Synthesis of 9-Deoxycotylenol Derivatives and Their Seed Germination-Stimulating Activity

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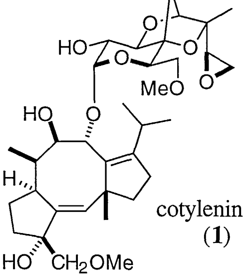
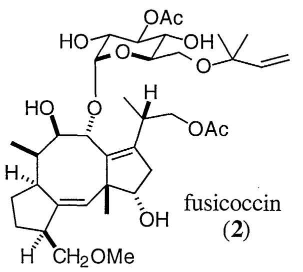
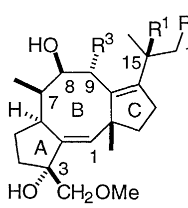
9-Deoxy-15-methoxymethoxycotylenol and 9-deoxy-19-methoxymethoxycotylenol were synthesized to clarify a role of the 9α-hydroxyl of cotylenol, a common aglycon of cotylenins possessing potent plant growth-regulating activities. Both of the 9-deoxycotylenol derivatives stimulated germination of lettuce seeds and therefore it has been clarified that the 9α-hydroxyl of cotylenol is not essential for the biological activities.


cotylenin A
(**1**)


fusicoccin
(**2**)



cotylenol (**3**) : $R^1 = H, R^2 = H, R^3 = OH$
4a : $R^1 = OMOM, R^2 = H, R^3 = H$
4b : $R^1 = H, R^2 = OMOM, R^3 = H$
5a : $R^1 = OH, R^2 = H, R^3 = H$
5b : $R^1 = H, R^2 = OH, R^3 = H$

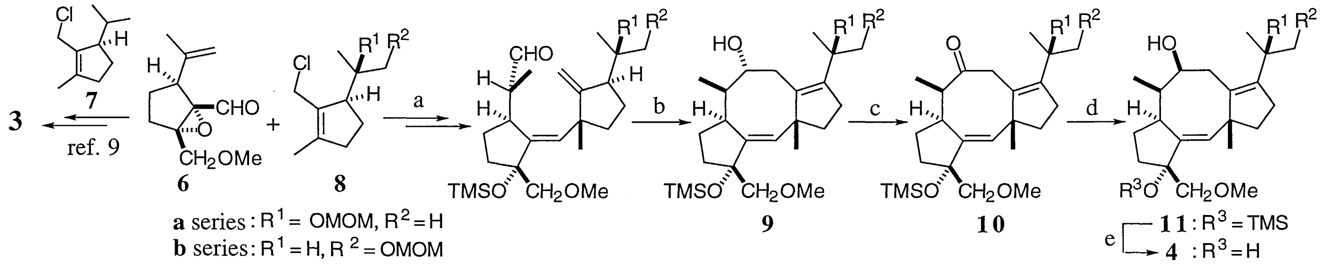
Cotylenins[1] and fusicoccins,[2] 5-8-5-membered tricyclic diterpenoid glycosides, are known to have identical yet unique plant growth-regulating activities.[3] More recently, a target protein of fusicoccin (**2**) has been purified and identified as a member of 14-3-3 protein family.[4] Since 14-3-3 proteins, originally identified as brain proteins of mammalian, have been considered to take an important role in intracellular signal transductions,[5] cotylenins and fusicoccins have now enhanced importance as probes of the intracellular processes.[6]

To exploit the unique properties of this class of compounds for the study of biological regulatory pathways, we planned to synthesize active analogs of cotylenol which can be easily accessible and carry an additional substituent for further functionalizations. It has been well demonstrated that cotylenol (**3**),[7] a common aglycon of cotylenins, exhibits potent plant growth-regulating activities as strong as cotylenins.[8] This fact suggested that 9α-hydroxyl of **3** might not be important for the activities, since it acts just as a linker for sugar moiety in cotylenins. Therefore, 9-deoxycotylenol derivatives, **4a**,**b** and **5a**,**b**, were selected for the synthetic targets in this study. These have an additional hydroxyl function at C-15 or C-19.

We have already reported the synthesis of **3** starting from the condensation of two parts of appropriately functionalized iridoid synthons, **6** and **7**.[9] Here, starting from the condensation of **6** and **8a**[10] or **8b**,[11] 8-*epi*-9-deoxycotylenol derivatives (**9a** and **9b**) were synthesized through eight-membered ring formation by means of thermally induced ene reaction, as has been reported. In order to convert the 8α-hydroxyl into its epimer, **9a** and **9b** were oxidized with PDC to the corresponding ketones, **10a** and **10b**. Then, these ketones were reduced with DIBALH. While **10a** was reduced to give **11a** stereoselectively, **10b** afforded **11b** and **9b** in a ratio of 3:1. Finally, **4a** and **4b** were obtained by removal of TMS protecting group.

It has been reported that the chair-sofa conformation of the central eight-membered ring is essential for the biological activities.[12] On an irradiation of the secondary methyl at C-7, an NOE enhancement was observed at the 9β proton in the case of **11**, but not observed in the case of **9**. These facts suggested that the eight-membered rings of **11a** and **11b** possess chair-sofa conformations like cotylenins[1] and fusicoccins,[12] while those of **9a** and **9b** have boat-sofa conformations.

