Centrally Acting Emetics. II.[1] Norapomorphine and Derivatives[2a]

MELVIN V. KOCH, JOSEPH G. CANNON,[2b]

Laboratory of Medicinal Chemistry, College of Pharmacy, The University of Iowa, Iowa City, Iowa 52240

AND ALLAN M. BURKMAN

Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210

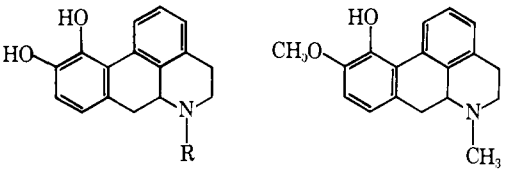
Received March 18, 1968

As a part of a continuing study of emetic agents related to apomorphine, norapomorphine and a series of N-alkylated norapomorphines have been prepared by rearrangement of the corresponding morphine derivatives. Considerable modification of literature methods for the rearrangement have been made; certain of the aporphine products were purified by gel filtration. Biological test data indicate that certain of the compounds are potent emetic agents. Apocodeine has been prepared for reevaluation of published reports of its emetic activity.

An earlier communication from this laboratory[1] reported preparation of N-allylnorapomorphine (**1a**) and its high potency as a centrally acting emetic. Accordingly, synthesis of a series of N-alkylated norapomorphines, all unknown, was undertaken in order to evaluate the effect of variation of nitrogen substituents on emetic activity (Table I). The substituents chosen (**1c–h**) were in general those which have been found to produce interesting or significant effects in the morphine series. It has been reported[3] that apocodeine (**2**) has a mild emetic activity; however, Sumwalt[4] has suggested that its emetic potency results from contamination by small amounts of apomorphine, and that pure apocodeine is inactive. Therefore, preparation of a pure sample of apocodeine was undertaken to resolve this question. Heimann[5] reported in 1915 that a sample of norapomorphine (**1b**) prepared by von Braun had emetic properties in dogs. von Braun and coworkers[6] had claimed preparation of norapomorphine by treatment of normorphine with hydrochloric acid; however, the identity and/or purity of this material are questioned, in that no melting point or elemental analytical data were reported for it. von Braun stated that the material rapidly turned a blue-green color, and he mentioned that it possessed similar physiological properties to apomorphine. In the present work, it was desired to prepare a pure, authentic sample of norapomorphine.

The apomorphine derivatives were prepared by acid-catalyzed rearrangement of the corresponding morphine congeners, which were synthesized by modifications of literature methods. Certain of the intermediate structures, namely N-propargylnormorphine and N,O,O′-tricyclopropylcarbonylnormorphine, have not been previously isolated nor characterized. Oparina and coworkers[7] and Hensiak, Cannon, and Burkman[1] rearranged morphine and N-allylnormorphine with orthophosphoric acid in a stream of HCl. The purpose of the HCl was apparently to purge water from the system; replacement of it with dry nitrogen resulted in successful, cleaner rearrangements. The N_2 provided an oxygen-free environment, diminishing the likelihood of oxidative side reactions, and exclusion of HCl prevented the formation of chloromorphide contaminants which have been shown to arise during treatment of morphine with HCl.[8] The chloromorphides as a class have been reported to possess antiemetic activity,[9] and thus are highly undesirable contaminants in material for biological testing.

Apomorphine derivative



1a, R = $CH_2CH{=}CH_2$
b, R = H
c, R = C_2H_5
d, R = n-C_3H_7
e, R = C≡CH
f, R = CH_2—cyclopropyl
g, R = $CH_2C_6H_5$
h, R = $CH_2CH_2C_6H_5$

2

Wright[10] had reported that apomorphine prepared by rearrangement of morphine contained "high molecular weight units." The technique of gel filtration, when applicable, simplified the removal of polymeric material from the aporphine products; Sephadex G-10, a cross-linked dextran, was most suitable in the present work. When the crude aporphine salts were subjected to gel filtration, the presence of high molecular weight material was indicated by a dark eluate which was not retarded on the column. This eluate gave a negative color test for apomorphine, and readily deposited solid material on standing. Those fractions of eluate which contained the aporphine monomers were retarded sufficiently to permit their separation from the dark eluate described above, and they gave a positive color test for apomorphine. Gel filtration could not be

(1) Part I: J. F. Hensiak, J. G. Cannon, and A. M. Burkman, *J. Med. Chem.*, **8**, 557 (1965).

