in parts per million (δ) relative to Me_4Si. Mass spectra were obtained on an LKB 9000A gas chromatography–mass spectrometer by direct probe. All optical rotations were obtained on a Perkin-Elmer 247 polarimeter using a 1 dm cell. The concentration of all samples was 1% and the solvent was benzene unless otherwise indicated. Elemental analyses were conducted by Galbraith Laboratories, Inc., Knoxville, Tenn. Hydrogenations requiring 3 atm of pressure were done on a Parr hydrogenator. Ultraviolet spectra were recorded on a Perkin-Elmer 124 recording spectrophotometer.

Crystal and Molecular Structure of *d,l*-Dethiobiotin. Role of Sulfur in Biotin Stereochemistry[1a]

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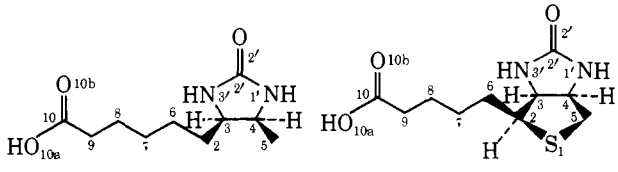
Abstract: The crystal structure of *d,l*-dethiobiotin ($C_{10}H_{18}N_2O_3$) has been accurately determined using x-ray diffraction techniques and refined to a residual of 0.055. The crystals are monoclinic, space group $P2_1/a$, with cell constants (at 22 ± 3 °C) a = 29.9166 (2) Å, b = 4.9990 (1) Å, c = 7.7561 (1) Å, β = 97.855 (2) °, and Z = 4. The ureido moiety in dethiobiotin displays unusual bond lengths for the C=O (1.244 (4) Å) and two C—N bonds (1.346 (4) and 1.347 (4) Å). The ureido oxygen accepts a strong hydrogen bond from the carboxyl group of a neighboring molecule (O···O, 2.633 Å; H···O, 1.83 Å) and is also hydrogen bonded to N(1′). These results lend support to the theory of activation of biotin by increased acidity of the NH groups and the increased nucleophilicity of nitrogen, stabilized by hydrogen bonds to the ureido oxygen. Since both the C–N bond lengths are very nearly equal, the reason for the preferential carboxylation at N(1′) is not electronic but steric, due to a close nonbonded contact (3.042 Å) of a carbon atom of the caproic acid chain to the proximal nitrogen. Lack of sulfur in dethiobiotin does not change the dimensions of the ureido moiety from those observed in biotin, indicating no dominant C···S transannular interaction as suggested by others. The 2-imidazolidone ring in dethiobiotin is markedly nonplanar, and the caproic acid chain is in the trans planar zig-zag conformation, indicating an important role for sulfur in stabilizing both the planarity of the imidazolidone ring and the twisted conformation of the side chain found in biotin.

Dethiobiotin (I) is an intermediate in the biosynthesis of biotin (II).[2] It has been demonstrated that dethiobiotin itself does not serve as a substitute for biotin in carboxylase, decarboxylase, or transcarboxylase systems, but rather is first converted to biotin which is then attached to the appropriate apoenzyme. As a biotin vitamer, it is capable of promoting growth in biotin-deficient systems by conversion to biotin, the essential coenzyme responsible for fixing CO_2 for eventual transfer. It has been observed that dethiobiotin counteracts the tumor promoting effects of biotin in certain systems.[3] It has been suggested[4] that nucleophilic activation of biotin is via hydrogen bond formation with an attendant increase in the double bond character of the carbonyl carbon–nitrogen bonds and a concomitant increase in NH acidity. In a recent paper,[5] structural evidence was presented for the stabilization of the polarization of the ureido carbonyl bond via hydrogen bond formation. In order to investigate the properties of the ureido function more fully, and to determine the role of sulfur on the basic vitamin conformation, the crystal structure of *d,l*-dethiobiotin was determined. This study revealed additional evidence regarding the polarization of the carbonyl bond and shed some light on the role of sulfur in biotin stereochemistry.



I

II

Experimental Section

Crystals of *d,l*-dethiobiotin (Nutritional Biochemical Corp.) were grown by slow evaporation from a saturated aqueous solution. A needle-shaped crystal, elongated along the b axis, of dimensions 0.10 × 0.21 × 0.13 mm, was mounted for data collection. Cell constants (Table I) were obtained by a least-squares fit of the 2θ values of 60 high angle reflections for which α_1,α_2 separation could be observed and measured on a GE XRD-5 diffractometer. Unique three-dimensional intensity data (2548 reflections) to the limit $\sin\theta/\lambda$ = 0.64 Å^{-1} for Cu Kα radiation (λ 1.540 51 Å) were measured on a GE XRD-5 diffractometer by the stationary crystal-stationary counter method[6] using a 5° take-off angle. Balanced Ni-Co Ross filters were used for effective monochromatization. Reflections (952) which had intensities less than twice the background value in that ($\sin\theta/\lambda$) range were given zero weight during the refinement and for the residual calculation. The anisotropy of absorption was checked for the 020 reflection (χ = 90°) in 10° steps of ϕ, and the crystal showed essentially no variation of absorption as ϕ varied. The data were corrected for Lorentz-polarization effects and processed in the usual way. No detailed absorption correction was applied (μ = 7.64 cm^{-1}).

Structure Determination and Refinement. The crystal structure was determined by routine application of the multi-solution tangent refinement technique, employing the program MULTAN.[7] The 15 nonhydrogen atoms were located in the first E map using the phase set with the highest figure of merit[7] (1.0159), yielding a structure with a residual of 0.26. The atomic positional and individual isotropic thermal parameters of the nonhydrogen atoms were refined by block-diagonal least-squares technique to an R of 0.10. An electron-density difference map at this stage clearly revealed the location of the 18 hydrogen atoms. Continuation of the refinement with individual anisotropic thermal parameters for nonhydrogen atoms and isotropic thermal parameters for hydrogen atoms led to the final R of $(\Sigma(\|F_o| - |F_c\|)/\Sigma|F_o|)$ = 0.055. Each of the shifts of the parameters in the final cycle were less than 0.2 of the esd of the corresponding parameter. The function minimized during the refinement was $[w(|F_o| - (1/k)|F_c|)^2]$, using the weighting scheme of Evans.[8] Atomic scattering factors for O, N, and C were those listed in International Tables;[9a] for the hydrogen atoms, the values given in ref 9b were used.

