Isolation, Synthesis, and Pharmacology of Metabolites of 1-(3,4-Dichlorobenzyl)-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone

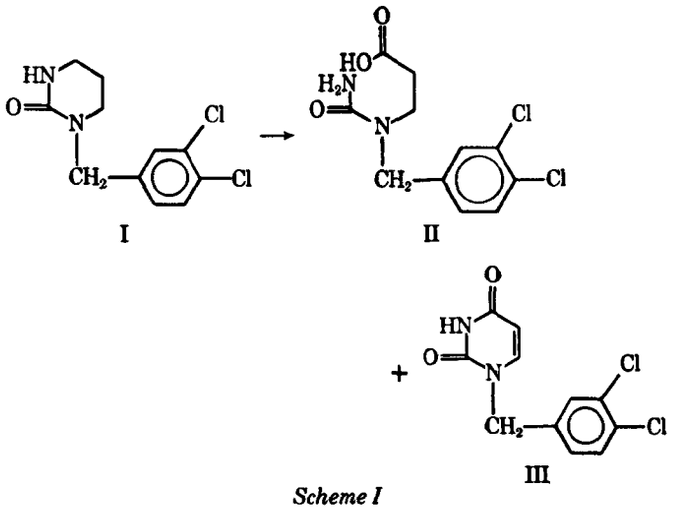
THOMAS J. SCHWAN ˣ, KEITH O. ELLIS, FRANK L. WESSELS, DONALD PUGH, and ROBERT BELL

Received January 21, 1980, from the *Scientific Affairs Department, Norwich-Eaton Pharmaceuticals, Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication May 1, 1980.

Abstract □ The metabolism of 1-(3,4-dichlorobenzyl)-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone, an antianxiety/antidepressant agent, in dogs is reported. Two metabolites, 3-[1-(3,4-dichlorobenzyl)-1-ureido]propanoic acid and 1-(3,4-dichlorobenzyl)uracil, were isolated, characterized, and synthesized. Neither metabolite was acutely toxic, and they did not exhibit antidepressant or antianxiety/anticonvulsant activity.

Keyphrases □ 1-(3,4-Dichlorobenzyl)-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone—isolation, synthesis, and biological activity of metabolites □ CNS agents—1-(3,4-dichlorobenzyl)-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone, isolation, synthesis, and biological activity of metabolites

1-(3,4-Dichlorobenzyl)-3,4,5,6-tetrahydro-2(1*H*)-pyrimidone (I) has been shown to possess antidepressant and antianxiety activity in animals (1). This report describes the isolation, characterization, synthesis, and pharmacological evaluation of two metabolites of this compound (II and III), which are referred to as Metabolites A and B, respectively (Scheme I).



Scheme I

RESULTS AND DISCUSSION

Isolation and Characterization of Metabolite A—Urine (pH 6.5–7.0), collected (2–24 hr) from female beagles administered 40 mg of I/kg po, was extracted with ether. Metabolite B was isolated from the ether extract. After the urine was adjusted to pH 3, further extraction with ether and concentration of the extract to near dryness gave a crystalline material (Metabolite A) which was washed with acetone.

Combustion analysis of Metabolite A indicated an empirical formula of $C_{11}H_{12}Cl_2N_2O_3$. The IR spectrum of this metabolite exhibited strong absorption at 2.95 and 3.05 (NH_2) μm, several bands in the 3.50–5.20-μm region, and a strong carbonyl band at 5.90 μm. Broad absorption was noted in the 6.20–6.50-μm region.

The NMR spectrum of Metabolite A was characterized by a multiplet in the δ 7.18–7.70 region (three aromatic protons), a singlet at δ 4.61 integrating for two protons (methylene group attached to aryl group and nitrogen), two triplets at δ 2.50 and 3.30, each of which integrated for two protons (two aliphatic methylene groups), a broad exchangeable singlet at δ 6.08 integrating for two protons (amino), and an exchangeable proton at δ ~9.0 (carboxylic acid).

