STUDIES ON DIENOMYCINS. II

CHEMICAL STRUCTURES OF DIENOMYCINS A, B AND C

SUMIO UMEZAWA, KUNIAKI TATSUTA,
YUKIO HORIUCHI and TSUTOMU TSUCHIYA

Department of Applied Chemistry, Faculty of Engineering,
Keio University, Koganei-shi, Tokyo, Japan

HAMAO UMEZAWA

Institute of Microbial Chemistry, Shinagawa-ku, Tokyo, Japan

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The chemical structures of dienomycins A, B and C were established mainly by the studies of their NMR spectra as follows: A, 4-isobutyroyloxy-3-methyl-2-(4-phenylbutadienyl)piperidine; B, 4-acetoxy-3-methyl-2-(4-phenylbutadienyl)piperidine; C, 4-hydroxy-3-methyl-2-(4-phenylbutadienyl)piperidine.

Isolation, characterization, biological activities and the gross chemical structures of dienomycins A, B and C were described in the preceding paper[1]. In this paper, determination of their chemical structures by their NMR spectra is reported.

Treatment of dienomycins A and B with sodium methoxide in methanol resulted in the formation of the same de-O-acylated product, which was identified as C by IR, UV and NMR spectra, thin-layer chromatographic pattern and other physical properties. Therefore, the NMR spectrum of C was subjected to detailed analysis.

Results and Discussion

The NMR spectra were measured by a Varian HA 100 spectrometer (100 MHz) in deuteriochloroform with a trace of deuterium oxide and tetramethylsilane was used as an internal reference. The NMR spectrum of dienomycin C free base is shown in Fig. 1 and that of A hydrochloride in Fig. 2.

In the preceding paper, we presented the gross structures for dienomycins A, B and C to be methyl-(4-phenyl-1,3-butadienyl)-piperidine bearing an acyloxyl (in A and B) or a hydroxyl group (in C). The remaining problems are to determine the positions of the methyl, 4-phenylbutadienyl and acyloxyl or hydroxyl groups in the piperidine ring and their configurations (Chart 1).

In the first place, the position connecting the phenylbutadienyl group to the piperidine ring was determined. In Fig. 1, the signals centered at δ 5.72 lie at the highest-field among the signals belonging to olefinic protons and have a quartet (J, 15.0 and 8.6 Hz; the lower-field two peaks are more intense and each

Chart 1.

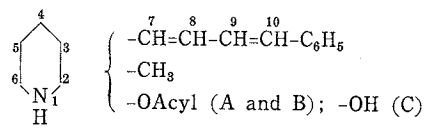
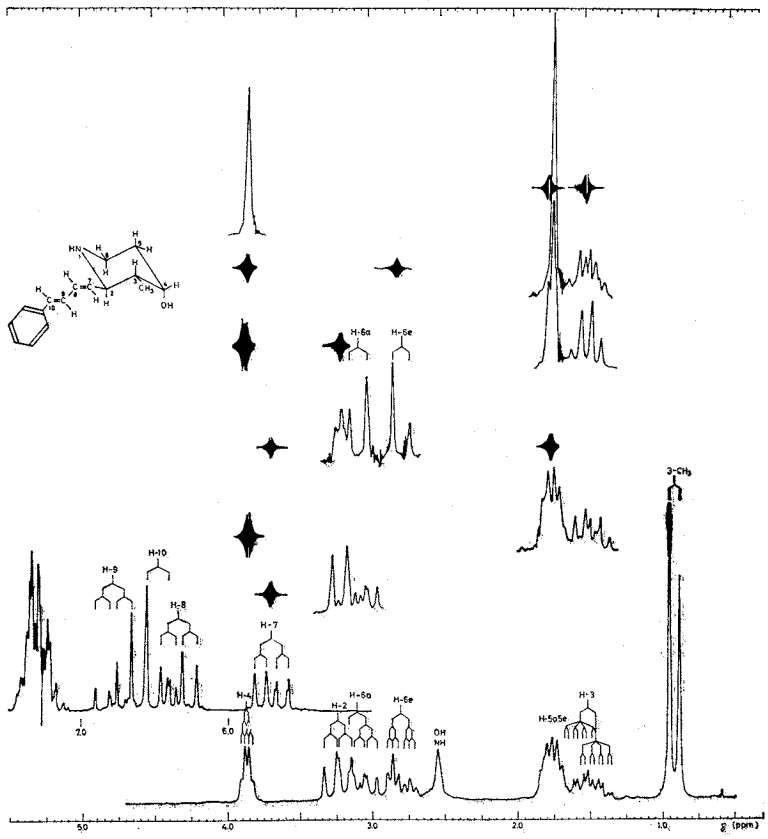


