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 aphidicolum 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.3 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.5–8

Within our programme to investigate the biotechnological use of fungi,9,10 we recently became interested in the metabolites produced under stressful conditions by Beauveria bassiana, an entomopathogenic deuteromycete9 which has found wide application as a whole-cell biocatalyst.11,12 Among the products excreted by this fungus to the broth culture of a low-nitrogen medium,6 we unexpectedly12 found cephalosporolides 113 and 214 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 C19H22O5 molecular formula corresponding to three double-bond equivalents, whereas its 13C NMR spectrum showed only two signals of sp2 carbons, assignable to a γ-lactone (109.6 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) with a chemical shift (1.28 ppm) indicating that the CH3 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 J1,2 (10 Hz) suggested a cis-disubstitution pattern for the γ-lactone ring.17 This stereochemistry, together with the 9R* relative configuration of 3, were subsequently confirmed by chemical correlation with 1 and 2 (see below).

We then determined the structures of metabolite 420 and its methyl ester 6.19 The HRMS of 4 revealed a C10H14O4 molecular formula corresponding to four elements of unsaturation, whereas its 13C 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) attached to an oxygenated CH appeared at 1.27 ppm whereas the multiplicity, coupling constant (J1,2 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.

When we passed bassianolone (3) through a pad of silica gel we obtained a mixture of spiroketalts 1 and 2 (Scheme 1). This result confirms the relative (3S*, 4S*, 9R*) 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.
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* (Scheme 2).

![Scheme 2 Proposed biogenesis of 3, 4 and 8 from pre-bassianolone (7).](image)

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

Finally, we tested the in vitro antimicrobial activity of compounds 1–4 (100 μg ml⁻¹) against gram-positive (*Bacillus megaterium* and *Staphylococcus aureus*), gram-negative (*Pseudomonas aeruginosa* and *E. coli*), 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*, *Candida lanuginosa*, 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 and antifungal drugs, we are currently engaged in the chemical synthesis of 3 in order to obtain enough product to complete its biological analysis.

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References

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*.
14. Compound 2 from *B. bassiana* golden syrup; δ<sub>1</sub>H = 33.3 (δ<sub>1</sub>H = 0.79 in *B. bassiana*); IR, 1749 (OH), 1357 (C=O), 1279 (C–OH); δ<sub>13</sub>C = 211.6 (C=O), 114.3 (C–OH), 57.7 (CH3 CO), 2.35 (2 H, m, 7-H), 2.17 (1 H, dd, J<sub>3,7</sub> = 12, J<sub>5,7</sub> = 2, 2-Ha), 2.67 (1 H, dd, J<sub>3,9a</sub> = 11, J<sub>5,9a</sub> = 4, 5-Ha), 2.81 (1 H, dd, J<sub>3,9a</sub> = 12, J<sub>5,9a</sub> = 3, 2-Ha), 3.02 (1 H, d, J<sub>5,7</sub> = 10, 7-Ha), 3.30 (1 H, m, 9a-H), 3.49 (1 H, dd, J<sub>3,7</sub> = 10, 2-Ha), 3.67 (2 H, s), 3.84 (1 H, sextuplet, J<sub>3</sub> = 6, 1-H), 5.97 (1 H, d, J<sub>1,3</sub> = 10, 3-H), 6.13 (1 H, d, J<sub>3,7</sub> = 10, 3-H), 6.29 (1 H, d, J<sub>1,3</sub> = 10, 3-H), 6.45 (1 H, d, J<sub>3,7</sub> = 10, 3-H), 6.67 (1 H, d, J<sub>1,3</sub> = 10, 3-H). 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* (Scheme 2).