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THE METABOLIC FATE OF ORALLY ADMINISTERED QUINIDINE GLUCONATE IN HUMANS*

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Abstract—Oral administration of quinidine (I) preparations to humans has been shown by mass spectroscopy (ms), gas–liquid chromatography/ms (GLC/ms) and other analytical techniques to give a wide range of urinary metabolites. These are found as the free, or to a minor extent, as the β-glucosiduronate conjugates. The major metabolites isolated and characterized represent two oxidative modifications of (I), and of 2′-quinolone and hydroxyquinuclidine derivatives. Also found were polyoxygenated quinidine derivatives. Current supplies of quinidine were found consistently to contain 10 to 30 per cent of an impurity.

THE CINCHONA alkaloid quinidine (I) (Fig. 1) is widely used as the hydrochloride or gluconate salt in the control of cardiac arrhythmias, but surprisingly little definitive information is available on its metabolic profile. This may be due to the fact that its metabolism has been linked by inference to that of the isomeric alkaloid quinine (II) (Fig. 1), since these compounds differ only in the configuration at C-8 in the quinuclidine ring. That significant differences do exist between the metabolism of the two bases has been shown in the only definitive work in this field, by Brodie *et al.*[1] in 1951. These workers showed that, while spectroscopically similar metabolic products can be obtained from each base, the relative amounts of phenolic and non-phenolic metabolites and also the amount of unchanged drug show wide differences (Table 1). The phenolic metabolites do not result from aromatic ring hydroxylation but are due rather to the facile enolization of a 2-quinolone system to give a base-soluble product, the so-called carbostyril compounds. Earlier workers[2, 3] had shown that from the metabolism *in vitro* of quinine with a rabbit liver homogenate a phenolic metabolite (carbostyril) could be isolated. This metabolite was examined by Mead and Koepfli,[4] who concluded that it was the 2-carbostyril of quinine. Brodie *et al.*[1] later found the same compound in the urine of a human subject taking quinine and also isolated a further metabolite, together with an appreciable amount of the unchanged drug. This second major metabolite, following a comparison of its ultraviolet spectrum with that of the phenolic metabolite, was considered to represent quinine hydroxylated in the quinuclidine ring. In contrast to quinine, quinidine was found[1] to be only partially

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metabolized (Table 1), with the major metabolite being a nonphenolic compound similar in ultraviolet spectral properties to the nonphenolic metabolite of quinine. The major phenolic metabolite resembled that found with quinine, but in contrast the amount found was considerably smaller. It therefore seemed of paramount interest to reinvestigate the metabolites of quinidine by using analytical methods not available to the previous investigators.

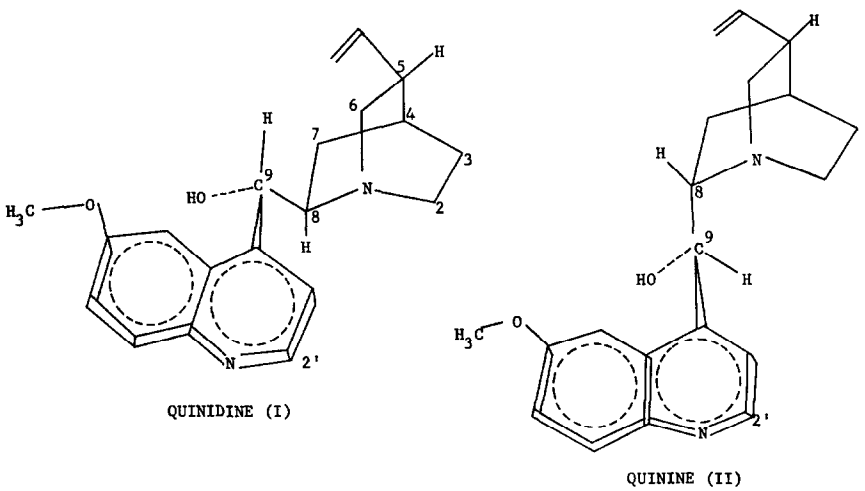


FIG. 1. Structural configuration of quinidine and quinine.

TABLE 1.

Base	Base (%)	Phenolic (%)	Nonphenolic (%)
Quinidine	77	9	14
Quinine	42	43	15

* After Brodie *et al.*[1]

EXPERIMENTAL

Ultraviolet absorption spectra (uv) were recorded in neutral 95% ethanol, basic 95% ethanol (pH > 10) and acidic 95% ethanol (pH > 1) on a Cary 14 spectrophotometer and are presented as λ_{max} in nm as the log of the molecular extinction coefficients. Infra-red spectra (i.r.) were measured in chloroform solution, as mineral oil mulls, or by the potassium bromide disc technique, on a Perkin–Elmer 221 instrument and are reported as ν_{max} in cm^{-1}. Proton nuclear magnetic resonance spectra (pmr) were measured by using a Varian Associates A-60 or HA-100* spectrometer on either deuterochloroform ($CDCl_3$) or hexadeuterodimethylsulfoxide (HDDMSO) solution containing tetramethylsilane as an internal reference standard, and are expressed in parts per million (δ). Mass spectra were obtained by the direct

* We wish to thank Dr. C. Moreland, North Carolina State University, Raleigh, N. C., for measuring the high resolution pmr spectra.

probe technique on an LKB-9000 or Perkin–Elmer Hitachi* instrument. Gas–liquid/mass spectrometry spectra were recorded by using the LKB-9000 instrument, and high† resolution mass measurements were made by using the peak matching technique on an Associated Electrical Industries model 902 mass spectrometer. Optical rotations in 95% ethanol were measured on a Rudolph model 80 polarimeter. Melting points (mp) were taken with a Kofler microscope melting point apparatus. Analytical thin-layer chromatography (TLC) employed silica gel H or HF (E. Merck, Darmstad) coated (0·2 mm thickness) glass plates using the saturated chamber technique with a solvent system composed of acetone:methanol:chloroform (2:2:6) presaturated with conc. ammonia solution (d 0·9). The various compounds were visualized by spraying with a modified Dragendorff's reagent, with which bases showed up as red spots on a yellow background. Preparative thin-layer chromatography (PTLC) separation used the same conditions but with a 1·0 mm layer of silica gel. Alumina column chromatography employed Brockmann alumina (Woelm) of various grades of activity. Partition columns were prepared by mixing the appropriate 1·0 M buffer and kieselguhr (Hyflo-supercel, Johns–Manville) in the ratio of 1 ml buffer solution to 1 g kieselguhr. The dry powder was packed into an appropriate sized glass column using a modification of the method of Martin,[5] with diethyl ether or heptane as the mobile phase. GLC studies were made with a Varian Aerograph model 2100-2 instrument using a flame ionization detection system with 4 ft × 0·25 in. glass column with either a 3·8% SE-30 or 3·8% OV-17 silicone phases on 100–120 mesh Chromasorb HP-AWS (Johns-Manville). The trimethylsilyl ether (tms) derivatives for GLC studies were made by heating a solution of the sample (100–200 μg) in a 1:2:9 mixture of trimethylchlorosilane:hexamethyldisilazane:anhydrous pyridine or in *bis*(trimethylsilyl)-acetamide or in *bis*-(trimethylsilyl)trifluoroacetamide (Regisil) at 100° for 10–15 min. After cooling, the solution (20–40 nl) was injected as such into the gas chromatograph. All evaporations were made under reduced pressure and all solvents used for recrystallization were freshly purified and distilled immediately before use. GLC/ms on the LKB-9000 studies used a 5 ft chromatographic column with the 3·8 per cent OV-17 packing as previously described. All separation and reaction procedures were routinely monitored by both TLC and GLC, enabling immediate combination of like materials prior to bulk evaporation or the product work-up.

