Oeschger, H., Siegenthaler, U., Schotterer, U., and Gugelmann, A., 1975. A box diffusion model to study the carbon dioxide exchange in nature. Tellus *27*, 168–192.
Peng, T.-H., and Broecker, W.S., 1978. Effect of sediment mixing on the rate of calcite dissolution by fossil-fuel CO_2. Geophys. Res. Lett. *5*, 349–352.
Pytkowicz, R.M., and Small, L.F., 1977. Fossil-fuel problem and carbon dioxide: an overview; in: The fate of fossil-fuel CO_2 in the oceans, p.7–31. Ed. N.R. Andersen and A. Malahoff. Plenum, New York.
Roether, W., 1979. Use of oceanic tracers to determine the uptake of excess CO_2 into the ocean; in: Man's impact on climate, p.109–114. Ed. W. Bach, J. Pankrath and W.W. Kellog. Elsevier, Amsterdam.
Rotty, R.M., 1980. Past and future emission of CO_2. Experientia *36*, 781–783.
Scope 13 'The global carbon cycle'. Ed. B. Bolin, E.T. Degens, S. Kempe and P. Ketner. J. Wiley, New York.
Takahashi, T., 1979. Carbon dioxide chemistry in ocean water; in: Carbon dioxide effects research and assessment program, p.63–71. Ed. W.P. Elliot and L. Machta. US Department of Energy, Washington DC.
Weiss, W., Roether, W., and Dreisigacker, E., 1979. Tritium in the North Atlantic ocean: inventory, input and transfer into the deep water; in: Behaviour of tritium in the environment, p.315–336. International Atomic Energy Agency, Vienna.
Wollast, R., and Reinhard-Derie, D., 1977. Equilibrium and mechanism of dissolution of Mg-calcites; in: The fate of fossil-fuel CO_2 in the oceans, p.479–493. Ed. N.R. Andersen and A. Malahoff. Plenum, New York.
Woodwell, G.M., and Houghton, R.A., 1977. Biotic influences on the world carbon budget; in: Global chemical cycles and their alterations by man, p.61–72. Ed. W. Stumm. Dahlem Konferenzen, Berlin.
Zimen, K.E., 1979. The carbon cycle, the missing sink, and future CO_2 levels in the atmosphere; in: Man's impact on climate, p.129–137. Ed. W. Bach, J. Pankrath and W.W. Kellog. Elsevier, Amsterdam.

SPECIALIA

The editors do not hold themselves responsible for the opinions expressed in the authors' brief reports. – Les auteurs sont seuls responsables des opinions exprimées dans ces brèves communications. – Für die Kurzmitteilungen ist ausschliesslich der Autor verantwortlich. – Per le brevi comunicazioni è responsabile solo l'autore. – Ответственность за короткие сообщения несёт исключительно автор. – Solo los autores son responsables de las opiniones expresadas en estas comunicationes breves.

The structure of a novel antitumor antibiotic, saframycin A

T. Arai, K. Takahashi, S. Nakahara and A. Kubo

Department of Antibiotics, Research Institute for Chemobiodynamics, Chiba University, Inohana, Chiba 280, Division of Chemotherapy, Chiba Cancer Center Research Institute, Nitona, Chiba 280, and Department of Organic Chemistry, Meiji College of Pharmacy, Nozawa, Setagaya, Tokyo 154 (Japan), 18 September 1979

Summary. A structure is assigned to saframycin A, a novel antitumor antibiotic from *Streptomyces lavendulae* No.314, on the basis of ^{13}C NMR-spectral data.

Streptomyces lavendulae is the well-known source of the classic antibiotic streptothricin[1]. During the course of screening for new antibiotics which are active against the established cell line L1210 mouse leukemia, we found that a strain of *Streptomyces lavendulae,* designated No.314, exhibited cytotoxicity against a line of tumor cells in addition to the production of streptothricin. Satellite antibiotics named mimosamycin and saframycin A to E were obtained from the organic solvent extract of the cultured filtrate[2]. Among these antibiotics, the structure of mimosamycin [$C_{12}H_{11}NO_4$] was determined as 2,6-dimethyl-7-methoxy-3,5,8-trioxo-2,3,5,8-tetrahydroisoquinoline (**1**)[3,4]. The structures of 2 major antibiotics, saframycin B [$C_{28}H_{31}N_3O_8$] and C [$C_{29}H_{33}N_3O_9$] were determined as **2** and **3,** respectively, by an X-ray crystallographic study and ^{13}C NMR-spectral data[5].

