(d) **Isomerization by Melting.**—A sealed tube containing 20 mg. of the carotenoid was maintained at 140–145° for five minutes. After plunging into ice water, the solidified mass was taken up in cold benzene and chromatographed (28 × 7 cm.), following dilution with petroleum ether.

100 colorless top section
60 pink: gazaniaxanthin: 494.5, 462.5, 434.5 mμ (with iodine, 490.5, 458.5 mμ)
5 brownish pink: 489, 457 mμ (490.5, 459 mμ)
20 orange brown: 488, 456.5 mμ (491, 458.5 mμ)
15 orange brown: 485, 454.5 mμ (490.5, 458.5 mμ)
15 yellow: 484, 453.5 mμ (491.5, 459 mμ)
15 yellow: 486, 454.5 mμ (491, 458.5 mμ)
12 pink: 489.5, 457.5 mμ (492, 459 mμ)
1 traces of color: 491, 460.5 mμ (491, 459 mμ)

Summary

1. Gazaniaxanthin,[3] the main pigment of *Gazania rigens* flowers, has been studied by means of methods causing *trans–cis* changes in solution and in melt. On the Tswett column two groups of neo-compounds appeared, the further differentiation of which was difficult.

2. The molecular extinction curve of all-*trans*-gazaniaxanthin shows a very flat maximum around 350 mμ. Upon heating or iodine catalysis, however, a marked maximum develops in this region ("*cis*-peak").[6]

3. Catalytic hydrogenation indicates that gazaniaxanthin may be dihydrorubixanthin.

4. The carotenoid mixture in *Gazania* flowers grown in Portugal[3] differs from that occurring in flowers grown in Southern California.

PASADENA, CALIFORNIA RECEIVED MARCH 4, 1943

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

The Effect of Oxygen on the Fluorescence of Certain Hydrocarbons[1]

BY J. A. MILLER AND C. A. BAUMANN

Oxygen is known to diminish the fluorescence of many compounds[2,3,4,5] including certain polycyclic hydrocarbons.[2,3,4] Weil-Malherbe and Weiss[2] observed that the fluorescence of benzpyrene was nearly the same in several solvents from which oxygen had been removed by suction, but that on oxygenation varying degrees of quenching resulted. The quenching effect was greater in oxygen than in air, and was completely and rapidly reversible. Recently we have observed that the fluorescence of several carcinogenic hydrocarbons varies markedly with the solvent employed.[6] The results did not, however, indicate to what extent the variations were due to dissolved oxygen or directly to the solvent itself.

Accordingly the effect of oxygen has been measured on the fluorescence of 3,4-benzpyrene (BP), 20-methylcholanthrene (MC), 9,10-dimethyl-1,2-benzanthracene (DMBA), 1,2,5,6-dibenzanthracene (DBA), 1,2-benzanthracene (BA), and anthracene (A), each in several representative solvents. Low concentrations of hydrocarbon were employed. The solutions were placed in long all-glass fluorometer tubes as previously described,[7] and the dissolved air removed at the pump. The intensity of fluorescence was measured in a Coleman photofluorometer[7] *in vacuo*[8] and again after shaking the solution with known mixtures of oxygen and nitrogen. Deductions were then made regarding the composition of a hydrocarbon–inhibitor complex assumed to be present in the quenched solutions. Other gases and other inhibitors of fluorescence were studied in a similar manner, and in certain cases the effect of the inhibitor on the hydrocarbon compared with its effect on the unsaponifiable matter of mouse tissues.

General Results

In vacuo, the fluorescence of dilute solutions of benzpyrene was nearly the same in the six solvents employed (Table I). In air, however, marked variations with solvent were observed, and usually the readings were less than half of those observed *in vacuo*. This parallels the results of Weil-Malherbe and Weiss.[2] However, in tetrahydrofurfuryl alcohol, the fluorescence observed in air was over 85% of that observed *in vacuo*, while in aniline or in carbon tetrachloride

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. We are indebted to the Wisconsin Research Foundation and to the Jonathan Bowman Cancer Research Fund for financial support.

