49. Yasuo Inubushi and Mutsuo Kozuka : Studies on the Alkaloids of Menispermaceous Plants. CXVIII[1]. On the Structure of Trilobine and Isotrilobine. (11)[2]. Synthesis of Trilobine-Type Alkaloid from Oxyacanthine*.

(*Pharmaceutical Institute, Medical Faculty, University of Kyoto***)

Recently, Tomita, Inubushi, and Kozuka[3,4] succeeded in deriving from isotetrandrine (I (−, +)) and tetrandrine (I (+, +)), belonging to the berbamine series (I), the bases of the trilobine-isotrilobine type (II) possessing a diphenylene dioxide nucleus in their molecules. As a result, it was elucidated that both O-methylanhydrodemethylisotetrandrine[3] (II (−, +)) and O-methylanhydrodemethyltetrandrine[4] (II (+, +)) thereby obtained, agreed with neither trilobine nor isotrilobine itself, and also their methyl methine bases[5] were not identical with any of those of trilobine and isotrilobine. These experimental results led to a suggestion that both trilobine and isotrilobine have the common structure of (VI)-type derivable from the oxyacanthine type of bases (III), and consequently, both optically inactive methyl methines of trilobine and isotrilobine were reinvestigated, with the result that they were confirmed to be identical. Thus it has become clear that the relationship between trilobine and isotrilobine is not that of structural isomerism, as so far considered, but that of optical isomerism. In other words, the difference between them is presumably due to the difference in the steric configurations of their two asymmetric centers.

The present paper deals with the synthesis of a new base of the trilobine type (VI) from oxyacanthine (III, R=H), and the data thereby obtained.

On being heated with hydrobromic acid (d=1.78) at 100° for 3 hrs., oxyacanthine (III, R=H) furnished demethyloxyacanthine (IV). This substance is a crystalline powder, m.p. 231~235° (decomp.) and shows $[\alpha]_D^{18}$: +291.9° (in pyridine). It contains no methoxyl groups, and gives a green color with ferric chloride, changing to violet on addition of alkali. After being dried *in vacuo*, it has a tendency to absorb moisture readily, and when it crystallizes from a mixture of water and methanol, it holds five molecules of water of crystallization, and its analytical values correspond to the composition of $C_{34}H_{34}O_6N_2 \cdot 5\,H_2O$. Methylation of this substance (IV) with diazomethane gave no crystalline methyl ether, but its hydrochloride crystallized in the form of microscopic needles, decomposing at 271~275°, $[\alpha]_D^6$: +261.3°, identical by direct comparison with the hydrochloride (m.p. 273~275° (decomp.), $[\alpha]_D^6$: +257.7°) of O-methyloxyacanthine[6].

Subsequently, demethyloxyacanthine (IV), when further heated with hydrobromic acid (d=1.78) in a sealed tube at 130° for 3 hrs., gave rise to anhydrodemethyloxyacanthine (V). This substance is phenolic, and crystallizes in the form of pillars, m.p. 292° (decomp.), $[\alpha]_D^{15}$: +278.7° (in pyridine). It gives no color with ferric chloride, and a blue color with sulfuric-nitric acid reagent, indicating the presence of a diphenylene dioxide system. It contains no methoxyl groups and its analytical values represent the composition of $C_{34}H_{32}O_5N_2 \cdot H_2O$.

