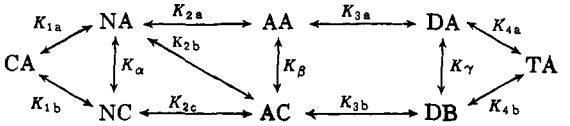
represents the highly improbable case of the phenolic proton being ionized in preference to both the primary phosphate and pyridinium protons. If the formation of a pyridinium dipolar form is to be favored, then NA must represent the predominant form. Form NC may also be present to at least a small extent, but no measurements could be made in this pD region owing to solubility difficulties. The solid state spectrum of pyridoxal phosphate does show a band at 1420 cm.$^{-1}$, so presumably NB is present to some extent in the crystalline material.

Forms AB and DA should have a band in the vicinity of the 1420 cm.$^{-1}$ band in TA. While the presence of the pyridinium proton might be expected to exert some influence against the electron enrichment of the ring owing to the ionization of the phenolic proton, no resonance forms can be written for such an interaction, and consequently this effect would be expected to be small. In the case of DA, the band due to the phenoxide form may be merged with that of TA; in the case of AB, however, this band has entirely disappeared. That is, the intensity of the 1420 cm.$^{-1}$ band correlates well with the fraction of TA present with a possibility that DA is contributing, but precludes any possibility of the presence of AB (see Fig. 10).

These considerations lead to the formulation of the equilibrium scheme



With the exception of TA, it is impossible to find the extinction coefficients of any of these species directly. The extinction coefficient for the C–O$^-$ group of TA may be calculated using the spectrum at pH 11.0, but this value is useless for calculation of other species—such as DA—because the band is obscured. The extinction coefficient of the 3-pyridol cation was used in making calculations for the 1490 cm.$^{-1}$ band. This extinction coefficient is, strictly, only applicable to the N$^+$–D group on other cations. Although some error would be expected if this extinction coefficient is transferred to other charge types, this provides the best approximation thus far available for pyridoxal phosphate in aqueous solution. The value of the extinction coefficient was 35.3 l./mole-mm.

On the basis of the above discussion, the constants for the system may now be calculated.

The microscopic constants are defined as

$$K_\alpha = [NA]/[NC], \quad K_\beta = [AA]/[AC], \quad K_\gamma = [DA]/[DB]$$

In the calculation of the constants, the sum of the concentration of any pair of tautomeric forms is obtained from Fig. 10. If two equations at two similar pH values are solved for the same species, divided by the respective total concentrations, and equated, it is possible to calculate the value of one of the constants. This procedure is based upon the assumption that at two similar pH values the mole fraction of a particular species will be constant. The above constant, together with mole fraction data and over-all dissociation constants, allows one to calculate the remainder of the constants by simple algebraic methods. It was not possible to calculate any values of K_α, but the predominant species in the acid regions, as has been pointed out above, are the pyridinium dipolar forms, such as NA, so this microscopic constant would be expected to be very large. The other equilibrium constants are defined as usual and are shown in the diagram of the equilibrium scheme. The values of these constants are

$K_\beta = 7.7 \pm 0.8$	$K_\gamma = 1.6 \pm 0.2$
$K_{3a} = 1.3 \times 10^{-6}$	$K_{3b} = 6.5 \times 10^{-6}$
$K_{4a} = 1.1 \times 10^{-8}$	$K_{4b} = 1.9 \times 10^{-8}$

The estimated errors in the values of these constants is 10–20% ($< \pm 0.1$ log value).

The calculation of the degree of hydration of the carbonyl function is based upon the assumption that the extinction coefficient of the carbonyl group in dimethyl sulfoxide is the same as the extinction coefficient of the unhydrated carbonyl in D_2O solution. Considering the highly polar nature of both solvents, this assumption seems reasonable.

The actual degree of hydration of the carbonyl function is almost constant over all pD values from 4.7 to 11.0. The degree of hydration at high pD values seems to be slightly higher than the intermediate pD values, but the scatter of the points was such that these higher values could be attributed to experimental errors.

If K_H is defined as

$$K_H = [\text{hydrated forms}]/[\text{nonhydrated forms}]$$

then K_H is found to be $+0.36 \pm 0.07$.

[CONTRIBUTION FROM THE ORGANIC CHEMISTRY DEPARTMENT, SCIENTIFIC DIVISION, ABBOTT LABORATORIES, NORTH CHICAGO, ILL.]

Direct Condensation of 2-Deoxy-D-ribose with Purines. Structure of the Products

BY JOHN A. CARBON

RECEIVED SEPTEMBER 19, 1963

The direct reaction of 2-deoxy-D-*erythro*-pentose (I) with any of various purines in hot polar solvents leads to the production of a diastereoisomeric pair of compounds with empirical formulas corresponding to the condensation of equimolar quantities of the sugar and purine with elimination of 1 mole of water. The products are thus isomeric with the corresponding 2'-deoxynucleosides. These compounds (IV and V) possess ultraviolet spectra only compatible with 9-substituted purines and were readily hydrolyzed by alkali, but not by acid, to the free purines. A possible formulation of these products as 2,3-dideoxy-3-(9-purinyl)-D-*erythro*- (and *threo*-) pentoses (IV and V) was proved by an unambiguous synthesis of one of the isomeric pairs. The products are thought of as arising from a nonstereospecific Michael addition of the purine to an intermediate α,β-unsaturated aldehyde (II), generated by loss of the C-3 hydroxyl of the 2-deoxy sugar. This sequence constitutes an hitherto unobserved reaction of 2-deoxy sugars.

The chemistry of 2-deoxy-D-*erythro*-pentose (2-deoxy-D-ribose) and the synthesis of 2'-deoxy-D-*erythro*-pentofuranosyl derivatives of purines and pyrimidines (2'-deoxynucleosides) has been a subject of great interest to many workers, not only because of the importance of this group of compounds in biological phenomena, but also because of the challenging synthetic problems encountered in this field.[1] It was

(1) For a recent review see T. L. V. Ulbricht, *Angew. Chem.*, **74**, 767 (1962).

recently noted in our laboratories that when aqueous solutions containing various 6-substituted purines and 2-deoxy-D-*erythro*-pentose were heated at 100°, ultraviolet-absorbing products were formed in substantial amounts, as revealed by paper chromatography. This paper is concerned with the isolation and proof of structure of these condensation products, which are formed by a new and hitherto unobserved reaction of 2-deoxy sugars.[2]

