theless yield a value of $\tau_{\text{pr(ii)}}$ of more than three hours, which is the same order of magnitude as the observed result. This case indicates that the $\tau_{\text{pr(ii)}}$ of Bond and Puls is sometimes a test of the application of the Gibbs theorem rather than a test of any diffusion hypothesis. The use of equation 8, however, which like equations 2 and 6 is based on diffusion only, and the obtaining therefrom of a value of n much greater than 5, would indicate that possibly other factors than diffusion are equally as responsible for the change of surface tension with time.

Acknowledgment.—The author is indebted to Professor J. W. McBain for discussing the subject matter of this paper with him.

Summary

1. A relation is pointed out between two published treatments of the hypothesis that the change of surface tension with time is due to the time required for solute molecules to diffuse to the surface.

2. The thermodynamic equation of Bond and Puls is supplemented by an equation based on a kinetic picture of a close-packed monomolecular layer.

3. Consideration of experimental data indicated that polymolecular surface layers, approximately five molecules deep, are present in solutions of short-chain soluble substances.

4. The present kinetic treatment offers a criterion of the diffusion mechanism which cannot be generally obtained from thermodynamic equations.

RECEIVED JANUARY 2, 1945

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

Phosphorescence in Fluid Media and the Reverse Process of Singlet–Triplet Absorption

BY GILBERT N. LEWIS AND M. KASHA

1. **Introduction.**—Our identification[1] of the phosphorescent state with the triplet state implies that this state is to be found in all media, and not merely in the rigid media which are so favorable to the display of phosphorescence. Indeed, although both lifetime and intensity are relatively small, phosphorescence can be observed in liquids at room temperature, especially in viscous liquids. Thus Boudin[2] found the phosphorescence of eosin in glycerol at room temperature, and Kautsky,[3] with a very rapid phosphoroscope, and with eyes conditioned by darkness, was able to see the phosphorescence of dyes in water and propyl alcohol. We shall also mention in our last section the phosphorescence of biacetyl in water. In that place we shall also discuss two cases of phosphorescence in the *gaseous* state.

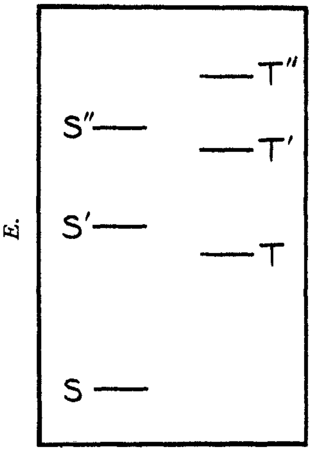

Fig. 1.—A schematic representation of energy levels: showing, left, the ground (stable) singlet state S and the electronically excited singlet states S′ and S″ and, right, the ground (metastable) triplet state T and the electronically excited triplet states T′ and T″.

However, the phosphorescence which accompanies the quasi-forbidden transition from triplet to singlet state (T → S in Fig. 1) is not the only means of studying the triplet state. We should look for the reverse process, namely, a quasi-forbidden transition from singlet to triplet state. Such transition should manifest itself by weak absorption bands which, in accordance with our previous discussion, should satisfy the following criteria.

I. If the bands of phosphorescence (T–S transition) and of the reverse absorption (S–T transition) are very narrow, the lowest frequency peak, at $\bar{\nu}_A$, of the expected absorption, should almost coincide with the highest frequency peak, at $\bar{\nu}_P$, of phosphorescence. On the other hand, if the bands are broad, $\bar{\nu}_A$ should be greater than $\bar{\nu}_P$ by an amount that, as a rough guess, may be taken as the sum of the half-widths of the absorption and emission bands. The energy difference, E_T, between triplet and singlet states, would then lie between $hc\bar{\nu}_P$ and $hc\bar{\nu}_A$, and provisionally, we may take it equal to the arithmetic mean.

II. Since the energy of S–T transition is in no way related to the energy of normal absorption (S–S′), the abnormal bands that we are seeking

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[1] Lewis and Kasha, THIS JOURNAL, **66**, 2100 (1944).

[2] S. Boudin, *J. chim. phys.*, **27**, 285 (1930).

[3] Kautsky, *Ber.*, **68**, 153 (1935).

may be in a quite different part of the spectrum from the bands of normal absorption.

III. In normal absorption the first band is always followed by an "octave" band (x' band), the frequency of the latter being roughly double that of the former.[4,5] When, however, there is transition between two states, neither of which has electronic oscillation, the corresponding absorption frequency would be connected in no known manner with the frequency of higher bands.

IV. One of the most useful criteria of S–T absorption bands concerns their width. We have seen in our preceding paper that the phosphorescence (T–S) bands are much narrower than the normal absorption (S–S′, S–S″), and the fluorescence (S′–S) bands of the same substance. Hence it was concluded that the S and T states are better defined, as to energy, than the S′ and S″ states. Thus we may predict that S–T absorption will also be characterized by narrow bands, and therefore by fine structure.

V. The separation of the individual vibrational bands of S–T absorption will be characteristic of vibrations in the triplet state, just as the bands of phosphorescence show the vibrational levels of the singlet state. Since, however, our measurements give the only present clue to the positions of these levels in the triplet state, we cannot actually use this criterion to prove S–T absorption, but we shall have plenty of proof based on the other five criteria.

VI. Since S–T absorption is the reverse of the phosphorescence process, the intensity of the S–T bands may be calculated, at least roughly, from the life of the phosphorescent state by the aid of a thermodynamic equation that we shall now discuss.

2. The Thermodynamic Equation Relating the Probability of Absorption and that of the Reverse Emission.—We may employ the following equation[6]

$$\int \epsilon' d\bar{\nu} = \frac{1}{8\pi c\bar{\nu}_A{}^2 n^2}\frac{g_u}{g_l}k_e \qquad (1)$$

where ϵ' is the extinction coefficient in natural units, and the integration is over the whole absorption band whose maximum is at $\bar{\nu}_A$; n is the refractive index of the medium; g_u and g_l are, respectively, the multiplicities of upper and lower states; k_e is the rate constant of the emission process.

If we substitute the ordinary molal extinction coefficient, ϵ, by the relation

$$\epsilon' = \frac{1000}{N}2.303\ \epsilon \qquad (2)$$

N being Avogadro's number, and introduce the values of the several numerical constants, we have

$$\int \epsilon d\bar{\nu} = 3.47 \times 10^8 \frac{1}{\bar{\nu}_A{}^2 n^2}\frac{g_u}{g_l}k_e \qquad (3)$$

In addition we must consider the equation

$$\tau \leq 1/k_e \qquad (4)$$

When the emission process alone takes molecules from the upper to the lower state, the mean life τ is by definition the reciprocal of k_e. When, however, there is some competing process, such as the thermal dissipation of the energy of the upper state, τ may be considerably less than the life that would be observed if emission of radiation were the sole process.

