(2) H. J. Muller-Eberhard, *Advan. Immunol.*, **8**, 1 (1968).
(3) F. Markwardt, P. Walsmann, and H. G. Kazmirowski, *Pharmazie*, **24**, 400 (1969).
(4) F. Markwardt, P. Walsmann, and H. Landmann *ibid.*, **25**, 551 (1970).
(5) F. Markwardt, H. Landmann, and P. Walsmann, *Eur. J. Biochem.*, **6**, 502 (1968).
(6) B. R. Baker and M. Cory, *J. Med. Chem.*, **12**, 1049 (1969).
(7) T. Fujita, J. Iwasa, and C. Hansch, *J. Amer. Chem. Soc.*, **86**, 5175 (1964).
(8) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
(9) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill, New York, N. Y., 1940.
(10) R. W. Taft, Jr., "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, p 598.
(11) D. Agin, L. Hersh, and D. Holtzmann, *Proc. Nat. Acad. Sci. U. S.*, **53**, 952 (1965).
(12) C. Hansch and E. Coats, *J. Pharm. Sci.*, **59**, 731 (1970).
(13) A. Leo, C. Hansch, and C. Church, *J. Med. Chem.*, **12**, 766 (1969).
(14) R. Shriner, R. Fuson, and D. Curtin, "The Systematic Identification of Organic Compounds," 4th ed, Wiley, New York, N. Y., 1956, p 50.
(15) E. Coats, W. R. Glave, and C. Hansch, *J. Med. Chem.*, **13**, 913 (1970).
(16) Reference 10, p 587.
(17) J. Hine, "Physical Organic Chemistry," 2nd ed, McGraw-Hill, New York, N. Y., 1962, p 96.
(18) E. Kutter, A. Herz, H. Teschemacher, and R. Hess, *J. Med. Chem.*, **13**, 801 (1970).
(19) M. Muramatu and S. Fujii, *J. Biochem. (Tokyo)*, **65**, 17 (1969).
(20) T. Chase, Jr., and E. Shaw, *Biochemistry*, **8**, 2212 (1969).
(21) G. E. Davies and J. S. Lowe, *Advan. Exp. Med. Biol.*, **8**, 452 (1970).
(22) J. D. Geratz, *Thromb. Diath. Haemorrh.*, **25**, 391 (1971).

In Vivo Metabolite Condensations. Formation of N^1-Ethyl-2-methyl-N^3-(2,6-dimethylphenyl)-4-imidazolidinone from the Reaction of a Metabolite of Alcohol with a Metabolite of Lidocaine†

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Isotopic labeling is used to demonstrate that N^1-ethyl-2-methyl-N^3-(2,6-dimethylphenyl)-4-imidazolidinone (2) is generated *in vivo* in the Rhesus monkey by condensation of ω-ethylamino-2,6-dimethylacetanilide (4), a metabolite of lidocaine, with acetaldehyde formed by ingestion of alcohol. This substance (2) was previously reported as a metabolite obtained from the urine of man after ingestion of lidocaine. Evidence now suggests that, under normal physiological conditions, the major portion of 2 originally determined was an artifact arising from the condensation of 4 with trace amounts of acetaldehyde present in the solvent used in the isolation procedure. The extreme ease, under a variety of mild nonenzymatic conditions, with which the condensation takes place demonstrates the care that must be exercised in interpreting data that potentially involve similar *in vivo* condensation reactions. The generality and potential importance of such reactions between aldehydes and amines are pointed out.

In the course of a study concerning the biotransformation of the potent antiarrhythmic agent lidocaine (1) in man, we discovered[1] a new metabolite, N^1-ethyl-2-methyl-N^3-(2,6-dimethylphenyl)-4-imidazolidinone (2). Further investigation also revealed the presence of a second new metabolite and it was subsequently shown to be N^1-ethyl-N^3-(2,6-dimethylphenyl)-4-imidazolidinone (3). Although the formation of 2 could readily be rationalized as proceeding *via* an intramolecular cyclization following initial oxidation (see Discussion), it was not apparent how 3 could be obtained from either 1 or 2 in any simple fashion.

In a series of papers in 1960, Hollunger[2] demonstrated that in rabbit, liver enzymes were largely responsible for the biotransformation of lidocaine and that a key metabolite was ω-ethylamino-2,6-dimethylacetanilide (4). Later Beckett and Boyes[3] demonstrated that 4 was a major biotransformation product of lidocaine in man and their report was subsequently confirmed by others[4] (Chart I).

Since 4 was known to be a major metabolic product in man, it seemed possible that 3 might be arising *via* an *in vivo* intermolecular condensation between 4 and some one carbon fragment at the oxidation level of formaldehyde. Similarly, the formation of 2 could be viewed as arising from an intermolecular condensation between 4 and acetaldehyde. To investigate the origin of these metabolites and the feasibility of such reactions *in vivo*, a series of labeling experiments was undertaken.

As will be shown, the results of these experiments clearly demonstrate the ease of such condensation reactions under very mild reaction conditions and, hence, the care that must be taken in interpretation of results. Further, they demonstrate that while the formation of 2 under normal physiological conditions cannot be unambiguously established at this time, 2 can be generated *in vivo* after lidocaine administration when the plasma level concentration of acetaldehyde is artificially increased by concomitant administration of ethanol.

Results

Human Studies. (a) Isolation of Metabolites. After oral administration of randomly tritiated lidocaine hydrochloride to three normal human volunteers, urine and feces were collected, frozen, and stored over a period of 72 hr. No attempt was made to control urinary pH during these studies. Of the radioactivity present in the urine 5–10% could be accounted for as extractable organic bases while the total radioactivity present represented 50% of the administered dose. Homogenization of the feces followed by oxidation of an aliquot and scintillation counting indicated that this route of elimination is of minor impor-