Formation and Isolation of the Products.—Paper chromatography of the solution obtained by heating a mixture of adenine and 2-deoxy-D-*erythro*-pentose in water at 100° for 24 hr. revealed unreacted adenine (R_{Ad} 1.00) and one other intense ultraviolet-absorbing spot (R_{Ad} 0.38). Maximal yields (71%) of the 0.38 material were obtained by using a 3:1 molar ratio of the sugar to adenine in an unbuffered aqueous solution. In the preliminary pilot runs of this type, approximate yields were obtained by elution of the spots and measuring their relative absorbancies at 260 mμ. A repetition of the reaction in aqueous buffers at various pH values revealed the reaction to be fastest in mildly alkaline solution (pH 8–9); however, the best yields of the 0.38 material were nevertheless obtained at pH 5 (Table II). Although the formation of the 0.38 material is apparently base-catalyzed, the products are unstable in alkaline solution (see below), thus explaining the lower yields at pH 8–9. The reaction also appears to proceed satisfactorily in dimethyl sulfoxide or N,N-dimethylformamide solution; however, these were not employed routinely since they appeared to offer little advantage over water.

The material of R_{Ad} 0.38 was isolated either by column chromatography of the reaction mixture over powdered cellulose or by partition chromatography over Hyflo Supercel[3] (see Experimental). It was soon apparent that the product as isolated from the columns consisted of a mixture of two compounds of λ_{max} 262 mμ in approximately equal proportions along with smaller quantities of λ_{max} 272 mμ material. This mixture was readily separated either by a repetition of the partition chromatography or, preferably, by adsorption chromatography over Florisil.[3] The two main products (IVa and Va) of λ_{max} 262 mμ were thus obtained as chromatographically pure crystalline solids.

Small pilot runs employing 6-methylthiopurine (IIIb) and 6-dimethylaminopurine (IIIc) as the purine components of the reaction gave good yields of similar products, as evidenced by paper chromatography (solvent B). In contrast to the products obtained from the adenine reaction, however, the reaction products were easily separated on paper and consisted of two well-defined spots of lower R_f than the starting purine. In each case, chromatography over silica gel resulted in a clean separation of the two products from unreacted starting material.

Determination of Structures.—Elemental analyses of the purified products gave empirical formulas compatible with the condensation of equimolar amounts of purine and 2-deoxy-D-*erythro*-pentose with the elimination of 1 mole of water, thus making them isomeric with 2′-deoxynucleosides. All of the products gave strongly positive Dische tests[4] characteristic of derivatives of 2-deoxy sugars. A comparison of the ultraviolet absorption spectra of the six compounds with those of appropriate model compounds gave data only in agreement with the formulation of the unknowns as 9-substituted purines[5] (Table I).

TABLE I

COMPARISON OF ULTRAVIOLET ABSORPTION SPECTRA

Compound	λ_{max}, mμ (ε) 0.1 *N* NaOH	H_2O	0.1 *N* HCl
IVa	262 (14,500)[a]	262 (14,300)	260 (13,950)
Va	262 (14,700)	262 (14,230)	260 (13,940)
1-Methyladenine[b]	270 (14,400)		259 (11,700)
3-Methyl-[b]	273 (13,300)		274 (17,000)
7-Methyl-[b]	270 (10,500)		272 (15,050)
9-Methyl-[b]	260 (14,700)		260 (14,200)
IVb	285 (19,200)	285 (19,200)	293 (17,400)
	292 (19,000)[c]	292 (19,200)	222 (10,600)
Vb	285 (19,200)	285 (19,400)	294 (17,500)
	292 (19,000)[c]	292 (19,300)[c]	222 (10,500)
9-Ethyl-6-methylthiopurine[d]	286 (17,700)	286 (17,600)	296 (16,500)
	290 (17,400)[c]	290 (17,100)[c]	222
7-Methyl-[e]	294 (16,300)		301 (14,500)
			225 (9,200)
3-Methyl-[f]	311 (17,200)	312 (20,900)	317 (25,200)
	237 (10,250)	236 (12,300)	235 (9,200)
IVc	277 (19,200)	275 (19,100)	268 (18,500)
	213 (15,300)	213 (17,100)	210 (17,600)
Vc	277 (19,000)	275 (18,900)	268 (18,200)
	213 (15,000)	213 (16,600)	210 (16,300)
6-Dimethylamino-9-methylpurine[g]	277 (18,100)	276 (18,100)	270 (17,500)
6-Dimethylamino-7-[g]	295 (15,600)	295 (17,400)	290 (19,800)

[a] Figures in parentheses are molar extinction coefficients. [b] Taken from ref. 5. [c] Shoulder. [d] J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **79**, 5238 (1957). [e] R. N. Prasad and R. K. Robins, *ibid.*, **79**, 6401 (1957). [f] J. W. Jones and R. K. Robins, *ibid.*, **84**, 1914 (1962); F. Bergmann, G. Levin, A. Kalmus, and H. Kivietny-Govrin, *J. Org. Chem.*, **26**, 1504 (1961). [g] B. R. Baker, R. E. Schaub, and J. P. Joseph, *ibid.*, **19**, 638 (1954).

A consideration of the above findings naturally led us to the supposition that the compounds in question were 2′-deoxynucleosides, a conclusion which was quickly shown to be erroneous by the simple expedient of comparing the physical constants of the two adenine derivatives IVa and Va with those of the known 9-(2′-deoxy-D-*erythro*-pentofuranosyl)- and 9-(2′-deoxy-D-*erythro*-pentopyranosyl) derivatives of adenine. Thus, of the four possible 9-substituted deoxynucleosides of adenine, three are known, *i.e.*, 9-(2′-deoxy-β-D-*erythro*-pentofuranosyl)-adenine (deoxyadenosine), 9-(2′-deoxy-α-D-*erythro*-pentofuranosyl)-adenine,[6] and 9-(2′-deoxy-β-D-*erythro*-pentopyranosyl)-adenine.[7] Neither of the two isomeric adenine derivatives obtained in our work were identical with any of these known materials, although marked similarities in the infrared spectra were apparent.

The most significant aspect of the chemistry of the unknown condensation products was their marked instability in aqueous alkali and contrasting high stability in dilute acid. This behavior is in direct opposition to the behavior of 2′-deoxynucleosides, which are well-known to be readily cleaved under acidic conditions, but which are quite stable in alkali.[8] Thus, solutions of either of the two products from adenine in 0.1 *N* sodium hydroxide were largely hydrolyzed to free adenine after 2–3 days at room temperature,

(2) It should be emphasized that the reaction discussed here is not related to the recently described condensation of adenine and 2-deoxy-D-*erythro*-pentose in the presence of polyphosphate ester: G. Schramm, H. Grötsch, and W. Pollmann, *Angew. Chem. Intern. Ed. Engl.*, **1**, 1 (1962). The Schramm condensation apparently results in the formation of a mixture of isomeric deoxynucleosides in which the purine is linked to C-1 of the sugar in the normal fashion: J. A. Carbon, *Chem. Ind.* (London), 529 (1963).

