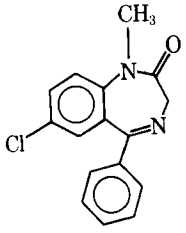
New Synthesis of Substituted Pyrrolo[1,2-*a*][1,3]diazepine and Its Pharmacological Activity

J. WALTER SOWELL, Sr. *, and C. DeWITT BLANTON, Jr. ˣ

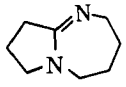
Abstract □ A facile route for the synthesis of the substituted pyrrolo[1,2-*a*][1,3]diazepine nucleus from readily available starting material is reported. The compound was tested for antimalarial activity in mice, antineoplastic activity in mice, acute hypotensive activity in rats and dogs, effect on cholesterol–lipoprotein levels in rats, anti-inflammatory activity in rats, antiviral activity in mice, CNS depressant or stimulant activity in mice, diuretic activity in fasted rats, and antidiabetic activity in rats. Hypotensive activity of relatively short duration was observed in rats. The compound lacked positive pharmacological activity in the remaining tests.

Keyphrases □ Pyrrolo[1,2-*a*][1,3]diazepine, substituted—synthesis, antimalarial, antineoplastic, antiviral, and CNS activity in mice, hypotensive, anti-inflammatory, diuretic, and antidiabetic activity in rats □ Hypotensive agents, potential—substituted pyrrolo[1,2-*a*][1,3]diazepine screened, rats

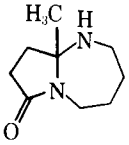
Since the first synthesis of chlordiazepoxide was reported (1, 2) and it was shown to possess antianxiety activity (3, 4), there has been a tremendous interest in the 1,4-benzodiazepines as potential medicinal agents. In addition to chlordiazepoxide, diazepam (I), oxazepam, and flurazepam have been marketed in the United States for their psychopharmacological properties. Research directed toward nuclear substitution of the 1,4-benzodiazepine nucleus has been prolific, but less attention (5–7) has been given to isosteric modifications of the benzene ring in this nucleus. A few reports (8–10) have described the amidine (II) and saturated derivative (III).



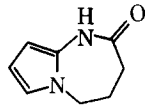
I



II



III



IV

This report describes a new synthesis for the 1*H*-pyrrolo[1,2-*a*][1,3]diazepine (IV) analog and preliminary pharmacology.

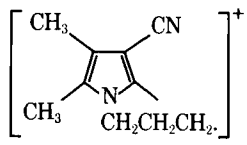
DISCUSSION

Chemistry—Acylation of 2-amino-3-cyano-4,5-dimethylpyrrole (V, Scheme I) (11) with 4-chlorobutyryl chloride in the presence of triethylamine gave the corresponding amide (VI). Upon treating a suspension of VI in absolute ethanol with potassium *tert*-butoxide, 9-cyano-7,8-dimethyl-2-oxo-1*H*-2,3,4,5-tetrahydropyrrolo[1,2-*a*]-[1,3]diazepine (VII) was obtained in good yields. One can visualize the diazepine (VII) formation proceeding through pyrrolyl anion formation followed by nucleophilic displacement of the halogen.

An alternative route to VII was investigated. Acylation of 1-acetyl-2-amino-3-cyano-4,5-dimethylpyrrole[1] (VIII) (12) with 4-chlorobutyryl chloride gave 1-acetyl-2-(4-chlorobutyramido)-3-cyano-4,5-dimethylpyrrole (IX, Scheme I). Ring closure to yield VII was achieved by refluxing IX in a hydroalcoholic solution containing sodium hydroxide. Reports (12, 13) indicated that 1-acetylpyrrole and derivatives thereof are susceptible to cleavage in alkaline media. Following the deacetylation, ring formation probably proceeds through pyrrolyl-anion formation and subsequent nucleophilic displacement of the halogen to yield VII.

Structural assignment was confirmed by elemental analysis and IR, NMR, and mass spectral data. The NMR spectrum of VII showed singlets at 2.03 and 2.10 ppm, which integrated for three protons each. These peaks were assigned to the protons on the methyl groups at the 7- and 8-positions. The complex multiplet between 2.2 and 2.7 ppm, integrating for four protons, was assigned to the protons of the methylenes at the 3- and 4-positions. A triplet centered at 4.15 ppm, integrating for two protons, was assigned to the protons of the methylene at the 5-position. A broad singlet centered at 10.55 ppm, integrating for one proton, was assigned to the proton of the nitrogen at the 1-position.

