with benzaldehyde (15 ml.) and ethanol (85 ml.). After 2 hr. the product was collected by filtration and recrystallized from ethanol; an analytical sample melted at 187.5–189.5°.

Anal. Calcd. for $C_{14}H_{12}ClN_3O$: C, 61.43; H, 4.42; Cl, 12.95; N, 15.35. Found: C, 61.14; H, 4.66; Cl, 12.93; N, 15.04.

1-Benzylideneamino-3-(3-chlorophenyl)hydantoin.—Benzaldehyde 4-(3-chlorophenyl)semicarbazone (5.46 g., 0.02 mole) and 2.44 g. (0.02 mole) of ethyl chloroacetate were added at 55–60° to an ethanolic sodium ethoxide solution (prepared from sodium (0.46 g.) and ethanol (15 ml.)), and the mixture was refluxed for 1 hr. After cooling, it was diluted with water (200 ml.) to separate the crude product which, air-dried, melted at 135–145°. An analytical sample (from ethyl acetate–ligroin) melted at 190.5–192.5°.

Anal. Calcd. for $C_{16}H_{12}ClN_3O_2$: C, 61.25; H, 3.86; Cl, 11.30; N, 13.39. Found: C, 61.01; H, 3.89; Cl, 11.15; N, 13.27.

1-(5-Nitrofurfurylideneamino)-3-(3-chlorophenyl)hydantoin (VII).—1-Benzylideneamino-3-(3-chlorophenyl)hydantoin (15.65 g.) in 200 ml. of diluted HCl was heated until all the benzaldehyde distilled; 5-nitro-2-furaldehyde (6 g.) was added and the solution was stirred at 70° for 15 min. and then cooled and filtered. The yellow crystals (from ethyl acetate–ligroin) melted at 156–161° dec.

Anal. Calcd. for $C_{14}H_9ClN_4O_5$: C, 48.21; H, 2.60; Cl, 10.16; N, 16.06. Found: C, 48.02; H, 2.55; Cl, 10.05; N, 15.95.

1-(5-Nitrofurfurylidene)-4-(3-chlorophenyl)semicarbazide.—This compound was prepared by the same procedure as described for benzaldehyde 4-(3-chlorophenyl)semicarbazone using 5-nitro-2-furaldehyde instead of benzaldehyde. After crystallization from ethanol–dimethylformamide the product melted at 226.5–232.5° dec.

Anal. Calcd. for $C_{12}H_9ClN_4O_4$: C, 46.68; H, 2.93; Cl, 11.81; N, 10.15. Found: C, 46.57; H, 2.89; Cl, 11.76; N, 10.02.

1-Amino-3-phenylhydantoin.—1-Benzylideneamino-3-phenylhydantoin (13.95 g.) was hydrolyzed by heating in HCl (125 ml.) and water (125 ml.) and distilling all the benzaldehyde. The hot solution was filtered and concentrated *in vacuo* to a small volume; on cooling at 0° for several hours the crude hydrochloride separated. It was dissolved in a little water and the solution made alkaline with ammonium hydroxide to the free base, which after recrystallization from water melted at 119–122°.

Anal. Calcd. for $C_9H_9N_3O_2$: C, 56.54; H, 4.75; N, 21.98. Found: C, 56.48; H, 4.71; N, 21.93.

1-Amino-3-(3-chlorophenyl)hydantoin.—This compound was prepared essentially by the same procedure as the one described above for 1-amino-3-phenylhydantoin, starting from 1-benzylideneamino-3-(3-chlorophenyl)hydantoin. After crystallization from water, the melting point was 108–109°.

Anal. Calcd. for $C_9H_8ClN_3O_2$: C, 47.90; H, 3.58; Cl, 15.71; N, 18.62. Found: C, 47.78; H, 3.52; Cl, 15.63; N, 18.48.

