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Phytochemistry 67 (2006) 1029–1033
PHYTOCHEMISTRY
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Clerodanes and other constituents of *Cleidion spiciflorum*

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Received 12 December 2005; received in revised form 22 February 2006
Available online 22 May 2006

Abstract

The polyoxygenated clerodane, spiciflorin (**1a**), was isolated from *Cleidion spiciflorum* (Burm. f.) Merr. (Euphorbiaceae). Other constituents were the glucoside of anol (**2**), columbin, scopoletin, 3,3′,4-*O*-trimethylellagic acid, acetylaleuritolic acid, common triterpenes and phenols.

Keywords: *Cleidion spiciflorum*; Euphorbiaceae; Clerodanes; Spiciflorin; Columbin; 3,3′,4-*O*-trimethylellagic acid; Acetylaleuritolic acid

1. Introduction

The tropical mainly Southeast Asian genus *Cleidion* (Euphorbiaceae) has been little investigated. A recent article (Li et al., 2005) reported the isolation of maslinic acid, α-amyrin, daucol, β-sitosterol and common benzenoids from *Cleidion brevipetiolatum* and an unpublished master's thesis (Menkham, 2001) described the isolation of tilianin, diosmetin 7-*O*-glucopyrananoside, 24*S*-methyl-5α-lanosta-9(11),25-dien-3β-ol, *trans*-phytol and anol glucopyranoside from the leaves of *Cleidion spiciflorum* (Burm. f.) Merr. The latter is the correct designation for the older binomial *Cleidion javanicum* Blume (Merrill, 1923). Its stems and roots are used locally to relieve fever and as a remedy for malaria while the bark serves as a cure for skin diseases and stomach ache. We now report the isolation and structure determination of a new polyoxygenated clerodane spiciflorin (**1a**) from the roots of *C. spiciflorum*. Other constituents of the roots were columbin,scopoletin, 3,3′,4-*O*-trimethylellagic acid, acetylaleuritolic acid, acetyloleanolic acid and its methyl ester, taraxerol, taraxerone, β-sitosterol, stigmasterol, 3,5-dimethoxy-4-hydroxybenzoic acid, vanillic acid, *trans*-4-propenylphenol (anol) glucoside (**2**) and 5-hydroxymethylfurfural.

2. Results and discussion

The ^{1}H and ^{13}C NMR spectra of spiciflorin (**1a**), $C_{20}H_{20}O_8$, and its monoacetate **1b**, $C_{22}H_{22}O_9$, are listed in Tables 1 and 2. They clearly show the presence of a 3-furoyl group attached to a methylene on C-9 as in many naturally occurring clerodanes (Merritt and Ley, 1992). Furthermore, in the spectra of **1a**, a group of three mutually coupled protons at δ 5.38, 3.82 and 3.73 attached to carbons at δ 72.84, 48.80 and 51.48

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doi:10.1016/j.phytochem.2006.03.024

Table 1
^{1}H and ^{13}C NMR spectroscopic data for spiciflorin (**1a**)[a]

Position	δH (*J*, Hz)	δC (DEPT)	COSY	NOESY	HMBC
1	5.38*brd* (3.0)	72.84*d*	H-2,3,10	H-2,6,10,20	C-3,5,9(w),18
2	3.82*dt* (3.9,3.3)	48.80*d*	H-1	H-1,3,10(w)	
3	3.73*d* (4.0)	51.48*d*	H-1	H-2, H-10(w),19	C-2,4,18
4		77.59*s*			
5		45.81*s*			
6	4.78*d* (5.4)	78.50*d*	H-7α,8	H-7α,β,19	C-4,10,17
7α	2.34*dd* (13.1, 5.4)	29.99*t*	H-7β	H-6,7β,8	C-5,6,8,9
7β	1.91*d* (13.1)		H-7α	H-6,7α,8,20	C-6,8,9
8	2.25*d* (5.4)	48.26*d*		H-7α,β,11b,20	C-6,7,9,10,17,20
9		37.04*s*			
10	2.45*brs*	49.68*d*	H-1	H-1,2,3(w),11a,19	C-1,2,3,5,6,9,19
11a	2.96*d* (17.9)	51.82*t*	H-11b,H-20	H-10,11b,4,15,19	C-9(s),10(s),12(s),20(s)
11b	3.25*d* (17.9)		H-11a	H-8,11a,14,15,20	C9(s),10(s),12(s),20(w)
12		193.65*s*			
13		128.05*s*			
14	6.75*dd* (1.8,0.8)	108.16*d*	H-15,16	H-11b(w)	C-13,15,16
15	8.11*brs*	147.87*d*	H-14,16	H-11a(w),11b	C-13,14,16
16	7.47*brs*	144.56*d*	H-14,15	H-14,H-15(w)	C-13,15
17		176.90*s*			
18		172.60*s*			
19	1.38*s*[b]	22.55*q*		H-3,6,10,11a(w)	C-4,5,6,10
20	1.44*s*[b]	25.72*q*		H-1,7β,8,11b	C-8,9,11

[a] Assignments based on HSQC, COSY, NOESY and HMBC experiments in $CDCl_3$ at 300, respectively, 75.47 MHz in ppm relative to TMS; coupling constants are in Hz. In the COSY and HMBC columns (s) or (w) refer to relatively strong or weak correlations.
[b] Intensity of three protons.