Equations (1) and (3) are valid only when the absorption and emission bands are single, very narrow, and coincide in frequency. When we are dealing with an organic molecule where the bands are broad, composed of various subordinate vibrational bands, and where the maxima of emission and absorption may be several thousand wave-numbers apart, the degree of validity of these equations can be determined only by experiment.

In the case of *fluorescence* of complex molecules, the only data by which equation (3) can be tested seem to be those of fluorescence lifetimes by Gaviola,[7] and the corresponding absorption measurements. The best cases to consider are rhodamine B and fluorescein in glycerol, where the fluorescence yield is nearly one hundred per cent., and therefore equation (4) is nearly valid. We have determined the absorption of rhodamine B in glycerol and obtained a curve[8] from which $\int \epsilon d\bar{\nu}$ is 1.52×10^8 and the frequency of the band maximum is 18050 cm.$^{-1}$ For our purpose the temperature and the wave length at which the refractive index is measured are unimportant and we may take for glycerol, $n = 1.47$. Finally for fluorescence $g_u = g_l$. Thus we find from equations (3) and (4) $\tau = 1/k_e = 3.2 \times 10^{-9}$ sec., while Gaviola found 4.2×10^{-9} sec. The surprisingly small discrepancy would be increased if the fluorescence efficiency were not one hundred per cent., and also if the rhodamine B is partly in the form of a colorless pseudoisomer, which is always present[1] to an extent that is unknown, though probably small. From this case, and from the similar case of fluorescein, where our data are not so definite, we may conclude that in such complex molecules equation (3), applied to the fluorescence process, is valid to within a factor of about 2.

Within such limits of error, we may expect equation (3) to be valid also for the phosphorescence process and the reverse absorption. Since we have identified the phosphorescent with the triplet state, we may here always take $g_u/g_l = 3$. Since the phosphorescence lives are usually much

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

[5] Lewis and Bigeleisen, THIS JOURNAL, **65**, 2107 (1943).

[6] The derivation of this equation will be given in another place. It differs only by the factor n^2, the square of the refractive index, from the one derived by Ladenburg (*Verh. d. D. Phys. Ges.*, **16**, 769 (1914); *Z. Physik*, **4**, 451 (1921)) from the absorption and emission coefficients of Einstein.

[7] Gaviola, *Z. Physik*, **42**, 853 (1927).

[8] This curve is almost identical with that found in aqueous solution by Auskaps (*Acta Universitatis Latviensis*, Riga, p. 279 (1930)).

longer than fluorescence lives, we must expect S–T absorption to be much smaller than the typical S–S′ absorption, being, however, most easily detectable for substances of the shortest phosphorescence lives.

3. Abnormal Colors of Nitroso Compounds.—There are a number of organic substances that show striking colors, unexpected in ordinary color theory. Chief among these are the green or blue nitroso compounds and the blue aryl-thioketones. We shall show that all of these abnormal colors are due to absorption from the singlet to the triplet state.

***p*-Nitrosodiethylaniline** gives the absorption curve shown in Fig. 2 which is characterized by lying largely in the infra-red, by the very small value of ϵ, and by vibrational structure which is evident even at room temperature. The value of $\int \epsilon d\bar{\nu} = 1700$ leads by equations (3) and (4) to a value of $\tau = 1.7 \times 10^{-3}$ sec. We looked for phosphorescence without success in this and the two following cases, nor did we expect to find it, since the phosphorescence bands must lie at lower frequencies than the absorption bands, and these stretch nearly to the limit of our photographic plate (about 11000 cm.$^{-1}$).

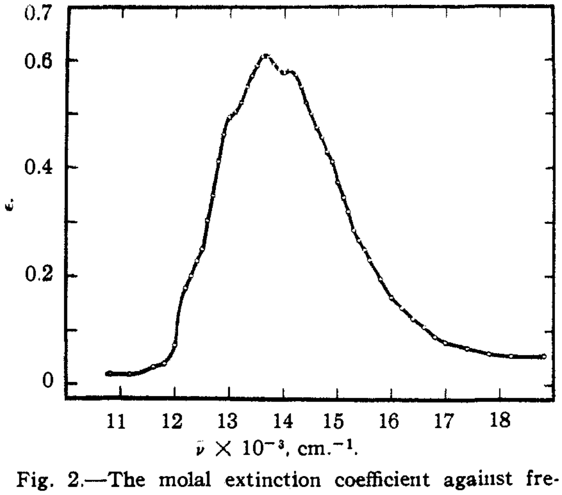

Fig. 2.—The molal extinction coefficient against frequency of *p*-nitrosodiethylaniline in hexane at room temperature.

Nitrosobenzene has an abnormal absorption, also lying chiefly in the infra-red. As is seen in Fig. 3, there is little observable structure at room temperature, but a pronounced structure at the temperature of liquid air, in EPA (5 ether, 5 isopentane, 2 ethanol, by volume). The individual vibrational bands are about equally spaced at intervals of 500 cm.$^{-1}$ We are going to conclude that all these nitroso colors are due to S–T absorption. Therefore this interval of 500 cm.$^{-1}$ must represent an important vibration of the triplet state, which we cannot at present identify further.

Dimethylbromonitrosomethane shows at room temperature the absorption curve given in Fig. 4.

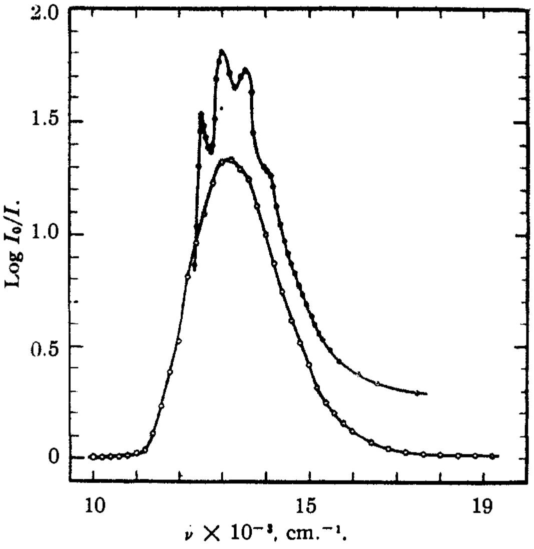

Fig. 3.—Extinction curve of nitrosobenzene; lower curve, in isopentane at room temperature; upper curve in EPA at 90°K.

