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CLERODENDRINS FROM *CLERODENDRON TRICHOTOMUM* AND THEIR FEEDING STIMULANT ACTIVITY FOR THE TURNIP SAWFLY

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Key Word Index—*Clerodendron trichotomum*; Verbenaceae; *Athalia rosae ruficornis*; turnip sawfly; Tenthredinidae; pharmacophagy; feeding stimulants; defense substance; *neo*-clerodane diterpenoids; Clerodendrin.

Abstract—Adults of the turnip sawfly, *Athalia rosae ruficornis*, are stimulated to feed pharmacophagously on the leaves of *Clerodendron trichotomum* by bitter *neo*-clerodane diterpenoids contained in glandular organs on the leaf surface. Four new *neo*-clerodane analogues, clerodendrins E, F, G and H, were isolated from leaf surface extracts of *C. trichotomum*. Clerodendrins E, F and G were characterized as analogues of clerodendrin A, in which the acyl moiety at the C-3β position was replaced by 2-acetoxy-2-methylpropanoyl, 2,3-diacetoxy-2-methylbutanoyl and 2-methylbutanoyl moieties, respectively. Clerodendrin H was shown to be the 7,8-dihydro-derivative of F. Of the seven clerodendrins (A–H) isolated from *C. trichotomum*, only the 7,8-dihydro analogues (clerodendrins B, D and H) exhibited distinct feeding stimulant activity to adults of the turnip sawfly.

INTRODUCTION

Adults of the turnip sawfly, *Athalia rosae ruficornis* (Jakovlev) (Tenthredinidae, Hymenoptera), frequently visit leaves of *Clerodendron trichotomum* (Thunb.) and feed voraciously on the leaf surface [1, 2]. Clerodendrins B (**IIa**) and D (**IId**) have been characterized as the specific feeding stimulants for the sawfly [1]. The insects sequester the bitter principles in their body tissues as defense substances by pharmacophagously feeding on the leaf tissues [3]. The sawflies, however, responded selectively to a few clerodendrin analogues, even though a number of analogues are present in *C. trichotomum* leaves. This paper reports on the structural elucidation of four new clerodendrins, E–H, and the feeding stimulant activity of the analogues for *A. rosae ruficornis*.

RESULTS

Feeding stimulants found in the glandular tissues present on the leaf surface of *C. trichotomum* were extracted by quickly rinsing the leaves with organic solvents after the leaf surface had been brushed. A series of *neo*-clerodane diterpenes were found in the silica gel column chromatographic fractions which elicited potent feeding response in *A. rosae ruficornis*. Besides clerodendrins A (**Ia**), B (**IIa**) and D (**IId**, ajugapitin or its 2′-epimer) reported previously [1, 4, 5], four new *neo*-clerodane analogues, which we have named here as clerodendrins E, F, G and H, were isolated from the chromatographic fractions.

Clerodendrins E, F and G were deduced to be analogues of clerodendrin A in which only the acyl side-chain at C-3 differed from that of A, because all of these compounds showed very similar ^{1}H and ^{13}C NMR signals for the clerodendrin skeleton with the 2α-hydroxy, 3β-acyloxy, 4α,17-epoxy, 6α,18-diacetoxy, 7,8-ene and 11,12,13,16-tetrahydrofurofuran functions as assigned in Tables 1 and 2.

Clerodendrin E gave the $[M]^+$ peak at m/z 592 (EIMS, corresponding to $C_{30}H_{40}O_{12}$). The acyl moiety at the C-3 position was verified to be an 2-acetoxy-2-methylpropanoyl from the ^{1}H and ^{13}C NMR spectra (Tables 1 and 2). Two prominent fragment ions (EIMS) due to the 2′-acetoxy-2′-

