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CO-OCCURRENCE OF PRENYLATED XANTHONES AND THEIR CYCLIZATION PRODUCTS IN CELL SUSPENSION CULTURES OF *HYPERICUM PATULUM**

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Key Word Index—*Hypericum patulum*; Guttiferae; xanthones; 5,9,11-trihydroxy-3,3-dimethyl-8-(3-methyl-2-butenyl)-3H,12H-pyrano[3,2-a]xanthen-12-one; garcinone B; 1,3,6,7-tetrahydroxy-8-(3-methyl-2-butenyl)-9H-xanthen-9-one; cell suspension cultures; elicitation.

Abstract—A new xanthone named paxanthone B, 5,9,11-trihydroxy-3,3-dimethyl-8-(3-methyl-2-butenyl)-3H,12H-pyrano[3,2-a]xanthen-12-one, has been isolated from callus tissues of *Hypericum patulum*, together with the known garcinone B and 1,3,6,7-tetrahydroxy-8-(3-methyl-2-butenyl)-9H-xanthen-9-one. Their structures were elucidated using spectral techniques.

INTRODUCTION

In our preceding communication, we reported the isolation and structural determination of paxanthone (**2**), toxyloxanthone B (**1**) and γ-mangostin (**5**) in cell suspension cultures of *Hypericum patulum* [1]. A further investigation of the methanol extract from the callus tissues has now provided three xanthones, including a new xanthone, paxanthone B (**3**). Of the three identified xanthones, two, i.e. garcinone B (**4**) from *Garcinia mangostana* (Guttiferae) [2] and 1,3,6,7-tetrahydroxy-8-(3-methyl-2-butenyl)-9H-xanthen-9-one (**6**) from *H. androsaemum* [3], have previously been recognized as natural products. These xanthones are distinguished by mono- or diprenylation, or by the presence of a dimethyl chromene ring.

Cyclization of the prenyl side-chain to an adjacent hydroxyl group could lead to the corresponding dimethyl chromene derivatives. The co-occurence of **6**, **1** and **2** is of biogenetic interest, **6** may act as a precursor for **1** and **2**. Also, **5** and **4** are interrelated in that cyclization of the prenyl side (3,3-dimethyl allyl)-chain of **5** with the neighbouring phenolic hydroxyl group results in the formation of **4**. Probable biogenetic relationships between the prenylated xanthones and their dimethyl chromene derivatives are briefly discussed.

RESULTS AND DISCUSSION

Callus tissue cultures were established from flowers of *H. patulum* and cultured in the dark on Linsmaier–Skoog medium containing 2,4-D and kinetin. Dried callus tissues were extracted with methanol. The methanol extract was dissolved in a small amount of water and partitioned between ethyl acetate and water. Three compounds, **3**, **4** and **6** were isolated from the ethyl acetate fraction by flash chromatography followed by recrystallization.

Compound **3** gave a peak at *m/z* 394 in the positive EI-mass spectrum. The IR spectrum suggested the presence of phenolic hydroxyl groups (3420 cm^{-1}) and a hydrogen bonded carbonyl group (1630 and 1610 cm^{-1}). The UV spectrum (244, 266, 315, 326sh, 383, 397sh nm) was that of an angular chromenoxanthone [4]. The ^1H NMR spectrum of **3** showed the presence of a 3,3-dimethylallyl substituent, which was confirmed by two singlets at δ 1.65 and 1.88 for the vinyl methyls, a triplet at δ 5.29 (1H) for the vinylic proton and a doublet at δ 3.49 (2H) for the benzylic protons. Furthermore, the two aromatic proton signals and a hydrogen bonded hydroxyl signal appeared as three singlets at δ 6.30 (H-10), 6.88 (H-6) and 13.30 (OH-11). The signals at δ 1.47 (6H, *s*), 5.93 (1H, *d*) and 8.07 (1H, *d*) in the spectrum were indicative of a 2,2-dimethyl pyran ring. The angular fusion of the chromene ring was deduced from the low field shift [5] of H-1 (δ 8.07), which is located in the deshielding area of the carbonyl group. Therefore, the possible structure of this compound was **3** or **4**. The spectral features of **3**, however, differed from the reported data of garcinone B (**4**) [2]. The chemical shift values of C-10 in the ^{13}C NMR spectra for paxanthone (**2**) and toxyloxanthone B (**1**) were δ 99.0 and 98.8, respectively, and those of C-8 were δ 94.0 in both compounds [1]. In the ^{13}C NMR spectrum of γ-mangostin (**5**), the signal at δ 93.0 was assigned to the unsubstituted C-4, and the signal due to the prenylated C-2 appeared at δ 111.3 in the same benzene ring [1]. On the basis of the above results, the signal at δ 98.4 of paxanthone B could be

*Part 2 in the series 'Xanthones in cell suspension cultures of *hypericum patulum*.' For Part 1, see ref. [1].

