Metabolic Fate of Methoxamine and N-Isopropylmethoxamine

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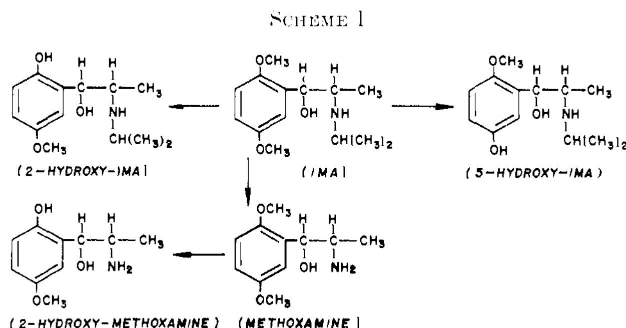
N-Isopropylmethoxamine has been shown to be O-dealkylated and N-dealkylated when given to rats and dogs. Methoxamine is O-dealkylated when given to dogs.

The properties of methoxamine as a pressor amine have been well investigated in man and animals. Recent studies indicate that methoxamine and its N-isopropyl derivative (IMA) are able to lower plasma free fatty acids (FFA) and to block the epinephrine-induced rise of plasma FFA and blood glucose in conscious dogs.[1,2] In view of the pharmacological interest in these compounds as a new type of adrenergic blocking agent, we studied various aspects of the metabolic fate of these drugs in dogs and rats. The results presented here show the conversion of IMA to methoxamine and the lack of conversion of the N-*t*-butyl analog (TMA or butoxamine) to methoxamine. Data are also given on O-methylated metabolites of IMA and methoxamine. The metabolic transformations of methoxamine and IMA are shown in Scheme I.

SCHEME I



Experimental Section

Chemicals.—IMA, 2-hydroxy-IMA, 5-hydroxy-IMA, 2-hydroxymethoxamine, and TMA were synthesized in these laboratories by Baltzly and Mehta[3] and were used as their hydrochlorides. In some experiments IMA was given as the lactate because of its greater solubility in this form. Methoxamine hydrochloride (Burroughs Wellcome & Co., Tuckahoe, N. Y.) was used.

Animal Experiments.—IMA (40 mg/ml) was administered as the lactate orally in a divided dosage of 400 mg/kg daily over a period of 4 days to 18 male, Sprague-Dawley rats which varied in weight from 205 to 265 g. (The total dose was 6.4 g.) Urine collected for the 24-hr period before drug administration to the rats was combined and used as control urine. The experimental urine collected for the 4 days of drug administration was combined.

IMA as the hydrochloride was administered orally to two dogs (10.7 and 12.0 kg) at a level of 10 mg/kg in divided doses for 3 and 2 days, respectively. Methoxamine hydrochloride was administered to two dogs (10.2 and 11.6 kg) at a dosage of 5 mg/kg for 1 day for the first dog and 10 mg/kg in divided doses for 2 days for the second dog. TMA as the hydrochloride was administered to two dogs (17.0 and 15.2 kg) at a dosage of 20 mg/kg in divided doses for 3 and 2 days, respectively. The dosages of all drugs were given to dogs orally in gelatin capsules. Collections of urine were made for 24-hr periods during administration of the drug to dogs and a control urine was collected for the day prior to drug administration.

Initial Studies with Rat Urine.—To 1.0 ml of rat urine, 0.1 ml of concentrated H_2SO_4 was added, and the solution was heated for 0.5 hr on a boiling-water bath. After allowing the solution to cool in an ice bath, 1 ml of 3.5 *N* NaOH and sufficient 1.0 *N* NaOH (about 0.4 ml) and 1.0 *N* $NaHCO_3$ (about 0.3 ml) was added to bring the pH of the solution to 10.0. After adjusting the volume of the solution to 4.0 ml with H_2O, 3 ml of the buffered mixture was extracted with 30 ml of CH_2Cl_2 and a 20-ml aliquot of the organic phase was evaporated to dryness. The residue was dissolved in 1.0 ml of methanol and a 20-μl. portion was applied to a paper chromatogram.

