w/v were prepared and the surface tensions measured at 22 ± 1 °C. Fourfold analyses were made for each concentration and the mean was taken. The surface tensions plus or minus the standard deviation of 0.1% aqueous solutions are reported in Table I.

Critical Micelle Concentration Determinations. The critical micelle concentrations of the *N'*-(pyridinioacetyl) fatty acid hydrazides have been determined by a surface-tension method.[8] The results were plotted as surface tension against log surfactant concentration. The cmc was interpreted from the intercept of two straight lines (Figure 1).

Antimicrobial Assays. In vitro antimicrobial activity was determined using the autotitration method.[7] The bacteria were inoculated into tryptose phosphate broth (Difco) and incubated at 37 °C for 18–20 h. The fungi were inoculated into proteose peptone no. 3 maltose and incubated at 23 °C for 3–5 days. Absence of growth (turbidity) was indicative of the activity of the drug being evaluated. The lowest level of drug which completely inhibited the development of growth is considered the MIC. Results are summarized in Table II.

Acknowledgment. We express our gratitude to Dr. S. M. Albónico for his interest and encouragement and to C. Weiss, B. Fernández, and R. Wagner for their excellent technical assistance. This work was supported in part by Research Grants from SECYT.

(±)-*trans*-2-(Aminomethyl)cyclobutanecarboxylic Acid Hydrochloride: A Rigid Analogue of γ-Aminobutyric Acid

John P. O'Donnell,* David A. Johnson, and Albert J. Azzaro

School of Pharmacy and the Department of Neurology, School of Medicine, West Virginia University, Morgantown, West Virginia 26506. *Received January 23, 1980*

The (±) title compound was prepared to evaluate prior observations that certain γ-aminobutyric acid (γAbu) congeners in the transoid disposition demonstrate γAbu receptor binding activity. It was prepared by a multistep sequence from (±)-methyl *trans*-2-(hydroxymethyl)cyclobutanecarboxylate. In a sodium-independent binding assay, the specific binding of (±)-1 to synaptic membranes prepared from rat brain tissue was $^{1}/_{14\,500}$ that of γAbu.

The conformational requirements necessary for biological activity of γ-aminobutyric acid (γAbu) have become the topic of several research studies. Although the conformational requirements of γAbu-ergic compounds have been inferred by physical analysis and theoretical calculations,[1-4] the preparation and subsequent pharmacological testing of structurally rigid molecules containing a segment of the γAbu molecule[5-8] have become the most useful approaches to investigations of this type. These latter studies with the rigid analogues have led to the suggestion that an extended conformation of γAbu is preferred for binding at the receptor and is required for pharmacological activity.[9] In order to further test this hypothesis, we have prepared (±)-2-(aminomethyl)cyclobutanecarboxylic acid hydrochloride (1), a molecule conformationally restricted to resemble the extended form of γAbu. We have tested this agent for its ability to compete with γAbu for sodium-independent synaptic membrane binding sites prepared from rat brain tissue.

Results and Discussion

Synthesis of (±)-1 from (±)-methyl hydrogen-*trans*-cyclobutane-1,2-dicarboxylate (2) is outlined in Scheme I.

