1,4-Di(D-glycosyl)piperazine Derivatives as Oxyuricidal Agents

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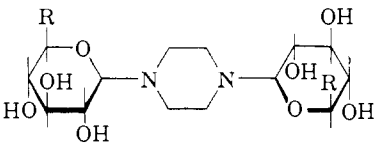
Two recent publications on N-glycosyl derivatives of piperazine by Catesby and Stephen[1] and Panagopoulos, Kovatsis and Sekeris[2] have prompted us to report additional results that were obtained in connection with the development of 1,4-di(D-glucosyl)piperazine (I) (DGP).[3]



I, R = $-CH_2OH$ (DGP)
II, R = —H

Piperazine, usually in the form of a salt, has been employed as the drug of choice in the treatment of infection by *Enterobius vermicularis* (human pinworm). However, particularly in the earlier use of the drug, a number of toxic side-effects had been noted in certain patients.[4] In a search for an effective oxyuricidal agent of low toxicity we prepared the compounds listed.

Experimental[5]

1,4-Di(D-glucosyl)piperazine (DGP)[1-3] (I) and **1,4-di(D-xylosyl)piperazine**[1] (**II**) were prepared similarly to the literature. **1,4-Bis(D-gluco-2,3,4,5,6-pentahydroxyhexyl)piperazine (III)** was prepared by subjecting a reaction mixture of 36 g. of D-glucose, 19.4 g. of piperazine hexahydrate, and 350 ml. of 68% methanol (after standing overnight) to a catalytic reduction with Raney nickel at 77 kg./cm.^{2} and 65–70° for 29 hr. The theoretical quantity of hydrogen was absorbed. The crude product was obtained by removing the catalyst and concentrating; yield, 3.1 g. (8%) of solid, m.p. 150–156°. The low yield was probably due to the formation of monosorbitylpiperazine and sorbitol in the reaction mixture. Two recrystallizations of the crude product from dilute methanol gave a white compound of m.p. 158.5–160.5°. An acidified portion of the purified product showed the absence of reducing sugar with Fehling solution. The infrared and n.m.r. spectra were found to be consistent with the structure.

Anal. Calcd. for $C_{16}H_{32}N_2O_{10}$: C, 46.37; H, 8.27; N, 6.76. Found: C, 46.46; H, 8.51; N, 6.74.

In a second example the reduction was attempted with a suspension of DGP in methanol. Here the hydrogen absorption was incomplete, and a large amount of the insoluble starting material was recovered. Only a 3% yield of the product was obtained.

1,4-Bis(tetra-*O*-acetyl-D-galactosyl)piperazine[1,2] (IV), **1,4-bis(tetra-*O*-acetyl-D-glucosyl)piperazine**[1-3] (V), and **1,4-bis(tri-*O*-acetyl-D-xylosyl)piperazine**[1] (VI) were obtained by known methods.

Oxyuricidal Evaluation.—Dose levels for DGP, anhydrous piperazine (the starting material) and commercial piperazine citrate are given in Table I. The remaining 5 compounds also were dosed as described in Table I at 2 or more levels within the range of 25 to 500 mg./kg./day. Doses were prepared daily, in 2% soluble starch, just prior to dosing. The procedure was adapted from that of Reinertson and Thompson[6] and Wells.[7]

Healthy, normal, male[8] mice, 18–24 g. weight, naturally infected with pinworms (*Syphacia obvelata* and *Aspiculuris tetraptera*) were used. For initial screening, 5 mice were dosed per level; for further evaluation 10 mice per level were used. Non-medicated controls and mice medicated with the reference agent were included in each run. Anhydrous piperazine and piperazine hexahydrate were the reference agents. Both are equipotent oxyuricides when dosed according to their piperazine content.

TABLE I

OXYURICIDAL ACTIVITY OF DGP, ANHYDROUS PIPERAZINE AND PIPERAZINE CITRATE IN MICE[a]

Compound	Dose[b,c] mg./kg.	No. mice	Reduction of adult worms: Mean (%)[d]	S.E.	ED$_{50}$[e] mg./kg.	Gross toxicity (died/dosed)
DGP (I)	17	10	50	±26		0/10
	50	10	74	±24	18	0/10
	150	10	90	±26		0/10
	450	10	91	±21		0/10
Anhydrous	17	10	37	±25		0/10
Piperazine	50	10	64	±24	28	0/10
	150	10	97	±21		1/10
	450	10	96	±23		2/10
Piperazine	17	10	10	±34		0/10
Citrate	50	9[f]	47	±33	33	0/9
	150	10	89	±28		0/10
	450	10	97	±20		0/10

[a] Summary of results from four sets of tests. Non-medicated controls included in each test (10 per test) had mean adult worm counts ranging from 69 ± 14 to 144 ± 30. [b] Dosage schedule: 1 oral dose/day for 4 consecutive days. [c] Dosage based on piperazine content. [d] Reduction based on worm burden of corresponding non-medicated controls. [e] Effective dose 50%, the quantity of drugs expected to produce a 50% reduction in worm burden, as calculated from standard dose response curves. [f] One mouse killed accidentally during test.

