crystallized again from the same solvents to give pure **13** (4.6 g, 37%), mp 311–320° dec. *Anal.* ($C_{34}H_{50}O_7$) C, H.

2β,3β-Dihydroxy-11-oxooleana-12,18-dien-30-oic Acid (14). **13** (11.2 g, 19.6 mmol) was treated as described for **7** through the decolorization step. After this, the decolorized solution was evaporated to a gum, which was dissolved in 0.5 *N* KOH–MeOH (100 ml) and refluxed 25 min. This reaction mixture was cooled, diluted to 1.1 l. (H_2O), acidified (4 *N* HCl), and extracted twice with EtOAc–Et_2O. The combined organic extracts were washed with H_2O, dilute brine, and saturated brine, decolorized, and evaporated to a solid which was crystallized (EtOH) to give three crops (2.93 g, 30%); mp 229–232°. *Anal.* ($C_{30}H_{44}O_5$) C, H.

Methyl 2α,3α-Dihydroxy-11-oxo-18β-olean-12-en-30-oate (16). **15**[2] (1 g, 2.15 mmol) was dissolved in 16 ml of a Py solution of OsO_4 (0.2 *M*) and heated on a steam bath for 70 min. The reaction mixture was allowed to stand overnight at room temperature, and then a solution of $NaHSO_3$ (0.1 g, 10 mmol) in H_2O (20 ml) was added.[10] The mixture was warmed on a steam bath for 10 min, cooled, and extracted twice with Et_2O. The combined organic phases were washed with 0.6 *N* HCl, H_2O, and then dilute K_2CO_3 solution, dried ($MgSO_4$), and evaporated to a gum. The gum was triturated with pentane to give 0.72 g of a solid which was crystallized (EtOH), giving 239 mg (22%) pure **16**: mp 238–239°; nmr (100 MHz, C_5D_5N) $J_{C(2)\text{-}H\text{-}C(3)\text{-}H}$ = 2.8 Hz; $J_{C(2)\text{-}H\text{-}C(1\beta)\text{-}H}$ = 4 Hz; $J_{C(2)\text{-}H\text{-}C(1\alpha)\text{-}H}$ = 12 Hz. *Anal.* ($C_{31}H_{48}O_5$) C, H. Assuming that the OsO_4 oxidation is a cis hydroxylation, and assuming that ring A is in a chair conformation, C_2H must be axial, because the large (12 Hz) *J* value cannot be derived from $J_{C(2)\text{-}H\text{-}C(3)\text{-}H}$ and thus must be from $J_{C(2)\text{-}H\text{-}C(1\alpha)\text{-}H}$. If C_2H is axial, C_2OH must be equatorial (α) and therefore C_3OH, being cis to C_2OH, must also be α, or axial. This calls for equatorial conformation for C_3H, and this is verified by the low $J_{C(2)\text{-}H\text{-}C(3)\text{-}H}$. See the preparation of compound **17** for decoupling experiments in a similar system.

Methyl 2β,3β-Dihydroxy-18β-olean-12-en-30-oate (17). **15**[2] (1.61 g) was treated as directly above again yielding a gum. This gum was hydrogenolyzed as in the preparation of **2**. The hydrogenolysis was repeated until tlc (7:3 PhH–EtOAc) showed very little unhydrogenolyzed material to be left. The material was then applied to a CC-7 column. Elution with 95:5 PhH–EtOAc gave **17** (634 mg, 38%), and a continuation of the column gave **18** (*vide infra*). **17** was crystallized from CH_2Cl_2–MeOH: mp 230–233° *Anal.* ($C_{31}H_{50}O_4$) C, H. Nmr (100 MHz, C_5D_5N): the C_2H is a quartet with a total width of 10 Hz. This *J* value is too small for an axial proton, so C_2H must be equatorial (α), meaning C_2OH must be β. C_3OH must thus also be β. To verify the assignment of peaks, the C_3H signal was irradiated, causing decoupling with the C_2H signal, which collapsed to a triplet (due to $C_{1\alpha}H$ and $C_{1\beta}H$ interactions with C_2H) as expected.

