METABOLIC PRODUCTS OF MICROORGANISMS. 245[†] COLABOMYCINS, NEW ANTIBIOTICS OF THE MANUMYCIN GROUP FROM *STREPTOMYCES GRISEOFLAVUS*

II. STRUCTURE OF COLABOMYCIN A

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The structure of colabomycin A (**1**) was elucidated by a detailed spectroscopic analysis. Two-dimensional NMR spectroscopy experiments provided assignments of the proton and carbon resonances of the tetraene carboxamide chains occurring in **1**. The configurations of eight out of nine double bonds were determined by analysis of their coupling constants. The absolute configurations of C-4 (4*S*), C-5 (5*R*) and C-6 (6*S*) were established from the CD spectra of the parent compound and of 2-(6-oxo-2,4-hexadienoylamino)-5,6-epoxy-1,4-benzoquinone (**2**), which was obtained from **1** by mild chromic acid oxidation.

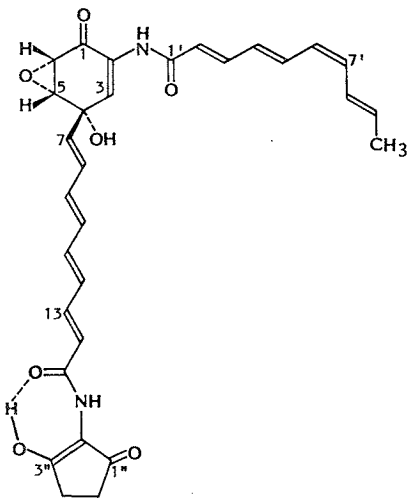
Colabomycin A (**1**), produced by *Streptomyces griseoflavus*, belongs to the manumycin group of antibiotics[1]. This paper deals with the determination of the chain lengths of the two polyene carboxamide units, the configurations of their double bonds, and the absolute configurations of the centers of chirality of **1**.

Structure of the Polyene Carboxamide Chains

A two-dimensional (2D) heteronuclear shift correlation (HETCOR) NMR experiment[2] (Fig. 1 and Table 1) provided correlations of resonances from seventeen olefinic protons with those of their corresponding directly bonded carbon atoms. From this experiment, assignments of ^{1}H and ^{13}C signals from oxirane carbons C-5 and C-6, as well as the terminal methyl group C-10′ were unambiguously confirmed. Two carbon signals were shown to coincide at δ 134 by their correlations with two proton resonances at δ 6.15 and δ 6.52. Signals for five of the olefinic protons composed a poorly-resolved envelope between δ 6.50 and δ 6.65. A long-range proton correlation detected in a COSY-90 experiment revealed a 2.5-Hz "W"-coupling between 5-H and 3-H (δ 7.33)[3]. In the C/H correlation experiment, the signal for 3-H correlated with a carbon signal at δ 129.5, thus providing the assignment of C-3. Most of the olefinic proton signals demonstrated extensive coupling, due to relatively large $^{3}J_{HH}$ couplings in the conjugated systems. The doublet character of three of the proton signals, at δ 6.71, 6.58, and 6.00 suggested that they represented protons at the end of chains. These three signals correlated to carbon signals at δ 122.5, 125.0, and 138.2, respectively.

Besides correlations attributed to vicinal couplings, a proton homonuclear shift correlation experi-

[†] See ref 1.



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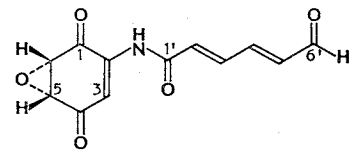

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Table 1. Summary of observed NMR connectivities in colabomycin A (**1**) with the ^{1}J- and ^{n}J-heteronuclear carbon-proton shift correlation methods.

