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Dioxadispiroketal Compounds and a Potential Acyclic Precursor from *Amomum aculeatum*

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Abstract

Four new compounds having an unusual 1,7-dioxadispiro[5.1.5.2]-12-ene-11-one tricyclic ring system (**1–4**), their potential precursor, 5R-hydroxy-1-(4-hydroxyl-phenyl)-eicosan-3-one (**5**), and two known compounds, aculeatins A (**6**) and B (**7**), have been isolated from *Amomum aculeatum*. All compounds were characterized by spectroscopic methods and the configurations were established by 2D NOE correlations. Compounds **1–4**, **6** and **7** showed cytotoxic activity against several human cancer cell lines.

Keywords

Amomum aculeatum; Zingiberaceae; Aculeatols A - D; Dioxadispiroketal; 5 R-Hydroxy-1-(4-hydroxyl-phenyl)-eicosan-3-one; NMR data

Amomum aculeatum Roxb. (Zingiberaceae) is a herbaceous plant distributed in Malaysia, Indonesia and Papua New Guinea. It is used as a folk medicine in Papua to treat fever and malaria.¹ Four novel compounds, aculeatins A–D, based on the previously unreported 1,7-dioxadispiro[5.1.5.2]pentadecane system, have been isolated from *A. aculeatum*.^{2–4} The unique dispiro skeleton of the aculeatins has prompted significant interest among the synthetic organic community, and aculeatins A, B, and D have been synthesized thus far.^{5–8} The absolute configurations of aculeatins A, B and D were finally established by enantioselective synthesis in combination with NOE measurements of the isomers obtained.^{7,8} Based on this synthetic work, the correct configurations of aculeatins A and B are *abs*-[2R,4R,6R] and *abs*-[2R,4R,6S], respectively.^{7,8} Aculeatins A–D were found to be cytotoxic against the KB cell line, antiprotozoal for *Plasmodium* and *Trypanosoma* species, and antibacterial for *Bacillus cereus* and *Escherichia coli*.^{2,3}

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As a part of a collaborative search for novel anticancer agents from plant origin,⁹ the hexane and chloroform extracts of the leaves and rachis of *A. aculeatum* collected in Indonesia¹⁰ were found to exhibit significant cytotoxic activity against several cancer cell lines. Bioassay-guided fractionation led to the isolation of four new aculeatin derivatives, aculeatols A – D (**1** – **4**), a new gingerol derivative (**5**), and the known compounds aculeatins A (**6**) and B (**7**). All of the isolated compounds, except **5**, were cytotoxic. The structural elucidation of the new compounds and the biological data of **1**– **7** are presented here.

Aculeatol A (**1**) was obtained as a white amorphous powder with a molecular formula of $C_{24}H_{40}O_5$ (HRESIMS m/z 431.27548 $[M+Na]^+$).¹¹ Comparison of the 1H and ^{13}C NMR spectra (Table 1) with those of aculeatin A (**6**) suggested that both compounds have a similar structure, except for the presence of an extra hydroxyl group in the cyclohexenone moiety of **1**.² HMBC correlations from the methylenes at δ_H 2.71 (H-10a) and 2.65 (H-10b) to δ_C 197.1 (C-11), from δ_H 5.98 (H-12) to δ_C 43.0 (C-10), and from δ_H 6.68 (H-13), 2.17 (H-14a) and 2.13 (H-14b) to δ_C 72.1 (C-9) confirmed the position of the hydroxyl group at C-9 (Fig. 1). The alkyl side chain of aculeatol A (**1**) was found to consist of 11 carbons, two methylenes less than aculeatin A (**6**), as determined from the molecular formula of aculeatol A.

