point, analysis and spectra usually can be obtained by using one-half to one-quarter of the amounts specified.

The infrared spectra of the derivatives of the following carbonyl compounds were determined (in KBr): acetaldehyde, acetone, chloroacetone, 1-phenyl-2-propanone, 1,1-diphenyl-2-propanone, acetophenone, *p*-nitroacetophenone, methyl thienyl ketone, fluorenone, methyl cyclopropyl ketone, methyl cyclopentyl ketone, diacetone alcohol and citral. These spectra support the proposed structure (II) for the mixed azines. None show any bands in the N–H stretching region or any bands in the free (non-hydrogen bonded) carbonyl region. All of the derivatives have a broad band of very high intensity in the 6.2–6.4 μ region that can be assigned to the hydrogen bonded enolic β-diketone structure stabilized by resonance.[6] All of these mixed azines have a strong, needle sharp band at 5.95–6.01 μ which is due to the C=N bonds of the azine structure. The derivatives also have the following bonds in common: 6.88–6.92, 7.15–7.30, 8.05–8.17, 9.20–9.27, 9.43–9.70, 11.08–11.24, 12.52–12.84, 13.14–13.34, 13.60–13.94 and 14.22–14.32 μ. In addition to these there are bands that are characteristic for each derivative in the 9 to 13 μ region.

2-Diphenylacetyl-1,3-indandione 1-dimethylhydrazone could not be prepared directly from the reaction of 2-diphenylacetyl-1,3-indandione and *unsym*-dimethylhydrazine but was successfully made in the following way. A mixture of 2-diphenylacetyl-1,3-indandione 1-azine with acetone (3.88 g., 0.02 mole) and 50 ml. of anhydrous ethanol was heated at reflux for five hours. The solution changed color from bright yellow to dark orange-red. The insoluble material was filtered, washed with small portions of ethanol and dried, yielding 1.93 g. of unreacted 2-diphenylacetyl-1,3-indandione 1-azine with acetone, m.p. 226–227°. The filtrate was evaporated to one-half its volume and cooled in Dry Ice. The orange crystals were collected and washed with ether; yielding 1.60 g. of III. After recrystallization from ethanol it melted at 221.5–222.5°. The orange crystals were fluorescent. The infrared spectrum was identical with that of 2-diphenylacetyl-1,3-indandione 1-azine with acetone with the exception of the intensity of the C=N band at 5.97 μ which is about one-half as intense as the corresponding band of 2-diphenylacetyl-1,3-indandione 1-azine with acetone. This would be expected since III has only one C=N bond while the azine has two.

Anal. Calcd. for $C_{25}H_{22}N_2O_2$: N, 7.33. Found: N, 7.29.

NEWARK, DELAWARE

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(6) (a) L. J. Bellamy and L. Beecher, *J. Chem. Soc.*, 4487 (1954); (b) THIS JOURNAL, **75**, 479 (1953).

[CONTRIBUTION FROM UNIVERSITY OF MICHIGAN, ROHM & HAAS CO., AND THE PENNSYLVANIA STATE UNIVERSITY]

Effect of Structure on the Stereochemistry of Electrode Reactions. Unsaturated C_4-Dibasic Acids and Esters. Stereospecific Reduction of the Double Bond

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Acetylenedicarboxylic acid (ADCA), dibromomaleic acid, and dibromofumaric acid have been coulometrically and polarographically reduced over a range of *p*H; the reduction products were identified polarographically, and, in the case of the macroscale electrolyses, by isolation and chemical and physical examination. Dibromomaleic and dibromofumaric acids are quantitatively reduced to ADCA in a two-electron process; their diesters behave similarly. Between *p*H 0.4 and 3.0 ADCA is reduced in a three-electron process to form *rac*-α,α'-dimethylsuccinic acid; its monoester yields diethyl *rac*-α,α'-dimethylsuccinate. However, the diethyl ester of ADCA is reduced to dimethyl fumarate. The stereospecificity of the reduction of ADCA and its esters is explained as being due to steric factors inherent in the compounds rather than to interaction between the reducible compounds and the electrode. Dimethylmaleic acid (anhydride) and dimethylfumaric acid are reduced by *trans* addition of hydrogens with single 2-electron waves to yield *rac*- and *meso*-dimethylsuccinic acids, respectively. The latter apparently represent the first clearly substantiated case of a stereospecific reduction of a double bond at the dropping mercury electrode.