Carbon No.	δ_C (ppm)	δ_H (ppm)	C,H-Coupling[a]	
			^{2}J	^{3}J
1	190.2		6-H	3-H/NH
2	129.1		3-H/NH	6-H
3	129.5	7.33		NH
4	71.6		5-H/7-H	
5	57.8	3.78		3-H
6	53.4	3.68		
7	138.2	6.00		
8	131.4	6.63[c]		10-H/12-H
9	136.7	6.58[c]		7-H/11-H
10[b]	134.1	6.52[c]	11-H	8-H/12-H
11	141.4	6.78		9-H/13-H
12	131.7	6.54[c]	13-H	14-H
13	143.3	7.35	12-H	11-H
14	122.5	6.71	13-H	12-H
15	167.1		14-H	13-H
1′	165.8		2′-H/NH	3′-H
2′	125.0	6.58[c]		4′-H
3′	142.1	7.31	4′-H	5′-H
4′	131.1	6.41		2′-H/6′-H
5′	136.1	7.23		3′-H/7′-H
6′	127.1	6.03		4′-H
7′[b]	134.1	6.15		5′-H/9′-H
8′	128.2	6.78	7′-H	6′-H/10′-H
9′	133.6	5.87		7′-H
10′	18.5	1.82	9′-H	

[a] Indicated protons show connectivities to the given carbon attributed to ^{2}J- or ^{3}J-coupling.
[b] Carbons show identical chemical shifts in the ^{13}C-dimension.
[c] Five proton signal cluster.

Fig. 1. Expansion of the downfield region of the ^1J-heteronuclear carbon-proton shift correlation NMR experiment of colabomycin A (**1**) in DMF-d_7 at 300 MHz.