Methyl 2α,3α-Dihydroxy-18β-olean-12-en-30-oate (18). Continued elution of the column described directly above gave **18** with 9:1 PhH–EtOAc. **18** (310 mg, 18%) resulted and was crystallized (CH_2Cl_2–MeOH); mp 270–271° *Anal.* ($C_{31}H_{50}O_4$) C, H (structure by elimination).

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Synthesis of Tritium-Labeled Chlorambucil and Aniline Mustard of High Specific Activity

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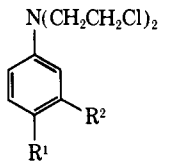
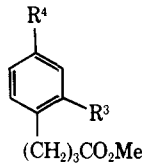
Two procedures are described for the synthesis of 4-[4-bis(2-chloroethyl)amino-2-iodophenyl]butyric acid, a potential precursor to tritium-labeled chlorambucil. The longer synthetic route involves the stepwise elaboration of the two nitro groups in methyl 4-(2,4-dinitrophenyl)butyrate and defines the meta orientation of the iodine substituent with respect to the alkylating function. An alternative, single stage procedure involves the direct iodination of chlorambucil, using iodine in oleum (20% SO_3). This reagent also converted aniline mustard and melphalan into analogous iodo derivatives, *N,N*-bis(2-chloroethyl)-3-iodoaniline and 3-[4-bis(2-chloroethyl)amino-2-iodophenyl]-L-alanine, respectively. The reductive tritiation of the iodo derivatives of chlorambucil and aniline mustard yielded the appropriate ^3H-labeled drugs with specific activities respectively of 8.3 and 20.3 Ci/mmol. The percentages of the ^3H-labeled compound present in admixture with the unlabeled analog were independently estimated in each case by mass spectrometry.

Growing interest in the mechanism of the antineoplastic action of *N,N*-bis(2-chloroethyl)arylamines[1-3] has led to a need for radioactively labeled drugs of this class. ^3H-Labeled aniline mustard [*N,N*-bis(2-chloroethyl)aniline, **1**],[4] melphalan [3-[4-bis(2-chloroethyl)aminophenyl]-L-alanine, **2**],[5] and chlorambucil [4-[4-bis(2-chloroethyl)aminophenyl]butyric acid, **3**][1] have been synthesized by catalyzed tritium–halogen exchange of suitably iodinated derivatives. Only **1** has hitherto been obtained with high specific activity, and this fact has been attributed[4] to the meta relationship between the iodo and alkylating substituents in the precursor to ^3H-labeled **1**. An ortho relationship, such as existed in the precursors of **2** and **3**, was believed to account for most of the loss of radioactivity during the acidic conditions used for the isolation of ^3H-labeled **2**[5] and **3**.[1]

The objectives of the present investigation were the synthesis of ^3H-labeled chlorambucil of higher specific ac-

tivity than hitherto obtained and the development of a potentially general approach to the synthesis of ^{3}H-labeled *N,N*-bis(2-chloroethyl)arylamines of high specific activity. The previously mentioned synthesis of ^{3}H-labeled aniline mustard[4] was not generally applicable, since it depended upon the lack of a para substituent in **1**.





1, $R^1 = R^2 = H$
2, $R^1 = CH_2CH(NH_2)CO_2H$; $R^2 = H$
3, $R^1 = (CH_2)_3CO_2H$; $R^2 = H$
9, $R^1 = (CH_2)_3CO_2H$; $R^2 = I$
10, $R^1 = H$; $R^2 = I$
11, $R^1 = CH_2CH(NH_2)CO_2H$; $R^2 = I$

4, $R^3 = R^4 = NO_2$
5, $R^3 = NO_2$; $R^4 = NH_2$
6, $R^3 = NH_2$; $R^4 = NO_2$
7, $R^3 = I$; $R^4 = NO_2$
8, $R^3 = I$; $R^4 = NH_2$

A direct route to suitably iodinated precursors was based on the observation that a solution of iodine in oleum (20% SO_3) can iodinate an aromatic nucleus at sites meta to electron-withdrawing substituents.[6] Chlorambucil, which resisted iodination by milder reagents, such as iodine monochloride in concentrated HCl, was thereby converted into a monoiodo derivative. The relative orientation of the iodo and alkylating substituents in this product was established by comparing it with the product from a lengthier synthesis, in which the desired meta relationship between these substituents was obtained by the separate elaboration of the two nitro groups in compound **4**. The partial reduction of **4** gave the aminonitro derivatives **5** and **6**. The isomer **6** was converted *via* the intermediates **7** and **8** into the iodochlorambucil **9**, identical with the product obtained from the single stage procedure. Catalytic hydrogenation of either product afforded chlorambucil.

