Pyrimidine and Triazine 3-Oxide Sulfates: A New Family of Vasodilators

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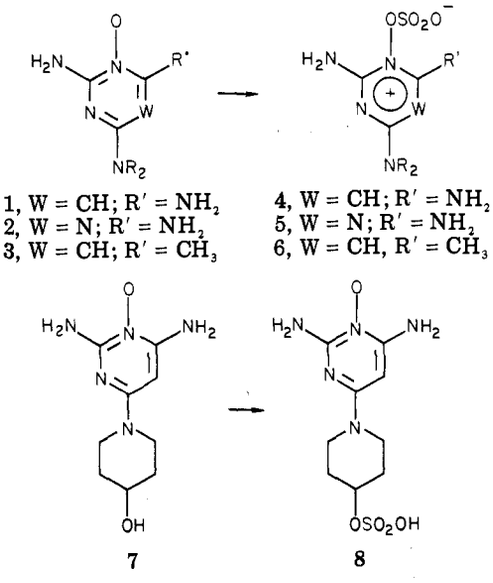
Di- and triaminopyrimidine 3-oxides (e.g., 2,4-diamino-6-piperidinylpyrimidine 3-oxide and 2,4-diamino-6-(diallylamino)triazine 3-oxide) react with sources of sulfur trioxide, such as sulfur trioxide trimethylamine or chlorosulfuryl chloride, to yield the corresponding heterocyclic *O*-sulfates. These sulfates are inner salts with unusual physical properties. The structure of the *O*-sulfate of 2,4-diamino-6-piperidinylpyrimidine 3-oxide was confirmed by X-ray. These *O*-sulfates are hypotensives. They apparently act by direct vasodilation.

Minoxidil (**1a**, the active component of Loniten tablets) is 2,4-diamino-6-piperidinylpyrimidine 3-oxide. It is a clinically effective hypotensive that lowers blood pressure by dilating peripheral arteriolar blood vessels. The onset of hypotensive activity is delayed by 30 to 90 min following drug administration.[1,2] Further, minoxidil does not exhibit a direct relaxant effect on vascular smooth muscle. For these reasons, we believed that minoxidil's hypotensive effects might be due to a metabolite. Minoxidil's metabolism has been extensively studied,[3,4] but none of the reported metabolites approach minoxidil in hypotensive activity. Subsequent to these reports, it was found that the sulfate scavenger 4-acetamidophenol blunted the hypotensive activity of minoxidil.[5] Because of this, we investigated the sulfate path of metabolism for minoxidil. Various routes to sulfate esters [e.g., reaction of purine *N*-oxides with PAPS (phosphoadenosine phosphosulfate) and sulfotransferase to form *O*-sulfate] have recently been reviewed.[6] We have recently reported that minoxidil (**1a**) is converted to minoxidil *O*-sulfate (**4a**) by PAPS and sulfotransferase.[5]

We now report the facile sulfation of the pyrimidine and triazine *N*-oxide oxygen and the isolation and characterization of the resultant *O*-sulfates. This is the first description of the chemical synthesis of triamino- and diaminopyrimidine or triaminotriazine *N*-oxide sulfates. The pyrimidine and triazine *N*-oxide sulfates that we describe are hypotensive in the rat. The mechanism by which **4a** relaxes vascular smooth muscle has been investigated but not yet defined.

Chemistry. We have investigated the reactions of 2,4,6-triaminopyrimidine and triazine 3-oxides with sources of sulfur trioxide. In principle, triaminopyrimidine 3-oxides of structure **1** (Scheme I) can react with sulfur trioxide or its amine conjugates in several ways: the 2- and 4-amino groups can form sulfamic acids, the electron-rich C-5 can be sulfonated, and the *N*-oxide oxygen can form a sulfate.[8] Only the last possibility was realized. When pyrimidine and triazine *N*-oxides of structure types **1–3** react with sulfur trioxide–pyridine, sulfur trioxide–trimethylamine, or chlorosulfonic acid, the only products that

Scheme I



are isolated are the *O*-sulfates **4–6** (see Scheme I). These compounds exist as inner salts. For example, 2,4-diamino-6-piperidinylpyrimidine 3-oxide (**1a**) reacts with sulfur trioxide sources to yield *O*-sulfate **4a** (NR_2 = piperidinyl, Scheme I). The structure of **4a** has been unambiguously assigned by X-ray (Figure 1).[9]