Isolation of 5-Hydroxy-IMA.—For the isolation of 5-hydroxy-IMA, 220 ml of rat urine was mixed with 22 ml of concentrated H_2SO_4 and heated on a boiling-water bath for 35 min. The urine was allowed to cool to room temperature and the pH was adjusted to 10 by the addition of 68 ml of 50% NaOH, 29.9 ml of 1 *N* NaOH, and 38.4 ml of 1 *N* $NaHCO_3$. After the volume was brought to 400 ml with water, the urine was extracted twice with 4 l. of CH_2Cl_2. The organic layers were combined and evaporated. The residue was dissolved in an ether–ethanol mixture and treated with ether saturated with dry HCl. The resulting precipitate was collected, washed with ether, and recrystallized several times from ethanol-benzene. The crystals obtained melted at 232.5–233°, alone or in mixture with an authentic sample of **5-hydroxy-IMA hydrochloride**. The isolated hydrochloride was chromatographed on paper and when the chromatogram was sprayed with the Pauly reagent, a spot with the same mobility as 5-hydroxy-IMA (R_f 0.25) was obtained. The ultraviolet spectrum of the metabolite, measured with a Cary Model 15 recording spectrophotometer, was identical with the spectrum of authentic 5-hydroxy-IMA in acid solution (0.5 *N* HCl), λ_{max} 290 and 224 mμ, and in basic solution (0.5 *N* NaOH), λ_{max} 306 and 236.5 mμ. The infrared spectra of the metabolite hydrochloride and 5-hydroxy-IMA hydrochloride measured with a Beckman Model IR4 were identical.

Chromatographic Methods. A. Paper Chromatography.—Paper chromatography was carried out in cylindrical glass jars (diameter 28 cm) with Whatman No. 1 paper using the ascending technique with the solvent system chloroform–methanol–water–acetic acid, 2:1:1:0.1 (v/v). The paper chromatograms were allowed to equilibrate overnight with the upper phase and then developed with the lower phase. After removing the organic phases by warming, the chromatograms were sprayed with the Pauly reagent.[4]

B. Preparation of Thin Layer Chromatograms.—Thin layer plates (200 × 200 mm) were prepared from aluminum oxide G according to Stahl (Brinkman Instruments Co., Westbury, L. I., N. Y.) by mixing 7 g of the dry powder with 9 ml of distilled water in a mortar and applying the slurry to the glass plates held by masking tape using the technique of Lees and DeMuria.[5] After air drying, the plates were activated in an oven for 30 min at 80°. Smaller plates were prepared by taping two (200 × 100 mm) or four (200 × 50 mm) plates together and coating them all at the same time.

All thin layer chromatograms were developed by the ascending method at room temperature in a solvent-saturated atmosphere. The reference compounds, standard solutions, or urinary extracts were spotted 1 cm from the edge of the adsorbent with usually ten spots on the larger plates (200 × 200 mm). The adsorbent layer was submerged in solvent to a depth of 0.5 cm, and the

(1) (a) J. J. Burns, K. I. Colville, L. A. Lindsay, and R. A. Salvador, *J. Pharmacol. Exptl. Therap.*, **144**, 163 (1964); (b) R. A. Salvador, K. I. Colville, S. A. April, and J. J. Burns, *ibid.*, **144**, 172 (1964).

(2) H. M. Maling, M. A. Williams, B. Highman, J. Garbus, and J. Hunter, *Arch. Exptl. Pathol. Pharmakol.*, **248**, 54 (1964).

(3) R. Baltzly and N. B. Mehta, to be published.

(4) I. Smith, "Chromatographic and Electrophoretic Techniques," Vol. 1, Interscience Publishers, Inc., New York, N. Y., 1960, pp 296, 297.

(5) T. M. Lees and P. J. DeMuria, *J. Chromatog.*, **8**, 108 (1962).

solvent was allowed to run a distance of 15 cm. After air drying, the finished chromatogram was heated in an oven at 80° for approximately 5 min to remove most of the acetic acid.

