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L-Chlorozotocin

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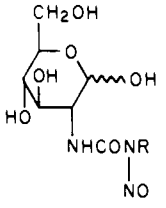
L-Chlorozotocin (2-[[[(2-chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-L-glucose) was synthesized in seven steps from L-arabinose for comparison with chlorozotocin, which is the D enantiomorph and an antineoplastic agent with clinical potential. Purification of the intermediate 2-amino-2-deoxy-L-glucose as the Schiff's base formed with 4-methoxybenzaldehyde ensured complete separation from the manno epimer. Comparative screening against leukemia L1210 with concurrent toxicity controls revealed no significant difference between D- and L-chlorozotocin in either activity or toxicity.

Current interest in chlorozotocin (1), an analogue of the

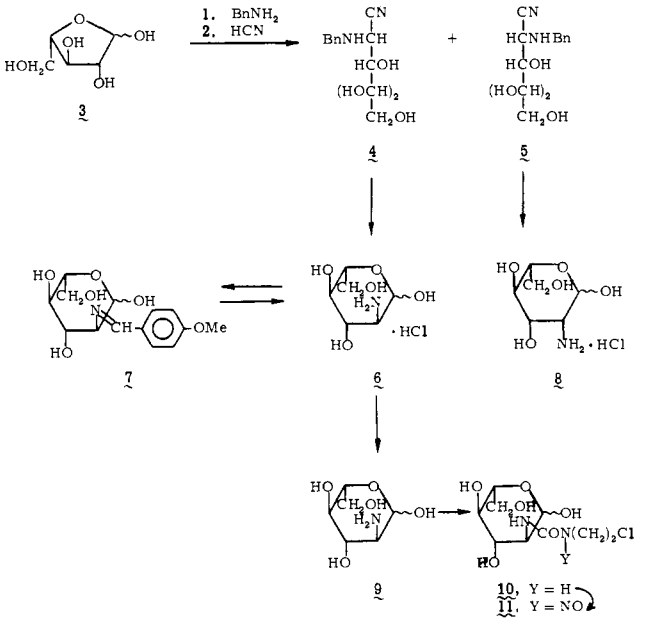


1, R = $(CH_2)_2Cl$
2, R = Me

antineoplastic antibiotic streptozotocin (2) and a prospective clinical drug, results from the observation of enhanced activity against leukemia L1210[1,2] and relatively low myelosuppression.[2] Derived from 2-amino-2-deoxy-D-glucose, 1 is in effect D-chlorozotocin. Since L-glucose is not actively transported in mammalian tissues,[3,4] the possible role of D-glucose-mediated transport of 1 was recently tested in a comparison of 1 and L-chlorozotocin (11) and found not to be evident: no significant difference in the antitumor and marrow-sparing effects of 1 and 11 was observed.[5] The synthesis of 11 (Scheme I) that enabled the above comparison is reported here along with prefatory screening against leukemia L1210.

The method used for the preparation of the intermediate 2-amino-2-deoxy-L-glucose hydrochloride (6) was an adaptation of the reported catalytic reduction of 2-deoxy-2-[(phenylmethyl)amino]-L-gluconononitrile (4) derived from L-arabinose (3).[6] Although the formation of 4 by the addition of hydrogen cyanide to *N*-(phenylmethyl)-L-arabinosylamine formed in situ is predominant,[7] this procedure prescribes a separation of 4 from its manno epimer 5 in order to avoid contamination of 6 with 2-amino-2-deoxy-L-mannose hydrochloride (8). We devised a convenient method that ensures a complete separation of 6 and 8, which is independent of the stereochemical purity of 4.

Scheme I



A basified aqueous solution of 2-amino-2-deoxy-D-glucose hydrochloride readily formed an insoluble Schiff's base with 4-methoxybenzaldehyde in near quantitative yield, whereas 2-amino-2-deoxy-D-mannose hydrochloride, whose free base is less basic than 2-amino-2-deoxy-D-glucose,[8-10] did not give evidence (TLC or precipitation) of reaction under the same conditions.[11] In a model experiment, a mixture of equal amounts of 2-amino-2-deoxy-D-glucose hydrochloride and 2-amino-2-deoxy-D-mannose hydrochloride gave only 2-deoxy-2-[[(4-methoxyphenyl)methylene]amino]-D-glucose under the above conditions. Thus, crude 6 containing 8 and ammonium chloride was purified as the Schiff's base 7, which was

