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# Isolation and structure elucidation of coleophomones A and B, novel inhibitors of bacterial cell wall transglycosylase

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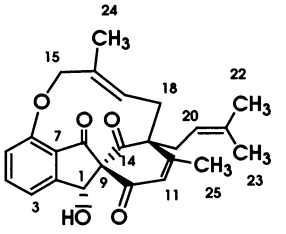
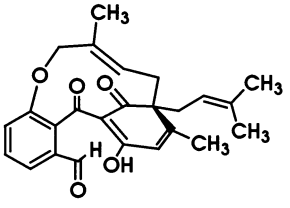
**Abstract**

The discovery, structure and absolute stereochemistry of coleophomones A and B are described, two structurally novel natural products that inhibit bacterial transglycosylase activity. Coleophomone A represents a new ring system, containing a highly condensed structure which undergoes a reversible retroaldol reaction to form coleophomone B. 

We report here the discovery of coleophomone A (**1**) and B (**2**), two structurally novel compounds that inhibit transglycosylase, an enzyme in bacterial cell wall synthesis that polymerizes disaccharide–pentapeptide units from lipid II to form uncrosslinked peptidoglycan.[1] Stereocenters are accurately depicted below. Coleophomone A contains an unusual spiro[cyclohex-3-ene-1,2′[2*H*]indene] moiety as part of a larger, rigid tetracyclic unit. The molecule appears to be derived from a mixed polyketide/isoprenoid biosynthesis. Interestingly, coleophomones A and B exist in equilibrium with each other under physiological conditions. For simplicity in the following discussion the numbering scheme indicated below has been adopted.




coleophomone A (**1**)




coleophomone B (**2**)

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Coleophomone A was obtained from bioassay-directed isolation of an acetone extract of a liquid fermentation of *Coleophoma* sp. (MF6338), a fungus isolated from unidentified plant litter collected in Sierra Villuercas (Cáceres, Spain). The extract was saturated with sodium chloride and extracted with ethyl acetate. The residue from the ethyl acetate layer was reconstituted in methanol and cooled to −80°C, out of which coleophomone A crystallized as a yellow-tan solid. Recrystallization from methanol afforded pure coleophomone A. The fermentation produced 330 mg/L of coleophomone A. The fermentation produced an equal titer of coleophomone B; however, it was more convenient to obtain coleophomone B directly from coleophomone A as described in the following example.[†] A solution of 12 mg of coleophomone A in 2 mL of methanol was treated at room temperature with 0.02 mL of triethylamine for 20 minutes. The reaction was quenched with potassium phosphate buffer (pH 2.5); extraction with ethyl acetate and concentration afforded a quantitative yield of coleophomone B.

**Structure of coleophomone A**. The molecular formula for coleophomone A was determined to be $C_{25}H_{26}O_5$ by EI-HRMS ($M^+$: 406.1802 observed, 406.1780 calcd for $C_{25}H_{26}O_5$), indicating 13 units of unsaturation. The $^{13}C$ NMR spectrum of **1** in $CDCl_3$ confirmed the carbon count and showed the presence of three carbonyl carbons at 205.26, 191.09 and 187.29 ppm, accounting for three of the five oxygens of the molecular formula. One of the two remaining oxygens was assigned to a hydroxyl group based on an IR stretching band for **1** at 3537 $cm^{-1}$.

Interpretation of NMR data from $^1H$, $^{13}C$, gCOSY, and HMQC experiments of **1** led to proton and carbon assignments of five partial structures, outlined below in bold face (see Fig. 1). The $^1H$ and $^{13}C$ chemical shifts and the $^1H$–$^1H$ coupling constants suggested that the three contiguous low field protons were part of an aromatic ring. The assignment of the remaining carbons of the aromatic ring was straightforward based on observed 3-bond proton/carbon HMBC correlations. HMBC correlations between fragments C and D and between C and E indicated that D an E were connected through a common carbon at 56.23 ppm to C. Additional correlations arising from C, D and E suggested an extension of fragment C as depicted in Fig. 2A.

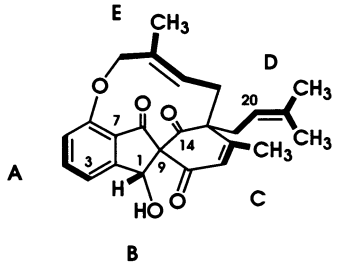


Figure 1. Partial structures of coleophomone A

Further elaboration of the partial structure of Fig. 2A to include fragments A and B was possible based on the 2- and 3-bond HMBC correlations shown in Fig. 2B. An observed C1–H3 correlation establishes the point of attachment of fragment B to the aromatic ring while H1 is correlated to the carbonyl carbons at 191.09 and 205.26 ppm, necessitating attachment of C1

[†] Compounds **1** and **2** exist in equilibrium in $CH_3CN$/water mixtures. The equilibrium strongly favors **2** at pH $\geq$ 7. The half-life of **1** at pH 7.5 is 5 min. Interconversion does not occur at pH<3. The aldol formation of **1** from **2** appears to proceed in a stereospecific manner since no evidence was seen of the other diastereomeric alcohol of **1**.