(2) (a) This investigation was supported in part by Grant NB-04349, National Institute of Neurological Diseases and Blindness. Abstracted in part from a thesis submitted by M. V. K. in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Iowa, 1967. (b) To whom all correspondence should be addressed.

(3) A. Matthiessen and W. Burnside, *Proc. Roy. Soc.* (London), **B19**, 71 (1870).

(4) M. Sumwalt, *U. S. Public Health Service, Public Health Rept. Suppl.*, **No. 165**, 953 (1943).

(5) H. Heimann, *Z. Exptl. Pathol. Therap.*, **17**, 343 (1915).

(6) J. von Braun, O. Kruber, and E. Aust, *Ber.*, **47**, 2312 (1914).

(7) M. P. Oparina, A. S. Karasina, and B. P. Smirnov, *Khim. Farm. Prom.*, **15**, 18 (1934); U.S.S.R. Patent 40,981 (Jan 31, 1935); *Chem. Abstr.*, **30**, 7285 (1936).

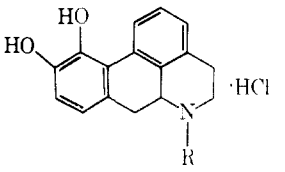
(8) L. Knorr and H. Hörlein, *Ber.*, **40**, 4883 (1907).

(9) C. H. DuToit and W. R. Christensen, Environmental Protection Series, Report No. 26. Office of the Quartermaster General, Department of the Army, 1948, p 26.

(10) C. R. A. Wright, *J. Chem. Soc.*, **25**, 652 (1872).

Table I

Norapomorphine and Derivatives



No.	R	Method	Yield, %	Dec pt, °C	$\alpha^{25}D$, deg (c 1.2)[a]	Formula	Analyses
1b	H	A	13	280–282	−82	$C_{16}H_{16}ClNO_2$	C, H, Cl, N
1c	C_2H_5	B	36	278–280	−43	$C_{18}H_{20}ClNO_2$	C, H, Cl, N
1d	n-C_3H_7	B	37	263–265	−55	$C_{19}H_{22}ClNO_2$	C, H, Cl, N
1e	$CH_2C{\equiv}CH$	A	20	245–250	[b]	$C_{19}H_{18}ClNO_2$	C, H
1f	CH_2— 	A	33	260–263	−44	$C_{20}H_{22}ClNO_2$	C, H, Cl, N
1g	$CH_2C_6H_5$	A	37	262–264	−19	$C_{23}H_{22}ClNO_2$	C, H, Cl, N
1h	$CH_2CH_2C_6H_5$	A	16	259–262	−32	$C_{24}H_{24}ClNO_2$	C, H, Cl, N

[a] The solvent was CH_3OH for all compounds except N-ethylnorapomorphine which was run in H_2O. [b] Insufficient material available for determination. [c] Recrystallized repeatedly from 2-PrOH–ether.

employed for some of the aporphine products, due to their extreme insolubility in the buffers used.

Pharmacology. Preparations.—Hydrochloride salts of all compounds were dissolved in appropriate volumes of physiological saline. For those studies requiring high concentrations of drug, suspensions were made by homogenizing[11] the compounds in an aqueous vehicle containing 0.25% methylcellulose (4000 cps).

Apomorphine Standard.—Apomorphine hydrochloride was included in the series of compounds examined and served as a reference standard. Activities of the norapomorphines and apocodeine were compared with those evoked by the standard.

