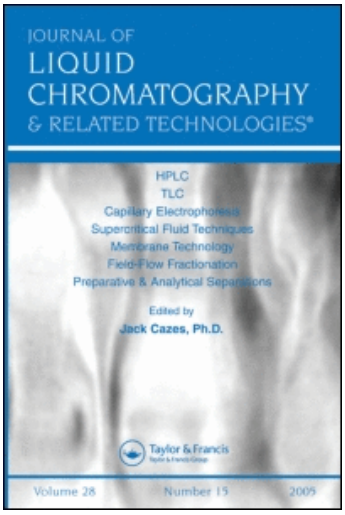

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

High Performance Liquid Chromatographic Separation of the Enantiomers and Diastereomers of Primaquine and Its Metabolites

John K. Baker[a]; Alice M. Clark[b]; Charles D. Hufford[b]
[a] Department of Medicinal Chemistry, School of Pharmacy University of Mississippi University, Mississippi [b] Department of Pharmacognosy, School of Pharmacy University of Mississippi University, Mississippi

To cite this Article Baker, John K. , Clark, Alice M. and Hufford, Charles D.(1986) 'High Performance Liquid Chromatographic Separation of the Enantiomers and Diastereomers of Primaquine and Its Metabolites', Journal of Liquid Chromatography & Related Technologies, 9: 2, 493 — 509
To link to this Article: DOI: 10.1080/01483918608076649
URL: http://dx.doi.org/10.1080/01483918608076649

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(2&3), 493-509 (1986)

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF THE ENANTIOMERS AND DIASTEREOMERS OF PRIMAQUINE AND ITS METABOLITES

John K. Baker[1], Alice M. Clark[2], and Charles D. Hufford[2]

[1]*Department of Medicinal Chemistry*
[2]*Department of Pharmacognosy*
School of Pharmacy
University of Mississippi
University, Mississippi 38677

ABSTRACT

Primaquine, which is marketed as a racemic mixture, was found to be metabolized by microorganisms to a biphenyl dimer, to a methylene linked dimer, and to a sulfur linked dimer. Using a conventional reversed-phase column, the biphenyl dimer could be resolved into three diastereomeric peaks while the methylene linked dimer and the sulfur linked dimer were each resolved into two diasteromeric peaks. Using a chiral Pirkle-1A column, primaquine could be resolved as two peaks, carboxyprimaquine as two peaks, the biphenyl dimer as six peaks, the methylene dimer as three peaks, and the sulfur dimer as three peaks.

INTRODUCTION

Primaquine (Fig. 1, I) is used as a racemic mixture of its two enantiomers for the treatment of the tissue forms of malaria.

 0148-3919/86/0902-0493$3.50/0

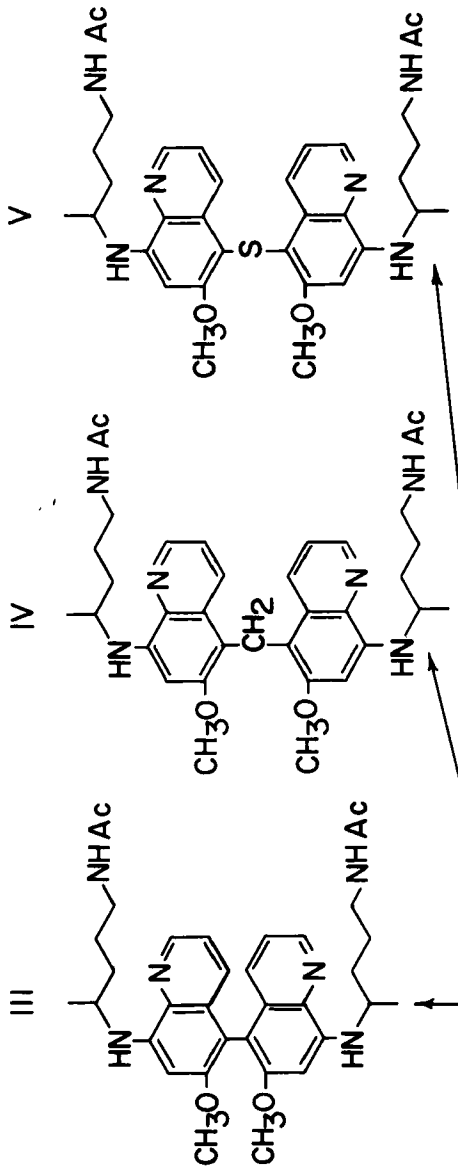

FIGURE 1

Metabolic Pathways for the Biotransformation of Primaquine with Mammalian or Microorganism Systems.

