

EMISSION SPECTROSCOPIC ANALYSIS OF ORGANIC COMPOUNDS*

É. V. SHPOL'SKIĬ

Usp. Fiz. Nauk 68, 51-69 (May, 1959)

1. The field of application of molecular spectroscopic analysis is unusually large, surpassing many-fold that of atomic spectroscopic analysis in its variety of possibilities of use. Physics and chemistry, especially biochemistry and petroleum geochemistry, medicine and biology, agriculture and the food industry—this is far from a complete list of the fields in which molecular spectroscopic analysis finds numerous and highly varied applications. However, although the tasks of molecular analysis are so great and so varied, the performance of these tasks lags far behind in the qualities of simplicity, definiteness, and reliability which are characteristic of atomic spectroscopic analysis. The reason for this lies in some very serious difficulties, which, in turn, originate in the very nature and character of molecular spectra. In particular, these difficulties are inherent in the character of the spectra of the class of molecules which is most interesting in practice, and in any case is the largest—the polyatomic molecules.

The features of molecular spectra which are unfavorable for analysis may be briefly summed up as: a) insufficient sharpness, or more strictly speaking, diffuseness, which prevents the determination of the band positions with the high degree of accuracy which is possible in the determination of the positions of atomic lines in gases; b) insufficient additivity, which is an obstacle to simultaneous analysis for many components, and is due in part to overlap of broad bands; c) insufficient individuality in a certain degree, which is sometimes, as we shall see, quite significant; this is the case even in the most individual spectra (and most often used in practice)—the vibration spectra.

As is known, the source of the complexity of molecular spectra is the wealth of degrees of freedom by which a molecule is characterized, in comparison with an atom. Further, as is known, in atoms the purely electronic states are the only type of states between which transitions occur to generate the spectrum. In a molecule, numerous vibration states and even more numerous rotation states are superimposed on each electronic state. At the present state of experimental technique, it is possible in principle to use each of these types of mo-

tion in order to obtain spectra, independently of the others. The transitions between purely rotational states correspond to the region of long-wavelength infrared or microwave radio; the transitions between vibrational states correspond to the region of infrared spectra, as well as the Raman spectra; finally, the transitions between electronic states give the visible and ultraviolet spectrum, which is actually a result of the combination of all of the forms of motion in the molecule.

2. Of the various methods of molecular analysis* which are associated with these different types of motion, each has its values and its defects. We shall hereinafter disregard the rotational states and their spectra, since in large polyatomic molecules the rotation levels are so close together that they actually merely coalesce, and as a consequence, "spoil" the electronic spectra in gases. From the viewpoint of the problems of qualitative analysis, the greatest possibilities are found in the vibration spectra, i.e., in the infrared and Raman spectra, since these spectra are the most individual and the least dependent on the external conditions. In the popular literature, these spectra are often even compared with fingerprints. The significance of vibration spectra in molecular analysis is indeed enormous, and I shall not try to belittle it, although it must not be exaggerated, either. We shall consider this question in somewhat more detail.

Infrared spectroscopic analysis is based on the existence of the so-called "characteristic frequencies." These, for example, are the frequencies of the valence-bond vibrations of certain atomic configurations ($C=O$, $C=H_2$, $C\equiv H_3$, etc.). They have definite values which are independent in the first degree of approximation of the molecule in which the given group occurs. The existence of such frequencies is the basis of "group analysis," i.e., the establishment of the presence of groups of atoms with certain bonds in the sample being studied from the presence of absorption bands lying in certain narrow frequency ranges in the infrared spectrum. Under certain limiting conditions (see reference 1, p. 527), infrared spectra permit one to solve the significantly more diffi-

*We shall consider hereinafter only the methods of qualitative molecular analysis.

*Expanded from a paper read on Nov. 23, 1958.

cult problem of the identity of individual substances, in the pure state or mixed with other substances. However, the matter is far from being so simple. If one disregards completely certain difficulties of method,* essential difficulties remain which are related to the nature of the spectra themselves. Thus, for example, the very concept of characteristic frequencies of certain atomic configurations is subject to a number of limitations: The characteristic frequency varies somewhat depending on the presence of other groups in the molecule (to give a concrete example, the frequency of the carbonyl bond $C=O$ in aldehydes is 1720 cm^{-1} , while in carboxylic acids it is 1652 cm^{-1}). Further, when resonance occurs with other groups in the given molecule, the frequency, which otherwise is "characteristic," may vary to the point of unrecognizability.†

With regard to the identification of individual substances by their infrared spectra, such an identification has been carried out in a limited number of cases with the aid of a compilation (atlas) of previously measured spectra of substances of maximum purity. It even appears that for some organic substances, the infrared spectra do not possess any marked individuality. The broad and important class of aromatic hydrocarbons shows this analytically unfavorable characteristic. In 1951 Cannon and Sutherland³ showed by measuring the infrared spectra of more than 120 aromatic compounds that these spectra differ only in very fine details, whereas with the compounds with many fused rings, the spectra are generally extremely poorly individualized. In summarizing their results, the authors emphasized that "the problem of distinguishing the various aromatic compounds by their infrared spectra is difficult in the highest degree," and indicated that the auxiliary method in this case must be the study of the ultraviolet absorption spectra.

Along with the infrared spectra of vibrational origin, there are also the Raman spectra. We shall not spend the time here to compare both methods from the viewpoint of their applications to chemical analysis (such a comparison is given, for example, in the book of Harrison, Loofbourow, and Lord,¹ § 276); each has its own advantages and its defects. However, insofar as both methods are based on the determination of the vibration frequencies of the normal state, the abovementioned difficulties in the identification of substances by their vibration

spectra are general for both methods. We must note that the availability of high quality photoelectric instruments must substantially facilitate the dissemination of this valuable method. An important role must also be played by the extensive series of studies on the Raman spectra of about 150 individual hydrocarbons which occur in light motor fuel, and by the development of methods of measuring the intensities of Raman lines. A detailed critical discussion of these methods is given in the monograph by G. S. Landsberg, P. A. Bazhulin, and M. M. Sushchinskii,⁴ in which the data on the intensities and other basic parameters of the Raman lines of about 300 individual hydrocarbons are compiled in tables.

Of great interest are the following conceptions of Bazhulin and Sushchinskii,⁵ leading members of the group under Landsberg, on the reasons for certain difficulties associated with analysis by means of Raman spectra: "The important characteristic of Raman spectra for analytical applications is the fact that all of the lines in these spectra are distributed within a relatively narrow spectral interval, amounting to not more than 300 Å for the most interesting spectral region. Within this spectral interval are distributed several tens of lines possessing appreciable widths (from 2 to 20 cm^{-1}). Hence, in the analysis of mixtures, the lines belonging to different components often overlap. Thus, the possibility is lost of using the lines which are analytically most interesting for quantitative analysis. It is also of importance in the analysis of mixtures of hydrocarbons that the lines of some classes of hydrocarbons are significantly more intense than those of other classes. For example, the lines of the aromatic hydrocarbons and the six-membered naphthenes are stronger than those of the paraffins and five-membered naphthenes by factors of tens or hundreds."

3. The preceding remarks, of course, did not have the purpose of "dethroning" the methods of molecular analysis based on the study of vibrational spectra. The fruitfulness of these methods is brilliantly illustrated by their widespread distribution. However, it is obvious that the variety of substances and of their properties is so large, particularly among the organic compounds, that it is unthinkable to conceive of a single method which is suitable for analysis in all cases. On the other hand, the possibility of applying any certain method is often limited by the optical properties of the given class of objects. For example, as has been mentioned, the use of infrared spectroscopy for qualitative analysis of aromatic hydrocarbons is clearly unsuitable. The application in this case of Raman spectroscopy is even less suitable, since, in addition to showing

*See, for example, Harrison, Loofbourow, and Lord, *Practical Spectroscopy* (Russ. trans.), IL, 1950, p. 524 ff.

†A detailed analysis of the far from simple concept of characteristic frequencies has been given by L. S. Mayants.²

common frequencies of their normal modes of vibration, these hydrocarbons commonly fluoresce strongly; this makes the application of Raman scattering generally impossible. Obviously, it is in general sound practice to make use of all the types of spectra, taking into account which of these are the most favorable in their properties for the identification of the given type of molecule.

