Synthesis of Potential Antimalarial Agents. IV. (1)

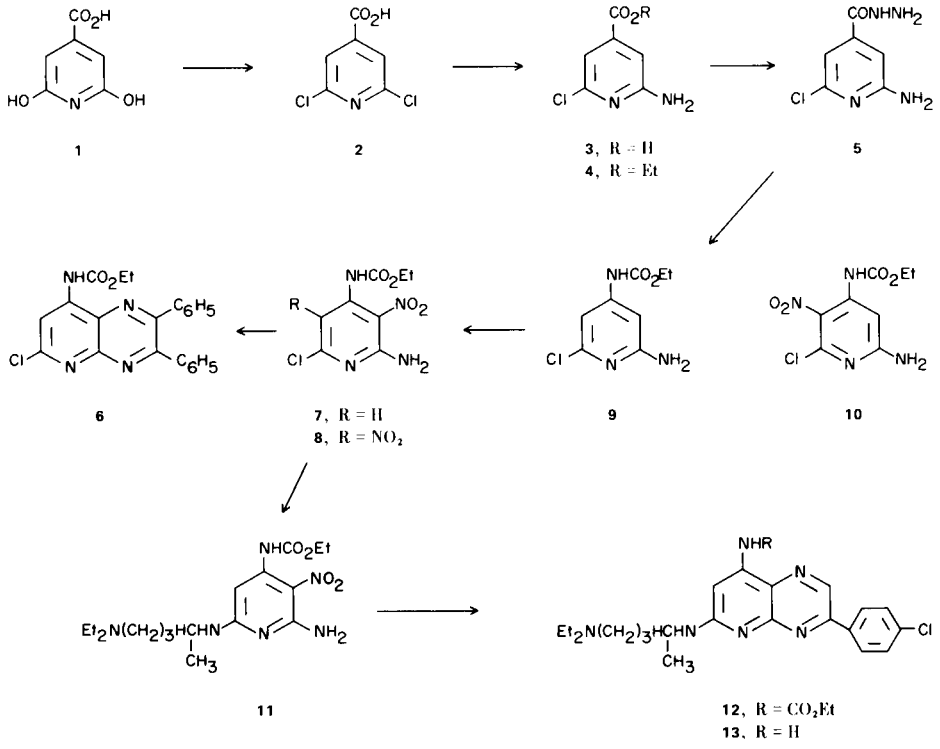
The Preparation of 8-Amino-3-(*p*-chlorophenyl)-6-[[4-(diethylamino)-1-methylbutyl]amino]pyrido[2,3-*b*]pyrazine

Carroll Temple, Jr., Robert D. Elliot, Jerry D. Rose, and John A. Montgomery

Kettering-Meyer Laboratory, Southern Research Institute

As part of our study of synthetic routes to pyridopyrazines (2), we wished to prepare 8-amino-3-(*p*-chlorophenyl)-6-[[4-(diethylamino)-1-methylbutyl]amino]-pyrido[2,3-*b*]pyrazine (**13**) to compare its antimalarial activity with that of the active, isomeric 6-amino-8-[[4-(diethylamino)-1-methylbutyl]amino] compound (2a.) In this paper we report the preparation and proof of structure of ethyl 2-amino-6-chloro-3-nitro-4-pyridinecarbamate (**7**), an intermediate from which the desired pyridopyrazine was obtained.

The chlorodehydroxylation of citrazinic acid (**1**) was carried out with phosphorus oxychloride in the presence of 2,6-lutidine to give the 2,6-dichloropyridine **2** (3). The amination of **2** with concentrated ammonium hydroxide at 200° gave the 2-amino-6-chloropyridine **3** (4), which was esterified with ethanolic hydrogen chloride to give the corresponding ethyl ester **4**. Hydrazinolysis of **4** with hot 95% hydrazine gave the acid hydrazide **5**. Nitrosation of **5** with isoamyl nitrite in ethanolic hydrogen chloride followed by *in situ* rearrangement of the acid azide gave a 49% yield of the 4-pyridinecarbamate **9**. This reaction also gave a 13% yield of the ethyl ester **4**, which probably was formed by the ethoxydeazidoation of the intermediate acid azide (5). Nitration of **9** in 96% sulfuric acid with approximately two molar equivalents of 70% nitric acid gave the 3,5-dinitropyridine **8**. With equimolar amounts of **9** and 70% nitric acid, however, a mononitropyridine was obtained in 77% yield. The proton magnetic resonance (pmr) spectrum indicated that this product was homogeneous and not a mixture of **7** and **10**. The structure of this pyridine was shown to be **7** by reduction of the nitro group, and the condensation of the resulting 2,3-diaminopyridine with benzil to give **6**. Since the nitration of 2-aminopyridine gave a mixture of 2-amino-3-nitro- and 2-amino-5-nitropyridines with the latter predominating (6), the formation of the 2-amino-3-nitropyridine **7** is