Biological Activities Investigated.—The substances were all examined for their ability to produce responses that were characteristically elicited by the reference standard apomorphine. These included (a) expressions of acute toxicity in mice (*i.e.*, convulsions and lethality), (b) compulsive behavioral responses (gnawing in mice, pecking in pigeons), and (c) emesis in pigeons and dogs.

Acute Toxicity in Mice.[12]—Graded doses of each compound were administered intraperitoneally into groups of mice and the animals were observed during a 6-hr period following injection. Median lethal doses (LD_{50}) and median convulsive doses (CD_{50}) and their respective standard errors were computed by the method of Miller and Tainter[13] using data available 2 hr postinjection. In virtually all instances, animals that failed to succumb to the toxic effects of the drugs within 2 hr, failed to respond thereafter. The single exception to this rule was seen during the LD_{50} determination of **1g**. Several deaths occurred in mice receiving this sparingly soluble compound sometime after the 6-hr observation period. A 12-hr LD_{50} for this substance[14] would, therefore, be somewhat lower than that recorded as the 2-hr LD_{50}. The 2-hr statistics are summarized in Table II along with estimates of convulsive and lethal potency relative to apomorphine. Doses are expressed in terms of micromoles of base per kilogram.

Apocodeine remains the most toxic of the compounds investigated, both in terms of lethality and convulsive activity. Among the norapomorphine series, **1c** ranked highest in the two parameters of toxicity. The availability of increased amounts of **1a**, a derivative whose pharmacological activities were described in an earlier report,[1] made possible a more accurate LD_{50} estimation than had been previously possible. Examination revealed that it was among the least toxic substances studied and was devoid of all convulsant activity.

Compulsive Gnawing in Mice.—The compulsive gnawing or chewing syndrome characteristically generated by low doses of apomorphine in rodents[15] was observed following intraperitoneal administration of five of the norapomorphine derivatives, but not apocodeine. Median gnawing doses (GD_{50}) and their standard errors were computed for these compounds and are presented in Table II. The most potent gnawing stimulant proved to be **1c**, while **1d** was approximately equivalent in potency to apomorphine. **1a** was somewhat less active than apomorphine, and **1b** and **1f** were only weakly active. The same five compounds elicited vigorous Straub tail responses when the doses administered exceeded the respective GD_{50}'s. Apocodeine failed to stimulate gnawing behavior, but provoked a conspicuous Straub tail response.

Compulsive Pecking in Pigeons.—Adult White Carneau pigeons of both sexes served as test subjects. These animals were selected from a colony of birds whose high sensitivity to apomorphine had been previously established. The stereotyped pecking behavior evoked in pigeons by low doses of apomor-

(11) Dispersion was facilitated either by use of ultrasound (Sonogen Model LG 40, Branson Ultrasonic Corp., Stamford, Conn.) or by triturating the compounds in a Ten Broeck tissue grinder.

(12) Mice used in this and subsequent experiments were female Harlan ICR albino animals, 18–24 g (Harlan Industries, Cumberland, Ind.).

(13) L. C. Miller and M. L. Tainter, *Proc. Soc. Exptl. Biol. Med.*, **57**, 261 (1944).

(14) The 12-hr LD_{50} of **1g** was estimated to be 1145 μM/kg.

(15) S. Morita, *Arch. Exptl. Pathol. Pharmakol.*, **78**, 188 (1915).

