A Spectroscopic Study of the Nature of the Complexes of Purines with Aromatic Compounds.

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The changes in infra-red absorption spectra observed on formation of crystalline complexes of caffeine and tetramethyluric acid with polycyclic hydrocarbons, dibenzocarbazoles, and other polycyclic aromatic compounds are only slight and comparable with those observed in the formation of the complex from diphenyl and 4 : 4′-dinitrodiphenyl by Burton and Richards (*J.*, 1950, 1316). They are, however, always in the same direction and can be related to structural changes; furthermore, comparable changes have been observed in concentrated chloroform solutions. These facts support the suggestion that the complexes owe their formation to forces of attraction between the two components arising from their mutual polarization.

THE ability of purines such as caffeine to form complexes with many other substances has long been known, and that aqueous solutions of purines can dissolve carcinogenic hydrocarbons including 3 : 4-benzopyrene was shown by Brock, Druckrey, and Hamperl (*Arch. Exp. Path. Pharm.*, 1938, **189**, 709). The quantitative aspect of the solubilization of aromatic hydrocarbons (both carcinogenic and non-carcinogenic) by purines in aqueous solution was studied by Weil-Malherbe (*Biochem. J.*, 1946, **40**, 351), who also prepared crystalline molecular compounds of pyrene, 3 : 4-benzopyrene, and coronene with 1 : 3 : 7 : 9-tetramethyluric acid; the forces responsible for the two effects were postulated by him as probably identical.

The solubilizing effect of aqueous solutions of purines has also been shown with carcinogenic aromatic amines such as benzidine and aminofluorene by Neish (*Rec. Trav. chim.*, 1948, **67**, 361) and for carcinogenic dibenzacridines and dibenzocarbazoles by Booth and Boyland (*Biochim. Biophys. Acta*, 1953, **12**, 75). The latter authors also showed that polycyclic compounds were dissolved by aqueous solutions of sodium deoxyribonucleate. In some of these instances solid molecular compounds are also formed, having been reported for caffeine and 1 : 3 : 5 : 7-tetramethyluric acid with a variety of the above-mentioned aromatic compounds. The infra-red spectra of some of these solid complexes, and both infra-red and ultra-violet spectra of their solutions have been obtained. The complexes studied were those of 1 : 2-5 : 6, 1 : 2-7 : 8-, and 3 : 4-5 : 6-dibenzocarbazole α-naphthol, and benzidine with both caffeine and tetramethyluric acid, and of pyrene, 3 : 4-benzopyrene, β-naphthylamine, and 3 : 4-6 : 7-dibenzacridine with tetramethyluric acid.

EXPERIMENTAL

Preparation of Molecular Complexes.—The molecular complexes formed by tetramethyluric acid with pyrene and 3 : 4-benzopyrene were kindly given by Dr. H. Weil-Malherbe.

The other complexes were prepared by shaking an excess of the solid aromatic compound (or a small volume of concentrated ethanolic solution) in a saturated aqueous solution of the purine and filtering off the excess of solid. A small amount of crystalline material was precipitated on storage and was separated by filtration (Booth and Boyland, *loc. cit.*).

Infra-red Spectra.—These were obtained on a Perkin-Elmer 12C spectrometer fitted with a 13-cycle amplifier. The spectra from 3600 to 2500 cm.$^{-1}$ were obtained witha lithium fluoride prism, and from 1900 to 650 cm.$^{-1}$ with a rock-salt prism. The accuracy of band position, expressed as wave-length deviation, remained approximately constant over the entire region at about 0·01 μ, and the reproducibility of successive runs was within about 0·003 μ. Materials in the solid state were measured as mulls in liquid paraffin or, where that medium absorbed, in a fully fluorinated oil. Chloroform was used as solvent for measurements on solutions, which were made up as follows. Saturated solutions of these substances in chloroform are about 0·5M. The aromatic compound was therefore dissolved in an equivalent amount of 0·5M-purine solution and made up to a known volume, giving about a 0·35M-solution. With tetramethyluric acid, 3 : 4-5 : 6-dibenzocarbazole and α-naphthol dissolved, but within a minute the solid complex was deposited from solution; from 0·12M-solution the carbazole complex was only slightly, and the α-naphthol complex not at all, precipitated. This precipitation is analogous to that observed in aqueous solutions by which method the majority of the complexes were prepared.

