

Proton-acceptor Properties of Carotene.

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As a preliminary to a study of proton-acceptor properties of deeply coloured polymers, an investigation is described dealing with carotene-acid adducts; and it is shown that equilibrium constants, K , characterising the basicity of carotene can be determined. A proton-transfer mechanism in the formation of these adducts is consistent with a relation of the Brønsted type between K and the dissociation constants of the three chloroacetic acids, and with the fact that addition of acids to carotene is prevented by basic solvents. The molar electrical conductivities, Λ , of the carotene-acid adducts in benzene solution are calculated and compared with Λ values of a previously investigated benzene-soluble salt.

THE experiments to be described deal with proton-acceptor properties of carotene, a deeply coloured hydrocarbon containing 10 conjugated double bonds in the α -modification and 11 in the β -form. The basicity of these polyenes is sufficiently large for proton-transfer reactions to be studied in an aprotic solvent, whereas in most previous investigations, pertaining to the basicity of hydrocarbons, the solvent acted as the proton donor (cf., e.g., Gold, Hawes, and Tye, *J.*, 1952, 2167, 2172, 2181). In the present case it was possible to vary the concentration of acids and carotene within wide limits, to establish the reversibility of the proton transfer, and to estimate equilibrium constants characterising the basicity of carotene with an accuracy of 25–30%. These measurements were done by a spectrophotometric method, which is based on the fact that a new absorption band appears immediately after the addition of acid to carotene, the maximum of this band being situated, in most cases, in the near infra-red region. The results of equilibrium measurements, in conjunction with determinations of the specific electrical conductance of benzene solutions containing carotene and acids, enabled the molar electrical conductivity of the adducts to be calculated. The numerical results provide independent evidence for the occurrence of proton-transfer processes.

EXPERIMENTAL

The mixture of α - and β -carotene used was recrystallised from carbon disulphide-ethyl alcohol to constant m. p., 172—173°, and stored in a high vacuum in the dark. Carotene stock solutions used for the spectrophotometric tests were stabilised by the antioxidant α -tocopherol, the molar ratio carotene: α -tocopherol being 10—20. Control experiments showed that under these conditions α -tocopherol had no influence on the equilibrium coefficients. The electrical-conductivity measurements were done with freshly dissolved carotene without α -tocopherol. Tri- and mono-chloroacetic acid were recrystallised from pure benzene, triisomyllammonium picrate from ethylene dichloride-light petroleum, and picric acid from water. Ethyl trichloroacetate and dichloroacetic acid were dried and redistilled at 1 mm. Hydrogen chloride vapour was dried before being dissolved in pure benzene, atmospheric moisture being excluded during storage and transfer to the optical or conductivity cells. The purity of dodecylbenzenesulphonic acid* was checked by analysis and equivalent-weight determinations. This acid had been prepared from the sodium salt by interaction with concentrated aqueous hydrochloric acid; after removal of sodium chloride by repeated shaking with the hydrochloric acid solution, the sulphonic acid was dried to constant weight *in vacuo* over potassium hydroxide. All solvents were carefully dried and redistilled. Benzene had been purified, moreover, by prolonged shaking successively with sulphuric acid, alkali, and water; it was stored over metallic sodium.

The spectrophotometric tests were done with a Unicam SP 500 instrument and cells varying in length from 0.2 to 10 cm. Some of the cells, of the type described by Smakula (*Z. physikal. Chem.*, 1934, 25, 94), were fitted with ground joints: solutions could thus be introduced without coming into contact with moisture. Cells up to a length of 3 cm. could be fitted into a housing, in which the temperature was kept constant within $\pm 0.1^\circ$ (cf. Wassermann, *Nature*, 1934, 134, 101), and in which a determination of optical densities within a temperature range of about 40° could be made. All tests were done with duplicate cells in order to eliminate the light absorption by added substances, e.g., acids or solvents.

