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CALOCALABAXANTHONE, THE PUTATIVE ISOPRENYL PRECURSOR OF CALABAXANTHONE FROM *CALOPHYLLUM CALABA*

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Key Word Index: *Calophyllum calaba* var. *calaba*; Guttiferae; xanthone; precursor.

Abstract—The bark of *Calophyllum calaba* var. *calaba* contains a new xanthone, 2,8-di-(3-methylbut-2-enyl)-1,3-dihydroxy-7-methoxyxanthone (calocalabaxanthone), the precursor of 5-hydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2H,6H-pyano-(3,2-b)-xanthen-6-one (calabaxanthone). In addition the bark contains calabaxthone and the other constituents isolated earlier from *Calophyllum calaba* var. *worthingtonii*.

INTRODUCTION

The bark and timber of *Calophyllum calaba* L., a Sri Lankan endemic species belonging to the Guttiferae, has been investigated by Somanathan and Sultanbawa [1]. *C. calaba* grows in both the dry and wet zones of Sri Lanka. Although the dry and wet zone's trees are similar in floral features and fruits, there is some difference in foliar structure, suggesting that the two varieties growing in ecologically diverse habitats may belong to separate subspecies or species. Stevens has recently classified [2] the dry zone plant as *C. calaba* var. *calaba* and the wet zone plant as *C. calaba* var. *worthingtonii*. The *C. calaba* studied by Somanathan and Sultanbawa [1] was obtained from the wet zone of Sri Lanka, and would be more properly named *C. calaba* var. *worthingtonii*. The present work on the bark of *C. calaba* var. *calaba* collected from Palai in the dry zone of Sri Lanka was investigated to determine whether any phytochemical differences exist between the two varieties. The triterpenes taraxerol, taraxerone and β-simiarenol and a diprenylated xanthone, calabaxanthone (**2**), were reported from the bark of the wet zone *C. calaba* whilst the timber extract yielded sitosterol and nine xanthones, scribilitifolic acid, 2-hydroxy-1,8-dimethoxyxanthone, 1,7-dihydroxyxanthone, 6-deoxyjacareubin, 1,6-dihydroxy-5-methoxyxanthone, jacareubin, guanandin, 2,8-dihydroxy-1-methoxyxanthone and 1,5,6-trihydroxyxanthone [1].

Apart from calabaxanthone (**2**) and the three triterpenes isolated from the wet zone *C. calaba* bark, *C. calaba* var. *calaba* yielded calocalabaxanthone (**1a**), a new xanthone, as a minor constituent. Its structure was elucidated to be that of 2,8-di-(3-methylbut-2-enyl)-1, 3-dihydroxy-7-methoxyxanthone (**1a**) by spectroscopic methods and by chemical transformation to calabaxanthone.

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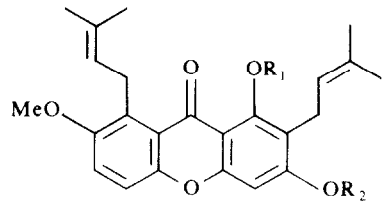
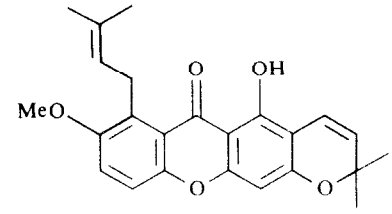
RESULTS AND DISCUSSION

