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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION AND ISOLATION OF QUINIDINE AND QUININE METABOLITES IN RAT URINE

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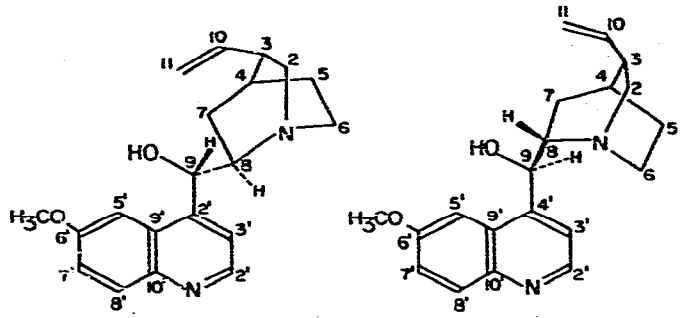
SUMMARY

A procedure for the separation and isolation of the urinary metabolites of quinidine and quinine by reversed-phase high-performance liquid chromatography is described. Nine metabolites of quinidine and eight metabolites of quinine were detected in the urine of male Sprague-Dawley rats after a single dose of quinidine or quinine (50 mg kg^{-1}). Following extraction from urine, the metabolites were separated on either an analytical or a semi-preparative reversed-phase column by gradient elution. After isolation and derivatization, the metabolites were analyzed by gas chromatography and gas chromatography—mass spectrometry.

INTRODUCTION

Quinidine (I) and quinine (II), members of the cinchona alkaloid family, have been widely used for the treatment of cardiac arrhythmias and malaria, respectively. Quinidine was introduced into medicine in 1918 [1] and is still used extensively in cardiac therapy, but quinine has been largely replaced by synthetic antimalarial drugs, including mepacrine and pamaquine. Nevertheless, quinine is still used as a bitter principle in the flavoring of carbonated table waters.

Quinidine and quinine are diastereoisomers that differ in their configuration at the C-8 and C-9 positions (Fig. 1). They are metabolized by oxidation of the quinoline and quinuclidine moieties to produce a series of phenolic and non-phenolic derivatives. The major metabolic products of quinidine found in man are (3*S*)-3-hydroxyquinidine [2—4], the carbostyril 2′-quinidinone [2, 3, 5] and O-desmethylquinidine [6, 7]. These metabolites have been detected by thin-layer chromatography (TLC) [2, 6—9] and gas chromatography—mass spectrometry (GC—MS) [2, 3] and characterized by ^{13}C NMR [3, 4], IR [3, 4]



(I) (II)

Fig. 1. Structures of quinidine (I) and quinine (II).

and UV absorption spectroscopy [2, 5] and mass spectrometry [2, 3, 6, 7]. Quinine has attracted less attention in recent years, presumably because of its declining therapeutic uses. The only significant work on quinine metabolism in man remains that of Brodie et al. [5]. These workers found that quinine is biotransformed primarily to the carbostyril and a product which was identified as 6-hydroxyquinine, but which may in fact be 3-hydroxyquinine by analogy with the metabolism of quinidine. A number of additional oxygenated metabolites of both quinidine and quinine have been suggested [2—5, 10—13] but have not been fully investigated. The identification of metabolites of quinidine and quinine by GC and GC—MS has been difficult, particularly when minor metabolites are of interest, because they are not fully resolved on packed columns. In addition, the interpretation of data is complicated by the presence of the corresponding dihydro analogs, dihydroquinidine (3—22%) or dihydroquinine (2—9%) in currently available samples [14]. These dihydro analogs are probably also metabolized by oxidative pathways. Because of these difficulties, investigators have often resorted to TLC for the separation of metabolites followed by direct inlet mass spectrometry for structural studies.

In this paper, we describe an high-performance liquid chromatographic (HPLC) method for the separation and isolation of nine metabolites of quinidine excreted in rat urine. We applied the HPLC method developed for quinidine to its isomer quinine, and eight metabolic products were isolated from rat urine.

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade. Glass-distilled acetonitrile, tetrahydrofuran and methanol were purchased from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). HPLC-grade acetic acid was obtained from J.T. Baker (Phillipsburg, N.J., U.S.A.). Column packings of 1% SE-30 coated on Gas-Chrom Q and bistrimethylsilylacetamide (BSA) were purchased from Applied Science Labs. (State College, Pa., U.S.A.). Glusulase was obtained from Endo Labs. (Garden City, N.Y., U.S.A.). Anhydrous quinidine (found by HPLC

to contain ca. 5% of dihydroquinidine) and quinine monohydrate (containing ca. 1% of dihydroquinine) were obtained from Aldrich (Milwaukee, Wisc., U.S.A.).