Results

The final positional and thermal parameters are collected in Tables II and III. The observed and calculated structure

Table I. Crystal Data for *d,l*-Dethiobiotin

Formula $C_{10}H_{18}N_2O_3$
a = 29.9166 (2) Å
b = 4.9990 (1) Å
c = 7.7561 (1) Å
β = 97.855 (2)°
Systematic absences $0k0$ with k odd; $h0l$ with h odd
Space group $P2_1/a$, Z = 4
ρ_{calcd} 1.238 g cm^{-3}
ρ_{obsd} 1.23 g cm^{-3}
μ(Cu Kα) 7.64 cm^{-1}
Temp 22 ± 3 °C, λ(Cu Kα) 1.54 051 Å

factors have been deposited (see paragraph at end of paper regarding supplementary material). Bond lengths and angles with their esd's are given in Tables IV and V. The average esd's are 0.004 Å for bond lengths and 0.2° in bond angles involving nonhydrogen atoms. The corresponding quantities when hydrogen is one of the atoms involved are 0.04 Å and 2°.

A view of the dethiobiotin molecule in the natural isomer (*d*) is given in Figure 1. The atomic numbering of the dethiobiotin molecule has been chosen to correspond to the biotin nomenclature, in particular ref 5, for ease of comparison with the results of that study. The bond distances and angles in the dethiobiotin and biotin molecules agree quite well, in most cases, within 2 or 3 esd's. The small ratio of measured data to parameters for the biotin determinations (5.85 and 4.56 for the two studies[5]) compared with dethiobiotin (12.25) leads to higher esd's for biotin; consequently, we did not attempt to interpret the differences in any detail.

Thermal Libration. The thermal motion of three groups of atoms were analyzed using the method and programs of Schomaker and Trueblood[10] to check whether the individual anisotropic thermal motions of the atoms in each group could be explained in terms of rigid-body librations. The three groups of atoms are: (1) the whole molecule; (2) the caproic acid chain: C(2), C(6), C(7), C(8), C(9), C(10), O(10a), and O(10b); and (3) the imidazolidone ring: N(1′), C(2′), N(3′), O(2′), C(3), C(4), C(2), and C(5). The root mean square differences between U_{ij}'s observed and calculated from rigid-body librations for the three groups are, respectively, 0.0077, 0.0032, and 0.0036 Å^2. The rigid body tensors for these three groups are given in Table VI. The maximum root mean square amplitude of vibration for the caproic acid chain is 14.6°, and the corresponding principal axis of libration is practically along the a axis and nearly along the length of the chain. Such a mode of thermal libration in which a long chain may be considered to vibrate rigidly has also been observed in other long-chain compounds, for example, in the 1,3-diglyceride of 11-bromoundecanoic acid.[11] The corrections due to the thermal libration for the bonds in the caproic acid chain are given in Table VII. The correction for thermal vibration

Table III. Coordinates (× 10^3) and Thermal Parameters for Hydrogen Atoms

	x	y	z	B (× 10^2)
H(10a)	−595 (1)	−32 (9)	−777 (5)	748
H(1′)	−987 (1)	−366 (7)	−852 (4)	542
H(3′)	−897 (1)	−47 (7)	−1048 (4)	575
H(3)	−908 (1)	234 (6)	−796 (3)	414
H(4)	−974 (1)	68 (7)	−745 (4)	453
H(5a)	−926 (1)	−303 (8)	−519 (5)	718
H(5b)	−974 (1)	−233 (8)	−524 (5)	696
H(5c)	−936 (1)	−6 (8)	−458 (5)	740
H(2a)	−866 (1)	−260 (7)	−715 (4)	545
H(2b)	−862 (1)	−12 (7)	−574 (4)	498
H(6a)	−822 (1)	237 (7)	−751 (4)	488
H(6b)	−827 (1)	43 (6)	−900 (4)	416
H(7a)	−774 (1)	−66 (7)	−577 (4)	520
H(7b)	−782 (1)	−275 (8)	−721 (4)	661
H(8a)	−736 (1)	230 (7)	−749 (4)	521
H(8b)	−745 (1)	36 (7)	−912 (4)	550
H(9a)	−690 (1)	−94 (9)	−576 (5)	733
H(9b)	−695 (1)	−280 (7)	−737 (4)	546

Table IV. Bond Lengths with Estimated Standard Deviations (Å)

O(2′)-C(2′)	1.244 (4)	N(3′)-H(3′)	0.84 (3)
C(2′)-N(3′)	1.346 (4)	N(1′)-H(1′)	0.79 (3)
N(3′)-C(3)	1.444 (4)	C(4)-H(4)	0.92 (3)
C(3)-C(4)	1.558 (4)	C(5)-H(5a)	0.97 (4)
C(4)-C(5)	1.504 (5)	C(5)-H(5b)	0.93 (4)
C(4)-N(1′)	1.450 (4)	C(5)-H(5c)	1.05 (4)
N(1′)-C(2′)	1.346 (4)	C(3)-H(3)	0.97 (3)
C(3)-C(2)	1.523 (4)	C(2)-H(2a)	0.98 (4)
C(2)-C(6)	1.533 (4)	C(2)-H(2b)	1.03 (3)
C(6)-C(7)	1.520 (4)	C(6)-H(6a)	0.96 (3)
C(7)-C(8)	1.516 (4)	C(6)-H(6b)	0.95 (3)
C(8)-C(9)	1.523 (4)	C(7)-H(7a)	0.96 (3)
C(9)-C(10)	1.493 (4)	C(7)-H(7b)	0.97 (4)
C(10)-O(10a)	1.307 (3)	C(8)-H(8a)	1.00 (3)
C(10)-O(10b)	1.202 (5)	C(8)-H(8b)	1.06 (3)
		C(9)-H(9a)	1.00 (4)
		C(9)-H(9b)	0.93 (4)
		O(10a)-H(10a)	0.84 (4)

