in water (20 ml.). The product from each aliquot was isolated by extraction with chloroform as previously described,[8] then dried *in vacuo* over P_2O_5 and NaOH for several days and assayed for radioactivity in the usual fashion.[8] The first-order rate constants for loss of Cl acetoxy were calculated for each aliquot by means of the usual equation[21]

$$k_{exch} = \frac{2.3}{t} \log \frac{a}{a - x}$$

where a is the specific radioactivity of the sample at zero time and $a - x$ that of the product from the aliquot removed at time t. The radio-chemical and rate constant data for each experiment are given in Table III.

TABLE III

ACETOXY EXCHANGE RATE DATA FOR VARIOUS C-1 ACETOXY LABELED ACETYLATED D-ALDOPYRANOSES IN 1:1 ACETIC ANHYDRIDE–ACETIC ACID AT 25°

Acetylated D-aldopyranose	[H_2SO_4], mole/l.	t, min.	Assay, mc./mole	k_{exch}, min.$^{-1}$
α-Glucose	0.50	0	0.500	.....
		60	.389	0.00418
		90	.341	.00429
		120	.303	.00417
		150	.265	.00423
			Av.	0.00422 ± 0.00004
β-Glucose	.50	0	1.849	.....
		1	1.168	0.459
		2	0.784	.428
		3	.489	.443
		4	.342	.422
		5	.235	.413
			Av.	0.433 ± 0.014
β-Glucose	.10	0	1.849	....
		3	1.559	0.0565
		6	1.345	.0531
		9	1.152	.0525
		12	0.970	.0536
		15	0.811	.0550
			Av.	0.0542 ± 0.0013
α-Mannose[8]	.50	..	...	
			Av.	0.0189 ± 0.0004
β-Mannose[8]	.50	..	...	
			Av.	0.0333 ± 0.0018
β-Galactose	.10	0	1.831	...
		3	1.250	0.127
		6	0.905	.120
		9	.656	.114
		12	.473	.113
		15	.344	.112
			Av.	0.117 ± 0.005
6-Deoxy-α-glucose	.10	0	0.486	....
		20	.392	0.0107
		35	.338	.0104
		50	.292	.0102
		70	.243	.0096
			Av.	0.0102 ± 0.0003
6-Deoxy-β-glucose	.10	0	1.867	...
		2	0.804	0.421
		4	.405	.382
		6	.203	.370
		8	.108	.357
		10	.0596	.344
			Av.	0.375 ± 0.021
β-Xylose	.05	0	1.903	...
		4	0.914	0.184
		6	.634	.183
		8	.455	.179
		10	.320	.178
			Av.	0.181 ± 0.003
β-Glucose[a]	.50	0	1.849	....
		30	0.339	0.0565
		60	.0709	.0545
		90	.0175	.0518
			Av.	0.0543 ± 0.0016

[a] In 100% acetic acid solvent testing <0.1% water and <0.5% acetic anhydride by vapor–liquid partition chromatographic analysis. Solvent kindly furnished and analyzed by Prof. R. H. Eastman.

This experimental method was adopted since Lemieux and co-workers have shown[6] that the Cl acetoxy exchange rate constants obtained by radio-activity assay of the crude reaction products accorded with those obtained by assay of the reaction products after separation and purification using column chromatography on Magnesol.

STANFORD, CALIF.

(21) W. A. Bonner and C. J. Collins, THIS JOURNAL, **77**, 102 (1955).

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

Potential Anticancer Agents.[1] XXIII. The Anomeric Ethyl 1-Thio-D-arabinofuranosides

BY ELMER J. REIST, PHILLIP A. HART, LEON GOODMAN AND B. R. BAKER

RECEIVED APRIL 25, 1959

The two crystalline anomeric ethyl 1-thio-D-arabinofuranosides (XV and XXIII) have been prepared. The β-anomer XV was synthesized through the cyclization of 5-O-benzoyl-D-arabinose diethyl mercaptal with mercuric chloride and cadmium carbonate. The synthesis of the α-anomer XXIII involved the stereospecific attack of ethyl mercaptide ion on 3,5-di-O-benzoyl-D-arabinofuranosyl chloride. A possible use of the α-anomer as a precursor in a deoxynucleoside synthesis is discussed.

