substituted nitrobenzenes. Each of these series will have to be extended and their $LD_{50}$ values determined before it can be established whether a parameter in addition to $E_R$, or an alternative parameter combination, is necessary in order to arrive at a correlation.

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# Carbon-13 Magnetic Resonance Study. Structure of the Metabolites of Orally Administered Quinidine in Humans


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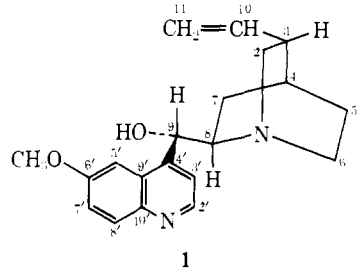
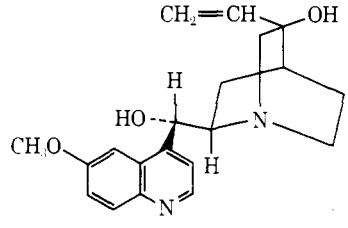
Carbon-13 pulsed Fourier transform nuclear magnetic resonance spectra of DMSO-$d_6$ solutions of the major metabolites of orally administered quinidine were obtained. The resonances were assigned by chemical shift comparisons and single-frequency off-resonance decoupling. The data have been used to establish the structure of the metabolites as 3-hydroxyquinidine and 2′-quinidinone.


The important cinchona alkaloid, quinidine (**1**), gives two major biotransformation products when administered orally to humans.[1] An analysis of the mass spectra of these metabolites showed that one of the metabolites (metabolite **5**)† resulted from oxidative modification of the quinoline ring of **1**, whereas the second metabolite (metabolite **4**)† resulted from hydroxylation of the quinuclidine ring of **1**.[1] An analysis of the mass spectrum and ir and uv properties of metabolite **5** indicated that it was the 2′-oxo derivative of quinidine; however, it was not possible to assign a structure to metabolite **4**. In the present paper we report our results on the application of carbon-13 nuclear magnetic resonance ($^{13}C$ nmr) spectroscopy for the structure elucidation of these metabolites of quinidine (**1**).

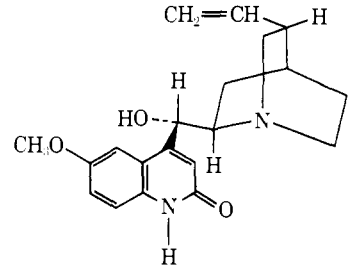


**1**

The structure assignments are based on an analysis of the natural abundance, proton noise decoupled and single frequency off-resonance decoupled $^{13}C$ nmr spectra of these metabolites and the parent drug. The chemical shift and multiplicity of the carbons for metabolite **4**, metabolite **5**, quinidine, and the maleate salts of quinidine and metabolite **4** are listed in Table I. Specific assignments were made with the use of reported chemical shift parameters for quinidine and other cinchona alkaloids[2] as well as the model compounds listed in Table I.



metabolite **4**



metabolite **5**

**Metabolite 4.** The observation that the mass spectrum of metabolite **4** gives a molecular ion at $m/e$ 340 and gives a quinuclidine fragment at $m/e$ 152 compared to $m/e$ 136 for quinidine is conclusive evidence that the metabolite contains a hydroxyl group in the quinuclidine ring.[1] Thus, the problem is to establish the position of attachment and stereochemistry of the hydroxyl group. Both quinidine and metabolite **4** and their maleate salts show nine signals in

†Metabolite **4** and metabolite **5** are the designation used by Palmer and coworkers for these metabolites.[1]