The ^{1}H NMR spectrum of calocalabaxanthone (**1a**) showed the presence of a chelated hydroxy group (δ 13.76), a methoxy group (δ 3.86), two isopentenyl side chains whose protons appeared as high field signals at δ 1.84 (6H), 1.76 (3H), 1.68 (3H), a triplet at δ 5.30 (2H), two doublets at δ 4.15 (2H, $J = 6$ Hz) and 3.46 (2H, $J = 6$ Hz) and aromatic protons as singlets at δ 6.28 (1H) and 7.25 (2H). High resolution mass spectrometry gave the molecular formula as $C_{24}H_{26}O_5$ (M^+ 394.1777). The formation of a diacetate, M^+ 478, indicated the presence of two hydroxy groups. The UV spectrum of calocalabaxanthone was unaltered by the addition of $NaOAc$–H_3BO_3 ruling out the possibility of an *ortho*-dihydroxy system. Although a chelated hydroxy group was present, the UV spectrum was unaffected by the addition of $AlCl_3$. This has been ascribed to crowding around the chelated hydroxy group due to isopentenyl substitution at C-2 [1,3]. Calocalabaxanthone (**1a**) on treatment with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) gave a cyclized product whose ^{1}H NMR spectrum showed the presence of two chromene doublets at δ 6.70 and 5.49 respectively. The benzylic protons of the second isopentenyl side chain in calocalabaxanthone appears at lower field (δ4.15) than those of the isopentenyl side chain which cyclizes (δ 3.46) and the chemical shift is almost unaltered by cyclization. The low field benzylic protons suggest that this side chain is at C-8 close to the xanthone carbonyl group. Furthermore, the C-2 isopentenyl side chain must be involved in the cyclization since the two chromene doublets appear at relatively high field. Cyclization of a C-8 isopentenyl group with a C-7 hydroxy group will give a chromene ring with its benzal proton appearing as a doublet at *ca* δ 8 [3–5]. The ^{1}H NMR of the cyclized product showed a chelated hydroxy group indicating that the side chain cyclizes with a hydroxy group at C-3. The one proton singlet at δ 6.28 appears at a high field for an aromatic proton in the xanthone



1a $R_1 = R_2 = H$

1b $R_1 = R_2 = Ac$



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ring system but this would be expected for the C-4 proton with phenolic groups *ortho* and *para* to it. This is further confirmed by the positive Gibbs test shown by the compound, and the striking ^1H NMR spectral paramagnetic shift of $\Delta\delta$ 0.71 seen for the C-4 proton on complete acetylation to the diacetate (**1b**). Although the other two aromatic protons coincide in a singlet at δ 7.25 in the ^1H NMR spectrum of calocalabaxanthone in $CDCl_3$, this signal appears as two doublets at δ 7.27 and 7.48 when the spectrum is run in acetone-d_6. The coupling constant of $J = 10$ Hz shown by these doublets indicates an *ortho*-relationship, which could be 5,6 with the methoxy group at C-7 or 6,7 with the methoxy group at C-5. If the former were the case the cyclized product would be identical to calabaxanthone (**2**). Our cyclized product was found to be identical with an authentic sample of this xanthone.

A single singlet for the two aromatic protons at C-5 and C-6 in the ^1H NMR spectra in $CDCl_3$ and CCl_4 when C-7 is oxygenated and C-8 prenylated has been generally observed in these systems [1, 4] and shown to be so in 1-hydroxy-6′6′-dimethyl pyrano-(2′,3′:7,8)-xanthone synthesized by Gabriel and Gottlieb [5]. Calocalabaxanthone should therefore have the structure 2,8-di-(3-methylbut-2-enyl)-1,3-dihydroxy-7-methoxyxanthone (**1a**). Though calabaxanthone has been reported in six *Calophyllum* species [6], this is the first report of calocalabaxanthone whose co-occurrence with calabaxanthone in *C. calaba* var. *calaba* strongly suggests it to be the putative isoprenyl precursor [7] of calabaxanthone. Calocalabaxanthone is a trioxygenated diprenylated xanthone. The only other known member of this class, 8-deoxygartanin, was isolated from the fruits of *Garcinia mangostana* [8].

EXPERIMENTAL

UV spectra were measured in EtOH and the IR spectra were obtained in KBr discs. ^1H NMR spectra were measured at 60 MHz. Low and high resolution mass spectra were obtained from the University of Tasmania. Analyt. TLC and prep. TLC were carried out with Si gel (Merck). Separation by column chromatography was carried out using Si gel (30–70 mesh) (Koch–Light). Mps are uncorr.

