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The Biosynthesis of the Fungal Meroterpenoids Boviquinone-3 and -4 follows Two Different Pathways

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Abstract

A key step in the biosynthesis of the fungal meroterpenoids boviquinone-3 and -4 is the prenylation of 3,4-dihydroxybenzoic acid. In fruit-bodies of *Suillus bovinus* boviquinone-4 is formed by geranylgeranylation of 3,4-dihydroxybenzoic acid at C-5, whereas in *Chroogomphus rutilus* the farnesyl side chain of boviquinone-3 is introduced at C-2. The biosynthesis of the terpenoid chain of boviquinone-4 follows the mevalonate route.

Keywords: biosynthesis; quinones; meroterpenoids; fungal pigments

Meroterpenoid quinones like the ubiquinones, plastoquinones and menaquinones are widespread in living organisms and are essential for several life processes. A group of structurally related 2,5-dihydroxy-1,4-benzoquinones with oligoprenyl side chains has been isolated from several basidiomycetes of the order Boletales [1]. Fruit-bodies of *Chroogomphus rutilus* and *C. helveticus* contain the farnesylated boviquinone-3 (helveticone) (**1**) [2] whereas *Suillus bovinus* produces its geranylgeranyl homologue boviquinone-4 (**2**) [3,4]. Structurally, the boviquinones resemble the ubiquinones whose quinone ring is known to originate from 4-hydroxybenzoic acid [5,6,7]. In a preliminary feeding experiment we have shown that ring ^{14}C-labelled 4-hydroxybenzoic is also incorporated into boviquinone-3 (**1**) by fruit-bodies of *Chroogomphus helveticus* [8]. In this publication we report on a more detailed investigation of boviquinone biosynthesis.

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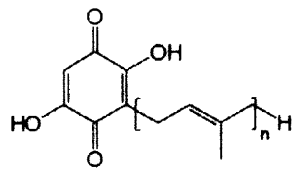
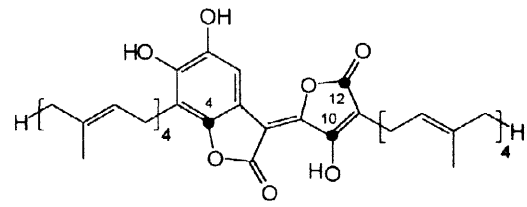


1: n = 3
2: n = 4



3 (^{13}C-label indicated by •)

Feeding of either 4-hydroxy[1-^{13}C]benzoic acid (**5**) or 3,4-dihydroxy[1-^{13}C]benzoic acid (**6**) [9] at low concentrations (0.04 mmol/fruit-body) to young specimens of *Suillus bovinus* gave rise to specific incorporation of these precursors into boviquinone-4 (**2**) [10]. Because of rapid tautomerization of the 2,5-dihydroxybenzoquinone system the sites of incorporation could only be determined after conversion of boviquinone-4 to its dimethyl ether **4** [4]. In both experiments C-2 and C-4 were equally enriched, and the incorporation was 1.1 and 1.3% (^{13}C atom% excess) for the feeding of **5** and **6**, respectively. The labelling pattern of **4** was confirmed by HMBC experiments summarized in Figure 1 in which 6-H as well as the methylene group attached to C-3 exhibit long range couplings to the ^{13}C enriched carbon atoms C-2 and C-4.

