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DISTRIBUTION OF PYRANOCOUMARINS IN *CALOPHYLLUM CORDATO-OBLONGUM*

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Key Word Index—*Calophyllum cordato-oblongum*; Cluciaceae; buds; twigs; pyranocoumarins; methyl ether of cordatolide **B**; cordatolides **A** and **B**; oblongulide; cordato-oblongic acid.

Abstract—Twigs of *Calophyllum cordato-oblongum* have been shown to contain a new pyranocoumarin, the methyl ether of cordatolide **B**, three reported pyranocoumarins, cordato-oblongic acid, friedelin, canophyllol and taraxerol. Buds of this species contained large quantities of pyranocoumarins and a small amount of sitosterol. This observation indicates that the coumarin-synthesising tissues are mainly located at the non woody young plant parts of *C. cordato-oblongum*. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Some pyranocoumarins isolated from *Calophyllum* species have been shown to inhibit HIV-1 replication and cytopathicity through their interaction with HIV-1 RT [1, 2]. Calanolides and inophyllums have shown activity against AZT-resistant viral strains, as well as the A 17 strain, which is known to be resistant to non-nucleoside RT inhibitors. Therefore, pyranocoumarins provide a new class of anti-HIV compounds. Prompted by these reports [1, 2] and, as a part of our continuing study on *Calophyllum* species [3–8], extracts of different parts of *C. cordato-oblongum* were investigated.

Calophyllum cordato-oblongum (local name, Kalu Keena) is a rare endemic plant growing in the lowland, evergreen, wet zone forests in Sri Lanka. Four xanthones, three coumarins, a chromene acid and three triterpenoids have been reported previously from the leaves [8], stem wood and stem bark [9] of this species. In this communication, we report the isolation of coumarins and triterpenoids from twigs and buds, and the distribution of coumarins in various plant parts of *C. cordato-oblongum*. Our research project set out specifically to determine anti-HIV activity of *Calophyllum* products. Cordatolide **A** (**1**) and cordatolide **B** (**2**) from the leaves of *C. cordato-oblongum* were found to be anti-HIV 1 RT-active and these results have been published elsewhere [10].

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RESULTS AND DISCUSSION

Plant materials were separately shade-dried and milled. Powdered twigs were successively extracted with hot hexane and EtOAc to give 2.9% of hexane extract and 1.9% of EtOAc extract. The hexane extract of twigs, when separated on a medium-pressure silica gel column (MPLC) using *n*-hexane–EtOAc yielded eight compounds, seven of which were identified as cordatolide **A** (**1**) (0.1%), cordatolide **B** (**2**) (0.34%), oblongulide (**3**) (0.26%), friedelin (0.3%), canophyllol (0.008%), sitosterol (0.09%) and cordato-oblongic acid (0.05%). The identities of these compounds were established by direct comparison of their spectral and physical data [8].

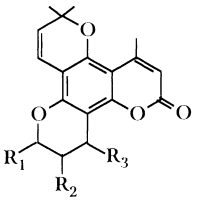
The remaining compound **4** (0.07%) showed a $[M]^+$ at m/z 356, with the base peak at m/z 341. The IR spectrum showed the presence of carbonyl (coumarin) and olefinic groups. The ^{1}H NMR data (Table 1) of **4** suggested that the 3H singlet at δ 3.58 is due to a methoxyl group. However, further comparison (Table 1) of its ^{1}H NMR data with cordatolide **A** (**1**) and cordatolide **B** (**2**), suggested that compound **4** was the 12-methyl ether of cordatolide **B** (**4**). The H-12 in the vicinity of the 12-HO group of **2** appeared at δ 4.96, while the same proton in the vicinity of the methoxyl group of **4**, was shifted to a higher field at δ 4.55. ^{13}C NMR data further confirmed structure **4** and the complete ^{13}C NMR (50 MHz) chemical shifts of **4** (in $CDCl_3$) are given in the Experimental.

