STRUCTURES OF NEW ANTIBIOTICS NAPYRADIOMYCINS

KAZURO SHIOMI, HIKARU NAKAMURA, HIRONOBU IINUMA,
HIROSHI NAGANAWA, KUNIO ISSHIKI, TOMIO TAKEUCHI
and HAMAO UMEZAWA

Institute of Microbial Chemistry
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

YOICHI IITAKA

Faculty of Pharmaceutical Sciences, University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

(Received for publication December 26, 1985)

Structures of novel antibiotics, napyradiomycins A, B1, B2, B3, C1 and C2 were determined. By X-ray crystallography, napyradiomycin B2 was determined to be (3*R*,10a*R*)-3-chloro-10a-[[(1*R*,3*S*)-3-chloro-2,2-dimethyl-6-methylenecyclohexyl]methyl]-3,10a-dihydro-6,8-dihydroxy-2,2-dimethyl-2*H*-naphtho[2,3-*b*]pyran-5,10-dione. The structures of other napyradiomycins were elucidated by NMR studies. Napyradiomycins C1 and C2 have unique structures which contain 14-membered ring cyclized by carbon-carbon bond.

Napyradiomycins A, B1, B2, B3, C1 and C2 (Fig. 1) were isolated from a culture broth of *Chainia rubra* MG802-AF1[1] and inhibited the growth of Gram-positive bacteria. We will report herein on the structure determination of these antibiotics.

The same chromophore was shown by their UV and NMR spectra. The structure of napyradiomycin B2 which was crystallized as pale yellow needles was determined by X-ray analysis. The assignment of signals on ^{1}H and ^{13}C NMR of B2 was established by the aid of ^{1}H-^{1}H shift correlation spectrum (^{1}H-^{1}H COSY) and ^{1}H-^{13}C shift correlation spectrum (^{1}H-^{13}C COSY). Based on the NMR data including NMR of B2, the structures of other napyradiomycins were determined.

The Structure of Napyradiomycin B2

The structure of B2 ($C_{25}H_{28}O_5Cl_2$) was determined by X-ray analysis to be (3*R*,10a*R*)-3-chloro-10a-[[(1*R*,3*S*)-3-chloro-2,2-dimethyl-6-methylenecyclohexyl]methyl]-3,10a-dihydro-6,8-dihydroxy-2,2-dimethyl-2*H*-naphtho[2,3-*b*]pyran-5,10-dione (Fig. 2). The X-ray crystallographic data were as follows.

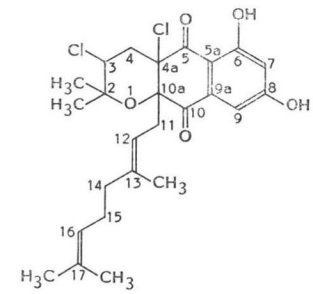
The crystals were grown in methanol solutions as aggregates of thin plates pale yellow in color. A small crystal of approximate dimensions $0.25 \times 0.08 \times 0.08$ mm was mounted on a Philips PW 1100 diffractometer and the diffraction intensities were measured with graphite monochromated CuKα radiation. Crystal data: Napyradiomycin B2, $C_{25}H_{28}O_5Cl_2 \cdot CH_3OH$, FW=511.4. Orthorhombic, space group $P2_12_12_1$, $Z=4$. Cell dimensions, $a=9.179$ (5), $b=37.939$ (20), $c=7.357$ (5) Å, $U=2562.0$ Å^3. $D_{calc}=1.326$ gcm^{-3}, μ for CuK$\alpha=26.29$ cm^{-1}.

Intensities of 1246 reflections were measured as above the 2σ(I) level out of 2264 within the 2θ range of 6° through 120°. A total of 220 Friedel reflections were measured for hk1 and hk2 at the medium 2θ angle. The Rf values for symmetry equivalent and Friedel pair reflections were 0.03 and 0.05, respectively.

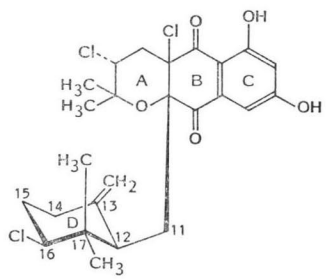
The structure was determined by the direct methods and refined by the block-diagonal least-squares

Fig. 1. The structures of napyradiomycins.

