[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF COLORADO]

The Synthesis of Nucleosides of Cytosine and 5-Methylcytosine[1]

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As part of a study on the relationships between structure and biological activity of the nucleic acid derivatives, a series of new cytosine nucleosides and their derivatives are reported. These are the 1-glycopyranosylcytosines of D-glucose, D-galactose, D-xylose and D- and L-arabinose; and the 1-glycopyranosyl-5-methylcytosines of D-xylose and D- and L-arabinose. In the synthesis of the above nucleosides it has been found that acetylglycopyranosyl chlorides are more suitable than the corresponding bromides as intermediates for condensation with the appropriate 2,4-diethoxypyrimidine. An improved synthesis for tetraacetyl-β-D-glucopyranosyl chloride is reported.

The importance of pyrimidine nucleosides as possible intermediates in nucleic acid synthesis is suggested not only by the work of Loring and Pierce[3] demonstrating the utilization of uridine and cytidine by a *Neurospora* mutant, but also by the more recent reports from Hammarsten's laboratory[4] showing the utilization of cytidine as a precursor of pyrimidines in the biosynthesis of pentosenucleic acid (PNA) and desoxypentosenucleic acid (DNA) in rats. The occurrence of 5-methylcytosine in DNA[5] further suggests that nucleoside derivatives of this pyrimidine might be involved in similar biosyntheses.

In connection with an investigation of the relationships between chemical structure and biological activity of nucleic acid derivatives, a series of synthetic nucleosides has been prepared in this Laboratory. Extending the studies of synthesis and biological testing of 1-glycopyranosyl nucleosides of uracil and thymine[6,7,8] several new 1-glycopyranosylcytosines and -5-methylcytosines and their derivatives are reported in this paper (Table I).

The pyrimidine nucleosides were synthesized by a modification of the method of Hilbert[9] using acetylglycopyranosyl chlorides (also called halogeno acetyl sugars, acetohalogeno sugars or halogenoses) in place of the corresponding bromides to react with 2,4-diethoxypyrimidine and 2,4-diethoxy-5-methylpyrimidine. Not only are the chloro halogenoses more stable and more readily prepared than the bromo analogs, but also they were found to produce higher yields of nucleosides when condensed with alkoxypyrimidines. These results indicate that acetylglycopyranosyl chlorides may have a wider usage in synthetic nucleoside chemistry. A comparison of yields obtained from the use of both types of halogenoses is given in Table II.

In the hope of obtaining two pyrimidine nucleosides of the same sugar but with opposite configuration at the glycosidic center and with different biological activities, we investigated the reaction between tetraacetyl-β-D-glucopyranosyl chloride (*trans*) and 2,4-diethoxypyrimidine. The preparation of this halogenose has been reported previously by Schlubach and co-workers[10] who treated acetobromoglucose with freshly prepared silver chloride. They obtained the same halogenose by the action of liquid hydrogen chloride on α-D-glucose pentaacetate in a sealed tube. We have found that this *trans* halogenose may be prepared easily and in higher yields from the interaction of ethereal