The Anomeric 5-Allyl-2′-deoxyuridines[1]

HARRY J. MINNEMEYER, PRISCILLA B. CLARKE, HOWARD TIECKELMANN,

Department of Chemistry, State University of New York at Buffalo, Buffalo, New York 14214

AND JAMES F. HOLLAND

Roswell Park Memorial Institute, Buffalo, New York 14203

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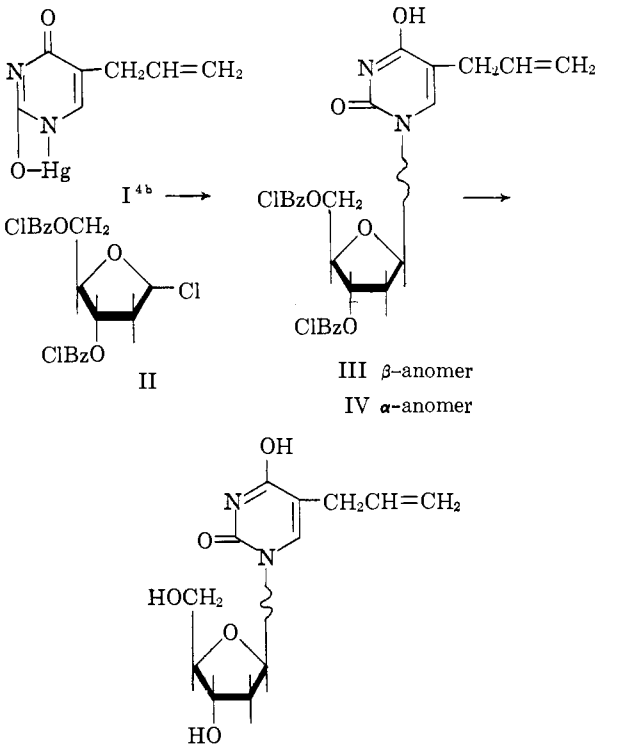
Interest in the chemotherapeutic properties of analogs of the naturally occurring pyrimidines and pyrimidine nucleosides of RNA and DNA in which changes have been made at the 5-position of the pyrimidine ring led us to prepare a number of 5-allylpyrimidines,[2] including 5-allyluridine.[3] We now wish to report the preparation of the anomeric 5-allyl-2′-deoxyuridines. While 5-allyluridine was reported as devoid of biological activity in several test systems,[3] the corresponding deoxyribosides reported here diminish catabolism of thymidine and 5-fluoro-2′-deoxyuridine in HeLa–PPLO (pleuropneumonia-like organisms) cultures.

In accordance with the structure of the mercury salt[4] one molar portion of 5-allyluracilmercury (I) was condensed with two molar portions of 3,5-(di-O-*p*-chlorobenzoyl)-2-D-deoxyribosyl chloride (II) in refluxing toluene to give a mixture of the blocked β- and α-nucleosides (III and IV) in 80% apparent yield as a tan glass. Condensations with a halogenose blocked with *p*-tolyl groups gave similar results. Alumina column chromatography showed that the crude material consisted of 21% of the mixed anomers (thus, 16% actual yield from I), the remainder being unidentified material.

CHART I



The anomers could not be separated by alumina column chromatography, although the mixed anomers

(1) This investigation was supported by Grant CA-02857 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) H. J. Minnemeyer, J. A. Egger, J. F. Holland, and H. Tieckelmann, *J. Org. Chem.*, **26**, 4425 (1961).

(3) H. J. Minnemeyer, H. Tieckelmann, and J. F. Holland, *J. Med. Chem.*, **6**, 602 (1963).

(4) (a) It has been reported that, in the synthesis of pyrimidine deoxyribosides, a "more reactive" mercury salt is required,[5] that is, one in which the pyrimidine and mercury are present in a 1:1 molar ratio. By chance, of three possible mercury salts,[6] this is the salt formed by the reaction of 5-allyluracil with mercuric chloride and sodium hydroxide. In contrast to thymine[7] it was not necessary to attempt the preparation of such a salt by other methods. (b) Structural formula I is intended only as a convenient representation which reflects the empirical formula of this compound.

(5) M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Am. Chem. Soc.*, **81**, 4112 (1959).

