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Chris R. Melville, and Steven J. Gould

J. Nat. Prod., **1994**, 57 (5), 597-601• DOI:
10.1021/np50107a005 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 5, pp. 597-601, May 1994

MURAYALACTONE, A DIBENZO-α-PYRONE FROM *STREPTOMYCES MURAYAMAENSIS*

CHRIS R. MELVILLE and STEVEN J. GOULD*

Department of Chemistry, Oregon State University, Corvallis, OR 97331-4003

ABSTRACT.—Murayalactone, **6**, has been isolated from *Streptomyces murayamaensis*, and its structure assigned with the use of inverse nmr spectroscopy. This constitutes the first occurrence of a dibenzo-α-pyrone in a *Streptomyces* species, and the first biogenesis of this ring system from a decaketide.

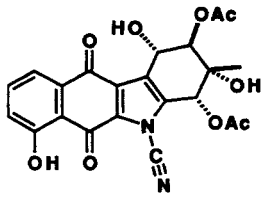
Streptomyces murayamaensis is known to produce metabolites derived from at least two independent decaketide pathways. The kinamycins, broad-spectrum antibiotics with modest cytotoxicity (1,2), were reported to be cyanamide derivatives of benzo[*b*]carbazolequinones (e.g., kinamycin D, **1**) (1–7). Recently, they have been shown to actually be diazo-substituted derivatives of benzo[*b*]fluorene (e.g., kinamycin D, **2**) (8). Biosynthetic studies of the kinamycins have shown that they are derived from a single decaketide precursor (9), and much of the present understanding of this pathway has been due to elucidation of the structures of minor metabolites (6, 8–12). This work has also led to the discovery of murayaquinone, **3**, the product of a second decaketide pathway (13). Compound **3** has antibacterial activity against *Mycoplasma galliseptica* and *Treponema hyodysenteriae* (14,15). We now report the characterization of the structurally related murayalactone, **6**, first detected and isolated from a uv-generated mutant of *S. murayamaensis*, and subsequently shown to be produced by the wild strain.

RESULTS AND DISCUSSION

An *S. murayamaensis* mutant, MC3, selected from the survivors of ultraviolet irradiation (M.C. Cone and S.J. Gould, unpublished results) does not produce the kinamycins, but does overproduce **3**. Extracts from fermentations in a glycerol-ammonium sulfate medium or a 2% farina cereal medium showed two blue spots by tlc. Although growth in the latter medium was better, extraction of the colored metabolites proved easier from the former one. During purification of the blue compounds by flash and medium-pressure liquid chromatography (mplc), a colorless compound was observed with uv detection during the latter step. This was collected and traces of an oily material removed by recrystallization.

The ^{1}H-nmr spectrum of the colorless compound contained resonances for an *n*-propyl moiety, an aryl methyl, and four aromatic protons (three contiguous), reminiscent of **3**. However, whereas two hydrogen-bonded phenols were observed in the spectrum of **3** (12.27 and 12.73 ppm), one hydrogen-bonded phenol at 11.62 ppm, and one additional exchangeable proton at 5.99 ppm were observed for the new compound.

High-resolution eims yielded a molecular ion at *m/z* 312.0997, corresponding to



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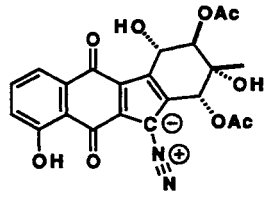

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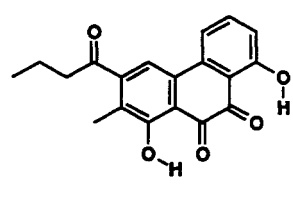

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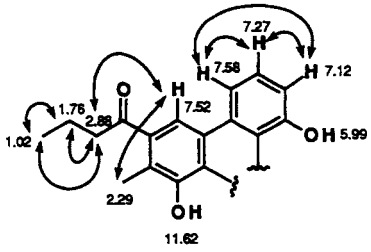

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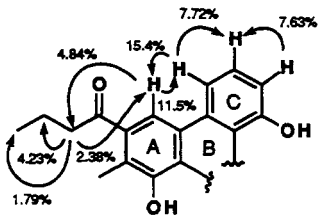


5

$C_{18}H_{16}O_5$, which differed from **3** by one less carbon. The low-resolution ms also contained an *m/z* 284 ion attributed to loss of ethylene by McLafferty rearrangement and an ion at *m/z* 269 (base peak) attributed to loss of a propyl radical. An *m/z* 241 ion corresponded to loss of a *n*-oxobutyl radical, presumably via a tropylium ion. These fragments supported the presence of a butyryl side-chain.

