

4,5-Disubstituted Primaquine Analogs as Potential Antiprotozoan Agents

HANNS BURGHARD and C. DeWITT BLANTON, Jr. *

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Abstract □ 4,5-Dimethylprimaquine and 5-fluoro-4-methylprimaquine were synthesized and evaluated against *Plasmodium berghei* in the mouse. Significant blood schizonticidal activity was observed. The 5-fluoro-4-methylprimaquine analog also was active as a tissue schizonticidal agent when evaluated against *P. cynomolgi* in the rhesus monkey, as an antileishmanial agent when evaluated against *Leishmania donovani* in the hamster, and as a causal prophylactic agent when evaluated against *P. berghei yoelii*.

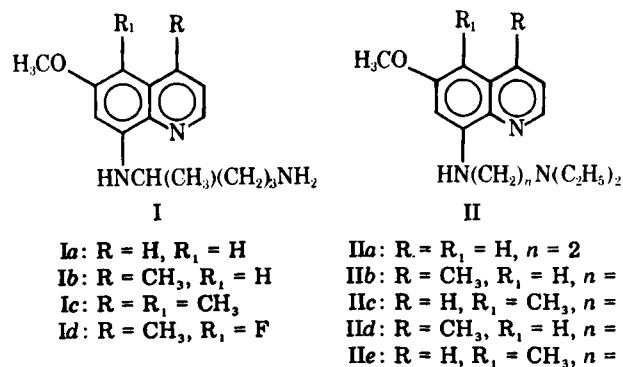
Keyphrases □ Primaquine analogs—synthesis and evaluation for antiprotozoan activity □ Antiprotozoan agents, potential—primaquine analogs, synthesis and evaluation for activity □ 8-Aminoquinolines, substituted—synthesis and evaluation for antiprotozoan activity

Although generally quite toxic, 8-aminoquinoline derivatives related to primaquine (Ia) are of interest as potential radical curative and causal prophylactic antimalarial agents (1). Primaquine, the tissue schizonticidal drug of choice, is used mainly with a strong blood schizonticide as a radical cure of relapsing malaria and for chemoprophylaxis or the interruption of transmission (2). A major drawback of primaquine is its relatively low therapeutic index, and modification of the side chain of the molecule exclusively has not resulted in any marked improvement of antimalarial activity combined with reduced toxicity (3, 4).

Substitution of a methyl group onto the 8-amino-6-methoxyquinoline nucleus offers potential for obtaining agents with high antimalarial activity and reduced toxicity relative to the unsubstituted nucleus. For example, 4-methylprimaquine (Ib) was reported (5, 6) to possess enhanced antimalarial activity and somewhat lower toxicity when evaluated against *Plasmodium cynomolgi* in the rhesus monkey (7, 8) and *P. berghei* in mice (9). Similar trends were reported when 8-aminoquinoline derivatives were screened against *P. gallinaceum* (10). Thus, 8-[(2-diethylaminoethyl)amino]-6-methoxyquinoline (IIa) had a therapeutic index of 1.8 (10) while the 4-methyl (IIb) and 5-methyl (IIc) analogs possessed therapeutic indexes of 20 and 9.1, respectively. Nuclear substitution and modification of the side chain at position 8 led to further improvement in the therapeutic index, as seen with IIId and IIe, which had therapeutic indexes of 59 and 33.5 (10). Based on these considerations, a program was initiated to synthesize and evaluate the potential antimalarial activity of 4,5-dimethylprimaquine (Ic).

DISCUSSION

Chemistry—The synthesis of the key intermediate (VII) required for the preparation of 4,5-dimethylprimaquine (Ic) is outlined in Scheme I. 4-Amino-2-fluoro-5-nitroanisole (III) was prepared by the procedure of Elderfield *et al.* (11). Reaction of III with methyl vinyl ketone under modified Skraup conditions (12) gave 4-methyl-5-fluoro-6-methoxy-8-nitroquinoline (IV). Attempts to displace the activated fluorine atom at position 5 with a methyl nucleophile [e.g., methylenetriphenylphosphorane (13)] failed. Similarly, attempts to displace the fluorine atom



with a cyanide ion (14) for subsequent reduction (15, 16) to a methyl group failed.

