[CONTRIBUTION FROM THE ORGANIC CHEMISTRY DEPARTMENT, RESEARCH DIVISION, ABBOTT LABORATORIES]

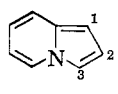
1-Indolizinealanine—A Possible Tryptophan Antimetabolite

JOHN A. CARBON AND SANDRA BREHM

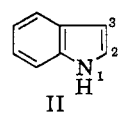
Received March 1, 1961

The title compound was prepared as a potential antimetabolite of tryptophan. 3-Acetylindolizine (III) readily underwent the Mannich reaction to form 3-acetyl-1-dimethylaminomethylindolizine (V). Conversion of V to the methiodide (VI), followed by alkylation of sodio ethyl acetamidocyanoacetate gave either 3-acetyl-2′-acetamido-1-indolizinepropionitrile (VIII) or ethyl 3-acetyl-2′-acetamido-2′-cyano-1-indolizinepropionate (IX), depending on the reaction conditions. Basic hydrolyses of either VIII or IX gave the 3-acetylamino acid (X), which was converted to the desired compound, 1-indolizinealanine (XI), by acid hydrolysis.

The preparation of analogs of the biologically important indole compounds in which the indole ring has been replaced by the indolizine[1] ring system (I) could conceivably give rise to compounds with potent pharmacological activity.[2] The indole (II) and indolizine (I) systems are alike in respects other than the obvious steric considerations. Positions 3 and 1 of the indolizine ring are positions of high electron density and are readily attacked by electrophilic reagents,[3] while it is well known that the 3-position of indole is also attacked by electrophilic groups with ease.[4] We have therefore prepared the tryptophan analog, 1-indolizinealanine (XI), as a possible antimetabolite of normal tryptophan or tryptamine metabolism.



I



II

Any attempt to build up an amino acid such as XI by substitution on the 1-position of indolizine (I) must be prefaced by the introduction of a suitable blocking group onto the more electron-rich 3-position, where electrophilic substitution first occurs.[3,5] A satisfactory blocking group was found in the acetyl group, which can be introduced into the 3-position merely by treating I with acetic anhydride and anhydrous sodium acetate,[6] and which can later be removed simply by hydrolysis in hot hydrochloric acid.[3,7] This method offers the added advantage that 3-acylindolizines are quite stable under highly alkaline conditions,[6] thus permitting alkaline hydrolyses on other portions of the molecule without removing the 3-acetyl function.

It was planned to build up the amino acid side chain by first forming the Mannich base (V), and converting this compound to the quaternary salt (VI), a substance which could conceivably serve to alkylate[8] sodio ethyl acetamidocyanoacetate to form IX. Suitable hydrolyses of IX should then give the desired amino acids (X and XI).

Treatment of 3-acetylindolizine (III) with dimethylamine and formaldehyde in glacial acetic acid gave an excellent yield of a single crystalline Mannich base (V). Although we were reasonably certain that the dimethylaminomethyl group had entered the 1-position of the ring,[9] it was necessary to prove this point as the methyl group in III could also serve as the reaction site. The absence of an unsubstituted 1 or 3 position in V was apparent, however, as this compound did not give a color with either *p*-dimethylaminobenzaldehyde or potassium iodate in acid solution,[10] while 3-acetylindolizine (III) gives strongly positive tests with these reagents. Furthermore, it is evident that the acetyl group remains unsubstituted since it can later be removed by acid hydrolysis without disrupting the amino acid side chain. The reaction of 3-acetylindolizine (III) with formaldehyde in glacial acetic acid gave 1,1′-methylenebis(3-acetylindolizine) (IV) as the main product. This same compound (IV) along with a small quantity of 3-acetyl-1-indolizinemethanol (VII) was also isolated

(1) *Indolizine* is the currently accepted Chemical Abstracts nomenclature for the pyrrolo[1,2-*a*]pyridine (I) ring system. However, the alternate name, *pyrrocoline*, is perhaps better known and was in fact preferred until 1957.

(2) This possibility was first suggested by Dr. James M. Price, University of Wisconsin Medical School. The biological studies with these compounds will be reported by Dr. Price's group elsewhere.

(3) E. T. Borrows and D. O. Holland, *Chem. Revs.*, **42**, 611 (1948).

(4) W. C. Sumpter and F. M. Miller, *Heterocyclic Compounds with Indole and Carbazole Systems*, Interscience, New York, 1954; P. L. Julian, E. W. Meyer, and H. C. Printy, in *Heterocyclic Compounds*, R. C. Elderfield, ed., Wiley, New York, 1952, Vol. 3, pp. 1–274.

