once from ethanol to give the N-benzoyl-2-amino-3-methylthianaphthene melting at 167.5–170°.

*Anal.* Calcd. for $C_{16}H_{13}NOS$: N, 5.24. Found: N, 5.37.

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## Studies on Carcinogenic Hydrocarbons: Dissociation Constants and Free Energies of Formation of Complexes with 2,4,7-Trinitrofluorenone[1]

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The reasons for the synthesis of appreciable amounts (about 20–25 g. of each) of all of the monomethyl-1,2-benzanthracenes and monomethylbenzo(c)phenanthrenes have been stated.[2] In an attempt to find some physical or chemical property of these hydrocarbons which might be correlated with their cancer producing activities, we have studied spectrophotometrically the equilibrium of the reaction between these hydrocarbons and 2,4,7-trinitrofluorenone[3] (TNF) in chloroform solution. From the data we have calculated the dissociation constants for the reaction

$$X\cdot TNF \rightleftharpoons X + TNF$$

We also have calculated the free energy of formation of the complexes X·TNF. The results of these calculations are summarized in Table I.

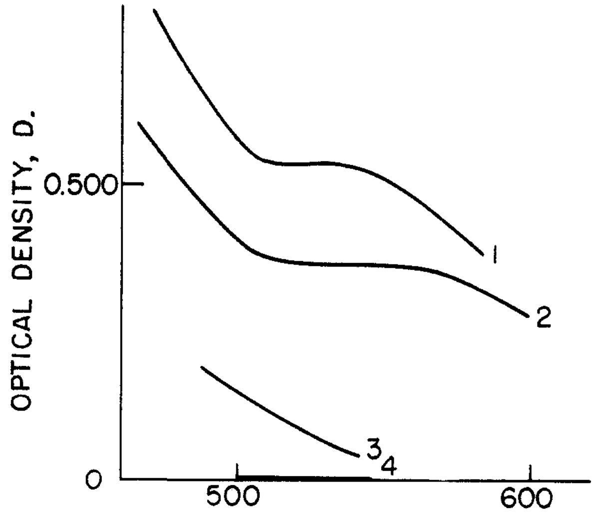

Fig. 1.—Typical absorption curves: initial concentrations in $CHCl_3$, 1, 6-methyl-1,2-benzanthracene 0.999 × $10^{-3}$ *M*, TNF 47.0 × $10^{-3}$ *M*; 2, 9-methyl-1,2-benzanthracene 1.045 × $10^{-3}$ *M*, TNF 46.7 × $10^{-3}$ *M*; 3, benzo(c)phenanthrene 0.750 × $10^{-3}$ *M*, TNF 38.4 × $10^{-3}$ *M*; 4, saturated solution of TNF.

TABLE I

DISSOCIATION CONSTANTS AND FREE ENERGIES OF FORMATION OF THE COMPLEXES OF 1,2-BENZANTHRACENES AND BENZO(C)-PHENANTHRENES IN CHLOROFORM, 25°

| 1,2-Benzanthracenes | Wave length,[a] mμ | *K*, mole/l. | *F*, cal./mole |
|---|---|---|---|
| 4-Methyl | 517 | 0.013 | −2600 |
| 9-Methyl | 546 | .019 | −2400 |
| 7-Methyl | 517 | .019 | −2400 |
| 6-Methyl[b] | 526 | .021 | −2300 |
| | 526 | .023 | −2200 |
| 8-Methyl | 517 | .023 | −2200 |
| 10-Methyl[b] | 546 | .023 | −2200 |
| | 546 | .024 | −2200 |
| 3-Methyl | 517 | .029 | −2100 |
| 3′-Methyl | 526 | .029 | −2100 |
| 4′-Methyl | 517 | .030 | −2100 |
| Unsubstituted | 517 | .034 | −2000 |
| 5-Methyl[b] | 517 | .032 | −2000 |
| | 526 | .036 | −2000 |
| 1′-Methyl | 517 | .040 | −1900 |
| 2′-Methyl | 526 | .059 | −1700 |
| Benzo(c)phenanthrenes | | | |
| 4-Methyl | 500 | 0.035 | −2000 |
| 2-Methyl[b] | 526 | .051 | −1800 |
| | 500 | .052 | −1800 |
| 6-Methyl | 500 | .054 | −1700 |
| 5-Methyl | 500 | .056 | −1700 |
| 3-Methyl | 500 | .087 | −1400 |
| Unsubstituted[b] | 500 | .12 | −1200 |
| | 500 | .12 | −1200 |
| 1-Methyl[b] | 500 | .15 | −1100 |
| | 500 | .15 | −1100 |

