and the reaction mixture was extracted with ethyl acetate four times. The organic layers were combined, washed with water, dried over Na_2SO_4, and evaporated to give a yellowish material. The crude product was purified by chromatography on silica gel (EtOAc) to afford pure **5** (75.0 mg, 91.1%): MS m/z 433 (M^+); IR ($CHCl_3$) ν_{max} 3440, 2940, 1720, 1685, 1540, 1440, 1325, 1155 cm^{-1}; ^{13}C NMR (125 MHz, pyridine-d_5) δ 24.5, 26.5, 26.7, 37.8, 39.5, 45.1, 47.6 (q, $^4J_{CF}$), 117, 158 (major conformer)/26.3, 27.1, 29.0, 37.4, 39.6, 45.5 (q, $^4J_{CF}$), 46.6, 117, 158 (minor conformer); ^1H NMR (500 MHz, pyridine-d_5) δ 1.4-1.6 (m), 1.86 (quint, H_2-3'), 1.87 (quint, H_2-3), 3.2-3.4 (m), 10.39 (br s), 10.58 (br s). ^{13}C NMR and ^1H NMR properties ($CDCl_3$) are listed in Table V.

Compound 11 was prepared from dipropylenetriamine (Tokyo Kasei No. D0090) by the same manner described above. **11**: ^{13}C NMR (125 MHz, $CDCl_3$) δ 26.6, 28.5, 36.6, 37.2, 43.9, 45.2 (q, $^4J_{CF}$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.88 (2 H, quint, J = 7 Hz), 1.96 (2 H, quint, J = 7 Hz), 3.33 (2 H, q, J = 7 Hz), 3.42 (2 H, q, J = 7 Hz), 3.46 (2 H, t, J = 7 Hz), 3.48 (2 H, t, J = 7 Hz), 6.67 (1 H, br s), 7.30 (1 H, br s); ^{13}C NMR (125 MHz, pyridine-d_5) δ 27.2, 29.1, 37.4, 37.8, 45.3, 45.8 (q, $^4J_{CF}$ = 2.8 Hz), 117.3 (q, $^1J_{CF}$ = 286 Hz), 117.1 (q, $^1J_{CF}$ = 286 Hz), 156.8 (q, $^2J_{CF}$ = 35 Hz), 158.0 (q, $^2J_{CF}$ = 36 Hz), 158.2 (q, $^2J_{CF}$ = 36 Hz); ^1H NMR (500 MHz, pyridine-d_5) δ 1.89 (2 H, quint, J = 7 Hz), 1.91 (2 H, quint, J = 7 Hz), 3.40 (2 H, q, J = 7 Hz), 3.42 (2 H, q, J = 7 Hz), 3.48 (4 H, t, J = 7 Hz), 10.33 (1 H, br s), 10.54 (1 H, br s).

Compound 12 was prepared from diethylenetriamine (Aldrich No. D9385-6) by the same manner described above. **12**: ^1H NMR (500 MHz, $CDCl_3$) δ 3.6-3.75 (8 H, m), 6.96 (1 H, br s), 7.05 (1 H, br s); ^{13}C NMR (125 MHz, pyridine-d_5) δ 37.0, 38.0, 46.2, 46.9 (q, $^4J_{CF}$ = 2.5 Hz), 117.0 (q, $^1J_{CF}$ = 286 Hz), 117.1 (q, $^1J_{CF}$ = 286 Hz), 157.8 (q, $^2J_{CF}$ = 35 Hz), 158.51 (q, $^2J_{CF}$ = 36 Hz), 158.53 (q, $^2J_{CF}$ = 36 Hz); ^1H NMR (500 MHz, pyridine-d_5) δ 3.70 (4 H, m), 3.80 (4 H, m), 10.84 (2 H, br s).

Compound 13 was prepared from pentylamine (Tokyo Kasei No. A0445) by the same manner described above. **13**: ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.7 (C-5), 22.2 (C-4), 28.5 (C-2), 28.8 (C-3), 40.0 (C-1), 116.0 (q, $^1J_{CF}$ = 285.5 Hz), 157.6 (q, $^2J_{CF}$ = 36.6 Hz); ^1H NMR (500 MHz, $CDCl_3$) δ 0.90 (3 H, t, J = 7 Hz, H_3-5), 1.32 (4 H, m, H_2-3, 4), 1.58 (2 H, quint, J = 7.5 Hz, H_2-2), 3.34 (2 H, q, J = 7.5 Hz, H_2-1), 6.42 (1 H, br s); ^{13}C NMR (125 MHz, pyridine-d_5) δ 14.1 (C-5), 22.5 (C-4), 29.1 (C-2), 29.2 (C-3), 40.2 (C-1); ^1H NMR (500 MHz, pyridine-d_5) δ 0.63 (3 H, t, J = 7 Hz, H_3-5), 1.08 (4 H, m, H_2-3, 4), 1.45 (2 H, quint, J = 7.5 Hz, H_2-2), 3.31 (2 H, q, J = 7.5 Hz, H_2-1), 10.3 (1 H, br s).