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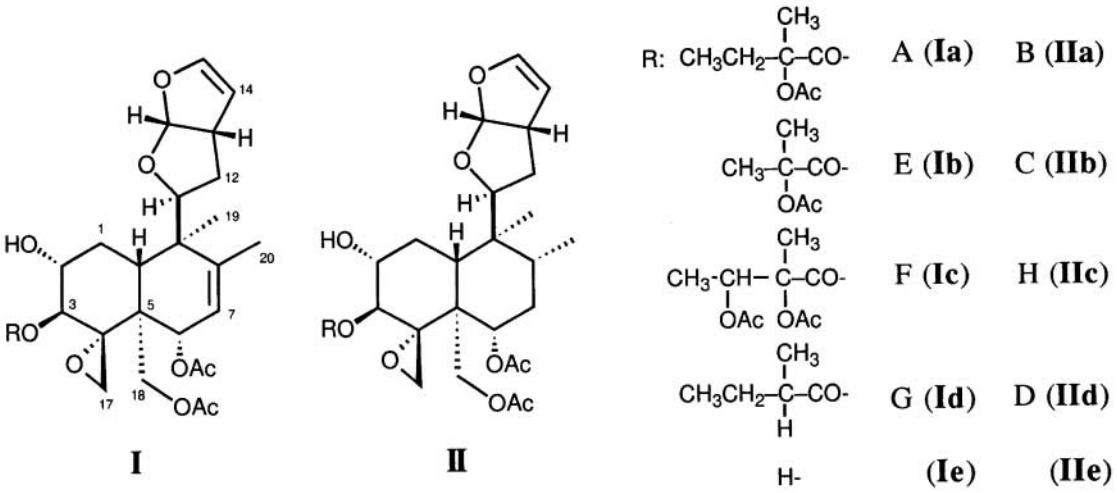

methylacyl moiety were found at *m/z* 129 and 101 in E while the corresponding ions in A were observed at *m/z* 143 and 115, indicating the 14 amu difference in the side chains between A and E. Upon mild alkaline hydrolysis, E afforded 2,3-diol **Ie**, which was also obtained by hydrolysis of A (**Ia**). Thus, the structure of clerodendrin E is as given by formula **Ib**.

Clerodendrin F gave the $[M]^+$ peak at *m/z* 664 (EIMS, corresponding to $C_{33}H_{44}O_{14}$). The ^1H and ^{13}C NMR assignments of compound F are listed in Tables 1 and 2 and were secured with the help of 2D-NMR techniques (H–H COSY, C–H COSY and NOESY). The ^1H NMR spectrum exhibited four acetoxy signals and a doublet methyl (δ1.27) coupled with a quartet methine (δ5.04) ($J_{3',4'} = 6.6$ Hz) and a singlet methyl (δ1.61) arisen from the 2,3-diacetoxy-2-methylbutanoyloxy moiety at the C-3β position. The two prominent fragment ions (EIMS) of F from the 2′,3′-diacetoxy-2′-methylbutanoyl moiety were found at *m/z* 201 and 173 and the corresponding ions due to loss of 42 amu (C_2H_2O) were observed at *m/z* 159 and 131. Upon alkaline hydrolysis, F gave 2,3,7,18-tetranol (**III**) [4] and 2,3-dihydroxy-2-methylbutanoic acid. On GC analysis of the methyl ester of the acid, the R_t coincided with that of methyl *erythro*-2,3-dihydroxy-2-methylbutanoate (prepared by oxidation of methyl angelate with $KMnO_4$–benzyltriethylammonium chloride in CH_2Cl_2), whereas the *threo*-isomer (prepared from methyl tiglate and $KMnO_4$) gave a different R_t. An acylation shift of the C-2β proton