[a] Wave length at which measurements were taken. [b] Two separate experiments were carried out and the values obtained in each are recorded.

### Experimental

The general procedure for spectrophotometric determination is described below.

A solution of the hydrocarbon of known concentration (0.7–1.0 × $10^{-3}$ *M*) in chloroform (C.P.) was prepared. This was transferred to a series of volumetric flasks containing weighed amounts of TNF. The TNF concentrations in the resultant solutions were from 35–47 × $10^{-3}$ *M*. The mixtures were shaken to dissolve the TNF and then were allowed to stand overnight at room temperature. Prior to the photometric measurements the solutions were set for several hours in the spectrographic laboratory where the room temperature was held at 25°.

The solutions of the TNF complexes with the methyl-1,2-benzanthracenes varied in color from a deep red to reddish purple while the methylbenzo(c)phenanthrenes gave orange solutions. For each of the methyl-1,2-benzanthracene solutions there was a region around 517–546 mμ where the optical densities of these solutions were constant (Fig. 1). No such plateaus were found in the absorption curves of benzo(c)phenanthrene and its methyl derivatives. The absorption of TNF alone was very small at the wave lengths studied.

The optical densities of the solutions were measured with a Beckman quartz spectrophotometer, model DU, in 1-cm. quartz cells. Measurements were made at a number of wave lengths in the region of 500–546 mμ. These were taken as rapidly as possible to minimize any temperature changes of the solution in the cell compartment.

The TNF complexes were made by the method described for 1,2-benzanthracene.[3] Recrystallization involved solution in acetone, addition of alcohol, and distillation to incipient crystallization. The melting points recorded in Table II are for once recrystallized samples. All of the TNF derivatives involved a 1:1 ratio of hydrocarbon and

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[1] This work was supported by a grant, C-483-C3,4 from the U. S. Public Health Service to whom grateful acknowledgment is made.

[2] M. S. Newman and R. Gaertner, THIS JOURNAL, **72**, 264 (1950); M. S. Newman, H. V. Anderson and K. H. Takemura, *ibid.*, **75**, 347 (1953).

[3] M. Orchin and O. Woolfolk, *ibid.*, **68**, 1727 (1946).

