ORGANIC LETTERS

2009
Vol. 11, No. 17
3970−3973

Synthesis of Sominone and Its Derivatives Based on an RCM Strategy: Discovery of A Novel Anti-Alzheimer's Disease Medicine Candidate "Denosomin"

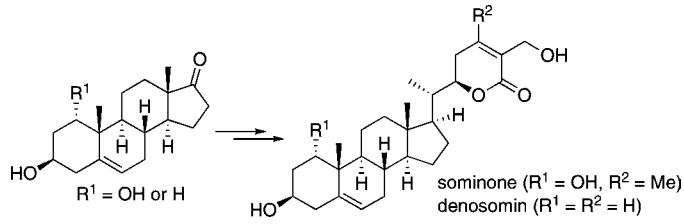
Yuji Matsuya,*,† Yu-ichiro Yamakawa,† Chihiro Tohda,‡ Kiyoshi Teshigawara,‡ Masashi Yamada,§ and Hideo Nemoto†

Graduate School of Medicine and Pharmaceutical Sciences and Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan, and Research Alliance & Portfolio Strategy, Taiho Pharmaceutical Co. Ltd., 1-2-4 Utikanda, Chiyoda-ku, Tokyo 101-0047, Japan

matsuya@pha.u-toyama.ac.jp

Received July 8, 2009

ABSTRACT



Synthesis of sominone was achieved starting from dehydroepiandrosterone on the basis of an RCM strategy for the construction of a δ-lactone side chain. This synthetic protocol was applied for the synthesis of several analogous derivatives including 1-deoxy-24-norsominone (denosomin), which was revealed to exhibit notable bioactivities for new antidementia chemotherapy, exceeding the original natural compound sominone.

In the present aging society, central nervous disorders such as dementia, Parkinson's disease and Alzheimer's disease represent serious medical problems, and the development of novel pharmaceuticals for such disorders has been hastened in order to improve therapeutic strategies. Enhancing brain function requires the reinforcement of neuronal networks, including neurite regeneration and synapse formation; therefore, we have been exploring antidementia drugs based on reconstructing neuronal networks in the damaged brain. We previously investigated the effects of extracts and constituents of Ashwagandha (root of *Withania somnifera* Dunal), an Ayurvedic tonic medicine, on neurite outgrowth in cultured neurons.[1] In the course of these studies, we isolated withanoside IV, a member of natural withanolides (ergostane-type steroids with a δ-lactone side chain), from methanol extracts of Ashwagandha, and found that this natural compound showed antidementia activity after oral administration to an Alzheimer's disease mouse model.[2] Furthermore, we noted that withanoside IV had significant function in enhancing

† Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama.
‡ Institute of Natural Medicine, University of Toyama.
§ Taiho Pharmaceutical Co. Ltd.

(1) (a) Tohda, C.; Kuboyama, T.; Komatsu, K. *Neuroreport* **2000**, *11*, 1981. (b) Zhao, J.; Nakamura, N.; Hattori, M.; Kuboyama, T.; Tohda, C.; Komatsu, K. *Chem. Pharm. Bull.* **2002**, *50*, 760. (c) Kuboyama, T.; Tohda, C.; Zhao, J.; Nakamura, N.; Hattori, M.; Komatsu, K. *Neuroreport* **2002**, *13*, 1715. (d) Kuboyama, T.; Tohda, C.; Komatsu, K. *Br. J. Pharmacol.* **2005**, *144*, 961.

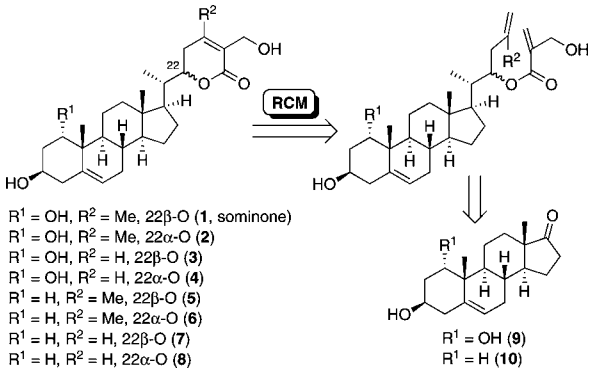
(2) Kuboyama, T.; Tohda, C.; Komatsu, K. *Eur. J. Neurosci.* **2006**, *23*, 1417.