Table 2
^{1}H and ^{13}C NMR spectroscopic data for acetate (**1b**)[a]

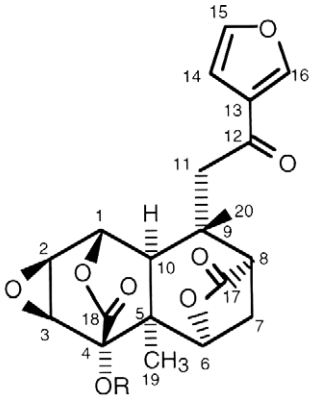
Position	δH (*J*, Hz)	δC (DEPT)	COSY	HMBC
1	5.39*brd* (2.9)	72.58*d*	H-2,3(w),10(w)	C-3,5,18
2	3.90*dd* (3.9,3.2)	50.44*d*	H-1(s),3	
3	3.78*dd* (3.9,0.9)	50.81*d*	H-1(w),H-2(s)	C-4,18
4		81.94*s*		
5		46.00		
6	4.85*d* (5.5)	78.28*d*	H-7α(s),8(w)	C-4(w),10,17
7α	2.36*ddd* (13.2,5.9,5.8)	29.90*t*	H-6(s),7β	C-5(s),6(s),8(s),9(s)
7β	2.04*d* (13.4)			C-8,9,17
8	2.26*d* (5.4)	48.03*d*	H-6(w)	C-6,7(w),9,10,17,20
9		36.96*s*		
10	2.46*brs*	49.69*d*	H-1	C-1,4(w),5(s),6,9(s),11(s),19,20
11a	2.95*d* (17.8)	51.81*t*	H-11b,20	C-9,12,20
11b	3.23*d* (17.8)		H-11a	C-9,10,12
12		193.45*s*		
13		128.09		
14	6.75*dd* (1.8,0.6)	108.21*d*	H-15,16	C-13,15,16
15	8.10*dd* (1.3,0.7)	147.78*d*	H-14,16	C-13,14,16
16	7.48*dd* (1.2,1.0)	144.62*d*	H-14,15	C-13,15
17		176.58*s*		
18		166.42*s*		
19	1.37*s*[b]	22.84*q*		C-4,5,6,10
20	1.46*s*[b]	25.79*q*	H-11	C-8,9,10,11
Ac	2.29*s*[b]	168.42*s*, 20.97*q*		CO(1Ac)

[a] Conditions identical with those specified in footnote of Table 1.
[b] Intensity of three protons.

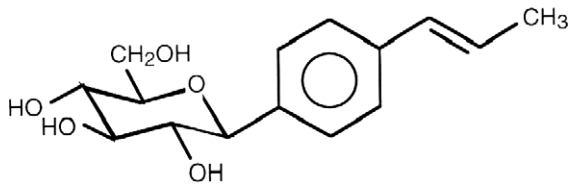
was characteristic of C-1, C-2 and C-3 of the 2β,3β-epoxy-4α-hydroxy-*cis*-δ-lactone system linking C-4 through C-18 (at δ 172.60) to C-1 of ring A in clerodanes like palmarin, chasmanthin and jateorin (Itakawa et al., 1987; Bhatt and Sabata, 1990), in which rings A and B are *cis*-fused and which like **1a** also carry an α-orientated hydroxyl group on C-4 as shown in the present instance by the NOESY cross-peak between H-10 and the methyl group on C-5 (Table 1) and the chemical shift of C-4 at δ 77.59 (Table 1).

Spiciflorin (**1a**), however, differs from clerodanes like palmarin, chasmanthin and jateorin in that due to hydroxylation at C-6, instead of at C-12, the second lactone function within the molecule involves lactone ring closure toward C-6 rather than C-12. The stereochemistry assigned to the second lactone ring in formula **1** is evidenced by the NOESY spectrum (Table 1). Thus, H-8 at δ 2.25, the β-orientated methyl group on C-9 (C-20) at δ 1.44 and H-7β at δ 1.91 are all in close proximity as shown in a Dreiding model and exhibit strong cross-peaks as they also did in our study of columbin. Hence H-8 is *cis* to the methyl group on C-9. In the model, H-6β is also close to the α-orientated methyl group on C-5, which accounts for the cross-peak between the two signals.

