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PREPARATION OF OPTICALLY ACTIVE 2,2-DISUBSTITUTED 5-HYDROXYCHROMENES BY ENZYMATIC RESOLUTION OF RACEMIC ESTERS

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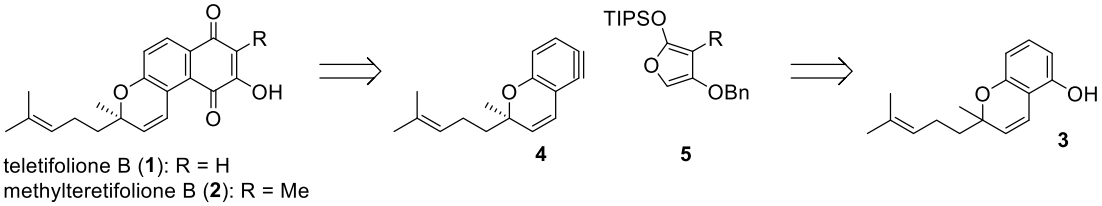
Dedicated with respect to Professor Kiyoshi Tomioka on the occasion of his 70th birthday

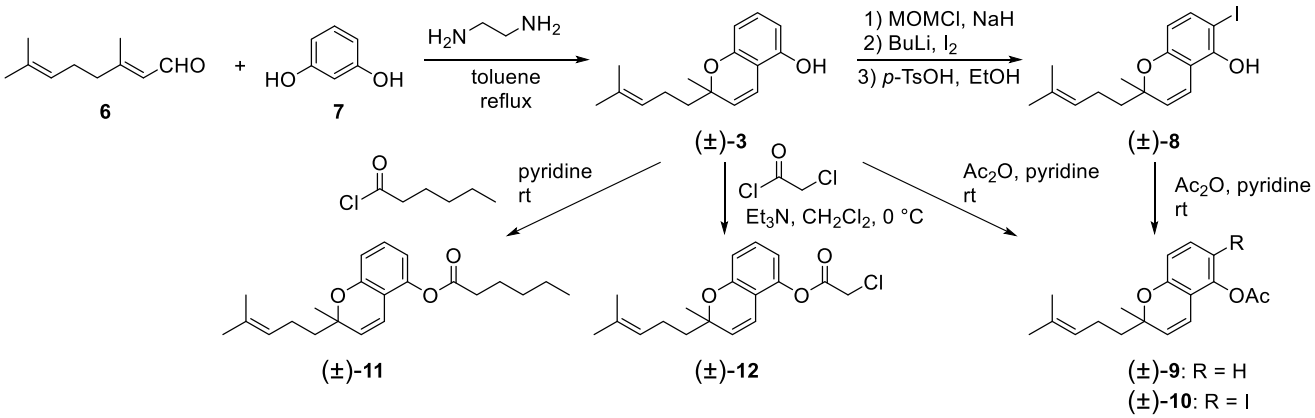
Abstract – Enzymatic kinetic resolution of racemic esters of 2,2-disubstituted 5-hydroxychromenes was examined. Transesterification of acetate using Amano Lipase PS in the presence of *t*-BuOH was most effective to give the corresponding optically active acetate in 18% yield and 95% *ee*. The absolute configuration of the acetate was determined as *R* based on the conversion to teretifolione B with natural absolute configuration.

Pyranonaphthoquinone natural products are widely distributed in plants and microorganisms, and they have diverse biological activities.[1] Angular benzochromenes are a small class of natural products isolated from *Conospermum* and *Pentas* plants.[2] We have reported asymmetric total synthesis of teretifolione B (**1**) and the corresponding methyl derivative **2**, monomeric benzochromenes isolated from *Conospermum* plants.[3] The key steps of this total synthesis are enzymatic resolution of racemic 2,2-disubstituted 5-hydroxychromene **3** and Diels-Alder reaction of optically active benzyne **4** and oxygen-substituted furans **5** (Scheme 1). In this paper, details of our trials for enzymatic resolution of racemic chromene **3** were reported.