10.1021/ol901553w CCC: $40.75
Published on Web 08/04/2009

the growth activity of neurites from injured and/or spared neurons and synaptogenesis in the brain.[2]

Withanoside IV is a steroidal saponin conjugated with two glucose residues at position C3 on the A-ring, and is speculated to be metabolized into a sapogenin, sominone (**1**, Scheme 1),[3] by enterobacterial β-glucosidases. As expected,

Scheme 1



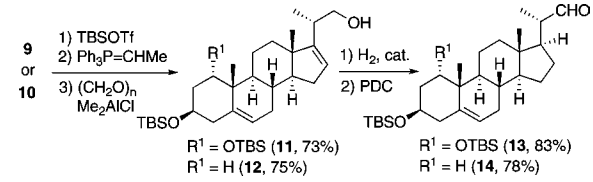
sominone was identified as the main metabolite in serum after oral administration of withanoside IV,[2] indicating the potential of withanoside IV as a prodrug for sominone activity on target organs. We found that sominone, prepared by enzymatic deglycosylation of withanoside IV, induced significant axonal and dendritic regeneration and synaptic reconstruction in cultured rat cortical neurons damaged by Amyloid $\beta(25-35)$.[2] In addition, in vivo experiments indicated that orally administered sominone enhanced memory and axonal density in the brains of normal mice.[4] In light of its high potential as a reconstructing agent of neuronal networks, sominone can be considered as an extraordinarily promising candidate for antidementia therapeutic agents.

To the best of our knowledge, no report on synthetic studies has focused on sominone itself as a synthetic target, despite such biological interest. Thus, we planned to develop an efficient synthetic method of sominone and its analogues, aiming at extensive SAR studies and in depth in vivo evaluations. Herein, we describe the synthesis of sominone utilizing a ring-closing metathesis (RCM) strategy and the discovery of a closely related analogue, 1-deoxy-24-nor-sominone (named "denosomin"), possessing much higher efficacy than sominone as an antidementia agent.

Our synthetic plan for sominone (**1**) and simplified derivatives **2**−**8** is depicted in Scheme 1. The key step of the synthesis is the construction of a δ-lactone moiety using the RCM reaction. Although synthetic studies of withanolides have been reported by several groups,[5] many of them required complex and multistep transformations in order to construct the δ-lactone moiety. On the other hand, recent advances in metathesis chemistry, including RCM, have allowed for the convenient construction of various ring systems.[6] In particular, continuous efforts exploring the efficient Ru-based catalysts have brought about significant improvements in the syntheses of sterically hindered cyclic compounds containing a tri- or tetra-substituted olefin structures.[7] We believed that a suitable choice of a catalyst could realize a concise δ-lactone formation of sominone and its derivatives via the RCM reaction. We prepared the RCM substrates from steroidal ketone compounds **9** and **10**.

The synthesis of sominone (**1**) and analogous compounds **2**−**8** commences with a standard transformation sequence starting from commercially available dehydroepiandrosterone (**10**) and its 1α-hydroxy derivative **9** (Scheme 2).[8] Three-

Scheme 2



step transformation of **9** and **10** into known alcohols **11** and **12** were achieved via Wittig olefination, followed by the ene reaction using modification of a reported method.[9] Stereoselective reduction and PDC oxidation efficiently proceeded to give the aldehydes **13** and **14** in high yield, respectively.[10]

Methallylation or allylation of aldehydes **13** and **14** was investigated to obtain a footing for the RCM substrates. Thus, methallylation was performed utilizing a Barbier-type addition reaction to afford alcohols **15a**,**b** and **16a**,**b** in good yields. Although scarcely any diastereoselectivity at the 22-position was observed, chromatographic separation was easily attained to give both diastereomers (**a** and **b**) in pure form. On the other hand, asymmetric allylation using (+)- or (−)-*B*-allyldiisopinocampheylborane[11] produced the corresponding allyl adducts **15c** and **16c** or **15d** and **16d**, respectively, with high stereoselectivity. In every case, only trace amounts of the opposite diastereomers were detected on TLC. Installation of the other olefin unit required for RCM, containing a hydroxymethyl substitu-

(3) (a) Sahai, M. *J. Nat. Prod.* **1985**, *48*, 474. (b) Atta-ur-Rahman; Jamal, S. A.; Choudhary, M. I. *Heterocycles* **1992**, *34*, 689.

(4) Tohda, C.; Joyashiki, E. *Br. J. Pharmacol.* **2009**, web-released.

