Communications to the Editor

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ISOLATION AND STRUCTURE OF CYCLONERVILASTEROL, 24-EPICYCLONERVILASTEROL, DIHYDROCYCLONERVILASTEROL, AND 24-EPIDIHYDROCYCLONERVILASTEROL. NOVEL METHYLSTEROLS FROM *NERVILIA PURPUREA* SCHLECHTER

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Four new methylsterols, cyclonervilasterol, 24-epicyclonervilasterol, dihydrocyclonervilasterol, and 24-epidihydrocyclonervilasterol, were isolated from *Nervilia purpurea* SCHLECHTER. The structures 1a, 2a, 3a, and 4a were proposed for these compounds, respectively, based on the chemical and spectroscopic evidence.

KEYWORDS —— methylsterol; cyclonervilasterol; 24-epicyclonervilasterol; dihydrocyclonervilasterol; 24-epidihydrocyclonervilasterol; *Nervilia purpurea*; Orchidaceae

In the preceding communication,[1] we reported the isolation and characterization of a sterol with non-conventional side chain, nervisterol, from *Nervilia purpurea* SCHLECHTER (Orchidaceae). This paper descrides the investigation on the methylsterol constituents in the ether extract of *N. purpurea*.[2]

The neutral fraction of the ether extract was chromatographed on silica gel with CH_2Cl_2-hexane to give a methylsterol fraction, which was found to be a mixture of six compounds (approximate ratio, 10:10:1:5:10:20) by HPLC analysis as shown in Fig. 1. Preparative HPLC of this mixture on a reversed phase column (TSK-GEL LS-410A ODS) using hexane-isopropanol-acetonitrile (5:15:80) gave cyclonervilasterol (1a) (3 mg),[3] mp 151-152°C, $[\alpha]_D$ +15.2°, $C_{29}H_{46}O$ (M^+, 410.3571; Calcd, 410.3548); 24-epicyclonervilasterol (2a) (1.7 mg),[4] mp 153-154°C, $[\alpha]_D$ -10.8°, $C_{29}H_{46}O$ (M^+, 410.3551; Calcd, 410.3548); dihydrocyclonervilasterol (3a) (2.3 mg),[5] mp 128-129.5°C, $[\alpha]_D$ +24.5°, $C_{29}H_{48}O$ (M^+, 412.3727; Calcd, 412.3705); and 24-epidihydrocyclonervilasterol (4a) (4.1 mg),[6] mp 129-130°C, $[\alpha]_D$ +11.8°, $C_{29}H_{48}O$ (M^+, 412.3712; Calcd, 412.3705).

Catalytic hydrogenation of 1a (0.5 mg) and 2a (0.3 mg) over PtO_2 gave the corresponding dihydro compounds, each showing the M^+ peak at m/z 412 ($C_{29}H_{48}O$), which were found to be identical with 3a and 4a, respectively, by HPLC, MS, and ^{1}H-NMR comparisons. Furthermore, compounds 1a and 2a, and accordingly, 3a and 4a were considered to be epimeric with each other from the close similarities of their spectral properties.

The ^{1}H-NMR spectrum of 1a exhibited signals for *trans* olefinic protons at δ 5.16 and 5.20 and the mass spectra of 1a and 3a showed significant peaks at m/z 285 (M^+- side chain) and 267 (M^+- side chain - H_2O), suggesting that the compound 1a has a double bond in the side chain. This was proved by the following experiment.

Treatment of cyclonervilasteryl acetate (5b) (a mixture of 1b and 2b) with OsO_4

in pyridine gave a tetraol (6), $C_{31}H_{52}O_6$ (M^+, 520.3753; Calcd, 520.3754). Oxidation of this tetraol (6)(2.7 mg) with $Pb(OAc)_4$, followed by Jones oxidation and methylation with CH_2N_2, gave a minute amount of methyl ester, the GC-MS of which showed the M^+ ion peak at m/z 130 ($C_7H_{14}O_2$). This was identified as methyl 2-methylisovalerate (7) by GC and GC-MS comparisons with a sample (7) prepared from ergosterol (Chart 2).

