seemed best not to use this procedure, for the question of denaturation of the protein in basic solution might be raised. As an alternative a number of ultrafiltration experiments was carried out. Two of these are summarized in Table II. In both cases, the ultrafiltrate in the presence of albumin had practically the same concentration of methyl orange as that obtained from the control tube containing no protein. Clearly no significant binding of methyl orange by bovine serum albumin occurs at *p*H's near 12.

TABLE II

ULTRAFILTRATION OF METHYL ORANGE–ALBUMIN SOLUTIONS

Expt.	*p*H	Conc. of albumin grams/liter	Concn. of M.O. in filtrate
1	11.73	..	2.04×10^{-5}
	11.85	1.90	2.09×10^{-5}
2	11.82	..	5.75×10^{-5}
	11.84	1.89	5.73×10^{-5}

These results indicate that insofar as binding of sulfonated compounds is concerned, the protein undergoes a critical, but reversible, change in the region near *p*H 10. Inspection of Table I reveals that this is also the region in which the ε-ammonium group of lysine is losing an H^+ ion, and, therefore, its positive charge. One is thus led to the belief that the stabilization of the protein–dye complex is strongly dependent on the electrostatic interaction with the cationic nitrogen of lysine. It is not clear, however, why lysine should occupy this special position. From the observation[2] that salmine gives a precipitate with either methyl orange or azosulfathiazole, it is evident that the guanidinium group is also capable of strong interaction with sulfonate anions, since this protamine contains over 87% arginine. Consequently, since an appreciable fraction of the guanidinium groups in albumin should still be in the cationic state at a *p*H near 11.5, one would expect some binding of the dye. The absence of spectral alterations at this *p*H must indicate that the arginine residues are in a relatively inaccessible position in the serum albumin molecule or that the guanidinium cations are bound to anionic groups within the protein.

Acknowledgment.—These investigations were supported by grants from the Permanent Science fund of the American Academy of Arts and Sciences and from the Office of Naval Research.

Summary

Investigations have been made of the binding of a triply-charged sulfonated dye (F D and C Red No. 2) by bovine serum albumin. The extent of binding can be correlated in terms of equations derived from the law of mass action.

Spectral methods and ultrafiltration experiments have been used to study the effect of *p*H on protein–anion interactions. Binding phenomena disappear near *p*H 12. Such behavior indicates that the ammonium group of lysine is strongly involved in these interactions.

EVANSTON, ILLINOIS RECEIVED FEBRUARY 20, 1947

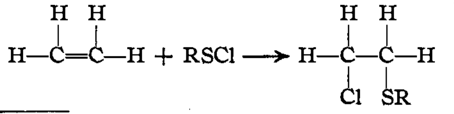
[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF NORTHWESTERN UNIVERSITY]

Derivatives of Sulfenic Acids. I. Reactions of 2-Nitrobenzenesulfenyl Chloride, 2,4-Dinitrobenzenesulfenyl Chloride and of the Corresponding Sulfenyl Thiocyanates with Olefins and with Methyl Ketones

BY NORMAN KHARASCH,[1] HERBERT L. WEHRMEISTER AND HENRY TIGERMAN

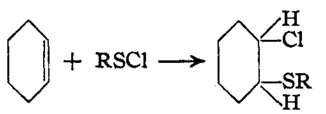
We have recently undertaken a series of studies concerning derivatives of sulfenic acids.[2] This paper describes certain new reactions of the 2-nitro- and 2,4-dinitrobenzenesulfenyl chlorides and thiocyanates.

Lecher and Stöcklin[3] reported three instances of the addition of sulfenyl chlorides to ethylene (equation (a), R = phenyl, *p*-tolyl or 2-nitrophenyl).



$$\mathrm{H{-}\overset{H}{C}{=}\overset{H}{C}{-}H + RSCl \longrightarrow H{-}\underset{Cl}{\overset{H}{C}}{-}\underset{SR}{\overset{H}{C}}{-}H} \qquad (a)$$

Fuson, Price and co-workers[3a] similarly added 2-chloroethanesulfenyl chloride to ethylene, cyclohexene and propylene. New examples of this interesting reaction were found in this study. Thus, 2-nitrobenzenesulfenyl chloride and 2,4-dinitrobenzenesulfenyl chloride add to cyclohexene to give corresponding 2-chlorocyclohexyl aryl sulfides (equation (b), R = 2-nitrophenyl or 2,4-dinitrophenyl).



