LVIII. THE SPECTRA OF CANCER-PRODUCING TARS AND OILS AND OF RELATED SUBSTANCES.

BY IZRAEL HIEGER.

From the Cancer Hospital Research Institute, London.

(Received March 3rd, 1930.)

THIS work deals with the application of optical methods to the identification of the cancer-producing agent present in tars and oils. The chemical separation of the cancer-producer will prove difficult since tars and oils are exceedingly complex mixtures, but the search has been narrowed by various investigations (see references in preceding paper) which suggest that the active agent is among the aromatic hydrocarbons with condensed rings. These compounds, represented by anthracene, possess the property of fluorescing intensely when exposed to ultra-violet light and the results and opinions expressed here are based on an examination of the fluorescence spectra and ultra-violet absorption spectra of several hundred substances, in part pure compounds, in part mixtures.

EXPERIMENTAL.

A quartz mercury vapour lamp (Kelvin, Bottomley and Baird) was used as a source of ultra-violet light. The lamp stood in an asbestos box in one side of which was a Wood's glass window enclosed by a fish-tail shaped tube, 3 cm. in diameter. Only filtered ultra-violet light emerged from the flattened aperture of the tube. The solutions were examined in corked tubes of non-fluorescing "monax" glass 70×6 mm. and of egg-shell thickness. The tube was clamped directly in front of the fish-tail aperture so as to prevent almost completely the escape of the exciting radiation. The spectroscope (Hilger, no. E 31) was placed with its slit about 1 cm. from the fluorescence tube and the axis of the collimator tube made an angle of about 80° with the normal of the incident rays. Such an arrangement reduced the tendency for the reception of the spectrum of the incident light reflected from the surface of the glass tube. The best position for the spectroscope was found by removing the plate holder and placing the eye in the focal plane and at a point corresponding with the focussed image of the visible part of the spectrum. A disc of bluish-green fluorescing light suddenly comes into view when the spectroscope is very slowly rotated about its central axis, indicating the optimal angle. For this sort of photography the extremely fast panchromatic plates now available possess no advantage over the panchromatic plates of ordinary speed. Correct exposure, determined by trial, varied from 5 seconds to an hour or more.

It is often difficult to avoid photographing the reflected spectrum of the incident light especially when the exposure is prolonged; the reflections appear as curious parabolic blurs, or as multiple fine lines, or as heavy fogging in the short wave-length region of the spectra.

Specificity of fluorescence spectra.

Fluorescence spectra do not distinguish between some of the aliphatic *meso*-derivatives of anthracene; 9-methylanthracene and 9-benzylanthracene give almost identical spectra. On the other hand the spectra of $\alpha\alpha$ -dinaphthyl and $\beta\beta$ -dinaphthyl can be easily distinguished. This experiment indicates the possibility of identifying closely related isomers. Commercial diphenylmethane is brightly fluorescent when appropriately excited, and its spectrum is identical with that of anthracene, but if diphenylmethane is synthesised in the laboratory the fluorescence is so faint that a 20 % solution of this hydrocarbon is as weakly fluorescent as a 0.002 % solution of anthracene. Such an experiment illustrates the use of a method of spectrum analysis in the detection of traces of highly fluorescent substances in the presence of large quantities of others not, or weakly, fluorescent.

Effect of substitution on fluorescence spectra.

When anthracene is substituted in the α - or β -position by Cl or CH₃ there is a shift of the spectrum in the direction of longer wave-length, the relative position of the bands and their general appearance remaining unchanged. But a tetramethylanthracene shows a spectrum only slightly different from that of the monomethyl derivative. This instance lends no support to any idea of proportionality between the amounts of substitution and of spectrum shift.

