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Studies on the Constituents of Orchidaceous Plants. IV.[1] Proton and Carbon-13 Signal Assignments of Cycloeucalenol-Type Triterpenes from *Nervilia purpurea* SCHLECHTER by Two-Dimensional Nuclear Magnetic Resonance Spectroscopy

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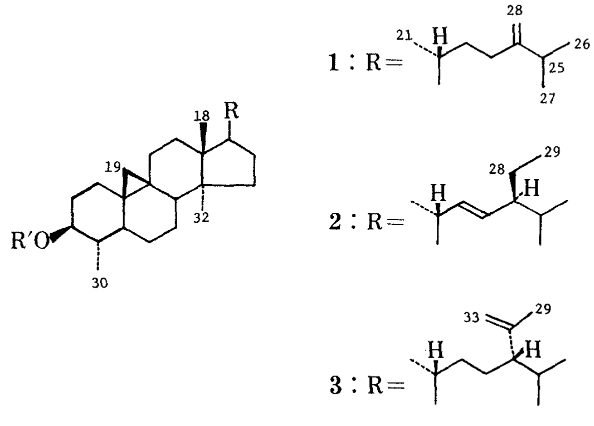
The proton and carbon-13 nuclear magnetic resonance (NMR) signals of cycloeucalenol (**1a**), cycloeucalenol acetate (**1b**), cyclonervilol (**2a**), cyclonervilol acetate (**2b**), and cyclohomonervilol acetate (**3b**), isolated from *Nervilia purpurea*, were assigned by means of two-dimensional NMR spectroscopy.

Keywords——*Nervilia purpurea*; triterpene; cycloeucalenol; cyclonervilol; cyclohomonervilol; ^{1}H-NMR; ^{13}C-NMR; 2-D NMR; ^{1}H–^{1}H shift-correlated NMR; ^{1}H–^{13}C shift-correlated NMR

The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of cycloeucalenol (**1a**) and cycloeucalenol acetate (**1b**) have already been investigated by Lukacs and co-workers,[2] who assigned all the ^{13}C-signals of these compounds. Later, Dahmen and Leander[3] pointed out that Lukacs' assignments of the C-7 and C-16 signals of cycloeucalenone should probably be reversed. In a previous paper,[4] we also reported the assignments of ^{13}C-signals of cycloeucalenol acetate (**1b**), cyclonervilol acetate (**2b**), and cyclohomonervilol acetate (**3b**),[5] isolated from *Nervilia purpurea* (Orchidaceae), based mainly on a comparison with those of **1b** reported by Lukacs *et al.*[2]

Recently we re-examined the ^{1}H- and ^{13}C-NMR signal assignments of cycloeucalenol (**1a**), cycloeucalenol acetate (**1b**), cyclonervilol (**2a**), cyclonervilol acetate (**2b**), and cyclohomonervilol acetate (**3b**) by application of two-dimensional (2-D) NMR spectroscopy,[6] and the results are reported in the present paper.