Fig. 1. NMR spectrum of dienomycin C free base in $CDCl_3$.



peak has a small splitting by a long range coupling). When irradiated at this point, a quartet centered at δ 3.22 (J, 10.0 and 8.6 Hz) collapsed to a doublet (J, 10 Hz) without disturbing the other signals in the higher-field than δ 5. Since the proton at δ 3.22 clearly belongs to piperidine ring from the shift-value and the splitting pattern, the proton at δ 5.72 is determined reasonably as H-7 (see Chart 1); the former proton (δ 3.22) is tentatively named as H-z for the convenience of our description. Therefore, a partial structure $>CH_z-CH_7=CH-$ is assumed.

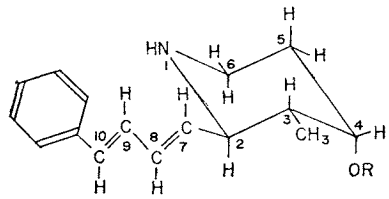
On looking through the NMR spectrum of dienomycin C, two characteristic signal-groups (one proton each) are recognized at δ 3.87 and 1.52. The former, which is isolated from the other signals and resonates at the lowest field other than olefinic and aromatic proton signals, will represent a methine proton bearing a hydroxyl group (this proton is tentatively named as H-x). When the NMR spectra of dienomycins A and B (see Figs. 4 and 5 in the preceding paper) are examined, very similar signals to that of H-x make their appearance at δ 5.1 (both A and B); this considerable down-field shift (1.2 ppm) from δ 3.87 is reasonable if the hydroxyl group in C is esterified into acyloxyl groups in A and B. This conclusion is further confirmed by decoupling technique as described later. Concerning the latter signal-group (δ 1.52), this is assigned to the methine proton bearing a methyl group ($-CH_y(CH_3)-$;

the proton is tentatively named as H-y) from the consideration of the shift-value and the multiple splitting pattern; this is further confirmed by irradiation at H-y, when the methyl doublet at δ 0.92 (J, 6.9 Hz) collapsed to a singlet.

In the next step, the isolated H-x (δ 3.87) was irradiated, when H-y having a doublet of double quartets (δ 1.52; J, 2.5, 6.9 and 10.0 Hz) collapsed to a double quartet (J, 6.9 and 10.0 Hz) and at the same time, methylene signals at δ 1.7~1.85 (2-protons, tentatively named as H-5′ and H-5″; these are later assigned to H-5_a and H-5_e, respectively) collapsed to a simpler pattern; and no change occurred in the other regions. From this observation, a sequence of $-CH_2-CH_x(OH)-CH_y(CH_3)-CH_{z'}$, in which H-x and H-y are vicinally situated, and H-y has another vicinally situated hydrogen (this is named H-z′), is suggested. Furthermore, from the fact that the coupling constants between three methine protons are $J_{x,y}$ 2.5 Hz and $J_{y,z'}$ 10.0 Hz and also on a reasonable assumption that the piperidine ring has the chair conformation, it can be assumed that H-z′ and H-y protons lie in a diaxial (*trans*) relationship, and H-y and H-x protons lie in an axial-equatorial (*cis*) relationship.

To confirm the presence of the proposed sequence, the following experiments were performed. Simultaneous irradiation at Hx (δ 3.87) and H-z (δ 3.22) caused H-y (δ 1.52) to change into a clear quartet (J, 6.9 Hz) and the methylene signals (H-5′, 5″, δ 1.7~1.85) to change into more decoupled peaks than the original. Also, simultaneous irradiation at H-x and at δ 2.82 (tentatively named H-6″; this is later assigned to H-6_e), caused H-5′, 5″ methylene signals to change into a singlet in appearance and H-y into a pattern more decoupled than the original but more complex than that obtained by the above irradiation experiment. Although the irradiation at δ 3.22 (H-z) or δ 2.83 (H-6″) inevitably causes the concommitant irradiation of the neighbouring protons preventing to give a definite answer, the comparison of both of the irradiation results leads to the conclusion that H-y is really coupled with H-z as well as with H-x and the methylene (H-5′, 5″) is at least coupled with H-6″. As the result, H-z equalls to H-z′ is concluded. To confirm further these relationships, simultaneous irradiation at H-y and the methylene (H-5′, 5″) was conducted, when H-x gave a sharp singlet. At the same time, from the small coupling constants of H-x with the methylene (H-5′, 5″) and with H-y (each of them has J ~2.5 Hz), equatorial H-x and consequently an axial OH are again ascertained. Thus, a partial structure $>CH_{6''}-CH_{5'}H_{5''}-CH_x(OH)-CH_y(CH_3)-CH_z(CH_7{=}CH-)-$ is established.

