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

Potential Anticancer Agents.[1] XVI. Synthesis of 2-Deoxy-β-D-ribofuranosides via the 2,3-Episulfonium Ion Approach

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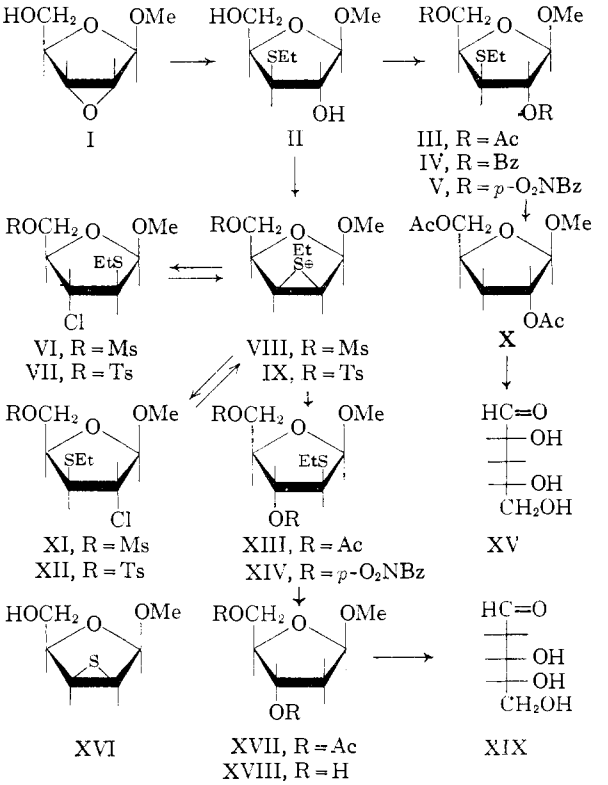
The ring-opening of methyl 2,3-anhydro-β-D-ribofuranoside (I) with sodium ethyl mercaptide occurred exclusively at C.3 to yield methyl 3-deoxy-3-(ethylthio)-β-D-xylofuranoside (II). Conventional tosylation of the 3-ethylthio compound II led to a chloro tosylate designated as methyl 3(2)-chloro-2,3-dideoxy-2(3)-(ethylthio)-5-*O*-tosyl-β-D-arabino(xylo)furanoside (VII/XII). Acetolysis of the chloro tosylate VII/XII gave a mixture of methyl 2,5-di-*O*-acetyl-3-deoxy-3-(ethylthio)-β-D-xylofuranoside (III) and methyl 3,5-di-*O*-acetyl-2-deoxy-2-(ethylthio)-β-D-arabinofuranoside (XIII) in a ratio of about 1 to 4, respectively. The 2-ethylthio compound XIII resulted from the migration of the ethylthio group from C.3 to C.2 *via* an episulfonium intermediate. Desulfurization of the mixture of diacetates III and XIII followed by hydrolysis gave 2-deoxy-D-ribose (XIX), identified as its anilide. This sequence of reactions from the anhydro sugar I to 2-deoxy-D-ribose (XIX), involving a novel rearrangement of a 3-(ethylthio)-xyloside (II) to a 2-(ethylthio)-arabinoside, is compatible with the chemistry of, and provides a new approach to, the synthesis of natural and artificial 2′-deoxynucleosides.

One of several approaches to the synthesis of 2′-deoxynucleosides under investigation in these laboratories involves the projected migration of a 3′-alkylthio group to C.2′ *via* a 2′,3′-episulfonium ion and subsequent desulfurization.[2] The well-known and highly effective participation of alkyl- and arylthio groups in the displacement of neighboring *trans*-halogens is explained by the initial formation of episulfonium ions,[3] and subsequent nucleophilic attack on these ions has frequently resulted in migration of the participating alkyl- or arylthio group.[4] It appeared that 2,3-episulfonium ions derived from 3-(alkylthio)- or 3-(arylthio)-furanosides would be particularly likely to yield rearranged sulfides on reaction with nucleophiles because of their close analogy to 2,3-anhydrofuranosides, which undergo nucleophilic attack predominantly at C.3.[5,6] In order to check the validity of these views, preparation and rearrangement of methyl 3-deoxy-3-(ethylthio)-β-D-xylofuranoside (II) were undertaken.

Reaction of methyl-2,3-anhydro-β-D-ribofuranoside (I)[6] with excess sodium ethyl mercaptide in refluxing methanol furnished the desired 3-(ethylthio)-furanoside II in essentially quantitative yield. The homogeneity of this non-crystalline compound was demonstrated readily by vapor-phase chromatography of its diacetate III, which failed to indicate the presence of any of the subsequently prepared 2-ethylthio isomer XIII, and also by its conversion in high yield to the crystalline dibenzoate IV and di-*p*-nitrobenzoate V. That II was indeed the expected 3-(ethylthio)-xyloside and not the isomeric 2-(ethylthio)-arabinoside was proved by desulfurization of its diacetate III and hydrolysis of the resulting 3-deoxyfuranoside X to 3-deoxy-D-xylose(ribose) (XV).[7] This sugar was identified by conversion to its known *p*-nitrophenylosazone.[7] The reaction of I with sodium ethyl mercaptide therefore provides an additional example of the very predominant C.3 attack of nucleophiles on 2,3-anhydrofuranosides that almost invariably takes place.