**Table I.** Carbon-13 Chemical Shifts for Quinidine and Its Metabolites in DMSO-$d_6$[a,b]

| Carbon atom | Quinidine[c,d] | Metabolite 4[d] | Metabolite 5[d] | Quinidine maleate[d] | Metabolite 4 maleate[d] | Quinuclidine[e] | 3-Quinuclidinol |
|---|---|---|---|---|---|---|---|
| 2 | (49.20) (t) | 57.08 (t) | (49.40) (t) | 48.52 (d) | 56.30 (t) | 48.2 | 57.90 |
| 3 | 39.89 (d) | 71.00 (s) | 39.89 (d) | 36.66 (d) | 69.63 (s) | 27.3 | 66.39 |
| 4 | 27.94 (d) | 33.62 (d) | 28.04 (d) | 26.86 (d) | 32.55 (d) | 21.5 | 28.05 |
| 5 | 26.37 (t) | 20.69 (t) | 26.47 (t) | 22.99 (t) | 17.89 (t) | 27.3 | 18.97 |
| 6 | (48.56) (t) | 49.25 (t) | (48.56) (t) | 27.58 (t) | 48.86 (t) | 48.2 | 47.17 |
| 7 | 23.28 (t) | 24.11 (t) | 22.74 (t) | 18.00 (t) | 19.27 (t) | 27.3 | 24.70 |
| 8 | 60.61 (d) | 59.29 (d) | 59.78 (d) | 59.29 (d) | 58.56 (d) | 48.2 | 46.15 |
| 9 | 70.91 (d) | 71.10 (d) | 71.49 (d) | 66.94 (d) | 66.89 (d) | | |
| 10 | 141.37 (d) | 144.24 (d) | 141.40 (d) | 137.97 (d) | 141.06 (d) | | |
| 11 | 114.41 (t) | 112.65 (t) | 114.60 (t) | 116.71 (t) | 115.24 (t) | | |
| $CH_3O$ | 55.47 (q) | 55.86 (q) | 55.67 (q) | 55.86 (q) | 55.77 (q) | | |
| 2′ | 147.52 (d) | 147.87 (d) | 161.74 (s) | 147.63 (d) | 147.33 (d) | | |
| 3′ | 120.97 (d) | 121.56 (d) | (118.67) (d) | 121.61 (d) | 122.54 (d) | | |
| 4′ | 149.46 (s) | 149.37 (s) | 153.70 (s) | 145.08 (s) | 146.05 (s) | | |
| 5′ | 102.50 (d) | 102.46 (d) | 106.96 (d) | 101.57 (d) | 102.01 (d) | | |
| 6′ | 156.83 (s) | 157.37 (s) | 154.04 (s) | 157.77 (s) | 158.40 (s) | | |
| 7′ | 119.81 (d) | 119.16 (d) | (119.06) (d) | 119.06 (d) | 119.50 (d) | | |
| 8′ | 131.16 (d) | 131.41 (d) | (119.06) (d) | 131.55 (d) | 130.77 (d) | | |
| 9′ | 127.10 (s) | 127.00 (s) | 117.20 (s) | 125.67 (s) | 126.02 (s) | | |
| 10′ | 143.95 (s) | 144.00 (s) | 133.56 (s) | 143.85 (s) | 142.97 (s) | | |

[a]Chemical shifts are in parts per million relative to tetramethylsilane. [b]Uncertain assignments are shown along with the next nearest uncertain chemical shift in parentheses. [c]Chemical shift data for quinidine were taken from ref 2. [d]Signal multiplicity obtained from single frequency off-resonance experiments: s = singlet, d = doublet, t = triplet, q = quartet. [e]Values taken from J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972, p 272.

the 20–72-ppm $^{13}C$ chemical shift region relative to internal TMS. These can be assigned to the seven carbons in the quinuclidine ring, the C-9 carbinol carbon, and the methyl of the $CH_3O$ group. Off-resonance experiments performed on metabolite 4 gave a $^{13}C$ nmr spectrum with one quartet, four triplets, three doublets, and one singlet for the eight signals. These results combined with the fact that an off-resonance spectrum of quinidine gave one quartet, four triplets, and four doublets for the same carbons show that the hydroxyl group is attached to a tertiary carbon. Of even greater importance is the absence of the 39.89-ppm resonance (doublet in off-resonance spectrum) present in the quinidine spectrum and the appearance of a new resonance at 71.00 ppm (singlet in the off-resonance spectrum) in the spectrum of metabolite **4**. These results combined with the observation that the C-4 carbons of metabolite 4 shift 5.68-ppm downfield ($\beta$ effect) relative to quinidine clearly show that the hydroxyl group is at position 3 of the quinuclidine ring of metabolite **4** (see structure). Similarly, the 36.66-ppm resonance in quinidine maleate is not found in the metabolite, but a new peak at 69.63 ppm (singlet in the off-resonance spectrum) is present. The downfield shift of carbons 2, 4, and 10 ($\beta$ effect) and the upfield shift of carbons 5 and 11 ($\gamma$ effect) are also consistent with this assignment.[2] Similar shifts of the $^{13}C$ resonances are observed in going from quinuclidine to 3-quinuclidinol (see Table I). The remaining carbons of metabolite **4** have essentially the same chemical shift as the corresponding carbons in quinidine but are different from those of other isomeric cinchona alkaloids.[2] These results, particularly the similarity of the chemical shifts of C-6 and C-4′, show that no epimerization at the C-8 and C-9 positions has accompanied the hydroxylation. With the present data it is not possible to rigorously establish the stereochemistry at the C-3 carbon. However, since most biological hydroxylations proceed with retention of configuration,[3] metabolite **4** has been assigned the structure shown.