Table 1. ^1H NMR Spectral assignments of clerodendrins

H	A (**Ia**)*	E (**Ib**)*	F (**Ic**)*	G (**Id**)	D (**IId**)*	H (**IIc**)
1β	2.67*m*	2.66*m*	2.66*m*	2.6*m*	2.58*m*	2.5*m*
2	3.61*m*	3.64*m*	3.61*m*	3.6*m*	3.65*m*	3.6*m*
3	5.48*d* (9.0)	5.47*d* (11.5)	5.44*d* (9.9)	5.33*d* (10.1)	5.24*d* (10.1)	5.38*d* (10.8)
6	5.23*br s*	5.23*br s*	5.21*br s*	5.24*br s*	4.69*dd* (4.7, 11.5)	4.70*dd* (4.5, 11.5)
7	5.03*br s*	5.03*br s*	5.03*br s*	5.07*br s*	*m*	*m*
11	4.07*dd* (4.7, 12.0)	4.08*dd* (5.0, 11.0)	4.06*dd* (4.9, 11.5)	4.10*dd* (5.4, 10.8)	4.03*dd* (4.4, 11.5)	4.04*m*
13	3.49*br t* (6.5)	3.47*br t* (6.5)	3.48*br t* (6.5)	3.5*m*	3.56*m*	3.53*m*
14	4.80*t* (2.5)	4.79*t* (2.2)	4.79*t* (2.5)	4.83*t* (2.5)	4.81*t* (2.7)	4.81*t* (2.5)
15	6.43*t* (2.5)	6.44*t* (2.2)	6.42*t* (2.5)	6.46*t* (2.5)	6.47*t* (2.7)	6.48*t* (2.5)
16	6.06*d* (6.6)	6.06*d* (6.0)	6.05*d* (6.1)	6.09*d* (5.4)	6.05*d* (6.1)	6.06*d* (6.1)
17a	2.82*d* (4.0)	2.84*d* (4.0)	2.78*d* (3.9)	2.83*d* (4.0)	2.81*d* (3.9)	2.79*d* (4.0)
17b	2.63*d* (4.0)	2.67*d* (4.0)	2.57*d* (3.9)	2.68*d* (4.0)	2.51*d* (3.9)	2.46*d* (4.0)
18a	4.61*d* (12.1)	4.61*d* (12.1)	4.60*d* (12.1)	4.68*d* (11.5)	4.79*d* (12.4)	4.82*d* (13.0)
18b	4.45*d* (12.1)	4.45*d* (12.1)	4.44*d* (12.1)	4.46*d* (11.5)	4.41*d* (12.4)	4.36*d* (13.0)
19	1.23*s*	1.24*s*	1.23*s*	1.23*s*	0.98*s*	0.96*s*
20	1.67*br s*	1.68*br s*	1.67*br s*	1.68*br s*	0.85*d* (6.0)	0.85*d* (6.0)
2′	–	–	–	2.32*sext* (7.2)	2.37*sext* (7.0)	–
3′	1.8*m*	1.49*s*	5.04*q* (6.6)	*m*	*m*	5.04*q* (7.0)
4′	0.94*t* (7.5)	–	1.27*d* (6.6)	0.89*t* (7.2)	0.90*t* (7.4)	1.26*d* (7.0)
2′Me	1.56*s*	1.57*s*	1.61*s*	1.15*d* (7.2)	1.12*d* (7.0)	1.60*s*
OAc	2.14*s*	2.13*s*	2.14*s*	2.14*s*	2.13*s*	2.14*s*
OAc	2.09*s*	2.09*s*	2.10*s*	2.00*s*	1.94*s*	2.12*s*
OAc	2.00*s*	2.01*s*	2.06*s*	–	–	2.06*s*
OAc	–	–	1.98*s*	–	–	1.92*s*

*Measured at 400 MHz. Otherwise measured at 90 MHz, $CDCl_3$. Chemical shifts in δ values with TMS as internal standard, *J* values in Hz.

