Table I—Recoveries of Pemoline Added to Human Urine Samples

Pemoline Added to 20 ml of Human Urine, μg	Amount Recovered, μg	Recovery, %
25.5	25.1	98.4
51	49.9	97.8
102	100	98.0
204	203.5	99.8
408	403.5	98.9
	Mean value ± *SD*	98.6 ± 0.80

analysis. Therefore, organic layers were dried by cooling for 15 min; remaining water droplets were removed with filter paper.

Pemoline could not be chromatographed even on nonpolar liquid phases. The product of acid hydrolysis, III, is more volatile, but peaks tend to tail on columns of various polarities. When 40–60-mesh polytetrafluoroethylene polymer[9] was used as support material, some improvement in peak symmetry was observed, but the columns were of low efficiency. Methylation with diazomethane to IV yielded a very volatile compound that demonstrated excellent peak symmetry on fairly nonpolar liquid phases.

Since the *N*-methyl derivative is insoluble in alkali, a final cleanup was possible by extracting the organic layer with sodium hydroxide.

Because of its similarity with the hydrolysis product of pemoline in chemical behavior, allobarbital was chosen as an internal standard. Both drugs are weak acids and can be reextracted into alkali, so allobarbital can be added immediately after hydrolysis. Furthermore, both products form *N*-methyl derivatives with diazomethane, so the final cleanup with sodium hydroxide solution gives no loss.

Recovery studies from urine and plasma were carried out by adding the internal standard before derivatization; in all other experiments, the internal standard was added immediately after hydrolysis. Addition of pemoline to five 20-ml urine samples in the range anticipated for therapeutic levels gave a recovery of 98.6 ± 0.80%. All determinations were carried out in duplicate, the lowest recovery from a single determination being 97.8% (Table I). Although recoveries were high, extreme care had to be taken during evaporation since IV and the methylated allobarbital are extremely volatile.

[9] Chromosorb T, Applied Science Laboratories.

Table II—Recoveries of 10 μg of Pemoline Added to Human Plasma Samples

Amount Recovered[a], μg	Recovery, %
10.20	101.5
9.70	96.3
9.80	97.4
9.90	98.2
9.90	98.6
Mean value ± *SD*	98.4 ± 1.94

[a] Values of the recovered IV without correction to I.

Addition of pemoline to human plasma yielded a recovery of 98.4 ± 1.94% (Table II). Since the normal range of plasma concentrations is 1.0 μg/ml, 10-ml samples were required when using a flame-ionization detector. Efficiency, however, could be improved markedly by using a nitrogen detector[10]. Although pemoline gave no increased nitrogen signal, the extreme stability and high noise-to-signal ratio of the detector allowed quantitative estimation of 20 ng of pemoline, which is sensitive enough to require 1 ml of plasma. Oral intake of 40 mg of pemoline, a therapeutic dose, gives a plasma concentration of 0.8 μg/ml at about 4 hr. Determination in urine is still possible 48 hr after ingestion due to the long elimination time of pemoline.

A complete elimination and metabolism pattern of pemoline is currently being investigated.

REFERENCES

(1) W. Traube and R. Ascher, *Chem. Ber.*, **46**, 2083 (1913).
(2) L. Schmidt, *Arzneim.-Forsch.*, **6**, 423 (1956).
(3) P. Bugard, *Thérapie*, **17**, 63 (1962).
(4) D. Duval and J. Sterne, *ibid.*, **15**, 1250 (1960).
(5) L. M. Cummins and J. E. Perry, *J. Pharm. Sci.*, **58**, 762 (1969).
(6) A. Slob, *Br. J. Ind. Med.*, **30**, 390 (1973).
(7) H. M. Fales and T. M. Jaouni, *Anal. Chem.*, **45**, 2302 (1973).
(8) J. C. Libeer and P. Schepens, *Pharm. Weekbl.*, **112**, 210 (1977).

[10] Hewlett-Packard 18789 A dual N-P-FID.