With regard to the electronic spectra lying in the visible and near ultraviolet regions of the spectrum, these have attained widespread application. Ordinarily, absorption spectra of the substance in solution at room temperature are used. When the qualitative composition is known, quantitative determinations by electronic spectra are possible with a high degree of accuracy, which apparently exceeds the accuracy of all other methods of spectroscopic analysis.^{7,8} However, a serious drawback for widespread use in qualitative analysis is the extreme diffuseness of the visible and ultraviolet absorption spectra of complex molecules, as had been mentioned at the beginning of this article. It is this diffuseness which is the reason for the insufficient definiteness of molecular analysis, whereby qualitative molecular analysis is not comparable with qualitative atomic spectroscopic analysis.

4. In speaking of molecular analysis we have heretofore had in mind the existing methods based on the use of absorption spectra; these methods of molecular analysis are the opposite, in a certain sense, of the emission methods of atomic analysis.

I wish to show that the neglect of emission spectra in molecular analysis is not only unjustified, but that there exist undoubtable opportunities for using emission spectra of molecules for the most delicate analysis of organic compounds.

In order that we may convince ourselves of this, we must examine the following questions:

1) Keeping in mind the topic of the luminescence of organic compounds, what are the most suitable methods of excitation of the luminescence?

2) How broad is the class of molecules which can be made to luminesce, and what are the conditions facilitating the appearance of luminescence or the increase in the luminescence yield?

3) Can conditions be established, such that the normally diffuse spectra of complex molecules will become as discrete as the atomic spectra in the gaseous state, or at least, as discrete as the Raman spectra?

a) The answer to the first question is quite definite. Although the luminescence of organic compounds may be elicited by different methods (electron bombardment, high-velocity particle radiation,

gamma-rays, x-rays, and finally, light), the most suitable excitation is that by light. The reason for this is the fact that optical excitation is not only the mildest, but also the most selective method of excitation, and in a number of cases may be used very conveniently in analytical applications. Thus, the luminescence in question is photoluminescence, and analysis by means of this luminescence is one of the types of luminescent analysis.

The simplest form of luminescent analysis is visual analysis according to the color of the luminescence. This has for a long time earned widespread use because of its unusual simplicity and its great sensitivity.⁹ However, although such a primitive method is in a number of cases very useful, it is not suitable for responsible identification and particularly for the distinguishing of substances which differ essentially in their nature and properties. This is because very many substances (especially organic ones) show the same color of luminescence. Hence, emission molecular analysis must necessarily be a spectroscopic analysis.

b) The ability to luminesce, and in particular to luminesce within a spectral region suitable for observation (the visible, the quartz ultraviolet, and the near infrared), is very widely distributed among organic compounds. However, it is known that organic compounds exist, including also colored compounds, which do not fluoresce at room temperature, either in the crystalline state or in solution. Nevertheless, it is possible in these cases, at least in some of them, to establish conditions, such that fluorescence is observed with a satisfactory quantum yield. A good illustration of such a case is the following observation of P. P. Feofilov.⁹ The triphenylmethane dyes (e.g., fuchsin) possess a structural skeleton containing three benzene rings connected by single bonds to a central carbon atom. They do not fluoresce in solution, apparently owing to the possibility of free rotation of the benzene rings about the single bonds. However, if such a dye is incorporated into a solid medium, it begins to luminesce with a good quantum yield. Favorable conditions for the appearance of luminescence may be attained also in solutions frozen to very low temperatures. Here the molecules become fixed in the matrix, so that the possibility is eliminated of radiationless redistribution of the excitation energy over an excessively large number of degrees of freedom. We shall see below that it is in frozen solutions, with the proper choice of solvent, that favorable conditions appear for the delicate qualitative analysis by emission methods of organic compounds.

From what has been said, it follows that under suitable conditions, the class of organic compounds

which are accessible for emission analysis is rather broad. It includes many important types of substances, especially those with unsaturated bonds, for example, the aromatic hydrocarbons, and many biochemically important substances.⁷

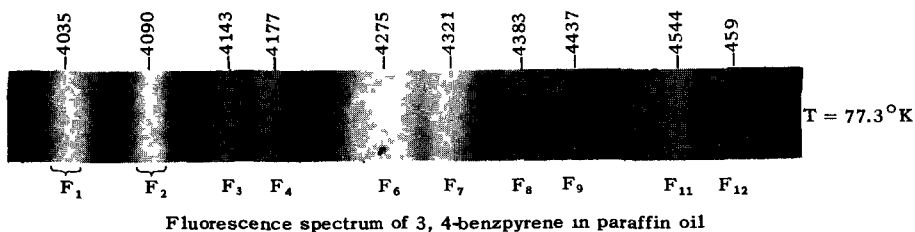
c) At first glance, the answer to the third question must be negative. In fact, it has been known for a long time that very many organic compounds (in particular, the dyes) give a broad structureless continuum in the fluorescence spectrum, extending over a large part or even all of the visible spectrum. Further, even in the 'twenties, S. I. Vavilov noted the fact that this spectrum essentially follows a standard statistical curve which is completely devoid of individuality. Great advances have been made in recent years in the interpretation of these continuous spectra of complex molecules, due to the studies of B. S. Neporent and B. I. Stepanov.¹⁰⁻¹² In particular, a convenient classification of molecules characterized by such types of diffuse spectra has been made. The basis of this classification is the fact that not all polyatomic molecules are subject to the law of mirror symmetry of their absorption and fluorescence spectra on the frequency scale. Molecules showing other relations between the absorption and fluorescence spectra also exist. Polyatomic molecules of this type were provisionally designated as "complex" by B. S. Neporent.

Extensive experimental and theoretical studies have been carried out by B. S. Neporent¹² and his associates on an example of the aromatic compounds belonging to the class of "complex" molecules—the derivatives of phthalimide. These studies have established the following important fact: the complex spectra of these molecules are characteristic of them not only in the condensed state, but also in the gaseous state. Here the spectrum does not depend on the vapor pressure, which was varied over wide limits, even to the point of such rarefaction that gas-kinetic collisions with other particles were eliminated. This shows that the occurrence of continuous spectra for the molecules belonging to this class is a result of intramolecular interactions, i. e., they are inherent in the molecules themselves. Obviously, the perspective of applying spectro-analytic methods to this type of polyatomic molecules is not favorable. I note parenthetically, however, that I am not at all convinced that the condition is completely hopeless, even in this clearly unfavorable case, or that there are no possibilities of "simplifying," so to speak, the spectra of these molecules. Here, conditions might be established in which the statistical curves of these spectra, which are completely devoid of

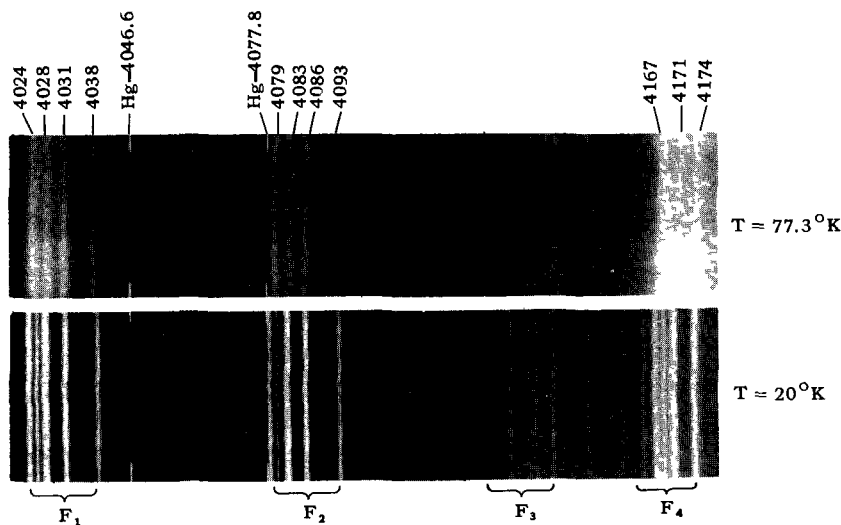
individuality, would be replaced by completely individual spectra.

