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Identification of Transformation Products Arising from Bacterial Oxidation of Codeine by *Streptomyces griseus*

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14-Hydroxycodeine and norcodeine were rigorously identified as products arising from codeine oxidation by *Streptomyces griseus* ATCC 10137. Both products were routinely detected in extracted culture filtrates after growth of cells in the presence of codeine for 1 week. Under these conditions, about 4 mol% of the codeine starting material was consumed, with norcodeine and 14-hydroxycodeine representing the only identifiable transformation products (molar ratio, 4:1, respectively). Extraction of a series of culture filtrates and purification of the pooled metabolites by thin-layer and high-pressure liquid chromatography led to the isolation of both biological products, the structures of which were verified by high-resolution mass spectrometry and proton nuclear magnetic resonance spectroscopy. The identities of both biological products were further confirmed by comparison of their spectral properties with those of authentic standards. This is the first report providing structural evidence for the biological formation of 14-hydroxycodeine from codeine and of codeine oxidation by *S. griseus*.

The metabolism of opium alkaloids including morphine, codeine, and thebaine by microorganisms has received limited attention. As these compounds, or derivatives thereof, are important therapeutic analgesics, their metabolism is of particular importance for two reasons. First, microbial transformations could serve as models by which to predict routes of mammalian metabolism (19), and second, microbes or enzymes therefrom may be useful as catalysts for arriving at more potent but less addictive structural analogs. Considerable work carried out in Japan in the early sixties showed that wood-rotting fungi identified as *Trametes sanguinea* were capable of oxidizing thebaine to mixtures of 14-hydroxycodeinone and 14-hydroxycodeine (8, 9, 20, 22). Similar results were reported in an independent study with a related fungus, *Trametes cinnabarina*, except that 14-hydroxycodeinone-*N*-oxide was described as an additional transformation product (7). However, when *T. sanguinea* was challenged with morphine or codeine, no identifiable products were observed (22). In later work, Liras and co-workers (11, 13) reported the bacterial conversion of morphine to 14-hydroxymorphine by an *Arthrobacter* sp., but rigorous chemical evidence for the identity of the reaction product was not provided. In a separate communication these same investigators described the conversion of morphine and codeine, respectively, to 14-hydroxymorphinone and 14-hydroxycodeinone by enzyme preparations of β-steroid dehydrogenase from *Pseudomonas testosteroni* (12); however, the identities of reaction products again were not established. In addition to these studies, recent reports have appeared that describe the N-demethylation of codeine by *Streptomyces paucisporogenes* and *Streptomyces lincolnensis* (18) and by the fungus *Cunninghamella baineri* (5, 6). The present work now provides the first evidence for codeine oxidation by the bacterium *Streptomyces griseus* and presents structural information on two biotransformation products formed (Fig. 1).

(A preliminary account of some of this work was reported at the 85th Annual Meeting of the American Society for Microbiology, Las Vegas, Nev., 3 to 7 March 1985 [D. A. Kunz, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, O73, p. 247].)

MATERIALS AND METHODS

Microorganisms and biotransformation conditions. All experiments were conducted with *S. griseus* ATCC 10137 obtained from the American Type Culture Collection, Rockville, Md. Cultures were maintained at 4°C on yeast malt extract agar slants (medium 200, American Type Culture Collection; Difco 0711, Difco Laboratories, Detroit, Mich.) or at −65°C by mixing a spore suspension obtained from cells grown on sporulation agar plates (medium 5, American Type Culture Collection) with dimethyl sulfoxide (American Type

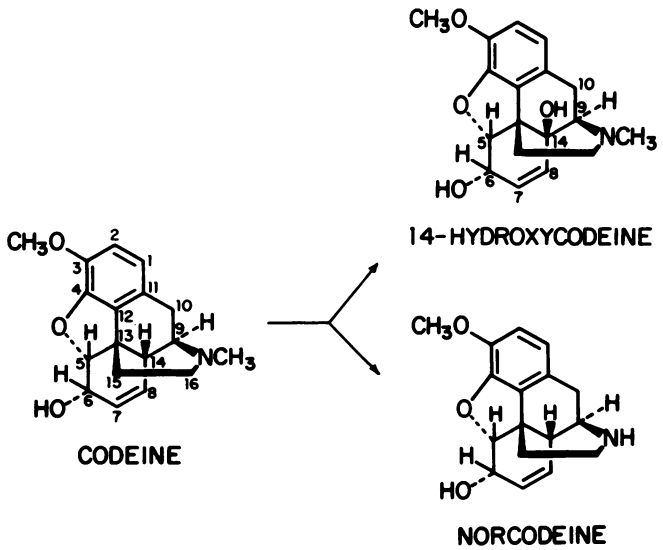

FIG. 1. Biotransformation products of codeine oxidation by *S. griseus* ATCC 10137.

