Journal of Natural Products
Vol. 53, No. 3, pp. 735-739, May-Jun 1990

APHIDICOLIN SYNTHETIC STUDIES, 2.[1] 2D NMR ANALYSIS OF APHIDICOLIN AND ITS DEGRADATION PRODUCTS 3α,18-DIHYDROXY-17-NORAPHIDICOLAN-16-ONE AND 3α,18-ISOPROPYLIDENEDIOXY-17-NORAPHIDICOLAN-16-ONE

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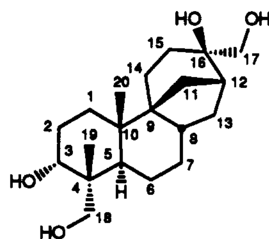
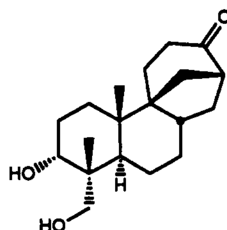
ABSTRACT.—The ^{1}H- and ^{13}C-nmr spectra of aphidicolin [**1**] and two degradation products, 3α,18-dihydroxy-17-noraphidicolan-16-one [**2**] and its isopropylidene derivative **3**, have been interpreted by 2D nmr analysis. The ^{1}H resonances were identified via phase-sensitive COSY experiments, whereas the ^{13}C assignments were based on one-bond and long-range ^{1}H/^{13}C heteronuclear correlation experiments. The ^{1}H spectra of compounds **1–3** and the ^{13}C spectrum of **3** have not been interpreted previously. Two carbon assignments reported earlier for **1** and **2** proved to be incorrect.

In 1972 Hesp and co-workers at ICI (1,2) announced the isolation and structure of the diterpene (+)-aphidicolin [**1**], produced by the fungus *Cephalosporium aphidicola*. The structure was deduced largely via chemical degradation studies and ultimately confirmed by X-ray crystallography. The unique architecture of aphidicolin and the novel mechanism whereby it inhibits DNA synthesis have attracted considerable interest, both chemical (3–16) and biological (17–21).

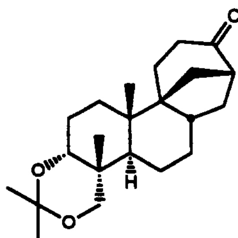
The biosynthesis of aphidicolin has been investigated extensively by Adams and Bu'Lock (22) and Hanson and coworkers (23–30). Their work entailed ^{13}C-nmr analyses of aphidicolin and several degradation products (24), including 3α,18-dihydroxy-17-noraphidicolan-16-one [**2**]. Assignments were based principally on ^{13}C multiplicities and chemical



1
(+)-aphidicolin



2



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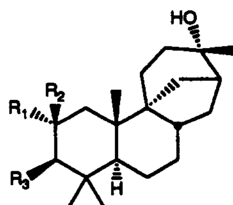

4 R_1=OH, R_2=R_3=H stemodin
5 R_1=R_2=H, R_3=OH maritimol
6 R_1,R_2=O, R_3=H stemodinone

[1]For Part 1, see Rizzo and Smith (16).

shifts, as well as spectroscopic comparisons with various chemically transformed intermediates, and were in accord with the earlier interpretations of Rosazza and co-workers (31,32). To date however, 2D nmr techniques have not been employed to confirm these assignments, and the ^{1}H spectra of **1–3** and ^{13}C spectrum of **3** remain uninterpreted.

We have previously reported the stereoselective synthesis of aphidicolin from the key precursor 3α,18-isopropylidenedioxy-17-noraphidicolan-16-one [**3**], a degradation product of **1** (16). During the course of this venture, we elected to undertake the unambiguous assignment of the ^{1}H- and ^{13}C-nmr spectra of **1** and several advanced synthetic intermediates. The recent report by Hufford (33), detailing ^{1}H- and ^{13}C-nmr analyses of the structurally related *Stemodia* diterpenes stemodin [**4**], maritimol [**5**], and stemodinone [**6**], prompts us to communicate our results at this time.

A phase-sensitive COSY experiment (34–37) readily elucidated the homonuclear proton couplings. Based on the couplings and chemical shifts, four important assignments were easily deduced for each compound. In the spectrum of **3**, for example, the resonances at δ 3.67, 2.63, and 2.39 correlated with the protons at C-3, C-12, and C-5, respectively. In addition, the AB quartet centered at δ 3.44 (J = 12.2 Hz, $\Delta\nu$ = 179.8 Hz, 2H) was readily assigned to the C-18 methylene moiety. The remaining ^{1}H resonances could then be identified via simple cross peak analysis of the COSY spectrum. We analyzed the ^{1}H spectra of **1** and **2** in similar fashion. The data and assignments are summarized in Table 1.

