$CHCl_3$, and dried (K_2CO_3), and solvent was removed under vacuum. The residue was crystallized from acetone and ether to yield 0.7 g (55%), mp 58–59 °C. Anal. ($C_{12}H_{16}N_2O_4 \cdot H_2O$) C, H, N.

4-Morpholino-2-formylpyridine *N*-Oxide. A solution of 0.506 g (2 mmol) of 4-morpholino-2-formylpyridine ethylene acetal *N*-oxide in 15 mL of 20% HCl was refluxed for 1.5 h. The solution was then made alkaline with Na_2CO_3 and water was removed under vacuum. The residue was extracted with $CHCl_3$, solvent was removed, and the residue was crystallized from acetone to yield 0.3 g (72%) of orange crystals, mp 180–182 °C. Anal. ($C_{10}H_{12}N_2O_3 \cdot H_2O$) C, H, N.

4-Nitro-2-formylpyridine *N*-Oxide. To a solution of 4.62 g (0.03 mol) of 4-nitro-2-picoline *N*-oxide in 100 mL of dioxane was added 3.68 g (0.033 mol) of freshly sublimed SeO_2 and the mixture was refluxed for 64 h. The precipitated selenium was removed by filtration and the filtrate was evaporated under vacuum. The residue was dissolved in 50 mL of water, neutralized with $NaHCO_3$ solution, and evaporated under vacuum to dryness. The remaining solids were extracted with $CHCl_3$ and the solvent was removed to leave a gummy residue which was subjected to silica gel column chromatography using $CHCl_3$ as the eluent. Initial fractions contained unreacted 4-nitro-2-picoline *N*-oxide which was then followed by the desired compound. The fractions containing the aldehyde were collected, the solvent was removed, and the residue was crystallized from benzene to yield 1.2 g (24%), mp 110–112 °C. Anal. ($C_6H_4N_2O_4$) C, H, N.

2-Picolinoylbenzenesulfonylhydrazide *N*-Oxide (16). Ethyl picolinate (4.53 g, 0.03 mol) was initially converted to its *N*-oxide with a molar equivalent of *m*-chloroperoxybenzoic acid, utilizing the procedure outlined for the synthesis of 4-chloro-2-formylpyridine ethylene acetal *N*-oxide. The *N*-oxide was obtained as an oil (3.02 g) that was dissolved in 15 mL of ethanol and directly reacted with 7.5 mL of hydrazine hydrate; this procedure immediately produced a white precipitate. The mixture was stirred for 1 h at room temperature and was filtered to yield 2.5 g (54%) of 2-picolinoylhydrazide *N*-oxide, mp 146–147 °C.

To a solution of 1.53 g (0.01 mol) of 2-picolinoylhydrazide *N*-oxide in 20 mL of pyridine was added slowly at 4 °C a solution of a molar equivalent of benzenesulfonyl chloride in 5 mL of pyridine. The solution was stirred for 1.5 h at room temperature and then the pyridine was removed under vacuum. The residue was washed with water and ethanol to yield 2.6 g (89%) of **16**, mp 210–212 °C.

References and Notes

(1) Presented in part before the fall meeting of American Society for Pharmacology and Experimental Therapeutics, New Orleans, La., Aug 1976; this work was supported by U.S. Public Health Service Grants CA-02817 and CA-16359 from the National Cancer Institute.
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Notes

Chemical Modification of 1,4-Diamino-1,4-dideoxy-3-*O*-(4-deoxy-4-propionamido-α-D-glucopyranosyl)-D-glucitol

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Chemical modification of the 4′-N position of 1,4-diamino-1,4-dideoxy-3-*O*-(4-deoxy-4-propionamido-α-D-glucopyranosyl)-D-glucitol (GlA_1) in the form of 4′-*N*-acyl analogues, e.g., **3**, led to no significant potency enhancement. The *n*-propylamino analogue **4** was more active against gram-positive bacteria but was less active vs. gram-negative bacteria. The intrinsic activity of the 6′-chloro analogue **15** like the antibiotic GlA_1 was not high, but the antibacterial spectrum was broad with moderate activity against most resistant organisms.

Antibiotic 1,4-diamino-1,4-dideoxy-3-*O*-(4-deoxy-4-propionamido-α-D-glucopyranosyl)-D-glucitol (GlA_1) is a component of a new complex of amino glycosides elaborated in fermentation broths by *Streptomycete*[1] and *Pseudomonas*[2] species. It has broad-spectrum activity[1,2] against bacteria including *Pseudomonas aeruginosa* and strains carrying amino glycoside resistance episomes.[1] The antibiotic is composed of 4-deoxy-4-propionamido-D-glucopyranose and 1,4-diamino-1,4-dideoxy-D-glucitol which were synthesized in our laboratories.[3] The structure has been established[3] as **1**, which is identical with the recently described Sorbistin antibiotics (Bristol),[4a] the LL-AM 31 antibiotic complex (Lederle),[4b] and antibiotic P2568 (Takeda).[2c] Recent publications[2c,5] on the preparation of many *N*-acyl derivatives of the natural product **6** prompted us to report on the chemical modification of **1**.