Scheme I

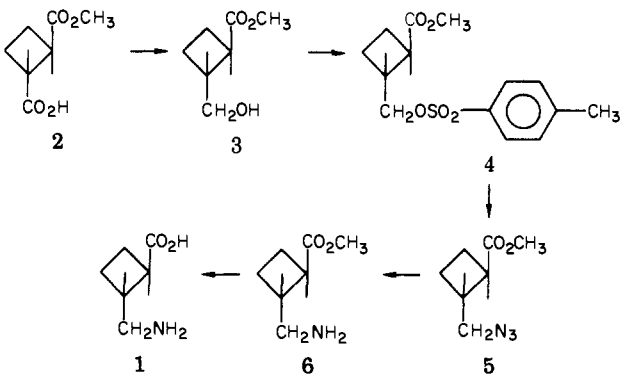


Table I. Sodium-Independent γAbu Receptor Binding in Rat Brain Tissue

agent tested	IC_{50} of [^{3}H]γAbu binding,[a] mol/L	relative affinity
muscimol	3.20 (±0.12) × 10^{-9} [b]	6.3
γAbu	2.00 (±0.03) × 10^{-8} [b]	1
bicuculline	3.80 (±0.27) × 10^{-6} [b]	$^{1}/_{190}$
(±)-*trans*-2-(aminomethyl)cyclobutanecarboxylic acid	2.90 (±0.14) × 10^{-4} [b]	$^{1}/_{14\,500}$

[a] Mean (± SEM) of four to six experiments performed in triplicate. [b] $p = 0.05$ as compared to γAbu.

Reduction of 2 with diborane gave the acid alcohol 3, which was readily converted to the tosylate 4. Displacement of the tosylate with sodium azide resulted in the azido ester 5, which due to its explosive nature was not purified. Reduction of 5 yielded the amino ester 6, which was subsequently hydrolyzed with hydrochloric acid to the amino acid 1.

The potency of (±)-1, relative to other γAbu agonists and antagonists, in displacing [^{3}H]γAbu from sodium-independent γAbu binding sites was examined in crude sy-

(1) E. G. Steward, R. Player, J. P. Puillian, D. A. Brown, and M. J. Pringle, *Nature (London)*, **233**, 87 (1971).
(2) E. G. Steward, R. Player, and D. Warner, *Acta Crystallogr., Sect. B*, **29**, 2825 (1973).
(3) L. B. Kier and E. B. Truitt, *Experientia*, **26**, 988 (1970).
(4) D. Warner and E. G. Steward, *J. Mol. Struct.*, **25**, 402 (1975).
(5) G. A. R. Johnson, D. R. Curtis, J. Davis, and R. M. McCulloch, *Nature (London)*, **248**, 804 (1974).
(6) G. A. R. Johnson, R. D. Allan, S. M. Kennedy, and B. Twitchen in "Gaba-Neurotransmitters: Pharmaco-chemical, Biochemical and Pharmacological Aspects", P. Krogsgaard-Larsen, J. Scheel-Krugar, and H. Kofod, Ed., Academic Press, New York, 1979, p 149.
(7) G. A. R. Johnson, D. R. Curtis, P. M. Beart, C. J. Game, R. M. McCulloch, and B. Twitchen, *J. Neurochem.*, **24**, 157 (1975).
(8) M. Segal, D. Sims, and E. Smissman, *Br. J. Pharmacol.*, **54**, 181 (1975).
(9) P. Krogsgaard-Larsen and G. A. R. Johnson, *J. Neurochem.*, **30**, 1377 (1978).

0022-2623/80/1823-1142$01.00/0

naptic membrane-rich fractions of rat brain as described by Enna and Snyder.[10] Sodium-independent binding sites for [^{3}H]γAbu possess the chemical specificity required of the physiological postsynaptic γAbu receptor[10-12] and, therefore, represent a useful model for determining the structural requirements for the agonist and antagonist that will interact at these sites. The results of the testing are summarized in Table I. Muscimol was the most potent of the agents tested, with a sixfold higher affinity for [^{3}H]γAbu binding sites than γAbu itself. On the other hand, bicuculline, an antagonist at the postsynaptic γAbu receptor, was about two orders of magnitude less potent than γAbu. Both of these findings are consistent with previous reports in the literature.[9,12-14]

