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31. **Tanezo Taguchi and Tatsuya Ishida**: Studies in Stereochemistry. XII.[1] Mechanism of Racemization in Schiff Bases of D-Methionine Ethyl Ester.

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Holland[2] has claimed that epimerization in ethyl N-benzylidene L(±)-*erythro*-*p*-nitrophenylserinate occurs through tautomerism in its azomethine structure. To ascertain the validity of his claim for a prototropic change in Schiff bases of α-amino acid esters, studies on the Schiff bases of D-methionine ethyl ester were undertaken.

The Schiff bases were prepared by condensation of D-methionine ethyl ester with aldehydes in ether in the presence of anhydrous sodium sulfate. As aldehydes were expected to affect the racemization velocity of the corresponding Schiff base, different kinds of aldehyde such as isobutyraldehyde, benzaldehyde, and *p*-nitrobenzaldehyde were used. Ethyl ester of N-benzylidene-D-methionine (I) was obtained as a colorless oil with unpleasant odor which was successfully distilled under reduced pressure accompanied by notable racemization. The change of optical rotation and the index of refraction before and after distillation of the compound are shown in Table I. The change of refractive index was negligible, and hence the decrease in the value of optical rotation was supposed to arise from racemization of the Schiff base (I).

TABLE I. The Change of Optical Rotation and Refractive Index before and after Distillation of Ethyl Ester of N-Benzylidene-D-methionine

	$[\alpha]_D^{30}$*	n_D^{21}
Before distillation	+125°	1.5407
After one distillation	+ 33°	1.5429
After two distillations	+ 11°	1.5431

* c=10, dehyd. EtOH.

The racemization was examined on the addition of a basic substance, potassium carbonate or triethylamine, in dehyd. ethanol at 25° (Fig. 1). The racemization velocity was found to be promoted by basic substances and was affected by potassium carbonate so greatly that the initial $[\alpha]_D^{18} = +60.7°$ of the solution decreased to approximate $[\alpha]_D^{20} = +5°$ after 32 days. When the reaction temperature rose to the boiling point of ethanol, the velocity became so fast that racemization in 10% ethanolic solution of (I) with 0.1 mole of triethylamine per mole of (I), for example, was completed after approximately 30 hours.

Ethyl esters of N-*p*-nitrobenzylidene- and N-isobutylidene-D-methionines (III and II) were also submitted to analogous treatment for racemization. The N-*p*-nitrobenzylidene derivative (III), m.p. 34~35°, was allowed to stand at 25° in dehyd. ethanol containing 0.1 mole of triethylamine per mole of (III). The recemization of the Schiff base (III) was completed after almost 24 hours (Fig. 2), but the N-isobutylidene derivative (II), $b.p_{0.02}$ 70~71°, racemized leaving 70% of initial $[\alpha]_D$ value of the solution even after 30 days under the same conditions (Fig. 2), and leaving 32% of the value after the same period when the reaction temperature rose to the boiling point of the solution. The racemization velocities of these two Schiff bases were compared with that of the N-benzylidene derivative (I) in Fig. 2 where the three compounds rank in the order of (II) $\ll$(I)$\ll$(III).

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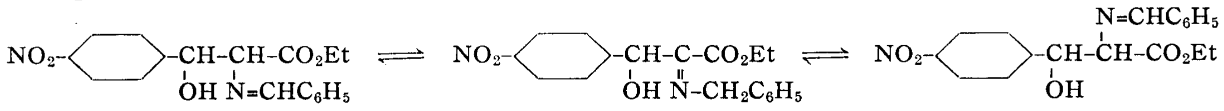
[1] Part XI. This Bulletin, 4, 487(1956),

[2] O.O. Holland: J. Chem. Soc., **1953**, 273.

