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Synthesis of 9-Deoxycotylenol Derivatives Carrying a Fluorescent Chromophore

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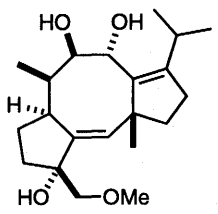
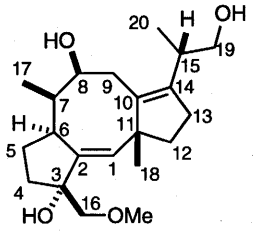
During the course of our study on the structure-activity relationships of cotylenol, a plant-growth regulating diterpenoid, 9-deoxycotylenol has been found to retain the biological activities. Here, aiming to create new tools for targeting 14-3-3 proteins which are the biding proteins of this class of molecules and have recently been regarded to be the key regulatory proteins in the intracellular signal transductions, the synthesis of 9-deoxycotylenol derivatives carrying a fluorescent chromophore is reported.

Introduction

Cotylenins were isolated as leaf growth substances from an unidentified species of *Cladosporium*,[1] while fusicoccins were originally isolated as phytotoxic substances responsible for a wilting disease of peach and almond trees caused by a phytopathogenic fungus, *Fusicoccum amygdali*.[2] In spite of these opposite effects on higher plants, their biological activities, *e.g.*, stimulation of seed germination, cell enlargement, and stomatal opening, are regarded to be identical in principle.[3] More recently, the binding protein of fusicoccin has been found to belong to the class of 14-3-3 proteins,[4] which were originally identified as brain proteins in mammals. Since 14-3-3 proteins have been considered to play an important role in intracellular signal transductions, cotylenins and fusicoccins have become increasingly important as tools for the study of biological regulatory pathways.[5]



A: cotylenol



1: 9-deoxy-19-hydroxycotylenol

As cotylenol (**A**),[6] a common aglycon of the cotylenins, possesses the same biological activities as the cotylenins themselves, the diterpenoid plays an essential role in the biological activity of the compounds. During the course of our study on the structure-activity relationships of this class of molecules, 9-deoxy-19-hydroxycotylenol (**1**) was found to retain potent germination-stimulating activity on lettuce seeds.[7] Here, we report the synthesis of fluorescent derivatives of **1**, aiming to develop new tools for targeting 14-3-3 proteins.

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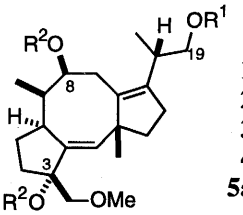
Results and Discussion

It is obvious that a fluorescent chromophore or a linker group should be attached on the 19-hydroxy group from the fact that a 19-*O*-methoxymethyl derivative of **1** (19-MOM-**1** in Table 1) has been found to be more active than **1** itself.[7]

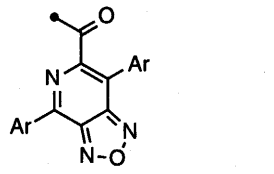


1 : R^1, R^2 = H — a
2 : R^1, R^2 = TMS — b
3 : R^1 = H, R^2 = TMS — c
4 : R^1 = **Fa**, R^2 = TMS — d
5a : R^1 = **Fa**, R^2 = H

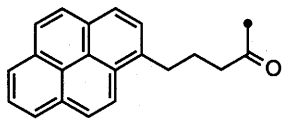
Scheme 1. [Reagents (yields)]. a: TMSCl / pyridine (73%), b: PPTS / aq. THF (85%), c: **Fa**-OH, EDCI, DMAP / CH_2Cl_2 (60%), b: Bu_4NF / THF (74%).

As a sure way to acylate the 19-hydroxy group selectively, **1**[7] was converted into **3** in a two-step procedure as shown in Scheme 1. Then, **3** was acylated with 4,7-bis(4-chlorophenyl)-[1,2,5]oxadiazolo[3,4-*c*]-pyridine-6-carboxylic acid (**Fa**-OH),[8] the ester derivatives of which are known to have a strong fluorescence in the visible region, with the aid of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP).[9] Deprotection of the TMS groups of the resulting **4** led to the fluorescent analog **5a**.

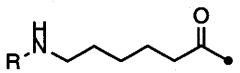
Instead of the multi-step manipulation mentioned above, we also tried the direct esterification of **1**.



Fa : Ar = 4-ClC$_6$H$_4$
Fb : Ar = C$_6$H$_5$



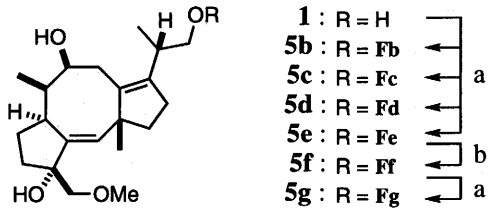
Fc



Fd : R = COOBu^t
Fe : R = COOCH$_2$C$_6$H$_5$
Ff : R = H
Fg : R = **Fa**

Fig. 1. Fluorescent and linker groups used in this study.

