Articles

3-Hydroxy-α-methyltyrosine Progenitors: Synthesis and Evaluation of Some (2-Oxo-1,3-dioxol-4-yl)methyl Esters

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The (5-methyl-2-oxo-1,3-dioxol-4-yl)methyl and (5-*tert*-butyl-2-oxo-1,3-dioxol-4-yl)methyl esters of 3-hydroxy-α-methyltyrosine (methyldopa) were prepared and evaluated as progenitors of the amino acid. ^{1}H NMR experiments reveal that the esters are converted cleanly to methyldopa and the corresponding α-diketone at pH 7.4, with the 5-methyl derivative undergoing hydrolysis faster than the 5-*tert*-butyl analogue. Bioavailability studies in dogs show that the esters, particularly the 5-methyl derivative, yield significant plasma levels of methyldopa. Both esters are orally effective antihypertensive agents in spontaneously hypertensive (SH) rats. These studies indicate that (2-oxo-1,3-dioxol-4-yl)methyl esters are viable prodrugs for the latentiation of methyldopa.

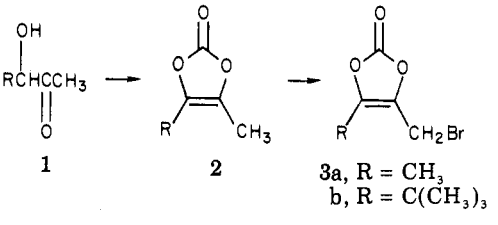
Methyldopa (4) is an effective, orally active antihypertensive agent. However, because the drug is poorly absorbed in some patients, prodrugs of methyldopa that would be more efficiently absorbed from the gastrointestinal tract and undergo conversion to the amino acid readily in the blood or target tissues continue to be of interest. Previous publications[1-3] from these laboratories have described the in vitro and in vivo evaluation of some methyldopa esters as progenitors of the amino acid.

A recent report[4] that certain 1,3-dioxol-2-one derivatives of ampicillin function as prodrugs to improve oral absorption suggested possible application of this prodrug strategy in the methyldopa series. In this work, we describe the syntheses of some (2-oxo-1,3-dioxol-4-yl)methyl esters of methyldopa and their evaluation as progenitors of this amino acid.

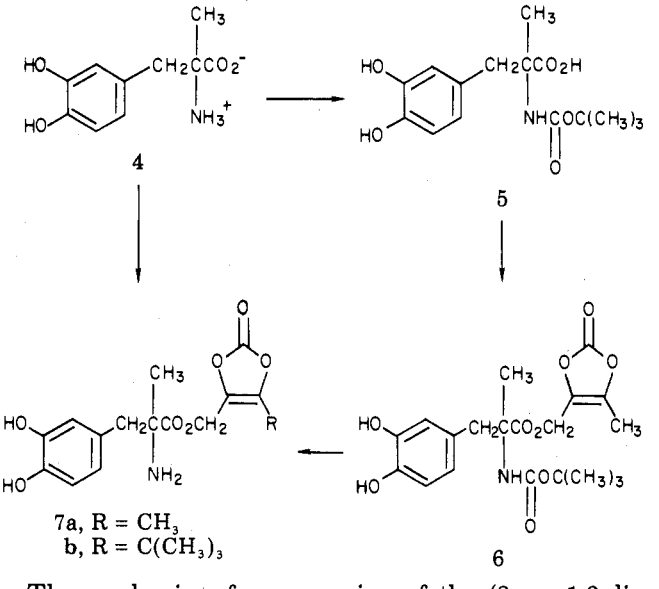
Chemistry. The bromomethyl intermediates **3** were obtained from the corresponding 1,3-dioxol-2-ones **2** by bromination with NBS according to published procedures[4] (Scheme I). Alkylation of the *N*-*t*-Boc derivative of methyldopa (**5**) as its triethylamine salt with **3a** in DMF led to the protected ester **6** (Scheme II). Subsequent removal of the *t*-Boc protective group of **6** with anhydrous HCl in EtOAc yielded the desired amino ester **7a**. Alternatively, direct alkylation of the unprotected amino acid, presumably as the dipolar ion **4**, with **3** in DMF proved to be a more convenient route to these amino esters.[5] The methyl (**7a**) and *tert*-butyl (**7b**) derivatives were prepared by this one-step procedure in 25 and 57% yields, respectively.

