RES-1149-1 and -2, Novel Non-peptidic Endothelin Type B Receptor Antagonists Produced by *Aspergillus* sp.

II. Structure Determination and Derivatization

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The structures of two novel non-peptidic endothelin type B receptor antagonists, RES-1149-1 and -2 were determined by spectroscopic methods. Several derivatives were synthesized from RES-1149-1 for biological assay.

In the preceding paper[1], we have described the taxonomy of the producing strain, fermentation, isolation, physico-chemical properties and biological properties of the novel non-peptidic endothelin type B (ET_B) receptor antagonists, RES-1149-1 and -2. In this paper, we present the structure determination and their derivatization.

Results

Structure Determination of RES-1149-1

The molecular formula of **1** was determined to be $C_{23}H_{30}O_5$ based on HRFAB-MS data (*m/z* 387.2190 $(M+H)^+$, $\Delta+1.8$ mmu). The IR spectrum suggested that **1** had a hydroxyl group (3452 cm^{-1}) and non-conjugated and conjugated carbonyl groups (1707 and 1616 cm^{-1}). The UV spectrum revealed that **1** had a trienone system (304 nm) and a simple enone (sh 218 nm). **1** was positive for 2,4-DNP color test, which showed the existence of aldehyde and/or ketone groups.

^1H and ^{13}C NMR spectra of **1** are summarized in Table 1. Two aldehydes, one ester, four olefins, three methylenes and four methyl groups were found. Protons of a 9-hydroxyl group (4.07 ppm) and C-11 aldehyde (9.77 ppm) were coupled ($J=1.3$ Hz). This showed the presence of a strong hydrogen bond[2]. Data from a ^1H-^1H COSY experiment, in conjunction with one-dimensional ^1H and ^{13}C NMR data, enabled the formulation of partial structures A~C (Fig. 2). An all-*E* configuration of conjugated olefins in partial structure A was deduced by ^1H-^1H coupling constants ($J=14.9\sim15.2$ Hz) and data from a NOESY experiment. Two methine protons (5-H and 7-H) in partial structure B showed a long-range coupling. An HSQC spectrum, which established all the

Fig. 1. Structure of RES-1149-1 (**1**), -2 (**2**) and related compounds.

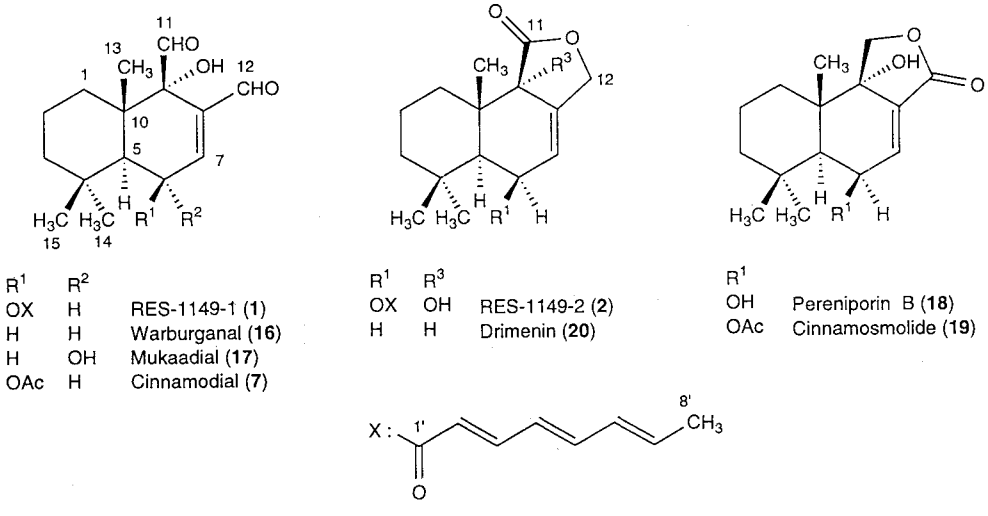
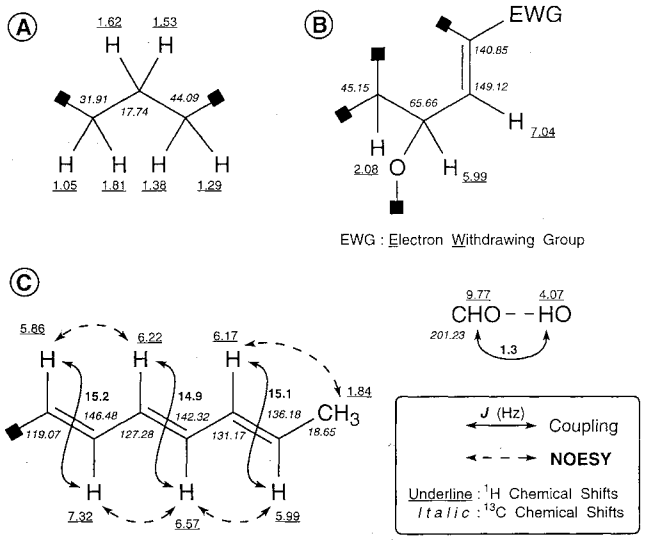


Fig. 2. Partial structures of **1**.



direct ^{1}H-^{13}C connectivities, facilitated the complete assignment of the carbons bearing hydrogens.

The two- and three-bond ^{1}H-^{13}C correlations were determined by an HMBC experiment, which established the connectivities of the partial structures and the other functional groups through oxygens and quaternary

Fig. 3. HMBC analysis of **1**.