TABLE II

BIOLOGICAL ACTIVITIES OF APOCODEINE AND NORAPOMORPHINE DERIVATIVES

No.	Compd	$GD_{50} \pm SE$,[a] $\mu M/kg$[b]	RP[c]	$CD_{50} \pm SE$,[d] $\mu M/kg$	RP	$LD_{50} \pm SE$,[e] $\mu M/kg$	RP	Compulsive pecking CPR ratio[f]	RP	Emesis in dogs TED ratio[g]	RP
1b	Norapomorphine	213 ± 18	0.04	441 ± 82	0.84	655 ± 34	0.90			0.36/36.0	0.01
	Apomorphine	8.4 ± 1.6	1.00	355 ± 29	1.00	592 ± 23	1.00	5,078/5,026	1.00	0.18/0.18	1.00
1c	N-Ethylnorapomorphine	2.6 ± 0.4	3.25	317 ± 19	1.17	421 ± 29	1.40	7,584/2,574	2.95	0.12/0.04	3.00
1d	N-*n*-Propylnorapomorphine	7.5 ± 0.7	1.14	467 ± 30	0.08	1024 ± 56	0.58	13,750/10,709	1.28	0.12/0.10	1.20
1a	N-Allylnorapomorphine[h]	31.8 ± 5.4	0.27			1818 ± 233	0.32	2,646/5,550	0.48	0.18/0.24	0.75
1e	N-Propargylnorapomorphine					>1829	<0.32				
1f	N-Cyclopropylmethylnorapomorphine	395 ± 34	0.02	447 ± 13	0.83	785 ± 55	0.75	323/5,257	0.06		
1g	N-Benzylnorapomorphine					>1315	<0.45				
1h	N-Phenethylnorapomorphine					>1015	<0.58				
2	Apocodeine			188 ± 37	1.98	402 ± 81	1.47				

[a] Median gnawing dose in mice ± standard error. [b] All doses × 10^{-6} *M* of base/kg. [c] Potency relative to apomorphine. [d] Median convulsive dose in mice ± standard error. [e] Median lethal dose in mice ± standard error. [f] Ratio of cumulative pecking response in pigeons evoked by 1.64 μM of base of test compound/kg to the CPR evoked by an equivalent amount of apomorphine; each value represents the mean of four determinations. [g] Estimated threshold emetic dose of apomorphine/TED of test drug; in units of μM/kg. [h] GD_{50} and CD_{50} values were extracted from an earlier report (see ref 1).

phine[16] was also displayed by birds receiving several of the norapomorphine derivatives (**1a**, **c**, **d**, **f**). In a preliminary screening maneuver, pigeons received varying doses of the test drugs intramuscularly. Drugs that failed to trigger the pecking syndrome in doses up to 70 μmoles/kg (approximately 20 mg/kg) were considered inactive. Those substances that exhibited stimulating actions were subsequently assayed by administering fixed doses (equivalent to 1.64 μmoles of base/kg) to pigeons whose responses to the same dose of apomorphine had been determined 8 days earlier. Responses, quantified by a method previously described,[17] yielded a cumulative pecking response (CPR) for each animal as an index of syndrome intensity. Four birds were used to assess the potency of each compound and each CPR value included in Table II represents a mean of four determinations. The relative potency of each compound was expressed as the mean intensity of response following test drug administration compared with the mean intensity of response following administration of an equimolar concentration of apomorphine. The most impressive effects were evoked by **1c**, whose potency was rated to be about three times that of apomorphine. **1d** also exceeded apomorphine in potency although by a less impressive margin. **1a** had about half the potency of the standard while **1f** proved to be an extremely weak pecking stimulant.

Emesis in Pigeons.—Although vomiting in pigeons in response to doses of apomorphine ranging up to 50 μmoles/kg (approximately 15 mg/kg) is usually negligible, two of the test compounds provoked persistent, sometimes extremely violent emesis in these animals. The most intense and prolonged vomiting was induced by **1b** in doses of 17–34 μmoles/kg (approximately 5–10 mg/kg). Considerably less intense was the vomiting provoked by a comparable dose of **2**. By comparison, the occasional emetic episode seen following injection of the other compounds was considered trivial.

