Synthesis of Some Carbon-3 Substituted 1,4-Benzodiazepin-2-ones and Their Central Nervous System Effects

Tomislav Kovač, Franjo Kajfež,* Vitomir Šunjić,

Department of Biomedical and Biochemical Research, Chemical Research Company, 33048 San Giovanni al Natisone (UD), Italy

Nikola Blažević, and Dragutin Kolbah

Faculty of Pharmacy and Biochemistry, University of Zagreb, 41000 Zagreb, Yugoslavia. *Received May 19, 1978*

Starting from 3-hydroxy-1,4-benzodiazepin-2-ones **1–3**, via 3-chloro derivatives **4–6**, 13 new C(3)-substituted 1,4-benzodiazepin-2-ones were synthesized. Reaction of **4–6** with ethylene glycol yielded 3-(β-hydroxyethyl) derivatives **7–9**. Similar reaction with the isopropylidene derivative of glycerol afforded **10–12**, which on hydrolysis of the isopropylidene group yielded glycerol derivatives **13–15**. Reaction of trichloroacetyl chloride with oxazepam and temazepam yielded the corresponding trichloroacetyl esters **16** and **17**. The β-hydroxyethyl derivative **7** was conjugated with an acetylated glucopyranose derivative to give isomeric **18** and **19**. Partition coefficients (log P_{oct}) and central nervous system activities (in six standard tests) were determined for **7–15** as well as several standard compounds. Most of the compounds exhibiting beneficial central nervous system activity had P_{oct} values between 1.71 and 2.48. No correlation between lipophilicity and central nervous system activity could be discerned for these compounds.

Drugs exhibiting CNS activity should have relatively high lipophilic character, i.e., high octanol–water partition coefficients P (usually, log $P \approx 2$), to permit their passage across the blood–brain barrier.[2a] However, injectable preparations of such highly lipid-soluble drugs can be obtained only with nonaqueous solvents or solvent aids, which may produce unwanted side effects.[2b]

Therapeutically useful 1,4-benzodiazepines are most often markedly lipophilic. The log P values are between 1.44 and 4.44.[3] These compounds undergo various biotransformations in vivo, as well as in vitro,[4,5] to give more hydrophilic 3-hydroxylated metabolites. Like their precursors, the 3-hydroxylated metabolites are active at central sites.[6,7] Thus, even more hydrophilic derivatives may retain the same biological activity toward the CNS. In pursuit of this observation, derivatives of 1,4-benzodiazepines with increased hydrophilicity, such as carboxylic acids,[8] phosphate esters,[9] tertiary amine salts,[10] quaternary ammonium salts,[11] and hydroxyalkyl derivatives[12,13] obtained by introducing the hydrophilic substituents at the N(1) or C(3) position of a parent 1,4-benzodiazepin-2-one, have been examined for antianxiety activity.

Such water-soluble derivatives of 1,4-benzodiazepine might find useful application in the treatment of tetanus seizures,[14] where oral administration is not convenient or possible and where injectable formulations of water-insoluble 1,4-benzodiazepines may cause venous inflammation.

For these reasons, we have synthesized various hydrophilic 1,4-benzodiazepin-2-ones and attempted to correlate their central effects with their octanol–pH 7.35 aqueous phosphate buffer partition coefficient, P_{oct}.

Chemistry. In a recent paper,[11] we described preparative procedures for 3-ammonium derivatives of 1,4-benzodiazepin-2-ones. The central activities of these compounds were characterized by the results of simple pharmacologic tests. A second group of hydrophilic derivatives, i.e., of hydroxyalkyloxy derivatives (**7–15**, Table I), have been prepared starting from the 3-hydroxy-1,4-benzodiazepin-2-ones **1–3** via 3-chloro derivatives **4–6**. The last step of the reaction was conducted using an excess of hydroxyalkylating reagent as the reaction medium, as described under the Experimental Section.