The use of 18-crown-6 ether (17) did not facilitate cyanide-ion displacement of the fluorine atom. However, by reaction of the anion of ethyl malonate and IV in the presence of copper(I) bromide (18) and 18-crown-6 ether, it was possible to displace the fluorine atom with formation of a carbon-carbon bond (V). Acid hydrolysis of the substituted malonate (V) led directly to the desired intermediate (VII). If the hydrolysis step was terminated too soon, the substituted acetic acid derivative (VI) could be isolated. Reduction of 4,5-dimethyl-6-methoxy-8-nitroquinoline (VII) with stannous chloride and alkylation of the amine (VIII) by 4-bromo-1-phthalimidopentane (19) in triethylamine (20, 21) followed by hydrazinolysis gave the desired analog (Ic).

Since IV then was readily available, 4-methyl-5-fluoroprimaquine (Id) also was prepared. Standard reduction of IV and alkylation of the amine (X) followed by hydrazinolysis gave the desired product (Id).

Direct synthesis of VII by the Skraup reaction of 2-nitro-4-methoxy-5-methylaniline (XI) (22) and methyl vinyl ketone (Scheme II) was attempted, but the expected intermediate (XII) was the only isolated product; various alterations in experimental conditions or reagents did not change the course of the reaction. (Several of these alterations are summarized in Scheme II, and the uncyclized intermediates XIII and XIV were isolated as in the case of XII.) Steric effects, as well as electronic effects, may prevent direct cyclization to VII. Therefore, Skraup cyclization of III and methyl vinyl ketone, although in low yield, was possible since the fluoro compound (III) presented less of a steric problem than XI.

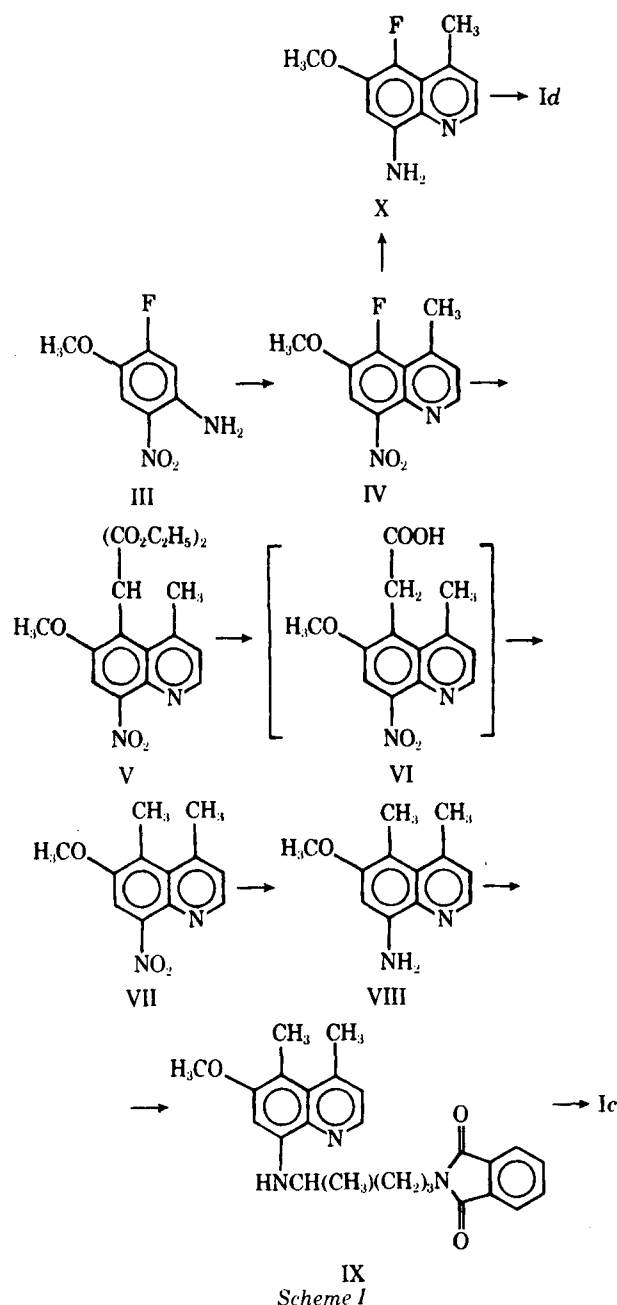
Antiprotozoan Activity¹—The blood schizonticidal activity of the potential antimalarials Ic and Id was assessed against *P. berghei* in mice by the method of Osden *et al.* (9). 4,5-Dimethylprimaquine (Ic) was active² in the Rane test: ΔMST = 7.6 at 160 mg/kg, 9.1 (IC, 2T) at 320 mg/kg, and 0.0 (3C, 2T) and 10.8 (IC, 3T) at 640 mg/kg. 5-Fluoro-4-methylprimaquine (Id) showed good activity against *P. berghei* at 10 mg/kg (ΔMST = 6.7). Doses higher than 40 mg/kg produced toxic deaths in all animals (5T). Primaquine diphosphate was active at 80 mg/kg (ΔMST = 9.4) with drug-related toxic deaths at 160 (ΔMST = 10.8, 2T) and 320 (ΔMST = 0.0, 5T) mg/kg.

