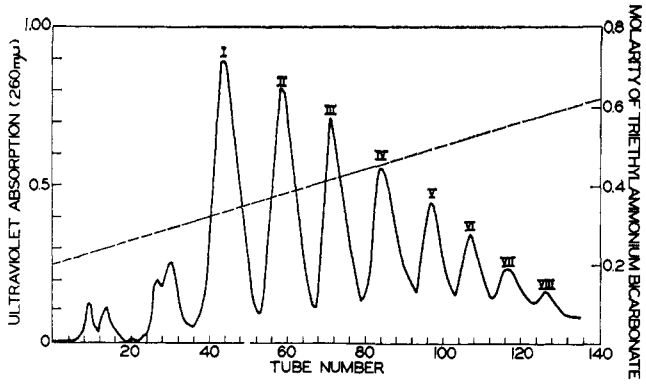

Fig. 2.—Fractionation of the degradation products of polyuridylic acid with micrococcal nuclease. The lower oligonucleotides up to the pentamer were removed by paper chromatography in solvent B. The rest of the degradation products (233 optical density units at 260 mμ) were fractionated on a DEAE-cellulose (carbonate) column (58 cm. × 0.6 cm. i.d.). A linear gradient of triethylammonium bicarbonate, pH 7.5, from 0.2 *M* (500 ml.) to 0.8 *M* (500 ml.) was used for elution and 5 ml./30 min. fractions were collected. Peak I consisted of pure UpUpUpUpUpUp, peak II of pure U(pU)$_6$p, peak III of pure U(pU)$_7$p, peak IV of pure U(pU)$_8$p, and peak V of pure U(pU)$_9$p. For further details see Experimental.

orated to a small volume and the residue (233 optical density units at 260 mμ) dissolved in water (1.0 ml.) was applied on a DEAE-cellulose (carbonate) column (58 cm. × 0.6 cm. i.d.) preequilibrated with 0.2 *M* triethylammonium bicarbonate, pH 7.5. The elution (3 ml. per 15 min.) with 0.2 *M* triethylammonium bicarbonate was continued until no ultraviolet-absorbing material appeared for a period of 4 fractions (approximately 100 ml. of buffer). A linear gradient was then applied, consisting of 500 ml. of 0.2 *M* triethylammonium bicarbonate, pH 7.5, in the mixing vessel and 500 ml. of 0.8 *M* of the same buffer in the reservoir. Uridine polynucleotides up to the tridecanucleotide were thus obtained (Fig. 2). Of these members, up to the decanucleotide were characterized with respect to chain length.

Experiments with Cell-Free Systems.—Extracts from *E. coli* B cultures, "washed ribosomes," and "supernatant" were prepared according to Nirenberg, *et al.*[4a] The incubation mixture (0.5 ml.) contained: 50 μmoles of Tris chloride buffer, pH 7.8; 25 μmoles of ammonium chloride; 5 μmoles of magnesium acetate; 5 μmoles of mercaptoethanol; 15 mμmoles of GTP; 0.5 μmole of ATP; 2.5 μmoles of trisodium phosphoenolpyruvate; 20 μg. of pyruvate kinase; 40 μg. of commercial polyuridylic acid, 0.4–2.0 optical density units at 260 mμ of oligouridylic acid (terminally cyclized or not); 15 mμmoles of C^{14}-phenylalanine (no additional amino acids were added), 20 μc. per μmole; 0.8 mg. of ribosomal protein; and 1.6 mg. of "supernatant" protein.[24] The radioactive amino acid was added last in the incubation mixture and the ribosomes and "supernatant" before it in that order. Incubations were carried out at 37° for 1 hr. An aliquot (0.1 ml.) of the incubation mixture was applied on a 3 MM paper disk[25] (2.3 cm. diameter). The paper disks were air-dried and then successively washed by soaking in 7% perchloric acid for 15 min., 3.5% perchloric acid for 5 min., and again in 3.5% perchloric acid which was heated in a boiling-water bath for 15 min. Volumes of 200 ml. of acid per 10–15 paper disks were used. Finally the paper disks were transferred in 3.5% perchloric acid for 15 min., ethanol–ether (2:1) for 1 min., ethanol–ether (1:2) for 1 min., and ether for 1 min. After the disks had been dried in the air they were transferred in separate scintillation vials containing 5.0 ml. of phosphor[26] and counted in a Packard Tricarb liquid scintillation counter. Typical results are given in Table V. No crosscontamination between the paper disks during the washing procedures could be detected. The amount of enzyme protein and the amount of polyuridylic acid needed for optimal incorporation are those mentioned in the incubation mixture.

