$H_{11}N_2O$), 119.0606 ($C_7H_7N_2$), 92.0498 (C_6H_6N).

***N'*-Nitrosonornicotine 1-*N*-Oxide (4).** A mixture of **1**[28] (1.68 g, 9.5 mmol) and *m*-chloroperbenzoic acid (1.9 g, 11 mmol) in 50 mL of $CHCl_3$ was stirred at 0–5 °C for 3 h. The resulting mixture was washed with saturated aqueous $NaHCO_3$ (2 × 25 mL), and the $CHCl_3$ solution was dried (Na_2SO_4) and concentrated to give 1.5 g of crude **4** as a yellow oil. Silica gel chromatography with elution by $CHCl_3$ and 15:1 $CHCl_3$/MeOH gave 1.3 g (70%) of **4** as an oil, a mixture of anti (55%) and syn (45%) isomers, pure by TLC [(silica; 10:1 $CHCl_3$/MeOH) R_f 0.25] and high-pressure LC (retention volume, 45 mL). Spectral properties of **4**: NMR ($CDCl_3$) δ 1.7–2.7 (4 H, m, CH_2CH_2), 3.5–3.8 (m, 1 H, *anti*-$CH_2NN{=}O$), 4.2–4.8 (m, 0.9 H, *syn*-$CH_2NN{=}O$), 4.8–5.2 (m, 0.45 H, *syn*-$CHNN{=}O$), 5.4–5.7 (m, 0.55 H, *anti*-$CHNN{=}O$), 6.8–7.5 (m, 2 H), 7.8–8.2 (m, 2 H, pyr H); IR (film) 1602, 1560, 1420, 1270, 1160, 1012 cm^{-1}; MS, *m/e* (relative intensity) 193 (M^+, 70), 163 (48), 145 (100), 118 (93). Anal ($C_9H_{11}N_3O_2$) C, H, N.

When the above reaction was carried out at 20 °C for 18 h, approximately 10% of *N'*-nitronornicotine 1-*N*-oxide was also formed: high-pressure LC retention volume, 47 mL; MS, *m/e* (relative intensity) 209 (69), 146 (68), 145 (100), 118 (83).

Analysis for 2–4 in Vitro. Liver microsomes from Aroclor 1254 pretreated rats were prepared as described previously.[29] Incubations were carried out at 37 °C for 60 min in five 25-mL Erlenmeyer flasks. Each flask contained NNN (18.4 mg, 0.104 mmol), NADPH (2.5 mg), glucose-6-phosphate dehydrogenase (50 units), glucose 6-phosphate (0.05 mmol), $MgCl_2$ (0.05 mmol), and microsomal suspension (2 mL; 9 mg/mL protein) brought to a total volume of 10 mL with 0.1 M pH 7.4 Tris-HCl buffer. In control incubations, heat-treated microsomes were used. Incubations were quenched by the addition of 10 mL of EtOH. Protein was removed by centrifugation and one-third of the supernatant was used for analysis of **4**. The remainder was analyzed for **2** and **3**.

To analyze for **4**, the supernatant was extracted five times with $CHCl_3$, and the combined $CHCl_3$ layers were dried (Na_2SO_4), concentrated and applied to two 0.5-mm silica gel TLC plates with elution by $CHCl_3$/MeOH, 10:1. The band corresponding to **4** (R_f 0.25) was eluted from the silica with MeOH and analyzed by MS. For quantitation of **4**, an aliquot of the $CHCl_3$ extract was analyzed by high-pressure LC.

To analyze for **2** and **3** the supernatant was concentrated and applied to three 0.5-mm silica gel TLC plates with elution by 10:1 $CHCl_3$/MeOH. The bands corresponding in R_f to standards were eluted from the silica with MeOH, concentrated to dryness, and silylated with bis(trimethylsilyl)trifluoracetamide and 1% trimethylchlorosilane (Regisil, Regis Chemical Co.). An aliquot was analyzed by GLC and combined GLC–MS with a program of 150 °C for 8 min and then 4 °C/min to 240 °C. Under these conditions, the retention time of the cis/trans isomers of the trimethylsilyl ethers of **2** were 21.8 and 22.3 min, and the retention time of the trimethylsilyl ethers of **3** was 22.8 min.

Analysis for 2–4 in Vivo. Male F-344 rats were given a sc injection of [2'-^{14}C]NNN[28] (1×10^7 dpm; 3, 10, 30, 100 or 300 mg/kg body weight) in 2 mL of 0.9% NaCl solution.[18] Urine was collected for 48 h. To assay for **4**, urine was lyophilized and then sonically dispersed with CH_3OH. An aliquot of the CH_3OH solution was analyzed by high-pressure LC. The fraction with the same retention time as **4**, obtained after a dose of 300 mg/kg, was collected and identified as **4** by its MS. Quantitation was accomplished by scintillation counting (all doses) and by comparison of UV detector response with reference **4** (100 and 300 mg/kg doses). To assay for **2** and **3**, urine from rats treated with 300 mg/kg NNN was extracted four times with $CHCl_3$. The $CHCl_3$ extracts were combined, dried, concentrated, and silylated with Regisil. An aliquot was analyzed by combined GLC–MS as described in the in vitro assay.

To analyze for glucuronides or sulfates of **2** and **3**, urine was extracted with $CHCl_3$, and the aqueous portion was incubated with β-glucuronidase (type IX, from *Escherichia coli*, 17 000 units) and sulfatase (type H-1, from *Helix pomatia*, 2460 units) for 16 h at 37 °C. The resulting mixture was extracted with $CHCl_3$, and the $CHCl_3$ extracts were dried, concentrated, and analyzed by high-pressure LC.

Acknowledgment. This study was supported by National Cancer Institute Grant CA 21393. Stephen S. Hecht is a recipient of NCI Research Career Development Award 5 KO4 CA 00124. Chi-hong B. Chen is a recipient of National Institute of Environmental Health Sciences Award ESO-2236. The authors thank Greg Cooper and Ms. Dorothy Lin for their expert technical assistance.

(29) Hecht, S. S.; Chen, C. B.; Hoffmann, D. *Cancer Res.* **1978**, *38*, 215–218.

Urinary Metabolites of Timolol from Humans and Laboratory Animals. Syntheses and β-Adrenergic Blocking Activities

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Syntheses are reported for three metabolites (**2–4**) of timolol (**1**) formed by oxidative metabolism of the morpholine ring. GLC–MS comparisons are presented which establish that the two metabolites whose structures were previously in question are identical with their synthetic counterparts **2** and **3**. In **2**, metabolic oxidation of the 4-morpholinyl group of **1** has occurred at the carbon next to oxygen to give the 2-hydroxy-4-morpholinyl moiety, whereas in **3**, the morpholine of **1** has been oxidized one step further and then ring opened to produce the *N*-(2-hydroxyethyl)glycine substituent. Biological testing of synthetic samples of the three major metabolites from human urine (**3**, **4**, and **6**) indicated that only **4**, in which the morpholine moiety has been degraded to a 2-hydroxyethylamino group, had significant β-adrenergic blocking activity (one-seventh that of timolol in anesthetized dogs).