Conjugation of various drugs with polyhydroxy compounds such as ethylene glycol, glycerol, pentoses, and hexoses is an established way for imparting hydrophilic properties to a drug molecule.[15,16] We devoted significant experimental effort to the attempted preparation of a third group of hydrophilic derivatives, i.e., C(3) ether glucosides of 1,4-benzodiazepin-2-ones, but without success. These failures might be explained by the aminic-like character of the hydroxy group in the starting compounds. Two vicinal heteroatoms, N and O, and an amide carbonyl group may cause a decrease in the acidity of the C(3)-OH group and, therefore, make the proton less prone to exchange by the sugar component. This rationalization is consistent with the observation that **7**, a β-hydroxyalkyl derivative of 3-hydroxy-1,4-benzodiazepin-2-one, was successfully conjugated with an acetylated sugar component, giving pairs of diastereomeric products **18** and **19** anomeric relative to the C(3) chiral center of the benzodiazepine. Selective deacetylation with dilute methanolic potassium hydroxide solution in preliminary experiments furnished free glucosides.

In one of the synthetic attempts, intermediates **16** and **17**, i.e., trichloroacetic acid esters of oxazepam **1** and temazepam **2**, were synthesized with the expectation that these esters should undergo the condensation reaction with the sugar component more easily than the parent compounds. These compounds produced marked CNS activity higher than compounds **1** and **2**. The compounds are very unstable and hydrolyzed to the starting compound even during determination of the partition coefficients.

Biological Activity. The compounds under investigation were administered orally, each to a group of six mice (male and female albino mice, Swiss-Webster) weighing 25–30 g. ED_{50} values were determined, and the results are given as relative potencies of the compounds compared with chlordiazepoxide, which is arbitrarily assigned a value of 1. The tests were performed according to widely accepted methods, i.e., pentylenetetrazole test,[17,18] maximal and minimal electroshock,[17,19] muscle relaxation and fighting test,[20] and hypnotic effect.,[21] The corresponding potencies obtained in these tests for C(3) *O*-acyl and *O*-hydroxyalkyl derivatives are presented in Table II.

Partition coefficients were obtained after equilibration of the compounds between 1-octanol and 0.15 M phosphate buffer, pH 7.35.[22] The log P_{oct} values are given in Table III.

Discussion

Esters and ethers of **1–3** hydrolyzed readily in dilute acid with release of 3-hydroxy-1,4-benzodiazepin-2-ones. Hydrolytic release of these moieties may be reasonably

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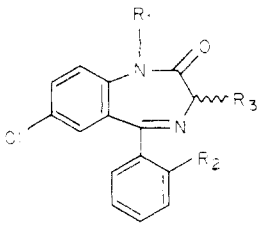
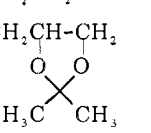
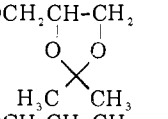
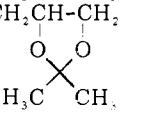
Table I. 3-O-Substituted Derivatives of 5-Substituted 7-Chlorophenyl-1,4-benzodiazepin-2-ones[a]



no.	R_1	R_2	R_3	recrystn solvent	yield, %	mp, °C	formula	anal.
7	CH_3	H	$-OCH_2CH_2OH$	chloroform-lt petr	85	217-219	$C_{18}H_{17}ClN_2O_3$	C, H, N
8	H	H	$-OCH_2CH_2OH$	ethanol	75	210-212	$C_{17}H_{15}ClN_2O_3$	C, H, N
9	H	Cl	$-OCH_2CH_2OH$	chloroform-acetone	79	115-117	$C_{17}H_{14}Cl_2N_2O_3$	C, H, N
10	CH_3	H		ethanol-water	75	150-152	$C_{22}H_{23}ClN_2O_4$	C, H, N
11	H	H		ether-lt petr	70	210-211	$C_{21}H_{21}ClN_2O_4$	C, H, N
12	H	Cl		chloroform-lt petr	75	164-168	$C_{21}H_{20}Cl_2N_2O_4$	C, H, N
13	CH_3	H	$-OCH_2CH(OH)CH_2OH$	ethanol-ether-lt petr	80	198-200	$C_{19}H_{19}ClN_2O_4$	C, H, N
14	H	H	$-OCH_2CH(OH)CH_2OH$	ethanol-ether-lt petr	70	166-168	$C_{18}H_{17}ClN_2O_4$	C, H, N
15	H	Cl	$-OCH_2CH(OH)CH_2OH$	ethanol-ether-lt petr	50	177-179	$C_{18}H_{16}Cl_2N_2O_4$	C, H, N
16	H	H	$OOCCCl_3$	benzene-lt petr	50	246-248	$C_{17}H_{10}Cl_4N_2O_3$	C, H, N
17	CH_3	H	$OOCCCl_3$	benzene-lt petr	58	194-196	$C_{18}H_{12}Cl_4N_2O_3$	C, H, N

