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The metabolic fate of quinine in rabbits

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Six metabolites of quinine were detected in the urine of rabbits given its monohydrochloride orally. Of the metabolites detected, three major ones were quantitatively separated and characterized as 2′-hydroxyquinine, 2′,3-dihydroxyquinine, and 2′,6′-dihydroxycinchonidine. No unchanged alkaloid was detected in the urine. The present results indicate that the most important reaction in the metabolism of the alkaloid in the rabbit is the formation of 2′-carbostyril derivatives.

Five metabolic products of quinine have been isolated from human urine. They are quinetine, a derivative in which the vinyl side-chain of the quinuclidine nucleus has been oxidized to a carboxyl group (Kerner, 1869), hemoquinic acid, which was isolated from the urine of subjects with blackwater fever and was characterized as 6-methoxyquinoline-4-ketocarboxylic acid (Nirenstein, 1918), 2′-hydroxyquinine, and two unidentified substances of which one was assumed to have a hydroxyl group at the 2-position and the other to have two hydroxyl groups at unknown positions of the quinuclidine nucleus (Brodie, Baer & Craig, 1951). Whether quinetine and hemoquinic acid are true metabolites of quinine is uncertain (Williams, 1959). The carbostyril type metabolite, 2′-hydroxyquinine, has also been isolated from a rabbit liver preparation incubated with quinine (Kelsey, Geiling & others, 1944). The enzyme which mediates 2′-hydroxylation of the alkaloid is present in the liver of other species (Lang & Keuer, 1957).

The potent inhibition of drug-metabolizing enzymes and the expected similarity of the metabolic pathway of quinine in man to that in the rabbit, in view of formation of 2′-hydroxyquinine, encouraged us to undertake further investigation of the metabolic fate of the alkaloid. We report the quantitative separation and characterization of three major urinary metabolites of quinine in rabbits.

METHODS

Quinine monohydrochloride dihydrate (200 mg/kg) was given as a 3% aqueous solution by stomach tube to seven male albino rabbits (2·5 to 2·8 kg). The total dose of the drug was 3·76 g. The 0–48 h urine was collected, pooled (3·6 litre) and hydrolysed (conc. HCl, 100 ml) by heating (100°, 40 min) and then made alkaline with 28% ammonia. It was extracted continuously with ethyl acetate for 20 h and a crude urinary extract obtained (8·84 g).

Column chromatography of the crude urinary extract

A silica gel column (Wakogel C-200, Wako Pure Chemicals, Tokyo; 884 g) was prepared using benzene saturated with 28% ammonia (column height : diameter 15 : 1). It was treated with the benzene–ammonia solvent (5 times bed volume). The urine extract was dissolved in the minimum volume of methanol, coated onto five times its weight of adsorbent by removing the solvent using a rotatory evaporator,

and then placed on the top of the column. This was eluted successively with benzene chloroform, and chloroform containing 5 to 15% ethanol. The benzene and chloroform used were saturated with 28% ammonia and left to stand as two layers for at least 20 h before use. The eluate was collected in 50 ml fractions and monitored by t.l.c.

Thin-layer chromatography

Silica gel plates containing an inorganic fluorescent agent (Wakogel UA–5; 0·25 mm in thickness) were used. The developing solvent systems consisted of chloroform saturated with ammonia and 1 to 35% ethanol in volume. The metabolites were detected by examination of the chromatograms beneath ultraviolet light (225 nm), by spraying with Dragendorff reagent, or with concentrated sulphuric acid followed by heating.

Spectra

Infrared absorption spectra were recorded on a Hitachi model EPI-03 IR spectrophotometer using the KBr pellet method, ultraviolet absorption spectra on a Beckman model DBG ultraviolet spectrophotometer, nmr spectra on a JEOL model JNM-4H-100 100 MHz nmr spectrometer after dissolution of samples in d_4-methanol containing TMS as an internal reference, except otherwise noted, and mass spectra on a Hitachi model RM-7L high resolution mass spectrometer.