(5) E. T. Borrows, D. O. Holland, and J. Kenyon, *Chem. Soc.*, 1069, 1075, 1077, 1083 (1946).

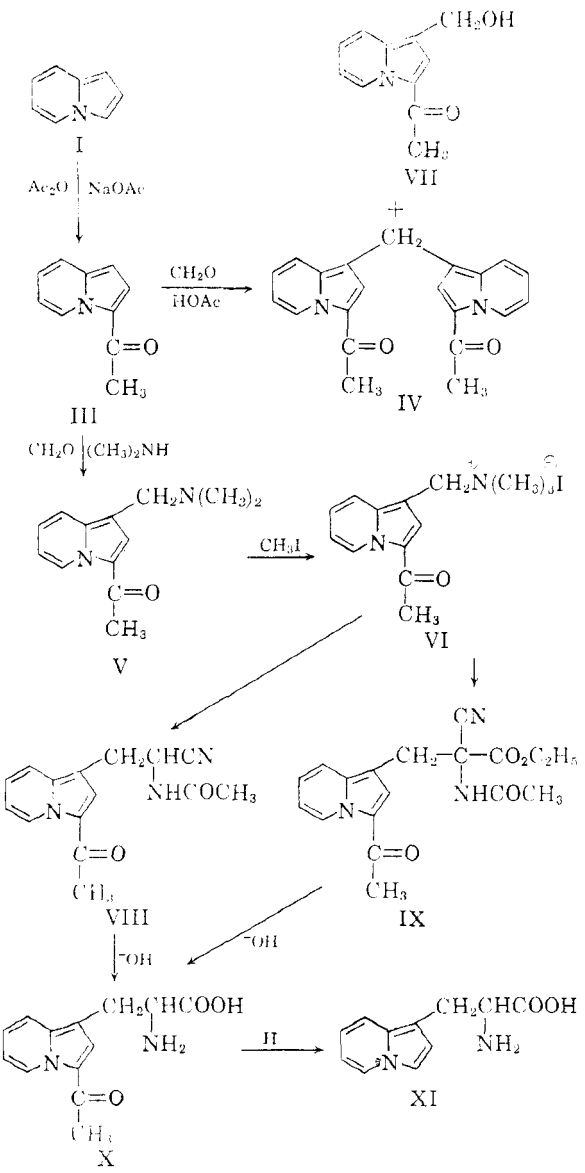
(6) M. Scholtz, *Ber.*, **45**, 1718 (1912).

(7) M. Scholtz, *Ber.*, **45**, 734 (1912); *Arch. Pharm.*, **251**, 266 (1913).

(8) The subject of carbon-carbon alkylations with amines and quaternary salts has been reviewed by J. H. Brewster and E. F. Eliel, *Organic Reactions*, **7**, 99 (1953).

(9) E. D. Rossiter and J. E. Saxton, *J. Chem. Soc.*, 3654 (1953) have prepared 1-dimethylaminomethyl-2,3-dimethylindolizine by a Mannich reaction on 2,3-dimethylindolizine. The quaternary salt from this base also served as an excellent alkylating agent.

(10) D. O. Holland and J. H. C. Nayler, *J. Chem. Soc.*, 1657 (1955).



when less than the theoretical quantity of dimethylamine was used in the Mannich step.

The preparation of the methiodide (VI) proceeded in virtually quantitative yield in absolute ethanol containing an excess of methyl iodide. Initial attempts to alkylate diethyl formamidomalonate in ethanolic sodium ethoxide with this material (VI) gave only oils which could not be purified, and which gave no detectable amino acids when hydrolyzed. However, the alkylation of sodio ethyl acetamidocyanoacetate in absolute ethanol gave a crystalline product, identified as the decarbethoxylated compound, 3-acetyl-2′-acetamido-1-indolizinepropionitrile (VIII). Base catalyzed decarbethoxylation of ethyl malonate or ethyl cyanoacetate derivatives has previously been observed when ethanolic sodium ethoxide is employed as the reaction medium.[11] However, when anhydrous *N*,*N*-dimethylformamide was employed as the solvent, the alkylation proceeded normally and in satisfactory yield to form ethyl 3 - acetyl - 2′ - acetamido - 2′ - cyano - 1 - indolizinepropionate (IX). Both of the alkylation products, VIII and IX, gave negative tests with *p*-dimethylaminobenzaldehyde or with potassium iodate,[10] thus establishing the absence of an unsubstituted 1 or 3 position. The infrared spectra were also compatible with the assigned structures.