Acknowledgment. This work was supported financially by H. B. O. I., Ft. Pierce, FL (Y.K). We are grateful to Drs. S. Cross, P. McCarthy, and N. Burres, H. B. O. I., for the antiviral, antimicrobial, and antitumor tests, respectively, and Dr. T. Sasaki, Kanazawa University, for an antitumor test. We thank Drs. T. Kinoshita and N. Nakayama for FAB-MS measurements and Dr. M. R. Wälchli for measuring HMBC spectrum.

Supplementary Material Available: ^1H NMR spectra of **2** and the complex of **2** and 2-methylpropionate; ^{13}C NMR spectra of **2**, measured in $CDCl_3$ and in the presence of CD_3OD (isotopic shift experiment); and Tables I and III of the NMR properties of ptilomycalin A (**1**) and its *p*-bromobenzoate (**4**) as well as compound **10** (7 pages). Ordering information is given on any current masthead page.

Thiotropocin Biosynthesis. Shikimate Origin of a Sulfur-Containing Tropolone Derivative

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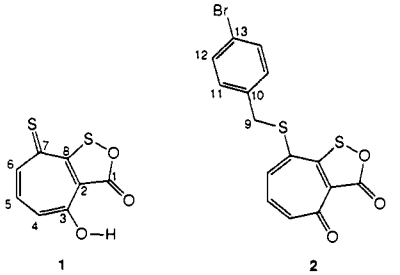
Contribution from the Department of Chemistry, Brown University, Providence, Rhode Island 02912. *Received May 4, 1992*

Abstract: Feeding of [U-$^{13}C_6$]glucose to *Pseudomonas* CB-104 gave labeled thiotropocin (**1a** and **1b**). The ^{13}C NMR spectrum of the derived *p*-bromobenzyl thioether (**2a** and **2b**) displayed a pattern of enhancements and couplings consistent with a shikimate origin for thiotropocin by way of a symmetrical phenylpyruvate (**11**) intermediate. The latter metabolite is proposed to undergo oxidative ring expansion followed by further oxidation and oxygen-sulfur exchange. These conclusions were further supported by specific and efficient incorporation of both [3-^{13}C]phenylalanine (**13**) and [1,2-$^{13}C_2$]phenylacetic acid (**12**) into thiotropocin.

Thiotropocin (**1**) is a novel antibiotic first isolated in 1984 from the fermentation broth of *Pseudomonas* sp. CB-104 by Harada and Ono and their colleagues at Takeda.[1] Thiotropocin and its sodium salt were found to exhibit antibacterial, antifungal, and antiprotozoal activities in vitro, with the observed antibiotic activity being strongest at low pH. The antibiotic caused morphological changes in both *Proteus mirabilis* and *Escherichia coli* and displayed immediate growth-inhibitory but only weak lytic activity against *P. mirabilis*.

The structure of thiotropocin was established by a combination of spectroscopic methods and X-ray crystallographic analysis of the *p*-bromobenzyl derivative **2**. Thiotropocin is the first example of a naturally occurring tropothione derivative and has a unique structure containing an -S-O-CO- moiety. Among the previously known naturally occurring aromatic tropolone derivatives are several fungal metabolites, including stipitatic (**4**) and stipitatonic (**5**) acids from *Penicillium stipitatum*.[2] Labeling studies have established the derivation of these latter compounds from acetate and methionine and led to the proposal that the common seven-membered tropolone ring could be formed by oxidative ring expansion of a methylated aromatic tetraketide (**3**) as illustrated in Scheme I.[3] By contrast, the alkaloidal tropolone derivative colchicine (**6**) has been shown to be biosynthesized from phenyl-



1 2

(1) (a) Kintaka, K.; Ono, H.; Tsubotani, S.; Harada, S.; Okazaki, H. *J. Antibiot.* **1984**, *37*, 1294. (b) Tsubotani, S.; Wada, Y.; Kamiya, K.; Okazaki, H.; Harada, S. *Tetrahedron Lett.* **1984**, *25*, 419.

(2) Pietra, F. *Chem. Rev.* **1973**, *73*, 293.

(3) Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic Press: New York, 1983; pp 78-80.