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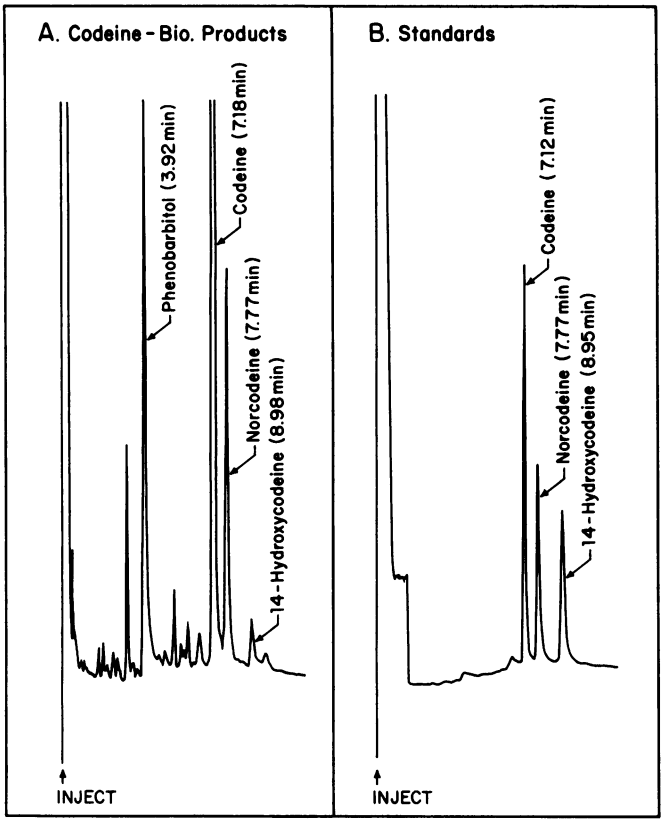

FIG. 2. Gas chromatography of extracted codeine transformation products produced by *S. griseus* ATCC 10137 (A) and standard samples of codeine, 14OHCOD, and norcodeine (B). The biological sample (A) was prepared after 10 days of cultivation, and extracted products were dissolved in 0.25 ml of methanol containing 20 mM phenobarbital before injecting 0.5 μl. Standard samples were prepared as a mixture of 3.3 mM each in methanol.

Culture Collection) (50:50, vol/vol). Starter cultures for small-scale biotransformation experiments were obtained by inoculating a single colony from a yeast malt extract agar plate to 10 ml of liquid yeast malt extract containing L-tyrosine at 2 to 5 mM. After growth at 30°C with rotary shaking, a second tube of the same medium was inoculated with 0.25 ml of cell suspension. This culture then was grown for 18 to 24 h before codeine phosphate was added to give a final concentration of 1.25 to 2.5 mM (0.37 to 0.75 mg/ml). Cultures were incubated for 7 days before cells were removed by centrifugation at 17,000 × *g* for 10 min and supernatants were analyzed for transformation products.

Chemicals. Narcotic drugs including codeine (7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan-6α-ol), phosphate, codeine (free base), 14-hydroxycodeine (oxycodeine) (14OHCOD), norcodeine (17-*N*-demethylcodeine), and morphine (7,8-didehydro-4,5-epoxy-17-methylmorphinan-3,6-diol) were provided by the Biomedical Products Department, Pharmaceutical Div., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del. All compounds were recrystallized before use and were judged to be 95 to 99% pure by high-pressure liquid chromatography (HPLC). *N*-Methyl-*N*-trimethylsilyl trifluoroacetamide used for preparation of narcotic trimethylsilyl (TMS) derivatives was purchased from Pierce Chemical Co., Rockford, Ill. Solvents for HPLC were purchased from Fisher Scientific Co., King of Prussia, Pa. All other chemicals were of the highest purity commercially available and were used without further purification.

Extraction and detection of codeine transformation products. Culture supernatants were adjusted to pH 9.6 with 50% (wt/vol) NH_4OH before extracting twice with an equal volume of CH_2Cl_2-ethanol (2:1). The solvent was evaporated under a stream of nitrogen at 46°C, and the crude organic residue was taken up in 0.25 ml of methanol and analyzed by thin-layer chromatography on precoated silica gel F254 sheets obtained from EM Reagents, Gibbstown, N.J. The solvent system contained ethyl acetate, methanol, and ammonium hydroxide (17:2:1). Compounds were viewed by UV absorbance and color formation after spraying with Dragendorff reagent. The latter reagent was prepared by the method of Munier (15) as described by Verpoorte and Baerheim Svendson (21) and gives a characteristic orange color with alkaloids.