Ultra-violet Spectra.—Absorption measurements were made with a Unicam S.P. 500 photoelectric spectrophotometer, readings being taken at intervals of 1 mμ. The spectra of the molecular compounds and their components were measured in ethanol or benzene. For examination of the effect on the spectra produced by a large excess of purine, 50% ethanol was used in most cases.

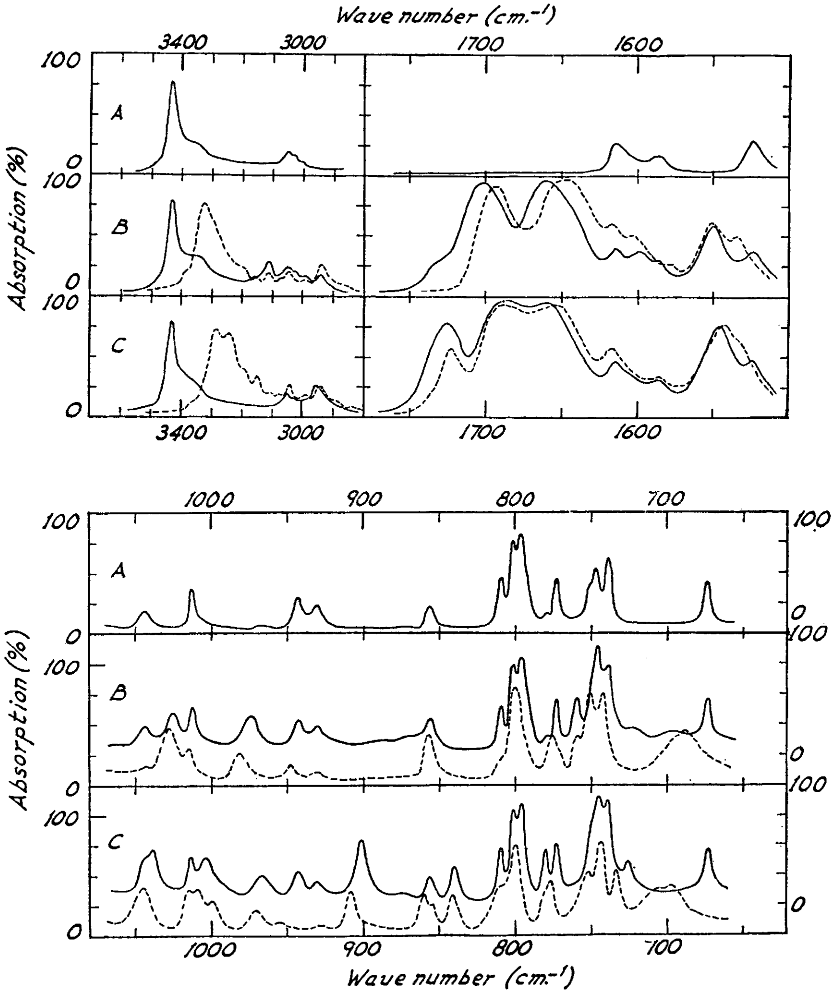
RESULTS AND DISCUSSION

The infra-red spectra of the pure components and the fourteen complexes were measured as solids under the conditions described. A typical set is shown in Fig. 1 where the spectra of the complexes of 3 : 4-5 : 6-dibenzocarbazole with caffeine and tetramethyluric acid may be compared with those of the mixtures of their components. From the spectrum of pure 3 : 4-5 : 6-dibenzocarbazole, it can be seen which part of the absorption of the mixture is due to it and which to the respective purine. The changes in absorption are relatively small and are typical of the effect obtained in all cases. It was in order to facilitate observation of these small changes that mixtures of the two components in proportions corresponding to each complex were made up as mulls in liquid paraffin and compared with the corresponding complex. If kept, such mixtures invariably formed complexes after times varying from 30 minutes to several weeks. The reaction time was reduced by heating and appeared to depend mainly on other external factors such as the extent of grinding and the amount of moisture or impurities present. The change did not occur on grinding of the dry materials together but it was always effected by melting, and these facts suggest that, as expected, complex formation takes place in solution, even though limited as in the case of the liquid paraffin. In all except one case, the complexes formed in this and the more conventional manner were identical. The exception occurred with 1 : 2-5 : 6-dibenzocarbazole and tetramethyluric acid which gave a 1 : 2 complex on isolation from solution. Heating a synthetic 1 : 2 mixture in liquid paraffin suspension sometimes gave the same complex, sometimes another form which appeared to have excess of free tetramethyluric acid. Heating a synthetic 1 : 1 mixture in a similar way gave this form without the free acid, showing the formation of a stable 1 : 1 complex. On storage at room temperature or only gentle heating two further 1 : 1 complexes were formed; these were metastable, however, and could always be transformed into the stable one. These forms must have different crystal structures whose relative stability is dependent on the amount of interaction possible between the two components in the particular geometrical arrangement.

The spectral changes observed are of the same order as those observed by Burton and Richards (*loc. cit.*) for complexes between certain polarizable aromatic compounds and some strongly polar polynitro-compounds. These authors pointed out that similar shifts have been observed during changes of state in pure compounds, and concluded that the complexes owe their stability, not to forces of attraction between the components, but rather to the lower energy of their crystal lattices compared with those of the component materials. The fact that a closely related series of complexes has been examined has, however, shown that the shifts are not purely random and suggests that attractive forces, though small, do exist between the components. Thus, it was found that the bands for caffeine at 1701 and 1658 and for tetramethyluric acid at 1688 and 1659 cm.