TABLE II

MELTING POINTS OF HYDROCARBONS, TNF COMPLEXES AND PICRATES

| Hydrocarbon | M.p.,[a] °C. | M.p. (TNF),[b] °C. | M.p. (picrate),[c] °C. |
|---|---|---|---|
| 1,2-Benzanthracene | 159.5–160.5[c] | 223.6–224.0[d] | 141.5–142.5 |
| 1′-Methylbenzanthracene | 138 | 241.1–241.4 | 119[e] |
| 2′-Methylbenzanthracene | 150.7–151.7 | 218.7–219.2 | 180 |
| 3′-Methylbenzanthracene | 163.0–163.9 | 239.0–239.6 | 146–146.8 |
| 4′-Methylbenzanthracene | 194 | 228.2–228.8 | 139 |
| 3-Methylbenzanthracene | 155.9–156.9 | 235.4–235.6 | 153[f] |
| 4-Methylbenzanthracene | 126.2–127.2 | 221.4–221.8 | 149–150 |
| 5-Methylbenzanthracene | 160.6–160.6 | 243.2–243.6 | 165.8–166.3 |
| 6-Methylbenzanthracene | 150.5–151.5 | 225.1–225.4 | 152–153 |
| 7-Methylbenzanthracene | 183.0–183.6 | 224.8–225.0 | 159.0–159.4 |
| 8-Methylbenzanthracene | 118.0–118.5 | 238.2–238.6 | 159.5–160.0 |
| 9-Methylbenzanthracene | 138.6–139.6 | 209.5–209.7 | 115 |
| 10-Methylbenzanthracene | 140.2–140.8 | 236.1–236.5 | 173.5–174.0 |
| Benzo(c)phenanthracene | 68[c] | 170.8–171.1 | 128–128.5[g] |
| 1-Methylbenzo(c)phenanthrene | 141.4–141.9[c] | 130.6–131.4[h] | None |
| 2-Methylbenzo(c)phenanthrene | 80–81[c] | 144.2–144.5 | 118–118.5 |
| 3-Methylbenzo(c)phenanthrene | 54–54.5[c] | 178.5–178.8 | 134–134.5 |
| 4-Methylbenzo(c)phenanthrene | 65–66[c] | 180.7–180.9 | 107–108 |
| 5-Methylbenzo(c)phenanthrene | 70.4–71.0[c] | 157.9–158.5 | 141.8–143.2 |
| 6-Methylbenzo(c)phenanthrene | 77–78[c] | 171.8–172.2 | 112.5–113.5 |

[a] See m.p.'s listed in M. S. Newman and R. Gaertner, THIS JOURNAL, **72**, 264 (1950). [b] Melting points taken on short range Anschütz thermometers certified by the U. S. Bureau of Standards. [c] Taken from M. Orchin, *J. Org. Chem.*, **16**, 1165 (1951). [d] M. Orchin and E. O. Woolfolk, THIS JOURNAL, **68**, 1727 (1946). [e] Dipicrate. [f] In the ref. in *a*, the m.p. was found to be 146.0–146.8°. [g] See W. E. Bachmann and R. O. Edgerton, THIS JOURNAL, **62**, 2970 (1940). [h] M. S. Newman and W. B. Wheatley, *ibid.*, **70**, 1913 (1948).

TNF, as all gave values for nitrogen which ranged from 7.2 to 7.8% N (calcd. for $C_{32}H_{19}O_7N_3$: N, 7.5). Microanalyses by the Galbraith Laboratories, Knoxville, Tennessee.

## Calculations

Calculation of the dissociation constants (Table I) of the complexes was based upon methods previously reported.[4]

For the dissociation X·TNF ⇄ X + TNF in dilute solutions, the dissociation constant, $K$, may be expressed as

$$K = \frac{[X][TNF]}{[X \cdot TNF]} \quad (1)$$

where [X], [TNF] and [X·TNF] are molar concentrations of hydrocarbon, TNF and complex, respectively. If $[X]_0$ be the initial molar concentration of hydrocarbon and $[TNF]_0$ be the initial molar concentration of TNF, then

$$K = \frac{([X]_0 - (X \cdot TNF))([TNF]_0 - [X \cdot TNF])}{[X \cdot TNF]} \quad (2)$$

From the expression for the molar extinction coefficient of the complex, $\epsilon = D/[X \cdot TNF]$, (where $D$ is the optical density of the solution corrected for the absorption of TNF and $l$ is the cell length), and for the case where $[TNF]_0 \gg [X \cdot TNF]$

$$K = \frac{([X]_0 - D/\epsilon l)([TNF]_0)}{D/\epsilon l} \quad (3)$$

$$1/D = (K/\epsilon l[X]_0)(1/[TNF]_0) + (1/\epsilon l[X]_0) \quad (4)$$

Equation (4) is of the form $y = mx + b$. Figure 2 illustrates the plot of equation (4) in the case of the 9-methyl-1,2-benzanthracene-TNF complex. The constants, $K/\epsilon l[X]_0$ and $1/\epsilon l[X]_0$, for the curve were determined by the method of least squares. From these $K$ is readily obtained. Since the pure hydrocarbon shows no absorption at 500–546 mμ and the absorption of TNF is very small (Fig. 1), the corrected optical density, $D$, of the solution is due to the complex alone.