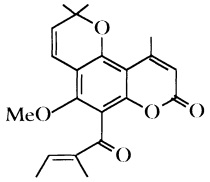
Milled buds were successively extracted with hot hexane, CH_2Cl_2, EtOH and MeOH to give 2.12% of hexane extract, 1.25% of CH_2Cl_2 extract, 5.57% of EtOAc extract and 2.12% of MeOH extract. The hex-

Table 1. ^{1}H NMR data for compounds **1**, **2** and **4** ($CDCl_3$)

Proton	Cordatolide **A** (**1**)	Cordatolide **B** (**2**)	Cordatolide **B**–OMe (**4**)
3-H	5.86, *s*	5.90, *s*	5.93, *d* (1.1)
4-Me	2.55, *s*	2.56, *s*	2.57, *d* (1.1)
6-Me	1.49, *s*	1.48, *s*	1.47, *s*
7-H	5.48, *d* (10)	5.51, *d* (10)	5.52, *d* (10.0)
8-H	6.58, *d* (10)	6.63, *d* (10)	6.62, *d* (10.0)
10-H	3.93, *m* (8)	4.30, *m* (10)	4.30, *m* (6.3, 12.6)
11-H	2.0, *m*	1.9, *m*	1.90, *m*
12-H	4.65, *d* (7)	4.96 *d* (3)	4.55, *d* (2.7)
10-Me	1.43, *d* (3.5)	1.43, *d* (5)	1.41, *d* (6.3)
11-Me	1.11, *d* (6.2)	1.15, *d* (7)	1.14, *d* (7.1)
12-OMe			3.58, *s*

Coupling constants (Hz) given in parentheses.





3 oblongulide

	R_1	R_2	R_3
1 cordatolide **A**	β -CH_3	α -CH_3	β-OH
2 cordatolide **B**	β -CH_3	α -CH_3	α-OH
4 cordatolide **B** - OMe	β -CH_3	α -CH_3	α-OMe

ane extract, when separated by MPLC using *n*-hexane–EtOAc, yielded a white crystalline solid (0.67%) with a $[M]^+$ of *m*/*z* 354 and the base peak at *m*/*z* 339. The IR spectrum of **3** showed the presence of carbonyl (coumarin) and olefinic groups. Further comparison of ^{1}H NMR data and TLC comparison of **3** with oblongulide, which has been reported from the leaves of this species, suggested that **3** was oblongulide [8]. The CH_2Cl_2 extract of the buds on MPLC yielded a mixt. of two compounds (0.2%) and were separated by prep. TLC to give cordatolide **A** (**1**) and cordatolide **B** (**2**). The identities of these two compounds were confirmed by direct comparison of spectral and physical data. The EtOAc extract of buds yielded sitosterol (0.017%). The above results show that the buds of *C. cordato-oblongum* consists of a considerable amount of the coumarin, oblongulide (**3**) and substantial amounts of cordatolide **A** (**1**) and cordatolide **B** (**2**). Except for a small amount of sitosterol, no triterpenoids were detected in the extracts of buds.

Our systematic investigation of various parts of *C. cordato-oblongum*, indicated (see Table 2) that the buds, twigs, leaves [8], stem bark and stem wood [9] of *C. cordato-oblongum* contain coumarins, the content of which decreases in the series going from buds to stem wood. No coumarins, but a considerable amount of xanthones were detected in the extracts of the stem wood of the above plant [9]. According to the anatomy of dicotyledons, the percentage of living cells gradually decreases from the apex toward the root collar [11, 12]. Our results show that the amount of coumarins present and the percentage of living cells in various plant parts has a significant correlation. This observation indicates that the coumarin-synthesizing tissues are mainly located in the non-woody young developing plant parts, particularly buds, twigs and leaves. It is well-known that coumarins play an important role in the natural defence mechanism of some plants [13]. Since young plant tissues are more vulnerable to external microbial attacks from bacteria, fungi and viruses, antimicrobial-active coumarins may be synthesised in young plant tissues. We

Table 2. Distribution of coumarins in *Calophyllum cordato-oblongum*

Plant part	% of coumarin (s)
Buds	0.87
Twigs	0.77
Leaves	0.43
Stem bark	0.04
Stem wood	0

have previously reported the antiviral activity of some of cordatolide **A** (**1**) and cordatolide **B** (**2**) from *C. cordato-oblongum* [10]; the present report further supports our hypothesis.

EXPERIMENTAL

General

Mps: uncorr; ^{1}H NMR: 200 MHz for solns in $CDCl_3$, reported in δ values relative to TMS as int. standard.

Plant material

Calophyllum cordato-oblongum Thw. was identified and collected in June 1996 from the Kanneliya forest in the Southern province of Sri Lanka. The plant specimen was compared with herbarium specimens (specimen No. 24771) at the Royal Botanic Gardens, Peradeniya, Sri Lanka.