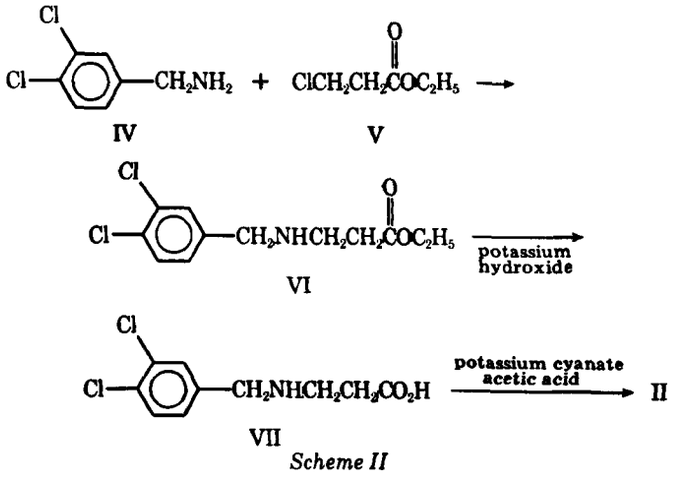
The combustion analysis and the IR and NMR spectra permitted a tentative characterization of Metabolite A as 3-[1-(3,4-dichlorobenzyl)-1-ureido]propanoic acid (II), which was reported previously (2).

Synthesis of Metabolite A—The structural assignment for Metabolite A was verified by independent synthesis of II in a three-step sequence originating with 3,4-dichlorobenzylamine (IV) (Scheme II). Reaction of IV with ethyl 3-chloropropionate (V) gave an ester (VI), which afforded the acid (VII) upon base hydrolysis. Acid-catalyzed treatment of VII with potassium cyanate resulted in the urea (II), whose IR and NMR spectra were identical to those of the isolated metabolite. Moreover, the synthetic material and the isolated metabolite were not separable when cochromatographed in four TLC solvent systems.

Isolation and Identification of Metabolite B—The residue remaining from evaporation of the ether extract of neutral urine was chromatographed on 2.0-mm thick TLC plates[1]. Development with ethyl acetate, elution of the R_f 0.48 zone with methanol, and subsequent separation using a Sephadex G-10 column gave a white residue. The NMR spectrum of this metabolite was characterized by a doublet centered at δ 7.83 (J = 8 Hz) (uracil H-6), a multiplet in the δ 7.25–7.68 region (three aromatic protons), a doublet centered at δ 5.65 (J = 8 Hz) (uracil H-5), a singlet at δ 4.93 integrating for two protons (methylene group attached to aryl group and nitrogen), and a broad exchangeable singlet at δ 11.36 integrating for one proton (uracil 3-NH). These data permitted Metabolite B to be tentatively identified as 1-(3,4-dichlorobenzyl)uracil (III).

Synthesis of Metabolite B—The structural assignment of Metabolite B was confirmed by synthesis of III from uracil (VIII) and 3,4-dichlorobenzyl chloride (IX) using the general procedure of Baker and Chheda (3) (Scheme III), who employed UV spectral data to prove the 1-substituted structure for their products. The NMR spectrum of synthetic III was identical to that of the isolated metabolite. Furthermore, the synthetic uracil and the biological material were not separable in four TLC solvent systems.

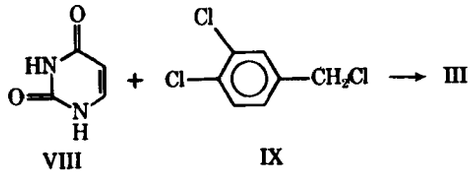
The reason why Metabolite B was extracted from urine at pH 6.5–7.0 while Metabolite A was extracted after the urine was adjusted to pH 3 is not known. It is unlikely that Metabolite A is generated from Metabolite B in acidic media because the latter product is isolated from an acidic medium (see *Experimental*). Furthermore, the dihydrouracil (X) is an unlikely hydrolytic precursor of Metabolite A because the former product



Scheme II

[1] Brinkmann type F-254.

