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Syntheses and Biological Evaluation of B-Ring-Modified Analogues of Dafachronic Acid A

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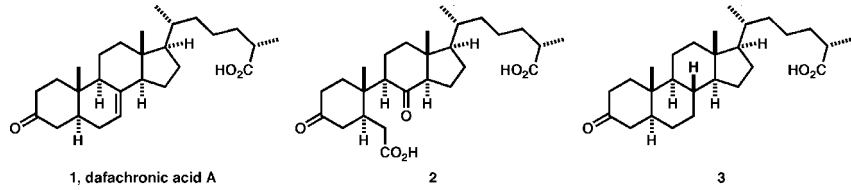
ABSTRACT



Synthesis and testing of dafachronic acid A (1) and its derivatives 2 and 3 have revealed that 1, and not a further oxidation product, is the natural ligand for the DAF-12 receptor of *Caenorhabditis elegans*.

Remarkably, the life span of the nematode *Caenorhabditis elegans* can be increased significantly by loss of function of a handful of genes that affect endocrine function. Among them, the daf-9 gene encodes a cytochrome P450 enzyme which is responsible for the biosynthesis of the bile acid-like steroid, dafachronic acid A (**1**). Based on various analytical techniques, it has been recently proposed by Mangelsdorf and Antebi that **1** is the major ligand for the nuclear receptor DAF-12, which in its ligand bound form regulates genes that prevent entry into the dauer stage, a long-lived quiescent mode.[1] However, synthesis of the proposed ligand remained elusive until a later work, in which the 25-(*S*) structure of **1** and its 25-(*R*)-diastereomer were made.[2,3]

In this research, we address the question of whether dafachronic acid A is the true ligand for the nuclear hormone receptor DAF-12 or just a precursor of a further biooxidation product which is the actual ligand. We were intrigued by the fact that dafachronic acid A, with its Δ^7-olefinic linkage, might be further oxidized biologically to a seco acid structure resembling that of glycinoeclepin A,[4,5] a potent hatching factor for the eggs of the nematode *Heterodera glycines*

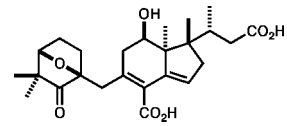


Figure 1. Structure of glycinoeclepin A.

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(1) (a) Motola, D. L.; Cummins, C. L.; Rottiers, V.; Sharma, K. K.; Li, T.; Li, Y.; Suino-Powell, K.; Xu, H. E.; Auchus, R. J.; Antebi, A.; Mangelsdorf, D. J. *Cell* **2006**, *124*, 1209–1223. (b) Gerisch, B.; Rottiers, V.; Li, D.; Motola, D. L.; Cummins, C. L.; Lehrach, H.; Mangelsdorf, D. J.; Antebi, A. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 5014–5019. (c) Rottiers, V.; Motola, D. L.; Gerisch, B.; Cummins, C. L.; Nishiwaki, K.; Mangelsdorf, D. J.; Antebi, A. *Developmental Cell* **2006**, *10*, 473–482. (d) (c) For an online resource on *C. elegans*, see: http://www.wormbook.org.

(2) Giroux, S.; Corey, E. J. *J. Am. Chem. Soc.* **2007**, *129*, 9866–9867.
(3) Giroux, S.; Corey, E. J. *Org. Lett.* **2008**, *10*, 801–802.
(4) Glycinoeclepin A, a natural product that is released into soil from the roots of the soybean plant, is active at 10^{-12} g/mL as a hatching factor for *H. glycines*; see: (a) Fukuzawa, A.; Furusaki, A.; Ikura, M.; Masamune, T *J. Chem. Soc. Chem. Commun.* **1985**, *221*−*222*, 748. (b) Masamune, T.; Anetai, M.; Takasugi, M.; Katsui, N. *Nature* **1982**, *297*, 495–496.