a: R′=H, **b**: R′=COCH$_3$

Chart 1

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

Compd. Proton	**1a**	**1b**	**2a**	**2b**	**3b**
1α-H	1.550 td (12.5, 3)	1.593 td (12, 4)	1.555 td (12, 3)	1.592 td (13, 4.5)	1.591 td (12.5, 4.5)
1β-H	1.283 dt (12.5, 3.5)	1.278 dt (11.5, 4)	1.285 dt (12, 4)	1.280 dt (13, 3.5)	1.281 dt (13, 4)
2α-H	1.983 ddt (11, 5, 3)	2.004 ddt (11.5, 5, 3)	1.985 ddt (11.5, 5, 3)	2.005 ddt (12, 5, 3)	2.003 ddt (11.5, 5, 3)
2β-H	1.423 dtd (13, 11, 3.5)	1.437 dtd (12.5, 11.5, 4)	1.424 dtd (12.5, 11, 3.5)	1.436 dtd (13, 11, 4)	1.435 dtd (13, 11.5, 4)
3-H	3.213 ddd (10.5, 9, 4.5)	4.507 td (10.5, 4.8)	3.215 ddd (11, 8.8, 5)	4.508 td (10.5, 5)	4.507 td (10.5, 5)
4-H	1.16 m	1.40 m	1.15 m	1.40 m	1.39 m
5-H	1.206 td (10.5, 4.5)	1.279 td (12, 4)	1.205 td (11.5, 4)	1.275 td (11.5, 5)	1.275 ddd (12.5, 11, 5)
6α-H	1.678 dtd (12.5, 4.5, 3.5)	1.683 dtd (12.5, 4.5, 3.5)	1.675 dtd (12.5, 4.5, 3.5)	1.683 dtd (13, 4.5, 3.5)	1.680 dtd (12.5, 4.5, 3.5)
6β-H	0.585 qd (12.5, 3)	0.595 qd (12.5, 3)	0.586 qd (12.5, 3)	0.599 qd (12.5, 3)	0.793 qd (12.5, 3)
7α-H	1.055 qd (12.5, 3.5)	1.071 qd (12.5, 3.5)	1.058 qd (12, 3.5)	1.068 qd (12.5, 3.5)	1.066 qd (12, 3)
7β-H	1.309 dtd (13, 5, 3.5)	1.313 dtd (13, 5, 3)	1.310 dtd (12, 5, 3)	1.310 dq (12.5, 4.5)	1.310 dtd (13, 5, 3.5)
8-H	1.583 dd (12, 5)	1.588 dd (12, 5.5)	1.585 dd (12, 5.5)	1.587 dd (12, 5.5)	1.575 dd (12, 5)
11-H	1.204 ddd (15, 8, 6.5)	1.197 ddd (15, 7, 6.3)	1.208 ddd (16, 9, 6)	1.198 ddd (15, 9, 5)	1.188 dt (15, 7)
11-H	1.969 ddd (15, 9, 7)	1.976 ddd (15, 9, 6.5)	1.978 ddd (15.5, 7.5, 6)	1.980 ddd (15, 10, 7)	1.966 dt (15, 8)
12-H	1.624 dd-like (9, 7)	1.623 t-like (8)	*ca.* 1.62 t-like (7)	*ca.* 1.62 m	1.606 t-like (8)
12-H			*ca.* 1.64 dd-like (9, 7)		
15-H	*ca.* 1.30 m	*ca.* 1.30 m	*ca.* 1.27 m	*ca.* 1.27 m	*ca.* 1.28 m
15-H					

16-H	*ca.* 1.30 m	*ca.* 1.30 m	*ca.* 1.27 m	*ca.* 1.27 m	*ca.* 1.25 m
16-H	*ca.* 1.92 m	*ca.* 1.92 m	1.755 dtd-like	*ca.* 1.75 m	1.880 m
17-H	*ca.* 1.62 m	*ca.* 1.62 m	*ca.* 1.63 m	*ca.* 1.63 m	*ca.* 1.59 m
20-H	1.40 m	1.41 m	2.043 ddq (10, 8.5, 7)	2.046 m	*ca.* 1.31 m
22-H	1.139 dddd (13, 11.5, 9, 5)	1.142 dddd (13, 11, 9, 5)	5.158 dd (15, 8.5)	5.158 dd (15, 8.5)	0.807 dddd (13, 11, 9, 5)
22-H	1.577 dddd (13, 11.5, 6, 3.5)	1.577 dddd (13, 11, 6, 3)			1.375 ddt (13, 11, 4)
23-H	1.890 ddd (14.5, 11.5, 5.5)	1.887 ddd (15, 11, 5.5)	5.035 dd (15, 8.8)	5.033 dd (15, 9)	1.020 dtd (13, 11, 5)
23-H	2.124 ddd (15, 11.5, 5)	2.126 ddd (15, 11, 4.5)			1.615 dddd (13, 11, 5.5, 4)
24-H	—	—	*ca.* 1.54 m	*ca.* 1.54 m	1.526 m
25-H	2.233 septet (7)	2.351 septet (7)	1.53 m	1.53 m	*ca.* 1.50 m
18-H	0.970 s	0.970 s	0.989 s	0.989 s	0.955 s
19α-H	0.143 d (4)	0.151 d (4)	0.145 d (4)	0.149 d (4)	0.143 d (4)
19β-H	0.387 br d (4)	0.401 br d (4)	0.394 br d (4)	0.407 br d (4)	0.397 br d (4)
21-H	0.898 d (6.5)	0.897 d (6)	0.970 d (6.5)	0.971 d (6.5)	0.872 d (6.5)
26-H[a]	1.026 d (7)	1.026 d (6.8)	0.800 d (6.5)	0.807 d (7)	0.807 d (6)
27-H[a]	1.031 d (6.7)	1.039 d (6.8)	0.850 d (6.5)	0.856 d (6.5)	0.916 d (6)
28-H	4.662 br d (1)	4.661 d (1.5)	1.170 ddq (14, 9, 6.5)	1.172 ddq (13, 9, 7)	—
28-H	4.714 br s	4.714 br s	1.423 dqd (13, 7, 6)	1.425 dqd (13, 7, 6)	—
29-H	—	—	0.806 t (6.5)	0.811 t (7)	1.591 d (0.5)
30-H	0.980 d (7)	0.844 d (6.5)	0.981 d (6)	0.844 d (6.5)	0.844 d (6.5)
32-H	0.893 s	0.902 s	0.895 s	0.902 s	0.891 s
33-H	—	—	—	—	4.608 br d (2.5)
33-H	—	—	—	—	4.742 dq (2.5, 1.2)
OAc	—	2.053 s	—	2.053 s	2.052 s