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Table I. Activity against Leukemia L1210[a]

compd	LD_{10},[b] mg/kg	dose,[c] mg/kg	60-day surviv/total	median day of death[d]	% ILS[e]	cell kill, logs	ED_{50},[f] mg/kg	ED_{50}/LD_{10}[g]
control				8				
1[h]	30	24	7/10	13	62	≥6	21	0.70
11	33	24	6/10	15	87	≥6	21	0.64

[a] CDF_1 male mice inoculated with 10^5 L1210 cells intraperitoneally (ip); drug in physiological saline administered ip on day 1 postimplant. [b] Single-dose LD_{10} as determined by concurrent toxicity controls with normal mice. [c] Maximum nontoxic dose relative to concurrent LD_{10}. [d] Based on dying mice. [e] Increase in life span, survivors excluded. [f] Dose for 50% 60-day survival in this experiment as determined from log-dose probit-survival plots. [g] Therapeutic index expressed as the fraction of the LD_{10} dose that effected 50% survival in this experiment. [h] Supplied by National Cancer Institute.

hydrolyzed and the resulting **6** converted to the free base **9**. The two-step conversion of **9** to **11** was modeled after the previously described preparation of chlorozotocin;[1] **1** and **11** were identical (TLC, IR, NMR, and mp) except for specific rotations, which varied with time owing to mutarotation and concurrent aqueous decomposition but approached a common magnitude of opposite sign (a difference in anomeric ratio would account for the 4° difference observed initially).

Comparative Biologic Actvity. The results of a concurrent comparison of the activity of the enantiomorphic chlorozotocins against leukemia L1210 are summarized in Table I. Testing was performed according to an established protocol[12] with 10 mice per test group and 30 mice in the control group. Both were highly active, and no significant differences between the two in either toxicity or antileukemic activity were discernible in this test.

As previously presented, both D- and L-chlorozotocin produced comparable inhibition of bone-marrow DNA synthesis, as measured by tritiated thymidine incorporation, and L-chlorozotocin also has relative bone-marrow sparing activity, as measured by circulating white blood cell counts, when administered at a maximally effective dose, $\leq LD_{10}$.[5] Thus, the L-glucose analogue appears to have comparable antitumor and toxicity characteristics, suggesting that active transport via the D-glucose moiety is not a significant factor in the structure–activity characteristics of D-chlorozotocin.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are uncorrected. IR spectra were determined with Perkin-Elmer 521 and 621 spectrophotometers, NMR spectra with a Varian XL-100-15 spectrometer, and specific rotations with a Rudolph 80 precision polarimeter. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Elemental analysis of **11** was performed by Galbraith Laboratories, Knoxville, Tenn.; spectral determinations and other elemental analyses were performed in the Molecular Spectroscopy Section of the Southern Research Institute under the direction of Dr. W. C. Coburn, Jr., and biological evaluation was performed by the Cancer Screening Division of the Southern Research Institute under the direction of Dr. W. R. Laster, Jr.

Evaporations were carried out under reduced pressure (water aspirator) with a rotary evaporator; products were dried in vacuo (oil pump) over P_2O_5. The final product was stored cold and dry. TLC was performed on Analtech silica gel GF plates.

Crude 2-Deoxy-2-[(phenylmethyl)amino]-L-gluconononitrile (4). A solution of L-arabinose (44.0 g, 0.293 mol; Sigma) in ethanol (200 mL) was heated with benzylamine (44 mL) to complete solution and cooled. Anhydrous hydrocyanic acid[13] (22 mL) was added dropwise, but fast, to the mixture, stirring of which was continued at room temperature overnight. The precipitate was washed with cold ethanol (3 × 25 mL) and digested with boiling ethanol (350 mL); the resulting mixture was cooled and the product collected: yield 62 g (80%); mp 129–131 °C (lit.[6] mp 130–132 °C); contamination with 2-deoxy-2-[(phenylmethyl)amino]-L-mannononitrile (**5**) indicated by TLC (3:1 $CHCl_3$–MeOH, ninhydrin).

