

Direct Evidence for Bromine-Olefin Charge-Transfer Complexes as Essential Intermediates of the Fast Ionic Addition of Bromine to Cyclohexene

Giuseppe Bellucci,*† Roberto Bianchini,† and Roberto Ambrosetti‡

Contribution from the Istituto di Chimica Organica della Facoltà di Farmacia, Università di Pisa, 56100 Pisa, Italy, and the Istituto di Chimica Quantistica ed Energetica Molecolare del C.N.R., 56100 Pisa, Italy. Received May 10, 1984

Abstract: The addition of molecular bromine to cyclohexene in 1,2-dichloroethane has been investigated spectrophotometrically with the stopped-flow technique in the 15–35 °C temperature range with and without cyclohexane added to the reaction medium. The third-order rate constant ($k_3 = 2.4 \times 10^5$ (0.1) $M^{-2} s^{-1}$ at 25 °C) decreased with increasing amounts of added cyclohexane, in agreement with a mechanism involving ionic intermediates, and with increasing temperature. Activation parameters were the following: $E_{a(\text{obsd})} = -7.78$ (0.20) kcal mol^{-1} , $\Delta H^\ddagger = -8.37$ (0.20) kcal mol^{-1} , $\Delta S^\ddagger = -64.0$ (0.7) eu. When the reaction was monitored near the UV absorption minimum of bromine, the formation of a highly absorbing 1:1 bromine-cyclohexene CTC became apparent from the increase in absorbances with increasing olefin at constant halogen concentration. The formation constant and the molar absorptivity at 287 nm of this CTC ($K_f = 0.47$ (0.08) M^{-1} , $\epsilon_{CT} = 5520$ (250) $M^{-1} \text{cm}^{-1}$ at 25 °C) have been simultaneously evaluated by the Scott equation and refined by a NLLSQ fitting method. Thermodynamic parameters of the 1:1 CTC, determined in the 15–35 °C range, were $\Delta H = -4.60$ (0.20) kcal mol^{-1} and $\Delta S = -17.0$ (0.6) eu. At sufficiently high cyclohexene concentration, the decay of the CT absorbances followed a clear second-order rate law with an observed rate constant, which decreased, as expected, with increasing olefin concentration. Values of the product $K_f \epsilon_{CT}$ consistent with those obtained from the initial absorbances of the reaction mixtures were obtained from the relation between the rate constants measured monitoring respectively the molecular halogen and the CTC. A simple, general equation is also proposed to evaluate the formation constants of highly reactive donor-acceptor complexes of the presently observed type from measurements of their rate of disappearance as a function of the donor concentration. The kinetic role and thermodynamic parameters obtained in this investigation show that the formation of the 1:1 CTC must be an essential step in the bromination mechanism, furthermore suggesting that a second bromine-olefin CTC, possibly of a 2:1 stoichiometry, should be involved on the reaction coordinate between the 1:1 CTC and the bromonium-tribromide ion pair.

Bromine-olefin charge-transfer complexes (CTC) have often been postulated as key intermediates in the mechanism of bromination of unsaturated compounds.¹⁻³ The overall second-order dependence of the bromination rate in polar hydroxylic solvents has been interpreted as the result of a fast, reversible formation of a transient CTC followed by a rate-determining solvent-assisted unimolecular ionization of its bromine-bromine bond (Ad_ECl mechanism).⁴ The third-order rate law (second order in bromine) observed in both protic and aprotic solvents of low or moderate dielectric constant has been likewise attributed⁵⁻⁷ to a bromine-assisted cleavage of the bromine-bromine bond in an olefin-bromine CTC to give a σ complex (bromonium or bromocarbenium ion) and a charge-dispersed tribromide ion. It was, however, emphasized⁸ that the intervention of a dimeric form of the halogen, more polarized and electrophilic than monomeric bromine, could equally well explain the kinetic results. Bromine-olefin CTC's as essential intermediates of an ionic mechanism have also been inferred from the concentration dependence of the third-order rate constants and from competitive brominations in a nonpolar solvent as carbon tetrachloride.⁹ Evidence has been produced,^{10,11} however, against pathways involving the intermediate formation of ionic species in the slow bromine additions occurring in nonpolar media, where a molecular mechanism involving a 2:1 bromine-olefin complex has been proposed instead.^{3,12}

In spite of this presumed widespread involvement of molecular complexes in the bromine addition reactions, bromine-olefin CTC's have so far been directly observed only in a very limited number of circumstances, all of which have in common a highly reduced reactivity of the olefin-bromine system. These include the following:

(i) Spectroscopic (UV) observation has been made of a 1:1 CTC of Br_2 with a deactivated compound, crotonic acid, in 1,2-dichloroethane,¹³ for which a formation constant K_f of 0.08 (0.08) M^{-1} has been reported. If one remembers, however, the criterion

stressed by Person,¹⁴ the existence of such a CTC ($K_f > 0$) can hardly be accepted as proved.

(ii) Spectroscopic (UV) observation has been made of 1:1 CTC's between Br_2 and a series of reactive olefins, including cyclohexene, in nonpolar solvents, like Freons 112 and 113,¹⁵ carbon tetrachloride,^{16,17} and hexane,^{3,18} that is under low reactivity conditions which are suspected to favor molecular over ionic mechanisms.³ Formation constants of these complexes have been reported to be too small to be measured ($K_f < 0.1 M^{-1}$ in CCl_4).¹⁶ Values of K_f at 25 °C of 0.145, 0.36, and 0.33 M^{-1} have been, however,

(1) De la Mare, P. B. D. "Electrophilic Halogenations"; Cambridge University Press: Cambridge, 1976.

(2) Schmid, G. H.; Garratt, D. G. "Chemistry of the Functional Groups, Double Bonded Functional Groups"; Patai, S., Ed.; Wiley: New York, 1977; Suppl. A, p 725.

(3) Sergeev, G. B.; Serguchev, Yu. A.; Smirnov, V. V. *Russ. Chem. Rev.* **1973**, *42* (9), 697. V'yunov, K.; Gynak, A. I. *Russ. Chem. Rev.* **1981**, *50*, 151.

(4) Dubois, J. E.; Garnier, F. *Bull. Soc. Chim. Fr.* **1968**, 3797. Garnier, F.; Donnay, R. H.; Dubois, J. E. *Chem. Commun.* **1971**, 829.

(5) (a) Yates, K.; McDonald, R. S.; Shapiro, S. A. *J. Org. Chem.* **1973**, *38*, 2460. (b) Modro, A.; Schmid, G. H.; Yates, K. *J. Org. Chem.* **1977**, *42*, 3673.

(6) Buckles, R. E.; Miller, J. L.; Thurmaier, R. J. *J. Org. Chem.* **1967**, *32*, 888.

(7) De la Mare, P. B. D.; Wilson, K. D. *J. Chem. Soc., Perkin Trans. 2* **1977**, 2048.

(8) Reference 1, p 131.

(9) Gebelein, C. G.; Frederick, G. D. *J. Org. Chem.* **1972**, *37*, 2211.

(10) Heublein, G.; Schutz, E. *Z. Chem.* **1968**, *9*, 147.

(11) Zalukaev, L. P.; Sergeev, G. B.; Smirnov, V. V.; Shmyzeva, Zh. V. *Kinet. Katal.* **1971**, *12*, 754.

(12) Sergeev, G. B.; Tung-ha, Ch'eng; Pokholok, T. V. *Kinet. Katal.* **1969**, *10*, 47.

(13) Buckles, R. E.; Yuk, J. P. *J. Am. Chem. Soc.* **1953**, *75*, 5048.

(14) Person, W. B. *J. Am. Chem. Soc.* **1965**, *87*, 167.

(15) Dubois, J. E.; Garnier, F. *Spectrochim. Acta, Part A* **1967**, *23A*, 2279.

(16) (a) Fukuzumi, S.; Kochi, K. *J. Am. Chem. Soc.* **1981**, *103*, 2783. (b) Fukuzumi, S.; Kochi, K. *J. Am. Chem. Soc.* **1982**, *104*, 7599.

(17) (a) Byrnell, C. J. A.; Coombes, R. G.; Hart, L. S.; Whiting, M. C. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1079. (b) Hart, L. S.; Whiting, M. C. *J. Chem. Soc., Perkin Trans. 2* **1983**, 1087.

(18) Sergeev, G. B.; Tung-ha, Ch'eng *Teor. Eksp. Khim.* **1967**, *3*, 422.

* Istituto di Chimica Organica della Facoltà di Farmacia.

