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ISOLATION AND STRUCTURAL ELUCIDATION OF TETRACENOMYCIN F2 AND TETRACENOMYCIN F1: EARLY INTERMEDIATES IN THE BIOSYNTHESIS OF TETRACENOMYCIN C IN *STREPTOMYCES GLAUCESCENS*[1]

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ABSTRACT.—This report describes the fermentation, isolation, and structural elucidation of tetracenomycin (Tcm) F2 [**2**], a metabolite produced by a blocked mutant strain WMH1092 of the Tcm C [**1**] producer *Streptomyces glaucescens* and by the recombinant strain *S. glaucescens* WMH1077(pWHM722). Elucidation of the Tcm F2 structure shows that **2** is the earliest intermediate identified to date in the biosynthesis of **1**. This is supported by the fact that **2** is very efficiently biotransformed to **1** by the *S. glaucescens* WMH1068 strain and is enzymatically converted to Tcm F1 [**3**] and to Tcm D3 [**4**], a known intermediate of Tcm C biosynthesis.

Tetracenomycin (Tcm) C [**1**] is an antitumor antibiotic produced by *Streptomyces glaucescens* GLA.0 (1). We have previously described the isolation of several groups of Tcm C-nonproducing mutants and the structural elucidation of metabolites isolated from them (2,3). From a consideration of the structures of these metabolites and the cosynthetic behavior of the corresponding mutants, we proposed that Tcm F2 [**2**] and Tcm F1 [**3**] are the immediate precursors of Tcm D3 [**4**], the earliest established intermediate of the biosynthetic pathway to **1** (Scheme 1) (2–4). We report here the isolation and structural elucidation of **2** and **3**. Their intermediacy in the biosynthetic pathway to **1** was confirmed by in vivo biotransformation of **2** to **1** and by in vitro enzymatic conversion of **2** to **3** and **3** to **4**.

RESULTS AND DISCUSSION

ISOLATION OF **2** AND **3**.—Initially, the *S. glaucescens* strain WMH1092 (formerly GLA.8-21) (2) grown in R2YENG medium (2) for 4 days was used for production of **2**. [The WMH1092 strain was isolated from mutagenesis of spores of the wild-type *S. glaucescens* GLA.0 strain (2) and recently was shown to have double mutations in the *tcmHI* genes, blocking the cyclization of **2** into more advanced Tcm metabolites (R.G. Summers and C.R. Hutchinson, unpublished data).] However, only limited spectroscopic data could be obtained because this strain produced very little **2** (<1 mg per 2.5 liters of culture). Recently it was found that *S. glaucescens* WMH1077(pWHM722) [formerly GLA.5-1(pWHM722)] (5), a recombinant strain that carries the *tcmKLMN* genes under the control of the *ermE** promoter in the high-copy number vector pIJ486, produces significantly more **2** than *S. glaucescens* WMH1092. Since **2** is the immediate product of the Tcm C polyketide synthase encoded by the *tcmKLMN* genes, the high production of **2** in the latter strain must have resulted from the overproduction of the Tcm C polyketide synthase. According to Scheme 2, 5 to 10 mg of **2** typically could be

