Expedited Articles

Synthesis and Antimalarial Activity in Vitro and in Vivo of a New Ferrocene−Chloroquine Analogue

Christophe Biot, Guillaume Glorian, Lucien A. Maciejewski, and Jacques S. Brocard*

Laboratoire de Catalyse Hétérogène et Homogène, Groupe de Synthèse Organométallique, URA CNRS 402, Université des Sciences et Technologies, 59655 Villeneuve d'ASCQ, France

Olivier Domarle, Georgette Blampain, Pascal Millet, and Alain J. Georges

Centre International de Recherches Médicales, BP 769 Franceville, Gabon

Huguette Abessolo and Daniel Dive

INSERM U.42, 369 rue Jules Guesde BP 39, 59651 Villeneuve d'ASCQ Cedex, France

Jacques Lebibi

Université de Masuku, BP 901 Franceville, Gabon

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The antimalarial activities of ferrocenic compounds mimicking chloroquine and active upon chloroquine-resistant strains of *Plasmodium falciparum* were evaluated. Four 7-chloro-4-[[[2-[(*N,N*-substituted amino)methyl]ferrocenyl]methyl]amino]quinoline derivatives have been synthesized; one of them, **1a**, showed high potent antimalarial activity in vivo on mice infected with *Plasmodium berghei* N. and *Plasmodium yoelii* NS. and was 22 times more potent against schizontocides than chloroquine in vitro against a drug-resistant strain of *P. falciparum*.

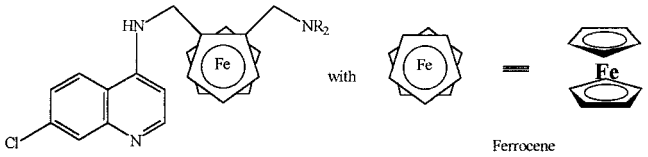
Introduction

According to the more recent estimates available regarding malarial mortality, 1.7−2.7 million deaths per year are reported up to the year 1993, the great majority of them occurring in Africa. Two major factors are responsible for the high level of mortality in Africa.[1] First, because of its chloroquine resistance, *Plasmodium falciparum* was able to spread throughout tropical Africa in the 1980s. And second, more recently, high levels of resistance to chloroquine have become common in some East African countries as well.[1] Interestingly, recent studies showed that the modification of the chloroquine lateral side chain allowed some improvement of the corresponding chloroquine derivative's activity upon both sensitive and resistant strains of *P. falciparum*.[2]

There is rapidly growing interest in the use of transition-metal complexes in medicine and other biological areas as well.[3,4] An example is the successful application of platinum complexes as antitumor agents.[5] Accordingly, much can be expected from organometallic chemistry when directed at therapeutic uses.

Following our interest in the synthesis of new analogues of chloroquine with potent activities, we have sought to develop approaches to such drugs involving ferrocenyl units.[6] Moreover, the stability and nontoxicity of the ferrocenyl moiety is of particular interest rendering such drugs compatible with almost any other treatment. Also, the wide range of ferrocenyl compounds available is quite promising in view of the numerous chloroquine equivalents accessible. In these new species, the carbon chain of chloroquine will be replaced by the hydrophobic ferrocenyl group and the 1−4 relative position of the two exocyclic N atoms is maintained in such derivatives.

Chart 1. 7-Chloro-4-[[[2-[(*N,N*-substituted amino)-methyl]ferrocenyl]methyl]amino]quinoline Derivatives



compd.	—NR$_2$
6a	—NMe$_2$
6b	—NEt$_2$
6c	—N⟨ ⟩
6d	—N⟨ ⟩N—Me

We report here the synthesis of 7-chloro-4-[[[2-[(*N,N*-dimethylamino)methyl]ferrocenyl]methyl]amino]quinoline (**1a**) which is active against a chloroquine-resistant strain of *P. falciparum* in vitro, as well as *Plasmodium berghei* N. and *Plasmodium yoelii* NS. in vivo. We have also synthesized a series of other compounds bearing various tertiary amino groups instead of the dimethy-