The potency of GlA_2 (**2**) is about 50% of **1**, whereas GlA_0 (depropionyl-GlA_1) (**6**) is totally inactive. The difference in activities of these natural products indicates that the acyl side chain on the 4-aminoglucose moiety is essential for bioactivity. Thus, the approach to enhance the antibiotic activity by introducing a new acyl group on the 4′-amino function would appear attractive. This line of approach has also been investigated independently by other workers.[2c,5]

The natural product **6**, obtained from the antibiotic complex by hydrazinolysis, was selectively reacted with

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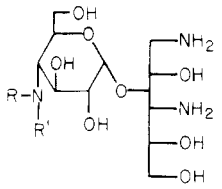
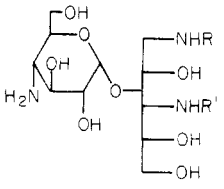


1, R = COC_2H_5; R′ = H
2, R = $COCH_3$; R′ = H
3, R = CO-*n*-C_3H_7; R′ = H
4, R = *n*-C_3H_7; R′ = H
5, R = *n*-C_3H_7; R′ = COC_2H_5



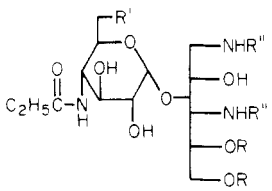
6, R = R′ = H
7, R = $COOCH_2C_6H_5$; R′ = H
8, R = $COOC(CH_3)_3$; R′ = H
9, R = H; R′ = CO-*n*-C_3H_7

N-(benzyloxycarbonyloxy)succinimide[6] and *tert*-butyloxycarbonyl azide to give **7** and **8**, respectively, which were reacylated with a wide variety of acids. After appropriate separation and deblocking, the new analogues were isolated by chromatography. This method was exemplified by the preparation of **3** and the isomeric **9**. The potency of the *n*-butyryl analogue **3**, which is identical with Sorbistin A_2,[4a] was about 37% of **1**, whereas the isomeric **9** was totally inactive. The 4′-*N*-propyl analogue **4**, prepared from the persilylated **1**, by reduction with lithium aluminum hydride in refluxing dioxane, showed enhanced activity against gram-positive bacteria (see Table I). However, it was less active against gram-negative bacteria. The analogue **5**, prepared by alkylation of **1**, has weak activity.

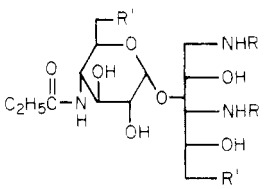
A large number of 4′-*N*-acyl analogues of **1** have already been reported[2c,5] either by using dimedone[5] or *N*-*p*-methoxybenzyl[2c] as protecting groups in their preparations. Thus far, no potency enhancement has been noted by us and others.[5] The minimum structural requirement for the antibiotic activity in this series of compounds is still **1** with the cyclopropylcarbonyl analogue[5b] having comparable bioactivities.

GlA_1 (**1**) is a novel antibiotic in that it has a diaminodideoxyhexitol as the aglycon which has recently been found also in related antibiotics.[4b,7] Classical amino glycoside antibiotics which have a deoxystreptamine or streptidine nucleus are known invariably to cause ototoxicity and nephrotoxicity as possible side effects, and many of these antibiotics are susceptible to R-factor inactivation.[8] In our further attempts to enhance the potency of **1**, we have elected to concentrate on modification of the pseudodisaccharide itself instead of coupling it with other aminoglycoside components to mimic the predominant features of kanamycins, gentamicins, etc. Following this strategy, we replaced the 6′-hydroxyl group of **1** with a chlorine or amino function. This was accomplished by the following sequence of reactions: carbobenzoxylation, acetonation, selective sulfonylation with 2-mesitylenesulfonyl chloride[9], nucleophilic displacement, and appropriate deblocking.

The 6′-chloro analogue **15** showed some improved bioactivity (see Table I). This is reminiscent with Upjohn's modifications of lincomycin;[10] the (7*R*)-7-chloro-7-deoxy analogue and the 7(*S*) epimer were twice and four times as active as lincomycin, respectively, and the 7(*S*) epimer also showed activity against some gram-negative bacteria.[11] The 6′-amino derivative **18** and the 6,6′-dichloro analogue **21** were less potent than GlA_1. They were about 20 and 10% of the activity of GlA_1, respectively, using agar diffusion assay on *Bacillus subtilis*. Compound **21** was prepared from **10** by selective sulfonylation with 2-mesitylenesulfonyl chloride, nucleophilic displacement with lithium chloride, and hydrogenolysis.



10, R = H; R′ = OH; R″ = $COOCH_2C_6H_5$
11, R, R = ; R′ = OH; R″ = $COOCH_2C_6H_5$
12, R, R = ; R′ = OSO_2-C_6H_2-2,4,6-Me_3; R″ = $COOCH_2C_6H_5$
13, R, R = ; R′ = Cl; R″ = $COOCH_2C_6H_5$
14, R = H; R′ = Cl; R″ = $COOCH_2C_6H_5$
15, R = R″ = H; R′ = Cl
16, R, R =  ; R′ = N_3; R″ = $COOCH_2C_6H_5$
17, R = H; R′ = N_3; R″ = $COOCH_2C_6H_5$
18, R = R″ = H; R′ = NH_2



19, R = $COOCH_2C_6H_5$; R′ = OSO_2-C_6H_2-2,4,6-Me_3
20, R = $COOCH_2C_6H_5$; R′ = Cl
21, R = H; R′ = Cl

As can be seen from Table I, the intrinsic activity of analogues **3** and **15** is not high. However, the antibacterial spectrum is broad like **1** with moderate activity against most resistant organisms. The *n*-propylamino analogue **4** is more active against gram-positive bacteria but is less active vs. gram-negative bacteria.

