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Synthesis and Antitumor Activity of Water-Soluble (2-Chloroethyl)nitrosoureas

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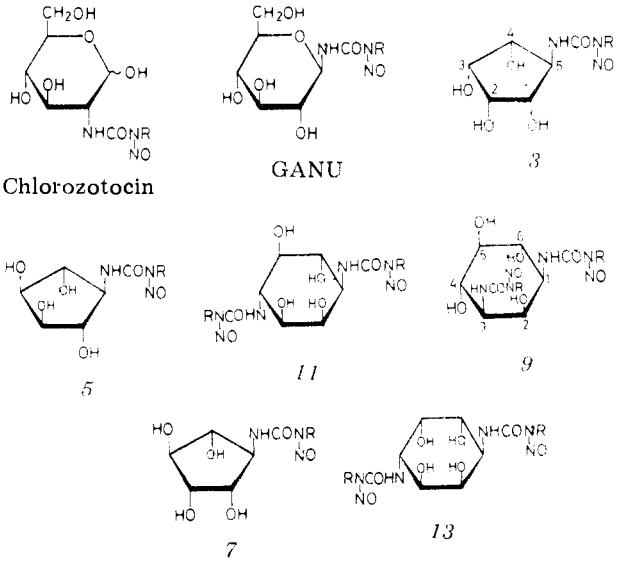
Six water-soluble (2-chloroethyl)nitrosoureido derivatives of cyclopentanetetrols and cyclohexanetetrols have been prepared. Their antitumor activities were determined against leukemia L1210 in mice.

Steptozotocin is a naturally occurring antitumor antibiotic[1-4] and has a unique structure: 2-(3-methyl-3-nitrosoureido)-2-deoxy-D-glucopyranose.[5,6] In this antibiotic, the methylnitrosoureido group seems to be an essential functional group for biological activity, and the carbohydrate moiety might play an important role as a carrier of the functional group. This has been verified by the fact that the introduction of the functional group into a polyhydroxycyclohexane system did not result in loss of activity against leukemia L1210.[7] Also, O-methylation of an anomeric hydroxyl group of streptozotocin[8] and its analogues[9] was not detrimental for activity.

Montgomery and his co-workers showed in their systematic studies on *N*-nitrosoureas[10,11] that a replacement of the methyl group in this functional group with a 2-chloroethyl group markedly enhanced the activity of the *N*-nitrosoureas against leukemia L1210. Accordingly, the clinically useful BCNU,[10] CCNU,[11] and MeCCNU[11] were synthesized. More recently, 2-[3-(2-chloroethyl)-3-nitrosoureido]-2-deoxy-D-glucopyranose (chlorozotocin)[12] exhibited strong antileukemic activity,[13-15] and its positional isomer, 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (GANU)[16] showed marked activity against leukemia L1210.[17]

Now we report a synthesis of six water-soluble (2-chloroethyl)nitrosoureido derivatives of cyclopentanetetrols and cyclohexanetetrols and their antileukemic activities against leukemia L1210. The melting points and yields of compounds **2-13** are listed in Tables I and II.



Chemistry. A synthesis of 5-[3-(2-chloroethyl)-3-nitrosoureido]-1,2,3,4-cyclopentanetetrols was exemplified in the case of its (1,2,3,4/5) stereoisomer.[18] When tetra-*O*-acetyl-(1,2,3,4/5)-5-acetamido-1,2,3,4-cyclo-

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Table I. Melting Points and Yields of 5-[3-(2-Chloroethyl)ureido]-1,2,3,4-cyclopentanetetrols **2**, **4**, and **6** and Bis[3-(2-chloroethyl)ureido]cyclohexanetetrols **8**, 10, and 12

compd	config	mp, °C	yield, %
2	1,2,3,4/5*N*	146-147	28
4	1,4/2,3,5*N*	129-130	68
6	1,2,4/3,5*N*	127-129	62
8	1*N*,2,3*N*,5/4,6	230-235 (dec)	75
10	1*N*,2,3,5/4*N*,6	202-215 (dec)	51
12	1*N*,2,3/4*N*,5,6	237-238 (dec)	84

