UDC 615.771.7:547.234'222

157. Yoshio Sakurai and Michiko Aoshima : Studies on Carcinostatic Substances. XLI.*1 Preparation of Azide Derivatives of 1,1-Bis(2-chloroethyl)hydrazine.

(*Sasaki Institute, Cancer Chemotherapy Section**2)

In the previous paper of the present authors,[1] some azide derivatives of 1,1-bis(2-chloroethyl)hydrazine were reported, and among them 1,1-bis(2-chloroethyl)-2-nicotinoylhydrazine was found to have an antitumor effect against Yoshida sarcoma.

This paper deals with the preparation of three azides of amino acid, including 1,1-bis(2-chloroethyl)-2-succinylhydrazine and 1,1-bis(2-chloroethyl)-2,2-dimethylhydrazine.

The compounds and their biological data so far obtained are demonstrated in the following Table I. In case hydrochloride or hydrobromide could not be obtained in crystalline state, they were identified as picrylsulfonate or picrolonate.

Table I.

Compound	m.p. (°C)	Yoshida Sarcoma *in vivo* LD_{50}	MTD	MED	Yoshida Sarcoma *in vitro* CE	MEC
$(ClCH_2CH_2)_2$-N•NH-CO-CH(NH$_2$)-CH_3•HBr	124~126	75	50	5	±	$5\cdot10^{-1}$
$(ClCH_2CH_2)_2$-N•NH-CO-CH_2NH_2•HBr	Powder	/	/	/	−	
$(ClCH_2CH_2)_2$-N•NH-CO-CH(NH$_2$)-$(CH_2)_3$-CH_2NH_2•HBr	Powder	/	/	/	/	/
$(ClCH_2CH_2)_2$-N•NH-CO-CH_2CH_2-COOH	64~65		>500	50	+	1
$(ClCH_2CH_2)_2N$•N-$(CH_3)_2$•HCl	Syrup	175	100	/	+	$5\cdot10^{-2}$

LD_{50} : Rat (i.p.) mg./kg.
MTD : Maximum Tolerance Dose on Rat (i.p.) mg./kg.
MED : Minimum Effective Dose on Yoshida Sarcoma *in vivo* (i.p.) mg./kg.
CE : Cell Effect, morphologically determined
MEC : Minimum Effective Concentration on Yoshida Sarcoma Cells *in vitro* m*M*

The derivatives of amino acid were prepared according to the following scheme :

$$\text{R-CH(NH-Cbz)-COCl} \xrightarrow[\text{in pyridine or triethylamine}]{(ClCH_2CH_2)_2NNH_2} \text{R-CH(NH-Cbz)-CONHN}(CH_2CH_2Cl)_2$$

$$\xrightarrow[\text{in AcOH}]{HBr} \text{R-CH(NH}_2\cdot\text{HBr)-CO-NHN}(CH_2CH_2Cl)_2$$

Cbz : $C_6H_5CH_2OCO$-

Succinyl derivative was obtained by reaction of succinic anhydride in pyridine with 1,1-bis(2-chloroethyl)hydrazine, and 1,1-bis(2-chloroethyl)-2,2-dimethylhydrazine was prepared by chlorination of 1,1-dimethyl-2,2-bis(2-hydroxyethyl)hydrazine with thionyl chloride.

Test of antitumor activity of the compounds has not yet been completed, however, as expected, 1-DL-alanyl-2,2-bis(2-chloroethyl)hydrazine seems to be a derivative

*1 This paper constitutes a part of series entitled "Studies on Carcinostatic Substances" by M. Ishidate and Y. Sakurai. Part XL : This Bulletin, **10**, 462 (1962).
*2 26, Nishigahara 1-Chome, Kita-ku, Tokyo (桜井欽夫, 青島迪子).
1) M. Ishidate, Y. Sakurai, Y. Kuwada : This Bulletin, 8, 543 (1960).

with latent activity, because it exhibits remarkable effect on Yoshida sarcoma *in vivo* but no effect on the same tumor *in vitro*. Complete biological data will be published later.

Experimental

1-(N-Carbobenzoxy-DL-alanyl)-2,2-bis(2-chloroethyl)hydrazine——Crude N-carbobenzoxy-DL-alanylchloride was prepared according to the method reported by Bergmann[2] from N-carbobenzoxy-DL-alanine (9.6 g.) and PCl_5 (9.6 g.). It was washed once with petr. ether and used as such without purification. The total amount of the chloride obtained was dissolved in dehyd. Et_2O (200 cc.), to which $(C_2H_5)_3N$ (24 g.) was added portionwise under cooling with cold brine. To this mixture, free base of 1,1-bis(2-chloroethyl)hydrazine obtained from its hydrochloride (6.0 g.), dissolved in dehyd. Et_2O (100 cc.), was added and the whole mixture was kept first in an ice-box for 3 hr. and then at room temperature for 1 hr. The precipitate was filtered off and the filtrate was washed first with 2% HCl, then with H_2O. After the Et_2O-layer was dried over anhyd. Na_2SO_4, the solvent was removed. The crystalline residue (6.3 g.) was recrystallized from EtOH to white prisms, m.p. 119~120°. *Anal.* Calcd. for $C_{15}H_{21}O_3N_3Cl_2$: C, 49.73; H, 5.84; N, 11.60. Found: C, 49.52; H, 5.75; N, 11.59.

