[Chem. Pharm. Bull. 33(5)1914—1929(1985)]

Studies on the Constituents of Orchidaceous Plants. II. Isolation, Structures, and Stereochemistry of Cyclonervilol, Cyclohomonervilol, and Dihydrocycloeucalenol C-24 Epimers, New Triterpenes from *Nervilia purpurea* SCHLECHTER[1)]

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(Received August 13, 1984)

Five new triterpenes, 24(R/α)- and 24(S/β)-dihydrocycloeucalenol, dihydrocyclonervilol, cyclonervilol, and cyclohomonervilol, were isolated along with cycloeucalenol and cyclofuntumienol from the triterpene fraction of *Nervilia purpurea* by the combination of chromatography on silver nitrate-impregnated silica gel and reversed-phase high-performance liquid chromatography (HPLC). The structures **4a**, **5a**, **7a**, **2a**, and **10a** are proposed for these compounds, respectively.

Keywords——*Nervilia purpurea*; triterpene; reversed-phase HPLC; cyclonervilol; cyclohomonervilol; 24-epimeric dihydrocycloeucalenol; 24-epimeric dihydrocyclonervilol; cycloeucalenol; cyclofuntumienol; ^{13}C-NMR

In a previous paper,[2)] we reported the characterization of the constituents of *Nervilia purpurea* SCHLECHTER and *N. aragoana* GAUD. (Orchidaceae), which are used as a folk medicine "I-tiam-hong" in Taiwan, and we also described the isolation of new triterpenes named cyclonervilol (**2a**) and cyclohomonervilol (**10a**) along with cycloeucalenol (**1a**) and related compounds from *N. purpurea*. This paper presents the full details of the structure elucidation of these triterpenes[1)] and the isolation of 24(R/α)- and 24(S/β)-dihydrocycloeucalenol (**4a** and **5a**), dihydrocyclonervilol (**7a**), and cyclofuntumienol (**3a**) from another lot of *N. purpurea*.[3)]

Dried whole plants of *N. purpurea*, collected in Pingtung Hsen, Taiwan, were extracted with dichloromethane at room temperature. The extract was subjected to alkaline hydrolysis and the neutral fraction was chromatographed on silica gel to give substance MA, substance MB, and a sterol mixture.[2)] Substance MA was then acetylated as usual and the resulting acetate mixture was chromatographed on 20% silver nitrate-silica gel with benzene–hexane mixture (1 : 9) to afford a mixture of A_{1b} acetate (**6b**) and A_3 acetate (**7b**) (approximate ratio 9 : 1),[2)] cyclonervilol acetate (**2b**), cyclohomonervilol acetate (**10b**), cyclofuntumienol acetate (**3b**), and cycloeucalenol acetate (**1b**). Identification of **3b** was done by comparing the spectral data with those reported in the literature.[4)]

The mixture of A_{1b} acetate and A_3 acetate (**6b** and **7b**) exhibited two peaks on gas chromatography (GC), and the mass spectra (MS) obtained by the GC-MS method showed the molecular ion peaks at m/z 470 and 484, respectively, together with several fragment peaks (Fig. 1). These fragmentation patterns resembled that of cycloeucalenol acetate (**1b**)[6)] and could be interpreted as shown in Chart 2, provided that the structures of A_{1b} acetate and A_3 acetate are **6b** and **7b**, respectively. This was shown to be the case by GC-MS comparisons with authentic samples of **6b** and **7b** prepared from **1b** and **2b**.

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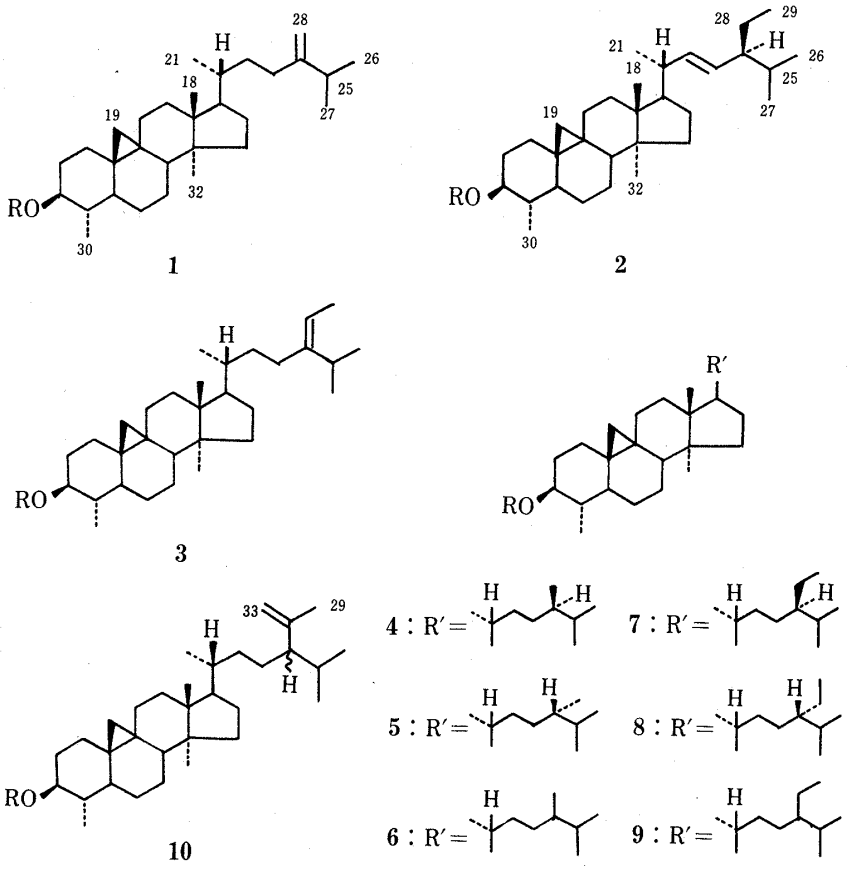

a: R=H **b**: R=$COCH_3$ **c**: R=COC_6H_5

Chart 1[5]

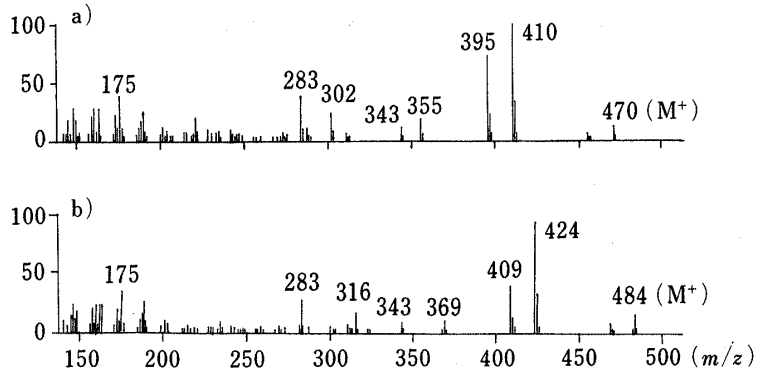

Fig. 1. Mass Spectra of the Triterpene Acetates from *N. purpurea* Obtained by GC-MS

a) A_{1b} acetate (**6b**), b) A_3 acetate (**7b**).

The above acetate mixture was then converted to the corresponding benzoate mixture (**6c** and **7c**), which unexpectedly revealed three peaks in high-performance liquid chromatography (HPLC) on a reversed-phase column with hexane–chloroform–acetonitrile (1 : 1 : 8) as the eluent (Fig. 2).

Application of preparative HPLC on a TSK-GEL ODS-120A column allowed us effectively to isolate each component.[7] Two major components obtained (**4c** and **5c**) were considered to be epimeric with each other, since in the MS they showed the same molecular ion peak at *m/z* 532 corresponding to the expected composition $C_{37}H_{56}O_2$ and their spectral

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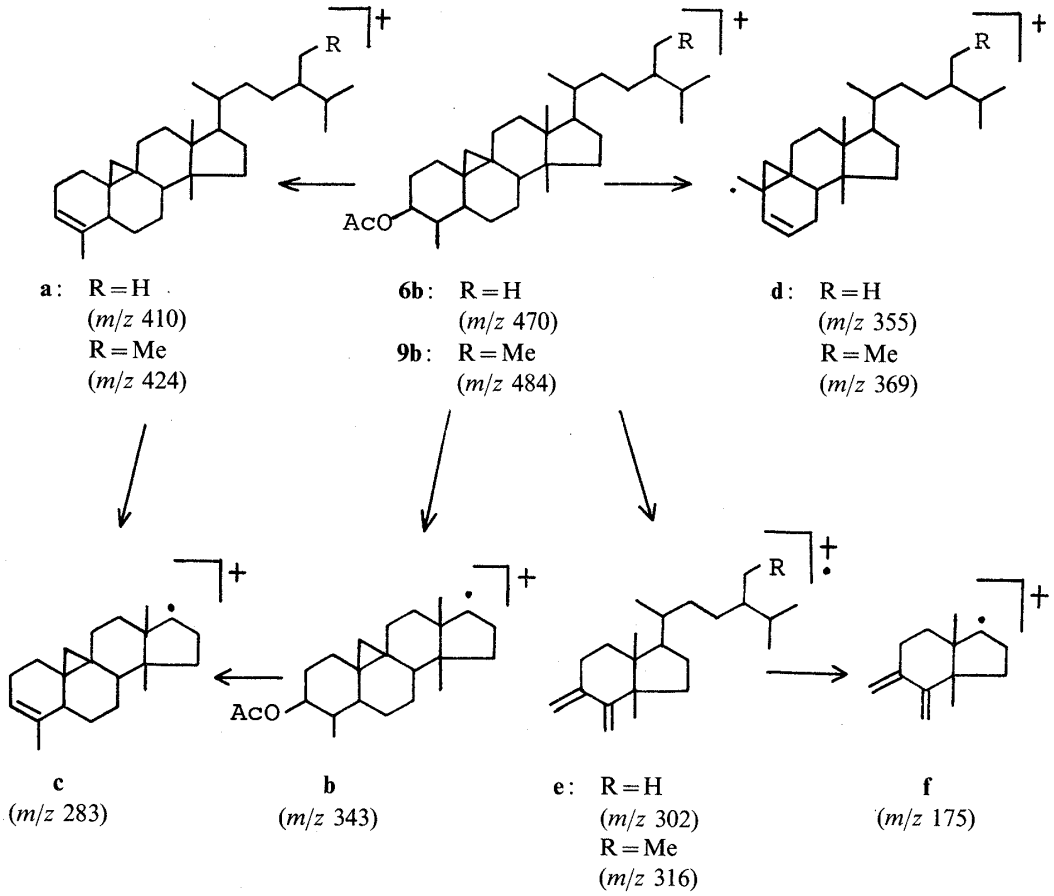

