Tetrahedron Letters,Vol.26,No.3,pp 331-334,1985
Printed in Great Britain

0040-4039/85 $3.00 + .00

THE STRUCTURE OF NEOCARZINOSTATIN CHROMOPHORE POSSESSING A NOVEL BICYCLO-[7,3,0]DODECADIYNE SYSTEM

Kiyoto Edo* and Michinao Mizugaki
Department of Pharmaceutical Sciences, Tohoku University Hospital
1-1 Seiryo-machi, Sendai 980, Japan

Yoshio Koide
Kayaku Antibiotics Research Laboratory, Funado, Itabashi-ku, Tokyo 174, Japan

Haruo Seto, Kazuo Furihata, Noboru Ōtake
Institute of Applied Microbiology, The University of Tokyo
Bunkyo-ku, Tokyo 113 Japan

Nakao Ishida
Department of Bacteriology, Tohoku University School of Medicine
2-1 Seiryo-machi, Sendai 980, Japan

Summary: The structure of the chromophore of an antitumor antibiotic neocarzinostatin has been elucidated as a bicycro[7,3,0]dodecadiyne system having naphthalenecarboxylic acid, aminosugar and ethylene carbonate units.

In 1979, a non-protein chromophore (NCS Chr) was discovered and isolated from an antitumor polypeptide antibiotic neocarzinostatin (NCS) by us[1] and others[2,3]. The accumulated evidence indicated that this chromophore is responsible for the biological activities such as DNA strand scission and for the cytotoxic properties of NCS against tumor and bacterial cells and that apoprotein, the other component of NCS, stabilizes this chromophore which is very labile against ultraviolet light and heating in the free form[4-6].

Until now, it was proposed that NCS Chr possesses 2-hydroxy-5-methoxy-7-methyl-1-naphthalenecarboxylic acid[7], an aliphatic aromatic diacyl peroxide moiety[8], N-methyfucosamine (MF), ethylene carbonate (EC)[10], and a highly strained ether ring (possibly an epoxide)[11]. Recently, Shibuya has revised the structure of the naphthalenecarboxylic acid residue to 2-hydroxy-7-methoxy-5-methyl-1-naphthalenecarboxylic acid (NA) based on its total synthesis[12].

In this communication, we report the total structure of NCS Chr (I) as well as its hydrogen chloride adduct (II).

Purified II was obtained from NCS powder by extraction with methanolic hydrogen chloride followed by purification by Lichrosorb RP-18 column chromatography. The physicochemical properties of II are as follows: amorphous powder, mp 125°C (dec.), $C_{35}H_{34}ClNO_{12}$, FAB-MS m/z 696 (MH^+), anal. found: C; 53.41, H; 5.00, N; 1.67, Cl; 9.20 %, calcd. for $C_{35}H_{34}ClNO_{12}\cdot HCl\cdot 3H_2O$: C; 53.44, H; 5.25, N; 1.78, Cl; 9.01 %, $[\alpha]_D^{20}$ -171°(c 0.1, MeOH) λ_{max}^{MeOH} (nm (ε)) 231 (24,600), 266 (7,600), 274 (7,930) 292 (6,960), 302 (6,760), 322 (4,830), 352 (3,600), ν_{max}^{KBr} (cm^{-1}) 3400, 2922, 2850, 1811, 1783, 1717, 965, 843 and 765.

The molecular formula of I prepared by treatment of NCS with glacial acetic acid was established as $C_{35}H_{33}NO_{12}$ by FAB-MS, PMR and CMR. This formula is in agreement with that reported by Hensens[11]. These data suggest that II is a hydrogen chloride adduct of native NCS Chr (I).

The 100 MHz CMR spectrum of II taken in $^{12}CD_3OD$ indicated the presence of 35 carbons. They are classified as 4 methyls, 1 methylene, 15 methines and 15 quaternary carbons based on off-resonance decoupled and INEPT experiments. The well-spaced 400 MHz PMR spectrum taken in CD_3OD showed the presence of 29 non-exchangeable protons, which were in accord with the proton number counted by INEPT. All of these proton signals were correlated to carbon resonances by selective proton decoupling (Table I). After the proton and carbon signals attributable to NA, MF and EC moieties had been excluded[13], 5 protons and 12 carbons present in a very highly unsaturated moiety common to I and II remained to be assigned. In order to reveal this moiety, NMR spectra were carefully analyzed to prove that both I and II have no aliphatic aromatic diacyl peroxide proposed previously by us[8].

