[Chem. Pharm. Bull. 34(8)3244—3248(1986)]

Studies on the Constituents of Orchidaceous Plants. VI.[1] Isolation and Structure Determination of Cyclohomonervilasterol and Neocyclonervilasterol, Novel Methylsterols from *Nervilia purpurea* SCHLECHTER

SHIGETOSHI KADOTA, SATOKO KANOMI (née MATSUDA), TAKEHIKO SHIMA and TOHRU KIKUCHI*

Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama 930-01, Japan

(Received February 20, 1986)

Two novel methylsterols, cyclohomonervilasterol and neocyclonervilasterol, were isolated from the methylsterol fraction of *Nervilia purpurea* by repeated preparative high-performance liquid chromatography. The structures **1** and **2** are proposed for these compounds, respectively.

Keywords——*Nervilia purpurea*; methylsterol; reversed-phase HPLC; cyclohomonervilasterol; neocyclonervilasterol; ^{1}H-NMR

In the preceding paper,[1] we reported the structure elucidation of four novel methylsterols, cyclonervilasterol (**3**), 24-epicyclonervilasterol (**4**), dihydrocyclonervilasterol (**5**), and 24-epidihydrocyclonervilasterol (**6**), which were isolated together with two minor components by preparative reversed-phase high-performance liquid chromatography (HPLC) of a methylsterol fraction (substance MB)[2] from *Nervilia purpurea* SCHLECHTER. Their ^{13}C-signal assignments were also performed by the use of two-dimensional nuclear magnetic resonance (NMR) spectroscopy. In a continuation of that work, we have examined the structures of the minor methylsterols, designated as cyclohomonervilasterol (**1**) and neocyclonervilasterol (**2**). The results are presented here.