Chart 2

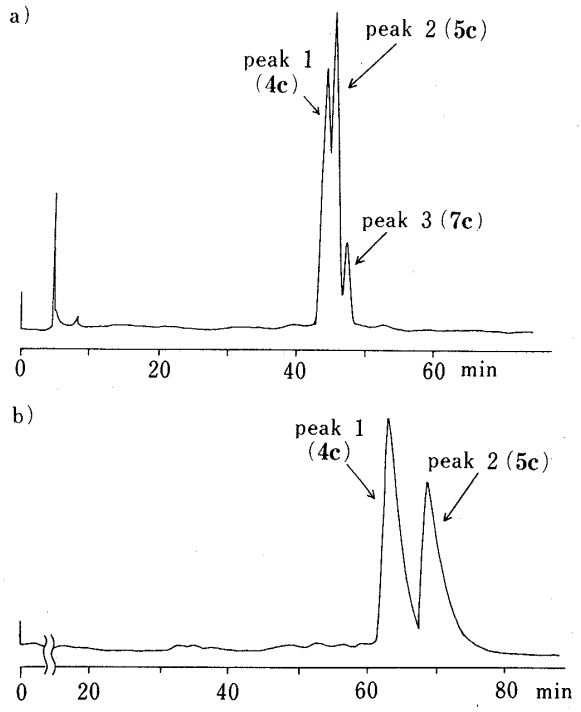

Fig. 2. HPLC Chromatograms of the Triterpene Benzoates from *N. purpurea*

a) A mixture of A_{1b} benzoate (**6c**) and A_3 benzoate (**7c**) (solvent, hexane–$CHCl_3$–CH_3CN 1:1:8), b) dihydrocycloeucalenol benzoate (**6c**) (solvent, $CHCl_3$–CH_3CN 2:8).

Conditions: column, TSK-GEL ODS-120A (25 cm × 4.6 mm i.d.); flow rate, 0.6 ml/min; detector setting, UV 240 nm; temperature, 20 °C.

patterns were identical. On the other hand, the minor component was identified as dihydrocyclonervilol benzoate (**7c**), as described later.

At this stage, catalytic reduction of authentic cycloeucalenol (**1a**) was examined.

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TABLE I. ^{1}H-NMR Spectral Data for Cycloeucalenol-Type Triterpenes from *Nervilia purpurea* and Their Derivatives

Compound (C24 config.)	3-H	18-H	19-H	21-H	26-H, 27-H[a)]	28-H	29-H	30-H	32-H	Others
2a	3.24	0.99	0.15, 0.40	0.975	0.855, 0.81		0.82	0.98	0.90	5.06, 5.17 (22, 23-H_2)
(*S*/α)	dt	s	d d	d	d d		t	d	s	dd dd
	(4.5, 10)		(4.0)	(6.0)	(6.0, 6.5)		(7.0)	(6.0)		(8.0, 15)
4a	3.20	0.971	0.14, 0.39	0.863	0.858, 0.811	0.784		0.984	0.896	
(*R*/α)	m	s	d d	d	d d	d		d	s	
			(4.0)	(6.5)	(6.8)	(6.6)		(6.1)		
4c	4.78	0.98	0.19, 0.45	0.862	0.859, 0.82	0.78		0.91	0.92	7.47, 8.08 (phenyl)
(*R*/α)	m	s	d d	d	d d	d		d	s	m m
			(4.0)	(5.4)	(6.8, 6.6)	(5.4)		(6.4)		
5a	3.20	0.964	0.14, 0.39	0.866	0.857, 0.78	0.78		0.979	0.889	
(*S*/β)	m	s	d d	d	d d	d		d	s	
			(4.0)	(6.5)	(6.8)	(6.8)		(6.1)		
5c	4.79	0.98	0.20, 0.46	0.87	0.86, 0.78	0.78		0.91	0.92	7.47, 8.08 (phenyl)
(*S*/β)	m	s	d d	d	d d	d		d	s	m m
			(4.0)	(6.4)	(6.8, 6.4)	(6.4)		(6.4)		
7a	3.21	0.966	0.14, 0.39	0.868	0.838, 0.816		0.85	0.981	0.89	
(*R*/α)	m	s	d d	d	d d		t	d	s	
			(4.0)	(6.5)	(7.0)		(7.5)	(6.5)		
7c	4.80	0.98	0.21, 0.46	0.88	0.84, 0.82		0.85	0.91	0.92	7.50, 8.08 (phenyl)
(*R*/α)	m	s	d d	d	d d		t	d	s	m m
			(4.0)	(6.6)	(6.8, 6.6)		(7.5)	(6.6)		
8a	3.21	0.97	0.14, 0.39	0.873	0.841, 0.816		0.86	0.985	0.893	
(*S*/β)	m	s	d d	d	d d		t	d	s	
			(4.0)	(6.5)	(7.0, 6.8)		(7.5)	(6.5)		
8c	4.80	0.98	0.19, 0.45	0.88	0.84, 0.82		0.86	0.91	0.92	7.50, 8.08 (phenyl)
(*S*/β)	m	s	d d	d	d d		t	d	s	m m
			(4.0)	(6.6)	(6.8)		(7.5)	(6.4)		
10a	3.23	0.96	0.14, 0.38	0.88[b)]	0.92, 0.81[b)]		1.57	0.98	0.89	4.63, 4.76 (33-H_2)
	dt	s	d d	d	d d		br s	d	s	m m
	(4.5, 10)		(4.0)	(6.5)	(6.5, 6.3)			(6.5)		

δ values in $CDCl_3$ and coupling constants in Hz. *a*) The higher-field signal was arbitrarily assigned to the 27-methyl group. *b*) Assignments may be interchanged, see ref. 16.

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Hydrogenation of **1a** on Adams catalyst and subsequent benzoylation yielded dihydrocycloeucalenol benzoate (**6c**), $C_{37}H_{56}O_2$, which was proved to be a mixture of C-24 epimers (approximate ratio of 51 : 49) by HPLC as illustrated in Fig. 2. Preparative HPLC of this mixture on a reversed-phase column with chloroform–acetonitrile (2 : 8) as the eluting solvent gave each epimer: one (peak 1) (**4c**) showed mp 130—132 °C and the other (peak 2) (**5c**), mp 133—135 °C.

Alkaline hydrolysis of these epimeric benzoates (**4c** and **5c**) afforded the corresponding alcohols: **4a**, mp 141—142 °C, and **5a**, mp 152—153 °C, respectively, whose MS were identical with each other. The proton nuclear magnetic resonance (^{1}H-NMR) spectra of **4a** and **5a**, and those of **4c** and **5c** were very similar and the signals were assigned as shown in Table I, based on comparisons with the ^{1}H-NMR spectrum of cycloeucalenol (**1a**)[8] and with those of sterols having an analogous side chain.[9] As to the stereochemistry of the C-24 position, the 24(R/α) configuration could be allotted to **4a**, and the 24(S/β) configuration to **5a**, based on the shielding values of the 27-methyl signals compared with those of the 24-epimeric sterols reported by Rubinstein *et al.*,[9] who found that the 27-methyl groups in campesterol (24R/α) and its acetate resonate at slightly lower field than their C-24(S/β) epimers. This conclusion was further supported by the HPLC behavior compared with that of analogous sterols.[10]

Eventually, the major components, **4c** and **5c**, obtained from the above-mentioned A_{1b}- and A_3-benzoate mixture were identified as 24(R/α)- and 24(S/β)-dihydrocycloeucalenol benzoate (**4c**, **5c**), respectively, on the basis of MS, ^{1}H-NMR, and HPLC comparisons.

Cyclonervilol acetate (**2b**), mp 140—143 °C, $[\alpha]_D$ +41 °, showed the molecular ion peak at m/z 482 in the MS and its molecular formula was determined to be $C_{33}H_{54}O_2$ by high-resolution MS. It showed strong infrared (IR) absorption bands at 1740 and 1250 cm^{-1} and ^{1}H-NMR signals at δ 2.05 (3H, s) and 4.52 (1H, dt, J=4.5, 10 Hz) arising from a secondary alcohol acetate grouping.

Alkaline hydrolysis of **2b** afforded cyclonervilol (**2a**), mp 166—169 °C, $[\alpha]_D$ +37.9 °, whose composition ($C_{31}H_{52}O$) was confirmed by the MS and high-resolution MS measurements. Its ^{1}H-NMR spectrum showed two quartets due to *trans*-oriented olefinic protons at δ 5.06 and 5.17 (J=8, 15 Hz), a double triplet due to a hydroxyl-bearing methine proton at δ 3.24 (J=4.5, 10 Hz), and a pair of doublets due to cyclopropyl methylene protons at δ 0.15 and 0.40 (J=4 Hz) which are characteristic of cycloeucalenol-type triterpene,[8] along with signals due to a primary methyl, four secondary methyls, and two tertiary methyls (Table I). The MS of **2a** showed significant signals at m/z 440 (M^+), 425, 422 (**a'**), 367 (**d'**), 314 (**e'**), 301 (**b'**), 283 (**c**), and 175 (**f**), which can be reasonably explained by the fragmentations shown in Chart 3.

From the above spectral data and the molecular formula, cyclonervilol was presumed to be a cycloeucalenol-type triterpene carrying an unsaturated side chain.

Treatment of cyclonervilol acetate (**2b**) with osmium tetroxide in pyridine gave a diol (**11**), $C_{33}H_{56}O_4$, mp 186—188.5 °C. Subsequent oxidation of this diol (**11**) with lead tetraacetate[11] led to a carboxylic acid (**12a**), $C_{26}H_{40}O_4$, and a small amount of another acid (**13**) which showed the ($M^+ + 1$) peak at m/z 131 ($C_7H_{15}O_2^+$) accompanied with peaks due to fragments such as ($M^+ - C_2H_5$) and ($M^+ - C_3H_7$) in the MS. Eventually the latter acid **13** was identified as 2-ethylisovaleric acid (**13**) by GC and GC-MS comparisons with a sample (**13**) prepared by ozonolysis of i-stigmasteryl methyl ether (**15**).[12] It should be noted here that in this ozonolysis the original product was the aldehyde **14**, which on standing was readily autooxidized to the acid **13** (see Experimental).