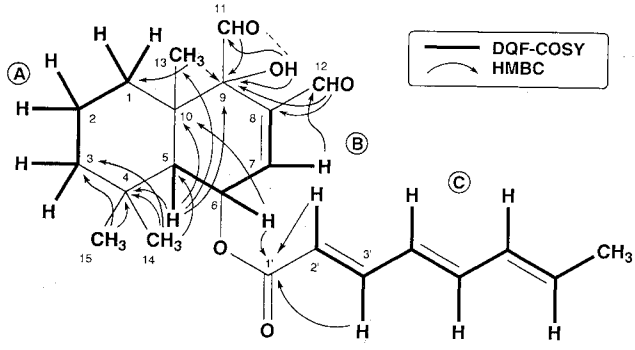


Table 1. NMR data of RES-1149-1 (**1**) and -2 (**2**).

Pos. No.	RES-1149-1 (**1**) [a), b)] ^{13}C	^{1}H	RES-1149-2 (**2**) [a), c)] ^{13}C	^{1}H
1	31.92 t	1.81 1H m	30.24 t	2.08 1H br.d 13.0
		1.05 1H m		1.79 1H ddd 13.0 , 13.0 , 3.6
2	17.76 t	1.62 1H m	17.80 t	1.71 1H dddt 13.0 , 13.0 , 13.0 , 2.7
		1.53 1H m		1.59 1H dq 13.0 , 3.4
3	44.11 t	1.38 1H m	44.87 t	1.42 1H br.d 13.0
		1.29 1H m		1.30 1H ddd 13.0 , 13.0 , 3.3
4	34.11 s		33.86 s	
5	45.18 d	2.08 1H d 4.6	44.84 d	2.04 1H d 4.8
6	65.66 d	5.99 1H t 4.6	66.28 d	5.71 1H m
7	149.12 d	7.04 1H d 4.8	123.71 d	5.89 1H m
8	140.88 s		135.05 s	
9	77.45 s		74.62 s	
10	41.74 s		37.88 s	
11	201.23 d	9.77 1H d 1.3	174.91 s	
12	193.13 d	9.48 1H s	68.96 t	4.96 1H dt 12.3 , 2.4
				4.72 1H dt 12.3 , 1.1
13	20.13 q	1.39 3H s	18.50 q	1.19 3H s
14	32.61 q	1.03 3H s	32.47 q	1.00 3H s
15	24.86 q	1.15 3H s	24.72 q	1.14 3H s
1'	166.13 s		166.30 s	
2'	119.07 d	5.86 1H d 15.2	119.66 d	5.82 1H d 15.3
3'	146.48 d	7.32 1H dd 15.2 , 11.4	145.88 d	7.26 1H dd 15.3 , 11.3
4'	127.28 d	6.22 1H dd 14.9 , 11.4	127.34 d	6.20 1H ddt 14.9 , 11.3 , 0.5
5'	142.32 d	6.57 1H dd 14.9 , 10.7	141.86 d	6.54 1H dd 14.9 , 10.7
6'	131.17 d	6.17 1H ddd 15.1 , 10.7 , 1.6	131.17 s	6.16 1H dddq 14.9 , 10.7 , 0.5 , 1.1
7'	136.18 d	5.99 1H dq 15.1 , 6.8	135.73 d	5.97 1H dq 14.9 , 6.9
8'	18.65 q	1.84 3H dd 6.8 , 1.6	18.56 q	1.83 3H dd 6.9 , 1.1
9-OH		4.07 1H d 1.3		3.19 1H br.s

a) Measured in $CDCl_3$; δ ppm from TMS as an internal standard.

b) 100 MHz for ^{13}C and 400 MHz for ^{1}H.

c) 125 MHz for ^{13}C and 500 MHz for ^{1}H.

carbons (Fig. 3). Crosspeaks between 2′-H, 3′-H, 6-H and C-1′ showed the connection of C-6 oxygen and C-2′ olefinic carbon with C-1′ carbonyl carbon. Correlations between 5-H, 14-H_3, 15-H_3 and C-3, C-4, C-5 confirmed the connectivity from C-3 to C-5 through C-4 quaternary carbon, which had *geminal* methyl groups. Long-range C-H couplings between 7-H, 9-OH, 11-H, 12-H, 13-H_3 and C-1, C-8, C-9, C-10 established a sequence of C-1, C-10, C-9 and C-8, which bore one hydroxy, one methyl and two aldehyde groups. Finally, C-5 and C-10 were bonded based on correlations between C-9, C-10, C-13 and 5-H.

Stereochemistry of RES-1149-1

The relative configuration of **1** was determined by a NOESY experiment (Fig. 4). Two NOE networks among 2-H_{ax}, 13-H_3 and 15-H_3, and among 1-H_{ax}, 3-H_{ax} and 5-H revealed 1,3-diaxial relationships of these protons in a chair-formed cyclohexane ring. This fact also indicated *trans* fusion of the decalin ring system. 5-H, 6-H and 14-H_3 resided on the same side of the rings. From these results all methylene protons and *geminal* methyl protons were stereospecifically assigned. NOEs between H-2′, H-3′ and 13-H_3, 15-H_3 suggested that the trienone sidechain stood upright in axial direction (data not shown).

Structure Determination of RES-1149-2

The structure of **2** was determined in the same way as **1**. In comparison with **1**, only the differences are described as follows. **2** had the same molecular formula as **1**, $C_{23}H_{30}O_5$, which was determined by HRFAB-MS (*m/z* 387.2173 ($M+H^+$) $\Delta+0.2$ mmu). In the UV spectrum, however, **2** showed no absorption maxima for a simple enone, but one for a trienone system (301 nm). On the other hand, an additional carbonyl absorption at 1778 cm^{-1} was observed in the IR spectrum, which revealed the presence of a γ-lactone ring in the molecule. In the NMR spectra (Table 1), a carbonyl (174.9 ppm) and an oxygenated methylene (^{13}C: 69.0 ppm, ^1H: 4.96 and 4.72 ppm), which was assigned to the γ-lactone, were observed instead of two aldehydes in **1**. NMR signals of both C-7 and 7-H were high-field shifted from those of **1**, which indicated that the lactone ring was fused in a non-conjugated form. This assignment is consistent with the UV and IR spectra. Long range C-H connectivities were confirmed by an HMBC experiment and stereochemistry was determined by a NOESY experiment (Fig. 5).