The mass spectrum of VII gave the parent ion at 203 mass units, corresponding to the molecular formula of $C_{11}H_{13}N_3O$. A strong peak at 160 amu (203 − 43 amu) showed the presence of X. A peak at 43 amu indicated the cleavage of —NHCO— from the parent structure.



X

Biological Activity—The new pyrrolodiazepine (VII) was submitted to a variety of biological screens. Antimalarial test results[2] showed that the compound was inactive against *Plasmodium berghei* in mice by the method of Osdene *et al.* (14). The diazepine (VII) was inactive when tested against L-1210 and P-388 leukemia according to standard protocol[3] (15, 16).

Pharmacological test data were obtained[4] for: (*a*) acute hypotensive action in rats, (*b*) effect on cholesterol–lipoprotein levels in rats by a modified procedure of Tensho *et al.* (17), (*c*) anti-inflammatory activity (hindpaw edema) in rats (18), (*d*) *in vivo* antiviral (encephalomycarditis) activity in mice[5], (*e*) preliminary central nervous system depressant or stimulant activity in mice[6], (*f*) diuretic activity

[1] Compound VIII was prepared by direct ring closure from 3-acetamido-2-butanone and malononitrile in the standard Gewald (11) condensation. The expected elemental analyses and NMR and IR spectra were obtained.

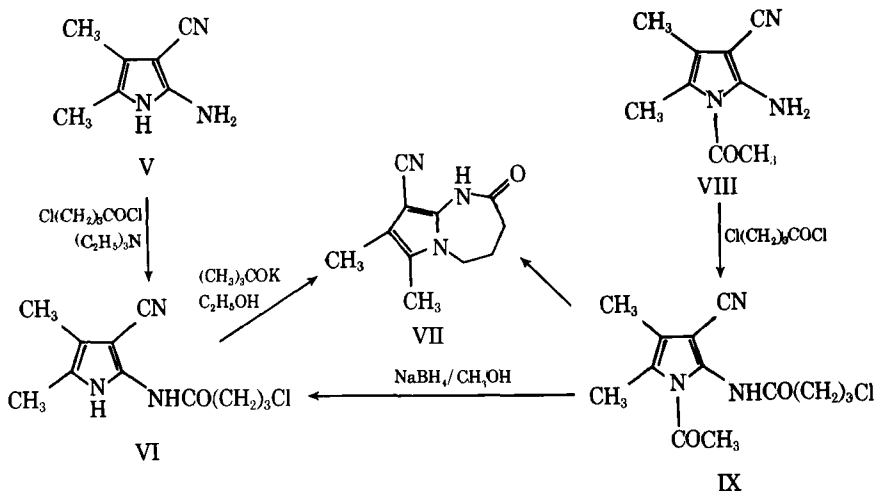
[2] Provided by the Walter Reed Army Institute of Research.

[3] The Division of Cancer Treatment, National Cancer Institute, National Institutes of Health.

[4] From The Upjohn Co.'s biological evaluation program.

[5] The compound is administered to mice, 100 mg/kg ip, before and after intranasal infection with encephalomycarditis virus. A three-stage sequential system is used to classify the compound as active or inactive on the basis of mortality in groups of 10 mice/stage.

[6] The compound is administered to a group of mice, 100 mg/kg ip. Observations, 30 min after dosing, are made on spontaneous effects (loss of righting reflex, depression, stimulation, or convulsions) and autonomic symptoms. Then nicotine sulfate is injected intravenously, and observations on tonic or running convulsions and lethality are recorded.



Scheme I

in fasted rats (19), and (*g*) antidiabetic activity in rats (20). Except for the acute hypotensive screen, VII did not exhibit significant activity in any of these tests. A single 50-mg/kg oral dose administered to two rats produced an average change in the mean arterial pressure from initial values of −17 and −6 mm Hg at 4 and 24 hr, respectively. The depressor activity was of relatively short duration. No hypotensive activity was observed when VII was evaluated in dogs, but further ring modification may yield compounds of more medicinal interest.

EXPERIMENTAL[7]

1-Acetyl-2-(4-chlorobutyramido)-3-cyano-4,5-dimethylpyrrole (IX)—A solution of VIII (12) (7.5 g, 0.0425 mole) in 75 ml of tetrahydrofuran and 45 ml of acetonitrile was continuously stirred in an ice bath while 4-chlorobutyryl chloride (3.3 g, 0.0234 mole) was added. After the addition was complete, the solution was stirred for an additional 30 min at this temperature. During this time, a heavy precipitate was formed. The reaction was allowed to continue for an additional hour at 50°.