Deprotection of MOM group of **9** leading to the triols (**5**), was somewhat troublesome, because the allylic and tertiary hydroxyl at C-3 was sensitive to an acid. The MOM group of the acetate (**12a**) derived from **9a** could be removed by an acetic acid-treatment to afford **13**. This deprotection proceeded most likely *via* an allylic cation intermediate, because a cyclic ether **15** was formed when the same conditions were directly employed on **9a**. In a similar way described above (from **9** to **4**), **13** was

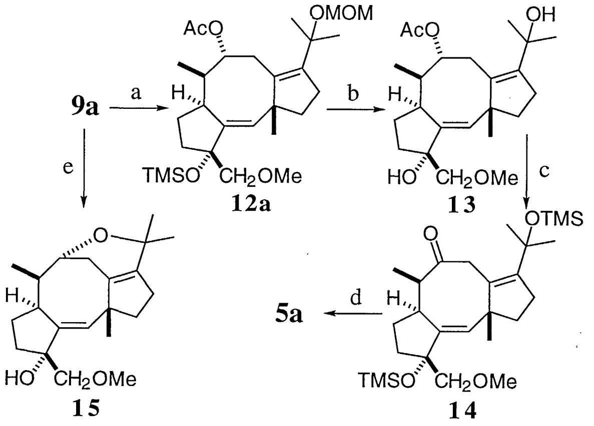


Scheme 1. [reagents (yields for **a** and **b** series, respectively)]. a: according to the method reported in ref. 9, 11 steps (9.5%, 15%), b: 160 °C/xylene (86%, 93%), c: PDC/CH_2Cl_2 (52%, 70%), d: DIBALH/toluene (92%, 65% (**9b**, 22%)), e: Bu_4NF/THF (93%, 95%).

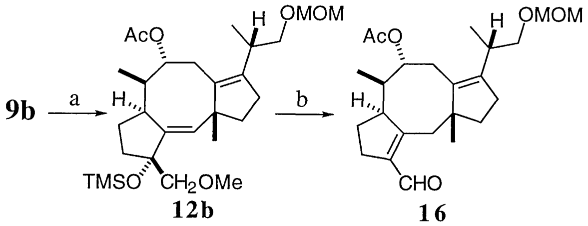
Table.[a] Stimulating activity on germination of lettuce seeds[b] (germination ratio, %) in the presence of (±)-abscisic acid (2 ppm)[c]

Time (hr)	Compounds and concentrations [ppm (nM)]															none
	4a			**5a**			**4b**			**5b**			**1**			
	10 (25)	50 (127)	100 (253)	10 (29)	50 (143)	100 (285)	1 (2.5)	5 (13)	10 (25)	1 (2.9)	5 (14)	10 (29)	1 (1.6)	5 (8.2)		
24	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0
48	0	2.5	7.5	0	0	0	0	72.5	95	0	0	35	27.5	90		0
72	0	2.5	12.5	0	0	7.5	0	82.5	95	0	15	60	75	97.5		0
96	0	15	25	0	2.5	37.5	0	85	95	0	25	67.5	80	100		0
120	0	30	52.5	0	2.5	50	—	—	—	—	—	—	—	—		0
144	0	67.5	95	0	2.5	90	—	—	—	—	—	—	—	—		0

[a]Experiments were carried out at 25 ℃ in the dark using 40 seeds. [b]'mamma lettuce', Nakahara Seed Production Co. [c]Without (±)-abscisic acid, 100% germination was observed within 48 hr.



Scheme 2. [reagents (yields)]. a: Ac_2O / Py (100%), b: aq.AcOH/THF (82%), c: i, TMSCl/Py, ii, $LiAlH_4$/THF, iii, PDC/CH_2Cl_2 (32%), d: i, DIBALH/toluene, ii, Bu_4NF/THF (82%), e: aq.AcOH/THF (~50%).



Scheme 3. [reagents (yields)]. a: Ac_2O/Py (83%), b: TMSBr/CH_2Cl_2 (30%).

converted into **5a** through a ketone **14**.

On the other hand, the MOM group of the acetate (**12b**) derived from **9b** was stable in same conditions. This fact also supported the intermediacy of the allylic cation in the conversion of **12a** to **13**. When TMSBr was employed on this procedure, **16** was obtained by elimination of the C-3 hydroxyl prior to deprotection of the MOM group. Eventually, TBS protection of the hydroxyl in the starting chloride has been necessary for the synthesis of the other triol **5b**.[13]

Evaluation of biological activities of 9-deoxycotylenol derivatives, thus obtained, was carried out by checking germination-stimulating activity using lettuce seeds. The results are summarized in the table. Although all synthetic analogs[14] were less active than cotylenin A (**1**),[1] a major constituent of natural cotylenins, **4b** still retained strong stimulating activity on lettuce seed germination. Also, **4a** retained the activity but at much higher threshold concentrations. The triols, **5a** and **5b**, were slightly less active than the corresponding MOM derivatives, **4a** and **4b**, respectively. This fact might correlate with higher polarity of the triols.[15]

As a conclusion, retention of the stimulating activity in 9-deoxycotylenol derivatives, especially in **4b**, clearly suggested that both the 9α-hydroxyl and the sugar moiety attached on it are not essential for the biological activities of this class of compounds. The difference observed in **4a** and **4b** revealed that the shape and/or the rotational conformation of the side chain on the C-ring strongly affect the degree of the stimulating activity.

Since further functionalization using the primary hydroxyl function of **5b**[13] is promising, studies to create new tools for targeting 14-3-3 proteins are currently in due course.

References and Notes

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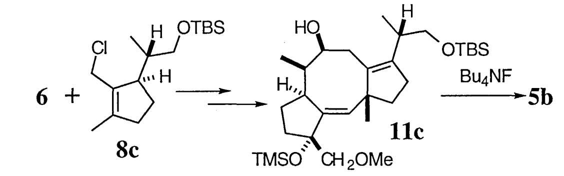
13 Starting from the condensation of **6** and **8c**, **11c** was prepared similarly. Removal of silyl-protecting groups in **11c** afforded **5b**. Details will be reported elsewhere.



14 The seed germination activities of the diols derived from **9a** and **9b**, C-8 epimers of **4a** and **4b**, were negligible.

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