Natural Products

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Benzopyrenomycin, a Cytotoxic Bacterial Polyketide Metabolite with a Benzo[*a*]pyrene-Type Carbocyclic Ring System**

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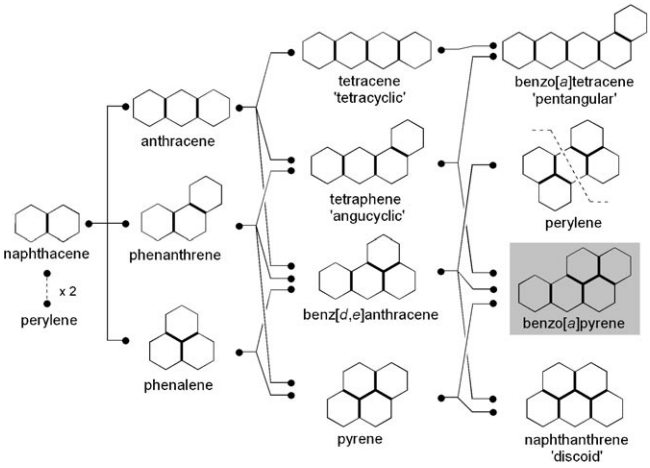
Polyketides form a major class of secondary metabolites of bacteria, fungi, and plants with broad structural diversity as a result of dense functionalization and spatial properties.[1] Many polyphenolic derivatives have found considerable interest as pharmaceuticals, biological tools, and dyes. A hallmark of aromatic polyketides is their common biogenesis from simple acyl and malonyl units through the action of different types of iterative polyketide synthases.[2–5] The orchestrated assembly and processing of the biosynthetic intermediates gives rise to polyphenolic compounds that differ widely in the number of carbocycles they contain, their topology, and the substitution of the rings.[4,6]

From a structural point of view, it is remarkable that only a limited number of carbocyclic (aromatic) polyketide frameworks occur naturally. Linear and monoangular polyphenolic ring systems are found almost exclusively (Scheme 1); *peri*-fused carbocycles, in which rings are fused through more than one face, are rarities. A possible rationale for this observation is the preferred U-shaped folding of a nascent poly-β-keto chain. S-shaped cyclization patterns, as in the biosynthesis of the pentacyclic "discoid" *Streptomyces* naphthanthrene metabolites resistomycin and resistoflavin, are clear exceptions.[7–11] Some phenalenes and benz[*d*,*e*]anthracenes from plants and fungi are probably also formed by an alternating polyketide folding pattern,[12,13] whereas the biosynthesis of phenylphenalenones involves intramolecular cycloaddition with a cinnamoyl-derived moiety.[14] Homologous tetracyclic pyrenes thought to be derived from phenanthrenes have been isolated from *Uvaria* and *Juncus* spp.[15,16] An important example of a perylene is altertoxin,[17] which is formed by naphthol dimerization in analogy with the hypericin[18] biosynthetic pathway. To date, however, a significant gap has remained between the pentacyclic pentangular[19,20] and



Scheme 1. Structure-based phylogeny of fundamental di- to pentacyclic ring systems found in natural aromatic polyketides; no natural product with a benzo[*a*]pyrene-type skeleton was known previously. Shared faces are highlighted in bold.

discoid[8] polyketide structures: the benzo[*a*]pyrene scaffold. Benzo[*a*]pyrenes are only known as notorious products of the pyrolysis of organic matter which are transformed into carcinogens upon epoxidation.[21–23] No example of the biogenesis of a related carbocyclic system has been described. Herein, we report the first discovery of a natural product with a benzo[*a*]pyrene framework.

During the course of metabolic profiling of the ketalin producer *Streptomyces lavendulae* (strain Tü 1668),[24] we noted the formation of minute amounts of a novel aromatic compound with UV absorptions at $\lambda_{max} = 417$, 251, and 217 nm when the strain was cultured on a large scale (2×50 L). High-resolution EIMS provided sufficient evidence that the compound had not been described previously. The crude extract was subjected to purification first with amberlite XAD16, then by reversed-phase flash chromatography (RP18) and subsequent open-column chromatography on Sephadex LH-20 and silica gel. The new compound **1** (7 mg in total) was isolated as a yellow solid. A series of biological assays with **1** revealed inhibitory activity against various tumor-cell lines. Compound **1** showed strong antiproliferative activity against the cell lines L-929 and K562 with GI_{50} values of 3.2 $\mu g\,mL^{-1}$ (8.2 μM) and 4.2 $\mu g\,mL^{-1}$ (10.8 μM), respectively, and moderate cytotoxicity against HeLa cells with a CC_{50} value of 26.4 $\mu g\,mL^{-1}$ (68.0 μM).