(2) Weil-Malherbe and Weiss, *Nature*, **149**, 471 (1942).
(3) Bowen and Norton, *Trans. Faraday Soc.*, **35**, 44 (1939).
(4) Bowen and Williams, *ibid.*, **35**, 765 (1939).
(5) Kautsky, *Ber.*, **64**, 2677 (1931).
(6) Miller and Baumann, *Cancer Research*, **3**, 223 (1943).
(7) Miller and Baumann, *ibid.*, **3**, 217 (1943).
(8) In this paper the phrase "*in vacuo*" refers to the absence of all gas but solvent vapor.

TABLE I

THE FLUORESCENCE OF HYDROCARBONS IN VARIOUS SOLVENTS *in Vacuo* AND IN AIR

Fluorescence, Ammeter Units[a]

Hydrocarbon..... μg./cc.	BP 0.02		MC 0.10		DMBA 0.10		DBA 2.00		BA .50		A .50	
	vac.	air	vac.	air	vac.	air	vac.	air	vac.	air	vac.	air
Petroleum ether	60.6	9.3	56.7	11.5	47.9	12.0	30.0	8.5	33.0	6.3	51.0	33.0
Acetone	63.0	17.2	88.4	27.5	120.6	19.5	45.7	11.5	58.9	15.8	57.2	41.0
Propionic acid	61.5	20.0	67.6	27.5	103.2	21.5	40.1	14.5	46.7	16.5	50.8	43.0
n-Butyl alcohol	59.6	27.0	75.0	36.0	113.6	29.0	43.6	17.5	50.2	23.5	52.8	41.0
Dioxane	70.6	34.8	90.0	53.0	126.3	36.5	54.3	24.0	74.7	40.0	56.6	51.8
Pyridine	60.2	34.8	105.2	62.5	130.5	41.5	59.7	39.5	117.2	63.0	79.0	76.0

[a] The intensity of the fluorescence was expressed in terms of the arbitrary 100 divisions of the ammeter scale, with the instrument set so that 0.0003% quinine sulfate gave a deflection of 50 units.[7]

fluorescence was negligible under both conditions. The fluorescence intensities of the other hydrocarbons were similarly depressed by oxygen, but the intensities *in vacuo* varied widely from one another for any given hydrocarbon (Table I). In every case the quenching was completely and rapidly reversible. The fluorescence of anthracene, the only compound studied lacking an angular benzene ring, was not so greatly affected by oxygen as the other hydrocarbons studied.

At a relatively low partial pressure of oxygen as in air, the fluorescence of benzpyrene in petroleum ether was 15.5% of that *in vacuo*, but for a further diminution much larger amounts of oxygen were required (Fig. 1). Thus fluorescence varied hyperbolically with oxygen tension. However, a change in air pressure of 10 mm. altered fluorescence by only 0.5 ammeter unit[7] or 1% in a reading of 50, the limit of accuracy of our instrument. Changes in temperature affected the fluorescence of the hydrocarbons more markedly. The solubility of oxygen in liquids such as petroleum ether decreased with increase in temperature, and the fluorescence of benzpyrene increased correspondingly. At ordinary room temperatures an increase of 10° increased fluorescence by approximately 6%. However, the combined changes in temperature and pressure within a room appeared to be of only minor consequence in the fluorometric determination of any of the hydrocarbons, since very reproducible readings have been obtained during the past 18 months, even with solvents such as petroleum ether in which the solubility of oxygen is high. *In vacuo*, temperature extremes from 0 to 50° affected the fluorescence of benzpyrene in petroleum ether by less than ± 1%.

In Table II representative solvents are listed according to their abilities to dissolve oxygen. Liquids such as petroleum ether, naphtha, ether and carbon tetrachloride dissolved large amounts of oxygen, and in these solvents the fluorescence of benzpyrene was low in the presence of air.