Instrumentation

HPLC. μBondapak C_{18} reversed-phase analytical columns (300 × 3.9 mm I.D.) and semi-preparative columns (300 × 7.8 mm I.D.) were obtained from Waters Assoc. (Milford, Mass., U.S.A.). HPLC analyses were carried out by gradient elution using a dual solvent delivery system (Waters 6000A), a solvent programmer (Waters 660) and a UV absorbance detector (Waters 440) at 254 nm. OmniScribe recorders (Houston Instruments, Austin, Texas, U.S.A.) were employed.

GC and GC—MS. Gas chromatographic separations were carried out using silanized glass capillary columns (45 m) coated with SE-30, prepared according to a procedure developed in this laboratory [15]. Analyses were temperature programmed from 180° at 2° min^{-1}. Methylene unit (MU) values were determined with *n*-alkanes as reference compounds.

GC—MS analyses were carried out using an LKB 9000-PDP/12 bioanalytical system equipped with silanized glass coil columns (1.85 m × 2 mm I.D.) packed with 1% SE-30 on Gas-Chrom Q (100—120 mesh). All separations were programmed from 180° at 3° min^{-1}.

Animal procedure

Male Sprague-Dawley rats (ca. 200 g) were injected with a single dose of quinidine or quinine (50 mg kg^{-1}; i.p.) in dimethyl sulfoxide (0.5 ml). A control rat was injected with an equal volume of dimethyl sulfoxide. The rats were housed individually in metabolism cages and 24-h urine samples were collected. The urines were stored at −20°.

Sample preparation

After enzymatic hydrolysis of the urine samples with Glusulase at pH 4.5—4.8 for 17 h at 37°, the metabolites were extracted by an ammonium carbonate—ethyl acetate procedure [16]. For profiling metabolites by HPLC on analytical columns, an aliquot (usually one tenth) of a diluted 24-h urine sample was used. Urine extractions were carried out in centrifuge tubes fitted with Teflon-lined screw-caps. For collection of metabolites from a semi-preparative HPLC column, an ammonium carbonate—ethyl acetate extract of a 24-h urine sample was used; the extraction was carried out in separating funnels.

The extracts were evaporated to dryness in a stream of nitrogen and the residue was dissolved in methanol and transferred into Reacti-vials. The final volume of an analytical sample was 25 μl and the final volume of sample for isolation with the semi-preparative column was 750 μl. Samples were centrifuged and aliquots were injected on to the HPLC columns; 1—2 μl was required for the analytical column and 100-μl injections were used for isolation of metabolites with the semi-preparative column.

HPLC analysis

When analyses were carried out on the analytical column, a 35-min gradient

system (profile 7) was used with a column pressure of 800—1000 p.s.i. and a flow-rate of 0.9 ml min^{-1}. The solvent system consisted of a mixture of solvent A (water—acetic acid, 99:1) and solvent B (water—acetonitrile—acetic acid, 40:59:1), in which the proportion of B varied from 10 to 85%. A modified solvent system was used with the semi-preparative column and consisted a mixture of solvent A (water—acetic acid, 99:1) and solvent B (water—acetonitrile—acetic acid—tetrahydrofuran, 40:59:1:0.1), in which the proportion of B varied from 10 to 80%. A 70-min gradient (profile 8) was used with a flow-rate of 1.8 ml min^{-1} and a column pressure of 1300—1500 p.s.i.

GC and GC—MS analyses

The individual fractions from two injections on the semi-preparative column were pooled, and the organic solvent was removed (Rotovap). After freeze-drying, the residues were transferred with methanol into Reacti-vials and evaporated to dryness in a stream of nitrogen. Each residue was dissolved in pyridine (10 μl) and silylated with BSA (10 μl). After heating at 60° for 2 h, an aliquot (1—5 μl) was analysed by GC and GC—MS.

Preparation of quinidine- and quinine-10,11-dihydrodiols

Quinidine (32 mg; 0.1 m*M*) or quinine monohydrate (34 mg; 0.1 m*M*) in pyridine (0.5 ml) was added to a solution of osmium tetroxide (28 mg; 0.11 m*M*) in benzene (2 ml). The resulting yellow solution was allowed to stand at ambient temperature for 2 days. Subsequent hydrolysis with a solution of *d*-mannitol (26 mg) and sodium hydroxide (70 mg) in water (0.5 ml) gave a dark brown solution, which was extracted with ethyl acetate (5 × 10 ml). After removal of solvent (Rotovap) the quinidine or quinine products were transferred into Reacti-vials. Samples were analyzed by HPLC using the solvent system described above. Two peaks were seen on each chromatogram. The products were collected from a semi-preparative column as before and, after removal of the solvent and derivatization with BSA, the products were analyzed by GC and GC—MS.