The reductive tritiation of the iodo derivative **9** gave labeled chlorambucil of specific activity 8.3 Ci/mmol, or 29% of the theoretical value, which represents a nearly threefold improvement on the incorporation obtained with an ortho-substituted precursor.[1] To prevent radiolytic decomposition, the chromatographically purified product was not crystallized, since even at a lower activity (586 mCi/mmol[2]) rapid darkening of crystals occurred whereas ^{3}H-labeled aniline mustard of high specific activity appeared to decompose rapidly in the solid state.[4]

Mass spectrometry provided a useful check on the chemical purity of the labeled chlorambucil, as well as providing an independent value for the percentage of the ^{3}H species present. Thus, no signals were present at *m/e* 429/431 (^{35}Cl- and ^{37}Cl-containing species) for the molecular ion of the iodo derivative **9**, showing that exchange of the iodine substituent was complete. The contribution of the tritiated species (mol wt 305/307) to the molecular ion complex (*m/e* 303/305/307) was estimated by subtracting the signals due to the molecular ion of the unlabeled derivative (see Figure 1). The content of ^{3}H-chlorambucil thus estimated, 31% of the total chlorambucil present, agreed well with the value conventionally obtained by scintillation counting.

Aniline mustard (**1**) and melphalan (**2**) also afforded monoiodo derivatives **10** and **11**, respectively, when treated with iodine in oleum, and these could be catalytically reduced to the parent drugs.

The catalyzed tritium–halogen exchange reaction, applied to the iodinated aniline mustard derivative **10**, yielded labeled aniline mustard of specific activity 20.3 Ci/mmol (70% of the theoretical value). The higher incorporation of tritium, compared with that in labeled chlorambucil, may reflect the absence in the aniline mustard structure of a labile proton, such as is present in the carboxyl group of chlorambucil (for a discussion of this point, see ref 5). The mass spectrum of tritiated aniline mustard similarly lacked signals appropriate to the molecular ion of the iodo derivative **10** at *m/e* 343/345. The molecular ion complex (*m/e* 217/219/221) likewise afforded an independent estimate (65%) of the percentage of the ^{3}H derivative present in the mixture (see Figure 1).

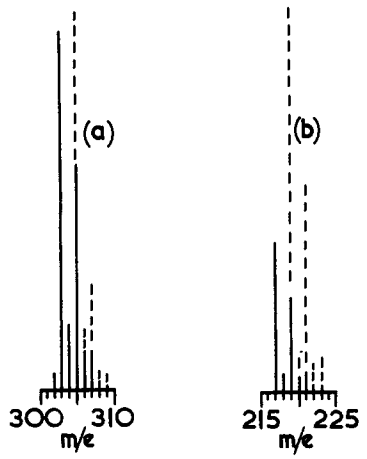


Figure 1. Molecular ion components in the mass spectra of (a) tritium-labeled chlorambucil and (b) tritium-labeled aniline mustard. Solid lines represent intensities for the unlabeled compounds; dotted lines represent the additional contribution due to the ^{3}H derivatives.

Although iodomelphalan (**11**) was smoothly converted into melphalan (**2**) by catalytic reduction in methanol, this solvent could not be used for the synthesis of [^{3}H]melphalan, since extensive radiolytic decomposition occurred therein. The polar nature of the melphalan precludes the use of solvents favorable to the stability of highly radioactive materials, and the problems presented thereby still await solution.

In summary, the direct iodination procedure is likely to be widely applicable to the preparation of potential precursors to tritiated aromatic nitrogen mustards of high specific activity and should replace the lengthier, individual synthetic routes hitherto employed.

