[CONTRIBUTION FROM THE NAVAL MEDICAL RESEARCH INSTITUTE]

On "High Energy Phosphate Bonds" of Biochemical Interest[1]

BY TERRELL L. HILL AND MANUEL F. MORALES

Relative resonance stabilization of the main hydrolysis product accounts for the unusual thermodynamic instability of arginine phosphate, creatine phosphate, acetyl phosphate and 1,3-phosphoglyceryl phosphate (Kalckar). The same explanation applies to amino phosphate. The low bond energy of the N–P bond does not appear to be important, for its contribution cancels out. In addition to relative resonance and tautomeric (in phospho-enol-pyruvate) stabilization of the hydrolysis product, there is an electrostatic "high energy" reactant effect in phospho-enol-pyruvate, ADP and ATP contributing to thermodynamic instability. This electrostatic effect is largest in ATP. In no case does it appear necessary to invoke the concept of some special kind of localized "high energy bond" in the reactant. Such terminology is therefore rather misleading if taken in its conventional meaning. It is suggested that the electrostatic repulsion in ATP, perhaps along with certain shape attributes, may confer upon this substance its special role as an energy donor. The Riseman–Kirkwood theory of energy transfer in muscle is discussed as an example.

I. Introduction

In the hydrolysis reaction which splits the O–P bond in, say, glycerol-α-phosphate or adenosine monophosphate (AMP) there is a decrease in standard free energy of about 2 kcal./mole.[2] We shall refer to this reaction as a "standard," in which there are no "special" effects. On the other hand, in the hydrolysis of the O–P or N–P bond in acetyl phosphate, 1,3-phosphoglyceryl phosphate, phospho-enol-pyruvate, amino phosphate, creatine phosphate, arginine phosphate, adenosine diphosphate (ADP) and adenosine triphosphate (ATP) there is a standard free energy decrease of the order of 10–12 kcal./mole.[2] These latter compounds are thus generally said to contain "high energy phosphate bonds." However, only ATP appears able[2] to convert part of this large free energy decrease into work, as, for example, in muscular function. In order to provide energy for useful work, the other "high energy compounds" must first transfer their "high energy" to ATP.

Kalckar[3] attributed the excess standard free energy decrease (*e.g.*, 12 − 2 = 10 kcal./mole), except in the case of phospho-enol-pyruvate (see below), primarily to the relative resonance[4] stabilization (low free energy) of the hydrolysis *product*. (Kalckar did not discuss the case of amino phosphate—see below.) For several compounds, we believe this to be the correct explanation, but in some cases—specifically in ATP—electrostatic forces may play an important role.

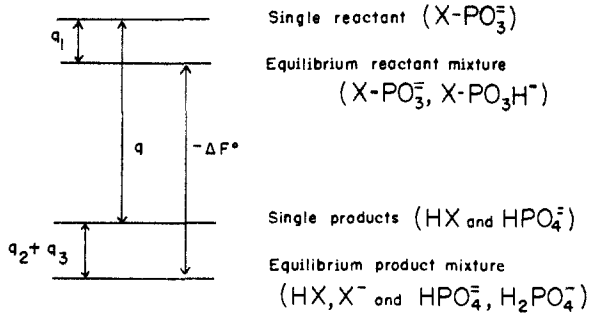


Fig. 1.—Free energy levels (equation 2).

II. The Hydrolysis Reactions

In this section we discuss the separate hydrolysis reactions (at *p*H 7), the principal reaction of interest being of the type

$$X\text{–}PO_3^- + H_2O \longrightarrow HX + HPO_4^- \qquad (1)$$

these particular species being chosen to conform with the standard reaction 5, below. The $-\Delta F^0$ values mentioned above have been estimated from equilibrium constants or by calculation.[2a] We wish to compare $-\Delta F^0$ for the standard reaction with the same quantity in the other examples and try to understand qualitatively the origin of the large value of $-\Delta F^0$ in the "high energy" cases.

Matters are somewhat complicated by the fact that $X\text{–}PO_3H^-$, X^- and $H_2PO_4^-$ may be present (there are also other species in some cases). $-\Delta F^0$ refers to the actual equilibrium reactant and product mixtures ($X\text{–}PO_3H^-$, $X\text{–}PO_3^-$), (HX, X^-) and ($H_2PO_4^-$, HPO_4^-), respectively

$$\begin{array}{ccc} X\text{–}PO_3^- + H_2O & \rightleftharpoons & HX + HPO_4^- \\ + & & \uparrow\downarrow K_2 \quad + \\ H^+ & & H^+ + X^- \\ K_1\uparrow\downarrow & & \downarrow\uparrow K_3 \\ XPO_3H^- & & H_2PO_4^- \end{array}$$