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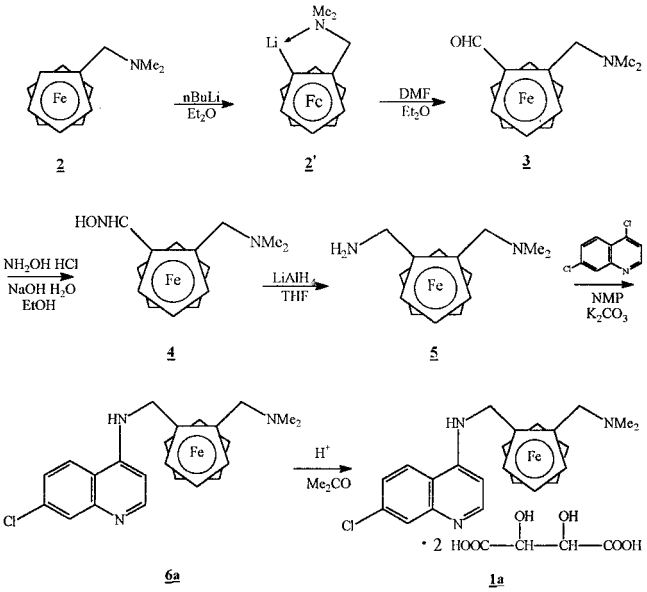
Scheme 1. Synthesis of 7-Chloro-4-[[[2-[(*N*,*N*-dimethylamino)methyl]ferrocenyl]methyl]amino]quinoline



lamino residue (Chart 1). However, with such different side chains lower activities were found.

Results and Discussion

Chemistry. The synthesis of 7-chloro-4-[[[2-[(*N*,*N*-dimethylamino)methyl]ferrocenyl]methyl]amino]quinoline (**1a**) has been carried out starting from the well-known precursor [(dimethylamino)methyl]ferrocene (Scheme 1). Thus, the [(dimethylamino)methyl]ferrocene (**2**) was first metalated in the presence of *n*-butyllithium.[7] The lithium derivative was then condensed with *N*,*N*-dimethylformamide at room temperature under nitrogen giving, after workup, 2-[(*N*,*N*-dimethylamino)methyl]ferrocenecarboxaldehyde (**3**) in respectable yields (70%).[8] The 1,2 orientation of the two substituents of the cyclopentadienyl ring in compound **3** was established unambiguously in agreement with reported data.[9] Next, aldehyde **3** was converted to the corresponding amine **5** in 96% yield via the oxime **4** following a known procedure.[10] Condensation of **5** with 4,7-dichloroquinoline in *N*-methyl-2-pyrrolidinone produced 7-chloro-4-[[[2-[(*N*,*N*-dimethylamino)methyl]ferrocenyl]methyl]amino]quinoline (**6a**) which was isolated in 60% yield after purification through chromatography.[11] Finally, the conversion of amine **6a** to the ammonium **1a** was achieved in acetone by acidification with 2 equiv of L-(+)-tartaric acid. The free base precursor **6a** of the biologically active **1a** has been fully characterized as reported in the Experimental Section.

It has been postulated that active antimalarials such as 4-aminoquinolines possess a common structural feature which is the distance between the two nitrogen atoms located in the side chain.[12] Such a hydrogen-bond existence could be compared to what is observed in amino alcohols such as mefloquine. In our case, the new complex **6a** possesses four carbon atoms between the two nitrogen atoms in the side chain, as is the situation in chloroquine. The infrared absorption at 3420 cm^{-1}, attributed to the N−H vibration, was quite broad and disappeared with dilution, indicative of the presence of intermolecular hydrogen bonds. Moreover, a second absorption was observed at 3680 cm^{-1} which is similar to the one reported for an intramolecular bond formation. We suggest that complex **6a** contains both intermolecular and intramolecular N−H hydrogen bonds.

Table 1. Mean and Standard Deviation of IC_{50} of Chloroquine and **1a** for Each Parasite Strain[a]

parasite strains	IC_{50} (μg/mL) CQDP	*n*	**1a**	*n*
SGE2	0.194 ± 0.175	8	0.160 ± 0.141	5
FG2	0.091 ± 0.065	3	0.059 ± 0.003	4
FG4	0.048 ± 0.009	4	0.057 ± 0.002	5
FG3	0.501 ± 0.139	3	0.095 ± 0.051	4
FCM6	1.241 ±0.937	9	0.061 ± 0.013	8
FCM17	2.813 ± 2.189	5	0.177 ± 0.151	3
FG1	2.620 ± 0.175	3	0.067 ± 0.008	3

[a] Values are the arithmetic mean IC_{50} (μg/mL) ± SD. Strains are regrouped according to their chloroquine susceptibility defined as susceptible for IC_{50} < 0.1 μg/mL, intermediate for IC_{50} between 0.1 and 1 μg/mL, and resistance for IC_{50} > 1 μg/mL.

