

STRUCTURE AND C-13 SIGNAL ASSIGNMENTS OF NEW METHYLSTEROLS FROM  
NERVILIA PURPUREA BY TWO-DIMENSIONAL NMR SPECTROSCOPY

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Abstract: The structures of cyclonervilasterol (1a) and its analogs (2a, 3a, and 4a), isolated from *Nervilia purpurea*, were confirmed by application of 2-D NMR spectroscopy including INADEQUATE and also their <sup>13</sup>C NMR signals were assigned.

In a previous paper,<sup>1)</sup> we reported the isolation and characterization of new methylsterols named cyclonervilasterol (1a), 24-epicyclonervilasterol (2a), dihydrocyclonervilasterol (3a), and 24-epidihydrocyclonervilasterol (4a) from *Nervilia purpurea* SCHLECHTER (Orchidaceae). In order to confirm the proposed structures of these compounds, we applied the two-dimensional (2-D) NMR spectroscopy<sup>2)</sup> to them.

First, the <sup>1</sup>H-<sup>13</sup>C shift correlated spectrum of cyclonervilasteryl acetate (5) (roughly 1:1 mixture of 1b and 2b)<sup>3)</sup> was measured and as shown in Fig. 1, the carbon signals due to an acetyl methyl (peak g), a carbinol methine (peak z), a disubstituted olefine (peak B and C), and a trisubstituted olefine (peak A and D) were readily assigned. In addition, the carbon j which is correlated with the proton at δ 0.63 (1H, d, J=4.0 Hz) was recognized to be the methylene carbon of a cyclopropane ring.

Then the 2-D INADEQUATE spectrum<sup>4)</sup> of 5 was measured to clarify the carbon-carbon connectivities in the molecule. The pulse system employed was the (90°)-τ-(180°)-τ-(90°)-t<sub>1</sub>/2-(135°)-t<sub>1</sub>/2-t<sub>2</sub> sequence,<sup>4)</sup> and the result was reproduced in Fig. 2 and 3, which exhibited the <sup>13</sup>C-<sup>13</sup>C correlated peaks of all the coupled <sup>13</sup>C pairs except those between the carbon k and h, and between the carbon k and j. On consideration of the fact that the carbons h and k are quaternary and the carbon j is secondary (cyclopropane methylene), the structure of cyclonervilasteryl acetate should be represented by the formula 5: hence, 1b and 2b for each epimer, except the stereochemistry.

Now, the <sup>13</sup>C signals of the methylsterols (1a and 2a) and their acetates (1b and 2b) are readily assigned as shown in Table 1 based on the comparison of the NMR data of 5 (Fig. 2 and 3) with the <sup>1</sup>H-decoupled <sup>13</sup>C spectra of 2a and 2b, together with the consideration of acetylation shifts. It should be noted that the chemical shifts for the side-chain carbons of the above compounds are consistent with those of brassicasterol (24R) and 24-epibrassicasterol (24S).<sup>5)</sup>