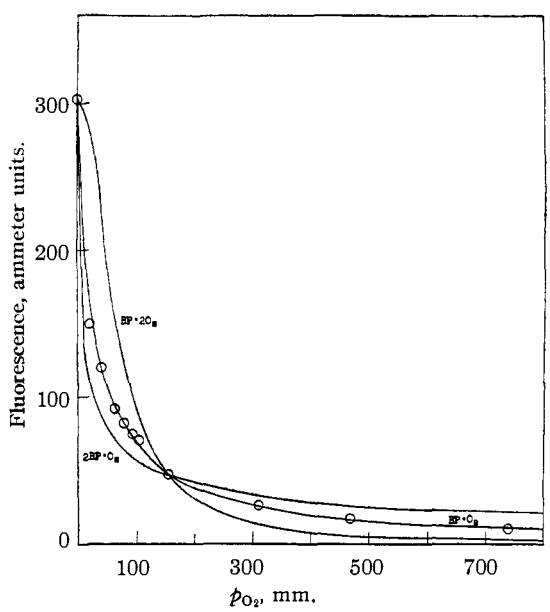


Fig. 1.—The fluorescence of benzpyrene in petroleum ether (1μg./cc.) at various partial pressures of oxygen. The circles indicate observed intensities of fluorescence; the curves are calculated curves for the possible combinations $2BP{\cdot}O_2$, $BP{\cdot}O_2$, $BP{\cdot}2O_2$. The derived equations are

$$\text{for } 2BP{\cdot}O_2,\ F = \frac{-nk_1(1 \pm \sqrt{1 + 8KkP_{O_2}}}{4kKP_{O_2}}$$

$$\text{for } BP{\cdot}O_2,\ F = \frac{nk_1}{1 + kKP_{O_2}}$$

$$\text{for } BP{\cdot}2O_2,\ F = \frac{nk_1}{1 + Kk^2P^2_{O_2}}$$

Each calculated curve passes through the point ($F = nk_1$, $P_{O_2} = 0$), the observed fluorescence *in vacuo*, and through the observed point chosen for the calculation of the Kk^y values, *viz.*, the fluorescence in air. The calculated curve that agrees with the observed data is thus common to all reliable observed points and is evidence for the presence of the assumed complex. However, a new family of curves for the other possible complexes is obtained for every point chosen for the evaluation of Kk^y.

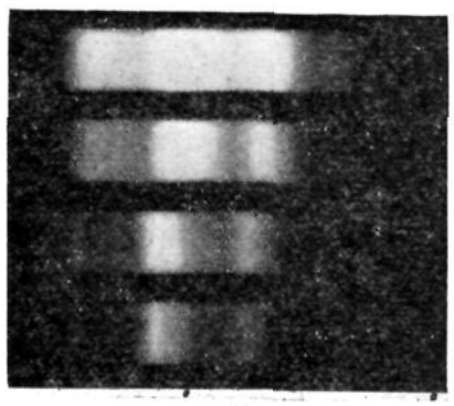
TABLE II

FLUORESCENCE OF BENZPYRENE AND SOLUBILITY OF OXYGEN IN VARIOUS SOLVENTS

Solvent	Fluorescence, ammeter units.[a] 0.1 μg. BP/cc. at 25°C.	Solubility of O_2; cc. O_2 at S. T. P./ cc. solvent at t, °C.[b]	
Ethyl ether	95.0	0.415 at	20.3°
Petroleum ether (up to 65°C., d. 0.668)	46.5[c]	.409	18.5
Petroleum ether (65–100°C., d. 0.709)	57.0[d]	.292	18
Carbon tetrachloride	8.0	.225	25
Naphtha (b. p. 106–117°)	57.0	.21+	28
Acetone	86.0	.207	19.3
Chloroform	115.0	.205	16.3
Methanol	79.0	.175	18.8
Xylene	104.0	.169	16
Toluene	105.0	.168	18
Benzene	120.0	.163	19
Ethyl acetate	77.0	.163	20
Isoamyl alcohol	145.0	.163	17.3
Ethanol, abs.	94.0	.143	19.8
Pyridine	175.0	.12†	28
Pyridine	175.0	.099	18.5
Ethanol, 95%	120.0	.12†	28
Dioxane	174.0	.12†	28
Methyl cellosolve	235.0	.12†	28
Tetrahydrofurfuryl alcohol	286.0	.10†	28
Nitrobenzene	0.0	.070	18.5

[a] Defined in Table I. [b] The solubilities of oxygen are those recorded in the "International Critical Tables," 1st ed. 1928, Vol. III, pp. 261–283, except the values marked †, which are approximations made in this Laboratory in a Van Slyke apparatus. [c] Fluorescence reading in Skelly Solve B, b. p. 66–67°. [d] Fluorescence reading in naphtha, b. p. 106–117°.