(b)

Only single modifications of the latter products were obtained, although geometric isomers are possible.

Reactions analogous to a and b, wherein sul-

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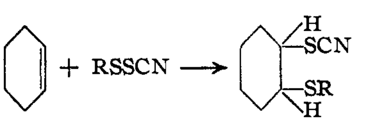
[1] Present address: Department of Chemistry, University of Southern California, Los Angeles 7, California.

[2] The sulfenic acids are generally designated as RSOH. Known derivatives include the sulfenyl halides (RSX), the sulfenyl thiocyanates (RSSCN), the sulfenamides ($RSNR_2$), the sulfenates (RSOR'), and sulfenic anhydrides (RSOSR). *Cf.*, Kharasch, Potempa and Wehrmeister, *Chem. Rev.*, **39**, 269–332 (1946).

[3] Lecher and Stöcklin, *Ber.*, **58**, 414 (1925).

[3a] Fuson, Price, *et al.*, *J. Org. Chem.*, **11**, 469–481 (1946).

fenyl thiocyanates add to olefins, have not been previously reported. We have found, however, that 2-nitrobenzenesulfenyl thiocyanate and 2,4-dinitrobenzenesulfenyl thiocyanate add smoothly to cyclohexene to give 2-arylthiocyclohexyl thiocyanates (equation (c), R = 2-nitrophenyl or 2,4-dinitrophenyl). Again, only single geometric modifications were found.


(c)

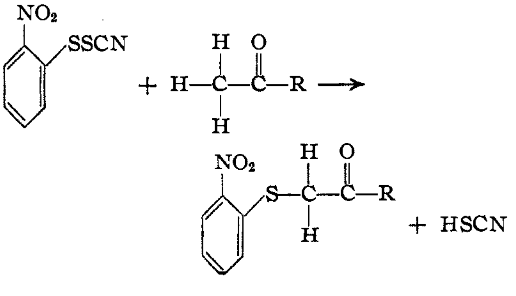
The addition of 2-nitrobenzenesulfenyl thiocyanate to styrene was also effected. Since this olefin is not symmetrical, two isomeric products of addition are possible, for the thiocyanate group may attach itself to either of the two carbons of the olefin bond. Only one of these possible products (m. p., 107–107.5°) was isolated in analytically pure condition; but since the unfractionated mixture also gave analytical results in agreement with the values calculated for the product of addition between styrene and 2-nitrobenzenesulfenyl thiocyanate, the inference that both isomers are formed simultaneously is being further investigated.

In our studies to determine whether the addition of sulfenyl thiocyanates to olefins is a general reaction, it was found that in some instances an important competing reaction is the decomposition of the sulfenyl thiocyanate into the disulfide and thiocyanogen

$$2RSSCN \longrightarrow RSSR + (SCN)_2 \qquad (d)$$

In the attempt to add benzenesulfenyl thiocyanate to cyclohexene, for example, the only product obtained was phenyl disulfide. Another interesting aspect of the addition of sulfenyl thiocyanates to olefins is that the reaction probably involves a free-radical mechanism, and that with unsymmetrical olefins the mode of addition of the thiocyanate may be subject to reversal, similarly as in the case of hydrogen bromide. This supposition, however, has no experimental basis as yet.

We have also found that 2-nitrobenzenesulfenyl thiocyanate and 2,4-dinitrobenzenesulfenyl thiocyanate react readily with acetone and with acetophenone, yielding the corresponding β-keto sulfides, as illustrated in equation (e), (R = methyl or phenyl).


(e)

These examples appear to be the first instances of the reactions of sulfenyl thiocyanates with ketones. Analogous reactions between sulfenyl chlorides and ketones have, however, been reported previously,[4] and additional examples of such reactions of sulfenyl chlorides were encountered in this study. Thus, 2,4-dinitrobenzenesulfenyl chloride reacts with acetone (or acetophenone) to form the corresponding 2,4-dinitrophenyl acetonyl (or phenacyl) sulfide. The β-keto sulfides serve as convenient derivatives for the 2-nitro- and the 2,4-dinitrobenzenesulfenyl chlorides and thiocyanates.