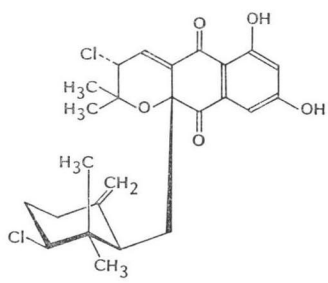
The carbon numbers apply to the assignment of NMR and they are not identical with the number of nomenclature or the description of the crystallographic analysis.



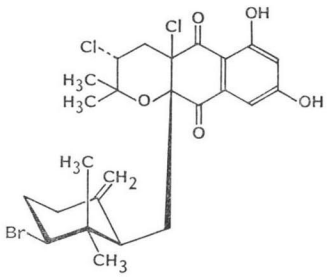
Napyradiomycin A



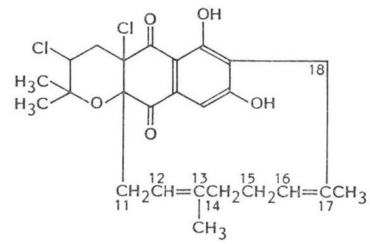
Napyradiomycin B1



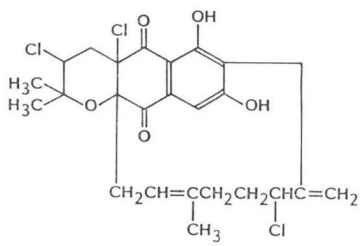
Napyradiomycin B2



Napyradiomycin B3



Napyradiomycin C1



Napyradiomycin C2

Fig. 2. Molecular structure drawn by PLUTO program.

PLUTO: "Cambridge Crystallographic Data Bese", Cambridge Crystallographic Data Center, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, England, 1983.

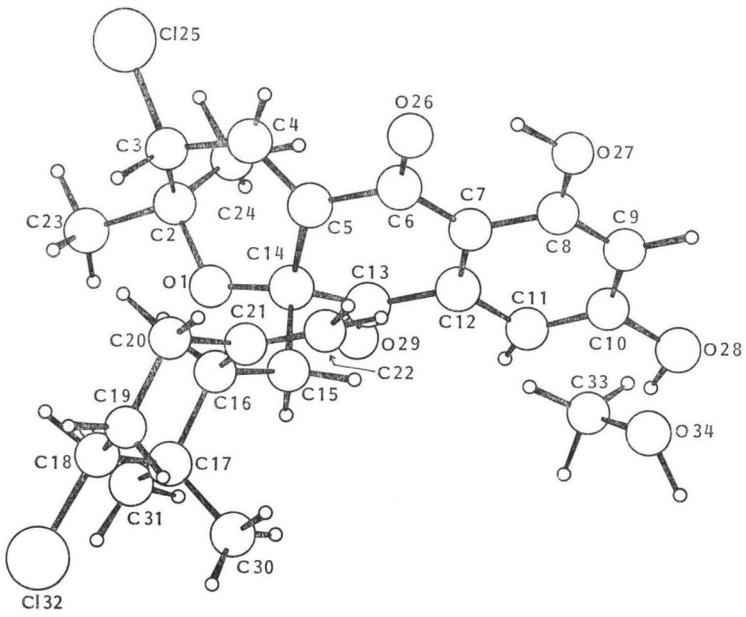


Fig. 3. Bond distances (Å) and atom numbering scheme.
The estimated standard deviations are 0.013 Å.

