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Citrusosides A−D and Furanocoumarins with Cholinesterase Inhibitory Activity from the Fruit Peels of *Citrus hystrix*

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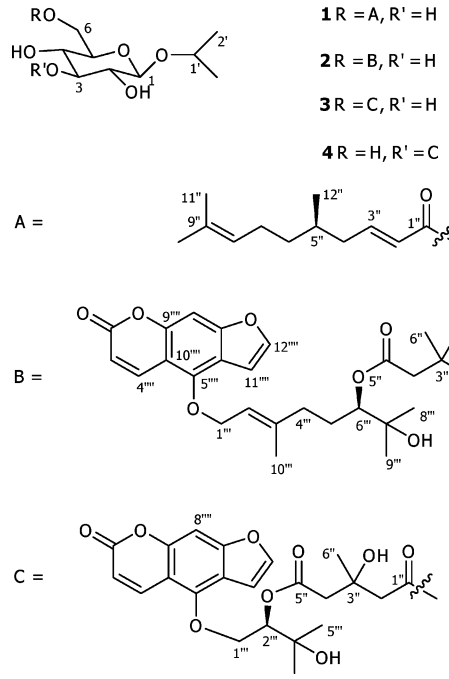
Four new compounds, citrusosides A−D (**1**−**4**), and 15 known compounds were isolated from the hexanes and CH_2Cl_2 extracts of the peels of *Citrus hystrix* fruits. Compound **1** is a 1-*O*-isopropyl-6-*O*-*β*-D-glucopyranosyl ester of 5″,9″-dimethyl-2″,8″-decadienoic acid. Compounds **2**−**4** possess a 1-*O*-isopropyl-*β*-D-glucopyranosyl and a dihydroxyprenylfuranocoumarin moiety conjugated to the 3-hydroxy-3-methylglutaric acid as diesters. Several furanocoumarins were evaluated for their cholinesterase inhibitory activity. (*R*)-(+)-6′-Hydroxy-7′-methoxybergamottin, (*R*)-(+)-6′,7′-dihydroxybergamottin, and (+)-isoimparatorin showed IC_{50} values of 11.2 ± 0.1, 15.4 ± 0.3, and 23 ± 0.2 *μ*M, respectively. Bioassay results indicated that the presence of a dioxygenated geranyl chain in the test compounds is crucial for the inhibitory activity.

Citrus hystrix DC (syn. *C. papeda* Miq.),[1] leech lime of the Rutaceae family, is commonly found in Southeast Asia. It is a small plant of about 3−8 m in height. The leaves and fruits of this plant have long been used as an ingredient in several Thai cooking recipes. The fruits are pear-shaped with a rough, warty skin. Its essential oil was reported to exhibit antibacterial activity.[2] Extracts of fruit peels were found to inhibit implantation, produce abortion, and slightly hasten labor time in pregnant rats.[3] Previous phytochemical investigations revealed the presence of terpenes,[4] phenolic compounds,[5] glyceroglycolipids,[6] and coumarins.[7] We report herein the isolation and spectroscopic data of compounds **1**−**4** from the fruit peels of this plant and cholinesterase inhibitory activity of some of the isolates.

Our study on bioactive compounds from *C. hystrix* led to the isolation of four new 1-*O*-isopropyl-*β*-D-glucopyranoside conjugates, citrusosides A−D (**1**−**4**), in addition to six known furanocoumarins, (+)-oxypeucedanin (**5**),[7,8] (*R*)-(+)-oxypeucedanin hydrate (**6**),[9] (+)-isoimparatorin (**7**),[10] (*R*)-(+)-6′-hydroxy-7′-methoxybergamottin (**8**),[10] (*R*)-(+)-6′,7′-dihydroxybergamottin (**9**),[7,11] and (+)-bergomottin (**10**),[7,11] a eudesmane sesquiterpene, (+)-4-*epi*-crytomeridiol (eudesmane-4*β*,11-diol),[12] five monoterpenes, citronellal,[13] citronellol,[13] citronellol acetate,[13] isopulegol,[14] and neo-isopulegol,[14] as well as 1-*O*-isopropyl-*β*-D-glucopyranoside,[15] sitosteryl-*β*-D-glucopyranoside,[16] and *β*-sitosterol.[16]