Experimental Section

Melting points were taken in a Thomas-Hoover Unimelt apparatus and are uncorrected. Optical rotations were measured at 27 °C with a Zeiss polarimeter. Thin-layer chromatography (TLC) was performed on silica gel G (analtech) plates, and the spots were detected by a ceric sulfate (1%)–sulfuric acid (10%) spray. Column chromatography was conducted on silica gel 60 (70–230 mesh ASTM). NMR spectra were recorded for solutions in chloroform-*d* (unless stated otherwise) at 60 or 100 MHz, with tetramethylsilane as the internal standard. Conventional processing consisted in drying organic solutions with anhydrous sodium sulfate, filtration, and evaporation of the filtrate under diminished pressure.

1,4-Diamino-1,4-dideoxy-3-*O*-(4-amino-4-deoxy-α-D-glucopyranosyl)-D-glucitol (GlA_0) (6). A solution of the antibiotic complex consisting of GlA_1, GlA_2, and GlA_0 (50 g) in hydrazine (250 mL) was heated under reflux in a nitrogen atmosphere for 48 h. The solution was concentrated to dryness and codistilled with MeOH (5 × 100 mL). The syrup was chromatographed on a column of silica gel (2.5 kg) with $CHCl_3$–MeOH–NH_4OH (2:2:1) as eluent. Fractions containing the product were pooled and evaporated to give a syrup which was dissolved in MeOH. Addition of 2-propanol to the methanolic solution gave **6** as solids (40 g): $[\alpha]_D$ +73.3° (*c* 1.02, H_2O) [lit.[26] $[\alpha]_D$ + 71.0° (*c* 1.0, H_2O)]; NMR (D_2O) δ 5.15 (d, $J_{1,2}$ = 2.0 Hz, H-1); MS (silylated) *m/e* 450. Anal. ($C_{12}H_{27}N_3O_8 \cdot CH_3OH$) C, H, N.

GlA_0 Nonaacetate. Acetylation of **6** (250 mg, 0.733 mmol) with acetic anhydride (0.5 mL) in pyridine (0.5 mL) gave a crystalline mass (420 mg) upon conventional workup. Recrystallization from EtOAc afforded pure material (400 mg, 76%): mp 187.5–188.5 °C; $[\alpha]_D$ +68.3° (*c* 1.0, $CHCl_3$). Anal. ($C_{30}H_{45}N_3O_{17}$) C, H, N.

1-*N*-Benzyloxycarbonyl-GlA_0 (7). A solution of *N*-(benzyloxycarbonyloxy)succinimide (30 g, 0.12 mol) in DMF (100 mL) was added dropwise to **6** (35 g, 0.103 mol) in H_2O (200 mL) and 2-propanol (200 mL) at −10 °C. The mixture was kept at 0–5 °C for 16 h and concentrated to dryness. The crude product was column chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (60:40:10) as eluent. Crystallization from MeOH–EtOAc afforded

Table I. In Vitro Antimicrobial Activity of GlA_1 Analogues[a]

Compd	MB no.	Strain	Resistance[b]	Agar dilution MIC values[c] GlA_1 (1), 200 μL/mL	GlA_2 (2), 200 μL/mL	3, 200 μL/mL	4, 200 μL/mL	15, 200 μL/mL	Kanamycin, 100 μL/mL
1	2985	*Staph. aureus*	Sensitive	100	200	200	50	50	<3.1
2	2314	*Staph. aureus*	*StrNeoKan*[b]	200	>200	*200*	100	200	>100
3	964	*B. subtilis*	Sensitive	50	100	100	50	50	<3.1
4	755	*Strep. faecalis*		200	>200	*200*	*200*	200	50
5	210	*Staph. aureus*		50	200	200	50	50	<3.1
6	2482	*E. coli*	Sensitive	50	100	200	200	100	<3.1
7	2964	*E. coli*	*StrNeoKanGen*[b]	50	100	200	200	100	100
9	2956	*Ps. aeruginosa*		50	*200*	*200*	*200*	100	50
10	3033	*Ps. aeruginosa*	*KanGen*	100	>200	*200*	>200	100	100
11	2921	*Klebsiella*	Sensitive	NR[d]	NR	200	*200*	50	NR
12	2922	*Klebsiella*	*StrNeoKanGenTob*[b]	NR	NR	200	200	50	NR
13	2840	*Serratia*	Unpigmented	200	>200	>200	200	100	>100
14	155	*Serratia*	Pigmented	>200	>200	>200	NR	200	<3.1
15	838	*Prot. vulgaris*	Sensitive	100	200	*25*	*100*	200	<3.1
16	1287	*Salmonella*	Sensitive	<3.1	200	*100*	100	50	<3.1
17	1159	*Erwinia*	Sensitive	<3.1	100	50	NR	<25	<3.1
18	60	*E. coli*	Sensitive	50	200	200	200	100	<3.1
19	965	*Brucella*	Sensitive	100	*200*	>200	200	100	6.25

[a] Tests conducted on Mueller-Hinton medium (inoculum 2×10^3). [b] Plasmid mediated; otherwise lab isolate. [c] The numbers in italics represent resisters at and above that concentration. [d] NR = not run.