Since the coupling constants of H-10 in II (see Fig. 2) with H-8, 11, 12 and 1' (anomeric proton of MF) were very small, distinction between vicinal and long range couplings could not be made with certainty. However, NOE experiments revealed spatial proximity of H-10 with H-8, 11 and 1'. The magnitude of the coupling constant between H-11 and 12 ($\underline{J}$=3.0 Hz) confirmed the vicinal

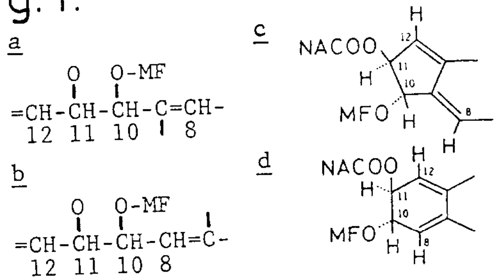
Table I. 100 MHz CMR AND 400 MHz PMR SPECTRAL DATA OF I AND II

		I (CD_3CO_2D,CD_3OD, 1:1)			II ($^{12}CD_3OD$)		
		^{13}C(ppm)	$^1J_{C-H}$	H(ppm)	^{13}C(ppm)	$^1J_{C-H}$	H(ppm)
	1	129.8(s)			130.9(s)		
	2	87.5(s)			89.9(s)		
	3	97.6(s)			99.9(s)		
	4	63.8(s)			80.1(s)		
	5	55.2(d)	196.6	4.14(1H,brs)	58.5(d)	162.6	5.20(1H,brs)
	6	99.7(s)			100.5(s)		
	7	90.7(s)			93.0(s)		
	8	106.5(d)	168.8	5.82(1H,brs)	107.8(d)	169.5	5.89(1H,d,J=1.2)
	9	160.2(s)			156.2(s)		
	10	82.2(d)	158.4	5.09(1H,brs)	82.5(d)	159.0	4.95(1H,brs)
	11	81.6(d)	156.7	6.25(1H,brs)	82.6(d)	150.0	6.14(1H,d,J=3.0)
	12	139.4(d)	176.0	6.79(1H,brs)	136.2(d)	174.9	6.58(1H,dd,J=3.0,1.2)
EC	13	76.1(d)	160.0	4.93(1H,dd,J=8.0,5.0)	78.0(d)	162.6	5.25(1H,dd,J=8.0,5.5)
	14	68.0(t)	156.3	4.54(1H,dd,J=5.0,9.0)	67.3(t)	157.5	4.61(1H,dd,J=5.5,9.0)
				4.75(1H,dd,J=9.0,8.0)			4.64(1H,dd,J=9.0,8.0)
	15	155.6(s)			156.1(s)		
MF	1'	95.4(d)	168.7	5.75(1H,d,J=3.0)	95.6(d)	174.0	5.63(1H,d,J=3.8)
	2'	59.5(d)	144.6	3.65(1H,dd,J=3.0,10.5)	59.2(d)	142.5	3.43(1H,dd,J=3.8,11.0)
	3'	68.2(d)	143.8	4.21(1H,dd,J=10.5,2.5)	68.1(d)	142.5	3.99(1H,dd,J=11.0,3.0)
	4'	72.4(d)	146.2	3.90(1H,d,J=2.5)	72.2(d)	142.5	3.76(1H,d,J=3.0)
	5'	69.1(d)	136.4	4.07(1H,d,J=6.5)	68.9(d)	144.6	4.07(1H,d,J=6.5)
	6'	16.6(q)	*	1.26(3H,d,J=6.5)	16.6(q)	127.3	1.26(3H,d,J=6.5)
	2'-NCH_3	32.7(q)	*	3.02(3H,s)	32.3(q)	142.8	2.92(3H,s)
NA	1"	106.2(s)			107.4(s)		
	2"	163.2(s)			161.8(s)		
	3"	116.4(d)	164.4	7.03(1H,d,J=9.0)	115.8(d)	163.8	6.98(1H,d,J=9.5)
	4"	133.6(d)	158.4	8.10(1H,d,J=9.0)	132.2(d)	159.0	8.00(1H,d,J=9.5)
	5"	138.1(s)			137.6(s)		
	6"	117.6(d)	160.4	6.90(1H,brs)	117.3(d)	155.7	6.84(1H,d,J=2.0)
	7"	160.4(s)			159.9(s)		
	8"	104.2(d)	160.4	7.76(1H,d,J=2.0)	103.1(d)	160.5	7.51(1H,d,J=2.0)
	4a"	124.0(s)			123.6(s)		
	8a"	134.9(s)			134.4(s)		
	1"-COO	172.1(s)			171.4(s)		
	5"-CH_3	20.0(q)	*	2.62(3H,s)	20.0(q)	127.3	2.56(3H,s)
	7"-OCH_3	55.9(q)	143.8	3.83(3H,s)	55.6(q)	143.4	3.83(3H,s)

