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PODOPHYLLOTOXIN-TYPE LIGNANS AS MAJOR CONSTITUENTS OF THE STEMS AND LEAVES OF *CASEARIA CLARKEI*

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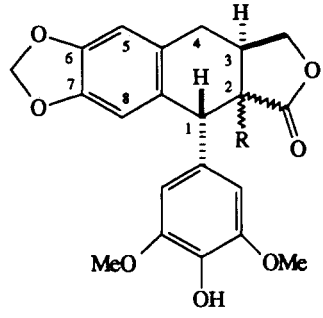
ABSTRACT.—The stems of *Casearia clarkei* have yielded the new lignan 2β-hydroxy-4′-demethyldesoxypodophyllotoxin together with 4′-demethyldesoxypodophyllotoxin, traces of 4′-demethyldesoxypicropodophyllotoxin, and a mixture of catechin and epicatechin. The leaves afforded large amounts of 4′-demethyldesoxypicropodophyllotoxin together with lupenone, friedelan-3β-ol and amentoflavone, but no trace of either of the other lignans obtained from the bark could be detected.

In continuation of our studies on Malaysian species of Flacourtiaceae (1), we wish to report the constituents of the stems and leaves of *Casearia clarkei* King, a small lowland rain forest tree endemic to Malaysia and Singapore (2). This genus attracted our attention because of reports of the isolation of unusual cytotoxic clerodane diterpenes from a number of neotropical species (3–5).

RESULTS AND DISCUSSION

The EtOAc-soluble extract of the stems (bark plus wood) was fractionated by vlc followed by prep. tlc to yield four bands. One of these was found to be a 1:1 mixture of catechin and epicatechin and was not further investigated. The remaining three all contained aryltetralin lignans. The most abundant was identified as 4′-demethyldesoxypodophyllotoxin [**1**] by comparison of spectral data with those previously published (6–8) (Table 1 and Experimental). The corresponding 2,3-cis compound 4′-demethyldesoxypicropodophyllotoxin [**2**] was obtained in trace amounts and identified by comparison of nmr data with those for **1** (Table 1). Non-equivalence of chemical shift values made the ^{1}H-nmr spectrum of **2** far easier to interpret than that of **1**, with the $J_{2,3}$ of 9.6 Hz typical of the cis-fused system (9) being clearly visible. The thermodynamically more stable picro-series of aryltetralins has been widely considered to be artifactual (10), but recent findings (8,11) suggest that they can occur naturally.



1 R=β-H
2 R=α-H
3 R=β-OH

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TABLE 1. ^{1}H- and ^{13}C-Nmr Shift Values for **1**, **2**, and **3** in $CDCl_3$.

Position	^{1}H			^{13}C		
	1	**2**	**3**	**1**	**2**	**3**
1	4.60 d 3.2	4.36 d 3.1	4.42 s	43.8	45.4	52.9
2	2.76 m	3.32 dd 9.6, 3.1	—	47.8	46.7	76.7
2a.....	—	—	—	175.2	178.7	175.0
3	2.76 m	3.01 m	2.78 m	32.9	33.3	35.9
3a.....	4.44 m	4.45 dd 9.0, 7.5	4.36 dd 8.2, 7.6	72.2	73.0	71.0
	3.91 m	3.98 dd 9.0, 3.2	4.23 dd 10.4, 8.2			
4	3.06 m	2.87 dd 15.3, 6.3	3.03 dd 16.0, 12.3	33.3	32.3	27.2
	2.76 m	2.49 dd 15.3, 5.7	2.93 dd 16.0, 6.1			
4a.....	—	—	—	128.5	128.6	128.6
5	6.67 s	6.67 s	6.71 s	108.6	109.0	108.6
6	—	—	—	147.2	147.0	147.2
7	—	—	—	147.9	147.1	147.3
8	6.52 s	6.59 s	6.52 s	110.7	110.1	111.4
8a.....	—	—	—	131.0	131.0	127.9
CH_2	5.95 ABq 1.2	5.96 ABq 1.2	5.97 ABq 1.2	101.4	101.2	101.4
	5.93	5.93	5.93			
1'.....	—	—	—	132.0	133.8	130.5
2'/6'	6.36 s	6.35 s	6.38 s	108.2	105.0	108.4
3'/5'	—	—	—	146.6	147.4	146.7
4'.....	—	—	—	132.1	133.7	134.4
OMe	3.79 s	3.82 s	3.78 s	56.6	56.7	56.7
OH	5.42 s	5.44 s	5.43 s			