(5) For example, see: (a) Hirayama, M.; Gamoh, K.; Ikekawa, N. *J. Am. Chem. Soc.* **1982**, *104*, 3735. (b) Perez-Medrano, A.; Grieco, P. *J. Am. Chem. Soc.* **1991**, *113*, 1057. (c) Tsubuki, M.; Kanai, K.; Keino, K.; Kakinuma, N.; Honda, T. *J. Org. Chem.* **1992**, *57*, 2930.

(6) For recent reviews, see: (a) Gradillas, A.; Perez-Castells, J. *Angew. Chem., Int. Ed.* **2006**, *45*, 6086. (b) Grubbs, R. H. *Tetrahedron* **2004**, *60*, 7117. (c) Nicolaou, K. C.; Bulger, P. G.; Sarlah, D. *Angew. Chem., Int. Ed.* **2005**, *44*, 4490. (d) Deiters, A.; Martin, S. F. *Chem. Rev.* **2004**, *104*, 2199.

(7) For reviews, see: Schrodi, Y.; Pederson, R. L. *Aldrichim. Acta* **2007**, *40*, 45.

(8) Dodson, R. M.; Goldkamp, A. H.; Muir, R. D. *J. Am. Chem. Soc.* **1960**, *82*, 4026.

(9) (a) Konno, K.; Ojima, K.; Hayashi, T.; Takayama, H. *Chem. Pharm. Bull.* **1992**, *40*, 1120. (b) Deng, L.; Wu, H.; Yu, B.; Jiang, M.; Wu, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2781.

(10) (a) Konno, K.; Ojima, K.; Hayashi, T.; Tanabe, M.; Takayama, H. *Chem. Pharm. Bull.* **1997**, *45*, 185. (b) Corey, E. J.; Grogan, M. J. *Tetrahedron Lett.* **1998**, *39*, 9351.

(11) For a review of allylborane reagents, see: Ramachandran, P. V. *Aldrichim. Acta* **2002**, *35*, 23.

ent, was carried out through a two-step sequence; esterification with PMP-protected acid chloride, followed by removal of the PMP group by CAN oxidation (accompanied by cleavage of the 3-TBS ether on the A-ring).[12] Thus, preparation of the requisite RCM substrates **19a**−**d** and **20a**−**d** from compounds **13** and **14** was accomplished. These transformations are summarized in Scheme 3 and Table 1.

Scheme 3

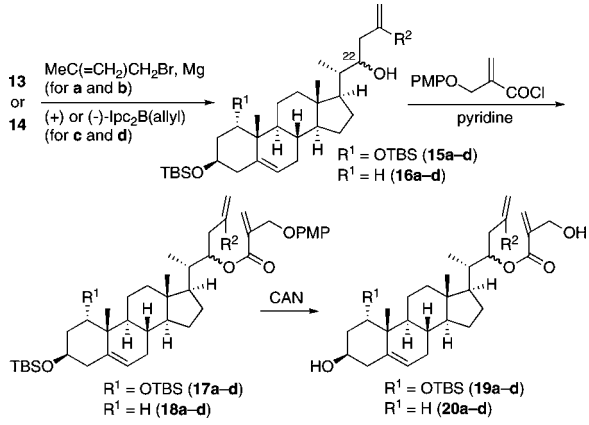


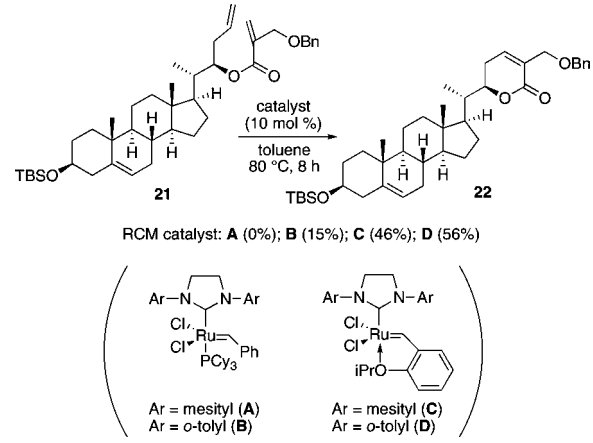
Table 1. Isolation Yields of the Compounds **15**−**20**

	yield (%)					
compd	**15**	**16**	**17**	**18**	**19**	**20**
a (R^2 = Me, 22β-O)	42[a]	44[b]	78	84	64	65
b (R^2 = Me, 22α-O)	32[a]	43[b]	76	86	54	60
c (R^2 = H, 22β-O)	61	78	70	75	57	67
d (R^2 = H, 22α-O)	65	73	84	88	47	74

[a] These diastereomers could be separated by column chromatography. [b] These diastereomers could be separated by column chromatography.

