Absorption Spectra of 3-4 Benzpyrene

EVIDENCE has been given[1] that benzpyrene emits various fluorescence spectra according to its physical state. Molecular benzpyrene, the monoclinic, the orthorhombic crystallized modification and the colloidal suspension in water fluoresce violet, green, blue and yellow respectively. The fluorescence spectrum of dissolved benzpyrene is unchanged in various solvents except for slight displacements of the entire group of bands[2]. We have now studied the absorption spectra with a low-voltage projection lamp as a light source for a continuous spectrum, which restricted the observation to the radiation transmitted by glass. It was found that the first broad absorption band of dissolved benzpyrene[3] has three components, α, β, γ, at 383, 380 and 377 mμ respectively in hexane (Fig. 1,*a*). They are of almost the same strength, but they behave differently in various solvents. While the α-component persists as the maximum, β and γ fuse and disappear more or less by polar effects due to the solvent.

The absorption spectrum of the blue fluorescing orthorhombic modification of solid benzpyrene (Fig. 1,*b*) is entirely different from that of the dissolved hydrocarbon. It shows a broad absorption band between 415 and 425 mμ which is accompanied by three narrower bands at 405–410, 392–396 and 384–387 mμ respectively. In order to delay the spontaneous transformation[1] of the metastable orthorhombic into the stable monoclinic crystals, very thin crystal films were prepared by evaporating a benzene solution of benzpyrene on cover glasses. A pile of six such films absorbs strongly enough to render visible the long wave-length portion of the absorption spectrum.

The monoclinic needles which fluoresce green, and their colloidal yellow fluorescent suspension in water, cannot easily be prepared in a state which is suitable for absorption spectroscopy. An indirect method was devised which allowed this difficulty to be overcome. It is based on the observation of Bowen and Sawtell[4] that the fluorescence efficiency of a fluorescent solution is usually independent of the wave-length of the exciting radiation within wide limits. Hence, if a continuous spectrum is focused on to the fluorescent substance in a layer so thin that the absorption is *feeble*, the absorption spectrum appears as fluorescent light. This can be recorded by a photographic plate if a thin film filter is placed between the fluorescent layer and the photographic emulsion, which completely absorbs the exciting short wave-length radiation and transmits the fluorescent light. Henri and Wurmser[5], by this technique, separated from a discontinuous spectrum the radiations which excite the fluorescence. Fig. 2 shows positives of 'fluorograms' of molecularly dissolved benzpyrene in Canada balsam (*a*), of the orthorhombic form as a single layer of sparsely distributed thin crystal squares of about 5 μ side under water (*b*), of the monoclinic form as a loose mosaic of microscopical crystal leaves on a cover glass (*c*) and of a colloidal suspension of benzpyrene in water (*d*). The fluorograms *a* and *b* repeat as luminous bands the dark bands of Fig. 1,*a* and 1,*b* although in a different strength. The same flat maxima of Fig. 2,*c* and 2,*d* at about 425 *mμ* establish the fact that the particles of the colloidal suspension are ultra-microscopical monoclinic crystals.

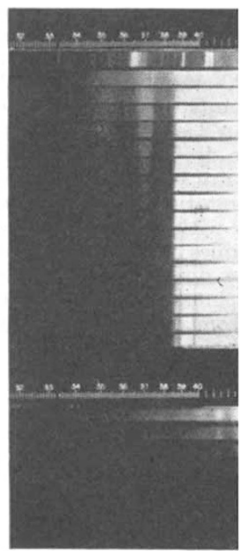

Fig. 1.
ABSORPTION SPECTROGRAMS (POSITIVES) OF BENZPYRENE.
(*a*) Dissolved in hexane, 1 : 100000 ; layers 2–32 mm. in thickness.
(*b*) Pile of 6 orthorhombic crystal films. Increasing exposures.

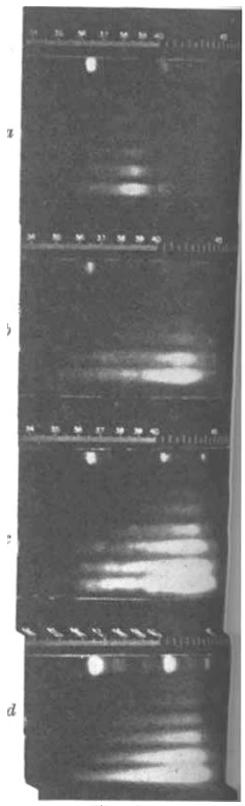

Fig. 2.
FLUOROGRAMS (POSITIVES) OF BENZPYRENE. INCREASING EXPOSURES.
(*a*) Dissolved in Canada balsam.
(*b*) Orthorhombic crystals in water.
(*c*) Mosaic of monoclinic crystals on glass.
(*d*) Colloidal suspension in water.

The method permits of recording—at the present stage, qualitatively—the absorption spectra of systems which are not suitable for the usual spectroscopic methods. This applies to colloid suspensions, microcrystals, and generally to mixed systems with a fluorescent component which must not or cannot be separated. Experiments are in progress to study the absorption spectra of biological fluorescent specimens in their original state, especially in connexion with problems of cancer research. It should be noted that a 'fluorogram' records the absorption spectrum of that component of a mixed fluorescent system (for example, a solid solution) which actually absorbs the light, irrespective of which component actually fluoresces. Thus colloidal suspensions of blue fluorescent pure anthracene and of green fluorescent anthracene which contains naphthacene both gave the fluorogram of anthracene only.

This research was carried out under a grant from the British Empire Cancer Campaign. I wish to thank Messrs. Adam Hilger Ltd. and Mr. F. Twyman for the loan of spectrographic equipment.

F. WEIGERT.

Mount Vernon Hospital,
Northwood, Middlesex.
June 13.

[1] Weigert, F., and Mottram, J. C., NATURE, **145**, 985 (1940).
[2] Chalmers, F. G., *Biochem. J.*, **32**, 271 (1938).
[3] Mayneord, W. V., and Roe, Edna M. F., *Proc. Roy. Soc.*, A, **152**, 323 (1935).
[4] Bowen, E. J., and Sawtell, J. W., *Trans. Faraday Soc.*, **33**, 1425 (1937).
[5] Henri, V., and Wurmser, R., *J. de Phys.* (6), **8**, 289 (1927).