The above observation together with the ^{1}H-NMR data[3] led us to suppose that cyclonervilasterol (1a) is a methylsterol having a cyclopropane and an ergosterol-type side chain. In consideration of the UV absorption band at 210 nm,[3] the other double bond must be conjugated with the cyclopropane ring in transoid orientation.[7]

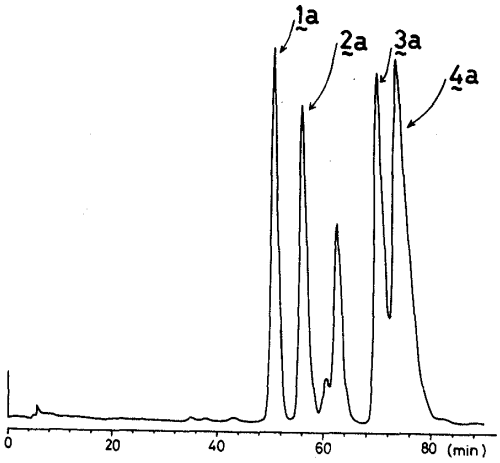

Fig.1. HPLC of Methylsterol Mixture

Conditions: column, TSK-GEL LS-410A ODS (30cm x 4mm i.d.); flow rate, 2.0 ml/min; eluant, hexane-isopropanol-acetonitrile (5:15:80); detector setting, UV 225 nm.

