Fig. 1.—Log n vs. log σ; silver nitrate added to silver bromide, circles or squares, 2 min. or 1 day, respectively, after mixing; ◐, ◨ in 10, 50, or 90 vol. % ethanol; ◓, ▣ in 50 vol. % acetone. Lines have unit slope.

silver was present as Ag^+, $AgBr(aq)$, or $AgBr_2^-$. The following equilibrium data were used to calculate the solubilities: $(Ag^+)(Br^-) = 5 \times 10^{-13}$; $(AgBr(aq)) = 1 \times 10^{-8}$; $(AgBr_2^-) = 1 \times 10^{-5}(Br^-)$. Ethanol (10, 50, or 90 vol. %) or acetone (50 vol. %), when present, was added both to the silver nitrate and potassium bromide solutions. The solubilities in these solvents were estimated from the work of Krahtovil and Tezak.[3] The equilibrium constant for formation of $AgBr_2^-$ increases with increasing content of ethanol or acetone, and the solubility at moderate $(10^{-3}-10^{-4}\ M)$ concentrations of bromide is larger than in the absence of ethanol or acetone, although the solubility product is much smaller than in water. The lowest calculated values of σ in the mixed solvents are uncertain because no data appear to be available for the concentration of undissociated silver bromide.

Figure 1 shows that at 2 min. after formation, n is remarkably close to proportional to σ. The relation, $\log n = 8.0 + \log \sigma$, applies over nearly five orders of magnitude and includes both aqueous and aqueous organic suspensions. As the sols age and the particles grow (with the exceptions mentioned below) the values of n decrease but n remains proportional to σ. This is illustrated in Fig. 1 by the data for 1 day after mixing, by which time the diameter in most sols is close to the final diameter.[2] At 1 day, $\log n = 6.1 + \log \sigma$; hence the values of n decrease about 80-fold from the values at 2 min. One-day old sols prepared at the lowest values of σ deviate from the linear relation, because in such sols there is practically no particle growth after 2 min. (compare circles and squares, Fig. 1, at $\log \sigma = 0.7$ and 1.3). Reversal of the order of addition (excess of bromide added to silver)

(3) J. Krahtovil and B. Tezak, *Arhiv kem.*, **26**, 243 (1954).

causes an increase in particle size, and hence a decrease in n, at 2 min.; however, at 1 day after mixing the relation between n and σ is the same as shown in Fig. 1. Sols prepared in the presence of ammonia or ethylenediamine tetraacetate generally deviate from the linear relations of Fig. 1, apparently because the initial growth pattern is changed drastically.

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Photochemical Addition of Benzo[a]pyrene to Pyrimidine Derivatives[1]

Sir:

We have found that the carcinogenic hydrocarbon benzo[a]pyrene (I) forms stable addition products with uracil, thymine, cytosine, 5-methylcytosine, guanine, and 6-azathymine upon irradiation with ultraviolet light.[2] The reaction proceeds even when wave lengths below 320 mμ are filtered out. Since the pyrimidine derivatives do not absorb above 320 mμ, the hydrocarbon is the primary chromophore. Pyrimidine, 2-aminopyrimidine, purine, adenine, dihydrouracil, and dihydrothymine do not react; thus, the presence of a conjugated carbonyl group within the pyrimidine ring system is required in order that the reaction may occur.

The reaction is carried out in 4% aqueous sodium dodecyl sulfate (SDS) solution (pH 6-7) containing 0.0001 M hydrocarbon and 0.032 M pyrimidine, in quartz cuvettes or (for large volumes) a continuous-flow irradiation device. During irradiation, the characteristic ultraviolet absorption peaks of the benzpyrene α-band at 404 mμ and p-band at 388, 367, 350, and 333 mμ disappear, and new peaks corresponding to those of the benzpyrene spectrum gradually appear at longer wave lengths. The exact positions of the new peaks depend to some extent on the pyrimidine derivative used (Table I). The reaction mixture itself turns orange due to photooxidation of the benzpyrene. Yields under these conditions vary from 2% or less for uracil to 50% for 5-methylcytosine. Yields improve and may exceed 95% if the reaction is carried out in 0.2 M NaOH.