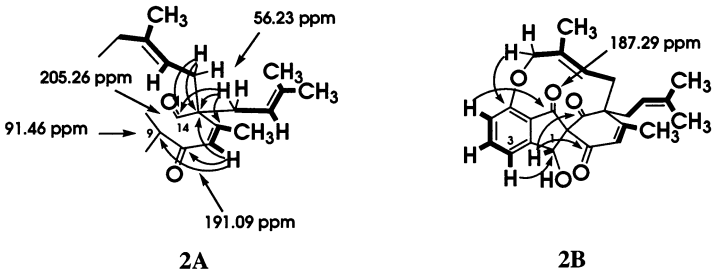


Figure 2. Selected HMBC correlations for coleophomone A

and C14 to the carbon at 91.46 ppm.‡ HMBC correlations between the methylene protons at C15 (4.942 and 4.740 ppm) and carbon 6 (153.81 ppm) indicate that the two centers are connected through an ether linkage. The remaining free valencies at C7 and at C9 are satisfied by inserting the final unplaced carbonyl (187.29 ppm) between C7 and C9, thus completing the structure of **1**.§ In support of this, a 4-bond HMBC correlation (W-coupling) is observed between H5 and C8=O (187.29 ppm). The geometry of the C17/C18 olefinic bond was established as *E* based on the NOESY correlations H18a–H24 and H17–H15a.

**Structure of coleophomone B.** High resolution EI-MS indicated that **2** was isomeric to coleophomone A (**1**). The $^1$H NMR of **2** differed most notably from that of **1** by the absence of the proton at C1 and by the presence of an apparent aldehyde singlet at 9.96 ppm. This suggested that **2** was formed by a base catalyzed retroaldol reaction of **1** with scission of the C1–C9 bond.

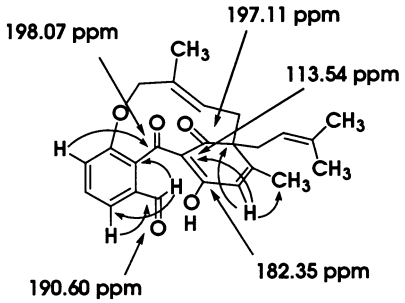


Figure 3. Selected HMBC correlations for coleophomone B

‡ Precedence for the downfield position of the non-oxygenated $sp^3$ C9 is found in the reported $^{13}$C assignment of an oxidation product of the plant natural product hypericum, in which a similarly substituted carbon appears at 83.2 ppm.[2] The further downfield position of C9 in **1** may be due to the additional strain introduced by the five-membered ring.

§ **1**: $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$ (C#): 69.33 (C1), 152.70 (C2), 115.91 (C3), 136.73 (C4 or C21), 120.42 (C5), 153.81 (C6), 121.77 (C7), 187.29 (C8), 91.46 (C9), 191.09 (C10), 131.91 (C11), 158.12 (C12), 56.23 (C13), 205.26 (C14), 76.77 (C15), 131.91 (C16), 132.84 (C17), 36.27 (C18), 35.84 (C19), 116.98 (C20), 136.67 (C21 or C4), 25.97 (C22), 18.13 (C23), 11.59 (C24), 19.73 (C25). $^1$H NMR (500 MHz, $CDCl_3$), $\delta$ (H#, *J*): 5.86 br s (H1), 7.31 ddd (H3, 8, 1, 1), 7.55 dd (H4, 8.5, 6.5), 6.95 ddd (H5, 8.5, 1, 1), 6.65 q (H11, 1), 4.94 d (H15a, 13), 4.74 (H15b, 13, 3, 3), 5.17 dddd (H17, 12, 4, 1, 1), 2.57 dd (H18a, 13, 13), 2.42 br d (H18b, 12), 2.22 br dd (H19a, 14, 6), 2.84 dd (H19b, 14, 8), 4.62 br dd (H20, 9, 7), 1.58 d (H22, 1), 1.52 br s (H23), 1.04 ddd (H24, 1, 1, 1), 2.12 d (H25, 1.5).