Even at this temperature the curve shows some structure. Making a rough estimate from the values of $\bar{\nu}_A$ and the apparent width of the bands, we obtain about the same value for the energy of the triplet state, E_T, for the three nitroso compounds so far considered, approximately 35 kcal.

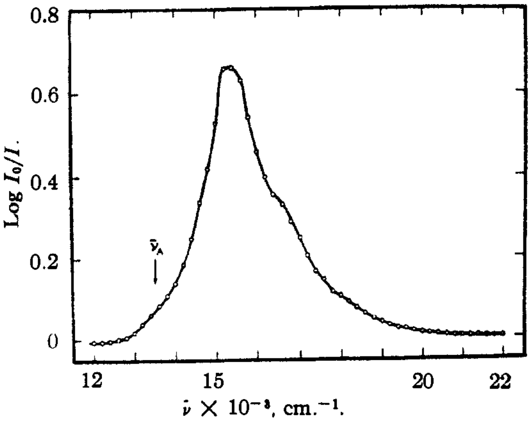

Fig. 4.—Extinction curve of dimethylbromonitrosomethane in isopentane at room temperature. The first vibrational band, not very prominent, is indicated as $\bar{\nu}_A$.

Diisopropylbromonitrosomethane, probably because of an extremely compact structure, whereby many of the rotations and vibrations of low energy are inhibited,[8a] has proved extraordinarily satisfactory for our purpose. Its ab-

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(8a) The sharpness of a low temperature absorption curve depends largely upon the limitation of the vibrations and rotations of small energy in the excited state. If scale models are made of our two aliphatic nitroso compounds, it appears that in the dimethyl compound the rotations around the —CH_3 bond and around the —NO bond are nearly free, whereas in the diisopropyl compound such rotations are inhibited and must be replaced by high frequency torsional vibrations.

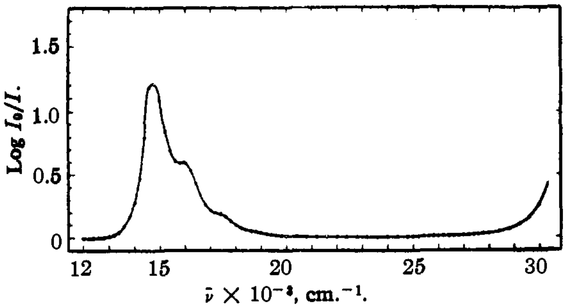

Fig. 5.—Extinction curve, over a wide spectroscopic range, of diisopropylbromonitrosomethane in isopentane at room temperature. The wide region of no absorption is noteworthy, especially as the absorption beginning at 30000 cm.$^{-1}$ is probably due to an impurity.

sorption curve in isopentane at room temperature is given in Fig. 5, over the range from 12000 to 30000 cm.$^{-1}$ After the main absorption band there is a remarkably long gap before further absorption begins to appear at 30000 cm.$^{-1}$, and even this last we suspect to be due to an impurity. Criterion III is therefore satisfied. The absorption at liquid air temperature has the remarkable structure shown in Fig. 6. We shall not attempt to analyze the band intervals, which must be attributed to vibrational levels of the triplet state.

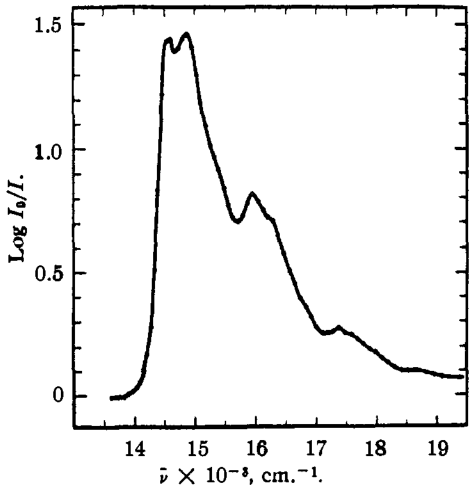

Fig. 6.—Extinction curve of diisopropylbromonitrosomethane in EPA at 90°K., showing the unusually narrow and well resolved bands of this very compact molecule.

The value of $\bar{\nu}_A$ for diisopropylbromonitrosomethane is about 1000 cm.$^{-1}$ greater than that for the dimethyl compound. For this reason and because of the extreme sharpness of the first band, it seemed possible that the corresponding phosphorescence could be obtained. Because of the short life that we have estimated for the nitroso compounds no phosphoroscope was used. The experiment was successful and the tracing of the photograph of phosphorescence emission is given in Fig. 7. It is evident that this emission spectrum is a very good "mirror image" of the absorption curve of Figs. 5 and 6. This indicates a similarity in the vibrational levels of singlet and triplet states, which may be connected with the peculiar type of resonance that we are about to discuss.

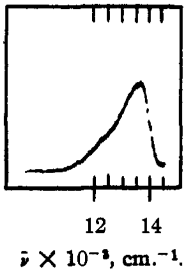

Fig. 7.—A tracing of a phosphorescence photograph of diisopropylbromonitrosomethane in EPA at 90°K. Because of the low dispersion of the quartz spectrograph in the infrared, and a wide slit width, any fine structure is obliterated.

While the absorption spectra of the other nitroso compounds satisfy many of the criteria of S–T transitions, it is remarkable that in diisopropylbromonitrosomethane every criterion is satisfied. For this substance, where we have studied both quasi-forbidden processes, namely, S–T absorption and T–S phosphorescence emission, we have found $\bar{\nu}_A$ = 14560 cm.$^{-1}$, and $\bar{\nu}_P$ = 13600 cm.$^{-1}$. Using the mean of these, we find for the difference in energy between triplet and singlet states, E_T = 40 kcal. This is a far more accurate value than the value of 35 kcal. estimated in the other three cases, but we think the difference is real.

As predicted in our preceding paper,[1] the nitroso group proves to be more powerful than any other group in lowering the energy of the triplet state. Indeed it is hardly correct to think of the nitroso group as lowering the triplet state energy of a molecule in which it is present; the most striking fact is that all four of our nitroso compounds have nearly the same value of E_T. In other words, it is nearly correct to say that E_T for R—N═O is independent of the nature of R.

We have noted that R—N═O is the nearest analog to O_2, and has similar possibilities of resonance. Unlike ethylene, in which the carbon atoms are held far apart by the triplet repulsion, we may presume that in the nitroso triplet state, as in that of O_2, the two atoms are held closely together by resonance involving dipole forms. This powerful resonance will nearly prevent one of the odd electrons from wandering into R, and therefore the constitution of R is unimportant.