Reaction of the 3-(ethylthio)-furanoside II with tosyl chloride in pyridine at room temperature afforded a sirup that analyzed correctly not for a ditosylate but for a monotosylate having the second hydroxyl replaced by chlorine. Apparently the initially formed 2,5-ditosylate undergoes facile elimination of its 2-tosyloxy group[8] to give the

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute. For the preceding paper in this series, *cf.* E. J. Reist, R. R. Spencer and B. R. Baker, *J. Org. Chem.*, **24**, in press (1959).

(2) L. Goodman, A. Benitez and B. R. Baker, THIS JOURNAL, **80**, 1680 (1958).

(3) H. L. Goering and K. L. Howe, *ibid.*, **79**, 6542 (1957); A. Streitwieser, Jr., *Chem. Revs.*, **56**, 571 (1956).

(4) *E.g.*, K.-D. Gundermann, *Angew. Chem.*, **69**, 726 (1957); *Ber.*, **88**, 1432 (1955); W. E. Parham, J. Heberling and H. Wynberg, THIS JOURNAL, **77**, 1169 (1955) and references therein.

(5) (a) J. Davoll, B. Lythgoe and S. Trippett, *J. Chem. Soc.*, 2230 (1951); (b) B. R. Baker, R. E. Schaub and J. H. Williams, THIS JOURNAL, **77**, 7 (1955); (c) B. R. Baker and R. E. Schaub, *ibid.*, **77**, 5900 (1955); (d) J. M. Anderson and E. Percival, *J. Chem. Soc.*, 819 (1956).

(6) C. D. Anderson, L. Goodman and B. R. Baker, paper VII of this series, THIS JOURNAL, **80**, 5247 (1958).

(7) P. W. Kent, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 1232 (1949).

(8) It was presumed that it is the 2-tosyloxy group that is replaced by chloride because *trans*-2-(methylthio)-cyclopentanol and 2-(methylthio)-ethanol react similarly with tosyl chloride to yield chlorides.[3]

episulfonium ion IX, which then reacts with the chloride ion present in the reaction mixture. The product may be either of the isomeric chloro tosylates VII and XII or both, depending on their relative rates of formation or, if the reaction of IX with chloride ion is reversible, on their relative thermodynamic stabilities. The isomeric composition of this rather unstable mixture of chloro tosylates was not experimentally amenable; since both isomers presumably undergo displacement reactions *via* the common episulfonium ion IX,[9] a structure proof was not considered important for the present purpose. The chloro tosylate VII/XII was formed in nearly quantitative yield. An analogous chloro mesylate VI/XI was obtained by the reaction of mesyl chloride with the 3-(ethylthio)-furanoside II, but in lower yield than the corresponding tosylate.

Acetolysis of the chloro tosylate VII/XII was accomplished by treatment with sodium acetate in refluxing methyl Cellosolve.[10] With a 1.5-hour reaction period,[11] the resultant crude product showed no tosylate absorption in the infrared. Since the infrared absorption spectrum also showed that some deacetylation had taken place, the crude product was reacetylated with acetic anhydride in pyridine. Evaporative distillation then afforded about a 70% yield of an oil that analyzed quite well for the desired product of ethylthio migration, namely, methyl 3,5-di-*O*-acetyl-2-deoxy-2-(ethylthio)-β-D-arabinofuranoside (XIII). The nonidentity of this diacetate XIII with the isomeric 3-ethylthio diacetate III was proved by methanolysis and conversion of the resulting crude diol to a crystalline di-*p*-nitrobenzoate XIV (in 55% yield) that was distinctly different from the isomeric di-*p*-nitrobenzoate V obtained earlier from the 3-(ethylthio)-xylofuranoside II. Raney nickel desulfurization of the 2-ethylthio diacetate XIII gave a 48% yield of a liquid analyzing correctly for the expected methyl 3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranoside (XVII).[12] Methanolysis of the deoxy diacetate XVII gave the corresponding diol XVIII[13] as an oil which consumed essentially no periodate, thus confirming its expected furanose structure. Mild acid hydrolysis of the deoxy furanoside XVIII[14] yielded the expected 2-deoxy-D-ribose (XIX). This sugar was isolated and characterized as its anilide,[15] which was obtained in 41% over-all yield from XVII.

A closer examination of the acetolysis product originally shown to contain the 2-ethylthio diacetate XIII, using vapor-phase chromatography, revealed that a substantial amount of the 3-ethylthio diacetate III was also present,[16] the ratio of XIII to III being approximately 4:1. Careful spectroscopic examination of a carbon tetrachloride solution of the chloro tosylate VII/XII in the hydroxyl region near 3 μ indicated that it contained, at most, 1% of unreacted or only partially reacted II. Thus the 3-ethylthio diacetate III present in the acetolysis product must have arisen by nonselective acetolysis of VII/XII and not by incomplete reaction of II with tosyl chloride. The fact that the attack of acetate ion on the episulfonium ion IX is less selective than the attack of ethyl mercaptide ion on the anhydrofuranoside I may possibly be attributed to the highly ionic character of the transition state through which the former reaction proceeds; this explanation is in accord with the general observation that nucleophilic attack on epoxides and episulfides becomes less selective the greater the ionic character of the transition state.[17-19]

Preliminary attempts to convert the anhydrofuranoside I to methyl 2,3-dideoxy-2,3-epithio-β-D-lyxofuranoside (XVI) by the *trans-O,S*-diacetate method developed in the cyclopentane series[2] were unpromising.[20] Since episulfides should

Subsequent application of this reaction to a 5-*O*-trityl-3-deoxy-3-(ethylthio)-xylofuranoside similarly resulted in replacement of the C.2-hydroxyl by chloride rather than in tosylation (to be published).

