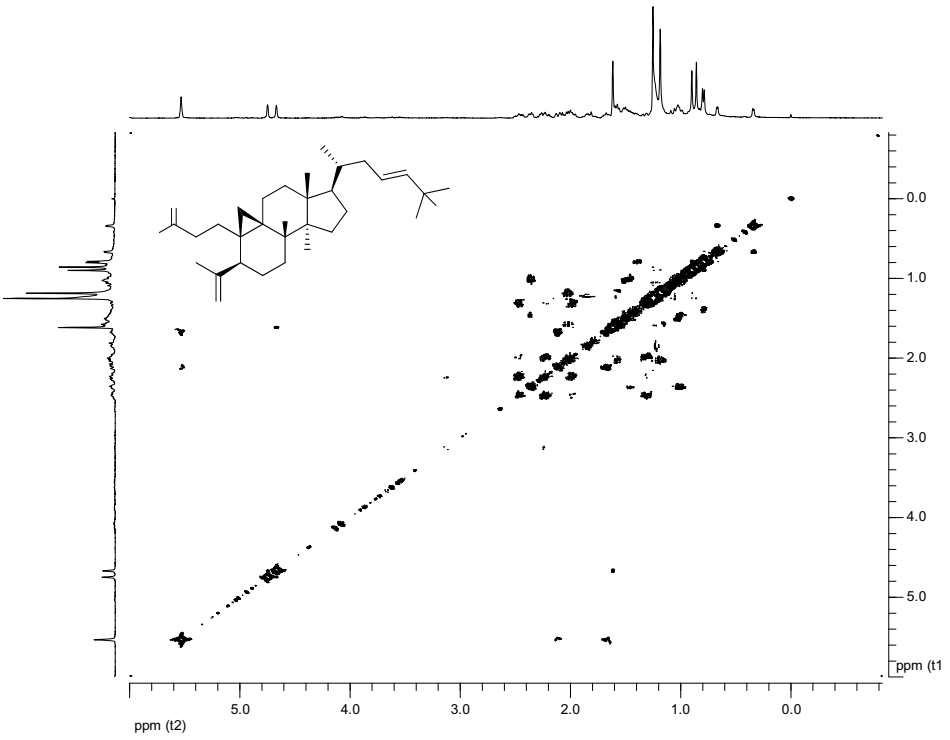
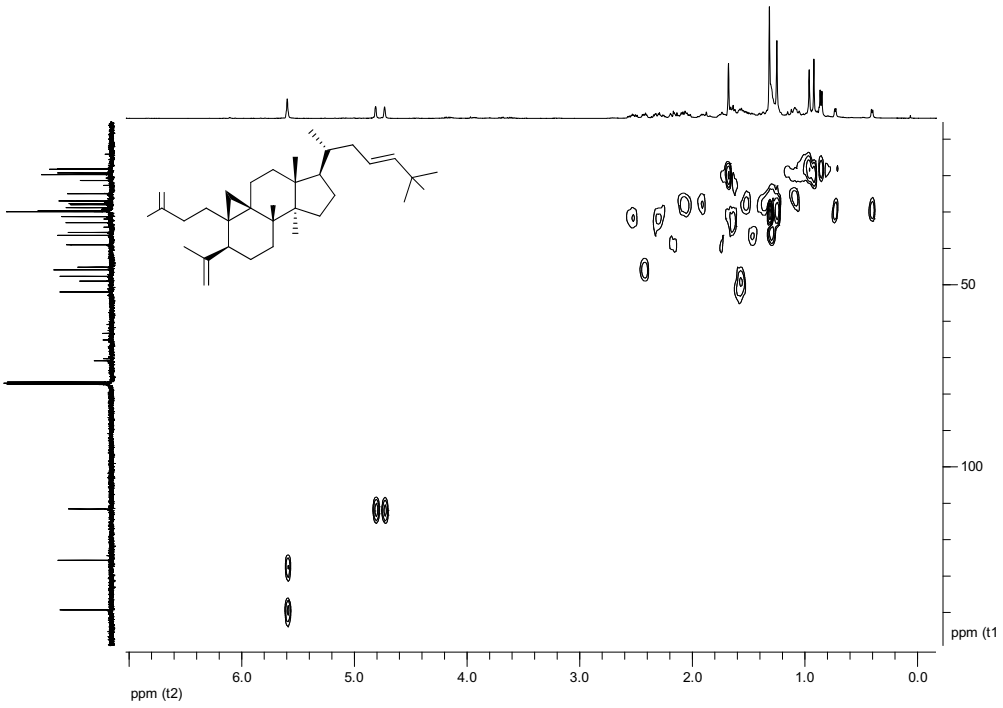


Figure S21. ^{1}H-^{1}H COSY spectrum of gardenoin H (**44**) ($CDCl_3$)



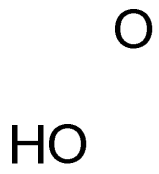


Figure S22. HSQC spectrum of gardenoin H (**44**) ($CDCl_3$)

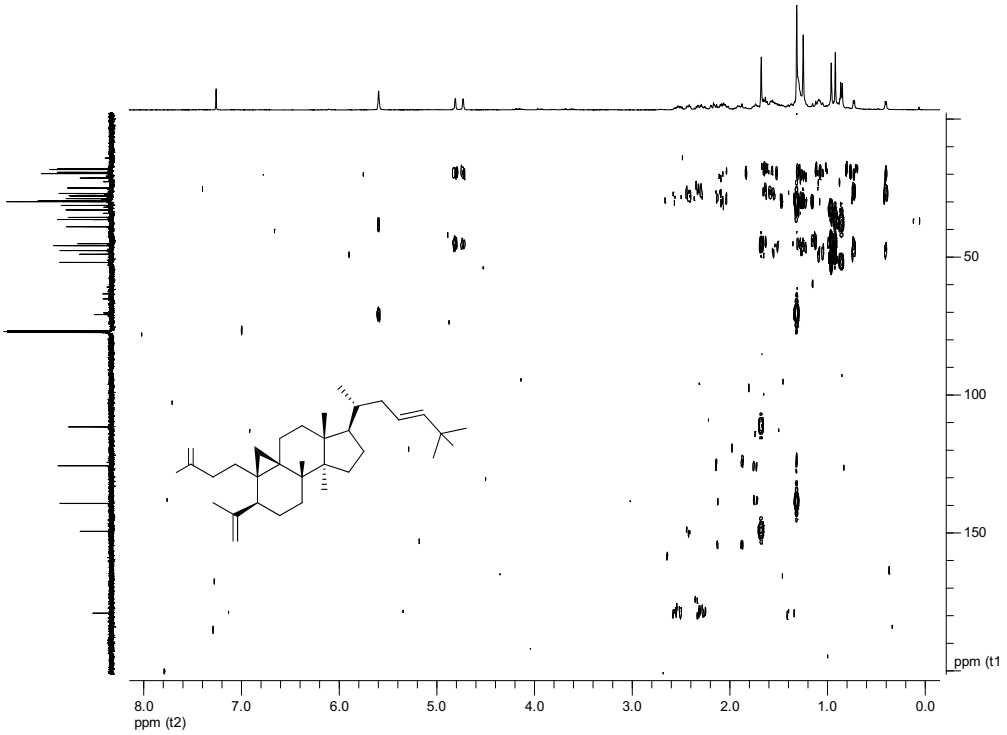


Figure S23. HMBC spectrum of gardenoin H (**44**) ($CDCl_3$)

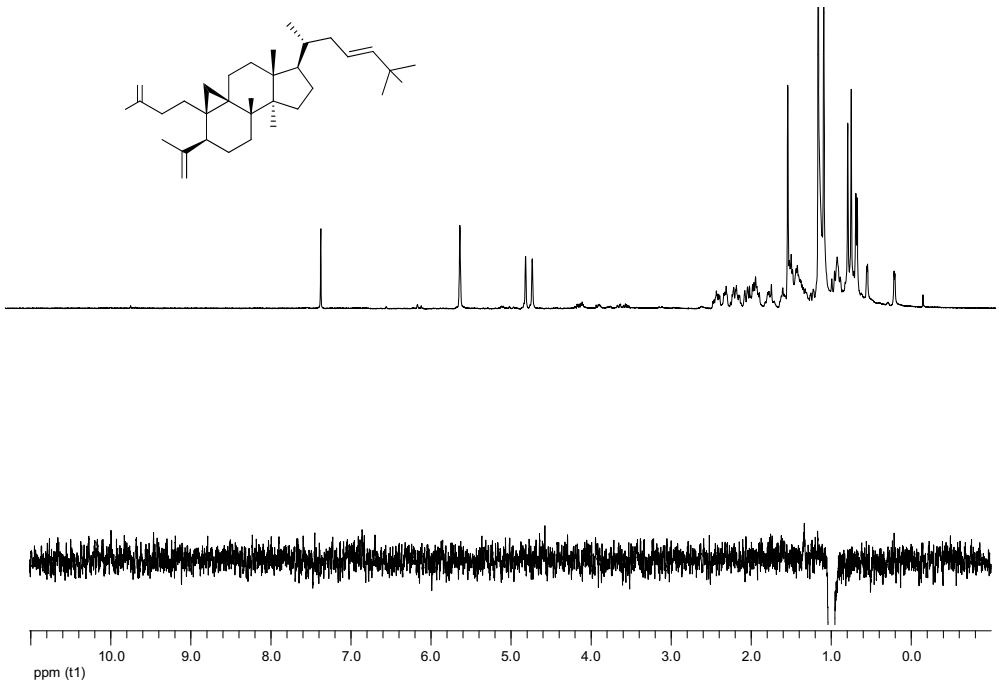
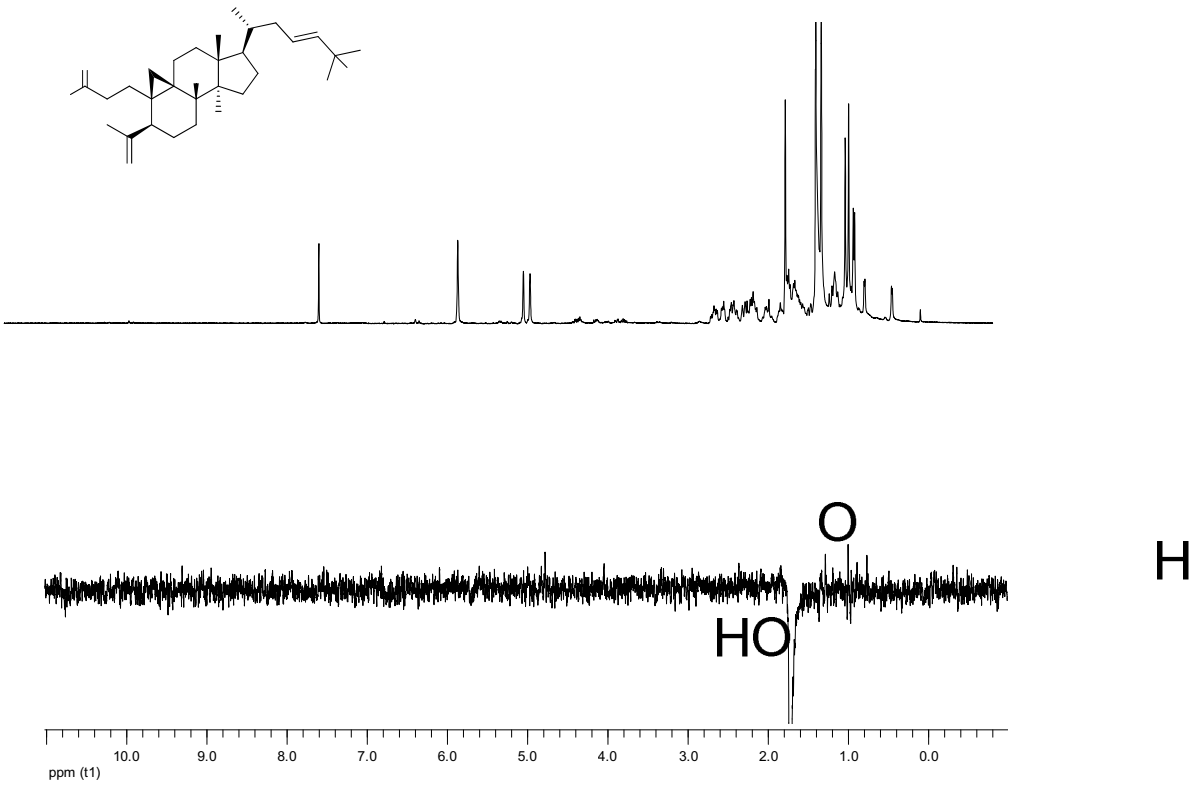


Figure S24. 1D NOE spectrum of gardenoin H (**44**) ($CDCl_3$)



H

Figure S24. 1D NOE spectrum of gardenoin H (**44**) ($CDCl_3$) (continue)

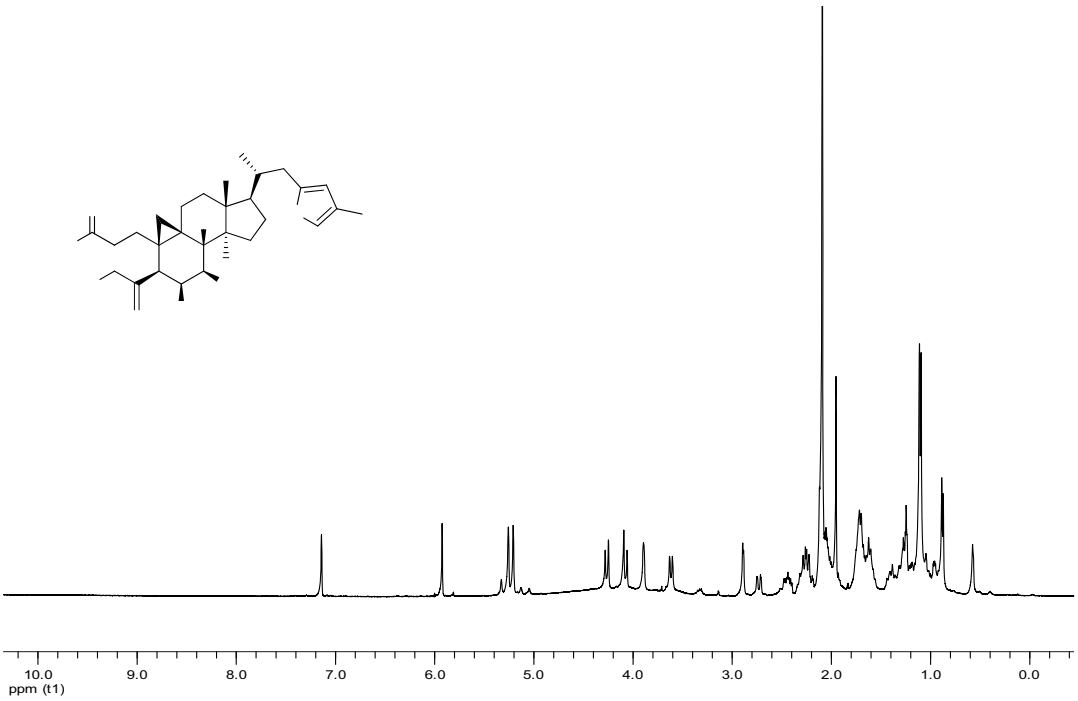


Figure S25. ^{1}H NMR spectrum of dikamakiartane A (**45**) (acetone-d_6, 400 MHz)

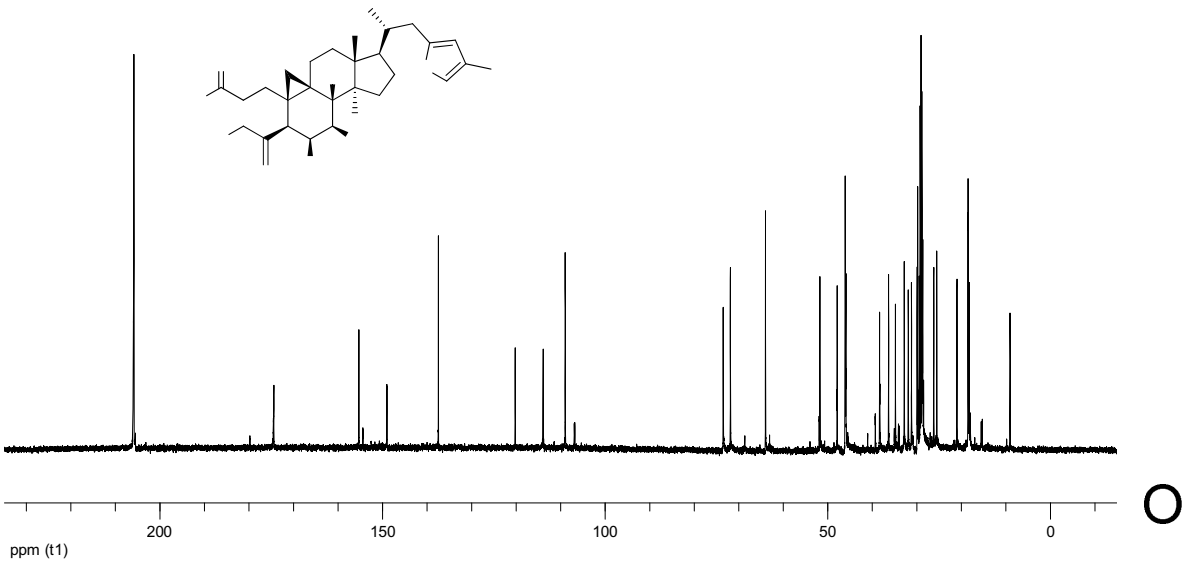


Figure S26. ^{13}C NMR spectrum of dikamakiartane A (**45**) (acetone-d_6, 100 MHz)

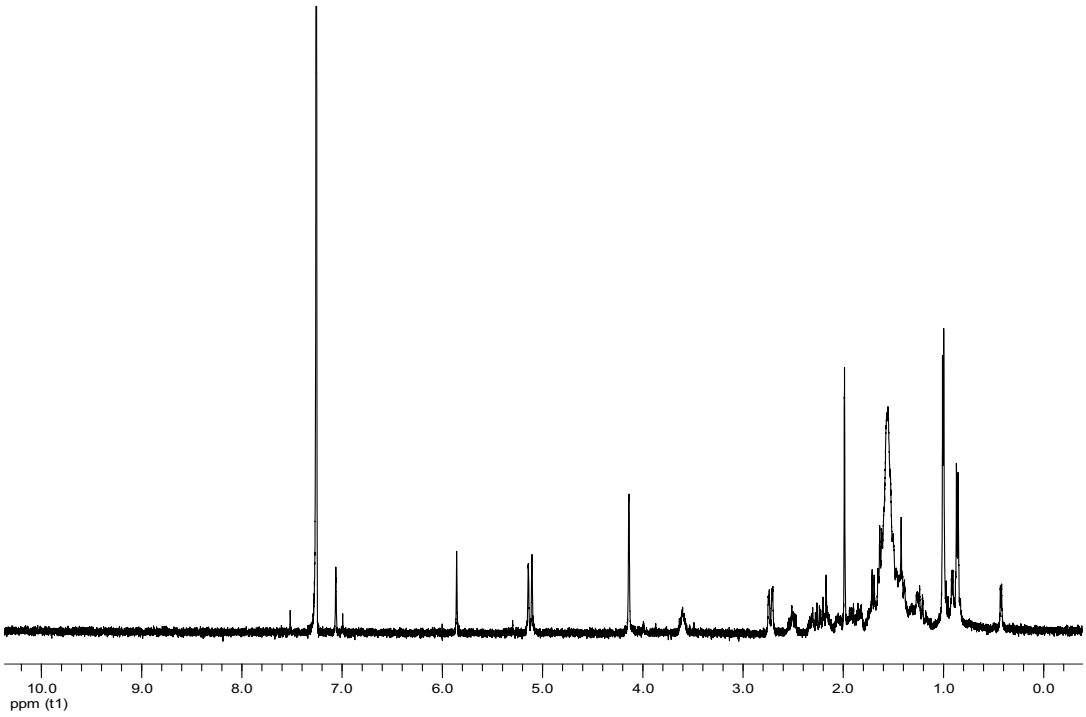


Figure S27. ^{1}H NMR spectrum of dikamakiartane C (**46**) (acetone-d_6, 400 MHz)

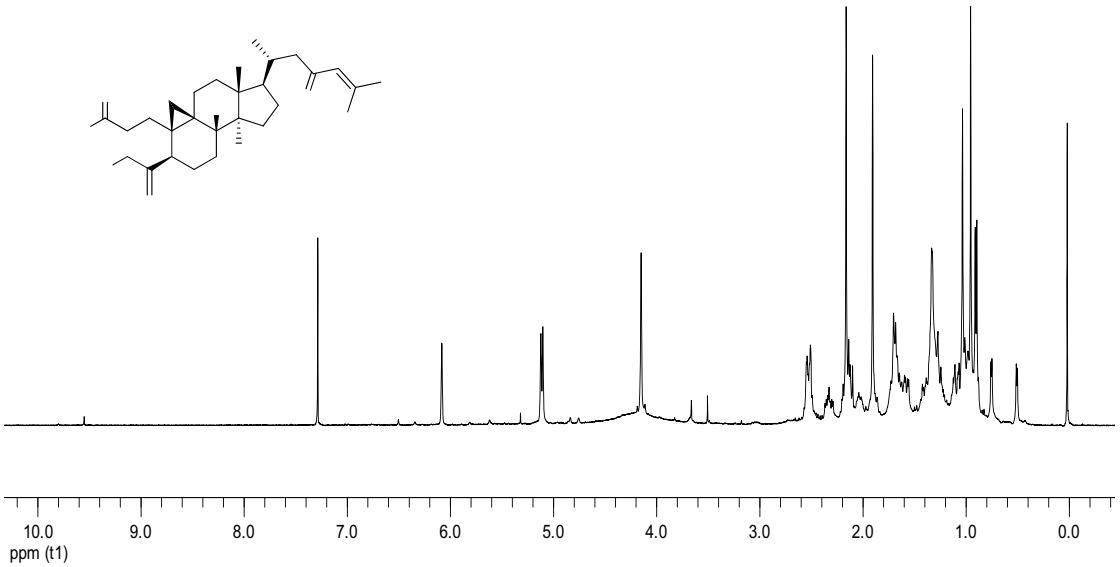


Figure S28. ^{1}H NMR spectrum of dikamakiartane D (**47**) ($CDCl_3$, 400 MHz)