In Vitro Hydrolysis Results. Hydrolytic stabilities of esters **7a** and **7b** at pH 7.4 and 37 °C were examined by 360-MHz ^{1}H NMR in deuterated buffer solutions. Under these conditions, both esters appear to be converted cleanly to methyldopa and the corresponding α-diketones **8a** and **8b** (Scheme III). Identification of these products in the hydrolysis mixtures was confirmed by spiking with authentic samples. Surprisingly, the ^{1}H NMR spectra obtained during the course of these experiments did not show significant formation of any other products or intermediates. This suggests that if intermediates are formed during hydrolysis of the dioxolone ring, they are relatively unstable and do not build in concentration.

Scheme I



Scheme II



The mechanism for conversion of the (2-oxo-1,3-dioxol-4-yl)methyl esters to methyldopa has not yet been

(1) Saari, W.; Freedman, M.; Hartman, R.; King, S.; Raab, A.; Randall, W.; Engelhardt, E.; Hirschmann, R.; Rosegay, A.; Ludden, C.; Scriabine, A. *J. Med. Chem.* **1978**, *21*, 746.
(2) Vickers, S.; Duncan, C.; White, S.; Breault, G.; Royds, R.; DeSchepper, P.; Tempero, K. *Drug Metab. Dispos.* **1978**, *6*, 640.
(3) Dobrinska, M.; Kukovetz, W.; Beubler, E.; Leidy, H.; Gomez, H.; Demetriades, J.; Bolognese, J. *J. Pharmacokinet. Biopharm.* **1982**, *10*, 587.
(4) Sakamoto, F.; Ikeda, S.; Tsukamoto, G.; Utsumi, I. *Eur. Pat. Appl.* EP 39477 and EP 39086; *Chem. Abstr.* **1982**, *96*, 85329*u*, 103976*j*.

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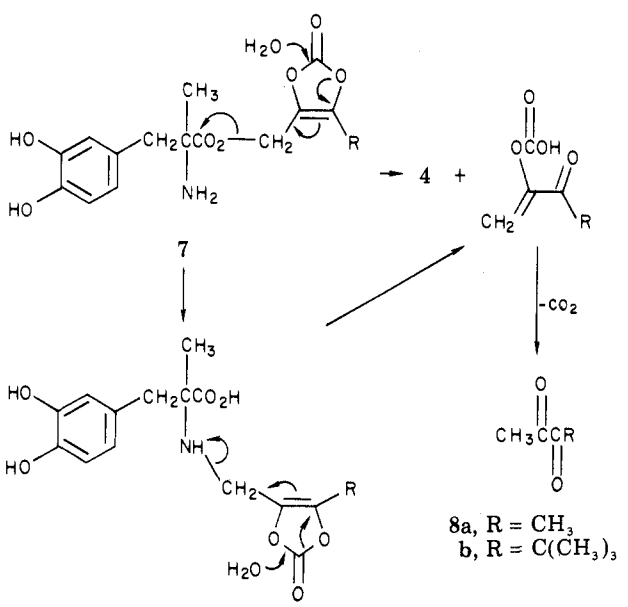
Table I. Pharmacokinetic Parameters for Methyldopa after Administration of Methyldopa and Esters **7a** and **7b** to Beagle Dogs[a]

			methyldopa	
	7a,[b] po	**7b**,[b] po	200 mg po	100 mg iv
area under plasma curve, mcg h/mL	61.7 ± 6.4	37.4 ± 10.3	58.3 ± 17.1	32.6 ± 9.7
urinary recovery, % dose	53.2 ± 6.6	31.4 ± 3.6	54.2 ± 14.3	57.7 ± 9.8
renal clearance, mL/min	29.2 ± 6.4	29.3 ± 6.8	31.9 ± 9.0	31.8 ± 11.8
terminal elimination half-life, h	3.1 ± 0.3	3.7 ± 0.4	3.6 ± 0.3	3.4 ± 0.5
systemic availability of methyldopa, % dose	94.2 ± 14.0	55.7 ± 7.6	92.1 ± 13.3	

[a] Four dogs, mean values plus or minus SD. [b] Oral dose of the ester equivalent to 200 mg of methyldopa.

Scheme III



determined. However, two possible routes that account for formation of the α-diketones **8** are indicated in Scheme III. It should be noted that the (2-oxo-1,3-dioxol-4-yl)-methyl esters are at the same oxidation level as the diketones. Rearrangement of **7** to **9** prior to opening of the dioxolone ring cannot be ruled out at this time.