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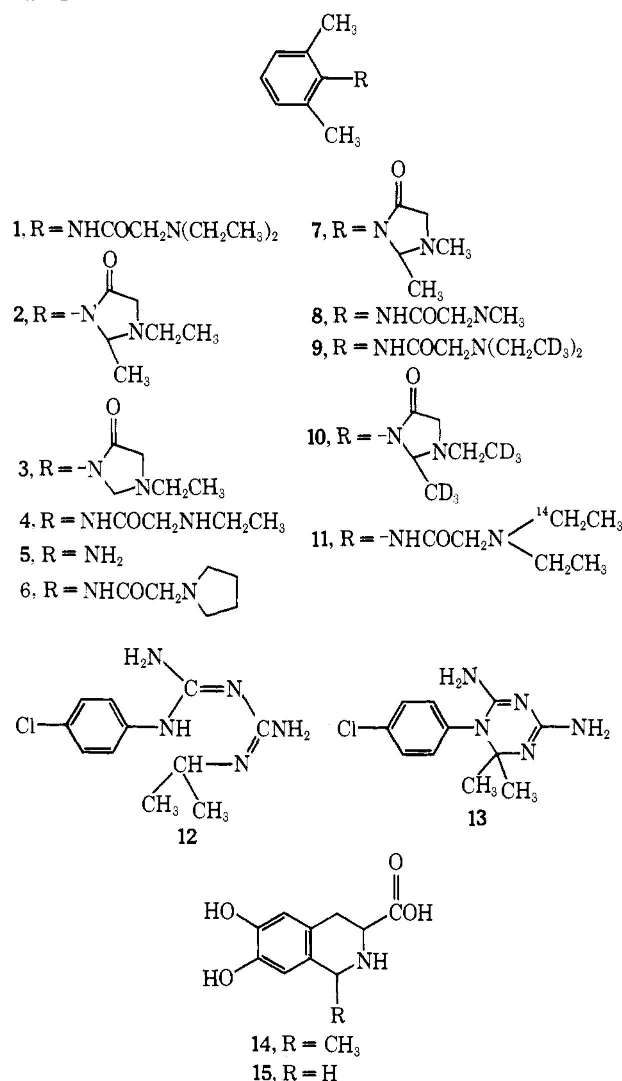
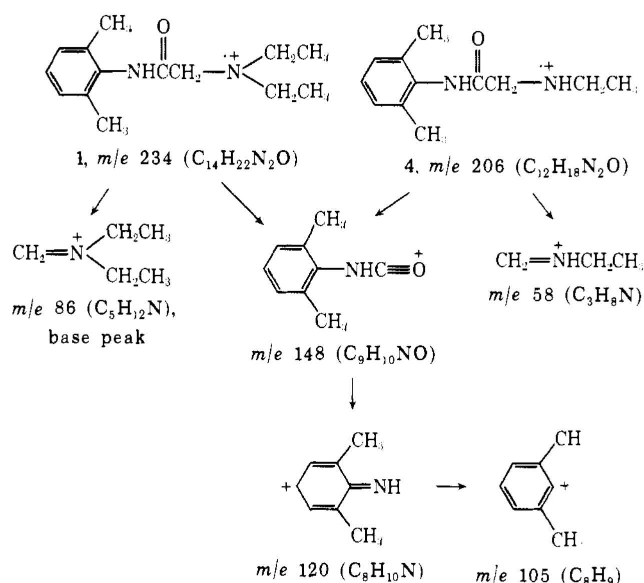
Chart I



Scheme I. Mass Spectral Fragmentation Pattern for 1 and 4



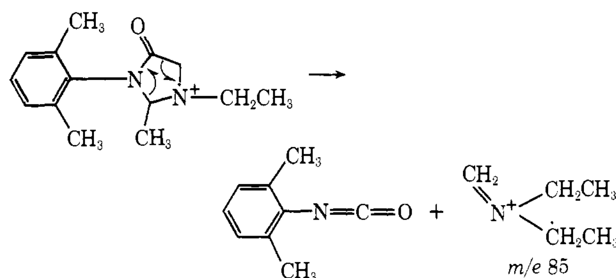
tance for lidocaine and its metabolites. Hence, at this point the feces were discarded. Since most of the radioactivity appeared in the urine 8 hr after administration, this sample was selected for initial investigation and isolation of the organic bases present. Glc separated the base isolate into five major components. Each component was collected from glc *via* a stream splitter and saved for analysis.

(b) **Mass Spectroscopy.** To establish a capability for elucidating the structure of any unknown metabolite, we undertook a mass spectral study of the behavior of lidocaine and one of its known metabolites, 4. The major fragmentation routes are depicted in Scheme I and are supported by exact mass measurement and metastable scanning. As expected,[5a] both compounds fragmented primarily by homolytic cleavage of the carbon-carbon bond between the carbonyl and methylene groups to generate immonium ions as the base peak ions at *m/e* 86 ($C_5H_{12}N$) and 58 (C_3H_8N), respectively. Exact mass measurement of the ions at *m/e* 148, 120, and 105 is consistent with the structures depicted in Scheme I and, as such, represents the other half of the molecule. Therefore, it appeared that metabolic transformations could conceivably be detected by changes in the atomic composition of these ions.

(c) **Identification of Metabolites and Proof of Structure.** Of the five major radioactive bases isolated, three proved to be known metabolites. These were lidocaine, 4, and 2,6-dimethylaniline (5). These structures were confirmed by retention times on two different glc systems, isolation of small quantities of each *via* glc using a stream splitter, scintillation counting, and high-resolution mass spectrometry.

The remaining two peaks were unknown, although collection and scintillation counting confirmed that they were lidocaine derived. One of these, the largest of all five peaks, was subjected to mass spectrometry and was found to have a parent ion at *m/e* 232 ($C_{14}H_{20}N_2O$), suggesting that the unknown contained one additional unit of unsaturation. Somewhat surprisingly, the base peak ion in the spectrum of this material occurred at *m/e* 85 ($C_5H_{11}N$). If the diethylaminomethylene portion of the molecule were intact, the compound should still give a base peak at *m/e* 86. However, if, unlikely as it may be, cyclization to a substituted pyrrolidine such as 6 or some isomeric substance had occurred, the base peak ion should be at *m/e* 84.

On the basis of chemical stability and the mass spectrum, the imidazolidinone 2 was considered a likely possibility. The base peak at *m/e* 85 can be rationalized mechanistically, as shown below.



The driving force for the fragmentation is presumably the generation of a neutral isocyanate and allylic radical cation.[5b] Certainly, this is a more reasonable process than generating a base peak ion at *m/e* 86 or 84 from such a molecule. Further, 2 was an attractive possibility because its formation could readily be rationalized as proceeding through a well-established metabolic pathway (see Discussion).

Compounds similar to 2 have been prepared by the condensation of an aldehyde with a suitable secondary amine.[6] Hence, acetaldehyde was condensed with 4. The reaction mixture was separated by glc and found to contain two peaks distinct from starting material. The major peak had a retention time identical with the unknown,

while the nmr was consistent with 2. Admixture of the synthetic material to the metabolite and recrystallization to constant specific activity confirmed the assigned structure.

The last of the five major peaks that was still unidentified was subjected to mass spectroscopy. The molecular ion occurred at *m/e* 218, $C_{13}H_{18}N_2O$, while the base peak ion occurred at *m/e* 71, C_4H_9N. The similarity in behavior of this material upon electron impact to that displayed by 2 immediately suggested either 3 or 7 as a possible structure for the metabolite. Both materials were synthesized, 3 by the condensation of 4 with formaldehyde and 7 by a similar condensation of ω-methylamino-2,6-dimethylacetanilide (8) with acetaldehyde. Mass fragmentation patterns and glc retention times indicated that the behavior of the metabolite was identical with 3 but different from 7. Admixture of the synthetic material to the urinary metabolite and recrystallization to constant specific activity established the structure as 3.

