Table III. Comparison of the Intravenous Effects of **20** and Hydralazine on Hemodynamic Parameters in the Pentobarbital Anesthetized Dog[a]

Compd	Dose, mg/kg iv[b]	Hemodynamic parameter, % Δ				
		MAP[c]	HR[d]	MFC[e]	CO[f]	CTPR[g]
20	0.1	−5.3	−6.3	+7.3	+10.0	−9.7
	0.3	−37.0	−5.0	+26.0	−21.0	−43.5
	1.0	−50.5	−9.3	+37.0	−23.3	−42.0
	3.0	−77.5	−10.0	+91.5	−69.0	−64.0
Hydralazine	0.1	−5.0	+6.3	+35.0	+15.8	−17.0
	0.3	−18.3	+14.0	+53.8	+25.0	−31.8
	1.0	−32.5	+9.8	+47.0	+25.5	−51.0
	3.0	−26.3	+5.3	+84.5	+91.0	−55.8
	10.0	−40.8	+29.8	+95.0	+74.5	−67.5

[a] Maximum effect occurring within 10 min after administration. [b] Four dogs per dose. [c] Mean arterial blood pressure, mm. [d] Heart rate, beats per minute. [e] Myocardial force of contraction, mm deflection. [f] Cardiac output, milliliters per minute. [g] Calculated total peripheral resistance, relative resistance units calculated by MAP/CO.

δ 7.80 (m, 11, Ar), 2.33 (s, 6, CH_3); mass spectrum 387 (M^+) and 389 ($M^+ + 2$) in the ratio of 9.8:1.

Determination of Hypotensive Activity. Compounds were evaluated in mongrel dogs and/or Wistar strain spontaneously hypertensive rats. Mongrel dogs, of either sex, were anesthetized with 35 mg/kg of sodium pentobarbital (Nembutal, Abbott Laboratories) intravenously *via* the cephalic vein. After tracheal intubation, femoral arterial blood pressure, the signal from lead II of the electrocardiogram, respiration and the blood pressure responses to bilateral common carotid occlusion, and intravenous norepinephrine were recorded on a Grass Model 7 polygraph. Aqueous solutions of the compounds to be tested were administered *via* the contralateral femoral vein. Compounds were administered to a minimum of two to four dogs and were considered active if systolic blood pressure was reduced by more than 20%.

Systolic blood pressure was recorded from the tails of spontaneously hypertensive rats. A photocell transducer was incorporated with a pneumatic pressure cuff for blood pressure measurement. The animals were warmed in a thermostatically controlled chamber (31.5 ± 0.5°) for 1 hr prior to obtaining blood pressure readings. The method for blood pressure measurement depends, essentially, on occlusion of the tail with the pneumatic cuff followed by a rapid release of the imposed pressure. The result is an abrupt rebound increase in the pressure head and blood flow in the tail. This increase is recorded as systolic blood pressure by a photocell transducer connected to a Grass Model 7 polygraph. Following 2 days of control readings, the compounds were administered orally at 25 mg/kg in 0.5% methocel (400 cps), four rats per compound, for 2 consecutive days. Blood pressure measurements were obtained 24 hr after the initial dose and 1, 2, 3, 4, and 24 hr after the second dose. Compounds were considered active if blood pressure was reduced by more than 15%.

Hemodynamic Evaluation of 20. Mongrel dogs, of either sex, were anesthetized with 35 mg/kg of sodium pentobarbital (Nembutal, Abbott Laboratories) intravenously *via* the cephalic vein. A tracheal cannula was inserted, artificial respiration applied, and the chest opened at the fifth right intercostal space. Cardiac output (CO) was obtained by affixing a Statham electromagnetic flow probe around the ascending aorta between the region of the aortic valve and the brachiocephalic artery. Right ventricular contractile force (MFC) was measured with a Walton-Brodie strain-gauge arch. Femoral arterial blood pressure (BP) was measured directly *via* a Statham P23Ac pressure transducer. The thorax was then closed and the animals were permitted to respire spontaneously. Heart rate (HR) was determined from the contractile force deflections. Relative calculated total peripheral resistance (CTPR) was calculated by the relationship of blood pressure to cardiac output. Aqueous solutions of the compounds were administered *via* the contralateral femoral vein at a rate of 2 mg/kg/min.