The configuration of aculeatol A (**1**) was determined from analysis of the splitting patterns and coupling constants of the 1H NMR signals, together with a 2D NOESY experiment. By analogy with aculeatin A (**6**), the downfield shift of H-2 (δ_H 4.04) is characteristic of the 1,3-diaxial relation with the anomeric oxygen atom,^{7,8} thus establishing an *R* configuration at the chiral carbon 6.¹² Both the configurations at C-2 and C-4 were determined to be *R*, based on the NOE cross peak observed between H-2 and OH-4. The coupling constant of H-4 (*p*, $J = 3.0$ Hz) is in agreement with the equatorial position of this proton in a six-membered ring that adopts a chair conformation. The configuration at C-8 was assigned as *R*, based on the NOE correlations from H-13 to H-17a, H-17b, H-14a and H-2 (Fig. 1). In the 2D NOESY spectrum, cross-peaks were also observed between H-9/H-14b, H-9/H-15b, and H-10b/H-14b. Examination of Dreiding models showed that these correlations were possible only if the cyclohexenone ring occurs in a half-chair conformation, with H-9 in the equatorial position and H-10b in the α -axial position. Moreover, the coupling constant values of H-9 (dd, $J = 6.8, 3.8$ Hz) were also consistent with the equatorial position of H-9 and an *S* configuration at C-9. From the above data, aculeatol A (**1**) was assigned as (2*R**,4*R**,6*R**,8*R**,9*S**)-4,9-dihydroxy-2-undecyl-1,7-dioxadispiro[5.1.5.2]pentadec-12-en-11-one.

The new compound **2**, aculeatol B, was isolated as a yellow oil and has the same molecular formula as aculeatol A (**1**) (HRESIMS m/z 431.27738 $[M+Na]^+$).¹³ The 1H and ^{13}C NMR spectra (Table 1) of aculeatols A and B were very similar, and HMBC NMR spectroscopic correlations confirmed the C-9 position of the hydroxyl group in the cyclohexenone ring, suggesting that compounds **1** and **2** are stereoisomers. Comparison of the 1H NMR signals $[(\delta_H$ 4.10, m, H-2) and (4.16, p, $J = 2.7$ Hz, H-4)] and 2D NOESY data of **2** with those of aculeatol A (**1**) indicated that the configurations at C-2, C-4 and C-6 are identical. In the 2D NOESY spectrum, cross peaks were observed between H-13/H-5b and H-13/H-15b, indicating an *S* configuration at the C-8 position (Fig. 2). NOE correlations between H-9/H-14a and H-10b/H-14a, together with the coupling constant values of H-9 (dd, $J = 6.5, 4.4$ Hz) established an *R* configuration at C-9. As in aculeatol A (**1**), the cyclohexenone ring adopts a half-chair conformation. Thus, aculeatol B (**2**) was assigned as (2*R**,4*R**,6*R**,8*S**,9*R**)-4,9-dihydroxy-2-undecyl-1,7-dioxadispiro[5.1.5.2]pentadec-12-en-11-one.

The HRESIMS of aculeatol C (**3**), a yellow oil, supported a molecular formula of $C_{26}H_{44}O_5$ (m/z 459.30827 $[M+Na]^+$).¹⁴ The 1D and 2D NMR spectra of aculeatol C were identical with those of aculeatol B (**2**), indicating that both compounds have the same configuration at all chiral centers. The only difference between these two compounds are the length of the alkyl side chain, as shown by the molecular formula of aculeatol C, which has two more methylenes in the alkyl chain than aculeatol B. Accordingly, aculeatol C (**3**) was assigned as (2*R**,4*R**,6*R**,8*S**,9*R**)-4,9-dihydroxy-2-tridecyl-1,7-dioxadispiro[5.1.5.2]pentadec-12-en-11-one.