Results and Discussion

Compound **1** was isolated as a liquid. The HRESIMS spectrum showed an $[M + Na]^+$ ion at *m/z* 423.2361 corresponding to an elemental formula of $C_{21}H_{36}O_7Na$. The FT-IR spectrum revealed absorption maxima for a hydroxy (3407 cm^{-1}) and double-bond (1652 cm^{-1}) functions. The ^{13}C NMR spectrum exhibited 21 carbon signals, comprising five methyl, four methylene, 10 methine, and two quaternary carbons, including one carbonyl and one olefinic carbon. The presence of a *β*-D-glucopyranosyl unit was evident from NMR signals of a dioxygenated methine group at δ_H 4.31 (1H, d, $J = 7.7$ Hz, H-1) and δ_C 101.2 (C-1), in addition to signals of four oxymethine groups at δ_H 3.53−3.33 and δ_C 76.1, 73.9, 73.4, and 70.3, and of an oxymethylene group at δ_H 4.38 (d, $J = 3.9$ Hz, H-6) and δ_C 63.5 (C-6). The low-field two-proton doublet at δ_H 4.38 was assigned to H_2-6 of the glucopyranose ring connected to an *O*-COR group. The two doublets at δ_H 1.21 and 1.17 (both $J = 6.2$ Hz), which coupled to a broad quintet at δ_H 3.95 ($J = 6.2$ Hz), indicated the presence of an *O*-isopropyl group. The 5″,9″-dimethyl-2″,8″-decadienoyl unit was detected from the $^1H-^1H$ COSY spectrum, which showed cross-peaks between two double triplet signals at δ_H 5.82 ($J = 15.6$, 1.4 Hz, H-2″) and 6.95 ($J = 15.3$, 7.5 Hz, H-3″) and sequential correlations from H-4″ to H-8″. Connectivity of an isopropyl group to an oxygen atom at C-1 and the presence of an ester linkage between the carboxylic C-1″ of the long-chain acid and an oxygen atom at C-6 of a *β*-D-glucopyranose were detected from HMBC correlations between H-1/C-1′ and H-6/C-1″, respectively. Compound **1** was named citrusoside A and identified as 1-*O*-isopropyl-6-*O*-(5″,9″-dimethyl-2″,8″-decadienoyl)-*β*-D-glucopyranoside. Reaction of **1** with Ac_2O in pyridine/DMAP gave a triacetate derivative of **1**. Full assignment of ^1H and ^{13}C NMR chemical shifts of **1** was as indicated in the Experimental Section (for the triacetate derivative, see Supporting Information, Table S2).

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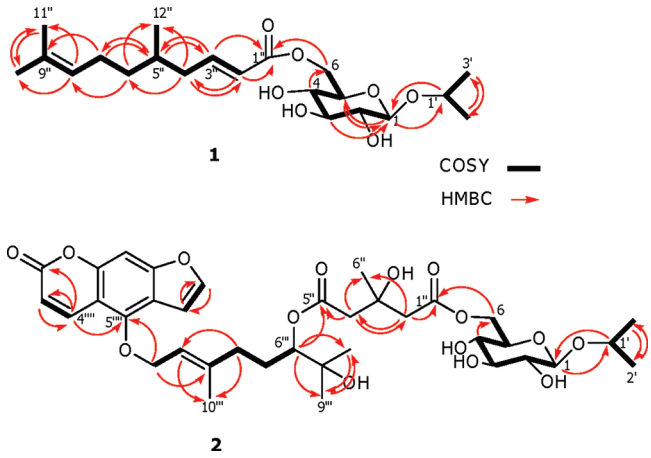

Figure 1. Selected HMBC and COSY correlations of **1** and **2**.

In order to clarify the configuration at C-5″ in **1**, compound **1** was transesterified using NaOEt/EtOH[17] to give 5,9-dimethyldeca-2*E*,8-dienoic acid ethyl ester (**1a**) and 1-*O*-isopropyl-*β*-D-glucopyranoside. The specific rotation of the pure **1a** in $CHCl_3$ was found to be $[\alpha]_D$ −7.83 (*c* 0.3, $CHCl_3$). The specific rotations of the 5*S*,9-dimethyldeca-2*E*,8-dienoic acid ethyl ester and 5*R*,9-dimethyldeca-2*E*,8-dienoic acid ethyl ester, prepared from Wittig reaction of the triphenylphosphonium salt of ethyl α-chloroacetate with *S*-(−)- and *R*-(+)-citronellal, respectively,[18] were found to be +2.40 (*c* 0.35, $CHCl_3$) and −8.65 (*c* 0.22, $CHCl_3$), respectively. On the basis of these data, the configuration at C-5″ in **1** was therefore concluded to be *R* (see Supporting Information, Table S1, for NMR data, and Scheme S1, Syntheses of **1a**). In addition the 2:1 mixture of *β*- and α-D-glucose detected by ^{1}H NMR, with $[\alpha]_D$ +34.5 (*c* 0.4, H_2O), was obtained after aqueous hydrolysis of 1-*O*-isopropyl-*β*-D-glucopyranoside using *β*-D-glucosidase (from almonds, EC 3.2.1.21).

