solid. Recrystallization from benzene–*n*-hexane gave 98 mg of **14** as colorless crystals: mp 151–152 °C; ir ν_{max} ($CHCl_3$) 1620 cm^{-1} (C=O); NMR ($CDCl_3$) δ 0.98 (3 H, d, J = 7 Hz, C_{11} Me), 1.33 (3 H, s, C_6 Me), 1.70 [6 H, s, $=C(Me)_2$], 2.43 (2 H, s, C_5CH_2), 2.87 (2 H, m, C_1CH_2), 3.53 (2 H, m, $NCH_2OCH=$), 4.43 (1 H, , C_2 CH), 5.02 (2 H, s, $PhCH_2O$), 6.83 (3 H, m, $C_{7,9}$ and C_{10} CH), and 7.35 (5 H, s, Ph).

Anal. Calcd for $C_{26}H_{31}NO_2$: C, 80.17; H, 8.02; N, 3.60. Found: C, 80.08; H, 8.09; N, 3.62.

1,2,3,4,5,6-Hexahydro-8-hydroxy-2,6-methano-6,11-dimethyl-3-(3-methyl-2-butenyl)-3-benzazocine (Pentazocine, 10). A. A mixture of 450 mg of the amide (**15**) and 3.6 g of 70% sodium bis(2-methoxyethoxy)aluminum hydride in 20 ml of dry xylene was heated under reflux and stirring in an oil bath for 42 h under a current of nitrogen. After the reaction mixture had been acidified with 10% hydrochloric acid while cooling, the organic layer separated was extracted with water. Both aqueous layers were combined and neutralized with 10% ammonia, and the separated free base was extracted with chloroform. The extract was dried over Na_2SO_4 and evaporated to give a caramel-like substance, which was purified on silica gel chromatography using benzene–methanol (98.5:1.5 v/v) to afford a caramel. Recrystallization from acetone gave 216 mg of pentazocine (**10**) as colorless crystals, mp 146–148 °C (lit.[8] mp 146–148 °C), which was identical with the authentic sample[8] from the ir and NMR spectra and TLC comparisons and mixture melting point test.

B. A mixture of 45 mg of the amide (**14**) and 350 mg of 70% sodium bis(2-methoxyethoxy)aluminum hydride in 3 ml of dry xylene was refluxed for 60 h with stirring under a current of nitrogen. The same workup as above gave a pale brown caramel, which was purified by preparative TLC on silica gel with methanol–ethyl acetate–benzene (1:5:4 v/v) to afford 18 mg of pentazocine (**10**).

C. A solution of 100 mg of the amide (**15**) and 900 mg of 70% sodium bis(2-methoxyethoxy)aluminum hydride in 5 ml of dry benzene was refluxed for 5 h with stirring under a current of nitrogen. The same workup as before gave 6 mg of pentazocine (**10**). The organic layer was washed with saturated aqueous sodium chloride solution, dried over Na_2SO_4, and evaporated to give a colorless caramel, which was solidified by triturating with ether. The resulting solid was recrystallized from methanol–ether to afford 70 mg of 1,2,3,4,5,6-hexahydro-2,6-methano-6,11-dimethyl-3-(3-methyl-2-butenyl)-8-(3-methyl-2-butenyloxy)-3-benzazocine (**16**) hydrochloride as colorless crystals: mp 117 °C; ir ν_{max} ($CHCl_3$) 1670 cm^{-1} (C=C); NMR ($CDCl_3$) δ 0.83 (3 H, d, J = 7 Hz, C_{11} Me), 1.34 (3 H, s, C_6 Me), 1.73 [12 H, s, 2 $=C(Me)_2$], 4.5 (2 H, d, J = 7 Hz, $ArOCH_2CH=$), and 6.78 (3 H, m, $C_{7,9}$ and C_{10} CH).

Anal. Calcd for $C_{24}H_{35}NO{\cdot}HCl{\cdot}\frac{1}{3}H_2O$: C, 72.79; H, 9.08; N, 3.54. Found: C, 72.65; H, 9.11; N, 3.29.

A mixture of 32 mg of the above amine (**16**) and 300 mg of 70% sodium bis(2-methoxyethoxy)aluminum hydride in 2 ml of dry xylene was refluxed for 42 h. The same workup as above gave 19 mg of pentazocine (**10**).