Detection of compounds in crude-culture filtrate extracts was further accomplished by gas chromatography. Compounds were analyzed either directly or after chemical preparation of the TMS derivatives. In the former procedure, 0.5 μl of crude organic residues in methanol containing 20 mM phenobarbital (Sigma Chemical Co., St. Louis, Mo.) as an internal standard was directly injected onto a 10-m series 530μ 50% phenylmethyl silicone column (Hewlett-Packard Co., Avondale, Pa.). The column was maintained at 200°C for 10 min, programmed to increase at a rate of 10°C/min up to 255°C, and held there for 5.0 min. Alternatively, compounds were derivatized by treatment with *N*-methyl-*N*-trimethylsilyl trifluoroacetamide. For this purpose, 75 μl of extract in methanol was transferred to a 100-μl-capacity crimp-sealed vial (Supelco, Inc., Bellefonte, Pa.), and the methanol was completely evaporated before adding 75 μl of *N*-methyl-*N*-trimethylsilyl trifluoroacetamide. The sealed vials then were heated at 80°C for 30 min before injecting 0.5-μl samples onto the same column as described above but programmed to remain at 150°C for 1.5 min before increasing the temperature to 235°C at 10°C/min and then maintained at 235°C for 8.0 min. Regardless of the method of analysis, injector and detector temperatures were 265 and 300°C, respectively, with He supplied as the carrier gas at a flow rate of 10 ml/min. The retention times for authentic codeine, 14OHCOD, and norcodeine analyzed directly are shown in Fig. 2; the corresponding TMS derivatives gave retention values of 12.9, 11.5, and 12.0 min, respectively (data not shown). Metabolic products were quantitated as TMS derivatives since this method was found to be more sensitive (9 ng or 1.5 nM detection limit as calculated in original culture broth) and allowed compounds to be better resolved than when injected directly. Estimations were made by comparing product peak retention times and areas with those of authentic standards. All estimates were based on an extraction efficiency of 90 ± 10%, with CH_2Cl_2-ethanol (2:1) as the solvent.

Isolation and identification of biological 14-hydroxycodeine and norcodeine. To obtain appreciable quantities of biological products for structural characterization, 50 separate 10-ml cultures of *S. griseus* ATCC 10137 were incubated with codeine phosphate for 1 week before culture supernatants were pooled and extracted. The extract was dried over anhydrous $MgSO_4$, and the solvent was removed by evaporation in vacuo. The crude organic residue then was subjected to preparative thin-layer chromatography on 500-μm silica gel GF plates (Analtech, Inc., Newark Del.) by the solvent system described earlier. Putative 14OHCOD and norcodeine bands were collected and further subjected to

preparative HPLC on a silica column (4.6 by 250 mm, Zorbax Sil; E. I. du Pont de Nemours & Co.) with 90% methanol, 6.6% of 2 N NH_4OH, and 3.4% water as the mobile phase; the elution rate was 1.0 ml/min, and the column was maintained at 35°C. Compounds were detected by UV light absorbance at 286 nm, and those fractions corresponding to 14OHCOD or norcodeine were collected and the solvent was removed by evaporation. A portion of each biological sample then was analyzed by mass spectrometry before and after TMS derivatization, whereas the remaining sample was subjected to 360-MHz ^1H-nuclear magnetic resonance (NMR) analysis.

Analytical methods. Samples for gas chromatography were run on a Hewlett-Packard 5880 gas chromatograph. A Du Pont 850 liquid chromatograph was used to separate and purify biological metabolites by HPLC. Electron impact mass spectra were recorded on a model 16F V.G. micromass spectrometer (Winsford, England) at 70 eV interfaced with a Varian 3700 gas chromatograph. ^1H-NMR spectra were obtained with a Nicolet 360 WB Fourier transform spectrometer with 100-μg samples dissolved in 0.6 ml of 100.0 atom% deuterated dimethyl sulfoxide (DMSO-d6; Aldrich Chemical Co., Inc., Milwaukee, Wis.). Samples were recorded at room temperature unless otherwise indicated, with DMSO-d6 as the lock signal in the absence of any additional internal standard. Authentic standards were analyzed similarly at 0.2% concentrations. Proton assignments were made by homonuclear decoupling experiments and shielding effects.