The tissue schizonticidal potential¹ of Id was evaluated in rhesus monkeys by a literature procedure (7, 8). Compound Id showed 3/3 cures³

¹ Antiprotozoan test results were provided by the Walter Reed Army Institute of Research. Tests for blood schizonticidal and causal prophylactic activity were carried out by the Rane Laboratory, University of Miami, Miami, Fla. Tests for tissue schizonticidal activity were carried out by Dr. L. H. Schmidt, Southern Research Institute, Birmingham, Ala. Tests for antileishmanial activity were carried out by Dr. W. L. Hanson, University of Georgia, Athens, Ga.

² The mean survival time (MST) of untreated mice is 6.1 days. A compound is active if ΔMST exceeds 6.1 days. Animals that survive 60 days postinfection are considered cured (C). Deaths on Days 2-5 after drug administration are attributed to drug toxicity (T).

³ Monkeys that do not relapse in 90 days are considered cured.



at 0.5 mg/kg. Primaquine diphosphate cured 90% of the monkeys in this test system when administered at a dose of 1.3 mg/kg for 7 days in combination with 5 mg of chloroquine/kg/day.

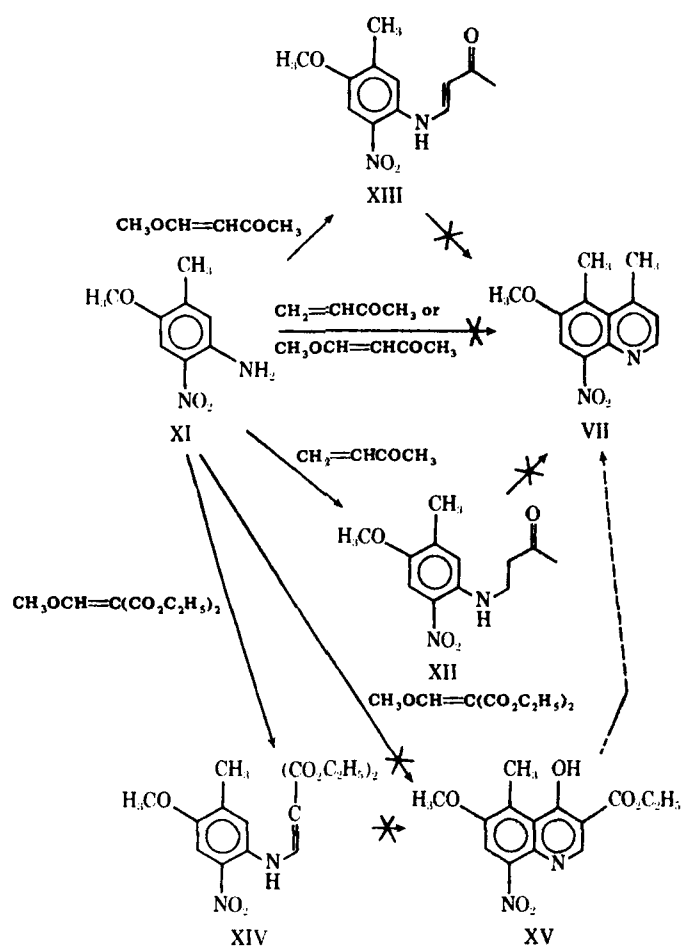
The antileishmanial activity¹ of Id against *Leishmania donovani* was determined in golden hamsters by the method of Hanson *et al.* (23). The suppressive effects of the test compound were compared to those of the reference compound, *N*-methylglucamine antimoniate⁴, and a G index⁵ was calculated (4, 23). 5-Fluoro-4-methylprimaquine (Id) showed significant activity in the antileishmanial screen with a G index of 10.98 at 3.25 mg/kg/day. Primaquine possessed a G index of 0.96 at the SD₉₀ level, whereas some 8-aminoquinolines with a methyl group at position 4 were reported to have enhanced antileishmanial properties (24); e.g., 8-[(6-diethylamino)hexyl]amino]-6-methoxy-4-methylquinoline had a G index of 472 at the SD₉₀ level (4).