D. To a solution of 91 mg of the quaternary ammonium salt (**17**) in 0.5 ml of hexamethylphosphoric triamide, 48 mg of 50% sodium hydride, and 76 mg of *n*-propyl mercaptan were added at 0 °C under a current of nitrogen. The reaction mixture was stirred for 15 min at 0 °C and then poured into ice–water, a mixture of which was washed with ether. The aqueous layer was neutralized with crystalline ammonium chloride and then extracted with chloroform. The extract was dried over Na_2SO_4 and evaporated. Purification of a pale brown caramel by preparative TLC on silica gel with methanol–ethyl acetate–benzene (1:5:4 v/v) gave 5 mg of pentazocine (**10**) and 12 mg of the amine (**18**), whose ir and NMR spectra and TLC behaviors were identical with those of **18**.

E. A mixture of 91 mg of the quaternary ammonium salt (**17**) and 58 mg of triphenylphosphine in 2 ml of acetonitrile was heated at 130–140 °C in a sealed tube for 12 h. After evaporation of the solvent, the residue was acidified with 10% hydrochloric acid and washed with ether. The aqueous layer was neutralized with 10% ammonia and extracted with chloroform. The extract was dried over Na_2SO_4 and evaporated to give a colorless caramel, which was purified by preparative TLC as above to give 10 mg of pentazocine (**10**) and 35 mg of the amine (**18**).

F. A mixture of 91 mg of the quaternary ammonium salt (**17**) and 576 mg of 70% sodium bis(2-methoxyethoxy)aluminum hydride in 4 ml of dry xylene was refluxed for 17 h with stirring under a current of nitrogen. The same workup as method A, followed by purification by preparative TLC, gave 6 mg of pentazocine (**10**) and 26 mg of the amine (**18**).

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Registry No.—**1**, 4397-53-9; **3**, 106-44-5; **4**, 2426-87-1; **6**, 93-51-6; **7**, 59069-55-5; **8**, 39595-83-0; **10**, 359-83-1; **11**, 59122-20-2; **12**, 59069-56-6; **13**, 59069-57-7; **14**, 59069-58-8; **15**, 59069-59-9; **16**, 59069-60-2; **17**, 59122-21-3; **18**, 57573-46-3; sodium bis(2-methoxyethoxy)aluminum hydride, 21608-56-0; dimethylallyl bromide, 870-63-3; benzyl chloride, 100-44-7.

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Microbial Transformations of Natural Antitumor Agents. 2. Studies with *d*-Tetrandrine and Laudanosine

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Microbial transformation studies have been conducted on benzyltetrahydroisoquinoline and bisbenzyltetrahydroisoquinoline alkaloids. The 4′-methyl ether of laudanosine (**2**) was cleaved in high yield by *Cunninghamella blakesleeana* (ATCC 8688a) to give pseudocodamine (**2b**) as the sole product. *Streptomyces griseus* (UI 1158) was used to chemically transform the natural antitumor alkaloid, *d*-tetrandrine (**1**). The metabolite was identified as *N*(2′)-nor-*d*-tetrandrine (**1a**) on the basis of NMR and mass spectral correlations, and was obtained in 50% yield. Both reactions represent highly selective dealkylations of polyfunctional molecules.

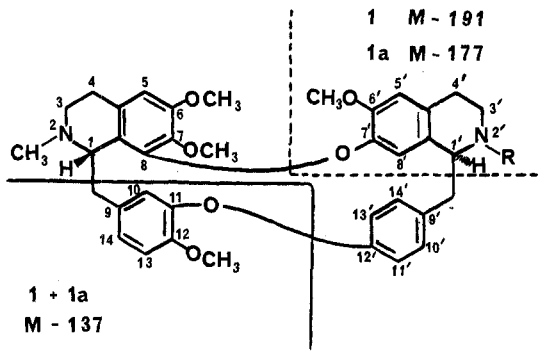
The successful application of microorganisms in the preparation of difficult-to-synthesize steroids has been well documented.[1] The use of microbial systems as tools for achieving chemical transformations of other classes of natural products has not been systematically exploited. Advantages of such microbial systems include mild reaction conditions, their se-

lectivity with polysubstituted organic compounds, and their potential for large-scale production of metabolites through routine fermentation scale-up techniques.