Acknowledgment. The authors are indebted to Mr. Martin J. Gordon and his associates for microanalytical services, Mr. Richard L. Stacey for technical assistance, and Mr. John Gogats for design of the photocell transducer and blood pressure screening equipment.

References

(1) (a) R. P. Mull, R. H. Mizzoni, M. R. Dapero, and M. E. Egbert, *J. Med. Chem.*, **5**, 944 (1962); (b) J. H. Short, U. Biermacher, D. A. Dunnigan, and T. D. Leth, *ibid.*, **6**, 275 (1963); (c) S. M. Gadekar, S. Nibi, and E. Cohen, *ibid.*, **11**, 811 (1968); (d) Netherlands Application 6,411,516; *Chem. Abstr.*, **63**, P18103*a* (1965); (e) W. C. Anthony and J. J. Ursprung, U.S. Patent 3,647,697 (1972).
(2) R. F. Hunter, *J. Chem. Soc.*, 125 (1930).
(3) R. F. Hunter and J. W. T. Jones, *J. Chem. Soc.*, 2190 (1936).
(4) R. Q. Brewster and F. B. Dains, *J. Amer. Chem. Soc.*, **58**, 1364 (1936).
(5) I. B. Douglass and F. B. Dains, *J. Amer. Chem. Soc.*, **56**, 719 (1934).
(6) W. König, W. Kleinst, and J. Götze, *Ber.*, **64**, 1664 (1931).

Etonitazene. An Improved Synthesis

Frank I. Carroll* and Michael C. Coleman

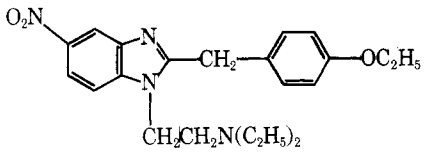
Chemistry and Life Sciences Division, Research Triangle Institute, Research Triangle Park, North Carolina 27709.
Received October 18, 1974

1-(β-Diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole (**1**, etonitazene) is a potent analgesic that has value in drug addiction studies. We have developed a simple high-yield synthesis of **1** that is adaptable to large-scale preparations. The synthesis involves the condensation of 2-(β-diethylaminoethylamino)-5-nitroaniline and *p*-ethoxyphenylacetic acid in THF in the presence of EEDQ.

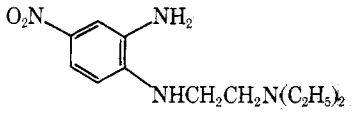
1-(β-Diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole (**1**, etonitazene) is a very potent analgesic.[1,2] However, it has a dependence potential comparable to that of morphine[3] and thus offers little advantage over morphine as an analgesic. Since experimental animals will not refuse to drink a solution of **1** as they will solutions of other analgesics, this analgesic has value in drug addiction studies.[4]

The reported synthesis of **1** involves the condensation of 2-(β-diethylaminoethylamino)-5-nitroaniline (**2**) as its hy-

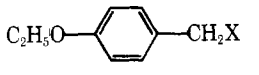
drochloride salt with ethyl *p*-ethoxyphenylacetamidate hydrochloride (**3**).[1] We have repeated this reaction several times and obtained 46–70% yields of **1**.[5] Recently we had a need to synthesize larger amounts of **1**. We found that **2** as well as its hydrochloride salt were stable compounds that could be prepared in large quantities (5 kg). However, due to the lability of **3** and the necessity of maintaining dry reactants and solvents in its preparation from *p*-ethoxyphenylacetonitrile (**4**), it was inconvenient to prepare large quantities of **3**.



