

## Stereochemistry of Dimeric Microbial Metabolites of Primaquine

Charles D. Hufford,\* Alice M. Clark, and  
James D. McChesney

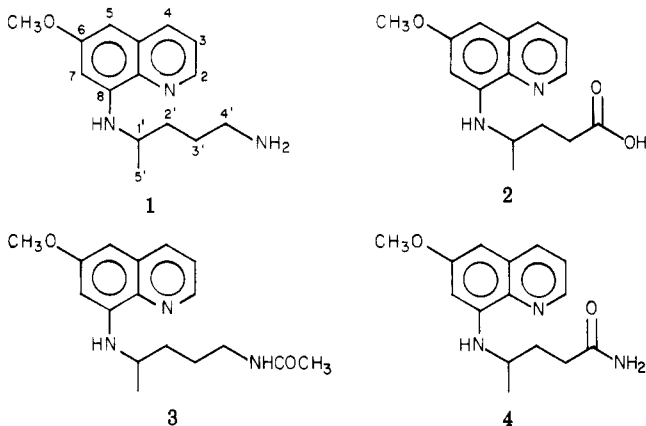
Department of Pharmacognosy, School of Pharmacy, The  
University of Mississippi, University, Mississippi 38677

John K. Baker

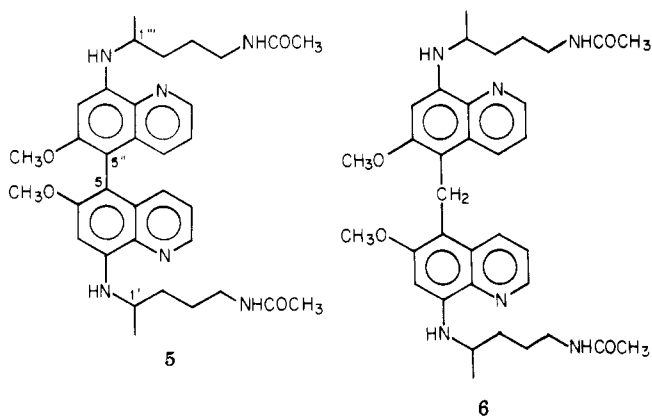
Department of Medicinal Chemistry, School of Pharmacy,  
The University of Mississippi, University,  
Mississippi 38677

Received December 15, 1983

Recent studies on the metabolism of the antimalarial drug, primaquine (1), have shown that the major fungal and mammalian metabolite is 8-(3-carboxy-1-methylpropylamino)-6-methoxyquinoline (2).<sup>1</sup> A second micro-



bial metabolite was identified as the *N*-acetyl derivative of 1 (3).<sup>1a</sup> Further microbial metabolism studies of 2 have led to the identification of 4 and to the speculation that 2 is excreted as a conjugate in mammals.<sup>2</sup> Additional microbial studies of 1 have led to the identification of two novel symmetrical dimeric derivatives 5<sup>3</sup> and 6,<sup>4</sup> both of which have been synthesized.



HPLC analyses of 5 obtained from both synthetic and microbial origin showed the presence of three peaks

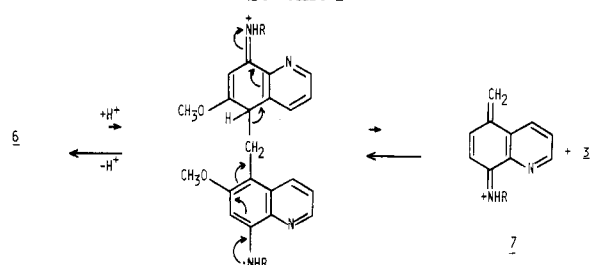
(1) (a) Clark, A. M.; Hufford, C. D.; McChesney, J. D. *Antimicrob. Agents Chemother.* 1981, 19, 337. (b) Baker, J. K.; McChesney, J. D.; Hufford, C. D.; Clark, A. M. *J. Chromatogr.* 1982, 230, 69.

(2) Hufford, C. D.; Clark, A. M.; Quinones, I. N.; Baker, J. K.; McChesney, J. D. *J. Pharm. Sci.* 1983, 72, 92.