TABLE I

PHYSICAL PROPERTIES OF SOME SYNTHETIC 1-GLYCOPYRANOSYLCYTOSINES

Compound	M.p., °C.[a]	$[\alpha]^{26}D$	Formula	Nitrogen, % Calcd.	Found
Xylosylcytosine	251–252 dec.	+24	$C_9H_{13}N_3O_5$	17.27	17.14
Xylosylcytosine·HCl	225–230 dec.	+21	$C_9H_{14}ClN_3O_5$	15.02	14.71
Xylosylcytosine·HNO_3	223–227 dec.	...	$C_9H_{14}N_4O_8$	18.29	18.03
Triacetyl-D-xylosyl-4-acetylaminouracil	277–278 dec.	...	$C_{17}H_{21}N_3O_9$	10.22	10.41
D-Arabinosylcytosine	265–267 dec.	−101	$C_9H_{13}N_3O_5$	17.27	17.22
D-Arabinosylcytosine·HNO_3	223–225 dec.	...	$C_9H_{14}N_4O_8$	18.29	17.97
L-Arabinosylcytosine	265–267 dec.	+100	$C_9H_{13}N_3O_5$	17.27	17.12
Glucosylcytosine·HCl	200–201 dec.	+20	$C_{10}H_{16}ClN_3O_6$	13.57	13.42
Galactosylcytosine·HCl·H_2O[b]	115–120 eff.	+48	$C_{10}H_{18}ClN_3O_7$	12.83	12.71
Galactosylcytosine·HNO_3	140–141 eff.	+49	$C_{10}H_{16}N_4O_9$	16.66	16.42
Xylosyl-5-methylcytosine	254–256 dec.	+14	$C_{10}H_{15}N_3O_5$	16.33	16.18
Xylosyl-5-methylcytosine·HCl	246–247 dec.	...	$C_{10}H_{16}ClN_3O_5$	14.31	14.43
Xylosyl-5-methylcytosine·HNO_3	231–232 eff.	...	$C_{10}H_{16}N_4O_8$	17.49	17.48
D-Arabinosyl-5-methylcytosine	290–291 dec.	−79	$C_{10}H_{15}N_3O_5$	16.33	16.36
D-Arabinosyl-5-methylcytosine·HNO_3	206–210 eff.	...	$C_{10}H_{16}N_4O_8$	17.49	17.46
L-Arabinosyl-5-methylcytosine	290–291 dec.	+78	$C_{10}H_{15}N_3O_5$	16.33	16.54

[a] Melting points are uncorrected and varied somewhat depending upon the rate of heating. Optical rotations were run in distilled water. [b] *Anal.* Calcd.: Cl, 10.82. Found: Cl, 10.80.

(1) This paper was presented in part at the 118th Meeting of the American Chemical Society in Chicago, Illinois, September, 1950.

(2) Université Libre de Bruxelles, Bruxelles, Belgium.

(3) H. S. Loring and J. G. Pierce, *J. Biol. Chem.*, **153**, 61 (1944).

(4) E. Hammarsten, P. Reichard and E. Saluste, *ibid.*, **183**, 105 (1950).

(5) R. D. Hotchkiss, *ibid.*, **175**, 315 (1948).

(6) D. W. Visser, K. Dittmer and I. Goodman, *ibid.*, **171**, 377 (1947).

(7) D. W. Visser, I. Goodman and K. Dittmer, THIS JOURNAL, **70**, 1926 (1948).

(8) K. Dittmer, I. Goodman, D. W. Visser and H. P. McNulty, *Proc. Soc. Exp. Biol. Med.*, **69**, 40 (1948).

(9) G. E. Hilbert, THIS JOURNAL, **59**, 330 (1937).

(10) H. H. Schlubach, P. Stadler and I. Wolf, *Ber.*, **61**, 287 (1928).

TABLE II

COMPARATIVE YIELDS OF ACETYLGLYCOPYRANOSYL-ETHOXYPYRIMIDINE NUCLEOSIDES FROM CHLORO AND BROMO ACETYL SUGARS

Halogenose	Yield with 2,4-diethoxypyrimidine, %	Yield with 2,4-diethoxy-5-methylpyrimidine, %
Acetobromoglucose	49[11a]	...
Acetochloroglucose	65	...
Acetobromogalactose	37[9]	...
Acetochlorogalactose	54	...
D- and L-acetobromoarabinose	38[9]	43[7]
D- and L-acetochloroarabinose	58	49
Acetobromoxylose	31[9]	36
Acetochloroxylose	54	48

[a] Figures in parentheses are literature references. All halogenoses are of the D-configuration unless otherwise specified.

hydrogen chloride on the readily available β-D-glucose pentaacetate at atmospheric pressure. It is interesting to note that the pentaacetate and the halogenose product were both of the beta (*trans*) configuration. This may be explained by assuming that with ethereal hydrogen chloride isomerization of the β-pentaacetate preceded halogenose formation.