Another stereoisomer of aculeatols A (**1**) and B (**2**), aculeatol D (**4**), was isolated as a yellow oil with a molecular formula of $C_{24}H_{40}O_5$ (HRESIMS m/z 431.27548 $[M+Na]^+$).¹⁵ The 1H NMR spectrum of aculeatol D (Table 1) was similar to those of aculeatols A and B, except for the more upfield signal of the methine proton H-2 (δ_H 3.91), indicating an *S* configuration at the stereocenter 6.^{7,8} Moreover, an NOE correlation between H-2 and a methylene proton at C-15 (δ_H 2.64) was observed, confirming the *R* and *S* configurations at C-2 and C-6, respectively (Fig. 2). By comparing the ^{13}C NMR spectra of aculeatols A–D and aculeatins A and B² of both 6*R*- and 6*S*-configurations, a pattern useful in determining the configuration at C-6 was observed. For the 6*R*-configuration, the C-2 and C-15 signals were at δ_C 65–66 and 39, respectively, while for the 6*S*-configuration, the signals for both carbons were shifted to δ_C 69 (C-2) and 35 (C-15) (Table 1). The small coupling constant of the methine H-4 (4.37, p, $J = 3.0$ Hz) confirmed the equatorial position of this proton and established the *R* configuration at C-4. The configuration at the chiral carbon 8 was determined as *R*, based on NOE correlations between H-13/H-15b and H-13/H-14b (Fig. 2). Analysis of the coupling constant values of H-9 (dd, $J = 8.8, 5.1$ Hz) and observed NOE correlations between H-9/H-14a and OH-9/H-14a revealed that proton 9 is in the α -axial position of the hexenone ring, thus establishing an *S* configuration at the C-9 position. Based on these data, aculeatol D (**4**) was assigned as (2*R**,4*R**,6*S**,8*R**,9*S**)-4,9-dihydroxy-2-undecyl-1,7-dioxadispiro[5.1.5.2]pentadec-12-en-11-one.

Compound **5** was isolated as an amorphous white powder from the rachis plant part, with a molecular formula of $C_{26}H_{44}O_3$ as determined by HRESIMS (m/z 427.31876 $[M+Na]^+$).¹⁶ The 1H NMR spectrum showed the presence of an AA' BB' pattern characteristics of a *p*-disubstituted benzene ring [δ_H 7.04 (2H, d, $J = 8.3$ Hz, H-2',6') and 6.75 (2H, d, $J = 8.3$ Hz, H-3',5')] and a methyl-terminated polymethylene chain [δ_H 1.10–1.38 (m) and 0.88 (3H, t, $J = 6.3$ Hz, H₃-20)]. In addition, the ^{13}C NMR spectrum also showed the presence of a nonconjugated ketone (δ_C 211.5), an oxygenated aromatic carbon (153.9), and an oxygenated methine (67.7). HMQC and COSY correlations established the connection of C-1–C-2 and C-4–C-6. The benzene ring and the ketone group were placed next to C-1 and C-2, respectively, based on the HMBC correlation from H₂-1 to δ_C 132.8 (C-1') and 129.4 (C-2',6'), and from H₂-2 to δ_C 211.5 (C-3). The absolute configuration at C-5 was determined as *R* by a convenient

Mosher ester method carried out in an NMR tube.^{17,18} Compound **5** resembles structurally gingerol-type compounds previously isolated from the rhizomes of ginger (*Zingiber officinale* Roscoe, Zingiberaceae),¹⁹ except for the lack of a *m*-methoxy group in the benzene ring and a longer alkyl chain, and was assigned as 5*R*-hydroxy-1-(4-hydroxyl-phenyl)-eicosan-3-one.

A plausible synthetic pathway for the formation of aculeatin-type compounds involving an intermediate, 5,7-dihydroxy-1-(4-hydroxyl-phenyl)-eicosan-3-one, has been proposed,^{5,6} and a biomimetic synthesis employing oxidative cyclization cascade reaction to generate aculeatin D has been conducted.⁶ The new compound **5** resembles the proposed intermediate, except for the lack of one hydroxyl group. Moreover, compound **5** has the correct configuration at OH-5 to generate aculeatins A (**6**) and B (**7**). Hence, it is possible that compound **5** is a precursor of the aculeatin-type compounds.