However, other types of polyatomic organic molecules exist, in particular those in which the diffuseness of the spectra in the condensed state (e. g., in solutions) is caused by the influence of the surrounding medium (e. g., the solvent). Among these molecules are the polycyclic aromatic hydrocarbons, certain simple substances containing the carbonyl group C=O (anthraquinone, benzophenone), and certain heterocyclic compounds. All these substances show the following interesting characteristic: if they luminesce in the crystalline state, their fluorescence spectra are continuous and strongly shifted toward longer wavelengths with respect to the spectra in the vapor or in solution. In solution, the spectrum possesses a certain degree of structure, consisting of several broad bands at room temperature. Obviously, in such cases the possibility of identifying the substance analytically by its spectrum is improved. Even more favorable conditions are realized on freezing the solution and chilling it to a low temperature, for example, that of liquid nitrogen (77.3°K). Then the bands become more narrow; their intensities increase, and the spectrum sometimes acquires a highly characteristic structure. In our laboratory the luminescence spectra of a number of such compounds have been studied: derivatives of pyrene, derivatives of anthracene with substituents in the 9, 10-positions, and certain derivatives of acridine. All of these studies have been made in alcohol or oil solutions at 77.3°K.^{14, 15} An example of an especially characteristic spectrum is that of the pentacyclic aromatic hydrocarbon 3, 4-benzopyrene, a very important carcinogenic substance found in various sorts of tars. The spectrum of this substance dissolved in paraffin oil, at 77.3°K, is shown in Fig. 1, and consists of an alternating series of strong and weak doublets, some of which are superimposed on a weak background. One must note that the width of the bands in this spectrum, even at low temperature, amounts to 100—150 cm⁻¹. That is, the width is quite significant, and as we shall see, in some cases creates conditions which are unfavorable for the exact identification of the substance by its spectrum.

5. In cases in which finer indications are needed for positive identification of a substance, one may make use of a phenomenon discovered several years ago by me in conjunction with A. A. Il'ina and L. A. Klimova. It happens that, if suitably chosen normal paraffins (from pentane to decane) are taken as the solvents, then in frozen solutions at 77.3°K the broad



Fluorescence spectrum of 3, 4-benzpyrene in paraffin oil



Part of the fluorescence spectrum of 3, 4-benzpyrene

FIG. 1a. The fluorescence spectrum of 3, 4-benzpyrene in paraffin oil at 77.3°K. Fused glass spectrograph. Linear dispersion in the original is 27 Å/mm at 4000 Å. The wavelengths of the band maxima and the designations taken from reference 14.

FIG. 1b. Part of the fluorescence spectrum of 3, 4-benzpyrene in n-heptane at 77.3°K and 20°K. Plane diffraction grating, second order. Linear dispersion in the original ~ 4.9 Å/mm. For designations of bands see Fig. 1a.

bands are split into series of narrow lines. The widths of these lines correspond approximately to the widths of the lines in Raman spectra, and sometimes even to those of the lines of the mercury spectrum in high-pressure mercury lamps (the PRK type of lamps). Without going into a detailed discussion of the phenomenon, we shall note that the solvent, when frozen in the form of a finely crystalline mass, plays here the role of a matrix in which the radiating molecules are fixed in a definite fashion. To a first approximation, these molecules form a "frozen" oriented gas at high rarefaction. We emphasize that the freezing of the solvent in the form of a crystalline mass (actually in the form of microcrystals) is the necessary condition for the appearance of discrete spectra. Conversely, in solvents which give transparent glasses on freezing (mixtures of alcohols, certain special solvent mixtures), the lines broaden and fuse together, with the result that broad bands are obtained, like the bands of benzpyrene in the spectrum in Fig. 1a.

In Figs. 1b and 2a, b are given several examples of quasilinear spectra which are of interest to us now. These were obtained at the temperatures of liquid nitrogen (77.3°K) and liquid hydrogen (20°K). One may see that the lines are so narrow that their positions may be determined without difficulty to a high degree of accuracy (at 77.3°K to 1 Å or better). When the temperature is lowered to 20°K, further

narrowing of the lines takes place, with a corresponding increase in the possibility of more accurate measurements. However, undoubtedly, it is quite sufficient for practical purposes to lower the temperature to 77.3°K.

The essential facts are that these spectra are absolutely reproducible, strictly individual, and possess complete additivity. Although the lowering of the temperature to 20°K introduces certain additional features in the form of extra lines, it does not change the individual character of the spectrum. As we shall see below, the strict individuality of these spectra has been excellently confirmed in a number of cases in which a substance identified in this way, obtained naturally or artificially, could be identified also in an independent manner (chemical or biological).

6. The obtaining of sharp, quasilinear spectra of complex molecules opens up possibilities of using them for the determination of the normal vibration frequencies of unexcited molecules (i.e., for study of the structure of molecules), as well as for analytical purposes. For both purposes, i.e., both in analytical applications and in the study of molecular structure, a certain characteristic of emission spectra of complex molecules plays an important role, and consists in the following. In atomic spectra, as well as the electronic spectra of simple diatomic molecules, any excited level

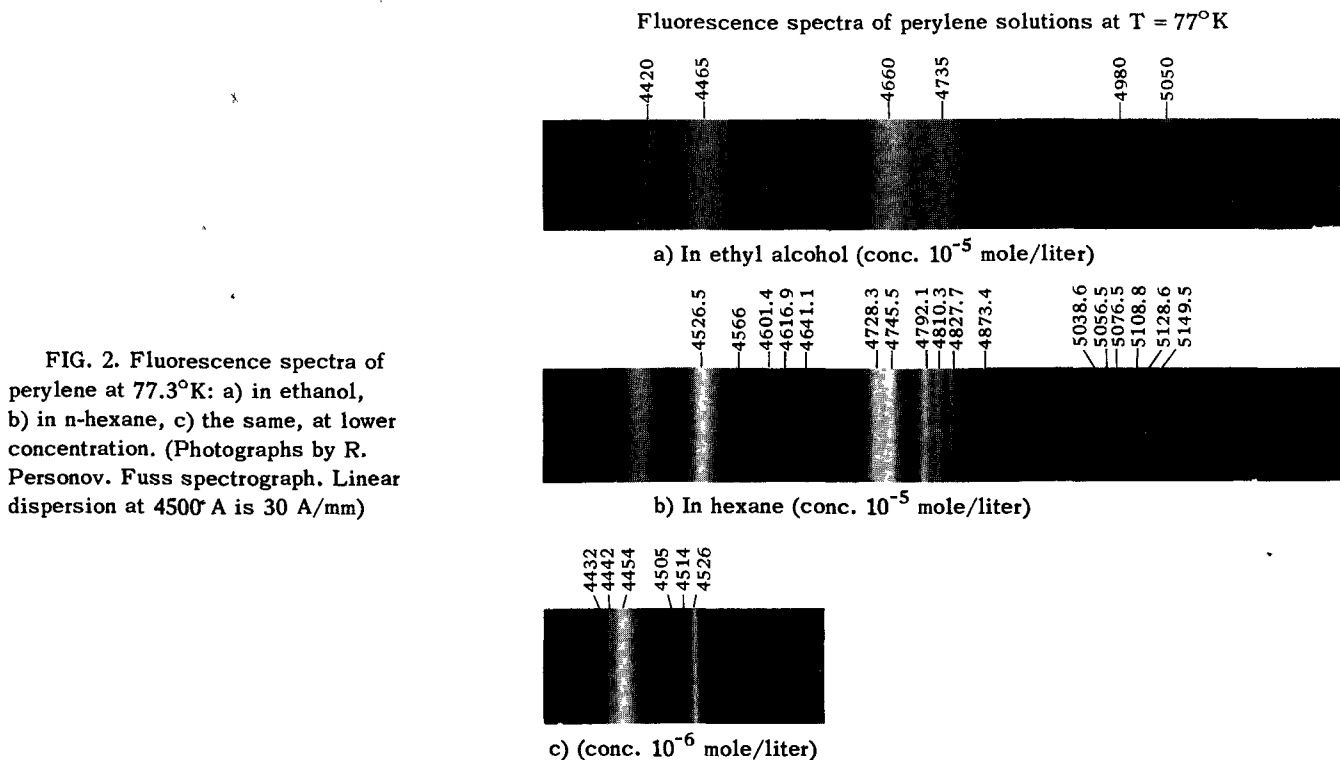
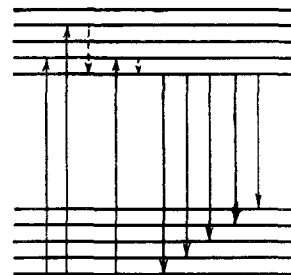