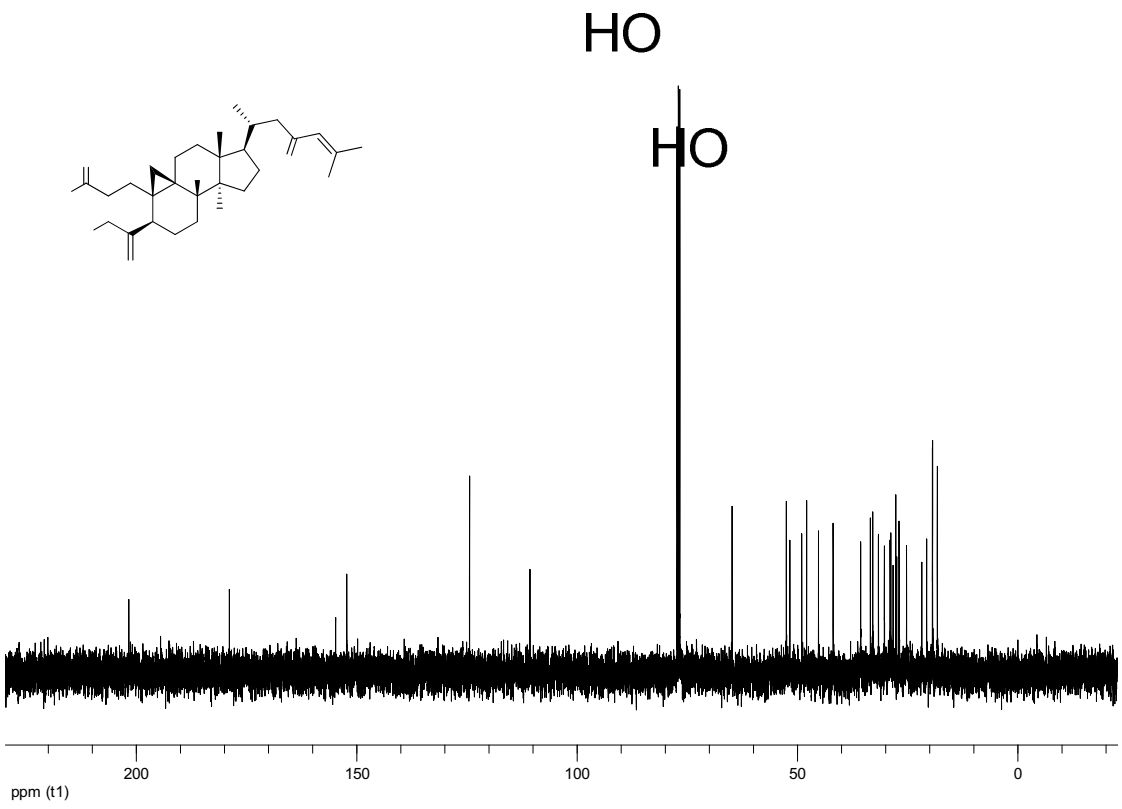


Figure S29. ^{13}C NMR spectrum of dikamakiartane D (**47**) ($CDCl_3$, 100 MHz)

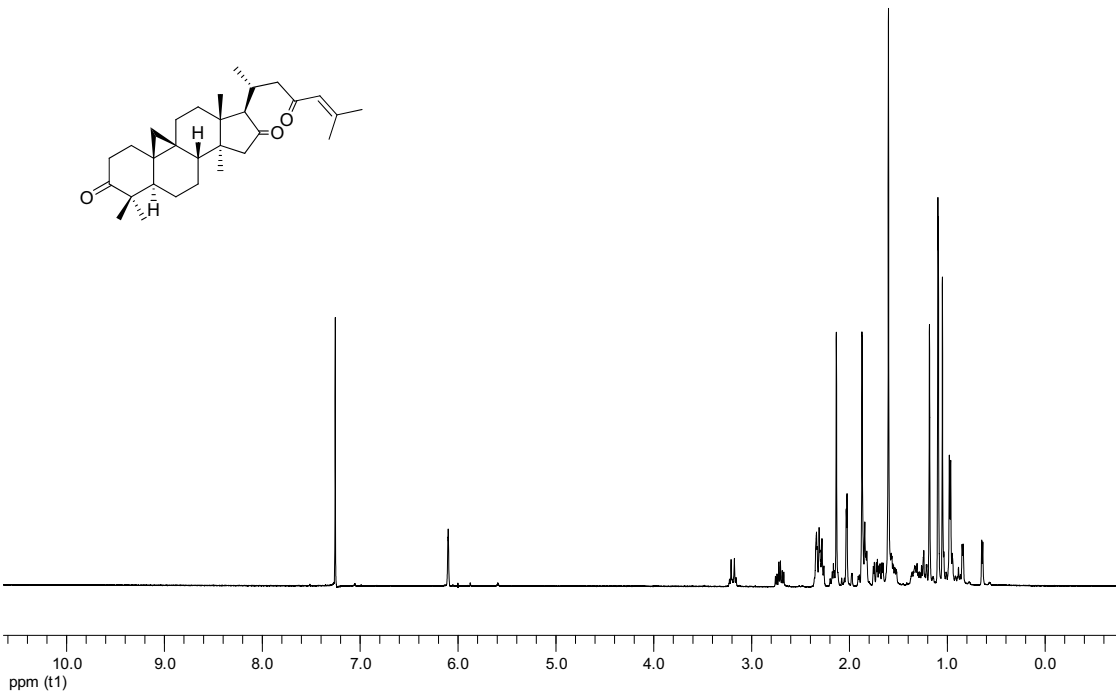


Figure S30. ^{1}H NMR spectrum of 5α-cycloart-24-ene-3,16,23-trione (**48**) ($CDCl_3$, 400 MHz)

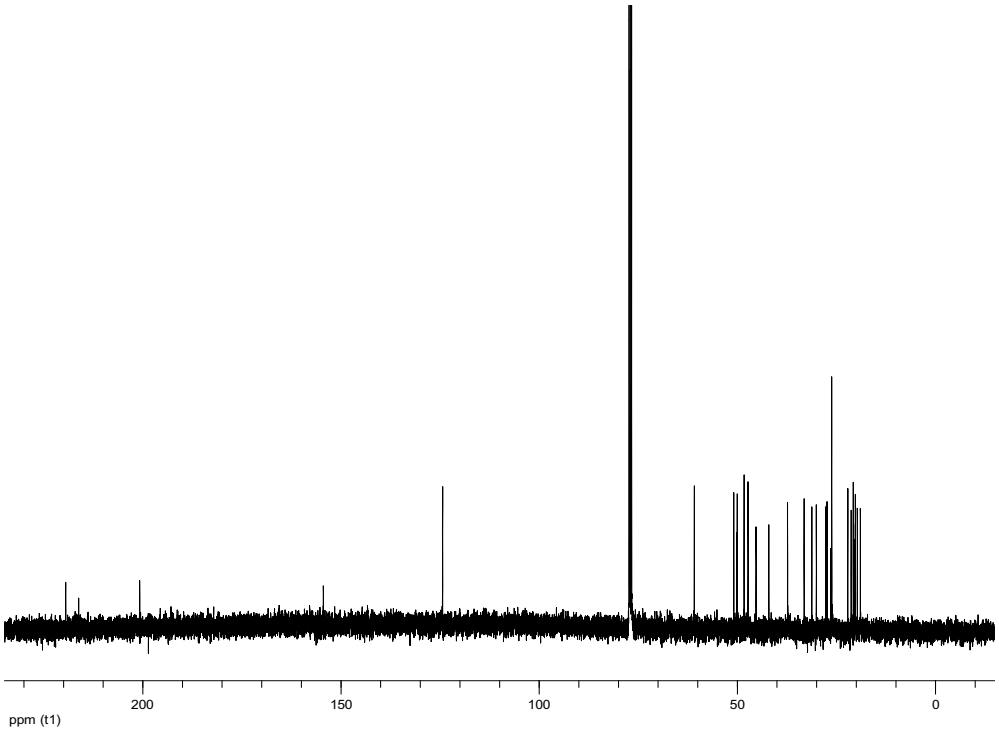


Figure S31. ^{13}C NMR spectrum of 5α-cycloart-24-ene-3,16,23-trione (**48**) ($CDCl_3$, 100 MHz)

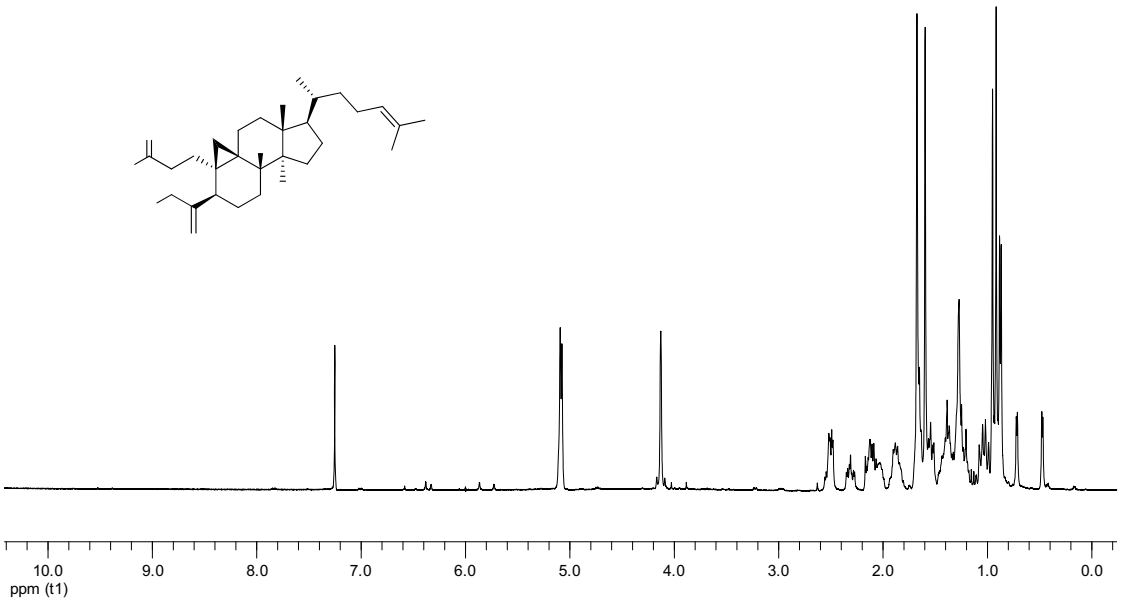


Figure S32. ^{1}H NMR spectrum of secaubryenol (**27**) ($CDCl_3$, 400 MHz)

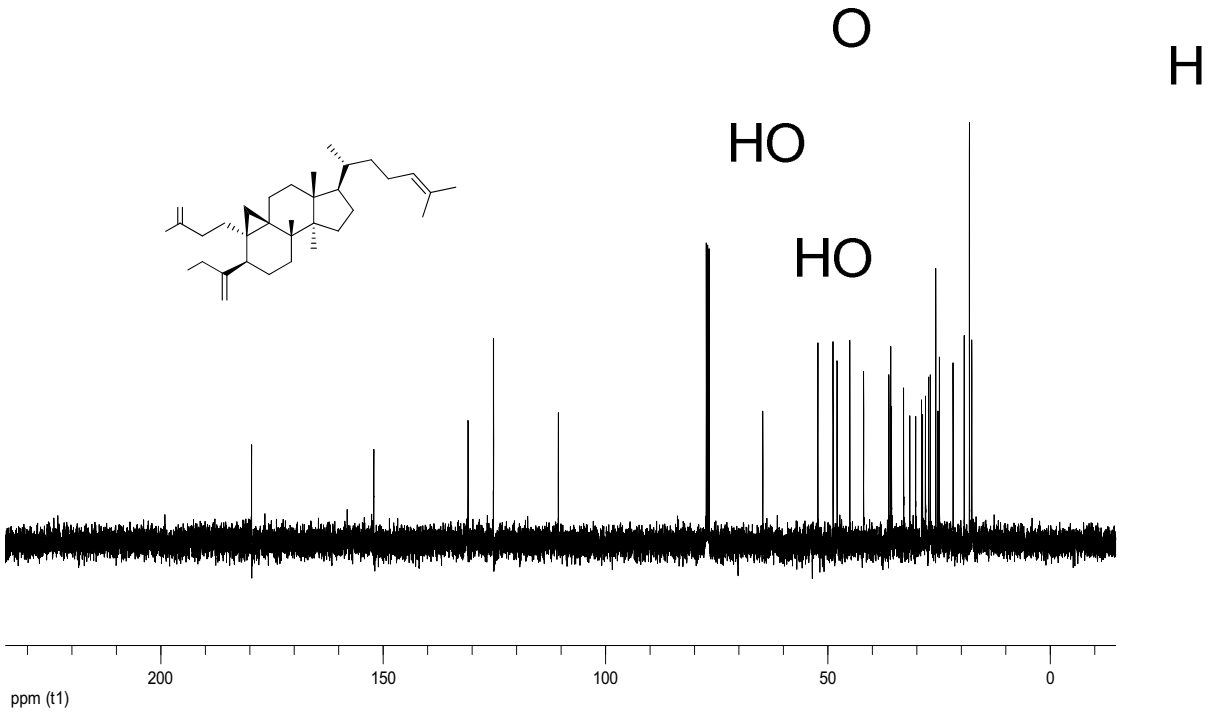


Figure S33. ^{13}C NMR spectrum of secaubryenol (**27**) ($CDCl_3$, 100 MHz)

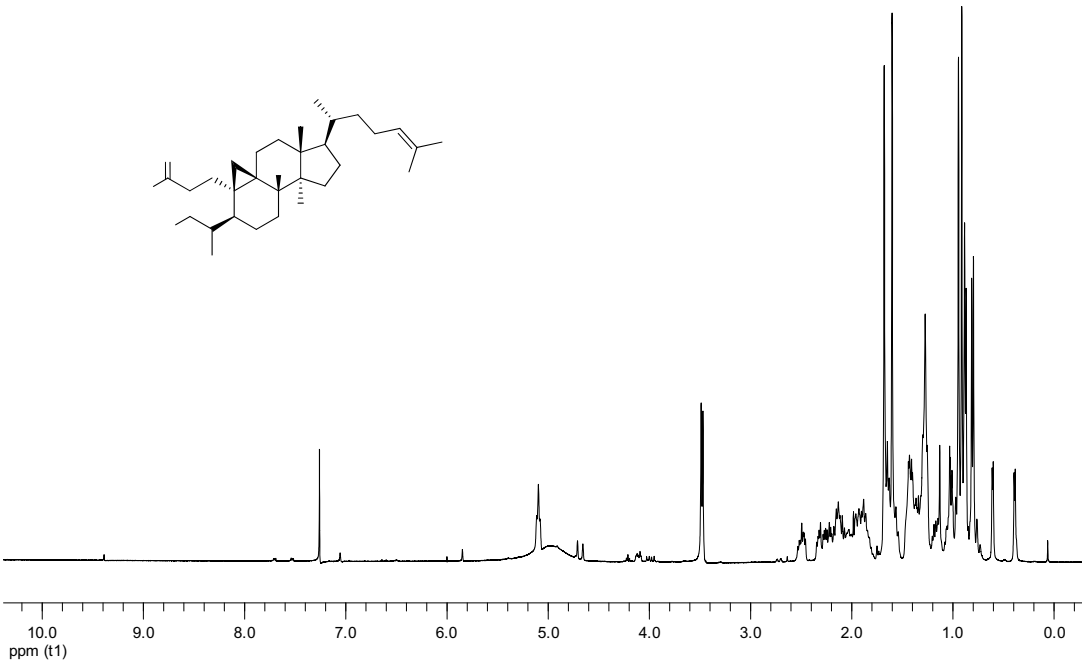


Figure S34. ^{1}H NMR spectrum of gardenoin I (**49**) ($CDCl_3$, 400 MHz)

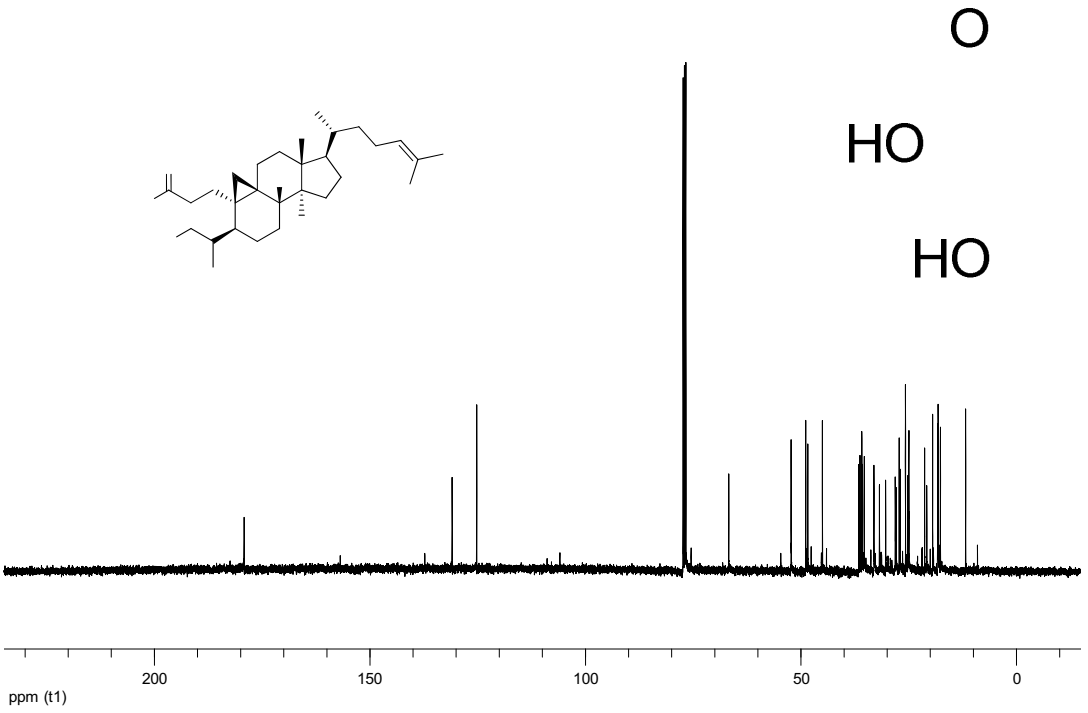


Figure S35. ^{13}C NMR spectrum of gardenoin I (**49**) ($CDCl_3$, 100 MHz)

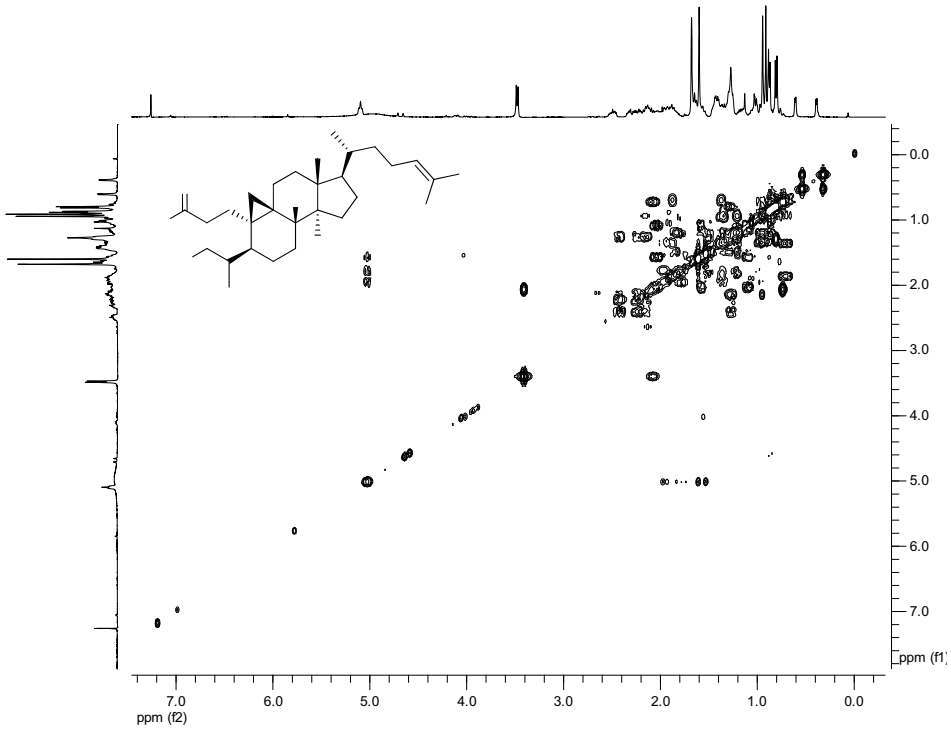
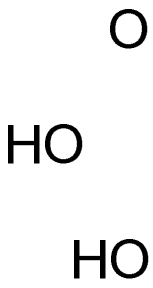


Figure S36. ^{1}H-^{1}H COSY spectrum of gardenoin I (**49**) ($CDCl_3$)



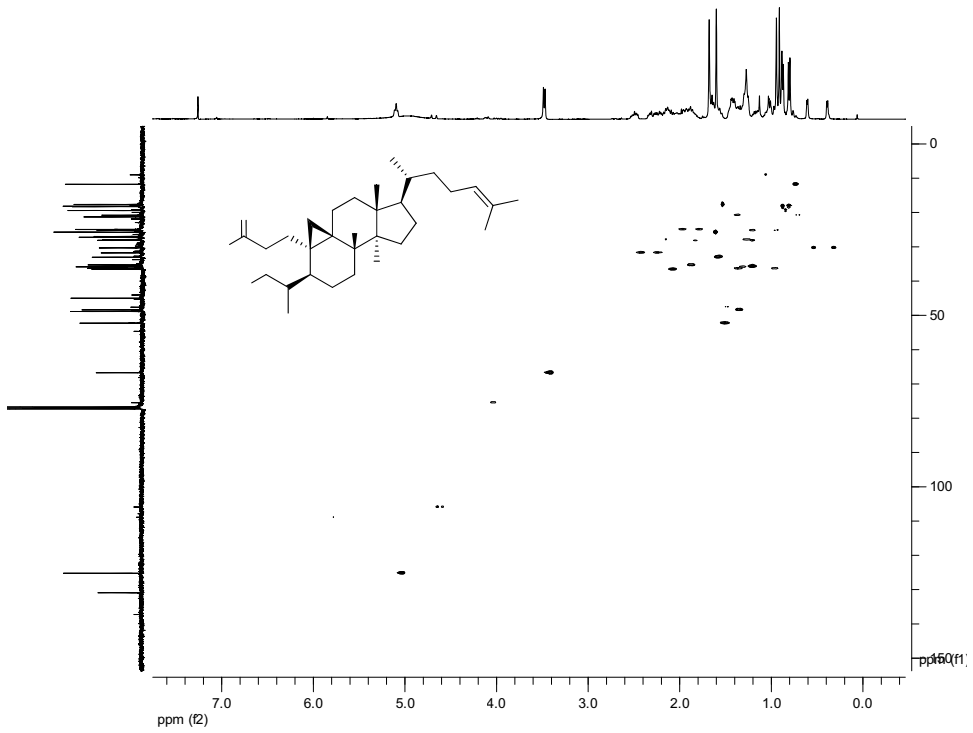


Figure S37. HSQC spectrum of gardenoin I (**49**) ($CDCl_3$)