† Istituto di Chimica Quantistica ed Energetica Molecolare del C.N.R.

Table I. Third-Order Rate Constants for the Bromination of Cyclohexene in 1,2-Dichloroethane^a

run	t, °C	10 ³ [Br ₂], M	10 ² [c-Hex], M	10 ⁻⁵ k ₃ , ^b M ⁻² s ⁻¹
1	15	1.41	2.68	3.64 (0.04)
2		0.85	8.07	3.75 (0.05)
3		0.74	1.83	4.07 (0.04)
4		0.70	6.60	3.72 (0.03)
5		0.52	8.45	4.11 (0.04)
6		0.47	9.70	3.59 (0.05)
7		0.45	20.0	4.00 (0.04)
				av 3.84 (0.21)
8	25	3.06	1.49	2.26 (0.01)
9		1.35	4.40	2.51 (0.03)
10		1.20	4.15	2.41 (0.02)
11		1.03	3.60	2.39 (0.02)
12		0.88	2.40	2.32 (0.01)
13		0.73	9.65	2.33 (0.03)
14		0.37	9.65	2.60 (0.02)
				av 2.40 (0.11)
15	35	2.41	19.75	1.55 (0.02)
16		1.10	3.56	1.67 (0.01)
17		1.02	3.20	1.60 (0.01)
18		0.82	12.51	1.59 (0.02)
19		0.67	4.01	1.60 (0.02)
20		0.65	1.43	1.60 (0.01)
21		0.41	12.05	1.52 (0.03)
				av 1.59 (0.05)

^aObtained monitoring Br₂ at 409 nm. ^bThe errors of the average rate constants are given as standard deviations from the average.

respectively reported^{3,18} for 1-hexene-, cyclohexene-, and 4-methyl-1-pentene-Br₂ CTC's in hexane. Reported absorption maxima for all observed 1:1 complexes are in the 250–350 nm range, the cyclohexene one having its λ_{max} between 290 and 300 nm in the three mentioned solvents. The detection of a 2:1 Br₂-cyclohexene complex by UV spectroscopy has also been reported.¹⁹

(iii) Observation has been made at the liquid nitrogen temperature of a Br₂-propene CTC by thermographic analysis²⁰ and of 1:1 and 2:1 Br₂-cyclohexene complexes by IR spectroscopy.²¹

No direct detection of CTC's of Br₂ has been reported so far for fast reacting systems of olefins and Br₂ in solvents of moderate or high dielectric constant, where ionic mechanisms are certainly involved. Under these conditions the existence and kinetic role of CTC's has been a matter of speculation, being supported only by largely circumstantial evidence.^{22,23} In this paper we report the detection of a 1:1 Br₂-cyclohexene CTC during the bromination of this cycloalkene in 1,2-dichloroethane, its spectral features, thermodynamic parameters, and kinetic fate, and its involvement in the bromination pathway.

Results and Discussion

Rate Constants and Activation Parameters for the Bromination of Cyclohexene in 1,2-Dichloroethane. The bromination of cyclohexene in 1,2-dichloroethane, an aprotic non-nucleophilic solvent of moderate dielectric constant (ε 10.36),²⁴ followed spectrophotometrically by the stopped-flow technique at the absorption maximum of bromine (409 nm), was found to be a very fast process obeying an overall third-order rate law (eq 1)²⁵ down to a 10⁻⁴ M concentration of the halogen. Table I shows the rate constants measured by fitting the absorbance/time data obtained under pseudo-second-order conditions to the integrated eq 2, where

Table II. Third-Order Rate Constants for the Bromination of Cyclohexene in 1,2-Dichloroethane in the Presence of Cyclohexane at 25 °C

run	cyclohexane mole fraction	10 ³ [Br ₂], M	10 ² [c-Hex], M	10 ⁻⁵ k ₃ , M ⁻² s ⁻¹
8–14				2.40 (0.11)
22	0.008	0.82	2.84	2.20 (0.03)
23	0.027	2.75	2.21	1.87 (0.02)
24	0.042	0.86	2.72	1.50 (0.02)
25	0.069	2.30	3.33	1.34 (0.01)
26	0.090	0.79	1.81	1.08 (0.02)
27	0.122	1.89	2.96	0.96 (0.01)
28	0.144	2.12	1.75	0.73 (0.01)
29	0.207	2.12	2.66	0.56 (0.02)

A is the absorbance for a 1-cm optical path and ε is the molar extinction coefficient of bromine.

$$-d[\text{Br}_2]/dt = k_3[\text{Br}_2]^2[\text{c-Hex}] \quad (1)$$

$$1/A = 1/A^0 + (k_3/\epsilon)[\text{c-Hex}]t \quad (2)$$

No evidence for the equilibrium formation of a highly reactive dimeric form of bromine, which could have been responsible for the observed rate law, was obtained by examining the concentration dependence of the absorption spectrum of the halogen in 1,2-dichloroethane. The molar absorptivities remained exactly unchanged in the 10⁻² to 10⁻⁵ M range over the entire spectrum. The temperature dependence of the extinction coefficient was likewise insignificant over the entire spectrum in the 15–35 °C range.

Reproducible values of k₃ were obtained at both the lowest and the highest halogen concentration. However, the bromination rate was very sensitive to slight changes in the reaction medium, as shown by the sharp drop in k₃ observed when mixtures of 1,2-dichloroethane and cyclohexane (ε 2.023)²⁶ were used as solvent (Table II). Occasional checks by GLC showed that *trans*-1,2-dibromocyclohexane was always the only reaction product.

The following activation parameters were obtained from a linearized fitting of all single values of k₃ at the three temperatures reported in Table I: E_a(obsd) = -7.78 (0.20) kcal mol⁻¹, ΔH[‡] = -8.37 (0.20) kcal mol⁻¹, ΔS[‡] = -64.0 (0.7) eu.²⁷

It must be emphasized that the rates exhibited a negative temperature coefficient, corresponding to a *negative* apparent activation energy, as observed for several alkenes,³ but not for aryl alkenes,²⁸ in apolar solvents like carbon tetrachloride. In the latter solvent the third-order bromination of cyclohexene, carried out in the presence of *N*-bromosuccinimide as an HBr scavenger, has been very recently reported^{17a} to exhibit a ΔH[‡] of -4.0 (0.1) kcal mol⁻¹ and a ΔS[‡] of -70 (1) eu. A controversy exists, however, as to the kinetic interpretation of the reaction in the absence of additives.^{16a,17} An apparent activation energy of zero had been found for the third-order bromination of allyl benzoate in chlorobenzene,²⁹ and very low activation enthalpies (0–1 kcal mol⁻¹) have also been reported for the third-order bromination of styrenes in acetic acid, in contrast with the second-order process, showing higher, positive ΔH[‡] values.^{5a}

The activation entropy for the third-order reaction of cyclohexene in 1,2-dichloroethane was large and negative, as in carbon tetrachloride,^{17a} in agreement with the expectation for a transition state involving three oriented reactant molecules. Similar results had been found for styrene bromination in acetic acid.^{5a}

Detection of a Cyclohexene-Bromine CTC. Under pseudo-second-order conditions, at initial 10⁻⁴ M Br₂ and 10⁻¹ M cyclohexene, the half-life of the halogen at 25 °C was 400 ms, a time fully suitable for reasonably accurate measurements of the

(19) Sergeev, G. B.; Smirnov, V. V.; Bakar'ina, G. A.; Vetrova, M. A. *Dokl. Akad. Nauk SSSR* **1972**, *203*, 394.

(20) Lishnevskii, V. A.; Sergeev, G. B. *Kinet. Katal.* **1964**, *5*, 407.

(21) Kil'mel'feld, Ya. M.; Mostovoi, A. B.; Fainshtein, L. I. *Dokl. Akad. Nauk SSSR* **1973**, *209*, 105.

(22) Dubois, J. E.; Garnier, F. *Tetrahedron Lett.* **1966**, 3047.

(23) Dubois, J. E.; Garnier, F. *Chem. Commun.* **1968**, 241.

(24) Riddick, J. A.; Toops, E. E. In "Technique of Organic Chemistry"; Weissberger, A., Ed.; Interscience: New York, 1955; Vol. VII, p 196.

(25) Bellucci, G.; Berti, G.; Bianchini, R.; Ingrassio, G.; Ambrosetti, R. J. *Am. Chem. Soc.* **1980**, *102*, 7480.

(26) Reference 24, p 52.