attributed to the greater inductive deactivation of the 5-position of **9** by the 6-chloro group than of the 3-position.

Reaction of **7** with 2-amino-5-diethylaminopentane replaced the chloro group to give **11**. Reduction of the nitro group of **11** with Raney nickel and condensation of the resulting 2,3-diamino-4-pyridinecarbamate with *p*-chlorophenylglyoxal gave a homogeneous sample of **12** (tlc). The assignment of the *p*-chlorophenyl group of this product to the 3- rather than the 2-position was based on the previous observation that the aldehyde moiety of a phenyl glyoxal preferentially reacts with the 3- rather than the 2-amino group of a 2,3-diaminopyridine (2b,7). In addition only one isomer was detected in the pmr spectrum of this product. The urethane group of **12** was cleaved with ethanolic potassium hydroxide to give the corresponding amino compound **13**. The pmr spectrum also indicated that this product was homogeneous.

EXPERIMENTAL

Melting points were determined on either a Kofler Heizbank or Mel-Temp apparatus and are corrected. The ultraviolet abosrption spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer, whereas the infrared absorption spectra were determined in pressed potassium bromide disks with a Perkin-Elmer Model 521 spectrophotometer. The pmr spectra were determined in deuterated DMSO with a Varian A-60A spectrometer at a probe temperature of about 40° using tetramethylsilane as an internal reference. The relative peak areas are given to the nearest whole number. Thin-layer chromatograms were prepared from silica gel H (Brinkmann) and were usually developed with mixtures of chloroform and methanol.

2,6-Dichloroisonicotinic Acid (**2**).

A stirred mixture of **1** (155 g., 1.00 mole) and phosphorus oxychloride (459 g., 3.00 moles) was heated under nitrogen in an oil bath at 93° for 5 minutes. The oil bath was removed and 2,6-lutidine (214 g., 2.00 moles) was added at such a rate that the generated white fumes refluxed gently in the lower portion of the attached Leibig condenser. The resulting brown mixture was stirred under nitrogen at 123° (oil bath temperature) for 18 hours. The reaction mixture at room temperature was added slowly to water (3 l.) which had been preheated to 80°. The hot mixture was cooled in an ice bath; the brown crystalline precipitate of crude acid was collected by filtration, washed with water, and air dried, yield 173 g. (90%). The solid was extracted with ether (1.2 l.) in a Soxhlet apparatus. The residue obtained by evaporation of the extract to dryness *in vacuo* was dissolved in 5% aqueous sodium hydroxide, and this solution was treated with charcoal. The filtrate at 0° was acidified with 10% hydrochloric acid (250 ml.). The light tan precipitate of product was collected by filtration, washed with water, and dried *in vacuo* over phosphorus pentoxide, yield 158 g. (82%), m.p. 211° with sublimation; λ max, nm (ϵ x 10^{-3}), 0.1 *N* hydrochloric acid 294 (4.11); ν max, in cm^{-1}, 3100, 3075 (CH), 1715 (C=O), 1593, 1543 (C=C, C=N).

Anal. Calcd. for $C_6H_3Cl_2NO_2$: Cl, 36.93; N, 7.30. Found: Cl, 37.2; N, 7.11.

Ethyl 2-Amino-6-chloroisonicotinate (**4**).