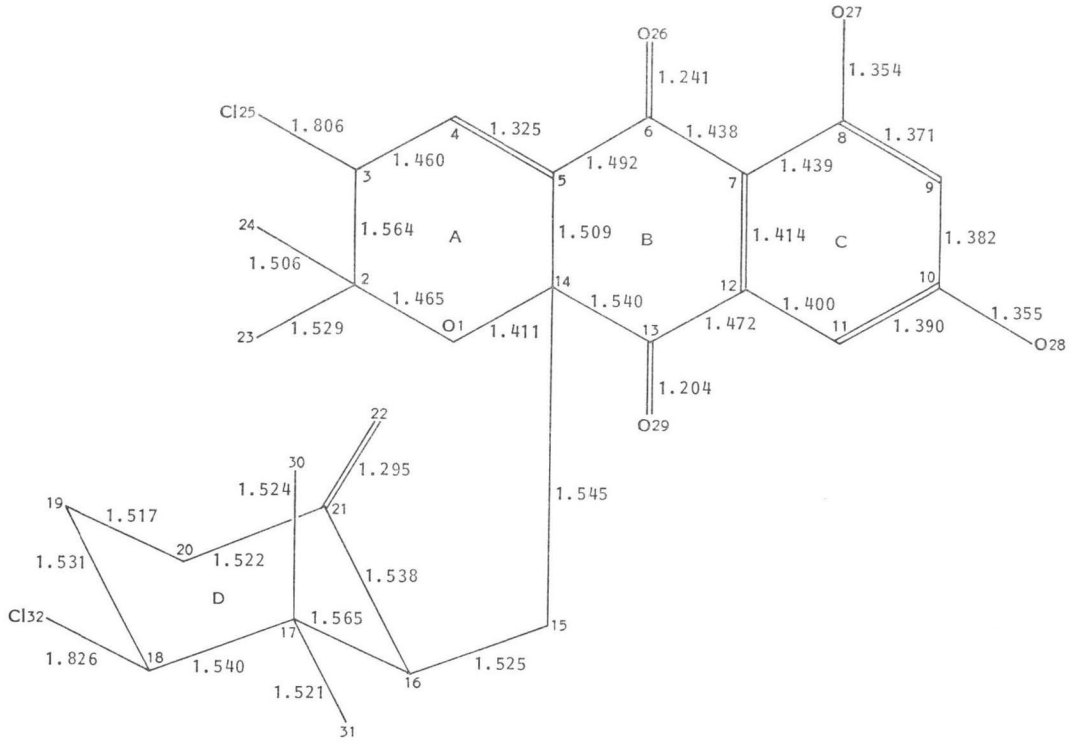


Table 1. Least-squares plane formed by planar group.

	Plane forming atoms				Not plane forming atom	
	Deviation (Å)				Deviation (Å)	
A ring	C3	0.013 (8)	C14	0.030 (7)	C2	−0.738 (8)
	C4	−0.002 (7)	O1	−0.023 (6)		
	C5	−0.018 (7)				
B and C rings	C5	−0.041 (8)	C10	−0.011 (8)	C14	0.452 (7)
	C6	0.041 (8)	C11	0.036 (8)	O29	−0.373 (7)
	C7	0.027 (8)	C12	0.034 (7)	O26	0.031 (7)
	C8	−0.007 (8)	C13	−0.037 (8)	O27	−0.054 (7)
	C9	−0.042 (8)			O28	0.014 (7)
D ring	C17	−0.006 (8)	C20	−0.006 (9)	C19	0.693 (9)
	C18	0.006 (9)	C21	0.006 (8)	C16	−0.707 (9)
Methylene bridge	C14	0.000 (7)	C16	0.000 (7)		
	C15	0.000 (7)				

calculations. All the heavier atoms of crystal solvent and 23 hydrogen atoms were located on the difference electron-density map. The least-squares calculation gave the R value of 0.053. The absolute structure was determined at this stage allowing for the dispersion corrections of the atomic scattering factor of chlorine. Of 48 Friedel pairs for which both the calculated and observed ratios of $|F(hkl)|/|F(\bar{h}\bar{k}\bar{l})|$ differ more than 3% from the unity, 44 pairs showed consistently the absolute configuration of the molecule as shown in Fig. 2.

Table 2. ^{1}H NMR chemical shifts of napyradiomycins.