Emesis in Dogs.—Adult female mongrel dogs weighing 8–10 kg were titrated with varying doses of apomorphine hydrochloride at 5–7-day intervals in order to establish, for each dog, a threshold emetic dose (TED). Intramuscular TED's usually ranged from 0.06–0.50 μmole/kg (approximately 0.02–0.15 mg/kg) and remained stable for each dog as long as the minimum interval of 5 days between injections was maintained. Test drugs were initially administered to these dogs in doses equal to one-half the TED of apomorphine. The doses were subsequently increased during the following test periods by geometrically spaced increments until vomiting occurred or until a dose equal to 100 times the apomorphine TED was administered. Compounds that failed to elicit vomiting in a dose 100TED were considered inactive. At least two dogs were used to assess the emetic potential of each drug. Relative emetic potency was expressed as the ratio of the mean TED for apomorphine to the mean TED for the test compound (see Table II).

Only **1c** exhibited emetic stimulant activity distinctly superior to apomorphine. **1d** was somewhat more potent than apomorphine while **1a** appeared to be slightly less potent than the standard. **1b** elicited an emetic

(16) A. M. Burkman, *J. Amer. Pharm. Assoc., Sci. Ed.*, **49**, 558 (1960).
(17) A. M. Burkman, *J. Pharm. Sci.*, **50**, 771 (1961).

response in only one animal and only at the highest dose of 36 μmoles/kg (approximately 11 mg/kg). The remaining compounds were devoid of activity as defined here. Although Matthiessen and Burnside[3] attributed mild emetic activity to apocodeine (**2**), none of the dogs in the present study vomited in response to maximum doses of the drug, and apocodeine TED's could not be established. Bergell and Pschorr[18] had reported that **2** was nauseant (but not emetic) but no prodromal symptoms were observed in the present study which could be construed as evidence of "nausea."

Experimental Section[19]

N,O,O′-Triacetylnormorphine was prepared by a modification of a method of von Braun and coworkers.[6] Normorphine[20] (20.0 g, 0.068 mole) was refluxed with 71 g (0.695 mole) of Ac_2O and 760 ml of pyridine for 3 hr, then the solvents were removed (steam bath) under reduced pressure. Residual volatile components were removed by repeated azeotroping with toluene (steam bath) under reduced pressure. Finally, the residue was heated with 300 ml of toluene and the resulting yellow solution was decanted from a dark, insoluble syrup. Evaporation of the toluene yielded 25.2 g (95%) of a light yellow solid, mp 167° (lit.[6] mp 164°).

N-Ethylnormorphine was prepared by a method utilized by Gates and Montzka[21] for preparation of N-cyclopropylmethylnormorphine. N,O,O′-Triacetylnormorphine (24.5 g, 0.062 mole) in 325 ml of purified THF was added dropwise to a stirred slurry of 7.2 g (0.190 mole) of $LiAlH_4$ in 75 ml of THF. The reaction mixture was stirred at room temperature for 24 hr, then the excess $LiAlH_4$ was decomposed with 40 ml of EtOAc followed by 35 ml of H_2O. A saturated solution of ammonium tartrate (400 ml) was added and the mixture was stirred for 4 hr. The aqueous suspension of aluminum salts was then separated and was washed with five 500-ml portions of EtOAc. The combined organic layers were washed with six 200-ml portions of saturated NaCl solution, dried (Na_2SO_4), and were taken to dryness under reduced pressure. The tan solid residue was recrystallized from EtOAc to yield 9.9 g (48%) of material, mp 200–204° (lit.[20] mp 195–198°).

N,O,O′-Tripropionylnormorphine was prepared by a modification of a method of von Braun and co-workers.[6] A mixture of 36.0 g (0.276 mole) of propionic anhydride and 10.0 g (0.034 mole) of normorphine[20] was refluxed 5 hr, then volatile materials were removed (steam bath) under reduced pressure. Residual volatile components were removed by repeated azeotroping with xylene from a steam bath under reduced pressure. Finally, the brown syrupy residue was crystallized from anhydrous ether to yield 13.2 g (90%) of acicular crystals, mp 117–119° (lit.[22] mp 215–217°). *Anal.* ($C_{23}H_{29}NO_6$) C, H, N, O.