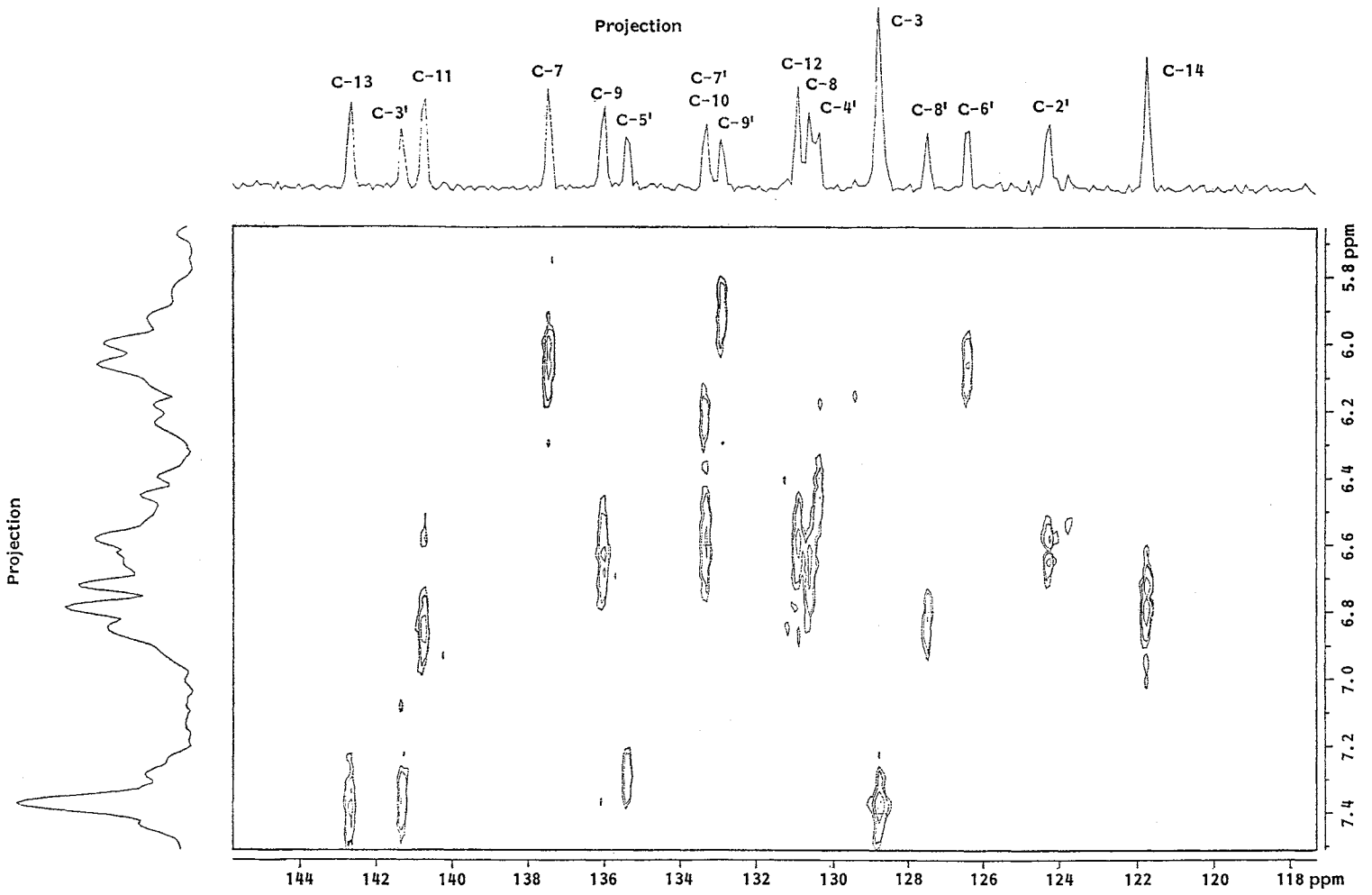


Fig. 2. Expansion of the downfield region of the 2D proton-proton double-quantum coherence (DQC) NMR experiment of colabomycin A (**1**) in DMF-d_7 at 500 MHz (straight lines; side-chain, broken lines; connecting chain).