Table II. Melting Points and Yields of 5-[3-(2-Chloroethyl)-3-nitrosoureido]-1,2,3,4-cyclopentanetetrols **3**, **5**, and **7** and Bis[3-(2-chloroethyl)-3-nitrosoureido]-cyclohexanetetrols **9**, 11, and 13

compd	config	mp, °C	yield, %	mp of tetra-*O*-Ac deriv, °C
3	1,2,3,4/5*N*	121-123 (dec)	40	
5	1,4/2,3,5*N*	110-111 (dec)	40	
7	1,2,4/3,5*N*	129-131 (dec)	66	
9	1*N*,2,3*N*,5/4,6	115 (dec)	94	148-150 (dec)
11	1*N*,2,3,5/4*N*,6	170 (dec)	56	
13	1*N*,2,3/4*N*,5,6	158-186 (dec)	88	

pentanetetrol[19] was hydrolyzed in 6 M hydrochloric acid and subsequently treated with Amberlite IRA-400 (OH^-) resin, (1,2,3,4/5)-5-amino-1,2,3,4-cyclopentanetetrol (**1**) was obtained as a syrup. N-Carbamoylation of **1** with 2-chloroethyl isocyanate gave (1,2,3,4/5)-5-[3-(2-chloroethyl)ureido]-1,2,3,4-cyclopentanetetrol (**2**). Nitrosation of **2** with sodium nitrite in formic acid afforded (1,2,3,4/5)-5-[3-(2-chloroethyl)-3-nitrosoureido]-1,2,3,4-cyclopentanetetrol (**3**) as crystals.

By the analogous reactions, (1,4/2,3,5)- (**5**) and (1,2,4/3,5)-5-[3-(2-chloroethyl)-3-nitrosoureido]-1,2,3,4-cyclopentanetetrol (**7**) were prepared from (1,4/2,3,5)-[20] and (1,2,4/3,5)-5-amino-1,2,3,4-cyclopentanetetrol,[21] respectively. Starting from *myo*-inosadiamine-1,3 dihydrochloride,[22] hexaacetyl-*myo*-inosadiamine-1,4[23] and *neo*-inosadiamine-1,4,[24] (1,2,3,5/4,6)-1,3-bis[3-(2-chloroethyl)-3-nitrosoureido]-2,4,5,6-cyclohexanetetrol (**9**), (1,2,3,5/4,6)-1,4-bis[3-(2-chloroethyl)-3-nitrosoureido]-2,3,5,6-cyclohexanetetrol (**11**), and (1,2,3/4,5,6)-1,4-bis[3-(2-chloroethyl)-3-nitrosoureido]-2,3,5,6-cyclohexanetetrol (**13**) were prepared, respectively, by the analogous reactions.

In all the cases, recrystallization of the nitroso derivatives from warm solvent caused spontaneous decomposition of the compounds with evolution of a gas. Therefore, unrecrystallized samples were submitted to biological tests.

It was notable that in the preparation of **13** the bisurea **12**, because of its insolubility, was nitrosated heterogenously in formic acid with sodium nitrite in fairly good yield.

Biological Results and Discussion. The results of in vivo tests against the transplanted mouse leukemia L1210 are shown in Tables III and IV. The methods used have been reported.[25,26] Preliminary experiments (W. T. Bradner, unpublished) suggested that the cyclopentanetetrol derivatives would be more effective given on a multiple rather than a single dose schedule. Accordingly, compounds **3**, **5**, and **7** were prepared fresh daily and treatment was administered for 8 days consecutively. BCNU was included for comparative purposes in Table III. All compounds tested were highly effective inhibitors of leukemia L1210; however, compounds **3** and **7** appeared to be somewhat superior to compound **5** in producing long-term survival.

Table III. Effect of Nitrosoureidocyclopentanetetrols on L1210 Leukemia[a]

dose, mg/kg	compd 3		compd 5		compd 7		BCNU	
	% T/C	surv	% T/C	surv	% T/C	surv	% T/C	surv
32	186	0	143	0	171	0	193	1
16	>471	6	171	0	>471	4	>471	6
8	>471	3	336	1	243	0	293	0
4	250	1	229	0	207	1	193	0
2	143	0	171	0	221	0	114	0
1	114	0	114	0	157	0	100	0
0.5	114	0	107	0	114	0	100	0
0.25	107	0	100	0	144	0	100	0

[a] Tumor inoculum: 10^6 leukemia cells per mouse implanted ip. Treatment: once daily for 8 days, starting on day 1, administered ip. Evaluation: median survival time (MST) results expressed as percent T/C = (MST treated/MST control) × 100. Criteria: T/C >125 considered significant tumor inhibition. Surv: number of mice surviving at 30 days (six mice/treatment dose).

Table IV. Effect of Nitrosoureidocyclohexanetetrols on L1210 Leukemia[a]

dose, mg/kg	compd 9		compd 11		compd 13		BCNU	
	% T/C	surv	% T/C	surv	% T/C	surv	% T/C	surv
128	86	0			293	0		
64	250	1			286	2		
32	214	0	136	0	>471	3	>471	5
16	179	0	>471	5	>243	0	271	2
8	171	0	214	0	171	0	193	1
4	114	0	171	0	136	0	143	0
2	121	0	150	0	129	0	114	0
1	100	0			114	0		

[a] Tumor inoculum: 10^6 leukemia cells per mouse implanted ip. Treatment: single dose, day 1 administered ip. Evaluation: median survival time (MST); results expressed as percent T/C = (MST treated/MST control) × 100. Criteria: T/C >125 considered significant tumor inhibition. Surv: number of mice surviving at 30 days (six mice/treatment dose).