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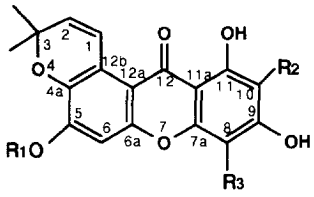
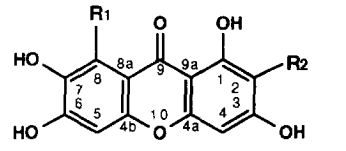


1 : R1 = H R2 = H R3 = H
2 : R1 = CH3 R2 = H R3 = H
3 : R1 = H R2 = H R3 =
4 : R1 = H R2 = R3 = H



5 : R1 = R2 =
6 : R1 = R2 = H

assigned to the unsubstituted carbon (C-10), indicating that a prenyl group was located at C-8. Hence, paxanthone B can be concluded to have structure **3**.

The other compounds, **4** and **6**, were identical with garcinone B [2] and 1,3,6,7-tetrahydroxy-8-(3-methyl-2-butenyl)-9H-xanthen-9-one [3], respectively, by comparison of their spectral data. Although they have been isolated from *G. mangostana* and *H. androsaemum*, respectively, these compounds have been isolated from cell suspension cultures of *H. patulum* for the first time.

HPLC protocols give high resolution chromatograms to allow the simultaneous analysis of the six xanthones (**1–6**) in unfractionated extracts (see Experimental). The methods are simple, sensitive and highly reproducible. The examination of such profiles over time would provide a highly dramatic view of the many concurrent molecular events which may relate to biological phenomena. Monoisoprenyl xanthones, 1,3,6,7-tetrahydroxy-8-prenyl xanthone, toxyloxanthone B and paxanthone, have R_ts of 9.6–15.9 min. Diisoprenyl derivatives, paxanthone B, γ-mangostin and garcinone B, have R_ts of 29.0–39.6 min. Among two groups of xanthones, the dimethyl chromene derivatives, toxyloxanthone B and garcinone B, have longer R_ts than their corresponding prenylated xanthones.

We now have the experimental tools to determine the levels of xanthones and their metabolic shifts induced by yeast elicitor (YE) (0.1 g 300 mol^{-1}) for 19 days of growth phase. Respective changes of the ratios of the integration units for the peak area at 262 nm between **6** and **2** (4:1), and that of the pair **5**/**4** (8.5:1), to (0.8:1) and (2.5:1) indicate that treatment of cultures with YE resulted in reductions of **6** and **5**, with a concomitant increase in **1** and **4** of R_ts of 10.7 and 39.6 min. These results show that the cyclization of the isoprenyl side-chain or the activation of cyclases are stimulated in response to environmental stress.

The elicitor-mediated induction or activation of cyclase which catalyses the transformation of prenyl to chromene, has been described in soybean tissue cultures [6, 7]. By analogy to glyceollin formation from its prenylated precursor, it seems likely that the conversion of the prenylated xanthones, **6** and **5** to **1** and **4**, respectively, involves cytochrome P450-dependent monooxygenase.

EXPERIMENTAL

Plant material. Hypericum patulum Thunb. was planted in our university medicinal plant garden and identified by Dr G. Yoneda (Faculty of Pharmaceutical Sciences, Osaka University). A voucher specimen is kept in our laboratory. Callus tissue cultures were established from flowers cultured in the dark on Linsmaier–Skoog medium containing 10^{-5} M 2,4-D and 10^{-7} M kinetin.

Extraction and isolation. Dried callus tissues (dry wt 525.8 g) were extracted with MeOH. The MeOH extract (185.7 g) was partitioned between EtOAc and H_2O. The EtOAc fr. (11.6 g) was subjected to repeated flash CC on silica gel using $CHCl_3$–MeOH and benzene–EtOAc gradient systems followed by LH-20 with MeOH to afford **3** (3.1 mg), **4** (2.2 mg) and **6** (6.5 mg).

