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DAPHNANE DITERPENOIDS FROM THE BARK OF *WIKSTROEMIA RETUSA*

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Key Word Index—*Wikstroemia retusa*; Thymelaeaceae; daphnane diterpenoid; macrocyclic daphnane diterpenoid.

Abstract—On examination of the less polar fraction from the bark of *Wikstroemia retusa*, nine daphnane-type diterpenoids, including four known ones, were isolated and the structures established. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Recently, we described the isolation and structure determination of four termitecidal daphnane-type diterpenoids [1, 2] from the bark of *Wikstroemia retusa* A. Gray, which is indigenous to the Ryukyu Islands and has been utilized locally as a source of paper-production from earlier times. In order to determine the pattern of diterpenoid constituents from this plant in detail and to compare it with those of other related plants, we analysed the diterpenoids in *W. retusa* by a standard fractionation procedure but without the biological activity test. This paper deals with the isolation and characterization of nine compounds including four reported in the preceding paper [1].

RESULTS AND DISCUSSION

Extraction and isolation of the diterpenoids were carried out by similar procedures to those described in the preceding paper. From the plant materials collected at Ishigaki Island, nine diterpenoids (**1**–**9**) were obtained after normal-phase and reversed-phase column chromatography and preparative HPLC. Among these compounds, **1**–**4** correspond to compounds **1**–**4** reported in the preceding paper [1], i.e. huratoxin (**1**) [1–4], 12β-acetoxyhuratoxin (wikstroelide A, **2**), the compound having a pentadecadienoic acid orthoester (12β-acetoxywikstrotoxin A) (wikstroelide B; **3**), and pimelea factor P_2 [5] (**4**) [1].

Based on FAB mass spectroscopy, **5** was assigned the molecular formula $C_{51}H_{76}O_{11}$. The ^1H and ^{13}C NMR signals showed a similar pattern as those of **2**, including signals for one acetyl group at C-12 and a tetradecadienoic acid in the *ortho*-ester form. However, the methylene proton signals of a primary carbinol at C-20 were shifted toward lowerfield in comparison with those of **2**, suggesting the presence of an acyl group at C-20. The fragment peak at *m/z* 239 in the negative FAB mass spectrum, as well as a comparison of the molecular formula of **5** with that of **2** ($C_{36}H_{50}O_{10}$) suggested that the acyl group was a pentadecenoic acid. The signals due to olefin protons at δ 5.36 and 5.40 were consistent with the presence of an unsaturated fatty acid. The olefinic linkage was assigned to be *trans*, based on the signals of neighbouring methylene carbons in the lower field at δ 31.9 and 32.6 [6]. In order to establish the location of the olefinic linkage, the mass spectrum of a dimethyl disulphide derivative of the pentadecenoic acid was examined [7, 8]. On deacylation with NaOMe/MeOH of **5**, **2** and 12-deacetyl-**2** were obtained along with a methyl ester of the pentadecenoic acid (**5a**) which was cleaved from C-20. When the dimethyl disulphide derivative of **5a** was subjected to EI mass spectroscopy, fragment peaks were observed at *m/z* 187 $[C_{11}H_{23}S]^+$ and 161 $[C_7H_{13}O_2S]^+$ as a result of cleavage between two olefinic carbons, and **5a** was assigned to be a methyl ester of *trans*-5-pentadecenoic acid. Finally, the location of the acid was confirmed by the HMBC response between H-20 (δ 3.89, 4.81) and the carboxyl carbon (δ 173.3) in the acid (Tables 1 and 2). **5** was named wikstroelide C.

Compound **6** (wikstroelide D) was considered to have the molecular formula $C_{52}H_{80}O_{11}$, i.e. 16 amu higher than **5**, based on a quasi-molecular peak at *m/z* 903.5601. In terms of the ^1H and ^{13}C NMR spectra, **6** seemed to have a similar structure to **2** or **5** with a