The primary hydroxy group (19-hydroxyl) of **1** could be transformed selectively in the presence of other secondary (8β-hydroxyl) and tertiary (3α-hydroxyl) hydroxy groups to afford the mono-acylated products in moderate yields. Thus, fluorescent analogs **5b** and **5c** were obtained easily by the condensation of **1** with 4,7-diphenyl-[1,2,5]oxadiazolo[3,4-*c*]pyridine-6-carboxylic acid (**Fb**-OH)[9] and 1-pyrenebutyric acid (**Fc**-OH), respectively. To attach the chromophore at the remote location from the diterpene nucleus, **5d** and **5e**, which have a versatile linker group for further functionalization, were also prepared using *N-tert*-butoxycarbonyl(BOC)- or *N*-benzyloxycarbonyl(CBZ)-6-aminohexanoic acid (**Fd**-OH and **Fe**-OH). CBZ-protected derivative **5e** was converted further, by the hydrogenolytic deprotection of the CBZ group, to the free amine **5f**, which was then condensed with **Fa**-OH leading to **5g**.



1 : R = H
5b : R = **Fb**
5c : R = **Fc**
5d : R = **Fd**
5e : R = **Fe**
5f : R = **Ff**
5g : R = **Fg**

Scheme 2. [Reagents (yields)]. a: **F**(**a**–**e**)-OH, EDCI, DMAP / CH_2Cl_2 (**5b**, 74%; **5c**, 82%; **5d**, 75%; **5e**, 75%; **5g**, 21%), b: H_2, Pd-C / MeOH (77%).

Preliminary evaluation of the biological activities of the 9-deoxycotylenol derivatives, thus obtained, was carried out by checking the germination-stimulating activity using lettuce seeds. The antagonistic nature of these compounds toward (±)-abscisic acid was evaluated and the results are summarized in Table 1.

Disappointingly, all the fluorescent analogs (**5a**, **5b**, **5c**, and **5g**) lost the germination-stimulating activity, whereas the variant **5d** with a tethered linker group retained a stimulating activity as strong as 19-MOM-**1**, which was the most potent 9-deoxycotylenol derivative among our synthetic variants.[8]

Table 1.[a] Stimulating activity on germination of lettuce seeds[b] (germination ratio, %) in the presence of (±)-abscisic acid (2 ppm)[c].

Time	Compounds and concentrations (nM)							
	1		19-MOM-**1**		**5a**		**5b**	
(hr)	14	29	13	25	7.0	14	15	31
24	0	35	72.5	95	0	0	0	0
48	15	60	82.5	95	0	0	0	0
72	25	67.5	85	95	0	0	0	0

Time	Compounds and concentrations (nM)						
	5c		**5d**		**5g**		none
(hr)	16	81	8.9	18	12	24	
24	0	0	0	10	0	0	0
48	0	0	37.5	80	0	0	0
72	0	0	97.5	100	0	0	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.

The retention of the biological activity in **5d** will potentially allow to derive other functional analogs such as polymer-supported derivatives for affinity chromatography. The reasons for the diminishment of the activity of the fluorescent analogs are not clear at this point. Because of the intracellular location of the 14-3-3 proteins,[6] their potential ligands must pass through the plasma membrane. The fluorescent analogs might not be able to reach their targets because of an interaction between their aromatic moiety and the plasma membrane. Therefore, the negative results obtained here do not necessarily mean that these compounds have lost the affinity towards 14-3-3 proteins. In this sense, for the proper evaluation of these compounds, assays using a cell-free system should be awaited.

Experimental

Melting points were measured with a Yanagimoto Micro Melting Point Apparatus. Elemental analyses were performed by the Institute of Central Analysis, Kyushu University. The NMR spectra were recorded on LA 400 and/or LA 600 in $CDCl_3$, unless otherwise noted. The mass spectra were measured with a JMS-70 spectrometer; among the data, only the molecular ion peak or the nearest peak as the alternative, and the base peak are recorded. The IR spectra were measured as KBr disks for crystalline compounds or as liquid films inserted between NaCl plates for oily compounds, using JASCO IR-A 102 spectrometer. Optical rotations were measured with a Union PM-101 apparatus. All solvents were pre-dried by standard methods unless stated otherwise. All reaction involving nonaqueous solutions were performed under an inert atmosphere. The

stationary phase for column chromatography was Wakogel C-300 and the eluent was a mixture of hexane and ethyl acetate. The organic extracts were dried over anhydrous magnesium sulfate unless otherwise stated.