A solution of **2** (50.0 g., 0.260 mole) in concentrated ammonium hydroxide (600 ml.) was heated in a stainless steal pressure vessel (glass lined) at 200° for 8 hours. The reaction mixture was poured into a beaker and boiled for 30 minutes to remove most of the ammonia. Next, the solution was treated with charcoal, cooled in an ice bath, and adjusted to *p*H 4 with 6 *N* hydrochloric acid. The precipitate of crude 2-amino-6-chloropyridine-4-carboxylic acid was collected by filtration, washed with water and air dried at 90° (heat lamp), yield 43.1 g. (96%). The acid was suspended in ethanol (500 ml.) (Waring blender), saturated with anhydrous hydrogen chloride with a spontaneous rise in temperature to the boiling point, and stirred for 18 hours at room temperature. The ethanol solution was poured into a mixture of water (2 l.) and ether (600 ml.) at 0° and made alkaline with sodium hydroxide pellets. The ether layer was removed in a separatory funnel, and the aqueous layer was further extracted with ether (3 x 300 ml.). The combined ether extract was dried over magnesium sulfate, treated with charcoal, and evaporated to dryness *in vacuo*. Recrystallization of the residue from boiling ethanol (450 ml.) gave pale yellow needles, which were collected by filtration, washed with cold ethanol, and dried *in vacuo* over phosphorus pentoxide, yield 28.7 g., m.p. 171°; λ max, nm (ϵ x 10^{-3}), 0.1 *N* hydrochloric acid 238 (7.44), 340 (5.13); ν max, in cm^{-1}, 3440, 3430, 3312, 3260, 3185 (NH), 2985, 2970, 2925, 2900 (CH), 1715, 1710 (CO), 1640 (NH), 1610, 1545 (C=C, C=N).

Anal. Calcd. for $C_8H_9ClN_2O_2$: C, 47.89; H, 4.52; N, 13.97. Found: C, 48.10; H, 4.47; N, 13.85.

Additional product, 2.30 g., m.p. 171°, crystallized from the concentrated mother liquors. The total yield was 31.0 g. (59%).

2-Amino-6-chloroisonicotinic Acid Hydrazide (**5**).

Finely powdered **4** (51.2 g., 255 mmoles) was stirred with 95% hydrazine (85 ml.) at 90-100° for 5 minutes. With stirring the resulting mixture was allowed to cool to 25°, then refrigerated, The tan product was collected by filtration, washed with water, and dried *in vacuo* over phosphorus pentoxide, yield 40.4 g. (85%); m.p. 244° dec. Recrystallization of this solid from 1:1 ethanol-water gave the pure product, m.p. 258° dec., in 74% yield; λ max, nm (ϵ x 10^{-3}), 0.1 *N* hydrochloric acid 238 (9.94), 331 (4.47); ν max, in cm^{-1}, 3385, 3293, 3180 (NH), 3085 (CH), 1665 (C=O), 1650, 1622 (NH), 1603, 1530 (C=C, C=N).

Anal. Calcd. for $C_6H_7ClN_4O$: C, 38.62; H, 3.78; N, 30.03. Found: C, 38.76; H, 4.12; N, 30.34.

Ethyl 6-Chloro-2,3-diphenylpyrido[2,3-*b*]pyrazine-8-carbamate (**6**).

A suspension of **7** (261 mg., 1.00 mmole) in ethanol (10 ml.) was hydrogenated in the presence of Raney nickel (870 mg. weighed wet with ethanol). The resulting mixture was filtered under nitrogen; the filtrate containing the diaminopyridine was treated with benzil (210 mg., 1.00 mmole), and stirred under nitrogen for 72 hours. The crystalline product was collected by filtration, washed with ethanol, and dried *in vacuo* over phosphorus pentoxide, yield 228 mg., m.p. 213°; λ max, nm (ϵ x 10^{-3}), 0.1 *N* hydrochloric acid 236 (19.8), 265 (19.8), 283 (19.5), 378 (17.4); ν max, in cm^{-1}, 3440, 3370 (NH), 3050, 3000, 2970, 2920 (CH), 1730 (C=O), 1580, 1550, 1535, 1550 (C=C, C=N), 1200 (COC), 735, 690 (monosubstituted phenyl).

Anal. Calcd. for $C_{22}H_{17}ClN_4O_2$: C, 65.27; H, 4.34; N, 13.86. Found: C, 65.43; H, 4.35; N, 13.84.

Additional product (57 mg., m.p. 213°) was obtained by concentration of the mother liquor. The total yield was 285 mg. (70%).

Ethyl 2-Amino-6-chloro-3-nitro-4-pyridinecarbamate (**7**).

