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Monacolin N, a compound resulting from derailment of type I iterative polyketide synthase function *en route* to lovastatin

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A novel compound, monacolin N, has been isolated from fermentation cultures of *Aspergillus nidulans* in which the lovastatin polyketide synthase genes *lovB* and *lovC* are heterologously expressed.

Lovastatin (**1**) is an inhibitor of HMG-CoA reductase, the key enzyme involved in cholesterol biosynthesis.[1] It is produced in cultures of the filamentous fungus *Aspergillus terreus via* a polyketide pathway.[2] Although some of **1** is used directly as a drug (Mevacor™), a major portion is chemically α-methylated in the butyryl side chain to generate the widely-prescribed analog, simvastatin (Zocor™). During formation of **1**, initial assembly by a type I iterative polyketide synthase (PKS) system yields the nonaketide dihydromonacolin L (**2**), which is further elaborated by a series of post-PKS transformations[3] into **1** (Scheme 1). It was demonstrated earlier that lovastatin nonaketide synthase (LovB) is capable of condensation of units from acetyl-CoA and malonyl-CoA, and also possesses ketoreductase (KR) and dehydratase (DH) activities.[4] In addition, purified LovB enzyme is capable of catalysing a Diels–Alder reaction on a hexaketide intermediate analog.[4b] Interestingly, an accessory protein (LovC) is essential for enoyl reductase (ER) activity and correct formation of the initial PKS product **2**.[4] In the absence of LovC, the failure of enoyl reduction at the tetraketide stage leads to formation of truncated pyrone metabolites **3** and **4** through condensation of additional acetate units from malonyl-CoA by the LovB enzyme. Two such building blocks are added to the incorrectly-reduced tetraketide without reduction followed by intramolecular cyclisation to form **3**. To generate **4**, three units are sequentially condensed on the incorrect tetraketide with keto reduction and dehydration occurring after condensation of the first C_2 unit to give an extra CH=CH moiety. We now report that failure of LovC to correctly reduce the enoyl functionality at the heptaketide stage leads to formation of a new dehydrogenated version of dihydromonacolin L, monacolin N (**5**) (Scheme 2).

Heterologous expression of *lovB* and *lovC* genes from *A. terreus* in *A. nidulans*, each on separate plasmids, affords primarily dihydromonacolin L (**2**) as expected.[4] However, small amounts of pyrone pigments **3** and **4** still appear to be produced, presumably due to incomplete association of LovB and LovC proteins and/or a mismatch in expression levels. It is also possible that in this heterologous system the absence of other proteins normally associated with post-PKS processes leads to weaker interaction. These pigments are the principal products from heterologous expression of *lovB* alone in *A. nidulans* or from inactivation of the *lovC* gene in the parent producer, *A.*

Scheme 1 Biosynthesis of lovastatin (**1**) and formation of pyrones.

Scheme 2 Possible formation of monacolin N (5).

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terreus.[4] During large scale isolation and purification of **2** from fermentation cultures of *A. nidulans lovB + lovC*, a new metabolite was also isolated. Named monacolin N (**5**), this compound is produced at a level *ca.* 1% (*ca.* 0.5 mg per litre of fermentation culture) that of dihydromonacolin L (**2**). Its structure, which has an additional double bond in the linker arm between the upper lactone ring and the lower decalin ring system, is confirmed by ^{1}H and ^{13}C NMR spectroscopy† as well as by X-ray crystallographic analysis (Fig. 1).‡

It appears that in extending the growing polyketide chain from hexaketide to heptaketide there is a failure by LovC to accomplish the enoyl reductase step necessary to reduce the double bond. As with the incorrectly reduced tetraketide, LovB can continue to add two carbon units from malonyl-CoA and extend the chain. However, in contrast to the pyrone formation from the incorrect tetraketide, in this case the additional units are added in the same fashion and with the same level of subsequent reduction as would be expected for normal formation of the natural PKS product, dihydromonacolin L (**2**). This may initially seem predictable as no further enoyl reduction capability is necessary at the octaketide or nonaketide stages. However, in contrast to bacterial *modular* type I PKS enzymes,[5] the fungal *iterative* type I PKS enzymes have but single copies of each enzymatic functionality, which are used repeatedly in assembly of a single molecule.[2,4,6] Hence, the operation of processing domains (*i.e.* ketoreductase, dehydratase, enoyl reductase, methyl transferase) and the level of reduction achieved at each stage of assembly must be controlled by the structure of the covalently-bound growing substrate. For example, the structure of the tetraketide initially formed after third malonyl condensation must enable the LovB PKS to accomplish the methylation, a functionality that remains unused before and after this stage. Interestingly, the recently reported compactin PKS, has a methylation domain that remains silent throughout.[6] Thus, it is not at all obvious *a priori* that an altered (*i.e.* unsaturated) tetraketide or heptaketide would be further processed in analogy to the correct reduced version by the lovastatin PKS system. The production of pyrones **3** and **4** and monacolin N (**5**) demonstrates that this fails to happen with the altered tetraketide but not with the heptaketide.