The electrical conductivities were determined with the help of a D.C. bridge, potential differences across the bridge mid-points being measured by an amplifying circuit, involving two ME 1400 valves and two pairs of EF 37 A valves, the second pair being connected with a micrometer. The standard resistors (10^3 — 10^{11} Ω) in one bridge arm were operated by means of a special high-resistance switch; a similar switch was used to reverse the leads to the conductivity cells. The valves and the heater currents were supplied (500 v, 20 mA) from a stabilised power unit. The potential across the conductivity cell, kept in an oil thermostat, could be varied from 48 to 120 v. In order to reduce polarisation effects no attempt was made to balance the bridge to zero ammeter deflection. The unknown resistance was measured by a technique in which minimum deflection was gradually achieved by successive adjustment of the standard resistors and of the decade boxes in the bridge arms. Each ammeter deflection was observed immediately after the closing of the circuit, the time during which the circuit was closed being as short as possible, and the potential across the conductivity cell being frequently reversed. Two cells of constants 0.0430 and 0.0161 cm^{-1} were used; the electrodes of the latter cell were three concentric platinum cylinders, two of which were connected with each other. Solutions could be introduced into one of the cells and diluted without coming into contact with atmospheric moisture. Control experiments showed that short exposure to atmospheric moisture had no significant effect, except in the tests with hydrochloric acid. The bridge was frequently tested with standard resistors in the range 10^3 — 10^{12} Ω ; in order to show that polarisation effects are negligible, further calibrations were carried out by means of seven benzene solutions of triisomyllammonium picrate, the concentration range being 0.01—0.1 mole/l. At the highest concentration the resistance could be measured both in the D.C. bridge and in a conventional A.C. bridge; furthermore, the molar electrical conductivities could be compared with those measured by Krauss and Fuoss (*J. Amer. Chem. Soc.*, 1933, 55, 25). The various tests agreed to within $\pm 5\%$.

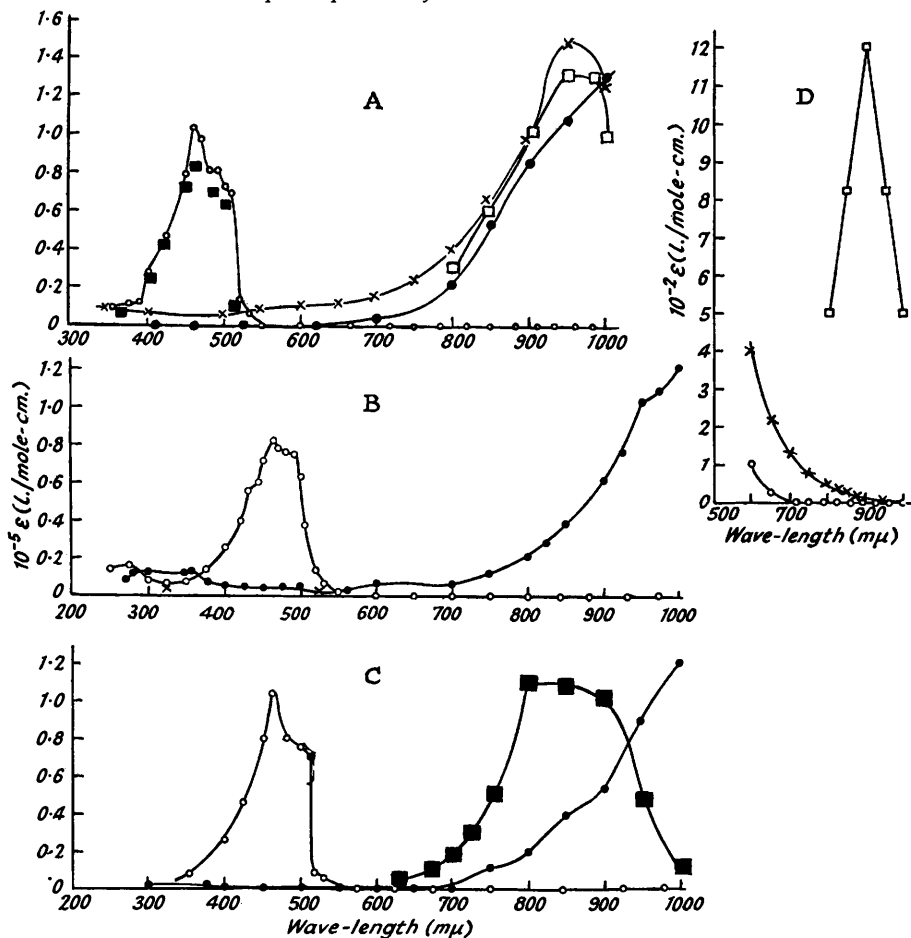
The optical densities and the electrical resistance of mixtures of carotene and acids in an aprotic solvent are time-dependent. In order to eliminate time effects, measurements were done at different times, and back-extrapolated to the time of mixing the acid with the carotene. If carefully purified reactants and solvents and freshly prepared acid solutions are used the experimental error, deduced with the help of the back-extrapolation method, is as specified.

