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Efficient synthesis of novel cytotoxic *cis*-fused α-methylene γ-lactones from 7,14-dihydroxy-*ent*-kaurenes by transformation under Mitsunobu reaction conditions

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Abstract—Facile transformation of 7,14-dihydroxy-*ent*-kaurenes such as excisanin A (**8**), kamebanin (**9**), and kamebakaurin (**10**), which are abundant in plants of the genus *Rabdosia* species (Labiatae), to *ent*-abietanes was accomplished under the Mitsunobu reaction conditions. The δ, ε-unsaturated *cis*-fused α-methylene γ-lactones (**17**, **18**, and **25**) thus prepared showed a moderate cytotoxic activity on P388 murine leukemia cells.

Several types of *ent*-abietane diterpenes have been isolated from plants of the genus *Euphorbia* (Euphorbiaceae) and the genus *Rabdosia* (Labiatae). Tetracyclic *ent*-abietanes jolkinolides A–E were isolated from *Euphorbia jolkini* by Uemura and Hirata,[1,2] and helioscopinolides A–F from *Euphorbia helioscopia* by Yamamura[3,4] and Crepsi-Perellino et al.,[5] respectively. Of them, jolkinolide D (**2**) is known to inhibit the tumor invasion into the basement membrane tissue and induce apoptosis in tumor cells.[6] Tricyclic *ent*-abietane diterpenoids, laxiflorins N (**5**), and O (**6**) were found in *Rabdosia erocalyx* var. *laxiflora*.[7] Recently, taibaihenryiin C (**7**), which has a unique diterpene skeleton, a δ, ε-unsaturated *cis*-fused α-methylene γ-lactone, was isolated from *Rabdosia henryi* by Li (Fig. 1).[8,9] As regards *ent*-kaurene diterpenoids, over 400 of them have been isolated from *Rabdosia* species and in those plants their contents are normally high.[10] In our previous study, we isolated 7,14-dihydroxy-*ent*-kaurene diterpenoids such as excisanin A (**8**, 0.007%), kamebanin (**9**, 0.002%), and kamebakaurin (**10**, 0.02%) from *Rabdosia excisa*.[11]

Previously, there was no efficient synthetic method for preparation of *ent*-abietanes. In the present study, we established a method of transformation of 7,14-dihydroxy-*ent*-kaurene diterpenoids occurring abundantly in *Rabdosia* plants to *ent*-abietanes having a δ, ε-unsaturated *cis*-fused α-methylene γ-lactone. As shown in Scheme 1, compound **A** may be derived from **B** under the Mitsunobu reaction conditions: conversion of 7-hydroxyl group to an oxyphosphonium ion intermediate, nucleophilic attack by the oxygen atom of 14-hydroxy group on the carbonyl carbon at C-15, and the elimination of triphenylphosphine oxide may occur in succession under the Mitsunobu reaction conditions to afford the corresponding *ent*-abietanes. In this case, usual intermolecular Mitunobu reaction would not take place, because of the steric bulkiness at C-7 and the difficulties of nucleophilic attack on C-7 by a carboxylate anion. Finally, selective protection of polyoxygenated *ent*-kaurenoids (**8**, **9**, and **10**) was to be performed. In this paper, we describe an efficient transformation of *ent*-kaurenes to *ent*-abietanes under the Mitsunobu reaction conditions and cytotoxic activities of the compounds thus prepared.

Synthetic procedure is given in Scheme 2. The substrates for the intramolecular Mitsunobu reaction, **15** and **16** were synthesized by first acetalizing 7,14-dihydroxy groups of **8** and **9** in the reported manner.[12,13] Acetylation of **11** and **12** proceeded smoothly to give **13** and **14**

