analysis had m.p. 168–195° and $\lambda_{max(\mu)}^{Nujol}$ 3.05 and 6.40 (NH), 3.6–3.9 (broad carboxyl OH), 5.95 (carboxyl C=O),[22] 6.05 (pyrimidine ring), 13.30 and 14.35 (monosubstituted phenyl). On paper chromatography in solvent C, the product moved as a single spot with R_{Ad} 1.50.

Anal. Calcd. for $C_{23}H_{24}N_4O_2$: C, 71.1; H, 6.23; N, 14.4. Found: C, 70.2; H, 6.29; N, 14.1.

(B) A better method of preparation of XXXV was available by the reaction of 0.50 g. (2.0 mmoles) of dichloro acid (XVI) with 2 ml. of benzylamine, the mixture heated for 17 hr. on the steam bath. Water (10 ml.) was added to the residue along with enough 10% aqueous sodium hydroxide to dissolve all the solid. The basic solution was extracted with two 10-ml. portions of ethyl ether and was neutralized with glacial acetic acid. The precipitate, 780 mg. (100%), was washed and dried and shown to be identical with the acid from the nitrile hydrolysis by identical infrared spectra[23] and paper chromatographic behavior.

When the mixture of dichloro acid (XVI) and benzylamine was refluxed for 3 hr., a solid product was obtained whose infrared spectrum suggested that it was the bis-(benzylamino) benzylamide (XXXVI). After recrystallization from ethyl alcohol the compound melted at 169–170°; $\lambda_{max(\mu)}^{KBr}$ 2.95–3.05 (NH), 6.05 (amide C=O), 6.32 (aryl and pyrimidine ring), 6.56–6.68 (aryl, pyrimidine ring and NH), 13.68 and 14.33 (monosubstituted phenyl). There was no broad carboxyl OH absorption in the 3.5 to 4.0 μ region and the intensity of the 13.7 and 14.3 μ bands was greater than in the acid (XXXV) spectrum. The material was not otherwise characterized.

2,4-Bis-(benzylamino)-N,N-diethyl-5,6,7,8-tetrahydro-6-quinazolinecarboxamide (XXXVII). A mixture of 3.0 g. (7.8 mmoles) of bis(benzylamino) acid (XXXV) and 7 ml. of thionyl chloride was heated under reflux for 45 minutes. The mixture was evaporated *in vacuo* and two 5-ml. portions of benzene were separately evaporated *in vacuo* from the residue. The final residue was dissolved in 20 ml. of methylene chloride and added dropwise to a stirred solution of 9 ml. of diethylamine in 10 ml. of methylene chloride. After the mixture had stood overnight, it was evaporated *in vacuo* and 25 ml. of water was added to the residue. The aqueous mixture was extracted with 25 ml. of methylene chloride, the organic solution was washed with 20 ml. of 0.1 *M* aqueous sodium hydroxide and two 20-ml. portions of water and was dried over magnesium sulfate. Evaporation of the methylene chloride solution left 3.1 g. of residue, which was recrystallized from 10 ml. of benzene to give 1.7 g. (50%) of product, m.p. 82–90°. A small amount of material was recrystallized from benzene-hexane (9:1) to give a solid, m.p. 82–85°; $\lambda_{max(\mu)}^{KBr}$ 3.00 and 6.52 (NH), 6.14 (amide C=O), 6.30 (aryl and pyrimidine ring), 6.89 (pyrimidine ring), 13.60 and 14.30 (monosubstituted phenyl). This material was not analytically pure.

Anal. Calcd. for $C_{27}H_{33}N_5O$: C, 73.1; H, 7.50; N, 15.8. Found: C, 74.1; H, 7.53; N, 15.3.

Attempts to cleave the benzyl groups of XXXVII by hydrogenolysis to give the diamino amide (XXV) were unsuccessful. The use of platinum oxide as catalyst gave an excessive uptake of hydrogen but infrared examination of the product showed no loss of benzyl groups. The use of 5% palladium-on-charcoal led to no uptake of hydrogen.

