hydroxamic acid, m.p. 152–154°, was obtained. A sample, recrystallized from isopropyl alcohol, melted at 148–150°.

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.73. Found: C, 59.98; H, 5.31; N, 12.91.

DL-α-**Benzamido-β-chlorobutyrohydroxamic Acid.**—To a stirred refluxing solution of 9.6 g. (0.044 mole) of 4-carbohydroxamido-5-methyl-2-phenyl-2-oxazoline in approximately 500 ml. of dry dioxane (distilled from sodium three times) was added dropwise over a period of 1 hr. 120 ml. of dioxane containing 1.8 g. (0.05 mole) of hydrogen chloride. During the addition of the hydrogen chloride solution, the reaction mixture became pink and a small quantity of a flocculent precipitate appeared. The solution was cooled, treated with Darco G-60 and filtered. The residue obtained from lyophilization of the filtrate was extracted with one 400-ml. and three 50-ml. portions of boiling ethyl acetate, and the combined extracts were diluted with an equal volume of chloroform. This solution was cooled and 6.6 g. (59%) of α-benzamido-β-chlorobutyrohydroxamic acid, m.p. 139–143°, was obtained. A sample, recrystallized from chloroform, melted at 141–142.5°. The ethyl acetate-insoluble portion of the residue was apparently unrearranged oxazoline hydrochloride and was not further investigated.

Anal. Calcd. for $C_{11}H_{13}N_2O_3Cl$: C, 51.47; H, 5.10; N, 10.89; Cl, 13.81. Found: C, 51.72; H, 5.40; N, 10.94; Cl, 13.72.

DL-**4-Benzamido-5-methyl-3-isoxazolidone.**—To a stirred slurry of 6.4 g. (0.025 mole) of α-benzamido-β-chlorobutyrohydroxamic acid in 100 ml. of water at 65–75°, 46 ml. (1.83 equivalents) of 1 *N* sodium hydroxide was added over a period of 1 hr. A permanent phenolphthalein end-point was obtained. The solution was cooled in an ice-bath and 21.4 ml. of 1.16 *N* (0.025 mole) hydrochloric acid was added. The acidic solution was extracted with three 250-ml. portions of chloroform, and the combined dried extracts were concentrated to approximately 225 ml. The DL-4-benzamido-5-methyl-3-isoxazolidone, 3.3 g., m.p. 188–194°, was filtered. The filtrate was concentrated and yielded another 1.0 g., m.p. 187–192°, bringing the total yield to 4.3 g. (79%). A small sample, recrystallized from chloroform, melted at 187–190°.

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 59.62; H, 5.14; N, 12.56.

Methyl DL-α-**Amino-β-aminoxybutyrate Dihydrochloride.**—A rapid stream of hydrogen chloride was passed into 250 ml. of dry methanol for approximately 40 minutes. To this solution was added 5 g. (0.02 mole) of 4-benzamido-5-methyl-3-isoxazolidone, and the solution was refluxed for 2 hr. The solvent was removed *in vacuo*. The crystalline residue was extracted with 150 ml. of boiling isopropyl alcohol leaving 2.1 g. (42%) of crystalline methyl α-amino-β-aminoxybutyrate dihydrochloride, m.p. 139–145°. A small sample, triturated with boiling isopropyl alcohol, melted at 136–138°.

Anal. Calcd. for $C_5H_{14}N_2O_3Cl_2$: C, 27.16; H, 6.38; N, 12.67; Cl, 32.07. Found: C, 27.44; H, 6.46; N, 12.46; Cl, 31.27.

In some runs only amorphous ester dihydrochloride was obtained. The amorphous product was used successfully in the next step.

DL-**4-Amino-5-methyl-3-isoxazolidone (XII).**—To a cold solution of 2.0 g. (9.1 mmoles) of methyl α-amino-β-aminoxybutyrate dihydrochloride in 2 ml. of water was added dropwise a cold solution of 1.8 g. (>3 equivalents) of potassium hydroxide in 2 ml. of water. During the addition of the alkali, the reaction mixture became pink and then colorless as the solution reached *p*H 11. The precipitate, after removal of the supernatant solution, was washed twice with 0.5 ml. of water. The washings and supernatant solution were combined and then diluted with 40 ml. of 1:1 ethanol–isopropyl alcohol. The resulting precipitate was filtered and was washed with a small volume of the 1:1 alcohol solution. The filtrate was cooled to 0–5° and was acidified to *p*H 6 by the dropwise addition of glacial acetic acid. The crystalline precipitate, 923 mg. (88%), was recrystallized in the same manner as DL-4-amino-3-isoxazolidone giving 761 mg. of DL-4-amino-5-methyl-3-isoxazolidone, m.p. 170–173°.

Anal. Calcd. for $C_4H_8N_2O_2$: C, 41.36; H, 6.94; N, 24.13. Found: C, 41.31; H, 6.68; N, 23.88.

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES DIVISION OF MERCK & CO., INC.]

Some Reactions of *erythro-* and *threo-β-p-*Nitrophenylserine

BY ARTHUR F. WAGNER

RECEIVED DECEMBER 29, 1956

Ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I) was converted to the corresponding 2-oxazoline, 4-carbethoxy-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (II), by an SNi displacement using *p*-toluenesulfonyl chloride in pyridine solution. The 4-carbethoxy-2-oxazoline II was converted to the 4-carbohydroxamido-2-oxazoline IV which was opened to the *erythro*-β-chlorohydroxamic acid V. Among the products resulting from the alkaline cyclization of the *erythro*-β-chlorohydroxamic acid V was the 4-carbohydroxamido-2-oxazoline IV. Ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I) reacted with thionyl chloride to yield the corresponding β-chloro compound III with retention of configuration. The corresponding *threo* isomer VI in the same reaction underwent nitrogen-to-oxygen acyl migration and inversion at the β-carbon to yield ethyl *erythro*-α-amino-β-benzoyloxy-β-*p*-nitrophenylpropionate hydrochloride (VII).

