Water-Soluble Derivatives of 3-Oxy-substituted 1,4-Benzodiazepines

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Abstract □ Several acid addition salts of amino ester derivatives of 3-oxy-substituted 1,4-benzodiazepines were prepared and tested for water solubility, stability, and pH. The monomethanesulfonic acid salts were the most suitable for parenteral administration.

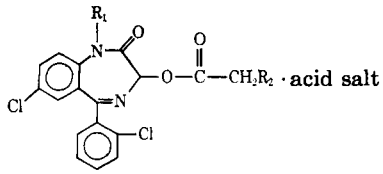
Keyphrases □ 1,4-Benzodiazepines, 3-oxy-substituted—water-soluble derivatives synthesized and tested for water solubility, stability, and pH □ Oxazepam—synthesis of aminoacetyl derivatives, tested for water solubility, stability, and pH □ Lorazepam—synthesis of aminoacetyl derivatives, tested for water solubility, stability, and pH □ Acid salts—synthesis of water-soluble derivatives of 3-oxy-substituted 1,4-benzodiazepines, tested for water solubility, stability, and pH

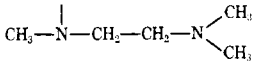
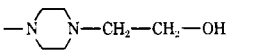
Recent efforts (1) in the area of 3-hydroxy-1,4-benzodiazepines have been directed to the preparation of water-soluble derivatives suitable for parenteral administration. Aqueous 1,4-benzodiazepine solutions would be expected to have the distinct advantage of diminishing the incidence of pain and the subsequent venous inflammation often encountered with injectable formulations of water-insoluble 1,4-benzodiazepines.

RESULTS AND DISCUSSION

The approach involved the preparation of derivatives of two known 3-hydroxy-1,4-benzodiazepines, oxazepam and lorazepam (I), whose pharmacological properties have been established and shown to be useful. The hydroxy groups used in the formation of the derivatives lent themselves to the formation of amino ester salts, which *in vivo* presumably hydrolyze to the starting 3-hydroxy-1,4-benzodiazepines. A number of derivatives that showed a range of water solubilities are listed in Table I.

Table I—Water-Soluble Derivatives of



Compound	R_1	R_2	Acid Salt
III	H		HCl
IV	CH_3		HCl
V (*a–d*)	H		2HCl[a], HCl[b], CH_3SO_3H[c], maleate[d]
VI (*a–c*)	CH_3		2HCl[a], HCl[b], CH_3SO_3H[c]
VII	H		2HCl
VIII	H		2HCl

The morpholino derivatives III and IV had somewhat lower solubilities and tended to hydrolyze rather readily. The diamine derivatives V–VIII as hydrochlorides showed considerably higher solubilities but had the disadvantage of forming very acidic aqueous solutions. This undesirable acidity was reduced to a large extent by the preparation of the monomethanesulfonic acid salts (Table II).

Table II—pH of Aqueous Solution of V at 5 mg/ml Concentration

Salt	pH
2HCl	2.3
HCl	3.7
CH_3SO_3H	4.9
Maleate	4.3

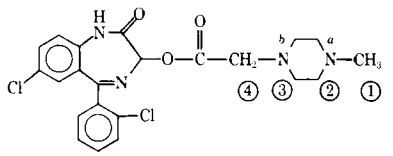
The methanesulfonic acid salts were also much more soluble than the hydrochlorides. Solutions of 200–300 mg/ml of either Vc or VIc could be prepared without difficulty.

The higher solubilities and stabilities of the monohydrochlorides and monomethanesulfonates of V and VI compared to the hydrochlorides of the morpholino derivatives III and IV may be attributed to the position of protonation (Table III). In the piperazine derivatives V and VI, protonation took place on the *a* nitrogen, as clearly indicated by the NMR spectra; in the morpholino derivatives, only the *b* nitrogen was available for protonation.

The synthetic sequences employed to prepare the compounds are outlined in Schemes I–IV. An efficient procedure for the methylation of I to II (Scheme I) was developed, in which equimolar amounts of base and methylating agent were used. When excess base and methyl iodide were used, two additional by-products (IX and X) were formed. The base-catalyzed tautomerization of I to XI in the presence of excess base also proceeded rapidly (2–4).

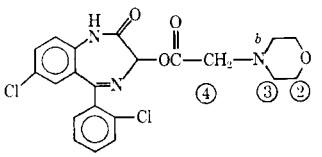
The intermediate Compounds XII and XIII were prepared by two alternative methods. Compound XII was obtained by an *N*-oxide rearrangement (2–5) of XIV (6, 7) when treated with chlo-

