Journal of Natural Products
Vol. 51, No. 6, pp. 1161-1165, Nov-Dec 1988

^{1}H-NMR ANALYSIS OF HERBIMYCINS AND DIHYDRO-HERBIMYCINS

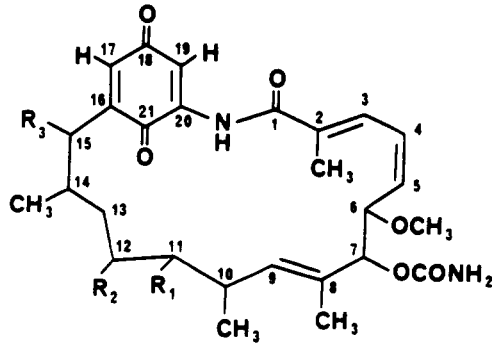
LONG-ZE LIN,[1] GÁBOR BLASKÓ,[2] and GEOFFREY A. CORDELL*

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

ABSTRACT.—Herbimycin A [**1**] and herbimycin C [**3**] and their dihydro-derivatives **5** and **6**, respectively, have been obtained from the cell broth of *Streptomyces hygroscopicus*, and the assignment of their ^{1}H-nmr has been completed. Strong cytotoxic activity of antibiotics **1**, **3**, **5**, and **6** against the P-388 and KB lymphocytic leukemia test systems has been demonstrated.

Herbimycin A [**1**] (1,2), herbimycin B [**2**] (3), and herbimycin C [**3**] (4) are benzoquinoid ansamycin antibiotics isolated from the culture broth of *Streptomyces hygroscopicus* AM-3675. They exert herbicidal, anti-tobacco-mosaic virus, and cytotoxic activity. The structure elucidation of herbimycins **1–3** has been completed by ir, uv, ^{1}H-, and ^{13}C-nmr spectroscopy and confirmed through single crystal X-ray crystallographic analysis (5). In 1984 a patent (6) claimed that fermentation of *S. hygroscopicus* produced the antibiotics TAN-420 A–F. According to the data reported, antibiotics TAN-420-F and TAN-420-D are identical with herbimycin A [**1**] and herbimycin C [**3**], respectively, and TAN-420-B is a new ansamycin and antibiotic characterized by structure **4**. The patent also claimed the production of the hydroquinoid ansamycins, TAN-420-A, -C, and -E (antibiotics **7**, **6**, and **5**, respectively). However, the new dihydro-herbimycin type antibiotics [**5–7**] were characterized only by their elemental analysis, mp, [α]D, uv, ir, and mass spectra. No nmr data were reported for antibiotics **5–7**. Moreover, the patent (6) did not mention any biological data for dihydro-herbimycins A [**5**] and B [**6**].

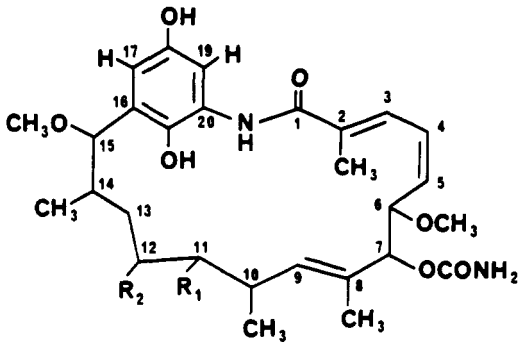
We report here on the isolation of herbimycins A [**1**] and C [**3**] and their dihydro-derivatives **5** and **6**, respectively, from the cultured cell broth of *S. hygroscopicus*, the assignment of their ^{1}H-nmr spectra, and some preliminary structure-activity relation-



	R_1	R_2	R_3
1	OMe	OMe	OMe
2	OH	OMe	H
3	OH	OMe	OMe
4	OH	OH	OMe

[1]On leave from the Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China.

[2]On leave from the Central Research Institute for Chemistry, Hungarian Academy of Sciences, Budapest, Hungary.



	R_1	R_2
5	OMe	OMe
6	OH	OMe
7	OH	OH

ships regarding their activity against the P-388 and KB lymphocytic leukemia systems in vitro.

Fermentation was carried out under the conditions reported in the literature (1). The cultured broth of *S. hygroscopicus* was centrifuged, and the supernatant was extracted with EtOAc. After evaporation of the combined organic phase, the residue was chromatographed on a Si gel column using $CHCl_3$-MeOH (95:5) as the eluent. Herbimycins A [**1**] and C [**3**] were obtained as dark yellow, amorphous materials, while dihydro-herbimycins A [**5**] and C [**6**] were light yellow powders. The isolates were characterized by their uv, ir, ^{1}H-nmr, and mass spectral data.