7 (32 g, 66%): mp 154–155.5 °C; $[\alpha]_D$ +67.2° (*c* 1.0, MeOH); NMR (D_2O) δ 7.37 (s, 5 H, aromatic), 5.13 (H-1), 5.03 ($-CH_2C_6H_5$). Anal. ($C_{20}H_{33}N_3O_{10}$) C, H, N.

1-*N*-*tert*-Butyloxycarbonyl-GlA_0 (8). Magnesium oxide (8.0 g) and *tert*-butyloxycarbonyl azide (7.5 mL) were added to a solution of **6** (16 g, 0.047 mol) in H_2O (80 mL) and dioxane (80 mL). The mixture was stirred at room temperature for 16 h, filtered, and concentrated to dryness. The residue was chromatographed on a column of silica gel with $CHCl_3$–MeOH–H_2O (60:40:10) as irrigant. Fractions containing the desired product were pooled and evaporated to give a powder. Crystallization from MeOH–EtOAc afforded **8** (10 g, 48%): mp 153.5–155 °C; $[\alpha]_D$ +71.7° (*c* 1.81, MeOH); NMR (D_2O) δ 5.12 (d, $J_{1,2}$ = 2.0 Hz, H-1), 1.41 [s, 9 H, $C(CH_3)_3$]. Anal. ($C_{17}H_{33}N_3O_{10}$) C, H, N.

4′-*N*-Butyryl-GlA_0 (3). A solution of butyryl chloride (0.044 mL, 0.43 mmol) in DMF (0.5 mL) was added dropwise to a stirred solution of **7** (200 mg, 0.42 mmol) in H_2O (0.5 mL) and DMF (0.5 mL). The progress of the reaction was monitored by TLC ($CHCl_3$–MeOH–H_2O, 60:40:10). After 1 h, the mixture was concentrated to dryness and the two major spots were separated by preparative chromatography. The faster moving component (R_f 0.4; 50 mg, 22%) and the slower one (R_f 0.29; 95 mg, 41%) were isolated as syrupy materials. Hydrogenolysis of these two intermediates in aqueous dioxane over 10% Pd/C gave the deblocked products which were purified by columns of CM-25 Sephadex (NH_4^+) with a linear gradient of 0.25 N NH_4OH as eluent. The title product **3** [19 mg (50%); MS *m/e* 232, 162, etc.] was obtained from the faster moving component (R_f 0.4) and exhibited antibacterial activity (see Table I). 4-*N*-Butyryl-GlA_0 (**9**) (46 mg, 64%), from the slower moving component, was devoid of biological activity. Compound **3** was characterized as 4′-*N*-butyryl-GlA_0 octaacetate: mp 178-179.5 °C; $[\alpha]_D$ +66° (*c* 1.03, $CHCl_3$); R_f 0.53 ($CHCl_3$–MeOH, 90:10); NMR ($CDCl_3$) δ 2.76 (m, $CH_3CH_2CH_2CO$), 2.08, 2.05, 2.03, 2.01, 1.98, 1.95 (24 H, N- and -OAc), 1.64 (t, *J* = 7.0 Hz, $CH_3CH_2CH_2CO$), 0.92 (t, $CH_3CH_2CH_2CO$); MS $(M + 1)^+$·, *m/e* 358. Anal. ($C_{32}H_{49}N_3O_{17}$) C, H, N.

4-*N*-Butyryl-GlA_0 (**9**) was also characterized as the peracetate: R_f 0.55 ($CHCl_3$–MeOH, 90:10); NMR ($CDCl_3$) δ 2.13, 2.09, 2.07, 2.04, 2.0, 1.95 (24 H, N- and -OAc), 1.67 (t, *J* = 7.0 Hz, $CH_3CH_2CH_2CO$), 0.92 (t, $CH_3CH_2CH_2CO$); MS $(M + 1)^+$·, *m/e* 330, etc.

Compound **3** can also be prepared from **8** in a similar manner. The blocking group was removed by brief treatment with CF_3COOH.