C. **Spray Reagents.**—For the detection of compounds, the following reagents were used to spray the developed thin layer chromatograms: (i) iodine reagent,[6] iodine–1% in CCl_4; (ii) Pauly reagent,[4] used to detect possible phenolic metabolites which yield strong reddish to brown colors when sprayed with this reagent; (iii) diazotized *o*-dianisidine reagent, similar to the Pauly reagent except that sulfanilic acid is replaced by *o*-dianisidine dihydrochloride (12 g) in reagent (a); used to detect possible phenolic metabolites; methoxamine yields a moderate brown color and IMA a faint yellow color when sprayed with this reagent; phenolic compounds yield stronger reddish to brown colors; (iv) diazotized benzidine reagent, similar to the Pauly reagent except that sulfanilic acid is replaced by benzidine dihydrochloride (9 g); used to locate methoxamine which yields a strong yellow color when sprayed with diazotized benzidine; phenolic compounds yield reddish to brown colors when sprayed with this reagent.

The R_f values of all reference compounds in the ten thin layer solvent systems used in this investigation are shown in Table I.

Analysis of Drugs by Thin Layer Chromatography. A. Analysis of Phenolic Metabolites in Urine.—Chromatography of the phenolic metabolites in the urine of drug-treated animals was done after acid hydrolysis of conjugates in the urine. For each 1.0 ml of rat urine or standard solution 0.3 ml of concentrated HCl was added, and the solution was heated for 1 hr on a boiling-water bath. After allowing the solution to cool in an ice bath, the pH was adjusted to 10 by the addition of 1.2 ml of concentrated NH_3 and 1 ml of pH 10 1 *M* ammonia buffer.[7] A 3-ml portion of aqueous phase was extracted with 30 ml of CH_2Cl_2, and a 20-ml aliquot of the organic phase was evaporated to dryness. The residue was dissolved in 1 ml of methanol and a 15-μl portion of the solution was applied to a thin layer plate.

Urine obtained from a dog was treated similarly except that the urinary residue was dissolved in a few drops of methanol, and the entire extract was applied to the thin layer plate. The conditions used for the confirmation and estimation of the O-dealkylated metabolites of IMA and methoxamine in dog are shown in Table II.

B. Analysis of Methoxamine in Urine of Drug-Treated Dogs.—To each milliliter of standard solution, control urine, or urine obtained from a methoxamine or IMA-treated animal, 2 ml of 0.5 *N* NaOH was added and the alkaline solution was extracted with 30 ml of a 1:1 mixture of CCl_4-$CHCl_3$. A 20-ml portion of the organic phase was washed with 0.1 vol. of 0.5 *N* NaOH; a 15-ml portion of the washed solvent was evaporated. The residue was chromatographed with system 3 (see Table I) and the thin layer plate was sprayed with diazotized benzidine.[8]

To 5 ml of urine obtained from a TMA-treated dog, 1 ml of 2 *N* NaOH was added and the alkaline urine was extracted with 60 ml of the CCl_4–$CHCl_3$ mixture. A 50-ml portion of the organic phase was washed with 0.1 vol. of 0.5 *N* NaOH and a 40-ml portion of the washed solvent was treated as described above.

Spectrophotometric Analysis of Metabolites of IMA. A. The Isolation of 2-Hydroxy-IMA for Spectrophotometric Analysis.—The CH_2Cl_2 extract of 2 ml of hydrolyzed rat urine was applied along with reference 2-hydroxy-IMA to three doubly layered thin layer plates[9] prepared as described in the chromatographic section. After the chromatograms were developed with solvent system 3, the reference 2-hydroxy-IMA on each plate was located by spraying with the iodine reagent. The adjacent area of the chromatographed urine extract was then eluted. The adsorbent was removed from the plate, packed in a thistle tube (6.5-mm diameter, supplied by Emil Greiner), and washed with 50 ml of distilled methanol. The solvent was removed by evaporation and the residue was dissolved in 3.0 ml of 0.1 *N* HCl. A 0.2-ml portion of the acidic solution was diluted with 3.8 ml of 1.0 *N* HCl, another 0.2-ml portion of the acidic solution was made alkaline with 3.8 ml of 1.0 *N* NaOH, and a 0.1-ml portion of the acidic solution was made alkaline with 3.9 ml of 1.0 *N* NaOH. The acidic and alkaline solutions were measured spectrophotometrically in a Cary Model 15 recording spectrophotometer. The absorbance was determined from 340 to 220 mμ against the appropriate reference solutions. The spectrum of the metabolite which had λ_{max} 290 and 224 mμ in acid and λ_{max} 306 and 236.5 mμ in base was identical with that of reference 2-hydroxy-IMA.