Hilbert[9] attempted the condensation between acetobromomannose (α-*trans*) and 2,4-diethoxypyrimidine and obtained a sirup from which no nucleoside was isolated. When we treated the *trans*-β modification of acetochloroglucose with 2,4-diethoxypyrimidine, a nucleoside was isolated which proved to be identical with that obtained from the reaction using the *cis* forms of acetobromo- and acetochloroglucose indicating that the reaction of the *trans* halogenose to isomerize to the more stable *cis* modification was faster than was either orthoester formation or reaction in the *trans* form with the alkoxypyrimidine.

1-D-Glucopyranosylcytosine was prepared by Hilbert and Jansen[11] by treatment of the 1,2-dihydro-2-oxo-4-ethoxy-1-tetraacetyl-D-glucopyranosylpyrimidine with ammoniacal alcohol in a sealed tube for several hours at 80°. The compound was extremely hygroscopic and in order to characterize it the nitrate and picrate salts as well as the completely acetylated derivative were prepared. This method proved quite satisfactory for the 1-D-glycopyranosylcytosines listed in Table I. In a similar way, Howard, Lythgoe and Todd[12] succeeded in the first synthesis of a naturally occurring pyrimidine nucleoside, cytidine. We found 1-D-galactopyranosylcytosine to be very hygroscopic and isolated it in the form of its hydrochloride. The pentosylcytosines of D-xylose and D- and L-arabinose, on the other hand, were anhydrous, though the hydrochlorides of the arabinosylcytosines were hygroscopic. In general, no over-all statement can be made as to the hygroscopic properties of synthetic cytosine nucleosides.

All these nucleosides were tested for biological activity on a natural and uracil-requiring strain of *Escherichia coli* and on the protozoan, *Tetrahymena geleii W.*, and showed activity only at very high concentrations. The detailed results of these studies are being published elsewhere. The syntheses of 1-glycofuranosyl nucleosides of cytosine, uracil and thymine are under way in this Laboratory. These compounds should be of especial interest because the lactol ring structure in the naturally occurring nucleosides is also of the furanose form.

(11) G. E. Hilbert and E. F. Jansen, THIS JOURNAL, **58**, 60 (1936).

(12) G. A. Howard, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 1052 (1947).

Experimental

Tetraacetyl-α-D-glucopyranosyl and galactopyranosyl chlorides were prepared by the method of Skraup and Kremann.[13] Triacetyl-α-D-xylopyranosyl and triacetyl-β-D-arabinopyranosyl chlorides were prepared according to Brauns.[14] The D-isomer of acetochloroarabinose, hitherto unreported, was prepared by the same method[13] and gave a melting point of 151–152° (cor.) and $[\alpha]^{26}D$ −243° (*c*, 2 in $CHCl_3$).

Preparation of Tetraacetyl-β-D-glucopyranosyl Chloride.—Twenty grams of β-D-glucose pentaacetate was added to a flask containing 250 ml. of anhydrous ether and equipped with a mechanical stirrer. Fifty grams of hydrogen chloride was passed into the stirred mixture at 5° during the course of 40 minutes. During this time most of the pentaacetate dissolved whereupon the flask was stoppered and placed in an ice-chest for two days. The clear, homogeneous solution was poured into a distilling flask and concentrated under vacuum. The precipitate which formed in the flask was filtered giving 9.5 g. of product, m.p. 95–96°. An additional 3 g. was obtained from the mother liquor. The total yield was 65%, $[\alpha]^{26}D$ −9° (*c*, 2 in anhydrous ether). One recrystallization from anhydrous ether gave pure product, m.p. 98–99°, $[\alpha]^{26}D$ −12°.