FIG. 2. Fluorescence spectra of perylene at 77.3°K : a) in ethanol, b) in n-hexane, c) the same, at lower concentration. (Photographs by R. Personov. Fuss spectrograph. Linear dispersion at 4500 \AA is 30 \AA/mm)

may be the final level for absorption, and likewise the initial level for emission if, of course, the transition is permitted by the selection rules. Here a downward transition, i.e., with emission, may take place either at once or in two stages: first a transition to an intermediate level, then from the intermediate level to the ground state, both transitions being accompanied by emission of radiation. Such stepwise transitions take place, for example, in mercury atoms excited either by electron impact or light, as has been shown by the well-known experiments of Wood. In complex molecules, transitions from the ground state with absorption, i.e., upward transitions, may also take place to any level for which the transition is permitted by the selection rules. To the contrary, radiative transitions, i.e., downward transitions, generally occur in two stages (Fig. 3). Here, in the first stage, a transition occurs without radiation (internal conversion) to the lowest excited level of the given multiplicity. Radiative transition only occurs from this lowest level.²⁰ This empirically established rule has only very rare exceptions. Thus, for example, azulene (an isomer of naphthalene), in which the lowest singlet level occurs at a height of $14,000\text{ cm}^{-1}$ above the ground state, does not, in general, show fluorescence from this level; such fluorescence should begin at 7100 \AA and extend into the infrared. On the contrary, azulene shows fluorescence in the ultraviolet, distributed in the range $3571\text{--}4000\text{ \AA}$. This observed fluorescence takes

FIG. 3. Diagram of the origin of fluorescence spectra of complex molecules. Dotted lines indicate radiationless transitions (internal conversion).



place not from the first, but from the second excited electronic level. However, from our present point of view, this and analogous exceptions do not play an essential role, since the rule whereby radiative transitions always take place from one definite level remains in effect. The number of possible combinations is thus sharply limited, and the spectrum of a complex molecule acquires a remarkable simplicity. It is easy to see that a spectrum of this type comprises an electronic vibrational sequence of levels of the lowest state. Its degree of complexity is determined by another set of selection rules, which permit transition to only a few of the very large number of levels of the normal modes of vibration. These selection rules (along with the selection rules which forbid combination of levels of different multiplicities and are associated with the law of conservation of angular momentum) are primarily the result of the symmetry properties of the molecule. Generally speaking, the higher the symmetry of the molecule, the greater the number of forbidden transitions, and the simpler the spec-

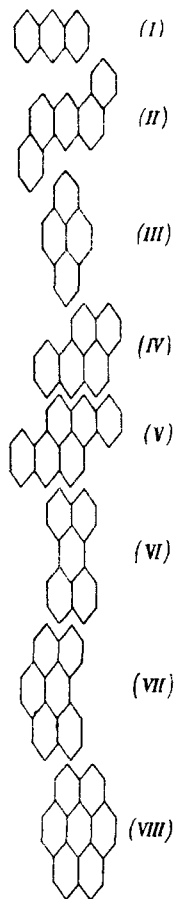


FIG. 3a. 1) Structural formulas of organic compounds mentioned in the text. Anthracene (I); 1, 2, 5, 6-dibenzanthracene (II); pyrene (III); 3, 4-benzpyrene (IV); 3, 4, 8, 9-dibenzpyrene (V); perylene (VI); 1, 12-benzperylene (VII); coronene (VIII).

trum. A graphic example lies in the comparison of the spectra of coronene (Fig. 3a, VIII) and 1, 12-benzperylene (VII). Coronene possesses a high degree of symmetry, and its spectrum is characterized by unusual simplicity. In fact, in the "permitted" singlet-singlet spectrum at the lowest temperatures, 40–50 lines of varying intensities may be counted; approximately the same number of lines may be counted in the "forbidden" triplet-singlet spectrum. The absence of one benzene ring in 1, 12-benzperylene, as compared with coronene, destroys the symmetry of the structure of the latter, and correspondingly, the number of lines for 1, 12-benzperylene increases to several hundred.³⁶ Analogous relations exist between the spectra of pyrene (III) and 3, 4-benzpyrene (IV), in that pyrene, whose molecule is more symmetrical than that of benzpyrene, has a correspondingly simpler spectrum. Besides, I wish to emphasize that, in all these cases, the spectra are fully resolved under the technique which we have used, i.e., the spectra consist of narrow lines rather than of broad bands.

7. The undoubted fact that the emission spectrum takes place from one single upper level has the consequence that the vibration frequencies of the ground state may be obtained by taking the differences between the frequencies of the lines observed in the luminescence spectrum and that of

one of the lines corresponding to the transition between the zero-point vibrational levels (0 — 0 transition). A vibrational analysis of a number of spectra of aromatic hydrocarbons has been carried out on the basis of this rule, and has revealed the occurrence of common frequencies for different molecules (see, for example reference 37).

Thus the peculiarity of the normal vibrations of polycyclic aromatic molecules, noted in the paper of Cannon and Sutherland mentioned above, has been confirmed by this method. Although this peculiarity causes a serious hindrance in analysis based on infrared spectra, no difficulties at all arise from the existence of common frequencies in the case of the electronic vibrational emission spectra. In fact, while the positions of the lines in the infrared spectrum are completely determined by the vibration frequencies of the molecule, these frequencies appear in the electronic spectra as the differences between the frequencies of the lines and that of the line for the 0 — 0 transition. Thus, these lines themselves are distributed in various regions of the spectrum; their position in the spectrum depends basically on the height of the lowest excited electronic level (0 — 0 transition), and is only secondarily determined the vibrational frequencies.

8. We shall discuss now several examples of analyses carried out by means of the emission spectra of organic compounds.

a) Carcinogenic substances, in particular 3, 4-benzpyrene

A series of chemical compounds is known which produces malignant tumors in man and in animals.²⁸ These so-called carcinogenic substances are found, for example, among the polycyclic aromatic hydrocarbons (special activity is shown by 20-methylcholanthrene, 3, 4-benzpyrene, 9-methyl-1, 2-benzanthracene, and some others). The reason why certain chemical compounds show carcinogenic properties is at present still unknown. Further, it sometimes happens that substances which are similar in structure or even isomeric are sharply distinguished in their carcinogenic activity. Examples of this are two isomers of benzpyrene, of which one (3, 4-benzpyrene) is highly carcinogenic, while the other (1, 2-benzpyrene) is not carcinogenic at all.⁴⁰ Finally, it is known that different carcinogenic substances are sometimes specific with regard to the type of malignant tumor which they produce.*

*The aromatic hydrocarbon 3, 4, 8, 9-dibenzpyrene is found in coal tar. Recently it was found to be present in tobacco smoke, in which 3, 4-benzpyrene also occurs. According to the data from the laboratory of Prof. Lacassagne in Paris,²⁵ 3, 4, 8, 9-dibenzpyrene causes sarcoma in mice.

possibility of applying it has been facilitated by the fact that most polycyclic aromatic hydrocarbons have fluorescence spectra consisting of several intense bands. An idea of the character of these spectra may be given by the spectrum of 3, 4-benzpyrene shown in Fig. 1. We note at this point that a precise analytical identification of a given substance is very important, in view of the specificity of the carcinogenic activity of various aromatic hydrocarbons, as indicated above. However, in solutions at room temperature, the substances of this class sometimes have similar spectra. Examples of this are 3, 4-benzpyrene, which has already been mentioned several times, and 3, 4, 6, 7-dibenzpyrene; they have very similar spectra, which are difficult to distinguish because of the great width of the spectral bands.

In view of the fact that 3, 4-benzpyrene is found rather often in tars, and even in certain food products, the development of highly sensitive and delicate methods of analysis for this hydrocarbon has become especially important. A method for detection of 3, 4-benzpyrene has been developed in detail by A. A. Il'ina.²⁰ To increase the selectivity and sensitivity of the method, the freezing of solutions in paraffin oil of the materials being studied was utilized. In order to establish the limits of sensitivity of the method, model experiments were carried out in which definite amounts of benzpyrene were added to bitumen (which shows a continuous fluorescence spectrum), and fluorescence spectra were taken. It was found that traces of the strongest band of benzpyrene at 4035 Å appeared even at a concentration of $10^{-3}\%$, while at a concentration of $5 \times 10^{-3}\%$, the presence of the band at 4035 Å and weak traces of the band at 4320 Å could be found. In Table I, taken from the paper by Il'ina,²⁰ an example is given of the analysis of a tar demonstrating the presence of 3, 4-benzpyrene and 1, 2, 5, 6-dibenzanthracene. It is not beside the point to mention here that, in this case, the positive identification of dibenzanthracene was made possible by means of the phosphorescence spectrum of this substance. A further perfection of the method of determination of 3, 4-benzpyrene was carried out by P. P. Dikun. He used a preliminary chromatographic fractionation to increase the sensitivity of the method, and in order to improve the reliability of the identification, he applied the method developed in our laboratory of obtaining line spectra

Spectro-fluorescent analysis has already been applied for a long time for the identification of carcinogenic substances (Hieger, Sannié, etc.). The from frozen solutions in normal paraffins. The latter improvement in the method is essential

TABLE I. An example of the analysis of a tar (extract from soot obtained by burning coal).

