THE MNIOPETALS, NEW INHIBITORS OF REVERSE TRANSCRIPTASES FROM A *Mniopetalum* SPECIES (BASIDIOMYCETES)

II. STRUCTURE ELUCIDATION

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The structures of six new drimance sesquiterpenoids, mniopetals A~F, were elucidated by a combination of chemical and spectroscopic methods. The mniopetals are inhibitors of RNA-directed DNA-polymerases.

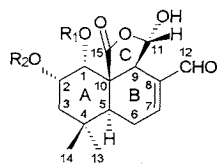
In our search for novel inhibitors of RNA-directed DNA-polymerases of human immunodeficiency, avian myeloblastosis and murine leukemia viruses[1~4], six new antibiotics, mniopetals A~F (**1~6**), as well as the biologically inactive sesquiterpenoids 1α,15-dihydroxymarasmene (**11**) and (−)-11,12-dihydroxydrimene (**14**) were isolated from fermentations of a Canadian *Mniopetalum* species. **11** had been previously found in cultures of *Marasmius oreades*[5] whereas **14** is a known intermediate in the total synthesis of polygodial[6,7].

The production, isolation and biological characterization of the mniopetals A~F has been described in the preceding paper[8]. In this study we report the structural elucidation of these compounds.

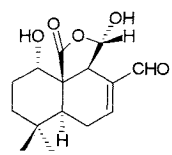
Structural Elucidation of the Mniopetals

Mniopetals A~F (**1~6**) are closely related to marasmal (**7**), a drimane derivative recently isolated by AYER *et al.*[5] from cultures of the basidiomycete *Marasmius oreades*.

Fig. 1. Structures of mniopetal A~F (**1~6**) and marasmal (**7**).



1~5



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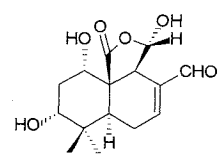

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	R_1	R_2
1	H	(*R*)-$H_3C(CH_2)_7CH(OAc)CO$
2	H	(*R*)-$H_3C(CH_2)_7CH(OH)CO$
3	H	$H_3C(CH_2)_5CH(OH)CO$
4	$H_3C(CH_2)_7CH(OH)CO$	H
5	H	H

Table 1. ^{1}H NMR spectral data for mniopetals A~F (**1**~**6**) (400 MHz, δ in ppm).

	1 (in $CDCl_3$)	**2** (in $CDCl_3$)	**3** (in $CDCl_3$)	**4** (in $CDCl_3$)	**5** (in CD_3OD)	**6** (in CD_3OD)
1-H	4.57 (s, br)	4.52 (s, br)	4.54 (s, br)	5.84 (d)	4.40 (s, br)	4.40 (s, br)
2α-H	—	—	—	—	—	1.66 (ddd)
2β-H	5.35 (ddd)	5.31 (ddd)	5.36 (ddd)	4.39 (ddd)	4.14 (ddd)	2.13 (dddd)
3α-H	2.07 (dd)	2.04 (dd)	2.02 (dd)	1.80 (dd)	1.92 (dd)	1.90 (ddd)
3β-H	1.47 (dd)	1.49 (dd)	1.53 (dd)	1.62 (dd)	1.45 (dd)	1.27 (m)
5-H	1.74 (dd)	1.72 (dd)	1.75 (m)	~1.7 (m)	1.69 (dd)	1.73 (dd)
6α-H	2.44 (dddm)	2.44 (dddm)	2.45 (dddm)	2.50 (dddm)	2.55 (dddm)	2.52 (dddm)
6β-H	2.22 (ddm)	2.23 (ddm)	2.23 (ddm)	2.24 (ddm)	2.18 (ddm)	2.20 (ddm)
7-H	7.10 (d, br)	7.14 (d, br)	7.13 (d, br)	7.14 (d, br)	7.27 (d, br)	7.26 (d, br)
9-H	3.89 (s, br)	3.77 (s, br)	3.80 (s, br)	3.24 (s, br)	3.78 (s, br)	3.72 (s, br)
11-H	5.49 (s, br)	5.54 (s, br)	5.49 (s, br)	5.51 (s)	5.46 (s, br)	5.43 (s, br)
12-H	9.42 (s)	9.49 (s)	9.44 (s)	9.43 (s)	9.48 (s)	9.47 (s)
13-H	1.03 (s)	1.02 (s)	1.03 (s)	1.06 (s)	1.07 (s)	1.04 (s)
14-H	1.32 (s)	1.29 (s)	1.31 (s)	1.30 (s)	1.32 (s)	1.27 (s)
2′-H	4.84 (dd)	4.17 (dd)	4.18 (dd)	4.30 (dd)	—	—
3′-H	1.80 (m)	1.69 (m)	1.69 (m)	~1.7 (m)	—	—
4′-H	1.38 (m)	1.38 (m)	1.39 (m)	~1.35 (m)	—	—
(5′~7′)-H	1.2~1.3 (m)	1.2~1.3 (m)	1.2~1.3 (m)	1.2~1.3 (m)	—	—
8′-H	1.2~1.3 (m)	1.2~1.3 (m)	0.86 (t)	1.2~1.3 (m)	—	—
9′-H	1.2~1.3 (m)	1.2~1.3 (m)	—	1.2~1.3 (m)	—	—
10′-H	0.86 (t)	0.85 (t)	—	0.86 (t)	—	—
2′-O_2CCH_3	2.12 (s)	—	—	—	—	—

1: J (Hz): 1,2β=2.2; 2β,3α=12.7; 2β,3β=4.1; 3α,3β=12.5; 5,6α=3.4; 5,6β=12.7; 6α,6β=19.2; 6α,7=6.7; 2′,3′a=6.5; 2′,3′b=6.5; 9′,10′=6.9.
2: J (Hz): *e.g.* 2′,3′a=7.8; 2′,3′b=4.2.
6: J (Hz): *e.g.* 1,2β=2.5; 2α,2β=14.1; 2α,3α=3.4; 2α,3β=7.1; 2β,3α=14.2; 2β,3β=2.5; 3α,3β=14.1.