* The position of the dodecyl group relative to the sulphonic acid group is not known.

Results.

Carotene Acid Adducts.—Carotene is characterised by a light-absorption band between 400 and 500 $m\mu$ (cf. Zechmeister, "Carotinoide," Springer, Berlin, 1934, p. 130), in which the molar light-absorption coefficient, ϵ , defined by $\epsilon = (1/cl) \log_{10} (I_0/I) = (\text{optical density})/(\text{carotene concentration} \times \text{optical path length})$, reaches values up to 1×10^6 l. mole⁻¹ cm.⁻¹. On addition of strong acids a new absorption band, at wave-length >500 $m\mu$, is immediately observed, which is due to an adduct C...D formed from n acid molecules, A, and carotene, B, according to (1), while the original band between 400 and 500 $m\mu$ disappears (see Fig. 1). The light

FIG. 1. Absorption spectrum of carotene without and with acids at 20°.



Solvent, benzene in A, C, and D; chloroform in B. \circ = carotene without acid.
 Symbols in A: \times , 1.85M with respect to trichloroacetic acid; complete conversion of the carotene into the adduct has taken place.*
 \bullet , 1.16M with respect to trichloroacetic acid and 2.00M with respect to nitrobenzene; \square , 1.16M with respect to trichloroacetic acid and 2.50M with respect to chloroform; \blacksquare , 1.33M with respect to ethyl trichloroacetate (molarity of carotene = 1.52×10^{-5}).
 Symbols in B: \bullet , 2.03M with respect to trichloroacetic acid; complete conversion of the carotene into the adduct has taken place.*
 Symbols in C and D: \bullet , 7.26M with respect to dichloroacetic acid; \blacksquare , 0.380M with respect to hydrochloric acid; \times , 0.244M with respect to picric acid; \square , 0.328M with respect to dodecylbenzenesulphonic acid.
 In all the experiments with acids the optical densities at each wave-length have been extrapolated to the time of mixing the carotene with the acid. In the experiments marked * the validity of Beer's law was tested by varying the carotene concentration in the range 7.04×10^{-7} to 1.52×10^{-5} mole/l.

absorption of the carotene-trichloroacetic acid adduct, for instance, is not significant, throughout the whole visible region of the spectrum. Some time after the formation of the adduct, however, the light absorption in the near ultra-violet and blue part of the spectrum increases



very markedly, as the result of consecutive, irreversible changes of unknown nature, thereby producing deep colour of the type previously noted (cf. references given by Zechmeister, *op. cit.*, p. 81). The latter effect is not shown in Fig. 1. Ethyl trichloroacetate, in contrast to the free acid, does not alter the light absorption of carotene to any marked extent.

Equilibria.—In the experiments with trichloroacetic, dichloroacetic, and hydrochloric acid, the proportion of B converted into C...D can be directly determined by measuring the optical density at a suitable wave-length, but the further interpretation of these results depends upon what assumption is made on the number of acid molecules combining with one carotene

FIG. 2. Influence of trichloroacetic acid concentration on optical density (920 m μ) at time of mixing the acid with the carotene solution. Optical path-length 10 cm.; carotene concn., 7.04×10^{-7} mole/l.; solvent, benzene; 21.5°.

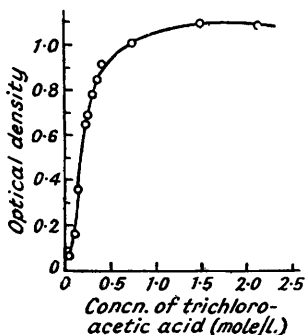
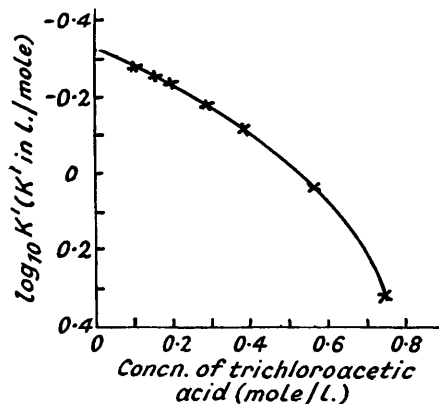


