Derivatives of 1,2,3,11a-Tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione as Anxiolytic Agents

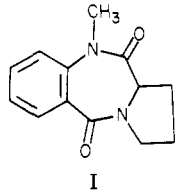
William B. Wright, Jr.,* Herbert J. Brabander, Eugene N. Greenblatt, Ivana P. Day, and Robert A. Hardy, Jr.

Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, New York 10965. *Received April 28, 1978*

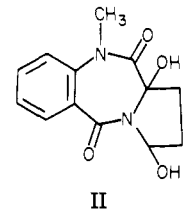
A study of the pharmacological properties of pyrrolo[2,1-*c*][1,4]benzodiazepine derivatives led to the choice of (+)-1,2,3,11a-tetrahydro-10-methyl-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione as a candidate for anxiolytic evaluation in a limited clinical trial in man. Metabolism studies in laboratory animals have pointed to rapid hydroxylation, possibly in the 3 and 11a positions. A series of compounds containing methyl groups in one or more of these positions has been prepared in an effort to block metabolism and thereby obtain more active or longer acting compounds. All of these derivatives were less active than the parent compound.

In recent years, compounds having the pyrrolo[2,1-*c*]-[1,4]benzodiazepine-5,11(10*H*)-dione moiety have been described in studies of anthramycin and related compounds as antitumor agents,[1-3] in compounds with antiphage activity,[4] and as intermediates for compounds with analgesic antagonist, antiinflammatory, and psychomotor depressant activity.[5]

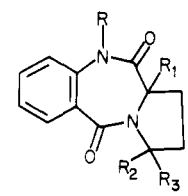
Our interest in compounds of this structure was directed to their anxiolytic activity. This led to the facile synthesis of a number of analogues from isatoic anhydrides and proline derivatives and the choice of (+)-1,2,3,11a-tetrahydro-10-methyl-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione (I; Table I, **1**),[6] derived from natural



I



II



III, R,R_1,R_2,R_3 = H or CH_3

L-proline, as a candidate for a limited clinical trial in man. In laboratory animals, **1** exhibited satisfactory anxiolytic activity while lacking the depression which accompanies the anxiolytic activity of chlordiazepoxide and diazepam. The activity of this series appeared to reside entirely in derivatives prepared from L-proline (e.g., **1** vs. **2** and **3**), and the N_{10}-methyl derivatives were invariably more active than the corresponding N_{10}-hydrogen analogues. Of the many compounds studied, only the 7-fluoro derivative **6** was more active than **1**.

Metabolism studies in laboratory animals pointed to rapid 10-demethylation and/or aliphatic hydroxylation.[7] Mono- and dihydroxylated derivatives were indicated, and the 3,11α-dihydroxy derivative II was suggested by GC–MS and NMR[8] as a possible structure for one of several hydroxylated metabolites. This suggested that a compound with one or more substituents in these positions (III) would be less readily oxidized and might be more active or longer acting. We, therefore, proceeded to prepare the compounds described in Table II.

Pharmacology. Anxiolytic screening was carried out by antagonism of pentylenetetrazole-induced seizures in rats.[9] Dose levels of the test compound were administered orally in 2% starch to groups of at least four rats. At the estimated time of peak effect, the rats were treated intravenously with 21–23 mg/kg of pentylenetetrazole (the dose estimated to produce clonic seizures in 99% of the unprotected rats). Protection (or lack thereof) was observed in each animal, and an ED_{50} was calculated based on 50% protection (see Table I). In follow-up tests, **1** was active in antagonizing pentylenetetrazole-induced convulsions in mice and dogs, increased conflict responding in the thirsty rat,[10] and was active in a squirrel monkey conflict procedure. In preliminary trials in humans, **1** did not appear to possess sufficient anxiolytic activity to warrant further development, compared to diazepam as a positive control.

Of the 3- and 11α-methyl derivatives (Table II), only **14** (ED_{50} = 59) and **15** (ED_{50} = 79) were active at the screening dose.

Experimental Section

All melting points were taken using a Mel-Temp apparatus with open capillaries and are uncorrected. Unless otherwise noted, microanalyses of all compounds were within ±0.4% of the theoretical values for C, H, N, and F (if present). Spectral data on selected compounds are on file at Lederle Laboratories.