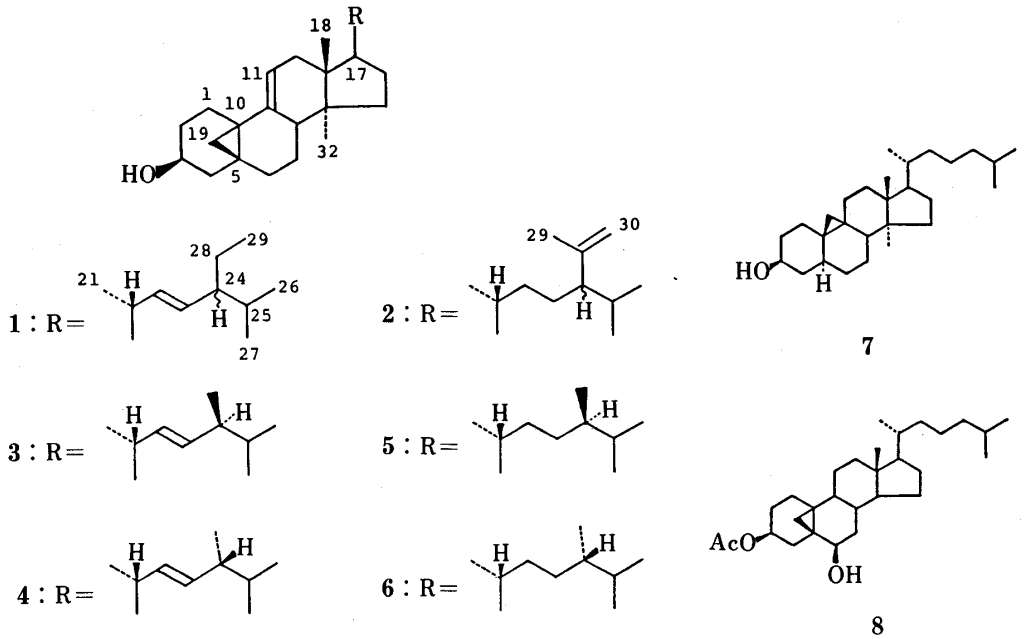

Chart 1

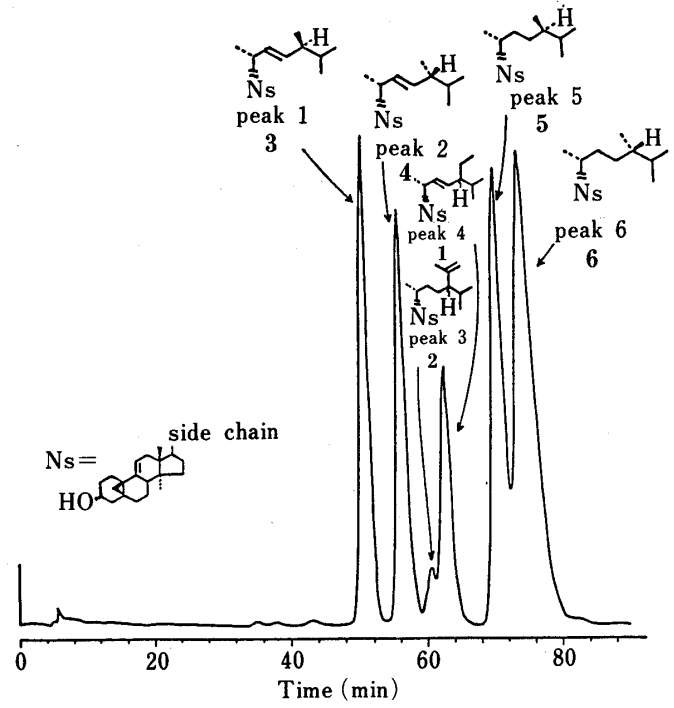

Fig. 1. HPLC Chromatogram of Substance MB (a Methylsterol Mixture) Obtained from *N. purpurea*

Conditions: column, TSK-GEL LS-410A ODS (30 cm × 7.5 mm i.d.); solvent, hexane–isopropanol–acetonitrile (5 : 15 : 80); flow rate, 2.0 ml/min; detector setting, UV 225 nm; temperature, 20 °C.

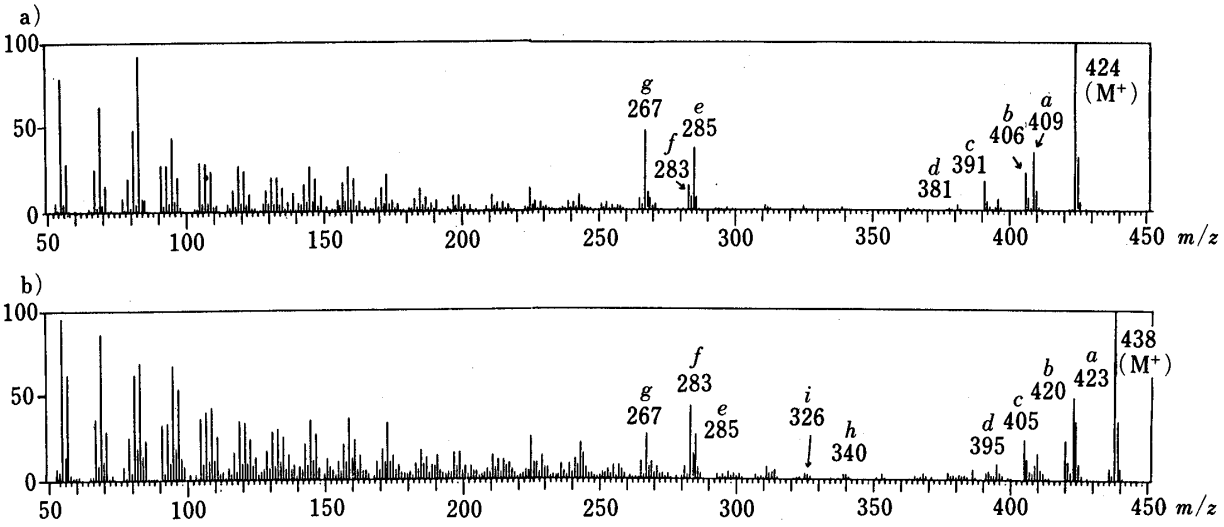

Fig. 2. Mass Spectra of Cyclohomonervilasterol (**1**) and Neocyclonervilasterol (**2**)

a) cyclohomonervilasterol (**1**), b) neocyclonervilasterol (**2**).

Cyclohomonervilasterol (**1**) (peak 4 in Fig. 1), mp 131—133 °C, showed the molecular ion peak at *m/z* 424 (base peak) in the mass spectrum (MS) and its molecular formula was determined to be $C_{30}H_{48}O$ by high-resolution MS measurement. The ultraviolet (UV) spectrum of **1** gave an absorption band at 211 nm (log ε 3.75), similar to that of cyclonervilasterol (**3**), suggesting the presence of the same conjugated system (a double bond conjugated with a cyclopropane ring).[1,3] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum (200 MHz) of **1** showed a multiplet due to an olefinic proton (δ 5.44), two quartets due to *trans*-oriented olefinic protons (δ 5.04, 5.18), a multiplet due to a hydroxy-bearing methine proton (δ 3.61), and a pair of doublets due to cyclopropyl methylene protons (δ 0.63, 0.67), along with signals arising from three secondary methyls and two tertiary methyls. The whole spectral pattern closely resembled that of cyclonervilasterol (**3**) except for the

appearance of a new triplet (δ 0.807) due to a primary methyl group instead of a doublet due to an additional secondary methyl group in **3** (Table I). The MS of **1** (Fig. 2a) showed significant fragment peaks at *m/z* 409 (*a*), 406 (*b*), 391 (*c*), 381 (*d*), 285 (*e*), 283 (*f*), and 267 (*g*), which could be reasonably explained by the fragmentations shown in Chart 2, based on comparison with the MS of **3**.

