son, P. Branc, B. Rockey, and A. J. Pohland, *J. Amer. Chem. Soc.*, **90**, 4706 (1968).
(8) J. Hlubucek, E. Ritchie, and W. C. Taylor, *Aust. J. Chem.*, **23**, 1881 (1970).
(9) C. Grunberg, H. N. Prince, E. Titsworth, G. Beskid, and M. D. Tendler, *Chemotherapia*, 11, 249 (1966).
(10) R. D. Brown, C. J. Drummond, F. N. Cahey, and W. C. Thomas, *Aust. J. Sci. Res., Ser. A*, 622 (1949).
(11) Chan Soo Oh and C. V. Greco, *J. Heterocycl. Chem.*, 261 (1970).

Metabolism of 1-[(5-Nitrofurfurylidene)amino]-2-imidazolidinone†

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The metabolism of nifuradene (1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone) (I), a urinary tract antibacterial agent, was investigated in the human. Two metabolites, 1-[(5-nitrofurfurylidene)amino]-hydantoin (nitrofurantoin) (II) and 4-hydroxy-1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone (III), were isolated and identified. A degradation product of the labile metabolite III was also isolated and identified as 1-[(5-nitrofurfurylidene)amino]-4-imidazolin-2-one (IV). An oxidative metabolic pathway of I through III to II is proposed.

Early work on the metabolism of nitrofuraldehyde derivatives emphasized reduction of the nitro group. A variety of evidence accumulated indicating the presence of the reduced form,[1] but isolation of the product was not achieved. Then Ebetino, *et al.*,[2] successfully synthesized a series of aminofurans and their acetylated derivatives after chemical reduction of the corresponding nitrofurans; and Olivard, *et al.*,[3] isolated the metabolic reduction product 5-acetamido-2-furaldehyde acetylhydrazone from urine of rabbits fed 5-nitro-2-furaldehyde acetylhydrazone.

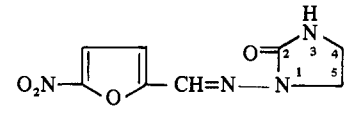
In this communication, we present evidence of a primary oxidative metabolic pathway involving the side chain of a nitrofuraldehyde derivative. The compound under investigation was nifuradene, I,[4] a promising urinary tract antibacterial agent.

Results

A. Isolation and Identification of Products. Earlier chromatographic work by D. Humphrey of these laboratories had indicated the presence of yellow metabolites of I in rat and dog urine. Their isolation was initiated in the present study, and prep tlc of the nitromethane extract of human urine from subjects administered I indicated the presence of 4 major bands. These bands had average R_f values of 0.96, 0.88, 0.80, and 0.68 in Me_2CO-MeOH (see Experimental Section). However, during preliminary investigations of these compounds, the material with R_f 0.80 would split into 2 bands upon being rechromatographed. The resulting second band had an R_f 0.88, corresponding to 1 of the original bands. Material from these R_f 0.88 bands had identical uv and ir spectra, and behaved identically during chromatography. The material with R_f 0.80 was labile, and was degrading to the material of R_f 0.88. Qualitative experiments indicated that thermolability was the major cause of conversion. Fresh urine samples showed no evidence of the R_f 0.88 material if they were rapidly processed and chromatographed at temps not exceeding 30°.

All 3 excretion products, and the degradation product, were isolated in small quantities in crystalline form. Because of the small quantities available, uv, ir, and nmr spectrometry were relied upon extensively for product identification.

†United States Adopted Name (USAN): nifuradene.



I

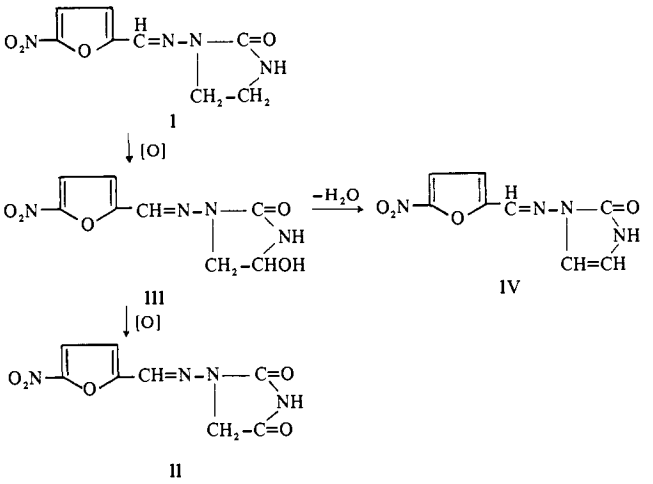
The excretion product of R_f 0.68 in solvent system 1 was unaltered I. The uv, ir, and nmr data for the excretion product all agreed with those for authentic I. Cochromatography of the product and authentic I in 2 paper and 2 tlc systems did not separate the components. The minimal inhibitory concn (MIC) of I against *Escherichia coli* was 0.19 μg/ml, which is equal to the MIC of authentic nifuradene.

