Synthesis of Xanthoxime and Pyridine-3-aldoxime Esters and Ethers as Potential Anticholinergics

By SUSHIL K. GOMER, EUGENE I. ISAACSON, ROBERT G. BROWN, and JAIME N. DELGADO

The synthesis of a series of oxime esters and ethers was accomplished by acylation and *O*-alkylations of 3-pyridinealdoxime and xanthoxime. The esterifications were effected by treating the oximes with benzoyl chloride whereas the *O*-alkylations were effected by treating the oximes with β-dimethylaminoethyl and γ-dimethylaminopropyl chlorides. In the course of this investigation, five compounds were synthesized and evaluated for their anticholinergic activity. This study reveals that the oxime esters and ethers possessing the bulkier xanthene moiety are, in general, more anticholinergic than those derived from 3-pyridinealdoxime. 9-(γ-Dimethylaminopropyloximino)-xanthene methyl bromide was found to be the most potent anticholinergic agent among the new compounds evaluated.

Previous work in these laboratories has led to the synthesis of oxime esters and ethers derived from 1-methyl-4-oximinopiperidine, 3-oximinotropane, benzophenone oxime, and dibenzosuberone oxime (1, 2). Since these studies have demonstrated that certain oxime derivatives possess interesting anticholinergic activity (1), it was decided to extend the series by preparing analogous compounds from pyridine-3-carboxaldehyde and xanthone as potential anticholinergic agents. These carbonyl compounds were selected as starting materials because they provide the basis for comparing the effect of a tricyclic moiety with the pyridine heterocycle on anticholinergic activity. The present work encompasses the synthesis and preliminary pharmacologic evaluation of the oxime derivatives delineated in Tables I and II.

The synthesis of 3-pyridinealdoxime presented no difficulties. For general preparative purposes the method of Ginsburg and Wilson (3) was employed. The utilization of this procedure entailed adjusting an aqueous solution of the aldehyde and hydroxylamine hydrochloride to neutrality by the addition of a 10% sodium hydroxide solution and heating for 20 min.

The synthesis of xanthoxime proceeded with greater difficulty than did the synthesis of 3-pyridinealdoxime. However, application of the method of Campbell *et al.* (4) proved to be successful. Refluxing the ketone together with hydroxylamine hydrochloride in anhydrous pyridine as base and solvent for 24 hr. gave the oxime in yields to 80% of theory.

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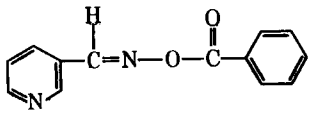
The oximes were converted to the ester derivatives by conventional acylation procedures employing the acyl chloride. 3-(Benzoyloximino)-pyridine was prepared using a number of solvents. The best yields were obtained when the solvent was anhydrous benzene or anhydrous ether. In the preparation of 9-(benzoyloximino)xanthene anhydrous ether was used as solvent.

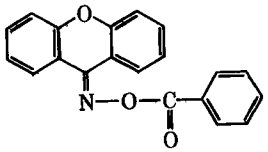
The preparation of the oxime ethers was accomplished by dissolving the oximes in ethanol, adding sodium hydride, and subsequently adding dropwise a solution of the respective aminoalkylhalides in ethanol. The ethers, all containing the aliphatic amino function, were characterized as the picrate or methyl halide derivatives.

The compounds were synthesized in order to evaluate the influence of certain groups on bioactivity, with a specific point in mind being that the oximino ester and oximino ether linkages could serve as groups favoring an interaction with the cholinergic receptor, provided that other groups are present. Thus, if the oximino linkage could serve as a binding group, then the presence of a cationic center, for example, the quaternary ammonium function, characteristic of many agents acting at cholinergic receptors, should promote affinity for the receptor. The result of interaction with the receptor could be either a cholinergic or anticholinergic action depending upon other molecular features. For example, relatively large nonpolar and semiplanar ring systems are frequently associated with anticholinergic activity. It would be interesting to contrast such a grouping with a smaller and more polar moiety.

