column chromatography using a mixture of chloroform and hexane (1: 1) gave the product **7**. Subsequent elution with ethyl acetate gave the product **5**.

Reaction of 1c with Malononitrile (4a)——Following the general procedure, compound **1c** (0.66 g) was allowed to react with malononitrile (0.2 g). The ethyl acetate fraction gave a residue, which was washed with ethyl acetate to give a crystalline substance (**10**).

Reaction of 1c with Ethyl Cyanoacetate——Compound **1c** (0.66 g) was allowed to react with ethyl cyanoacetate (0.35 g) according to the general procedure. A mixture of ether and pertoleum ether (1: 3) was added to the ethyl acetate extract, and the whole was allowed to stand overnight in a refrigerater at −15°C to give a crystalline substance. Recrystallization from benzene gave the product **11**.

Acknowledgement A part of the cost of this work was defrayed by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan, which is gratefully acknowledged. We are indebted to Mrs. C. Koyanagi for elemental analysis, and Mr. K. Kawamura for the spectral data.

References

1) T. Kato and N. Katagiri, *Chem. Pharm. Bull.*, **21**, 729 (1973).
2) T. Kato, N. Katagiri, and R. Sato, *J. Chem. Soc. Perkin I*, **1979**, 525.
3) T. Kato, N. Katagiri, and R. Sato, *Chem. Pharm. Bull.*, **27**, 1176 (1979).
4) T. Kato, T. Chiba, R. Sato, and T. Yashima, *J. Org. Chem.*, **45**, 2020 (1980).
5) T. Kato, N. Katagiri, and R. Sato, *J. Org. Chem.*, **45**, 2587 (1980).

[Chem. Pharm. Bull. 29(8)2366—2369(1981)]

Antitumor Activity of 3-Nitroso-2-oxazolidones

Michiko Miyahara*, Makoto Miyahara, Shozo Kamiya and Akihiko Maekawa

National Institute of Hygienic Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo, 158, Japan

(Received December 19, 1980)

2-Oxazolidones (Ia→f) were not effective against rat ascites hepatoma AH13 or mouse lymphoid leukemia, L1210. However, among 3-nitroso-2-oxazolidones (IIa→f), compounds IIa, IIb, IIc and IId were active against AH13, and compounds IIa, IIb and IIf were active against L1210. Cyclic N-nitrosocarbamates and N-nitrosoureas showed greater antitumor effects than the corresponding acyclic N-nitroso compounds.

Since the reaction of compounds IIa→f with 4-(*p*-nitrobenzyl)pyridine gave a purple color, their antitumor mechanism presumably involves alkylation, but there was no correlation between the antitumor activities and the color intensities.

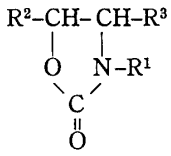
Keywords——2-oxazolidones; cyclic carbamates; 3-nitroso-2-oxazolidones; acyclic nitroso carbamates; antitumor activity; AH13; L1210; alkylating activity; NBP reagent

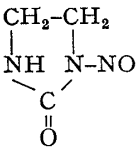
Ethyl carbamate (urethan) had been used for the treatment of chronic myeloid leukemia and related blood disease.[1] Antitumor antibiotics, mitomycin C and bleomycins, which are clinically useful antitumor agents, also have a carbamate partial structure with other antitumor chemical functional groups. Antitumor plant components, maytansinoids, have a cyclic carbamate partial structure with other antitumor chemical functional groups. These findings prompted us to test the antitumor activities of 2-oxazolidones (I) as cyclic carbamates and their N-nitroso derivatives (II).

Some of 3-nitroso-2-oxazolidones (IIa→f) were active against rat ascites hepatoma AH13 and mouse lymphoid leukemia L1210. Their alkylating activities were shown to be involved in the antitumor mechanism.

As shown in Table I, 2-oxazolidones (Ia→f) were all inactive against AH13 and L1210.

