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A PRENYLATED XANTHONE FROM CELL SUSPENSION CULTURES OF *HYPERICUM PATULUM**

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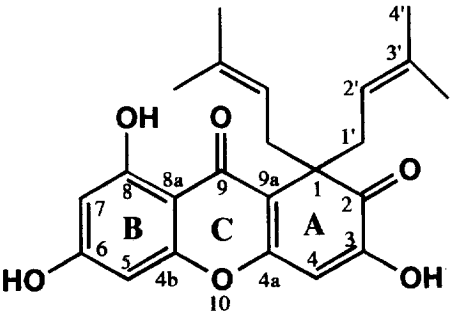
Key Word Index—*Hypericum patulum*; Guttiferae; xanthones; cell suspension culture; 1,2-dihydro-3,6,8-trihydroxy-1,1-bis(3-methylbut-2-enyl)-xanthen-2, 9-dione.

Abstract—A new xanthone patulone, 1,2-dihydro-3,6,8-trihydroxy-1,1-bis(3-methylbut-2-enyl)-xanthen-2,9-dione, has been isolated from the callus tissues of *Hypericum patulum* together with the known compounds 1,3,5,6-tetrahydroxanthone and (−)-epicatechin. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Previously we have reported the isolation and structural determination of eleven prenylated xanthones or their dimethylchromene derivatives from chloroform or methanol extracts of callus tissues of *Hypericum patulum* [1–4]. Further investigation of chloroform and methanol extract from the callus tissues of *Hypericum patulum* has now led to the isolation of a new xanthone, patulone (**1**), together with a known 1,3,5,6-tetrahydroxanthone (**2**) and (−)-epicatechin (**3**).



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RESULTS AND DISCUSSION

Compound **1**, bright yellow needles, has the molecular formula, $C_{23}H_{24}O_6$, which was established by high resolution EI-mass spectrum. The IR spectrum suggested the presence of phenolic hydroxyl group (3300 cm^{-1}), conjugated carbonyl group (1670 cm^{-1}) and xanthone carbonyl group (1643 cm^{-1}). The ^1H NMR spectrum of **1** showed the presence of a hydrogen-bonded hydroxyl (δ 13.89) and three aromatic protons. One of the three protons gave rise to a singlet at δ 6.53, while the other two appeared as a pair of *meta*-coupled protons (δ 6.26 and 6.38). Furthermore, the signals at δ 4.74 (2H, t) for two vinylic protons, at δ 3.43 and 2.80 (each 2H) for two methylene groups, and at δ 1.47 (12H) for four methyl groups, revealed the presence of two identical prenyl substituents. This was confirmed by the mass spectral fragmentation of **1**, which exhibited base peak ions at *m/z* 327 $[M - 69]^+$ representing a loss of a C_5H_9 unit, and at *m/z* 272 $[M - 69 - 55]^+$ due to a further loss of a C_4H_7 fragment. The two prenyl groups were suggested to attach to sp^3 carbon (δ 56.7) since cross-peaks displayed between the methylene protons in the H–H COSY spectrum.

In addition, the ^{13}C and C–H COSY NMR spectra indicated the presence of two carbonyls (δ 180.2 and 201.2) and eight other quaternary carbons. The ^1H and ^{13}C NMR signals for ring B and C (γ-pyrone ring) conform closely to those of 1,3,6,7-tetrahydroxy-8-isoprenyl xanthone, previously isolated from the same culture [3, 5].

The substitution pattern of ring A was established with a aid of HMBC experiment (Fig. 1). The carbonyl group at δ 201.2 was elucidated to be at 2 or 3 position since the correlation was observed with the proton at δ 6.53 which was ascribed to H-4 in ring A on the bases of the 1,3,6,7-tetrahydroxy xanthone derivatives [3, 5]. Cross-peaks were also found between the methylene protons (1′-H) of the isoprenyl groups and this

*Part 5 in the series 'Xanthones in Cell Suspension Cultured of *Hypericum patulum*'. For part 4, see ref. [1]

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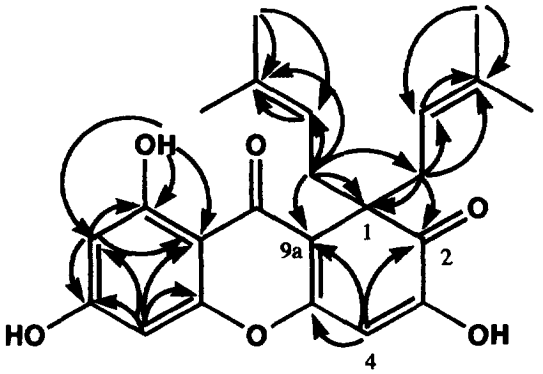

Fig. 1. Long-range correlation in the HMBC spectrum of compound **1**.

carbonyl group, C-9a and C-1. The carbonyl group was therefore assumed to be attached to C-2 and the isoprenyl groups to be C-1.