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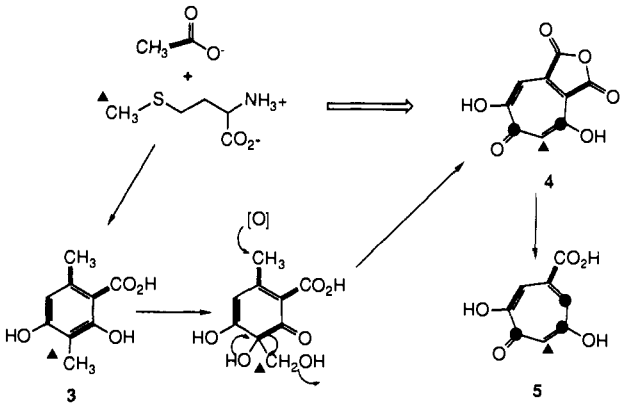
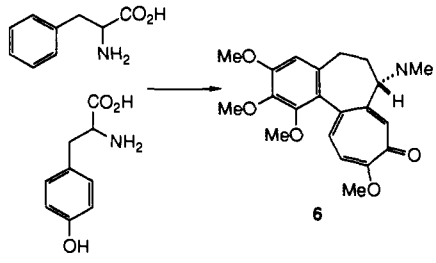
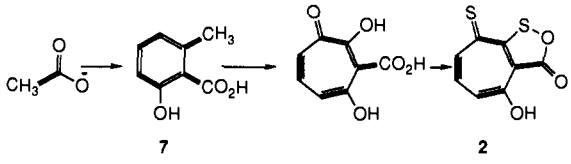
Scheme I



Scheme II



Scheme III



alanine and tyrosine[4] (Scheme II). Several alkyltropolones, such as nookatinol,[5a] have been found in the heartwood of various *Cupressales* and are of possible terpenoid biogenetic origin.[5b]

Based on analogy to the known origins of structurally similar fungal metabolites, we initially considered the possibility that thiotropocin might be formed by simple oxidative ring expansion of the aromatic tetraketide 6-methylsalicylic acid (**7**) (Scheme III). In fact, investigation of the biosynthesis of **1** has revealed an unexpected shikimate origin for this novel metabolite.

Assignment of the ^1H and ^{13}C NMR Spectra of Thiotropocin and Its *p*-Bromobenzyl Derivative. Although Harada et al. had reported ^{13}C NMR data for both thiotropocin and its *p*-bromobenzyl derivative, several of these assignments were in fact only tentative. Since we required unambiguous NMR signal assignments for our biosynthetic investigations, we therefore reanalyzed the ^1H and ^{13}C NMR spectra of both **1** and **2**.

In the ^{13}C NMR spectrum of biosynthetically derived [2-^{13}C]thiotropocin enriched with 70 atom % ^{13}C, three signals were coupled to C-2, as evidenced by the appropriate ^{13}C–^{13}C satellite peaks.[6] These three signals were assigned to the carboxyl carbon C-1 (170.6 ppm, J_{CC} = 61.4 Hz), C-8 (167.7 ppm, J_{CC} = 71.7 Hz), and the enolic carbon C-3 (182.6 ppm, J_{CC} = 57.7 Hz)[7] (Table I). The ^1H NMR chemical shifts of H-4 and H-6 were assigned by selective ^1H decoupling of the ^{13}C NMR spectrum of [2-^{13}C]thiotropocin obtained in 1:1 $CDCl_3$:DMSO-d_6. Thus irradiation of the proton resonance at δ 7.47 resulted in collapse of the 6.2-Hz C-2 doublet to a singlet, whereas control irradiation at δ 7.62 had no effect, leading to the assignment of δ 7.47 and 7.62 to H-4 and H-6, respectively, with H-5 being assigned to δ 7.67 (dd, J_{45} = 11.8 Hz, J_{56} = 9.0 Hz). A ^1H-^{13}C HETCOSY experiment on unlabeled thiotropocin in the same solvent led readily to the corresponding assignments of C-4 (136.2 ppm), C-5 (135.5 ppm), and C-6 (131.8 ppm). These assignments were correlated with the corresponding signals in DMSO-d_6 through the use of solvent mixtures with different proportions of $CDCl_3$.

Thiotropocin is readily converted to the *p*-bromobenzyl thioether **2**.[1b] Not only is this derivative more soluble in $CDCl_3$, but also the ^{13}C NMR signals for C-4 and C-5 are much better resolved than the corresponding signals in the parent thiotropocin. The ^1H NMR spectrum of **2** was readily assigned with the aid of selective NOE experiments. Thus irradiation of the H-9 methylene protons at δ 4.08 in acetone-d_6 led to enhancement of the signals at δ 7.31 (H-11) and 7.21 (H-6), which were readily distinguished from one another by their characteristic coupling patterns. Assignment of the remaining proton signals followed in an unexceptional manner. These assignments could then be correlated with the corresponding ^1H NMR spectrum of **2** in $CDCl_3$, summarized in Table II. The assignments were fully confirmed by computer simulation[8] using the coupling constants measured in acetone-d_6 in combination with the observed $CDCl_3$ chemical shifts. The corresponding ^{13}C NMR spectrum of **2** could then be completely assigned by a long-range ^{13}C–^1H HETCOSY experiment. All one-bond and three-bond ^1H–^{13}C couplings were apparent in the resultant spectrum, leading to unambiguous identification of six quaternary carbon resonances, seven methines, and one methylene carbon. Only the signal for the carboxyl carbon, C-1, was absent from the COSY spectrum. The assigned chemical shifts are summarized in Table I.