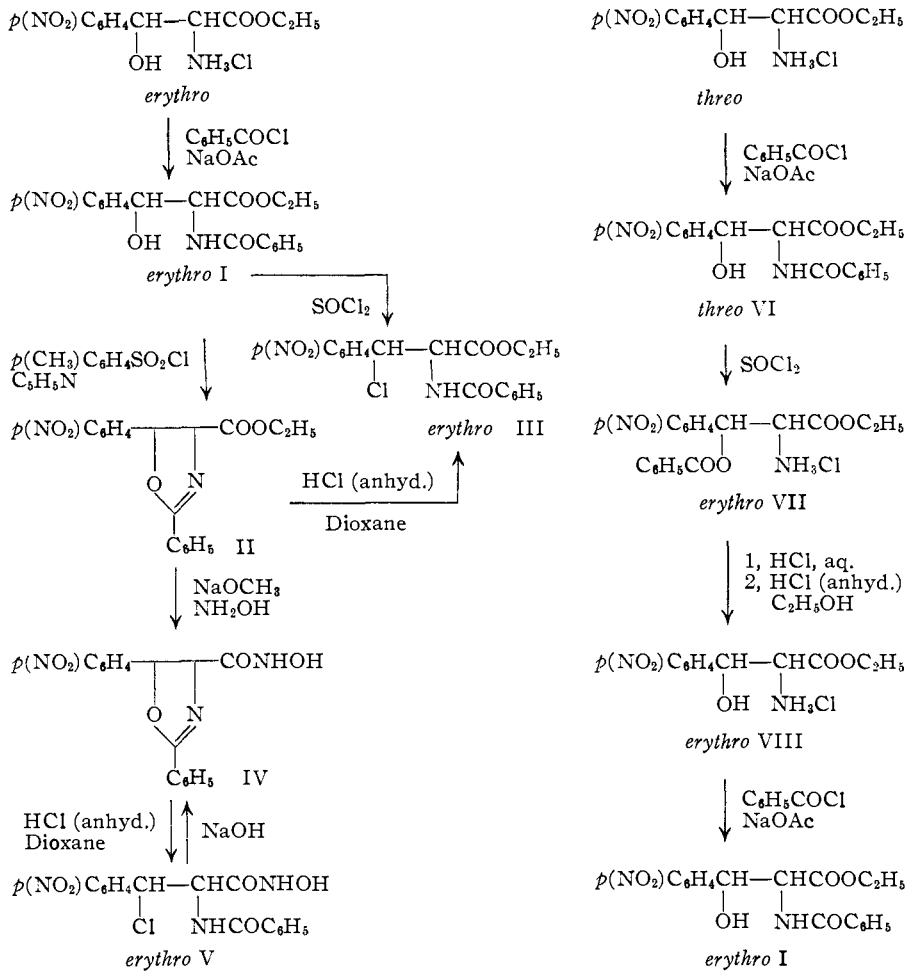
The characterization and synthesis of chloramphenicol[1] did much to stimulate interest in the stereochemistry and reactivity of the β-*p*-nitrophenylserines. More recently publications from these laboratories[2] described the synthesis of the antibiotic cycloserine[3] using serine as the precursor for the 3-isoxazolidone nucleus. This synthesis used a 4-carbethoxy-2-oxazoline intermediate to protect the α- and β-positions of the original serine molecule while the carbethoxy group was converted to a carbohydroxamido group. In contrast to the alternative of converting a β-chloroester to a β-chlorohydroxamic acid directly, the alkaline stability of the 2-oxazoline ring effectively protected the α- and β-positions of serine from undergoing any reaction during the conversion of the ester to a hydroxamic acid. The carbohydroxamido-2-oxazoline was opened to a β-chlorohydroxamic acid which was then cyclized to a 3-isoxazolidone.

This paper describes the behavior of *erythro-* and *threo-β-p-*nitrophenylserine in a similar sequence of reactions designed to synthesize 2-oxazolines and

(1) M. C. Rebstock, H. M. Crooks, Jr., J. Controulis and Q. R. Bartz, THIS JOURNAL, **71**, 2458 (1949).

(2) C. H. Stammer, A. N. Wilson, F. W. Holly and K. Folkers, *ibid.*, **77**, 2346 (1955); C. H. Stammer, A. N. Wilson, C. F. Spencer, F. W. Holly and K. Folkers, *ibid.*, **79**, 3236 (1957).

(3) D-4-Amino-3-isoxazolidone. Our generic name for this antibiotic has been changed from oxamycin to cycloserine. Oxamycin is now the registered trade-mark of Merck & Co., Inc., for this antibiotic.



3-isoxazolidones with a *p*-nitrophenyl substituent. Striking differences were observed in the behavior of *erythro-* and *threo-β-p*-nitrophenylserine derivatives at two steps of the reaction sequence. For the preparation of 2-oxazolines it was noted that "*cis* effects" of the substituents dictate the choice of reaction for the cyclization of either isomer. In reactions with thionyl chloride, ethyl *erythro-α*-benzamido-*β*-hydroxy-*β-p*-nitrophenylpropionate (I) gave a different type of product from that isolated from the corresponding *threo* isomer II in the same reaction. A difference in reaction mechanism is clearly indicated here. All of these observations are presented below and some of the reactions are discussed in the light of recently confirmed configurations of the two diastereomers.[4]

