220. *Fluorescence Spectrography as an Aid in the Identification of Compounds.*

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The fluorescence spectra of a group of polycyclic hydrocarbons, isomeric substitution products, and related compounds, were examined, using modifications in the standard technique, with the incorporation of a "moving wedge" near the slit of the spectrograph. The work provides additional data for judging the reliability of fluorescence spectrography in identifying certain types of compound.

FLUORESCENCE excited by ultra-violet light has wide applications, *e.g.*, in chromatography, quantitative analysis, and certain biological studies. The use of fluorescence spectrography by Mayneord played an important part in the isolation of 3 : 4-benzpyrene from tar (Cook, Hewett, and Hieger, *J.*, 1933, 395; Hieger, *Amer. J. Cancer*, 1937, **29**, 709), and has since been used in metabolic studies (Peacock, *Brit. J. Exp. Path.*, 1936, **17**, 164; Chalmers, *Biochem. J.*, 1938, **32**, 271; Berenblum *et al.*, *Cancer Res.*, 1943, **3**, 151; Doniach *et al.*, *Brit. J. Exp. Path.*, 1943, **24**, 1; and others). But its scope for wider use in the identification of compounds will depend on the accumulating evidence as to the specificity of fluorescence spectra in relation to molecular structure.

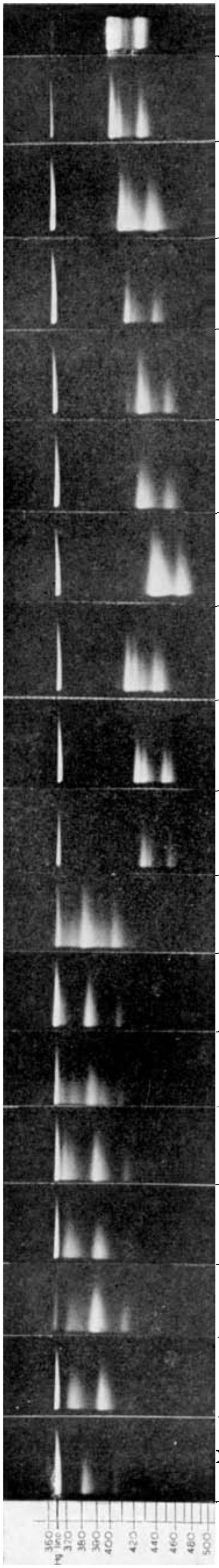
Hieger (*Biochem. J.*, 1930, **24**, 505) found characteristic banded fluorescence spectra a common feature among polycyclic hydrocarbons. The present use of a spectrograph of wider dispersion, and incorporation of a "moving wedge," provided improved facilities for assessing the characteristics of the band systems (compare I and II, or XIX and XX, Plate).

Characteristics of Fluorescence Spectra.—Though influenced by many factors (such as light source, optical system, plate sensitivity, and solvent), reproducible characteristic patterns for particular groups of compounds are obtainable. Under the conditions used in the present work, the spectra of anthracene and its methyl derivatives (XX—XXV) exhibited 4 single wide bands (the fourth too faint to be visible in the reproductions), of which the second band is the most intense, each band exhibiting maximal intensity at about its middle. Those of 3 : 4-benzpyrene and its derivatives (II—X) had very narrow bands, the first being most intense, with rapid rise and gradual fall in intensity of each band, the system displaying a "fluted" character (see Hieger, *loc. cit.*), *i.e.*, each major band being followed by one or two of weaker intensity. The spectra of 1 : 2-benzanthracene and its derivatives (XXVI—XXX) were similar to that of 3 : 4-benzpyrene, except for less tendency for duplication. Those of chrysene and its derivatives (XI—XVIII) resembled that of anthracene, but with weak bands in intermediate positions. The fluorescence spectrum of pyrene, consisting of multiple fine bands very close together (Hieger, *loc. cit.*; Mischer *et al.*, *Biochem. Z.*, 1936, **287**, 189), is an example of another characteristic pattern.

Derivatives with methyl, hydroxy-, methoxy-, acetoxy-, chloro-, and other substituents, have been examined. Though their introduction leads to a shift in the positions of the bands, to the longer wave-length, the patterns remained similar to those of the respective parent hydrocarbons. Less shift occurred with dimethyl substitution in the β- than in the α-positions of anthracene (compare XXI with XXV). Similarly, with the dibenzfluorenes, the bands of 2 : 3 : 6 : 7- (XXXI) were nearest to the shorter wave-length, while those of 3 : 4 : 5 : 6- (XXXVI) were furthest away. The chrysene series (XI—XVIII) illustrates the effect of methoxy-substitution in different positions of the molecule, the series being arranged, in the Plate, according to the degree of shift. Isomeric monomethoxybenzpyrenes (III—V) also show differences in the positions of the bands.