TABLE V

EFFECT OF OLIGONUCLEOTIDES ON THE TEMPLATE ACTIVITY OF HIGH MOLECULAR WEIGHT POLYURIDYLIC ACID

Oligonucleotide additions	O.D. units at 260 mμ of oligonucleotide added	μμmoles of C^{14}-L-phenylalanine incorporated[a] In the absence of high molecular weight polyuridylic acid	In the presence of 40 μg. of high molecular weight polyuridylic acid
No oligonucleotide added	0	2.9	95.3
UpUpUpUpUpUp[b]	0.4	3.8	66.5
UpUpUpUpUpUp[b]	1.2	2.9	66.0
UpUpUpUpUpU-cyclic-p[c]	0.4	3.5	96.5
UpUpUpUpUpU-cyclic-p[c]	1.2	3.8	113.0
UpUpUpUpUpUp[d]	0.4	3.5	65.0
UpUpUpUpUpUp[d]	1.2	2.5	66.0
UpUpUpUpUpUp-cyclic-p[e]	0.4	2.9	100.0
UpUpUpUpUpUp-cyclic-p[e]	1.2	3.2	96.0

[a] Per 0.16 mg. of "ribosomal" and 0.32 mg. of "supernatant" protein; incubation at 37° for 1 hr. [b] Enzymatically prepared. [c] From enzymatically prepared oligonucleotide. [d] Chemically synthesized. [e] From chemically synthesized oligonucleotide.

For cyclization of the 3'-terminal phosphate group, the oligonucleotide (10 optical density units at 260 mμ) in methanol solution (0.5 ml.) was treated with tri-*n*-butylamine (3 μmoles) and DCC (100 μmoles) for 6 hr. at room temperature.[27] Methanol was removed by blowing nitrogen gas near the surface of the solution. Water (1.0 ml.) was added and the excess of DCC was extracted with cyclohexane (three 0.5-ml. portions). The water phase was evaporated as above to the desired volume. This solution was used directly in the incubation experiments.

(24) The protein was determined by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(25) R. J. Mans and G. D. Novelli, *Arch. Biochem. Biophys.*, **94**, 48 (1961); F. J. Bollum, *J. Biol. Chem.*, **234**, 2733 (1959).

(26) The phosphor consisted of 4 g. of 2,5-diphenyloxazole (PPO) and 100 mg. of 1,4-*bis*-2-(5-phenyloxazolyl)benzene (POPOP) per liter of reagent grade toluene.

(27) H. G. Khorana, *J. Am. Chem. Soc.*, **81**, 4657 (1959).

COMMUNICATIONS TO THE EDITOR

Excited State p*K*'s. I. Azobenzene and Azoxybenzene

Sir:

Since the discovery by Förster[1] and Weller[2] that fluorescence spectra in buffered solution permit the determination of the p*K*'s of acids and bases in excited singlet states, a number of such p*K**'s have been reported.[1-3] In almost every case, the p*K** has been substantially different than the ground state p*K*, usually by some five units.

Also, since the beginning of the investigations into p*K**'s, it has been recognized that an estimate of Δp*K* can be made from the shift in transition energy of a given absorption or fluorescence band in going from

(1) T. Förster, *Z. Elektrochem.*, **54**, 42 (1950).

(2) A. Weller in "Progress in Reaction Kinetics," Vol. 1, Pergamon Press, London, 1961, p. 187.

(3) W. Bartok, P. J. Lucchesi, and N. S. Snider, *J. Am. Chem. Soc.*, **84**, 1842 (1962); J. C. Haylock, S. F. Mason, and B. E. Smith, *J. Chem. Soc.*, 4897 (1963).