Extraction and isolation

A portion (20 g) of the hexane extract of twigs was separated on a column of silica gel (220 g, Merk Art 9385) by MPLC with hexane and EtOAc as eluants. Further purification of column frs by prep. TLC (Merck Kieselgel 60 F_{254}) and flash and medium pressure CC (Merck Kieselgel 60, 230–300 mesh ASTM) with hexane and EtOAc as solvents gave **1** (1.775 g), **2** (0.6723 g), **3** (2.9144 g), **4** (0.476 g), friedelin (2.062 g), canophyllol (54 mg), sitosterol (587 mg) and cordato-oblongic acid (335 mg). The hexane extract (1.28 g) of buds was separated on a column of silica gel (20 g, Merk Art 9385) by MPLC with hexane and EtOAc as eluants. Further purification of column frs by prep. TLC (Merck Kieselgel 60 F_{254} and flash and medium pressure CC (Merck Kieselgel 60, 230–400 mesh ASTM) with hexane and EtOAc as solvents gave **3** (0.402 g). The CH_2Cl_2 extract (3.45 g) of buds on MPLC yielded a mixt. of two compounds (0.133 g). Further separation of frs by prep. TLC (Merck Kieselgel 60 F_{254}) and flash and medium pressure CC (Merck Kieselgel 60, 230–400 mesh ASTM) with hexane and EtOAc as solvents gave cordatolide **A** (**1**) and cordatolide **B** (**2**). The EtOAc extract (1.27 g) of buds yielded sitosterol (10 mg).

Oblongulide (**3**). White needles, mp 134–135°, lit. [8] 126°. ^{1}H NMR (200 MHz): 6.56 (1H, *d*, $J = 10.06$ Hz), 6.46 (1H, *m*, $J = 6.98$ Hz and $J = 1.33$ Hz), 6.00 (1H, *m*, $J = 1.20$ Hz), 5.72 (1H, *d*, $J = 10.06$ Hz), 3.8 (3H, *s*), 2.56 (3H, *d*, $J = 1.20$), 1.96 (3H, *m*, $J = 1.33$ Hz and $J = 1.03$ Hz), 1.86 (3H, *m*, $J = 6.98$ Hz and $J = 1.03$ Hz), 1.38 (6H, *s*). EIMS *m*/*z* (rel. int.): $[M]^+$ 354 (36), 339 (100), 309 (15), 299 (9), 281 (13), 253 (6), 241 (2), 213 (2), 185 (2), 149 (38), 128 (6), 115 (5).

Cordatolide **A** (**1**). White needles, mp 106–107°, lit. [8] 85°. EIMS *m*/*z* (rel. int.): $[M]^+$ 342 (40), 327 (100), 309 (36), 271 (100), 255 (11), 243 (9), 149 (2), 115 (9).

Cordatolide **B** (**2**). White needles, mp 217–218°, lit. [8] 178°. EIMS *m*/*z* (rel. int.): $[M]^+$ 342 (34), 327 (100), 309 (32), 271 (100), 255 (9), 243 (6), 115 (6).

Cordatolide **B** *methyl ether* (**4**). Yellow solid, mp 112–113°. UV λ_{max}^{MeOH} nm: (log ε): 219 (3.70), 229 (3.70), 248 (3.43), 273 sh, 282 (3.81), 325 (3.42). IR ν_{max}^{KBr} cm^{-1}: 2998, 1740, 1720, 1700, 1680, 1645, 1635, 1600, 1580, 1560, 1540, 1520, 1500, 1460, 1440, 1420, 1400, 1380, 1360, 1340, 1300, 1280, 1250, 1230, 1200, 1140, 1080, 1040, 990, 960, 910, 900, 860, 840, 780, 750, 680. ^{13}C NMR (50 MHz, $CDCl_3$): δ 160.7 (C-2), 154.7 (C-5a), 153.4 (C-1a), 153.2 (C-9a), 151.9 (C-4), 126.8 (C-8), 116.5 (C-7), 110.9 (C-3), 106.2 (C-8a), 104.5 (C-4a), 103.7 (C-12a), 72.6 (C-6), 73.3 (C-10), 70.6 (C-12), 59.2 (MeO-12), 38.5 (C-11), 27.8 (Me-6), 27.7 (Me-6), 24.4 (Me-11), 19.1 (Me-10), 13.3 (Me-4). EIMS *m*/*z* (rel. int.): $[M]^+$ 356 (40), 342 (36), 341 (100), 325 (49), 309 (26), 285 (85), 255 (11), 243 (2), 115 (4).

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