Synthesis of RES-1149 Derivatives

To obtain a preliminary information on structure-activity relationships, several derivatives were synthesized from **1** (Scheme 1). The dialdehyde part of **1** was so labile in both acidic and basic conditions, that we deemed it necessary to protect 1 as the diacetal KT-7619 (**4**) prior to further chemical manupulation. When the reaction time was shortened, the monoacetal KT-7624 (**3**) was a main product. After acetalization, these compounds were still too unstable for chromatography on silica gel. Accordingly, a purification step in the subsequent reactions was excluded as far as possible.

Next, the acyl group of **4** was hydrolysed with alkali, and the resulting diol KT-7620 (**5**) was fully deprotected with acid, to give dial-diol KT-7766 (**6**). Physico-chemical data for **6** agreed well with those reported in the literature[3]. Acylation of **6** with corresponding acid anhydrides afforded KT-7767 (**7**, =cinnamodial) and cinnamoyl derivative KT-7913 (**8**). Physico-chemical properties of **7**, including the specific rotation, agreed

Fig. 4. Relative configuration of **1**.

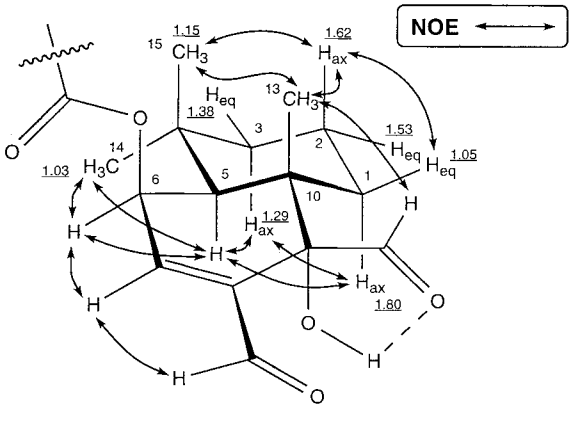
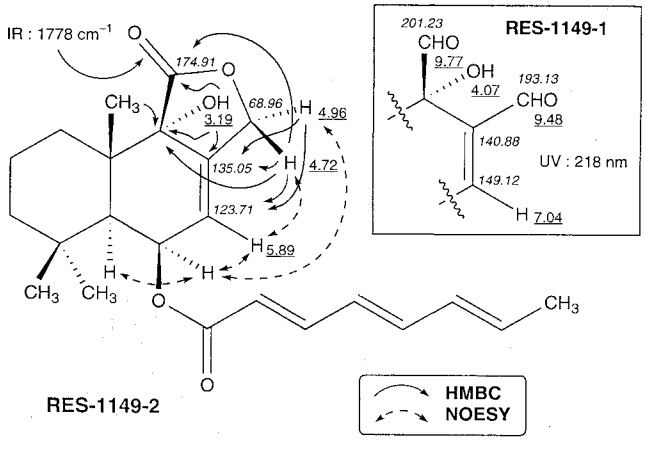
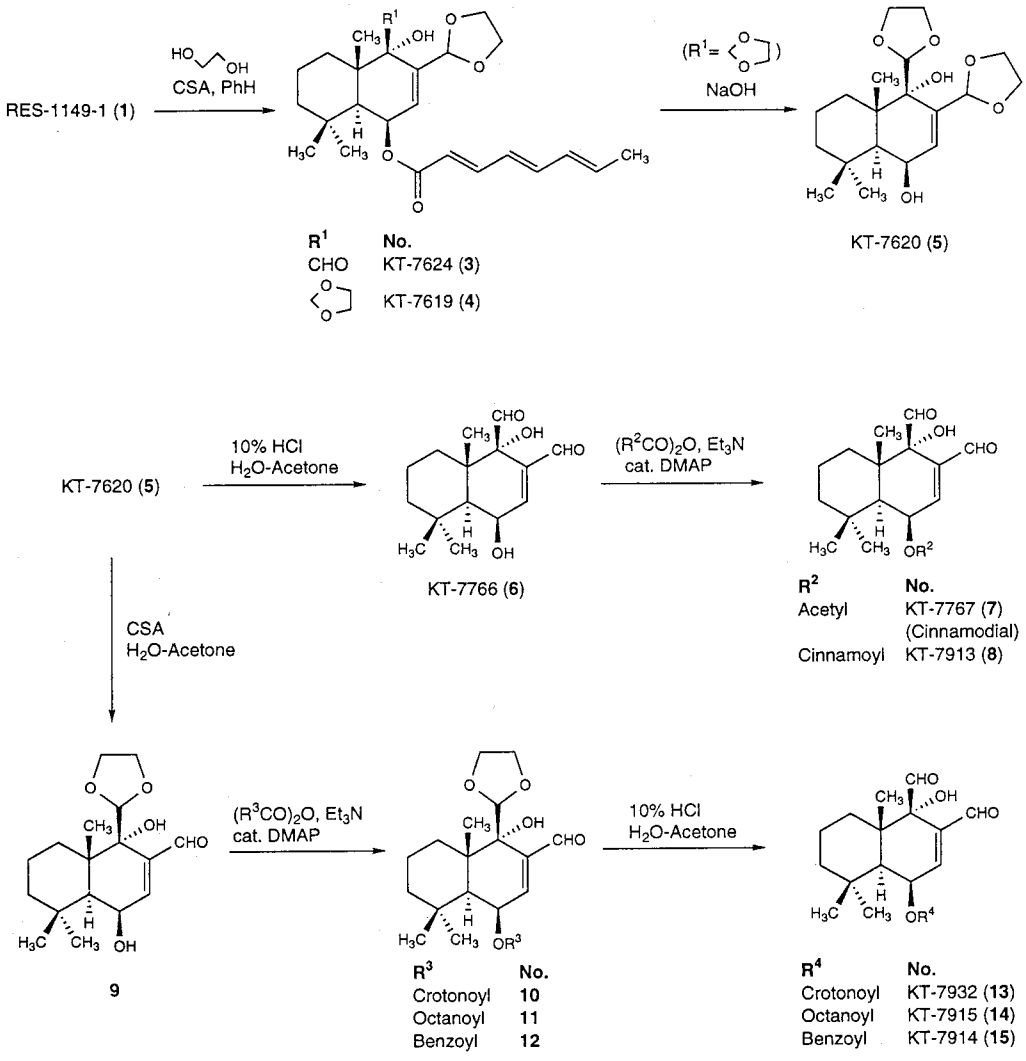