Compound Id was tested for causal prophylactic activity^{1,6} against sporozoite-induced *P. berghei yoelii* in rodents (25, 26). This agent was active at 10 mg/kg when administered subcutaneously (5/5 cures).

⁴ Glucantime.

⁵ The glucantime index, G, was calculated using $G = \text{dose (SD}_x\text{) of glucantime/dose (SD}_x\text{) of test drug}$.

⁶ Prophylactic activity is evidenced by survival of drug-treated mice for 30 days. Survival of 40% or more of the treated mice indicates activity.



Compound Id given orally was active at 10 and 40 mg/kg (5/5 cures).

Although Id was not tested for tissue schizonticidal, antileishmanial, or causal prophylactic activity, the data presented confirm that appropriate substituents at the 4- and 5-positions can alter significantly the antiprotozoan activity of 8-aminoquinolines. In the blood schizonticidal test (9), Id was more active at lower doses than primaquine, but it also was more toxic. Compound Id was not as active as primaquine in this test system, but it was significantly less toxic.

EXPERIMENTAL⁷

4-Methyl-5-fluoro-6-methoxy-8-nitroquinoline (IV)—4-Amino-2-fluoro-5-nitroanisole (III) was prepared by the method of Elderfield *et al.* (11). Compound III (37.23 g, 0.2 mole), 34.96 g of arsenic pentoxide (0.15 mole), and 45 ml of 85% phosphoric acid were mixed to form a viscous slurry. This slurry was stirred vigorously until a homogeneous paste formed. Then 200 ml of 85% phosphoric acid was added gradually while stirring. The suspension was heated to 90°. This mixture was treated with 16.8 g (0.24 mole) of methyl vinyl ketone at such a rate as to maintain the temperature at 90°; the addition required 0.5 hr.

