no longer active against *Candida albicans* even at the concentration of 1000 μg/ml, however, it showed about twofold stronger cytotoxicity in L5178Y cells than that of bredinin as shown in Fig. 1.

Although there is no apparent evidence, it is stated that nucleotides in general do not enter directly into cells, they can enter as nucleosides after their hydrolytic conversion by probably a phosphatase.[2b] However, the fact that bredinin · 5'-monophosphate showed enhanced cytotoxicity suggested direct penetration by itself. If it were subject to hydrolysis, the cytotoxicity should be the same as that of bredinin.

Unlike the virazole 5'-monophosphate which has been reported as the active metabolite,[4] no phosphate derivatives of bredinin have been detected in L5178Y cells or a rat liver suggesting that bredinin is not phosphorylated in the cells or a rat, and it is also found that bredinin is not incorporated into nucleic acid of L5178Y cells.[7] The discrepancy between bredinin and its 5'-monophosphate in the cytotoxicity in the cells is taken into account of the presence of different cytocidal mechanisms. The loss of the anticandida activity would most likely be due to the permeability change.

On the comparison of anti-L 1210 activity between the phosphate and bredinin, they showed just the same dose response effect in molar level on life prolongation in mice. This suggested that bredinin 5'-monophosphate is converted to bredinin in the animal.

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Facile Thermal Dimerization of a photochemically Isomerized Olefin

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In sharp contrast to 4-methyl-2-methoxycarbonylmethylene-3,4-dihydro-3-oxo-2H-1,4-benzothiazine (Z-form), the photochemically produced isomer (E-form) underwent a facile thermal dimerization to form a cyclobutane derivative. The present observation is notable for demonstrating that the apparent photodimerization of the open-chain olefin could involve primarily the thermal dimerization of its isomer formed photochemically.

A number of thermal dimerization of open-chain olefins appropriately substituted to form cyclobutanes are well-known.[2] To our best knowledge, however, the distinct difference

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[2] For a review, see J.D. Roberts and C.M. Sharts, "Organic Reactions," Vol. 12, Willey, New York, N.Y., 1962, p 1.

of reactivity in the thermal dimerization between two possible geometric isomers of the open-chain olefins has not been reported.[3,4]

We found that a photo-isomerized olefin causes the facile thermal dimerization in sharp contrast to a parent olefin. The present observation is notable for demonstrating that the apparent photodimerization of the open-chain olefin could involve primarily the thermal dimerization of its isomer formed photochemically.

4-Methyl-2-methoxycarbonylmethylene-3,4-dihydro-3-oxo-2H-1,4-benzothiazine (IIz) was easily prepared by methylation of the corresponding 1,4-benzothiazine (I).[5] The stereochemistry of IIz was deduced from the spectral comparison with the photochemically produced isomer (*vide infra*) and is compatible with the stereochemistry (Z-form) of 2-alkylidene-3,4-dihydro-3-oxo-2H-1,4-benzothiazines recently assigned on the basis of the nuclear magnetic resonance (NMR) spectral data.[6]

A solution of IIz in benzene (4×10^{-3}M) was irradiated with a 400 W high-pressure mercury arc lamp through Pyrex filter under nitrogen for 5 hr. The reaction mixture was concentrated under reduced pressure and the residue was carefully chromatographed on silica gel to separate IIz (16%), an isomer (IIe) (20%, contaminated with small amounts of IIz and III) and a dimer (III) (62%) together with two other dimers (mp 229—230°, 1%, and 220—221°, 1%). The high reactivity of the isomer IIe prevented the isolation of a completely pure sample.

Previously, we have reported that irradiation of I leads to the formation of its isomeric compound as a major product (reference 5*c*). On the basis of the NMR spectral comparison with IIe and III, and its mass spectral data ($M^+=470$), however, a dimeric structure should be assigned for the photoproduct. In the case of I, isolation of other products was unsuccessful.

TABLE I. Some Physicochemical Data of a Pair of Isomeric 1,4-Benzothiazines (IIz and IIe) and a Dimer (III)

	(IIz)	(IIe)[a]	(III)
mp (°C)	147—148	—	237—238
Mass m/e M^+	249	—	498
NMR ($CDCl_3$) δ			
vinyl or cyclobutane proton	7.15(s, 1H)	6.34(s, 1H)	3.68(s, 2H)
N-C$\underline{H}_3$	3.59(s, 3H)	3.50(s, 3H)	3.28(s, 6H)
COOC$\underline{H}_3$	3.81(s, 3H)	3.82(s, 3H)	3.74(s, 6H)
IR (KBr) cm^{-1}			
$\underline{CO}OCH_3$	1680	—	1740
$\underline{CO}NCH_3$	1630	—	1635
UV λ_{max}^{MeOH} (ε) nm	368 (6×10^3)	328 (—)	292 (3.4×10^3)

a) The spectral data listed was obtained from those of the sample contaminated with small amounts of IIz and III.