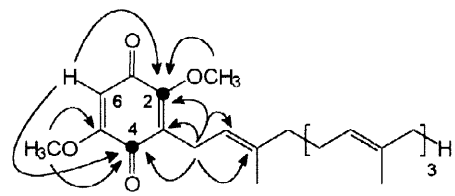


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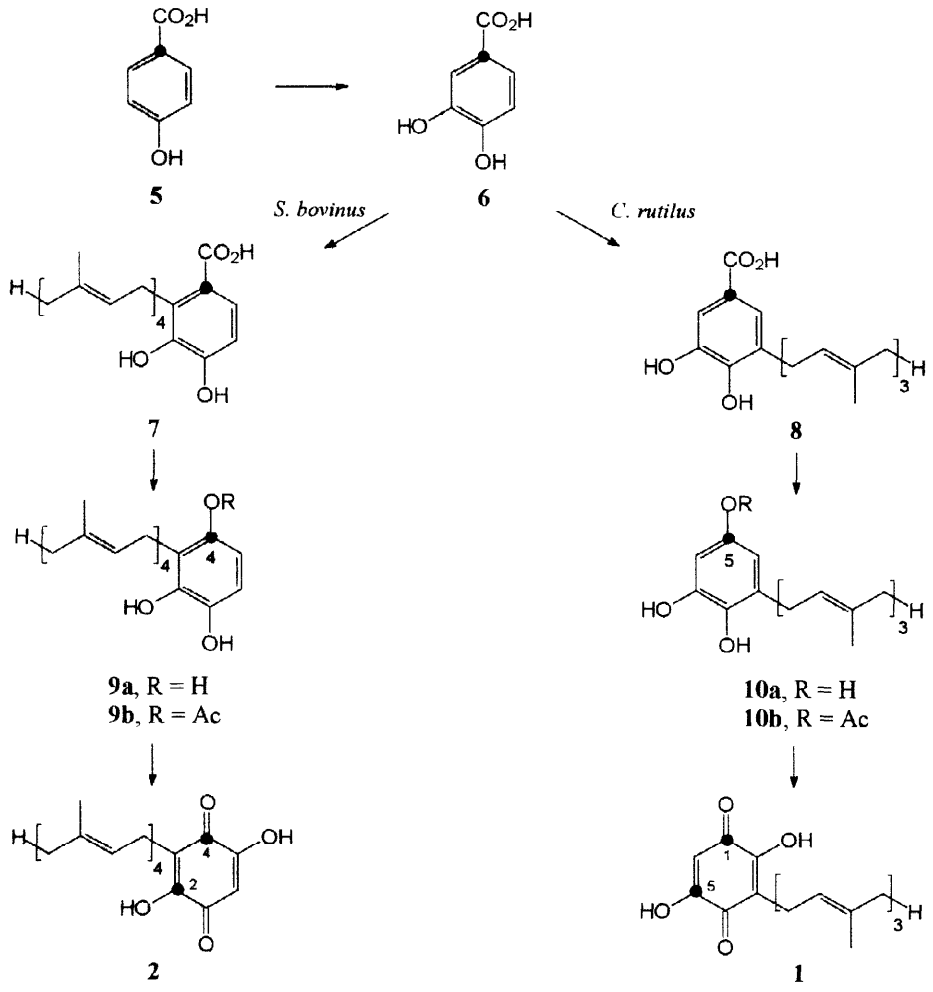
Figure 1. HMBC correlations of labelled boviquinone-4 dimethyl ether (**4**) after feeding of 4-hydroxy- or 3,4-dihydroxy[1-^{13}C]benzoic acid to fruit-bodies of *Suillus bovinus*.

The feeding experiments indicate that boviquinone-4 (**2**) is formed by prenylation of 3,4-dihydroxybenzoic acid (**6**) at C-2 to yield 2-geranylgeranyl-3,4-dihydroxybenzoic acid (**7**) (Scheme 1). Oxidative decarboxylation of 7 then affords the trihydroxy intermediate **9a** which is further hydroxylated and oxidized to boviquinone-4. 4-Hydroxybenzoic acid (**5**) acts as the precursor of 3,4-dihydroxybenzoic acid.

Interestingly, the application of higher doses of the precursors **5** and **6** (~0.2 mmol/fruit-body) or even other aromatic acids such as benzoic or salicylic acid induces a change of metabolism. Instead of boviquinone-4 (**2**) the fruit-bodies of *S. bovinus* produce large amounts of bovilactone-4,4 (**3**) [11], whose labelling pattern indicated in formula **3** is in agreement with its formation by coupling of trihydroxy compound **9a** with boviquinone-4 (**2**) with concomitant ring cleavage and formation of the lactone rings [2].