TABLE I. Toxicity and Anti AH13 and L1210 Actiyity of 2-Oxazolidones and 3-Nitroso-2-oxazolidones (*ip*—*ip* System)



Compd. No.	R^1	R^2	R^3	Donryu-AH13 Single MTD[a] (mg/kg)	Donryu-AH13 Single MED[b] (mg/kg)	Donryu-AH13 Daily administration Dose (mg/kg)	T/C (%)	60-Days survivors	CDF_1-L1210 Dose (mg/kg)	CDF_1-L1210 T/C (%)
Ia	H	H	H	>500	>500	50	94	0/5	100	101
						100	84	0/5	200	98
									400	98
Ib	H	CH_3	H	>250	>250	25	127	0/6	100	109
						50	102	0/6	200	113
									400	104
Ic	H	$ClCH_2$	H	>500	>500	50	95	0/5	100	98
						100	88	0/6	200	100
									400	100
Id	H	$BrCH_2$	H	>500	>500	50	86	0/6	100	102
						100	88	0/6	200	100
									400	102
Ie	H	C_6H_5	H	>500	>500	50	100	0/6	100	91
						100	104	0/6	200	109
									400	106
If	H	H	C_6H_5	>250	>250	50	103	0/6	100	102
						100	109	0/6	200	102
									400	98
IIa	NO	H	H	100	25	5	173	0/6	25	128
						10	289	1/6	50	113
						20	673	4/6	100	124
									200	64
IIb	NO	CH_3	H	>250	>250	25	80	0/6	50	102
						50	210	1/6	100	119
									200	139
IIc	NO	$ClCH_2$	H	250	10	12.5	168	0/5	100	114
						25	165	0/6	200	114
						50	174	0/6	400	71
IId	NO	$BrCH_2$	H	250	2.5	12.5	157	0/6	50	109
						25	265	0/6	100	106
						50	182	0/6	200	94
									400	79
IIe	NO	C_6H_5	H	>500	>500	50	140	0/5	100	104
						100	140	0/5	200	107
									400	115
IIf	NO	H	C_6H_5	250	>250	50	98	0/6	100	121
						100	117	0/6	200	125
									400	121
Reference compounds										
III	$C_2H_5N(NO)COOC_2H_5$			>500	>500	Not tested			50	100
									100	116
									200	109
									400	113
IV	$C_2H_5N(NO)CONH_2$			>250	>250	Not tested			50	94
									100	129
									200	121
V				50	50	2.5	205	0/6	6.25	133
						5	290	0/6	12.5	129
						10	>389	2/6	25	168

a) Maximum tolerated dose.
b) Minimum effective dose.

These compounds were not toxic to Donryu rats and CDF_1 mice at the maximum dose tested, 250 or 500 mg/kg to rats and 400 mg/kg to mice.

Compounds IIa, IIb, IIc and IId were active against AH13. Among them, compound IIa was the most active, and its administration at a dose of 20 mg/kg for 5 days prolonged the life span of rats bearing AH13. Compounds IIa, IIb and IIf were active against L1210, though the activities were only borderline, and among them, IIb was the most active (max. T/C%: 139). The 3-nitroso-2-oxazolidones active against AH13 were not necessarily active against L1210.

Ethyl N-ethyl-N-nitrosocarbamate (III), 1-ethyl-1-nitrosourea (IV) and a cyclic nitrosourea, 1-nitroso-2-imidazolidone (V), were tested for antitumor activities against both tumors in order to compare the activities of cyclic and acyclic nitrosocarbamates, and of cyclic and acyclic nitrosoureas. Since the acyclic compounds III and IV did not show any cytological effect as judged by microscopic observation after single administration to rats bearing AH13, daily administration test of these compounds to rats was not carried out. However, the cyclic nitrosocarbamate IIa was active against AH13, and also against L1210. The cyclic nitrosourea V was active against both tumors. Consequently, the cyclic nitroso compounds appeared to have a stronger antitumor effect and to be more toxic than the corresponding acyclic nitroso compounds. Hassner and Reuss[2] reported that some carbonium cations were formed in the decomposition of 3-nitroso-2-oxazolidones in alkaline solution. It is therefore suggested that some active intermediates are also formed *in vivo* by ring-opening of the 3-nitroso-2-oxazolidones.