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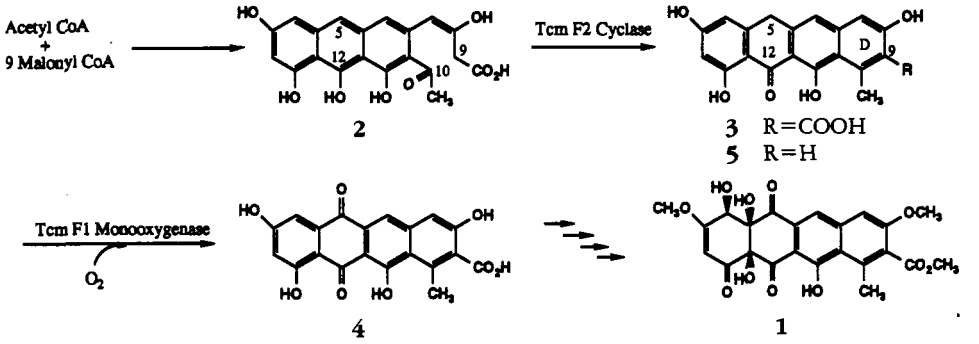
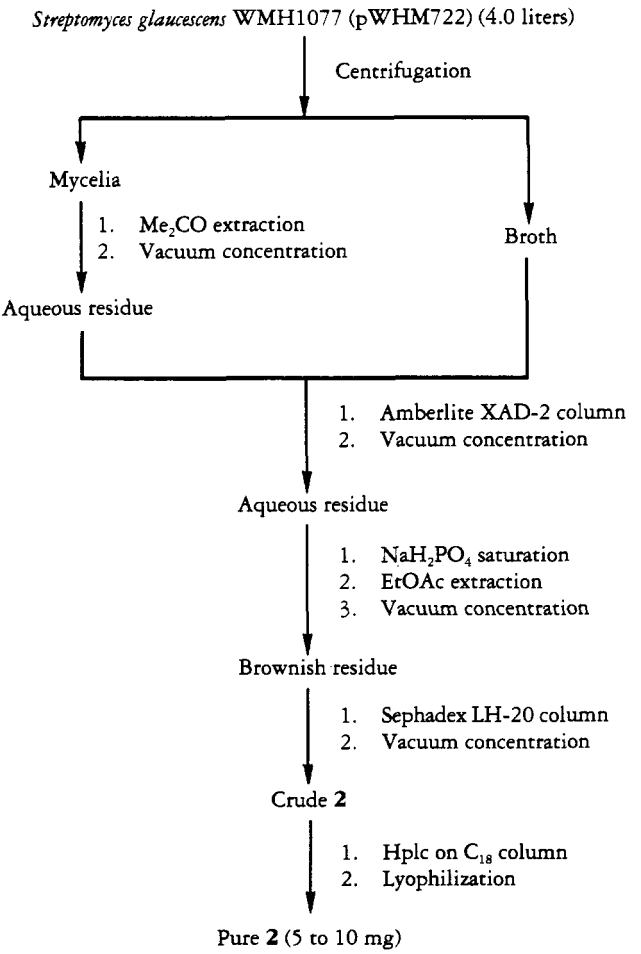


SCHEME 1. Biosynthetic pathway of Tcm C in *Streptomyces glaucescens* [the Tcm C numbering system (3) is used].

isolated from 4 liters of culture of the WMH1077(pWHM722) strain grown in R2YENG medium in the presence of thiostrepton (10 μg/ml).

We reported earlier that none of the 34 *S. glaucescens* blocked mutants accumulated **3**, despite the fact that collectively they produced various biosynthetic intermediates (2,3). This may have been the result of the small size of the *tcmH* gene (6), which encodes



SCHEME 2. Scheme for isolation of Tcm F2 from *Streptomyces glaucescens* WMH1077 (pWHM722).

the 12.8 KDa Tcm F2 monooxygenase that oxidizes **3** to **4**, and consequently a random *tcmH* mutation would be expected to occur at a low frequency. Therefore, we turned our attention to an in vitro system with the intention to synthesize **3** enzymatically. When **2** was incubated in tris-HCl buffer, pH 8.0, containing 1.0 mM dithiothreitol in the presence of the Tcm F2 cyclase (7), **3** was the predominant product observed (>90% by hplc analysis). In a typical experiment, 1.0 to 1.5 mg of **3** could be enzymatically synthesized from 2.0 mg of **2**.

STRUCTURAL ELUCIDATION OF **2** AND **3**.—The exact molecular formula of **2** was derived from hreims analysis on the assumption that the ion of *m/z* 340.09545 (9.6%) was the $[M-CO_2]^+$ fragment (calcd 340.09469) because the $[M]^+$ is too weak to be detected. This assumption was validated by analyzing the same sample by electron spray ms analysis where the molecular ion of **2** was evident as a pair of ions with mass of 402 (70%) and 385 (50%) for $[M+NH_4]^+$ and $[M+H]^+$, respectively. Therefore, it was concluded that **2** has the molecular formula $C_{20}H_{16}O_8$, consistent with its polyketide biogenesis.