As noted earlier, the key intermediate of the 3-isoxazolidone synthesis is a 2-oxazoline. Two courses were open for the synthesis of 4-carbethoxy-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (II). The first involves reaction of ethyl α-amino-*β*-hydroxy-*β-p*-nitrophenylpropionate hydrochloride with ethyl iminobenzoate[5,6] and the second consists of an SNi displacement[7] at the *β*-carbon in ethyl *α*-benzamido-*β*-hydroxy-*β-p*-nitrophenylpropionate. In the former case the participating groups should be *cis* oriented, while in the latter case they should be in a *trans* orientation. In the reaction of ethyl *erythro-α*-amino-*β*-hydroxy-*β-p*-nitrophenylpropionate hydrochloride with ethyl iminobenzoate at room temperature, no 2-oxazoline could be isolated.[8] When ethyl *erythro-α*-amino-*β*-hydroxy-*β-p*-nitrophenylpropionate hydrochloride was first benzoylated on nitrogen and the resulting amide I then allowed to react with *p*-toluenesulfonyl chloride in pyridine,[7] the 2-oxazoline II was isolated in yields up to 70%.

Considering the *cis* orientation requirements for cyclization with ethyl iminobenzoate and the *trans* orientation necessary for SNi cyclization, it can be seen that a "*cis* effect"[9] will play an important role in the transition state of each of these diastereomers

(4) For a comprehensive review of the data describing the synthesis and stereochemistry of the two diastereoisomers see D. O. Holland, P. A. Jenkins and J. H. C. Naylor, *J. Chem. Soc.*, 273 (1953).

(5) D. F. Elliott, *ibid.*, 589 (1949).

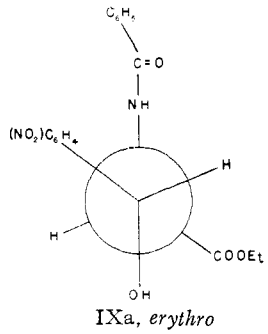
(6) W. S. Johnson and E. N. Schubert, THIS JOURNAL, **72**, 2187 (1950).

(7) R. N. Boyd and R. H. Hansen, *ibid.*, **75**, 5896 (1953).

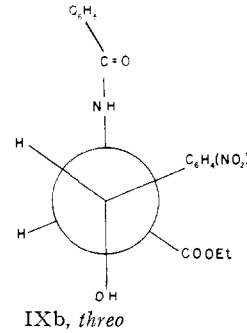
(8) A similar observation has been reported by E. D. Bergmann, H. Bendas and W. Taub, *J. Chem. Soc.*, 2673 (1951). These workers report no 2-oxazoline isolated from the reaction between *erythro-β-p*-nitrophenylserine ethyl ester and ethyl iminobenzoate in refluxing dichloroethane, tetrachloroethane or dibromoethane. With the corresponding *threo* isomer, however, I. Elphimoff-Felkin, H. Felkin and Z. Welvart, *Compt. rend.*, **234**, 1789 (1952), report a facile conversion to the 2-oxazoline with ethyl iminobenzoate. With the *β*-phenylserine ethyl esters, however, both the *erythro* and *threo* isomers yield 2-oxazolines on treatment with ethyl iminobenzoate either in the presence or absence of solvent. See M. Viscontini and E. Fuchs, *Helv. Chim. Acta*, **36**, 1 (1953), and I. Elphimoff-Felkin, H. Felkin, B. Tchoubar and Z. Welvart, *Bull. soc. chim. France*, 252 (1952).

(9) D. Y. Curtin, *Record Chem. Progr.* (Kresge–Hooker Sci. Lib.), **15**, 111 (1954).

prior to cyclization. With the *erythro* isomer the necessary conditions at the transition stage for SNi displacement place the bulky *p*-nitrophenyl group between the hydrogen atom and the benzamido group (IXa). In the *threo* series the same conditions put the *p*-nitrophenyl group between the carbethoxy and benzamido groups in the transition state (IXb). Since the relative positions of the bulky groups are more favorable in case IXa, the *erythro* isomer should give the 2-oxazoline more readily by SNi displacement.

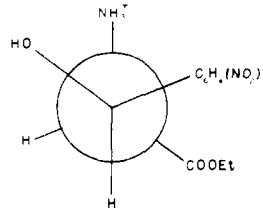


IXa, *erythro*

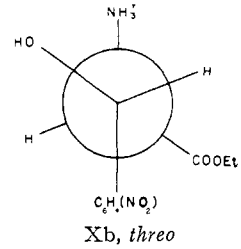


IXb, *threo*

The conversion of ethyl α-amino-β-hydroxy-β-*p*-nitrophenylpropionate hydrochloride to the corresponding 2-oxazoline with ethyl iminobenzoate should proceed more readily with the *threo* isomer. This cyclization requires a *cis* orientation of hydroxyl and amino groups in the transition state. Such a *cis* arrangement is spatially more compatible with the *threo* isomer Xb when the influence of the "*cis* effect" of the bulky groups is considered. The preferred orientations of the groups for this *cis* transition state are illustrated below.[10]



Xa, *erythro*



Xb, *threo*

The 2-oxazoline II was converted in 60% yield to 4-carbohydroxamido-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (IV) with sodium methoxide and hydroxylamine.[11] The 2-oxazoline hydroxamic acid IV on treatment with anhydrous hydrogen chloride in dioxane solution yielded the β-chloro acid, *erythro*-α-benzamido-β-chloro-β-*p*-nitrophenylpropionohydroxamic acid (V).[12] When the hydroxamic acid V was cyclized with alkali at about 70°, the 2-oxazoline IV was the only crystalline product isolated (30% yield). As reported,[2] the course of the alkaline cyclization of α-benzamido-β-chloropropionohydroxamic acid was dependent upon the temperature. A lower temperature favored the 2-oxazoline while a higher temperature favored the 3-isoxazolidone. When the alkaline cyclization of the *erythro*-β-*p*-nitrophenyl analog V was carried out at 100°, the yield of 2-oxazoline IV dropped to 20%.