4. Abnormal Colors of Thioketones.—Another group of compounds showing anomalous colors are the thioketones, the aryl thioketones being blue.[9] We have chosen for study *thiobenzophenone*, the phosphorescence of which we have already investigated.[1] Its absorption at room temperature is shown by the broad smooth curve of Fig. 8, showing little resemblance to the bands of phosphorescence emission. However, at liquid air temperature a striking structure appears (Fig. 9), although the bands are by no means as narrow as those, for example, in Fig. 6. At the

(9) Bost and Cosby, THIS JOURNAL, **57**, 1404 (1935).

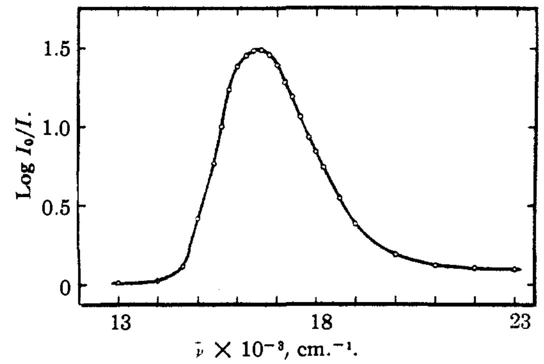

Fig. 8.—The extinction curve of thiobenzophenone in toluene at room temperature.

left of Fig. 9, with the same frequency scale, is the tracing of the phosphorescence spectrum. In this case the spacing of the bands is wider in the emission than in the absorption spectrum. If corresponding vibrational levels are concerned, these levels are farther apart in the singlet than in the triplet state.

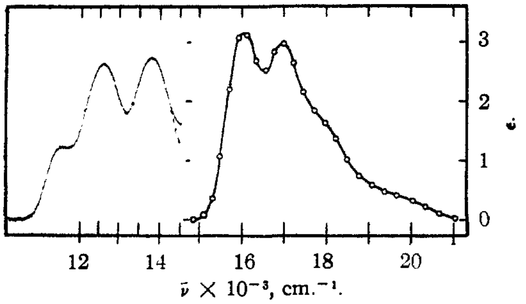

Fig. 9.—Right, molal extinction curve of thiobenzophenone in EPA at 90°K.; left, tracing of photograph of phosphorescence emission of thiobenzophenone in EPA at 90°K. The termination of the phosphorescence spectrum at the left is due to the fact that the photographic plate becomes insensitive for frequencies below about 11000 cm.$^{-1}$. The small tail at the right of the tracing is probably due to an impurity of benzophenone.

Here there is a wide separation between $\bar{\nu}_A$ = 16000 cm.$^{-1}$ and $\bar{\nu}_P$ = 13800 cm.$^{-1}$. Using once more the average, we find E_T = 43 kcal., which coincides with the value that we obtained previously from phosphorescence alone. Here again many of the criteria of S–T absorption are satisfied. The absorption is in a part of the spectrum far from that of its expected normal absorption; a marked structure is observed; and the individual bands, although broad, are by no means as broad as the normal absorption bands of substances such as ketones. The lowest absorption frequency is about that to be predicted from the phosphorescence bands. The area under the absorption curve, $\int \epsilon d\bar{\nu}$, is about 9300, corresponding by equation (3) to 2.3×10^{-4} sec. for τ, if we assume equation (4). Experimentally we have no knowledge regarding the half-life except that the phosphorescence could not be observed with our phosphoroscope, and therefore τ is not greater than 10^{-3} sec.

Aliphatic thioketones have not been studied spectroscopically. These, together with thiophosgene, are red liquids of weak absorption, and we believe this absorption will prove to be of the S–T type, with higher values of E_T than in the case of the aryl compounds. We have concluded that in RNO, the resonance of the odd electrons in the triplet state is confined to the NO groups. In $R_2C{=}S$ it may be assumed that the corresponding resonance penetrates farther into the R groups, so that E_T is less when one of these is aromatic.

Dimers and polymers of nitroso compounds and thioketones frequently occur. When two molecules in the triplet state come together, they tend to associate by forming a bond with one electron of each molecule; or two bonds, using all four of the odd electrons. Higher polymers and especially trimeric rings may be formed similarly. Considering, for simplicity, only the case of dimers, from two like molecules, and writing $E_{\rm Dim}$ for the energy of association, we may note that when $E_{\rm Dim}$ is greater than $2E_T$, then, neglecting entropy changes, the dimer is thermodynamically stable with respect to the molecules in the singlet state. In all cases therefore, in which E_T is small, we may expect dimerization, unless $E_{\rm Dim}$ becomes small, as for example, through a steric hindrance to association. In order to exhibit the abnormal color of monomeric aliphatic nitroso compounds, we have had to use those in which the nitroso group is attached to a tertiary carbon. Similar conditions are met in the aliphatic thioketones, where it has been shown that all $R_2C{=}S$ molecules polymerize, except when both R's are tertiary butyl.[10] In both types association is evidently hindered sterically.

5. S–T Absorption in Other Substances of Short-lived Phosphorescence.—So far we have considered substances with very short phosphorescence lives, and therefore relatively high S–T absorption, which gives rise to known anomalous colors. We shall now mention a few substances, also of short-lived phosphorescence, which do not, however, show a notably anomalous color because their S–T bands fall in the same spectral region as their much stronger normal S–S′ bands.

Phenazine gave the not entirely satisfactory curve shown in Fig. 10. Superposed upon the beginning of the normal broad absorption band, we see the smaller but sharper S–T band at 18200 cm.$^{-1}$. This is a case in which the two phosphorescence bands of highest frequency are much weaker than the third band (Plate II, 7),[1] as we have indicated by the vertical bars. If the absorption bands are a sort of mirror image of the

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[10] Kretov and Komissarov, *J. Chem. Soc.* USSR, **5**, 388 (1935).

emission bands, it may be that a more careful study would show two weak absorption bands to the left of the main S–T band. If we assume that the S–S′ curve is given by the dotted line, the area above gives $\int \epsilon d\bar{\nu} = 620$, from which, by equation (3), assuming equation (4), we find $\tau = 2.8 \times 10^{-3}$ sec., whereas our former rough estimate was of the order of 10^{-2} sec.

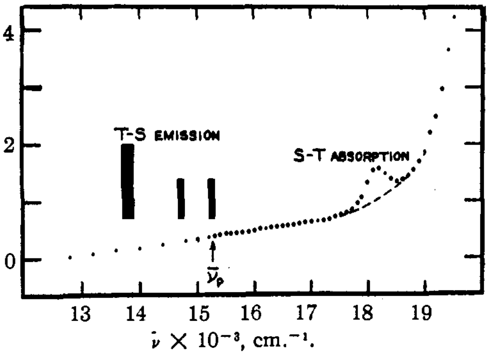

Fig. 10.—Molal extinction curve of phenazine in ether at room temperature. The main curve is due to the beginning of S–S′ absorption, but the superposed hump is due to S–T absorption, and there may be smaller peaks to the left. The vertical bars show the positions and, roughly, the intensities of the phosphorescence emission bands, terminating at $\bar{\nu}_P$.