(3) Hyflo Supercel, a brand of diatomaceous silica, is a product of the Johns-Manville Co. Florisil was obtained from the Floridin Co., Tallahassee, Fla.

(4) Z. Dische, *Mikrochem.*, **8**, 4 (1930); R. E. Deriaz, M. Stacey, E. G. Teece, and L. F. Wiggins, *J. Chem. Soc.*, 1222 (1949).

(5) For excellent examples and a thorough discussion of the use of ultraviolet spectra to determine the position of substitution on the purine nucleus, see N. J. Leonard and J. A. Deyrup, *J. Am. Chem. Soc.*, **84**, 2148 (1962).

(6) R. K. Ness and H. G. Fletcher, Jr., *ibid.*, **82**, 3434 (1960). The author is indebted to Dr. Fletcher for a sample of this compound.

(7) H. Zinner and E. Wittenburg, *Ber.*, **95**, 1866 (1962). The author is indebted to these workers for a sample of their deoxyribopyranoside.

(8) For a thorough, but unfortunately, out-dated review on 2-deoxy-D-ribose chemistry, see W. G. Overend and M. Stacey, *Advan. Carbohydrate Chem.*, **8**, 45 (1953).

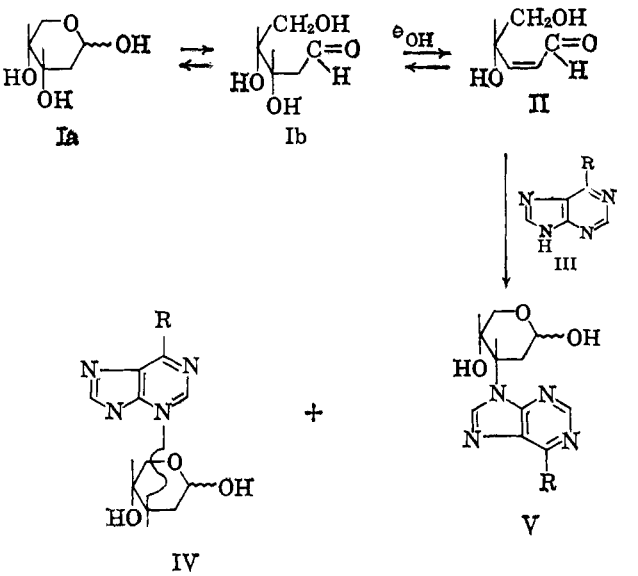
while solutions in 0.1 *N* hydrochloric acid were stable for at least 1 month. Hydrolysis of any of the unknown condensation products to the free purine was readily effected by warming an alkaline solution on the steam bath for 5–10 min. 2′-Deoxyadenosine, the corresponding α-anomer,[6] or 9-(2′-deoxy-β-D-*erythro*-pentopyranosyl)-adenine[7] were not hydrolyzed under the above conditions, but were readily cleaved in hot 0.1 *N* hydrochloric acid.

Although the presence of the free purine was easily shown in the alkaline hydrolysates by paper chromatography, we were unable to isolate other cleavage products. This is not surprising considering the well-known alkaline lability of 2-deoxy-D-*erythro*-pentose,[8] and, in fact, we were unable to detect the presence of more than a trace of undestroyed material when 2-deoxy-D-*erythro*-pentose was warmed in alkaline solution under conditions identical with those used for the hydrolysis of the unknown compounds.

Quantitative periodate oxidation studies on the two adenine products IVa and Va revealed a slow uptake of oxidant which leveled off after 7 hr. at the calculated value for the consumption of 1 mole. Although the 6-methylthio derivatives IVb and Vb readily consumed periodate, the reactions could not be quantitated because of the easily oxidized methylthio group. An investigation of the reaction mixture from periodate oxidations of each of the six unknowns revealed the presence of approximately 1 mole of formaldehyde per mole of starting material.

The optical rotations of the three pairs of products were strongly indicative of diastereoisomeric pairs. For example, in each case the first of the two unknown products to come off the column (the least polar substance) possessed a weakly positive rotation, while the second material (the most polar) possessed a strongly negative rotation (see Experimental). The compounds exhibited a slow mutarotation in N,N-dimethylformamide solution, a fact which again seemed to preclude their formulation as C_1-deoxynucleosides, since the latter compounds do not mutarotate.[9] The presence of a free reducing center at C-1 was considered likely because of positive tests with Tollens and Fehling reagents. These tests are by no means conclusive, however, since they are both run in alkaline solution, conditions which are likely to hydrolyze the purine–sugar linkage.[10] The uptake of 1 mole of periodate accompanied by the formation of formaldehyde can be interpreted as the oxidation of a 2-deoxy aldose such as IV and V, in the open chain form, at C_4–C_5, releasing C-5 as formaldehyde.

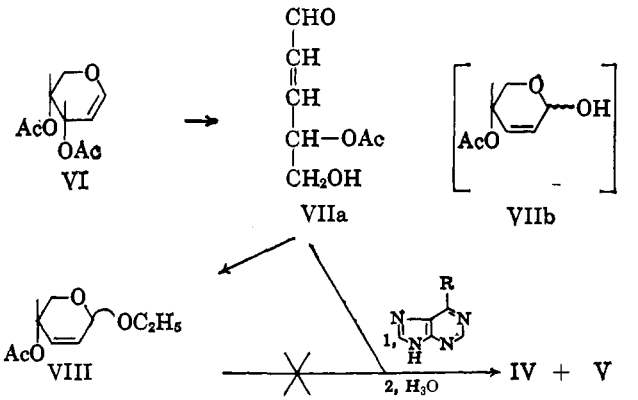
At this point we were fairly certain that we were dealing with diastereoisomeric pairs in which the 9-purinyl residue was located at other than C-1 of the sugar. The most likely position of attachment of the purine residue was felt to be at C-3 of a 2,3-dideoxy sugar because (1) a basic group at C-3 of an aldose is known to be readily eliminated in alkaline solution,[11] in line with their possible formulation as β-substituted aldehydes; (2) a likely mechanism could be written for the production of compounds of this type (IV and V) by postulating a Michael addition of the purine to an intermediate 2-deoxy-2,3-dehydro sugar (II) (see below); and (3) the formation of formaldehyde by periodate oxidation argued for the presence of a *vic*-diol grouping at C-4 and C-5.



a series, R = NH_2
b series, R = SCH_3
c series, R = $N(CH_3)_2$

Although the structures IV and V seemed highly likely for the various isomeric pairs we had isolated, the data were insufficient to be considered a rigorous proof.[12] Therefore, the most direct proof was sought by an independent and unambiguous synthesis of one of the pairs (IVb and Vb) by direct Michael addition of 6-methylthiopurine (IIIb) to a 2,3-double bond in a compound closely related to II.

