in the availability of the various tyrosine groups in the different proteins, as observed in the cases of pepsin and albumin by Li.[16]

Since, at least in the case of human proteins,[19] the albumin contains less tyrosine, 4.6%, than does the γ-globulin, 6.7%, we would expect the γ-globulin to iodinate more rapidly on the basis of the tyrosine concentration alone. However, this is not the case here, since antibody iodinates more slowly than the albumin.

These differences in rates of iodination are especially of significance in connection with studies on antitissue sera.[5] It has been shown that when the radioiodinated globulin fraction of antiserum prepared against rat kidney was injected into rats, radioiodine localized in the kidney to the extent of about 1% of the radioactivity in the injected material. The localized radioactivity was apparently attached to antibody specific to rat kidney. Since the amount of antibody localized was taken to be equivalent to the amount of protein associated in the globulin fraction with the amount of iodine localized, we must now revise the figure by a factor of 2, to take into account the difference of iodination—thus: 2% of the injected protein was antibody which localized in the kidney.

The Effect of Iodination on Antibody Activity.—The decrease in precipitability of antibodies observed here was due to the iodination of the antibody and the oxidation of the antibody with either a decrease in the combining constant for the antigen or an increase in the solubility of the antigen–antibody complex. It is more probable that a decrease in combining constant was the reason for the reduction in the amount of precipitate. It is reasonable that any iodination or oxidation in the specific region would greatly affect the combination of antigen and antibody, while alteration elsewhere in the molecule would affect the combination but slightly.

Acknowledgment.—We wish to thank Miss B. Sherman and Messrs. R. Clark, R. Medina and A. Silverstein for assistance in this study.

Summary

It was shown that, by immunochemical methods, it is possible to determine relative rates of iodination of proteins in a mixture of proteins. Thus antibody to ovalbumin iodinates at one-third the rate of the proteins of whole serum and at one-half the rate of the proteins of the globulin fraction separated at 1.75 molar ammonium sulfate. The globulin fraction iodinated at one-half the rate of the albumin fraction. Antibody to beef serum albumin and antibody to ovalbumin iodinate at the same rate. The introduction of even 18 iodine atoms per antibody molecule does not destroy its ability to undergo specific precipitation.

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[CONTRIBUTION NO. 741 FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

Electric Moments and Structure of Substituted Thiophenes. III. Polysubstituted Thiophenes[1,2,3]

BY ROBERT G. CHARLES AND HENRY FREISER

The work described herein is an extension of a study of the effect of substitution on the character of the thiophene nucleus. Measurements described in the previous papers of this series have indicated the likelihood of resonance interaction of some substituents in the 2-position and the thiophene nucleus. A possible or partial explanation of the results was advanced which involved a decreased participation of the sulfur electrons in the thiophene nucleus resonance. Accordingly, compounds were chosen for study which might exhibit this change in the role of sulfur in the thiophene ring resonance to a more marked degree. Measurements were made of the dielectric constants, refractive indices and densities of benzene solutions of benzothiophene, dibenzothiophene, 2,5-dimethylthiophene, tetrachlorothiophene and tetraphenylthiophene at 30°. The dielectric polarization of dinaphthylenethiophene at 30° was determined from measurements in *p*-xylene solutions, since this compound was not sufficiently soluble in benzene. The moment values are of some significance in explaining certain aspects of thiophene chemistry.

Experimental

Purification of Materials.—Jones and Laughlin reagent-grade benzene was used after purification *by careful fractionation* as described previously.[4] Paragon *p*-xylene was recrystallized and fractionally distilled to give a product that boiled at 136.4–137.6° at 733 mm. and had a melting point of 13.0°, d^{30}_4 0.852116 g./ml. Its dielectric constant was determined at 30° by comparison with that of benzene and air and found to be 2.2497.

The 2,5-dimethylthiophene was prepared by ring closure

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(1) For Paper II, see Keswani and Freiser, THIS JOURNAL, **71**, 1789 (1949).

(2) This work is abstracted from a portion of the thesis to be submitted by Robert G. Charles to the University of Pittsburgh in partial fulfillment of the requirements for the Master of Science degree.

(3) This paper was presented before the Physical and Inorganic Division at the Atlantic City A. C. S. convention, September 22, 1949.

(4) Keswani and Freiser, THIS JOURNAL, **71**, 218 (1949).

of acetonylacetone with phosphorus pentasulfide.[5] The crude product was freed of hydrogen sulfide by shaking exhaustively with dilute sodium hydroxide until a negative test was obtained with lead acetate paper. The compound was then washed, dried and finally purified by fractional distillation; b. p. 134.9–135.2° at 737.2 mm.

Benzothiophene was kindly supplied by both the Jefferson Chemical Company and the Koppers Chemical Company and was recrystallized to constant melting point at 31.0°.