One week after the first dose, the mice were etherized, the ceca and colons removed, and each intestinal segment placed in separate, small Petri dishes. The contents of each segment were dispersed in 30% zinc sulfate[9] and examined under magnification for pinworms. All adult worms were counted and the presence of microscopic-sized, immature worms was noted and their numbers estimated. The percentage reduction of adult worms per dose level in the medicated mice was based on the adult worm burden of the corresponding control mice.

Table I presents a summary of representative results obtained in evaluating the oxyuricidal effectiveness of DGP, anhydrous piperazine and piperazine citrate.

Observations on the microscopic-sized immatures suggested that DGP and anhydrous piperazine have about the same degree of activity, with both being slightly the more effective against the immature forms. The gross toxicity observed with the latter compound appears consistent with the results of the toxicological studies.

The other 5 derivatives were screened for their oxyuricidal action with these results. The practically insoluble and difficultly hydrolyzable derivative (V) showed merely a trace of activity; the soluble derivative (II) approximated the potency of DGP, but it showed twice the toxicity. Activities of the two derivatives IV and VI were intermediate and corresponded to their relative solubility and hydrolyzability. Derivative III did not follow the same pattern, for although soluble, it was inactive. Presumably, its lack of activity is due to its being a stable tertiary amine and not a labile glycosyl derivative.

Results obtained with a mechanical mixture of 1 mole of piperazine and 2 moles of glucose provide further evidence to suggest that piperazine must be present as "free piperazine" or an "active

(1) C. G. C. Catesby and A. M. Stephen, *J. Org. Chem.*, **26**, 601 (1961).

(2) C. Panagopoulos, A. Kovatsis, and C. Sekeris, *Arzneimittel-Forsch.*, **11**, 629 (1961).

(3) T. A. Martin, U. S. Patent 2,910,465 (Oct. 27, 1959).

(4) R. H. R. White and O. D. Standen, *Brit. Med. J.*, **2**, 1272 (1953); J. P. Hanzlik, *J. Lab. Clin. Med.*, **2**, 308 (1917).

(5) Melting points are corrected (Thomas-Hoover capillary apparatus). Microanalyses, infrared and nuclear magnetic resonance spectra are by Dr. Francis G. McDonald and staff.

(6) J. W. Reinertson and P. E. Thompson, *J. Parasitol.*, **37**, 15), Sec. 2, 28 (1951); *Exp. Parasitol.*, **1**, 384 (1952).

(7) H. S. Wells, *J. Infectious Diseases*, **89**, 190 (1951).

(8) A. W. Mathies, *J. Parasitol.*, **40**, 702 (1954).

(9) E. C. Faust, "Human Helminthology," Lea and Febiger, Philadelphia, 3rd ed., 1949, p. 593.