Proton	Chemical shifts (δ) in ppm relative to TMS						
	A($C_{25}H_{30}O_5Cl_2$)	Na salt of A	B1($C_{25}H_{29}O_5Cl_3$)	B2($C_{25}H_{28}O_5Cl_2$)	B3($C_{25}H_{29}O_5Cl_2Br$)	C1($C_{25}H_{28}O_5Cl_2$)	C2($C_{25}H_{27}O_5Cl_3$)
2-CH_3	1.50 s	1.40 s	1.37 s	1.53 s	1.38 s	1.54 s	1.53 s
2-CH_3	1.18 s	1.16 s	1.18 s	1.07 s	1.20 s	1.32 s	1.28 s
3-H	4.42 dd (4.8, 11.2)	4.41 dd (7.2, 9.6)	4.44 dd (4.0, 12.0)	4.46 d (2.0)	4.45 dd (4.0, 12.0)	4.52 dd (4.4, 11.6)	4.48 dd (4.0, 12.0)
4-H or H_2	2.48 dd (4.8, 14.0), 2.41 dd (11.2, 14.0)	2.45 m	2.52 dd (4.0, 13.6), 2.34 dd (12.0, 13.6)	6.85 d (2.0)	2.53 dd (4.0, 14.0), 2.35 dd (12.0, 14.0)	2.6 m, 2.54 dd (11.6, 14.0)	2.60 dd (4.0, 14.0), 2.50 dd (12.0, 14.0)
6-OH	11.84 s	12.38 br s				12.46 br s	12.68 br s
7-H	6.73 d (2.4)	5.96 d (2.4)	6.72 br s	6.65 br s	6.74 br s		
8-OH	3.6 br s			3.5 br s			
9-H	7.22 d (2.4)	6.66 d (2.4)	7.14 br s	7.11 br s	7.17 br s	7.25 s	7.11 s
11-H_2	2.70 br d (8.0)	2.60 dd (8.0, 13.6), 2.52 dd (8.0, 13.6)	1.99 br d (8.4), 1.61 br d (15.6)	2.0 m, 1.9 m	2.04 br d (8.4), 1.63 br d (15.6)	2.6 m	2.68 m, 2.54 m
12-H	4.70 br t (8.0)	4.82 dd (8.0, 8.0)	2.64 dd (8.4, 15.6)	1.9 m	2.67 dd (8.4, 15.6)	4.70 m	4.55 m
13-CH_3 or CH_2	1.31 s	1.34 s	4.78 s, 4.75 s	4.81 s, 4.30 s	4.78 s, 4.75 s	1.14 s	1.10 s
14-H_2	1.6 m	1.68 m	2.25 ddd (3.6, 3.6, 12.4), 1.93 ddd (3.6, 12.4, 12.4)	2.33 ddd (4.0, 4.0, 12.4), 1.96 ddd (4.0, 12.4, 12.4)	2.2 m, 1.9 m	2.0 m, 1.55 m	2.1 m, 1.3 m
15-H_2	1.6 m	1.76 m	2.03 dddd (3.6, 3.6, 4.0, 12.4), 1.72 dddd (3.6, 11.6, 12.4, 12.4)	2.08 dddd (4.0, 4.0, 4.0, 12.4), 1.74 dddd (4.0, 11.6, 12.4, 12.4)	2.2 m, 1.9 m	2.0 m, 1.75 m	1.6 m, −0.01 m
16-H	4.89 br s	4.98 br t (8.0)	3.78 dd (4.0, 11.6)	3.82 dd (4.0, 11.6)	4.02 dd (4.1, 11.2)	3.16 br d (10.8)	4.07 dd (4.0, 10.0)
17-CH_3 or CH_2	1.50 s	1.52 s	0.57 s	0.61 s	0.62 s	1.75 s	5.38 s, 5.33 s
17-CH_3	1.62 s	1.60 s	0.69 s	1.04 s	0.73 s		
18-H_2						3.41 s	3.98 br d (14.0), 3.57 d (14.0)

Solvent: $CDCl_3$ except Na salt of A (acetone-d_6). Coupling constants (Hz) are in parentheses.

Table 3. ^{13}C NMR chemical shifts of napyradiomycins.

