

Active Antitumor Components in a Decomposed Amino Sugar I: Effect of Sugar Structure on Activity

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Abstract □ While pure methyl 5-(2-chloroethylamino)-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside hydrochloride has no L-1210 leukemia activity, a decomposed sample was found to be very active. One of several approaches taken to determine the nature of the active component involved a study of how sugar structure affects antitumor activity. A number of aminoribose derivatives were prepared and tested against the murine L-1210 and P-388 leukemia and the B-16 melanoma tumor systems. Compounds were tested as pure materials and as synthetically degraded mixtures. Both the β-haloethyl group and a secondary amine were required for highest activity.

Keyphrases □ Amino sugar, decomposed—active antitumor components, effect of sugar structure on activity □ Sugars, amino—active antitumor components in decomposed samples, effect of structure on activity □ Antitumor activity—decomposed amino sugar □ Structure-activity relationships—decomposed amino sugar—antitumor activity

The one-arm sugar mustard, methyl 5-(2-chloroethylamino)-5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranoside hydrochloride (I), was prepared during studies of the antitumor activity of ribose alkylating agents (1). Although I was inactive in the murine L-1210 leukemia system when first tested, it was discovered later that a decomposed sample of I was highly active ($T/C > 200\%$)¹ in that system. The entire sample was utilized, however, in the testing program. Resynthesis of I again confirmed the inactivity of the pure material.

A two-pronged approach was taken to determine the structure(s) of the active component(s) in the decomposed mixture of I. One study attempted purposely to degrade pure I and isolate and identify the active components². The second approach, the subject of the study described here, was an investigation of the effect of chemical structure on the activity of purposely degraded samples. A method was devised to accelerate the production of active material from 5 years to 5 hr, and it was planned to prepare and degrade certain aminoribose derivatives to provide information about the structural characteristics required to produce active material from I.

RESULTS AND DISCUSSION

The first series of compounds prepared (Scheme I) was designed to answer the following two questions:

1. Was the nitrogen mustard group necessary for activity since it imparted no activity to I?
2. Was a secondary amine required or would a tertiary amine produce activity?

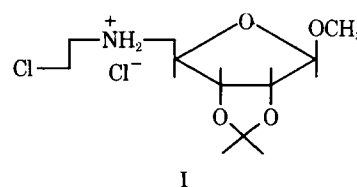
In an attempt to answer the first question, Compounds VI–IX were synthesized. The first key intermediate prepared was the tosylate (IV). It was so poorly reactive with the required amine nucleophiles, however, that the mesylate (V) had to be synthesized to obtain the desired amino sugars. The *N*-propyl derivative (VI) was synthesized, because in this compound the reactive chlorine was replaced with a completely inert methyl group while leaving the partition coefficient relatively undisturbed ($\pi_{CH_3} = 0.5$; $\pi_{Cl} = 0.39$) (3). The other three derivatives should be somewhat more hydrophilic than I. The bromo analog of I (XV) was also synthesized.

Compounds VI–IX and XV were tested in the leukemia L-1210 system. They were tested both as analytically pure materials and as the product from the synthetic degradation process shown to produce an active mixture from pure, inactive I. None of the non-halogen-containing derivatives were L-1210 active in either form (pure or decomposed) when tested at 400, 200, and 100 mg/kg QD 1-9 or Q4D (2). The bromo analog (XV), however, was active both as a pure compound and after degradation (Table I). This activity is in contrast to that of the chloro compound (I), which was active only after degradation. Pure XV was highly active in the leukemia P-388 system (2). Since the yield of XV was low (Table II), it was not tested in the degraded form in the P-388 system and was tested only on the Day 1 schedule in L-1210.

A comparison between pure and degraded XV (Day 1 only) in L-1210 shows the degraded form to be more active. It is also less toxic based on mouse weight loss. While pure XV is L-1210 active and pure I is not, there are not enough data on degraded XV to make a definitive comparison of the two materials. It appears, however, that a halogen atom is required in the molecule to produce L-1210 activity in a degraded sample.