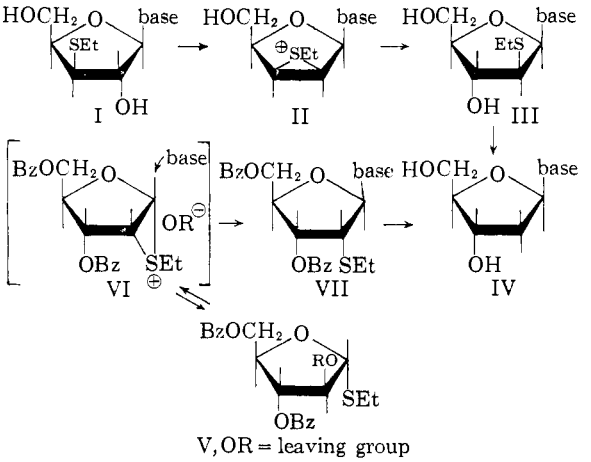
Although the important aspects of the structure of purine deoxynucleosides have been known for thirty years,[2] efforts toward their synthesis by conventional methods[3] have failed. The recent suc-

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, Contract No. SA-43-ph-1892. For the preceding paper of this series *cf.* L. Goodman and B. R. Baker, THIS JOURNAL, **81**, 4294 (1959).

(2) (a) P. A. Levene and F. S. London, *J. Biol. Chem.*, **81**, 711 (1929); **83**, 793 (1929); (b) W. Klein, *Z. physiol. Chem.*, **224**, 244 (1934); **255**, 81 (1938).

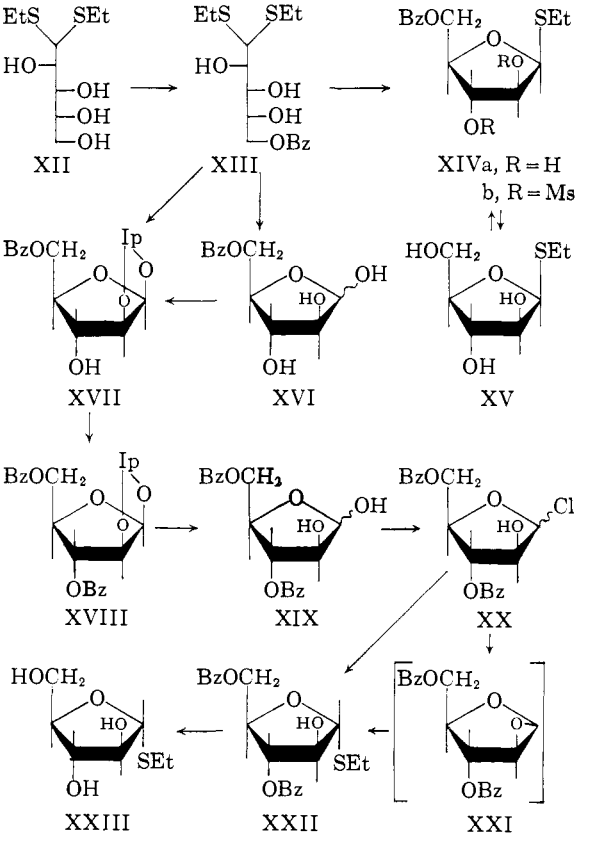
(3) For a summary of this work, see L. Goodman, A. Benitez and B. R. Baker, paper I of this series, THIS JOURNAL, **80**, 1680 (1958).

cessful synthesis of 2′-deoxyadenosine in this Laboratory[4] utilized as the key reaction an ethylthio migration from C_3' of 6-amino-9-[3′-deoxy-3′-(ethylthio)-β-D-xylofuranosyl]-purine (I) to C_2' by a nucleophilic attack on the 2,3-episulfonium ion II, thus forming 6-amino-9-[2′-deoxy-2′-(ethylthio)-β-D-arabinofuranosyl]-purine (III). Desulfurization of III with Raney nickel then gave 2′-deoxyadenosine (IV).