A previous study[1] on vicinal dibromo acids and esters showed that such compounds could undergo two-electron reduction to form the corresponding olefinic compounds and that under some conditions one geometrical isomer was produced preferentially. The present study is a continuation of this work and presents another system in which over-all stereospecific reductions occur.

Polarographic data for the acids studied—acetylenedicarboxylic (ADCA), dibromofumaric, dibromomaleic, dimethylfumaric and dimethylmaleic—and their ethyl esters have not been previously reported, except for a decomposition potential for ADCA[2a] and mention of the use of the polarographic determination of ADCA[2b] to follow its catalytic hydrogenation. Polarography of phenyl-substituted acetylenes and ethylenes[3] in neutral or basic solution showed the triple bonds to be more difficult to reduce than the corresponding double bonds. Since the acetylene derivatives yield a single polarographic wave involving 4 electrons, it was assumed that as soon as the double bond was formed on the reduction of the triple bond, it too was reduced.

Summary of Experimental Behavior

Acetylenedicarboxylic acid and a number of related dicarboxylic acids and their monoethyl and diethyl esters have been reduced over a range of *p*H at a micro mercury dropping electrode, using polarographic technique, and at a massive stirred mercury cathode; coulometric measurements were also made using the massive electrode. The reduction products were identified polarographically, and by isolation and chemical and physical examination. The experimental data are summarized in Tables I and II. The polarographic currents were diffusion-controlled.

Dimethylmaleic Acid (Anhydride)[4] and Dimethylfumaric Acid.—These acids undergo two-electron reduction at more negative potentials than maleic or fumaric acids. Melting points of the residues

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(1) P. J. Elving, I. Rosenthal and A. J. Martin, THIS JOURNAL, **77**, 5218 (1955).

(2) (a) L. Schwaer, *Collection Czechoslov. Chem. Communs.*, **7**, 326 (1935); *Chem. Listy*, **26**, 485 (1932); (b) A. L. Markman, *J. Gen. Chem. U.S.S.R.*, **24**, 67 (1954); *Zhur. Obshchei Khim.*, **24**, 65 (1954); *C. A.*, **49**, 8001 (1955).

(3) (a) H. A. Laitinen and S. Wawzonek, THIS JOURNAL, **64**, 1765 (1942); (b) S. Wawzonek, E. W. Blaha, R. Berkey and M. E. Runner, *J. Electrochem. Soc.*, **102**, 235 (1955); (c) G. J. Hoijtink, J. van Schooten, E. de Boer and W. I. Aalbersberg, *Rec. trav. chim.*, **73**, 355 (1954); G. J. Hoijtink, *ibid.*, **73**, 895 (1954); **74**, 1525 (1955).

(4) See subsequent discussion.

TABLE I

EFFECT OF *p*H ON THE POLAROGRAPHIC PROPERTIES OF UNSATURATED C_4-DIBASIC ACIDS AND RELATED COMPOUNDS[a]