In view of the above spectral data and the molecular formula, the structure of cyclohomonervilasterol was assigned as **1**.

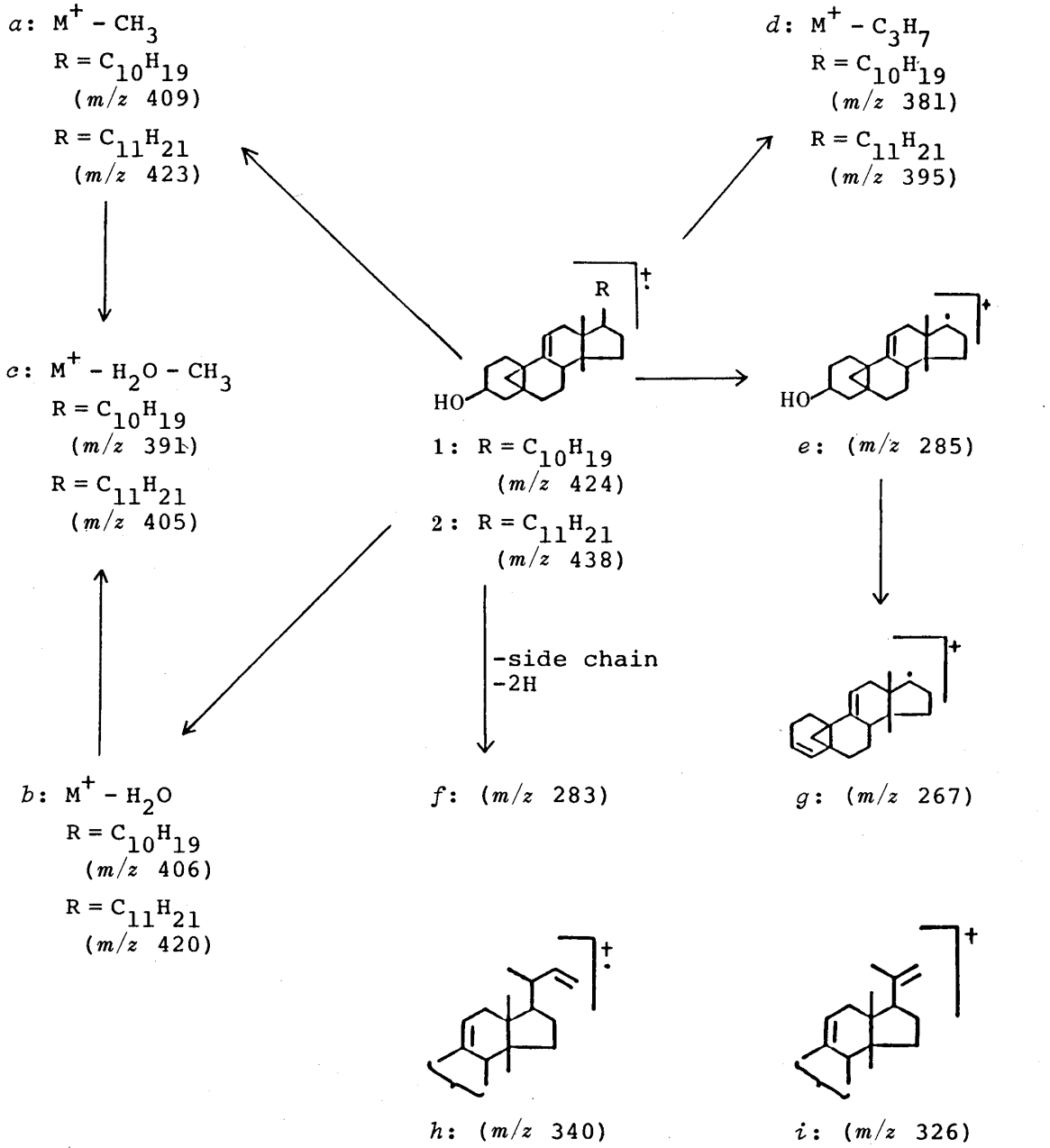

Chart 2

Neocyclonervilasterol (**2**), a very minor component (peak 3 in Fig. 1), was obtained as colorless needles, mp 121—123 °C, after repeated HPLC of substance MB.[2] It showed the molecular ion peak at *m/z* 438 (base peak) in the MS and its molecular formula $C_{31}H_{50}O$ was confirmed by high-resolution MS measurement. The UV spectrum of **2** exhibited a maximum absorption at 209 nm (log ε 3.74) and the ^{1}H-NMR exhibited signals ascribable to a vinyl