It is conceivable that a 2′-alkylthio nucleoside could be created through a nucleophilic attack on a 1,2-episulfonium ion in a manner somewhat similar to that used on the 2,3-episulfonium ion,[2] in which a purine (or pyrimidine) base is the attacking nucleophile (V → VII). The stereochemistry of such a migration requires that the 1-alkylthio group have a *trans* configuration with respect to the C_2-leaving group. This requirement plus the additional feature that the ultimate substituent on C_1 must have the β-configuration common to the naturally occurring nucleosides restricts the choice of starting sugar to a suitably substituted ethyl 1-thio-α-D-arabinofuranoside (V). The stereochemically controlled synthesis of ethyl 3,5-di-O-benzoyl-1-thio-α-D-arabinofuranoside (XXII), a material which is suitably blocked to insert an appropriate leaving group on C_2, is the subject of this paper.

There are numerous references to the conversion of glucose diethyl mercaptal (VIII) to ethyl 1-thio-α-D-glucofuranoside (IX)[5] by the use of 1 mole equivalent of mercuric chloride. That this reaction did not always proceed in this desired fashion was shown by Green and Pacsu,[5b] who, in attempting the same transformation on D-galactose diethyl mercaptal and on L-arabinose diethyl mercaptal in ethyl alcohol, were able to isolate only the oxygen glycoside (as illustrated by X → XI). It has now been found that when 5-O-benzoyl-D-arabinose diethyl mercaptal (XIII)[6] was treated with 1 mole equivalent of mercuric chloride in acetone in the presence of excess cadmium carbonate[5c] as an acid acceptor, yields up to 67% of a crystalline anomer of ethyl 5-O-benzoyl-1-thio-D-arabinofuranoside (XIVa) were formed. Treatment of XIVa with an excess of mesyl chloride at 0° gave a 68% yield of a crystalline dimesylate XIVb. Debenzoylation of XIVa with methanolic sodium methoxide gave the unblocked ethyl 1-thio-D-arabinofuranoside (XV)



in 31% yield after three recrystallizations. The optical rotations of the monobenzoate XIVa and the unblocked thioglycoside XV were −96.5 and −130°, respectively, thus suggesting strongly that the configuration of the ethylthio group at C_1 was

(4) C. D. Anderson, L. Goodman and B. R. Baker, THIS JOURNAL, **80**, 6453 (1958); paper XIX of this series, *ibid.*, **81**, 3967 (1959).

(5) (a) W. Schneider and J. Sepp, *Ber.*, **49**, 2054 (1916); (b) J. W. Green and E. Pacsu, THIS JOURNAL, **59**, 1205 (1937); **60**, 2056 (1938); (c) M. L. Wolfrom, D. I. Weisblat and A. R. Hanze, *ibid.*, **66**, 2065 (1944).

(6) Selective benzoylation of D-arabinose diethyl mercaptal (XII)[7] by the procedure used by Lieser and Schweizer[8] on L-arabinose diethyl mercaptal gave a 72% yield of 5-O-benzoyl-D-arabinose diethyl mercaptal (XIII).

(7) (a) M. L. Wolfrom, D. I. Weisblat, W. H. Zophy and S. W. Waisbrot, *ibid.*, **63**, 201 (1941); (b) E. Fischer, *Ber.*, **27**, 673 (1894).

(8) T. Lieser and R. Schweizer, *Ann.*, **519**, 271 (1935).

β. This supposition was further borne out by comparison with the corresponding α-ethylthio arabinoside XXIII, which was synthesized by a different route described below.

Since the preceding sequence of reactions gave a thioglycoside of arabinose of undesired anomeric configuration, an alternate synthesis of the thioglycoside was devised which was expected to give exclusively the desired α-anomer. This sequence is outlined in XVI → XXII.

Earlier experiments on the cyclization of the mercaptal XIII to the thioglycoside XIVa, in which mercuric oxide was used as the acid acceptor in place of cadmium carbonate, gave the expected thioglycoside XIVa when run on a relatively small scale. Scale-up of the reaction, however, gave a product which was contaminated with considerable amounts of 5-O-benzoyl-1,2-O-isopropylidene-D-arabinofuranose (XVII). The probable explanation for this anomaly is that mercuric oxide, due to its low solubility in the reaction medium, cannot take up the hydrogen chloride liberated in the reaction at a rate sufficient to maintain neutrality. The resulting slightly acid conditions are adequate to remove the remaining ethylthio group of XIVa and cause reaction with the acetone solvent to give the observed 1,2-O-isopropylidene derivative XVII. Repetition of this reaction with excess mercuric chloride in the absence of an acid acceptor was found to give yields up to 50% of XVII, in contrast to the 40% over-all yield of XVII obtained by the conventional two-step method of removal of the ethylthio groups followed by mineral acid-catalyzed acetonation of the resulting 5-O-benzoyl-D-arabinose (XVI).