The β-adrenergic antagonist drug timolol (**1**), like propranolol and a number of other β-blockers, has been shown to be effective in humans for the treatment of hypertension and angina pectoris.[1] In addition, timolol has been

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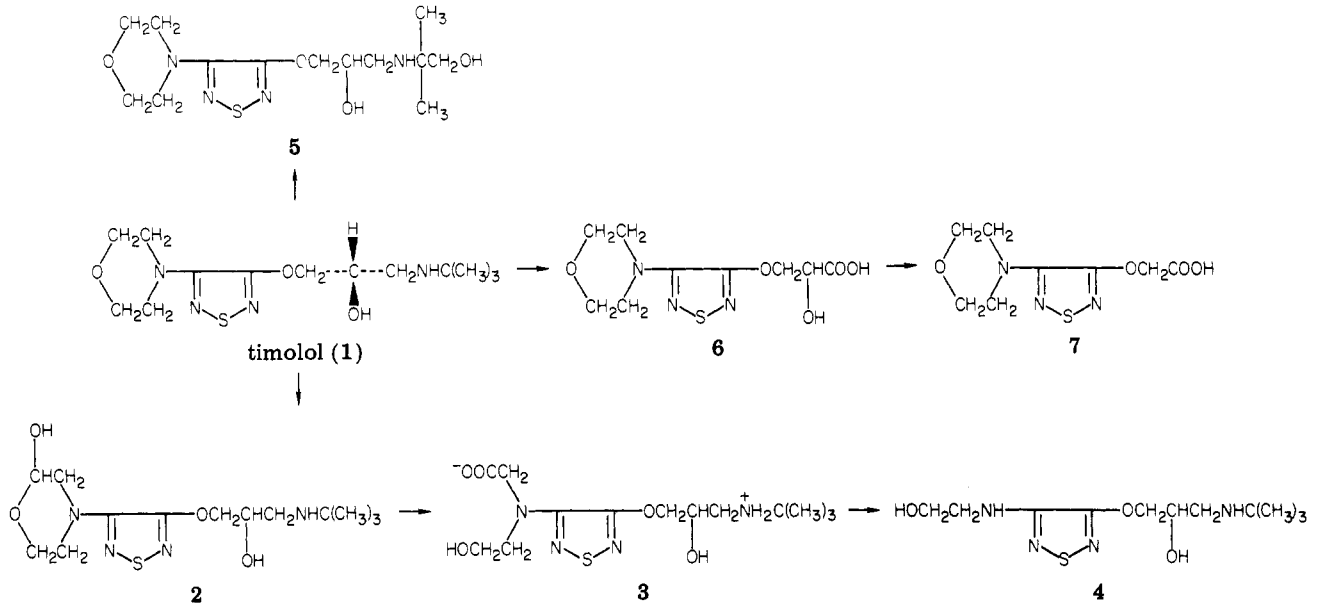
Scheme I. Metabolites of Timolol in Humans, Rodents, and Dogs[a] Resulting from Oxidation of the Oxypropanolamine or Morpholine Substituents

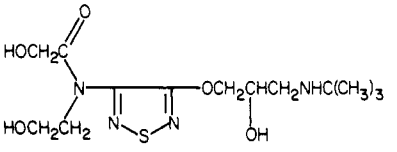


[a] References 3 and 4.

marketed recently for the treatment of glaucoma, based on its ability to lower intraocular pressure when administered directly into the eye.[2] This drug appears to represent a major advance in therapy of this disease. Timolol was designed as a novel heterocyclic-substituted oxypropanolamine derivative in a synthetic program, the aim of which was the discovery of β-blocking agents more specific in action than propranolol. Indeed, for the ophthalmic application of timolol the absence of the significant local anesthetic effect, present with propranolol, is an important asset.

The clinical introduction of timolol in a number of countries has necessitated a detailed search for metabolites, as well as the determination of whether or not these metabolites contribute significantly to its β-adrenergic blocking activity. Studies on metabolites from radioactively labeled **1** in several species have now been described.[3,4] Metabolic oxidation was shown to occur not only on the basic oxypropanolamine side chain but also at the morpholine moiety, resulting in the formation of at least six distinct products as shown in Scheme I. The morpholine ring-modified metabolites *N*-[4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,2,5-thiadiazol-3-yl]-*N*-(2-hydroxyethyl)glycine (**3**) and 1-[(1,1-dimethylethyl)amino]-3-[[4-[(2-hydroxyethyl)amino]-1,2,5-thiadiazol-3-yl]oxy]-2-propanol (**4**) represent major metabolic products (30 and 10%, respectively, of the administered dose) present in human urine. Metabolite **6** [2-hydroxy-3-[[4-(4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-propanoic acid], which in human urine accounts for only 6% of the dose, is the most important metabolite from dog urine (55%). In this paper we present the detailed syntheses of metabolites **3** and **4**, along with the synthesis of the presumed metabolic precursor of **3**, 1-[(1,1-dimethylethyl)amino]-3-[[4-(2-hydroxy-4-morpholinyl)-1,2,5-thiadiazol-3-yl]oxy]-2-propanol (**2**). Compound **2** has been detected as a minor metabolite in urine from rats and mice.[4] Syntheses of **6** and **7** have been reported previously by Bélanger,[5] while the synthesis of metabolite **5** (a minor metabolite in man) has not yet been described. The results of β-adrenergic blocking activity determinations in dogs for compounds **3**, **4**, and **6**, the major metabolites from human urine, are also presented.

Chemical Synthesis. In the initial report[3] on the results of GLC–MS examination of the metabolic products derived from **1**, it was possible to assign structures unequivocally to metabolites **4** and **6**. The structure **8** was



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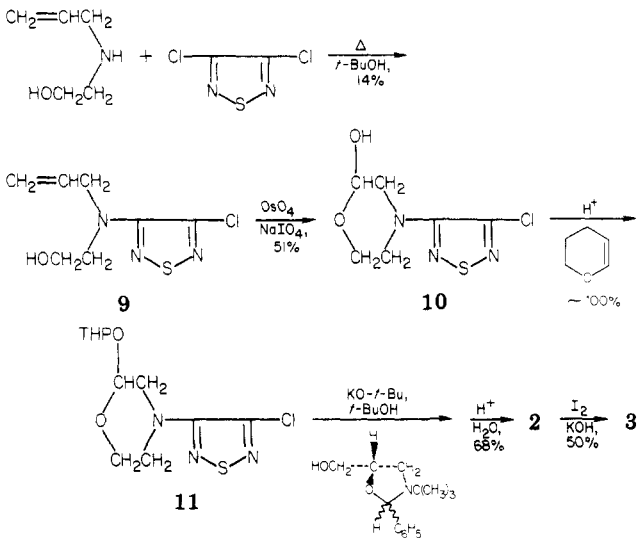
suggested, but not proven, for a major metabolite resulting from oxidative attack at the morpholine ring. Our preliminary synthetic studies[6] demonstrated that this hydroxyacetamide type of derivative in the 1,2,5-thiadiazole series was not stable to the basic conditions employed for isolation of the metabolites. Consequently, the alternative isomeric structure **3** was deemed the only reasonable one; therefore, its synthesis was undertaken.

