A New 2,6-Difluorobenzodiazepinone

THOMAS L. LEMKE*▲, JACKSON B. HESTER, and ALLAN D. RUDZIK

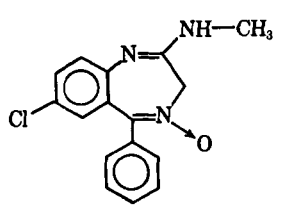
Abstract □ The synthesis of 7-chloro-1,3-dihydro-5-(2,6-difluorophenyl)-2*H*-1,4-benzodiazepin-2-one is reported. The compound was tested and found to be very potent when compared to chlordiazepoxide and diazepam in mice.

Keyphrases □ 2,6-Difluorobenzodiazepinone—synthesis, potency compared to chlordiazepoxide and diazepam, mice □ 7-Chloro-1,3-dihydro-5-(2,6-difluorophenyl)-2*H*-1,4-benzodiazepin-2-one—synthesis, potency compared to chlordiazepoxide and diazepam, mice

The discovery of chlordiazepoxide (1), 7-chloro-2-methylamino-5-phenyl-3*H*-1,4-benzodiazepine 4-oxide (I), a potent tranquilizer and muscle relaxant, has been of considerable importance to the psychopharmacological field. Molecular modification of the basic 5-phenyl-1,4-benzodiazepine moiety has led to several additional clinically useful agents: diazepam (II, R_1 = CH_3, R_2 = H, R_3 = Cl), oxazepam (II, R_1 = H, R_2 = OH, R_3 = Cl), and nitrazepam (II, R_1 = H, R_2 = H, R_3 = NO_2) (2). The large number of derivatives prepared since the initial discovery of biological activity has led to certain patterns of structure–activity relationships and even some general rules applicable to this type of structure (3).

Our interest was in the effect on biological activity of substituents on the 5-phenyl ring. As reported by Sternbach *et al.* (3), substitution in the 2′-position gave the most significant results. Substitution of halogen at the 2′-position generally increased overall activity, while introduction of other substituents usually resulted in decreased activity (*i.e.*, R_2' = OCH_3; CH_3; CF_3; NO_2). It was suggested that the effect was mainly influenced by the size of the substituent and did not depend on its electroinductive properties, since the electron-releasing as well as electron-withdrawing groups reduced potency. Furthermore, substituents in the 3′- or 4′-position had an unfavorable effect. Recently, several disubstituted 5-phenyl analogs were reported (only one substituent in the *ortho*-position), but no biological results were included (4, 5).

There was also interest in the effect of multiple substitution in the 5-phenyl ring and it was decided to investigate the effect of 2,6-difluoro substitution. This compound would have two substituents with a considerable electronic change from hydrogen but without much steric change.



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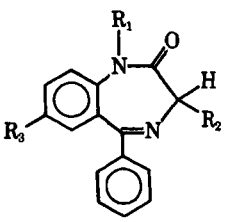

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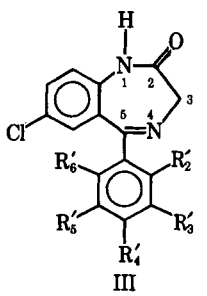
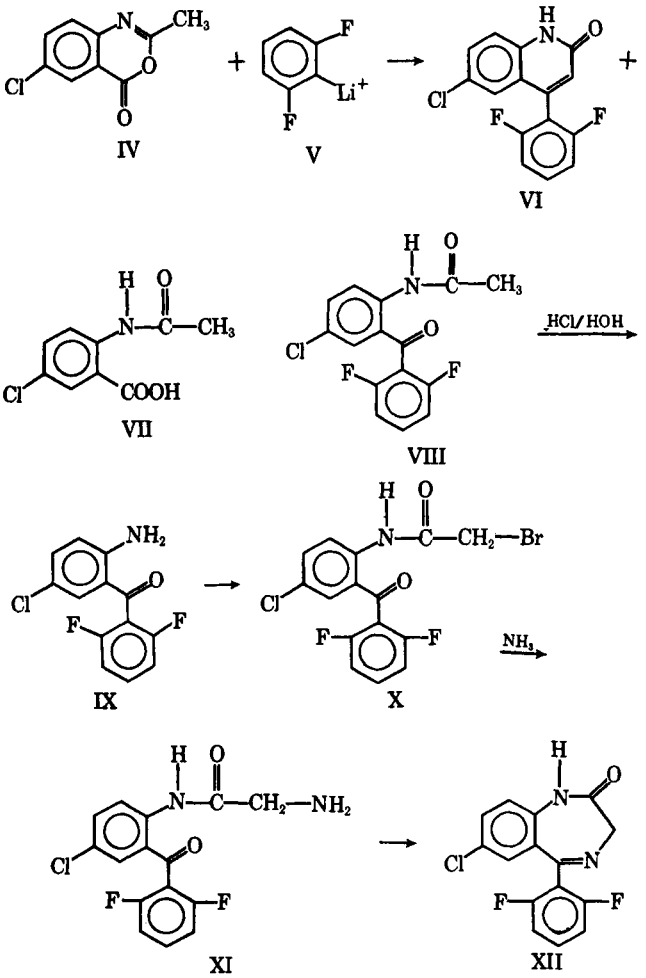