Further benzoylation of the monobenzoate XVII under standard conditions gave an 80% yield of crystalline 3,5-di-O-benzoyl-1,2-O-isopropylidene-D-arabinofuranose (XVIII). Treatment of XVIII with 50% aqueous acetic acid containing hydrochloric acid gave a quantitative yield of 3,5-di-O-benzoyl-D-arabinose (XIX) as an uncrystallizable oil.

Treatment of XIX with hydrogen chloride in methylene chloride[9] gave crude 3,5-di-O-benzoyl-D-arabinofuranosyl chloride (XX) as a gum which was treated directly with sodium ethyl mercaptide in ethyl mercaptan to give an uncrystallizable oil whose analysis and infrared spectrum were in agreement with those expected for ethyl 3,5-di-O-benzoyl-1-thio-D-arabinofuranoside (XXII). The rotation of this oil was +159°, thus indicating that this material was indeed the α-ethyl anomer. Debenzoylation of XXII with methanolic sodium methoxide under standard conditions gave, after three recrystallizations, 44% of a crystalline ethyl 1-thio-α-D-arabinofuranoside (XXIII), as shown by its analysis and rotation. The rotation of XXIII was +240° as compared with −130° observed for XV, thus substantiating the postulate concerning the anomeric configurations of the benzoylated thioglycosides XIVa and XXII.

The apparent exclusive formation of the α-thioglycoside XXII from a chloro sugar XX, which must undoubtedly be a mixture of α- and β-anomers, could be expected. Micheel and Klemer[10] have found that treatment of either α- or β-1-fluoro derivatives of aldoses with sodium methoxide gave a methyl glycoside whose configuration at C_1 was *trans* with respect to the C_2-hydroxyl. This observation was explained by the formation of an intermediate 1,2-epoxide as illustrated by XXI when the starting fluorine atom is *trans* to the C_2-hydroxyl. Subsequent cleavage of the epoxide by methoxyl generates the C_1–C_2 *trans*-glycoside. When the starting fluorine atom is *cis* to the C_2-hydroxyl, epoxide formation is impossible and the C_1–C_2 *trans*-glycoside is formed by a simple SN2 displacement of fluoride by methoxide. This same principle should hold in the reaction of the chloro sugar XX with sodium ethyl mercaptide and both anomers of XX should give only the α-anomer of the thioglycoside XXII.

This use of a free hydroxyl at C_2 of a sugar to accomplish the synthesis of an essentially anomerically pure product with a C_1–C_2 *trans* configuration from an anomerically mixed starting material could have further utility in sequences calling for subsequent selective reaction at the C_2 position of the sugar molecule.

Experimental[11]

5-O-Benzoyl-D-arabinose Diethyl Mercaptal (XIII).—D-Arabinose diethyl mercaptal (XII)[7] (10 g.) was benzoylated by the procedure described by Lieser and Schweizer[8] for the benzoylation of L-arabinose diethyl mercaptal to give 10.0 g. (72%) of crystalline XIII, m.p. 117–118°, $[\alpha]^{27}D$ −52.8° (1% in chloroform); λ_{max}^{Nujol} (μ) 5.88 (benzoate C=O), 7.80 (benzoate C—O—C). Lieser and Schweizer[8] reported a yield of 73%, m.p. 119°, and $[\alpha]^{22}D$ +49.5° (2% in chloroform) for 5-O-benzoyl-L-arabinose diethyl mercaptal.

Anal. Calcd. for $C_{16}H_{24}O_5S_2$: C, 53.3; H, 6.71; S, 17.8. Found: C, 53.1; H, 6.60; S, 18.1.