Direct Synthesis of IVb and Vb.—The extremely facile rearrangement of D-*erythro*-3,4-diacetoxy-2,3-dihydro-4H-pyran (VI) (diacetyl-D-arabinal) to form D-4-acetoxy-5-hydroxy-2-pentenal (VIIa) (acetyl-pseudo-arabinal) has been known for many years.[13] The latter compound has always been written in the cyclic hemiacetal form (VIIb), however.[14] A study of



the infrared and n.m.r. spectra of a sample of VIIa, prepared by the method of Gehrke,[13] indicated the compound to exist entirely in the *aldehydo* form VIIa.

(9) J. Baddiley in "The Nucleic Acids," Vol. I, E. Chargaff and J. N. Davidson, Ed., Academic Press, Inc., New York, N. Y., 1955, pp. 137–190.

(10) The compounds all gave light brown spots on paper when treated with an aniline hydrogen phthalate spray, indicative of a free 2-deoxy aldose [*cf.* S. M. Partridge, *Nature*, **164**, 443 (1949); L. Hough, J. K. N. Jones, and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950)]. The absence of a typical aldehyde carbonyl absorption in the infrared spectra did not preclude the presence of a free reducing center at C-1 because of the possibility of cyclic hemiacetal forms such as IV and V.

(11) (a) C. D. Anderson, W. W. Lee, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **83**, 1900 (1961); E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, and K. Gerzon, *ibid.*, **76**, 3121 (1954).

(12) The nuclear magnetic resonance spectra of the compounds of type IV and V yielded little additional information. Because of their solubility characteristics it was necessary to run the spectra in deuterated dimethyl sulfoxide. Unfortunately, the resulting spectra were of a high degree of complexity owing in large part to the ready anomerization of the compounds at C-1. The possibility of the sugar residue being on the 6-amino group in IVa and Va was excluded by the presence of a signal at 2.60 τ of integrated intensity of two, which disappeared upon the addition of deuterium oxide.

(13) M. Gehrke and F. X. Aichner, *Ber.*, **60**, 918 (1927).

(14) B. Helferich, *Advan. Carbohydrate Chem.*, **7**, 209 (1952).

The significant signals in the n.m.r. spectrum of VIIa were a doublet at 0.10 τ ($J_{1,2}$ = 8 c.p.s.), assigned to the aldehydic proton, and a rather broad signal centered at 6.58 τ, disappearing upon the addition of deuterium oxide, assigned to the hydroxyl proton at C-5. The other features of the spectrum were completely compatible with structure VIIa. The infrared spectrum of VIIa displayed two strong carbonyl absorptions at 5.73 and 5.90 μ, assigned to the ester and aldehyde carbonyls, respectively.

Preliminary experiments with the α,β-unsaturated aldehyde VIIa indicated that simply heating it under reflux in aqueous solution in the presence of 6-methylthiopurine (IIIb) was sufficient to bring about addition of the purine to the double bond and partial hydrolysis of the acetyl group. Paper chromatography (solvent B) of the crude reaction mixture indicated the presence of two spots of R_f identical with those obtained with IVb and Vb, along with spots corresponding to 6-methylthiopurine and acetylated material. Better results could be obtained by carrying out the reaction in N,N-dimethylformamide using triethylamine as catalyst, followed by deacetylation in dilute acid. In this manner a 78% yield of combined IVb and Vb was obtained. Isolation of the products by chromatography over silica gel gave materials identical in all respects with compounds IVb and Vb as obtained from the reaction of 6-methylthiopurine (IIIb) with 2-deoxy-D-*erythro*-pentose.

The presence of the intact aldehyde function in VIIa was necessary for addition of purines to the double bond to occur. For example, the cyclic ethyl glycoside VIII, prepared from VIIa with ethyl orthoformate in ethanol,[13] did not react with 6-methylthiopurine (IIIb). This can be taken as further evidence that the actual reacting species in the condensation of purines with 2-deoxy-D-*erythro*-pentose (I) or with VII is an α,β-unsaturated aldehyde.

Absolute Configuration at C-3.—Each of the isomeric pairs IV and V derived from the three purines consists of (1) a relatively high-melting substance, fairly insoluble in water, with a small positive rotation, plus (2) a lower-melting material, quite soluble in water, with a large negative rotation. We have assigned the D-*xylo* (C_3–C_4-*trans*) configuration to the former compounds, and the D-*ribo* (C_3–C_4-*cis*) configuration to the latter. This is in accord with the generalization that *of two aldopyranose derivatives which differ only in their relative configuration at C-3, the one having a* D-*carbon at C-3 will be more levorotatory.*[15] It should also be noted that 3-amino-2,3-dideoxy-D-*erythro*-pentose, a compound closely related to structure V, exhibits a negative rotation, $[\alpha]^{25}D$ −77°, of the same order of magnitude as the members of series V.[11a] The assignment of configurations at C-3 to these compounds purely on the basis of optical rotation is not entirely rigorous, and should be considered tentative until further proof is forthcoming.

Biological Implications.—The ease with which the compounds of type IV and V are formed from adenine and 2-deoxy-D-*erythro*-pentose suggests that they might occur naturally. 2,3-Dideoxy 3-substituted sugars are unknown, however, with the exception of 2,3-dideoxy-3-amino-*erythro*-D-pentose and its derivatives, recently synthesized by Anderson, *et al.*[11a] Since the usual procedure for the isolation of nucleosides and related compounds from natural sources involves an alkaline hydrolysis, compounds of the type discussed here would not be expected to survive.[15a]

(15) For examples see A. K. Bhattacharya, R. K. Ness, and H. G. Fletcher, Jr., *J. Org. Chem.*, **28**, 428 (1963); also *cf.* A. K. Bose and B. G. Chatterjee, *ibid.*, **28**, 1425 (1958).