Dibenzalacetone has a small S–T absorption band at 22200 cm.$^{-1}$ making a noticeable hump on the main S–S′ absorption band. It is to be seen in the published curve of Radulescu.[11] The area under the S–T band is hard to estimate, but is approximately that calculated from the half-life of phosphorescence, which is between 10^{-1} and 10^{-2} sec.

Nitrobenzene and **α-nitronaphthalene** were purified with great care in a search for the S–T band, but in the calculated region for $\bar{\nu}_A$ the main S–S′ absorption is rising so rapidly that no S–T hump could be observed.

6. Search for S–T Absorption in Substances of Long Phosphorescence Life.—So far we have considered substances with $\tau < 10^{-1}$ sec. and with values of ε lying between 0.1 and 3. Turning now to substances of much longer phosphorescence life, experiments were made at 90° with pure liquid naphthalene (phosphorescence half-life about 10 sec.) and with *anthracene* dissolved in a small amount of toluene at 80°, both in 5-cm. cells. In neither case were S–T bands observed, although with anthracene a sharp band at about 6500 Å. was to be expected if the phosphorescence life, which we had been unable to measure experimentally, were of the same order of magnitude as that of the very similar phenazine. The negative result of our absorption measurements shows that the phosphorescence half-life must be at least 1000 times as great for anthracene as for phenazine. It is not true that we found no absorption in the experiments with naphthalene and anthracene, but the small absorption found was due to bands of another sort that we must mention briefly.

Vibrational–Rotational bands are alien to our subject, but are important experimentally whenever measurements are made with long cells. These bands, starting in the far infrared and appearing periodically with greatly diminishing intensity at increasing frequencies, are still appreciable in the visible. Figure 11 shows the extinction due to these bands in the visible and near infrared, for various solvents, all in 75-cm. cells, except in the case of dioxane, which was measured in a 40-cm. cell. The positions of many of these bands are in the literature, but it seems well to show here their positions and intensities. For it is evident that even with a 10-cm. cell, these bands are observable and must be taken into account in exact spectroscopy, although they become less important, especially with dilute solutions, when a solution is compared directly with the pure solvent, as in some instruments. Such bands account wholly for the very small absorption found in the experiments with naphthalene and anthracene.

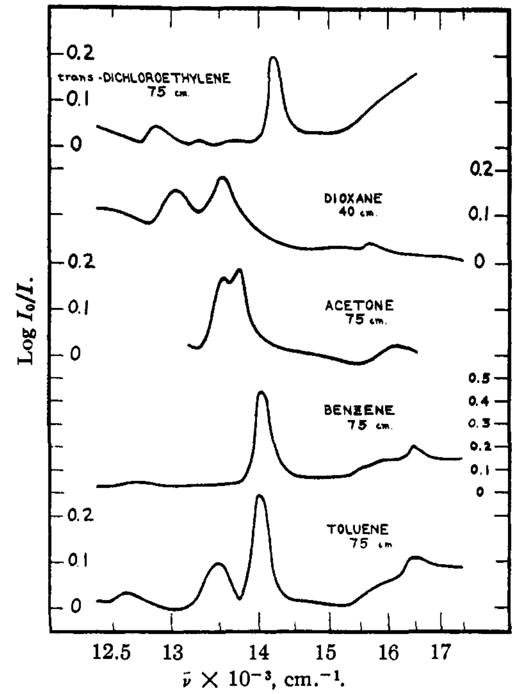

Fig. 11.—Extinction curves due to pure vibrational-rotational bands in various pure liquids in the orange-red and near infrared.

We next proceeded to a 10-cm. cell and studied ***p*-dichlorobenzene** dissolved in a relatively small amount of benzene. The absorption curve is given in Fig. 12; the S–T absorption is small but noticeable on account of the sharpness of the bands, whose positions are indicated by the three arrows, and which are superposed on general S–S′ absorption. The value of $\int \epsilon d\bar{\nu}$ can be estimated only roughly but agrees in order of magnitude with that calculated by equation (3) from the phosphorescence life of about 10^{-1} sec. The first of the S–T bands gives $\bar{\nu}_A = 26400$ cm.$^{-1}$,

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(11) D. Radulescu, *Ber.*, **64**, 2243 (1931).

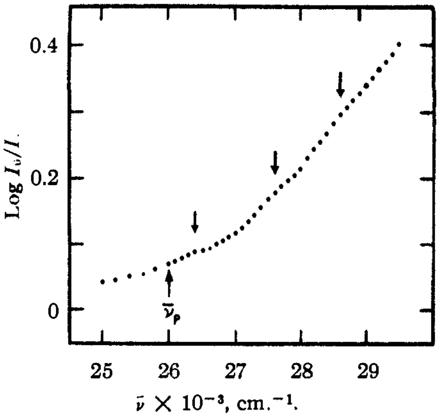

Fig. 12.—Extinction curve of 10 cm. of *p*-dichlorobenzene dissolved in a minimal amount of benzene, at room temperature. The main curve is that of S–S′ absorption, upon which are superposed the small S–T humps at about 26400, 27600, and 28700 cm.$^{-1}$, marked by downward arrows. The upward arrow shows the position of the highest band of phosphorescence emission, from our preceding paper.

which is extremely close to the highest phosphorescence band for which we found[1] $\bar{\nu}_P = 26000$ cm.$^{-1}$. The average leads to $E_T = 74.5$ kcal. as against our previous estimate of 77 kcal. Apparently we overestimated the width of the phosphorescence bands.

7. The Complete Absorption Spectrum of Liquid Benzene from 12500 to 2200 Å.—Fortunately, in benzene, the vibrational–rotational bands are widely separated from the electronic bands, and of the latter, the S–T bands are situated where the S–S′ bands have only begun to appear. Figure 13 shows all the absorption bands of benzene over a wide spectral range. The left-hand set are all due to pure vibration–rotation, the right-hand set, on a very different scale, belong to the known S–S′ absorption of benzene, while the intermediate section shows four S–T bands superimposed upon the tail of the first S–S′ band. It may be noted that Sklar[12] observed very weak bands in this region which he also ascribed to a singlet–triplet transition.