λ_{max} at 18°C	λ_{max} at -18°C	Identification*	Remarks
—	396	DBA (394.6) γ	Fluorescence spectrum
—	399		
405	404	BP (403.5)	
—	407	DBA (406.2) γ	
—	409		
—	412		
415	416	DBA (417.2) γ	
420	420		
—	424		
—	428	BP (427.5)	
432	432	BP (432.1)	Phosphores- cence spec- trum
—	435		
—	439		
—	445	BP (445)	
—	549	DBA (548)	
—	561	DBA (560.7)	
—	573		
—	605	DBA (603.6)	
—	620		
—	635		

*Abbreviations: DBA — 1, 2, 5, 6-dibenzanthracene; BP — 3, 4-benzpyrene.

since, as has been indicated, the bands are still rather broad ($100 - 150 \text{ cm}^{-1}$) in frozen solutions in paraffin oil or ethanol. Thus, at low concentrations of the product being sought, it is possible to confuse the strongly carcinogenic 3, 4-benzpyrene with related carcinogenic or noncarcinogenic products having similar spectra. We quote from the paper of P. P. Dikun²¹ at the Sixth All-Union Conference on Luminescence (Leningrad, February, 1958) the following ideas: "Even with the application of preliminary fractionation, doubts sometimes arise as to the correctness of the identification of 3, 4-benzpyrene. This is associated with the relatively great width of the bands in the fluorescence spectrum of 3, 4-benzpyrene, with the presence in some products of compounds having fluorescence spectra similar to that of benzpyrene, and also with the fact that one cannot always isolate a pure enough 'benzpyrene' fraction. Some investigators in such cases state that they have shown the presence of a 'benzpyrene-like substance'.* However, from the standpoint of the oncologist, it is absolutely necessary to know whether we are dealing in a given case with 3, 4-benzpyrene or with a similar substance, since a compound with the most insignificant difference in chemical structure may be completely noncarcinogenic.

*Latarget et al., Bulletin Cancer, 43/2 (1956).

TABLE II. Examples of the analysis for 3, 4-benzpyrene by means of the quasilinear fluorescence spectra in frozen (77° K) hexane solutions. (P. P. Dikun,²¹)

Wavelengths of the lines in the fluorescence spectrum of 3, 4-benzpyrene (A)	Wavelengths of the lines in the fluorescence spectra of benzpyrene fractions (A)		
	Fraction from polluted air	Fraction from the tar component of tobacco smoke	Fraction from shale tar
4015.7	4015.7	4015.6	4015.7
4021.3	4021.2	4021.5	4021.4
4024.7	4024.7	4025.0	4024.7
4028.5	4027.9	4028.5	4028.3
4031.2	4031.5	4031.4	4030.8
4070.8	4070.4	4071.0	4071.1
4076.3	4076.4	4077.1	4076.9
4080.3	4080.1	4080.0	4080.9
4160.1	4159.9	4159.1	4160.0
4163.7	4163.7	4163.9	4163.7
4167.8	4167.7	4168.3	4167.8
4218.4	4218.7		4218.7
4221.7	4222.3		4221.9
4225.6	4225.5		4225.6
4252.4	4252.4	4252.3	4252.2
4258.6	4258.6	4259.1	4259.2
4264.0	4263.8	4263.6	4263.5
4268.9	4268.7	4268.7	4268.4
4294.8	4294.4	4294.8	4294.6
4298.9	4299.0	4299.0	4298.9
4303.7	4303.3	4305.4	4304.8
4308.2	4308.0	4309.3	4308.5

In order to solve the problem in such doubtful cases, we use the fluorescence spectra of solutions of 3, 4-benzpyrene in n-hexane and n-octane frozen in liquid nitrogen, as described by É. V. Shpol'skiĭ and his associates.⁵ In order to test the reliability of this method, we have compared the wavelengths of the principal lines in the fluorescence spectrum of frozen solutions of 3, 4-benzpyrene in n-hexane and n-octane with the wavelengths of the lines obtained under the same conditions in the fluorescence spectra of the 'benzpyrene' fractions from five different products."

In Table II an example is given of the qualitative analysis for benzpyrene by means of preliminary chromatography and freezing of the solution in n-hexane. It may be seen that the lines of the "benzpyrene fractions" coincide with the lines of pure benzpyrene to an accuracy of tenths of an Angstrom unit, such that the spectroscopic identification of 3, 4-benzpyrene is absolutely reliable. S. G. Bogomolov has successfully applied the same methods in the analysis of the smoke discharged from factory chimneys for carcinogenic hydrocarbons. According to his data, benzpyrene may be detected even at a concentration of 10^{-9} g per gram of material.³⁸

b) The detection of aromatic hydrocarbons in bitumens and in petroleum fractions

The study of the individual compounds found in petroleum or in the organic material in sedimentary strata is highly important in the general plan of the studies on the genesis of petroleum. In this respect, the presence in extracts of tertiary clays of appreciable amounts of the individual pentacyclic aromatic hydrocarbon perylene, which was found by A. A. Il'ina,²² is of interest. The essential point for us in this case is the methodology of this work. In view of the fact that the bands of this hydrocarbon under ordinary conditions (solutions in chloroform, ethanol, and other solvents) are very broad even at low temperatures (Fig. 4), the original identification by the fluorescence spectrum of the chloroform extract could not be considered completely reliable.

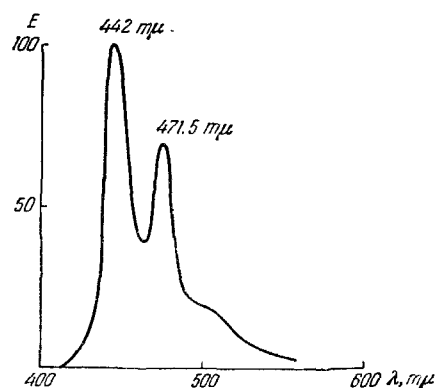


FIG. 4. Fluorescence spectrum of chloroform extracts of bitumens from clays of the Upper Maikop deposits.²³ The numbers indicate the wavelengths of the fluorescence band maxima, which are close to those of perylene in alcohol solution at 77.3°K.

Hence, Il'ina made use of the fact that frozen solutions of perylene in normal paraffins give a line spectrum. From the coincidence of the wavelengths of 14 lines in the fluorescence spectra of extracts from oil-bearing clays (transferred to solution in n-hexane) with the corresponding lines in the spectrum of pure perylene, she was able to prove the presence of perylene with absolute assurance.