RESULTS

Detection and separation of urinary metabolites

Silica gel t.l.c. showed the presence of six metabolites which were visualized either under ultraviolet light or by Dragendorff reagent; no unchanged alkaloid was detected (Fig. 1A). Only very small amounts of metabolite I and the minor meta-

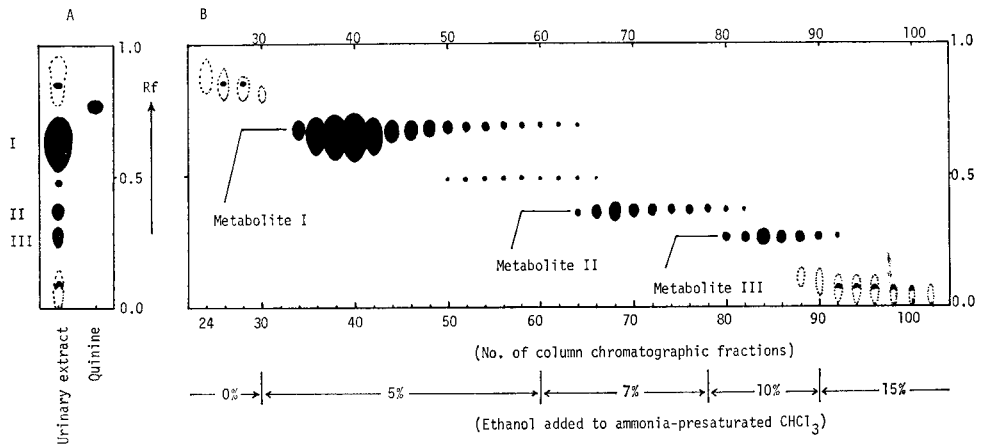

FIG. 1. T.l.c. of the urinary extract obtained after administration of quinine monohydrochloride to rabbits (A) and a t.l.c.-monitored diagram of column chromatographic separation of the metabolites (B). t.l.c. was carried out in ammonia-presaturated chloroform and ethanol (4:1) on plates coated with silica gel containing an inorganic fluorescent agent, and the chromatograms were visualized under ultraviolet light (255 nm) and spraying with Dragendorff reagent or with sulphuric acid followed by heating. Spots circled with a dotted line represent urinary components visualized by the sulphuric acid method but not by the former two. For the preparation of the ammonia-pretreated silica gel column and the application of the sample, see the METHODS. The column chromatogram was developed successively with benzene (500 ml), chloroform, and chloroform containing ethanol shown in %. The effluent was collected into 50 ml fractions. There were insufficient minor metabolites with highest and lowest polarities to be isolated pure. The metabolite between I and II in A could not be isolated.

bolite with the highest R_F value were extracted from the urine when acid hydrolysis was omitted. Control t.l.c. experiments with extracts obtained similarly from the urine of the same animals untreated showed that no urinary component was present that was extractable with ethyl acetate and visualized by both methods. The adequacy of the continuous extraction method was tested by the re-extraction of residual urine with n-butanol after saturation with sodium chloride when no polar metabolites nor any polar materials were detected.

The metabolites were separated on the ammonia-pretreated silica gel column using mixtures of chloroform saturated with ammonia and ethanol as developing solvents (Fig. 1B). Eluates containing only metabolite I (as shown by t.l.c.) were combined and the same was done for eluates containing metabolites II and III. Other eluates were combined and re-chromatographed. T.l.c. with mixtures of chloroform saturated with ammonia and various ratios of ethanol and subsequent visualization by the sulphuric acid method showed that the combined fractions containing the major metabolites were practically free from contamination with urinary components.

Evaporation of the solvent from the three combined fractions gave crystalline solids in yields of 1085 mg (33·5% of quinine monohydrochloride dihydrate administered), 260 mg (7·7%), and 189 mg (6·1%) for metabolites I, II and III, respectively.