Synthesis of 4,5-Dimethoxykynuramine and Its *In Vivo* Conversion to 6,7-Dimethoxy-4-quinolinol

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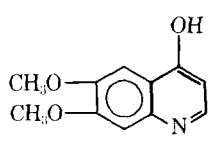
Abstract □ 4,5-Dimethoxykynuramine was synthesized in a three-step sequence originating with veratrole. Indirect evidence indicates that the drug was converted *in vivo* to the hypotensive agent 6,7-dimethoxy-4-quinolinol by the action of monoamine oxidase.

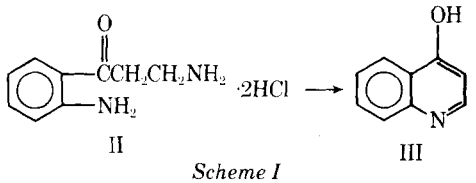
Keyphrases □ 4,5-Dimethoxykynuramine—synthesized, *in vivo* conversion to 6,7-dimethoxy-4-quinolinol □ 6,7-Dimethoxy-4-quinolinol—formed *in vivo* from 4,5-dimethoxykynuramine □ Antihypertensive agents—6,7-dimethoxy-4-quinolinol, formed *in vivo* from 4,5-dimethoxykynuramine

The hypotensive and antihypertensive activities of 6,7-dimethoxy-4-quinolinol (I) were demonstrated previously (1).



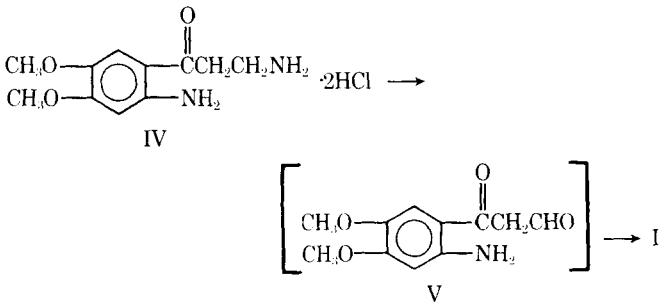
I

Since it had been shown that the *in vitro* incubation of kynuramine (II) with monoamine oxidase gave 4-quinolinol (III) (Scheme I) (2), the possibility of the conversion of 4,5-dimethoxykynuramine (IV) to the aldehyde V and then the quinolinol I (Scheme II) was considered. Should this transformation occur *in vivo*, IV might be considered a useful prodrug for I.



II III

Scheme I



IV V I

Scheme II

This report presents the synthesis and pharmacology of IV as well as a study of its *in vivo* conversion to I.

RESULTS AND DISCUSSION

Friedel–Crafts acylation of veratrole (VI) with β-nitropropionyl chloride (VII) (3) gave 3′,4′-dimethoxy-3-nitropropiophenone (VIII), apparently the first example of the utilization of VII in this type of synthesis (Scheme III). Treatment of VIII with concentrated nitric acid gave the dinitro compound IX.

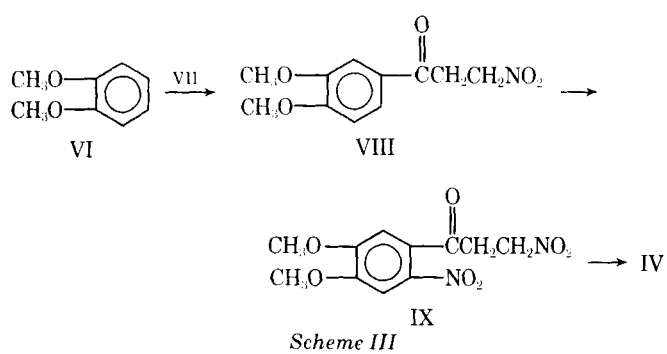
The NMR spectrum of IX exhibited two aromatic singlets, thereby eliminating the possibility of a 1,2,3,4-arrangement of substituents (an *ortho*-orientation of protons). However, a 1,2,3,5-arrangement containing a *meta*-orientation of protons with an expected J of 2–3 Hz (4) could not be completely ruled out. The lack of any splitting pattern in the aromatic protons of IX prompted assignment of the 1,2,4,5-orientation of substituents to IX. Subsequence demonstration of the conversion of IX → IV → I verified this structural assignment.