Compound[b]	Wave and values[c]	*p*H (actual experimental value is usually within ±0.1 *p*H unit)									
		0.5	1.5	2.0	2.5	3.0	4.2	5.0	6.7	8.2	9.5
ADCA	$-E_{1/2}$	0.56	0.75	0.91	1.05^d	1.16^d					
	I	7.1	7.1	6.8	5.7	4.7					
MEE-ADCA	I: $-E_{1/2}$	0.56		0.71	0.80		0.91		0.98		
	I	5.0		4.8	4.8		4.0		0.7		
	II: $-E_{1/2}$								1.12	1.24	1.32
	I								3.0	3.9	3.9
DEE-ADCA	I: $-E_{1/2}$	0.45	0.48	0.52	0.57		0.69		0.73	0.78^e	$0.80^{e,f}$
	I	4.5	4.3	4.2	4.1		3.9		3.6		
	II: $-E_{1/2}$	0.55	0.63	0.67	0.75		0.88			0.99^e 1.24^e	$1.01^{e,f}$ $1.32^{e,f}$
	I	4.4	4.3	4.3	4.0		3.9				
MEE-FA	$-E_{1/2}$	0.55			0.76		1.10		1.19	1.22	
MEE-MA	$-E_{1/2}$	0.03					1.02		1.06	1.04	
DBFA	I: $-E_{1/2}$	0.37	0.39^g	0.52	0.70	0.83		1.11	1.17	1.03	1.04
	I	3.9	3.7	3.9	3.9	3.5		3.2	2.7	3.5	3.5
	II: $-E_{1/2}$	0.57	0.64^g	0.83	1.04	1.23					
	I	5.4	5.2	5.3	5.0	4.2					
DEE-DBFA	I: $-E_{1/2}$	0.24		0.24	0.24		0.23		0.23	0.25^e	
	I	3.5		3.6	3.4		3.4		3.4		
	II: $-E_{1/2}$	0.44		0.52	0.58		0.70		0.74	0.78^e	
	I	4.4		4.2	4.2		4.0		3.9		
	III: $-E_{1/2}$	0.55		0.68	0.75		0.87		0.99	1.00^e 1.32^e	
	I	4.3		4.1	4.1		4.0		3.9		
DBMA	I: $-E_{1/2}$	0.37^h		0.43	0.66		0.89		0.99	0.95	0.95^f
	I	5.4		5.5	5.1		4.4		2.7	4.0	4.0
	II: $-E_{1/2}$	0.56		0.77	1.06						
	I	6.8		7.0	6.7						
DEE-DBMA	I: $-E_{1/2}$	0.12		0.12	0.12		0.12		0.13	0.13^e	
	I	3.5		3.8	3.4		3.3		3.3		
	II: $-E_{1/2}$	0.43		0.53	0.57		0.70		0.73	0.78^e	
	I	4.4		4.1	4.0		3.9		3.9		
	III: $-E_{1/2}$	0.55		0.68	0.76		0.89		0.99	1.02^e 1.30^e	
	I	4.2		4.0	4.0		3.9		3.8		
DMFA	I: $-E_{1/2}$	0.62		0.77	0.79		0.89	0.95	1.02		
	I	3.5		3.4	3.4		3.2	2.7	1.3		
	II: $-E_{1/2}$							1.36	1.47	1.57	1.57
	I							0.3	1.7	2.5	2.5
DMMA	I: $-E_{1/2}$	0.52		0.69	0.70		0.80	0.84			
	I	3.7		3.7	3.6		1.1	0.3			
	II: $-E_{1/2}$						1.29	1.38			
	I						2.2	3.0			

[a] Temperature 25° for the acids and 2° for the esters; concentration generally 0.2 to 0.8 m*M*. Data for a number of runs at intermediate *p*H values have not been included. [b] ADCA, acetylenedicarboxylic acid; FA, fumaric acid; MA, maleic acid; DBFA, dibromofumaric acid; DBMA, dibromomaleic acid; DMFA, dimethylfumaric acid; DMMA, dimethylmaleic acid; MEE-, monoethyl ester of, DEE-, diethyl ester of. [c] $E_{1/2}$ is in volts; I is the diffusion current constant in customary units. [d] Polarographic wave was very close to the background discharge wave. [e] Rapid hydrolysis in basic media renders current values relatively meaningless. [f] *p*H was 9.0. [g] *p*H was 1.0. [h] *p*H was 0.75.

obtained on evaporating the ether extracts from coulometrically reduced solutions (HCl–KCl buffer, *p*H 1.9) proved the *racemic* and *meso* forms of α,α'-dimethylsuccinic acids to be the respective reduction products of DMMA and DMFA.

Acetylenedicarboxylic Acid; Its Mono- and Diethyl Esters.—$E_{1/2}$ values for the single ADCA wave are more negative than those of maleic or fumaric acids[5] and are strongly *p*H-dependent. The number of electrons involved in the over-all current-producing process, *n*, is estimated from I (diffusion current constant) as being 3, which value is confirmed coulometrically. Due to merging of the ADCA wave with that due to buffer-cation electrolysis, no ADCA wave was resolvable above *p*H 3. Ether extraction of a coulometrically reduced solution of 0.730 g. of ADCA in HCl–KCl buffer gave 0.447 g. of product,[6] which, after recrystallization, melted at 132°, and had a neutral equivalent of 73.8 and a molecular weight (Rast method) of 143; m.p. of the anhydride and diamide derivatives of this product (87–89° and 246°, re-

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(5) P. J. Elving and I. Rosenthal, *Anal. Chem.*, **26**, 1454 (1954).

(6) Weight of product calculated on the basis of two molecules of ADCA producing one of dimethylsuccinic acid is 0.468 g. The neutral equivalent and molecular weight of dimethylsuccinic acid are 73 and 146, respectively.