Table III—NMR Chemical Shifts (δ) of III and V



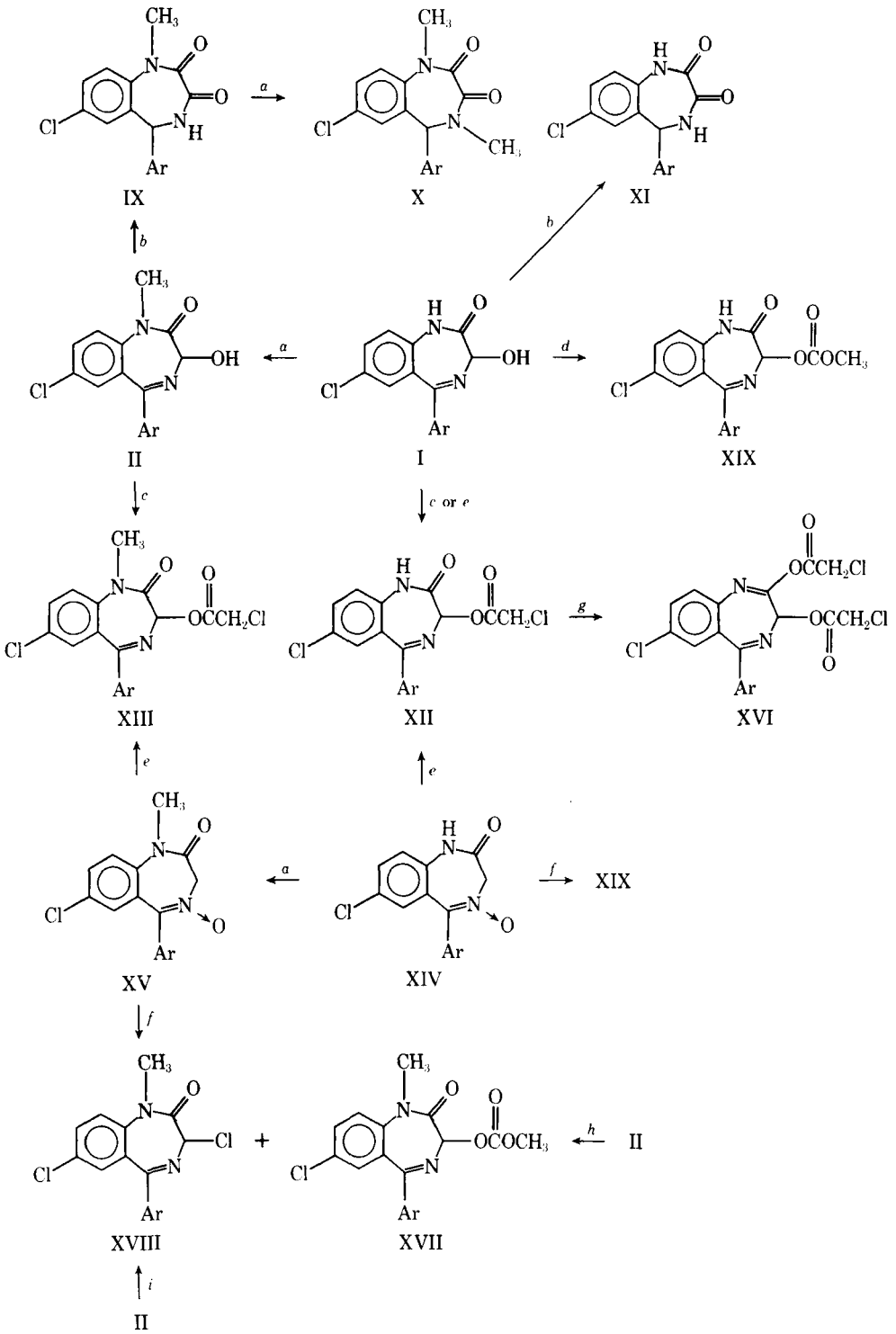
V

	①	②	③	④
Free base	2.27	2.55	2.70	3.57
Monohydrochloride	2.81	3.35	3.05	3.67
Dihydrochloride	2.80	3.40	3.40	4.20



III

Monohydrochloride	3.5	4.0	4.67



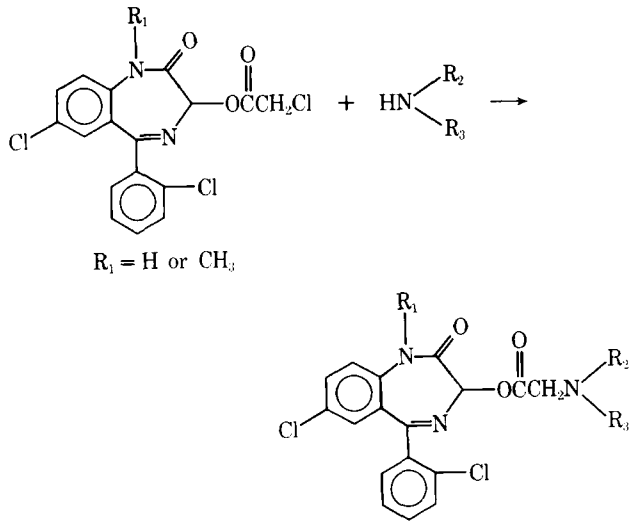
Scheme I—Key: Ar, o-chlorophenyl; a, *potassium hydroxide and methyl iodide;* b, *potassium hydroxide;* c, *chloroacetyl chloride and 4-dimethylaminopyridine;* d, *methyl chloroformate and 4-dimethylaminopyridine;* e, *chloroacetic anhydride;* f, *methyl chloroformate;* g, *chloroacetyl chloride;* h, *methyl chloroformate and lutidine; and* i, *thionyl chloride.*

roacetic anhydride. Methylation of XIV (8) followed by an analogous rearrangement gave XIII. Alternatively, XII and XIII were obtained from I and II by chloroacetylation with chloroacetyl chloride in the presence of 4-dimethylaminopyridine (9) or directly with chloroacetic anhydride. When an excess of chloroacetyl chloride was used, an additional product, XVI (derived from diacylation), was obtained.

When the analogous *N*-oxide rearrangement of XV with methyl chloroformate was carried out, a mixture of the carbonate XVII and the chloro Compound XVIII (1) was obtained. The carbonate, uncontaminated with XVIII, could be prepared from the 3-hydroxy Compound II upon treatment with methyl chloroformate. The 3-chloro derivative was alternatively prepared from II and thionyl chloride (1).

The final step in the preparation of the amine derivatives III–VIII involved the treatment of XII and XIII with the secondary amines (Scheme II).