Spiciflorin (**1a**) underwent surprisingly facile acylation to form monoacetate **1b** whose spectroscopic data (Table 2) differed significantly from those of **1a** only in the chemical shifts of C-2 through C-4. COSY and HMBC data corroborated the conclusions reached earlier from study of **1a**.



1a R = H
b R = Ac



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3. Experimental

3.1. General

^{1}H and ^{13}C NMR spectra were recorded at ambient temperature on a Bruker AMC instrument operating at 300.13, respectively, 75.47 MHz or a Bruker DRX instrument operating at 500, respectively, 125 MHz. EI mass spectra were measured on a Hitachi Perkin–Elmer RMV-6M instrument, whereas HRMS mass spectra were obtained on a Kratos Concept II 2 sector mass spectrometer. Rotations were determined on a Polax-2 L instrument. Si gel for CC was Si gel 60 (0.2–0.5 mm) and Si 60 GF 254 Merck for preparative TLC.

3.2. Plant material

Cleidion spiciflorum (Burm. f.) Merr. was collected in Chiang Rai Province, Northern Thailand, in May 2004. The plant material was identified by Dr. Tawatchai Wongprasert; a voucher specimen (BFK 131868) was deposited in the Royal Forestry Department, Paholyothin Road, Bangkok, Thailand.

3.3. Extraction and isolation of constituents

Dried and powdered roots (6 kg) were percolated by hexane (3 × 20 L) at rt and filtered. Evaporation of the filtrate under reduced pressure provided the crude hexane extract (20 g). Percolation of the solid material extracted by hexane with EtOH (3 × 20 L) at room temperature followed by filtration and evaporation of the filtrate under reduced pressure provided the crude EtOH extract (203 g). The latter was dissolved in $CHCl_3$ (3 × 500 ml) with the aid of an ultrasound bath; filtration followed by evaporation of the $CHCl_3$ filtrate under reduced pressure gave the crude $CuCl_3$ extract (13 g).

The crude hexane extract (20 g) was applied to a Si gel column (250 g) and eluted with hexane and hexane–EtOAc, with 200 ml fractions being eluted as follows: Fractions 1–5 (hexane), 6–10 (hexane–EtOAc, 9:1), 11–16 (hexane–EtOAc, 4:1), 17–22 (hexane–EtOAc, 7:3), 23–30 (hexane–EtOAc, 3:2), 31–36 (hexane–EtOAc, 1:1), 37–42 (hexane–EtOAc, 2:3), 43–50 (hexane–EtOAc, 3:7), 51–60 (hexane–EtOAc, 1:4), 61–70 (hexane–EtOAc, 1:9). Fractions 6–10 (1.3 g) were combined and subjected to chromatography over Si gel (50 g) using ten 125 ml subfractions of hexane–EtOHAc (9:1). Subfraction 3 (380 mg) on recrystallization from hexane furnished taraxerone (200 mg) identified by MS, ^{1}H and ^{13}C NMR spectrometry (Kiem et al., 2004; Mahato and Kundu, 1994). Recrystallization of subfraction 6 afforded methyl acetyloleanate (78 mg) identified by MS, ^{1}H and ^{13}C NMR spectrometry (Chakravarty et al., 1991; Mahato and Kundu, 1994). Fractions 11–16 (1.4 g) of the original chromatogram were combined and applied to a Si gel column (50 g), eight 125 ml subfractions of hexane–EtOAc (4:1) being collected. Recrystallization of subfraction 4 (120 mg) gave 64 mg of taraxerol, identified by MS, ^{1}H and ^{13}C NMR spectrometry (Corbett and Cumming, 1972; Mahato and Kundu, 1994). Recrystallization of subfraction 5 (436 mg) from hexane gave 327 mg of the acetate of oleanolic acid identified by MS, ^{1}H and ^{13}C NMR spectrometry (Chakravarty et al., 1991; Mahato and Kundu, 1994). Fractions 17–22 (900 mg) of the original chromatogram were combined and recrystallized from hexane to give 120 mg of a mixture

of β-sitosterol and stigmasterol (Akihisa et al., 1992; Klass et al., 1992). Fractions 22–30 (2 g) of the original chromatogram were combined and subjected to chromatography over Si gel (60 g) using four 125 ml subfractions of hexane EtOAc (4:1) to give from subfraction 2 (103 mg) more of the β-sitosterol-stigmasterol mixture. Rechromatography of fractions 31–36 (2.4 g) over Si gel (100 g) using four 125 ml subfractions of hexane–EtOAc (3:1) gave from subfraction 3 (700 mg) 236 mg of acetylaleuritolic acid identified by MS, ^{1}H and ^{13}C NMR spectrometry (McLean et al., 1987).