It has been shown that the major metabolite of primaquine by microorganisms (1) and mammalian systems (rat (2), monkey (3), and man (4)) is carboxyprimaquine (II). It has also been shown that microorganisms can metabolically convert primaquine to an N-acetylated metabolite (1) and this metabolite was further transformed to either a biphenyl metabolite (Fig. 1, III)(5), or a methylene linked dimer (IV)(6), or a sulfur linked dimer (V)(7).

Though the primaquine diphosphate in current clinical use is a racemic mixture, it has been shown that both the therapeutic activity and toxicity of the two enantiomers are not the same (8). It has been proposed that the difference in biological activity of the two isomers was due to differences in either the rate of formation of biologically active metabolites of the two primaquine stereoisomers or differences in the inherent activity of the isomers of the metabolites. It was also found that III produced by either synthesis or metabolism existed as 6 stereoisomers and IV existed as 3 stereoisomers (9). It was also found that some of the isomers of IV would spontaneously interconvert on standing in solution (9) and it has also been shown that some of the isomers of V would interconvert (7).

The major objectives of the present study were to develop a method for quantitating the relative concentrations of R- and S-primaquine and to develop assay methods for the various enantiomers and diastereomers of metabolites II, III, IV, and V. It was also anticipated that trends in the elution order of the enantiomers might prove useful in the assignment of the stereochemistry of new synthetic primaquine analogs or metabolites.

EXPERIMENTAL

Materials

Racemic primaquine diphosphate was utilized as obtained from the Aldrich Chemical Company. The individual (+) and (-)-isomers

of primaquine diphosphate were obtained from the Walter Reed Army Institute of Research. Though the enantiomers of primaquine have been separated, the absolute stereochemistry of the compound has not been established. To simplify the discussion of the results, it was arbitrarily assumed that (+)-primaquine was the R-enantiomer.

Metabolic Transformations

R-carboxyprimaquine (II) was prepared by fermentation from (+)-primaquine using a previously described procedure (1). A mixture of the six isomers of the biphenyl dimer (III) was prepared from racemic primaquine diphosphate by fermentation (5) using Streptomyces rimosus. A mixture of SSS- and SRS-biphenyl dimer was also prepared using the same procedure using S. rimosus with S-primaquine diphosphate.

A mixture of the three isomers of the methylene dimer (IV) was prepared from racemic primaquine diphosphate by fermentation (6) using Candida tropicalis. Fermentation of racemic primaquine diphosphate with S. roseochromogenus was used to produce mixtures of the methylene dimer (IV) and the sulfur dimer (V)(7). Early in the incubation with S. roseochromogenus approximately equal quantities of the methylene dimer and the sulfur dimer were produced while late in the incubation, the sulfur dimer predominated.

Preparative Scale Synthesis of VI

The carbonylimidazole derivative of primaquine (VI) was prepared by taking 1.0 g of primaquine diphosphate in water, then extracting the free base into ethyl acetate following the addition NH_4OH. After drying the solution with Na_2SO_4, the solvent was removed by evaporation under vacuum. The residue was redissolved in 5.0 ml of dry tetrahydrofuran along with 0.5g N,N'-carbonyldi-

imidazole (CDI) at room temperature. After the reaction had proceeded 1.0 hr., the solvent was removed under vacuum then the residue was partitioned between H_2O and ethyl acetate. The ethyl acetate fraction was purified by preparative chromatography (Silica gel, ethyl acetate) to give 350 mg of VI as a very light yellow glass. C-13 NMR: C-2, 144.4 ppm; C-3, 122.0; C-4, 134.9; C-4a, 130.1; C-5, 92.1; C-6, 159.5; C-7, 97.2; C-8, 144.9; C-8a, 135.4; CH_3O, 55.2, C-1', 40.8; C-2', 33.9; C-3', 26.1; C-4', 40.9; C-5, 20.7; imidazole, 116.3, 129.8, and 129.8. H-1 NMR: H-2, 8.5 ppm (d); H-3, 7.3 (dd); H-4, 7.9 (d); H-5, 6.2 (d); H-7, 6.3 (d); CH_3O, 3.9 (s); CH_3CH, 1.25 (d); imidazole, 6.95, 7.35, and 8.1. Infrared ($CHCl_3$ sol.): C=O, 1,715 cm^{-1}.

Analytical Scale Formation of VI and VII

Using 1.0 ml of freshly dried tetrahydrofuran; 1.0 mg of racemic primaquine, R-primaquine, S-primaquine, racemic carboxyprimaquine, or R-carboxyprimaquine were each reacted with 5.0 mg of carbonyldiimidazole at room temperature. For the kinetic study, the reaction was monitored for a total of 4.5 hr., but for the other assays, the mixture was chromatographed on the Pirkle-1A system after the sample had been allowed to react for one hour.