(9) *Cf.* the reaction of hydrochloric acid with the isomeric 1,2-(ethylthio)-propanols, both of which yield the same product, ethyl 2-chloro-1-propyl sulfide (R. C. Fuson, C. C. Price and D. M. Burness, *J. Org. Chem.*, **11**, 475 (1946)).

(10) B. R. Baker and R. E. Schaub, *ibid.*, **19**, 646 (1954).

(11) Considerable darkening of the reaction mixture occurred during this period, and carbonization when the reflux period was extended to 3–4 hours.

(12) In addition, a 16% yield of a lower-boiling fraction analyzing correctly for a methyl dideoxypentoside acetate was obtained. A chlorine analysis on the starting material used in this desulfurization indicated the presence of 2.22% chlorine, and therefore it appears that this dideoxy acetate was derived from chlorine-containing product(s) of the incomplete acetolysis of the chloro tosylate VII/XII.

(13) This compound, as an anomeric mixture, has been prepared from 2-deoxy-D-ribose; see R. E. Deriaz, W. G. Overend, M. Stacey and L. F. Wiggins, *J. Chem. Soc.*, 2836 (1949).

(14) *Cf.* the hydrolysis of the corresponding pyranoside.[7]

(15) E. Hardegger, M. Schellenbaum, R. Huwyler and A. Züst, *Helv. Chim. Acta*, **40**, 1815 (1957).

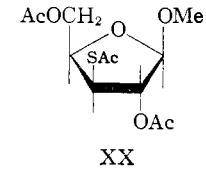
(16) Three minor contaminants were also present. These impurities were probably products of the apparent slow decomposition of the chloro tosylate VII/XII and the chlorine-containing product(s) of partial acetolysis, the latter affording the methyl dideoxyfuranoside[12] on hydrogenolysis with Raney nickel.

(17) Methyl 2,3-anhydro-4,6-*O*-benzylidene-α-D-allopyranoside undergoes ≥93% C.2-attack by ammonia (W. H. Myers and G. J. Robertson, THIS JOURNAL, **65**, 8 (1943)), a 4:1 ratio of C.2- to C.3 attack by *O,O*-dibenzylphosphoric acid in carbon tetrachloride (W. E. Harvey, J. J. Michalski and A. R. Todd, *J. Chem. Soc.*, 2271 (1951)) and a 1:3 ratio of C.2- to C.3- attack by hydrogen bromide in acetone (F. H. Newth, W. G. Overend and L. F. Wiggins, *ibid.*, 10 (1947)). The course of ring-opening of episulfides has been found to be more subject to changes from basic to acidic media than that of epoxides; see W. Davies and W. E. Savige, *ibid.*, 774 (1951).

(18) *Cf.* E. L. Eliel in "Steric Effects in Organic Chemistry," ed. by M. S. Newman, John Wiley and Sons, Inc., New York, N. Y., 1956, pp. 106–114.

(19) The change in nucleophilic reagent from ethyl mercaptide ion to acetate ion may also be significant. *E.g.*, methyl 2,3-anhydro-α-D-lyxofuranoside reacts more selectively with ammonia than with methoxide ion (*cf.* E. E. Percival and R. Zobrist, *J. Chem. Soc.*, 564 (1953), and ref. 5d. It is possible, therefore, that use of a nucleophile other than acetate ion (and/or a less polar reaction medium) would increase the selectivity of attack on IX.

(20) The considerably lower reactivity of the anhydro sugar I relative to cyclopentene oxide complicated the preparation of the required starting material, methyl 2,5-di-*O*-acetyl-3-(acetylthio)-3-deoxy-β-D-xylofuranoside (XX). Reaction of I with thiolacetic acid required the use of reflux temperature and long reaction periods, conditions under which thiolacetic acid decomposes to difficultly separable products. Under the conditions required for reaction of I with sodium hydrogen sulfide, considerable reaction of the initially formed mercaptan with a second mole of I occurred. The triacetate XX could, however, be obtained *via* either route. Simple hydrolysis of XX to the parent mercaptofuranoside appeared to be the main course of reaction on treatment with either aqueous sodium hydroxide or methanolic sodium methoxide. Use of a better leaving group than acetate ion[2] or other basic



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undergo nucleophilic attack *via* transition states which are more S_N2-like[16] than those derived from episulfonium ions, 2,3-epithiofuranosides may prove to react with the high selectivity characteristic of the reaction of 2,3-anhydrofuranosides. Consequently, methods for the synthesis of epithiofuranosides are still under investigation.