Table II. Positional and Thermal Parameters (× 10^4)[a]

	x	y	z	β_{11}[b]	β_{22}	β_{33}	β_{12}	β_{13}	β_{23}
O(2′)	−9532 (1)	−4011 (5)	−11300 (3)	71 (2)	602 (12)	206 (4)	−29 (3)	17 (1)	−66 (12)
O(10a)	−6179 (1)	−1144 (5)	−7490 (3)	78 (2)	542 (12)	364 (6)	23 (3)	33 (2)	166 (15)
O(10b)	−6549 (1)	2597 (6)	−8173 (4)	112 (3)	624 (15)	567 (10)	32 (3)	76 (3)	416 (20)
N(1′)	−9655 (1)	−2761 (6)	−8541 (3)	79 (2)	560 (14)	229 (5)	−40 (3)	30 (2)	−52 (15)
N(3′)	−9162 (1)	−509 (6)	−9770 (3)	87 (2)	516 (13)	221 (5)	−37 (3)	19 (2)	53 (14)
C(2′)	−9458 (1)	−2554 (6)	−9993 (4)	57 (2)	415 (13)	196 (5)	5 (3)	10 (2)	82 (14)
C(3)	−9086 (1)	408 (6)	−7990 (4)	79 (3)	361 (13)	243 (6)	−2 (3)	16 (2)	−19 (15)
C(4)	−9520 (1)	−632 (7)	−7311 (4)	71 (3)	481 (15)	238 (6)	15 (3)	20 (2)	−20 (17)
C(5)	−9471 (1)	−1601 (10)	−5459 (5)	109 (4)	889 (26)	234 (7)	−19 (5)	29 (3)	27 (23)
C(2)	−8642 (1)	−649 (7)	−7034 (4)	74 (3)	517 (16)	226 (6)	−11 (4)	15 (2)	9 (17)
C(6)	−8232 (1)	502 (7)	−7762 (4)	74 (3)	488 (16)	245 (7)	−15 (4)	11 (2)	−9 (17)
C(7)	−7790 (1)	−831 (7)	−7022 (4)	78 (3)	470 (15)	254 (7)	−13 (4)	19 (2)	4 (17)
C(8)	−7388 (1)	349 (7)	−7748 (4)	75 (3)	455 (14)	226 (6)	−6 (3)	16 (2)	28 (16)
C(9)	−6942 (1)	−994 (7)	−7066 (4)	82 (3)	439 (15)	271 (7)	6 (4)	28 (2)	56 (17)
C(10)	−6544 (1)	319 (7)	−7676 (4)	85 (3)	438 (15)	233 (6)	10 (4)	17 (2)	35 (16)

[a] TF = exp{−($\beta_{11}h^2 + \beta_{22}k^2 + \beta_{33}l^2 + \beta_{12}hk + \beta_{13}hl + \beta_{23}kl$)}. Estimated standard deviations given in parentheses. [b] (β_{11} × 10^5).

Table V. Bond Angles with Estimated Standard Deviations (deg)

O(2')-C(2')-N(3')	126.2 (2)	N(1')-C(4)-C(5)	112.0 (3)
O(2')-C(2')-N(1')	126.0 (2)	C(3)-C(2)-C(6)	112.3 (3)
N(3')-C(2')-N(1')	107.8 (3)	C(2)-C(6)-C(7)	113.3 (3)
C(2')-N(3')-C(3)	112.3 (2)	C(6)-C(7)-C(8)	112.4 (3)
C(2')-N(1')-C(4)	112.3 (2)	C(7)-C(8)-C(9)	113.7 (3)
N(3')-C(3)-C(4)	100.8 (2)	C(8)-C(9)-C(10)	113.3 (3)
N(3')-C(3)-C(2)	111.7 (2)	C(9)-C(10)-O(10a)	114.2 (3)
C(4)-C(3)-C(2)	115.6 (3)	C(9)-C(10)-O(10b)	122.9 (3)
C(3)-C(4)-N(1')	101.1 (2)	O(10a)-C(10)-O(10b)	122.7 (3)
C(3)-C(4)-C(5)	117.0 (2)		

is significant for all these bonds and is as large as 0.04 Å for C(10)-O(10b).

The thermal libration for the 2-imidazolidone ring is comparatively smaller than the caproic acid chain. The maximum root mean square amplitude is 7.3°. The orientation of the principal axes of libration for the ureido group differs from that of the caproic acid, indicating a different libration for the two groups. Also, the principal axes of libration are not oriented in any special way with respect to the ureido ring. The correction of thermal vibration for the bonds in the ring ranges from 0.004 Å for the C(2')-N(3') bond to 0.015 Å for C(2)-C(3). In the following discussion, the bond distances corrected for thermal vibrations are also indicated, where appropriate, within squared parentheses.

Molecular Features. The ureido moiety in dethiobiotin displays somewhat unusual bonds for a ureido group; the C=O bond is 1.244 [1.252] Å long and the two C-N bonds are 1.346 [1.357] Å and 1.347 [1.351] Å long. These values may be compared on the one hand with the average values 1.209 (6) Å and 1.370 (7) Å for the C=O and C-N bonds in 12 barbiturate structures[12] and, on the other hand, with values of 1.270 (7) Å and 1.326 (6) Å for the corresponding bonds in urea.[13] Values for comparable bond lengths in uronium nitrate have been determined by both Cu and Mo x-ray diffraction[14] studies and by neutron[15] studies. They are:

	Cu	Mo	Neutron
C=O	1.302 (3) Å	1.307 (3) Å	1.298 (2) Å
C-N_1	1.315 (3)	1.309 (3)	1.315 (1)
C-N_2	1.297 (3)	1.306 (3)	1.312 (1)

It has been pointed out[15] that these results indicate a partial double bond character in each of the C-O and C-N bonds. The unexpected feature in dethiobiotin is the redistribution of the bonding electrons (as judged by bond distances) in the ureido group though the ring is not aromatic. It seems likely that in dethiobiotin there is an appreciable contribution of the valence bond structures III or IV which endow the carbonyl group with a partial negative charge. This has important consequences in

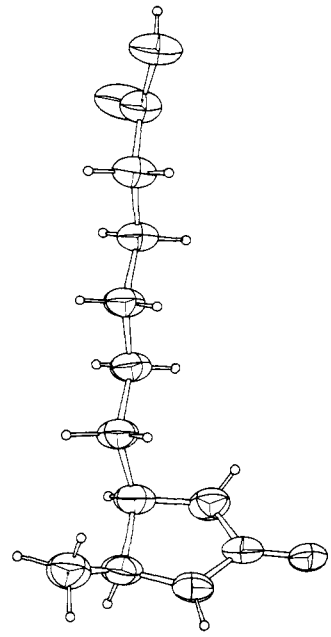


Figure 1. View of dethiobiotin as a natural (*d*) isomer.