The structure of the cytotoxic metabolite was resolved fully by MS and NMR spectroscopy. High-resolution EIMS

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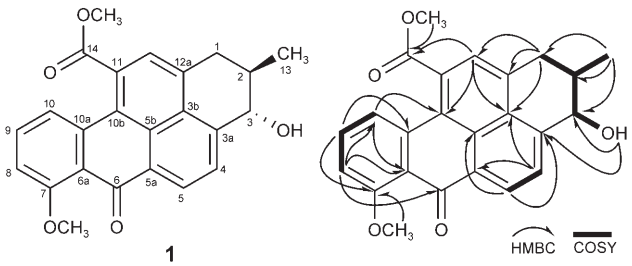
revealed the molecular formula of **1** to be $C_{24}H_{20}O_5$ (*m/z*: calcd: 388.1311; found: 388.1300). The ^{13}C NMR and DEPT spectra of **1** contained signals for all 24 carbon atoms and revealed the presence of two methoxy groups, a methyl group, a methylene group, a methine carbon atom, an oxymethine carbon atom, six aromatic methine carbon atoms, ten aromatic quaternary carbon atoms, the carbonyl group of an ester ($\delta_C = 170.9$ ppm), and a conjugated keto group ($\delta_C =$ 181.5 ppm; Table 1). The ^1H NMR spectrum confirmed these findings with signals for two methoxy groups ($\delta_H = 3.86$ and 3.92 ppm) and one secondary methyl group ($\delta_H = 1.17$ ppm (d, $J = 6.5$ Hz)), as well as six hydrogen atoms attached to aromatic rings (four signals appear as doublets, one as a doublet of doublets, and one as a singlet). Furthermore, the expected methylene hydrogen atoms ($\delta_H = 2.93$ and 3.25 ppm), oxymethine hydrogen atom ($\delta_H = 4.58$ ppm), and methine hydrogen atom ($\delta_H = 2.02$ ppm) were identified together with the hydrogen atom of a hydroxy group ($\delta_H =$ 5.79 ppm).

Table 1: NMR spectral data for compound **1** in [D_6]DMSO.[a]

Position	δ_C [ppm]	δ_H [ppm]
1	35.5 (t)	3.25 (dd, obscured, 1 H, H^{β})[b]
		2.93 (dd, $J = 16.0$, 10.5 Hz, 1 H, H^{α})
2	36.8 (d)	2.02 (m, 1 H) β
3	73.1 (d)	4.58 (dd, $J = 8.1$, 6.9 Hz, 1 H)
3a	146.6 (s)	–
3b	127.9 (s)	–
4	124.6 (d)	8.01 (d, $J = 7.6$ Hz, 1 H)
5	128.8 (d)[c]	8.43 (d, $J = 7.6$ Hz, 1 H)
5a	128.7 (s)[c]	–
5b	126.6 (s)	–
6	181.5 (s)	–
6a	119.8 (s)	–
7	160.5 (s)	–
8	112.6 (d)	7.24 (d, $J = 8.2$ Hz, 1 H)
9	133.8 (d)	7.70 (dd, $J = 8.2$, 8.0 Hz, 1 H)
10	119.5 (d)	7.28 (d, $J = 8.0$ Hz, 1 H)
10a	137.1 (s)	–
10b	123.6 (s)	–
11	130.4 (s)	–
12	124.4 (d)	7.53 (s, 1 H)
12a	138.2 (s)	–
13	17.9 (d)	1.17 (d, $J = 6.5$ Hz, 3 H)
14	170.9 (s)	–
7-OCH_3	56.1 (q)	3.92 (s, 3 H)
14-OCH_3	52.9 (q)	3.86 (s, 3 H)
3-OH	–	5.79 (d, $J = 6.9$ Hz, 1 H)[d]