When the *meso*-hydrogens of anthracene are substituted by aliphatic groups like methyl or benzyl the spectrum resembles the ordinary three-banded anthracene spectrum, but in the spectrum of *meso*-phenyl- and *meso*-naphthyl-anthracene the resemblance falls off and in *meso*-diphenylanthracene is gone. Benzene has been shown to possess fluorescence bands themselves situated in the ultra-violet region, and on general principles it may be justifiable to conclude that the curious spectrum of 9 : 10-diphenylanthracene is in part, at least, due to its behaviour as a compound which can be regarded as anthracene-substituted benzene. Some of the methylated polybenzanthracenes give fluorescence spectra identical with that of the parent polybenzanthracene. One of these is represented in Plate III (no. 278, 1 : 2 : 5 : 6-dibenzanthracene). Probably the disproportion in relative mass of the small methyl group and the large dibenzanthracene nucleus accounts for the stability of the spectrum.

Effect of concentration, solvent, and irradiation.

Strong and weak solutions of 9 : 10-dibenzylanthracene and 9 : 10-dimethylanthracene give slightly different spectra, but this effect has not been observed

with varying dilutions of other compounds where the concentrations were chosen at random. The concentration effect observed with these two anthracene derivatives is perhaps the result of a swamping action of one band upon another connected with their remarkably powerful fluorescence; one part in two hundred million of solvent still shows fluorescence.

Whether the solvent has any influence on the nature of the fluorescence has been tested in only one case. Solutions of AlCl_3 -treated tetralin in chloroform, light petroleum, and alcohol gave identical spectra.

When the chloroform solution of AlCl_3 -treated tetralin was exposed to ultra-violet light for more than about 30 minutes, the beautiful violet-blue fluorescence slowly changed to a pale grey-blue. Solutions of the tetralin preparation in light petroleum and alcohol can be irradiated for 3 hours without the least change in the original colour or spectrum. Fading effects are also observed with xylene solutions of stilbene and with a chloroform solution of a mixture of naphthacene and dihydronaphthacene. The fading of chloroform solutions led to the use of light petroleum as a more inert solvent and later to the use of benzene, because of the insolubility in light petroleum of some of the more complex hydrocarbons.

Spectra of cancer-producing substances, and especially of that obtained from tetralin.

Among the first few substances to be examined were some cancer-producing tars and oils, a number of which show similar bands (see Plate III). The bands were in position 4000, 4180, 4400 Å., sharply defined on the short wave-length side, and grading less abruptly into the background on the long wave-length side. Such graded bands will be referred to as "fluted."

For purposes of comparison, we have found it necessary to use a pair of dividers to contrast or compare spectrum bands on the paper photographs with one another or with the standard 4000, 4180, 4400 Å. bands or with the benzanthracene bands, the importance of which is discussed later. We have used the very strong 3680 Hg line as a suitable fixed wave-length from which measurements can be made. In Plates III and IV the photographs have been so arranged that the 3680 Hg lines of the spectra are in alignment.

For the comparison of spectra on glass plates, the method used is simply to superimpose a test plate on the standard 4000, 4180, 4400 spectrum plate, or on the standard benzanthracene spectrum plate. The five variables consist of number and nature (fluting) of bands, absolute wave-length of bands, their position relative to one another and their individual intensity.

An interesting cancer-producing substance is that obtained as the high-boiling fraction from the mixture resulting from the action of AlCl_3 on commercial tetrahydronaphthalene (tetralin). This type of reaction and the study of the products as carcinogenic agents are described in the preceding paper. Tetrahydronaphthalene is allowed to react with 2 % AlCl_3 for 9 hours at 65°,

the crude mixture decomposed with water, dried and fractionated. The fraction distilling at 260–320° at 14 mm. is a powerful cancer-producing agent even in concentration of only 4 %. It is a clear viscid syrup, yellow or orange in colour, and intensely fluorescent; one part in 40 millions of ether can still be distinguished from pure ether. The fluorescence spectrum is very sharply defined and identical with the spectrum so frequently found in cancer-producing tars and oils. One or two of the numerous subsequent batches of the 260–320° fraction did not give spectra exactly like the standard. One of these aberrant spectra was given by a fraction made from tetralin, prepared by Technical Research Works, Ltd., by a method which ensures that the final product is free from decalin; but the usual 4000, 4180, 4400 Å. spectrum was obtained from the 260–320° fraction prepared from pure tetralin made by another method, namely the sulphonate separation from commercial tetralin. The former of these preparations is under test on mice; the latter was carcinogenic.