Characterization and identification of urinary metabolites

Metabolite I. This was recrystallized from methanol and acetone to give needles, m.p. 247–249°; MS *m/e* 340 (M^+); λ_{max}0·1 N H_2SO_4 nm (log ϵ): 212 (4·55), 236 (4·50), 255 (4·20), 284 (3·75), 353 (3·88); ν_{max}cm^{-1}: 3534, 3484, 3205, 3106, 1667, 1626, 1506, 1451, 1425, 1272, 1236, 1163, 1103, 1038, 993, 917, 838, 680. The mass spectrum indicated that the metabolite was a quinine derivative with one additional oxygen atom probably in the quinoline nucleus but not in the quinuclidine nucleus; that the latter was intact was shown by the appearance of the most intense fragment ion peak due to a vinylquinuclidinium ion (*m/e* 136). Location of the biologically introduced oxygen atom was shown by the nmr spectrum to be at the 2′-position in which aromatic proton signals were integrated to correspond to four hydrogens and appeared at δ 6·86 (singlet, 3′-H), 7·18 (double doublet, $J_{7'-8'}$ 8·7 and $J_{5'-7'}$ 2·0 Hz, 7′-H), 7·33 (unresolved doublet, 5′-H), and 7·35 ppm (doublet, J 8·7 Hz, 8′-H). The nmr spectrum also showed that both methoxyl and vinyl groups were retained in the molecule. Therefore, the structure of the metabolite was assigned to be 2′-hydroxyquinine, the same substance as has been isolated from an incubation of quinine with a rabbit liver preparation (Kelsey & others, 1944). The earlier reported melting point and partial ultraviolet spectrum (Brodie & others, 1951) recorded in the region of wavelengths longer than 220 nm were in accordance with our findings. As postulated from the spectral data, the metabolite exists as a 2-quinolone either in a solid state or in solution.

Metabolite II. This was also recrystallized from methanol and acetone to give prisms, m.p. 244–245°; MS *m/e* 356 (M^+); λ_{max}0·1 N H_2SO_4 nm (log ϵ): 212 (4·53), 236 (4·48), 255 (4·18), 284 (3·73), 353 (3·86); ν_{max}cm^{-1}: 3367, 1658, 1621, 1504, 1471, 1416, 1267, 1250, 1168, 1134, 1099, 1036, 1004, 967, 928, 894, 871, 838, 679. The mass spectrum indicated that the metabolite was a quinine derivative with two additional oxygen atoms, of which one was shown to be possibly localized in the quinuclidine nucleus by the appearance of the most intense fragment ion peak due

to a vinylquinuclidinium ion with one oxygen (*m/e* 152) and the other probably in the quinoline nucleus. Superimposability of the ultraviolet spectrum of this metabolite on that of metabolite I suggested that both aromatic moieties were the same, and this was confirmed by the nmr spectrum in which aromatic proton signals were integrated to correspond to four hydrogens and appeared with similar features to those of metabolite I. Therefore, it was postulated that one of the biologically introduced oxygen atoms was located at the 2′-position. Location of another oxygen atom incorporated in the quinuclidine nucleus was also established from the nmr spectrum to be at the 3-position to form a hydroxyl group, i.e. a remarkable spectral change was observed in signals due to the vinyl protons compared with the ones arising from quinine. The vinyl protons of quinine acetate (in $CDCl_3$) resonated at δ 4·95 and 4·98 ppm (2H, partially overlapped two quartets, $J_{10-11trans}$ 17·5 and $J_{10-11cis}$ 10·5 Hz, 11-H) and 5·70 ppm (1H, septet, J_{3-10} 6·7 Hz, 10-H). However, in metabolite II, the 10-proton signal appeared at δ 5·99 ppm as a quartet ($J_{10-11trans}$ 17·5 and $J_{10-11cis}$ 10·5 Hz), indicating replacement of the 3-hydrogen by a hydroxyl group.

Thus, the structure of the metabolite was shown to be 2′,3-dihydroxyquinine.