4-(*p*-Nitrobenzyl)pyridine (NBP reagent) has been used for the assay of alkylating agents and for comparison of the alkylating activities of various types of alkylating agents.[3] The structures of the colored products have not always been elucidated. We examined the reactions of the non-nitroso compounds (Ia→f) and the nitroso compounds (IIa→f) with the NBP reagent by the modified method reported by Preussmann *et al.*[3b] Compounds Ia—f did not give any color reaction, whereas all of the nitroso compounds (IIa→f) gave a purple color. The color intensities are tabulated in the experimental section. These results suggest that these compounds, similar to other nitrosourea antitumor agents, are alkylating agents. However, there was no correlating between the antitumor activities and the color intensities.

Experimental

2-Oxazolidones (Ia→f) and 3-Nitroso-2-oxazolidones (IIa→f)——2-Oxazolidone (Ia): Purchased from Wako Junyaku Co., mp 89°C. 5-Methyl-2-oxazolidone (Ib): Colorless prisms. mp 113°C (lit.,[4] mp 111—113°C). 5-Chloromethyl-2-oxazolidone (Ic): Purchased from Aldrich Chemical Co., mp 104—105°C. 5-Bromomethyl-2-oxazolidone (Id): Colorless prisms, mp 106—107°C (lit.,[5] mp 107°C). 5-Phenyl-2-oxazolidone (Ie): Colorless leaflets (from ethanol), mp 89—90°C (lit.,[6] mp 88—90°C). 4-Phenyl-2-oxazolidone (If): Colorless leaflets (from ethanol), mp 127—128°C. *Anal.* Calcd for $C_9H_9NO_2$; C, 66.24; H, 5.56; N, 8.58. Found: C, 66.16; H, 5.54; N, 8.56. 3-Nitroso-2-oxazolidone (IIa): Pale yellow prisms (from a mixture of ether and n-hexane), mp 41°C. *Anal.* Calcd for $C_3H_4N_2O_3$: C, 31.04; H, 3.47; N, 24.14. Found: C, 30.85; H, 3.72; N, 24.26. 5-Methyl-3-nitroso-2-oxazolidone (IIb): Pale yellow, crystalline powder (from ether), mp 30—32°C. IR ν_{max}^{Nujol} cm^{-1}: 1815 (C=O), 1495, 1345, 1160. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, d, CH_3), 3.42 (1H, q, $-CH_2-$), 4.08 (1H, q, $-CH_2-$), 4.87 (1H, q, $-CH\langle$). 5-Chloromethyl-3-nitroso-2-oxazolidone (IIc): Pale yellow, crystalline powder (from ether), mp 15—20°C. IR ν_{max}^{Nujol} cm^{-1}: 1795, 1500, 1340, 1150. ^{13}C-NMR($CDCl_3$) δ; 151.431 (C=O), 44.712 (C_3), 73.100 (C_4), 43.275 (C_6). ^{1}H-NMR($CDCl_3$) δ: ABX, 5.31—5.42 (1H, Mul, $-CH\langle$), 4.00—4.36 (4H, Mul, $ClCH_2-$, $-CH_2-$). 5-Bromomethyl-3-nitroso-2-oxazolidone (IId): Pale yellow, crystalline powder, mp 41—42°C. IR ν_{max}^{Nujol} cm^{-1}: 1790, 1490, 1345, 1120. ^{1}H-NMR ($CDCl_3$) δ: ABX, 3.73—4.26 (2H, Mul, $-CH_2-$), 4.79—5.20 (1H, Mul, $-CH\langle$), 3.65 (2H, d, $ClCH_2-$). 3-Nitroso-5-phenyl-2-oxazolidone (IIe): Yellow, crystalline powder (from a mixture of acetone and *n*-hexane), mp 76—79°C (lit.,[7] mp 76.5—77.5°C). 3-Nitroso-4-phenyl-2-oxazolidone (IIf): Yellow needles (from a mixture of acetone and *n*-hexane), mp 105—107°C. *Anal.* Calcd for $C_9H_8N_2O_3$: C, 56.25; H, 4.20; N, 14.58. Found: C, 56.17; H, 4.26; N, 14.68.