δ values in $CDCl_3$ and coupling constants in Hz. *a*) The higher-field signal is arbitrarily assigned to the 26-methyl, and the lower-field signal to the 27-methyl group.

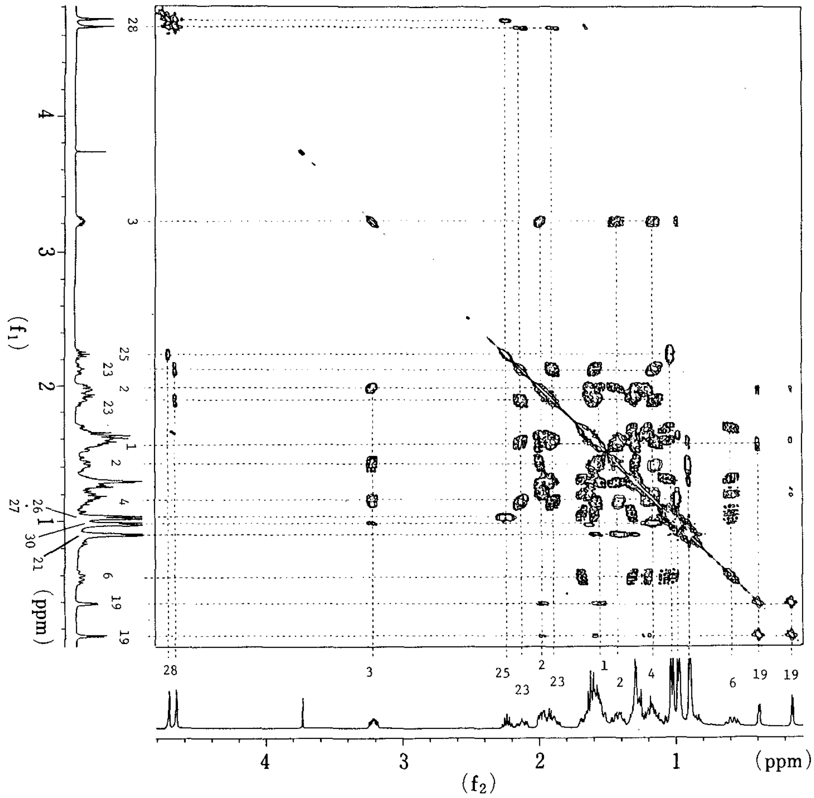


Fig. 1. Contour Map of the ^1H–^1H Shift-Correlated Spectrum of Cycloeucalenol (**1a**)