0022-3549/80/1000-1203$01.00/0



VIII IX

Scheme III

is generated in acidic media (2). The presence of appreciable quantities of carboxylate ion at near-neutral pH conditions could account for failure of ether extraction of the product under these conditions.

Compounds II and III, the identified metabolites of I, were evaluated in selected pharmacological tests to determine their contribution to the pharmacological activity of the parent compound. The two metabolites did not exhibit pharmacological activity in gross observation tests. At doses up to 800 mg/kg po in mice, there were no changes in behavior or autonomic signs, and no deaths occurred within 72 hr.

The major pharmacological effect of the parent compound (I) is antidepressant/antianxiety activity (1) (Table I). Therefore, these metabolites were evaluated for such activity using anti-tetrabenazine-induced ptosis and the prevention of pentylenetetrazol-induced tonic extensor seizures. In addition, other chemical convulsants were included. These two metabolites were inactive in all of the antidepressant and anticonvulsant/antianxiety tests conducted.

It is concluded that II and III are not acutely toxic and do not contribute to the antidepressant/antianxiety activity of I.

EXPERIMENTAL[2]

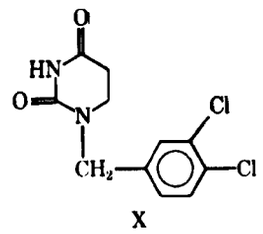
Isolation and Identification of Metabolite A—Two female beagles were administered I at a dose of 40 mg/kg/day once daily for 4 days. Urine was collected at 0–8- and 8–24-hr intervals until 20 liters of urine was accumulated. After each aliquot of urine was collected, it was stored at −10°.

The frozen urine samples were thawed under warm water, and an aliquot was taken for pH determination. The pH of the urine samples ranged from 6.5 to 7.0. The urine was extracted three times with equal volumes of ether. The ether extracts were combined and evaporated, and the residue was stored at −10°.

The pH of the ether-extracted urine was adjusted to ~3.0 with 1 *N* HCl, and the urine was extracted again three times with equal volumes of ether. The ethereal extracts were concentrated to near dryness on a rotating evaporator[3]. Just prior to dryness, a small amount of water was added to the flask. Crystalline material began to separate after the flask was allowed to stand at room temperature for 15 min. The crystalline material was separated by filtration and washed 12 times with 2-ml portions of acetone. Approximately 135 mg of the crystalline material remained after this washing. This crystalline precipitate was used to obtain the physical data on Metabolite A; NMR (dimethyl sulfoxide-d_6): δ 7.18–7.70 (m, 3H, aromatic CH), 4.61 (s, 2H, aromatic CH_2N), 2.50 and 3.30 (two t, 4H, aliphatic CH_2), 6.08 (broad s, 2H, exchangeable, NH_2), and 9.0 (broad s, 1H, exchangeable, CO_2H); IR (KBr): 2.95, 3.05 (NH_2), 3.50–5.20 (H-bonded acid), 5.90 (C=O), and 6.20–6.50 (C=C) μm.

Anal.—Calc. for $C_{11}H_{12}Cl_2N_2O_3$: C, 45.38; H, 4.16; N, 9.62. Found: C, 45.35; H, 4.07; N, 9.58.

Synthesis of Metabolite A—*Ethyl 3-(3,4-Dichlorobenzylamino)-propanoate (VI)*—A mixture of 17.6 g (0.10 mole) of IV, 13.6 g (0.10 mole) of V, 13.8 g (0.10 mole) of potassium carbonate, and 15.0 g (0.10 mole) of



X

[2] Melting points were determined on a Mel-Temp apparatus and are uncorrected. NMR spectra were determined on a Varian A-60A instrument using tetramethylsilane as the internal standard, and IR spectra were determined on a Perkin-Elmer model 137B spectrophotometer.

[3] Buchler Instruments.