The X-ray structures of **4a** and **1a** reveal that in both **4a** (minoxidil *O*-sulfate) and **1a** (minoxidil) ring atoms N-1, C-2, C-4, C-5, and C-6 lie in a plane. However, in **4a**, N-3 is 0.13 Å below the plane, and the amino groups at C-2 and C-4 are, respectively, 0.06 and 0.14 Å above this plane. In **1a**, the analogous N-3 and the amino groups lie in the plane. In both **1a** and **4a**, the piperidino nitrogen is planar. Thiessan et al.[7] have noted that in the case of 3-hydroxyxanthine, the nitrogen to which hydroxy is attached is displaced 0.19 Å from the best plane of the aromatic ring. This result is consistent with that observed for **4a** ($NOSO_2^-$) but not for **1a** (N → O).

The physical properties of these inner-salt *O*-sulfates are surprising. They are insoluble in water (less than 1 mg/mL) for **4a**) and they are more soluble in dipolar aprotic solvents (e.g., dimethylformamide or acetonitrile) than their precursor *N*-oxides. They also move more rapidly on silica gel than their precursors with a variety of eluents. In contrast, the purine *N*-oxide *O*-sulfates that are reported in the literature are water soluble and polar on silica gel.[6]

In an internal competition experiment between an aliphatic alcohol and a pyrimidine *N*-oxide, sulfur trioxide–pyridine exclusively sulfates the aliphatic alcohol. Thus,

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(9) This work will be reported elsewhere along with other pyrimidine *N*-oxide X-ray structures by D. W. DuChamp and C. Chidester.
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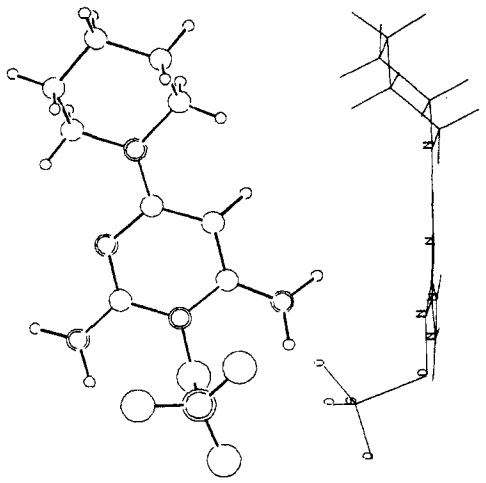

Figure 1. X-ray structure of 4a.

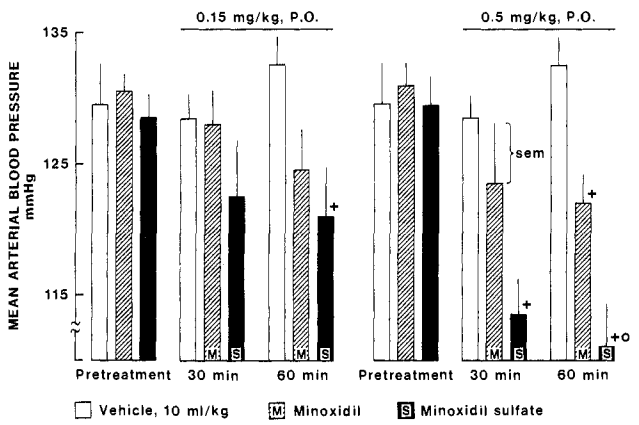

Figure 2. Hypotensive activity of minoxidil and minoxidil sulfate. Minoxidil (M) and minoxidil sulfate (S) were given orally to Sprague–Dawley rats: "+" indicates a significant drop in mean arterial blood pressure; "+" indicates a significantly greater drop than the comparable "+" designated pressure. SEM is standard error of the mean. See text and Experimental Section.

pyrimidine 7 yields exclusively the piperidine *O*-sulfate 8 under the usual reaction conditions. For pyrimidine 7, the aliphatic alcohol is more reactive than the *N*-oxide function.

The stability in aqueous solution of pyrimidine *O*-sulfate **4a** was determined. A saturated solution (1 mg/mL) of pH 7 was monitored by HPLC. Compound **4a** is hydrolyzed to the parent pyrimidine, 2,4-diamino-6-piperidinylpyrimidine 3-oxide, with a half life of 48 h.

