TABLE I

SOLUBILITY OF CARBON DIOXIDE IN ACID SOLUTIONS AT 25°

H_2SO_4, m	Mole %	α	S	$S_{calcd.}$
0.0	0	0.7565	0.7587	
0.5	0.8928	.6983	.7127	0.7183
1.0	1.7697	.6650	.6911	.6911
2.0	3.4779	.6132	.6610	.6642
3.0	5.1277	.5854	.6546	.6619
4.0	6.7220	.5740	.6659	.6757
6.0	9.7551	.5878	.7332	.7332
8.0	12.597	.6159	.8238	.8154
10.0	15.266	.6337	.9053	.9116
14.15	20.32	.6404	1.0372	1.1372
18.86	25.37	.6225	1.1453	
28.29	33.77	.5840	1.3386	
37.72	40.48	.5659	1.5573	
56.58	50.49	.5741	2.1232	
94.30	62.95	.645		
188.6	77.26	.753		
282.9	83.60	.813		
565.8	91.07	.880		
1131.6	95.32	.920		
	100	.960		
$HClO_4$				
0.0	0.0000	0.7565	0.7587	
.25	.4484	.753	.764	0.771
.50	.8928	.759	.778	.783
.75	1.3332	.765	.793	.796
1.00	1.7697	.772	.809	.809
1.50	2.6313	.785	.840	.835
2.00	3.4779	.798	.865	.861
4.00	6.7220	.835	.984	.973
6.00	9.7551	.863	1.091	1.091
10.00	15.266	.866	1.239	1.343
15.47	21.79	.762	1.264	
22.84	29.15	.718	1.426	

Constants for Equation 1

	a	b
H_2SO_4	0.0885	0.2159
$HClO_4$	.107	.04284

has been plotted against the weight and mole per cent. of acid in Fig. 1.

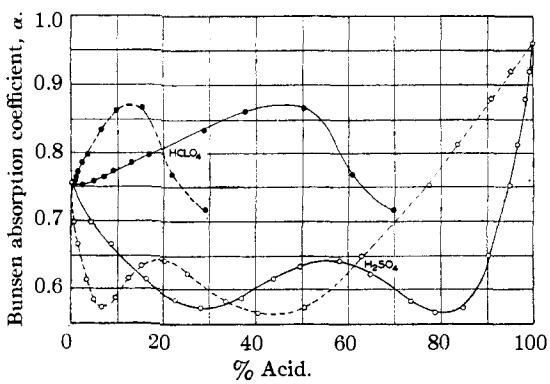

Fig. 1.—Bunsen coefficient for carbon dioxide in solutions of sulfuric and perchloric acids at 25°: ——, weight per cent.; – – –, mole per cent.

The solubility of carbon dioxide in concentrations of perchloric acid up to six molal, and in sulfuric acid up to ten molal, fits equation 1 with a maximum error of about 1%. The values calculated are given in Table I as $S_{calcd.}$. From the curves it is seen that the equation no longer fits the data after the solubility passes through the first maximum point, though the first minimum point in the sulfuric acid solution is fitted well by the calculated values. The maxima and minima in the gas solubility curves do not correspond to simple compounds of the acid with water.

Equation 1 represents a hyperbola which goes to positive infinity at $-1/b$ and becomes tangent to the line S_0am at the other extremity; the equation can account only for a minimum, as with sulfuric acid, but not for a maximum, in any actual isotherm.

DEPARTMENT OF CHEMICAL ENGINEERING
UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON RECEIVED DECEMBER 10, 1940

Recovery of the Cottonseed Allergenic Protein from its Picrate by Electrophoresis

BY JOSEPH R. SPIES

In a recent communication a chemical method for recovering the cottonseed allergenic protein CS-13A from its picrate was described.[1]

Incidental to a large scale electrophoretic separation of the allergenic fraction CS-1A it was found that the protein CS-13A could be recovered from its picrate by high voltage electrophoresis.[2,3,4] Advantage was taken of the fact that the protein picrate was soluble in 50% dioxane, in which the freed protein was insoluble. In this solution picric acid migrated toward the anode cell and the protein moved to the cathode cell where it precipitated.

Experimental

The electrophoresis apparatus used for the separation consisted of a series of six cells made from 125 ml. Erlenmeyer flasks with 10 mm. side tubes sealed on 25 mm. above the bottom. Cells were joined by 25 mm. lengths of heavy walled gum rubber tubing. Temperature was

(1) Spies, Coulson, Bernton and Stevens, THIS JOURNAL, **62**, 1420 (1940).