The excretion product having R_f 0.96 in solvent system 1 was nitrofurantoin, *i.e.*, 1-[(5-nitrofurfurylidene)amino]-hydantoin (II). In the form of the hydrate, both the product and authentic II yielded identical uv, ir, and nmr data. No separation of components was observed during cochromatography in 2 paper and 2 tlc systems.

The third excretion product of R_f 0.80 in solvent system 1 had ir absorption bands at 1250 and 1015 cm^{-1} indicating that the furan ring remained intact.[5] Bands at 1505 and 1335 cm^{-1} further indicated that NO_2 was still present,[6] and led to the conclusion that metabolic attack on I had been initiated in the imidazolidinone ring portion of the compound. Since both the C=O stretching band (1705 cm^{-1}) and the NH stretching band (3380 cm^{-1})[6] appeared in the spectrum, the reaction appeared to be initiated at the 4 or 5 CH_2 group (see Scheme I).

Further data concerning the structure of this compound, and the other isolated urinary materials, were obtained by nmr spectrometry. The nmr signals obtained with the R_f 0.80 compound are listed in Table I under number III. The doublets at δ 7.01 and 7.71 ppm are attributed to the 2 furan ring protons. Their location agrees well with those of the other nitrofurans listed. The singlet at δ 7.59 must result from the azomethine proton, since no protons are present on adjacent atoms and no splitting of the peak is expected or found. This confirms the ir data on the integrity of the 2-furaldehyde moiety. There are 3 bands (quadruplets; δ 3.50, 3.88, 5.27) representing the imidazolidinone ring in the spectrum of III, whereas only 2 bands (δ 3.50, 3.76) are found in the spectrum of I. These 3 sets of quadruplets are typical of a CH_2CH= group corresponding to an

Scheme I. Proposed Metabolic Pathway for Nifuradene (I)



AMX system. The δ position of the bands for the CH_2 group in III (3.50, 3.88) correlates well with the same group in I (3.50, 3.76). It is probable that the singlet at δ 8.06 is produced by an undissociated proton of an OH. A broad singlet at δ 8.17 is attributed to the proton of the NH group found in the ir spectrum. The evidence thus indicates that III is 4-hydroxy-1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone. The instability of III under all but the mildest conditions precluded synthesis of the compound. The MIC of product III is 1.4 μg/ml.

The degradation product of III had R_f 0.88 in solvent system 1. Again, the ir spectrum indicated that the nitrofuran ring remained intact. The C=O (1710 cm^{-1}) and the NH (3350 cm^{-1}) stretching bands were also present, leading to the conclusion that further change had occurred in the CH_2 portion of the imidazolidinone ring.

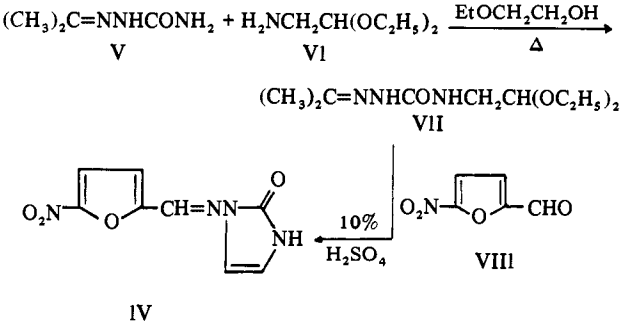
The nmr signals (Table I) from the degradation product (IV) confirm that the 2-furaldehyde portion of the molecule remains intact, as indicated by the furan signals at δ 7.11, 7.73 and the azomethine signal at δ 8.92.