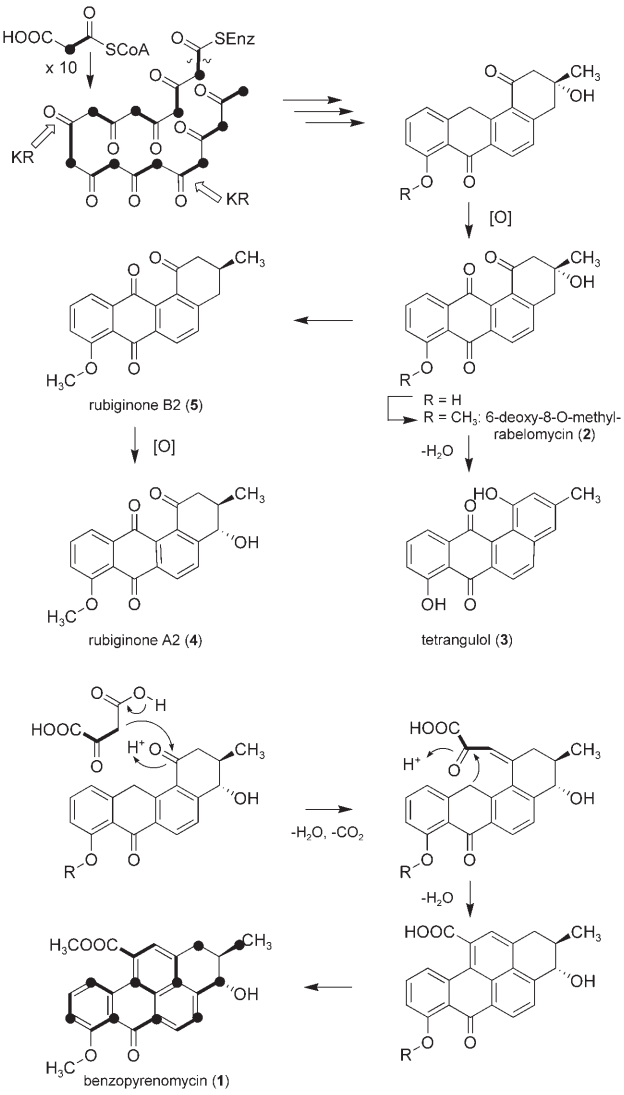
[a] The ^1H NMR spectrum was recorded at 300 MHz, the ^{13}C NMR spectrum at 75 MHz. [b] The signal is obscured by a signal due to water. In $CDCl_3$, $\delta = 3.32$ (dd, $J = 16.5$, 4.2 Hz). [c] The two signals are interchangeable in terms of their assignment. [d] The 3-OH hydrogen atom is exchangeable with CD_3OD in $CDCl_3$.

A double INEPT spectrum enabled the assignment of all hydrogen atoms to the directly bonded carbon atoms (INEPT = insensitive nuclei enhanced by polarization transfer). Three segments of the structure, the moieties OH/3-H/2-H(2-Me)/1-H_2, 4-H/5-H, and 8-H/9-H/10-H, were established through correlations detected in a $^1H,^1H$ COSY experiment (Scheme 2). On the basis of the spectral evidence gathered by this stage, -CH(OH)CH(CH_3)CH_2-, a 1,2,3-trisubstituted phenyl group, a 1,2,3,4-tetrasubsituted phenyl group, and a pentasubstituted phenyl group were identified as partial structures. The positions at which these partial structures were fused and the substituents attached were determined by $^1H,^{13}C$ long-range correlations revealed in the HMBC spectrum (Scheme 2). A few two-bond correlations (OH/C3, 2-Me/C2, and 1-H_2/C12a) and many three-bond correlations were observed. These correlations were the key to determining the connectivity of the majority of the substructures. The linkages through C6a/C6/C5a were assigned by a four-bond HMBC correlation between 8-H and C6 and a $^3J_{C,H}$ coupling between 5-H and C6. C11 was the only position for which no long-range coupling was observed; however, a strong $^3J_{C,H}$ coupling between the attached ester carbonyl group and 12-H supported its location. Additional proof was provided by an NOE correlation (NOESY) between the ester methoxy group and 10-H. Likewise, the methoxy group at C7 showed an NOE correlation with 8-H. An NOE between 3-H and 13-H_3, in combination with the large vicinal coupling constant between 3-H and 2-H ($J = 8.1$ Hz) indicated a *trans* configuration at C3/C2 with the two hydrogen atoms in a quasi-bisaxial conformation. This assignment was supported further by a strong NOE correlation between the signal for 1-H_{ax} at $\delta_H = 2.93$ ppm (dd, $J = 16.0$, 10.5 Hz) and that for 3-H at $\delta_H =$ 4.58 ppm (dd, $J = 6.9$, 8.1 Hz), which indicated that both hydrogen atoms are in an axial position. All physicochemical data are in full agreement with the proposed structure of **1** as methyl 1,2,3,6-tetrahydro-3-hydroxy-7-methoxy-2-methyl-6-oxobenzo[*pqr*]tetraphene-11-carboxylate (Scheme 2). This novel compound, named benzopyrenomycin, is the first known natural product with a benzo[*a*]pyrene-derived scaffold.



