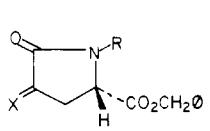
purified by high-pressure liquid chromatography (LC) collection.

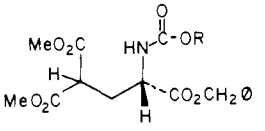
This reaction also afforded a 10% yield of a 3:2 mixture of *trans*- and *cis*-pyro-Gla derivatives **8**[10a] and **9**,[10a] which were purified by LC collection. These compounds do not noticeably react further with benzyl alcohol under the above-described conditions. Thus, the first step in the reaction of **6** with benzyl alcohol is an exchange of the trichloroethyl ester for a benzyl ester. This is then followed by opening of the pyrrolidine ring with minor loss from competitive decarbobenzyloxylation[11] of the N-Cbz function.

Hydrogenolysis of **7** in methanol over 10% palladium/charcoal afforded a 93% yield of L-Gla: mp 154–155 °C dec;[12] $[\alpha]_D$ +33.9° (*c* 1.2, 6 N HCl); lit.[5] $[\alpha]_D$ +34.6°; mp 167–167.5 °C dec. Its NMR spectrum (600 MHz) was identical with that of racemic material prepared from racemic **7** and, in accord, with the published spectrum[5] at 360 MHz.

This methodology lends itself very nicely to the preparation of analogues of L-Gla. Thus, reaction of **6** with methanol in the presence of triethylamine (room temperature, 2.5 h) afforded a 62% yield of **10**:[10a] $[\alpha]_D$ +9.5° (*c* 1.0, $CHCl_3$). A more differentiated analogue was prepared as follows.

Reaction of benzylpyroglutamate (**11**) with **5** in the presence of pyridine–DMAP afforded **12**: mp 61–63 °C; $[\alpha]_D$ −46.2° (*c* 1.03, ethanol). The latter reacts with the Bredereck reagent **3**[8,9] at *room temperature* in dimethoxyethane, providing a 43%[13] yield of **13**:[10a,b] mp 118.5–119 °C; $[\alpha]_D$ −66.7° (*c* 1.0, CH_2Cl_2). Treatment of **13** with **5** in toluene at 95 °C, followed





11 R = H, X = H_2

12 R = C(O)OCH_2CCl_3, X = H_2

13 R = C(O)OCH_2CCl_3, X = C(H)N(Me)$_2$

14 R = C(O)OCH_2CCl_3, X = H,C(O)OCH_2CCl_3

6 → **10** R = $CH_2\emptyset$

14 → **15** R = CH_2CCl_3

by chromatography on silica gel, gave a 41% yield of the trans:cis (ca. 2:1) diastereomers **14**.[10a] Reaction of **14** with methanol, as above, gave **15**:[10a] $[\alpha]_D$ +7.8° (*c* 5.2, CH_2Cl_2); 35%[13] yield.

It is expected that this methodology will find ready application in the synthesis of peptides containing modified L-Gla residues.

Acknowledgments. This research was supported on by Public Health Service Grant No. CA-12107-14. NMR spectra at 250 MHz were measured on facilities supported by RR-00296-11. We also acknowledge a National Institutes of Health Postdoctoral Fellowship (CA 06196-01) to L.A.C.

References and Notes

(1) (a) Stenflo, J.; Fernlund, P.; Egan, W.; Roepstorff, P. *Proc. Natl. Acad. Sci. U.S.A.* **1974,** *71,* 2730; (b) Nelsestuen, G. L.; Zytkovicz, T. H.; Howard, J. B. *J. Biol. Chem.* **1974,** *249,* 6347; (c) Magnusson, S.; Sottrup-Jensen, L.; Petersen, T. E.; Morris, H. R.; Dell, A. *FEBS Lett.* **1974,** *44,* 189.

(2) Howard, J. B.; Nelsestuen, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1975,** *72,* 1281; (b) Howard, J. B.; Fausch, M. D.; Nelsestuen, G. L. *J. Biol. Chem.* **1975,** *250,* 6178.