**Metabolite 5.** The mass spectrum of metabolite **5** showed a molecular ion at $m/e$ 340 and showed a peak at $m/e$ 205 for the quinoline fragment, representing the addition of 16 mass units or one oxygen atom to the corresponding fragment in quinidine ($m/e$ 189).[1] The ir spectrum showed absorption at 2970 and 1683 $cm^{-1}$, and the uv spectrum showed a bathochromic shift in base (350 → 361 nm).[1] Based on this information, metabolite **5** was assigned the 2′-quinidinone structure shown. The $^{13}C$ nmr spectrum of metabolite **5** shows a resonance at 161.74 ppm for the C-2′‡ and gives chemical shifts for carbon 2–11 which are almost identical with those of quinidine. These results are in agreement with the 2′-quinidinone structure and strongly indicate that no isomerization has accompanied the metabolic oxidation of the quinoline ring of quinidine. The remaining $^{13}C$ nmr chemical shift assignments for the 2′-quinolinone ring of metabolite **5** were based on correlations with quinidine.

## Experimental Section

The $^{13}C$ nmr spectra were determined at 24.92 MHz on a modified JEOL JNM-PS-100 FT-NMR interfaced with a Nicolet 1085 Fourier transform computer system. Spectra were obtained in dimethyl sulfoxide-$d_6$ (DMSO-$d_6$) in a 5-mm tube inserted inside a 10-mm tube. The spectra were recorded at ambient temperature by using the deuterium resonance of DMSO-$d_6$ as the internal lock signal. All proton lines were decoupled by a broad-band (~2500 Hz) irradiation from an incoherent 99.075-MHz source. Interferograms were stored in 8K of computer memory (4K output data points in the transformed phase corrected real spectrum), and chemical shifts were measured on 5000-Hz sweep width spectra. Typical pulse widths were 12 $\mu$sec, and the delay time between pulses was fixed at 1.0 sec. In the case of metabolites 4 and 5, 32 and 64K (twice as many for single frequency off-resonance experiments) data accumulations were obtained on 15 mg and 6 mg/0.3 ml of solvent sample. The chemical shifts reported are believed accurate to within ±0.1 ppm.

Melting points were determined on a Kofler hot-stage microscope using a calibrated thermometer. Ir spectra were measured with a Perkin-Elmer Model 467 grating infrared spectrophotometer.

Combined gas–liquid chromatography mass spectrometry was carried out on an LKB-9000 (LKB, Proclukter, Sweden) spectrometer. Microanalyses were carried out by Micro-Tech laboratories, Skokie, Ill. Where analyses were indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of theoretical values.

‡The $^{13}C$ nmr spectrum (DMSO-$d_6$) of 2-quinolinone showed a resonance at 162.08 ppm relative to TMS for the C-2 carbon.

The two metabolites were isolated and purified as previously reported.[1] Metabolite 4, recrystallized from a methanol and ethyl ether mixture, had mp 211–212° (lit.[1] mp 226–228°).§ *Anal.* ($C_{20}H_{24}N_2O_3$) C, H, N.

Metabolite 4 maleate salt, recrystallized from an methanol and ether mixture, had mp 225–226°. *Anal.* ($C_{24}H_{28}H_2O_7$) C, H, N.

Metabolite 5 had mp 230–232° dec (lit.[1] mp 229–232°).

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§The melting point reported in ref 1 for metabolite 4 may have actually been the melting point of the maleate salt.

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# Stereochemistry and Absolute Configuration of the Analgesic Agonist-Antagonist (−)-5-*m*-Hydroxyphenyl-2-methylmorphan

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The absolute configuration of the title compound was established to be 1*R*,5*S* by single-crystal X-ray analysis of its hydrobromide salt (−)-1·HBr. Both rings of the 2-azabicyclo[3.3.1]nonane system exist in chair conformations with the phenyl and methyl substituents equatorial. The distance between the cationic nitrogen and the aromatic ring is 5.66 Å, which is 1.0 Å greater than that in morphine and other axially oriented phenylpiperidine analgesics. The analgetically more potent enantiomers of 1 [(+)-1*S*,5*R*] and of the α- and β-prodines have substitution on the same enantiotopic edge of the piperidine ring, suggesting a similar stereochemical preference in the interaction of these molecules with the analgetic receptor.

