Journal of Natural Products
Vol. 47, No. 5, pp. 882-884, Sep-Oct 1984

MICROBIOLOGICAL TRANSFORMATION OF QUINIDINE

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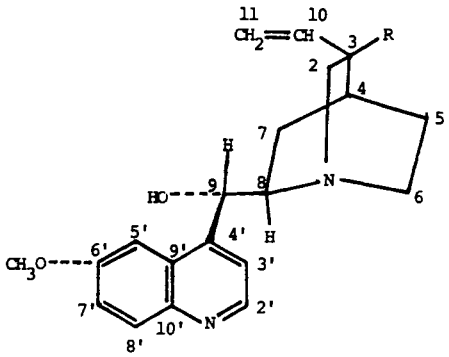
Quinidine (**1a**), a cinchona alkaloid used in the treatment of cardiac arrhythmias, is extensively metabolized in man. Three metabolites have been isolated and characterized: (3*S*)-3-hydroxyquinidine, 2′-quinidone, and *O*-desmethylquinidine (1-4). Additional metabolites were also isolated in small quantities, and spectral data suggested that they were free and conjugated products of oxidative-type transformations (2). Since microbial transformations have demonstrated usefulness in the preparation of metabolites of structurally complex natural products (5), this approach was used with quinidine in an attempt to produce larger quantities of the unidentified metabolites.

Screening scale experiments were conducted to identify organisms capable of transforming quinidine. By thin layer chromatographic observation, the following organisms were found to convert quinidine to a single, more polar metabolite: *Aspergillus fumigatus* (MR 51), *Cunninghamella elegans* (UI-Sih 1393), *Cunninghamella blakesleeana* (NRRL 1369), *Cunninghamella baineri* (SC 3065), *Cunninghamella echinulata* (NRRL 3655), *Stemphylium consortiale* (Wisc 4136), *Streptomyces griseus* (ATCC 10137), and *Streptomyces griseus* (ATCC 13273). A preparative scale incubation using *S. griseus* (ATCC 13273) with 1.44 g of quinidine (**1a**) resulted in the isolation of the metabolite in approximately 3% yield after column chromatography and preparative tlc.

The ms of the metabolite (**1b**) provided a molecular ion 16 mass units higher than that of quinidine (**1a**) and gave a quinuclidine fragment at *m/z* 152 compared to *m/z* 136 for **1a** (2). The characteristic fragment at *m/z* 189 corresponding to the quinoline portion plus



1a R=H
1b R=OH

the C-9 substituent was present in the metabolite spectrum. These data suggested that the metabolite contained a hydroxyl group in the quinuclidine ring. The position of attachment and the stereochemistry of the hydroxyl group were established by further spectral evaluation.

The pmr spectra of quinidine (**1a**) and the metabolite (**1b**) were similar in the aromatic and aliphatic regions. However, changes in the vinyl proton signals were evident in the metabolite spectrum. Quinidine exhibits complex multiplets in the region of 4.8 ppm to 6.2 ppm, attributed to the hydrogen atoms on carbons 10 and 11 (6). In the metabolite (**1b**), the H-10 signal appears as a doublet of doublets at 6.27 ppm (J=10.8 Hz and 17.4 Hz, 1H) while the H-11 signals appear as doublets at 5.35 ppm (J=17.4 Hz, 1H) and 5.20 ppm (J=10.8 Hz, 1H). This simplified first-order splitting pattern for the vinyl proton signals reflects a loss of the hydrogen atom on C-3 and indicates the addition of the hydroxyl group at this position.