Many active antitumor compounds have been isolated from a variety of natural sources. These compounds are often structurally complex, and serious difficulties are encountered in the preparation of potentially active derivatives, and in the study of their metabolism. Microbial transformation systems are being developed to accomplish such goals.

This report is concerned with microbial transformation studies on the bisbenzyltetrahydroisoquinoline alkaloid, *d*-tetrandrine (1) (Chart I), a compound which has demonstrated potent antitumor[2] and cytotoxic[3,4] activity. The use of this compound may be limited by a multitude of dose dependent toxic effects[5-7] some of which may be related to the production of metabolites in mammalian systems. In this regard, the availability of N- and O-demethylated derivatives of 1 for antitumor and/or toxicity studies, and as intermediates in the synthesis of potentially less toxic derivatives would be of particular interest. De novo synthesis of such derivatives is a laborious and low-yielding process, and requires the judicious application of suitable protecting groups.[8,9]

Chart I



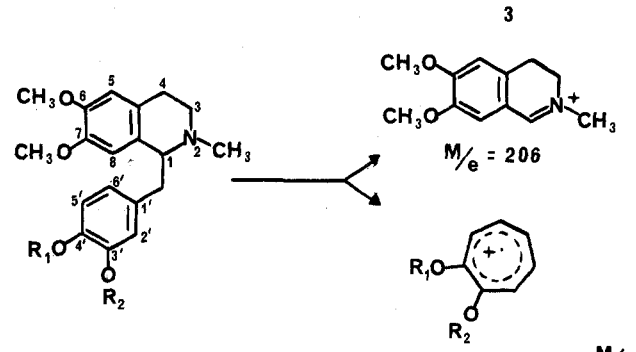
1 R = CH_3
1a R = H

d-Tetrandrine was available in limited supply for our initial work. In addition, microbial transformation studies had not been reported for monomeric or dimeric benzylisoquinoline alkaloids like 1 and laudanosine (2). Laudanosine represents one-half of the dimeric structure of 1, and it could be prepared in quantity. Thus, initial microbial transformation experiments were performed with laudanosine (2) in the hope that microorganisms metabolizing this "model" compound would also metabolize 1.

Some 60 cultures were used to conduct small-scale screening experiments with (*RS*)-laudanosine. Several of these organisms had previously achieved O- and N-dealkylations, or hydroxylations with other substrates.[10-17] (*RS*)-Laudanosine metabolites were produced by the following cultures: *Cunninghamella blakesleeana* (ATCC 8688a), *Stysanus microsporus* (UI 2833), *Aspergillus niger* (ATCC 10581), *Cunninghamella echinulata* (ATCC 9244), and *Microsporum gypseum* (ATCC 11395). Yields of metabolites obtained with *C. blakesleeana* were reproducible and were considerably higher than those obtained with other cultures. (*RS*)-Pseudocodamine (1.20 g) was obtained when *C. blakesleeana* was incubated with 2.7 g of (*RS*)-laudanosine.

The mass spectrum of the metabolite (**2b**, Chart II) was typical of benzyltetrahydroisoquinolines[18,19] and indicated that O-demethylation had occurred in the benzylic portion of laudanosine. The NMR spectrum was of limited value in distinguishing between the two possible O-dealkylation products, (*RS*)-pseudocodamine (**2b**) or (*RS*)-laundanine (**2a**), since only the 7-methoxy signal is sufficiently shielded and separated from the remaining methoxyl signals.[20] The metabolite had the same melting point as pseudocodamine,[21] some 30 °C lower than that reported for the isomeric compound (**2a**). Final proof of structure was obtained by alkaline permanganate degradation of the ethyl ether derivative of the metabolite (**2c**) to 4-ethoxy-3-methoxybenzoic acid by the method of Späth.[22]

Chart II



		M/e
2 $R_1, R_2 = CH_3$	4, $R_1, R_2 = CH_3$	151
2a $R_1 = CH_3, R_2 = H$	4a, $R_1 = H, R_2 = CH_3$	137
2b $R_1 = H, R_2 = CH_3$	4b, $R = CH_3, R = H$	137
2c $R_1 = C_2H_5, R_2 = CH_3$		

Unreacted laudanosine recovered from the fermentation was not optically active, and neither was the metabolite. Thus, the O-demethylation reaction was nonstereoselective. When the yield of **2b** is corrected for recovered starting material, (*RS*)-pseudocodamine was obtained in 89% yield.