TABLE I—PHYSICAL CONSTANTS AND ANALYTICAL DATA OXIME ESTERS



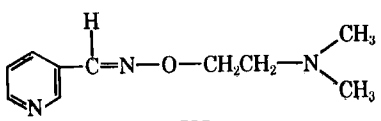
I



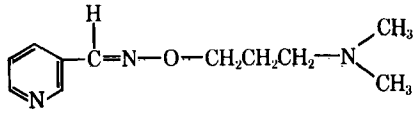
II

Compd. No.	Derivatives	M.p., °C.	% Yield	Empirical Formula	Anal. Calcd.	Anal. Found
I	—	—	—	—	—	—
I-*A*	Hydrochloride salt	124–124.5	62	$C_{13}H_{11}ClN_2O_2$	C, 59.46 H, 4.22	59.59 4.35
II	—	126.5–127	98	$C_{20}H_{13}NO_3$	C, 76.17 H, 4.15	75.98 4.13

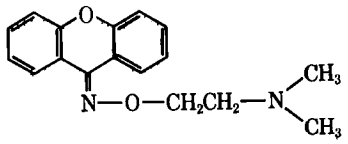
TABLE II—PHYSICAL CONSTANTS AND ANALYTICAL DATA OXIME ETHERS



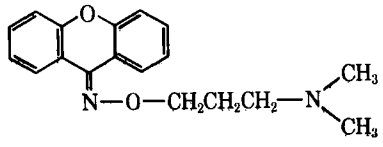
III



IV



V



VI

Compd. No.	Derivatives	M.p., °C.	% Yield	Empirical Formula	Anal. Calcd.	Anal. Found
III	—	—	67	$C_{10}H_{15}N_3O$	—	—
III-*A*	Picrate	134.5–135	—	$C_{16}H_{18}N_6O_8$	C, 45.50 H, 4.29	45.54 4.50
III-*B*	Dimethyl dibromide	226–226.5	—	$C_{12}H_{21}Br_2N_3O$	C, 37.61 H, 5.52	37.67 5.35
IV	—	—	56.4	$C_{11}H_{17}N_3O$	—	—
IV-*A*	Picrate	130	—	$C_{17}H_{20}N_6O_8$	C, 46.78 H, 4.62	46.87 4.75
IV-*B*	Dimethyl diiodide	166–166.5	—	$C_{13}H_{23}I_2N_3O$	C, 31.78 H, 4.72	32.37 4.78
V	—	—	60	$C_{17}H_{18}N_2O_2$	—	—
V-*A*	Picrate	177–178.5	—	$C_{23}H_{21}N_5O_9$	C, 54.01 H, 4.14	54.26 4.38
VI	—	—	65.8	$C_{18}H_{20}N_2O_2$	—	—
VI-*A*	Picrate	134–135	—	$C_{24}H_{23}N_5O_9$	C, 54.86 H, 4.41	54.84 4.39
VI-*B*	Methyl bromide	240	—	$C_{19}H_{23}BrN_2O_2$	C, 58.32 H, 5.92	58.20 5.88

EXPERIMENTAL[1]

Synthesis of Oximes

3-Pyridinealdoxime—This compound was prepared according to the method of Ginsburg and Wilson (3). The oxime was recrystallized from 95% ethanol. The analytically pure sample melted at 150°; reported m.p. 150–151° (3). The yield was 1.8 g. (85% of theory).

Xanthoxime—The procedure of Campbell *et al.* (4) was adapted to the synthesis of this compound. The oxime was recrystallized from anhydrous benzene. An analytically pure sample of this compound melted at 161°; reported m.p. 161° (4). The yield was 8.5 g. (80% of theory).

[1] Reported melting points are uncorrected. A Thomas-Hoover Unimelt apparatus was used for melting point determinations. Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., conducted the elemental analysis.