The remaining signals to be analyzed are the signals ranging between δ 2.7~3.2 (2-protons), although the signals centered at δ 2.8 (H-6″, doublet of triplets, J, 11.5, 3.6, 3.6 Hz) was proved already to be vicinal to H-5′, 5″-methylene. On irradiation at δ 1.77 (H-5′, 5″) the protons (δ 2.7~3.2, tentatively named H-6′ and H-6″) of this region gave 2-proton AB quartet ($J_{6',6''}$ 11.5 Hz). This finding and the rather high δ-values of H-$_a$



A: R = $OCCH(CH_3)_2$
B: R = $OCCH_3$
C: R = H

Table 1. Analyses of NMR spectra of dienomycin C free base and A hydrochloride in $CDCl_3$ and a trace of D_2O (100 MHz)

Assignment	Dienomycin C free base			Dienomycin A hydrochloride		
	δ	Signal pattern	J (Hz)	δ	Signal pattern	J (Hz)
H-2	3.22	q	$J_{2,3}=10.0$ $J_{2,7}=8.6$	3.62	q	$J_{2,3}=11.0$ $J_{2,7}=8.9$
H-3	1.52	m	$J_{3,3CH_3}=6.9$ $J_{3,4}=2.5$	2.37	m	$J_{3,4}=2$ $J_{3,3CH_3}=6.9$
H-4	3.87	q	$J_{4,5e}$ and $J_{4,5a}=$ 2.5	5.06	m	
H-5e H-5a	1.77(2H)	m		1.88 2.1~2.6	d(not sharp)	$J_{5e,5a}=\sim14$
H-6e	2.82	dt	$J_{5e,6e}$, $J_{5d,6e}=3.6$ $J_{6e,6a}=11.5$	3.31		$J_{5a,6a}$, $J_{6e,6a}=\sim13$
H-6a	3.12	dq	$J_{5e,6a}=\sim7$ $J_{5a,6a}=\sim9$	3.08	m	
H-7	5.72	q	$J_{2,7}=8.6$ $J_{7,8}=15.0$	5.87	q	$J_{7,8}=14.2$ $J_{2,7}=8.9$
H-8 H-9 H-10	6.31 6.74 6.50	q q d	$J_{8,9}=10.0$ $J_{9,10}=15.0$	6.4~7.0 (3H)	m	
C3-CH_3	0.92	d	$J_{3,3CH_3}=6.9$	0.84	d	$J_{3,3CH_3}=6.9$
Phenyl	7.1~7.45	m(5H)		7.1~7.5	m(5H)	
4-OH 1-NH	2.55	s(2H)				
4-OCOCH(CH_3)$_2$				CH_3 CH	1.14, 1.16 2.57	each doublet $J=7.1$ septet

Abbreviations: s, singlet; d, doublet; q, quartet; m, multiplet; dt, doublet of triplets; dq, doublet of quartets.

6′ (δ 3.12) and H_e–6″ (δ 2.82) suggest that H-6′ and H-6″ are geminal protons situated vicinally to H-5′ and 5″ and also to the imino group of piperidine ring. Coupling constants relating to H-6′ are estimated to be $J_{5'',6'}\sim7$ and $J_{5',6'}\sim9$ Hz.

From all of the results described above, the protons of H_x, H_y, H_z, H-5′, 5″, H-6′ and 6″ will prove to be H-4, 3, 2, 5a, 5e, 6a and 6e, respectively (see Chart 1) and the above structures or their enantiomers are assigned to dienomycins A, B and C.

Incidentally we will mention briefly the olefine region of the NMR spectrum. Although spin-decoupling studies were not made in this region, relative signal intensities and clear-cut coupling patterns made the analysis easy and the assignments as shown in Fig. 1 were made. By taking the large coupling constants ($J_{7,8}$ 15 Hz, $J_{8,9}$ 10 Hz and $J_{9,10}$ 15 Hz) into consideration, *trans-trans* configuration of the diene group is confirmed in agreement with the result derived from UV spectrum.