Analysis of the ^{1}H-nmr and homonuclear COSY spectra of herbimycin A [**1**] indicated meta coupling between H-19 (δ 7.34, d) and H-17 (δ 6.63, t), thereby establishing the assignment of the hydrogens attached to the quinone moiety. A long-range coupling of H-17 (δ 6.63, t) with H-15 (δ 4.51, bd) was also observed. Vinylic protons at δ 6.98 (bd), 6.51 (t), and 5.86 (dd) exhibited an ABC coupling pattern as expected for H-3, H-4, and H-5, respectively. The long range coupling of H-3 (δ 6.98, bd) with methyl resonance at δ 2.02 (bs) permitted the assignment of 2-Me, and similar coupling was observed between H-9 (δ 5.50, m) and 8-Me (δ 1.66, bs). Methine protons attached to oxygenated carbon atoms; H-6 (δ 4.50, bd), H-7 (δ 5.65, bs), H-11 (δ 3.51, m), H-12 (δ 3.45, m), and H-15 (δ 4.51, bd) were assigned according to their observed vicinal couplings in the COSY spectrum. The coupling constants of H-11 and H-12 could not be determined because of their overlap with other signals. Finally, the chemical shift values of H-10 (δ 2.66 m) and H-14 (δ 1.61) were determined according to their coupling with the protons of the attached methyl group.

The signals in the ^{1}H-nmr spectrum of herbimycin C [**3**] were even better resolved than those of **1**. The ^{1}H-^{1}H COSY spectrum of **3** showed groups of coupled subunits similar to those in the COSY spectrum of **1**. Thus, the protons of the disubstituted *p*-quinone nucleus appeared at δ 7.47 (d) and δ 6.63 (t) and were assigned to H-19 and H-17, respectively, and the vinylic hydrogens H-3, H-4, and H-5, appeared at δ 6.95 (dd), 6.58 (t), and 5.87 (t), respectively. Methine protons attached to oxygenated carbon atoms were well separated and their chemical shift values and assignments were established to be as follows: H-6 (δ 4.37, bd), H-7 (δ 5.26, bs), H-11 (δ 3.57, dd), H-12 (δ 3.43, dd), and H-15 (δ 4.35, m). The four methyl groups were assigned on the basis of their geminal or long-range couplings: 2-Me (δ 2.03, bs), 8-Me (δ 1.78, s), 10-Me (δ 1.02, d), and 14-Me (δ 0.87, d). From these data the coupling pattern for all of the hydrogens in herbimycin C [**3**] could be established (Figure 1). Assignments of the ^{1}H-nmr spectra of herbimycins A [**1**] and C [**3**] are shown in Table 1. Our data confirmed

TABLE 1. ^{1}H-Nmr Assignments for the Herbimycins.[a]

Proton	Compound			
	1	**3**	**5**	**6**
2-Me	2.02 (bs)	2.03 (bs)	2.05 (bs)	2.01 (bs)
H-3	6.98 (bd, 11.5)	6.95 (dd, 11.6, ~1)	7.01 (m)	6.94 (dd, 11.5, 1)
H-4	6.51 (t, 11.5)	6.58 (t, 11.6)	6.54 (t, 11.5)	6.49 (t, 11.9)
H-5	5.86 (dd, 11.5, 10.5)	5.87 (t, 11.6)	5.79 (t, 11.5)	5.84 (t, 11.5)
H-6	4.50 (bd, 10.5)	4.35 (bd, 11.5)	4.32 (bd, 11.5)	4.29 (bd, 11.5)
H-7	5.65 (bs)	5.26 (bs)	5.07 (bs)	5.14 (bs)
7-$OCONH_2$	4.86 (bs)	4.99 (bs)	4.97 (bs)	5.06 (bs)
8-Me	1.66 (bs)	1.78 (s)	1.75 (bs)	1.75 (s)
H-9	5.50 (m)	5.79 (bd, 8.6)	5.54 (m)	5.71 (bd, 8.5)
H-10	2.66 (m)	2.78 (ddd, 8.6, 7.0, 5.8)	2.87 (m)	2.80 (m)
10-Me	1.09 (d, 6.8)	1.02 (d, 7.0)	1.06 (d, 7.0)	1.07 (d, 7.0)
H-11	3.51 (m)	3.57 (dd, 8.2, 5.8)	3.50 (m)	3.48 (m)
H-12	3.45 (m)	3.43 (dd, 8.2, 3)	3.40 (m)	3.45 (m)
H-13	1.76 (m)	1.94 (ddd, 16.5, 10.0, 3)	2.01 (m)	1.95 (m)
H-14	1.61 (m)	1.74 (m)	1.65 (m)	1.67 (m)
14-Me	0.82 (d, 6.8)	0.87 (d, 6.8)	0.89 (d, 6.8)	0.80 (d, 6.8)
H-15	4.51 (bd, 8.5)	4.37 (m)	3.85 (m)	3.78 (m)
H-17	6.63 (t, 2.4)	6.63 (t, 2.4)	6.34 (bs)	6.37 (bs)
H-19	7.34 (d, 2.4)	7.47 (d, 2.4)	8.12 (bs)	8.08 (bs)
NH	8.81 (s)	8.60 (s)	8.42 (bs)	8.31 (bs)
OMe	3.54 (s), 3.35 (s)	3.37 (s), 3.33 (s)	3.36 (s) 2×	3.36 (s), 3.33 (s)
	3.35 (s), 3.32 (s)	3.28 (s)	3.30 (s) 2×	3.30 (s)

[a]Recorded in $CDCl_3$.