The alternative direct acylation of I with the activated amino acid XXII (10) was also feasible (Scheme III). The required amino acid XXI could be prepared *via* the ester XXIV or directly from chloroacetic acid and the amine XX. When a large excess of chloroacetic acid was used, Compound XXIII was the only product.



Scheme II

However, equimolar quantities of chloroacid and amine afforded a mixture of XXI and XXIII.

The formation of XXIII could be avoided by the initial reaction of XX with ethyl chloroacetate to form the ester XXIV (11), which readily hydrolyzed in hot water.

The methanesulfonates Vc and VIc and the maleate V*d* were obtained by treatment of V and VI with equimolar amounts of the appropriate acids. The dihydrochlorides were best prepared by treating the free base with an excess of ethereal hydrogen chloride. The simplest procedure for the preparation of the monohydrochlorides involved the admixture of equimolar amounts of the dihydrochlorides and their respective free bases (Scheme IV).

The pharmacological activity of III–VIII is similar to that of I and II (12).

EXPERIMENTAL

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2*H*-1,4-benzodiazepin-2-one (II)—A 3-liter erlenmeyer flask equipped with a magnetic stirrer was charged with 160 g (0.5 mole) of I, 1500 ml of tetrahydrofuran, and a solution of 33.0 g (0.5 mole) of potassium hydroxide (85%) in 300 ml of water. Iodomethane (75.0 g, 5.3 moles) was added to the reaction and stirring was continued for 1.5 hr. Since the reaction mixture was still alkaline, 15 ml of dimethyl sulfate was added. The mixture was stirred for an additional hour, filtered, and concentrated *in vacuo*. The residue was filtered and washed successively with 500 ml of water and 300 ml of ethanol. The product, mp 205–207° [lit. (1) mp 205–207°], after drying in a vacuum oven overnight, weighed 104 g.

Concentration of the filtrate afforded an additional 42.0 g of crude product, mp 180–195°, which was recrystallized by adding a warm tetrahydrofuran solution of the crude material to 600 ml of boiling ethanol and concentrating the resulting solution until crystals began to separate (300 ml). The recrystallized product, mp 209–211°, weighed 25.3 g. Further concentration of the filtrate afforded 6.7 g of crystalline material, mp 190–194°. The total yield was 81%.

7-Chloro-5-(*o*-chlorophenyl)-1-methyl-4,5-dihydro-2*H*-1,4-benzodiazepine-2,3(1*H*)-dione (IX) and 7-Chloro-5-(*o*-chlorophenyl)-4,5-dihydro-1,4-dimethyl-1*H*-1,4-benzodiazepine-2,3-dione (X)—When the previous reaction was carried out in the presence of excess base and excess methylating agent, two additional products (IX and X) were isolated in low yield. Compound IX had mp 239–241°; NMR [$(D_3C)_2SO$]: ppm (δ) 3.28 (s, 3), 5.82 (s, 1), and 7.2–7.7 (m, 7).

Anal.—Calc. for $C_{16}H_{12}Cl_2N_2O_2$: C, 57.32; H, 3.60; N, 8.36. Found: C, 57.52; H, 3.66; N, 8.41.

Compound X had mp 255–258°; NMR [$(D_3C)_2SO$]: ppm (δ) 3.10 (s, 3), 3.20 (s, 3), 6.00 (s, 1), and 6.9–8 (m, 7).

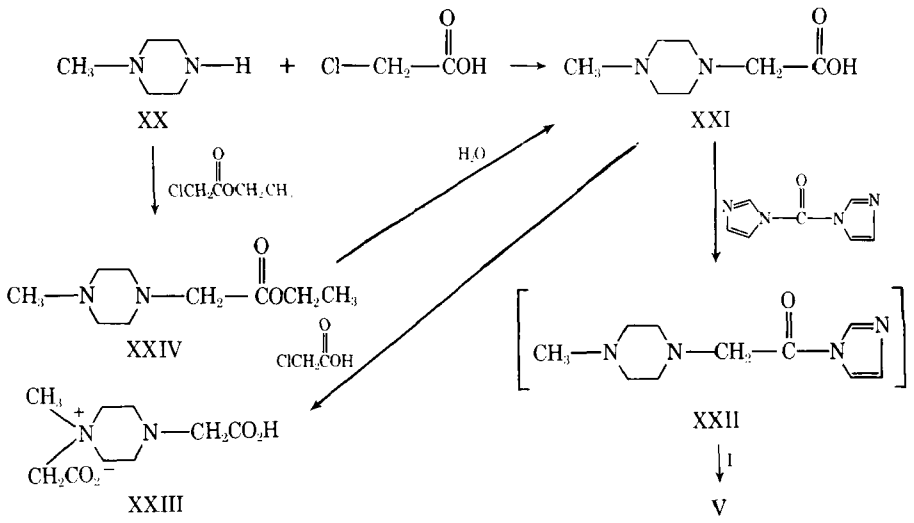
Anal.—Calc. for $C_{17}H_{14}Cl_2N_2O_2$: C, 58.47; H, 4.04; Cl, 20.31; N, 8.02. Found: C, 58.62; H, 4.11; Cl, 20.31; N, 8.07.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2*H*-1,4-benzodiazepin-2-one Chloroacetate (XIII)—*Method I*—This compound was prepared by the same procedure as described for XII but from 7-chloro-5-(*o*-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one 4-oxide (XV) (6.7 g, 0.02 mole) and chloroacetic anhydride (12 g, 0.07 mole), giving 6.8 g (82.6% yield), mp 172–174°; NMR ($CDCl_3$): ppm (δ) 3.51 (s, 3), 4.41 (s, 2), 6.15 (s, 1), 7.19 (d, 1), and 7.4–8 (m, 6).