(27) It will be clear, in the light of the results discussed later on, that these values are not to be considered as true activation parameters but as combinations of kinetic and thermodynamic quantities involved in a multistep reaction.

(28) Heublein, G. Z. *Chem.* **1969**, *9*, 28.

(29) De la Mare, P. B. D.; Scott, R. A.; Robertson, P. W. *J. Chem. Soc.* **1945**, 509.

Table III. Formation Constants and Molar Absorptivities of the 1:1 Cyclohexene-Br₂ CTC from Initial Absorbances of Mixtures of Bromine and Cyclohexene in 1,2-Dichloroethane

run	<i>t</i> , °C	10 ⁴ [Br ₂], M	[c-Hex], M	<i>A</i> ^{0a} (2 cm)	<i>K</i> _f ^b M ⁻¹	ε _{CTC} ^b M ⁻¹ cm ⁻¹
30	15	5.23	0.084	0.300	0.60 (0.07)	5640 (545)
31		9.88	0.097	0.710		
32		4.70	0.097	0.340		
33		2.84	0.151	0.265		
34		4.70	0.234	0.651		
35		1.25	0.234	0.185		
36		1.25	0.407	0.287		
37		0.59	0.562	0.176		
38		0.59	0.773	0.243		
39		0.59	0.852	0.255		
40		0.59	0.998	0.262		
41		0.59	1.367	0.267		
42	25	7.34	0.096	0.420	0.47 (0.08)	5520 (271)
43		3.74	0.096	0.215		
44		3.74	0.262	0.470		
45		0.95	0.386	0.165		
46		0.95	0.503	0.210		
47		0.95	0.635	0.245		
48		0.95	0.894	0.323		
49		0.95	0.995	0.335		
50		0.85	0.995	0.305		
51		0.85	1.267	0.355		
52		0.85	1.497	0.400		
53	35	8.20	0.125	0.460	0.37 (0.05)	5375 (585)
54		4.12	0.125	0.248		
55		4.12	0.241	0.380		
56		4.12	0.385	0.545		
57		2.72	0.385	0.390		
58		2.72	0.532	0.500		
59		2.72	0.686	0.572		
60		2.72	0.868	0.651		
61		1.61	0.868	0.460		
62		1.61	0.998	0.501		
63		1.61	1.271	0.585		
64		1.61	1.681	0.650		

^a Measured at 287 nm, 2 cm optical path. ^b Calculated by eq 25.

absorbances obtained immediately after mixing the reagents in the stopped-flow apparatus before the start of the bromination reaction, if an intensely absorbing species is involved and monitored at the appropriate wavelength. No definite evidence for the immediate formation of species different from free bromine could be obtained, even when working at the highest alkene concentration when the reaction was monitored at the absorption maximum of the halogen, or at higher wavelengths, as is usual for brominations with molecular bromine. The very low absorbances measured at the low bromine concentrations employed prevented the evaluation of small differences with respect to the values anticipated by assuming that bromine was totally present as free halogen. On the other hand, an increase of the bromine concentration caused a decrease in the half-life, making the evaluation of the initial absorbances, immediately after mixing with the alkene, problematic.

However, when the reaction of 10⁻⁴–10⁻⁵ M bromine with a large excess of cyclohexene was monitored around 290 nm, initial absorbances much higher than those expected for the free halogen were clearly measured. Furthermore, for the same starting bromine concentration these initial absorbances increased with increasing cyclohexene concentration (see Table III). These observations indicated the instantaneous formation of a new species, whose electronic absorption spectrum was hypsochromically and hyperchromically shifted with respect to that of free bromine, as expected for an olefin-Br₂ CTC.^{15,16}

This interpretation was confirmed by the examination of the UV spectrum of the CTC in the 250–350 nm range (Figure 1), that was obtained from the initial absorbances at several wavelengths, corrected for the contribution of free bromine with use of the formation constant (*K*_f) of the CTC determined as described below. The λ_{max} was in agreement with the values reported for

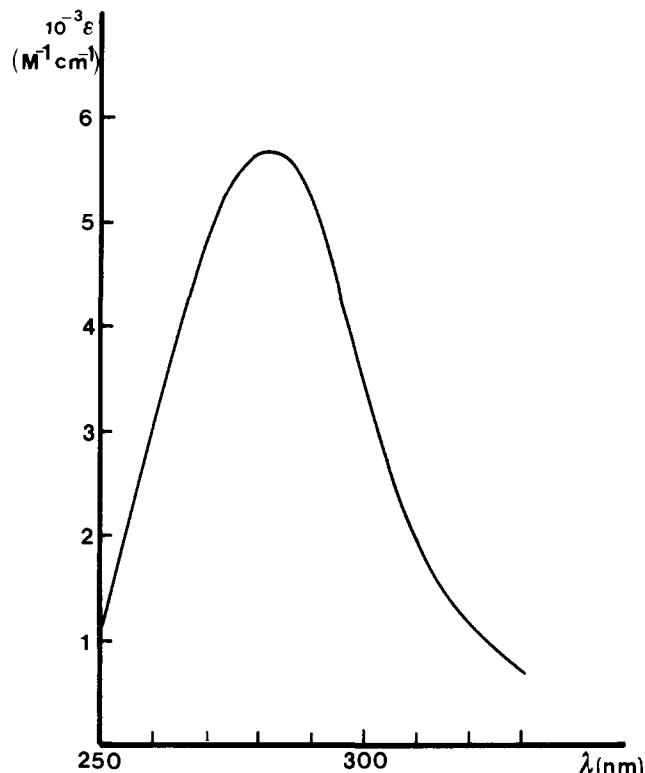


Figure 1. UV spectrum of the bromine-cyclohexene CTC in 1,2-dichloroethane at 25 °C.

the cyclohexene-Br₂ CTC in apolar solvents.^{15,16,18}

Stoichiometry and Thermodynamic Parameters of the Cyclohexene-Br₂ CTC. Although the cyclohexene-Br₂ CTC has been recently reported to have a very low formation constant in CCl₄ around room temperature (<0.1 M⁻¹),¹⁶ an attempt at a precise determination was made in 1,2-dichloroethane by measuring the initial absorbances (*A*⁰) at 287 nm, near the absorption maximum of the CTC, in a set of reactions carried out in the temperature range of 15–35 °C. The reagent concentrations were combined so as to obtain both reasonably long half-lives and reasonably high initial absorbances (Table III). These requirements made the investigation of a wider temperature range hardly possible since, at the useful bromine concentrations, the rate below 15 °C was too high while over 35 °C the extent of complexation of the halogen was too low for accurate measurements. As a first approximation, the contribution of the absorption of free Br₂ to the *A*⁰ values was ignored, and the data obtained at each temperature were fitted to the Scott equation for 1:1 complexes,³⁰ in a form containing the total initial concentrations of bromine, [Br₂]_{tot}⁰, and alkene, the path length, *l*, and the initial absorbances due only to the CTC, *A*⁰, and where the alkene concentration is in large excess with respect to that of the halogen (eq 3).

$$[\text{Br}_2]_{\text{tot}}^0 [\text{c-Hex}] / A^0 = 1 / (K_f \epsilon_{\text{CTC}}) + (1 / \epsilon_{\text{CTC}}) [\text{c-Hex}] \quad (3)$$

Preliminary values of the formation constant, *K*_f, and the molar absorptivity, ε_{CTC}, were so simultaneously obtained (Figure 2). These values of *K*_f ensured that the reagent concentrations employed met the criterion stressed by Person¹⁴ for the evaluation of formation constants of weak complexes, requiring that the donor concentration must be comprised between 0.1 (1/*K*_f) and 9(1/*K*_f).

However, bromine was not completely transparent at 287 nm at the concentrations of Table III, and its contribution to the *A*⁰ values, although small, had to be taken into account, in order to get a more accurate evaluation of the thermodynamic and spectral parameters of the CTC. This required a nonlinear least-squares fitting procedure (see Experimental Section). Since the fitting function (eq 25) was derived for a 1:1 complex, the excellent

(30) Scott, R. L. *Recl. Trav. Chim. Pays-Bas* 1965, 75, 787.