The cmr chemical shift assignments for the metabolite (**1b**) were based on those reported for quinidine and the mammalian 3-hydroxyquinidine metab-

olite (7,8). The resonance at 39.84 ppm for C-3 of quinidine is not found in the metabolite spectrum, and a new singlet at 70.74 ppm is present and attributed to C-3. The downfield shifts of carbons 2, 4, and 10 are attributed to the β-effect and verify substitution at the 3 position. The 3-hydroxyquinidine epimers have been prepared synthetically, and the stereochemistry about C-3 was assigned based on the ^{13}C-chemical shifts of the C-5 and C-7 methylenes (8). (3*S*)-3-Hydroxyquinidine has the 3-hydroxyl group *syn* to C-5 and was reported to show a 5.68 ppm upfield shift and a 0.83 ppm downfield shift for the C-5 (20.69 ppm) and C-7 (24.11 ppm) resonances, respectively, when compared with quinidine. The (3*R*)-3-hydroxy epimer, which contains the 3-hydroxyl group *syn* to C-7, was reported to have resonances at 23.86 ppm and 22.39 ppm for C-5 and C-7. The upfield shift of C-5 (5.63 ppm) and the downfield shift of C-7 (1.34 ppm) of the *S. griseus* metabolite (**1b**) indicate that the 3-hydroxy group is *syn* to C-5 and verify the structure of this metabolite as (3*S*)-3-hydroxyquinidine.

TABLE 1. Cmr Chemical Shifts in DMSO-d^6

	1a[a]	**1b**
C(2) . . .	49.20 (t)	56.9309 (t)
C(3) . . .	39.89 (d)	70.7409 (s)
C(4) . . .	27.94 (d)	33.3926 (d)
C(5) . . .	26.37 (t)	20.5817 (t)
C(6) . . .	48.56 (t)	49.0914 (t)
C(7) . . .	23.28 (t)	24.6192 (t)
C(8) . . .	60.61 (d)	59.3708 (d)
C(9) . . .	70.91 (d)	70.9139 (d)
C(10) . . .	141.37 (d)	143.8656 (d)
C(11) . . .	114.41 (t)	112.0267 (t)
OCH_3 . . .	55.47 (q)	55.4103 (q)
C(2′) . . .	147.52 (d)	147.4741 (d)
C(3′) . . .	120.97 (d)	120.9036 (d)
C(4′) . . .	149.46 (s)	149.1568 (s)
C(5′) . . .	102.50 (d)	102.3947 (d)
C(6′) . . .	156.83 (s)	156.8149 (s)
C(7′) . . .	119.81 (d)	118.8329 (d)
C(8′) . . .	131.16 (d)	131.1418 (d)
C(9′) . . .	127.10 (s)	126.8251 (s)
C(10′) . . .	143.95 (s)	144.2328 (s)

[a]See Carroll, *et al.* (3).

As with most biological hydroxylations, the hydroxylation of quinidine by *S. griseus* (ATCC 13273) has proceeded with retention of configuration about the substituted carbon. The product produced by *S. griseus* is identical to one of the primary mammalian metabolites of quinidine and is another excellent example of the microbial model's approach to the study of mammalian metabolism.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined in open-ended capillaries with a Thomas-Hoover apparatus and are uncorrected. Ir spectra were obtained on a Perkin Elmer 237B spectrophotometer with KBr discs, and uv spectra were recorded in a Beckman model 24 spectrophotometer. Pmr spectra were recorded with a Nicolet NT 300 spectrometer with TMS as an internal standard. Off-resonance cmr spectra were obtained on a Bruker WM-360 FTQNMR operating at 90.5 MHz with decoupler continuous wave offset at 5500 Hz (0.17 mM; 3,961 scans). Broadband decoupled cmr were obtained on the instrument described above with a decoupler offset at 7300 Hz. High resolution mass spectral data were provided by the Midwest Center for Mass Spectrometry, University of Nebraska, Lincoln, Nebraska. Quinidine was obtained from the Aldrich Chemical Co., Milwaukee, Wisconsin.

CHROMATOGRAPHY.—Tlc was performed on 0.25 mm thick layers of silica gel GF_{254} (Merck) on glass plates. Prior to use, plates were activated at 50° for 30 min. The developing solvent for tlc plates was $CHCl_3$-Me_2CO-diethylamine(30:60:10)-quinidine (**1a**) Rf=0.75, (3*S*)-3-hydroxyquinidine (**1b**) Rf=0.55. Visualization of developed tlc plates was accomplished by spraying with Dragendorff's reagent.