For practical purposes it is desirable that D-methionine racemizes via its own Schiff base without conversion to its ester, but in a study on relationship between pH and Schiff base formation of free amino acids, Gulland[3] concluded that the formation reaction occured above pH 7 and was reversible. The formation of sodium salt of N-benzylidene-D-methionine was tried by boiling sodium salt of D-methionine and benzaldehyde in dehyd. ethanol. The disappearance of benzaldehyde on boiling suggested the possible existence of the corresponding Schiff base in the solution (pH 8.0~8.5), which was further boiled for 48 hours in expectation of the occurance of racemization. However, unchanged D-methionine and a small amount of polymerization product were recovered from the solution without racemization. The formation of sodium salt of N-*p*-nitrobenzylidene-D-methionine was also tried by the analogous treatment where the corresponding Schiff base precipitated in solid state from the solution. The compound was found to be unstable in water and fairly stable in anhydrous methanol and ethanol. It was recrystallized from methanol, m.p. 210°(decomp.). After boiling of the Schiff base in anhydrous methanol for 6 hours, the racemization was accomplished and DL-methionine was recovered in 50% yield. Addition of a basic substance as a catalyst is not recommended because polymerization is also accelerated with racemization.

Discussion

In the Schiff bases of D-methionine ethyl ester, the tendency to racemize might originate from the activation of H at the asymmetric carbon by the electron-attracting character of the adjacent ethoxycarbonyl group. The azomethine group seems to play a supporting part. The racemization velocity of sodium salt of N-*p*-nitrobenzylidene-D-methionine was found to be lower than that of the corresponding Schiff base of D-methionine ethyl ester. The decrease of velocity may be due to the decrease of electron-attracting character of the carboxyl anion in the molecule of the salt. The N-*p*-nitrobenzylidene derivative of D(+)-*threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol was found to remain unchanged on several trials for racemization. The failure of racemization is presumably due to the fact that the compound lacks an electron-attracting group adjacent to the aminated asymmetric carbon except the azomethine group. These findings give support to the above consideration for the racemization of Schiff bases. It has been reported that prototropy catalyzed by basic substances in compounds with a powerful electron-approoriating group occurs through tautomerism which was explained as a bimolecular reaction by Ingold, *et al*[4] and as a synchronous termoleular reaction by Ossorio and Hughes.[5] Holland[2] found that the treatment of the N-benzylidene derivative of ethyl L(±)-*erythro*-*p*-nitrophenylserinate with triethylamine gave a mixture of *erythro* and *threo* epimers. He suggested that the isomerization occurs through tautomerism as shown in the following :



erythro *threo*

In the present study the racemization velocities of ethyl esters of N-isobutylidene-, N-benzylidene-, and N-*p*-nitrobenzylidene-D-methionine (II, I, and III) were compared and the relative velocities were found to be in the order of (II)≪(I)≪(III). If the racemization resulted through tautomerism in the azomethine structure as Holland claimed, the relative velocities might be in the order of (II)>(I)>(III). The latter order is deduced

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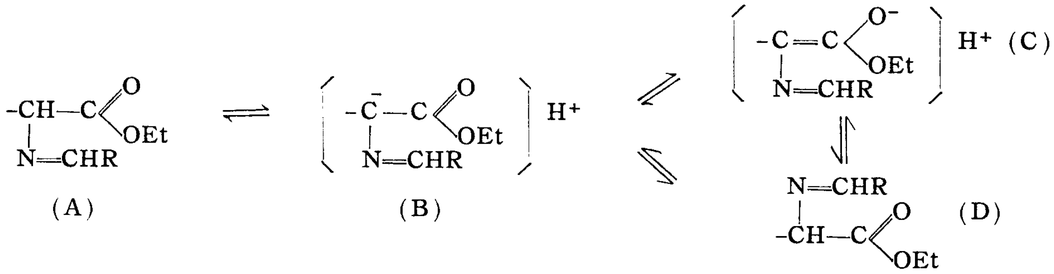
[3] Gulland, Mead : J. Chem. Soc., **1935**, 210.

[4] Ingold, Schoppee, Thorpe : J. Chem. Soc., **1926**, 1477.