Let K_1, K_2 and K_3 be the three acid dissociation constants, respectively, and let q be the standard free energy decrease for Equation 1 *as written*. Then one finds (Fig. 1), taking all activity coefficients as unity

$$-\Delta F^0 = q + q_2 + q_3 - q_1 \qquad (2)$$

$$q_i = RT \ln [(1 + K_i^*)/K_i^*] \geq 0 \; (i = 1,3) \qquad (3)$$

$$q_2 = RT \ln (1 + K_2^*) \geq 0 \qquad (4)$$

$$K_i^* = K_i/(H^+) \quad (i = 1,2,3)$$

where (H^+) is the hydrogen ion concentration (10^{-7} mole/liter). Our procedure will be to write all reactions as in Equation 1, discussing q_1 and q_2 essentially as corrections (q_3 cancels below).

AMP (Standard).—In the reaction

$$A\text{—}O\text{—}P^+(O^-)_2\text{—}O^- + H_2O \longrightarrow AOH + HPO_4^- \qquad (5)$$

AOH represents adenosine (the adenine amino group is uncharged[5,6] at *p*H 7). There is, of course, resonance between the phosphate structure written and others involving double bonds,[7] for example

(1) The opinions contained herein are the writers' and are not necessarily those of the Navy Department. This paper was presented at a meeting of the Washington, D. C., Section of the American Chemical Society, October 12, 1950, and a preliminary account was given at the 18th International Physiological Congress in Copenhagen, August 15, 1950. Not copyrighted.

(2) (a) F. Lipmann, *Advances in Enzymol.*, **1**, 99 (1941); (b) E. Baldwin, "Dynamic Aspects of Biochemistry," Cambridge University Press, 1948.

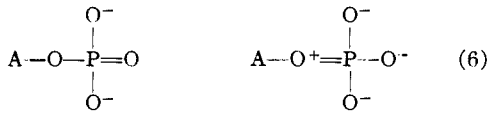
(3) H. M. Kalckar, *Chem. Revs.*, **28**, 71 (1941).

(4) G. W. Wheland, "The Theory of Resonance," John Wiley and Sons, Inc., New York, N. Y., 1944.

(5) H. Wassermeyer, *Z. physiol. Chem.*, **179**, 238 (1928).

(6) A. G. Ogston, *J. Chem. Soc.*, 1713 (1936).

(7) L. Pauling, "The Nature of the Chemical Bond," Cornell University Press, Ithaca, N. Y., 1940.



(6)

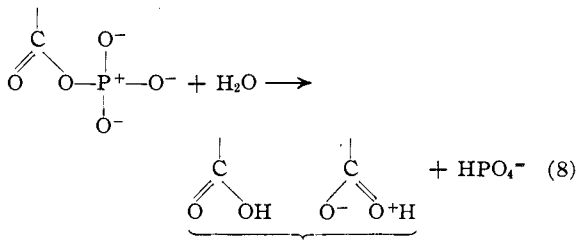
However, the success of theories of electrostatic effects on dissociation constants,[8,9] using only single bond forms as in Equation 5, indicates that single bond structures are probably most important.

For AMP,[5,10] $pK_1 = 6.2$ (notation above) so that, at 25°, q[10] (superscript zero for "standard reaction") = 0.1 kcal./mole. Also, $q_2^0 = 0$ since no species other than AOH makes an appreciable contribution to adenosine. Hence, on cancelling q_3

$$(-\Delta F^0) - (-\Delta F^0)^0 = (q - q^0) + (q_2 - q_1) + 0.1 \text{ kcal./mole} \quad (7)$$

This is the equation we wish to use for comparison purposes.

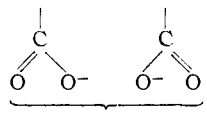
Acetyl Phosphate and 1,3-Phosphoglyceryl Phosphate. Because of "opposing resonance,"[3] (a) the resonance in the phosphate group of the reactant in Equation 8 is less strong



(8)

than in the same group in Equation 5, and (b) the resonance in the carboxyl group of the product is stronger than in the carboxyl group of the reactant. Both of these effects contribute to $q > q^0$.

The resonance between equivalent forms[3,4] in the acetate

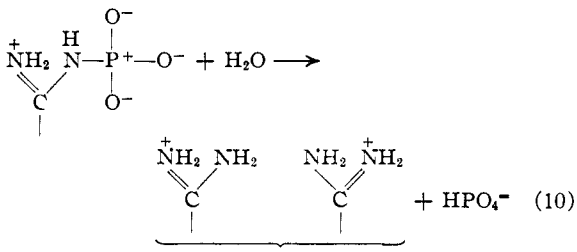


(9)

ion is very strong and is primarily responsible for the fact that the acid is almost completely dissociated at pH 7 ($pK_2 = 4.73$, $q_2 = 3.1$ kcal./mole). Also, $pK_1 = 4.7$[11] and $q_1 = 0$.