The above synthesis of methyl 3,5-di-*O*-acetyl-2-deoxy-β-D-ribofuranoside (XVII) represents the first successful conversion of an anhydropentoside to a 2-deoxypentoside[21] and indicates the feasibility of 2′-deoxynucleoside preparation *via* alkylthio migration. A synthesis of 2′-deoxyadenosine based on the present method is currently under investigation. Such a synthesis should be adaptable to the preparation of a variety of non-naturally occurring 2′-deoxynucleosides to be tested as possible anticancer agents.

Experimental[22]

Methyl 2,3-anhydro-β-D-ribofuranoside (I), prepared as previously described,[6] was shown by vapor-phase chromatography at 190°[23] to contain 1.5% of the corresponding α-anomer as its only contaminant.

Methyl 3-Deoxy-3-(ethylthio)-β-D-xylofuranoside (II).—A solution of 25.0 g. (0.171 mole) of I in 15 ml. of methanol was added to a sodium ethyl mercaptide solution prepared from 27.5 g. (0.51 mole) of sodium methoxide, 110 ml. of methanol and 41.3 ml. (0.56 mole) of ethanethiol. The mixture was refluxed under nitrogen for 19 hours, cooled, and evaporated *in vacuo* to a viscous sirup. The residue was dissolved in 350 ml. of water, and the solution was swirled in a spin evaporator with 100 g. of Amberlite IRC-50(H)[24] at about 35° (bath temperature) and 30–40 mm. pressure for 3 hours. The then neutral mixture was filtered, and the resin was washed with hot water (3 × 60 ml.). The combined filtrate and washings were evaporated to dryness *in vacuo*. The brownish residue was dissolved in 500 ml. of hot 3:1 benzene–methanol, decolorized with Norit A, and filtered. Evaporation of the filtrate, finally at 35° and 0.5 mm., gave 35.8 g. (100%) of II as an amber oil whose infrared spectrum was essentially identical with that of the analytical sample. The product of a preliminary small-scale run, obtained in 84% yield, was dried 2 hours at 100° and 0.1 mm. and used for analysis. It had $[\alpha]^{24}D$ −26° (4% in $CHCl_3$) and λ_{max}^{film} 2.94 (OH), 7.86 μ (EtS).[25] The absence of unreacted I was indicated by the lack of epoxide absorption at 11.6 μ.[6]

Anal. Calcd. for $C_8H_{16}O_4S$: C, 46.1; H, 7.74; S, 15.4. Found: C, 45.6; H, 7.61; S, 15.2.

The Methyl 2,5-Di-*O*-acetyl-3-deoxy-3-(ethylthio)-β-D-xylofuranoside (III).—A solution of 1.56 g. (7.50 mmoles) of II and 3.0 ml. (32 mmoles) of acetic anhydride in 15 ml. of reagent pyridine was allowed to stand at room temperature, protected from moisture, for 16 hours. Water (0.6 ml.) was then added with stirring and cooling. After 1 hour, the mixture was diluted with 60 ml. of chloroform, washed with 1 *M* aqueous sodium bicarbonate (4 × 20 ml.), and dried over magnesium sulfate. Filtration and evaporation, finally at 30° and 0.8 mm., afforded 2.09 g. of crude III. A 2.03-g. portion, evaporatively distilled at 90–100° (bath temperature) and 0.1 mm., gave a colorless liquid (1.89 g., 89% yield) having $n^{20}D$ 1.4778, $[\alpha]^{26}D$ −38° (1% in $CHCl_3$); and λ_{max}^{film} 5.71 (acetate C=O), 8.11 μ (acetate C–O–C).

Anal. Calcd. for $C_{12}H_{20}O_6S$: C, 49.3; H, 6.90; S, 11.0. Found: C, 49.2; H, 7.03; S, 10.7.

A vapor-phase chromatogram[23] run on this material showed the presence of two minor, unknown contaminants, but the major peak represented 94% of the total area under the curve. There was no indication of the peak characteristic of the 2-ethylthio isomer XIII, and it was possible to estimate that the maximum limit of contamination of III by XIII was about 3%.

Methyl 2,5-Di-*O*-benzoyl-3-deoxy-3-(ethylthio)-β-D-xylofuranoside (IV).—A solution of 5.6 g. (27 mmoles) of II in 100 ml. of reagent pyridine was cooled to 0°, and 9.4 ml. (81 mmoles) of benzoyl chloride was added dropwise with stirring. After 1 hour at 0°, the mixture, protected from moisture, was allowed to stand at room temperature for 47 hours. Water (1.0 ml.) then was added with stirring, and the mixture was evaporated *in vacuo* to a volume of about 35 ml. The concentrate was diluted with 100 ml. of chloroform, washed with 1 *M* aqueous sodium bicarbonate (6 × 35 ml.) and 35 ml. of water, and dried over magnesium sulfate. Filtration and evaporation, finally at 40° and 0.5 mm., afforded 10.2 g. of crude product, m.p. 67–72°. Recrystallization from ethyl acetate–hexane afforded three crops of white needles: (1) 5.59 g., m.p. 70–74°; (2) 0.93 g. m.p. 74–75°; and (3) (after recrystallization from heptane) 0.96 g., m.p. 74–75°; total yield 67%. The analytical sample, m.p. 74–75°, $[\alpha]^{25}D$ +2.5° (1% in $CHCl_3$), was prepared by recrystallization of the first crop from heptane. It had λ_{max}^{KBr} 5.80 (benzoate C=O), 7.88, 8.97 μ (benzoate C–O–C).