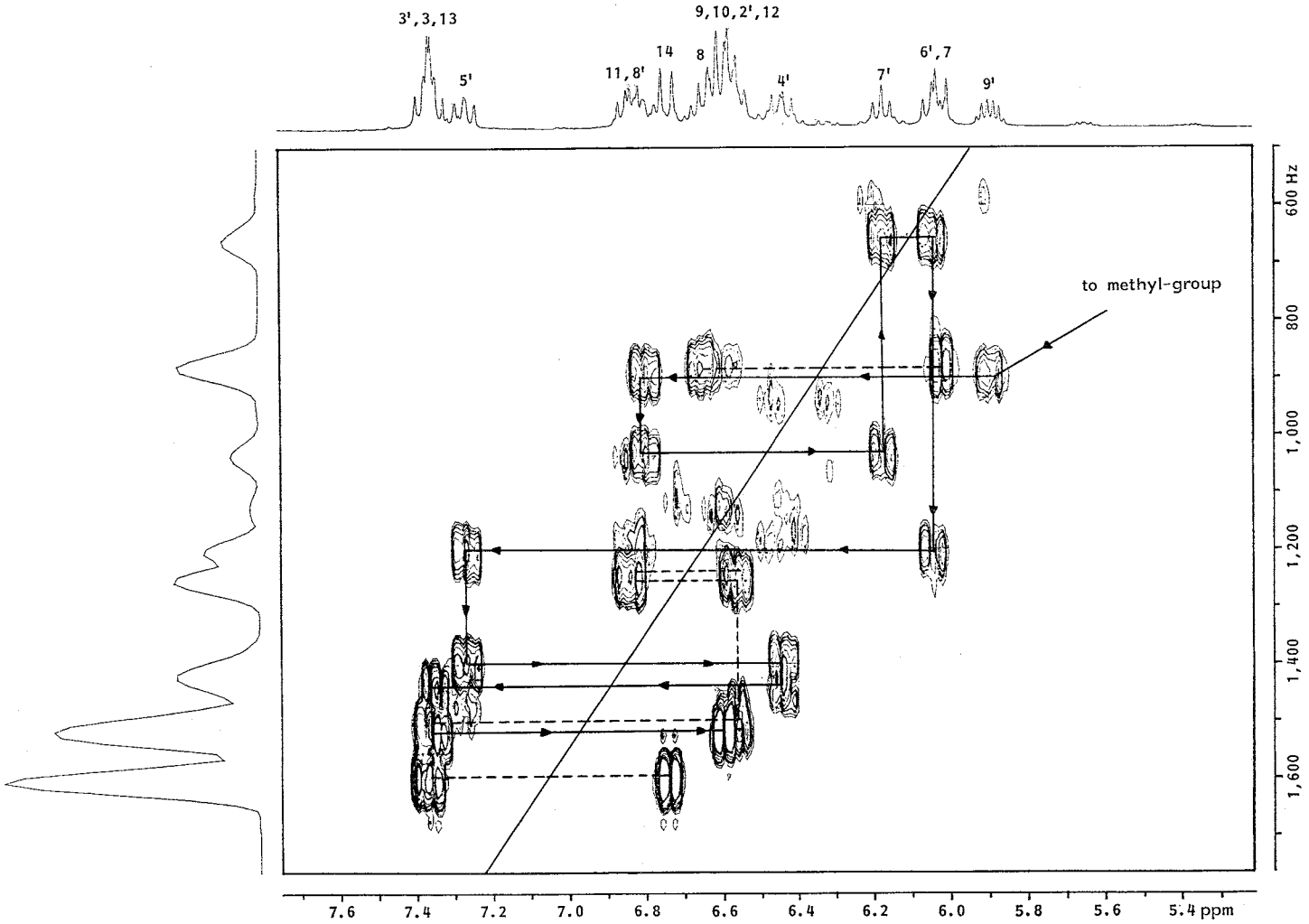


Table 2. Chemical shifts, double- and triple-quantum frequencies and virtual vicinal coupling constants of the olefinic protons in colabomycin A (1).

Proton No.	δ_H (ppm)	H (Hz)	(Hz)	DQC[a] (Hz)	TQC[a] (Hz)	TQC[b] (Hz)	$^3J_{H,H}$ (Hz)
9′	5.87	2,960	222	−1,580/894	−905	−910	nr
7	6.00	3,020	286	875			14.8
6′	6.03	3,030	297	655/1,203	1,561	1,560	12.6/11.1
7′	6.15	3,110	358	1,030/655	1,327	1,330	12.6/10.3
4′	6.41	3,225	492	1,398/1,438	2,345	2,350	14.8/11.4
10	6.52	3,295	552	1,127/1,246	1,821	—	nr
12	6.54	3,295	552	1,246/1,498	2,192	—	nr
9	6.58	3,310	575	1,164/1,127	1,716	—	nr
2′	6.58	3,315	575	1,521			14.8
8	6.63	3,325	589	875/1,164	1,450	—	nr
14	6.71	3,375	639	1,585			14.7
8′	6.78	3,410	672	894/1,030	1,252	1,245	15/11
11	6.78	3,425	694	1,246/1,246	1,798	1,800	14.6/11.1
5′	7.23	3,645	906	1,203/1,398	1,695	1,700	14.8/11.1
3′	7.31	3,680	946	1,498/1,585	2,137	2,140	nr
3	7.33	3,685	946				nr
13	7.35	3,690	946	1,438/1,521	2,014	2,010	nr

[a] Calculated.
[b] Observed.
—: Not interpretable due to higher order spin systems.
nr: Not resolved.