TABLE I

PRINCIPAL ULTRAVIOLET ABSORPTION MAXIMA OF
BENZPYRENE AND ITS PHOTOPRODUCTS IN 4% SDS SOLUTION

	λ_{max}, mμ			
Benzpyrene	404	388	367	350
Photoproduct with				
Uracil	406	397	376	357
Thymine	406	397	377	358
Cytosine	406	397	376	357
5-Methylcytosine	406	397	377	358
6-Azathymine	406	395	375	357
Guanine	409	397	376	357

The reaction product can be isolated by lyophilizing large volumes of the reaction mixture, dissolving the residue in hot 95% ethanol, crystallizing out as much

TABLE II

MOLAR ABSORBANCY INDEXES FOR BENZPYRENE AND BENZPYRENE–CYTOSINE PHOTOADDUCT[a]

	—α-band—		p-band			—β-band—	
Benzpyrene	404 (3850)	385 (29,000)	365 (25,800)	347 (12,700)	332 (5250)	297 (61,500)	285 (47,600)
Benzpyrene–cytosine	405 (9660)	393 (27,600)	373 (23,400)	354 (12,600)	338 (2800)	301 (44,900)	288 (37,100)

[a] In 95% ethanol, based on the calculated molecular weight of 363 for the adduct.

detergent as possible, and chromatographing the mother liquor on Dowex 1-X8 anion-exchange resin (chloride form). Unchanged pyrimidine and NaCl are eluted immediately with 95% ethanol; the photoproduct is

I

II
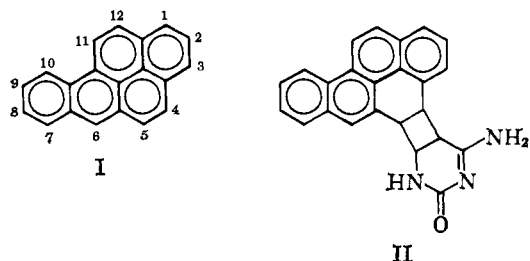
obtained next in essentially pure form, followed by and well separated from unreacted benzpyrene. The dodecyl sulfate anion and most of the hydrocarbon oxidation products remain on the column. The benzpyrene–cytosine adduct, isolated in this manner, can be crystallized with difficulty from 95% ethanol in the form of yellow needles, m.p. above 300° (sealed capillary), but is generally obtained in amorphous form. Although it is more soluble in pyridine or acetic acid than in ethanol, no n.m.r. spectrum could be recorded from solutions in pyridine-d_5 or acetic acid-d_4 even at 110°, because of the low concentrations obtained.

Thymine, cytosine, uracil, and related compounds are known to undergo both ground-state and excited-state addition reactions across the 5,6 double bond.[3,4] The requirement that the 5,6 double bond be present in reactive pyrimidines, and be activated by conjugation to a carbonyl group, suggests that the photochemical reaction consists of addition of the hydrocarbon across this bond. Comparison of the infrared spectra (KBr pellet) of the cytosine, thymine, and 6-azathymine adducts with spectra of the parent pyrimidines and dihydrothymine support this conclusion. When the thymine 5,6 bond is saturated, the strong band at 11.85 μ shifts to the red, and the medium-intensity pair at 8.04 and 8.25 μ shift to the blue and change in relative intensity (Fig. 1). This pattern also occurs in the benzpyrene–pyrimidine adducts. Weak bands at 11.90, 12.10, and 12.45 μ also occur in the spectra of the adducts, but may be obscured by other bands. These are probably due to C–H out-of-plane deformation vibrations of the hydrocarbon; their constancy of position is evidence for the identity of the hydrocarbon portion of the various adducts.