Similar analysis was applied to determine the molecular formula of **3**. From the hreims analysis, the molecular ion of *m/z* 366.07372 indicated that **3** has the molecular formula of $C_{20}H_{14}O_7$ (calcd 366.07396), although this ion had a very low intensity (0.2%). Yet, the $[M-CO_2]^+$ fragment (calcd 322.08412) was very abundant at *m/z* 322.08413 (20%). Electron spray ms analysis of **3** further confirmed its molecular formula by the observation of a pair of ions with mass of 384 (100%) and 367 (63%) for $[M+NH_4]^+$ and $[M+H]^+$, respectively.

The structures of **2** and **3** as shown in Scheme 1 fully accommodate all the other spectroscopic data. The uv spectrum of **2** [λ max (ε) in MeOH 376 (5.600), 268 (20,900)] agrees with its tricyclic anthrone backbone (8,9), whereas that of **3** [λ max (ε) in MeOH 414 (12,200), 356 (11,500)] gave the expected red shift from the anthrone skeleton of **2** to the cyclized tetracyclic naphthacenone backbone of **3** (3,8). The anthrone nature of **2** was further supported by its ^{1}H- and ^{13}C-nmr data. Three singlets and two doublets (1H each) were observed in the ^{1}H-nmr spectrum, representing the four aromatic and one olefinic protons. Of the twenty carbons in the ^{13}C-nmr spectrum, sixteen of them were identified as aromatic or olefinic carbons. One side chain for the MeCO- group is evident from the singlet (3H) at 1.90 ppm in the ^{1}H-nmr spectrum and the ^{13}C resonances at 29.9 ppm and 193.5 ppm in the ^{13}C-nmr spectrum, while the terminal $-CH_2COOH$ moiety of the other side chain was derived from the singlet (2H) at 4.17 ppm in the ^{1}H-nmr spectrum and the ^{13}C resonances at 39.0 ppm and 171.0 ppm in the ^{13}C-nmr spectrum. Furthermore, the presence of ir bands at 3800–2400 and 1664 cm^{-1} are consistent with the phenolic -OH's and the carboxylic acid groups, whereas the MeCO- group is evident from ir bands at 1632, 1445, and 1377 cm^{-1}.

The structure of **3** was derived mainly by comparision of its spectroscopic data to those of **2**. Cyclization of **2** at C-9 and C-10 to form the D ring of **3** is consistent with the ^{1}H-nmr spectrum of **3**. Unlike **2**, whose -CO- at C-12 exists in the =C-OH form, the corresponding -CO- of **3** is observed in the C=O form. This is evident from the ^{1}H-nmr spectrum of **3**, where the protons of the two neighboring -OH's display sharp resonances at 14.88 and 12.38 ppm, characteristic of -OH groups hydrogen-bonded to the C=O group as observed in many other quinones (4,10). This deduction is further supported by the singlet (2H) at 4.34 ppm which accounts for the $-CH_2-$ group at C-5 of **3**. Finally, the singlet (3H) at 2.88 ppm is assigned to the -Me group, whose chemical shift is almost identical to those observed for the same group in other Tcm C biosynthetic intermediates (3,4).

The above discussion concentrated more on establishing individual structural

fragments than on making connections among these fragments. The unstable nature of **2**, which decomposes rapidly in either acidic or basic conditions and whose solution turns dark brown upon exposure to air, made it difficult for us to carry out further studies. Attempts were made to convert **2** into more stable derivatives or known compounds, but, unfortunately, all failed. However, with the Tcm F2 cyclase (7) and Tcm F1 monooxygenase (8), purified recently in our lab from *S. glaucescens* WMH1068 (formerly GLA.11-47), we successfully converted **2** to **3** or **3** to **4** enzymatically. It is interesting to point out that **3** is the predominant product produced upon incubation of **2** in the presence of the Tcm F2 cyclase if the reaction is carried out in buffer of pH≥8.0. Incubation of the same reaction mixture in buffer of pH≤6.5 results in a new compound, 9-decarboxy-Tcm F1 [**5**]. We have reported the isolation of **5** from a *S. glaucescens* mutant strain (3), and its structure was confirmed as follows. Hreims analysis of **5** gave the $[M]^+$ of 322.08382 (66.5%, calcd 322.08413), assigning it a molecular formula of $C_{19}H_{14}O_5$. As expected, **5** displays a ^{1}H-nmr spectrum almost identical to that of **3**, except for an extra, broad singlet (1H) at 6.86 ppm that reflects the decarboxylation of **3** at C-9. A substantial amount of **4** was also synthesized from **2** by the Tcm F2 cyclase and Tcm F1 monooxygenase catalyzed reactions and purified. Hrfabms analysis of the so-derived **4** gave the $[M+H]^+$ of 381.05938 (25%) (calcd 381.06104), consistent with the molecular formula of $C_{20}H_{12}O_8$, and the eims analysis gave the $[M-CO_2]^+$ of 336.07273 (93%) (calcd 336.06339), with the molecular ion too weak to be detected. The ^{1}H-nmr, uv, and ir spectra of **4** were all identical to those reported in the literature (4), thus providing supplementary evidence to support the structures of **2** and **3**.