1. *Extraction of quinidine metabolites from urine*

Urine from a patient receiving orally 330 mg quinidine gluconate twice a day, under long-term preventive therapy, was used in these studies. The urine was extracted in 2- to 3-l. batches and was preserved prior to extraction by addition of chloroform and was also kept at 0° in the dark.

Ammonium hydroxide solution was added to the urine (2–3 l.) to give pH > 10, the aqueous alkaline liquors were extracted in a continuous extraction apparatus with chloroform for 48 hr, after which time no further basic material was extractable with chloroform (or ethyl acetate). The chloroform extracts were evaporated to dryness to give the total unconjugated metabolite fraction and the aqueous liquor was concentrated by freeze-drying to one-tenth the original volume (200–300 ml,

* We are grateful to Dr. M. Bursey, University of North Carolina at Chapel Hill, Chapel Hill, N.C., for these measurements.

† These measurements were made by Dr. D. Rosenthal of our laboratory.

adjusted to pH 5–6). A precipitate of inert material was removed from the concentrated liquors; to these was added an equal volume of 0·2 M acetate buffer (pH 4·5), also 500,000 units of bovine β-glucuronidase (Ketodase, Warner–Chilcott), 2 ml ethyl acetate, and the resulting mixture was incubated at 38°. After 3 days the incubation mixture was brought to pH > 10 with ammonium hydroxide solution, and then continuously extracted with chloroform as previously described. Evaporation of the chloroform gave the hydrolyzed β-glucosiduronate quinidine metabolite fraction. A portion of the above extracted aqueous liquors was freeze-dried and the residue dissolved in aqueous hydrochloric acid. The resultant solution gave a negative test when examined for basic material by the Dragendorff and Meyer tests. When an ultraviolet spectrum of part of the above solid residue dissolved in 0·1 N sulfuric acid, none of the very characteristic quinine/quinidine chromophore could be detected. Since this ruled out the possibility of appreciable amounts of sulfate-conjugated metabolites, the final aqueous liquors from all extraction procedures were not further investigated.

2. *Separation of metabolite fractions from section* 1 *into phenolic and nonphenolic basic fractions*

The total unconjugated quinidine metabolite fraction was dissolved in chloroform (100 ml) and successively extracted with aliquots of 1% (v/v) aqueous sulfuric acid (30 ml) until the acid extract no longer gave a positive Meyer's or Dragendorff's test for basic material. The acidic extracts were combined and the remaining chloroform solution was washed with a saturated solution of sodium bicarbonate, followed by water, and then it was evaporated to dryness. The combined acid extracts were made alkaline (pH > 10) and extracted seven times with chloroform followed by concentration of the combined chloroform extracts to a small volume (100 ml). This was next extracted ten times, under a nitrogen atmosphere, to minimize air oxidation of the phenolic compounds, with an aqueous solution of sodium hydroxide (2·5 N, 20 ml). The remaining chloroform solution was then washed with water and evaporated to dryness to give quinidine nonphenolic metabolite fraction (6·2 g). The combined sodium hydroxide extracts were brought to pH 4·0 by addition of acid, and then to pH > 10 with ammonium hydroxide; this was then extracted with chloroform (× 7). The combined chloroform extracts were evaporated to dryness to give the quinidine phenolic metabolite fraction (1·0 g). The hydrolyzed β-glucosiduronate quinidine metabolite fraction obtained from part of the urine was similarly processed to give comparable phenolic (0·286 g) and non-phenolic (0·289 g) fractions.

3. *Fractionation of the quinidine nonphenolic metabolite fraction by partition column chromatography*

The fraction was subjected to partition column chromatography using 400 g kieselguhr and 400 ml of 1·0 M Tris-acid maleate buffer, pH 5·2. The column was eluted with diethyl ether, followed by chloroform, and finally all basic material was stripped from the column by elution with chloroform saturated with ammonia. The 180 eluate fractions (40 ml each) were combined into eight large fractions and evaporated to dryness: (I) 1–8 (1·351 g); (II) 9–13 (0·421 g); (III) 14–37 (1·339 g); (IV) 38–53 (0·386 g); (V) 54–62 (0·162 g); (VI) 63–73 (0·495 g); (VII) 74–79 (0·16 g);

(VIII) 80–180 (0·701 g). These fractions were then separately processed to give individual metabolites.

4. *Fraction I, section* 3

The dark-colored solution of fraction I (in benzene) was subjected to "clean-up" alumina column chromatography (10 g, activity IV), with benzene and then diethyl ether as eluents. The combined eluates were evaporated to dryness and the residue was repeatedly crystallized from benzene/heptane until pure material, as indicated by TLC and GLC analysis, was obtained. Metabolite I crystallized in colorless needles, m.p. 178–181°. U.v., λ_{max} 235 (4·22); λ_{max}^{acid} 239 (4·21); λ_{max}^{basic} 235 (4·26); in addition all had end absorption <210 nm. Ir, ν_{max}^{KBr} 3470 (OH); 3182 (NH); 1650 (> N—C=O); 1625, 1590, 1500, 1039, 842, 788 (C=C aromatic). Mol. wt. found by ms, 193·07; calculated for $C_{10}H_{11}NO_3$, 193·0738. Metabolite I did not give a positive basic test with either Dragendorff's or Meyer's reagents.