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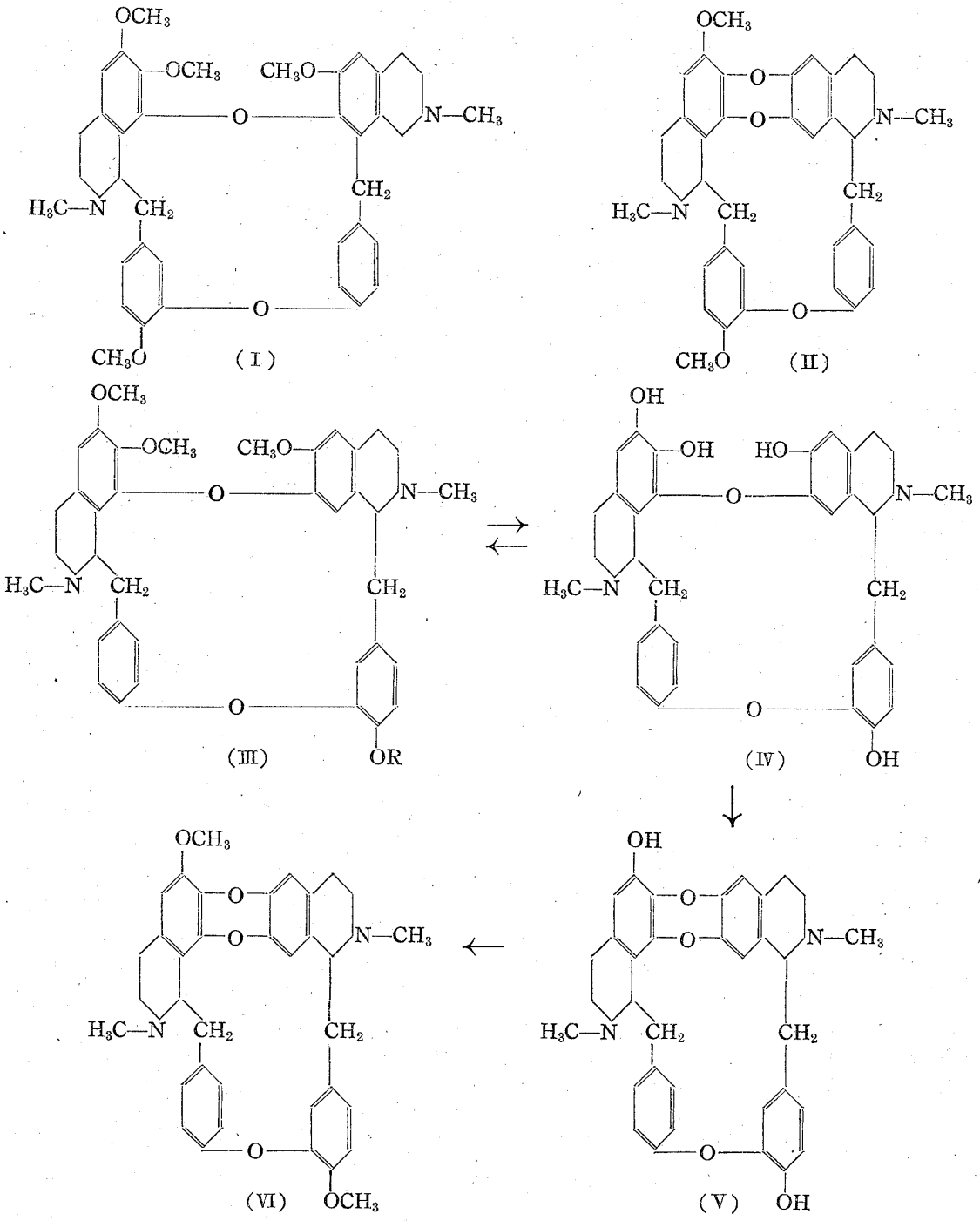
* Masao Tomita : Studies on the Alkaloids of Menispermaceous Plants. CXVIII.

