Table II. Substrates. Substituted Anilides of N-Acetyl-L-tyrosine

Substituent	Formula	Compn., % (calcd. / found) C	H	N	M.p., °C.
m-Cl	$C_{17}H_{17}N_2O_3Cl$	61.34	5.15	8.42	192–193
		61.19	5.34	8.57	
p-Cl	$C_{17}H_{17}N_2O_3Cl$	61.34	5.15	8.42	224–225
		61.14	5.18	7.97	
m-CH_3O	$C_{18}H_{20}N_2O_4 \cdot 0.5H_2O$	64.10	6.24	8.31	161–162
		64.77	6.20	8.27	
p-CH_3	$C_{18}H_{20}N_2O_3$	69.21	6.45	8.97	204–205
		69.06	6.60	9.24	
p-CH_3O	$C_{18}H_{20}N_2O_3$	65.84	6.14	8.56	220–221
		65.58	6.68	8.21	

The k_2 value for N-acetyl-L-tyrosine *m*-chloroanilide is constant within experimental error between pH 6.2 and 8.0. It decreases at more alkaline values of pH, its profile being represented by a pK' value of 9.7. On the acidic side, there is a suggestion of a decrease in k_2 at pH 6.0. However, the increase in K_m and the limited solubility of the substrate made it impossible to show unequivocally the decrease of k_2 at lower values of pH. On the other hand, the pH profile of the ratio k_2/K_m is a bell-shaped curve with pK' = 6.8 for the acidic side and 9.3 for the basic side. These values are in fair agreement with the values obtained by Sager and Parks[7] for the second-order rate constant of the hydrolysis of the substituted anilides of N-benzoyl-L-tyrosine. The pH profile of k_2/K_m may be considered to represent the prototropic behavior of unbound enzyme and substrate, while the pH dependence of k_2 reflects the ionization of the initial enzyme–substrate complex.[8] As the substrate has no ionizable group over the pH range studied, the binding of the substrate, N-acetyl-L-tyrosine *m*-chloroanilide, seems to have a remarkable effect on the ionization of the catalytically important group of the enzyme. This effect has not been observed in other specific substrates with a smaller ester or amide structure[8] and seems to be due to a bulky *m*-chloroanilide structure.

In D_2O, the plateau value of k_2 for the *m*-chloroanilide, as determined at pD 8.2,[9] is less than that in H_2O by a factor of 3.4. This kinetic isotopic effect and the large negative ρ-value for the rate constant of the acylation suggest that a proton transfer from an acidic group of the enzyme to the anilide substrate occurs in the transition state of the acylation step. Whether this electrophilic nature of the catalysis is a general characteristic of the acylation step specific to the anilide substrates is a problem yet to be elucidated. However, the present data clearly show that an acidic group in the active site of chymotrypsin plays a part in the enzymic catalysis. The fact that k_2 varies depending on the structure of the leaving group allows us to generalize the previous conclusion[6] that the acylation step is rate limiting in the hydrolysis of N-acetyl-L-tyrosine anilides.

The substrates used and listed in Table II were synthesized by the azide method. N-Acetyl-L-tyrosine hydrazide was converted to the azide by reaction with nitrous acid. The azide was then treated with an appropriately substituted aniline in ethyl acetate.

(7) W. F. Sager and P. C. Parks, *Proc. Natl. Acad. Sci. U. S.*, **52**, 408 (1964).

(8) M. L. Bender, G. E. Clement, F. J. Kezdy, and H. d'A. Heck, *J. Am. Chem. Soc.*, **86**, 1680 (1964).

(9) pD was defined by assuming that the relationship, pD = meter pH + 0.4 found by K. Glason and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960), holds for a 5% dimethylformamide–deuterium oxide mixture.