5. *Isolation of unchanged quinidine from eluate fractions II, III and IV*

Fractions II–IV (from section 3) were crystallized from methanol or methanol/hexane to give a single compound (as indicated by GLC and TLC analysis) as colorless lathes, m.p. 170–171° (d), identical in all respects (i.r., u.v., m.s., p.m.r., TLC, GLC, m.p. and mixed m.p.) to authentic quinidine recrystallized in the same manner. The $[\alpha]_D^{26}$ was + 248·05 (C = 0·23) measured in neutral freshly distilled 95% ethanol. Only one sample of commercial quinidine or its salts was found to be free of an impurity which was not removable by recrystallization (see next section).

6. *Examination of commercial samples of quinidine and its salts*

During the purification of authentic quinidine for comparison with that obtained in section 5, it was found that all commercial samples (all together 20 were examined) of quinidine or quinidine salts (hydrochloride, sulfate or gluconate), available as such or in various pharmaceutical preparations, showed by TLC an impurity of slightly lower R_f value than quinidine. The amount of this impurity varied from 5 to 30 per cent of the total free bases. Extensive PTLC failed to remove all traces of the impurity from the quinidine. Smaller traces of an impurity of similar TLC characteristics was found in commercial samples of quinidine whose date of manufacture/supply was prior to 1940. Only one sample (100 mg), obtained from a commercial source prior to 1920, was found by TLC to be free from the impurity. The quinidine gluconate medication received by the patient in the present study contained, by TLC, 20–30 per cent of the unknown impurity. A detailed study of the impurity will be reported in a further communication.

7. *Separation of metabolites from fraction VI, section* 3

The material from fraction VI was subjected to repeated PTLC and the resultant fractions combined to give five major separate fractions. Fractions 1 and 4 were found to be unchanged quinidine. Fractions 2 and 5 were identical to the metabolite isolated from grouped eluate fraction VIII, section 3. Fraction 3 was again rechromatographed to remove traces of quinidine and of fraction 2 to give pure metabolite 3 as shown by GLC and TLC.

Metabolite 3 crystallized from acetone/hexane to give fine colorless needles, m.p. 225–230° (d) or 233–234° (d), (sealed tube). U.v., λ_{max} 271 (3·58), λ_{max}^{acid} 269 (3·79),

λ_{max}^{base} 271 (3·65), end absorption <210 nm occurs in all above spectra. Ir, ν_{max}^{KBr} 3450 (OH), 3150 (NH) 2970 (C—CH_3), 1710–1700 (C=O), 1665 (>N—C=O lactam), 1600 and 1505 (C=C aromatic). Pmr, ($CDCl_3$), 7·5, (1H, aromatic), 3·98, (3H, Ar—OCH_3), 3·57, (3H, N—CH_3), 3·43, (3H, Ar—OCH_3), 1·28 (3H, >—C—CH_3). Ms, mol. wt. 193. This compound gave a positive test with Dragendorff's reagent.

8. *Isolation of metabolite* 4 *from fraction VIII, section* 3

Repeated crystallization of fraction VIII from methanol/hexane gave short colorless needles, m.p. 226–228°. The mother liquors, together with material isolated from fraction VI, were rechromatographed by PTLC to give a further amount of TLC and GLC pure material for a total yield of 0·401 g of metabolite 4. U.v. λ_{max} 207 (4·38), 229 s (4·29), 233 s (4·27, 278 (3·31), 321 (3·39), 332 (3·43); λ_{max}^{acid} 252 (4·23), 313 (3·36), 347 (3·49); λ_{max}^{basic} 329 (4·24), 319 (3·66), 332 (3·69). The i.r. and p.m.r. spectra were similar to but not identical with those of quinidine. Ms, gave mol. wt. of 340·1783, calculated for $C_{20}H_{24}N_2O_3$, 340·1787.

9. *Fractionation of quinidine phenolic metabolites*

The phenolic metabolites (1·65 g) were separated into four main fractions by silica gel (220 g) column chromatography using chloroform, chloroform saturated with ammonia, chloroform/methanol and methanol as eluents. Only fraction 2 (0·45 g) contained basic material; fractions 1 (0·56 g), 3 (0·153 g) and 4 (0·321 g) were highly colored and were not further investigated at the time. PTLC of fraction 2 gave four further fractions 2-I (0·16 g), 2-II (0·075 g), 2-III (0·047 g) and 2-IV (0·055 g).

Repeated PTLC of subfraction 2-I gave metabolite 5 (0·078 g) which crystallized from benzene/methanol in long colorless lathes, m.p. 229–232° (d). U.v., λ_{max} 208 (4·47), 234 (4·50), 279 s (3·62), 350 (3·77); λ_{max}^{acid} 233 (4·53), 253 s (3·95), 281 s (3·64), 354 (3·77); λ_{max}^{base} 233 (4·59, 361 (3·95). I.r., ν_{max}^{KBr} 3450 (OH), 2970–3010 (NH bonded), 1683 [>N—C=O; 2 quinolone[6]], 1648, 1580 and 1530 (C=C aromatic). The p.m.r. spectrum was similar to that of quinidine but only showed four aromatic protons in a complex multiplet below 7·4δ. Mol. wt. (m.s.), 340·1785; $C_{20}H_{24}N_2O_3$ requires 340·1787.

After removal of some nonbasic material which had been introduced as a result of multiple PTLC fractionations, subfraction 2-III was crystallized from methanol/benzene to give the following further PTLC metabolite 6 (7·7 mg) and metabolite 7 (3·3 mg). PTLC of fractions 2-II and 2-IV similarly yielded very small amounts of GLC and TLC pure minor metabolites.

10. *Fractionation of the quinidine conjugated metabolites*

PTLC of both the phenolic and nonphenolic fractions have yielded minor metabolites. The major nonphenolic metabolite was identical, as shown by GLC and TLC, with the major nonphenolic metabolite 4. There were, however, marked differences between the TLC patterns of the above metabolites and their corresponding compounds obtained from the nonconjugated fractions. A more detailed examination using m.s. and GLC/m.s. of the above and other minor metabolite fractions will be reported in a further communication.