Table 2. ^{13}C NMR spectral assignments of clerodendrins

C	A(**Ia**)	E(**Ib**)	F(**Ic**)	G(**Id**)	D(**IId**)	F(**IIc**)
1	31.8	31.4	31.6	33.0	30.5	29.2
2	70.9*	70.6*	71.1	70.8*	71.7*	71.2*
3	73.8	73.3	74.5	72.3	72.3	74.5
4	62.1	61.9	62.1	62.3	62.8	62.8
5	44.3	43.9	44.1	42.1	46.1	46.1
6	71.4*	71.3*	70.8	71.7*	71.4*	71.4*
7	124.4	124.6	124.6	124.6	33.4†	33.3†
8	139.3	139.8	139.6	139.2	36.0	35.7
9	42.3	42.0	42.0	44.3	40.1	40.1
10	38.0	37.7	37.7	37.9	43.9	43.7
11	85.8	85.6	85.8	86.0	84.2	83.9
12	33.2	33.0	33.0	33.2	31.6†	31.6†
13	45.7	45.3	45.5	45.7	45.7	45.7
14	102.1	102.1	102.3	102.1	101.8	101.7
15	146.5	147.0	146.9	146.5	147.1	147.0
16	109.0	108.9	109.1	109.0	107.8	107.8
17	43.7	43.6	43.4	43.4	42.5	42.6
18	62.6	62.6	62.5	62.5	61.5	61.5
19	19.7	18.9†	19.5	19.9	13.9	13.8
20	19.4	19.1†	19.2	19.3	16.4	16.6
2′	82.1	78.6	82.3	41.2	41.2	82.3
3′	31.9	23.5	72.2	26.8	26.8	72.1
4′	7.4	–	14.5	11.3	11.3	14.3
2′-Me	20.8	24.6	17.7	16.4	16.5	18.0
Ac–Me	21.0	20.7	20.8	21.0	21.0	21.0
Ac–Me	21.1	20.7	20.8	21.1	21.0	21.0
Ac–Me	21.3	20.8	20.8	–	–	21.0
Ac–Me	–	–	21.1	–	–	21.3
CO	170.1	170.1	168.8	170.3	170.1	168.2
CO	170.5	170.7	170.2	170.9	171.0	169.6
CO	170.7	171.2	170.6	172.7	175.7	169.8
CO	171.8	171.2	171.3	–	–	170.7
CO	–	–	172.0	–	–	171.5

Measured at 22.5 MHz, $CDCl_3$. Chemical shifts in δ values with TMS as an internal standard.
*,†These assignments may be reversed.

(from δ3.16 to 5.00) was observed in the ^1H NMR spectrum of the 2α-acetoxy derivative of F (see Section 4). Further, a significant NOE correlation was observed between the hydroxy proton at C-2α and 2′-methyl (δ1.61) in the NOESY spectrum of F. Thus, the structure of clerodendrin F is as given in formula **Ic**. The absolute configuration of the 2′,3′-diacetoxy-2′-methylbutanoyl moiety remains to be clarified.

Clerodendrin G gave the $[M]^+$ peak at m/z 548 (EIMS, corresponding to $C_{29}H_{40}O_{10}$). The ^1H and ^{13}C NMR spectra indicated the presence of a 2-methylbutanoyloxy moiety (Tables 1 and 2). The presence of prominent mass fragmentation ions at m/z 85 and 57 also supported the presence of the moiety, exhibiting prominent ions of m/z 85 and 57. Upon mild alkaline hydrolysis, G gave 2,3-diol **Ie** and 2-methylbutanoic acid. Thus, clerodendrin G was determined to be a 7,8-dehydro-derivative of D (**Id**).

The molecular weight of clerodendrin H was determined to be 666 (corresponding to $C_{33}H_{46}O_{14}$) from the secondary ion mass spectrum (SIMS: m/z 667 $[M + H]^+$; 689 $[M + Na]^+$), which is 2 amu unit greater than the Mr of F (**Ic**). The ^1H NMR spectrum assigned four acetoxy methyls, a tertiary methyl (δ1.60) and a secondary methyl (δ1.26) coupled with a quartet methine (δ5.04) ($J_{3',4'} = 7.0$ Hz), indicating the presence of a 2,3-diacetoxy-2-methylbutanoyloxy moiety as found in F. On the other hand, clerodendrin H gave very similar ^1H and ^{13}C NMR signals of the diterpene skeleton to those present in the spectrum of clerodendrin D (**IId**) (Tables 1 and 2), which indicated a 7,8-dihydro structure in H. Upon alkaline hydrolysis, in common with F, *erythro*-2,3-dihydroxy-2-methylbutanoic acid was recovered from the acidic fraction. Further, the partial hydrolysis of H yielded 2α,3β-diol-6α,18-diacetate (**IIe**). Thus the structure of clerodendrin H is as shown in formula **IIc**.