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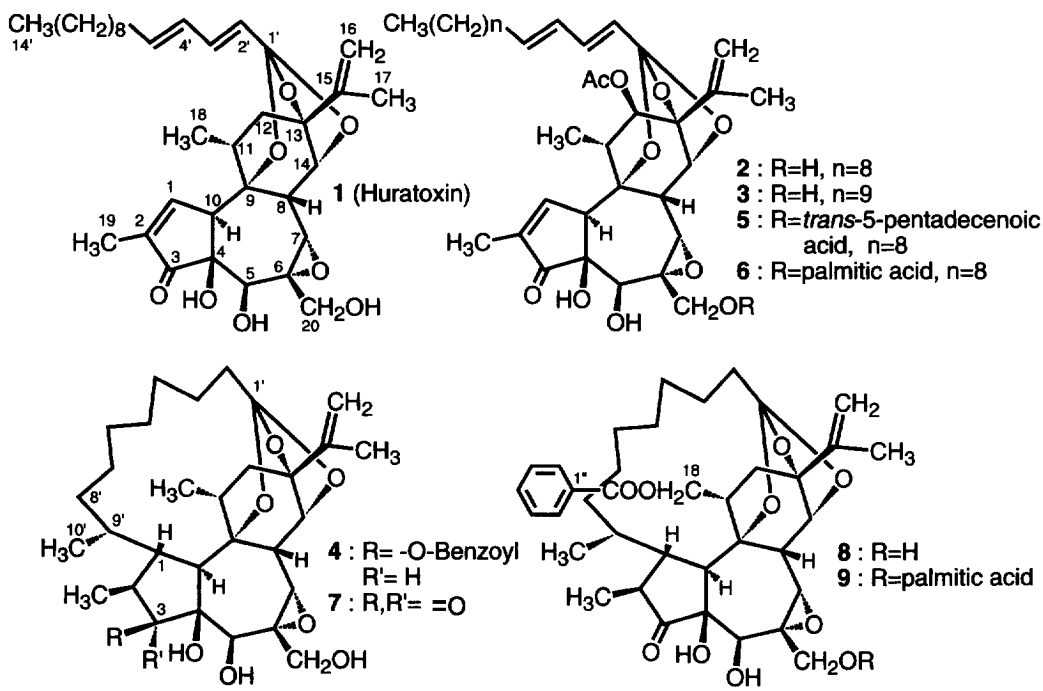
tetradecadienoic acid moiety in the orthoester and one acetyl group at C-12. The fragment peak at *m/z* 255 in the negative FAB mass spectroscopy suggested the presence of palmitic acid at C-20, instead of the pentadecenoic acid in **5**. The linkage of palmitic acid at C-20 was confirmed by the cross peak between H-20 and the carbonyl carbon of palmitic acid in the HMBC spectrum. The structure of **6** was thus characterized as the 20-palmitate of **2**.

Compound **7** (wikstroelide E) was the dominant diterpenoid in the fresh bark. Since, as in the ^{1}H NMR spectrum of **4**, the 10′-methyl group was observed as a doublet and H-1 as a multiplet signal at δ 2.10, **7** was considered to retain the carbon bridge between C-1 and the orthoester function at C-9, 13, 14; these elements forming a macrocyclic ring as in **4**. However, neither the proton signal due to H-3 nor those due to a benzoyl group in **4** were present in **7**, but a carbonyl carbon signal appeared at δ 219.8, suggesting that ring A of **7** was a saturated 3-oxo-structure. Based on the cross peaks between H-19/C-1 and C-3, H-1/C-9 and C-10′, and H-10′/C-1 and C-8′ in the HMBC spectrum, the structure of ring A was confirmed.

The NMR data of **8** ($C_{37}H_{48}O_{10}$) showed the presence of the same functions as in ring A of **7**, a carbonyl carbon (δ 219.8) and a C-19 methyl group (δ_H, 1.13 (J = 6 Hz); δ_C, 13.7). The macrocyclic structure was also suggested by the doublet signal of the 10′-methyl group (δ_H, 0.91 (J = 6 Hz); δ_C, 12.5), although H-1 was shifted towards lowerfield in comparison with that of **7**. The presence of a benzoyl group was suggested based on the ^{1}H signals at δ 7.43, 7.55, and 8.04, and the ^{13}C signals at δ 128.3–132.9. A notable difference between **8** and **7** was the presence in **8** of a primary hydroxyl group represented by the ^{1}H signals at δ 4.69 and 4.89 and the ^{13}C signal at δ 69.3(t), while a 3H doublet signal due to H-18 in **7** was no longer observed in **8**. Other primary carbinol proton signals at δ 3.78 and 3.87 were assignable to H-20, as were those of **7**. The location of the benzoyl group was determined to be at C-18, based on the HMBC coupling between H-18 and the carboxyl carbon of the benzoyl group. The structure of **8** was thus characterized as 18-benzoyloxy-7, and **8** was named wikstroelide F.