*Silylation of 9-deoxy-19-hydroxycotylenol (**1**). Formation of 16-methoxy-3α,8β,19-tris(trimethylsilyloxy)fusicocca-1,10(14)-diene (**2**).* An anhydrous pyridine solution (2.0 cm^3) of 9-deoxy-19-hydroxycotylenol (**1**, 62 mg, 0.18 mmol) and TMSCl (0.5 cm^3) was stirred at room temperature for 12 h and the mixture was diluted with aqueous $NaHCO_3$ and extracted with ether. The organic layers were washed successively with water and brine, dried, and concentrated under reduced pressure. The residue was chromatographed to give **2** (72 mg, 73%) as a colorless oil.

2: Found: C 63.63, H 10.21%; Calcd for $C_{30}H_{58}O_4Si_3$: C 63.54, H 10.31%; $[\alpha]_D^{25}$ –8.7 (*c* 1.26, $CHCl_3$); EIMS: *m/z* 551 (M^+–15, 11) and 521 (base peak); IR (NaCl): ν 2954, 2876, 1452, 1383, 1311, 1249, 1204, 1101, 1070, 985, 867, 838, and 752 cm^{-1}; ^{1}H-NMR (600 MHz): δ 0.077 (9H, s), 0.082 (9H, s), 0.14 (9H, s), 0.78 (3H, d, *J*=7.0 Hz), 0.99 (3H, d, *J*=7.0 Hz), 1.13 (3H, s), 1.18 (1H, m), 1.41 (1H, dtd, *J*=13.0, 7.3, 1.5 Hz), 1.59-1.64 (2H, m), 1.79 (1H, ddd, *J*=12.1, 7.1, 1.5 Hz), 1.84-1.93 (2H, m), 2.05-2,11 (2H, m), 2.14 (1H, dd, *J*=13.4, 11.7 Hz), 2.30 (1H, m), 2.72 (1H, dm, *J*=2.4 Hz), 2.79 (1H, sext., *J*=7.0 Hz), 2.93 (1H, dd, *J*=10.6, 1.5 Hz), 3.31 (1H, d, *J*=10.4 Hz), 3.37 (3H, s), 3.45 (1H, dd, *J*=9.7, 7.0 Hz), 3.47 (1H, dd, *J*=9.7, 7.0 Hz), 3.80 (1H, dt, *J*=11.5, 4.0 Hz), and 5.43 (1H, d, *J*=2.7 Hz); ^{13}C-NMR (150 MHz): δ –0.4 (3C), 0.3 (3C), 2.3 (3C), 7.6, 15.1, 27.3, 27.4, 29.0, 31.7, 33.7, 36.1, 40.1, 42.2, 44.8, 52.6, 59.4, 65.9, 76.8, 77.4, 85.4, 134.5, 136.2, 139.4, and 141.5.

*Selective desilylation of **2**. Formation of 16-methoxy-3α,8β-bis(trimethylsilyloxy)fusicocca-1,10(14)-dien-19-ol (**3**).* A moistened THF solution (50 cm^3) of **2** (60 mg, 0.11 mmol) and PPTS (a catalytic amount) was stirred at 0 ℃ for 5 h. After the usual workup, the chromatographic purification of the mixture on silica-gel afforded **3** (45 mg, 85%) as a colorless oil.

3: $[\alpha]_D^{26}$ –53.4 (*c* 0.58, $CHCl_3$); IR (NaCl): ν 3446, 2954, 2874, 2820, 1667, 1452, 1312, 1249, 1066, 985, 891, 839, and 756 cm^{-1}; ^{1}H-NMR (600 MHz): δ 0.08 (9H, s), 0.14 (9H, s), 0.78 (3H, d, *J*=7.1 Hz), 1.00 (3H, d, *J*=7.0 Hz), 1.17 (3H, s), 1.20 (1H, m), 1.40 (1H, ddm, *J*=7.3, 1.5 Hz), 1.64 (1H, m), 1.83-1.89 (2H, m), 1.96 (1H, dm, *J*=7.9 Hz), 2.06-2.10 (2H, m), 2.21 (1H, dd, *J*=13.6, 11.5 Hz), 2.30 (1H, m), 2.70 (1H, dm, *J*=2.4 Hz), 2.89 (1H, qm, *J*=6.8 Hz), 2.93 (1H, dd, *J*=10.6, 1.5 Hz), 3.31 (1H, d, *J*=10.6 Hz), 3.37 (3H, s), 3.4-3.52 (2H, m), 3.79 (1H, dt, *J*=11.5, 4.0 Hz), and 5.44 (1H, d, *J*=2.7 Hz); ^{13}C-NMR (150 MHz): δ 0.3 (3C), 2.3 (3C), 7.6, 14.7, 26.7, 27.5, 29.1, 31.6, 33.7, 36.1, 40.2, 42.4, 44.7, 52.9, 59.4, 65.7, 76.7, 77.3, 85.4, 133.9, 139.7, 139.8, and 140.4.