Table VII. Bond Distances Corrected for Thermal Libration (Å)

	Corrected	Uncorrected
C(2)-C(6)	1.546	1.533
C(6)-C(7)	1.539	1.520
C(7)-C(8)	1.530	1.515
C(8)-C(9)	1.541	1.522
C(9)-C(10)	1.507	1.493
C(10)-O(10a)	1.323	1.306
C(10)-O(10b)	1.242	1.202

Table VI. Results of Rigid-Body Analysis of Thermal Parameters

	Group 1[a]				Group 2[b]				Group 3[c]			
	EA[d]	EV[e]			EA[d]	EV[e]			EA[d]	EV[e]		
L[f]	26.2	−9768	843	−1969	214.2	9987	329	−388	53.6	4409	2564	8601
	2.6	−361	−9717	−2334	4.7	506	−5643	8240	13.0	8972	−1536	−4141
	0.1	−2111	−2211	9521	0.6	51	−8249	−5653	4.3	258	9543	−2976
T[g]	0.0654	784	4855	8707	0.0660	−844	2697	−9592	0.0644	550	2503	9666
	0.0532	2525	−8546	4538	0.0503	−431	−9628	−2669	0.0459	1090	−9638	2433
	0.0317	9644	1843	−1897	0.0343	−9955	188	929	0.0265	9925	920	−803
S[h]		−50	−14	110		−443	−167	−67		−72	−26	−120
			9	27			171	36			208	−70
				41				272				−136
R[i]		0.83*a*	23.91 Å			0.73*a*	20.99 Å			0.93*a*	26.98 Å	
		0.08*b*	0.38			0.0*b*	−0.01			0.15*b*	0.77	
		0.80*c*	6.16			0.75*c*	5.76			0.84*c*	6.47	
ΔU_{ij}[j] (Å²)		0.0077				0.0032				0.0036		
σU_{ij}[k] (Å²)		0.0087				0.0041				0.0048		

[a] All nonhydrogen atoms. [b] O(10a), O(10b), C(2), C(6), C(7), C(8), C(9), C(10). [c] N(1'), O(2'), N(3'), C(3), C(4), C(5), C(2). [d] Eigenvalues (mean square amplitude) in degrees squared (*L*) or Å² (*T*). [e] Eigenvectors (direction cosines × 10^4) relative to orthogonal vectors **a**, **b**, and **c***. [f] Librational tensor. [g] Translational tensor. [h] Symmetrized screw tensor (× 10^5 rad Å) expressed relative to principal axes of *L*. [i] The unweighted centroid or center of gravity of rigid body is used as origin. **R** relates the fractional and orthogonalized (Å) coordinates to the first origin. [j] The root mean square difference of observed and calculated U_{ij}. [k] The estimated standard deviation of U_{ij}.

Table VIII. Torsion Angles for *d*-Dethiobiotin[a] (deg)

(a) Ureido Ring, Ring Angles	
C(2′)-N(3′)-C(3)-C(4)	−21.7
N(3′)-C(3)-C(4)-N(1′)	22.2
C(2′)-N(1′)-C(4)-C(3)	−17.8
C(4)-N(1′)-C(2′)-N(3′)	5.1
C(3)-N(3′)-C(2′)-N(1′)	11.7
(b) Exocyclic Angles	
N(3′)-C(3)-C(4)-C(5)	144.1
C(2′)-N(1′)-C(4)-C(5)	−143.1
C(3)-N(3′)-C(2′)-O(2′)	−167.6
C(4)-N(1′)-C(2′)-O(2′)	−175.6
C(2)-C(3)-C(4)-N(1′)	−98.3
C(2)-C(3)-N(3′)-C(2′)	101.5
C(2)-C(3)-C(4)-C(5)	23.5
(c) Valeryl Side Chain	
N(3′)-C(3)-C(2)-C(6)	66.5
C(4)-C(3)-C(2)-C(6)	−179.1
C(3)-C(2)-C(6)-C(7)	−171.2
C(2)-C(6)-C(7)-C(8)	−179.6
C(6)-C(7)-C(8)-C(9)	−178.5
C(7)-C(8)-C(9)-C(10)	−175.4
C(8)-C(9)-C(10)-O(10a)	−163.5
C(8)-C(9)-C(10)-O(10b)	21.9

[a] Values are given for the *d* isomer in the structure.

III IV

connection with the nucleophilicity and hydrogen bonding of O(2′) as pointed out in a later section.

The 2-imidazolidone ring is substantially puckered. The torsion angles around the ring are given in Table VIII. These torsion angles show that the pucker is not symmetric. The best four-atom plane (Table IX) omits C(3) which is 0.367 Å away from this plane through C(2′), N(1′), N(3′), and C(4). With reference to the plane through N(1′), C(2′), and N(3′), C(3) and C(4) have opposite and unequal deviations (Table IX), resulting in a "twist" conformation. This conformation may also be described as a hybrid between a C_3 envelope (asymmetry parameter,[16] $\Delta C_s = 4.3°$) and a $C_{2'}$ half-chair ($\Delta C_2 = 5.4°$). The pucker of the 2-imidazolidone ring in dethiobiotin (as compared with the planar ring in biotin) does not cause any significant change in the bond distances of the atoms in the ring and has caused only a slight change in the bond angles. On the other hand, the exocyclic angles C(2)-C(3)-C(4) (115.6°) and C(3)-C(4)-C(5) (117.0°) have expanded by more than 6°, as compared with biotin (109.7° and 108.6°, respectively). Presumably, the 1,4 interactions between C(2) and C(5) (2.949 Å) and N(3′) and C(6) (3.042 Å compared with 2.881 Å in biotin) are somewhat relieved both by the pucker and the expansion of the exocyclic angles.