The resulting mixture was kept at 85–95° for 1.5 hr and then was poured into 800 ml of ice-cold water and slowly made basic with 450 ml of concentrated ammonium hydroxide. The dark solid was collected, washed with water, and dried at 50° overnight. The product was dissolved in chloroform (200 ml), but some tar and by-products remained undissolved. A volume of silica gel approximately equal to the volume of crude product was added, and the chloroform mixture was removed *in vacuo*. The brown silica gel complex was transferred onto a short column containing approximately a double volume of fresh silica gel. This column

⁷ Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. NMR spectra were determined on a Hitachi Perkin-Elmer R20A high-resolution NMR spectrometer using tetramethylsilane as the internal reference. IR spectra were determined on a Perkin-Elmer 467 grating spectrophotometer using the potassium bromide technique. Elemental analyses were determined by Atlantic Microlab, Atlanta, Ga. Mass spectra were determined on a DuPont 21-490 low-resolution mass spectrometer.

was eluted with chloroform, and the solvent was evaporated *in vacuo* to give a red impure product.

Recrystallization from ethyl acetate gave 11.8 g (25% yield) of product, mp 142°; NMR⁸: δ 2.81 (d, 3H, CH₃ at C-4, J = 7 Hz), 3.90 (s, 3H, CH₃O at C-6), 7.26 (d, H at C-3, J = 5 Hz), 8.67 (d, H at C-2, J = 5 Hz), and 8.95 (d, H at C-7, J = 8 Hz). Concentration of the mother liquor gave another 4.3 g (9% yield) for an overall yield of 34%. If the quantity of methyl vinyl ketone is doubled, the yield can be raised to 60–70%.

Anal.—Calc. for C₁₁H₉FN₂O₃: C, 55.93; H, 3.84; N, 11.85. Found: C, 55.97; H, 3.88; N, 11.80, 11.82.

Diethyl α -(4-Methyl-6-methoxy-8-nitroquinolyl)malonate (V)—Sodium hydride (0.7 g of 50% dispersion in paraffin oil) was added over 10 min to a stirred mixture of IV (2.95 g, 12.5 mmoles) and copper(I) bromide (0.1 g, 0.7 mmole) in 25 ml of ethyl malonate. The mixture was maintained in an oil bath at 40–45° during the addition of sodium hydride. Ten drops of 18-crown-6 ether were added to the mixture, and the temperature was raised to 80°. Stirring and heating (60–80°) under a nitrogen atmosphere was continued for 6 hr.

The red solution was cooled and diluted with 600 ml of water, and the pH of the aqueous mixture was adjusted to 10 with 10% NaOH. After standing for 48 hr at room temperature, a fluffy yellow solid (5.1 g) was collected by filtration. The material was recrystallized from benzene-petroleum ether (30–60°), mp 162–163° (4.1 g, 87% yield); NMR (CDCl₃): δ 1.28 (t, 6H, CH₃ groups of esters), 2.88 (s, 3H, CH₃ at C-4), 3.98 (s, 3H, CH₃O at C-6), 4.25 (q, 4H, CH₂ groups of esters), 5.7 (s, 1H, CH at C-5), 7.28 (broad s, 1H, H at C-3), 7.75 (s, 1H, H at C-7), and 8.65 (broad s, 1H, H at C-2); mol. wt. (mass spectra): calc., 376, and found, 376.

Anal.—Calc. for C₁₈H₂₀N₂O₇: C, 57.44; H, 5.36; N, 7.44. Found: C, 57.42; H, 5.38; N, 7.39.

4,5-Dimethyl-6-methoxy-8-nitroquinoline (VII)—Compound V (2.9 g, 7.7 mmoles) was suspended in 50 ml of water and treated with 10 ml of acetic acid and 5 ml of concentrated sulfuric acid. The temperature of the mixture was raised slowly to reflux. The dark mixture was stirred and refluxed for 4 hr (oil bath temperature was maintained between 130 and 150°), cooled, and poured into 250 ml of water containing sufficient 10% NaOH to give a pH of 10. A greenish-gray product separated.

The product was collected by filtration and recrystallized from aqueous dimethylformamide after treatment with charcoal to yield fine yellow needles, mp 177–179° (1.5 g, 83.7% yield); NMR (dimethyl sulfoxide-*d*₆): δ 2.66 (s, 3H, CH₃ at C-5), 2.85 (s, 3H, CH₃ at C-4), 3.98 (s, 3H, CH₃O at C-6), 7.35 (d, 1H, H at C-3, J = 4.5 Hz), 8.15 (s, 1H, H at C-7), and 8.60 (d, 1H, H at C-2, J = 4.5 Hz).