[a] All compounds listed exhibited in the NMR spectrum a characteristic singlet for the 3-C(H)-O proton at 4.9-5.0 ppm. Characteristic bands in the infrared spectra were regularly found at 1695, 1610-1620, 1595, 1565, 1498, 1450, 1330, 748, and 700 cm^{-1}.

assumed to occur in vivo, and the pharmacological effects observed are probably caused in the majority of the times by the parent 3-hydroxy compounds liberated after administration of the conjugates. Among the compounds investigated, appreciable activity was demonstrated by β-hydroxyethyl derivatives and their conjugates with acetylated sugar. Trichloroacetyl esters were also highly effective compounds. As a consequence of their higher acute toxicity, they have a decreased therapeutic index. The conjugates with glycerol were also active, but the intermediary acetonides **10–12** were inactive. They have the highest log P values among compounds described in this paper, however. The measured partition coefficient of compounds **7–9** is roughly 2, a value that is considered optimal for the CNS-active agents. The glycerol conjugates **13–15** had the lowest log P_{oct} values (about 1.7–1.9).

Since we have not been able to find a correlation between the partition coefficient and the results of any individual pharmacological test, we conclude that these pharmacological effects are not determined by water–lipid distribution but rather by other molecular properties.

Experimental Section

Melting points (uncorrected) were determined on a Kofler microheating stage. NMR spectra were recorded with a Varian T-60 and a Perkin-Elmer R17 instrument using Me_4Si as an internal standard, and IR spectra were recorded on a Perkin-Elmer M-137 instrument. UV spectra, as well as C_{oct} and C_w concentrations for P_{oct} determination, were derived with a Varian Techtron M 165 instrument. TLC chromatograms were obtained on Merck Fertigplatten F 254 coated plates (0.25-mm thickness, silica gel). Column chromatography was run on Merck's chromatographic silica gel (0.05–0.25-mm grains). Light petroleum ether used as a component of eluting solvents was the fraction boiling at 40–60 °C.

Preparation of Compounds 7–12. Starting compounds **4–6**[11] were added gradually, with vigorous stirring, to warm (50–60 °C) 1,2-ethanediol or isopropylidene glycerol (20 mL). Stirring and heating were maintained for 15 min after the addition was completed; then the mixture was allowed to cool to ambient temperature, and stirring was continued for another 4 h. The resulting solution was mixed with ice–water (100 mL), and the resulting slurry was extracted with chloroform (3 × 50 mL). The combined extracts were washed with water (2 × 30 mL), dried (Na_2SO_4), and evaporated. Residues were dissolved in the solvent mixture listed in Table I. The pure compounds crystallized upon chilling and standing for extended periods in an ice bath. Alternatively, the crude residues were dissolved in the most polar solvent of the system indicated in Table I, and crystallization was induced in the chilled solution by addition of a less polar solvent. Characteristic data for compounds **7–15** are presented in Table I.

Preparation of Compounds 13–15. Crude **10–12**, respectively (20 mmol), were dissolved by heating in methanol (70 mL). Compound **11** was dissolved in a mixture of methanol (70 mL) and acetone (40 mL). Without discontinuing the stirring, these solutions were allowed to assume ambient temperature, whereupon 30% aqueous formic acid (170 mL) was added and stirring was continued for another 5–6 h. The reaction mixtures were then neutralized (solid $NaHCO_3$), and the organic solvents were evaporated under reduced pressure. The remaining aqueous suspensions were extracted with chloroform (3 × 50 mL). Extracts