FERMENTATION PROCEDURES.—*S. griseus* (ATCC 13273) was maintained on ATCC medium no. 5. Methods used in the cultivation of microorganisms have been described elsewhere (9). A two-stage incubation procedure using a soybean meal-glucose medium was employed. Incubations were conducted on rotary shakers (model G-25 New Brunswick Scientific Co.) operating at 250 rpm and 27°. Screening experiments were carried out in 125-ml steel-capped DeLong flasks containing 25 ml of medium. Quinidine was added to the 24-hour-old stage II cultures as a solution in DMF to a final concentration of 400 μg/ml of culture medium. Controls consisted of cultures grown without quinidine and of incubations containing medium and substrate without microorganisms. Samples were

withdrawn after 24, 48, 72, 96, 168, 240, and 336 h, adjusted to pH 9.0 with 58% NH_4OH, and extracted with EtOAc for tlc analysis. Conversions were detected at 72 h and maximum conversions were observed between 240 and 336 h.

PREPARATION AND CHARACTERIZATION OF (3S)-3-HYDROXYQUINIDINE (**1b**).—A total of 1.44 g of quinidine in 14.4 ml DMF was distributed evenly among 18 1-liter DeLong flasks, each containing 200 ml of soybean meal-glucose medium. After 336 h, the incubation mixtures were pooled, adjusted to pH 9.0 with 58% NH_4OH, and extracted with 3000 ml EtOAc three times. The extracts were dried over anhydrous Na_2SO_4 and concentrated to a viscous brown oil (2.9 g). The oil was applied to a silica gel column (2×60 cm, 60 g) and eluted with CH_2Cl_2 (500 ml) followed by CH_2Cl_2-MeOH (100:10) at a flow rate of 5 ml/min, while 20-ml fractions were collected. Fractions 26-65 contained quinidine (**1a**). Fractions 66-126 contained a mixture of quinidine and the metabolite (**1b**); these were combined, dried over anhydrous Na_2SO_4, and concentrated. The metabolite was further purified by dissolving in a small amount of CH_2Cl_2 and applying to 5.0-mm silica gel preparative layer tlc plates. The band between Rf 0.5 and 0.6 was scraped from the plates, mixed with 20-ml portions of Me_2CO three times, stirred, and filtered. Evaporation of the combined filtrates yielded 47 mg of pure (3S)-3-hydroxyquindine. The product, recrystallized from a MeOH and Et_2O mixture, had mp 205-207° [lit (3) mp 211-212°]; ir (KBr) 3400-3200, 2930, 2860, 1735, 1625, 1515, 1240 cm^{-1}; uv (MeOH) λ max 281 (log ε 3.60), 333 (log ε 3.69); pmr ($CDCl_3$) ppm, 1.0-3.84 (11 H, multiplets, H-2,4,5,6,7,8,9), 3.89 (1 H, s, -OCH_3), 5.20 (1 H, d, J=10.8 Hz, H-11), 5.35 (1 H, d, J=17.4 Hz, H-11), 5.75 (1 H, broad singlet, -OH), 6.27 (2 H, dd, J=10.8 Hz, 17.4 Hz, H-10), 7.17 (1 H, d, J=2.5 Hz, H-5′), 7.34 (1 H, dd, J=2.5 Hz, 9.2 Hz, H-7′), 7.55 (1 H, d, J=4.4 Hz, H-3′), 8.01 (1 H, d, J=9.2 Hz, H-8′), 8.72 (1 H, d, J=4.4 Hz, H-2′); ms *m/z* 340.1786 (5.10%, calcd. for $C_{20}H_{24}N_2O_3$ 340.1787); 189.0791 (14.42%, calcd. for $C_{11}H_{11}NO_2$ 189.0790); 152.1070 (100%, calcd. for $C_9H_{14}NO$ 157.1075).

ACKNOWLEDGMENTS

This research was supported by a grant from the American Heart Association, Oregon Affiliate, Inc. The author thanks Pamela Uchiyama, Yan Ki Lau, and Dan Colley for their excellent assistance.

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Received 29 December 1983