III

CHEMISTRY

The synthetic sequence for the synthesis of 7-chloro-1,3-dihydro-5-(2,6-difluorophenyl)-2*H*-1,4-benzodiazepin-2-one (XII) is shown in Scheme I. The 2-lithio-1,3-difluorobenzene (5), prepared from 1,3-difluorobenzene and *n*-butyllithium (7), upon addition to 6-chloro-2-methyl-4*H*-3,1-benzoxazin-4-one (IV) gave 6-chloro-4-(2,6-difluorophenyl)carbostyril (VI) in addition to 2-acetamido-5-chloro-2′,6′-difluorobenzophenone (VIII). Compound VI may arise from base-catalyzed cyclization of VIII. Unreacted IV, after acid workup,



Scheme I

Table I—Administration of the Compounds to Mice

Compounds	LD_{50}	LRR_{50}	Tr_{50}	Ch_{50}	D_{50}	P_{50}	Antagonism of: Nicotine TE	Nicotine L	Thiosemicarbazide	Strychnine	Electroshock	Pentylenetetrazol	Potentiation of Ethanol
Desmethyldiazepam (II) ($R_1 = R_2 = H$, $R_3 = Cl$)	>200	>200	18	1.3	1.4	2	0.28	0.22	0.7	6	142	2.5	3
Chlordiazepoxide (I)	>200	63	14	4.5	2.2	3.6	1.6	1.4	2.8	32	28	5.0	9
Diazepam (II) ($R_1 = CH_3$, $R_2 = H$, $R_3 = Cl$)	>200	>50	7	2.0	0.7	1.3	0.28	0.28	0.7	8	50	0.8	0.9
III ($R_2' = F$, $R_3' = R_4' = R_5' = R_6' = H$)	>25	>25	4	0.36	0.04	0.32	0.01	0.01	0.16	1.6	50	0.32	2
III ($R_2' = Cl$, $R_3' = R_4' = R_5' = R_6' = H$)	>100	>100	22	0.5	0.16	0.4	0.08	0.08	0.11	45	23	0.17	1.8
XII	>1000	>50	8	0.5	0.06	0.11	0.02	0.02	0.18	2	63	0.14	0.18
VI	>200	>200	>200	142	32	63	63	79	—	—	—	—	—

was recovered as 2-acetamido-5-chlorobenzoic acid (VII). The remaining sequence uses procedures previously reported by Sternbach *et al.* (8, 9).

PHARMACOLOGY

Male, albino mice (CF-1)[1], weighing 18–22 g., were used for all studies reported here. The test compounds were dissolved or suspended in 0.25% aqueous methylcellulose solution and administered intraperitoneally. Procedures for measuring: (*a*) acute toxicity (LD_{50}), (*b*) the effect of the compound on overt behavior, loss of righting reflex (LRR_{50}), traction (Tr_{50}), chimney (Ch_{50}), dish (D_{50}), and pedestal (P_{50}), and (*c*) antagonism of nicotine-induced tonic extensor convulsions (TE) and death (L) were described previously, as was the antagonism of thiosemicarbazide and strychnine convulsions and lethality and electroshock convulsions (10).

Other test procedures used for this series of compounds are described here.

Potentiation of Ethanol Narcosis—A subhypnotic dose of ethanol (10 ml./kg. of a 50% aqueous solution) was administered orally to groups of six mice 30 min. after the test compound. Thirty minutes later, each mouse was examined for loss of righting reflex. The dose of test compounds was decreased in 0.3 log intervals; the number of animals in each group that exhibited loss of righting reflex was used as a quantal response parameter for calculating the effect dose (ED_{50}) of the test compound.