TABLE II

PRODUCTS OF ELECTROLYTIC REDUCTION OF UNSATURATED C_4-DIBASIC ACIDS AND THEIR ETHYL ESTERS AT A MASSIVE MERCURY CATHODE

		Applied potential,	*n*	Reduction product[b]	
Compound[a]	*p*H	−v.	Found	Compound[a]	Yield, %
ADCA	0.5	0.80	3.0	[c]	[c]
	2.3	1.10	3.1	[c]	[c]
MEE-ADCA	0.6	0.60	3.0	[c]	[c]
	4.2	1.10	3.0	[c]	[c]
	6.7	1.30	3.1	[c]	[c]
	8.2	1.40	2.7[d]	[c]	[c]
DEE-ADCA	0.6	0.48	2.0	DEE-FA	91[d]
	2.6	.65	2.0	DEE-FA	92[d]
	6.8	.80	1.9	DEE-FA	93[d]
DBFA	0.5	0.43	2.0	ADCA	100
	2.5	0.90	2.0	ADCA	97
	4.2	1.10	2.0	ADCA	101
	6.7	1.30	1.9	ADCA	96
	8.2	1.25	1.9	ADCA	95
DEE-DBFA	0.5	0.32	2.0	DEE-ADCA	96
	2.5	.40	2.0	DEE-ADCA	94
	6.8	.40	1.9	DEE-ADCA	94
DBMA	0.5	0.43	2.0	ADCA	98
	2.5	0.80	2.0	ADCA	100
	4.2	1.00	2.1	ADCA	95
	6.7	1.20	2.0	ADCA	96
	9.5	1.20	2.1	ADCA	100
DEE-DBMA	0.5	0.20	2.1	DEE-ADCA	97
	2.5	.30	2.0	DEE-ADCA	100
	4.2	.30	1.9	DEE-ADCA	98
	6.7	.30	1.9	DEE-ADCA	94

[a] See Table I for meaning of compound abbreviations. [b] The reduced solution was polarographed over the entire *p*H range to analyze for the product. Yield figures are estimated to be good to ±2%. [c] The reduction product was not polarographically reducible. [d] The low values are probably due to hydrolysis of the ester.

spectively) correspond very closely with those of *rac*-α,α'-dimethylsuccinic acid.[7] For the latter compound to be formed, decarboxylation and dimerization would have to occur. When the escaping gas from the coulometer cell solution in which ADCA was being reduced, which was largely the nitrogen used to exclude oxygen, was bubbled through $Ba(OH)_2$ solution, a white precipitate formed which was found to be $BaCO_3$. The yield of CO_2 approximated that expected for a loss of one CO_2 per molecule of ADCA.

Furthermore, the reduction product of dimethylmaleic acid, which had been shown to be the *rac*-α,α'-dimethylsuccinic acid, was identical to the reduction product of ADCA as shown by mixed melting point determination.

In acidic solution the monoethyl ester of ADCA gives only one polarographic wave whose *n* value is estimated from the *I* value to be 3, which is confirmed coulometrically. A large sample of the ester was reduced at 2° in HCl–KCl buffer, *p*H 1.60. The reduction product was extracted with ether, washed, and dried; its b.p. was 220° at atmospheric pressure (diethyl *rac*-dimethylsuccinate, b.p. 221–222°). This ester was hydrolyzed with sodium hydroxide; the solution was then made acidic and extracted with ether; the purified product melted at 129–130° and was *rac*-dimethylsuccinic acid.

In acidic solution the diethyl ester of ADCA gives two 2*e* polarographic waves, in contrast to the single 3*e* wave of the acid and half-ester. The $E_{1/2}$ values of the second wave closely parallel those of diethyl fumarate; coulometric examination proves that the latter is the reduction product for the first wave at all *p*H levels. In basic solution more than two polarographic waves appear due to rapid hydrolysis of the diethyl acetylenedicarboxylate to the monoester, which appears to be quite stable; the latter procedure presents a good method for preparing the monoester.

Ethyl Hydrogen Fumarate and Maleate. By partially hydrolyzing the diethyl esters of fumaric and maleic acids in basic solution, the monoethyl esters were prepared. A single polarographic wave, giving an S-shaped *p*H–$E_{1/2}$ relation, was found for each compound over the *p*H range available.

Dibromofumaric and Dibromomaleic Acids, and Their Diesters.—Below *p*H 3.3, dibromofumaric acid (DBFA) gives two polarographic waves; the second is identical to the ADCA wave. The *I* value of wave II is approximately 1.5 times that of wave I; the waves are close together, making accurate current measurement difficult. Estimation from the *I* values yields *n* values of 2 for wave I and 3 for wave II. Coulometric data confirm these *n* values and prove that the macroscale reduction produces ADCA as the first product.

