Communications to the Editor

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OCCURRENCE OF NON-CONVENTIONAL SIDE CHAIN STEROLS IN AN ORCHIDACEOUS PLANT, NERVILIA PURPUREA SCHLECHTER AND STRUCTURE OF NERVISTEROL

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From *Nervilia purpurea* SCHLECHTER (Orchidaceae), were isolated unusual side chain sterols, 5a, 6a, and 7a, which had been isolated from marine invertebrates. A new sterol (8a), named nervisterol, was also isolated and its structure elucidated.

KEYWORDS —— *Nervilia purpurea*; Orchidaceae; non-conventional side chain sterol; nervisterol; 22-dehydro-24-isopropylcholesterol; 24-isopropylcholesterol; 24-isopropenylcholesterol; GC-MS

In the preceding paper,[1] we reported the isolation and characterization of new triterpenes, cyclonervilol and cyclohomonervilol, from *Nervilia purpurea* SCHLECHTER. This paper describes the investigation of the sterol constituents in the etherial extract of *N. purpurea*.

The Neutral fraction of the ether extract was chromatographed on silica gel with CH_2Cl_2-hexane to give a sterol fraction, which was found to be a mixture of eight sterols as shown in Fig. 1 by GC-MS analyses.[2] This was acetylated as usual and the resulting acetate mixture (100 mg) was carefully separated by column chromatography on 20% $AgNO_3$-SiO_2 (160 g) using benzene-hexane (1:5), followed by preparative TLC on 20% $AgNO_3$-SiO_2 plates, to afford 24-*epi*brassicasteryl acetate (1b)[3] (7 mg), 24ξ-methylcholesteryl acetate (2b, impure)[4] (1 mg), stigmasteryl acetate (3b)[5] (28 mg), ergosteryl acetate (4b)[5] (0.5 mg), S_4-OAc (5b)[6] (12 mg), S_5-OAc (6b)[7] (3.2 mg), S_{5B}-OAc (7b)[8] (1 mg), and nervisteryl acetate (8b)[9] (5.3 mg).

Free sterols: S_4-OH (5a),[6] S_5-OH (6a),[7] and S_{5B}-OH (7a)[8] were obtained by alkaline hydrolyses of 5b, 6b, and 7b, respectively. Of these, S_4-OH and S_5-OH were identified as 22-dehydro-24-isopropylcholesterol (5a)[10] and 24-isopropylcholesterol (6a),[10] respectively, on the basis of spectroscopic evidence and by direct comparisons with authentic samples by means of GC and GC-MS.

On the other hand, S_{5B}-OH was determined to be 24-isopropenylcholesterol (7a) by comparing the spectral data with those reported in the literature,[11] although direct comparison could not be performed.

Nervisterol (8a),[9] mp 175–177°C, $C_{30}H_{48}O$ (M^+: 424.3749. Calcd: 424.3705), was obtained by alkaline hydrolysis of 8b. It showed a characteristic IR (KBr) band at 890 cm^{-1} (=CH_2) and PMR signals[2] for a vinyl methyl at δ1.65 (s) and two olefinic protons at δ4.68 (br. s), suggesting that nervisterol has an isopropenyl

