the $F_3$, and loss of tails in the $F_4$ generation of Sprague–Dawley strain rats.[3]

Condensation of 7-methoxy-β-tetralone with ethyl cyanoacetate in the Guareschi reaction[2] gave II in low yield (20–35%) and the ammonium salt of II was obtained only after the addition of a large excess of ether to the reaction mixture. Attempted hydrolysis of II by the usual procedure, employing 60–70% sulfuric acid, to the corresponding *gem*-diacetic acid III gave largely decomposition and intractable tars. However, it was found that prolonged refluxing of the imide II with a large excess of concentrated hydrochloric acid smoothly hydrolyzed II to III in fair yield. The desired *gem*-diacetic acid III was converted to the corresponding anhydride IV, which was obtained as a glass; without further purification IV was converted directly to the imide V. The imide V was isolated in pure state by vacuum distillation. Reduction of V with lithium aluminum hydride in the usual manner gave the desired base VI, which was isolated as the dihydrochloride.

A comparison of the two compounds for inhibitory effects ($ED_{50}$ in μg/ml) on the growth of KB cells in culture gave the following results: I, <1.0, 2.8, <1.0, 0.63; VI, 2.5, 2.6. The inhibitory effects on the growth of this cell line were of the same order of magnitude, although I seems to be the more potent. The 7-methoxytetraline analog (VI) of I is at present undergoing tests to determine its effects, if any, on the fertility and offspring of Sprague–Dawley rats. These results will be published later.

### Experimental Section

Elemental microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside 77, N. Y., and the Microanalytical Laboratory of the National Institutes of Health. All melting points were determined on a Thomas–Hoover capillary-type melting point apparatus and are corrected.

**3,4-Dihydro-7-methoxynaphthalenespiro-2(1H),4′-piperidine-3′,5′-dicyano-2′,6′-dione (II).**—A solution of 125 g (0.68 mole) of 7-methoxy-β-tetralone, of 96.5% purity by a vapor phase chromatogram, in 160 g of ethyl cyanoacetate was prepared and cooled to 0°. This solution was mixed with 450 ml of absolute alcohol that had previously been saturated with anhydrous $NH_3$ at 0°. After the mixture had been allowed to stand in the refrigerator for 3 weeks at 5°, it was diluted with 1500 ml of anhydrous ether and allowed to stand an additional 2 days. The precipitate was filtered, washed with anhydrous ether, and dried. It was dissolved in boiling water (approximately 5 l.), filtered, and acidified with 400 ml of concentrated HCl. The mixture was placed in the refrigerator overnight and the precipitate was filtered, washed with water, and dried at 90° for several days. It weighed 41.5 g (20%). Recrystallization from ethyl acetate gave analytical material, mp 233.5–234.5°.

*Anal.* Calcd for $C_{17}H_{15}N_3O_3$: C, 66.01; H, 4.89; N, 13.59. Found: C, 66.26; H, 4.93; N, 13.68.

**7-Methoxytetralin-2,2-diacetic Acid (III).**—The imide II (25 g, 0.08 mole) was refluxed in a 5-l. flask with 1500 ml of concentrated HCl for 52 hr. After 36–40 hr of refluxing the imide had all dissolved and a clear solution was obtained. On cooling, a first crop of 11 g was obtained. The filtrate was concentrated to 400 ml and, on cooling, yielded an additional crop of 4 g. Both crops melted at 198–201°. The combined crops (15 g, 60%) were dissolved in $KHCO_3$ solution, decolorized three times with carbon and acidified with 10% HCl. The product (13 g) melted at 201–202°, unchanged on recrystallization from water.

*Anal.* Calcd for $C_{15}H_{19}O_5$: C, 64.74; H, 6.52. Found: C, 64.71; H, 6.43.

Examination of the infrared spectrum showed no OH absorption and indicated that the methoxyl group was intact.