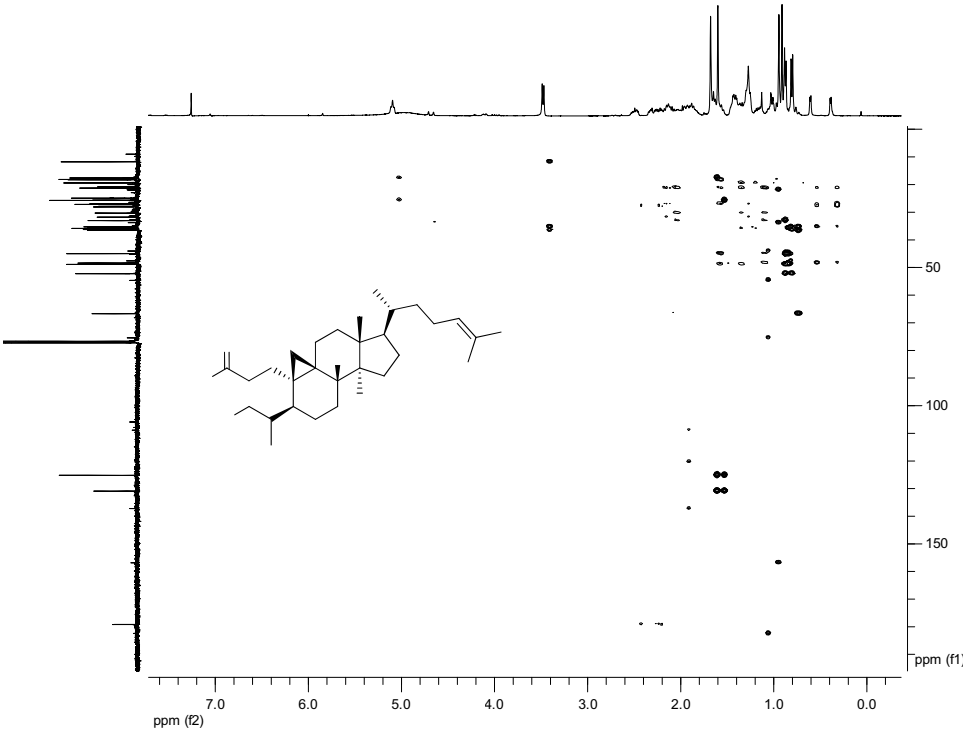
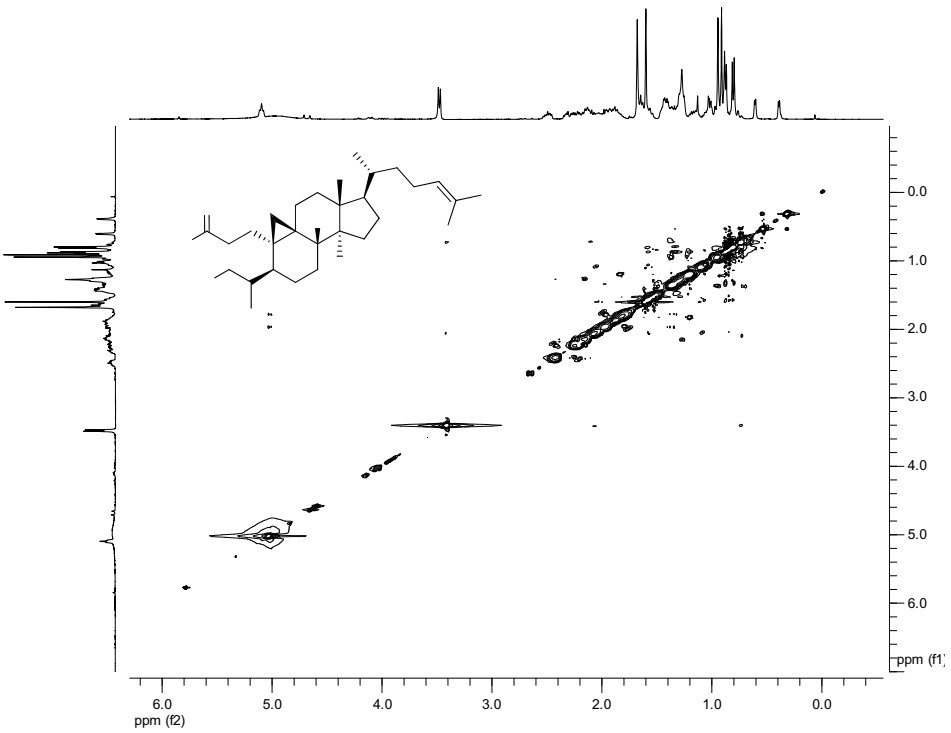


Figure S38. HMBC spectrum of gardenoin I (**49**) ($CDCl_3$)



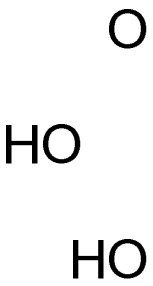


Figure S39. NOESY spectrum of gardenoin I (**49**) ($CDCl_3$)

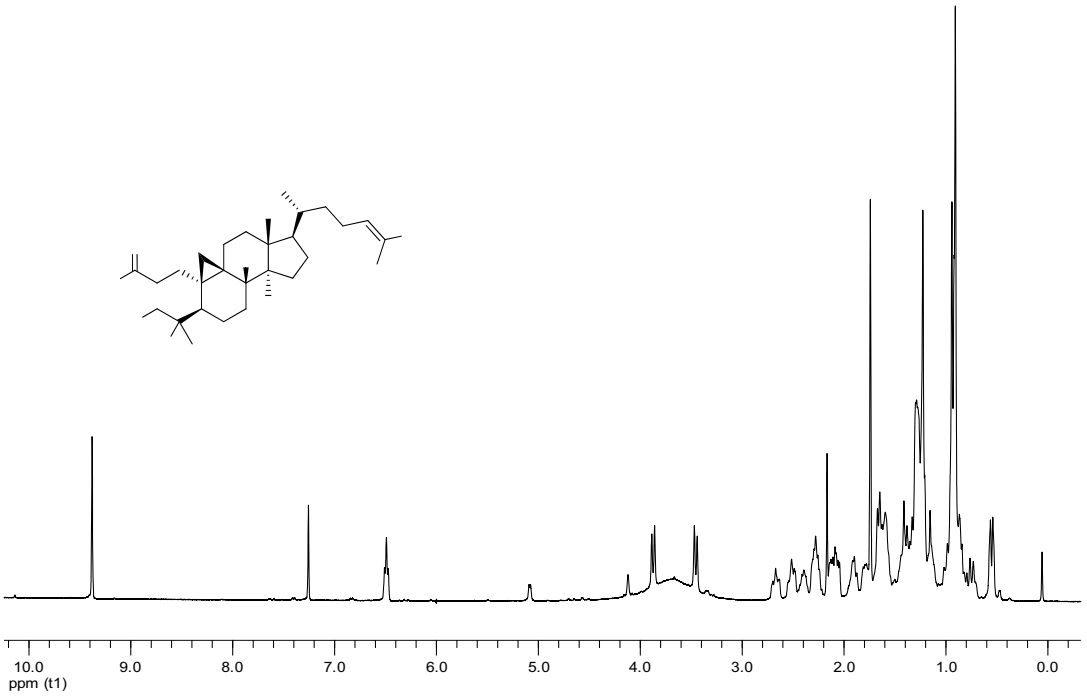


Figure S40. ^{1}H NMR spectrum of gardenoin J (**50**) ($CDCl_3$, 400 MHz)

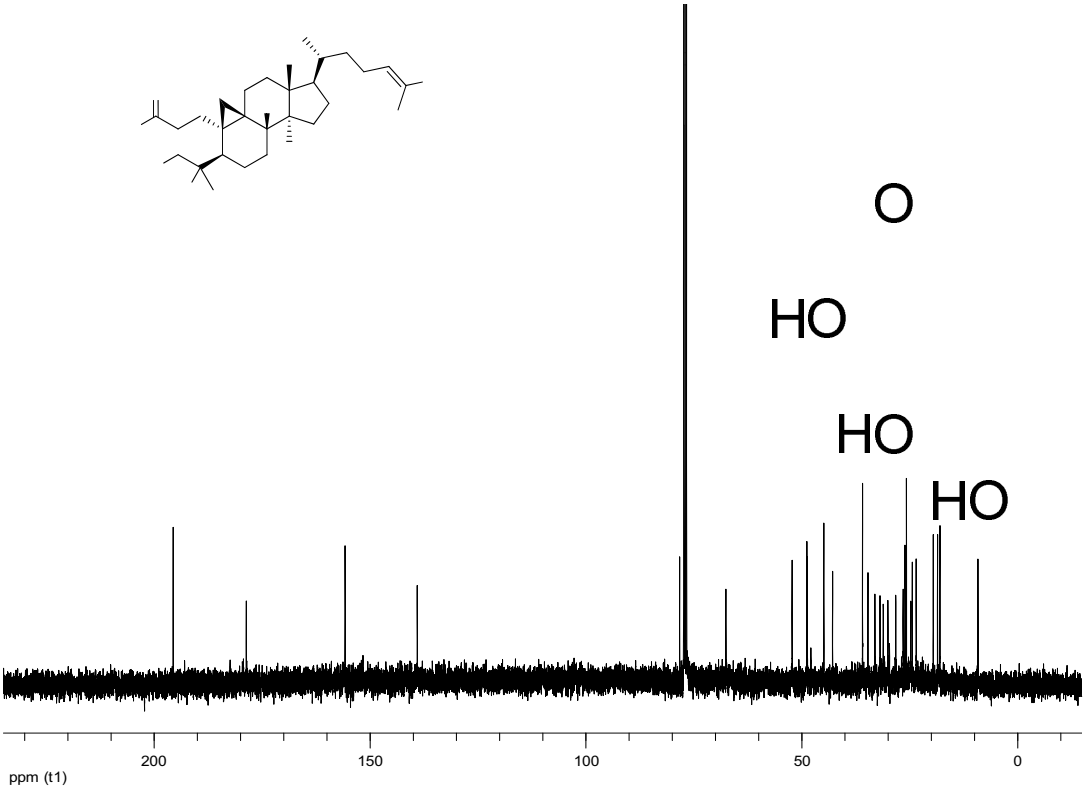


Figure S41. ^{13}C NMR spectrum of gardenoin J (**50**) ($CDCl_3$, 400 MHz)

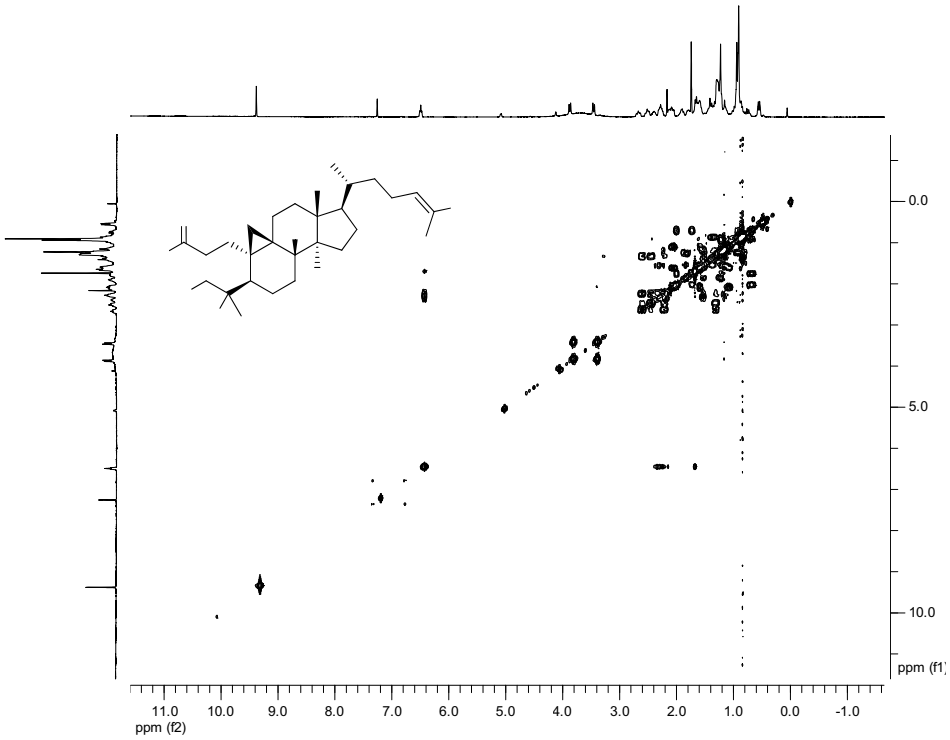
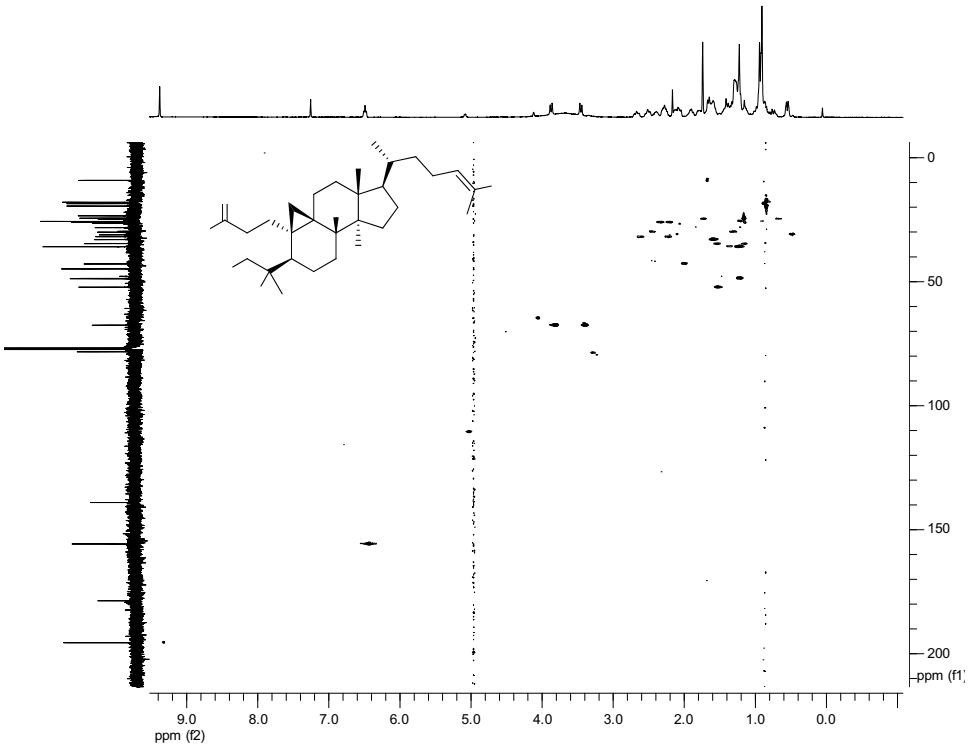


Figure S42. ^{1}H-^{1}H COSY spectrum of gardenoin J (**50**) ($CDCl_3$)



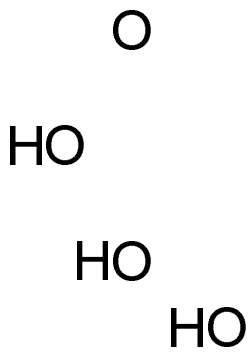


Figure S43. HSQC spectrum of gardenoin J (**50**) ($CDCl_3$)

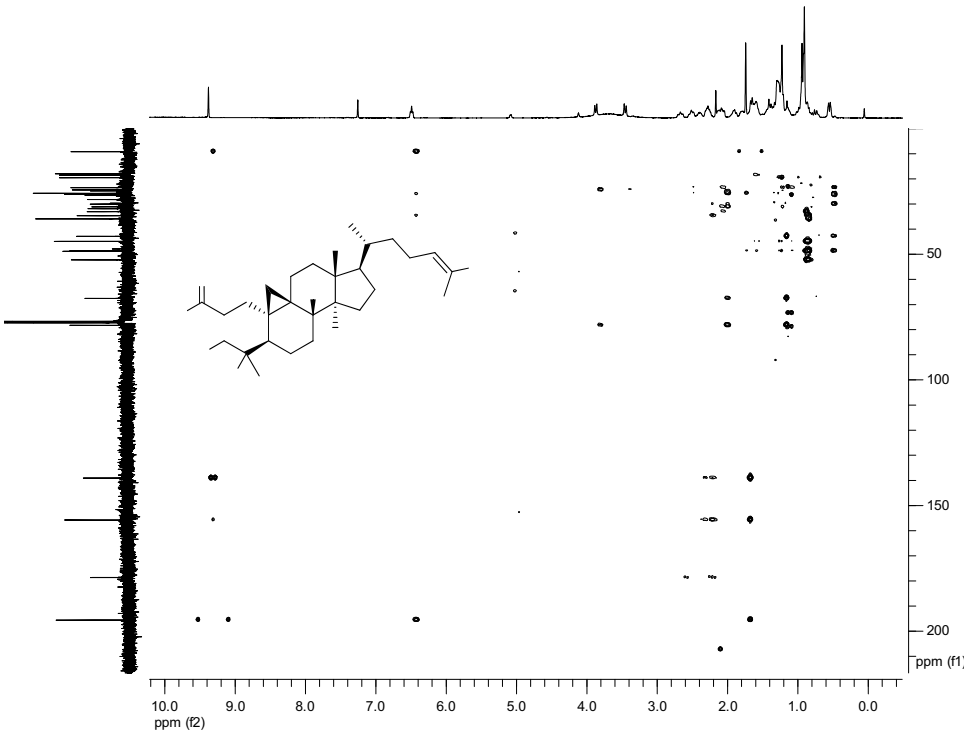
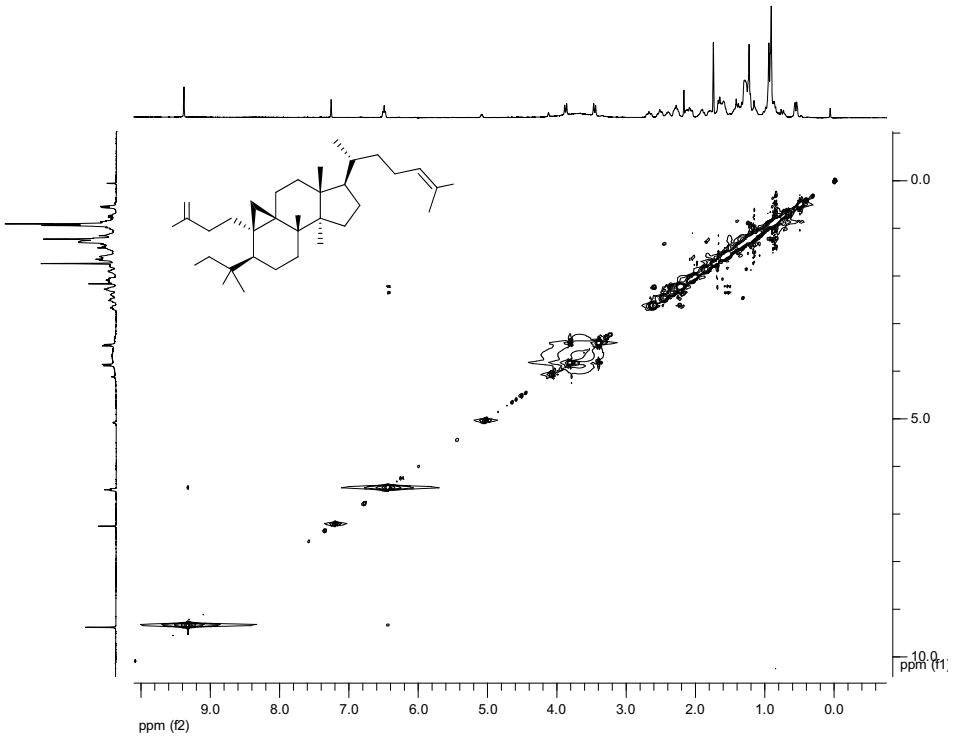


Figure S44. HMBC spectrum of gardenoin J (**50**) ($CDCl_3$)



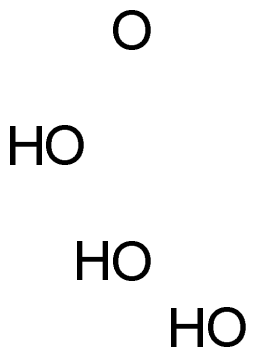


Figure S45. NOESY spectrum of gardenoin J (**50**) ($CDCl_3$)

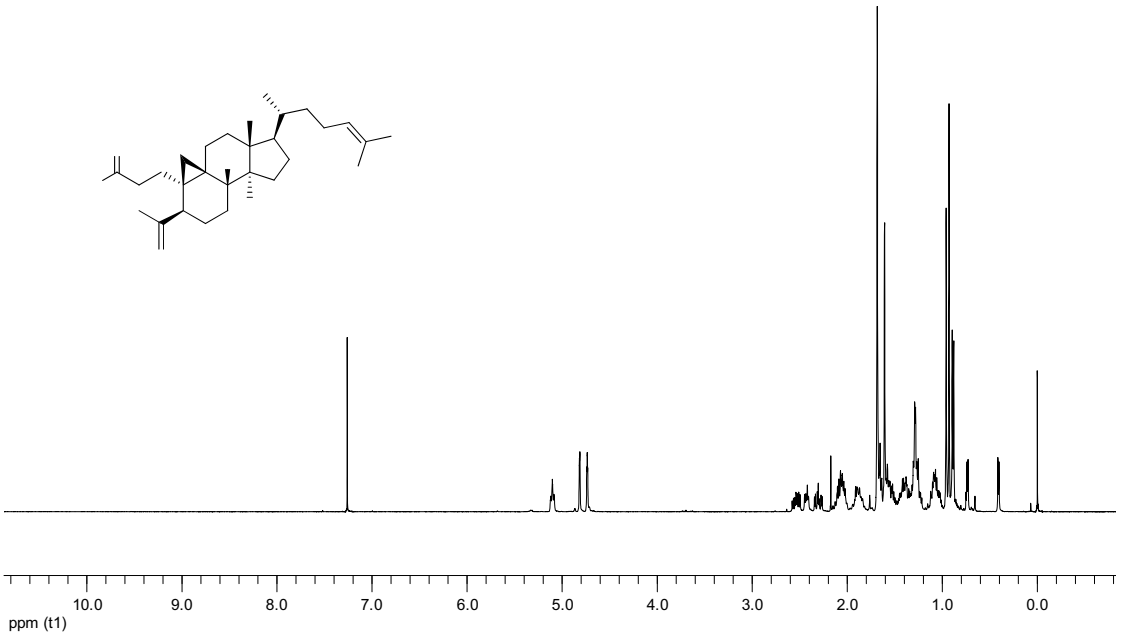


Figure S46. ^{1}H NMR spectrum of sootepin E (**34**) ($CDCl_3$, 400 MHz)

