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A XANTHONE SUBSTITUTED WITH AN IRREGULAR MONOTERPENE IN CELL SUSPENSION CULTURES OF *HYPERICUM PATULUM**

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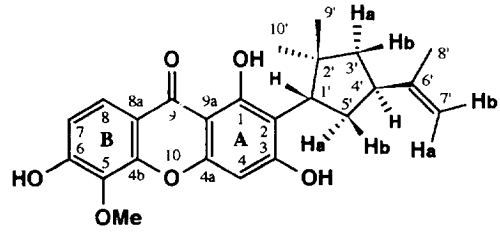
Key Word Index—*Hypericum patulum*; Guttiferae; xanthones; 1,2,6-trihydroxy-8-methoxy-2- (2',2'-dimethyl-4'-isopropenyl)-cyclopentanyl-xanthone; paxanthonin; morusignin D.

Abstract—A new xanthone named paxanthonin, 1,2,6-trihydroxy-8-methoxy-2-(2',2'-dimethyl-4'-isopropenyl)-cyclopentanyl-xanthone, has been isolated from the callus tissues of *Hypericum patulum* together with morusignin D. The structures of both compounds were elucidated by spectroscopic methods.

INTRODUCTION

Previously we have reported on the isolation and structure elucidation of six prenylated xanthones from the methanol extract of the callus tissues of *Hypericum patulum* [1, 2], and discussed the possible biosynthetic relationships between these xanthones [2]. A further investigation of the chloroform extract from the callus tissues has now revealed two 1, 3, 5, 6-tetraoxygenated xanthones including a new xanthone, paxanthonin (**1**). We report now on the isolation and structural elucidation of **1** together with known morusignin D (**2**).



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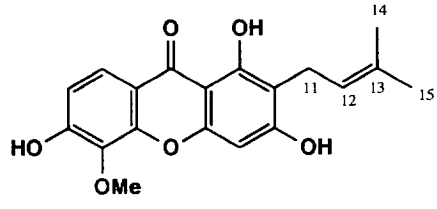

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RESULTS AND DISCUSSION

Compound **1** gave a peak at *m/z* 410 in the positive EI-MS spectrum. The IR spectrum suggested the presence of phenolic hydroxyl groups (3550 and 3490 cm^{-1}) and a hydrogen bonded carbonyl group (1638 cm^{-1}). The ^1H NMR spectrum of **1** showed the presence of three aromatic protons. One gave rise to a singlet at δ6.59, while the other two appeared as a set of one-proton doublets at δ7.00 and 7.85 showing an *ortho*-coupling (J = 8.6 Hz). The other proton signals were observed at δ3.99 (OMe) and 13.8 (hydrogen bonded OH).

The ^{13}C NMR spectrum of **1** was analysed by comparing it with the spectra of paxanthone, γ-mangostin and 1,3,6,7-tetrahydroxy-8-prenylxanthone. The chemical shift values of the carbon atoms in the A ring were similar to those of the relevant carbon atoms of γ-mangostin, suggesting that C-2 was substituted. HMBC cross-peaks were observed between H-4 (δ6.59) and C-2 (δ111.6), C-3 (δ165.0), C-4a (δ156.4) and C-9a (δ103.0). These two and three-bond HMBC correlations unambiguously indicated that C-2 was attached to the other carbon unit. However, irradiation of the lowest field signal (δ_H13.80) owing to the chelated hydroxy group which must be located at C-1 unexpectedly enhanced H-4 by 1.51% and H-1' (δ3.70) by 0.74% in a NOED experiment. This remains a problem to be clarified.

In the ^{13}C NMR spectrum of **1**, the lower chemical shift value of the methoxyl carbon atom (δ61.8) indicated the methoxyl group to be di-*ortho*-substituted [3]. The HMBC spectrum of **1** showed correlation of the methoxyl protons (δ3.99) with the aromatic carbon (δ135.5), and H-7 (δ7.00) showed cross-peaks with the aromatic carbon (δ135.5) and C-8a (δ115.2). HMBC cross-peaks were also

*Part 3 in the series 'Xanthones in cell suspension cultures of *Hypericum patulum*'. For Part 2 see ref. [2].

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observed between H-8 (δ7.85), which *ortho*-coupled with H-7 (δ7.00), and C-6 (δ156.9) and C-4b (δ151.4), establishing the location of the methoxy group at position C-5 (δ135.5). These results indicated that **1** is a 1,3,5,6-tetrahydroxyxanthone derivative having a substituted group at C-2.