Table I. ^{13}C NMR Assignments for Thiotropocin (**1**) and its *p*-Bromobenzyl Thioether (**2**)

C	**1**, ppm DMSO	**1**, ppm $CDCl_3$–DMSO	**2**, ppm $CDCl_3$
1	170.60		168.24
2	120.04		117.48
3	182.62		178.63
4	137.74	136.23 (H-4, 7.47)	144.21
5	137.64	135.48 (H-5, 7.67)	131.19
6	133.80	131.76 (H-6, 7.62)	142.76
7	150.09		130.72
8	167.65		177.67
9			42.56
10			134.00
11			130.86
12			132.18
13			122.70

Table II. ^1H NMR Assignments for Thiotropocin *p*-Bromobenzyl Thioether (**2**) in $CDCl_3$

H	δ (m, J (Hz))
4	6.99 (dd, $J_{4,5}$ = 11.3, $J_{4,6}$ = 2.0)
5	6.85 (dd, $J_{4,5}$ = 11.3, $J_{5,6}$ = 8.4)
6	6.82 (dd, $J_{4,6}$ = 2.0, $J_{5,6}$ = 8.4)
9	4.08 (s)
11	7.07 (br d, $J_{11,12}$ = 8.4, $J_{11,11'}$ = 4.5)
12	7.48 (br d, $J_{11,12}$ = 8.4, $J_{12,12'}$ = 4.5)

Shikimate Origin of Thiotropocin. Based on the expected polyketide derivation of thiotropocin, we initially attempted to label the antibiotic with [1-^{14}C]- and [2-^{14}C]acetate and with [1,2-$^{13}C_2$]acetate. After repeated failure to obtain labeled thiotropocin, we next attempted incorporation of synthetically prepared [3,5-^3H]-6-methylsalicyclic acid[9] but with no better success. We

(4) Battersby, A. R.; Dobson, T. A.; Foulkes, D. M.; Herbert, R. B. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1730.

(5) (a) Hirose, Y. *Agric. Biol. Chem.* **1963**, 795. (b) Erdtman, H.; Norin, T. *Fortschr. Chem. Org. Naturst.* **1966**, *24*, 216.

(6) The assignment of C-2 itself is completely unambiguous since this is the only site in thiotropocin with three contiguous carbon atoms capable of giving rise to the observed couplings.

(7) These assignments reverse those originally attributed to C-3 and C-7.[1b]

(8) PANIC 851 program, Bruker Instruments NMR software.

(9) Abell, C. Ph.D. Thesis, University of Cambridge, Cambridge, England, 1982.

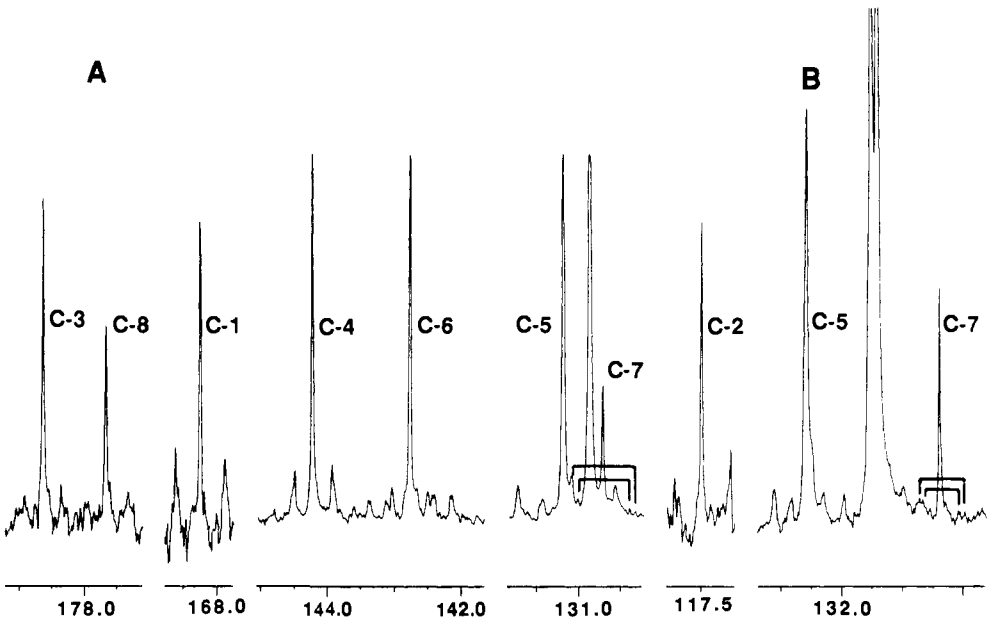
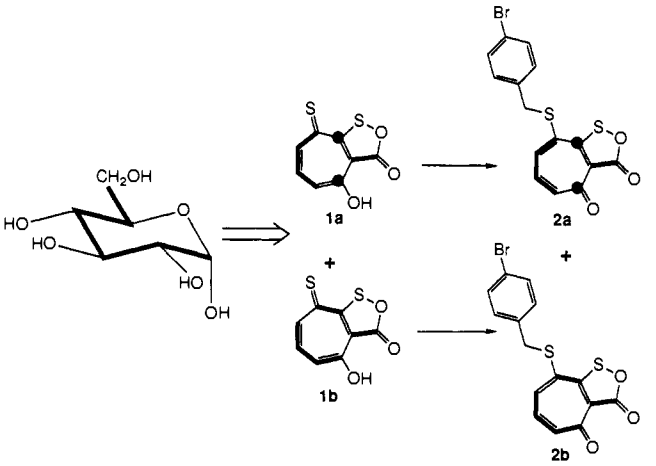