Compound **2** was obtained as a sticky liquid, and its HRESIMS spectrum revealed a molecular formula of $C_{36}H_{48}O_{15}$. The FT-IR spectrum exhibited absorption maxima for OH (3368 cm^{-1}) and carbonyl (1731, 1626 cm^{-1}) groups. The ^{1}H NMR signals of an anomeric proton at δ_H 4.33 (d, J = 7.7 Hz, H-1), oxymethine protons at δ_H 3.47−3.30, and oxymethylene protons at δ_H 4.40 (H-6) indicated the presence of a *β*-D-glucopyranosyl moiety, and signals at δ_H 1.20 and 1.16 (both d, J = 6.4 Hz) and a quintet at δ_H 3.94 (J = 6.2 Hz) could be assigned to an *O*-isopropyl group, as found in **1**. The ^{1}H NMR spectrum, which showed two pairs of doublet signals at δ_H 8.15 and 6.25 (both with J = 9.9 Hz), and another two pairs at δ_H 7.58 and 6.93 (both with J = 2.3 Hz), in addition to ^{13}C NMR signals (Table 1) at δ_C 161.4, 158.1, 152.6, 148.9, 114.3, 107.6 (all qC), 145.1, 139.8, 112.6, 105.0, and 94.4 (all CH), indicated compound **2** to possess a furanocoumarin unit.[7] The ^{1}H−^{1}H COSY spectrum, which showed sequential connectivity from H-1‴ to H-6‴, and HMBC correlations of H-1‴/C-5⁗, C-3‴; H-5‴/C-3‴, C-6‴ (δ_C 80.0); and H_3-8‴, H_3-9‴/C-6‴, C-7‴ (δ_C 72.3) indicated the presence of a 6‴,7‴-dioxygenated geranyl moiety connected to *O*-C-5⁗ of the furanocoumarin nucleus, as found in 6′,7′-dihydroxybergamottin,[7,11] which was also isolated in this study. The less-shielded ^{1}H NMR resonances at δ_H 4.83 (dd, J = 9.9 and 2.6 Hz) assigned to H-6‴ required the bonding of C-6‴ to an *O*-acyl group. Key NMR signals of two methylene groups at δ_H 2.68 and 2.63 (both doublets with J = 14.7 Hz, H-2″), δ_C 46.0 (C-2″) and at δ_H 2.79 and 2.54 (both doublets with J = 14.6 Hz, H-4″), δ_C 45.1 (C-4″) with methyl signals at δ_H 1.36 (H-6″), δ_C 27.4 (C-6″), and three quaternary carbon signals at δ_C 171.6 (C-1″), 171.9 (C-5″), and 70.1 (C-3″), in conjunction with HMBC correlations between H-2″/C-1″, C-3″, C-4″, and C-6″ and between H-4″/C-2″, C-3″, C-5″, and C-6″, indicated the presence of a 3-hydroxy-3-methylglutaryl unit. Connectivities from C-1″ to *O*-C-6‴ and C-5″ to *O*-C-6‴ were established from HMBC correlations of H-6/C-1″ and of H-6‴/C-5″, respectively. The structure of compound **2** was assigned accordingly as shown and was given the name citrusoside B. Upon acetylation, a triacetate derivative with well-defined ^{1}H NMR signals of H-2 (δ_H 4.90, t, J = 8.4 Hz), H-3 (δ_H 5.17, t, J = 9.6 Hz), and H-4 (δ_H 5.01, t, J = 9.8 Hz) was obtained (for ^{1}H and ^{13}C NMR data of triacetate of **2**, see Table S3, Supporting Information).