Preliminary experiments had shown that heating crystalline III produced the degradation product with a concomitant loss in weight approximately equal to 1 mole of H_2O. An unsaturated ring could be formed, with dehydration occurring across the 3-4 or 4-5 positions. If dehydration occurred 3-4, the ring would contain a CH_2CH= group. The nmr spectrum would be higher order, and contain 8 or more lines. If dehydration occurred 4-5, the ring should contain a CH=CH group and the nmr spectrum, 2 doublets. Inspection of the spectrum showed doublets at δ 6.63 and at 7.22 which result from side-chain ring protons and are typical of the CH=CH group. It is not known whether the initial dehydration occurs 3-4 or 4-5, since rearrangement may occur. The evidence thus indicates that degradation product IV is 1-[(5-nitrofurfurylidene)amino]-4-imidazolin-2-one. Independent synthesis gave a product that confirmed this structure, yielding uv, ir, and nmr data identical with those of the metabolic product. The MIC of product IV was 0.06 μg/ml.

Table I. Nmr Signals of Nifuradene and Related Products

Assignment	Appearance	Ppm δ Compound I	Compound II	Compound III	Compound IV
CH_2CH_2 (imidazolidinone)	Multiplet	3.50			
	Multiplet	3.76			
CH_2 (4-hydroxyimidazolidinone)	Quadruplet			3.50	
	Quadruplet			3.88	
CH_2 (hydantoin)	Singlet		4.36		
NCHO (4-hydroxyimidazolidinone)	Quadruplet			5.27	
CH=CH (4-imidazolin-2-one)	Doublet				6.63
	Doublet				7.22
CH=CH (furan)	Doublet			7.01	
	Doublet	7.01			
	Doublet		7.11		
	Doublet				7.11
	Doublet			7.71	
	Doublet	7.72			
	Doublet		7.72		
	Doublet				7.73
CH=N	Singlet	7.54			
	Singlet			7.59	
	Singlet		7.74		
	Singlet				8.92
OH	Singlet			8.06	
NH	Singlet			8.17	

The synthesis of 1-[(5-nitrofurfurylidene)amino]-4-imidazolin-2-one (IV) was accomplished by the following sequence of reactions. Acetone semicarbazone (V) was treated with aminoacetaldehyde diethylacetal (VI) in 2-ethoxyethanol to form acetone 4-(2,2-diethoxyethyl)semicarbazone (VII). The acid hydrolysis of VII in the presence of 5-nitro-2-furaldehyde (VIII) gave the desired product IV.



B. Proposed Metabolic Pathway. The initial portion of a metabolic pathway of nifuradene can be theorized from the identity of the excretion products. The proposed metabolic sequence and the nonmetabolic degradation of the labile intermediate are shown in Scheme I. Nifuradene (I) is oxidized to 4-hydroxy-1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone (III), which is further oxidized to nitrofurantoin (II). The intermediate (III) may also be readily degraded to 1-[(5-nitrofurfurylidene)amino]-4-imidazolin-2-one (IV) through a spontaneous, relatively slow, dehydration step.

To test the second metabolic step (III → II), a rat was administered a suspension of III perorally. An extract of the urine from the rat was chromatographed by tlc (both systems) and by paper chromatography (basic system). Reference I, II, III, and IV were run concurrently on each chromatogram. The urine extract yielded 2 spots on all chromatograms, corresponding to the administered compound (III) and to its proposed metabolic product (II). No spots corresponding to I or IV were found on any chromatogram.

A qualititative experiment on the metabolism of IV was also run. Two rats were administered IV, po to one and iv to the other. Nitromethane extracts of the urine from the rats were chromatographed by tlc (two systems) and by paper chromatography (two systems). Reference II, III, and IV were run concurrently on each chromatogram. Only a very low proportion of the dose was recovered as yellow,

nitromethane-extractable products. The urine extract yielded two distinct and several faint spots, which did not correspond to any of the 3 standards. Since neither II nor III was found, it appears that the dehydration III → IV is metabolically irreversible.

The products described in this report do not account for all of the nifuradene dosage which is apparently absorbed, and it should not be inferred that this is the exclusive metabolic route. It is probable that NO_2 reduction is the initial process in an alternative pathway[1a-g,3] beginning with I and perhaps with III and/or II. A minor pathway undoubtedly leads to a labile metabolite absorbing at 415 mμ, which has been previously reported in the urine of animals fed other nitrofuran derivatives.[1a-g,3] Small amounts of this metabolite were detected chromatographically as the 2,4-dinitrophenylhydrazine derivative in human urines from these trials.