Figure 1. Partial 100.6-MHz ^{13}C NMR spectrum of thiotropocin *p*-bromobenzyl thioether (**2**) showing splittings of individual resonances. Peaks used to calculate coupling constants for C-7 are indicated. Panel A: spectrum of **2** in $CDCl_3$. Panel B: resonances for C-5 and C-7 in DMSO-d_6.

therefore turned to the use of [U-$^{13}C_6$]glucose, a precursor which we had earlier introduced for the examination of polyacetate, terpenoid,[10] or shikimate[11] metabolism in cases in which other precursors had proven to be ineffective. Through well-known pathways of intermediary metabolism, glucose can be converted into two-carbon (e.g., acetate), three-carbon (e.g., pyruvate), and four-carbon (e.g., erythrose 4-phosphate) intermediates carrying contiguous carbon atoms from the original sugar precursor. Intact incorporation of these various intermediates can be recognized by analysis of the pattern of enhancements and couplings in the ^{13}C NMR spectrum of any derived metabolite. Thus an intact two-carbon unit will give rise to the well-known pair of enhanced and coupled doublets approximately centered about the natural abundance singlet. We have previously termed this three-signal pattern a trio.[11] Incorporation of an intact three-carbon unit gives rise to a more complex but nonetheless easily recognized pattern. Each site derived from a carbon at the end of the three-carbon chain will give rise to the trio of signals associated with a single carbon–carbon coupling. The signal derived from the central carbon, on the other hand, results from the superposition of a doublet of doublets, arising from the presence of two labeled neighbors, and a pair of doublets, resulting from those species in which either one or the other of the adjacent carbon atoms is labeled, plus the natural abundance signal. In the simplest case, in which the two J_{CC} coupling constants are identical, this pattern simplifies to a characteristic quintet of five peaks. By extension, four contiguous ^{13}C atoms give rise to a pattern of a trio coupled to a quintet coupled to another quintet coupled to a trio. In most cases, two-bond J_{CC} couplings have a negligible effect on the appearance of the multiplets.

For the actual incorporation experiment, 100 mg of [U-$^{13}C_6$]glucose diluted with 200 mg of unlabeled glucose was administered to 100 mL of a 12-h replacement culture of *Pseudomonas* CB-104. After an additional 24 h, the cultures were harvested and the resultant extract was subjected to purification by preparative TLC to yield 2.7 mg of labeled thiotropocin. Analysis of the ^{13}C NMR spectrum of labeled **1** in DMSO-d_6 indicated that each carbon atom was coupled to at least one of its neighbors. Unfortunately, the overlap of the critical C-4 and C-5 signals precluded complete analysis of the labeling pattern. The labeled thiotropocin was therefore converted to the corresponding *p*-bromobenzyl thioether **2**, which was analyzed by ^{13}C NMR in both $CDCl_3$ and DMSO-d_6 in order to allow maximum resolution of all peaks of interest. The labeling patterns for **1** and **2**, illustrated in Scheme IV, corresponded to the superposition of two isotopomerically labeled species, **1a** or **2a**, respectively containing two-carbon (C-1,2) and four-carbon (C-4,5,6,7) patterns plus a pair of enhanced singlets (C-3 and C-8) and **1b** or **2b**, each containing a pair of intact two-carbon units (C-1,2 and C-7,8) plus a four-carbon unit (C-3,4,5,6). For example, in the spectrum of labeled **2** (Figure 1), the signals for C-1 and C-2 appeared as a simple pair of enhanced and coupled doublets. The corresponding ^{13}C–^{13}C satellites for C-8 were less intense relative to the central resonance, consistent with the notion that in half the thiotropocin *p*-bromobenzyl thioether (**2b**) C-8 would be coupled to its neighbor, C-7, while in the remaining **2a** C-8 would give rise to an enhanced singlet. A similar analysis can be applied to the signals for C-3. Both C-6 and C-5 were clearly coupled to both of their neighboring carbon atoms, as evidenced by the characteristic doublet of doublets observed for both sets of NMR signals. Note that in half the samples of thiotropocin and its *p*-bromobenzyl thioether **2b**, C-6 was coupled only to C-5, resulting in a diminished relative intensity for the outermost peaks of the doublet of doublets and a compensating increase in the intensity of one of the inner doublets. The coupling pattern for C-5 was most evident in the spectrum of labeled **2** recorded in DMSO-d_6. C-7, which was separately coupled to either one or the other of

