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Isao Aiko and Tsuneo Gōno : On the Optical Isomers of α-〔N-Bis(β-chloroethyl)amino〕propionamide.

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Recently Izumi reported on the anti-cancer action of racemic N-bis(β-chloroethyl)-α-amino acids.[1]

An attempt is reported here to resolve this derivative of α-amino acid into optical isomers and to compare their biological activity. However, resolution of racemic N-bis-(β-chloroethyl)alanine failed, because its brucine salt was not crystalline, while the crystalline salt of α-chloro-*d*-camphor-π-sulfonic acid could not be separated into the isomers by recrystallization. It was then succeeded to some extent to prepare the optically antipodal isomers of α-〔N-bis(β-chloroethyl)amino〕propionamide by recrystallization of the *d*-camphor-β-sulfonate of the racemate using a mixture of acetone and ethanol.

Effectiveness on the Yoshida sarcoma cells and toxicity of both isomers are demonstrated in Table I.**

Table I. $Cl\text{-}CH_2\text{-}CH_2 \atop Cl\text{-}CH_2\text{-}CH_2 \rangle N\text{-}\overset{CH_3}{\overset{|}{C}}H\text{-}CONH_2 \cdot HCl$

	Toxicity			Effection Yoshida sarcoma MED (mg./kg.)
	LD_{50} (mg./kg.)		MTD (mg./kg.)	
	Rat	Mouse	Rat	
l-Form	0.7	2.9	0.5	0.05
d-Form	0.7	3.1	0.5	0.05

LD_{50} : Intraperitoneal
MTD : Max. tolerable Dose (intraperitoneal)
MED : Min. effective Dose (intraperitoneal) to induce the characteristic morphological aberration in the mitotic process of Yoshida sarcoma cells in the peritoneal cavity of rat 48 hrs. after injection.

Bergel, *et al.* once reported that the *l*-form of *p*-bis(2-chloroethyl)phenylalanine surpassed its antipode as regards activity against the Walker rat carcinoma.[2]

It is interesting that, as seen in the table, both isomers of α-〔N-bis(β-chloroethyl)-amino〕propionamide, which had no primary amino group in α-position, exhibited almost the same grade of toxicity and effectiveness on the tumor, regardless of their optical property.

The acid obtained from the *d*- or *l*-amide by hydrolysis with concentrated hydrochloric acid seemed to agree by analysis with *d*- or *l*-bis(β-chloroethyl)alanine, but the yield from the reaction was also poor. The experiment is now being continued.

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Experimental

***l*-α-〔N-Bis(β-chloroethyl)amino〕propionamide *d*-Camphor-β-sulfonate** (I)—Acetone (350 cc.) was

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** Animal experiments were carried out by Dr. H. Satoh of Iatrochemical Institute of Pharmacological Research Foundation, Tokyo.

1) M. Izumi : This Bulletin, **2**, 279(1954).
2) F. Bergel : J. Pharm. Pharmacol., **7**, 308(1955); J. Chem. Soc. **1954**, 2409.

added to MeOH solution of *dl*-α-[N-bis(β-chloroethyl)amino]propionamide (39 g.) and *d*-camphor-β-sulfonic acid (42.5 g.) and the mixture was kept in ice-box over night. The precipitated product (47 g.; m.p. 135~137°) was recrystallized from MeOH-acetone (80 cc. : 300 cc.) to plates, m.p. 150~151° (decomp.); $[\alpha]_D^{21}$ +3.3°(c=3.0, *l*=1, H_2O). *Anal.* Calcd. for $C_{17}H_{30}O_5N_2Cl_2S$: C, 45.84; H, 6.79. Found: C, 45.64; H, 6.73.

***l*-α-[N-Bis(β-chloroethyl)amino]propinamide (II)**—When a concentrated solution of (I) (9.3 g.) was added with a solution containing an equivalent amount of K_2CO_3, (II) separated as crystals (3.4 g.) melting at 70~72°; $[\alpha]_D^{16}$ −57° (c=3.0, *l*=1, EtOH). *Anal.* Calcd. for $C_7H_{14}ON_2Cl_2$: C, 39.45; H, 6.63; N, 13.15. Found : C, 39.43; H, 6.45; N, 13.00.