Cultures capable of metabolizing 2 and other natural products such as dimethoxyaporphine,[11] acronycine,[17] and L-tyrosine[23] were examined for their potential to transform *d*-tetrandrine. Those yielding metabolites of 1 were *Cunninghamella blakesleeana* (ATCC 8688a), *Cunninghamella echinulata* (NRRL 3655), *Mucor mucedo* (UI4605), *Penicillium brevi-compactum* (ATCC 10418), *Streptomyces griseus* (UI 1158), *Streptomyces punipalus* (NRRL 3529), and *Streptomyces lincolnensis* (ATCC 25466). All except for *C. blakesleeana* gave high yields of a common metabolite. *S. griseus* was selected for a preparative scale fermentation.

The metabolite **1a** was obtained in 50% yield from a 2.0-g incubation of *d*-tetrandrine. Although the classical structure proof of compounds like **1a** involves Hofmann degradation, or Na/liquid ammonia cleavage of the aryl ethers to yield known benzyltetrahydroisoquinolines,[24] it is possible to characterize them by mass spectral and NMR methods. Bick et al.[25] found that all resonances due to heteroatom methyl substituents in 1 are well separated and easily distinguished in the NMR spectrum. Correlations between *d*-tetrandrine (1) and the metabolite **1a** are shown in Table I. The only signal absent in **1a** is that attributable to the *N*(2′)-methyl group of 1. The spectrum of **1a** is nearly identical with that of (*RS*)-(**1a**) obtained synthetically,[25] and of cycleanorine (*SS*)-(**1a**), a minor alkaloid isolated from *Cyclea peltata*.[26]

The elemental composition of the metabolite **1a**, 608.2938 (calcd for $C_{37}H_{40}N_2O_6$, 608.2886) indicated the loss of a single methyl group from *d*-tetrandrine. Other mass spectral fragments were consistent with the published spectrum of **1a**,[26] and that of an authentic sample of 1.

In the low-resolution spectrum, weak but diagnostic peaks occur at M − 191 and M − 137 for 1, and M − 177 and M − 137 for **1a** as illustrated in Chart I. These peaks support the NMR spectral correlation which indicated that the *N*(2′)-methyl group was selectively cleaved by the microorganism.

Table I. Chemical Shifts[a] (ppm) of the *N*- and *O*-Methyl Groups of *d*-Tetrandrine (1) and the Metabolite 1a

	C-12	C-6	C-6′	C-7	N(2)	N(2′)
d-Tetrandrine (1)	3.93	3.75	3.37	3.20	2.33	2.62
1 literature[25]	3.9	3.73	3.35	3.18	2.3	2.59
Metabolite (1a)	3.92	3.73	3.35	3.23	2.32	
(+/−)-1a[9]	3.95	3.75	3.35	3.22	2.33	
(+/+)-1a[26]	3.88	3.70	3.33	3.22	2.33	

[a] Spectra obtained in $CDCl_3$ with Me_4Si as internal standard.

This microbial reaction provides a simple and rapid route to the preparation of *N*(2′)-nor-*d*-tetrandrine (1a) which may serve as a useful intermediate in the synthesis of potentially active tetrandrine derivatives. The reaction is accomplished in high yield with no complicating side products, and may be conducted in large scale using suitable fermentation apparatus. When corrected for unreacted starting material recovered from the fermentation, the N-demethylation reaction is essentially quantitative. The identified metabolite is also produced by the *Streptomyces, Penicillium,* and *Mucor* species described earlier, but in somewhat lower yields as estimated by TLC. *Cunninghamella blakesleeana* gave a different and currently unidentified metabolite, but in lower yield. Efforts are being made to improve metabolite yields before preparative scale fermentations are conducted to isolate and characterize this and possibly other metabolites of **1**.