The FAB mass spectrum of **9** (wikstroelide G) showed a quasi molecular ion peak at *m/z* 913.5441, suggesting the molecular formula $C_{53}H_{78}O_{11}$. In the ^{1}H and ^{13}C NMR spectra, the essential signals due to the structure of **8** were observed. Additionally, the presence of a fatty acid was suggested. Based on the molecular formula, the acid was considered to be palmitic acid. Since this proton signals due to two primary carbinols were shifted to lowerfield and the C-20 carbinol proton signals and the carboxyl carbon of palmitic acid showed correlation with each other in the HMBC spectrum, the linkage of palmitic acid was assigned to be at C-20.



In this paper, daphnane-type diterpenoids including three macrocyclic forms and two tetradecadienoic acid orthoesters were identified from the bark in addition to the four described previously. Diterpenoids with daphnane skeleton are known to have many biological activities, e.g. antileukaemic [3, 4], piscicidal [9] irritant [10] or antifertility [11].

EXPERIMENTAL

General. ^{1}H NMR: 400 or 500 MHz, and ^{13}C NMR: 100 MHz, $CDCl_3$, TMS as int. standard. UV: MeOH.

Table 1. ^{1}H NMR spectral data for diterpenoids **5**–**8** and **9** [δ ppm in $CDCl_3$ (500 MHz)]

H	5	6	7	8	9
1	7.55 *br s*	7.55 *d*(1)	2.10 *m*	2.40 *m*	2.39 *m*
2			2.35 *m*	2.38 *m*	2.38 *m*
5	4.25 *d*(2)	4.26 *br s*	4.07 *s*	4.09 *br s*	4.03 *br s*
7	3.42 *s*	3.42 *s*	3.41 *s*	3.44 *s*	3.35 *s*
8	3.50 *d*(2)	3.50 *d*(2)	2.86 *d*(2)	2.92 *d*(2)	2.94 *d*(2)
10	3.86 *d*(2)	3.86 *d*(2)	3.31 *d*(11)	3.40 *d*(11)	3.41 *d*(11)
11	2.37 *q*(7)	2.40 *q*(7)	2.63 *qui*(7)	2.83 *t*(8)	2.84 *t*(7)
12	4.98 *s*	4.99 *s*	1.67 *d*(14)	2.27 *d*(14)	2.27 *d*(15)
			2.18 *dd*(14,7)	2.16 *dd*(14,8)	2.15 *dd*(15,7)
14	4.71 *d*(2)	4.71 *d*(2)	4.33 *d*(2)	4.39 *d*(2)	4.37 *d*(2)
16	4.96 *br s*	4.96 *br s*	4.88 *br s*	4.92 *br s*	4.92 *br s*
	5.00 *br s*	5.01 *br s*	4.99 *br s*	5.04 *br s*	5.04 *br s*
17	1.83 *d*(1)	1.83 *d*(1)	1.77 *br s*	1.80 *br s*	1.80 *br s*
18	1.29 *d*(7)	1.29 *d*(7)	1.38 *d*(7)	4.69 *dd*(12,8)	4.69 *dd*(11,8)
				4.89 *d*(12)	4.87 *d*(11)
19	1.79 *br s*	1.79 *br s*	1.10 *d*(7)	1.13 *d*(6)	1.12 *d*(6)
20	3.89 *d*(12)	3.89 *d*(12)	3.75 *d*(12)	3.78 *d*(12)	3.87 *d*(12)
	4.81 *d*(12)	4.82 *d*(12)	3.86 *d*(12)	3.87 *d*(12)	4.73 *d*(12)
2′	5.63 *d*(16)	5.63 *d*(15)			
3′	6.65 *dd*(16,10)	6.65 *dd*(15,10)			
4′	6.03 *dd*(15,10)	6.03 *dd*(15,10)			
5′	5.84 *dt*(15,7)	5.84 *dt*(15,7)			
10′			0.88 *d*(7)	0.91 *d*(6)	0.89 *d*(6)
14′	0.88 *t*(7)	0.88 *t*(7)			
others	1.99 *s*	1.99 *s*		8.04 *dd*(8,1)	8.03 *br d*(8)
	(OAc)	(OAc)		7.55 *td*(8,1)	7.55 *br t*(8)
	0.88 *t*(7)	0.88 *t*(8)		7.43 *t*(8)	7.43 *t*(8)
	(15″)	(16″)		(benzoyl)	(benzoyl)
	5.36, 5.40 *m*				0.88 *t*(7)
	(5″, 6″)				(16‴)
	1.98, 2.02 *m*				
	(4″, 7″)				

Plant materials. Wikstroemia retusa A. Gray were collected in Ishigaki Island in July 1995. A sample is deposited in the Herbarium of Fukuoka University (Voucher FUK950724S).