Dibenzothiophene was prepared by fusion of diphenyl with sulfur[6] and was purified by fractional crystallization and distillation; b. p. 133–136° at 2 mm., m. p. 99.5–100.0°.

Dinaphthylenethiophene was prepared by fusion of acenaphthene with sulfur,[7] and was also purified by recrystallization, m. p. 276–278°.

A sample of tetrachlorothiophene was kindly sent by the Socony-Vacuum Oil Company. It was purified by fractional distillation followed by fractional crystallization; b. p. 96.6–97.5° at 7 mm., m. p. 28.82°.

Tetraphenylthiophene was prepared by heating phenylacetic acid with sulfur[8] and purified by recrystallization, m. p. 188.0–188.5°.

Measurements and Calculations.—The procedure has been described previously.[4] The results are summarized in Table I where the dielectric constants, ϵ, and the densities, d, of benzene (p-xylene in the case of dinaphthylenethiophene) solutions containing mole fraction, f_2 of the substance indicated are given along with the molar polarization and molar refraction. Unless otherwise indicated, the molar refraction was obtained from solution measurements. The table also includes the polarization, P, obtained by extrapolation to $f_2 = 0$ of the P_2 values and the dipole moment, μ, calculated therefrom in the usual manner.

TABLE I

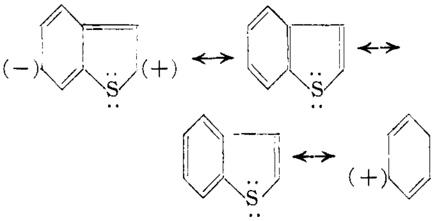
DIELECTRIC POLARIZATION DATA AT 30°

f_2	ϵ	d	P_2	MR_D	P_∞	$\mu \times 10^{18}$
			Benzothiophene			
.000000	2.2627	0.86823				
.02372	2.2881	.87736	49.81	41.82	49.6	0.62
.03146	2.2956	.88034	49.42	41.85		
.04485	2.3110	.88556	49.78	41.81		
.06005	2.3256	.89127	49.49	41.88		
			Dibenzothiophene			
.01291	2.2871	.87600	72.41		73.7	.83
.01908	2.2983	.87960	72.17			
.02692	2.3129	.88477	71.51	59.81		
.03327	2.3223	.88800	71.07	59.70		
.04398	2.3390	.89437	70.14	59.66		
			2,5-Dimethylthiophene			
.02155	2.2694	.87108	38.9	34.2^a	39.5	.51
.02714	2.2710	.87185	38.7			
.04437	2.2745	.87402	38.3			
			Tetrachlorothiophene			
.01594	2.2870	.88677	62.41	44.05^a	61.39	.93
.03229	2.3103	.90578	61.45			
.04453	2.3294	.92029	61.45			
.07031	2.3661	.94955	61.28			
			Tetraphenylthiophene			
.003310	2.2730	.87186	145.3	135.5	141.6	.60
.004664	2.2753	.87332	139.1	134.4		
.006284	2.2816	.87504	144.1	134.6		
.007911	2.2836	.87669	138.9	133.0		
.008523	2.2862	.87734	140.6	134.0		
			Dinaphthylenethiophene			
.00000	2.2497	.85212				
.0007363	2.2521	.85309	122.2	106.4^b	119.7	.8
.0009134	2.2524	.85335	117.3			

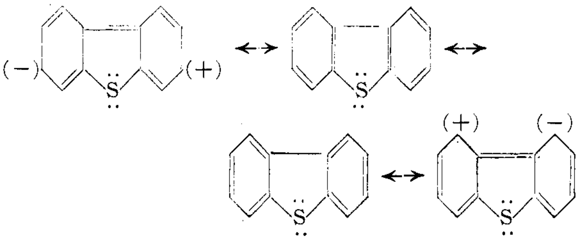
[a] Calculated from measurements of pure liquid solute.
[b] Computed from bond refraction data.

Discussion

The increase of the moments of benzothiophene and dibenzothiophene over that of thiophene itself cannot be attributed to inductive effects but must be an indication of resonance interaction between the nuclei of these polynuclear compounds. The increase can be ascribed to the increasing significance of resonance structures used to describe the ground states of thiophene, benzothiophene and dibenzothiophene which do not involve the unshared pairs of electrons of the sulfur atom, such as



and



If the sulfur electrons are indeed less involved in the ring resonance than in thiophene, then the moment of the S should increase as it does from 0.53 D in thiophene to 1.97 in thiophane (tetrahydrothiophene) to some intermediate value. A comparison between these polynuclear thiophene derivatives and thiophane seems profitable. Thiophene with two –C═C– bonds and thiophane with these bonds completely saturated may be considered as two extremes. In benzothiophene, one of the –C═C– bonds of thiophene changes from a double bond to one of somewhat lower order. Since in dibenzothiophene both such bonds are similarly involved, its resemblance to thiophane is even more marked than that of benzothiophene. The observed increase in the moments may not indicate the full significance of the decreasing part played by the sulfur electrons since structures in which these electrons participate have a greater charge separation in the polynuclear derivatives than in thiophene and hence a larger effect on the over-all moment.