We had initially thought that biotransformations observed on monomeric benzyltetrahydroisoquinolines would also occur on the dimeric molecule (1). Instead, N-demethylation, not O-demethylation, was the predominant mode of biotransformation of **1**. O-Dealkylation was the major path of metabolism with (*RS*)-laudanosine (**2**). Microorganisms capable of metabolizing **1** did not metabolize **2** well either. Obvious differences in the two substrates which might shift dealkylation pathways are molecular size, and the bulk of the bisaryl ether couplings of **1** relative to **2**. The nature of the ether substituents might also influence the mode of biotransformation of compounds like **1** and **2**. Steric factors appear to be important in directing O-dealkylations with other alkaloids.[11]

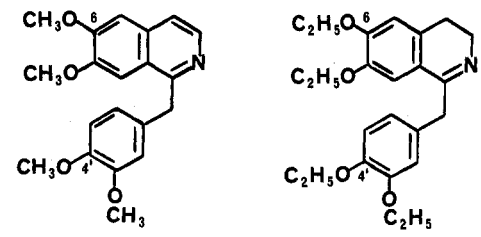
Studies on the microbial[10,30] and mammalian metabolism of papaverine (**5**) have been conducted. The major metabolic products obtained with this fully aromatic benzylisoquinoline alkaloid derivative (**5**) are the 4′- and 6-O-demethylated derivatives. Gyarmati et al.[32] found that a dihydrobenzylisoquinoline alkaloid derivative (**6**) also underwent O-dealkylation at the 4′ and 6 positions. It appears that O-dealkylation will occur at the 6 position of isoquinolines lacking a basic nitrogen atom, and/or an *N*-methyl substituent. Further studies are in progress to elucidate the importance of these features in directing O-dealkylation reactions with isoquinolines.

The microbial reactions observed with both laudanosine and *d*-tetrandrine were highly selective dealkylations of polyfunctional molecules, similar to those observed with microbial transformations of other natural products.[11,16,27-29] The yields of both transformations were high but they could be improved.

In an earlier report, microorganisms gave high yields of 9-hydroxyacronycine, a metabolite also produced in mammals.[17] Microorganisms may serve as useful metabolic models to predict ways in which mammals metabolize natural products like **1** and **2**.[10] N- and O-dealkylation reactions are common mammalian monooxygenase reactions, and it is plausible that the metabolites identified in this work will also be obtained in mammalian systems. Other metabolites of both of the alkaloids studied have been observed with other cultures. These metabolites will be isolated and identified once conditions for their production have been optimized.

To our knowledge, this is the first report of microbial transformations of mono- or dimeric benzyltetrahydroisoquinolines.

Chart III



5 6

Experimental Section

NMR spectra were obtained with a Varian T-60 spectrometer with Me_4Si as an internal standard. Low-resolution mass spectra were taken on a Finnigan Model 3200 instrument. High-resolution mass spectral data were obtained through the services of Battelle Columbus Laboratories, Columbus, Ohio. Melting points were obtained with a Thomas-Hoover melting point apparatus and are corrected. Ir spectra were obtained with a Perkin-Elmer 267 instrument, and optical rotations were determined with a Perkin-Elmer Model 141 polarimeter. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, Ind.

Chromatographic Procedures. Thin layer chromatography (TLC) was performed on 0.25 mm thick layer plates of silica gel GF_{254} prepared with a Quickfit Industries speader. Plates were air dried for 1 h, and oven activated at 120 °C for 30 min prior to use. Solvent systems used in developing TLC plates were A, C_6H_6–MeOH–58% NH_4OH (80:30:0.1); B, C_6H_6–MeOH–58% NH_4OH (40:12:0.1); C, C_6H_6–MeOH–HOAc (40:2:1). Visualization usually involved viewing developed plates under short (254 nm) and long (365 nm) wavelength ultraviolet light, and by later spraying them with Dragendorff's reagent.[33] Column chromatography was conducted with silica gel (Baker 3405) which was slurried and wet packed in the developing solvents.

Fermentation Procedures. All cultures described in this work are maintained in the culture collection of the University of Iowa, College of Pharmacy. Those bearing the designation (UI) have been maintained in our collection for some time, while those bearing designations of (NRRL) and (ATCC) were obtained from the Northern Regional Research Laboratories of the Agriculture Research Service of the USDA in Peoria, Ill., and the American Type Culture Collection, Rockville, Md., respectively.