On the other hand, methylation of the former acid (**12a**) with diazomethane gave a methyl ester (**12b**), $C_{27}H_{42}O_4$, mp 160—169 °C, whose ^{1}H-NMR spectrum showed the signals due to cyclopropyl methylene protons (δ 0.15 and 0.41) as well as an acetoxyl-bearing methine, an acetyl, two secondary methyl, and two tertiary methyl groups. The MS showed

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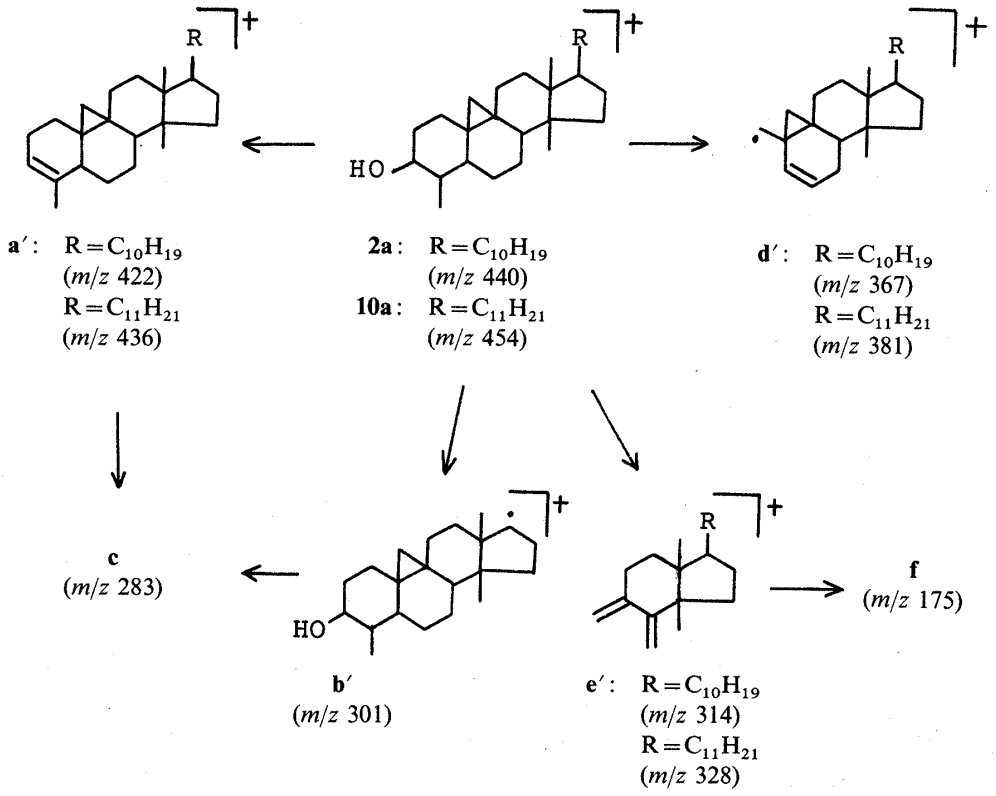

Chart 3

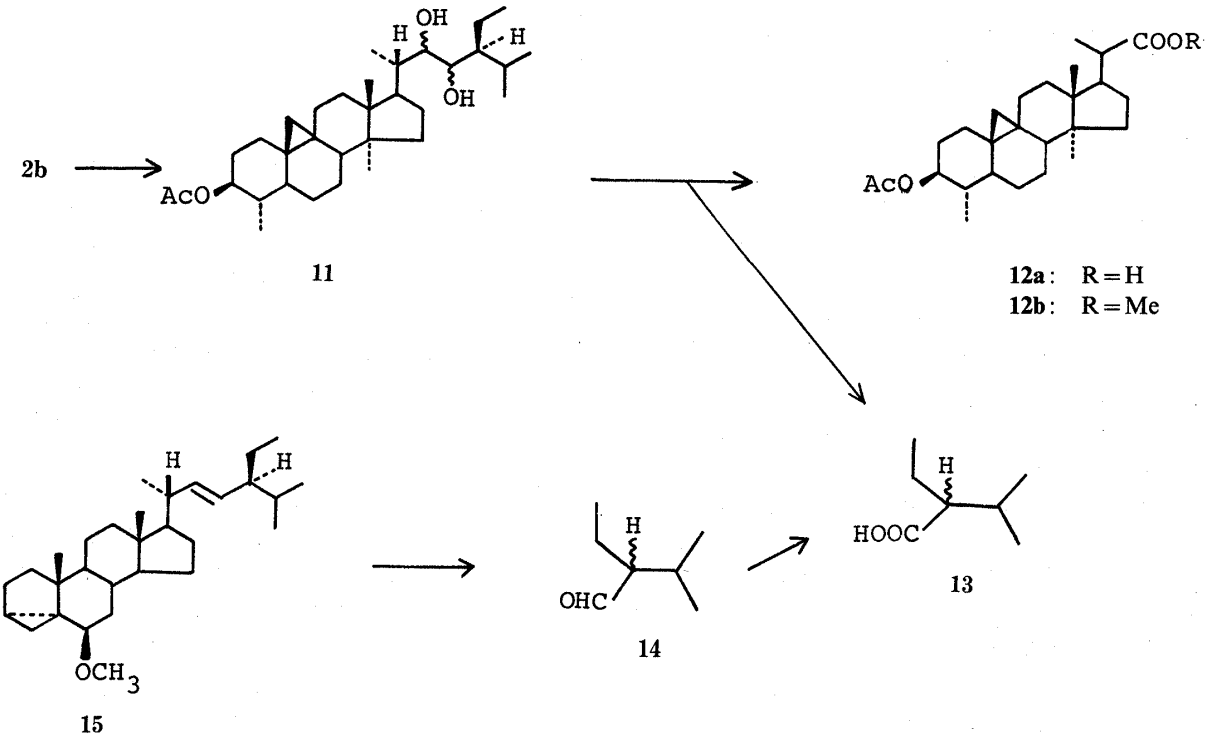

Chart 4

the molecular ion peak (*m/z* 430) and fragment peaks attributable to ($M^+ - 60$), **c**, and **f** (see Chart 2).

On the basis of these spectral data, this ester could be assigned the structure **12b**. Thus, the structure of cyclonervilol was deduced to be **2a**. This was confirmed by chemical

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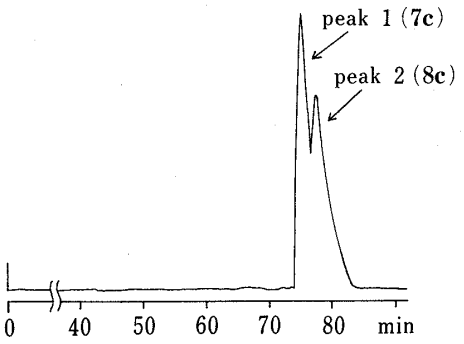

Fig. 3. HPLC Chromatogram of Dihydrocyclofuntumienol Benzoate (**9c**)

Conditions: the same as given in Fig. 2 (solvent, $CHCl_3$–CH_3CN 2:8).

correlation with cyclofuntumienol (**3a**).

Medium-pressure catalytic hydrogenation of cyclofuntumienol acetate (**3b**) on Adams catalyst, followed by alkaline hydrolysis and subsequent benzoylation, yielded dihydrocyclofuntumienol benzoate (**9c**), $C_{38}H_{58}O_2$, which was indicated to be a mixture of C-24 epimers (approximate ratio of 51:49) by HPLC as illustrated in Fig. 3. Each of these epimers was isolated by repeated preparative HPLC: the more mobile epimer (**7c**) showed mp 148—149.5 °C and the less mobile one (**8c**), mp 149—151 °C.

Alkaline hydrolysis of each epimeric benzoate (**7c** and **8c**) afforded the corresponding triterpene: **7a**, mp 154—156 °C, and **8a**, mp 151—153 °C, respectively, whose MS were superimposable on each other. The ^{1}H-NMR spectra of **7a** and **8a** and also those of **7c** and **8c** were very similar and the signals were assigned as given in Table I, based on comparisons with the spectra of sitosterol (24*R*/α) and clionasterol (24*S*/β), having the same side chain of established stereochemistry. Here, assignments of the stereochemistry at the C-24 position of **7a** and **8a** were based on the chemical shift difference of the 29-methyl signals.[9,13] The relative HPLC mobilities of **7c** and **8c** compared with those of the epimeric 24-ethyl sterols also supported this conclusion.[10]

Next, benzoylation of cyclonervilol (**2a**) followed by catalytic hydrogenation on Adams catalyst gave dihydrocyclonervilol benzoate (**7c**), $C_{38}H_{58}O_2$, mp 150—151 °C, which showed a single peak on HPLC. This compound was proved to be identical with 24(*R*/α)-dihydrocyclofuntumienol benzoate (**7c**), described above, by MS, ^{1}H-NMR, and HPLC comparisons. Therefore the structure of cyclonervilol was established to be **2a**.

It should be mentioned here that the minor component, A_3 benzoate, was identified as **7c** (24*R*), and no trace of its epimeric counterpart could be found in substance MA. It is of particular interest from the biogenetic view point that in the case of the 24-ethyl triterpene, only one of the 24-epimers exists in the plant, whereas in the 24-methyl series, both of the 24-epimers occur.[14]

Cyclohomonervilol acetate (**10b**), mp 149—151 °C, showed $[\alpha]_D$ +37.3° and its molecular formula $C_{34}H_{56}O_2$ was established by MS and high-resolution MS measurements. The IR and ^{1}H-NMR spectra clearly showed the presence of a secondary acetoxy grouping, and the presence of a cyclopropane, a vinyl methyl, and a terminal methylene was also indicated by the ^{1}H-NMR spectrum.

Alkaline hydrolysis of **10b** gave cyclohomonervilol (**10a**), mp 166—167 °C, $[\alpha]_D$ +40.5°, which had the molecular formula $C_{32}H_{54}O$ and showed characteristic ^{1}H-NMR signals due to cyclopropyl methylene protons at δ 0.14 and 0.38 (d, J=4 Hz) and terminal methylene protons at δ 4.63 and 4.76 (m) along with signals due to a vinyl methyl, two tertiary methyl, and four secondary methyl groups (Table I). The MS of **10a** showed the molecular ion peak at m/z 454 and significant peaks ascribable to the fragment ions **a′**, **d′**, **e′**, **b′**, **c**, and **f** (see Chart 3).