**3,4-Dihydro-7-methoxynaphthalenespiro-2(1H),4′-piperidine-2′,6′-dione-1′-(3-dimethylaminopropyl) (V).**—The diacetic acid III (11 g, 0.04 mole) was refluxed for 5 min with 25 ml of acetic anhydride. The acetic anhydride was stripped off *in vacuo* and the crude anhydride cooled. It became a viscous glassy material. 3-Dimethylaminopropylamine (5 g) was added and the mixture refluxed until a clear melt was obtained. The melt was heated at 180° for 1 hr and vacuum distilled. The product was obtained as a glass, bp 226–233° (0.1 mm) (7 g, 51%) based on the diacid III.

*Anal.* Calcd for $C_{20}H_{28}N_2O_3$: C, 69.74; H, 8.19; N, 8.13. Found: C, 69.52; H, 8.47; N, 9.02.

The **imide methiodide** was formed in alcohol and melted at 206–208°, and at 211–213° after recrystallization from alcohol.

*Anal.* Calcd for $C_{21}H_{31}IN_2O_3$: I, 26.09. Found: I, 26.38.

**3,4-Dihydro-7-methoxynaphthalenespiro-2(1H),4′-piperidine-1′-(3-dimethylaminopropyl) Dihydrochloride (VI).**—The imide V (5 g, 0.014 mole), dissolved in 50 ml of tetrahydrofuran (THF), was added to a solution of 5 g of $LiAlH_4$ in 250 ml of THF and stirred and refluxed for 1 hr. After decomposition with water, filtering, and stripping the solvent, it was apparent that part of the product was contained in the inorganic cake. The cake was extracted twice with boiling ethyl acetate. These extracts and the orginal filtrate were combined and dried ($Na_2SO_4$). After filtering the $Na_2SO_4$, the addition of alcoholic HCl precipitated the product (4 g, 71%), mp 275–279°. Two recrystallizations from ethanol–methanol–ether raised the melting point to 284–287° dec.

*Anal.* Calcd for $C_{20}H_{34}Cl_2N_2O$: C, 61.68; H, 8.80; Cl, 18.21; N, 7.19. Found: C, 61.46; H, 8.85; Cl, 18.19; N, 7.39.

**Acknowledgment.**—The authors wish to thank Dr. Everette L. May of the National Institute of Arthritis and Metabolic Diseases for kindly furnishing the 7-methoxy-β-tetralone used in this investigation.

(3) C. F. Geschickter, 8th Annual Clinical Conference on Cancer, University of Texas, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, 1963.

## 1-Amino-2-(4-imidazolyl)cyclopropanecarboxylic Acid[1a]

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The inhibition of specific histidine decarboxylase,[2] or other less specific enzymes which decarboxylate histidine as a source of intracellular histamine,[3-5] has long been regarded as a potentially favorable alternative to the therapeutic competition with histamine by histamine antimetabolites. Among inhibitors of specific histidine decarboxylase, which have been found of particular interest, are α-hydrazinohistidine,[3] 4-bromo-3-hydroxybenzyloxyamine,[3] and α-methylhistidine.[5,6] It had previously been observed, in other pharmacodynamic types, that replacement of a metabolically

(1) (a) Supported in part by Grant GM-01-001882 from the Institute of General Medicine, National Institutes of Health, U. S. Public Health Service. (b) Du Pont Teaching Fellow, 1963–1964; NASA trainee, 1965–1966.

(2) R. J. Levine, T. L. Sourkes, and A. Sjoerdsma, *Biochem. Pharmacol.*, **14**, 139 (1965).

(3) R. W. Schayer, *J. Biol. Chem.*, **199**, 245 (1952).

(4) L. Kameswaran and G. B. West, *Intern. Arch. Allergy Appl. Immunol.*, **21**, 347 (1962).

(5) G. Kahlson, E. Rosengren, and R. Thunberg, *J. Physiol.* (London), **169**, 467 (1963).

