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XANTHONES FROM ROOT BARK OF *CALOPHYLLUM THWAITESII*

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Key Word Index—*Calophyllum thwaitesii*; Guttiferae; root bark; xanthones; thwaitesixanthone; 11,12-dihydrothwaitesixanthone; calothwaitesixanthone; demethylcalabaxanthone; 6-deoxy-γ-mangostin; trapezifolixanthone.

Abstract—The root bark of *Calophyllum thwaitesii* has been shown to contain six xanthones, calozeylanic acid, friedelin and sitosterol. Two of the xanthones have been identified as demethylcalabaxanthone and trapezifolixanthone, another as the novel compound, 11,12-dihydrothwaitesixanthone. The other three xanthones are thwaitesixanthone, calothwaitesixanthone and 6-deoxy-γ-mangostin, which have previously been reported from this species.

INTRODUCTION

Calophyllum species are well known for their medicinal uses [1–3]. Although a variety of interesting secondary metabolites from these plants have been already isolated and characterized [4–6], little attention has been focused on their biological activity. Recently, several coumarins isolated from two *Calophyllum* species were found to inhibit HIV-1 replication and cytopathicity through their interaction with the HIV-1 RT [5]. Therefore, a complete anti-HIV bioassay of all the coumarins from *Calophyllum* species is a pressing need.

Our research project is set out specifically to determine the potent anti-HIV activity of *Calophyllum* products. In this investigation, the root bark of *C. thwaitesii* was found to contain demethylcalabaxanthone, trapezifolixanthone, three xanthones, thwaitesixanthone, calothwaitesixanthone and 6-deoxy-γ-mangostin, earlier reported from the same species [6], and a new xanthone with M_r *m/z* 378. Other than xanthones, calozeylanic acid [7], friedelin and sitosterol, earlier reported from the same species [8], have been isolated. The new xanthone was identified as 11,12-dihydrothwaitesixanthone (**1**) using spectroscopic data and partial synthesis. Demethylcalabaxanthone and trapezifolixanthone have been reported earlier from other species [9, 10], but are rare.

RESULTS AND DISCUSSION

Root bark of *C. thwaitesii* was successively extracted with cold hexane, dichloromethane, ethyl acetate and methanol. The hexane extract when separated on a medium-pressure silica gel column yielded nine compounds, eight of which were identified as thwaitesixanthone, calothwaitesixanthone, 6-deoxy-γ-mangostin, trapezifolixanthone [10], demethylcalabaxanthone [9] friedelin, sitosterol and calozeylanic acid [7]. A new compound (**1**) shown by UV and IR spectra to be a xanthone of M_r *m/z* 378 was isolated as a minor constituent. Hydrogenation of **1** gave tetrahydrothwaitesixanthone (**2**) [8], M_r *m/z* 380. The ^{1}H NMR spectrum (200 MHz) of **1** showed the presence of a chelated hydroxyl group at δ 12.05 (1H, *s*). The singlet at δ 1.55 (6H, 2 × Me) and doublets at δ 8.05 (1H, J = 10 Hz) and 5.80 (1H, J = 10 Hz) suggested the presence of a 2,2-dimethyl-2*H*-pyrano ring. The singlets at δ 1.47 (3H, Me) and 1.37 (3H, Me), and triplets at δ 2.72 (2H) and 1.82 (2H) also indicated the presence of a dihydropyrano ring. The xanthone also had three aromatic protons and these appeared as two singlets at δ 7.24 (2H) and 6.28 (1H). The high field aromatic proton should be in an electron-rich environment such as that found in the phloroglucinol ring of the xanthone. The presence of a dihydropyrano ring and a 2,2-dimethyl-2*H*-pyrano ring was confirmed by the hydrogenation of **1** to yield **2**. The position of the 2,2-dimethyl-2*H*-pyrano ring in the structure **1** was confirmed by direct comparison of ^{1}H NMR spectra with those of thwaitesixanthone and calothwaitesixanthone. From these observations, **1** was identified as

It is biogenetically significant that thwaitesixanthone (**6**) co-occurs with 6-deoxy-γ-mangostin (**3**), calothwaitesixanthone (**4**) and demethylcalabaxanthone (**5**) in the root bark of *C. thwaitesii*. Thwaitesixanthone (**6**) is probably the end product of biosynthetic conversion **3**→**4**→**6** [6] and **3**→**5**→**6**. So far, no methylated xanthone has been isolated from *C. thwaitesii* [6, 8] and this confirms the absence of a methylating enzyme system in this species. This allows the biosynthesis of **4**, **5** and **6** from **3**.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR spectra were recorded at 200 or 60 MHz for solns in $CDCl_3$ and are reported in δ (ppm) values relative to TMS as int. standard.

Plant material. Calophyllum thwaitesii Planch and Triana was identified and collected in February 1994 from the Kaneliya forest in the Galle district of Sri Lanka by Mr Shantha Ekenayake (Institute of Fundamental Studies, Kandy, Sri Lanka).

Extraction and isolation. Shade-dried plant material (0.07 kg) was milled and then extracted with hexane to yield 0.0037 kg (5.3%) of extract. A portion (12.5 g) was sepd into acidic (0.690 g) and neutral (9.18 g) frs by washing with 5% $NaHCO_3$ soln. The neutral fr. (9.18 g) was separated on a column of silica gel (120 g, Merk Art 9385) by MPLC with hexane, EtOAc and MeOH as eluents. Further purification of column frs gave **1** (10 mg), **3** (10 mg), **4** (200 mg), **5** (40 mg), **6** (480 mg) and trapezifolixanthone (75 mg). The acidic fr. obtained from the $NaHCO_3$ wash was purified by prep. TLC to give calozeylanic acid (600 mg).

11,12-*Dihydrothwaitesixanthone* (**1**). Yellow needles, mp 258–259°. UV λ_{max} (MeOH) (log ε) nm: 223.5 (2.465), 272.0 *sh* (1.005), 284.0 *sh* (1.104), 290.0 (1.132), 328.5 (0.611), 404.0 (0.175). IR ν_{max}^{KBr} cm^{-1}: 3505, 2975, 1735, 1650, 1610, 1580, 1470, 1410, 1390, 1370, 1332, 1310, 1295, 1260, 1164, 1140, 1125, 1098, 1070, 950, 905, 890, 840, 820, 770, 750, 718. ^{1}H NMR (200 MHz): δ 12.05 (1H, *s*, 3-OH), 8.05 (1H, *d*, *J* = 10 Hz, H-5), 7.24 (2H, *s*, 9-H, 10-H), 6.28 (1H, *s*, 12-H), 5.80 (1H, *d*, *J* = 10 Hz, 6-H), 2.72 (2H, *t*, 2-H), 1.82 (2H, *t*, 1-H), 1.55, 1.47, 1.37 (6H, 3H, 3H, each *s*, 7-Me_2, 14-Me_2). ^{13}C NMR (50 MHz): δ 26.7 (C-11), 31.9 (C-12), 94.0 (C-8), 103.7 (C-13a), 116.5 (C-15), 117.5 (C-2), 121.0 (C-6), 124.0 (C-5), 132.5 (C-1). MS *m/z* 378.146774, $C_{23}H_{22}O_5$ requires 378.146724, (rel. int.): $[M]^+$ 378 (8), 364 (12), 362 (26), 360 (7), 307 (6), 293 (11), 279 (16), 169 (63), 162 (7), 152 (8), 140 (6), 115 (7), 93 (6), 69 (10), 65 (13), 63 (12), 55 (16), 43 (24).

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