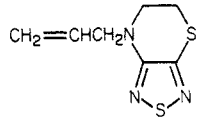
The synthesis of timolol is readily accomplished by reaction of 3,4-dichloro-1,2,5-thiadiazole with morpholine, followed by introduction of the suitably protected, optically active oxypropanolamine side chain, and finally by removal of the protecting group.[7] Analogous pathways were followed in the syntheses of both **3** and **4** (as their *S* isomers). The overall route of synthesis of **3** starting from allylhydroxyethylamine is shown in Scheme II. The olefinic group of the latter served as a synthon for the acidic group in **3**. This route of synthesis also permitted isolation of the hemiacetal **2**, identified as a minor metabolite in rodent urine.

(1) Brogden, R. N.; Speight, T. M.; Avery, G. S. *Drugs* **1975**, *9*, 164.
(2) Kosman, M. E. *J. Am. Med. Assoc.* **1979**, *241*, 2301.
(3) Tocco, D. J.; Duncan, A. E. W.; Deluna, F. A.; Hucker, H. B.; Gruber, V. F.; VandenHeuvel, W. J. A. *Drug Metab. Dispos.* **1975**, *3*, 361.
(4) Tocco, D. J.; Duncan, A. E. W.; Deluna, F. A.; Smith, J. L.; Walker, R. W.; VandenHeuvel, W. J. A. *Drug Metab. Dispos.* **1980**, *8*, 236.
(5) Bélanger, P. C. *Can. J. Chem.* **1978**, *56*, 722.
(6) Wasson, B. K.; Scheigetz, J.; Rooney, C. S., unpublished.

Scheme II. Synthetic Route to 2 and 3



The reaction of excess morpholine with 3,4-dichloro-1,2,5-thiadiazole goes rapidly at 120 °C to give 3-chloro-4-(4-morpholinyl)-1,2,5-thiadiazole in high yield.[7] In contrast, reaction of 3,4-dichloro-1,2,5-thiadiazole neat with *N*-allylhydroxyethylamine gave very low yields of the desired **9**. The best conditions found for this reaction were refluxing *n*-PrOH for several days or refluxing *t*-BuOH for 4 weeks. Significant thiadiazole ring destruction occurred under these conditions, as evidenced by the production of elemental sulfur and the diminution of the characteristic UV absorption of the 1,2,5-thiadiazole. From the reaction with *N*-allylethanolamine, the byproduct **12**, containing



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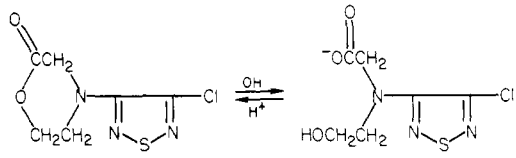
an additional sulfur, was unexpectedly isolated. The mechanism of its formation presumably involves chlorination of the hydroxyl of **9** by HCl, produced from the initial condensation, and reaction with sulfur. This bicyclic ring system has not been reported previously.

Oxidation of **9** using $OsO_4/NaIO_4$[8] in a two-phase Et_2O–H_2O system gave the hemiacetal intermediate **10**. Conversion to the tetrahydropyranyl (THP) ether **11**, followed by introduction of the optically active (*S*)-3-(*tert*-butylamino)-2-hydroxypropoxy side chain by the procedure of Weinstock,[7] and then stepwise hydrolysis of the benzylidene and THP blocking groups afforded **2**. Compound **2**, which has a second chiral center at the hemiacetal carbon, was expected to exist as a mixture of two diastereomers. While no evidence for two diastereomers was seen by TLC, 60-MHz ^1H NMR, or ^{13}C NMR, careful analysis of the 300-MHz ^1H NMR of its hemifumarate salt indicated the presence of two isomers in a ratio of 1:1. Using high-resolution ^1H NMR, the CH_2N and CH_2O resonances from optically active (1,2,5-thiadiazol-3-yloxy)propanolamine derivatives are normally seen as pairs of doublets of doublets, due to the nonequivalence of the two protons in each of the side-chain methylene groups. In the 300-MHz ^1H NMR spectrum of **2**, even greater multiplicity was observed to be present for the side-chain CH_2N and CH_2O resonances (for the latter this was seen only in the free base form). Spin-decoupling experiments confirmed that the increased complexity of these multiplets was attributable to the presence of two diastereomers, and not to proton–proton couplings within a single isomer. The *J* value of less than 3 Hz estimated for the proton attached to the hemiacetal carbon of **2** suggests that the hydroxyl at this position in both diastereomers is in the axial orientation as predicted by the anomeric effect.[9]

Oxidation of the hemiacetal intermediate **10** with $Br_2/BaCO_3/H_2O$ gave, after acidification, the crystalline lactone **13**. Addition of **13** to dilute aqueous NaOH resulted in rapid opening of the lactone ring, as indicated by the inability to extract the thiadiazole derivative from the basic solution with organic solvents. Acidification resulted in rapid reversal to the easily extractable lactone form.



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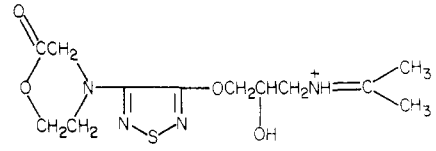
For oxidation of the penultimate intermediate **2** in the synthesis of **3**, $I_2/KOH/MeOH$ was utilized. Compound **3** was easily isolated as an optically active, water-soluble, crystalline zwitterion. Its structure was assigned based on ^1H and ^{13}C NMR, elemental analysis, as well as the lack of a lactone carbonyl in the IR spectrum. A major peak in the low-resolution mass spectrum [$M^+ - (18 + 15)$] corresponded to the lactone **14**. Attempts to convert **3**



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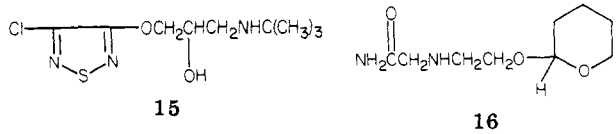
to its corresponding lactone by treatment with aqueous mineral acid were unsuccessful. While the lactone may be the initially formed product of oxidation of the hemiacetal **2**, the open-chain, zwitterionic form is clearly preferred at neutral and moderately acidic pH's.

A shorter alternative route to **3** was attempted without success. Timolol can be prepared by the reaction of morpholine with **15**.[10] A number of attempts to react the protected *N*-(hydroxyethyl)glycine derivative **16** with **15**, either neat or in solvent, failed to give the desired condensation product in significant yield.



15 **16**

The synthesis of **4** followed a course analogous to that described for **2**, except that no oxidation step was required. The reaction of ethanolamine with 3,4-dichloro-1,2,5-thiadiazole in refluxing *t*-BuOH gave 3-chloro-4-[(2-hydroxyethyl)amino]-1,2,5-thiadiazole (**17**). Conversion

(7) Weinstock, L. M.; Mulvey, D. M.; Tull, R. *J. Org. Chem.* **1976**, *41*, 3121.
(8) Pappo, R.; Allen, D. S., Jr.; Lemieux, R. U.; Johnson, W. S. *J. Org. Chem.* **1956**, *21*, 478.
(9) Hall, S. S.; Weber, G. F.; Duggan, A. J. *J. Org. Chem.* **1978**, *43*, 667 and references therein.
(10) Wasson, B. K.; Gibson, W. K.; Stuart, R. S.; Williams, H. W. R.; Yates, C. H. *J. Med. Chem.* **1972**, *15*, 651.