The possibility must be considered that the substance responsible for the bands may not be identical with the cancer-producer but is some closely allied compound, frequently, if not invariably, produced when the cancer-producer is formed, either by low temperature catalysis or at high temperature. The carcinogenic agent in tars and oils may be only one of a group of such compounds.

The very frequent recurrence of the 4000, 4180, 4400 Å. bands in carcinogenic agents and the delicacy and class specificity (see previous section and Plates III and IV) of the fluorescence test justify the use of the fluorescence spectrum to indicate the probability of carcinogenic activity and to assist in directing the preparation of appropriate hydrocarbons. The test on mice is, of course, necessary for conclusive proof.

The fluted bands (4000, 4180, 4400 Å.) are shown more distinctly by AlCl_3 -treated tetralin [see Kennaway, 1930] than by any other cancer-producing substance. Now very similar bands are given by 1:2-benzanthracene; these bands also are strongly fluted and only in their absolute position in the wavelength scale do they differ from the 4000, 4180, 4400 Å. bands.

On generally accepted grounds one would expect that it might be possible, by increasing the mass of the benzanthracene molecule by suitable substitution, to produce a compound with a corresponding shift of the spectrum in the direction of increased wave-length possessing a spectrum with bands at 4000, 4180, 4400 Å. To this end a number of benzanthracene derivatives have been made in this laboratory; many of the new compounds have been synthesised by Dr J. W. Cook (see Plate III). A compound with a fluorescence spectrum intermediate between those of 1:2-benzanthracene and AlCl_3 -treated tetralin, namely, 1:2:7:8-dibenzanthracene, has yielded 2 cancers in 10 mice.

A spectrum having bands at 4000, 4180, 4400 Å., has been obtained from the following cancer-producing materials (Plate IV).

			Fluorescence spectrum				Fluorescence spectrum
Substance	Carcino-genic activity			Substance	Carcino-genic activity		
101 AlCl ₃ -treated tetralin	Active			101 AlCl ₃ -treated tetralin	Active
102 1 : 2-Benzanthracene	1/50			407 Acenaphthanthracene	Under test
225 4-Chloro-1 : 2-benzanthracene	Not tested			438 10-Benzylacenaphthanthracene	Under test
399 3-Methyl-1 : 2-benzanthracene	Under test			445 Chrysene	? See text
396 6-Phenyl-1 : 2-benzanthracene	Under test			449 Benzonaphthochrysene	Under test
456 6-isoPropyl-1 : 2-benzanthracene	Under test			431 Dinaphthochrysene	Under test
250 6-Methyl-1 : 2-benzanthracene	Under test			57 Picene	Inactive, see text
398 9 : 10-Diphenyl-1 : 2-benzanthracene	Under test			62 Anthracene	Inactive
415 10-Benzyl-1 : 2-benzanthracene	Under test			A35 Phenanthrene	Inactive
425 Tetrahydro-1 : 2-benzanthracene	Under test			439 2-Phenylanthracene	Under test
276 1 : 2 : 3 : 4-Dibenzanthracene	Under test			419 Triphenylene	? See text
278 1 : 2 : 5 : 6-Dibenzanthracene	1/10			430 Pyrene	Under test
331 3'-Methyl-1 : 2 : 5 : 6-dibenzanthracene	4/10			89 Perylene	Under test
284 1 : 2 : 7 : 8-Dibenzanthracene	4/10			446 ββ-Dinaphthyl	Inactive
371 1 : 2 : 3 : 4 : 5 : 6-Tribenzanthracene	Under test			A37 Fluorene	Inactive
94 Naphthacene-dihydronaphthacene	Inactive
219 Tetrahydro-2 : 3-benzanthracene	Under test
426 Dihydroindinaphthanthracene	Under test
A64 Acenaphthene	Inactive

The fractions in column 3 represent the ratio of tumour-bearing mice to total mice tested.

Table I.