In 1,3-phosphoglyceryl phosphate the two phosphate groups are sufficiently far apart so that we may neglect their interaction. Hence the acetyl phosphate discussion applies here also to a good approximation.

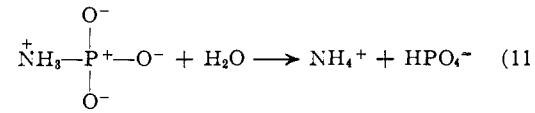
Arginine Phosphate, Creatine Phosphate and Amino Phosphate.—We consider the two guanidine phosphates together and ignore the influence of distant charges at the carboxyl end of these molecules. We have



(10)

The dissociation constants[10] are such that $q_1 = q_2 = 0$. There is strong resonance between equivalent forms in the product. There is a weaker resonance between nonequivalent forms in the reactant. The strength of this latter resonance and of the resonance in the phosphate group of the reactant (compare Equation 5) is further reduced by opposing resonance.[3] All of these effects tend to make $q > q^0$. Acting in the opposite direction, but presumably overbalanced (if the observed result is to be explained in this way—see footnote 25) by the resonance effects, there is a net electrostatic attraction between $=NH_2^+$ and $-PO_3^=$ tending to stabilize the reactant.

The amino phosphate reaction is

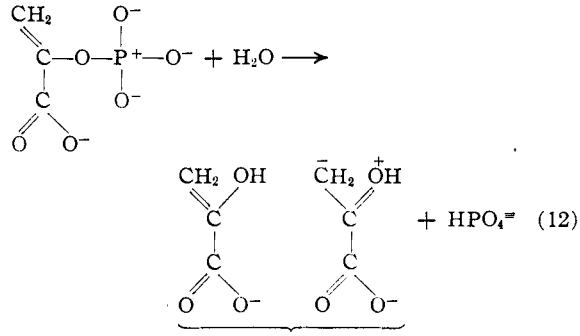


(11)

Again, the dissociation constants[10] give $q_1 = q_2 = 0$. Opposing resonance and neighboring positive charges make the resonance in the phosphate group of the reactant less strong than in the standard reactant. The same effects reduce the resonance in the $-NH_3^+$ group of the reactant compared to the resonance in the product NH_4^+. In addition, there is an increase in the number of equivalent resonance structures in going from $-NH_3^+$ to NH_4^+. These effects contribute to $q > q^0$. As above, there is an opposite stabilization of the reactant owing to the net electrostatic attraction between $-NH_3^+$ and $-PO_3^=$.

Meyerhof and Lohmann[2a,12] attribute the large free energy decrease in the present three cases to the relative instability of the N–P bond compared to the O–P "standard" bond. Using the bond energies and electronegativities given by Pauling[7] one does indeed find that the P^+–O bond is more stable (higher bond energy) than the P^+–N bond by about 28 kcal./mole. However, one must also consider the difference in N–H and O–H bond energies in the *product*. The O–H bond is more stable by 26.5 kcal./mole, so this rough calculation shows that the *net* effect is probably small or negligible; consequently the "high energy" character cannot be explained on such grounds.

Phospho-enol-pyruvate.—The first step in the hydrolysis is



(12)

Again, opposing resonance reduces the resonance in the reactant phosphate group relative to the standard and in the $CH_2{=}C(-O-)-$ group relative to the product (this latter effect is not large because the two resonance forms in Equation 12 are not equivalent). These give $q > q^0$. Acting in the same direction ($q > q^0$) is the net electrostatic repulsion between $-COO^-$ and $PO_3^=$ in the reactant, to be discussed below in connection with ADP and ATP.

There is presumably a further decrease in free energy here[3] owing to a tautomeric shift in the product from enol-pyruvate ion to keto-pyruvate ion. In simple cases of keto–enol equilibrium the keto form is favored[13] by a factor which may be of the order of 10^4 (this would give a free energy decrease of about 5.5 kcal./mole) or even more. We are not aware of experimental evidence on the keto–enol equilibrium for pyruvate ion in *aqueous* solution, but know of no reason to expect it to be unusual. Müller and Baumberger[14] made extensive polarographic measurements on pyruvic acid in aqueous solution over a wide pH range and concluded that below pH 5.8 the keto form is more stable but that the reverse is true above this pH. However, more recently Brdička[15] has shown rather conclusively that Müller and Baumberger's measurements have nothing to do with the keto–enol equilibrium but rather pertain to the dissociation of the carboxyl group of pyruvic acid (an explanation is

(8) G. E. K. Branch and M. Calvin, "The Theory of Organic Chemistry," Prentice-Hall Co., New York, N. Y., 1941.