$^{-1}$, due to the C=O groups, are invariably reduced in frequency on complex formation, usually by 5—10 cm.$^{-1}$. Similarly the strong bands for caffeine at 1026 and 974 cm.$^{-1}$ and for tetramethyluric acid at 1003 and 901 cm.$^{-1}$ (and, less consistently, at 1039 cm.$^{-1}$), which are almost certainly due to the C–N bonds contiguous to the C=O groups, are shifted to higher frequencies by 4—7 cm.$^{-1}$. Again, the strong bands of the aromatic compound component at 750—850 cm.$^{-1}$, due to the out-of-plane C–H deformation modes, are invariably increased in frequency in the complex relative to their position in the parent; the increase averages about 8 cm.$^{-1}$, although in some cases it is about 12 cm.$^{-1}$.

Now these shifts are exactly those which would accompany the particular force of attraction between the two components postulated by Pauling (*Proc. Nat. Acad. Sci.*, 1939, **25**, 577) to explain the formation of complexes such as these between a polar and a polarizable molecule. This force of attraction is supposed to arise in the following way. The aromatic compounds have large polarizabilities in the planes of their molecules. Hence, a molecule of the polarizable component lying close to and parallel to one of the polar molecules is polarized and stabilizes those structures of the polar molecule which bear separated charges. This increases their contribution to the resonance hybrid, and gives rise to an interaction potential corresponding to a force of attraction, thus explaining the formation of a stable complex. Furthermore, a modification of the electronic distribution in the molecules is involved and this should be detectable in their spectra; in particular, the C–O bonds of the purine have less, and the C–N bonds more, double-bond

FIG. 1. *Infra-red spectra as mulls in liquid paraffin or fully fluorinated oil in regions where differences are most marked between complexes and their components.*



A, ——— 3 : 4-5 : 6-*Dibenzocarbazole.*
B, ——— 1 : 1 *Mixture of* 3 : 4-5 : 6-*dibenzocarbazole and caffeine.*
– – – – *Corresponding complex.*
C, ——— 1 : 1 *Mixture of* 3 : 4-5 : 6-*dibenzocarbazole and tetramethyluric acid.*
– – – – *Corresponding complex.*

In B and C, from 1060 *to* 750 *cm.*$^{-1}$, *the curve of the mixture is displaced upwards corresponding to* 25% *absorption.*

character and the frequency of the bands in the infra-red characteristic of their stretching modes are respectively lowered and raised. These are indeed the directions of the small shifts observed and strongly suggest that an interaction of this type occurs. Briegleb (*Z. physikal. Chem.*, 1935, **31**, *B*, 58) had suggested a similar theory, envisaging the polar molecule as inducing dipole moments in the polarizable one and the attraction resulting from the interaction of the permanent and the induced dipole.