The present communication is concerned with the structural elucidation of saframycin A (**4**) which possesses the highest antitumor activity among these satellite antibiotics against mouse leukemia L1210 and P388[6].

It was also reported that saframycin A (**4**) was found to bind to DNA and to inhibit both DNA and RNA syntheses[7].

The physical constants of saframycin A (**4**), a yellow amorphous powder (cold ether), are as follows: m.p. 122–126 °C (ether), $[\alpha]_D + 18.2°$ (MeOH); $C_{29}H_{30}N_4O_8$; mass spectrum m/e (%): 562 (M^+, 8), 462 (M–100, 30), 243 (83), 220 (100), 218 (54); UV λ_{max}^{MeOH} nm (log ε): 267 (4.34), 370 (inf.); CD (MeOH): 278 nm ($\Delta\varepsilon$ −28.5); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1716, 1685, 1660, 1615.

The ^1H NMR-spectrum ($CDCl_3$, 100 MHz) showed signals at δ1.90 (s), 1.98 (s), 2.24 (s), 2.30 (s), 4.04 (2×s) for 6 methyl groups. The ^{13}C NMR-data ($CDCl_3$) revealed the nature of all the methyl groups [δ8.8 (2×C−CH_3), 24.3 (COCH), 41.7 (N−CH_3), 61.0 and 61.1 (2×OCH_3)] and displayed the characteristic 6 pairs of signals at δ128–187 due to the quaternary aromatic and carbonyl carbons, and an unusual signal at δ116.7 (s) (table). The above spectroscopic data indicate that saframycin A (**4**) must have the same carbon skeleton as saframycin B (**2**) and C (**3**).

Comparison of the empirical formula of saframycin A (**4**) with that of saframycin B (**2**) [$C_{28}H_{31}N_3O_8$] suggests that 1 cyano group is substituted for 1 hydrogen atom of **2.** This assignment was substantiated by the ^{13}C NMR-spectrum (δ116.7). However, in the IR-spectrum, no absorption band was observed in the C≡N region.

This discrepancy could be explained by assuming that in the IR-spectrum the introduction of the oxygenated groups into the molecule results in a quenching of the C≡N absorption intensity[8].

In confirmation of our tentative assignment, ^{13}C NMR-spectra of 3 model compounds, **5, 6** and **7,** were examined and their nitrile signals were observed at δ116.7, 116.6 and 116.4, respectively.

Furthermore, acid hydrolysis of saframycin A (**4**) was carried out in 0.05 N H_2SO_4 at 100 °C for 40 min and **4** released about 1 mole equivalent HCN which was determined by the Epstein method[9].

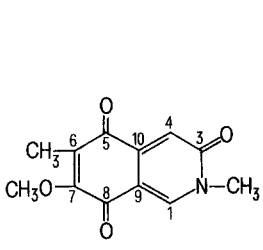
This presents additional evidence for the cyano group in the molecule. The remaining problem was to determine the position of substitution by the cyano group.

The PND and off-resonance decoupling ^{13}C NMR-data of **4** excluded the possibility of the cyano group being localized at the C-4, C-14, C-22 methylene carbons or the C-1, C-11, C-3, C-13 methine carbons adjacent to tertiary nitrogens. The above results and the absence of a signal corresponding to the C-21 methylene carbon [δ58.7 (t) in **2**] and the presence of a methine carbon at δ58.3 (d) enabled the localization of the cyano group at the C-21 position in **2** (table).

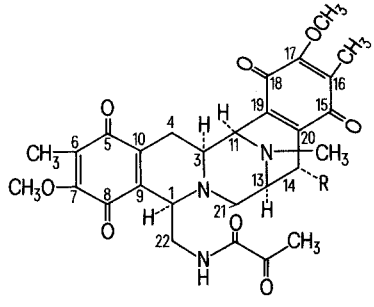
^{13}C chemical shifts (δ) of saframycin B(**2**) and A(**4**) in $CDCl_3$*