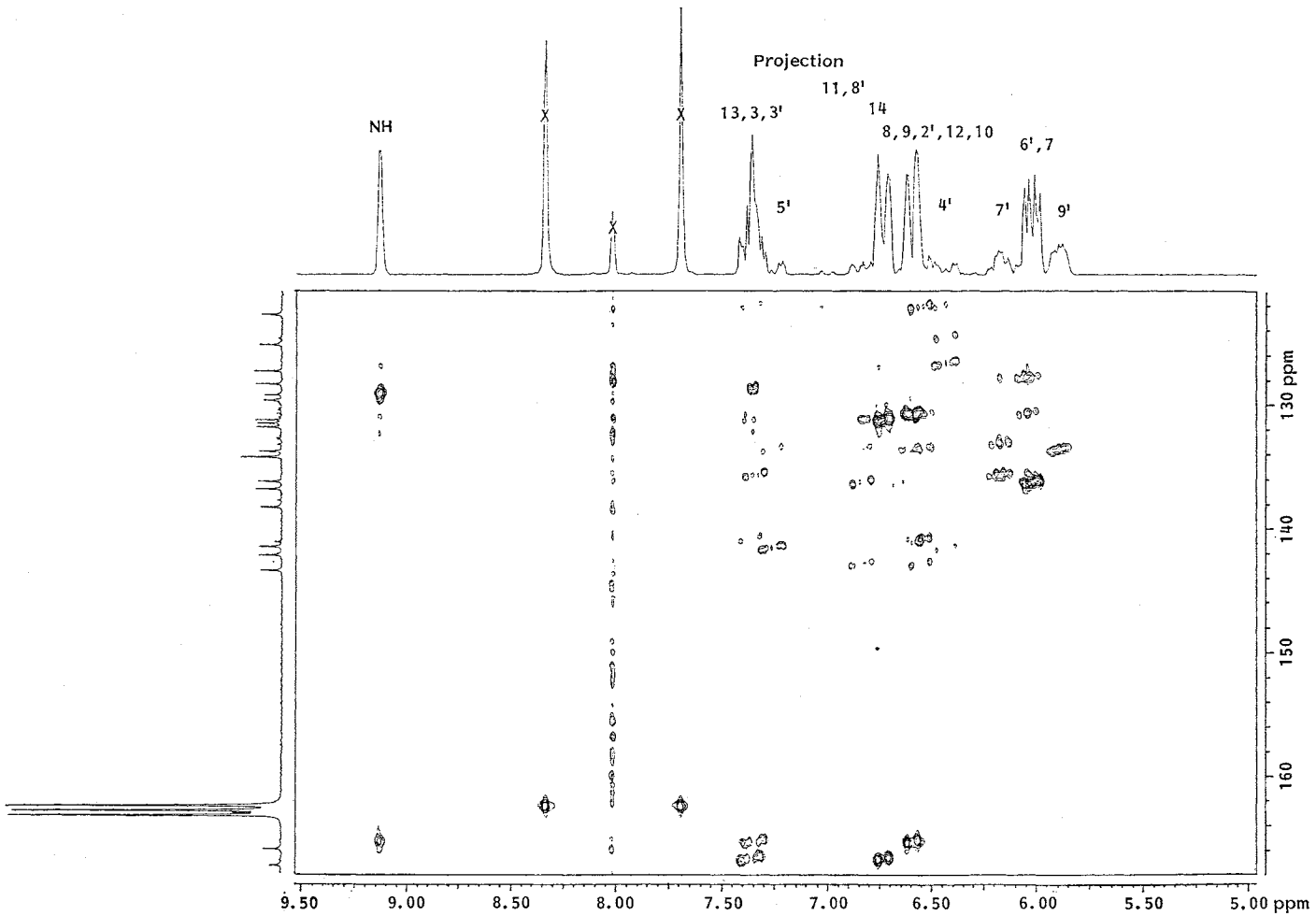
ment[4,5] (COSY) revealed additional cross peaks due to long-range couplings. The olefinic methyl group at δ 1.82 was found to correlate with signals at δ 5.87, 6.15, and 6.78. These signals represent olefinic methine protons one, two, and three carbon atoms away.

Determination of total proton connectivity patterns through the polyene carboxamide chains was not possible from the COSY data alone, due to the congested nature of the olefinic region and the limited resolution of the experiment. To accomplish this goal it was necessary to employ 2D multiple quantum NMR spectroscopy.

A proton homonuclear double-quantum coherence (DQC) experiment[6,7] (Fig. 2 and Table 2) allowed sequential connectivity patterns through vicinal olefinic protons to be traced relatively directly. Coupled protons share a unique double-quantum coherence with a frequency equal to the algebraic sum of the chemical shifts of the coupled protons relative to the transmitter. The double-quantum frequencies are given by the F1 dimension in a 2D experiment. In the F2 dimension, correlations for two coupled protons are found to lie equidistant from a center of gravity around a line with slope +/−2. By following these patterns, a connectivity map of the olefinic chains is readily generated. Chain termini are obvious by breaks in connectivity at doublet signals. A single chain ideally will give a single, unbroken map.