Although this substituted group had 10 carbon atoms and 17 hydrogen atoms (C_{10} H_{17}), it was not a geranyl group because only one double bond was detected in this fragment indicating the presence of a five- or six-membered ring structure.

The ^1H, ^{13}C and ^1H–^{13}C COSY NMR spectra of **1** showed the presence of three tertiary methyl groups [δ_H1.78 (H-8′), 1.11 (H-9′) and 0.97 (H-10′); δ_C 21.3, 30.6 and 25.1 (C-8′, C-9′ and C-10′)], two quaternary carbons [δ45.9 (C-2′) and 150.3 (C-6′)], a $>C=CH_2$ unit [δ_H 4.67 (H-7′b) and 4.77 (H-7′a); δ_C108.3 (C-7′)], two methylenes [δ_H1.61 (H-3′b) and 1.72 (H-3′a), and δ_H1.71 (H-5′a) and 3.00 (H-5′b); δ_C48.4 (H-3′) and 33.3 (C-5′)] and two methines [δ_H3.70 (H-1′) and 3.08 (H-4′); δ_C44.3 (C-1′) and 44.9 (C-4′)]. These data were consistent with the presence of a five-membered ring. Two methyl signals as singlets (δ_H1.11 and 0.97) and a quaternary carbon singlet (δ45.9) suggested the presence of a *gem*-dimethyl group. Consequently, it appeared that the remaining methyl group (δ_H1.78) and a $>C=CH_2$ unit comprised a $Me-C=CH_2$ group.

From the above NMR data, the nature of the substitutions attached to the five-membered ring was established. The orientation of the substituents and the stereochemistry of the monoterpene moiety were deduced based on the following results.

The presence of a carbon unit made up of a quaternary carbon (δ45.9), one of two methine carbons (δ_H3.70, δ_C 44.3) and of two methylene carbons (δ33.3) in a cyclopentane ring was shown by the double doublet proton signal (δ3.70) due to the methine proton. The multiplicity of the proton signal [δ1.61 (H-3′b)] showed the presence of another carbon unit composed of a methine carbon (δ44.9), a methylene carbon (δ48.4) and a quaternary carbon (δ45.9). The ^1H–^1H correlation data (Table 1) confirmed the assignment of all the protons belonging to the monoterpene moiety. HMBC cross-peaks were observed between the methyl proton (δ0.97), one of *gem*-methyls, and C-1′ (δ44.3), C-2′ (δ45.9), C-3′ (δ48.4) and C-10′ (δ25.1), and another *gem*-methyl proton (δ1.11) also showed the same cross-peaks. These two and three-bond HMBC correlations allowed placement of C-2′ adjacent to C-1′ and C-3′. H-3′b (δ1.61) showed HMBC correlations to C-2′, C-4′ (δ44.9), C-9′ (δ30.6) and C-10′ (δ25.1). HMBC cross-peaks were observed between Me-8′ (δ_H1.78) and C-4′ (δ44.9), C-6′ (δ150.3) and C-7′ (δ108.3), indicating the location of the isopropenyl group at C-4′. H-1′ (δ3.70) gave cross-peaks with C-1 (δ163.2), C-2, C-3, C-2′, C-5′ (δ33.3), C-9′ and C-10′, indicating that C-2 was attached to C-1′ and that the *gem*-dimethyl group was located at C-2′. The correlations described above led to extensive tracing of the carbon skeleton as shown in formula **1.**

Table 1. ^1H–^1H correlation data of the monoterpene moiety of compound **1**

H		Correlation with δ
1′	3.70 *dd* (7.9, 11)	1.77/3.00
3′b	1.61 *t* (11)	1.72/3.08
4′	3.08 *m*	1.61/1.72/3.00
5′b	3.00 *m*	1.72/3.08/3.70
8′	1.78 *s*	4.67/4.77
7′a	4.67 *br s*	1.78/4.67
7′b	4.77 *br s*	1.78/4.77
9′	1.11 *s*	0.97
10′	0.79 *s*	1.11
3′a/5′a	1.72 *m*	1.61/1.72/3.00/3.08/3.70

Coupling constants (J in Hz) are given in parentheses.