Anal. Calcd. for $C_{22}H_{24}O_6S$: C, 63.4; H, 5.81; S, 7.69. Found: C, 63.5; H, 6.03; S, 7.60.

Methyl 3-Deoxy-3-(ethylthio)-2,5-di-*O*-(*p*-nitrobenzoyl)-β-D-xylofuranoside (V).—A 0.58-g. sample of II was esterified by the procedure described for IV, except that *p*-nitrobenzoyl chloride was substituted for benzoyl chloride. The crude product was a tan glass (1.32 g.) that crystallized on trituration in ethyl acetate. Recrystallization from ethyl acetate gave three crops: (1) 880 mg., m.p. 119–122°; (2) 220 mg., m.p. 125–128°; and (3) 96 mg., m.p. 115–124°; total yield, 85%. Two recrystallizations of the first two crops from ethyl acetate–hexane and one from benzene–hexane afforded nearly white crystals (0.53 g.), m.p. 127–128°, $[\alpha]^{24}D$ +12° (2% in $CHCl_3$); λ_{max}^{KBr} 5.78 (aromatic ester C=O), 6.55, 7.42 μ (aromatic NO_2).

Anal. Calcd. for $C_{22}H_{22}N_2O_{10}S$: C, 52.2; H, 4.38; S, 6.32. Found: C, 52.1; H, 4.35; S, 6.43, 6.60.

Methyl 2,5-Di-*O*-acetyl-3-deoxy-β-D-xylo(ribo)furanoside (X).—A solution of 1.06 g. (2.76 mmoles) of III in 60 ml. of absolute ethanol containing 10 g. (wet) of suspended Raney nickel[26] was refluxed for 5 hours with stirring, then filtered hot through Celite. The catalyst and Celite were washed with hot ethanol (4 × 15 ml.), and the combined filtrate and washings were evaporated to dryness *in vacuo*. The residue, re-evaporated from toluene, was reacetylated with excess acetic anhydride in pyridine as described for the preparation of III, affording 0.73 g. of crude X. This was evaporatively distilled at 50–60° (bath temperature) and 0.15 mm., yielding 0.45 g. (54%) of colorless oil that had $n^{20}D$ 1.4496, $[\alpha]^{26}D$ −37° (2% in $CHCl_3$); λ_{max}^{film} 3.52 (methoxyl CH), 5.70 (acetate C=O), 8.08 μ (acetate C–O–C).

Anal. Calcd. for $C_{10}H_{16}O_6$: C, 51.7; H, 6.94. Found: C, 51.4; H, 7.03.

The above product gave positive tests with both the cysteine (in 75% sulfuric acid) and diphenylamine Dische reagents,[27] in contrast to blank tests run simultaneously. The tests were not run quantitatively, but it was apparent that neither test could qualitatively differentiate between 2- and 3-deoxypentoses.[28]

conditions may, however, prove effective for the synthesis of the desired episulfide.

(21) For a review of the earlier attempts to obtain 2-deoxyribosides from 2,3-anhydroribosides, see ref. 2 and W. G. Overend and M. Stacey in "The Nucleic Acids," Vol. I, ed. by E. Chargaff and J. N. Davidson, Academic Press, Inc., New York, N. Y., 1955, p. 9.

(22) Melting points were taken on a Fisher-Johns apparatus and, like boiling points, are uncorrected. Optical rotations were measured with a calibrated Standard polarimeter model D attachment to the Beckman DU spectrophotometer; see A. S. Keston, Abstracts of 127th Meeting, American Chemical Society, 18 C (1955).

(23) The vapor-phase chromatography was performed on a 6-foot column containing Dow-Corning 710 fluid on Johns-Manville Chromosorb at a temperature of 240° (unless otherwise noted) by W. E. Wilson of the Analytical Chemistry Section of Stanford Research Institute.

(24) A weak-acid cation exchange resin manufactured by Rohm and Haas Company, Philadelphia, Pa.

(25) A. Menefee, D. O. Alford and C. B. Scott, *J. Org. Chem.*, **22**, 792 (1957).

(26) "Sponge nickel catalyst" obtained from the Davison Chemical Co., Cincinnati 29, Ohio.

(27) Z. Dische in "The Nucleic Acids," Vol. I, ed. by E. Chargaff and J. N. Davidson, Academic Press, Inc., New York, N. Y., 1955, p. 286.

(28) *Cf.* R. Allerton, W. G. Overend and M. Stacey, *J. Chem. Soc.*, 255 (1952).