Scheme 1. Retrosynthesis of teretifolione B (**1**) and the methyl derivative **2**

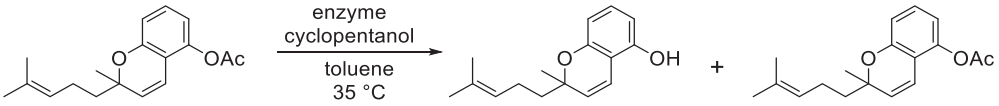
Racemic hydroxychromene (±)-**3** was synthesized by cyclocondensation reaction of citral (**6**) and resorcinol (**7**) in the presence of ethylenediamine as a catalyst. Iodochromene (±)-**8** was prepared by MOM protection of (±)-**3**, ortholithiation-iodination, and deprotection.[3] Hydroxychromenes (±)-**3** and (±)-**8** were acetylated to give the corresponding acetate (±)-**9** and (±)-**10**. (±)-**3** was also acylated with hexanoyl chloride or chloroacetyl chloride to give esters (±)-**11** and (±)-**12** (Scheme 2).



Scheme 2. Preparation of substrates (±)-**3**, (±)-**9**-**12** for enzymatic resolution

At first, hydrolysis of (±)-**9** and (±)-**10** in wet *i*-Pr_2O[4] was examined. A mixture of (±)-**9** and Amano Lipase PS in *i*-Pr_2O (saturated with H_2O) was stirred at rt for 40 min to give a *ca*. 2 : 1 mixture of (+)-**3** and (-)-**9** (35% and 18% enantiomeric excess (*ee*), respectively).[5] No reaction was observed when iodochromene (±)-**10** was applied with using Amano Lipase PS and lipase from porcine pancreas (PPL) as an enzyme. Next (±)-**9** was subjected to transesterification condition using cyclopentanol as an alcohol.[6] Reaction using Amano Lipase PS at 35 °C for 30 min gave a *ca*. 1 : 1 mixture of (+)-**3** (57% *ee*) and (-)-**9** (46% *ee*) (Table 1, run 1). Similar selectivity was observed when Lipase TL was applied (run 2). When Novozyme 435 was used, the antipodes (-)-**3** (49% *ee*) and (+)-**9** (56% *ee*) were obtained as a major isomer (run 3). Reactions with Lipase OF and PPL gave low selectivity (runs 4 and 5).

Table 1. Effects of enzymes on transesterification of (±)-**9** with cyclopentanol[a]



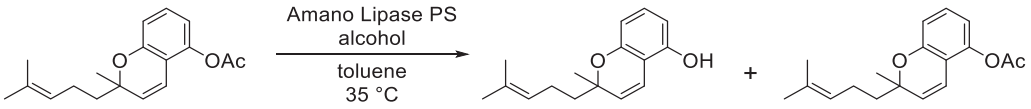
(±)-**9** **3** **9**

run	enzyme	time	ratio (**3** : **9**)	% *ee* (**3**, **9**)
1	Amano Lipase PS	30 min	44 : 56	57, 46
2	Lipase TL	40 min	55 : 45	45, 58
3[b]	Novozyme 435	15 min	51 : 49	49, 56
4	Lipase OF	12 h	56 : 44	2, 3
5	PPL	19 d	22 : 78	15, 5

[a] (+)-**3** and (-)-**9** were obtained as a major isomer otherwise noted. [b] (-)-**3** and (+)-**9** were obtained as a major isomer.

We next examined the effect of alcohols using Amano Lipase PS. Almost similar selectivity was observed when 1º (Table 2, runs 2-4), 2º (run 5), and 3º alcohol (run 6) were applied. In further trials using *t*-BuOH, the optical purity of **9** was improved to 73% *ee* in 2 h (run 7), 90% *ee* in 3 h (run 8), and

Table 2. Effects of alcohols and times on transesterification of (±)-**9**

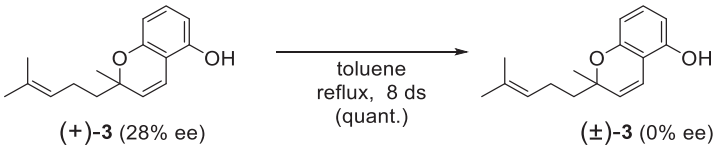


(±)-**9** (+)-**3** (-)-**9**

run	alcohol	time	crude[a]		isolated (%)	
			ratio (**3** : **9**)	% *ee* (**3**, **9**)	yield (**3** : **9**)	*ee* (**3**, **9**)
1	cyclopentanol	30 min	44 : 56	57, 46	- [a]	
2	MeOH	60 min	52 : 48	57, 62	- [a]	
3	EtOH	60 min	53 : 47	56, 51	- [a]	
4	1-BuOH	50 min	49 : 51	55, 53	- [a]	
5	2-PrOH	50 min	43 : 57	61, 48	- [a]	
6	*t*-BuOH	50 min	41 : 59	- [b]	42, 58	59, 40
7	*t*-BuOH	2 h	55 : 45	- [b]	55, 43	52, 67
8	*t*-BuOH	3 h	64 : 36	- [b]	62, 36	45, 90
9	*t*-BuOH	5.5 h	74 : 26	23, 97	- [a]	

[a] Not isolated. [b] Not determined.