Antagonism of Pentylenetetrazol-Induced Clonic Convulsions—Pentylenetetrazol (85 mg./kg.) in aqueous solution was administered subcutaneously to groups of six mice 30 min. after the test compound. The mice were observed for 20 min. for symptoms of clonic convulsions. The dose of test compound was decreased in 0.3 log intervals; the number of animals in each group protected against the pentylenetetrazol-induced clonic convulsions was used as a quantal response parameter for calculating the effect dose (ED_{50}) of the test compound.

Result—The pharmacologic properties of desmethyldiazepam, diazepam, chlordiazepoxide, and the experimental compounds are shown in Table I. In general, diazepam is more active than the desmethyl derivative on all end-points. Diazepam is also 2–10 times more active than chlordiazepoxide in these test systems. Halogen substitution on the *ortho*-position of the 5-phenyl ring markedly increases the activity of the compound. Compound XII, the 2,6-difluoro derivative, is 2–20 times more active on antagonizing pentylenetetrazol-induced clonic convulsions and in potentiating ethanol narcosis. There appears to be little difference in the activity of the *ortho*-fluoro and chloro derivative.

EXPERIMENTAL[2]

2-Acetamido-5-chloro-2′,6′-difluorobenzophenone (VIII)—To a solution of 114 g. (1.0 mole) of *m*-difluorobenzene (V) in 800 ml. of previously distilled tetrahydrofuran, cooled to −50° and maintained under N_2, was added 320 ml. (1.0 mole) of *n*-butyllithium in *n*-heptane with stirring. The addition took about 50 min. and was followed by stirring for an additional 2 hr. at −50°. The cold lithio compound was then added with stirring over a 40–50-min. period to a solution of 187.8 g. (0.97 mole) of IV in 1400 ml. of benzene and 1000 ml. of tetrahydrofuran at 25°. The reaction was allowed to stir at 25° under N_2 for 20 hr., at which time 1000 ml. of 2 *N* HCl was added. The layer was separated and discarded. A yellow suspended solid, present in the organic layer, was removed by filtration. The solid amounted to 42.7 g., m.p. 310–315° dec., of VI. An analytic sample was prepared by recrystallation from dimethyl sulfoxide.

Anal.—Calc. for $C_{15}H_8ClF_2NO$: C, 61.76; H, 2.76; Cl, 12.15; N, 4.80. Found: C, 61.87; H, 2.72; Cl, 12.74; N, 4.74.

The organic layer was washed with cooled, dilute NaOH and dried. Acidification of the aqueous extract led to recovery of VII. Upon concentration of the organic layer, 101 g. of a semisolid was recovered. The semisolid was partially dissolved in 2100 ml. of hot Skellysolve B; on concentration and cooling, 40 g. of crude product (VIII) was recovered. The material, which was insoluble in Skellysolve B, amounted to 38 g.; this material was chromatographed on 2.5 kg. of silica gel[3] using ethyl acetate–cyclohexane (1:1) as the eluting solvent (fraction size 175 ml. each). Fractions 18–26 contained 18.1 g. of product, VIII, which was combined with the above material and recrystallized from cyclohexane, m.p. 118–120°.

Anal.—Calc. for $C_{15}H_{10}ClF_2NO_2$: C, 58.17; H, 3.25; Cl, 11.45; F, 12.27; N, 4.52. Found: C, 58.11; H, 3.38; Cl, 11.53; F, 12.24; N, 4.20.

2-Amino-5-chloro-2′,6′-difluorobenzophenone (IX)—A suspension of 4.2 g. (0.014 mole) of VIII in 350 ml. of concentrated HCl and 350 ml. of H_2O was heated on a steam bath with stirring and under N_2 until complete solution resulted. The solution was cooled and made basic with 50% NaOH. The solid was removed by extraction with CH_2Cl_2. The combined organic extract was dried and concentrated. The residue was recrystallized from cyclohexane to give 2.4 g., m.p. 103–105°, of product, IX.

Anal.—Calc. for $C_{13}H_{10}ClF_2NO$: C, 58.33; H, 3.01; Cl, 13.24; F, 14.20; N, 5.23. Found: C, 58.33; H, 3.29; Cl, 13.31; F, 14.87; N, 5.14.

2-Bromo-4′-chloro-2′-(2,6-difluorobenzoyl)acetanilide (X)—To a solution of 2.7 g. (0.01 mole) of IX in 100 ml. of benzene, through which a rapid stream of N_2 was passed, was added 3.03 g. (0.015 mole) of bromoacetyl bromide. A precipitate formed shortly after the addition was complete, and TLC of the benzene layer showed loss of starting material. The benzene was removed and the white solid was recrystallized from cyclohexane to give 3.4 g. of X, m.p. 146–147.5°.