Condensation Reactions.—In the interactions of halogenoses with alkoxypyrimidines the acetochloro sugars (*cis*) of D-glucose, D-galactose, D-xylose, and D- and L-arabinose were treated with 2,4-diethoxypyrimidine, and the acetochloro sugars of D-xylose and D- and L-arabinose with 2,4-diethoxy-5-methylpyrimidine. Equal weights of pyrimidine and halogenose were used. In all cases the reactions proceeded smoothly at elevated temperatures. The products were identified by mixed melting points with those obtained from the use of corresponding acetobromo sugars. The condensation reaction of acetochloroxylose with 2,4-diethoxy-5-methylpyrimidine described below is general for all the others.

Preparation of 1,2-Dihydro-2-oxo-4-ethoxy-5-methyl-1-triacetyl-D-xylopyranosylpyrimidine.—Twenty-two grams of acetochloroxylose was added to 22 g. of 2,4-diethoxy-5-methylpyrimidine in a 50-ml. flask fitted with an outlet tube and protected from moisture. (It is necessary that all of the halogenose be immersed in the pyrimidine to avoid charring. This can be accomplished by washing undissolved halogenose down the sides of the flask with anhydrous ether.) The flask was heated in an oven at 85° for one-half hour after which the temperature was raised to 100° and maintained at that level for 24 hours. The temperature was then raised to 110–115° for another 24 hour period. The reaction mixture usually darkened during this time. Upon cooling to room temperature, adding an equal volume of anhydrous ether, and stirring with a glass rod, a white precipitate formed which was filtered, washed with anhydrous ether, and dried. The yield was 14.3 g. (48%), m.p. 188–189°. One recrystallization from 95% ethanol gave a pure product, m.p. 189–190° (uncor.).

Anal. Calcd. for $C_{18}H_{24}N_2O_9$: N, 6.79. Found: N, 6.68.

The above reaction was also carried out with acetobromoxylose. The identical product obtained in 34% yield was identified by its melting point and a mixed-melting point with the above material.

Preparation of 1-D-Xylopyranosylthymine.—From the reaction of 4 g. of 1,2-dihydro-2-oxo-4-ethoxy-5-methyl-1-D-xylopyranosylpyrimidine with methanolic hydrogen chloride by the method of Hilbert[9] 2.2 g. of nucleoside was obtained. Recrystallization from a 50% alcohol–water solution gave pure compound, m.p. 284–285° (dec.).

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: N, 10.85. Found: N, 11.00.

(13) H. Skraup and R. Kremann, *Monatsh.*, **22**, 379 (1901).

(14) D. H. Brauns, THIS JOURNAL, **46**, 1484 (1924); **47**, 1280 (1925).

Preparation of 1-D-Glycopyranosylcytosines.—The compounds listed in Table I were prepared by the method of Hilbert and Jansen[11] with a slight change in conditions. It was found that if the reactants were heated in a sealed tube at 90° instead of 80°, crystallization occurred on the walls of the hot tube. When crystals ceased to form the reaction was considered complete. The yields in these reactions were practically quantitative. The free cytosine nucleosides of xylose and arabinose were crystallized from 95% ethanol. Galactopyranosylcytosine was isolated as the hydrochloride in the following manner: The sealed tube was cooled, opened, and the contents poured into a flask and concentrated to a sirup which was taken up in hot 95% ethanol. Concentrated hydrochloric acid was added dropwise until the solution was decidedly acidic whereupon it was cooled slowly and placed in an ice-box overnight. Precipitation occurred in the form of large, colorless crystals. A similar procedure was used for the isolation of the hydrochloride of 1-D-glucopyranosylcytosine. The hydrochloride salts of the other nucleosides listed in Table I were made directly from the free cytosine nucleosides by dissolving them in hot aqueous alcohol, adding concentrated hydrochloric acid dropwise, and cooling. The products crystallized immediately.