The structure of monacolin N (**5**) and its resemblance to **2**, suggested that perhaps **5** could be transformed by the *A. terreus* post-PKS enzymatic system into an unsaturated analog **6** of lovastatin (**1**) (Scheme 3). Previous studies with a mutant of *A. terreus* blocked at the critical lovC gene have established that this organism, which is incapable of production of **2**, contains fully functional post-PKS enzymes necessary to form lovastatin (**1**) if exogenous dihydromonacolin L (**2**) is added.[3] Therefore ^{13}C-labelled **5** was generated by adding sodium 1-[^{13}C] sodium acetate to a fermentation of *A. nidulans lovB + lovC*, and the purified labelled **5** was added to a culture of *A. terreus lovC* mutant known to transform **2** to **1**.[3] However, no trace of **6** could be detected and >25% of **5** was recovered unchanged. It

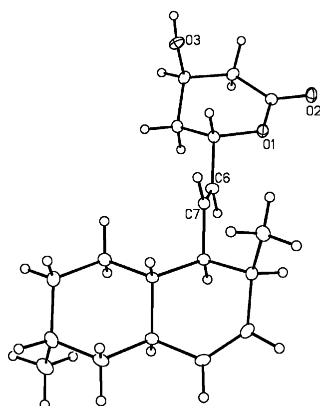
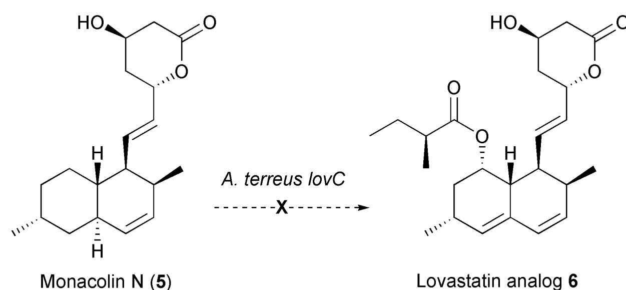


Fig. 1 X-ray structure of monacolin N (**5**). Numbering is arbitrary.



Scheme 3 Attempted biotransformation of **5**.

appears that the post-PKS enzyme system is not willing to accept **5** as a substrate in place of the normal intermediate **2**.

In summary, a new product, monacolin N (**5**), has been isolated from fermentation cultures of *A. nidulans lovB + lovC* which probably results from a mis-match in the expression levels or association of the LovB and LovC proteins. Despite its structural similarity to the natural PKS product, dihydromonacolin L (**2**), it is not detectably transformed to the unsaturated analog **6** of lovastatin (**1**) by post-PKS enzymes. Further studies on the function of the iterative type I PKS system and the post-PKS enzymes are in progress.

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Notes and references

† *Spectral data* for **5**: R_f = 0.5 (EtOAc, 100%); HREIMS [*M*]$^+$ 304.20435 (304.20386 calcd. for $C_{19}H_{28}O_3$) 286.2 (2.6 %), 275.0 (2.3%), 216.2 (7.0%), 161.1 (10.6%), 91.0 (100.0%); ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.71, (ddd, 1H, J = 15.2, 9.8, 0.8 Hz), 5.55 (ddd, 1H, J = 9.8, 4.4, 3.1 Hz), 5.49 (dd, 1H J = 15.4, 6.8 Hz), 5.30 (d, 1H, J = 10.0 Hz), 5.15 (ddd, 1H, J = 10.0, 6.8, 3.1 Hz) 4.38 (m, 1H), 2.75 (dd, 1H, J = 17.7, 5.1 Hz), 2.60 (ddd, 1H, J = 17.7, 4.0, 1.6 Hz), 2.25–2.18 (m, 2H), 2.05–1.97 (m, 2H), 1.92–1.85 (m, 2H), 1.52–1.44 (m, 4H), 1.33 (dd, 1H, J = 12.8, 3.1 Hz), 1.28 (dd, 1H, J = 13.2, 4.8 Hz), 1.26–1.22 (m, 2H), 0.96 (d, 3H, J = 7.2 Hz) 0.90 (d, 3H, J = 6.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.1, 136.8, 132.4, 131.2, 128.1, 76.1, 62.8, 47.0, 39.1, 38.9, 38.8, 36.9, 36.6, 35.8, 32.2, 27.7, 25.2, 18.4, 16.6.

‡ *Crystal data* for **5**: $C_{19}H_{28}O_3$: M = 304.41, 0.42 × 0.28 × 0.05 mm, orthorhombic, a = 5.5364(8), b = 9.9736(14), c = 31.173(4) Å, V = 1721.3(4) Å^3, T = 193 K, space group $P2_12_12_1$ (No. 19), Z = 4, μ(Mo–Kα) = 0.077 mm^{-1}, 8084 reflections measured, 3504 unique (R_{int} = 0.0413) which were used in all least squares calculations, $R_1(F)$ = 0.0444 (for 2789 reflections with $F_o^2 \geq 2\sigma(F_o^2)$), $wR_2(F^2)$ = 0.1111 (for all unique reflections). CCDC 208957. See http://www.rsc.org/suppdata/cc/b3/b304252a/ for crystallographic data in .cif or other electronic format.The absolute stereochemistry is assigned based on its biosynthetic relationship to dihydromonacolin L (**2**) and its known configuration.

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