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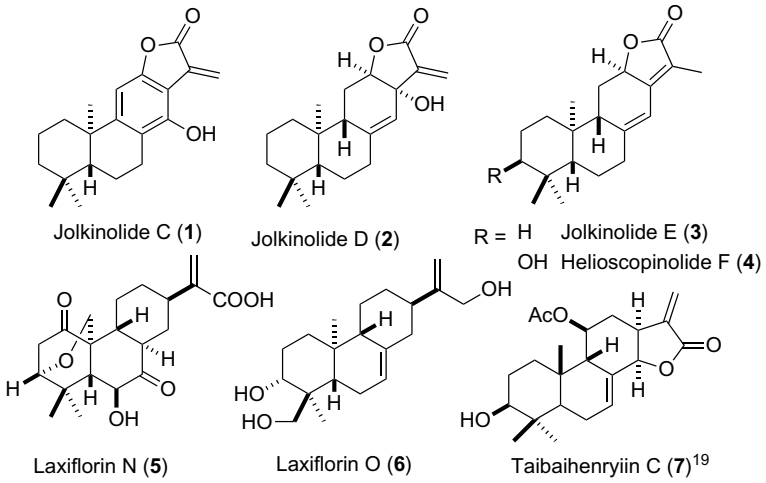
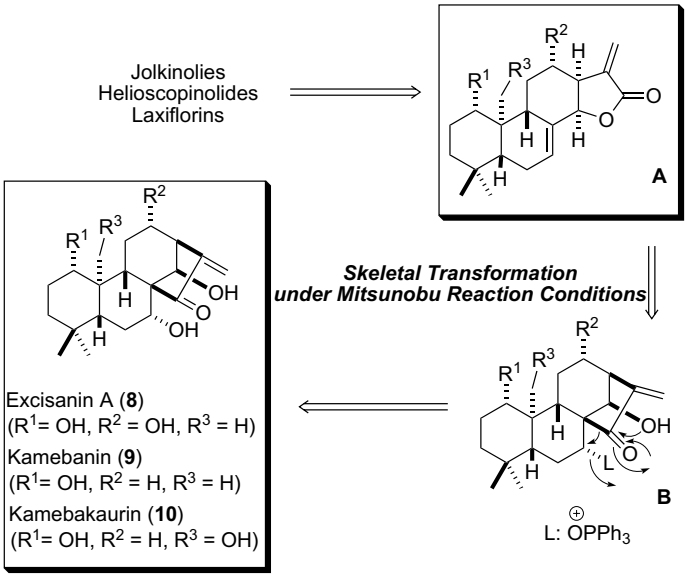


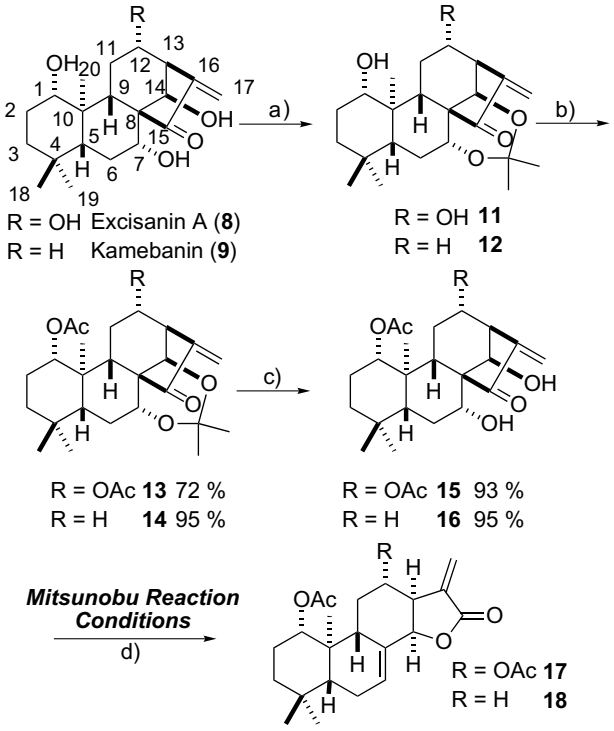
Figure 1. Structures of naturally occurring diterpenes having *ent*-abietane skeletons (**1**–**7**).