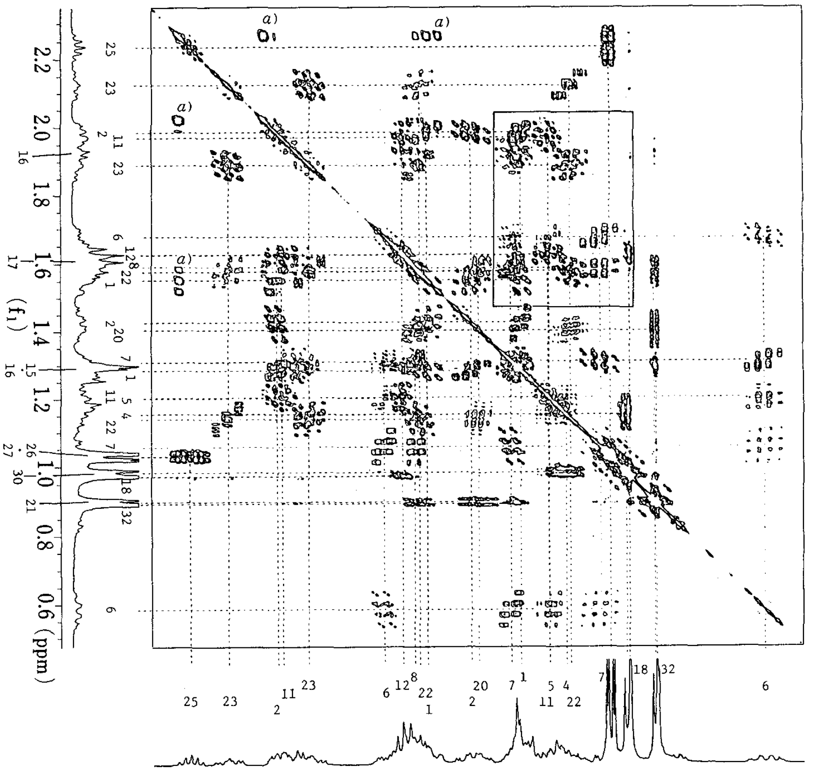


Fig. 2. Contour Map of the Highly Resolved ^1H–^1H Shift-Correlated Spectrum of Cycloeucalenol (**1a**) in the Upfield Region

The rectangular region at the upper right is enlarged and reproduced in Fig. 3. *a*) Folded cross-peaks of one of the 19-methylene protons.