The feeding stimulant activity of the seven clerodendrin analogues (A–H) isolated from *C. trichotomum* leaves were evaluated using adult turnip sawflies as shown in Fig. 1. Clerodendrins B (**IIa**), H (**IIc**) and D (**IId**) induced a 100% response at 10^{-7} g/filter paper. On the other hand, clerodendrins A (**Ia**), E (**Ib**), F (**Ic**) and G (**Id**) induced much lower responses and failed to give in 100% response even at the highest doses (10^{-5} g/filter paper).

DISCUSSION

Seven clerodendrins (A–H) have been isolated from leaves of *C. trichotomum*. These are *neo*-clerodane analogues either with 7,8-unsaturation

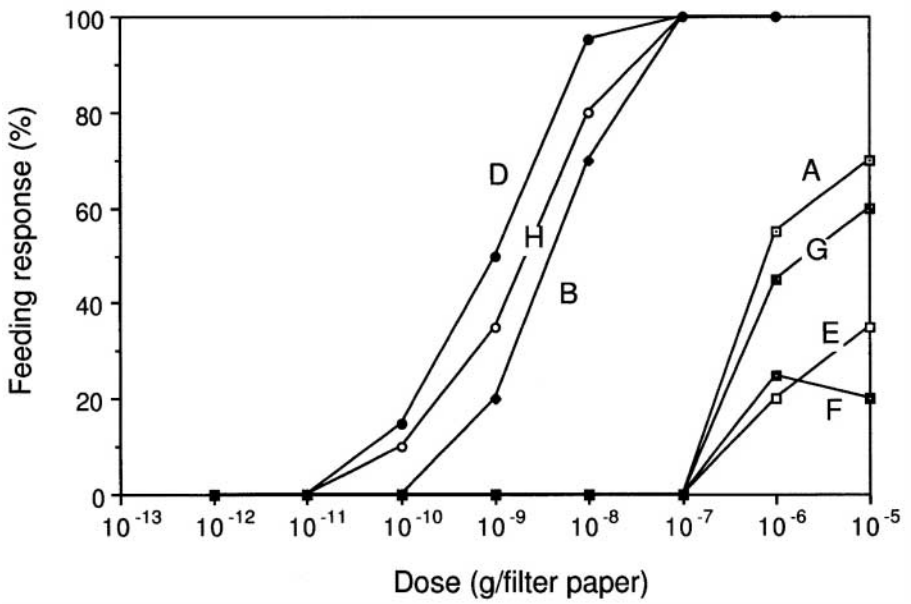

Fig. 1. Dose responses for clerodendrins A (**Ia**), B (**IIa**), D (**IId**), E (**Ib**), F (**Ic**), G (**Id**) and H (**IIc**) in the feeding stimulant bioassay using females of *Athalia rosae ruficornis* ($N = 20$ for each plot).

(type **I**) or saturation (type **II**) in combination with various acyloxy moieties at C-3. We were unable to detect clerodendrin C (**IIb**), the structure of which had been originally reported as a component of *C. fragrance* Vent. together with B and D [6, 7].

Amongst the seven analogues, clerodendrins B (**IIa**), D (**IId**) and H (**IIc**) were found to be distinctively bioactive, provoking several orders of magnitude higher activity than that of the type **I** compounds [A (**Ia**), E (**Ib**), F (**Ic**) and G (**Id**)] in the feeding stimulant assay (Fig. 1). Although compounds **Ia** and **Ic** used in this bioassay were carefully purified by a combination of HPLC and recrystallization, it is not certain whether these compounds (A and F) are in fact bioactive or whether the marginal activity is due to the presence of very small quantities of type **II** contaminants. It should be noted that a large proportion of the clerodendrin analogues in the leaf surface rinse was found to be less active type **I** compounds, in which the content of clerodendrin A accounted for 80–90% of the total analogues, while the total amounts of type **II** compounds accounted for approximately 10% (see experimental part). No significant increase of activity was observed when A was mixed with B. Therefore, there seemed to be no synergistic action between type **I** and type **II** compounds.