Consideration of molecular models of both *erythro*- and *threo*-α-benzamido-β-chloro-β-*p*-nitrophenylpropionohydroxamic acid (V) shows that a *threo* isomer should offer the better opportunity for cyclization to a 3-isoxazolidone. Accordingly steps were undertaken to synthesize this isomer. The key compound in this sequence was ethyl *threo*-α-benzamido-β-chloro-β-*p*-nitrophenylpropionate. Ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I) was used to study the course of displacement of the hydroxyl with halogen, and the following observation was made. When I (*erythro*) was allowed to react with thionyl chloride in the absence of solvent, the corresponding β-chloro compound III was obtained in *ca.* 60% yield. The *erythro* configuration of III was established by identity with an authentic specimen of ethyl *erythro*-α-benzamido-β-chloro-β-*p*-nitrophenylpropionate prepared from I (*erythro*) by first converting to the 2-oxazoline II using *p*-toluenesulfonyl chloride in pyridine and then opening the 2-oxazoline ring with anhydrous hydrogen chloride in dioxane. An inversion at the β-carbon in each step[7,12] of the latter synthesis leads to over-all retention of configuration. This result is in accord with the conclusion reached earlier[13] that the displacement of the hydroxyl of methyl *threo*-α-acetamido-β-hydroxy-β-phenylpropionate with halogen using thionyl chloride proceeds with retention of configuration.

When ethyl *threo*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (VI) was allowed to react with thionyl chloride in the absence of solvent, the only product which could be isolated was ethyl *erythro*-α-amino-β-benzoyloxy-β-*p*-nitrophenylpropionate hydrochloride (VII) (*ca.* 50% yield). The identity of the rearranged and inverted product was confirmed by the conversion of VII to ethyl *erythro*-α-amino-β-hydroxy-β-*p*-nitrophenylpropionate hydrochloride (VIII) by acid hydrolysis followed by esterification. The configuration was further confirmed by the conversion of VIII to ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I) by benzoylation in the presence of sodium acetate.

The displacement of hydroxyl with halogen by thionyl chloride in the *erythro*-isomer with retention of configuration can occur in either of two ways. In one instance an SNi displacement by the halogen of the intermediate ester halide[14] would yield the corresponding halide with retention of configuration. On the other hand, the same intermediate ester halide can cyclize to the corresponding oxazoline which in turn can be converted to the chlorobenz-

(10) The stereochemical notations follow the suggestion of M. S. Newman, *Record Chem. Progr.*, **13**, 111 (1952).

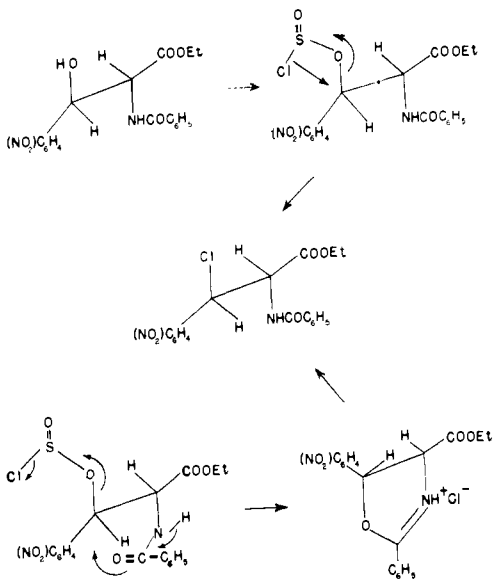
(11) The racemization of optically active esters by bases is well known (see C. L. Bickel, THIS JOURNAL, **60**, 927 (1938)). Under these conditions, therefore, one would expect racemization at the α-carbon to give a mixture of *cis*- and *trans*-oxazolines. It has been found, however (D. F. Elliott, *J. Chem. Soc.*, 62 (1950)), that *cis*-oxazolines mutarotate at the α-carbon in alkali to give *trans*-oxazolines. The rate of mutarotation is even faster in alcoholic sodium ethoxide solution. Thus the *trans*-oxazoline ester II on treatment with sodium methoxide and hydroxylamine will yield the *trans*-oxazoline hydroxamic acid IV.

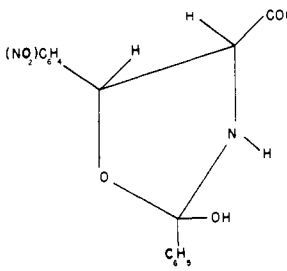
(12) For a discussion of the rearrangements of oxazoline hydrochlorides see E. M. Fry, *J. Org. Chem.*, **14**, 887 (1949); **15**, 438, 802 (1950).

(13) D. O. Holland, P. A. Jenkins and J. H. C. Naylor, *J. Chem. Soc.*, 273 (1953).

(14) E. R. Alexander, "Ionic Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 94.

amido compound with over-all retention of configuration.[15] With the *threo* isomer in thionyl chloride, however, there is both benzoyl migration and inversion at the β-carbon rather than displacement of hydroxyl with halogen.[16] Since 1,2-*trans*-cyclization of the *threo* isomer to the 2-oxazoline II is unfavorable because of the role of "*cis*-effects," a mechanism involving cleavage of a 2-oxazoline to a rearranged and inverted product would not apply. Nitrogen-to-oxygen acyl migrations proceed by way of a cyclic transition state.[17] In this instance a 1,2-*cis*-cyclization[18] would lead to the oxazolidine XI; therefore, the acyl migration would not necessarily in itself be attended by inversion.[17] A suggested mechanism then in the case of the *threo* isomer might first involve migration of the benzoyl group from nitrogen to oxygen through 1,2-*cis*-cyclization and subsequent epimerization at the β-carbon to give the thermodynamically more stable[19] *erythro* configuration.