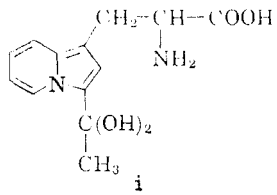
Although it should be possible theoretically to convert VIII or IX directly to the desired amino acid (XI) by acid-catalyzed hydrolysis and decarboxylation, attempts along these lines gave only intensely colored deep blue solutions, from which no pure compounds could be isolated. Base-catalyzed hydrolyses of either compound, however, resulted in a smooth conversion to the 3-acetylamino acid (X). This compound was obtained as a pale yellow to greenish solid from water, which gave a satisfactory analysis for the monohydrate of X. This water proved to be bound very tenaciously, since it could not be removed even by heating a sample at 140° at 0.05 mm. for twenty-four hours. The assigned structure (X) is considered correct, however, since the substance gave an ultraviolet absorption spectra typical of a 3-acetyl-1-substituted indolizine[12]; gave negative *p*-dimethylaminobenzaldehyde and potassium iodate tests[10]; and was converted to the desired amino acid (XI) by acid hydrolysis.[13] The presence of the indolizine ring system in X was also shown by treatment with nitric acid in glacial acetic acid at 100° to give a small yield of 1,3-dinitroindolizine, a known compound.[6]

The tryptophan analog, 1-indolizinealanine (XI), was obtained by hydrolysis of the 3-acetyl compound (X) in boiling 10% hydrochloric acid. The isolation and purification of XI are complicated by the fact that the acid treatment results in the formation of a deep blue dye which is exceedingly difficult to separate from the product. Our best samples of the free amino acid (XI) are colored a pale bluish green. In contrast to the 3-acetyl derivative (X), compound XI gives positive

(11) For examples see A. C. Cope, H. L. Holmes, and H. O. House, *Org. Reactions*, 9, 127 (1957).

(12) All of the 3-acetylindolizines prepared in this work gave ultraviolet absorption maxima at 360–370 mμ, 262–266 mμ, and 225–227 mμ. Also see ref. 3, p. 635.

(13) The possibility that compound X actually exists as the *gem*-diol (i) has been considered. The infrared spectrum does not give a conclusive answer to this question since the



i

area where 3-indolizinyl ketones normally absorb (6.1–6.2 μ) is covered by the typical amino acid absorptions.

p-methylaminobenzaldehyde and potassium iodate tests,[10] indicating a free 1 or 3 position. The ultraviolet absorption spectrum of XI is typical of a 1-substituted indolizine (see Experimental).

EXPERIMENTAL[14]

Indolizine (pyrrocoline) (I) was prepared both by the acid hydrolysis of 1,3-diacetylindolizine[7,15] and by the catalytic dehydrogenation and cyclization of 3-(2-pyridyl)-1-propanol.[16] The latter method was preferable although we were consistently unable to achieve the reported yield (50%).[16]

3-Acetylindolizine (III) was obtained by the treatment of I with acetic anhydride and anhydrous sodium acetate as directed by Scholtz.[6] The product was a very highly refractive yellow oil (n_D^{25} above 1.70) which crystallized when chilled. Recrystallization from petroleum ether (b.p. 60–68°) gave pale yellow prisms, m.p. 38.0–38.5° (Scholtz[6] apparently did not obtain this compound in the crystalline state). This material gave highly colored solutions with *p*-dimethylaminobenzaldehyde or potassium iodate in aqueous acid.[10] The carbonyl absorption in the infrared occurred at 6.2 μ.

3-Acetyl-1-dimethylaminomethylindolizine (V). A mixture of 16.9 g. (0.15 mole) of 40% aqueous dimethylamine, 15 ml. of glacial acetic acid, and 12.2 g. (0.15 mole) of formalin (37%) was prepared at 5°. There was added, in one portion, 15.9 g. (0.10 mole) of 3-acetylindolizine (III), and the resulting mixture was allowed to stand with occasional stirring at room temperature for 24 hr. The clear yellow solution was poured with stirring into a solution of 14 g. of sodium hydroxide in 100 ml. of water causing the separation of a pale yellow oil which slowly solidified on standing. The mixture was filtered with suction to give 21.2 g. of product, m.p. 72–73°. Recrystallization from petroleum ether (b.p. 60–68°) gave 19.4 g. (89.8%) of large colorless prisms, m.p. 73.0–73.5°.