If the formation of the solid complex is due to the type of interaction described above, the effect should be observed in solution. However, as the interaction is a short-range effect, one can only expect its detection in concentrated solutions. Chloroform was chosen as solvent; it dissolves either component to a reasonable extent and, in the 0·1-mm. thickness used, is sufficiently transparent to give the complete spectrum from 820 to 3600 cm.$^{-1}$ except for a narrow region at 1195—1245 cm.$^{-1}$. The interactions of 3 : 4-benzopyrene with tetramethyluric acid and of 3 : 4-5 : 6-dibenzocarbazole, α-naphthol and pyrene with both caffeine and the acid were studied in this solvent. The most concentrated solutions possible were used, and comparisons of the spectra were made between those of the mixed solutions and of the components separately at the same concentration. An effect comparable to that observed with the solid materials was found; thus, the purine bands at 1660—1700 cm.$^{-1}$ were shifted to lower frequencies, and those at 900—1100 cm.$^{-1}$ upwards, as were the bands due to the C–H deformation modes of the aromatic compounds which were not obscured by the chloroform.

By studying the effect of dilution, and using the equilibrium concentration relations, it should be possible to calculate the equilibrium constant, the degree of association in any solution, and hence the spectrum of the undissociated complex. In general, however, the spectral changes, especially in the diluted solutions, cannot be measured sufficiently accurately to afford these quantities with significance. In the case of the interaction of caffeine with 3 : 4-5 : 6-dibenzocarbazole and α-naphthol, the spectral changes were greatest and the extent of association could be approximately determined; a 0·348M-solution of caffeine and the carbazole had a degree of association of 0·44, and a 0·338M-solution of caffeine and α-naphthol one of 0·90.

As it has not been possible to obtain accurate values of the degree of association of most of the solutions and therefore of the spectral changes which would be shown by the undissociated complex, these two factors have been combined by comparing the spectral changes actually observed for various complexes at the same concentration. This has shown the associating effect of tetramethyluric acid to be greater than that of caffeine, and the effect of the aromatic compounds to be α-naphthol $\gg$3 : 4-5 : 6-dibenzocarbazole $>$ 3 : 4-benzopyrene $>$ pyrene. In approximately 0·35M-solutions, allowance being made for the two cases where precipitation occurred, the shift to lower frequencies of the purine bands at 1660—1700 cm.$^{-1}$ varied from about 12 to 3 cm.$^{-1}$, that to higher frequencies of the purine bands at 900—1100 cm.$^{-1}$ from about 6 to 2 cm.$^{-1}$ with a similar shift in the aromatic bands observable. For α-naphthol and 3 : 4-5 : 6-dibenzocarbazole, where hydrogen bonding effects are possible, part of the interaction must be of that type. The extent of hydrogen bonding, as deduced from the ratio of intensities of the associated and non-associated X–H bands, is the same in an equimolar solution of purine and aromatic compound as in a solution of the aromatic compound of the same total concentration; the associated X–H band is, however, displaced about 25 cm.$^{-1}$ less to lower frequencies, indicating a slightly weaker hydrogen bond in the complex than in the compound alone. Even where hydrogen bonding does occur, further interaction must be taking place, for certain shifts such as those in the aromatic C–H frequencies cannot be caused by that phenomenon; furthermore, in the case of pyrene and 3 : 4-benzopyrene, hydrogen bonding seems unlikely and all shifts must be due to the other type of interaction.