N-n-Propylnormorphine.—N,O,O′-Tripropionylnormorphine (13.2 g, 0.030 mole) in 300 ml of purified THF was added dropwise to a stirred slurry of 3.0 g (0.079 mole) of $LiAlH_4$ in 100 ml of THF. After addition was complete, the mixture was refluxed 8 hr and cooled, and the excess $LiAlH_4$ was decomposed by dropwise addition of 20 ml of cold H_2O. The heavy gray-white mixture was treated with 200 ml of 10% NaOH and to the resulting orange solution was added an excess of solid CO_2, resulting in separation of a heavy precipitate. This material was collected on a filter; it was dried and extracted 70 hr with $CHCl_3$ in a Soxhlet apparatus in which the thimble chamber was heated with an ir lamp. The $CHCl_3$ extract was taken almost to dryness on a steam bath, and the residue was rubbed with anhydrous ether to induce formation of 7.0 g (68%) of a tan solid, mp 225–229° dec (lit.[20] mp 225–228°).

N-Propargylnormorphine was prepared by a method utilized by Clark and coworkers[22] for certain other morphine congeners. A mixture of propargyl bromide (5.2 g, 0.044 mole), 12 g (0.040 mole) of normorphine,[20] 5.3 g (0.063 mole) of $NaHCO_3$, and 200 ml of absolute EtOH was refluxed 5 hr, then was filtered while still hot. The filtrate was taken to dryness under reduced pressure and the residue was extracted 24 hr with $CHCl_3$ in a Soxhlet apparatus in which the thimble chamber was heated with an ir lamp. The $CHCl_3$ extract was taken almost to dryness on a steam bath and the residue was rubbed with anhydrous ether to induce formation of a light yellow solid. A solution of this material in 20 ml of 2-PrOH was diluted with 3 l. of anhydrous ether and the resulting mixture was filtered. The filtrate was evaporated to approximately 50 ml, which caused separation of 5.7 g (46%) of a solid, mp 182–185°. A Karl Fischer determination indicated the presence of no chemically or physically bound H_2O. *Anal.* ($C_{19}H_{21}NO_4$) C, H, N.

N,O,O′-Tricyclopropylcarbonylnormorphine.—A mixture of 5.0 g (0.017 mole) of normorphine,[20] 8.6 g (0.082 mole) of cyclopropylcarbonyl chloride (Aldrich Chemical Co.), and 200 ml of pyridine was refluxed for 9 hr. The solvent was removed from a steam bath under reduced pressure, and the residual volatile components were removed by repeated azeotroping with xylene from a steam bath under reduced pressure. Finally, the dark residue was heated with 900 ml of toluene and the resulting yellow solution was decanted from an insoluble syrup. The toluene solution was evaporated under reduced pressure; the yellowish white solid residue was washed with 600 ml of anhydrous ether, resulting in 5.1 g (64%) of white crystals, mp 183–185°. *Anal.* ($C_{28}H_{29}NO_6$) C, N, O.

N-Cyclopropylmethylnormorphine was prepared by the method of Gates and Montzka,[21] mp 189–191° (lit.[21] mp 190–192°).

N-Benzylnormorphine was prepared from normorphine[20] by the method of Clark and coworkers,[22] mp 226–227° (lit.[22] mp 230–231°).

N-Phenethylnormorphine was prepared from normorphine[20] by the procedure of Clark and coworkers,[22] mp 246–247° dec (lit.[23] mp 250–253° dec).

Norapomorphine and N-Substituted Norapomorphines.—A suspension of 10 g of normorphine or of the N-alkylated normorphine in 56 ml of 85% H_3PO_4 was immersed in an oil bath at 120° while passing N_2 through the mixture at a rapid rate. The temperature was raised to 145–150° over 20 min, during which time most of the morphine derivative dissolved. After 1 hr, the hot reaction mixture was diluted with 100 ml of cold H_2O and was placed in a cold room overnight. The cold reaction mixture was treated with 25 g of NaCl, added in small portions with stirring; a tan solid separated. This was collected on a filter and was treated according to method A or B.