In the NOED experiment, irradiation of methyl proton (δ1.11), one of *gem*-methyls, enhanced H-1′ by 7.22% and the H-3′b intensity by 4.73%, suggesting their *syn* relationship and the proximity of this methyl group to H-1′ and H-3′b. These results also indicated that C-1′ should be connected to C-2 of the xanthone nucleus. A 6.02% intensity enhancement of H-4′ (δ3.08) upon irradiation of another *gem*-methyl proton (δ0.97) revealed their *syn* relationship. Irradiation of methyl proton (δ1.78) enhanced H-7′b (2.34%) and the H-5′b (δ3.00) (1.50%). The lack of enhancement of H-4′ (δ3.08) suggested that this methyl group was *anti* to H-4′.

Furthermore, in the decoupling difference spectrum, irradiation at the frequency of H-1′ (δ3.70) collapsed H-5′b to a triplet. Although H-3′a was overlapped with H-5′a, a clear doublet (J = 11 Hz) could be seen in a decoupling difference experiment involving irradiation of H-4′ (δ3.08, *m*). In addition, a triplet (δ1.61, H-3′b) was also collapsed to a doublet (J = 11 Hz). Irradiation at the frequency of H-3′b (δ1.61, *t*) collapsed the H-4′ (δ_H3.08, *m*) to a quartet, and a multiplet (δ_H3.00, H-5′b) was changed to a quartet, indicating the presence of a ^4J-coupling between H-3′b and H-5′b (W-arrangement).

From the above results, the structure of the monoterpene moiety was confirmed to be (1*R**,4*S**)-2,2-dimethyl-4-isopropenyl cyclopentane. Thus, the formula **1** was proposed which has not been reported previously in the literature and which we have named paxanthonin.

Compound **2** gave a peak at *m/z* 342 in the positive EI-mass spectrum, and had UV and IR spectra which were in agreement with a xanthone nucleus. The ^1H and ^{13}C NMR spectra for the xanthone skeleton resembled those of paxanthonin (**1**), but differed in the signals of the substituted fragment at C-2. Compound **2** had signals characteristic of two vinyl methyl groups [δ1.79 (H-15) and 1.65 (H-14); δ 25.9 (C-15) and 17.9 (C-14)], a trisubstituted double bond [δ 5.29 (H-12); δ123.3 (C-11) and 131.6 (C-13)] and a methylene group [δ3.38 (H-11); δ22.0 (C-11)]. These values were in agreement with the presence of a 3,3-dimethyl allyl group. Spectral analysis of **2** showed that the structure of **2** was identical to that of morusignin D previously isolated from *Morus insignis* Bur. (Moraceae) [4].

Co-occurence of 1,3,5,6- and 1,3,6,7-tetraoxygenated xanthones has been reported from Guttiferae [5] and Moraceae [6], and in this study their co-existence in cell suspension cultures of *H. patulum* has been shown for the first time. On the basis of the isolation of maclurin, 2,4,6,3′,4′-pentahydroxy benzophenone, together with 1,3,5,6- and 1,3,6,7-tetrahydroxyxanthone from the heartwood of *Symphonia globulifera* (Guttiferae), the biogenetic role of maclurin as a precursor for xanthones has been discussed [7]. The first tracer studies on the biosynthesis of xanthones in the Guttiferae have been reported [8]. Cinnamic acid, benzoic acid, *m*-hydroxybenzoic acid and 4′-deoxymaclurin as well as malonic acid were efficient precursors to mangostin in *Garcinia mangostana*, suggesting that the 1,3,5,6- and 1,3,6,7-tetraoxygenated xanthones may be formed by the 6-oxygenation of either the 1,3,5- or the 1,3,7-trioxygenated xanthone derived from 4′-deoxy maclurin [9]. Although it remains to be seen whether prenylation or cyclopentane ring addition occurs at the benzophenone or xanthone stage. The prenylated xanthones [1, 2], paxanthonin and morsignin D, might be biosynthesized from 4′-deoxymaclurin and / or maclurin in *H. patulum* callus.

EXPERIMENTAL

Spectral data. NMR: 125 MHz for ^{13}C and 500 MHz for ^{1}H in $(CD_3)_2CO$ (TMS as an int. standard) unless otherwise stated. HMBC: 125 MHz with $J = 9$ Hz in $(CD_3)_2CO$.