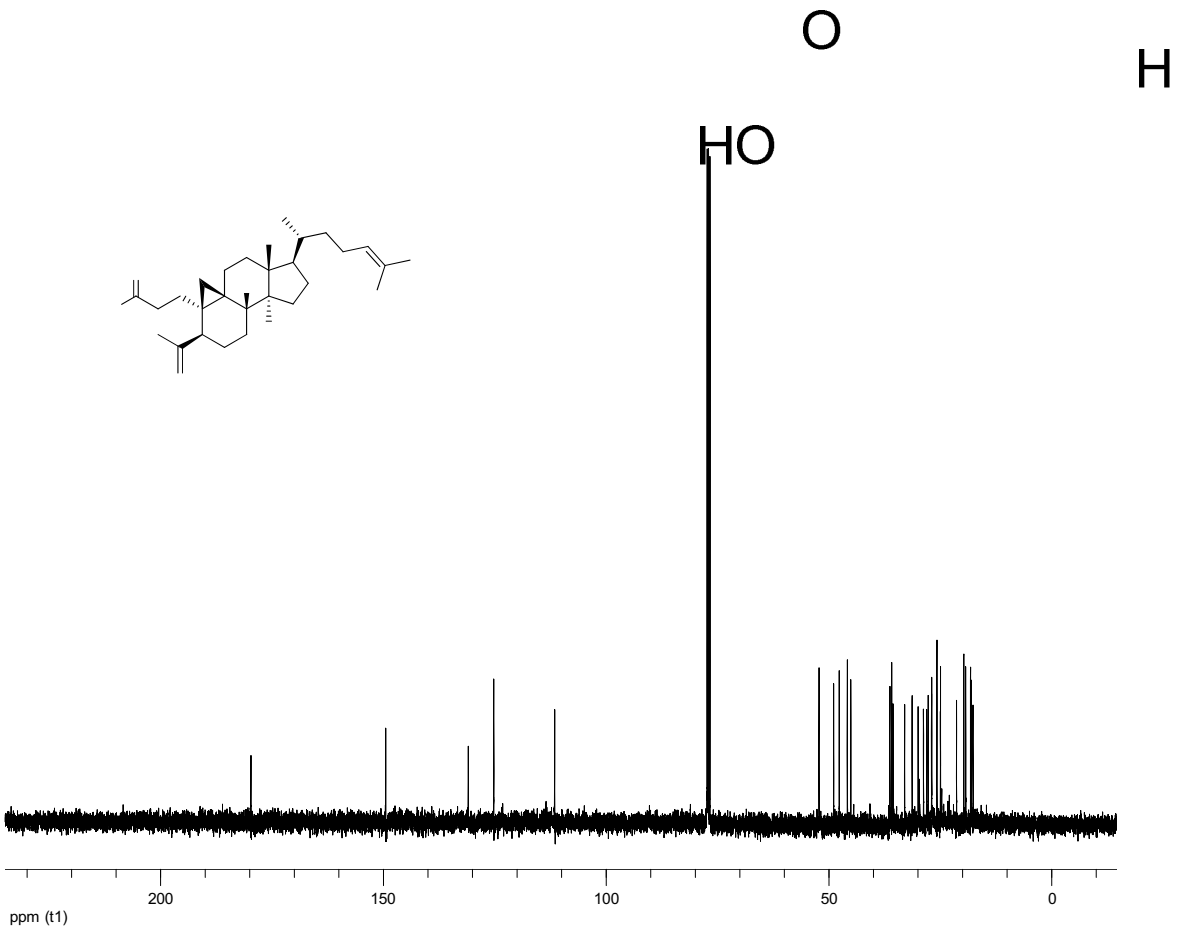


Figure S47. ^{13}C NMR spectrum of sootepin E (**34**) ($CDCl_3$, 100 MHz)

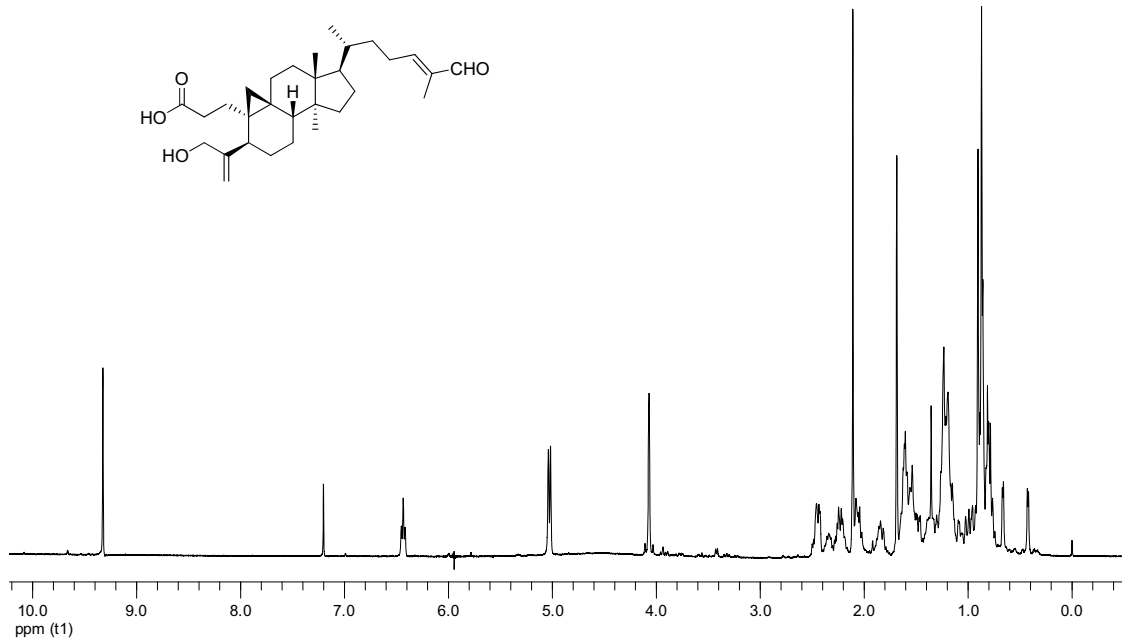


Figure S48. ^{1}H NMR spectrum of coronaloic acid (**51**) ($CDCl_3$, 400 MHz)

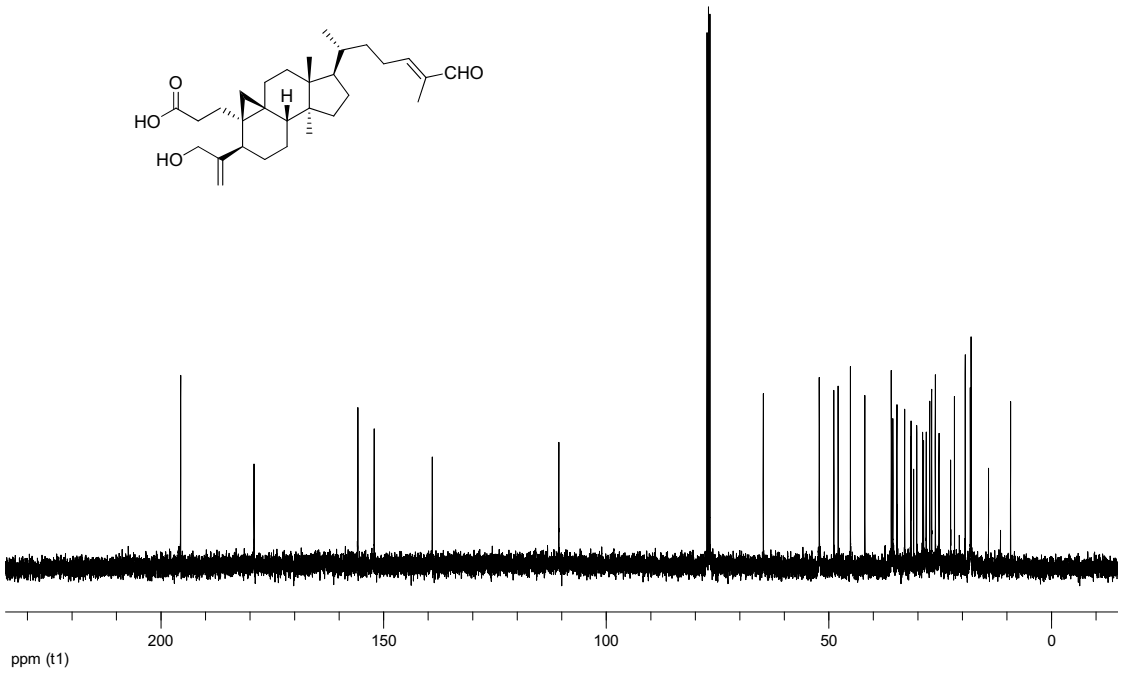


Figure S49. ^{13}C NMR spectrum of coronaloic acid (**51**) ($CDCl_3$, 100 MHz)

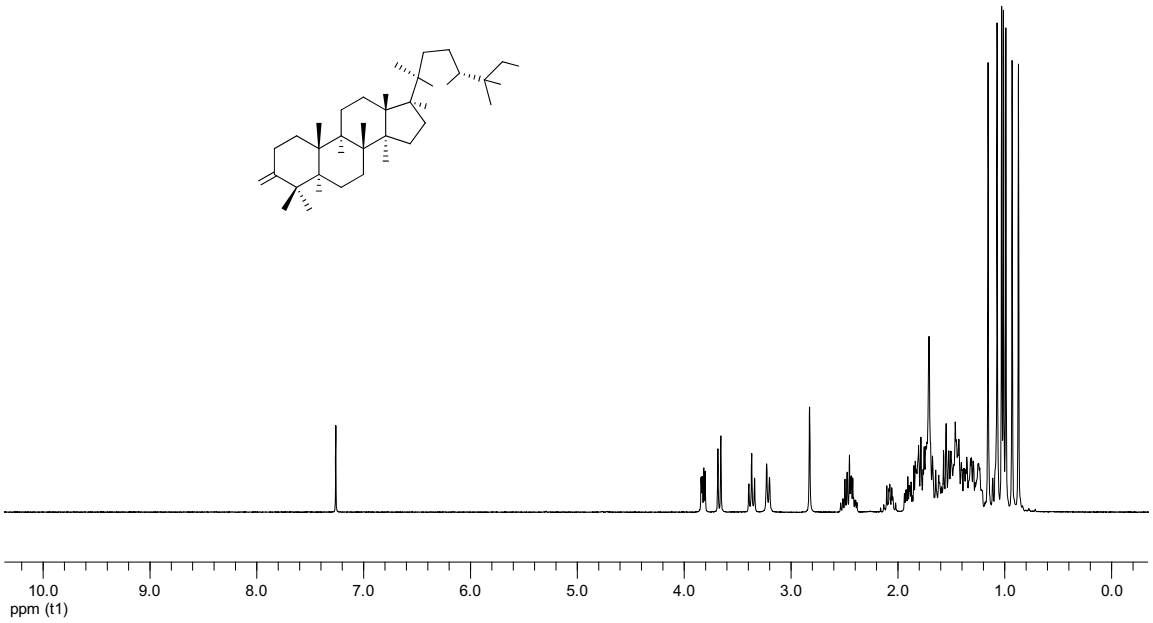


Figure S50. ^{1}H NMR spectrum of (*20R,24R*)-epoxy-3-oxo-dammarane-25$\mathcal{E}$,26-diol (**52**) ($CDCl_3$, 400 MHz)

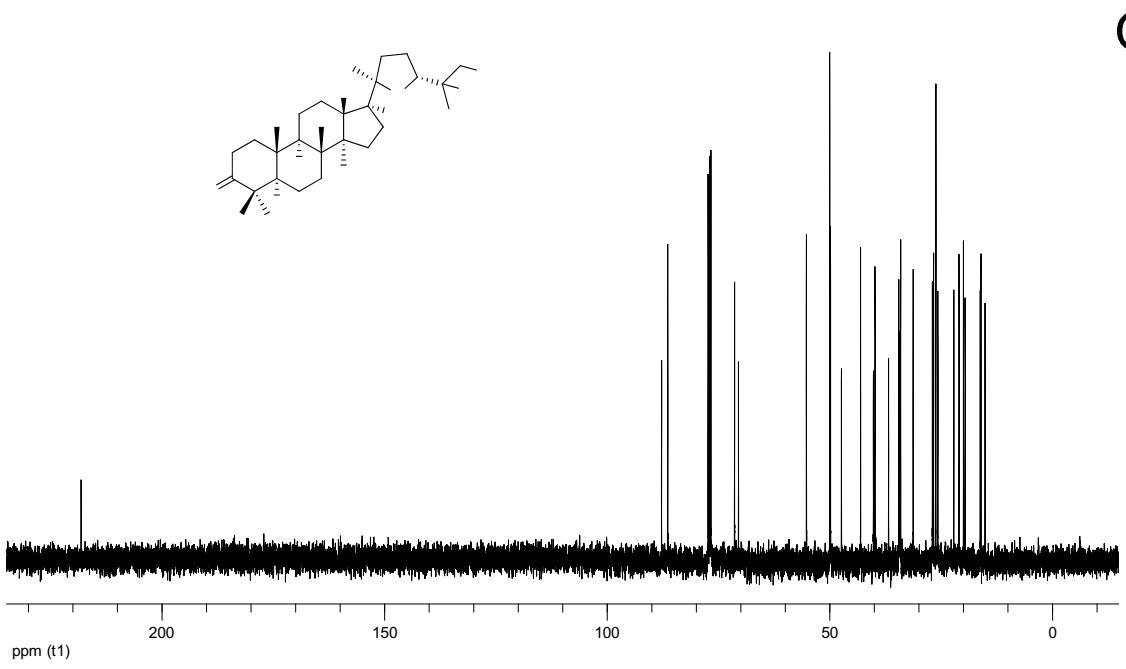


Figure S51. ^{13}C NMR spectrum of (*20R,24R*)-epoxy-3-oxo-dammarane-25$\mathcal{E}$,26-diol (**52**) ($CDCl_3$, 400 MHz)

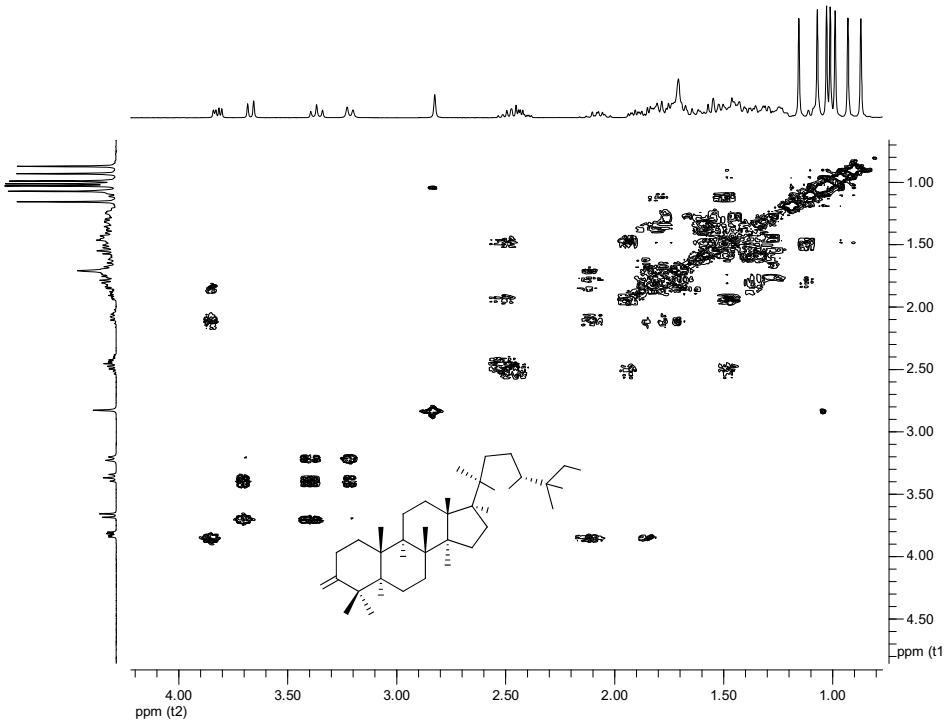


Figure S52. ^{1}H-^{1}H COSY spectrum of (*20R,24R*)-epoxy-3-oxo-dammarane-25$\mathcal{E}$,26-diol (**52**)

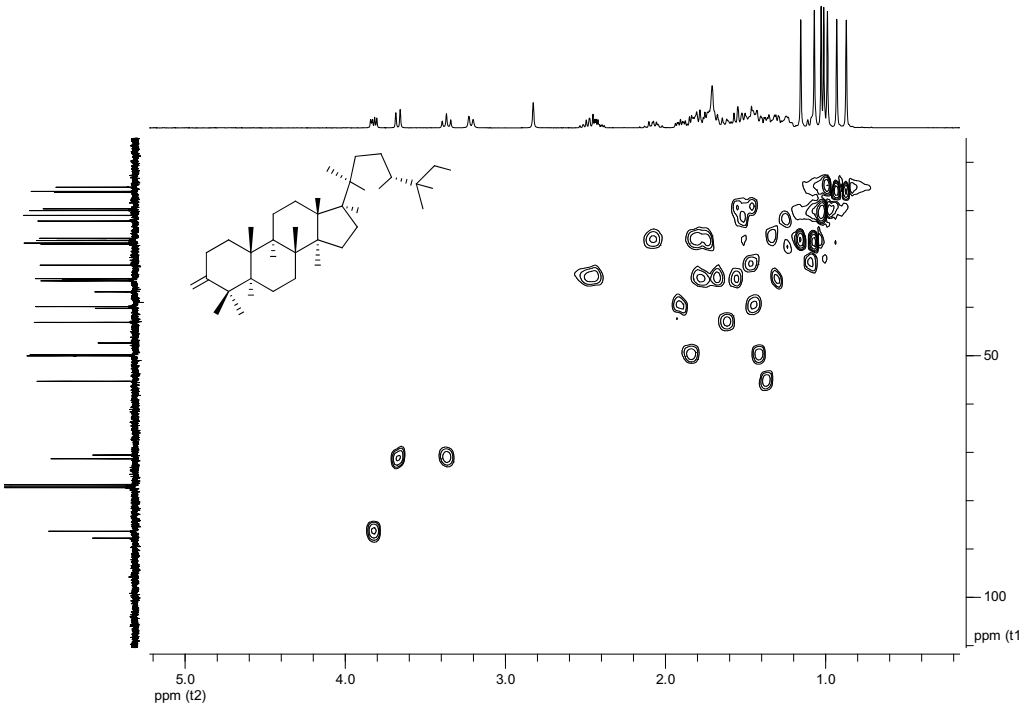


Figure S53. HSQC spectrum of (*20R,24R*)-epoxy-3-oxo-dammarane-25$\mathcal{E}$,26-diol (**52**)

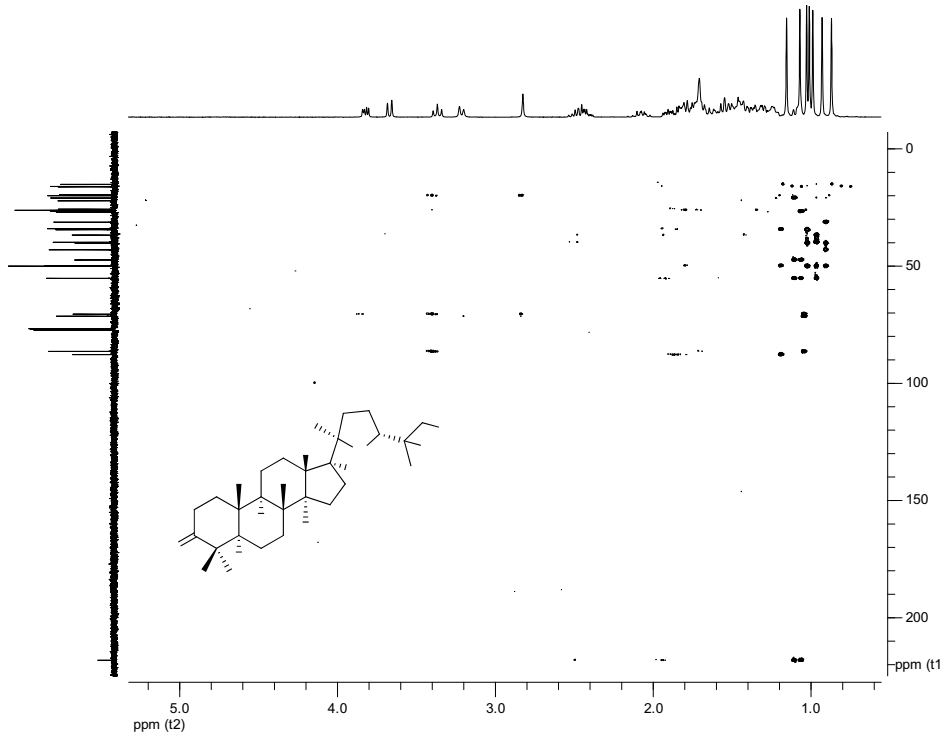


Figure S54. HMBC spectrum of (*20R,24R*)-epoxy-3-oxo-dammarane-25*E*,26-diol (**52**)