(9) A. Kossiakoff and D. Harker, THIS JOURNAL, **60**, 2047 (1938).

(10) See also W. D. Kumler and J. J. Eiler, *ibid.*, **65**, 2355 (1943).

(11) F. Lipmann, *Advances in Enzymol.*, **6**, 264 (1946).

(12) O. Meyerhof and K. Lohmann, *Biochem. Z.*, **196**, 49 (1928).

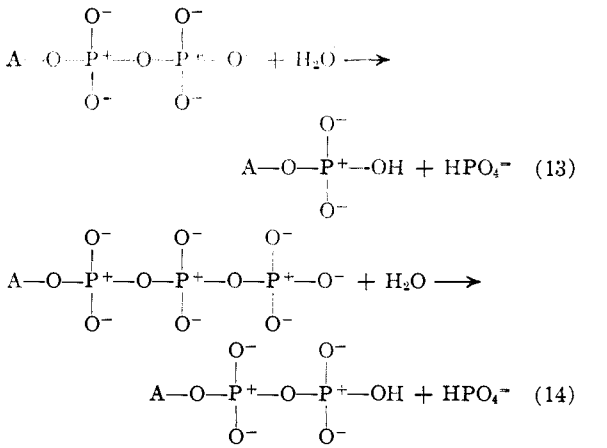
(13) G. W. Wheland, "Advanced Organic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1949, Second Edition, pp. 593 and 613; see also ref. 8, Table 33, and pp. 286–297.

(14) O. H. Müller and J. P. Baumberger, THIS JOURNAL, **61**, 590 (1939).

(15) R. Brdička, *Coll. Czech. Chem. Comm.*, **12**, 212 (1947).

given by Brdička of the "discrepancy" between pH 5.8 and the carboxyl dissociation constant $pK \cong 2.4$). The value of q_1 is 0.1 kcal./mole, from[10] $pK_1 = 6.4$; $q_2 = 0$.

ADP and ATP.—The reactions to be compared with Equation 5 are



(13)

(14)

There are two "opposing resonance" effects (as above) contributing to $q > q^0$: (a) resonance in the terminal phosphate of the reactant is less than in the standard reactant; and (b) resonance in the next to terminal phosphate of the reactant is less than in the product.

The pK's for the dissociation of the last phosphate hydrogen in AMP, ADP and ATP are[5,16] 6.2, 6.6 and 6.6 (hence a completely ionized phosphate chain is the most prevalent species in every case at pH 7). These give $q_1 = 0.2$ kcal./mole and $q_2 = 1.2$ kcal./mole for Equation 13, and $q_1 = 0.2$ kcal./mole and $q_2 = 0.7$ kcal./mole for Equation 14. The ionization of the products (AMP, ADP) in Equations 13 and 14 is aided somewhat by an increase in the number of equivalent resonance forms in the terminal phosphate group. However, this effect may not be large in view of the successful theories of acid strength[8,9] already mentioned, which ignore resonance.

On the basis of the effects discussed above, Kalckar[3] classified ADP and ATP as cases whose decomposition products were stabilized by resonance, just as in arginine phosphate, acetyl phosphate, etc. However, in ADP and particularly in ATP there is also a net electrostatic repulsion between phosphate groups which tends to split off the terminal phosphate.[17] A similar effect has already been mentioned in phospho-enol-pyruvate.

From Equations 5, 13 and 14 it is clear that the net contribution of this electrostatic effect to $q - q^0$ is determined, to a good approximation, by the inter-phosphate group repulsion ($-PO_2^-$– and $-PO_3^=$) in the reaction of Equation 13 and two such repulsions (terminal $-PO_3^=$ with the two $-PO_2^-$– groups) in the reactant of Equation 14.

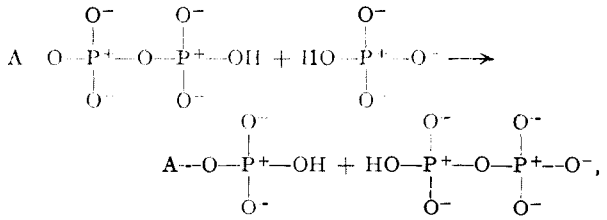
It would seem worthwhile to try to compute the magnitude of these electrostatic effects using the best theoretical model available (Kirkwood–Westheimer[20]), and taking into account different ionic species, rotation about bonds, variation of effective dielectric constant with configuration, etc. We propose to do this later, along the lines of an earlier treatment of pyrophosphoric acid by one of us.[21] For the present, it is of interest to obtain an order of magnitude estimate of the electrostatic contribution to $q - q^0$. We do this by assuming: (a) a formal charge distribution (single bond structures) as in the reactants of Equation 12, 13 and 14 (replacing $-\overset{|}{\underset{|}{P}}{}^+-O^-$ by $-\overset{|}{\underset{|}{P}}{}=O$ does not change the result appreciably); (b) an average effective dielectric constant $D_E = 50$ for *all* interactions, which is a reasonable[20,21,22] guess; and (c) an intercharge distance for each interaction which is the average between the approximate minimum and maximum intercharge distance (allowing rotation about bonds), obtained from known or estimated bond angles and distances.[21] We find the *approximate* values of about 5 or 6 kcal./mole for ATP and about 3 or 4 kcal./mole for ADP and phosopho-enol-pyruvate. It therefore seems probable that the electrostatic contribution to $q - q^0$ in ATP (at least) is an important part of the total free energy decrease.[23]