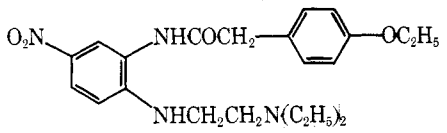
1



2



3, X = C(=NH)OC$_2$H$_5$·HCl
4, X = CN
5, X = CO$_2$H



6

Due to the difficulties involved in the literature synthesis of **1**, we decided to investigate the feasibility of preparing **1** by a two-step procedure involving first the condensation of **2** with the readily available and stable *p*-ethoxyphenylacetic acid (**5**) to give 4-nitro-2-(*p*-ethoxyphenylacetamino)-*N*-(2-diethylaminoethyl)aniline (**6**), followed by the cyclization of **6** to the imidazole **1**. We found that the amide **6** could be obtained in low yield by treating **2** with *p*-ethoxyphenylacetyl chloride. However, if the condensation of **2** with **5** were conducted with 1.2–1.5 equiv of EEDQ or DCI in THF or other solvents (see Table I) at 25°, the amide **6** was isolated in reasonable yield. More important, we noted that the reaction mixtures contained small amounts of the imidazole **1**. Indeed, if the condensation of **2** with **5** were carried out using 2 or more equiv of EEDQ in tetrahydrofuran (THF) at 50°, **1** was obtained in essentially quantitative yield. In addition to the improvement in yield, the work-up procedure is greatly facilitated since quinoline, carbon dioxide, and ethanol are the only by-products formed. The imidazole **1** could also be prepared by treating the amide **6** with EEDQ in CH_2Cl_2 at 35° or PCl_5 in refluxing chloroform.

The coupling reagent EEDQ has been extensively used for the preparation of amides;[6] however, to our knowledge this agent has not been previously used for the preparation of benzimidazoles. Thus, the synthesis of **1** from **2** and **5** with or without the isolation of the intermediate **6** represents a potentially new method for the preparation of other benzimidazoles.

Experimental Section

Melting points were determined on a Kofler hot stage microscope using a calibrated thermometer. Ir spectra were measured with a Perkin-Elmer Model 467 grating infrared spectrophotometer. Nmr spectra were recorded on a Varian Model HA-100 spectrometer with tetramethylsilane as an internal standard. Mass spectra were determined on an AEI-MS 902 spectrometer. The spectral properties of etonitazene and the amide **6** are in agreement with the structures shown. Microanalyses were carried out by Micro-Tech Laboratories, Skokie, Ill. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.5% of theoretical values.

Table I. Preparation of 4-Nitro-2-(*p*-ethoxyphenylacetylamino)-*N*-(2-diethylaminoethyl)aniline (**6**) by the EEDQ or DCI Method

Reagent (equiv)	Solvent (ml)	Yield (%) of **6**
EEDQ (1.2)	CH_2Cl_2 (2)	83
EEDQ (1.2)	THF (10)	56
DCI (1.1)	EtOAc (10)	31
EEDQ (1.5)	THF (2.5)	70
EEDQ (1.5)	Benzene–ethanol (1:1) (2)	85
DCI (1.5)	EtOAc (2)	75
DCI (1.5)	CH_2Cl_2 (2)	68

1-(β-Diethylaminoethyl)-2-(*p*-ethoxybenzyl)-5-nitrobenzimidazole (1, Etonitazene). To a stirred solution of 2-(β-diethylaminoethylamino)-5-nitroaniline (**2**,[1] 12.6 g, 0.05 mol) and *p*-ethoxyphenylacetic acid (**5**, 9.91 g, 0.055 mol) in THF (50 ml) heated at 50° was added EEDQ (12.4 g, 0.05 mol). After 24 hr, more EEDQ (6.18 g, 0.025 mol) was added with a final addition of EEDQ (12.4 g, 0.05 mol) after 72 hr. The mixture was allowed to react at 50° for another 120 hr. Solvent was removed *in vacuo*, and approximately 6.6 ml of 12 *N* HCl was added with stirring. The solution was extracted with $CHCl_3$ (2 × 200 ml) and dried (Na_2SO_4), and solvent was evaporated *in vacuo* to give 21.6 g (100%) of etonitazene hydrochloride: mp 161–164°. The hydrochloride recrystallized from absolute C_2H_5OH had mp 163–164.5° (lit.[1] mp 162–164°). The free base recrystallized from ether–petroleum ether (1:2) as needles: mp 77–78° (lit.[1] 75–76°).