Synthesis of Oxime Esters

3-(Benzoyloximino)pyridine (I)—This compound was prepared from 3-pyridinealdoxime (3.05 g., 0.025 mole) and benzoyl chloride (4.21 g., 0.03 mole) in 1,4-dioxane according to the procedure of Gass and Bope (5). The yield was improved when anhydrous benzene was the solvent. However, anhydrous ether was found to be the most suitable reaction solvent. The hydrochloride salt (I-*A*) was prepared and recrystallized from ethanol to give the analytically pure sample, m.p. 124°. The yield was 3.2 g. (62% of theory).

9-(Benzoyloximino)xanthene (II)—Xanthoxime (3.37 g.; 0.016 mole) in anhydrous ether (200 ml.) was placed in a 500-ml., 3-necked round-bottom flask fitted with a stirrer, dropping funnel, and a condenser with a drying tube. Benzoyl chloride (2.24 g., 0.01 mole) dissolved in anhydrous ether (2.5 ml.) was added dropwise with stirring at room

temperature. Anhydrous benzene (100 ml.) was added twice at 15-min. intervals. The reaction mixture was washed with 10% potassium carbonate solution. The nonaqueous layer was dried over anhydrous sodium sulfate and filtered. The solvents were distilled off under reduced pressure. The solid residue was recrystallized from absolute ethanol. The analytically pure sample melted at 126.5–127°; the yield was 4.9 g. (98% of theory).

Synthesis of Oxime Ethers

3-(β-Dimethylaminoethyloximino)pyridine (III)—3-Pyridinealdoxime (6.1 g., 0.05 mole) was placed with ethanol (75 ml.) in a 3-necked round-bottom flask fitted with stirrer, a dropping funnel, and a water condenser with a drying tube. Sodium hydride (4.70 g. of a 51.6% mineral oil dispersion; 0.01 mole) was added slowly in small portions. The reaction mixture was stirred for 30 min. β-Dimethylaminoethylchloride hydrochloride (7.25 g., 0.05 mole) in ethanol (50 ml.) was added dropwise with stirring to the refluxing reaction mixture. The mixture was refluxed and stirred for 4 hr. Strictly anhydrous conditions were observed throughout the period. The reaction mixture was filtered, and the solvent was distilled off under reduced pressure. The product was obtained as an orange, viscous oil. The yield was 6.4 g. (67% of theory). The oxime ether was characterized as the picrate (III-*A*) and dimethyl dibromide (III-*B*) derivatives.

The other oxime ethers, 3-(γ-dimethylaminopropyloximino)pyridine (IV), 9-(β-dimethylaminoethyloximino)xanthene (V), and 9(γ-dimethylaminopropyloximino)xanthene (VI) were synthesized according to the method described for 3-(β-dimethylaminoethyloximino)pyridine with reflux periods of 6, 30, and 48 hr., respectively. Each of these was characterized as the picrate derivative, IV-*A*, V-*A*, and VI-*A*, respectively. 3-(γ-Dimethylaminopropyloximino)pyridine (IV) also was characterized as the methyl iodide derivative (IV-*B*) and 9(γ-dimethylaminopropyloximino)xanthene as the methyl bromide derivative (VI-*B*).

PHARMACOLOGICAL EVALUATION

The pharmacological evaluation of the synthesized compounds was undertaken to assess their spasmolytic activity against the spasm induced by predetermined doses of the spasmogens, acetylcholine bromide and methacholine[2] chloride, on the excised strips of smooth muscle of rat ileum. The method involves a quantitative comparison, *in vitro*, of the spasmolytic activity of each experimental compound in parallel with a reference drug, atropine sulfate, under reproducible conditions.

The smooth muscle employed as test tissue was a strip of 1–2 cm. in length from an anesthetized male or female albino rat which had been recently sacrificed. The strip was suspended in an 80-ml. bath chamber filled to a constant volume (70 ml.) with the Sollmann-Rademaeker solution at a constant temperature of 37°. The muscle contractions were amplified and recorded on an E and M Physiograph through a myograph attachment.