TABLE II

ACUTE, LETHAL TOXICITY OF PIPERAZINE-CONTAINING COMPOUNDS IN MICE AND RATS

Compound and Vehicle	Dose (mg./kg.)	Total no. dead / No. tested 24 hr.	48 hr.	LD_{50} (mg./kg.)[a] As compound	As piperazine
		Mouse			
DGP (I), 10% gum acacia[b]	33,000	19/30	19/30	32,000	6,720
	30,250	3/10	5/10	(33,280–30,866)	(6,989–6,482)
	27,500	0/10	0/10		
1,4-Di(D-xylosyl)-piperazine (II), distilled H_2O	18,857	12/15	12/15	14,388	3,525
	13,469	5/10	5/10	(16,953–12,384)	(4,153–3,034)
	9,620	1/20	1/20		
	3,204	0/10	0/10		
Anhydrous piperazine HCl + H_2O (pH 7.0)	3,360	7/10	7/10	2,730	2,730
	2,240	3/10	3/10	(3,331–2,238)	3,331–2,238)
	1,120	0/10	0/10		
Piperazine Hexahydrate HCl + H_2O (pH 7.0)	8,000	9/10	9/10	5,300	2,385
	6,000	9/10	10/10	(6,042–4,648)	(2,719–2,092)
	4,000	1/10	2/10		
	3,000	0/10	0/10		
Piperazine citrate HCl + H_2O (pH 6.0)	12,000	10/10	10/10	8,500	3,400
	8,000	7/20	8/20	(9,860–7,328)	(3,822–2,931)
	6,000	1/10	2/10		
	4,000	0/10	0/10		
		Rat			
DGP (I) 10% gum acacia	33,000	7/10	8/10	30,000	6,300
	27,500	3/10	3/10	(33,900–26,544)	(7,119–4,585)
	22,000	0/10	1/10		
Piperazine citrate HCl + H_2O (pH 6.0)	15,000	10/10	10/10	11,200	4,502
	12,000	5/10	6/10	(12,678–9,825)	(5,097–3,950)
	9,000	2/10	3/10		
	6,000	0/10	0/10		

[a] Figures in parentheses represent 95% confidence limits. [b] Acute LD_{50} values in distilled water were 29,900 mg./kg. (22,800–34,700), as piperazine 6,279 mg./kg. (4788–7287).

form" thereof for it to exert oxyuricidal activity. The potency of the freshly prepared mixture was related directly to its piperazine content and was essentially the same as DGP at corresponding concentrations of piperazine.

Although adult pinworms are more sensitive to the action of piperazine than are immature worms,[10] all three compounds evaluated (Table I) showed significant activity against the immature worms, but DGP and anhydrous piperazine tended to show slightly more effect. Since the mouse pinworm is reportedly more resistant to oxyuricides than the human pinworm,[11] results of chemotherapeutic tests of such agents in mice are suggestive of their clinical activity.

Based on piperazine content, DGP has essentially the same oxyuricidal potency as anhydrous piperazine and piperazine citrate. This equivalent activity with equivalent levels of piperazine has proved true with all of the many soluble and insoluble piperazine derivatives[12] that we have tested, provided that the compounds are altered in the body so as to release piperazine or an "active form" thereof. Results obtained with the other glycosyl derivatives suggest that their solubility and/or hydrolyzability are of significance in their oxyuricidal activities.

Toxicology.—Acute toxicity values were determined in mice and rats for DGP and piperazine citrate and in mice only for 1,4-di(D-xylosyl)piperazine (II), anhydrous piperazine and piperazine hexahydrate. The toxicity of the several agents was calculated in terms of the piperazine content which was 21% for DGP; 40% for piperazine citrate; 45% for piperazine hexahydrate and 100% for anhydrous piperazine.

A repeated dose study using DGP as the test material was conducted in 6 week old albino rats. One of 4 groups, each consisting of 6 males and 6 females, served as controls. The remaining three groups represented dose levels of 3000, 2000, and 1000 mg. of DGP/kg. which are equivalent to doses of 630, 420 and 210 mg. of piperazine/kg., respectively. DGP suspended in 10% gum acacia was administered orally once each day, 7 days a week for 10 weeks. Both weights and food consumption values were recorded each week. Complete hematologic studies were conducted after 6 weeks of drug administration. Acute, lethal toxicity of DGP in mice and rats is summarized in Table II. Additional values for reference agents also are given.

In general, acute, lethal doses of the piperazine derivatives produced ataxia, diarrhea and depression. Some deaths occurred between 24 and 48 hr. after dosing, but do not significantly alter the acute LD_{50} values. Repeated oral administration of DGP had no deleterious effect on food consumption or weight gain. Hemograms for the treated animals revealed no marked differences from those of the control animals. Survival was not altered and gross examination of necropsy material revealed no pathologic changes attributable to the drug.

In order to show further that the low order of toxicity exhibited by DGP is an inherent characteristic of the compound, some work[3] was done with a mechanical mixture of 1 mole of piperazine and 2 moles of glucose. The LD_{50} value of the freshly prepared mixture as determined in white mice was found to be 2646 mg./kg. (based on the piperazine content) which is in the general range characteristic of piperazine and its salts.

DGP was acutely less toxic than 1,4-di(D-xylosyl)piperazine, anhydrous piperazine, piperazine hexahydrate and piperazine citrate in mice and piperazine citrate in rats. Repeated doses of the product equivalent to 630 mg./kg. of piperazine each day were well tolerated in rats.

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(12) T. A. Martin, P. S. Prickett, and W. T. Sumerford, unpublished results.