The bark of *C. calaba* was obtained from Palai, from the northern dry zone of Sri Lanka. The air-dried, chipped and milled bark was successively extracted with petrol, $CHCl_3$ and MeOH. The $CHCl_3$ extract (14 g) was chromatographed over Si gel (420 g). Elution of the column with petrol–C_6H_6 (9:1) afforded deep yellow crystals (from EtOH) of calabaxanthone (188 mg), mp 172° (lit. [1] mp 172°), whilst petrol–C_6H_6 (7:3) gave taraxerol (142 mg), mp 278–279° (lit. [1] mp 279–280°) and taraxerone (116 mg), mp 238° (lit. [1] mp 239–240°); confirmed by comparison with authentic material (mmp, IR and Co-TLC).

Isolation of calocalabaxanthone. Elution with C_6H_6–petrol (2:1) gave calocalabaxanthone contaminated with taraxerol. Repeated prep. TLC gave calocalabaxanthone (84 mg) as yellow-orange needles from EtOH, mp 164–166°, M^+ 394.1777 ($C_{24}H_{26}O_5$, requires M^+ 394.1780). UV λ_{max} nm (log ϵ). 243 (3.9), 263 (3.8), 315 (3.6), 375 (3.1); IR ν_{max}^{KBr} cm^{-1}: 3425, 1630. δ ($CDCl_3$) ppm: 13.76 (1H, *s*, OH), 7.25 (2H, *s*, 5- and 6-H), 6.28 (1H, *s*, 4-H), 5.30 (2H, *t*, CH_2–C<u>H</u>=), 4.15 (2H, *d*, Ar–C<u>H</u>$_2$ $J = 6$ Hz), 3.86 (3H, *s*, OMe), 3.46 (2H, *d*, Ar–CH_2, $J = 6$ Hz), 1.84 (6H, *s*, 2Me), 1.76 (3H, *s*, Me), 1.68 (3H, *s*, Me); δ (acetone-d_6) ppm: 7.48 (1H, *d*, $J = 10$ Hz), 7.27 (1H, *d*, $J = 10$ Hz), 6.43 (1H, *s*), 5.3 (2H, *m*), 4.15 (2H, *d*, $J = 8$ Hz), 3.93 (*s*, OMe), 3.46 (2H, *d*, $J = 8$ Hz), 1.83 (3H, *s*), 1.66 (3H, *s*), 1.30 (6H, *s*). MS *m/z* (rel. int.) 394 (100), 379 (2), 351 (72), 339 (27), 338 (48), 323 (38), 317 (20), 295 (26).

Diacetoxycalocalabaxanthone. Calocalabaxanthone (50 mg) was refluxed with Ac_2O–pyridine (1:3, 4 ml) at 100° for 18 hr. Work-up and prep. TLC gave diacetoxycalocalabaxanthone, mp 132–134° (from MeOH), M^+ 478 (100); IR ν_{max}^{KBr} cm^{-1}: 1775, 1760, 1645, 1600, 1260, 1180 (*br*). ^1H NMR δ ($CDCl_3$) ppm: 7.02 (2H, *s*), 6.99 (1H, *s*), 5.05 (2H, *m*), 3.97 (2H, *d*, $J = 7$ Hz), 3.72 (3H, *s*, OMe), 3.19 (2H, *d*, $J = 7$ Hz), 2.43 (3H, *s*, OCOMe), 2.22 (3H, *s*, OCOMe), 1.79 (3H, *s*, Me), 1.65 (9H, *s*, 3Me).

Cyclization of calocalabaxanthone (**1a**). **1a** (30 mg) was refluxed with DDQ (30 mg) in C_6H_6 (5.0 ml) for 15 hr. The product was concd and subjected to prep. TLC to give calabaxanthone (**2**) (21 mg) as yellow needles from EtOH, mp 171–173° (lit. [1], mp 172°). Identical to authentic sample (mmp, IR, ^1H NMR, MS and co-TLC).

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A BERBINE ALKALOID, LIENKONINE FROM *CORYDALIS OCHOTENSIS**

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Key Word Index—*Corydalis ochotensis*; Fumariaceae; alkaloid; berbine-type; lienkonine.