Metabolism. In vitro studies have shown that soluble rat-liver sulfotransferase enzyme will transfer the sulfate from phosphoadenosine phosphosulfate to minoxidil (**1a**). This enzymatic product is identical with minoxidil *O*-sulfate (**4a**) by HPLC and mass spectral criteria (see Scheme I).[5]

Pharmacology. The pyrimidine and triazine sulfates that we have described are potent hypotensives in the conscious rat (See Table I). Minoxidil sulfate is more potent and has a faster onset of activity than minoxidil. This is illustrated by Figure 2. At 0.15 mg/kg minoxidil sulfate, but not minoxidil, treated rats developed significant (+ = $p \leq 0.05$) hypotension within 1 h (reference is vehicle-treated rats). At 0.5 mg/kg, only minoxidil sulfate treated rats had developed significant hypotension within 30 min. These sulfate-treated rats also developed significantly greater (O = $p \leq 0.05$) decreases in blood pressure at 1 h than the minoxidil-treated rats (see Figure 2 and legend).

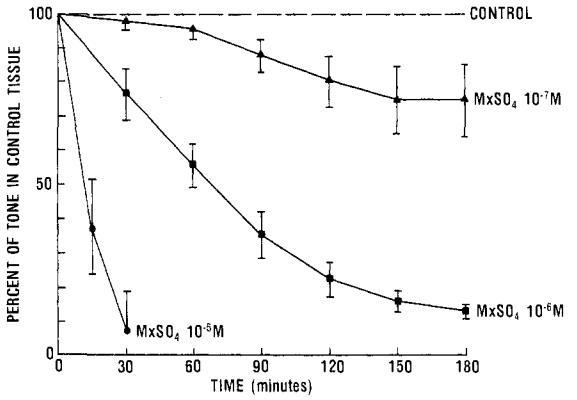

Figure 3. Direct relaxant effects on arterial rings of minoxidil sulfate. Relaxation of meserteric arterial rings of rabbit in vitro induced by minoxidil sulfate ($MxSO_4$) at either 10^{-5}, 10^{-6}, or 10^{-7} M. Results are expressed as the percent of the tone in the untreated arterial ring. Tone was induced in all tissues with phenylephrine, 1 μg/mL. $N = 4$ at each concentration.

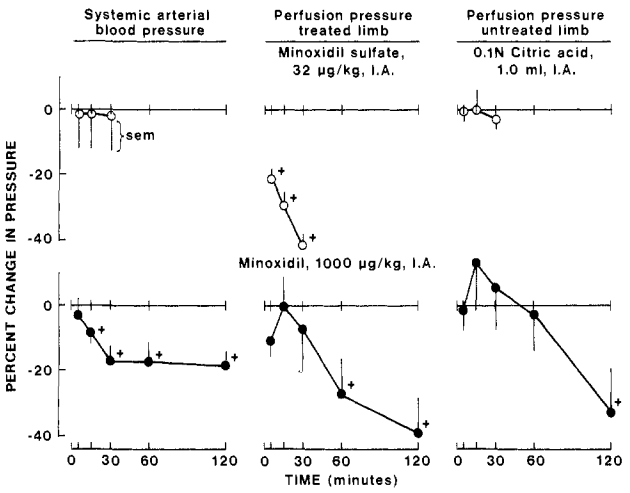

Figure 4. Minoxidil and minoxidil sulfate in the isolated vascular bed of dog. Changes in mean systemic arterial blood pressure and perfusion pressure of the canine hindlimbs after local intraarterial injections of minoxidil or minoxidil sulfate into a perfused limb. SEM is the standard error of the mean. Vehicle is 1.0 M citric acid.

Figure 3 illustrates the relaxing effect of minoxidil sulfate (**4a**) on mesenteric arterial rings from the rabbit. Four arterial rings were studied in several experiments. Three of the four arterial rings were treated with either 10^{-7}, 10^{-6}, or 10^{-5} M minoxidil sulfate, and the fourth ring was a control. Data are expressed as tone in the artery as a percent of the tone in the control vessel. During 180 min, the minoxidil sulfate treated tissues showed both a concentration and time related relaxation. This shows that minoxidil sulfate is active in vitro as a vasodilator. The parent compound, minoxidil, was not effective at concentrations up to 10^{-5} M (not shown).