For the purpose of seeking an efficient RCM catalyst of the present synthesis, an initial survey was performed using several Ru-based catalysts (**A**−**D**)[6,7] and *O*-benzylated substrate **21** (Scheme 4).[13] RCM of homoallyl acrylates to form δ-lactone compounds has been recently reported, albeit in a much more simple compound system, in which the second-generation Grubbs' catalyst (**A**) most efficiently gave rise to ring-closure, particularly in the cases of tri- or tetrasubstituted olefin formations.[14] However, cyclization of substrate **21** did not occur in the presence of catalyst **A** at 80 °C. In light of a recent report regarding the effects of *N*-aryl substituents of NHC ligands on the catalyst activity,[15] we next attempted the *o*-tolyl variant **B** for the RCM of **21**, leading to the formation of desired δ-lactone **22** in low yield (15%). However, employment of the second-generation Hoveyda−Grubbs' catalyst (**C**) significantly improved the cyclization yield (46%), and the corresponding *o*-tolyl variant **D** gave the best result (56% yield). Therefore, catalyst **D** was selected for further investigation.

Scheme 4



Less sterically hindered substrates **19c**,**d** and **20c**,**d** (R^2 = H) were first subjected to the RCM reaction using catalyst **D** in toluene at 80 °C. Excellent results were obtained for **19c** and **20c**, possessing a natural-type *R* configuration at the 22-position, while non-natural diastereomers **19d** and **20d** gave moderate yields and required a somewhat prolonged reaction time. Although the rationale for the differences in RCM reactivity between these diastereomers due to the configurations at the 22-position remained unclear, construction of the δ-lactone with the trisubstituted olefin structure was satisfactorily accomplished by means of the RCM strategy (Scheme 5, Table 2). For the synthesis of sominone,

Scheme 5

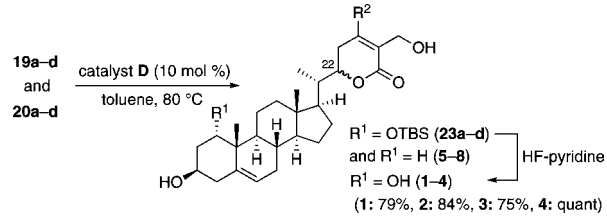


Table 2. RCM of the Substrates **19a**−**d** and **20a**−**d**

substrate		time (h)	product	yield[a] (%)
19a	R^2 = Me, 22β-O	2	**23a**	22(92)
19b	R^2 = Me, 22α-O	2	**23b**	24(95)
19c	R^2 = H, 22β-O	2	**23c**	85(85)
19d	R^2 = H, 22α-O	10	**23d**	58(70)
20a	R^2 = Me, 22β-O	3	**5**	17(64)
20b	R^2 = Me, 22α-O	3	**6**	15(75)
20c	R^2 = H, 22β-O	2	**7**	88(98)
20d	R^2 = H, 22α-O	5	**8**	52(61)

[a] Isolation yields. Yields in parentheses are brsm.

we attempted challenging RCM with the substrate **19a** involving tetrasubstituted olefin formation (R^2 = Me) under

the same reaction conditions. Although the reaction was revealed to be rather sluggish as expected, the required cyclization afforded δ-lactone **23a** in a 22% isolation yield after 2 h without any side reactions (92% yield based on the recovered starting material). Because the longer reaction time caused a considerable decrease in substrate recovery with an almost equal isolation yield of product, a recycling process with a shorter reaction time (2−3 h) was considered to be better suited for this RCM transformation. Similarly, substrates **19b** and **20a**,**b** could be transformed into the cyclization products **23b**, **5**, and **6**, respectively, using catalyst **D** (Scheme 5, Table 2). For compounds **23a**−**d**, the TBS group at the 1-position was removed upon treatment with HF−pyridine in high yield. Thus, the synthesis of the biologically important withanolide, sominone (**1**) and related compounds **2**−**8**, was accomplished through a concise and efficient synthetic route. The NMR data for the synthetic sample of sominone were in good agreement with those reported in the literature.[3]