Anal. Calcd. for $C_{13}H_{16}N_2O$: C, 72.18; H, 7.46; N, 12.95. Found: C, 71.97; H, 7.39; N, 12.78.

This compound did not give a color with acidic *p*-dimethylaminobenzaldehyde or potassium iodate,[10] indicating the lack of a free 1 or 3 position. The carbonyl absorption in the infrared occurred at 6.2 μ. The ultraviolet maxima were at 365, 262, and 227 mμ.

In a similar experiment, in which only 73% of the theoretical amount of dimethylamine was used, the crude product did not crystallize. The oil was extracted into ether and the ether extracts dried over anhydrous potassium carbonate. After evaporation of the ether, the residual oil was distilled *in vacuo* to give three fractions (1) b.p. 106–121° at 0.25 mm., $n_D^{25} > 1.70$; (2) b.p. 121–132° at 0.25–0.15 mm., n_D^{25} 1.6425; (3) b.p. 132–137° at 0.15 mm, n_D^{25} 1.6292; plus a dark solid residue. Fraction 1 was identified as mainly 3-acetylindolizine (III) by the infrared spectrum. Fraction 3, which crystallized on standing, consisted mainly of the desired product, 3-acetyl-1-dimethylaminomethylindolizine (V) (m.p. 73°), plus a small quantity (5%) of a compound insoluble in hot petroleum ether (b.p. 60–68°). This compound could be obtained as colorless needles from water, m.p. 131–133°, and was identified as *3-acetyl-1-indolizinemethanol* (VII). The ultraviolet absorption maxima occurred at 370, 264, and 227 mμ.

Anal. Calcd. for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40; O, 16.91. Found: C, 69.55; H, 5.81; N, 7 71; O, 17.08.

The residue from the distillation was washed with ethanol, and the resulting tan solid recrystallized from Methyl Cellosolve containing a drop of 2*N* sodium hydroxide to obtain almost colorless scales, m.p. 217–219° (dec.). It was necessary to recrystallize this compound from alkaline media to avoid the formation of violet dyes. This material was identified as *1,1'-methylenebis(3-acetylindolizine)* (IV) (see below).

Anal. Calcd. for $C_{21}H_{18}N_2O_2$: C, 76.34; H, 5.49; N, 8.48. Found: C, 76.17; H, 5.67; N, 8.42.

This same material (IV) was readily prepared by allowing a mixture of 4.77 g. (0.030 mole) of 3-acetylindolizine (III), 3.6 g. (0.045 mole) of formalin (37%), and 6 ml. of glacial acetic acid to stand overnight at room temperature. The mixture, containing a yellow precipitate, was poured into 35 ml. of water and the product filtered and washed with water. The air-dried product weighed 3.5 g. (70%), m.p. 217–219° (dec.). Recrystallization from Methyl Cellosolve did not change the decomposition point.

(3-Acetyl-1-indolizinylmethyl)trimethylammonium iodide (VI). A solution of 18.6 g. (0.086 mole) of 3-acetyl-1-dimethylaminomethylindolizine (V) and 12.8 g. (0.090 mole) of methyl iodide in 200 ml. of absolute ethanol was allowed to stand at room temperature for 24 hr. The colorless crystalline product was isolated by suction filtration and washed with ethanol. After air drying the product weighed 30.6 g. (99.3%), m.p. 249–255° dec., and turned a deep violet color above 170°. This material was analytically pure, but could be recrystallized from water to obtain colorless needles with the same melting behavior. The absorption maxima in the ultraviolet were at 360, 262, and 227 mμ.

Anal. Calcd. for $C_{14}H_{19}IN_2O$: C, 46.94; H, 5.35; N, 7.82. Found: C, 47.03; H, 5.62; N, 7.88.

3-Acetyl-2'-acetamido-1-indolizinepropionitrile (VIII). To a solution of 0.21 g. (0.0092 g.-atom) of sodium in 35 ml. of absolute ethanol was added 1.56 g. (0.0092 mole) of ethyl acetamidocyanoacetate and 3.0 g. (0.0084 mole) of (3-acetyl-1-indolizinylmethyl)trimethylammonium iodide (VI), and the resulting mixture was refluxed under a slow stream of dry nitrogen for 18 hr. Trimethylamine was continuously evolved during this period. The clear brown solution was evaporated to dryness *in vacuo*, and the residual oil treated with 50 ml. of water. After standing for 4 days at room temperature the oil had solidified to a brown amorphous solid. This material was recrystallized from methanol to obtain 0.60 g. (27%) of tan needles, m.p. 189–191°. An analytical sample was obtained as clumps of tiny needles from methanol, m.p. 192.5–193.0°.