The hydrochloride of **9** (30 g., 0.12 mole) was added in small

portions to 96% sulfuric acid (60 ml.) at 25° under nitrogen. The mixture was stirred under nitrogen until the solid dissolved, and the resulting solution was stirred an additional 0.5 hours to drive off the evolved hydrogen chloride gas. This solution at 0° was treated dropwise with a solution of 70% nitric acid (7.5 ml.) and 96% sulfuric acid (52.5 ml.). After stirring at 0° for 10 minutes, the orange solution was stirred at 25° for 30 minutes and at 35° for 15 minutes, then poured slowly over crushed ice (~600 g.). The yellow precipitate was collected by filtration, washed with cold water and dried *in vacuo* over phosphorus pentoxide. The crude product was recrystallized from ethanol (3 l.) yield 24 g. (77%), m.p. 191-192°; λ max, nm (ε x 10^{-3}), 0.1 *N* hydrochloric acid 217 (37.8), 260 (5.87), 318 (5.74), 392 (6.86); ν max, in cm^{-1}, 3450, 3255, 3143 (NH), 2980 (CH), 1750 (C=O), 1620 (NH_2), 1575, 1520 (C=C, C=N); pmr (7% w/v), δ, 1.25 (3, CH_3), 4.2 (2, CH_2), 7.3 (1, 5-H), 8.05 (2, NH_2), 10.4 (1, NH).

Anal. Calcd. for $C_8H_9ClN_4O_4$: C, 36.89; H, 3.48; N, 21.50. Found: C, 36.70; H, 3.67; N, 21.64.

Ethyl 2-Amino-6-chloro-3,5-dinitro-4-pyridinecarbamate (**8**).

Similarly, treatment of the hydrochloride of **9** (1.0 g., 4.0 mmoles) in 96% sulfuric acid (2 ml.) with a solution of 70% nitric acid (0.50 ml.) and 96% sulfuric acid (0.50 ml.) gave crude **8**. Two recrystallizations of this product from ethanol gave the analytical sample, yield 0.78 g. (64%), m.p. 253°; λ max, nm (ε x 10^{-3}), 0.1 *N* hydrochloric acid 215 (26.6), 271 (7.59), 379 (6.22); ν max, in cm^{-1}, 3435, 3290, 3180 (NH), 2985, 2910 (CH), 1710 (C=O), 1635 (NH_2), 1583, 1555, 1533, 1510 (C=C, C=N); pmr (10% w/v), δ, 1.22 (3, CH_3), 4.13 (2, CH_2), 8.2 (2, NH_2), 10.5 (1, NH).

Anal. Calcd. for $C_8H_8ClN_5O_6$: C, 31.44; H, 2.64; N, 22.91. Found: C, 31.69; H, 2.71; N, 22.73.

Ethyl 2-Amino-6-chloro-4-pyridinecarbamate Hydrochloride (**9**).

A 0.835 *N* solution of anhydrous hydrogen chloride in ethanol (49.2 ml., 41.0 mmoles) was added dropwise (1 drop/6 sec) to a stirred suspension of finely powdered **5** (3.73 g., 20.0 mmoles) and isoamyl nitrite (2.58 g., 22.0 mmoles) in ethanol (110 ml.) at 0°. The mixture was stirred an additional 18 hours at 0°, then heated at reflux for 5 hours under nitrogen. The resulting solution was evaporated to dryness *in vacuo*, and the residue was stirred for 18 hours under ether (85 ml.). The white precipitate was collected by filtration, washed with ether, and dried *in vacuo* over phosphorus pentoxide. This crude product was extracted with hot water (45 ml.) to give **4** (0.54 g.), m.p. 170°, identified by elemental analysis and tlc. The hot aqueous extract was cooled to 50° and treated with charcoal. The title compound crystallized from the solution as colorless needles which were collected by filtration, washed with cold water, and dried *in vacuo* over phosphorus pentoxide, yield 2.47 g. (49%), m.p. 190-191°; λ max, nm (ε x 10^{-3}), 0.1 *N* hydrochloric acid 230 (44.2), 252 (12.1), 300 (7.04); ν max, in cm^{-1}, 3330, 3170, 3100 (NH), 3040, 2980 (CH), 1700 (C=O), 1658, 1625 (NH), 1600, 1570, 1520 (C=C, C=N).