Experimental Section‡

Acetone 4-(2,2-Diethoxyethyl)semicarbazone (VII). To a soln of V (115 g, 1 mole) in 2-ethoxyethanol (600 ml) was added VI (133 g, 1 mole). The reaction mixt was heated at reflux temp for 6 hr. The solution was concd under reduced pressure. The residue was poured over ice where it slowly crystd into a thick solid. The product was collected and dried to yield 74 g (31.8%), mp 45–47°. An analytical sample was prepared by recrystn from hexane (charcoal), mp 50–52°. *Anal.* ($C_{10}H_{21}N_3O_3$) C, H, N.

1-[(5-Nitrofurfurylidene)amino]-4-imidazolin-2-one (IV). To a soln of VII (74 g, 0.32 mole) in 10% H_2SO_4 (500 ml) was added VIII (42 g, 0.3 mole) dissolved in abs EtOH. The soln was heated on a steam bath for 2 hr and then cooled. The brick-red ppt was filtered, washed (H_2O, Et_2O), and dried to yield 55 g (84%), mp 240–242°. An analytical sample was prepd by recrystn from DMF–H_2O (charcoal) (Note: a DMF complex is formed which can be broken by heating the material at 110°), mp 243–245°. *Anal.* ($C_8H_6N_4O_4$) C, H, N.

Isolation of Metabolites from Human Urine. Nifuradene was administered orally to each of 10 human volunteers as a single 300-mg capsule. Urine voided 0.5 hr after dosing was discarded. Urine voided during the succeeding 5.5 hr was collected, and the total vol detd. It was then extd twice with 2 vol of $MeNO_2$. The combined extracts were concd in a rotary evaporator until a ppt began to form. Abs MeOH was added until the soln cleared, then evapn was contd until the vol was roughly 0.01 the original $MeNO_2$ vol.

Tlc plates (200 × 200 × 0.5 mm) prepd from silica gel G (Brinkmann Instrument Co.) were activated for 20 min at 110°. The concd extract was streaked on the plates and developed with Me_2CO–abs MeOH (19:1) (solvent system 1). After air-drying, the yellow bands were scraped from the plates and corresponding bands from each plate were pooled, poured into 0.9-cm chromatographic columns, and eluted with abs MeOH. The pooled eluates of each band were concd with a rotary evaporator.

A second set of tlc plates (200 × 200 × 0.5 mm) was prepd, this time with silica gel N (Brinkmann Instrument Co.). After air-drying, the plates were activated for 20 min at 110°. The MeOH concentrates from the original bands were streaked on separate sets of plates, and developed with $CHCl_3$–$MeNO_2$–abs MeOH (5:4:1) (solvent system 2). After air-drying the bands were again scraped from the plates, poured into chromatographic columns, and eluted with chromatographic grade Me_2CO. The pooled Me_2CO eluates of each product were concd under N_2 at room temp. Hexane was added dropwise to the concd solution until the cloud point was reached. The samples were then held at 0° overnight. The tubes were centrifuged, the supernatant liquid was removed, and the crystals were washed with hexane. Concn of the supernatant liquid yielded a second crop of crystals. The products were dried *in vacuo* at room temp.

Cochromatography. The 2 tlc systems described were also used for cochromatography. Paper chromatography was carried out on Whatman No. 1 paper using an acidic (H_2O–satd *n*-BuOH–88% HCO_2H) (19:1) or a basic (*n*-BuOH–95% EtOH–0.5 *N* NH_4OH) (4:1:1) solvent system. The excretion product and the known compound were each spotted separately, and as a mixt of the 2 materials, on the same chromatogram. Spots were examined both visually and under uv light. They appeared yellow visually, and had a yellow fluorescence under uv.

Instrumental Analysis of Metabolic Products. All uv spectra were obtd with the Perkin-Elmer Model 202 spectrophotometer as MeOH solns of the products. The ir spectra were obtd with the Perkin-Elmer Model 21 ir spectrophotometer from KBr pellets of the products. The nmr spectra were obtd on a Varian HA-100 nmr spectrometer. The compounds were dissolved in DMSO-*d* for nmr spectrometry.

The uv max of I and authentic nifuradene were 372 (ε 18,800 for I; 19,500 for authentic nifuradene) and 272 mμ. Identical ir spectra of both samples contd bands above 1000 cm^{-1} at 3425, 2960, 1730, 1600, 1545, 1450, 1375, 1340, 1245, 1165, 1065, and 1020 cm^{-1}.