Anal.—Calc. for C₁₂H₁₂N₂O₃: C, 62.06; H, 5.21; N, 12.06. Found: C, 62.12; H, 5.26; N, 12.04.

α -(4-Methyl-6-methoxy-8-nitroquinolyl)acetic Acid (VI)—When the oil bath temperature was not maintained above 130°, hydrolysis and decarboxylation of V was incomplete, and VI was obtained in near quantitative yield, mp 115–117°; NMR (dimethyl sulfoxide-*d*₆): δ 2.90 (s, 3H, CH₃ at C-4), 4.00 (s, 3H, CH₃O at C-6), 4.30 (s, 2H, CH₂ at C-5), 7.45 (broad s, 1H, H at C-3), 8.3 (s, 1H, H at C-7), and 8.7 (broad s, 1H, H at C-2).

When VI was refluxed with the aqueous acetic acid-sulfuric acid mixture at 130–150° for 4 hr, VII was obtained in a 97.6% yield.

8-Amino-4,5-dimethyl-6-methoxyquinoline (VIII)—A solution of stannous chloride hydrate (31.6 g, 0.14 mole) and 15 ml of concentrated hydrochloric acid was cooled to –5–0°. After the addition of 0.5 g of mossy tin, the mixture was stirred as a precooled solution of VII (8.2 g, 0.035 mole) in 25 ml of concentrated hydrochloric acid was added at a rate such that the temperature remained below 6°. The addition required ~45 min. The brown suspension was stirred for an additional 15 min at 0°, and the ice bath was removed.

After stirring at room temperature for 5 hr, the brown suspension was poured into 200 ml of water. The yellow solid which precipitated became gray when the acidic mixture was made basic with 9 *N* NaOH. The basic solution was covered with 200 ml of ether-ethyl acetate (1:1) and filtered through diatomaceous earth⁹ to remove some black insoluble material. The organic layer was separated from the water layer, and the filter cake was washed with ethyl acetate (200 ml) and acetone (200 ml). The aqueous layer was extracted with ethyl acetate (2 \times 100 ml). All organic layers were combined and dried (magnesium sulfate).

Removal of the solvents *in vacuo* gave a gold-colored solid. The analytical sample was obtained from aqueous ethanol (4.5 g, 63.6% yield), mp 127–128°; NMR (CDCl₃): δ 2.6 (s, 3H, CH₃ at C-4), 2.8 (s, 3H, CH₃ at C-5), 3.80 (s, 3H, CH₃O at C-6), 4.8 (broad s, 2H, NH₂, exchanged by

deuterium oxide), 6.7 (s, 1H, H at C-7), 7.05 (d, 1H, H at C-3, J = 4.5 Hz), and 8.39 (d, 1H, H at C-2, J = 4.5 Hz).

Anal.—Calc. for C₁₂H₁₄N₂O: C, 71.26; H, 6.98; N, 13.85. Found: C, 70.99; H, 6.98; N, 13.85.

4,5-Dimethyl-6-methoxy-8-[(4-amino-1-methylbutyl)amino]quinoline (Ic) Maleate—A mixture of VIII (4 g, 0.0198 mole), 4-bromo-1-phthalimidopentane (8.79 g, 0.0297 mole), and triethylamine (3 g, 0.0297 mole) was stirred and heated at 110–135° for 5 hr. Additional 4-bromo-1-phthalimidopentane (2.93 g, 0.0099 mole) and triethylamine (1 g) were added, and heating was continued for 6 hr. The dark reaction mixture was digested in 200 ml of benzene and filtered hot to remove triethylamine hydrobromide. The benzene was removed *in vacuo* to give a dark blood-red oil. This oil was treated with ethanol (150 ml) and 15 ml of 85% hydrazine hydrate.