Table 2. Effect of CQDP and **1a** on *P. berghei* N. and *P. yoelii* NS.[a]

parasite strain	CQ 1 mg/kg	CQ 10 mg/kg	**1a** 1 mg/kg	**1a** 10 mg/kg
P. berghei N.				
expt 1	69.2	2.08	75.54	0
expt 2	60.1	0.59	75.8	0
P. yoelii NS.				
expt 1	64.7	0	125.2	0.3
expt 2	76.6	0	109.1	0

[a] Percent of parasitemia in control mice observed at the end of a 4-day test. Concentrations indicated in chloroquine base equivalents.

Table 3. Antimalarial Activity of CQDP and **1a** on *P. berghei* N. and *P. yoelii* NS. Mortality in Mice

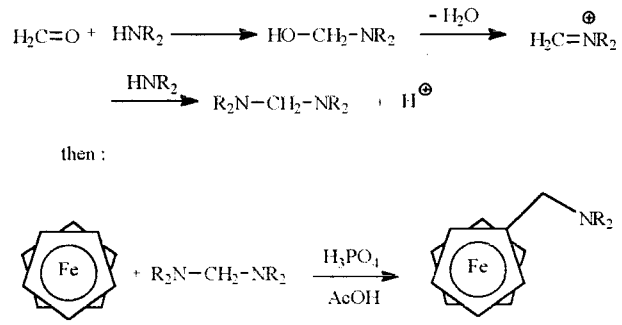
parasite strain	CQ 1 mg/kg	CQ 10 mg/kg	**1a** 1 mg/kg	**1a** 10 mg/kg	none
P. berghei N.					
expt 1	5/5	5/5	5/5	0/5	5/5
expt 2	5/5	5/5	5/5	0/5	5/5
P. yoelii NS.					
expt 1	5/5	5/5	5/5	0/5	5/5
expt 2	5/5	5/5	5/5	2/5	5/5

Biological Activities. The screening procedure is described in the Experimental Section, and the antimalarial activity of the compound described herein is given in Tables 1−3.

1. In Vitro Assessments of Antimalarial Activity. The results summarized in Table 1 indicated that, against the chloroquine-sensitive lineage SGE2, FG2, and FG4, the new water-soluble ferrocene analogue and chloroquine had a similar level of activity. Against the semi-chloroquine-resistant FG3 strain, **1a** was found to be more active than CQDP, the IC_{50} ratio being about 5. Also, **1a** proved to be far more active against the highly chloroquine-resistant lineages FCM6, FCM17, and FG1. In fact, the ratio of IC_{50} indicated that compound **1a** was about 22 times more effective against the parasite than CQDP.

2. Blood Schizontocidal Activity in Mice. Table 2 shows the results of preliminary in vivo tests. Concentrations are indicated in chloroquine base equivalents. We observed that **1a** appears as efficient as CQDP upon the two strains in a 4-day test. Survival study up to 60 days after the end of a 4-day test (Table 3) indicates that for **1a** at the highest dose all mice were cured from *P. berghei* N. infection and only 20% of mice

Scheme 2. Synthesis of [(*N,N*-Substituted amino)methyl]ferrocenes



showed a recrudescence of infection with *P. yoelii* NS. Recrudescence occurred in all cases in chloroquine-treated mice.

The structure−activity relationships demonstrate that the antimalarial effect increased due to the presence of ferrocene. The main criteria for effectiveness of our new drug **1a** against the malaria parasite could be its ability to penetrate infected cells.

Many observations showed that CQ accumulation and efflux kinetics are different in sensitive and resistant *P. falciparum* strains.[13] As **1a** totally inhibits the resistance to CQ, the mechanism of action of the coupled molecules should be different from that of chloroquine: the reason for this difference has been unknown until now. But we could suggest that **1a** was not released (or was slower than CQ) by resistant *P. falciparum*. Further studies to examine the kinetics of **1a** accumulation and release by the parasite are in progress.