GlA_1 (1). Compound **1** was prepared from **7** and propionyl chloride in a similar manner as **3**. The synthetic product had $[\alpha]_D$ +87.1° (*c* 1.0, H_2O); NMR (D_2O) δ 5.17 (d, $J_{1,2}$ = 2.0 Hz, H-1), 2.30 (q, *J* = 7.0 Hz, CH_3CH_2CO), 1.11 (t, CH_3CH_2CO); MS *m/e* 319, 218, 162. Anal. ($C_{15}H_{31}N_3O_9$) C, H, N. The synthetic GlA_1 was further characterized as the peracetate: mp 197–198 °C; $[\alpha]_D$ +68.5° (*c* 1.01, $CHCl_3$); R_f 0.52 ($CHCl_3$–MeOH, 90:10); NMR ($CDCl_3$) δ 6.80 (br, 4′-NH), 6.35 (br, 4-NH), 6.14 (br, 1-NH), 5.03 (d, $J_{1,2}$ = 3.5 Hz, H-1), 2.20 (q, *J* = 7.0 Hz, CH_3CH_2CO), 2.13, 2.10, 2.07, 2.05, 2.01, 1.96 (24 H, N- and -OAc), 1.15 (t, CH_3CH_2CO); MS $(M + 1)^+$·, *m/e* 588, 529, 344. Anal. ($C_{31}H_{47}N_3O_{17}$) C, H, N.

4′-*N*-Propyl-GlA_0 (4). Hexamethyldisilazane (10 mL) and trimethylsilyl chloride (1.0 mL) were added to a solution of **1** (2.0 g, 5.04 mmol) in pyridine (20 mL), and the mixture was heated at 80 °C for 2 h, filtered, and concentrated to dryness. Dioxane (50 mL) and $LiAlH_4$ (0.5 g) were added to the residue, and the mixture was heated at 90 °C for 1 h. Another batch of $LiAlH_4$ (0.3 g) was added and the mixture was again heated at 90 °C for 16 h. The reaction was cooled and excess $LiAlH_4$ was quenched with MeOH (50 mL) and H_2O (50 mL). The mixture was filtered and concentrated to a cake (10 g) which was taken up in H_2O (50 mL) and heated on a steam cone for 15 min, filtered, and evaporated to dryness. Isolation by column chromatography on silica gel ($CHCl_3$–MeOH–NH_4OH, 2:2:1) gave **4** (0.5 g, 26%): $[\alpha]_D$ +72.8° (*c* 1.0, H_2O); R_f 0.26 (GlA_1, R_f 0.21); NMR (D_2O) δ 5.10 (d, $J_{1,2}$ = 2.5 Hz, H-1), 1.50 (m, $CH_3CH_2CH_2$), 0.90 (t, *J* = 6.0 Hz, $CH_3CH_2CH_2$); MS *m/e* 305, 204. Anal. ($C_{15}H_{33}N_3O_8 \cdot H_2CO_3$) C, H, N.

The acetylated product had MS *m/e* 761 (M^+·), 616, etc., which confirmed the structure of **4**.

4′-*N*-Propyl-GlA_1 (5). Sodium hydride (50 mg) was added to a solution of **1** (397 mg, 1.0 mmol) in DMF (4 mL) and the suspension was stirred at room temperature for 20 min before gradual addition of *n*-propyl iodide (180 mg, 1.06 mmol). The mixture was heated at 50 °C with stirring for 16 h and cooled, and excess NaH was quenched with MeOH (10 mL). The filtered solution was concentrated to a residue which was column chromatographed on silica gel with $CHCl_3$–MeOH–NH_4OH (3:3:1) as eluent. The title compound **5** was isolated as a foam (60 mg, 14%): R_f 0.48 ($CHCl_3$–MeOH–NH_4OH, 2:2:1) (GlA_1, R_f 0.21); NMR (D_2O) δ 5.16 (d, $J_{1,2}$ = 3.0 Hz, H-1), 2.89 (q, *J* = 7.0 Hz, $CH_3CH_2CH_2$), 2.31 (q, *J* = 7.5 Hz, CH_3CH_2CO), 1.64 (m, $CH_3CH_2CH_2$), 1.13 (t, CH_3CH_2CO), 0.95 (t, $CH_3CH_2CH_2$).

1,4-Di-*N*-benzyloxycarbonyl-GlA_1 (10). A solution of *N*-(benzyloxycarbonyloxy)succinimide (13.2 g, 53 mmol) in DMF (100 mL) was added dropwise to a stirred solution of **1** (10 g, 25 mmol) in H_2O (100 mL) and DMF (100 mL) at 0 °C. The mixture was stirred at room temperature for 16 h and concentrated to a residue which was partitioned between *n*-BuOH and H_2O. The two layers were back-washed several times with a saturated solution of the other. The organic layer contained **10** and traces

of *N*-hydroxysuccinimide as indicated by TLC and NMR. The dried *n*-BuOH solution was evaporated to give a powder (15 g, 89%). An analytical sample was obtained by preparative chromatography ($CHCl_3$–MeOH–H_2O, 80:30:2): $[\alpha]_D$ +53.3° (*c* 1.02, MeOH); NMR (MeOH-d_4) δ 7.20 (s, 10 H, aromatic), 5.03 (5 H, H-1 and $C_6H_5CH_2O$), 2.21 (q, J = 7.8 Hz, CH_3CH_2CO), 1.10 (t, CH_3CH_2CO). Anal. ($C_{31}H_{43}N_3O_{13}$) C, H, N.