Table 2. ^{13}C NMR spectral data for mniopetals A (**1**), B (**2**) and F (**6**) (100.62 MHz, δ in ppm, J in Hz).

	1 (in $CDCl_3$)	**2** (in $CDCl_3$)	**6** (in CD_3OD)		**1** (in $CDCl_3$)	**2** (in $CDCl_3$)	**6** (in CD_3OD)
C-1	67.42	68.15 (dm, 150[a])	68.84	C-14	23.27	23.25 (qm, 124[a])	23.44
C-2	70.82	70.78 (dm, 147[a])	26.71	C-15	176.34	176.45 (m)	179.42
C-3	37.06	37.41 (tm, 129[a])	34.45	C-1′	169.80	174.56 (m)	—
C-4	33.44	33.60 (m)	33.42	C-2′	72.91	71.22 (dm, 148[a])	—
C-5	39.15	39.54 (dm, 127[a])	41.80	C-3′	30.71	34.29 (tm, 126[a])	—
C-6	24.52	24.72 (tm, 130[a])	26.43	C-4′	25.12	24.90 (tm, 125[a])	—
C-7	155.84	155.47 (dm, 158[a])	156.77	C-5′	31.77	31.83 (tm, 124[a])	—
C-8	138.02	138.13 (d, 26[b])	140.73	C-(6′~8′)	29.06	29.22 (tm, 124[a])	—
C-9	45.69	46.18 (dm, 141[a])	48.10		29.21	29.30 (tm, 124[a])	—
C-10	53.31	53.70 (m)	54.44		29.30	29.42 (tm, 124[a])	—
C-11	99.49	100.40 (dm, 179[a])	102.29	C-9′	22.68	22.66 (tm, 124[a])	—
C-12	194.01	193.84 (dd, 177[a], 9[c])	195.57	C-10′	14.27	14.11 (qm, 124[a])	—
				CH_3CO_2-2′	172.44	—	—
C-13	33.14	33.19 (qm, 126[a])	34.50	CH_3CO_2-2′	20.94	—	—

[a] $^1J_{C,H}$ (Hz).
[b] $^2J_{C,H}$ (Hz).
[c] $^3J_{C,H}$ (Hz).

As is indicated by the ^{1}H and ^{13}C NMR data (Tables 1 and 2) all mniopetals contain the same structural pattern at rings B and C. The presence of the α,β-unsaturated aldehyde unit is confirmed by a strong IR absorption at ~1675 cm^{-1} (KBr), signals at δ ~9.45 (CHO) and ~7.2 (br d, 7-H) in the

^{1}H NMR spectrum, and signals at δ ~194 (C-12), ~156 (C-7), and ~139 (C-8) in the ^{13}C NMR spectrum. The lactone and lactol groups of the mniopetals give rise to ^{13}C NMR signals and δ ~177 (C-15) and ~100 (C-11), respectively, and absorptions at ~1760 and ~3430 cm^{-1} in the IR spectrum. In all cases, the 11-H signal in the ^{1}H NMR spectrum appears as a broad singlet, which is characteristic for an α-orientation of the 11-hydroxy group[5].

In mniopetal A (**1**), $C_{27}H_{40}O_9$, the presence of a sequence (C)–CH(OH)–CH(OCOR)–CH_2–(C) can be deduced from the ^{1}H NMR spectrum. It forms part of ring A, which is connected with the rest of the molecule by the ^{1}H-^{13}C long range correlations given in Fig. 2.

The stereochemistry of ring A follows from the ^{1}H NMR spectrum. Since 2-H at δ 5.35 shows a diaxial coupling of J=12.7 Hz to H-3α, the acyloxy substituent must occupy an equatorial position. The 1-H resonance appears as a broad singlet indicating that the hydroxy group at this carbon must be axial. This is supported by the strong deshielding of the protons in 3- and 9-positions, due to 1,3-interaction with the axial hydroxy group[9].

The remaining ^{1}H and ^{13}C NMR data of mniopetal A (**1**) are consistent with the presence of a 2-acetoxydecanoyloxy residue at C-2. The absolute configuration at the side chain stereogenic center was determined by methanolysis of **1** to methyl 2-hydroxydecanoate (**8**) which subsequently was converted into the (*S*)-MTPA ester **9** [MTPA=α-methoxy-α(trifluoromethyl)-(phenylacetic acid)] by treatment with

Fig. 2. Important ^{1}H-^{13}C long-range couplings (COLOC experiments) of mniopetal A (**1**), arrows are directing from H to C.

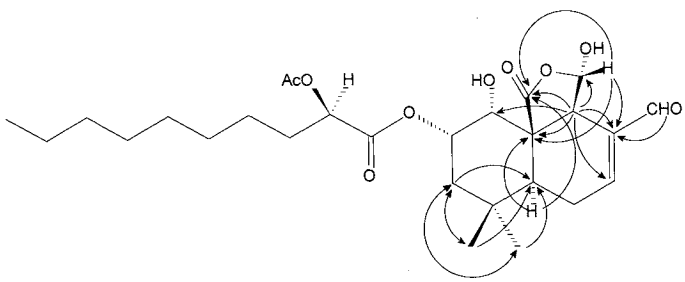


Fig. 3. Conversion of mniopetal A (**1**) into the (*S*)-MTPA ester of methyl (*R*)-2-hydroxydecanoate [(*R*,*S*)-**9**].