The mass spectra indicated that the two products were stereoisomers of quinidine-10,11-dihydrodiol or quinine-10,11-dihydrodiol. Each pair of stereoisomers had essentially identical mass spectra and assigment of *R* and *S* configurations could not be made.

The methylene unit (MU) values of the quinidine-10,11-dihydrodiols with a 45-m glass capillary column were 30.8 and 31.0. The quinine dihydrodiols were not resolved on the capillary column and a single MU value of 30.9 was found.

RESULTS

Figs. 2 and 3 show the separation of the urinary metabolites of quinidine and quinine, respectively, on a semi-preparative reversed-phase HPLC column. The dotted lines show where fractions were collected. The major metabolites of quinidine, (3*S*)-3-hydroxyquinidine and O-desmethylquinidine, were found in fractions 16 and 17, respectively, and their identities were confirmed by GC—MS. 2'-Quinidinone was not detected in rat urine. Two additional major metabolites, not previously reported, were found in fractions 9 and 11. These

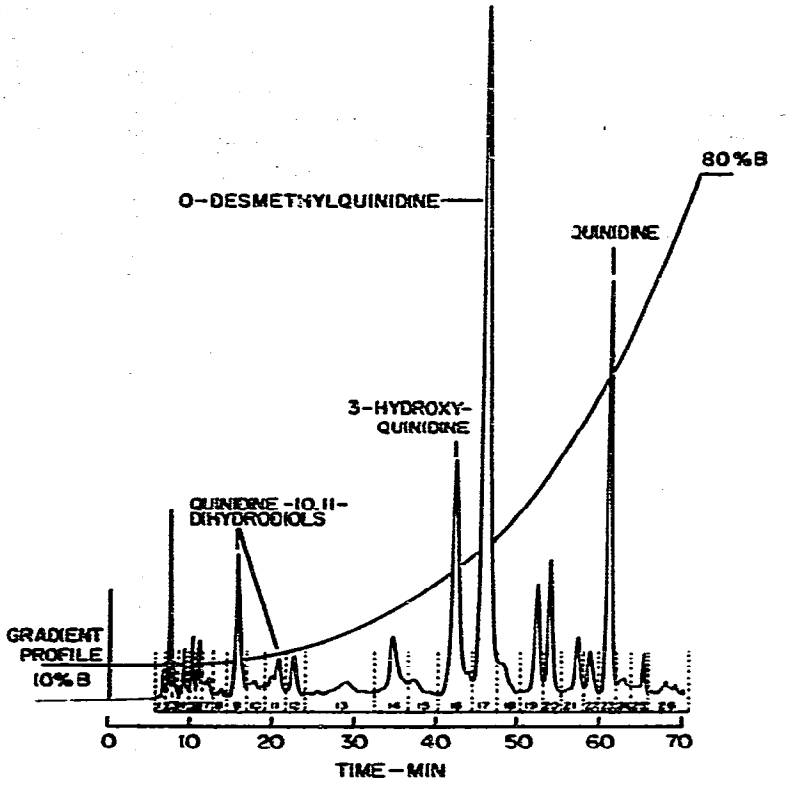


Fig. 2. Reversed-phase HPLC separation of quinidine metabolites on a semi-preparative column by gradient elution.