Table I. Differential β-Adrenergic Blocking Activity of Timolol and Its Metabolites in the Anesthetized Dog. Inhibition of Isoproterenol-Induced Hypotension, Tachycardia, and Bronchodilatation

compd	no. of tests	blockade of isoproterenol effects: ED_{50}, μg/kg iv		
		hypotension	tachycardia	bronchodilatation
timolol[a]	4	2.8 ± 0.63[b]	5.5 ± 1.06[b]	0.9 ± 0.4[b]
metabolite 4	4	19.9 ± 3.46[b]	38.1 ± 6.51[b]	5.4 ± 0.33[b]
metabolite 3	2	NE[c]	NE	NE, 3.0 mg/kg
metabolite 6	4	NE	NE	NE

[a] References 7 and 10. [b] Data presented as the mean ± SEM. [c] NE = no effect at doses up to 1 or 3 mg/kg iv.

to the tetrahydropyranyl ether 18, followed by introduction of the benzylidene protected side-chain moiety, and then removal of the protecting groups with acid (as described for the synthesis of 2) gave the *S* isomer of 4. Mass spectral evidence that synthetic 4 and the natural metabolite are identical has been reported previously.[3]

Gas Chromatographic–Mass Spectral Comparisons of 2 and 3 with Authentic Timolol Metabolites. Samples of synthetic 3 and the analogous metabolite from rat urine[3,4] were compared in derivatized form by GLC–MS. (Authentic samples of metabolite 3 from human, rat, and mouse urine have been shown previously to be identical.[3,4]) The samples were (a) subjected to methylation with diazomethane, followed by trimethylsilylation using BSA or BSA-d_{18}, and (b) subjected to trimethylsilylation only. A comparison of the retention times and mass spectra of the various derivatives of synthetic 3 and the metabolite 3 showed them to be identical.

A urinary metabolite of timolol in rodents has been shown to be the compound resulting from monohydroxylation of the morpholine ring.[4] As 2 (the synthetic precursor of 3) was now available for direct comparison with this minor metabolite, the metabolite-containing fraction and 2 were trimethylsilylated and examined by GLC–MS. The resulting chromatograms displayed identical retention times. In addition, the mass spectra of the two derivatives were essentially identical. These data are strong evidence for the rodent metabolite possessing structure 2. It is presumed that the chiral center in the oxypropanolamine side chain remains unchanged in metabolites 3 and 2 but, because of the small quantities of materials, it has been impossible to measure optical rotation values.

Biological Results. Timolol and three of its urinary metabolites prepared by chemical synthesis were tested for differential β-adrenergic blocking activities in anesthetized dogs vs. isoproterenol-induced hypotension, tachycardia, and bronchodilatation using a protocol worked out in our laboratories. The detailed procedures are described under Experimental Section and the data presented in Table I. Timolol effectively antagonized isoproterenol-induced hypotension, tachycardia, and bronchodilatation with an ED_{50} of 2.8, 5.5, and 0.9 μg/kg iv, respectively. The corresponding ED_{50} values for synthetic metabolite 4 were 19.9, 38.1, and 5.4 μg/kg iv. The results suggest that at the 50% level of activity metabolite 4 is ~7 times less potent than timolol. Synthetic metabolite 6 had no β-adrenergic blocking activity at doses up to 1 (one experiment) or 3 mg/kg iv (three experiments). Synthetic metabolite 3 did not block isoproterenol-induced hypotension or tachycardia in doses up to 3 mg/kg iv but had some moderate blocking activity (22–50%) against isoproterenol-induced bronchodilatation at high doses (0.3–3 mg/kg iv).

Discussion

Metabolite 4, which represents 10% of the administered does of timolol in humans, was the only one of the three synthetic metabolites tested which showed significant β-adrenergic blocking activity (one-seventh that of timolol in anesthetized dogs). Metabolite 3 (accounting for 30% of the administered drug in humans) presumably is rendered inactive by the presence of the polar hydroxyethylglycine substituent. It was anticipated that 6, which has lost the amine function from the side chain, would be inactive as a β-blocker.

The pathways to metabolites 2–4 suggested in Scheme I are speculative. Although 2 has been detected only in rodent urine at low levels, it seems reasonable, based on literature precedent for metabolic O-dealkylation,[11] that 2 will be the first product of oxidative metabolism of the morpholine ring in all species studied. Compound 2 is presumably then oxidized further to 3. Some highly relevant literature[12] now exists concerning the metabolism of other morpholine-containing molecules. Metabolic oxidation may take place at either the oxygen or nitrogen end of the ring. In the majority of published examples, as in this work, oxidative attack at the carbon adjacent to oxygen is preferred. However, our work provides the only example known to us in which degradation of the morpholine ring proceeds as far as a single ethanolamine residue (metabolite 4). As indicated in Scheme I, we suggest metabolite 4 may be formed by further metabolic oxidation of 3. Although there is, at present, no direct evidence from animals bearing on this question, we obtained some indication that this reaction can occur during work on the synthesis of 3. Mass spectral examination of the crude product from the hypoiodite oxidation of 2 showed peaks in the low-resolution mass spectrum which were not present in the spectrum of pure 3, which corresponded to M^+ and $M^+ - CH_3$ ions from compound 4. In addition, from an oxidation of 10 using Ag_2O, 3-chloro-4-[(2-hydroxyethyl)amino]-1,2,5-thiadiazole (17) was identified as a byproduct by IR, UV, and TLC comparison with authentic 17.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were obtained with a Perkin-Elmer 257 grating spectrophotometer and ultraviolet spectra with a Perkin-Elmer 202 spectrophotometer. ^1H NMR, 60 MHz, spectra were determined with a Varian EM360 spectrometer,[13] C NMR with a Varian CFT-20 instrument, and ^1H NMR, 300 MHz, with a Varian SC-300 spectrometer. The last two instruments were equipped with Fourier-transform accessories. ^{13}C NMR spectral data for 1–4 and key intermediates are included as supplementary material (see paragraph at the end of this paper

(11) Brodie, B. B.; Gillette, J. R.; LaDu, B. N. *Annu. Rev. Biochem.* **1958**, *27*, 427.
(12) (a) Chatfield, D. H. *Xenobiotica* **1976**, *6*, 509. (b) Tanayama, S.; Nakai, Y.; Fujita, T.; Suzuoki, Z.; Imashiro, Y.; Masuda, K. *Xenobiotica* **1974**, *4*, 175. (c) Nakai, Y.; Shirakawa, Y.; Fugita T. *Jpn. J. Pharmacol.* **1972**, *22* (Suppl), 102 S (Abstract 164). (d) Prox, A.; Zimmer, A.; Machleidt, H. *Xenobiotica*, **1973**, *3*, 103. (e) Tosolini, G. P.; Moro, E.; Giraldi, P. N. *J. Chromatogr.* **1971**, *57*, 417.

concerning supplementary material). Elemental analyses were performed by Dr. C. Daessle, Montreal, and are within ±0.4% of the theoretical values when indicated by symbols of the elements. TLC were run using Eastman chromagram sheets, no. 13181, precoated with silica gel. Spots were visualized by UV and exposure to iodine vapor. Optical rotations were determined with a Zeiss Model LAP photoelectric precision polarimeter. Routine low-resolution mass spectra on synthetic compounds were obtained from the Morgan-Schaffer, Corp., Montreal.