Experimental[5]

2-Chlorocyclohexyl *o*-Nitrophenyl Sulfide.—2-Nitrobenzenesulfenyl chloride (m. p. 74.5–75°) was obtained in 71% yield by scission of 2-nitrophenyl disulfide with chlorine in carbon tetrachloride solution.[6] This product (2.4 g.) and 2 ml. of cyclohexene were dissolved in 15 ml. of ethylene chloride. After refluxing for two hours, the solvent was evaporated, and the residue was recrystallized from a mixture of 20 ml. of ether with 30 ml. of ethanol. A 64% yield of yellow crystals, m. p. 97–101°, was obtained. A sample for analysis was thrice crystallized from ethanol; m. p., 100–102°.

Anal. Calcd. for $C_{12}H_{14}O_2NSCl$: C, 53.03; H, 5.19. Found: C, 53.09; H, 5.27.

2-Chloroethyl *o*-Nitrophenyl Sulfide.—The formation of this sulfide, by addition of *o*-nitrobenzenesulfenyl chloride to ethylene, as described by Lecher and Stöcklin,[3] was confirmed; yield 74%; m. p. 49–50°.

2,4-Dinitrobenzenesulfenyl Chloride.—The preparation of this sulfenyl chloride, by reaction of chlorine with a suspension of 2,4-dinitrophenyl disulfide in nitrobenzene, has been mentioned in the literature.[7] Following this suggestion, we found that scission of the disulfide by chlorine proceeded smoothly at 130°, but that the yield of 2,4-dinitrobenzenesulfenyl chloride was considerably reduced because of charring which occurred during removal of the nitrobenzene by vacuum distillation. By replacing nitrobenzene with ethylene bromide, as described below, the desired sulfenyl chloride was obtained in 69% yield. The use of carbon tetrachloride as solvent for this reaction was also attempted, but was found to be unsatisfactory, for it does not permit attainment of a sufficiently high temperature for the cleavage of 2,4-dinitrophenyl disulfide by chlorine.

2,4-Dinitrophenyl disulfide (dec. 240–280°) was prepared in good yield by the reaction of 2,4-dinitrochlorobenzene with sodium disulfide, following the procedure described for the preparation of *o*-nitrophenyl disulfide.[8] The disulfide (15.2 g.) was suspended in 200 ml. of ethylene bromide in a three-necked, one-liter flask, fitted with a reflux condenser. A crystal of iodine was added, the reaction mixture was heated to 120–130°, and chlorine was passed in, until a clear solution resulted (eight hours). The latter was filtered to remove a trace of suspended material, and the bulk of the ethylene bromide was removed by distillation at reduced pressure. On cooling the residual liquid, a solid precipitated. This was recrystallized from hot carbon tetrachloride; yield 69%; m. p.

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[4] Zincke, *et al.*, *Ann.*, **406**, 103 (1914), and earlier papers in the same series. See also reference 2.

[5] The microanalyses reported in this paper were made by Margaret M. Ledyard of Northwestern University. All melting points reported are uncorrected.

[6] "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 455.

[7] Billman, Garrison, Anderson and Wolnak, THIS JOURNAL, **63**, 1920 (1941).

[8] "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 220.

91–93°. A third crystallization yielded excellent yellow crystals; m. p. 94–95°.

2-Chlorocyclohexyl 2′,4′-Dinitrophenyl Sulfide.—One gram of 2,4-dinitrobenzenesulfenyl chloride (m. p. 91–93°) and 2 ml. of cyclohexene were dissolved in 15 ml. of ethylene bromide. The clear yellow solution was heated on the steam-bath for two hours. The solvent was evaporated, and the residual oil was dissolved in 30 ml. of hot ethanol. On cooling, excellent yellow needles were obtained; yield 67%; m. p. 115–117°.

Anal. Calcd. for $C_{12}H_{13}O_4N_3SCl$: C, 45.50; H, 4.14. Found: C, 45.84; H, 4.31.

2-Nitrobenzenesulfenyl Thiocyanate.—The preparation of this compound was accomplished by shaking, for twenty hours, a mixture of 45 g. (0.24 mole) of 2-nitrobenzenesulfenyl chloride, dissolved in 400 ml. of dry benzene, with 23.5 g. (0.24 mole) of finely powdered, anhydrous potassium thiocyanate, and working up the product as directed by Lecher and Simon[9]; yield 71%; m. p. 91–92°.