Ethyl 5-O-Benzoyl-1-thio-β-D-arabinofuranoside (XIVa).—To a stirred solution of 5.0 g. (14 mmoles) of 5-O-benzoyl-D-arabinose diethyl mercaptal (XIII) in 60 ml. of acetone was added 4.5 g. (26 mmoles) of cadmium carbonate followed by 3.75 g. (13.8 mmoles) of mercuric chloride. The suspension was stirred for 18 hours, then filtered. The precipitate was washed with 10 ml. of acetone. To the combined filtrate and washings was added 140 ml. of 10% aqueous potassium iodide. The solution immediately turned cloudy and was held at 0° for 1 hr., then filtered. The precipitate was washed first with a solution of 40 ml. of acetone and 110 ml. of 10% aqueous potassium iodide, then 100 ml. of water. The solid, on recrystallization from benzene, gave 1.58 g. (38%) of white crystals, m.p. 117–118°, $[\alpha]^{22}D$ −96.5° (2.2% in chloroform). This material had an infrared spectrum identical with that of the analytical sample prepared in a different run.

Anal. Calcd. for $C_{14}H_{18}O_5S$: C, 56.4; H, 6.08; S, 10.7. Found: C, 56.7; H, 6.27; S, 10.3.

(9) This procedure was described by R. K. Ness and H. G. Fletcher, Jr., THIS JOURNAL, **78**, 4710 (1956), for the preparation of 3,5-di-O-benzoyl-D-ribofuranosyl chloride from 1,3,5-tri-O-benzoyl-D-ribofuranose.

(10) F. Micheel and A. Klemer, *Ber.*, **91**, 663 (1958).

(11) Melting points were taken on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Standard polarimeter model D attachment to the Beckman DU spectrophotometer calibrated with standard sucrose solutions. Paper chromatograms were run with water-saturated butyl alcohol by the descending procedure on Whatman No. 1 paper. The spots were located by a bromine spray described by Weygand, Bestmann and Ziemann[12] for the detection of mercaptals, which had been modified as follows: solution A consisted of 10 ml. of methyl Cellosolve, 10 ml. of water and 0.03 ml. of bromine; solution B consisted of a 0.01% solution of methyl orange in water. Adenine was used as a standard and spot locations were expressed as *R*Ad units, with adenine at 1.00.

(12) F. Weygand, H. J. Bestmann and H. Ziemann, *Ber.*, **91**, 1040 (1958).

The combined filtrate and washings above were extracted with three 60-ml. portions of chloroform. The chloroform extracts were washed with two 50-ml. portions of water, then combined and dried over magnesium sulfate. The chloroform was removed *in vacuo* to yield a white solid. Recrystallization from benzene gave 1.20 g. (total 67%) of crystalline material, m.p. 112–114°, whose infrared spectrum was identical with that of crop 1 and the analytical sample.

Ethyl 5-O-Benzoyl-2,3-di-O-mesyl-1-thio-β-D-arabinofuranoside (XIVb).—A solution of 1.35 g. (4.5 mmoles) of ethyl 5-O-benzoyl-1-thio-β-D-arabinofuranoside (XIVa) in 11 ml. of pyridine was cooled to 0°. To this solution was added 0.75 ml. (9.5 mmoles) of mesyl chloride dropwise with stirring. The reaction mixture was kept at 0° for 18 hr., then decomposed by the addition of 5 drops of water to the cold, stirred solution. After 15 min., the solution was poured into 10 ml. of saturated aqueous sodium bicarbonate solution. The mixture was extracted with two 10-ml. portions of chloroform. The chloroform extracts were washed in succession with 10 ml. of saturated aqueous sodium bicarbonate and two 10-ml. portions of water, then combined and dried over magnesium sulfate. Removal of the chloroform *in vacuo* yielded 2.0 g. of an oil which crystallized on addition of methanol. Recrystallization from methanol gave 1.4 g. (68%) of white crystals, m.p. 75–76°; $[\alpha]^{27}D$ −79.2° (1% in chloroform); $\lambda_{max}^{Nujol}(\mu)$ 5.82 (benzoate C=O), 7.33 (CH_3, sulfonate), 8.50 (sulfonate).

Anal. Calcd. for $C_{16}H_{22}O_9S_3$: C, 42.3; H, 4.88; S, 21.2. Found: C, 42.4; H, 4.96; S, 21.0.