The rates of conversion of the esters to methyldopa in these in vitro hydrolysis experiments could be determined conveniently by monitoring either decreases in intensity of selected ^{1}H NMR signals of the ester or increases in signals attributable to methyldopa. Plots of the integrals for these signals vs. time allowed calculation of the corresponding hydrolysis rates. In this way, $t_{1/2}$ for conversion of the 5-methyl ester **7a** to methyldopa under these conditions was found to be 3.7 ± 2.3 h, while that for the 5-*tert*-butyl analogue **7b** proved to be significantly slower, 11.6 ± 4.1 h.

Bioavailability Studies. Bioavailability of methyldopa to the systemic circulation following oral administration of **7a** and **7b** was determined in male beagle dogs. In a randomized, cross-over study, four dogs received single oral doses of methyldopa and esters equivalent to 200 mg of methyldopa. A 100-mg intravenous (iv) dose of methyldopa was used as a standard. Plasma and urine specimens were analyzed for methyldopa by the HPLC method described in the Experimental Section. Representative plasma profiles of methyldopa in the four treatments are compared in Figure 1. Mean pharmacokinetic parameters are summarized in Table I.

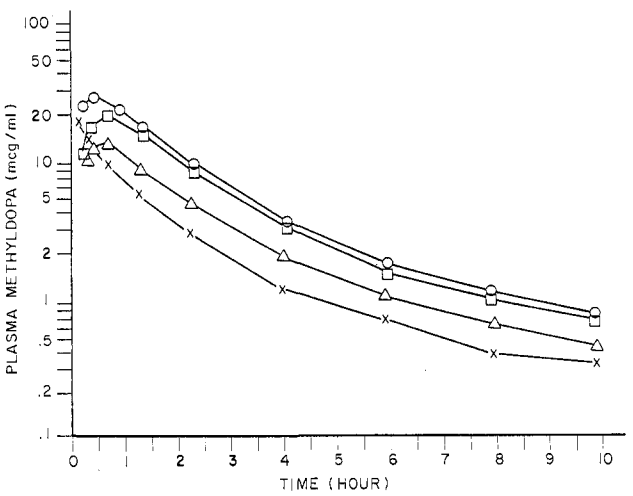


Figure 1. Representative plasma levels of methyldopa in the dog after administration of the following compounds: **7a**, equivalent to 200 mg of methyldopa, po (○); **7b**, equivalent to 200 mg of methyldopa, po (△); methyldopa, 200 mg, po (□); methyldopa, 100 mg, iv (X).

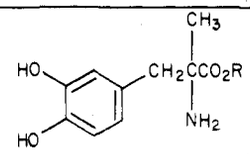
As can be seen from Figure 1, peak plasma concentrations of methyldopa were attained within 1 h after each oral dose treatment. Thereafter, the rate of decline in plasma methyldopa concentrations was similar among all treatments, including the iv dose. Renal clearances and terminal half-lives of methyldopa were similar among all treatments.

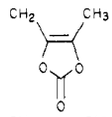
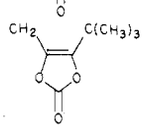
About 60% of the iv dose of methyldopa was recovered in urine as unchanged amino acid, indicating that in the dog, as in man,[3] renal excretion is the major route of elimination of the drug. Orally administered methyldopa was well absorbed, with 92% of the dose available to the systemic circulation in unchanged form. Similar results were obtained with the 5-methyl ester **7a** indicating that it was completely available as methyldopa prior to reaching the systemic circulation.

In contrast, absorption and/or hydrolysis of the 5-*tert*-butyl derivative **7b** to methyldopa was substantially lower, only ~56% of the dose being available as methyldopa. It is possible that the lower plasma levels of methyldopa observed for **7b** compared to those for **7a** may result, at least in part, from slower conversion of **7b** to the amino acid, as observed in the vitro hydrolysis experiments. This would afford more opportunity for the catechol ring of intact ester **7b** to undergo metabolism and for the formation of products other than methyldopa after

(5) For other examples of this direct esterification of amino acids, see ref 1 and 6.