(6) J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *ibid.*, **79**, 5060 (1957).

(7) M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960).

could be separated from major impurities by such a process. The β-anomer (III) was obtained in analytical purity through a series of fractional crystallizations. The α-anomer (IV) could not be crystallized from the filtrates. In many runs fractional crystallizations were of limited value.

Deacylation of the blocked nucleosides by heating in sealed tubes at 100° in ethanol previously saturated with ammonia at 0° consistently gave quantitative recoveries of the crude mixed anomeric nucleosides V and VI. Additional purification by partition chromatography on Celite® columns using an ethyl acetate–water system also consistently gave recoveries of above 85% of the mixed anomers. The yield of purified mixed anomers from I was about 14%. Optical rotation data indicated that the mixture consisted of about 35% α-anomer and 65% β-anomer.

The synthesis of mixtures of the nucleosides V and VI was not troublesome. However, a reproducible procedure for the separation of the anomers was not achieved. The crystalline β-anomer (V) was obtained by deacylating the pure crystalline sample of the blocked nucleoside (III) obtained through fractional crystallization. In another experiment the α-anomer (VI) was obtained through fractional crystallization of a mixture of the deacylated nucleosides.

Although anomers V and VI have significantly different solubilities in several solvents, extensive paper chromatography experiments showed that the anomers always traveled with identical R_f values. Separations were achieved by thin layer chromatography. Because the difference in R_f values of the anomers was small, the separation could not be duplicated in corresponding column operations due to extensive overlap of bands.

As required, anomers V and VI gave identical ultraviolet absorption spectra over a range of pH values. Within experimental error, the spectra obtained were identical with those published for thymidine[8] and the extinction coefficients were consistent for the proposed structures.

Assignments of the anomeric configurations were based on the 60 Mc./sec. n.m.r. spectra determined in deuterium oxide. It has been shown[9] that there are only small changes in the fine structure and chemical shifts for the protons of the sugar moiety of configurationally related glycosides provided no substantial changes have been made in the aglycon which would change the conformation of the sugar. Lemieux has published and analyzed the n.m.r. spectra of the anomeric thymidines.[10] The spectra of the americon 5-allyl-2′-deoxyuridines, determined under similar conditions, resemble these spectra point for point, except the added presence of signals due to the allyl group at the 5-position of the pyrimidine ring. These signals have chemical shifts that do not interfere with the proton signals due to the sugar moiety, and their locations have been verified by comparison with other 5-allylpyrimidines. The results indicate that the conformations of the carbohydrate portion of the 5-allyl-2′-deoxyuridines are similar to those of the corresponding thymidines.

(8) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).
(9) R. U. Lemieux and M. Hoffer, *Can. J. Chem.*, **39**, 110 (1961).
(10) R. U. Lemieux, *ibid.*, **39**, 116 (1961).

TABLE I[a]

	——M 5-allyl-2′-deoxyuridine——		
M thymidine	0	5×10^{-5}	10^{-4}
0	1.4	0.9	1.2
5×10^{-5}	1.5	5.6	5.8
10^{-4}	1.9	6.1	6.4

[a] Growth of HeLa cells contaminated with pleuropneumonia-like organisms in Eagle's tissue culture medium containing methotrexate 10^{-6} *M*, hypoxanthine 10^{-4} *M*, and glycine 10^{-3} *M*. Growth, after 6 days, is expressed in multiples of the cell inoculum planted, measured as protein.

