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NEW TRITERPENES FROM NERVILIA PURPUREA SCHLECHTER, AN ORCHIDACEOUS PLANT STRUCTURES OF CYCLONERVILOL AND CYCLOHOMONERVILOL AND CHEMICAL CORRELATION WITH CYCLOEUCALENOL

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Summary: Two new triterpenes, cyclonervilol and cyclohomonervilol, were isolated from *Nervilia purpurea* SCHLECHTER. The structure 7a and 1a were proposed for these compounds, respectively, based on the chemical and spectroscopic evidence.

Dried herbs of *Nervilia purpurea* SCHLECHTER are used as a folk medicine for treatment of confused wound and hypertention in Formosa. From this plant we have isolated two new triterpenes and their structures were elucidated.

Dried whole plant of *N. purpurea* (540 g), collected in Pingtung Hsen, Formosa, was extracted with ether and the extract was separated into the neutral and the acidic fractions in the usual manner. The neutral fraction was chromatographed on silica gel with CH_2Cl_2-hexane to give a mixture of triterpenes which was acetylated as usual. Chromatography of the acetate mixture (170 mg) on 20% $AgNO_3$-SiO_2 (300 g) using benzene-hexane (1:5) gave cyclonervilol acetate (7b) (25 mg), mp 129-131°, $[\alpha]_D$ +41°($CHCl_3$), $C_{33}H_{54}O_2$ (M^+: 482.4173. Calcd: 482.4121), MS m/z: 482 (M^+), 467, 422, 343, 314, 283; NMR($CDCl_3$) δ: 0.14, 0.41 (each 1H, d, J=4 Hz), 0.8-1.0 (CH_3x7), 2.05 (3H, s, Ac), 4.5 (1H, m, C<u>H</u>-OAc), 5.1 (2H, m, -C<u>H</u>=C<u>H</u>-); and cyclohomonervilol acetate (1b)(45 mg), mp 149-151°, $[\alpha]_D$ +37.3°($CHCl_3$), $C_{34}H_{56}O_2$ (M^+: 496.4272. Calcd: 496.4277), MS m/z: 496 (M^+), 481, 436, 343, 328, 283; NMR ($CDCl_3$) δ: 0.14, 0.39 (each 1H, d, J=4 Hz), 0.78-0.98 (CH_3x6), 1.57 (3H, s, C=C-CH_3), 4.5 (1H, br, -C<u>H</u>-OAc), 4.60, 4.73 (each 1H, m, C=C<u>H</u>$_2$), together with a small amount of cycloeucalenol acetate (6 mg), mp 87-90°.

Cyclohomonervilol (1a), mp 166-167°, $[\alpha]_D$ +40.5°($CHCl_3$), $C_{32}H_{54}O$ (M^+: 454.4199. Calcd: 454.4174), was obtained by alkaline hydrolysis of 1b. It showed

NMR signals for two cyclopropane protons at δ: 0.14 and 0.38 (d, J=4 Hz), suggesting that the compound is a member of cycloeucalenol type triterpenes.[1)]