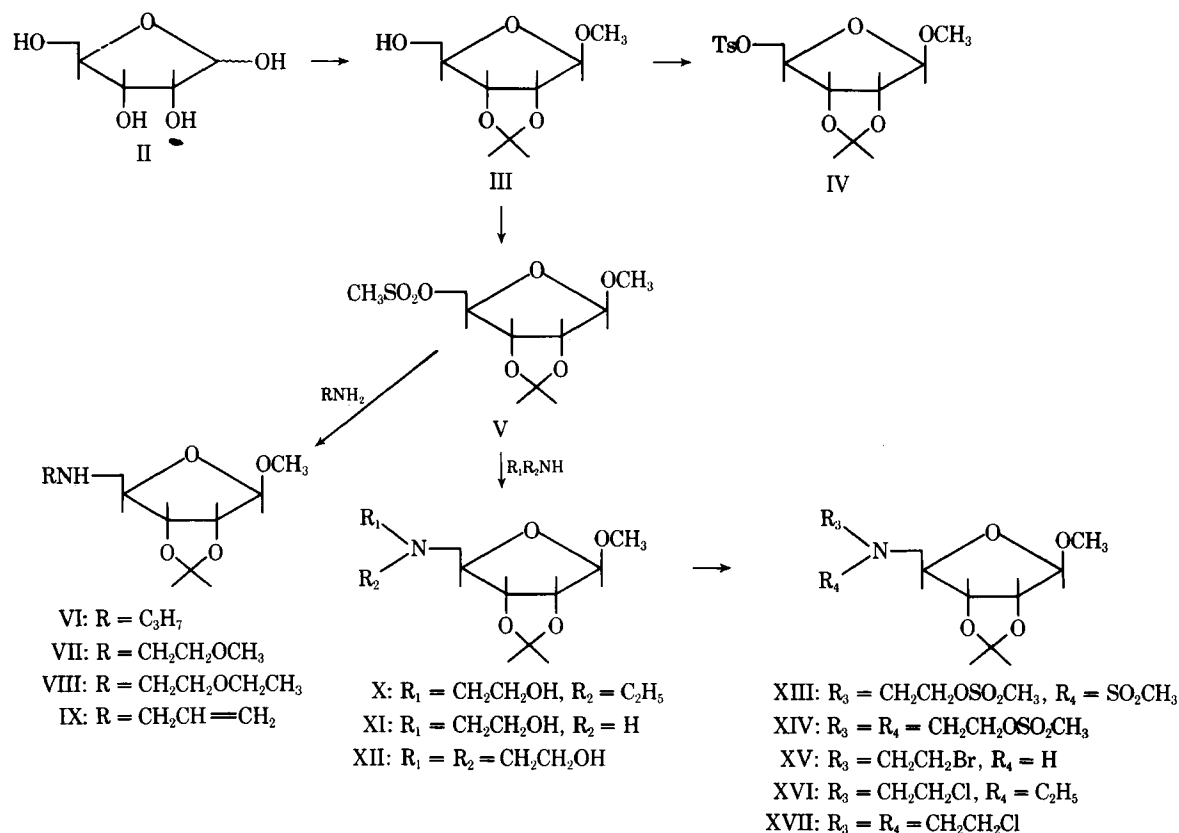
Compound XVI was prepared to explore the effect of additional substitution on the amine nitrogen. Since compounds of general type XVIII were possible degradation products based on analogy with the aminoxylose system (4), it appeared mechanistically impossible to produce XVIII from a tertiary amine such as XVI. Pure XVI had been prepared and tested (L-1210) in 1960, but that sample was inactive in limited testing. When the isopropylidene blocking group was removed from XVI, an active compound resulted (1). Compound XVI was resynthesized and a retest of the pure material showed reproducible L-1210 activity (Table I). Activity was also found in the leukemia P-388 and in the B-16 melanoma test systems. Degradation under standard conditions did produce a marginally L-1210 active mixture, but the activity was reduced, not enhanced as was found with secondary amines I and XV. Doses of degraded XVI higher than 3.1 mg/kg were toxic. A conclusion can be made that the change from a secondary to a tertiary amine in this series adversely affects the L-1210 activity of the degraded product by destroying the pure active compound, by non-production of an active intermediate, or both.

The two-arm mustard (XVII) also had been prepared and tested (1). Although the initial test of XVII showed L-1210 activity, confirmation testing was negative. Compound XVII was resynthesized for this study but in considerably lower yield than XVI. For this reason, only the pure, nondegraded material was tested (Table I). The bis(β-chloroethyl) compound was confirmed active in both the



¹ T/C = treated $\times$ 100%/control. For protocol details, see Ref. 2.

² This will be described in a subsequent report.