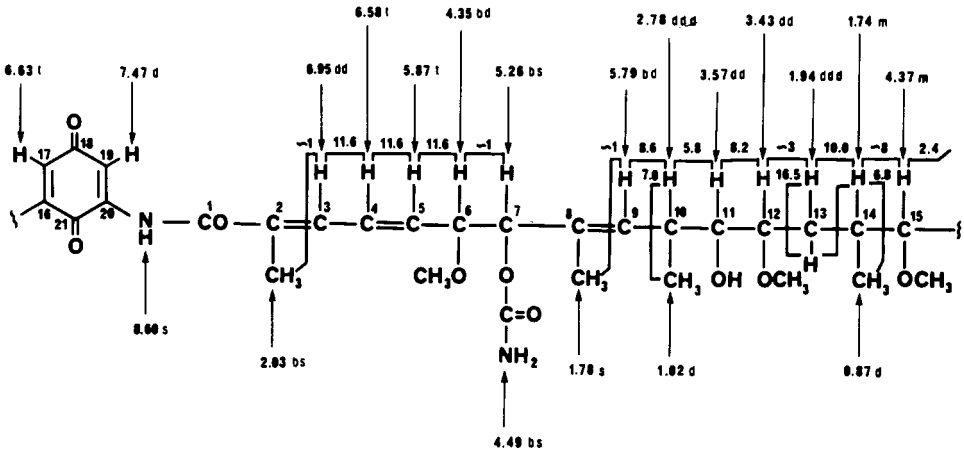


FIGURE 1. ^{1}H-^{1}H coupling pattern of herbimycin C [**3**].

the majority of the previous ^{1}H-nmr assignments for **1** (2) and **3** (4), although revisions were necessary for the assignments of H-11 and H-12.

The ^{1}H-nmr spectrum of dihydro-herbimycins A [**5**] and C [**6**] showed significant shifts for H-17 and H-19 as expected for the change of the *p*-quinone moiety to an aromatic system (Table 1). But resonances of the macrocyclic nucleus were essentially unaffected on replacement of the *p*-quinone by a *p*-hydroquinone moiety. Besides spectroscopic investigation, the structures of dihydro-herbimycins A [**5**] and C [**6**] were supported by chemical evidence. Herbimycins A [**1**] and C [**3**] were reduced to **5** and **6**, respectively, with $Na_2S_2O_4$, and, on the other hand, dihydro-herbimycins A [**5**] and C [**6**] were oxidized to **1** and **3**, respectively, using $FeCl_3$ in aqueous media. Products of these transformations were identified by co-tlc, ^{1}H-nmr, and by uv measurements.

It is known that herbimycins A [**1**] and C [**3**] exert strong herbicidal and cytotoxic activity (1,2,4). No biological data were previously reported (6) for dihydro-herbimycins A [**5**] and C [**6**]. In our laboratory all four isolated antibiotics [**1**, **3**, **5**, and **6**] were evaluated in the P-388 and KB lymphocytic leukemia test systems in vitro according to established protocols (7,8), and the data obtained are shown in Table 2. The replacement of a methoxy group at C-11 for a hydroxy group resulted in a decrease in activity in both the P-388 and KB test systems for the herbimycin and the dihydro-herbimycin series. Furthermore, the *p*-quinone type antibiotics exerted higher activity than that of their corresponding dihydro-derivatives in both the P-388 and KB test systems.

TABLE 2. Cytotoxicity Testing of the Herbimycins.

Compound	ED_{50}(μg/ml)	
	P-388	KB
1	0.0051	0.12
3	0.17	0.12
5	0.022	0.30
6	0.34	3.49

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—Melting points were determined using a Kofler hot-stage apparatus and are uncorrected. Uv spectra were recorded on a Beckman DU-7 spectrophotometer and ir spectra on a Nicolet MX-1 FT-IR interferometer. Mass spectra were recorded on a Varian MAT 112S in-

strument operating at 70 eV. Nmr spectra were collected on a Varian XL-300 instrument. Chemical shifts are reported in δ values. TMS was used as an internal standard. Homonuclear COSY spectra were recorded on the same instrument using standard Varian pulse programs.