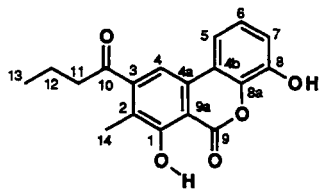
A ^{1}H-^{1}H COSY 45 spectrum showed all of the vicinal couplings expected for **3**, as well as a four-bond coupling between doublets at 7.12 and 7.58 ppm and a five-bond coupling between singlets at 2.29 and 7.52 ppm. A long-range ^{1}H-^{1}H COSY experiment provided two additional couplings to 2.88 ppm; one from 1.02 ppm and one from 7.52 ppm. These results led to the partial structure **4**, and 1D difference nOe experiments confirmed the relationship between the A and C rings (enhancements between the aromatic protons at 7.52 and 7.58 ppm). These and other enhancements are summarized on structure **5**.

In addition to resonances for the five carbons of the side-chains, the ^{13}C-nmr spectrum of the new metabolite contained thirteen resonances between 106 and 165 ppm but lacked the quinone resonances of **3**. The cumulative data supported either of the lactone regioisomers, **6** or **7**. A pair of closely related compounds, **8** and **9**, have been reported from the chemical oxidation of piloquinone (16). Although comparison of the uv spectra supported the structural similarity of **8**, **9**, and the new metabolite, this failed to distinguish **6** from **7**.

An HMQC (17) experiment provided all the one-bond ^{1}H-^{13}C connectivities, while long-range ^{1}H-^{13}C correlations were detected with the HMBC experiment [**10**] (18). In addition to 2- and 3-bond correlations, three 4-bond couplings were also observed (19,20). These data allowed direct assignment of all carbons except the lactone carbonyl, which was then assigned by default to 164.7 ppm. Of critical importance were assignments for C-8a and C-9a, which were consistent with **6** rather than **7**. Thus, three-bond correlations to 139.0 ppm from H-5 (7.58 ppm) and H-7 (7.12 ppm) identified C-8a, and a three-bond correlation from H-4 (7.52 ppm) and a four-bond correlation from the aryl methyl (2.29 ppm) to 123.4 ppm identified C-9a. The chemical shifts for C-8a, the ring carbon substituted with oxygen rather than a carbonyl, and C-8 are also consistent with calculated values (21).

Crude extracts of the mutant organism grown in three media were analyzed by hplc with photodiode array detection. Each showed a peak with the identical retention time and uv/visible absorbance spectrum of **6**. Co-injection with authentic **6** in each case gave symmetrical enhancement of the peak and an unchanged spectrum. Extracts saved from earlier fermentations of wild type *S. murayamaensis* were next examined. Of six extracts from three fermentation media, four contained **3**, and two of the latter also contained **6**. The other two extracts containing **3** also contained an unknown metabolite that obscured the region of the chromatogram where **6** would elute.

Several dibenzo-α-pyrones have been reported. These include the plant products



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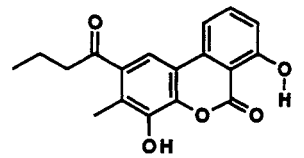

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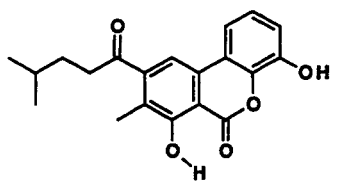

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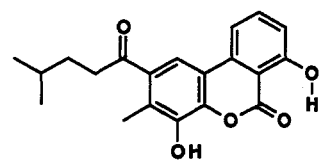

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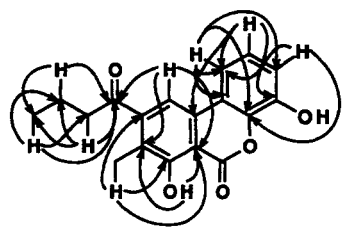

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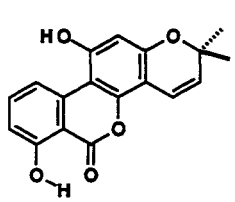
sarolactone, **11**, from *Hypericum japonicum* (22), sabilactone, **12**, from *Sabina vulgaris* (23), and autumnariol, **13**, and autumnariniol, **14**, from the bulbs of *Eucomis autumnalis* (24,25), as well as the mycotoxins alternariol, **15**, and alternariol monomethyl ether, **16**, from the fungus *Alternaria tenuis* (26), and *A. dauci* (27), and altenuisol, **17**, from *A. tenuis* (28) and *A. alternata* (29). The present study is the first report of this ring system from a prokaryote.

