CH_2Cl_2–hexane gave **7b** (740 mg, 85%) as fine colorless needles: mp 196.5–198°; $[\alpha]^{26}D$ +27°; ir 1715 (20-C=O), 1695 cm^{-1} (3, C=O); NMR δ 0.65 (s, 3 H, 18-CH_3), 0.73 (t, J = 7.5 Hz, 3 H, 17α-CH_2CH_3), 1.02 (s, 3 H, 19-CH_3), 2.04 (s, 3 H, 21-CH_3). Anal. ($C_{23}H_{36}O_2$) C, H.

17α-Ethylpregn-5-ene-3,20-dione (9b). A solution of **8b**[11b] (860 mg, 2.51 mmol) in anhydrous t-BuOH (20 ml) under N_2 was treated with t-BuOK (2.82 g, 25.1 mmol). The mixture was stirred for 1.5 hr and quenched with 10% HOAc (94 ml). The mixture was then diluted with 10% $NaHCO_3$ (150 ml) and extracted with CH_2Cl_2. The organic extracts were washed with H_2O, dried (Na_2SO_4), and evaporated to give a yellow oil. The oil was added to a silicic acid (30 g) column prepared in hexane and eluted with EtOAc–hexane (1:9). The eluate was evaporated and the residue recrystallized from Et_2O–pentane to afford **9b** (406 mg, 47%) as colorless needles: mp 167–169°; $[\alpha]^{25}D$ −19°; ir 1715 (20-C=O), 1695 cm^{-1} (3-C=O); NMR δ 0.68 (s, 3 H, 18-CH_3), 0.72 (t, J = 8 Hz, 3 H, 17α-CH_2CH_3), 1.18 (s, 3 H, 19-CH_3), 2.08 (s, 3 H, 21-H), 5.38 (m, 1 H, 6-H). Anal. ($C_{23}H_{34}O_2$) C, H.

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2-(3,4-Dichloroanilino)quinolizinium Bromide, a Unique Antispasmodic, Antisecretory, and Antiulcerogenic Agent

Robert J. Alaimo*

Chemistry Division

and Marvin M. Goldenberg

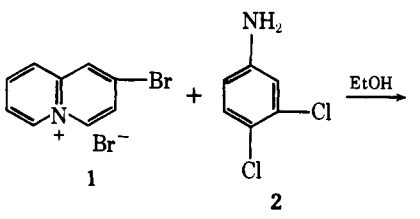
Pharmacometrics Division, Norwich Pharmacal Company, Division of Morton-Norwich Products, Inc., Norwich, New York 13815. *Received March 13, 1975*

2-(3,4-Dichloroanilino)quinolizinium bromide (**3**) was prepared by reaction of 2-bromoquinolizinium bromide with 3,4-dichloroaniline in ethanol. This compound possesses unique antispasmodic, antisecretory, and antiulcerogenic properties.

Earlier we reported[1,2] the synthesis and biologic activity of a number of substituted quinolizinium salts. As a continuing part of this investigation, we now wish to describe the synthesis of and report some preliminary pharmacologic testing results on 2-(3,4-dichloroanilino)quinolizinium bromide (**3**).[3] This compound when evaluated in a number of pharmacologic testing procedures was found to possess unique and potent properties. Among these are potent antispasmodic action of long duration on colonic contractions in response to pelvic nerve stimulation, gastric antisecretory activity, and antiulcerogenic action.

To the best of our knowledge, this is the first example of a nonanticholinergic antispasmodic agent which possesses gastric antisecretory and antiulcerogenic activity.

