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Prenylfuranocoumarin–HMGA–flavonol glucoside conjugates and other constituents of the fruit peels of *Citrus hystrix* and their anticholinesterase activity

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ABSTRACT

Sixteen compounds including dihydroxy prenylfuranocoumarins/3-hydroxy-3-methylglutaric acid conjugates and dihydroxy prenylfuranocoumarins/3-hydroxy-3-methylglutaric acid/1-*O*-flavonyl-*β*-D-glucopyranoside conjugates, together with other dihydroxyprenylfuranocoumarins conjugates, were isolated from the ethyl acetate extract of the fruit peels of *Citrus hystrix*. Some of the isolates were evaluated for their cholinesterase inhibitory activity, but only one compound possessing a 3-*O*-*β*-D-glucopyranosyl-3,5,7,4′-tetrahydroxy-6,8,3′-trimethoxyflavonol nucleus in the prenylfuranocoumarin–HMGA conjugate showed strong activity.

1. Introduction

Citrus hystrix DC (syn. *Citrus papada* Miq.), known in Thailand as "Makruut", is a Rutaceae plant commonly found in Southeast Asia (Smitinand, 1980). Previous work reported the isolation of 4 new compounds, citrusosides A–D, together with six furanocoumarins, a sesquiterpene (eudesmane-4*β*,11-diol), 5 monoterpenes, and 1-*O*-isopropyl-*β*-D-glucopyranoside from the hexane and CH_2Cl_2 extracts of the fruit peels (Youkwan et al., 2010). Later, two new coumarins, hystrixarin and hopeyhopin; a benzenoid derivative, hystroxene-1 and a quinolinone alkaloid, hystrolinone, along with 33 known compounds, were obtained from the roots of this plant (Panthong et al., 2013). Cardioprotective and hepatoprotective effects of a *C. hystrix* peel extract on a rat model (Putri et al., 2013) were also reported. Extraction and analysis of volatile compounds, flavonoids and limonin from *C. hystrix* (Kasuan et al., 2013 and Chinapongtitiwat et al., 2013), as well as synergistic interaction and mode of action of *C. hystrix* essential oil against bacteria causing periodontal diseases (Wongsariya et al., 2014), were also investigated. In the present study, as a continuation of a search for anticholinesterase constituents of *C. hystrix*, sixteen new compounds (**1**–**16**) together with 13 known compounds were obtained from the ethyl acetate extract of the fruit peels. Reported herein are the spectroscopic identification of compounds **1**–**16** and cholinesterase inhibitory activity of some isolates.

2. Result and discussion

Purification of the ethyl acetate extract of the fruit peel of *C. hystrix* using a combination of chromatographic techniques led to isolation of sixteen new compounds (**1**–**16**) (Figs. 1 and 2). The known compounds, bergaptol, limocitrin (Horowitz and Gentili, 1964), umbelliferone (Kong et al., 1996), oxypeucedanin hydrate (Harkar et al., 1984; Ryu et al., 2001), 6′,7′-dihydroxybergamottin (Murakami et al., 1999; Ohta et al., 2002), citrusosides A–D (Youkwan et al., 2010), limocitrol-3-D-glucoside (Gentili and Horowitz, 1964), tschimganic ester B (Shikishima et al., 2001), 1-*O*-isopropyl-glucopyranoside (Sigurskjold et al., 1992; Du et al., 1998; Kitajima et al., 1998) and neohesperidin (Vasconcelos et al., 1998; Kazuma et al., 2003) were identified using spectroscopic methods and by comparison with previous reported data.

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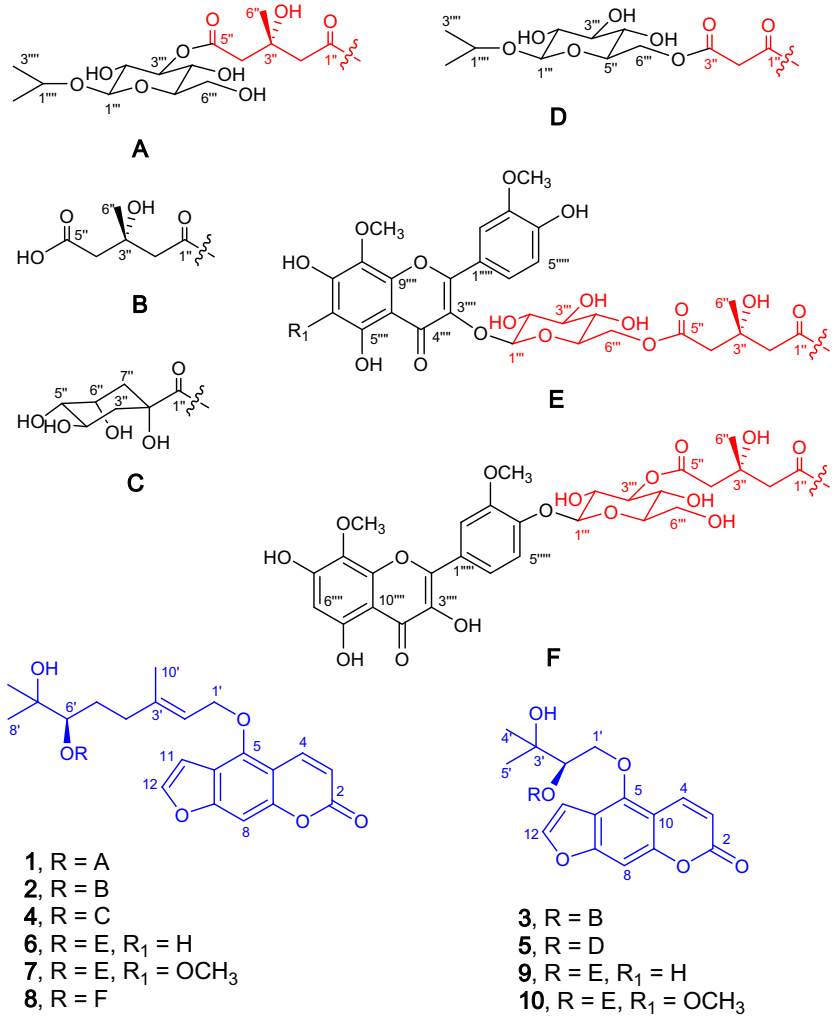