It is remarkable that the first of the S–T bands with $\bar{\nu}_A = 29400$ cm.$^{-1}$ lies actually below the first phosphorescence band,[12a] $\bar{\nu}_P = 29800$ cm.$^{-1}$. It is possible that the first absorption band is due to an anti-Stokes effect, or more likely there is a solvent effect, such that the energy interval between singlet and triplet benzene is about 1 kcal. higher in EPA at 90°K. than in pure benzene at room temperature. We studied the absorption curve at room temperature of a mixture of 1 mole benzene to 2 moles ethanol and obtained the identical S–T bands shown in Fig. 13. The separation of each S–T band from the next is approximately 900 cm.$^{-1}$, and this must represent an important vibration in the triplet state of benzene.

8. Phosphorescence in the Gaseous State; The Absorption and So-Called "Delayed Fluorescence" of Biacetyl Vapor.—In the well studied case of biacetyl there is a luminescence[13] with a

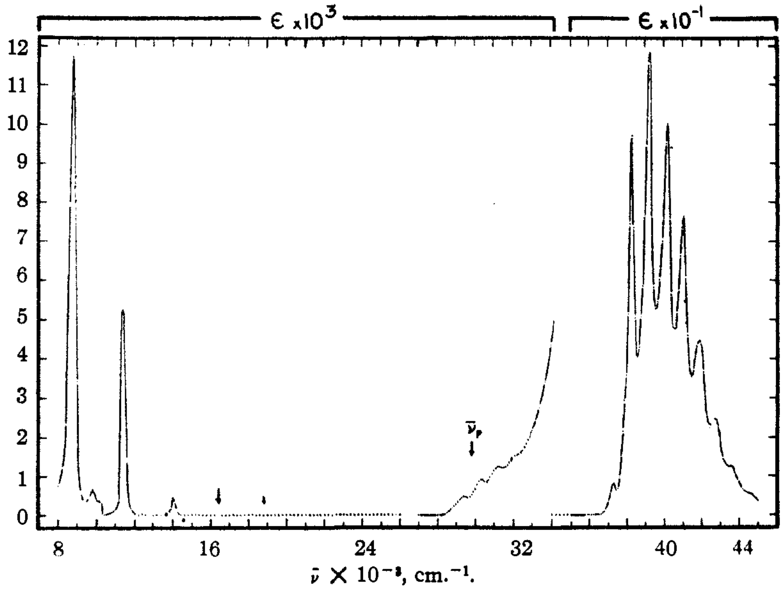

Fig. 13.—Complete molal extinction curve of liquid benzene (and at right, dilute solution of benzene in ethanol) at room temperature, from 12500 to 2200 Å. At the left are the pure vibrational-rotational bands of which the last two, indicated by arrows, are detectable only in long tubes. The central section shows the S–T bands at 29400, 30300, 31200, and about 32100 cm.$^{-1}$. These are superposed on the extreme tail of the bands at about 37000 cm.$^{-1}$. The right-hand section shows, with a very different ε scale, the S–S′ band with its vibrational structure. The first small band of this set is some 20000 times as intense as the S–T bands. The arrow at $\bar{\nu}_P$ shows the position of the highest phosphorescence band of benzene in EPA at 90°K.

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(12) Sklar, *J. Chem. Phys.*, **5**, 669 (1937).

(12a) Added in proof: New experiments on the phosphorescence of benzene by Mr. R. V. Nauman lead us to suspect that the 0-band obtained in our one experiment with thirty-hour exposure was illusory and due to a mercury line from the Midget Sun leaking into the spectroscope. If this suspicion is verified, the above discrepancy between $\bar{\nu}_A$ and $\bar{\nu}_P$ disappears.

(13) So drastic a definition as we gave in our preceding paper, namely, that every emission with change of multiplicity is to be classed as phosphorescence, regardless of the mean life of the emitting substance, occasionally conflicts with previous common usage Thus the characteristic triplet-singlet emissions of atomic Mg, Zn, Cd, and Hg, with lives ranging from 10^{-8} to 10^{-7} sec., including the 2537 Å. "fluorescence" of Hg, must all be classed as phosphores-

mean life of 1.65×10^{-3} sec.,[14] or 1.47×10^{-3} sec.[15] in the vapor state. In water the same luminescence has been observed, but very weak, and with only one-thirtieth as long a life.[14] We shall now show that this luminescence is phosphorescence; in other words, it accompanies a transition from the triplet to the singlet state.

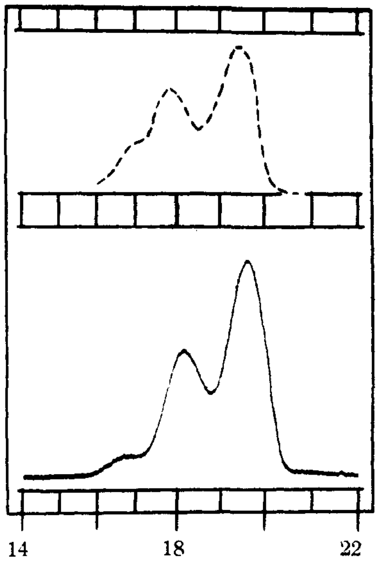

Fig. 14.—The phosphorescence emission spectrum of biacetyl. Lower curve is a tracing of our phosphorescence photograph in EPA at 90°K. The upper curve is redrawn from the work of Matheson and Zabor on the luminescence of biacetyl vapor at room temperature. Left-hand portions of both curves are considerably affected by photographic plate peculiarities.

The upper curve of Fig. 14 reproduces the luminescence spectrogram (redrawn) of biacetyl, in the vapor state at room temperature, obtained by Matheson and Zabor.[16] The lower curve is a tracing of our photograph of the phosphorescence of biacetyl in rigid EPA at 90°K. The resemblance is remarkable, except for the band farthest to the left, where the apparent maximum is largely influenced by plate sensitization. *In* the rigid EPA the life of the phosphorescence is of the order of 5×10^{-3} sec. That we are dealing with the same emission process in the two cases cannot be doubted.

The absorption spectrum of biacetyl in hexane was studied by Lardy,[17] and of the vapor by Almy and Anderson.[14] The vapor spectrum at 27° we have redetermined with the results shown in Fig. 15, in which the crosses indicate the individual measurements of Almy and Anderson. These authors, using an equation identical with equation (1) (since in this case $n = 1$), concluded that the luminescence process could not be the direct reverse of the absorption process, since the large area under the absorption curve leads to a calculated value of τ one hundredth or one thousandth as long as that which they measured.