The uv max of II and authentic nitrofurantoin were 358 and 266 mμ. Identical ir spectra of both samples contd bands above 1000 cm^{-1} at 3700, 3525, 2970, 2770, 1755, 1735, 1610, 1565, 1520, 1440, 1400, 1370, 1345, 1260, 1210, 1140, and 1020 cm^{-1}.

The uv max of IV and authentic 1-[(5-nitrofurfurylidene)-amino]-4-imidazolin-2-one were 377 (ε 14,100 for IV; 14,774 for authentic 1-[(5-nitrofurfurylidene)amino]-4-imidazolin-2-one), 289, and 229.5 mμ. The ir spectrum contd bands above 1000 cm^{-1} at 3350, 3100, 2900, 1710, 1610, 1550, 1505, 1485, 1405, 1390, 1350, 1270, 1250, 1195, 1115, and 1030 cm^{-1}.

Product III had uv max at 368 (ε 19,600) and 267.5 mμ. The ir spectrum contd bands above 1000 cm^{-1} at 3380, 3090, 1705, 1580, 1505, 1445, 1410, 1370, 1335, 1250, 1180, 1145, 1070, and 1015 cm^{-1}. *Anal.* ($C_8H_8N_4O_4$) C, H, N.

Determination of Minimal Inhibitory Concentration. The minimal inhibitory concn (MIC) of each compound was detd against *Escherichia coli* (Es-2) by an adaptation of the Microtiter (Cooke Engineering Co., Alexandria, Va.) method to MIC determination by Curtis and Heotis.§ This microscale serial dilution procedure allows multiple assays on a minimum amount of compound. The assay requires 0.025 ml of test soln for each serial dilution. Each compd was dissolved in DMF and dild to 200 μg/ml with distd H_2O. A max of 1% DMF was present in the dild soln. A minimum of 2 assays was performed on each of 7 dilutions.

Metabolic Tests. In the first experiment, 2 mg of 4-hydroxy-1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone (III) was suspended in 1% methylcellulose and administered po to a 210-g rat. The rat was placed in a metabolism cage and urine was collected for 6 hr.

In the second experiment, 2 rats received 10 mg/kg doses of 1-[(5-nitrofurfurylidene)amino]-4-imidazolin-2-one (IV), one po and one iv. The po dose was administered as a 1% methylcellulose suspension, while the iv dose was infused as a 3% solution in DMSO. The rats were placed in metabolism cages and urine was collected for 6 hr.

Urine from each metabolic trial was extd 3 times with 2 vol of $MeNO_2$. The extracts were concd to approx 0.5 ml and chromatographed in the systems described for cochromatography.

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References

(1) (a) D. L. Cramer, *J. Bacteriol.*, 54 119 (1947); (b) J. D. Taylor, H. E. Paul, and M. F. Paul, *J. Biol. Chem.*, 191, 223 (1951); (c) A. H. Beckett and A. E. Robinson, *J. Pharm. Pharmacol.*, 8, 1072 (1956); (d) R. E. Asnis, *Arch. Biochem. Biophys.*, 66, 208 (1957); (e) A. H. Beckett and A. E. Robinson, *J. Med. Pharm. Chem.*, 1, 135 (1959); (f) A. H. Beckett and A. E. Robinson, *ibid.*, 1, 155 (1959); (g) H. E. Paul, V. R. Ells, F. Kopko, and R. C. Bender, *ibid.*, 2, 563 (1960).
(2) F. F. Ebetino, J. J. Carroll, and G. Gever, *J. Med. Pharm. Chem.*, 5, 513 (1962).
(3) J. Olivard, S. Valenti, and J. A. Buzard, *ibid.*, 5, 524 (1962).

‡Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for these elements or functions were within ±0.4% of the theoretical values.

§T. E. Curtis and J. P. Heotis, unpublished data.

(4) J. G. Michels and G. Gever, *J. Amer. Chem. Soc.*, 78, 5349 (1956).
(5) A. H. J. Cross, S. G. E. Stevens, and T. H. E. Watts, *J. Appl. Chem.*, 7, 562 (1957).
(6) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd ed, Wiley, New York, N. Y., 1958, pp 250 and 300.