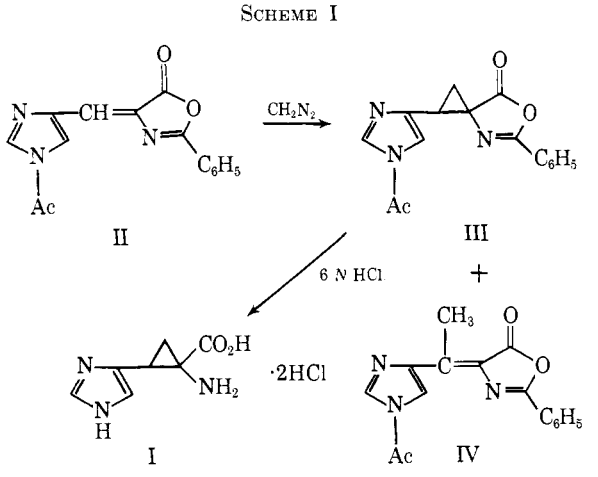
(6) B. Robinson and D. H. Shepherd, *J. Chem. Soc.*, 5037 (1961).

blocking methyl group by cyclopropyl[7,8] or ethynyl[9] groups alters the biochemical profile of the α-methyl-substituted compound in an interesting manner, and therefore the synthesis and evaluation of 1-amino-2-(4-imidazolyl)cyclopropanecarboxylic acid (I) was undertaken.

An attractive pathway to I appeared in the addition of diazomethane to 4-[(1-acetyl-4-imidazolyl)methylene]-2-phenyl-2-oxazolin-5-one (II) followed by hydrolytic cleavage of the oxazolone ring. The choice of this route was justified by the findings of Awad, *et al.*,[10] and of Mustafa, *et al.*,[11] that carbon-to-carbon double bonds exocyclic to certain hetero rings, including oxazolones, react with diazomethane to give cyclopropane derivatives. Moreover, Awad, *et al.*,[10] had cleaved the azlactone, 1,5-diphenyl-6-oxa-4-azaspiro-[2.4]hept-4-en-7-one, to 1-benzamido-2-phenylcyclopropanecarboxylic acid, and it appeared probable that the N-benzoyl group could be hydrolyzed under conditions which would not affect the cyclopropane ring. However, the formation of the spirooxazolone structures was by no means a foregone conclusion. In one case, *i.e.*, 4-ethylidene-2-phenyl-2-oxazolin-5-one, C-alkylation with resulting methylene insertion had been observed.[11] The considerable variation of structures around the reactive double bond did not permit elaboration of rules as to when one of these two types of reaction with diazomethane would be favored.

Compound II was treated with diazomethane in chloroform–ether and a colorless spirooxazolone (III) was obtained, the structure of which was verified by its analysis and spectra (see Scheme I). In addition to III, an equal amount of a yellow compound was obtained to which structure IV was assigned based on its spectral properties (see Experimental Section). The analogous case of the 4-ethylideneoxazolone mentioned above[11] exhibited a similar methylene insertion.

SCHEME I




Acid hydrolysis of III led directly to I. The structure of I was supported by its analytical and spectral data, particularly its nmr spectrum which is in agreement with three-membered ring systems,[10] revealing an ABX pattern in the region 2.0–3.5 ppm, with the observed constants $J_{AX} = J_{BX} = 9$ cps and $J_{AB} = 7.5$ cps. The stereochemistry of I has not been determined in the absence of a second isomer which would serve for comparison.[12]

Compound I, as the dihydrochloride, was assayed by Professor Victor H. Cohn for its ability to inhibit the activity of fetal rat liver histidine decarboxylase by the general procedure of Burckhalter,[13] except that histidine-$^{14}$COOH was used as a substrate and the evolution of $^{14}CO_2$ was measured. At $10^{-3}$ *M*, I inhibited the enzyme approximately 30%. This compares with an inhibition of approximately 50% by $10^{-5}$ *M* 4-bromo-3-hydroxybenzyloxyamine dihydrogen phosphate.[2] The amino acid I did not inhibit imidazole N-methyltransferase.

### Experimental Section

Melting points were taken in a capillary melting point apparatus preheated to about 15° below the reported values and are corrected. Infrared spectra were determined on a Perkin-Elmer Model 337 spectrophotometer as KBr pellets. Ultraviolet spectra were recorded on a Beckman DU spectrophotometer. Nmr spectra were run with a Varian A-60 spectrometer and are reported as parts per million units downfield from tetramethylsilane used as an internal standard unless indicated otherwise. Analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn.