Scheme 2. Chemical structure of compound **1** with HMBC and $^1H,^1H$ COSY correlations.

The occurrence of the benzopyrene-type carbocyclic system is particularly startling from a biosynthetic point of view. It is unlikely that a novel polyketide folding pattern gives rise to this particular ring system.[4,25] As isotope-labeling experiments were hampered by the minute yields of **1** (93 $\mu g\,L^{-1}$) and by the fact that **1** was only detected following fermentation on a large scale (2×50 L), we sought to obtain further clues with respect to the cometabolites of *S. lavendulae*. Four other aromatic polyketides, **2**–**5** (Scheme 3), were isolated from the *S. lavendulae* fermentation broth and characterized fully. The comparison of HRMS and NMR



Scheme 3. Structures of angucyclic cometabolites of *S. lavendulae* and model for the formation of **1** with a putative acetate pattern. KR = keto reductase.

spectra with literature data indicated that these four compounds were identical to the known angucyclic polyketides 6-deoxy-8-methylrabelomycin[26] (**2**, 0.2 mg L^{-1}), tetrangulol[27] (**3**, 0.05 mg L^{-1}), rubiginone A2[28] (**4**, 0.04 mg L^{-1}), and rubiginone B2[28] (**5**, 0.19 mg L^{-1}). The structural relationship of **2**–**5** to **1** is remarkable; in particular, **4** displays the same *trans*-3-hydroxy-2-methyl substitution pattern.[29] Thus, the most plausible model for the biogenesis of **1** is the condensation of an angucyclic anthrone intermediate with a C_3 or C_4 building block. A likely scenario is the reaction of an anthrone precursor with oxaloacetate (C_4) through two aldol condensations (Scheme 3).

An analogous reaction with the less reactive pyruvate (C_3) is also conceivable. However, the alternative reaction of the quinone **4** with succinate is unlikely, as these compounds are significantly less reactive than the anthrone and oxaloacetate. Therefore, the pathway leading to the pentacycle **1** seems to branch off early in the biosynthetic scheme, before quinine formation. Although the route that leads to the angucyclinone congeners appears to be the dominant pathway, one may speculate that particular conditions facilitate the formation of **1**. First, an excess of glucose or glycerol leads to increased titers of pyruvate and/or oxaloacetate, the product of the tricarboxylic acid (TCA) cycle. Second, oxygen limitation during fermentation would affect electron transport as well as the 12-oxygenase that introduces the quinone oxygen atom. In this case, the reactive anthrone intermediate would branch to the benzopyrene product, possibly without the need for enzyme catalysis. Angucyclines have been reported to be versatile precursors in the biosynthesis of kinamycins,[30,31] jadomycins,[32,33] and gilvocarcins.[34,35] Angucycline-modifying reactions were described previously in the context of the formation of other frameworks, specifically urdamycins C, D, and H.[36–39] However, they have not yet been implicated in the formation of extended and *peri*-fused carbocycles. The heteroaromatic framework of chartreusin[40] has a benzo[*a*]-pyrene-like ring topology, which results from an oxidative rearrangement of an anthracyclic precursor.[41]

In conclusion, by chemical metabolite profiling we have identified a trace metabolite from a large-scale fermentation of *S. lavendulae* as a novel aromatic polyketide and solved its structure by 2D NMR spectroscopy. The new compound, benzopyrenomycin, is the first natural product with a carbocyclic benzo[*a*]pyrene ring system to be discovered. One of four angucyclic congeners identified in the broth of *S. lavendulae*, rubiginone A2, has an identical exocyclic substitution pattern. This finding provides strong evidence for a model according to which benzopyrenomycin is biosynthesized by the condensation of an angucyclic anthrone precursor with a C_3/C_4 building block, such as oxaloacetate. A biological evaluation of **1** revealed significant activity against various tumor-cell lines. Both the novel structure and the cytotoxic activity of **1** encourage future investigations of cometabolites that occur in minute amounts in large-scale fermentations.

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