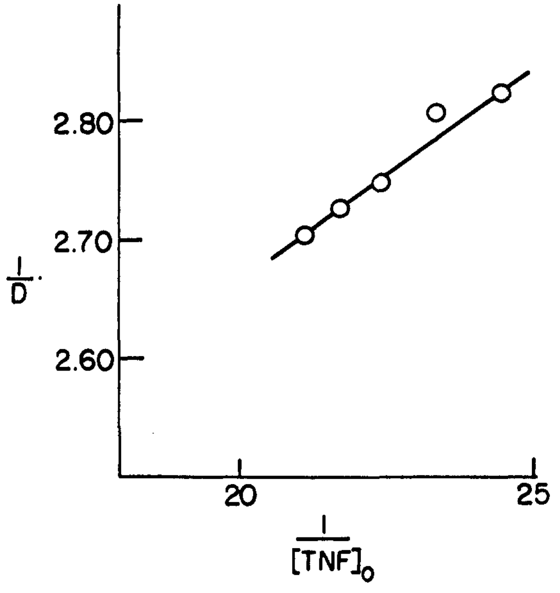

Fig. 2.—Plot of equation (4) for the 9-methyl-1,2-benzanthracene–TNF complex. Initial concentration of hydrocarbon 1.003 × $10^{-3}$ $M$. $D$ = optical density of solutions at 546 mμ. $[TNF]_0$ = initial molar concentration of TNF.

## Discussion

In attempting to correlate any physical or chemical property of a compound with its carcinogenic activity it is essential to have at least a semiquantitative measure of this activity. In spite of the large amount of biological testing which has been

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(4) H. A. Benesi and J. H. Hildebrand, THIS JOURNAL, **71**, 2703 (1949); J. Landauer and H. McConnell, *ibid.*, **74**, 1221 (1952).

done, no study has as yet been reported in which all of the monomethyl-1,2-benzanthracenes and/or -benzo(c)phenanthrenes have been tested under reasonably constant conditions. The difficulties inherent in the attempt to assign the degree of carcinogenic potency to any hydrocarbon are well summarized in the Introduction to the latest survey on carcinogenic activity.[5] Fortunately, work which will provide some of the necessary data[6] on the methyl-1,2-benzanthracenes and -benzo(c)-phenanthrenes is now in progress in the laboratories of E. von Haam (Ohio State University) and I. Berenblum (Weizmann Institute).

Despite this present lack of complete quantitative data on the carcinogenic properties of these hydrocarbons, it is fairly well established that among the monomethyl-1,2-benzanthracenes, the 10-methyl is the most active cancer producing agent, the 5- and 9-methyl derivatives are slightly less active, while the 1′-derivative is comparatively inactive.[5,6]

From this qualitative information it is apparent that there is no correlation between carcinogenic activity of the hydrocarbon and the dissociation constant (or free energy of formation) of its TNF complex. For example, the TNF complex of the 5-methyl derivative is less stable than that of the 10-methyl while the 9-methyl complex is more stable. Furthermore, relatively inactive carcinogens form complexes which are about as stable as the 10- (*e.g.*, the 8-methyl), more stable (*e.g.*, the 7-methyl), or even less stable (*e.g.*, the 1′- and 2′-methyl). There are not enough data on the carcinogenic activity in the benzo(c)phenanthrene series to make any comparisons.