5-O-Benzoyl-1,2-O-isopropylidene-D-arabinofuranose (XVII). A.—A mixture of 5.0 g. (14 mmoles) of 5-O-benzoyl-D-arabinose diethyl mercaptal (XIII) and 10.0 g. (37 mmoles) of mercuric chloride in 60 ml. of acetone was stirred at 50–55° for 2 hr. Toward the end of the reaction, the mixture turned very dark. After being cooled, the reaction mixture was filtered and the precipitate was washed with three 10-ml. portions of acetone. After the addition of 2 ml. of pyridine, the filtrate was concentrated to dryness *in vacuo*. The residue was partitioned between 50 ml. of chloroform and 75 ml. of 10% aqueous potassium iodide solution. The chloroform layer was washed with an additional 75 ml. of 10% aqueous potassium iodide, then with two 25-ml. portions of water. The aqueous phases were back-extracted with two 40-ml. portions of chloroform. The combined chloroform extracts were dried over magnesium sulfate, then evaporated to dryness *in vacuo* to yield a brown oil which crystallized on standing. Recrystallization from toluene yielded 2.0 g. (49%) of a white product, m.p. 145°, in two crops. The infrared spectrum was identical with that of the analytical sample prepared by procedure B and the mixed melting point of the two gave no depression.

B.—Treatment of 30 g. of 5-O-benzoyl-D-arabinose diethyl mercaptal (XIII) with mercuric chloride and cadmium carbonate in water, a procedure described by Lieser and Schweizer[8] for the preparation of 5-O-benzoyl-L-arabinose, gave 19 g. of 5-O-benzoyl-D-arabinose (XVI) as a brown sirup.

Crude XVI was dissolved in 600 ml. of dry acetone and filtered to remove insoluble impurities. The filtrate was stirred for 20 hr. with 39.0 g. of anhydrous copper sulfate and 1.2 ml. of 96% sulfuric acid. The mixture was filtered and the filtrate was neutralized with calcium hydroxide. The resultant calcium sulfate was removed by filtration and the filtrate was concentrated to dryness. The residue was dissolved in 210 ml. of chloroform, then the chloroform solution was washed with two 70-ml. portions of water. The chloroform layer was dried over magnesium sulfate, filtered, then evaporated to dryness to give 21 g. of crystalline solid. Recrystallization from ethyl acetate–cyclohexane gave 11 g. (45% based on XIII) of crystalline material, m.p. 148–149°, $[\alpha]^{27}D$ + 24.0° (1% in chloroform); $\lambda_{max}^{KBr}(\mu)$ 2.95 (OH), 5.82 (benzoate C=O), 7.25 (*gem*-dimethyl).

Anal. Calcd. for $C_{15}H_{18}O_6$: C, 61.2; H, 6.17. Found: C, 60.8; H, 6.05.

3,5-Di-O-benzoyl-1,2-O-isopropylidene-D-arabinofuranose (XVIII).—A solution of 0.5 g. (2 mmoles) of 5-O-benzoyl-1,2-O-isopropylidene-D-arabinofuranose (XVII) in 3 ml. of dry pyridine was cooled in an ice-bath and 0.5 ml. of benzoyl chloride (3 mmoles) was added dropwise with stirring. The mixture was stored at 3° for 21 hr., protected from moisture, then diluted with 3 ml. of chloroform. This solution was washed with ice-water and with aqueous sodium bicarbonate, then dried over magnesium sulfate. The solution was evaporated to dryness and the last traces of pyridine were removed by the addition and evaporation of 3 portions of toluene. The crude product (0.84 g.) was recrystallized from methanol to yield 0.50 g. (75%) of white needles, m.p. 81–82°, $[\alpha]^{26}D$ +20.4° (1% in chloroform); $\lambda_{max}^{KBr}(\mu)$ 5.83 (benzoate C=O), 7.26 (*gem*-dimethyl).

Anal. Calcd. for $C_{22}H_{22}O_7$: C. 66.3; H, 5.57. Found: C, 66.2; H, 5.70.

Ethyl 3,5-Di-O-benzoyl-1-thio-α-D-arabinofuranoside (XXII).—A solution of 1.0 g. (2.5 mmoles) of 3,5-di-O-benzoyl-1,2-O-isopropylidene-D-arabinofuranose (XVIII) in 10 ml. of 50% aqueous acetic acid containing 0.22 ml. of concentrated hydrochloric acid was heated in a bath at 80° for 40 min. This solution was cooled and extracted with two 15-ml. portions of chloroform. The chloroform extracts were washed with saturated aqueous sodium bicarbonate, then water, dried over magnesium sulfate and evaporated to dryness *in vacuo* to yield 0.94 g. of 3,5-di-O-benzoyl-D-arabinose (XIX) as an oil.