Studies of the biosynthesis of **15** and **16** have indicated their formation from simple folding and cyclization of a heptaketide intermediate (30–34). By contrast, the co-occurrence of the phenanthraquinone **3** suggests that **6** is derived from a decaketide with a subsequent decarboxylation. Oxidation of the central ring of a phenanthrene intermediate could lead to **3**, or oxidative cleavage and loss of a second carbon could yield **6**. This latter process would presumably require monooxygenase cleavage of the K region



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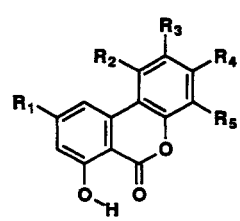

12 $R_1=R_3=OMe$, $R_2=R_4=H$, $R_5=Me$
13 $R_1=R_3=R_5=H$, $R_2=Me$, $R_4=OH$
14 $R_1=R_3=H$, $R_2=Me$, $R_4=OH$, $R_5=OMe$
15 $R_1=R_4=OH$, $R_2=Me$, $R_3=R_5=H$
16 $R_1=OMe$, $R_2=Me$, $R_3=R_5=H$, $R_4=OH$
17 $R_1=R_3=OH$, $R_2=R_5=H$, $R_4=OMe$

carbon-carbon double-bond, analogous to the proposed steps in the conversion of the benzanthraquinone dehydrorabelomycin to the kinamycins in the same organism (11,35,36).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The solvents CH_2Cl_2 and EtOAc were distilled, and Me_2CO was of chemically pure grade. Kieselgel 60 (230–400 mesh) Si gel was used for flash chromatography, and 10 μm Analtech Sorbent Si gel was used for medium-pressure chromatography. All nmr shifts are reported for CD_2Cl_2 although some experiments were run in $CDCl_3$ and these gave slightly different chemical shifts. The melting point determination utilized a Kofler hot-stage microscope.

FERMENTATION.—A soy-glucose seed medium (10) was incubated for 5.5 days at 27° and 280 rpm. Glycerol-ammonium sulfate medium consisting of 3% glycerol, 0.3% NH_4Cl, 0.2% $CaCO_3$, 0.1% $K_2HPO_4 \cdot 3H_2O$, 0.04% $MgSO_4 \cdot 7H_2O$, and 0.01% $FeSO_4 \cdot 7H_2O$ (400 ml/2-liter flask) in deionized H_2O, were inoculated with the seed culture (10% v/v), and were similarly grown for 5.5 days, 27°, 280 rpm. A farina medium consisting of 2% farina cereal and 2 ml/liter trace metal salts solution (10), was grown as above and harvested at 3.5 days.