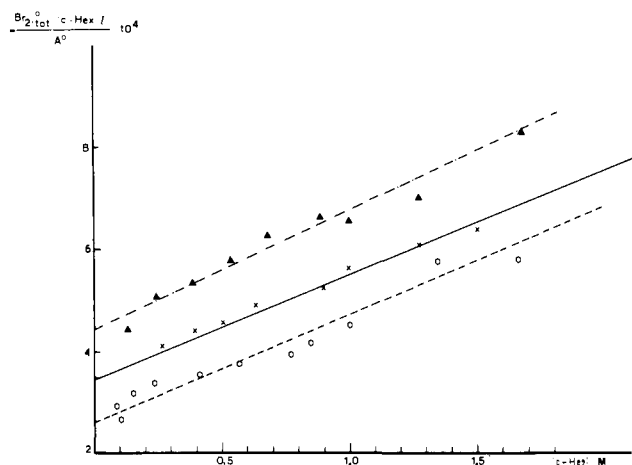


Figure 2. Scott plots (eq 3) of the absorbances (at 287 nm) and reagent concentrations for bromine and cyclohexene mixtures in 1,2-dichloroethane: 15 °C (O); 25 °C (X); 35 °C (▲). Calculated formation constants and molar absorptivities were 15 °C, 0.80 M⁻¹, and 4800 M⁻¹ cm⁻¹; 25 °C, 0.60 M⁻¹, and 4890 M⁻¹ cm⁻¹; and 35 °C, 0.51 M⁻¹, and 4350 M⁻¹ cm⁻¹.

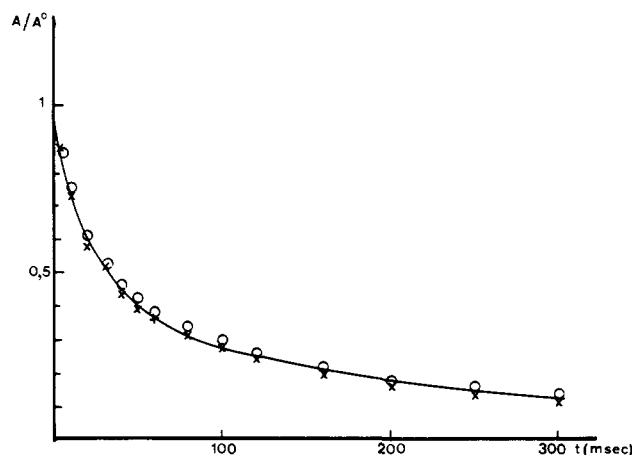


Figure 3. Decay of the absorption of the free Br₂ at 409 nm (X) and of the cyclohexene-Br₂ CTC at 287 nm (O) in the reaction of 4.51 × 10⁻⁴ M Br₂ and 0.20 M cyclohexene in 1,2-dichloroethane at 15 °C.

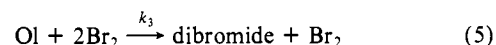
convergence of these fittings provided a further confirmation of the 1:1 stoichiometry of the observed complex. The final values of K_f and ϵ_{CT} so obtained are reported in Table III.

A fitting of $\ln K_f$ against $1/T$ gave the following values of the enthalpy and entropy of formation of the cyclohexene-Br₂ CTC: $\Delta H = -4.27$ (0.11) kcal mol⁻¹, $\Delta S = -15.82$ (0.37) eu. Since the molar absorptivities of the CTC proved to be temperature independent in the investigated range within the limits of the experimental error, the thermodynamic parameters were then refined by a nonlinear least-squares fitting of the initial transmittance, concentration, and temperature data of Table III (see Experimental Section). The following final values were obtained: $\Delta H = -4.60$ (0.20) kcal mol⁻¹, $\Delta S = -17.0$ (0.6) eu. It is to emphasize that these enthalpy and entropy values are very similar to those ($\Delta H = -4.0$ kcal mol⁻¹, $\Delta S = -15.7$ eu) found for the cyclohexene-Br₂ CTC in hexane,^{18,31} thus showing that the solvent polarity, at least in the limited 1.89–10.36 range of dielectric constant, does not affect the physical properties of this weak π - σ complex, in agreement with its expected low extent of charge transfer. In particular it must be stressed, for the purpose of the subsequent discussion, that the formation of the CTC is an *exothermic* process.

Kinetics of Disappearance of the Cyclohexene-Br₂ CTC. The formation of an alkene-bromine CTC during the fast bromination of cyclohexene having been firmly established, two alternative roles can be envisaged for this transient intermediate. First, the CTC can be an unreactive species whose only effect is that of reducing

the concentrations of the actual reactants involved in the third-order step of Scheme I (eq 5). Under this hypothesis, the rate

Scheme I



of consumption of the total bromine should be expressed by eq 6. At sufficiently high alkene concentrations, the CTC concentration is simply given by eq 7 and that of the total halogen by eq 8. Introducing expression 8 in eq 6 gives eq 9, where the observed rate constant, $k_{3(\text{obsd})}$, is related to the actual third-order rate constant by eq 10. It can be observed that, at alkene concentration such that $K_f[\text{c-Hex}] \ll 1$, eq 9 coincides with eq 1, and the $k_{3(\text{obsd})}$ obtained by monitoring the reaction at the absorption maximum of Br₂ is the true third-order constant.

$$-d[\text{Br}_2]_{\text{tot}}/dt = k_3[\text{Br}_2]_{\text{free}}^2[\text{c-Hex}] \quad (6)$$

$$[\text{CTC}] = K_f[\text{Br}_2]_{\text{free}}[\text{c-Hex}] \quad (7)$$

$$[\text{Br}_2]_{\text{tot}} = [\text{Br}_2]_{\text{free}} + [\text{CTC}] = (1 + K_f[\text{c-Hex}])[\text{Br}_2]_{\text{free}} \quad (8)$$

$$-d[\text{Br}_2]_{\text{free}}/dt = k_{3(\text{obsd})}[\text{Br}_2]_{\text{free}}^2[\text{c-Hex}] \quad (9)$$

$$k_{3(\text{obsd})} = k_3/(1 + K_f[\text{c-Hex}]) \quad (10)$$

On the other hand, the decay of the free Br₂ absorption and that of the CTC absorption were coincident during the entire course of the reaction, as shown in Figure 3, so that the kinetics of bromination of the alkene could be measured by monitoring either species. The expressions of the free Br₂ and of the total Br₂ concentrations as a function of the CTC concentration are given by eq 11 and 12. Introducing these expressions in eq 6 gives eq 13, in which the linear dependence on the alkene concentration of eq 9 has disappeared and the observed second-order rate constant, $k_{2(\text{obsd})}$, is related to the third-order constant, measured by monitoring free Br₂, by eq 14. Integration of eq 13 and introduction of the CT absorbances (A_{CT} , 1 cm optical path) leads to eq 15, and a plot of $1/A_{CT}$ against time should give a straight line whose slope gives $k_{2(\text{obsd})}/\epsilon_{CT}$.

$$[\text{Br}_2]_{\text{free}} = [\text{CTC}]/(K_f[\text{c-Hex}]) \quad (11)$$

$$[\text{Br}_2]_{\text{tot}} = ((1 + K_f[\text{c-Hex}])/K_f[\text{c-Hex}])[\text{CTC}] \quad (12)$$

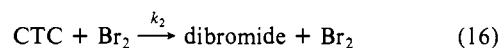
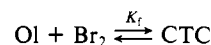
$$-d[\text{CTC}]/dt = k_{2(\text{obsd})}[\text{CTC}]^2 \quad (13)$$

$$k_{2(\text{obsd})} = k_3/(K_f(1 + K_f[\text{c-Hex}])) \quad (14)$$

$$1/A_{CT} = 1/A_{CT}^0 + (k_{2(\text{obsd})}/\epsilon_{CT})t \quad (15)$$

Alternatively, if the alkene-Br₂ CTC is a reactive intermediate of the bromination, being involved together with free Br₂ in the second-order step of Scheme II (eq 16), the rate of disappearance of the total Br₂ should be given by eq 17. Introducing eq 7 and

Scheme II



8, or eq 11 and 12, in eq 17 gives for the observed third-order and second-order rate constants, measured by monitoring respectively the free Br₂ and the CTC, expressions 18 and 19, kinetically indistinguishable from the corresponding eq 10 and 14.

$$-d[\text{Br}_2]_{\text{tot}}/dt = k_2[\text{CTC}][\text{Br}_2]_{\text{free}} \quad (17)$$

$$k_{3(\text{obsd})} = k_2K_f/(1 + K_f[\text{c-Hex}]) \quad (18)$$

$$k_{2(\text{obsd})} = k_2/(1 + K_f[\text{c-Hex}]) \quad (19)$$

All these expressions anticipate a progressive decrease in the observed rate constant for the bromination with increasing alkene at sufficiently high alkene concentrations. This effect is best