Carbon No.	2(B)	4(A)
5 or 15	185.7 or 187.0	185.2 or 186.5
6 or 16	127.7 or 129.2	128.3 or 129.2
7 or 17	155.5 or 156.1	155.6 or 155.9
8 or 18	181.3 or 182.8	180.8 or 183.4
9 or 19	136.3 or 136.6	135.6 or 135.6
10 or 20	141.6 or 142.8	141.2 or 141.6
6 or 16 $-CH_3$	8.6 or 8.6	8.7 or 8.7
7 or 17 $-OCH_3$	60.9 or 60.9	61.0 or 61.1
$N-CH_3$	41.2	41.6
1 or 11	52.2 or 54.8	54.0 or 54.3
3 or 13	56.9 or 57.4	54.6 or 56.3
4	25.6 (t)	25.1 (t)
14	22.7 (t)	21.6 (t)
21	58.7 (t)	58.3 (d)
22	40.4 (t)	40.7 (t)
NHCO	160.1	160.2
$COCH_3$	24.2, 196.5	24.3, 196.7
CN		116.7 (s)

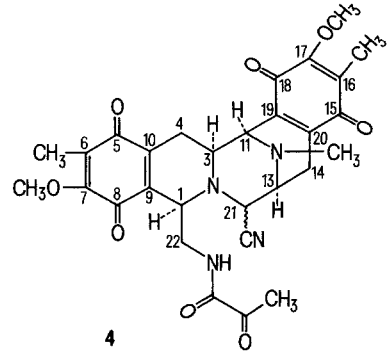
* Natural-abundance ^1H noise decoupled (PND) and off-resonance ^{13}C FT NMR-spectra were recorded on a Jeol FX-60 and FX-100 FT NMR-spectrometer. TMS served as an internal reference (δ 0).



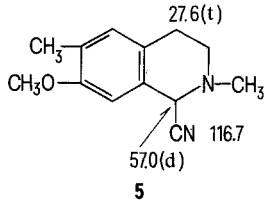
1



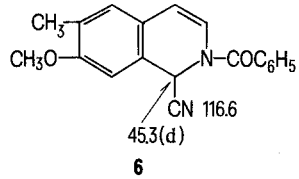
2 R=H
3 R=OCH_3



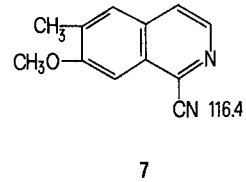
4



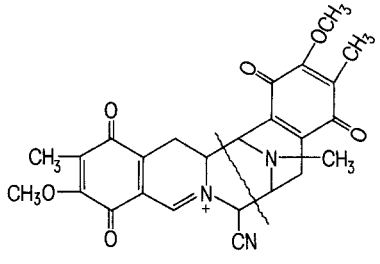
5



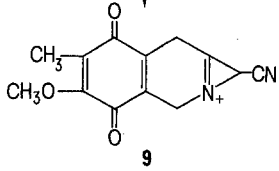
6



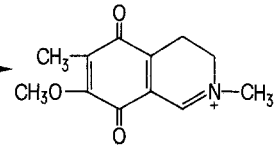
7



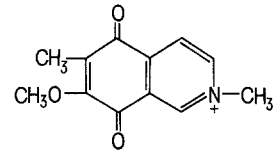
8(M^+-100)



9



10



11

		Calculated	Found
8	$C_{25}H_{24}N_3O_6$	462.1629	462.1679
9	$C_{13}H_{11}N_2O_3$	243.0790	243.0809
10	$C_{12}H_{14}NO_3$	220.0974	220.0978

The shift assignment of the C-21 carbon can be rationalized by the observation that the substitution of a proton by a cyano group results in only a slight down-field shift at the attached carbon[10].

Moreover, the high resolution mass spectrum confirmed the above conclusion. The fragment ions m/e 462 (M^+ -100) (**8**), 220 (**10**) and 218 (**11**) were derived from the basic skeleton of saframycin group antibiotics. Of particular significance was the presence of the fragment ion m/e 243 (**9**) corresponding to the ion containing a cyano group at C-21 in the molecule.

Saframycin A therefore has the structure depicted in **4**, 21-cyanosaframycin B, or its antipode. However, assignment of the configuration of the cyano group has not been made.

1 S.A. Waksman, Proc. exp. Biol. Med. *49*, 207 (1942).
2 T. Arai, K. Takahashi and A. Kubo, J. Antibiot., Tokyo *30*, 1015 (1977).
3 H. Fukumi, H. Kurihara, T. Hata, C. Tamura, H. Mishima, A. Kubo and T. Arai, Tetrahedron Lett. *1977*, 3825.
4 H. Fukumi, H. Kurihara and H. Mishima, Chem. pharm. Bull. *26*, 2175 (1978).
5 T. Arai, K. Takahashi, A. Kubo, S. Nakahara, S. Sato, K. Aiba and C. Tamura, Tetrahedron Lett. *1979*, 2355.
6 T. Arai, K. Takahashi, K. Ishiguro and Y. Mikami, Gann *71*, (1980).
7 K. Ishiguro, S. Sakiyama, K. Takahashi and T. Arai, Biochemistry *17*, 2545 (1978).
8 L.J. Bellamy, in: The Infra-red Spectra of Complex Molecules, p.264. Methuen, London 1957.
9 J. Epstein, Anal. Chem. *19*, 272 (1947).
10 G.C. Levy and G.L. Nelson, in: Carbon-13 Nuclear Magnetic Resonance for Organic Chemists, p.48. Wiley-Interscience, New York 1972.