Table II. Pharmacological Potencies of 3-O-Substituted Derivatives of 1,4-Benzodiazepin-2-ones and Selected Reference Agents (Relative to Chlordiazepoxide)[a]

compd	anticonvulsant effect: pentylenetetrazole[d]	anticonvulsant effect: electro shock[e] max	anticonvulsant effect: electro shock[e] min	muscle relax.[f]	fighting test[f]	hypnotic effect[g]	LD_{50}, mM/kg po
7	12.5 (10.8-14.5)[b]	0.80 (0.73-0.88)	8.30 (0.28-0.31)	1.10 (1.02-1.18)	1.50 (1.30-1.73)	0.40 (0.36-0.44)	17.40
8	10.3 (8.6-12.3)	0.58 (0.53-0.63)	0.40 (0.37-0.43)	1.3 (1.15-1.40)	1.7 (1.44-2.00)	0.35 (0.33-0.37)	18.14
9	33.6 (29.3-38.5)	1.20 (0.96-1.5)	2.8 (2.48-3.16)	1.86 (1.52-2.28)	17.8 (15.1-21.0)	0.65 (0.58-0.73)	9.36
10	inactive	inactive	inactive	inactive	1.90 (1.60-2.26)	2.30 (2.05-2.58)	9.16
11	inactive	inactive	inactive	inactive	1.70 (1.43-2.01)	0.52 (0.49-0.55)	9.98
12	inactive	inactive	inactive	inactive	2.3 (2.15-2.60)	2.0 (1.80-2.22)	7.59
13	10.3 (8.6-12.3)	0.38 (0.35-0.41)	inactive	0.80 (0.71-1.20)	1.0 (0.83-1.20)	0.42 (0.36-0.49)	16.00
14	12.5 (10.6-14.8)	2.3 (2.20-2.40)	0.52 (0.48-0.56)	0.70 (0.61-0.80)	1.4 (1.14-1.72)	0.51 (0.42-0.62)	16.63
15	36.8 (31.9-42.4)	4.68 (4.10-5.34)	0.86 (0.72-1.03)	1.25 (1.07-1.46)	15.3 (12.6-18.58)	0.58 (0.49-0.69)	7.82
16	16.5 (13.6-19.9)	5.7 (4.8-6.8)	1.32 (1.21-1.44)	1.60 (1.35-1.90)	1.92 (1.66-2.28)	0.73 (0.68-1.07)	6.85
17	15.3 (13.1-17.9)	5.2 (4.41-6.10)	1.62 (1.41-1.86)	1.93 (1.65-2.25)	1.30 (1.17-1.44)	0.90 (0.85-0.95)	5.60
18	13.4 (11.6-15.5)	3.6 (2.7-4.8)	0.98 (0.68-1.41)	0.68 (0.51-0.91)	1.78 (1.47-2.15)	0.70 (0.63-0.78)	17.34
19	13.8 (12.1-15.74)	3.4 (2.8-4.1)	1.02 (0.91-1.14)	0.75 (0.66-0.95)	1.83 (1.54-2.17)	0.76 (0.65-0.89)	17.15
D[c]	5.8 (4.70-7.15)	6.2 (4.10-9.40)	1.73 (1.42-1.89)	3.30 (3.10-3.51)	4.30 (3.93-4.70)	1.75 (1.57-1.95)	2.81
M[c]	6.2 (5.1-7.5)	0.81 (0.71-0.92)	0.32 (0.28-0.37)	1.30 (1.18-1.43)	1.0 (0.9-1.11)	0.30 (0.27-0.33)	5.24
O[c]	12.3 (10.1-15.0)	2.1 (1.91-2.31)	0.77 (0.71-0.84)	0.44 (0.42-0.46)	1.3 (1.05-1.61)	0.48 (0.48-0.58)	13.95

[a] ED_{50} of chlordiazepoxide/ED_{50} of compound tested = value in Table II. [b] 95% confidence limits are given in parentheses, calculated according to the method of ref 18. [c] D = diazepam, M = madazepam, O = oxazepam. [d] Biological tests were done according to ref 17 and 18. [e] Biological tests were done according to ref 17 and 19. [f] Biological tests were done according to ref 20. [g] Biological tests were done according to ref 21.