The bond distances and angles for the fatty acid-like chain are within the normal range for similar compounds, and, after correction for thermal vibration, the bond distances are close to 1.54 Å. The C-Ĉ-C bond angles (average, 113.2°) display the typical expansion from the tetrahedral value observed for hydrocarbons such as pentanes and octanes.[17] The caproic acid chain has the fully extended trans planar zig-zag conformation found in *n*-alkanes and fatty acids. In contrast, the valeryl side chain in biotin is severely twisted around the C(6)-C(7) bond. The torsion angles describing the side chain conformation of dethiobiotin are given in Table VIII. As the chain progresses from the ring to the carboxyl terminal, the torsion angle progressively decreases from −179.6° for the C(6)-C(7) bond to −175.4° for C(8)-C(9) bond, indicating a slight helical twist. A more pronounced twist is observed about the C(9)-C(10) bond, resulting in a significant deviation of the planar carboxyl group from the all-trans zig-zag arrangement. Such deviations from all-trans zig-zag arrangements have been observed in long-chain fatty acids and their esters.[11,18]

Hydrogen Bonding and Packing. There are three hydrogen bonds in this structure, O(10a)-H···O(2′), N(3′)-H···O(10b), and N(1′)-H···O(2′) (for distances and angles of hydrogen bonds, see Table X). The hydrogen bond from the carboxyl group to the ureido carbonyl oxygen is a short one [O(10a)-O(2′), 2.663 Å, H(10a)···O(2′), 1.83 Å] but not as short (O(10a)···O(2′), 2.55 Å) as in biotin. The hydrogen bond from N(3′) to O(10b) is very similar to that found in biotin. The

Table IX. Least-Squares Planes[a]

Plane	Atoms	*l*	*m*	*n*	*d*, Å	Rms dev, Å
1	N(1′), N(3′), C(2′)	−0.672	0.631	−0.389	20.473	0.0
2	N(1′), N(3′), C(2′), C(4)	−0.671	0.598	−0.438	20.854	0.021
3	O(2′), C(2′), N(1′),[b] C(2′),[b] O(2′),[b] N(1′)	0.021	0.962	−0.271	0.0	0.2
4	O(2′), C(2′), N(3′), O(10a),[c] O(10b),[c] C(10)[c]	−0.252	0.518	−0.817	12.690	0.166

Atoms	Plane 1	Atoms	Plane 2	Atoms	Plane 3	Atoms	Plane 4
N(1′)	0.0	N(1′)	−0.025	O(2′)	−0.147	O(2′)	0.262
N(3′)	0.0	N(3′)	−0.015	C(2′)	0.284	C(2′)	−0.203
C(2′)	0.0	C(2′)	0.026	N(1′)	−0.133	N(3′)	−0.030
C(3)	−0.271	C(4)	0.015	N(1′)[b]	0.133	O(10a)[c]	−0.099
C(4)	0.120	C(3)	−0.367	C(2′)[b]	−0.284	O(10b)[c]	0.179
				O(2′)[b]	0.147	C(10)[c]	−0.109

[a] The equation of the plane is $lX + mY + nZ = d$; *X*, *Y*, and *Z* are cartesian coordinates along **a**, **b**, and **c***; *l*, *m*, and *n* are the direction cosines. [b] Molecule at $-2 - x, -1 - y, -2 - z$. [c] Molecule at $-\frac{3}{2} - x, -\frac{1}{2} + y, -2 - z$.

Table X. Hydrogen Bonds

D-H···A	D-H, Å	D···A, Å	H···A, Å	D-H···A, Å	A in position
N(1′)-H(1′)···O(2′)	0.79	2.908	2.12	174.8	$-2 - x, -1 - y, -2 - z$
O(10a)-H(10a)···O(2′)	0.84	2.663	1.83	169.3	$-\frac{3}{2} - x, \frac{1}{2} + y, -2 - z$
N(3′)-H(3′)···O(10b)	0.84	2.985	2.22	151.4	$-\frac{3}{2} - x, -\frac{1}{2} + y, -2 - z$