Centrally Acting Emetics. 5. Preparation and Pharmacology of 10-Hydroxy-11-methoxyaporphine (Isoapocodeine). *In Vitro* Enzymatic Methylation of Apomorphine[1,†]

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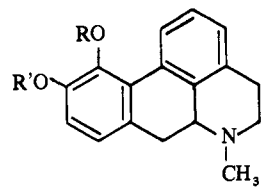
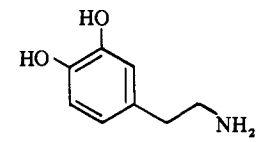
The title compound has been prepared by direct etherification of apomorphine. It was inert as an emetic in pigeons and dogs and as an inducer of the gnawing response in mice and of the "pecking syndrome" in pigeons. Tlc procedures for the 3 possible ether derivatives of apomorphine have been devised. Incubation of apomorphine with COMT preparation produced a mixture of isomeric monomethyl ethers, with the 10-methylated isomer greatly predominating. Possible significance of these data is cited.

The current interest in apomorphine (1) in the study and treatment of parkinsonism,[2] and the reports[2-5] of physiologic similarity and/or relationship of apomorphine to dopamine (2) suggested an investigation of the metabolic fate of apomorphine, with emphasis on possible parallels to the metabolism of dopamine. Kaul, *et al.*,[6-9] reported that the rabbit, the rat, and the horse excrete mixtures of apomorphine *O*-glucuronides, with the 10-glucuronide isomer predominating. The fraction of the total dose of apomorphine accounted for as excreted "bound" apomorphine in these studies varied from 13.6 to 71.9%. Under certain special experimental conditions, small percentages of unaltered apomorphine could be detected in rabbit urine.

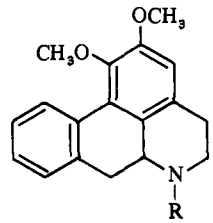
Smith and Sood[10] have described N-dealkylation of *N*-alkylnornuciferine systems 3 by guinea pig microsomal preparations. The literature has revealed no additional studies of the biological fate of apomorphine or of other aporphine derivatives. Accordingly, the effect of a catechol *O*-methyltransferase (COMT) preparation on apomorphine *in vitro* was investigated. For comparison purposes, the 3 possible O-methylation products of apomorphine, 10,11-dimethoxyaporphine (apomorphine dimethyl ether) (4), 10-methoxy-11-hydroxyaporphine (apocodeine) (5), and 10-hydroxy-11-methoxyaporphine ("isoapocodeine") (6), were required. Of these, the last-named, isoapocodeine (6), is unknown and its preparation was undertaken, beginning with apomorphine. Knorr and Raabe[11] had concluded that O-monomethylation of apomorphine leads exclusively to the 10-methylated product (apocodeine, 5), and this contention was supported by Borgman's report[12] that the sole isolable side product in preparation of the dimethyl ether of apomorphine with methyl tosylate and NaH was apocodeine. It seemed reasonable to presume that treatment of apomorphine with 1 equiv of base, followed by 1 equiv of benzyl bromide, would afford exclusively the 10-benzyl ether 7 which would be a key intermediate in preparation of 10-hydroxy-11-methoxyaporphine, according to Scheme I.





2

1, R = R' = H
4, R = R' = Me
5, R = H; R' = Me
6, R = Me; R' = H



3

Tlc analysis of the reaction mixture resulting from benzylation of apomorphine showed 3 spots, in addition to 1 spot for apomorphine itself; it was concluded that these 3 spots represented the 2 isomeric monobenzyl ethers and the dibenzyl ether. Treatment of a mixture of monobenzyl ethers with base and methyl tosylate induced formation of a monobenzyl monomethyl ether of apomorphine which, upon treatment under reductive debenzylation conditions, permitted isolation of 10-methoxy-11-hydroxyaporphine (apocodeine) (5). This finding leads to the conclusion that the sterically hindered, presumably unfavored 11-OH position of apomorphine can be benzylated to the exclusion of the 10 position, and that O-benzylation of apomorphine results in mixtures of products. Therefore, Scheme I seemed unsuited for preparation of isoapocodeine.

Treatment of apomorphine with 1 equiv of base followed by 1 equiv of methyl tosylate resulted in formation of a 3-component product mixture, from which the 2 monomethyl ethers and the dimethyl ether could be isolated, identified, and characterized. The yield of isoapocodeine was poor, but the method is sufficiently good to be considered to be of preparative value. The results of this work indicate

†This investigation was supported in part by Grant NS-04349, National Institute of Neurological Diseases and Stroke. Abstracted in part from portions of theses submitted by R. J. B. (1970) and A. M. (1971) in partial fulfillment of the requirements for the Ph. D. degree, University of Iowa.