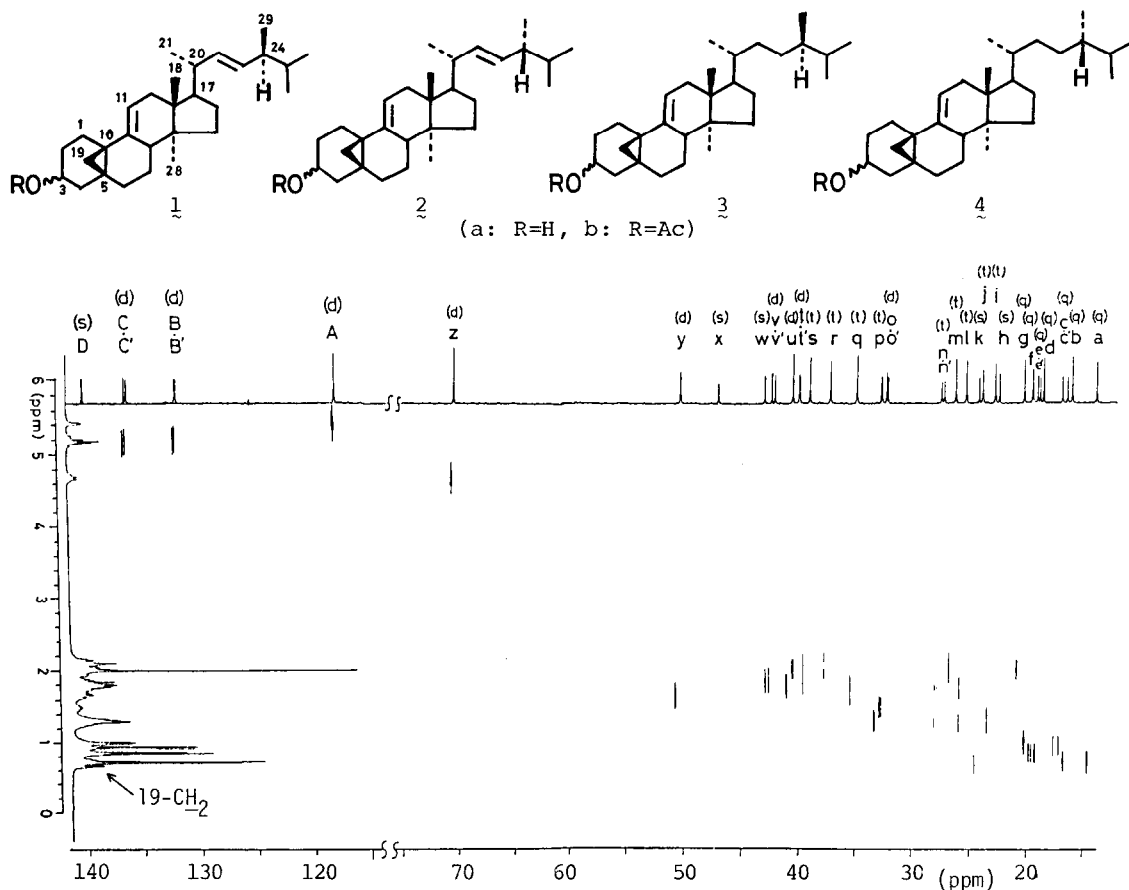


Fig. 1. The  $^1\text{H}$ - $^{13}\text{C}$  shift correlated spectrum of cyclonervilasteryl acetate (5) (in  $\text{CDCl}_3$ , Nicolet NT-300 spectrometer). The  $^1\text{H}$  shifts are the ordinate and the  $^{13}\text{C}$  shifts are the abscissa, and  $\text{sp}^2$  carbons are marked with capital letters A-E and  $\text{sp}^3$  carbons with small letters a-z in the order of increasing  $\delta$  value. The carbonyl carbon signal ( $\delta$  170.5) is outside of the spectrum. Some of the signals appear as double lines because the sample is the 24-epimeric mixture. The multiplicities of carbon signals were determined by means of off-resonance and INEPT methods, and are indicated as (s), (d), (t), and (q).

With the 3-keto derivatives, the 2- and 4-carbons showed down-field shifts as expected,<sup>6)</sup> but some of carbon signals, especially the 19-methylene carbon, exhibited considerable up-field shifts. The assignment of the 19-carbon signal was done with the aid of  $^1\text{H}$ - $^{13}\text{C}$  shift correlated spectrum of 6 (a mixture of the 24-epimers) as shown in Fig. 4. The other carbon signals were assigned by comparison of their chemical shifts with those of the parent methylsterols (Table 1).

Next, the  $^{13}\text{C}$  NMR spectra of dihydro compounds: 3a, 4a, 3b, and 4b, were examined and the assignments of  $^{13}\text{C}$  signals were performed as shown in Table 1, based on the comparisons of their chemical shifts with those of 1a, 2a, 1b, and 2b, respectively, and also with those of sterols reported in the literature.<sup>5)</sup>

The stereochemistry of their skeletal structures followed from the biogenetic analogy with the related compounds obtained from this plant,<sup>1)</sup> but its investigation is now in progress.