RESULTS AND DISCUSSION

The present study on the isolation and fractionation of the human urinary excreted metabolites of quinidine after the oral administration of a commercial preparation of quinidine gluconate has revealed a vast array of metabolites. These are mainly present in the unconjugated or free state but a minor, yet significant, amount is excreted as the previously unreported β-glucosiduronate conjugates. Sulfate conjugation was not found, although this could have been due to the initial basic isolation conditions, resulting in their hydrolysis; they would have then been found in the free metabolite fraction. Significantly, conjugation of unmetabolized quinidine did not take place, suggesting that in the conjugated fraction the 9-hydroxy group is not the principal site of conjugation. The interpretation of the findings concerning the metabolic products of quinidine is rendered somewhat more difficult by the presence of a persistent impurity in many currently available samples of quinidine. While it is dangerous to generalize on the limited number (twenty) of samples examined, there appeared to be a relationship between their dates of preparation and the amount of the impurity present. In samples presumably obtained prior to 1945, TLC analysis clearly showed relatively smaller amounts of the impurity. The source of the impurity may be due to a current method[7] of preparation of quinidine, in which it is manufactured via a base-catalyzed isomerization of quinine. In this procedure, not only is quinidine produced, but also a complex mixture of the epi quinine and quinidine bases, together with unchanged quinine. The presence of material other than quinidine in commercial preparation was first reported in 1922 by Lewis,[8, 9] who believed it to be dihydroquinidine. The identity of the quinidine contaminant(s) will be reported in a later paper in this series.

Mass spectroscopy has played a crucial role in the determination of the structures of the quinidine metabolites because of its ability not only to provide precise information on their structures but also to determine extremely accurately their molecular weights and empirical formulae. Detailed information regarding the mass spectral characteristics of quinidine was not available,[10] but partial information on the mass spectral fragmentation modes of the related alkaloid, cinchonine, are recorded by Spiteller and Spiteller-Friedmann.[11] When the mass spectrum of quinidine was taken by using various electron beam energies, it was found that spectra obtained at 20 eV and at low direct probe inlet temperatures (<70°) were the most informative. Above 20 eV, the relative abundance of the molecular ion signal decreases rapidly in comparison to that of the principle (base peak) fragmentation ion at m/e 136. Mass spectra obtained at 20 eV for quinidine, and for metabolites 4 and 5, are shown in Figs. 2–4. The mass spectrum of quinidine shows that the C—C bonds β to the quinuclidine nitrogen (C8-9 and C5-6) are cleaved in an analogous manner to that observed in cinchonine[10, 11] to give two principal fragments, one consisting of the quinuclidine part of the molecule (m/e 136 base peak) and another corresponding to quinoline moiety plus the C-8 substituents (m/e 189). Further fragmentation of these two primary ion fragments can be seen and are summarized in Fig. 5. The mass spectra of the metabolites 4 and 5 show a molecular ion (M^+) at 340 in contrast to that of quinidine at 324. The metabolites therefore differ from quinidine only by the addition of one oxygen atom. The base peak in metabolite 5, like that of quinidine, is at m/e 136 showing that in metabolite 5 the oxygen is located in the quinoline part of the molecule. Confirmation of this is that there is no signal at m/e 189 in metabolites

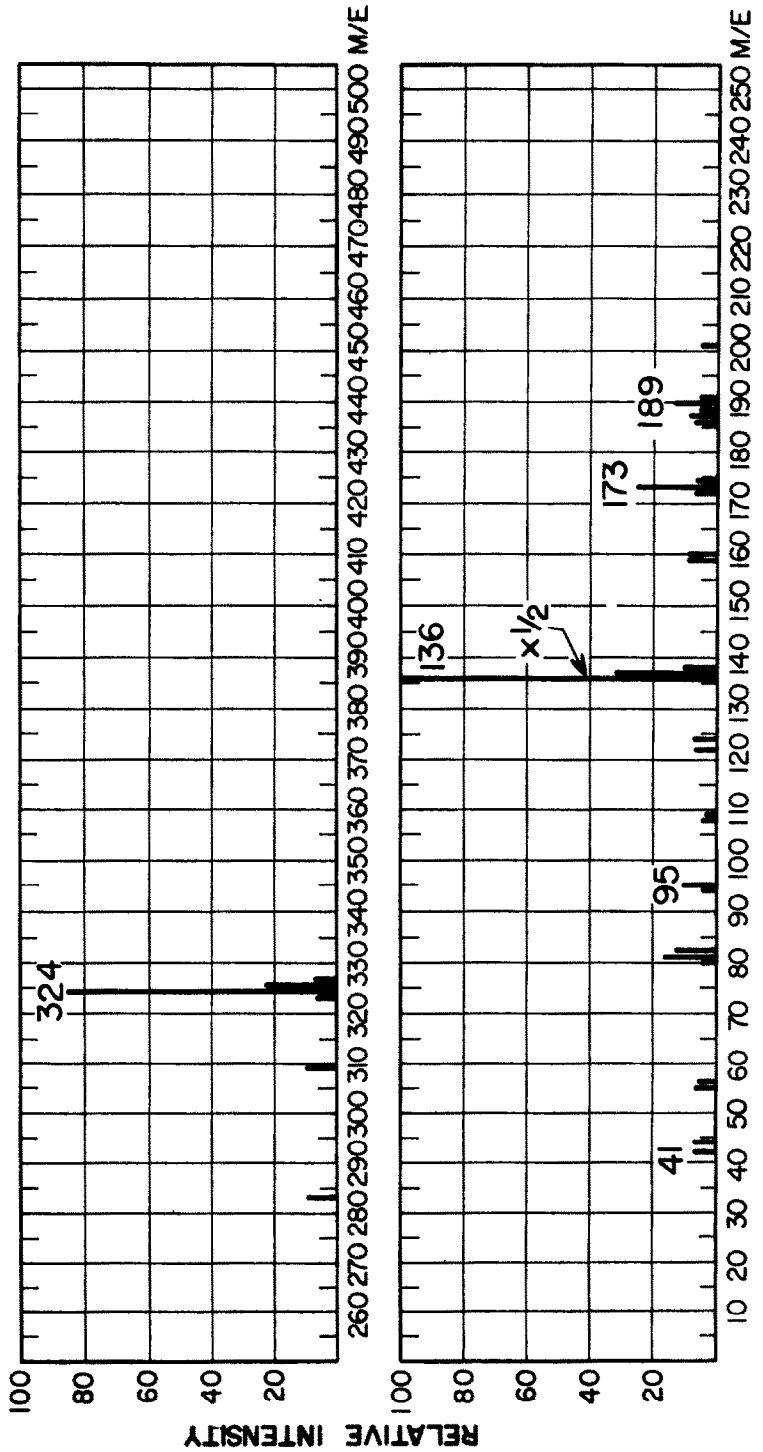


FIG. 2. Mass spectrum of quinidine.