The method was essentially that of Magnus as modified by Ariens (6). This method uses logarithmic dilutions of spasmogens. The Ariens method yields an S-shaped cumulative dose-response curve when percent contraction (response) is plotted against log millimole dose. The potencies of the experimental compounds relative to the reference drug were determined in a series of experiments. The five compounds reported in Table III include one (II) that was markedly inactive against the spasmogens. A second compound (III-*B*) exhibited cholinomimetic activity as reported in Fig. 1. The remaining three compounds showed antispasmodic activity as summarized in Figs. 2, 3, and 4. Relative potencies of the antispasmodics are presented in Table III.

STRUCTURE-ACTIVITY RELATIONSHIPS

The pharmacological evaluation of the experimental compounds reveals that most possess perceptible antispasmodic activity, although the order of acetylcholine antagonism is low as compared with atropine sulfate. Compound VI-*B* is the most active of the experimental compounds. It is $^1/_{62}$ as active as atropine sulfate. It possesses the relatively large xanthene group. Replacement of the large and bulky xanthene group in VI-*B* with a methylpyridinium group yields com-

TABLE III—RELATIVE POTENCIES OF DERIVATIVES OF OXIME ESTERS AND OXIME ETHERS

Compound No.	Activity	Relative Potency Compared with Atropine Sulfate
I-*A*	Anticholinergic	1/40,000
II	Inactive	—
III-*B*	Cholinomimetic	—
IV-*B*	Anticholinergic	1/666
VI-*B*	Anticholinergic	1/62.5

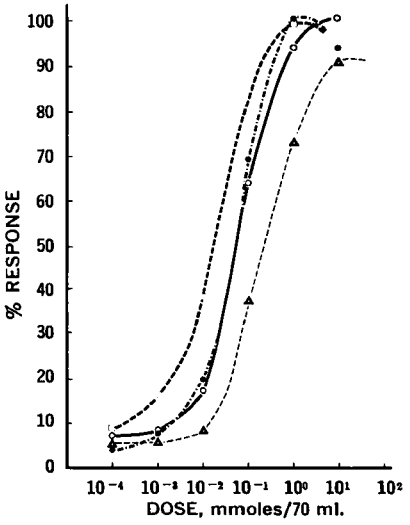


*Fig. 1—Investigation of activity of Compound III-*B *on rat ileum. Key: cumulative dose response curves. ○, acetylcholine bromide (control); ●, acetylcholine bromide (control after pretreatments); △, pretreatment with 1.0 × 10⁻³ mmoles atropine sulfate; □, pretreatment with 1.0 × 10 mmoles III-*B.

[2] Mecholyl chloride, Merck & Co., Rahway, N. J.

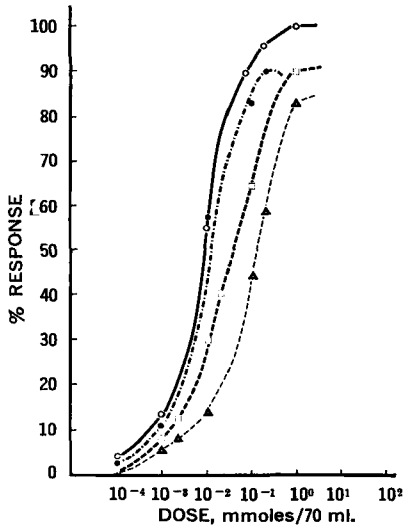


Fig. 2—Investigation of spasmolytic activity *of compound I-*a *on rat ileum. Key: Cumulative dose-response curves.* ○, *methacholine chloride (control);* △, *pretreatment with 1.0 × 10^{-4} mmoles atropine sulfate;* □, *pretreatment with 1.0 × 10^{0} mmoles I-*A*;* ●, *methacholine chloride (control after pretreatments).*