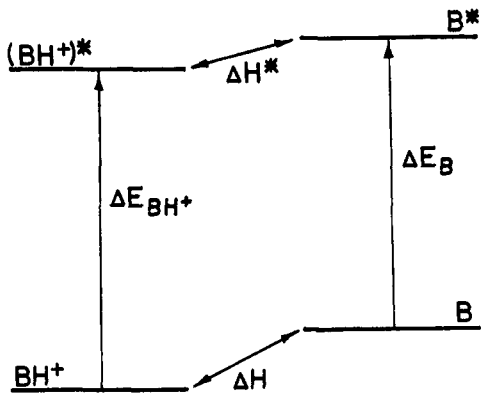

Fig. 1.—The Förster cycle for absorption (for fluorescence arrows must be reversed).

the acid to the conjugate base or base to conjugate acid. Such estimates are based on the energy cycle shown in Fig. 1, hereafter called the "Förster cycle."[1] Corresponding transition energies of a base and its conjugate acid, ΔE_B and ΔE_{BH^+} respectively, are related by eq. 1

$$\Delta E_{BH^+} + \Delta H^* = \Delta H + \Delta E_B \qquad (1)$$

where ΔH is the enthalpy difference between the prototropic species in the ground state and ΔH^* that in the excited state. This permits evaluation of pK^* and ΔpK from the expression

$$\Delta pK = pK^* - pK = \frac{\Delta E_B - \Delta E_{BH^+}}{2.303RT} = \frac{(\nu_B - \nu_{BH^+})Nhc}{2.303RT} \qquad (2)$$

where ν represents the frequency of the transition in wave numbers and h is Planck's constant. The validity of eq. 2 is based on the assumptions that (1) the entropy changes for the acid–base equilibria in the ground and excited states are equal, (2) the transition considered is free from Franck–Condon effects in that the same vibrational sub-band is involved in both prototropic species and the spacing of the vibrational sub-bands in the two species is about the same, and (3) the transitions in both prototropic species must be corresponding. This means they must involve the same configurations, and if the two species belong to the same point group the two transitions must belong to the same irreducible representation. If different point groups are involved, the transition must belong to the same irreducible representation in the highest common subgroup.

Almost without exception, fluorescence occurs only from the lowest excited singlet state. Thus for only this one state can pK^* values be obtained from fluorescence measurements, and even these values may not be meaningful since the lowest excited singlets of the two prototropic species may not be corresponding. These same limitations do not apply to absorption spectra. Thus, providing a correspondence of transitions between the species in equilibrium may be established, pK^* values can be derived for *any* excited state for which absorption data can be obtained. Such values may not reflect any physically attainable equilibrium because of the short lifetimes of the excited states involved, but nevertheless have the same thermodynamic significance as ground state pK values, subject of course to the approximations involved in obtaining them.

A particularly favorable case for study is *trans*-azobenzene. The acid–base behavior of this compound and of its derivatives has been extensively studied in this laboratory,[4] and a detailed analysis of the absorption spectra of both free base and conjugate acid has been proposed, identifying six separate and assigned bands. From this analysis, the shifts in transition energies of corresponding bands in free base and conjugate acid have been obtained and are used to calculate the ΔpK values in Table Ia. The transitions involving lone pair (n) levels are not considered since the n levels are involved in the acid–base reaction. The notation for the bands follows Platt's nomenclature.[5]

TABLE I

Transition Orbitals	Transition States	ν_B, cm.$^{-1}$ × 10^{-2}	ν_{BH^+}, cm.$^{-1}$ × 10^{-2}	Δ_pK	Δq[a]
		a. Azobenzene			
f → g	^{1}B ← ^{1}A	318	239	16.6	0.08
v → g	^{1}G ← ^{1}A	356	262	19.7	0.12
e → g	^{1}C ← ^{1}A	388	284	21.8	0.10
v → w	^{1}H ← ^{1}A	424	424	0.0	0.00
e → w		448	455	−1.5	−0.02
		b. Azoxybenzene			
f → g	^{1}B ← ^{1}A	311	261	10.5	0.06
v → g	^{1}G ← ^{1}A	355	265	18.9	0.26
e → g	^{1}C ← ^{1}A	385	336	10.3	0.25
e → w		420	431	−2.3	−0.01
v → w	^{1}H ← ^{1}A	433	433	0.0	0.00

[a] Based on simple LCAO–MO wave functions. The Δq values in azobenzene refer to the nitrogen atom, in azoxybenzene to the oxygen atom.