A number of *neo*-clerodane derivatives with the 11,12,13,16-tetrahydrofurofuran moiety are known from plants in the Verbenaceae and Labiatae [1, 7–12]. We have found that clerodin and 3-epicaryoptin had feeding stimulant activity as potent as that of 7,8-saturated clerodendrins (unpublished data), further supporting the structural requirement of B-ring saturation while the A-ring substituents did not greatly influence the activity. However, none of the *neo*-clerodane analogues with an α,β-unsaturated γ-lactone structure in place of a tetrahydrofurofuran moiety (ajugamarins and ajugatakasins from *Ajuga decumbens*), exhibited activity, even though the B-ring forms a 7,8-saturated structure with the same configurational arrangements of substituents as those present in type **II** clerodendrins [12].

Adults of *A. rosae ruficornis* selectively sequester clerodendrin D by feeding on *C. trichotomum* [3]. We have also identified ajugachin (a 2-methylpropanoyl ester of **IIe** at C-3 position) and **IIe** from the body tissues of the adult females which fed voraciously on the glandular trichomes on the leaves of *C. trichotomum* (unpublished data) [13]. These bitter compounds have been shown to play a defensive role against a predatory bird [3]. Interestingly, adults of *A. rosae ruficornis* do not seem to sequester the major clerodendrins such as A and B [3]. The host plants of the turnip sawfly larvae are restricted within the Cruciferae. They seem to have obtained an ecological advantage by pharmacophagously acquiring specific bitter principles from *neo*-clerodane-containing plants during the adult stage [3].

EXPERIMENTAL

General

M.p.s: uncorr.; EIMS: 70 eV; SIMS: 8 kV with Xe as the primary beam gas, glycerol matrix. Plant materials were collected in September 1989 and October 1995 in Sakyo-ku, Kyoto (Japan) and

voucher specimens were deposited in the Herbarium of Pesticide Research Institute, Kyoto University.

Feeding bioassay

Adult females of *A. rosae ruficornis* (3–10 days after adult eclosion) were used for the feeding stimulant assay. A soln of a test sample of given concentration was applied to a square piece of filter paper (Toyo filter paper, qualitative No. 2, 8×8 mm^2) and allowed to dry. The treated paper was brought into contact with the sawfly's mouthparts and a positive response was scored when the insect fed on the paper for more than 10 s. Twenty insects were used for each compound, testing different concentrations of a compound on the same insects in a sequence from the lowest to the highest doses.

Extraction and isolation of clerodendrins

The leaf surfaces on both sides of fresh leaves of *C. trichotomum* (5.7 kg) were gently rubbed with a brush and immersed twice in a mixture of hexane–Me_2CO (1:1) within 10 min in total and the extracts were concentrated under reduced pressure (20 mm Hg, ~38°) to give a solid mass. The organic layer (13.5 g) obtained on partition between water and EtOAc was chromatographed on a silica gel column (Wako gel C-200, 350 g, 370×55 mm i.d.) eluted with 30% benzene in hexane (Fr. 1), 40% EtOAc in hexane (Fr. 2), 60% EtOAc in hexane (Fr. 3), EtOAc (Fr. 4). A crystalline mass of clerodendrins A and B precipitated from fraction 3. After filtration of the crystals, portions of the filtrate were rechromatographed on a silica gel column (Wako gel C-300, 20 g, 385×12.5 mm i.d.), eluted with increasing concentrations of EtOAc in hexane. Fractions eluted with 50–60% EtOAc in hexane were subjected to HPLC (YMC-Pack SIL S-5, 300×10 mm i.d., eluting with 70% EtOAc in hexane, 2.5 ml/min). Clerodendrins A and H were isolated at R_t 10.0 and 15.5 min, respectively. Mixtures of B + G and D + E + F were eluted at around R_t 11.8 and 13.2 min, respectively, and were rechromatographed on the same column eluting with 20% EtOH in hexane (2.5 ml) [R_t (min): D, 14.4; G, 14.4; B, 15.6, E, 17.3; F, 17.8]. Each component was purified further by recrystallization and successive HPLC purification. The amounts of each compound per g fresh leaf (surface rinse) were estimated to be as follows: A, 263 μg; B, 21 μg; D, 9 μg; E, 4.2 μg; F, 5.4 μg; G, 1.3 μg; H, 1.1 μg.