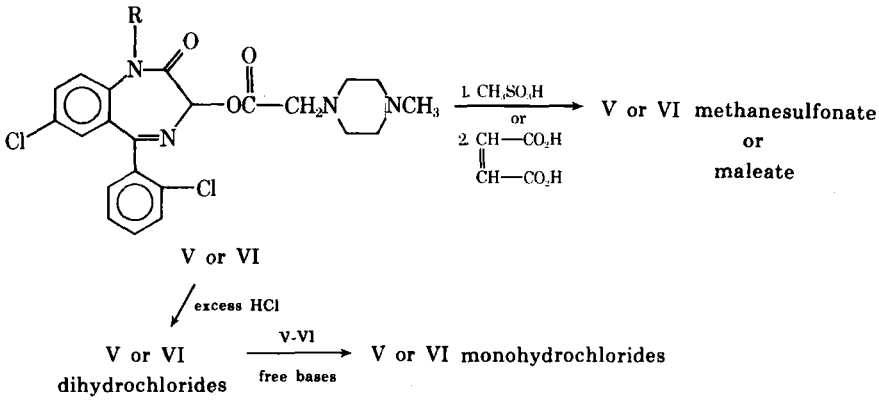
Anal.—Calc. for $C_{18}H_{13}Cl_3N_2O_3$: C, 52.52; H, 3.18; Cl, 25.84; N, 6.80. Found: C, 52.27; H, 3.37; Cl, 25.72; N, 6.61.

Method II—To a solution of II (10 g, 0.03 mole) and 4-dimethylaminopyridine (3.66 g, 0.03 mole) in 200 ml of tetrahydrofuran was added chloroacetyl chloride (3.39 g, 0.03 mole). A very heavy precipitate formed instantly. After 10 min of stirring, no trace of starting material could be detected by TLC (silica plates using ether as eluent). The precipitate was filtered off and the filtrate was flash evaporated, giving an oil which crystallized upon addition of ether, yielding 12.1 g (99%) of product. The melting point and the IR and NMR spectra were identical to those described above.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one Chloroacetate (XII)—*Method I*—A mixture of 7-chloro-5-(*o*-chlorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one 4-oxide (XIV) (16 g, 0.05 mole) and chloroacetic anhydride (25 g, 0.145 mole) was heated, with stirring, at 100–110° for 2.5 hr. Ether was then added and the mixture was stirred for 10 min. The solid obtained (14.7 g, 75% yield) was filtered, washed with ether, and recrystallized from methylene chloride–ether, giving XII, mp 232–234°; NMR [$(D_3C)_2SO$]: ppm (δ) 4.72 (s, 2), 6.10 (s, 1), 7.17 (d, 1), and 7.4–8 (m, 6).



Scheme III



Scheme IV

Anal.—Calc. for $C_{17}H_{11}Cl_3N_2O_3$: C, 51.35; H, 2.79; N, 7.05. Found: C, 51.26; H, 2.83; N, 6.94.

Method II—Compound XII was prepared from I by a procedure similar to that described for the preparation of XIII. The product had properties identical to those of XII obtained from the *N*-oxide XIV.

7-Chloro-5-(*o*-chlorophenyl)-3*H*-1,4-benzodiazepine-2,3-diol Bis(chloroacetate) (XVI)—When XIV was treated with an excess of chloroacetyl chloride, the dichloroacetylated compound XVI was obtained in low yield. Recrystallization from acetone gave a product with a melting point of 212–213.5°; NMR [$(D_3C)_2SO$]: ppm (δ) 4.69 (s, 2), 5.05 (s, 2), 6.31 (s, 1), 7.3 (broad s, 1), and 7.4–7.9 (m, 6).

Anal.—Calc. for $C_{19}H_{12}Cl_4N_2O_4$: C, 48.13; H, 2.55; Cl, 29.91; N, 5.91. Found: C, 48.23; H, 2.62; Cl, 30.25; N, 5.89.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one Carbonate Methyl Ester (XIX)—*Method I*—A mixture of XIV (5 g, 0.0155 mole) and 100 ml of methyl chloroformate was refluxed for 3 hr. A small amount of undissolved material was filtered off and the filtrate was flash evaporated. The residue was dissolved in 10 ml of methylene chloride and allowed to crystallize. The obtained solid, 2 g (30% yield), was filtered, washed with a small amount of cold methylene chloride, and dried, giving XIX, mp 223–225°; NMR [$(D_3C)_2SO$]: ppm (δ) 3.82 (s, 3), 5.82 (s, 1), 7.05 (d, 1), and 7.3–7.9 (m, 6).

Anal.—Calc. for $C_{17}H_{12}Cl_2N_2O_4$: C, 53.84; H, 3.19; N, 7.39. Found: C, 53.40; H, 3.22; N, 7.19.

Method II—To a solution of 3.21 g (0.01 mole) of I and 1.5 g (0.0123 mole) of dimethylaminopyridine in 100 ml of anhydrous tetrahydrofuran was added 2 ml of methyl chloroformate. A heavy precipitate formed immediately. The mixture was stirred at room temperature for 18 hr. The solid was filtered and the filtrate was flash evaporated to a light-yellow foam. The foam was dissolved in 15 ml of methylene chloride and allowed to crystallize. The obtained solid, 2.8 g (72% yield), had the same melting point and IR and NMR spectra as those obtained for the product prepared by Method I.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2*H*-1,4-benzodiazepin-2-one Carbonate Methyl Ester (XVII)—To a solution of II (16.7 g, 0.05 mole) in 300 ml of tetrahydrofuran were added 10 ml of methyl chloroformate and 10 ml of 2,6-lutidine. The reaction mixture was stirred overnight. TLC on silica gel, with ether as eluent, indicated the presence of unreacted starting material. The precipitate was filtered and a fresh excess of methyl chloroformate and 2,6-lutidine was added.