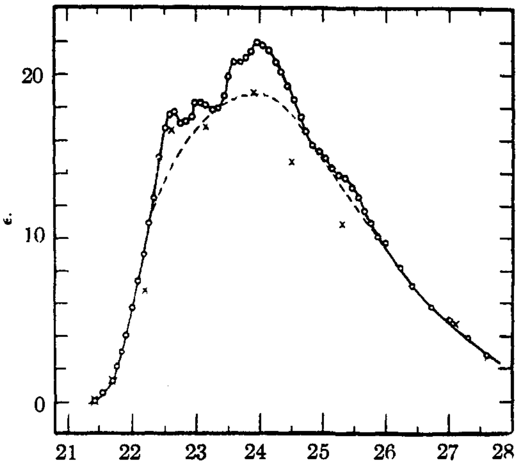

Fig. 15.—Molal extinction curve of biacetyl vapor at 27°. It is assumed that something like the dotted curve represents the S–S′ absorption, while the area above this dotted curve is due to S–T absorption. The crosses are points obtained by Almy and Anderson.

After seeing the numerous examples that we have already given, in which the finer structure of the S–T bands is superposed upon the normal S–S′ absorption, we have no difficulty in recognizing the curve of Fig. 15 as a composite of S–T bands and the very broad and smooth S–S′ band that is characteristic of all ketones. We have attempted, rather arbitrarily, to picture the S–S′ band by the dotted curve, and it is the area between the two curves that we must evaluate in making an estimate of τ. Furthermore Almy and Anderson took $g_u/g_l = 1$, whereas, now recognizing the upper state as triplet, we take $g_u/g_l = 3$. Finally, Almy and Anderson introduced a factor of 30, since they found 0.03–0.035 as the ratio between the number of photons reëmitted to the number absorbed. But this may mean that only 3% of the molecules that have absorbed light ever reach the triplet state, and does not imply that nearly all of the molecules in the triplet state may not reach the ground state by the phosphorescence process. The fact that τ changes very little, in going from the vapor to a rigid solvent at liquid air temperature, indicates that equation (4) is nearly valid.

Calculating from the area between our two

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cence, if our definition is to be extended to such simple particles. On the other hand a quasi-forbidden emission, without change of multiplicity, is not to be classed as phosphorescence; although, for example, the true fluorescence corresponding to the S–S′ absorption bands of benzene (Fig. 13, right) should, according to equation (3), show a faint afterglow with the most rapidly rotating phosphoroscopes that have been used.

(14) Almy and Anderson, *J. Chem. Phys.*, **8**, 805 (1940).

(15) Rawcliffe, *Phys. Rev.*, **59**, 915 (1941).

(16) Matheson and Zabor, *J. Chem. Phys.*, **7**, 536 (1939).

(17) Lardy, Dissertation, Zürich, 1924.

curves we find for τ a value about 2.5 times as short as the experimental value. The agreement is not perfect and is worse if we place the dotted curve lower than is done in Fig. 15. It is to be noted, however, that the separation of $\bar{\nu}_P$ and $\bar{\nu}_A$ of 3000 cm.$^{-1}$ is greater than in any other case that we have studied, and equation (3) can hardly be expected to retain its full validity under such extreme conditions.

From the average of $\bar{\nu}_P$ and $\bar{\nu}_A$, in the vapor state, we find E_T = 60 kcal., as compared with the 58 kcal. previously estimated merely from the phosphorescence in EPA at low temperature.

Acetyl propionyl was investigated in the hope that a better separation of S–T and S–S′ absorption would be found. In fact, the phosphorescence bands are at lower frequency, $\bar{\nu}_P$ = 18600 cm.$^{-1}$; but the absorption is also at lower frequencies, $\bar{\nu}_A$ in the vapor being 22500 cm.$^{-1}$. Otherwise the absorption and emission spectra are so nearly a duplication of those of biacetyl, that we shall not reproduce them here. From the average of $\bar{\nu}_P$ and $\bar{\nu}_A$, we find E_T = 59 kcal., as compared with our previous estimate of 55 kcal.

The phosphorescence of the vapors of biacetyl and acetyl propionyl suggests that similar phosphorescence can be found in the vapors of other organic substances of short phosphorescence life, especially at low pressure. With a longer life, the triplet molecule has a greater chance of experiencing some quenching collision before it emits phosphoresence. Concerning these quenching collisions, the study of which would be of great practical and theoretical interest, we know little at present. They seem to be nearly absent in rigid media,[18] judging by the several rough agreements that we have obtained between observed and calculated values of τ. They also seem to be rare in a vapor at low pressure. On the other hand, these quenching collisions are responsible for the loss of nearly all the triplet molecules in a fluid solvent, although such loss appears to be considerably diminished in solvents as viscous as glycerol and triethanolamine.

Experimental Part

Most of the substances were the same as those used in our preceding paper. The nitroso compounds, including the substance that we believe to be new, diisopropylbromonitrosomethane, were prepared by standard methods. Of these, the two aliphatic compounds are the least stable, but even these can be kept at room temperature in a solvent such as isopentane, in the presence of air, for a week or two, without appreciable decomposition.

The absorption measurements were made either with the spectrophotometer built in this Laboratory and previously described,[19] or with a Beckman Quartz Photoelectric Spectrophotometer Model DU. The former instrument was used with long cells and also for low temperatures. The latter was used for measurements of a wider spectroscopic range, at room temperature.

Summary

This paper deals mainly with the quasi-forbidden absorption bands corresponding to the emission bands of phosphorescence, the former being singlet–triplet as the latter are triplet–singlet transitions. As predicted, our experiments show that these new absorption bands differ markedly from ordinary bands of absorption, which involve only singlet–singlet transitions, and that they satisfy six criteria. These criteria deal with the position and width of S–T absorption bands, with the separation of their vibrational structure, and finally with the intensity of absorption. This intensity should be calculable from the lifetime of the corresponding phosphorescence emission, by a thermodynamic equation (3) which is novel only in that the refractive index of the medium is introduced.

This equation should be valid for any absorption and corresponding emission when the bands are narrow and single, but need not be so with the broad and multiple bands characteristic of the absorption and luminescence of organic molecules. It was tested for the case of ordinary absorption and fluorescence by means of the fluorescence lives of rhodamine B and fluorescein obtained by Gaviola, and of our own absorption measurements. Equation (3) was found to be valid within a factor of about 2. Within such tolerance we find, in numerous cases, that the intensity of S–T absorption may be calculated from the lives of the phosphorescent states.

When the phosphorescence life is short, S–T absorption may give rise to noticeable color and is shown to be the cause of the abnormal colors of thioketones and of nitroso compounds. Four compounds of the latter type were investigated and their S–T absorption satisfies most, and one, diisopropylbromonitrosomethane, satisfies all, of the criteria of such transitions. It is shown that the resonance of nitroso compounds in the triplet state is similar to that of molecular oxygen.