The 4-*O*-acetyl derivative of intermediate **9a**, suillin (**9b**), occurs in fruit-bodies of *Suillus variegatus* and several other *Suillus* species [1,12]. Feeding of 4-hydroxy[1-^{13}C]benzoic acid and 3,4-dihydroxy[1-^{13}C]benzoic acid to young fruit-bodies of *S. variegatus* gave [4-^{13}C]-labelled **9b** as expected from Scheme 1 [13].

Surprisingly, feeding of 4-hydroxy- or 3,4-dihydroxy[1-^{13}C]benzoic acid to *Chroogomphus rutilus* yielded boviquinone-3 (**1**) with equal distribution of the label at C-1 and C-5 (Scheme 1) [14]. This implies that in the biosynthesis of boviquinone-3 prenylation of 3,4-dihydroxybenzoic acid takes place at C-5 to yield 5-farnesyl-3,4-dihydroxybenzoic acid (**8**) as the first intermediate. Subsequent oxidative decarboxylation, hydroxylation at



Scheme 1. Proposed pathways for the biosyntheses of boviquinone-4 (**2**) and boviquinone-3 (**1**).

C-4 and oxidation affords boviquinone-3 (**1**). This pathway is supported by the isolation of 5-acetoxy-3-farnesyl-1,2-dihydroxybenzene (**10b**), the 5-*O*-acetyl derivative of the postulated intermediate **10a** [15] from fruit-bodies of *C. rutilus*. The acetate **10b** was obtained from both feeding experiments and exhibited ^{13}C enrichments at C-5 from **5** and **6** of 1.3 and 3.6%, respectively, in agreement with the proposed biosynthesis [14].

Feeding of [1-^{13}C]- or [2-^{13}C]glucose to fruit-bodies of *Suillus bovinus* resulted in complimentary labelling patterns of the side chain in boviquinone-4 (**2**) in accord with the formation of isopentenyl pyrophosphate *via* the classical mevalonate route (Figure 2) [16]. This result is supported by the incorporation of ^{14}C-labelled mevalonate in boviquinone-3 (**1**) in fruit-bodies of *Chroogomphus helveticus* [8].

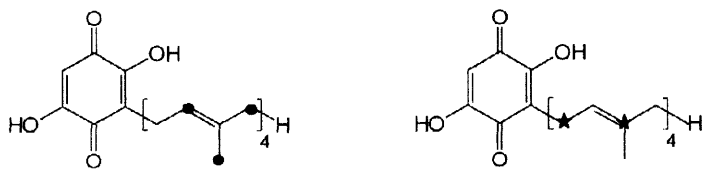


Figure 2. Labelling patterns of boviquinone-4 (**2**) after feeding of [1-^{13}C]- and [2-^{13}C]glucose. Enriched carbon atoms from [1-^{13}C]glucose are indicated by • and enriched carbon atoms from [2-^{13}C]glucose by *.

When [1'-^{13}C]tyrosine [10] was added to cultures of *Suillus bovinus*, the bovilactone-4,4 (**3**) isolated after two months disclosed the same ^{13}C enrichments as in the feeding experiment with 4-hydroxy[1-^{13}C]benzoic acid (**5**) [17]. This proves the role of tyrosine as precursor of 4-hydroxybenzoic acid in this mushroom [18].

Our results show that the biosynthesis of the boviquinones differs from that of the ubiquinones in the prenylation step, which requires 3,4-dihydroxybenzoic acid instead of the 4-hydroxy derivative. Moreover, the structurally homologous fungal metabolites boviquinone-3 (**1**) and boviquinone-4 (**2**) are produced by two different pathways in closely related mushrooms. Studies on the biosynthesis of tridentoquinone and related meroterpenoids from basidiomycetes will be reported in due course.

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References and Notes

[1] Gill, M.; Steglich, W. *Progress Chem. Org. Nat. Prod.* **1987**, *51*, 98.

[2] Steglich, W.; Esser, F.; Pils, I. *Z. Naturforsch.* **1971**, *26b*, 336.

[3] Beaumont, P. C.; Edwards, R. L. *J. Chem. Soc. (London) C* **1969**, 2398 .

[4] Beaumont, P. C.; Edwards, R. L. *J. Chem. Soc. (London) C* **1971**, 2582.

[5] Gibson, F. *Biochem. Soc. Trans.* **1973**, *1*, 317.