We then examined the effects of the sominone-related compounds on axonal extensions in amyloid β (Aβ)-damaged neurons (Alzheimer's disease model) as an index of neuronal network reconstructing activity. Test compounds **1**−**8** were administered to rat cortical neurons 3 days after treatment with Aβ(1−42), and axon length (Figure 1, left) was measured after an additional 5 days. Axon length was shorter in the cells treated with 5 μM Aβ(1−42) followed by vehicle (0.1% DMSO) than in control cells (without Aβ treatment). On the other hand, axon length was significantly longer in the Aβ(1−42)-treated cells treated in combination with compounds **1**, **4**−**7** (1 μM), or nerve growth factor (NGF) (100 ng/mL) than cells treated with vehicle alone. In particular, compound **7** (denosomin) exhibited the most potent activity for axonal regeneration among the new derivatives, and the potency reached a level comparable to that of sominone (**1**).

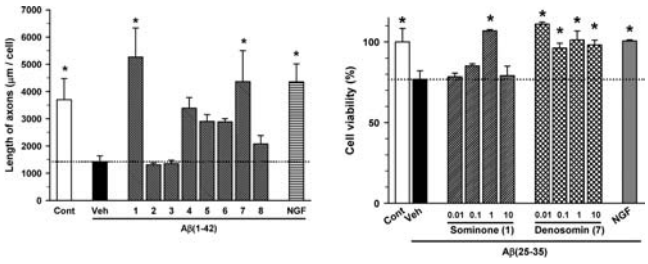


Figure 1. Effects of compounds **1**−**8** (1 μM) on axonal extensions following Aβ(1−42)-induced atrophy (left) and protective effects of compounds **1** and **7** (0.01−10 μM) on Aβ(25−35)-induced cell death (right) in rat cortical neurons. Positive control: NGF (100 ng/mL). The results are presented as the mean ± SD *P < 0.05 vs Veh/Aβ.

Encouraged by these results, we further investigated the protective effects of sominone (**1**) and denosomin (**7**) on Aβ(25−35)-induced cell death. Rat cortical neurons were treated with various concentrations of each compound or vehicle (0.1% DMSO), simultaneously with 20 μM Aβ(25−35), and cell viability was determined after 2 days. The rate of cell survival was significantly decreased by Aβ(25−35) treatment compared with controls. As shown in Figure 1 (right), sominone (**1**) increased cell viability up to control levels at a dose of 1 μM, but lower doses resulted in a considerable decrease in the effects. On the other hand, the protective effects of denosomin (**7**) on Aβ(25−35)-induced cell death were revealed to be much more striking, with peak activity seen at 0.01 μM. The effects of denosomin (**7**) were more potent than those of NGF, thus suggesting that denosomin is an attractive target molecule in the development of novel anti-Alzheimer's disease medicines.

In this paper, we describe the synthesis of sominone and related compounds utilizing the RCM reaction to construct the δ-lactone side chain. During the study, we found a novel artificial compound, denosomin, to be a highly promising candidate for new antidementia chemotherapy, with activity exceeding that of the original natural compound, sominone. It is important to note that the efficiency of denosomin synthesis is superior to that of sominone, as (1) availability of the starting material (dehydroepiandrosterone) is good, (2) stereochemistry of the 22-position can be completely controlled using the Ipc_2B(allyl) reagent, and (3) the key RCM reaction proceeds more smoothly due to the lack of the 24-methyl group. Thus, the synthesis of denosomin was achieved in 9 steps with 20.2% overall yield starting from commercially available dehydroepiandrosterone (**10**). Taking advantage of the synthetic efficiency, large-scale preparation and in-depth in vivo studies of denosomin, as well as elucidation experiments of the mechanism of action, have been performed in our laboratory, and their results will be reported in due course.

Supporting Information Available: Experimental procedures and compound characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL901553W

(12) Cyclization of the compound **17a** (precursor of sominone) did not proceed under any RCM conditions .

(13) Although this substrate was first prepared prior to **19** and **20**, deprotection of the cyclized product **22** by hydrogenolysis was found to be troublesome.

(14) D'Annibale, A.; Ciaralli, L.; Bassetti, M.; Pasquini, C. *J. Org. Chem.* **2007**, *72*, 6067.

(15) (a) Berlin, J. M.; Campbell, K.; Ritter, T.; Funk, T. W.; Chlenov, A.; Grubbs, R. H. *Org. Lett.* **2007**, *9*, 1339. (b) Stewart, I. C.; Ung, T.; Pletnev, A. A.; Berlin, J. M.; Grubbs, R. H.; Schrodi, Y. *Org. Lett.* **2007**, *9*, 1589.