** Yoshida-konoe-cho, Sakyo-ku, Kyoto (犬伏康夫, 小塚睦夫).

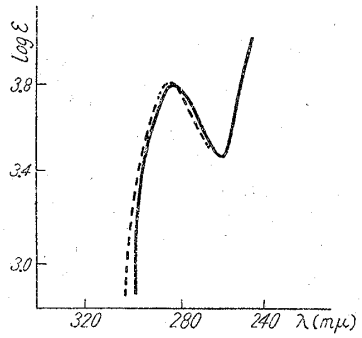
1) Part CXVII : H. Kondo, K. Takeda : Ann. Repts. ITSUU Lab. Japan, 5, 1(1953).
2) Part 10 : M. Tomita, Y. Inubushi : This Bulletin, **2**, 6 (1954).
3) M. Tomita, Y. Inubushi, M. Kozuka : *Ibid.*, **1**, 360 (1953).
4) Y. Inubushi : *Ibid.*, **2**, 1 (1954).
5) M. Tomita, Y. Inubushi, M. Kozuka : *Ibid.*, **1**, 368 (1953).
6) H. Kondo, M. Tomita : J. Pharm. Soc. Japan, **50**, 309 (1930).



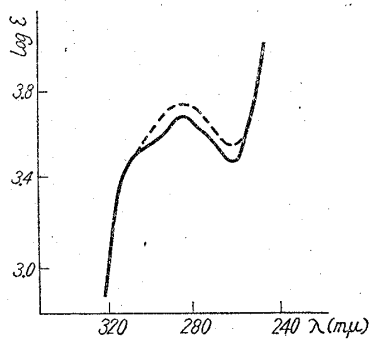
Anhydrodemethyloxyacanthine (V), on methylation with diazomethane, afforded O-methylanhydrodemethyloxyacanthine (VI), which crystallized in the form of colorless pillars, m.p. 182~183°, $[\alpha]_D^{12}$: +256.2° (in chloroform). The test for a diphenylene dioxide group with sulfuric-nitric acid reagent was positive. By analyses, it contains two methoxyl groups and two methylimino groups, and has the composition of $C_{36}H_{36}O_5N_2$.

The ultraviolet spectra of oxyacanthine (III, R=H) and demethyloxyacanthine (IV) are shown in Fig. 1, and exhibit a close relationship with those of the oxyacanthine-berbamine series. The spectra of anhydrodemethyloxyacanthine (V), O-methylanhydrodemethyloxyacanthine (VI), trilobine, and isotrilobine, shown in Fig. 2, are in contrast with those in Fig. 1. That is to say, the difference between the spectra of demethyloxyacanthine (IV) and anhydrodemethyloxyacanthine (V) is, as reported by the authors[3]

in the previous paper, that the absorption maximum of the latter has shifted to a longer wave-length range than that of the former, and produces a wide absorption band. This difference closely resembles that between the spectra of the oxyacanthine-berbamine and the trilobine-type bases. O-Methylanhydrodemethyloxyacanthine (VI) shows a complete similarity with trilobine and isotrilobine in the absorption spectra.

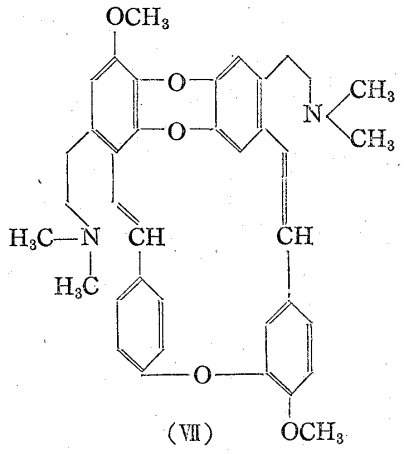


—— Oxyacanthine
---- Demethyloxyacanthine
Fig. 1.



—— Trilobine; Isotrilobine; O-Methyl-anhydrodemethyloxyacanthine
---- Anhydrodemethyloxyacanthine
Fig. 2.

Anhydrodemethyloxyacanthine (V) →
Trilobine (VI) →
Isotrilobine (VI) →



(VII)

Anhydrodemethyloxyacanthine (V), after conversion into its O-methyl ether methosulfate, was submitted to the first stage of the Hofmann degradation, and gave the methyl methine base (VII), which crystallized from acetone in the form of colorless pillars, m.p. 106～107°. After being dried *in vacuo*, it melted at 114° (sint. 110°), $[\alpha]_D^{13}$: ±0°. Furthermore, the properties of this substance agree reasonably well with those given for the optically inactive methyl methine base[7] derived from trilobine and isotrilobine, the identity of which was established by the mixed melting point determination.