Conversely, tetrahydrofurfuryl alcohol, pyridine, and dioxane dissolved lesser amounts of oxygen, and they permitted much greater intensities of fluorescence in the presence of air. Furthermore, within the series—benzene, toluene, xylene—fluorescence varied inversely as the solubility of oxygen. This relationship, however, was not an exact one. Ethyl ether and petroleum ether absorbed nearly the same amounts of oxygen, yet the fluorescence of benzpyrene in the former was double that in the latter. Isoamyl alcohol is reported to dissolve as much oxygen as ethyl acetate, yet fluorescence in the two solvents was markedly different (Table II). Nitrobenzene dissolved little oxygen, but it completely prevented the fluorescence of the hydrocarbon. Evidently solubility of oxygen was not the only factor through which solvents influenced the intensity of the fluorescence of dissolved hydrocarbons. The ether linkage appeared to be one of these other factors: fluorescence was higher in tetrahydrofurfuryl alcohol, methyl cellosolve, dioxane, pyridine (N-analog of an ether), and diethyl ether than the small solubilities of oxygen might lead one to expect.



Solvent	State	Exposure time, min.
Pyridine	Vacuum	1
Pyridine	Air	1
Petroleum ether	Vacuum	1
	Air	4

4358 Å. 5461 Å.

Fig. 2.—Fluorescence spectra of benzpyrene in vacuum and in air.

Benzpyrene is probably less subject to such solvent effect *per se* than the other hydrocarbons, for the intensity of its fluorescence *in vacuo* was approximately the same for several solvents. Methylcholanthrene and the other carcinogens, on the contrary, yielded fluorescence intensities *in vacuo* which varied widely from solvent to solvent. These differences must have been due to the solvent rather than to variable traces of oxygen, since the values observed were readily reproducible, and essentially the same results were obtained whether the "vacuum" was produced with an oil pump, with a water pump or whether the oxygen was displaced by prolonged bubbling of nitrogen through the solution.

Further evidence for solvent effects *per se* was the observation that the position and character of the fluorescence bands of benzpyrene varied with the solvent both in air and *in vacuo*. Oxygen reduced the intensity of the bands without, however, altering either their position or discreteness (Fig. 2). Oxygen failed to alter either the intensity or the character of the absorption spectrum of benzpyrene.

Other Inhibitors and the Fluorescence of Hydrocarbons.—Sulfur, like oxygen, inhibited the fluorescence of benzpyrene in solution. Quenching, which amounted to 21.7%, was observed with saturated solutions of rhombic sulfur in petroleum ether *in vacuo;* continued illumination resulted in the appearance of a yellow precipitate, which was also noted upon irradiation in the absence of benzpyrene. Nitrobenzene and tetranitromethane, previously reported as

fluorescence inhibitors[6] in aerated solvents, also inhibited the fluorescence of benzpyrene *in vacuo*. Both in air and *in vacuo* the quenching produced by very small amounts of tetranitromethane was markedly and quickly enhanced by short exposures to ultraviolet light. Apparently the inhibition of fluorescence by these substances was a reversible process. Solutions exhibiting quenched fluorescence were passed through a column of aluminum oxide, and under ultraviolet light a fluorescent band of hydrocarbon appeared in each case when the column was washed with solvent.

In previous experiments[6] the addition of water to a solution of hydrocarbon in a miscible aerated solvent considerably enhanced the fluorescence of the solution. At least part of this effect was due to the lowered solubility of oxygen in the aqueous solvent. *In vacuo*, however, added water diminished the fluorescence somewhat; *e. g.*, an inhibition of 1.6% was observed for benzpyrene in dioxane containing 10% water. In contrast to these various inhibitors, naphthacene consistently failed to inhibit the fluorescence of these hydrocarbons in liquid solution whether in the presence of air[7] or *in vacuo*.