3-Chloro-4-[*N*-allyl-*N*-(2-hydroxyethyl)amino]-1,2,5-thiadiazole (9). A mixture of 3,4-dichloro-1,2,5-thiadiazole[13] (125 g, 0.81 mol), *N*-allylethanolamine[14] (325.8 g, 3.23 mol) and *t*-BuOH (3 L) was heated at reflux for 25–30 days under N_2. Formation of product was monitored at 315 nm. The solvent was removed by evaporation, and the residue was extracted with 3 × 1 L of EtOAc. The combined EtOAc extract was washed with H_2O (1 L), 1 N HCl (0.5 L), and H_2O (1 L). After drying over Na_2SO_4 and evaporation under vacuum, 55 g of crude **9** was obtained. Purification was effected by chromatography (800 g of silica gel, CH_2Cl_2) to give 25 g (14%) of **9** as an oil: R_f (10% EtOAc/$CHCl_3$) 0.3. Anal. ($C_7H_{10}ClN_3OS$) C, H, Cl, N, S.

7-Allyl-5,6-dihydro-7*H*-1,2,5-thiadiazolo[3,4-*b*][1,4]thiazine (12). Forerun material from the chromatographic purification of **9** was rechromatographed (40 g of silica gel, CCl_4) to give crude **12** (3.7 g) as a dark oil, which on distillation gave 3.3 g of **12**, as a light yellow oil, bp 106 °C (0.07 mm). Anal. ($C_7H_9N_3S_2$) C, H, N, S.

3-Chloro-4-(2-hydroxymorpholin-4-yl)-1,2,5-thiadiazole (10). To **9** (15 g, 68 mmol) in Et_2O (375 mL) was added a 0.5% solution of OsO_4 in *t*-BuOH (30 mL). The mixture was stirred for 15–30 min until black in color. $NaIO_4$ (40.19 g, 187 mmol) in H_2O (330 mL) was added, followed by NaOAc (15.33 g, 187 mmol) in H_2O (60 mL). The two-phase system was stirred vigorously for 20–23 h. The Et_2O was separated and the aqueous phase washed with Et_2O (2 × 250 mL). The combined Et_2O solutions, after passage through a short column of Al_2O_3 to remove OsO_4, were evaporated to a brown oil (13 g). Decolorization of an Et_2O solution (100 mL) with charcoal and evaporation afforded a brown solid (11.9 g). Recrystallization from cyclohexane (950 mL) gave 7.7 g (51%) of **10**, mp 78–78.5 °C. Anal. ($C_8H_8ClN_3O_2S$) C, H, Cl, N, S.

3-Chloro-4-(2-oxomorpholin-4-yl)-1,2,5-thiadiazole (13). To a mixture of **10** (1 g, 4.5 mmol), $BaCO_3$ (3.54 g, 18 mmol), and CH_3OH (60 mL) at 0–5 °C was added dropwise a 2.3% solution of bromine water (62 mL, 13 mmol). The ice bath was removed, the mixture stirred for 15 min, and then the solvents were removed under vacuum. The residue was extracted with $CHCl_3$ (3 × 50 mL). Evaporation of the $CHCl_3$ extracts afforded a colorless solid (0.81 g). Recrystallization from cyclohexane (100 mL) gave **13** (0.62 g, 62%), mp 118–119 °C. Anal. ($C_6H_6ClN_3O_2S$) C, H, Cl, N, S.

In an examination of other conditions for this oxidation, the following experiment was run: To a mixture of **10** (101 mg, 0.455 mmol) and $AgNO_3$ (193 mg, 1.14 mmol) in H_2O (5 mL) was added dropwise 1 N NaOH (1.36 mL, 1.36 mmol) at room temperature. After 30 min, additional 1 N NaOH (1 mL) was added, followed by $AgNO_3$ (30 mg) at 45 min. After 60 min, it was established by TLC (silica gel, EtOAc/$CHCl_3$ 1:1) that starting material had essentially disappeared. Extraction of the filtered solution with Et_2O (10 mL) gave a neutral fraction (23 mg) with IR, UV, and TLC behavior identical to **17**.

3-Chloro-4-[2-(3,4,5,6-tetrahydro-2*H*-pyran-2-yloxy)-morpholin-4-yl]-1,2,5-thiadiazole (11). To a solution of **10** (32.3 g, 150 mmol) in alcohol-free $CHCl_3$ (445 mL) was added *p*-toluenesulfonic acid (0.2 g) and then dihydropyran (15.4 g, 180 mmol). After 1 h, when TLC and NMR indicated reaction to be complete, the $CHCl_3$ solution was washed with 5% Na_2CO_3 solution (2 × 50 mL) and H_2O (3 × 50 mL). After drying over $MgSO_4$ and evaporation, **11** was obtained as an oil: R_f [$CHCl_3$/EtOAc (9:1)] 0.6.

1-[(1,1-Dimethylethyl)amino]-3-[[3-(2-hydroxymorpholin-4-yl)-1,2,5-thiadiazol-4-yl]oxy]-2-propanol (2). A solution of potassium *tert*-butoxide [prepared from potassium (7.13 g, 0.18 mol) and 238 mL of anhydrous *t*-BuOH] was added to (*S*)-(−)-3-*tert*-butyl-5-(hydroxymethyl)-2-phenyloxazolidine[7] (42.9 g, 0.18 mol), and the mixture was stirred for 10 min under argon. Compound **11** (47.2 g, 0.15 mol) was then admixed. After the mixture stirred overnight under argon, Et_2O (2 L) was added. The solids which formed were removed by filtration and washed thoroughly with Et_2O. The combined filtrate was washed with H_2O (5 × 80 mL and 2 × 100 mL) and then dried ($MgSO_4$). Evaporation afforded an oil (81.7 g), which was dissolved in Et_2O (2.2 L). The solution was cooled to 0 °C and extracted with cooled 0.05 N HCl (2 × 625 mL and 1 × 312 mL) to remove unreacted oxazolidine starting material. The combined HCl extracts were back-extracted with Et_2O (300 mL). The combined Et_2O extracts were washed with 5% Na_2CO_3 solution (2 × 80 mL), H_2O (4 × 100 mL), and dried ($MgSO_4$). Evaporation afforded a clear orange-yellow gum (61.4 g, 83.4%): R_f [hexane/$CHCl_3$ (3:1) plus 2 drops of concentrated NH_4OH] 0.25. This intermediate (61.4 g) was dissolved in a mixture of THF/HOAc/H_2O (3:1:1) (1.25 L) and stirred at room temperature for 1 h under argon to hydrolyze the benzylidene group. The solvent was removed on a rotary evaporator under vacuum at 50 °C and then at high vacuum, leaving an oil (73.5 g): R_f [hexane/$CHCl_3$ (1:1) plus 1% concentrated NH_4OH] 0.2.

Removal of the tetrahydropyranyl ether blocking group was effected by dissolving the above intermediate (73.5 g) in 0.1 N HCl (1.6 L) and allowing the solution to stand at room temperature for 5 h. The solution was then extracted with Et_2O (3 × 350 mL and 1 × 250 mL). The combined Et_2O extracts were washed with H_2O (100 mL). The combined aqueous solutions after addition of 10% Na_2CO_3 solution (550 mL) were extracted with $CHCl_3$ (2 × 500 mL, 2 × 400 mL, and 300 mL). The $CHCl_3$ extracts were washed with H_2O (5 × 75 mL), dried ($MgSO_4$), and evaporated to give **2** (33.2 g, 68.4% overall from **11**) as a reddish gum: R_f [MeOH/$CHCl_3$ (1:4) plus 1% concentrated NH_4OH] 0.5. This material, although not completely pure, was suitable for use in the subsequent oxidation step.