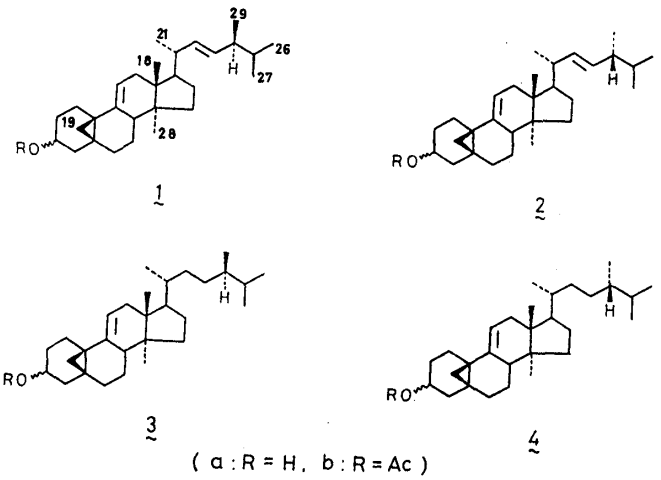

Chart 1

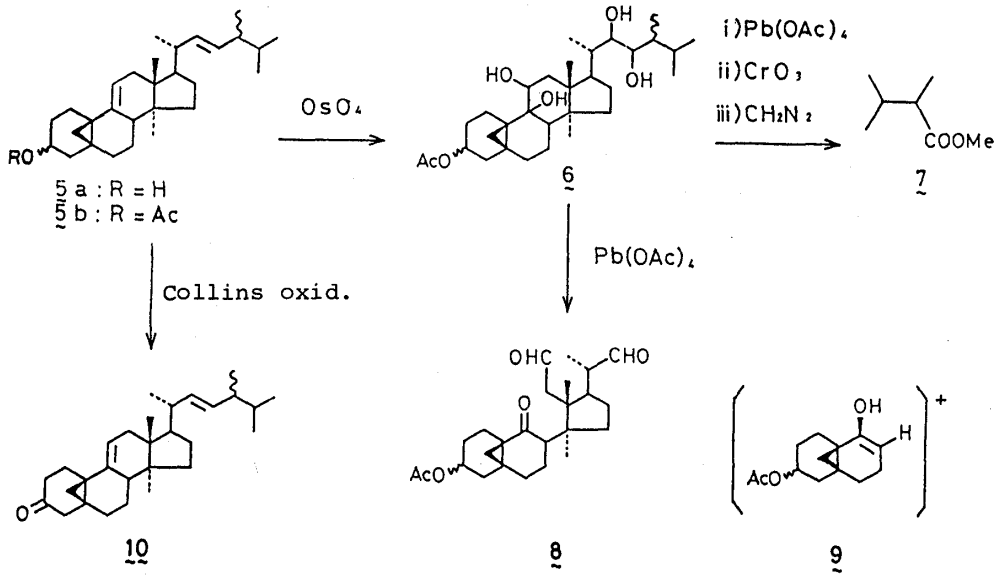

Chart 2

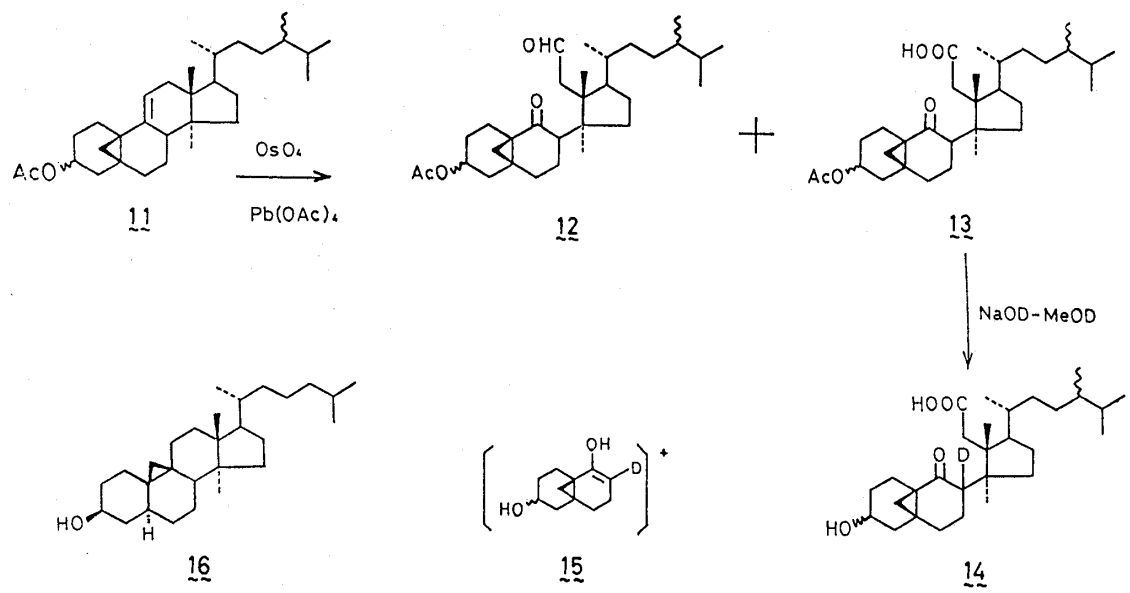

Chart 3

Then, we carried out several chemical reactions in order to obtain more detailed information about this conjugated system.

Collins oxidation of 5a (a mixture of 1a and 2a) gave a 3-keto derivative (10), mp 140-141°C, $C_{29}H_{44}O$ (M^+, 408.3344; Calcd, 408.3348), IR ν 1700 cm^{-1}. This compound (10) did not isomerize under various alkaline conditions and its ^{1}H-NMR spectrum showed AB quartet signals at δ 2.55 and 2.65 (each 1H, J=17.5 Hz, $C_{(4)}$-H_2), suggesting that the $C_{(5)}$ would be a quaternary sp^3-carbon rather than a sp^2 -carbon.

Oxidation of the tetraol (6) (3.7 mg) with $Pb(OAc)_4$ afforded mainly an oily keto dialdehyde (8) (0.8 mg). The mass spectrum of 8 showed the M^+ ion peak at m/z 416 and the base peak at m/z 222 ($C_{13}H_{18}O_3$, 222.1247; Calcd, 222.1255) which could be ascribed to the McLafferty fragment ion (9) (Chart 2). Similarly, osmium tetroxide oxidation of dihydrocyclonervilasteryl acetate (11) (a mixture of 3b and 4b) (7 mg), followed by $Pb(OAc)_4$ oxidation, afforded a keto aldehyde (12) (2.4 mg), MS m/z: 486 ($C_{31}H_{50}O_4$, 486.3717; Calcd, 486.3708) and 222 (base peak, 9) ($C_{13}H_{18}O_3$, 222.1247; Calcd, 222.1255), and a keto acid (13) (1 mg), MS m/z: 502 ($C_{31}H_{50}O_5$, 502.3644; Calcd, 502.3655) and 222 (base peak, 9) ($C_{13}H_{18}O_3$, 222.1252). Treatment of 13 with NaOD in MeOD gave a mono-deuterated product (14), which showed the M^+ ion peak at m/z 461 ($C_{29}H_{47}O_4D$, 461.3603; Calcd, 461.3614) and the base peak (McLafferty fragment peak, 15) at m/z 181 ($C_{11}H_{15}O_2D$, 181.1189; Calcd, 181.1211) in the mass spectrum (Chart 3).