Metabolite III. This highly polar material was obtained as a coloured crystalline solid which showed an intense yellow fluorescent spot under ultraviolet light on thin-layer chromatograms treated with ammonia vapour. It was unstable in solution, gradually turning into more polar materials, especially on exposure to daylight. The metabolite was sparingly soluble in acetone and highly soluble in alcohols. Recrystallization failed to remove the coloured impurities. For further purification it was treated with benzoyl chloride in anhydrous pyridine at 60° for 1 h. The mixture contained a non-fluorescent benzoate appearing as a single spot having an R_F value of 0·70 on thin-layer chromatograms developed with chloroform saturated with ammonia and containing 1% ethanol. However, during the purification of its reaction product on an ammonia-pretreated silica gel column, it was converted to a more polar compound whose R_F value (0·38) was larger than that of metabolite III (0·07). The material had yellow fluorescent properties and was unstable in solution; it was recrystallized from acetone and methanol to give prisms, m.p. 207–208°; MS *m/e* 430 (M^+); ν_{max} cm^{-1}: 3400, 3145,3050, 2626, 2530, 1729, 1649, 1626, 1604, 1547, 1510, 1425, 1380, 1263, 1104, 1088, 1065, 1020, 828, 712. The nmr spectrum showed that it had nine aromatic protons but no methoxyl group, indicating it to be a mono-benzoate of demethylquinine with one additional oxygen atom in the quinoline nucleus. That was also indicated by the mass spectrum in which the most intense fragment ion peak with *m/e* 136 was assigned to arise from the unmodified vinyl- quinuclidine moiety by the splitting between the 8- and 9-positions as similarly observed in the spectra of metabolites I (*m/e* 136) and II (*m/e* 152 (136 + 16)). Since it is well known that a benzoate of a phenol is susceptible to ammonolysis, the alkali-labile, less polar material, first observed in the reaction mixture was assumed to be a dibenzoate of the metabolite.

Treatment of the metabolite with diazomethane in methanol and ether led to the formation of two materials resolvable by column chromatography of which the more polar one was confirmed to be metabolite I by mixed melting point, t.l.c., and comparison of the infrared and ultraviolet spectra with those of the authentic specimen. The other material, fine needles from acetone and methanol, m.p. 220–221°, showed a molecular ion peak of *m/e* 354 in the mass spectrum, suggesting it

to be a monomethyl ether of metabolite I, namely 2′-methoxyquinine. The latter was identical with the product obtained by the treatment of metabolite I with diazo methane.

Thus, the structure of metabolite III was shown to be 2′,6′-dihydroxycinchonidine.

A possibility that the metabolite might be derived artificially from metabolite I during acid hydrolysis of the urine, was excluded by t.l.c. examination of an ethyl acetate extract, obtained from rabbit urine containing metabolite I alone after the hydrolysis under conditions stronger (2N HCl, 100°, 2 h) than those above were applied: no trace amount of metabolite III was detected on the chromatograms.

DISCUSSION

To our knowledge the present investigation has shown several previously unreported metabolites to be excreted in the urine after the administration of quinine to rabbits. It also provides the first quantitative data about urinary excretion of the major ones. The urinary metabolites isolated are all 2′-oxygenated derivatives of quinine, of which 2′-hydroxyquinine (metabolite I) was present in greatest amount. The difference in amounts and the number of metabolites extracted before and after the acid hydrolysis of the urine of animals dosed with the alkaloid indicates most metabolites occur as acid-labile precursors such as glucuronides and/or sulphates in the untreated urine.

There is a remarkable difference in the metabolism of quinine in man and the rabbit. Brodie & others (1951) claimed that in man, oxidation of the quinuclidine nucleus was the major reaction and carbostyril formation was less important. The two major metabolites isolated by them from human urine are unidentified but were assumed, mainly from the results of ultraviolet spectrometry, to be oxidation products of the quinuclidine nucleus. We found all major urinary metabolites of quinine in the rabbit to be phenolic derivatives.