Scheme IV



(10) Cane, D. E.; Rossi, T.; Pachlatko, J. P. *Tetrahedron Lett.* **1979**, 3639. Cane, D. E.; Rossi, T.; Tillman, A. M.; Pachlatko, J. P. *J. Am. Chem. Soc.* **1981**, *103*, 1838.

(11) Gould, S. J.; Cane, D. E. *J. Am. Chem. Soc.* **1982**, *104*, 343.

Scheme V

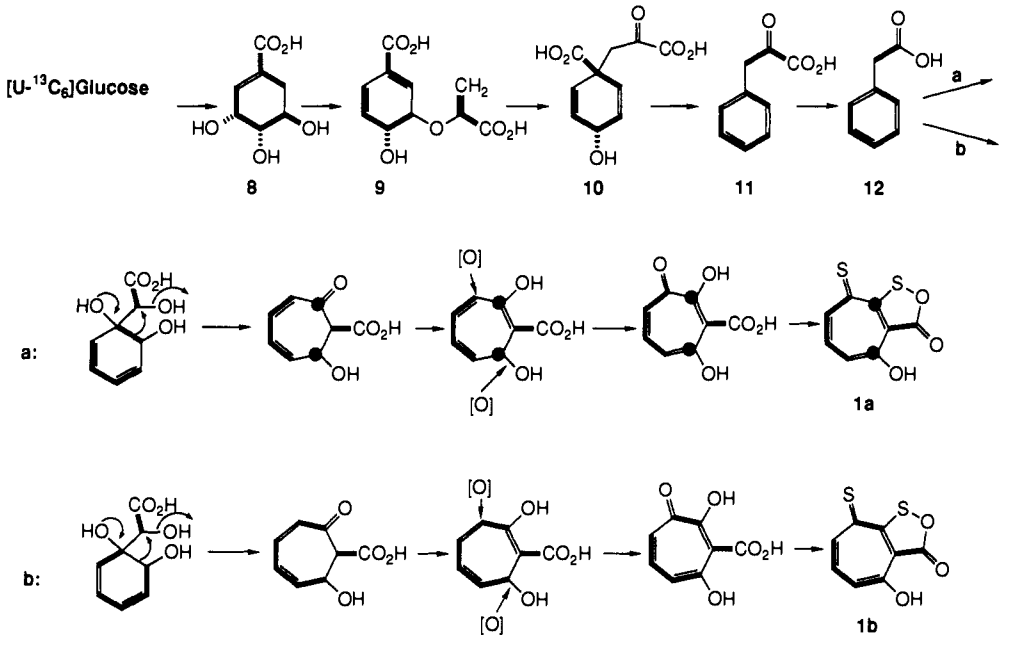


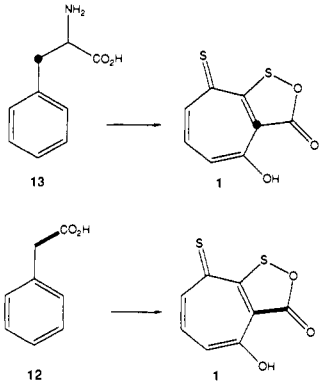
Table III. ^{13}C NMR Assignments for the Labeling of **2** by [U-$^{13}C_6$]Glucose

	$CDCl_3$			DMSO		
C	δ (ppm)	pattern	J_{CC} (Hz)	δ (ppm)	pattern	J_{CC} (Hz)
1	168.24	d	73.1	167.8	d	72.3
2	117.48	d	73.1	116.5	d	72.3
3	178.63	s + d	56.2	178.4	s + d	56.2
4	144.21	dd	56.2, 63.5	142.8	dd	(overlap)
5	131.19	dd	53.6, 62.6	132.5	dd	53, 62.7
6	142.76	ddd	53.8, 69.9, 3.2	142.8	dd	(overlap)
7	130.72	d + d	61.8, 70 (est)	130.4	d + d	68, 58
8	177.67	s + d	60.8	177.8	s + d	57.0

its neighboring carbon atoms, gave rise to a pair of doublets of low intensity relative to the (partially enhanced) natural abundance signal. These data are summarized in Table III.