Table II. Effects of Methyldopa Esters on Blood Pressure of SH Rats



compd	structure	dose, mmol/kg, po	initial mean arterial pressure ± SE, mmHg	max reduction in mean arterial pressure ± SE,[a,b] mmHg
7a		0.12[c]	180 ± 8	29 ± 5
		0.24[d]	172 ± 6	38 ± 6
		0.47[e]	164 ± 10	52 ± 4
7b		0.12[c]	182 ± 8	24 ± 4
		0.24[d]	172 ± 5	45 ± 11
		0.47[e]	185 ± 6	93 ± 18
	$CH(CH_3)O_2CC(CH_3)_3$[f]	0.12[c]	181 ± 3	23 ± 4
		0.24[d]	181 ± 11	60 ± 8
		0.47[e]	179 ± 5	83 ± 11
methyldopa	H	0.24[d]	174 ± 11	37 ± 6

[a] Six rats in each treatment group. [b] All values significantly different from pretreatment values; $p < 0.05$. [c] Equivalent to 25 mg/kg of methyldopa. [d] Equivalent to 50 mg/kg of methyldopa. [e] Equivalent to 100 mg/kg of methyldopa. [f] (*S*)-α-(Pivaloyloxy)ethyl (*S*)-3-hydroxy-α-methyltyrosinate hydrochloride dihydrate.

hydrolysis of the ester function.

Antihypertensive Activity. The effects of esters **7a** and **7b** on the mean arterial pressure of SH rats following oral administration are compared with those of methyldopa and its α-(pivaloyloxy)ethyl ester in Table II. This latter ester had been shown previously to be at least twice as potent as methyldopa in lowering blood pressure of SH rats.[1] Both (2-oxo-1,3-dioxo-4-yl)methyl esters **7a** and **7b**, as well as the α-(pivaloyloxy)ethyl ester, were observed to lower blood pressure in a dose-related manner. All three esters exhibited similar onset and duration of action, with mean arterial pressure beginning to decline 1 h after dosing with the maximum effect observed at 4–5 h after treatment.

Although the 5-methyl derivative **7a** does not appear to be as potent as the α-(pivaloyloxy)ethyl ester, the 5-*tert*-butyl analogue **7b** is equipotent with the α-(pivaloyloxy)ethyl ester, with a relative potency estimate of 0.95 (0.67–1.35); $p = 0.50$. A unique estimate of the relative potency of **7a** compared to the α-(pivaloyloxy)ethyl ester is not possible, since in this case dose–response lines are not parallel.

The greater antihypertensive activity of **7b** compared to **7a** seems unexpected when it is recalled that in the dog bioavailability studies the 5-methyl derivative **7a** afforded higher plasma levels of methyldopa than the *tert*-butyl compound. These blood pressure effects may be attributable to absorbed intact ester **7b** or may reflect a species difference in the biotransformation of the esters. Similar species differences have been observed previously for metabolism of the α-succinimidoethyl and α-(pivaloyloxy)ethyl esters of methyldopa.[2]

In summary, (2-oxo-1,3-dioxol-4-yl)methyl esters such as **7** appear to be viable candidates for the latentiation of methyldopa. Bioavailability studies in dogs indicate that these esters, particularly the 5-methyl derivative, yield significant plasma levels of methyldopa. In addition, the esters are orally effective antihypertensive agents in SH rats.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus using open capillaries and are uncorrected. ^{1}H NMR were recorded for all intermediate and final products on a Nicolet NT-360 instrument using tetramethylsilane as an internal standard and are consistent with assigned structures. TLC's were performed on Analtech fluorescent silica gel plates, and spots were detected by UV, exposure to I_2 vapor, or spraying with Dragendorff reagent. Microanalytical results are indicated by symbols of the elements and are within ±0.2% of theoretical values.

(*S*)-*N*-(*tert*-Butoxycarbonyl)-3-hydroxy-α-methyltyrosine (5). A mixture of (*S*)-3-hydroxy-α-methyltyrosine sesquihydrate (methyldopa; 5.0 g, 21 mmol), Et_3N (2.1 g, 21 mmol), and di-*tert*-butyl dicarbonate (4.9 g, 23 mmol) in DMF (50 mL) was stirred under N_2 at 20–25 °C for 1 h and then at 60 °C for 18 h. After most of the DMF was removed at 50–60 °C and 0.1 mm, the residue was partitioned between a 5% citric acid solution (25 mL) and EtOAc (75 mL). The organic extract was washed with two 10-mL portions of H_2O, saturated with NaCl, dried (Na_2SO_4), and filtered. The filtrate was concentrated at 40 °C under reduced pressure. The residue was dried further at 0.1 mm to give 6.5 g (quant) of the *N*-Boc derivative of methyldopa as a foam.

