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MURAYAANTHRAQUINONE, A HYBRID BENZ[*a*]ANTHRAQUINONE FROM A UV MUTANT OF *STREPTOMYCES MURAYAMAENSIS*

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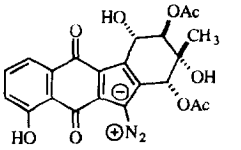
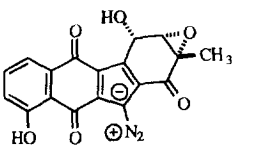
Abstract: An X-ray crystallographic analysis revealed that a new metabolite of *Streptomyces murayamaensis*, murayaanthraquinone, contains a 5-azo-16-oxo-dibenzo[*b*,*k*]chrysene ring system. This hexacyclic structure has been shown to be a hybrid derived from a benz[*a*]anthraquinone and a 3-amino-4-hydroxybenzoic acid.

In our continuing studies of kinamycin biosynthesis (e.g. kinamycin D, **1**[1]), in *Streptomyces murayamaensis*,[2,3] we have generated a number of UV mutants that accumulate a variety of colored metabolites that are either trace components or are undetectable in extracts of the wild-type organism. Diode array-detected HPLC of EtOAc extracts from the wild-type strain revealed a new metabolite with a UV chromophore (260, 304, 420 nm) similar to ketoanhydrokinamycin, **2**.[4] Extracts of blocked strain MC2[5] also contained the new metabolite, but in greater amount. We now report that this new orange-yellow metabolite, **3**, is a hybrid composed of a benz[*a*]anthraquinone and 3-amino-4-hydroxybenzamide, **4**.[6]



1



2

Two liters of a 7-day fermentation of strain MC2 in 7% farina/0.2% trace metals medium were worked up to the EtOAc extract.[5] Concentration afforded a solid residue (750 mg), which was chromatographed in thirds on phosphate-buffered (pH 7.0) flash grade silica gel (3 x 16 cm, 3% MeOH/CH_2Cl_2). Elution with the same solvent yielded a fraction (~12 mg) enriched in **3**. This was purified on a column of Sephadex LH-20 (1.8 x 27 cm, 50% MeOH/CH_2Cl_2) and afforded a bright orange-yellow fraction containing ~2 mg of pure **3**.

High resolution positive ion FABMS furnished the formula $C_{26}H_{17}N_2O_7$ ($M + H^+$, *m/z* 469.1030, calcd 469.1024). Absorptions at 1709, 1669 and 1629 cm^{-1} in the IR spectrum indicated a ketone and a quinone (one carbonyl hydrogen-bonded). The ^1H- and $^1H\{^1H\}$COSY NMR spectra yielded two partial structures, **5** and **6**, in addition to a quaternary methyl singlet (δ1.30), a diastereotopic methylene (δ3.15 and 3.90, J = 13 Hz), an aromatic singlet (δ8.10), and a broad exchangeable signal centered at δ11.9. During an attempt to obtain a ^{13}C NMR spectrum in DMSO-d_6, the sample crystallized. It was re-dissolved by heating, and then slowly cooled over a 48-hour period to furnish larger crystals. X-ray diffraction analysis of a single triclinic needle was performed to a maximum $\sin\theta/\lambda = 0.5436$ $Å^{-1}$. Using 1336 observed reflections, the space group P-1 (#2), and the direct methods program SHELXS (TEXAN crystallographic software package)[7] the positions of a $C_{26}N_2O_7$ molecule, along with a molecule of DMSO were determined. Hydrogen atoms were

placed in calculated positions, and a DIFABS[8] absorption correction (transmission factors 0.81 to 1.28) applied. Anisotropic refinement of all non-hydrogen atoms gave R = 0.041 and R_w = 0.044. Successful refinement in the centrosymmetric space group P-1 demonstrates the crystal contains a racemic mixture of **3**.

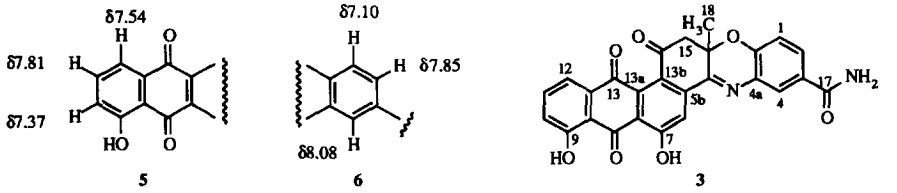
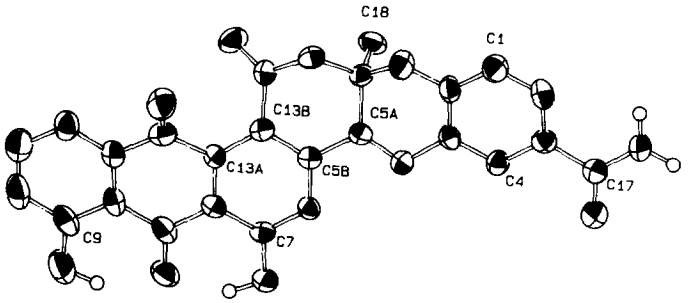


Figure 1. ORTEP drawing from the single-crystal X-ray structure determination of **3**. Hydrogens on carbon have been omitted for clarity.