Amino acid composition of hemocyanin monomers from the horseshoe crab, *Tachypleus tridentatus*[1]

H. Sugita and K. Sekiguchi

Institute of Biological Sciences, the University of Tsukuba, Sakura-mura, Niihari-gun, Ibaraki 305 (Japan), 7 December 1979

Summary. The amino acid compositions of the 4 kinds of hemocyanin monomers from *Tachypleus tridentatus* are similar to each other. Whole hemocyanin from *T. tridentatus* is remarkably similar to *Limulus polyphemus* hemocyanin in the content of all the amino acids examined.

The minimum molecular weight of arthropod hemocyanins has been reported recently to be in the range from 65,000 to 83,000[2-5]. The electrophoretic patterns of hemocyanins from 4 kinds of horseshoe crabs revealed that each hemocyanin consisted of 4 or more kinds of monomers, and that there were electrophoretic similarities among the hemocyanin monomers from 4 Xiphosuran species, that is, *Tachypleus tridentatus*[4], *T. gigas*[6], *Carcinoscorpius rotundicauda*[6], and *Limulus polyphemus*[7]. In our preliminary study some hemocyanin monomers from *T. gigas, C. rotundicauda,* and *L. polyphemus* reacted with anti-*Tachypleus tridentatus* hemocyanin antiserum. In immuno-diffusion tests, they formed precipitin lines partially or totally identical with some immunoprecipitin lines caused by the reaction of the antiserum with hemocyanin monomers from *Tachypleus tridentatus*[8]. To compare the characteristics of hemocyanin monomers from the 4 kinds of horseshoe crabs is very interesting for the study of the systematic relationship of all 4 species in the order Xiphosura.

Amino acid composition of arthropod hemocyanins has already been reported[2,5,9]. However, mixtures of hemocyanin monomers were analyzed as a whole in those experiments. Recently, Jeffrey et al.[10] have reported the amino acid composition of 1 of 2 hemocyanin monomers from the Australian freshwater crayfish. Sullivan et al.[7] have reported the amino acid composition of 5 fractions of

Amino acid composition of hemocyanin samples

Amino acid[a]	Monomer of *Tachypleus* hemocyanin				Whole hemocyanin	
	HT 1	HT 2	HT 3	HT 4	*Tachypleus*[b]	*Limulus*[c]
Lysine	7.15	6.90	6.52	7.83	6.65	6.80
Histidine	9.78	9.07	9.91	7.28	9.04	9.26
Arginine	7.07	8.38	8.09	6.69	7.73	7.16
Aspartic acid	14.84	13.92	12.42	13.33	13.36	12.16
Threonine	3.89	5.08	4.36	5.06	4.46	4.73
Serine	4.42	4.14	4.02	4.42	3.91	3.88
Glutamic acid	11.07	12.85	13.93	13.47	12.89	13.44
Proline	4.50	4.01	3.83	4.21	4.02	3.79
Glycine	3.53	3.40	3.51	4.31	3.30	3.00
Alanine	3.68	3.14	3.51	3.48	3.35	3.13
Valine	6.14	5.51	5.48	6.73	5.75	6.02
Isoleucine	5.25	5.70	6.39	6.25	5.86	5.50
Leucine	9.38	9.79	9.58	9.67	9.61	8.71
Tyrosine	2.10	1.92	1.54	Trace	3.18 (4.97)[d]	5.39
Phenylalanine	7.20	6.18	6.89	7.25	6.92	7.02

HT 2 consists of 2 kinds of immunologically distinct molecules[4] which cannot be separated by the electrophoretic method of Davis[11].
[a] Calculated from the analytical data of hydrolysis for 72 h and reported as g/100 g protein. [b] Average values of 4 hydrolysates. [c] Calculated from data for *Limulus* from Ghiretti-Magaldi et al.[9] and expressed as percents of 15 kinds of amino acids for comparison with *Tachypleus*. [d] A value from hydrolysis for 24 h.