

Chemical Constituents of the Bark of *Ryparosa kunstleri* (Flacourtiaceae)

KHOZIRAH SHAARI and PETER G. WATERMAN¹

Forest Research Institute Malaysia
52109 Kepong, Kuala Lumpur, Malaysia

¹Phytochemistry Research Laboratories
Department of Pharmaceutical Sciences
University of Strathclyde
Glasgow G1 1XW, Scotland, UK

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ABSTRAK

Tiga triterpena telah dapat diasingkan daripada ekstrak kulit pokok spesies *Ryparosa kunstleri* (Flacourtiaceae) sebagai kandungan sebatian kimia major, iaitu friedelin, acid trikadenik B, dan sebatian baru, asid 3 β -*p*-koumaroiltrikadenik. Asid stiktik, sejenis sebatian depsidon, juga telah diasingkan tetapi kewujudannya di dalam ekstrak tersebut dianggap sebagai sebatian cecair.

ABSTRACT

The bark of *Ryparosa kunstleri* yielded the triterpenes friedelin, trichadenic acid B, and the new 3 β -*p*-coumaroyltrichadenic acid as its major constituents. Stictic acid, a depsidone, was also isolated but was deduced to be a contaminant.

INTRODUCTION

Ryparosa kunstleri King (Flacourtiaceae) is an uncommon, medium-sized, rainforest tree found in lowland and mountainous areas of Peninsular Malaysia (Whitmore 1973). As part of an ongoing study on the chemistry of Malaysian species of the Flacourtiaceae, we examined extracts from the bark of this species.

MATERIAL AND METHODS

Bark of *Ryparosa kunstleri* was collected from the controlled plots of Pasoh Forest Reserve, Negeri Sembilan, Malaysia in February 1993. Specimens were identified by comparison with specimens in the herbarium of the Forest Research Institute Malaysia obtained previously from the same source.

The powdered bark (3.5 kg) was extracted successively with petroleum ether and EtOAc. The petrol extract (20 g) was fractionated by

VLC over Si gel 60 H eluting with petrol and then petrol containing increasing amounts of EtOAc. The 10% EtOAc fraction yielded friedelin (713 mg). Subsequent column chromatography of the 30% EtOAc fraction over Sephadex eluting with 100% CHCl₃ yielded trichadenic acid B **1** (43 mg) after recrystallization of the precipitate obtained from the column fractions 7 to 12. The EtOAc extract (40 g) was also fractionated by VLC in the same manner. The 10-20% EtOAc fractions yielded more friedelin (200 mg). The 50% EtOAc fraction was columned over Sephadex eluting with CHCl₃ followed by CHCl₃:MeOH (9:1). Subsequent PTLC (Si gel 60F₂₅₄, CHCl₃, 3x) of column fractions 8-13 yielded 3 β -*p*-coumaroyltrichadenic acid **2** (108 mg). Finally the 75-100% EtOAc fractions were recombined and concentrated down to a small volume to yield stictic acid **3** (32 mg).

Structure elucidation of these compounds was achieved by 1D and 2D NMR with particular emphasis on Heteronuclear Multiple Bond Coherence (HMBC) spectroscopy (Bax and Summers 1986). Melting points were obtained on a Gallenkamp melting point apparatus and are uncorrected. UV spectra were run in EtOH and IR spectra were recorded as KBr discs. NMR spectra were run on a Bruker AMX-400 instrument using standard microprograms (HMBC with d_6 set for $J=7$ Hz) and in C_5D_5N . High-resolution EIMs were obtained on an AEI-MS902 spectrometer using direct-probe insertion at 120° and with 70 eV.