* not determined

relationship of these two protons. The CMR data of II indicated that C-8 (107.8 ppm) and C-12 (136.2 ppm) are sp^2 carbons and that C-10 (82.5 ppm) and C-11 (82.6 ppm) are oxygenated methines. Thus, the relationship of these carbons is represented by either one of the two partial structures (*a* and *b*) show in Fig. 1.

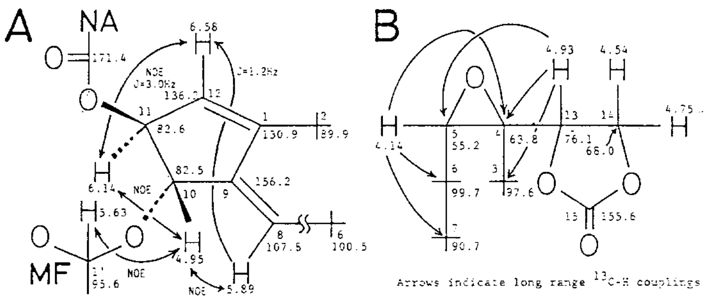
Fig. 1.



Since H-8 and H-12 are long-range-coupled with each other (J=1.2 Hz) without showing NOE enhancement, *a* and *b* must be extended to *c* and *d* respectively, in Fig. 1. However, accommodation of the remaining acetylenic units (*vide infra*) to the partial structure *d* would give a symmetric structure which could not account for the large ^{13}C-chemical shift differences between C-8 and C-12, and C-1 and C-9. Thus the partial structure *d* was excluded. The similar observations were made with I. The relationship shown in Fig. 1*c* was confirmed by LSPD experiment irradiating H-12 of II which resulted in the collapse of the C-2, 9 and 10 resonances. When H-10 and 11 of II were irradiated, C-1' and 1"-COO (171.4 ppm) were affected, respectively. On the basis of these results, we propose the 2-cyclopentene-1-ylidene unit indicated in Fig. 2A as the common partial structure for the unknown moiety in I and II. The relative stereochemical relationship between H-10 and 11 is assumed to be *trans* due to the very small coupling between them.

The linkage of the epoxide and ethylene carbonate moieties was established also by NMR analysis. In the CMR spectrum of I, the very large coupling constant (J_{C-H} =197 Hz) of C-5 revealed the presence of the epoxide as reported by Hensens[11]. Disappearance of the epoxide in II was shown by comparison of PMR and CMR data of I and II (in II, C-4 80.1 ppm, C-5 58.5 ppm, J_{C-H}=163 Hz). The ^{13}C-chemical shifts of the remaining carbons in I and II are very similar as shown in Table I. These results suggest that II is a hydrogen chloride adduct of I. When H-13 of ethylene carbonate of I was irradiated by LSPD experiment, C-3 and both the epoxide carbons C-4 and 5 were decoupled. On the other hand, when the epoxide proton H-5 was irradiated, three quaternary carbons C-4, 6 and 7 were affected. Thus the remaining partial structure has been elucidated as indicated in Fig. 2B.