4-Nitro-2-(*p*-ethoxyphenylacetylamino)-*N*-(2-diethylaminoethyl)aniline. A. Acid Chloride Method. To 15.1 g (0.06 mol) of 2-(2-diethylaminoethyl)-4-nitroaniline (**2**) in 15.0 ml of dimethylformamide was added dropwise 12.5 g (0.063 mol) of *p*-ethoxyphenylacetyl chloride (prepared by treating *p*-ethoxyphenylacetic acid with $SOCl_2$ in ClC_6H_5). After the addition was completed, the reaction mixture was heated 6 hr at 78°. The solvent was removed *in vacuo*, and the residue was made basic with ammonium hydroxide and extracted with chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate and then concentrated *in vacuo* to give a residue which on recrystallization from ethyl acetate yielded 6.4 g (26%) of 4-nitro-2-(*p*-ethoxyphenylacetylamino)-*N*-(2-diethylaminoethyl)aniline (**6**): mp 121–125°. *Anal.* ($C_{20}H_{26}N_4O_4$) C, H, N.

B. EEDQ or DCI Method. To a stirred solution of **2** (0.253 g, 0.001 mol) and **5** (0.180 g, 0.001 mol) in the appropriate solvent was added EEDQ or DCI. The mixture was allowed to stir at room temperature for 12 hr. Solvent was removed *in vacuo* and the residue made alkaline with 6 *N* NH_4OH. The solution was extracted with $CHCl_3$ (3 × 15 ml) and dried (Na_2SO_4), and solvent was evaporated *in vacuo*. The residue was dissolved in hot ethyl acetate and allowed to crystallize at 0°. The results obtained are shown in Table I.

Preparation of Etonitazene from 6. A. Using EEDQ in CH_2Cl_2. To a stirred solution of **6** (345 mg, 0.8 mmol) in CH_2Cl_2 (5 ml) was added EEDQ (205 mg, 0.8 mmol). The mixture was allowed to stir at 35° for 72 hr. Solvent was removed *in vacuo* and the residue crystallized from ethyl acetate–petroleum ether to give 0.329 g (99%) of **1**: mp 70–76°. Recrystallization from ether–petroleum ether raised the melting point to 75–76° (lit.[1] 75–76°).

B. Using PCl_5 in $CHCl_3$. A mixture of 345 mg (0.8 mmol) of **6** and 208 mg (0.8 mmol) of PCl_5 in 10 ml of $CHCl_3$ was refluxed for 3 hr. The mixture was cooled and made basic with 14% aqueous ammonia solution. The $CHCl_3$ layer was separated, dried (Na_2SO_4), and concentrated *in vacuo*. The remaining residue was recrystallized from ether–petroleum ether to give 0.28 g (85%) of **1**: mp 75–76° (lit.[1] 75–76°).

Acknowledgment. This work was supported under Contract No. HSM-42-73-228 with the National Institute on Drug Abuse, Division of Research, Biomedical Research Branch.

References

(1) A. Hunger, J. Keberle, A. Rossi, and K. Hoffmann, *Helv. Chim. Acta*, **43**, 1032 (1960).
(2) H. B. Murphree in "Drills Pharmacology in Medicine," 3rd ed, J. R. Dipalma, Ed., McGraw-Hill, New York, N.Y., 1965, p 266.
(3) H. F. Fraser, H. Isbell, and R. Wolback, Bulletin of Drug Addiction and Narcotics, addendum 2, 1960, p 35.
(4) S. J. Mulé and L. A. Woods, *J. Pharmacol. Exp. Ther.*, **136**, 232 (1962).
(5) F. I. Carroll, R. W. Handy, J. A. Kepler, and J. A. Gratz, *J. Heterocycl. Chem.*, **4**, 262 (1967).
(6) M. Fieser and L. F. Fieser, "Reagents for Organic Synthesis," Vol. 4, Wiley-Interscience, New York, N.Y., 1974, p 223.