I should remark that the line spectrum of pure perylene in frozen hexane solution was first measured at Oxford by E. Bowen,³⁶ who in two papers has confirmed and expanded our results with regard to the possibility of obtaining line spectra of aromatic hydrocarbons in frozen paraffin solutions. At present, the emission and absorption spectra of pure perylene, a sample of which was kindly given to us by Prof. I. Ya. Postovskiĭ, are being studied by Graduate Student R. I. Personov. In particular, R. I. Personov has repeated the comparison of the spec-

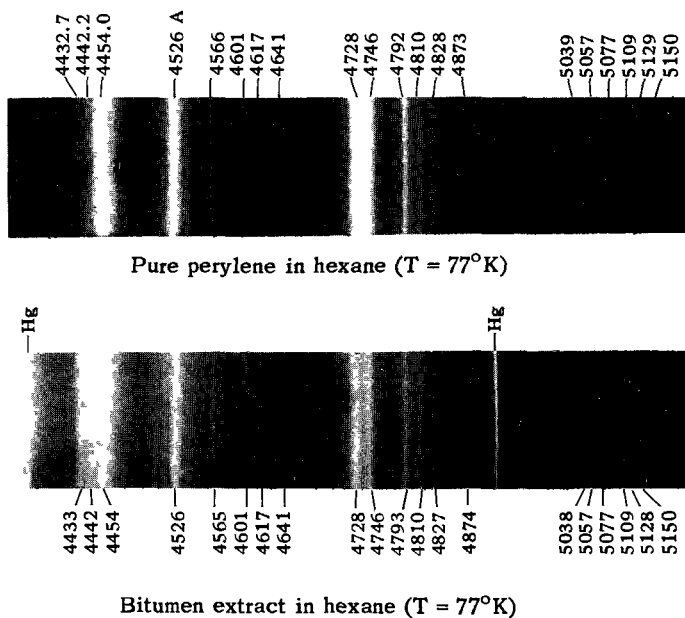
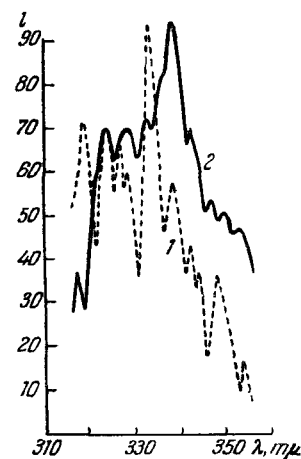


FIG. 5. Comparison of fluorescence spectra of bitumen extract and pure perylene at 77.3°K. Solvent is n-hexane. (Photographs by R. Personov.)

tra of the extracts obtained by A. A. Il'ina with the spectrum of pure perylene. His results are given with the wavelengths in Fig. 5. The coincidence of the wavelengths leaves no doubt as to the reliability of the identification.

Kh. I. Mamedov²³ has applied spectro-luminescent analysis for the identification of aromatic substances in petroleum fractions. The fluorescence spectra of narrow cuts (196–220°, 225–250°) were taken in iso-octane at room temperature and at liquid-nitrogen temperature. Selective excitation of the total aromatics by means of the mercury lines 313 and 365 μ was applied in order to make possible a separation of the spectra of different types of compounds. Further, an attempt was made to identify the individual aromatic compounds in the cited petroleum fractions. Thus, the comparison of the fluorescence spectra of the petroleum fractions with that of β -methylnaphthalene in iso-octane revealed a similarity between the two spectra, even at room temperature. Hence the author concluded that β -methylnaphthalene was present in the petroleum which was being studied. Unfortunately, the bands at room temperature are too broad for a reliable identification to be made on the basis of similarity of spectra. Lowering the temperature to that of liquid nitrogen leads to a great increase in the sharpness of the bands (Fig. 6). However, in iso-octane (as in iso-paraffins in general), line spectra of the type described above are not observed. It would be very interesting to use the corresponding normal paraffin as the solvent with the aim of obtaining a line spectrum. In such a case, a subsequent vibrational analysis would make it

FIG. 6. Fluorescence spectra: 1 – β -methylnaphthalene, 2 – the fraction (225–250) of petroleum in iso-octane at 77.3°K according to Kh. I. Mamedov.²⁴



possible to carry out the identification with complete reliability.*

c) The determination of coronene and 1, 12-benzperylene

After what has been said, it should be sufficient to demonstrate in Fig. 7 the comparison of the spectrum of a byproduct of petroleum refining with that of pure coronene. The series of lines occurring in the spectrum of the byproduct but not coinciding with the coronene lines turned out to coincide with the wavelengths of 1, 12-benzperylene, which had been measured by Bowen³⁶ at Oxford in hexane at 77.8°K. This example is interesting as an illustration of the complete additivity of the spectra of substances occurring in a complex mixture.

*The latter remark is even more applicable to the interesting new paper by Kh. I. Mamedov³⁹ (added in proof).

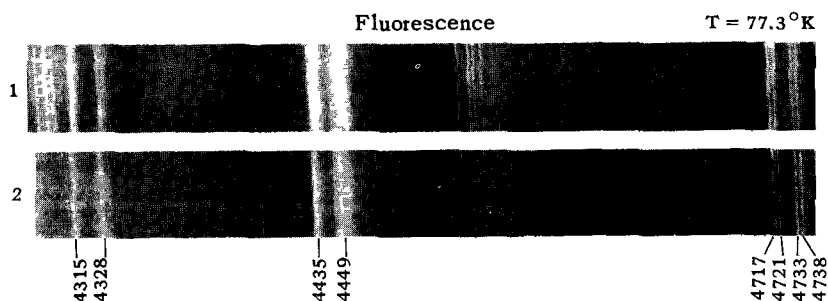


FIG. 7. Fluorescence spectra: 1 – by-product from petroleum refining, 2 – pure coronene in n-hexane at 77.3°K.

9. An additional and also highly significant advantage of the freezing of solutions consists in the fact that here, along with fluorescence, one may observe a persistent luminescence, or phosphorescence. The study of the spectrum of this persistent luminescence may play a substantial auxiliary role. In a number of important cases, as we shall see below, it may play even an independent role in the spectroscopic identification of complex organic compounds.

First let us consider some of the most important characteristics of this luminescence. The phosphorescence of organic compounds is observed, generally speaking, in rigid media.* Here, in solid or highly viscous solutions, as in vitrified sugar or in boric acid, phosphorescence is observed both at room temperature or at low temperatures. (For a review of the older literature see the monographs of P. Pringsheim²⁹ and V. L. Levshin.³⁰)

The phosphorescence observed in frozen solutions in organic solvents is commonly characterized by long lifetimes (from 10^{-3} sec to some seconds; in the case of coronene, about 10 sec). The spectrum of this phosphorescence is strongly shifted to longer wavelengths with respect to the fluorescence spectrum. The magnitude of the relative displacement of the two spectra (that is, the distance between the short-wavelength limits, or between the band heads of the two spectra, if they are discrete) is sometimes as much as several thousand Angstrom units.

The Polish physicist A. Jablonski was the first to indicate that the appearance of this luminescence is evidence of the existence of a metastable level occurring between the ground state and the lowest excited electronic level. According to the interpretation which is generally accepted at present, and was independently proposed by Gilbert, N. Lewis and A. N. Terenin, this metastable level T (Fig. 8) is a triplet state, while the ground state S and the first excited level S' are singlet states. The transition from the excited singlet level S' to the nearby

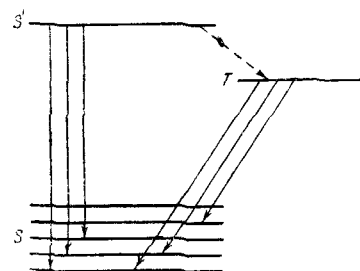


FIG. 8. Diagram of the origin of phosphorescence in frozen solutions: S and S' are singlet levels, the ground state and the excited level, respectively. T is a triplet level. The dotted line indicates a radiationless transition.

triplet level belongs to the category of processes of internal conversion: it takes place without emission and is accomplished within a short time interval. This interval is in any case less, and probably even significantly less than the lifetime of 10^{-8} sec of the excited state for the process of fluorescence (M. Kasha¹⁹).

After falling into the triplet level T, the molecule is in a metastable state, since the transition to the electronic-vibrational levels of the lower singlet state requires a 180° reversal of the spin of one of the electrons for dipole emission. Thus it is strictly forbidden (the so-called rule against intercombination). This explains the fact that the molecule in the level T may remain there for time intervals as much as 10^9 times longer than the normal lifetime of the excited state. The reason why the transition $T \rightarrow S$ occurs nevertheless, in spite of the rule against intercombination, consists in the fact that the metastable level T is actually not a pure triplet state. Due to the interaction between the spin and orbital moments (spin-orbit interaction), it is a superposition of triplet and singlet states. Hence, there is a non-zero, although small, probability for the transition from the nominally triplet state to the lower singlet level. The probability of such "forbidden" transitions, due to spin-orbit interaction, rapidly increases with the atomic number Z in atomic spectra (in proportion to Z^8). This explains the following apparently paradoxical fact: while in

*P. P. Dikun²⁸ has shown, however, that a weak persistent emission is observed even in the vapors of organic compounds.

helium the intercombination line 591.6 \AA ($^3P_{2,1} - ^1S_0$) is highly forbidden and correspondingly very weak, in mercury ($Z = 80$), the same type of transition leads to the resonance line, very strong in emission and absorption, at $\lambda = 2537 \text{ \AA}$.