Fig. 1. Structures of compounds **1–10**.

Compound **1** was isolated as a yellow sticky liquid with a molecular formula of $C_{36}H_{48}O_{15}$ based on the HRESIMS spectrum. Its ^{1}H NMR spectrum showed sets of signals indicative of a furanocoumarin with an 6′-*O*-acylated-6′, 7′-dioxygenated geranyl group [δ_H 8.16 and 6.26 (both as d, J = 9.7 Hz, H-4 and H-3, respectively), 7.59 d (J = 2.2 Hz, H-12), 7.14 s (H-8), 6.94 d (J = 2.2 Hz, H-11), 5.51 t (J = 6.8 Hz, H-2′), 4.82 dd (J = 9.7, 2.6 Hz, H-6′), 1.65 s (H_3-10′), 1.18 s (H_3-8′) and 1.19 s (H_3-9′)], a 3-hydroxy-3-methylglutaryl (HMGA) group [δ_H 2.89 d (J = 15.3 Hz, H-2″), 2.53 d (J = 15.3 Hz, H-2″), 2.77d (J = 14.0 Hz, H-4″), 2.67 d (J = 14.0 Hz, H-4″), and 1.41 s (H_3-6″)], and an *O*-isopropyl-*β*-glucopyranosyl moiety [δ_H 4.43 (d, J = 7.7 Hz, H-1‴), 3.88–3.39 (H-2‴, H-4‴-H-6‴) and δ_H 1.23 and 1.17 (both d with J = 6.1 Hz, H_3-2⁗ and H_3-3⁗, respectively) with a quintet at δ_H 3.99 (J = 6.1 Hz, H-1⁗)] as found in citrusoside B (Youkwan et al., 2010). However, a low-field signal for H-6‴ at *ca.* δ_H 4.83 was replaced by a resonance at δ_H 4.98 (t, J = 9.5 Hz, H-3‴) instead (Tables 2 and 3). The important HMBC cross-peak between H-3‴/C-5″ established connectivity between the oxygen atom at C-3‴ and C-5″ of the HMGA group (see Supplementary data). Compound **1** was thus elucidated as an isomer of citrusoside B with C-5″ of the HMGA attached to the *O*-C-3‴ of the *β*-D-glucopyranose ring and named citrusoside E.

Compound **2** was isolated as a yellow amorphous solid with molecular formula of $C_{27}H_{32}O_{10}$ based on HRESIMS. The ^{1}H and ^{13}C NMR spectra (Table 1) showed sets of signals of a furanocoumarin with 6′,7′-dioxygenated geranyl, and 3-hydroxy-3-methylglutaryl groups as found in compound **1**. No signals of a *β*-D-glucopyranosyl moiety were observed, indicating that the other carboxyl group of the 3-hydroxy-3-methylglutaric acid is free. Connectivities of an oxygen atom at C-5 to C-1′, and of another oxygen atom at C-6′ to C-1″ of the HMGA group, were based on HMBC correlations between H-1′/C-5 and C-3′, and between H-6′/C-8′, C-9′ and C-1″, respectively. Compound **2** could thus be elucidated as 6′-*O*-(3″-hydroxy-3″-methylglutaryl)-6′,7′-dihydroxybergamottin.

Compound **3** was isolated as a colorless amorphous solid with a molecular formula of $C_{22}H_{24}O_{10}$ based on the HRESIMS spectrum showing $[M+Na]^+$ ion at m/z 471.1267 (calcd for $C_{22}H_{24}O_{10}Na$, 471.1260). The ^{1}H and ^{13}C NMR spectra displayed sets of signals of a furanocoumarin and of a HMGA group as in compound **2**. The absence of an olefinic proton signal at ca. δ_H 5.48 with the presence of resonances at δ_H 5.30 (dd, J = 8.4 and 2.6 Hz, H-2′), 4.67 (dd, J = 10.2 and 2.6 Hz, H-1′ a), 4.54 (dd, J = 10.2 and 8.4 Hz, H-1′ b), 1.26 (s, H_3-4′), and 1.26 (s, H_3-5′) indicated compound **3** to possess a 2′, 3′-dihydroxyprenyl group instead of a 6′,7′-dioxygenated geranyl group as found in **2**. No resonances of a glucopyranosyl moiety were observed thus indicating that the other carboxyl group of the 3-hydroxy-3-methylglutaric acid is free. The HMBC correlations between H-2′/C-1′, C-3′, C-4′, C-5′ and C-1″ thus led to the elucidation of compound **3** as 2′-*O*-(3″-hydroxy-3″-methylglutaryl)-oxypeucedanin hydrate.

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