Polarographic reduction of the diethyl ester of dibromofumaric acid (DEE-DBFA) produces three waves in acidic solution, the second and third of which correspond to those of diethyl acetylenedicarboxylate. In basic solution some hydrolysis occurs. Coulometric reduction showed an *n* value of 2 for the first-wave process. Consequently, the ester is successively reduced to diethyl acetylenedicarboxylate, diethyl fumarate and, finally, diethyl succinate. In no case was 100% recovery of diethyl acetylenedicarboxylate made; the loss can be attributed to hydrolysis.

Below *p*H 2.5, dibromomaleic acid (DBMA) gives two waves; the *I* value of the second is approximately 1.5 times that of the first (estimated *n* values are 2 and 3); wave II corresponds to the ADCA wave. Coulometric data prove *n* for wave I to be 2 and the reduction product to be ADCA. Polarographic reduction of the diethyl dibromomaleate produces three waves in acidic solution, the last two being coincidental with those of diethyl acetylenedicarboxylate; coulometric reduction shows *n* for the first-wave process to be 2, and the product to be the latter ester. In basic solution, hydrolysis is noted.

Reduction Paths and Mechanisms

The discussion which follows, is summarized in Fig. 1 which describes the postulated reduction mechanisms for the compounds studied and their interrelation.

Study[1] of the reduction of the α,α'-dibromosuccinates demonstrated that the configuration of reduction products could not be predicted on the

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(7) I. Heilbron, ed., "Dictionary of Organic Compounds," Vol. I, Oxford University Press, New York, N. Y., 1946, p. 955.