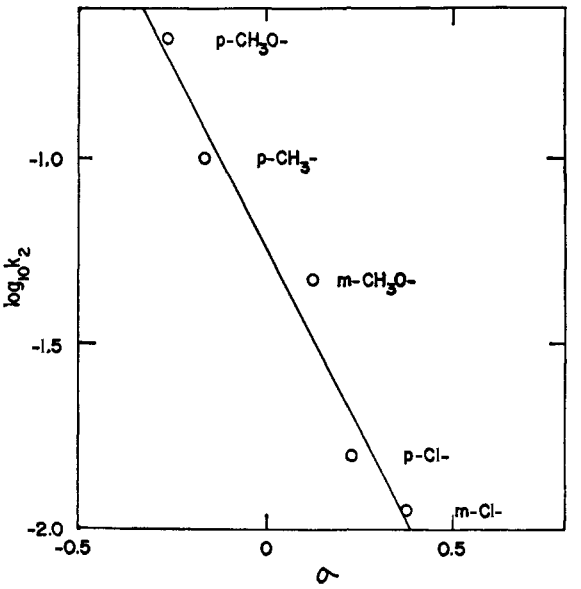

Figure 1. ρ–σ plot of the first-order rate constants of the chymotrypsin-catalyzed hydrolyses of *meta*- and *para*-substituted anilides of N-acetyl-L-tyrosine in 5% (v./v.) dimethylformamide–water at 25°, pH 8.0.

The k_2 and K_m values were read from Eadie plots of the initial rates (8% or less completion of the reaction) of hydrolysis determined by means of a pH-stat. Each reaction solution contained 0.1 *M* KCl and 5% (v./v.) dimethylformamide. Enzyme concentrations ranged between 3 and 4 × 10^{-5} *M* and the substrate concentration, well in excess of that of the enzyme, was varied approximately 10-fold.

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The Structure of a Pentaoxyphosphorane by X-Ray Analysis[1]

Sir:

Triisopropyl phosphite and phenanthrenequinone form a crystalline *1:1 adduct* $(C_{14}H_6O_2)P(OC_3H_7)_3$ which is representative of a new type of phosphorus compound.[2] This type of compound is characterized by a large positive chemical shift in the P^{31} n.m.r.

(1) This work was performed in part under the auspices of the U. S. Atomic Energy Commission, the National Cancer Institute of the Public Health Service (Grant No. CA-04769-05), and the National Science Foundation (GP-3341).

(2) F. Ramirez, S. B. Bhatia, R. B. Mitra, Z. Hamlet, and N. B. Desai, *J. Am. Chem. Soc.*, **86**, 4394 (1964), which contains complete documentation.

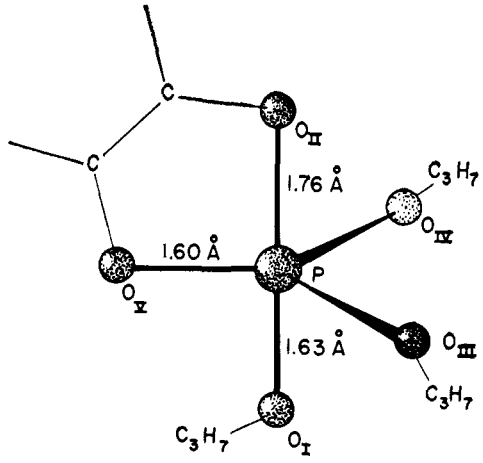

Figure 1. The molecular structure of $(C_{14}H_6O_2)P(OC_3H_7)_3$. The complete phenanthrene ring is not shown. The errors in the P–O distances are about 0.02 Å. The three P–O distances in the basal plane do not differ significantly; the mean value is indicated. The distances and angles in the isopropoxy groups and in the phenanthrene ring do not differ significantly from those expected.

spectrum (between +44 and +62 p.p.m. relative to 85% H_3PO_4), which was taken as strong evidence that the phosphorus atom is covalently bound to five oxygen atoms. We have recently determined the crystal structure of this adduct and find that the conclusion of the previous study is well founded.