(2) The theory and an application of high-voltage electrophoresis have been described by R. J. Williams and J. H. Truesdail, *ibid.*, **53**, 4171 (1931). See also later papers by Williams and co-workers.

(3) V. du Vigneaud, G. W. Irving, H. Dyer and R. R. Sealock, *J. Biol. Chem.*, **123**, 45 (1938), have used high voltage electrophoresis in fractionating the posterior pituitary hormone.

(4) E. Gebauer-Fuelnegg and A. I. Kendall, *Ber.*, **64**, 1070 (1931), separated the strongly basic histamine from the dipicrate by electrodialysis using direct current at 110 v.

maintained below 35° by circulating water through a copper trough holding the apparatus.

To carry out the recovery of the protein, 1 g. of protein picrate (CS-5-7)[5] dissolved in 100 ml. of 50% dioxane, was placed in the third cell from the cathode and 100 ml. of 50% dioxane was placed in each of the other cells. Direct current at 1500–2500 volts was applied at the platinum electrodes in terminal cells for four hours, and the voltage was then increased to 4000 volts for sixty-eight hours. The initial current of 1.1 milliamperes gradually increased to 6.0 and then dropped to 2.9 where it remained constant. At the end of this operation the cathode cell contained a gummy precipitate and nearly colorless solution. The other cells contained picric acid as indicated by the yellow color which was progressively more intense toward the anode cell. The supernatant liquid in the cathode cell was decanted and the precipitate was dissolved in 30 ml. of water. This solution, decolorized by boiling with activated charcoal, was centrifuged and filtered through a Seitz sterilizing pad. The clear colorless filtrate was poured into 100 ml. of cold ethanol and precipitated by adjusting the *p*H to 6.3 with dilute acetic acid. A yield of 180 mg. of a white powder was recovered by centrifuging followed by drying in a vacuum over phosphorus pentoxide. The recovered solid contained 19.8% (ash-water free basis) nitrogen and gave protein color tests like CS-13A. The solid diluted to 1:10^6 induced strongly positive cutaneous reactions on cottonseed sensitive patients.

(5) Spies, Bernton and Stevens, THIS JOURNAL, **62**, 2793 (1940).

ALLERGEN INVESTIGATIONS
BUREAU OF AGRICULTURAL CHEMISTRY AND ENGINEERING
U. S. DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C. RECEIVED JANUARY 24, 1941

COMMUNICATIONS TO THE EDITOR

ULTRAVIOLET ABSORPTION OF THE DIPHENIC ACIDS

Sir:

Recent studies have emphasized the fact that absorption in the ultraviolet is a measure of resonance or conjugation and that structure is indicated only incidentally as it contributes to this phenomenon. Recently in this Laboratory the absorption curves have been obtained for ortho-ortho′-, meta-meta′- and para-para′-diphenic acids. It is interesting to report the radically different behavior of these three compounds in respect to their ultraviolet absorption.

The *o-o′*-diphenic acid shows an absorption which differs but little from that of benzoic acid. Evidently the carboxyl groups in the *o*-position interfere to such an extent that the coplanar position is impossible and resonance between the rings completely disappears.

On the other hand, the *p-p′*-diphenic acid (in this case the methyl ester) shows a very great absorption with the maximum near 2800 Å. Here we have exact coplanarity with complete resonance throughout.

By far the most interesting of these compounds, however, is the *m-m′*-diphenic acid. While the absorption is much greater than that of the *o-o′*-compound, it is much less than that of the *p-p′*-acid and the maximum is shifted toward shorter wave lengths so that it lies beyond the range of the medium quartz spectrograph. It is clear that it is not possible for a structure to exist which involves double bonds between the rings and between the ring and the carbon of the carboxyl group at the same time and this is the important structure for total resonance. Hence competition between these structures results in a decreased amount of conjugation.

It is interesting to note that a similar situation arises in the case of diphenylmethane. *p*-Cresol shows a marked increase in absorption and a shift of the maximum toward longer wave lengths. On the other hand, *p*-hydroxydiphenylmethane shows little difference from the diphenylmethane. This is not difficult to understand when we recognize that the principal resonance structure which contributes to the absorption in diphenylmethane is one in which the *p*-carbon acquires a negative charge. This structure does not involve conjugation with the hydroxyl group.

The details of this research will be published later but meanwhile we wish to acknowledge our indebtedness to Dr. N. Kornblum and Messrs. L. Brooks and J. C. Robinson for the preparation of the several acids.

NOYES CHEMICAL LABORATORY
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

E. C. DUNLOP
BARBARA WILLIAMSON
W. H. RODEBUSH
A. M. BUSWELL

RECEIVED MARCH 6, 1941