The compounds obtained in the present investigation were evaluated for their cytotoxicity activity against several human cancer cell lines *in vitro* (Table 2).²⁰ Among the new isolates, aculeatols A–D (**1–4**) were found to be significantly active, while compound **5** was inactive.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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- Yellow oil; [α]_D²⁰ -33 (c 1.6, CHCl₃); UV (MeOH) $\lambda_{\max}$ (log ϵ) 220 (4.54) nm; IR (film) $\nu_{\max}$ 3401, 2925, 2854, 1685, 1049 cm⁻¹; ¹H and ¹³C NMR data, see Table 1; EIMS *m/z* 208 (18), 165 (100), 123 (36), 107 (67); HRESIMS *m/z* 431.27548 [M + Na]⁺ (calcd for C₂₄H₄₀O₅Na, 431.27679).
- This was confirmed by the absence of any NOE correlations between the methine proton H-2 and one methylene proton at C-15. This enhancement was observed for aculeatin B (**7**), which has the opposite configuration at C-6.

13. Yellow oil; $[\alpha]_D^{20} +52$ (c 0.4, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 221 (4.59) nm; IR (film) ν_{max} 3425, 2922, 2851, 1669, 1098 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; EIMS m/z 390 (63), 180 (41), 165 (83), 123 (76), 107 (89), 95 (100); HRESIMS m/z 431.27738 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{24}\text{H}_{40}\text{O}_5\text{Na}$, 431.27679).
14. Yellow oil; $[\alpha]_D^{20} +45$ (c 0.2, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 220 (4.51) nm; IR (film) ν_{max} 3423, 2924, 2853, 1684, 1106 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; EIMS m/z 418 (40), 180 (32), 165 (44), 123 (60), 107 (83), 95 (100); HRESIMS m/z 459.30827 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{26}\text{H}_{44}\text{O}_5\text{Na}$, 459.30809).
15. Yellow oil; $[\alpha]_D^{20} +11$ (c 3.2, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 221 (4.50) nm; IR (film) ν_{max} 3455, 2922, 2851, 1694, 1065 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; EIMS m/z 390 (63), 180 (40), 165 (84), 123 (76), 107 (79), 95 (100); HRESIMS m/z 431.27579 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{24}\text{H}_{40}\text{O}_5\text{Na}$, 431.27679).
16. White amorphous powder; $[\alpha]_D^{20} -4.5$ (c 0.3, CHCl_3); UV (MeOH) λ_{max} (log ϵ) 225 (3.27), 270 (2.68) nm; IR (film) ν_{max} 3422, 2917, 2849, 1697, 1521, 1472, 914 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz, TMS) δ 7.04 (2H, d, $J = 8.3$ Hz, H-2', 6'), 6.75 (2H, d, $J = 8.3$ Hz, H-3', 5'), 4.82 (1H, br s, OH-4'), 4.02 (1H, m, H-5), 2.83 (2H, t, $J = 7.3$ Hz, H₂-1), 2.72 (2H, t, $J = 7.3$ Hz, H₂-2), 2.57 (1H, dd, $J = 17.4, 2.8$ Hz, H-4a), 2.48 (1H, dd, $J = 17.4, 8.9$ Hz, H-4b), 1.48 (1H, m, H-6a), 1.10-1.38 (H-6b, H₂-7 – H₂-19), 0.88 (3H, t, $J = 6.3$ Hz, H₃-20); ^{13}C NMR (CDCl_3 , 100 MHz, TMS) δ 211.5 (C-3), 153.9 (C-4', s), 132.8 (C-1', s), 129.4 (2C, C-2', 6', d), 115.3 (2C, C-3', 5', d), 67.7 (C-5, d), 49.3 (C-4, t), 45.3 (C-2, t), 36.4 (C-6, t), 31.9 (C-18, t), 29.7-29.4 (C-8–C-17, t), 28.7 (C-1, t), 25.5 (C-7, t), 22.7 (C-19, t), 14.1 (C-20, q); EIMS m/z 295 (89), 175 (43), 164 (29), 107 (100); HRESIMS m/z 427.31876 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{26}\text{H}_{44}\text{O}_3\text{Na}$, 427.318263).
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Figure 1.
Key HMBC and NOESY correlations for **1**