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl (*S*)-*N*-(*tert*-Butoxycarbonyl)-3-hydroxy-α-methyltyrosinate (6). A solution of *N*-(*tert*-butoxycarbonyl)-3-hydroxy-α-methyltyrosine (760 mg, 2.4 mmol), Et_3N (263 mg, 2.6 mmol), and 4-(bromomethyl)-5-methyl-1,3-dioxol-2-one,[4] (**3a**; 500 mg, 2.6 mmol) in DMF (10 mL) was stirred under N_2 at 60 °C for 3 h. Most of the DMF was then removed at 55–60 °C and 0.1 mm, and the residue was partitioned between saturated $NaHCO_3$ solution (10 mL) and EtOAc (50 mL). The organic extract was washed with H_2O saturated with NaCl (10 mL), dried (Na_2SO_4), and filtered, and the filtrate was concentrated under reduced pressure at 40–45 °C. The residue was flash chromatographed over silica gel (230–400 mesh, 40 g) with 3% MeOH–97% $CHCl_3$ as eluant to afford 320 mg (31%) of ester **6** as a foam: homogeneous on TLC (5% MeOH–95% $CHCl_3$), R_f 0.35; ^{1}H NMR ($CDCl_3$) δ 1.46 [s, $C(CH_3)_3$], 1.52 (s, α-CH_3), 2.20 (s, 5-CH_3), 3.05 (m, CH_2), 4.87 (dd, OCH_2), 5.08 (s, NH), 6.48 (dd, aromatic CH), 6.60 (d, aromatic CH), 6.75 (d, aromatic CH).

(5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl (*S*)-3-Hydroxy-α-methyltyrosinate (2*R*,3*R*)-Hydrogen Tartrate Hemihydrate (7a). A. From the *N*-*t*-Boc Derivative 6. A solution of the *N*-*t*-Boc-protected amino ester **6** (1.24 g, 2.9 mmol) in EtOAc (40 mL) was cooled with an ice bath and saturated with anhydrous HCl over 10 min. After the solution was warmed slowly to 20–25 °C over 1.5 h, solvent was removed under reduced pressure at 30–40 °C. Saturated $NaHCO_3$ solution, 10 mL, was added to the residue, and the deblocked ester was extracted into EtOAc (50 mL). The EtOAc extract was washed with H_2O saturated with NaCl, 10 mL, dried (Na_2SO_4), and filtered, and the filtrate was concentrated under reduced pressure. Treatment of a solution

of the crude base in 95% EtOH–5% H_2O with L-tartaric acid, followed by the addition of EtOAc, gave 370 mg (26%) of the crystalline hydrogen tartrate hemihydrate salt of the amino ester: mp 123–128 °C dec; homogeneous on TLC (equal volumes of toluene, *n*-BuOH, HOAc, H_2O, and acetone), R_f 0.79; ^1H NMR (CD_3OD) δ 1.60 (s, α-CH_3), 2.18 (s, 5-CH_3), 3.04 (dd, CH_2), 4.42 (s, C*H*OH), 5.08 (s, OCH_2), 6.46 (dd, aromatic CH), 6.61 (d, aromatic CH), 6.71 (d, aromatic CH). Anal. ($C_{15}H_{17}NO_7 \cdot C_4H_6O_6 \cdot {}^1/_2H_2O$) C, H, N.

B. From Methyldopa Sesquihydrate (4). A mixture of (*S*)-3-hydroxy-α-methyltyrosine sesquihydrate (990 mg, 4.14 mmol) and 4-(bromomethyl)-5-methyl-1,3-dioxol-2-one[4] (**3a**; 800 mg, 4.14 mmol) in DMF (10 mL) was stirred under N_2 at room temperature for 3 h. After the mixture was concentrated at 45 °C under reduced pressure, the residue was partitioned between a saturated $NaHCO_3$ solution and EtOAc. The EtOAc extract was washed with H_2O saturated with NaCl, dried (Na_2SO_4), and filtered, and the filtrate was concentrated. The residue was converted to the hydrogen tartrate hemihydrate as in method A to give 500 mg (25%) of product, mp 124–128 °C dec, identical with the product of method A by mixture melting point, TLC, and ^1H NMR.