The mixture was refluxed for 3 hr, cooled to room temperature, and concentrated *in vacuo*. The residue was treated with methylene chloride (200 ml) and filtered to remove phthalhydrazide. The methylene chloride filtrate was dried (magnesium sulfate) and concentrated to an oil. The residual oil was treated with 50 ml of ethanol and 3 g of maleic acid. Addition of ether precipitated a voluminous yellow solid. After recrystallization from ethanol-ether, 1.9 g of the maleate of Ic was obtained (23.8% yield), mp 135–137°.

Anal.—Calc. for C₂₁H₂₉N₃O₅: C, 62.51; H, 7.24; N, 10.41. Found: C, 62.49; H, 7.27; N, 10.41.

4-Methyl-5-fluoro-6-methoxy-8-aminoquinoline (X)—Compound IV was reduced by the procedure described for the preparation of VIII. Compound X was obtained in a 91% yield (ethanol) as greenish-white crystals, mp 129°; NMR (dimethyl sulfoxide-*d*₆): δ 2.64 (d, 3H, CH₃ at C-4, J = 7.0 Hz), 3.9 (s, 3H, CH₃O at C-6), 6.8 (d, H at C-7, J = 8.0 Hz), 7.18 (d, H at C-3, J = 5.0 Hz), and 8.39 (d, H at C-2, J = 5.0 Hz).

Anal.—Calc. for C₁₁H₁₁FN₂O: C, 64.06; H, 5.37; N, 13.58. Found: C, 64.22; H, 5.40; N, 13.56.

4-Methyl-5-fluoro-6-methoxy-8-[(4-amino-1-methylbutyl)amino]quinoline (Id) Maleate—The maleate of Id was prepared in a 59% yield (ethanol) by the procedure described for Ic, mp 131°.

Anal.—Calc. for C₂₀H₂₆FN₃O₅: C, 58.95; H, 6.43; N, 10.31. Found: C, 58.70; H, 6.48; N, 10.25.

2-Nitro-4-methoxy-5-methyl-*N*-(3-oxobutyl)aniline (XII)—2-Nitro-4-methoxy-5-methylaniline (1.82 g, 0.01 mole), prepared by the procedure of Carmack *et al.* (22), and 40 g of 85% phosphoric acid were heated at 85° while 1.05 g (0.015 mole) of methyl vinyl ketone was added dropwise with vigorous stirring. After 30 min, the addition was completed and the mixture was stirred for an additional 30 min. The brown solution was poured into ice-water and filtered, and the orange product was dried. After recrystallization from ethanol (1.84 g, 73% yield), XII melted at 138°. [This material was identical to that isolated from the Skraup reaction, and no evidence for quinoline (VII) formation was observed.]

Anal.—Calc. for C₁₂H₁₆N₂O₄: C, 57.13; H, 6.39; N, 11.10. Found: C, 57.11; H, 6.43; N, 11.12.

4-(2-Nitro-4-methoxy-5-methylanilino)but-3-en-2-one (XIII)—2-Nitro-4-methoxy-5-methylaniline (3.64 g, 0.02 mole) (22) was mixed with 4.0 g (0.04 mole) of 4-methoxybut-3-en-2-one and fused at 150° for 3 hr. The flask was fitted with a condenser to remove methanol. Red crystals were collected when the reaction mixture was cooled and the residue recrystallized from ethanol (71% yield), mp 177°. [This product was identical to that obtained when Skraup reaction conditions were employed, and no evidence of quinoline (VII) formation was obtained.]

Anal.—Calc. for C₁₂H₁₄N₂O₄: C, 57.59; H, 5.63; N, 11.19. Found: C, 57.55; H, 5.66; N, 11.22.