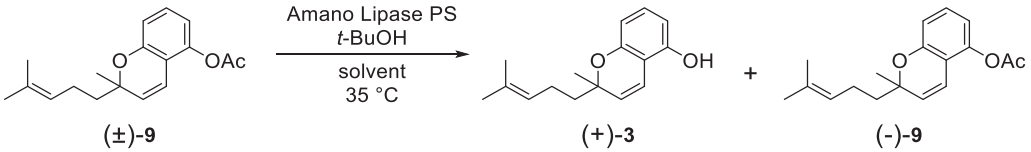
97% *ee* after 5.5 h (run 9). (±)-**3** can be recovered after the complete racemization of (+)-**3** under thermal conditions (Scheme 3).[4]



Scheme 3. Epimerization of (+)-**3** under thermal condition

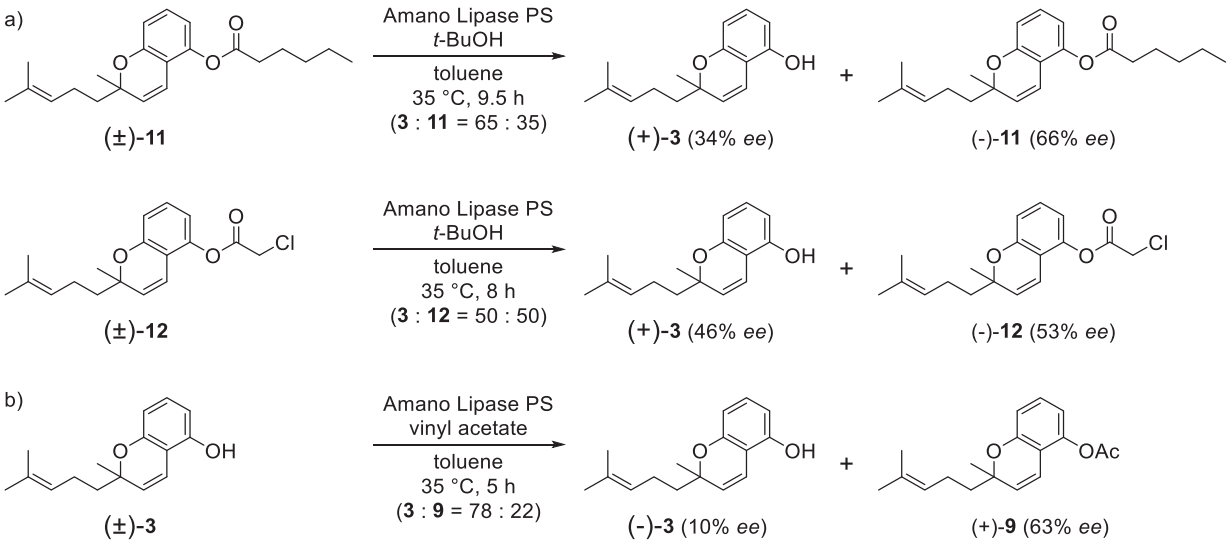
Using solvent other than toluene, TBME and acetonitrile showed the reaction faster than those of toluene and similar selectivity (Table 3, runs 3-6). Acetone showed similar result to toluene but low efficiency (run 7). It could be because essential water molecules would be stripped out from the enzyme by using hydrophilic solvent like acetone. On the examination of acyl group on **9**, both of hexanoyl[7] **11** and chloroacetyl esters **12** showed slower reaction rate and lower enantioselectivity. Application of cyclopentanol instead of *t*-BuOH for hexanoyl ester **11** slightly improved the selectivity (**3** : **11** = 54 : 46, 43% *ee* for (+)-**3**, 62% *ee* for (-)-**11**) (Scheme 4a). The lower reactivity and selectivity could be due to the steric bulkiness of the acyl group rather than acetyl group. Trial of acylation condition of (±)-**3** using vinyl acetate in toluene gave (+)-**9**, antipode for transesterification condition, with lower enantioselectivity (Scheme 4b). Irreversible process using vinyl acetate gave better result than transesterification on alcoholic substrates in most cases, however, transesterification gave better results in