III. Discussion

Although we have introduced a rather formal approach (Equations 2, 3, 4, 7) necessary for quantitative or semi-quantitative discussion, we do not wish to obscure the conclusion that, as suggested by Kalckar,[3] strong resonance[25] between equivalent forms (Equations 9, 10) in the main hydrolysis product (thus lowering its free energy) together with opposed resonance in the reactant

(16) K. Lohmann, *Biochem. Z.*, **254**, 391 (1932); **282**, 120 (1935).

(17) The products AMP and pyrophosphate are also possible in reaction 14. We have examined the reaction



taking into account all four q's and the inter-phosphate electrostatic repulsions (in the approximate manner described below), and find that the products ADP and phosphate in reaction 14 are thermodynamically favored over AMP and pyrophosphate by about 1 kcal./mole at pH 7. Bond energy and resonance effects, of the type discussed above, cancel. Other possible effects, such as entropy changes, have not been taken into account. Hence the AMP-pyrophosphate reaction is also a "high energy" reaction (though perhaps slightly less so than the ADP–phosphate reaction) and these products should be observed under conditions giving an appreciable reaction rate. In fact, there is some recent experimental evidence[18,19] showing that ATP can indeed split off pyrophosphate in the presence of appropriate enzymes. It might also be mentioned that the increase in instability of ATP on addition of acid should be attributed to an increase in *rate* of decomposition (a common effect of acid in hydrolysis reactions).

(18) L. A. Heppel and R. J. Hilmoe, personal communication.

(19) E. A. Zeller, *Arch. Biochem.*, **28**, 138 (1950); G. H. Hitchings and H. S. Fuller, *J. Biol. Chem.*, **128**, xlv (1939).

(20) J. G. Kirkwood and F. H. Westheimer, *J. Chem. Phys.*, **6**, 506, 513 (1938).

(21) T. L. Hill, *ibid.*, **11**, 552 (1943); **12**, 147 (1944).

(22) Accurate measurements have not been made of *all* of the phosphate dissociation constants, using pure AMP, ADP and ATP (however, see the note added at the end of the paper). The most reliable information available at present on interphosphate electrostatic repulsions comes from the successive dissociation constants of pyrophosphoric acid. The use of successive constants in the *same* acid to obtain information about electrostatic effects is much more reliable than a comparison of related constants in *different* acids (because of greater cancellation of other effects in the former case), *e.g.*, the constants for the dissociation of the last hydrogen in AMP, ADP and ATP.

In Table II of reference 21 (1944), the best theoretical values of the successive ΔpK's of pyrophosphoric acid are 1.8, 4.6 and 1.9. The experimental values (given incorrectly in Table II) are 1.1, 4.6 and 1.9. This is excellent agreement, especially for the more highly dissociated species (these are the ones we are most interested in). Implicit in the calculations leading to the above theoretical results is an ADP type of repulsion ($-PO_2H^-$ and $-PO_3^=$) of average magnitude about 3.3 kcal./mole. We have chosen $D_E = 50$ primarily because this value of D_E in the present relatively simple treatment gives approximately the above value for the ADP repulsion.

We have ignored salt effects but the measurements of Schmidt, *et al.*, on amino acids [*J. Phys. Chem.*, **43**, 1121 (1939); **44**, 880, 893 (1940); *Arch. Biochem.*, **1**, 473 (1943)] suggest that this is not serious at ionic strengths of biological interest.

(23) The reaction 2ADP $\rightleftarrows$ AMP + ATP (with myokinase as catalyst) apparently has an equilibrium constant of the order of unity.[24] On the other hand, according to the electrostatic calculation and the q's (above), ATP would have a greater free energy decrease on hydrolysis than ADP by about 1.5 kcal./mole. However, this discrepancy cannot be considered serious at the present time because we have ignored differences arising from resonance, entropy and other effects.