TABLE I

AVERAGE $R_f \times 100$

Compound	System[a] 1	2	3	4	5	6	7	8	9	10
Methoxamine	71	17	28	53	0	15	12	10	37	58
2-Hydroxy-methoxamine	33	0	6	17	0	0	0	0	20	34
IMA	89	52	73	85	29	47	34	30	70	77
2-Hydroxy-IMA	84	38	51	77	19	37	22	16	70	70
5-Hydroxy-IMA	76	23	28	57	7	17	12	9	51	63

[a] 1: $CHCl_3$–EtOH–H_2O–AcOH (20:10:15:1), lower phase; 2: $CHCl_3$–EtOH–H_2O–AcOH (20:10:35:1), lower phase; 3: $CHCl_3$–EtOH–H_2O–AcOH (20:10:35:2), lower phase; 4: $CHCl_3$–EtOH–H_2O–AcOH (20:10:35:4), lower phase; 5: $CHCl_3$–MeOH–H_2O–AcOH (20:10:25:1), lower phase; 6: $CHCl_3$–MeOH–H_2O–AcOH (20:10:25:2), lower phase; 7: ethylene dichloride–MeOH–H_2O–AcOH (20:15:10:1), lower phase; 8: benzene–EtOH–H_2O–AcOH (20:10:10:1), upper phase; 9: benzene–PrOH–H_2O–AcOH (20:10:20:1), upper phase; 10: benzene–PrOH–H_2O–AcOH (20:10:10:1), upper phase.

TABLE II

Drug given	Compd estd	Vol. of urine for anal.	Chromatographic solvent system[a]	Spray reagent
Methoxamine	2-Hydroxy methoxamine	1 ml	1	Diazotized *o*-dianisidine
IMA	2-Hydroxy-IMA	2 ml	3	Diazotized *o*-dianisidine
IMA	5-Hydroxy-IMA	1 ml	2	Pauly reagent[b]
IMA	2-Hydroxy-methoxamine	3 ml	1	Diazotized *o*-dianisidine

[a] See Table I. [b] Methoxamine, with mobility similar to that of 5-hydroxy-IMA, does not react with the Pauly reagent.

B. Measurement of O-Dealkylated Metabolites in Rat Urine.—The amount of phenolic metabolites in rate urine was determined spectrophotometrically. A 3-ml portion of the hydrolyzed, buffered (pH 10) urine was extracted with 30 ml of ethylene dichloride and a 20-ml portion of the organic phase was reextracted with 10 ml of 0.5 *N* NaOH. The absorbance of the alkaline solution at 306 mμ was measured in a Beckman Model DU spectrophotometer and the total phenolic metabolites were calculated using the extinction coefficient of 5-hydroxy-IMA.

Analysis of Methoxamine in Dog Urine.—To 5 ml of urine from a dog dosed with IMA, 1 ml of 2 *N* NaOH was added, and the alkaline urine was extracted with 60 ml of the CCl_4–$CHCl_3$ mixture (1:1). The aqueous layer was discarded, and the organic layer was washed with 6 ml of 0.5 *N* NaOH. The alkaline wash was discarded and the organic layer was evaporated. The residue was dissolved in a small volume of methanol and applied to a doubly layered thin layer plate[9] and chromatographed along with a sample of authentic methoxamine with system 3. Authentic methoxamine was located on the thin layer plate with the iodine reagent and the adjacent metabolite area was eluted as described for the elution of 2-hydroxy-IMA except that 20 ml of methanol was used to elute the urinary extract. After the solvent was removed by evaporation, the residue was dissolved in 1.5 ml of 0.1 *N* HCl. A 0.5-ml portion of the acidic solution was diluted with 1 ml of 1 *N* HCl and another 0.5-ml portion of the acidic solution was made alkaline with 1 ml of 1 *N* NaOH. The acidic and alkaline solutions were measured spectrophotometrically in a Cary Model 15 recording spectrophotometer. The absorbance was determined from 340 to 250 mμ against the

(6) Reference 4, p 217.