**4-[(1-Acetyl-4-imidazolyl)methylene]-2-phenyl-2-oxazolin-5-one (II),** prepared by the method of Pyman,[14] had mp 188–190° (lit.[14] mp 191°); infrared, 1790, 1770, 1760, and 1740 ($\nu_{C=O}$ oxazolone and N-acetyl), 1660 cm$^{-1}$ ($\nu_{C=N}$ oxazolone); ultraviolet ($CH_2Cl_2$), 392 mμ (ε 20,900), 374 (28,700), 359 (sh) (23,300), 271 (17,700), and 247 (sh) (11,400); nmr ($CF_3CO_2H$), δ 9.50 (1 H, doublet, $J$ = 2 cps, imidazole H), 8.40 (1 H, doublet, $J$ = 2 cps, imidazole H), 7.85 (6 H, multiplet), 2.90 (3 H, singlet, NCOC$H_3$).

**Addition of Diazomethane to II.**—A solution of 25.7 g (0.0915 mole) of II in 2 l. of chloroform was treated with a dry solution of diazomethane in 2.5 l. of ether prepared from 48 g (0.466 mole) of N-nitroso-N-methylurea. The mixture was stirred for 4 hr at room temperature and filtered to yield 5.26 g (19%) of 4-[1-(1-acetyl-4-imidazolyl)ethylidene]-2-phenyl-2-oxazolin-5-one (IV), mp 223–228°. An analytical sample prepared by recrystallization from ethyl acetate consisted of yellow crystals: mp 230–232°; infrared, 1785 ($\nu_{C=O}$ oxazolone), 1730 ($\nu_{C=O}$ N-acetyl), 1635 cm$^{-1}$ ($\nu_{C=N}$ oxazolone); ultraviolet ($CH_2Cl_2$), 392 mμ (sh) (ε 17,800), 375 (25,200), 359 (sh) (21,900), 272 (19,900), 247 (sh) (11,700); nmr ($CF_3CO_2H$), δ 9.60 (1 H, doublet, $J$ = 2 cps, imidazole H), 8.46 (1 H, doublet, $J$ = 2 cps, imidazole H), 7.85 (5 H, multiplet), 2.77 (3 H, singlet, C$H_3$), 2.60 (3 H, singlet, C$H_3$).

*Anal.* Calcd for $C_{16}H_{13}N_3O_3$: C, 65.08; H, 4.44; N, 14.23. Found: C, 64.82; H, 4.49; N, 14.34.

(7) C. L. Zirkle, C. Kaiser, D. H. Tedeschi, R. E. Tedeschi, and A. Burger, *J. Med. Pharm. Chem.*, **5**, 1265 (1962).

(8) C. Kaiser and C. L. Zirkle, Belgian Patent 648,020 (1964); *Chem. Abstr.*, **63**, 14979*b* (1965).

(9) A. Burger, S. E. Zimmerman, and E. J. Ariëns, *J. Med. Chem.*, **9**, 469 (1966).

(10) W. I. Awad, A. K. Fateen, and M. A. Zayed, *Tetrahedron*, **20**, 891 (1964).

(11) A. Mustafa, W. Asker, A. H. Harhash, and A. M. Fleifel, *ibid.*, **21**, 2215 (1965).

(12) The Erlenmeyer–Plöchl oxazolone synthesis usually yields only one compound; in those cases in which both geometrical isomers have been synthesized, the less stable isomer could be transformed into the more stable one under mild conditions [H. E. Carter and W. C. Risser, *J. Biol. Chem.*, **139**, 255 (1941); R. E. Buckles, R. Filler, and L. Hilfman, *J. Org. Chem.*, **17**, 233 (1952); T. Kaneko, K. Oizumi, and H. Katsura, *Nippon Kagaku Zasshi*, **79**, 91 (1958); *Chem. Abstr.*, **54**, 5485*h* (1960); R. Filler, K. B. Rao, and Y. S. Rao, *J. Org. Chem.*, **27**, 1110 (1962)]. The second governing factor, the mechanism of the addition of diazomethane to the double bond and the stereochemistry of cyclopropane formation, is equally uncertain. The experiments of Awad, *et al.*,[10] and of Mustafa, *et al.*,[11] shed no light on this question, although it has been suggested that pyrazolines are probable intermediates[10] based on the isolation of such compounds when diazomethane reacts with other ethylenic derivatives [W. I. Awad, S. M. Abdel, R. Omran, and M. Sobhy, *J. Org. Chem.*, **26**, 4126 (1961)]. The formation of the same cyclopropane derivative III (and of IV) in the presence or absence of catalytic amounts of copper powder does not prove or disprove a carbene mechanism. Indeed, the results of the latter experiment may point to the operation of more than one mechanism (W. Kirmse, "Carbene Chemistry," Academic Press Inc., New York, N. Y., 1964, p 12).