HPLC with Non-Chiral Columns

Standard reversed-phase analyses of the diastereomeric mixtures were conducted using a Whatman PXS 5/25 ODS column utilizing a 5 um, C-18 packing material. The mobile phase (1.0 ml/min) was prepared using 8.4g of KH_2PO_4, 6.6g of K_2HPO_4, 4.0g of N,N-dimethyloctylamine, 2.8L of CH_3OH, and 1.2L of water. The chromatographic peaks were detected using a dual wavelength unit (Waters Assoc. Model 440) operating at 254 nm and 280 nm.

HPLC with Chiral Columns

Pirkle type 1-A columns (4.6 mm x 250 mm) packed with spherical 5 micron aminopropyl packing modified with the N-3, 5-dinitrobenzoyl derivative of D-phenylglycine was utilized as obtained from the Regis Chemical Company. The mobile phase (1.0 ml/min) was prepared using 850 ml hexane, 105 ml 2-propanol, and 75 ml of acetonitrile. The chromatograms were obtained using a 254 nm UV-detector.

RESULTS AND DISCUSSION

Using the non-chiral, reversed-phase columns, the chromatograms of the biphenyl dimer (III) obtained from the fermentation of racemic primaquine were found to consist of three peaks (at 7 min, 9 min, and 12 min; Fig. 2). When the biphenyl dimer obtained by chemical synthesis from racemic primaquine was chromatographed, it was also found to consist of the same three peaks and the peak areas were found to have a 1:2:1 ratio. When the biphenyl dimer (III) was synthesized from R-primaquine, only the peaks at 7 minutes and 12 minutes were obtained. Because of the chiral centers in the side chains, one might have expected to observe only two chromatographic peaks (the RR, SS pair and the SR=RS pair). However, the restricted rotation of the hindered biphenyl systems introduced a third chiral center. Thus, when III was prepared from R-primaquine, the peak observed at 7 minutes would correspond to RSR-III and the other peak at 12 minutes would correspond to RRR-III (the biphenyl system's stereochemistry is represented by the middle letter and the stereochemistry of the side chain is represented by the first and last letter). When the peak at 7 minutes was isolated using preparative HPLC, the material was found to be very stable at room temperature, but when heated to 192^{o} the system would equilibrate to a mixture of the peak at 7 minutes and 12 minutes. When the peak at 9 minutes was isolated and then heat-

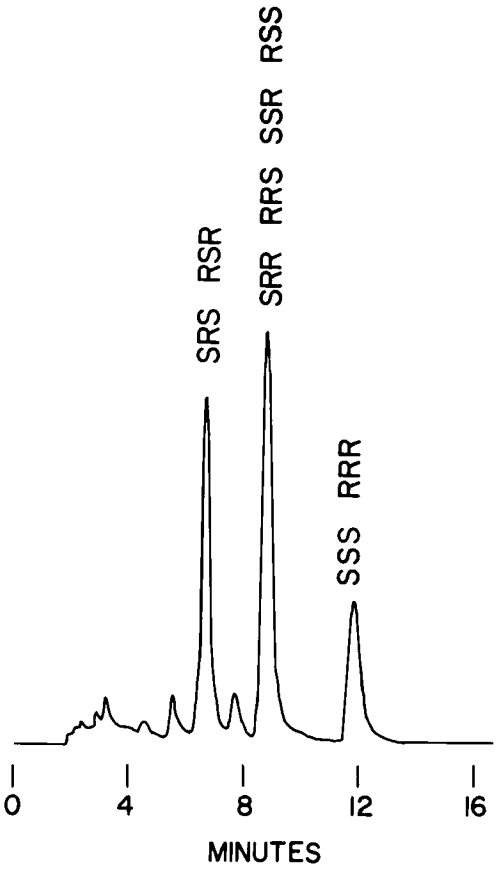

FIGURE 2

The Reversed-Phase Chromatogram of the Biphenyl Dimer (III) obtained from the Fermentation of Racemic Primaquine.

ed, it remained unchanged. These findings were consistent with the thermal interconversion of the stereochemistry of the biphenyl rings at elevated temperatures leading to an equilibration of the RRR-isomer with the RSR-isomer while the interconversion of the RRS-isomer to the RSS-isomer would not have been detected because of their enantiomeric relationship(9).