2-(*o*-Nitrophenylthio)-cyclohexyl Thiocyanate.—A solution of 2-nitrobenzenesulfenyl thiocyanate (5.8 g., 0.027 mole) and cyclohexene (2.8 g., 0.034 mole), in 35 ml. of ethylene chloride, was heated at 90–100° for two hours. The solvent was evaporated and the residual oil was dissolved in 25 ml. of hot absolute alcohol. On cooling to room temperature an oil separated. This solidified when allowed to stand for several days at −10°. A yield of 4.8 g. (62.3%) was obtained by recrystallization from 20 ml. of ether as bright yellow crystals; m. p. 68–70°.

Anal. Calcd. for $C_{13}H_{14}O_2N_2S_2$: C, 53.03; H, 4.79. Found: C, 52.87; H, 4.55.

The presence of the thiocyanate group in this product was shown by the facts (1) that heating a small quantity of the product with aqueous ferric chloride solution resulted, after a few minutes, in the development of the characteristic color associated with the test for the thiocyanate ion, and (2) oxidation of the product (1 g.), at 90°, with aqueous nitric acid (2 ml. of water, 5 ml. of nitric acid, sp. g., 1.42) yielded a sulfonic acid which was not isolated as such, but was converted to a corresponding S-*p*-chlorobenzylthiuronium salt; m. p. 168–169°.

2,4-Dinitrobenzenesulfenyl Thiocyanate.—Five grams of 2,4-dinitrobenzenesulfenyl chloride (m. p. 94–95°) was dissolved in 85 ml. of dry benzene. To this was added 2.5 g. of anhydrous, finely powdered potassium thiocyanate. The mixture was shaken for twenty hours, filtered and the filter cake was washed with 20 ml. of hot benzene. When the solvent was evaporated, an oil remained which solidified partially after standing for some time at room temperature. The crude product was repeatedly crystallized from hot carbon tetrachloride, yielding finally 0.8 g. of yellow crystals, melting at 74.5–76°. (NOTE ADDED IN PROOF: C. M. Buess, extending the work, reports that the melting point is raised to 83–84° by repeated crystallization from glacial acetic acid.

Anal. Calcd. for $C_7H_3O_4N_3S_2$: C, 32.66; H, 1.17. Found: C, 32.88; H, 1.46.

2-(2′,4′-Dinitrophenylthio)-cyclohexyl Thiocyanate.—A mixture containing 0.5 g. of 2,4-dinitrobenzenesulfenyl thiocyanate and 2 ml. of cyclohexene in 15 ml. of ethylene chloride was refluxed on the steam-bath for two hours. After filtering to remove a trace of solid, the ethylene chloride was evaporated at 90°. The residual oil solidified when cooled to 0°. Two crystallizations from hot absolute alcohol yielded 0.3 g. of yellow crystals which melted at 139–139.5°.

Anal. Calcd. for $C_{13}H_{13}O_4N_3S_2$: C, 46.01; H, 3.86. Found: C, 46.10; H, 3.64.

Addition of 2-Nitrobenzenesulfenyl Thiocyanate to Styrene.—A solution of 2.7 g. (0.013 mole) of 2-nitrobenzenesulfenyl thiocyanate and 2 ml. (0.017 mole) of stabilized styrene (Eastman Kodak 1465), in 15 ml. of ethylene chloride, was heated on the steam-bath for one and three-quarter hours. The solvent was then removed by heating at reduced pressure. On cooling the residue, a yellow solid was obtained. This was dissolved in 20 ml. of boiling carbon tetrachloride, and the slightly cloudy solution was filtered, yielding a clear filtrate. On cooling to 0°, yellow crystals precipitated. These were collected, washed with two ten-milliliter portions of carbon tetrachloride, and dried at 50°. The product weighed 3.1 g. and melted at 88–94°. A 0.2-g. sample was recrystallized from a mixture of 10 ml. of ethanol and 3 ml. of ether. The product then melted at 99–103°. A mixed melting point determination with 2-nitrobenzenesulfenyl thiocyanate (m. p. 91–92°) gave a value of 76–90°. The mixed melting point sample resolidified at about 90°, and remelted again only when heated to 194–205°. The once recrystallized sample was now twice more crystallized from ethanol, yielding a product which melted at 103–106°. The main portion of the original product was twice crystallized from ethanol, and once from ether. The product obtained melted at 100–105° and gave the following analysis.