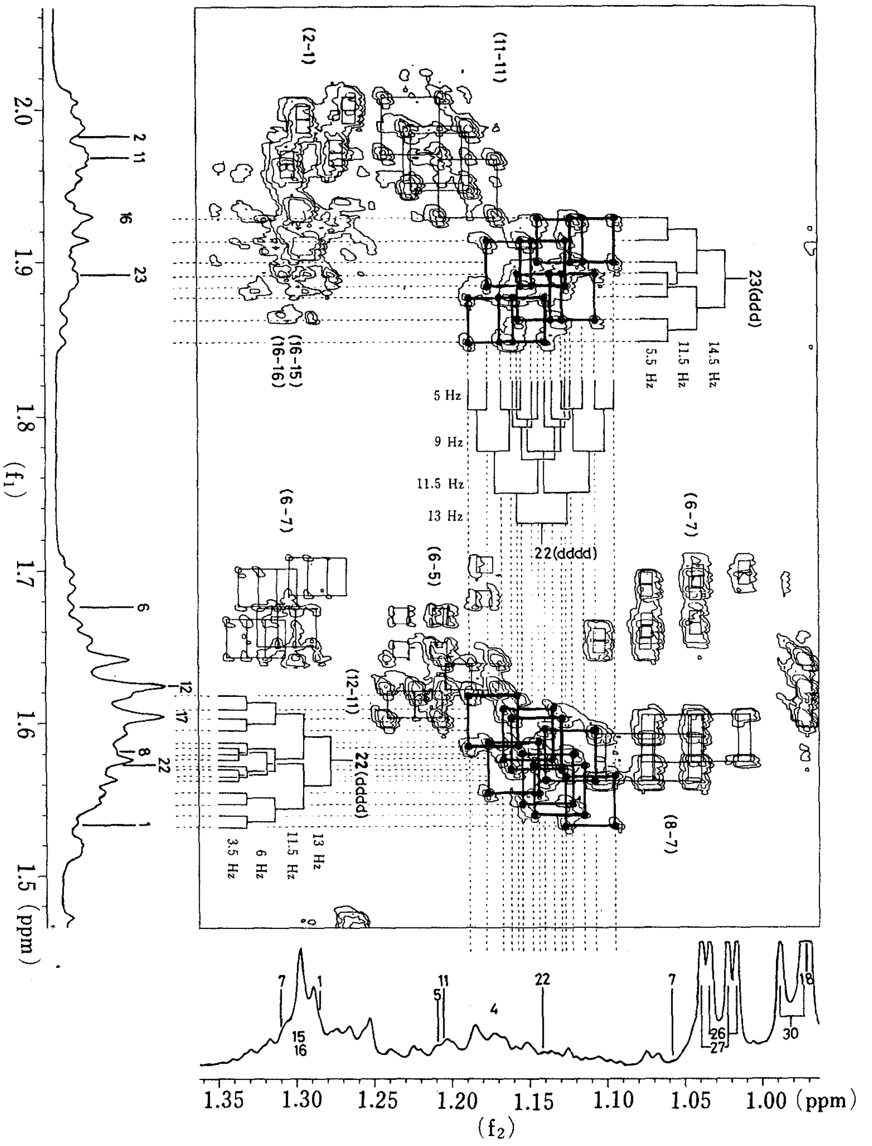


Fig. 3. Enlarged Contour Map of the Rectangular Region of the Upper Right of Fig. 2

First, the ^1H–^1H shift-correlated spectrum of **1a** was measured in order to assign the proton signals accurately. As shown in Fig. 1, the proton signals due to terminal methylene (δ 4.714 and 4.662), hydroxy-bearing methine (δ 3.213), cyclopropane methylene (δ 0.143 and 0.387), 6β-H (δ 0.585), 21-methyl (δ 0.898), 30-methyl (δ 0.980), and 26- and 27-methyls[7] (δ 1.026 and 1.031) are readily assigned. Figure 1 illustrates the cross-peaks corresponding to the coupling between, *inter alia*, 28-H and 25-H (long-range coupling), 28-H and 23-H (long-range coupling), 3-H and 2-H_2, 3-H and 4-H, and 19β-H and 1α-H (long-range coupling).

For more detailed analysis, a highly resolved spectrum was measured in the region of δ 0.48—2.33 and the result is reproduced in Fig. 2. It shows the cross-peaks corresponding to the coupling between 25-H and 26- and 27-methyls, 23-H_2 and 22-H_2, and 20-H and 21-methyl. Moreover, there are cross-peaks arising from the coupling between 21-methyl and 22-H (long-range coupling), 1-H_2 and 2-H_2, 18-methyl and 12α-H (long-range coupling), 12-H_2 and 11-H_2, 6-H_2 and 5-H and 7-H_2, 7-H_2 and 8-H, 32-methyl and 15-H (long-range coupling), 15-H and 16-H, and 16-H and 17-H.

In order to clarify the cross-peaks corresponding to the coupling between 22-Ḣ and 22-H,