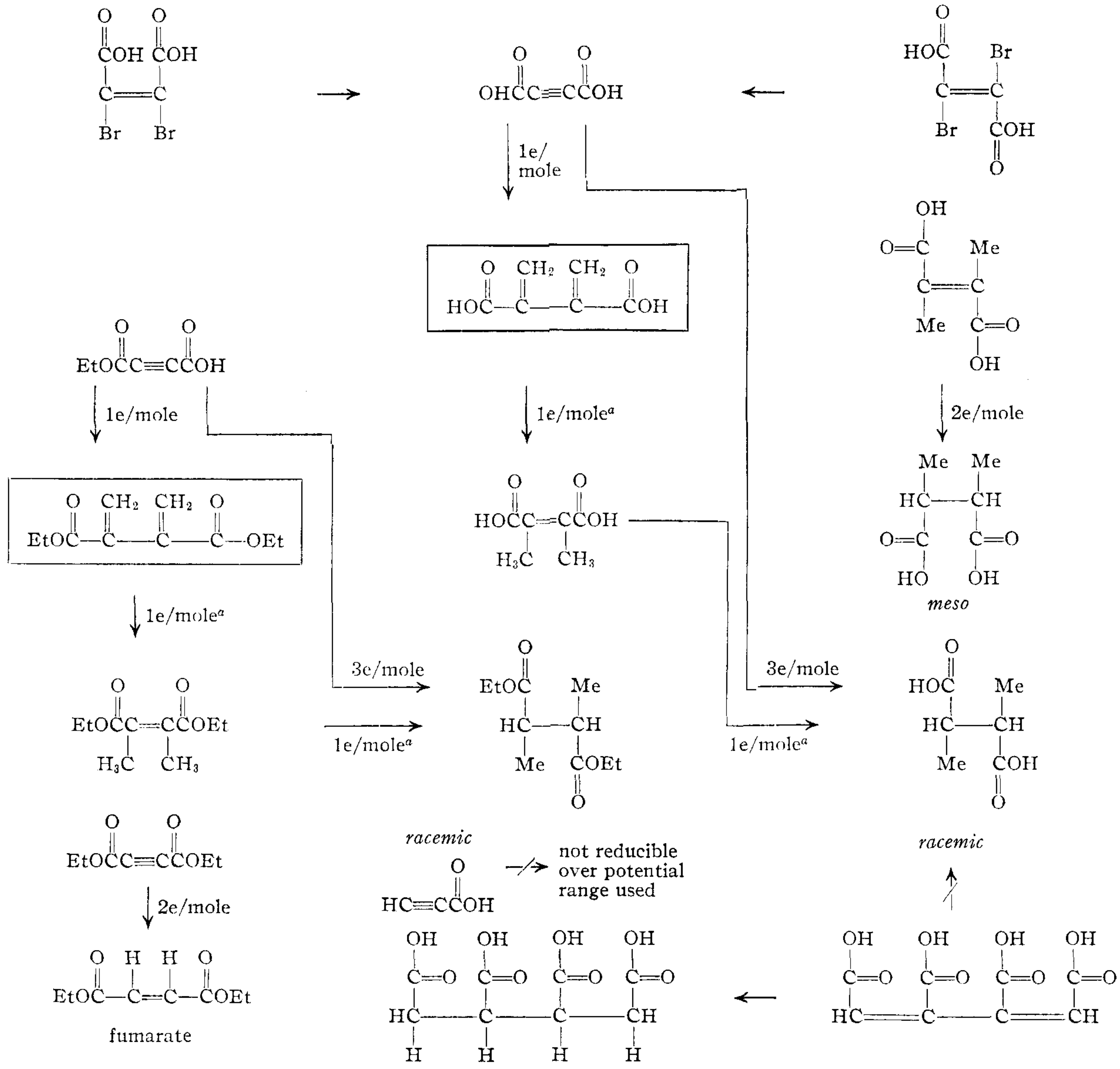

Fig. 1.—Interrelation and postulated mechanisms for the electrochemical reduction of acetylenedicarboxylic acid and related compounds. Compounds enclosed in rectangles are postulated intermediates. The superscript a indicates that the electron transfer shown is calculated on the basis of the constituent ACDA acid or ester. A crossed arrow indicates that the reaction does not proceed under normal polarographic conditions.

basis of the mechanism of outwardly analogous chemical reductions, *i.e.*, action of zinc on dibromobutanes. In addition, both the *meso* and *racemic* forms of dibromosuccinic acid yielded fumaric acid except in a limited *p*H region, where hydrogen bonding constrained the *racemic* form so that the carboxyl groups remained in a *cis* position and the reduction product was consequently maleic acid. The general mechanism for the addition of electrons to the carbon-halogen bond proposed in that study[1] needs no revision to cover the behavior of the compounds described in the present paper.

Before discussing the most interesting series of reductions, *i.e.*, that of acetylenedicarboxylic acid to yield *rac*-dimethylsuccinic acid, certain aspects of the reduction of the half- or monoesters of maleic and fumaric acids should be emphasized. Previous work[6] on the latter acids revealed the existence of a consecutive set of two S-shaped pH–$E_{1/2}$ relations for each acid. It was postulated that the upper flat branch of the first S curve corresponded to the reduction (kinetically controlled) of the half-dissociated acid and, likewise, the top of the second S curve corresponded to the *p*H-independent reduction of the completely dissociated acid. Comparison of the data for the esters (Table I) to those for the acids[5] shows that in the *p*H-independent region of $E_{1/2}$ values the half-esters have $E_{1/2}$ values very close to those for the high *p*H part of the first S-shaped curve for the corresponding acids, thus substantiating the nature of the curve as postulated.[5] The slightly lower $E_{1/2}$ values for the half-esters as compared to the acids is a general case, which has been discussed.[8]

The over-all reduction of both diethyl dibromomaleate and dibromofumarate to yield diethylsuc-

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[8] I. Rosenthal, C. S. Tang and P. J. Elving, THIS JOURNAL, **74**, 6112 (1952).

cinic acid follows the course one would have predicted, based on earlier work.[1] However, closer examination indicates an interesting point. There is not a great energy difference between the elimination electrochemically of *cis*- or *trans*-halogens with diethyl dibromomaleate and dibromofumarate. In the case of the dibromosuccinates, however, *racemic* and *meso* forms have preferred configurations giving *cis* and *trans* type orientation of the bromines. In both cases, however, reduction could take place when both forms gave a common orientation of bromine through rotation. It was, therefore, difficult to establish whether *cis* or *trans* or neither was a preferred type of reduction. With the diethyl dibromomaleate and dibromofumarate, the orientation of the bromines is fixed and there is still not a great deal of difference in reduction potentials. This is evidence against two simultaneous one-electron attacks on the two carbon–halogen bonds to lead to the formation of a double bond.

We have with ADCA and its half-ester the case of a stereospecific over-all reduction to *rac*-dimethylsuccinic acid. The evidence indicates that the decarboxylation must occur in the structure before dimerization. The evidence for this is that 1,2,3,4-tetracarboxy-1,3-butadiene (the probable product of the dimerization without decarboxylation) is stable and reducible without decarboxylation.[9] Since ADCA itself is stable in aqueous solution at 25°, the decarboxylation must occur during an electrode process. If the electrical field or the mercury interface itself could deform the molecule sufficiently to cause decarboxylation, propiolic acid could be formed, which could then go on to dimerize and to be reduced. To prove that the decarboxylation was caused during a reduction process rather than by the effect of an electrical field, propiolic acid was prepared[10] and purified. Its failure to give a polarographic wave in any of the buffers used proves that propiolic acid is not a stable intermediate in any step of the reduction of ADCA. The extent of dimerization is strong but not conclusive evidence for the formation of a relatively stable free radical during the decarboxylation step.