Table I—Pharmacology of the Metabolites of I Compared to the Parent Compound

Compound	Antitetrabenazine Activity[a] at 50 mg/kg po, mg/kg	Percent Protection in Anticonvulsant/Antianxiety Tests at 200 mg/kg po				
		PTZ[b]	ISON[c]	PTZ[d]	PICRO[e]	STRY[f]
I	17[g]	23[g]	146[g]	0	0	0
II	>50	0	0	0	20	10
III	>50	0	0	10	30	0

[a] Prevention of tetrabenazine-induced (35 mg/kg ip) ptosis in mice. [b] Prevention of pentylenetetrazol-induced (45 mg/kg iv) tonic extensor seizure. [c] Prevention of isoniazide-induced (340 mg/kg ip) tonic extensor seizure. [d] Prevention of pentylenetetrazol-induced (112.5 mg/kg ip) clonic convulsions. [e] Prevention of picrotoxin-induced (15 mg/kg ip) tonic extensor seizure. [f] Prevention of strychnine-induced (2.5 mg/kg ip) tonic extensor seizure. [g] ED_{50} data taken from Ref. 1.

sodium iodide in 150 ml of absolute ethanol was stirred and refluxed for 18 hr, cooled, and poured into 250 ml of cold tap water. The mixture was extracted with chloroform (3 × 125 ml) and the combined extracts were washed with 200 ml of water, dried over magnesium sulfate, and concentrated to dryness *in vacuo* to give 33.8 g (>100%) of a crude oily product; IR (film): 3.0 (NH), 5.80 (C=O), and 8.0–8.10 (COC ester) μm.

3-(3,4-Dichlorobenzylamino)propanoic Acid (VII)—A solution of 23 g of potassium hydroxide dissolved in 120 ml of water was added to VI (0.10 mole). The mixture was stirred and refluxed for 3.0 hr, cooled, and made slightly acidic with acetic acid. Then the solution was extracted with ethyl acetate (2 × 75 ml), and the product was separated from the aqueous phase after the second extraction. The product was filtered, washed with water, and dried at 60° for 18 hr to give 19.0 g (76%) of the product, mp 181–185°. Recrystallization from distilled water gave an analytical sample, mp 188–191°; IR (mineral oil): 3.7–4.8 (N^+H), 6.09 (C=O), and 6.35–6.45 (COO^-) μm.

Anal.—Calc. for $C_{10}H_{11}Cl_2NO_2$: C, 48.41; H, 4.47; N, 5.68. Found: C, 48.12; H, 4.59; N, 5.60.

3-[1-(3,4-Dichlorobenzyl)-1-ureido]propanoic Acid (II)—To a solution of 5.87 g (0.0236 mole) of VII in 30 ml of acetic acid was added quickly a solution of 3.72 g (0.0472 mole) of potassium cyanate in 8 ml of water. The mixture was stirred at ambient temperature for 19 hr and filtered to give 5.00 g (73%) of the product, mp 138–147°. An analytical sample, mp 162–164° [lit. (2) mp 153–156°], was obtained by recrystallization from absolute ethanol.

Anal.—Calc. for $C_{11}H_{12}Cl_2N_2O_3$: C, 45.38; H, 4.16; N, 9.62. Found: 45.56; H, 4.19; N, 9.38.

The IR and NMR spectra of the biological metabolite and synthetic II were identical. The two materials were not separable in the following TLC solvent systems: chloroform–methanol–water–acetic acid (85:12:2:2), chloroform–methanol (50:50), benzene–methanol–acetone–acetic acid (80:10:5:5), and benzene–dioxane–acetic acid (90:25:4).

Isolation and Identification of Metabolite B—Isolation of Metabolite B was initiated using the residue remaining from the neutral ether extract, which was prepared as already described. The residue was dissolved in absolute methanol and applied to 2.0-mm thick TLC plates[1]. The plates were developed with ethyl acetate for 1 hr. The R_f 0.48 zone was removed from the plate, and the silica was pulverized. The resultant silica was divided into 5-g aliquots, and each aliquot was added to a column (2.5 × 17 cm) containing 20 ml of absolute methanol.