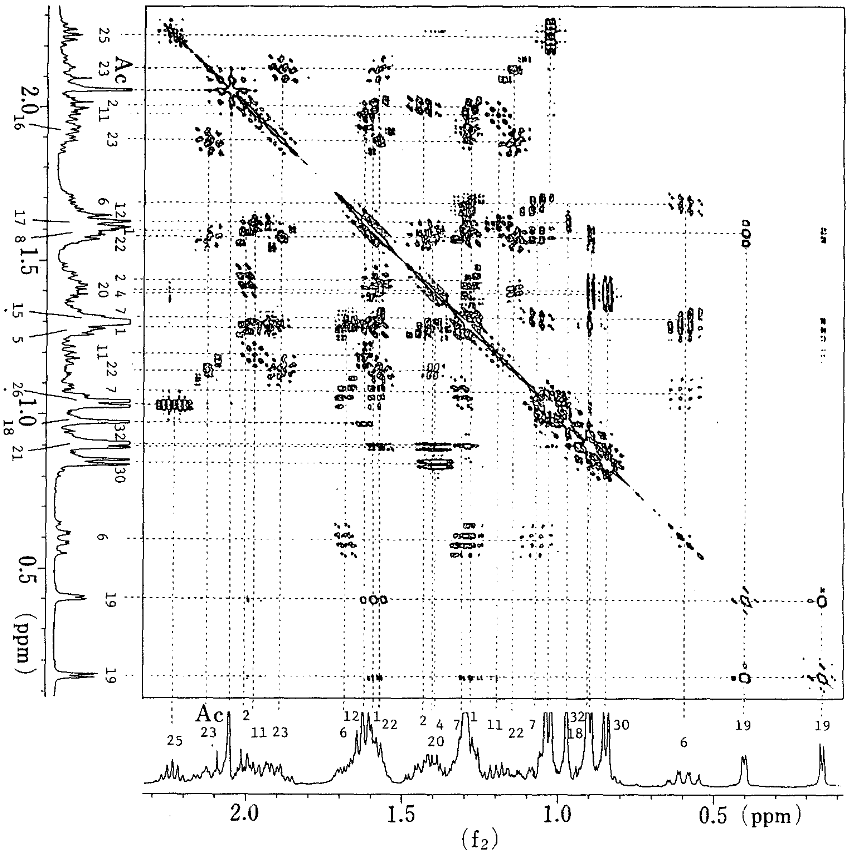


Fig. 4. Contour Map of the Highly Resolved ^1H–^1H Shift-Correlated Spectrum of Cycloeucalenol Acetate (**1b**) in the Upfield Region

Table II. ^{13}C-NMR Spectral Data for Cycloeucalenol-Type Triterpenes and Their Derivatives from *Nervilia purpurea*

Compd. ^{13}C	**1a**	**1b**	**2a**	**2b**	**3b**
C-1	30.88 (t)	30.54 (t)	30.86 (t)	30.48 (t)	30.50 (t)
C-2	34.90 (t)	31.00 (t)	34.90 (t)	30.94 (t)	30.94 (t)
C-3	76.64 (d)	78.79 (d)	76.71 (d)	78.72 (d)	78.72 (d)
C-4	44.69 (d)	41.59 (d)	46.69 (d)	41.53 (d)	41.53 (d)
C-5	43.43 (d)	43.49 (d)	43.41 (d)	43.44 (d)	43.44 (d)
C-6	24.73 (t)	24.72 (t)	24.72 (t)	24.65 (t)	24.70 (t)
C-7	25.22 (t)[a]	25.08 (t)[a]	25.22 (t)	25.03 (t)[a]	25.04 (t)[a]
C-8	46.90 (d)	46.91 (d)	46.89 (d)	46.85 (d)	46.90 (d)
C-9	23.64 (s)	23.75 (s)	23.64 (s)	23.66 (s)	23.72 (s)
C-10	29.65 (s)	29.44 (s)	29.68 (s)	29.40 (s)	29.38 (s)
C-11	27.08 (t)[a]	27.05 (t)[a]	27.07 (t)	26.99 (t)[a]	26.99 (t)[a]
C-12	32.99 (t)[a]	32.91 (t)[a]	32.88 (t)	32.76 (t)[a]	32.80 (t)[a]
C-13	45.45 (s)	45.43 (s)	45.30 (s)	45.20 (s)	45.29 (s)
C-14	49.00 (s)	48.95 (s)	49.17 (s)	49.07 (s)	48.85 (s)
C-15	35.42 (t)[a]	35.40 (t)[a]	35.52 (t)	35.46 (t)[a]	35.38 (t)[a]
C-16	28.18 (t)[a]	28.15 (t)[a]	28.89 (t)	28.83 (t)	28.06 (t)
C-17	52.31 (d)	52.26 (d)	52.17 (d)	52.07 (d)	52.13 (d)
C-18	17.84 (q)	17.83 (q)	18.01 (q)	17.97 (q)	17.77 (q)
C-19	27.28 (t)	27.14 (t)	27.31 (t)	27.17 (t)	27.14 (t)
C-20	36.21 (d)	36.17 (d)	40.94 (d)	40.91 (d)	36.65 (d)

TABLE II. (continued)