(±)-1 was also effective in displacing [^{3}H]γAbu from the sodium-independent binding sites; however, its relative affinity for these sites was particularly low. The low affinity of (±)-1 for sodium-independent γAbu binding sites illustrates the delicate balance between the role of electronic and steric effects in determining the biological activity of γAbu analogues. Incorporation of a 2- or a 3-methyl group into the γAbu molecule reduces the relative affinity of these agents, with respect to γAbu, by three orders of magnitude.[14] In addition, restricted rotation, by incorporation of the γAbu molecule into the *trans*- and *cis*-3-aminocyclopentanecarboxylic acid, reduces the affinity for γAbu binding sites by two and three orders of magnitude, respectively.[15]

Enhanced affinity for sodium-independent binding sites has been observed for both muscimol and *trans*-4-aminocrotonic acid (TACA),[6] both of which are six- and twofold more potent than γAbu, respectively, and are extended planar analogues of γAbu but differ in electronic nature. Recently,[16] (±)-*trans*-2-(aminomethyl)cyclopropanecarboxylic acid was found to closely approximate γAbu at its sodium-independent binding sites. It is of interest to note that the nonplanar nature of the extended substituted cyclopropane moiety appears to be unfavorable for γAbu binding compared to muscimol and TACA. However, the additional sp^2 nature of the cyclopropane moiety appears to compensate for the nonplanar methylene interaction.

If an explanation for the lack of γAbu-binding activity of (±)-1 is attempted on the basis of structural comparison, it is found that the overall molecular dispositions of muscimol, TACA, and/or γAbu in the extended conformation approach planarity. However, in its extended conformation, (±)-1 has the 3,4-methylene bridge of the cyclobutane ring system residing orthogonal to the plane of the γAbu molecule. This additional protrusion and the lack of sp^2 character of the cyclobutane ring system may, in fact, account for the lack of effective drug–receptor interaction in this binding assay. These data are consistent with the proposal that a planar transoid disposition of the γAbu molecule is significant in interactions with the γAbu receptor.

Experimental Section

All melting points and boiling points are uncorrected. Melting points were determined in open glass capillaries using a Thomas-Hoover Uni-melt apparatus. IR spectra were recorded with a Beckmann IR-18 spectrophotometer, and NMR spectra were recorded with Varian Associates T-60 and EM-360 spectrometers using tetramethylsilane as an internal standard. Elemental analysis were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated by symbols of the elements, the analytical results were within ±0.4% of theoretical values.

(±)-Methyl *trans*-2-(Hydroxymethyl)cyclobutanecarboxylate (3). Following an analogous method of Schroff et al.,[17] diborane gas, generated by the dropwise addition of a diglyme solution of sodium borohydride (4.26 g, 0.113 mol) to a stirred mixture of boron trifluoride etherate (58.1 g, 0.2 mol) and diglyme (20 mL), was swept by a slow stream of N_2 into a solution of 8.0 g (0.05 mol) of (±)-methyl hydrogen-*trans*-cyclobutane-1,2-dicarboxylate in 50 mL of Et_2O at room temperature. Generation of BH_3 gas was continued until a dense turbidity appeared, and the material precipitated out giving a clear solution (1 h). The solution was stirred at room temperature for an hour, followed by methanolysis and the removal of volatiles at 45–50 °C in vacuo. The residue was taken up in Et_2O and extracted with 5% $NaCO_3$. The ethereal layer was separated, dried (A_2SO_4), and concentrated in vacuo to an oil, which was distilled through a Vigruex column to give 5.4 g (75%) of a colorless liquid: bp 78–80 °C (0.35 mmHg) [lit.[16] bp 81–82 °C (1.0 mmHg)].

(±)-*trans*-2-(Carbomethoxy)cyclobutanemethyl *p*-Toluenesulfonate (4). To a stirred solution of 5.0 g (0.035 mol) of **3** in 35 mL of pyridine at 0 °C was added 13.34 g (0.07 mol) of *p*-toluenesulfonyl chloride over 20 min. The solution was permitted to stand (10 °C) with occasional swirling for 72 h. The resultant solution was poured over 40 mL of ice–water to give an oily precipitate. The mixture was extracted with Et_2O, and the combined extracts were washed with cold 5% HCl and H_2O and dried (K_2CO_3–Na_2SO_4). The volatiles were removed under reduced pressure to leave a liquid residue, which was crystallized from petroleum ether and acetone. The white solid was collected and dried in vacuo at room temperature to yield 8.54 g (83%) of tosylate, mp 44–45 °C. Anal. ($C_{14}H_{18}O_5S$) C, H, N.