This process was repeated five times until only traces of 3-hydroxy compound could be detected. The solution was then flash evaporated and the residue was taken up in ethyl acetate and washed several times with water. The solution was concentrated to 50 ml, whereupon it crystallized, yielding 12.5 g (64%) of product, mp 193–194.5; NMR ($CDCl_3$): ppm (δ) 3.50 (s, 3), 3.89 (s, 3), 5.90 (s, 1), 7.08 (d, 1), and 7.3–7.9 (m, 6).

Anal.—Calc. for $C_{18}H_{14}Cl_2N_2O_4$: C, 54.98; H, 3.59; Cl, 18.03; N, 7.13. Found: C, 55.08; H, 3.63; Cl, 18.12; N, 7.15.

3,7-Dichloro-5-(*o*-chlorophenyl)-1,3-dihydro-1-methyl-2*H*-1,4-benzodiazepin-2-one (XVIII)—A mixture of II (22 g, 0.066 mole) and 50 ml of thionyl chloride was refluxed for 0.5 hr. The excess thionyl chloride was flash evaporated and the residue was recrystallized from acetonitrile, giving 21 g (90% yield) of XVIII, mp 217–219° [lit. (1) mp 217–219°]; NMR ($CDCl_3$): ppm (δ) 3.58 (s, 3), 5.80 (s, 1), 7.18 (d, 1), and 7.2–8 (m, 6).

XVIII (1) and XVII from XV—A mixture of XV (2.5 g, 7.5 mmoles) and methyl chloroformate (50 ml) was refluxed for 2 hr, whereupon no trace of starting *N*-oxide XV could be detected by TLC [ether–pentane (8.5:1.5) on silica gel plates]. The TLC showed the presence of two compounds in approximately equal ratio. The faster moving compound had an R_f value identical to that of XVIII (1). The solvent was flash evaporated and the residue was chromatographed on 100 g of silica gel. The 3-chloro Compound XVIII was eluted initially, followed by the 3-methyl carbonate XVII, 0.4 g (13% yield), mp 193–194.5°; NMR ($CDCl_3$): ppm (δ) 3.56 (s, 3), 3.96 (s, 3), 6.01 (s, 1), 7.24 (d, 1), and 7.4–8 (m, 6).

Anal.—Calc. for $C_{18}H_{14}Cl_2N_2O_4$: C, 54.98; H, 3.59; N, 7.13. Found: C, 54.90; H, 3.76; N, 7.27.

7-Chloro-5-(*o*-chlorophenyl)-4,5-dihydro-2*H*-1,4-benzodiazepine-2,3(1*H*)-dione (XI)—The title compound was obtained in low yield upon treatment of I with an excess of base, analogous to the conversion of II to IX, mp 330–331°; NMR [$(D_3C)_2SO$]: ppm (δ) 5.92 (s, 1) and 7.1–7.9 (m, 7).

Anal.—Calc. for $C_{15}H_{10}Cl_2N_2O_2$: C, 56.10; H, 3.14; N, 8.72. Found: C, 55.91; H, 3.29; N, 9.03.

4-Methyl-1-piperazineacetic Acid (XXI)—*Method I*—A solution of *N*-methylpiperazine (XX) (15 g, 0.15 mole) and chloroacetic acid (4.7 g, 0.05 mole) in 50 ml of water was stirred for 18 hr. The solution was then placed on a column containing 300 g of resin[1] in its basic form. The column was washed with distilled water until the pH of the eluent was neutral; then the column was eluted with 10% hydrochloric acid. A total of 900 ml of the acid eluent was collected and rechromatographed through a column containing 300 g of resin[2] in its acidic form.

The column was washed with distilled water until no more chloride ion was eluted (as indicated by the absence of a precipitate with silver nitrate) and was then eluted with 10% ammonium hydroxide until 600 ml of basic eluent was collected. The eluted solution, when flash evaporated, gave 3.7 g (46% yield) of crystalline product (recrystallized from methanol–ether), mp 159.5–161°; NMR [$(D_3C)_2SO$]: ppm (δ) 2.25 (s, 3), 2.5 (broad m, 4), 2.7 (broad m, 4), and 3.15 (s, 2).

Anal.—Calc. for $C_7H_{14}N_2O_2 \cdot \frac{1}{4}H_2O$: C, 51.67; H, 8.97; N, 17.22. Found: C, 51.69; H, 8.98; N, 16.81.

Method II—A solution of ethyl 4-methyl-1-piperazineacetate (XXIV) (3.2 g, 0.0172 mole) in 75 ml of water was refluxed for 2 hr. The solvent was flash evaporated and the residue was crystallized from methanol–ether, giving 2.6 g (95%) of the free acid, with melting point and NMR spectra identical to those reported in Method I.

1,4-Dicarboxymethyl-1-methylpiperazinium Hydroxide Inner Salt and Its Sodium Salt (XXIII)—To a solution of chloroacetic acid (19 g, 0.02 mole) in 10 ml of water was added a solution of sodium hydroxide (8 g, 0.02 mole) in 10 ml of water. The solution was stirred for 0.5 hr, and XX (10 g, 0.01 mole) was added. The solution was stirred for 18 hr at room temperature and was

[1] Dowex 1-X 8.
[2] Dowex 50 W-X2.

then chromatographed on 900 g of resin[2] (acidic form). The column was washed with distilled water until no more chloride ion was obtained, as indicated by silver nitrate treatment, and was then eluted with 3 *N* ammonium hydroxide until 1800 ml of basic eluent was collected. Flash evaporation gave a foam which, when mixed with absolute ethanol and added to methylene chloride, gave 9.2 g (42% yield) of product as a white crystalline solid. Recrystallization from water–methanol afforded pure product, mp 306–307°; NMR (D_2O): ppm (δ) 3.43 (s, 3), 3.7 (broad m, 4) [superimposed at 3.8 (s, 2)], 4 (broad m, 4), and 4.12 (s, 2).