The following chemical intermediates were prepared by literature procedures: 2-methylproline,[11] 2,5-dimethyl-1-pyrroline-5-carboxylic acid hydrochloride,[12] 5,5-dimethylproline,[13] *cis*-5-methylproline hydrochloride,[14] *trans*-5-methylproline,[14] and 6-fluoroisatoic anhydride.[15]

General Procedure A. A small round-bottom flask containing 0.01 mol of the isatoic anhydride, 0.011 mol of the amino acid, and 1.1 mL of Me_2SO was immersed in an oil bath at 100–110 °C. The temperature of the bath was gradually increased to about 155–160 °C and held until the reaction appeared by TLC to be over (1–6 h). As the temperature was increased, solution gradually occurred and fine bubbles of CO_2 were evolved. The reaction mixture was partially cooled, taken up in C_6H_6, washed with H_2O, concentrated, and recrystallized from a suitable solvent. In some cases the product was insoluble in the C_6H_6–H_2O mixture and was filtered off. When crystallization was difficult, final purification was by partition chromatography on Celite diatomaceous earth.

TLC was measured using acetone–hexane (40:60) and silica gel plates. Under UV light, the bright fluorescent spot around R_f 0.6–0.7, due to the isatoic acid, disappeared and a darker absorbing spot at a slightly lower R_f, due to the desired product, appeared. Spots near the origin were due to the open amino acid intermediate or undesirable by-products.

General Procedure B. A mixture of 0.1 mol of the isatoic anhydride, 0.11 mol of the proline derivative, and about 50 mL of Me_2SO was heated on the steam bath for 2–4 h and diluted with H_2O. The precipitate which separated was filtered off, dried, and recrystallized from a suitable solvent.

Procedure C. A mixture of 0.02 mol of the isatoic anhydride, 3.0 g (0.021 mol) of 5,5-dimethylproline, and 10 mL of Me_2SO was heated on the steam bath for 20 h and diluted with H_2O and C_6H_6, and the layers were separated. The C_6H_6 layer was washed with H_2O and concentrated to an oily residue which would not crystallize. Partition chromatography (Celite diatomaceous earth column) resulted in a crystalline product which was collected with Et_2O.

Procedure D. ***cis*-1,2,3,11a-Tetrahydro-3,10-dimethyl-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione (13).**

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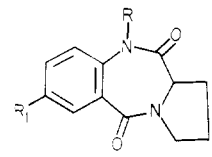
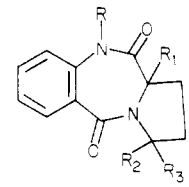
Table I. Derivatives of 1,2,3,11a-Tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione



compd	R	R_1	$[\alpha]^{25}_D$[a]	yield, %	mp, °C	formula	Anti PTZ[b] ED_{50}
1	CH_3	H	+486	70[c,d]	121-122	$C_{13}H_{14}N_2O_2$	9.9 (5.5-17.8)
2	CH_3	H	0	76[c,d]	124-126	$C_{13}H_{14}N_2O_2$	20.2 (11.4-35.5)
3	CH_3	H	-477	35[c,d]	120-122	$C_{13}H_{14}N_2O_2$	>200
4	H	H	+523	82[e,f]	220-222[g]	$C_{12}H_{12}N_2O_2$	35.0 (17.0-74.0)
5	H	F	+443	67[e,d]	199-201	$C_{12}H_{11}FN_2O_2$	23.0 (17.9-29.9)
6	CH_3	F	+417	68[h]	131-132	$C_{13}H_{13}FN_2O_2$	3.9 (2.7-5.4)
chlordiazepoxide							2.5 (1.7-3.8)
meprobamate							21.5 (13.0-34.4)

[a] About 1% in methanol. [b] Antagonism of pentylenetetrazole-induced seizures in the rat, mg/kg, oral (95% confidence limits). [c] Procedure A. [d] Recrystallized from ethyl acetate. [e] Procedure B. [f] Recrystallized from ethanol. [g] Reference 2b reports mp 220-222 °C. [h] Procedure F.

Table II. Derivatives of 1,2,3,11a-Tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione Which Contain Methyl Groups in the Pyrrolidine Ring



compd	R	R_1	R_2	R_3	procedure	yield, %	mp, °C	formula
7	CH_3	CH_3	H	H	A	40[a]	122-124	$C_{14}H_{16}N_2O_2$
8	H	CH_3	H	H	A	44[b]	242-244	$C_{13}H_{14}N_2O_2$
9	CH_3	CH_3	CH_3	H	A	36[c]	175-177	$C_{15}H_{18}N_2O_2$
10	H	CH_3	CH_3	H	A	38[b]	187-189	$C_{14}H_{16}N_2O_2$
11	CH_3	H	CH_3	CH_3	C	50[c]	93-95	$C_{15}H_{18}N_2O_2$
12	H	H	CH_3	CH_3	C	14[d]	191-193	$C_{14}H_{16}N_2O_2$
13	CH_3	H	CH_3	H	D	82[a,e]	148-150	$C_{14}H_{16}N_2O_2$
14	CH_3	H	CH_3	H	E	56[f]	132-135	$C_{14}H_{16}N_2O_2$
15	H	H	CH_3	H	D	50[a,e]	169-171	$C_{13}H_{14}N_2O_2$