Anal. Calcd. for $C_8H_{10}ClN_3O_2 \cdot HCl$: C, 38.12; H, 4.40; N, 16.67. Found: C, 38.15; H, 4.27; N, 16.65.

Ethyl 2-Amino-6-[[4-(Diethylamino)-1-methylbutyl]amino]-3-nitro-4-pyridinecarbamate Monohydrochloride (**11**).

A mixture of **7** (6.10 g., 23.4 mmoles), 2-amino-5-diethylaminopentane (3.96 g., 25.0 mmoles), and absolute ethanol (200 ml.) was refluxed under nitrogen for 75 hours, and the resulting solution was evaporated to dryness *in vacuo*. The residual gum was dissolved in chloroform and poured into a short silica gel H column (150 g.). Elution with chloroform gave unreacted **7** (1.1 g., 18% recovery), and elution with chloroform-methanol (95:5) gave the hydrochloride salt of **11**. This solid was dissolved in warm ethanol (50 ml.), and the solution was diluted very slowly with ether (500 ml.) to deposit fluorescent yellow crystals of **11**, which were dried *in vacuo* over phosphours pentoxide at 65°, yield 7.0 g. (71%). This sample sintered at about 114° and foamed at 121-123°; λ max, nm (ε x 10^{-1}), 0.1 *N* hydrochloric acid 269 (8.5), 382 (17.5); ν max, in cm^{-1}, 3440, 3230, 3170 (NH), 2970, 2935 (CH), 1735 (C=O), 1605, 1570, 1520 (C=C, C=N), 1260 (COC).

Anal. Calcd. for $C_{17}H_{30}N_6O_4 \cdot HCl$: C, 48.74; H, 7.46; Cl, 8.50. Found: C, 48.53; H, 7.55; Cl, 8.60.

Ethyl 3-(*p*-Chlorophenyl)-6[[4-(diethylamino)-1-methylbutyl]-amino]pyrido[2,3-*b*]pyrazine-8-carbamate Dihydrochloride (**12**).

A solution of the hydrochloride of **11** (6.50 g., 15.5 mmoles) in ethanol (200 ml.) was hydrogenated over Raney nickel catalyst (*ca.* 15 g.) at an intial hydrogen pressure of 3.5 kg-cm^{-2}. The catalyst was removed under nitrogen, and the colorless filtrate was treated with solid *p*-chlorophenylglyoxal monohydrate (3.14 g., 17.0 mmoles). The resulting yellow solution was stirred at room temperature for 96 hours, refluxed under nitrogen for 2 hours, then treated with charcoal, and evaporated to dryness *in vacuo*. An aqueous suspension (200 ml.) of the residue was adjusted to *p*H 10 with 50% sodium hydroxide solution and extracted with chloroform (3 x 200 ml.). The combined chloroform extract was washed with water, dried over sodium sulfate, and evaporated to dryness. The orange gum was dissolved in dry ethanol (100 ml.) containing 3 *N* ethanolic hydrogen chloride (15 ml.), and the resulting solution was diluted with ether (250 ml.) with stirring. The yellow solid that deposited during 18 hours was collected by filtration under nitrogen and reprecipitated from ethanol with ether. The hygroscopic yellow solid was dried *in vacuo* over phosphorus pentoxide at 78°, yield 6.3 g. (73%). This sample sintered above 145° and foamed at 152-154°; λ max, nm (ε x 10^{-3}), *p*H 7, 253 (30.1), 394 (20.5); ν max, in cm^{-1}, 3375, 3200 (NH), 2975, 2930 (CH), 1750 (C=O), 1640 (NH), 1605, 1590 (C=C, C=N), 1210 (COC), 840 (1,4-disubstituted phenyl), pmr (25% w/v), δ, 1.3 (m, 12, CH_3), 1.85 (broad, 4, CCH_2CH_2C), 3.1 (broad, 6, NCH_2), 4.31 (q, 2, OCH_2), 4.62 (broad, 1, NCH), 7.83 (q, 4, C_6H_4), 7.92, 9.28 (1, 1, ring CH), 10.3 (broad, 4, NH, HCl).

Anal. Calcd. for $C_{25}H_{33}ClN_6O_2 \cdot 2HCl$: C, 53.82; H, 6.32; N, 15.06; Cl, 19.06. Found: C, 53.68; H, 6.27; N, 14.96; Cl, 18.82.