Anal. Calcd. for $C_{15}H_{12}O_2N_2S_2$: C, 56.94; H, 3.82. Found: C, 56.67; H, 3.86.

The above experiment was repeated, using 2.1 g. of 2-nitrobenzenesulfenyl thiocyanate, 1.6 ml. of styrene and 15 ml. of ethylene chloride. The crude reaction product was obtained, as above. Recrystallization from hot carbon tetrachloride yielded 2.2 g. of yellow crystals which melted at 89–99°. Repeated crystallization from hot carbon tetrachloride finally yielded an excellent yellow product which melted at 107–107.5° (product A), and a second, bright yellow solid, melting at 89–92° (product B). The latter was obtained only in very small amount, and was not sufficiently pure for analysis.

Anal. (product A). Calcd. for $C_{15}H_{12}O_2N_2S_2$: C, 56.94; H, 3.82. Found: C, 56.63; H, 3.94.

Further experiments to establish the exact structures of the products in this reaction are now in progress.

Attempt to Add Benzenesulfenyl Thiocyanate to Cyclohexene.—A solution containing 0.05 mole (8.3 g.) of benzenesulfenyl thiocyanate in absolute ether was prepared as directed by Lecher and Wittwer.[10] Since benzenesulfenyl thiocyanate decomposes when distilled even at very low pressures, the product was used in solution directly after preparation. The ether solution of benzenesulfenyl thiocyanate was added to 75 ml. of ethylene chloride and 8 ml. of cyclohexene. The resulting clear mixture was refluxed for two and one-half hours and the solvent was then evaporated. The oily residue solidified on cooling. Recrystallization from absolute alcohol yielded 3 g. of excellent, colorless crystals which melted at 56–58°. The suspicion that this product was phenyl disulfide was confirmed by the facts that a mixed melting point determination with an authentic sample of phenyl disulfide caused no lowering of the melting point, and that the analysis for carbon and hydrogen agreed with the values calculated for phenyl disulfide.

Anal. Calcd. for $C_{12}H_{10}S_2$: C, 66.05; H, 4.59. Found: C, 65.90; H, 4.92.

Some additional phenyl disulfide was obtained by working up the mother liquors, but no other product could be found.

2-Nitrophenyl Acetonyl Sulfide.—One gram of 2-nitrobenzenesulfenyl thiocyanate was refluxed for one hour with 10 ml. of acetone. The acetone was evaporated, and the residue was twice crystallized from absolute alcohol, yielding 0.9 g. of yellow crystals. The melting point of the product (81–82°) corresponded to that of the known 2-nitrophenyl acetonyl sulfide.[11]

2-Nitrophenyl Phenacyl Sulfide.—A mixture of 1 g. of 2-nitrobenzenesulfenyl thiocyanate, 10 ml. of ethylene bromide and 2 g. of acetophenone was refluxed for two and one-half hours. When the reaction mixture was cooled to

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[9] Lecher and Simon, *ibid.*, **54**, 632 (1921).

[10] Lecher and Wittwer, *ibid.*, **55**, 1474 (1922).

[11] Zincke and Farr, *Ann.*, **391**, 55 (1912).

room temperature, a small quantity of brown solid separated. This was collected (0.01 g.) and found to decompose above 250°. It was not further investigated. After standing for one day, crystals appeared in the filtrate. These were collected, dried at 50°, and found to weigh 0.92 g. Recrystallization from a mixture of chloroform with a small amount of added hexane yielded a product which melted at 140.5–141°.

Anal. Calcd. for $C_{14}H_{11}O_3NS$: C, 61.54; H, 4.03. Found: C, 61.41; H, 4.33.

2,4-Dinitrophenyl Acetonyl Sulfide.—This was prepared by refluxing 2 g. of 2,4-dinitrobenzenesulfenyl chloride with 20 ml. of acetone for several hours. Evaporation of the excess acetone and two crystallizations from absolute alcohol gave a good yield of lustrous brown crystals; m. p., 140–140.5°.

Anal. Calcd. for $C_9H_8O_5N_2S$: C, 42.18; H, 3.16. Found: C, 42.52; H, 3.42.