Scheme 1. Synthetic strategy of *ent*-abietanes from *ent*-kaurenes (**8**–**10**).

in 72% and 95% yields, respectively. Then, deacetalization of **13** and **14** was carried out in 1 M HCl–THF–MeOH to afford the corresponding 7,14-dihydroxy-*ent*-kaurenes **15** and **16** in high yields, respectively. The skeletal transformation of **15** was studied under different Mitsunobu reaction conditions (Table 1). When the reaction was carried out at room temperature, PNB (*p*-nitrobenzoic acid) and 3,5-DNB (3,5-dinitrobenzoic acid) gave better yields than other acids (entries 1–4 in Table 1). When the reaction was carried out under refluxing, the yield of the desired **17** was higher than at room temperature (entries 5 and 6 vs 1 and 2 in Table 1). When formic acid, benzoic acid, or 2,6-di-*tert*-butylphenol was used as a proton source at room temperature, apparently the reaction proceeded less efficiently. Thus, the best result was obtained under the following conditions [diethyl-azodicarboxylate (DEAD): 2.2 equiv; concentration of the substrate: 0.0058 M; proton source: PNB: 2.4 equiv; time: refluxing for 1 h (entry 6 in Table 1)].[14] When treated in the same way by using the same reaction conditions as entry 6 in Table 1, **16** gave the desired *ent*-abietane **18** in 54% yield.[14] The structural identification of **17** was made by the 1H and 13C NMR, and 2D NMR spectral studies, and finally by X-ray crystallographic analysis (Fig. 2).

Then, we synthesized *ent*-kaurenoid diterpene **25** from kamebakaurin (**10**) following the analogous reaction scheme (Scheme 3). Acetalization of **10** in the reported manner[15] produced the desired **21** only in 20% yield (entry 1 in Table 2). Acetalization of **10** in different reaction conditions failed to produce **21**, and gave diacetonide **19** (entry 2 in Table 2) or diacetonide **19** and monoacetonide **20** (entry 3 in Table 2). Acid-catalyzed regioselective demonoacetalization of the major product of acetalization, diacetonide **19**, however, was found to proceed to give **21** in 85% yield. The structures of **20** and **21** were identified after conversion to diace-



Scheme 2. Preparation of **17** and **18**. (a) Refs. 10 and 11; (b) Ac_2O/Py/room temperature; (c) 1 M HCl/THF/MeOH/room temperature; (d) See Table 1.

tates **22** and **23**, respectively. In the HMBC spectral data of **22**, the correlations between the acetal carbon and C-20 methylene protons, and between 7- and 14-methine protons and the carbonyl carbons of acetyl groups were observed to show that it was a 1,20-acetonide. On the other hand, in that of **23**, the correlations between 7-methine proton and the acetal carbon, and 1-methine proton and 20-methylene protons and the carbonyl carbons of acetyl groups were observed. Consequently, **23** was shown to be the desired 7,14-acetonide. The deacetalization of the acetonide **23** proceeded to give the corresponding diol **24** in 92%. Finally, the skeletal transformation of **24** under the conditions described for entry 6 in Table 1 gave the desired *ent*-abietane **25** in 50%.[14] Although the S_N2' reactions of glycals[16,17] and the rearrangement of an isocaryolane sesquiterpenoid[18]