Experimental Section†

Methyl 4-(2,4-Dinitrophenyl)butyrate (4). 4-(2,4-Dinitrophenyl)butyric acid[7] (18 g, 71 mmol) was converted into its methyl ester (19 g, 100%), bp 172–174° at 0.5 mm, by the procedure previously applied[7] to the 4-nitro analog. *Anal.* ($C_{11}H_{12}N_2O_6$) C, H, N.

Methyl 4-(4-Amino-2-nitrophenyl)butyrate (5) and Methyl 4-(2-Amino-4-nitrophenyl)butyrate (6). A mixture of methyl 4-(2,4-dinitrophenyl)butyrate (4, 20 g, 75 mmol) and 5% Pd/C (1 g) was stirred under H_2 at room temperature until 5 l. of gas was consumed (*ca.* 4 days). PhH (50 ml) was added and the filtered solution was applied to a column of silicic acid (300 g, 45 cm × 15 cm[2]) which was eluted with PhH. Starting material (2.6 g) eluted in 4 l.; after a further 1 l., the 2-amino-4-nitro derivative **6** eluted in 6 l. Recrystallization of the solid concentrate from PhMe gave orange granules (2.5 g, 14%), mp 94–95°. The uv spectrum (in EtOH) showed λ_{max} 230, 250, and 288 nm. 4-Nitro-o-toluidine[8] exhibited the same maxima. *Anal.* ($C_{11}H_{14}N_2O_4$) C, H, N. A further 1.5 l. of mixed fractions from the column contained **6**, together with the 4-amino-2-nitro analog **5** which was eluted pure with a further 10 l. of PhH, followed by 1 l. of *i*-Pr_2O. Recrystalli-

† Melting points, which were corrected, were determined with a Kofler hot-stage apparatus. Merck Kieselgel G was used for column chromatography and for thin-layer chromatography (tlc) on coated glass plates (20 × 5 cm). For nmr spectra, a Perkin-Elmer R-10 spectrometer operating at 60 MHz was used, and for uv spectra, a Unicam SP-800 spectrometer. Mass spectra were determined by the direct insertion method with an ionizing voltage of 70 eV and ion-source temperature of 120°.

zation of the solid concentrate from cyclohexane–PhMe (1:2, 78 ml) gave orange granules (3.8 g, 21%), mp 45–48°. The uv spectrum contained a single maximum (λ_{max} 240 nm). 2-Nitro-*p*-toluidine[9] similarly exhibited a single maximum (λ_{max} 238 nm). *Anal.* ($C_{11}H_{14}N_2O_4$) C, H, N.

Methyl 4-(2-Iodo-4-nitrophenyl)butyrate (7). 4-(2-Amino-4-nitrophenyl)butyrate (**6**, 3 g, 12.6 mmol) was converted into the corresponding iodonitro derivative by conventional reaction with acidic $NaNO_2$ followed by treatment with aqueous KI. A solution in PhH of the crude product was applied to a column of silicic acid (35 g, 20 cm × 4.5 cm²) which was eluted with PhH (600 ml). Crystallization of the concentrate from petroleum ether (bp 60–80°, 100 ml) gave the required product as colorless needles (2.6 g, 61%), mp 52–53°. *Anal.* ($C_{11}H_{12}INO_4$) C, H, N.

Methyl 4-(4-Amino-2-iodophenyl)butyrate (8). A solution of methyl 4-(2-iodo-4-nitrophenyl)butyrate (**7**, 2.35 g, 7 mmol) in cyclohexane (100 ml) containing Adams PtO_2 catalyst (0.3 g) (*cf.* ref 10 and 11) and anhydrous $MgSO_4$ (0.5 g) to prevent adhesion of catalyst to the liberated water was stirred under H_2 at room temperature for 1 hr, when 500 ml was consumed. The filtered solution was concentrated and a solution of the residue in PhH was applied to a column of silicic acid (40 g, 25 cm × 4.5 cm²) which was eluted with PhH (10-ml fractions). Fractions 15–40 contained starting material (0.085 g). Fractions 80–150 were combined with a subsequent $CHCl_3$ eluate (260 ml) and the concentrate was crystallized from cyclohexane (20 ml) to give the required product as colorless needles (0.95 g, 43%), mp 46–47°. *Anal.* ($C_{11}H_{14}INO_2$) C, H, I, N.