Experimental

Paper chromatography was carried out on Whatman No. 1 paper using (a) solvent A, isopropyl ether–ethanol–water (25:7:-saturated); or (b) solvent B, isopropyl ether–ethanol–water (25:5:saturated). These solvents were prepared by adding water slowly with stirring to the appropriate isopropyl ether–ethanol mixture until a permanent turbidity was evident. The slight excess of water was allowed to settle out before using. Isopropyl ether was purified by washing with ferrous sulfate solution and with wate , and distilling. Spots were visualized under ultraviolet light.

Condensation of Adenine with 2-Deoxy-D-*erythro*-pentose.—Pilot experiments were carried out by mixing adenine (IIIa) and 2-deoxy-D-*erythro*-pentose (I) in a 100-fold quantity of the appropriate solvent and heating at 100° for various lengths of time (see Table II). Small aliquots were spotted on Whatman No. 1 paper and chromatographed descending using solvent A for at least 36 hr. (solvent allowed to drip off the end of the paper). The products appeared as a single spot of R_{Ad} 0.36–0.38, λ_{max} 262 mμ (H_2O). Small quantities of ultraviolet-absorbing material of lower R_{Ad} were invariably obtained, but were not investigated. Approximate yields were obtained by cutting out the spots, eluting overnight in 5 ml. of water, and checking the absorbancy at 260 mμ. The results are summarized in Table II.

TABLE II

EFFECT OF SOLVENT ON THE ADENINE-2-DEOXY-D-RIBOSE CONDENSATION

Solvent	Time, hr.	Moles 2-deoxy-D-ribose per mole of adenine	Yield, %[a]
Water	25	1.0	34
Water	24	2.0	54
Water	24	3.0	71
10% pyridine (aq.)	6	1.0	16
0.1 *N* HCl	21	1.0	0
.01 *N* HCl	5	1.0	7
.01 *N* HCl	21	1.0	28
.1 *M* phthalate, pH 4.0	5	1.0	10
.1 *M* phthalate, pH 4.0	21	1.0	29
.1 *M* phosphate, pH 5.0	5	1.0	15
.1 *M* phosphate, pH 5.0	21	1.0	35
.1 *M* Tris, pH 7.0	5	1.0	14
.1 *M* Tris, pH 7.0	21	1.0	32
.1 *M* Tris, pH 8.0	5	1.0	23
.1 *M* Tris, pH 8.0	21	1.0	25
.1 *M* Tris, pH 9.0	5	1.0	20
.1 *M* Tris, pH 9.0	21	1.0	24
N,N-Dimethylformamide	20	3.0	80
Dimethyl sulfoxide	20	3.0	82

[a] All reactions were run at 100°. Approximate yields were determined spectrophotometrically assuming the extinction coefficients of the products to be the same as that of adenine. The figures represent combined yields of both anomers.

For the isolation of the products, a reaction mixture, obtained by refluxing a solution of 1.0 g. of adenine and 1.0 g. of 2-deoxy-D-*erythro*-pentose in 100 ml. of water for 24 hr., was evaporated to dryness *in vacuo* and the material of R_{Ad} 0.38 separated from unreacted adenine by chromatography over powdered cellulose. A column (6 × 40 cm.) of powdered cellulose was prepared in water, washed with 75% ethanol until the absorbancy of the eluent at 260 mμ was below 0.05, and then with 300 ml. of solvent A. The residue from the reaction mixture was taken up in 50 ml. of warm 80% methanol, filtered to remove a small quantity of adenine (80 mg.), and applied to the column. The column was eluted with solvent A, collecting 25-ml. fractions automatically and following the optical density of the fractions at 260 mμ. Pure adenine (510 mg.) was eluted in tubes 55–154 and the main batch of material of R_{Ad} 0.38 appeared in tubes 179–333 (420 mg.), λ_{max} 262 (H_2O), 260 (pH 1), 262 mμ (pH 13). This material was rechromatographed either by absorption chromatography over Florisil[3] or by partition chromatography on Hyflo Supercel.[3] For example, a column (6 × 58 cm.) of Florisil was prepared in ethyl acetate (wet-packed). A solution of 436 mg. of crude R_{Ad} 0.38 material (from another run similar to that described above) was dissolved in 75 ml. of methanol, 35 g. of

(15a) NOTE ADDED IN PROOF.—The structure of rhodosamine, an amino sugar from the antibiotic rhodomycin, has recently been shown to be 2,3-dideoxy-3-dimethylamino-L-fucose [see H. Brockmann, E. Spohler, and T. Waehneldt, *Ber.*, **96**, 2925 (1963)].

Florisil was added, and the mixture was evaporated to dryness *in vacuo*. The resulting solid was packed on top of the column, and elution was carried out using 7.5% methanol in ethyl acetate, collecting 25-ml. fractions automatically. Compound IVa appeared in tubes 50–98 (140 mg.) and compound Va in tubes 104–208 (149 mg.) with a clean separation between the two peaks. The combined fractions were evaporated to dryness *in vacuo*. **2,3-Dideoxy-3-(6-amino-9-purinyl)-D-*threo*-pentose (IVa)** was thus obtained as colorless needles of the monohydrate from water, m.p. 203–205° dec., $[\alpha]^{25}D$ +3.1° [*c* 0.715, DMF–water (3:1)]. It was necessary to dry the sample at 100° *in vacuo* for 24 hr. to obtain anhydrous material.

Anal. Calcd. for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.21; N, 27.87. Found: C, 47.78; H, 5.32; N, 27.99.

2,3-Dideoxy-3-(6-amino-9-purinyl)-D-*erythro*-pentose (Va) was recrystallized by dissolving in a small quantity of absolute ethanol and adding 2 volumes of ethyl acetate. After standing overnight in the cold, the product (Va) was obtained as granules with a slight pinkish cast. This material, although chromatographically pure, possessed no definite melting point, but seemed to decompose gradually at 120–130° to a viscous mass. After drying for 2 days at 100° *in vacuo*, the compound still retained ethanol of crystallization; $[\alpha]^{25}D$ −109° (*c* 0.45, water).

Anal. Calcd. for $C_{10}H_{13}N_5O_3 \cdot C_2H_5OH$: C, 48.48; H, 6.45; N, 23.56. Found: C, 48.47; H, 6.31; N, 23.57.