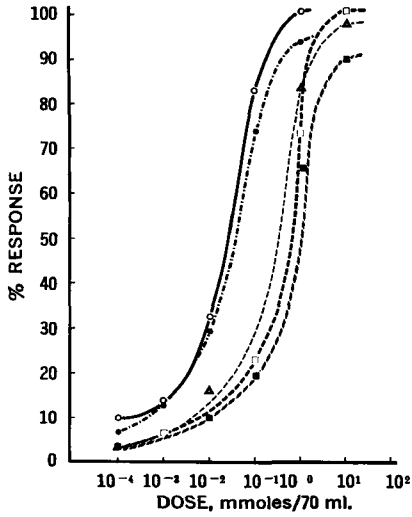


*Fig. 3—Investigation of spasmolytic activity of Compound IV-*B *on rat ileum. Key: cumulative dose-response curves* ○, *acetylcholine bromide (control);* ●, *acetylcholine bromide (control after pretreatments);* △, *pretreatment with 1.0 × 10^{-3} mmoles atropine sulfate;* □, *pretreatment with 1.0 × 10 mmoles IV-*B*;* ■, *pretreatments with 2.0 × 10 mmoles IV-*B.

pound IV-*B* which is about 10 times less active than the former.

Compound III-*B* somewhat resembles acetylcholine in structure. It has a *N*-methyl-3-pyridinealdoximino moiety replacing the acetoxy group. In broad qualitative terms, it thus has structural features related to a second choline moiety, *i.e.*, a quaternary ammonium function, a three carbon chain, and a site of high electron density. This compound shows cholinomimetic activity. It is

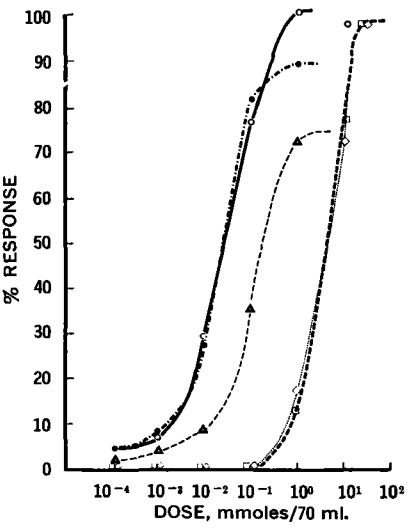
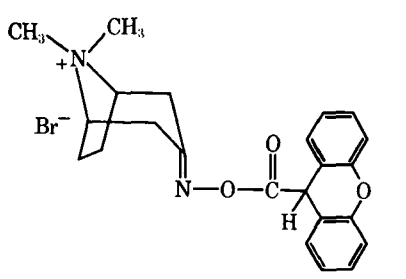


*Fig. 4—Investigation of spasmolytic activity of Compound VI-*B *on rat ileum. Key: cumulative dose-response curves.* ○, *acetylcholine bromide (control);* △, *pretreatment with 1.0 × 10^{-3} mmoles atropine sulfate;* □, ◇, *pretreatment with 1.0 × 10 mmoles, VI-*B*;* ●, *acetylcholine bromide (control after pretreatments).*

interesting to note that IV-*B*, differing from III-*B* by the insertion of a —CH_2— group, possesses discernible anticholinergic activity.

Compound II is poorly soluble in an aqueous medium. Lack of distribution and accessibility to the site of action may partly account for its inactivity, but it should be noted that this compound does not possess a cationic head, one of the pharmacophoric moieties commonly implicated in the drug–receptor interaction, which also should be important. Compound I-*A* would be present to some extent in the protonated form under the test conditions. Compounds III-*B*, IV-*B*, VII have the quaternary ammonium function and are active. These studies indicate that although an ester function is not absolutely essential (7), the presence of a related site of polarity is important. Many potent anticholinergic drugs possess interatomic distances between the cationic nitrogen and a polar and/or polarizable group (*e.g.*, ester) comparable to those of acetylcholine, in addition to large hydrophobic and semirigid moieties. In this connection, it is noteworthy that compound VI-*B* is less active than the oxime ester 3-(xanthene-9-acyloximino)tropane methyl bromide (VII), reported previously (1). The compound VII is six times more active than atropine. This difference



VII

in anticholinergic activity between VII and the experimental compound VI-*B* might be due in part to the difference in steric rigidity and bulkiness of the tropane N-function and the acyclic N-function of VI-*B*.