Chemistry. The synthesis of **3** was accomplished by the reaction of 2-bromoquinolizinium bromide (**1**) with 3,4-dichloroaniline (**2**) in ethanol. The general reaction procedure has been described previously.[1]



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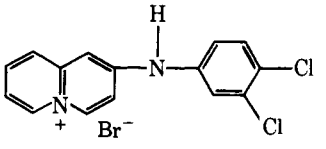

3

Compound **3** is an odorless, off-white crystalline solid with a melting point of 264–265°. It has water solubility to the extent of 2.4 g/l. at room temperature. The NMR spec-

trum (dimethyl sulfoxide-d_6) of 3 shows a broad, exchangeable band centered at 10.85 ppm assignable to the aniline NH. A triplet centered at 9.15 ppm and integrating for two protons has been assigned to the protons at positions 4 and 6 of the quinolizinium ring. The remaining eight aromatic protons occur as a complex multiplet between 7.42 and 8.33 ppm. Specific peak assignments for these eight protons are not possible at this time. The infrared, ultraviolet, and mass spectra of 3 are consistent with the assigned structure and the data are included in the Experimental Section. Microanalytical data for C, H, N, ionic Br, and total halogen are within ±0.30% of the calculated values.

Pharmacologic Testing. The antispasmodic action of 3 was studied via the intravenous route of administration in the anesthetized dog.[4] Contractile responses of the distal colon to intermittent pelvic nerve stimulation and to acetylcholine were observed along with blood pressure changes. Compound 3 at 5 and 10 mg/kg iv exerted moderate to marked inhibitory action of long duration (2.5–3.5 hr) on contractile responses of the colon to both pelvic nerve stimulation and acetylcholine. No significant changes in blood pressure were observed at these doses; 3 also failed to inhibit the fall in blood pressure in response to acetylcholine.

The pylorus-ligated standard antisecretory testing procedure[5] was used to measure the effect of 3 on acid concentration and volume of gastric secretions. Compound 3 was administered perorally 1 hr prior to pylorus ligation and secretions were collected and volumes recorded 4 hr later. At doses of 50 and 100 mg/kg, 3 depressed free acid concentration and total titratable acid content of the gastric secretions while providing little reduction in gastric secretory volumes.

A slightly modified procedure of Shay et al.[6] was used to determine the antiulcerogenic activity of 3. Pyloric ligation of 18-hr duration in Sprague–Dawley rats previously fasted 48 hr was the only modification. Compound 3 was given perorally 1 hr before pylorus ligation and 18 hr later the rats were sacrificed and the stomachs examined for the degree of ulceration. At doses of 100 and 150 mg/kg po, compound 3 diminished ulcer formation as evidenced by the presence of less severe rumenal ulcers. No perforated ulcers were observed in the stomachs of the rats dosed with 3, while in the control stomachs perforation occurred in six of ten rats.

The results of the pharmacologic testing of 3 as well as the comparative drugs, papaverine and atropine, are given in Table I.

Table I. Evaluation of 3, Papaverine, and Atropine along the Gastrointestinal Tract

Drug	Dose, mg/kg iv	Antispasmodic[a] evaluation	Dose, mg/kg po	Antisecretory[b] evaluation	Antiulcerogenic[c] evaluation
3			0	—	4.7
	5	++[d]	50	++	—
	10	++++	100	+++	2.6
			150	—	1.5
Papaverine	5	+	100	0	—
	10	++			
Atropine	0.5	+	10	+++	2.5
	1.0	++	25	++++	1.1

[a] As determined by the drugs' inhibitory action on colonic contractions to pelvic nerve stimulation in the dog. Testing performed on 12 dogs at the 10 mg/kg dose level and 4 dogs at the 5 mg/kg dose level. The experimental procedure has been published previously.[4] [b] As revealed by the effect of the drug on gastric acid concentration in the pylorus-ligated rat preparation, ten rats per control group and five rats per test drug level. [c] As determined by the effect of the drug on ulcer formation in the pylorus-ligated rat test. Grading system: 0, normal stomach; 0.5, gray discoloration and thinning of mucosa; 1, hemorrhagic spots; 2, hemorrhagic suffusion; 3, 1–5 small ulcers (<3 mm); 4, many small ulcers (or 1 marked ulcer); 5, many ulcers (marked size); 6, perforated ulcer. Ten rats per control group and five rats per test drug level. [d] Grading system: ++++, marked (85–100%) inhibition; +++, moderate (60–84%) inhibition; ++, slight (45–59%) inhibition; +, little (11–44%) inhibition; 0, no (0–10%) inhibition; —, not determined.