Further NMR studies (COSY, HMQC, and HMBC) confirmed this and established that the C10 carbonyl was present as the enol form (see Fig. 3).¶

**Absolute stereochemistry of coleophomone A**. The absolute stereochemistry was determined by single crystal X-ray analysis on the 4-bromophenylcarbamate derivative (**3**) of **1**.‖ The results, shown as a perspective drawing of **3** in Fig. 4, confirmed the overall structure of coleophomone A and established the absolute stereochemistry of centers C1, C9 and C13 to be *R*, *R* and *S*, respectively.††

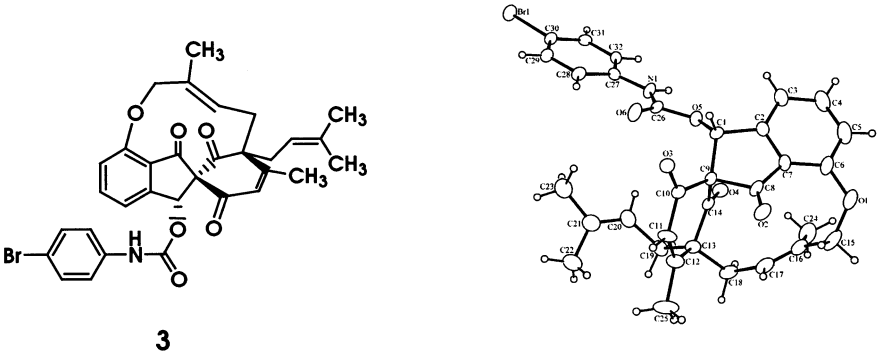


Figure 4. Perspective view (ORTEP) of **3**

**Biological properties**. Coleophomone A and B were tested for inhibition of the four final steps of peptidoglycan synthesis in ether-treated *E. coli* W7.[5] Only the transglycosylase step was inhibited ($IC_{50}=62$ μM). However, the compounds are not specific inhibitors of peptidoglycan synthesis since they simultaneously inhibited the syntheses of peptidoglycan, RNA, DNA and protein in *Bacillus megaterium* MB410.[6] Coleophomones A and B show weak antibacterial activity against aerobic bacteria.

¶ **2**: $^{13}$C NMR (125 MHz, $CDCl_3$), $\delta$ (C#): 190.60 (C1), 136.12 (C2), 126.12 (C3), 129.66 (C4), 125.23 (C5), 154.04 (C6), 133.64 (C7), 198.07 (C8), 113.54 (C9), 182.35 (C10), 122.68 (C11), 164.95 (C12), 58.78 (C13), 197.11 (C14), 77.01 (C15), 135.17 (C16), 129.35 (C17), 38.66 (C18), 34.56 (C19), 118.69 (C20), 134.57 (C21), 25.93 (C22), 18.32 (C23), 20.06 (C24), 20.44 (C25). $^1$H NMR (500 MHz, $CDCl_3$), $\delta$ (H#, *J*): 9.96 s (H1), 7.59 dd (H3, 7.5, 1.5), 7.48 dd (H4, 8.5, 7), 7.44 dd (H5, 8, 1.5), 6.37 ddd (H11, 1, 1, 1), 4.57 d (H15a, 11.5), 4.41 br d (H15b, 12), 5.44 br m (H17), 2.29 ddd (H18a, 13, 4, 1), 2.38 dd (H18b, 13, 12), 2.26 dd (H19a, 15, 8), 2.92 dd (H19b, 15, 5), 1.59 q (H22, 1), 1.54 br s (H23), 1.32 dd (1, 1), 2.08 d (H25, 1).

‖ 0.14 mmol of 4-bromophenylisocyanate was added to 0.07 mmol of **1** and 0.07 mmol of CuCl in 0.5 ml DMF; 18 h rt.[3] After workup, prep TLC afforded **3** in 59% yield. Recrystallized from MeOH.

†† Crystal data and experimental conditions: formula $C_{32}H_{30}BrNO_6$, $M_r=604.508$, monoclinic, *P*2l, $a=7.198(3)$, $b=22.945(3)$, $c=17.360(2)$ Å, $\beta=94.38(2)°$, $V=2859(2)$ Å$^3$, $Z=4$, $D_x=1.404$ g cm$^{-3}$, $\mu$(CuKα)$=2.32$ mm$^{-1}$, $F(000)=1248$, $T=294$ K. The final agreement statistics are $R=0.056$ (based on 4323 reflections with $I\geq 2\sigma(I)$), $wR=0.097$, $S=1.01$ with $(\Delta/\sigma)_{max}=0.01$. The Flack parameter for absolute structure determination is 0.01(2).[4] See supplementary data for full details and atomic coordinates.

## References


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