Hence, **1** is 1,2-dihydro-3,6,8-trihydroxy-1,1-bis(3-methylbut-2-enyl)-xanthen-2,9-dione and is named patulone. This isolation should be of biogenetic and chemotaxonomic interest, since a similar compound (3,4-dihydro derivative of **1**), tomentonone, have been isolated from the stem bark of *Calophyllum tomentosum* T. of the same Guttiferae family [6].

Compound **2** was identical with 1,3,5,6-tetrahydroxyxanthone by comparison of its spectral data, which was isolated from the methanol extract of the fresh herb of *Tripterospermum taiw anense* (Guttiferae) [7]. Its pharmacological effect on anti-platelet aggregation was also reported [8]. Compound **3** was identical with (−)-epicatechin by comparison of its spectral data [9, 10]. Compounds **2** and **3** have been isolated from the cell suspension culture of *Hypericum patulum* for the first time.

EXPERIMENTAL

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

Extraction and isolation. Dried callus tissues (dry wt 1.2 g) were extracted successively with $CHCl_3$ and MeOH. The $CHCl_3$ extract (27 g) was fractionated on silica gel flash chromatography using a step-gradient $CHCl_3$–MeOH followed by purification on Sephadex LH 20 (MeOH) to give **1** (21 mg). Separately, the MeOH extract (283 g) was dissolved in least amount of water and was partitioned with EtOAc and water, respectively. The EtOAc extract (18 g) was fractionated on silica gel flash chromatography using a $CHCl_3$–MeOH gradient system repeatedly. Compound **2** (26 mg) and **3** (38 mg) were purified on Sephadex LH 20 (MeOH) and crystallization from benzene–MeOH, respectively.

Patulone (**1**). Yellow needles, mp 140 ~ 144° (*n*-hexane–acetone). High resolution EIMS: $[M]^+ = 396.1474$. Positive EIMS *m/z*: 396 $[M]^+$ (57), 353 $[M—C_3H_7]^+$ (84), 327 $[M—C_6H_9]^+$ (100), 285 $[M—C_3H_7—C_6H_9]^+$ (88), 69 $[C_6H_9]^+$ (45), 45(64). UV λ_{max}^{MeOH} nm: 244sh (log ε 3.76), 280sh (log ε 3.61), 305 (log ε 3.68), 417 (log ε 3.52). ^1H NMR (500 MHz, $(CD_3)_2CO$): δ 1.47 (12H, *s*, Me × 4), 2.73 (2H, *dd*, *J* = 13.7 and 7.6, 1′-H), 3.43 (2H, *dd*, *J* = 13.7 and 7.6, 1′-H), 4.74 (2H, *t*, *J* = 7.6, 2′-H), 6.26 (1H, *d*, *J* = 1.83, 7-H), 6.38 (1H, *d*, *J* = 1.83, 5-H), 6.53 (1H, *s*, 4-H), 9.30 (1H, *br*, 3-OH), 13.20 (1H, *s*, 8-OH). ^{13}C NMR (125 MHz, $(CD_3)_2CO$): δ 18.0 (C-5′ × 4), 25.8 (C-4′ × 4), 38.5 (C-1′ × 2), 56.7 (C-1), 94.2 (C-5), 100.0 (C-7), 105.0 (C-8a), 109.4 (C-4), 116.6 (C-9a), 119.2 (C-2′ × 2), 135.3 (C-3′ × 2), 155.1 (C-4a), 157.9 (C-4b), 161.2 (C-8), 163.9 (C-6), 164.8 (C-3), 180.2 (C-9), 201.2 (C-2).

1,3,5,6-*Tetrahydroxyxanthonin* (**2**). Yellow powder, mp > 300° (dec. 239°), EIMS *m/z*: 260 $[M]^+$.

(−)-*Epicatechin* (**3**). Mp 239–241, $[\alpha]_D$ − 58.2° (MeOH), EIMS *m/z*: 290 $[M]^+$.

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