Tcm F2 and Tcm F1 are the earliest intermediates identified in Tcm C biosynthesis. The fact that **2** can be enzymatically converted to **3** and **4** establishes unambiguously their intermediacy in the biosynthetic pathway of **1**. Further evidence for this was obtained from feeding of the purified **2** in vivo to *S. glaucescens* WMH1068 (2). We chose *S. glaucescens* WMH1068 because it has a deletion mutation in the *tcmL* gene and, therefore, produces a nonfunctional Tcm C polyketide synthase. Consequently this strain cannot synthesize **2** from acetyl CoA and malonyl CoA, but since the rest of its biosynthetic machinery is intact, it is able to metabolize an early intermediate to the final product **1** (5). When **2** was fed to *S. glaucescens* WMH1068, it was very efficiently biotransformed to give **1** in more than 50% yield, confirming **2** as the earliest intermediate identified to date in the Tcm C biosynthetic pathway.

The structural novelty of **2** and **3** is their anthrone and naphthacenone backbones. Although these types of intermediates have been proposed in the biosynthesis of many, if not all, anthracyclines of polyketide origin (11–14), no examples of such compounds have been identified so far. Therefore, the isolation of **2** and **3** provides the first direct evidence of the involvement of such intermediates in the biosynthesis of anthracyclines.

It is interesting to point out the biosynthetic sequence established here for a naphthacenequinone like **4**. The quinone moiety of **4** is introduced after the complete formation of the naphthacenone backbone, i.e., in the order **2**→**3**→**4**. Similar biosynthetic events also have been proposed for the biosynthesis of tetracycline in *Streptomyces aureofacieus*. Although the corresponding anthrone intermediate has never been identified, two hypothetical anthraquinone-type intermediates, i.e., protetrone (15) and its methylanthrone analogue (9), were identified in the form of shunt metabolites, suggesting that the cyclized naphthacenone is the substrate likely to be oxidized to yield the naphthacenequinone intermediate. In contrast, in a parallel anthracycline biosynthetic pathway in *Streptomyces peucetius*, the earliest intermediate identified is an anthraquinone, aklanonic acid (10), which is cyclized to the tetracyclic aklaviketone (10, 16–18). The latter biosynthetic sequence, therefore, shows that in *S. peucetius* the quinone moiety of

the naphthacenequinone intermediate is introduced before complete formation of the naphthacenone backbone.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr and ^{13}C-nmr spectra were taken on a Bruker Aspect 3000 spectrometer, using 5-mm tubes, and both ^{1}H- and ^{13}C-nmr samples were referenced to TMS. Ir spectra were recorded on a Mattson Polaris FT-IR spectrometer. Eims were carried out on a Kratos MS-80RFA spectrometer, electron spray ms on a Sciex API3 spectrometer, and fabms on a VG Analytic ZAB-SE organic mass spectrometer. Uv spectra were recorded on a Cary 14/Olis UV/VIS spectrophotometer. Analytic tlc was done with precoated Keiselgel 60 F_{254} glass plate (0.25 mm) and visualized by long- and/or short-wave uv. Hplc was done on a Waters 501 instrument with a Waters 484 tunable absorbance detector and a Waters Radi-Pak C_{18} (Novapak, 4 μM, 8×100 mm) column.