Initial Monkey Experiments. To investigate the genesis of the cyclic metabolites by utilization of isotopically labeled materials, it was necessary to have an animal model that would emulate the fate of the drug in man. To this end we repeated our experiments in the Rhesus monkey. The monkey was administered an isotonic solution of lidocaine hydrochloride and the urine collected and worked up in a fashion identical with that which we had used previously.[1] Under these conditions both 2 and 3 were isolated and identified.

Since we could reproduce our experiments in the monkey, we next attempted to determine whether these apparent metabolites were in fact artifacts. The solvents, either ether and/or methylene chloride, that we had used for extractions were spectral grade and reputedly contained less than 0.001% of either acetaldehyde or formaldehyde as contaminants. Both solvents were meticulously purified and freed of aldehydes, using the method outlined by Schwartz and Parks.[7]

Control Experiments. Compound 4 was added to samples of both human and monkey urine and duplicate experiments were run using "unpurified" and purified spectral solvents for extraction. The results of these experiments indicated that most, if not all, of 2 and 3 was the result of a reaction between 4 and the aldehydes contaminating the solvent (Figure 1a). However, glc peaks corresponding to both 2 and 3 could consistently be detected (Figure 1b) from the control experiment. Conversely, if the experiments were repeated and lidocaine was added to the urine instead of 4, these peaks could not be detected (Figure 1c).

These data imply that 2 and 3 result from either residual contamination in the purified solvents or that they arise from reaction with minute quantities of aldehydes present in urine.

To investigate these possibilities, 4 was allowed to sit in purified methylene chloride for 24 hr. The methylene chloride was evaporated and the residue analyzed by glc. No cyclic metabolites could be detected. The control experiments were repeated again by adding 4 to both monkey and human urine. The urines were allowed to sit for 48 hr, each was divided into four aliquots, and each aliquot was separately extracted with purified CH_2Cl_2, $CHCl_3$, Et_2O, and CCl_4. In all cases, both cyclic metabolites could be detected on analysis and the results from all four experiments were identical within experimental error.

Since both metabolites could be detected after using purified solvents, it seemed possible that at least some small portion of these materials could be arising *in vivo*

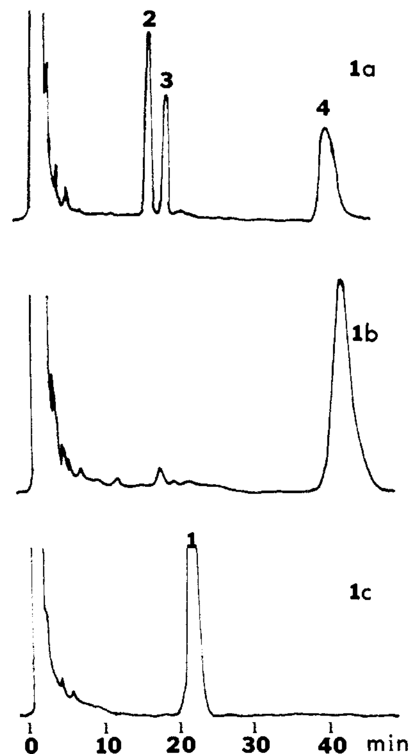


Figure 1. Glc trace of the basic metabolites obtained from (a) 4 added to urine and extracted with unpurified CH_2Cl_2; (b) 4 added to urine and extracted with purified CH_2Cl_2; (c) 1 added to urine and extracted with purified CH_2Cl_2.

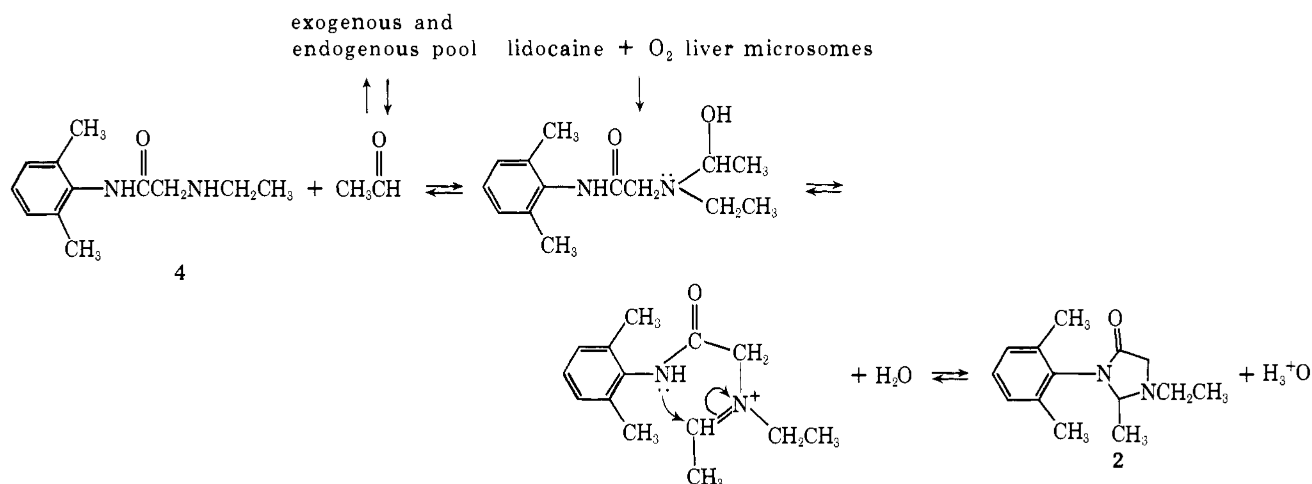
even though there were no readily apparent differences in concentration between the results of these experiments and the controls.

Labeling Experiments under Normal Physiological Conditions. In order to determine if some small quantity of 2 was arising *via* an *in vivo* intramolecular process, we synthesized specifically labeled lidocaine-d_6 (9) and administered it to the monkey. We assumed that if any of 2 was being generated by an intramolecular process, then a parent ion at *m/e* 238 should be detectable in its mass spectrum even if 10 were only a tiny fraction of the isolated material. To ease the isolation problems, nonpurified methylene chloride was used for extraction, the metabolites were isolated, and the mass spectrum was determined. No ion at *m/e* 238 could be detected, although a large parent ion was found at *m/e* 235.

In a similar experiment, lidocaine containing ^{14}C in the methylene group of one of the *N*-ethyl groups (11) was synthesized. Its specific activity was determined, it was administered to the monkey, and the experimental protocol described above was repeated. The cyclic metabolites 2 and 3 were isolated and their specific activities were determined. Within experimental error, the specific activity had fallen to 50% of the administered lidocaine. Clearly, this result is consistent with the deuterium experiment. Unfortunately, these experiments do not exclude the possibility of the labels being lost *via* equilibrium with aldehydes present in plasma, urine, and/or solvent. In fact, the following experiment where purified solvents were used demonstrates that the aldehydes present in urine are sufficient to equilibrate with 2 and 4.

