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Bassianolone: an antimicrobial precursor of cephalosporolides E and F from the entomoparasitic fungus *Beauveria bassiana*

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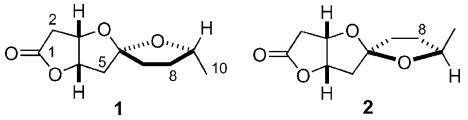
We have established the chemical structure of (+)-bassianolone (3), the antimicrobial compound precursor of cephalosporolides E and F, and that of the furan metabolite 4 from the entomopathogenic fungus *Beauveria bassiana*.

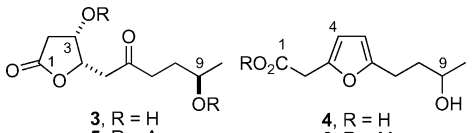
In 1985, Hanson and co-workers isolated two rare metabolites, cephalosporolides E (**1**) and F (**2**), from an industrial fermentation of the fungus *Cephalosporium aphidicola* grown under sulfur limiting conditions.[1] The authors established the chemical structure of compounds **1** and **2** with the aid of X-ray analysis and suggested that these products might arise from another fungal metabolite, cephalosporolide C, *via* a process involving hydrolysis, relactonization and acetal formation.[1] Nevertheless, they could not mimic this process in the laboratory. Intriguingly, cephalosporolides E and F were (to date) never again detected in nature, despite intense research devoted to fungal secondary metabolism in recent years.[2–5]



1 **2**

Within our programme to investigate the biotechnological use of fungi,[6–8] we recently became interested in the metabolites produced under stressful conditions by *Beauveria bassiana*, an entomoparasitic deuteromycete[9] which has found wide application as a whole-cell biocatalyst.[10,11] Among the products excreted by this fungus to the broth culture of a low-nitrogen medium,[6] we unexpectedly[12] found cephalosporolides **1**[13] and **2**,[14] together with a third metabolite of a previously unknown chemical structure, which we called (+)-bassianolone (**3**),[15] and a new furan compound **4**, apparently derived from the same biogenetic pathway as **3**.

The HRMS of **3** indicated a $C_{10}H_{16}O_5$ molecular formula corresponding to three double-bond equivalents, whereas its ^{13}C NMR spectrum showed only two signals of sp^2 carbons, assignable to γ-lactone (169.6 ppm) and ketone (214.4 ppm) groups, thus revealing the monocyclic nature of this fungal metabolite. To find out more about its structure, **3** was treated with acetic anhydride and pyridine, thus obtaining acetyl derivative **5**.[16] The ^1H NMR spectrum of **5** showed two acetate-group signals (2.02 and 2.04 ppm) together with a methyl doublet (10–H_3) with a chemical shift (1.28 ppm) indicating that the CH_3 group was attached to an oxygenated carbon. Moreover, three proton signals at 5.05 (9-H), 5.45 (3-H) and 5.16 ppm (4-H) revealed the positions of the two acetate groups and the closure of the γ-lactone ring respectively. The COSY spectrum showed three bond correlations between 2-H and 3-H, 3-H and 4-H, and 4-H and 5-H, which were confirmed by analysis of the coupling-constant values in the ^1H NMR spectrum. Furthermore, correlations between 7-H and 8-H, 8-H and 9-H, and 9-H and 10-H were also observed in the COSY spectrum. Moreover, the HMBC spectrum showed long-range heteronuclear correlations between 1-C and 2-H, and between 6-C and 5-H and 7-H, definitively establishing the carbon skeleton of **5** and consequently that of **3**. The relatively high value of the coupling constant $J_{3,4}$ (10 Hz) suggested a *cis*-disubstitution pattern for the γ-lactone ring.[17] This stereochemistry, together with the 9*R** relative configuration of **3**, were subsequently confirmed by chemical correlation with **1** and **2** (see below).