Fermentations were conducted using the two-stage procedure and medium previously described.[11,17] Small-scale fermentation screening experiments were conducted in 25 ml of sterile medium in 125-ml Erlenmeyer flasks shaken at 250 rpm, at 27 °C. Larger scale fermentations were done in 500-ml or 1000-ml Erlenmeyer flasks containing ⅕ of their volumes of sterile medium. The progress of microbial transformation reactions was routinely monitored by TLC as follows: 4-ml samples of incubations were withdrawn at time intervals, adjusted to pH 8.5 with saturated $NaHCO_3$, and extracted with 1.0 ml of ethyl acetate, and 30 μl of the extracts were spotted on TLC plates.

Preparation of (*RS*)-Laudanosine (2). (*RS*)-Laudanosine was prepared by $NaBH_4$ reduction of papaverine methiodide by literature procedures.[34] The product obtained was comparable in all physical properties to an authentic sample of racemic laudanosine (Sigma Chemical Co.): mp 114–116 °C (reported[34] 114–116 °C); picrate, mp 176–177 °C (reported[35] 174–176 °C).

Preparation of 3-Ethoxy-4-methoxybenzoic Acid (*O*-Ethylisovanillic Acid). *O*-Ethylisovanillic acid was prepared from isovanillic acid by the method of Späth,[22] mp 164–165 °C (reported[22] 164–165 °C). Anal. Calcd for $C_{10}H_{12}O_4$: C, 61.22; H, 6.16. Found: C, 61.34; H, 6.47.

Preparation of 4-Ethoxy-3-methoxybenzoic Acid (*O*-Ethylvanillic Acid). *O*-Ethylvanillic acid was prepared from vanillic acid by the procedure of Späth,[22] mp 195–196 °C (reported[36] 195–196 °C). Anal. Calcd for $C_{10}H_{12}O_4$: C, 61.22; H, 6.16. Found: C, 61.10; H, 6.40.

Microbial Conversion of (*RS*)-Laudanosine (2) to (*RS*)-Pseudocodamine (2b) with *Cunninghamella blakesleeana* (ATCC 8688a). Second-stage cultures of *C. blakesleeana* were grown in 5.1 l. of medium in 500-ml Erlenmeyer flasks. A total of 2.7 g of **2** was converted to its HCl salt with 6 N HCl, and was dissolved in 220 ml of sterile deionized water. After neutralizing the laudanosine **2** solution with 5% NaOH, it was distributed evenly among the culture flasks. Formation of the metabolite **2b** was followed by TLC using solvent system A. After 5 days of incubation, the fermentation was harvested, all cultures were pooled and adjusted to pH 8.5 with saturated $NaHCO_3$, and this mixture was extracted with ether in a continuous liquid–liquid extractor. The combined ether extracts were dried over anhydrous Na_2SO_4 and evaporated to a gold oil (4.5 g). The oil was applied to a silica gel column (400 g, 53 × 5.5 cm), and eluted with C_6H_6–MeOH–58% NH_4OH (300:20:0.1) at a rate of 2 ml/min, while 15-ml fractions were collected. Fractions 56–140 yielded 1.35 g of (*RS*)-laudanosine as unreacted starting material, mp 115 °C. Fractions 164–400 gave pure (*RS*)-pseudocodamine (**2b**), 1.20 g (46% yield): mp 132–133 °C (reported[21] 131–132 °C); mass spectrum *m/e* (rel abundance) 343 (1), 342 (1), 206 (100), 191 (9), 190 (21), 137 (7); NMR ($CDCl_3$) 2.55 (s, 3 H, NCH_3), 3.57 (s, 3 H, 7-OCH_3), 3.77 (s, 3 H, OCH_3), 5.92 (s, 1 H, OH), 6.05 (s, 1 H, Ar), 6.83 ppm (m, 4 H, Ar). Anal. Calcd for $C_{20}H_{25}NO_4$: C, 69.95; H, 7.34; N, 4.08. Found: C, 70.47; H, 7.11; N, 4.08.