5-*tert*-Butyl-4-methyl-1,3-dioxol-2-one (2b). A 12.5% solution of phosgene in toluene (44 g) was added over 30 min to a well-stirred solution of 4,4-dimethyl-2-hydroxy-3-pentanone[8] (4.85 g, 37.3 mmol) in toluene, 90 mL, at 0–5 °C. Following this addition, a solution of pyridine (11.4 mL) in toluene (25 mL) was added over 30 min to the cooled reaction mixture. After stirring at 20–25 °C for 20 h, the reaction mixture was washed with 3 N HCl and H_2O saturated with NaCl. The toluene extract was dried (Na_2SO_4) and filtered, and the filtrate was concentrated under reduced pressure. *p*-Toluenesulfonic acid hydrate (100 mg) was added to the residue, which was then stirred neat at 160 °C for 24 h. The resulting black liquid was dissolved in EtOAc, washed with saturated $NaHCO_3$ and H_2O saturated with NaCl, dried (Na_2SO_4), and filtered, and the filtrate was concentrated under reduced pressure. The residue was distilled to give 3.5 g (60%) of the 1,3-dioxol-2-one **2b**: bp 82–84 °C (3 mm); ^1H NMR ($CDCl_3$) δ 1.25 [s, $C(CH_3)_3$], 2.15 (s, CH_3).

4-(Bromomethyl)-5-*tert*-butyl-1,3-dioxol-2-one (3b). A mixture of 5-*tert*-butyl-4-methyl-1,3-dioxol-2-one (4.13 g, 26.4 mmol), *N*-bromosuccinimide (5.16 g, 29 mmol), and dibenzoyl peroxide (10 mg) in CCl_4 (120 mL) was heated at reflux for 1.5 h. After the mixture was cooled and filtered, the filtrate was concentrated under reduced pressure to give 6.5 g of the bromomethyl derivative **3b**: ^1H NMR ($CDCl_3$) δ 1.3 [s, $C(CH_3)_3$], 4.3 (s, CH_2).

(5-*tert*-Butyl-2-oxo-1,3-dioxol-4-yl)methyl (*S*)-3-Hydroxy-α-methyltyrosinate (2*R*,3*R*)-Hydrogen Tartrate Hemihydrate (7b). A mixture of 4-(bromomethyl)-5-*tert*-butyl-1,3-dioxol-2-one (5.27 g, 22.4 mmol) and (*S*)-3-hydroxy-α-methyltyrosine sesquihydrate (5.3 g, 22.4 mmol) in dry DMF (35 mL) was stirred under N_2 at 20–25 °C for 3 h. After the mixture was concentrated under reduced pressure at 40–50 °C, the residue was partitioned between saturated $NaHCO_3$ solution and EtOAc. The EtOAc extract was washed (H_2O saturated with NaCl), dried (Na_2SO_4), and filtered, and the filtrate was concentrated. Treatment of a solution of the impure ester base in 95% EtOH–5% H_2O with L-tartaric acid, followed by the addition of EtOAc, gave 6.75 g (57.4%) of the crystalline hydrogen tartrate hemihydrate salt of the amino ester: mp 113–121 °C dec; homogeneous on TLC (equal volumes of toluene, *n*-BuOH, HOAc, H_2O, and acetone), R_f 0.75; ^1H NMR (CD_3OD) δ 1.31 [s, $C(CH_3)_3$], 1.59 (s, α-CH_3), 3.04 (dd, CH_2), 4.41 (s, C*H*OH), 5.17 (s, OCH_2), 6.46 (dd, aromatic CH), 6.62 (d, aromatic CH), 6.71 (d, aromatic CH). Anal. ($C_{18}H_{23}NO_7 \cdot C_4H_6O_6 \cdot {}^1/_2H_2O$) C, H, N.

^1H NMR Studies. Deuterated phosphate buffer was prepared by the lyophilization of pH 7.4 phosphate buffer (0.1 μ) and reconstitution in D_2O. Solutions of 3–4 mg of the 1,3-dioxol-2-one esters **7a** and **7b** in 0.5 mL of this deuterated buffer were maintained at 37 ± 1 °C for the duration of the study, which was 12 h. Spectra were recorded on a Nicolet NT-360 instrument at preset intervals ranging from every 5 min at the beginning of the experiment to every hour at the end of the study. Disappearance of ester **7a** was followed by decreases in intensity of both the CH_3 signal of the 5-methyldioxolone and the CH_2 signal of the amino acid portions of the molecule. Hydrolysis of ester **7b** was determined by similar decreases in intensity of the CH_2 signals of the dioxolone and amino acid portions of the molecule. Formation of methyldopa was assessed by increases in intensity of aromatic CH and CH_2 signals attributable to this amino acid. Integrals of these peaks were plotted vs. time by the following equation: $f(x) = K_1(e^{-X/t_2}) + t_3$ and $f(x) = K_1(1 - e^{-X/t_2}) + t_3$. Half-lives were obtained from a nonlinear least squares program; error = $\pm 3\sigma$.