(±)-Methyl *trans*-2-(Azidomethyl)cyclobutanecarboxylate (5). To compound **4** (2.0 g, 0.067 mol) in 20 mL of 60% (v/v) acetone–water was added 0.91 g (0.014 mol) of sodium azide. The mixture was heated on reflux on a steam bath for 18 h. The acetone was removed at reduced pressure and the cooled aqueous solution was extracted with $CHCl_3$. The combined extracts were dried ($MgSO_4$) and concentrated in vacuo to leave a clear liquid residue: IR (neat) 2100 cm^{-1} (N=N), 1740 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.6–2.4 (m, 4 H), 2.5–3.1 (m, 2 H), 3.4 (d, 2 H), 3.73 (s, 3 H).

(±)-Methyl *trans*-2-(Aminomethyl)cyclobutanecarboxylate (6). Compound **5** (4.8 g, 0.028 mol) in 200 mL of 10% (v/v) chloroform–methanol was hydrogenated with 0.15 g of 10% Pd/C in a Parr shaker at an initial pressure of 30 psig. The hydrogenation flask was swept out intermittently with fresh hydrogen. After 20 h, the reaction mixture was filtered and the volatiles were removed under reduced pressure. The residue was taken up in 100 mL of methanol and refluxed with 2.7 g of H_2SO_4 (concentrated) for 12 h. The cooled reaction mixture was concentrated in vacuo to about 15 mL, taken up in 25 mL of 1% HCl, and extracted with $CHCl_3$. The aqueous acid solution was then adjusted to pH 10 with 5% Na_2CO_3 and extracted with $CHCl_3$. Combined $CHCl_3$ extracts were dried ($MgSO_4$) and concentrated in vacuo to leave an oil, which was distilled to give 1.76 g (44%) of a colorless liquid, bp 49–50 °C (0.15 mmHg). Anal. ($C_7H_{13}NO_2$) C, H, N.

(±)-*trans*-2-(Aminomethyl)cyclobutanecarboxylic Acid Hydrochloride (1). Compound **6** (1.75 g, 0.012 mol) was refluxed with 1 N HCl (20 mL) for 2 h. The volatiles were removed under reduced pressure to leave a white solid residue, which was recrystallized from Me_2CO–H_2O to yield 1.45 g (72%) of the product, mp 144–146 °C. Anal. ($C_6H_{12}NO_2Cl$) C, H, N.

(10) S. J. Enna and S. H. Snyder, *Brain Res.*, **100**, 81 (1975).
(11) S. R. Zukin, A. B. Young, and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, 71, 4802 (1974).
(12) D. V. Greenlee, P. C. VanNess, and R. W. Olsen, *J. Neurochem.*, 31, 933 (1978).
(13) S. J. Enna and S. H. Snyder, *Mol. Pharmacol.*, 13, 442 (1977).
(14) S. H. Nicholson, C. H. Suckling, and L. L. Iversen, *J. Neurochem.*, **32**, 249 (1979).
(15) R. J. Hitzemann and H. H. Loh, *Brain Res.*, 144, 63 (1978).
(16) Since the submission of this manuscript, the synthesis and pharmacology of (±)-*trans*-2-(aminomethyl)cyclopropanecarboxylic acid have been reported. R. D. Allan, D. R. Curtis, P. M. Headley, G. A. R. Johnson, D. Lodge, and B. Twitchen, *J. Neurochem.*, **34**, 652 (1980).
(17) C. C. Shroff, W. S. Stewart, S. Uhn, and J. W. Wheeler, *J. Org. Chem.*, **36**, 3356 (1971).