Doubly labeled 4 containing ^{14}C in the carbonyl group and tritium in the aromatic ring of known specific activity was added to an 8-hr urine collection from a human volunteer who had received lidocaine and the urine allowed to stand for 12 hr. The urine was processed as previously described and extracted with purified solvents. The isolat-

Scheme II. Proposed Mechanism for the Formation of 2



ed cyclic metabolite 2 as well as labeled 4 now had the same specific activity, although it was different from and less than the starting specific activity. This result implies that 2 and 4 must be in equilibrium. Hence, the rate of equilibrium probably limits the possibility of detecting an intramolecular process.

Thus, the reaction appeared efficient enough to scavenge minute quantities of aldehydes under very mild conditions. To investigate just how efficient the reaction might be, 1 μg/ml of 4 was allowed to react with 1 μg/ml of acetaldehyde in a phosphate buffer at room temperature. The reaction mixture was concentrated and injected directly into a gas chromatograph. The resultant glc trace indicated a 20% conversion of 4 to 2 under these conditions. Hence, it appeared that if the *in vivo* concentration of acetaldehyde could be increased, the formation of the metabolite might possibly be induced.

Labeling Experiments with Concomitant Administration of Ethanol. To determine if this were indeed the case, ethanol-d_5 was infused into the monkey along with lidocaine in phosphate buffer. The urine was collected and processed with purified solvents as described previously. A glc trace indicated that the peak attributable to 2 from the alcohol experiment was approximately 25 times as great as that obtained from a simultaneously run control. The metabolite was collected and subjected to mass spectroscopy and was found to have 39% containing one or more atoms of deuterium. This result is based on the abundance of the M + 1, M + 2, and M + 3 ions minus the contribution of M + 1 due to the natural abundance of ^{13}C. No ion at M + 4 could be detected. This result indicated that the acetaldehyde produced *in vivo* from the alcohol was losing some of its deuterium *via* enolization prior to condensation and the acetaldehyde was exchanging *via* equilibrium in the normal aldehyde pool.

To gain confirmation for this result and unambiguously determine whether 2 was being produced from an *in vivo* source of acetaldehyde, the experiment was repeated using ethanol-*1-*^{14}*C*. The metabolite was isolated, subjected to scintillation counting, and found to be radioactive. Mass spectroscopy confirmed its structure. Determination of its specific activity revealed that approximately 35% dilution had occurred. Several explanations seem possible for this observation. Either the labeled acetaldehyde is being diluted in the normal metabolic pool prior to condensation and/or the presence of excess acetaldehyde is enhancing formation of 2 *via* an intramolecular reaction. The difference in the amount of label incorporated in the two experiments, 39% in the deuterium experiment and 65% in the ^{14}C experiment, is intriguing. It may simply be biological variability, and/or it may mean that the deuteron at the 2 position (aldehydic deuteron) of 2 is acidic enough to exchange under our experimental conditions and/or it may be due to different rates of equilibration in the two experiments. Studies designed to answer these questions are currently being investigated.

Discussion

A generalized mechanism for the formation of 2 consistent with all our results is depicted in Scheme II. There is considerable evidence that enzymatic N-dealkylations proceed by way of a carbinolamine intermediate such as that shown above, which then either spontaneously or enzymatically disassociates to an amine and an aldehyde.[8] In the case of lidocaine, because of the availability of a nonbonded lone pair of electrons five atoms away from the electrophilic site, a second reaction becomes theoretically possible, that of an intramolecular nucleophilic attack by the amide nitrogen on the immonium cation carbon‡ resulting in ring closure and the generation of a stable noncharged molecule. Moreover, there is precedent[9] for a similar intramolecular reaction occurring *in vivo* in the course of the metabolism of the antimalarial agent proguanil (**12**) to generate the biologically active triazine **13**. From a strictly chemical viewpoint, this latter reaction can be viewed as a Mannich type of reaction.[10] Thus, the secondary amine 4 and the cyclic metabolite 2 are related through a common intermediate, the carbinolamine, obtained from reaction with acetaldehyde. It could, therefore, be anticipated that the three substances might be in measurable equilibrium depending on their relative stabilities and reaction conditions. That this is indeed the case and that equilibrium is extremely facile under very mild conditions are apparent from the results obtained. Indeed the facile nature of the reaction precludes the possibility in this report of determining just how much, if any, of 2 might arise from the originally administered lidocaine *via* an initial intramolecular cyclization. Even presupposing significantly large amounts of 2 were generated in this fashion, this would not be detectable on urine analysis because equilibrium between 2, 4, and the endogenous or

‡ We have invoked attack by the amide nitrogen on an immonium ion intermediate rather than directly on the carbinolamine because there is precedence for such intermediates in Mannich reactions and intuitively a nucleophilic attack by a sterically hindered amide on a "stabilized carbonium ion" seems a much more reasonable process than direct S_N2 displacement of OH^- from the carbinolamine carbon.

exogenous (urine, solvents) aldehyde pool would have already occurred (Scheme II).

Recently Freund[11] has reported that lidocaine is an effective agent in preventing ethanol withdrawal seizures in addicted mice. It may be that the drug is exerting its pharmacological action *via* its membrane-stabilizing properties. However, one cannot help but speculate that its preventative effect may in some way be associated with the propensity of its metabolite 4 to react with aldehydes.

These results would appear to have bearing on three general areas of current research interest.

(1) Beckett[12] has coined the term "metabonate" for a substance which appears to be a metabolite but which in reality is formed during isolation and/or storage. The ease by which **2** and **4** can be artifactually formed certainly illustrates the problem and opens to question the genesis of natural products formed by similar reactions. For example, while isolating L-Dopa from velvet beans, one group of workers[13] reported finding **14** as a new naturally occurring amino acid. Similarly, a second group[14] reported finding **15** as a naturally occurring amino acid from the seeds of *Mucuna mutisiana*. Since both compounds could result chemically[15] by condensation of acetaldehyde with L-Dopa in the case of **14** and from formaldehyde in the case of **15**, the validity of these compounds as natural products is open to some question.

(2) Much attention has been focused recently on the *in vivo* and *in vitro* generation of substituted tetrahydroisoquinolines *via* the condensation of exogenous or endogenously induced levels of aldehydes and the hormonal amines.[15-21] These substances are known to be pharmacologically active in their own right and several groups of workers have speculated as to their possible involvement in the dependence and withdrawal phenomena of morphine addition[16,19,20] and alcoholism.[21] Our results would indicate that quantification of levels of certain of these condensation products might be extremely difficult. For example, Sandler, *et al.*,[22] have recently reported the *in vivo* formation of aldehyde condensation products in patients being treated for Parkinsonism with L-Dopa. As pointed out in their paper, they could not meaningfully interpret their quantitative data on urine levels because of great variability from determination to determination.