*Esterification of **3** with 4,7-bis(4-chlorophenyl)-[1,2,5]oxadiazolo[3,4-c]pyridine-6-carboxylic acid (**Fa**-OH). Formation of 16-methoxy-3α,8β-bis(trimethylsilyloxy)-fusicocca-1,10(14)-dien-19-yl 4,7-bis(4-chlorophenyl)-[1,2,5]oxadizolo[3,4-c]pyridine-6-carboxylate (**4**).* To a solution of **3** (20 mg, 0.040 mmol) and 4,7-bis(4-chlorophenyl)-[1,2,5]oxadiazolo-[3,4-*c*]pyridine-6-carboxylic acid (**Fa**-OH, 19 mg, 0.049 mmol) in CH_2Cl_2 (1.5 cm^3) was added 4-(dimethylamino)pyridine (11.4 mg, 0.093 mmol) and EDCI (12 mg, 0.063 mmol). After the mixture was stirred at room temperature for 7 h, it was diluted with aq. $NaHCO_3$ and extracted with ethyl acetate. The organic extract was washed with brine, dried and evaporated. Chromatography of the residue afforded **4** (21 mg, 60%) as yellow crystals and the starting material **3** (5 mg, 25%).

4: mp. 148-149 ℃; Found: C 62.77, H 6.67%; Calcd for $C_{45}H_{57}N_3Cl_2O_6Si$: C 62.63, H 6.66%; $[\alpha]_D^{25}$ –2.1 (*c* 0.94, $CHCl_3$); FABMS: *m/z* 818 (M^+-45, 5.6) and 73 (base peak); IR (KBr): ν 2954, 2876, 1740, 1595, 1539, 1487, 1408, 1250, 1160, 1093, 1068, 1015, 993, 892, 864, 839, and 754 cm^{-1}; ^{1}H-NMR (600 MHz): δ 0.08 (9H, s), 0.09 (9H, s), 0.75 (3H, d, *J*=7.0 Hz), 0.98 (3H, d, *J*=7.0 Hz), 1.04 (3H, s), 1.18 (1H, m), 1.39 (1H, ddm, *J*=7.5, 1.3 Hz), 1.55-1.63 (2H, m), 1.79 (1H, ddd, *J*=12.1, 7.5, 1.6 Hz), 1.86 (1H, m), 1.90 (1H, m), 2.06-2.09 (2H, m), 2.12 (1H, dd, *J*=13.4, 11.4 Hz), 2.20 (1H, m), 2.65 (1H, dm, *J*=2.4 Hz), 2.91 (1H, dd, *J*=10.6, 1.3 Hz), 3.03 (1H, sext., *J*=7.0 Hz), 3.29 (1H, d, *J*=10.6 Hz), 3.36 (3H, s), 3.72 (1H, dm, *J*=11.4 Hz), 4.11 (1H, dd, *J*=10.6, 7.0 Hz), 4.24 (1H, dd, *J*=10.6, 7.5 Hz), 5.42 (1H, d, *J*=2.7 Hz), 7.51 (2H, ddd, *J*=8.6, 2.6, 2.0 Hz), 7.57 (2H, dm, *J*=8.6 Hz), 7.60 (2H, dm, *J*=8.4 Hz), 8.69 (2H, ddd, *J*=8.8, 2.6, 2.0 Hz); ^{13}C-NMR (150 MHz): δ 0.24 (3C), 2.3 (3C), 7.5, 15.1, 27.2, 27.3, 29.2, 31.7, 32.5, 33.7, 40.2, 42.1, 44.7, 52.6, 59.4, 68.9, 76.7, 77.3, 85.4, 120.5, 129.2 (2C), 129.4 (2C), 130.4, 130.6 (2C), 130.9 (3C), 132.8, 134.1, 136.2, 138.0, 139.0, 139.5, 139.6, 143.7, 144.7, 150.1, 151.5, and 166.1.

*Desilylation of **4**. Formation of 3α,8β-dihydroxy-16-methoxyfusicocca-1,10(14)-dien-19-yl 4,7-bis(4-chlorophenyl)-[1,2,5]oxadizolo[3,4-c]pyridine-6-carboxylate (**5a**).* A solution of **4** (17 mg, 0.020 mmol) in THF (2.0 cm^3) was treated with Bu_4NF (1 M solution in THF; 1.0 cm^3) at room temperature for 12 h. The mixture was then diluted with aq. $NaHCO_3$ and extracted with ether. The organic extract was washed with brine, dried and evaporated. Chromatography of the residue on silica-gel afforded **5a** (5 mg, 35%) as yellow crystals.