The final lignan analyzed by eims for M^+ $C_{21}H_{20}O_8$, with one oxygen more than **1** and **2**. The ^{1}H- and ^{13}C-nmr spectra (Table 1) indicated substitution patterns on the two aromatic nuclei identical to those in **1** and **2**. However, the ^{13}C-nmr spectrum revealed an additional quaternary carbinol (76.7 ppm) and the ^{1}H-nmr spectrum showed loss of a methine proton. This missing proton must be that normally found at C-2, as H-1 now appeared as a sharp singlet, while the spin system associated with the C3a-C3-C4 protons was still visible. The assignments of the carbinol and all the other ^{13}C-nmr resonances were confirmed by an HMBC study (Table 2), and on the basis of these data structure **3** was assigned. There was no direct nmr evidence to support trans stereochemistry between the hydroxyl and H-3 across C-2/C-3 in **3**, but the similarity of chemical shift values for C-2'/C-6', C-3'/C-5' and H-3, and the occurrence of trans-diaxial coupling between H-3 and one H-4 and one H-3a proton are all factors strongly indicative of similar stereochemistry in **1** and **3**. This was confirmed by their cd spectra (Figure 1), which showed a positive Cotton effect at 286 nm and a negative Cotton effect at 272 nm, in agreement with observations found for other lignans with the C-1/C-2/C-3 absolute stereochemistry of the podophyllotoxin-type lignans (9,12). Compound **3**, 2β-hydroxy-4'-demethyldesoxypodophyllotoxin, appears to be a new natural product.

A petroleum ether extract of the leaves yielded only the common triterpenes lupenone and friedelan-3β-ol, which were identified by direct comparison with literature data and an authentic sample, respectively. The EtOAc extract gave the biflavonone amentoflavone, again confirmed by comparison with authentic material, together with large amounts of **2** (0.175% of leaf dry weight). Neither **1** nor **3** could be detected, even at the level of tlc analysis. The occurrence of **2** in such large amounts and without detectable amounts of **1** suggests that in this case the picro compound occurs naturally. The leaf and stem samples were collected at the same time and treated in the same manner so it would be difficult to explain why conversion of **1** to **2** should be minimal in the stems

TABLE 2. Long-range C-H Connectivities in **3**, Established by HMBC.[a]

Position	δ_H	δ_C[b]	^2J	^3J
1	5.07 s	53.3	131.3/4 (C-8a), 76.2 (C-2)	129.6 (C-4a), 111.7 (C-8), 110.6 (C-2/6), 37.3 (C-3)
2	—	76.0		
2a.....	—	175.6		
3	3.20 m	37.3		
3a.....	4.48 m 4.41 m	71.4		175.6 (C-2a), 76.0 (C-2)
4	3.30 dd 14.7, 12.0	27.8	129.6 (C-4a), 37.3 (C-3)	131.3/4 (C-8a), 109.2 (C-5), 76.0 (C-3)
4a.....		129.6		
5	6.89 s	109.2		147.3 (C-7), 131.3/4 (C-8a), 27.8 (C-4)
6	—	147.3		
7	—	147.3		
8	6.83 s	111.7		147.3 (C-6), 129.6 (C-4a), 53.3 (C-1)
8a.....	—	134.3/4		
CH_2...	5.97 s	101.8		147.3 (C-6/7)
1'.....		137.3		
2'/6' ..	6.92 s	110.6	148.9 (C-3'/5'), 137.3 (C-1)	131.3/4 (C-4'), 110.6 (C-6'/2'), 53.3 (C-1)
3'/5' ..	—	148.9		
4'.....	—	131.3/4		
OMe ..	3.77 s	56.8		148.9 (C-3'/5')

[a]Spectra were run in pyridine-d_5.
[b]^1J correlations were established by means of HC-COBldec.