[5] R. P. Ossorio, E. G. Hughes : *Ibid.*, **1952**, 426.

from the consideration that tautomerism which arises from the azomethine structure is promoted by the N-isobutylidene group with $-I$ effect and inhibited by the N-*p*-nitrobenzyliden group with $+I$ effect in comparison with the effect of N-benzylidene group. However, the racemization velocities of the Schiff bases were found to be in the reverse order, $II \ll I \ll III$. This finding denies Holland's proposal of tautomerism arising from the azomethine structure as the isomerization mechanism. Hence it was postulated that the racemization occurs through the separation and recombination of H^+ from and with the asymmetric carbon, $(A) \rightleftarrows (B) \rightleftarrows (D)$, in the process of which intermediate (C) may also be involved, $(A) \rightleftarrows (B) \rightleftarrows (C) \rightleftarrows (D)$.



The azomethine group affects racemization not through tautomerism arising from itself but in the manner now to be described.

Base-catalyzed prototropy in such a series is activated by the electron-attracting character of the adjacent ethoxycarbonyl group and probably also by the stabilization of the intermediate (C) due to the conjugation of double bonds. In the N-isobutylidene derivative (II), H^+ dissociation at the asymmetric carbon is inhibited to some degree by the $-I$ effect of the isobutylidene radical. On the other hand, in the N-*p*-nitrobenzylidene derivative (III) the dissociation is promoted by the $+I$ effect of *p*-nitrobenzylidene group and additionally by the attainable resonance energy of the molecule after the dissociation. Therefore, the racemization velocities seem to vary in the order of $(II) \ll (I) \ll (III)$. In general it is doubtful that prototropy in Schiff bases of amines with an adjacent carbonyl group occur through tautomerism in $>C^{*}H-N=C<$ structure, but in Schiff bases of amines without another electron-appropriating group in their neighborhood, it is naturally supposed that prototropy may occasionally occur through tautomerism due to the azomethine structure under the additional influence of other factors. For example tautomerism in the following type of compounds is well known.

(IV) (V)

In both of the components (IV and V) of the tautomeric system, the C=N double bond can alternatively conjugate with one of the two benzene rings. Therefore, the two components are stabilized and can be isolated. The isomerism of Schiff bases derived from pyridoxal and pyridoxamine[6] or of substituted methylene-azomethines[5] belongs to this type of tautomerism. The failure in the racemization of D(+)-*threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol shows that the azomethine group does not cause tautomerism by itself unless it is accompanied by some activation factors (e.g. conjugation with benzene ring). On looking back upon the whole discussion, it is suggested that the prototropic changes in Schiff base with and without an electron-appropriating group occur through different mechanisms.

The authors are indebted to the Microanalytical Section of this Institute and the Center of Microanlyses of this University for microanalyses and also to Takeda Pharmaceutical Industries, Ltd. for supplying D-methionine.

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6) B. Witkop, T. W. Beiler: J. Am. Chem. Soc., **76**, 5589(1954).

Experimental[7]

D-Methionine—Supplied by the Takeda Pharmaceutical Industries, Ltd. Colorless scales, m.p. 278~280°(decomp.), $[\alpha]_D^{21}$ +7.2.

D-Methionine Ethyl Ester—Prepared from D-methionine and dehyd. EtOH with HCl by the usual method, b.p$_{15}$ 123~126°; $[\alpha]_D^{30}$ −9.1°.

Schiff Bases of D-Methionine Ethyl Ester: General Procedure—To 5 parts of D-methionine ethyl ester dissolved in 15 parts of dehyd. ether were added 3 parts of anhyd. Na_2SO_4 and 1 mole of aldehyde per mole of D-methionine ethyl ester. The mixture was boiled for about 1 hr. After cooling, Na_2SO_4 was filtered off and the filtrate was evaporated to dryness, leaving the crude product. The microanalytical data, yield, and physical constants are given in Table II.

TABLE II.