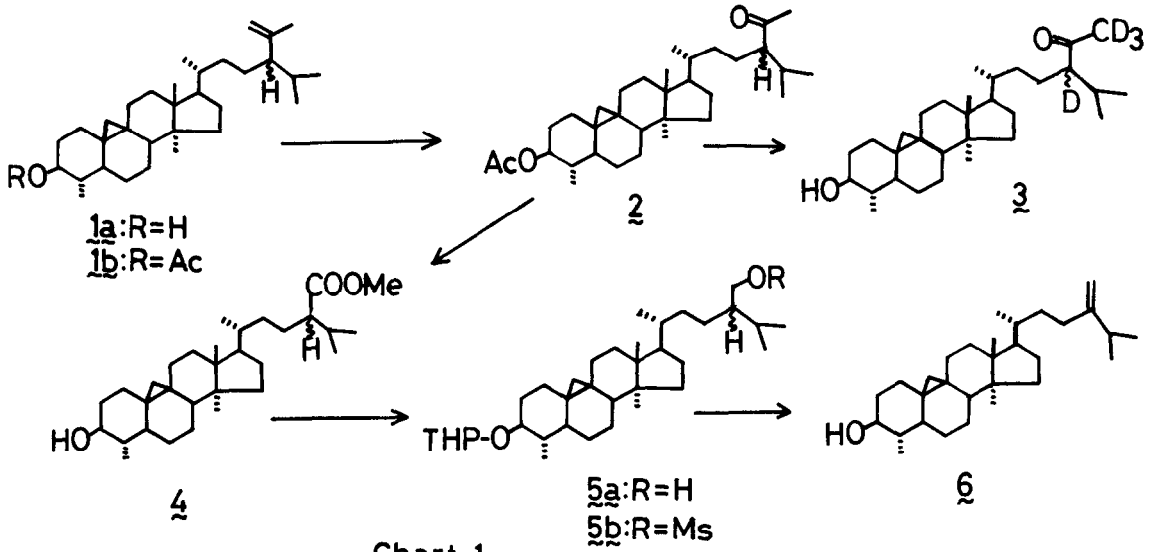

Chart 1

Osmium tetroxide oxidation of 1b, followed by HIO_4 oxidation, afforded a methyl ketone (2), mp 120-123°, MS m/z: 498 (M^+, $C_{33}H_{54}O_3$), NMR($CDCl_3$) δ: 2.01, 2.07 (each 3H, OAc and $COCH_3$), which on treatment with NaOD in MeOD gave a tetradeuterated product (3), mp 137-138°, MS m/z: 460 (M^+, $C_{31}H_{48}O_2D_4$), 427 ($M-CD_3$), 301 ($M-C_{10}H_{15}OD_4$). Thus, the compound 2 must have a methine and a methyl group adjacent to the carbonyl group.

The NMR spectrum of 2 in the presence of $Eu(DPM)_3$ (ca. 1 mol eq.) showed two tert-methyl and four sec-methyl signals. Among them, two sec-methyl signals (δ 2.95 and 3.30) changed to singlets on irradiation at δ 5.6, suggesting that the compound 2 has an isopropyl group (see Fig. 1).

Thus the structure of cyclohomonervilol might be assigned to the formula 1a. In order to confirm this assumption, we then examined the transformation of 1b into cycloeucalenol (6) as shown in Chart 1.

Haloform reaction of the methyl ketone (2)(20 mg) followed by methylation gave a methyl ester (4)(13 mg), mp 125-126°, MS m/z: 472 (M^+, $C_{31}H_{52}O_3$), which was converted to a tetrahydropyranyl ether and subsequently reduced with $LiAlH_4$ to give an alcohol (5a)(8 mg). The mesylate (5b) derived from 5a was heated at 170-180° in DMSO and then treated with aq. AcOH to yield an alcohol (6)(1.5 mg), mp

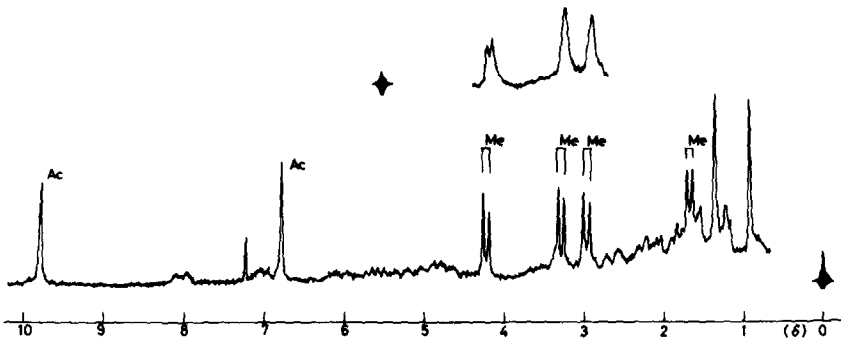

Fig. 1. NMR spectrum of the methyl ketone (2) in $CDCl_3$ with added $Eu(DPM)_3$ (ca. 1 mol. eq.).

122-123°, MS m/z: 426 (M^+, $C_{30}H_{50}O$). This alcohol (6) was proved to be identical with an authentic sample of cycloeucalenol (6) by GC, IR(KBr), and MS comparisons.