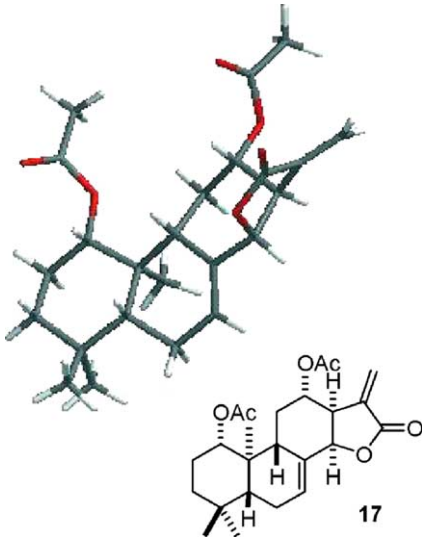
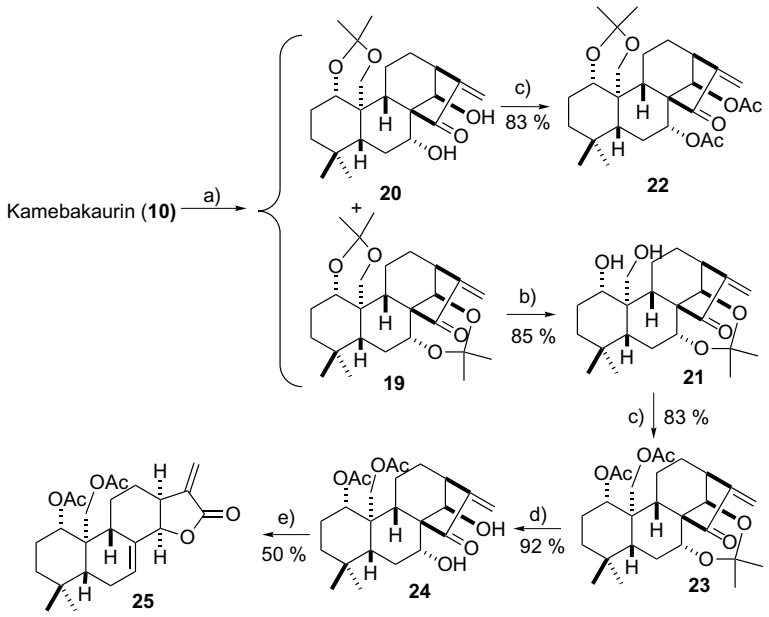


Figure 2. Stereodiagram of **17** as determined by single crystal X-ray analysis.[20]

Table 1. Intramolecular Mitsunobu reactions of **15** and **16** under different reaction conditions

Entry	Substrate	Eq.[a]	Conc. (mM)[b]	Acids	(Eq.)[c]	Temperature[d]	Time (h)[e]	Products	(%)[f]
1	**15**	2.2	23	PNB	(2.4)	rt	24	**17**	(24)
2	**15**	2.2	23	PNB	(2.4)	rt	48	**17**	(13)
3	**15**	2.2	23	3,5-DNB	(2.4)	rt	48	**17**	(27)
4	**15**	2.2	23	BA	(2.4)	rt	48	**17**	(–)[g]
5	**15**	2.2	12	PNB	(2.4)	Reflux	1	**17**	(52)
6	**15**	2.2	5.8	PNB	(2.4)	Reflux	1	**17**	(58)
7	**15**	1.1	5.8	PNB	(3.0)	Reflux	1	**17**	(47)
8[h]	**15**	1.1	5.8	PNB	(3.0)	Reflux	1	**17**	(–)[g]
9	**15**	1.1	23	HCOOH	(2.0)	Reflux	6	**17**	(23)
10	**15**	2.2	21	3,5-DNB	(2.4)	Reflux	1	**17**	(43)
11[i]	**15**	2.2	5.8	PNB	(2.4)	Reflux	1	**17**	(46)
12[j]	**15**	4.5	5.8	PNB	(2.4)	Reflux	9	**17**	(–)[k]
13	**15**	2.2	5.8	2,6-DTBP	(2.4)	Reflux	6	**17**	(–)[k]
14	**16**	2.2	5.8	PNB	(2.4)	Reflux	1	**18**	(54)

[a] Equimolar amount of diethyl azodicarboxylate (DEAD).
[b] Concentration of substrate.
[c] PNB (*p*-nitrobenzoic acid); 3,5-DNB (3,5-dinitrobenzoic acid); BA (benzoic acid); 2,6-DTBP (2,6-di-*tert*-butylphenol).
[d] Reaction temperature.
[e] Reaction time (h).
[f] Isolated yields of desired products.
[g] Inseparable mixture.
[h] Diisopropyl azodicarboxylate (DIAD) was employed instead of DEAD.
[i] Benzene was used as solvent instead of THF.
[j] DEAD on polystylene (1.2 mmol/g) was employed instead of DEAD.
[k] Starting material recovered.