For molecules the rule against intercombination and the consequences of the conditions existing in the electron cloud (e.g., the dependence of the transition probability on Z) remain in effect. In particular, the rapid increase in the probability of a "forbidden" transition at large values of Z may be illustrated very graphically by the interesting observations of Kasha and Becker on the triplet-singlet transitions in molecules of porphyrins, phthalocyanines, and chlorophylls. These observations were made both with and without the presence of central heavy and paramagnetic metal atoms. It was found that when atoms of high Z value were present in the molecule, the probability of triplet-singlet intercombination was greatly increased.

For molecules, the rule against intercombination (connected with the law of conservation of angular momentum) may be combined with other selection rules based on symmetry; this actually takes place, for instance, in benzene and coronene. Here the transition probability must decrease greatly, while the lifetime is correspondingly increased. For example, in coronene the lifetime is increased by a factor of 10^9 , and the triplet state becomes metastable in the full sense of the word.

We note the following interesting and important fact. The detection of absorption in cases of infinitesimally small transition probabilities, such as those mentioned above, is an exceedingly difficult problem. Particularly, it requires very thick layers (of the order of meters) of substance, or high pressures (for gases). To the contrary, for emission the existence of a metastable level affects only the lifetime of the excited state; this only facilitates the observations, which in any case are experimentally just as simple as in the observation of luminescence of short lifetime (i.e., fluorescence). Here it is found that, even when heavy atoms are absent in the molecule (the Z values are small), the luminescence intensity may be very large. For example, in the case of coronene, the ratio of the fluorescence yield to the phosphorescence yield is approximately unity, that is, the phosphorescence yield amounts to about 50% of the total luminescence yield. This apparent paradox has the following explanation. Let us consider a molecule excited to a certain level S' , which is the lowest excited level in the singlet system. There are two pathways for the downward transition: (1) A transition with emission, $S' \rightarrow S$ (fluorescence),

to the ground (stable) singlet state is possible; the average lifetime of the excited state for this transition is $\tau = 10^{-8}$ sec, while the probability of the transition $A = 1/\tau = 10^8 \text{ sec}^{-1}$. (2) A transition without emission, $S' \rightarrow T$, to the lower triplet state is possible. Since this transition takes place without emission, it is accomplished more quickly (probably even considerably more quickly) than the transition $S' \rightarrow S$; the lifetime for this transition is certainly some orders of magnitude shorter than 10^{-8} sec, for instance, $\tau \sim 10^{-12} - 10^{-13}$ sec, such that $A_1 = 10^{12} - 10^{13} \text{ sec}^{-1}$ (see reference 19). Since the transition $T \rightarrow S$ is forbidden, the state T is metastable, so that the excited molecules will accumulate in this state. Then, since the emission intensity is proportional to the number of excited molecules, the intensity of the forbidden emission (phosphorescence) may be very great. It may even happen that the phosphorescence intensity is greater than the fluorescence intensity. This will be the case when the molecules leave the excited state S' by other radiationless processes (quenching of fluorescence) with high probabilities, besides the radiationless transition $S' \rightarrow T$.

The chain of spontaneous processes $S' \rightarrow T \rightarrow S$ is completely analogous to the chain of radioactive processes $P \rightarrow Q \rightarrow R$: the substance Q arises from P , and in turn, is transformed into R . If, as before, we take as the measure of the transition probability the reciprocal of the average lifetime $A = 1/\tau$, then, as is shown in the theory of radioactive processes,* the number of molecules in the triplet state at the moment t will be

$$N_2 = N_1(0) \cdot \frac{A_1}{A_1 - A_2} (e^{-A_2 t} - e^{-A_1 t}),$$

where A_1 and A_2 are, respectively, the probabilities of the transitions $S' \rightarrow T$ and $T \rightarrow S$. However, since $A_1 \gg A_2$ (e.g., for coronene, $A_1 = 10^{12} - 10^{13}$, and $A_2 = 0.1$), we obtain from the previous formula

$$N_2 = N_1(0) \cdot e^{-A_2 t} = N_1(0) \cdot e^{-\frac{t}{\tau_2}}.$$

One may see that the number of molecules in the triplet state at moment t does not depend at all on the probability of the radiationless conversion $S' \rightarrow T$, and gradually decreases with the relaxation time τ_2 corresponding to the triplet transition $T \rightarrow S$. Thus we see that, in distinction from absorption, which is exceedingly difficult to observe for forbidden transitions, the observation of emission from the triplet state presents no difficulties in comparison with fluorescence. The observation of phosphorescence is in some respects even preferable from the experimental point of view. Thus,

*See, for example, É. V. Shpol'skiĭ, *Атомная физика* (Atomic Physics), Vol. II, Gostekhizdat 479 ff (1951).

in photographing the spectra, both spectra are commonly recorded simultaneously on the plate. However, if we are interested only in the phosphorescence spectrum, then by virtue of the persistence of the emission in this case, we may make use of the phosphoscope. This filters out both the fluorescence spectrum and the exciting light. Hence one may use the full light output of the source to excite the emission, without resorting to filters or monochromators, so that the exposure is considerably shortened.

10. With regard to the suitability of using phosphorescence for spectro-analytical purposes, insofar as I know, this was first indicated by A. A. Il'ina.²⁰ She considered the fact that in some cases in which the fluorescence spectra of structurally similar substances overlap, the phosphorescence spectra may be distinct. An example of this are the spectra of two dibenzacridines, which are shown together with the formulas of these substances in Fig. 9. Here one may clearly see that the phosphorescence spectra, taken simultaneously with the fluorescence spectra but lying at $\lambda > 500 \text{ m}\mu$, are clearly distinct from each other both in position and in the distribution of intensity among the sharp bands.

Another example of the suitability of using phosphorescence spectra for the definite identification of substances is shown in Table I. Here one may see that in the fluorescence spectrum of the sample, along with a series of distinct bands which definitely belong to benzpyrene, there are three weak bands which may be tentatively ascribed to 1, 2, 5, 6-dibenzanthracene (DBA). This assumption, however, becomes a certainty, since three other bands definitely belonging to DBA are observed in the phosphorescence spectrum. With regard to the phosphorescence of 3, 4-benzpyrene, this spectrum, as was shown in a recent paper by Muel,³¹ is strongly shifted to longer wavelengths,

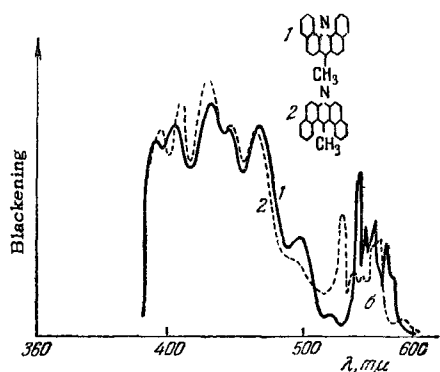
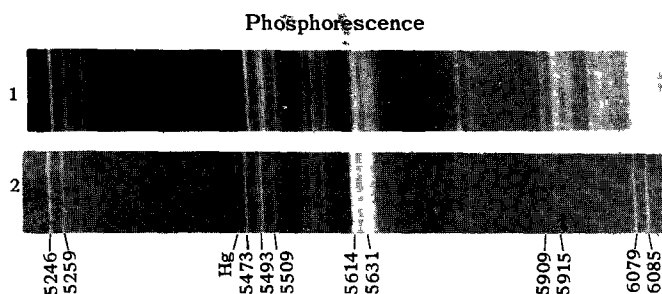


FIG. 9. Microphotometer traces of fluorescence ($\lambda < 500 \text{ m}\mu$) and phosphorescence ($\lambda > 500 \text{ m}\mu$) spectra of two dibenzacridines.²¹



1. Mixture being studied in n-heptane
2. Coronene in n-heptane

FIG. 10. Phosphorescence spectra of an unknown product and of pure coronene.

and is situated in the near infrared (8000–10,000 Å).

Application of the conditions under which quasi-linear spectra are obtained naturally expands the possibilities of spectroscopic phosphorescent analysis. The type of phosphorescence spectrum under these conditions may be seen in Fig. 10. Here a comparison is made of the phosphorescence spectra of an unknown product and of pure coronene; the identification of the unknown as coronene may be made reliably on the basis of this comparison. I wish to remark that, as our experience has shown, the linear phosphorescence spectra are ordinarily simpler than the fluorescence spectra, and hence may be subjected more easily to vibrational analysis, which may be an essential aid in the identification of an unknown substance.