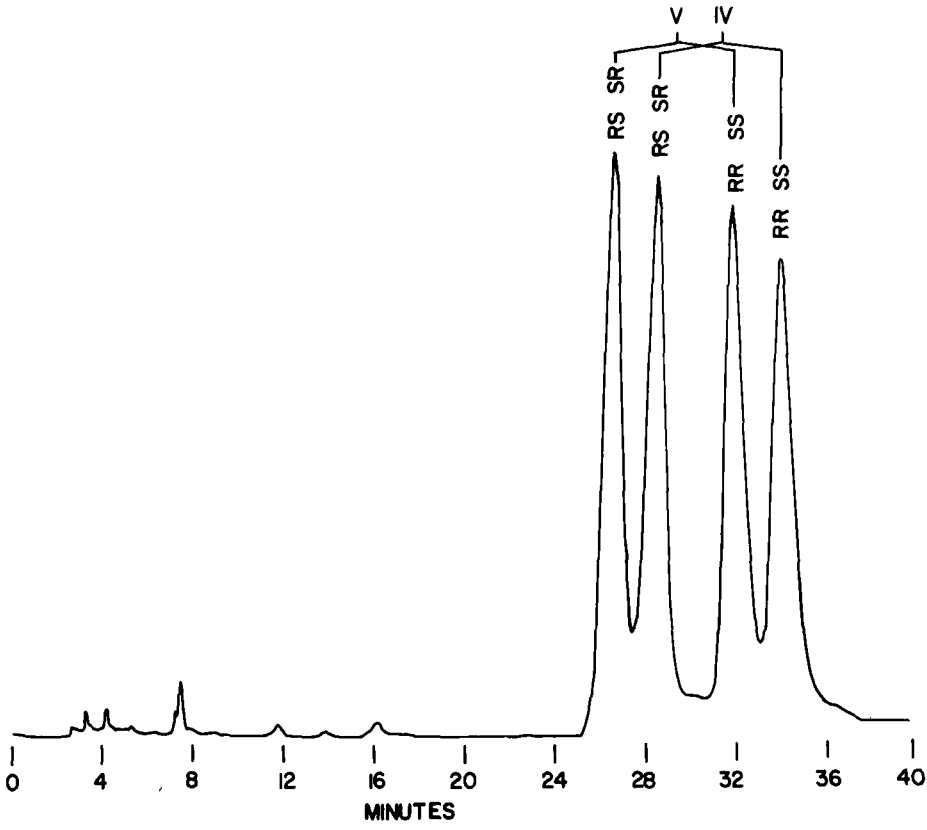

FIGURE 3

The Reversed-Phase Chromatogram of the Methylene Dimer (IV) and the Sulfur Dimer (V) obtained from the Fermentation of Racemic Primaquine by S. roseochromogenus.

With the methylene dimer (IV) the aromatic rings would be free to rotate at room temperature and only three isomers would be expected: the RR-isomer, the SS-isomer and the RS = SR meso pair. When the methylene dimer (IV) obtained from fermentation of racemic primaquine was chromatographed on the reversed-phase system, a peak at 26 minutes and a peak at 32 minutes was obtained (Fig. 3) which corresponded to the pair of meso isomers(RS and SR) and to

the pair of enantiomers(RR and SS). When the methylene dimer was prepared by chemical synthesis a 1:1 area ratio was observed by the 26 minutes and 32 minutes peak. When the methylene dimer was synthesized from R-primaquine or from S-primaquine, only the peak at 32 minutes was observed.

When the sulfur dimer (V) was prepared by fermentation (Fig. 3) from racemic primaquine, peaks at 28 and 34 minutes were observed. When either R-primaquine or S-primaquine was used for the starting material, only the peak at 34 minutes was observed. Thus the peak at 28 minutes could be assigned to the meso pair (RS-V and SR-V) and the peak at 34 minutes could be assigned to the mixture of the two enantiomers.

When the sample of the biphenyl dimer (III) obtained by chemical synthesis from racemic primaquine was chromatographed on the Pirkle 1-A column, all six isomers were observed and the peak areas were in the 1:2:2:1 ratio that would be expected from theory (Fig. 4, second from top). When the biphenyl dimer was prepared by chemical synthesis from S-primaquine, only two peaks were observed which would correspond to the SSS-isomer and the SRS-isomer (Fig. 4, third from top). When the biphenyl dimer obtained by fermentation starting from racemic primaquine was chromatographed, all six isomers were observed, but the isomers with R-stereochemistry in the side chain were metabolically favored over the isomers with S-configuration (Fig. 4, top).