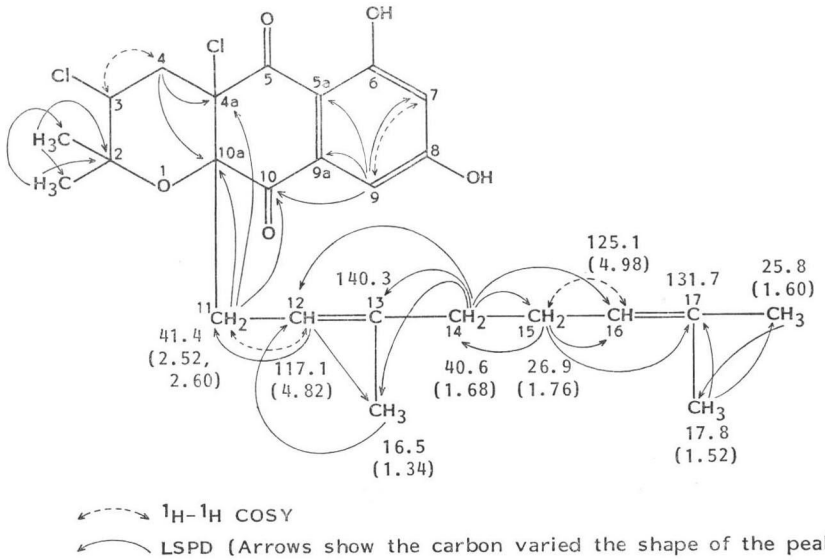
Carbon	Chemical shifts (δ) in ppm relative to TMS						
	A	Na salt of A	B1	B2	B3	C1	C2
2	(79.0) s	78.6 s	(80.9) s	76.6 s	(80.9) s	79.2 s	79.1 s
2-CH_3	28.8 q	29.5 q	29.0 q	27.1 q	29.0 q	29.2 q	29.1 q
2-CH_3	22.3 q	22.4 q	22.4 q	20.3 q	22.5 q	22.4 q	22.3 q
3	58.8 d	60.7 d	58.7 d	59.5 d	58.7 d	58.8 d	58.6 d
4	42.8 t	44.3 t	42.7 t	136.8 d	42.8 t	42.3 t	42.1 t
4a	(78.8) s	81.2 s	(78.8) s	136.8 s	(78.8) s	77.8 s	77.7 s
5	(193.7) s	188.5 s	(193.5) s	(188.1) s	(193.7) s	194.0 s	194.7 s
5a	110.2 s	103.7 s	108.8 s	111.2 s	108.6 s	111.0 s	111.0 s
6	(163.9) s	(167.0) s	(164.2) s	(164.7) s	(164.2) s	164.6 s	(162.2) s
7	109.6 d	110.5 d	109.5 d	109.2 d	109.6 d	124.2 s	120.0 s
8	(164.8) s	(179.7) s	(165.5) s	(165.7) s	(165.6) s	162.5 s	(162.7) s
9	107.8 d	115.4 d	108.5 d	108.9 d	108.5 d	108.0 d	107.5 d
9a	135.3 s	135.1 s	135.1 s	135.6 s	135.1 s	132.1 s	133.9 s
10	(196.2) s	196.6 s	(193.8) s	(195.4) s	(193.9) s	196.1 s	195.9 s
10a	83.6 s	84.5 s	84.3 s	82.3 s	84.3 s	84.9 s	84.8 s
11	41.3 t	41.4 t	35.6 t	36.6 t	35.5 t	42.1 t	41.2 t
12	114.9 d	117.1 d	45.9 d	48.1 d	45.8 d	118.3 d	116.6 d
13	142.8 s	140.3 s	145.3 s	145.6 s	145.3 s	139.6 s	141.0 s
13-CH_3 or CH_2	16.5 q	16.5 q	110.2 t	109.6 t	110.2 t	13.5 q	14.5 q
14	39.8 t	40.6 t	35.0 t	35.6 t	(37.3) t	39.8 t	38.1 t
15	26.0 t	26.9 t	34.5 t	34.5 t	(35.9) t	23.0 t	39.8 t
16	123.7 d	125.1 d	70.7 d	70.6 d	66.6 d	122.0 d	64.0 d
17	131.8 s	131.7 s	41.8 s	42.0 s	41.8 s	133.4 s	145.4 s
17-CH_3 or CH_2	17.5 q	17.8 q	15.5 q	15.8 q	16.4 q	18.3 q	116.8 t
17-CH_3	25.6 q	25.8 q	26.4 q	27.1 q	27.8 q		
18						31.3 t	29.5 t

Solvent: $CDCl_3$ except Na salt of A (acetone-d_6).
The chemical shifts in parentheses were not clearly assigned.