3-Deoxy-D-xylose(ribose) (XV) *p*-Nitrophenylosazone.—A solution of 99 mg. (0.43 mmole) of X in 3 ml. of reagent methanol containing 0.10 ml. of 1 *M* methanolic sodium methoxide was allowed to stand at room temperature for 16 hours in a stoppered flask. Neutralization with 3 drops of 2 *M* aqueous acetic acid and evaporation *in vacuo* afforded a colorless oil, which was refluxed in 4.0 ml. of 0.25 *M* aqueous sulfuric acid (under nitrogen) for 1.0 hour.[29] The cooled solution was neutralized by stirring with 1.0 g. of Dowex 2 (CO_3), then filtered and evaporated to dryness *in vacuo*. The residual crude XV was converted to its *p*-nitrophenylosazone,[7] a red, microcrystalline solid of m.p. 249–252° dec., yield 51 mg. (30%). One recrystallization from 95% ethanol gave material of constant m.p. 253.5–255.5° dec. (reported m.p. 253–255°[7] and, for the enantiomer, 254–256°[30]); λ_{max}^{KBr} 2.90 with shoulder at 3.03 (OH, NH, H_2O), 6.23 (C=N), 6.29 (aromatic C=C), 6.62 μ (NO_2, NH, phenyl). The analytical sample was dried 1 hour at 100° and 0.2 mm.

Anal. Calcd. for $C_{17}H_{18}N_6O_6 \cdot H_2O$: C, 48.6; H, 4.80; N, 20.0. Found: C, 48.4, 48.9; H, 4.72, 4.66; N, 19.8.

The enantiomer,[30] recrystallized from "ethanol" and dried by an unspecified procedure, had analyzed correctly for the anhydrous compound.

The product of the alternative ethyl mercaptide ring-opening of I could theoretically have been 2-deoxy-D-ribose, which forms a yellow *p*-nitrophenylhydrazone, m.p. 160°.[31]

Methyl 3(2)-Chloro-2,3-dideoxy-2(3)-(ethylthio)-5-*O*-tosyl-β-D-arabino(xylo)furanoside (VII/XII).—To a solution of 2.63 g. (12.6 mmoles) of II in 62 ml. of reagent pyridine was added 9.65 g. (50 mmoles) of *p*-toluenesulfonyl chloride with stirring, using a 20–25° water-bath for cooling. The mixture, protected from moisture, was left at room temperature for 68 hours, 0.62 ml. of water was added, and stirring was resumed for 2 hours. The mixture was evaporated to about 25-ml. volume *in vacuo* (bath temperature 35°) and poured into 100 ml. of water. The product was extracted with one 60-ml. and two 45-ml. portions of chloroform. The combined extracts were washed with water (2 × 25 ml.) and dried over magnesium sulfate. Filtration and evaporation *in vacuo* (bath temperature 35°) afforded an oil which was dissolved in about 15 ml. of toluene and re-evaporated as before. The residue was dissolved in about 15 ml. of benzene and again evaporated, finally at 30° and 0.5 mm. This gave 4.36 g. (91%) of VII/XII as a light brown oil; λ_{max}^{film} 6.27 (phenyl), 7.32 (CH_3 and $-OSO_2-$), 7.90 (SEt[25]), 8.42, 8.50 μ ($-OSO_2-$). There was no absorption near 3.0 μ.[32] This product gave a hot ethanolic silver nitrate test for chloride. A portion dried at 56° and 0.1 mm. for 1.5 hours was analyzed.

Anal. Calcd. for $C_{15}H_{21}ClO_5S_2$: C, 47.3; H, 5.56; Cl, 9.31; S, 16.8. Found: C, 47.4; H, 5.62; Cl, 9.47; S, 16.9.

The product of a preliminary run, obtained in 98% yield, had $[\alpha]^{24}D$ −14° (2% in $CHCl_3$).

Methyl 3(2)-Chloro-2,3-dideoxy-2(3)-(ethylthio)-5-*O*-mesyl-β-D-arabino(xylo)furanoside (VI/XI).—Treatment of 0.64 g. of II as described above but substituting mesyl chloride for tosyl chloride gave, in the one run made, a 69% yield of VI/XI as an amber oil having λ_{max}^{film} 7.36 (CH_3 and $-OSO_2-$), 7.89 (SEt[25]), 8.50 μ ($-OSO_2-$). There was no absorption in the region of 3.0 μ. This material gave a positive Beilstein test. For analysis, a portion was dried at 56° and 0.1 mm. for 1 hour.

Anal. Calcd. for $C_9H_{17}ClO_5S_2$: C, 35.5; H, 5.62; S, 21.0. Found: C, 35.7; H, 5.61; S, 21.0.

Methyl 3,5-Di-*O*-acetyl-2-deoxy-2-(ethylthio)-β-D-arabinofuranoside (XIII).—A solution of 2.43 g. (6.38 mmoles) of chloro tosylate VII/XII and 1.95 g. (24 mmoles) of anhydrous sodium acetate in 20 ml. of 95% aqueous methyl Cellosolve was refluxed for 1.5 hours, during which time it became dark in color. The mixture, which contained some precipitated salts, was cooled, diluted with 60 ml. of chloroform, washed with water (3 × 25 ml.), and dried over magnesium sulfate. On filtration and evaporation *in vacuo*, an oil was obtained that showed some infrared hydroxyl absorption. Reacetylation, using excess acetic anhydride in pyridine as described for III, gave 1.29 g. of residual brown oil which was evaporatively distilled at 90–100° (bath temperature) and 0.10 mm. and collected in three fractions: (1) 0.22 g., $n^{20}D$ 1.4826; (2) 0.47 g., $n^{20}D$ 1.4786, $[\alpha]^{26}D$ −55° (1% in $CHCl_3$); and (3) 0.59 g., $n^{20}D$ 1.4768; total yield of yellow oil, 71%. This second fraction, which was used for analysis, had λ_{max}^{film} 3.52 (methoxyl CH), 5.70 (acetate C=O), 8.09 μ (acetate C—O—C), and, like the crude product, showed no tosylate absorption.