Paxanthone B (**3**). Yellow needles, mp 239–241° (benzene–Me_2CO). Positive EIMS: *m/z* 394 $[M]^+$. IR ν_{max}^{KBr} cm^{-1}: 3420, 2950, 2900, 1630, 1610, 1570, 1490, 1450, 1420. UV λ_{max}^{MeOH} nm (log ε): 244 (4.35), 266 (4.38), 315 (4.21), 326 (4.22), 383 (3.71), 397sh (3.69). ^{1}H NMR [500 MHz, $(CD_3)_2CO$]: δ 1.47 (6H, *s*, 2 × Me), 1.65 (3H, *s*, Me-16), 1.88 (3H, *s*, Me-17), 3.49 (2H, *d*, *J* = 7.3 Hz, H-13), 5.29 (1H, *t*, *J* = 7.3 Hz, H-14), 5.93 (1H, *d*, *J* = 10.4 Hz, H-2), 6.30 (1H, *s*, H-10), 6.88 (1H, *s*, H-6), 8.07 (1H, *d*, *J* = 10.4 Hz, H-1), 13.30 (1H, *s*, OH). ^{13}C NMR [125 MHz, $(CD_3)_2CO$]: δ 18.0 (Me, C-16), 22.2 (C-13), 25.9 (Me, C-17), 27.2 (2 × Me, C-3), 76.8 (C-3), 98.4 (C-10), 103.5 (C-6), 104.1 (C-11a), 106.5 (C-8), 108.3 (C-12a), 120.9 (C-12b), 121.6 (C-1), 123.5 (C-14), 131.6 (C-15), 133.5 (C-2), 138.9 (C-4a), 153.8 (C-6a), 154.2 (C-7a), 155.3 (C-5), 162.4 (C-9), 162.9 (C-11), 183.4 (C-12).

Garcinone B (**4**). Yellow needles, mp 185–187° (benzene–Me_2CO). Positive EIMS: *m/z* 394 $[M]^+$. IR ν_{max}^{KBr} cm^{-1}: 3450, 3050, 2900, 1645, 1625, 1595, 1570, 1490, 1450, 1410. UV λ_{max}^{MeOH} nm (log ε): 243 (4.42), 263 (4.42), 316 (4.29), 323 sh (4.29), 332 (4.31), 378 (3.71), 394sh (3.68). ^{1}H NMR [500 MHz, $(CD_3)_2CO$]: δ 1.46 (6H, *s*, 2 × Me), 1.65 (3H, *s*, Me-16), 1.78 (3H, *s*, Me-17), 3.36 (2H, *d*, *J* = 7.3 Hz, H-13), 5.29 (1H, *t*, *J* = 7.3 Hz, H-14), 5.93 (1H, *d*, *J* = 10.4 Hz, H-2), 6.42 (1H, *s*, H-8), 6.80 (1H, *s*, H-6), 8.07 (1H, *d*, *J* = 10.4 Hz, H-1), 9.30 (2H, *s*, OH), 13.67 (1H, *s*, OH-11).

1,3,6,7-*Tetrahydroxy*-8-(3-*methyl*-2-*butenyl*)-9*H*-*xanthene*-9-*one* (**6**). Yellow powder, mp 203–206° (benzene–

Me_2CO). EIMS: *m/z* 328 $[M]^+$. IR ν_{max}^{KBr} cm^{-1}: 3400–3300, 2970, 2900, 1650, 1605, 1570, 1500. UV λ_{max}^{MeOH} nm (log ε): 240 (4.40), 254 (4.48), 305 (4.28), 360 (3.95). ^1H NMR [500 MHz, $(CD_3)_2CO$]: δ 1.64 (3H, *s*, Me-14), 1.84 (3H, *s*, Me-15), 4.18 (2H, *d*, J = 6.7 Hz, H-11), 5.33 (1H, *t*, J = 6.7 Hz, H-12), 6.17 (1H, *d*, J = 1.8 Hz, H-2), 6.28 (1H, *d*, J = 1.8 Hz, H-4), 6.79 (1H, *s*, H-5), 13.70 (1H, *s*, OH). ^{13}C NMR [125 MHz, $(CD_3)_2CO$]: δ 18.3 (Me, C-15), 26.0 (Me, C-14), 26.4 (C-11), 93.7 (C-4), 98.5 (C-2), 101.2 (C-5), 103.9 (C-9a), 111.4 (C-8a), 124.7 (C-12), 128.3 (C-8), 131.2 (C-13), 142.3 (C-7), 154.0 (C-4b), 154.0 (C-6), 158.1 (C-4a), 164.6 (C-1), 165.0 (C-3), 183.1 (C-9).

HPLC analysis. Cells were harvested 19 days after addition of yeast elicitor. Dried callus tissues were extracted with $CHCl_3$. The $CHCl_3$ extract was evapd to dryness under red. pres. and then dissolved in MeOH. The MeOH soln was filtered and the filtrate used directly for HPLC. Injections of 5 μl were made on a reverse-phase column (μ-Bondasphere RP-18, 3.9 mm i.d. × 150 mm 5 μm particle size, Waters). Chromatography was achieved using MeOH–5% aq. HOAc, gradient from 68 to 75% MeOH in 10 min. A flow rate of 0.9 ml min^{-1} was used. Spectrophotometric detection was at 262 nm. Under these conditions, base line resolution was achieved on all of the metabolites of interest.

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