A number of interesting features arise in interpreting the results. For the transition v → w, $\Delta\nu$ is zero, implying a ΔpK of zero. This is perfectly consistent with the band assignment, since both the v and w levels are physically far removed from the azo group. Even more interesting, the shift $\Delta\nu$ and hence the implied ΔpK for the unequivocally assigned excited states varies roughly as the change in charge density (Δq) at the azo nitrogen for the transition, consistent with the widely held view that electron density and basicity are closely related. The data presented tend to confirm the proposed assignments, and to provide a basis for distinguishing between alternate possibilities for bands not conclusively assigned.

A similar but less firmly established analysis of the absorption spectrum of azoxybenzene[6] permits similar conclusions, summarized in Table Ib. The hypsochromic shift of the band previously given the tentative assignment v → w suggests that this band should be assigned to the e → g transition, and that the v → w band lies below, possibly at 43,300 cm.$^{-1}$.

The analysis of the $\Delta\nu$ values for substituted azobenzenes are quite similar. $\Delta\nu$ for the v → w bands are generally small for *para*-substituted derivatives and larger for *meta*-substituted derivatives, where the

(4) H. H. Jaffé, S. J. Yeh, and R. W. Gardner, *J. Mol. Spectry.*, **2**, 120 (1958).

(5) J. R. Platt, *J. Chem. Phys.*, **18**, 1168 (1950); *J. Opt. Soc. Am.*, **43**, 252 (1953).

(6) C. S. Hahn, Ph.D. Thesis, University of Cincinnati, Cincinnati, Ohio, 1961.

reduced molecular symmetry tends to mix the v and w orbitals with the e, f, and g orbitals.

Thus, the estimation of several excited state pK's for each compound considered seems established, and may provide an additional tool useful in band assignment.

(7) Procter and Gamble Co. Research Fellow, 1963–1964.

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RECEIVED MAY 11, 1964

Excited State pK's. II. $\Delta\nu$–σ Relations[1]

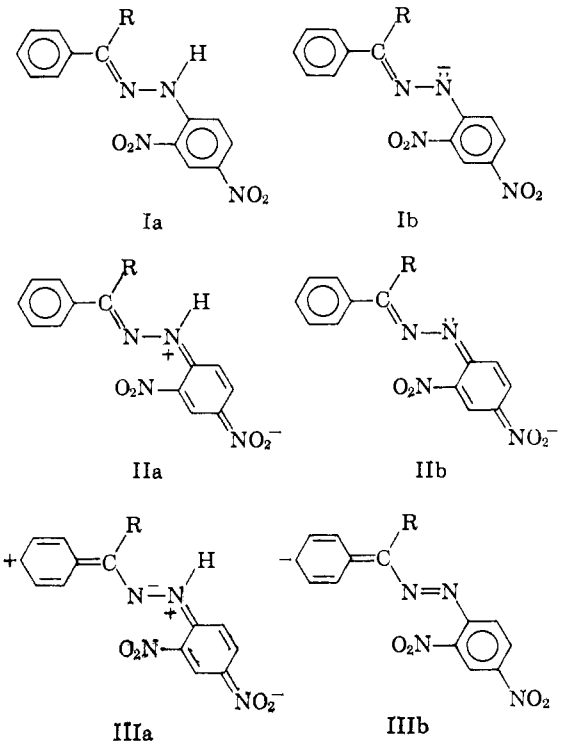
Sir:

C. K. Hancock and his students[2] have observed that $\Delta\nu$, the difference between absorption frequency of free acid and conjugate base, can be correlated with Hammett substituent constants (σ) in some favorable cases. Examples were the 2,4-dinitrophenylhydrazones of a series of substituted benzaldehydes and acetophenones and 4-substituted 2-nitrophenols. No adequate explanation for this observation has been advanced.

According to the Förster cycle,[3] this $\Delta\nu$ represents the difference, $\Delta pK = pK^* - pK$, between the pK of the ground state and the pK^* of the excited singlet reached by the absorption process. It then appears that this ΔpK correlates with substituent constants. Since it has been shown in all of the above cases that the ground state pK's correlate with the *same* substituent constants, it follows that the same is true for the pK^*'s. This is the first instance of such a correlation to have been encountered.