Fig. 5. Structure of **2** and comparison of spectroscopic data with those of **1**.



Scheme 1. Derivatization of **1**.



well with those reported for cinnamodial[4]. This showed that the absolute configuration of RES-1149-1 (**1**) was the same as that of cinnamodial (as shown in the Figures).

Except for acetic and cinnamic anhydrides, **6** could not be acylated. Acylation with various acid chlorides was also unsuccessful. So, partially deprotected mono-acetal (**9**) was utilized with other anhydrides. After the acylation, compounds **10**, **11** and **12** were fully deprotected, to afford crotonoyl derivative KT-7932 (**13**), octanoyl derivative KT-7915 (**14**) and benzoyl derivative KT-7914 (**15**), respectively. These derivatives were submitted to the biological assay for endothelin receptor binding.

Discussion

The structure of RES-1149-1 (**1**) and -2 (**2**), including the absolute configuration of **1**, were determined. These compounds belong to the drimane sesquiterpenoids. As for **1**, structurally related drimane dials are well known. Warburganal (**16**)[8] has no oxygen, mukaadial (**17**)[9] has an *epi*-hydroxyl group, and cinnamodial (**7**) has an acetoxyl group at C-6, respectively. But there are no compounds with an *E,E,E*-2,4,6-octatrienoyloxy group at this position. As for **2**, a majority of drimane γ-lactones such as cinnamosmoliode (**18**)[10] and pereniporin B (**19**)[11], are of the conjugated type. But the non-conjugated types, such as drimenin (**20**) and **2** are in the minority. No other compound of this type with an *E,E,E*-2,4,6-octatrienoyloxy group at C-6, is known. Presumably, **2** was derived from **1** by intramolecular Cannizzaro or Tischtschenko reaction. The similar conversion of a dial to a γ-lactone has been reported[9,10]. We don't know whether **2** is biosynthetically generated or is a chemical rearrengement product of **1**.

The known drimane sesquiterpenoids mentioned above, show a variety of biological activities, for example, insect antifeedant, hot-tasting[12] and cytotoxic activities[4,11]. However, no ET_B receptor antagonist activity like that of **1** and **2**, has been reported. The

structure-activity relationships for **1**, **2** and related derivatives are of considerable interest. The results of the biological assays will be reported and discussed in the following paper[13].

Experimental

General

Materials were obtained from commercial suppliers, except for RES-1149-1 and were used without further purification. Unless otherwise noted, the organic layer after extraction of the reaction mixture was washed with saturated NaCl solution, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* with a rotary evaporater and a vacuum pump. Column chromatography was performed on Wakogel C-200 100~200 mesh silica gel (WAKO Pure Chemical Ind., Ltd., Osaka, Japan). Physico-chemical and spectral data were measured on following instruments: MP, Yanaco micro melting point apparatus; $[\alpha]_D$, Jasco DIP-370 digital polarimeter; UV, Shimadzu UV-2200 UV-VIS spectrophotometer; IR, Jeol JIR-RFX3001 spectrophotometer; ^1H and ^{13}C NMR, Bruker AM500, Jeol JMN-α400 and JMN-FX100 spectrometers; FAB-MS, Jeol JMS-HX110/110A spectrometer.

Preparation of Monoacetal KT-7624 (3)

To a solution of RES-1149-1 (**1**) (139 mg, 0.36 mmol) in benzene (30 ml), ethyleneglycol (3 ml) and *dl*-camphor-10-sulfonic acid (6.0 mg, 0.026 mmol) were added. The solution was refluxed for 3.5 hours, removing water with a Dean-Stark water separator. After cooling, the reaction mixture was mixed with saturated aq $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was washed, dried and concentrated. Chromatography of a part (46 mg) of the residual oil (179 mg) with *n*-hexane - EtOAc (9:1→7:3, stepwise elution) afforded monoacetal KT-7624 (**3**) (11.9 mg, 30%) and diacetal KT-7619 (**4**) (4.0 mg, 9.1%).

3: FAB-MS *m/z* 431 $(M+H)^+$; IR ($CHCl_3$) ν_{max} 3475, 2952, 1709, 1616, 1269, 1124, 1086, 1005; ^1H NMR (100 MHz, $CDCl_3$) δ 9.82 (1H, s), 7.22 (1H, dd, J=15.1, 10.7 Hz), 6.48 (1H, dd, J=15.1, 8.8 Hz), 6.3~5.8 (4H, m), 5.78 (1H, d, J=14.4 Hz), 5.71 (1H, t, J=4.8 Hz), 5.11 (1H, s), 4.0~3.5 (4H, m), 2.01 (1H, d, J=4.6 Hz), 2.0~1.0 (6H, m), 1.77 (3H, d, J=5.9 Hz), 1.46 (3H, s), 1.07 (3H, s), 0.93 (3H, s).