Reference Compounds——Ethyl N-ethyl-N-nitrosocarbamate (III): Pink liquid, bp 52—53°C (5 mmHg) (lit.,[8] bp 90°C (42 mmHg)). 1-Ethyl-1-nitrosourea (IV): mp 101—102°C (lit.,[9] mp 103—104°C). 1-Nitroso-

2-imidazolidinone (V): mp 98—100°C (lit.,[10] mp 95—100°C).

Screening Methods——AH13 Test System: AH13 cells donated by the Sasaki Institute, Tokyo, were propagated by intraperitoneal inoculation in Donryu rats (Nihon Rat Co., Tokyo).

Single Administraiton Test: Details have been described in our previous paper.[11]

Daliy Administration Test: The test compound was intraperitoneally administered every day for 5 days, starting on day 3 after tumor inoculation. Each compound was tested at two or three dose levels ranging from one-twelfth to one-fifth of MTD, and six animals were used at each dose level. Antitumor activity was evaluated in terms of T/C% (T=mean survival time of treated animals, C=mean survival time of control ainmals) and survival ratio at the 60th day after inoculation.

L1210 Test System——L1210 cells donated by the Cancer Chemotherapy Center, Tokyo, were propagated by intraperitoneal inoculation in DBA/2 mice, and CDF_1 mice supplied from the Institute of Medical Science, University of Tokyo, were used as test animals. The test compounds were intraperitoneally administered on days 2 and 6 after intraperitoneal inoculation of 10^5 L1210 cells per mouse. Antitumor activity was evaluated in terms of T/C%. A value of $125 \leqq T/C\%$ was considered effective against L1210.

Test Method for Alkylating Activity——Each compound (0.03 m mol) was dissolved in 1.4 ml of ethylene glycol monomethyl ether, and the solution was mixed with 1.4 ml of a 5% 4-(*p*-nitrobenzyl)pyridine solution in ethylene glycol monomethyl ether. The mixture was allowed to stand for 15 minutes at 37°C, then 0.2 ml of piperidine was added (final concentration: 0.01 m mol/3.0 ml). After 5 minutes, the absorbance was determined at the maximum wavelength. The relative activity is presented as a percentage of the activity of compound IIa.

Table II. Relative Alkylating Activity in the Color Reaction of 3-Nitroso-2-oxazolidones (IIa→f) with the NBP Reagent

Compd. No.	λ_{max}	Relative alkylating activity
IIa	561	100
IIb	564	48
IIc	547	55
IId	533	38
IIe	561	20
IIf	548	154
III	568	45
IV	563	258
V	561	151

Acknowledgement We are grateful to Dr. Shigeru Tsukagoshi, Cancer Chemotherapy Center, Tokyo, for providing animals bearing tumors and for helpful advice. We are also grateful to Dr. Yuzo Hayashi of this Institute for helpful advice.

References and Notes

1) A.P. Dustin, "Ciba Foundation Sym., Leukemia Resi.", p. 244. (1954).
2) A. Hassner and R.H. Reuss, *J. Org. Chem.*, **39**, 553 (1974).
3) *a*) J. Epstein, R.W. Rosenthal and R.J. Ess, *Anal. Chem.*, **27**, 1435 (1955); *b*) R. Preussmann, H. Schneider and F. Epple, *Arzneim. Forsch.*, **19**, 1059 (1969); *c*) G.P. Wheeler and S. Chumley, *J. Org. Chem.*, **10**, 259 (1967).
4) H. Najer, P. Chabrier, R. Giudicelli, J. Menin and J. Duchemin, *Bull. Chem. Soc., France*, 1841 (1959).
5) E. Katchalski and D.B. Ishai, *J. Org. Chem.*, **15**, 1067 (1950).
6) W.J. Close, *J. Am. Chem. Soc.*, **73**, 95 (1951).
7) M.S. Newman and A. Kutner, *J. Am. Chem. Soc.*, **73**, 4199 (1951).
8) H.V. Pechmann, *Ber.*, **28**, 2641 (1895).
9) E.A. Werner, *J. Chem. Soc.*, **115**, 1100 (1919).
10) M.W. Kirkwood and G.F. Wright, *J. Am. Chem. Soc.*, **76**, 1836 (1954).
11) Mi. Miyahara, S. Kamiya, A. Maekawa and S. Odashima, *Gann*, **70**, 731 (1979).