(3) For a review of the biochemistry of γ-carboxyglutamic acid, see: Stenflo, J.; Suttie, J. W. *Annu. Rev. Biochem.* **1977,** *46,* 157.

(4) (a) Fernlund, P.; Stenflo, J.; Reopstorff, P.; Thomsen, J. *J. Biol. Chem.* **1975,** *250,* 6125; (b) Morris, H. R.; Thompson, M. R.; Dell, A. *Biochem. Biophys. Res. Commun.* **1975,** *62,* 856; (c) Boggs, N. T., III; Gawley, R. E.; Koehler, K. A.; Hiskey, R. G. *J. Org. Chem.* **1975,** *40,* 2850; (d) Marki, W.; Schwyzer, R. *Helv. Chim. Acta* **1975,** *58,* 1471; (e) Marki, W.; Oppliger, M.; Schwyzer, R. *Helv. Chim. Acta* **1976,** *59,* 901; (f) Weinstein, B.; Watrin, K. G.; Loie, H. J.; Martin, J. C. *J. Org. Chem.* **1976,** *41,* 3634.

(5) Marki, W.; Oppliger, M.; Thanei, P.; Schwyzer, R. *Helv. Chim. Acta* **1977,** *60,* 798.

(6) Oppliger, M.; Schwyzer, R. *Helv. Chim. Acta* **1977,** *60,* 43.

(7) Berenbom, M.; White, J. *J. Am. Chem. Soc.*, **1949,** *71,* 2246.

(8) (a) Bredereck, H.; Simchen, G.; Rebsdat, S.; Kantlehner, W.; Horn, P.; Wahl, R.; Hoffmann, H.; Grieshaber, P. *Chem. Ber.* **1968,** *101,* 41. (b) For a review see: Simchen, G. *Adv. Org. Chem.* **1979,** *9*(2), 393–527.

(9) In spite of numerous publications by Bredereck and his associates[8b] on the reactions of orthoformamide derivatives with "active" carbon–hydrogen bonds, the value of this beautiful methodology has perhaps still not been fully comprehended. For some recent applications of Brederecks findings to general synthetic problems, see: (a) secoalkylation: Trost, B. M.; Preckel, M. *J. Am. Chem. Soc.* **1973,** *95,* 7862; (b) formation of α-dicarbonyl compounds: Wasserman, H. H.; Ives, J. L. *J. Org. Chem.* **1978,** *43,* 3238; (c) formation of α-methylene lactones: Danishefsky, S.; Hirama, M.; Gombatz, K.; Harayama, T.; Berman, E.; Schuda, P. F. *J. Am. Chem. Soc.* **1978,** *100,* 6536; (d) formation of α-phenylthiomethylene lactones: Martin, S. F.; Moore, D. R. *Tetrahedron Lett.* **1976,** 4459; (e) formation of α-formyl lactones: Gutzwiller, J.; Pizzolato, G.; Uskokovic, M. *J. Am. Chem. Soc.* **1971,** *93,* 5907; Ban, Y.; Taga, N.; Oishi, T. *Tetrahedron Lett.* **1974,** 187.

(10) The structure of this compound is consistent with (a) its infrared, NMR, and mass spectra and (b) its combustion analysis.

(11) Small amounts of dibenzyl carbonate were isolated by chromatography.

(12) The racemate of Gla is reported[4f] to melt from 90 to 92 °C. In our hands, L-Gla decomposes when heated at 154–155 °C by decarboxylation. This decomposition point does not change materially on recrystallization. The discrepancy between our decomposition temperature and that reported might be a consequence of a small amount of racemate in our material, but more likely it is a consequence of subtle differences in heating.

(13) The lower yields of the *N*-trichloroethoxycarbonyl series arise from the greater lability of this group toward nucleophiles in the sequence.

Samuel Danishefsky,* Ellen Berman
Lane A. Clizbe, Masahiro Hirama
Department of Chemistry, University of Pittsburgh
Pittsburgh, Pennsylvania 15260
Received March 5, 1979

Structure of Elasnin, a Novel Elastase Inhibitor

Sir:

A novel human granulocyte elastase inhibitor, elasnin, has been isolated from the culture broth of *Sm. noboritoensis* KM-2753.[1] This inhibitor with low toxicity is of interest in respect to the control of pathological processes such as acute arthritis, various inflammations, pulmonary emphysema, and pancreatitis, which are known to be caused by elastase.[2] In the present report, we describe the structural elucidation of elasnin based on chemical degradations and biosynthetic means using ^{13}C-labeled precursor.