Optical-rotation data indicated that the α-anomer ($[\alpha]^{25}D$ +20.5°) was more dextrorotatory than the β-anomer ($[\alpha]^{25}D$ +11.9°). This result is in agreement with Hudson's rules of isorotation. Exceptions to these rules have been reported for other pyrimidine deoxyribosides.[9]

Biological Activity.—5-Allyl-2′-deoxyuridine has been studied in cultures of HeLa cells growing in Eagle's medium. No growth inhibition was produced in concentrations up to 10^{-4} *M*. These cultures have been found to be contaminated with pleuropneumonia-like organisms (PPLO), and thereby to have acquired an enhanced activity to destroy pyrimidine deoxynucleosides.[11] In a special medium incorporating hypoxanthine, glycine, and the folic acid antagonist, methotrexate, thymidine becomes the limiting nutrient for reversal of the growth inhibition.[12] The contaminated cultures, HeLa–PPLO, were found to grow poorly on thymidine. Thymine, the product of the enzyme nucleoside phosphorylase, is unable to support growth. 5-Allyl-2′-deoxyuridine cannot substitute for thymidine in supporting growth. In the presence of 5-allyl-2′-deoxyuridine, however, there is sparing of thymidine, and growth occurs. Both α- and β-anomers demonstrate activity in this system (Table I).

In other experiments to be reported elsewhere[13] in which labeled thymidine has been used, the thymidine sparing has been found to be due, in part at least, to inhibition of nucleoside phosphorylase activity.

In the same HeLa–PPLO system (in standard Eagle's medium) and presumably because of the same enzyme, 5-fluoro-2′-deoxyuridine is catabolized to 5-fluorouracil rapidly. Addition of 5-allyl-2′-deoxyuridine to media containing 5-fluoro-2′-deoxyuridine caused enhanced growth inhibition. This is consistent with the known greater growth-inhibitory activity of the deoxynucleoside when contrasted to the free pyrimidine base. Incubations of labeled 5-fluorodeoxyuridine with HeLa–PPLO demonstrated diminished catabolism to 5-fluorouracil in the presence of 5-allyldeoxyuridine.[13]

Experimental[14]

1-(3,5-Di-O-[*p*-chlorobenzoyl)-2-deoxy-α,β-ribosyl]-4-hydroxy-5-allyl-2(1H)-pyrimidone (III and IV).—5-Allyluracil-

(11) M. T. Hakala, J. F. Holland, and J. S. Horoszewicz, *Biochem. Biophys. Res. Commun.*, **11**, 466 (1963).
(12) M. T. Hakala and E. Taylor, *J. Biol. Chem.*, **234**, 126 (1959).
(13) J. F. Holland, unpublished observations.
(14) Melting points are corrected and were determined on a Frechsl melting point apparatus. Analyses were by Galbraith Laboratories, Knoxville, Tenn., and Alfred Bernhardt, Mulheim (Ruhr), Germany. Ultraviolet absorption data were determined on a Beckman DK-2 spectrophotometer. Optical rotations on 10-mg. samples were measured by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y.

mercury[3] (3.52 g., 0.01 mole) mixed with 3.52 g. of Celite was suspended in 300 ml. of toluene by vigorous stirring and dried azeotropically by distilling 100 ml. of the toluene. 3,5-Di-O-(*p*-chlorobenzoyl)-2-deoxy-D-ribosyl chloride[15] (8.60 g., 0.02 mole) was then added in one portion and the mixture refluxed 8 min. After chilling to room temperature, the mixture was filtered through a small plug of glass wool and 1.5 l. of petroleum ether (b.p. 35–60°) was added. The clear supernate was decanted from the precipitated gum and discarded. The gum was dissolved in chloroform, washed with 30% potassium iodide solution, then water, and dried over magnesium sulfate. After filtration, the chloroform was evaporated and the resulting sirup heated at 100° *in vacuo* to leave the crude product as a tan glass, 4.37 g. (80%).

Isolation of the β-Anomer III.—The glass (4.2 g.) resulting from a condensation reaction was dissolved in methylene chloride and concentrated to a volume of 10 ml. A white powdery precipitate was removed (0.17 g.) which was identified as *p*-chlorobenzoic acid. After the solvent was removed *in vacuo* the sirup was dissolved in hot absolute ethanol, and on cooling an oil separated. After freezing in Dry Ice, the mixture was set aside for 16 hr. at room temperature. A fine white crystalline precipitate was separated from gummy material. This weighed 0.44 g. (8%), m.p. 145–152°. After recrystallization from 50 ml. of hot absolute ethanol, it melted at 153–155° (0.33 g.). An additional crystallization gave the analytical sample in white needles, m.p. 154–155°.