TABLE 1. ^{1}H-nmr (500 MHz) Data and Assignments.[a]

Proton	Compound		
	1 (C_5D_5N)	**2** ($CDCl_3$)	**3** ($CDCl_3$)
H-1 . . .	1.05–1.00 (m)	1.03–0.094 (m)	1.00 (td, J = 12.9, 2.3)
	2.51 (m)	2.10–2.03 (m)	2.14–2.02 (m)
H-2 . . .	2.06–1.95 (m)	2.00–1.90 (m)	1.94–1.91 (m)
	1.84–1.61 (m)	1.52–1.40 (m)	1.80–1.51 (m)
H-3 . . .	3.93 (br s)	3.70 (s)	3.67 (dd, J = 2.8, 2.1)
H-5 . . .	2.90 (dd, J = 12.7, 2.8)	2.24 (d, J = 8.6)	2.39 (dd, J = 12.9, 2.8)
H-6 . . .	1.29 (dq, J = 12.6, 4.1)	1.52–1.40 (m)	1.80–1.51 (m)
	1.84–1.61 (m)	1.34–1.26 (m)	1.31 (ddd, J = 12.7, 12.0, 4.7)
H-7 . . .	1.84–1.61 (m)	1.76–1.69 (m)	1.77–1.72 (m)
	1.43 (dq, J = 12.4, 3.9)	1.52–1.40 (m)	1.46 (dd, J = 12.6, 4.4)
H-8 . . .	2.06–1.95 (m)	2.00–1.90 (m)	2.14–2.02 (m)
H-11 . . .	2.35 (d, J = 10.9)	1.76–1.69 (m)	1.68 (dd, J = 12.0, 5.1)
	1.51–1.46 (m)	1.58 (d, J = 12.0)	1.60 (d, J = 12.0)
H-12 . . .	2.58 (dd, J = 6.7, 6.5)	2.61 (dd, J = 7.6, 6.2)	2.63 (dd, J = 5.8, 5.6)
H-13 . . .	1.84–1.61 (m)	2.10–2.03 (m)	2.14–2.02 (m)
	1.15 (dd, J = 13.2, 8.0)	1.14–1.07 (m)	1.12–1.86 (m)
H-14 . . .	1.84–1.61 (m)	2.10–2.03 (m)	2.14–2.02 (m)
	1.84–1.61 (m)	2.00–1.90 (m)	1.90–1.86 (m)
H-15 . . .	2.30 (dd, J = 12.0, 7.4)	2.36 (ddd, J = 17.1, 7.3, 6.5)	2.37–2.31 (m)
	2.24–2.20 (m)	2.23–2.18 (m)	2.27 (dd, J = 17.4, 8.6)
H-17 . . .	3.85–3.80 (m)		
	3.73 (dd, J = 12.7, 3.0)		
H-18 . . .	3.85–3.80 (m)	3.36 (m)	3.60 (d, J = 12.2)
	3.64 (dd, J = 10.9, 2.8)		3.25 (d, J = 12.2)
H-19 . . .	0.80 (s)	0.69 (s)	0.73 (s)
H-20 . . .	1.04 (s)	1.03 (s)	1.08 (s)

[a]Chemical shifts are measured relative to TMS (δ = 0). Coupling constants are given in Hz.

Following analysis of the ^{1}H spectra, we turned to the assignment of the ^{13}C resonances. Although the INADEQUATE (38,39) pulse sequence has been established as the least ambiguous method for determining carbon-carbon connectivity, the poor sensitivity of this technique necessitates large sample sizes and prolonged experiment times. These drawbacks led us to explore the use of ^{13}C-^{1}H heteronuclear correlation experiments.

For **1** and **2**, all carbon resonances were resolved in C_5D_5N and $CDCl_3$, respectively. However, for **3** only 22 of the 23 carbons were resolved in $CDCl_3$. The numbers of attached hydrogens were determined by using the INEPT pulse sequence. This indicated that the unresolved resonance for **3** corresponded to one of the three quaternary carbons. A *J*-modulated spin-echo pulse sequence (JMODXH) (40) then established the coincidence of the missing quaternary carbon signal with the methylene resonance at δ 34.5.

One-bond heteronuclear correlation spectra (XHCORR) (41) permitted the identification of most of the carbon resonances for **1–3** (Table 2). However, assignments for the C-19 and C-20 methyl groups as well as the C-4, C-9, and C-10 quaternary centers remained ambiguous. Fortunately, the C-16 quaternary carbon of aphidicolin could be differentiated on the basis of chemical shift. In addition, the C-17 and C-18 hydroxymethyl resonances of **1** were identified by comparison with degradation product **2**.