Both compounds IVa and Va gave strongly positive Dische tests.[4] In quantitative periodate oxidations,[16] IVa consumed 0.89 mole in 5 hr., 0.97 mole in 7 hr., and 0.99 mole in 24 hr.; similarly, Va consumed 0.90 mole after 24 hr. Formaldehyde was determined by the dimedone method.[16]

Alternatively, compounds IVa and Va could be isolated in a pure state by partition chromatography of the crude mixture of R_{Ad} 0.38. This method was only satisfactory for relatively small quantities of the mixture, however. Hyflo Supercel (150 g.), previously washed with 6 *N* hydrochloric acid and water, and dried at 100°, was mixed thoroughly with 75 ml. of water saturated with ethyl acetate, and then packed into a column of 3 × 44 cm. dimensions. After washing with ethyl acetate (saturated with water) until the optical density at 260 mμ was below 0.05, 100 mg. of the mixture of IVa and Va was dissolved in 5 ml. of water, mixed well with 10.5 g. of Supercel, and packed onto the top of the column. The compounds were eluted with ethyl acetate (saturated with water), collecting 20-ml. fractions. After a small quantity of adenine appeared in tubes 40–52, compound IVa was eluted in tubes 100–150, and compound Va in tubes 154–208. The compounds thus obtained were identical with those obtained by the Florisil chromatography.

Basic Hydrolysis of IVa and Va.—It was noted that the ultraviolet absorption maxima of IVa and Va in 0.1 *N* sodium hydroxide solution (λ_{max} 262 mμ) shifted to 269 mμ after standing for a few days at room temperature, suggesting hydrolysis to adenine (λ_{max} 269 mμ at pH 12). Similar alkaline solutions of 2'-deoxyadenosine, 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)-adenine,[6] and 9-(2-deoxy-β-D-*erythro*-pentopyranosyl)-adenine,[7] remained unchanged after standing 1 month.

Samples (5 mg.) of IVa and Va were heated at 100° with 2 ml. of 0.1 *N* sodium hydroxide or with 2 ml. of 0.1 *N* HCl. Paper chromatography of the reaction mixtures (solvent A) revealed complete hydrolysis to adenine by the alkali, but the compounds were unchanged by the acid. In similar experiments with the model deoxynucleosides, the compounds were unaffected by alkali, but were almost completely hydrolyzed by acid.

Several attempts to demonstrate the presence of products other than adenine in the alkaline hydrolysates of IVa and Va were unsuccessful. These hydrolysates were dark brown in color, indicating extensive degradation of the sugar portions of the molecules.

Condensation of 6-Methylthiopurine with 2-Deoxy-D-*erythro*-pentose.—One gram of 6-methylthiopurine (IIIb) and 3 g. of 2-deoxy-D-*erythro*-pentose were mixed with 100 ml. of water and refluxed for 24 hr. An aliquot of the yellow solution was analyzed by ascending paper chromatography (solvent B, 7 hr.). Visualization of the spots by ultraviolet light revealed spots of $R_{6\text{-MTP}}$ 1.0 (bright yellow fluorescence), $R_{6\text{-MTP}}$ 0.65 (light blue fluorescence), and $R_{6\text{-MTP}}$ 0.49 (light blue fluorescence).[17]

The reaction mixture was evaporated to dryness, the sirupy residue dissolved in 75 ml. of methanol, and 30 g. of silica gel carefully added. After evaporation to dryness *in vacuo*, the resulting solid was packed onto the top of a silica gel column (6 × 55 cm.), which had been prepared using solvent B. Elution was with solvent B, collecting 22-ml. fractions, and was followed at 290 mμ. Unreacted 6-methylthiopurine (IIIb) was eluted in tubes 165–250; the material of $R_{6\text{-MTP}}$ 0.65 appeared in tubes 260–350, and the material of $R_{6\text{-MTP}}$ 0.49 appeared in tubes 370–485. After recovery of the solids by evaporation *in vacuo* of the combined fractions, **2,3-dideoxy-3-(6-methylthio-9-purinyl)-D-*threo*-pentose (IVb)** was obtained as colorless tiny needles from water (sparingly soluble) (203 mg.), m.p. 210–212° dec., $[\alpha]^{23}D$ +18.4 → +25.9° (72 hr., *c* 1.58, N,N-dimethylformamide). This material gave a single spot of $R_{6\text{-MTP}}$ 0.65 (solvent B).

Anal. Calcd. for $C_{11}H_{14}N_4O_3S$: C, 46.80; H, 5.00; N, 19.85; S, 11.36. Found: C, 47.08; H, 5.25; N, 19.96; S, 11.24.

Recrystallization of the combined solids from tubes 370–485 gave 212 mg. of **2,3-dideoxy-3-(6-methylthio-9-purinyl)-D-*erythro*-pentose (Vb)** as hydrated colorless needles from water or anhydrous crystals from ethanol–ether; m.p. 154–156°, $[\alpha]^{23}D$ −141° → −128° (72 hr., *c* 1.59, N,N-dimethylformamide). Paper chromatography (solvent B) showed only a single spot of $R_{6\text{-MTP}}$ 0.49. This material was extremely difficult to obtain in a completely anhydrous state. The analytical results are on material dried at 100° *in vacuo* for 24 hr., conditions which converted the crystals to a glass ($R_{6\text{-MTP}}$ 0.49).

Anal. Calcd. for $C_{11}H_{14}N_4O_3S$: C, 46.80; H, 5.00; N, 19.85; S, 11.36. Found: C, 46.48; H, 5.09; N, 19.56; S, 10.82.

Basic Hydrolysis of IVb and Vb.—Samples (3 mg.) of IVb and Vb were hydrolyzed in 0.5 ml. of 0.1 *N* sodium hydroxide at 100° for 5 min. Paper chromatography (ascending, solvent B) showed strong spots corresponding to 6-methylthiopurine ($R_{6\text{-MTP}}$ 1.0) and, in each case, two weak spots of $R_{6\text{-MTP}}$ 0.65 and 0.49. Apparently, some anomerization of IVb and Vb occurred in basic solution along with considerable hydrolysis.