Scheme 3. Preparation of **25**. (a) See Table 2; (b) 1 M HCl/THF/MeOH/rt; (c) Ac_2O/Py; (d) 1 M HCl/THF/0 °C; (e) DEAD/PPh_3/PNB/THF/reflux.

Table 2. Acetalization of Kamebakaurin (**10**)

Entry	Reaction conditions	Yields (%)[a]		
		19	**20**	**21**
1	Me_2CO/*c*-H_2SO_4/rt/3 h	80	—	20
2	DMP/PSA[b]/Me_2CO/rt/0.5 h	92	—	—
3	Me_2CO/PSA[b]/0 °C/1 h	11	83	—

[a] Isolated yields.
[b] *p*-Toluene sulfonic acid.

Table 3. Cytotoxic activities of **8**–**25** on P388 murine leukemia cells

Compounds	IC_{50} (μg/mL)	Compounds	IC_{50} (μg/mL)
8	1.80	**17**	0.56
9	0.69	**18**	0.64
10	0.82	**19**	2.05
11	0.92	**20**	0.62
12	1.22	**21**	2.58
13	0.087	**22**	0.19
14	2.18	**23**	0.78
15	0.46	**24**	0.68
16	0.23	**25**	2.61

under Mitsunobu reaction conditions have been reported, the skeletal transformation of *ent*-kaurenes to *ent*-abietanes has not been described. The efficient transformation reaction found in the present study suggests that naturally occurring *ent*-abietanes including **7** may be biogenetically synthesized from *ent*-kaurenes, and also that **7**, whose absolute configuration has not been reported, have the *ent*-kaurenoid type absolute configuration (Scheme 3).

Natural *ent*-kaurene diterpenes **8**, **9**, **10** and the synthesized compounds **11**–**25** including 7,8-unsaturated *cis*-fused α-methylene γ-lactones (**17**, **18**, **25**) were tested for their cytotoxitic activities on P388 murine leukemia cells, and the results are given in Table 3. All the compounds showed low to moderate cytotoxic activities. IC_{50} of *ent*-abietanes **17** and **18** were 0.56 and 0.64 μg/mL, respectively.

We established an efficient method of transformation of 7,14-dihydroxy-*ent*-kaurenes to *ent*-abietanes under Mitsunobu reaction conditions, which enables us to synthesize *ent*-abietane analogues easily. The yield of each step was moderate to good and 7,14-dihydroxy-*ent*-kaurenes, the starting material, are abundant in nature. The synthesized *ent*-abietanes were shown to have a cytotoxic activity. Thus, the present method gives a useful approach to the structure–activity relationship studies.