In attempting to arrive at some knowledge of the intermediate steps in the ADCA reduction, we run into difficulty. Dimethylmaleic anhydride in acid solution reduces to yield the *rac*-dimethylsuccinic acid as does ADCA; dimethylfumaric acid gives the *meso*-acid. This points to the maleic acid as an intermediate. Dimethylmaleic acid is isolatable only as the anhydride[11] (as is the case with fulgenic acid[12]) and, indeed, when acidified with strong mineral acid in solution, comes out directly as the anhydride. There is then some question as to whether in the conditions used for coulometry we were not in fact dealing with the anhydride, *i.e.*, the postulated fulgenic acid would exist as the anhydride and, due to the anhydride ring, maintain its configuration and be reduced to dimethylmaleic anhydride. The latter, in turn, would be reduced as such to the dimethylsuccinic anhydride which, being less stable, could convert to the acid. The same process cannot apply in an obvious way to the reduction of the half-ester of acetylenedicarboxylic acid to yield the diester of *rac*-dimethylsuccinic acid. However, this predominant tendency to form anhydrides may also indicate a general interaction between carboxyl groups that persists in some form even with the esters.

The detailed mechanism of reduction and dicarboxylation for acetylenedicarboxylic acid is complex. The conversion of the starting acid to the intermediate fulgenic acid involves the loss of CO_2, and the addition of two H^+ ions (one of which is formally the original carboxyl group hydrogen) and one electron per mole. The nature of the activated state cannot be defined with the evidence on hand. However, comparison of acid, monoester and diester shows that the second carboxyl group of the acid plays no direct role in the decarboxylation. A possible path would be addition of one electron and one hydrogen ion to form the free radical, $HOOC\text{-}\underset{\cdot}{C}{=}CHCOOH$, which could then undergo the dicarboxylation often encountered with such structures to yield the free radical, $HOOC\underset{\cdot}{C}{=}CH_2$; dimerization of the latter would produce fulgenic acid.

The reduction of dimethylmaleic and dimethylfumaric acids to form *rac*- and *meso*-α,α'-dimethylsuccinic acids, respectively, is direct evidence that the reduction of double bonds at the electrode proceeds through *trans* addition. This appears to be the first experimental evidence on this point.

In this connection, diethyl acetylenedicarboxylate also undergoes *trans* addition to yield diethyl fumarate. Examination of $E_{1/2}$ *vs.* pH variation is difficult here because of the closeness of the two waves. The average shift in $E_{1/2}$ with pH is higher than 0.059 v. per pH unit but less than two times that value. Again data at high pH are suspect because of the possibility of hydrolysis.

Comparison of Coulometric (Macroscale Electrolytic) and Polarographic Reduction.—It is significant that whenever positive identification of polarographic reduction products formed in the present investigation could be made, the polarographic and coulometric reduction processes resulted in the same products, formed by processes involving the same number of electrons and, as far as can be ascertained, by the same mechanism. However, a cautioning factor should be mentioned. Due to the instability of the esters of ADCA and the merging of the reduction wave of the ADCA itself with the background discharge at relatively low pH values, the coulometric runs were performed at low pH values. The reduction products could be different at higher pH values, where the carboxyl groups are highly dissociated.

Experimental

Chemicals.—The monopotassium salt of ADCA (Eastman Kodak Co.) was converted to the acid by extraction from acidic solution with ether. After recrystallization from water, melting point and polarographic analysis showed it to be pure. The diethyl ester of ADCA was prepared by treating the monopotassium salt with absolute

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[9] I. Rosenthal, A. J. Martin, J. R. Hayes and P. J. Elving, unpublished work.

[10] W. H. Perkin and J. L. Simsonsen, *J. Chem. Soc.*, **91**, 834 (1907).

[11] E. H. Rodd, "Chemistry of Carbon Compounds," Vol. 1B, Elsevier Press, Houston, Texas, 1952, p. 1000.