5a: ^{1}H-NMR (600 MHz): δ 0.78 (3H, d, *J*=7.1 Hz), 0.90 (3H, d, *J*=7.0 Hz), 1.06 (3H, s), 1.26 (1H, m), 1.42 (1H, m), 1.62 (1H, ddd, *J*=12.3, 8.6, 8.2 Hz), 1.77-1.82 (2H, m), 1.90-2.01 (3H, m), 2.08 (1H, dm, *J*=11.9 Hz),

2.13 (1H, m), 2.28 (1H, m), 2.49 (1H, s), 2.80 (1H, m), 2.93 (1H, qm, *J*=7.1 Hz), 3.13 (1H, dd, *J*=9.5, 0.9 Hz), 3.37 (1H, d, *J*=9.5 Hz), 3.41 (3H, s), 3.79 (1H, m), 4.04 (1H, dd, *J*=10.6, 7.7 Hz), 4.25 (1H, dd, *J*=10.6, 7.0 Hz), 5.57 (1H, d, *J*=2.6 Hz), 7.55 (2H, ddd, *J*=8.8, 2.4, 2.2 Hz), 7.58 (2H, ddd, *J*=8.8, 2.6, 2.0 Hz), 7.62 (2H, ddd, *J*=8.8, 2.4, 2.2 Hz), and 8.70 (2H, ddd, *J*=8.8, 2.6, 2.0 Hz); ^{13}C-NMR (150 MHz): δ 7.9, 15.1, 27.1, 27.4, 28.5, 31.9, 32.2, 35.5, 40.5, 41.7, 44.5, 52.8, 59.3, 69.0, 76.1, 77.5, 81.9, 120.7, 129.3 (2C), 129.4 (2C), 130.6, 130.7 (2C), 130.9 (2C), 132.8, 135.0, 136.2, 137.5, 139.1, 139.5, 139.7, 143.6, 144.7, 150.2, 151.5, and 166.2.

Direct esterification of the triol ***1*** *with 4,7-diphenyl-[1,2,5]oxadiazolo[3,4-c]pyridine-6-carboxylic acid (****Fb****-OH). Formation of 3α,8β-dihydroxy-16-methoxy-fusicocca-1,10(14)-dien-19-yl 4,7-diphenyl-[1,2,5]oxa-diazolo[3,4-c]pyridine-6-carboxylate (****5b****).* To a solution of **1** (8 mg, 0.023 mmol) and 4,7-diphenyl-1,2,5-oxazolo[3,4-c]pyridine-6-carboxylic acid (**Fb**-OH, 3.9 mg, excess) in CH_2Cl_2 (1.5 cm^3) was added DMAP (2.0 mg) and EDCI (3.5 mg). After stirred at room temperature for 2 h, the mixture was diluted with aq. $NaHCO_3$ and extracted with ethyl acetate. The organic extract was washed with brine, dried and evaporated in vacuo. Chromatography of the residue on silica-gel afforded **5b** (11 mg, 74%) as yellow crystals.

5b: ^{1}H-NMR (600 MHz): δ 0.78 (3H, d, *J*=7.1 Hz), 0.87 (3H, d, *J*=6.8 Hz), 1.06 (3H, s), 1.26 (1H, m), 1.43 (1H, m), 1.63 (1H, dm, *J*=12.3 Hz), 1.76-1.79 (2H, m), 1.89-1.99 (3H, m Hz), 2.07-2.14 (2H, m), 2.26 (1H, m), 2.46 (1H, s). 2.79 (1H, m), 2.88 (1H, m), 3.12 (1H, d, *J*=9.5 Hz), 3.78 (1H, d, *J*=9.5 Hz), 3.40 (3H, s), 3.37 (1H, m), 4.02 (1H, dd, *J*=10.6, 7.7 Hz), 4.20 (1H, dd, *J*=10.6, 7.0 Hz), 5.56 (1H, d, *J*=2.6 Hz), 2.54-7.56 (2H, m), 7.61-7.63 (2H, m), 7.67-7.69 (2H, m), and 8.71-8.73 (2H, m); ^{13}C-NMR (150 MHz): δ 7.9, 15.1, 27.1, 27.4, 28.6, 31.9, 32.2, 35.5, 40.5, 41.6, 44.3, 52.8, 59.3, 68.9, 76.1, 77.6, 81.9, 121.6, 128.3, 128.9, 129.0, 129.2, 129.37, 129.39, 129.42, 129.7, 129.8, 132.3, 132.4, 134.6, 135.1, 137.4, 139.5, 139.9, 143.7, 145.0, 151.2, 151.7, and 166.6.

Direct esterification of the triol ***1*** *with 1-pyrenebutyric acid (****Fc****-OH). Formation of 3α,8β-dihydroxy-16-methoxyfusicocca-1,10(14)-dien-19-yl 1-pyrenebutyr-ate (****5c****).* To a solution of **1** (20 mg, 0.057 mmol) and 1-pyrenebutyric acid (**Fc**-OH, 18 mg, 0.062 mmol) in CH_2Cl_2 (1.5 cm^3) was added DMAP (7.0 mg) and EDCI (15 mg). After stirred at room temperature for 2 h, the mixture was diluted with aq. $NaHCO_3$ and extracted with ethyl acetate. The organic extract was washed with brine, dried and evaporated in vacuo. Silica-gel chromatography of the residue afforded **5c** (29 mg, 82%) as greenish solids.