Other Gases and the Fluorescence of Hydrocarbons.—Bowen and Norton[3] first showed that nitrogen had no effect on the fluorescence of certain hydrocarbons and that the quenching of fluorescence by oxygen could be easily and completely reversed by displacement of this gas from solution with a stream of pure nitrogen. In the present experiments the quenching power of a series of gases was tested. Purified samples of nitrogen, hydrogen, carbon dioxide, carbon monoxide, hydrogen sulfide, sulfur dioxide, hydrogen chloride, ammonia, methylamine, and trimethylamine at atmospheric pressure were passed through evacuated solutions of benzpyrene in petroleum ether or ethanol until saturation occurred and an oxygen-free atmosphere was assured. The maintenance of the fluorescence intensity observed *in vacuo* was taken as evidence of non-inhibition. Inhibition occurred only with hydrogen chloride, trimethylamine, and sulfur dioxide. The hydrogen chloride was freed of oxygen with chromous chloride and dried with concd. sulfuric acid. A consistent and reversible inhibition of fluorescence to the extent of 12% was observed at 740 mm. in a solution of 0.02 μg. benzpyrene per cc. of petroleum ether. Pure trimethylamine hydrochloride was prepared[9] and the free base liberated with excess sodium hydroxide. Saturation at 740 mm. produced an inhibition of 96.4% in a solution of 0.1 μg. of benzpyrene per cc. of petroleum ether. Sulfur dioxide under the same conditions, completely and reversibly inhibited the fluorescence of benzpyrene, and fluorescence could only be observed at relatively low values of P_{SO_2}. At a partial pressure of 149 mm. sulfur dioxide was 25 times as potent as oxygen in quenching the fluorescence of benzpyrene; at higher pressures the ratio increased until at 740 mm. no fluorescence was observed in a solution of 10.0 μg. of benzpyrene per cc. of petroleum ether. This concentration of hydrocarbon would yield a calculated 30,000 ammeter units of fluorescence *in vacuo*.

Inhibitors and the Fluorescence of Tissue Extracts.—In a fluorometric determination of benzpyrene in the tissues of animals developing cancer, the effect of oxygen on the fluorescence of the natural substances from the tissues could become as important on the net result as the effect of oxygen on benzpyrene itself. Accordingly the non-saponifiable fraction from normal mouse tissue was prepared and its fluorescence in petroleum ether and in pyridine observed at various partial pressures of oxygen. The fluorescent materials in the tissue extracts proved to be much less sensitive to dissolved oxygen than the hydrocarbons. Ratios of fluorescence intensities in air and *in vacuo* ranged from 1:1.08–1.13 for pyridine, and 1:1.40–1.69 for petroleum ether (Table III). Corresponding ratios for the hydrocarbons averaged approximately 1:2 in pyridine and 1:4 in petroleum ether. It has previously been noted that the fluorescence of the tissue substances is relatively insensitive to changes in solvent.

Theory of Fluorescence Quenching by Oxygen.—The partial pressure of oxygen necessary to give a molecular concentration of dissolved oxygen in petroleum ether equivalent to that of 0.1 μg. of benzpyrene per cc., as in our experiments, can be calculated to be only 3×10^{-3} mm., a pressure well within our experimental error. It is doubtful whether this amount of oxygen affects fluorescence at all. Conversely, it can be calculated that petroleum ether in equilibrium

(9) Adams and Marvel, "Organic Syntheses," John Wiley and Sons, Inc., New York, N. Y., 1941, Coll. Vol. 1, p. 531.

TABLE III

THE EFFECT OF OXYGEN ON THE FLUORESCENCE OF NON-SAPONIFIABLE MATTER[a] FROM MOUSE TISSUE

Tissue	Wet weight equivalents of extract aliquot,[b] g.	Petroleum ether			Ammeter Units,[c] Wet weight equivalents	Pyridine		
		Air	Vacuum	Ratio		Air	Vacuum	Ratio
Liver	2.4	25.1	35.5	1.41	1.2 g.	15.8	17.3	1.09
Organs	1.8	20.1	34.0	1.69	0.9 g.	13.2	14.7	1.11
GI Tract	2.6	14.0	19.5	1.40	1.3 g.	11.8	12.7	1.08
Carcass	20.4	33.1	52.3	1.58	10.2 g.	28.9	32.8	1.13