A ^{13}C-enriched sample **3a**, was later prepared biosynthetically by feeding [U-$^{13}C_6$]-D-glucose,[9-11] **7**, (98+% enriched, 1.0 g in 25 mL H_2O) in thirds at 24, 48, and 72 hours after inoculation, distributed amongst 5 200-mL fermentations. Work-up afforded 1.6 mg of **3a**. ^{13}C NMR analysis revealed ~2% enrichments at each of the benzanthraquinone carbons, but extremely low enrichments for those of the aminohydroxybenzamide moiety. Six pairs of ^{13}C-^{13}C coupled resonances were readily matched from the $^1J_{CC}$ values and their assignments then made from the chemical shifts. These were confirmed and all but four others assigned (Table 1) from HMQC and HMBC NMR experiments (Scheme 1).

Scheme 1

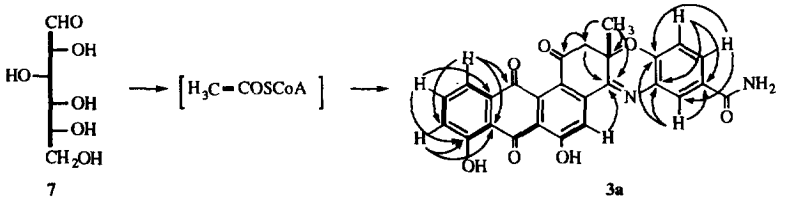


Table 1. ^{1}H- and ^{13}C NMR spectroscopic data for **3** and **3a**.

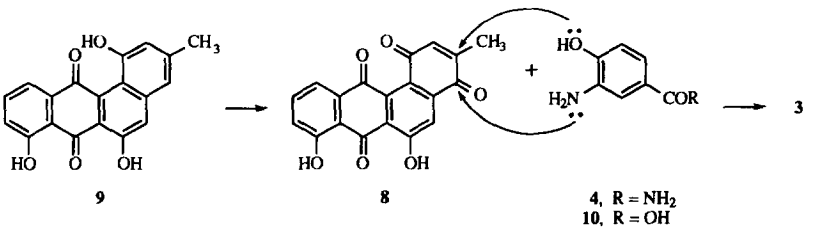
Pos	^{1}H NMR	^{13}C NMR	$^{1}J_{CC}$	Pos	^{1}H NMR	^{13}C NMR	$^{1}J_{CC}$
1	7.10, 1H, d, *J*=8.4 Hz	116.3 CH	*	12	7.54, 1H, d, *J*=7.2 Hz	118.7 CH	61.4
2	7.85, 1H, dd, *J*=8.4, 2.0 Hz	131.4 CH	*	12a		135.0 C	61.2
3		129.0 C	*	13		182.6 C	53.2
4	8.08, 1H, d, *J*=2.0 Hz	127.0 CH	*	13a		** C	
4a		132.0 C	*	13b		** C	
5a		157.1 C	55.7	14		192.6 C	52.7
5b		** C		15	3.15, 3.90, 2H, d, *J*=13 Hz	51.5 CH_2	
6	8.1, 1H, s	117.5 CH	68.6	15a		74.3 C	37.5
7		162.5 C	70.2	16a		148.0 C	*
7a		121.1 C	55.5	17		166.7 C	*
8		190.0 C	55.7	18	1.3, 3H, s	20.7 CH_3	37.5
8a		115.9 C	65.0	17-NH_2	8.00, 2H, br, exchangeable		
9		160.7 C	64.6	7-OH	11-12, br, exchangeable		
10	7.37, 1H, d, *J*=8.6 Hz	124.0 CH	57.0	9-OH	11-12, br, exchangeable		
11	7.81, 1H, dd, *J*=8.2, 8.2 Hz	137.6 CH	56.9				

* The enrichment was very low and J_{CC} could not be measured.

** ^{13}C chemical shift could not be identified by the HMBC experiment.

The formation of compound **3** can be rationalized by addition of an *o*-aminophenol to a *p*-quinone **8** (Scheme 2). The latter is presumably derived from dehydrorabelomycin, **9**, previously identified as an *S. murayamaensis* metabolite.[12] Indeed, **8** (PD 116744) and **9** have been isolated from *Streptomyces* sp. WP 3668.[13,14] The coupling partner (either the benzoic acid **10** or the benzamide **4**[6]) represents a new naturally-occurring regioisomer of aminohydroxybenzoic acid. The 2,3-,[15] 2,6-,[16] and 3,5-isomers[17,18] have all been shown to be derived from the shikimic acid pathway. The origin of **4** will be reported in due course.

Scheme 2



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