Acknowledgments: The authors are indebted to Peter Lim for infrared interpretations, to his group for paper chromatography, and to O. P. Crews, Jr., and group for the large-scale preparation of intermediates.

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(22) When the spectrum was run in KBr, the acid carbonyl occurred at 6.05 μ.

(23) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley, New York, N. Y., 1958.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XXVI. Synthesis of Nucleosides Derived from D-Fructose

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The reaction of chloromercuri derivatives of purines with the appropriately blocked derivatives of D-fructose has been utilized to synthesize 9-α-D-fructofuranosyladenine (II) and 9-β-D-fructopyranosyladenine (III). The stereochemistry of these ketose nucleoside condensations is discussed.

As part of an intensive program on the synthesis of C′-methyl- and C′-hydroxymethylpentofuranosyl nucleosides, the syntheses of a number of C_5′-methylpentofuranosyl nucleosides have been reported from this Laboratory.[2–6] A logical continuation of this work involves the syntheses of C_1′-methyl- and C_1′-hydroxymethyl nucleosides (I, R + H or OH). As the majority of the naturally occurring nucleosides contain the β-D-ribofuranose configuration,[7] it was most desirable to prepare the C_1′-substituted nucleosides in which the sugar

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, Contract No. SA-43-ph-1892. For the preceding paper of this series, *cf.* W. A. Skinner, M. G. M. Schelstraete, and B. R. Baker, *J. Am. Chem. Soc.*, in press.

(2) E. J. Reist, L. Goodman, R. R. Spencer, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 3962 (1958).

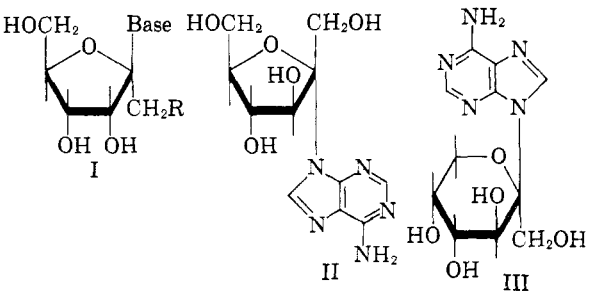
(3) E. J. Reist, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 5775 (1958).

(4) E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Org. Chem.*, **23**, 1753 (1958).

(5) E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Org. Chem.*, **23**, 1757 (1958).

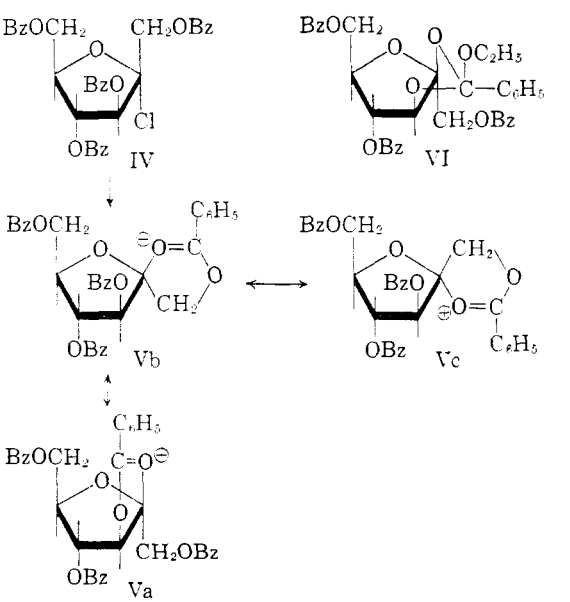
(6) E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Org. Chem.*, **23**, 1958 (1958).