For convenience of comparison, the data of a trilobine type base derived from oxyacanthine, and trilobine and isotrilobine as well as their corresponding derivatives, are summarized in Table I.

In spite of the fact that O-methylanhydrodemethyloxyacanthine (VI) derived from oxyacanthine (III, R=H) furnished the analytical data, the ultraviolet absorption spectrum, as well as the color reaction etc., which are completely similar to those given for trilobine and isotrilobine, it is not identical, as is apparent from the foregoing results, with any of them, though the optically inactive methyl methines from these three bases were confirmed to be quite identical with each other. This fact suggests that O-methyl-anhydrodemethyloxyacanthine (VI) is optically isomeric with trilobine or isotrilobine.

7) H. Kondo, M. Tomita: J. Pharm. Soc. Japan, **52**, 356 (1932); Ann., **497**, 104 (1932); M. Tomita, T. Tani: J. Pharm. Soc. Japan, **62**, 468 (1942).

TABLE I.

Anhydrodemethyloxyacanthine (V)	Demethyltrilobine	Demethylisotrilobine
Microscopic pillars m.p. 292°(decomp.) $[\alpha]_D^{15}$: +278.7° (pyridine) $C_{34}H_{32}O_5N_2$	Pillars m.p. 290°(decomp.) $[\alpha]_D^{18}$: +229.3°(CH_3CO_2H) $C_{34}H_{32}O_5N_2$	Microscopic crystals m.p. 270°(decomp.) $C_{34}H_{32}O_5N_2$
O-Methylanhydrodemethyloxyacanthine (VI)	Trilobine	Isotrilobine
Pillars (acetone) m.p. 181~182° $[\alpha]_D^{12}$: +256.2°($CHCl_3$) $C_{36}H_{36}O_5N_2$	Pillars or Needles (C_6H_6) m.p. 238° $[\alpha]_D^{8}$: +302.8°($CHCl_3$) $C_{36}H_{36}O_5N_2$	Pillars or Needles m.p. 215°(acetone) $[\alpha]_D^{8}$: +314.8°($CHCl_3$) $C_{36}H_{36}O_5N_2$
O-Methylanhydrodemethyloxyacanthine methyl methine (VII)	Trilobine methyl methine (VII)	Isotrilobine methyl methine (VII)
Pillars (acetone) m.p. 106~107° m.p. 114°(sint. 110°) (dried *in vacuo* at 80°) $[\alpha]_D^{13}$: ±0° $C_{38}H_{40}O_5N_2$	Pillars (acetone) m.p. 105~107° m.p. 115°(sint. 113°) (dried *in vacuo* at 80°) $[\alpha]_D$: ±0° $C_{38}H_{40}O_5N_2$	Pillars (acetone) m.p. 105~107° m.p. 114°(sint. 110°) (dried *in vacuo* at 80°) $[\alpha]_D$: ±0° $C_{38}H_{40}O_5N_2$

In this investigation, experimental evidence was also presented that both trilobine and isotrilobine should be represented by the same structural formula (VI), as suggested in the previous paper of this series. Thus, it has been elucidated that the relationship between trilobine and isotrilobine is not that of structural isomerism (one is represented by (II) and the other, by (VI), and vice versa), as so far considered, but of optical isomerism, and that the difference between them must be due to the difference in the stereochemical arrangements about the two asymmetric centeres in their molecules. However, the experimental data so far obtained are insufficient to discuss the steric configurations of the two asymmetric centers in these bases, and this problem must await a further investigation accompanied by comparison with a trilobine-type base derivable from repandine[8], an optical isomer of oxyacanthine.

Moreover, the present experiment gave support to the view[9] that the bases of the oxyacanthine-berbamine series which arose by dehydrogenation from the two molecules of the coclaurine type bases, may undergo subsequent dehydration to form the trilobine-isotrilobine type bases.