4-[4-Bis(2-chloroethyl)amino-2-iodophenyl]butyric Acid (9). (**a**) A solution of methyl 4-(4-amino-2-iodophenyl)butyrate (**8**, 0.96 g, 3 mmol) and ethylene oxide (2 g) in glacial AcOH–water (9:1 v/v, 10 ml) was left at room temperature for 3 hr. The solution was concentrated under reduced pressure and the residue partitioned between PhH (100 ml) and saturated aqueous $NaHCO_3$ (100 ml). The organic phase was dried ($MgSO_4$) and 20 ml of PhH removed by distillation. The remainder was refluxed with $POCl_3$ (4 ml) for 1 hr and then, after further concentration, for 2 hr with *n*-$Bu_4N^+OH^-$ in MeOH–PhH (0.1 *M*, 20 ml). The solution was concentrated and the residue partitioned between PhH (20 ml) and HCl (1 *N*, 20 ml). The dried ($MgSO_4$) organic phase was applied to a column of silicic acid (30 g, 25 cm × 4.5 cm²) which was eluted with $CHCl_3$ (10-ml fractions). On crystallization of the concentrate from fractions 26–75 from petroleum ether (bp 80–100°)–PhMe (1:2, 5 ml), the required product formed colorless granules (0.56 g, 43%), mp 98–100°. *Anal.* ($C_{14}H_{18}Cl_2INO_2$) C, H, Cl, I, N.

(**b**) Chlorambucil[7] (**3**, 1 g, 3.3 mmol) was added to the mixture formed by stirring I_2 (2 g) in 20% oleum (10 ml) for 1 hr. The mixture was stirred for a further 1 hr and then poured slowly into stirred ice–water (100 ml). $CHCl_3$ (50 ml) was added and the mixture stirred vigorously with passage of SO_2 until all solid (I_2) had dissolved. The pale red organic phase was dried ($MgSO_4$) and a solution of the concentrate in PhH was applied to a column of silicic acid (30 g, 16 cm × 4.5 cm²) and eluted with PhH (500 ml) and then with petroleum ether (bp 30–40°)–ether (1:1, 500 ml). On crystallization of the concentrate from the latter solvent (10 ml), the required product formed colorless needles (0.3 g, 22%), mp and mmp (with the product from method a) 98–100°. The nmr spectra (in $CDCl_3$) of the products from the two reactions were also identical. *Anal.* C, H, Cl, I, N.

4-[4-Bis(2-chloroethyl)aminophenyl]butyric Acid (3). A solution of 4-[4-bis(2-chloroethyl)amino-2-iodophenyl]butyric acid (**9**, 0.096 g, 0.22 mmol) in dry dioxane (25 ml) containing Et_3N (0.15 ml), 5% Pd/C (0.15 g), and Adams PtO_2 catalyst (0.005 g) was stirred, in the dark, under H_2 overnight at room temperature. The catalyst was filtered off (Hyflo) and the concentrated filtrate was partitioned between $CHCl_3$ (10 ml) and HCl (0.1 *N*, 10 ml). The dried ($MgSO_4$) organic phase was applied to a column of silicic acid (7 g, 10 cm × 0.75 cm²) and eluted with $CHCl_3$ (10-ml fractions). On crystallization from petroleum ether (bp 30–40°)–PhMe (1:1, 1 ml) of the concentrate from fractions 4–13, colorless needles (0.05 g, 75%) separated, mp and mmp (with authentic chlorambucil[7]) 64–66°. The nmr spectrum ($CDCl_3$, 10% w/v solution) was also identical. The aromatic H signals form an AB quartet, the asymmetrical doublet (separation 9 Hz) components centered on τ 2.84 and 3.31.

Reductive Tritiation of 4-[4-Bis(2-chloroethyl)amino-2-iodophenyl]butyric Acid. Preparation of 4-[4-Bis(2-chloroethyl)aminophenyl-2-*t*]butyric Acid. The foregoing hydrogenation conditions and reagent quantities were used for reduction by $^3H_2$, except that 0.31 g (0.72 mmol) of the iodo derivative **9** was employed. After removal of catalyst, the filtrate was diluted with EtOAc (10 ml) and washed with HCl (1 *N*, 10 ml) and water (10 ml). The dried ($MgSO_4$) organic phase was diluted to 100 ml with EtOAc before despatch (see Acknowledgments). On receipt, the solution was concentrated and then applied to a column of silicic acid (20 × 3 cm²) which was eluted with EtOAc (200 ml). The eluate was concentrated and the residue dissolved in PhH (50 ml) and stored at room temperature in the dark.