To a solution of 0.85 g. of crude XIX in 3 ml. of methylene chloride and 10 ml. of carbon tetrachloride was added 1.0 g. of Drierite. This mixture was cooled to 0° in an ice-bath, then treated with hydrogen chloride gas for 1 hr. The mixture was kept at 0° for 2 hr., then filtered and evaporated to dryness *in vacuo*. Dry benzene (1 ml.) was added and removed *in vacuo* to remove all traces of hydrogen chloride, leaving the crude chloro sugar XX as an oil.

To 10 ml. of ethanethiol was added in portions 0.2 g. of sodium hydride with stirring. After hydrogen evolution had ceased, the resultant suspension of white sodium ethyl mercaptide in ethanethiol was poured into the flask containing the chlorosugar XX. The reaction was stirred at room temperature for 15–20 min. (by which time all the chloro sugar had dissolved), then placed in an ice-bath for an additional 40 min. with continued stirring.

The reaction mixture was worked up by the addition of ice and 15 ml. of saturated aqueous sodium bicarbonate solution. After being stirred for 5 min., this mixture was extracted with three 15-ml. portions of water (the aqueous phase was neutral to *p*H paper), combined and dried over magnesium sulfate, then evaporated to dryness *in vacuo* to yield 0.83 g. (87%) of a yellow oil which gave a negative Benedict test for reducing sugar: $[\alpha]^{23.5}D$ +134° (1.81% in chloroform); $\lambda_{max}^{film}(\mu)$ 5.82 (benzoate C=O), 7.26 (CH_3).

Anal. Calcd. for $C_{21}H_{22}O_6S$: C, 62.7; H, 5.51; S, 7.95. Found: C, 62.8; H, 5.83; S, 7.91.

The rotations observed for the crude product XXII in repetitions of this reaction varied from +130 to +159°. The fact that the product was essentially all in the α-configuration was indicated by the high positive rotation as compared to the strongly negative rotation observed for the β-anomers XIVa, XIVb, XV.

Ethyl 1-Thio-β-D-arabinofuranoside (XV).—Ethyl 5-O-benzoyl-1-thio-β-D-arabinofuranoside (XIVa) (1.0 g., 3 mmoles) was dissolved in 16 ml. of absolute methanol containing 0.6 ml. of 1 *N* methanolic sodium methoxide. After standing 18 hr. at room temperature, the solution was stirred with ~0.25 g. of Dowex 50 (H ion) until the solution became neutral to *p*H paper. The resin was removed by filtration and the filtrate was concentrated to dryness *in vacuo*. The residue was triturated with ether to give 0.34 g. of a stiff, white gum. The infrared spectrum confirmed that the 5-benzoate had been completely removed (by the absence of any carbonyl band at 5.8 μ). The gum crystallized on standing and was recrystallized from ethyl acetate–Skellysolve B to give white crystals, m.p. 49–50°, $[\alpha]^{27}D$ −130° (1% in methanol). The paper chromatogram[11] showed a single spot at R_{ad} 2.63.

Anal. Calcd. for $C_7H_{14}O_4S$: C, 43.2; H, 7.26; S, 16.5. Found: C, 43.2; H, 7.41; S, 16.5.

Ethyl 1-Thio-α-D-arabinofuranoside (XXIII).—Treatment of 1.0 g. (2.5 mmoles) of ethyl 3,5-di-O-benzoyl-1-thio-α-D-arabinofuranoside (XXII), $[\alpha]D$ +159°, with methanolic sodium methoxide as described in the preparation of ethyl 1-thio-β-D-arabinofuranoside gave 0.5 g. (100%) of a yellow sirup which crystallized on standing. Recrystallization from ethyl acetate–Skellysolve B gave white crystals, m.p. 62–63°, $[\alpha]^{27}D$ +240° (1% in methanol). The paper chromatogram[11] showed a single spot at R_{ad} 2.69.

Anal. Calcd. for $C_7H_{14}O_4S$: C, 43.2; H, 7.26; S, 16.5. Found: C, 43.4; H, 7.34; S, 16.0.