Preparation of Diacetal KT-7619 (4)

To a solution of RES-1149-1 (**1**) (978 mg, 2.53 mmol) in benzene (60 ml), ethyleneglycol (6 ml) and *dl*-camphor-10-sulfonic acid (24 mg, 0.10 mmol) were added. The solution was refluxed for 5 hours, removing water with a Dean-Stark water separator. After cooling, the reaction mixture was mixed with saturated aq $NaHCO_3$ solution, and extracted with ether. The organic layer was washed, dried and concentrated. The residual oil (1.53 g) containing diacetal KT-7619 (**4**) was used for the next step without purification: FAB-MS *m/z* 475 $(M+H)^+$; IR ($CHCl_3$) ν_{max} 3516, 1699, 1616, 1269, 1134, 1070, 1005; ^1H NMR (100 MHz, $CDCl_3$) δ 7.17 (1H, dd, J=15.2, 11.6 Hz), 6.46 (1H, dd, J=15.9, 9.0 Hz), 6.3~5.8 (4H, m), 5.74 (1H, d, J=15.2 Hz), 5.73 (1H, s), 5.67 (1H, t, J=5.6 Hz), 4.93 (1H, s), 4.1~3.6 (8H, m), 2.0~1.0 (6H, m), 1.92 (1H, d, J=4.2 Hz), 1.77 (3H, d, J=5.6 Hz), 1.25 (3H, s), 1.04 (3H, s), 0.94 (3H, s).

Preparation of Diol KT-7620 (5)

To a solution of crude **4** (1.53 g) in DMSO (35 ml), a solution of NaOH (506 mg, 12.7 mmol) in water (8 ml) was added and stirred for 16 hours at 50°C. After cooling, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed, dried and concentrated. The residual oil (509 mg) containing diol KT-7620 (**5**) was used for the next step without purification: FAB-MS *m/z* 377 $(M+Na)^+$, 355 $(M+H)^+$; High resolution (HR) FAB-MS *m/z* 355.2133 Δ+1.2 mmu for $C_{19}H_{30}O_6+H$; IR $CHCl_3$) ν_{max} 3514, 3015, 2954, 1603, 1462, 1367, 1076, 1055, 945; ^1H NMR (400 MHz, $CDCl_3$) δ 6.40 (1H, d, J=5.5 Hz), 5.76 (1H, s), 4.99 (1H, s), 4.49 (1H, dd, J=5.5, 4.2 Hz), 4.16~3.72 (8H, m), 2.07 (1H, dt, J=3.6, 14.3 Hz), 1.78~1.22 (5H, m), 1.75 (1H, d, J=4.2 Hz), 1.36 (3H, s), 1.28 (3H, s), 1.08 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 135.29 (s), 129.22 (d), 104.27 (d), 102.01 (d), 76.73 (s), 65.87 (t), 65.34 (t), 65.24 (d), 63.88 (t), 63.08 (t), 46.03 (d), 44.00 (t), 40.66 (s), 34.20 (s), 33.18 (q), 32.63 (t), 25.24 (q), 19.56 (q), 18.51 (t).

Preparation of Dial-diol KT-7766 (6)

To a solution of crude **5** (161 mg) in acetone (6 ml), water (3 ml) and 10% HCl (0.3 ml) were added. After stirring for 4 hours at room temperature (RT), 10% HCl (0.3 ml) was added again and stirred for additional 3 hours at the same temperature. After cooling, the reaction mixture was mixed with saturated aq $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was washed, dried and concentrated. Chromatography of the residual oil (133 mg) with *n*-hexane - EtOAc (7:3) afforded dial-diol KT-7766 (**6**) (22.6 mg, 11% from **1**). Physico-chemical and spectral data of **6** agreed well with those previously reported[3]: MP 122.0~125.0°C; $[\alpha]_D^{28}=-264°$ (*c* 0.172, $CHCl_3$); FAB-MS *m/z* 267 $(M+H)^+$; HRFAB-MS *m/z* 267.1618 Δ+2.1 mmu for $C_{15}H_{22}O_4+H$; IR (KBr) ν_{max} 3647, 3627, 1718, 1678, 1051, 802; ^1H NMR (400 MHz, $CDCl_3$) δ 9.77 (1H, s), 9.50 (1H, s), 7.05 (1H, d, J=4.6 Hz), 4.84 (1H, t, J=4.7 Hz), 4.08 (1H, br s), 1.88 (1H, d, J=4.9 Hz), 1.76 (1H, dt, J=4.4, 13.3 Hz), 1.63 (1H, dtt, J=13.3, 13.3, 3.2 Hz), 1.52 (1H, m), 1.38 (1H, m), 1.35 (6H, s), 1.29 (1H, m), 1.12 (3H, s), 0.99 (1H, m).