Experimental Section

Chemistry. NMR spectra were recorded on a Brucker AC 300 spectrometer, IR spectra on a Perkin-Elmer 1420 instrument, and UV spectra on a Uvikon 930 spectrometer. EI mass spectra were acquired with a quadrupole instrument (Nermag R 10-10 H). Melting points are uncorrected. Merck's Kieselgel 60 PF254 was used for layer chromatography.

[(Dimethylamino)methyl]ferrocene is available commercially. The other [(*N,N*-substituted amino)methyl]ferrocenes have to be prepared as described below for [(diethylamino)-methyl]ferrocene (Scheme 2).

Synthesis of [(Diethylamino)methyl]ferrocene. Diethylamine (830 μL, 8 mmol) was added dropwise to a stirred solution of formaldehyde (300 μL, 4 mmol) in water (37%) at 0 °C. The reaction mixture was then stirred at room temperature for 45 min. Pellets of potassium hydroxide were next added until saturation. Diethyl ether (25 mL) was added, and the resulting organic layer was dried over Na_2SO_4 and distilled (T = 107 °C; P = 17 mmHg). *N,N,N′,N′*-tetraethylmethylenediamine was isolated as an oil (190 mg, 30%).

A mixture of ferrocene (2.79 g, 15 mmol), *N,N,N′,N′*-tetraethylmethylenediamine (2.37 g, 15 mmol), and phosphoric acid (1.3 mL) was heated in acetic acid for 5 h. After the mixture cooled to room temperature, a solution (20 mL) of H_2O/Et_2O (1:1) was added, the aqueous extract was made alkaline, and the free base was extracted with CH_2Cl_2 (2 × 100 mL). The organic layers were combined, dried over Na_2SO_4, and evaporated to dryness. The red oil was purified by TLC (elution with Et_2O/hexane/triethylamine) (1.504 g, 37%): ^{1}H NMR ($CDCl_3$) δ 4.15 (m, 2H), 4.10 (m, 7H), 3.50 (s, 2H), 2.42 (m, 4H), 1.02 (m, 6H).

The two other derivatives were synthesized following an identical procedure.

Lithiation of [(Dimethylamino)methyl]ferrocene (2) To Form 2′. Under nitrogen, a stirred solution of [(dimethylamino)methyl]ferrocene (**2**; 2.43 g, 10 mmol) in 20 mL of anhydrous diethyl ether was treated with *n*-butyllithium in hexane (5 mL, 12.5 mmol). Metalation was completed by stirring for 16 h at room temperature, and the crude solution was employed as described below.

Condensation of 2′ with *N,N*-Dimethylformamide. The solution described above was reacted with *N,N*-dimethylformamide (0.8 mL, 12.5 mmol) under nitrogen at room temperature. After 4 h the compound was hydrolyzed by addition of water (20 mL). The organic layer was separated, and the remaining aqueous phase was washed with small portions of diethyl ether (2 × 20 mL). The Et_2O extracts were combined, dried over Na_2SO_4, and evaporated to dryness to give a red oil which was purified through silica gel chromatography. Elution with Et_2O/hexane/triethylamine (70:20:10) gave 2-[(*N,N*-dimethylamino)methyl]ferrocenecarboxaldehyde (**3**; 1.9 g, 70%): ^{1}H NMR ($CDCl_3$) δ 10.10 (s, 1H), 4.81 (m, 1H), 4.61 (m, 1H), 4.56 (m, 1H), 4.21 (s, 5H), 3.85 (d, 1H, J = 13 Hz), 3.35 (d, 1H, J = 13 Hz), 2.23 (s, 6H); MS (EI) *m/e* 271 ($M^{\bullet+}$), 256 ($M^{\bullet+}$ − CHO), 227 ($M^{\bullet+}$ − NMe_2), 198 ($M^{\bullet+}$ − (CHO + NMe_2)), 163, 121, 95, 58, 56.