ISOLATION OF MURAYALACTONE [**6**].—On two separate occasions, 6.8 liters of glycerol-ammonium sulfate fermentation, and on one occasion, 3.4 liters of farina fermentation, were each processed as follows. Filtration of the whole broth through Celite on cheese cloth gave a gray cell mass that was sequentially extracted with Me_2CO (200 ml) and EtOAc (200 ml). After drying with Na_2SO_4 and concentration, the crude residue (0.91 g) was washed with hexane to remove oily materials, dissolved in CH_2Cl_2, filtered and concentrated to afford 0.44 g of dried material. Chromatography on a flash column (2.5×15 cm, prepared with CH_2Cl_2, and eluted with 2% EtOAc in CH_2Cl_2) first yielded fractions containing primarily **3** (112 mg, total purified production of **3** was ca. 23 mg/liter) and then fractions containing **3**, **6**, and the two blue compounds (49.3 mg). The latter were combined and rechromatographed on a second column (1×11 cm) now yielding a mixture of the blue compounds and **6** (16.8 mg). This was applied to a third column (1×12 cm) eluted with 3% EtOAc in $CHCl_3$, and yielded the less polar blue compound (4.2 mg, impure), a mixture of the blue compounds and **6** (2.8 mg), and the more polar blue compound (4.6 mg). The fractions from each batch of mixed fractions were combined (8.2 mg) and purified by mplc (2×30 cm) eluted with 30% EtOAc in hexane at 12 ml/min, 80–100 psi. Uv detection at 300 nm showed a peak centered at 4.6 min that was collected (25 ml). Concentration gave 2.7 mg of **6**, which was recrystallized from $CHCl_3$ by addition of pentane to give pure **6** (1.8 mg) as a colorless powder: mp 158.8–161.5°; ir ν max (KBr pellet) 3546, 3443, 2963, 2930, 1699, 1676, 1195, 1127, 781 cm^{-1}; uv λ max (ε) 200 sh (23,600), 228 (22,700), 246 (16,000), 266 (10,400), 284 sh (7,820), 359 nm (5,390); ^1H nmr (CD_2Cl_2, 300 MHz) δ 11.62 (1H, s), 7.58 (1H, dd, J=8.1 and 1.2 Hz), 7.52 (1H, s), 7.27 (1H, dd, J=8.1 and 8.1 Hz), 7.12 (1H, dd, J=8.0 and 0.9 Hz), 5.99 (1H, s), 2.88 (2H, t, J=7.2 Hz), 2.29 (3H, s), 1.76 (2H, tq, J=7.3 and 7.3 Hz), 1.02 (3H, t, J=7.4 Hz); ^{13}C nmr (CD_2Cl_2, 75 MHz) δ 206.1 (C-10), 164.7 (C-9), 161.6 (C-1), 149.6 (C-3), 144.5 (C-8), 139.0 (C-8a), 133.1 (C-4b), 125.8 (C-6), 123.4 (C-9a), 119.0 (C-4a), 117.0 (C-7), 114.6 (C-5), 110.1 (C-4), 106.1 (C-2), 45.4 (C-11), 17.6 (C-12), 13.9 (C-13), 12.4 (C-14); eims *m/z* 312 (55), 284 (1), 269 (100), 241 (17), 128 (6); hreims calcd for $C_{18}H_{16}O_5$ 312.0998, found 312.0997.

DIODE ARRAY HPLC.—Fermentation samples (10 ml, including mycelia) were mixed with an equal volume of EtOAc, and immediately frozen until analyzed. After thawing, the organic layer was concentrated to near dryness, redissolved in CH_2Cl_2 (1.00 ml), and 10-μl aliquots were analyzed on a Waters NovaPak C_{18} radial compression column (0.8×10 cm) eluting at 1.50 ml/min over 20 min with a linear gradient from 5 to 95% MeCN in H_2O (each solvent contained 0.1% HOAc) with a Waters 600E hplc. A Waters 990+ photodiode array detector provided absorbance spectra from 200 to 650 nm with 2 nm resolution of all analyte peaks, and allowed comparisons with an in-house database of *S. murayamaensis* metabolites.

NMR EXPERIMENTS.—A Bruker AM 300 spectrometer was used for two-dimensional experiments, with the long-range COSY spectra being acquired with the following parameters: D_1 1.50 sec, D_0 3 μsec, NE 256, SI_2 1024 word, SI_1 512 word, SW_2 2702.7 Hz, SW_1 675.7 Hz; and 64 scans per transient. HMQC spectra were obtained using: D_1 2.0 sec, D_0 3 μsec, D_5 135 μsec, L2 36, NE 128, SI_2 2048 word, SI_1 512 word, NS 144, SW_2 2336.4 Hz, SW_1 6443.3 Hz; using the BIRDDP9 Bruker pulse program. HMBC spectra were obtained using: D_1 2.0 sec; D_0 3 μsec; NE 128; SI_2 2048 word; SI_1 512 word; SW_2 3448.3 Hz; SW_1 7987.2 Hz; the data were acquired in 128 experiments of 112 scans each using the INVDR2LP pulse program.

ACKNOWLEDGMENTS

This research was supported by U.S. Public Health Service Grant GM 31715 to S.J.G. The N.L. Tartar

Charitable Trust to Oregon State University provided partial support for C.R.M. Dr. Martha Cone is thanked for preparing and fully characterizing the mutant organism used for the initial isolation, and for developing the hplc analysis protocol. Mr. Roger Kohnert is thanked for help with the inverse nmr experiments, and Mrs. Lilo Barofsky for ms services. The Bruker AM 400 nmr spectrometer was purchased in part through grants from the National Science Foundation (CHE-8216190) and from the M.J. Murdock Charitable Trust to Oregon State University, and the Bruker AC 300 spectrometer was purchased in part through grants from the Public Health Service Division of Research Resources (RR04039-01) and the National Science Foundation (CHE-8712343) to Oregon State University.

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Received 26 October 1993