Bioavailability Studies. Four male beagle dogs received single oral doses of methyldopa, **7a**, and **7b**, equivalent to 200 mg of methyldopa, and a 100 mg iv dose of methyldopa in a four-period, randomized cross-over study with a minimum 1-week washout period between treatments. Dogs were fasted overnight and for 6 h after each dose; water was allowed ad libitum. Blood samples were obtained from the jugular vein at frequent intervals through 10-h postdose, and spontaneously voided urine was collected through 48 h.

Plasma and urine specimens were analyzed for methyldopa by an HPLC method using amperometric detection. The procedure for the analysis of methyldopa in plasma samples involved the addition of ethyldopa (internal standard) and $HClO_4$ (0.010 mL, 70%) to plasma (0.200 mL), followed by centrifugation and filtration (0.45 μm Acrodisk-CR). The supernatant (0.020 mL) was injected directly into the HPLC system. Urine specimens were processed in the following manner. Free levels: urine (0.050 mL) was diluted by the addition of internal standard (0.050 mL) and mobile phase (0.900 mL). The sample was mixed on a Vortex mixer, and an aliquot (0.020 mL) was injected directly into the HPLC system. Total levels: to an aliquot (0.200 mL) of the "free" solution, discussed above, were added $HClO_4$ (0.010 mL, 70%) and HCl (0.200 mL, 37%). The sample was mixed on a Vortex mixer and heated (99 ± 1 °C, 15 min) to promote hydrolysis of the drug conjugates; $HClO_4$ alone at this concentration would not hydrolyze the drug conjugates. The sample was cooled to room temperature, and an aliquot (0.020 mL) was injected directly into the HPLC system. The HPLC instrumentation consisted of a Waters pump (6000A), a Waters autosampler (WISP 710B), a Waters column (Microbondapak C-18, 4.6 × 250 mm), a Bioanalytical Systems Tl-8A KEL-F thin-layer detection cell (0.5V), a Bioanalytical Systems LC-4A amperometric detector (50 nA/V), an Eldex Model III temperature control unit (25 °C), and a Hewlett-Packard integrater (3390A). The mobile phase (flow rate 1.5 mL/min) consisted of Milli-Q water (18 M cm^{-1} resistivity, 3835 mL), $KHPO_4$ (108.8 g), disodium EDTA (0.40 g), and H_3PO_4 (54.4 mL, 85%).

The total area under the methyldopa plasma concentration–time curve was obtained from the sum of the trapezoidal area to 10 h, and the residual area was obtained by extrapolation of the terminal log linear slope (0.693/half-life). The renal clearance of methyldopa was determined from the ratio of its total urinary recovery and the area under the curve. The availability of methyldopa to the systemic circulation after oral dose treatments relative to the iv standard was calculated by the method of Kwan and Till[7] under the constant nonrenal clearance assumption.

Antihypertensive (SH Rat) Assay. Compounds were evaluated for their effect on the mean arterial pressure of unanesthetized Wistar–Okamoto spontaneously hypertensive rats purchased from Charles River/Lakeview Laboratories, Wilmington, MA. Only male rate of 290 to 350 g body weight and 16–24 weeks of age were used. Under ether anesthesia, the caudal artery of the rat was cannulated (approximately 1 cm beyond the anus) with PE-10 tubing. The rostral end of the catheter was advanced into the abdominal aorta just below the left renal artery. The PE-10 tubing was flared at the other end and connected to a specially designed "y"-shaped 20-g needle connector, which was anchored in the caudal groove of the tail. The fascia and skin were then sutured. AWG-20 tubing with a tubular spring guard was fastened to the connector that was subsequently attached to a water-tight swivel positioned above the rat cage. The swivel, in turn, was connected to a P-23Gb Statham pressure transducer. Associated with the pressure transducer was a three-way stopcock

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and adjustable needle valve system, which permitted a continuous, sterile, pyrogen-free, 0.225% saline infusion. The animals were allowed to recover from anesthesia overnight before the start of drug treatments. Arterial pressure was recorded continuously through Statham P-23Gb transducers on a Honeywell 906C Visicorder. Mean arterial pressures were printed at $^1/_2$-h intervals through a data acquisition system (Data Graphics Corp., San Antonio, TX) by means of ASR-33 teletype units. The drug doses were computed on the basis of the base weight. Six rats were used in each treatment group.