Preparation of a New Immunosuppressant, 4,5-Bis(aminomethyl)acridine

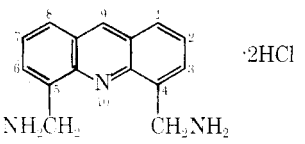
F. K. Hess* and P. B. Stewart

Pharma Research Canada Ltd., Pointe Claire, Quebec, Canada.

Received August 23, 1974

4,5-Bis(aminomethyl)acridine, useful as an immunosuppressant, was prepared in 31% overall yield by the reaction of acridine with *N*-(hydroxymethyl)phthalimide and subsequent decomposition with excess 6 *N* HCl. The drug was found to produce a suppression of the humoral antibody response comparable to several known immunosuppressive agents.

Certain acridine derivatives have been shown to have immunosuppressive properties.[1,2] In the course of studies on new compounds which can alter the immune response and avoid the disadvantages of drugs now in use,[3] we have synthesized and screened the title compound.



1

As can be seen from Table I, the compound in the maximum tolerated doses produced a suppression of the humoral antibody response comparable to several of the known immunosuppressive agents. Significant reduction in the plaque-forming cells (PFC) followed the *in vivo* administration, both by the intraperitoneal and oral route.

Chemistry. Initially, the bis(aminomethyl)acridine was obtained from the 4-aminomethyl derivative, which in turn had been prepared earlier by a novel route from acridine under the conditions of the Tscherniac–Einhorn reaction.[4] However, it could be obtained more conveniently from acridine and an excess of the Tscherniac–Einhorn reagent (see Experimental Section). The structure was confirmed by elemental analysis and the nmr spectrum.

Biological Testing. The compounds were tested for their immunosuppressive properties by the hemolysin plaque-forming cell (PFC) test as described by Jerne.[5] A group of six mice were used for the test and control groups and the results of the drug-tested group were expressed as the mean percentage suppression of the total PFC/spleen as compared to the untreated immunized controls. All determinations were done in triplicate.

The question of dosage is difficult for immunosuppressive agents. For the known compounds with extensive pharmacological data, the dosage was chosen after reference to the published literature.[6-8] In the case of the new compounds with only limited toxicity data, it was chosen with reference to the acute LD_{50} and was, therefore, somewhat arbitrary. Usually we chose 20% of the acute LD_{50} by that route with a maximum single dose of 200 mg/kg if not found toxic on repeated application.

Experimental Section

Melting points were obtained on an Electrothermal melting point apparatus. Nuclear magnetic resonance spectra were recorded on a Varian T-60 instrument in the solvent stated with tetramethylsilane (TMS) as an internal standard. Microanalyses were performed by Micro-Tech Laboratories, Inc., Skokie, Ill., and agreed with the theoretical values to within ±0.4%.

4,5-Bis(aminomethyl)acridine (1). Into 500 ml of concentrated H_2SO_4, cooled to 10–15°, *N*-hydroxymethylphthalimide (177 g, 1 mol) was introduced in small portions. After the compound was dissolved, acridine (90 g, 0.5 mol) was gradually added and the

Table I. Plaque-Forming Cell (PFC) Test in Mice

Compound	Dose, mg/kg (day −1 to day +3)	Route	% suppression of total PFC/spleen ± S.E.M.	LD_{50} ± S.E.M., mg/kg
4,5-Bis(aminomethyl)-acridine	10	ip	88 ± 4.4	54 ± 9 ip
	75	po	66.6 ± 8.6	>1000 po
	100	po	86 ± 3.7	
3,6-Diaminoacridine	200	po	20 ± 5.8	>1000 po
3-Methyl-3-hydroxy-1-(*p*-isopropylcarbamoyl-phenyl)triazene	150	ip	96.6	2505.4 ± 87 ip
	200	po	96.0	2747.0 ± 112 po
Cyclophosphamide	100	ip	99.9 ± 0.02	210 sc
Azathioprine	35	ip	39.2 ± 10.2	
	50	po	59.0 ± 20.0	350 sc
6-Mercaptopurine	50	ip	44.0 ± 9.5	100 sc
Antilymphocyte serum	3 ml/kg	ip	98 ± 0.4	