(7) "Biochemists Handbook," Van Nostrand Co., Inc., Princeton, N. J., 1961, p 35.

(8) The phenolic compounds present in the urine from drug-treated animals that react with the benzidine spray are not extracted by the above procedure. In fact, 5-hydroxy-IMA (200 μg/ml added to control urine) was not detected on the tlc plates that were processed as described for methoxamine.

(9) A double strip of masking tape and twice the amount of absorbent was used to prepare a plate.

appropriate reference solutions. The reference methoxamine compound and the metabolite had λ_{max} 290 mμ when the ultraviolet spectrum was measured in acid or base.

Results and Discussion

Urines for drug-treated animals were acid hydrolyzed and chromatographed along with hydrolyzed control urines and standards (which consisted of the appropriate reference compound dissolved in a control urine sample). The standards in the control urine were similar concentration to that of the urinary metabolite so that after the thin layer chromatogram was sprayed with a dye reagent, the phenolic metabolite in urine could be estimated by a visual comparison with the standards. The identity of several metabolites was established by chromatography of these substances in the urine along with the known or reference compound in several thin layer solvent systems.[10]

Metabolism of IMA in Rats. The rat was selected for the initial studies because this species had been shown to metabolize IMA at a much more rapid rate than the dog.[1b] Analysis of the rat urine by the methyl orange procedure of Burns, *et al.*,[11] showed that only about 2.0% of the dose was excreted unchanged.

For the initial chromatographic studies, the rat urine was hydrolyzed with H_2SO_4. Methylene dichloride extracts of the hydrolyzed urines were chromatographed on paper. Two spots were located (R_f 0.25, 0.75) when the developed chromatogram was sprayed with the Pauly reagent. These results suggested the presence of two phenolic metabolites of IMA. The intensity of the color of the spot with R_f 0.25 was much stronger than the other spot (R_f 0.75). Authentic 5-hydroxy-IMA had R_f 0.25 and reference 2-hydroxy-IMA had R_f 0.75 when chromatographed with the paper chromatographic system. In order to accumulate sufficient material so that the identity of the chromatographically slower moving metabolite (with R_f 0.25) could be definitely established, a large volume of urine from a drug-treated rat was hydrolyzed with acid. A crystalline metabolite was isolated from the hydrolyzed urine and conclusively identified as 5-hydroxy-IMA as described in the Experimental Section.

Identification of 2-Hydroxy-IMA as a Metabolite of IMA.—During the more recent studies it was determined by the spectrophotometric methods described earlier that conjugates present in the rat urine were hydrolyzed more completely with HCl. The hydrolyzed urine extracts were chromatographed on thin layer plates with solvent system 3, and two spots were located (R_f 0.28, 0.51) after spraying the plates with the Pauly reagent or diazotized *o*-dianisidine. The metabolite with R_f 0.28 corresponded to 5-hydroxy-IMA whose isolation was described, and the metabolite with R_f 0.51 corresponded to the 2-hydroxy-IMA. The metabolite and the reference 2-hydroxy-IMA also had similar mobilities when chromatographed in three other solvent systems (2, 5, and 6). To measure the ultraviolet spectrum of this metabolite, sufficient material was isolated by thin layer chromatography (tlc) from extracts of the rat urine. The metabolite corresponding to 2-hydroxy-IMA was eluted from the tlc plate with methanol, and the ultraviolet spectrum was measured. The ultraviolet spectra of 2-hydroxy-IMA and the metabolite were similar in acid (λ_{max} 290 and 224 mμ) and in base (λ_{max} 306 and 236.5 mμ).