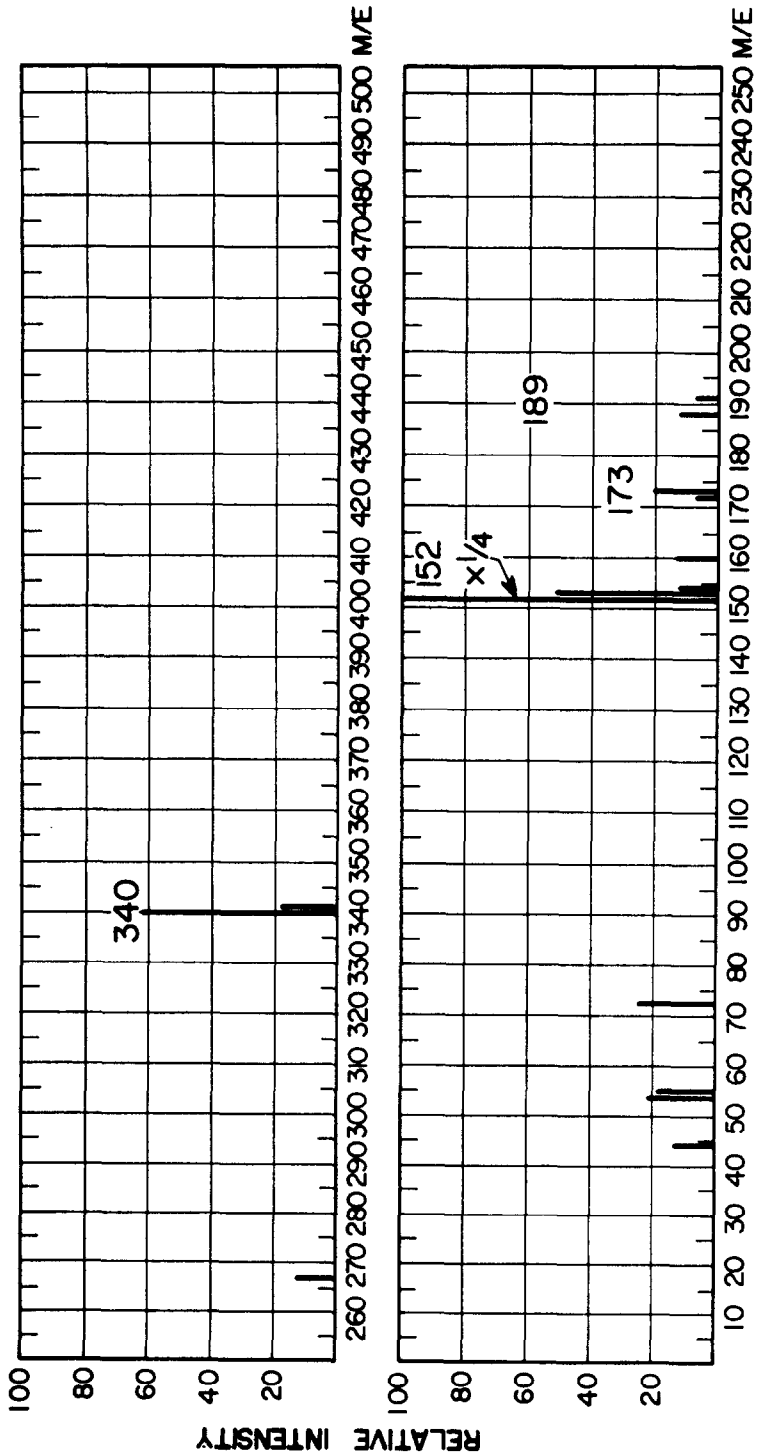


FIG. 3. Mass spectrum of quinidine metabolite 4.

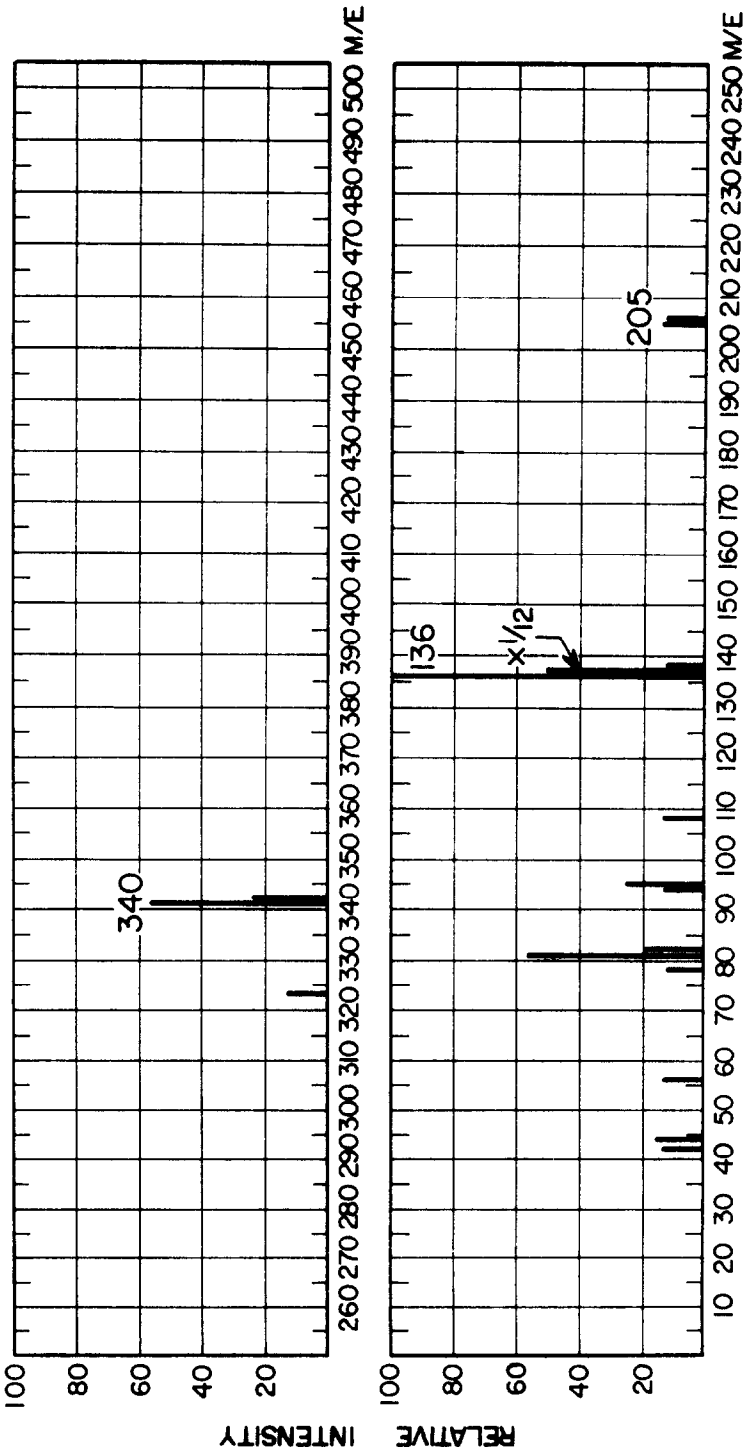


FIG. 4. Mass spectrum of quinidine metabolite 5.