FIG. 3. Influence of trichloroacetic acid concentration on logarithm of equilibrium coefficient, K' , calculated according to eqn. (2). Carotene concn., 2.82×10^{-6} mole/l.; solvent, benzene; 21.5°.



molecule and on the state of dissociation of the adduct. The present equilibrium coefficients K and K' , calculated on the assumption that $n = 1$ and that C and D are held firmly together, are defined by

$$K = \frac{[C \dots D]}{[A][B]} \cdot \frac{fC \dots D}{fA \times fB} = K' \frac{fC \dots D}{fA \times fB} = \frac{d_A - d_B}{d_\infty - d_A} \cdot \frac{1}{[A]} \cdot \frac{fC \dots D}{fA \times fB} \quad (2)$$

where the expressions in brackets are concentrations, the f 's are activity coefficients, d_∞ is the optical density in the presence of sufficient acid to give rise to complete conversion of B into C...D, d_A is the optical density of a mixture of B and C...D, and d_B is the optical density of B without A. Whenever eqn. (2) was applied the ratio $[C \dots D]/[B]$ could be varied and it was noted that the coefficient K' increases with increasing acid concentration, probably owing to a decrease of fA ; the change of fA , which has to be postulated in order to account for the observed variation of K' , is large compared to changes of activity coefficients in systems in which the dielectric constant is larger than that of benzene. In order to estimate K an empirical extrapolation method was used in which $\log K'$ was plotted against $[A]$, the resulting graph being extrapolated to $[A] \rightarrow 0$. As the carotene concentration was below 10^{-5} mole/l. it is justified to assume that the ratio $(fC \dots D/fB)_{[A] \rightarrow 0}$ approaches unity. It is consistent with this assumption that in the experiments with trichloroacetic acid and varying carotene concentration, the K' values, extrapolated to $[A] = 0$, are independent of the carotene concentration, within the limits of the specified experimental errors.

In the experiments with picric and dodecylbenzenesulphonic acid complete conversion of B into C...D was not possible, because these acids could not be used in sufficiently high concentration and, therefore, d_∞ could not be determined directly, as in the case of the other acids. The equilibrium coefficients, K' , were calculated as follows (cf., e.g., Page, *Trans.*

Faraday Soc., 1953, 49, 637): the ratio $[A][B_i]/d_A$ (where $[A]$ = acid concentration, $[B_i]$ = total concentration of carotene in reaction mixture, and d_A = optical density of the solution containing carotene and acid) was plotted against $[A]$, the resulting graph being a straight line, within the limits of the estimated inaccuracy of d_A . The intercept of this line on the $[A][B_i]/d_A$ axis and its slope being designated by i and s , respectively, K' is given by

$$K' = s/i \quad \dots \dots \dots (3)$$

This relation is again based on the assumption that n in (1) is unity. The complete conversion of B into C...D, as indicated by a limiting value of the optical density, at sufficiently large acid concentrations is shown in Fig. 2. Similar results were obtained with dichloroacetic acid and hydrochloric acid. With trichloroacetic acid, experiments at 460 $m\mu$ were also carried out. At this wave-length a minimum value of the optical density is reached if the acid concentration is sufficiently large. Equilibrium constants, K , deduced from experiments at 920 $m\mu$ and 460 $m\mu$ agreed to within ± 0.15 l./mole; the $K(920 \text{ m}\mu)$ values are more accurate, however, because at this wave-length d_B in eqn. (2) is zero. Typical graphs showing the dependence of $\log K'$ on $[A]$ and that of $[A][B_i]/d_A$ on $[A]$ are in Figs. 3 and 4, and the results