Figure 2.
Key NOESY correlations for **2** and **4**

Table 1
 ^1H and ^{13}C NMR Data for Compounds **1** – **4** Acquired in CDCl_3^a

	aculeatol A (1)		aculeatols B (2) and C (3)		aculeatol D (4)	
	δ_{C}	δ_{H} (J Hz)	δ_{C}	δ_{H} (J Hz)	δ_{C}	δ_{H} (J Hz)
2	65.0	4.04 m	66.5	4.10 m	69.7	3.91 m
3	37.7	1.77 br d (13.0)	37.5	1.82 br d (13.5)	37.8	1.62 m
		1.44 m		1.43 m		1.52 m
4	64.8	4.14 p (3.0)	64.6	4.16 p (2.7)	65.3	4.37 p (3.0)
5	38.3	1.97 dd (14.3, 3.3)	39.6	2.02 dd (11.8, 2.7)	40.4	2.03 dd (13.9, 3.0)
		1.93 m (14.3)		1.99 dd (11.8, 2.7)		1.86 m
6	108.2		108.5		106.7	
8	83.9		85.4		82.0	
9	72.1	3.98 dd (6.8, 3.8)	72.8	4.07 dd (6.5, 4.4)	69.7	3.93 dd (8.8, 5.1)
10	43.0	2.71 dd (16.7, 6.8)	42.9	2.72 dd (16.7, 6.5)	44.2	2.76 dd (16.2, 8.8)
		2.65 dd (16.7, 3.8)		2.67 dd (16.7, 4.4)		2.71 dd (16.2, 5.1)
11	197.1		196.7		198.6	
12	128.6	5.98 d (10.1)	129.2	5.98 d (10.1)	129.6	5.98 d (10.1)
13	151.3	6.68 d (10.1)	148.6	6.64 d (10.1)	148.1	6.61 d (10.1)
14	32.0	2.17 m (8.4, 7.8)	32.3	2.45 ddd (17.8, 11.8, 9.4)	31.8	2.54 dd (11.6, 7.3)
		2.13 m		1.97 ddd (17.8, 8.5, 3.2)		1.87 m
15	39.0	2.15 m	39.3	2.25 ddd (12.8, 11.8, 8.5)	34.6	2.64 dd (11.4, 7.3)
		1.91 m (11.7)		2.05 dd (12.9, 9.5)		1.88 m
16	35.8	1.48 m	35.8	1.50 m	35.7	1.57 m
		1.43 m		1.44 m		1.44 m
17	25.6	1.44 m	25.4	1.44 m	25.5	1.44 m
		1.29 m		1.29 m		1.30 m
18–21 ^b	29.7–29.3	1.33–1.23 m	29.7–29.3	1.33–1.23 m	29.7–29.3	1.33–1.23 m
22 ^c	31.9	1.33–1.23 m	31.9	1.33–1.23 m	31.9	1.33–1.23 m
23 ^d	22.7	1.28 m	22.7	1.28 m	22.7	1.28 m
24 ^e	14.1	0.88 t (6.8)	14.1	0.88 t (6.8)	14.1	0.88 t (6.8)
OH-4		3.33 br s				4.00 br s
OH-9		3.33 br s				3.98 br s

^a ^1H and ^{13}C NMR spectra were acquired at 600 and 100 MHz, respectively, TMS was used as internal standard

^bPositions 18–23 for compound **3**.

^cPosition 24 for compound **3**.

^dPosition 25 for compound **3**.

^ePosition 26 for compound **3**.

Table 2Cytotoxic Activity of Compounds **1** – **7**^a

compound	cell line ^b		
	Lu1	LNCaP	MCF-7
1	1.5	0.6	1.1
2	0.6	0.5	1.1
3	1.2	0.5	0.7
4	2.2	0.9	0.7
6	0.4	0.2	0.1
7	1.3	0.5	0.8

^a Compound **5** was inactive (ED₅₀ >5 μ g/mL) against the tested cell lines.

^b Results are expressed as ED₅₀ values μ g/mL). Key to cell lines used: Lu1 = human lung cancer; LNCaP = hormone-dependent human prostate cancer; MCF-7 = human breast cancer.