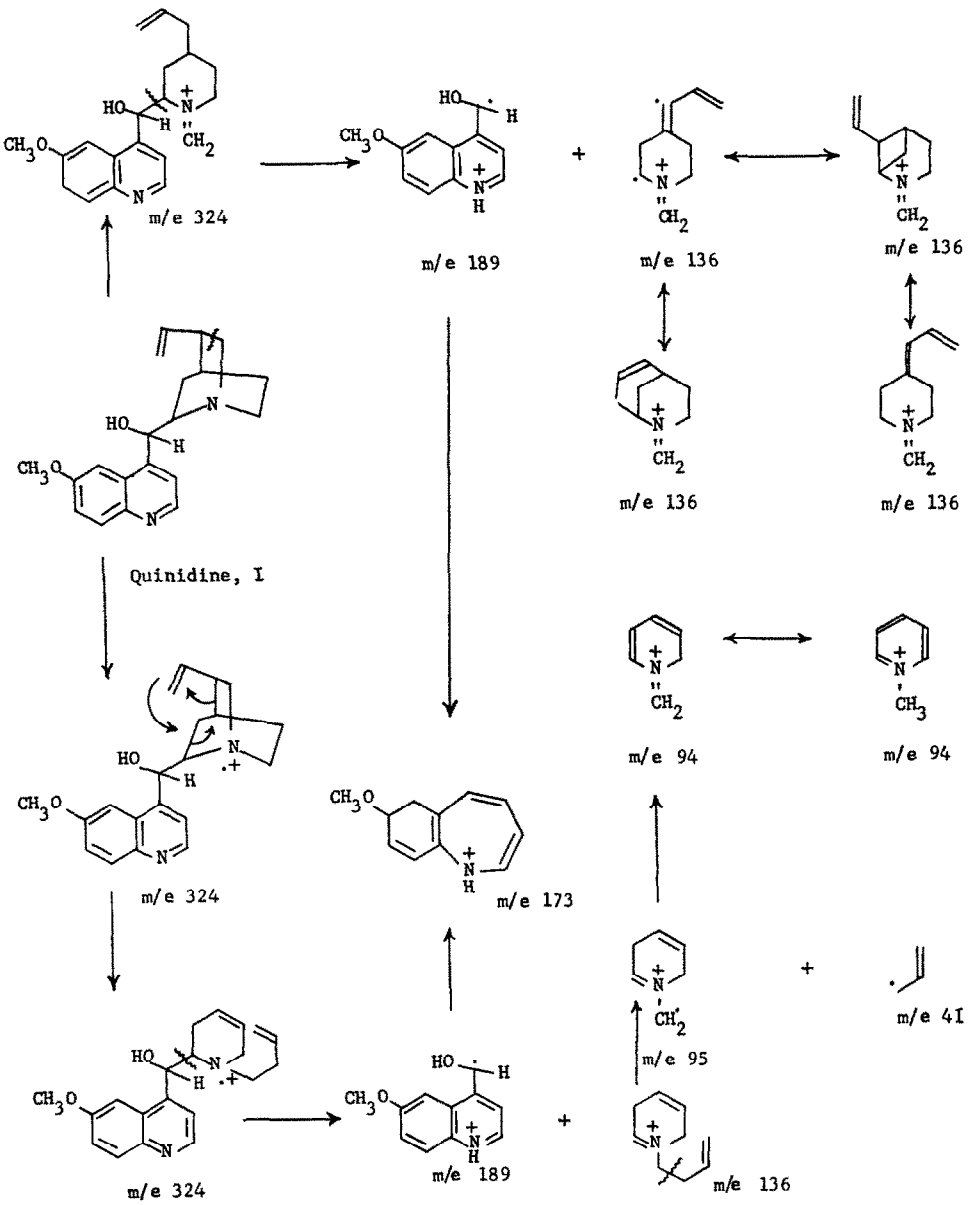


FIG. 5. Mass spectral fragmentation patterns for quinidine (I).

corresponding to the quinoline fragment, as in quinidine, but a signal absent in the latter is found at 205, representing the addition of 16 mass units or one oxygen atom. By contrast, the base peak due to the quinuclidine fragment is shifted in the ms spectrum of metabolite 4 from m/e 136 to m/e 152, showing that in this metabolite the additional oxygen is located in the quinuclidine ring, while the quinoline fragment appears normally at m/e 189. The i.r. spectrum of metabolite 5 shows a ν_{max} 2970 and 1683 cm^{-1}, and the uv spectrum shows a bathochromic shift in base (350→