The adduct crystallized in the orthorhombic space group Pbca with eight molecules in a cell with dimensions $a = 18.18 \pm 0.01$, $b = 25.03 \pm 0.01$, and $c = 10.14 \pm 0.01$ Å. The X-ray density is 1.193 g./cc. The structure was determined by the usual Patterson, Fourier, and least-squares methods from data obtained from integrated Weissenberg photographs taken with Cu Kα radiation. The present agreement factor $\Sigma||F_o| - |F_c||/\Sigma|F_o|$ is 0.13 for the 754 observed reflections. The details of the structure determination as well as the results of a refinement of a set of data obtained with a scintillation counter will be reported later.

In agreement with the earlier work,[2] we find that the phosphorus atom is indeed five-coordinated and is at the center of a nearly perfect trigonal bipyramid (Figure 1) with one isopropyl group in an apical position (O_I) and two isopropyl groups in equatorial positions (O_{III} and O_{IV}). One of the oxygens attached to the aromatic system (O_{II}) is in an apical position, while the other (O_V) occupies the third equatorial position.

Several significant points are:

(1) The apical P–O bond leading to the aromatic carbon is somewhat longer than the apical P–O bond joined to the aliphatic carbon, the latter being approximately equal in length to the three P–O bonds in the equator of the bipyramid.

(2) The five-membered ring is planar (and is in fact coplanar with the phenanthrene system) but is not a regular pentagon. The two P–O–C angles in this ring are equal and are less obtuse than the other P–O–C angles not involved in ring formation. The two O–C–C angles in the ring are not equal, and one of them (108°) is significantly different from the ideal aromatic angle of 120°.

(3) The isopropyl groups are oriented in such a way as to minimize intramolecular carbon–carbon and carbon–oxygen repulsions; even so, the molecule is rather crowded, the closest nonbonded distances between oxygen atoms and carbon atoms in neighboring groups being 2.64, 2.73, 2.79, and 2.65 Å. We feel that the presence of a five-membered ring joining one of the equatorial (O_V) and one of the apical (O_{II}) oxygens contributes significantly to the stability of this compound by minimizing the number of short *nonbonded interactions*. This feature may be responsible for the stability of other cyclic unsaturated[2] and saturated[3] pentaoxyphosphoranes.

(3) F. Ramirez, N. Ramanathan, and N. B. Desai, *J. Am. Chem. Soc.*, 85, 3465 (1963).

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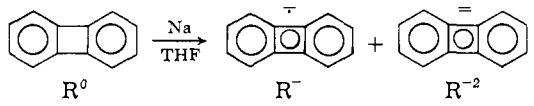
The Dibenzocyclobutadiene Dianion

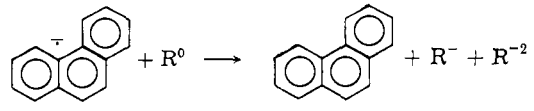
Sir:

Biphenylene reacts with sodium or potassium in tetrahydrofuran forming two species which can be shown to be the anion radical (λ_{max} 588 mμ) and dianion (λ_{max} 565 mμ) of biphenylene (1). At low conversions R^-



(1)

is predominant (see Figure 1), but already at 30% conversion to the anion radical (titration[1]) a dianion shoulder is clearly visible. At slightly less than stoichiometric conversion to R^- the dianion peak is quite prominent, indicating extensive disproportionation ($2R^- \rightleftarrows R^0 + R^{-2}$). As metal uptake continues to two atoms, the R^- peak disappears completely. Though the positioning of the peaks (anion radicals and their dianions generally absorb near one another in the visible region) and their response to metal uptake are alone suggestive of the structures assigned, further verification is available. To eliminate the possibility that the 565 mμ absorption arises from a spurious decomposition product of R^-, the latter was generated instantaneously from the naphthalene and also phenanthrene anion radicals (2). The same mixtures of R^- and R^{-2} as before were obtained. Also, the relative peak intensities did not change with time in the absence of excess alkali metal. Further, after com-



(2)

plete two-atom metal uptake a 40% yield of biphenylene could be isolated by electron transfer to benzil, quenching the benzil dianion with excess benzoyl chloride, and chromatographing the hexane-soluble fraction. A 50% yield of 1,2-dibenzoyloxystilbene was also ob-

(1) Aliquots of the solutions were added, with care, directly to water and titrated with acid to a phenolphthalein end point.