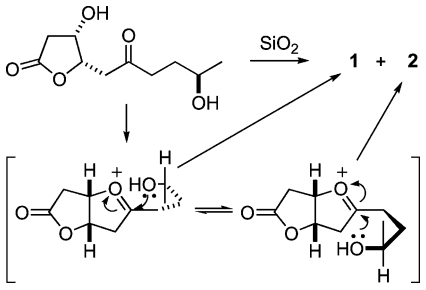


3, R = H
5, R = Ac

4, R = H
6, R = Me

We then determined the structures of metabolite **4**[18] and its methyl ester **6**.[19] The HRMS of **4** revealed a $C_{10}H_{14}O_4$ molecular formula corresponding to four elements of unsaturation, whereas its ^{13}C NMR spectrum showed only four signals assignable to olefin carbons (106, 109, 145.4 and 155.6 ppm) and a carbonyl group (174.3 ppm), thus indicating the monocyclic character of this metabolite. Moreover, the IR spectrum showed a group of bands between 2500 and 3000 cm^{-1} and carbonyl absorption at 1722 cm^{-1}, characteristic of carboxylic acids. Therefore we treated **4** with diazomethane, obtaining the corresponding ester **6**. In the ^1H NMR spectrum, the doublet of a methyl group (10-H_3) attached to an oxygenated CH appeared at 1.27 ppm whereas the multiplicity, coupling constant ($J_{4,5}$ 3 Hz) and chemical shift values (5.97 and 6.13 ppm) of 4-H and 5-H were assignable to a 2,5-disubstituted furan ring,[17] thus finally establishing the structure of **6** and consequently that of **4**. We are currently trying to determine the absolute configuration of compounds **1**–**6**.[20]

When we passed bassianolone (**3**) through a pad of silica gel we obtained a mixture of spiroketals **1** and **2** (Scheme 1). This result confirms the relative (3*S**, 4*S**, 9*R**) configuration of bassianolone and suggests that, in contrast with Hanson's proposal,[1] it is the true chemical parent of cephalosporolides E and F, which are possibly simple artefacts formed during



Scheme 1 Silica-promoted spirocyclization of **3**.

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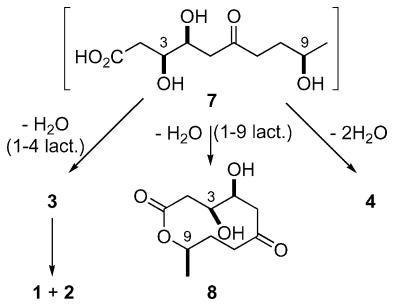
the isolation process. Moreover, the co-occurrence of **3** and **4** in *B. bassiana* suggests the existence of a common biogenetic precursor, probably of short lifetime, which we have called pre-bassianolone (**7**). This metabolic intermediate, containing an even number of carbon atoms, might derive from the polyketide pathway and might also be the precursor of cephalosporolide C (**8**) and related metabolites from *C. aphidicola*[1] (Scheme 2).



Scheme 2 Proposed biogenesis of **3**, **4** and **8** from pre-bassianolone (**7**).

Cases of a common biogenetic precursor for diverse metabolites are numerous[21] and probably constitute one of the devices employed by nature to reduce the number of genes required for the biosynthesis of natural products.[22]

Finally, we tested the *in vitro* antimicrobial activity[23] of compounds **1**–**4** (100 μg ml^{-1}) against gram-positive (*Bacillus megaterium* and *Staphylococcus aureus*), gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and fungal (*Candida albicans*) species. Cephalosporolides E and F, and the furan metabolite **4** showed no antimicrobial activity, whilst (+)-bassianolone (**3**) completely inhibited the visible growth of *S. aureus* and *C. albicans*. Therefore, we subsequently centred our attention on **3**, which also blocked the growth of *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Micrococcus luteus*, *Mucor rouxii* and *Schizosaccharomyces pombe*, and drastically reduced the growth of *Saccharomyces cerevisiae* and *Yarrowia lipolytica.*

In summary, we have established the chemical structures of (+)-bassianolone (**3**) and the furan derivative **4**, two unprecedented metabolites from the fungus *B. bassiana*. Bassianolone has proved to be the true precursor of cephalosporolides E (**1**) and F (**2**) and showed selective antimicrobial activity against gram-positive cocci and fungi. The antimicrobial activity of bassianolone requires further studies and, as there is a clinical need for novel antibacterial[24] and antifungal[25] drugs, we are currently engaged in the chemical synthesis of **3** in order to obtain enough product to complete its biological analysis.