The total O-dealkylated metabolites of IMA excreted by the rat was estimated by the spectrophotometric method described in the Experimental Section. These results indicated that 60% of the dose of IMA was excreted in the urine as conjugates of O-dealkylated metabolites,[12] and 5% of the dose could be accounted for as the unconjugated O-dealkylated metabolites.

IMA Metabolism in Dogs. Urine obtained from dogs given IMA was hydrolyzed as described in the Experimental Section. When the extract of hydrolyzed urine was chromatographed, the two phenolic compounds already described in hydrolyzed rat urine were located. The amounts of 2-hydroxy-IMA and 5-hydroxy-IMA in dog urine were estimated using tlc. The presence of 2-hydroxy-IMA in the urine of dogs dosed with IMA was confirmed by chromatographic means as described above for IMA-treated rats.

Excretion of Methoxamine in Urine of IMA-Treated Animals.—The early experiments revealed O-demethylation as a major metabolic pathway of IMA, but examination of urine from IMA-treated animals showed that N-dealkylation was also a metabolic route for transformation of IMA. The presence of methoxamine in the urine of an animal treated with IMA was confirmed by chromatography of urine extracts in four thin layer solvent systems (1, 3, 4, and 9). In each system, a spot with the same R_f and yellow color as methoxamine was detected by the benzidine spray.

Whereas there are bathochromic shifts in the spectra of the phenolic analogs of IMA when the spectra are measured in acid and then in alkaline solution, there is no shift in the spectrum of methoxamine in going from acid to alkaline solution.[13] To obtain sufficient material to compare the ultraviolet spectrum of the metabolite with that of methoxamine, dog urine was extracted with a CCl_4–$CHCl_3$ mixture, and the extract was separated on thin layer plates. The methoxamine metabolite was eluted from the chromatographic adsorbent with methanol and the ultraviolet spectrum was measured. The reference methoxamine compound and the chromatographically isolated metabolite has similar spectra in acidic and in basic solution.

The amount of methoxamine in the urine was estimated chromatographically with the method described in the Experimental Section. When the extract of hydrolyzed urine, obtained from an animal given IMA, is chromatographed with solvent system 1, a third spot of a slow-moving compound can be located with the *o*-dianisidine reagent.

(10) Internal standards were also chromatographed in order to eliminate doubt caused by variation in R_f values of a particular substance chromatographed not only at different times, but even when chromatographed at different locations on the same plate. This involved the chromatography of an extract of drug urine to which reference compound had been added.

(11) J. J. Burns, B. L. Berger, P. A. Lief, A. Wollack, E. M. Papper, and B. B. Brodie, *J. Pharmacol. Exptl. Therap.*, **114**, 289 (1955).

(12) Since both 2-hydroxy-IMA and 5-hydroxy-IMA have adsorption maxima at 306 mμ, this procedure could be used to quantify accurately total phenolic metabolites. The above figures include a correction factor, because the recovery of reference 5-hydroxy-IMA through this procedure is 66%. When a known amount of IMA was subjected to acid hydrolysis, it was determined by the above procedure that only 1% of reference IMA is cleaved by the hydrolytic procedure. Tlc studies indicated that the 1% of degraded IMA was roughly half 2-hydroxy-IMA and half 5-hydroxy-IMA.

(13) This is also true of IMA.

TABLE III
THE ESTIMATED METABOLIC PRODUCTS OF IMA IN DOG URINE

Compound	Dog 1 Concn, μg/ml	Dog 1 % of dose recovered	Dog 2 Concn, μg/ml	Dog 2 % of dose recovered
Methoxamine	30	8	25	5
2-Hydroxy-IMA	20	5	15	3
5-Hydroxy-IMA	100	26	110	22
2-Hydroxy-methoxamine	8	2	14	3

TABLE IV
THE ESTIMATED METABOLIC PRODUCTS OF METHOXAMINE IN DOG URINE

Compound	Dog 1 Concn, μg/ml	Dog 1 % of dose recovered	Dog 2 Concn, μg/ml	Dog 2 % of dose recovered
Methoxamine	30	38	50	32
2-Hydroxy-methoxamine	10	12	15	10

The mobility of this metabolite and 2-hydroxymethoxamine in system 1 was similar. The presence of this metabolite in the urine of IMA-treated dogs was confirmed by chromatographic means with 1, 4, and 10; the mobility of the unknown and reference compound was the same in all systems. The identity of this metabolite could not be definitely established because the 5-O-demethylated methoxamine was not available and could not be compared with the possible metabolite, but the chromatographic evidence definitely showed the presence of an O-demethylated methoxamine derivative in the urine. The results of the estimation, in urine, of the metabolites of IMA are shown in Table III.