The specific activity of the adduct between benzpyrene and cytosine-2-C^{14} showed that the stoichiometry of the reaction is 1:1. Without n.m.r. evidence we cannot specify the points of attachment of the pyrimidine to the hydrocarbon, but benzpyrene undergoes ground-state addition of osmium tetraoxide across the 4,5

bond,[5] and MO theory predicts that addition reactions should occur at this position (the K region of Pullman and Pullman[6]). We therefore suggest that the photoproducts may consist of one or more of the eight isomers of structure II (drawn for cytosine). X-Ray crystallographic analysis is being initiated to determine the structure of the benzpyrene–cytosine adduct.
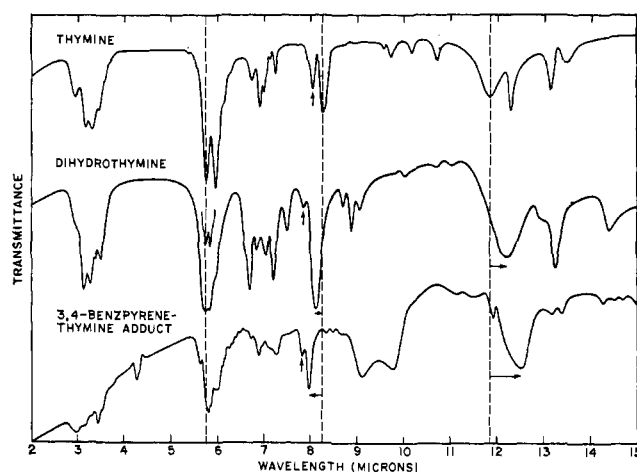

Fig. 1.—Infrared spectra of thymine, dihydrothymine, and benzo[a]pyrene–thymine adduct (KBr pellets); dashed lines are drawn at 5.75, 8.25, and 11.85 μ.

It may be noted that formation of a cyclobutane ring would introduce strain into the benzpyrene molecule, which may be expected to produce a bathochromic shift in its ultraviolet spectrum.[7] This would compensate for the hypsochromic shift expected from the saturation of the hydrocarbon 4,5 bond. From Table II it can be seen that all the adduct bands are bathochromic relative to benzpyrene, and the relative intensities of the α and β bands are markedly different.

The reactions proceed much more readily at pH values above the pK_a values of the pyrimidines. The photoaddition of benzpyrene to ribo- and deoxyribonucleosides and denatured salmon sperm DNA has been demonstrated directly by observation of the ultraviolet absorption spectrum of the pyrimidine photoproducts in SDS reaction systems containing 0.2 M NaOH. It is probable that the photochemical binding of tritiated benzpyrene to denatured calf thymus DNA recently observed by Ts'o, et al.,[8] is due, at least in part, to this reaction.

We are investigating the photochemical reactions of other hydrocarbons with pyrimidines in SDS solution to determine whether the ability to form photo-

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(4) R. Beukers and W. Berends, Biochim. Biophys. Acta, 41, 550 (1960).

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(6) A. Pullman and B. Pullman, Advan. Cancer Res., 3, 117 (1955).

(7) H. H. Jaffe and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley and Sons, Inc., New York, N. Y., 1962, p. 449.

(8) P. O. P. Ts'o, P. Lu, and A. E. Smith, Abstracts of the 8th Annual Meeting of the Biophysical Society, Feb. 26–28, 1964.

adducts correlates with carcinogenic activity of the hydrocarbons.

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Electron Spin Resonance Study of Iminoxy Free Radicals

Sir:

The existence of nitroxides as a class of stable free radicals[1-3] suggests that a related class of radicals derived from oximes should also exist. This note reports the preparation of such species, iminoxy radicals (or alkylidene nitric oxides), as transient intermediates in the oxidation of oximes with ceric ammonium nitrate in methanol. The radicals were detected by e.s.r. using flow techniques suitable for observing transient species.[4] The procedures used have been previously described.[5]
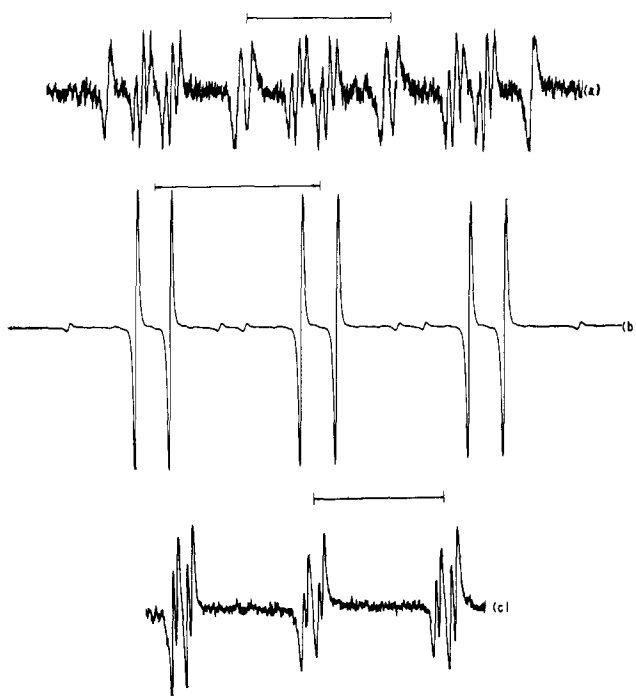

Fig. 1.—First derivative e.s.r. spectra of iminoxy radicals derived from (a) *syn*-benzaldoxime, (b) phenylglyoxaldoxime, and (c) *p*-nitrosophenol. The scale is 29.2 for a, 29.3 for b, and 33.0 gauss for c.

Iminoxy radicals exhibit two interesting features. First, their nitrogen coupling constants are large and characteristic. Values, ranging from 28–33 gauss, are listed in Table I for a variety of radicals and are considerably greater than the nitroxides (10–16),[2] NH_3^+ (18.1),[6] NH_2 (10.3),[7] and $N_2H_4^+$ ($2a_N = 23.0$ gauss).[5] Even though a significant portion of the electron density must reside upon the oxygen atom,

(1) J. R. Thomas, *J. Am. Chem. Soc.*, **82**, 5955 (1960).
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(5) J. Q. Adams and J. R. Thomas, *J. Chem. Phys.*, **39**, 1904 (1963).
(6) T. Cole, *ibid.*, **35**, 1169 (1961).
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the measured coupling constants are comparable to those estimated for unit electron density on nitrogen in *para*[8,9] and *ortho*[10] azine anions. This suggests that the unpaired spin on nitrogen is not in a pure $2p_z$ orbital, as it is presumed to be in the azine anions whose nitrogen splittings are explained in terms of π–σ interactions,[10] but occupies a hybrid orbital containing some s character.

TABLE I

HYPERFINE COUPLING CONSTANTS FOR IMINOXY FREE RADICALS

Radical	a_N	a_H*[a]	$a_{ring\ H}$
Me, Me \ C = N–O	33		
Me, H \ C = N–O	32.5	5.2	
C₆H₅, H \ C, O–N (*anti*)	29.2	26.9	
C₆H₅, H \ C = N–O (*syn*)	31.6	6.2	1.4 (2)
O= (ring) =N–O	33.0		1.2 (1) / 3.7 (1)
O= HO (ring) N–O	27.9		1.7 (1)
C₆H₅, C₆H₅ \ C = N–O	31.6		
C₆H₅, CH₃ \ C = N–O	30.6		

[a] The asterisk denotes hydrogen attached to carbon of imino group.

The second interesting aspect of iminoxy radicals is the detection of *cis* and *trans* isomers. One clear example of this occurs with the iminoxy derived from benzaldoxime. Figure 1a shows the e.s.r. spectrum obtained from the oxidation of *syn*-benzaldoxime (hydrogen attached to imino carbon and oxime hydroxyl *cis*) which consists of the pattern 1,3-3,2,3-3,2,3-3,1 where the intensities of the paired triplet lines are in the ratio 1:2:1. The observed spectrum is interpreted as arising from approximately equal concentrations of *syn* and *anti* isomers. The 1,2,2,1

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