RESULTS AND DISCUSSION

Examination of extracted culture filtrates by thin-layer chromatography revealed the presence of two metabolites after growth of *S. griseus* ATCC 10137 with codeine for 7 days. Both metabolites (R_f, 0.18 and 0.58) along with unconsumed codeine (R_f, 0.39) were detected on visual inspection of chromatograms by UV light absorbance and after color development with Dragendorff reagent. The fast migrating compound (R_f, 0.58) had similar chromatographic properties as authentic 14OHCOD, whereas the slower moving metabolite (R_f, 0.16) was similar to standard norcodeine. Analysis of incubation products by gas chromatography gave similar results (Fig. 2). That is, the major metabolite had a retention time similar to authentic norcodeine (7.77 min), whereas the minor compound cochromatographed with 14OHCOD (8.98 min). Although estimates of the amount of each metabolite varied, the concentrations of putative norcodeine and 14OHCOD in 7-day-old cultures were found to be approximately 25 and 75 μM, respectively. With substrate concentrations of 2.5 mM codeine, this represents an overall 4 mol% conversion yield. It is noteworthy that while substrate conversion was low, no products other than 14OHCOD and norcodeine could be detected. In addition, extending incubation periods to 10 days gave even slightly higher yields of metabolites (~7 mol%), making detection and quantitation more reliable. Control reaction mixtures containing codeine without cells but adjusted to pH 8.5 to mimic experimental samples in which the pH had increased from 6.1 to 8.5 during cultivation showed no evidence for 14OHCOD. In contrast, traces of norcodeine could be detected, but this amounted to only 1% of the total norcodeine accumulated, indicating that while some chemical N-demethylation does occur, it is relatively insignificant in comparison with biological catalysis by *S. griseus* ATCC 10137.

Further identification and structural characterization of metabolic products were achieved after their purification.

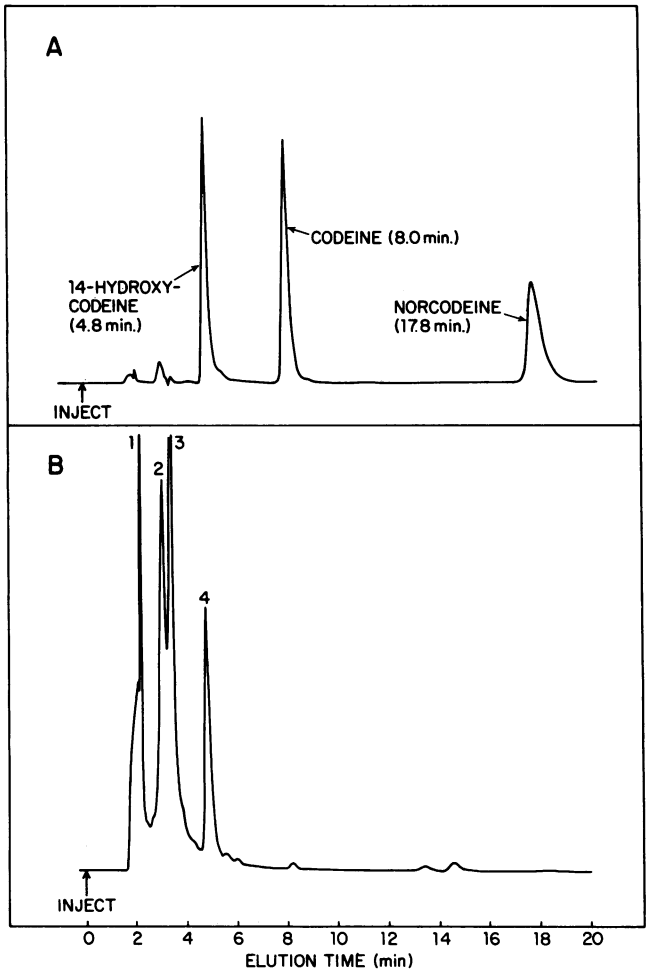

FIG. 3. HPLC elution profile of a standard mixture of codeine, 14OHCOD, and norcodeine (A) and putative biological 14OHCOD (B) produced by *S. griseus* ATCC 10137. A 100-μl partially purified sample obtained by preparative thin-layer chromatography was injected as shown in panel B, and peak 4 was collected for further characterization. Standard samples were prepared as a mixture at 2 mg/ml each in elution solvent, and 100 μl was injected.