Different substituents in the same position of the molecule resulted in different degrees of shift, as seen in the 1 : 2-benzanthracene series (XXVI—XXIX), where less shift is seen with acetoxy- than with hydroxy- or methoxy-substitution. Mono-, di-, and tri-chlorobenzpyrenes (VIII—X) show progressive shift with multiple substitution; yet the position of substitution seems to be a dominant factor, since mono-substitution in some positions (*e.g.*, 8-methoxybenzpyrene, V) produces as much shift as di-substitution in other positions (*e.g.*, 5 : 10-dimethoxybenzpyrene, VI).

Intensity of Fluorescence.—This is strongly influenced by the set-up of the apparatus (light source, filter, dispersion of the spectrograph, size and position of tube, etc.), so that comparisons are valueless except under standard conditions. The nature of the solvent also affects fluorescence intensity (Miller and Baumann, *Cancer Res.*, 1943, **3**, 223; Weil-Malherbe, *ibid.*, 1944, **4**, 102), the position of the bands (Chalmers, *loc. cit.*; Sambursky and Wolfsohn, *Trans. Faraday Soc.*, 1940, **36**, 427), and their sharpness (definition being good in hexane, less good in benzene or ethanol, and poor in chlorine-containing solvents). Reduced intensity may result from oxygen-solubility in the solvent, leading to corresponding degrees of oxygen quenching (Bowen and Williams, *Trans. Faraday Soc.*, 1939, **35**, 765; Weil-Malherbe, *Cancer Res.*, 1944, **4**, 102), or from photo-oxidation, formation of polymers, or chemical interaction with the solvent, and may accordingly be affected



I. 3 : 4-*Benzpyrene* (*slit*).

II. 3 : 4-*Benzpyrene* (*moving wedge*).

III. 5-*Methoxy*-3 : 4-*benzpyrene*.

IV. 10-*Methoxy*-3 : 4-*benzpyrene*.

V. 8-*Methoxy*-3 : 4-*benzpyrene*.

VI. 5 : 10-*Dimethoxy*-3 : 4-*benzpyrene*.

VII. 5 : 8-*Dimethoxy*-3 : 4-*benzpyrene*.

VIII. 5-*Chloro*-3 : 4-*benzpyrene*.

IX. *Dichloro*-3 : 4-*benzpyrene*.

X. *Trichloro*-3 : 4-*benzpyrene*.

XI. *Chrysene*.

XII. 3-*Methoxychrysene*.

XIII. 4-*Methoxychrysene*.

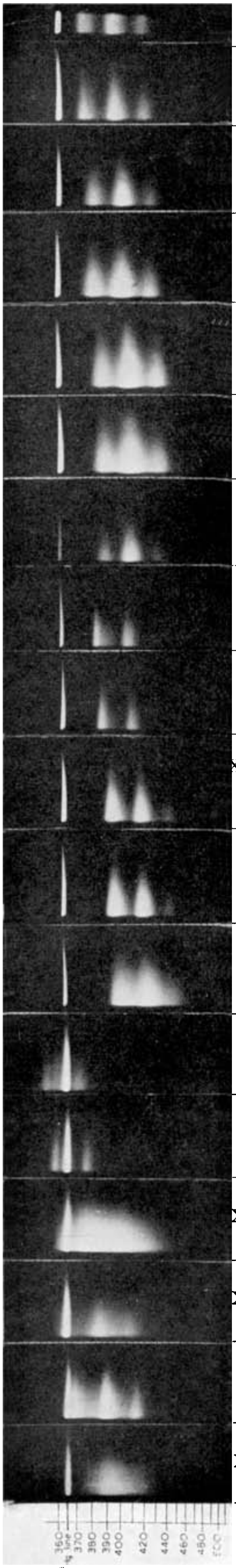
XIV. 2-*Methoxychrysene*.

XV. 5-*Methoxychrysene*.

XVI. 6-*Methoxychrysene*.

XVII. 1-*Methoxychrysene*.

XVIII. 1 : 2-*Dimethoxychrysene*.



XIX. *Anthracene* (*slit*).

XX. *Anthracene* (*moving wedge*).

XXI. 2 : 3-*Dimethylanthracene*.

XXII. 2-*Methylanthracene*.

XXIII. 1 : 2-*Dimethylanthracene*.

XXIV. 1 : 3-*Dimethylanthracene*.

XXV. 1 : 4-*Dimethylanthracene*.

XXVI. 1 : 2-*Benzanthracene*.

XXVII. 4′-*Acetoxy*-1 : 2-*benzanthracene*.

XXVIII. 4′-*Methoxy*-1 : 2-*benzanthracene*.

XXIX. 4′-*Hydroxy*-1 : 2-*benzanthracene*.

XXX. 9 : 10-*Dimethoxy*-1 : 2-*benzanthracene*.

XXXI. 2 : 3 : 6 : 7-*Dibenzfluorene*.

XXXII. 1 : 2 : 6 : 7-*Dibenzfluorene*.

XXXIII. 1 : 2 : 5 : 6-*Dibenzfluorene*.

XXXIV. 1 : 2 : 7 : 8-*Dibenzfluorene*.

XXXV. 1 : 2 : 3 : 4-*Dibenzfluorene*.

XXXVI. 3 : 4 : 5 : 6-*Dibenzfluorene*.

by the viscosity of the solvent, through influence on the collision of molecules (Bowen, " The Chemical Aspect of Light," 1946).