In other experiments we showed that the relaxing effect also occurred when arteries were contracted with a thromboxane-like agonist, the prostaglandin endoperoxide analogue, U-46,619.[11] This indicates that the relaxing effect shown in Figure 3 was not due to specific antagonism of the α-adrenergic stimulant effects of phenylephrine. Since relaxations occurred in indomethacin-treated tissues (see above), the effect of minoxidil sulfate on arterial muscle was not dependent on endogenous prostaglandin production.

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Table I. *O*-Sulfate Physical Data and Hypotensive Activity

no.[a]	NR_2	method[b]	yield,[c] %	formula	anal.[d]	mp, °C	ΔBP (dose)[i]
4a	c-NC_5H_{10}	A	51[e]	$C_9H_{15}N_5SO_4$	C, H, N, S	188–189 dec	−16 (0.15)
		B	83[e]			188–190 dec	
		C	49[e]			188–189 dec	
		D	48[f]			191 dec	
4b	$NHCH_3$	A	44[f]	$C_5H_9N_5O_4S \cdot H_2O$	C, H, N, H_2O	136–319	−8 (50)
5	$N(CH_2CH{=}CH_2)_2$	A	71[g]	$C_9H_{14}N_6SO_4$	C, H, N, S	258 dec	−21 (5)
6a	c-NC_4H_8	B	26[e,f]	$C_9H_{14}N_4O_4S$	H, N; C[j]	143 dec	−39 (50)
6b	c-$N(CH_2CH_2)_2O$	A	28[e,g]	$C_9H_{14}N_4SO_5$	C, H, N, S	232–233 dec	−37 (14)
8		A	83[f,h]	$C_9H_{15}N_5SO_5$	C, H, S; N[k]	251 dec	

[a] See Scheme I. 4a = minoxidil *O*-sulfate. [b] Described under Synthesis of 4a in Experimental Section. [c] Based on isolated, pure *O*-sulfates. Other product was unreacted starting material. [d] Unless otherwise noted, elemental analyses are ±0.4% from theory. [e] Crystallized from DMF/H_2O. [f] From CH_3CN. [g] From EtOH/water. [h] From EtOH. [i] ΔBP (dose) refers to the average change in mean arterial blood pressure (mmHg) observed at 4 h after oral administration (mg/kg). Pressures are measured in female Sprague–Dawley rats. [j] C: calcd, 39.40; found, 38.95. [k] N: calcd, 22.94; found, 21.59.

Figure 4 illustrates that in a whole animal, minoxidil sulfate (**4a**) is a direct vasodilator, whereas minoxidil is not. The hindlimb of a dog was perfused at constant flow via an aortic to iliac arterial shunt, and perfusion pressure was monitored. Changes in perfusion pressure reflected changes in hindlimb vascular resistance. When minoxidil sulfate (32 μg/kg) was injected directly into the arterial supply of the perfused limb, a prompt and significant (+ = $p \leq 0.05$) decrease in perfusion pressure occurred, although mean systemic arterial pressure was not altered. In contrast, when minoxidil (1 mg/kg) was injected into the perfused limb, significant decreases in perfusion pressures were delayed and were associated with a comparable decrease in systemic arterial pressures (Figure 4).

Conclusion

These data support the idea that minoxidil *O*-sulfate is a direct-acting vasodilator and is, thus, unlike the parent drug. This is the first example of a new class of vasodilators: aminopyrimidine and aminotriazine *O*-sulfates.

Experimental Section

Melting points were determined in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded on a Varian HFT80 and are consistent with assigned structure. Yields, physical data, and methods of preparation are summarized in Table I.

2,4-Diamino-6-piperidinyl-3-sulfoxypyrimidinium Hydroxide Inner Salt (4a). Method A. A mixture of 1.00 g (4.8 mmol) of 2,4-diamino-6-piperidinylpyrimidine 3-oxide and 1.50 g (9.43 mmol) of pyridine–sulfur trioxide was stirred for 2.5 h at room temperature in 15 mL of dimethyl formamide. The mixture was shaken with 60 mL of water for 2 min. The solid was filtered and recrystallized from DMF and water to yield 0.71 g (51%) of **4a**: ^1H NMR δ 7.62–7.25 (br, s, 2 H, NH_2), 3.62–3.35 [br, s, 4 H, $N(CH_2)_2$], 1.70–1.25 [br, s, 6 H, $(CH_2)_3$].