A crystalline hemifumarate salt was readily formed by the addition of fumaric acid in EtOH to crude **2** in EtOH in a yield, after recrystallization from hot *i*-PrOH, of 57%: mp 182–184 °C; IR (KBr) 3395–3480, 1633, 1506, 1380, 1360, 1240, 1213 cm^{-1}; UV (EtOH) λ_{max} 215 nm (ϵ 11 799), 300 (7616); ^1H NMR (300 MHz, D_2O) δ 1.42 (s, 9 H, *t*-Bu), 3.20, 3.39 (each dd, 2 H, J = 13.0 and 10.0 Hz, J = 13.0 and 3.0 Hz, CH_2NH-*t*-Bu), 3.47–3.68 (m, 4 H, CH_2NCH_2), 3.83, 4.18 (each m, 2 H, OCH_2CH_2), 4.38 [m, 1 H, $OCH_2CH(OH)$], 4.50, 4.61 [each dd, 2 H, J = 11.5 and 5.5 Hz, J = 11.5 and 4.0 Hz, $OCH_2CH(OH)$], 5.18 [m, 1 H, $J \approx 2$, $OCH(OH)CH_2$]; mass spectrum, m/e 332 (M^+); $[\alpha]^{RT}_D$ −9.33 (5.03%, H_2O). Anal. ($C_{13}H_{24}N_4O_4S \cdot 0.5C_4H_4O_4$) C, H, N, S.

(*S*)-(−)-*N*-[4-[3-[(1,1-Dimethylethyl)amino]-2-hydroxypropoxy]-1,2,5-thiadiazol-3-yl]-*N*-(2-hydroxyethyl)glycine (3). A solution of I_2 (4.48 g, 25.5 mmol) in MeOH (45.4 mL) cooled to 0 °C was added dropwise during 11 min to aqueous 4% KOH solution (107 mL, 76.5 mmol). After 5 min this reagent was added during 17 min to **2** free base (8.9 g, 26.8 mmol) in MeOH (88 mL) maintained at 0 °C. The resulting solution was stirred for 21 min, at which point the MeOH was removed under vacuum. After addition of H_2O (300 mL) and extraction with Et_2O (2 × 30 mL), the aqueous solution was acidified with HOAc (19 mL). The opaque red mixture was extracted with Et_2O (3 × 100 mL) and the aqueous phase evaporated to dryness on a rotary evaporator. The solid residue, after pumping at high vacuum, was extracted with refluxing 2% EtOH–$CHCl_3$ (3 × 700 mL). The $CHCl_3$ solution was partially evaporated, filtered, and then taken to dryness. The crude residue was dissolved in MeOH (75 mL) and the solution partially evaporated. *i*-PrOH (40 mL) was added and the solution evaporated to 40–50 mL, at which point crystals appeared. After standing in the refrigerator, **3** (5.34 g, 57.1%), mp 193.5–194.5 °C (softening at 190 °C), was obtained on filtration. The product from four batches (21.7 g) was dissolved in MeOH (900 mL) and the solution filtered hot. To the filtrate, after evaporation to about 250 mL, was added EtOH (100 mL). The solution was reduced in volume further on the rotary evaporator until crystallization began. After the mixture was left

(13) Weinstock, L. M.; Davis, P.; Handelsman, B.; Tull, R. *J. Org. Chem.* **1967**, *32*, 2823.
(14) Falbe, J.; Schulze-Steinen, H. J.; Korte, F.; Hoerhold, K.; Mandelartz, L.; Zimmermann, D. *Chem. Ber.* **1965**, *98*, 1923.

standing overnight at ambient temperature, pure **3** (19.0 g, 87.7%) was obtained, mp 196–197 °C (dec): IR (KBr) 3320–3420, 3026, 2980, 2930, 2860, 2800–2460, 1586, 1549, 1505, 1396, 1318, 1221 cm^{-1}; UV (MeOH) λ_{max} 205 nm (ϵ 4966), 310 (9497); ^1H NMR (300 MHz, D_2O) δ 1.41 (s, 9 H, *t*-Bu), 3.13, 3.33 (each dd, 2 H, J = 13.0 and 10.2 Hz, J = 13.0 and 2.2 Hz, CH_2NH-*t*-Bu), 3.74 (m, 2 H, OCH_2CH_2N), 3.81 (m, 2 H, OCH_2CH_2N), 4.15 (s, 2 H, $^-OOCCH_2N$), 4.29 [m, 1 H, $OCH_2CH(OH)$], 4.40, 4.48 [each dd, 2 H, J = 12.0 and 5.5 Hz, J = 12.0 and 4.5 Hz, $OCH_2CH(OH)$]; mass spectrum, m/e 348 ($M^+ - CH_3$, weak), 330 ($M^+ - H_2O$), 315 ($M^+ - H_2O - CH_3$); $[\alpha]^{RT}_D$ −6.56° (5.01%, H_2O). Anal. ($C_{13}H_{24}N_4O_5S$) C, H, N, S.

***N*-[2-(3,4,5,6-Tetrahydro-2*H*-pyran-2-yloxy)ethyl]glycinamide (16).** To a solution of *N*-(2-hydroxyethyl)glycine ethyl ester hydrochloride[15] (14.13 g, 77 mmol) in alcohol-free $CHCl_3$ (275 mL) was added dihydropyran (7.11 g, 84.6 mmol) in $CHCl_3$ (30 mL). After stirring for 30 min at room temperature, ^1H NMR indicated reaction was complete. Following evaporation, concentrated NH_4OH (1.2 L) was added to the residue and the solution stirred for 20 h. After evaporation to a small volume, concentrated NH_4OH (10 mL) was added, and the mixture was extracted with $CHCl_3$ (5 × 100 mL). Evaporation of the $CHCl_3$ afforded an oil (13.7 g), which partially crystallized on standing overnight. Trituration with Et_2O gave **16** (6.7 g, 43%), mp 58–60 °C. Recrystallization (Et_2O) gave mp 60–60.5 °C. Anal. ($C_9H_{18}N_2O_3$) C, H, N.

3-Chloro-4-[(2-hydroxyethyl)amino]-1,2,5-thiadiazole (17). A mixture of 3,4-dichloro-1,2,5-thiadiazole (50 g, 0.32 mol), ethanolamine (58.9 g, 0.97 mol), and *t*-BuOH (2.4 L) was heated at reflux for 28 days under N_2. Formation of product was monitored at 310 nm. After evaporation of solvent, the residue was extracted with EtOAc (4 × 250 mL). The residue from evaporation of the EtOAc was chromatographed (300 g of silica gel, EtOAc) to give 36.3 g (61.6%) of **17**, mp 63–66 °C. Recrystallization from cyclohexane gave mp 65–66 °C. Anal. ($C_4H_6ClN_3OS$) C, H, Cl, N, S.

