (0.15 mg). Their identities were confirmed by MS and HPLC comparisons with authentic samples.

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References and Notes


5) The stereochemistry at the C-24 position of 9 and its epimer was determined from the HPLC behavior compared with that of analogous sterols, the 24α-epimers of which are usually eluted faster than the 24β-epimers; see ref. 8.