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3) In $2\pi+4\pi$ cycloaddition of olefins, the increased reactivity of the *trans*-isomer comparing with the *cis*-isomer has been observed. (*cf.* R. Huisgen, H.J. Sturm, and H. Wagenhofer, *Z. Natureforsh.*, **17b**, 202 (1962); J. Sauer, D. Lang, and H. Weist, *ibid.*, **17b**, 206 (1962)).

4) It has been reported that the highly strained *trans*-cycloheptenone, *trans*-cyclooctenone and *cis, trans*-cycloocta-1,5-diene which are formed photochemically undergo $2\pi+2\pi$ and $2\pi+4\pi$ cycloadditions even at low temperature. (*cf.* P.E. Eaton and K. Lim, *J. Am. Chem. Soc.*, **86**, 2087 (1964); E.J. Corey, M. Tada, R. LaMahieu, and L. Libit, *ibid.*, **87**, 2051 (1965); K. Ziegler, H. Sauer, L. Bruns, H. Froitzheim-Kühlhorn, and T. Schneider, *Ann. Chem.*, **589**, 122 (1954); A.C. Cope, C.H. Howell, and A. Knowles, *J. Am. Chem. Soc.*, **84**, 3190 (1962)).

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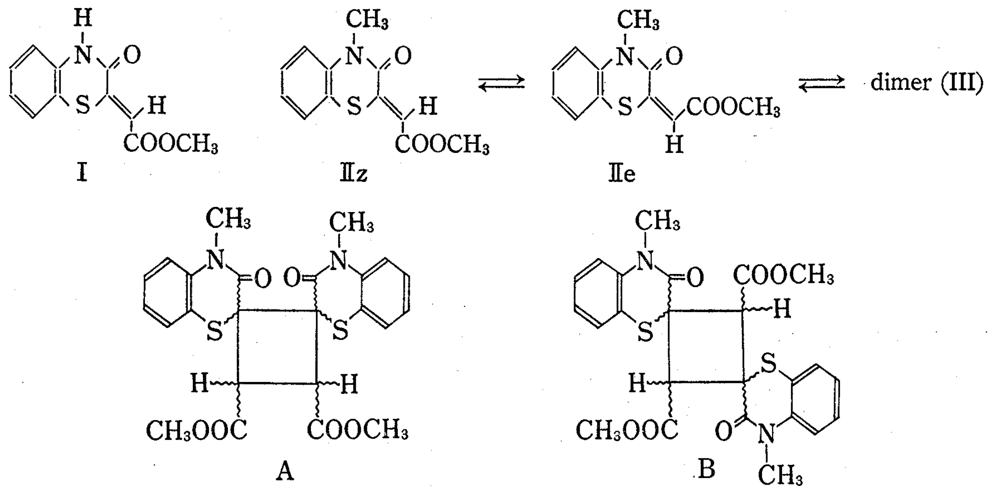

Chart 1

The table summerizes some physicochemical data of a pair of isomeric 1,4-benzothiazines (IIz and IIe) and the dimer (III). The spectral data fully support the isomeric and dimeric structures, IIe and III.

The thin-layer chromatographic (TLC) analysis of the irradiated solution of IIz prior to chromatographic separation showed the presence of IIe as a major product. The isomer IIe in benzene (ca 4×10^{-3}M) was fairly stable on standing in an ice box for a week, whereas concentration of the solution of IIe under reduced pressure at 50° in the dark caused the smooth conversion into III and IIz. The conversion was monitored by taking NMR spectra and TLC. The dimer III thus formed was identical in every respect with the dimer obtained as a major product in the experiment above mentioned. Contrary to instability of IIe, IIz was quite stable, *i.e.*, IIz did not change significantly when heated in dimethylsulfoxide (DMSO) at 150° for 3 hr.

Thus, the thermal dimerization of IIe to give III with ease was proved. In particular, it should be noted that the thermal dimerization is regio- and stereospecific.

Upon heating in DMSO at 150° for 10 hr, the dimer III was converted into IIz in 50% yield. Beside unchanged IIz, IIe and other products were not detected in the reaction mixture. This fragmentation could involve the thermal isomerization of the unstable isomer IIe initially formed as the result of cleavage of the cyclobutane ring.

At present, the precise structure of the dimer III is not clear. The presence of two equivalent cyclobutane protons in the NMR spectrum of III, however, suggests that III adopts a symmetrical structure among a number of possible head-to-head and head-to-tail structures depicted as (A) and (B).

The remarkable reactivity of IIe appears to be not limited to the dimerization. Extension of the present observation is now in progress.