Acknowledgements

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13 Spectroscopic properties, including optical rotation, of product **1** isolated from the broth culture of *B. bassiana* were in accordance with those reported for (+)-cephalosporolide E excreted by *C. aphidicola*[1].
14 Compound **2** from *B. bassiana*: golden syrup; $[\alpha]_D^{25} = -33.3$ (*c* 0.79 in $CHCl_3$); IR, ^1H and ^{13}C NMR, and mass spectra were in accordance with those reported for cephalosporolide F from *C. aphidicola*[1].
15 Data for **3**: white powder; mp = 82–84 °C; $[\alpha]_D^{25} = +97.0$ (*c* 2.70 in MeOH); δ_C (100 MHz, $CDCl_3$) 19.4 (q), 33.7 (t), 38.9 (t), 40.7 (t), 43.4 (t), 68.8 (d), 72.1 (d), 75.0 (d), 169.6 (s), 214.4 (s); *m*/*z* (FAB) 239.0897 $[M + Na]^+$ ($C_{10}H_{16}O_5Na$ requires 239.0895).
16 Data for **5**: golden syrup; δ_H (400 MHz, $CDCl_3$) 1.28 (3 H, d, $J_{10,9}$ 6, 10-H_3), 2.02 (3 H, s, CH_3CO), 2.04 (3 H, s, CH_3CO), 2.35 (2 H, m, 7-H), 2.57 (1 H, dd, $J_{2a,3}$ 12, J_{gem} 17, 2-Ha), 2.67 (1 H, dd, $J_{5a,4}$ 3, J_{gem} 18, 5-Ha), 2.81 (1 H, dd, $J_{2b,3}$ 3, J_{gem} 17, 2-Hb), 3.02 (1 H, dd, $J_{5b,4}$ 7, J_{gem} 18, 5-Hb), 5.05 (1 H, m, 9-H), 5.16 (1 H, ddd, $J_{4,5a}$ 3, $J_{4,5b}$ 7, $J_{4,3}$ 10, 4-H), 5.45 (1 H, ddd, $J_{3,2b}$ 3, $J_{3,4}$ 10, $J_{3,2a}$ 12, 3-H); δ_C (100 MHz, $CDCl_3$) 19.7 (C-10), 20.9 (*C*H_3CO), 21.0 (*C*H_3CO), 33.4 (C-8), 38.2 (C-2), 40.0 (C-7), 43.5 (C-5), 68.7 (C-3), 71.0 (C-4), 72.6 (C-9), 167.9 (C-1), 169.7 (CH$_3$*C*O), 170.2 (CH$_3$*C*O), 206.7 (C-6). NMR peak assignments were made with the aid of 2D NMR experiments (COSY, HMQC and HMBC). The numbering system follows that employed in ref. 1 for cephalosporolides E and F.
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18 Spectroscopic data for **4**: IR υ_{max}/cm^{-1} 3403 (OH), 2966, 2931, 2635, 1722 (CO), 1561; δ_H (300 MHz, $CDCl_3$) 1.21 (3 H, d, *J* 6), 1.76 (2 H, q, *J* 6), 2.70 (2 H, m), 3.66 (2 H, s), 3.84 (1 H, sextuplet, *J* 6), 5.94 (1 H, d, *J* 2.5), 6.11 (1 H, d, *J* 2.5); δ_C (75 MHz, $CDCl_3$) 23.4 (q), 24.4 (t), 33.8 (t), 37.3 (t), 67.5 (d), 106.0 (d), 109.0 (d), 145.4 (s), 155.6 (s), 174.3 (s); *m*/*z* (EI) 198.0898 $[M]^+$ ($C_{10}H_{14}O_4$ requires 198.0892).
19 Spectroscopic data for **6**: $[\alpha]_D^{25} = +46.0$ (*c* 0.13 in MeOH); δ_H (300 MHz, $CDCl_3$) 1.27 (3 H, d, *J* 7), 1.80 (2 H, q, *J* 7), 2.74 (2 H, m), 3.67 (2 H, s), 3.80 (3 H, s), 3.86 (1 H, sextuplet, *J* 7), 5.97 (1 H, d, *J* 3), 6.13 (1 H, d, *J* 3); δ_C (75 MHz, $CDCl_3$) 23.6 (q), 24.5 (t), 34.1 (t), 37.5 (t), 52.3 (q), 67.4 (d), 106.0 (d), 108.7 (d), 145.9 (s), 155.5 (s), 170.2 (s).
20 Using X-ray analysis, Hanson and co-workers were able to establish the relative stereochemistry of cephalosporolide E but not the absolute configuration[1].
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