REFERENCES

(1) Kochhar, M. M., Brown, R. G., and Delgado, J. N., *J. Pharm. Sci.*, **54**, 393(1965).
(2) Wylie, B. B., Isaacson, E. I., and Delgado, J. N., *ibid.*, **54**, 1373(1965).
(3) Ginsburg, S., and Wilson, I. B., *J. Am. Chem. Soc.*, **79**, 481(1957).
(4) Campbell, N., McCallum, S. R., and McKenzie, D. J., *J. Chem. Soc.*, **1957**, 1922.
(5) Gass, L., and Bope, F. W., *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 186(1959).
(6) Ariens, E. J., Presented to the Scientific Section, American Pharmaceutical Association, Detroit meeting, April 1965.
(7) Winder, C. V., Kaiser, M. E., Anderson, M. M., and Glassco, E. M., *J. Pharmacol. Exptl. Therap.*, **87**, 121(1946).

Keyphrases

Xanthoxime esters, ethers—synthesis
Pyridine-3-aldoxime esters, ethers—synthesis
Anticholinergic activity—screening
Ileum strips—spasmolytic testing

Morphine-Induced Fetal Malformations I

Exencephaly and Axial Skeletal Fusions

By HOWARD S. HARPEL, JR.* and RONALD F. GAUTIERI

High subcutaneous doses of morphine sulfate, 100–500 mg./kg., administered to CF-1 mice on Day 8 or Day 9 of gestation are teratogenic and result in a large number of fetuses with exencephaly and axial skeletal fusions. Retardation in food consumption is not primarily responsible for these effects even though fasting alone affects embryonic development. Based upon the narrow range between the maternal LD_{50} and the teratogenic doses in this species, the teratogenic potential of morphine sulfate is low.

With the current interest in drug-induced embryopathies, it is surprising that the recent literature is devoid of teratogenic studies involving the narcotic analgesics. In 1931, Myers (1) reported that daily morphine administration beginning 3–5 months before mating and continuing throughout gestation had little effect upon the growth and reproduction of the albino rat. Although the litters were of average size and composed of young that appeared normal, the method of examination was not as extensive as the techniques currently employed in teratogenic studies. Moreover, ample time was available for the female rats to become tolerant to morphine before conception, and consequently, all development occurred in a morphine-adapted environment.

Therefore, it was imperative to determine the consequences of morphine sulfate administration during the critical stages of development in a common laboratory mammal by administering high subcutaneous doses to nontolerant CF-1 mice on the eighth or ninth day of gestation. Moreover, during the investigation it became clear that drug administration produced a temporary decrease in food intake, and for this reason the effect of food deprivation on each of these 2 days also was investigated.

EXPERIMENTAL

CF-1 albino mice weighing between 20–25 g. were obtained from Carworth Farms, Inc., New City, N. Y. Females were caged in groups of 25 to 30 for at least 2 weeks after arrival and were not mated until they weighed at least 25 g. Males and gravid females were caged individually in metal cages measuring 12.5 × 15 × 10 cm. with a wire mesh front and floor. The colony was maintained on Purina laboratory chow and tap water *ad libitum*. Sodium chloride (0.9%) and morphine sulfate[1] (4.0%) prepared weekly in distilled water were

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* Present address: Menley and James Laboratories, 1500 Spring Garden Street, Philadelphia, PA 19102

[1] Morphine sulfate, USP, Merck Co., Rahway, N. J.