Method B. In an alternate method, 1.05 g (5.02 mmol) of 2,4-diamino-6-piperidinylpyrimidine 3-oxide and 2.78 g of trimethylamine–sulfur trioxide were stirred in 50 mL of $CHCl_3$ and 4 mL of DMF for 2.5 h. The mixture was concentrated, and the residue was crystallized from DMF and water to yield 1.20 g (83%) of pure **4a** (mp, NMR).

Method C. In this method, 1.00 g (4.8 mmol) of 2,4-diamino-6-piperidinylpyrimidine 3-oxide, 1.11 g (9.6 mmol) of chlorosulfonic acid, and 2.47 g (19.1 mmol) of *N,N*-diisopropylethylamine in 25 mL of chloroform was stirred at room temperature overnight. The residue was concentrated. The residue was shaken with aqueous sodium bicarbonate. The solid was filtered and rinsed with ether to yield 1.2 g of solid, which was recrystallized from DMF and water to yield 0.69 g (49%) of **4a** (mp, NMR).

Method D. A suspension of 1.04 g (5.0 mmol) of 2,4-diamino-6-piperidinylpyrimidine 3-oxide, 0.70 g of trimethylamine–sulfur trioxide, and 50 mL of acetonitrile were stirred vigorously for 2 days. The mixture was concentrated. The residue was heated with 80 mL of acetonitrile and filtered hot. The filtrate was concentrated to 20 mL, cooled, and filtered to yield 700 mg (48%) of **4a** (mp, NMR), 190–195 °C dec.

2,4-Diamino-6-(4-sulfoxy-1-piperidinyl)pyrimidine 3-Oxide (8). A solution of 2.20 g (9.78 mmol) of 2,4-diamino-6-(4-hydroxy-1-piperidinyl)pyrimidine 3-oxide and 2.00 g (12.6 mmol) of pyridine–sulfur trioxide complex was stirred for 1 h at room temperature. The mixture was concentrated. The residue was stirred with 10 mL of water for 3 min and then concentrated in vacuo. The residue was triturated with hot acetonitrile to yield 2.47 g (83%) of compound 8, 251 °C dec.

Hypotensive Activity in Rat. The blood pressure of restrained female Sprague–Dawley rats was measured directly from chronic indwelling aortic cannulas exteriorized from the nape of the neck.[10] The rats were restrained in a towel during the test. Blood pressure was measured via Statham transducer (P23G) and Grass Model 5 polygraph. Measurements were made before and after oral administration of each compound. Compounds were suspended in a carboxymethylcellulose vehicle at 10 mL/kg.

Methods. Mesenteric Artery Relaxation. Mesenteric arteries were removed from male rabbits and cross-sectional cuts were made to give four rings, each 2-mm wide. The rings were placed in tissue baths containing Krebs solution at 37 °C, mounted so that isomeric tension of the circular smooth muscle could be monitored continuously. Muscle tone was induced by adding phenylephrine to the bath, 1 μg/mL. The baths contained indomethacin (1 μg/mL) to block endogenous prostaglandin synthesis.

Perfused Hindlimbs. Mongrel dogs of either sex (15–22 kg) were pretreated with morphine sulfate (2 mg/kg sc) and anesthetized 30 min later with an intravenous injection of chloralose (50 mg/kg) and urethane (60 mg/kg). The hindlimbs were perfused independently at constant flow with a pump (Sigma motor pump) interposed on tubing that led from the aorta to the iliac arteries. The preparation has been described in detail elsewhere.[12] Perfusion pressures were recorded from the extracorporeal tubing distal to the pump. Systemic arterial pressure (brachial artery) and perfusion pressures were monitored with transducers (Statham P23G) connected to a Grass Model 7 polygraph. Intraarterial injections were made downstream from the pump into the perfusion tubing. The limb to receive drug was determined by coin toss, and the contralateral limb received a comparable volume injection of the vehicle alone (0.1 N citric acid). The Student's *t* test was used to test for statistical significance of differences ($p < 0.05$) that occurred after treatment.[13]

Registry No. **1a**, 38304-91-5; **1b**, 55973-02-9; **2**, 6494-76-4; **3a**, 80142-47-8; **3b**, 80142-49-0; **4a**, 80142-45-6; **4b**, 87190-06-5; **5**, 80142-50-3; **6a**, 80142-46-7; **6b**, 80142-48-9; **7**, 56828-37-6; **8**, 87190-07-6.

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