Liquid paraffin, having a low oxygen solubility, high viscosity, and no halogen or other disturbing group, has proved to be a highly suitable solvent for fluorescent work, exhibiting maximal intensity and good resolution of the bands (their positions corresponding to those in benzene, *i.e.*, slightly shifted to the longer wave-length as compared with those in hexane). All spectra in the Plate were obtained from solutions in liquid paraffin.

Semi-quantitative Estimations of Fluorescence Intensity.—The method of fluorimetric estimation by matching intensities of bands on photographic plates (Berenblum and Schoental, *Biochem. J.*, 1942, **36**, 86) was applied to the present series of compounds. Comparisons were made of the concentrations required to produce intensities comparable to that of a standard solution of 0·3 μg. of 3 : 4-benzpyrene per ml. of benzene (the values are obviously only approximate, in view of the different positions of the respective bands).

Hydrocarbons.—(*a*) 3 : 4-Benzpyrene and 1 : 2-benzpyrene were the most fluorescent of the series; (*b*) 20-methylcholanthrene, 1 : 2-benzanthracene, and anthracene required 15—30 times the concentration to produce comparable intensity; (*c*) 1 : 2 : 5 : 6-dibenzanthracene required about 200 times; and (*d*) chrysene, 3 : 4-benzphenanthrene, and pyrene required 2000 to more than 6000 times the concentration (the above estimations were in benzene; in other solvents, the ratios can be different).

Substitution Products.—The intensities of 5- and 8-methoxy-3 : 4-benzpyrene were about the same as that of 3 : 4-benzpyrene. With weaker fluorescing hydrocarbons, introduction of methoxy-groups actually increased the intensity of fluorescence, *e.g.*, 1- and 5-methoxychrysenes required 100—200 times lower concentration, 3-, 2-, and 6-methoxychrysenes required 30—40 times lower, and 4-methoxychrysene required 5 times lower concentration than chrysene to produce comparable intensities. With different substituents of 1 : 2-benzanthracene, the 4′-acetoxy- and the 9 : 10-dimethoxy-derivatives were only slightly more fluorescent than the parent hydrocarbon, while the 4′-hydroxy- and 4′-methoxy- were 4—5 times more intense. The finding that 1 : 2-dimethoxychrysene was about 20 times more fluorescent than chrysene, despite the close similarity in their spectra, is an example where quantitative values may be of help in identification.

Conclusions.—Differences in fluorescence spectra between an unknown and a reference substance can be taken as proof of non-identity. Similarity in spectrum, though not absolute proof of identity, represents a valuable addition to other methods of identification, and is specially useful when only minute amounts of material are available, or when still dealing with impure products. But as many factors influence fluorescence spectrography, comparisons must be made with reference to the particular set-up of apparatus, etc., rather than with published data of other workers.

EXPERIMENTAL.

The apparatus included a Bellingham & Stanley Intermediate Quartz Spectrograph (2000—8000 A. = about 15 cm.); Kodak I–F plates (sensitivity to about 6900 A.); Mercra Lamp (British Thomson-Houston Co. Ltd.); and Wood's glass filter. The lamp, with focusing quartz lens, was housed in a box with a small window, in front of which was the tube (thin-walled Monax, 7—8 mm. internal diameter) containing the test solution. Beyond the tube was the spectrograph, with its axis at about 110° to the beam of incident light. Slit : 0·05—0·2 mm.; exposures : 1—5 minutes, according to intensity of fluorescence.

The " moving wedge " consisted of a small elongated metal plate with a central opening, 4 cm. long, with one edge horizontal and the other cut according to a logarithmic scale. This was mechanically driven at a uniform rate (12 or 16 mm. per minute) perpendicular to the length of the slit, behind which it was situated.

Liquid paraffin (*d* 0·880—0·895) was used as solvent for investigation of characteristics of spectra by the "moving wedge " method. For quantitative estimations, benzene (and for some series, hexane) was used as solvent and diluent, 10 solutions (including one or more standards) being photographed on one plate. Exposures : 3 minutes without moving wedge.

The preparations of some of the compounds studied have previously been described (Berenblum and Schoental, *Cancer Res.*, 1943, **3**, 145, 686; Cook and Schoental, *J.*, 1945, 288), while the synthesis of 10-methoxy- and di- and tri-chloro-benzpyrenes, by Cook and Schoental, will be published elsewhere.

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