Extraction and isolation. The fresh bark (8 kg) was cut into small pieces and percolated with MeOH. The concentrate of the MeOH percolate was partitioned with $CHCl_3$. The $CHCl_3$ extract (12 g) was chromatographed on a silica gel column with $CHCl_3$-MeOH-H_2O (7:1:1.6, bottom layer), benzene-Me_2CO (10:1–5:1), hexane-Me_2CO (4:1–3:1), and hexane-EtOAc (1:2–1:3), successively, and finally subjected to HPLC (ODS; 75% ~ 90% MeCN, 100% MeOH) to give compounds **1** (2.4 mg), **2** (3.2 mg), **3** (2.0 mg), **4** (19.0 mg), **5** (11.2 mg), **6** (18.3 mg), **7** (106.9 mg), **8** (10.6 mg), and **9** (3.9 mg). Compounds **1**–**4** were identified as **1**–**4** in the preceding paper [1], respectively, by NMR, MS, TLC and HPLC.

Compound **5** (*wikstroelide C*). Solid, $[\alpha]_D^{24}$ +15.5° (MeOH, *c* 0.65). UV λ_{max} nm (log ε): 230 (4.48); FAB MS *m/z*: 887.5286, $C_{51}H_{76}O_{11}Na$ requires 887.5285, *m/z*: 887 $[M+Na]^+$, 865 $[M+H]^+$, 805 $[M\text{-}AcO]^+$, 643 $[M\text{-}C_{14}H_{25}CO]^+$, 207 $[C_{13}H_{23}CO]^+$; negative FAB MS *m/z*: 239 $[C_{14}H_{27}COO]^-$, 223 $[C_{13}H_{23}COO]^-$, 59 $[CH_3COO]^-$; ^{1}H and ^{13}C NMR: Tables 1 and 2. HMBC cross peaks (3-bond): H-7/C-9, H-9/C-7, H-12/C-9,-$COCH_3$, H-14/C-1′, H-17/C-13, H-16/C-13, H-17/C-13, H-19/C-1,3, H-20/C-1″. **5** (5.7 mg) in CH_2Cl_2 (0.5 ml) was allowed to stand at room temp. for 3 hr with 0.05 M NaOMe in MeOH (0.5 ml). The mixture was neutralized and subjected to silica gel CC with hexane-Me_2CO (20:1–1:1) to give a methyl ester of pentadecenoic acid (**5a**) (2.0 mg), **2** (0.5 mg) and 12-deacetyl-**2** (1.2 mg). To a soln of **5a** in CS_2 (0.2 ml), dimethyldisulphide (0.2 ml) and I_2 (1 mg) were added and the mixture was warmed for 40 hr at 60° in a sealed vial. After dilution with 5% $Na_2S_2O_3$, the mixture was extracted with hexane, and the hexane extract was then subjected to EI MS: *m/z* (rel.int) 348 (21); 348.2138. Calcd for $C_{18}H_{36}O_2S_2$: 348.2156, *m/z*: 187 (100); 187.1525. Calcd for $C_{11}H_{23}S$: 187.1521, *m/z*: 161 (81); 161.0650. Calcd for $C_7H_{13}O_2S$: 161.0637.

Compound **6** (*wikstroelide D*). Solid, $[\alpha]_D^{26}$ +11.4° (MeOH, *c* 0.40). UV λ_{max} nm (log ε): 231 (4.12); FAB MS *m/z*: 903.5601, $C_{52}H_{80}O_{11}$ Na requires 903.5599. *m/z*: 881 $[M+H]^+$, 821, 207, 81; negative FAB MS *m/z*: 255 $[C_{15}H_{31}COO]^-$, 223 $[C_{13}H_{23}COO]^-$, 59 $[CH_3COO]^-$; ^{1}H and ^{13}C NMR: Tables 1 and 2.