Compd. ^{13}C	**1a**	**1b**	**2a**	**2b**	**3b**
C-21	18.42 (q)	18.39 (q)	20.91 (q)	20.84 (q)	18.74 (q)
C-22	35.13 (t)	35.10 (t)	138.63 (d)	138.58 (d)	34.51 (t)
C-23	31.41 (t)	31.38 (t)	129.39 (d)	129.34 (d)	26.79 (t)
C-24	156.89 (s)	156.95 (s)	51.32 (d)	51.25 (d)	55.54 (d)
C-25	33.90 (d)	33.88 (d)	31.93 (d)	31.87 (d)	30.94 (d)
C-26[b)]	22.06 (q)	22.05 (q)	19.07 (q)	19.00 (q)	21.47 (q)
C-27[b)]	21.94 (q)	21.92 (q)	21.10 (q)	21.06 (q)	20.84 (q)
C-28	106.05 (t)	106.00 (t)	25.44 (t)	25.39 (t)	147.34 (s)
C-29	—	—	12.26 (q)	12.21 (q)	19.03 (q)
C-30	14.46 (q)	14.46 (q)	14.43 (q)	14.39 (q)	14.45 (q)
C-32	19.21 (q)	19.18 (q)	19.18 (q)	19.18 (q)	19.13 (q)
C-33	—	—	—	—	111.86 (t)
O$\underline{C}$OCH$_3$	—	170.94 (s)	—	170.88 (s)	170.87 (s)
OCO$\underline{C}$H$_3$	—	21.36 (q)	—	21.31 (q)	21.33 (q)

δ values in CDCl$_3$. The multiplicities of carbon signals are indicated as (s), (d), (t), and (q). *a)* Previous assignments (ref. 2 and 4) are revised. *b)* Signals of C-26 and C-27 are arbitrarily assigned, but they are correlated to the signals of the 26-methyl and 27-methyl protons, respectively.

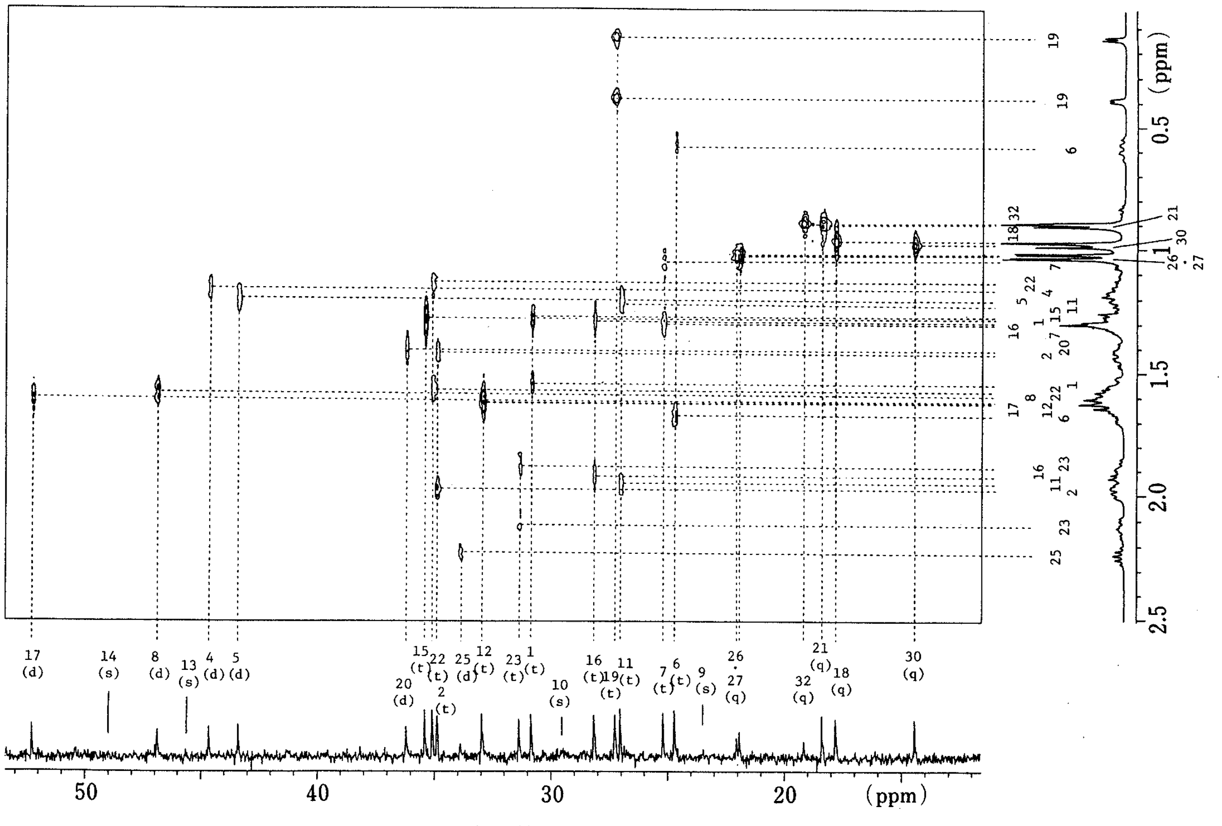


Fig. 5. Contour Map of the ^{1}H–^{13}C Shift-Correlated Spectrum of Cycloeucalenol (**1a**) in the Upfield Region