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Diels–Alder Reactions of α,β-Unsaturated Sulfonyl Compounds

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Ethylenesulfonic acid, ethylenesulfonyl chloride and methyl vinyl sulfone have been studied as dienophiles in the Diels–Alder reaction. Ethylenesulfonic acid does not react with anthracene; as a strong acid it induces the polymerization of cyclopentadiene and 2,3-dimethyl-1,3-butadiene, rather than reacting with these substances in the Diels–Alder sense. Ethylenesulfonyl chloride is a very active dienophile; it enters into a rapid exothermic reaction with cyclopentadiene, 2,3-dimethyl-1,3-butadiene and isoprene. It reacts with butadiene under somewhat more vigorous conditions. The yields are excellent. The isoprene adduct is shown to be 1,2,5,6-tetrahydro-4-methylbenzenesulfonyl chloride by conversion of the morpholide to 4-toluenesulfomorpholide. Ethylenesulfonyl chloride is somewhat less reactive than maleic anhydride as shown by its failure to combine with anthracene or furan. Methyl vinyl sulfone is a less reactive dienophile than ethylenesulfonyl chloride, but it reacts with 1,3-butadiene, isoprene, 2,3-dimethyl-1,3-butadiene and cyclopentadiene to form the corresponding cyclohexene derivatives in good yields.

Previous reports of Diels–Alder condensations in which α,β-unsaturated sulfonyl compounds serve as dienophiles have concerned only *p*-tolyl vinyl sulfone and 1,1-dioxathia-2-cyclopentene[1,2]; the temperatures employed in reactions with such active dienes as cyclopentadiene and dimethylbutadiene were so high (about 150°) as to suggest that the sulfonyl function does not greatly enhance the dienophilic activity of an adjacent ethylenic group. Since theoretical considerations lead to the prediction of a substantial enhancement, we have examined the behavior of some of the simplest unsaturated sulfonyl compounds in the diene reaction; the present report deals with ethylenesulfonic acid, ethylenesulfonyl chloride and methyl vinyl sulfone.

Cyclopentadiene and 2,3-dimethyl-1,3-butadiene polymerized rapidly when ethylenesulfonic acid was added to their solutions in dioxane or glacial acetic acid. The polymerizations were not inhibited by hydroquinone. It is, of course, not surprising that the strong acid causes the polymerization of the active dienes. There was no evidence of reaction between anthracene and ethylenesulfonic acid, even when mixtures in acetic acid solution were heated for seven days.

In the absence of solvents, ethylenesulfonyl chloride entered into rapid exothermic reaction with cyclopentadiene, 2,3-dimethyl-1,3-butadiene and isoprene. The isoprene adduct was found to be the expected 1,2,5,6-tetrahydro-4-methylbenzenesulfonyl chloride, whose structure was proved by bromination and subsequent dehydrobromination of the morpholide to the known 4-toluenesulfomorpholide. The substituted cyclohexenesulfonyl chlorides which were formed by these reactions could be distilled *in vacuo*, without decomposition, to separate them from the small amount of hydroquinone which was employed to inhibit polymerization of the reactants. The yields, after distillation, were in the neighborhood of 90%. The reaction of ethylenesulfonyl chloride with butadiene proceeded in similar yield but required more strenuous conditions.

Several attempts to obtain an adduct from ethylenesulfonyl chloride and anthracene were made. The conditions tried ranged from refluxing in xylene solution for three hours to standing at room temperature in xylene solution for several weeks, but only anthracene and ethylenesulfonyl chloride were found in the reaction mixtures. No adduct could be isolated by distillation from an equimolar mixture of furan and ethylenesulfonyl chloride which had remained at room temperature for 24 hours. An attempt to dehydrate[3] any adduct which might have formed in such a reaction resulted in the formation of an intractable tar.

(1) K. Alder, H. F. Rickert and E. Windemuth, *Ber.*, **71**, 2451 (1938).

(2) The present work was almost complete when the condensation of methyl ethylenesulfonate with cyclopentadiene was reported by A. Lambert and J. D. Rose [*J. Chem. Soc.*, 46 (1949)]. For examples of condensations in which the dienophile contains both a carbonyl and a sulfonyl group see E. A. Fehnel and M. Carmack, THIS JOURNAL, **70**, 1813 (1948).

(3) M. G. Van Campen and J. R. Johnson, THIS JOURNAL, **55**, 430 (1933).