Table III. Partition Coefficients (1-Octanol, Aqueous Phosphate Buffer, pH 7.35) for 3-O-Substituted 1,4-Benzodiazepin-2-ones **7-15** and Selected Reference Compounds

compd	log P_{oct}
7	1.98
8	1.99
9	2.04
10	2.37
11	2.14
12	2.48
13	1.74
14	1.71
15	1.96
oxazepam	2.17
medazepam	4.05
chlordiazepoxide	2.50
diazepam	2.66

were combined, washed with water (2 × 30 mL), dried (Na_2SO_4), and evaporated. The crude products were recrystallized from solvent mixtures as indicated in Table I.

Preparations of compounds 16 and 17. To the compound 1 or **2** (10 mmol), dissolved on brief heating in a mixture of dry pyridine (18 mL) and dry acetone (8 mL) and then cooled in an ice bath (ca. −15 °C), a solution of trichloroacetyl chloride (3.5 mL) in dry ether (5 mL) was added dropwise, during 5 min. The reaction mixture was left in ice bath for 30 min, and then dry ether (150 mL) was added, and the mixture was allowed to stand for an additional 30 min. The pyridine hydrochloride crystals obtained were filtered, washed with dry ether, and discarded. The filtrate was washed with HCl (2 × 50 mL) and with water (2 × 50 mL). The organic layer was dried (Na_2SO_4) and evaporated to dryness, and the residue was crystallized from benzene–light petroleum ether (Table I).

3-*O*-α- and 3-*O*-β-1′-Deoxy-2′,3′,4′,6′-tetraacetyl-D-glucopyranosido-1,4-benzodiazepin-2-ones (18 and 19). Compound **8** (10.3 g, 30 mmol) was dissolved in dry benzene (150 mL), and then silver oxide (20 g) and 1-bromo-1-deoxy-2,3,4,6-tetra-acetyl-α-D-glucopyranose (11.8 g, 35 mmol) were added. The mixture was vigorously stirred for 48 h and heated to 70 °C. Thereafter, precipitated solids were filtered off, the filtrate was evaporated, and the residual amorphous material was chromatographed (600 g of silica gel), using benzene–acetone (9:1) as the eluant. The faster moving zone gave 6.8 g of pure **18** (epimeric mixture); an analytical sample was obtained by repeated chromatography, using the same solvent system: IR (KBr) 3245 (NH), 1695 and 1715 (CO, amide), 1748 (CO, acetyl), 1610 (C=N), 2969 and 1480 (CH_2), 1227 (COC, acetyl), 890 cm^{-1} (β-anomeric C—C), 740, 705 cm^{-1}; NMR ($CDCl_3$) δ 2.0 (s, 4 × $COCH_3$), 3.7–5.2 (m, 10 H), 5.75 (d, J = 4.0 Hz, β-anomeric 1 H), 7.2–7.6 (m, 9 H), 10.1 (s, 1 H). Anal. ($C_{31}H_{33}ClN_2O_{12}$) C, H, N.

The slower moving zone gave 7.3 g of pure **19** (epimeric structure); an analytical sample was obtained as with compound **18**: IR (KBr) 3240, 1750, 1715, 1690, 1612, 1480, 1225, 850 (α-anomeric C–C), 735, 705 cm^{-1}; NMR ($CDCl_3$) δ ~2.0 (s, 4 × $COCH_3$), 3.7–5.2 (m, 11 H, including α-anomeric C_1-H), 7.2–7.8 (m, 9 H), 10.4 (s, 1 H). Anal. ($C_{31}H_{33}ClN_2O_{12}$) C, H, N.

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(1) Corresponding address: Chemical Research Company, 6830 Chiasso, Corso San Gottardo 35, Switzerland.
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Synthesis and Biological Activities of Ftorafur Metabolites. 3′- and 4′-Hydroxyftorafur

Al Jeng Lin,* Robert S. Benjamin, Potu N. Rao, and Ti Li Loo

Department of Developmental Therapeutics, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, Texas 77030. *Received February 6, 1979*