ISOLATION OF **2**.—Frozen cell suspensions of *S. glaucescens* WMH1077(pWHM722) were used to inoculate 50 ml of R2YENG medium with thiostrepton (10 μg/ml) in a 250-ml baffled Erlenmeyer flask and incubated for 40 h at 30° and 300 rpm in a rotary shaker to produce a vegetative inoculum. The R2YENG fermentation medium with thiostrepton (10 μg/ml), 500 ml in a 2-liter baffled Erlenmeyer flask, was inoculated with the above seed inoculum (20 ml of inoculum/500 ml of medium) and incubated under the same conditions for 24 to 28 h. The fermentation culture (usually 4 liters) was centrifuged (4°, 20 min, 17,700×*g* in a Sorvall RC-5B refrigerated centrifuge) to separate the mycelia from the broth. The mycelia were extracted wth Me_2CO (1 liter), and the Me_2CO extract was vacuum-concentrated (<40°) to give an aqueous residue. This residue was combined with the broth and loaded onto an Amberlite XAD-2 (Mallinckrodt) column (3.5×25 cm). The column was washed with H_2O and eluted with 1.0 liter of a linear gradient from H_2O to MeOH. Fractions were monitored by tlc, developed in $CHCl_3$-MeOH-HOAc (85:15:0.25). Under these conditions, **2** has an R_f of 0.57. Fractions containing **2** were vacuum-concentrated (<40°) to remove as much MeOH as possible, and after saturation with NaH_2PO_4, the aqueous residue was extracted with EtOAc (4×100 ml). The combined EtOAc extract was vacuum-concentrated, and the brownish residue was loaded on a Sephadex LH-20 (Pharmacia) column (1.5×75 cm), developed with MeOH. Fractions containing **2** were vacuum-concentrated to give a brownish oil of crude **2**. This crude **2** was finally purified by hplc on a C_{18} column developed with a linear gradient from MeCN-H_2O-HOAc (80:20:0.1) to MeCN in 12 min at a flow rate of 2 ml/min with an uv detection at 280 nm. Under these conditions, **2** has a retention time of 5.1 min. Fractions containing **2** were vacuum-concentrated to remove as much MeCN as possible, and the aqueous residue was lyophilized to yield pure **2** as a fluffy yellow powder (5 to 10 mg): *ir* (KBr) 3800–2400 (br), 1664, 1632, 1566, 1445, 1377, 1252, 1166 cm^{-1}; uv λ max (ε) (MeOH or MeOH/HCl) 376 (5,600), 268 nm (20,900), (MeOH/NaOH) 353 (4,050), 292 (14,400), 252 nm (13,700); ^{1}H nmr (300.1 MHz, DMSO-d_6) δ 1.90 (s, 3H), 4.17 (s, 2H), 5.18 (d, 1H, J=2.1 Hz), 5.51 (d, 1H, J=2.0 Hz), 6.43 (s, 1H), 6.59 (s, 1H), 7.09 (s, 1H), 9–11 (br s, 2 to 4H, OH's) (the chemical shifts and multiplicity of the ^{1}H resonances of **2** in DMSO show some concentration and temperature dependency); ^{13}C nmr (75.5 MHz, DMSO-d_6) δ 29.9, 39.0, 87.5, 97.7 (2 carbons), 99.3 (2 carbons), 112.1, 114.1 (2 carbons) 123.0, 134.0, 138.9, 164.5, 165.4 (2 carbons), 167.9, 171.2 (2 carbons), 193.5; electron spray ms *m/z* (rel. int.) $[M+NH_4]^+$ 402 (70), $[M+H]^+$ 385 (50); hreims 340.09545 (9.6%, $C_{20}H_{16}O_8$-CO_2, calcd 340.09469); eims *m/z* (rel. int.) 340 (9.6), 322 (38.6), 298 (37), 282 (9.5), 257 (36.3), 256 (74.1), 255 (35.9), 216 (32.3), 126 (31.4), 111 (11.0).