Yield, Chemical and Radiochemical Purity, Specific Activity, and Stability of Tritiated Chlorambucil. To estimate the yield of tritiated chlorambucil, 0.5 ml of the stock PhH solution was diluted to 50 ml with PhH to give solution A. Solution A (5 ml) was concentrated and the residue reconcentrated with 95% EtOH (3 × 5 ml) before dissolution in this solvent (10 ml). The quantitative uv spectrum of this solution was compared with that of a standard solution of (unlabeled) chlorambucil containing 0.015 mg/ml, which gave an absorbance A_{258} of 0.98 in a 1-cm cell. The yield was 0.173 g (80%).

A sample of the stock PhH solution was subjected to tlc alongside authentic chlorambucil, with $CHCl_3$–MeOH (25:3) as developing solvent, using plates coated with fluorescent Kieselgel (GF_{254}). A single uv detectable spot was observed at the R_f value of the authentic material. For estimation of the radiochemical purity, 1 ml of the stock solution was diluted with PhH to 10 ml to give solution B. Aliquots (10 μl) of solution B were subjected to tlc as above. After development, the silicic acid was removed at 1-cm intervals into HCl (0.1 *N*, 0.5 ml) and then diluted with phosphor (10 ml) and assayed by scintillation counting. The radioactivity (92%) occurred in the region corresponding to the authentic material. After storage of the original stock PhH solution for 9 months in the dark at 20°, the radiochemical purity had fallen to 82% of the initial value. For the determination of the specific activity (8.3 Ci/mmol), 1 ml of the solution B was further diluted with PhH to 200 ml, and 1-ml aliquots were used for scintillation counting.

***N,N*-Bis(2-chloroethyl)-3-iodoaniline (10).** The title compound was prepared from aniline mustard (**1**, 1 g, 4.6 mmol) using the reagent quantities and reaction conditions described in method b above for the synthesis of iodochlorambucil (**9**). For column chromatography, petroleum ether (bp 40–60°) was the eluent. From this solvent, the required product gave white prisms (0.3 g, 16%), mp 39–40°. *Anal.* ($C_{10}H_{12}Cl_2IN$) H, Cl, I, N; C: calcd, 34.9; found, 34.3.

Reductive Tritiation of *N,N*-Bis(2-chloroethyl)-3-iodoaniline. Preparation of *N,N*-Bis(2-chloroethyl)aniline-3-*t*. The iodoaniline **10** (0.343 g, 1 mmol) was reduced with $^3H_2$ using the same reaction and isolation conditions and reagent quantities described above for the reductive tritiation of iodochlorambucil (**9**), except that PhH was the eluent for column chromatography of the product. The same methods were used to estimate the yield (0.144 g, 66%), chemical and radiochemical purity (93%), and specific activity (20.3 Ci/mmol), except that the developing solvent for tlc was petroleum ether (bp 60–80°)–PhH (1:1).

3-[4-Bis(2-chloroethyl)amino-2-iodophenyl]-L-alanine (11). The title compound was prepared from melphalan[12] (**2**, 1 g, 3.3 mmol) using the reagent quantities and reaction conditions described for iodochlorambucil (**9**, method b). After the passage of SO_2, the solution was neutralized ($NaHCO_3$). The precipitated solid was recovered by filtration, dissolved in AcOH, and then reprecipitated with saturated aqueous NaOAc, and the resulting solid was recrystallized from *n*-BuOH saturated with H_2O to give the required product (0.45 g, 32%) as white, microcrystalline needles, mp 203–204°. *Anal.* ($C_{13}H_{17}Cl_2IN_2O_2$) C, H, Cl, N; I: calcd, 29.45; found, 29.0.