The ultra-violet absorption spectra also showed interaction in solution, but only in concentrated solutions. Thus, the spectra of the molecular complexes were found to be equal to the sum of the absorptions of the two components at the concentrations used for these measurements (usually approx. 4×10^{-5}M). When however the spectrum of the aromatic component was examined in the presence of a large excess of purine, a reduction in the height of the maxima and a bathochromic shift were observed (Booth and Boyland, *loc. cit.*). Results and a typical example of the changes produced are shown in the Table and Fig. 2.

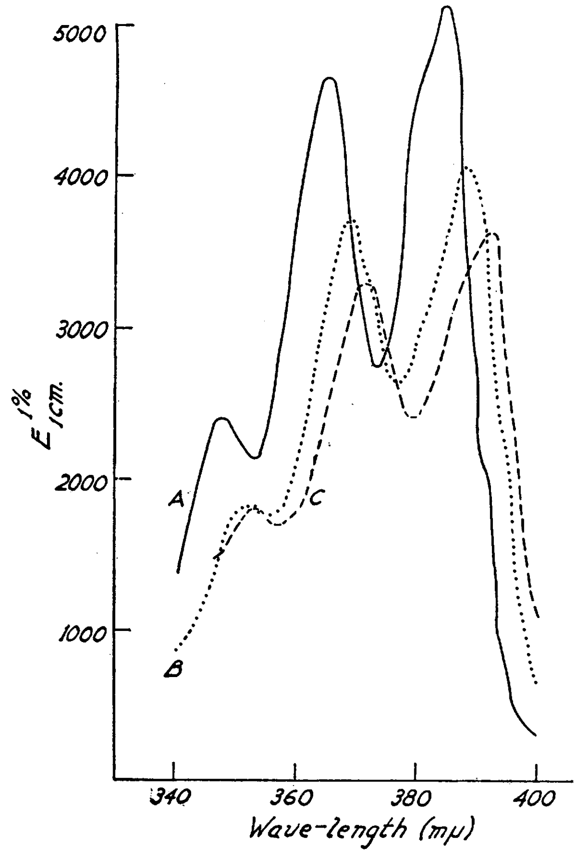


FIG. 2. *Ultra-violet absorption data.*
A, 3 : *4-Benzopyrene in* 50% EtOH.
B, 3 : *4-Benzopyrene in* 50% EtOH *saturated with caffeine.*
C, 3 : *4-Benzopyrene in* 50% EtOH *saturated with tetramethyluric acid.*

Aromatic component	Concn. (mg./l.)	Solvent *	$\lambda_{max.1}$ (mμ)	Reduction in $E_{max.1}$ (%)	$\lambda_{max.2}$ (mμ)	Reduction in $E_{max.2}$ (%)
Phenanthrene	212	50% EtOH	339	—	347	—
	144	1·5% Aq. caffeine	340	33	349	32
Pyrene	3	50% EtOH	320	—	336	—
	3	1·5% Aq. caffeine	325	30	341	31
3 : 4-Benzopyrene	9	50% EtOH	365	—	384	—
	9	50% EtOH satd. with caffeine	369	21	388	21
	9	50% EtOH satd. with T.M.U.	371	29	391	29
3 : 4-5 : 6-Dibenzocarbazole	10	50% EtOH	348	—	365	—
	10	50% EtOH satd. with caffeine	350	14	367	12
	10	50% EtOH satd. with T.M.U.	350	20	367	20
3 : 4-6 : 7-Dibenzacridine	15	50% EtOH	377	—	397	—
	15	50% EtOH satd. with caffeine	379	16	400	15
	15	50% EtOH satd. with T.M.U.	380	26	401	26

* T.M.U. = tetramethyluric acid.

From both ultra-violet and infra-red spectra, therefore, an interaction between the purines and the polycyclic compounds is found to occur in concentrated solutions. Furthermore, the shifts in the infra-red spectra are the same in solution as in the solid state, and can be correlated with specific changes in electronic distribution in the molecules. These facts all support the explanation of the formation of these complexes as due to forces of attraction between the two components arising from their mutual polarization.

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