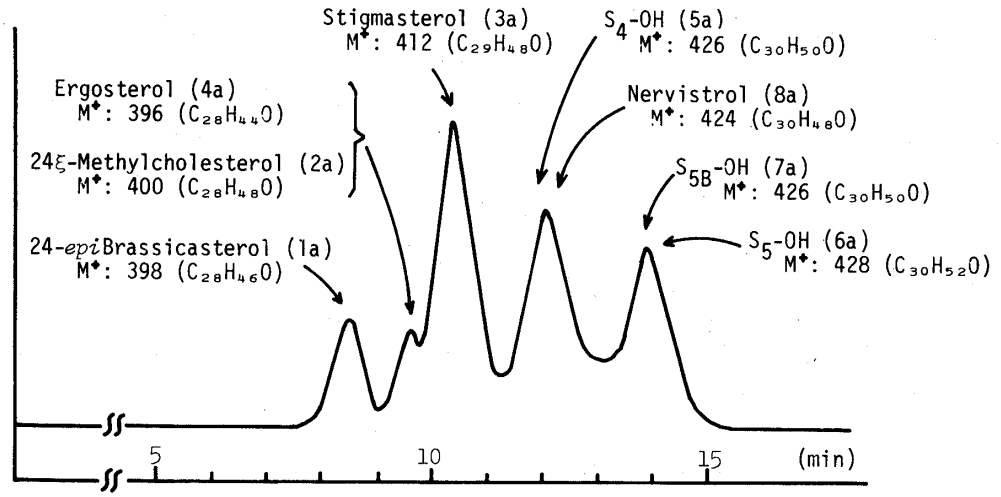
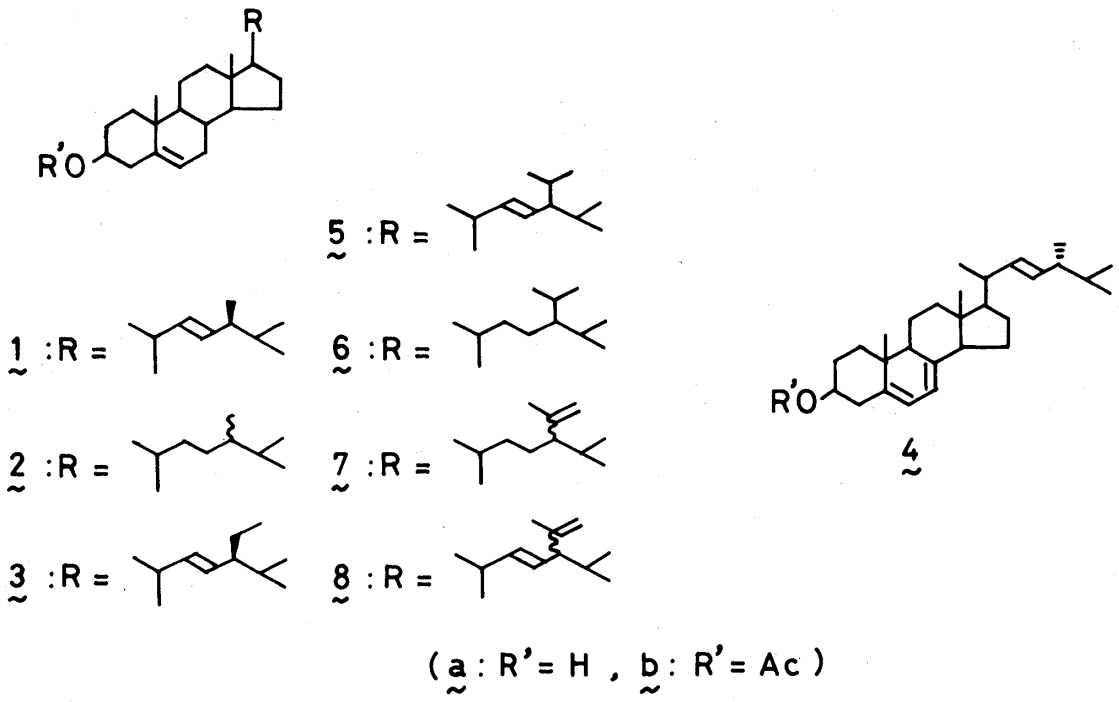


Fig. 1 Gas chromatogram of the sterol mixture obtained from *N. purpurea* (2% OV-17 column).



grouping. Furthermore, the PMR spectrum exhibited a one-proton signal at δ5.39 (br. d), typical for the olefinic proton of Δ^5-sterols,[12] and a two-proton signal at δ5.16-5.37 (m) for the double bond at C(22)-C(23).

Selective hydrogenation of nervisteryl acetate (8b) (0.9 mg) in the presence of tristriphenylphosphinerhodium chloride[13] in benzene gave a dihydro compound (5b) (0.5 mg), mp 147-150°C, which was proved to be identical with S_4-OAc (5b) by GC, GC-MS, and PMR comparisons.