(3) The generality of the binding of low molecular weight drugs or their degradation products to tissue protein components is becoming increasingly recognized.[23] In case of lidocaine, its metabolite, **4**, readily condenses with acetaldehyde to form **2**, even though a sterically hindered amide nitrogen must function as a nucleophile in this reaction. This implies that good nucleophiles such as the sulfhydryl group of cysteine, or the ring nitrogen of histidine, could effectively compete for reaction with a reactive intermediate formed in the process of dealkylation or in the reverse reaction with aldehyde. If such reactive electrophilic intermediates are a general phenomena in N-dealkylation processes or in the presence of alcohol ingestion, then perhaps the biological responses, either efficacious or toxicological, of various amine drugs may, at least partially, be explained in terms of a reaction of such an intermediate with a nucleophile of a critical macromolecule. This possibility is under current investigation.

Experimental Section

Melting points were taken by capillary on a Thomas-Hoover Uni-Melt instrument and are uncorrected. Uv spectra were recorded on a Cary 16 spectrophotometer; ir on a Perkin-Elmer 337 spectrophotometer; nmr on a Varian A-60A (δ) or a JEOL 100 MHz (δ^{100}); mass spectra were taken on an AEI MS 902 (direct inlet, 70 eV). Vpc analyses were obtained on a Varian 2100 gas chromatograph equipped with flame ionization detectors using a 5.5 ft × 0.25 in. × 2 mm i.d. glass U column freshly packed with 2% Carbowax 20M on 80-100 mesh KOH-washed Chromosorb W. Conditions employed were: column temperature 160°; injector 250°; detector 250°; N_2 carrier gas flow 40 ml/min; hydrogen and air adjusted to give maximum flame response; chart speed (Varian A-25 recorder) 0.1 in./min. The column was equipped with a 10:1 splitter for collection of samples in capillary tubes for mass spectral analysis or scintillation counting. A Tri-Carb Model 3375 scintillation counter was used for radioactivity determinations. All determinations were made in 10-cc Aquasol (NEN) and corrected for quenching using internal standardization with either toluene-^{14}C standard (NEN) or toluene-^{3}H standard (NEN).

Isolation of Basic Metabolites from Human and Monkey Urine. Administration of lidocaine (1) to human volunteers was accomplished *via* the oral route with 500-mg encapsulated doses of 1 as the hydrochloride.§ Male Rhesus monkeys, restrained in metabolism chairs, were administered by iv infusion 100 mg of 1 dissolved in sterile water for injection made acidic with a small amount of HCl, neutralized with NaOH, made isotonic with NaCl, made to a volume of 40 ml, and filtered through a millipore filter. Infusion was maintained for 20 hr at a rate of 2 ml/hr. Aliquots of the total urine collected for 36 hr were extracted (pH 11) with equal volumes of solvent‡ by shaking for 30 min on an automatic shaker in centrifuge tubes. The samples were then spun down for 20 min at 1000g to help break emulsions. Combined extracts were extracted with two 0.5 vol of 10% HCl which were combined and basified to pH 11 with 6 *N* NaOH followed by back-extraction with 2 vol of solvent. After drying (Na_2SO_4), solvent was removed at room temperature by rotovac. Residue was taken up in 1 ml of the solvent used for extraction and subjected to glc. Retention times on a freshly packed column are: 5, 1.2 min; **2**, 17 min; 3, 19 min; 1, 21.2 min; 4, 41 min. Retention times on a column packed with 3% OV-17 on A/W Chromosorb W 80-100 mesh are: 5, 1 min; 2, 8 min; 3, 7.9 min; 1, 9.5 min; 4, 8.8 min (185°).

ω-Ethylamino-2,6-dimethylacetanilide (4). To a solution of ω-chloro-2,6-dimethylacetanilide[24,25] (51.5 g, 0.25 mol) in 250 ml of C_6H_6 was added ethylamine, 60 ml of a 70% solution (0.5 mol), and the two-phase system was refluxed for 5 hr. After cooling the reaction mixture was poured into 1 vol of H_2O and the C_6H_6 layer separated and extracted with two 0.5 vol of 10% HCl. Combined acid extracts were back-washed with 1 vol of C_6H_6, basified with NaOH to pH 11, and back-extracted with three ⅓ vol of purified CH_2Cl_2. Combined organic extracts were washed with 1 vol of H_2O and dried (Na_2SO_4), and solvent was removed by rotovac at room temperature to yield a crystalline mass. Recrystallization from purified hexane gave 4 (25 g, ~50%) as white needles: mp 49-50.5° (lit.[24] 47-49°); nmr δ ($CDCl_3$, TMS) 1.18 (t, J = 7 Hz, NCH_2CH_3), 1.63 (s, NH), 2.25 (s, aromatic Me's), 2.82 (q, J = 7 Hz, NCH_2CH_3), 3.48 (s, $O{=}CCH_2N$), 7.1 (s, aromatics), 8.8 ppm (broad s, $HNC{=}O$); mass spectrum calcd for $C_{12}H_{18}N_2O$ 206.14191, found 206.14228; mass fragments m/e 191, 163, 148, 132, 120, 105, 91, 77, 53. *Anal.* ($C_{12}H_{18}N_2O$) C, H, N.

N^1-Ethyl-2-methyl-N^3-(2,6-dimethylphenyl)-4-imidazolidinone (2). To an ice-bath cooled, stirred solution of 4 (20.6 g, 0.1 mol) in 2 l. of Et_2O was added two 10-ml portions of acetaldehyde. The reactants were warmed slowly to room temperature and allowed to stir for 5 hr after which time solvent was removed by rotovac yielding an orange oil. Recrystallization from petroleum ether (bp 30-60°) gave **2**: 9.8 g (48%); mp 62-63°; ir (KBr) 1715 cm^{-1} (lactam C=O); nmr δ^{100} (C_6H_6-d_6, TMS)** 0.87 (d, J = 5.5 Hz, C-2 methyl), 0.86 (t, J = 7.3 Hz, NCH_2CH_3), 2.05 and 2.56 (nonequivalent s, aryl Me's), 2.49 and 1.95 ppm (nonequivalent octets, J_{gem} = 14.5 Hz, J_{Me} = 5.5 Hz, ring methine); mass

§ The original studies were carried out in three normal human male volunteers using randomly tritiated lidocaine. Once the cyclic metabolites were identified, additional studies in both man and monkey employed normal lidocaine. We wish to thank Dr. C. T. Peng of the School of Pharmacy, University of California, San Francisco, Calif., for a generous gift of the randomly tritiated lidocaine. The randomness of the tritiated label and the possibility of exchange *in vivo* prevents the possibility of obtaining hard quantitative data. Hence, the figure of 3-5% of the administered dose appearing as extractable bases in the urine must be taken as a lower limit. Lidocaine and all its known metabolites are currently being quantitated in our laboratories utilizing chemical ionization mass spectrometry and stable isotopes. These data will be the subject of a future report.