Table IV. Rates of Disappearance of the Charge-Transfer Absorbance at 287 nm in 1,2-Dichloroethane^a

run	<i>t</i> , °C	[c-Hex], M	10 ⁴ [Br ₂], M	<i>k</i> _{2(obsd)} /ε _{CT}	<i>K</i> _f ε _{CT}	
					<i>b</i>	<i>c</i>
65	15	0.355	1.12	105	3042	3384
66		0.486	0.62	97	3065	
67		0.571	0.62	97.5	2933	
68		0.685	0.62	90	3028	
69		0.792	0.62	86	3098	
70		0.928	0.62	84	2936	
71		1.090	0.62	73	3184	
72		1.350	0.57	60	3524	
73		1.663	0.62	54	3560	
74	25	0.298	1.74	84	2507	2594
75		0.375	0.91	81	2511	
76		0.499	0.91	82	2370	
77		0.602	0.94	77	2432	
78		0.692	0.94	74	2450	
79		0.820	0.94	70	2478	
80		0.988	0.94	61	2690	
81		1.235	0.76	52	3018	
82		1.502	0.76	44	3200	
56	35	0.385	4.12	69	2017	1988
57		0.385	2.72	69	2017	
58		0.532	2.72	67	1982	
59		0.686	2.72	65	1950	
60		0.867	2.72	56	2150	
61		0.867	1.61	58	2075	
62		0.998	1.61	48.5	2394	
63		1.271	1.61	41	2637	
64		1.681	1.61	34.5	2841	

^aTreatment of the initial absorbance-concentration data of runs 65–73 and 74–82 according to eq 25 gave for the formation constant of the CTC at 15 and 25 °C, respectively, values in excellent agreement with those obtained from the data of Table III. ^bCalculated by eq 20 with use of the average value of *k*₃ of Table I. ^cCalculated from the individual values of *K*_f and ε_{CT} obtained at each temperature by eq 25.

checked by monitoring the CTC, since its high absorption at sufficiently high alkene concentration allows one to reduce the bromine concentration while increasing that of cyclohexene, so as to keep the rate within accurately measurable values. Furthermore, the experimentally accessible kinetic parameters, i.e., the third-order rate constant *k*₃ measured according to eq 2 and the *k*_{2(obsd)}/ε_{CT} values obtained at each alkene concentration by eq 15, should be related to the product of the formation constant and the molar absorptivity of the CTC by eq 20, thus providing a final test for the consistency of the entire picture.

$$k_3 / ((k_{2(\text{obsd})} / \epsilon_{\text{CT}})(1 + K_f[\text{c-Hex}])) = K_f \epsilon_{\text{CT}} \quad (20)$$

Table IV shows the results of an investigation of the kinetics of disappearance of the cyclohexene–Br₂ CTC in reactions of bromine with up to 1.7 M alkene monitored at 287 nm, near the absorption maximum of the CTC. Of course, good fittings of the absorbance–time data to eq 15 are to be expected, and actually found, only for reactions carried out under conditions in which the contribution to the 287-nm absorption due to the free halogen was negligible with respect to that of the alkene–Br₂ CTC, that is at relatively low bromine concentrations and cyclohexene concentrations sufficiently high to ensure a relatively high concentration of the CTC. When these conditions were fulfilled, each run exhibited very clean second-order kinetics up to over 90% conversion, as exemplified by Figure 4. It must be stressed that the lack of any deviation from the linearity in these plots exclude any contribution to the rate equation by a first-order term in bromine down to about 5 × 10^{−6} M Br₂.

At all three examined temperatures the trend in *k*_{2(obsd)} expected on the basis of eq 14, or of its kinetic equivalent eq 19, was actually observed. At alkene concentration below 1 M and at each temperature, the product *K*_fε_{CT} derived kinetically by eq 20 by using the average value of *k*₃ of Table I, was in good agreement with the one obtained from the individual values of *K*_f and ε_{CT} calculated on the basis of the initial absorbances.

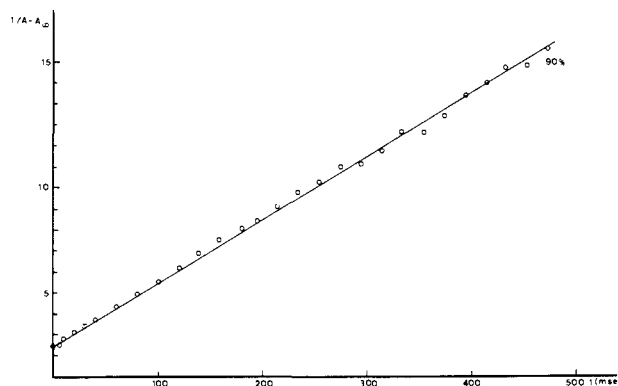


Figure 4. Fitting of the charge-transfer absorbance/time data (287 nm, 2 cm optical path) to the second-order integrated equation (eq 15) for the reaction of 7.0 × 10^{−5} M Br₂ with 0.998 M cyclohexene in 1,2-dichloroethane at 25 °C.

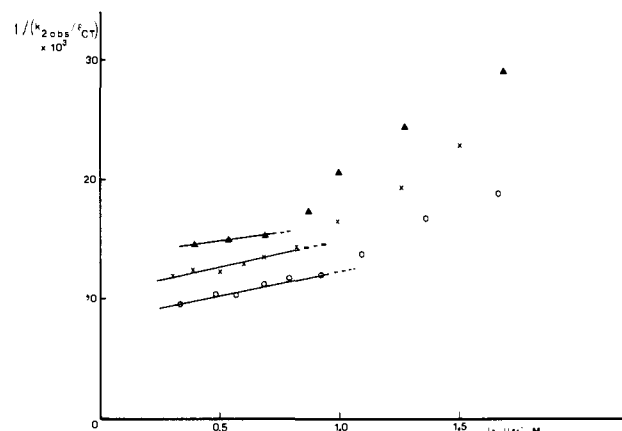


Figure 5. Plots of 1/(*k*_{2(obsd)}/ε_{CT}) (from data of Table IV) against cyclohexene concentration (eq 21) for the reactions of Br₂ and cyclohexene at 15 °C (O), 25 °C (X), and 35 °C (▲).

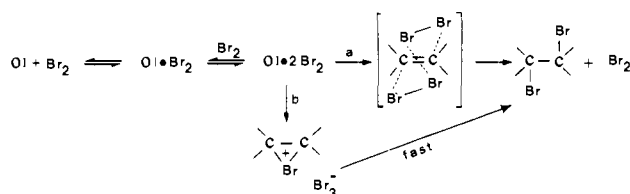
In principle, the dependence of *k*_{2(obsd)}/ε_{CT} on the alkene concentration could be used as the basis of a method for the determination of the formation constant of the alkene–Br₂ CTC. Rearranging eq 20 gives eq 21, and a plot of 1/(*k*_{2(obsd)}/ε_{CT}) as a function of the alkene concentration should give a straight line, the ratio of the slope to the intercept of which gives the formation constant of the complex.

$$1 / (k_{2(\text{obsd})} / \epsilon_{\text{CT}}) = K_f \epsilon_{\text{CT}} / k_3 + (K_f^2 \epsilon_{\text{CT}} / k_3) [\text{c-Hex}] \quad (21)$$

Unfortunately, all sets of data of Table IV led to nonlinear plots, the slopes of which tended to increase with increasing alkene, as anticipated also by the increase of the product *K*_fε_{CT} calculated by eq 20 at the highest cyclohexene concentrations. Figure 5 shows these plots. This failure is almost surely due to the concurrence of a solvent effect by high concentrations of the non polar olefin, of the type of the above mentioned effect by cyclohexane (Table II), tending to depress the bromination rate more than expected on the basis of complex formation alone.

When restricted to alkene concentrations below about 1 M, the fittings of the data of Table IV to eq 21 gave values of *K*_f (0.53 M^{−1} at 15 °C, 0.42 M^{−1} at 25 °C, 0.22 M^{−1} at 35 °C) in fair agreement with those obtained by the NLLSQ fitting of the absorbance–concentration data of Table III. However, being based on too narrow ranges of both alkene concentration and *k*_{2(obsd)}/ε_{CT}, they alone could not be considered as fully reliable. We suggest that eq 21 may be used most advantageously for the determination of *K*_f of highly reactive donor–acceptor complexes, when their reaction rates are too high to allow a precise evaluation of the starting absorbances, while the rate constants can still be measured. The condition to be fulfilled is that the formation constant be high enough to produce a decrease in rate constants observable at donor concentrations sufficiently low so that the properties of the reaction medium need not be modified.