Sodium-Independent [^{3}H]γAbu Binding Assay. The Na-independent binding of [^{3}H]γAbu was determined in crude synaptic membrane-rich fractions of rat brain as described by Enna and Snyder[10] with only minor modifications. Briefly, whole rat brain was homogenized in 15 volumes of ice-cold 0.32 M sucrose using a Teflon–glass homogenizer. By differential centrifugation,[10] the homogenate was fractionated into a crude synaptic membrane pellet that was frozen at −20 °C for at least 18 h prior to use.

The frozen synaptic membrane pellet was resuspended (1 mg/1 mL) in 0.05 M Tris–citrate buffer (pH 7.1) containing 0.05% Triton X-100.[13] The suspension was incubated at 37 °C for 30 min and then centrifuged at 48000*g* for 10 min (4 °C). The 48000*g* pellet was resuspended in 0.05 M Tris–citrate buffer (pH 7.1), and aliquots of the suspension were incubated with 8.0 nM [^{3}H]γAbu (36.1 Ci/mmol) for 5 min at 4 °C in the presence and absence of various concentrations (concentration range of each agent tested was 1 nM to 1 mM) of known γAbu receptor agonists, antagonists, or (±)-1. Following incubation, the suspension was centrifuged at 48000*g* for 15 min. The resulting pellets were dissolved in Soluene-350 (Packard Instrument Co.), and the radioactivity was assayed in Dimilume-30 (Packard Instrument Co.) with a Packard liquid scintillation spectrometer equipped with automatic standardization.

Nonspecific Na-independent binding was determined in the presence of excess unlabeled γAbu (5×10^{-5} M). Total specific Na-independent γAbu binding was obtained by reducing the total bound radioactivity by an amount equal to that not displaced by excess unlabeled γAbu. The IC_{50} value for each agonist, antagonist, or (±)-1 was obtained by plotting the total specific Na-independent binding (as a percentage of control) at each concentration of agonist, antagonist, or (±)-1.[18] All experiments were performed in triplicate. Total protein in the synaptic membrane fraction was determined by the biuret method.[19] Statistical significance ($p = 0.05$) was determined by Student's t test.

Acknowledgment. The authors express their appreciation to Mrs. Kathleen Ice for her excellent technical assistance in the pharmacological studies.

(18) S. J. Enna in "Neurotransmitter Receptor Binding", H. I. Yamamura, Ed., Raven Press, New York, 1978, p 127.
(19) E. Layne, *Methods Enzymol.*, **3**, 447 (1967).

Structure–Activity Relationships of Pyrrole Amidine Antiviral Antibiotics. 2. Preparation of Mono- and Tripyrrole Derivatives of Congocidine

Meir Bialer,*[1] Boris Yagen, Raphael Mechoulam,

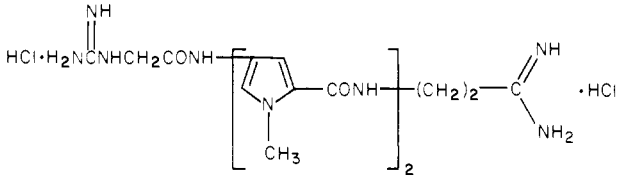
Department of Natural Products, Hebrew University, Pharmacy School

and Yechiel Becker

Department of Molecular Virology, Hebrew University, Medical School, Jerusalem, Israel. *Received December 26, 1979*

Representatives of three types of congocidine (1) analogues were synthesized. These were tested for cytotoxicity, inhibition of herpes simplex virus (HSV) replication in cultured cells, and effects on the synthesis of HSV DNA in isolated nuclei in vitro, as well as on DNA synthesis by purified HSV DNA polymerase. All synthesized tripyrrole derivatives of congocidine were less cytotoxic and more active than the parent drug in all the three antiviral tests.