RESULTS AND DISCUSSION

By VLC (vacuum liquid chromatography) and PTLC (preparative thin layer chromatography), the three triterpenes were isolated from the petrol and EtOAc extracts of the bark of *R. kunstleri*. The first of these was identified as friedelin by comparison with an authentic sample. High resolution EI-mass spectrometry of compound 1 suggested an empirical formula of $C_{30}H_{50}O_3$. The ^{13}C NMR spectrum showed resonances attributable to a carboxylic acid ($\delta_c 179.8$) and a carbinol doublet ($\delta_c 71.4$) which was assigned to C-3 on biogenetic grounds. The 1H NMR spectrum exhibited seven methyl singlets, one of which was broad ($\delta 1.14$). An HC-COBI (Bax 1983) study revealed this broad methyl signal to have $\delta_c 11.0$ and is magnetically equivalent with a methine ($\delta_c 54.3$). The methine proton ($\delta 1.14$) was observed to be coupled to H-3 in the COSY spectrum, therefore it was assignable as H-4. The configuration of H-3 had to be equatorial (α) due to the small half-height width ($W_{1/2}$ 14 Hz) observed for its signal which indicated an equatorial-equatorial and axial-equatorial coupling. Using HMBC spectroscopy, compound 1 exhibited long range H-C connectivities indicative of the friedelane skeleton by identifying 2J and 3J connectivities associated with the methyl resonances. Assuming that the broad methyl signal was attributable to Me-23, C-3, C-6, C-8, C-9, C-10, C-13, C-14, C-15 could all be unambiguously assigned by this procedure. The methine proton H-18 (1.96, dd, J 11.9, 5.6 Hz) could be identified and was shown to be 2J coupled to C-13, 3J to the carboxylic acid and a methyl ($\delta_c 31.9$) which had to be Me-28, thus confirming the placement of the carboxylic acid at C-27. By default the

remaining methyls were assigned as C-29 ($\delta_c 31.5$) and C-30 ($\delta_c 36.0$), both showing 2J couplings to the quaternary carbon C-20 ($\delta_c 29.2$). These data characterised the isolate as the known compound trichadenic acid B or 3β -hydroxy-D:A-friedooleanan-27-oic acid and was confirmed by comparison with published physical and spectral data (Gunasekara and Sultanbawa 1977a; Tanaka *et al.* 1988).

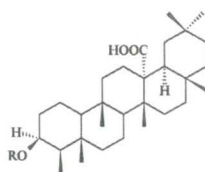
Compound 2 had a 1H NMR spectrum typical of an esterified triterpene. Careful analysis revealed the parent triterpene to be trichadenic acid and comparison of the chemical shift values provided confirmation. The carbinol proton H-3a showed a 3J correlation to the ester carbonyl ($\delta_c 167.4$) thus placing the ester group at this position. Examination of the remaining proton and carbon signals indicated a *p*-coumaroyl group. Proton signals observed for the group included two olefinic *trans*-related protons (J 15.9 Hz) and four aromatic protons exhibiting an AB spin system. The presence of an oxygenated quaternary carbon ($\delta_c 161.8$) which was 3J correlated to the aromatic protons on C-2' and C-6' confirmed placement of a hydroxyl group on C-4'. On the above evidence, compound 2 is assigned as the novel 3β -*p*-coumaroyltrichadenic acid. Through an HMBC experiment (Table 1) the tracking of resonances coupling to the methyl groups was achieved in a manner analogous to that in compound 1, thus allowing the unambiguous assignment of all the carbon atoms except C1, C2, C7, C11, C12, C16,

TABLE 1
 2J and 3J couplings for compound 2

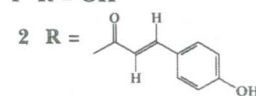
C-H	^{13}C cross peaks	
	2J	3J
C3a-H	-	167.6(C9')
C18-H	55.1 (C13)	31.7(C28), 179.8 (C27)
C23-H ₃	50.5 (C4)	39.0(C5), 74.9 (C3)
C24-H ₃	39.0 (C5)	41.6(C6), 50.4 (C4), 60.4 (C10)
C25-H ₃	37.8(C9)	60.4 (C10)
C26-H ₃	39.9(C14)	34.0(C15), 55.1 (C13)
C28-H ₃	31.4(C17)	43.8(C18)
C29-H ₃	29.0(C20)	36(C30)
C30-H ₃	29.0(C20)	31.4(C29)
C8'-H	167.6(C9')	126.6 (C1')
C7'-H	116.2(C8')	131.0(C2',6')
C2',6'-H	-	131.0(C6',2'), 145.3(C7'), 161.8(C4')
C3',5'-H	-	117.2(C5',3'), 126.6(C1')