5c: HREIMS Found: *m/z* 620.3505; Calcd for $C_{41}H_{48}O_5$: *m/z* 620.3502; $[\alpha]_D^{30}$ +24.8 (*c* 1.37, $CHCl_3$); FABMS: *m/z* 620 (M^+, 8.9), 154 (base peak); IR (KBr): ν 3454, 3040, 2950, 2870, 1730, 1603, 1587, 1452, 1371, 1299, 1246, 1185, 1099, 1035, 1014, 844, and 754 cm^{-1}; ^{1}H-NMR (600 MHz): δ 0.71 (3H, d, *J*=7.1 Hz), 0.96 (3H, s), 0.99 (3H, d, *J*=7.0 Hz), 1.25 (1H, m), 1.39-1.44 (2H, m), 1.57-1.63 (2H, m), 1.73-1.78 (2H, m), 1.93-2.01 (4H, m), 2.10 (1H, m), 2.16 (2H, quint., *J*=7.5 Hz), 2.27 (1H, m), 2.41 (2H, t, *J*=7.3 Hz), 2.43 (1H, br), 2.80 (1H, dm, *J*=1.8 Hz), 3.01 (1H, dm, *J*=8.2 Hz), 3.11 (1H, dd, *J*=9.5, 0.9 Hz), 3.35 (1H, d, *J*=9.5 Hz), 3.38 (2H, td, *J*=7.5, 2.4 Hz), 3.41 (3H, s), 3.79 (1H, dm, *J*=11.4 Hz), 3.92 (1H, dd, *J*=10.6, 6.6 Hz), 4.07 (1H, dd, *J*=10.6, 8.4 Hz), 5.52 (1H, d, *J*=2.4 Hz), 7.85 (1H, d, *J*=7.9 Hz), 7.99 (2H, t, *J*=7.7 Hz), 8.03 (2H, m), 8.11 (1H, d, *J*=7.7 Hz), 8.12 (1H, d, *J*=9.2 Hz), 8.16 (2H, m), and 8.29 (1H, d, *J*=9.2 Hz); ^{13}C-NMR (150 MHz): δ 7.8, 15.0, 26.8, 26.9, 27.2, 28.4, 31.6, 32.5, 32.8, 33.9, 35.5, 40.4, 41.7, 44.3, 52.8, 59.3, 66.9, 76.2, 77.6, 81.9, 123.3, 124.79, 124.82, 124.95, 125.01, 125.13, 125.9, 126.7, 127.37, 127.42, 127.5, 128.7, 130.0, 130.9, 131.4, 135.1, 135.6, 137.2, 139.5, 140.4, and 173.2.

Direct esterification of the triol ***1*** *with 6-(tert-butoxycarbonylamino)hexanoic acid (****Fd****-OH). Formation of 3α,8β-dihydroxy-16-methoxyfusicocca-1,10(14)-dien-19-yl 6-(tert-butoxycarbonylamino)-hexanoate (****5d****).* To a solution of **1** (15 mg, 0.043 mmol) and 6-(*tert*-butoxycarbonylamino)hexanoic acid (**Fd**-OH, 19 mg, 0.082 mmol) in CH_2Cl_2 (1.5 cm^3) was added DMAP (5 mg) and EDCI (12 mg, excess). After stirred at room temperature for 30 min, the mixture was diluted with aq. $NaHCO_3$ and extracted with ethyl acetate. The organic extract was washed with brine, dried and evaporated in vacuo. Chromatography of the residue afforded **5d** (18 mg, 75%) as white solids.

5d: $[\alpha]_D^{18}$ +16.6 (*c* 1.32, $CHCl_3$); FABMS: *m/z* 564 (M^++1, 9.9), 185 (base peak); IR (NaCl): ν 3370, 2936, 2868, 1705, 1693, 1640, 1526, 1453, 1391, 1366, 1273, 1250, 1169, 1101, 1038, 1013, 950, and 754 cm^{-1}; ^{1}H-NMR (600 MHz): δ 0.81 (3H, d, *J*=7.0 Hz), 1.01 (3H, d, *J*=7.0 Hz), 1.13 (3H, s), 1.26-1.36 (2H, m), 1.44 (9H, s), 1.48 (2H, m), 1.66 (1H, dt, *J*=12.1, 8.4 Hz), 1.79-1.84 (2H, m), 1.94-2.02 (4H, m), 2.13 (1H, m), 2.14 (1H, dd, *J*=12.8, 12.1 Hz), 2.27 (2H, t, *J*=7.3 Hz), 2.37 (1H, d, *J*=13.4 Hz), 2.48 (1H, s), 2.85 (1H, dm, *J*=7.5 Hz), 3.04 (1H, dqm, *J*=8.4, 7.0 Hz), 3.11 (2H, t, *J*=6.4 Hz), 3.15 (1H, dd, *J*=9.5, 0.9 Hz), 3.39 (1H, d, *J*=9.5 Hz), 3.42 (3H, s), 3.84 (1H, dm, *J*=11.5 Hz), 3.92 (1H, dd, *J*=10.6, 6.4 Hz), 4.05 (1H, dd, *J*=10.6, 8.6 Hz), 4.57 (1H, br), and 5.59 (1H, d, *J*=2.4 Hz); ^{13}C-NMR (150 MHz): δ 7.9, 15.0, 24.6, 26.3, 27.1, 28.4 (3C), 28.5, 29.8, 31.9, 32.5, 34.2, 35.5, 40.3, 40.5, 41.8, 44.4, 52.9, 59.3 (2C), 66.7, 76.2, 77.5, 81.9, 135.1, 137.2, 139.6, 140.4, and 173.3.