From the above spectral properties and the molecular formula, cyclohomonervilol was

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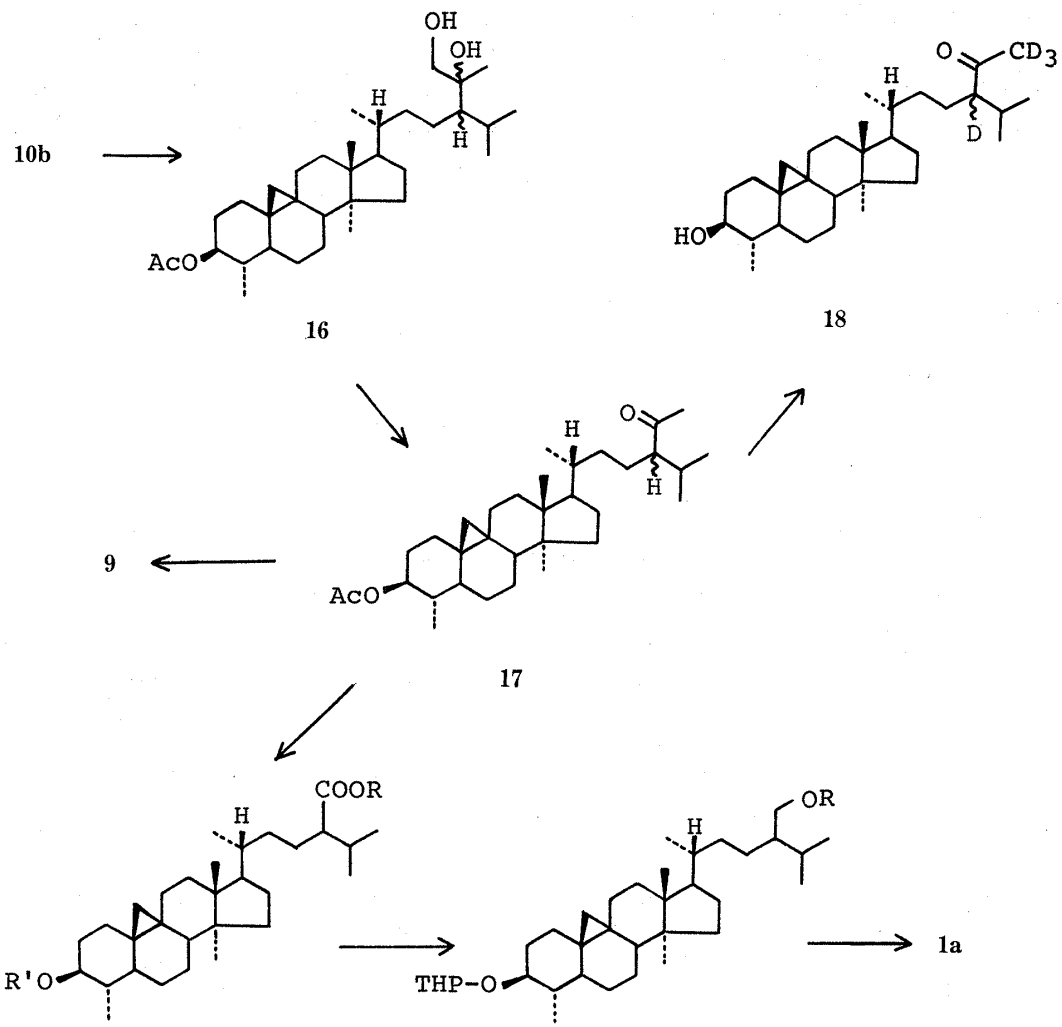

19a: R=R′=H
19b: R=Me, R′=H
19c: R=Me, R′=THP

20a: R=H
20b: R=Ms

Chart 5

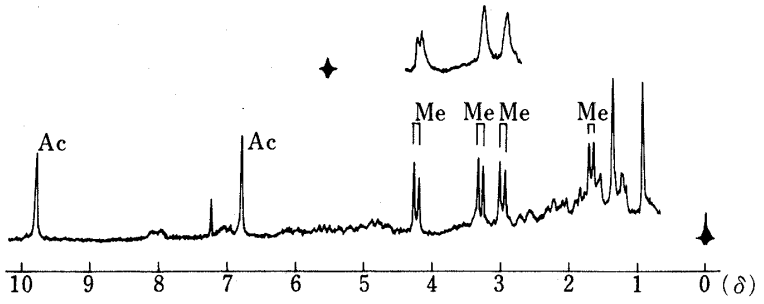

Fig. 4. ^{1}H-NMR Spectrum (90 MHz) of the Methyl Ketone (**17**) in $CDCl_3$ with Added Eu(DPM)$_3$ (*ca.* 1 mol eq)

also believed to be a cycloeucalenol-type triterpene possessing a homologous side chain. Thus, we examined the reaction at the side chain of **10b**.

Osmium tetroxide oxidation of cyclohomonervilol acetate (**10b**) followed by periodate oxidation afforded a methyl ketone (**17**), mp 120—123 °C, whose MS showed the molecular ion peak at *m/z* 498 ($C_{33}H_{54}O_3$). Its IR spectrum exhibited strong absorptions at 1735 and

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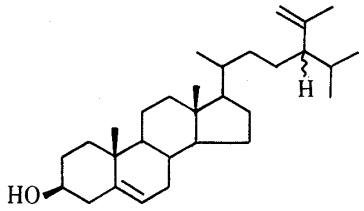
1705 cm^{-1} and the ^1H-NMR spectrum showed signals due to an acetoxyl group and a newly introduced acetyl group at δ 2.01 and 2.07. Treatment of **17** with sodium deuteroxide in methanol-d_1 gave a tetradeuterated product (**18**), mp 137—138 °C. The MS of **18** exhibited the molecular ion peak at m/z 460 ($C_{31}H_{48}D_4O_2$) accompanied with peaks at m/z 427 ($M^+ - CD_3$)



21

Chart 6

TABLE II. ^{13}C-NMR Spectral Data for the Triterpene Acetates from *Nervilia purpurea*

Carbon	**1b**	**2b**	**10b**
C1	30.5 (t)	30.5 (t)	30.5 (t)
C2	30.9 (t)	31.0 (t)	30.9 (t)
C3	78.7 (d)	78.7 (d)	78.7 (d)
C4	41.5 (d)	41.5 (d)	41.5 (d)
C5	43.4 (d)	43.4 (d)	43.4 (d)
C6	24.7 (t)	24.7 (t)	24.7 (t)
C7	27.0 (t)[a]	27.0 (t)	27.0 (t)
C8	46.8 (d)	47.0 (d)	46.9 (d)
C9	23.7 (s)	23.7 (s)	23.7 (s)
C10	29.4 (s)	29.4 (s)	29.4 (s)
C11	25.0 (t)	25.1 (t)	25.0 (t)
C12	35.3 (t)	35.5 (t)	35.4 (t)
C13	45.4 (s)	45.2 (s)	45.3 (s)
C14	48.9 (s)	49.1 (s)	48.9 (s)
C15	32.9 (t)	32.8 (t)	32.8 (t)
C16	28.1 (t)[a]	28.9 (t)	28.1 (t)
C17	52.2 (d)	52.1 (d)	52.1 (d)
C18	17.7 (q)	18.0 (q)	17.8 (q)
C19	27.1 (t)	27.2 (t)	27.1 (t)
C20	36.1 (d)	41.0 (d)	36.7 (d)
C21	18.3 (q)	20.9 (q)	18.7 (q)
C22	35.1 (t)	138.6 (d)	34.5 (t)
C23	31.3 (t)	129.3 (d)	26.8 (t)
C24	156.8 (s)	51.3 (d)	55.5 (d)
C25	33.8 (d)	31.9 (d)	30.3 (d)
C26	21.9 (q)	19.0 (q)	20.8 (q)
or			
C27	22.0 (q)	21.2 (q)	21.5 (q)
C28	106.0 (t)	25.4 (t)	147.3 (s)
C29	—	12.3 (q)	19.0 (q)
C30	14.4 (q)	14.4 (q)	14.4 (q)
C32	19.1 (q)	19.2 (q)	19.1 (q)
C33	—	—	111.9 (t)
$OCOCH_3$	170.8 (s)	171.0 (s)	170.9 (s)
$OCOCH_3$	21.3 (q)	21.4 (q)	21.3 (q)

δ values in $CDCl_3$. The multiplicities of carbon signals are indicated as (s), (d), (t), and (q). *a*) Assignments in ref. 19 were reversed.

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and 301 ($M^+ - C_{10}H_{15}D_4O$). Thus the ketone **17** must have a methine group and a methyl group adjacent to the carbonyl group.

The 90 MHz ^{1}H-NMR spectrum of **17** showed overlapped signals corresponding to six methyl groups in the δ 0.77—0.96 region, but in the presence of tris(dipivaloylmethanato)-europium(III) [Eu(DPM)$_3$] they were clearly separated to show two singlets due to tertiary methyl groups and four doublets due to secondary methyl groups as illustrated in Fig. 4. Among them, two of the secondary methyl signals at δ 2.95 and 3.30 changed to singlets on irradiation at δ 5.60, suggesting that the ketone **17** still has an isopropyl group.

Thus the structure of cyclohomonervilol might be assigned as **10a**. In order to confirm this, we examined the transformation of **10b** into cycloeucalenol (**1a**) as shown in Chart 5.

Haloform reaction of the methyl ketone (**17**) followed by methylation with diazomethane gave a methyl ester (**19**), mp 125—126 °C, $C_{31}H_{52}O_3$, which was converted to a tetrahydropyranyl ether and subsequently reduced with lithium aluminum hydride to yield an alcohol (**20a**). The mesylate (**20b**) derived from **20a** was heated at 170—180 °C in dimethyl sulfoxide and then treated with aqueous acetic acid to yield a small amount of the alcohol (**1a**) melting at 122—123 °C. This alcohol (**1a**) gave the molecular ion peak at m/z 426 ($C_{30}H_{50}O$) and was proved to be identical with an authentic sample of cycloeucalenol (**1a**) by GC, IR, and MS comparisons.