In a similar manner, the somewhat less soluble cyclohexanetetrol derivatives were compared using a single dose treatment on day 1. Compounds **11** and **13** were superior to compound **9** in producing long-term survival (Table IV). A greater difference in toxicity was apparent in this series of cyclohexanetetrols (compared to the cyclopentanetetrols), as indicated by the fourfold difference in an optimum dose (maximum T/C) between compounds **9** and **11**.

It is clear that small positional changes between the isomers can effect antitumor activity and toxicity in these series. Since hematologic toxicity is a major side effect of (2-chloroethyl)nitrosoureas, these compounds are being studied further for comparative leukopenic effects.

Experimental Section

Melting points were determined in capillary tubes in a liquid bath and are uncorrected. Solutions were concentrated under reduced pressure below 30 °C.

Preparation of (2-Chloroethyl)ureido Derivative. A free base of aminocyclopentanetetrol or inosadiamine was treated with 2-chloroethyl isocyanate (1.5–2.2 mol per each amino group) in

cold water with agitation. The reaction mixture settled overnight in a refrigerator and was then concentrated. The residue was washed with methanol or recrystallized from an appropriate solvent to give the product (Table I).

Preparation of (2-Chloroethyl)nitrosoureido Derivative. A urea was dissolved or suspended in 80–99% formic acid. To the mixture, sodium nitrite (2.6–3.1 mol per each ureido group) was added under ice cooling with agitation. After 1 h, the reaction solution was diluted with an equal volume of water and subsequently treated with Amberlite IR-120 (H^+) resin, except in the case of **12**. The solution was concentrated and the residue was washed with an organic solvent to give the product (Table II). In the case of **12**, the reaction mixture was settled overnight in a refrigerator and the precipitated product, **13**, was collected by filtration.

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5-Substituted Uracil Arabinonucleosides as Potential Antiviral Agents

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Four 5-substituted analogues of 1-(β-D-arabinofuranosyl)uracil were prepared and evaluated as antiviral agents. 1-(β-D-Arabinofuranosyl)-5-(propynyloxy)uracil (**5**) was prepared by the propargyl bromide alkylation of 1-(β-D-arabinofuranosyl)-5-hydroxyuracil (**6**) which was synthesized by reaction of 1-(β-D-arabinofuranosyl)uracil with bromine–water–pyridine. Compound **6** could also be prepared by bromine–water–pyridine treatment of 1-(2,-3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)uracil, followed by removal of the acetyl groups by NH_3–CH_3OH. 1-(β-D-Arabinofuranosyl)-5-cyanouracil (**4**) was synthesized by basic cleavage of O^2-2′-anhydro-5-cyanouridine which was prepared by reaction of 5-cyanouridine with diphenyl carbonate in hexamethylphosphoramide. 1-(β-D-Arabinofuranosyl)-5-nitrouracil (**1**) was obtained by nitration of 2′,3′,5′-tri-*O*-(3,5-dinitrobenzoyl)uridine (**2**) with fuming HNO_3–H_2SO_4, followed by removal of the protecting groups with NaOEt–EtOH. Compounds **1**, **4**, and **6** were devoid of significant antiviral activity against herpes simplex (type 1) virus, vaccinia virus, and vesicular stomatitis virus in primary rabbit kidney cell cultures and human skin fibroblasts. The propynyloxy analogue, **5**, showed an anti-herpes virus activity comparable to 1-(β-D-arabinofuranosyl)uracil but was substantially less active than 1-(β-D-arabinofuranosyl)thymine.

Recently, the syntheses and antiviral activities of three new 5-substituted thymidine analogues have been reported, namely, 5-nitro-[1], 5-cyano-[2], and 5-(propynyloxy)-2′-deoxyuridines.[3] These substitutents endow 2′-deoxyuridine with unusual properties: 5-cyano-2′-deoxyuridine may be regarded as a specific anti-vaccinia virus agent[2b] that is not incorporated into DNA;[2a] 5-propynyloxy-2′-deoxyuridine possesses potent in vitro anti-herpes activity with remarkably low toxicity toward the replicating host cell;[3,4] 5-nitro-2′-deoxyuridine shows potent inhibitory activity toward both vaccinia virus and herpes simplex virus, and its mode of action seems to be targeted at thymidylate synthetase.[1b,c] *ara*-T [1-(β-D-arabinofuranosyl)thymine] recently has been the subject of a number of papers describing its selective inhibitory effects against herpes simplex and zoster viruses,[5–7] while *ara*-U[8] itself and 5-