[a] The material used had been stored in petroleum ether in the dark for two weeks. Fresh solutions yielded somewhat higher ratios, 1.8–1.9 in petroleum ether and 1.2–1.3 in pyridine. [b] Equivalent to petroleum ether extract of saponified tissue from one 27 g. adult albino mouse. [c] Defined in Table I.

with air at 25° and 740 mm. dissolves approximately 10,000 times as many molecules of oxygen as there are of benzpyrene at this concentration. Under these conditions 15.5% of the original fluorescence still remains. Thus it follows that at any instant only a small fraction of the total oxygen in the solvent can be actually reacting with the hydrocarbon.

Complex formation, however transient, is often assumed to be a basic step in the quenching of fluorescence, and the theory of its mechanism has been explored in some detail.[10] Bowen and Williams[4] have concluded that for certain hydrocarbons fluorescence quenching by oxygen and photo-oxidation are similar but alternative processes, and may involve a dissociable collision complex of hydrocarbon and oxygen. Weil-Malherbe and Weiss[2] have suggested that the quenching of hydrocarbon fluorescence by oxygen may be represented by a chemical reaction in which an electron is transferred from the excited hydrocarbon to the oxygen molecule of the complex. However, whether the essential quenching reaction by oxygen involves a complex or a collision does not appear to have been determined.

The hyperbolic relationship existing between the fluorescence intensity of benzpyrene and the partial pressure of oxygen (Fig. 1) may be explained in at least two ways. On the basis of mass action it may be assumed that in the quenching of fluorescence a non-fluorescent complex $x\text{HC}\text{–}y\text{O}_2$ is formed. Since the observed quenching is easily and completely reversible, it would follow that this complex is easily dissociated as in

$$x\text{HC} + y\text{O}_2 \rightleftarrows x\text{HC}\text{–}y\text{O}_2$$

The equilibrium constant of this reaction is expressed by

$$K = \frac{(x\text{HC}\text{–}y\text{O}_2)}{(\text{HC})^x (\text{O}_2)^y} \qquad (1)$$

If the concentration of hydrocarbon is n moles per liter and

$$\alpha = \text{moles of } x\text{HC}\text{–}y\text{O}_2 \text{ per liter}$$

then

$$n - \alpha x = \text{moles of free HC per liter}$$

According to the assumption that the fluorescence of a solution is proportional to the concentration of free hydrocarbon, the fluorescence

$$F = k_1(\text{HC}) = k_1(n - \alpha x)$$

By means of these values, and the Henry's law equivalent, $(\text{O}_2) = kP_{\text{O}_2}$, equation 1 can be reduced to

$$xK(kP_{\text{O}_2})^y F^x + k_1{}^{x-1} F - nk_1{}^x = 0 \qquad (2)$$

a general equation relating F and P_{O_2} in terms of the constants as defined. For the simplest complex, $\text{HC}\text{–}\text{O}_2$, x and y both equal 1, and equation 2 becomes

$$F = \frac{nk_1}{1 + kKP_{\text{O}_2}} \qquad (3)$$

This is similar in form both to the Stern–Volmer equation[11] for the quenching by foreign gases of the fluorescence of vapors at low pressures, and also to the equation used by Bowen and Norton[3] to express the dependence of the "fluorescence efficiency" (quanta emitted/quanta absorbed) of hydrocarbons upon the concentrations of such quenchers as dissolved oxygen or the hydrocarbon itself at high concentrations.

It is not necessary to know k and K separately for a given solvent, since

$$Kk^y = \frac{nk_1{}^{x-1}k_1{}^{x-1} F}{xF^x P_{\text{O}_2}^y}$$

an expression in terms of observable and assumed quantities. Thus it is possible to calculate the F *vs.* P_{O_2} curve for any assumed complex of a given hydrocarbon and oxygen. All such calculated curves must pass through two experimentally determined points. One convenient point is ($F = k_1$, $P_{\text{O}_2} = 0$), characteristic of the

(10) Bowen, Weiss and others. A general discussion of luminescence held by the Faraday Society in Sept., 1938. Part I. Luminescence of Liquids and Vapours, *Trans. Faraday Soc.*, **35**, 15 (1939).