Thus the structure of nervisterol was established to be 22-dehydro-24-isopropenylcholesterol (8a) except for the stereochemistry at the C-24 position.

These isopropyl-containing sterols have recently been isolated from marine sponges, *Pseudaxinyssa* sp.[10] and *Verongia cauliformis*,[11] and other unusual side chain sterols have so far been found in marine sources.[14] Our present result provided the first example of isolation of non-conventional sterols from the terrestrial source. Distribution of this kind of sterols in terrestrial plant species would be a problem of particular interest.

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2) MS measurements were done on a JEOL D-300 mass spectrometer using a direct inlet system or a GC injection system. GC-MS operating conditions were as follows: column, 2% OV-17 on Gas-Chrom Q (2m x 3mm i.d. glass tube); column temp., 280°C; injection temp., 300°C; carrier gas, helium; ionization energy, 70 eV; accelerating voltage, 3 kV. ^{1}H-NMR (PMR) spectra were measured on a Varian Associates XL-200 spectrometer in $CDCl_3$ solutions using tetramethylsilane as an internal standard. Optical rotations were measured in $CHCl_3$ solutions at 24-25°C.

3) 1a: mp 147-149°C, $C_{28}H_{46}O$ (M^+: 398.3585. Calcd.: 398.3548).
1b: mp 157-158°C, $[\alpha]_D$ -54.4°, MS m/z: 380 (M^+-60) (base peak), 365, 337, 282, 255. PMR δ: 0.69 (3H, s, 18-Me), 0.82, 0.84 (each 3H, d, J=6.7 Hz, isopropyl), 0.91, 1.00 (each 3H, d, J=6.8 Hz, 28- and 21-Me's), 1.02 (3H, s, 19-Me), 2.03 (3H, s, OAc), 4.64 (1H, m, C<u>H</u>-OAc), 5.20 (2H, m, 22- and 23-H_2), 5.41 (1H, br. d, J=5.5 Hz, 6-H). Identity of 1b was confirmed by direct GC and PMR comparisons with an authentic sample of 24-*epi*brassicasteryl acetate (1b).

4) Further purification of 2b was not possible because of the small amount available. The compound 2b was found to be indistinguishable from campesteryl (=24R-methylcholesteryl) acetate by direct GC and GC-MS comparisons, but the configuration at C(24)-position could not be ascertained.

5) The configuration at C(24)-position of 3b and 4b was confirmed by direct 200 MHz PMR comparison with the respective authentic sample of stigmasteryl acetate and ergosteryl acetate. See C. Delseth, Y. Kashman, and C. Djerassi, Helv. Chim. Acta, 62, 2037 (1979).

6) 5a: mp 168-171°C, $[\alpha]_D$ -45.7°, $C_{30}H_{50}O$ (M^+: 426.3878. Calcd.: 426.3861), MS m/z: 426 (M^+), 383 (base peak), 365, 314, 300, 271, 255. PMR δ: 0.70 (3H, s, 18-Me), 0.77, 0.78 (each 3H, d, J=6.7 Hz, isopropyl), 0.84 (6H, d, J=6.7 Hz, isopropyl), 1.01 (3H, s, 19-Me), 1.05 (3H, d, J=6.7 Hz, 21-Me), 3.52 (1H, m, C<u>H</u>-OH), 5.08 (2H, m, 22- and 23-H_2), 5.36 (1H, br. d, J=5.5 Hz, 6-H).
5b: mp 153-156°C, $[\alpha]_D$ -51.3°, MS m/z: 408 (M^+-60) (base peak), 365, 282, 255. PMR δ: 0.70 (3H, s, 18-Me), 0.76, 0.78 (each 3H, d, J=6.7 Hz, isopropyl), 0.83 (6H, d, J=6.7 Hz, isopropyl), 1.02 (3H, s, 19-Me), 1.03 (3H, d, J=6.7 Hz, 21-Me), 2.04 (3H, s, OAc), 4.62 (1H, m, C<u>H</u>-OAc), 5.08 (2H, m, 22- and 23-H_2), 5.39 (1H, br. d, J=5.5 Hz, 6-H).