Fig.2. Partial Structure of NCS Chr



Arrows indicate long range ^{13}C-H couplings

The final problem is to connect partial structure A and B. In the FT-IR spectrum (KBr) of II, twin acetylenic bands at 2187 and 2194 cm^{-1} were observed. In addition, there remained only four unassigned carbons in II with the degree of unsaturation being five which could be only explained by the presence of two acetylenic bonds and one ring structure. The relationship between structure A and B was examined by long range spin decoupling and LSPD experiments. The epoxide methine proton H-5 of I was long-range-coupled with H-8. LSPD experimental results (Fig. 2B) suggested the connection pattern indicated in Fig. 3, unequivocally. The quaternary carbon resonances of I were assigned to cyclic diacetylenic carbons in a highly strained system^{14} by LSPD experimental data (C-2, 3, 6 and 7; 87.4, 97.6, 99.7 and 90.7, respectively). The structure in Fig. 3 was supported by the molecular and fragment ion species at m/z 696 (MH^+), 482 (MH^+ - $C_{13}H_{10}O_3$), 215 ($C_{13}H_{10}O_3$, NA), 160 ($C_7H_{14}O_3$, MF), and 88 ($C_3H_4O_3$, EC) obtained by linked scan SIMS.

On these results we propose the relative chemical structure of NCS Chr (I) and its hydrogen chloride adduct (II) indicated in Fig. 3.

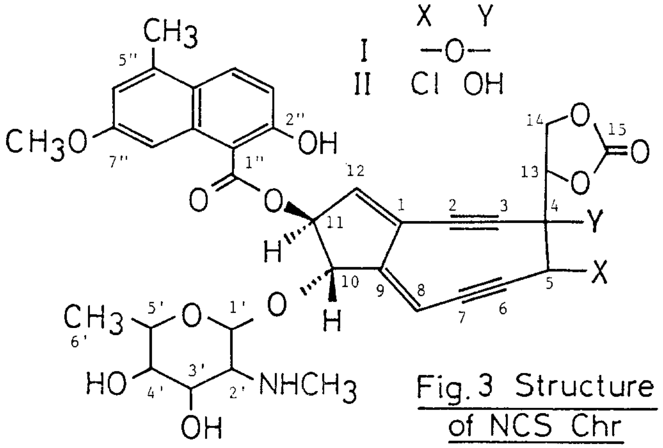


Fig. 3 Structure of NCS Chr

ACKNOWLEDGEMENTS

The authors wish to thank JEOL Co. Ltd. for FAB-MS measurement and Hitachi Co. Ltd. for SIMS measurement. The authors are grateful to Dr. G. Kusano, Tohoku University, for helpful discussion and Dr. M. Shibuya, University of Tokushima, for a gift of the CMR data of the naphthalenecarboxylic acid. This work was partially supported by Grands-in-Aid for Cancer Research from the Ministry of Health and Welfare and the Ministry of Education, Science and Culture, and by a Grant-in-Aid for Developmental Scientific Research 59860012.

REFERENCES AND NOTES

1) Y. Koide et al. J. Antibiot. 33, 342 (1980). 2) M. A. Napier et al. Biochem. Biophys. Res. Commun. 89, 635 (1979). 3) H. Suzuki et al. Biochem. Biophys. Res. Commun. 94, 225 (1980). 4) I. H. Goldberg, Neocarzinostatin: Antibiotics Vol. 2. F. H. Hahn (Ed.), Springer-Verlag, Berlin, Heiderberg, p. 267 (1979). 5) K. Ohthuki and N. Ishida, Protein, Nucleic Acid & Enzyme 26, 937 (1981) (in Japanese). 6) K. Edo and N. Ishida, Igaku no Ayumi 129, 373 (1984) (in Japanese). 7) K. Edo et al. J. Antibiot. 33, 347 (1980). 8) K. Edo et al. J. Antibiot. 35, 106 (1982). 9) G. A. Schonberg et al. Biochem. Biophys. Res. Commun. 95, 1351 (1980) 10) M. A. Napier et al., Biochem. Biophys. Res. Commun. 100, 1703 (1981). 11) O. D. Hensens et al. Biochim. Biophys. Res. Commun. 113, 538 (1983). 12) M. Shibuya et al. Tetrahedron Lett. 25, 1171 (1984). 13) Assigned by comparison with fucosamine (Chem. Pharm. Bull. 22, 2196 (1974)), propylene carbonate (L.F. Johnson et al., Carbon-13 NMR spectra (1972) Wiley-Interscience, p. 62) and 2-hydroxy-7-methoxy-5-methyl-1-naphthalenecarboxylic acid (Dr. Shibuya's data). 14) The CMR spectrum of 1,5-cyclooctadiyne exhibited a signal at 95.8 ppm for sp carbon (Helv. Chim. Acta. 58, 162 (1975)).

(Received in Japan 4 September 1984)