(11) Hirschlaff, "Fluorescence and Phosphorescence," Chemical Publishing Co., Inc., New York, N. Y., 1942, p. 18.

TABLE IV

THE INTENSITY OF FLUORESCENCE OF BENZPYRENE IN VARIOUS SOLVENTS FOR DIFFERENT PARTIAL PRESSURES OF OXYGEN; OBSERVED AND CALCULATED[a]

Intensity of fluorescence of 0.1 μg. BP/cc., ammeter units[b]

P_{O_2}, mm.	Cyclohexane Obs.	Cyclohexane Calcd.	Methyl cellosolve Obs.	Methyl cellosolve Calcd.	Pyridine Obs.	Pyridine Calcd.	Tetrahydrofurfuryl alcohol Obs.	Tetrahydrofurfuryl alcohol Calcd.	P_{O_2}	Tricaproin 0.2 μg. BP/cc. Obs.	Tricaproin 0.2 μg. BP/cc. Calcd.
0	304	(304)[c]	314	(314)	301	(301)	334	(334)	0	36.3	(36.3)
21	226	235	...	...	281	274	..	...	79	32.7	33.3
42	190	204	...	...	253	252	..	...	157	30.9	(30.9)
63	162	170	...	...	238	233	..	...	373	24.5	25.6
116	120	115	225	228	200	196	..	...	447	23.8	24.2
155	95.5	(95.5)	209	(209)	175	(175)	286	(286)	747	18.0	19.8
310	57.8	56.5	156	157	130	123	250	247			
466	39.4	40.2	125	125	101	95.0	220	219			
742	21.0	27.4	85.5	92.4	72.0	67.8	178	178			

[a] All calculated for the complex BP·O_2. [b] Defined in Table I. [c] Figures in parentheses were used to calculate constants.

TABLE V

OBSERVED AND CALCULATED FLUORESCENCE INTENSITIES OF HYDROCARBONS FOR COMPLEX H·CO_2

Fluorescence, Ammeter Units.[a] Hydrocarbon, μg./cc. petroleum ether

P_{O_2}, mm.	MC, 0.1 μg. Obs.	MC, 0.1 μg. Calcd.	DMBA, 0.1 μg. Obs.	DMBA, 0.1 μg. Calcd.	DBA, 2.0 μg. Obs.	DBA, 2.0 μg. Calcd.	BA, 1.0 μg. Obs.	BA, 1.0 μg. Calcd.	A, 0.5 μg. Obs.	A, 0.5 μg. Calcd.
0	56.0	(56.0)	47.9	(47.9)	30.0	(30.0)	66.8	(66.8)	51.0	(51.0)
21	37.1	36.9	31.0	33.9	21.3	22.3	42.0	41.9	47.9	47.5
63	22.7	21.9	22.4	21.5	14.0	14.8	25.3	24.1	42.0	41.7
116	14.7	14.5	14.4	14.7	9.50	10.4	16.5	15.7	35.2	36.3
155	11.6	(11.6)	11.9	(11.9)	8.50	(8.50)	12.5	(12.5)	33.0	(33.0)
310[b]	6.26	6.47	7.80	6.80	4.34	4.96	6.64	6.89	23.8	24.4
466[b]	4.40	4.48	4.59	4.74	3.04	3.49	4.55	4.74	18.6	19.3
742[b]	2.58	2.90	2.74	3.10	1.69	2.29	2.71	3.06	11.6	14.1

[a] Defined in Table I. [b] More concentrated solutions were used to obtain readings at these pressures.

free hydrocarbon in the oxygen-free solvent. Another is observed fluorescence in air. These numerical values plus trial values of x and y serve for the determination of the Kb^y value, and the fluorescence at any other partial pressure of oxygen can then be calculated.