XI

Acknowledgment.—The author is indebted to Mr. R. N. Boos and associates for elemental analyses and to Dr. N. R. Trenner and Mr. R. W. Walker for infrared spectral data.

Experimental

Ethyl *erythro*-α-Benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I).—Five grams (0.017 mole) of *erythro*-β-*p*-nitrophenylserine ethyl ester hydrochloride[20] was suspended in a mixture of 50 ml. of water and 50 ml. of chloroform. Then 3.4 g. (0.043 mole) of sodium acetate and 2.4 g. (0.018 mole) of benzoyl chloride were added. The mixture was shaken for 2 hr. and then acidified by the addition of 1 ml. of concentrated hydrochloric acid. The product was isolated by filtration. The chloroform phase was separated, dried over magnesium sulfate and concentrated *in vacuo*. Recrystallization of the combined solid products from ethanol yielded 3.6 g. (60%) of ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate, m.p. 158.5–160.5°; $\lambda_{max}^{Nujol\ mull}$ 2.99, 5.75, 6.07, 6.2–6.28, 6.6 and 7.4 μ.

Anal. Calcd. for $C_{18}H_{18}N_2O_6$ (358.34): C, 60.33; H, 5.06; N, 7.82. Found: C, 60.31; H, 5.17; N, 7.65.

4-Carbethoxy-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (II).—Ten grams (0.028 mole) of ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I) was dissolved in 40 ml. of anhydrous pyridine. The solution was cooled and 5.3 g. (0.028 mole) of *p*-toluenesulfonyl chloride was added. After standing overnight at room temperature, the solution was poured into 100 ml. of 10% aqueous sodium bicarbonate, and the product was isolated by chloroform extraction. The chloroform extract was washed with water, dried over anhydrous magnesium sulfate and concentrated *in vacuo*. Removal of the pyridine by distillation *in vacuo* at 75–80° yielded a 9.1-g. residue.

The residue was triturated with 100 ml. of dry ether and the mixture was filtered; 2.5 g. of starting material, m.p. 158–160°, was isolated. Concentration of the ether solution *in vacuo* yielded 6 g. of the 2-oxazoline. Recrystallization of the product from 20 ml. of ethanol gave 4.2 g. (45%) of 4-carbethoxy-5-*p*-nitrophenyl-2-phenyl-2-oxazoline, m.p. 88–91°, λ_{max}^{EtOH} 255 mμ (log ε 4.240); $\lambda_{max}^{CCl_4}$ 5.77, 6.04, 6.2, 6.28, 6.5, 6.6 and 7.4 μ.

Anal. Calcd. for $C_{18}H_{16}N_2O_5$ (340.33): C, 63.51; H, 4.74; N, 8.23. Found: C, 63.29; H, 4.39; N, 8.21.

Ethyl *erythro*-α-Benzamido-β-chloro-β-*p*-nitrophenylpropionate (III). (a) From 4-Carbethoxy-5-*p*-nitrophenyl-2-phenyl-2-oxazoline.—One and nine-tenths milliliters of an anhydrous dioxane solution of hydrogen chloride (0.776 *N*) was added to 500 mg. (1.47×10^{-3} mole) of 4-carbethoxy-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (II) dissolved in 10 ml. of anhydrous dioxane. The solution was heated on the steam-bath and concentrated *in vacuo*. Final traces of dioxane were removed by dissolving the residue in ether and concentrating *in vacuo* several times. Crystallization of the product from ethanol–ether–petroleum ether yielded 280 mg. of material melting at 120–122°. The product was taken up in chloroform and the solution was washed with water, dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo*. Recrystallization of the product from ethanol–ether–petroleum ether gave 140 mg. of ethyl *erythro*-α-benzamido-β-chloro-β-*p*-nitrophenylpropionate, m.p. 121–124°.

(15) The experimental conditions do not allow a choice between these two mechanisms.

(16) The difference in the course of reaction of the two diastereomers is not unusual. Thus, J. Weijlard, K. Pfister, E. F. Swanezy, C. A. Robinson and M. Tishler, THIS JOURNAL, **73**, 1216 (1951), report that N-formyl-*erythro*-2-amino-1,2-diphenylethanol is 1,2-*trans*-cyclized with inversion to the oxazoline salt by thionyl chloride under conditions which do not affect the N-formyl derivative of the *threo* isomer. On the other hand, G. Fodor, V. Bruckner, J. Kiss and G. Ohegyi, *J. Org. Chem.*, **14**, 337 (1949), showed that N-acetyl-*threo*-2-amino-1,2-diphenylethanol undergoes nitrogen-to-oxygen acyl migration through 1,2-*cis*-cyclization to the oxazolidine with alcoholic hydrogen chloride, while similar conditions leave the *erythro*-isomer unchanged.

(17) L. H. Welsh, THIS JOURNAL, **71**, 3500 (1949).

(18) The rate of 1,2-*cis*-cyclization will be greater for the *threo*-isomer since the smaller rather than the large groups are eclipsed in the intermediate or planar transition state (see D. H. R. Barton and R. C. Cookson, *Quart. Revs.* (*London*), **10**, 51 (1956)). Examples of this effect are found in the slower rates of acyl migration observed in *erythro*-1,2-amino-alcohols as compared to the rates with corresponding *threo* isomers (see L. H. Welsh, ref. 17, and G. Fodor, *et al.*, ref. 16).