Anal. Calcd. for $C_{26}H_{22}Cl_2N_2O_7$: C, 57.26; H, 4.07; N, 5.14. Found: C, 57.09; H, 4.14; N, 4.77.

Although the crystallizations described above were not always successful it was the only technique found which permitted the isolation of a pure anomeric blocked nucleoside. During one series of experiments, 1.51 g. of crystalline material consisting mostly of the β-anomer III was collected and used in the following experiment.

Isolation of the β-Anomer V, 5-Allyl-2′-deoxyuridine.—The "purified" β-anomer III (1.51 g., 2.77 mmoles) was placed in a tube containing 60 ml. of absolute ethanol previously saturated with ammonia at 0°. The tube was sealed and heated overnight at 100°. After cooling, the tube was opened and the ethanol and ammonia were evaporated to leave a crystalline mass. Water (75 ml.) and chloroform were added and the aqueous layer extracted at least 5 times with 50-ml. portions of chloroform. Evaporation of the aqueous layer gave a tan gum (0.74 g., 100%) which could not be crystallized.

The gum (0.74 g.) was subjected to partition column chromatography on a Celite® 545 column using an ethyl acetate–water system according to a previously described procedure.[3] On the column used (45 × 2.7 cm.), materials which were colored and absorbed ultraviolet light were eluted with the first 15 ml. of eluent and discarded. The next 170 ml. of eluent contained nothing. Material absorbing ultraviolet light at 267 mμ was eluted during the passage of an additional 150 ml. of organic phase. Evaporation of the solvent gave 0.65 g. (87%) of pale yellow sirup. This was dissolved in 10 ml. of hot ethyl acetate and on cooling an oil separated which crystallized on standing during 16 hr. The tiny white crystals weighed 0.45 g. (61%), m.p. 116–118°. An additional crystallization gave the analytical sample, m.p. 116–118°.

N.m.r. spectra demonstrated that this was the β-anomer of 5-allyl-2′-deoxyuridine, $[\alpha]^{25}D$ +11.9° (*c* 0.44, water); $\lambda_{max}^{pH\ 7}$ 268 mμ (ε 9450); $\lambda_{min}^{pH\ 7}$ 236 mμ (ε 2440); $\lambda_{max}^{pH\ 13\ (0.1\ N\ NaOH)}$ 267 mμ (ε 6920); $\lambda_{min}^{pH\ 13\ (0.1\ N\ NaOH)}$ 246 mμ (ε 4390); $\lambda_{max}^{pH\ 14\ (1\ N\ NaOH)}$ 267 mμ (ε 7260); $\lambda_{min}^{pH\ 14\ (1\ N\ NaOH)}$ 246 mμ (ε 4440).

Anal. Calcd. for $C_{12}H_{16}N_2O_5$: C, 53.72; H, 6.01; N, 10.44. Found: C, 53.29; H, 6.01; N, 10.36.

Alumina Column Chromatography of Anomers III and IV.—The crude blocked nucleosides from three condensation reactions were combined (13.1 g. total) and dissolved in benzene. The solution was applied to a column containing 400 g. of alumina[16] in benzene. Additional benzene (14.5 l.) was passed through the column until the eluent was free from solids or substances which absorbed ultraviolet light. From the benzene fractions there was obtained 6.9 g. (54% of the material applied to the column) of sirup that contained no nucleosides and this was discarded.

It had been observed that the passage of graded mixtures of benzene–methylene chloride eluted anomers III and IV but failed to effect a separation. Other combinations of solvents gave similar results. After anomers III and IV were eluted, solvents with higher eluting power did not remove additional material.

Therefore, on the column described here, benzene was replaced by ethyl acetate as eluent and the anomers III and IV were eluted and recovered as a tan glass (2.9 g., 21% of the sample applied to the column). The remaining 3.3 g. (25%) of the sample could not be recovered.