Figure 1 shows the calculated curves of various possible complexes of benzpyrene with oxygen in petroleum ether, as well as the observed fluorescence at various pressures of oxygen. The curve obtained experimentally corresponds closely to the calculated curve for the complex BP-O_2; it does not correspond to any of the curves for the other possible simple complexes of BP and O_2. In Table IV data are given for observed and calculated curves relating the partial pressure of oxygen to the fluorescence of benzpyrene in five other solvents including the triglyceride tricaproin. Experiments also have been performed with benzpyrene in purified cottonseed oil and in lard. The fluorescent materials in the crude fat were eliminated by treatment with tetranitromethane, after which colored materials were adsorbed on charcoal. In Table V the results of similar experiments are presented for each of the other hydrocarbons in petroleum ether. In every case the agreement between the observed curves and the calculated curves for the complex HC-O_2 was sufficiently close to exclude other possible combinations.

This agreement, however, does not necessarily prove that a combination between hydrocarbon and oxygen actually exists as such. It may merely express the probability of bimolecular collisions, and an equation identical with equation 3 has been derived from a collision hypothesis.[12] An attempt was made to distinguish between the mechanisms implicit in the two derivations by measuring the volume of air confined above pyridine before and after solid benzpyrene was dissolved in the liquid. The experiment was performed in a Barcroft respirometer, and the amounts of hydrocarbon dissolved were 10.16 and 0.68 mg. per 5 cc. Since 58% of the fluorescence of the hydrocarbon is quenched in pyridine at the P_{O_2} of air, 526 and 35 μl. of oxygen, respectively, would have been absorbed if, in

(12) M. J. Johnson, personal communication.

quenching, the hydrocarbon had combined with the calculated amount of oxygen firmly enough to remove it effectively from solution. However, no gaseous uptake was observed. Since no errors in combustion have been reported, it may be assumed that no oxygen was combined with the benzpyrene in the solid state. Hence within the limitations of the experiment, no evidence of firm combination was observed, and the collision hypothesis would appear to be favored if an essential difference actually exists between a collision and a short-lived complex.

But, however interpreted, the data on the quenching of fluorescence by oxygen indicate that benzpyrene somehow reacts with dissolved oxygen, and that the reaction is not only regular and reproducible but even predictable from fundamental considerations. The results deny the popular impression of the capriciousness of fluorometric measurements. From a physiological point of view it is of interest that the reaction between dissolved oxygen and the carcinogenic hydrocarbon proceeds in glycerides or in natural fats much as in any other solvent. While the significance of the reaction is as yet obscure, it is possible that it may be involved in the carcinogenic process itself. Another possibility is that the reaction is the first step in a sequence by which the hydrocarbons are rendered biologically inactive.

Summary

1. The intensity of fluorescence was measured for 3,4-benzpyrene, 20-methylcholanthrene, 9,10-dimethyl-1,2-benzanthracene, 1,2,5,6-dibenzanthracene, 1,2-benzanthracene and anthracene, each in several solvents in air and *in vacuo*. In air the intensities usually ranged from one-half to one-sixth of those observed *in vacuo* (absence of all gas but solvent vapor). Most of the difference was due to dissolved oxygen, although in some cases the degree of quenching did not parallel oxygen solubility. In the presence of oxygen the fluorescence bands of benzpyrene were diminished in intensity, but both the fluorescence and absorption spectra of benzpyrene were qualitatively the same in air and *in vacuo*.

2. Sulfur dioxide was many times as effective an inhibitor of fluorescence as oxygen; hydrogen chloride and trimethylamine were less effective. The inhibition was reversible by changing the partial pressure of the gas. Sulfur, nitrobenzene, and tetranitromethane inhibited the fluorescence of benzpyrene solutions both in air and *in vacuo*. This inhibition could be reversed by passing the quenched solution through a column of aluminum oxide. Nitrogen, hydrogen, carbon dioxide, hydrogen sulfide, methylamine, and ammonia failed to affect the intensity of fluorescence of benzpyrene.

3. The fluorescence intensities of the hydrocarbons varied hyperbolically with the partial pressure of oxygen. Identical equations relating these two quantities were derived on the alternate assumptions that a non-fluorescent dissociable complex formed between oxygen and the hydrocarbon or that the quenching of fluorescence by oxygen was a collision phenomenon. Observed values agreed well with calculated values from the derived equations.

4. The fluorescence of the non-saponifiable matter from mouse tissue did not vary greatly with P_{O_2} either in petroleum ether or in pyridine.

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