From the foregoing evidence, the structure of cyclohomonervilol was established to be 1a except for the stereochemistry at the C-24 position. It is worth to note here that a sterol possessing the same side-chain structure has recently been isolated from a sponge, *Verongia cauliformis*.[2)]

Cyclonervilol (7a), mp 166-169°, $[\alpha]_D$ +37.9°($CHCl_3$), $C_{31}H_{52}O$ (M^+: 440.4023. Calcd: 440.4018), was also obtained by alkaline hydrolysis of 7b. Its NMR spectrum (200 MHz) showed a pair of doublets at δ 0.15 and 0.40 for cyclopropane protons and two quartets for olefinic protons at about δ 5.06 and 5.17 (each 1H, J=8, 15 Hz), indicating that both the allylic carbons adjacent to the double bond have a hydrogen atom.

Treatment of cyclonervilol acetate (7b)(5.5 mg) with OsO_4 in pyridine gave a diol (4.5 mg), mp 186-188.5°, MS m/z: 516 (M^+, $C_{33}H_{56}O_4$). Subsequent oxidation of the diol (2 mg) with $Pb(OAc)_4$ afforded a carboxylic acid (8a), MS m/z: 416 (M^+, $C_{26}H_{40}O_4$), along with a small amount of another acid (9), MS m/z: 131 (M+1, $C_7H_{15}O_2$). The latter (9) was identified as 2-ethylisovaleric acid (9) by GC and GC-MS comparisons with a sample (9) prepared from i-stigmasteryl methyl ether.[3)] On the other hand, methylation of the former acid (8a) gave a methyl ester (8b), mp 160-169°, $C_{27}H_{42}O_4$ (M^+: 430.3081. Calcd: 430.3081), NMR($CDCl_3$) δ: 0.15, 0.41 (each 1H, d, J=4 Hz, cyclopropane H_2), 0.85-0.98 (CH_3x4), 2.05 (3H, s, Ac), 3.66 (3H, s, $COOCH_3$), 4.50 (1H, br, CH-OAc).

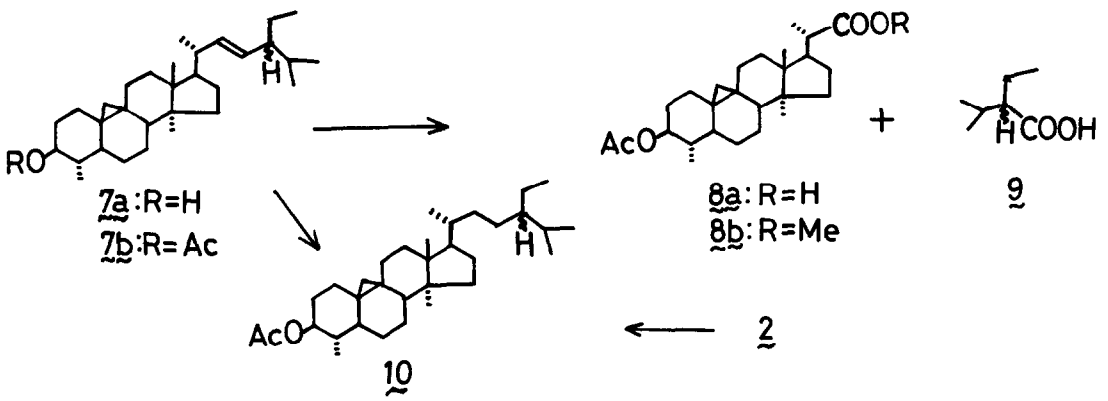

Chart 2

The above findings led us to suppose that the structure of cyclonervilol might be 7a. This was confirmed by the chemical correlation with cyclohomonervilol (1a) as shown in Chart 2.

Catalytic hydrogenation of 7b (1 mg) gave a dihydro compound (10)(0.5 mg), MS m/z: 484 (M^+, $C_{33}H_{56}O_2$). On the other hand, Wolff-Kishner reduction of the methyl ketone (2)(15 mg) and subsequent acetylation gave a crystalline substance (3 mg), which was indicated to be a mixture of two compounds (about 1:2) by GC examination. Eventually the major product (10) was found to be identical with dihydrocyclonervilol acetate (10), described above, by GC and GC-MS comparisons.

On the basis of the above result, the structure of cyclonervilol was proved to be 7a except for the stereochemistry at the C-24 position.

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