Scheme I—Synthesis of amino sugars

L-1210 and P-388 systems. This finding contrasts with the lack of L-1210 activity for the mono(β -chloroethyl) derivative, I. Compounds II–V and X–XIV (Scheme I) were evaluated both pure and as degraded materials in the L-1210 system and were inactive ($T/C < 125\%$).

NMR evidence² indicates that acetone and methanol (loss of isopropylidene and methylglycoside) are liberated early in the acidic degradation procedure. The initial product of these reactions should have structure XXIV (Scheme II). This made the prepara-

tion of XXIV for testing desirable. Among all the amino sugars initially prepared, however, only I could not be hydrolyzed to a stable deblocked product (1).

Because of the suspected instability of XXIV, a reaction sequence was devised (Scheme II) that allowed the final step to proceed under mild conditions. The debenzoylation of XXIII by hydrogen over palladium-on-charcoal in the aqueous acidic solution gave the reduced, hydrolyzed product, XXIV. A periodate oxidation was carried out to characterize this reaction product (11). Methyl

Table I—Murine Antitumor Activity

Compound	Form	Tumor System	Treatment Schedule ^a	Dose, mg/kg/day	Activity (T/C), %
XV	Pure	L-1210	Day 1 only	100	125
		L-1210	Days 1, 5, 9	100	157
	Pure	L-1210	QD 1-9	50	131
				36	137
		P-388	QD 1-9	36	250
				18	205
		L-1210	Day 1 only	9	188
				400	144
				200	126
				18.5	144
XVI	Pure	L-1210	QD 1-9	12.5	126
		P-388	QD 1-9	12.5	188
	Pure	B-16	QD 1-9	8.2	211
				4.0	195
				2.0	158
				7.2	145
				4.3	129
				3.1	125
XVII	Degraded	L-1210	QD 1-9	6.2	139
		L-1210	Days 1, 5, 9	4.1	130
	Pure	P-388	QD 1-9	2.7	138
				1.7	133
				3.1	227
				1.0	241
				0.3	154

^a Reference 2.

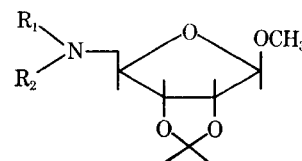


Table II—Physical Constants for Some Aminoribose Derivatives

Compound	R ₁	R ₂	Melting Point	Yield, %	Formula	Analysis, %	
						Calc.	Found
VI	CH ₂ CH ₂ CH ₃	H	145°	67	C ₁₂ H ₂₃ NO ₄ ·HCl	C 51.16 H 8.52 Cl 12.61	51.46 8.80 12.56
VII	CH ₂ CH ₂ OCH ₃	H	104°	76	C ₁₂ H ₂₃ NO ₅ ·HCl	N 4.97 C 48.35 H 8.10 Cl 11.93	4.82 48.10 8.31 11.70
VIII	CH ₂ CH ₂ OC ₂ H ₅	H	150°	36	C ₁₃ H ₂₅ NO ₅ ·HCl	N 4.70 C 50.08 H 8.35 Cl 11.40	4.65 50.10 8.20 11.20
IX	CH ₂ CH=CH ₂	H	135°	37	C ₁₂ H ₂₁ NO ₄ ·HCl	N 4.49 C 51.62 H 7.88 Cl 12.73	4.55 51.93 8.03 12.52
X	CH ₂ CH ₂ OH	CH ₂ CH ₃	135°	59	C ₁₃ H ₂₅ NO ₅ ·HCl	N 5.02 C 50.10 H 8.35 Cl 11.49	4.93 49.80 8.29 11.73
XI	CH ₂ CH ₂ OH	H	142°	86	C ₁₁ H ₂₁ NO ₅ ·HCl	N 4.49 C 46.56 H 7.76 Cl 12.52	4.41 46.54 7.57 12.50
XII	CH ₂ CH ₂ OH	CH ₂ CH ₂ OH	148°	33	C ₁₃ H ₂₅ NO ₆ ·HCl	N 4.94 C 47.63 H 7.93 Cl 10.84	4.90 47.56 8.06 10.76
XIII	SO ₂ CH ₃	CH ₂ CH ₂ OSO ₂ CH ₃	89°	52	C ₁₃ H ₂₅ NO ₉ S ₂	N 4.27 C 38.70 H 6.25 S 15.90	4.21 38.69 6.23 15.80
XIV	CH ₂ CH ₂ OSO ₂ CH ₃	CH ₂ CH ₂ OSO ₂ CH ₃	80°	36	C ₁₅ H ₂₉ NO ₁₀ S ₂	N 3.47 C 40.27 H 6.48 S 14.32	3.24 40.24 6.28 14.62
XVI	CH ₂ CH ₂ Cl	C ₂ H ₅	117°	82	C ₁₃ H ₂₄ ClNO ₄ ·HCl	N 4.24 C 47.28 H 7.57 Cl 21.52	3.98 47.13 7.87 21.98
XVII	CH ₂ CH ₂ Cl	CH ₂ CH ₂ Cl	128°	32	C ₁₃ H ₂₃ Cl ₂ NO ₄ ·HCl	N 3.84 C 42.80 H 6.58 Cl 29.22	3.62 42.78 6.83 29.24