Hydrogenation of IX using palladium-on-carbon gave IV, isolated as a dihydrochloride salt.

Preliminary screening of IV *via* the CARDAMAP (5) procedure revealed hypotensive activity of long duration (5 hr) at 50 mg/kg iv, but lower doses (10 mg/kg iv) yielded no effect on blood pressure. Urine from the experimental animals was retained for detection of the presence of I. The results of TLC indicated that I was excreted as a metabolite of IV in the dog and that parent IV was also present in the final extract of dog urine.

This publication presents quantitative estimates of the excretion of I in rat urine following orally administered IV. Additionally, the effects on excretion (and, therefore, presumably conversion) of animals pretreated with a monoamine oxidase inhibitor, pheniprazine[1], are discussed.

With the assumptions of complete absorption of IV from the GI tract and a subsequent 100% conversion to I, on a weight basis 76.7% of an administered dose of IV is available for conversion to I. The percent recovery as presented is calculated on this stoichiometric equivalent dose of I.



VI VIII IX IV

Scheme III

Urine samples collected up to 2 hr postdrug from the first group (A), who received only IV, contained an average of 0.063 ± 0.015 mg of I, representing 2.2% of the available IV administered. A second group (B), pretreated with pheniprazine prior to administration of IV, did not excrete any urine during the initial 2-hr period, so no values were available for comparison. The 2–4-hr period for Group A yielded 0.131 ± 0.012 mg of I, or 4.5% of the administered dose of IV recoverable as I, compared to 0.005 ± 0.002 mg, or 0.2%, from Group B. This difference was significant (t test) at $p < 0.001$.

The cumulative percentage recoveries over 6 hr following drug administration amounted to 7.9% in Group A as compared to 0.4% in Group B. The urine from the pheniprazine-only treated animals, Group C, averaged 5.3, 7.0, and 13.1 fluorescent units at the 2-, 4-, and 6-hr time periods, respectively. These figures were applied as a correction factor for the Group B animals prior to the determination of the concentration of I.

These results indicated that 6 hr was probably not a sufficient time for near complete excretion of I. Therefore, a second experiment was run, identical to the first, except that urine collections were made at 4 and 24 hr. An additional group (Group D) of animals was included and received only I to obtain data for comparison to groups treated with IV. Four hours following only the administration of IV, an average of 0.173 ± 0.032 mg, or 6.8% of the equivalent dose of I, was recovered in the urine. Twenty hours later, a mean additional 0.0992 ± 0.023 mg (3.9%) was recovered from this group, yielding a cumulative total of 10.7%.

In the animals pretreated with pheniprazine (Group B), no I could be detected in the urine 4 hr after IV administration; at 24 hr, an average of only 0.017 mg (0.7%) of the dose was recovered (this average was supplied by detectable levels in only two of the five animals tested). Group D, which received only I, yielded an average of 0.223 ± 0.045 mg (5.4%) of the administered dose at 4 hr and an additional 0.640 ± 0.066 mg at 24 hr for a total of 21.3%.

The possibility was considered that pheniprazine pretreatment could interfere with excretion and/or detection of I. Therefore, an additional group of animals (Group E) was treated with 20 mg of pheniprazine/kg po 2 hr prior to 10 mg of I/kg po. Urine samples were collected and analyzed at 4 and 24 hr as previously described. At the respective time intervals, urinary excretion of I averaged 0.417 ± 0.084 mg (11.6%) and 1.215 ± 0.085 mg (33.9%), approximately double that of the animals receiving I alone (Group D).