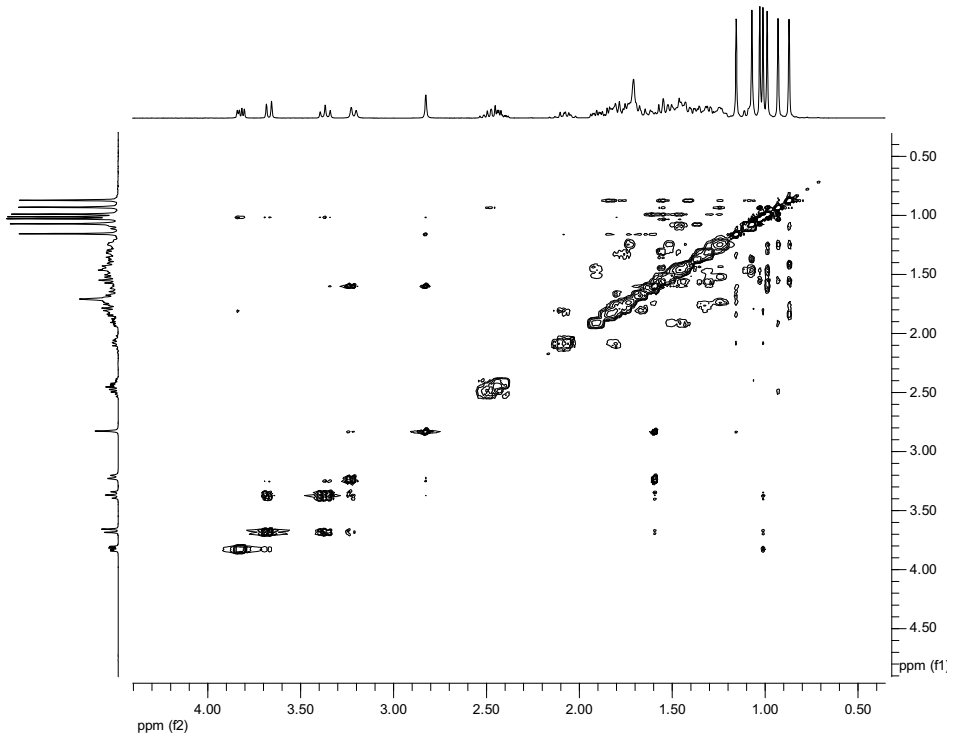


Figure S55. NOESY spectrum of (*20R,24R*)-epoxy-3-oxo-dammarane-25*E*,26-diol (**52**)

O

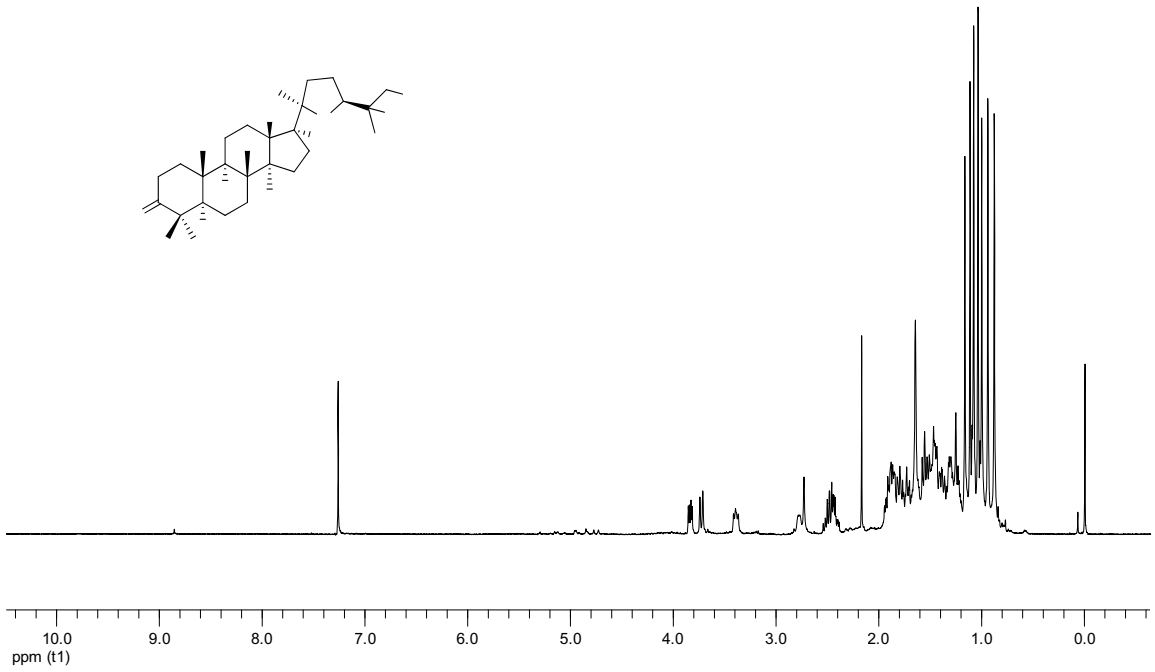


Figure S56. ^{1}H NMR spectrum of (*20R,24S*)-epoxy-3-oxo-dammarane-25$\mathcal{E}$,26-diol (**53**) ($CDCl_3$, 400 MHz)

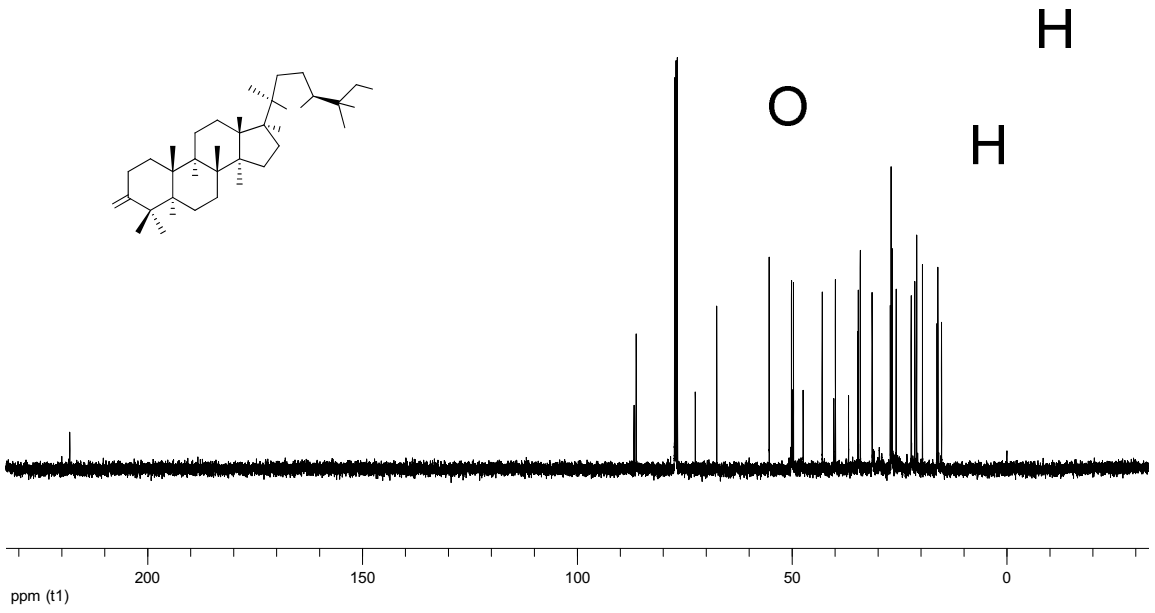


Figure S57. ^{13}C NMR spectrum of (*20R,24S*)-epoxy-3-oxo-dammarane-25$\mathcal{E}$,26-diol (**53**) ($CDCl_3$, 100 MHz)

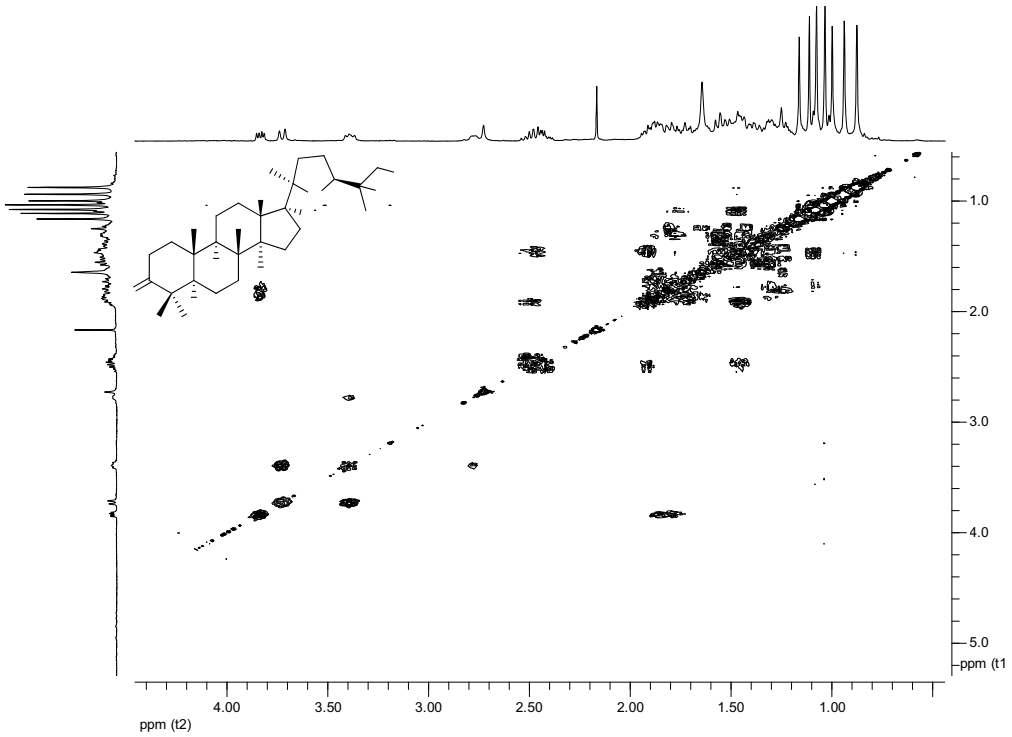


Figure S58. ^{1}H-^{1}H COSY spectrum of (*20R,24S*)-epoxy-3-oxo-dammarane-25$\mathcal{E}$,26-diol (**53**)

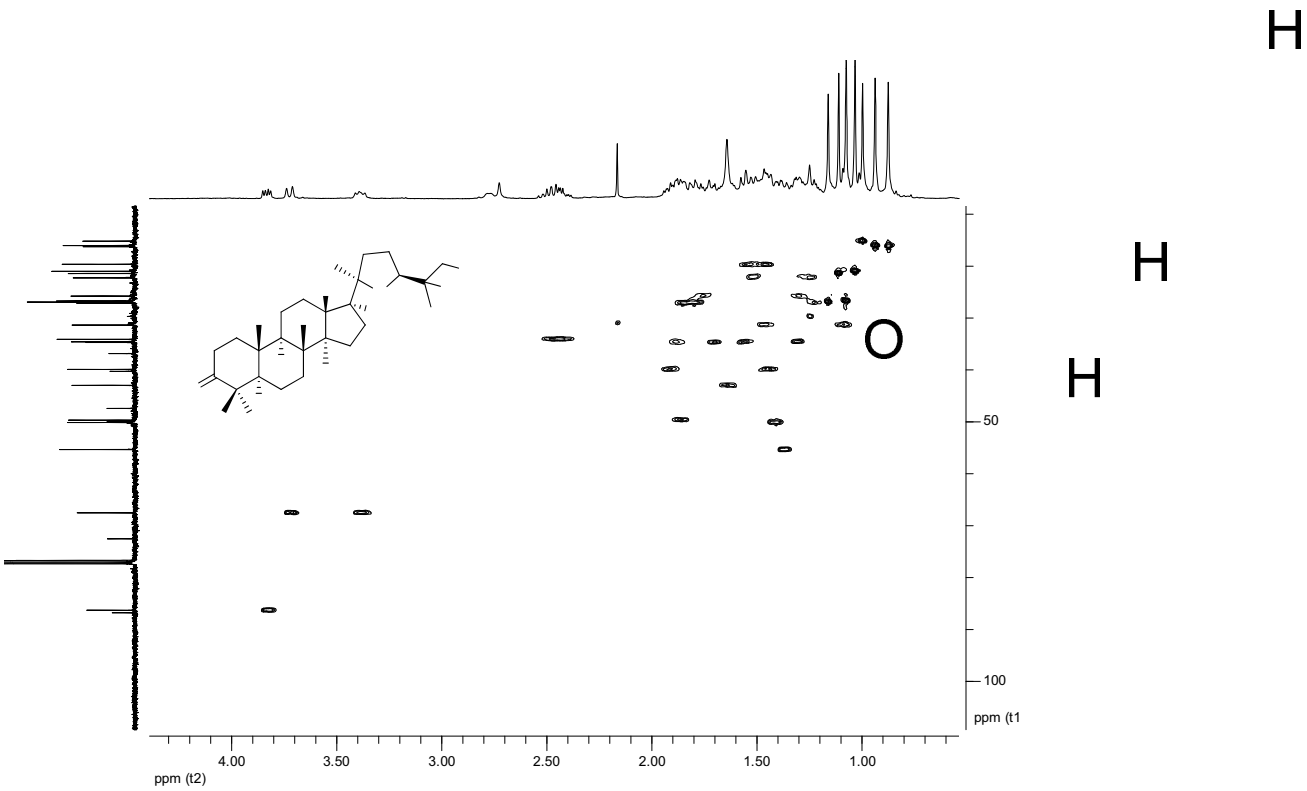


Figure S59. HSQC spectrum of (*20R,24S*)-epoxy-3-oxo-dammarane-25$\mathcal{E}$,26-diol (**53**)

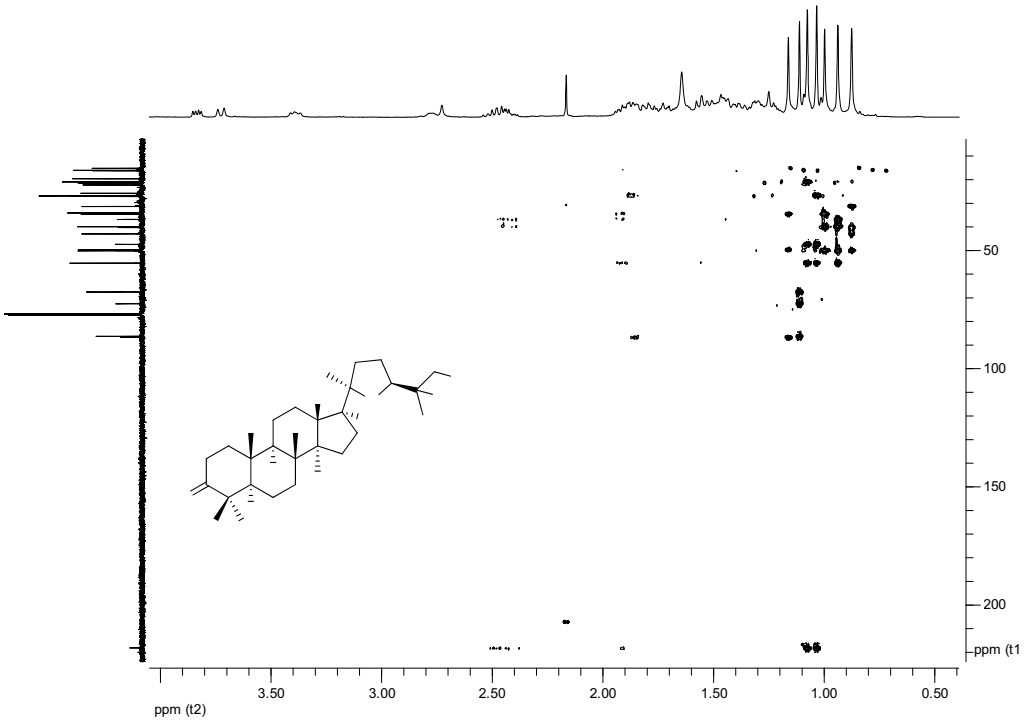


Figure S60. HMBC spectrum of (*20R,24S*)-epoxy-3-oxo-dammarane-25*E*,26-diol (**53**)

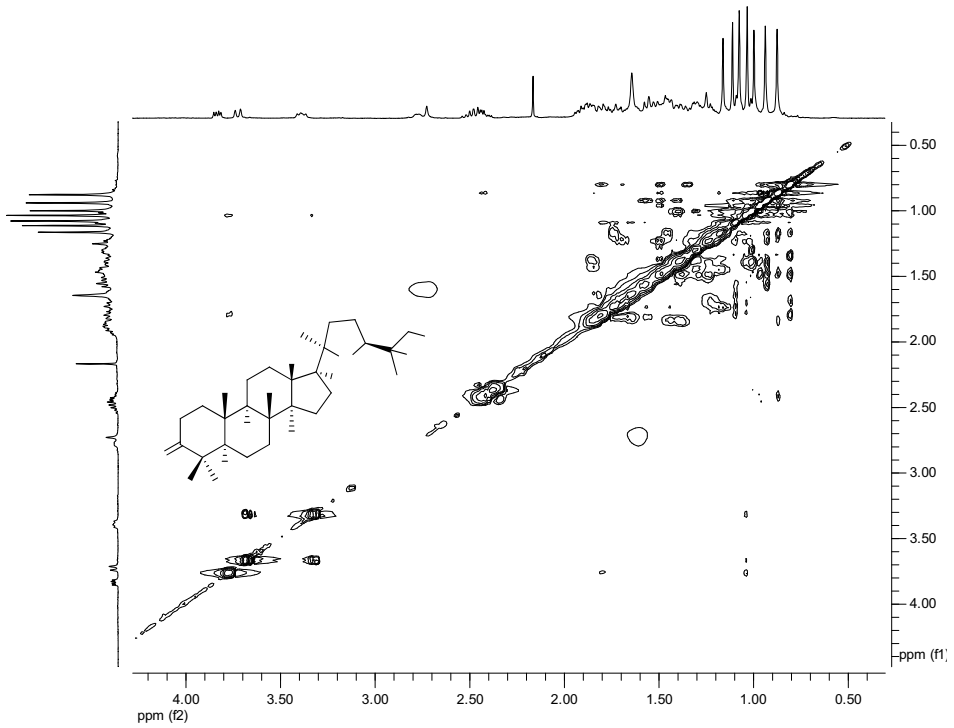


Figure S61. HMBC spectrum of (*20R,24S*)-epoxy-3-oxo-dammarane-25*E*,26-diol (**53**)

H

O

H

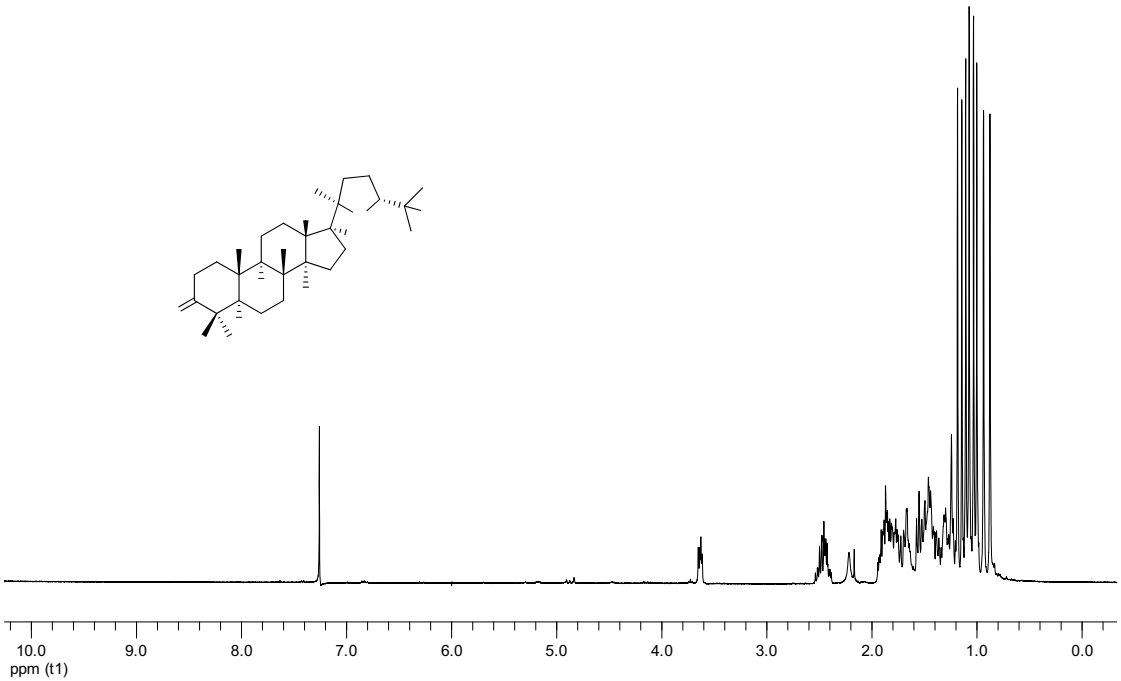


Figure S62. ^{1}H NMR spectrum of (*20R,24R*)-ocotillone (**54**) ($CDCl_3$, 400 MHz)

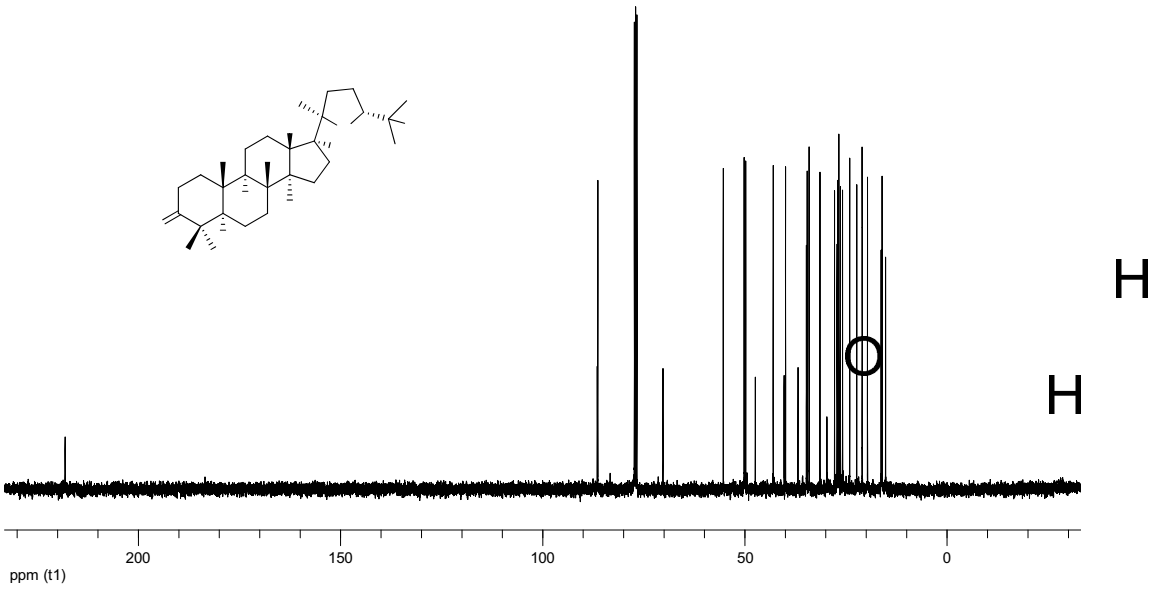


Figure S63. ^{13}C NMR spectrum of (*20R,24R*)-ocotillone (**54**) ($CDCl_3$, 400 MHz)

ภาคผนวก ข

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Cycloartane triterpenes from the exudate of *Gardenia thailandica*

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ABSTRACT

Two new cycloartane triterpenes, named gardenoins I - J (**1**–**2**), were isolated from the exudate of *Gardenia thailandica*, along with three known compounds, secaubryenol (**3**), sootepin E (**4**) and coronaloic acid (**5**). The structures were elucidated on the basis of spectroscopic evidence. Their cytotoxic effect on five human tumor cell lines was examined.