[6] Olson, R.E.; Rudney, H. *Vitam. Horm.* **1983**, *40*, 1.

[7] Clarke, C.F.; Williams, W.; Teruya, J. H. *J. Biol. Chem.* **1991**, *266*, 16636.

[8] Arnold, R.; Steglich, W. unpublished experiments.

[9] Beyer, J.; Lang-Fugmann, S.; Mühlbauer, A.; Steglich, W. *Synthesis*, in press.

[10] [1-^{13}C]-**6** (100 mg) in DMSO (1 ml) was injected in 18 young fruit-bodies. After 3 days in their natural habitat the mushrooms were harvested, worked up, and the resulting boviquinone-4 (50 mg) was converted into its dimethyl ether **4** [4], which was subjected to ^{13}C NMR spectroscopy. The ^{13}C enrichment (atom% excess) at C-2 (δ 155.5) and C-4 (δ 183.7) was 2.6% for each position. Similarly, feeding of [1-^{13}C]-**5** yielded **4** with a ^{13}C enrichment of 1.1%.

[11] Jägers, E.; Steglich, W. *Angew. Chem.* **1981**, *93*, 1105; *Angew. Chem. Int. Ed. Engl.* **1981**, *20*, 1016. After feeding labelled **5** or **6** for 3 days, **3** showed the following ^{13}C enrichments: C-4 (δ 146.9) 50%, C-10 (δ 161.3) 25% and C-12 (δ 167.1) 25%.

[12] Jägers, E.; Pasupathy, V.; Hovenbitzer, A.; Steglich, W. *Z. Naturforsch.* **1986**, *41b*, 645.

[13] [1-^{13}C]-**6** (200 mg) in DMSO (300 µl) was administered *via* syringe to 5 young fruit-bodies. After 3 days the mushrooms were worked up and **9b** (47 mg) was isolated. **9b** showed a ^{13}C enrichment of 36% at C-4 (δ 142.1).

[14] [1-^{13}C]-**6** (90 mg) in acetone (200 µl) was applied to 3 young fruit-bodies. After 4 days, **1** (40 mg) and **10b** (12 mg) were isolated and the following ^{13}C enrichments (atom% excess) were determined: **1** (dimethyl ether derivative) 1.3% at C-1 (δ 183.73) and C-5 (δ 158.8); **10b** 3.6% at C-5 (δ 143.81). Similarly [1-^{13}C]-**5** (61 mg) yielded **1** (64mg) and **10b** (35 mg) with ^{13}C enrichments of 0.5 and 1.1%, respectively.

[15] Piattelli, M.; Geraci, C.; Lambusta, D.; Cianci, P. *Planta Med.* **1992**, *58*, 383.

[16] [1-^{13}C]- or [2-^{13}C]Glucose (200 mg) in H_2O (500 µl) were injected into 3 young fruit-bodies. After 3 days, **2** was isolated and converted into **4**. In the [1-^{13}C]glucose experiment, ^{13}C enrichments of 2-3% were observed for the 4 $\underline{C}H$ carbons (δ 119.7-124.4), the 4'-, 8'- and 12'-$\underline{C}H_2$ carbons (δ ~39.74) and the 5 $\underline{C}H_3$ carbons (δ 2×16.0, 16.2, 17.7, 25.7) of the geranylgeranyl chain of **4**. [2-^{13}C]Glucose experiment: ^{13}C enrichments of 0.8-1.6% for C-3', C-7', C-11' and C-15' (δ 131.2-137.3) and the 1'-, 5'-, 9'- and 13'-$\underline{C}H_2$ carbons (δ 22.3-26.8). The labelling patterns are in accord with the glycolysis of [1-^{13}C]- and [2-^{13}C]glucose to [2-^{13}C]- and [1-^{13}C]acetate, respectively, before entering the mevalonate pathway.

[17] Labelling of **3** after administering of [1'-^{13}C]-tyrosine: C-4 (^{13}C enrichment 9%), C-10 and C-12 (each 5%).

[18] Compare e.g. Strack, D. *Phenolic Metabolism*, Academic Press, San Diego **1997**.