Table 2. ^{13}C NMR spectral data for diterpenoids **5**–**8** and **9** [δ ppm in $CDCl_3$ (100 MHz)]

C	5	6	7	8	9
1	160.1	160.1	49.0	48.2	48.1
2	136.8	136.8	42.5	42.6	42.6
3	209.2	209.2	219.8	219.8	219.3
4	72.3	72.3	75.8	75.7	75.7
5	69.9	70.0	71.7	71.7	70.0
6	59.4	59.4	60.9	60.9	59.6
7	64.0	64.0	64.3	63.9	64.0
8	35.3	35.3	36.7	36.1	35.9
9	78.2	78.2	80.8	79.9	79.8
10	47.3	47.3	43.3	43.1	43.1
11	44.0	44.0	35.1	41.7	41.6
12	78.3	78.3	36.3	32.0	31.9
13	83.6	83.6	83.4	83.5	83.4
14	80.4	80.4	80.8	82.6	82.5
15	143.2	143.2	146.6	146.0	146.1
16	113.3	113.3	110.9	111.3	111.3
17	18.7	18.7	18.8	18.8	18.8
18	18.2	18.2	21.3	69.3	69.3
19	9.9	9.9	13.5	13.7	13.6
20	65.5	65.5	65.3	65.2	66.2
1′	117.1	117.1	119.8	119.9	120.0
2′	122.3	122.3	33.6	33.5	33.4
	(-OAc)	(-OAc)		(-*O*-benzoyl)	(-*O*-benzoyl)
	169.6	169.6		166.5	166.5
	21.1	21.1		130.4	130.4
				129.6 (×2)	129.6 (×2)
				128.3 (×2)	128.4 (×2)
				132.9	132.9
others	135.0 (3′)	135.0 (3′)	38.2 (8′)	38.1 (8′)	38.1 (8′)
	128.6 (4′)	128.6 (4′)	29.7 (9′)	30.1 (9′)	30.0 (9′)
	139.2 (5′)	139.2 (5′)	12.5 (10′)	12.5 (10′)	12.6 (10′)
	32.7 (6′)	32.7 (6′)	28.4–20.7	29.3–20.5	173.6 (1‴)*
	14.1 (14′, 15″)	14.1 (14′, 16″)	(3′–7′)	(3′–7′)	34.1 (2‴)
	173.3 (1″)	173.4 (1″)			14.1 (16‴)
	33.5 (2″)	34.2 (2″)			29.7–20.5
	131.8, 128.6	31.9–22.7			(3′–7′,
	(5″, 6″)	(7′–13′,			3‴–15‴)
	31.9, 32.6	3″–15″)			
	(4″, 7″)				
	32.6–22.7				
	(7′–13′,				
	3″–14″)				

* Carbonyl carbon of palmitic acid.

HMBC cross peaks (3-bond): H-5/C-7, H-7/C-14, H-8/C-6, H-12/-$COCH_3$, H-14/C-15,1′, H-16/C-17, H-17/C-13,16, H-19/C-1,3, H-20/C-1″, H-3′/C-1′.

Compound **7** (*wikstroelide E*). Solid, $[\alpha]_D^{24}$ +98.8° (MeOH, *c* 0.25). FAB MS *m/z*: 533.3116, $C_{30}H_{44}O_8$+H requires 533.3114. ^1H and ^{13}C NMR: Tables 1 and 2. HMBC cross peaks (3-bond): H-1/C-9,10′, H-5/C-7, H-7/C-9,14,20, H-8/C-6, H-10/C-5,11,9′, H-11/C-13, H-12/C-9,18, H-18/C-9, H-19/C-1,3, H-10′/C-1,8′.

Compound **8** (*wikstroelide F*). Solid, $[\alpha]_D^{24}$ +68.1° (MeOH, *c* 0.53). UV λ_{max} nm (log ε): 228 (4.18); FAB MS *m/z*: 675.3149, $C_{37}H_{48}O_{10}Na$ requires 675.3145. *m/z*: 675, 653 $[M+H]^+$, 154, 136, 105; ^1H and ^{13}C NMR: Tables 1 and 2. HMBC cross peaks (3-bond): H-5/C-7, H-8/C-6,11, H-10/C-11, H-14/C-7, H-16/C-17, H-18/C-7″, H-19/C-1,3, H-10′/C-1,8′, H-2″,6″/CO(benzoyl).

Compound **9** (*wikstroelide G*). Solid, $[\alpha]_D^{21}$ +68.1° (MeOH, *c* 0.49). UV λ_{max}: 226(sh) (4.08); FAB MS *m/z*: 913.5441, $C_{53}H_{78}O_{11}Na$ requires 913.5442. *m/z* 657, 639, 477, 105; ^1H and ^{13}C NMR: Tables 1 and 2. HMBC cross peaks (3-bond): H-5/C-3,7, H-7/C-9,14,20, H-8/C-6, H-14/C-9,15,1′, H-17/C-13, H-20/C-1‴, H-2″,6″/CO(benzoyl), H-4″/C-2″,6″, H-3″,5″/C-1″.

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