2-Deoxy-2-[[(4-methoxyphenyl)methylene]amino]-L-glucose (7). A solution of **4** (17.9 g, 67.5 mmol; contaminated with **5**) in 1 N HCl (156 mL) was hydrogenated (Parr shaker) over palladium chloride[14] (1.7 g, MC/B) at an initial pressure of 51 psi. Hydrogen uptake ceased after ~80 min. The catalyst was removed and the filtrate evaporated to dryness, leaving a residue of 18.0 g [a mixture of **6**, NH_4Cl, and **8** according to TLC (H_2O, ninhydrin)]. A 6.24-g sample of the residue was made basic with 1 N NaOH (47 mL) and treated with 4-methoxybenzaldehyde (2.8 mL, Eastman). This mixture was stirred for 5 h at room temperature and chilled. The product that precipitated was washed successively with cold water (3 × 2 mL) and ether (2 × 5 mL): yield 4.1 g (~49% from **3**); mp 161–163 °C dec (lit.[15] mp of the D-isomer 166 °C dec); homogeneous by TLC (1:1 $CHCl_3$–MeOH, ninhydrin); IR (KBr) 1635 cm^{-1} (C=N), identical with the D isomer. Anal. ($C_{14}H_{19}NO_6$) C, H, N.

2-Amino-2-deoxy-L-glucose Hydrochloride (6). A mixture of **7** (10.0 g, 33.7 mmol) and 1 N HCl (50 mL) was refluxed for 3 min, cooled, and extracted with ether (2 × 50 mL). The aqueous layer was evaporated to dryness with two additions of ethanol (20 mL) and the residue further dried: yield 6.7 g (92%); mp ~190 °C; IR (KBr) identical with the D isomer; homogeneous by TLC (H_2O, ninhydrin); $[\alpha]^{25}_D$ -81° (20 min) (*c* 1.05, H_2O) → -70° (24 h)[16] [lit.[6] $[\alpha]^{22}_D$ -94° (3 min) (*c* 1.0, H_2O) → -70° (2 h)] [D isomer: $[\alpha]^{25}_D$ +93° (20 min, *c* 1.0, H_2O) → +73° (24 h)[16]]. Anal. ($C_6H_{13}NO_5$·HCl) C, H, N.

2-Amino-2-deoxy-L-glucose (9). A suspension of **6** (6.60 g, 30.7 mmol) in absolute ethanol (52 mL) was stirred with diethylamine (4.5 mL) for 7 h at room temperature. The insoluble solid was collected; treated as above three times more with the following ratios of ethanol and diethylamine in milliliters: (1) 52:3.5, (2) 50:3.0, (3) 50:3.0; and washed with ether: yield 4.1 g (74%); mp 109–110 °C (lit.[1,17] mp of the β-D isomer 110–111 °C); IR (KBr) comparable to the D isomer. Anal. ($C_6H_{13}NO_5$) C, H, N.

2-[[[(2-Chloroethyl)amino]carbonyl]amino]-2-deoxy-L-glucose (10). 2-Chloroethyl isocyanate (2.3 mL, Eastman) was added gradually to a stirred suspension of **9** (4.8 g, 26.8 mmol) in *N*,*N*-dimethylformamide (62 mL) maintained at room temperature with a water bath. Complete solution occurred within a few minutes, and precipitation began after ~1 h. The mixture was diluted with ether (470 mL) and stirred at 5 °C for 30 min. The precipitated **10** was washed with ether (3 × 30 mL): yield 7.4 g (97%); mp 158–160 °C dec (lit.[1] mp of the D isomer 160–161 °C); homogeneous by TLC [2:1 $CHCl_3$–MeOH, $(NH_4)_2SO_4$–H_2SO_4 char]; IR (KBr) identical with the D isomer. Anal. ($C_9H_{17}ClN_2O_6$) C, H, N.