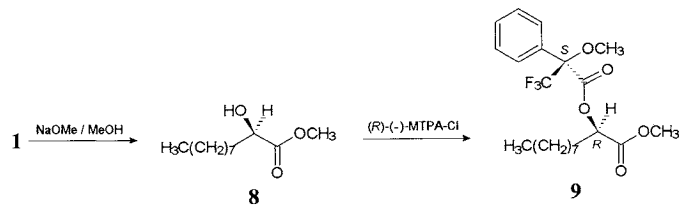
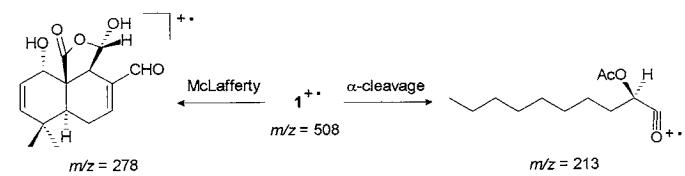


Fig. 4. Characteristic fragmentations of **1** in the EI-MS.



(*R*)-(−)-MTPA-Cl[10,11]. For comparison, a mixture of (*S*,*R*)-**9** and (*S*,*S*)-**9** was prepared by esterification of racemic **8** with (*R*)-(−)-MTPA-Cl. As was demonstrated by YASUHARA and YAMAGUCHI[11], the ^{1}H NMR data of the MTPA-derivatives of α-hydroxycarboxylic acid esters allow an unambiguous assignment of their absolute configuration. In the present case, the (*S*, *R*)-diastereomer of MTPA ester **9** was obtained, which proves the (2′*R*)-configuration of the acetoxyacyl side chain in mniopetal A (**1**).

On electron impact, mniopetal A (**1**) and other acylated compounds of this series undergo McLafferty rearrangement with loss of the acyloxy chain yielding an intense 'drimane' ion at *m/z* 278 ($C_{15}H_{18}O_5$) in the MS. In the case of **1**, α-cleavage of the 2-acetoxydecanoyl side chain leads to a diagnostic fragment ion *m/z* 213 ($C_{12}H_{21}O_3$) as shown in Fig. 4.

Mniopetal B (**2**) shows a peak at *m/z* 448 ($C_{25}H_{36}O_7$) due to the loss of water from the molecular ion. The presence of a strong $[M+Na]^+$ peak at *m/z* 489 in the (+)-FAB-MS confirms $C_{25}H_{38}O_8$ as the molecular formula. The ^{1}H and ^{13}C NMR spectra of **2** lack the signals of the acetyl residue, and show an upfield shift of the 2′-H resonance to δ 4.17. Therefore, mniopetal B (**2**) is the deacetyl derivative of **1**.

The MS and NMR spectra of mniopetal C (**3**), $C_{23}H_{34}O_8$, demonstrate that this compound is the lower homologue of mniopetal B (**2**) and contains a 2-hydroxyoctanoyloxy residue at C-2.

Mniopetal D (**4**), $C_{25}H_{38}O_8$, is an isomer of mniopetal B (**2**) in which the 2-acetoxydecanoyl residue is attached to the OH-group in 1-position. This cases deshielding of 1-H to δ 5.84 and an upfield shift of the 2-H resonance to δ 4.39. The acyl residue in 1-position effects an upfield shift of 9-H to δ 3.24 whereas in the 2-acylated compounds **1**～**3** the corresponding signal occurs at δ ～3.8.

Mniopetal E (**5**), $C_{15}H_{20}O_6$, is the basic diol from which the mniopetals **1**～**4** are derived by esterification. In the ^{1}H NMR spectrum of **5** the resonances of 1-H and 2-H appear at δ 4.40 and 4.14, respectively.

Mniopetal F (**6**), $C_{15}H_{20}O_5$, contains only one axial hydroxy group at ring A. The location of this substituent and the relative stereochemistry of **6** were established by NOE experiments given in Fig. 5. It should be noted that during NMR measurements in CD_3OD the pseudoaxial 6β-proton in **6** was smoothly exchanged against deuterium. Kuehneromycin A (**10**), the 1-oxo derivative corresponding to mniopetal F (**6**) has been recently found in cultures of a Tasmanian *Kuehneromyces* sp.[3].

One of the major metabolites of *Mniopetalum* sp., 1α,15-dihydroxymarasmene (**11**), has already been described as a cometabolite of marasmal (**7**) from *Marasmius oreades*[5]. **11**, $C_{15}H_{22}O_4$, shows complex ^{1}H NMR and ^{13}C NMR spectra reflecting an equilibrium between the two epimeric hemiacetals. On acetylation, **11** yielded a mixture of

Fig. 5. NOE correlations for mniopetal F (**6**).

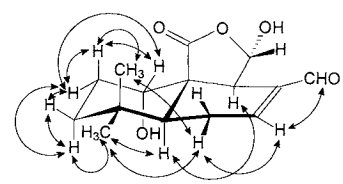
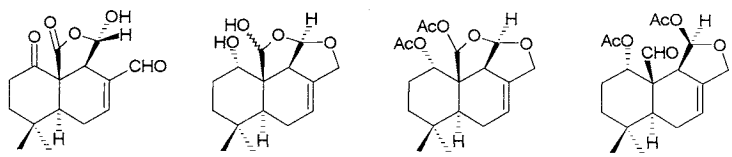


Fig. 6. Structures of kuehneromycin (**10**), 1α,15-dihydroxymarasmene (**11**), diacetate **12**, and diacetoxyaldehyde **13**.