(7) R. S. Tipson, "Advances in Carbohydrate Chemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1945, p. 193.



possessed the β-configuration and which maintained the stereochemistry of D-ribofuranose at the remaining carbon atoms of the sugar moiety. The requisite sugar for nucleosides of this type is D-allulose (D-psicose). Because at the inception of this work there had been no cases reported of the use of ketose sugars in nucleoside condensations[8] and because D-allulose is very difficultly available, it seemed logical to initiate model studies of a nucleoside condensation which utilized a readily available ketose sugar such as D-fructose. The syntheses and stereochemistry of 9-α-D-fructofuranosyladenine (II) and 9-β-D-fructopyranosyladenine (III) are the subjects of this paper.

Normally, the condensation between a heavy metal salt of a purine or pyrimidine and an acylated glycosyl halide will form a nucleoside with a C_1'-C_2' *trans* configuration, regardless of the original configuration at C_1-C_2.[9] This result has been attributed[9] to a neighboring group participation by the 2-acyl group *via* an ortho ester ion which then reacts with the purine moiety to give the *trans* nucleoside. As in the aldose sugars there is only one acyloxy group vicinal to the reactive center, the stereochemical directive influence is relatively simple. In the case of the ketose sugars, however, the situation is more complex, for there are now two vicinal acyloxy groups, both of which should be capable of forming an ortho-ester ion. In addition, the C_1 group can be either α or β with respect to C_2. Thus, there is the possibility of three ortho-ester ions (Va, b, and c) with which to contend. Structures Va and Vb would be expected to yield the α-nucleoside, whereas Vc should yield the β-nucleoside.



Ness and Fletcher[10] reported that the reaction of 1,3,4,6-tetra-*O*-benzoyl-α-D-fructofuranosyl bromide[11] with ethanol and zinc oxide gave a 50% yield of a material which they identified as 1,4,6-tri-*O*-benzoyl-2,3-*O*-(1-ethoxybenzylidene)-β-D-fructofuranose (VI). A structure of this type could only have arisen from reaction of the ethanol with Va. Assuming from this information that Va is the favored ortho-ester ion, it is reasonable to expect that condensation of the halo sugar (IV) with a purine should give predominantly the α-nucleoside.

When the chloro sugar (IV) was condensed with chloromercui-6-benzamidopurine in the usual fashion,[2] a yield of 17% of crude nucleoside was obtained upon regeneration of the picrate. This crude nucleoside had a rotation $[\alpha]_D$ +42.6° (1% in water) and could be easily recrystallized from absolute ethanol to give a crystalline nucleoside with $[\alpha]_D$ +46.8° (1.03% in water). The close agreement in rotation between the crude and purified nucleosides indicates that only one anomer was formed. The positive value of the rotation suggests that the nucleoside has the α-configuration. This is further borne out by comparison with the rotations of the glycosides of fructofuranose. Thus, methyl α-D-fructofuranoside tetraacetate has a rotation $[\alpha]_D$ +88.1° (chloroform),[12] whereas methyl β-D-fructofuranoside tetraacetate has a rotation $[\alpha]_D$ −26.2° (methanol).[13]

In the case of fructopyranose, presumably the same arguments should hold true. Certainly, it would appear that three different ortho-ester ions (VIIIa, b, and c) are possible. Ions VIIIa and b should favor the formation of an α-nucleoside, whereas VIIIc should favor β-nucleoside formation. Although the bromo sugar exists in the β-configuration[14] (on the basis of rotation) and the

(8) A recent communication by W. Schroeder and W. Hoeksema, *J. Am. Chem. Soc.*, **81**, 1767 (1959) reported the synthesis of 6-amino-9-D-psicofuranosylpurine, as part of the structure proof of an antibiotic which has marked antibacterial and antitumor activity *in vivo*.

(9) B. R. Baker, Ciba Foundation Symposium on "The Chemistry and Biology of Purines," J. and A. Churchill Ltd., London, 1957, pp. 120–130.