TABLE I. ^{1}H-NMR Spectral Data for Methylsterols from *Nervilia purpurea*

Compounds	3-H	18-Me	19-CH_2	21-Me	26- and 27-Me
3	3.60 m ($W_{1/2}=16$)	0.686 s	0.63 d, 0.67 d (4.0) (4.0)	0.966 d (6.51)	0.836 d, 0.818 d (6.76) (6.76)
1	3.61 m ($W_{1/2}=16$)	0.685 s	0.63 d, 0.67 d (4.0) (4.0)	0.988 d (6.46)	0.848 d, 0.798 d (6.54) (6.54)
2	3.60 m ($W_{1/2}=16$)	0.657 s	0.62 d (4.0)	0.889 d (6.2)	0.912 d, 0.803 d (6.5) (6.5)

Compounds	32-Me	28-Me	29-Me	11-H	Others
3	0.700 s	0.914 d (6.76)		5.43 m	5.14 dd, 5.20 dd (22, 23-H) (15, 7) (15, 7)
1	0.704 s		0.807 t (7.27)	5.44 m	5.04 dd, 5.18 dd (22, 23-H) (15, 7) (15, 7)
2	0.688 s		1.57 br s	5.41 m	4.61 m, 4.74 m (30-H_2)

δ values in $CDCl_3$ and coupling constants in Hz.

methyl (δ 1.57) and a terminal methylene (δ 4.61, 4.74), along with signals due to a cyclopropane methylene,[4] two tertiary methyls, three secondary methyls, a hydroxy-bearing methine proton, and a olefinic proton (Table I), suggesting the presence of an isopropenyl group in **2**. In the MS (Fig. 2b) it showed fragment peaks due to *a*, *b*, *c*, *d*, *e*, *f*, and *g*. In addition, there were weak fragment peaks at *m/z* 340 and 326, which may be assignable to ions *h* and *i*, respectively, characteristic of C-25 unsaturated sterols (Chart 2).[5]

From the above spectral properties and the molecular formula, the structure of neocyclonervilasterol was deduced to be **2**.

Both of the above compounds, **1** and **2**, are believed to be homogeneous since each of the HPLC chromatograms gave a single peak, but the stereochemistry at the C-24 position in **1** and **2** is uncertain. The stereochemistry at the C(10), C(13), C(14), C(17), and C(20) positions followed from the biogenetic analogy with the sterols and triterpenes contained in this plant[2,6] and pollinastanol (**7**),[7] which is a well-known methylsterol. The configuration of the C(3)-hydroxy group was presumed to be β-equatorial based on the NMR behavior of C(3)-H (δ 3.60, m, $W_{1/2}$ about 16 Hz), which is comparable with that of 3β-acetoxy-6β-hydroxy-5β,19-cyclocholestane (**8**)[8] (δ 4.72, m, $W_{1/2}$ about 16 Hz).

It is of interest from the biogenetic viewpoint that in the case of (24-ethyl)- and (24-isopropenyl)methylsterol, only one of the 24-epimers exists in the plant, whereas in the 24-methyl series, both of the 24-epimers occur,[9] as in the cases of triterpenes[2] and sterols.[6]

Experimental

Melting points were determined on a Kofler-type apparatus and are uncorrected. UV spectra were taken in EtOH solution. ^{1}H-NMR spectra were measured on a Varian XL-200 spectrometer in $CDCl_3$ solutions using tetramethylsilane as an internal standard and chemical shifts are recorded in δ values. MS and high-resolution MS were obtained with a JEOL JMS-D 300 spectrometer (ionization voltage, 70 eV; accelerating voltage, 3 kV) using a direct inlet system. HPLC and preparative HPLC were performed on a Waters Associates ALC/GPC 201 D compact-type liquid chromatograph using a TSK-GEL LS-410A ODS column (column size, 30 cm × 7.5 mm i.d.; detector setting, UV 225 nm) with hexane–isopropanol–acetonitrile (5 : 15 : 80) as the eluent (flow rate, 2.0 ml/min).