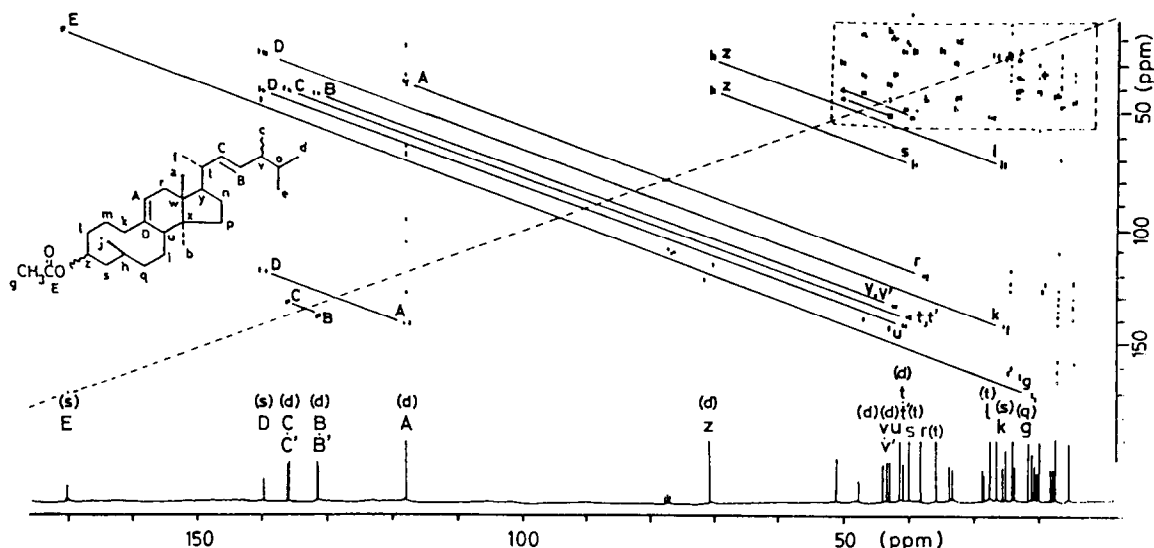


Fig. 2. The 2-D INADEQUATE spectrum (contour map) of cyclonervilasteryl acetate (5) (Nicolet NT-300 spectrometer, sample: 150 mg in  $\text{CDCl}_3$ , 65 hr run). The conventional  $^1\text{H}$ -decoupled  $^{13}\text{C}$  spectrum runs along the down margin. Coupled  $^{13}\text{C}$ - $^{13}\text{C}$  pairs are joined by the slanting lines. The rectangular region at the upper right is enlarged and reproduced in Fig. 3.

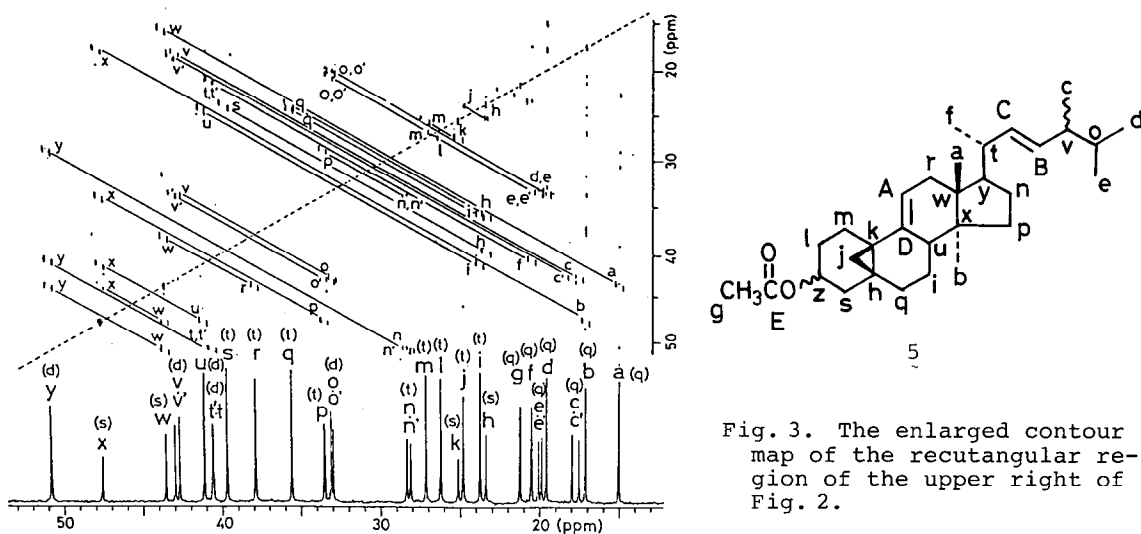


Fig. 3. The enlarged contour map of the rectangular region of the upper right of Fig. 2.