After the silica was wetted thoroughly with methanol, the column was drained slowly. The methanol was evaporated, and a white residue was found covering the sides of the flask. This residue was dissolved in 5 ml of dimethyl sulfoxide, and this mixture then was added to 95 ml of water. The dimethyl sulfoxide–water mixture was added to a Sephadex G-10 column (2.5 × 5 cm), and the column was washed with distilled water (200 ml). During the column washing, the water eluates (20-ml aliquots) were examined for traces of the metabolite by UV absorption.

The metabolite then was eluted from the column with ~200 ml of methanol–water (50:50). Elution with methanol–water was continued until only insignificant absorption was found at 264 nm. The methanol–water mixture was evaporated to dryness on the rotating evaporator. The white crystalline residue that remained was used to obtain physical data on Metabolite B; NMR (dimethyl sulfoxide-d_6): δ 7.83 (d, 1H, J = 8 Hz, uracil H-6), 7.25–7.68 (m, 1H, aromatic CH), 5.68 (d, 2H, J = 8 Hz, uracil H-5), 4.93 (s, 2H, aromatic CH_2N), and 11.36 (broad s, exchangeable, 1H, NH).

Synthesis of Metabolite B: 1-(3,4-Dichlorobenzyl)uracil (III)—A mixture of 33.6 g (0.30 mole) of uracil (VIII), 41.4 g (0.30 mole) of potas-

sium carbonate, 19.5 g (0.10 mole) of 3,4-dichlorobenzyl chloride (IX) and 10 g of sodium iodide in 300 ml of dimethyl sulfoxide was stirred at 95–100° for 16 hr, cooled, and poured into 1400 ml of cold distilled water with stirring. The mixture was acidified with concentrated hydrochloric acid and immediately (within 15 min) extracted with chloroform (3 × 400 ml). The combined extracts were washed with 200 ml of water, dried over magnesium sulfate, and concentrated to dryness *in vacuo*.

A solid separated during extraction; within 30 min after the acidification step, this solid was filtered and combined with the residue from the extraction. Recrystallization of the combined product from 850 ml of ethanol gave 13.85 g (51%) of the product, mp 215–218°.

An analytical sample, mp 215–218°, was obtained by drying the product at 100° for 2.5 hr over sodium hydroxide *in vacuo*; IR (mineral oil): 5.80–5.95 (C=O) μm.

Anal.—Calc. for $C_{11}H_8Cl_2N_2O_2$: C, 48.73; H, 2.97; N, 10.34. Found: C, 48.71; H, 2.99; N, 10.19.

The NMR spectra of Metabolite B and synthetic III were identical. The two materials were not separable in the following TLC solvent systems: ethyl acetate, ethyl acetate–methanol (90:10), chloroform–methanol–water (85:15:2), and dichloroethylene–methanol (95:5).

Gross Observation in Mice—Groups of three unfasted male mice (TAC:SW/fBr[4]), 20–26 g, were administered oral doses (50–800 mg/kg) of the test compounds as a 2% suspension in 0.5% methylcellulose 4000 cps[5]. Gross pharmacological effects were rated over a 2-hr observation period by methods similar to those reported by Irwin (4). Pharmacological effects, including skeletal muscle relaxation, behavioral, central nervous system (CNS), and autonomic effects, were rated on a scale from 0 to 4, with 0 = no effect, 1 = slight, 2 = moderate, 3 = extreme, and 4 = severe. The animals were returned to their cages; any deaths occurring with 72 hr were recorded, and the highest tolerated dose was determined.

Tetrabenazine Antagonism—The method used was similar to that described by Barnett *et al.* (5). Groups of five unfasted male mice (TAC:SW/N fBr), 20–27 g, were pretreated with the test compounds at 50 mg/kg po as a 0.5% suspension in 0.5% methylcellulose or with 0.5% methylcellulose alone. Thirty minutes later, each animal received tetrabenazine methanesulfonate[6] (35 mg/kg ip in saline) or saline. After an additional 30 min, the degree of palpebral narrowing was estimated as a measure of ptosis and compared to 0.5% methylcellulose and tetrabenazine control groups. The degree of ptosis that developed was evaluated using a rating scale of 0 to 4, with a score of 4 representing a normal palpebral opening and scores of 3, 2, 1, and 0 representing slight, moderate, marked, and complete active closure of the palpebral opening, respectively. The percent prevention of ptosis was calculated as described by Barnett *et al.* (5).