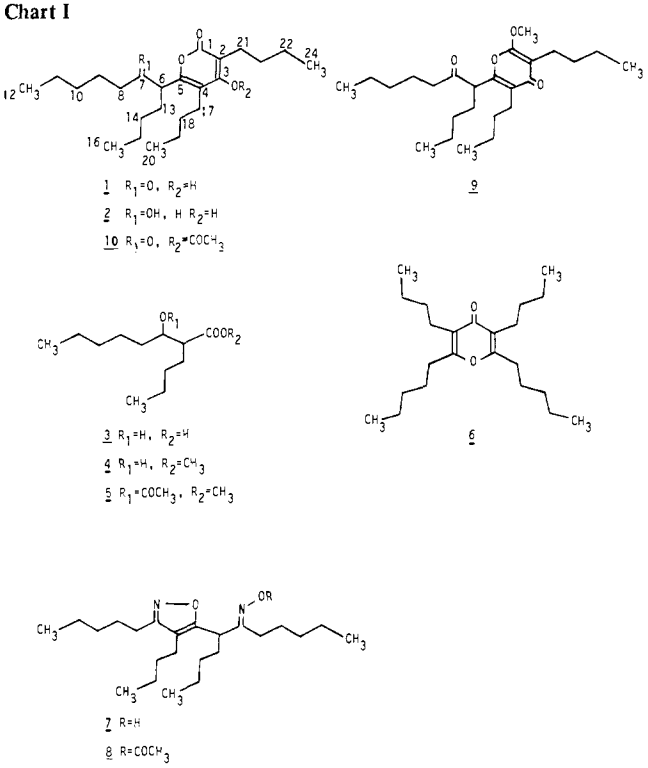
Elasnin (**1**) is a lipophilic colorless and viscous oil possessing the following physical and spectroscopic properties: n^{17}_D 1.4983; $[\alpha]^{18}_D$ −0.9° (*c* 1, EtOH); λ^{EtOH}_{max} 291 nm (ε 7760); ν 3430 (OH), 2960 and 2860 (CH_2, CH_3), 1715 (ketone carbonyl), 1665 (conjugated ester carbonyl), and 1636 cm^{-1} (double bond). The molecular formula $C_{24}H_{40}O_4$ (M^+, *m/e* 392) was established for **1** by high-resolution mass spectral and elemental analyses. The 25.2-MHz ^{13}C NMR spectrum indicated the presence of a ketone carbonyl (δ 207.0), an ester carbonyl (either δ 165.5 or 164.7), and four quarternary olefinic carbons (either δ 165.7 or 164.7, 153.8, 115.0, 104.3). In addition, four methyl carbons (completely overlapped at δ 13.9), several methylene carbons (δ 22.4–40.3), and a methyne (δ 54.7) in the high-field region, as shown by an off-resonance decoupling experiment, were consistent with the presence of four linear C_4 and C_5 alkyl chains in **1.**

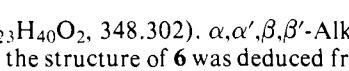
Reduction of **1** with $NaBH_4$ afforded dihydroelasnin (**2**) [$C_{24}H_{42}O_4$; M^+, *m/e* 394; λ^{EtOH}_{max} 292 nm (ε 6400)], indicating the presence of a ketone carbonyl at the position isolated from the chromophore in **1.** Ozonolysis of **2** followed by oxidative degradation with 30% H_2O_2 and glacial acetic acid gave an oily acid **3,** which was then treated with CH_2N_2 to give methyl ester **4:** $C_{12}H_{24}O_3$; *m/e* 213 (M^+ − OH), 199 (M^+ − OCH_3); ν 3520 (OH), 1730 cm^{-1} (ester carbonyl). The ^{13}C NMR and mass spectral analyses[3] of **3** and the corresponding monoacetate **5,** obtained by acetylation of **3,** suggested 2-butyl-3-hydroxyoctanoic acid for the structure of **3.**