1,4-Di-*N*-benzyloxycarbonyl-5,6-*O*-isopropylidene-GlA$_1$ (11). Anhydrous cupric sulfate (20 g) was added to a vigorously stirred solution of **10** (13 g, 19.5 mmol) in dry acetone (200 mL). After 24 h, the inorganic salt was filtered and washed with acetone. The combined filtrates were concentrated to a syrup which contained a diacetonide in addition to **11** [TLC, $CHCl_3$–MeOH (14:1); multiple developments]. Separation of the mixture by column chromatography on silica gel with $CHCl_3$–MeOH (90:10) as eluent gave **11** (11.3 g, 82%) [$[\alpha]_D$ +45° (*c* 1.03, MeOH). Anal. ($C_{34}H_{47}N_3O_{13}$) C, H, N] and 1,4-di-*N*-benzyloxycarbonyl-2′,3′;5,6-di-*O*-isopropylidene-GlA$_1$ (0.43 g, 3%) [$[\alpha]_D$ 38.7° (*c* 1.0, $CHCl_3$). Anal. ($C_{37}H_{51}N_3O_{13}\cdot CH_3OH$) C, H, N].

1,4-Di-*N*-benzyloxycarbonyl-2′,3′;5,6-di-*O*-isopropylidene-GlA$_1$. 2,2-Dimethoxypropane (20 mL) and *p*-toluenesulfonic acid (0.08 g) were added to a stirred solution of **11** (3.5 g, 4.96 mmol) in DMF (20 mL), and the mixture was heated at 60 °C with stirring for 3 h. The cooled solution was neutralized with basic resin AG 1-X8 (–OH), filtered, and concentrated to a residual glass (3.8 g) which contained two components [R_f 0.28 and 0.37, $CHCl_3$–MeOH–H_2O (90:10:1)]. A stirred solution of this crude mixture in pyridine (30 mL) was treated with pyridine hydrochloride (0.45 g) at 50 °C for 2 h. TLC indicated a complete conversion of the more mobile by-product (R_f 0.37) into the title compound (R_f 0.28). The mixture was concentrated to a syrup which was taken up in $CHCl_3$ (100 mL) and washed with H_2O (3 × 50 mL). The dried solution was evaporated to give a powder (3.4 g, 88%): $[\alpha]_D$ +39° (*c* 1.0, $CHCl_3$). Anal. ($C_{37}H_{51}N_3O\cdot CH_3OH$) C, H, N.

1,4-Di-*N*-benzyloxycarbonyl-5,6-*O*-isopropylidene-6′-*O*-mesitylenesulfonyl-GlA$_1$ (12). 2-Mesitylenesulfonyl chloride (3.42 g, 15.6 mmol) was added in portions to a solution of **11** (10 g, 14.2 mmol) in dry pyridine (50 mL), and the solution was kept at room temperature for 24 h. The mixture was concentrated to a syrup which was taken up in dichloromethane (250 mL) and washed successively with H_2O, dilute acid, aqueous $NaHCO_3$, and H_2O. The dried solution was evaporated to a syrup which was column chromatographed on silica gel (500 g) with $CHCl_3$–MeOH (95:5) as eluent. Fractions containing the desired product were pooled and concentrated to give **12** (6.3 g, 50%): $[\alpha]_D$ +45.6° (*c* 1.08, $CHCl_3$). Anal. ($C_{43}H_{57}N_3SO_{15}$) C, H, N, S.

1,4-Di-*N*-benzyloxycarbonyl-6′-chloro-6′-deoxy-5,6-*O*-isopropylidene-GlA$_1$ (13). Lithium chloride (1.0 g, 23.5 mmol) was added to a solution of **12** (1.0 g, 1.13 mmol) in dry DMF (20 mL), and the mixture was heated at 95 °C (bath temperature) with stirring for 16 h and concentrated to dryness. The residue was taken up in $CHCl_3$ and washed several times with H_2O, dried, and evaporated to give **13** (0.7 g, 86%). An analytical sample was obtained by preparative chromatography ($CHCl_3$–MeOH, 90:10): $[\alpha]_D$ +66.5° (*c* 1.06, $CHCl_3$). Anal. ($C_{34}H_{46}N_3ClO_{12}$) C, H, N, Cl.

1,4-Di-*N*-benzyloxycarbonyl-6′-chloro-6′-deoxy-GlA$_1$ (14). A solution of **13** (700 mg, 0.97 mmol) in aqueous MeOH (20 mL) containing acidic ion-exchange resin AG 50W-X2 (100–200 mesh, 5 mL) was stirred at room temperature for 3 h, filtered, and concentrated to give **14** (630 mg, 95%). An analytical sample had $[\alpha]_D$ +57.8° (*c* 0.76, $CHCl_3$). Anal. ($C_{31}H_{42}N_3ClO_{12}$) C, H, N, Cl.

6′-Chloro-6′-deoxy-GlA$_1$ (15). A solution of **14** (500 mg, 0.73 mmol) in aqueous dioxane (5 mL) was hydrogenated over 10% Pd/C (400 mg) for 2 h. The mixture was filtered through Celite and washed with aqueous dioxane (10 mL). The combined filtrates were concentrated to a syrup (380 mg) which was purified by a column of CM-25 Sephadex (NH_4^+) with a linear gradient of 0.25 N NH_4OH as irrigant. The product was detected by TLC ($CHCl_3$–MeOH–NH_4OH, 2:2:1) in fractions 70–83 with 5 mL in each tube. These fractions were pooled and concentrated to give **15** (222 mg, 60%): $[\alpha]_D$ +54.8° (*c* 1.03, MeOH); MS *m/e* 301, 200. Anal. ($C_{15}H_{30}N_3ClO_8\cdot H_2CO_3$) C, H, N, Cl.