Pentylenetetrazol Antagonism—The method used was a modification of that described by Goodman *et al.* (6). Groups of five unfasted male mice (TAC:SW/N fBr), 19–27 g, were pretreated with the test compounds at a dose of 200 mg/kg po as a 2% suspension in 0.5% methylcellulose or with 0.5% methylcellulose alone at 10 ml/kg. One hour later, each animal received pentylenetetrazol[7] (45 mg/kg iv in saline). Seizures usually occurred within 1 min, and mice that failed to exhibit tonic extensor seizures were recorded as protected. The results were expressed as the percent protected in any treatment group.

Other Convulsant Antagonism—The method used was a modification of that described by Costa *et al.* (7). Groups of 10 male mice (TAC:SW/N fBr), 19–27 g, were pretreated with the test compounds at a dose of 200 mg/kg po as a suspension triturated in 0.5% methylcellulose or with 0.5% methylcellulose alone at 10 ml/kg. Thirty minutes later, each animal received one of the following agonists intraperitoneally: strychnine sulfate[8], 2.5 mg/kg as a 0.02% solution in saline; pentylenetetrazol, 112.5 mg/kg as a 1.125% solution in saline; isoniazid[9], 340 mg/kg as a 3.4% solution in saline; picrotoxin[10], 15 mg/kg as a 0.15% solution in saline; and harmaline[10], 50 mg/kg as a 0.5% solution in saline.

Strychnine-, pentylenetetrazol-, and picrotoxin-induced seizures usually occurred within 5–15 min, and mice that failed to exhibit these seizures within 30 min were considered protected. Isoniazid-induced seizures usually occurred within 30–50 min, and mice that failed to exhibit these seizures within 90 min were considered protected. Harmaline caused a sustained tremor of the head and limbs usually within 15 min, and mice that failed to exhibit this tremor within 30 min were considered protected. The results were expressed as the percent protected in each treatment group.

[4] Taconic Farms, Germantown, N.Y.
[5] Dow Chemical Co.
[6] Hoffmann-La Roche.
[7] Knoll.
[8] Humco Laboratories.
[9] City Chemical Corp.
[10] Aldrich Chemical Co.

REFERENCES

(1) K. O. Ellis, T. J. Schwan, F. L. Wessels, and N. J. Miles, *J. Pharm Sci.*, **69**, 1194 (1980).

(2) A. R. Surrey and G. Y. Lesher, U.S. pat. 3,194,808 (July 13, 1965); through *Chem. Abstr.*, **63**, 13283g (1965).

(3) B. R. Baker and G. Chheda, *J. Pharm. Sci.*, **54**, 25 (1965).

(4) S. Irwin, *Psychopharmacologia*, **13**, 322 (1968).

(5) A. Barnett, R. I. Taber, and D. D. Greenhouse, *Int. J. Neuropharmacol.*, **8**, 353 (1969).

(6) L. S. Goodman, M. S. Grewal, W. C. Brown, and E. A. Swinyard, *J. Pharmacol. Exp. Ther.*, **108**, 168 (1953).

(7) E. Costa, A. Guidotti, C. C. Mao, and A. Suria, *Life Sci.*, **17**, 167 (1975).

ACKNOWLEDGMENTS

The authors thank Ms. Patricia Curtis for the NMR spectra, Ms. Cora Jeffrey and Ms. Donna George for the microanalyses, Mr. Donald Deyo for technical assistance, and Mr. Richard Burns and Mr. W. F. Dauchy for technical assistance in the pharmacological evaluation of these compounds. They also thank Nancy C. Maginsky for typing the manuscript.