*Clerodendrin E (**Ib**)*

Needles (hexane–C_6H_6), m.p. 168–171°. EIMS (direct inlet) *m/z* (rel. int.): 592 $[M]^+$ (0.3), 547 (0.5), 496 (0.8), 405 (10), 391 (49), 129 (57), 111 (100), 101 (49), 83 (23); ^{1}H and ^{13}C NMR: Tables 1 and 2.

*Clerodendrin F (**Ic**)*

Needles (hexane–C_6H_6), m.p. 219°. EIMS (direct inlet) *m/z* (rel. int.): 664 $[M]^+$ (0.1), 605 (0.4), 463 (51), 361 (14), 201 (84), 186 (53), 173 (38), 159 (66), 131 (100), 111 (81), 83 (26); ^{1}H and ^{13}C NMR: Tables 1 and 2.

Clerodendrin F acetate

^{1}H NMR (90 MHz, $CDCl_3$): δ 1.23 (3H, *s*, H-19), 1.26 (3H, *d*, J = 6.6, H-4′), 1.57 (3H, *s*, 2′-Me), 1.66 (3H, *br s*, H-20), 1.97 (3H, *s*, Ac–Me), 1.98 (3H, *s*, Ac–Me), 2.02 (3H, *s*, Ac–Me), 2.11 (3H, *s*, Ac–Me), 2.15 (3H, *s*, Ac–Me), 2.62 (1H, *m*, H-1β), 2.63 (1H, *d*, J = 3.9, H-17b), 2.78 (1H, *d*, J = 3.9, H-17a), 3.50 (1H, *m*, H-13), 4.07 (1H, *dd*, J = 4.9, 11.5, H-11), 4.45 (1H, *d*, J = 12.0, H-18b), 4.62 (1H, *d*, J = 12.0, H-18a), 4.81 (1H, *t*, J = 2.5, H-14), 4.91 (1H, *q*, J = 6.6, H-3′), 5.00 (1H, *m*, H-2β), 5.06 (1H, *br s*, H-7), 5.21 (1H, *br s*, H-6), 5.56 (1H, *d*, J = 9.9, H-3α), 6.09 (1H, *d*, J = 7.0, H-16), 6.43 (1H, *t*, J = 2.5, H-15).

*Clerodendrin G (**Id**)*

Amorphous solid. $[\alpha]_D^{24}$ = + 60.9° ($CHCl_3$, *c*, 0.23). EIMS (direct inlet) *m/z* (rel. int.): 548 $[M]^+$ (0.1), 438 (2.6), 347 (100), 288 (7), 157 (36), 111 (70), 85 (22), 57 (21); ^{1}H and ^{13}C NMR: Tables 1 and 2.

*Clerodendrin H (**IIc**)*

Amorphous solid. SIMS *m/z*: 689 $[M + Na]^+$, 667 $[M + H]^+$, 607 $[M–OAc]^+$; ^{1}H and ^{13}C NMR: Tables 1 and 2.