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(5) Paal, *Ber.*, **18**, 2251 (1885).

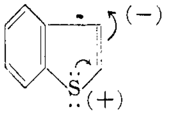
(6) Gilman and Jacoby, *J. Org. Chem.*, **3**, 111 (1938).

(7) Dziewonski and Bachmann, *Ber.*, **36**, 965 (1903).

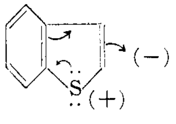
(8) Ziegler, *ibid.*, **23**, 2473 (1890).

This interpretation is strengthened by chemical evidence. Thiophane will readily form a sulfone while thiophene does not, because the unshared electron pairs of the thiophene sulfur, involved in the nuclear resonance, are not available for the coördination of oxygen. It is significant therefore that benzothiophene and dibenzothiophene both form sulfones, attesting the greater availability of the sulfur electron pairs in these compounds than in thiophene.

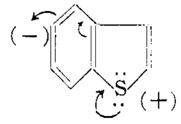
The fact that the moment increase of these compounds over the thiophene moment, while real, is only a small fraction of the increase observed in thiophane is of significance. This indicates that structures in which the sulfur electrons are involved in the resonance are still very important in these polynuclear derivatives and helps to explain orientation in benzothiophene. Benzothiophene is less active than thiophene and substituents enter predominantly in the β-position (thiophene substituents go to the α-position). Structures such as I and IIa are similar to those in thiophene with IIa being more important for the description of thiophene and responsible for α-orientation. In benzothiophene, while the structure making β-orientation possible (I) is not changed, structure IIb making IIa less important results in a lowered electron concentration in the hetero-ring at the expense of the α-carbon. On this basis, one can predict that the benzene portion of the molecule, being electron-enriched should be activated. Dibenzothiophene should be more easily substituted than benzene.



I

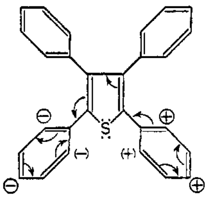


IIa

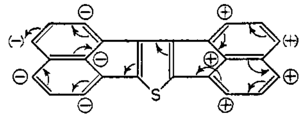


IIb

These considerations lead one to the search for mononuclear thiophene derivatives which are capable of sulfone formation. The main requirement of such a derivative is that the substituents be of a nature permitting resonance with the thiophene nucleus without involving the unshared electron pairs of sulfur. Tetraphenylthiophene meets these requirements. Its moment is greater than that of thiophene and it is known to form a sulfone. The resonance forms in which the sulfur electrons do not participate may be more significant than indicated by the small increase of the tetraphenylthiophene moment since there is a great increase in charge separation in those sulfur-participating structures resulting in a greater effect upon the moment in the opposite direction. This is particularly true for the structures involving the phenyl groups in the 3 and 4 positions as the bonding angle of substituents in these positions is closer to the vertical.[4] In this regard, dinaphthylenethiophene might be expected to show a higher moment than the tetraphenylthiophene since the phenyl groups are fused together as it were. This not only decreases to some extent the large charge separation possible in the tetraphenyl derivatives but the number of resonance structures not involving the sulfur electrons increases significantly as indicated by



9 possibilities



25 possibilities

The moment of 2,5-dimethylthiophene has been found to be larger than that calculated on the basis of vector addition of group moments by 0.11 *D*. This is similar to what was found in the case of 2-methylthiophene[1] and suggests that both of these substances might be capable of sulfone formation.

The moment of tetrachlorothiophene, like that of the tetrabromo derivative,[4] is considerably lower than the calculated value (1.50 *D*) which is due to the neutral inductive effects of the four adjacent chlorine atoms. It is noteworthy that the negative deviation is significantly less than that observed in the tetrabromothiophene which lends weight to the possibility of greater resonance interaction between chlorine and the thiophene nucleus previously postulated.[4]

Summary

1. Dielectric constants, densities and refractions at 30° are reported for benzene solutions of benzothiophene, dibenzothiophene, 2,5-dimethylthiophene, tetrachlorothiophene and of tetraphenylthiophene.

2. The dielectric constants and densities at 30° are reported for *p*-xylene and solutions of dinaphthylenethiophene in *p*-xylene.

3. The dipole moments for the thiophene derivatives, calculated by use of the Debye equation, have been found to be 0.62, 0.83, 0.51, 0.93, 0.60 and 0.8, respectively.

4. The dipole moment values are discussed in terms of the light shed on various aspects of thiophene chemistry.

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