6′-Azido-1,4-di-*N*-benzyloxycarbonyl-6′-deoxy-5,6-*O*-isopropylidene-GlA$_1$ (16). Sodium azide (1.0 g, 15.4 mmol) was added to a solution of **12** (1.0 g, 1.13 mmol) in dry DMF (20 mL), and the suspension was heated at 100 °C (bath temperature) with stirring for 16 h. The mixture was concentrated to a residue which was taken up in $CHCl_3$ and washed several times with H_2O. The dried solution was evaporated to a syrup which was column chromatographed on silica gel ($CHCl_3$–MeOH, 90:10) to give **16** (0.62 g, 75%): $[\alpha]_D$ +62.1° (*c* 1.02, $CHCl_3$); ν_{max}^{Nujol} 2090 cm^{-1} (N_3). Anal. ($C_{34}H_{46}N_6O_{12}$) C, H, N.

6′-Azido-1,4-di-*N*-benzyloxycarbonyl-6′-deoxy-GlA$_1$ (17). A solution of **16** (500 mg, 0.68 mmol) in aqueous MeOH (10 mL) containing acidic ion-exchange resin AG 50W-X2 (100–200 mesh, 4 mL) was stirred at room temperature for 3 h, filtered, and concentrated to give **17** (455 mg, 96%): R_f 0.22 ($CHCl_3$–MeOH, 90:10) (**16**, R_f 0.62).

6′-Amino-6′-deoxy-GlA$_1$ (18). A solution of **17** (300 mg, 0.43 mmol) in aqueous dioxane (4 mL) was hydrogenated over 10% Pd/C (250 mg) for 2 h. The mixture was filtered through Celite and washed with aqueous dioxane (10 mL). The combined filtrates were evaporated to a syrup which was purified by a column of CM-25 Sephadex (NH_4^+) with a linear gradient of 0.25 N NH_4OH as irrigant to give **18** (100 mg, 54%): R_f 0.14 ($CHCl_3$–MeOH–NH_4OH, 2:2:1).

1,4-Di-*N*-benzyloxycarbonyl-6,6′-di-*O*-mesitylenesulfonyl-GlA$_1$ (19). 2-Mesitylenesulfonyl chloride (0.67 g, 3.07 mmol) was added in portions to a cooled solution of **10** (1.0 g, 1.5 mmol) in dry pyridine (10 mL), and the solution was kept at room temperature. The progress of the reaction was monitored by TLC ($CHCl_3$–MeOH, 90:10). The two monosulfonates (R_f 0.40 and 0.44) which formed initially were gradually converted into the disulfonate **19** (R_f 0.54). After 2 days, the mixture was concentrated to a syrup which was partitioned between $CHCl_3$ and H_2O. The organic layer was washed with dilute acid, aqueous $NaHCO_3$, and H_2O. The dried solution was evaporated to a syrup which was column chromatographed on silica gel ($CHCl_3$–MeOH, 95:5) to give **19** as a powder (0.9 g, 58%): $[\alpha]_D$ +49.3° (*c* 1.04, $CHCl_3$). Anal. ($C_{49}H_{63}N_3S_2O_{17}$) C, H, N, S.

1,4-Di-*N*-benzyloxycarbonyl-6,6′-dichloro-6,6′-dideoxy-GlA$_1$ (20). Lithium chloride (300 mg, 7.06 mmol) was added to a solution of **19** (300 mg, 0.29 mmol) in dry DMF (10 mL), and the mixture was heated at 80–85 °C (bath temperature) with stirring for 16 h and concentrated to dryness. The crude product was purified by preparative chromatography ($CHCl_3$–MeOH–H_2O, 80:20:2) to give **20** (153 mg, 75%), R_f 0.34 ($CHCl_3$–MeOH, 90:10) (**19**, R_f 0.54).

6,6′-Dichloro-6,6′-dideoxy-GlA$_1$ (21). A solution of **20** (120 mg, 0.17 mmol) in aqueous dioxane (5 mL) containing a few drops of acetic acid was hydrogenated over 10% Pd/C (100 mg) for 2 h. The mixture was filtered and the filtrate was concentrated to a syrup which was purified by a column of CM-25 Sephadex (NH_4^+) with a linear gradient of 0.2 N NH_4OH as eluent to give **21** (48 mg, 65%): R_f 0.36 ($CHCl_3$–MeOH–NH_4OH, 5:5:1).