7) 6a: mp 134-136°C , $[\alpha]_D$ -42.0°, $C_{30}H_{52}O$ (M^+: 428.4032. Calcd.: 428.4018), MS m/z: 428 (M^+) (base peak), 413, 410, 395, 343, 317, 273, 255. PMR δ: 0.68 (3H, s, 18-Me), 0.83, 0.84 (each 3H, d, J=6.7 Hz, isopropyl), 0.86 (6H, d, J=6.7 Hz, isopropyl), 0.94 (3H, d, J=6.4 Hz, 21-Me), 1.01 (3H, s, 19-Me), 3.53 (1H, m, CH-OH), 5.37 (1H, br. d, J=5.5 Hz, 6-H).
6b: mp 108-110°C , MS m/z: 410 (M^+-60) (base peak), 395, 302, 289, 255. PMR δ: 0.68 (3H, s, 18-Me), 0.82, 0.84 (each 3H, d, J=6.7 Hz, isopropyl), 0.86 (6H, d, J=6.7 Hz, isopropyl), 0.94 (3H, d, J=6.4 Hz, 21-Me), 1.02 (3H, s, 19-Me), 2.03 (3H, s, OAc), 4.63 (1H, m, CH-OAc), 5.39 (1H, br. d, J=5.5 Hz, 6-H).

8) 7a: mp 126-128°C , $C_{30}H_{50}O$ (M^+: 426.3908. Calcd.: 426.3861), MS m/z: 426 (M^+) (base peak), 411, 408, 393, 328, 314, 299, 271, 255, 253. PMR δ: 0.67 (3H, s, 18-Me), 0.82, 0.91, 0.92 (each 3H, d, J=5.8 Hz, 21-Me and isopropyl), 1.01 (3H, s, 19-Me), 1.56 (3H, s, vinyl Me), 3.52 (1H, m, CH-OH), 4.60, 4.73 (each 1H, s, $C=CH_2$), 5.35 (1H, br. d, J=5.5 Hz, 6-H).
7b: MS m/z: 408 (M^+-60) (base peak), 393, 255, 253. PMR δ: 0.66 (3H, s, 18-Me), 0.80, 0.91, 0.92 (each 3H, d, J=5.8 Hz, 21-Me and isopropyl), 1.02 (3H, s, 19-Me), 1.56 (3H, s, vinyl Me), 2.03 (3H, s, OAc), 4.60 (1H, m, CH-OAc), 4.60, 4.77 (each 1H, s, $C=CH_2$), 5.41 (1H, br. d, J=5.5 Hz, 6-H).

9) 8a: mp 175-177°C , $[\alpha]_D$ -47.9°, $C_{30}H_{48}O$ (M^+: 424.3695. Calcd.:424.3705), MS m/z: 424 (M^+, weak), 381, 363, 300, 271, 255, 109 (base peak). PMR δ: 0.70 (3H, s, 18-Me), 0.82, 0.84 (each 3H, d, J=6.8 Hz, isopropyl), 1.00 (3H, d, J=6.7 Hz, 21-Me), 1.01 (3H, s, 19-Me), 1.65 (3H, s, vinyl Me), 3.56 (1H, m, CH-OH), 4.68 (2H, br. s, $C=CH_2$), 5.27 (2H, m, 22- and 23-H_2), 5.39 (1H, br. d, J=5.5 Hz, 6-H).
8b: mp 187-189°C , $[\alpha]_D$ -55.2°, MS m/z: 406 (M^+-60), 363 (base peak), 282, 255, 253. PMR δ: 0.69 (3H, s, 18-Me), 0.80, 0.82 (each 3H, d, J=6.7 Hz, isopropyl), 1.00 (3H, d, J=6.7 Hz, 21-Me), 1.02 (3H, s, 19-Me), 1.65 (3H, s, vinyl Me), 2.03 (3H, s, OAc), 4.67 (1H, m, CH-OAc), 4.69 (2H, br. s, $C=CH_2$), 5.23 (2H, m, 22- and 23-H_2), 5.37 (1H, br. d, J=5.5 Hz, 6-H).

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