‡ As stated in the text, spectral grade solvents were used and in some cases these solvents were further purified to remove all traces of aldehydes.

** The nmr for 2 given in ref 1, note 4, was determined in C_6H_6 d_6 and not $CDCl_3$ as reported.

spectrum calcd for $C_{14}H_{20}N_2O$ 232.15782, found 232.15756; mass fragments *m/e* 217, 160, 147, 105, 91, 85, 70. *Anal.* ($C_{14}N_{20}N_2O$) C, H, N.

N^1-Ethyl-N^3-(2,6-dimethylphenyl)-4-imidazolidinone (3). To a solution of 4 (1.0 g, 4.85 mmol) in 100 ml of C_6H_6 was added a twofold excess of 35% aqueous formaldehyde. The reaction was refluxed for 3 hr, cooled, washed with saturated NaCl, and dried (Na_2SO_4). Removal of C_6H_6 with a stream of N_2 gas yielded a white crystalline mass which was recrystallized from petroleum ether (bp 30–60°) to give white needles of **3**: 0.6 g (56.5%); mp 100–101°; ir (KBr) 1715 cm^{-1} (lactam C=O); nmr δ ($CDCl_3$, TMS) 1.16 (t, *J* = 7 Hz, NCH_2CH_3), 2.27 (s, aryl Me's), 2.74 (q, *J* = 7 Hz, NCH_2CH_3), 3.47 (m, C-5 methylenes), 4.20 (m, C-2 methylenes), 7.13 ppm (s, aromatics); mass spectrum calcd for $C_{13}H_{18}N_2O$ 218.14191, found 218.14127; mass fragments *m/e* 160, 147, 105, 90, 71, 58. *Anal.* ($C_{13}H_{18}N_2O$) C, H, N.

N^1-Methyl-2-methyl-N^3-(2,6-dimethylphenyl)-4-imidazolidinone (7). (a) ω-Chloro-2,6-dimethylacetanilide (10 g, 0.05 mol) in 50 ml of dry C_6H_6 was added to a fourfold excess of CH_3NH_2 in a stainless steel bomb cooled in Dry Ice-acetone. The bomb was sealed and heated for 18 hr at 100°. After cooling, the bomb was unsealed and warmed to room temperature. The reaction mixture was extracted twice with 5% HCl; the combined acid extracts were back-washed with Et_2O, basified with KOH pellets, and extracted twice with CH_2Cl_2. The organic layers were washed with saturated NaCl solution and dried over Na_2SO_4, and solvent was evaporated under N_2 to give a pale yellow liquid which was recrystallized from CH_2Cl_2 to give white crystals (2.7 g) of ω-*N*-methyl-2,6-dimethylacetanilide, mp 41° (lit.[25] 41°).

(b) A solution of ω-*N*-methyl-2,6-dimethylacetanilide (1 g, 4.9 mmol) in 100 ml of C_6H_6 was refluxed with excess acetaldehyde for 1 hr. After cooling, the reaction mixture was washed with saturated aqueous NaCl and dried over Na_2SO_4. Removal of solvent under N_2 gave an oil which was crystallized from petroleum ether (bp 30–60°) to yield white crystals: mp 139–142°; nmr δ ($CDCl_3$, TMS) 1.03 (m, C-2 methyl), 2.20 (s, aryl methyls), 2.45 (s, NCH_3), 3.25 (m, 1 H), 3.70 (m, 1 H), 3.80 (m, 1 H), 7.07 ppm (s, aromatics); mass spectrum *m/e* 218, 203, 147, 132, 117, 105, 91, 71, 57 (base peak).

ω-[β,β-d_6-Diethylamino]-2,6-dimethylacetanilide or Lidocaine-d_6 (9). (a) A mixture of Ac_2O-d_6 (Stohler, 5.0 g, 46 mmol) and acetonitrile-d_3 (Stohler, 1.5 g, 34 mmol) was hydrogenated (30 psi, H_2) on a Parr shaker over 60 mg of Adam's catalyst for 4 hr. An additional 30 mg of Adam's catalyst was then added and the hydrogenation continued an additional 2 hr. After removal of reduced Pt by suction filtration, the filtrate was distilled through a short-path distillation head to yield *N*-ethylacetamide-d_6, 2.2 g (71%).

(b) To an ice-bath cooled solution of AlH_3 (~31 mmol) in THF was added dropwise a THF solution of *N*-ethylacetamide-d_6 (1.5 g, 16.5 mmol) over 45 min. After 18 hr excess AlH_3 was destroyed with 2 ml of 1:1 THF-H_2O followed by hydrolysis of aluminate complexes with 8 ml of 15% NaOH. The liberated diethylamine-d_6 was codistilled with THF at 59–64°.

(c) To the 60 ml of THF solution containing diethylamine-d_6 was added ω-chloro-2,6-dimethylacetanilide[24] (1.20 g, 60 mmol), and the solution stirred at reflux for 2 hr. To the solution was added 300 mg of NaOH in 1.5 ml of H_2O and the reaction refluxed an additional 4 hr. Rotovap of solvent yielded a pale yellow liquid which was taken up in Et_2O and washed with H_2O followed by extraction with 10% HCl. The acid extract was basified with 3 *N* NaOH and back-extracted into Et_2O. Ether back-extract was washed with H_2O, filtered through Na_2SO_4, and dried over Drierite. Removal of solvent and recrystallization from petroleum ether (bp 30–60°) yielded **9** (780 mg): mp 65–67°; nmr δ ($CDCl_3$, TMS) 2.23 (s, aryl Me's), 2.66 (broad m, [$N(CH_2CD_3)_2$]), 3.20 (s, $O=CCH_2N$), 7.07 ppm (s, aromatics); mass spectrum calcd for $C_{14}H_{16}N_2O(-d_6)$ 228.12625, found 228.12703; mass fragments *m/e* 220, 148, 132, 120, 105, 92.

Metabolic Studies with 9. A 4.8-kg Rhesus monkey was administered iv a total of 100 mg of **9** over 20 hr and aliquots of 36-hr urine were extracted as previously described using reagent grade Et_2O. Mass spectral analysis of **2** collected from glc showed that only three deuteriums were retained in the metabolite on the *N*-ethyl side chain: mass fragments *m/e* 235, 220, 160, 147, 132, 117, 105, 91, 88 (base peak), 70.

ω-[α-^{14}C-Diethylamino]-2,6-dimethylacetanilide (11). This compound was synthesized by the procedure described above for **9** from acetonitrile-^{14}C (Amersham-Searle) and redistilled Ac_2O. The product was recrystallized five times from petroleum ether (bp 30–60°) to constant specific activity and the radiochemical purity determined by glc and tlc (cyclohexane-$CHCl_3$-MeOH 70:20:10) with the aid of a radiochromatographic scanner. A final specific activity of 0.060 μCi/mg was obtained.