The ^{1}H shifts are given on the ordinate and the ^{13}C shifts on the abscissa. The multiplicities of carbon signals were determined by means of off-resonance and INEPT methods, and are indicated as (s), (d), (t), and (q).

and 22-H and 23-H, the rectangular region at the upper right (Fig. 2) was enlarged and is illustrated in Fig. 3, in which the squares are connected with full lines in the cross-peaks. The sizes of these squares indicate the splitting patterns and the coupling constants between 22-H and 22-H, and 22-H and 23-H.

The other cross-peaks could be analyzed in similar ways except for a few protons which gave complicated cross-peaks because of mutual overlapping. Final assignments for cycloeucalenol (**1a**) are given in Table I.

Then, we applied the same method to **1b** (Fig. 4). The ^{1}H–^{1}H shift-correlated spectra of **2a**, **2b**, and **3b** were also measured and analyzed in detail, and the signal assignments for these triterpenes are listed in Table I.

Next, the ^{13}C-signals of **1a** were assigned through measurement of the ^{1}H–^{13}C shift-correlated spectrum, which enabled accurate analysis on the basis of the proton chemical shift data of **1a**, as shown in Fig. 5. The ^{13}C-signals of **1b**, **2a**, **2b**, and **3b** were analyzed in the same manner and the results are summarized in Table II.

In our present experiment, Lukacs' assignments of the C-7, C-11, C-12, C-15, and C-16 signals of cycloeucalenol (**1a**) and cycloeucalenol acetate (**1b**)$^{2)}$ were corrected. Assignments of the C-7, C-11, C-12, and C-15 signals of cyclonervilol acetate (**2b**) and cyclohomonervilol acetate (**3b**) reported in our previous paper$^{4)}$ were also revised.

It should be noted that combined use of ^{1}H–^{1}H and ^{1}H–^{13}C shift-correlation spectroscopy is a powerful tool for determination of the precise frequencies of proton and carbon signals in complicated NMR spectra.

Experimental

NMR spectra were taken on JEOL FX-90Q and JNM-GX 400 spectrometers in $CDCl_3$ solutions with tetramethylsilane as an internal standard.

The ^{1}H–^{1}H shift-correlated spectra were measured by the use of a ^{1}H–^{1}H shift correlation sequence with a 45° mixing pulse, and N-type peak selection. Data processing was carried out with the standard JEOL software. An f_2 spectral width of 1900 Hz (4.75 ppm) over 1024 data points gave a digital resolution of 3.7 Hz. A number of 512 spectra, each of 16 transients, gave, with appropriate incrementing of the evolution delay, an f_1 width of 1900 Hz and a digital resolution of 3.7 Hz (with zero filling). The sample concentration was 8—10 mg in $CDCl_3$; total acquisition time was about 12 h, and processing time *ca.* 20 min. In highly resolved spectra measurements, an f_2 spectral width of 750—900 Hz (1.88—2.25 ppm) over 1024 data points gave a digital resolution of 1.5—1.76 Hz. A number of 512 spectra, each of 16 transients, gave, with appropriate incrementing of the evolution delay, an f_1 width of 750—900 Hz and a digital resolution of 1.5—1.76 Hz (with zero filling).

The ^{1}H–^{13}C shift-correlated spectra were obtained with the usual pulse sequence. Data processing was performed with the standard JEOL software. The spectral widths were 1900 Hz in f_1 and 10000 Hz in f_2, giving digital resolutions of 7.4 and 9.8 Hz with a 512×2048 data point matrix. The amount of sample used was 8—10 mg in $CDCl_3$. Acquisition of 16 transients for each of the 256 FID's required 13 h, and processing took about 1 h.

References and Notes

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7) The higher-field signal was arbitrarily assigned to the 26-methyl group.