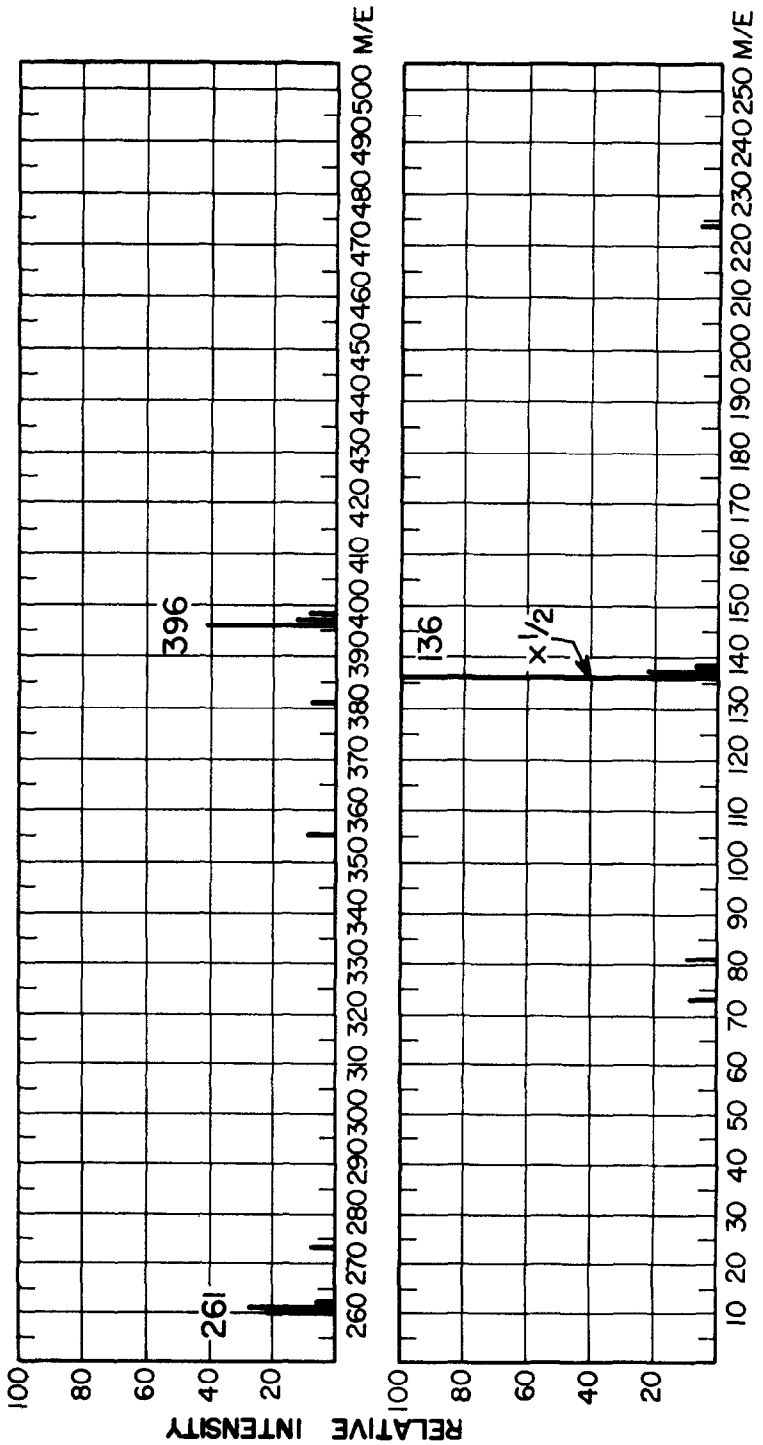


FIG. 6. Mass spectrum of quinidine tms ether.

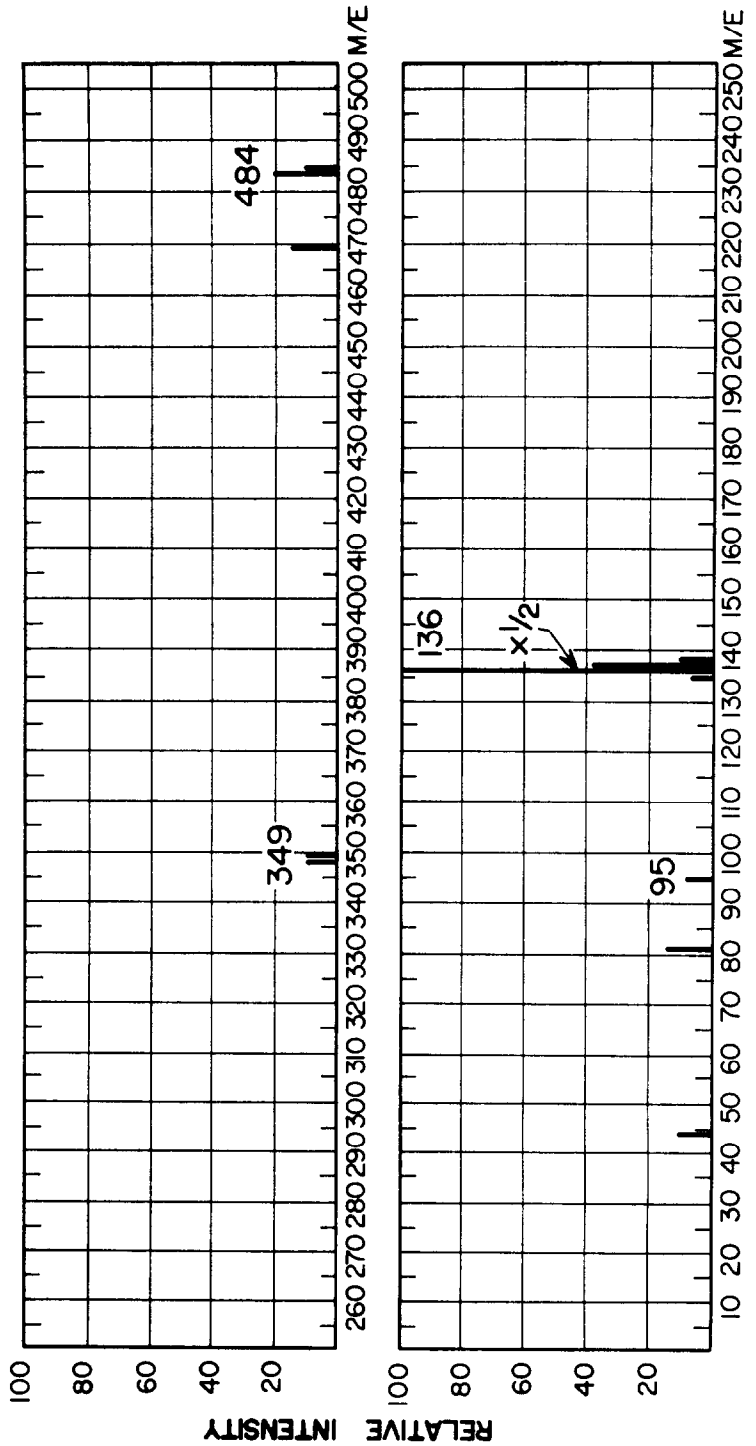
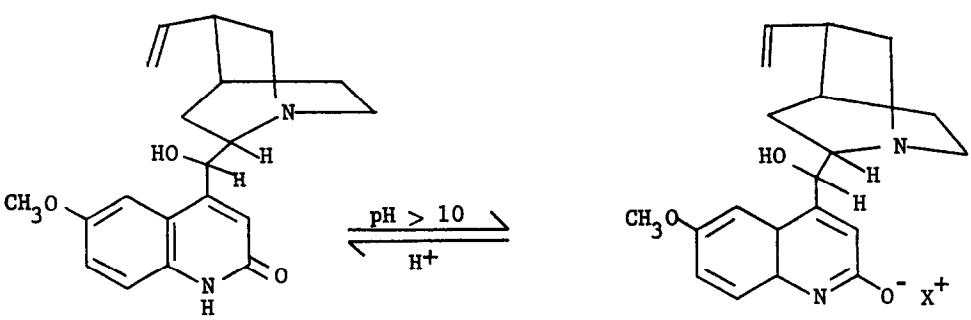


FIG. 7. Mass spectrum of quinidine metabolite 5 tms ether.