Interesting applications of phosphorescence analysis were recently made in the identification of biologically important substances. S. Freed and W. Salmre³² in the Chemistry Department of Brookhaven National Laboratory applied phosphorescent spectroscopic analysis for the identification of indole derivatives which play an important role in processes occurring in the central nervous system. The phosphoscope was used to eliminate the exciting light and the fluorescence spectrum. Solutions of the substances being studied in a 9:1 methanol-ethanol mixture (which gives a transparent glass on freezing) were frozen at 77.3°K. In Fig. 11 are shown the photoelectrically recorded spectra of indole and two of its derivatives, of which tryptophan is one of the aromatic amino acids occurring in proteins, and serotonin is a substance which plays a large role in the therapy of certain mental diseases. As one may see, the spectra have a distinct structure, which, moreover, is strongly marked by compression of the scale on the wavelength axis. In any case, the spectra are individual enough so that

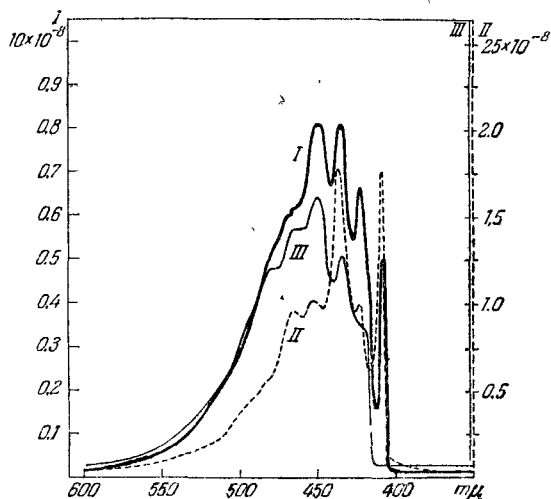


FIG. 11. Phosphorescence spectra of indole derivatives in a mixture of alcohols at 77.3°K: I – indole (2.5 mg/ml), II – tryptophan (0.5 mg/ml), III – 5-hydroxytryptamine (serotonin) creatinine sulfate (0.1 mg/ml). (Taken from Freed and Salmre.³²)

one may distinguish the spectra of each of the compounds without difficulty.

By an analogous spectroscopic method, Steele and Szent-Györgyi³³ have established the presence of tryptophan in the protein of the crystalline lens of cattle, and conversely, its absence in insulin.

¹Harrison, Lord, and Loofbourow, Practical Spectroscopy (Russ. transl. ILM, 1950).

²L. S. Mayants, The Theory of Characteristic Frequencies and Some of Its Applications, Труды ФИАИ Trans. Phys. Inst. Acad. Sci. V, 1950, pp 65-183.

³C. G. Cannon and G. B. B. M. Sutherland, Spectrochimica Acta 4, 373 (1951).

⁴Landsberg, Bazhulin, and Sushchinskii, Основные параметры спектров комбинационного рассеяния углеводородов, (Fundamental Parameters of the Raman Spectra of Hydrocarbons), U.S.S.R. Acad. Sci. Press, M., 1956.

⁵P. A. Bazhulin and M. M. Sushchinskii, Usp. Fiz. Nauk 43, 380 (1951).

⁶A. E. Gillam and E. S. Stern, An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry. (Russ. transl. ILM., 1957).

⁷Абсорбционная спектроскопия (Absorption Spectroscopy), Collection of articles edited by E. V. Shpol'skii, IL, 1953.

⁸M. A. Konstantinova-Shlezinger, Люминесцентный анализ (Luminescence Analysis) U.S.S.R. Acad. Sci. Press, M., 1948.

⁹P. P. Feofilov, Dokl. Akad. Nauk SSSR 45, 387 (1944).

¹⁰B. S. Neporent and B. I. Stepanov, Usp. Fiz. Nauk 43, 380 (1951).

¹¹B. I. Stepanov, Флуоресценция сложных молекул, (The Fluorescence of Complex Molecules), Minsk, 1955.

¹²B. S. Neporent, Труды ГОИ (Trans. State Opt. Inst.) 35, No. 150, 3 (1957); J. Exptl. Theoret. Phys. (U.S.S.R.) 21, 172 (1951).

¹³A. A. Il'ina and E. V. Shpol'skii, Izv. Akad. Nauk SSSR, Ser. Fiz. 15, 766 (1951).

¹⁴E. V. Shpol'skii and A. A. Il'ina, J. Exptl. Theoret. Phys. (U.S.S.R.) 21, 142 (1951).

¹⁵Shpol'skii, Il'ina, and Klimova, Dokl. Akad. Nauk SSSR 87, 935 (1952).

¹⁶Shpol'skii, Klimova, and Girdzhiyanskaite, Материалы X Всесоюз. совещ. по спектроскопии, Том I, Молекулярная спектроскопия (Proceedings of the Tenth All-Union Conference on Spectroscopy, Vol. I, Molecular Spectroscopy), p. 24, L'vov, 1957.

¹⁷E. V. Shpol'skii and L. A. Klimova, Dokl. Akad. Nauk SSSR 111, 1227 (1956), Soviet Phys. "Doklady" 1, 782 (1956).

¹⁸E. V. Shpol'skii, Сб., посвященный памяти Г. С. Ландсберга (Collected Volume in Memory of G. S. Landsberg), in press.

¹⁹M. Kasha, Disc. Faraday Soc. 9, 14 (1950).

²⁰A. A. Il'ina, Izv. Akad. Nauk SSSR, Ser. Fiz. 15, No. 6, 771 (1951).

²¹P. P. Dikun, Paper at the 6th Conference on Luminescence, Leningrad, Feb. 17-23, 1958.

²²A. A. Il'ina, Люминесцентный анализ, Материалы IV Совещания по люминесценции Минск, 20-25 июня 1955 (Proceedings of the 4th Conference on Luminescence, Minsk, June 20-25, 1955), p. 41, Minsk, 1956.

²³Kh. I. Mamedov, *ibid.*, p. 47.

²⁴Shpol'skii, Il'ina, and Bazilevich, Izv. Akad. Nauk SSSR, Ser. Fiz. 12, 519 (1948).

²⁵Lacassagne, Vuu-Hoi, and Zaidela, Compt. rend. 245, 991 (1955).

²⁶N. P. Vuu-Hoi, Nature 182, 1158 (1958).

²⁷L. M. Shabad, Очерки экспериментальной онкологии (Outlines of Experimental Oncology), M., 1947.

²⁸P. P. Dikun, Труды ГОИ-памяти С. И. Вавилова (Transactions of the State Optical Institute, in memory of S. I. Vavilov), M., 1953, p. 77.

²⁹P. Pringsheim, Fluorescence and Phosphorescence (Russ. transl. edited by S. I. Vavilov), ILM., 1949.

³⁰V. L. Levshin, Флуоресценция и фосфоресценция жидких и твердых веществ, (Fluorescence and Phosphorescence of Liquid and Solid Materials), M., 1951.

³¹B. Muel and M. Hubert-Habart, J. Chim. Physique 55, 377 (1958).

³²S. Freed and W. Salmre, Science 128, 1341 (1958).

³³R. H. Steele and A. Szent-Györgyi, Proc. Nat. Acad. Sci. USA **43**, 477 (1957); **44**, 540 (1958).

³⁴T. N. Bolotnikova, Izv. Akad. Nauk SSSR, Ser. Fiz. **23**, 29 (1959).

³⁵É. V. Shpol'skiĭ and L. A. Klimova, Izv. Akad. Nauk SSSR **23**, (1959).

³⁶E. J. Bowen and B. Brocklehurst, J. Chem. Soc. (London) 3875 (1954); 4320 (1955).

³⁷É. V. Shpol'skiĭ and É. A. Girdzhiyauskaite,

(Optics and Spectroscopy) **4**, 620 (1958).

³⁸S. G. Bogomolov, Paper at the 6th Conference on Luminescence, Leningrad, Feb. 17-23, 1958.

³⁹Kh. I. Mamedov, Izv. Akad. Nauk SSSR, Ser. Fiz., **23**, (1959).

⁴⁰E. Clar, Aromatische Kohlenwasserstoffe, 2 Aufl., 1952, p. 33.

Translated by M. V. King