Preparation of Cinnamodial, KT-7767 (7)

To a solution of **6** (10.6 mg, 0.040 mmol) in benzene (0.2 ml), acetic anhydride (11 μl, 0.12 mmol), triethylamine (10 μl, 0.072 mmol), and a catalytic amount of 4-dimethylaminopyridine (4-DMAP) were added. After

stirring for 18 hours at RT, the reaction mixture was dried under reduced pressure to give KT-7767 (**7**) (10.3 mg, 84%). Physico-chemical and spectral data of **7** agreed well with reported data for cinnamodial[2~7]: $[\alpha]_D^{27} = -284°$ (*c* 0.205, $CHCl_3$) (*lit.*[2] $-407°$); FAB-MS *m/z* 309 $(M+H)^+$; HRFAB-MS *m/z* 309.1695 $\Delta -0.7$ mmu for $C_{17}H_{24}O_5+H$; IR ($CHCl_3$) ν_{max} 3469, 2950, 1735, 1718, 1691; ^1H NMR (400 MHz, $CDCl_3$) δ 9.76 (1H, d, $J=1.5$ Hz), 9.48 (1H, s), 7.00 (1H, d, $J=4.6$ Hz), 5.90 (1H, t, $J=4.8$ Hz), 4.07 (1H, d, $J=1.5$ Hz), 2.15 (3H, s), 2.05 (1H, d, $J=4.9$ Hz), 1.78 (1H, dt, $J=4.5$, 13.3 Hz), 1.62 (1H, dtt, $J=13.3$, 13.3, 3.2 Hz), 1.51 (1H, m), 1.40 (1H, m), 1.34 (3H, s), 1.30 (1H, dd, $J=12.6$, 4.1 Hz), 1.17 (3H, s), 1.04 (1H, m), 1.03 (3H, s).

Preparation of KT-7913 (**8**)

To a solution of **6** (5.5 mg, 0.021 mmol) in benzene (0.3 ml), cinnamic anhydride (16 mg, 0.058 mmol), triethylamine (5 μl, 0.036 mmol), and a catalytic amount of 4-DMAP were added and stirred for 3 hours under N_2 at RT. The reaction mixture was mixed with 2 N HCl and extracted with EtOAc. The organic layer was washed with saturated aq $NaHCO_3$ and saturated aq NaCl solutions, dried, and concentrated to give a crude product (20 mg). Two steps of chromatography (1st: *n*-hexane-EtOAc (10:1→1:1, stepwise elution); 2nd: *n*-hexane-acetone (10:1→5:1, stepwise elution)) afforded KT-7913 (**8**) (4.0 mg, 49%): FAB-MS *m/z* 397 $(M+H)^+$; HRFAB-MS 397.1998 $\Delta -1.7$ mmu for $C_{24}H_{28}O_5+H$; IR ($CHCl_3$) ν_{max} 3471, 1716, 1691, 1635, 1306, 1159, 1126; ^1H NMR (400 MHz, $CDCl_3$) δ 9.79 (1H, d, $J=1.5$ Hz), 9.50 (1H, s), 7.74 (1H, d, $J=15.9$ Hz), 7.59~7.51 (2H, m), 7.43~7.37 (3H, m), 7.08 (1H, d, $J=4.9$ Hz), 6.45 (1H, d, $J=15.9$ Hz), 6.06 (1H, t, $J=4.8$ Hz), 4.09 (1H, d, $J=1.5$ Hz), 2.12 (1H, d, $J=4.6$ Hz), 1.82 (1H, dt, $J=4.5$, 13.3 Hz), 1.65 (1H, dtt, $J=13.3$, 13.3, 3.2 Hz), 1.54 (1H, m), 1.44 (3H, s), 1.42 (1H, m), 1.31 (1H, dt, $J=3.3$, 13.0 Hz), 1.20 (3H, s), 1.08 (1H, m), 1.06 (3H, s).

Preparation of Monoacetal (**9**)

To a solution of crude **5** (509 mg) in acetone (30 ml), water (20 ml) and *dl*-camphor-10-sulfonic acid (10 mg, 0.043 mmol) were added and stirred for 4 hours at RT. The reaction mixture was mixed with saturated aq $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed, dried, and concentrated to give crude **9** (450 mg), which was used for the next step without purification: MP 87.0~88.5°C; $[\alpha]_D^{20} = -53.5°$ (*c* 0.33, CH_3CN); FAB-MS *m/z* 311 $(M+H)^+$; HRFAB-MS *m/z* 311.1850 $\Delta -0.9$ mmu for $C_{17}H_{26}O_5+H$; UV (CH_3CN) ν_{max} 302 (ε 740), 217 (ε 6800); IR (KBr) ν_{max} 3442, 2920, 1697, 1637, 1464, 1074; ^1H NMR (400 MHz, $CDCl_3$) δ 9.67 (1H, s), 6.64 (1H, d, $J=5.4$ Hz), 5.02 (1H, s), 4.63 (1H, t, $J=4.8$ Hz), 4.05 (2H, m), 3.91 (2H, m), 3.46 (1H, br s), 2.04 (1H, dt, $J=3.9$, 13.5 Hz), 1.76 (1H, d, $J=4.6$ Hz), 1.68 (1H, dtt, $J=13.5$, 13.5, 3.5 Hz), 1.54 (1H, m), 1.43 (1H, m), 1.37 (1H, m), 1.36 (3H, s), 1.32 (1H, m), 1.22 (3H, s), 1.09 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.70 (d), 140.83 (s), 140.79 (s), 102.81 (d), 76.50 (s), 66.50 (t), 64.87 (d), 63.77 (t), 46.02 (d), 43.91 (t), 40.14 (s), 34.20 (s), 32.88 (q), 31.96 (t), 25.29 (q), 18.96 (q), 18.26 (t).