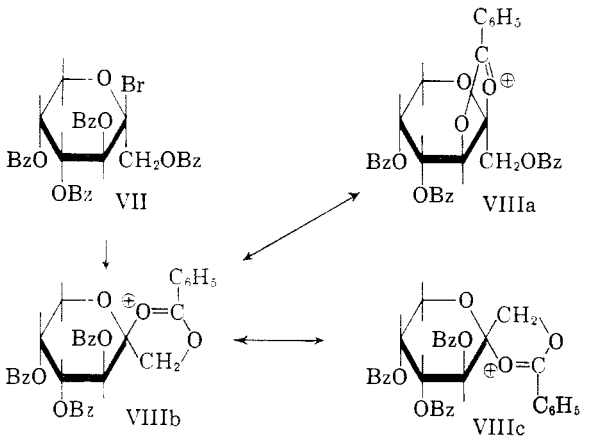
(10) R. K. Ness and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **78**, 1001 (1956).

(11) B. Helferich and L. Bottenbruch, *Ber.*, **86**, 651 (1953), assumed from the rotation of this bromo sugar that it was an α-D-halide.

(12) C. B. Purves and C. S. Hudson, *J. Am. Chem. Soc.*, **59**, 49 (1937).

(13) H. H. Schlubach and E. Bartels, *Ann.*, **541**, 76 (1939).

(14) R. K. Ness and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **75**, 2169 (1953).



ortho-ester ion VIIIc is the one that can form initially, one cannot necessarily expect that the β-nucleoside would be the sole product of the reaction, since the ortho-ester ions (VIII) can equilibrate.

Condensation of the bromo sugar (VII) with chloromercuri-6-benzamidopurine in the usual fashion[2] gave, on regeneration from the picrate, a 50% yield of crude amorphous nucleoside III which had a rotation $[\alpha]_D$ −75° (1% in methanol). Treatment of this crude nucleoside with hot absolute ethanol caused the crystallization of the β-anomer, which had a rotation of $[\alpha]_D$ −171° (1% in water). As the crude nucleoside was free of adenine, as shown by paper chromatography, it seems most likely that the very large change in rotation between the crude and recrystallized nucleoside indicates the presence of large amounts of α-nucleoside in the crude product. On the basis of the rotational values reported for various α- and β-fructopyranosides,[15] a rough estimate can be made that the crude nucleoside from the condensation is an approximately equal mixture of the α and β forms. This is further borne out by the fact that a 43% recovery of crystalline nucleoside III was obtained from crude III.

The formation of anomerically pure nucleoside in the furanose condensation from the halo sugar (IV), while pyranosyl bromide (VII) gave an anomeric nucleoside mixture, is somewhat surprising. That the pyranose condensation gave a mixture of anomers can only be interpreted on the basis of participation by the C_1 benzoate to give the ortho-ester ions VIIIb and VIIIc, the latter reacting with the purine to form the β-anomer. The complete absence of isolable amounts of the β-furanose nucleoside suggests that either the ortho-ester ion Vc did not form in appreciable amounts, or if it did, there was some factor, possibly the steric hindrance of the 3-benzoate, which prevented reaction between Vc and the purine base.

(15) C. P. Barry and J. Honeyman, "Advances in Carbohydrate Chemistry," Vol. VII, Academic Press, Inc., New York, N. Y., 1952, p 86.

On the basis of the present information, it is not possible to draw any conclusions as to the relative effects of ortho-ester ions from C_3 versus C_1 benzoate. However, further work in progress on other appropriate ketose sugars should do much to clarify the stereochemistry of these condensations and afford a possible chemical proof of the configuration of the products.