The crude organic residue (325 mg) obtained after extracting culture filtrates from 50 separate 10-ml culture broths was first subjected to preparative thin-layer chromatography. This led to the removal of most of the unconsumed codeine starting material and gave approximately 300 μg and 1 mg of crude biological 14OHCOD and norcodeine, respectively. Both biological compounds were further purified by preparative HPLC as described in Materials and Methods. Putative biological 14OHCOD could be effectively separated from minor amounts of codeine still present in the sample as well as from other impurities (Fig. 3). Collection of the 14OHCOD fraction yielded approximately 150 μg of the pure biological compound. The metabolite had the same retention time on gas chromatography (Fig. 4) and gave the identical mass spectrum as that of authentic 14OHCOD. Figure 4 shows the mass spectrum of the TMS derivative which gave a molecular ion of 459 *m/e* (315 *m/e*, underivatized, data not shown), a base peak at 229 *m/e*, and a fragmentation pattern indistinguishable from that of authentic material. Similar purification of putative biological norcodeine by HPLC (elution time 17.8 min) afforded about 700 μg of the pure

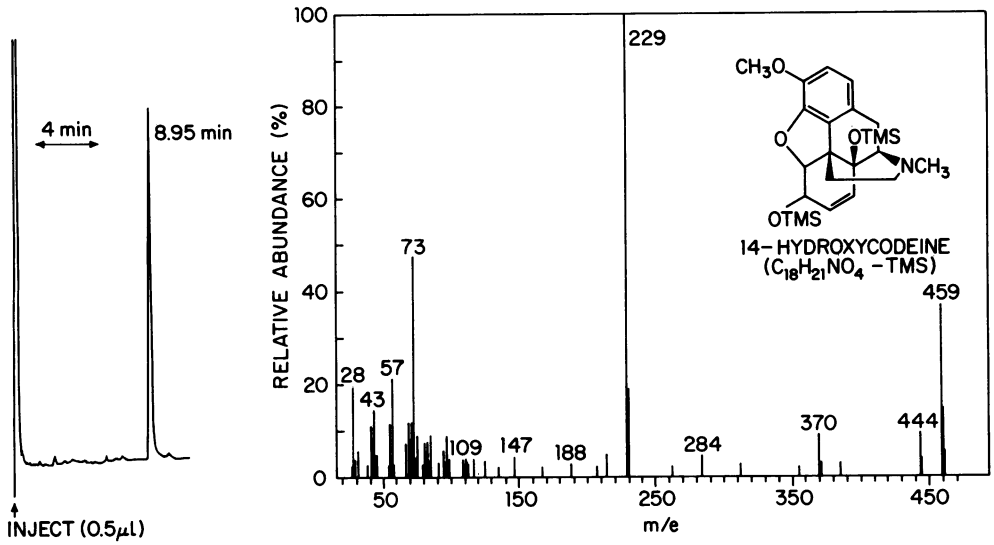

FIG. 4. Gas chromatography and electron impact mass spectrum of purified 14OHCOD obtained from pooled cultures of *S. griseus* ATCC 10137 incubated with codeine. 14OHCOD purified as described in the legend to Fig. 3 was taken up in methanol, and 0.5 μl was injected as shown. A separate portion was evaporated to dryness, and the TMS derivative was prepared by treatment with *N*-methyl-*N*-trimethylsilyl trifluoroacetamide before determining the mass spectrum.

metabolite. It also gave the identical mass spectrum as that of authentic material (Fig. 5), yielding molecular ions of 285 and 357 *m/e*, respectively, before and after (data not shown) TMS derivatization. These results are consistent with the presence of a single derivatizable hydroxyl group in norcodeine (C-6) versus two in 14OHCOD (C-6 and C-14).

Unequivocal structure identification of *S. griseus* ATCC 10137 metabolites was accomplished by high resolution ^{1}H-NMR analysis. Both putative biological 14OHCOD and authentic standard gave identical spectra in DMSO-d6 (Fig. 6A and B). In addition, these spectra were different from that of codeine as described previously (2, 3), and as determined here in which protons H_7 (5.49 ppm) and H_8 (5.20 ppm) each were coupled to H_{14} (2.50 ppm) and gave identical coupling constants (J = 2.8 ± 0.2 Hz) (Fig. 6D). In 14OHCOD, these couplings were absent and H_{14} was replaced with hydroxyl,