The precipitate was removed by filtration and washed with 100 ml of tetrahydrofuran, and the combined filtrates were evaporated *in vacuo* to yield an oily, light-tan residue. This residue was shaken with 50 ml of cyclohexane, and solidification of the oil was obtained. The crude product (4.5 g, 75%) was recrystallized from benzene–cyclohexane to yield a white product homogeneous on TLC (ethyl acetate, R_f 0.53; tetrahydrofuran, R_f 0.59; chloroform, R_f 0.34), mp 120–121°; IR (potassium bromide): 3200, 2215, 1720, 1665, 1600, 1570, 1485, 1310, and 985 cm^{-1}; NMR (deuterochloroform): δ 2.03 (s, 3H, CH_3 at C-4 or C-5), 2.29 (s, 3H, CH_3 at C-5 or C-4), 2.00–2.40 (m, 2H, H's on the methylene that are beta to the carbonyl of the amide at C-2), 2.57 (s, 3H, CH_3 of acetyl), 2.60 (t, 2H, —$COCH_2$—), 3.62 (t, 2H, J = 6.0 Hz, —CH_2Cl), and 8.88 (s, 1H, NH of amide); UV λ_{max} (methanol): 209 (ϵ 13,300) and 284 (9800) nm.

Anal.—Calc. for $C_{13}H_{16}ClN_3O_2$: C, 55.42; H, 5.72; N, 14.91. Found: C, 55.28; H, 5.81; N, 14.86.

2-(4-Chlorobutyramide)-3-cyano-4,5-dimethylpyrrole (VI)—*Method I*—A solution of V (11) (6.75 g, 0.05 mole) and triethylamine (5.05 g, 0.05 mole) in 150 ml of tetrahydrofuran was cooled to −50° in a dry ice–acetone bath, and 4-chlorobutyryl chloride (7.05 g, 0.05 mole) was added. Immediately upon addition of the acid chloride, a heavy precipitate formed. The dry ice–acetone bath was removed, and the contents of the vessel were stirred vigorously as the mixture warmed to room temperature. After 30 min at room temperature, the reaction mixture was heated at 40° for 30 min.

The triethylamine hydrochloride was removed by filtration and washed with tetrahydrofuran. The combined filtrates were diluted with 300 ml of water to yield a light-tan solid, which was collected by filtration, washed with water, and air dried. The crude amide (9.8 g, 81.7%) was recrystallized three times from benzene–cyclohexane to yield white, fluffy crystals [homogeneous on TLC (ethyl acetate, R_f 0.57)], mp 170–171°; IR (potassium bromide): 3250, 3160, 2220, 1670, 1620, 1600, 1510, 1470, 1450, 1330, 1250, 1225, and 1125 cm^{-1}; NMR (deuterochloroform): δ 2.05 (s, 3H, CH_3 at C-4 or C-5), 2.10 (s, 3H, CH_3 at C-5 or C-4), 2.20–2.80 (m, 4H, —$COCH_2CH_2$—), 3.65 (t, 2H, J = 6.0 Hz, —CH_2Cl), 9.75 (s, 1H, —HNCO—), and 10.30 (s, 1H, N_1H); UV λ_{max} (methanol): 210 (ϵ 9990) and 284 (8190) nm.

Anal.—Calc. for $C_{11}H_{14}ClN_3O$: C, 55.12; H, 5.89; N, 17.53. Found: C, 54.97; H, 6.00; N, 17.58.

Method II—A solution of IX (2 g, 0.007 mole) in 25 ml of absolute methanol was treated with sodium borohydride (1.0 g, 0.027 mole) with stirring. After the addition was complete, the solution was stirred for 3 min and then poured into an ice–water mixture. The pH was adjusted to 7 with acetic acid, and the precipitate was collected, washed with water, and air dried.

The crude product (1.55 g, 91%) was recrystallized from benzene–cyclohexane and characterized as VI by the IR spectrum and melting point (168–169°). [The lability of the N^1-acetyl group under alkaline or alcoholic conditions (13) apparently accounts for the product obtained in this reaction.]

9-Cyano-7,8-dimethyl-2-oxo-1*H*-2,3,4,5-tetrahydropyrrolo-[1,2-*a*][1,3]diazepine (VII)—*Method I*—A suspension of IX (1.0 g, 0.00355 mole) in 75 ml of water and 25 ml of 95% ethanol was treated with sodium hydroxide (4.7 ml of 0.75 *N*, 0.00355 mole). The mixture was refluxed with continuous stirring for 20 hr, during which time a clear solution was obtained. The ethanol was removed, and a solid precipitated from the aqueous solution. This solid was collected by filtration, washed with water, and air dried.