The observed labeling patterns indicate the intermediacy of a symmetrical shikimate-derived intermediate (Scheme V). Thus [U-$^{13}C_6$]glucose would be converted by well-established pathways to shikimate (**8**) and then to chorismate (**9**) derived from erythrose 4-phosphate and 2 equiv of phosphoenolpyruvate.[12,13] Chorismate mutase-catalyzed rearrangement to prephenate (**10**) followed by decarboxylation and loss of water will give symmetrical phenylpyruvate (**11**) containing intact four-carbon and two-carbon units in the aromatic ring and a three-carbon unit in the side chain. Formation of phenylacetic acid (**12**), presumably in a thiamin-dependent process, will leave an intact two-carbon glucose-derived unit in the side chain. Oxidative ring expansion would involve either of the ortho carbon atoms of the aromatic ring with an equal probability of either cleaving the bond between the two-carbon and four-carbon units or scission of the two-carbon unit itself, resulting in the generation of a pair of carbon atoms without labeled neighbors.

Scheme VI



The proposed biosynthetic pathway was further tested by carrying out two additional incorporation experiments. Thus, administration of 50 mg of [3-^{13}C]phenylalanine (**13**) to 100 mL of a 12-h-old production culture of *Pseudomonas* CB-104 gave 3.2 mg of thiotropocin labeled with ca. 70 atom % ^{13}C at C-2, as expected (Scheme VI). This sample was used in the ^{13}C NMR assignment experiments described above.[6] Although phenylalanine itself is probably not a true intermediate in the formation of thiotropocin, it should be readily converted to phenylpyruvate (**11**) by transamination. We next administered 50 mg of [1,2-$^{13}C_2$]-phenylacetic acid (**12**) as the sodium salt to 100 mL of a 12-h production culture. The resulting labeled thiotropocin exhibited the predicted pair of enhanced and coupled doublets, corresponding to labels at C-1 and C-2 (25 atom % ^{13}C, J_{CC} = 61.4 Hz).

Conclusion

Taken together, these results are fully consistent with the formation of thiotropocin by the oxidative ring expansion of phenylacetic acid, itself biosynthesized in the expected manner by the shikimate–chorismate pathway.[15] A plausible route to thiotropocin is illustrated in Scheme V, involving ring expansion and further oxidation to generate a 4-hydroxytropolone carboxylic acid. Sulfur–oxygen exchange would be expected to take place by attack of a nucleophilic sulfur species on the tropolone carbonyl followed by tautomerization and analogous introduction of the

(12) Haslam, E. *The Shikimate Pathway*; Halsted Press: New York, 1974.

(13) Herbert has recently used [U-$^{13}C_6$]glucose to study the biosynthesis of another shikimate-derived antibiotic, obafluorin, a metabolite of *Pseudomonas fluorescens*. (Herbert, R. B.; Knaggs, A. R. *J. Chem. Soc., Perkin Trans. 1* **1992**, 103.) It was reported that the erythrose 4-phosphate-derived portions of both aromatic rings in obafluorin displayed labeling patterns characteristic of an isolated labeled carbon atom (C-1) and an intact three-carbon unit corresponding to C-2, C-3, and C-4 of the erythrose intermediate. Only a minor amount of the labeled obafluorin consisted of intact four-carbon units. This observation was ascribed to catabolism of glucose to glyceraldehyde 3-phosphate and pyruvate by the Entner–Doudoroff pathway known to predominate in *Pseudomonas*[14] species. Similar depletions in the extent of C-1–C-2 coupling have previously been observed in *Streptomyces*[11] and, in fact, can be used to identify the orientation of the erythrose 4-phosphate moiety in shikimate-derived metabolites.

(14) Lessie, T. G.; Phibbs, P. V., Jr.; *Ann. Rev. Microbiol.* **1984**, *219*, 213.

(15) The biosynthesis of hydroxyphenylacetic acid in higher plants has been described: Kindl, H. *Eur. J. Biochem.* **1969**, *7*, 340.

second sulfur atom. Further oxidation to the sulfenic acid derivative and lactonization would then yield thiotropocin.