Scheme III



The CTC's Role in the Bromination Mechanism. It has been previously emphasized that the two mechanistically alternative processes sketched in Schemes I and II for the bromination of an alkene, assuming the CTC as an unessential or essential intermediate, respectively, are kinetically indistinguishable. The comparison of the apparent activation energy of the overall reaction with the formation enthalpy of the CTC allows, however, a clear-cut distinction between these two possibilities.

In fact, introducing eq 10 or eq 18 in eq 22, and recalling that

$$E_{a(\text{obsd})} = -R \partial \ln (k_{\text{obsd}}) / \partial T^{-1} \quad (22)$$

the formation enthalpy of the CTC is given by $\Delta H = -R \partial \ln (K_f) / \partial T^{-1}$, one obtains respectively the expressions for the apparent activation energy for a termolecular scheme not involving the CTC (eq 23) and for a bimolecular scheme involving it (eq 24), where $E_{a(3)}$ is the activation energy for step 5 of Scheme I and $E_{a(2)}$ that for step 16 of Scheme II.

$$E_{a(\text{obsd})} = E_{a(3)} - \Delta H(K_f[\text{c-Hex}] / (1 + K_f[\text{c-Hex}])) \quad (23)$$

$$E_{a(\text{obsd})} = E_{a(2)} + \Delta H(1 / (1 + K_f[\text{c-Hex}])) \quad (24)$$

Since the formation of the alkene- Br_2 CTC has been shown to be an exothermic process ($\Delta H < 0$), eq 23 must always give a positive apparent activation energy, irrespective of the value of K_f and of the alkene concentration, whereas eq 24 can give a negative value of $E_{a(\text{obsd})}$, provided that $|\Delta H| > E_{a(2)}$ and $K_f[\text{c-Hex}] < |\Delta H| / E_{a(2)}$, that is for reactions carried out at not too low temperature (or high K_f) and not too high alkene concentration.

The negative value of the apparent activation energy ($E_{a(\text{obsd})} = -7.78$ (0.20) kcal mol⁻¹) found in this investigation for the third-order bromination of cyclohexene in 1,2-dichloroethane excludes therefore a reaction pathway not involving the CTC as a key intermediate and is consistent instead with a mechanism in which the CTC lies on the reaction coordinate for the bromination. Furthermore, introducing the experimental values of $E_{a(\text{obsd})}$, ΔH , and K_f in eq 24 gives an evaluation of the $E_{a(2)}$ term, which, for alkene concentrations below ~ 0.1 M and in the explored temperature range, is found to be again a negative amount, $E_{a(2)} \approx -3.2$ kcal mol⁻¹. This suggests that the reaction shown in eq 16 of Scheme II is actually not a single step but involves the equilibrium formation of a second complex with enthalpy higher, in absolute value, than the true activation energy for its conversion into products.

Unfortunately, no direct spectral evidence for the formation of a second cyclohexene- Br_2 complex was obtained under the conditions of the present study, possibly because of its too low equilibrium concentration, or unfavorably shifted absorption spectrum, so that its stoichiometry could not be established.³²

(31) A ΔH of -12.5 (0.3) kJ mol⁻¹ (-2.95 (0.1) kcal mol⁻¹) has been reported in ref 17a for complex formation between cyclohexene and Br_2 in carbon tetrachloride. However, this value was obtained from the temperature dependence of the slopes of linear plots reporting the increment in absorption near the maximum of free bromine on addition of cyclohexene to solutions of bromine and *N*-bromosuccinimide, while the cyclohexene- Br_2 CTC alone is not expected to have an absorptivity higher than that of the free halogen above 400 nm.

(32) The formation of a Br_2 -cyclohexene complex between more than two molecules, even if about as exothermic as that of the 1:1 CTC, should involve a more adverse entropy change and therefore a lower K_f . Furthermore, even with similar K_f , the amount of 2:1 complex at equilibrium should be much lower than that of the 1:1 one at the low bromine concentrations employed in this investigation. Finally, the excellent fittings of the absorbance-concentration data to eq 25, even at the higher bromine concentrations examined, exclude any significant interference by absorptions from complexes different from the 1:1 one.

However, a 2:1 Br_2 -cyclohexene complex has been actually observed spectrophotometrically under conditions of highly reduced reactivity^{19,21} and regarded as the key intermediate involved in the molecular mechanism proposed for the slow bromination of alkenes in apolar solvents. In this mechanism the 2:1 complex is envisaged³ to rearrange to products through the nonpolar six-membered transition state shown in path a of Scheme III.

The high reaction rate and its marked dependence on the solvent polarity observed for the third-order bromination in solvents of moderate dielectric constant in the present and in previous investigations,^{5b,33,34} as well as the formation of 1,4-dibromo adduct as the main product of the bromination of a conjugated diene,³³ exclude, however, the incursion of such a nonpolar mechanism and point instead to one involving ionic intermediates in 1,2-dichloroethane. In this solvent, as generally in aprotic ones of moderate polarity, a more probable evolution of a 2:1 Br_2 -alkene complex should consist in a slow cleavage of a $\text{Br}-\text{Br}$ bond to an ion pair formed of a bromonium (or bromocarbenium) ion and a charge-dispersed tribromide ion (path b of Scheme III). A fast collapse of this ion pair should finally give the product dibromide and molecular Br_2 . The function of the second alkene- Br_2 complex might therefore be that of bringing the 1:1 complex and a second molecule of Br_2 rapidly together, for a reaction involving a low enthalpy (charge delocalization in the TS) and a relatively high entropy of activation (fairly rigid arrangement of three molecules in the TS).³⁵

Conclusions. The results of this investigation definitely show that the bromine addition to cyclohexene, and presumably to other reactive alkenes, in solvents of moderate dielectric constant as 1,2-dichloroethane, where very high reaction rates are observed, occurs through an ionic mechanism involving the formation of charge-transfer complexes as key intermediates. While the formation of a 1:1 CTC and its implication in the bromination pathway has been unambiguously demonstrated, a second bromine-olefin CTC, involved along the reaction coordinate between the 1:1 CTC and the ionic intermediate of the reaction, although not directly observed, is suggested by the comparison of the kinetic and thermodynamic data of reaction steps. In conclusion, the present data, while not leading to a radically new mechanistic proposal for the reaction under investigation, put on a more solid experimental basis a repeatedly formulated hypothesis, which had so far never been supported by direct evidences, at least for fast-reacting systems.

Experimental Section

Materials. 1,2-Dichloroethane and bromine (both C. Erba RPE >99.5%) were treated as previously reported.²⁵

Cyclohexene free from aromatic and 1,3-cyclohexadiene impurities present in commercial samples, which were very difficult to remove completely and interfered with the measurements of the absorption maximum of the cyclohexene-bromine CTC, was prepared by dehydration of pure cyclohexanol (Schuchardt, >99.8%) with sulfuric acid. Traces of peroxides and moisture, affecting the bromination rates, were removed by refluxing over LiAlH_4 followed by fractionation on a Vigreux column immediately before use.

Analytical Procedures. Stock solutions of bromine in 1,2-dichloroethane were prepared and stored for at most 3 h under protection from external light. Concentrations were determined from the UV spectra taken in a Pye Unicam SP8-400 UV-VIS spectrophotometer equipped with a thermostated cell-holder, using the previously determined value of the molar absorptivity ($\epsilon_{\text{max}} 211 \text{ M}^{-1} \text{ cm}^{-1}$) at the absorption maximum ($\lambda_{\text{max}} 409 \text{ nm}$).²⁵ These solutions were checked initially and then occasionally during the storage by measuring the UV absorption in the 280–350 nm range, where the absorption maximum of the halogen oc-

(33) Bellucci, G.; Berti, G.; Bianchini, R.; Ingrosso, G.; Yates, K. *J. Org. Chem.* **1981**, *46*, 2315.

(34) Results to be published from this laboratory.

(35) In other words, this means that the energy profile for the bromination reaction should show a minimum along the reaction coordinate between the 1:1 CTC and the bromonium-tribromide ion pair. In our view this should be due to a slight polarization of the Br_2 molecule in the 1:1 CTC, causing the formation of a relatively stable aggregate with a second Br_2 molecule having the linear $\text{Br}-\text{Br}-\text{Br}$ arrangement required for the formation of tribromide ion.

curs, and discarded when the molar extinction coefficients at 287 and 350 nm exceeded respectively the values of 35 and $10 \text{ M}^{-1} \text{ cm}^{-1}$, this being taken as an indication for the presence of trihalide ions deriving from hydrogen halide present in the solution. It was found that the addition of very small amounts of water to these solutions caused a marked increase in absorption in the 240–370 nm range, with a shoulder at the typical λ_{max} for the tribromide ion (270 nm), the latter probably being formed by association of Br_2 with HBr arising from its reaction with water. Particular care was therefore devoted to handle all solutions under anhydrous conditions.