Condensation of 6-Dimethylaminopurine (IIIc) with 2-Deoxy-D-*erythro*-pentose.—A mixture of 1.5 g. of 6-dimethylaminopurine (IIIc)[18] and 4.5 g. of 2-deoxy-D-*erythro*-pentose in 125 ml. of water was refluxed for 24 hr. and then evaporated to dryness *in vacuo* to obtain a light yellow sirup. Paper chromatography (ascending, 7 hr., solvent B) showed three main ultraviolet-absorbing spots of $R_{6\text{-DMAP}}$ 1.0, 0.68, and 0.40, corresponding to IIIc, IVc, and Vc, respectively. The mixture was chromatographed over silica gel using solvent B as eluent, essentially as described for the isolation of IVb and Vb. The elution pattern was followed at 275 mμ. Recovered 6-dimethylaminopurine (366 mg.) appeared in tubes 305–435, material of $R_{6\text{-DMAP}}$ 0.68 in tubes 540–695, a mixture of the two compounds of $R_{6\text{-DMAP}}$ 0.68 and 0.40 in tubes 696–724, and pure 0.40 material in tubes 725–885. The combined contents of tubes 540–695 were evaporated to dryness *in vacuo* to leave a colorless solid (842 mg.). **2,3-Dideoxy-3-(6-dimethylamino-9-purinyl)-D-*threo*-pentose (IVc)**, was thus obtained as colorless needles (640 mg.) from water, m.p. 212–213° dec., $[\alpha]^{23}D$ −14.1° → +17.2° (48 hr., *c* 2.08, N,N-dimethylformamide). This material gave a single spot on paper chromatography ($R_{6\text{-DMAP}}$ 0.68) using solvent B.

Anal. Calcd. for $C_{12}H_{17}N_5O_3$: C, 51.60; H, 6.14; N, 25.07. Found: C, 51.43; H, 6.14; N, 25.28.

The material in tubes 696–724, which consisted of 85 mg. of a mixture of IVc and Vc, was discarded. Evaporation *in vacuo* of the solvent from the combined tubes 725–885 left a light yellow gum (1.32 g.). The gum was crystallized by dissolving in warm ethanol and chilling to obtain 920 mg. of colorless crystals, m.p. 183–184.5°. This material, **2,3-dideoxy-3-(6-dimethylamino-9-purinyl)-D-*erythro*-pentose (Vc)**, was recrystallized for analysis from ethanol containing a little water; m.p. 184.5–185.5°, $[\alpha]^{23}D$ −151 → −112° (48 hr., *c* 1.51, N,N-dimethylformamide). Paper chromatography (solvent B) showed only one spot ($R_{6\text{-DMAP}}$ 0.40).

Anal. Calcd. for $C_{12}H_{17}N_5O_3$: C, 51.60; H, 6.14; N, 25.07. Found: C, 51.77; H, 6.35; N, 24.97.

The treatment of 3 mg. of IVc or Vc with 0.5 ml. of 0.1 *N* sodium hydroxide solution resulted in complete hydrolysis to 6-dimethylaminopurine (IIIc), as evidenced by paper chromatography (solvent B).

Direct Synthesis of IVb and Vb by Michael Addition of 6-Methylthiopurine (IIIb) to VIIa.—Diacetyl-D-arabinal (VI) was prepared by the method of Karrer[19a] as modified by Deriaz, *et al.*[19b] This material (VI) was rearranged to VIIa in boiling water for 15 min. as directed by Gehrke.[13] In some cases, VIIa was distilled *in vacuo*, b.p. 112–120° at 0.1 mm., $n^{25}D$ 1.4800; however, this was not necessary for the condensation with IIIb. An alternate method of synthesis of VIIa, by a similar rearrangement of diacetyl-D-xylal,[20] was also used. Compound VIIa dis-

(16) J. R. Dyer, *Methods Biochem. Anal.*, **3**, 111 (1956).

(17) 6-Methylthiopurine on paper chromatograms exhibits a marked yellow fluorescence when viewed under ultraviolet light. In contrast 9-substituted 6-methylthiopurines exhibit a light blue fluorescence. It is of interest that 3-substituted 6-methylthiopurines fluoresce bright yellow in a similar fashion to the unsubstituted material.

(18) (a) G. B. Elion, E. Burgi, and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952); (b) B. R. Baker, J. P. Joseph, and R. E. Schaub, *J. Org. Chem.*, **19**, 631 (1954); (c) L. Goldman, J. W. Marsico, and A. L. Gazzola, *ibid.*, **21**, 599 (1956).

(19) (a) P. Karrer, B. Becker, F. Benz, P. Frei, H. Solomon, and K. Schöpp, *Helv. Chim. Acta*, **18**, 1435 (1935); (b) R. E. Deriaz, W. G. Overend, M. Stacey, E. G. Teece, and L. F. Wiggins, *J. Chem. Soc.*, 1879 (1949).

played significant infrared absorptions at 2.89 (m), 3.40 (m), 5.73 (s), 5.90 (s), 6.99 (w), 7.23 (m), and 8.05 μ (s).

One gram (6.0 mmoles) of 6-methylthiopurine (IIIb) and 3.0 g. (19.0 mmoles) of D-4-acetoxy-5-hydroxy-2-pentenaldehyde (VIIa) were dissolved in 100 ml. of N,N-dimethylformamide containing 2.0 ml. of triethylamine. The solution was heated at 60–70° for 16 hr. and finally evaporated *in vacuo* at 50° to remove the solvent. The sirupy residue was taken up in 100 ml. of 0.1 *N* hydrochloric acid and heated on the steam bath for 30 min. An examination of the brown solution at this point by ascending paper chromatography (solvent B) revealed the presence of unreacted 6-methylthiopurine ($R_{6\text{-MTP}}$ 1.0) plus strong light blue fluorescent spots at $R_{6\text{-MTP}}$ 0.65 and 0.49, at identical positions with IVb and Vb. Small quantities of light blue fluorescent material which ran ahead of IIIb were present, probably corresponding to acetylated IVb and Vb. The combined yield of IVb and Vb was 78%, estimated spectrophotometrically from eluents of the chromatograms.

A portion of the crude mixture from above was chromatographed over silica gel using solvent B as eluent, as described previously, collecting 23-ml. fractions. Tubes 130–220 contained recovered 6-methylthiopurine (IIIb); tubes 235–310 contained the material of $R_{6\text{-MTP}}$ 0.65; and tubes 335–445 contained the material of $R_{6\text{-MTP}}$ 0.49. A work-up of the combined tubes within each fraction gave compounds IVb and Vb, identical in all respects (infrared spectra, melting points, paper chromatography) with the compounds obtained by direct condensation of 2-deoxy-D-*erythro*-pentose (I) with 6-methylthiopurine (IIIb).

(20) (a) M. Gehrke and F. Obst, *Ber.*, **64**, 1724 (1931); (b) P. A. Levene and T. Mori, *J. Biol. Chem.*, **83**, 803 (1929).