Metabolic Studies with 11. A 4.8-kg Rhesus monkey was administered iv a total of 100 mg of **11**, sp act. 0.060 μCi/mg, over 20 hr. The metabolites **2** and **3** were isolated from an aliquot of the 36-hr urine as previously described and collected from glc. Reinjection was used to verify their purity. Two independent methods were then used to determine the specific activity of the isolated metabolites. In the first method, **2** was taken up in 300 μl of pure EtOH and its uv spectrum determined at 264 nm. The absorbance obtained was compared to that obtained from the spectrum of a known concentration of an authentic sample. Having determined the concentration, we used a 100-μl aliquot for scintillation counting. The specific activity was determined and found to be 0.029 μCi/mg or ~49% that present in the originally administered lidocaine (**11**). In the second method, standard curves of known concentration of cyclic metabolites **2** and **3** *vs.* lidocaine were determined by glc. A known amount of lidocaine was added to 100-μl aliquots of each of the metabolites in pure EtOH, the samples were subjected to glc, and the concentration of each was determined from the standard curves. Another 100-μl aliquot was counted and again the specific activity in both cases was found to be 49% of that of the administered lidocaine (**11**).

Reaction of 4 with Acetaldehyde under Conditions of "Physiological" Concentration and pH. To 1 l. of Na phosphate buffer solution (pH 7.4) was added, with stirring, 1 ml of a 1 mg/ml solution of acetaldehyde in distilled H_2O followed 15 min later by 1 ml of a 1 mg/ml solution of **4** in phosphate buffer. The buffer solution was stirred for 4 hr at room temperature, followed by a 4-hr period of bubbling N_2 gas through the solution in order to remove as much of the remaining acetaldehyde as possible. The buffered reaction mixture was evaporated (rotovac) at room temperature under reduced pressure (~5 mm) to a volume of 50 ml and a 10-μl sample was subjected to glc. Glc analysis indicated 20% conversion of **4** to **2** based on standard curves of the two compounds on the Carbowax column used.

Study with EtOH-d_6. A 5.2-kg Rhesus monkey was infused iv with a 40-ml solution containing 10 ml of xylocaine (Astra) (10 mg/ml), plus 10 ml of 95% EtOH-d_6 (Stohler Isotope), plus 20 ml of normal saline. Infusion was maintained at 2 ml/hr for 20 hr. The total 36-hr urine was combined and extracted with aldehyde-free CH_2Cl_2 as previously described. Metabolite **2** was collected from glc and subjected to EI mass spectral analysis which showed peaks at *m/e* 232 (61), 233 (23), 234 (11.5), 235 (4.5), 236 (0). The following controls were used. (a) Two weeks before coadministration of EtOH-d_6 and lidocaine, the same Rhesus monkey was administered 100 mg of lidocaine by iv infusion in the usual manner. The total 36-hr urine was combined and extracted with aldehyde-free CH_2Cl_2 as described. Comparative glc analysis of the basic metabolites from EtOH-d_6 *vs.* control showed enhanced levels (~25 times) of **2** in the EtOH-d_6 study. (b) One week prior to infusion with EtOH-d_6, the same monkey was infused with 10 ml of unlabeled 95% ethanol in 30 ml of normal saline and 3 mg of **4** added to the urine collection pail. Urine was collected for 36 hr and extracted with aldehyde-free CH_2Cl_2 in the same manner. Comparative glc analysis of the basic metabolites from the EtOH-d_6 *vs.* control extract showed enhanced levels (~20 times) of **2** in the EtOH study, based on relative peak areas of **2** to **4**. This indicates that the enhanced levels of **2** found under conditions of ethanol administration do not arise from the collected urine.

Study with EtOH-*1*-^{14}C. A 4.7-kg Rhesus monkey was infused iv with a 40-ml solution containing 10 ml of xylocaine (Astra) (10 mg/ml), plus 10 ml of 95% EtOH containing 500 μCi of EtOH-*1*-^{14}C (NEN), and 20 ml of normal saline. The experimental and isolation conditions were the same as those used in the EtOH-d_6 study. The collected sample of **2** was taken up in 200 μl of pure EtOH and a 100-μl aliquot counted. A 10-μl aliquot was reinjected to indicate the chromatographic purity of the collected metabolite. Another 2 × 10 μl aliquot was cochromatographed on the Carbowax system with known concentrations of lidocaine to determine the concentration of **2** using a standard curve of peak area ratios. The specific activity of **2** was 1.81 μCi/mmol. The specific activity of the administered EtOH was 2.96 μCi/mmol. This indicates a 35–40% dilution of radioactive label in the metabolite **2**. The remaining sample was evaporated under a stream of N_2 and subjected to mass spectral analysis where the fragmentation was found to be identical with synthetic **2**.

Doubly Labeled 4. For future studies, 4 labeled randomly with tritium in the aromatic ring and specifically with ^{14}C in the carbonyl group was synthesized.

(a) Tritiated water, 2 ml (~500 μCi), was distilled twice *in vacuo* using a high vacuum manifold. To this was added freshly prepared platinum black (50 mg) and freshly distilled 2,6-xylidine (1.0 g, 8.2 mmol). Concentrated H_2SO_4 was added dropwise with stirring to solubilize the amine. The contents of the tube were frozen in liquid N_2 and the tube was sealed. The reaction was run for 36 hr with the sealed-glass tube encased in a metal tube immersed in an oil bath maintained at 90-95°. The dark purple reaction mixture was then refrozen in liquid N_2 and the sealed tube broken. Impurities were extracted with four 5-ml portions of CH_2Cl_2 and remaining contents basified with KOH pellets. The product was extracted with three 10-ml portions of CH_2Cl_2; combined extracts were washed with H_2O and filtered through Na_2SO_4. Solvent was removed under a stream of N_2 and product vacuum distilled at room temperature (5-10 μ) over 8 hr using the high vacuum manifold to give 785 mg of clear liquid, specific activity ~0.80 mCi/mmol.

(b) To tritiated 2,6-xylidine (700 mg, 5.8 mmol) in 40 ml of CH_2Cl_2 was added chloroacetic acid-*1*-^{14}C (Amersham-Searle, 550 mg, 5.8 mmol, sp act. 0.15 mCi/mmol). DCC (1.24 g, 6 mmol) in 10 ml of CH_2Cl_2 was added dropwise to the stirred solution over 20 min and the reaction stirred 3 hr at room temperature. White precipitate of dicyclohexylurea was filtered off and filtrate washed with 2 × 10 ml of 10% Na_2CO_3 and 10 ml of H_2O and dried (Na_2SO_4). Removal of solvent and recrystallization to constant specific activity from CCl_4 gave doubly labeled ω-chloro-2,6-dimethylacetanilide (698 mg, ^3H specific activity corrected for quenching and crossover using internal standards ~0.82 mCi/mmol, ^{14}C specific activity corrected for quenching and crossover using internal standards ~0.13 mCi/mmol).