On the basis of the configuration at C-6′ in 6′,7′-dihydroxybergamottin as *R* using the Mosher ester method (see Figure 2),[19,20] and on the proposal that **2** was biosynthesized from 3-hydroxy-3-methylglutaric acid, 6′*R*,7′-dihydroxybergamottin, and 1-*O*-isopropyl-*β*-D-glucopyranoside, the absolute configuration at C-6‴ in **2** could thus be assigned as *R*.

Compound **3** was obtained as a liquid with the molecular formula $C_{31}H_{40}O_{15}$ by HRESIMS. The ^{1}H and ^{13}C NMR spectra showed sets of signals of a 1-*O*-isopropyl-*β*-D-glucopyranosyl moiety, as well as of a furanocoumarin and a 3-hydroxy-3-methylglutaryl moiety, as found in **2**. The presence of a 2‴,3‴-dihydroxyprenyl group, in place of the 6‴,7‴-dioxygenated geranyl group, was implied from the absence of an olefinic proton signal at ca. δ_H 5.51 with the presence of ^{1}H NMR signals at δ_H 5.34 (dd, J = 8.4, 2.5 Hz, H-2‴), 4.67 (dd, J = 10.0, 2.5 Hz, H-1‴a), 4.56 (dd, J = 10.0, 8.4 Hz, H-1‴b), 1.31 (s, H_3-4‴), and 1.30 (s, H_3-5‴) and ^{13}C NMR signals at δ_C 78.2, 71.5, 71.0, 25.9, and 26.5. The long-range HMBC correlations of the less-shielded carbinolic proton at δ_H 5.34 with carbon signals of C-1‴, C-3‴, C-4‴, C-5‴, and C-5″ at δ_C 71.0, 71.5, 25.9, 26.4, and 171.1, respectively, revealed connectivity of the oxygen atom at C-2‴ to a carboxylic carbon (C-5″) of the 3″-hydroxy-3″-methylglutaric acid moiety. The ether linkage between C-1‴ and C-5⁗ was detected from HMBC correlation between H-1‴/C-5⁗. The structure of compound **3** (citrusoside C) was therefore proposed as shown, and the assigned ^{1}H and ^{13}C NMR chemical shifts of **3** are given in Table 1 (for ^{1}H and ^{13}C NMR data of the triacetate of **3**, see Table S3, Supporting Information).

On the basis of the configuration at C-2′ in (+)-oxypeucedanin hydrate, which was found to be *R* using the Mosher ester method (see Figure 2),[19,21] and on the proposal that **3** was biosynthesized from 3-hydroxy-3-methylglutaric acid, 2′*R*-oxypeucedanin hydrate, and 1-*O*-isopropyl-*β*-D-glucopyranoside, it could be concluded that the absolute configuration at C-2‴ in **3** was also *R*.

Compound **4** was isolated as a colorless liquid with a similar elemental formula, $C_{31}H_{40}O_{15}$, to **3**. The ^{1}H and ^{13}C NMR spectra exhibited similar sets of signals for a furanocoumarin, a 1-*O*-isopropyl-*β*-D-glucopyranosyl, a 2,3-dihydroxyprenyl, and a 3-hydroxy-3-methylglutaryl unit. Connectivity among units was detected by HMBC correlations and found to be rather similar to those in compounds **2** and **3**. The ^{1}H,^{1}H−COSY spectrum indicated, however, that H-3 resonated at a less-shielded position (δ_H 4.98, t, J = 9.5 Hz) and for H-6 at higher field (δ_H 3.87 and 3.81, both as dd, J = 12.0, 3.8 Hz) than those of the corresponding protons in **3**, thus indicating the bonding of an *O*-acyl group at C-3, instead of at C-6 as in **3**. The presence of an ester linkage between the carboxylic C-1″ of the 3″-hydroxy-3″-methylglutaric acid and an oxygen atom at C-3 of a *β*-D-glucopyranose moiety was further supported by HMBC correlations between H-3/C-4, C-1″. The structure of **4** (citrusoside D) was thus elucidated as shown. Full assignments of ^{1}H and ^{13}C NMR resonances are given in Table 1.