[12] Reference 11, p. 1009.

alcohol in the presence of sulfuric acid.[13] Fractional distillation under vacuum yielded the pure ester. Preparation of the monoethyl ester of ADCA could not be found in the literature; the method used, which has been mentioned, involved partial hydrolysis of the diester. The usual procedure of preparing diethyl acetylenedicarboxylate was employed. After refluxing the potassium salt of ADCA in alcohol, the resulting solution was decanted, diluted with an equal volume of water, cooled and made slightly basic to phenolphthalein with cold 15% NaOH solution. Saponification of the first ethyl group is extremely rapid and, as soon as a lasting red color appears, the hydrolysis is stopped by making the solution acidic again with sulfuric acid. The saponification can be followed polarographically (see data on ADCA and its esters). Ether extraction removes the ester from water and inorganic materials; the ether solution is washed with water, dried and evaporated on a steam-bath. The residue can be distilled in vacuum; the ester distils at 134–135° at 6 mm.

Dibromofumaric acid, prepared by the controlled addition of bromine to ADCA,[14] was recrystallized twice from water; its purity was shown by melting point and polarographic analysis. Its diethyl ester was prepared by treating the silver salt with excess ethyl bromide[15]; fractional freezing followed by recrystallization gave the pure solid ester. The anhydride of dibromomaleic acid (Metro Chemical Co.) was purified by sublimation; the diethyl ester, prepared by treating this anhydride with absolute alcohol containing dry HCl,[16] was purified by vacuum distillation.

The anhydride of α,α'-dimethylmaleic acid was prepared[17] and purified by sublimation. The *trans*-dimethylfumaric acid, prepared by heating the *cis*-acid with HCl, was purified by ether extraction and water recrystallization.

Buffers were prepared as previously described[1]: HCl–KCl, *p*H 0.4 to 2.0; Na_2HPO_4–citric acid, *p*H 2.0 to 7.0; NH_3–NH_4Cl, *p*H 8.2 to 9.7.

Apparatus.—A Sargent Model XXI polarograph was used in conjunction with a potentiometer. A Beckman Model G *p*H meter was used for *p*H measurement. The coulometric apparatus has been described.[1]

Procedures.—The polarographic and coulometric procedures were the same as those previously reported.[1] The acids were studied at 25 ± 0.1°. The esters were studied at 2 ± 0.5°; the test solutions had 4 volume % of alcohol to ensure solubility of the esters. This concentration of alcohol seemed to have no effect on the polarographic results.

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Electrokinetic Changes in the Starch Medium During Zone Electrophoresis

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The present investigation was undertaken in order to determine the factors influencing the determination of mobilities on starch. It was found that marked changes took place in the conductivity as well as the *p*H of the buffer in the starch medium, which in turn caused changes in the electrical quantities. These changes can be accounted for by the ion-exchange properties of starch and can for the most part be avoided by washing the starch with buffer before the experiment. It was found that the most reproducible mobility measurements were obtained when the field strength was taken as V/l, and it is suggested that corrections for the higher ionic strength in the solid medium be made if comparison with mobilities in free solution is desired.

The introduction of a supporting medium in zone electrophoresis has created many electrokinetic problems which are not encountered with electrophoresis in free solution. Some of these problems have been discussed in connection with paper electrophoresis[1–4] but have received only scant attention in connection with supporting media other than paper.[5–8] It is the purpose of this report to present data on some of the changes that occur during zone electrophoresis when starch is used as the supporting medium[9] and to elucidate some of the observed effects in terms of the ion-exchange properties of starch.

Apparatus and Materials

The electrode vessels are made of plastic (Perspex) and separated into several compartments by means of baffles. Each vessel measures 17 × 17 × 17.5 cm. and holds 2 l. of buffer. The rear compartment contains a well filled with KCl into which silver–silver chloride electrodes are dipped. In the front compartment electrical contact is made by pressing the ends of the trough against a thick wad of filter paper saturated with buffer. A connection of rubber tubing between the vessels ensures hydrostatic equilibrium. A more complete description of the apparatus can be found elsewhere.[15]

Commercial potato starch, trademark "Swan," was used for all reported experiments. This starch contained 15.7% moisture; when dissolved in distilled water it gave a *p*H of 7.00. The starch was packed into semicylindrical glass troughs with open ends, previously described by Fønss-Bech and Li.[10] Three sizes of troughs were used, measuring 3.8 × 1.8 × 40 cm., 3.6 × 1.7 × 60 cm. and 1.5 × 0.7 × 40 cm., respectively.

The compositions of the buffers most commonly used are given in Table I.

Procedure

The starch is mixed with enough buffer to give a viscous paste which is then poured into the troughs. The escape

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