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Registry No. **2** (R = *t*-Bu), 86005-11-0; **3a**, 80715-22-6; **3b**, 86005-12-1; **4**, 555-30-6; **5**, 62631-37-2; **6**, 86005-08-5; **7a** tartrate, 86005-10-9; **7b** tartrate, 86016-63-9; **8a**, 431-03-8; **8b**, 40898-19-9; di-*tert*-butyl dicarbonate, 24424-99-5; 4,4-dimethyl-2-hydroxy-3-pentanone, 52279-28-4.

Inhibition of Arabinose 5-Phosphate Isomerase. An Approach to the Inhibition of Bacterial Lipopolysaccharide Biosynthesis

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Arabinose 5-phosphate (A5P) isomerase is a key enzyme in the biosynthesis of lipopolysaccharide, an essential component of the outer membrane of Gram-negative bacteria. The mechanism of the isomerase is envisioned to involve an enediol intermediate. A series of compounds, which are analogues of the substrates or intermediate, were tested as inhibitors of A5P isomerase with the belief that a good inhibitor would stop bacterial growth or render the cells more susceptible to other antibiotics or natural defenses. In a series of phosphorylated sugars, the order of isomerase inhibitory activity was as follows: aldonic acids > alditols > aldoses. Nonphosphorylated sugars were much less inhibitory. The best inhibitor was erythronic acid 4-phosphate (**54**), which had $K_m/K_i = 29$. None of the compounds displayed antibacterial activity in vitro.

The rise of antibacterial resistance in pathogenic bacteria is a continuing medical problem. One approach to the attack of this problem is to develop a new antibacterial agent that has a completely novel mode of action with the expectation that no cross-resistance with older drugs would be found. Bacterial cell wall peptidoglycan has been the primary target for research of this type, but we report here a new approach to the inhibition of the formation of the outer membrane of Gram-negative bacteria.

The envelope of Gram-negative bacteria has three principal parts: the outer membrane (OM), peptidoglycan, and the cytoplasmic membrane (CM).[1] The functions of the outer membrane, in part, are to retain proteins and enzymes in the periplasmic space (between OM and CM), to protect the cell from undesirable enzymes and chemicals, to provide channels for nutrients and ions,[2] and to facilitate cell–cell interactions, such as adhesion, conjugation, and chemotaxis.

The primary components of the outer membrane are phospolipids, lipopolysaccharide, and proteins. The lipopolysaccharide (LPS) is found on the outside of the unsymmetrical lipid bilayer, with the polysaccharide chains exposed on the cell surface. The LPS determines the antigenicity, toxicity, adhesiveness, invasiveness, and penetrability of the cell. The structure of *Salmonella* LPS is shown in Figure 1. Much of the present knowledge of the structure and biosynthesis of LPS has come from the work of Osborn and co-workers.[3]

Lipopolysaccharide is an attractive target for chemotherapy because of three components unique to bacteria: the β-hydroxymyristoyl groups in lipid A, D-*manno*-3-deoxyoctulosonic acid (KDO), and L-*glycero*-D-*manno*-heptose. Mutants that are defective in LPS biosynthesis are usually less pathogenic and more susceptible to antibiotics.[4] Importantly, mutants that are defective in KDO biosynthesis are not viable. Hence, the inhibition of KDO biosynthesis became our primary target.

The biosynthesis of KDO has been discussed by Heath and co-workers,[5] and a schematic of KDO biosynthesis is shown in Figure 2. The biological significance of KDO was recognized by Unger and co-workers, who have thoroughly explored the chemistry of KDO. Their work has been summarized in a recent review.[6] All of the enzymes in this pathway have been isolated and characterized by Ray and co-workers,[7] and the isomerase was an early focal point.

The isomerase enzymes that interconvert aldoses and ketoses are found in both mammals and microorganisms. The function of the isomerases is to synthesize cell components rather than produce energy. The characteristics of a number of these enzymes were discussed by Topper[19] and later by Noltman.[20] More recently, Rose has discussed the mechanisms,[21] and, fortunately, much of this work has now been consolidated in the chapter by Walsh.[22]

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