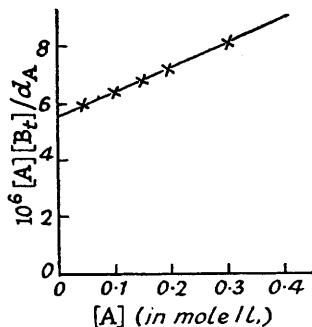


FIG. 4. Interaction of carotene and hydrochloric acid in benzene solution at 19°.
 Conc'n. of carotene = 15.2×10^{-6} mole/l. = $[B_i]$.
 Conc'n. of hydrochloric acid = $[A]$.
 Optical density, 920 $m\mu$ (10-cm. cell), at time of mixing the carotene and the acid = d_A .
 Intercept, $i = (5.6 \pm 0.2) \times 10^{-6}$ (mole/l.)²; slope, $s = (9.0 \pm 0.5) \times 10^{-6}$ mole/l.

of all the equilibrium measurements are in Table I. The figures in the first nine lines show that the K values of the reaction between trichloroacetic acid and carotene appear to decrease somewhat with increasing temperature, but the effect is not substantially larger than the experimental error. The equilibrium coefficient being represented by $K = \exp \Delta S/R \cdot \exp -\Delta H/RT$, it follows that ΔS , the entropy change accompanying the proton transfer to the carotene, is -9 ± 7 cal./degree, and $-\Delta H$, the heat change, is 2.0 ± 1.8 kcal./mole. Attempts have

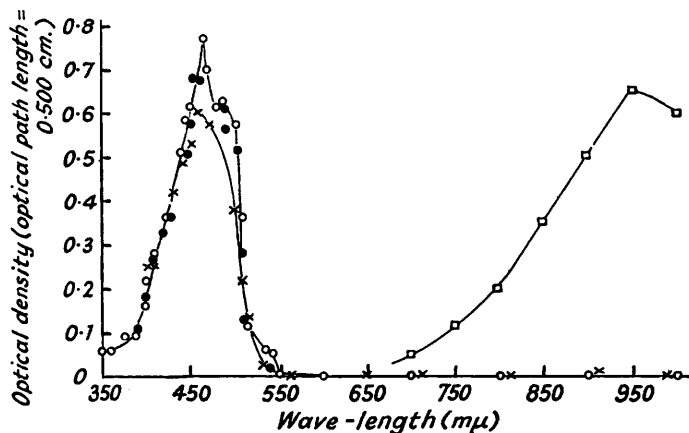
TABLE I. Equilibrium between carotene and acids (in C_6H_6 , except as shown).

		Concentration		No. of runs	Wave-length (m μ)	K (l./mole)	K'	Calc. by eqn.:		
Acid	Temp.	Acid (mole/l.)	Carotene ($10^6 \times$ mole/l.)							
CCl ₃ -CO ₂ H	11.0°	0.18—1.5	2.30	9	920	0.40 \pm 0.10	—	}		
		0.046—1.5	0.704	16		0.40 \pm 0.10	—			
	20 \pm 1	0.09—2.2	1.41	11		0.45 \pm 0.10	—			
		0.09—1.1	2.30	9		0.35 \pm 0.10	—			
		0.09—2.8	2.82	11		0.50 \pm 0.15	—			
		0.09—2.0	5.64	10		0.30 \pm 0.15	—			
		0.30—3.2	15.2	59		0.40 \pm 0.10	—			
	31.0	0.18—1.1	2.30	8		920	0.33 \pm 0.10		—	}
	48.0	0.18—1.1	2.30	8		0.26 \pm 0.10	—			
21	0.3—3.1	8.49	9 †	0.30 \pm 0.10	—					
CHCl ₂ -CO ₂ H	21	1.2—9.7	15.2	11	920	0.02 \pm 0.01	—	}		
HCl	19	0.042—0.38	15.2	6	920	—	2.0 \pm 0.5			
D.B.S.A.*	19	0.021—0.17	15.2	6	1000	—	1.6 \pm 0.5	}		
		0.025—0.24	231	5	650	—	7 \pm 3			
	Picric	18	—	—	6	—	—		3 \pm 2	

* D.B.S.A. = Dodecylbenzenesulphonic acid. † Expts. in CHCl₃.

also been made to measure the equilibrium coefficient of the reaction between monochloroacetic acid and carotene, but only the order of magnitude, $K' \approx 10^{-5}$ l./mole (benzene solution, 20°), could be estimated with the help of eqn. (2), and assuming that the light-absorption

FIG. 5. Influence of dioxan on the light absorption of a mixture of carotene and trichloroacetic acid in benzene solution at 22°.