(19) According to D. H. R. Barton and R. C. Cookson, *Quart. Revs.* (*London*), **10**, 49 (1956), the *erythro* isomer is the more stable isomer of diastereoisomeric pairs of non-polar compounds in which differences of free energy are mainly due to differences in compression energy. This order of stability has been established experimentally by direct equilibration or by the introduction of a second asymmetric center in reactions known to result (by mechanism) in mixtures approximating to the equilibrium mixture.

(20) E. D. Bergmann, H. Bendas and W. Taub, *J. Chem. Soc.*, 2676 (1951).

Anal. Calcd. for $C_{18}H_{17}ClN_2O_5$ (376.80): C, 57.36; H, 4.55; Cl, 9.41; N, 7.44. Found: C, 57.14; H, 4.37; Cl, 9.96; N, 7.58.

(b) **From Ethyl *erythro*-α-Benzamido-β-hydroxy-β-*p*-nitrophenylpropionate.**—One gram (2.8×10^{-3} mole) of ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I) was dissolved in 3 ml. of thionyl chloride. The mixture was cooled until solution was complete. After standing at room temperature for 3 hr., the solution was concentrated *in vacuo*. Ether was added to the residue and final traces of thionyl chloride were removed by concentration *in vacuo*. The residue was triturated with ether and the product was isolated by filtration; 650 mg. of product, m.p. 122–124°, was obtained.

Recrystallization of a portion of the product (250 mg.) from ethanol–ether–petroleum ether yielded 150 mg. of ethyl *erythro*-α-benzamido-β-chloro-β-*p*-nitrophenylpropionate, m.p. 122–124°. Admixture with an authentic specimen of this compound (IIIa) caused no depression in the melting point of the specimen.

The infrared spectrum of the compound in chloroform solution showed functionality consistent with the proposed structure; $\lambda_{max}^{CHCl_3}$ 2.93, 5.76, 6.01, 6.2 and 6.28, 6.5–6.6 and 7.4 μ.

4-Carbohydroxamido-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (IV). (a) From 4-Carbethoxy-5-*p*-nitrophenyl-2-phenyl-2-oxazoline.—One gram of hydroxylamine hydrochloride was dissolved in 25 ml. of warm ethanol. When the solution had cooled to room temperature, 13.8 ml. of methanolic 1.16 *N* sodium methoxide was added. The mixture was cooled and filtered. The filtrate was added to a solution of 4.06 g. (0.012 mole) of 4-carbethoxy-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (II) in 50 ml. of ethanol. The reaction mixture was cooled to *ca.* 0°, and 10 ml. of methanolic 1.16 *N* sodium methoxide was added slowly. The solution turned red; in the course of ten minutes the color of the solution faded to pale yellow.

After standing at room temperature for 1 hr., the solution was concentrated *in vacuo* to a volume of *ca.* 20 ml. The mixture was diluted with 50 ml. of water and acidified with 14.2 ml. of 0.99 *N* hydrochloric acid. The product was isolated by filtration and dried *in vacuo* over phosphoric anhydride. Recrystallization of the product from dioxane–ether yielded 2.25 g. (60%) of 4-carbohydroxamido-5-*p*-nitrophenyl-2-phenyl-2-oxazoline, m.p. 201–203° dec., λ_{max}^{EtOH} 255 mμ (log ε 4.255); $\lambda_{max}^{Nujol\ mull}$ 3.1, 5.95, 6.05, 6.2, 6.28 and 6.54 μ.

Anal. Calcd. for $C_{16}H_{13}N_3O_5$ (327.28): C, 58.73; H, 3.88; N, 12.84. Found: C, 58.74; H, 3.88; N, 12.80.

(b) **From *erythro*-α-Benzamido-β-chloro-β-*p*-nitrophenylpropionohydroxamic Acid.**—A suspension of 500 mg. (1.4×10^{-3} mole) of *erythro*-α-benzamido-β-chloro-β-*p*-nitrophenylpropionohydroxamic acid (V) in 5 ml. of ethanol was gently refluxed and titrated with 0.99 *N* sodium hydroxide until the phenophthalein end-point was permanent for several minutes (2×10^{-3} equivalent of alkali was consumed). The solution was cooled in an ice-bath and 10 ml. of water was added. The mixture was acidified with 1 ml. of 0.99 *N* hydrochloric acid and stirred. The product was isolated by filtration and dried *in vacuo;* 320 mg. of material melting at 175–178° dec. was obtained.

The product was boiled with 25 ml. of ethanol and the mixture was filtered. The residue (90 mg.) melted at 194° dec. The ethanolic filtrate was cooled and filtered; an additional 30 mg. of product, m.p. 194° dec., was obtained. The two crops of crystalline product (120 mg.) were combined and characterized as 4-carbohydroxamido-5-*p*-nitrophenyl-2-phenyl-2-oxazoline, m.p. 194° dec.; λ_{max}^{EtOH} 250 mμ (log ε 4.297).

The infrared spectrum of the product was identical to that of an authentic specimen of 4-carbohydroxamido-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (IVa). Admixture of the product with the same authentic specimen (IVa) gave no depression in the melting point.

***erythro*-α-Benzamido-β-chloro-β-*p*-nitrophenylpropionohydroxamic Acid (V).**—Two milliliters of an anhydrous dioxane solution of hydrogen chloride (1.09 *N*) was added to a suspension of 640 mg. (0.002 mole) of 4-carbohydroxamido-5-*p*-nitrophenyl-2-phenyl-2-oxazoline (IVa) in 6 ml. of anhydrous dioxane. The mixture was warmed on the steam-bath for five minutes. The solution was cooled and the product which precipitated was collected by filtration; 690 mg. of hydroxamic acid, m.p. 179–182° dec., was obtained. Recrystallization of the product from 40 ml. of hot isopropyl alcohol yielded 200 mg. (28%) of *erythro*-α-benzamido-β-chloro-β-*p*-nitrophenylpropionohydroxamic acid, m.p. 182–183° dec.; λ_{max}^{EtOH} 235 mμ (log ε 4.129) and 260 mμ (log ε 4.099); λ_{max}^{solid} 3.12, 6.09, 6.2, 6.9, 6.52 and 6.67 μ.