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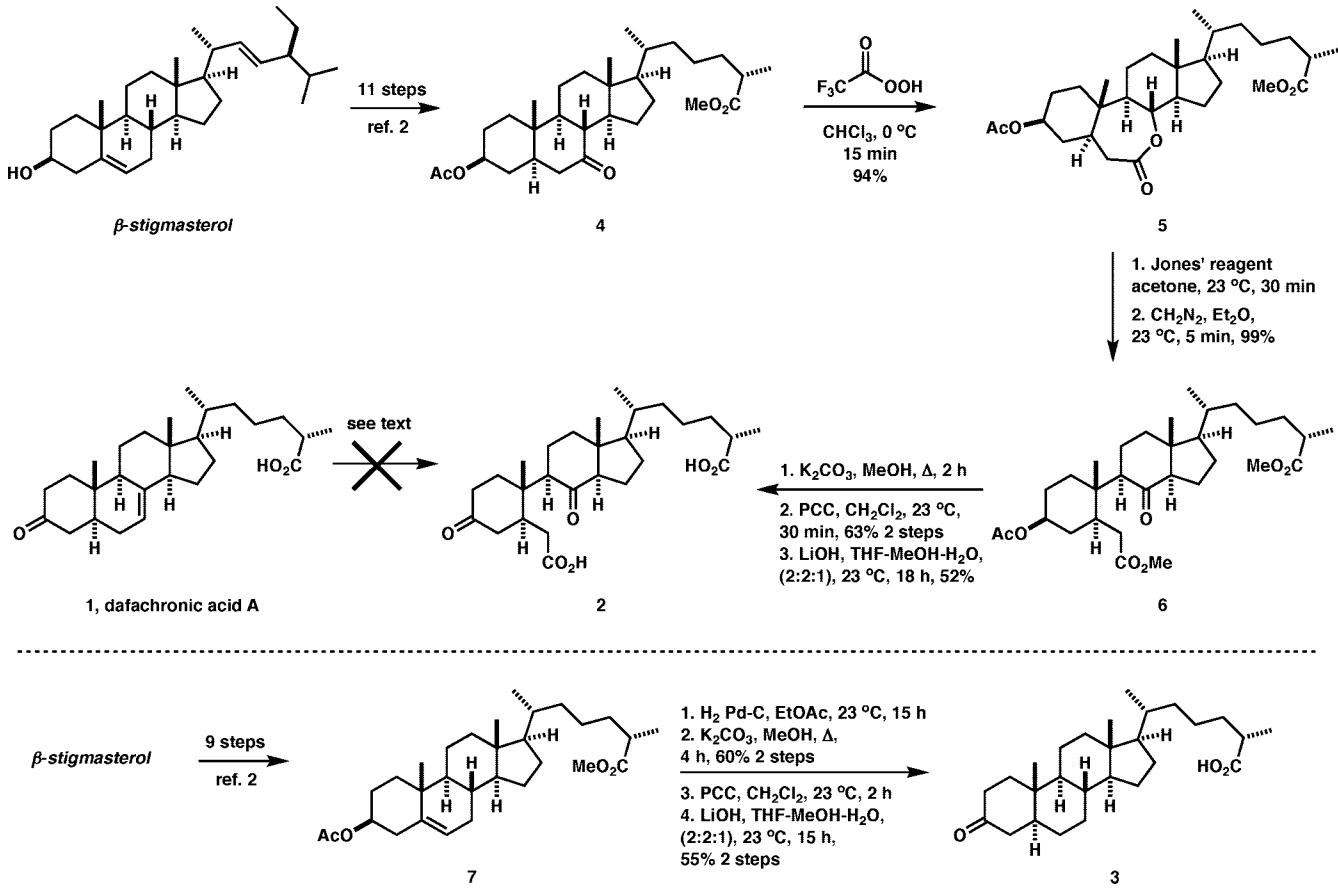
Scheme 1. Synthesis of Analogues **2** and **3** from β-Stigmasterol



(Figure 1). Consequently, we became interested in exploring the biological activity of the β-seco dafachronic acid A derivative **2**, as an analogue of glycinoeclepin A, which might even be a more active metabolite of **1**. In this paper, we describe the synthesis and biological evaluation of **2**. For comparison, we have also synthesized the 7,8-dihydro derivative of dafachronic acid A, **3**, which would be expected to be devoid of activity if the seco acid **2** were the real ligand for DAF-12, rather than dafachronic acid A (**1**).

The synthesis of the diketo diacid **2** started with the previously reported 6-keto steroid **4**.[2] Baeyer−Villiger oxidation of **4** with trifluoroperacetic acid ($(CF_3CO)_2O$, H_2O_2, 0 °C, $CHCl_3$) afforded the desired 7-membered lactone **5** in 94% yield and as a sole regioisomer. Lactone **5** was cleaved to a ketoacid intermediate by treatment with Jones' reagent (2 equiv, 23 °C, acetone) which was esterified by diazomethane (CH_2N_2, Et_2O) to give ketoester **6** in essentially quantitative yield over two steps. Saponification of the 3β-acetate, oxidation of the resulting alcohol to the ketone, and hydrolysis gave the diketo diacid **2** in 52% overall yield (three steps, Scheme 1). Our initial strategy for the synthesis of **2** involved the oxidation of the Δ^7-olefinic linkage in **1** by various methods. Surprisingly, all attempts to directly oxidize the Δ^7 bond to the diketo diacid **2** using O_3 then H_2O_2, $KMnO_4$, NBu_4MnO_4, and $RuCl_3-NaIO_4$ were unsuccessful.