Hydrochloride (III) of (II)—Dry HCl was passed into a solution of (II) (3 g.) and the separated product was recrystallized from MeOH, m.p. 189~191°(decomp.). Yield : 3.0 g. $[\alpha]_D^{15}$ −17° (c=3.0, *l*=1, H_2O). *Anal.* Calcd. for $C_{17}H_{15}ON_2Cl_3$: C, 33.69; H, 6.05. Found : C, 33.68; H, 5.90.

***d*-α-[N-Bis(β-chloroethyl)amino]propionamide (IV)**—The whole filtrate, from which (I) was removed, was concentrated. The crude precipitate thus obtained was recrystallized 5 times from dehyd. EtOH. Plates, m.p. 151~152°(decomp.). Yield, 7.0 g. (IV) was obtained by treating this *d*-camphor-β-sulfonate with conc. KOH, as scales, m.p. 71~73°; $[\alpha]_D^{21}$ +40.5° (c=3.0, *l*=1, EtOH).

***dl*-N-Bis(β-chloroethyl)alanine α-Chloro-*d*-camphor-π-sulfonate**—*dl*-N-Bis(β-chloroethyl)alanine hydrochloride (5.0 g.) and ammonium α-chloro-*d*-camphor-π-sulfonate (2.8 g.) were dissolved in hot H_2O (24 cc.) and the solution was kept in a cool place over night. A crystalline salt precipitated which melted at 172~175°. $[\alpha]_D^{8}$ +33.3°(c=3.0, *l*=1, H_2O). Its specific rotation did not change after repeated recrystallization from H_2O, acetone-ether, or hydr. acetone. *Anal.* Calcd. for $C_{17}H_{28}O_6NCl_3S$: C, 42.46; H, 5.87. Found : C, 42.62; H, 6.10.

***l*-N-Bis(β-chloroethyl)alanine Hydrochloride**—(III) (5.2 g.) was added into conc. HCl (d=1.19) (31 cc.) and heated at 80° for 1 hr. After evaporation *in vacuo* to dryness, a crystalline residue was extracted with hot acetone. The cooled extract was added with ether and a crystalline precipitate was obtained. Yield, 3.4 g. of m.p. 86~87°. It was recrystallized from acetone, m.p. 90°. $[\alpha]_D^{25}$ −22° (c=3.0, *l*=1, H_2O), $[\alpha]_D^{25}$ −30° (c=1.5, *l*=1, H_2O+1*M* HCl). *Anal.* Calcd. for $C_7H_{14}O_2NCl_3$: C, 33.55; H, 5.63; N, 5.59. Found : C, 32.99; H, 6.31; N, 5.13.

Summary

Racemic α-[N-bis(β-chloroethyl)amino]propionamide was resolved into the optical isomers. There was however found no difference between the two isomers in toxicity or anticancer activity against the Yoshida sarcoma.

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Shoji Shibata : On the Structure of Strepsilin. II.[1]

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Previously I proposed that strepsilin, $C_{15}H_{10}O_5$, m.p. 324°, a lichen substance isolated from *Cladonia strepsilis* (ACH.) WAIN., is a dibenzofuran derivative, which should be represented by the formula (Ia). Evidence for the structure (Ia) was provided chiefly by the conversion of strepsilin by alkaline degradation into 1-methyl-3,7-dihydroxydibenzofuran (III), m.p. 212°, which was synthetically obtained. For an acidic intermediate product of the alkaline degradation, $C_{14}H_{10}O_5$, m.p. 308°, the structure (II) was adopted mainly by its blue coloration with ferric chloride.

The compound (III) might be formed by the reactions which involved oxidation of the phthalide ring followed by decarboxylation at the 9-position.

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1) S. Shibata : Acta Phytochim., **14**, 177(1944) (C. A., **45**, 5677(1951)).