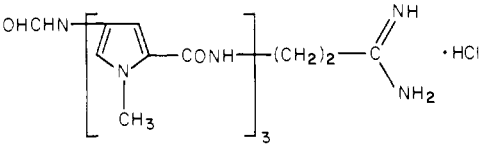
Congocidine (1) is a basic oligopeptide isolated from the fermentation medium of *Streptomyces chromagens* and *Streptomyces umbifaciens*.[2,3] The chemical structure of 1 was established by a series of degradations and a total



congocidine (1)

synthesis.[4,5] An antibiotic named netropsin was isolated from a fermentation medium of *Streptomyces netropsis*[6] and different chemical structures were assigned to it.[7-9] In 1964, the correct chemical structure of netropsin was established by proving its identity to congocidine.[10] Still, there are several cases (e.g., ref 11) where a wrong chemical structure is given for congocidine (or netropsin).[11]

Congocidine has very interesting biological activities, such as antibacterial,[12] antiparasitic,[2,3,10] and antiviral. As an antiviral drug, congocidine inhibits the multiplication of DNA viruses, such as vaccinia virus,[5,13] HSV,[14] and SFV,[15] and reteroviruses, such as RSV,[16] RuLV,[17] and FeLV.[18] Congocidine and distamycin A[19] (2) are the major



distamycin A (2)

(1) Corresponding address: Pharmacy Department, School of Pharmacy, Hebrew University, Jerusalem, Israel.

(2) C. Cosar, L. Ninet, S. Pinnert-Sindico, and J. Preudhomme, *C. R. Hebd. Seances Acad. Sci.*, **234**, 1498 (1952).
(3) C. Depois and L. Ninet, *Abstr. Int. Congr. Microbiol. (Rome), 6th*, **162**, 241 (1953).
(4) M. Julia and N. Preau Joseph, *C. R. Hebd. Seances Acad. Sci.*, **243**, 961 (1956).
(5) M. Julia and N. Preau Joseph, *C. R. Hebd. Seances Acad. Sci.*, **257**, 1115 (1963).
(6) A. C. Finaly, F. A. Hochstein, B. A. Sobin, and F. X. Murphy, *J. Am. Chem. Soc.*, **73**, 341 (1951).
(7) C. W. Waller, C. F. Wolf, W. J. Stein, and B. L. Hutchings, *J. Am. Chem. Soc.*, **79**, 1265 (1957).
(8) M. J. Weiss, J. S. Webb, and J. M. Smith, *J. Am. Chem. Soc.*, **79**, 1266 (1957).
(9) E. E. Van Tamelen and A. D. G. Powell, *Chem. Ind. (London)*, 365 (1957).
(10) S. Nakamura, H. Yonehara, and H. Uemezawa, *J. Antibiot., Ser. A*, **17**, 220 (1964).
(11) "The Merck Index", 9th ed., Merck and Co., Inc., Rahway, NJ, 1976, p 2466.
(12) H. Thrum, *Naturwissenschaften*, **46**, 87 (1959).
(13) G. H. Werner and R. Marel, *Actual. Pharm.*, **21**, 133 (1963).
(14) Y. Becker, S. Newman, and J. L. Levitt-Hadar, *Isr. J. Med. Sci.*, **8**, 1225 (1972).
(15) Y. Becker, Y. Asher, and Z. Zakay-Rones, *Antimicrob. Agents Chemother.*, 438 (1972).
(16) M. Kotler and Y. Becker, *FEBS Lett.*, **22**, 222 (1972).
(17) M. A. Chirigos, F. J. Ruscher, I. A. Kamel, G. R. Fanning, and A. Goldin, *Cancer Res.*, **23**, 1646 (1963).
(18) K. Sugiura, *Gann*, **50**, 251 (1959).

0022-2623/80/1823-1144$01.00/0