These observations indicate that the dispositions of the cyclopropane and the double bond are at $C_{(5)}$-$C_{(10)}$ and $C_{(9)}$-$C_{(11)}$, respectively. Thus, we propose the structure 1a, 2a, 3a, and 4a for cyclonervilasterol, 24-epicyclonervilasterol, dihydrocyclonervilasterol, and 24-epidihydrocyclonervilasterol, respectively.

The stereochemistry at $C_{(10)}$, $C_{(13)}$, $C_{(14)}$, and $C_{(17)}$ positions followed from the biogenetic analogy with the sterols and triterpenes contained in this plant[1,8] and with pollinastanol (16)[9] which is a well-known methylsterol. As to the stereochemistry of the side chain grouping, 24S configuration could be assigned to 1a and

4a, and 24R configuration to 2a and 3a, by comparison of the shielding value of the 21-CH_3 signal with those in dihydrobrassicasterol and campesterol reported in the literature.[10]

A support for these structures was provided by the 2-D NMR experiments including INADEQUATE,[11] which will be reported in the forthcoming paper.

Our present result provided the first example of methylsterols having a cyclopropane ring at the $C_{(5)}$-$C_{(10)}$ position. These compounds are of interest in the viewpoint of biogenetic relation with natural sterols and triterpenes.

REFERENCES AND NOTES

1) T. Kikuchi, S. Kadota, H. Suehara, and T. Namba, *Chem. Pharm. Bull.*, 30, 370 (1982).

2) Melting points are uncorrected. Optical rotations were taken in $CHCl_3$ solutions (c = 0.43-0.80) at 22°C. Mass spectra were measured on a JEOL D-300 mass spectrometer and ^{1}H-NMR spectra on a Varian XL-200 spectrometer in $CDCl_3$ solutions with TMS as the internal standard.

3) 1a. UV λ_{max}^{EtOH}nm (log ε): 210 (3.72); MS m/z: 410(M^+), 395(M^+-15), 392(M^+-18), 377, 285(M^+-side chain), 267; ^{1}H-NMR δ: 0.63 and 0.67 (each 1H, d, J=4.0 Hz, 19-CH_2), 0.68 and 0.70 (each 3H, s, 18- and 28-CH_3), 0.82 and 0.84 (each 3H, d, J=6.3 Hz, 26- and 27-CH_3), 0.91 (3H, d, J=6.8 Hz, 29-CH_3), 0.97 (3H, d, J=6.4 Hz, 21-CH_3), 3.60 (1H, m, $W^{1/2}$=16 Hz, C<u>H</u>-OH), 5.16 and 5.20 (each 1H, dd, J=15, 7 Hz, -C<u>H</u>=C<u>H</u>-), 5.43 (1H, m, C=C<u>H</u>-).

4) The MS and UV spectra of 2a are virtually the same as those of 1a. Also the ^{1}H-NMR spectrum is almost identical with that of 1a except for the 21-CH_3 (δ 0.98, d, J=6.4 Hz) and the CH=CH signals (δ 5.18 and 5.23, each 1H, dd, J=15, 7 Hz).

5) 3a. UV λ_{max}^{EtOH}nm (log ε): 211 (3.75); MS m/z: 412(M^+), 397(M^+-15), 394(M^+-18), 379, 285(M^+-side chain), 267; ^{1}H-NMR δ: 0.63 and 0.67 (each 1H, d, J=4.0 Hz, 19-CH_2), 0.68 and 0.70 (each 3H, s, 18- and 28-CH_3), 0.77 and 0.80 (each 3H, d, J=6.3 Hz, 26- and 27-CH_3), 0.85 (3H, d, J=6.8 Hz, 29-CH_3), 0.87 (3H, d, J=6.5 Hz, 21-CH_3), 3.60 (1H, m, $W^{1/2}$=16 Hz, C<u>H</u>-OH), 5.43 (1H, m, C=C<u>H</u>-).

6) The MS and UV spectra of 4a are virtually identical with those of 3a. Also the ^{1}H-NMR spectrum is almost identical with that of 3a except for the 21-CH_3 signal (δ 0.89, d, J=6.5 Hz).

7) J. W. Rowe, 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).

8) Probably the $C_{(3)}$-OR grouping has the β-equatorial configuration in view of the $C_{(3)}$-H NMR behavior, which is comparable with that of 3β-acetoxy-6β-hydroxy-5β,19-cyclocholestane. See 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) A. Ducruix, C. Pascard-Billy, 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).

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