Due to a previous report on the anticholinasterase properties of several coumarins,[22] butyrylcholinesterase inhibitory activity of the isolates was investigated. The results are shown in Table 2. Compound **8** was found to possess the highest potency, showing an IC_{50} value of 11.2 ± 0.1 μM, whereas compounds **9** and **7** showed IC_{50} values of 15.4 ± 0.3 and 23 ± 0.2 μM, respectively. Galanthamine, a positive control, showed an IC_{50} value of 3.2 ± 0.2 μM. This bioassay indicated the presence of a dioxygenated

Table 1. ^{1}H and ^{13}C NMR Data of **2**−**4** in $CDCl_3$[a]

	2		**3**		**4**	
position	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.33 d (7.7)	101.1	4.30 d (7.8)[e]	101.1	4.42 d (7.7)	101.2
2	3.47	76.3	3.48	76.2	3.41[g]	75.5
3	3.30 t (8.1)	73.4	3.30 t (8.2)	73.5	4.98 t (9.5)	78.0
4	3.47	70.2	3.46[f]	70.1	3.67 t (9.5)	69.0
5	3.47	73.5	3.45[f]	73.3	3.40[g]	71.9
6	4.42 d (12.0)	63.7	4.39 d (10.9)	63.7	3.87 dd (12.0, 3.4), 3.81 dd (12.0, 4.3)	62.2
	4.38 d (12.0)		4.30 d (10.9)[e]			
1′	3.94 quint (6.4)	72.2	3.90 quint (6.2)	72.3	3.99 quint (6.2)	72.5
2′	1.20 d (6.4)	22.0[d]	1.12 d (6.2)	21.9	1.19 d (6.2)	21.9
3′	1.16 d (6.4)	23.4[d]	1.15 d (6.2)	23.4	1.23 d (6.2)	23.4
1″		171.6[b]		171.6[c]		171.8[d]
2″	2.68 d (14.7)	46.0[e]	2.62 d (14.7)	45.7	2.73 d (14.2)	46.1
	2.63 d (14.7)		2.60 d (14.7)		2.69 d (14.2)	
3″		70.1		70.1		70.4
4″	2.79 d (14.6) 2.54 d (14.6)	45.1[e]	2.79 d (14.8)	45.4	2.90 d (15.1) 2.57 d (15.1)	44.6
			2.72 d (14.8)			
5″		171.9[b]		171.1[c]		171.5[d]
6″	1.36 s	27.4	1.34 s	27.2	1.39 s	26.8
1‴	4.91 brd (6.8)	69.6	4.67 dd (10.0, 2.5) 4.56 dd (10.0, 8.5)	71.0	4.64 dd (10.3, 2.9)	71.5
					4.58 dd (10.3, 8.0)	
2‴	5.51 t (6.8)	119.7	5.34 dd (8.5, 2.5)	78.2	5.34 dd (8.0, 2.9)	78.1
3‴		141.8		71.5		71.2
4‴	2.04	35.9	1.31 s	25.9	1.32 s	25.8
5‴	1.65[h]	27.9	1.30 s	26.5	1.32 s	27.7
6‴	4.83 dd (9.9, 2.6)	80.0				
7‴		72.3				
8‴	1.169	26.5[f]				
9‴	1.173	24.1[f]				
10‴	1.65[h]	16.6				
2⁗		161.4		161.4		161.5
3⁗	6.25 d (9.9)	112.6	6.24 d (9.8)	112.8	6.29 d (9.8)	113.2
4⁗	8.15 d (9.9)	139.8	8.03 d (9.8)	139.4	8.07 d (9.8)	139.1
5⁗		148.9		148.1		148.4
6⁗		114.3		113.1		113.3)
7⁗		158.1		158.1		158.2
8⁗	7.12 s	94.4	7.05 s	94.2	7.14 s	94.6
9⁗		152.6		152.4		152.5
10⁗		107.6		106.7		106.8
11⁗	6.93 d (2.3)	105.0	6.95 d (2.4)	104.9	6.93 d (2.3)	104.7
12⁗	7.58 d (2.3)	145.1	7.57 d (2.4)	145.3	7.59 d (2.3)	145.0

[a] Coupling constants are listed in parentheses in Hz. [b−d] Interchangeable signals. [e−h] Overlapping signals.

geranyl chain in the test compounds to be crucial for the inhibitory activity. Our study is among the few reports on the occurrence of 1-*O*-isopropyl-*β*-D-glucopyranoside[15] in Nature.

Experimental Section

General Experimental Procedures. Melting points were measured using an Electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO DIP 1020 polarimeter. The IR spectra were obtained on a Perkin-Elmer 1760x FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AVANCE 400 MHz spectrometer. Chemical shifts are referenced to the residual solvent signals ($CDCl_3$: δ_H 7.24 and δ_C 77.0 ppm). HRESIMS was recorded on a Bruker Daltonics microTOF mass spectrometer.