In the 2,4-dinitrophenylhydrazones[2a,c] of the benzaldehydes and acetophenones, the pK^*'s are highly satisfactorily correlated with σ's, correlation coefficients running in the order of 0.98. One possible explanation for this behavior might be that the substituted ring is not strongly involved in the chromophore and the acidic center. This explanation appears highly improbable when one examines either resonance structures or the ρ-value (ρ^*) for the pK^*'s. Conversion of the $\Delta\nu$'s into pK units leads to a value of ρ^* of 4.00 for the aldehyde and of 4.88 for the ketone series, some ten times larger than in the ground state. This implies a very strong interaction between substituent and acidic center.

Maybe the situation is best interpreted in terms of the following resonance structures (Ia–IIIb). IIIa must be important in the excited state of the free acid since the *p*-amino group produces a *strong* bathochromic shift, the *p*-nitro group a much lesser one. On the other hand, IIIb is important in the conjugate base since here the *p*-nitro group produces the strong bathochromic shift, the *p*-amino group a much lesser one. No reasonable structure analogous to IIIa can be written in the base, nor one analogous to IIIb in the acid. Structure IIIa, however, apparently makes practically no contribution to the ground state of the acid, since the ground state pK does not require



σ^+-values, for *p*-alkoxy and *p*-amino groups. Since a σ^--value is needed for the *p*-nitro group one must conclude that IIIb is involved importantly in the ground state of the conjugate base. All these relations are consistent with the intuitive energy orders Ia < IIa < IIIa and Ib < IIb ≈ IIIb.

In series of 4-substituted phenols,[4] 2,6-di-*t*-butylphenols,[4] and 2-nitrophenols,[2b] the $\Delta\nu$'s for the ^1L_a band are not too well correlated with σ's, but the pK^* values for the ^1L_a state calculated by use of the Förster cycle correlate reasonably well; ρ^*-values of −4.97, −8.36, and −2.62, larger than the ground state ρ's by about a factor of two and with r values of 0.94, 0.97, and 0.99, respectively, are obtained. These correlations required the use of σ^+ and σ^- constants for mesomerically electron-donating and -withdrawing substituents, respectively, presumably because of the high polarity of the ^1L_a state of the phenols. Use of the data from the same work[4] for the ^1L_b band yields quite small $\Delta\nu$, and consequently a ρ-value not very different from that of the ground state. Bartok, *et al.*,[5] using absorption and fluorescence data obtained widely differing results for three of the same phenols in the ^1L_b state.

Spectra and pK's of azo- and azoxybenzene derivatives have been extensively investigated in this laboratory.[6a–c] A fair pK^*–(σ^+, σ^-) correlation is observed for the monosubstituted azobenzenes.[6a] In the *p*-N,N-dimethylaminoazobenzene[6b] series, reasonable correlations between pK_1^* and pK_2^* are found while pK_3^* and pK_4^* are substantially insensitive.[7] For the azoxy-

(1) Part I: *J. Am. Chem. Soc.*, **86**, 2982 (1964).

(2) (a) L. A. Jones and C. K. Hancock, *J. Org. Chem.*, **25**, 226 (1960); (b) M. Rapoport, C. K. Hancock, and E. A. Meyers, *J. Am. Chem. Soc.*, **83**, 3489 (1961); (c) L. A. Jones and N. L. Mueller, *J. Org. Chem.*, **27**, 2356 (1962).

(3) T. Förster, *Z. Elektrochem.*, **54**, 42 (1950).

(4) L. A. Cohen and W. M. Jones, *J. Am. Chem. Soc.*, **85**, 3397 (1963); L. A. Cohen and W. M. Jones, *ibid.*, **85**, 3402 (1963).

(5) W. Bartok, P. J. Lucchesi, and N. S. Snider, *ibid.*, **84**, 1842 (1962), have obtained data which are not in agreement with this finding; repetition of their experiments (W. Bartok, private communication), however, has brought their results in line with the above statement.

(6) (a) H. H. Jaffé and R. W. Gardner, *ibid.*, **80**, 319 (1958); (b) M. Isaks and H. H. Jaffé, *ibid.*, **86**, 2209 (1964); (c) C.-S. Hahn and H. H. Jaffé, *ibid.*, **84**, 949 (1962).

(7) The labeling pK_1, pK_2... etc. follows the diagram of S. J. Yeh and H. H. Jaffé, *ibid.*, **81**, 3283 (1959).