Four isomers of ftorafur were synthesized as authentic samples of possible ftorafur (FT) metabolites. 2,3-Dihydrofuran was treated with perbenzoic acid in MeOH to give 2-methoxy-3-hydroxytetrahydrofuran, which upon treatment with Ac_2O/pyridine yielded the key intermediate 2-methoxy-3-acetoxytetrahydrofuran. The other intermediate, 2-ethoxy-4-acetoxytetrahydrofuran, was prepared by acid hydrolysis (HCl/50% EtOH) of 1,1-diethoxy-3,4-dihydroxybutane, followed by acetylation (Ac_2O/pyridine). Treatment of 2,4-bis(trimethylsilyl)-5-fluorouracil with either 2-methoxy-3-acetoxytetrahydrofuran or 2-ethoxy-4-acetoxytetrahydrofuran in 1,2-dichloroethane at room temperature using $SnCl_4$ as catalyst afforded *cis*- and *trans*-3′-OAc-FT or 4′-OAc-FT, respectively. However, *trans*-3′-OAc-FT and *cis*-4′-OAc-FT were the major condensation products. In each case, separation of these cis and trans isomers was achieved by silica gel column chromatography. Treatment of 3′- or 4′-OAc-FT with NH_3/CH_3OH at 5 °C overnight yielded the desired hydroxylated FT. Both *trans*-3′-OH-FT and *cis*-4′-OH-FT showed no significant activity against L1210 up to 100 mg/kg. These two agents produced an inhibitory effect on HeLa cell growth equal to that of ftorafur, with ID_{50} = 200 μg/kg.

(*R*,*S*)-1-(Tetrahydro-2-furanyl)-5-fluorouracil (ftorafur or FT), a pyrimidine antimetabolite, has shown significant antitumor activity in several adenocarcinomas with a spectrum of activity similar to, but less toxic than, 5-fluorouracil (5-FU).[1-3] It was considered a prodrug of 5-FU,[4-7] and microsomal enzymes[8] were probably involved in the conversion of FT to 5-FU in vivo. However, studies of the pharmacologic fate of FT in several species suggested the presence of other metabolites in addition to 5-FU.[9,10] Although, Smolyan-Skaya and Tugarino[7] first demonstrated the in vivo formation of a microbiologically active metabolite from FT, we were the first to report the detection and isolation of hydroxylated FT metabolites from patients' urine after FT administration.[10] These findings received support from other investigators, who described similar metabolites in the urine of rabbits treated with FT.[11] Further, these authors suggested that the structures of the metabolites were 3′-OH- and 4′-OH-FT, based on NMR evidence. However, in their work no authentic samples were available for comparison and the absolute configurations of the hydroxyl group in these metabolites remained undetermined.

The synthesis of the hydroxylated FT metabolites received added impetus from observation in our clinical pharmacological studies of FT. In man, apparently a single intravenous administration of FT at a nontoxic therapeutic dose elicits a sustained plasma concentration of 5-FU several times higher than that achievable by the direct continuous infusion of 5-FU at its maximum tolerated dose, without causing serious effects, particularly serious mucositis.[12] Clearly, FT is not merely a depot form of 5-FU, but most important, our clinical studies suggest that perhaps FT, and more likely its hydroxylated metabolite(s), may selectively protect the gastrointestinal mucosa from 5-FU toxicity.[12] This hypothesis must be verified biochemically, enzymatically, and pharmacologically with synthetic FT metabolites. We now describe the synthesis of the four pairs of diastereoisomers of 3′- and 4′-OH-FT.

Chemistry. Since four pairs of diastereoisomers of hydroxylated FT metabolites may exist, a nonstereospecific approach was used in the synthesis of these isomers. The synthesis of the two isomeric 3′-OH-FT is outlined in Scheme I.

The preparation of *trans*-3-hydroxy-2-methoxytetrahydrofuran (**3**) followed a previously described procedure[13] that involved treatment of 2,3-dihydrofuran with perbenzoic acid in methanol at 5 °C. The acetylation of compound **3** was achieved by using Ac_2O/pyridine as the acetylating agent at room temperature to give compound **4** in good yield. Treatment of 2,4-bis(trimethylsilyl)-5-fluorouracil with compound **4** in 1,2-dichloroethane under the catalysis of stannic chloride[14] at room temperature yielded a mixture of compounds **5** and **6** which were separated on a silica gel column using THF–petroleum

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