To synthesize the 7,8-dihydro analogue **3**, we have also used an intermediate from our synthesis of **1**.[2] Thus, the Δ^5-double bond in **7** was reduced (H_2, 1 atm, Pd−C, EtOAc) to give the fully saturated steroid, and the same three steps as above were performed to give analogue **3** in 33% overall yield for the four steps. It should also be mentioned that the hydrogenation of **1** to **3** failed under several conditions.[6]

Next, samples of the synthetic dafachronic acid A **1**, the *seco*-diacid **2**, and 7,8-dihydrodafachronic acid A **3** were evaluated for their bioactivity. First, the ability of synthetic ligands to rescue daf-9 hormone biosynthetic mutants from the dauer state was measured. Consistent with **1** being a natural ligand for DAF-12, dafachronic acid A rescued dauer formation in the nanomolar range, with half-maximal activity of 18.5 nM (Figure 2). Similarly, the 7,8-dihydrodafachronic acid A also gave substantial rescue with half-maximal rescue at 292 nM. By contrast, the *seco*-diacid **2** was found *not* to rescue *C. elegans* from the dauer state, indicating that it is not a ligand. Second, the ability of synthetic ligands to activate DAF-12 in transcriptional assays on a target gene,

(5) For the syntheses of glycinoeclepin A, see: (a) Murai, A; Tanimoto, N.; Sakamoto, N.; Masamune, T. *J. Am. Chem. Soc.* **1988**, *110*, 1985–1986.

(6) To the best of our knowledge, no successful hydrogenation of isolated Δ^7 double bonds has been reported in the literature. (b) Mori, K.; Watanabe, H. *Pure Appl. Chem.* **1989**, *61*, 543–546. (c) Corey, E. J.; Houpis, I. N. *J. Am. Chem. Soc.* **1990**, *112*, 8997–8998.

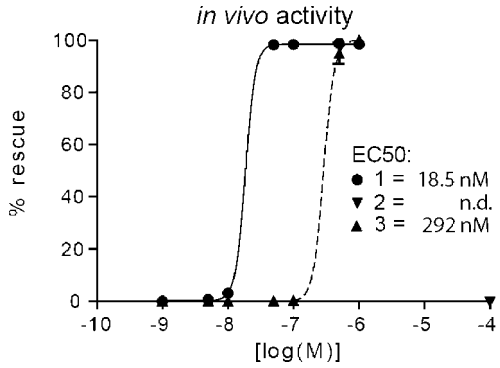


Figure 2. In vivo activity of sterols **1**−**3** measured as the percentage of rescue of *daf-9(dh6)* null worms from dauer to wild-type gravid adults.

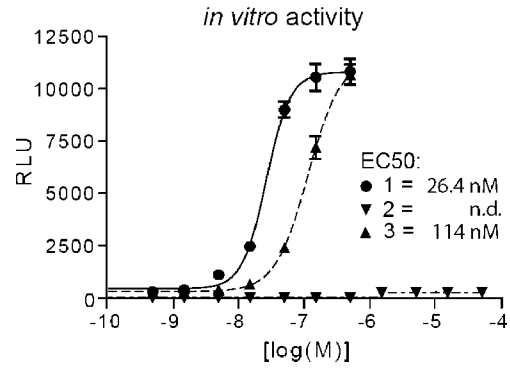


Figure 3. Transcriptional activation of DAF-12 by **1**−**3** on lit-1::ptk-luciferase reporter constructs, measuring relative luciferase units with and without ligand (RLU) vs concentration.

lit-1, was measured. To do this, plasmid constructs containing the daf-12 gene and the lit-1 gene fused to a luciferase reporter were cotransfected into human embryonic kidney cells (HEK293T) and treated with various doses of the compounds and luciferase induction measured by light emission.[1] In accord with the dauer rescue results, **2** showed *no activity* even at 100 μM concentration (Figure 3), whereas 7,8-dihydrodafachronic acid A (**3**) showed activity similar to that of dafachronic acid A (**1**). Specifically, measurement of the dose−response revealed EC_{50} values for daf-12 activation to be 114 nM for 7,8-dihydrodafachronic acid A and 26 nM for dafachronic acid A.

These results taken together allow the following conclusions: (1) dafachronic acid A is a natural ligand for DAF-12 nuclear receptor, (2) in contrast to the soybean nematode case, ring B oxidative cleavage products are not the active agents for gene activation of *C. elegans* DAF-12, and (3) the $\Delta^{7,8}$ double bond is not essential for dafachronic acid activity on *C. elegans*.

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Supporting Information Available: Experimental protocols, characterization for all new compounds, and methods for performing the dauer assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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