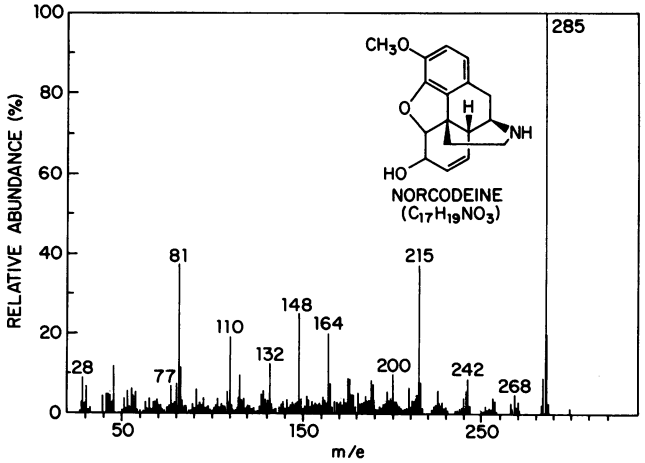

FIG. 5. Electron impact mass spectrum of biologically formed norcodeine obtained after cells of *S. griseus* ATCC 10137 had oxidized codeine.

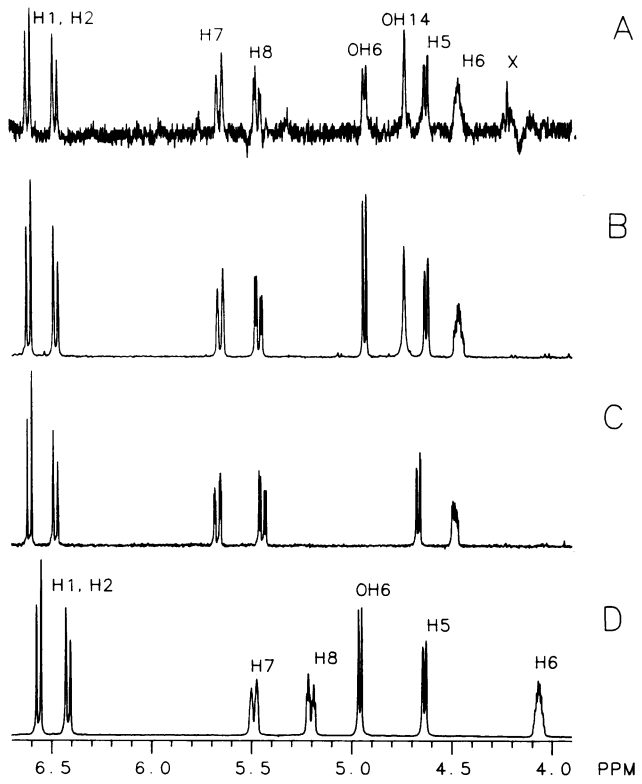

FIG. 6. Partial ^{1}H-NMR spectra of purified biological 14OHCOD (A) generated from codeine by *S. griseus* ATCC 10137 and authentic standards (B to D). All spectra were recorded in DMSO-d6 at ambient temperature, except that shown in panel C, which was obtained at 100°C. Partial spectra of 14OHCOD before and after exchange with 0.1% D_2O are shown in panels B and C, respectively. Panel D shows the spectrum of codeine, illustrating the difference in chemical shifts as compared with 14OHCOD for H_7 and H_8, both absent in 14OHCOD. Protons and their relative molecular positions are as indicated in Fig. 1. X indicates minor impurity.