Concs. (mole/l.) in final reaction mixture: carotene, 1.52×10^{-5} ; trichloroacetic acid, 1.16.

Graph (1). \square , Carotene + $\text{CCl}_3\text{CO}_2\text{H}$ without dioxan; optical densities between 350 and 650 $\text{m}\mu$ < 0.05 ; all optical densities extrapolated to time of mixing the carotene with the acid.

Graph (2). \times , Carotene + $\text{CCl}_3\text{CO}_2\text{H}$ + dioxan; dioxan (2.34M) had been added shortly after mixing the carotene with the acid; before addition of the dioxan the solution was $1.60 \times 10^{-5}\text{M}$ in carotene and 1.30M in $\text{CCl}_3\text{CO}_2\text{H}$.

Graph (3). \circ , Carotene alone.

Graph (4). \bullet , Carotene + dioxan; dioxan concn. 2.34M; no acid present.

FIG. 6. Specific electric conductance, κ , of carotene in 1.00M-trichloroacetic acid; solvent, benzene; 25.0°.

Ordinate is the observed specific conductance, calc. from resistance (back-extrapolated to the time of mixing the carotene with the acid) and corrected for the κ value of the acid without the carotene ($\kappa = 3.4 \times 10^{-10} \Omega^{-1} \text{cm}^{-1}$).

The accuracy of the various points on the graph is $\pm 10-20\%$.

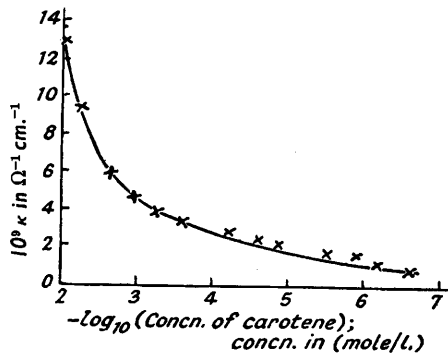
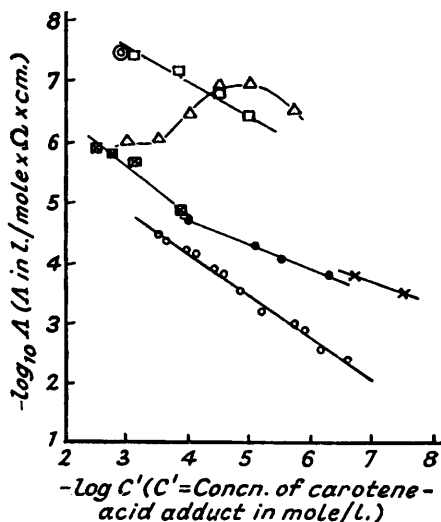


FIG. 7. Molar electrical conductivities of carotene acid adducts and of tetraisoamylammonium picrate in benzene solution at 25.0°.



Δ = Tetraisoamylammonium picrate; values taken from Fuoss and Krauss (J. Amer. Chem. Soc., 1933, 55, 3611). The other symbols relate to the carotene acid adducts: \circ = Trichloroacetic acid; \bullet = dichloroacetic acid; \times = monochloroacetic acid; \boxtimes = dodecylbenzenesulphonic acid; \square = picric acid; \odot = hydrochloric acid.

coefficient of the carotene-monochloroacetic acid adduct is the same as that of the dichloroacetic acid adduct. A more accurate estimation of K' was not possible in this case, because a sufficiently large conversion of B into C...D could not be achieved, owing to the limited solubility of monochloroacetic acid.

Fig. 1(A) indicates that the formation of the carotene-trichloroacetic acid adduct in benzene solution is not prevented by the polar aprotic solvent nitrobenzene, and some of the figures in Table 1 show that the K values relating to a non-polar and a polar aprotic solvent (benzene and chloroform) are similar. Basic solvents, on the other hand, give rise to a marked shift of the equilibrium. A typical experiment was as follows. Carotene and trichloroacetic acid were mixed, under concentration conditions indicated in the legend to Fig. 5, the light absorption being as shown by graph (1). If the solution, shortly after mixing of the carotene with the acid, is made 2.34M with respect to the basic solvent dioxan, the absorption curve is represented by graph (2). It will be seen that the addition of this basic solvent brings about the disappearance of the band in the near infra-red region, owing to the formation of the carotene-acid adduct, and the reappearance of the original carotene band, thereby excluding the possibility that the band at the relatively long wave-lengths is due to some irreversible chemical change. Control experiments were carried out, solutions of carotene alone and of carotene with dioxan (graphs 3 and 4) being used. Similar effects were observed with other basic solvents, namely, piperidine, acetone, and ethyl alcohol. Some equilibrium coefficients, as obtained in the absence and presence of basic solvents, are in Table 2; the K' values in lines 2—9 were estimated from eqn. (2) on the assumption that d_{∞} can be computed from the light-absorption coefficient of the carotene-trichloroacetic acid adduct, as determined in the absence of basic solvent.