α -D-mannopyranoside was used to standardize the procedure (12). Compound XXIV consumed 2 moles of periodate with the formation of 1 mole of formic acid. This result is consistent with the structure shown for XXIV but inconsistent with the structures expected for a hydroxypiperidine rearrangement product or an open chain sugar.

Compounds XIX–XXIII were inactive in the L-1210 system. Enough XXIV was obtained to carry out an L-1210 dose–response test (800, 400, 200, and 100 mg/kg) on the Day 1 treatment schedule (2). This test was negative at all doses. Decomposed samples of I have given T/C values > 200% under the same treatment conditions. The search for the active component in decomposed I is continuing.

EXPERIMENTAL³

Decomposition Studies—The deionized water used was deoxygenated for 24 hr with nitrogen prior to being warmed to 60° in a three-necked, round-bottom flask equipped with a reflux condenser, magnetic stirrer, and a gas inlet tube. A slow stream of nitrogen was passed into the flask during the reaction period. The material

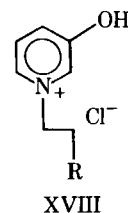
to be degraded was added to the warm solution to give a concentration of 100 mg/ml. The stirred solution was maintained at 60° for 5.0 hr, cooled, and lyophilized to give the test mixture.

Methyl 2,3-O-Isopropylidene- β -D-ribofuranoside (III)—This compound was prepared as a yellow syrup [method of Leonard and Carraway (5)] in 62% yield on a 30-g scale.

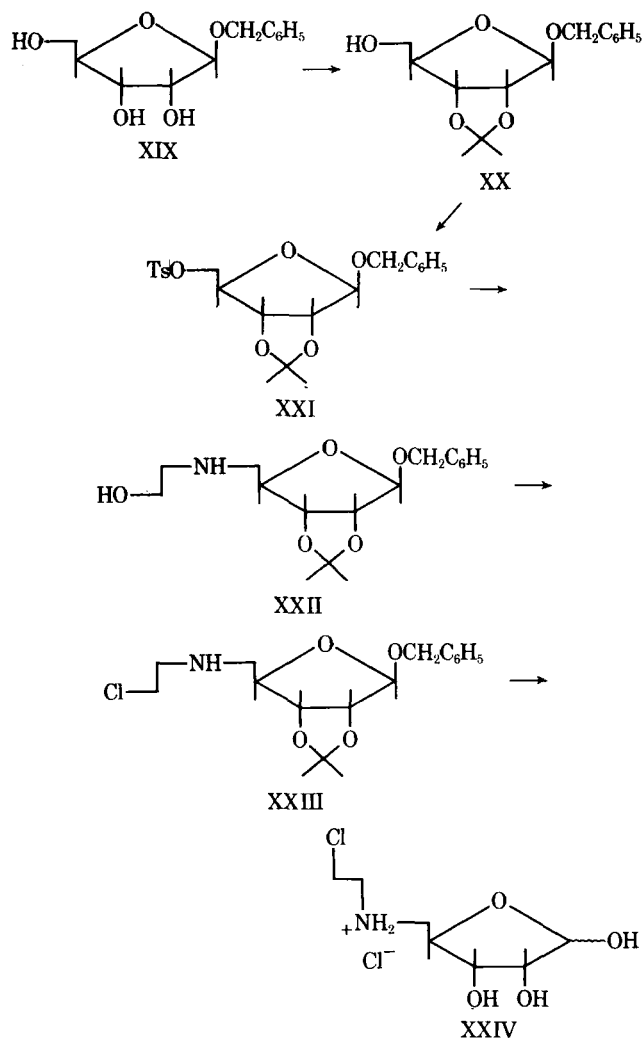
Methyl 2,3-O-Isopropylidene-5-O-*p*-toluenesulfonyl- β -D-ribofuranoside (IV)—This material was prepared as white needles (14.9 g, 82%), mp 82–83° [lit. (6) mp 83.5–84.5°] by the method of Shunk *et al.* (6).