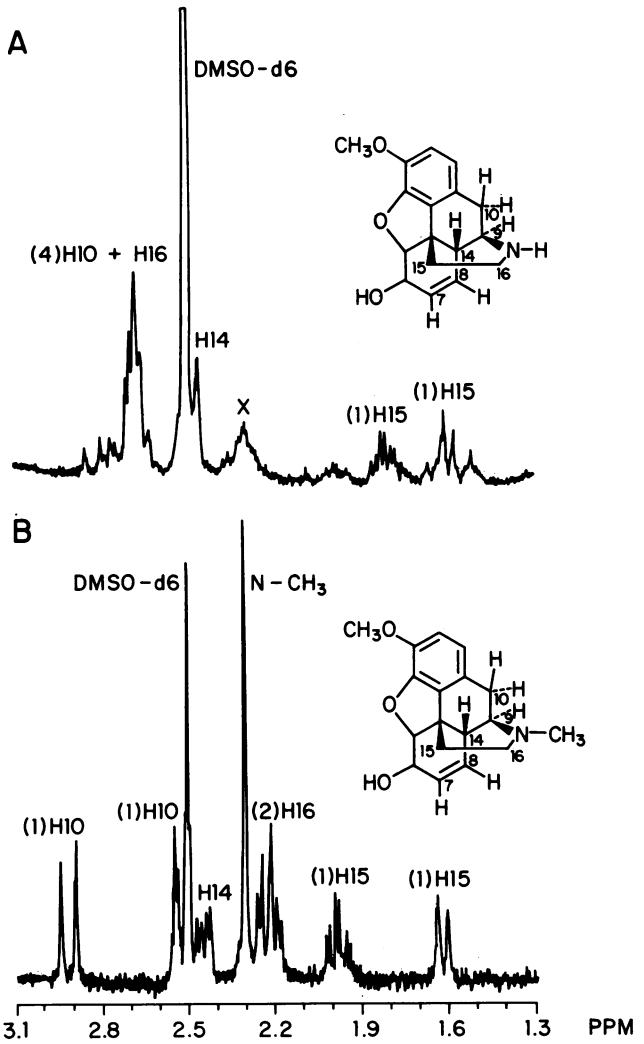

FIG. 7. Partial ^{1}H-NMR spectra of biologically formed norcodeine (A) obtained from pooled cultures of *S. griseus* ATCC 10137 incubated with codeine, and authentic codeine standard (B). Proton signals shown are those principally assigned to the N-containing (piperidine) ring with the N-CH_3 resonance (2.30 ppm) absent in the biological sample (A). Numbers within parentheses refer to proton integrals. X indicates minor impurity.

as evidenced by the appearance of a singlet at 4.73 ppm. The identities of both the C-14 and C-6 hydroxyl protons in 14OHCOD were further confirmed by deuterium exchange (Fig. 6C) and decoupling experiments. For example (Fig. 6C), signals at both 4.73 and 4.93 ppm collapsed after treatment of 14OHCOD with D_2O, thus allowing the unequivocal assignment of the C-6 and C-14 hydroxyl protons to be made. Similar 360-MHz analysis of the second metabolite led to its structural characterization as norcodeine. In this case, the biological compound could be easily distinguished from codeine starting material by the absence of a strong singlet at 2.30 ppm, consistent with the loss of the N-CH_3 substituent from codeine (Fig. 7). In addition, the chemical shifts of resonances assigned to the C-10, C-15, and C-16 protons by decoupling experiments were different from those observed for the corresponding protons in codeine. Other signals recorded for the metabolite and authentic norcodeine were further found to be identical.

The structural identification of 14OHCOD as a product of codeine oxidation by *S. griseus* provides strong evidence that 14-hydroxylation of this alkaloid can be accomplished biologically (Fig. 1). This finding is consistent with earlier reports describing similar microbial conversions for both codeine and morphine in which the chemical identities of reaction products were not established (11–13). Indeed, the bacterium-facilitated C-14 hydroxylation of codeine appears to be unique since this conversion has apparently never been accomplished chemically. The unreactive chemical nature of codeine can probably be attributed to the tertiary nature of carbon at position 14, whereas thebaine, which is vinylic at C-14, readily undergoes hydroxylation in acidic hydrogen peroxide (4, 14, 17). Similarly, biological hydroxylation of thebaine at C-14 also has been reported (1, 7–9, 20, 22). Since introduction of a hydroxyl substituent at C-14 of the morphinan structure has been shown to dramatically increase analgetic potency (10), biocatalytic methods that permit the utilization of codeine and morphine as starting materials for 14-hydroxylation may serve to complement existing chemical routes that rely exclusively on thebaine. For this reason, studies are being continued in an effort to define optimal physiological conditions and the nature of the enzymes responsible for both codeine hydroxylation and N-demethylation. The latter transformation giving norcodeine represents the major reaction catalyzed by *S. griseus* (Fig. 1) and is analogous to that reported for two other *Streptomyces* (18), for fungal (5, 6), and for mammalian (16) systems. These findings could indicate that N-dealkylation represents a common mode of opium alkaloid metabolism, whereas 14-hydroxylation may be unique to bacteria.