1-DL-Alanyl-2,2-bis(2-chloroethyl)hydrazine Hydrobromide——1-(N-Carbobenzoxy-DL-alanyl)-2,2-bis(2-chloroethyl)hydrazine (0.9 g.) was dissolved in glacial AcOH (4 cc.) which was previously saturated with HBr[3] and the solution was kept at 20° for 30 min. with occasional stirring. After being added with dehyd. Et_2O (10 cc.) and kept overnight in an ice-box, the crystalline precipitate (0.75 g.) was filtered and washed with Et_2O. It was recrystallized by keeping its EtOH solution in an ice-box for a week after addition of adequate amount of Et_2O. Hygroscopic crystalline powder, m.p. 124~126°, was obtained. *Anal.* Calcd. for $C_7H_{16}ON_3Cl_2Br$: C, 27.20; H, 5.22; N. 13.60. Found: C, 27.20; H, 5.17; N, 13.33. Picrolonate, yellow needles, m.p. 169~170° (decomp.). *Anal.* Calcd. for $C_{17}H_{23}O_6N_7Cl_2$: C, 41.48; H, 4.71; N, 19.91. Found: C, 41,71; H. 4.66; N, 19.88.

1-(N-Carbobenzoxyglycyl)-2,2-bis(2-chloroethyl)hydrazine——Preparation procedure is the same as in case of the foregoing alanine derivative. Yield, 0.3 g. from 1,1-bis(2-chloroethyl)hydrazine hydrochloride (4 g.). Colorless needles, m.p. 126~127°. *Anal.* Calcd. for $C_{14}H_{19}O_3N_3Cl_2$: C, 48.26; H, 5.50; N, 12.07. Found: C, 48.52; H, 5.76; N, 12.22.

1,1-Bis(2-chloroethyl)-2-glycylhydrazine Hydrobromide——The hydrobromide was too hygroscopic to be obtained as pure specimen. Picrolonate, yellow needles, m.p. 183~184° (decomp.), was prepared. *Anal.* Calcd. for $C_{16}H_{21}O_6N_7Cl_2$: C, 40.18; H, 4.43; N, 20.51. Found: C, 40.40; H, 4.32; N, 20.28.

1,1-Bis(2-chloroethyl)-2-(N-α,ε-dicarbobenzoxy-L-lysyl)hydrazine——N-α,ε-dicarbobenzoxy-L-lysylchloride was prepared by a method of Bergmann from N-α,ε-dicarbobenzoxy-L-lysine[4] (3.0 g.) and PCl_5 (1.6 g.) and dissolved as such without purification in dehyd. Et_2O (70 cc.). To the mixture, pyridine (2 g.) was first added under strong cooling with brine and then the free base of 1,1-bis(2-chloroethyl)hydrazine obtained from its hydrochloride (1.0 g.). The solution was treated similarly as in case of preparation of the above stated alanine derivatives. Yield, 0.1 g. White crystalline powder, m.p. 134~135°. *Anal.* Calcd. for $C_{26}H_{34}O_5N_4Cl_2$: C, 56.42; H, 6.19; N, 10.12. Found: C, 56.30; H, 6.11; N, 9.89.

1,1-Bis(2-chloroethyl)-2-L-lysylhydrazine Hydrobromide——The hydrobromide was not obtained as pure specimen because of its strong hygroscopicity. Crystalline picrolonate, m.p. 158~159°, was obtained, but not perfectly pure. *Anal.* Calcd. for $C_{20}H_{30}O_6N_8Cl_2$: C, 43.72; H, 5.50; N, 20.40 Found: C, 44.49; H, 4.38; N, 20.04.

1,1-Bis(2-chloroethyl)-2-succinylhydrazine——Succinic anhydride (0.5 g.), dissolved in pyridine (4 cc.), was mixed with dehyd. Et_2O solution of 1,1-bis(2-chloroethyl)hydrazine prepared from its hydrochloride (1.0 g.). Et_2O was removed by slight warming and the residual solution was kept overnight at 24°. Pyridine was distilled off *in vacuo* and the solid residue obtained after being kept *in vacuum* H_2SO_4-desiccator for 3 days was extracted with $CHCl_3$. $CHCl_3$ was distilled off from the extract and the residue was again dissolved in H_2O and extracted with AcOEt. On removing AcOEt, a crystalline solid was obtained, which was recrystallized from H_2O. Fresh crystals were colorless prisms, melting at 64~65°, but, when dried these changed to white powder melting at 88~89°. *Anal.* Calcd. for $C_8H_{14}O_3N_2Cl_2$: C, 37.37; H, 5.48; N, 10.90. Found: C, 37.42; H, 5.67; N, 10.93.