Method A.—The precipitate was dissolved in H_2O at 50–60°, and this temperature was maintained (see Table I).

Method B.—The precipitate was dissolved in 150 ml of H_2O at 50–60°, and the solution was allowed to cool to room temperature. It was filtered and the dark filtrate was placed on a 5.0 × 63.5 cm Sephadex G-10 (lot 9493, Pharmacia Chemicals) column wet with 0.1 *M* acetate buffer of pH 4.0. This buffer was used to elute the sample into 20-ml fractions, from 150 to 200 being collected, depending upon the retardation of the sample on the column. The detection of aporphine material in the eluate was accomplished by use of the nitric acid color test for apomorphine,[24] a positive test being the development of a purple color. Those fractions which gave a positive test were pooled (see Table I).

The aqueous solution obtained by method A or B was treated with excess Na_2SO_3 added in small amounts with stirring; the free aporphine base separated as a finely divided solid. This material was extracted in a separatory funnel with 6–8 l. of ether in 500–700-ml portions. (In the case of norapomorphine **1b**, a continuous liquid-liquid extraction for 24 hr was necessary.) The ether extracts were combined, dried ($MgSO_4$), and filtered,

(18) P. Bergell and R. Pschorr, *Therap. Gegenwart*, **45**, 247 (1904).

(19) Melting points were determined on a Thomas–Hoover apparatus and were corrected. Elemental analyses and Karl Fischer determinations were performed by Huffman Microanalytical Laboratory, Wheatridge, Colo. and Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Where analyses are indicated only by symbols of the elements, the analytical results obtained for those elements were within ±0.4% of the theoretical values.

(20) A. F. Green, G. K. Ruffell, and E. Walton, *J. Pharm. Pharmacol.*, **6**, 330 (1954).

(21) M. Gates and T. A. Montzka, *J. Med. Chem.*, **7**, 127 (1964).

(22) R. L. Clark, A. A. Pessolano, J. Weijlard, and K. Pfister, *J. Amer. Chem. Soc.*, **75**, 4963 (1953). It is assumed that this melting point is a misprint.

(23) L. F. Small, N. B. Eddy, J. H. Ager, and E. L. May, *J. Org. Chem.*, **23**, 1387 (1958).

(24) "The Pharmacopoeia of the United States of America," 15th ed., Mack Publishing Co., Easton, Pa., 1960, p 63.

and the filtrate was treated with ethereal HCl, resulting in a dense white precipitate which was collected on a filter (see Table I).

Apocodeine (2).—Codeine phosphate (10.0 g, 0.0236 mole) was rearranged as described for the morphine series. The dark reaction mixture was diluted with 300 ml of H_2O and extracted with ether. The aqueous layer was basified with concentrated NH_4OH and extracted repeatedly with ether. The combined ethereal extracts were evaporated on a steam bath, and small amounts of residual H_2O were removed by azeotroping with benzene. The solvents were completely removed under reduced pressure, the residue was taken up in ether–benzene (10:90), and this solution was chromatographed on neutral alumina. Elution with the same solvent system, with ether, and finally with ether–CH_3OH (90:10) permitted collection of fractions which formed a salt with ethereal HCl and were pooled. The HCl salt was recrystallized from C_2H_5OH–ether (charcoal) to afford 1.5 g (20%) of white crystals, mp 260–265° dec (lit.[25] mp 260–263°). *Anal.* ($C_{18}H_{20}ClNO_2$) C, H, Cl; N: calcd, 4.42; found, 3.71.

Apocodeine was freed from its HCl salt with Na_2CO_3, mp 120–123° (lit.[25] mp 122.5–124.5°).

(25) K. Folkers, *J. Amer. Chem. Soc.*, **58**, 1814 (1936).