Synthesis of 2-[(*N,N*-Dimethylamino)methyl]ferrocenecarboxaldehyde Oxime (4). A solution of sodium hydroxide (0.48 g, 12.2 mmol) in water (3 mL) was added to a stirred mixture of **3** (1 g, 3.7 mmol) and hydroxylamine hydrochloride (0.42 g, 6 mmol) in EtOH (25 mL) at room temperature. The resulting solution was stirred under reflux for 2 h, quenched by the addition of water, neutralized by treatment with CO_2 gas, and extracted with portions of CH_2Cl_2 (2 × 25 mL). The combined extracts were dried over Na_2SO_4 and evaporated to dryness under reduced pressure to give **4** (1.03 g, 98%):^{1}H NMR ($CDCl_3$) δ 8.03 (s, 1H), 4.54 (m, 1H), 4.35 (m, 1H), 4.29 (m, 1H), 4.13 (s, 5H), 3.85 (d, 1H, J = 13.07 Hz), 3.33 (d, 1H, J = 13.07 Hz), 2.22 (s, 6H); MS (EI) *m/e* 286 ($M^{\bullet+}$), 269 ($M^{\bullet+}$ − OH), 226, 176, 163, 121, 95, 58, 56.

Reduction of 4 To Form 2-[(*N,N*-Dimethylamino)-methyl]ferrocenylmethylamine (5). $LiAlH_4$ (0.1 g, 2.6 mmol) and **4** (0.066 g, 0.8 mmol) were combined in THF under nitrogen at room temperature. The reaction mixture was then heated under reflux for 6 h. The cooled solution was diluted with Et_2O, washed with brine, dried over K_2CO_3, and evaporated to dryness to obtain pure **5** as an oil (0.060 g, 95%):^{1}H NMR ($CDCl_3$) δ 4.13 (m, 1H), 4.11 (m, 1H), 4.04 (s, 5H), 4.01 (m, 1H), 3.65 (d, 1H, J = 13.87 Hz), 3.61 (d, 1H, J = 12.48 Hz), 3.44 (d, 1H, J = 13.87 Hz), 2.87 (d, 1H, J = 12.48 Hz), 2.14 (s, 6H); MS (EI) *m/e* 272 ($M^{\bullet+}$), 255 ($M^{\bullet+}$ − NH_3), 227 ($M^{\bullet+}$ − $HNMe_2$), 199, 161, 134, 121, 77, 58, 56.

Condensation of 5 with 4,7-Dichloroquinoline To Form 6a. A mixture of **5** (0.55 g, 2 mmol), 4,7-dichloroquinoline (2 g, 10 mmol), triethylamine (2 mL, 14.4 mmol), and K_2CO_3 (0.4 g, 2.9 mmol) in *N*-methyl-2-pyrrolidinone (7 mL) was stirred under nitrogen at 135 °C for 4 h and, after cooling to room temperature, diluted with CH_2Cl_2 (50 mL). The reaction mixture was washed with brine (10 × 50 mL) and dried over Na_2SO_4. The organic phase was then reduced under vacuum, and the resulting oil was purified by TLC (silica gel, using AcOMe/hexane/triethylamine, 45:50:5) (0.520 g, 60%): mp 193−195 °C; IR (KBr) ν_{max} 3680, 3420, 3020, 2400, 1220, 930, 750, 670 cm^{-1}; UV ($CHCl_3$) λ_{max} 217, 255, 334 nm; ^{1}H NMR ($CDCl_3$) δ 8.53 (d, 1H, J = 5.39 Hz), 7.91 (d, 1H, J = 2.14 Hz), 7.61 (d, 1H, J = 8.96 Hz), 7.26 (dd, 1H, J = 2.14, 8.96 Hz), 6.46 (d, 1H, J = 5.39 Hz), 4.35 (d, 1H, J = 13.12 Hz), 4.28 (m, 1H), 4.17−4.15 (m, 2H), 4.15 (s, 5H), 4.09 (m, 1H), 3.80 (d, 1H, J = 12.57 Hz), 2.88 (d, 1H, J = 12.57 Hz), 2.22 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 152.2 (CH), 150.1 (C^{IV}), 149.3 (C^{IV}), 134.6 (C^{IV}), 128.3 (CH), 124.7 (CH), 122.2 (CH), 117.9 (C^{IV}), 98.9 (CH), 83.9 ($2C^{IV}$), 71.4 (CH), 70.5 (CH), 69.2 (5CH), 65.9 (CH), 58.1 (CH_2), 44.9 ($2CH_3$), 42.5 (CH_2); MS (EI) *m/e* 435 ($M^{\bullet+}$ ^{37}Cl), 433 ($M^{\bullet+}$ ^{35}Cl), 390 ($M^{\bullet+}$ ^{37}Cl − ($HNMe_2$)), 388 ($M^{\bullet+}$ ^{35}Cl − ($HNMe_2$)), 271, 256, 213, 134, 121, 91, 77, 58, 56. Anal. ($C_{23}H_{24}N_3FeCl$) C, H, N.