Using the Pirkle 1-A column, the sulfur dimer (V) could be resolved into all three of the isomers expected from theory. Using racemic primaquine in the fermentation, the chromatogram of the isolated product showed a peak at 51 minutes for RR-V, 52 minutes for SS-V, and 60 minutes for the RS-V meso isomer (Fig. 5, bottom). To make the assignments of the stereochemistry of the three peaks, the sulfur dimer was prepared from S-primaquine by fermentation and only the peak at 52 minutes for SS-V was observed (Fig. 5, third from top) and co-injection of the two samples (Fig. 5, top) was also found to be consistent with the assignment. When the sulfur dimer was prepared from R-primaquine by fermentation, only

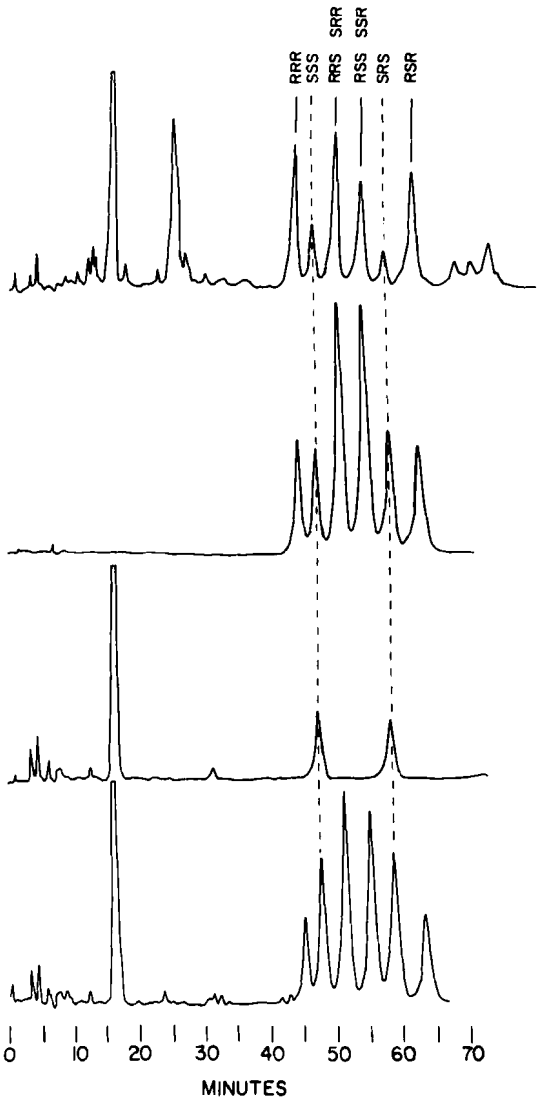

FIGURE 4

The Pirkle 1-A Chromatograms obtained for the Biphenyl Dimer (III). Top: III obtained from fermentation of racemic primaquine. Second: III obtained by synthesis from racemic primaquine. Third: III obtained from fermentation of S-primaquine. Bottom: Co-injection of second and third products.

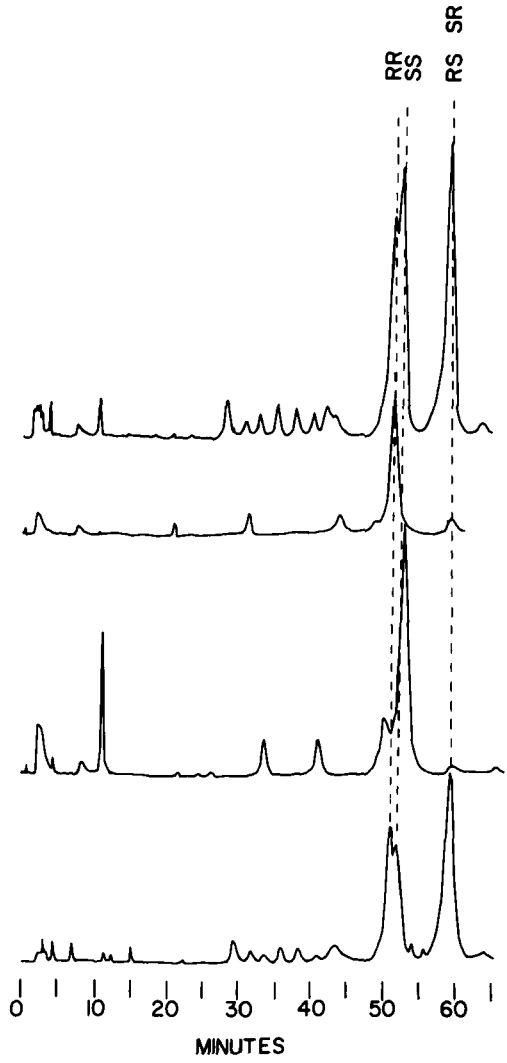

FIGURE 5

The Pirkle 1-A Chromatograms obtained for the Sulfur Dimer (V). Top: Co-injection of third and fourth products. Second: V obtained from the fermentation of R-primaquine. Third: V obtained from the fermentation of S-primaquine. Fourth: V obtained from the fermentation of racemic primaquine.