Experimental

All melting points were measured on a Yanagimoto micro-melting point apparatus (a hot-stage type) and are uncorrected. IR spectra were recorded on a Hitachi 215 spectrometer in KBr disk. NMR spectra were obtained on a Hitachi R 20-B(60 MHz) spectrometer using $CDCl_3$ as solvent. Chemical shift values are given in δ (ppm) relative to tetramethylsilane as an internal standard. Abbreviations are as follows: s=singlet, d=doublet, m=multiplet. Ultraviolet (UV) spectra were recorded on a Hitachi 323 spectrometer in MeOH solution. Mass spectra were run on a Hitachi RMU-6L. *Rf* values in TLC were obtained by using silica gel plates (Tokyokasei spotfilm, Silica gel f) and $CHCl_3$-$(CH_3)_2CO$ (30: 1) as developing solvent.

Preparation of 4-Methyl-2-methoxycarbonylmethylene-3,4-dihydro-3-oxo-2H-1,4-benzothiazine (IIz)——To a solution of I (1.0 g, 4.25 mmol) in dimethylformamide (DMF) (10 ml) was added K_2CO_3 (3.0 g, 21.7 mmol) and CH_3I (10 ml). The reaction mixture was vigorously stirred at room temperature for 7 hr and then poured into 2 N HCl on cooling. The product collected by filtration was recrystallized from ether to give IIz, mp 147—148°, as yellow crystals in 70% yield. *Anal.* Calcd. for $C_{12}H_{11}O_3NS$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.77; H, 4.35; N, 5.38

Irradiation of (IIz)——A solution of IIz (0.5 g) in benzene (500 ml) was irradiated under a nitrogen atmosphere with a 400 W high-pressure mercury arc lamp surrounded with a water-jacket through a Pyrex filter for 5 hr. Thin-layer chromatographic analysis of the reaction mixture showed the presence of five compounds (IIz: *Rf*=0.79, IIe: *Rf*=0.70 (a major product), III: *Rf*=0.32, dimers: *Rf*=0.41 and *Rf*=0.24). Removal of the solvent under reduced pressure at 20° as soon as possible left an oily residue which was chromatographed on silica gel (solvent: $CHCl_3$: $(CH_3)_2CO$=30: 1). From the first fraction, the starting material IIz was recovered in 16% yield (81.6 mg). The second fraction gave an isomer IIe in about 20% yield, as a paleyellow oil. This oily substance IIe, however, was contaminated with small amounts of IIz and a dimer III (detected by NMR and TLC). The third fraction gave III, mp 237—238°, as colorless crystals in 62% yield (311.7 mg). *Anal.* Calcd. for $C_{24}H_{22}O_6N_2S_2$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.40; H, 4.38; N, 5.19.

From other fractions, small amounts of two dimers, mp 229—230° and mp 220—221°, as colorless crystals were isolated. Dimer (mp 229—230°, *Rf*=0.41): NMR ($CDCl_3$) δ: 2.88 (6H, s, two N-$C\underline{H}_3$), 3.74 (6H, s, two $COOC\underline{H}_3$), 5.02 (2H, s, two cyclobutane ring protons), 6.20—7.40 (8H, m, aromatic protons). IR (KBr) cm^{-1}: 1730, 1720 ($\underline{CO}OCH_3$), 1650 ($\underline{CO}NCH_3$). UV λ_{max}^{MeOH} (ε) nm: 280 (sh. 3000). Mass Spectrum *m/e*: 498 (M^+). Dimer (mp 220—221°, *Rf*=0.24): *Anal.* Calcd. for $C_{24}H_{22}O_6N_2S_2$: C, 57.83; H, 4.45; N, 5.62. Found: C, 57.82; H, 4.48; N, 5.72. NMR ($CDCl_3$) δ: 3.17 (3H, s, $NC\underline{H}_3$), 3.45 (3H, s, $NC\underline{H}_3$), 3.60 (1H, d, J=11 Hz, a cyclobutane ring proton), 3.66 (3H, s, $COOC\underline{H}_3$), 3.78 (3H, s, $COOC\underline{H}_3$), 4.98 (1H, d, J=11 Hz, a cyclobutane ring proton), 6.20—7.50 (8H, m, aromatic protons). IR (KBr) cm^{-1}: 1745, 1730 ($\underline{CO}OCH_3$), 1660 ($\underline{CO}NCH_3$). UV λ_{max}^{MeOH} (ε) nm: 280 (sh, 3000). Mass Spectrum *m/e*: 498 (M^+).

Thermal Dimerization of Isomer (IIe)——A solution of IIe (contaminated with small amounts of IIz and III) in $CDCl_3$ was heated in a NMR tube at 50° in the dark for 5 hr. Conversion of IIe into IIz and III was monitored by taking NMR spectra and TLC. Other products were not detected by NMR spectra and TLC. Analogous conversion of IIe in benzene was observed when the irradiated solution of IIz was concentrated under reduced pressure in the dark.

Thermolysis of Dimer (III)——A solution of (III) (30 mg) in DMSO-d_6 (0.4 ml) was heated in a NMR tube at 150°. About 50% conversion of (III) into (IIz) was observed after 10 hr. which was monitored by taking NMR spectra. In this conversion, IIe and other products were not detected by NMR spectra and TLC.