Abstract—Lienkonine, a further alkaloid from *Corydalis ochotensis*, has been isolated and elucidated as (−)-2,3,10-trimethoxy-8α-methyl-13aαH-berbine-9-ol by the spectral data and chemical properties.

INTRODUCTION

Besides protopine, an ordinary alkaloid of *Corydalis* spp., several alkaloids from *Corydalis ochotensis* Turcz (Fumariaceae) have been isolated previously[1]. There are yenhusomine, yenhusomidine and ochotensimine which belong to the spirobenzylisoquinoline type; corytenchine (**1**), corytenchirine (**2**) and didehydrocheilantifoline which belong to the berbine type and adlumidine which belongs to the phthalideisoquinoline type. Here we report a further new berbine alkaloid, lienkonine, isolated from this plant.

RESULTS AND DISCUSSION

The base D, a molecular compound of corytenchine (**1**) and corytenchirine (**2**) formerly separated from part A shown in the scheme of the previous paper [1] was also found in part B. After separating base D from part B, the HCl salt was obtained while the mother liquid was acidified with HCl. When this salt was converted into free base, lienkonine was produced.

Owing to the positive Gibbs test and the information from IR and UV spectra, lienkonine was indicated as a phenolic berbine derivative. The ^{1}H NMR (60 MHz, $CDCl_3$) spectrum shows the presence of three methoxyl groups at δ3.85 (6H, *s*, 2×OMe) and 3.88 (3H, *s*, 1×OMe), four aromatic protons at δ6.65 (1H, *d*, J = 6 Hz), 6.62 (1H, *s*), 6.69 (1H, *s*) and 6.77 (1H, *d*, J = 6.5 Hz), one methyl group centred at δ1.43 (3H, *d*, J = 6.5 Hz) and one hydroxyl group at δ5.75 (1H, *br s*); thus lienkonine is of the 2,3,9,10-oxygenated berbine type. Methylation of lienkonine with diazomethane produced a colourless, oily base whose ^{1}H NMR spectrum shows four methoxyl groups which progressively supported one phenolic group in the lienkonine molecule. The mass spectrum (70 eV) exhibited a molecular ion at *m/z* 355 and fragment ions at *m/z* 340, 192 [ion (**6**)], 190 [ion (**7**)] and 164 [ion (**8**)] which indicated that one hydroxyl group must be attached to ring D [2]. On the other hand, the signals for methyl groups centred at δ1.43 in the ^{1}H NMR spectrum and the fragment at *m/z* 164 [ion (**8**)] in the mass spectrum of lienkonine suggested that a methyl group may be located at C-8 or C-13.

The stereochemistry of the structures of 2,3,9,10-tetramethoxyberbine and 13-methylberbine (corydaline and mesocorydaline) have already been studied extensively. The spectral data of these diastereomers have been reported [3–7] but the IR ($CHCl_3$) and ^{1}H NMR spectra of *O*-methyllienkonine are different from those of (±)-corydaline and (±)-mesocorydaline which have been synthesized by the methods reported in the literature [8, 9].

In order to determine the stereochemistry of *O*-methyllienkonine, the IR and ^{1}H NMR spectra were compared with *O*-methylcorytenchirine whose steric structure has already been decided [1]. Signals due to the 8-methyl group of *O*-methyllienkonine appeared at δ1.43 (3H, *d*, J = 6.5 Hz) and are similar to those of *O*-methylcorytenchirine (δ1.40, 3H, *d*, J = 6.5 Hz), but are different from the chemical shifts of aromatic protons. In the IR spectra, both *O*-methylcorytenchirine and *O*-methyllienkonine show no Bohlmann band absorption in the 2800–2700 cm^{-1} regions. In accordance with these facts, the B/C ring junction of *O*-methyllienkonine must be a *cis*-fused system similar to *O*-methylcorytenchirine. Therefore, the steric relationship of hydrogens at the C-8 and C-13a

*Part VII in the series "Studies on the Alkaloids of Formosan *Corydalis* Species". For Part VI see ref. [1].