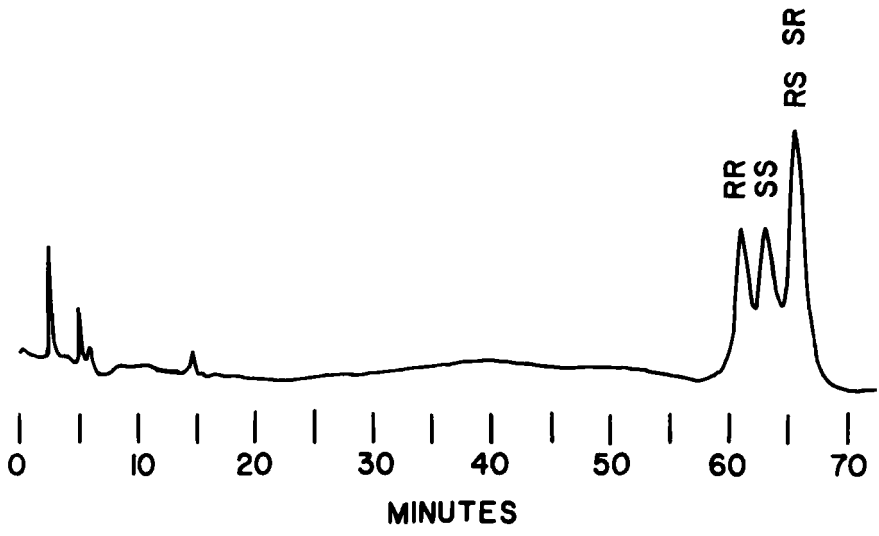

FIGURE 6

The Pirkle 1-A Chromatogram obtained for the Methylene Dimer (IV) prepared by Synthesis from Racemic Primaquine.

the peak at 51 minutes for RR-V was observed (Fig. 5, second from top).

The chromatogram of the methylene dimer (IV) obtained by chemical synthesis from racemic primaquine was found to have peaks at 61, 64, and 66 minutes in a peak area ratio of 1:1:2 (Fig. 6). It was also found that the methylene dimer prepared from R-primaquine exhibited only the peak at 61 minutes, while the dimer prepared from S-primaquine exhibited only the peak at 64 minutes. The methylene dimer obtained by fermentation showed all three peaks, but the peak area ratio was not exactly in the 1:1:2 ratio that would be expected on a purely statistical distribution of the isomers.

One of the objectives of the project was to develop methods for the analysis of the individual enantiomers of primaquine itself. Using the most polar mobile phase that was compatible with the chiral column, underivatized primaquine could not be eluted

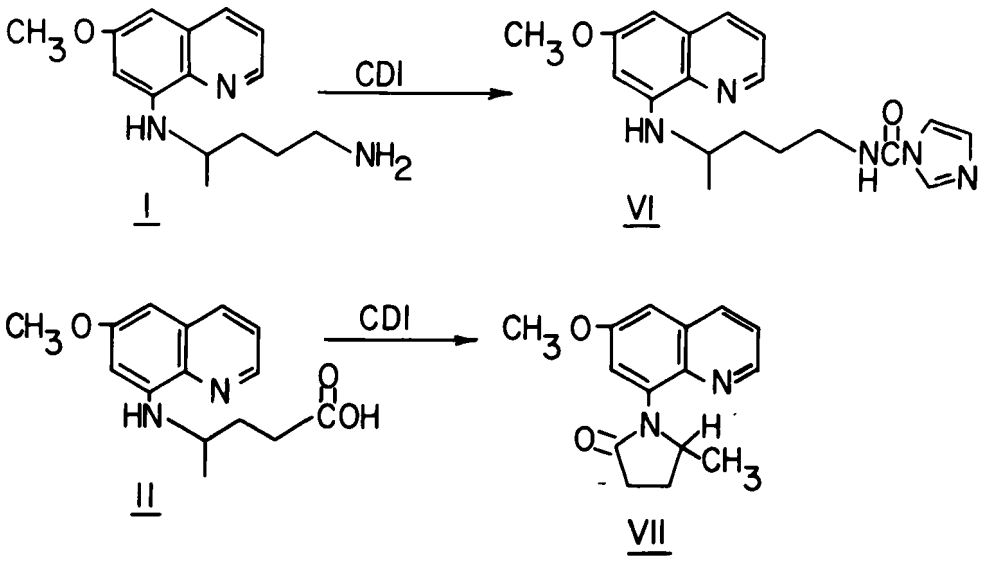

FIGURE 7

The Derivatization of Primaquine and Carboxyprimaquine with N,N-carbonyldiimidazole (CDI).