Conclusions

A composite view of the findings obtained indicates that compound 3 exerts several unique pharmacologic activities as opposed to papaverine, a nonspecific smooth muscle antispasmodic, or to atropine, a specific anticholinergic drug. Compound 3 is an effective inhibitor of smooth muscle contractile activity as well as a gastric secretory and ulcerogenic antagonist, whereas papaverine possesses only the antispasmodic property. Unlike atropine, compound 3 evokes marked antispasmodic activity. The doses of atropine used in this study were greater than those required to nearly completely suppress acetylcholine-induced contractions.[4] In addition, compound 3 does not inhibit the fall in blood pressure of the dog in response to acetylcholine, as does atropine, suggesting a lack of specific anticholinergic action.

It may be concluded that the uniqueness of compound 3 lies in its ability to be an effective gastrointestinal antispasmodic, antisecretory, and antiulcerogenic agent without possessing specific atropine-like or anticholinergic activity.

Experimental Section

The melting point was determined in an open capillary using a Mel-Temp melting point apparatus and is uncorrected. The NMR spectrum was obtained on a Varian A-60A instrument using tetramethylsilane as an internal standard. The infrared spectrum was recorded on a Perkin-Elmer Model 137 Infracord. The ultraviolet spectrum was determined on a Perkin-Elmer Model 350 spectrophotometer. The mass spectrum was obtained on a Hitachi Perkin-Elmer RMV-7 mass spectrometer at Northern Illinois University, DeKalb, Ill.

2-(3,4-Dichloroanilino)quinolizinium Bromide (3). To 600 ml of ethanol were added 2-bromoquinolizinium bromide (1, 45 g, 0.15 mol) and 3,4-dichloroaniline (2, 49 g, 0.30 mol). The mixture was stirred and heated under reflux for 4 hr and then treated with charcoal and filtered. The hot solution was chilled to precipitate the product (35 g, 64%).

Recrystallization from ethanol (charcoal) gave analytical material which melted at 264–265°: λ_{max} 345 nm ($E_{1\,cm}^{1\%}$ 576) (5% EtOH–H_2O); ν_{max} (Nujol) 1667, 1595, 887, 846 cm^{-1}; δ (Me_2SO-d_6) 7.42–8.33 (m, 8, aromatic H's), 9.15 (t, 2, 4- and 6-quinolizium ring H's), 10.85 (broad, exchangeable, 1, NH); *m/e* (rel abundance) 292 (5), 290 (28), 288 (40), 254 (2), 252 (5), 218 (7), 155 (43), 129 (33), 128 (14), 117 (100), 82 (35), 80 (35). Anal. ($C_{15}H_{11}BrCl_2N_2$) C, H, N, Br, total halide.

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Structure–Activity Relationships in Reactivators of Organophosphorus-Inhibited Acetylcholinesterase. 10. Hydroxyiminomethylarylethenylpyridine Methiodides†

Francesca Arena, Fedele Manna, Cosimo Pizza, Maria Luisa Stein,

Institute of Pharmaceutical and Toxicological Chemistry, University of Naples, Italy

and Mario Grifantini*

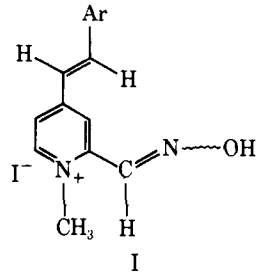
Institute of Pharmaceutical and Organic Chemistry, University of Camerino, Italy. *Received April 29, 1975*

The synthesis of styrylpyridine methiodides where a hydrogen of the pyridyl moiety was replaced by the hydroxyiminomethyl group produced highly effective inhibitors of acetylcholinesterase. As starting materials 4-methylpyridine-2-aldoxime and 2-methylpyridine-4-aldoxime methiodides were prepared which, together with 4-imidazolylethenylpyridine-2-aldoxime methiodide, were the only substances for which some activity as reactivators of phosphorylated electric eel cholinesterase in vitro could also be found.

In a previous communication[1] we reported the synthesis of some heterocyclic acraldoximes methiodides, vinyl homologs of effective reactivators of acetylcholinesterase (AChE) inhibited by diisopropylphosphorofluoridate (DFP). The ethenyl group increased the binding to the enzyme and the compounds showed some inhibitory activity but still were fairly good reactivators.