The authors wish to express their appreciation to Mr. T. Komiyama in Gotemba-machi, Shizuoka Prefecture, for his assistance in collecting the plant material, and to Dr. S. Katoh, Dr. M. Hiyama, and Mr. N. Tanno of the Osaka Factory of the Dainippon Pharmaceutical Industries for the facilities for preparing the methanol extract. This work was carried out under the guidance of Prof. Dr. M. Tomita, and was supported by a Grant from the Scientific Research Fund of the Ministry of Education, to which the authors are also indebted.

Experimental[10]

Isolation and Purification of Oxyacanthine (III, R=H)[11]—1.3 kg. of the sticky residual mass prepared from the MeOH extract of the root and stem of *Berberis Thunbergii* DC., was extracted several times with 2% aq. AcOH solution. The extracts were combined, made alkaline with aq. NH_3, and the depositing precipitate was extracted by means of a large amount of ether. The combined ether extracts were concentrated, and shaken with 2% aq. H_2SO_4 solution, into which the basic portion was transferred.

8) F. v. Bruchhausen, H. Schultze : Arch. Pharm., **267**, 623 (1929); I. R. C. Bick, A. R. Todd : J. Chem. Soc., **1948**, 2170; E. Fujita, T. Saijoh : J. Pharm. Soc. Japan, **72**, 1232 (1952).
9) M. Tomita, T. Tani : J. Pharm. Soc. Japan, **62**, 468 (1942); M. Tomita : Fortschr. Chem. org. Naturstoffe, **9**, 175 (1952).
10) All melting points are uncorrected. The authors are indebted to Messrs. Hozumi, Imaeda, and Kinoshita for the microanalyses.
11) H. Kondo, M. Tomita : J. Pharm. Soc. Japan, **50**, 309 (1930); K. Tanaka : *Ibid.*, **64**, 27 (1944); E. Fujita : *Ibid.*, **72**, 213 (1952).

Subsequently, this acid extract was saturated with Na_2SO_4 and allowed to stand overnight, after which the deposited precipitate of the sulfate was filtered with suction. This was dissolved in water, made alkaline with aq. NH_3, and the depositing base was taken up in ether. The ether extract was dried over anhyd. K_2CO_3, and concentrated until crystals appeared. Upon standing overnight, colorless needles crystallized out in large quantities. The yield was 5.6 g. By recrystallization from a mixture of acetone and ether, they showed m.p. 210~213°, m.p. 214~216° (in vac.). $[\alpha]_D^{24}$: +276.1° (in $CHCl_3$, l=0.947 dm., c=1.035). The mother liquor separated from oxyacanthine was freed from ether, and after addition of a small portion of benzene, was kept standing, yielding 2.5 g. of berbamine benzene adduct, m.p. 127°.

Demethyloxyacanthine (IV)—1 g. of oxyacanthine was heated with 10 cc. of HBr (d=1.78) at 100° for 3 hrs. After the reaction was complete, the content was poured into 30 cc. of water, whereby the hydrobromide separated out. This was collected, dissolved in MeOH, and filtered from insoluble impurities. The filtrate was made alkaline with aq. NH_3, and the impurities which deposited were removed by filtration. Subsequently, the MeOH solution was warmed gently, and water was added, whereupon yellow precipitates deposited out. They were dissolved in a mixture of $CHCl_3$ and MeOH, filtered from insoluble impurities, and water added, yielding 0.5 g. of crystalline powder, m.p. 231~235° (decomp). This substance yielded a green color with $FeCl_3$, changing to violet on addition of alkali. It contains no methoxyl group. $[\alpha]_D^{15}$: +291.9° (in pyridine, l=0.5 dm., c=0.507). *Anal.* Calcd. for $C_{34}H_{34}O_6N_2 \cdot 5H_2O$: C, 62.19; H, 6.70. Found: C, 61.84; H, 6.95.