ENZYMATIC SYNTHESIS OF **3** AND **5** FROM **2**.—In a 100-ml round-bottom flask, **2** (3.1 mg, 8.07 μmol) was dissolved in 30 ml of 0.1 M tris-HCl buffer, pH 8.0, containing 1.0 mM dithiothreitol. The flask was capped with a septum stopper and flushed with N_2. A solution of the partially purified Tcm F2 cyclase (2 ml, 1.0 mg of proteins) was introduced via the septum stopper, and the resulting solution was incubated at 30° for 90 min. The reaction mixture was saturated with NaH_2PO_4 and extracted with EtOAc (5×10 ml). The combined EtOAc extract was vacuum-concentrated to give crude **3** as a yellow residue. The crude **3** was further purified by hplc on a C_{18} column under the same conditions as those used for **2** where **3** has a retention time of 7.3 min. Fractions containing **3** were vacuum-concentrated to remove as much MeCN as possible, and the aqueous residue was finally lyophilized to give pure **3** as a fluffy yellow powder (1.83 mg, 62%): ir (KBr) 3800–2600 (br), 1611, 1576, 1447, 1367, 1277, 1156 cm^{-1}; uv λ max (ε) (MeOH or MeOH/HCl) 414 (12,200), 356 (11,500), 286 (12,600), 252 nm (20,800), (MeOH/NaOH) 446 (19,900), 312 (4,800), 262 nm (16,000); ^{1}H nmr (300.1 MHz, DMSO-d_6) δ 2.88 (s, 3H), 4.34 (s, 2H), 6.23 (d, 1H, J=2.2 Hz), 6.43 (brs, 1H), 6.90 (s, 1H), 7.06 (s, 1H), 12.38 (s, 1H), 14.88 (s, 1H); electron spray ms *m/z* (rel. int.) $[M+NH_4]^+$ 384 (100), $[M+H]^+$ 367 (63); hreims 366.07372 (0.2%, $C_{20}H_{14}O_7$, calcd 366.07396), 322.08413 (20%, $C_{20}H_{14}O_7$-CO_2, calcd 322.08412); eims *m/z* (rel. int.) 366 (0.2), 322 (20), 235 (5), 119 (8).

For the synthesis of **5** from **2**, the same reaction described above for **3** was carried out in 0.1 M bis-

tris-HCl buffer, pH 6.5, and the reaction mixture was incubated under the same conditions as above. Hplc analysis showed complete conversion of **2** with production of **5** and **3** in ratio of 10:1. Workup similar to that described above yielded pure **5**, which has a retention time of 8.2 min under the given hplc conditions. Compound **5**: yellow powder; hreims 322.08382 (66.5%, $C_{19}H_{14}O_5$, calcd 322.08413); ^{1}H nmr (300.1 MHz, DMSO-d_6) δ 2.89 (s, 3H), 4.37 (s, 2H), 6.27 (d, 1H, J=2.3 Hz), 6.47 (d, 1H, J=2.2 Hz), 6.86 (brs, 1H), 6.91 (d, 1H, J=2.3 Hz), 7.11 (s, 1H), 12.47 (s, 1H), 14.69 (s, 1H).

ENZYMATIC SYNTHESIS OF **4** FROM **2**.—In a 100-ml Erlenmeyer flask, compound **2** (7.5 mg, 19.5 μmol) was dissolved in 60 ml of 0.1 M sodium phosphate buffer, pH 7.5, containing 2 mM dithiothreitol. A solution of the partially purified Tcm F2 cyclase and Tcm F1 monooxygenase (10 ml, 420 mg of proteins) was added, and the resulting solution was incubated at 30° for 2 h. The reaction was monitored by tlc developed as described for the isolation of **2** and, under these conditions, **2**, **3**, and **4** have R_f of 0.57, 0.38, and 0.29, respectively. The reaction mixture was worked up as described for the enzymatic synthesis of **3** from **2**: the reaction solution was saturated with NaH_2PO_4 and extracted with EtOAc (5×20 ml), and the EtOAc extract was vacuum-concentrated to give the crude **4**, which was further purified by hplc on the C_{18} column. Under the given conditions, **3** and **4** have the same retention time of 7.3 min. Lyophilization of the fractions containing **4** finally yielded pure **4** (4.8 mg, 65%) as a fluffy red-wine colored powder: ir (KBr) 3800–2400 (br), 1617, 1589, 1436, 1399, 1268, 1178 cm^{-1}; uv λ max (ε) (MeOH or MeOH/HCl) 483 (9,390), 338 (10,960), 312 (15,590), 277 (23,190), 243 nm (15,800) (MeOH/NaOH) 381 (18,600), 303 nm (20,000); hrfabms 381.05938 (25%, $C_{20}H_{12}O_8$, calcd 381.06104); hreims 336.07273 (93%, $C_{20}H_{12}O_8$-CO_2, calcd 336.06339).