Anal.—Calc. for $C_9H_{16}N_2O_4$: C, 49.99; H, 7.46; N, 12.96. Found: C, 50.13; H, 7.64; N, 12.73.

Treatment of this 1,4-dicarboxymethyl-1-methylpiperazinium hydroxide inner salt with an equivalent amount of sodium hydroxide, followed by recrystallization from water–methanol, gave the corresponding sodium salt in 90% yield, mp 159–162°; NMR (D_2O): ppm (δ) 3.1 (broad m, 4), 3.25 (s, 2), 3.35 (s, 2), 3.8 (broad m, 4), and 4.0 (s, 2).

Anal.—Calc. for $C_9H_{15}N_2NaO_4{\cdot}2H_2O$: C, 39.42; H, 6.98; N, 10.21. Found: C, 39.28; H, 7.06; N, 10.13.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one 4-Methyl-1-piperazineacetate Dihydrochloride (V*a*)—*Method I (from XII)*—A solution of XII (3 g, 7.5 mmoles) and XX (3 ml) in 50 ml of anhydrous tetrahydrofuran was stirred for 16 hr at room temperature. The solvent was flash evaporated and the residue, dissolved in methylene chloride, was washed repeatedly with saturated aqueous sodium chloride. The organic phase was dried and concentrated to a total volume of 5 ml, which was then added to a vigorously stirred solution of gaseous hydrogen chloride in ether. The obtained solid was recrystallized from methanol–ether, giving 1.5 g of V*a* (35% yield), mp 202–204° dec.; NMR [$(D_3C)_2SO$]: ppm (δ) 2.80 (s, 3), 3.40 (broad s, 8), 4.23 (s, 2), 5.98 (s, 1), 6.96 (d, 1), and 7.2–7.8 (m, 6).

Anal.—Calc. for $C_{22}H_{22}Cl_2N_4O_2{\cdot}2HCl{\cdot}2H_2O$: C, 46.33; H, 4.95; H_2O, 6.32; N, 9.82. Found: C, 46.11; H, 4.34; H_2O, 6.40; N, 9.85.

Method II (from XXI)—To a solution obtained by warming a mixture of 4-methyl-1-piperazineacetic acid (0.79 g, 5 mmoles) and anhydrous dimethylformamide (10 ml) was added 1,1-carbonyldiimidazole (0.81 g, 0.5 mmole). A solution of I (1.6 g, 0.5 mmole) in 50 ml of anhydrous tetrahydrofuran was added. The reaction mixture was stirred at room temperature for 80 hr and then flash evaporated, giving an oil. The residue was mixed with ethyl acetate and washed three times with 100 ml of water.

The organic phase was dried and evaporated, giving an oil. The oil was dissolved in methylene chloride (3 ml), and the solution was added to a solution of gaseous hydrogen chloride in ether. A precipitate was obtained which, when recrystallized from methanol–ether, gave 1.2 g (42% yield) of the desired hydrochloride, mp 218–220°; NMR [$(D_3C)_2SO$]: ppm (δ) 2.80 (s, 3), 3.40 (broad s, 8), 4.23 (s, 2), 5.98 (s, 1), 6.96 (d, 1), and 7.2–7.8 (m, 6).

Anal.—Calc. for $C_{22}H_{22}Cl_2N_4O_3{\cdot}2HCl{\cdot}H_2O$: C, 47.84; H, 4.75; N, 10.15. Found: C, 47.51; H, 4.57; N, 9.85.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2*H*-1,4-benzodiazepin-2-one 4-Methyl-1-piperazineacetate Dihydrochloride (VI*a*)—This compound was prepared as described in the synthesis of V*a* but from XIII (5 g, 0.012 mole) and *N*-methylpiperazine. The product (2.5 g, 36% yield) decomposed above 210°; NMR [$(D_3C)_2SO$]: ppm (δ) 2.82 (s, 3), 3.43 (broad s, 11), 4.27 (s, 2), 6.00 (s, 1), 7.07 (d, 1), and 7.4–8 (m, 6).

Anal.—Calc. for $C_{23}H_{24}Cl_2N_4O_3{\cdot}2HCl{\cdot}1\frac{1}{2}H_2O$: C, 48.02; H, 5.08; N, 9.74. Found: C, 47.88; H, 4.89; N, 9.78.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one 4-Morpholineacetate Ester Hydrochloride (III)—This compound was prepared by a procedure similar to the one described in the synthesis of V but from XII (3 g, 7.5 mmoles) and morpholine. The product obtained (1.3 g, 40% yield) had a melting point of 255–257° dec.; NMR [$(D_3C)_2SO$]: ppm (δ) 3.5 (broad s, 4), 4.0 (broad s, 4), 4.67 (s, 2), 6.17 (s, 1), 7.11 (d, 1), and 7.5–8 (m, 6).