The crude product (0.075 g, 10.4%)[8] was recrystallized three times from benzene–cyclohexane to yield a pale-pink powder [homogeneous on TLC (ethyl acetate, R_f 0.42; tetrahydrofuran, R_f 0.55)], mp 181–182°; IR (potassium bromide): 3300, 2200, 1670, 1600, 1550, 1450, 1400, 1280, 1225, 1120, and 750 cm^{-1}; NMR (deuterochloroform): δ 2.03 (s, 3H, CH_3 at C-8 or C-7), 2.10 (s, 3H, CH_3 at C-7 or C-8), 2.20–2.70 (m, 4H, —$COCH_2CH_2$—), 4.15 (t, 2H, J = 6.7 Hz, —NCH_2—), and 10.55 (broad s, 1H, amide NH); mol. wt. (mass spectra): calc., 203, and found, 203.

Anal.—Calc. for $C_{11}H_{13}N_3O$: C, 65.01; H, 6.45; N, 20.67. Found: C, 65.08; H, 6.49; N, 20.84.

Method II—A suspension of VI (4.8 g, 0.02 mole) in 75 ml of absolute ethanol was treated with potassium *tert*-butoxide (2.45 g, 0.022 mole). The suspension was heated to reflux and then stirred at room temperature for 10 days. (Subsequent experiments revealed that heating was not necessary to obtain similar results. Furthermore, stirring the reaction mixture overnight at room temperature gave

[7] Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. The NMR spectra were determined on a Hitachi Perkin-Elmer R 20A high-resolution NMR spectrometer, using tetramethylsilane as the internal reference. IR spectra were determined on a Perkin-Elmer 237B grating spectrophotometer, using the potassium bromide technique. UV spectra were determined in methanol solution with a Perkin-Elmer 202 UV–visible spectrophotometer. Elemental analyses were determined by Atlantic Microlab, Inc., Atlanta, Ga. TLC was performed on Eastman chromatogram sheets, type 6060 (silica gel).

[8] The low yield may be rationalized as a result of the release of acetate not being sufficiently basic to form the pyrrolyl anion for subsequent ring closure.

identical results.) The alcoholic suspension was diluted with 25 ml of water (starting material and other by-products are soluble in the aqueous alkaline medium), and the precipitate was collected by filtration, washed with water, and air dried. The compound (3.1 g, 76.2%) melted at 181–182° and exhibited identical spectral and physical data as the material from Method I.

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Partition Coefficients of Selected Pyridine Carbamates and Comparisons with Their Acetylcholinesterase and Butyrylcholinesterase Inhibitory Potencies

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Abstract □ Partition coefficients of a series of 2-substituted 3-(*N,N*-dimethyl)carbamyloxypyridines were determined in an octanol–buffer (pH 7.4) system. The values obtained were compared with the inhibitory potencies of acetylcholinesterase and butyrylcholinesterase. No significant difference was found for the role of hydrophobicity in the two enzyme systems.

Keyphrases □ Partition coefficients—substituted pyridine carbamates, compared with acetylcholinesterase and butyrylcholinesterase inhibitory potencies □ Pyridine carbamates, substituted—partition coefficients compared with acetylcholinesterase and butyrylcholinesterase inhibitory potencies □ Acetylcholinesterase—inhibition by substituted pyridine carbamates compared to their partition coefficients □ Butyrylcholinesterase—inhibition by substituted pyridine carbamates compared to their partition coefficients □ Enzymes—acetylcholinesterase and butyrylcholinesterase, inhibition by substituted pyridine carbamates compared to their partition coefficients

Previously (1), the synthesis and enzymatic evaluation of some pyridine carbamates were reported, and the compounds were used as enzyme inhibitors in a comparative study of the active centers of acetylcholinesterase and butyrylcholinesterase. Fundamental differences in acetylcholinesterase and butyrylcholinesterase regarding their interaction with the selected carbamate inhibitors were presented (1). No results were included, however, on the effect of any chemical or physical properties of the inhibitor molecules on inhibitory activity. The purpose of this paper is to report results of a study on the contribution of relative hydrophobicities to the inhibition of acetylcholinesterase and butyrylcholinesterase by the selected carbamates (1).

Limited literature results comparing the active