Also in the case of thiobenzophenone the group of S–T absorption bands and the group of T–S emission bands are both shown. But here the two groups are widely separated, the bands are very broad, and the spacing between the individual vibrational bands is different in the two groups, showing a different spacing of vibrational levels in singlet and triplet states. The color of other thioketones is discussed. The polymeriza-

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(18) Further evidence that in rigid media most of the molecules in the triplet state leave that state by phosphorescence, is afforded by the fact that we have studied a number of substances both at 90° and at 65°K. without noticeable change in life. However, in the case of the long lived phosphorescence of diphenylamine, the life was increased some 50% by going to the lowest temperature, showing that when the life of the triplet state is long the measured life may be considerably less than that which would be observed if there were no dissipative or quenching processes, even in a rigid medium.

(19) Lewis, Lipkin and Magel, THIS JOURNAL, **63**, 3005 (1941).

tion of thioketones and nitroso compounds is correlated with the energy of the triplet state.

Other substances of short lived phosphorescence, phenazine and dibenzalacetone, showed S–T absorption bands, but superposed upon the large bands of normal absorption. In looking for the extremely weak S–T bands of substances of long phosphorescence life, it was necessary to make an incidental study of some of the pure rotation–vibration bands that extend into the visible (Fig. 11). The whole absorption spectrum of liquid (and dissolved) benzene was determined between 12,500 Å. and 2200 Å., including four weak S–T bands. Similar S–T bands were found for *p*-dichlorobenzene.

The phenomenon of phosphorescence in nonrigid solvents is discussed, and in the last section it is shown that the luminescence of the vapors of biacetyl and acetyl propionyl is true phosphorescence. It is almost identical with the phosphorescence in a rigid medium at very low temperature. The absorption of light by these vapors was redetermined and is shown to be due to the superposition of narrow S–T bands upon the broad band of normal absorption.

BERKELEY, CALIFORNIA RECEIVED MARCH 7, 1945

[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 997]

The Serological Properties of Simple Substances. IX. Hapten Inhibition of Precipitation of Antisera Homologous to the *o*-, *m*-, and *p*-Azophenylarsonic Acid Groups

BY LINUS PAULING AND DAVID PRESSMAN

During their extensive studies of the properties of antisera prepared by injecting animals with artificial conjugated antigens containing groups of known chemical structure, Landsteiner and his collaborators investigated the effect of position of substituents in the benzene ring of haptens and haptenic groups on the precipitation reactions of antisera and azoproteins and the inhibition of precipitation by haptens.[1] They found that combination of antiserum and antigen or hapten is decreased by the presence of substituent groups in the precipitating antigen or the hapten in positions other than those occupied in the immunizing antigen. Similar results have been obtained in our studies.[2,3,4,5] In order to learn more about the nature of the intermolecular forces operative in these serological systems, we have extended our quantitative studies of hapten inhibition to include the reactions of antisera homologous to the *o*-, *m*-, and *p*-azophenylarsonic acid groups (hereafter called anti-R_o serum, anti-R_m serum, and anti-R_p serum, respectively) with the corresponding azo-ovalbumins R_o-ovalbumin, R_m-ovalbumin, and R_p-ovalbumin, and have obtained the results reported in this paper. The hapten-inhibition data have been interpreted by use of the heterogeneity theory[4] to provide values of the heterogeneity index σ and the hapten inhibition constant K_0' for each hapten with each system of antiserum and precipitating antigen, and it has been found possible to formulate a detailed discussion of the effective intermolecular forces which accounts in the main for the observed relative values of K_0'.

Experimental Methods

Haptens.—The *o*-chlorophenylarsonic, *m*-chlorophenylarsonic, 2,4-dinitrophenylarsonic, and 2,4-dichlorophenylarsonic acids were prepared by the Bart synthesis by Mr. George Cleland. The *o*- and *m*-(*p*-hydroxyphenylazo)-phenylarsonic acids were prepared by coupling diazotized arsanilic acid with a ten-mole excess of phenol at *p*H 9.5–10. The acids were finally purified by crystallization from 50% alcohol and washing the crystals with water acidified with hydrochloric acid. The rest of the haptens used have been reported previously.[6]

Protein Antigens.—The immunizing antigens used for inoculations except for diazotized *p*-arsanilic acid coupled with sheep serum were prepared by Mr. Allan Grossberg by coupling diazotized *o*-arsanilic acid or *m*-arsanilic acid with whole sheep serum. For each antigen 0.1, 0.2, and 0.3-g. portions of the amine were diazotized and coupled with three 70-ml. portions of sheep serum at *p*H 8.5. After the coupling was complete, the three products containing different amounts of hapten were mixed and purified by the method of Landsteiner and van der Scheer.[7] This was done to increase the possibility of using an azoprotein of optimum antigenicity. The antigen made by coupling diazotized *p*-arsanilic acid with sheep serum has been described previously.[8]

The azoprotein test antigens were made by diazotizing 0.2 g., 0.2 g., and 0.15 g. of *o*-, *m*-, and *p*-arsanilic acid, respectively, and coupling individually with 0.1-g. portions of crystallized hen ovalbumin at *p*H 10.0. The solutions were dialyzed overnight against running tap water and then precipitated at *p*H 3.0–3.5, redissolved at *p*H 10, reprecipitated at *p*H 3.0–3.5, and dissolved in 50 ml. saline to *p*H 7. The antigens thus prepared when analyzed were found to contain approximately 2.5% arsenic.

Antisera.—The preparation of anti-R_p serum has been described previously.[8] The anti-R_o and anti-R_m sera were prepared by a similar method. A single pool of each antiserum was used.

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(1) K. Landsteiner, "The Specificity of Serological Reactions," Charles C. Thomas, Springfield, Ill. 1936.

(2) D. Pressman, D. H. Brown and L. Pauling, THIS JOURNAL, **64**, 3015 (1942).

(3) D. Pressman, J. T. Maynard, A. L. Grossberg, and L. Pauling, *ibid.*, **65**, 728 (1943).

(4) L. Pauling, D. Pressman, and A. L. Grossberg, *ibid.*, **66**, 784 (1944).

(5) D. Pressman, S. M. Swingle, A. L. Grossberg, and L. Pauling, *ibid.*, **66**, 1731 (1944).

(6) D. Pressman and D. H. Brown, *ibid.*, **65**, 540 (1943).

(7) K. Landsteiner and J. van der Scheer, *J. Exptl. Med.*, **55**, 781 (1932).

(8) L. Pauling, D. Pressman, D. H. Campbell, C. Ikeda, and M. Ikawa, THIS JOURNAL, **64**, 2994 (1942).