Direct esterification of the triol **1** *with 6-(benzyloxycarbonylamino)hexanoic acid (***Fe-OH***). Formation of 3α,8β-dihydroxy-16-methoxyfusicocca-1,10(14)-dien-19-yl 6-(benzyloxycarbonylamino)-hexanoate (***5e***).* To a solution of **1** (55 mg, 0.157 mmol) and 6-(benzyloxycarbonylamino)hexanoic acid (**Fe**-OH, 50 mg, 0.188 mmol) in CH_2Cl_2 (2.0 cm^3) was added DMAP (10 mg) and EDCI (50 mg, excess). After stirred at room temperature for 30 min, the mixture was diluted with aq. $NaHCO_3$ and extracted with ethyl acetate. The organic extract was washed with brine, dried and evaporated in vacuo. Chromatography of the residue afforded **5e** (70 mg, 75%) as a colorless oil.

5e: $[\alpha]_D^{23}$ +15.8 (*c* 0.57, $CHCl_3$); HRFABMS Found: *m/z* 598.3745; Calcd for $C_{35}H_{51}NO_7$: *m/z* 598.3744; FABMS: *m/z* 530 (M^+-51, 8.9) and 91 (base peak); IR (NaCl): ν 3368, 3064, 3032, 2946, 2868, 1706, 1536, 1455, 1165, 1153, 1100, 1037, 1016, 740, and 698 cm^{-1}; ^{1}H-NMR (600 MHz): δ 0.80 (3H, d, *J*=7.1 Hz), 1.01 (3H, d, *J*=7.0 Hz), 1.12 (3H, s), 1.25-1.35 (3H, m), 1.44 (1H, m), 1.49-1.52 (2H, m), 1.59-1.63 (2H, m), 1.65 (1H, dt, *J*=12.1, 8.4 Hz), 1.79-1.83 (2H, m), 1.97-2.02 (3H, m), 2.11-2.15 (2H, m), 2.26 (2H, t, *J*=7.3 Hz), 2.36 (1H, m), 2.51 (1H, br), 2.84 (1H, m), 3.03 (1H, m), 3.14 (1H, dd, *J*=9.5, 0.9 Hz), 3.18-3.21 (2H, m), 3.39 (1H, d, *J*=9.5 Hz), 3.42 (3H, s), 3.84 (1H, dm, *J*=11.5 Hz), 3.92 (1H, dd, *J*=10.6, 6.6 Hz), 4.04 (1H, dd, *J*=10.6, 8.8 Hz), 4.86 (1H, br), 5.09 (2H, s), 5.59 (1H, d, *J*=2.6 Hz), and 7.31-7.36 (5H, m); ^{13}C-NMR (150 MHz): δ 7.9, 15.0, 24.5, 26.2, 27.1, 28.4, 29.6, 32.0(2C), 34.2, 35.5, 40.5, 40.8, 41.8, 44.4, 52.8, 59.3, 66.6, 66.8, 76.2, 77.6, 81.9, 128.1(2C), 128.5(2C), 135.1, 136.6, 137.2, 139.6, 140.4, 156.4, and 173.3.

Deprotection of the CBZ group of **5e**. *Formation of 3α,8β-dihydroxy-16-methoxyfusicocca-1,10(14)-dien-19-yl 6-aminohexanoate (***5f***).* To a rapidly stirred solution of **5e** (20 mg, 0.0335 mmol) in 10 cm^3 of methanol at room temperature was added 5 mg of 5% palladium on activated carbon, and the resulting mixture was stirred under 1 atom of hydrogen atmosphere. After 30 min, the mixture was filtered, the catalyst was washed with methanol, and the solvent was removed at reduced pressure. The desired amine **5f** (12 mg, 77%) was obtained as a colorless oil.