The preparation of compounds **6b**−**d** is analogous to the preparation of **6a**.

Conversion of 6a to the Ammonium 1a. A solution of L-(+)-tartaric acid (0.15 g, 1 mmol) in acetone was added dropwise to a solution of **6a** (0.21 g, 0.5 mmol) in acetone at room temperature. The resulting precipitate was collected by filtration and washed with Et_2O giving **1a** (0.33 g, 0.45 mmol,

90%): UV (H_2O) λ_{max} 204, 218, 252, 311 nm; ^1H NMR (D_2O) δ 8.40 (d, 1H, J = 7.08 Hz), 8.18 (d, 1H, J = 9.01 Hz), 7.92 (s, 1H), 7.67 (d, 1H, J = 9.01 Hz), 6.98 (d, 1H, J = 7.08 Hz), 4.75−4.33 (m, 12H), 4.31 (m, 4H), 2.85 (s, 3H), 2.75 (s, 3H). Anal. ($C_{31}H_{36}N_3O_{12}FeCl$) C, H, N.

Biology. 1. In Vitro Activity of Chloroquine Analogues. Culture-adapted parasites were maintained in continuous culture by a modified method of Trager and Jessen.[14] The FCM6 and FCM17 clones come from Thailand. The SG2 strain was preformed from Zaire. The FG1, FG2, FG3, and FG4 uncloned lineages were isolated from Gabonese individuals[15] (Domarle et al., in press). The drug activity was evaluated by using a modification of the proliferation test described by Desjardin et al.[16] based on hypoxanthine incorporation, a metabolite of the purine base synthesis. At the young tropozoite stage, parasites were treated by chloroquine and **1a** for 48 h (necessary time for the parasite to grow from tropozoite stage to schizonte) at the concentration ranged 0.001−10 μg/mL in triplicate.

The [*G*-^{3}H]hypoxanthine (Amersham, U.K.) incorporation by schizontes was measured in liquid a scintillation counter (Beckman). Cultures of parasites without drugs were used as control. With the aim of comparing the chloroquine and **1a** activity, the 50% inhibitory concentration (IC_{50}) was calculated of the regression line by projection of a straight line through the mean dpm (radioactivity level) between maximum and minimum values. We have designated the strain as chloroquine-sensitive if IC_{50} is lower than 0.1 μg/mL, semichloroquine-resistant if IC_{50} ranges from 0.1 to 1 μg/mL, and chloroquine-resistant if IC_{50} averages above 1 μg/mL.

2. In Vivo Antimalarial Studies. Chloroquine diphosphate (Sigma) and **1a** were administered in solution in PBS (NaCl, 136.9 mM; KCl, 2.7 mM; KH_2PO_4, 1.47 mM; Na_2HPO_4, 8 mM). Doses were calculated in chloroquine base equivalents for the two products. *P. berghei* strain N. (sensitive to chloroquine) and *P. yoelii* NS. (nonsensitive to chloroquine) were used for experiments.[17] In vivo antimalarial activity was measured using the 4-day test.[17] Female Swiss mice (Janvier) weighing 25 g were infected at day 0 with 10^7 infected RBC from a donor exhibiting 20−30% parasitemia. Drugs were administered subcutaneously once daily from day 0 to day 3 (4 × 1 or 4 × 10 mg/kg, respectively). Control mice received an identical volume of PBS. Thin blood films were made at day 4 and stained with Giemsa. Parasitemia were recorded on 1000 RBC. Survival of mice at the end of the test was studied up to 60 days, and controls for parasitemia were done on stained thin blood films.

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Supporting Information Available: Extra experimental data (Tables 4, 5) (3 pages). Ordering information is given on any current masthead page.

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