Starting with the terminal methyl group (10′-H_3, δ 1.82) eight resonances from 9′-H (δ 5.87) to the doublet of 2′-H (δ 6.58) could be assigned in continuity, allowing designation of the side-chain as a tetraene. Hence, it followed that the remaining eight olefinic protons composed a second tetraene, connecting the 5,6-epoxycyclohex-2-enone and 2-amino-3-hydroxycyclopent-2-enone moieties. It was possible to unambiguously assign signals for 7-H and 8-H, and the 10-H to 14-H segment (broken line, Fig. 2). A break in connectivity was found between 8-H and 10-H, so the assignment of 9-H

Fig. 3. Expansion of the downfield region of the ^{1}H-detected multiple bond heteronuclear multiple-quantum coherence (HMBC) NMR experiment of colabomycin A (**1**) in DMF-d_7 at 300 MHz.



was not readily obvious. Protons 7-H and 11-H gave correlations into the five-proton signal envelope around δ 6.6. The small differences in chemical shift between 8-H, 9-H, and 10-H provided a highly second order spin system, through which transmission of double-quantum coherence presumably was inefficient.

A proton heteronuclear triple-quantum coherence (TQC) NMR experiment[7] (Table 2) was employed to resolve the discontinuity. This experiment provided correlations among three continuous protons, and the overlapping patterns provided confirmations of the assignments made from double-quantum data. By analogy with the double-quantum experiment, the triple-quantum sequence correlates three-proton spin systems with their unique triple-quantum frequencies given by the algebraic sums of their chemical shifts relative to the transmitter. As shown in Table 2, double- and triple-quantum frequencies calculated from the chemical shift values are in good accordance with those observed along the F1 axes of the two experiments. The pairs of olefinic protons at the α- and β-positions to the amide carbonyls are more easily assigned using the TQC experiment, because of the significant differences of the chemical shifts of the α- and especially γ-protons (2′-H and 14-H, 4′-H and 12-H).

A ^{1}H-detected inverse heteronuclear multiple bond (multiple-quantum) coherence (HMBC) NMR experiment (Fig. 3 and Table 1) using the pulse sequence proposed by BAX and SUMMERS[8~10] allowed assignment of the amide carbonyl resonances for C-1′ (δ 165.8) and C-15 (δ 167.1) from the observed $^2J_{C,H}$- and $^3J_{C,H}$-couplings of 2′-H and 3′-H or 14-H and 13-H, respectively. The amide proton at δ 9.15 was assigned to the C-1′ amide group because of its $^2J_{C,H}$- and $^3J_{C,H}$-couplings to C-2/C-1′ and C-1/C-3, respectively. The observed $^2J_{C,H}$-coupling of 7-H to C-5 (δ_C/δ_H: 71.6/6.00) confirmed the point of attachment of the tetraene chain as C-4.

The configurations of most of the double bonds were assignable from their $^3J_{H,H}$-coupling constants, which were obtained from a resolution enhanced ^{1}H NMR spectrum and/or a 2D ^{1}H J-resolved spectrum. Comparison of the coupling constants within the doublet-of-doublets for 4′-H (J=14.8 and 11.4 Hz) observed on the one-dimensional ^{1}H NMR spectrum to those observed in the J-resolved experiment, clearly indicated that differences attributable to methodological errors were negligible. Table 2 presents the observed multiplicities and coupling constants. Four different types of vicinal couplings have to be considered for the tetraene units: The couplings *via* the double bonds (*E*: 14~16 Hz, *Z*: 10~12 Hz) and *via* single bonds (*s-trans*: 10.0~11.5 Hz, *s-cis*: 4~6 Hz)[11,12]. The observed coupling constants on 7-H, 11-H, 14-H, 2′-H, 4′-H, 5′-H, and 8′-H allowed assignment of the *E*-configuration to the corresponding double bonds. The 12.6 Hz coupling observed on 6′-H and 7′-H is much more likely for a *Z*-configuration. The *J*-resolved experiment revealed artefactual signals between these protons, indicating a non-first order spin system, although the difference in chemical shift relative to coupling constant meets the usually-prescribed first-order prerequisite ($\Delta\delta/J>5$; δ 0.12, 500 MHz; J=12.6). In a similar case BOLAND *et al.*[11] interpreted a 14.8-Hz coupling as the *E*-configuration ($\Delta\delta$ 0.13, 400 MHz). The configuration of the 9-H/10-H double bond could not be assigned from this data due to coincidental chemical shifts of both protons. Taken together, the coupling constants *via* single bonds indicate predominantly *s-trans* configurations.