Anal.—Calc. for $C_{21}H_{19}Cl_2N_3O_4{\cdot}HCl{\cdot}H_2O$: C, 50.17; H, 4.41; Cl, 21.15; N, 8.36. Found: C, 50.43; H, 4.06; Cl, 21.39; N, 8.55.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2*H*-1,4-benzodiazepin-2-one 4-Morpholineacetate Hydrochloride (IV)—This compound was prepared by a procedure similar to the one described in the synthesis of V but from XIII (3.5 g, 8.5 mmoles) and morpholine. The product (2.7 g, 61% yield) had a melting point of 237–238°; NMR [$(D_3C)_2SO$]: ppm (δ) 3.52 (broad s, 7), 4.05 (broad s, 4), 4.75 (s, 2), 6.26 (s, 1), 7.27 (d, 1), and 7.7–8 (m, 6).

Anal.—Calc. for $C_{22}H_{21}Cl_2N_3O_4{\cdot}HCl{\cdot}H_2O$: C, 51.13; H, 4.68; N, 8.13. Found: C, 51.41; H, 4.60; N, 8.11.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one 4-Methyl-1-piperazineacetate Hydrochloride (V*b*)—An aqueous solution of V*a* (29 g, 0.05 mole) was neutralized with an excess of 1 *N* sodium hydroxide and extracted with methylene chloride. The organic phase was dried and evaporated, giving V as a fine white powder, 18.5 g (80% yield); NMR ($CDCl_3$): ppm (δ) 2.27 (s, 3), 2.55 (broad s, 4), 2.70 (broad s, 4), 3.57 (s, 2), 6.08 (s, 1), 7.08 (d, 1), and 7.2–7.9 (m, 6).

A solution of equimolar quantities of V*a* and the above-obtained free base (2.3 mmoles of each) in methanol was stirred at room temperature for 30 min. The solvent was flash evaporated and the residual foam was recrystallized from methanol–ether, giving 2 g (82% yield) of white solid, mp 233–234°; NMR [$(D_3C)_2SO$]: ppm (δ) 2.81 (s, 3), 3.05 (broad m, 4), 3.35 (broad m, 4), 3.67 (s, 2), 5.95 (s, 1), 7.00 (d, 1), and 7.3–7.8 (m, 6).

Anal.—Calc. for $C_{22}H_{22}Cl_2N_4O_3{\cdot}HCl{\cdot}H_2O$: C, 51.22; H, 4.89; Cl, 20.62; N, 10.86. Found: C, 51.44; H, 4.56; Cl, 20.91; N, 10.74.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2*H*-1,4-benzodiazepin-2-one 4-Methyl-1-piperazineacetate Hydrochloride (VI*b*)—An aqueous solution of VI*a* (11.6 g, 0.02 mole) was neutralized with an excess of 1 *N* sodium hydroxide and extracted with methylene chloride. The organic phase was dried and evaporated, giving VI as a fine powder, 9.1 g (94.6% yield); NMR ($CDCl_3$): ppm (δ) 2.27 (s, 3), 2.57 (broad m, 4), 2.65 (broad m, 4), 3.46 (s, 3), 3.55 (s, 2), 6.10 (s, 1), 7.10 (d, 1), and 7.2–7.9 (m, 6).

A solution of equimolar quantities of VI*a* and the above-obtained free base (20 mmoles of each) in methanol was stirred at room temperature for 30 min. The solvent was flash evaporated and the residual foam was recrystallized from methanol–ether, giving 15 g (71% yield) of solid, mp 270–271°; NMR [$(D_3C)_2SO$]: ppm (δ) 2.87 (s, 3), 3.1 (broad m, 4), 3.4 (broad m, 4), 3.49 (s, 3), 3.70 (s, 2), 6.07 (s, 1), 7.12 (d, 1), and 7.5–7.9 (m, 6).

Anal.—Calc. for $C_{23}H_{24}Cl_3N_4O_3{\cdot}HCl{\cdot}H_2O$: C, 51.13; H, 5.14; Cl, 20.08; N, 10.57. Found: C, 52.34; H, 4.75; Cl, 20.64; N, 10.56.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one 4-Methyl-1-piperazineacetate Methanesulfonate (V*c*)—To a solution of 7-chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one 4-methyl-1-piperazineacetate (V) (2.3 g, 5 mmoles), prepared as described, was added methanesulfonic acid (0.48 g, 5 mmoles). The solution was stirred at room temperature for 30 min. The solvent was then flash evaporated and the residue was recrystallized from methanol–ether, giving 2.3 g (80% yield) of white crystalline product, which did not melt sharply but slowly decomposed above 160°; NMR [$(D_3C)_2SO$]: ppm (δ) 2.42 (s, 3), 2.82 (s, 2), 3.0 (broad m, 4), 3.4 (broad m, 4), 3.65 (s, 2), 5.95 (s, 1), 7.05 (d, 1), and 7.2–7.9 (m, 6).

Anal.—Calc. for $C_{22}H_{22}Cl_2N_4O_3{\cdot}CH_4SO_3{\cdot}H_2O$: C, 48.00; H, 4.90; Cl, 12.32; N, 9.74. Found: C, 48.08; H, 4.71; Cl, 12.62; N, 9.41.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2*H*-1,4-benzodiazepin-2-one 4-Methyl-1-piperazineacetate Methanesulfonate (VI*c*)—To a solution of 7-chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-1-methyl-2*H*-1,4-benzodiazepin-2-one 4-methyl-1-piperazineacetate (VI) (197.5 g, 0.4 mole), prepared as described, was added methanesulfonic acid (38.8 g, 0.4 mole). The solution was stirred at room temperature for 30 min. The solvent was then flash evaporated and the residue was recrystallized from methanol–ether, giving 196 g (85.5% yield) of white crystalline product, mp 238–241°; NMR [$(D_3C)_2SO$]: ppm (δ) 2.48 (s, 3), 2.87 (s, 3), 3 (broad m, 4), 3.3 (broad m, 4), 3.48 (s, 3), 3.68 (s, 2), 6.02 (s, 1), 7.10 (m, 1), and 7.5–7.95 (m, 6).