Acidic degradation of **1** with 2.5 N HCl in acetic acid gave a colorless oil, **6:** $C_{23}H_{40}O_2$; M^+ *m/e* 348 (348.300; calcd for

0002-7863/79/1501-4386$01.00/0

Chart I





$C_{23}H_{40}O_2$, 348.302). $\alpha,\alpha',\beta,\beta'$-Alkyl-substituted γ-pyrone[4] as the structure of **6** was deduced from the following spectral data: λ_{max}^{EtOH} 258 nm (ϵ 7800); ν 1645 ($\alpha,\beta,\alpha',\beta'$-unsaturated ketone carbonyl), 1610 cm^{-1} (double bond); δ 179.0 (γ-pyrone carbonyl carbon), 163.6 (two α carbons of γ-pyrone), 123.3 (two β carbons of γ-pyrone). With regard to the substitution pattern of alkyl groups of the γ-pyrone nucleus, two symmetrical positional isomers, α,α'-*n*-butyl-β,β'-*n*-pentyl-γ-pyrone and α,α'-*n*-pentyl-β,β'-*n*-butyl-γ-pyrone for **6** were proposed on the basis of the number of its ^{13}C NMR signals as well as the chemical shift of alkyl carbons:[5] δ 13.9 (four methyls), 22.4, 23.0, 24.4, 27.2, 31.0 (each two methylenes), and 31.4 (four methylenes). Taking into account the structural features[6] of ozonolysis product **3**, the *n*-pentyl group must be substituted to the α position of γ-pyrone nucleus, and, therefore, α,α'-*n*-butyl-β,β'-*n*-pentyl-γ-pyrone for the structure of **6** can be ruled out.

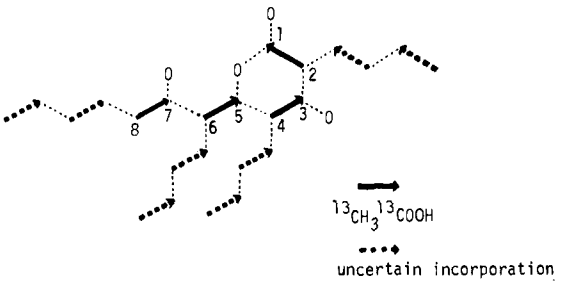
The facile transformation accompanied by the acid-catalyzed decarboxylation of **1** into **6** demonstrates the interpretation of "triketide" as an intermediate.[7] More explicit evidence for the existence of "triketide intermediate" was obtained by the transformation of **1** into isoxazole derivative. Namely, mild reflux of **1** with hydroxylamine hydrochloride in acetic acid afforded a colorless oil, **7**: $C_{23}H_{42}N_2O_2$; M^+ *m/e* 378 (378.320; calcd for $C_{23}H_{42}N_2O_2$, 378.324); δ 2.58 (CH_2, triplet), 3.66 (CH, triplet), 9.00 (=NOH, exchanges with D_2O). Compound **7** was acetylated with acetic anhydride and pyridine to give a monoacetate **8**: $C_{25}H_{44}N_2O_3$; M^+ *m/e* 420; ν 1768 cm^{-1} (=$NOCOCH_3$). The characteristic ^{13}C NMR signals due to isoxazole carbons at δ 165.7, 163.4, and 116.0 and an oxime carbon at δ 160.6 and the observation of two mass fragment peaks at *m/e* 194 (base peak; $C_{12}H_{20}NO$) and 264 ($C_{17}H_{30}NO$), corresponding to the isoxazole ring moiety containing alkyl chains, indicated the structure **7** as shown in Chart I. Taking into consideration the reactivities described above and the spectral evidence of **1**, it was concluded that 4-hydroxy-α-pyrone would be the most suitable for the skeletal structure of **1**.