Further, the chemical correlation of cyclohomonervilol (**10a**) with cyclonervilol (**2a**) was attempted as follows. Wolff–Kishner reduction of the methyl ketone (**17**) followed by acetylation gave a crystalline substance. Although this substance could not be isolated in a pure state, the major product was proved to be identical with dihydrocyclonervilol acetate (**9b**) by GC and GC-MS analyses. However, the reversed-phase HPLC measurement of the benzoate derived from the above product indicated it to be a mixture of 24(R/α)- and 24(S/β)-epimers (**7c** and **8c**).[15]

On the basis of the foregoing result, the structure of cyclohomonervilol should be

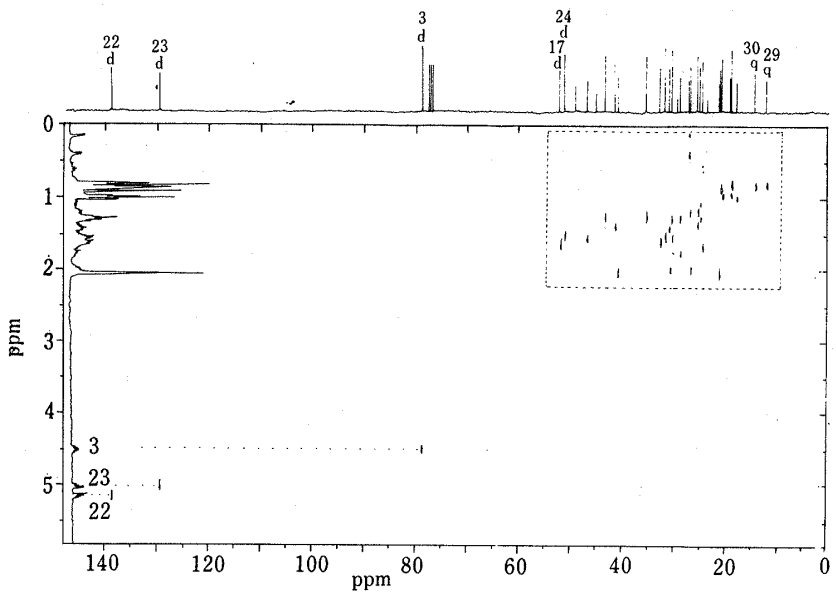

Fig. 5. The ^{1}H–^{13}C Shift Correlated Spectrum of Cyclonervilol Acetate (**2b**) in $CDCl_3$

The ^{1}H shifts are the ordinate and the ^{13}C shifts are the abscissa. The carbonyl carbon signal (δ 171.0) is outside of the spectrum. The multiplicities of carbon signals were determined by means of the off-resonance and INEPT methods, and are indicated as s, d, t, and q. The carbon signals due to a carbinol methine (C3) and a disubstituted olefin (C22 and C23) were assigned. The rectangular region at the upper right is enlarged and reproduced in Fig. 6.

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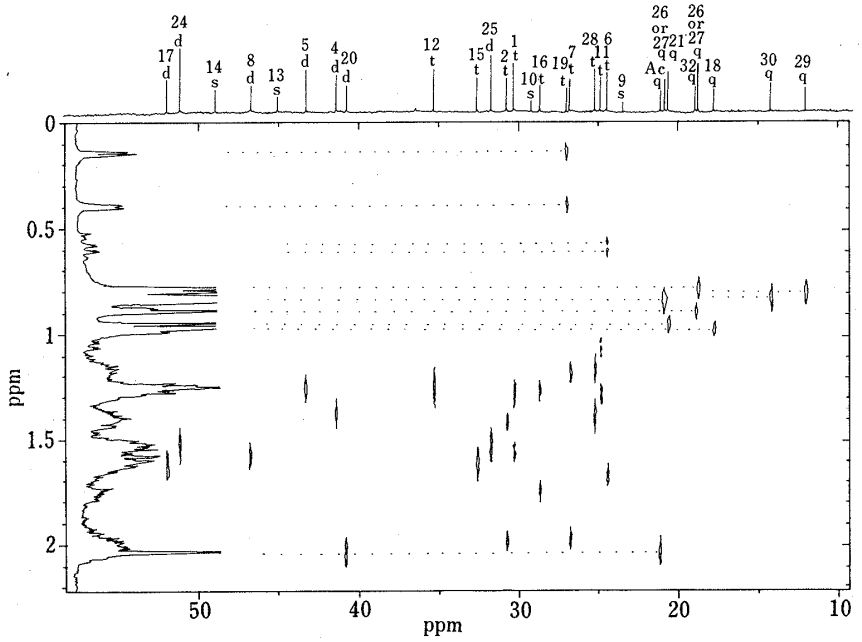

Fig. 6. Enlarged ^{1}H–^{13}C Shift Correlated Spectrum of the Rectangular Region in the Upper Right Part of Fig. 5

represented by the formula **10a**. The stereochemistry at the C-24 position in **10a** is uncertain, and this problem is currently under investigation.

It is worth noting here that a sterol (**21**) possessing the same side chain structure as **10a** has recently been isolated from a marine sponge, *Verongia cauliformis*.[16]

Next, we examined the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of a series of cycloeucalenol-type triterpenes isolated from this plant by application of the INEPT method[17] and two-dimensional NMR spectroscopy.[18] The ^{13}C-NMR spectra of cycloeucalenol (**1a**), cycloeucalenol acetate (**1b**), and cycloeucalenone have already been investigated by Lukacs and coworkers,[19] who assigned all the ^{13}C-signals of these compounds. Later, Dahmen *et al.*[20] pointed out that Lukacs' assignments of the C-7 and C-16 signals (δ 28.0 and 26.9, respectively) of cycloeucalenone should probably be reversed. In our present experiment, the spectra of cyclonervilol acetate (**2b**) and cyclohomonervilol acetate (**10b**) exhibited the corresponding ^{13}C-signals at δ 28.9 and 27.0 and at δ 28.1 and 27.0, respectively. The observed downfield shift (0.8 ppm) of the lower ^{13}C-signal of **2b** compared with that of cycloeucalenol acetate (**1b**) supported Dahmen's suggestion, because C-16 would be more sensitive to the magnetic effect due to the structural difference of the side chain than C-7.

The ^{1}H–^{13}C shift correlated NMR measurements[18] of **2b** (Figs. 5 and 6) and **10b** led readily to the assignments of the signals due to the cyclopropyl methylene carbon, methyl carbons, and olefinic carbons in these compounds as shown in Table II. Assignments of the other ^{13}C-signals were done based on a comparison with those of **1b** reported by Lukacs *et al.*[19] (Table II).

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. IR spectra were recorded in KBr discs on a JASCO IRA-2 instrument and NMR spectra were taken on Hitachi R-22, Varian Associates EM-390 and XL-200, and Bruker AM-400 spectrometers in $CDCl_3$ solutions with tetramethylsilane as an internal standard; chemical shifts are recorded in δ values. NMR measurements in the presence of $Eu(DPM)_3$ were made after adding a weighed amount of the reagent to a solution of the substrate in $CDCl_3$. GC analyses were done on a Shimadzu GC-

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6A gas chromatograph using a 2 m glass column (3 mm i.d.) packed with either 2% OV-17 on Gas-Chrom Q or Thermon-1000 + H_3PO_4 (10 + 1%) on Chromosorb W at a column temperature of 280 °C (OV-17) or 150 °C (Thermon-1000 + H_3PO_4). Nitrogen was employed as a carrier gas at a flow rate of 40 ml/min. 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 (2 m × 2 mm i.d. glass tube) or Thermon-1000 + H_3PO_4 (10 + 1%) on Chromosorb W (2 m × 2 mm i.d. glass tube); column temperature, 280 °C (OV-17) or 170 °C (Thermon-1000 + H_3PO_4); injection temperature, 300 °C (OV-17) or 200 °C (Thermon-1000 + H_3PO_4); ionization energy, 70 eV; accelerating voltage, 3 kV. HPLC and preparative HPLC were performed on a Waters Associates ALC/GPC 201D 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 $CHCl_3$–CH_3CN (2 : 8) or hexane–$CHCl_3$–CH_3CN (1 : 1 : 8) as the eluent (flow rate 0.6 ml/min). Preparative thin layer chromatography (TLC) was carried out on Merck Kieselgel GF_{254} with $CHCl_3$, and plates were examined under ultraviolet (UV) light (for UV-absorbing material). Extraction of substances from the silica gel was done with MeOH–$CHCl_3$ (1 : 9) and solutions were concentrated *in vacuo*. Mallinckrodt silica gel was used for ordinary column chromatography, and 20% $AgNO_3$–silica gel was prepared according to Ghosh's description.[21] For drying of organic solutions, anhydrous $MgSO_4$ was used.

Extraction and Separation of the Constituents of *Nervilia purpurea* ——Dried herbs of *N. purpurea* (580 g), collected in Pingtung Hsen, Taiwan, in August, 1978, were extracted with CH_2Cl_2 (5 l × 3) for 2 d at room temperature. Concentration of the CH_2Cl_2 extracts *in vacuo* afforded a dark green residue (*ca.* 40 g), which was refluxed with 10% KOH–MeOH (150 ml) for 6 h. After concentration *in vacuo*, the residue was extracted with CH_2Cl_2 and the CH_2Cl_2 solution was washed with water, dried, and concentrated to leave an oily residue (6.3 g), which was chromatographed on silica gel (400 g) with CH_2Cl_2–hexane mixture (1 : 9, 3 : 7, and 1 : 1) as the eluent. Earlier fractions eluted with CH_2Cl_2–hexane (1 : 1) yielded a crystalline material, which was recrystallized from acetone to give substance MA (0.34 g).[2] Subsequent fractions eluted with the same solvent gave substance MB (0.8 g) after recrystallization from acetone, and the final eluate with CH_2Cl_2–hexane (1 : 1) was a mixture (0.7 g), which was re-chromatographed on silica gel (120 g) with CH_2Cl_2–hexane (1 : 1) to give an additional crop of substance MB (0.1 g) and a sterol mixture (0.15 g).[2]