Absolute Configuration at C-4, C-5 and C-6

The configuration at C-4 was obtained by the same method as published for asukamycin[13] and manumycin[14] using the exciton chirality method[15,16]. All prerequisites for employing this method

are fulfilled as in the case of these antibiotics. For **1** in acetonitrile an extremely strong positive CD-couplet ($[\theta]^{22}$ +313,400 at 370 nm, −354,200 at 328 nm) was observed, which is consistent only with the 4*S*-configuration. Thus, colabomycin A was shown to possess the same configuration at C-4 as asukamycin and the opposite of manumycin.

Because of the dominating exciton-coupling of the tetraene carboxamide chains, the CD spectrum of **1** did not allow any predictions about the stereochemistries of the centers of chirality of the oxirane ring. To eliminate this effect, **1** was degraded by a chromic acid oxidation[3,14,17]. Even after optimization of the conditions the oxidation resulted in a variety of products out of which **2** was isolated in a yield of 1.3%. The molecular formula ($C_{12}H_9NO_5$) was derived from the high-resolution electron impact mass spectrum (HREI-MS) (*m/z* 247, M^+), and a fragment ion was observed (*m/z* 109, $C_6H_5O_2$) indicating the side-chain residue. The ^1H NMR spectrum of **2** exhibited the resonances typical of 5-H (δ 3.88, J=3.5 and 2.0 Hz) and 6-H (δ 3.97, J=3.5 Hz)[14,17], as well as five olefinic resonances (3-H, 2′-H to 5′-H), a D_2O-exchangeable signal of an amide proton at δ 8.04, and an aldehyde proton at δ 9.74 (6′-H, J=7.5 Hz). These data confirmed the structure of **2** as 2-(6-oxo-2,4-hexadienoylamino)-5,6-epoxy-1,4-benzoquinone. Two cotton effects with opposite signs were observed at 370 nm (+15,200) and 322 nm (−26,500) as predicted for conjugated enediones[14]. Comparison with the CD data of similar oxidation products of manumycin[14,17] allowed assignment of **2** to the 5*R*/6*S*-configuration. Since degradation of colabomycin A to **2** does not effect the centers of chirality in the oxirane ring, the absolute configuration of **1** at C-5 and C-6 has also been assigned as 5*R*/6*S*.

Discussion

From the NMR spectroscopist's point of view colabomycin A (**1**) with its two asymmetric, independent tetraenes represents one of the most complicated polyene systems ever studied. All known reports on the NMR spectroscopic behavior of polyene natural products have dealt with simpler substances containing fewer total protons (*e.g.* giffordene[11]) or alkyl-branched chains (*e.g.* β-carotene[18] or all-*trans*-retinal[19]) with or without symmetry in the polyenic chains. Using recently-developed 2D NMR pulse sequences, it was possible to assign most of the olefinic methine protons of **1**. The observed vicinal coupling constants provided considerable evidence for the configuration of eight out of nine double bonds. Only the configuration of the 9-H/10-H double bond still remains uncertain. Further investigations were limited by coincidental signals resulting in unresolved non-first order spin systems, on one hand, and the instability and poor solubility of colabomycin A in solvents other than DMF on the other. According to the chirality at C-4, colabomycin A (**1**) resembles asukamycin[13] more closely than manumycin[14].

Experimental

General and Analytical

See ref 1.

NMR Measurements

All 2D NMR experiments were performed with 13 mg **1** dissolved in 0.7 ml DMF-d_7 at 23°C. Experiments were performed either with an IBM AF-300 instrument operating at a field strength of 7.1T and equipped with an Aspect 3000 data system with array processor and a process controller capable on non-90 degree RF phase shifts, or a similarly equipped Bruker AM-500 instrument operating at 11.83T. In the COSY-90 experiment at 11.83T, a 4K×2K data matrix was accumulated at a sweep width of 5,700 Hz. Acquisition times were therefore 0.36 second in both dimensions. The spectrum was processed with nonshifted sine-bell windows, and a magnitude calculation was employed.