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

MENLO PARK, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE MOUNT SINAI HOSPITAL, AND FROM THE LABORATORY OF MARINE BIOCHEMISTRY AND ECOLOGY, N. Y. ZOOLOGICAL SOCIETY]

Holothurin. I. The Isolation, Properties and Sugar Components of Holothurin A[1]

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The isolation, properties and sugar components of holothurin A, the toxic principle of *Actinopyga agassizi*, a sea-cucumber found in the Bahama Islands are described. Elementary analysis of holothurin A leads to the empirical formula $C_{50-2}H_{81-5}O_{25-6}SNa$. The hydrolysis products derived from holothurin A suggest that it is a mixture of several glycosides, each of which contains a steroid aglycone of *ca.* 26–28 carbon and 4–5 oxygen atoms, one molecule each of four different sugars and one molecule of sulfuric acid as the sodium salt. The separation and identification of the monoses derived from holothurin A has been achieved. They were D-glucose, D-xylose, D-glucomethylose (quinovose) and 3-O-methylglucose. The neurotoxic, hemolytic and cancerostatic properties of holothurin A are referred to.

Some species of sea-cucumbers (class Holothuroidea) contain a poisonous principle. The ecological significance of this poison is not clear. It has been suggested that it protects these animals against predators. Sea-cucumbers of various species are eaten in various parts of the world, but occasional poisoning by this article of food is presumably not related to the toxin under discussion, which is ineffective by oral ingestion. A peculiar gland, found in this species and named after the zoologist Cuvier, is particularly rich in poison, which is also found in the blackish slimy integument. We have studied the chemical composition of the toxic substance from Cuvier's gland of *Actinopyga agassizi*, a holothuria found in the Bahama Islands.[2] In two preliminary notes[3,4] we reported that the toxic material holothurin, obtained from the water extract of Cuvier's gland, appeared to consist of a few steroid aglycones, bound individually to 4 molecules of monosaccharides. Further purification of holothurin and analysis of its hydrolytic products, as described below, is in accord with this view. This would place holothurin, as the substance has been designated,[2] into the class of cardiac glycosides or steroid saponins such as have hitherto been found in plants, especially monocotyledons, only. It shares with the plant drugs its saponin-like character and neurotoxic and hemolytic properties, which will be described elsewhere.[5,6] But, besides, holothurin contains one molecule of sulfuric acid, bound in ester linkage, which suggests a relationship with steroid alcohols, *e.g.*, scymnol and ranol in the bile of the most primitive vertebrates.[7]

Some of the holothurin glycosides resemble digitonin and other saponins in forming a complex with cholesterol. The glycosides which enter into such combination comprise over 60% of the total glycosides; they may be recovered from the complex by treatment with pyridine. Our experiments do not favor the assumption of a 1:1 complex with cholesterol, such as is formed by plant saponins; the complex is richer in cholesterol and the electrostatic situation at the sulfate ester site may be assumed to influence the composition of the adduct. We have designated the cholesterol precipitable fraction as holothurin A.

Holothurin A showed no absorption in the ultraviolet region. The infrared spectrum showed bands at 5.72 and 6.14 μ indicative of a five or six-membered ring lactone and one double bond. The absence of a positive Legal test in conjunction with the ultraviolet spectrum excluded the presence of an α,β-unsaturated lactone. Hydrogenation of the double bond failed under a variety of conditions. Elementary analysis of holothurin A leads to the formula $C_{50-2}H_{81-5}O_{25-6}SNa$. Methoxyl determination indicated the presence of one such group in holothurin A. Acid hydrolysis of the neutral non-reducing holothurin A yielded water insoluble aglycones, sulfuric acid and water-soluble reducing sugars. At least 4 individual steroid aglycones are obtainable from the hydrolysis of holothurin A. The elementary analysis and molecular weight determination[8] of these aglycones show all to have between 26 and 28 carbons and 4–5 oxygen atoms, while none of the aglycones contained a methoxyl group. The observed rotation of the total sugar mixture, obtained from the hydrolysis of holothurin A, has been found to be the same as that which would be calculated for an equimolecular mixture of the four sugars isolated and described below. These observations are consistent with the hypothesis that

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(4) *Cf.* T. Yamanouchi, *Publ. Seto Marine Biol. Lab.* 4 (Parts 2–3), 183 (1955).

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(7) G. D. Haslewood, *Physiol. Rev.*, **35**, 178 (1955).

(8) To be reported in a separate communication.