Anal. Calcd. for $C_{16}H_{14}ClN_3O_5$ (363.75): C, 52.84; H, 3.88; Cl, 9.75; N, 11.55. Found: C, 52.84; H, 3.47; Cl, 9.5; N, 11.55.

Ethyl *threo*-α-Benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (VI).—A suspension of 49 g. of ethyl *threo*-α-acetamido-β-acetoxy-β-*p*-nitrophenylpropionate[21] in 500 ml. of 2.5 *N* hydrochloric acid was warmed on the steam-bath for 2.5 hr. The solution was concentrated *in vacuo* and the residue was dried *in vacuo*. The product was dissolved in 250 ml. of ethanol, and the solution was saturated with anhydrous hydrogen chloride. After standing overnight at room temperature, the solution was concentrated *in vacuo*. Forty grams of crude *threo*-β-*p*-nitrophenylserine ethyl ester hydrochloride was obtained.

The product was dissolved in 300 ml. of water, and 28 g. of sodium acetate, 19.2 g. of benzoyl chloride and 300 ml. of chloroform were added. The mixture was shaken for 2 hr. at room temperature. The chloroform phase was separated and the aqueous phase was extracted with 200 ml. of chloroform. The combined chloroform solutions were washed with 350 ml. of 0.3 *N* hydrochloric acid, then with water and finally dried over anhydrous magnesium sulfate. The chloroform solution was filtered and concentrated *in vacuo*. Crystallization of the residue (44 g.) from 100 ml. of ethanol yielded 16 g. of product melting at 126–134°. Recrystallization of this fraction from ethanol yielded 11 g. of ethyl *threo*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate, m.p. 136–138°. The infrared spectrum in chloroform solution confirms the presence of an N-benzoyl function; $\lambda_{max}^{CHCl_3}$ 2.98, 5.78, 6.0, 6.2, 6.3, 6.55 and 7.4 μ.

Anal. Calcd. for $C_{18}H_{18}N_2O_6$ (358.34): C, 60.33; H, 5.06; N, 7.82. Found: C, 60.59; H, 5.13; N, 7.77.

Reaction of Ethyl *threo*-α-Benzamido-β-hydroxy-β-*p*-nitrophenylpropionate with Thionyl Chloride. (a) Ethyl *erythro*-α-Amino-β-benzoyloxy-β-*p*-nitrophenylpropionate Hydrochloride (VII).—A mixture of 4 g. of ethyl *threo*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate and 4 g. of anhydrous calcium carbonate[22] was added to 25 ml. of thionyl chloride. After standing at room temperature for three days, the reaction mixture was filtered and concentrated *in vacuo*. Trituration of the residue with ether and filtration yielded 3.45 g. of product. Recrystallization of the product from ethanol–petroleum ether (1:1) gave 1.9 g. of product melting at 180° dec. The infrared spectrum of the product in the solid state was consistent with a structure containing two ester functions and an amine hydrochloride function.

Recrystallization of the product (660 mg.) from ethanol yielded 250 mg. of ethyl *erythro*-α-amino-β-benzoyloxy-β-*p*-nitrophenylpropionate hydrochloride, m.p. 197° dec.; λ_{max}^{solid} 3.5–4.4, 5.70, 5.85, 6.21, 6.35, 6.57 and 7.38 μ.

Anal. Calcd. for $C_{18}H_{19}ClN_2O_6$ (394.81): C, 54.75; H, 4.85; Cl, 8.98; N, 7.10. Found: C, 54.81; H, 4.75; Cl, 9.03; N, 7.09.

(b) Confirmation of Configuration of VII. (i) Ethyl *erythro*-α-Amino-β-hydroxy-β-*p*-nitrophenylpropionate Hydrochloride (VIII).—A mixture of 900 mg. of VII and 15 ml. of 2.5 *N* hydrochloric acid was warmed on the steam-bath. Ethanol was added to make the mixture homogeneous, and the solution was warmed on the steam-bath for 2 hr.

The reaction mixture was concentrated *in vacuo*, and the residue was leached with hot benzene to remove benzoic acid. The residue was taken up in ethanol, and the solution was saturated with anhydrous hydrogen chloride.

After standing at room temperature overnight, the solution was concentrated *in vacuo* and the residue was crystallized from ethanol; 350 mg. of ethyl *erythro*-α-amino-β-hydroxy-β-*p*-nitrophenylpropionate hydrochloride, m.p.

(21) B. N. Feitelson, J. T. Gunner, R. J. Moualim, V. Petrow, O. Stephenson and S. W. F. Underhill, *J. Pharm. Pharmacol.*, **3**, 154 (1951).

(22) The same product is obtained when anhydrous calcium carbonate is omitted.

177° dec., was obtained. A second crystallization yielded 170 mg. of the compound melting at 188° dec. (lit.[23] *erythro-*, m.p. 182–184° dec.; *threo-*, m.p. 153–155° dec.

(ii) **Ethyl *erythro*-α-Benzamido-β-hydroxy-β-*p*-nitrophenylpropionate (I).**—A mixture of 40 mg. of VIII, 28 mg. of sodium acetate and 19 mg. of benzoyl chloride in 4 ml. of water and 4 ml. of chloroform was shaken for 2 hr. About 20 ml. of chloroform was added to the reaction mixture, and the aqueous phase was acidified with a few drops of 2.5 *N* hydrochloric acid. The chloroform phase was separated and dried over anhydrous magnesium sulfate. The chloroform solution was filtered and concentrated *in vacuo*. Crystallization of the residue from ethanol gave 30 mg. of ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate, m.p. 154–156°. A second crystallization from ethanol yielded 18 mg. of product melting at 158–160°. There was no depression in melting point on admixture of the product with an authentic specimen of ethyl *erythro*-α-benzamido-β-hydroxy-β-*p*-nitrophenylpropionate.