Concerning the low-field position of the axial H-6a relative to the equatorial H-6e, this unusual position will be explained[2] in terms of the proximity of H-6a to the axial hydroxyl group at C-4.

All data concerning the NMR spectrum are described in Table 1.

In the next step, the NMR spectrum of dienomycin A was considered, in which the rationale employed in the analysis of the spectrum of C was applied, and the assignments of all protons were made as shown in Fig. 2. Some irradiation results

Fig. 2. NMR spectrum of dienomycin A hydrochloride in $CDCl_3$.

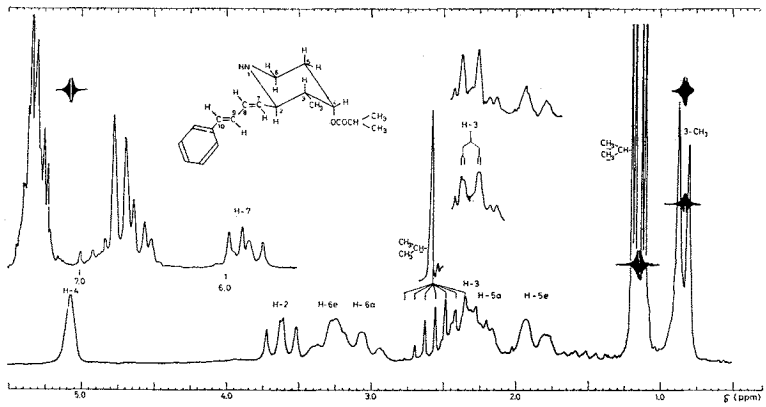
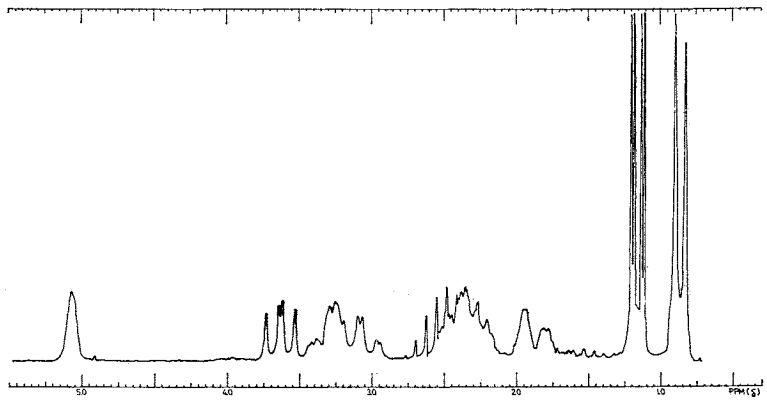


Fig. 3. NMR spectrum of dienomycin A hydrochloride in $CDCl_3$ at 70°C.



will be described below. Irradiation at δ 0.84 (C3-CH_3) caused the multiplet at δ 2.37 to change into a deformed doublet, which, on simultaneous irradiation at δ 0.84 and 5.06 (H-4), gave a sharpened doublet (J 11 Hz), the value being in good agreement with that of axial-axial (H-2, 3) coupling. Considerable downfield shift (0.85 ppm) of H-3 from that of C may be caused by C-4 ester group. Irradiation at δ 1.15 ($(C\underline{H}_3)_2CH$) collapsed the septet at δ 2.57 (J 7 Hz) to a singlet, indicating that this may be the methine signal of a isopropyl group.

Although the spectrum of A hydrochloride showed rather broad and overlapped pattern at room temperature, elevation of temperature to 70°C improved the situation (see Fig. 3); this suggests that the slow interconversion between conformations of the piperidine ring is effected at room temperature.

In order to confirm the piperidine structure, B was acetylated to give an N-acetylated derivative, in which no absorption band at ~3300 and ~1550 cm^{-1} in the IR spectrum was found, indicating the absence of any amide N-H.

Experimental

De-O-acylation of dienomycins A and B: To a solution of dienomycin A hydrochloride (1.16 g) in dry methanol (25 ml) was added a solution (6 ml) of 2 N sodium