(3) Clark, A. M.; Hufford, C. D.; Puri, R. K.; McChesney, J. D. *Appl. Environ. Microbiol.* 1984, 47, 540.

(4) Clark, A. M.; Hufford, C. D.; Gupta, R. C.; Puri, R. K.; McChesney, J. D. *Appl. Environ. Microbiol.* 1984, 47, 537.

Scheme 1<sup>a</sup>



<sup>a</sup> R = -(CH<sub>2</sub>)<sub>3</sub>NHCOCH<sub>3</sub>.

(designated I, II, and III in order of elution; 1:2:1, respectively). Since (±)-primaquine was used as starting material and the possibility of atropisomerism exists about the biphenyl-like linkage and since the molecule is symmetrical, a total of six stereoisomers (three enantiomeric pairs of diastereoisomers) would be expected which would result in three chromatographic peaks with area ratios of 1:2:1.<sup>5</sup> The three peaks I, II, and III have been separated by preparative HPLC and shown to be C-5-C-5' linked dimers by <sup>1</sup>H NMR and mass spectroscopy.<sup>3</sup>

Biphenyl-like compounds with ortho-substituted methoxy groups have been shown to exhibit atropisomerism with different degrees of stability.<sup>6</sup> In order to verify atropisomerism of 5 each of the isomers I, II, and III was heated (192 °C) in separate sealed tubes in 1-octanol. After 2.5 h the solutions were examined by HPLC. Upon heating<sup>7</sup> 5-I was epimerized to 5-III and 5-III to 5-I as would be expected if restricted rotation about the biphenyl-like linkage occurred.<sup>8</sup> The HPLC analysis of 5-II after heating showed no change.<sup>9</sup> Finally, 5 was prepared from (-)-primaquine.<sup>10</sup> Examination by HPLC showed only the presence of I and III.<sup>11</sup> These experiments confirm that the isomers of 5 obtained are the result of the chirality of the two asymmetric carbons in the side chain, restricted rotation about the biphenyl-like linkage, and the symmetrical nature of the dimer.

HPLC analysis of 6 obtained from both synthetic and microbial origin<sup>4</sup> showed the presence of two peaks (designated A and B in order of elution; 1:1). Since (±)-primaquine was used as starting material in both cases, a total of three stereoisomers of 6 would be expected; a pair of enantiomers and a diastereoisomer (meso form) which would lead to two separable peaks observed by HPLC.<sup>12</sup>

(5) The possible stereoisomers would be the *RRR* and its enantiomer *SSS* (peak I or III), *SRS* and its enantiomer *RSR* (peak I or III), and *RSS* (≡*SRR*) and its enantiomer *SSR* (≡*RSS*) (peak II). The first letter listed is arbitrarily assigned to the configuration of C-1'', the second letter to the configuration of the biphenyl linkage and the third letter to the configuration of C-1'. The area for peak II would be twice that for I and III since it can form in two different ways.

(6) Kawano, N.; Okigawa, M.; Hasaka, N.; Kouno, I.; Kawahara, Y.; Fujita, Y. *J. Org. Chem.* 1981, 46, 389. Rahman, W.; Ilyas, M.; Okigawa, M.; Kawano, N. *Chem. Pharm. Bull.* 1982, 30, 1491 and the references cited therein.

(7) The isomers were stable indefinitely at room temperature. The *t*<sub>1/2</sub> for the conversions was 132 min at 192 °C.

(8) As an example, the *RRR* would be converted to the *RSR* and the *SSS* to the *SRS*; likewise the *SRS* would be converted to the *SSS* and the *RSR* to the *RRR*. (See ref 5.) This results in interconversions between isomers I and III.

(9) In this case, *RSS* is converted to *RSS* but by symmetry this is identical with *SSR*, the enantiomer of *RRS*. Thus, in the case of 5-II, conversion between enantiomers is occurring but this is not detected by HPLC.

(10) (±)-Primaquine has been resolved into its optically active forms. Carroll, F. I.; Berrang, B.; Linn, C. P. *J. Med. Chem.* 1978, 21, 326. The absolute stereochemistry of the (+) and (-) forms has not been established.