Anal.—Calc. for $C_{15}H_9BrClF_2NO_2$: C, 46.36; H, 2.33; Br, 20.56; Cl, 9.12; F, 9.78; N, 3.60. Found: C, 46.46; H, 2.48; Br, 20.68; Cl, 9.21; F, 9.49; N, 3.82.

2-Amino-4′-chloro-2′-(2,6-difluorobenzoyl)acetanilide (XI)—To a solution of 26 g. (0.067 mole) of X in 350 ml. of CH_2Cl_2 was added 350 ml. NH_3. The solution was stirred under reflux for 5 hr. and then allowed to stir overnight while the excess NH_3 evaporated.

[1] Carworth Farms.

[2] Melting points are uncorrected and were determined using a Thomas-Hoover melting-point apparatus.

[3] Brinkmann.

The resulting CH_2Cl_2 solution was filtered to remove the NH_4Br and concentrated, and the residue was recrystallized from 2 l. of cyclohexane to give 19.4 g. of product, m.p. 133–135°. The product appeared to contain cyclic XII as reflected in the analysis.

Anal.—Calc. for $C_{15}H_{11}ClF_2N_2O_2$: C, 55.48; H, 3.42; Cl, 10.92; F, 11.70; N, 8.63. Found: C, 56.69; H, 3.99; Cl, 11.19; F, 11.06; N, 8.34.

1,3-Dihydro-5-(2,6-difluorophenyl)-7-chloro-2*H*-1,4-benzodiazepin-2-one (XII)—A solution of 21.0 g. (0.065 mole) of XI in 300 ml. of pyridine was heated under reflux under N_2 for 18 hr. The reaction mixture was concentrated and the residue was washed with Skellysolve B and recrystallized from ethyl acetate–Skellysolve B and then ethyl acetate to give 11.7 g. of product in Crop 1, m.p. 248–249°, and 2.3 g. in Crop 2, m.p. 244–246°. This material was found to contain solvated ethyl acetate; therefore, an additional recrystallization of a 10-g. sample from EtOH was performed to give 7.0 g. in Crop 1, 1.3 g. in Crop 2, and 1.0 g. in Crop 3, m.p. 251–253°.

Anal.—Calc. for $C_{15}H_9ClF_2N_2O$: C, 58.74; H, 2.96; Cl, 11.56; F, 12.39; N, 9.14. Found: C, 58.84; H, 2.89; Cl, 11.56; F, 12.01; N, 9.11.

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Characterization of Sulfonamides by TLC Using Metal Ions▲

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Abstract □ The reactions of metal ions such as copper (II), cobalt (II), nickel (II), and cerium (IV) with 15 sulfonamides on TLC plates were examined to obtain a suitable reagent for the visualization of these drugs. A solution of copper acetate in methanol produces spots of varying colors, whereas an acidic solution of ceric sulfate gives yellow or purple spots with all the sulfonamides studied. Cobalt (II) and nickel (II) are specific for a limited number of sulfonamides.

Keyphrases □ Sulfonamides—characterization by TLC, using metal ions □ TLC—characterization of 15 sulfonamides using metal ions

Various chromatographic procedures have been proposed for the separation and identification of sulfonamides. The literature on such methods employing TLC was recently reviewed (1, 2). *p*-Dimethylaminobenzaldehyde, coupling with *N*-(1-naphthyl)ethylenediammonium dichloride after nitrous acid treatment, vanillin, and 1-phenyl-3-methyl-2-pyrazoline-5-one in pyridine with potassium cyanide after chlorine treatment have been generally employed as locating agents for sulfonamides. These are either nonspecific, because they give colors with other compounds containing the same functional groups, or are unable to distinguish between various sulfonamides. The use of metal ions as characterizing agents for sulfonamides has not been studied in detail. Güven and Pekin (3) used an ammoniacal solution of copper and, more recently, Clarke and Humphreys (4) employed aqueous copper sulfate as a visualizing agent for sulfonamides on alkaline TLC plates.

The present study describes the reactions of 15 sulfonamides on chromatoplates with several metal ions such as copper (II), nickel (II), cobalt (II), and cerium (IV) in an attempt to evolve a suitable agent for characterizing individual sulfonamides by different color spots.

EXPERIMENTAL

Materials—Pure drug samples were obtained from various pharmaceutical companies. Silica gel F 254 was used[1]. Metal salts, solvents, and other reagents were analytical grade.

TLC Plates—The 20 × 20-cm. glass plates were coated with a 250-μm. layer of silica gel slurry in water and dried for 1 hr. at 110°. The dried plates were cooled to room temperature before use.

[1] E. Merck, Germany.