2,4-Dinitrophenyl acetonyl sulfide was also obtained by carrying out a similar reaction between 2,4-dinitrobenzenesulfenyl thiocyanate and acetone.

2,4-Dinitrophenyl Phenacyl Sulfide.—One gram of 2,4-dinitrobenzenesulfenyl chloride was dissolved in 10 ml. of ethylene chloride, 3.0 g. of acetophenone was added and the mixture was refluxed for two hours. An excellent yellow solid precipitated on cooling. This was collected, dried at 50° and found to weigh 1.3 g., (95% yield). After recrystallization from absolute alcohol, the product melted at 168–170°. The identical substance was also obtained by reacting, in a similar manner, 2,4-dinitrobenzenesulfenyl thiocyanate with acetophenone.

Anal. Calcd. for $C_{14}H_{10}O_5N_2S$: C, 52.82; H, 3.17. Found: C, 53.26; H, 3.31.

Summary

2-Nitrobenzenesulfenyl chloride and 2,4-dinitrobenzenesulfenyl chloride add to cyclohexene to yield the corresponding 2-chlorocyclohexyl aryl sulfides.

The reactions of 2-nitrobenzenesulfenyl thiocyanate and 2,4-dinitrobenzenesulfenyl thiocyanate with cyclohexene proceed in a manner similar to those of the corresponding sulfenyl chlorides, yielding 2-(arylthio)-cyclohexyl thiocyanates. The addition of 2-nitrobenzenesulfenyl thiocyanate to styrene has also been effected. In the attempted addition of benzenesulfenyl thiocyanate to cyclohexene, however, only phenyl disulfide was isolated.

The reactions of the 2-nitro- and the 2,4-dinitrobenzenesulfenyl chlorides and thiocyanates with acetone and with acetophenone, yielding β-keto sulfides, are also described.

LOS ANGELES, CALIFORNIA RECEIVED NOVEMBER 6, 1946

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA]

The Spectra of the p-Dimethylaminochalcones and of their Ions

BY ESTELLA R. KATZENELLENBOGEN AND GERALD E. K. BRANCH

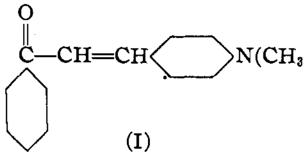
Lewis and Calvin[1] were able to interpret the spectra of a considerable number of organic molecules on the basis of the assumption that the electrons in these molecules act as quantized oscillators. They also postulated that the absorption spectrum of a molecule of appreciable polarizability in two dimensions should show two bands: the band at longer wave length being due to absorption of light vibrating along the direction of maximum polarizability (x-axis), and the second band being due to absorption of light vibrating along the direction of minimum polarizability (y-axis) which we shall assume to be perpendicular to the x-axis. These bands have been called x- and y-bands, respectively.

It should be noted that this theory accepts the basic concept of light absorption being due to the transition of the molecule from one quantum state to another. Further, the theory does not deny the possibility of calculating the energy difference of these states. It merely attempts to avoid the difficulties of such a calculation and yet to predict the spectrum of a molecule with some accuracy by making use of a classical picture and of some empirical facts, such as the magnitudes of the bathochromic or hypsochromic effects of certain groups.

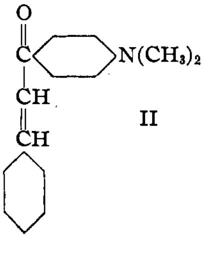
The temptation to avoid the calculation of the spectrum of an organic molecule becomes obvious if one considers that even such a relatively simple substance as a p-aminochalcone is a hybrid of more than fifty resonating forms, without invoking Dewar structures or structures with more than one separation of charge.

In this paper we shall show that the spectra of the p-dimethylaminochalcones and of their three positive ions can be predicted and interpreted by the theory of Lewis and Calvin.

4-Dimethylaminochalcone (I) and 4′-dimethylaminochalcone (II) were chosen for this study since they are both vinylogs of p-dimethylaminobenzophenone (III) and differ only in the position of the vinyl group.



(I)



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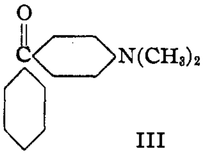

III

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[1] Lewis and Calvin, *Chem. Rev.*, **25**, 273 (1939).