The desired bromine concentrations were then obtained by dilution and checked again by UV with use of 5-cm cells when necessary.

At 287 nm, the wavelength at which the absorption of the cyclohexene- Br_2 CTC was measured, the molar absorptivity of bromine was $\epsilon = 35 \text{ M}^{-1} \text{ cm}^{-1}$. When 0.1 M solutions of bromine in 1,2-dichloroethane were examined in the 250–480 nm range in 1-mm cells and then progressively diluted down to $5 \times 10^{-5} \text{ M}$ with use of 5-cm cells, no changes in the molar absorptivities were observed, within the limits of experimental errors. No appreciable change was likewise found for each solution by changing the temperature from 15 to 35 °C.

Working with cyclohexene/bromine molar ratios as high as 5×10^4 , the purity of the alkene was of utmost importance. It was checked by GLC on a DANI 3800 gas chromatograph which was equipped with a 20-m (i.d. 0.2 mm) capillary column, filled with SE52 silicon oil, and by UV absorption. Only samples over 99.9% in purity (detection limit of the GLC analysis) and showing no absorption bands above the solvent cutoff (225 nm) at 1 M cyclohexene concentration in 1,2-dichloroethane were employed for the kinetic measurements.

The products of several kinetic runs were examined by GLC on a 1.5-m (i.d. 2.5 mm) column packed with diethylene glycol-succinate on silyanized Chromosorb W (80–100 mesh): only *trans*-1,2-dibromocyclohexane was found, besides the excess alkene.

Kinetic Measurements. Absorbances of reaction mixtures were recorded on a Durrum Model D-110 stopped-flow apparatus equipped with a 2-cm observation cell and coupled to a Tektronix 5103 storage oscilloscope and a data acquisition system built around a 12-bit A/D converter interfaced to a Commodore 4032 personal computer. The data acquisition system was able to take transmittance measurements with 1:3000 resolution at time intervals, varying on the different parts of the kinetic curve, down to a minimum of 100 μs .

Transmittances could be measured with total noise and drift not exceeding $\pm 0.5\%$ at any wavelength used, with a time constant of 0.1 ms; however, sample-to-sample reproducibility was worse by at least a factor of 2.

The dead time of the mixing system was about 3 ms at all examined temperatures, although deviations due to mixing were detectable in the residuals of the fittings up to about 5 ms. Attempts at including the actual start time as a fitting parameter in the data treatment, although feasible in principle for a higher-than-first-order reaction, were unsuccessful because of the very large correlation of such parameter with the kinetic constant. Initial reagent concentrations had therefore to be adjusted so that half-lives were at least 50 ms (10 times the total duration of the initial deviations of the experimental measurements), and most of the initial absorbance data were obtained from reactions with much longer half-lives. The highest data acquisition rate available was used during the first 10 ms of reaction, and the maximum absorbance (minimum transmittance) value recorded after the 3-ms mixing time was taken as the initial absorbance.

Temperature control of the reagent solutions (± 0.05 °C) was achieved with a Lauda MK70 constant-temperature circulating bath; however, fluctuations of ± 0.1 °C were measured inside the observation cell with a Comark thermocouple thermometer. The instrument was allowed to stabilize and the system equilibrated thermally before each kinetic measurement. A few preliminary runs were monitored on the oscilloscope, and only when they were found to be exactly reproducible were the data acquired and logged on disc for subsequent processing.

Numerical Procedures for Data Fitting. The usual slope-and-intercept methods, either numerical or graphical, were used both for the determination of the rate constants and activation parameters and for a preliminary evaluation of the thermodynamic and spectral parameters of the CTC; the latter were then refined by a nonlinear least-squares fitting (NLLSQ) program coded in BASIC for the Commodore 4032 computer used for data acquisition.

In the NLLSQ fittings, care was taken to write the fitting function with use of variables on which the accidental errors could be reasonably assumed as constant (such as transmittance for photometric measurements); this allowed a simple constant-weight scheme to be adopted.

The standard deviations, given in parentheses in the text, were obtained as $(s^2 \cdot \mathbf{B}^{-1})^{1/2}$, where the scalar quantity s^2 is the variance of the fitting, which was also used to assess the relative quality of different

fittings obtained with different parameter sets. \mathbf{B}^{-1} denotes the inverse of the matrix \mathbf{B} , whose elements b_{ij} are given by^{36,37}

$$b_{ij} = \sum_k (\partial f_k / \partial p_i \cdot \partial f_k / \partial p_j)$$

Here, f_k denotes the value of the fitting function at the k th experimental point and p_i the value of the i th parameter; the sum runs over all experimental points.

The \mathbf{B} matrix yielded the correlation coefficients (r) between the parameters. Thus, for instance,

$$r(p_i, p_j) = -b_{ij} / (b_{ii} b_{jj})^{-1/2}$$

defines the partial correlation coefficient between the parameters p_i and p_j ; the same formula, without the minus sign, applied to the elements of \mathbf{B}^{-1} , gives the total correlation.³⁸

Pronounced nonlinearity of the fitting function against the parameters gives rise to convergence difficulties, which, however, in themselves do not imply that the data cannot define the parameters accurately: it is only necessary to start from parameter values quite close to the final ones, and care has to be exercised in evaluating the required derivatives. This was particularly important for the fitting discussed below under (b), owing to the marked nonlinearity of the transmittance against ΔH and ΔS .

(a) Formation Constant of the Cyclohexene-Bromine CTC. The fitting function is best defined in successive steps. The formation constant is given by the following equation

$$K_f = x / ([\text{c-Hex}]_{\text{tot}} - x) ([\text{Br}_2]_{\text{tot}} - x)$$

where x is the concentration of the CTC formed. Its acceptable solution is the smallest one

$$x = 0.5([\text{c-Hex}]_{\text{tot}} + [\text{Br}_2]_{\text{tot}} + 1/K_f) - (0.25([\text{c-Hex}]_{\text{tot}} + [\text{Br}_2]_{\text{tot}} + 1/K_f)^2 - [\text{c-Hex}][\text{Br}_2]_{\text{tot}})^{1/2}$$

The initial transmittance (T°) of bromine-cyclohexene mixtures, at wavelengths where the alkene is transparent, is given by the equation

$$-\log(T^\circ) = \epsilon_{\text{Br}_2}([\text{Br}_2]_{\text{tot}}^\circ - x) + \epsilon_{\text{CT}}x \quad (25)$$

where $[\text{Br}_2]_{\text{tot}}^\circ$ is the total initial concentration of bromine. This equation contains implicitly K_f through x . It contains as measured variables the total concentrations and the transmittance; the fitting parameters are K_f and ϵ_{CT} , whose starting values were obtained from the Scott plots of Figure 2. ϵ_{Br_2} was also treated as a parameter, whose value was measured independently on bromine solutions. The calculated transmittance value was compared to the experimental one in the NLLSQ procedure.

In all actual fittings, $r(\epsilon_{\text{CT}}, K_f)$ was close to 0.985, working with between 5% and 45% complexed bromine. This r value would have decreased on introducing measurements carried out with a larger fraction of the available bromine (or cyclohexene) present as the CTC; however, it was hardly possible to take accurate measurements by working at cyclohexene/bromine molar ratios higher than 5×10^4 , so that the total range measured was the maximum accessible to experiments.

(b) Thermodynamic Parameters of the Cyclohexene-Bromine CTC. The numerical treatment described under section (a) was followed again, but the thermodynamic expression for the formation constant was used instead

$$K_f = \exp(\Delta S/R) \exp(-\Delta H/(RT))$$

The resulting fitting function included the total concentrations of reagents, the transmittance, and the temperature as measured variables and ΔH , ΔS , and ϵ_{CT} as fitting parameters. All parameters were taken as temperature independent, at least in the narrow temperature range studied. For ϵ_{CT} , in particular, this was substantiated by part (a) type fittings at separate temperatures.

The only high r value was $r(\Delta H, \Delta S) = 0.98$. As far as we know, this is typical of all determinations of thermodynamic equilibrium parameters, although the usual log plots tend to obscure this effect. The r value could have been decreased by increasing the temperature range of the mea-

(36) Wentworth, W. E. *J. Chem. Educ.* **1965**, *42*, 96.