1. Introduction

Plants belonging to the genus *Gardenia* have proved to produce a variety of cycloartane triterpenoids, some of which display interesting biological activities (Grougnet et al., 2006; Reutrakul et al., 2004; Silva et al., 1999; Tuchinda et al., 2002, 2004). Additionally, extracts of various species exhibiting anti-implantation and abortifacient effects (Lu et al., 1981), and antiulcer (Takase et al., 1989), antibacterial (Laurens et al., 1985), diuretic, analgesic, hypertensive and larvicidal activities (Hussian et al., 1991; Manson, 1939) have been reported. Previous investigations on the plants in this genus have led to the isolation of an array of structurally diverse cycloartanes with a wide range of biological activities, particularly cytotoxic and anti-HIV effects (Reutrakul et al., 2004; Tuchinda et al., 2002). Recently, we have also reported the isolation and identification of a number of cytotoxic 3,4-*seco*-cycloartane triterpenoids from two species found in Thailand, *G. sootepensis* and *G. tubifera* (Nuanyai et al., 2009, 2010). This prompted us to investigate another plant in this genus, *Gardenia thailandica*. In this paper, we describe the isolation, structural characterization and cytotoxicity of two new cycloartane triterpenes (**1**–**2**) from the exudate of *G. thailandica*, together with three known compounds, secaubryenol (**3**), sootepin E (**4**) and coronaloic acid (**5**).

2. Results and discussion

The exudate collected from the aerial parts of *G. thailandica* was dissolved in a 1:1 mixture of CH_2Cl_2 and MeOH, which was then subjected to silica gel column chromatography using acetone–hexane mixtures of increasing polarity as eluent. Further purification by repeated normal phase column chromatography gave two new 3,4-*seco*-cycloartane triterpenes (**1** and **2**) and three known compounds, secaubryenol (**3**), sootepin E (**4**) and coronaloic acid (**5**). The structures of **3**–**5** were determined by comparison of their NMR spectroscopic data with those in the literature (Grougnet et al., 2006; Nuanyai et al., 2009; Tuchinda et al., 2002).

Compound **1** was obtained as colorless gum. Its molecular formula was established as $C_{30}H_{50}O_3$ by the HRESIMS ion at *m*/*z* 481.3655 $[M+Na]^+$ (calcd 481.3658), indicating six degrees of unsaturation. The IR absorption bands at 3443 and 1704 cm^{-1} implied the presence of hydroxyl and carbonyl functionalities. The 1D NMR data (Table 1) indicated that two of the six units of unsaturation came from one carbon–carbon double bond and a carboxylic acid group. Therefore, the remaining degrees of unsaturation required **1** to have a tretracyclic core. Analysis of the ^{13}C NMR and HSQC spectra revealed the presence of 30 nonequivalent carbons including one carboxylic carbonyl, six methyls (four tertiary and two secondary), 12 methylenes (one oxygenated), six methines (one olefinic) and five quaternary carbons (one olefinic). The ^1H NMR spectrum (Table 1) displayed typical signals associated with a 3,4-*seco*-cycloartane triterpenoid including two tertiary methyl singlets at δ_H 0.91 and 0.95, one secondary methyl doublet at 0.81 (d, J = 7.0 Hz), and a pair of doublets at δ_H 0.39 and 0.61 (J = 4.2 Hz), characteristic of the C-19

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Table 1
NMR spectroscopic data (400 MHz, $CDCl_3$) for compounds **1** and **2**.

Position	**1**		**2**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	27.9 CH_2	1.34 m	29.9 CH_2	1.37 m
				2.51 m
2	31.8 CH_2	2.32 m	31.9 CH_2	2.28 m
		2.49 m		2.68 m
3	179.2 C	–	178.7 C	–
4	36.6 CH	2.15 m	78.3 C	–
5	35.3 CH	1.94 m	42.9 CH	2.08 m
6	25.2 CH_2	1.01 m	24.8 CH_2	1.79 m
		1.28 m		2.06 m
7	25.0 CH_2	1.28 m	25.8 CH_2	1.27 m
8	48.4 CH	1.42 m	48.7 CH	1.29 m
9	21.3 C	–	23.5 C	–
10	27.2 C	–	29.7 C	–
11	27.0 CH_2	1.17 m	26.5 CH_2	1.14 m
		2.11 m		2.12 m
12	33.1 CH_2	1.65 m	33.1 CH_2	1.65 m
13	45.0 C	–	44.9 C	–
14	48.9 C	–	47.9 C	–
15	35.9 CH_2	1.39 m	35.9 CH_2	1.30 m
16	28.1 CH_2	1.28 m	28.2 CH_2	1.27 m
				1.91 m
17	52.3 CH	1.58 m	52.2 CH	1.60 m
18	18.3 CH_3	0.95 s	18.6 CH_3	0.94 s
19	30.3 CH_2	0.61 d (4.2)	31.1 CH_2	0.54 br s
		0.39 d (4.2)		0.56 br s
20	35.8 CH	1.28 m	35.9 CH	1.42 m
21	18.2 CH_3	0.88 d (6.3)	18.0 CH_3	0.91 m
22	36.3 CH_2	1.03 m	34.7 CH_2	1.22 m
		1.42 m		1.60 m
23	24.9 CH_2	1.86 m	26.1 CH_2	2.27 m
		2.04 m		2.40 m
24	125.2 CH	5.10 t (6.9)	155.8 CH	6.50 t (7.1)
25	130.9 C	–	139.1 C	–
26	25.7 CH_3	1.68 s	195.6 CH	9.38 s
27	17.6 CH_3	1.60 s	9.2 CH_3	1.74 s
28	11.8 CH_3	0.81 d (7.0)	24.4 CH_3	1.22 s
29	66.7 CH_2	3.48 d (7.1)	67.6 CH_2	3.46 d (11.0)
				3.88 d (11.0)
30	19.4 CH_3	0.91 s	19.5 CH_3	0.90 s

methylene protons of a cyclopropane ring of a cycloartane triterpene (Cantillo-Ciau et al., 2001; Chen et al., 1990; Sun et al., 1996; Tan et al., 1991). Both ^1H and ^{13}C NMR signals of **1** were very similar to those of secaubryenol (**3**) (Grougnet et al., 2006), with the marked difference being the appearance of a three-proton doublet at δ_H 0.81 due to an additional secondary methyl (C-28) and a methine (C-4) proton at δ_H 2.15 instead of signals observed for a terminal alkene moiety in **3**. This was confirmed by HMBC correlations of H_3-28 to C-4, C-29, and by ^1H–^1H COSY correlations of H_2-29/H-4 and H-4/H_3-28 (Fig. 2). The relative configuration of **1** was established to be the same as secaubryenol on the basis of NOE correlations. Observed NOESY correlations of H-5/Me-30, Me-30/H-17 and H-17/Me-21 indicated an α-orientation of these protons, while the correlations of H-8/Me-18, H-8/H_2-19 and Me-18/H-20 suggested that they were β-oriented (Fig. 3). Thus, **1** was determined as a new 29-hydroxy-3,4-*seco*-cycloartane and given the name as gadenoin J.

Compound **2** was obtained as colorless needles, and its molecular formula $C_{30}H_{48}O_5$ was deduced from the HRESIMS ion at m/z 511.3403 $[M+Na]^+$ (calcd 511.3399). Obvious in the ^1H NMR spectrum were a cyclopropane methylene (C-19) at δ_H 0.54 and 0.56, a pair of broad singlets (Lee et al., 2001), five methyls due to four tertiary and one secondary methyls, and an aldehyde δ_H 9.38. Comparison of the ^1H and ^{13}C NMR spectra of **2** with those of **1** revealed them to be very similar, except for the presence of an aldehyde group (δ_H 9.38, δ_C 195.6), and the absence of a vinylic methyl in **1**. Additionally, one more significant difference between **1** and **2** was found at the side chain connected to C-5 according to the appearance of an oxygenated quaternary carbon at δ_C 78.3 (C-4) instead of a methine carbon in **2**. This was clarified by no vicinal ^1H–^1H COSY correlations observed for H_2-29 through H_3-28 which indicated the quaternary center adjacent to them. The full assignment and connectivity were determined by ^1H–^1H COSY and HMBC correlations as shown in Fig. 2. The relative configuration of **2** was deduced to be the same as that of **1** on the basis of the NOESY correlations. Thus, the structure of **2** was as indicated (Fig. 1) and it was named gardenoin J.

The cytotoxicity of compounds **1–5** was tested in vitro against five human tumor cell lines. Only compound **4** was shown to be

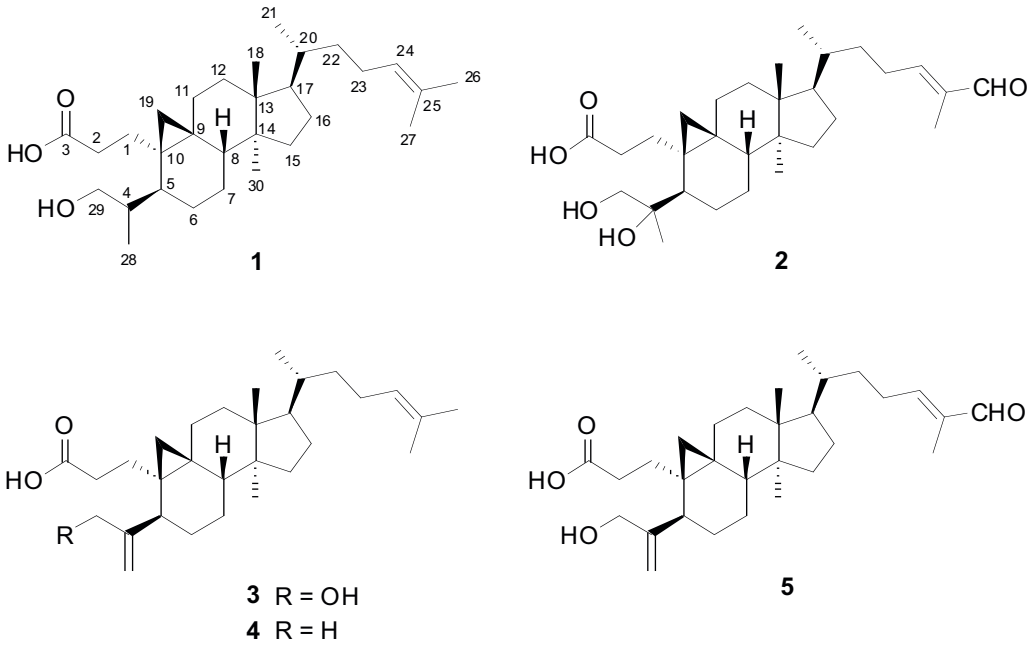

Fig. 1. Structures of cycloartane triterpenes isolated from *G. thailandica*.

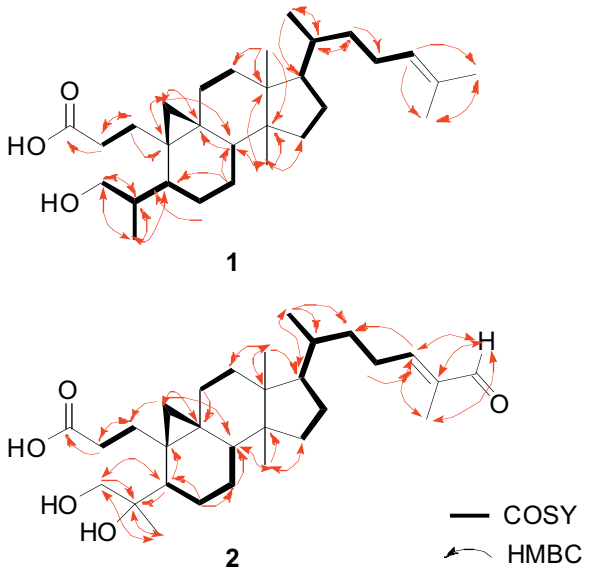

Fig. 2. ^{1}H–^{1}H COSY and key HMBC correlations of **1** and **2**.

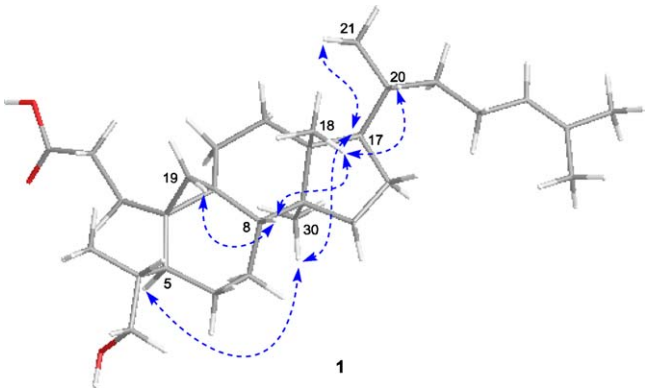

Fig. 3. Diagnostic NOE correlations of **1**.

cytotoxic against all cell lines tested as previously reported (Nuanyai et al., 2009), while compounds **1–3** and **5** did not display any cytotoxic effect at a dose of 10 μg/mL. Interestingly, the active compound **4** differed from the other compounds in the absence of a 29-hydroxy unit. This unit might cause the loss of biological activity of the compounds in this series.

3. Experimental

3.1. General experimental procedures

Melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. Optical rotations were measured on a PerkinElmer 341 polarimeter at a wavelength of 589 nm. UV data were recorded on Shimadzu UV-160 spectrophotometer. IR spectra were recorded on Bruker vector 22 Fourier transform infrared spectrophotometer. The NMR spectra were recorded on a Varian YH400 spectrometer at 400 MHz for ^{1}H NMR and at 100 MHz for ^{13}C NMR using TMS (tetramethylsilane) as the internal standard. HRESIMS were obtained using a Bruker micrOTOF mass spectrometer.

3.2. Plant material

The exudate was manually collected from the fresh aerial parts of *G. thailandica* from Bangkhan, Bangkok, Thailand, in July 2009. A voucher specimen (BKF 159039) has been deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

3.3. Extraction and isolation

The exudate (10 g) of *G. thailandica* was dissolved in a 1:1 mixture of CH_2Cl_2 and MeOH (20 mL). This solution was subsequently subjected to passage over a silica gel column eluted with a gradient system of acetone–hexane (from 1:4 to 1:0) to yield 11 fractions (I–XI). Fraction III (453.7 mg) was subjected to silica gel column chromatography (CC) using a mixture of acetone–hexane (1:4) as an eluent to afford **4** (53 mg). Fraction V was further purified using a silica gel column eluted with acetone–hexane (1:4) to give **1** (9.0 mg). Fraction VI was rechromatographed on a silica gel column, eluting with acetone–hexane (1:2) to afford **3** (255.6 mg) and **5** (29.2 mg), while fraction VII was separated by CC eluting with acetone–hexane (1:3) to yield **2** (10.0 mg).

3.4. Gardenoin I (1)

Colorless gum; $[\alpha]^{25}_{D}$ +29.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 329.9 (2.37), 275.1 (2.49) nm; IR (KBr) 3443, 2934, 1704, 1600, 1452, 1370, 1108, 1046 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 481.3655 $[M+Na]^{+}$ (calcd for $C_{30}H_{50}O_3Na$, 481.3658).

3.5. Gardenoin J (2)

Colorless needles; mp 110–111 °C; $[\alpha]^{25}_{D}$ +32.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 231.1 (3.62), 227 (3.64) nm; IR (KBr) 3439, 2939, 1712, 1617, 1443, 1378, 1117, 1034 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 511.3403 $[M+Na]^{+}$ (calcd for $C_{30}H_{48}O_5Na$, 511.3399).

3.6. Cytotoxicity assay

Cytotoxicity was evaluated against five human tumor cell lines using MTT colorimetric method as previously reported (Nuanyai et al., 2009, 2010). The following tumor cell lines were used in the assay; human breast ductal carcinoma ATCC No. HTB 20 (BT474), undifferentiated lung carcinoma (CHAGO), liver hepatoblastoma (Hep-G2), gastric carcinoma ATCC No. HTB 103 (KATO-3), and colon adenocarcinoma ATCC No. CCL 227 (SW-620). All cell lines were obtained from the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, and were cultured in RPMI-1640 supplemented with 25 mM HEPES, 0.25% (w/v) sodium bicarbonate, 5% (v/v) fetal bovine serum, and 100 μg/mL kayamycin.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2010.10.003.

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Antiangiogenic activity of 3,4-*seco*-cycloartane triterpenes from Thai *Gardenia* spp. and their semi-synthetic analogs

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ABSTRACT

Twelve naturally occurring 3,4-*seco*-cycloartane triterpenes (**1**–**12**) isolated from *Gardenia sootepensis* and *Gardenia obtusifolia*, and eight semi-synthetic derivatives (**13**–**20**) were evaluated for their antiangiogenic activity on a rat aortic sprouting assay, an ex vivo model of angiogenesis. Among these compounds, sootepin B (**1**) displayed the most potent activity in terms of the inhibition of microvessel sprouting from rat aortic rings in a dose-dependent manner with IC_{50} value of 4.46 μM. Its angiogenic effect was found to occur via suppression of endothelial cell proliferation and tubular formation, and was likely mediated by regulation (inhibition) of the Erk1/2 signaling pathway.

Cancer is one of the important public health problems all over the world and continues to cause a large number of present deaths, including pre-reproductive deaths, and the per capita population level appears to be increasing in the affected populations. Despite many anticancer drugs having been discovered and developed for use in clinical treatments over the last few decades, most of available drugs are non-selective cytotoxic molecules that provoke severe adverse effects, as well as acquiring drug resistance. Therefore the discovery and development of new effective chemotherapeutic agents is still required in the fight against cancer.

The use of antiangiogenic therapy as an alternative treatment for cancer patients has attracted considerable attention from researchers in recent years since the development and progression of tumors is crucially dependent upon angiogenesis.[1,2] Indeed, angiogenesis, the formation of new blood vessels from the pre-existing vasculature, is not only required for tumor growth and survival, but also for the transplantation and metastasis of tumors.[3,4] The newly formed blood vessels promote tumor progression by supplying oxygen and nutrients in sufficient amounts and by removing the waste metabolites. Thus the inhibition of tumor angiogenesis is one of the most promising current approaches in the cancer prevention and treatment.[5] Furthermore, therapy based upon targeting angiogenesis can be applied for a broad spectrum of tumors because angiogenesis is required for all of them, yet it has a low potential for the evolution of resistance due to the genetic stability of endothelial cells whose migration and proliferation are involved in the angiogenesis process.

Ring-A 3,4-*seco*-cycloartane type triterpenes have been mainly found in plants belonging to the genus *Gardenia* from family Rubiceae.[6–11] Many of them have been found to exhibit cytotoxic activities towards various tumor cell lines.[6–10] Recently we reported the isolation and characterization of a number of these triterpenoids from the apical buds and their exudate of some Thai *Gardenia* species that exhibit in vitro cytotoxicity against five human cancer lines.[12–15] However, the effect of these triterpenes on angiogenesis is seldom investigated. In this Letter, we focus on the assessment of the antiangiogenic activity of 3,4-*seco*-cycloartanes obtained from *Gardenia sootepensis* and *Gardenia obtusifolia* and their semi-synthetic analogs in order to gain more information about the structure–activity relationship (SAR).

The twelve naturally occurring 3,4-*seco*-cycloartane triterpenes used in this study were isolated from the apical buds of *G. sootepensis* and the exudate of *G. obtusifolia* as previously reported.[12,13] These are sootepin B (**1**), coronalolide (**2**), turbiferolide methyl ester (**3**), sootepin A (**4**), coronalolide methyl ester (**5**), gardenoins B and C (**6**, **7**), sootepins C and E (**8**, **9**), secaubryenol (**10**), and dikamakiartanes A and C (**11**, **12**). As for the semi-synthetic derivatives, the first compound series (**13**–**17**) were prepared from coronalolide (**2**) by modification of the COOH at C-1 to ester and amide functions using the combination of DCC (EDC for **17**) and HOBt as the coupling agent. The second series of compounds (**18**–**20**) were obtained by modification of the primary alcohol of sootepin A (**4**). The benzoate **18** was prepared by treating **4** with benzoyl chloride in the presence of Et_3N. Reactions of **4** with the corresponding acids, pretreated with

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oxalyl chloride in the presence of a catalytic amount of DMF to generate the acid chlorides, afforded **19** and **20**. The structures of the modified compounds, shown in Figure 1, were confirmed by spectroscopic data (see Supplementary data).

To investigate the SARs of both the natural and the semi-synthetic derivatives, their effect on angiogenesis was evaluated by monitoring the inhibition of microvessel sprouting from rat aortic rings (an ex vivo model) at a single dose of 25 μM.[16] Among compounds possessing an exomethylene γ-lactone (**1**–**7** and **13**–**20**), the naturally occurring sootepin B (**1**), bearing a free COOH at C-1 and Me at C-26, showed the strongest antiangiogenic activity, completely suppressing the microvessel sprouting, followed by coronalolide (**2**) at ~85% inhibition, but their semi-synthetic derivatives were all less active. For the five natural compounds without an exomethylene γ-lactone group, sootepin E (**9**) showed the highest anti-angiogenic activity, at ~92% inhibition, which was the second highest of all 20 compounds after that of **1**. The esterification and amidation of the C-1 carboxylic acid (**3**–**5**, **13**–**20**), as well as the presence of highly oxidized functionalities in place of the methyl group at C-26 (**2**, **4**, **5**, **13**–**20**) caused a significant loss of activity, revealing from only 40–60% and 10–50% inhibition, respectively (Fig. 2). Similar results were obtained from the derivatives with a cleaved C-6–C-29 bond (**8**–**12**). The free COOH at C-1 is also required for activity as observed in **9** and **10**, but they exhibited a lower activity than **1**, which suggests that the exomethylene γ-lactone (formed by lactonization of C-29 onto C-6) plays a crucial role for the antiangiogenic effect. Furthermore, there was no apparent significant or only a slight inhibition of angiogenesis by the four derivatives having a furan ring in the side chain (**6**–**7**, **11**–**12**).