Anal. Calcd. for $C_{15}H_{15}N_3O_2$: C, 66.89; H, 5.61; N, 15.60; O, 11.88. Found: C, 66.80; H, 5.34; N, 15.68; O, 11.71.

Ethyl 3-acetyl-2'-acetamido-2'-cyano-1-indolizinepropionate (IX). To a suspension of 2.29 g. (0.054 mole) of sodium hydride (56.5% suspension in mineral oil) in 90 ml. of anhydrous, redistilled *N,N*-dimethylformamide was carefully added with stirring 8.5 g. (0.050 mole) of dry ethyl acetamidocyanoacetate. After hydrogen evolution had ceased, 16.1 g. (0.045 mole) of the quaternary salt (VI) was added, and the stirred mixture was heated at 110–120° under a stream of dry nitrogen for 2.5 hr. Most of the *N,N*-dimethylformamide was removed *in vacuo*, and the oily residue was treated with 100 ml. of water. The resulting gummy oil, which solidified on standing several hours, was filtered, washed with water, and dried *in vacuo* at 55°. Recrystallization of this crude product (12.9 g.) from ethyl acetate gave 9.5 g. (62%) of a tan solid, m.p. 175–177°. An analytical sample was obtained as colorless leaflets from ethyl acetate, m.p. 179–180°.

Anal. Calcd. for $C_{18}H_{19}N_3O_4$: C, 63.32; H, 5.61; N, 12.31; O, 18.76. Found: C, 63.20; H, 5.34; N, 12.37; O, 19.01.

3-Acetyl-1-indolizinealanine (X). One gram (2.9 mmoles) of IX was mixed with 10 ml. of 10% sodium hydroxide solution, and refluxed gently for 16 hr. Ammonia was evolved during most of the reflux period. The clear brown solution was neutralized to *p*H 4–5 with glacial acetic acid at 50–60°,

(14) The melting points are uncorrected and were taken on a Fisher-Johns melting point apparatus unless stated otherwise in the Experimental.

(15) A. E. Chichibabin and F. N. Stepanow, *Ber.*, **62**, 1068 (1929).

(16) V. Boekelheide and R. J. Windgassen, Jr., *J. Am. Chem. Soc.*, **81**, 1456 (1959).

filtered quickly to remove silicates, and the filtrate cooled to 5°. The product slowly separated as a yellow curdy precipitate which quickly turned blue-green on exposure to the air. This material was filtered with suction and washed with water and ethanol to obtain 0.63 g. (82%) of a pale bluish green solid, with no definite melting point (decomposed gradually above 200°). For analysis a sample was recrystallized from water to obtain bluish needles, gradual decomposition above 200°. The sample was dried at 100° *in vacuo* for 48 hr. before analysis.

Anal. Calcd. for $C_{13}H_{14}N_2O_3 \cdot H_2O$: C, 59.07; H, 6.10; N, 10.60. Found: C, 59.04; H, 6.14; N, 10.64.

The analysis did not change significantly upon drying the sample at 140° at 0.05 mm. for 24 hr.

This compound gave negative tests with *p*-dimethylaminobenzaldehyde or potassium iodate,[10] indicating a lack of free 1 or 3 positions. The ultraviolet spectra was typical of a 3-acetylindolizine (peaks at 372, 264, and 226 mμ). The infrared spectrum (Nujol mull) was compatible with structure X.[13]

3-Acetyl-1-indolizinealanine (X) formed a sparingly soluble *monohydrochloride* when treated with 10% hydrochloric acid at room temperature. This salt was extremely difficult to purify, however, readily decomposing to deep blue solutions when dissolved in water. The best sample was obtained by dissolving in warm water, adding 2 drops of 2*N* hydrochloric acid, and then adding several volumes of acetone to precipitate bluish prisms, m.p. 265–267° dec. (cap.).

Anal. Calcd. for $C_{13}H_{15}ClN_2O_3$: Cl, 12.54; N, 9.91. Found: Cl, 12.23; N, 9.44.