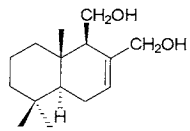


10 **11** **12** **13**

diacetate **12** and diacetoxyaldehyde **13** which could be separated by chromatography. In contrast to the free hemiacetal **11**, only one single epimer of **12** was observed.

The absolute stereochemistry of **11** given in the formula, was determined by high field NMR application of the Mosher method and will be topic of a separate publication[12].

Fig. 7. Structure of (−)-11,12-dihydroxy-7-drimene (**14**).



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A second biologically inactive compound from *Mniopetalum* was identified as the known (−)-11,12-dihydroxy-7-drimene (**14**), $C_{15}H_{26}O_2$. Its spectral and physical data were in agreement with those reported in the literature[7,8]. Since the absolute configuration of (−)-11,12-dihydroxy-7-drimene has been established by total syntheses[7,8], the natural product **14** possesses the absolute stereochemistry given in the formula. It corresponds to that of **11** and most of the natural occurring drimane derivatives of known absolute configuration[13]. Since the mniopetals A ~ F (**1** ~ **6**) are produced by the same fungus, an identical stereochemistry can be assumed for these compounds. All mniopetals exhibit nearly the same CD curves which resemble closely that of kuehneromycin A (**10**)[3].

Experimental

General

Spectral data were recorded on the following instruments: ^1H and ^{13}C NMR, Bruker AC-200, AMXR-300 and AM-400; EI-MS, A.E.I. MS-50 and Finnigan MAT 90 and 95Q; FAB-MS, Kratos Concept H-System; IR, Bruker FT-IR IFS 48 and Perkin-Elmer 1420; UV, Perkin-Elmer Lambda 16 and Varian Cary 17; CD, Jobin Yvon CNRS Roussel-Jouan Dichrographe III. Optical rotations were recorded with a Perkin Elmer 241 polarimeter. The mp's were determined with a Reichert hot-plate microscope and are uncorrected. Merck silica gel 60 (230 ~ 400 mesh) was used for flash chromatography. TLC was carried out on aluminium foils coated with silica gel Merck 60 F_{254}. Solvent systems used for flash chromatography and TLC: I, toluene - acetone - HOAc, 70 : 30 : 1; II, petroleum ether$_{40\sim60}$ - EtOAc, 5 : 1; III, petroleum ether$_{40\sim60}$ - EtOAc, 10 : 1; IV, petroleum ether$_{40\sim60}$ - EtOAc, 2 : 1. All solvents were distilled prior to use.

Mniopetal A (**1**)

Colorless oil; Rf 0.53 (I); $[\alpha]_D^{20}$ −63° (*c* 1.33, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε) 228 (3.70); CD λ_{max}^{MeCN} nm ($\Delta\varepsilon$) 230 (−5.83), 263 (0), 323 (+0.87), 370 (0); IR (KBr) cm^{-1} 3440, 2956, 2928, 2857, 1750, 1674, 1645, 1373, 1202, 1117, 1094, 1059, 947; ^1H NMR, Table 1; ^{13}C NMR, Table 2; EI-MS (direct inlet, 180°C) *m/z* (relative intensity %) 508.2669 (1, M^+, calcd for $C_{27}H_{40}O_9$ 508.2672), 490 (15, $C_{27}H_{38}O_8$), 278 (41, $C_{15}H_{18}O_5$), 260 (20, $C_{15}H_{16}O_4$), 234 (36, $C_{14}H_{18}O_3$), 216 (54, $C_{14}H_{16}O_2$), 215 (47, $C_{14}H_{15}O_2$), 213 (65, $C_{12}H_{21}O_3$), 205 (22, $C_{13}H_{17}O_2$), 204 (37, $C_{13}H_{16}O_2$), 188 (29, $C_{13}H_{16}O$), 187 (22, $C_{13}H_{15}O$), 186 (57, $C_{13}H_{14}O$), 185 (24, $C_{11}H_{21}O_2$), 125 (60, C_9H_{17}), 69 (24), 43 (100).

Determination of the Absolute Configuration at C-2′ of Mniopetal A

To a stirred solution of **1** (5.0 mg) in THF (4 ml) and MeOH (2 ml) were added five drops of 30% methanolic NaOMe. After stirring at 20°C for 1 hour, the reaction mixture was diluted with $CHCl_3$ (30 ml) and washed successively with saturated aqueous NH_4Cl (20 ml) and brine (25 ml). The organic layer was dried over Na_2SO_4 and evaporated to dryness to give an oil, which was chromatographed on a silica gel column. Elution with solvent system II (Rf 0.40) afforded methyl 2-hydroxydecanoate (**8**) (1.5 mg, 75%).