EXPERIMENTAL[16]

9-α-D-Fructofuranosyladenine (II). To a mixture of 2.0 g. (3.35 mmoles) of 1,3,4,6-tetra-*O*-benzoyl-D-fructofuranose[17] in 60 ml. of anhydrous ether which had been saturated with dry hydrogen chloride at 0° was added 2.25 ml. of acetyl chloride. The solution was stored at 0° for 2 days, by which time the tetrabenzoate had dissolved. The solution was concentrated to dryness *in vacuo* at 30° and the last traces of acetic acid were removed by the addition and removal *in vacuo* of two 5-ml. portions of dry benzene to yield the chloro sugar IV as a white foam whose infrared absorption spectrum showed the essential absence of hydroxyl absorption at 2.9 μ; $[\alpha]_D^{30}$ +8.8 ± 2.8° (0.89% in methylene chloride).

A solution of IV in 200 ml. of dry xylene was condensed with 1.82 g. of chloromercuri-6-benzamidopurine[18] in the usual manner.[2] Evaporation of the organic phase gave 2.3 g. of crude blocked II as a foam; λ_{max}^{film} 3.25 μ (NH), 5.77 μ (benzoate C=O), 7.9 μ (O=C—O), 9.0 μ and 9.75 μ (C—O—C). A solution of 2.3 g. of blocked II in 45 ml. of reagent methanol and 4.2 ml. of *N* methanolic sodium methoxide was heated at reflux for 40 minutes. The solution was neutralized with Dowex 50(H), the resin was removed by filtration, and the filtrate was concentrated to dryness *in vacuo*. The residue was dissolved in 25 ml. of water and extracted with two 15-ml. portions of ether. The aqueous phase was concentrated to dryness *in vacuo* and the residue was dissolved in 18 ml. of methanol and treated with 18 ml. of 10% methanolic picric acid. After the mixture had stood for 1 hr. at 0°, the amorphous picrate was filtered and then washed with cold methanol. The free nucleoside was regenerated by treating a suspension of the above picrate in 20 ml. of water with a total of 1.0 g. of Dowex 2(CO_3) added in small portions with stirring over 1 hr. After the picrate had all dissolved, the resin was removed by filtration and the aqueous filtrate was concentrated to dryness *in vacuo* to yield 0.17 g. (17%) of an off-white solid which showed one spot at R_{Ad} 0.43 in solvent A and R_{Ad} 1.68 in solvent B; $[\alpha]_D^{31}$ +42.6° (1% in water). Recrystallization from absolute ethanol gave white crystals, m.p. 234–235° (dec.); $[\alpha]_D^{31}$ +46.8 ± 3.1° (1.03% in water).

(16) Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with a Standard Polarimeter Model D attachment to the Beckman DU spectrophotometer calibrated with standard sucrose solutions. Paper chromatograms were run with water-saturated butyl alcohol (solvent A) and 5% aqueous sodium phosphate (solvent B) by the descending procedure on Whatman No. 1 paper. The spots were located by visual examination with an ultraviolet lamp. Adenine was used as a standard and spot locations were expressed as R_{Ad} units, with adenine at 1.00.

(17) P. Brigl and R. Schinle, *Ber.*, **67**, 127 (1934). We wish to thank Dr. H. G. Fletcher, Jr., of the National Institutes of Health for supplying seed crystals of 1,3,4,6-tetra-*O*-benzoyl-D-fructofuranose.

(18) Prepared from mercuric chloride and 6-benzamidopurine as described for chloromercuri-2,6-diacetamidopurine.[19]

(19) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 950 (1957).

Anal. Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.5; H, 5.08; N, 23.6. Found: C, 44.5; H, 5.17; N, 23.4.

9-β-D-Fructopyranosyladenine (III). A solution of 8.2 g. of 1,3,4,5-tetra-O-benzoyl-D-fructopyranosyl bromide (VII)[14] in dry xylene was treated with 8 g. of chloromercuri-6-benzamidopurine and the nucleoside was isolated through the picrate as described for 9-α-D-fructofuranosyladenine (II) to yield 1.7 g. (46%) of a pale yellow foam which showed one spot at R_{Ad} 0.20 in solvent A and R_{Ad} 1.63 in solvent B; $[\alpha]D$ $-75 \pm 3°$ (1% in methanol).