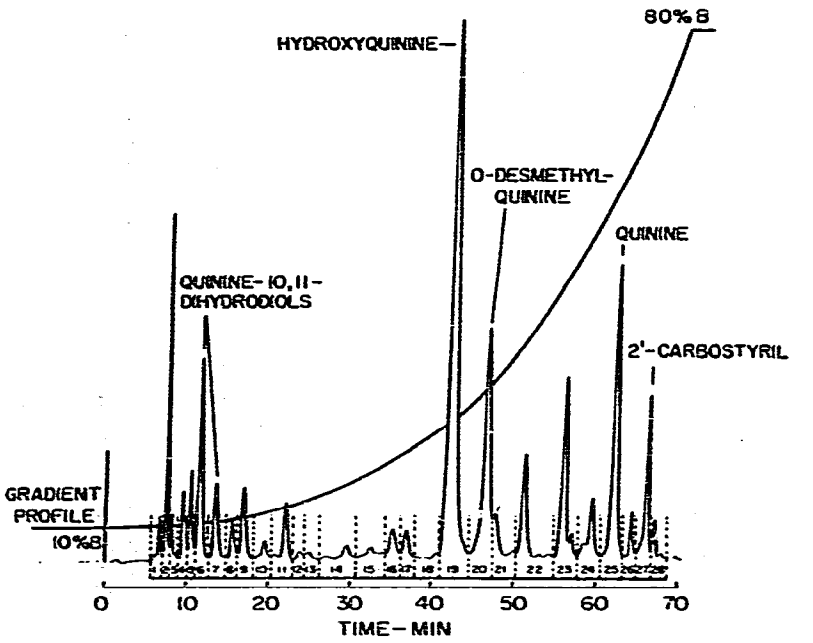


Fig. 3. Reversed-phase HPLC separation of quinine metabolites on a semi-preparative column by gradient elution.

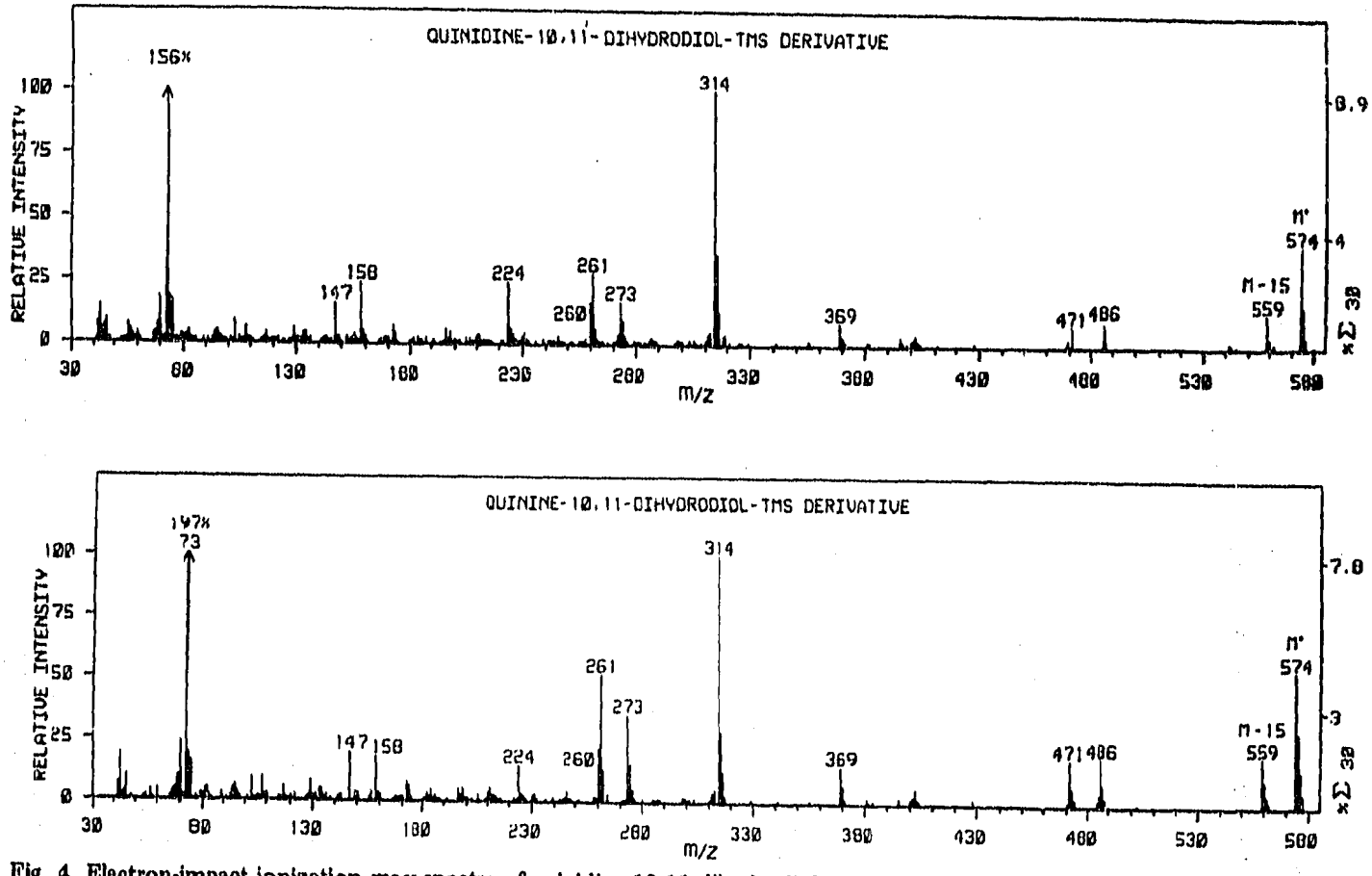


Fig. 4. Electron-impact ionization mass spectra of quinidine-10,11-dihydrodiol (TMS derivative) (upper) and of quinine-10,11-dihydrodiol (TMS derivative) (lower).

were identified as diastereoisomers of quinidine-10,11-dihydrodiol by GC—MS and by comparison with synthesized reference compounds. The mass spectrum of the quinidine-10,11-dihydrodiol collected in fraction 9 is shown in Fig. 4 (upper).