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14. General procedure for transformation of *ent*-kaurenes to *ent*-abietanes **17**, **18**, and **25**. DEAD (27 mL, 0.149 mmol) was added to a mixture of PPh_3 (54 mg, 0.206 mmol), 7,14-dihydroxy-*ent*-abietane (0.069 mmol), and PNB (28 mg, 0.168 mmol) in dry THF (12 mL) under an Ar atmosphere. The mixture was refluxed for 1 h and then evaporated in vacuo to give an oily residue, which was purified by MPLC (Hexane/AcOEt = 2:1) and HPLC (ODS, MeCN/H_2O = 55:45) to give the corresponding *ent*-abietane.
Compound 17: 58%; Colorless needles, 207–209 °C; $[\alpha]_D$ −11.4° ($c = 0.26$, $CHCl_3$); ^{1}H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.18 (1H, d, $J = 0.6$), 6.08 (1H, d, $J = 5.9$), 5.65 (1H, s), 4.79 (1H, d, $J = 5.9$), 4.65 (1H, dd, $J = 4.2$, 1.3), 4.56 (1H, ddd, $J = 12.0$, 10.0, 3.5), 2.93 (1H, dd, $J = 10.0$, 5.9), 2.39 (1H, ddd, $J = 12.0$, 3.5, 3.5), 2.30 (1H, br d, $J = 12.0$), 2.13 (1H, m), 2.06–1.99 (1H, m), 2.03 (3H, s), 2.02 (3H, s), 1.71–1.66 (1H, m), 1.65–1.56 (1H, m), 1.47 (1H, m), 1.45–1.44 (1H, m), 1.30 (1H, dd, $J = 4.2$, 1.7), 1.18 (1H, ddd, $J = 12.0$, 12.0, 12.0), 0.93 (6H, s), 0.90 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 170.8, 170.2, 169.5, 138.8, 134.1, 130.9, 123.1, 83.9, 82.7, 74.0, 49.2, 45.72, 45.66, 39.9, 39.6, 33.3, 33.2, 31.9, 25.1, 23.7, 22.9, 21.8, 21.3, 9.8; IR (film) 1766, 1738 (C=O), 1671 (C=C); HRMS (ESI): Calculated for $C_{24}H_{32}O_6$ Na: 439.2097 (M^++Na). Found: 439.2126.
Compound 18: 54%; Colorless amorphous solid, mp 150–151 °C; $[\alpha]_D$ −93.7° ($c = 0.27$, $CHCl_3$); ^{1}H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.12 (1H, s), 6.00 (1H, dd, $J = 2.6$, 2.6), 5.53 (1H, s), 4.68 (1H, d, $J = 5.5$), 4.67 (1H, dd, $J = 12.0$, 5.4), 2.90 (1H, ddd, $J = 10.5$, 5.7, 5.7), 2.16 (1H, m), 2.13 (1H, m), 2.05–1.98 (1H, m), 2.02 (3H, s), 1.81–1.75 (2H, m), 1.67–1.58 (3H, m), 1.46–1.41 (2H, m), 1.39–1.22 (3H, m), 0.94 (3H, s), 0.93 (3H, s), 0.89 (3H, s); ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 170.2 (overlapped), 141.7, 133.0, 131.7, 120.2, 83.1, 82.6, 49.0, 47.6, 40.7, 39.6, 39.4, 32.7, 32.747, 32.699, 29.2, 25.0, 24.4, 23.5, 22.2, 22.7; IR (film) 1762, 1737 (C=O), 1667 (C=C); HRMS (ESI): Calculated for $C_{22}H_{30}O_4$ Na: 381.2042 (M^++Na). Found: 381.2026.
Compound 25: 50%; Colorless amorphous solid, mp 166–167 °C; $[\alpha]_D$ −89.1° ($c = 0.10$, $CHCl_3$); ^{1}H NMR (500 MHz, 300 K, $CDCl_3$) δ 6.12 (1H, d, $J = 0.9$), 6.06 (1H, dd, $J = 2.3$, 2.3), 5.55 (1H, br s), 4.74 (1H, dd, $J = 11.4$, 4.8), 4.70 (1H, d, $J = 5.4$), 4.51 (1H, d, $J = 13.2$), 4.48 (1H, d, $J = 13.2$), 2.93 (1H, ddd, $J = 9.7$, 6.2, 6.2), 2.31 (1H, m), 2.27–2.14 (2H, m), 2.02 (3H, s), 2.01 (3H, s), 1.94–1.90 (1H, m), 1.86–1.83 (2H, m), 1.74–1.66 (2H, m), 1.55–1.47 (3H, m), 1.38–1.32 (1H, m), 0.99 (3H, s), 0.94 (3H, s).; ^{13}C NMR (125 MHz, 300 K, $CDCl_3$) δ 170.7, 170.1, 169.9, 141.6, 133.6, 131.7, 120.3, 82.6, 82.3, 63.0, 49.2, 46.5, 41.8, 40.9, 39.4, 33.0, 32.7, 29.4, 25.6, 25.1, 24.5, 21.8, 21.5, 21.2; IR (film) 1761, 1738 (C=O), 1667 (C=C); HRMS (ESI): Calculated for $C_{24}H_{32}O_6$Na: 439.2097 (M^++Na). Found: 439.2098.
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19. The relative configuration of **7** has been determined on the basis of X-ray crystallographic analysis.[7,8] but its absolute configuration is not reported.
20. Crystallographic data for compound **17** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 220809. Copies of the data can be obtained, free of charge, on application to the Director, CCDC (e-mail: deposit@ccdc.cam.ac.uk).