A mixture of (*R*)-(−)-MTPA-Cl (15 mg), **8** (1.5 mg) and pyridine (0.3 ml) in CCl_4 (0.8 ml) was stirred at 20°C for 5 hours. The mixture was poured into Et_2O (40 ml) and the solution washed consecutively

with saturated aqueous NH_4Cl (2×30 ml), saturated aqueous $NaHCO_3$ (30 ml) and brine (30 ml). The organic phase was dried over Na_2SO_4 and evaporated *in vacuo*. The resulting oil was chromatographed on a silica gel column. Elution with solvent system III afforded (*S*, *R*)-**9** (2.3 mg, 74%) as colorless oil; Rf 0.57 (III); $[\alpha]_D^{20}$ 0° (*c* 0.10, $CHCl_3$); UV λ_{max}^{MeCN} nm (log ε) 206 (sh, 4.88), 262 (sh, 3.69); IR (KBr) cm^{-1} 2950, 2925, 1750, 1450, 1269, 1215, 1168, 1115, 1074, 1013, 760, 716, 692; ^1H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, *J*=7.0 Hz, 10-H), 1.15~1.45 (12H, m), 1.90 (2H, m, 3-H), 3.56 (3H, q, *J*=1.1 Hz, OCH_3), 3.74 (3H, s, CO_2CH_3), 5.17 (1H, t, *J*=6.3 Hz, 2-H), 7.40 (3H, m, Ph), 7.58 (2H, m, Ph); EI-MS (180 °C) *m/z* (%) 418.1956 (2, M^+, calcd for $C_{21}H_{29}F_3O_5$ 418.1967), 349 (6), 216 (12), 190 (50), 189 (100), 186 (5), 185 (43), 153 (48), 149 (24), 135 (25), 105 (28), 83 (24), 55 (28).

(*S*)-MTPA-esters (*S*,*R*)-**9** and (*S*,*S*)-**9** from Racemic Methyl 2-Hydroxydecanoate (**8**)

A mixture of the diastereomeric MTPA-esters (*S*,*R*)-**9** and (*S*,*S*)-**9** (8.6 mg, 83%) was obtained from *rac.* methyl 2-hydroxy-decanoate (**8**) (5.0 mg) and (*R*)-(−)-MTPA-Cl according to the previous procedure. The ^1H NMR signals of the diastereomers were assigned according to Lit.[8,9].

Colorless oil; Rf 0.57 [(*S*,*R*)-**9**] and 0.62 [(*S*,*S*)-**9**] (III); ^1H NMR (200 MHz, $CDCl_3$) δ 0.87 (3H, t, *J*=7.0 Hz, 10-H), 1.15~1.45 (12H, m), 1.77~1.98 (2H, m, 3-H), 3.56 (3/2 H, q, *J*=1.1 Hz, OCH_3 of [(*S*,*R*)-**9**]), 3.65 (3/2H, q, *J*=1.1 Hz, OCH_3 of [(*S*,*S*)-**9**]) 3.74 (3/2H, s, CO_2CH_3 of [(*S*,*R*)-**9**]), 3.77 (3/2H, s, CO_2CH_3 of **9**), 5.15 (1/2H, t, *J*=6.3 Hz, 2-H of [(*S*, *S*)-**9**]), 5.17 (1/2H, t, *J*=6.3 Hz, 2-H of [(*S*,*R*)-**9**]), 7.40 (3H, m, Ph), 7.58 (2/2H, m, Ph of [(*S*,*R*)-**9**]), 7.63 (2/2H, m, Ph of [(*S*,*S*)-**9**]).

Mniopetal B (**2**)

Colorless oil; Rf 0.45 (I); $[\alpha]_D^{20}$ −46° (*c* 0.28, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε) 226 (4.00); CD λ_{max}^{MeCN} nm (Δε) 230 (−7.32), 265 (0), 323 (+1.07), 380 (0); IR (KBr) cm^{-1} 3410, 2955, 2927, 2856, 1763, 1735, 1676, 1653, 1458, 1370, 1244, 1203, 1166, 1123, 1096, 1057, 945; ^1H NMR, Table 1; ^{13}C NMR, Table 2; EI-MS (180°C) *m/z* (%) 448.2478 (2, $M^+ - H_2O$, calcd for $C_{25}H_{36}O_7$ 448.2461), 278 (38), 261 (27), 234 (65), 216 (60), 215 (42), 205 (29), 204 (55), 188 (44), 186 (39), 159 (28), 148 (33), 83 (42, C_6H_{11}), 69 (100, C_5H_9), 57 (43), 55 (43); (+)-FAB-MS (mNBA = 3-nitrobenzoic acid) *m/z* 489 $(M+Na)^+$ 449 $(M-H_2O+H)^+$, 279, 261.

Mniopetal C (**3**)

Colorless oil; Rf 0.41 (toluene - HCO_2Et - HCO_2H, 10 : 5 : 3); $[\alpha]_D^{20}$ −45° (*c* 0.05, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε) 228 (3.91); IR (KBr) cm^{-1} 3430, 2955, 2928, 2857, 1770, 1749, 1676, 1649, 1207, 1184, 1123, 1094; ^1H NMR, Table 1; EI-MS (180°C) *m/z* (%) 420.2153 (3, $M^+ - H_2O$, calcd for $C_{23}H_{32}O_7$ 420.2148), 278 (72), 261 (34), 234 (41), 233 (33), 216 (69), 215 (100), 205 (37), 204 (63), 188 (55), 187 (34), 186 (42), 159 (35), 148 (25), 97 (36), 83 (42), 69 (40), 57 (52), 55 (64), 44 (31), 43 (67), 41 (27); (+)-FAB-MS (mNBA) *m/z* 461 $(M+Na)^+$, 421 $(M-H_2O+H)^+$, 279, 261.

Miniopetal D (**4**)

Colorless oil; Rf 0.39 (I); $[\alpha]_D^{20}$ −40° (*c* 0.05, $CHCl_3$); UV λ_{max}^{MeOH} nm (log ε) 228 (3.95); IR (KBr) cm^{-1} 3420, 2956, 2928, 2856, 1765, 1735, 1676, 1649, 1371, 1202, 1166, 1117, 1095, 1059, 948; ^1H NMR, Table 1; EI-MS (180°C) *m/z* (%) 448.2474 (1, $M^+ - H_2O$, calcd for $C_{25}H_{36}O_7$ 448.2461), 278 (15), 234 (21), 216 (21), 215 (24), 91 (27), 83 (38), 69 (100), 57 (67), 55 (53); (+)-FAB-MS (mNBA) *m/z* 489 $(M+Na)^+$, 449 $(M-H_2O+H)^+$, 279, 261.