361 nm), which is characteristic of a 2-quinolone structure.[7] This is consistent with the mass spectral data, which show the addition of one oxygen atom with no concomitant hydrogen loss. Additional evidence for the location of the additional oxygen was found when the ms of the tms ether derivatives of quinidine and metabolite 5 were measured (Figs. 6 and 7): M^+ ions corresponding to *mono-* and *bis*-tms ethers of quinidine and metabolite 5 were observed at m/e 396 and 484 respectively. The base peak in both metabolite 5 and quinidine was at m/e 136, indicating the same unchanged quinuclidine fragment. The quinoline fragment derived from quinidine gave the expected signal at m/e 261, corresponding to a *mono*-tms ether derivative, but in sharp contrast the corresponding fragment in metabolite 5 was at m/e 349. This fragment corresponds to a *bis*-tms ether derivative of the m/e 205 unsilylated quinoline part of metabolite 5 and is due to tms ether of the enolized 2-quinolone system. Metabolite 5, therefore, can now be firmly assigned structure IV (Fig. 8), with V being its corresponding enolic form responsible for the observed base-induced bathochromic shift. Structure IV is analogous to that tentatively proposed by Mead and Koepfli[4] for the quinine "*in vitro*" phenolic metabolite of Kelsey *et al.*[2]

The oxygen function in the quinuclidine part of metabolite 4 could be present as an *N*-oxide, a hydroxyl group, or more unlikely in the ring following a rearrangement of the *N*-oxide. The i.r. of metabolite 4 does not show a $\nu_{max}^{cm^{-1}}$ 970–950 characteristic of *N*-oxide[12] and the ms does not show a fragment at m/e 136 (142–16), which would result from the facile loss of oxygen from an *N*-oxide as in that of the alkaloid olivacine.[13] A comparison of the mass spectrum of the tms derivative of metabolite 4 (Figs. 9 and 6) to that of quinidine showed that it formed a *bis*-tms derivative (M^+ 484), in contrast to the mono-tms derivative (M^+ 396) formed by quinidine. In addition, the principal fragments of metabolite 4 each had one tms group (m/e 224 and 261) as opposed to the m/e 136 and 261 fragments found in quinidine. The formation of a *bis*-tms derivative by metabolite 4 is conclusive evidence that the oxygen is present as a hydroxyl group in the quinuclidine ring sustem. Preliminary ms and GLC/ms studies on the minor metabolites showed that some of these represented the addition of two oxygen atoms. The examination of the properties of these polyoxygenated quinidine metabolites, metabolites 1 and 3, and the position of substitution of the hydroxyl group in the quinuclidine ring of metabolite will be reported in further communications.



IV V

FIG. 8. Configuration of structure IV (metabolite 5) and structure V (enolic form of metabolite 5).

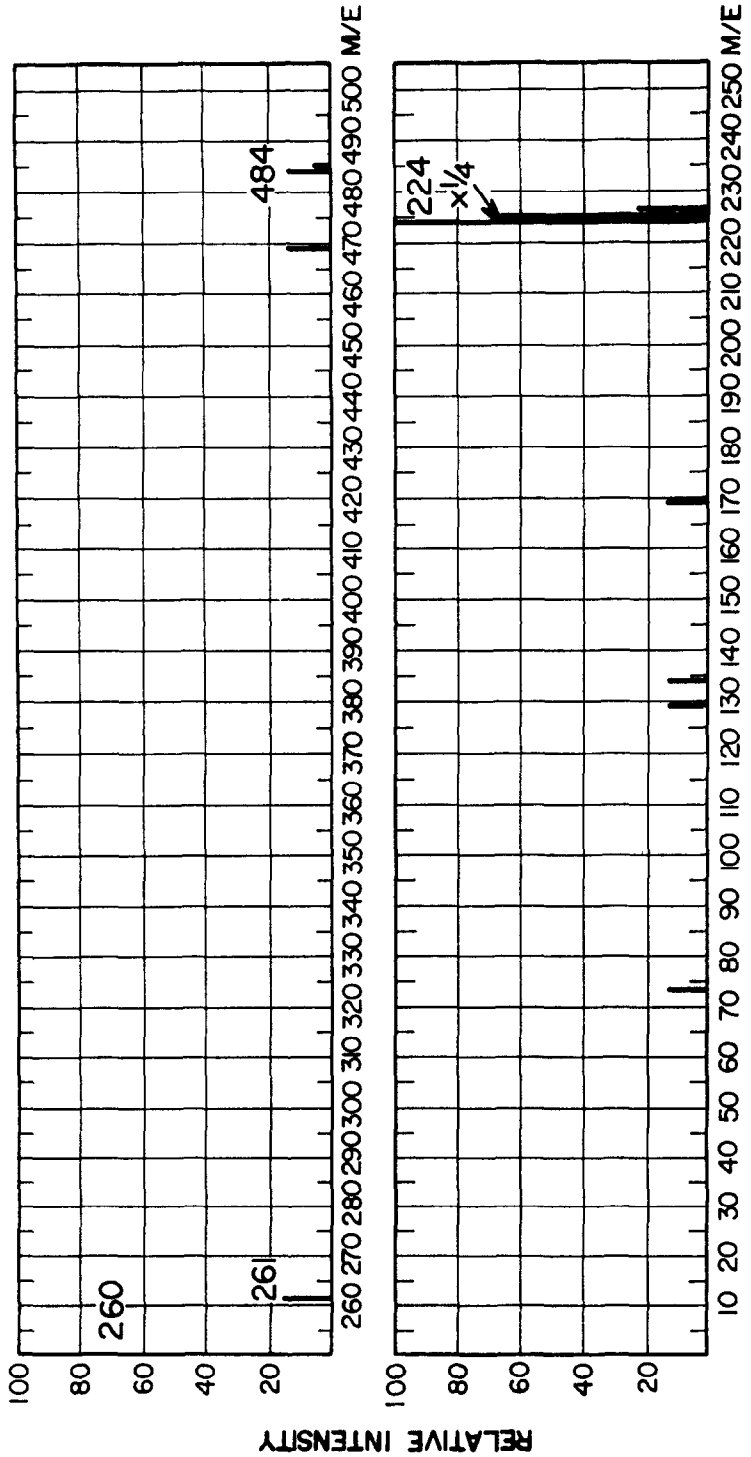


FIG. 9. Mass spectrum of quinidine metabolite 4 tms ether.

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