Schiff base	Formula	Analyses (%)		m.p. or b.p.	$[\alpha]_D$ (c=10, EtOH)	Yield
		Calcd.	Found			
Ethyl ester of N-benzylidene-D-methionine (I)	$C_{14}H_{19}O_2NS$	C, 63.44 H, 7.28 N, 5.28	C, 63.04 H, 7.08 N, 5.51	b.p$_{0.004}$ 130~131°	cf. Table I	96%
Ethyl ester of N-isobutylidene-D-methionine (II)	$C_{11}H_{21}O_2NS$	N, 6.06	N, 5.89	b.p$_{0.02}$ 70~71°	$[\alpha]_D^{10}$ +61.5° a) $[\alpha]_D^{10}$ +56.3° b)	83.5%
Ethyl ester of *p*-nitrobenzylidene-D-methionine (III)	$C_{14}H_{18}O_4N_2S$	C, 54.19 H, 5.81 N, 9.03	C, 54.32 H, 5.82 N, 8.83	m.p. 34~35° c)	$[\alpha]_D^{15}$ +68.8° a) $[\alpha]_D^{15}$ +48.0° b)	97%

a) Values of crude product.
b) Values after purification.
c) Recrystallized from ether-petroleum ether.

Sodium Salt of N-*p*-Nitrobenzylidene-D-methionine—A mixture of 5 g. of sodium salt of D-methionine and 4.5 g. of *p*-nitrobenzaldehyde in 50 cc. of dehyd. EtOH was warmed on a water bath until in solution. After cooling, the precipitated yellow crystals were collected by filtration and dried *in vacuo*; yield, 3.8 g. On concentration of the filtrate, an additional 2 g. was obtained. The product was easily soluble in water decomposing to sodium salt of D-methionine and *p*-nitrobenzaldehyde and scarcely soluble in EtOH and sparingly soluble in MeOH with slow decomposition.

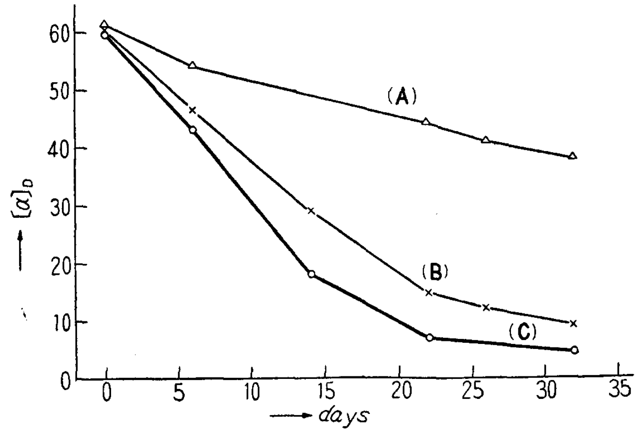

Fig. 1. Velocity of Racemization in Ethyl Ester of N-benzylidene-D-methionine (I) with or without a Catalyst at 25°

Test solutions contained 10 g. of (I) per 100 cc. EtOH; without catalyst (A), with 0.1 mole of triethylamine per mole of (I)(B), and 0.1 mole of K_2CO_3 per mole of (I)(C). The solutions themselves were used for the measurement of optical rotation.

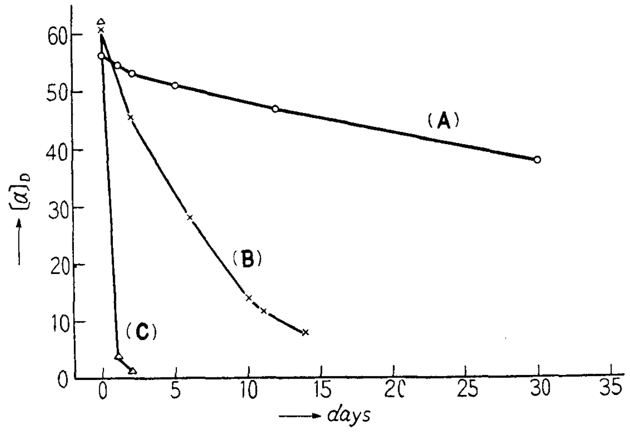

Fig. 2. Velocitiy of Racemization in Schiff Bases of D-Methionine Esters with Triethylamine at 25°