Preparation of Crotonoyl Derivative **10**

To a solution of crude **9** (116 mg) in benzene (2 ml), crotonic anhydride (0.32 ml, 2.2 mmol), triethylamine (0.30 ml, 2.2 mmol), and 4-DMAP (46 mg, 0.38 mmol) were added and stirred for 17 hours under N_2 at RT. The reaction mixture was mixed with 2 N HCl and extracted with EtOAc. The organic layer was washed with saturated aq $NaHCO_3$ and saturated aq NaCl solutions, dried, and concentrated to give a crude product (350 mg). Which was chromatographed with *n*-hexane-EtOAc (7:3) to afford crotonoyl derivative **10** (89 mg, 36% from **1**): FAB-MS (NBA) *m/z* 379 $(M+H)^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 9.65 (1H, s), 6.96 (1H, dq, $J=15.5$, 6.9 Hz), 6.61 (1H, d, $J=5.5$ Hz), 5.83 (1H, dq, $J=15.5$, 1.7 Hz), 5.05 (1H, s), 3.51 (1H, br s), 1.99 (1H, d, $J=4.3$ Hz), 1.89 (3H, dd, $J=6.9$, 1.7 Hz), 2.00~1.05 (6H, m), 1.25 (3H, s), 1.13 (3H, s), 1.01 (3H, s).

Preparation of Octanoyl Derivative **11**

In the same manner as the preparation of **10**, acylation of crude **9** (96.2 mg) with octanoic anhydride gave a crude product (524 mg). Which was chromatographed with *n*-hexane-EtOAc (9:1→1:1, stepwise elution) to afford octanoyl derivative **11** (23.7 mg, 10% from **1**): FAB-MS *m/z* 437 $(M+H)^+$; ^1H NMR (500 MHz, $CDCl_3$) δ 9.64 (1H, s), 6.59 (1H, d, $J=5.4$ Hz), 5.75 (1H, t, $J=4.9$ Hz), 5.04 (1H, s), 4.05 (2H, m), 3.91 (2H, m), 3.51 (1H, br s), 2.35 (2H, t, $J=7.5$ Hz), 2.04 (1H, m), 1.96 (1H, d, $J=4.5$ Hz), 1.64 (2H, m), 1.28 (8H, m), 1.21 (3H, s), 1.14 (3H, s), 1.01 (3H, s), 0.88 (3H, t, $J=7.2$ Hz).

Preparation of Benzoyl Derivative **12**

In the same manner as the preparation of **10**, acylation of crude **9** (109 mg) with benzoic anhydride gave a crude product (426 mg). Which was chromatographed with *n*-hexane-EtOAc (8:2→1:1, stepwise elution) to afford benzoyl derivative **12** (40 mg, 16% from **1**): FAB-MS *m/z* 415 $(M+H)^+$; IR ($CHCl_3$) ν_{max} 3527, 3008, 2954, 1705, 1452, 1271, 1109, 1070, 1026; ^1H NMR (500 MHz, $CDCl_3$) δ 8.12~7.36 (5H, m), 6.71 (1H, d, $J=5.4$ Hz), 6.04 (1H, t, $J=4.9$ Hz), 5.09 (1H, s), 4.05 (2H, m), 3.92 (2H, m), 3.56 (1H, br s), 2.13 (1H, dt, $J=4.0$, 13.3 Hz), 2.09 (1H, d, $J=4.3$ Hz), 1.71 (1H, dtt, $J=13.3$, 13.3, 3.4 Hz), 1.58 (1H, m), 1.53 (1H, m), 1.41 (3H, s), 1.39 (1H, m), 1.35 (1H, m), 1.15 (3H, s), 1.06 (3H, s).

Preparation of KT-7932 (**13**)

To a solution of **10** (51 mg, 0.13 mmol) in acetone (1 ml), water (0.2 ml) and six drops of 10% HCl were added. The solution was stirred for 2 hours at RT and for 3 hours at 50°C. After cooling, the reaction mixture was mixed with saturated aq $NaHCO_3$ solution and

extracted with EtOAc. The organic layer was washed, dried, and concentrated to give crude product (34.4 mg). Which was chromatographed with *n*-hexane - EtOAc (8 : 2→7 : 3, stepwise elution) to afford KT-7932 (**13**) (1.8 mg, 4.0%): FAB-MS *m/z* 335 $(M+H)^+$; HRFAB-MS *m/z* 335.1865 $\Delta+0.7$ mmu for $C_{19}H_{26}O_5+H$; IR ($CHCl_3$) ν_{max} 3473, 3022, 2929, 1716, 1691, 1655, 1261, 1182, 1103, 1041; ^1H NMR (500 MHz, $CDCl_3$) δ 9.77 (1H, d, $J=1.4$ Hz), 9.47 (1H, s), 7.03 (1H, dq, $J=15.6$, 6.9 Hz), 7.02 (1H, d, $J=4.7$ Hz), 5.97 (1H, dd, $J=4.7$, 4.7 Hz), 5.89 (1H, dq, $J=15.6$, 1.7 Hz), 2.08 (1H, d, $J=4.7$ Hz), 1.93 (3H, dd, $J=6.9$, 1.7 Hz), 1.89 (1H, m), 1.80 (1H, m), 1.62 (1H, m), 1.52 (1H, m), 1.39 (1H, m), 1.37 (3H, d, $J=0.3$ Hz), 1.30 (1H, m), 1.15 (3H, s), 1.03 (3H, s).

Preparation of KT-7915 (**14**)

In the same manner as the preparation of **13**, deprotection of **11** (19.2 mg) with 10% HCl gave a crude product (17.7 mg), which was chromatographed with *n*-hexane - EtOAc (9 : 1→7 : 3, stepwise elution) to afford KT-7915 (**14**) (4.2 mg, 24%): FAB-MS *m/z* 393 $(M+H)^+$; HRFAB-MS *m/z* 393.2641 $\Delta\pm0.0$ mmu for $C_{23}H_{36}O_5+H$; ^1H NMR (400 MHz, $CDCl_3$) δ 9.76 (1H, d, $J=1.0$ Hz), 9.48 (1H, s), 7.00 (1H, d, $J=4.8$ Hz), 5.91 (1H, dd, $J=4.8$, 4.8 Hz), 4.07 (1H, d, $J=1.0$ Hz), 2.35 (2H, t, $J=7.6$ Hz), 2.05 (1H, d, $J=4.8$ Hz), 1.79 (1H, dt, $J=4.2$, 13.2 Hz), 1.64 (2H, m), 1.70～1.20 (5H, m), 1.34 (3H, s), 1.28 (8H, m), 1.16 (3H, s), 1.02 (3H, s), 0.88 (3H, t, $J=7.0$ Hz).