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LITERATURE CITED

1. **Aida, K., K. Uchida, K. Iizuka, S. Okuda, K. Tsuda, and T. Uemura.** 1966. Incorporation of molecular oxygen into a morphine alkaloid, 14-hydroxycodeinone by *Trametes sanquiuea*. Biochem. Biophys. Res. Commun. **22**:13–16.
2. **Batterham, T. J., K. H. Bell, and U. Weiss.** 1965. The nuclear magnetic resonance spectra of the codeine isomers and their derivatives. Aust. J. Chem. **18**:1799–1806.
3. **Crabb, T. A.** 1975. Nuclear magnetic resonance of alkaloids, p. 249–387. *In* E. F. Mooney (ed.), Annual reports on NMR spectroscopy, vol. 6A. Academic Press, Inc., New York.
4. **Freund, M., and E. Speyer.** 1916. Über die Umwandlung von Thebain in Oxycodeinon und Dessen Derivate. J. Prakt. Chem. **94**:135–178.
5. **Gibson, M., C. J. Soper, and R. T. Parfitt.** 1984. Application of ^{13}C-n.m.r. spectroscopy to study the mechanism of N-demethylation of [N-Me-^{13}C] codeine by cell-free extracts of *Cunninghamella baineri*. Anal. Chim. Acta **163**:175–183.
6. **Gibson, M., C. J. Soper, R. T. Parfitt, and G. J. Sewell.** 1984. Studies on the mechanism of microbial N-demethylation of codeine by cell-free extracts of *Cunninghamella baineri*. Enzyme Microb. Technol. **6**:471–475.
7. **Gröger, D., and H. P. Schmauder.** 1969. Mikrobielle Umwandlung von Thebain. Experentia **25**:95–96.
8. **Iizuka, K., S. Okuda, K. Aida, T. Asai, K. Tsuda, M. Yamada, and I. Seki.** 1960. Mikrobiologische Verwandlung von Thebain in das 14-Hydroxycodeinon Mittels Eingeger Pilze. Chem. Pharm. Bull. **8**:1056–1057.
9. **Iizuka, K., M. Yamada, J. Suzuki, I. Seki, K. Aida, S. Okuda, T.**

Asai, and K. Tsuda. 1962. Untersuchungen auf dem Gebiet der Mikrobiologishcen Umsetzung. XVI. Umwandlung von Alkaloiden in der Morphin-Reihe Durch *Trametes sanguinea*. Chem. Pharm. Bull. **10**:67–70.

10. **Johnson, M. R., and G. M. Milne.** 1981. Analgetics, p. 699–758. *In* M. E. Wolff (ed.), Burger's medicinal chemistry, 4th ed. (part 3). John Wiley & Sons, Inc., New York.
11. **Liras, P., T. Atherholt, and W. W. Umbreit.** 1975. Transformation of morphine and codeine by bacteria. Dev. Ind. Microbiol. **16**:401–405.
12. **Liras, P., S. S. Kasparian, and W. W. Umbreit.** 1975. Enzymatic transformation of morphine by hydroxysteroid dehydrogenase from *Pseudomonas testosteroni*. Appl. Microbiol. **30**:650–656.
13. **Liras, P., and W. W. Umbreit.** 1975. Transformation of morphine by resting cells and cell-free systems of *Arthrobacter* sp. Appl. Microbiol. **30**:262–266.
14. **Lutz, R. E. and L. Small.** 1939. Reduction studies in the morphine series. IX. Hydroxycodeinone. J. Org. Chem. **4**: 220–233.
15. **Munier, R.** 1953. Séparation des alcaloïdes de leurs N-oxydes par Microchromatographie sur Papier. Bull. Soc. Chim. Biol. **35**:1225–1231.
16. **Scrafani, J. T., and D. H. Clouet.** 1971. Biotransformations, p. 137–158. *In* D. H. Clouet (ed.), Narcotic drugs biochemical pharmacology. Plenum Publishing Corp., New York.
17. **Seki, I.** 1960. Improvement in preparation of 14-hydroxycodeinone. Takamine Kenkusho Nempo **12**:52–55.
18. **Sewell, G. J., C. J. Soper, and R. T. Parfitt.** 1984. The screening of some microorganisms for their ability to N-dealkylate drug molecules. Appl. Microbiol. Biotechnol. **19**:247–251.
19. **Smith, R. V., and J. P. Rosazza.** 1982. Microbial transformations as a means of preparing mammalian drug metabolites, p. 1–42. *In* J. P. Rosazza (ed.), Microbial transformation of bioactive compounds. CRC Press, Inc., Boca Raton, Fla.
20. **Tsuda, K.** 1964. Microbial transformation of steroids and alkaloids, p. 167–193. *In* I.A.M. Symposium on Microbiology, Institute of Appl. Microbiol, no. 6. University of Tokyo, Tokyo, Japan.
21. **Verpoorte, R., and A. Baerheim Svendson.** 1983. Chromatography of alkaloids. Part A: thin-layer chromatography. J. Chromatogr. Libr. **23a**:11–18 and 502.
22. **Yamada, M., K. Iizuka, S. Okuda, T. Asai, and K. Tsuda.** 1962. Untersuchungen auf dem Gebiet der Microbiologischen Umsetzung. XVII. Umwandlung von Alkaloiden in der Morphin-Reihe Durch *Trametes sanguinea*. Chem. Pharm. Bull. **10**:981–984.