Oxidation of quinoline and its derivatives, including quinine, to the corresponding carbostyrils has been demonstrated by Knox (1946) to be effected by rabbit liver extract which contains an enzyme named quinoline oxidase or quinine oxidase. Comparative studies on the enzymic formation of 2-carbostyrils from quinolines have demonstrated that only the liver of rabbits has the enzyme activity (Lang & Keuer, 1957) which is localized in the supernatant fraction (Sax & Lynch, 1964). The enzyme that oxidizes the other positions of the quinoline nucleus is in the microsomal fraction of the liver (Sax & Lynch, 1964; Mitoma, Posner & others, 1956). The earlier suggestion that, in the metabolism of quinolines by rabbit liver preparations, carbostyril formation reaction occurs before aromatic hydroxylation (Sax & Lynch, 1964), is in agreement with the present results showing the predominant formation of 2′-hydroxyquinine, since hydroxylation of aliphatic carbons is well known to be catalysed by the same type of microsomal enzyme as that acting on aromatic compounds (Brodie, Gillette & La Du, 1958). Hence, it appears that, in the rabbit, oxidation at the 2′-position of quinine occurs first.

O-Demethylation of quinine has been demonstrated by Axelrod (1956) in a study of the dealkylation of a series of ethers by the microsomal mixed function oxidase. It appears from the present data that the *O*-demethylation product, 6′-hydroxycinchonidine, is not excreted in rabbit urine, at least in appreciable amounts. It

may, however, be one of the three minor metabolites detected on thin-layer chromatograms, but no direct evidence has been obtained for their structures.

The previously isolated polar material, quinetine (Kerner, 1869), which could be extracted, if present, by the continuous extraction method or the subsequent butano extraction of the residual treated urine, also appears to be absent in the urine of rabbits, since our preliminary t.l.c. examination with the urinary extract indicated that no spot due to a quinine derivative disappeared after the treatment of its solution in chloroform with 3% aqueous sodium carbonate solution.

Formation of the 3-hydroxylation product (metabolite II) as one of the major urinary metabolites is in accordance with the previously established principle that the allylic position of drugs is susceptible to hydroxylation as observed in the *in vivo* metabolism of hexenylbarbiturates (Bush, Butler & Dickson, 1953; Tsukamoto & Yoshimura, 1955) and ionones (Prelog & Meier, 1950; Prelog, Wursch & Meier, 1951; Bielig & Hayashida, 1940).

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REFERENCES

Axelrod, J. (1956). *Biochem. J.*, **63**, 634–639.
Bielig, H. J. & Hayashida, A. (1940). *Hoppe-Seyler's Z. physiol Chem.*, **266**, 99–111.
Brodie, B. B., Baer, J. E. & Craig, L. C. (1951). *J. biol. Chem.*, **188**, 567–581.
Brodie, B. B., Gillette, J. R. & La Du, B. N. (1958). *Ann. Rev. Biochem.*, **27**, 427–454.
Bush, M. T., Butler, T. C. & Dickson, H. L. (1953). *J. Pharmac. exp. Ther.*, **108**, 104–111.
Kelsey, F. E., Geiling, E. M. K., Oldham, F. K. & Dearborn, E. H. (1944). *Ibid.*, **80**, 391–392.
Kerner, J. (1869). *Pflügers Arch. ges. Physiol.*, **2**, 200–243.
Knox, W. E. (1946). *J. biol. Chem.*, **163**, 699–712.
Lang, K. & Keuer, H. (1957). *Biochem. Z.*, **329**, 277–282.
Mitoma, C., Posner, H. S., Reitz, H. C. & Udenfriend, S. (1956). *Arch. Biochem.*, **61**, 431–441.
Nirenstein, M. (1918). *J. Roy. Army med. Corps*, **32**, 218–219.
Prelog, V. & Meier, H. L. (1950). *Helv. chim. Acta*, **33**, 1276–1284.
Prelog, V., Wursch, J. & Meier, H. L. (1951). *Ibid.*, **34**, 859–861.
Sax, S. M. & Lynch, H. J. (1964). *J. Pharm. exp. Ther.*, **145**, 113–121.
Tsukamoto, H. & Yoshimura, H. (1955). *Chem. pharm. Bull., Tokyo*, **3**, 397–398.
Williams, R. T. (1959). *Detoxication Mechanisms*, p. 657. London: Chapman & Hall.