Table I. ^{13}C Chemical Shifts and Coupling Constants ($J_{^{13}C,^{13}C}$) of [1,2-^{13}C]Acetate-Enriched Elasnin **1** and Its Methyl Ether **9**

carbon	chemical shift, δ_C[a]			$J_{^{13}C,^{13}C}$, Hz	
	1	**9**	**10**	**1**	**9**
1	165.5[b]	162.6	163.1	76.3	85.4
2	104.3	104.6	116.4[c]	76.3	85.4
3	164.5[b]	179.6	158.5	61.0	47.3
4	114.9	125.3	118.7[c]	61.0	47.3
5	154.8	154.3	155.4	51.9	51.9
6	54.7	54.1	54.9	51.9	51.9
7	206.9	207.0	206.2	37.9	37.9
8	40.2	40.2	40.2	37.9	37.9
$OCOCH_3$			167.0		

[a] Relative to internal Me_4Si. [b,c] Assignments may be reversed.

Chart II. Biosynthetic Pattern of Elasnin



uncertain incorporation

The structural correlation between **1** and **9** was also supported by biosynthetic investigation using ^{13}C-labeled precursor and ^{13}C NMR spectroscopy. [1,2-^{13}C]Acetate (90% enriched in ^{13}C) was fed to the fermentation culture of *Sm. noboritoensis* KM-2753 as a precursor, and ^{13}C-labeled elasnin was isolated from the culture broth. The ^{13}C-labeled elasnin was treated with CH_2N_2 to afford the ^{13}C-labeled methyl ether **9**. The ^{13}C NMR spectrum showed satellite resonances due to ^{13}C–^{13}C couplings. The couplings of 76.3 Hz (between C-1 at δ 165.6 and C-2 at δ 104.3) and 61.0 Hz (C-3 at δ 164.7 and C-4 at δ 115.0) in **1** altered to those of 85.4 Hz (C-1 at δ 162.6 and C-2 at δ 104.6) and 47.3 Hz (C-3 at δ 179.6 and C-4 at δ 125.0) in **9**, respectively. The difference in the ^{13}C–^{13}C coupling and ^{13}C chemical shift values between **1** and **9** is strongly consistent with the structural change from α-pyrone to γ-pyrone, as suggested earlier by their UV and IR spectral data. Furthermore, the ^{13}C NMR evidence suggested that **1** was biosynthesized via *polyketide* from 12 molecules of acetate as shown in Chart II. It should be noted that **1** is a novel inhibitor that has a highly alkylated pyrone skeleton differing from other protease inhibitors[8] isolated from *Streptomyces*.

Acknowledgments. The authors are indebted to Miss H. Umezawa for excellent technical assistance and Dr. K. Miyano for helpful discussions.

References and Notes

(1) (a) S. Ōmura, H. Ohno, T. Saheki, M. Yoshida, and A. Nakagawa, *Biochem. Biophys. Res. Commun.*, **83**, 704 (1978); (b) H. Ohno, T. Saheki, J. Awaya, A. Nakagawa, and S. Ōmura, *J. Antibiot.*, **31**(11), 1116 (1978).

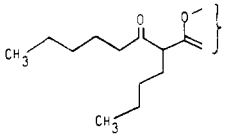
(2) (a) A. Janott, *Annu. Rev. Med.*, **23**, 117 (1972); (b) M. C. Geokas, *Arch. Pathol.*, **86**, 117 (1968).

(3) The structural evidence of **3** was obtained by the following spectral data: δ 176.1 (ester carbonyl carbon), 72.3 (hydroxy carbon), 50.9 (methyne), 22.6–35.6 (seven methylene carbons), 13.9 (two methyl carbons) in the ^{13}C NMR of **4**; typical fragment of *m/e* 241 ($M^+ - OCH_3$), 229 ($M^+ - COCH_3$), 130 (base peak; $CH_3CH_2CH_2CH_2CH{=}C(OH)(OCH_3)$, 87 ($CH_2{=}CHC(=O^+H)(OCH_3)$ in the mass spectrum of **5**.