(13) A. Burckhalter, *Biochem. Pharmacol.*, **11**, 315 (1962).

(14) F. L. Pyman, *J. Chem. Soc.*, **109**, 186 (1916).

The filtrate from IV was returned to the reaction flask and stirred overnight (~12 hr) at 26°. The clear yellow solution was concentrated *in vacuo* to 125 ml and cooled, and the crystals were filtered and washed with ether to give 5.75 g (21%) of nearly colorless 1-(1-acetyl-4-imidazolyl)-5-phenyl-6-oxa-4-azaspiro[2.4]hept-4-en-7-one (III), mp 167–168°. Recrystallization from ethyl acetate gave colorless material: mp 170.5–171°; infrared, 1815 ($\nu_{C=O}$ oxazolone), 1735 ($\nu_{C=O}$ N-acetyl), 1635 cm$^{-1}$ ($\nu_{C=N}$ oxazolone); ultraviolet ($CH_2Cl_2$), 263 mμ (ε 18,100); nmr ($CF_3CO_2H$), δ 9.48 (1 H, doublet, $J$ = 2 cps, imidazole H), 8.00 (6 H, multiplet, imidazole H and phenyl H), 3.25 (1 H, multiplet, cyclopropane H), 2.75 (5 H, singlet superimposed on a multiplet, $NCOCH_3$ and cyclopropane $CH_2$).

*Anal.* Calcd for $C_{15}H_{13}N_3O_3$: C, 65.08; H, 4.44. Found: C, 64.86; H, 4.43.

**1-Benzamido-2-(4-imidazolyl)cyclopropanecarboxylic acid** was obtained by alkaline hydrolysis of the azlactone III. A mixture of 1.35 g (4.6 mmoles) of III and 0.64 g (6 mmoles) of $Na_2CO_3$ in 10 ml of water was heated on a steam bath until a clear solution resulted (~15 min). The hot solution was treated with activated charcoal (Darco), filtered, and neutralized to pH 5 by dropwise addition of glacial acetic acid. On cooling at 4° for 48 hr and filtering, 0.5 g (40%) of product was obtained. Recrystallization from $H_2O$ and drying *in vacuo* over $P_2O_5$ gave colorless crystals, mp 263–264°.

*Anal.* Calcd for $C_{14}H_{13}N_3O_3$: C, 61.99; H, 4.83. Found: C, 61.82; H, 4.95.

**1-Amino-2-(4-imidazolyl)cyclopropanecarboxylic Acid (I) Dihydrochloride.**—A solution of 1.35 g of III in 30 ml of 18% HCl was refluxed for 12 hr. On cooling 0.3 g of benzoic acid (as shown by its infrared spectrum and mixture melting point) separated. This was removed by filtration and the filtrate was extracted with three 50-ml portions of benzene to yield an additional 0.15 g of benzoic acid (total 80%).

The yellow aqueous layer was concentrated to dryness *in vacuo* and the residue dissolved in the minimum amount of warm absolute methanol. The yellow solution was diluted with anhydrous acetone until it became turbid and then allowed to stand at −17° for 48 hr. The nearly colorless crystals were filtered, washed with acetone, and air dried to give 0.6 g (54%) of I. Careful recrystallization from methanol–acetone gave crystals of mp 192° after sintering and softening at 186°; nmr ($D_2O$), δ (relative to sodium 3-(trimethylsilyl)-1-propanesulfonate as internal standard) 8.93 (1 H, singlet, imidazole H), 7.74 (1 H, singlet, imidazole H), 3.30 (1 H, triplet, X portion of ABX system, $J_{AX}$ = $J_{BX}$ = 9 cps, cyclopropane H), 2.23 (2 H, eight-line multiplet, AB portion of ABX system, $J_{AB}$ = 7.5 cps, cyclopropane $CH_2$).