methoxide in methanol and allowed to stand at 37°C for 5 days. Evaporation *in vacuo* gave a residue, which was extracted with benzene (80 ml). The extracts, which showed virtually a single spot (Rf 0.35) on thin-layer chromatography with silica gel (methanol – benzene – ethyl acetate (5 : 2 : 2) with coloration by sulfuric acid), were evaporated and the residue was chromatographed on a silica gel column (Mallinckrodt 60 g, 2.8×22 cm) with the same solvent system. The substance having an Rf value of 0.35 appeared in the fraction of 170~500 ml, which was evaporated to give a residue. The solution of the residue in benzene was filtered from any insoluble materials and concentrated; addition of isopropylether gave a de-O-acylated product; yield 620 mg (72 %); which is positive for WOOD, EHRLICH and RYDON-SMITH reagents; mp 130~131°C, $[\alpha]_{589}^{20}$ +85° (*c* 1.0, methanol); IR spectrum (KBr disk): ~3400 (OH); 3320 (NH); 3100~2800 (CH); 1640, 998, 985, 965 (diene); 1595, 747, 692 cm^{-1} (phenyl); UV spectrum: λ_{max}^{MeOH} (ε) 210.5 (15,800), 221 (14,200), 227 (14,200), 234 (9,600), 280 (sh., 36,900), 287 (39,400), 297 (sh., 30,300), 307 mμ (sh., 17,100). Found: C 78.62, H 9.08, N 5.64, O 6.68. Calcd. for $C_{16}H_{21}NO$: C 78.97, H 8.70, N 5.76, O 6.58 %.

Dienomycin B hydrochloride was treated in a similar manner and gave a de-O-acylated product which has the same physical characteristics as that derived from dienomycin A. Both de-O-acylated products showed chemical and physical properties identical with dienomycin C free base.

Acetylation of dienomycins B and C into 4-acetoxy-N-acetyl-3-methyl-2-(4-phenylbutadienyl)piperidine (N-acetyldienomycin B): A suspension of dienomycin B hydrochloride (1.38 g) in pyridine (10 ml) and acetic anhydride (7 ml) was stirred at room temperature overnight. To the resultant clear solution was added a small volume of water and the mixture was evaporated *in vacuo* to give a brown syrup, which showed virtually a single spot (Rf 0.41) on thin-layer chromatogram of silica gel (benzene – ethyl acetate 2 : 1). The syrup was then chromatographed on a silica gel column (85 g, 2×26 cm) with the same solvent system. The substance (Rf 0.41) eluted between 240~460 ml was evaporated to give a pale yellow syrup, which was dissolved in ethyl acetate and treated with charcoal. Evaporation of the solution at 50°C under reduced pressure (1 mmHg) gave a colorless syrup, 1.27 g (91 %); $[\alpha]_{589}^{25}$ −38° (*c* 1, methanol); IR spectrum (KBr disk): 3040~2875 (CH); 1738, 1370, 1240 (OAc); 1655, 1645 (amide I); 752, 690 cm^{-1} (phenyl); NMR ($CDCl_3$): δ 2.06 (3-proton singlet, OAc), δ 2.15 (3-proton singlet, NAc). Found: C 72.95, H 7.82, N 4.05. Calcd. for $C_{20}H_{25}NO_3$: C 73.36, H 7.70, N 4.28 %.

A suspension of dienomycin C hydrochloride (96 mg) was acetylated with acetic anhydride (0.5 ml) in pyridine (1 ml) as above to give a syrup, 93 mg (83 %), $[\alpha]_{589}^{25}$ −38° (*c* 1, methanol). The product showed identical Rf-value (0.41), IR and NMR spectra with those of the product derived from dienomycin B.

Benzoylation of dienomycin B: To a suspension of dienomycin B hydrochloride (400 mg) in pyridine (2 ml) was added benzoyl chloride (1 ml) and the mixture was stirred at room temperature overnight. The reaction mixture was filtered to remove some insoluble matter, and to the filtrate was added a small volume of methanol. Evaporation of the mixture gave a syrup (790 mg), which showed virtually a single spot (Rf 0.54) on thin-layer chromatogram of silica gel (benzene – ethyl acetate 8 : 1). The syrup was then chromatographed on a silica gel column (80 g, 3×22 cm) with the same solvent system. The substance (Rf 0.54) eluted between 220~380 ml was evaporated to give a solid, which was dissolved in ethyl acetate and treated with charcoal. After removal of the charcoal, the solution was concentrated. Cooling at 5°C overnight gave the titled compound, 320 mg (66 %), m. p. 41~42°C $[\alpha]_{589}^{23}$ −181° (*c* 1, methanol); IR spectrum (KBr disk): 3020~2900 (CH); 1738, 1370, 1235 (OAc); 1640, 1630 (amide I); 1600, 750, 700~690 cm^{-1} (phenyl). Found: C 76.80, H 6.94, N 3.74. Calcd. for $C_{25}H_{27}NO_3$: C 77.09, H 6.99, N 3.60 %.

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