5f: ^{1}H-NMR (400 MHz): δ 0.80 (3H, d, *J*=7.1 Hz), 1.02 (3H, d, *J*=6.8 Hz), 1.13 (3H, s), 1.24-1.44 (6H, m), 1.55-1.68 (5H, m), 1.79-1.84 (2H, m), 1.95-2.02 (4H, m), 2.09-2.18 (2H, m), 2.27-2.35 (3H, m), 2.84 (?H, br), 3.03 (1H, qm, *J*=7.1 Hz), 3.14 (1H, d, *J*=9.5 Hz), 3.39 (1H, d, *J*=9.5 Hz), 3.41 (1H, m), 3.42 (3H, s), 3.83 (1H, ddd, *J*=11.2, 3.9, 3.4 Hz), 3.94-4.04 (2H, m), and 5.59 (1H, d, *J*=2.4 Hz); ^{13}C-NMR (100 MHz): δ 8.0, 15.1, 24.4, 26.1, 27.1, 28.5, 32.0, 32.6, 34.0, 35.5, 40.5, 41.7, 44.3, 52.9, 59.3, 66.9(2C), 76.1, 77.2, 77.6, 81.9, 135.0, 137.4, 139.6, 140.3, and 173.5.

Condensation of **5f** *with 4,7-bis(4-chlorophenyl)-[1,2,5]oxadizolo[3,4-c]pyridine-6-carboxylic acid (***Fa-OH***). Formation of 3α,8β-dihydroxy-16-methoxyfusicocca-1,10(14)-dien-19-yl 6-[4,7-bis(4-chlorophenyl)-[1,2,5]oxadiazolo[3,4-c]pyridine-6-carbonylamino]hexanoate (***5g***).* To a solution of **5f** (12 mg, 0.0259 mmol) and 4,7-bis(4-chlorophenyl)-[1,2,5]oxadiazolo[3,4-*c*]pyridine-6-carboxylic acid (**Fa**-OH, 12 mg, 0.0311 mmol) in CH_2Cl_2 (1.5 cm^3) was added DMAP (5 mg) and EDCI (15 mg, excess). After stirred at room temperature for 30 min, the mixture was diluted with aq. $NaHCO_3$ and extracted with ethyl acetate. The organic extract was washed with brine, dried and evaporated in vacuo. Chromatography of the residue afforded **5g** (4.5 mg, 21%) as yellow crystals.

5g: mp. 68-69 ℃; HRFABMS Found: *m/z* 830.3215; Calcd for $C_{45}H_{52}Cl_2N_4O_7$: *m/z* 830.3213; FABMS: *m/z* 830 (M^+, 0.28) and 154 (base peak); ^{1}H-NMR (400 MHz): δ 0.76 (3H, d, *J*=7.0 Hz), 1.00 (3H, d, *J*=6.8 Hz), 1.11 (3H, s), 1.24-1.28 (2H, m), 1.39-1.43 (2H, m), 1.60-1.82 (8H, m), 1.94-2.04 (3H, m), 2.07-2.17 (2H, m), 2.28 (2H, t, *J*=7.2 Hz), 2.33 (1H, dm, *J*=113.5 Hz), 2.47 (1H, s), 2.82 (1H, m), 3.01 (1H, m), 3.13 (1H, d, *J*=9.4 Hz), 3.38 (1H, d, *J*=9.7 Hz), 3.41 (3H, s), 3.42-3.48 (2H, m), 3.80 (1H, dm, *J*=8.5 Hz), 3.90 (1H, dd, *J*=10.6, 6.8 Hz), 4.04 (1H, dd, *J*=10.6, 8.5 Hz), 5.57 (1H, d, *J*=2.4 Hz), 7.49-7.54 (3H, m), 7.57-7.60 (2H, m), 7.65-7.67 (3H, m), 7.88 (1H, m), and 8.63-8.65 (2H, m); ^{13}C-NMR (100 MHz): δ 7.9, 14.2, 15.1, 21.0, 24.6, 26.5, 27.14, 27.17, 28.5, 29.3, 32.0, 34.2, 35.5, 39.5, 40.5, 41.8, 44.4, 52.9, 59.3, 60.4, 66.8, 76.2, 77.6, 81.9, 123.6, 128.2 (2C), 129.17, 129.23 (2C), 129.34 (2C), 129.4 (2C), 132.4, 135.1, 137.3, 139.6, 140.4, 142.0, 145.0, 150.0, 153.3, 163.9, and 173.3.

Germination tests. The study on the effect of the compounds on the germination of lettuce seeds was carried out in the presence of 2 ppm of (±)-abscisic acid at 25 ℃ in the dark. The number of germinated seeds was counted after the indicated period of time and the value was expressed as a percentage based on the number of seeds tested (40 seeds were used in each experiment). Without abscisic acid, a germination inhibitor, 100% of germination was observed within 48 h. The results are summarized in Table 1.

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