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1,4-Benzodioxanes as Reversible and Irreversible Antagonists at Adrenergic Receptors

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A series of 1,4-benzodioxanes has been synthesized and examined for activity at adrenergic receptors. The prototype of this series is 2-*N*,*N*-diethylaminomethyl-1,4-benzodioxane (I). The corresponding *N*-2-chloroethyl derivative was found to be an irreversible α-receptor antagonist, and this irreversible activity was maintained or abolished by the 6- and 7-$MeSO_2NH$ substituents, respectively. I, its 6- and 7-$MeSO_2NH$ derivatives and related nonalkylating compounds showed competitive or potentiating activity toward norepinephrine (NE) in the rat and guinea pig vas deferens. I was the most active competitive antagonist. Additionally, I and some analogs increased the maximum contractile response to NE. Since a similar effect was also exerted by certain compounds on carbachol-induced responses in the guinea-pig vas deferens, it is unlikely that this augmenting effect is exerted at the receptor level and it is speculated that it may be related to increased Ca^{2+} mobilization in the E–C coupling process.

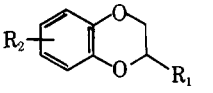
Our continuing concern with structure–activity relationships of ligands active at adrenergic receptors[1,2] and of the mechanisms by which ligand-receptor interactions are linked to the physiological response[3,4] has prompted us to examine a series of 1,4-benzodioxanes. These were chosen because this structure includes well-known α-receptor antagonists as Prosympal (I). However, as with many other α-receptor antagonists the structural relationship of I and its analogs to norepinephrine is far from clear[5] and it appeared of interest to determine whether I could, like Dibenamine and related 2-halogenoethylamine antagonists, be exhibiting its antagonism, in whole or in part, by interacting at a site other than the norepinephrine recognition site of the α receptor.[2,3,6]

To pursue this aim we have synthesized a number of potential irreversibly acting analogs of I. Additionally, we have attempted to determine the binding requirements of the benzodioxane antagonists relative to the catecholamine agonists at the α receptor. Methanesulfonamido derivatives both of I and its alkylating analog have been prepared and examined to determine whether this substitution produces effects in these compounds that parallel the effects in the substituted phenylethanolamines.[5,7,8]

Results and Discussion

A number of the compounds prepared exhibited irreversible α-adrenergic receptor antagonism in the rat vas deferens (Table I) and this was of long duration. This activity is, however, substantially lower than that of phenoxybenzamine, one of the best known of this class of antagonists.[5,9,10] Despite this limitation it is of obvious interest that 2-*N*-ethyl-*N*-(2-chloroethyl)aminomethyl-6-methanesulfonamido-1,4-benzodioxane (VIII) and not the corresponding 7-substituted isomer VI produces irreversible antagonism (Table I); this finding is highly reminescent of the work of Larsen, *et al.*,[7,8] showing a similar de-

Table I. Duration of Irreversible Blockade in Rat Vas Deferens α-Receptor Preparation



No.	R_1	R_2	Block (90–100%)	$t_{1/2}$,[a] min	Blocking concn (M)/ time, min	$t_{1/2}$ (DMPEA),[b] min
II	$CH_2N(C_2H_5)C_2H_4Cl$	H	Yes	337 ± 40	10^{-4}/12	37 ± 5
III	H	6-$CH_2N(C_2H_5)C_2H_4Cl$	Yes	310 ± 30	10^{-4}/11	45 ± 5
VIII	$CH_2N(C_2H_5)C_2H_4Cl$	6-$NHSO_2CH_3$	Yes	250 ± 20	10^{-4}/11	48 ± 7
VI	$CH_2N(C_2H_5)C_2H_4Cl$	7-$NHSO_2CH_3$	No		10^{-4}/15	
X	H	6-$CHClCH_2N(CH_3)_2$	No		10^{-4}/15	

[a] $t_{1/2}$ ± S.E.M. There was no significant difference between these values as determined by Student's t test at $p < 0.01$. [b] $t_{1/2}$ following tissue pretreatment with DMPEA (10^{-6} M, 5 min). This concentration neither affected the NE dose-response curve nor changed the initial degree of blockade produced by the benzodioxane antagonist. There was no significant difference between these values at the $p = 0.01$ level.