Based on the results obtained from the screening using the ex vivo microvessel sprouting model, only sootepin B (**1**) was chosen for further evaluation, starting with its potency to suppress microvessel outgrowth by treating aortic rings with various doses

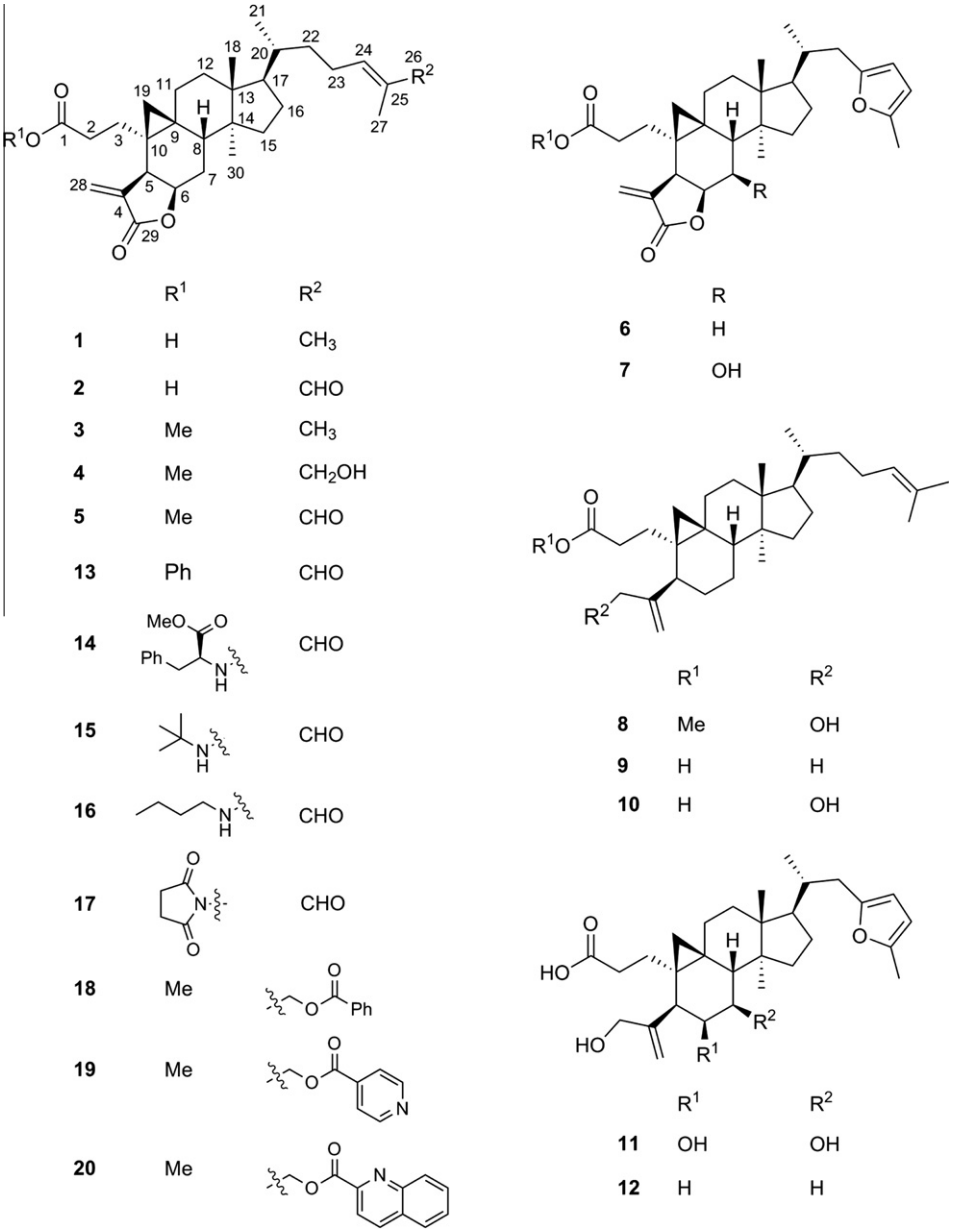

Figure 1. Structures of the naturally occurring 3,4-*seco*-cycloartanes (**1**–**12**) and their semisynthetic derivatives (**13**–**20**).

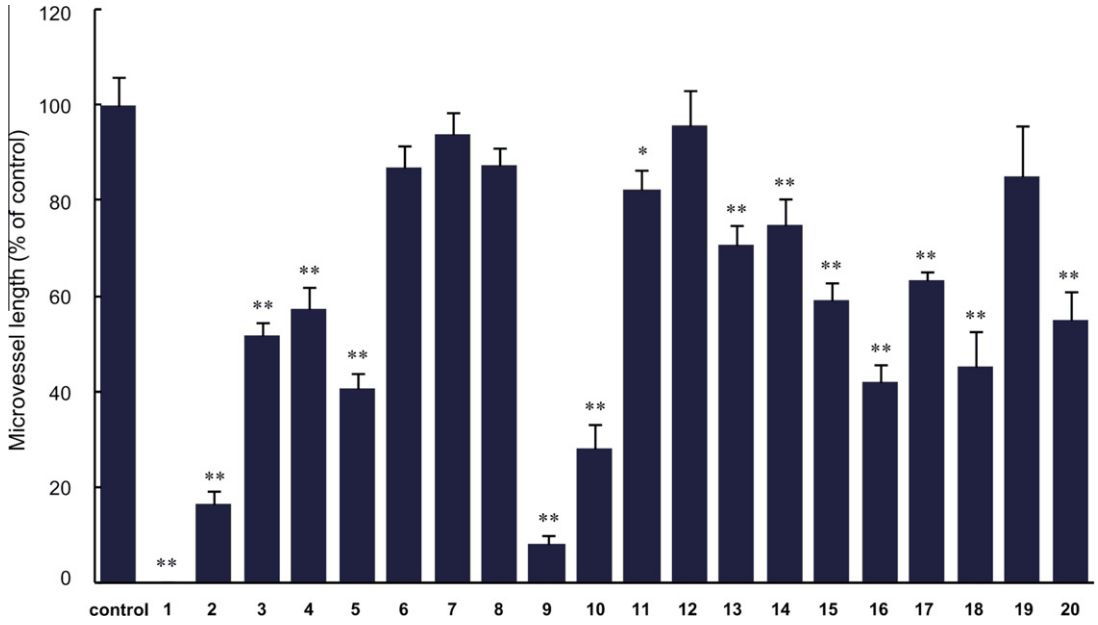

Figure 2. Effect of each of 3,4-*seco*-cycloartanes (**1**–**20**) at 25 μM on the ex vivo angiogenesis rat aortic sprouting assay as an angiogenesis model. Values are the mean ± SD ($n = 6$). Significantly different from the control: $^{*}p < 0.05$ and $^{**}p < 0.01$.

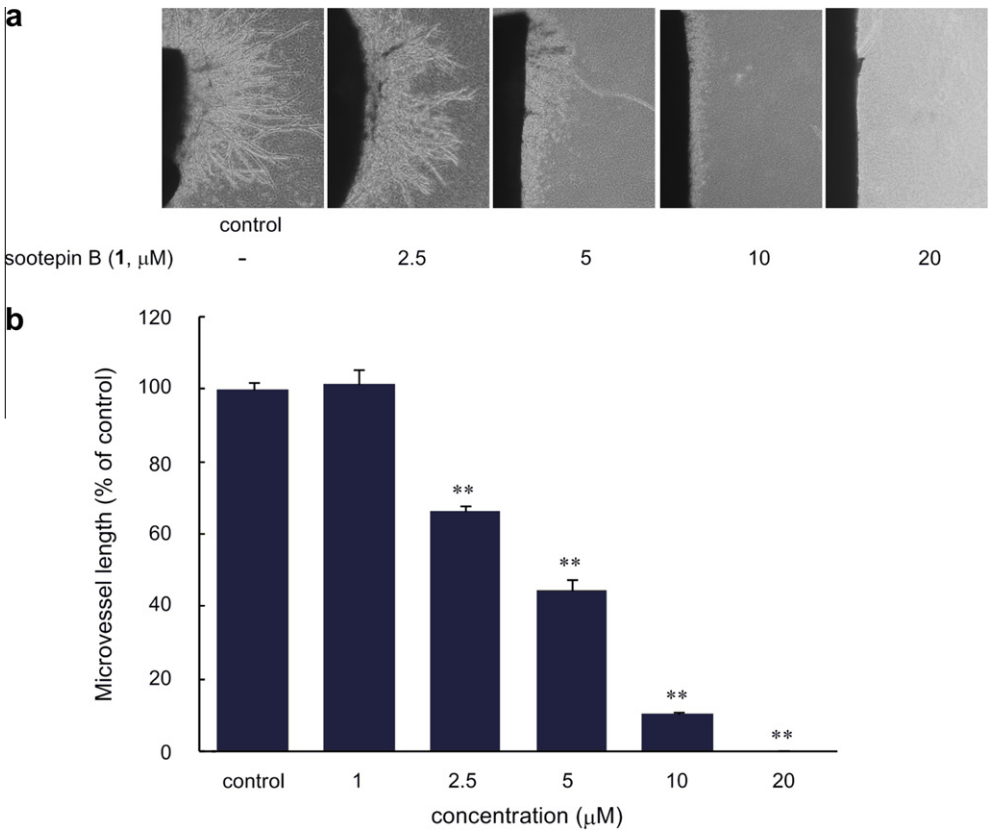

Figure 3. Inhibitory effect of sootepin B (**1**) at various concentrations on the ex vivo rat aortic sprouting assay. (a) Microscopic photograph of microvessel formation. (b) The average microvessel length on the seventh day of culture. Values are the mean ± SD ($n = 6$). Significantly different from the control: $^{**}p < 0.01$.

of the compound. As shown in Figure 3, the inhibitory effect of **1** was found to be dose-dependent manner with an IC_{50} value of 4.46 μM.

To investigate how sootepin B (**1**) could exert the anti-angiogenic effect, its in vitro inhibitory activity towards the functions of human umbilical vein endothelial cells (HUVECs), in terms of the in vitro cell proliferation, tubular formation and migration, were evaluated. The effect of **1** on the proliferation of HUVECs was evaluated by using the WST-8 assay,[17] following treatment of HUVECs with various concentrations of **1** or vehicle (DMSO)

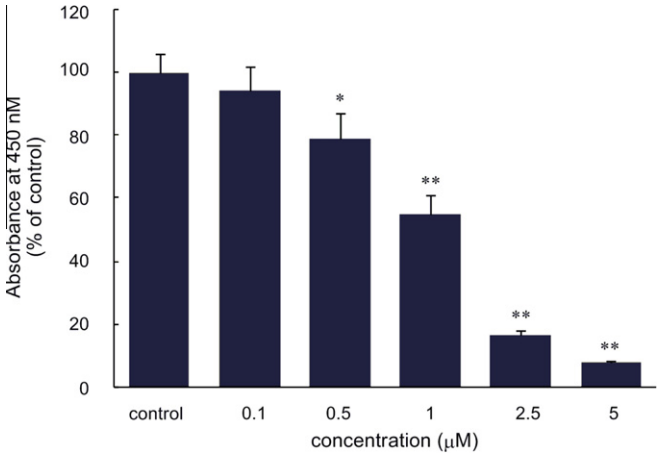

Figure 4. Effect of sootepin B (**1**) on the HUVEC proliferation. Values are the mean ± SD (n = 5). Significantly different from the control: $^{*}p$ <0.05 and $^{**}p$ <0.01.

alone for 72 h. The results (Fig. 4) indicated that sootepin B (**1**) inhibited the proliferation of the endothelial cells in a dose-dependent manner with an IC_{50} value of 1.20 μM. Next, whether sootepin B (**1**) could affect the HUVECs tubule formation was investigated,[18] by inoculating HUVECs onto reconstituted basement membranes (Matrigel) and then allowing them to migrate, attach to each other and then form capillary-like structures.[19,20] The effect of the compound was assessed by measuring the length of tube-structured cells compared to the vehicle control after incubation for 12 h. Sootepin B (**1**) was found to suppress tubule formation in the endothelial cells in a dose dependent manner with a significant inhibition being observed at 5 μM, an IC_{50} of 11.20 μM and the complete inhibition at 20 μM (Fig. 5). Finally, the effect of sootepin B (**1**) on the VEGF-induced migration of HUVECs was examined using a modified Boyden chamber assay.[21] VEGF is known as a specific and pivotal growth factor involved in endothelial cell proliferation, migration and survival during blood vessel formation.[22,23] HUVECs were seeded on cell culture inserts with sootepin B (**1**) at concentrations ranging from 5 to 50 μM and VEGF (10 ng/mL). After incubation for 6 h, the membranes containing migrated cells were stained and cut off from the inserts. The number of migrated cells was then counted under a microscope. As seen in Supplementary Figure S9, VEGF strongly stimulated HUVECs migration and sootepin B (**1**) did not display any detectable effect upon this, even at a dose as high as 50 μM. Thus **1** might not be involved in the VEGF-induced endothelial cell migration.

Based on these results from the above ex vivo and in vitro assays, sootepin B (**1**) would appear to be a potent angiogenic inhibitor, and that it mainly functions by suppression of endothelial cell proliferation and tubule formation, but has no effect upon cell migration.

The mechanism by which sootepin B (**1**) regulates angiogenic inhibition was then investigated by western blot analysis.[24] In angiogenesis there are two known important pathways responsible for VEGF stimulus on endothelial cells, the phosphoinositide 3-kinase/Akt and the extracellular signal-regulated kinase (Erk1/2) pathways. Both of these pathways are reported to be involved in endothelial cell proliferation, migration and survival.[25] The treatment of HUVECs with sootepin B (**1**) suppressed the phosphorylation of Erk1/2 in both a time- and dose-dependent manner (Fig. 6), but did not affect the expression of phosphorylated Akt (data not shown). This suggested that the antiangiogenic property of sootepin B (**1**) is mediated by alteration of the Erk1/2 signaling pathway. The strong suppressive effect of sootepin B (**1**) on the

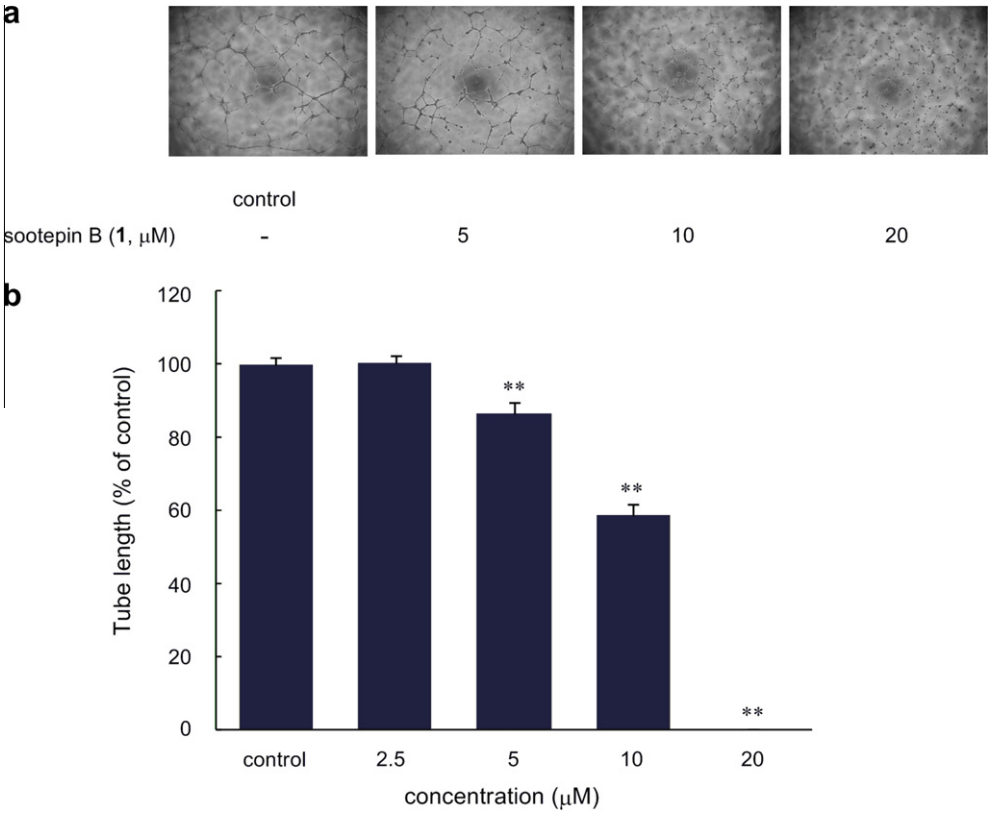

Figure 5. Effect of sootepin B (**1**) on the HUVEC tubular formation. (a) Microscopic photograph of tubular formation on the reconstituted gel after incubation for 12 h. (b) The average capillary length. Values are the mean ± SD (n = 5). Significantly different from the control: $^{**}p$ <0.01.

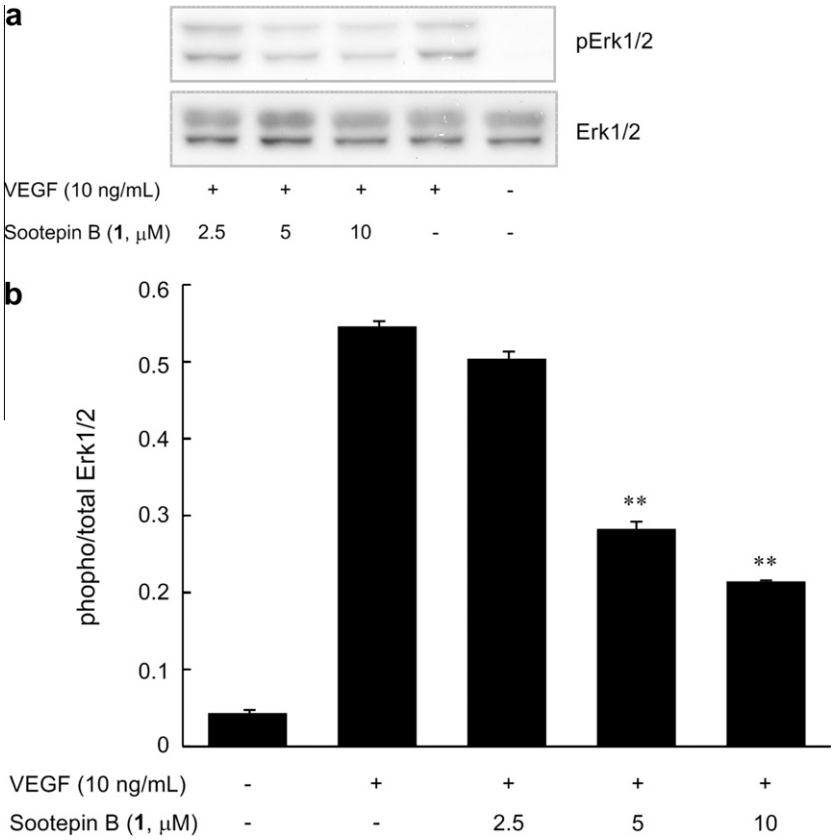

Figure 6. Effect of sootepin B (**1**) concentration on the phosphorylation level of Erk1/2 in HUVECs. (a) HUVECs were pretreated with various doses of sootepin B (**1**) or vehicle (DMSO) alone for 1 h. (b) The level of phosphorylated (pErk1/2) and total Erk1/2 protein was determined by western blot analysis. Values are the mean ± SD ($n = 3$). Significantly different from the control: $^{*}p < 0.05$.

phosphorylation of Erk1/2 is in line with a previous report in which inhibitors for Erk1/2 signaling pathway suppress endothelial cell proliferation.[26] Furthermore the inactive effect of sootepin B (**1**) on migration is consistent with the absence of any detectable effect on the phosphorylation of Akt since this pathway plays an important role in endothelial cell migration.[27]

In summary, a series of 3,4-*seco*-cycloartane triterpenes, comprised of twelve naturally occurring and eight semi-synthetic derivatives, were evaluated for their antiangiogenic activity. The results indicated that the natural compound sootepin B (**1**) possesses a potent angiogenic activity, presumably mediated via suppression of endothelial cell proliferation and tubular formation. Its effect on angiogenesis might be mediated by regulation of the Erk1/2 signaling pathway. Therefore sootepin B (**1**) might be potentially useful in cancer therapy as an angiogenic inhibitor.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.128.

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16. Ex vivo angiogenesis assay. The rats were maintained according to the guide for the care and use of laboratory animals established by Hiroshima University. All experiments using animals were approved by Hiroshima University Animal Research Committee. A six-week-old specific pathogen free male Wistar rat was sacrificed by bleeding from the right femoral artery under anesthesia with diethyl ether. The thoracic aorta was removed, washed with RPMI 1640 medium, turned inside out, and cut into 1-mm lengths. The aortic rings were then placed on 6-well culture plate and covered with 0.5 mL of gel matrix solution (8 volumes of porcine tendon collagen solution, 1 volume of 10× Eagle's MEM and 1 volume of reconstitution buffer), and then allowed to gel at

37 °C for 30 min. Two millilitre of RPMI 1640 medium containing 1% (v/v) of TIS+ with the designated doses of the test compounds or vehicle (DMSO) were added to the wells. After incubation for 7 days at 37 °C in 5% CO_2, the capillary length was estimated under phase-contrast microscopy by measuring the distance from the cut end of the aortic segment to the approximate mid-point of the capillary.