Test solutions contained 10 g. of the Schiff base per 100 cc. EtOH and 0.1 mole of triethylamine per mole of the Schiff base; (A) ethyl ester of N-isobutylidene-D-methionine, (B) ethyl ester of N-benzylidene-D-methionine, and (C) ethyl ester of N-*p*-nitrobenzylidene-D-methionine. The solutions themselves were used for the measurement of optical rotation.

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[7] All m.p.s and b.p.s are uncorrected.

Recrystallization from dehyd. MeOH showed m.p. 210°(decomp.); $[\alpha]_D^{20}$ +20.5°(c=2, MeOH at pH 8.0[8] with triethylamine). *Anal.* Calcd. for $C_{12}H_{13}O_4N_2SNa$: N, 9.20. Found : N, 9.13.

N-*p*-Nitrobenzylidene Derivative of D(+)-*threo*-1-*p*-Nitrophenyl-2-amino-1,3-propanediol—A mixture of 2.5 g. of D(+)-*threo*-1-*p*-nitrophenyl-2-amino-1,3-propanediol and 1.5 g. of *p*-nitrobenzaldehyde in 50 cc. of MeOH was boiled on a water bath for 0.5 hr. and concentrated. After cooling, the precipitated colorless crystals were collected; m.p. 175~176° after recrystallization from MeOH. $[\alpha]_D^{20}$ +142°(c=1, MeOH). *Anal.* Calcd. for $C_{16}H_{15}O_6N_3$: N, 12.17. Found : N, 12.22.

Attempted Racemization in the Schiff Bases of D-Methionine Ethyl Ester under Various Conditions—The methods and results were described in the text and shown in Figs. 1 and 2. The recovery of methionine from solutions treated for racemization is as follows :

a) **N-Benzylidene Derivative** (I)—i) From the dehyd. EtOH solution of 10% of (I) with 0.1 mole of triethylamine per mole of (I)($[\alpha]_D$ of which decreased from +60.7° to +2.5° after boiling for 24 hrs.) : 30 cc. of the solution was evaporated *in vacuo*, 15 cc. of 10% HCl added, and shaken. After allowing to stand for 3 hrs., the separated benzaldehyde was extracted with benzene. The aqueous solution was boiled for 1 hr., evaporated to dryness, 7 cc. of EtOH added, and neutralized with pyridine to pH 5~6. On setting aside, colorless scales precipitated, m.p. 265~275°. Yield, 1.20 g. (70%). $[\alpha]_D^{15}$ −0.01(c=10, EtOH). The product was identical with DL-methionine by a mixed m.p. determination.

ii) From the dehyd. EtOH solution of 10% of (I) with 0.1 mole of K_2CO_3 per mole of (I)($[\alpha]_D$ of which decreased from +60.3° to +4.5° after allowing to stand at 25° for 32 days)(see Fig. 1) : After filtration of K_2CO_3 the filtrate was treated just as in (i) to obtain DL-methionine, m.p. 263~269°. Yield, 60%. $[\alpha]_D^{15}$ −0.47 (c=10, EtOH).

iii) From the dehyd. EtOH Solution of 10%, (I) without catalyst ($[\alpha]_D$ of which decreased from +61.0° to +38° on allowing to stand at 25°)(see Fig. 1) : The solution was treated as in (i) to obtain partially racemized D-methionine, m.p. 265~269°. Yield, 67%. $[\alpha]_D^{18}$ +3.11 (c=10, EtOH).