from the column. Previous reports (10) have also indicated that drugs containing free amine groups usually can not be chromatographed on these columns. Our initial effort at resolving the isomers of primaquine utilized acetic anhydride to form the amide of the primary amine group of the drug. It was found that the N-acetyl derivative could be eluted from the column, however the racemic mixture showed only one sharp peak.

While the N-acetyl derivative was not satisfactory, it was found that primaquine could easily be derivatized with carbonyldiimidazole to give a carbonylimidazole derivative (VI, Fig. 7) that could be resolved into the two enantiomeric peaks. Compound VI was also synthesized on a preparative scale and ^{1}H-NMR, ^{13}C-NMR, and IR spectral data were found to be consistent with the proposed structure. When carboxyprimaquine (II) was reacted with carbonyldiimidazole, a lactam (VII) was formed and the

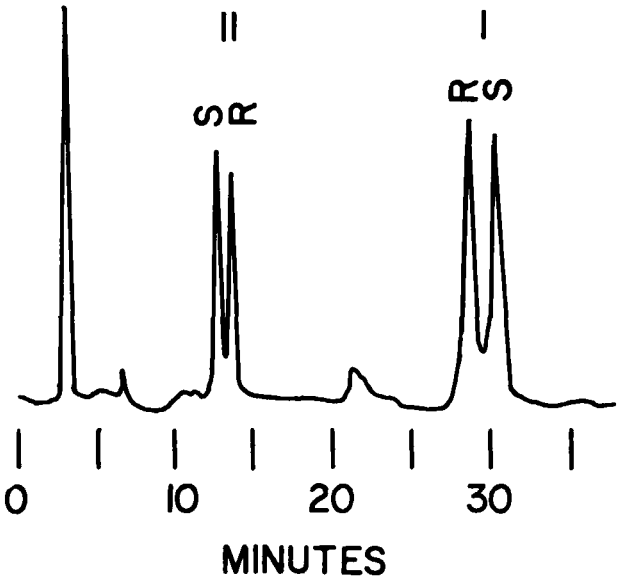

FIGURE 8

The Pirkle 1-A Chromatograms of the CDI Derivatives of Racemic Primaquine (I) and Racemic Carboxyprimaquine (II).

chromatograms of this product on the Pirkle 1-A column were found to be identical to that of a reference sample of VII previously synthesized (11).

The chromatograms of the CDI derivatives of racemic primaquine and racemic carboxyprimaquine were found to give very well resolved peaks for the pairs of enantiomers (Fig. 8). The assignments of the pairs of peaks were made by derivatizing separate samples of R-primaquine, S-primaquine, and R-carboxyprimaquine. The kinetics of the reaction of primaquine and carboxyprimaquine with CDI at room temperature were also followed (Fig. 9). It was found that the reaction was at 90% completion within 45 minutes.

In summary, it was found that all of the isomers primaquine (2 isomers), carboxyprimaquine (2 isomers), the biphenyl dimer (6 isomers), the methylene dimer (3 isomers), and the sulfur dimer (3 isomers) could be resolved with the Pirkle 1-A column. With the