A62'''	Gas works tar made at 1250°
A62'	Gas works tar made at 450°
85	Low temperature tar, 350°
A48	Creosote oil
A22	Green oil
A32	Anthracene oil
A21	Pitch distillate
A19	Heated Californian petroleum
A4	Acetylene tar
A72	Yeast tar
A73	Muscle tar
A25	Cholesterol tar
A23	Hair tar
101	AlCl ₃ -treated tetralin
100	1 % gas works tar
A3	Ether extract of gas works tar
A8	AlCl ₃ -treated naphthalene
244	Residue from preparation of α -phenylnaphthalene

One of the weakest of these agents was the tar made from human hair, which produced 2 cancers in 94 mice. The ether extract of gas works tar and a 1 % dilution of this (A3 and 100) were used in a quantitative study of the effect of dilution on cancer-producing activity [Hieger, 1929]. 101 is the "Schroeter" described in the preceding paper. The bands in A8 are extremely faint, and the material was feebly carcinogenic, producing 1 cancer in 50 mice. 244 requires special mention. It was strongly carcinogenic and gives the bands at 4000, 4180 and 4400 Å., together with another band of shorter wave-length.

The following materials gave a diffuse spectrum covering very roughly the whole area 4000–4400 Å. without differentiation into bands (Table II and Plate IV).

Table II.

A14	Shale oil (blue oil)
A16	Refined lubricating oil
A18	Blast furnace tar
A31	Unheated Californian petroleum
A50	Pitch (medium soft)
A62''	Gas works tar 600°
86	Spindle oil

Of these A14, A50, A62'' and 86 are carcinogenic. A16 and A18 were inactive. A31 yields when heated a strongly carcinogenic material A19 (Table I).

Only one material giving no bands in the region in question has produced a tumour, namely commercial tetralin (A6) which gave 1 cancer in 30 mice (see preceding paper).

The 32 hydrocarbons of which the fluorescence spectra are shown in Plate III fall into three classes.

(1) Those, 20 in number, which are still under test for carcinogenic activity.

(2) Those which have hitherto been found to be non-carcinogenic (see Table I in preceding paper); namely, anthracene, phenanthrene, fluorene, acenaphthene, pyrene, triphenylene, $\beta\beta$ -dinaphthyl, naphthacene and dihydronaphthacene. None of these gives the bands shown by the carcinogenic substances in Table I. Picene (a pure specimen obtained through the kindness of Dr Chattaway) gives two bands with rather diffuse edges, and of shorter

wave-length than 4000 and 4180 Å. It was tested here on 30 mice in suspension in dermoid oil; further tests will be made with it in other media.

(3) Those which have given tumours. Chrysene (445) which has produced cancers and papillomata in the experiments of Twort and Fulton [1930], but not in this laboratory, gives a spectrum very similar to that of 1:2-benzanthrene, but displaced considerably towards the region of shorter wave-length. 1:2-Benzanthracene gave 1 transient papilloma in 50 mice. 1:2:7:8-Dibenzanthracene has given 2 cancers in 10 mice¹; it is noteworthy that the spectral bands of this compound lie about half-way between those of 1:2-benzanthracene and those of AlCl_3 -treated tetralin and the other carcinogenic materials.

The following materials gave the same spectrum (Plate IV) as those in Table I, but did not produce cancer; β -tetroylpropionic acid mother-liquor (A26), undistilled AlCl_3 -treated tetralin (A100), benzyl oleate (60), oleic acid (207). One may suggest two possible explanations for these discrepancies. (1) That the effect of the AlCl_3 on tetralin is only to prepare an intermediate substance which is rendered carcinogenic during distillation. It would be interesting to see if tetralin heated (under pressure) to the temperature of distillation of the AlCl_3 -treated mixture became carcinogenic. It is conceivable that the spectrum (bands at 4000, 4180, 4400 Å.) is produced by the precursor of the carcinogenic agent. (2) Since many of the derivatives of anthracene remain fluorescent when diluted to one part in 200 millions of solvent it is possible that the minute quantity of active substance present may be sufficient for the fluorescence test yet be well below the carcinogenic threshold. Benzyl oleate gave no tumours in an experiment on 20 mice, although Twort and Twort [1928] obtained several papillomata in mice with oleic acid.