Mniopetal E (**5**)

Colorless oil; Rf 0.19 (I); $[\alpha]_D^{20}$ −57° (*c* 0.10, $CHCl_3$); UV λ_{max}^{MeCN} nm (log ε) 228 (3.84); CD λ_{max}^{MeCN} nm (Δε) 230 (−5.01), 250 (0), 320 (+0.94), 380 (0); IR (KBr) cm^{-1} 3400, 2934, 1769, 1676, 1649, 1172, 1116, 1096, 1052; ^1H NMR, Table 1; EI-MS (180°C) *m/z* (%) 296.1263 (0.2, M^+, calcd for $C_{15}H_{20}O_6$ 296.1260), 278 (5), 234 (23), 205 (24), 204 (45), 148 (100, $C_9H_8O_2$), 121 (36, C_8H_9O), 120 (37, C_8H_8O), 105 (23, C_8H_9), 91 (40), 57 (27), 43 (58).

Mniopetal F (**6**)

Colorless oil; Rf 0.45 (I); $[\alpha]_D^{23}$ −29° (*c* 0.22, MeOH); UV λ_{max}^{MeOH} nm (log ε) 228 (4.26); CD λ_{max}^{MeCN} nm

($\Delta\varepsilon$) 207 (−5.26), 232 (−4.57), 258 (+0.05), 326 (+0.79); IR (KBr) cm^{-1} 3429, 2930, 2860, 1769, 1677, 1647, 1454, 1369, 1226, 1170, 1115, 1089, 1058; ^1H NMR, Table 1; ^{13}C NMR, Table 2; EI-MS (190°C) *m/z* (%) 280.1273 (3, M^+, calcd for $C_{15}H_{20}O_5$ 280.1311), 262 (44, $C_{15}H_{18}O_4$), 244 (10, $C_{15}H_{16}O_3$), 234 (22, $C_{14}H_{18}O_3$), 217 (52, $C_{14}H_{17}O_2$), 206 (67, $C_{13}H_{18}O_2$), 188 (64, $C_{13}H_{16}O$), 159 (31, $C_{12}H_{15}$), 132 (64, C_9H_8O), 117 (40, C_9H_9), 105 (54, C_8H_9), 91 (100, C_7H_7), 79 (52), 69 (35), 55 (30).

1α,15-Dihydroxymarasmene (**11**)

Colorless microcristals: MP 150~154°C, MP[5] 152~155; Rf 0.35 (I); $[\alpha]_D^{20}$ +92° (*c* 0.60, MeOH); UV λ_{max}^{MeOH} no absorption above 220 nm; IR (KBr) cm^{-1} 3400, 2931, 2867, 1457, 1391, 1367, 1170, 1124, 1059, 1044, 1028, 1003, 970, 956, 923; ^1H NMR (400 MHz, CD_3OD) δ 0.93~1.08 (6H, m), 1.23 (1H, m), 1.55~1.90 (3H, m), 1.95~2.55 (3H, m), 3.25~3.55 (1H, m), 3.90~4.60 (3H, m), 5.10~5.90 (3H, m); ^{13}C NMR (100.6 MHz, CD_3OD) δ 20.32, 21.79, 31.91, 32.45, 39.68, 40.67, 49.36, 52.08, 66.56, 69.47, 99.18, 103.78, 105.02, 107.74, 121.93, 123.55 (CH, CH_3); 24.41, 26.60, 27.16, 28.33, 36.33, 72.24, 73.67 (CH_2); 33.57, 34.14, 134.94, 135.64 (C); EI-MS (180°C) *m/z* (%) 266.1521 (2, M^+, calcd for $C_{15}H_{22}O_4$ 266.1518), 248 (43, $C_{15}H_{20}O_3$), 220 (31, $C_{14}H_{20}O_2$), 219 (100, $C_{14}H_{19}O_2$), 201 (62, $C_{14}H_{17}O$), 173 (50, $C_{13}H_{17}$), 149 (78, $C_9H_9O_2$), 131 (32, $C_{10}H_{11}$), 119 (32, $C_8H_7O_9$), 118 (25, C_9H_{10}), 117 (25), 105 (35), 91 (38), 81 (35, C_6H_9), 69 (28).

Acetylation of 1α,15-Dihydroxymarasmene (**11**)

Treatment of 1α,15-dihydroxymarasmene (**11**, 35 mg) with acetic anhydride (1.0 ml) in pyridine (2.0 ml) for 24 hours, followed by removal of the solvents, gave an oil, which was chromatographed on a silica gel column. Elution with petroleum ether $_{40\sim60}$ - EtOAc (3 : 1) afforded 1α,15-diacetoxymarasmene (**12**, 15 mg, 33%), followed by diacetoxyaldehyde **13** (18 mg, 39%).