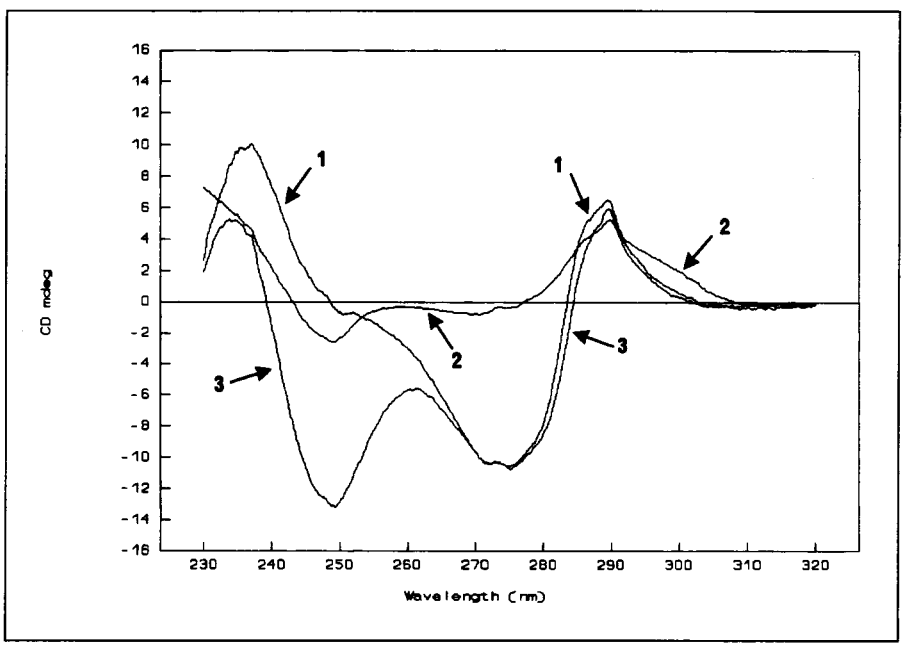

FIGURE 1. Cd Spectra of **1** to **3**. [Samples (0.5 mg ml^{-1}) were run in MeOH and spectra recorded in cells of pathlength 0.5 cm.]

but complete in the leaf. Furthermore, subsequent recollections of further leaf samples and their tlc analysis consistently revealed **2** to be by far the most abundant lignan.

This is the first record of podophyllotoxin-type lignans in the Flacourtiaceae, although an arylnaphthalene derivative has been reported (13). It is also clear that this Asian *Casearia* species is chemically very different from those neotropical taxa that have previously been investigated (3–5). The only other phytochemical report for an Asiatic species (14) records the presence of 6-*C*-prenyl and 8-*C*-prenyl 7-methoxycoumarins in *C. graveolens*, another class of secondary metabolite that has not as yet been found among neotropical *Casearia*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were obtained on a Gallenkamp melting point apparatus and are uncorrected. Uv spectra were run in EtOH and ir spectra were recorded as KBr disks. Nmr spectra were run on a Bruker AMX-400 instrument using standard microprograms (HMBC with d_6 set for $J=7$ Hz) and in $CDCl_3$ unless otherwise stated. High-resolution eims were obtained on an AEI-MS902 spectrometer using direct-probe insertion at 120° and with 70 eV.

PLANT MATERIAL.—Stems and leaves of *Casearia clarkei* were collected from the controlled plots at Pasoh Forest Reserve, Negeri Sembilan, Malaysia in February 1992. The plant material was identified by comparison with specimens in the Forest Institute's Herbarium obtained previously from the same source.

EXTRACTION AND ISOLATION.—The powdered stems (1.8 kg) were extracted successively with petroleum ether and EtOAc. Tlc analysis of the two extracts showed them to be identical and they were bulked. The combined extract (7.0 g) was fractionated by vlc over Si gel 60 H eluting with petroleum ether and then petroleum ether containing increasing amounts of EtOAc. Subsequent prep. tlc of the fraction eluted with 35% EtOAc in petroleum ether [Si gel $60F_{254}$; $CHCl_3$-MeOH (95:5), three developments] to give **1** (120 mg; R_f 0.60), **2** (4.5 mg; R_f 0.56) and **3** (31 mg; R_f 0.41). Prep. tlc of the 40% EtOAc fraction [Si gel $60F_{254}$; $CHCl_3$/MeOH (90:10)] yielded an equimolar mixture of catechin and epicatechin (60 mg) as a red gum.