Isolation and Properties of Triterpenes from Substance MA ——Substance MA (195 mg) was treated with acetic anhydride (1 ml) and pyridine (1 ml) overnight at room temperature. Usual work-up and recrystallization from ether–MeOH yielded an acetate mixture (200 mg), which was chromatographed on 20% $AgNO_3$–silica gel (210 g) with benzene–hexane (1 : 9). The first eluate was a mixture of A_{1b} acetate (**6b**) and A_3 acetate (**7b**) (approximate ratio 9 : 1, 45 mg), whose identities were confirmed by GC and GC-MS comparisons with authentic dihydrocycloeucalenol acetate (**6b**: C-24 epimeric mixture) and dihydrocyclonervilol acetate (**7b**), respectively. The second eluate, upon recrystallization from MeOH, gave cyclonervilol acetate (**2b**) (10 mg), colorless needles, mp 140—143 °C, $[\alpha]_D$ +41° (c = 1.0). MS m/z: 482 (M^+), 467, 422 (**a'**), 407, 367 (**d'**), 343 (M^+ − side chain), 314 (**e'**), 283 (**c**, base peak), and 175 (**f**). High-resolution MS: Found 482.4173, Calcd for $C_{33}H_{54}O_2$ (M^+) 482.4121. IR ν cm^{-1}: 1740, 1250, and 975 (*trans* CH=CH). ^{1}H-NMR δ: 0.15, 0.40 (each 1H, d, J = 4 Hz, 19-CH_2), 0.82 (3H, t, J = 7 Hz, 29-CH_3), 0.80, 0.85, 0.855, 0.98 (each 3H, d, J = 6 Hz; 27-, 30-, 26-, and 21-CH_3, respectively), 0.91, 1.00 (each 3H, s; 32-, 18-CH_3), 2.05 (3H, s, OAc), 4.52 (1H, dt, J = 4.5, 10 Hz, C<u>H</u>–OAc), 5.05, and 5.17 (each 1H, dd, J = 8, 15 Hz, C<u>H</u>=C<u>H</u>). The third eluate, upon recrystallization from MeOH, gave cyclohomonervilol acetate (**10b**) (34 mg), colorless needles, mp 149—151 °C, $[\alpha]_D$ +37.3° (c = 0.48). MS m/z: 496 (M^+), 481, 436 (**a'**, base peak), 421, 381 (**d'**), 343 (M^+ − side chain), 328 (**e'**), 283 (**c**), and 175 (**f**). High-resolution MS: Found 496.4272, Calcd for $C_{34}H_{56}O_2$ (M^+) 496.4277. IR ν cm^{-1}: 1740, 1250; 1645 and 890 (C=CH_2). ^{1}H-NMR δ: 0.14, 0.40 (each 1H, d, J = 4 Hz, 19-CH_2), 0.81, 0.85, 0.88, 0.92 (each 3H, d, J = 6 Hz; 27-, 30-, 21-, and 26-CH_3, respectively), 0.90, 0.96 (each 3H, s; 32- and 18-CH_3, respectively), 1.57 (3H, br s, 29-CH_3), 2.06 (3H, s, OAc), 4.53 (1H, dt, J = 4.5, 10 Hz, C<u>H</u>–OAc), 4.63, and 4.76 (each 1H, m, C=CH_2). The fourth eluate, upon recrystallization from MeOH, gave cyclofuntumienol acetate (**3b**) (7 mg), mp 100—102 °C. MS m/z: 482 (M^+), 467, 422 (base peak), 407, 367, 343, 324, 314, 283, and 175. High-resolution MS: Found 482.4107, Calcd for $C_{33}H_{54}O_2$ (M^+) 482.4121. ^{1}H-NMR δ: 0.16, 0.41 (each 1H, d, J = 4 Hz, 19-CH_2), 0.84, 0.88 (each 3H, d, J = 6.4 Hz; 30- and 21-CH_3, respectively), 0.99 (6H, d, J = 6.4 Hz, 26- and 27-CH_3), 0.91, 0.97 (each 3H, s, 32- and 18-CH_3, respectively), 1.59 (3H, d, J = 7 Hz, 29-CH_3), 2.85 (1H, septet, J = 7 Hz, C<u>H</u>(CH_3)$_2$), 4.53 (1H, dt, J = 5, 10 Hz, C<u>H</u>–OAc), and 5.14 (1H, q, J = 7 Hz, C=C<u>H</u>–CH_3). The final eluate gave, after recrystallization from MeOH, cycloeucalenol acetate (**1b**) (20 mg), mp 103—105 °C, $[\alpha]_D$ +63.3° (c = 0.5), which was identified by IR and ^{1}H-NMR, and GC-MS comparisons with an authentic sample.

Cyclonervilol (2a) ——Cyclonervilol acetate (**2b**) (7.5 mg) was refluxed with 5% KOH–MeOH (1.5 ml) for 1 h and the reaction mixture was worked up as usual. Recrystallization of the product from MeOH gave cyclonervilol (**2a**) (5.2 mg), colorless needles, mp 166—169 °C, $[\alpha]_D$ +37.9° (c = 0.52). MS: see Chart 3. High-resolution MS: Found 440.4023, Calcd for $C_{31}H_{52}O$ (M^+) 440.4018. ^{1}H-NMR: see Table I.

Cyclohomonervilol (10a) ——The acetate **10b** (10 mg) was hydrolyzed in the same manner as above and the product was recrystallized from MeOH to give cyclohomonervilol (**10a**) (7 mg), colorless needles, mp 166—167 °C, $[\alpha]_D$ +40.5° (c = 0.6). MS m/z: 454 (M^+), 439 (M^+ − 15), 436 (**a'**), 421, 381 (**d'**), 356, 328 (**e'**), 313, 301 (**b'**), 283 (**c**), and 175 (**f**). High-resolution MS: Found 454.4199, Calcd for $C_{32}H_{54}O$ (M^+) 454.4174. ^{1}H-NMR: see Table I. IR

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νcm^{-1}: 3500 and 890.

Separation of the Mixture of A_{1b} and A_3 by HPLC——1) Conversion of the Acetate Mixture (**6b** and **7b**) to the Benzoate Mixture (**6c** and **7c**): The acetate mixture (30 mg) was hydrolyzed with 2% KOH–MeOH (1.5 ml) in the usual manner to give the alcohol mixture (**6a** and **7a**) (28 mg), which was treated with benzoyl chloride (0.005 ml) in pyridine (0.6 ml) under stirring for 16 h at room temperature. Usual work-up yielded a crystalline product, which was purified by preparative TLC to give the benzoate mixture (**6c** and **7c**) (28 mg).

2) Separation of the Benzoate Mixture by HPLC: The benzoate mixture (10 mg) was subjected repeatedly to preparative HPLC on a TSK-GEL ODS-120A column with $CHCl_3$–CH_3CN (2:8) at 26 °C and hexane–$CHCl_3$–CH_3CN (1:1:8) at 20 °C as the eluting solvents to give 24(R/α)-dihydrocycloeucalenol benzoate (peak 1, **4c**) (2.4 mg), mp 129—131 °C, MS *m/z*: 532 (M^+, $C_{37}H_{56}O_2$); and 24(S/β)-dihydrocycloeucalenol benzoate (peak 2, **5c**) (3.5 mg), mp 134—135 °C, MS *m/z*: 532 (M^+, $C_{37}H_{56}O_2$); and dihydrocyclonervilol benzoate (peak 3, **7c**) (0.7 mg), mp 148—149 °C, MS *m/z*: 546 (M^+, $C_{38}H_{58}O_2$). The identities of these compounds were confirmed by MS, ^{1}H-NMR, and HPLC comparisons with the corresponding authentic samples.

Catalytic Hydrogenation of Cycloeucalenol and Separation of the C-24 Epimers of Dihydrocycloeucalenol——1) Hydrogenation of Cycloeucalenol (**1a**) with Adams Catalyst: Cycloeucalenol (**1a**) (6 mg), mp 137—139 °C, $[\alpha]_D$ +39.3° (c=0.5), was hydrogenated on PtO_2 (1 mg) in AcOEt (1 ml) and MeOH (3 ml) at room temperature for 48 h. After removal of the catalyst by filtration, the filtrate was concentrated under reduced pressure and the residue was chromatographed on silica gel. Elution with MeOH–CH_2Cl_2 (5:95) gave a mixture of dihydro compounds (**6a**) (6 mg). MS *m/z*: 428 (M^+), 413, 410, 395, 355, 302, 283, and 175.

2) Hydrogenation of Cycloeucalenol Acetate (**1b**): The acetate (**1b**) (2 mg) was hydrogenated on PtO_2 in MeOH (1 ml) for 36 h. Usual work-up and recrystallization from ether–MeOH gave the acetate mixture (**6b**) (1.2 mg), mp 98—100 °C. MS *m/z*: 470 (M^+), 455, 410 (**a**), 395, 355 (**d**), 343 (**b**), 302 (**e**), 283 (**c**), and 175 (**f**).

3) Benzoylation of **6a** and Separation of the C-24 Epimers (**4c** and **5c**) by HPLC: A sample of **6a** (6 mg) was treated with benzoyl chloride (0.04 ml) in pyridine (0.6 ml) for 16 h at room temperature, and the reaction mixture was diluted with water and extracted with CH_2Cl_2. The extract was washed successively with 5% HCl and dil. Na_2CO_3, then dried, and concentrated. The residue (6.3 mg) was purified by preparative TLC to give dihydrocycloeucalenol benzoate (**6c**) (5.8 mg). MS *m/z*: 532 (M^+). This product was subjected to preparative HPLC on TSK-GEL ODS-120A with $CHCl_3$–CH_3CN (2:8) as the eluent. The more mobile fraction afforded 24(R/α)-dihydrocycloeucalenol benzoate (**4c**) (3.0 mg), small needles (from ether–MeOH), mp 130—132 °C. MS *m/z*: 532 (M^+), 517, 410, 395, 355, 302, 283, 175, and 105. High-resolution MS: Found 532.4288, Calcd for $C_{37}H_{56}O_2$ (M^+) 532.4277. ^{1}H-NMR: see Table I. The less mobile fraction gave 24(S/β)-dihydrocycloeucalenol benzoate (**5c**) (2.2 mg), small needles (from ether–MeOH), mp 133—135 °C. MS: identical with that of **4c**. High-resolution MS: Found 532.4272, Calcd for $C_{37}H_{56}O_2$ (M^+) 532.4277. ^{1}H-NMR: see Table I.