Finally, the least-squares refinement in which all 34 heavier atoms and 32 hydrogen atoms were included and the dispersion corrections for C1 were allowed for, gave the R value of 0.048. The final atomic parameters were sent to Cambridge Crystallographic Center†. The molecular structure is illustrated in Fig. 2. Bond lengths shown in Fig. 3 are in agreement with the chemical structure. Ring A adopts a distorted envelope conformation with a flag-pole atom C2. The naphthoquinone ring (B and C rings) is nearly coplanar but C14 atom is twisted out from the plane and the deviation of O29 is remarkable. Ring D adopts the usual chair conformation. The plane formed by C17, C18, C20 and C21 makes an angle of 71.6° with the plane of methylene bridge formed by C14, C15 and C16. The planarity of each group is shown in Table 1. All oxygen atoms except ether oxygen in ring A form hydrogen bonds. All hydroxyl groups including those of solvation molecules donate hydrogen and all carbonyl oxygen atoms receive hydrogen bond. Thus the following three are noticed; one is intramolecular O27-HO27···O26, 2.559(10) Å and the other two are intermolecular connecting the methanol molecules, O28-HO28···O34, 2.609(11) Å and O34-HO34···O29^{i}, 2.779(11) Å, where (i) is at $\frac{1}{2}+x$, $\frac{1}{2}-y$, $-z$ and others are at x, y, z.

† The final thermal parameters and the list of Fo and Fc may be obtained from one of the authors (HIKARU NAKAMURA) upon request.

Fig. 4. The structure of napyradiomycin A demonstrated by ^{1}H-^{1}H COSY and LSPD.
The values beside the carbons indicate the chemical shifts of ^{13}C NMR, and the values in the parentheses indicate the chemical shifts of ^{1}H NMR.



The Structure of Napyradiomycin B1

About 50% of B1 ($C_{25}H_{29}O_5Cl_3$) was converted to form B2 when it was kept in a refrigerator for 40 days in 85% methanol solution; that is, each one of H and C1 was released resulting in the formation of a double bond. As shown in Tables 2 and 3, B1 has the same D ring of B2, and the saturated bond of C4-C4a in B1 was unsaturated in B2. The stereo-chemistry of C4a could not be determined. Based on this conversion of B1 to B2, their NMR and B2 structure, the structure of B1 was proposed as follows; (3*R*,10a*R*)-3,4a-dichloro-10a-[[(1*R*,3*S*)-3-chloro-2,2-dimethyl-6-methylenecyclohexyl]methyl]-3,4,4a,10a-tetrahydro-6,8-dihydroxy-2,2-dimethyl-2*H*-naphtho[2,3-*b*]pyran-5,10-dione (Fig. 1).

The Structure of Napyradiomycin B3

B3 ($C_{25}H_{29}O_5Cl_2Br$) has one atom of bromine in the molecule and its ^{1}H NMR and ^{13}C NMR were similar to those of B1 except C16. The signals of ^{13}C NMR of C16 shifted to 4.1 ppm (higher field) compared with that of B1.

It was therefore, elucidated that chlorine in D ring of B1 was substituted by bromine in that of B3 (Fig. 1).

The Structure of Napyradiomycin A

On ^{1}H NMR spectrum of A ($C_{25}H_{30}O_5Cl_2$) measured in $CDCl_3$, the signals of methyl and methylene groups could not be assigned because of overlapping of their signals. But the signals of sodium salt of A were assigned as shown in Tables 2 and 3. Geometrical isomerism was estimated by the chemical shifts of ^{13}C NMR[2]. From these data the structure of A was determined to be 3,4a-dichloro-10a-[(*E*)-3,7-dimethylocta-2,6-dien-1-yl]-3,4,4a,10a-tetrahydro-6,8-dihydroxy-2,2-dimethyl-2*H*-naphto[2,3-*b*]pyran-5,10-dione (Fig. 1). The data of ^{1}H-^{1}H COSY and long range selective proton decoupling (LSPD) shown in Fig. 4 also supported this structure.