Table 3. Effects of solvent on transesterification of (±)-**9**



run	solvent	time	ratio (**3** : **9**)	% ee (**3**, **9**)
1 [a]	toluene	50 min	41 : 59	63, 42
2 [b]	toluene	5.5 h	74 : 26	23, 97
3	TBME	15 min	50 : 50	40, 41
4	TBME	2 h	94 : 9	5, 94
5	MeCN	60 min	56 : 44	46, 45
6	MeCN	3.5 h	93 : 7	6, 96
7	acetone	6.5 h	88 : 12	11, 95

[a] Run 6 in Table 2. [b] Run 9 in Table 2.



Scheme 4. a) Trials for enzymatic resolution of esters (±)-**11** and (±)-**12**. b) Trials for enzymatic resolution in acylation condition of (±)-**3**.

in this trial, probably because of lower reactivity of phenols than alcohol. Regarding to the result, most effective reaction system was identified as the reaction of (±)-**9** with Amano Lipase PS in the presence of *t*-BuOH in toluene. Eventually (-)-**9** (95% *ee*) was prepared in 18% yield on a large scale by this method for further synthetic study to be converted to (*R*)-(+)-teretifolione B (**1**). Thus, the absolute configuration of (-)-**9** was determined as *R*.[3]

In summary, enzymatic kinetic resolution of racemic esters of 2,2-disubstituted 5-hydroxychromenes was examined. Transesterification condition of acetate (±)-**9** using Amano Lipase PS in the presence of *t*-BuOH was most effective to give the corresponding optically active acetate in 18% yield and 95% *ee*. The absolute configuration of the acetate was determined as *R* based on the conversion to teretifolione B with natural absolute configuration. Studies of the resolution of other hydroxychromenes and total synthesis of related pyranonaphthoquinone natural products are now underway in our laboratory.

EXPERIMENTAL

Commercially available reagents and anhydrous solvents were used without further purification. Anhydrous solvents (CH_2Cl_2 and THF) were purchased from Wako chemicals. Analytical thin layer chromatography was performed on silica gel 60 F_{254} plate from Merck KGaA. Flash chromatography was carried out with Silica gel 60 (40-50 μm) from Kanto Chemical Co. Amano Lipase PS, from *Burkholderia cepacia*, was purchased from Aldrich. IR spectra were recorded on a JASCO FT/IR-4100 spectrophotometer with Attenuated Total Reflectance Unit ATR PRO450-S and shown in cm^{-1}. Chiral HPLC analysis was performed on JASCO 875-UV Intelligent UV/VIS Detector and 880-PU Intelligent

HPLC pump with LA soft CDS system. EI-MS was recorded on a JEOL GC-Mate II and ESIMS with DART system was recorded on a JEOL JMS-T100LP in positive ion mode. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra were recorded on a JEOL ECX 400 spectrometer with deuterated chloroform ($CDCl_3$) as a solvent and tetramethylsilane as an internal reference at room temperature (rt). Chemical shifts were reported in ppm and *J* in Hz. Abbreviations were used for multiplicity: s = singlet, d = doublet, t = triplet, sept. = septet, m = multiplet. Synthetic procedure and spectral data of (±)-**3** and (±)-**9** were reported previously.[3]

6-Iodo-2-methyl-2-(4-methylpent-3-en-1-yl)-2*H*-chromen-5-yl acetate [(±)-**10**]

To a solution of (±)-**3** (103 mg, 0.28 mmol) in pyridine (0.2 mL), acetic anhydride (0.20 mL, 2.12 mmol) was added and the whole was stirred at rt for 3.5 h. The mixture was diluted with AcOEt (20 mL) and washed with sat. aq. $NaHCO_3$ (1 x 2 mL), 2 M HCl (1 x 2 mL) and brine (1 x 2 mL), and dried over Na_2SO_4. The solvent was evaporated *in vacuo* and the residue was purified by CC (SiO_2, hexane – AcOEt = 95 : 5 – 85 : 15) to give (±)-**10** as a yellow oil (81 mg, 71%).