(c) To doubly labeled ω-chloro-2,6-dimethylacetanilide (198 mg, 1 mmol) in 20 ml of C_6H_6 was added a 70% aqueous solution of ethylamine (161 mg, 2.5 mmol). After 3 days at 60° the reaction was washed with H_2O and extracted with 2 × 50 ml of 3 *N* HCl and the combined acid extracts were back-washed with 25 ml of CH_2Cl_2. Acid extract was basified (pH 11) with 20% KOH and back-extracted with 3 × 50 ml of CH_2Cl_2. Combined CH_2Cl_2 extracts were washed with 2 × 20 ml of H_2O and dried (Na_2SO_4). Evaporation of solvent gave a 175-mg crystalline mass of doubly labeled 4 which was recrystallized three times from hexane to constant sp act. 112 mg (^3H corrected sp act. 0.65 mCi/mmol, ^{14}C corrected sp act. 0.125 mCi/mmol). The purity of the product was confirmed by glc analysis and radiochromatographic analysis.

Reverse-Isotope Dilution Study with Doubly Labeled 4. To 10 ml of pH 7.4 phosphate buffer was added doubly labeled 4 (~10 μg, sp act. ^3H 0.65 mCi/mmol) and 50 μl of the resulting solution added to a 1-l. aliquot of a total 36-hr human urine sample from a normal male subject dosed orally with 500 mg of lidocaine. The sample was placed in a refrigerator (10°) for 12 hr. Extraction and isolation of metabolites were carried out as previously described using aldehyde-free CH_2Cl_2 and the Carbowax glc system. Metabolite 2 was collected and taken up in 1.5 ml of purified hexane, and its concentration was determined by uv absorbance at 264 nm. Solvent was then evaporated under a stream of N_2 and radioactivity determined by scintillation counting using the tritium channel. Specific activity found for 2 was 2.20 μCi/mmol. Metabolite 4 was collected and diluted to 2 ml in purified hexane. A 100-μl aliquot was counted using the tritium channel and 1.5-ml aliquot used for uv determination of concentration at 264 nm. Specific activity found for 4 was 1.90 μCi/mmol.

References

(1) G. D. Breck and W. F. Trager, *Science*, **173**, 544 (1971).
(2) (a) G. Hollunger, *Acta Pharmacol. Toxicol.*, **17**, 356 (1960); (b) *ibid.*, **17**, 365 (1960); (c) *ibid.*, **17**, 374 (1960); (d) *ibid.*, **17**, 384 (1960).
(3) A. H. Beckett, R. N. Boyes, and P. J. Appleton, *J. Pharm. Pharmacol.*, **18**, 76 S (1966).
(4) (a) J. B. Keenaghan and R. N. Boyes, *J. Pharmacol. Exp. Ther.*, **180**, 454 (1972); (b) J. M. Strong, M. Parker, and A. J. Atkinson, Jr., *Clin. Pharmacol. Exp. Ther.*, **14**, 67 (1973).
(5) (a) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, San Francisco, Calif., 1967, pp 297-299; (b) p 79 and Chapters 4 and 5.
(6) F. Kusada, M. Murayama, Y. Matsuo, H. Okumura, and N. Tatewaki, *Chem. Abstr.*, **66**, 115707r (1967).
(7) D. P. Schwartz and D. W. Parks, *Anal. Chem.*, **33**, 1396 (1961).
(8) R. E. McMahon, *J. Pharm. Sci.*, **55**, 457 (1966).
(9) (a) H. C. Carrington, A. F. Crowther, D. G. Davey, A. A. Levi, and F. L. Rose, *Nature (London)*, **168**, 1080 (1951); (b) A. F. Crowther and A. A. Levi, *Brit. J. Pharmacol.*, **8**, 93 (1953); (c) H. C. Carrington, A. F. Crowther, and G. J. Stacy, *J. Chem. Soc.*, 1017 (1954).
(10) B. B. Thompson, *J. Pharm. Sci.*, **57**, 715 (1968).
(11) G. Freund, *Neurology*, **23**, 91 (1973).
(12) (a) A. H. Beckett, J. M. VanDyke, H. H. Chissick, and J. W. Gorrod, *J. Pharm. Pharmacol.*, **23**, 812 (1971); (b) A. H. Beckett and S. Al-Sarraj, *ibid.*, **24**, 176 (1972).
(13) M. E. Daxenbichler, R. Klumin, D. Weisleder, C. H. Van Etter, and K. D. Carlson, *Tetrahedron Lett.*, 1801 (1972).
(14) E. A. Bell, J. R. Nulu, and C. Cone, *Phytochemistry*, **10**, 2191 (1971).
(15) G. Cohen and M. Collins, *Science*, **167**, 1749 (1970).
(16) V. E. Davis, M. J. Walsh, and Y. Yamanaka, *J. Pharmacol. Exp. Ther.*, **174**, 401 (1970).
(17) (a) P. Holtz, K. Stock, and E. Westermann, *Nature (London)*, **203**, 656 (1964); (b) *Arch. Pharmakol. Exp. Pathol.*, **248**, 387 (1964).
(18) (a) P. V. Halushka and P. C. Hoffman, *Biochem. Pharmacol.*, **17**, 1873 (1968); (b) W. M. McIssaac, *Biochem. Biophys. Acta*, **52**, 607 (1961).
(19) (a) P. V. Halushka and P. C. Hoffman, *Science*, **169**, 1104 (1969); (b) M. H. Seevers, *ibid.*, **170**, 1113 (1970).
(20) A. Goldstein and B. A. Judson, *ibid.*, **172**, 290 (1971).
(21) G. Cohen, *Biochem. Pharmacol.*, **20**, 1757 (1971).
(22) M. Sandler, S. B. Carter, K. R. Hunter, and G. M. Stern, *Nature (London)*, **241**, 439 (1973).
(23) (a) B. B. Levine, *Annu. Rev. Med.*, **17**, 23 (1966); (b) R. O. Neri, S. Tolksdorf, S. M. Berser, F. B. Erlanger, F. J. Agate, and S. Treberman, *Endocrinology*, **74**, 593 (1964); (c) D. H. Schmidt and V. P. Butler, *Program Amer. Soc. Clin. Invest.*, 74 (1969); (d) T. R. A. Davies and T. L. Goodfriend, *Amer. J. Physiol.*, **217**, 73 (1969); (e) D. E. Okan and T. U. L. Biber, *ibid.*, **214**, 791 (1968); (f) A. L. Misra, C. L. Mitchell, and L. A. Woods, *Nature (London)*, **232**, 48 (1971).
(24) N. Lofgren and B. Lundquist, *Svensk Kem. Tidskr.*, **58**, 206 (1946).
(25) N. Lofgren and G. Widmark, *ibid.*, **58**, 323 (1946).