Experimental Section

Methods and Materials. NMR spectra were obtained at 400.13 or 250.13 MHz (^{1}H) and 100.6 or 62.9 MHz (^{13}C). ^{1}H/^{13}C long-range HETCOSY spectral acquisition parameters: 20 000-Hz sweep width in the F_2 dimension; 128 spectra (860 scans each) accumulated with a 2.0-s relaxation delay. Resolution was 9.766 Hz per point in the F_2 dimension and 13.378 Hz per point in the F_1 dimension. [1,2-$^{13}C_2$]Phenylacetic acid (99 atom %), DL-[3-^{13}C]phenylalanine (99 atom %) and D-[U-$^{13}C_6$]-glucose (90 atom %) were purchased from ISOTEC, Inc. Thiotropocin (**1**) was converted to the *p*-bromobenzyl thioether derivative **2** as previously described.[1b]

Fermentation of *Pseudomonas* CB-104. A loopful of cells from a cultivated slant was inoculated into 50 mL of seed medium in a 250-mL flask. The seed medium contained fructose (10 g), polypepton (BBL) (5 g), meat extract (Wako Pure Chemical Industries) (5 g), and NaCl (5 g) (pH 7.0) per liter. The inoculated seed culture was incubated at 28 °C for 2 days with reciprocal shaking at 100 rpm. The inoculum (0.8 mL) was transferred to 50 mL of the same seed medium and incubated at 24 °C for 1 day with 20 mL/min of aeration. The resulting culture (2.5 mL) was then transferred to 100 mL of production medium containing fructose (5 g) (sterilized separately), polypepton (0.5 g), meat extract (0.5 g), NaCl (0.5 g), and $Na_2S_2O_3$ (0.05 g) (pH 7.0). The production medium was incubated at 24 °C for 36 h with 44 mL/min of aeration and 100-rpm agitation.

Isolation and Purification of Thiotropocin. Cells from the above production medium were removed by centrifugation at 4000*g* for 15 min. The supernatant was then adjusted to pH 3.0 with sulfuric acid and extracted four times with ethyl acetate. The product was transferred to the aqueous phase by extraction with three portions of 2% $NaHCO_3$. The aqueous phase was then adjusted to pH 3.0 and reextracted three times with ethyl acetate. The crude product obtained after evaporation of the solvent was subjected to purification by preparative TLC (toluene–dioxane–acetic acid, 45:10:2) to yield 4 mg of thiotropocin crystals per 100 mL of fermentation medium.

Administration of Labeled Precursors. [U-$^{13}C_6$]Glucose. Feedings of labeled glucose were carried out with replacement cultures. Cells from a 12-h production culture were collected by centrifugation at 4000*g* for 15 min and resuspended in the same volume of a replacement medium of the same composition as the production medium except for glucose, which was at a concentration of 300 mg per 100 mL. After incubation of the resuspended cells at 24 °C for 0.5 h, 100 mg of [U-$^{13}C_6$]glucose and 200 mg of unlabeled glucose in 5 mL of water were introduced by sterile filtration. After an additional 24 h, the culture broth was extracted in the usual manner to yield, after purification, 2 mg of thiotropocin.

[3-^{13}C]Phenylalanine. A solution of 50 mg of [3-^{13}C]phenylalanine in 5 mL of nano-pure water, pH adjusted to pH 7.0, was administered by sterile filtration to 100 mL of a 12-h production culture. Incubation for an additional 24 h yielded 3.2 mg of purified thiotropocin.

[1,2-$^{13}C_2$]Phenylacetic Acid. A solution of 50 mg of [1,2-$^{13}C_2$]phenyl acetic acid in 5 mL of nano-pure water, pH adjusted to pH 7.0, was administered by sterile filtration to 100 mL of a 12-h production culture. Incubation for an additional 24 h yielded 6.7 mg of purified thiotropocin.

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(−)-Cryptaustoline: Its Synthesis, Revision of Absolute Stereochemistry, and Mechanism of Inversion of Stereochemistry

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Abstract: The asymmetric synthesis of (*S*)-(+)-cryptaustoline was accomplished and found to differ in sign of rotation with the natural "*S*"-(−)-material. The previously assigned absolute configuration was found to be incorrect and is now corrected. The reversal in stereochemistry came about through an unusual manner involving (*S*)-(+)-laudanosoline (**3**) cyclizing to (*R*)-(−)-cryptaustoline ((−)-**1c**). The mechanism was explored by oxidation of a series of deuterio derivatives of laudanosoline, all of which retained deuterium in the resultant cryptaustoline. Both chemical and enzymatic oxidations yield the same stereochemical result which is dictated simply by the stereochemistry of the starting material **3**. A reverse Michael addition followed by readdition is considered the most plausible route to the stereochemical inversion of (+)-**3** to (−)-**1c**.

Introduction

As part of a program designed to further demonstrate the synthetic utility of chiral formamidines[1] toward the total synthesis of a variety of indole[2] and isoquinoline alkaloids,[3] we undertook the seemingly simple target in the dibenzopyrrocoline series, (−)-cryptaustoline (**1a**). Isolated[4a] in 1952 from *Cryptocarya bowiei* (Hook) Druce, indigenous to Queensland, **1a** was one of only two dibenzopyrrocoline alkaloids obtained from this plant, the other being (−)-cryptowoline (**2**). The water-soluble alkaloids were isolated as their sparingly soluble iodides and were both levorotatory.[4c] Biologically, their mode of action is still uncertain,

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