4) 24(R/α)-Dihydrocycloeucalenol (**4a**): The benzoate **4c** (1.2 mg) was heated with 2% KOH–MeOH (0.3 ml) under stirring for 2 h. After evaporation of the solvent, the product was taken up in CH_2Cl_2 and the solution concentrated. The residue was further purified by preparative TLC with $CHCl_3$ as the eluent and then crystallized from MeOH to afford colorless needles (**4a**) (0.8 mg), mp 141—142 °C. MS *m/z*: 428 (M^+), 413, 410 ($M^+ - H_2O$), 395, 355, 302, 301 (M^+ − side chain), 283, and 175. ^{1}H-NMR: see Table I.

5) 24(S/β)-Dihydrocycloeucalenol (**5a**): The benzoate **5c** (0.6 mg) was treated with 2% KOH–MeOH (0.2 ml) in the same manner as above. After purification by preparative TLC and crystallization of the product from MeOH, it gave colorless needles (**5a**) (0.3 mg), mp 152—153 °C. MS: identical with that of **4a**. ^{1}H-NMR: see Table I.

Osmium Tetroxide Oxidation of Cyclonervilol Acetate (2b)——Osmium tetroxide (20 mg) was added to a solution of **2b** (7 mg) in pyridine (1 ml) and the mixture was allowed to stand overnight at room temperature. Thereafter, a solution of sodium bisulfite (1.5 g) in water (2 ml) was added to the reaction mixture and the whole was stirred for 30 min. The mixture was then poured into ice-water, basified with Na_2CO_3, and extracted with CH_2Cl_2. The extract was washed successively with 2% HCl and dil. Na_2CO_3, dried, and concentrated. Silica gel column chromatography of the residue with MeOH–$CHCl_3$ (5:95) gave a crystalline mass, which was recrystallized from ether to afford colorless needles (**11**) (6 mg), mp 186—188.5 °C. MS *m/z*: 516 (M^+, $C_{33}H_{56}O_4$), 456, 441, 423, 401, 383, 343, 283, and 175. ^{1}H-NMR δ: 0.14, 0.40 (each 1H, d, J=4 Hz, 19-CH_2), 0.8—1.0 (CH_3 × 7), 2.03 (3H, s, OAc), 3.65 (2H, m, C<u>H</u>–OH × 2), and 4.50 (1H, m, C<u>H</u>–OAc).

Lead Tetraacetate Oxidation of the Diol (11)——Lead tetraacetate (15 mg) was added to a stirred solution of **11** (2.5 mg) in dry benzene (0.8 ml) and stirring continued for 12 h at room temperature. The reaction mixture was diluted with water, and extracted with ether. The extract was dried and concentrated. The residue (1.5 mg) was chromatographed on silica gel with ether–pentane (1:9) as the eluting solvent. The first eluate gave a fatty acid (**13**), which was identified as 2-ethylisovaleric acid (**13**) by GC and GC-MS comparisons with a sample prepared from i-stigmasteryl methyl ether (**15**). Subsequent elution with ether–pentane (2:8) afforded a crystalline substance, which was recrystallized from MeOH to give a carboxylic acid (**12a**) (1.2 mg), mp 228—230 °C. MS *m/z*: 416 (M^+, $C_{26}H_{40}O_4$), 401, 356 ($M^+ - 60$), 341, 301, 283 (**c**), 248, and 175 (**f**). This product was dissolved in ether and treated with excess diazomethane at room temperature. Evaporation of the solvent and recrystallization of the residue from MeOH gave the methyl ester (**12b**) (1 mg), mp 160—169 °C. MS *m/z*: 430 (M^+), 415, 370 ($M^+ - 60$), 355, 315, 283,

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and 175. High-resolution MS: Found 430.3087, Calcd for $C_{27}H_{42}O_4$ (M^+) 430.3083. ^{1}H-NMR δ: 0.15, 0.41 (each 1H, d, J=4 Hz, cyclopropyl CH_2), 0.84 (3H, d, J=6 Hz, *sec*-CH_3), 0.93, 0.99 (each 3H, s, *tert*-CH_3), 1.15 (3H, d, J=6.6 Hz, *sec*-CH_3), 2.05 (3H, s, OAc), 3.65 (3H, s, $COOCH_3$), and 4.52 (1H, m, C<u>H</u>–OAc).

Ozonolysis of i-Stigmasteryl Methyl Ether (15)——Ozone was passed into a solution of **15** (3.0 g) in CH_2Cl_2 (36 ml) and pyridine (0.7 ml) at −40 °C for 6.5 h. Then glacial acetic acid (3.9 ml) and zinc powder (1.86 g) were added, and the mixture was vigorously stirred for 2 h under argon gas at room temperature. The reaction mixture, after removal of the zinc powder by filtration, was steam-distilled and the distillate (300 ml) was saturated with NaCl and extracted with CH_2Cl_2. The CH_2Cl_2 solution was washed successively with 5% HCl, water, and dil. $NaHCO_3$ and then dried and concentrated to leave an oily residue (250 mg), which showed ^{1}H-NMR signals at δ 9.60 (CHO) and 9.86 (COOH) (relative intensity 3 : 1).[12] After standing at room temperature overnight, this residue showed no aldehyde proton signal, but only the carboxyl proton signal in the ^{1}H-NMR spectrum. Distillation of this residue gave 2-ethylisovaleric acid (**13**) (200 mg), bp_{26} 205 °C (bath temperature). MS *m/z*: 131 (M^++1), 115 ($M^+ - CH_3$), 113, 102, 101 ($M^+ - C_2H_5$), 87 ($M^+ - C_3H_7$), and 85 (M^+ −COOH). IR ν cm^{-1} ($CHCl_3$): 3000—2500, 1700 (COOH). ^{1}H-NMR (60 MHz) δ: 0.88—1.00 ($CH_3 \times 3$) and 9.86 (1H, br s, COOH). ^{1}H-NMR (200 MHz) δ: 0.94 (3H, t, J=7.2 Hz, CH_2–C<u>H</u>$_3$), 0.97 (6H, d, J=7.0 Hz, isopropyl), 1.66 (2H, quintet, J=7.2 Hz, CH–C<u>H</u>$_2$–CH_3), 1.90 (1H, octet, J=7.0 Hz, C<u>H</u>$(CH_3)_2$), and 2.09 (1H, q, J=7.2 Hz, C<u>H</u>–COOH).

Catalytic Hydrogenation of Cyclonervilol Derivatives——1) Dihydrocyclonervilol Acetate (**7b**): The acetate **2b** (1.5 mg) was hydrogenated on PtO_2 (1 mg) in MeOH (1.5 ml) for 5 d. After removal of the catalyst, the solution was concentrated and the residue was chromatographed on 20% $AgNO_3$–silica gel (4 g). Elution of the column with benzene–hexane (1 : 19) gave dihydrocyclonervilol acetate (**7b**) (0.4 mg). MS *m/z*: 484 (M^+), 469, 424 (**a**), 409, 369 (**d**), 343 (**b**), 316 (**e**), 301, 283 (**c**), and 175 (**f**). Further elution with the same solvent afforded the starting material (**2b**) (0.7 mg).

2) Dihydrocyclonervilol Benzoate (**7c**): Cyclonervilol (**2a**) (2 mg) was treated with benzoyl chloride (0.03 ml) in pyridine (0.4 ml) for 16 h at room temperature. Usual work-up and purification by preparative TLC gave the benzoate **2c** (2 mg), which was hydrogenated on PtO_2 in AcOEt (2 ml) and MeOH (3 ml) for 48 h at room temperature. The catalyst was filtered off and the filtrate was concentrated *in vacuo*. The residue was purified by preparative TLC and then recrystallized from MeOH to give the dihydro compound (**7c**) (1.5 mg), needles, mp 150—151 °C. This compound was proved to be identical with 24(R/α)-dihydrocyclofuntumienol benzoate (**7c**) by MS, ^{1}H-NMR, and HPLC comparisons.

Preparation and Separation of the C-24 Epimers of Dihydrocyclofuntumienol——1) Medium-Pressure Catalytic Hydrogenation of Cyclofuntumienol Acetate (**3b**): The acetate **3b** (3 mg) was hydrogenated on PtO_2 (1 mg) in AcOEt (1 ml) and MeOH (3 ml) at 3 atm and room temperature for 17 h. The reaction mixture was worked up as usual and the product was purified by preparative TLC and then recrystallized from MeOH to give the dihydro compound (**9b**) (2 mg). MS: identical with that of **7b**. This product was hydrolyzed with 2% KOH–MeOH (1 ml) as usual, giving the corresponding alcohol mixture (**9a**) (1.5 mg) after purification by preparative TLC.

2) Benzoylation and Separation of the C-24 Epimers (**7c** and **8c**) by HPLC: The above **9a** (1.5 mg) was allowed to react with benzoyl chloride (0.03 ml) in pyridine (0.4 ml) for 16 h at room temperature. Usual work-up gave the benzoate mixture (**9c**) (1 mg). MS *m/z*: 546 (M^+, $C_{38}H_{58}O_2$), which was subjected to preparative HPLC under the same conditions as used for **6c**. The more mobile fraction gave 24(R/α)-dihydrocyclofuntumienol benzoate (**7c**) (0.5 mg), needles (from MeOH), mp 148—149.5 °C. MS *m/z*: 546 (M^+), 531, 424 (**a**), 409, 369 (**d**), 316 (**e**), 283 (**c**), 175 (**f**), and 105. ^{1}H-NMR: see Table I. The less mobile fraction afforded the 24(S/β)-epimer (**8c**) (0.3 mg), needles (from MeOH), mp 149—151 °C. MS: identical with that of **7c**. ^{1}H-NMR: see Table I.

3) 24(R/α)-Dihydrocyclofuntumienol (**7a**): The above benzoate **7c** (0.5 mg) was heated with 2% KOH–MeOH (0.3 ml) under stirring for 2 h. After evaporation of the solvent, the product was taken up in CH_2Cl_2 and the solution was concentrated. The residue was further purified by preparative TLC (developed with $CHCl_3$) and the product was recrystallized from MeOH to afford colorless needles (**7a**) (0.3 mg), mp 154—156 °C. MS *m/z*: 442 (M^+, $C_{31}H_{54}O$), 427, 424 (**a**), 409, 369 (**d**), 316 (**e**), 301 (M^+ −side chain), 283 (**c**), and 175 (**f**). ^{1}H-NMR: see Table I.