Plant material. Hypericum patulum Thunb. was planted and grown in our university medicinal plant garden and was verified by Dr G. Yoneda (Faculty of Pharmaceutical Sciences, Osaka University, Japan). 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 (910 g) were extracted with $CHCl_3$. The $CHCl_3$ extract (15.6 g) was fractionated by silica gel CC eluting with $CHCl_3$–MeOH. A fraction eluted with $CHCl_3$–MeOH (30:1) was further flash chromatographed on silica gel using a *n*-hexane–EtOAc gradient system followed by Sephadex LH-20 with MeOH to afford **1** (6.1 mg) and **2** (1.5 mg).

Paxanthonin (**1**). Yellow powder. Positive EI-MS: *m/z* 410 $[M]^+$. IR ν_{max}^{KBr} cm^{-1}: 3550, 3490, 3150–3300, 2920, 1638, 1605, 1585; UV λ_{max}^{MeOH} nm (log ε): 246 (4.12), 259sh (3.87), 281 (3.43), 320 (3.89), 363sh (3.27). $[\alpha]_D$ − 26.22° [$(Me)_2CO$; *c* 0.305]; ^{1}H NMR [500 MHz, $(CD_3)_2CO$]: δ0.97 (3H, *s*, Me-10′), 1.11 (3H, *s*, Me-9′), 1.61 (1H, *t*, $J_{a,b} = J_{3'b,4'} = 11$, H-3′b), 1.71 (H, *m*, H-5′a), 1.72 (H, *d*, $J_{a,b} = 11$, H-3a′), 1.78 (3H, *s*, H-8′), 3.00 (1H, *m*, H-5′b), 3.08 (1H, *m*, H-4′), 3.70 (1H, *dd*, $J_{1',5'b} = 7{,}9$, $J_{1',5'a} = 11$), 3.99 (3H, *s*, OMe-S), 4.67 (1H, *br s*, H-7′a), 4.77 (1H, *br s*, H-7′b), 6.59 (1H, *s*, H-4), 7.00 (1H, *d*, $J_{7,8} = 8.6$, H-7), 7.85 (1H, *d*, $J_{8,7} = 8.6$, H-8), 13.80 (1H, *s*, OH-1). (The assignments and coupling constants were determined by decoupling difference spectra); ^{13}C NMR [125 MHz, $(CD_3)_2CO$]: δ21.3 (Me, C-8′), 25.1 (Me, C-10′), 30.6 (Me, C-9′), 33.3 (C-5′), 44.3 (C-1′), 44.9 (C-4′), 45.9 (C-2′), 48.4 (C-3′), 61.8 (OMe-5), 94.7 (C-4), 103.0 (C-9a), 108.3 (C-7′), 111.6 (C-2), 114.2 (C-7), 115.2 (C-8a), 122.1 (C-8), 135.5 (C-5), 150.3 (C-6′), 151.4 (C-4b), 156.4 (C-4a), 156.9 (C-6), 163.2 (C-3), 165.0 (C-1), 181.0 (C-9).

Morusignin D (**2**). Yellow needles, mp 220–224° [$(Me)_2CO$]. Positive EI-MS: *m/z* 342 $[M]^+$. IR ν_{max}^{KBr} cm^{-1}: 3050–3150, 2910, 1640, 1610, 1570, 1450; UV λ_{max}^{MeOH} nm (log ε): 245 (4.56), 258sh (4.23), 280 (3.88), 319 (4.27), 360sh (3.74); ^{1}H NMR [500 MHz, $(CD_3)_2CO$]; δ1.65 (3H, *s*, Me-14), 1.79 (3H, *s*, Me-15), 3.38 (2H, *d*, $J_{11,12} = 7.3$, H-11), 3.99 (3H, *s*, OMe-5), 5.29 (1H, *t*, $J = 7.3$, H-12), 6.57 (1H, *s*, H-4), 7.00 (1H, *d*, $J_{7,8} = 9.0$, H-7), 7.83 (1H, *d*, $J_{8,7} = 9.0$, H-8), 13.36 (1H, *s*, 1-OH); ^{13}C NMR [125 MHz, $(CD_3)_2CO$]: δ17.9 (Me, C-14), 22.0 (C-11), 25.9 (Me, C-15), 61.7 (5-OMe), 94.4 (C-4), 103.0 (C-9a), 111.6 (C-2), 114.2 (C-7), 115.2 (C-8a), 122.0 (C-8), 123.3 (C-12), 131.6 (C-13), 135.6 (C-5), 151.5 (C-4b), 156.4 (C-4a), 156.9 (C-6), 163.5 (C-3), 161.6 (C-1), 180.9 (C-9).

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