Absorption spectra.

AlCl_3 -treated tetralin showed two bands at about 3700 and 3900 Å. and between these limits of wave-length the curve exactly followed the absorption curve of benzantracene.

SUMMARY.

The fluorescence spectra of many cancer-producing substances show the same bands at wave-lengths 4000, 4180, 4400 Å. These bands are remarkably like those of the fluorescence spectrum of 1:2-benzanthracene, apart from their absolute wave-length. A study of the fluorescence spectra of a large number of condensed ring aromatic hydrocarbons shows that the spectrum is characteristic for closely related derivatives of a hydrocarbon. Absorption

¹ (Note added at correction of proof.) Two more cancers have been obtained from the mice of the 1:2:7:8-dibenzanthracene series; thus 4 of the 10 mice painted with this material have given cancers. Four of the 10 mice painted with impure 3'-methyl-1:2:5:6-dibenzanthracene have developed papillomata and papillomata have been obtained in 1 mouse from the series of 10 painted with 1:2:5:6-dibenzanthracene.

			Fluorescence spectrum				Fluorescence spectrum
Substance	Carcino-genic activity			Substance	Carcino-genic activity		
101 AlCl ₃ -treated tetralin	Active			101 AlCl ₃ -treated tetralin	Active
A62 Durham Holmside gasworks tar 450°	Active			86 Spindle oil	Active
A62'' Durham Holmside gasworks tar 600°	Active			A31 Unheated Californian petroleum	Inactive
A62''' Durham Holmside gasworks tar 1250°	Active			A19 Heated Californian petroleum 880°	Active
A 3 Ether extract of gasworks tar	Active			A4 Acetylene tar	Active
100 1 % gasworks tar	Active			A59 Chlorinated acetylene tar	Active
A18 Blast furnace tar	Inactive			A72 Yeast tar	Active
A48 Creosote oil	Active			A73 Muscle tar	Active
A32 Anthracene oil	Active			A23 Hair tar (800°)	2/94
A22 Green oil	Active			A25 Cholesterol tar	Active
A34 Anthracene 85 %	?			A8 AlCl ₃ -treated naphthalene	1/50
A50 Pitch (medium soft)	Active			A100 Undistilled AlCl ₃ -treated tetralin	Inactive
A21 Pitch distillate	Active			A6 Commercial tetralin	1/30
441 Pitch distillate 300-345°/4 mm.	Under test			A26 Tetroyl-propionic acid mother liq.	Inactive
A13 Shale oil (Pumpherston S)	Inactive			244 Residue from preparation of phenylnaphthalene	Active
A14 Shale oil (blue oil)	Active			207 Oleic acid ("redistilled")	Inactive
A15 Shale oil (Pumpherston R)	Inactive			60 Benzyl oleate	Inactive
A16 Refined lubricating oil	Inactive
85 Low temperature tar 350°	Active

The fractions in column 3 represent the ratio of tumour-bearing mice to total mice tested.

spectra in the ultra-violet also indicate, so far as the experiments have been carried, some connection between at least one powerfully cancer-producing material (*i.e.* the fraction boiling at 260–320°/14 mm. from the mixture obtained by the action of AlCl_3 on commercial tetrahydronaphthalene) and 1:2-benzanthracene.

The author is indebted to the British Empire Cancer Campaign for a research grant; to Prof. Leitch, the Director of this Institute, for the use of its facilities; to Dr Kennaway, who supervised the animal experiments; to W. V. Mayneord, who photographed many of the spectra; to Dr E. de Barry Barnett, for the loan of specimens of pure hydrocarbons; and to Dr J. W. Cook for a number of pure synthetic compounds.

REFERENCES.

- Hieger (1929). *J. Path. Bact.* **32**, 419.
Kennaway (1930). *Biochem. J.* **24**, 497.
Twort and Fulton (1930). *J. Path. Bact.* **33**, 119.
—— C. C. and Twort, J. M. (1928). *J. Hyg.* **28**, 219.