[a] Recrystallized from ethyl acetate. [b] Recrystallized from ethanol. [c] Purified by partition chromatography (heptane-methanol). [d] Purified by partition chromatography [heptane-CH_2Cl_2-$CH_3O(CH_2)_2OH$-H_2O]. [e] Cis isomer. [f] Trans isomer.

A mixture of 15.0 g (0.091 mol) of *cis*-5-methylproline hydrochloride, 9 mL (0.09 mol) of *N*-methylmorpholine, and 50 mL of Me_2SO was stirred, 14.1 g (0.08 mol) of *N*-methylisatoic anhydride was added, and the reaction mixture was heated at reflux temperature for 6 h. Water and C_6H_6 were added and the layers were separated. The C_6H_6 layer was washed with H_2O and concentrated and the residue was treated with Et_2O. Crystals separated and were recrystallized from EtOAc.

***cis*-1,2,3,11a-Tetrahydro-3-methyl-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione (15).** This compound was prepared as above but precipitated when H_2O was added at the end of the reflux period, and C_6H_6 extraction was not required.

Procedure E. *trans*-1,2,3,11a-Tetrahydro-3,10-dimethyl-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione (14). A flask containing 1.68 g (0.095 mol) of *N*-methylisatoic anhydride and 1.29 g (0.01 mol) of *trans*-5-methylproline was immersed in an oil bath at 130 °C and left for 95 min while the temperature was gradually raised to 180 °C. The reaction mixture was boiled with 20 mL of EtOAc and some dark insolubles were filtered off. The filtrate was concentrated and the residue was mostly dissolved in 80 mL of Et_2O. After filtration, the Et_2O was concentrated to a low volume, and crystals separated and were filtered off.

Procedure F. (+)-7-Fluoro-1,2,3,11a-tetrahydro-10-methyl-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione (6). A mixture of 5.0 g (0.018 mol) of (+)-7-fluoro-1,2,3,11a-tetrahydro-5*H*-pyrrolo[2,1-*c*][1,4]benzodiazepine-5,11(10*H*)-dione, 1.19 g (0.022 mol) of $NaOCH_3$, and 60 mL of EtOH was stirred for 2 h, and 3.4 mL (0.054 mol) of MeI was added. The clear solution was allowed to stand overnight, concentrated to remove the solvent, extracted with C_6H_6, washed with H_2O, and again concentrated. The oily residue was triturated with hexane and the crystalline product was filtered off and recrystallized from EtOAc.

2,5-Dimethylproline. A mixture of 9.6 g (0.07 mol) of 2,5-dimethyl-1-pyrroline-5-carboxylic acid hydrochloride,[13] 200 mL of MeOH, and 200 mg of platinum oxide was shaken in a Parr hydrogenator under a hydrogen pressure of about 45 psi until reduction was complete. The catalyst was filtered off and the mother liquor was concentrated. The crystalline residue was washed onto a filter with Et_2O. The yield of 2,5-dimethylproline hydrochloride was 9.4 g (97%), mp 175-178 °C. Anal. Calcd for $C_7H_{14}ClNO_2$: C, 46.80; H, 7.86; Cl, 19.74; N, 7.80. Found: C, 45.90; H, 7.82; Cl, 20.54; N, 7.98.

The above hydrochloride salt (8 g) was dissolved in a little H_2O and passed through a column of Amberlite IR-45. The eluent was concentrated to remove the H_2O and the crystalline 2,5-dimethylproline was collected with Et_2O and dried in a vacuum oven at 65 °C: yield, 6.5 g; mp 235 °C dec. Anal. Calcd for $C_7H_{13}NO_2$: C, 58.72; H, 9.15; N, 9.78. Found: C, 57.32; H, 8.95; N, 9.53.

The above base was successfully used as an intermediate without further purification.

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(7) Metabolism studies were performed by Dr. John A. Morrison.

(8) Spectral studies on metabolites were performed by Messrs. William Fulmor and George O. Morton.