When the two separate studies involving administration of IV are compared to one another, the percentage recoveries as I at the 4-hr level are remarkably similar, adding credence that the values are reliable. The slight difference in the percentage recovery of I in the animals treated with IV as compared to those dosed with I was not significant at the 4-hr interval; however, at 24 hr the percentage recovery was double in the latter group. This result may indicate that IV is rapidly absorbed and partially converted to I, so that blood levels of I may not be distinguishable between the two groups over the relatively short time period of 4 hr. However, over the longer period (24 hr), I is more completely absorbed than IV and/or the rate and degree of conversion of IV → I decreases with time. Since parent IV was found in the urine of dogs treated intravenously with the compound, it would not be surprising to find unconverted compound in the urine of rats in the present study.

Although it has been shown that pheniprazine pretreatment does not block absorption, excretion, or detection of I in rat urine, the possibility still exists that pheniprazine may block or markedly reduce IV absorption. This premise has not been tested.

The 100% increase of I in the urine of animals pretreated with pheniprazine and I as compared to those treated with I alone may suggest one or both of the following: (*a*) pheniprazine pretreatment increases absorption and/or excretion of I and/or (*b*) I is metabolized through enzyme systems susceptible to inactivation by pheniprazine. Additional work is necessary to clarify this finding.

EXPERIMENTAL[2]

Pharmacology—Urine from experimental animals dosed with IV was found to contain I along with IV by TLC in the following solvent systems: 1-butanol–acetic acid–water (5:4:1), methanol–ethyl acetate–water (5:

[1] Catron, Lakeside Laboratories.

[2] The NMR spectrum was determined on a Varian A-60A spectrometer in deuterated dimethyl sulfoxide using tetramethylsilane as an internal standard. IR spectra were determined as mineral oil mulls with a Perkin-Elmer 137B spectrophotometer. Melting points were taken on a Mel-Temp block and are corrected.

4:1), methanol, and 2-propanol–ethyl acetate–water (5:4:1).

In an initial experiment, 15 male Charles River rats, 350–425 g, were divided into three groups of five animals each. Each animal was placed in an individual metabolism cage, and food was withheld for 24 hr prior to dosing. Water was provided *ad libitum*. Group A was dosed with IV, 10 mg/kg po; Group B was pretreated with pheniprazine, 20 mg/kg po, 2 hr prior to the administration of IV, 10 mg/kg po. Group C served as a pheniprazine control; *i.e.*, these animals received 20 mg of pheniprazine/kg po only.

The urine from each animal was collected separately at 2, 4, and 6 hr following IV administration. Each sample was analyzed for I according to a reported spectrophotofluorometric method (6) in which the I concentration is determined from standard curves constructed from fluorescence readings (activating wavelength of 320 nm and fluorescent wavelength of 370 nm) obtained with control urine to which known concentrations of I have been added and subsequently extracted. Compound IV exhibits no fluorescence between 200 and 600 nm.

A second experiment involving 15 rats was conducted as already described, except that urine collections were made at 4 and 24 hr following IV. To this experiment was added, for comparison, a fourth group (D), which was dosed with I (10 mg/kg po).

Chemistry—*β-Nitropropionyl Chloride (VII) (3)*—To a stirred suspension of 70 g (0.59 mole) of β-nitropropionic acid, 0.5 ml of pyridine and 500 ml of chloroform was added slowly, at room temperature, 210 ml (339 g, 2.85 moles) of thionyl chloride. The mixture was stirred and refluxed for 2.5 hr and cooled. The solvents were removed *in vacuo* to give 91 g (>100%) of the crude acid chloride, VII, which was used directly in the next step.

3′,4′-Dimethoxy-3-nitropropiophenone (VIII)—Aluminum chloride (173 g, 1.30 moles) was added quickly to 700 ml of vigorously stirred nitrobenzene. While the temperature was maintained between 10 and 15°, a solution of 91 g (0.59 mole) of VII in 50 ml of nitrobenzene was added followed by a solution of 73 g (0.53 mole) of veratrole (VI) in 50 ml of nitrobenzene. The mixture was stirred at room temperature for 6 hr and then poured cautiously into a mixture of 300 ml of concentrated hydrochloric acid and 2 kg of iced water.