2-[[[(2-Chloroethyl)nitrosoamino]carbonyl]amino]-2-deoxy-L-glucose (11). Dinitrogen trioxide (Matheson) was bubbled into a cold (0–5 °C), stirred suspension of **10** (3.90 g, 13.7 mmol) in 1 N HCl (39 mL) at a moderately fast rate for short intervals during 1 h. The mixture was diluted with ethanol (20 mL) and stirred at 0 °C for 1 h. The light-yellow product was collected: yield 2.7 g; mp 143–144 °C dec (lit.[1] mp of the D isomer, 140–141 °C dec). A second crop (150 mg) with the same melting point was recovered by concentrating the filtrate to ~15 mL, diluting with ethanol (20 mL), and chilling: total yield 66%; homogeneous by TLC [5:1 $CHCl_3$–MeOH, UV and $(NH_4)_2SO_4$–H_2SO_4 char]; IR (KBr) and NMR (Me_2SO-d_6) identical with the D isomer;[1] $[\alpha]^{25}_D$ -53° (0 h) (*c* 1.0, H_2O) → -36° (2.5 h) → -19° (20 h)[18] [D isomer: $[\alpha]^{25}_D$ +57° (0 h) (*c* 1.0, H_2O) → +35° (2.5 h) → +20° (20 h)[18]]. Anal. ($C_9H_{16}ClN_3O_7$) C, H, N. (The nitrosation of **10** in concentrated hydrochloric acid with subsequent

addition of ether as in the preparation of the D isomer[1] and recrystallization from ethanol gave a 36% yield of 11.)

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1-(Dichloroacetyl)-1,2,3,4-tetrahydro-6-quinolinol Esters. New Potent Antiamebic Agents

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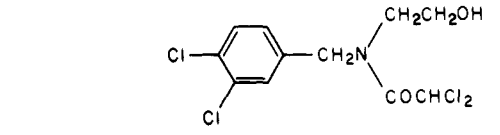
A series of 1-(dichloroacetyl)-1,2,3,4-tetrahydro-6-quinolinols and certain *O*-acyl derivatives thereof have been prepared and shown to be potent antiamebic agents in the *Entamoeba criceti* infected hamster model. Compounds were compared with etichlordifene and diloxamide and one of them, 1-(dichloroacetyl)-6-(2-furoyloxy)-1,2,3,4-tetrahydroquinoline (4), was selected for human trial.

The first dichloroacetamide antiamebic agent to receive extensive animal testing and clinical trial was chlorbetamide[1] (I; Mantomide®). Later additions to this clinically effective class of compounds were teclozan[2] (II; Falmonox®), etichlordifene[3] (III; Kitnos®), and diloxamide[4] (IV; Furamide®). We now describe a new class of dichloroacetamide, 1-(dichloroacetyl)-1,2,3,4-tetrahydro-6-quinolinol, derivatives (V), several of which are more potent than any of the above reference drugs when screened against an *Entamoeba criceti* model in hamsters.



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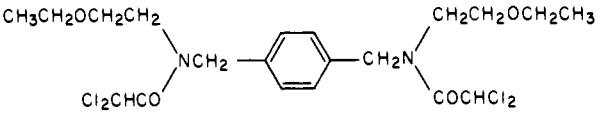

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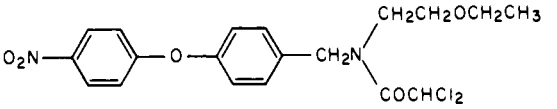

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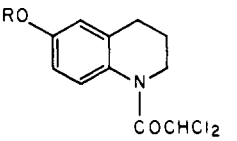

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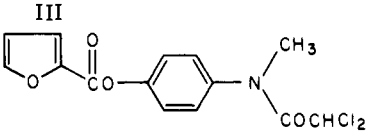

IV

Chemistry. Compounds **2** through **13** of Table I were prepared from the free phenol **1**, which in turn was prepared by direct dichloroacetylation of the known[5] 1,2,-3,4-tetrahydro-6-quinolinol. The halogenated phenols **8** and **9** were prepared by direct interaction of **1** with sulfuryl chloride and elemental bromine, respectively, and were determined to be the 5-halo isomers by inspection of their NMR spectra. Esterification of the phenolic amides was accomplished using the appropriate acyl chloride and triethylamine in an appropriate solvent.

Screening. Young adult female Sprague–Dawley hamsters weighing 100–120 g and harboring trophozoites of the naturally occurring *Entamoeba criceti* in the cecum and colon were used in the studies. The animals had a rate of spontaneous infection with *E. criceti* of nearly 100% in the breeding colony. Examinations for infectivity were made in ten randomly chosen animals weekly, and in no

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