The methyl groups and the quaternary centers were next assigned by taking advantage of the COLOC (42) pulse sequence, which provided long-range heteronuclear correlations. The C-19 methyl resonances were distinguished by the observation of three-bond couplings of H-3 to the carbon resonances at δ 18.1, 17.6, and 17.1 for **1**, **2**, and **3**, respectively. The XHCORR spectra showed that these carbon signals corre-

TABLE 2. ^{13}C-nmr (125 MHz) Assignments.[a]

Carbon	Compound			
	1 (C_5D_5N)	**2** ($CDCl_3$)	**3** ($CDCl_3$)	Method
C-1	27.2	26.7	26.9	XHCORR
C-2	27.5	26.6	23.8	XHCORR
C-3	76.3	76.8	73.4	XHCORR
C-4	41.0	40.2	34.5	COLOC
C-5	34.1	32.9	32.9	XHCORR
C-6	23.5	21.7	21.3	XHCORR
C-7	27.4	26.0	25.9	XHCORR
C-8	40.5	41.2	41.4	XHCORR
C-9	49.7	49.0	49.0[b]	COLOC
C-10	40.5	39.6	39.5[b]	COLOC
C-11	33.4	34.0	33.2	XHCORR
C-12	42.2	48.3	48.1	XHCORR
C-13	31.8	31.6	31.4	XHCORR
C-14	29.9	22.7	21.7	XHCORR
C-15	25.6	34.4	34.6	XHCORR
C-16	74.2	215.9	215.5	^{13}C
C-17	68.2			^{13}C
C-18	71.9	71.6	68.5	XHCORR
C-19	18.1	17.6	17.1	COLOC
C-20	15.4	15.6	16.0	COLOC

[a]Chemical shifts are measured relative $CDCl_3$ (δ = 77.0).
[b]For these assignments the HMBC pulse sequence was used; see Bax and co-workers (44,45).

lated to the upfield methyl singlets in the ^{1}H spectra of all three compounds, thus securing the assignments of the methyl resonances in both the ^{1}H and ^{13}C spectra. The C-4 quaternary centers of **1**–**3** were then assigned by observation of strong two-bond couplings involving the protons at C-3, C-5, C-18, and C-19. The vicinal quaternary centers at C-9 and C-10 proved more problematic. For each compound, the C-20 methyl singlet showed strong heteronuclear correlation to both of these carbon resonances. The observation of cross peaks from H-12 to the carbon resonance at δ 49.7 and 49.0 for **1** and **2**, respectively, differentiated C-9 and C-10. These resonances were assigned to C-9 for each compound. For **3**, however, the H-12 to C-9 coupling was not observed, possibly due to one-bond *J*-modulation effects (43). Using the inverse long range heteronuclear correlation (HMBC) experiment of Bax and co-workers (44,45), the H-12 to C-9 cross peak was observable for **3**, correlating to the carbon resonance at 49.0. In all three cases, no cross peak was observed from H-12 to the carbon resonances at δ 40.5, 39.6, and 39.5 for **1**, **2**, and **3**, respectively. This is not unusual because this would entail four-bond coupling.

In summary, we have unambiguously assigned the complete ^{1}H and ^{13}C spectra of aphidicolin [**1**] and two of its degradation products (**2** and **3**) via 2D nmr techniques, including ^{1}H-^{1}H homonuclear correlation (COSY) and one-bond (XHCORR) and long-range (COLOC, HMBC) ^{13}C-^{1}H heteronuclear correlation. [For a review on long-range heteronuclear correlation see Martin and Zektzer (43)]. The ^{1}H spectra of **1**–**3** and the carbon spectrum of **3** were previously unassigned. The carbon assignments for aphidicolin agreed with those previously published except for the C-14 and C-15 resonances, which were found to be reversed. The reported chemical shifts for C-6 and C-14 of **2** should also be interchanged. These corrections demonstrate the utility of one-bond heteronuclear correlation for the assignment of carbon spectra. Likewise, the unambiguous differentiation of the quaternary and angular methyl carbon resonances underscores the usefulness of the COLOC and HMBC techniques.

EXPERIMENTAL

All experiments were conducted at 300°K with a Bruker AM500 spectrometer. A 35-mg sample of aphidicolin was dissolved in 0.5 ml of C_5D_5N. For **2** and **3**, 70- and 85-mg samples were dissolved in 0.5-ml portions of $CDCl_3$. For **1**, **2**, and **3**, the ^{1}H sweep widths were 3125, 2250, and 1750 Hz, respectively; the ^{13}C sweep widths were 14,460, 10,690, and 8620 Hz. For XHCORR and COLOC experiments, J_{CH} = 125 Hz and 10 Hz were used, respectively. For **3**, the HMBC experiment was performed using a ^{1}H sweep width of 6000 Hz and a ^{13}C sweep width of 30,000 Hz. The one-bond carbon/proton coupling constant (Δ_1) was set to 125 Hz, and Δ_2 was set to 70 msec.

ACKNOWLEDGMENTS

Support for this investigation was provided by the National Institutes of Health (National Cancer Institute) through grant CA-22807 and by Merck Sharp and Dohme Research Laboratories. We also thank ICI Pharmaceuticals Division for a generous gift of natural aphidicolin and Dr. Christopher S. Shiner for helpful suggestions and critical comments.

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Received 14 November 1989