Recently the bathochromic shifts in the ultraviolet absorption of 1,2-benzanthracene produced by the substitution of a methyl group for each of the twelve hydrogens have been determined.[7] A comparison of the observed shifts with the conjugating power (calculated both by the molecular orbital and valence bond methods) of the hydrocarbons was made. The stabilities of the hydrocarbon–TNF complexes given in Table I show no correlation with either the bathochromic shifts or the conjugating power of the hydrocarbon.

Orchin[8] has discussed steric effects in the formation of complexes of tetracyclic compounds. He states that "although substitution of a methyl group on the hydrocarbon usually enhances the ease of formation and stability of the picrate, if the methyl group is located where steric interference (methyl, hydrogen overlap) is possible, complex formation is often made difficult or the complex forms in other than a 1:1 ratio." With the methyl-1,2-benzanthracenes such steric interference is possible in the case of the 9-methyl (overlap with 1′-hydrogen) and 1′-methyl (overlap with the 9-hydrogen); in 1-methylbenzo(c)phenanthrene considerable overlap with the 1′-hydrogen is possible. Orchin used the melting point of the picrate as a criterion of its stability,[9] concluding that the 9-methyl-1,2-benzanthracene picrate, because of its low melting point (see Table II), is unstable; he noted that the 1′-methyl was not a 1:1 complex and that the 1-methylbenzo(c)phenanthrene complex did not form.

Since the TNF compounds with the methyl-1,2-benzanthracenes and -benzophenanthrenes are all formed in a ratio of 1:1, an evaluation of the general applicability of the melting point as a criterion of complex stability is afforded. While these compounds may be arranged into groups which melt fairly closely and have comparable stability, there are notable exceptions. The 9-methyl complex is the lowest melting (209°) of the 1,2-benzanthracenes, but is the second most stable; the 1′-methyl is the second highest melting, but is the next to the least stable.

The effect of the methyl, hydrogen overlap on the stability of the TNF complexes is of interest. The 1′-methyl-1,2-benzanthracene complex is, indeed, relatively unstable, but the 9-methyl complex is the second most stable. As would be expected from steric considerations, the complex compound with 1-methylbenzo(c)phenanthrene is the most unstable of its series.

We are presently engaged in determining the rate of reaction of the hydrocarbons of the 1,2-benzanthracene series with maleic anhydride and the equilibrium constants for these reactions.

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[5] "Survey of Compounds which Have Been Tested for Carcinogenic Activity," 2nd Ed., Federal Security Agency, Public Health Service Publication No. 149, U. S. Government Printing Office, Washington, D. C., 1951.

[6] *Ibid.*, 1st Ed. On p. 9 J. L. Hartwell says, "The most obvious need, as is shown by the table, is for more complete investigation of compounds already studied in a preliminary fashion."

[7] G. M. Badger, R. S. Pierce and R. Pettit, *J. Chem. Soc.*, 1112 (1952).

[8] M. Orchin, *J. Org. Chem.*, **16**, 1165 (1951).

[9] See also C. Shinomiya, *Bull. Chem. Soc. Japan*, **15**, 92, 137, 281 (1940).

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## Densities and Freezing Points of Sodium Permanganate Solutions

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In the use of strong solutions of sodium permanganate it became necessary to know the densities and phase diagram for the system sodium permanganate–water. The temperature at which a solid phase appears was determined for solutions from 0 to 96 weight per cent. sodium permanganate. The salt received for this work analyzed 88 weight per cent. and when dried *in vacuo* at 100° the analysis rose to 98.5% sodium permanganate. Further drying did not improve the purity. All compositions are given on the weight basis and corrected to the $NaMnO_4$ and water content only. The 1.5% is considered inert and as playing no part on freezing point curves. Solutions were made up by mixing weighed amounts of salt and water and titrations against standard sodium oxalate were used to check the concentration. The freezing points composition curves were obtained by recording the cooling curves for various concentrations of sodium permanganate in the standard way; *i.e.*, the solutions were mechanically stirred during cooling and seed crystals added where any tendency to under-