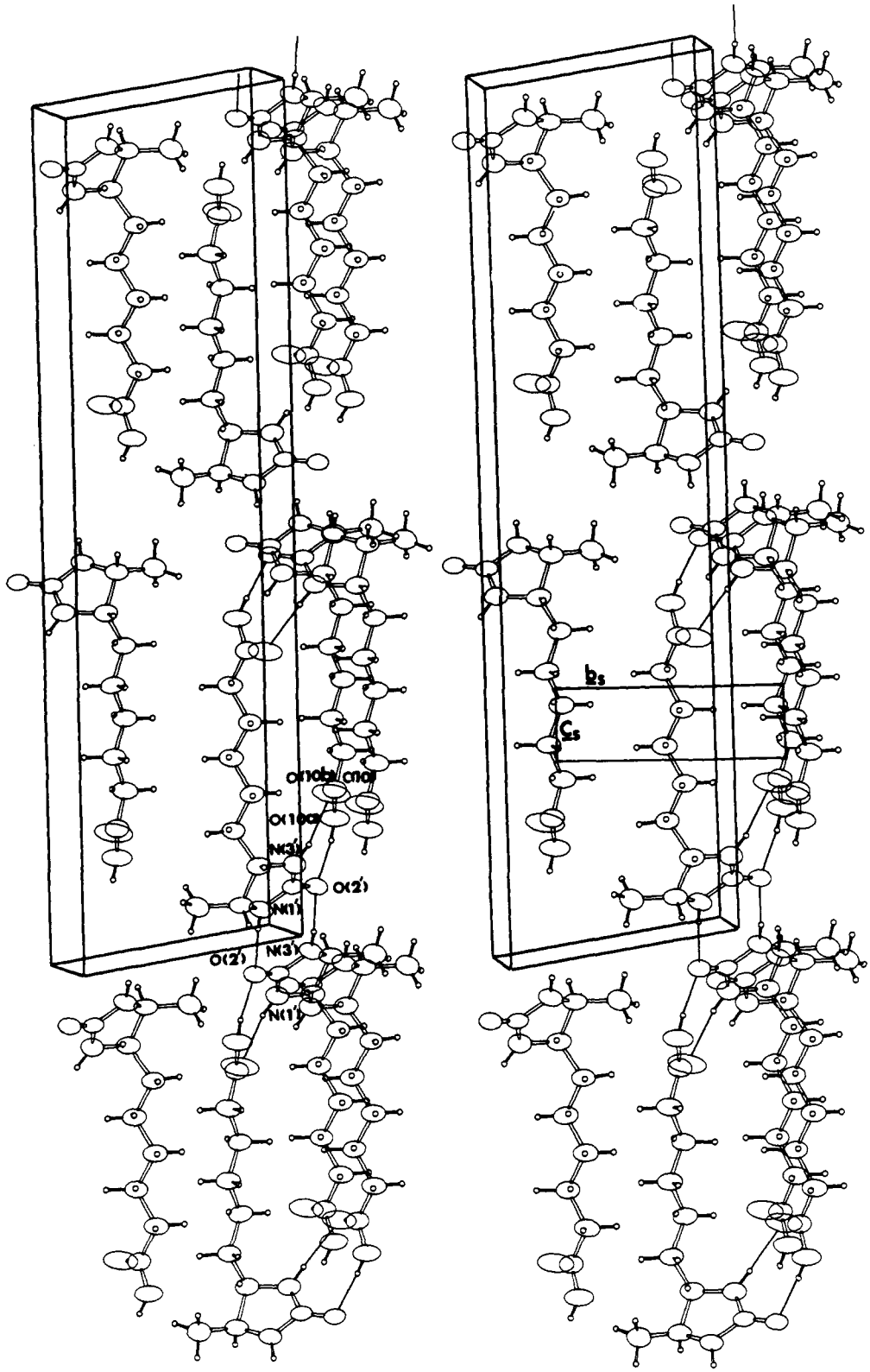


Figure 2. A stereoview of the crystal structure, hydrogen bonding, and orthorhombic perpendicular subcell.

third one involving N(1′)-H(1′) is very different, since the acceptor in dethiobiotin is O(2′) whereas in biotin it is O(10b). The O(2′) in biotin makes a rather long contact (2.77 Å) to H(1′) in such a way that one of the lone-pair sp^2 orbital points towards H(1′) and the other to the hydrogen bonding H(10a). In dethiobiotin, O(2′) also has contacts to the same atoms H(10a) and H(1′), but both contacts in dethiobiotin are shorter and interpretable as hydrogen bonds. The angles around O(2′) [C(2′)-O(2′)···H(1′), 116°; C(2′)-O(2′)···H(10a), 118°; H(1′)···O(2′)···H(10a), 119°] clearly show that the sp^2 orbitals are pointing directly to the two hydrogen bonding atoms.

The hydrogen bonding (Figure 2) is localized about the inversion center at (000) and (½00) and forms planar (see Table IX) six-membered rings {···O(2′)-C(2′)-N(1′)-H(1′)···O(2′)-C(2′)-N(1′)-H(1′)···O(2′)}. The sequence {···O(2′)-C(2′)-N(3′)-H(3′)···O(10b)-C(10)-O(10a)-H(10a)···}, not

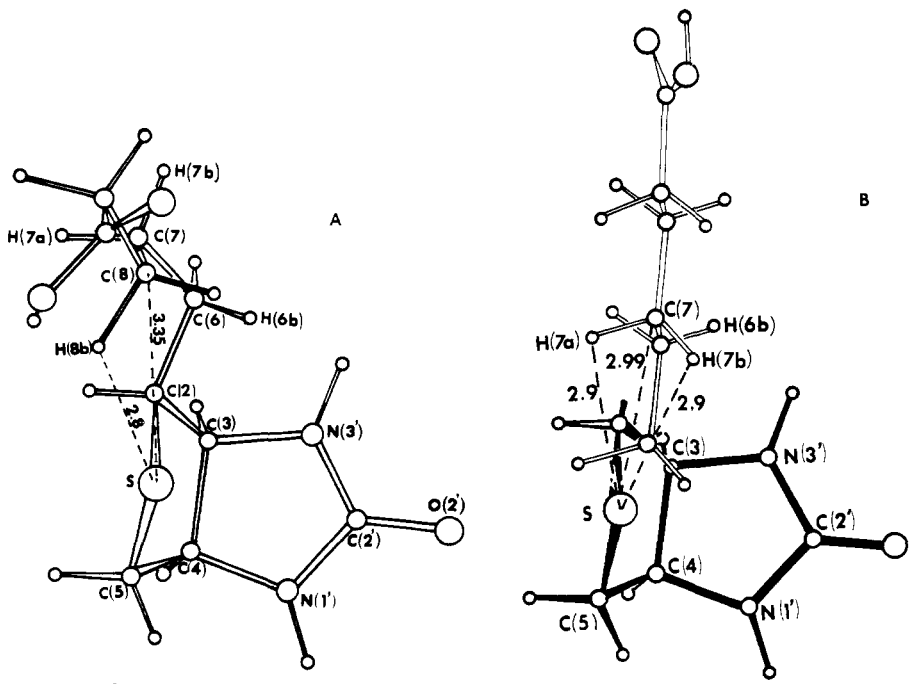


Figure 3. (A) The structure of biotin[5] and the contacts to sulfur. (B) The structure of biotin in which the conformation of the valeryl side chain as found in dethiobiotin is added in thin lines for comparison. The short contacts which *would* result if the side chain of biotin assumes a conformation similar to that of dethiobiotin are indicated by dotted lines.

about the center of inversion, also forms planar rings (see Table IX). These two rings connect the molecules in a head-to-tail fashion and form a helical arrangement.

The packing of the fatty acid-like chain resembles the orthorhombic perpendicular packing observed by von Sydow[19] for normal-chain fatty acids. The orthorhombic subcell, indicated in bold line in Figure 2, is seen as projected down the *b* axis. The subcell measures $a_s = b = 4.999$ Å, $b_s = c = 7.756$ Å, and $c_s = 2.535$ Å. The volume per CH_2 (24.57 Å^3) is in the upper range of observed methylene volumes for the normal-chain fatty acids in the orthorhombic cell. The crystal stability along the *b* direction is additionally due to the van der Waals' forces between the carbon chains and dispersion forces between polarized carbonyls.