Methyl 2,3-O-Isopropylidene-5-O-methanesulfonyl- β -D-ribofuranoside (V)—This material was prepared by the procedure of Kissman and Weiss (7), as a white crystalline solid (49 g, 88%), mp 75–76° [lit. (7) mp 73–74°].

Methyl 5-Alkylamino-5-deoxy-2,3-O-isopropylidene- β -D-ribofuranosides (General Procedure for VI–IX)—A solution of



³ Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were carried out by Dr. W. C. Alford, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.



Scheme II—Synthesis of deblocked I

V (5.0 g) in 25 ml of the appropriate amine was heated in a pressure bottle at 115° for 17 hr. The solution was cooled and concentrated *in vacuo*. The residue was dissolved in water and extracted with ether (3 × 100 ml). The ether layer was washed with water and dried (sodium sulfate). Addition of dry hydrogen chloride to the ether solution caused the product to precipitate as the hydrochloride salt. The products were recrystallized from methanol-ether (Table II).

Methyl 5-(2-Hydroxyethylamino)-5-deoxy-2,3-O-isopropylidene-β-D-ribofuranosides (General Procedure for X–XII)—A stirred mixture of V (10 g) and the appropriate ethanolamine derivative (30 ml) was heated at 150° for 4 hr. The solution was cooled and diluted with chloroform (100 ml). The chloroform solution was washed with four 10-ml portions of water, and the chloroform was removed *in vacuo*, and the residual oil was dissolved in dry ether to which dry hydrogen chloride was added. The resulting white crystalline solids were isolated (Table II).

Methyl 2,3-O-Isopropylidene-5-deoxy-5-[(2-hydroxyethyl)(methylsulfonyl)amino]-β-D-ribofuranoside Methanesulfonate (Ester) (General Procedure for XIII and XIV)—To a stirred, dry ice-acetone cooled solution of XI (6.7 g) in dry pyridine (30 ml) was added, dropwise, methanesulfonyl chloride (5 ml). After the addition was complete (0.5 hr), the reaction mixture was refrigerated overnight. The mixture was added slowly to a vigorously stirred ice-water mixture. The resulting solution was extracted with chloroform. The chloroform layer was washed with water, dried (sodium sulfate), and concentrated to dryness. The resulting solid was recrystallized from ethanol (Table II).

Methyl 5-(2-Bromoethylamino)-5-deoxy-2,3-O-isopropyl-

idene-β-D-ribofuranoside Hydrobromide (XV)—A solution of XI (1.5 g) in dry methylene chloride (20 ml) and 2,6-lutidine (0.7 ml) was heated gently on a steam bath under anhydrous conditions. To the refluxing solution was added a solution of thionyl bromide (1.0 ml) in dry methylene chloride (10 ml). After the addition was complete (1 hr), the solvent was distilled and the residual solid was poured into a stirred ice-water mixture containing 10% sodium bicarbonate. The solution was extracted with ethyl acetate, and the extract was dried (sodium sulfate) and passed through a column of alumina. The eluate was distilled under reduced pressure to remove the solvent, and the residue was dissolved in dried ether. A white crystalline solid precipitated (0.4 g, 26%, mp 138°) upon the addition of dry hydrogen bromide gas.

Anal.—Calc. for $C_{11}H_{21}Br_2NO_4$: C, 33.70; H, 5.37; Br, 40.92; N, 3.58. Found: C, 33.30; H, 5.59; Br, 40.79; N, 3.45.

Methyl 2,3-O-Isopropylidene-5-[N-ethyl-(2-chloroethyl)amino]-5-deoxy-β-D-ribofuranoside Hydrochloride (General Procedure for XVI and XVII)—A solution of X (9.48 g) in dry methylene chloride (25 ml) was saturated with dry hydrogen chloride under anhydrous conditions. Thionyl chloride (10 ml) was added dropwise to the refluxing solution. After the addition was complete (2 hr), the solution was refluxed for 1 hr. Excess solvent was removed *in vacuo*. The residue was dissolved in dry ether (1 liter) and saturated with dry hydrogen chloride. On standing overnight, a white crystalline solid separated from the solution (Table II).