(23) C. G. Alberti, B. Camerino and A. Vercellone, *Experientia*, **8**, 261 (1952).

RAHWAY, NEW JERSEY

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

A Study of the Action of Acid and Alkali on Certain Purines and Purine Nucleosides[1]

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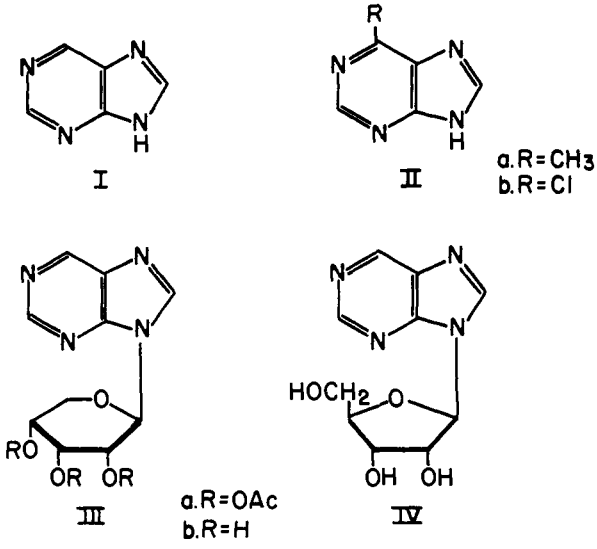
A series of glycosyl derivatives of various purines was found to be extremely labile toward dilute alkali at room temperature. In studies with 9-β-D-ribofuranosylpurine two products resulting from cleavage of the imidazole ring, with loss of carbon-8 but without loss of the ribosyl group, were partially characterized. Purine, its ribofuranosyl and ribopyranosyl derivatives evolve formic acid when heated with *p*-toluenesulfonic acid. Studies of the reaction indicate a complex decomposition pattern, including some loss of carbon-8. Syntheses of 9-D-ribopyranosylpurine and 6-methyl-D-ribofuranosylpurine are described.

In the course of studies of the properties of several derivatives of purine (I to IV), it was observed that the spectrum of 9-β-D-ribofuranosylpurine (IV, nebularine)[3] and of related glycosyl derivatives, underwent drastic and irreversible changes in dilute aqueous alkali at room temperature. It was also observed that 9-(2′,3′,4′-tri-*O*-acetyl-D-ribopyranosyl)-purine (IIIa) and other derivatives of purine yielded anomalous "acetyl" analyses. Such chemical behaviors have not previously been observed with any nucleosides of purines.



One nucleoside, namely, IV, which possesses both of these unusual properties occurs in the mushroom *Agaricus* (*Clitocybe*) *nebularis* Batsch.[4a,b,5] It is inhibitory toward *Mycobacteria*,[4a,b] is highly toxic to mammals[3,6a,b] and exhibits selective toxicity toward cells of mouse sarcoma 180 in culture.[7] Purine I, IV and other purine derivatives, 6-methylpurine (IIa) and 6-chloropurine (IIb), have some selective inhibitory effects on the growth of experimental tumors.[8]

Investigations of these unusual chemical behaviors, including attempts to elucidate the structural factors responsible and of possible correlations with the pharmacodynamic properties of certain of these compounds are in progress.[9] Consideration of these newly recognized chemical behaviors was necessary in studies of the metabolic fates of IV[10a] and I[10b] as well as in the work described in the following three papers; these properties must be considered when investigating new purine derivatives from natural sources.

Results and Discussion

Acid Instability.—The analysis for the formation of volatile acids involved heating the compound in question with a 25% aqueous solution of *p*-toluenesulfonic acid at 100° for 4 hr., steam distillation

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(2) Post-doctoral Research Fellow of the National Cancer Institute, 1953–1955.

(3) G. B. Brown and V. S. Weliky, *J. Biol. Chem.*, **204**, 1019 (1953).

(4) (a) N. Löfgren and B. Lüning, *Acta Chem. Scand.*, **7**, 225 (1953); (b) N. Löfgren, B. Lüning and H. Hedström, *ibid.*, **8**, 670 (1954).

(5) E. Fischer, "Untersuchungen in der Purin Gruppe," Springer, Berlin, 1907, p. 68, suggested that purine (I) would probably be found in nature.

(6) (a) F. S. Philips and D. A. Clarke, personal communication; (b) A. P. Truant and H. E. D'Amato, *Federation Proc.*, **14**, 391 (1955).

(7) J. J. Biesele, M. C. Slautterback and M. Margolis, *Cancer*, **8**, 87 (1955).

(8) (a) D. A. Clarke, F. S. Philips, S. S. Sternberg and C. C. Stock, *Ann. N. Y. Acad. Sci.*, **60**, 235 (1954); (b) F. S. Philips, S. S. Sternberg, L. Hamilton and D. A. Clarke, *ibid.*, **60**, 283 (1954); (c) K. Sugiura, Special Lectures of the 14th Japan Medical Congress, Kyoto, Japan, 697–714 (1955).

(9) G. B. Brown, M. P. Gordon, A. Hampton and D. I. Magrath, "The Chemistry and Biology of Purines," CIBA Symposia, Churchill, London, 1957, p. 192.

(10) (a) M. P. Gordon and G. B. Brown, *J. Biol. Chem.*, **220**, 927 (1956); (b) M. P. Gordon, in preparation.