Fig. 5. The structure of napyradiomycin C1 demonstrated by ^{1}H-^{1}H COSY and long range ^{1}H-^{13}C COSY.

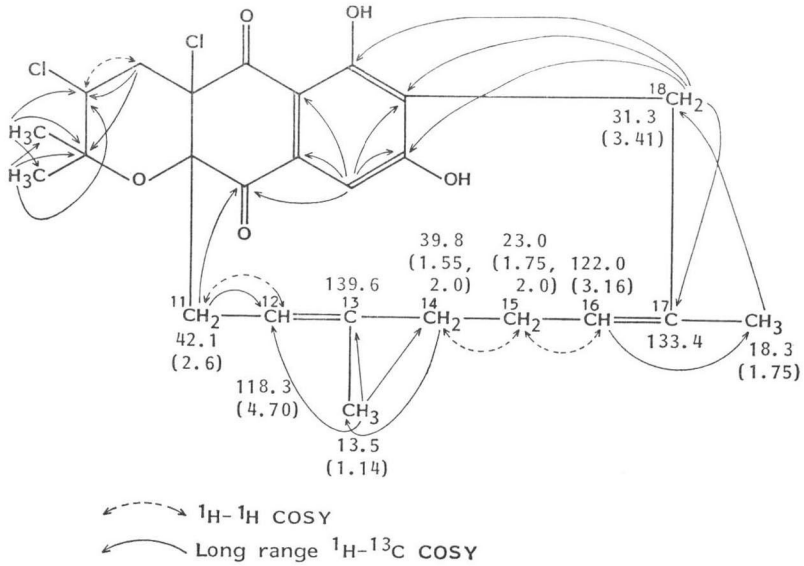
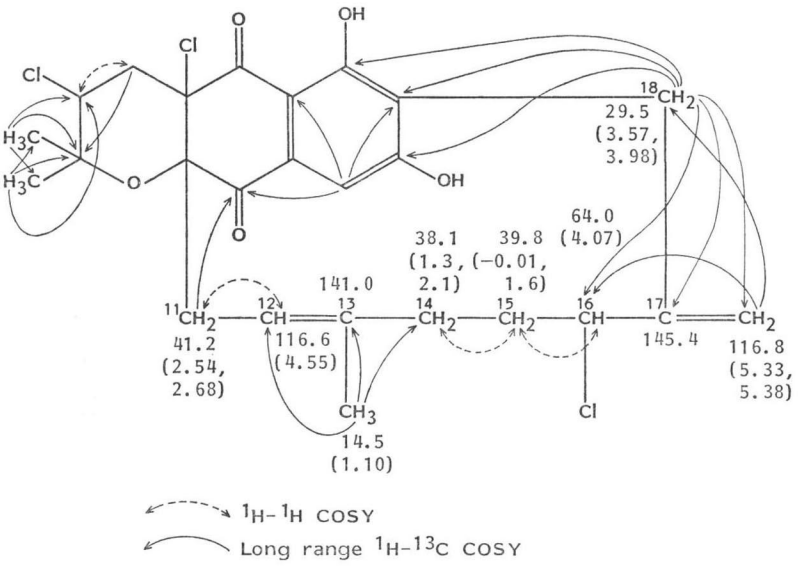


Fig. 6. The structure of napyradiomycin C2 demonstrated by ^{1}H-^{1}H COSY and long range ^{1}H-^{13}C COSY.