Benzyl β-D-Ribofuranoside (XIX)—This compound was prepared by the method of Ness *et al.* (8) in 69% yield. The resulting semisolid syrup was recrystallized from ethyl acetate to give white prisms, mp 103° [lit. (8) mp 105°].

Benzyl 2,3-O-Isopropylidene-β-D-ribofuranoside (XX)—A mixture of XIX (6.6 g), anhydrous acetone (90 ml), 2,2-dimethoxypropane (10 ml), and anhydrous copper sulfate (5 g) was stirred at room temperature overnight. The solution was filtered through a layer of charcoal, and the solvent was removed under reduced pressure. The residual solid was crystallized from ether to give 6.9 g (88%) of white microneedles, mp 105° [lit. (9) mp 104–105°].

Benzyl 2,3-O-Isopropylidene-5-p-toluenesulfonyl-β-D-ribofuranoside (XXI)—This compound was prepared by the method of Hanessian and Haskell (10) to give 10.2 g (66%) of white needles, mp 95° [lit. (10) mp 93–95°].

Benzyl 2,3-O-Isopropylidene-5-[(2-hydroxyethyl)amino]-5-deoxy-β-D-ribofuranoside (XXII)—A suspension of XXI (10.0 g) in ethanolamine (40 ml) was heated at 160° for 4 hr. The cooled solution was diluted with chloroform (100 ml) and washed with three 10-ml portions of water. After drying (sodium sulfate), the solvent was removed *in vacuo* and the residual oil was distilled (175°/0.2 torr). The distillate solidified on standing overnight to give 6.2 g (86%) of white crystals, mp 45°.

Anal.—Calc. for $C_{17}H_{25}NO_5$: C, 63.14; H, 7.74; N, 4.33. Found: C, 63.26; H, 7.89; N, 4.11.

Benzyl 2,3-O-Isopropylidene-5-[(2-chloroethyl)amino]-β-D-ribofuranoside Hydrochloride (XXIII)—A solution of XXII (2.0 g) in dry methylene chloride (15 ml) was saturated with dry hydrogen chloride. Thionyl chloride (5 ml) in methylene chloride (20 ml) was added dropwise to the refluxing solution. After addition was complete (1 hr), the solution was heated under reflux for 3 hr. The solvent was removed *in vacuo*, and the residue was dissolved in chloroform. The solution was washed once with a 10% ice-cold solution of sodium carbonate and twice with water and was dried (sodium sulfate). The chloroform was removed *in vacuo*, and the residue was dissolved in dry ether (100 ml). An oily residue resulted upon addition of dry hydrogen chloride. This residue solidified on standing overnight. Recrystallization from dimethylformamide-ether gave 1.2 g (51%) of yellow prisms, mp 148°.

Anal.—Calc. for $C_{17}H_{25}Cl_2NO_4$: C, 53.96; H, 6.61; Cl, 18.78; N, 3.70. Found: C, 53.85; H, 6.58; Cl, 18.39; N, 3.71.

5-[(2-Chloroethyl)amino]-5-deoxy-β-D-ribofuranoside Hydrochloride (XXIV)—A solution of XXIII (0.3 g) in water (20 ml) containing concentrated hydrochloric acid (0.3 ml) was reacted with hydrogen over 10% palladium-on-charcoal catalyst (0.2 g) for 22 hr at 23° in a Parr apparatus. The solution was filtered and lyophilized to give 0.2 g (88%) of a highly hygroscopic white crystalline solid.

Anal.—Calc. for $C_7H_{15}Cl_2NO_4 \cdot 2H_2O$: C, 29.58; H, 6.59; Cl, 25.00; N, 4.93. Found: C, 29.70; H, 6.46; Cl, 24.60; N, 4.84.

The periodate oxidation of XXIV was carried out using the

method of Jackson and Hudson (11, 12). Titration over 5 hr showed that 2.1 moles of periodate was consumed and that 0.98 mole of formic acid had been produced by the oxidation, indicating a ribofuranose ring structure.