4-[3(5)-Pyrazolyl]pyridinium Salts. A New Class of Hypoglycemic Agents

VICTOR J. BAUER,[1] HARRY P. DALALIAN, WILLIAM J. FANSHAWE, S. R. SAFIR,

Organic Chemical Research Section

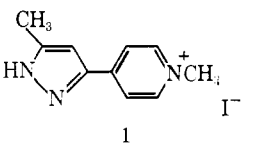
E. C. TOCUS, AND C. R. BOSHART

Experimental Therapeutics Research Section,
Lederle Laboratories, A Division of American Cyanamid Company, Pearl River, New York 10965

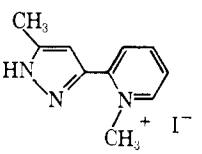
Received April 24, 1968

A series of 4-[3(5)-pyrazolyl]pyridinium salts has been synthesized. Many of these compounds display interesting hypoglycemic activity in alloxan-diabetic mice; a structure–activity relationship is derived.

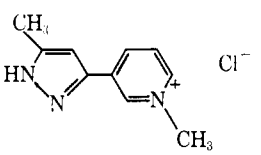
During the course of screening of randomly selected compounds for oral hypoglycemic activity, it was discovered that 1-methyl-4-[5(3)-methyl-3(5)-pyrazolyl]pyridinium iodide (**1**) markedly lowered the blood sugar levels of fasted normal chicks. Comprehensive development of the lead was begun when it was demonstrated that this effect was just as pronounced in alloxan-diabetic mice (up to 95% reduction of blood glucose values). In this paper we delineate the structural requirements for hypoglycemic activity of the pyrazolylpyridinium salts.



1



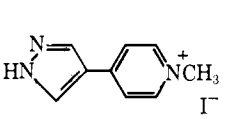
2



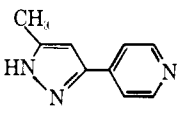
3

Structure–Activity Correlation.—Attention was first directed to the specificity of the location of the pyrazole–pyridinium ring attachment. Compounds **2** and **3**, the 2-pyridinium and 3-pyridinium analogs of **1**, were found to be inactive, as was **4**, in which the 4-pyrazolyl position is bonded to the 4-pyridinium position. Thus, the 4-[3(5)-pyrazolyl]pyridinium structure is required.

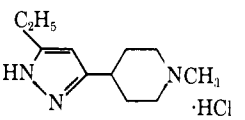
The presence of the pyridinium salt moiety of **1** was shown to be necessary by the absence of hypoglycemic activity in the related tertiary base **5** and piperidine salt **6**. Variations in the nature of the five-membered



4



5



6

heterocyclic ring will be considered in subsequent papers.[2]

The effect upon activity of substituents on the 4-[3(5)-pyrazolyl]pyridinium nucleus was then explored by the synthesis and testing of an extensive series of analogs of **1** (Table I). It was found that compounds containing a hydrogen atom (**7**, **8**), alkyl group (**9**–**14**), benzyl group (**15**), or cyclopropyl ring (**16**) at the 5(3)-pyrazolyl position were active, but that the activity was destroyed by the introduction of certain electronegative substituents (**17**–**19**) or a phenyl group (**20**) at this site.

The hydrogen atom at the 4-pyrazolyl or 3-pyridyl position could be replaced by a methyl group (**21**, **22**) with retention of activity.

When the N-methyl substituent of **1** was replaced with larger alkyl groups (**23**–**29**), activity was retained. Alkenyl substituents on the pyridine nitrogen gave **30**–**34** which displayed hypoglycemic activity. Compound **35**, in which the N-methyl had been replaced by cyclopropylmethyl, was active, but **36** with a phenacyl and **37**, with an ethoxycarbonylmethyl substituent, were inactive.

Since alkyl groups at the 5(3)- and 4-pyrazolyl positions led to active compounds, the tetrahydroindazole

(1) Author to whom inquires should be addressed.

(2) V. J. Bauer, W. J. Fanshawe, H. P. Dalalian, and S. R. Safir, *J. Med. Chem.*, **11**, 984 (1968).