(24) H. M. Kalckar, *J. Biol. Chem.*, **148**, 127 (1943).

(25) Resonance energies up to 50 kcal./mole or more are common, so that effects of the present order of magnitude are not surprising. We have made no attempt in the present paper to estimate resonance energies for individual cases.

is the essential explanation of the "high energy phosphate bond" in arginine phosphate, creatine phosphate, acetyl phosphate and 1,3-phosphoglyceryl phosphate. We believe that amino phosphate should be added to this list, by virtue of the resonance in the ammonium ion. In disagreement with Kalckar,[3] however, we attribute the large free energy decrease in phospho-enol-pyruvate, ADP and ATP not just to relative resonance and tautomeric[26] stabilization of the hydrolysis product but also to electrostatic repulsion in the reactant.[27] The electrostatic effect appears to be especially important in ATP.

It seems worth emphasizing that, on the basis of the above conclusions, the widely accepted concept of some special kind of *localized* "high energy (or energy-rich) phosphate bond" (O–P or N–P) *in the reactant* is misleading if taken literally for: (1) we are actually interested in "free energy" and not just "energy"; (2) the principal result of resonance in the reactions discussed is in fact a "low energy" effect *in the product* (which is thermodynamically indistinguishable from a "high energy" reactant effect); and (3) as is well known, the usual concept of localized "bond energies"[7] does *not* include electrostatic and resonance effects[28] of the type described above since these are *non-localized* properties of the *molecule (or group) as a whole* (but these are precisely the effects primarily responsible for "high energy phosphate bonds"!). These points should be explicitly understood in using the terminology "high energy phosphate bond," which, however, is convenient and vivid.

An interesting possibility which we believe should be kept in mind in future work is that the relatively large electrostatic repulsion and/or net negative charge make ATP, rather than other unstable compounds, the substance of choice in energy transfer. At least if the energy acceptor is a charged, flexible enzyme it is easy to visualize how the transfer might be accomplished (see below). This is not to minimize the possible importance[29] of factors such as molecular shape (which is, incidentally, also affected by charge, *i.e.*, the phosphate chain tends to be straightened out by the repulsion just discussed); however, such factors in an evolutionary sense may be secondary.[30]

We are thus essentially suggesting that it may be relatively easy to transfer *electrostatic* free energy in ATP into available energy. For example, Riseman and Kirkwood[31] have made the interesting suggestion that relaxation of actomyosin involves a transfer of the terminal phosphate in ATP molecules to alcoholic groups in actomyosin. The repulsion between negative charges[32] of the attached groups would cause extension of the actomyosin chain, thus storing free energy in the form of negative configurational entropy. Contraction is supposed to result from removal of the negatively charged phosphate groups,[33] allowing a return to the original state, with conversion of the stored free energy into work.

In some ways the electrostatic properties of ATP would fit in satisfactorily with the Riseman–Kirkwood picture: (1) possible attraction of ATP to –OH sites by neighboring positive charges on the protein; (2) after attaching the terminal phosphate of ATP to the –OH group, splitting of the phosphate chain by internal electrostatic repulsion, leaving behind on actomyosin a charged phosphate group, and returning ADP to the surrounding medium.

In spite of the attractiveness of the Riseman–Kirkwood model, however, it leaves unexplained the mechanism whereby ATP *specifically* initiates the fast *contraction* of extended actomyosin.[34]

We have mentioned the smaller electrostatic repulsions in ADP and phospho-enol-pyruvate. It would be of interest to look carefully for small ATP-like effects of these compounds on actomyosin,[36] although molecular shape considerations may eliminate this possibility, especially for phospho-enol-pyruvate.

Note Added October 21, 1950.—After completion of this paper an article was published on the same subject by P. Oesper.[39] We have commented on Oesper's paper elsewhere.[40] Also, Alberty, *et al.*,[41] have recently redetermined some of the dissociation constants of a number of phosphate compounds including ATP, ADP and AMP. Computations of the variation of ionic charge and free energy of hydrolysis with *p*H are given, using the new dissociation constants. The latter calculation is in agreement with our Equations 2–4 and in disagreement with Oesper's equations

(26) In the phospho-enol-pyruvate case.

(27) Dr. Koloman Laki has pointed out to us that, although precise measurements are not available, it would appear that the rate of dephosphorylation (in the absence of any enzyme) is appreciably slower in the three "electrostatic" cases than in the other "high energy" compounds. This may not be a coincidence.

(28) Incidentally, resonance is an "energy" effect while electrostatic repulsion is a "free energy" effect (see, for example, ref. 4, pp. 165–167).

(29) Kinetically rather than thermodynamically.