4) 24(S/β)-Dihydrocyclofuntumienol (**8a**): The benzoate **8c** (0.3 mg) was treated with 2% KOH–MeOH (0.2 ml) in the same manner as above. After purification by preparative TLC, the product was recrystallized from MeOH to give the alcohol **8a** (0.1 mg), colorless needles, mp 151—153 °C. MS: identical with that of **7a**. ^{1}H-NMR: see Table I.

Osmium Tetroxide Oxidation of Cyclohomonervilol Acetate (10b) Followed by Periodate Oxidation——Osmium tetroxide (15 mg) was added to a solution of **10b** (10 mg) in pyridine (1 ml). The mixture was allowed to stand overnight at room temperature, then a solution of sodium bisulfite (2 g) in water (1.3 ml) was added and the whole was stirred for 30 min, poured into ice water, basified with Na_2CO_3, and extracted with CH_2Cl_2. The extract was washed successively with 2% HCl and dil. Na_2CO_3, dried, and concentrated *in vacuo*. Silica gel column chromatography of the residue with MeOH–CH_2Cl_2 (5 : 95) gave a crystalline substance (11 mg), which was recrystallized from pentane to give colorless needles (**16**) (9 mg), mp 155—156.5 °C. MS *m/z*: 530 (M^+, $C_{34}H_{58}O_4$), 512, 497, 470 (M^+ −AcOH), 452, 437, 362, 344, 343 (**b**), 283 (**c**), and 175 (**f**). IR ν cm^{-1}: 3440 (OH), 1735 and 1250 (OAc).

The above diol (**16**) (9 mg) was dissolved in dioxane (0.5 ml) and then a solution of HIO_4 (5 mg) in H_2O (0.05 ml) was added under vigorous stirring. After the stirring had been continued for 10 min, the reaction mixture was

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extracted with ether and the ether extract was washed with brine, dried, and concentrated. The residue was chromatographed over silica gel (1 g) and the material (6 mg) eluted with CH_2Cl_2 was recrystallized from MeOH to give colorless needles (**17**) (5 mg), mp 120—123 °C. MS *m/z*: 498 (M^+), 483, 455 ($M^+ - 43$), 438 ($M^+ - AcOH$), 423, 383 (**d'**, R = $C_{10}H_{19}O$), 283 (**c**), and 175 (**f**). High-resolution MS: Found 498.4068, Calcd for $C_{33}H_{54}O_3$ (M^+) 498.4073. IR ν cm^{-1}: 1735, 1705, and 1250. ^{1}H-NMR δ: 0.14, 0.41 (each 1H, d, J=4 Hz, 19-CH_2), 0.77—0.96 ($CH_3 \times 6$), 2.01, 2.07 (each 3H, s, OAc and $COCH_3$), and 4.52 (1H, m, C<u>H</u>–OAc).

Methyl Ketone-24,29,29,29-d_4 (18)——To a solution of the methyl ketone **17** (3 mg) in MeOD (0.8 ml) was added 7.5 N NaOD–D_2O solution (0.25 ml) and the mixture was gently refluxed for 5 h. After concentration *in vacuo*, the mixture was diluted with D_2O and extracted with CH_2Cl_2. Drying and concentration of the extract gave a crystalline residue (3 mg), which was purified by silica gel column chromatography to give the methyl ketone-24,29,29,29-d_4 (**18**). Recrystallization from MeOH afforded a pure sample (2 mg), mp 137—138 °C. MS *m/z*: 460 (M^+, $C_{31}H_{48}D_4O_2$), 445, 427, 387 (**d'**, R = $C_{10}H_{15}D_4O$), 334 (**e'**, R = $C_{10}H_{15}D_4O$), 319, 301 (**b'**), 283 (**c**), and 175 (**f**).

Haloform Reaction of the Methyl Ketone (17)——A chilled solution of NaOBr (NaOH 430 mg and Br_2 0.14 ml) in H_2O (3.6 ml) and dioxane (2 ml) was added to an ice-cooled solution of the methyl ketone **17** (28 mg) in dioxane (2.8 ml) and the mixture was stirred at 8 °C for 3 h. Then, 10% Na_2SO_3–H_2O (2 ml) was added and the mixture was refluxed in an oil bath for 15 min. After cooling, the reaction mixture was diluted with H_2O and extracted with CH_2Cl_2. The CH_2Cl_2 solution was extracted with 5% aq. NaOH and the aqueous layer was acidified with HCl, then extracted again with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with brine, dried, and concentrated to afford a crystalline product, which was chromatographed on silica gel with acetone–CH_2Cl_2 (5 : 95) as the eluent and then recrystallized from MeOH to give the carboxylic acid (**19a**) as colorless needles (20 mg), mp 190—191.5 °C. IR ν cm^{-1}: 3430, 3000—2600, and 1700. High-resolution MS: Found 458.3814, Calcd for $C_{30}H_{50}O_3$ (M^+) 458.3760. This product was dissolved in ether and treated with excess CH_2N_2 at room temperature. Removal of the solvent and recrystallization of the residue from pentane afforded the methyl ester (**19b**) (20 mg), mp 125—126 °C. MS *m/z*: 472 (M^+), 454, 439, 399, 346, 301, 283, and 175. High-resolution MS: Found 472.3891, Calcd for $C_{31}H_{52}O_3$ (M^+) 472.3916. IR ν cm^{-1}: 3440 and 1735. ^{1}H-NMR δ: 0.14, 0.42 (each 1H, d, J=4 Hz, 19-CH_2), 0.83—1.03 ($CH_3 \times 6$), 3.20 (1H, m, C<u>H</u>–OH), and 3.63 (3H, s, $COOCH_3$).

Conversion of the Methyl Ester (19b) to Cycloeucalenol (1a)——Dihydropyran (0.1 ml) was added to a solution of the methyl ester **19b** (20 mg) in $CHCl_3$ (pre-dried with phosphorus pentoxide) (1.5 ml) and the mixture was stirred for 30 min at room temperature. The mixture was then made alkaline by the addition of aq. Na_2CO_3 and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with brine, dried, and concentrated *in vacuo* to afford a crystalline residue, which was purified by silica gel column chromatography with CH_2Cl_2 as the solvent, giving the pyranyl ether (**19c**) (20 mg), mp 159—164 °C. IR ν cm^{-1}: 1735, 1200—1050. This was treated with excess $LiAlH_4$ (30 mg) in boiling ether (2 ml) for 5 h and the reaction mixture was worked up in the usual way to afford an oily residue (20 mg), which was purified by chromatography on silica gel with CH_2Cl_2 as the solvent to give the corresponding alcohol (**20a**) (14 mg), oil. IR ν cm^{-1}: 3600. This alcohol (**20a**) was dissolved in pyridine (0.5 ml), then methanesulfonyl chloride (0.5 ml) was added, and the mixture was allowed to stand at room temperature for 30 min. The reaction mixture was diluted with ice-water and extracted with ether. The ether solution was washed successively with 3% HCl and dil. Na_2CO_3, and dried. Evaporation of the solvent *in vacuo* gave an oily residue (19 mg), which was chromatographed on silica gel. Elution with CH_2Cl_2 gave the mesylate (**20b**) (12 mg), oil. IR ν cm^{-1}: 1360, 1330, 1175 (OSO_2CH_3), and 1200—1030 (acetal). ^{1}H-NMR δ: 0.14, 0.40 (each 1H, d, J=4 Hz, 19-CH_2), 2.97 (3H, s, S–CH_3), and 4.13 (2H, br d, J=5.6 Hz, C<u>H</u>$_2$–OMs).

The above mesylate (**20b**) (12 mg) was dissolved in dimethylsulfoxide (DMSO, 1 ml) and then anhydrous $NaHCO_3$ (10 mg) was added. The mixture was heated at 178 °C for 6 h in an oil bath, and then poured into ice-water, and the whole was extracted with ether. The ether solution was washed with water, dried, and concentrated *in vacuo* to afford an oily substance (7 mg), which was purified by silica gel column chromatography. Recrystallization of the product obtained from the CH_2Cl_2 eluate (2 mg) from MeOH gave colorless needles (**1a**) (1.5 mg), mp 120—123 °C. MS *m/z*: 426 (M^+). IR ν cm^{-1}: 3350 (OH), 1640 and 890 (C=CH_2). This product was identified as cycloeucalenol (**1a**) by GC, IR (KBr), MS, and ^{1}H-NMR comparisons with an authentic sample.

Wolff–Kishner Reduction of the Methyl Ketone (17)——A mixture of the methyl ketone **17** (17 mg), anhydrous hydrazine (18 mg), triethylene glycol (1 ml), and Na (7 mg) was heated at 185 °C for 7 h in an oil bath. Then the temperature of the oil bath was elevated to 225—230 °C and maintained at this temperature for 2 h. After further heating at 185 °C for 8 h, the mixture was diluted with water and extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with water, dried, and concentrated. Silica gel column chromatography of the residue using CH_2Cl_2 as the eluent afforded a crystalline substance (3 mg).

A half of the above product was treated with Ac_2O (0.5 ml) in pyridine (0.5 ml). Usual work-up of the reaction mixture and recrystallization from MeOH gave an acetate (1.2 mg), which showed two peaks on GC. The major component (*ca.* 70%) of this acetate was proved to be **9b** by GC and GC-MS analyses.

Next, another portion of the Wolff–Kishner product (0.8 mg) was allowed to react with benzoyl chloride (0.02 ml) in pyridine (0.2 ml) in the usual way. The resulting benzoate (0.6 mg) was subjected to preparative HPLC on a TSK-GEL ODS-120A column with $CHCl_3$–CH_3CN (2 : 8) as the eluent to give two components (**7c** and **8c**, *ca.*

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0.1 mg each), which were identified as 24(R/α)-dihydrocyclofuntumienol benzoate (**7c**) and the 24(S/β)-epimer (**8c**), respectively, by MS and HPLC comparisons.

Acknowledgment The authors are grateful to Dr. S. Matsunaga of Osaka College of Pharmacy for a gift of cycloeucalenol, to Dr. T. Shingu and Dr. T. Yokoi of Kobe Gakuin University for performing the NMR pseudo-contact shift experiments, to Dr. K. Matsushita of JEOL Ltd. and Mr. M. Morikoshi of this University for the ^{13}C-NMR measurements, and to Dr. M. Imachi of Bruker Japan Ltd. for the measurements of two-dimensional NMR spectra.

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