Methoxamine Metabolism in Dogs.—Methoxamine and the O-dealkylated metabolite of methoxamine were detected and estimated in urine samples from dogs treated with methoxamine by the technique described above for IMA-treated animals. The estimation of methoxamine and the O-demethylated methoxamine in the urine of two dogs treated with methoxamine is shown in Table IV.

Studies on the Possible N-Dealkylation of N-*t*-Butylmethoxamine.—The possible dealkylation of TMA to methoxamine was investigated. The urine from two TMA-treated dogs was analyzed for the presence of methoxamine. Methoxamine in the urine of these animals could not be detected by chromatographic means even though the method was sensitive to less than 0.2% of the dose. These results for the *t*-butyl derivative of methoxamine are in contrast to the results obtained for the isopropyl derivative.

Whether the presence of a hydrogen on the α carbon atom of the substituted alkyl group is a requirement for N-dealkylation was not investigated further. However, a wide variety of compounds have been demonstrated to undergo N-dealkylation in the intact animal as well as *in vitro*. For an excellent review of this subject, see the recent report of McMahon.[14]

Pharmacological Effects of Metabolites.—Methoxamine and IMA were rapidly converted to O-dealkylated metabolites in the animals studied. The 2-O-demethylated and 5-O-demethylated IMA both have activity in blocking the epinephrine-induced lipolysis in the rat epididymal fat pad.[1b] However, the compounds are not active *in vivo* presumably because they are rapidly conjugated and excreted.

N-isopropylmethoxamine undergoes cleavage of the N-alkyl group. This reaction is of considerable interest, since it can furnish an explanation of some of the side effects exhibited by animals that have been dosed with IMA. Animals that have received the N-*t*-butyl derivative of methoxamine, which is not dealkylated to methoxamine, do not exhibit these side effects.[15] Therefore, substitution of a *t*-butyl group for the isopropyl group blocked N-dealkylation as a metabolic pathway in this compound and eliminated the toxicity attributed to methoxamine.

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(14) R. E. McMahon, *J. Pharm. Sci.*, **55**, 457 (1966).
(15) J. J. Burns, S. A. April, and R. A. Salvador, *Progr. Biochem. Pharmacol.*, **3**, 248 (1966).

Local Anesthetics with Enhanced Affinity for Proteins

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2-Dialkylaminoaceto-2',6'-xylides bearing groups with some affinity for protein, such as CH_2SH, $CH_2CH{=}CH_2$, $CH_2C{\equiv}CH$, CH_2OH, $COOC_2H_5$, CH_2OOCCH_3, and $CONH_2$, in the 2 position have been synthesized and evaluated as local anesthetics.

Many local anesthetics, *e.g.*, lidocaine (**1**), have in common the molecular feature, $R_2N \cdots CO$, in which the basic nitrogen atom is separated from the carbonyl group by a chain of 1–4 atoms,[1] but we are aware of no specific attempt to increase the affinity of the molecule for tissue protein by attaching thereto potential binding groups such as SH (to form a disulfide bond), $CH{=}CH_2$ or $C{\equiv}CH$ (sulfide bond), OH (hydrogen bond), or dipoles like $COOC_2H_5$, $OOCCH_3$, and $CONH_2$ (van der Waals forces). Such groups, we conceived, by helping to retain the drug at the injection site, might both prolong its duration of action and reduce side effects on the central nervous system. We report here upon the

(1) A. Burger in "Medicinal Chemistry," A. Burger, Ed., 2nd ed, Interscience Publishers, Inc., New York, N. Y., 1960, p 441.