IR ν_{max} 1762. ^{1}H-NMR δ: 7.47 (1H, d, *J* = 8.7 Hz, C7-H), 6.50 (1H, dd, *J* = 8.6, 0.6 Hz, C8-H), 6.28 (1H, d, *J* = 10.1 Hz, C4-H), 5.61 (1H, d, *J* = 10.1 Hz, C3-H), 5.07 (1H, t sept., *J* = 7.1, 1.4 Hz, CH=), 2.38 (3H, s, CH_3), 2.15 – 2.00 (2H, m, CH_2), 1.73 (1H, ddd, *J* = 13.9, 10.4, 6.2 Hz, CH) 1.66 (3H, s), 1.64 (1H, ddd, *J* = 14.0, 10.8, 6.1 Hz, CH), 1.57 (3H, s, CH_3), 1.38 (3H, s, CH_3). ^{13}C-NMR δ: 168.1, 154.5, 146.8, 137.8, 131.9, 131.2, 123.7, 116.9, 116.3, 116.0, 79.2, 78.9, 41.1, 26.4, 25.6, 22.6, 21.0, 17.6. HRESIMS *m/z* 413.0600 (M + H, calcd. for $C_{18}H_{22}O_3I$ 413.0614).

2-Methyl-2-(4-methylpent-3-en-1-yl)-2*H*-chromen-5-yl hexanoate [(±)-**11**]

To a solution of (±)-**3** (201 mg, 0.82 mmol) in pyridine (0.53 mL), hexanoyl chloride (0.23 mL, 1.65 mmol) was added and the whole was stirred at rt for 1 h. The mixture was diluted with AcOEt (20 mL) and washed with sat. aq. $NaHCO_3$ (1 x 20 mL). Aqueous layer was extracted with AcOEt (1 x 10 mL). The combined organic layer was washed with 2 M HCl (1 x 5 mL) and brine (1 x 5 mL), and dried over Na_2SO_4. The solvent was evaporated *in vacuo* and the residue was purified by CC (SiO_2, hexane – AcOEt = 97 : 3) to give (±)-**11** as a yellow oil (206 mg, 73%).

IR ν_{max} 1762. ^{1}H-NMR δ: 7.07 (1H, t, *J* = 8.1 Hz, C7-H), 6.66 (1H, d, *J* = 8.0 Hz, C8-H), 6.58 (1H, dd, *J* = 8.2, 0.7 Hz, C6-H), 6.35 (1H, d, *J* = 10.1 Hz, C4-H), 5.60 (1H, d, *J* = 10.1 Hz, C3-H), 5.09 (1H, t sept., *J* = 7.3, 1.3 Hz, CH=), 2.57 (2H, t, *J* = 7.6 Hz, CH_2), 2.17-2.02 (2H, m, CH_2), 1.81-1.62 (4H, m, CH_2 x 2), 1.66 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.44-1.36 (4H, m, CH_2 x 2), 1.39 (3H, s, C2-CH_3), 0.93 (3H, t, *J* = 7.1 Hz, CH_3). ^{13}C-NMR δ: 171.9, 154.0, 146.4, 131.8, 130.2, 128.6, 124.0, 116.6, 114.2, 114.0, 113.8, 78.5, 41.1, 34.20, 31.3, 26.3, 25.6, 24.7, 22.7, 22.3, 17.6, 13.9. HREIMS *m/z* 342.2212 (calcd. for

$C_{22}H_{30}O_3$: 342.2195). HPLC conditions: column CHIRALPAK IC-3 (DAICEL, 2.1 mm x 250 mm), solvent hexane – *i*-PrOH : = 300 : 1, detection 254 nm, flow rate 0.2 mL/min, 15.1 min and 17.1 min. The former was obtained as a major isomer with enzymatic resolution with Amano Lipase PS (Scheme 4a).

2-Methyl-2-(4-methylpent-3-en-1-yl)-2*H*-chromen-5-yl chloroacetate [(±)-**12**]

To a solution of (±)-**6** (304 mg, 1.24 mmol) in CH_2Cl_2 (1.2 mL), chloroacetyl chloride (0.13 mL, 1.63 mmol) and Et_3N (0.26 mL, 1.87 mmol) were added successively at 0 °C and the whole was stirred at 0 °C for 1 h. The mixture was diluted with CH_2Cl_2 (10 mL), washed successively with H_2O (1 x 2 mL), sat. aq. $NaHCO_3$ (1 x 2 mL) H_2O (1 x 2 mL), and brine (1 x 2 mL) and dried over Na_2SO_4. The solvent was evaporated *in vauo* and the residue was purified by CC (SiO_2, hexane – AcOEt = 95 : 5) to (±)-**12** give as a yellow oil (262 mg, 66%).