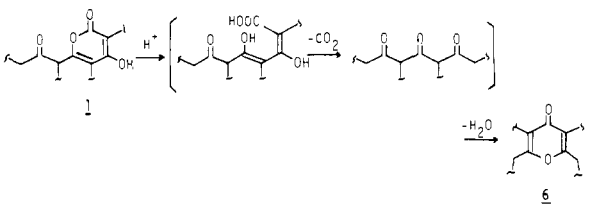
(4) (a) R. H. Wiley and C. H. Jarboe, *J. Am. Chem. Soc.*, **78**, 624 (1956); (b) D. Herbst, W. B. Mors, O. R. Gottlieb, and C. Djerassi, *ibid.*, **81**, 2427 (1959); (c) J. D. Bu'Lock and H. G. Smith, *J. Chem. Soc.*, **1**, 502 (1960); (d) Y. Koyama, Y. Fukakusa, N. Kyomura, S. Yamaguchi, and T. Arai, *Tetrahedron Lett.*, 355 (1969).

(5) ^1H NMR and ^{13}C NMR spectra of **1** and its derivatives were measured in deuteriochloroform, and IR spectra were obtained in carbon tetrachloride. The assignment of each alkyl carbon in the ^{13}C NMR spectra of all compounds was mainly carried out on the basis of the data listed in J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, 1972.

(6) Since the hydroxyl carbon of **3** should correspond to the ketone carbonyl of **1**, the following partial structure must be present in **1**.



(7) (a) L. C. Dorman, *J. Org. Chem.*, **32**, 4105 (1967). (b) The following mechanism for the production of **6** was speculated:



(8) Studies on the protease inhibitors arising from *Streptomyces* have been summarized in the following reviews. (a) T. Aoyagi and H. Umezawa, "Protease and Biological Control", Cold Spring Harbor Laboratories, Cold Spring Harbor, N.Y., 1975, pp 429–454; (b) H. Umezawa, *Methods Enzymol.*, 458, 678–695 (1976); (c) H. Umezawa and T. Aoyagi, "Proteinases of Mammalian Cells and Tissues", A. J. Barrett, Ed., ASP Biological and Medical Press, Amsterdam, 1977, pp 637–662.

S. Ōmura,* A. Nakagawa, H. Ohno

School of Pharmaceutical Sciences
Kitasato University and The Kitasato Institute
Minato-ku, Tokyo 108, Japan

Received February 12, 1979

Upper Excited Triplet State Mechanism in the Trans → Cis Photoisomerization of 4-Bromostilbene

Sir:

The direct trans → cis photoisomerization of stilbene occurs by twisting from 0 to 90° in the lowest excited singlet state, $^1t^* \rightarrow {}^1p^*$, followed by internal conversion, $^1p^* \rightarrow {}^1p$, and twisting from 90 to ~180°, eq 6, in the ground state[1] (for definitions see the explanation of Scheme I). This singlet mechanism for the photoisomerization of stilbene in solution at room temperature is widely accepted.[1-7] However, the discovery of a triplet state of stilbene in rigid matrices[8-10] raises the question as to whether or not a triplet mechanism, as favored by Fischer et al.,[11-13] may contribute to the trans → cis photoisomerization at low temperatures. For 4-bromostilbene, involvement of the lowest triplet state mechanism was postulated by Fischer[11-13] and by Saltiel.[3,5,14] In contrast to unsubstituted stilbene, the quantum yield of fluorescence, ϕ_f, is smaller than 0.2 for *trans*-4-bromostilbene even in rigid glasses at −196 °C.[5,11,15,16]

Using laser flash photolysis,[17-19] we now measured the triplet-triplet absorption spectrum, the triplet lifetime, τ, and the quantum yield of the triplet state, $\phi_{triplet}$, of 4-bromostilbene as a function of temperature in different solvents. The triplet-triplet absorption spectrum is similar to the known spectra of stilbene[8,9] and 4,4′-dichlorostilbene[9] and could be observed below a certain maximum temperature, t_m, without any significant change in shape and λ_{max} down to −196 °C (−80 °C in glycerol and glycerol triacetate (GT), Table I). From this it is concluded that the observed triplet state has the planar trans configuration ($^3t^*$) and that $\phi_{triplet}$ is identified with the yield of formation of $^3t^*$, $\phi_{^3t^*}$.