Preparation of KT-7914 (**15**)

In the same manner as the preparation of **13**, deprotection of **12** (32.4 mg) with 10% HCl gave a crude product (24.2 mg). Which was chromatographed with *n*-hexane - EtOAc (8 : 2→1 : 1, stepwise elution) to afford KT-7914 (**15**) (3.7 mg, 13%): FAB-MS *m/z* 371 $(M+H)^+$; HRFAB-MS *m/z* 371.1848 $\Delta-1.0$ mmu for $C_{22}H_{26}O_5+H$; ^1H NMR (400 MHz, $CDCl_3$) δ 9.80 (1H, d, $J=1.4$ Hz), 9.49 (1H, s), 8.05 (2H, m), 7.61 (1H, m), 7.48 (2H, m), 7.12 (1H, d, $J=4.8$ Hz), 6.21 (1H, t, $J=4.7$ Hz), 4.10 (1H, d, $J=1.4$ Hz), 2.18 (1H, d, $J=3.6$ Hz), 1.86 (1H, dt, $J=4.4$, 13.3 Hz), 1.66 (1H, m), 1.56 (1H, m), 1.53 (3H, s), 1.41 (1H, m), 1.33 (1H, m), 1.18 (3H, s), 1.10 (1H, m), 1.07 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 201.04 (d), 193.08 (d), 165.64 (s), 148.47 (d), 141.17 (s), 133.63 (d), 129.91 (d)×2, 128.75 (d)×2, 77.51 (s), 66.43 (d), 45.32 (d), 44.09 (t), 41.83 (s), 34.11 (s), 32.65 (q), 32.01 (t), 25.20 (q), 20.45 (q), 17.76 (t).

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References

1) OGAWA, T.; K. ANDO, T. TANAKA, Y. UOSAKI & Y. MATSUDA: RES-1149-1 and -2, novel non-peptidic endothelin type B receptor antagonists produced by *Aspergillus* sp. I. Taxonomy of producing strain, fermentation, isolation, and phisico-chemical and biological properties. J. Antibiotics 49: 1～5, 1996
2) BROOKS, C. J. W. & G. H. DRAFFMAN: Sesquiterpenoids of *Warburgia* species-II ugandensolide and ugandensidial (cinnamodial). Tetrahedron 25: 2887～2898, 1969
3) WHITE, J. D. & L. P. J. BURTON: Synthesis of the insect antifeedant (±)-cinnamodial and the drimane sesquiterpenoids (±)-isodrimenin and (±)-fragrolide. J. Org. Chem. 50: 357～364, 1985
4) MAHMOUD, I. I.; A. D. KIGHORN, G. A. CORDELL & N. R. FARNSWORTH: Potential anticancer agents. XVI. Isolation of bicyclofarnesane sesquiterpenoids from *Capsicodendron Dinisii*. J. Natl. Prod. 43: 365～371,1980
5) VIDARI, G. & L. GARLASCHELLI: Synthetic studies on biologically active natural compounds. part I: Stereospecific transformation of uvidin A into (−)-cinnamodial. Tetrahedron 45: 7371～7378, 1989
6) CORTES, M.; I. RAZMILIC & J. LOPEZ: Synthesis of (−)-ugandensidial. J. Natl. Prod 53: 1369～1371, 1990
7) KIOY, D.; A. I. GRAY & P. G. WATERMAN: A Comparative study of the stem-bark drimane sesquiterpenes and leaf volatile oils of *Warburgia Ugandensis* and *W. Stuhlmannii*. Phytochemistry 29: 3535～3538, 1990
8) KUBO, I.; Y.-W. LEE, M. PETTEI, F. PILKIEWICZ & K. NAKANISHI: Potent army worm antifeedants from the east African *Warburgia* plants. J. Chem. Soc. Chem. Commun. 1976: 1013～1014, 1976
9) KUBO, I.; T. MATSUMOTO, A. B. KAKOOKO & N. K. MUBIRU: Structure of mukaadial, a molluscicide from the *Warburgia* plants. Chem. Lett. 1983: 979～980, 1983
10) CANONICA, L.; A. CORBELLA, P. GARIBOLDI, G. JOMMI, J. KREPINSKY & G. F. C. CASAGRANDE: Sesquiterpenoids of *Cinnamosma Fragrans* Baillon: Structure of cinnamolide, cinnamosmolide and cinnamodial. Tetrahedron 25: 3895～3902, 1969
11) KIDA, T.; H. SHIBAI & H. SETO: Structure of new antibiotics, pereniporins A and B, from a Basidiomycete. J. Antibiotics 39: 613～615, 1986
12) KUBO, I. & I. GANJIAN: Insect antifieedant terpenes, hot-tasting to humans. Experientia 37: 1063～1064, 1981
13) OGAWA, T.; Y. UOSAKI, T. TANAKA, E. TSUKUDA, A. MIHARA & Y. MATSUDA: RES-1149-1 and -2, novel non-peptidic endothelin type B receptor antagonists produced by *Aspergillus* sp. III. Biochemical properties of RES-1149-1, -2 and structure-activity relationships. J. Antibiotics 49 (2): 1996, in press