The Structures of Napyradiomycins C1 and C2

^{1}H NMR and ^{13}C NMR spectra of C1 ($C_{25}H_{28}O_5Cl_2$) and C2 ($C_{25}H_{27}O_5Cl_3$) (Tables 2 and 3) showed that one aromatic proton (H7) of others disappeared. From ^{1}H-^{1}H COSY and long range ^{1}H-^{13}C COSY[3] shown in Figs. 5 and 6, the possible structures of C1 and C2 were estimated to be 3,4a-dichloro-3,4,4a,10a-tetrahydro-6,8-dihydroxy-2,2-dimethyl-7,10a-(13,17-dimethylocta-12,16-dieno)-2*H*-naphtho[2,3-*b*]pyran-5,10-dione (Fig. 5) and 3,4a-dichloro-3,4,4a,10a-tetrahydro-6,8-dihydroxy-2,2-dimethyl-7,10a-(16-chloro-13-methyl-17-methyleneocta-12-eno)-2*H*-naphtho[2,3-*b*]pyran-5,10-dione (Fig. 6) respectively. One of the chemical shifts of H15 in C2 was measured at high field (−0.01 ppm) and its

location on C ring was suggested by HGS biochemistry molecular model (Maruzen). Stereo-isomerism of 12 and 16 positions of C1 and C2 was not determined.

Any antibiotics which have the same chromophore as do napyradiomycins were not known in the products of actinomyces. The chromophore of napyradiomycins has been found in fungus products such as cryptosporin[4]. However any compounds which have the side chain at C10a position have not been known. 3-Bromo-α-lapachone (3-bromo-3,4-dihydro-2,2-dimethyl-2*H*-naphtho[2,3-*b*]pyran-5,10-dione)[5] which is synthesized from lapachol is more analogs to the chromophore of napyradiomycins than natural products.

Experimental

NMR spectra were recorded at 400 MHz on Jeol JNM-GX400. Long range ^{1}H-^{13}C COSY of napyradiomycin C1 were measured by both $\frac{1}{4}J$=25 ms (J_{CH}=10 Hz) and 50 ms (5 Hz). And that of napyradiomycin C2 was measured by 25 ms (10 Hz).

Preparation of Sodium Salt of Napyradiomycin A

To the solution of 20 mg of A in 10 ml of MeOH, 290 μl of 0.1 M NaOH was added and kept for 20 minutes at room temp. The solution was concd to dryness and chromatographed on Sephadex LH-20 (20 ml) using MeOH as eluant. A and its Na salt were eluted and separated yielding yellow brownish oil of A (8.9 mg) and yellow powder of A Na salt (12.5 mg). MP 122~130°C. *Anal* Calcd for $C_{25}H_{29}O_5Cl_2Na$: C 59.65, H 5.81, O 15.89, Cl 14.09, Na 4.51. Found: C 59.09, H 5.76, O 16.53, Cl 13.57, Na 4.80. UV λ_{max}^{MeOH} nm (log ε) 204 (4.41), 254 (4.27), 265 (sh 4.23), 298 (4.31), 365 (sh 4.08), 377 (4.12), 400 (sh 4.00). IR(KBr) cm^{-1} 3400, 2980, 1680, 1610, 1480, 1380, 1310, 1190, 1080, 870, 740. Data of ^{1}H NMR and ^{13}C NMR are shown in Tables 2 and 3, respectively.

References

1) SHIOMI, K.; H. IINUMA, M. HAMADA, H. NAGANAWA, M. MANABE, C. MATSUKI, T. TAKEUCHI & H. UMEZAWA: Novel antibiotics napyradiomycins. Production, isolation, physico-chemical properties and biological activity. J. Antibiotics 39: 487~493, 1986
2) BARLOW, L. & G. PATTENDEN: Synthesis of poly-*Z*-isomers of 2,6,11,15-tetramethylhexadeca-2,6,8,10,14-pentaene, a C_{20} analogue of phytoene. Re-examination of the stereochemistry of a new isomer of phytoene from *Rhodospirillum rubrum*. J. Chem. Soc. Perkin Trans. I 1976: 1029~1034, 1976
3) SHOOLERY, J. N.: Recent developments in ^{13}C- and proton-NMR. J. Nat. Prod. 47: 226~259, 1984
4) CLOSSE, A. & H.-P. SIGG: Isolierung und Strukturaufklärung von Cryptosporin. Helv. Chim. Acta 56: 619~625, 1973
5) KAPOOR, N. K.; R. B. GUPTA & R. N. KHANNA: Reaction of lapachol with lead tetraacetate & *N*-bromosuccinimide. Indian J. Chem. 20B: 508~509, 1981