The Michael addition of IIIb to the α,β-unsaturated aldehyde VIIa could also be carried out in water, although in lower yield. Thus, 0.10 g. of 6-methylthiopurine and 0.30 g. of VIIa were added to 10 ml. of water and the solution was refluxed for 16 hr. Ascending paper chromatography (solvent B) revealed the desired products (IVb and Vb) to be present, along with a considerable quantity of acetylated material. One ml. of 1 *N* hydrochloric acid was added and the mixture was allowed to stand overnight at room temperature. A second chromatography revealed the acetylated material had been largely hydrolyzed by this treatment. The combined yield of compounds IVb and Vb was approximately 54%, estimated spectrophotometrically on eluents of the chromatograms.

Attempted Condensation of 6-Methylthiopurine (IIIb) with D-5-Acetoxy-5,6-dihydro-2-ethoxy-2H-pyran (VIII).—Compound VIII was prepared by the treatment of VIIa with ethyl orthoformate in ethanolic ammonium chloride solution, as described by Gehrke.[18] 6-Methylthiopurine (IIIb, 50 mg.) and VIII (150 mg.) were dissolved in 10 ml. of dry N,N-dimethylformamide containing 0.1 ml. of triethylamine. The resulting solution was heated at 60–70° for 16 hr. Paper chromatography of the colorless solution (solvent B) revealed only unreacted IIIb as a single bright yellow fluorescent spot ($R_{6\text{-MTP}}$ 1.00).

Acknowledgment.—The author is indebted to Miss Diane Jones for her excellent technical assistance; to Dr. Jack Tadanier for many helpful discussions; to Mr. O. Kolsto and staff for the microanalyses; to Mr. W. Washburn and staff for the infrared spectra; and to Dr. R. W. Mattoon and Mr. R. Kriese for the n.m.r. spectra.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM, MASSACHUSETTS]

Hydrolysis of D(−)-Ethyl β-Phenyl-β-hydroxypropionate and D(−)-Ethyl β-Phenyl-β-acetamidopropionate by α-Chymotrypsin[1]

BY SAUL G. COHEN AND SANDRA Y. WEINSTEIN

RECEIVED SEPTEMBER 3, 1963

Hydrolysis of DL-ethyl β-phenyl-β-hydroxypropionate by α-chymotrypsin leads to high yields of L(+)-ethyl β-phenyl-β-hydroxypropionate and D(−)-β-phenyl-β-hydroxypropionic acid. Hydrolysis of DL-ethyl β-phenyl-β-acetamidopropionate by α-chymotrypsin leads also to the corresponding L(+) ester and D(−) acid. Kinetic parameters for hydrolysis of D(−)-ethyl-β-phenyl-β-hydroxypropionate are K_m = 0.0062 *M*, k_3 = 0.032 sec.$^{-1}$. Hydrolysis of the L-enantiomorph is much slower and not dependent on concentration. Factors affecting the D-specificity and rates of hydrolysis of these β-substituted esters and the L specificity and rates of hydrolysis of diethyl β-acetamido- and β-hydroxyglutarates and diethyl N-acetylaspartate are discussed. The mechanism previously proposed for preferential hydrolysis of ethyl D-α-acetoxypropionate may not be applied to the D specificity of hydrolysis of the title compounds.

Introduction

The stereospecificity of reactions of α-chymotrypsin has been described in terms of sites on the enzyme oriented complementarily to the four groups oriented tetrahedrally about the α-carbon atom of substrates which are derivatives of α-substituted carboxylic acids.[2–5a,6] While the composition of the complementary sites is uncertain, it has been proposed[4,6] that one is much restricted[5b] and requires a small substrate group, the α-hydrogen; a second may make use of hydrogen bonding, as from the α-acylamido group; a third is relatively nonpolar, associating strongly with β-aryl or other large nonhydrophilic substituent; the fourth may be the reaction site at which presumably is located a serine hydroxyl, which attacks the carbonyl carbon of the group which is undergoing hydrolysis. In these terms, substitution of the α-acetoxyl group of ethyl α-acetoxypropionate for the acetamido group of the corresponding derivative of alanine leads, in the absence of a nonhydrophilic β-substituent, to association of the acetoxyl group at the nonpolar site of the enzyme, inversion of the usual stereospecificity, and more rapid hydrolysis of the D rather than of the L enantiomorph.[4,6] A similar interpretation, also involving productive and nonproductive binding interactions, has been applied to the hydrolysis of D-1-keto-3-carbomethoxytetrahydroisoquinoline by α-chymotrypsin[3,5,7a]; nonproductive, competitive binding interactions had been envisaged previously.[7b]

In our investigation of structural requirements for stereospecificity in these reactions, we found that both the symmetric molecules diethyl α-acetamidomalonate[8] and diethyl β-acetamidoglutarate,[9] and the asymmetric molecules ethyl α-acetamidopropionate[10] and ethyl β-phenyl-β-acetamidopropionate[10] were hydrolyzed stereospecifically by α-chymotrypsin. It appeared that the presence of an α- or β-acetamido group at a center or

(1) Requirements for Stereospecificity in Hydrolysis by α-Chymotrypsin. VII. For paper VI see ref. 26. We are pleased to acknowledge generous support for this work by the Division of Research Grants, The National Institutes of Health, RG4584.

(2) F. Westheimer, *Proc. Natl. Acad. Sci. U. S.*, **43**, 969 (1957).

(3) G. E. Hein and C. Niemann, *ibid.*, **47**, 1341 (1961).

(4) S. G. Cohen, J. Crossley, E. Khedouri, and R. Zand, *J. Am. Chem. Soc.*, **84**, 4163 (1962).

(5) (a) G. E. Hein and C. Niemann, *ibid.*, **84**, 4495 (1962); (b) H. R. Almond, Jr., D. T. Manning, and C. Niemann, *Biochemistry*, **1**, 243 (1962).

(6) S. G. Cohen, J. Crossley, E. Khedouri, R. Zand, and L. H. Klee, *J. Am. Chem. Soc.*, **85**, 1685 (1963).

(7) (a) G. E. Hein, R. B. McGriff, and C. Niemann, *ibid.*, **82**, 1830 (1960); (b) R. L. Bixler and C. Niemann, *ibid.*, **80**, 2716 (1958).

(8) S. G. Cohen and L. H. Klee, *ibid.*, **82**, 6038 (1960).

(9) S. G. Cohen and E. Khedouri, *ibid.*, **83**, 1093 (1961).

(10) S. G. Cohen, Y. Sprinzak, and E. Khedouri, *ibid.*, **83**, 4225 (1961).