REFERENCES

- (1) E. J. Reist, R. R. Spencer, M. E. Wain, I. G. Junga, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 2821(1961).
- (2) R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1(1972).
- (3) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525(1971).
- (4) H. Paulsen and K. Todt, in "Advances in Carbohydrate Chemistry," vol. 23, M. Wolfson and R. Tipson, Eds., Academic, New York, N.Y., 1968, p. 115.
- (5) N. J. Leonard and K. L. Carraway, *J. Heterocycl. Chem.*, **3**, 487(1966).
- (6) C. S. Shunk, J. B. Lavigne, and K. Folkers, *J. Amer. Chem. Soc.*, **77**, 2210(1955).

- (7) H. M. Kissman and M. J. Weiss, *ibid.*, **80**, 5559(1958).
- (8) R. K. Ness, H. W. Diehl, and H. G. Fletcher, *ibid.*, **76**, 763(1954).
- (9) E. R. Rauch and D. Lipkin, *J. Org. Chem.*, **27**, 403(1962).
- (10) S. Hanessian and T. H. Haskell, *ibid.*, **28**, 2604(1963).
- (11) E. L. Jackson and C. S. Hudson, *J. Amer. Chem. Soc.*, **59**, 994(1937).
- (12) *Ibid.*, **61**, 959(1939).

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Effect of Ethanol on Theophylline Absorption in Humans

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Abstract □ This study was carried out to determine if ethanol, which enhances theophylline absorption from the rat small intestine, has a similar effect when administered orally to human subjects. Seven normal adults received 200 mg of theophylline/m² of body surface area, in 50 ml of either aqueous solution or hydroalcoholic solution containing 20% ethanol. There was no significant difference in the average plasma concentrations of theophylline produced by these two solutions, but three subjects (all female) experienced nausea after taking the aqueous solution while none became nauseous after taking theophylline in the hydroalcoholic solution.

Keyphrases □ Theophylline—effect of ethanol on absorption, humans □ Ethanol—effect on absorption of theophylline, humans □ Absorption—theophylline, effect of ethanol, humans

Theophylline is an effective bronchodilator used widely for the treatment of asthma. It is desirable to have oral dosage forms available from which this drug is rapidly absorbed so that acute attacks of asthma can be treated and relieved promptly at home. There have been claims that the absorption of theophylline is enhanced if administered in hydroalcoholic solution, but the evidence is conflicting (1-4).

Recently, an initial concentration of 5% ethanol was found to increase significantly the absorption of theophylline from a ligated segment of small intestine of anesthetized rats, and a constant concentration of 2% ethanol (and even lower concentrations in unpublished studies) increased appreciably the absorption of theophylline from the perfused small intestine of anesthetized rats (5). This absorption-enhancing effect of ethanol was associated with an increase in the net flux of water from the intestinal

lumen and may be due to solvent drag (5). The study described here was initiated to determine if the rate of absorption of theophylline can be increased in humans by administering the drug in hydroalcoholic rather than in aqueous solution.

EXPERIMENTAL

Seven healthy volunteers (four females and three males), 24-33 years old and capable by education and background (registered nurses and graduate students in pharmaceuticals) to give informed consent, participated in the study. They were instructed to abstain from coffee, tea, chocolate, cola drinks, and alcoholic beverages for 24 hr before and during the study and to take no drugs (except oral contraceptives if these were used regularly) for 3 days before and during the study.

Theophylline, 200 mg/m² body surface area, dissolved either in 50 ml of water or in 50 ml of hydroalcoholic solution containing 20% (v/v) ethanol, was administered in the morning 1 hr after a light breakfast. The two preparations were given in crossover fashion, 1 week apart. Fifteen milliliters of blood was withdrawn through an indwelling venous catheter into a heparinized syringe immediately before drug administration, and 10-ml blood samples were obtained at 10, 30, 60, 120, 240, 360, and 480 min thereafter. Plasma was separated and stored in a freezer pending assay.

At the end of the experiment, each subject was given a card with the questions: "Did you notice any pharmacologic effects during the day? If so, what and when?" There was no additional questioning, elaboration, or discussion to prevent any biased suggestive influences on the response to the question.

Theophylline concentrations in plasma were determined by the spectrophotometric method of Schack and Waxler (6), modified for smaller sample volumes and greater sensitivity (7). Blank values and recovery of theophylline were determined for each subject by assaying the zero-time plasma as such and after adding theophylline to yield a concentration of 10 µg/ml. The blank values ranged from 1.48 to 3.47 µg apparent theophylline/ml, and the recovery of added drug ranged from 85.1 to 97.7%. The analytical re-