Anal. Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.5; H, 5.08; N, 23.6. Found: C, 43.8; H, 5.63; N, 21.6.

Treatment of 1.4 g. of this material with 20 ml. of hot ethanol caused crystallization to take place. Recrystallization from absolute ethanol gave 0.6 g. (16%) of material, m.p. 227–228° (dec.); $[\alpha]D$ $-171 \pm 4°$ (1% in water).

Anal. Calcd. for $C_{11}H_{15}N_5O_5$: C, 44.5; H, 5.08; N, 23.6. Found: C, 44.6; H, 5.12; N, 23.7.

Acknowledgments. The authors are indebted to Dr. Peter Lim and staff for the chromatograms and optical rotations as well as the interpretation of infrared absorption spectra, and to Mr. O. P. Crews, Jr., and staff for large-scale preparation of intermediates.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MICHIGAN STATE UNIVERSITY]

Tetrazole Analogs of Amino Acids[1]

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Received May 15, 1959

The synthesis of analogs of several amino acids in which the carboxyl group is replaced by the acidic 5-tetrazolyl group is described. Tetrazole analogs of glycine, D,L-alanine, β-alanine, D,L-phenylalanine and D,L-tryptophan have been prepared. With the exception of the tryptophan analog each was prepared by at least two independent methods. Apparent dissociation constants of the tetrazole analogs were determined and are comparable to those of the respective amino acids. The tetrazole analogs were further characterized as phenylureas and as acetyl and benzoyl derivatives.

Numerous examples of metabolite antagonism have been noted for compounds that bear various relationships to the naturally occurring α-amino acids. One of the most thoroughly investigated is phenylalanine. Various changes in its structure have transformed phenylalanine into an inhibitor of bacterial growth. Among the changes sufficient to interfere with the nutritional effect of this amino acid are introduction of an amino group[4] or a fluorine atom[5] in the *para* position of the benzene ring. Substitution of certain heterocyclic rings for the phenyl group, such as 2-pyridyl,[6] 2-thienyl,[7] 2-furyl,[8] and 2-pyrryl,[9] has also resulted in analogs which exhibit specific antagonism for phenylalanine. 5-Methyltryptophan[10] and β-3-indolylacrylic acid[11] act as antimetabolites for tryptophan. The changes necessary to develop antimetabolite activity are not restricted to any one portion of the amino acid structure. Analogs of glycine, alanine, valine, and leucine with the sulfonic acid residue replacing the carboxyl group have shown specific inhibition of the utilization of these amino acids as measured by interference with bacterial growth.[12]

In view of the acidic character of the 5-substituted tetrazoles it has been suggested that analogs of biologically active carboxylic acids in which the carboxyl group is replaced by a 5-tetrazolyl group might interfere with the normal utilization of the respective carboxylic acids.[13] Tetrazole analogs of 3-indolylacetic acid and 2,4-dichlorophenoxyacetic acid antagonize the plant growth regulatory effects of these compounds,[14,15] and there are indications that the tetrazole analog of nicotinic acid will prevent growth of certain bacteria.[16]

These observations have encouraged us to prepare analogs of several amino acids in which the 5-tetrazolyl group replaces the carboxyl group. Analogs of glycine, D,L-alanine, β-alanine, D,L-phenylalanine and D,L-tryptophan are described in the following. The synthesis of each 5-amino-

(1) Based on the doctoral thesis submitted to Michigan State University in 1958 by James M. McManus.

(2) White Laboratories Fellow, 1956–1958.

(3) Present address: Chas. Pfizer & Co., Inc., Brooklyn, N. Y.

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(11) P. Fildes, *Biochem. J.*, **32**, 1600 (1938).

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(15) R. M. Hamilton, A. Kivilaan, and J. M. McManus, *Plant Physiol.* In press.

(16) J. M. McManus and R. M. Herbst, *J. Org. Chem.* In press.