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Book Reviews

Biochemistry of Mental Disorders: New Vistas. Edited by Earl Usdin and Arnold J. Mandell. Marcel Dekker, New York, N.Y. 1978. xviii + 268 pp. 15.5 × 23.5 cm. $29.50.

Each year the Intrascience Foundation conducts a symposium on a timely topic and awards a gold medal to a leader in the field. Individuals previously honored by the Foundation include Carl Djerassi, Russ Merrifield, Manfred Eigen, Bernard Brodie, Sol Spiegelman, and Donald Frederickson. In 1975 Professor Seymour S. Kety received the gold medal and the symposium dealt with "New Vistas in the Biochemistry of Mental Disorders". This book represents the proceedings of the symposium. An introductory chapter by Robert Felix, the first Director of the National Institute of Mental Health, reviews the dramatic changes in psychiatric research over the years and focuses on the crucial role that Seymour Kety played as the first scientific Director of the NIMH in fostering the rigorous, fundamental scientific endeavors which set the stage for the explosive growth in our understanding of brain function. The chapter by Seymour Kety summarizes his own investigations into brain blood flow and psychiatric genetics.

The majority of the book's chapters deals with a variety of basic and clinical approaches to mental illness. Floyd Bloom elegantly reviews "Modern Concepts in Electrophysiology for Psychiatry", focusing on his work mapping out the norepinephrine pathway with cell bodies in the locus coeruleus. Arnold Mandell's chapter deals with effects of drugs on serotonin metabolism in animal brain. William Bunney reviews his clinical research clarifying how drugs act in affective disorders. George Aghajanian writes of his pioneering work clarifying how psychedelic drugs act via serotonin neurons. The following chapter by William Dement summarizes both basic and clinical research relating to sleep, a condition which depends in part on the serotonin systems whose function has been clarified by Aghajanian. Other chapters on serotonin include one on animal effects by Mark Geyer and studies on levels of serotonin metabolites in spinal fluid of psychotic patients by Malcolm Bowers. Biological research in psychiatric patients is also highlighted by studies of lithium reported in chapters by Ole Rafaelsen and Lewis Judd as well as the elegant chapter on spinal fluid metabolites of biogenic amines authored by Frederick Goodwin.

Areas covered in this book represent a broad panorama of research focused upon the biogenic amines and conducted both in animals and man. This theme reflects the major thrust of most research in biological psychiatry. While a great deal is now known of how biogenic amines function as neurotransmitters in the brain and how numerous psychotropic drugs exert their pharmacological effects by altering amine disposition, we still have yet to find the "cause" of any major mental illness in a specific biochemical defect.

The individual speakers at the symposium describe their most up to date research. Unfortunately, even though the book was produced by photocopy from the typed manuscripts, its publication still lags more than 2 years following the symposium. Nonetheless, the high quality of much of the research described in the book makes it a valuable volume for those interested in recent advances in biological psychiatry.

Johns Hopkins University **Solomon H. Snyder**

Psychopharmacology of Thiothixene. By Thomas A. Ban. Raven Press, New York, N.Y. 1978. 236 pp. 15.5 × 24 cm. $12.50.

Thiothixene is the Pfizer analogue of the phenothiazine derivative thioproperazine. Neither the latter compound, nor its structural relative, pipotiazine, was marketed by Rhone-Poulenc, developer of both compounds, nor has that company published on their pharmacology or clinical utility (the pharmacology and clinical use of the long-chain fatty acid esters of pipotiazine have been reported extensively). Thus, a strict comparison of these three structural analogues, pharmacologically and clinically, is not possible.

The motivation for the replacement of the phenothiazine heterocycle by the thioxanthine tricycle originated in the hope that such a substitution might result in an antipsychotic drug with a decreased incidence of extrapyramidal side effects in humans. While thiothixene has been shown clinically to be as effective an agent as chlorpromazine or trifluperazine, it has no demonstrable superiority over those older drugs. Furthermore, its extrapyramidal side effects are quite general and severe and, in the one major study, were seen in 43% of 359 patients. In addition, the drug has already produced at least one case of tardive diskinesia.

The literature up to 1975 (245 research papers; 43 reviews) on the synthesis, metabolism, biochemistry, physiology, pharmacology, and clinical applications of thiothixene comprises the scope of this monograph. The synthesis section can be faulted for its naivete and the extraordinary number of chemical errors and the incorrect use of language present on every page; certainly, the statements (p 17) "Thiothixene may be identified chemically by the orange color produced when 10 mg of the compound is