b) **N-Isobutylidene Derivative** (II)—The material used was the dehyd. EtOH solution of 10% of (II) with 0.1 mole of triethylamine per mole of (II)($[\alpha]_D$ of which showed +38° dropping from initial +56.2° after being allowed to stand at 25° for 30 days)(see Fig. 2). After evaporation of 15 cc. of the solution, the residue was distilled under reduced pressure to give partially racemized (II) b.p$_{0.025}$ 71~73°. Yield, 1.2 g.(80%). To the product was added 7 cc. of 10% HCl and the solution was boiled for 1 hr. The aqueous solution was evaporated to dryness *in vacuo*, 5 cc. of EtOH added and neutralized with pyridine to pH 5~6 to cause the precipitation of partially racemized D-methionine, m.p. 262~268°. Yield, 560 mg.(60%). $[\alpha]_D^{18}$ +4.31°(c=10, EtOH).

c) **N-*p*-Nitrobenzylidene Derivative** (III)—The sample used was the dehyd. EtOH solution of 10% of (III) with 0.1 mole of triethylamine per mole of (III)($[\alpha]_D$ of which decreased from +62.0° to +1° after being allowed to stand at 25° for 24 hrs.)(see Fig. 2). After evaporation of 15 cc. of the solution, 7 cc. of 10% HCl was added and the mixture shaken to cause the precipitation of *p*-nitrobenzaldehyde. After filtration, the filtrate was washed with benzene, boiled for 1 hr., and then evaporated to dryness under reduced pressure. To the residue was added 5 cc. of EtOH and the solution neutralized with pyridine to pH 5~6, causing precipitation of colorless scales which were identical with DL-methionine by a mixed m.p. determination, m.p. 268~270°. Yield, 450 mg.(45%). $[\alpha]_D^{18}$ −0.20°(c=10, EtOH).

Attempted Racemization in Sodium Salt of N-*p*-Nitrobenzylidene-D-methionine—Two grams of sodium salt of N-*p*-nitrobenzylidene-D-methionine dissolved in dehyd. MeOH (pH 7.2)[9] was boiled for 6 hrs. and evaporated to dryness under reduced pressure. To the residue was added 10% HCl and the mixture was shaken to cause the precipitation of *p*-nitrobenzaldehyde. After filtration the filtrate was washed with benzene and upon evaporation, NaCl precipitated. After filtration, to the filtrate 5 cc. of EtOH was added and the solution was neutralized with pyridine to pH 5~6, precipitating colorless scales, m.p. 268~275°. Yield, 480 mg. (50%). $[\alpha]_D^{18}$ −1.2°(c=10, 10% HCl).[10] A mixed m.p. with an authentic sample of DL-methionine showed no depression.

Attempted Racemization in the N-*p*-Nitrobenzyliden Derivative of L(+)-*threo*-1-*p*-Nitrophenyl-2-amino-1,3-propanediol—A dehyd. MeOH solution containing 1% each of the material and triethylamine was boiled on a water bath for 10 hrs. After evaporation to dryness, the residue showed m.p. 175° and $[\alpha]_D^{20}$ +142°(c=1, MeOH), identical with those of the starting material.

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[8] Below pH 8, decomposition occurs slowly to disturb the measurement by turbidity.

[9] Addition of basic catalyst promotes not only racemization but also polymerization.

[10] An authentic sample of D-methionine : $[\alpha]_D^{18}$ −13.4°(c=10, 10% HCl).

Summary

Ethyl esters of N-isopropylidene-, N-benzylidene-, and N-*p*-nitrobenzylidene-D-methionine (II, I, and III) were prepared. The relative velocities of racemization of the Schiff bases were measured resulting in the order of (II)$\ll$(I)$\ll$(III). It has been postulated by Holland that prototropy in Schiff bases derived from α-amino acid esters occurs through tautomerism in the $>C^*H-N=C<$ structure. The relative velocities found experimentally were in reverse of the theoretical assumption that they would be (II)$>$(I)$>$(III) by the electronic effect of the N-substituent, if the said postulation were correct. The findings support the view that the prototropic change occurs originally in the $>C^*H-\overset{|}{C}=O$ structure and is additionally helped by the azomethine structure in the molecule.

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