The presence of the intact indolizine ring system in X was also shown by conversion to the known compound, *1,3-dinitroindolizine*.[6] A suspension of 0.50 g. of 3-acetyl-1-indolizinealanine (X) in 5 ml. of glacial acetic acid was treated cautiously with 3 ml. of concd. nitric acid (sp. gr. 1.42). The clear, dark yellow solution was heated on the steam bath for 1 hr., evaporated *in vacuo* until most of the acetic acid was removed, and then treated with 7 ml. of water. The bright yellow precipitate (0.1 g.) was isolated by suction filtration and washed with water. Recrystallization of this material (m.p. 230–231°) from Methyl Cellosolve gave yellow leaflets, m.p. 232–233°. A mixed melting point with an authentic sample of 1,3-dinitroindolizine[5] (m.p. 234–235°) was undepressed. Scholtz[6] reported m.p. 229° for this material.

1-Indolizinealanine (XI). 3-Acetyl-1-indolizinealanine (X) (11.4 g.; 0.0432 mole) was mixed with 115 ml. of 10% hydrochloric acid and heated under reflux for 20 hr. (nitrogen atmosphere). The deep blue solution was cooled, neutralized to *p*H 8–9 with ammonium hydroxide with cooling, and evaporated the solution (now dark yellow) to a small volume *in vacuo*. After chilling to 5°, the pale yellow solid was isolated by suction filtration and washed thoroughly with ice water to remove ammonium chloride. The solid thus obtained weighed 6.41 g. (72.9%), m.p. (cap.) 233–235° (dec.). This material was very difficult to handle, since it quickly turned deep bluish green upon exposure to the air and light. Attempts to recrystallize the material from water gave only blue solutions from which the amino acid was difficult to recover.

This compound is best characterized and purified as the *dihydrochloride* salt. Two-tenths gram was dissolved in a minimum quantity of warm water and 10 drops of concentrated hydrochloric acid added. The deep green solution was treated with 6 volumes of absolute ethanol, and the precipitated solid purified by reprecipitation from dilute hydrochloric acid with ethanol. The dihydrochloride was thus obtained as bluish green prisms, m.p. (cap.) 240–243° dec.

Anal. Calcd. for $C_{11}H_{14}Cl_2N_2O_2$: C, 47.66; H, 5.09; Cl, 25.58. Found: C, 47.41; H, 5.14; Cl, 25.28.

Compound XI gave strongly positive tests with both acidic *p*-dimethylaminobenzaldehyde and potassium iodate, indicating an unsubstituted 1 or 3 position.[10] The ultraviolet spectrum was typical of that of a simple alkyl-substituted indolizine,[3] showing maxima at 296, 285, and 233 mμ in neutral or basic solutions, with a shift in acid to maxima at 307 and 235 mμ with strong end absorption. The infrared spectrum (Nujol mull) was that of a typical α-amino acid.

Acknowledgment. The authors are indebted to Mr. E. F. Shelberg and his staff for the microanalyses, to Mr. F. Chadde for the ultraviolet absorption spectra, and to Mr. W. Washburn and his staff for the infrared absorption spectra.

NORTH CHICAGO, ILL.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF PITTSBURGH]

The Chemistry of Pyrazine and Its Derivatives. IV. The Alkylation and Arylation of Methylpyrazine[1]

JOHN D. BEHUN[2] AND ROBERT LEVINE

Received February 24, 1961

Methylpyrazine has been alkylated at its side chain with a series of alkyl halides and benzyl chloride and arylated with bromobenzene using the sodium amide-liquid ammonia method. It has also been alkylated with benzyl alcohol using potassium hydroxide as the condensing agent. The alkylpyrazines have been reduced to 2-alkylpiperazines, which have been converted to the corresponding bis(benzenesulfonamides).

In the earlier papers in this series, we reported that pyrazylmethylsodium, prepared from methylpyrazine and sodium amide in liquid ammonia, can be acylated with a series of esters to give a variety of pyrazylmethyl ketones,[3] $PzCH_2COR$, and condensed with several aldehydes and ketones to give the corresponding pyrazylmethylcarbinols, $PzCH_2C(OH)RR'$.[4]

(1) This work was performed under Contract No. AT-(30-1)-670 between the U. S. Atomic Energy Commission and the University of Pittsburgh.

(2) This paper is based on part of the thesis presented by J. D. Behun to the Graduate Faculty of the University of Pittsburgh in partial fulfillment of the requirements for the Ph.D. degree.

(3) J. D. Behun and R. Levine, *J. Am. Chem. Soc.*, **81**, 5157 (1959).