(11) Peak II which represents opposite stereochemistries in the side chain cannot be generated from optically active primaquine.

While 6-A and -B could be separated by preparative HPLC, both A and B equilibrated back to the original mixture (1:1).<sup>13</sup> This behavior seemed difficult to rationalize based on structure 6. The isolation of A and B and equilibration back to the original mixture was somewhat like that observed for syn and anti isomerisms of oximes and nitrosamines. Another explanation would be the possible interconversions via the intermediates shown in Scheme I which are also the presumed intermediates formed in the synthesis of 6 from 3 and formaldehyde/formic acid.<sup>4</sup> Addition of 7 to 3 could explain the conversions between 6-A and -B since both the (+) and (-) forms of 7 and 3 would be present.

Synthesis of 6 from both (+)- and (-)-primaquine was accomplished by using the same procedure as for the ( $\pm$ ) form.<sup>4</sup> HPLC analyses of the products from both showed only one peak (6-B) even after standing in solution at room temperature for several days. This result rules out any explanation invoking syn-anti-type isomerism. By mixing equal quantities of 6 prepared from (+)-primaquine with that prepared from (-)-primaquine, the formation of peak 6-A could be followed by HPLC and was complete after 3 days. This last experiment provides convincing evidence that 6-A and -B<sup>14</sup> are interconverted as shown in Scheme I.

The formation of the novel dimeric compounds 5 and 6 from microbial enzyme systems may have implications for the complete understanding of the metabolism of primaquine. In addition, the stereochemical studies described here show that dimers like 5 do show atropisomerism and that 6-A and -B can accomplish epimerization without direct involvement of the asymmetric carbon atoms.<sup>15</sup>

### Experimental Section

**General HPLC Methods.** For the quantitative analysis of the three isomers of 5 and the two isomers of 6, a C-18 reversed-phase, 5  $\mu$ m-particle size HPLC system (Whatman PXS 5/25 ODS column) was utilized. The mobile phase (1.0 mL/min) was prepared by using 8.4 g of  $\text{KH}_2\text{PO}_4$ , 6.6 g of  $\text{K}_2\text{HPO}_4$ , 4.0 g of *N,N*-dimethyloctylamine, 2.8 L of  $\text{CH}_3\text{OH}$ , and 1.2 L of water. The chromatographic peaks were detected by using a dual wavelength unit (Waters Assoc. Model 440) operating at 254 and 280 nm in conjunction with a dual-pen strip chart recorder. Identifications of the components of the mixtures were based on a comparison of the retention time and the  $A_{254}/A_{280}$  ratio of each component.

**Equilibration Studies for 5-I, -II, and -III.** The isomers I, II, and III (retention times 6.5, 8.7, 11.8 min, respectively) obtained in pure form as described previously<sup>3</sup> were dissolved in 1-octanol (a few  $\mu$ g per 20  $\mu$ L) in separate capillary tubes which were then sealed. The sealed tubes were placed in a sand bath at 192 °C. The capillary was removed at the specified times and broken, a few microliters withdrawn, resealed and then replaced in the sand bath. The initial solution ( $t = 0$ ) and samples at  $t = 60, 150,$  and 210 min were analyzed by HPLC. The conversion of 5-I to a 1:1 equilibrium mixture of 5-I and 5-III was found to follow simple first-order kinetics. Linear regression analysis of the data gave a half-life of 132 min. Essentially the same results were found from following the conversion of 5-III to the equilibrium mixture of the two isomers.

(12) The peaks would be expected to have the same area ratios since one peak would represent the pair of enantiomers ( $\text{RCH}_2\text{R}$  and  $\text{SCH}_2\text{S}$ ) while the other one would represent the meso form ( $\text{RCH}_2\text{S}$ ) which could form in two ways.

(13) The  $t_{1/2}$  for interconversion was 10.6 h at room temperature in the mobile phase used for HPLC.

(14) Peak A thus represents the  $\text{RCH}_2\text{S}$  dimer while peak B is an enantiomeric mixture of  $\text{RCH}_2\text{R}$  and  $\text{SCH}_2\text{S}$ .