The powdered leaves (800 g) were also extracted successively with petroleum ether and EtOAc. The petroleum ether extract (25 g) was concentrated and subjected to vlc eluting with hexane and then hexane containing increasing amounts of EtOAc. The 10% and 20% EtOAc fractions yielded friedelan-3β-ol (290 mg) and lupenone (198 mg), respectively. The EtOAc extract (60 g) was similarly treated and then elution from vlc carried out first with hexane and then increasing amounts of EtOAc. This was then replaced with 100% EtOAc and subsequently increasing proportions of MeOH in EtOAc. Fractions eluted with 100% EtOAc and up to 50% MeOH in EtOAc all had identical tlc profiles and were bulked. These combined extracts were resubjected to vlc, eluting with hexane-$CHCl_3$ (20:80) and then $CHCl_3$. On concentration, a large amount of a solid separated out from the former fraction. This was filtered and subsequent prep. tlc [Si gel $60F_{254}$; $CHCl_3$, three developments] yielded **2** (1.4 g). The $CHCl_3$ fraction gave amentoflavone [80 mg; R_f 0.32; $CHCl_3$-MeOH (80:20)] after repeated prep. tlc and then recrystallization from MeOH.

4′-Demethyldesoxypodophyllotoxin [**1**].—Amorphous solid, mp 251–253° [lit. (6) 234–237°]; $[\alpha]_D$ −122° (c=0.14, $CHCl_3$) [lit. (15) −128°]; Uv, ir, eims in agreement with published data (6–8). ^1H and ^{13}C nmr, see Table 1.

4′-Demethyldesoxypicropodophyllotoxin [**2**].—Amorphous solid, mp 174–175°; $[\alpha]_D$ +37° (c=0.05, $CHCl_3$); uv λ max (log ε) 348 (2.82), 289 (3.62), 240 sh (3.88), 207 (4.55) nm; ir ν max 3400, 1759, 1615, 1518 cm^{-1}; ^1H and ^{13}C nmr, see Table 1; hreims *m/z* M^+ 384.1200 (calcd 384.1209 for $C_{21}H_{20}O_7$) (100%), 353 (16), 339 (11), 325 (23), 324 (11), 312 (26), 299 (10), 293 (10), 269 (18), 230 (32), 186 (13), 185 (11).

2′β-Hydroxy-4′-demethyldesoxypodophyllotoxin [**3**].—Needles from $CHCl_3$, mp 255–256°; $[\alpha]_D$ −239° (c=0.05, $CHCl_3$); uv λ max (log ε) 294 (3.22), 245 sh (3.58), 207 (4.22) nm; ir ν max 3460, 1777, 1607, 1516 cm^{-1}; ^1H and ^{13}C nmr, see Table 1; hreims *m/z* M^+ 400.1200 (calcd 400.1209 for $C_{21}H_{20}O_8$) (100%), 270 (12), 269 (57), 239 (9), 201 (18), 200 (24), 185 (11), 181 (15), 174 (15), 173 (43), 167 (17), 139 (11).

Amentoflavone.—Yellow powder from EtOAc, mp 249–251° [lit. (16) 247–249°]. Ir, ^1H and ^{13}C nmr in agreement with published data (16).

Lupenone.—Amorphous solid, mp 192–195° [lit. (17) 170°]; $[\alpha]_D$ +68° (c=0.11, $CHCl_3$) [lit. (17) +63.5°]. Ir, ^1H and ^{13}C nmr in agreement with published data (18).

Friedelan-3β-ol.—Fine needles from hexane, mp 268–270° [lit. (19) 282–284°]; $[\alpha]_D$ +20° (c=0.08, $CHCl_3$) [lit. (19) +25°]. Ir, ^1H and ^{13}C nmr were in agreement with published data (19).

Catechin/epicatechin Mixture.—The sample gave a ^{1}H-nmr spectrum ($CHCl_3$) for a 50:50 mixture. Spectra were compared with those of pure catechin and epicatechin and spin systems confirmed by means of a COSY-45 nmr experiment.

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