FERMENTATION AND ISOLATION.—*S. hygroscopicus* AM 3672 was transferred into a 500-ml flask containing the following seed medium (100 ml): glucose 2.0%, starch 1.0%, soybean meal 2.0%, dry yeast 1.0%, fish flour 0.2%, $CaCO_3$ 0.6%, NaCl 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, K_2HPO_4 0.02%, pH adjusted to 7.2 before sterilization) and cultivated at 28° for 48 h. Seed culture (1 liter) was transferred into a stainless steel fermentor (100 liter) containing the following production medium (50 liters): glucose 1.0%, starch 4.0%, fish meal 0.4%, soybean oil 0.3%, dry yeast 0.2%, $CaCO_3$ 0.6%, NaCl 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, NH_4NO_3 0.2%, pH adjusted to 7.8 with 1 N NaOH before sterilization), and the fermentation was carried out at 28° for 4 days with agitation of 200 rpm and with forced aeration. The cultured cell broth was centrifuged and the supernatant was concentrated under reduced pressure to 20 liters. The antibiotics were extracted with EtOAc (30 liters), and the organic phase was washed with 5% $NaHCO_3$ solution and H_2O, dried, and evaporated. The residue was chromatographed on Si gel with $CHCl_3$-MeOH (95:5) to yield, in the order of elution, **1** (2.0 g), **3** (370 mg), **5** (190 mg), and **6** (40 mg). Mp, uv, and ir of the compounds isolated were in good agreement with the data reported for **1** (1,2), **2** (3), **5**, and **6** (6).

Compound **5**.—Mp 163–165°; uv λ max (MeOH) (log ε) 254 (4.25), 310 (3.92) nm; ir (KBr) ν max 1720, 1640, 1605 cm^{-1}; ^{1}H nmr see Table 1.

Compound **6**.—Mp 155–157°; uv λ max (MeOH) (log ε) 254 (4.23), 310 (3.89) nm; ir (KBr) ν max 1720, 1650, 1610 cm^{-1}; ^{1}H nmr see Table 1.

REDUCTION OF HERBIMYCINS.—Antibiotic **1** (or **3**) (10 mg) in EtOAc (10 ml) was added to a stirred 10% $Na_2S_2O_4$ solution (10 ml) for 5 min. Extraction with $CHCl_3$ and purification by preparative tlc resulted in **5** (or **6**) (6–7 mg), respectively. The isolated products were identified by co-tlc and ^{1}H-nmr spectral data.

OXIDATION OF DIHYDROHERBIMYCINS.—Antibiotic **5** (or **6**) (10 mg) in MeOH (10 ml) was added to 1% $FeCl_3$ solution (5 ml) and kept at room temperature for 1 h. The reaction mixture was extracted with $CHCl_3$ and purified by preparative tlc to give **1** (or **3**) (7–8 mg), identified by co-tlc and ^{1}H-nmr measurements.

ACKNOWLEDGMENTS

This work was supported, in part, by grant CA 20164 from the Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. The authors thank the Research Resources Center of the University of Illinois at Chicago for the provision of spectroscopic facilities and Dr. J.M. Pezzuto for cytotoxicity data.

LITERATURE CITED

1. S.Omura, Y. Ywai, Y. Takahashi, N. Sadakane, A. Nakagawa, H. Oiwa, Y. Hasegawa, and T. Ikai, *J. Antibiot.*, **32**, 255 (1975).
2. S. Omura, A. Nakagawa, and N. Sadakane, *Tetrahedron Lett.*, 4323 (1979).
3. Y. Iwai, A. Nakagawa, N. Sadakane, S. Omura, H. Oiwa, S. Matsumoto, M. Takahashi, T. Ikai, and Y. Ochai, *J. Antibiot.*, **33**, 1114 (1980).
4. K. Shibata, S. Satsumbayashi, A. Nakagawa, and S. Omura, *J. Antibiot.*, **39**, 1630 (1986).
5. A. Furusaki, T. Matsumoto, A. Nakagawa, and S. Omura, *J. Antibiot.*, **33**, 781 (1980).
6. T. Seiichi, M. Masayuki, and T. Hasegawa (Takeda Chemical Industries, Ltd.) Eur. Pat. Appl., EP 110,710 (1984); *Chem. Abstr.*, **101**, 108940z (1984).
7. R.I. Geran, N.H. Greenberg, M.M. McDonald, A.M. Schumacher, and B.J. Abbott, *Cancer Chemother. Rep. Part 3*, **2**, 1 (1972).
8. M. Arisawa, C.A. Bevelle, J.M. Pezzuto, and G.A. Cordell, *J. Nat. Prod.*, **47**, 453 (1984).

Received 4 April 1988