Isolation and Properties of Methylsterols from Substance MB (Methylsterol Fraction)——Substance MB (mixture) (50 mg) was subjected repeatedly to preparative HPLC on a TSK-GEL LS-410A ODS column with hexane–isopropanol–acetonitrile (5 : 15 : 80) as the eluting solvent at 20 °C to give cyclohomonervilasterol (peak 4, **1**)

(2 mg) and neocyclonervilasterol (peak 3, **2**) (0.6 mg), along with **3** (peak 1), **4** (peak 2), **5** (peak 5), and **6** (peak 6).

Cyclohomonervilasterol (1)——Colorless needles from ether–MeOH, mp 131—133 °C. UV λ_{max}^{EtOH} nm (log ε): 211 (3.75). High-resolution MS: Found 424.3698, Calcd for $C_{30}H_{48}O$ (M^+) 424.3703; Found 406.3646, Calcd for $C_{30}H_{46}$ 406.3597; Found 391.3318, Calcd for $C_{29}H_{43}$ 391.3363; Found 381.3200, Calcd for $C_{27}H_{41}O$ 381.3155; Found 285.2253, Calcd for $C_{20}H_{29}O$ 285.2217; Found 283.2043, Calcd for $C_{20}H_{27}O$ 283.2061; Found 267.2153, Calcd for $C_{20}H_{27}$ 267.2112. ^{1}H-NMR: see Table I.

Neocyclonervilasterol (2)——Colorless needles from ether–MeOH, mp 121—123 °C. UV λ_{max}^{EtOH} nm (log ε): 209 (3.74). High-resolution MS: Found 438.3842, Calcd for $C_{31}H_{50}O$ (M^+) 438.3859; Found 423.3588, Calcd for $C_{30}H_{47}O$ 423.2625; Found 420.3746, Calcd for $C_{31}H_{48}$ 420.3754; Found 405.3555, Calcd for $C_{30}H_{45}$ 405.3519; Found 395.3332, Calcd for $C_{28}H_{43}O$ 395.3312; Found 340.2744, Calcd for $C_{24}H_{36}O$ 340.2764; Found 285.2163, Calcd for $C_{20}H_{29}O$ 285.2217; Found 283.2065, Calcd for $C_{20}H_{27}O$ 283.2061; Found 267.2104, Calcd for $C_{20}H_{27}$ 267.2111. ^{1}H-NMR: see Table I.

References and Notes

1) Part V: T. Kikuchi, S. Kadota, S. Matsuda and H. Suehara, *Chem. Pharm. Bull.*, **34**, 3183 (1986).
2) T. Kikuchi, S. Kadota, H. Suehara and T. Shima, *Chem. Pharm. Bull.*, **33**, 1914 (1985).
3) J. W. Rawe, A. Melera, D. Arigoni, O. Jeger and L. Ruzicka, *Helv. Chim. Acta*, **40**, 1 (1957); L. H. Knox, E. Velarde, S. Berger, I. Delfin, R. Grezemkovsky and A. D. Cross, *J. Org. Chem.*, **30**, 4160 (1965).
4) The ^{1}H-NMR signal due to one of the 19-methylene protons was obscured by overlapping with the methyl proton signals.
5) I. J. Massey and C. Djerassi, *J. Org. Chem.*, **44**, 2448 (1979).
6) T. Kikuchi, S. Kadota, H. Suehara and T. Namba, *Chem. Pharm. Bull.*, **33**, 2235 (1985).
7) A. Ducruix, C. Pascard-Bill, M. Devys, M. Barbier and E. Lederer, *J. Chem. Soc., Chem. Commun.*, **1973**, 929. Also see M. J. Thompson, S. R. Dutky, Y. Lehner, L. N. Standifier and E. W. Herbert, Jr., *Phytochemistry*, **17**, 1053 (1978).
8) T. Kobayashi, M. Maeda, H. Komatsu and M. Kojima, *Chem. Pharm. Bull.*, **30**, 3082 (1982). We are indebted to Dr. Maeda, Kyushu University, for the ^{1}H-NMR data.
9) Co-occurrence of brassicasterol and its C-24 epimer in the seeds of some *Brassica* and *Raphanus* species, sitosterol and its C-24 epimer (clionasterol) in the seeds of *Cucumis sativus*, and 24-isopropenylcholesterol and its C-24 epimer in the Caribbean sponge *Verongia cauriformis* has been reported. See T. Matsumoto, N. Shimizu and T. Itoh, *Phytochemistry*, **22**, 1830 (1983); T. Matsumoto, T. Shigemoto and T. Itoh, *ibid.*, **22**, 2622 (1983); W. C. M. C. Kokke, C. S. Pak, W. Fenical and C. Djerassi, *Helv. Chim. Acta*, **62**, 1310 (1979).