Methylation of Demethyloxyacanthine (IV) with Diazomethane—To 0.3 g. of demethyloxyacanthine in alcohol, diazomethane (from 1.5 g. nitrosomethylurea) in ether was added and the mixture was set aside for 3 days, after which a further similar quantity of diazomethane was added. The reaction mixture was dissolved in benzene, chromatographed on alumina, and the slightly yellowish oil thus obtained was difficult to crystallize. This was converted into its crystalline hydrochloride by dissolving in a small portion of EtOH, and by making acid by addition of a few drops of HCl. Upon recrystallization from a small amount of water, it showed the decomposing point of 271~275°; yield, 0.03 g. $[\alpha]_D^6$: +261.3°(in MeOH, l=0.25 dm., c=0.82). *Anal.* Calcd. for $C_{38}H_{42}O_6N_2 \cdot 2HCl \cdot 2H_2O$: C, 62.38; H, 6.56. Found: C, 62.13; H, 6.51.

Meanwhile, the free base prepared by methylating 0.5 g. of oxyacanthine with diazomethane in like manner, did not crystallize after purification, but it gave a crystalline hydrochloride decomposing at 273~275°; yield, 0.3 g. $[\alpha]_D^6$: +257.7° (in MeOH, l=0.25 dm., c=0.80). *Anal.* Calcd. for $C_{38}H_{42}O_6N_2 \cdot 2HCl \cdot 2H_2O$: C, 62.38; H, 6.56. Found: C, 62.10; H, 6.43.

Anhydrodemethyloxyacanthine (V)—1.8 g. of demethyloxyacanthine (IV) was heated with 10 cc. of HBr (d=1.78) in a sealed tube at 130° for 3 hrs. After completion of the reaction, the reaction mixture was flown into a small amount of water, and the depositing hydrobromide was filtered with suction. This was dissolved in 20 cc. of MeOH by warming, filtered, and the filtrate was made alkaline with aq. NH_3 and upon warming gently, deposited minute pillars, decomposing at 292° and weighing 0.85 g. They yielded no color with $FeCl_3$, and a blue color with H_2SO_4 and HNO_3 reagent. $[\alpha]_D^{15}$: +278.7° (in pyridine, l=0.5 dm., c=0.43). *Anal.* Calcd. for $C_{34}H_{32}O_5N_2 \cdot H_2O$: C, 72.08; H, 6.00. Found: C, 72.10; H, 5.94.

O-Methylanhydrodemethyloxyacanthine (VI)—0.5 g. of anhydrodemethyloxyacanthine (V) was dissolved in $CHCl_3$ and treated with excess ethereal diazomethane. The mixture was kept standing with occasional shaking for 1 week. After repeating one more such manipulation, the reaction mixture was filtered, and the solvent removed. The residue was dissolved in aq. HBr solution, made alkaline with aq. NaOH solution, and the depositing base taken up in $CHCl_3$. The chloroform extract was dried over anhydrous K_2CO_3 and the solvent removed. The residue was dissolved in benzene, passed through an alumina column (1×5 cm.), and developed with the same solvent. A colorless oil obtained from the initial eluate fraction was moistend with a small portion of acetone, and upon standing, solidified. By recrystallization from acetone, it crystallized in the form of pillars, m.p. 181~182°; yield, 0.3 g. $[\alpha]_D^{12}$: +256.2° (in chloroform, l=0.5 dm., c=0.56). *Anal.* Calcd. for $C_{36}H_{36}O_5N_2$: C, 74.96; H, 6.30; OCH_3, 10.76; NCH_3, 5.20. Found: C, 74.63; H, 6.26; OCH_3, 10.32; NCH_3, 5.07.