Anal. Calcd. for $C_{12}H_{20}O_6S$: C, 49.3; H, 6.90; S, 11.0. Found: C, 48.9; H, 6.94; S, 11.3, 11.4.

Vapor-phase chromatography[23] of fraction 2 indicated the presence of five components. The fourth and fifth peaks, which were assigned to XIII and III, respectively, represented 83% of the total area under the curve and had an area ratio of 4.2:1.0.

In a second preparation, the crude reacetylated product obtained from 4.56 g. of VII/XII was evaporatively distilled at 90° (bath temperature) and 0.10 mm., affording a 67% yield of pale yellow oil collected as a single fraction, $n^{20}D$ 1.4810. Elemental analysis indicated the presence of 2.22% chlorine. Vapor-phase chromatography[23] of this total distillate indicated the presence of six components. The fifth and sixth peaks, which were assigned to XIII and III, respectively, represented 74% of the total area under the curve and had an area ratio of 3.5:1.0. In a third experiment, the acetolysis period was extended to 2.25 hours with no significant change in the yield, the ratio of XIII to III, or the chlorine content of the product.

Methyl 2-Deoxy-2-(ethylthio)-3,5-di-*O*-(*p*-nitrobenzoyl)-β-D-arabinofuranoside (XIV).—A solution of 0.36 g. (1.2 mmoles) of distilled acetolysis product from the preceding experiment in 15 ml. of reagent methanol containing 0.2 ml. of 1 *M* methanolic sodium methoxide was refluxed for 0.5 hour and evaporated to dryness *in vacuo*. The residue was dissolved in benzene, re-evaporated *in vacuo*, and esterified with a 100% excess of *p*-nitrobenzoyl chloride in pyridine as described for IV; yield 0.56 g., m.p. 135–141°. A 0.52-g. portion of the brownish crude product was recrystallized from ethyl acetate–hexane, using Norit A for decolorization, affording 214 mg. of XIV, m.p. 147–148°. Evaporation of the mother liquor *in vacuo* and two recrystallizations of the resultant residue from benzene–heptane afforded an additional 101 mg., m.p. 142–147°, total yield 55%. Recrystallization of the first crop from benzene–heptane gave 190 mg. of off-white crystals, m.p. 149–150°, $[\alpha]^{24}D$ −64° (1% in $CHCl_3$); λ_{max}^{KBr} 5.79 (aromatic ester C=O), 6.54, 7.40 μ (aromatic NO_2).

Anal. Calcd. for $C_{22}H_{22}N_2O_{10}S$: C, 52.2; H, 4.38; S, 6.32. Found: C, 52.5; H, 4.47; S, 6.28, 6.08.

Methyl 3,5-Di-*O*-acetyl-2-deoxy-β-D-ribofuranoside (XVII).—Distilled 2-ethylthio acetate XIII (2.43 g., 8.33 mmoles) from the second preparation described above was desulfurized by the procedure described for the isomer III, but using the more active Raney nickel "C" of Hurd and Rudner.[33] This afforded 1.89 g. of reacetylated crude product which was distilled at 2 μ pressure and collected as two fractions. Fraction 1, which analyzed for a methyl dideoxypentoside acetate, was a colorless liquid (235 mg., 16%), b.p. ≤ room temperature (condensed in an ice-cooled receiver), $n^{20}D$ 1.4333, $[\alpha]^{31}D$ −50° (1% in $HCCl_3$); λ_{max}^{film} 3.51 (methoxyl CH), 5.71 (acetate C=O), 8.05 μ (acetate C–O–C).

Anal. Calcd. for $C_8H_{14}O_4$: C, 55.2; H, 8.10. Found: C, 54.7; H, 8.23; S, 0.29.

Fraction 2, the desired XVII, was also a colorless liquid (934 mg., 48%), b.p. 62–72° (mainly 70–72°), $n^{20}D$ 1.4440; $[\alpha]^{31}D$ −55° (2% in $CHCl_3$); λ_{max}^{film} 3.52 (methoxyl CH), 5.72 (acetate C=O), 8.07 μ (acetate C–O–C).

Anal. Calcd. for $C_{10}H_{16}O_6$: C, 51.7; H, 6.94. Found: C, 51.7; H, 6.78; S, 0.50.

Fraction 2 gave positive tests with both the cysteine (in 75% sulfuric acid) and diphenylamine Dische reagents.[27]

2-Deoxy-D-ribose (XIX) Anilide.—A solution of 809 mg. (3.48 mmoles) of XVII in 6 ml. of reagent methanol containing 0.01 ml. of 1 *M* methanolic sodium methoxide was left

(29) *Cf.* the hydrolysis of the corresponding D-[7] and L-pyranosides.[30]

(30) S. Mukherjee and A. R. Todd, *J. Chem. Soc.*, 969 (1947).