(15) In some related studies using chiral HPLC stationary phases, details of which will be published later, we have now separated 5 into its six stereoisomers and 6 into its three stereoisomers.

**HPLC Separation of 6-A and -B and Determination of Half-Life.** A 1:1 mixture of 6-A and 6-B was injected onto a 10  $\mu$ m, C-18, reversed-phase column (Waters,  $\mu$ -Bondapak C-18) by using the same mobile phase; however, a 1.5 mL/min flow rate was used. The chromatographic experiment showed base-line separation of 6-A and 6-B (retention times 29.1, 35.2 min, respectively). The pure fractions were stored at room temperature in the mobile phase. A second C-18 reversed-phase HPLC system was then used to follow the kinetics of the conversion of 6-A to the 1:1 equilibrium mixture of 6-A and 6-B. A least-squares regression analysis of the data showed a half-life of 10.6 h for the interconversion at room temperature in the methanol-phosphate buffer system.

**Synthesis of 6 from Optically Active Primaquine (1) and Equilibration of 6-A and -B.** A 5-mg sample each of (+)- and (-)-primaquine (1) diphosphate was acetylated by using acetic anhydride-pyridine at room temperature for 0.5 h (no starting material, TLC). The evaporated residue was then converted to 6 by using the same procedure as previously described.<sup>4</sup> The evaporated residues from this reaction were purified by flash chromatography over alumina (Woelm, N, III) using ethyl acetate-*n*-hexane (1:1). About 1 mg of purified 6 was obtained from each isomer. These were then analyzed by HPLC. Samples of 6 prepared from (+)- and (-)-primaquine were then mixed (equal quantities) and allowed to stand at room temperature in methanol and the equilibration followed by HPLC.

**Synthesis of 5 from (-)-Primaquine (1).** (-)-Primaquine (1) diphosphate (4 mg) was acetylated as described previously for 6 and converted to 5 by using the procedure outlined previously.<sup>3</sup> The HPLC analysis was performed on the residue after workup.

**Acknowledgment.** This investigation received the financial support of the UNDP/World Bank/World Health Organization Special Programme for Research and Training in Tropical Diseases. We also gratefully acknowledge Walter Reed Army Institute of Research for the samples of (+)- and (-)-primaquine diphosphate.

### Synthesis of *N*-(3-(Dimethylamino)-2-(substituted thio)-2-propenylidene)-*N*-methylmethanaminium Salts via 2-(Alkylthio)- and 2-(Arylthio)-3-(dimethylamino)acroleins

Donald P. Matthews

Pharmaceutical Research and Development, Medicinal Chemistry Group, Merrell Dow Research Institute, Indianapolis, Indiana 46268

Received February 6, 1984

Derivatives of *N*-(3-(dimethylamino)-2-propenylidene)-*N*-methylmethanaminium salts (4) are well-known in the literature as intermediates for the synthesis of 5-substituted pyrimidines and other heterocyclic systems.<sup>1</sup> Although 2-aryl, alkyl, alkoxy, and alkylamino derivatives are known, the 2-substituted thio derivatives have not been reported. Our initial attempts at preparing these 2-thio analogues using standard literature procedures<sup>2</sup> for known methanaminium derivatives were unsuccessful.

We report herein a novel two-step synthetic procedure (Scheme I) to these compounds based on the known addition of sulfonyl halides to enamines.<sup>3</sup> The sulfonyl

(1) (a) Wagner, R. M.; Jutz, C. *Chem. Ber.* 1971, 104, 2975. (b) Zschacke, H. Z. *Chem.* 1977, 17, 63. (c) Jutz, C.; Kuchlechner, R.; Seidel, H. J. *Chem. Ber.* 1969, 102, 2301. (d) Lloyd, D.; Tucker, K. S. *J. Chem. Soc., Perkin Trans. 1* 1981, 3, 726.

(2) (a) Arnold, Z.; Holy, A. *Collect. Czech. Chem. Commun.* 1963, 78, 869. (b) Jutz, C.; Lobering, H.; Trinkel, K. *Synthesis* 1977, 332. (c) Kucera, J.; Arnold, Z. *Collect. Czech. Chem. Commun.* 1967, 32, 3793.