1α,15-Diacetoxymarasmene (**12**)

Colorless oil; Rf 0.50 (IV); $[\alpha]_D^{20}$ +66° (*c* 0.70, $CHCl_3$); UV λ_{max}^{MeCN} nm (log ε) 228 (sh, 3.63), 274 (3.16); IR (KBr) cm^{-1} 2945, 2920, 1747, 1730, 1366, 1237, 1221, 1210, 1199, 1152, 1030, 985, 931; ^1H NMR (200 MHz, $CDCl_3$) δ 0.73 (3H, s, 14-H), 0.97 (3H, s, 13-H), 1.27 (1H, ddd, *J*=13.0, 4.5 and 2.5 Hz), 1.56 (1H, dd, *J*=14.1 and 4.0 Hz), 1.60~1.80 (2H, m), 2.00~2.27 (2H, m) 2.10 (3H, s, CH_3CO_2), 2.12 (3H, s, CH_3CO_2), 2.36 (1H, m), 2.95 (1H, m, 9-H), 4.41 (2H, m, 12-H), 5.32 (1H, dd, *J*=2.8 and 2.8 Hz, 1-H), 5.67 (1H, d, *J*=3.7 Hz, 11-H), 5.79 (1H, m, 7-H), 6.08 (1H, s, 15-H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 19.03, 21.23, 21.26, 23.20, 24.81, 31.56, 32.39, 35.35, 40.31, 47.97, 50.90, 69.13, 71.43, 95.44, 103.85, 121.84, 133.19, 169.25, 170.11; EI-MS (180°C) *m/z* (%) M^+ not detected, 290.1510 (13, M^+-HOAc, calcd for $C_{17}H_{22}O_4$ 290.1518), 248 (13, $C_{15}H_{20}O_3$), 230 (30, $C_{15}H_{18}O_2$), 203 (49, $C_{14}H_{19}O$), 202 (100, $C_{14}H_{18}O$), 187 (57, $C_{13}H_{15}O$), 174 (40, $C_{13}H_{18}$), 173 (42, $C_{13}H_{17}$), 146 (54, $C_{10}H_{10}O$), 145 (41, $C_{10}H_9O$), 118 (44, C_9H_{10}), 117 (35, C_9H_9), 43 (77); (+)-FAB-MS (mNBA + NaOAc) *m/z* 723 $(2M+Na)^+$, 373 $(M+Na)^+$, 313 $(M-HOAc+Na)^+$, 291 $(M-HOAc+H)^+$, 231 $(M-2HOAc+H)^+$.

Diacetoxyaldehyde **13**

Colorless oil; Rf 0.44 (IV); $[\alpha]_D^{20}$ +47° (*c* 0.85, $CHCl_3$); UV λ_{max}^{MeCN} nm (log ε) 228 (sh, 3.89); IR (KBr) cm^{-1} 2950, 1738, 1703, 1370, 1240, 1224, 1033, 1003, 960, 922; ^1H NMR (200 MHz, $CDCl_3$) δ 0.80 (3H, s, 14-H), 0.99 (3H, s, 13-H), 1.23 (1H, dm, *J*=13.2 Hz), 1.54 (1H, dd, *J*=13.6 and 4.0 Hz), 1.60~1.89 (2H, m), 2.00 (3H, s, CH_3CO_2), 2.09 (3H, s, CH_3CO_2), 2.05~2.19 (1H, m), 2.30~2.65 (2H, m), 3.22 (1H, m, 9-H), 4.30 (1H, dm, *J*=11.7 Hz, 12-H), 4.47 (1H, dddd, *J*=11.7, 3.2, 2.0 and 2.0 Hz, 12-H), 5.33 (1H, dd, *J*=2.8 and 2.8 Hz, 1-H), 5.70 (1H, m, 7-H), 5.79 (1H, d, *J*=4.9 Hz, 11-H), 9.75 (1H, s, 15-H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 20.14, 20.93, 21.11, 23.37, 23.51, 30.99, 33.31, 35.13, 44.07, 48.17, 49.97, 68.32, 69.89, 97.12, 117.26, 134.05, 169.44, 169.82, 203.77; EI-MS (180°C) *m/z* (%) M^+ not detected, 290.1509 (13, M^+-HOAc, calcd for $C_{17}H_{22}O_4$ 290.1518), 262 (29, $C_{16}H_{22}O_3$), 230 (40, $C_{15}H_{18}O_2$), 203 (30, $C_{14}H_{19}O$), 202 (78, $C_{14}H_{18}O$), 201 (74, $C_{14}H_{17}O$), 187 (40, $C_{13}H_{15}O$), 174 (59, $C_{13}H_{18}$), 173 (64, $C_{13}H_{17}$), 159 (30, $C_{11}H_{11}O$), 146 (46, $C_{10}H_{10}O$), 145 (55, $C_{10}H_9O$), 131 (41, $C_{10}H_{11}$), 118 (49, C_9H_{10}), 117 (37, C_9H_9), 105 (46, C_8H_9), 69 (40), 43 (100); (+)-FAB-MS (mNBA + NaOAc) *m/z* 723 $(2M+Na)^+$, 373 $(M+Na)^+$, 313 $(M-HOAc+Na)^+$, 291 $(M-HOAc+H)^+$, 231 $(M-2HOAc+H)^+$.