The series of strong analgesics known as the phenylmorphans possesses significant enantiomeric stereoselectivity in their biological actions. May and coworkers[1] reported that (±)-5-*m*-hydroxyphenyl-2-methylmorphan (1) possesses an analgesic potency nearly equivalent to that of morphine. Its enantiomers, however, have a fourfold difference in analgesic activity, with the (+) isomer being the more active.[2] More importantly, it was found that (−)-1 exhibits a weak narcotic antagonist activity and only a mild physical dependence capacity,†[3] while (+)-1 has no antagonist activity and has a high physical dependence capacity.[4] Recently, the *N*-propyl, allyl, and cyclopropylmethyl derivatives of (+)-1 and its racemate were shown to have about 1/5–1/10 the analgesic activity of the parent *N*-methyl compound and to have only a very weak antagonist activity.[4] Because of this demonstrated antipodal stereoselectivity it became of interest to establish the absolute configuration of 1. This has been accomplished by single-crystal X-ray analysis of (−)-5-*m*-hydroxyphenyl-2-methylmorphan hydrobromide (−)-1·HBr.

## Experimental Section

The hydrobromide salt of (−)-1 ($C_{15}H_{22}NOBr$) crystallizes from methanol–acetone as colorless needles: mp 232–233°; $[\alpha]^{20}D$ −4.2° ($H_2O$). The space group is $P2_12_12_1$ with unit cell parameters $a$ = 10.347, $b$ = 22.215, $c$ = 6.213 Å, $z$ = 4, and $d$ (calcd) = 1.45 g/cm³. Intensity data were collected from a 0.25 × 0.20 × 0.35 mm crystal on a computer controlled Picker FACS 1 diffractometer in a $\theta$–$2\theta$ scan mode using graphite monochromated Mo K$\alpha$ radiation. Two octants of data were collected, $hkl$ to $2\theta$ = 45° and $hk\bar{l}$ to $2\theta$ = 55°, to give 2219 independent observed reflections (intensity > $2\sigma$). The data were corrected for Lorentz and polarization factors but no corrections were made for absorption. The structure was solved by Patterson and Fourier methods using the $hkl$ data set and was refined by full-matrix least-squares procedures. The 22 hydrogen atoms were located in a difference Fourier and the complete structure was refined (nonhydrogen atoms anisotropically) to an agreement residual $R$ of 0.047 for the 1000 observed $hkl$ reflections, using weights based on counting statistics and without correction for anomalous dispersion. The absolute configuration of the molecule was established from the anomalous dispersion of the bromine atom by use of the Hamilton $R$ factor ratio[5] and by comparison of Friedel pairs. The $R$ factor ratio of the enantiomers was 0.041/0.059 for the $hkl$ data set and 0.049/0.063 for the $hk\bar{l}$ data set. The 1*R*,5*S* enantiomer gave the lower $R$ value with both data sets and is thus unequivocally shown to be the correct absolute configuration. Two final cycles of full-matrix least-squares refinement on the combined interscaled data sets using correction for the anomalous dispersion of the bromine atom produced a final $R$ of 0.039 for the 2219 independent observed reflections. (See paragraph at end of paper regarding supplementary material.)

Hydrogen bonding between the proton on the piperidine nitrogen and the bromide ion and between the phenolic hydroxyl proton and the bromide ion is indicated by the following parameters: N···Br, 3.22 Å; N–H, 0.91 Å; H···Br, 2.36 Å; <N–H···Br, 160°; O···Br, 3.38 Å; O–H, 0.71 Å; H···Br, 2.67 Å; <O–H···Br, 176°.

## Results and Discussion

The absolute configuration of (−)-1·HBr, as determined from the anomalous dispersion of the bromine atom, is 1*R*,5*S* and is shown correctly in Figures 1 and 2.‡ From these drawings it is apparent that both rings of the 2-azabicyclo[3.3.1]nonane system are in chair conformations with the phenyl and methyl substituents equatorial. However, these chairs have become somewhat distorted in order to relieve the steric interaction between carbon atoms 3 and 7. If both rings were in true chair conformations these two atoms would be separated by a distance of approximately 2.5 Å, which is the same as the sterically unfavorable 1-4 distance in the boat conformation of cyclohexane. The steric interaction is relieved in this molecule by bending the ends of the chairs outward so that the actual C(3)–C(7) distance is 3.08 Å. This deformation causes the interplanar angles of the chairs to be in-

†E. L. May, personal communication.

‡Computer controlled perspective drawing using the three-dimensional atomic coordinates from the X-ray data: C. K. Johnson, ORTEP, Oak Ridge National Laboratory Report ORNL-3794, 1965.