(30) In this connection, one cannot fail to be impressed by the unusual combination of properties of inorganic phosphate, making its biological roles possible: (a) dissociation constants (one very strong; one in the physiological *p*H range); (b) reversible attachment to organic structures through alcoholic groups (with the aid of enzymes), in some cases forming a bridge between two organic structures; and (c) reversible polymerization (with the aid of enzymes). One result of these properties is the existence in ATP of an unusual concentration of electrical charge which can hardly be unrelated to the biological uniqueness of this substance.

(31) J. Riseman and J. G. Kirkwood, THIS JOURNAL, **70**, 2820 (1948).

(32) According to available dissociation constants[5,10] the predominant ionic species is *doubly* charged $-O-PO_3^=$ at *p*H 7.

(33) According to this, inorganic phosphate is not released with ADP on relaxation, but later on contraction.

(34) That is, the Riseman–Kirkwood suggestions apply to the relatively slow extension (of actomyosin) and dephosphorylation (of ATP) processes, and not to the fast contraction of actomyosin, also induced by ATP.[35] The next to last paragraph in Riseman and Kirkwood's paper is misleading since the experiments referred to concern the contraction and not the extension process.

(35) For recent discussions, see M. F. Morales, *Biochim. et Biophys. Acta*, **2**, 618 (1948); also, D. J. Botts and M. F. Morales, *J. Cell. and Comp. Physiol.*, (in press).

(36) Actually, there are definite reductions in the visual opacity of actomyosin threads when they come in contact with solutions of ADP.[37] This is also true of tripolyphosphate,[37] and tripolyphosphate is readily dephosphorylated by actomyosin.[38] The electrostatic properties of tripolyphosphate are of course closely related to those of ATP.

(37) D. J. Botts and M. F. Morales, unpublished.

(38) M. E. Tarver and M. F. Morales, *J. Cell. and Comp. Physiol.*, in press.

(39) P. Oesper, *Arch. Biochem.*, **27**, 255 (1950).

(40) T. L. Hill and M. F. Morales, *ibid.*, **29**, 450 (1950).

(41) R. A. Alberty, R. M. Smith and R. M. Bock, Chicago meeting of the American Chemical Society, September, 1950.

on pp. 259 and 269. The new constants do not alter any of our qualitative arguments.

Acknowledgment.—We are indebted to Drs. F. Lipmann and K. Laki for a number of very helpful comments and references.

BETHESDA, MD. RECEIVED AUGUST 28, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, TENNESSEE A. AND I. STATE COLLEGE]

The Synthesis, Properties and Dehydrohalogenation of Some α-Phenoxy and 2,4-Dichlorophenoxy Substituted Acid Chlorides

BY CARL M. HILL, HELEN I. SCHOFIELD, ALFRED S. SPRIGGS AND MARY E. HILL

This paper describes the synthesis and chemistry of several α-phenoxy and α-2,4-dichlorophenoxy substituted fatty acid chlorides; namely, propionyl, butyryl, valeryl, isovaleryl, caproyl and enanthyl. Although the acid chlorides of both series reacted smoothly with such reagents as ammonia, aniline, phenylhydrazine, ethanol and phenol, there was a noticeable difference in the reactivity of the chlorides of each series with a given reagent.

A previous paper[1] reported the dehydrohalogenating action of triethylamine on several phenoxy and chlorine-substituted phenoxyacetyl chlorides. 2,4-dichlorophenoxy substituted fatty acid chlorides. It was observed that the nature of the alkyl and phenoxy group attached to the alpha carbon of

TABLE I

PHYSICAL CONSTANTS AND ANALYTICAL DATA OF ACID CHLORIDES

α-Phenoxy	B.p.[a] °C.	Mm.	d^{20}_4	$n^{20}D$	MR_D Found	MR_D Calcd.	Yield, %	Formula	Chlorine,[b] % Found	Chlorine,[b] % Calcd.
Propionyl[c]	105–106	5	1.1865	1.5184	47.15	47.16	73	$C_9H_9O_2Cl$	19.28	19.20
Butyryl[c]	104–105	6	1.1355	1.5138	52.60	51.78	74	$C_{10}H_{11}O_2Cl$	18.21	17.85
Valeryl	109–110	7	1.1127	1.5087	57.00	56.40	88	$C_{11}H_{13}O_2Cl$	16.64	16.67
Caproyl[d]	126–127	11	1.0885	1.5048	61.69	61.02	88	$C_{12}H_{15}O_2Cl$	15.99	15.64
Enanthyl	115–117	3	1.0643	1.5025	66.75	65.63	82	$C_{13}H_{17}O_2Cl$	14.20	14.77
α-2,4-Dichlorophenoxy										
Propionyl	137–139	9	1.3857	1.5475	58.05	56.90	84	$C_9H_7O_2Cl_3$	13.91	13.98
Butyryl	145–147	5	1.3516	1.5397	62.05	61.52	90	$C_{10}H_9O_2Cl_3$	12.85	13.25
Valeryl	160–162	10	1.3078	1.5350	67.01	66.14	54	$C_{11}H_{11}O_2Cl_3$	11.80	12.59
Isovaleryl	145–147	4	1.3028	1.5338	67.15	66.14	98	$C_{11}H_{11}O_2Cl_3$	12.45	12.59
Caproyl	150–153	3	1.2732	1.5301	71.70	70.76	96	$C_{12}H_{13}O_2Cl_3$	12.01	12.00
Enanthyl	175–176	7	1.2392	1.5268	76.76	75.38	98	$C_{13}H_{15}O_2Cl_3$	11.17	11.46