Diethyl (3-Methyl-4-methoxy-6-nitroanilino)methylenemalonate (XIV)—2-Nitro-4-methoxy-5-methylaniline (0.91 g, 0.005 mole) (22) and 1.08 g (0.005 mole) of diethyl methoxymethylenemalonate ester were fused (27) and kept at 150° for 45 min. The crude product (XIV) was recrystallized from ethanol (1.61 g, 92% yield, mp 127–128°). [No evidence for quinoline (XV) formation was obtained.]

Anal.—Calc. for C₁₆H₂₀N₂O₇: C, 54.54; H, 5.72; N, 7.95. Found: C, 54.41; H, 5.72; N, 7.96.

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⁸ The solvent was Unisol-D, a blended mixture of deuterated chloroform and deuterated dimethyl sulfoxide, Norell Chemical Co., Landisville, N.J.

⁹ Celite.

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Aporphines XXVI: GLC and Mass Spectrometric Properties of Trifluoroacetyl Derivatives of *N*-Methyl-, *N*-Propyl-, and Noraporphines

J. F. GREEN*, G. N. JHAM†, J. L. NEUMEYER*, and PAUL VOUROŠ‡*

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Abstract □ The *O*-trifluoroacetyl and *N,O*-trifluoroacetyl derivatives of a series of aporphine and noraporphine alkaloids were prepared, and their GLC and mass spectrometric characteristics were determined. The positional isomers apocodeine and isoapocodeine were resolved chromatographically as their trifluoroacetyl derivatives, and their mass spectra were distinctly different. Mass spectrometric fragmentation processes apparently unique to *N*-trifluoroacetyl derivatives of noraporphines were observed. Plausible mechanisms for the formation of the major ions in the mass spectra of the various compounds are given.

Keyphrases □ Aporphines—trifluoroacetyl derivatives, GLC and mass spectrometry □ Noraporphines—trifluoroacetyl derivatives, GLC and mass spectrometry □ GLC—trifluoroacetyl derivatives of aporphines and noraporphines

The need for sensitive and quantitative methods for elaborating the metabolism of apomorphine (*Ia*) (Scheme I) has increased due to the biological activity of apomorphine and related alkaloids (1–3). Although conjugation was reported as a primary route for the metabolism of *Ia* in the rat (4), apocodeine (*Id*) and isoapocodeine (*Ie*) formed from *O*-methylation have been reported as possible metabolites in rat liver preparations (5). *N*-Dealkylation also was reported to give an *in vitro* metabolite of several naturally occurring aporphine alkaloids (6). More recent studies in liver microsomal preparations indicated that

several aporphine analogs could be converted metabolically to their *O*-demethylated derivatives (*i.e.*, *Id* → *Ia* and *Ie* → *Ia*) (7).

BACKGROUND

N-n-Propylnorapomorphine (*Ib*) was reported to be several times more potent than *Ia* in several biological systems (3). Monohydroxyaporphines substituted in either the 10- or 11-position were found to be active dopamine agonists, although they were less potent than *Ib* (8, 9). It is generally accepted that catechol analogs of apomorphine are more potent dopamine agonists than are the monohydroxy analogs (10). However, the possibility that monohydroxy analogs such as *Ij* and *Ik* are converted *in vivo* into a catechol-like compound (*e.g.*, *Ib*) cannot be excluded (11). Preliminary studies showed no evidence for the microsomal hydroxylation of the monohydroxy aporphines *Ij* and *Ik* to the corresponding catechol, *Ib*, using TLC to distinguish monophenols from catecholic metabolites (7).

To develop more definitive techniques, specifically GLC–mass spectrometric methods for the detection, identification, and quantitation of aporphines, the *O*-trifluoroacetyl derivatives of a series of *N*-methyl- and *N*-propylaporphines were prepared. Since *N*-dealkylation is a common metabolic process, the mass spectra of a series of trifluoroacetyl noraporphine derivatives also were considered. It was hoped that this examination might reveal unique fragmentation processes which could aid in the recognition of noraporphine metabolites or natural products in general.

Paper chromatography, GLC, and TLC were used previously to define