Degradation of Anhydrodemethyloxyacanthine (V) by Hofmann Method—0.3 g. of anhydrodemethyloxyacanthine (V) was dissolved in 10 cc. of 5% aq. NaOH, and shaken with 1 cc. of $(CH_3)_2SO_4$. Then, a further 0.5 cc. of $(CH_3)_2SO_4$ was added and the mixture was shaken for 40 minutes, during which period the medium was kept alkaline. The reaction mixture was extracted with ether to remove excess $(CH_3)_2SO_4$, and the aqueous portion was diluted to 30 cc. by addition of water, and heated with 6 g. of KOH for 30 minutes. The deposited oil was taken up in ether, the ether extracts dried over anhyd. K_2CO_3 and the ether distilled off, leaving a yellow oily product. This was induced to crystallize by moistening it with acetone and upon standing it formed colorless pillars, m.p. 106~107°; further recrystallization did not raise the m.p. When it was dried *in vacuo*, however, the melting point was raised to 114° (sint. at 110°), undepressed by admixture with the methyl methine, m.p. 114° (sint. at 110°) of either trilobine or isotrilobine. $[\alpha]_D^{13}$: ±0° (in chloroform, l=0.5 dm., c=0.49).

Anal. Calcd. for $C_{38}H_{40}O_5N_2$: C, 75.49; H, 6.62. Found : C, 75.06; H, 6.84.

Summary

Upon the assumption that if any base of the trilobine type (VI) is derived from oxyacanthine (III, R=H), this base must reasonably be identical with either trilobine or isotrilobine, O-methylanhydrodemethyloxyacanthine (VI) was prepared. It was found, however, that the base (VI) so obtained agreed with neither trilobine nor isotrilobine itself, and was an optical isomer of these. On comparison of the optically inactive methyl methine base (VII), obtained by the elimination of the two asymmetric centers in the molecule of (VI), with the optically inactive methyl methine base derived from either trilobine or isotrilobine, both were confirmed to be identical. Accordingly, it is suggested that trilobine and isotrilobine must be optically isomeric with each other, both having the same structure (VI).

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50. **Kozo Maruyama** : Effect of Nitrogen Mustard N-Oxides and the Related Compounds upon Viscosity of a Sodium Polymetacrylate Solution*.

(*Iatrochemical Institute of the Pharmacological Research Foundation***)

In 1952, the paper had been published by Alexander[1] which described that viscosity of the neutral solution of polyacrylic acid, like desoxyribonucleic acid, fell markedly when it was kept with the bi- or poly-functional alkylating agents such as nitrogen mustard (HN_2) or tris(ethylenimino)-S-triazine (TEM) at 19° for a certain length of time.

It was also suggested that such decrease in viscosity was caused by the simultaneous alkylation of the neighbouring two acid residues of a desoxyribonucleic acid molecule or a polyacrylic acid molecule by the twin β-chloroethyl groups of the compound under formation of ring esters. On the contrary, in case of the monofunctional reagent, the esterification may occur at random on acid residues and so they had less influence on viscosity of the solution. This fact was supposed to have a certain relation with the biological activity of the compounds.

Nitrogen mustard N-oxides, which had been found by us to have remarkable anticancer effectiveness, were already shown to react with anions as monofunctional alkylating agents *in vitro*, forming N,N-alkyl-β-chloroethyl-O-(A)-ethylhydroxylamines as end products (A : anion)[2].

The aim of this experiment was to decide whether the nitrogen mustard N-oxides belong to the monofunctional alkylating agent or not from the view point of viscosity depression.

As a reaction mixture, a dilute solution containing polymetacrylic acid equivalent to 0.1 *mM* calculated as a monomer and 0.05 *mM* of the alkylating agent was tested in this experiment. After the solution was incubated at 18° for 24 hrs., its relative visco-

* M. Ishidate, Y. Sakurai : Studies on Cancerocidal Substances. IX.

** 1-26, Nishigahara, Kita-ku, Tokyo (丸山幸三).

1) P. Alexander : Nature, **169**, 226, 572 (1952).

2) I. Aiko, S. Owari, M. Torigoe : J. Pharm. Soc. Japan, **72**, 1297 (1952); S. Owari : This Bulletin, **1**, 353 (1953).