Permanganate Degradation of 2b to 4-Ethoxy-3-methoxybenzoic Acid. (*RS*)-Pseudocodamine (**2b**), 75 mg, isolated from the fermentation was suspended in 10 ml of dry dimethylformamide with 50 mg of NaH, and 17.4 μl of ethyl iodide was added to the mixture with a microsyringe over a 30-min period. Upon completion of the addition of ethyl iodide, TLC (solvent B) indicated that the reaction was complete. The product (**2c**) was identical with **2** on TLC. The reaction mixture was stirred with 10 g of ice, extracted exhaustively with ethyl acetate, and dried over anhydrous Na_2SO_4 before evaporation to an oil: NMR ($CDCl_3$) 1.43 (t, 3 H, $-CH_2CH_3$), 3.78 (s, 3 H, $-OCH_3$), 3.83 (s, 3 H, $-OCH_3$), 6.12 (s, 1 H, Ar), 6.83 ppm (m, 4H, Ar).

A solution of 450 mg of $KMnO_4$ in 25 ml of H_2O was added dropwise to a suspension of 62 mg of **2c** in 10 ml of H_2O until the reaction mixture remained purple (15 ml of $KMnO_4$ solution). TLC (solvent C) indicated that the reaction was nearly complete. Addition of 1 ml more of the permanganate solution followed by stirring for 1 h completed the reaction. The mixture was acidified with concentrated H_2SO_4 and extracted with ethyl acetate. The extract was purified by preparative TLC (1.0 mm thick layers, solvent C). Elution of the product from the silica gel with methanol, and crystallization from methanol gave 25 mg of an analytical sample (75% yield) of 4-ethoxy-3-methoxybenzoic acid, mp 192–193 °C (reported[36] 195–196 °C). Anal. Calcd for $C_{10}H_{12}O_4$: C, 61.22; H, 6.16. Found: C, 61.10; H, 6.40.

Production of *N*(2′)-Nor-*d*-tetrandrine (1a) from *d*-Tetrandrine (1) by *Streptomyces griseus* (UI 1158). *S. griseus* was grown in 9 l. of medium held in 1.0-l. flasks, and 2 g of *d*-tetrandrine (1) which was converted to the HCl salt with 6 N HCl and dissolved in 40 ml of H_2O was distributed evenly among them. Fermentation monitoring with TLC (solvent A) indicated that the biotransformation was completed after 7 days. After adjusting the combined fermentation beers to pH 8.5 with saturated $NaHCO_3$, this solution was exhaustively extracted with ether in a continuous liquid–liquid extractor. Evaporation of the combined ether extracts gave a residue of 3.05 g which was subjected to column chromatography on silica gel (310 g, 5.5 × 43 cm) using C_6H_6–MeOH (20:1) as the developing solvent. Fractions of 17 ml were collected at a flow rate of 2 ml/min, and fractions 131–335 gave 1.0 g of unreacted *d*-tetrandrine, while fractions 390–1050 gave 1.0 g of the metabolite **1a** as a dry, pure glass upon evaporation of the eluting solvent. Attempts to crystallize the metabolite failed, and analytical data were obtained on the amorphous material: NMR ($CDCl_3$) 2.32 (s, 3 H, NCH_3), 2.6–4.2 (complex signals representing benzylic and other nonaromatic H, 14 H), 3.23 (s, 3 H, 7-OCH_3), 3.35 (s, 3 H, 6′-OCH_3), 3.73 (s, 3 H, 6-OCH_3), 3.92 (s, 3 H, 12-OCH_3), 6.00 (s, 1 H, Ar), 6.30 (m, 2 H, Ar), 6.50 (m, 2 H, Ar) 6.73 (d, 1 H, Ar), 6.87 (s, 2 H, Ar), 7.33 ppm (m, 2 H, Ar); mass spectrum *m/e* (rel abundance) 608 (62), 471 (6), 431 (10), 382 (67), 381 (68), 368 (48), 350 (12), 335 (8), 191 (100); elemental composition (by high-resolution mass spectroscopy) 608.2938 (calcd for $C_{37}H_{40}N_2O_6$, 608.2886).

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Registry No.—**1,** 518-34-3; **1a,** 38769-08-3; **2,** 1699-51-0; **2b,** 6391-58-8; **2c,** 59069-53-3; *O*-ethylisovanillic acid, 2651-55-0; isovanillic acid, 645-08-9; *O*-ethylvanillic acid, 3535-30-6; vanillic acid, 121-34-6.

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