[a] Boiling and melting points are corrected. [b] Analyzed by potentiometric titration using silver, silver–silver chloride electrodes; %'s refer to ionizable chlorine. [c] Reported: R. Stoermer and P. Atenstadt, *Ber.*, **35**, 3565 (1902); b.p. 115–117° (10 mm.). [d] Reported: C. A. Bischoff, *ibid.*, **34**, 2127 (1901); b.p. 128–131° (38 mm.).

TABLE II

PHYSICAL CONSTANTS AND ANALYTICAL DATA FOR AMIDES AND ANILIDES OF ACID CHLORIDES

α-Phenoxy chloride	Amides[a] Yield, %	M.p., °C.	Formula	Nitrogen, % Found	Nitrogen, % Calcd.	Anilides Yield, %	M.p., °C.	Formula	Nitrogen, % Found	Nitrogen, % Calcd.
Propionyl[b]	90	128–129	$C_9H_{11}O_2N$	8.51	8.49	31	116–116.5	$C_{15}H_{15}O_2N$	6.13	5.80
Butyryl[c]	45	118–119	$C_{10}H_{13}O_2N$	8.30	7.82	30	79–81	$C_{16}H_{17}O_2N$	5.38	5.49
Valeryl	44	114–115	$C_{11}H_{15}O_2N$	7.65	7.25	28	98–99	$C_{17}H_{19}O_2N$	5.18	5.20
Caproyl	57	100–102	$C_{12}H_{17}O_2N$	6.98	6.76	24	93–93.5	$C_{18}H_{21}O_2N$	4.43	4.95
Enanthyl	87	106–108	$C_{13}H_{19}O_2N$	6.12	6.34	65	84–85	$C_{19}H_{23}O_2N$	4.65	4.71
α-2,4-Dichlorophenoxy										
Propionyl	97	81–82	$C_9H_9O_2Cl_2N$	5.96	5.98	98	138–139	$C_{15}H_{13}O_2Cl_2N$	4.50	4.52
Butyryl	94	108–109	$C_{10}H_{11}O_2Cl_2N$	5.76	5.65	66	126–127	$C_{16}H_{15}O_2Cl_2N$	4.48	4.32
Valeryl	94	86–87	$C_{11}H_{13}O_2Cl_2N$	5.54	5.34	99	149–150	$C_{17}H_{17}O_2Cl_2N$	4.11	4.14
Isovaleryl	93	94–96	$C_{11}H_{13}O_2Cl_2N$	5.38	5.34	83	123–124	$C_{17}H_{17}O_2Cl_2N$	4.11	4.14
Caproyl	98	101–102	$C_{12}H_{15}O_2Cl_2N$	5.10	5.07	59	136–137	$C_{18}H_{19}O_2Cl_2N$	4.03	3.98
Enanthyl	97	98–99	$C_{13}H_{17}O_2Cl_2N$	4.83	4.83	37	107–108	$C_{19}H_{21}O_2Cl_2N$	4.00	3.83

[a] Nitrogen analyses by semi-micro Dumas method. [b] Amide and anilide reported: C. A. Bischoff, *Ber.*, **34**, 1837 (1901); *ibid.*, **34**, 1839 (1901), m.p. 132–133° and 118.5–119°, respectively. [c] Amide and anilide reported: C. A. Bischoff, *ibid.*, **34**, 1837 (1901) and *ibid.*, **34**, 1840 (1901), m.p. 123° and 93–94°, respectively.

In an effort to study the influence of different alkyl radicals upon the properties and character of alkyl-phenoxy disubstituted ketenic substances, these studies were extended to several α-phenoxy and α-

(1) C. M. Hill, G. W. Senter and M. E. Hill, THIS JOURNAL, **72**, 2286 (1950).

an acid chloride apparently influences the extent and mode of polymerization of the disubstituted ketenes formed upon dehydrohalogenation with triethylamine.

Dehydrohalogenation of propionyl and butyryl chlorides of the α-phenoxy series produced two iso-