(31) R. Allerton and W. G. Overend, *ibid.*, 1480 (1951).

(32) A 0.95 *M* solution of this product in carbon tetrachloride examined in a 0.55-mm. cell showed approximately half the hydroxyl absorption exhibited at 2.80 and 2.89 μ by a 0.010 *M* carbon tetrachloride solution of II under the same conditions.

(33) C. D. Hurd and B. Rudner, THIS JOURNAL, **73**, 5157 (1951).

at room temperature for 23 hours (stoppered), then evaporated to dryness *in vacuo*. The residue was dissolved in 15 ml. of water, neutralized by stirring with 50 mg. of Amberlite IRC-50(H),[34] filtered, and evaporated to dryness *in vacuo*. The residual crude XVIII was a colorless oil which, after solution in absolute ethanol and re-evaporation (finally at 35° and 0.9 mm.), weighed 528 mg. (102%) and showed no carbonyl absorption in the infrared. On periodate oxidation, this oil consumed only 0.042 ± 0.005 equivalent of oxidant. The remaining 499 mg. was dissolved in 50 ml. of 0.01 *M* aqueous acetic acid, and the *p*H (measured potentiometrically) was adjusted to that of 0.01 *M* aqueous acetic acid by the addition of 0.3–0.4 ml. of 2 *M* aqueous acetic acid. The resulting solution was diluted with an additional 12.5 ml. of 0.01 *M* aqueous acetic acid, then refluxed under nitrogen for 1.75 hours.[14] The cooled, colorless solution, which gave a positive Benedict test, was evaporated to dryness *in vacuo* (bath 40°). The residual crude XIX, after solution in methanol and re-evaporation *in vacuo*, was converted to its anilide (402 mg.), m.p. 150–153° dec., by the method of Hardegger, *et al.*[15] Recrystallization from methanol gave 304 mg. (41%) of white crystals, m.p. 173–175° dec. A mixture with authentic anilide[34] gave no depression in m.p.; the infrared spectra of the two samples were identical. The product of a preliminary run had m.p. 175–177° dec. and $[\alpha]^{27}D$ +175° (1% in pyridine); reported[15] m.p. 172–173° dec. and $[\alpha]D$ +171° (1% in pyridine).

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

Sedimentation Studies of Fractions of Deoxyribonucleic Acid[1]

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Eight fractions of calf thymus DNA obtained by chromatography on a column of the anion-exchanger ECTEOLA have been examined in the analytical ultracentrifuge in dilute solution (0.003%). These fractions, amounting to 56.5% of the DNA recovered from the column with eluents of increasing ionic strength and then increases in *p*H, showed progressive increases in $s_{50\%}$ from 10.9 to 24.1 *S* (compared with 15.8 *S* for the original). The original DNA showed the usual wide spread in sedimentation coefficients. The spread was very narrow for the early fractions but broadened considerably for the later ones. Evidence is presented that the fractionation procedure discriminates among DNA's, in part, on the basis of properties related to their sedimentation coefficients and that the method does not lead to measurable physical alterations.

It has been shown[3-6] that the deoxyribonucleic acid (DNA) of various sources could be separated into different fractions by means of chromatography on the substituted cellulose anion exchanger ECTEOLA.[7] These fractions were found to differ in their biological activity and base composition.[4,6,8,9] From a study of the behavior of various nucleotides and DNA specimens on columns of ECTEOLA, it was inferred that the chromatography could discriminate among DNA molecules on the basis of size.[5,6,10] The present report deals with a study of the sedimentation behavior of such chromatographic fractions in the analytical ultracentrifuge.[11]

Earlier studies have indicated that DNA was heterodisperse with respect to sedimentation coefficient.[12,13] In order to assess conclusions drawn from physico-chemical measurements on such inhomogeneous material, the validity of which might be influenced by the polydispersity,[14,5] a number of approaches have been made to obtain more homogeneous specimens. Schumaker and Schachman[13] obtained a fraction of DNA with a narrower sedimentation distribution than the original by applying the technique of zone centrifugation. Shooter and Butler[15,16] (*cf.* also[17]), using moving boundary centrifugation, obtained a fraction of DNA with a lower average sedimentation coefficient than the original.

Experimental

Nucleic Acid.—The DNA was prepared from fresh calf thymus glands by the Schwander and Signer method.[18] Several of the properties of this preparation (designated as "S-II" in a previous publication[19] have been reported.[6,19]

Chromatography.—An adaptation of the previously described[3,6] chromatographic method was used to fractionate 78.5 mg. of the DNA (1 mg. per ml. of 0.001 *M* NaCl) adsorbed on a column (7.4 × 2.8 cm.) of ECTEOLA-SF-1[6] in the cold room. The column was washed with 425 ml. of tris buffer (tris-(hydroxymethyl)-aminomethane), 0.01 *M*, *p*H 7. A gradient elution system with two mixing chambers was attached to the column. The description and mathematical treatment of this arrangement have been discussed previously.[6] The stock solution, saturated with chloroform and consisting of 0.5 *M* NaCl (in 0.01 *M* tris), was allowed to drip into the first mixing chamber containing 250 ml. of

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