8-Amino-3-(*p*-chlorophenyl)-6-[[4-(diethylamino)-1-methylbutyl]-amino]pyrido[2,3-*b*]pyrazine Dihydrochloride Dihydrate (**13**).

A solution of **12** (3.90 g., 6.70 mmoles) and potassium hydroxide pellets (2.80 g. 50.0 mmoles) in absolute ethanol (200 ml.) was refluxed under nitrogen for 9 hours, then cooled, and evaporated to dryness *in vacuo*. An aqueous suspension (200 ml.) of the gummy residue was adjusted to *p*H 1 with concentrated hydrochloric acid, stirred for about 5 minutes, and readjusted to *p*H 11 with 50% sodium hydroxide. The mixture was extracted with chloroform (3 x 100 ml.), and the combined extract was washed with water, dried over sodium sulfate, and evaporated to dryness. The glassy residue was dissolved in warm ethanol (100 ml.), and the solution was diluted with 3 *N* ethanolic hydrogen chloride (5 ml.) to deposit a heavy precipitate. The thick slurry was diluted to 500 ml. with ether and filtered under nitrogen. This solid was recrystallized from boiling ethanol (100 ml.), and when crystallization appeared to be complete, the mixture was diluted to 500 ml. with ether. The resulting hygroscopic cream-

colored solid was collected by filtration under nitrogen and dried *in vacuo* over phosphorus pentoxide at 78°, yield 3.2 g. (92%), m.p. 250-252° dec; λ max, nm (ϵ x 10^{-3}), 0.1 *N* hydrochloric acid 275 (11.1), 370 (29.4), *p*H 7, 274 (25.8), 386 (19.7), 0.1 *N* sodium hydroxide 274 (29.0), 393 (19.5); ν max, in cm^{-1}, 3390, 3220, 3120 (braod, NH), 2970, 2925 (CH), 1650 (NH_2), 1595, 1515 (C=C, C=N), 840 (1,4-disubstituted phenyl); pmr (15% w/v), δ, 1.27 (m, 9, CH_3), 1.78 (broad, 4, CCH_2CH_2C), 3.12 (broad, 6, NCH_2), 3.87 (broad, 1, NCH), 6.43 (1, ring CH), 7.95 (q, 6, C_6H_4, NH_2), 9.3 (2, ring CH, NH), 11.9 (broad, 2, HCl).

Anal. Calcd. for $C_{22}H_{29}ClN_6 \cdot 2H_2O$: C, 50.63; H, 6.76; N, 16.10; Cl, 20.38. Found: C, 50.92; H, 6.65; N, 16.08; Cl, 20.35.

Acknowledgments.

The authors are indebted to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute who performed most of the microanalytical and spectral determinations reported.

REFERENCES

(1) This work was carried out for the Division of Medicinal Chemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Contract No. DA-49-193-MD-2999. This is Contribution No. 714 from the Army Research Program on Malaria.

(2a) C. Temple, Jr., J. D. Rose, R. D. Elliot, and John A. Montgomery, J. Med. Chem., 11, 1216 (1968); (b) R. D. Elliott, C. Temple, Jr., and J. A. Montgomery, *J. Org. Chem.*, **33**, 2393 (1968).

(3) A. Bavley and M. Harfenist, U. S. Patent 2742478 (1956).

(4) H. Meyer and E. Ritter v. Beck, *Monatsh. Chem.*, **36**, 740 (1915).

(5) T. Curtius and A. Reidel, *J. Prakt. Chem.*, **76**, 238 (1907).

(6) W. T. Caldwell and E. C. Kornfeld, *J. Am. Chem. Soc.*, **64**, 1696 (1942).

(7) Similar results are obtained in the condensation of 4,5-diaminopyrimidine with glyoxals to give a preponderence of the 7-substituted pteridine. See reference 8.

(8) D. R. Seeger, D. B. Cosulich, J. M. Smith, Jr., and M. E. Hultquist, *J. Am. Chem. Soc.*, 71, 1753 (1949); H. S. Forrest and J. Walker, *J. Chem. Soc.*, 2077 (1949); A. Albert, *Quart. Rev.*, **6**, 197 (1952).

Received November 28, 1969 Birmingham, Alabama 35205