IR ν_{max} 1780. ^{1}H-NMR δ: 7.09 (1H, t, *J* = 8.0 Hz, C7-H), 6.70 (1H, dt, *J* = 8.2, 0.9 Hz, C8-H), 6.63 (1H, dd, *J* = 8.2, 0.9 Hz, C6-H), 6.38 (1H, dd, *J* = 10.1, 0.9 Hz, C4-H), 5.63 (1H, d, *J* = 10.1 Hz, C3-H), 5.09 (1H, t sept., *J* = 7.1, 1.4 Hz, CH=), 4.31 (2H, s, CH_2Cl), 2.17-2.02 (2H, m, CH_2), 1.78-1.62 (2H, m, CH_2), 1.66 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.39 (3H, s, CH_3). ^{13}C-NMR δ: 165.5, 154.2, 145.8, 131.9, 130.8, 128.7, 123.9, 116.1, 114.5, 113.9, 113.5, 78.7, 41.1, 40.7, 26.3, 25.6, 22.7, 17.6. HRESIMS *m/z* 321.1250 (M+H, calcd. for $C_{18}H_{22}ClO_3$ 321.1258). HPLC conditions: column CHIRALPAK IC-3 (DAICEL, 2.1 mm x 250 mm), solvent hexane – *i*-PrOH : = 300 : 1, detection 254 nm, flow rate 0.2 mL/min, 38.7 min and 44.7 min. The former was obtained as a major isomer with enzymatic resolution with Amano Lipase PS (Scheme 4a).

Racemization experiment under thermal condition.

A solution of **3** (503 mg, 2.06 mmol, 28% ee (*S*)) in toluene (10 mL) was refluxed for 8 days. The solvent was evaporated *in vacuo* to recover (±)-**3** (a brown oil, 501 mg, 99%, 0% ee).

General procedure for enzymatic resolution of (±)-**9**

To a stirred solution of (±)-**9** (4.74 g, 16.9 mmol) in toluene (126 mL), *t*-BuOH (4.8 mL, 50.2 mmol) and Amano Lipase PS from *Burkholderia cepacia* (Aldrich, 6.10 g) were added and the mixture was stirred at 35 °C for 5.5 h. The whole was filtered through Celite® pad, and the filtrate was concentrated *in vacuo*. The residue was purified over CC (SiO_2, hexane – AcOEt = 95 : 5) to afford (-)-**9** (a yellow oil, 847 mg, 18%, 95% *ee*) and (+)-**3** (3.11 g, 75%).

$[\alpha]_D^{23}$ -66.2 (*c* 1.0, $CHCl_3$). IR ν_{max} 1769. ^{1}H-NMR δ: 7.07 (1H, t, *J* = 8.1 Hz, C7-H), 6.66 (1H, dt, *J* = 8.1, 0.9 Hz, C8-H), 6.58 (1H, dd, *J* = 8.1, 0.9 Hz, C6-H), 6.36 (1H, dd, *J* = 10.1, 0.9 Hz, C4-H), 5.60 (1H,

d, J = 10.1 Hz, C3-H), 5.08 (1H, t sept., J = 7.1, 1.3 Hz, CH=), 2.32 (3H, s, Ac), 2.17-2.02 (2H, m, CH_2), 1.74 (1H, ddd, J = 14.0, 10.5, 6.0 Hz, CH), 1.65 (1H, ddd, J = 14.0, 10.5, 6.0 Hz, CH), 1.66 (3H, d, J = 1.3 Hz, CH_3), 1.57 (3H, s, CH_3), 1.39 (3H, s, CH_3). ^{13}C-NMR δ: 169.1, 154.1, 146.3, 131.8, 130.3, 128.6, 123.9, 116.6, 114.1, 113.9, 78.5, 41.1, 26.3, 25.6, 22.7, 20.8, 17.6 (One sp^2 carbon is missing). HREIMS *m/z* 286.1568 (calcd. for $C_{18}H_{22}O_3$ 286.1569).

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