3-Chloro-4-[[2-(3,4,5,6-tetrahydro-2*H*-pyran-2-yloxy)ethyl]amino]-1,2,5-thiadiazole (18). A mixture of **17** (30.0 g, 0.17 mol), dihydropyran (16.0 g, 0.19 mol), *p*-toluenesulfonic acid (1.5 g), and tetrahydrofuran (1600 mL) was stirred at room temperature for 20 h. After evaporation of solvent, the residue was chromatographed (200 g of silica gel, $CHCl_3$) to give 37.4 g of **18**, mp 49–51 °C. Recrystallization from *n*-pentane gave mp 51–53 °C. Anal. ($C_9H_{14}ClN_3O_2S$) C, H, Cl, N, S.

(*S*)-(−)-1-[(1,1-Dimethylethyl)amino]-3-[[4-[(2-hydroxyethyl)amino]-1,2,5-thiadiazol-3-yl]oxy]-2-propanol (4). A solution of potassium *tert*-butoxide [prepared from potassium (7.94 g, 0.20 mol) and *t*-BuOH (246 mL)] was added to (*S*)-(−)-3-*tert*-butyl-5-(hydroxymethyl)-2-phenyloxazolidine (45.0 g, 0.19 mol).[7] After complete solution had occurred (10 min), **18** (40.8 g, 0.155 mol) was added in one portion. After being stirred at room temperature for 20 h under nitrogen, Et_2O (1 L) was added and the mixture filtered to remove unwanted solids. The filtrate (1.3 L), after washing with H_2O (3 × 250 mL), was evaporated to give an oil (77.1 g). An Et_2O (2.26 L) solution of the residue was shaken for 1 min with 0.1 N HCl (740 mL × 2) to remove unreacted oxazolidine starting material. The Et_2O phase was separated, immediately washed with 5% Na_2CO_3 solution (740 mL), and then evaporated to an oil (41.4 g). The oily residue was redissolved in Et_2O (1 L) and stirred with 1 N HCl (370 mL) for 10 min. After removal of the organic phase and washing with additional Et_2O (500 mL), the aqueous solution was heated at 50–60 °C for 15 min. When the solution cooled, 6 N NaOH solution (62 mL) was added, and the mixture was extracted with Et_2O (3 × 1300 mL). Evaporation of the Et_2O extract gave crude **4** as an oil (14.8 g). To the crude **4** in EtOH (110 mL) was added fumaric acid (2.96 g, 0.025 mol) in EtOH (110 mL). Et_2O (440 mL) was added gradually and the mixture stirred overnight. Compound **4** hemifumarate (7.9 g) was obtained on filtration. Additional product (5.2 g) was recovered from the mother liquors, giving a total of 13.1 g (23.3% overall), mp 194–196 °C. Recrystallization from boiling *i*-PrOH gave pure **4** hemifumarate (10.2 g, 18.3% overall): mp 195–197 °C; IR (KBr) 3430, 3300, 1644, 1590, 1550, 1540, 1370, 1240, 662 cm^{-1}; UV (EtOH) λ_{max} 206 nm (ϵ 17 153), 296 (10 452); ^1H NMR (300 MHz, D_2O) δ 1.40 (s, 9 H, *t*-Bu), 3.21, 3.36 (each dd, 2 H, J = 13.0 and 10.0 Hz, J = 13.0 and 3.0 Hz, CH_2NH-*t*-Bu), 3.54 (t, 2 H, J = 6 Hz, OCH_2CH_2N), 3.80 (t, 2 H, J = 6 Hz, OCH_2CH_2N), 4.36 [m, 1 H, $OCH_2CH(OH)$], 4.49, 4.58 [each dd, 2 H, J = 11.0 and 5.5 Hz, J = 11.5 and 4.0 Hz, $OCH_2CH(OH)$]; mass spectrum, m/e 290 (M^+); $[\alpha]^{RT}_D$ −5.55° (2.0%, H_2O). Anal. ($C_{13}H_{24}N_4O_5S$) C, H, N, S.

Gas Chromatographic–Mass Spectral Comparisons of Synthetic 2 and 3 with Timolol Metabolites. Combined GLC–mass spectrometry was done using an LKB-9000 instrument operated under the following conditions: 70-eV ionizing potential, 60-μA trap current, 3.5-kV accelerating potential, and 250 °C source temperature. Trimethylsilylations were effected by treating each sample (~5 μg) with ~20 μL of a 2:1 mixture of bis(trimethylsilyl)acetamide (BSA) and pyridine for 1 h at 65 °C. Methylation of **3** was carried out by treating the sample of **3** (dissolved in methanol) with an excess of an ether solution of diazomethane (prepared from Diazald). The isolation of metabolites **2** and **3** has been described.[3,4] In these experiments the source of metabolites was rat urine.

Trimethylsilylation of synthetic **3** and metabolite **3** resulted in the introduction of three $(CH_3)_3Si$ groups: mass spectrum, m/e 564 (M^+), 549 ($M^+ - CH_3$), 448 ($M^+ - CH_2CHOMe_3Si$), 363 [$M^+ - CHCH(OMe_3Si)CH_2NHC(CH_3)_3$], 246 ($m/e$ 363 − $COOMe_3Si$). GLC retention times for the two samples were identical (3.6 min). Operating conditions for the GLC determinations were as follows: a 5 ft × 3.0 mm i.d. spiral glass column; 3% OV-101 on 80–100 mesh Supelcoport; column temperature 241 °C; injection port temperature 260 °C; helium carrier gas 25 mL/min. Methylation of synthetic **3** and metabolite **3**, followed by trimethylsilylation, resulted in the introduction of two $(CH_3)_3Si$ groups and one CH_3: mass spectrum, m/e 506 (M^+), 491 ($M^+ - CH_3$), 390 ($M^+ - CH_2CHOMe_3Si$), 305 [$M^+ - CHCH(OMe_3Si)CH_2NHC(CH_3)_3$], 246 ($m/e$ 305 − $COOCH_3$). GLC retention time for the two samples, employing the above conditions, were identical (3.1 min).

Trimethylsilylation of synthetic **2** and metabolite **2** resulted in the introduction of two $(CH_3)_3Si$ groups: mass spectrum, m/e 476 (M^+), 461 ($M^+ - CH_3$), 386 ($M^+ - Me_3SiOH$), 371 ($M^+ - CH_3 - Me_3SiOH$), 360 ($M^+ - CH_2CHOMe_3Si$), 275 [$M^+ - CHCH(OMe_3Si)CH_2NHC(CH_3)_3$]. GLC retention times for the two samples were identical (4.2 min). The operating conditions were the same as above, except that a 6 ft × 3.0 mm i.d. spiral column, with 3% SP2100 on 80–100 mesh Supelcoport, was used at a temperature of 215 °C. Detailed mass spectra of Me_3Si derivatives of **2** and **3** are included as supplementary material.

Differential β-Adrenergic Blocking Activity in Anesthetized Dogs. Dogs of either sex in the weight range of 5–10 kg were anesthetized with vinbarbital, 60 mg/kg iv supplemented with 20 mg/kg im, and succinylcholine chloride, 2 mg/kg im. All dogs were bilaterally vagotomized and artificially ventilated at a rate of 22 breaths per minute, with volume adjusted to provide an intrapulmonary pressure of 15 cm of water. Arterial pressure was monitored from a cannulated femoral artery, heart rate from bilateral needle chest electrodes through a Beckman cardiotachometer, and intrapulmonary pressure from a pressure transducer inserted into the respiratory system. All parameters were recorded on a Beckman dynograph, and injections were made into a cannulated femoral vein.