In the DQC and TQC experiments (11.83T), F_1 and F_2 spectral widths were set to 5,100 Hz with

the carrier frequency positioned at 5.41 ppm. 2K data points were recorded in t_2 in the quadrature detection mode, and 256 FIDs (zero-filled to 1K) were recorded in t_1 (16 scans each, preceeded by 2 dummy scans). Acquisition times were 0.2 second in F_2 and 0.1 second in F_1. A relaxation delay of 2 seconds between pulses was employed. The multiple-quantum evolution delay was set at 9.62 mseconds. In both multiple-quantum experiments nonshifted sine-bell multiplication was applied before Fourier transformation in both time domains. In both cases a power spectrum presentation was selected.

The data matrix of the $^1J_{C,H}$-shift correlation (7.1T) experiment consisted of 4K×1K data points in f_2 (14,286 Hz) and f_1 (1,322 Hz). Acquisition times were 0.14 second in F_2 and 0.19 second in F_1. For each t_1 increment, 256 scans (preceeded by 2 dummy scans) were recorded and the delay time between scans was 0.5 second.

The HMBC (7.1T) experiment employed a 2K×1K data matrix with 80 scans per t_1 increment (preceeded by 2 dummy scans) and a delay time between the scans of 1 second. The spectral width in F2 (proton dimension) was 2,747.3 Hz, and in F1 (^{13}C dimension) was 14,285.714 Hz. Acquisition times therefore were 0.072 and 0.37 seconds in t_1 and t_2 dimensions, respectively. The ^{13}C carrier was generated by a PTS-160 synthesizer operating at 75.4767 MHz, and ^{13}C pulses were amplified and controlled by the BSV-3 X-nucleus decoupler. The Δ_1 and Δ_2 durations were set to 3.3 and 60 msecconds, respectively. Pi/3-shifted sine-bell squared multiplication was employed in both time domains before Fourier transformation.

2-(6-Oxo-2,4-hexadienoylamino)-5,6-epoxy-1,4-benzoquinone (2)

At room temperature 20 ml of 90% aqueous acetic acid and 1 ml of THF were added to a mixture of 250 mg of colabomycin-complex[1] and 109 mg of CrO_3. After 2 minutes the stirred mixture exhibited a red-brown color. One ml of THF was added 8 minutes later and after 10 minutes the reaction mixture was treated in an ultrasonic bath for 3 minutes. Ten minutes later 40 mg CrO_3 was added, and after 50 minutes total the solution was quenched with 40 ml 2 N HCl, stirred for 5 minutes and extracted four times with 40 ml ether. The combined organic layers were washed with water, dried over sodium sulfate and evaporated. To remove traces of acetic acid 5 ml of toluene were added twice and the solution evaporated *in vacuo*. The brown oily residue was chromatographed on a low-pressure (1.7 bar) silica gel column (15×2.5 cm, $CHCl_3$ - MeOH, 98 : 2). Repeated chromatography on silica gel (20×1.5 cm, hexane - EtOAc, 2 : 1) and Sephadex LH-20 (20×1 cm, $CHCl_3$) yielded 1.5 mg **2**: Rf 0.42 ($CHCl_3$ - MeOH, 9 : 1); UV $\lambda_{max}^{CH_3CN}$ nm (ε) 323 (12,000), 283 (15,000), 242 (6,500); ^{1}H NMR (200 MHz, $CDCl_3$) δ 3.88 (1H, dd, J=3.5 and 2.0 Hz, 5-H), 3.97 (1H, d, J=3.5 Hz, 6-H), 6.45 (1H, d, J=15 Hz, 2′-H), 6.52 (1H, dd, J=15 and 7.5 Hz, 5′-H), 7.21 (1H, dd, J=15 and 11 Hz, 4′-H), 7.55 (1H, dd, J=15 and 11 Hz, 3′-H), 7.67 (1H, d, J=2.0 Hz, 3-H), 8.04 (1H, b, NH, exchangeable with D_2O), 9.74 (1H, d, J=7.5 Hz, 6′-H); EI-MS (70 eV) m/z (abundance) 247 (6%, M^+, HR calcd for $C_{12}H_9NO_5$ and found: 247.0481), 109 (100%, HR calcd for $C_6H_5O_2$ and found: 109.0289); CD $\lambda_{extreme}^{CH_3CN}$ nm ($[\theta]^{22}$) 370 (+15,200), 322 (−26,500), 283 (+10,600), 232 (−3,300).

Acknowledgment

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