1,1-Dimethyl-2,2-bis(2-hydroxyethyl)hydrazine——Solution of *asym*-dimethylhydrazine (10 g.) in 2*N*

2) M. Bergmann, *et al.*: Ber., **65**, 1192 (1932); Org. Syntheses., **23**, 13 (1943).
3) N. F. Albertson, *et al.*: J. Am. Chem. Soc., **75**, 5323 (1953); Dov Ben-Ishai, *et al.*: J. Org. Chem., **17**, 1564 (1952); *ibid.*, **19**, 62 (1954).
4) M. Bergmann, *et al.*: J. Biol. Chem., **111**, 245 (1935).

AcOH (120 cc.) was adjusted to pH 5～6 by addition of glacial AcOH. Into this solution, ethylene oxide (23 g.) was passed at room temperature and the mixture was kept overnight in an ice-box. Solvent and reagent were removed *in vacuo* and the syrupy residue was used as such for the next process without purification.

1,1-Bis(2-chloroethyl)-2,2-dimethylhydrazine Hydrochloride——Into suspension of 1,1-dimethyl-2,2-bis(2-hydroxyethyl)hydrazine (5 g.) in $CHCl_3$ (15 cc.), $SOCl_2$ (28 g.) dissolved in $CHCl_3$ (15 cc.) was added dropwise under ice-cooling. After addition of $SOCl_2$ was completed, temperature was gradually raised to 40～50° and the whole solution was kept at the same temperature with stirring for 2 hr. The solution separated in two layers, when cooled. The upper-layer was removed off and the lower one was distilled. The residue was poured on ice-water, extracted with Et_2O, and the H_2O-layer was distilled *in vacuo*. Hydrochloride was not obtained in crystalline state. Picrylsulfonate, pale yellow prisms. m.p. 188～189°, was prepared. *Anal.* Calcd. for $C_{12}H_{17}O_9N_5Cl_2S$: C, 30.13; H, 3.58; N, 14.64. Found : C, 30.30; H, 3.22; N, 14.45.

The authors are indebted to Dr. H. Satoh and Dr. H. Imamura for their collaboration in biological experiments.

Summary

Derivetives of 1,1-bis(2-chloroethyl)hydrazine, especially of amino acid hydrazine type, were prepared for the purpose of testing their latent antitumor activity.

(Received August 14, 1961)

UDC 547.94[615.783.19].07:542.98:582.28

158. Michihiro Yamada, Kazuaki Iizuka, Shigenobu Okuda, Toshinobu Asai, und Kyosuke Tsuda : Untersuchungen auf dem Gebiet der Mikrobiologischen Umsetzung. XVII.[1] Umwandlung von Alkaloiden in der Morphin-Reihe durch *Trametes sanguinea* (2).

(*Institut für angewandte Mikrobiologie,*[*1] *Universität Tokio*)

Die Beobachtung, daß eine Kultur von *Trametes sanguinea* einerseits Thebain (I) in 14β-Stellung zu hydroxylieren und andererseits die Carbonylgruppe des 14β-Hydroxycodeinons (IV) zu reduzieren vermag, veranlaßte uns, das Verhalten dieses Mikroorganismus gegenüber Codeinon (II) bzw. Neopinon (VII) zu studieren.

Die Versuche wurden wie bei der Umsetzung mit Thebain[1] bei 30° unter aeroben Bedingungen in Schüttelkulturen durchgeführt. Die Umsetzungen wurden zunächst papierchromatographisch verfolgt und dann in präparativem Maßstab ausgeführt.

Codeinon (II)[2] lieferte bei der Umsetzung in Nährlösung Nr. 2[*2] zwei Stoffe, nämlich Codein (III)[2] und 14β-Hydroxycodein (V),[2,3] mit einer Ausbeute von 24.5 bzw. 7.6%. Wenn diese Umsetzung in Nährlösung Nr. 1[*2] durchgeführt wurde, so ließ sich auch ein dritter Stoff, nämlich 14β-Hydroxycodeinon (IV)[2,4] neben den obengenannten Stoffen

*1 Yayoicho, Bunkioku, Tokio (山田道弘, 飯塚和明, 奥田重信, 朝井勇宜, 津田恭介).

*2 Nährlösung Nr. 1 : H_2O Lösung von 1% Glukose, 0.2% Pepton, 0.1% Rindfleisch-Extrakt, 0.1% Hefe-Extrakt, 0.3% Corn-steep-liq.; Nährlösung Nr. 2 : Koji-Extrakt mit 0.3% Corn-steep-liq. Siehe auch Fußnote 1.

1) XVI Mitteil. Dieses Bulletin, **10**, 67 (1962).
2) R.H.F. Manske, H.L. Holmes : The Alkaloids Vol. II, p. 91 (1952), Academic Press Inc., New York.
3) A.C. Currie, J. Gillon, G.T. Newbold, F.S. Spring : J. Chem. Soc., **1960**, 773.
4) Fel' Dman, Lyutenberg : J. Applied Chem., (U.S.S.R.) **18**, 715 (1945).