TABLE 2
 ^{13}C and ^1H NMR spectral data for
 compounds 1 and 2

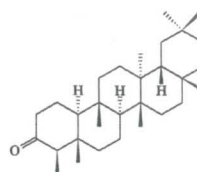
C	^{13}C		^1H	
	1	2	1	2
1	20.6	20.0		
2	38.1	33.4		
3a	71.4	74.9	3.55m	4.99dt (11.0, 5.0)
4	54.3	50.5	1.14m	
5	38.9	39.0		
6	42.2	41.6		
7	19.1	18.9		
8	53.7	53.4		
9	38.1	37.8		
10	61.3	60.4	1.10m	1.0m
11	39.1	37.8		
12	28.9	28.7		
13	55.3	55.1		
14	40.1	39.9		
15	34.1	34.0		
16	37.0	36.8		
17	31.6	31.4		
18	44.0	43.8	1.96 dd (11.9, 5.6)	1.94 dd (11.7, 5.4)
19	37.0	36.8		
20	29.2	29.0		
21	33.6	33.4		
22	39.0	38.8		
23	11.0	10.5	1.14brs	0.83 d(6.6)
24	15.4	14.9	0.86 s	0.80 s
25	19.4	19.1	0.96 s	0.88 s
26	23.4	23.2	1.22 s	1.18 s
27	179.8	179.4	8.54	8.00 (OH)
28	31.9	31.7	1.33 s	1.31
29	31.5	31.4	1.14 brs	1.12 s
30	36.0	36.0	1.06 s	1.04 s
1'	-	126.6	-	-
2'	-	131.0	-	7.65 d (8.5)
3'	-	117.2	-	7.17 d (8.5)
4'	-	161.8	-	-
5'	-	117.2	-	7.17 d (8.5)
6'	-	131.0	-	7.65 d (8.5)
7'	-	145.3	-	8.00 d (15.9)
8'	-	116.2	-	6.64 d (15.9)
9'	-	167.6	-	-



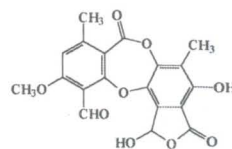
1 R = OH



2 R =



Friedelin



Stictic acid

Trichadenic acid B [1].-Needles from petrol, mp 328-330° (dec.) [lit. 333-334° (Gunasekara and Sultanbawa 1977a)]; $[\alpha]_D +37^\circ$ (c, 0.5 in CHCl_3) [lit. $+40.1^\circ$ (Gunasekara and Sultanbawa 1977a)]; IR ν_{max} 3420, 1687, 1387, 1256, 1168, 1028 cm^{-1} . ^1H and ^{13}C NMR (see Table 2); EIMS m/z (rel. int) 458 (7), 425 (6), 372 (4), 307 (35), 289 (13), 259 (100, 220 (7), 203 (19), 165 (26), 152 (100).

3β -*p*-coumaroyltrichadenic acid [2].-Amorphous solid, mp 196-199° (dec.); $[\alpha]_D +18^\circ$ (c, 0.5 in CHCl_3); IR ν_{max} 3380, 1680, 1604, 1514 cm^{-1} , ^1H and ^{13}C NMR (Table 2).

Stictic acid.-Amorphous solid, 255-258° (dec.) [lit. 259-262° (Ingolfssdottir *et al.*, 1986)]; IR ν_{max} cm^{-1} 3409, 3255, 2360, 1741, 1689, 1604, 1550; ^1H NMR ($\text{C}_5\text{D}_5\text{N}$) δ 11.1, 1H, s; 7.43, 1H, s; 6.87, 1H, s; 3.84, 3H, s; 2.62, 3H, s; 2.42, 1H, s. ^{13}C NMR ($\text{C}_5\text{D}_5\text{N}$) δ c s at 187.9, 169.0, 164.8, 163.8, 162.2, 154.5, 151.9, 149.5, 139.0, 137.0, 122.2, 116.2, 114.9, 110.8, 57.1, 22.5, 10.3; EIMS m/z M^+ 386 (100%), 369 (24), 368 (60), 341 (42), 314 (23), 312 (27), 286 (21), 259 (923), 193 (76), 191 (53), 184 (33).

Friedelin.-Needles from petrol, mp 257-259° [lit. 264-265° (Gunasekara and Sultanbawa 1977b)]; IR and ^1H NMR identical with an authentic sample.

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C19, C21, C22 (Table 2). The assignments of these carbons were, however, made by comparison with those of compound 1.

The final compound stictic acid, a depsidone, was isolated only in a small amount. It was identified by comparison of its NMR and mass spectral data with those found in the literature (Ingolfssdottir *et al.* 1986; Huneck *et al.* 1968). Being of a class of compounds that is usually derived from lichens and since the extract was from bark material, this compound was assumed to be a contaminant.

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