Scheme I. Upper Excited Triplet Path for Trans → Cis Photoisomerization

$$^1t + h\nu \rightarrow {}^1t^* \quad (1)$$

$$^1t^* \rightarrow {}^3t_h^* \quad (2)$$

$$^3t_h^* \rightarrow {}^3b_h^* \quad (3)$$

$$^3b_h^* \rightarrow {}^3b^* \rightarrow {}^3p^* \quad (4)$$

$$^3p^* \rightarrow {}^1p \quad (5)$$

$$^1p \rightarrow \alpha\,{}^1t + (1-\alpha)\,{}^1c \quad (6)$$

In Figure 1, $\phi_{triplet}$ and τ^{-1} are plotted vs. T^{-1} in comparison with ϕ_f and the quantum yield for the direct trans → cis photoisomerization, $\phi_{t\rightarrow c}$, in a 1:1 mixture of methylcyclohexane and isohexane, MCH-1H. $\phi_{t\rightarrow c}$ does not change from room temperature down to −155 °C, but falls off drastically by further lowering the temperature, and at −185 °C it is too small to be measured. At −155 °C the $^3t^*$ state of 4-bromostilbene appears. The yield of $^3t^*$ increases with decreasing temperature and reaches a constant value at $t_0 = -173$ °C. A comparison of t_m and t_0 values in glycerol and GT with those in the other solvents indicates that viscosity rather than temperature determines the formation of $^3t^*$ (Table I). The lifetime of $^3t^*$ increases in MCH-1H with decreasing temperature from 330 ns at −159 °C reaching a constant value of 0.45 ms below $t_0 = -173$ °C (Figure 1). This is explained by the assumption that the increase in viscosity hinders the $^3t^*$ state from twisting. Therefore, it is suggested that the sharp decrease of $\phi_{t\rightarrow c}$ below −155 °C results from viscosity dependent barriers in both, the upper excited and the lowest triplet states. As shown in Table I, almost the same lifetime of $^3t^*$ is found below t_0 in all solvents examined. Below t_0 no twisting occurs, since $\phi_{t\rightarrow c}$ is zero.

Above −155 °C, trans → cis photoisomerization cannot proceed in the lowest triplet state via $^3t^* \rightarrow {}^3p^*$ since no $^3t^*$ is formed. This follows from the decrease of $\phi_{^3t^*}$ with increasing

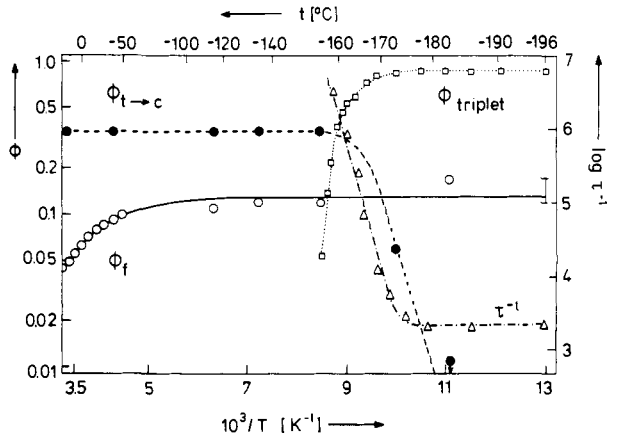


Figure 1. Semilogarithmic plot of $\phi_{t\rightarrow c}$, ϕ_f, $\phi_{triplet}$, and τ^{-1} (in s^{-1}) vs. T^{-1} for *trans*-4-bromostilbene in MCH-1H. ϕ_f values above −50 °C in *n*-pentane are from ref 5 and 21; ϕ_f below −50 °C and $\phi_{t\rightarrow c}$ in MCH-1H are from ref 11–13. At −183 °C, $\phi_{t\rightarrow c} = 0.003$; at 30 °C, a somewhat greater value of $\phi_{t\rightarrow c} = 0.48$ has been found in *n*-pentane.[5] $\phi_{triplet}$ is obtained from optical densities, and a value of $1 - \phi_f = 0.87$ is assumed at −196 °C.

0002-7863/79/1501-4388$01.00/0