Discussion

The formation of hydrogen bonds between the oxygen of the ureido group and an H donor is interesting for several reasons.[4] Lynen and co-workers[20] developed a reaction scheme for the enzymatic carboxylation of biotin in which the first step entails the nucleophilic attack of a nitrogen of the ureido group at bicarbonate. But the nucleophilicity of this nitrogen is very low. To facilitate such a nucleophilic attack, an activation of the nitrogen has to take place during the enzymatic process. A possible mode of activation would be the polarization of the carbonyl double bond of the urea part by formation of a hydrogen bond or protonation of the oxygen, leading to an increase in the double bond character of the carbonyl carbon–nitrogen bond and to a concomitant increase in the acidity of the NH group.[4] Such conditions will favor the deprotonation of NH and the attack by the resulting nitrogen anion. Mildvan and Scrutton[21] suggested a mechanism for the carboxylation half-reaction where an activation of the ureido group occurs by polarization of the CO group by a phenolic or similar group present at a suitable location in the enzyme. Bruice and Hegarty[22] favor a mechanism for the carboxylation half-reaction at O(2′) rather than N(1′) due to the nucleophilicity of O(2′). Infrared studies by Griesser et al.[4] show that hydrogen bond formation takes place between the CO group of 2-imidazolidone and the OH group of phenol, supporting the polarizability of the ureido C=O bond.

Our studies on dethiobiotin are complementary to that of biotin[5] and clearly demonstrate that the ureido oxygen of dethiobiotin takes part in strong hydrogen bonds to appropriate donors. A delocalization of the electronic charge occurs in the ureido part of the ring, resulting in the polarization of the carbonyl bond. The delocalization of the charge in dethiobiotin leads to an equal change in both of the C–N distances of the ureido moiety as already pointed out and does not confer an increased nucleophilicity, as judged by the shortening of the C–N bonds, for N(1′) over N(3′). The two C–N bonds of the ureido moieties are nearly equal in several structures containing this group, except in ureidopurines[23] and in N^1-(*N*-methylcarbamoyl)-N^3-methyl-5,6-dihydrouracil[24] where the two bonds are asymmetric and differ by values ranging from 0.04 Å to as much as 0.11 Å. Though the ureido group exhibits a high degree of delocalizability and under appropriate conditions can assume a highly unsymmetrical form, the primary reason for the carboxylation at N(1′) rather than N(3′) seems to be steric,[25] and not electronic. Our studies clearly show the nucleophilic qualities and polarizability of the ureido carbonyl group and the associated hydrogen bonding to it, but do not indicate whether such mechanisms are actually used in the biological situation.

The function of sulfur in biotin has been studied,[26] and three suggestions have been made regarding its role. A transannular interaction between the carbonyl carbon and sulfur has been proposed,[26a] but experimental[26b] and theoretical[26b,27] studies do not support this hypothesis. Our results clearly show that the bond distances and angles in the ureido ring of dethiobiotin and biotin[5] are nearly identical, and hence the sulfur does not have any pronounced interaction with the carbonyl group. The possibility of hydrogen bonding between sulfur and biotin-dependent enzymes was suggested[26c] and was further examined by studying the protonation of sulfur.[28] Hydrogen bonding to sulfur in thioethers has not been observed; the only structure, to our knowledge, in which a hydrogen is bonded to a divalent sulfur is that of 3,3,3′,3′-tetramethyl-D-cystine (D-penicillamine disulfide) dihydrochloride.[29] In this structure, a hydrogen of the NH_3^+ group is hydrogen bonded to the proximal sulfur, such that the N–H···S contact is nearly normal to the plane through the sulfide and nearly along the unshared p_z

orbitals of sulfur. If this result may be extended[30] to biotin, any hydrogen bonding to the sulfur from an appropriate donor of the enzyme would be directed nearly normal to the plane of the sulfide and, for steric reasons, trans to the valeric acid chain. Another possible function of sulfur is to complex with divalent cations to stabilize the enzyme-(metal ion)-substrate complex.[26] If such a complex is formed, the divalent cation to sulfur direction will be nearly normal to the plane of the sulfide. Our analysis[30] of the directionality of the nonbonded contacts with sulfur has shown that atoms that accept electrons from a sulfide S approach it in a direction nearly normal to the sulfide plane. Thus, the orientation of the tetrahydrothiophene ring will be similar and somewhat specific with respect to the hydrogen of the donor or divalent cation if hydrogen bonding or coordination occurs.

A comparison of our results on dethiobiotin with that of biotin suggests an important role for sulfur in maintaining the planarity of the 2-imidazolidone ring and in stabilizing the twisted conformation of the valeryl side chain in biotin. As already discussed, the 2-imidazolidone ring in dethiobiotin is substantially puckered compared with the nearly planar ring in biotin. The pucker of this ring is not due to any change in electron delocalization, since the bond distances and angles are nearly the same in both biotin and dethiobiotin. Consequently, the planarity of the 2-imidazolidone ring is primarily due to the sulfur. Though it has been demonstrated[31] that an intact ureido ring is important for the binding of biotin and its analogues to the tryptophan residues of avidin, the influence, if any, of the nonplanarity of the ring on this association has not been evaluated.

The tetrahydrothiophene ring in biotin puckers with sulfur endo to the ureido ring, rather than exo. The latter pucker will orient the C(2)-C(6) bond nearly axial to the tetrahydrothiophene ring and will give rise to an energetically unfavorable N(3′)···C(6) contact, shorter than the 2.881 Å found in biotin; consequently, the S-exo pucker in biotin is not possible. The S-endo pucker of the ring imposes conformational restrictions on the valeryl side chain of biotin. If the side chain in biotin were to assume the fully extended trans planar zig-zag conformation similar to that in dethiobiotin, short contacts between sulfur and C(7) and the hydrogens on C(7) would result (Figure 3). To avoid such contacts, especially the S···C(7) contact, the fatty acid chain is twisted about the C(6)-C(7) bond by 73.4°; this twist results in contacts of 3.497 Å for S···C(7), 3.352 Å for S···C(8), and 2.77 Å for both S···H(6b) and S···H(8b), all nearly in the sulfide plane. The direction of these four contacts make angles of 101.4, 102.5, 68.5, and 117.6°, respectively, with respect to the normal of the plane through C(2), S, and C(5). We have shown elsewhere[29,30,32] that sulfur may be considered to have a smaller radius (1.625 Å) in the plane of the sulfide than in the direction normal to this plane. Electron density maps of sulfides and disulfides[33] all show the electron cloud to be more extensive normal to the sulfide plane than in the plane. Consequently, the four contacts mentioned above are not energetically unfavorable and are sterically allowed.[34] The structure of di-*p*-bromoanilide of CO_2-biotin also shows a twist in the valeric acid side chain, presumably to avoid a contact with sulfur. The conformation of this valeric side chain is different from that in biotin and from the trans planar zig-zag conformation in dethiobiotin; the C(3)-C(2)-C(6)-C(7) torsion angle is −62°, compared with 172° for biotin and −171° for dethiobiotin. These results suggest that sulfur may play a role in imposing conformational restrictions on the valeryl side chain. It is known that the length of the side chain is important for carboxylation reactions; an increase or decrease of the side chain length by one carbon, as in homobiotin or norbiotin, respectively, results in a 90% decrease in the carboxylation rate and a 50% reduction in phosphoryl transfer rate. In this connection, it is interesting to note that the valeryl chain is in the twisted rather than the straight zig-zag conformation, and sulfur seems to have a role in stabilizing this twisted conformation.