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Dehydrative Metabolites of 1-(3-Chlorophenyl)-1-methyl-2-phenyl-2-(2-pyridine)ethanol as Potential Hypocholesteremic Agents

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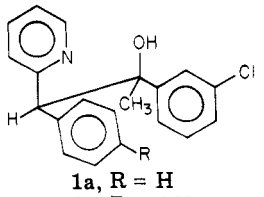
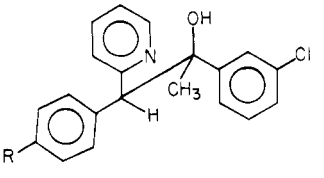
The *E* and *Z* isomers of 2-[2-(3-chlorophenyl)-1-phenyl-1-propenyl]pyridine (**2a,b**) and 2-[2-(3-chlorophenyl)-1-(4-hydroxyphenyl)-1-propenyl]pyridine (**4a,b**) were synthesized and separated as possible metabolites of 1-(3-chlorophenyl)-1-methyl-2-phenyl-2-(2-pyridine)ethanol (**1a**). Following administration of **1a** to rats, a HPLC system was used to examine urine and serum specimens for the less polar metabolites of **1a**. Isomers **2a** and **2b** were not detected but their hydroxylated derivatives **4a** and **4b** were observed as minor metabolites. Compounds **2a,b** and **4a,b** exhibited hypocholesteremic activity in rats; compounds **4a** and **4b** are of special interest because they possessed relatively low estrogenicity.

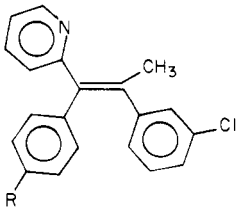
The compound 1-(3-chlorophenyl)-1-methyl-2-phenyl-2-(2-pyridine)ethanol (**1a**) has shown promising hypocholesteremic activity in rats but not in rhesus monkeys or humans.[1] Studies are in progress in these laboratories to determine if species differences in the metabolic disposition of **1a** could be responsible for the species-specific hypocholesteremic activity. It has been reported that the 2-(4-hydroxyphenyl) derivative **3a** is a pharmacologically active metabolite of **1a**[2] in rats, and previous chemical studies indicated that dehydration of **1a** yielded the unsaturated isomers **2a** and **2b**.[3] Therefore, it was postulated that isomers **2a** and **2b** and their hydroxylated derivatives **4a** and **4b** could be metabolites of **1a** and might be involved in the species-specific activity of **1a**.



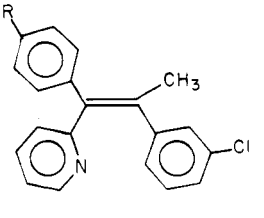
1a, R = H
3a, R = OH



1b, R = H
3b, R = OH



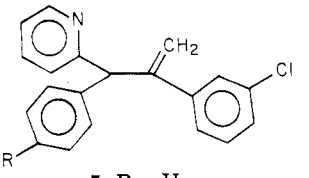
2a, R = H
4a, R = OH



2b, R = H
4b, R = OH

This paper reports the synthesis of compounds **2a,b**, **4a,b**, **5**, and **8** together with the determination of their hypocholesteremic and estrogenetic activities. Their importance as metabolites of compound **1a** in rats also has been evaluated.

Chemistry. As previously reported, dehydration of the higher melting racemate **1a** with 85% H_3PO_4 at 110 °C first yields the terminal olefin **5** which was followed by a slow conversion to the more thermodynamically stable conjugated *E* and *Z* isomers **2a** and **2b**.[3] However, dehydration of the lower melting diastereomer **1b** led largely



5, R = H
8, R = OH

to decomposition to 2-benzylpyridine (**6**) and 3-chloroacetophenone (**7**).

In the present investigation it was found that **1b**, produced in amounts equivalent to that of **1a** in its synthesis, but which was less hypocholesteremic, could be used as a source for **2a** and **2b** or **5** in a modified procedure. Dehydration of **1b** to the *E* and *Z* mixture **2a,b** was accomplished in 65% yield by limiting the temperature to 75–80 °C for 72 h to give primarily **5** and then heating at 110 °C to obtain the isomerized products **2a** and **2b**. Similarly, dehydration of **3b** gave a 91% yield of (*E*)- and (*Z*)-hydroxyphenyl compounds **4a** and **4b**. The terminal olefin compounds **5** and **8** could be isolated if the respective dehydration reactions were limited to 75–80 °C.

The presence of two singlets in the NMR for methyl groups at δ 2.19 and 2.23 ($CDCl_3$) for the mixture of compounds **2a,b** and at δ 2.08 and 2.20 (Me_2SO-d_6) for the mixture of compounds **4a,b** was the initial indication of the presence of *E* and *Z* mixtures. Both mixtures could be separated by TLC on 15% $AgNO_3$ impregnated silica gel with ethyl acetate–acetic acid as developing solvent. Preparative separation was by column chromatography with 20% $AgNO_3$ on silica gel and with ethyl acetate–acetic acid (9:1) as eluting solvent. The *E* and *Z* isomers of **4** could also be conveniently separated by trituration of the mixture in acetone at room temperature, as **4b** was the more soluble isomer.

The assignment of *E* and *Z* stereochemistry was on the basis of NMR comparisons to triprolidine hydrochloride (**9**) for which the effective antihistamine has been established as the *E* isomer while the less active isomer (**10**) is its *Z* isomer.[4] Of interest was a comparison of the NMR chemical shifts for the methylene and vinyl protons for compounds **9** and **10**. For triprolidine hydrochloride (**9**), which has the 2-pyridyl system (and thus the unshared pair

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