BIOTRANSFORMATION OF **2** TO **1** IN *S. GLAUCESCENS* WMH1068.—*S. glaucescens* WHM1068 was grown in the R2YENG medium (10 ml in a 1.5×15 cm culture tube) for 48 h at 30° and 300 rpm in a rotary shaker. An MeOH solution of **2** (0.5 mg in 200 μl) was added via a 0.45 μm sterile acrodisc (GelmanScience) and the incubation was continued for additional 48 h. The culture was saturated with NaH_2PO_4 and extracted with EtOAc (4×2 ml). The EtOAc extract was analyzed by hplc; **1** was the only Tcm metabolite detected and represented more than a 50% conversion from **2**. The hplc conditions described above were used, and **1** had a retention time of 6.7 min.

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LITERATURE CITED

1. W. Weber, H. Zahner, J. Siebers, K. Schroder, and A. Zeeck, *Arch. Microbiol.*, **121**, 111 (1979).
2. H. Motamedi, E. Wendt-Pienkowski, and C.R. Hutchinson, *J. Bacteriol.*, **167**, 575 (1986).
3. S. Yue, H. Motamedi, E. Wendt-Pienkowski, and C.R. Hutchinson, *J. Bacteriol.*, **167**, 581 (1986).
4. J. Rohr, S. Eick, A. Zeeck, P. Reuschenbach, H. Zahner, and H.-P. Fiedler, *J. Antibiot.*, **41**, 1066 (1988).
5. R.G. Summers, E. Wendt-Pienkowski, H. Motamedi, and C.R. Hutchinson, *J. Bacteriol.*, **174**, 1810 (1992).
6. B. Shen and C.R. Hutchinson, *Biochemistry* (in press).
7. B. Shen and C.R. Hutchinson, *Biochemistry* (in press)
8. R.M. Silverstein, G.C. Bassler, and T.C. Morrill, "Spectrometric Identification of Organic Compounds," John Wiley & Sons, New York, 1981, pp. 321–326.
9. J.R.D. McCormick, E.R. Jensen, N.H. Arnold, H.S. Corey, U.H. Joachim, S. Johnson, P.A. Miller, and N.O. Sjolander, *J. Am. Chem. Soc.*, **90**, 7127 (1968).
10. K. Eckardt, D. Tresselt, G. Schumann, W. Ihn, and C. Wagner, *J. Antibiot.*, **38**, 1034 (1985).
11. T.J. Simpson, *Nat. Prod. Rep.*, **8**, 537 (1991).
12. P.L. Bartel, N.C. Connors, and W.R. Strohl, *J. Gen. Microbiol.*, **136**, 1877 (1990).
13. M.G. Anderson, C.L.-Y. Khoo, and R.W. Richards, *J. Antibiot.*, **42**, 640 (1989).
14. J. Mann, "Secondary Metabolism," Clarendon Press, Oxford, 1987, pp. 70–81.
15. J.R.D. McCormick and E.R. Jensen, *J. Am. Chem. Soc.*, **90**, 7126 (1968).
16. C. Wagner, K. Eckardt, G. Schumann, W. Ihn, and D. Tresselt, *J. Antibiot.*, **37**, 691 (1984).
17. K. Eckardt, G. Schumann, U. Grafe, W. Ihn, C. Wagner, W.F. Fleck, and H. Thrum, *J. Antibiot.*, **38**, 1095 (1985).
18. N.C. Connors, P.L. Bartel, and W.R. Strohl, *J. Gen. Microbiol.*, **136**, 1887 (1990).

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