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Supplementary Material Available: A listing of observed and calculated structure factor magnitudes and weak reflections (16 pp). Ordering information is given on any current masthead page.

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Mechanism of Phosphorylation by *N'*-Phosphorocreatine. Concurrent Formation of Creatine and Creatinine[1,2]

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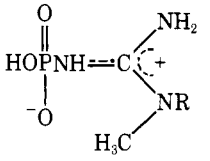
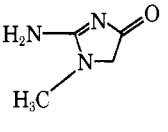
Abstract: The mechanism of phosphorylation by phosphorocreatine and the mechanism of creatinine formation have been investigated in aqueous solution. The hydrolysis of phosphorocreatine (PC) was found to occur via two pathways: pathway A leads to the production of creatine and inorganic phosphate; pathway B leads to the production of creatinine and inorganic phosphate. At pH values above 1.0, pathway A accounts for greater than 90% of the hydrolysis; pathway B becomes predominant only in strongly acidic solutions. For pathway A, the bell-shaped, pH-rate profile with a rate maximum at pH 1-2 (k_{max} = 1.77×10^{-2} min^{-1}), $\Delta S^{*} = -2$ eu, and $k(H_2O)/k(D_2O) = 0.86$ strongly support a metaphosphate mechanism for phosphorylations by phosphorocreatine. The biological implications with regard to the action of ATP:creatine *N*-phosphotransferase (creatine kinase, E.C. 2.7.3.2) and the origin of urinary creatinine are discussed.

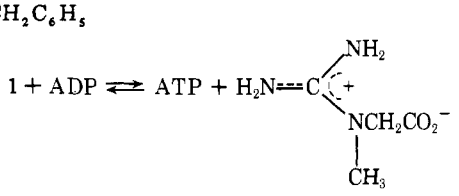
N'-Phosphorocreatine (**1**, PC), a phosphoroguanidine,[3] plays an essential role by phosphorylating ADP (eq 1) during periods of rapid utilization of ATP in muscle. Our studies of the mechanism of phosphorylation by the simple phosphoroguanidines, **2** and **3**, appears to involve proton transfer leading to unimolecular cleavage to the protonated guanidine and the reactive intermediate, monomeric metaphosphate ion, PO_3^-,[2,4] which is the actual phosphorylating agent. Phosphoroguanidines appear to be the most reactive precursors of metaphosphate.[4] The presence of the carboxylate group in PC presents possibilities for functional group and ionization effects which could lead to special reactivity in this natural phosphorylating agent. Therefore, in order to assess the significance of the metaphosphate mechanism with respect to the in vivo phosphorylation reaction involving PC, the in vitro mechanism of phosphorylation of water (hydrolysis) by PC has been studied. This paper is addressed to the chemistry of hydrolysis of **1**, the implications for biological phosphorylation by **1**, and the mechanism of formation of creatinine (**5**).



1, R = CH_2CO_2H (*N'*-phosphorocreatine)
2, R = CH_3
3, R = $CH_2C_6H_5$



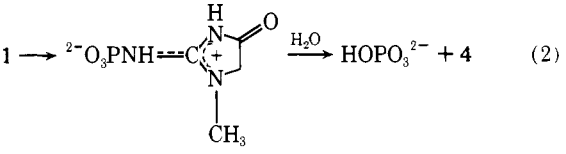
5 (creatinine)



4 (creatine)

Previous investigations on PC are inconclusive. PC has been shown to be stable in alkaline solution[5] but to undergo rapid hydrolysis in acid with a rate maximum at pH 1-3.[6,7] It has also been reported that PC hydrolyzes in acid some 30 times faster than an unsubstituted phosphoroguanidine,[8] therefore, indicating possible intramolecular catalysis by the carboxylate group.

The products of hydrolysis of PC are reported to be creatine (**4**) and a small amount of creatinine (**5**).[9] A mechanism for formation of **5** involving cyclization to **6** followed by P-N cleavage and ring opening (eq 2) has been proposed;[10] evidence for this mechanism includes the report of an induction period.[11] However, hydrolysis in ^{18}O-enriched water has demonstrated that this mechanism cannot be correct since no label was found in the creatine isolated from the reaction.[12] Recently, it has also been shown that **5** hydrolyzes to creatine more slowly than **1** does.[13]



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Results

Potentiometric Titration. A potentiometric titration was carried out on the disodium salt of PC under the same conditions as those employed in the kinetic studies above pH 0.78, that is, T = 30.5 °C and μ = 0.20 N in added NaCl. The overlapping dissociation constants were calculated by the method of Noyes.[14] The dissociation constants (Table I) are assigned in eq 3; the bases for the assignments have been discussed.[4,15]

Products of the Hydrolysis of PC. The rates of hydrolysis of PC were measured at 30.47 ± 0.05 °C in the pH range of 0.78-5.58 in buffered solutions with the ionic strength maintained constant at 0.20 N by the addition of NaCl or $NaClO_4$, and in 0.40-4.00 N $HClO_4$. On hydrolysis, PC produces inorganic phosphate and both **4** and **5**. Both products were detected by paper chromatography for the hydrolysis of PC in 1.0 N $HClO_4$. The Jaffé-Folin determination[16] demonstrated conclusively that **5** is ca. 5% of the products of the hydrolysis of PC at pH 5.22 and 5.88.

A quantitative measure of the product composition is afforded by the absorbance at 225.0 nm at the end of the hy-