*Anal.* Calcd for $C_7H_{11}Cl_2N_3O_2$: C, 35.02; H, 4.62. Found: C, 34.99, 35.10; H, 4.90, 4.75.

**Acknowledgment.**—The authors are grateful to Professor Victor H. Cohn of the Department of Pharmacology, the George Washington University School of Medicine, Washington, D. C., for carrying out the enzyme inhibition tests.

## Central Nervous System Depressant Imides of Cyclobutanecarboxylic Acid[1a]

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Our research[2] on those aspects of picrotoxinin's structure which are important to its activity as a competitive antagonist at inhibitory synapses has made necessary the synthesis of various model lactones. Bridged lactones, especially those with a degree of internal strain as is characteristic of picrotin and picrotoxinin, are difficult to synthesize. One recent and reasonably promising route to compounds of the desired type involves the photolytic internal chlorination of N-chloro-N-acetylamides,[3] and N-chloroamides.[4,5] The products of this reaction may be converted to lactones by hydrolysis and cyclization. To test applicability of this scheme to the production of cogent lactones we synthesized the imides reported in Table I. These compounds were prepared by conventional routes which involved either the neat reaction of a suitable amide with excess acetylating agent[6] or acetylation of an amide in pyridine solvent. The former procedure always gave rise to varying quantities of nitrile, and reaction times in excess of 1 hr gave nitrile as major product. Work currently in progress indicates nitrile formation to involve intramolecular elimination by the product imide and not dehydration of the starting amide. The reaction is strongly subject to steric effects and is viewed as involving an enol-type four-centered transition state.

When the first four members of Table I were tested for acute toxicity and general biological activity N-acetylcyclobutanecarboxamide was found to exert an unusual sedative effect. This depressant action was peculiar to the derivative of cyclobutanecarboxylic acid, although several other imides in the series were quite toxic. In view of this apparent selectivity in action the several other imides in Table I were prepared and tested. In addition to **1** those showing central depressant properties were **7** and **10–12**. The only compound derived from cyclobutanecarboxylic acid which did not show the effect was **5**. This substance is also unique in having low toxicity, whereas the other derivatives of cyclopropanecarboxylic acid have $LD_{50}$ values of 250 mg/kg (**8**) and 350 mg/kg (**9**).

The depressant effect produced by these compounds was striking due to the speed with which it developed. Intraperitoneal doses of 300 mg/kg and higher caused loss of the righting reflex in from 30 to 60 sec. Lower doses produced an immediate and marked lowering of spontaneous activity. Preliminary pharmacodynamic data for these substances are given in Table II. The $LD_{50}$ for each of these compounds is so high that doses of 1000 mg/kg produced no deaths although the total period of depression usually exceeded 10 hr.

It is well known that imides are hydrolyzed with ease. In dealing with the biological activity of such compounds it is important to know whether they are serving only as a pro-drug. To test this possibility cyclobutanecarboxamide and cyclobutanecarboxylic acid, the hydrolysis products of dicyclobutanecarboximide, were evaluated for depressant properties. The amide was found to be active. However, even at a dose of 500 mg/kg the loss of spontaneous activity took 10 min to develop. For this reason the rate-limiting step in the response of the rapidly acting compounds is not associated with hydrolysis to the amide.

(1) (a) Supported in part by a research grant (MH 08348) from the National Institutes of Health, U. S. Public Health Service. (b) To whom inquiries should be directed.

(2) C. H. Jarboe and L. A. Porter, *J. Chromatog.*, **19**, 427 (1965).

(3) R. C. Petersen and A. Wambsgans, *J. Am. Chem. Soc.*, **86**, 1648 (1964).

(4) D. H. R. Barton, A. L. J. Beckwith, and A. Goosen, *J. Chem. Soc.*, 181 (1965).

(5) A. L. J. Beckwith and J. E. Goodrich, *Australian J. Chem.*, **18**, 747 (1965).

(6) P. Dunn, E. A. Parkes, and J. B. Polya, *Rec. Trav. Chim.*, **71**, 675 (1952).