In four drug vehicle (1 N saline) control preparations, bronchoconstriction was induced with histamine (base), 10 μg/kg iv administered at 10-min intervals, and recorded as millimeters of pen excursion over base line. When these responses had stabilized, isoproterenol (base), 0.4 μg/kg iv, was administered 30 s prior to each histamine challenge. The fall in blood pressure and increase in heart rate from base levels were recorded in mmHg and beats per minute, respectively, and inhibition of histamine-induced bronchoconstriction was calculated as a percentage of control responses.

When the three recorded responses to isoproterenol (vasodepression, tachycardia, and inhibition of histamine bronchoconstriction) were consistent, within limits, 1 N saline, 0.1 mL/kg iv, was injected 3 min before each isoproterenol administration over a period of 90 min. Any changes in the recorded isoproterenol responses were calculated as a percentage of control responses.

(15) Jones, E. R. H.; Wilson, W. *J. Chem. Soc.* **1949**, 547.

For evaluation of test drugs, dogs were prepared as described above. Cumulative drug doses were administered intravenously 3 min prior to each standard dose of isoproterenol, as described for the drug vehicle control experiments, until graded inhibition of the isoproterenol responses was achieved for all parameters, where possible, within a reasonable drug dose range. Percent inhibition (isoproterenol controls = 100) to drug dose was used to compute the drug ED_{50} for each parameter. All drug solutions were made in 1 N saline using base weights and injected in volumes of 0.1 mL/kg iv or less.

Acknowledgment. We wish to acknowledge the encouragement and helpful suggestions of Dr. E. L. Engelhardt during the chemical synthesis portion of this work. The authors express their appreciation to Mr. S.-C. Ho for the ^{13}C NMR spectra, to Dr. D. W. Cochran for useful discussions concerning the interpretation of NMR spectra, and to Dr. P. C. Bélanger for the supply of compound **6** used in this work.

Supplementary Material Available: ^{13}C NMR spectral data for **1–4, 5, 6, 8, 9,** and **17** (Table II) and mass spectra of the Me_3Si derivatives of synthetic **2** and **3** and of metabolites **2** and **3** (Figures 1–4) (6 pages). Ordering information is given on any current masthead page.

Glycolipids as Potential Immunologic Adjuvants

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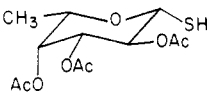
A group of 1-thio-β-L-fucopyranosides containing hexadecane, 9-octadecene, adamantane, 1,2-diphenyltetrafluoroethane, and 3-hexynyl- and 3,6-dioxaoctylcholesterols were synthesized as potential immunologic adjuvants. Many of these fucosyl lipids and 6-(5-cholesten-3β-yloxy)hexyl 1-thioglycosides were found to give good response to subunit A/Victoria influenza virus. Carbohydrates with L-fucose and D-galactose backbones appeared essential for adjuvant activity. Lactose which has a terminal D-galactose moiety was found to be active, whereas L-arabinose which lacks a 5-(hydroxymethyl) group was inactive.

Bacterial glycolipids are known to have adjuvant activity; e.g., trehalose mycolate[1] is active as an adjuvant for the induction of delayed-type hypersensitivity to bovine serum albumin in guinea pigs. However, this glycolipid was shown to be highly toxic in mice ($LD_{50} \simeq 1.5$ mg/kg). The adjuvant activities of various bacterial glycolipids containing α-branched β-hydroxy fatty acids and mono- or disaccharides, such as L-rhamnose, D-glucose, fructose, and sucrose, were also reported.[1] Synthetic glycolipids such as N-acylated D-glucosamine derivatives[2] were shown to have adjuvant activity in the immune response to human γ-globulin and sheep red blood cells (SRBC) in mice. Esters prepared from palmitoyl chloride and maltose, cellobiose, D-galactose, or L-arabinose were reported[3] to be mitogenic for spleen lymphocytes of Wistar rats, Swiss mice, and nude mice. These active glycolipids also enhanced antibody production against SRBC in Wistar rats and Swiss mice.[3] In this paper, we report the synthesis and biological evaluation of a group of novel 1-thio-β-L-fucopyranosyl lipids as potential immunologic adjuvants. The saccharide determinant L-fucose was chosen for its involvement in many cell-membrane functions.[4] The lipid moiety was derived from saturated and unsaturated aliphatics, adamantane, 1,2-diphenyltetrafluoroethane, and 3-hexynyl- and 3,6-dioxaoctylcholesterols which have different stereochemical, electronic, and in vivo distribution characteristics.

Chemistry. The 1-thio-β-L-fucopyranosides **2–7** were synthesized in good yields from 2,3,4-tri-*O*-acetyl-1-thio-β-L-fucopyranose[5] (**1**) and 1-bromohexadecane, oleyl iodide, 2-(1-adamantyl)ethyl iodide, 3-[(*p*-tetrafluorophenethyl)-phenyl]propyl iodide (**13**), 8-(5-cholesten-3β-yloxy)-3,6-dioxaoctyl iodide (**17**), and 6-(5-cholesten-3β-yloxy)-3-hexyne iodide (**25**), respectively, in dichloromethane containing triethylamine or in dry tetrahydrofuran using

Chart I[a]



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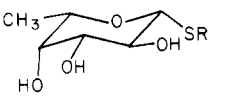
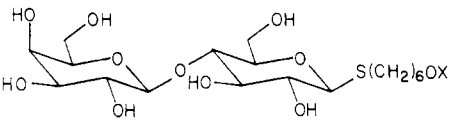


2, R = $(CH_2)_{15}CH_3$
3, R = $(CH_2)_8CH{=}CH(CH_2)_7CH_3$

4, R = CH_2CH_2

5, R = $(CH_2)_3$-C_6H_4-CF_2CF_2-C_6H_5
6, R = $(CH_2CH_2O)_3X$
7, R = $(CH_2)_2C{\equiv}C(CH_2)_2OX$
8, R = $(CH_2)_6OX$



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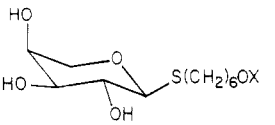

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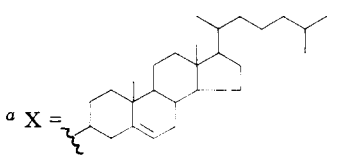

[a] X =

(1) I. Azuma, K. Sugimura, S. Itoh, and Y. Yamamura, *Jpn. J. Microbiol.*, **20**, 465 (1976).
(2) U. H. Behling, B. Campbell, C. M. Change, C. Rumpf, and A. Nowotny, *J. Immunol.*, **117**, 847 (1976); Girpi Inst. Rech. Pro., German Patent, 2708–667 (1978).
(3) V. N. Nigam and C. A. Brailovsky, "Abstracts of Papers", The Am. Soc. for Cell Bio., 17th Annual Meeting, San Diego, Calif., Nov 15–18, abstr CI-99a (1977); *Cancer Res.*, **38**, 3315 (1978).
(4) J. M Mckibbin, *J. Lipid Res.*, **19**, 131 (1978).
(5) K. L. Matta, R. N. Girotra, and J. J. Barlow, *Carbohydr. Res.*, **43**, 101 (1975).

0022-2623/80/1823-1184$01.00/0