The organic layer was separated, and the aqueous material was extracted with 3 × 200-ml portions of chloroform. The combined organic layers were washed with 300 ml of water, 300 ml of 10% sodium carbonate, and 2 × 300-ml portions of water. After the solution was dried over magnesium sulfate, the solvents were removed *in vacuo* to give the oily product. Crystallization from ethyl acetate gave 45.5 g (36%) of VIII, mp 113–118°. The analytical sample, mp 126–128°, was obtained by recrystallization from ethanol; IR: 6.08 (C=O, ketone), 6.31 (C=C), 6.49, and 7.41 (NO_2) μm.

Anal.—Calc. for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.86. Found: C, 55.11; H, 5.78; N, 5.59.

3′,4′-Dimethoxy-3,6′-dinitropropiophenone (IX)—To 300 ml of concentrated nitric acid, stirred and maintained at 5–10°, was added 45.5 g (0.19 mole) of VIII in small portions over 20 min. The resulting solution was stirred at 5° for 10 min and then added slowly to 2.5 kg of iced water. The suspension was stirred at room temperature for 20 min and filtered. The solid was washed with 3 × 200-ml portions of water, air dried, and dried at 60° to give 48 g of crude IX, mp 120–127°. Recrystallization from 2 liters of ethanol gave 34 g (63%) of IX, mp 135–140°.

The analytical sample, mp 140–142°, was obtained by recrystallization from alcohol; IR: 5.88 (C=O, ketone), 6.20 (C=C), 6.55–6.70, and 7.35 (NO_2) μm; NMR: δ 7.67 and 7.28 (2 s, 2H, aromatic), 3.68 and 3.43 (2 t, 4H, methylene), 3.98, and 3.96 (2 s, 6H, CH_3O) ppm.

Anal.—Calc. for $C_{11}H_{12}N_2O_7$: C, 46.48; H, 4.26; N, 9.86. Found: C, 46.62; H, 4.39; N, 9.78.

3′,4′-Dimethoxy-3,6′-diaminopropiophenone Dihydrochloride (4,5-Dimethoxykynuramine) (IV)—A 17.3-g (0.61-mole) portion of IX, 500 ml of methanol, 240 ml of methanol saturated with hydrochloric acid, and 22 g of 5% palladium-on-carbon (50% moisture) were shaken on a Parr apparatus at room temperature until the theoretical quantity of hydrogen was consumed (5.5 hr). The catalyst was filtered, and the resulting solution was concentrated to dryness at 35–45° *in vacuo*. The residue was slurried with ethanol, and the solid was filtered to give 13.5 g of the crude product, mp 204–209°.

The material was recrystallized from ethanol, and the solid was washed with 70 ml of ethanol to afford 5.3 g (29%) of the product, mp 208–214°.

The analytical sample, mp 202–207°, was recrystallized from ethanol containing hydrochloric acid; IR: 6.01 (C=O, ketone) and 6.21 (C=C) μm.

Anal.—Calc. for $C_{11}H_{18}N_2O_3 \cdot 2HCl$: C, 44.45; H, 6.10; N, 9.43. Found: C, 44.63; H, 6.35; N, 9.21.

REFERENCES

(1) R. K. Bickerton, R. F. Dailey, W. T. Rockhold, and R. H. Buller, *J. Pharmacol. Exp. Ther.*, **144**, 218 (1964).

(2) H. Weissbach, T. Smith, J. Daly, B. Witkop, and S. Udenfriend, *J. Biol. Chem.*, **235**, 1160 (1960).

(3) G. Barger and F. Tutin, *Biochem. J.*, **12**, 402 (1918).

(4) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon, New York, N.Y., 1959, p. 85.

(5) G. O'Bleness, R. K. Bickerton, and W. T. Rockhold, *Comput. Appl. Serv.*, **4**, 69 (1964).

(6) P. L. Cox, J. P. Heotis, and J. A. Buzard, *Arch. Int. Pharmacodyn. Ther.*, **168**, 450 (1967).

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