TABLE 2. Influence of basic solvents on the equilibrium coefficient, K' (l./mole), characterising the interaction between carotene and trichloroacetic acid in benzene at 20—21°.

Concns. (mole/l.): carotene, 15.2×10^{-6} ; trichloroacetic acid, 1.10.					
Basic solvent and concn. (mole/l.)		K'	Basic solvent and concn. (mole/l.)		K'
None	—	1.0	Piperidine	1.31	<0.02
Dioxan	2.34	<0.02		0.660	0.04
	1.17	0.13	Acetone	2.73	0.10
	0.889	0.60	Ethyl alcohol	3.44	<0.02
	0.783	0.75			

Electrical Conductivities.—Typical results are shown in Fig. 6. The ordinate is the observed specific conductivity, at the time of mixing, corrected for the specific conductivity of the acid without the carotene. A correction for the conductivity of the carotene without acid was not necessary, because the specific conductivity of the most concentrated carotene solution was less than $10^{-11} \Omega^{-1} \text{ cm.}^{-1}$. By using the results of spectrophotometric measurements, the concentration, c' , of the carotene acid adduct can be calculated. By dividing the specific conductance by c' , the molar conductivity, Λ , of the carotene-acid adduct is obtained. The Λ values are shown in Fig. 7, together with the molar conductivity of a previously investigated benzene-soluble salt.

DISCUSSION

When the logarithm of the equilibrium constant, K , relating to the interaction of carotene with the three chloroacetic acids, is plotted against the logarithm of the dissociation constants, K_a , of these acids in water, an approximately linear relation of the Brønsted type holds, namely :

$$\log_{10} K \approx m \log_{10} K_a + n \dots \dots \dots (4)$$

where m and n are respectively 1.5 and -0.8 if K and K_a are expressed in terms of moles and litres. Such a functional relation is compatible with the supposition that carotene, owing to the presence of π -electrons in the conjugated double bonds, acts as a proton-acceptor and that the adduct C...D in eqn. (1) is a salt, the proton of the acid having been transferred to the polyene, while the acid anion is held in close proximity by electrostatic forces. A proton-transfer mechanism of eqn. (1) is consistent with the observation that the equilibrium coefficients of the relatively strong hydrochloric, dodecylbenzenesulphonic, and picric acids are larger than the K values of the chloroacetic acids (see Table 1),

although a relation similar to (4) does not appear to hold with those acids which are not chemically related to each other. Furthermore, the suggested mechanism is indicated by the fact that the formation of the carotene-trichloroacetic acid adduct is prevented by the basic solvents dioxan, piperidine, acetone, and ethyl alcohol, while no such "competitive" inhibition occurs with polar aprotic solvents.

The occurrence of a proton transfer is proved by the marked electrical conductivity of the carotene-acid adducts in benzene, control experiments having established that the conductivity of the acid alone or of carotene alone is not significant in most experiments. The graphs in Fig. 7 for the adducts show that there is an approximately linear functional relation between the logarithm of the molar electrical conductivity, Λ , and the logarithm of the concentration, c' , of the various carotene salts. For some previously investigated benzene-soluble salts a conductance minimum occurs, which is due (Fuoss and Krauss, *J. Amer. Chem. Soc.*, 1933, **55**, 2387) to the formation of triple ions. There is no evidence that such effects play a rôle in the carotene-acid adducts which are here investigated. Comparison of the graph for the ammonium salt with those for the adducts in Fig. 7 is nevertheless of some interest, because it shows that, on the whole, the Λ values of the carotene acid adducts are larger than those of the authentic salt measured in the same solvent.

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