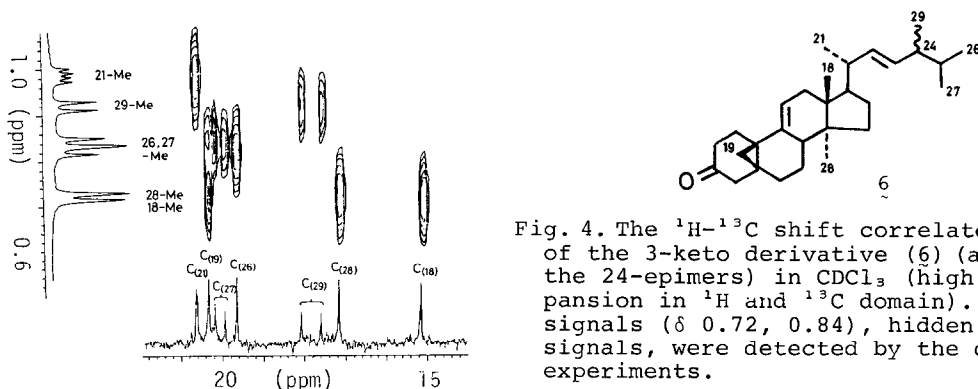


Fig. 4. The  $^1\text{H}$ - $^{13}\text{C}$  shift correlated spectrum of the 3-keto derivative (6) (a mixture of the 24-epimers) in  $\text{CDCl}_3$  (high field expansion in  $^1\text{H}$  and  $^{13}\text{C}$  domain). The  $19\text{-H}_2$  signals ( $\delta$  0.72, 0.84), hidden by the  $\text{CH}_3$  signals, were detected by the decoupling experiments.

Table 1.  $^{13}\text{C}$  NMR data of the methylsterols and derivatives ( $\delta$  in  $\text{CDCl}_3$ ).

	1a		2a		3-keco deriv. of 1a and 2a		3a		4a		3b		4b		
	24 S	24 R	24 S	24 R	24 S	24 R	24 R	24 S	24 R	24 S	24 R	24 R	24 S	24 S	24 R
1		27.99		27.27		25.17 *		27.96						27.27	
2		30.18		26.40		36.32		30.18						26.40	
3		68.40		70.81		215.59		68.39						70.81	
4		44.64		39.83		47.00		44.62						39.83	
5		24.02		23.59		22.84 *		23.96						23.57	
6		36.17		35.71		32.05 *		36.18						35.74	
7		24.02		23.89		23.33		24.02						23.89	
8		41.39		41.35		41.24		41.37						41.35	
9		140.54		140.16		139.14		140.43						140.04	
10		25.48		25.29		24.60 *		25.44						25.26	
11		117.84		118.14		119.39		117.92						118.23	
12		38.09		38.08		38.13		38.19						38.20	
13		43.79		43.77		43.80		43.88						43.86	
14		47.73		47.71		47.98		47.57						47.57	
15		33.68		33.64		33.70		33.64						33.61	
16	28.53	28.24	28.49	28.24	28.40	28.14	27.84	27.89						27.83	
17		51.05		51.01		51.06		51.28	51.16			51.25	51.11		
18		15.18		15.19		15.21		14.97				14.98			
19		25.07		24.97		20.46		25.05				24.97			
20		40.62	40.70	40.62	40.60	40.67	36.28	36.63			36.30	36.61			
21		20.62		20.59		20.64	18.34	18.52			18.34	18.57			
22	136.31	136.11	136.30	136.10	136.20	136.00		34.08			34.08				
23	132.19	131.90	131.97	131.86	132.17	132.07	30.59	30.99			30.69	31.00			
24	43.15	42.86	43.13	42.84	43.11	42.85	38.94	39.19			38.93	39.17			
25	33.30	33.16	33.28	33.14	33.29	33.16	32.47	31.52			32.47	31.50			
26		19.67		19.66		19.67	18.39	17.58			18.34	17.59			
27		20.19	19.95	20.18	19.95	20.07	20.21	20.52			20.21	20.53			
28		17.28		17.26		17.22		17.26				17.23			
29	18.20	17.62	18.06	17.61	17.91	17.48	15.46	15.54			15.45	15.50			
$\text{CH}_3$				21.38								21.38			
$\text{C=O}$				170.52								170.55			

\* Assignments are tentative.

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#### REFERENCES AND NOTES

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6. Assignments of the 2- and 4-carbon signals were supported by the  $^{13}\text{C}$  NMR spectrum of 2,2,4,4-tetradeuterated derivative of **6**.

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