The mass spectra of quinidine, quinine and their metabolites exhibit characteristic ions formed by cleavage of the 8,9-carbon bond. In the mass spectra of the TMS derivatives of the dihydrodiols (mol. wt. = 574), these ions are observed at *m/z* 314 (base peak), corresponding to the addition of two OTMS groups to the vinyl side-chain of the quinuclidine moiety, and at *m/z* 260 and 261, corresponding to the unchanged quinoline portion of the molecule. The dihydrodiol metabolites can be distinguished from a dihydroxyquinidine substituted on the quinuclidine ring, rather than on the side-chain, by the molecular ion at *m/z* 572 (TMS derivative) and a base peak at *m/z* 312.

The two major metabolites of quinine excreted by man, hydroxyquinine and 2′-quininone [5], were found in fractions 20 and 27, respectively, and their identities were confirmed by GC—MS. 2′-Quininone was eluted from the reversed-phase column after quinidine. Drayer et al. [17] found that the corresponding quinidine metabolite, 2′-quinidinone, was eluted after quinidine from a μBondapak C_{18} column. The diastereoisomers of the 10,11-dihydrodiol of quinine and O-desmethylquinine were eluted in fractions 6, 7 and 19, respectively. The metabolites were identified by GC—MS, and in the case of the dihydrodiols, by comparison with synthesized reference compounds. The MU value of O-desmethylquinine was 27.7. The dihydrodiols were not resolved on the GC column and their MU value was 30.9. The mass spectral fragmentation of these metabolites was essentially the same as that of their quinidine analogs, but the relative peak intensities of the ions differed. The mass spectrum of the quinine-10,11-dihydrodiol collected in fraction 6 is shown in Fig. 4 (lower).

DISCUSSION

The reversed-phase HPLC procedure described here, based on gradient elution, can be used to study the metabolism of quinidine or quinine. The separation of quinidine, quinine and their metabolites on a reversed-phase (μBondapak C_{18}) HPLC column is influenced by the concentration of acetic acid in the eluting solvents. In the absence of acetic acid, quinidine and quinine were retained on the column. In the presence of 0.1% of acetic acid, both quinidine and quinine were eluted as poorly defined peaks with very long retention times. By increasing the concentration of acetic acid to 1%, both quinidine and quinine and their metabolites were eluted as well defined peaks. Comparable results were obtained by using a solvent system containing 0.1% of acetic acid and one of a series of alkylsulphonic acids (C_6—C_9), reagents which form paired ions (PIC reagents). However, it is more convenient to use acetic acid alone to generate a counter ion for the elution and separation of quinidine, quinine and their metabolites.

When the separations were carried out on an analytical column, profiles of the urinary metabolites were obtained that could be used to study the parameters affecting human metabolism and species differences in metabolism. For example, the urinary profiles of the rat and man are different. 2′-Quinidinone, a

major metabolite in man, was not detected in the rat. We found this metabolite in a urine sample obtained from a patient maintained on quinidine.

When the analyses were carried out with a semi-preparative column, nine metabolites of quinidine and eight metabolites of quinine were isolated and characterized. The time required for separation and collection of fractions for subsequent GC and GC—MS analysis was about 3 h. In addition to the dihydrodiols of quinidine and quinine and O-desmethylquinine which have not been described previously, additional oxygenated metabolites of both drugs have been characterized. The GC and GC—MS properties of these new metabolites will be reported separately.

Hydroxylation in the quinuclidine part of quinidine was shown to yield primarily (3*S*)-3-hydroxyquinidine [2—4]. Reaction at a hindered position is unusual, but in this instance the vinyl group presumably determines the site of hydroxylation. The corresponding metabolite of quinine (Fig. 3) is probably (3*S*)-3-hydroxyquinine; the isomeric 6-hydroxy structure [5] may have been incorrectly assigned. Further studies may, however, result in the identification of 6-hydroxy compounds in the group of monohydroxy metabolites derived from both quinine and quinidine.

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