17. HUVEC proliferation assay. A HUVEC suspension in HuMedia EG2 (1.5×10^4 cells/mL) was seeded into each well of 96-well plate (100 μL) and incubated for 24 h at 37 °C in 5% CO_2. The medium was then removed and replaced with fresh HuMedia EG2 containing various doses of sootepin B (**1**) or vehicle (DMSO) and incubated for 72 h at 37 °C in 5% CO_2. Cell proliferation was detected using the WST-8 reagent, and the inhibition of proliferation was measured at 450 nm using a microplate reader.
18. HUVEC tube formation assay. HUVEC tube formation was assayed using a BD Matrigel.[19,20] The solid gel was prepared in a 96-well tissue culture plate according to the manufacture's instruction. HUVECs (1×10^5 cells/mL) in HuMedia EG2 medium containing various doses of sootepin B (**1**) or vehicle (DMSO) alone were seeded onto the surface of the solid BD Matrigel and incubated for 12 h at 37 °C in 5% CO_2. Tube formation was then observed under an inverted light microscope at 40× magnification. Microscopic fields were photographed with a digital camera (OLYMPUS DSE330-A system).
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21. HUVEC migration assay. HUVEC migration was assayed using a modified Boyden chamber.[19,20] A microporous membrane (8 μm) as an insert inside each well of 24-well cell culture plates was coated with 0.1%(w/v) gelatin. The HUVEC suspension (2.5×10^5 cells/mL) in Medium 199 with 0.1% (w/v) bovine serum albumin was seeded into each chamber (400 μL) and to each well was added with 400 μL of Medium 199 containing 0.1% (w/v) BSA and 10 ng/mL of human recombinant VEGF with or without sootepin B (**1**). The assembled chamber was incubated for 6 h at 37 °C in 5% CO_2. Non-migrated cells on the surface of the membrane were removed by scrubbing with a cotton swab. The migrated cells were then fixed with methanol, stained with Diff-Quik stain (Sysmex, Kobe, Japan), and then counted on three randomly selected fields of view for each membrane under a light microscope at 200× magnification.
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24. Western blot analysis. HUVECs were grown to confluence and then starved for 16 h in RPMI 1640 containing 5%(v/v) FBS. Cells were pretreated for 60 min with sootepin B (0–10 μM) or vehicle (DMSO) alone and stimulated by the addition of VEGF (10 ng/mL) for 10 min. After stimulation, cells were washed twice with PBS and then lysed with mammalian protein extraction reagent (M-PER; Pierce, Rockford, IL, USA). Lysates were clarified by centrifugation for 15 min at 12000 rpm at 4 °C. The protein concentration was determined using the microBCA protein assay kit (Pierce, Rockford, IL, USA) with BSA as standard. For each sample, 50 μg of total protein was separated by SDS polyacrylamide gel electrophoresis under reducing conditions and then transferred to a nitrocellulose membrane. After blocking, the membranes were incubated with primary antibody for phopho-Erk1/2 or phospho-Akt (Cell Signaling Technology, Tokyo, Japan) at 4 °C overnight and for Erk1/2 or Akt at room temperature, respectively. Immunoreactive bands were visualized by chemiluminescence using a WesternBreeze (Invitrogen) with a ChemiDoc XRS (BIO-RAD).
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Dammarane triterpenes from the apical buds of *Gardenia collinsae*

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ABSTRACT

Two new dammarane triterpenes, named 20*R*,24*R*-epoxy-3-oxo-dammarane-25ξ,26-diol (**1**) and its C-24 epimer (**2**), were isolated from the apical buds of *Gardenia collinsae*, along with a known compounds (20*R*,24*R*)-ocotillone (**3**). The structures were elucidated on the basis of spectroscopic evidence. Their cytotoxic effect on five human tumor cell lines was examined.

1. Introduction

The genus *Gardenia* (Rubiaceae) comprises more than 80 species distributed over the tropical forests in various parts of the world and 15 species of them were reported to be found in Thailand (Smitinand, 2001). Various species of *Gardenia* plants have been used ethnomedically for abortifacient and contraceptive purposes (Woo et al., 1981; Mbela et al., 1992; Jain, 1989), and for the treatment of headaches, asthma and malaria (Croft and Tu'Ipulotu, 1980; Chhabra et al., 1991; Gessler et al., 1995). Additionally, extracts of several species exhibiting anti-implantation and abortifacient effects (Lu et al., 1981), and antiulcer (Takase et al., 1989), antibacterial (Laurens et al., 1985), diuretic, analgesic, hypertensive and larvicidal activities (Hussian et al., 1991; Manson, 1939) have been reported. Previous investigations on the plants in this genus have led to the isolation of an array of structurally diverse flavonoids and cycloartane triterpenoids, with a wide range of biological activities, particularly cytotoxic and anti-HIV effects (Reutrakul et al., 2004; Tuchinda et al., 2002). Recently, we have also reported the isolation and identification of a number of cytotoxic 3,4-*seco*-cycloartane triterpenoids from two species found in Thailand, *Gardenia sootepensis* and *Gardenia tubifera* (Nuanyai et al., 2009, 2010). In the continuing search for potential anti-cancer drug leads from Thai *Gardenia* plants, a new dammarane triterpene (**1**) and its epimer (**2**) were isolated from the apical buds of *G. collinsae*, together with one known compound (20*R*,24*R*)-ocotillone (**3**), as well as their cytotoxicity was also evaluated.

2. Results and discussion

The MeOH-soluble fraction of the fresh apical buds of *G. collinsae* was partitioned between EtOAc and H_2O to afford an EtOAc extract, which was then subjected to silica gel column chromatography using EtOAc-hexane mixtures of increasing polarity as eluent. Further purification by repeated normal-phase column chromatography yielded a new dammarane-type triterpene (**1**) and its C-24 epimer (**2**), along with (20*R*,24*R*)-ocotillone (**3**) (Fig. 1).

Compound **1**, a white amorphous powder, had the molecular formula $C_{30}H_{50}O_4$ as established by the HRESIMS ion at *m/z* 497.3609 $[M+Na]^+$ (calcd 497.3607). Its IR spectrum showed absorption bands at 1710 and 3466 (broad) cm^{-1}, which indicated the presence of carbonyl and hydroxyl groups. Inspection of the ^{13}C NMR and HSQC spectra revealed the presence of 30 nonequivalent carbons consisting of one ketone carbonyl, seven tertiary methyls, 11 methylenes (one oxygenated), five methines (one oxygenated) and six quaternary carbons (two oxygenated). The ^1H NMR spectrum of **1** (Table 1) displayed seven singlet signals of tertiary methyls at δ_H 0.87, 0.93, 0.99, 1.01, 1.03, 1.08 and 1.16, associating with dammarane-type triterpenoid. The planar structure of **1** was elucidated on the basis of 2D NMR (^1H–^1H COSY, HSQC and HMBC) spectroscopic data (Fig. 2). The tetrahydrofuran ring was evident from a key HMBC correlation from an oxygenated methine proton at δ_H 3.82 (H-24) to C-20. An oxygenated methylene group (δ_H 3.37 and 3.67; δ_C 71.3) was located as C-26 which was addressed by HMBC correlations from H-26 to C-24 and C-25. Detailed analyses of the NMR spectroscopic data of **1** as described above led to the establishment of the same planar structure as 20*S*,24*R*-epoxy-3-oxo-dammarane-25ξ,26-diol (**4**) (Anjaneyulu et al., 1993). The NMR data of **1** were also similar to those of **4**, with the marked difference being the ^{13}C resonances of Me-21 (δ_C 22.5 for **4**) and oxygenated carbons of the tetrahydrofuranyl unit [δ_C 86.4 qC

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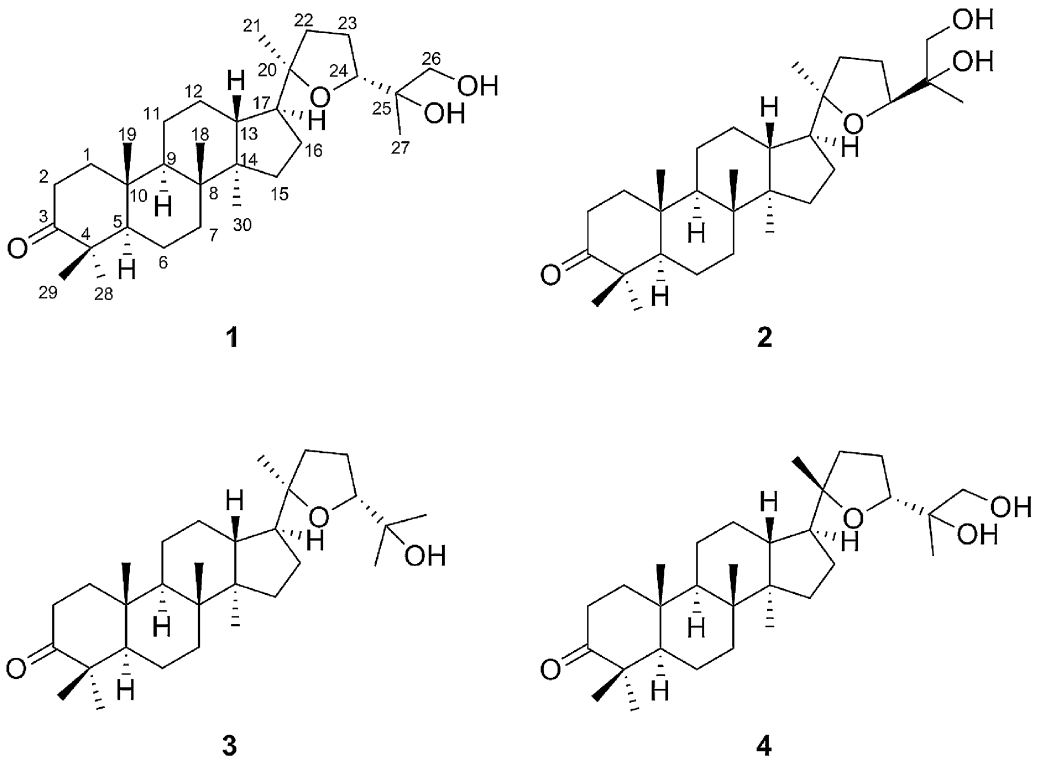

Fig. 1. Structures of the isolated dammarane triterpenes.

Table 1
NMR spectroscopic data (400 MHz, $CDCl_3$) for compounds **1**–**3**.

Position	**1**		**2**		**3**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1a	39.9 CH_2	1.91 m	39.9 CH_2	1.91 m	39.9 CH_2	1.93 m
1b		1.45 m		1.44 m		1.47 m
2	34.1 CH_2	2.46 m	34.1 CH_2	2.46 m	34.1 CH_2	2.47 m
3	218.0 C	–	218.1 C	–	218.1 C	–
4	47.4 C	–	47.4 C	–	47.4 C	–
5	55.3 CH	1.36 m	55.3 CH	1.37 m	55.3 CH	1.37 m
6a	19.6 CH_2	1.55 m	19.7 CH_2	1.55 m	19.7 CH_2	1.56 m
6b		1.45 m		1.46 m		1.46 m
7a	34.5 CH_2	1.56 m	34.6 CH_2	1.56 m	34.6 CH_2	1.56 m
7b		1.30 m		1.31 m		1.31 m
8	40.2 C	–	40.3 C	–	40.3 C	–
9	49.9 CH	1.41 m	49.7 CH	1.42 m	49.8 CH	1.42 m
10	36.8 C	–	36.9 C	–	36.9 C	–
11a	22.1 CH_2	1.52 m	22.3 CH_2	1.51 m	22.3 CH_2	1.52 m
11b		1.25 m		1.24 m		1.26 m
12a	25.7 CH_2	1.75 m	25.8 CH_2	1.76 m	25.8 CH_2	1.77 m
12b		1.33 m		1.29 m		1.32 m
13	43.1 CH	1.61 m	43.0 CH	1.64 m	43.0 CH	1.68 m
14	50.0 C	–	50.0 C	–	50.0 C	–
15a	31.3 CH_2	1.47 m	31.4 CH_2	1.46 m	31.4 CH_2	1.46 m
15b		1.10 m		1.09 m		1.09 m
16	26.1 CH_2	1.80 m	27.1 CH_2	1.82 m	27.0 CH_2	1.84 m
17	50.0 CH	1.85 m	50.1 CH	1.86 m	50.2 CH	1.88 m
18	16.0 CH_3	0.99 s	16.1 CH_3	0.99 s	16.1 CH_3	1.01 s
19	15.1 CH_3	0.93 s	15.2 CH_3	0.93 s	15.2 CH_3	0.94 s
20	87.8 C	–	86.8 C	–	86.5 C	–
21	26.7 CH_3	1.16 s	26.7 CH_3	1.16 s	27.2 CH_3	1.15 s
22a	34.4 CH_2	1.78 m	34.7 CH_2	1.88 m	34.8 CH_2	1.88 m
22b		1.68 m		1.70 m		1.68 m
23a	26.2 CH_2	2.08 m	27.1 CH_2	1.81 m	26.4 CH_2	1.79 m
23b		1.81 m		1.23 m		1.32 m
24	86.3 CH	3.82 dd (4.9, 10.4)	86.3 CH	3.83 dd (4.7, 10.2)	86.4 CH	3.63 m
25	70.6 C	–	72.5 C	–	70.2 C	–
26a	71.3 CH_2	3.67 t (10.8)	67.5 CH_2	3.73 d (11.0)	24.1 CH_3	1.11 s
26b		3.37 d (10.8)		3.39 t (11.0)		
27	20.0 CH_3	1.01 s	21.4 CH_3	1.11 s	27.8 CH_3	1.19 s
28	27.0 CH_3	1.08 s	26.9 CH_3	1.07 s	26.7 CH_3	1.08 s
29	21.0 CH_3	1.03 s	21.0 CH_3	1.04 s	21.0 CH_3	1.04 s

Table 1 (*Continued*)

Position	1		2		3	
	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)
30	16.2 CH_3	0.87 s	16.3 CH_3	0.87 s	16.3 CH_3	0.88 s
26-OH		3.21 br d (10.56)				

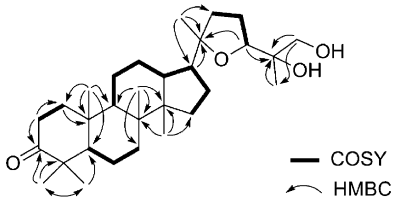

Fig. 2. ^1H–^1H COSY and key HMBC correlations of **1**.

(C-20), 82.7 CH (C-24) for **4**]. These results implied that compounds **1** and **4** are diastereomers differing from each other by the relative configurations of the C-20 and C-24 chiral centers. Comparison of the ^{13}C NMR data of **1** with those of (20*R*,24*R*)-ocotillone (**3**) and 20*S*,24*R*-epoxy-3-oxo-dammarane-25ξ,26-diol (**4**) at C-20 and C-24 (Table 1) indicated that compound **1** should have the 20*R*,24*R* configuration. Furthermore, this was also supported by the lack of NOESY correlation between Me-21 and H-24 (Fig. 3). Thus, compound **1** was determined to be a new dammarane triterpene, 20*R*,24*R*-epoxy-3-oxo-dammarane-25ξ,26-diol.

Compound **2** was obtained as colorless gum, and it had the same molecular formula and gross structure as compound **1** by HRESIMS and interpretation of its 2D NMR spectroscopic data. The NMR spectra of **2** (Table 1) were very similar to those of **1**. The only marked difference was the intense NOESY correlation between H-24 and Me-21 (Fig. 3) observed in **2**, but lacked in **1**. This supported that compound **2** should have the 20*R*,24*S* configuration.

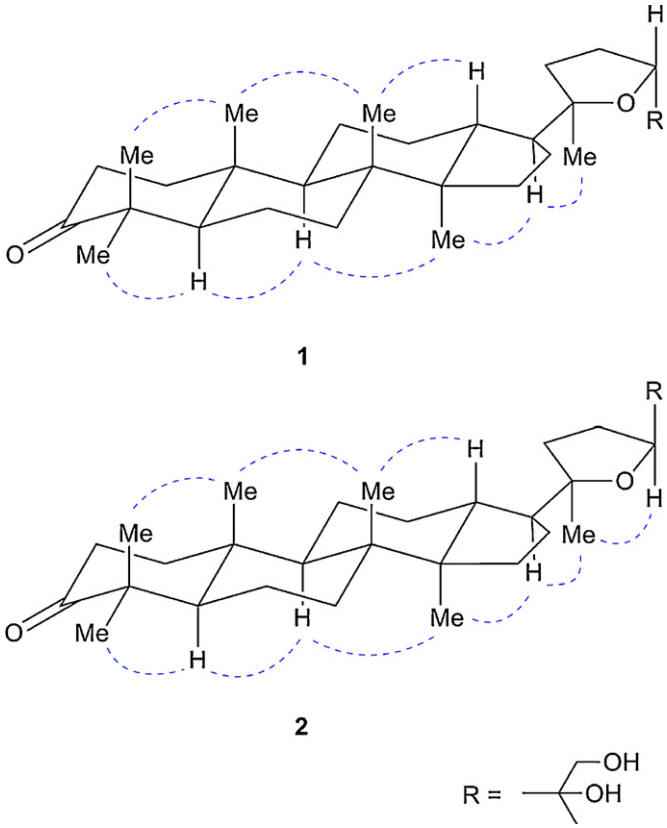

Fig. 3. Key NOESY correlations of **1** and **2**.

Compound **3** was determined to be (20*R*,24*R*)-ocotillone by means of the comparison with the reported spectral data (Betacor et al., 1983; Wahlberg and Enzell, 1971).

The cytotoxicity of compounds **1**–**3** was tested in vitro against five human tumor cell lines. Only compound **3** was shown to be moderately cytotoxic against SW-620 and CHAGO cell lines with the same IC_{50} value of 6.25 μM, while compounds **1** and **2** did not display any cytotoxic effect at a dose of 10 μg/mL. Interestingly, the active compound **3** differs from the other compounds in the absence of a C-26 oxygenated methylene group. The increasing of hydrophilic functionality at this position might cause the loss of biological activity of the compounds in this series.

3. Experimental

3.1. General experimental procedures

Melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter at a wavelength of 589 nm. UV data were recorded on Shimadzu UV-160 spectrophotometer. IR spectra were recorded on Bruker vector 22 Fourier transform infrared spectrophotometer. The NMR spectra were recorded on a Varian YH400 spectrometer at 400 MHz for ^1H NMR and at 100 MHz for ^{13}C NMR using TMS (tetramethylsilane) as the internal standard. HRESIMS were obtained using a Bruker micrOTOF mass spectrometer.

3.2. Plant material

The fresh apical buds of *G. collinsae* were collected from Nakorn Sawan Province, Thailand, in February 2010. A voucher specimen (BKF 159041) has been deposited at the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

3.3. Extraction and isolation

The fresh apical buds (53 g) of *G. collinsae* were extracted with MeOH (500 mL ×2, each overnight). After removing the solvent under reduced pressure, the combined MeOH crude extract was suspended in H_2O (200 mL), then partitioned with EtOAc (200 mL ×3), to afford an EtOAc crude extract (11 g). The EtOAc extract was fractionated by a silica gel column eluted with a gradient system of EtOAc-hexane to yield 25 pooled fractions (I–XXV). Fractions IX and X were combined together and then recrystallized with a 1:4 mixture of acetone–hexane to give **3** (950 mg). Fraction XXI afforded **1** (35.2 mg) after precipitation and filtration. Fraction XXII was rechromatographed on a silica gel column, eluting with MeOH–CH_2Cl_2 (2:98) system to afford nine subfractions (XXIIa–XXIIi). Subfraction XXIId was further purified by column chromatography on silica gel with a mixture of acetone–hexane (1:3) to yield **2** (8.0 mg).

3.4. 20R,24R-epoxy-3-oxo-dammarane-25ξ,26-diol (1)

White amorphous powder; mp 165–166 °C; $[\alpha]_D^{25}$ +31.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 334.0 (1.36), 232.0 (2.20) nm; IR (KBr) 3426, 2939, 1708, 1456, 1378, 1043 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 497.3609 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3607).

3.5. 20R,24S-epoxy-3-oxo-dammarane-25ξ,26-diol (2)

Colorless gum; $[\alpha]_D^{25}$ +37.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 243.9 (3.12) nm; IR (KBr) 3426, 2921, 1708, 1460, 1365, 1040 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 497.3611 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_4Na$, 497.3607).

3.6. Cytotoxicity assay

Cytotoxicity was evaluated against five human tumor cell lines using MTT colorimetric method as previously reported (Nuanyai et al., 2009, 2010). The following tumor cell lines were used in the assay; human breast ductal carcinoma ATCC No. HTB 20 (BT474), undifferentiated lung carcinoma (CHAGO), liver hepatoblastoma (Hep-G2), gastric carcinoma ATCC No. HTB 103 (KATO-3), and colon adenocarcinoma ATCC No. CCL 227 (SW-620). All cell lines were obtained from the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, and were cultured in RPMI-1640 supplemented with 25 mM HEPES, 0.25% (w/v) sodium bicarbonate, 5% (v/v) fetal bovine serum, and 100 μg/mL kayamycin.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytol.2011.03.001.

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SOOTEPENDIAL, A NEW 3,4-SECO CYCLOARTANE FROM A THAI GARDENIA PLANT, INDUCES APOPTOSIS IN LIVER CANCER CELL AND ANGIOGENIC INHIBITION

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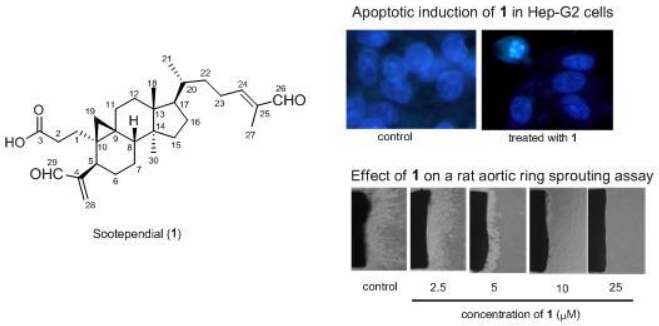
Ring-A 3,4-*seco*-cycloartane triterpenes have been mainly found in plants belonging to the genus Gardenia (Rubiceae). Many of them have been found to exhibit cytotoxic activity towards various cancer cell lines. We aimed to determine 3,4-*seco*-cycloartane from Thai *Gardenia* spp. potential for cancer leads and to explore the detailed mechanism. In this study, a new 3,4-*seco*-cycloartane triterpenes, sootependial (**1**) was isolated from bud exudate of Thai *G. sootepensis* and its structure was elucidated on the basis of spectroscopic data. Sootependial (**1**) showed potent cytotoxicity selective to human liver cancer cells (Hep-G2) in MTT assay and antiangiogenic activity in ex vivo model (a rat aortic ring sprouting) assay. Treatment with **1** exerted growth inhibition through G1 arrest and actively induced apoptosis of Hep-G2 cells. Its induction of apoptosis was accompanied by a reduction of Bcl-2 level. Moreover, its angiogenic effect was found to occur mainly by suppressing endothelial cell proliferation and tubule formation, suggesting the potential of **1** as a lead compound for cancer treatment.



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