(37) Hamilton, W. C. "Statistics in Physical Science"; Ronald Press: New York, 1965.

(38) By definition r is in the range -1 to $+1$, but is not to be confused with the more commonly quoted correlation coefficient (r_c) between measured variables, which indicates the amount of linear dependence of a variable upon another. As a matter of fact, we should look for experimental conditions yielding a high value of r_c and a low value of r the larger the range of the measured variables, the lower the r values.

surements; however, this hardly would have been possible in the present case, as explained previously. Furthermore, a wider temperature interval would introduce even more complex problems, due to the temperature dependence of the fitting parameters.

A solid convergence was obtained, even allowing all three parameters to vary freely. The final ϵ_{CT} value ($5520 (250) \text{ M}^{-1} \text{ cm}^{-1}$) was close to the average of the individual values obtained at different temperatures.

Acknowledgment. This work was supported by a grant from the Consiglio Nazionale delle Ricerche and from the Ministero della Pubblica Istruzione. We thank Mr. E. Fontana for technical assistance.

Registry No. Cyclohexene, 110-83-8; cyclohexene-bromine (1:1 CTC), 16489-73-9.

Biosynthesis of Macrolide Antibiotics. 6. Late Steps in Brefeldin A Biosynthesis

Yoshikuni Yamamoto, Akira Hori, and C. Richard Hutchinson*

Contribution from the School of Pharmacy, University of Wisconsin, Madison, Wisconsin 53706.
Received September 10, 1984. Revised Manuscript Received January 1, 1985

Abstract: Brefeldin A (**1**), a macrolide antibiotic produced by several fungi, contains a cyclopentanol ring as part of its 16-membered lactone. The role that the oxygen and double bond functionality at positions 2, 3, 4, 7, 10, and 11 play in the mechanism of 5-membered ring formation is examined by comparing the efficiency of incorporation of several isotopically labeled compounds into **1** which are putative intermediates of the late part of the biosynthetic pathway. Brefeldin C (7-deoxybrefeldin A, **3**) and 7-oxobrefeldin A (**2**) were efficiently metabolized to **1**, 4-deoxybrefeldin C (**8**) was inefficiently metabolized to **1**, but the 3,4-double bond isomers of 4-deoxybrefeldin C and 2,3,10,11-tetrahydrobrefeldin C were not metabolized to **1** by intact cells of *Eupenicillium brefeldianum*. 7-Oxobrefeldin A was found in the fungal cells, and a small amount of exogenously added brefeldin A was converted to **2** in vivo. These results support the intermediacy of **3** in the biosynthetic pathway of **1** and suggest that **1** and **2** can be interconverted by a biochemical redox process. The low conversion of **8** to **1**, however, suggests that it is not an intermediate of the main biosynthetic pathway to **1** but possibly only metabolized to **1** by a shunt pathway or by a nonspecific hydroxylase.

Brefeldin A (**1**), a macrolide antibiotic produced by several fungi,¹ resembles the prostaglandins structurally, and for this reason, its biosynthesis has attracted considerable interest.¹ The central issue is the timing and mechanism of the formation of its cyclopentanol ring. Does this occur before or after the closure of the larger macrolide ring, and does the functionality at positions 2, 3, 4, 7, 10, and 11 have a role in the cyclization process? From the results of isotopic labeling experiments with ¹⁸O-¹ and ²H-labeled²⁻⁴ acetate, we have proposed that closure of a 16-membered macrolide generates the 5-membered ring of **1** by an epoxyolefin cyclization mechanism which would create the C-4 hydroxyl and C-10 olefin simultaneously.¹ Therefore, we needed to determine when these and the other functionality surrounding the 5-membered ring are introduced during the biosynthesis of **1**. The information presented below shows that in the main pathway to brefeldin A, introduction of the C-7 hydroxyl onto an existing cyclopentane ring is an efficient, terminal step but that the introduction of the C-4 hydroxyl and C-2 and C-10 double bonds is not. Furthermore, the C-7 functionality can interconvert between the hydroxyl and ketone oxidation states as an incidental part of the metabolism of **1**.

The isotopically labeled compounds to be tested as biosynthetic precursors of **1** were synthesized from brefeldin A as shown in Scheme I. The preparation of [7-²H,7-³H]brefeldin C (**3**) was adapted from the methods used by Sunagawa et al. in their work on the identification of **3**,⁵ the preparation of [7-²H,7-³H]-2,3,10,11-tetrahydrobrefeldin C (**4**) from the method of Harri et al. for the reduction of **1**,⁶ and the preparation of [4-²H,4-³H]-

brefeldin A and -7-oxobrefeldin A from the methods of Corey and Wollenberg.⁷ The synthesis of 4-deoxybrefeldin C (**8**) was complicated by preferred isomerization of the 2,3-double bond into the unconjugated 3,4-position.⁸ The most successful among several procedures we tried for C-4 deoxygenation⁹ was an adaptation of the method of Keinan and Greenspoon¹⁰ in which the addition of a catalytic amount of Pd(PPh₃)₄ was found to promote formation of the desired 2,3-double bond isomer.¹¹ Deoxygenation of 7-oxobrefeldin A gave two isomeric products (**6** and **7**) in about equal yield which were converted into [7-³H]-**8** and its 3,4-double bond isomer **9** by the methods used for the preparation of [7-²H,7-³H]-**3**. All the isotopically labeled compounds were purified chromatographically and had the same physical and spectral characteristics as their unlabeled counterparts except, of course, for the absence of certain resonances in the proton NMR spectra of the corresponding deuterium-labeled compounds.

We examined the incorporation of each of these labeled compounds into brefeldin A by separate feeding experiments with *Eupenicillium brefeldianum* in which the putative precursor was fed on the third or fourth day of the fermentation period followed by three more days of growth. The isolated **1** was recrystallized to constant specific radioactivity and the site of its expected isotopic labeling confirmed by degradation or ²H NMR spectroscopy. The

(7) Corey, E. J.; Wollenberg, R. H. *Tetrahedron Lett.* 1976, 4701.

(8) The 3,4-double bond isomer is the exclusive product when the mixture of 2,3/3,4-double bond isomers are treated with *t*-BuOH/*t*-BuOH-Me₂SO at room temperature or LDA/THF at -78 °C.

(9) We could not repeat the work of Entwistle, Howard, and Johnstone (Entwistle, I. D.; Howard, C. C.; Johnstone, R. A. W. *Phytochemistry* 1974, 13, 173) in which they prepared 4-deoxybrefeldin A in a 44% yield by Li/liquid NH₃ reduction of 4,7-diacetyl-brefeldin A.

(10) Keinan, E.; Greenspoon, N. *Tetrahedron Lett.* 1982, 23, 241.

(11) The intermediate π -allylpalladium(0) complex presumably is formed in the reduction of 4,7-diacetyl-brefeldin A or 4-acetyl-7-oxobrefeldin A and then is reduced at C-2 or C-4 or eliminates AcOH to form a 2,3,4,5,10,11-triene derivative of brefeldin A.¹² We are not certain that **5** forms the same intermediate Pd(0) complex although unpublished results from Prof. Barry Trost's laboratory support this possibility.

(12) Tsuji, J.; Yamakawa, T. *Tetrahedron Lett.* 1979, 613. Hutchins, R. O.; Learn, K.; Fulton, R. P. *Tetrahedron Lett.* 1980, 27.

(1) Mabuni, C. T.; Garlaschelli, L.; Ellison, R. A.; Hutchinson, C. R. *J. Am. Chem. Soc.* 1979, 101, 707 and references therein.

(2) Hutchinson, C. R.; Kurobane, I.; Mabuni, C. T.; Kumola, R. W.; McInnes, A. G.; Walter, J. A. *J. Am. Chem. Soc.* 1981, 103, 2474.

(3) Hutchinson, C. R.; Kurobane, I.; Cane, D. E.; Hasler, H.; McInnes, A. G. *J. Am. Chem. Soc.* 1981, 103, 2477.

(4) Hutchinson, C. R.; Shu-Wen, Li; McInnes, A. G.; Walter, J. A. *Tetrahedron* 1983, 39, 3507.

(5) Sunagawa, M.; Ohta, T.; Nozoe, S. *Heterocycles* 1979, 13, 267.

(6) Harri, E.; Loeffler, W.; Sigg, H. P.; Stahelin, H.; Tamm, C. *Helv. Chim. Acta* 1963, 46, 1235.