*Preparation of **Ie***

Clerodendrins A, E and G were individually hydrolyzed with K_2CO_3 in MeOH–H_2O (100:1) to yield **Ie** as a common component. Crystalline solid. EIMS (direct inlet) *m/z* (rel. int.): $[M]^+$-absent, 373 (13), 186 (40), 173 (100), 145 (65), 111 (61), 43 (30); ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.22 (3H, *s*, H-19), 1.68 (3H, *br s*, H-20), 2.01 (3H, *s*, Ac-Me), 2.09 (3H, *s*, Ac–Me), 2.65 (1H, *m*, H-1β), 2.87 (2H, *s*, H-17a,b), ~3.5 (1H, *m*, H-13), ~3.6 (1H, *m*, H-2β), 3.93 (1H, *d*, J = 9.0, H-3α), 4.08 (1H, *dd*, J = 5.0, 12.0, H-11), 4.39 (1H, *d*, J = 12.0, H-18b), 4.70 (1H, *d*, J = 12.0, H-18a), 4.80 (1H, *t*, J = 2.5, H-14), 5.07 (1H, br *s*, H-7), 5.29 (1H, *br s*, H-6), 6.06 (1H, *d*, J = 6.2, H-16), 6.43 (1H, *t*, J = 2.5, H-15).

*Hydrolysis of clerodendrin F (**Ic**)*

Clerodendrin F was treated with MeONa in MeOH, and the acidified reaction mixture purified through a Sep-pak cartridge C_{18} (Waters Associates), by eluting with water (3 ml) and then with MeOH (1 ml). Tetraol **III** was recovered from the MeOH eluate. EIMS (direct inlet) *m/z* (rel. int.): $[M]^+$-absent, 314 (9), 204 (32), 186 (48), 111 (100),

83 (49), 41 (66); ^{1}H NMR (90 MHz, DMSO-d_6): δ 1.01 (3H, *s*, H-19), 1.60 (3H, *br s*, H-20), 2.76 (1H, *d*, J = 4.0, H-17b), 2.93 (1H, *d*, J = 4.0, H-17a), 3.39 (1H, *d*, J = 12.0, H-18b), 3.95 (1H, *d*, J = 12.0, H-18a), 4.01 (1H, *m*, H-7), 4.85 (1H, *t*, J = 2.5, H-14), 5.19 (1H, *br s*, H-6), 6.00 (1H, *d*, J = 6.5, H-16), 6.51 (1H, *br t*, J = 2.5, H-15). *Erythro*-2,3-dihydroxy-2-methylbutanoic acid was recovered from the water eluate by extracting with MeOAc. ^{1}H NMR (90 MHz, CD_3OD): δ 1.15 (3H, *d*, J = 6.8, H-4), 1.34 (3H, *s*, 2-Me), 3.36 (3H, *s*, OMe), 3.82 (1H, *q*, J = 6.8, H-3). Methyl ester (treated with CH_2N_2): GC (HP-101)-MS *m/z* (rel. int.): $[M]^+$-absent, 104 (21), 89 (14), 71 (9), 45 (25), 43 (100).

Preparation of ***IIe***

Clerodendrin H was hydrolyzed with K_2CO_3 in MeOH–H_2O (100:1) to yield *erythro*-2,3-dihydroxy-2-methylbutanoic acid (identified same as above) and the 2,3-diol-6,18-diacetyl derivative (**IIe**). EIMS *m/z* (rel. int.): $[M]^+$ 466 (43), 376 (14), 304 (15), 111 (60), 83 (48), 43 (100); ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.84 (3H, *d*, J = 6.0, H-20), 0.97 (3H, *s*, H-19), 1.94 (3H, *s*, Ac–Me), 2.10 (3H, *s*, Ac–Me), ~2.6 (1H, *m*, H-1β), 2.78 (1H, *d*, J = 4.0, H-17b), 2.87 (1H, *d*, J = 4.0, H-17a), ~3.6 (1H, *m*, H-2β), ~3.6 (1H, *m*, H-13), 3.91 (1H, *d*, J = 9.0, H-3α), 4.02 (1H, *dd*, J = 4.7, 11.5, H-11), 4.27 (1H, *d*, J = 12.0, H-18b), 4.70 (1H, *m*, H-6), 4.81 (1H, *t*, J = 2.5, H-14), 4.94 (1H, *d*, J = 12.0, H-18a), 6.04 (1H, *d*, J = 6.1, H-16), 6.47 (1H, *t*, J = 2.5, H-15).

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