Cavallito and coworkers[2–4] demonstrated a high inhibition of choline acetyltransferase (ChA) by *trans*-4-styrylpyridine methiodides and later Baker and coworkers[5,6] found this activity, although in lower degree, in nonquaternized stilbazoles; the authors agree on the importance of the trans vinyl bridge for binding to ChA either by direct interaction of the double bond with the enzyme or by maintenance of a coplanar molecule which can act both as donor and acceptor in a charge-transfer complex with the enzyme.

For our purpose it was very interesting that the abovementioned stilbazoles inhibited AChE only slightly or not at all. For this reason we decided to synthesize derivatives of 2-pyridinecarbaldoxime and 4-pyridinecarbaldoxime methiodides (2-PAM and 4-PAM) having arylethenyl residues in the 4 and 2 positions, respectively. Compounds of general formula I and II might have more or less affinity for AChE, depending on the kind of substituent on the aryl, but could theoretically have reactivating properties.



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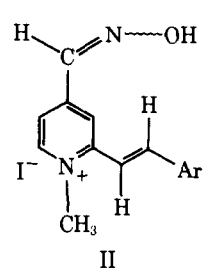


II

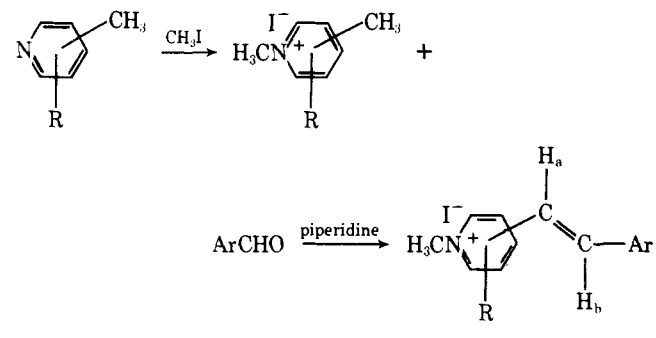
The substituents on Ar were chosen among those increasing the activity of the simple stilbazole as an inhibitor of ChA and not of AChE; imidazolyl groups in the place of Ar were taken into account for reasons given elsewhere.[7,8]

Chemistry. 2-Hydroxyiminomethyl-4-methylpyridine (1) was prepared by reaction of 4-methylpyridine-2-carboxaldehyde diacetate[9] with hydroxylamine hydrochloride. The *E* configuration was assigned to the oxime 1 since it gave a red-brown color with ferrous salts[10] and green copper chelate with cupric ions;[11] furthermore, the ir spectrum in $CHCl_3$ showed a sharp band at 3570 cm^{-1}, characteristic of a free OH group, and in the NMR spectrum the difference Δ [δ(OH) − δ(CH=N)] was 3.58 ppm.[12]

Forman's synthesis[13] was followed for the preparation of 4-hydroxyiminomethyl-2-methylpyridine (**15**) and the *Z* configuration of the oxime was confirmed by the signals at δ 12.20 (OH) and 7.40 (CH=N) of the NMR spectrum in Me_2SO-d_6 (Δ = 4.8 ppm).

In the reaction of **1** and **15** with methyl iodide the aldoxime group seems to retain its configuration since in the NMR spectra Δ is 4.35 ppm for **2** and 5.15 ppm for **16** (Scheme I).

Scheme I



R = 2- or 4-CH=NOH, respectively, in the case of 4-methyl or 2-methyl derivatives

The quaternary salts **2** and **16** were condensed with aldehydes in methanol, using piperidine as catalyst.[14]

The trans configuration of the ethenyl group was proved by uv and NMR spectra; all compounds had λ_{max} between 352 and 400 nm and, when they were exposed in EtOH solution to sunlight, a rapid shift of the maxima to lower wavelengths occurred, showing photoisomerization from trans to cis configuration.[15] In the NMR spectra the protons H_a and H_b had a trans coupling constant ranging from 15.6 to 18 Hz.[16] The trans configuration of compounds **3**, **4**, **9**, and **17–19** was assigned by uv spectra only, because of the low solubility of **4**, **18**, and **19** in Me_2SO-d_6 and of the

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