(−)-11,12-Dihydroxydrimene (**14**)

Colorless oil; Rf 0.47 (I); $[\alpha]_D^{23}$ −6.5° (*c* 0.16, $CHCl_3$); UV λ_{max}^{MeOH} no absorption above 220 nm; IR (KBr) cm^{-1} 3390, 2924, 2849, 1631, 1459, 1441, 1388, 1366, 1346, 1209, 1167, 1119, 1079, 1041, 996; ^1H NMR (400 MHz, CD_3OD) δ 0.85 (3H, s, 15-H), 0.93 (3H, s, 14-H), 0.96 (3H, s, 13-H), 1.20 (1H, ddd, J=13.2, 13.0 and 3.9 Hz, 1α-H), 1.27 (1H, ddd, J=13.6, 13.0 and 3.6 Hz, 3α-H), 1.29 (1H, dd, J=12.3 and 4.6 Hz, 5-H), 1.48 (1H, dddd, J=13.0, 3.2, 3.1 and 1.8 Hz, 3β-H), 1.53 (1H, dddd, J=13.8, 7.1, 3.6 and 3.2 Hz, 2α-H), 1.66 (ddddd, J=13.8, 13.6, 13.2, 3.4 and 3.1 Hz, 2β-H), 1.98 (1H, m, 6β-H), 2.06 (1H, dddd, J=13.0, 3.5, 3.4 and 1.8 Hz, 1β-H), 2.10 (1H, m, 9-H), 2.12 (1H, m, 6α-H), 3.66 (1H, dd, J=11.0 and 7.5 Hz, 11-H), 3.89 (1H, dd, J=11.0 and 2.6 Hz, 11-H), 3.99 (1H, d, J=12.7 Hz, 12-H), 4.30 (1H, ddd, J=12.7, 2.2 and 1.1 Hz, 12-H), 5.83 (1H, dm, J=5.7 Hz, 7-H); ^{13}C NMR (75 MHz, CD_3OD) δ 14.94 (C-15), 19.85 (C-2), 22.32 (C-13), 24.56 (C-6), 33.77 (C-4[a]), 33.90 (C-14[a]), 36.81 (C-10), 40.62 (C-1), 43.27 (C-3), 51.08 (C-5), 55.88 (C-9), 61.36 (C-11), 67.04 (C-12), 126.42 (C-7), 138.20 (C-8), [a]assignments may be interchanged; EI-MS (70 °C) *m/z* (%) 238.1952 (6, M^+, calcd for $C_{15}H_{26}O_2$ 238.1933), 220 (6, $C_{15}H_{24}O$), 207 (4, $C_{14}H_{23}O$), 205 (2, $C_{14}H_{21}O$), 190 (47, $C_{14}H_{22}$), 175 (11), 124 (24), 109 (100, C_8H_{13}), 105 (15), 91 (14), 81 (12), 69 (12), 55 (10).

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References

1) ERKEL, G.; T. ANKE, R. VELTEN & W. STEGLICH: Podoscyphic acid, a new inhibitor of avian myeloblastosis virus and moloney murine leukemia virus reverse transcriptase from a *Podoscypha* Species. Z Naturforsch. 46c: 442~450, 1991
2) ERKEL, G.; T. ANKE, A. GIMENEZ & W. STEGLICH: Antibiotics from Basidiomycetes XLI. Clavicoronic acid, a novel inhibitor of reverse transcriptases from *Clavicorona pyxidata* (PERS: EX FR.) DOTY. J. Antibiotics 45: 29~37, 1992
3) ERKEL, G.; K. LORENZEN, T. ANKE, R. VELTEN, A. GIMENEZ & W. STEGLICH: Kuehneromycins A and B, two new biological active compounds from Tasmanian *Kuehneromyces* sp. (Strophariaceae, Basidiomycetes). Z. Naturforsch. 1994, in press
4) ERKEL, G.; T. ANKE, R. VELTEN, A. GIMENEZ & W. STEGLICH: Hyphodontal, a new antifungal inhibitor of reverse transcriptases from *Hyphodontia* sp. (Corticiaceae, Basidiomycetes). Z. Naturforsch. 1994, in press
5) AYER, W. A. & P. A. CRAW: Metabolites of the fairy ring fungus, *Marasmius oreades*. Part 2. Norsequiterpenes, further sesquiterpenes, and agrocybin. Can. J. Chem. 67: 1371~1380, 1989
6) MORI, K. & H. WATANABE: Synthesis of both enantiomers of polygodial, an insect antifeedant sesquiterpene. Tetrahedron 42: 273~281, 1986
7) HE, J.-F. & Y-L. WU: Synthesis of drimane sesquiterpenes. An intramolecular Diels-Alder approach. Tetrahedron 44: 1933~1940, 1988
8) KUSCHEL, A.; T. ANKE, R. VELTEN, D. KLOSTERMEYER, W. STEGLICH & B. KÖNIG: The mniopetals, new inhibitors of reverse transcriptases from a *Mniopetalum* species (Basidiomycetes). I. Producing organism, fermentation, isolation and biological activities. J. Antibiotics 47: 733~739, 1994
9) CARR, J. B. & A. C. HUITRIC: Synthesis, proton magnetic resonance, and stereochemistry of certain *o*-tolylcyclohexanediols. J. Org. Chem. 29: 2506~2510, 1964
10) DALE, J. A. & H. S. MOSHER: Nuclear magnetic resonance enantiomer reagents. Configurational correlations *via* nuclear magnetic resonance chemical shifts of diastereomeric mandelate, *O*-methylmandelate, and α-methoxy-α-trifluoromethylphenylacetate (MPTA) esters. J. Am. Chem. Soc. 95: 512~519, 1973
11) YASUHARA, F. & S. YAMAGUCHI: Determination of absolute configuration and enantiomeric purity of 2- and 3-hydroxycarboxylic acid esters. Tetrahedron Lett. 21: 2827~2829, 1980
12) VELTEN, R.; W. STEGLICH & T. ANKE: Determination of the absolute configuration of a tetra-cyclic drimane sesquiterpenoid by Mosher's method. Tetrahedron Asymmetry 5: 1229~1232, 1994
13) JANSEN, B. J. M. & A. DE GROOT: The occurrence and biological activity of drimane sesquiterpenoids. Nat. Prod. Rep. 8: 309~318, 1991