Curiously, this purified mixture of anomers III and IV could not be fractionally crystallized in a satisfactory manner.

Isolation of the α-Anomer VI.—The mixture of anomers III and IV obtained in the preceeding experiment (2.90 g.) was deacylated with ethanolic ammonia by the procedure used for the preparation of the β-anomer. The crude nucleosides weighed 1.44 g. (101%). The mixture was chromatographed on a Celite column by the usual procedure, and evaporation of the solvent gave a white crystalline mass which was recrystallized from 100 ml. of hot ethyl acetate. Clusters of white needles (0.65 g.) separated; m.p. 161–165°. An additional crystallization from 90 ml. of ethyl acetate gave 0.42 g. of analytically pure material, m.p. 164–167°; $[\alpha]^{25}D$ +20.5° (*c* 0.56, water); $\lambda_{max}^{pH\ 7}$ 268 mμ (ε 9900); $\lambda_{min}^{pH\ 7}$ 236 mμ (ε 2290); $\lambda_{max}^{pH\ 13\ (0.1\ N\ NaOH)}$ 267 mμ (ε 7350); $\lambda_{min}^{pH\ 13\ (0.1\ N\ NaOH)}$ 245 mμ (ε 4380) $\lambda_{max}^{pH\ 14\ (1\ N\ NaOH)}$ 267 mμ (ε 7660); $\lambda_{min}^{pH\ 14\ (1\ N\ NaOH)}$ 245 mμ (ε 4530).

Anal. Calcd. for $C_{12}H_{16}N_2O_5$: C, 53.72; H, 6.01; N, 10.44. Found: C, 53.45; H, 5.94; N, 10.33.

Thin Layer Chromatography.—Glass plates were coated with silica gel G, dried at 110° for 0.5 hr., then cooled and exposed to the atmosphere for 2 hr. or more. Maximum separation was achieved using ethyl methyl ketone as solvent, which gave R_f values of 0.45 (α-anomer) and 0.57 (β-anomer). Detection was by means of a 2% aqueous potassium permanganate spray The compounds appeared instantly as cream-colored spots on purple background. The color fades after 5–10 min.

Acknowledgment.—We thank Dr. James G. Colson of the Buffalo Medical Group for assistance in the determination of the n.m.r. spectra.

(15) J. J. Fox, N. Yung, I. Wempen, and M. Hoffer, *J. Am. Chem. Soc.*, **83**, 4066 (1961). It is important that pure halogenose be used in the condensation reaction. Approximately 45 g. of crude halogenose can be dissolved in 500 ml. of hot carbon tetrachloride. A dark insoluble oily impurity is removed by several filtrations. Because of the instability of the halogenose, the recrystallization should be carried out as rapidly as possible.

(16) Woelm® alumina was purchased from Alupharm Chemicals, P. O. Box 755, New Orleans, La. Neutral alumina of activity grade IV (contains 10% water) was used. Directions for the preparation are supplied with the product.

Preparation of D- and L-Octopamine[1]

THOMAS KAPPE AND MARVIN D. ARMSTRONG

The Fels Research Institute, Yellow Springs, Ohio

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The natural occurrence of octopamine (norsympatol, norsynephrine, α-(aminomethyl)-4-hydroxybenzyl alcohol) was first reported by Erspamer[2] who identified it as a constituent of extracts of salivary glands of the octopus. He showed that natural octopamine has the same configuration as natural D-(−)-norepinephrine (*l*-noradrenaline). The pharmacological responses given by purified octopamine were qualitatively the same as those of racemic octopamine, while quantitatively it appeared to be about twice as active as the synthetic compound. The recent demonstration that octopamine occurs in tissues of mammals[3] made it desirable to separate synthetic material into its optical isomers so that their physical properties might be de-

(1) This work was supported in part by Research Grant MH-02278 from the National Institute of Mental Health, U. S. Public Health Service.

(2) V. Erspamer, *Nature*, **169**, 375 (1952).

(3) Y. Kakimoto and M. D. Armstrong, *J. Biol. Chem.*, **237**, 422 (1962).