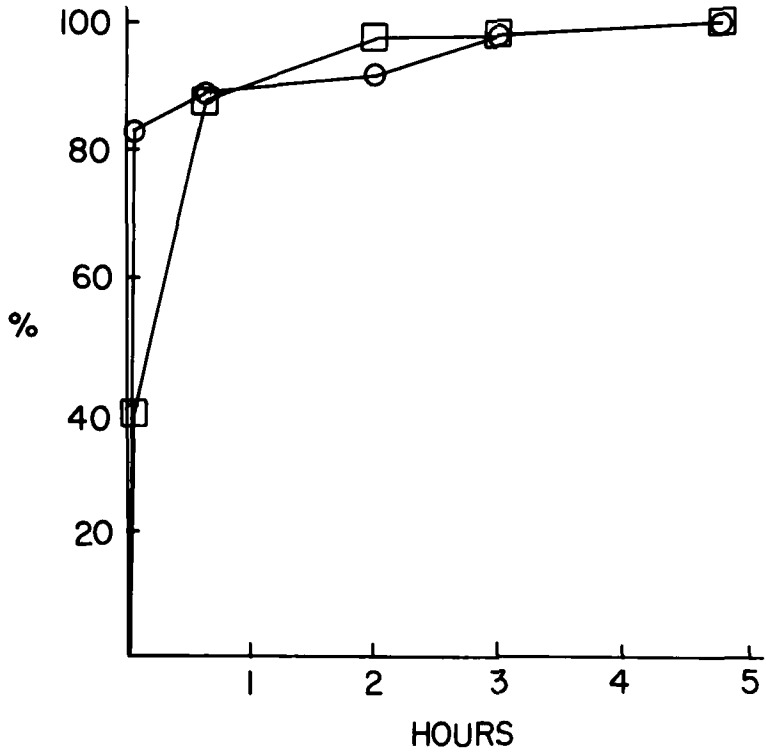

FIGURE 9

The Kinetics of the CDI Derivatization of Primaquine and Carboxyprimaquine at Room Temperature. Percent reaction vs. reaction time for primaquine (squares) and carboxyprimaquine (circles).

simple exception of carboxyprimaquine, it was always found that the isomer with the side chain methyl group with the R-configuration had shorter retention times compared to the S-isomer (Note: the assigment of the absolute stereochemistry was based on the assumption that (+)-primaquine had R-configuration). It was also found that the microbial metabolism of primaquine to carboxyprimaquine, the methylene dimer, and the sulfur dimer appeared to have little stereoselectivity. A significant amount of stereoselectivity was observed only for the metabolism of primaquine to the biphenyl dimer.

ACKNOWLEDGEMENT

This investigation received the financial support of the UNDP/-World Bank/World Health Organization Special Programme for Reseach and Training in Tropical Diseases.

REFERENCES

1. Clark, A.M., Hufford, C.D., and McChesney, J.D., Primaquine: Metabolism by Microorganisms and Carbon-13 Nuclear Magnetic Resonance Assignments, Antimicrob. Agents Chemother., 19, 337, 1981.

2. Baker, J.K., McChesney, J.D., Hufford, C.D., and Clark, A.M., High-Performance Liquid Chromatographic Analysis of The Metabolism of Primaquine and the Identification of a New Mammalian Metabolite, J. Chromatogr., 23, 69, 1982.

3. Baker, J.K., Bedford, J.A., Clark, A.M., and McChesney, J.D., Metabolism and Distribution of Primaquine in Monkeys, Pharm. Research, 98, 1984.

4. Mihaly, G.W., Ward, S.A., Edwards, G., Orme, M.L., and Breckenridge, Pharmacokinetics of Primaquine in Man: Identification of the Carboxylic Acid Derivative as a Major Plasma Metabolite, Br. J. Clin. Pharmac., 17, 441 (1984).

5. Clark, A.M., Hufford, C.D., Puri, R.K., and McChesney, J.D., Production of a Novel Dimeric Metabolite of Primaquine by Streptomyces rimosus, App. Microb. Environ. Microbiol., 47, 540, 1984.

6. Clark, A.M., Hufford, C.D., Gupta, R.C., Puri, R.K., and McChesney, J.D., Microbial Transformation of Primaquine by Candidia tropicalis, App. Microb. Environ. Microbiol., 47,. 537, 1984.

7. Hufford, C.D., Baker, J.K., McChesney, J.D., and Clark, A.M., A Novel Sulfur-Containing Microbial Metabolite of Primaquine, J. Org. Chem., submitted for publication, 1984.

8. Schmidt, L.H., Alexander, S., Allen, L., and Rasco, J., Comparison of the Curative Antimalarial Activities and Toxicities of Primaquine and its D and L Isomers, Antimicrob. Agents Chemother., 12, 51, 1977.

9. Hufford, C.D., Clark, A.M., McChesney, J.D., and Baker, J.K., Stereochemistry of Dimeric Microbial Metabolites of Primaquine, J. Org. Chem., 49, 2822, 1984.

10. Wainer, I.W., Doyle, T.D., Hamidzadeh, Z., and Aldridge, A., Application of High-Performance Liquid Chromatographic Chiral Stationary Phases to Pharmaceutical Analysis, J. Chromatogr., 261, 123, 1983.

11. Hufford, C.D., Clark, A.M., Quinones, I.N., Baker, J.K., and McChesney, J.D., Microbial Metabolism Studies on the Major Microbial and Mammalian Metabolite of Primaquine, J. Pharm. Sci., 72, 92, 1983.