The crude $CHCl_3$ extract (13 g) was applied to a Si gel column (120 g) and eluted with petrol–$CHCl_3$, $CHCl_3$–acetone and $CHCl_3$–MeOH, with 200 ml fractions being collected as follows: fractions 1–9 (petrol–$CHCl_3$, 1:1), 10–29 (petrol–$CHCl_3$, 3:7), 30–41 (petrol–$CHCl_3$, 1:9), 42–58 ($CHCl_3$–acetone, 9:1), 59–64 ($CHCl_3$–acetone, 7:3), 65–68 ($CHCl_3$–MeOH, 9:1). Fractions 9 and 10 (623 mg) were combined; removal of solvent gave a yellow precipitate suspended in a yellowish mother liquor. Recrystallization of the ppt from $CHCl_3$–MeOH gave 220 mg of 3,3′,4-*O*-trimethylellagic acid (Khae et al., 1990) identified by MS, ^{1}H and ^{13}C NMR spectrometry. TLC of the mother liquor (Si gel, $CHCl_3$–acetone–HCO_2H, 9:1:0.1) gave scopoletin (52 mg, Murray, 2002) columbin (12 mg), spiciflorin (**1a**, 12 mg) and 20 mg of a mixture of 3-methoxy-4-hydroxybenzoic acid (Sukushima et al., 1995) and 3,5-dimethoxy-4-hydroxybenzoic acid. Although the small amount of columbin obtained from the chromatogram could not be induced to crystallize its ^{1}H and ^{13}C NMR spectra in $CDCl_3$ were identical with those reported in the literature (Waterman et al., 1985; Hungerford et al., 1998); HMBC, COSY and NOESY experiments supported the structure assignment. The HRMS corresponded to the loss of H_2O; FAB HRMS *m/z* 359.14942, calcd for M-H2O + H^+ 359.14946. Fractions 11 and 12 were combined; after removal of a precipitate of 3,3′,4-*O*-trimethylellagic acid (122 mg) purification of the mother liquor by TLC (Si gel, $CHCl_3$–acetone–HCO_2H, 9:1:01) afforded **1a** (36 mg), hydroxymethylfurfural (23 mg, Jabbar et al., 1995), and a mixture of 3-methoxy-4-hydroxy-benzoic acid and 3,5-dimethoxy-4-hydroxybenzoic acid (93 mg). Fractions 62–64 (86 mg) were combined and purified by TLC (Si gel, $CHCl_3$–MeOH–HCO_2H, 85:15:01) to give the glucoside of anol (**2**) (37 mg) as a colorless gum (Menkham, 2001). Since the ^{1}H and ^{13}C NMR spectroscopic data of this non-crystalline substance are not available in the open literature they are listed below.

3.3.1. Spiciflorin (*1a*)

Colorless gum, $[\alpha]_D^{20}$ −55 ($CHCl_3$, C = 0.1 g/100 ml); for ^{1}H and ^{13}C NMR spectra see Table 1, +FAB M + H *m/z* 389 ($C_{20}H_{21}O_8$), FAB HRMS *m/z* 389.12344, calcd for $C_{20}H_{20}O_8 + H^+$ 389.12344. Acetylation of 15 mg of **1a** (Ac_2O-pyridine) followed by the usual work-up afforded **1b** (11 mg) as a gum for ^{1}H and ^{13}C NMR spectra see Table 2, FAB HRMS 431.13411 $(M + H)^+$, calcd for $C_{22}H_{22}O_9 + H^+$ 431.13421.

3.3.2. Anol glucoside (*2*)

^{1}H and ^{13}C NMR spectra of *trans*-4-propenylphenol glucoside (anol glucoside) (**2**), ($CDCl_3$). H-2,6 *δ* 6.95 *d* (8.7), H-3, 5 7.29 *d* (8.7), H-α *δ* 6.34 *brd* (15.8), H-β 6.15 *dd* (15.8, 6.6), H-γ 1.81 *brd* (6.6, 3 p), H-1′ 4.28 *d* (7.6, 3p), H-2′ 3.2 *m*, H-3′ 3.25 *m*, H-4′ 3.15 *m*, H-5′ 3.3 *m*, H-6′ 3.7 *m*, 3.47 *m*, 2′-OH 5.32 *d* (4.9), 3′-OH 5.11 *d* (3.4), 4′-OH 5.04 *d* (4.9), 6′-OH 4.59+ (5.7); C-1 *δ* 156.47, C-2, δ 116.31, C-3,5 126.73, C-4 131.30, C-α 130.27, C-β 123.56, C-1′ 100.41, C-2′ 73.27, C-3′ 76.64, C-4′ 69.74, C-5′ 55.07, C-6′ 60.73.

Acknowledgments

We thank Fundação para a Ciência e Tecnologia of Portugal (I&D No. 226/94) POCTI (QCAIII), CIIMAR (Programa Plurianual) and FEDER for support. The Faculty of Science, Burapha University, supported the work in Thailand.

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