Anal.—Calc. for $C_{23}H_{24}Cl_2N_4O_3{\cdot}CH_4SO_3$: C, 50.44; H, 4.94; Cl, 12.41; N, 9.80; S, 5.61. Found: C, 50.32; H, 4.94; Cl, 12.41; N, 9.77; S, 5.67.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one 4-Methyl-1-piperazineacetate Maleate (V*d*)—To a methanolic solution of V (2.3 g, 5 mmoles), prepared as described, was added maleic acid (0.58 g, 5 mmoles). The solution was stirred at room temperature for 30 min. The solvent was then flash evaporated and the residue was recrystallized from methanol–ether, giving 1.5 g (51% yield) of white crystalline product, which did not melt sharply but slowly decomposed above

125°; NMR [$(D_3C)_2SO$]: ppm (δ) 2.92 (s, 3), 3.0 (broad s, 4), 3.4 (broad s, 4), 3.63 (s, 2), 6.02 (s, 1), 6.30 (s, 2), 7.1 (d, 1), and 7.3–7.9 (m, 6).

Anal.—Calc. for $C_{22}H_{22}Cl_2N_4O_3 \cdot C_4H_4O_4 \cdot \frac{1}{2}H_2O$: C, 53.25; H, 4.64; Cl, 12.09; N, 9.55. Found: C, 52.96; H, 4.53; Cl, 11.96; N, 9.46.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one *N*-(2-Dimethylaminoethyl)-*N*-methylglycinate Dihydrochloride (VII)—The title compound was prepared by the same procedure as that described in the preparation of V*a* but from XII and *N,N,N'*-trimethylethylenediamine. The product was obtained from ethanol–ether in 20% yield, mp 221–223° dec.; NMR [$(D_3C)_2SO$]: ppm (δ) 2.95 (s, 6), 3.00 (s, 3), 3.65 (broad s, 4), 4.52 (broad s, 2), 6.10 (s, 1), 7.02 (d, 1), and 7.4–7.8 (m, 6).

Anal.—Calc. for $C_{22}H_{24}Cl_2N_4O_3 \cdot 2HCl \cdot 2H_2O$: C, 46.17; H, 5.28; N, 9.79. Found: C, 45.96; H, 5.36; N, 9.85.

7-Chloro-5-(*o*-chlorophenyl)-1,3-dihydro-3-hydroxy-2*H*-1,4-benzodiazepin-2-one 4-(Hydroxyethyl)-1-piperazineacetate Dihydrochloride (VIII)—The title compound was prepared as described for VII but from XII and *N*-(2-hydroxyethyl)piperazine. The product, obtained in 46% yield from ethanol–ether, became dark above 195° and decomposed above 202°; NMR [$(D_3C)_2SO$]: ppm (δ) 3.1–4.2 (broad m, 16), 4.55 (broad s, 2), 6.03 (s, 1), 7.01 (d, 1), and 7.3–7.8 (m, 6).

Anal.—Calc. for $C_{23}H_{26}Cl_4N_4O_4 \cdot 2HCl \cdot 2\frac{1}{2}H_2O$: C, 45.33; H, 5.13; H_2O, 7.39; N, 9.29. Found: C, 45.34; H, 4.70; H_2O, 7.39; N, 9.33.

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GLC Determination of Propranolol, Other β-Blocking Drugs, and Metabolites in Biological Fluids and Tissues

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Abstract □ A highly sensitive and specific electron-capture GLC method was developed for β-blocking drugs, including propranolol, oxprenolol, alprenolol, pronethalol, dichloroisoproterenol, practolol, and sotalol, and several of their metabolites. The drugs are separated and detected as their trifluoroacetyl derivatives. Minimum detectable amounts ranged from 0.1 to 1.1 pg. The chemical structures of the derivatives were confirmed by GLC–mass spectrometry. Propranolol was extracted from plasma by either a single- or a back-extraction procedure, with oxprenolol as the internal standard. The minimum detectable concentration was 0.1 ng/ml plasma. The standard curve was linear from 0.5 to 500 ng/ml. Applications of this method demonstrated quantitatively detectable plasma propranolol levels 24 hr after a small (0.05 mg/kg) intravenous dose. Accurate plasma determinations could be made in patients receiving numerous other drugs. The method was shown to be applicable to propranolol and two metabolites, 1-(α-naphthoxy)-2,3-propylene glycol and *N*-desisopropylpropranolol, in tissue. Determinations of picogram amounts of oxprenolol and its four major urinary metabolites demonstrate the general applicability of the method to all β-blocking drugs and most of their metabolites.

Keyphrases □ Propranolol and two metabolites—GLC determination in biological fluids and tissues □ β-Blocking drugs—electron-capture GLC determination of propranolol, oxprenoiol, alprenolol, pronethalol, dichloroisoproterenol, practolol, sotalol, and several metabolites in biological fluids and tissues □ GLC—determination of propranolol, other β-blocking drugs, and metabolites in biological fluids and tissues

Large variations in response to β-blocking drug therapy have been demonstrated in numerous clinical studies, making the task of evaluating the clinical efficacy of this chemical and pharmacological class of compounds extremely difficult. These variations have been attributed in part to individual differences in the metabolic disposition of these drugs.

A better understanding of variations in the metabolic disposition of β-blocking drugs during treatment requires reliable measurements of these drugs in plasma, other biological fluids, and tissues. Most β-blocking drugs are extensively metabolized (1, 2),