Plant Material. The fruits of *Citrus hystrix* DC. (Rutaceae), known in Thailand as "Makruut", was collected from Bangkunkong subdistrict, Bangkruai district, Nonthaburi Province, Thailand, in June 2003. The plant was identified by Assoc. Prof. Dr. Nijsiri Ruangrangsi, Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand. A voucher specimen (SSCH/2003) is maintained at the Chemistry Department, Ramkhamhaeng University.

Extraction and Isolation. The dried, ground fruit peels (5.7 kg) were extracted successively with hexanes, CH_2Cl_2, and MeOH at room temperature to obtain, after solvent evaporation under reduced pressure, hexanes (240.2 g), CH_2Cl_2 (155.0 g), and MeOH (384.8 g) extracts, respectively.

The hexanes extract (201.3 g) was fractionated using silica gel column chromatography (CC) with a gradient of hexanes−CH_2Cl_2 (100:0) to CH_2Cl_2−MeOH (70:30) to obtain eight fractions. Fraction 1 was separated by CC using hexanes−CH_2Cl_2 (90:10 to 80:20) to give three subfractions (1.1−1.3). Subfraction 1.2 after CC (silica gel, hexanes−CH_2Cl_2, 90:10 to 40:60) gave five subfractions (1.2.1−1.2.5). Isopulegol (48.9 mg) was obtained from subfraction 1.2.5. Purification of subfraction 1.2.2 using silica gel CC (hexanes−CH_2Cl_2, 85:15) gave citronellal (21.7 mg). Subfraction 1.2.3 after CC (silica gel, hexanes−CH_2Cl_2, 85:15 to 80:20) gave citronellyl acetate (27.5 mg) and neo-isopulegol (8.3 mg). Fraction 3 (6.88 g) was purified by CC (silica gel, hexanes−EtOAc, 96:4 to 93:7) to give isoimperatorin (**7**, 55.1 mg), $[\alpha]^{29}_D$ +0.41 (*c* 0.53, $CHCl_3$), and bergomottin (**10**, 7.0 mg), $[\alpha]^{28}_D$ +2.89 (*c* 0.36, $CHCl_3$), after CC (silica gel, CH_2Cl_2, then reversed-phased C_{18}, MeOH−H_2O, 85:15). Fraction 5 was purified (silica gel CC, hexanes−EtOAc, 90:10 to 50:50) and yielded five subfractions (5.1−5.5). Subfraction 5.2 gave *β*-sitosterol (21.9 mg) after CC (CH_2Cl_2−MeOH, 100:0 to 97:3). Subfraction 5.4 was fractionated by CC (silica gel, CH_2Cl_2−MeOH, 99:1) to give three subfractions (5.4.1−5.4.3). Subfraction 5.4.2 after further CC (reversed-phase C_{18}, MeOH−H_2O, 70:30, then silica gel, CH_2Cl_2−MeOH, 99:1 to 97:3) gave (*R*)-(+)-6′-hydroxy-7′-methoxybergamottin (**8**, 16.6 mg), $[\alpha]^{28}_D$ +8.7 (*c* 0.68, $CHCl_3$), and (+)-6′,7′-dihydroxybergamottin (**9**, 9.2 mg), $[\alpha]^{28}_D$ +6.1 (*c* 0.42, $CHCl_3$). Fraction 7 (1.18 g) was purified using CC (silica gel, CH_2Cl_2−MeOH, 99:1 to 85:15) to give five subfractions (7.1−7.5). Subfraction 7.2 gave citronellol (39.8 mg). Subfraction 7.4 (780.5 g) was purified using silica gel CC (hexanes−EtOAc, 60:40 to 50:50) to give three subfractions (7.4.1−7.4.3). Subfraction 7.4.2 (190.5 g) after CC (reversed-phase C_{18}, MeOH−H_2O, 60:40 to 100:0) yielded oxypeucedanin hydrate (**6**, 54.9 mg), $[\alpha]^{28}_D$ +20.4 (*c* 0.41, $CHCl_3$), and an additional quantity of dihydroxybergamottin (**9**, 17.4 mg). Subfraction 7.5 (466 mg) after CC (silica gel, hexanes−EtOAc, 70:30 to 0:100,

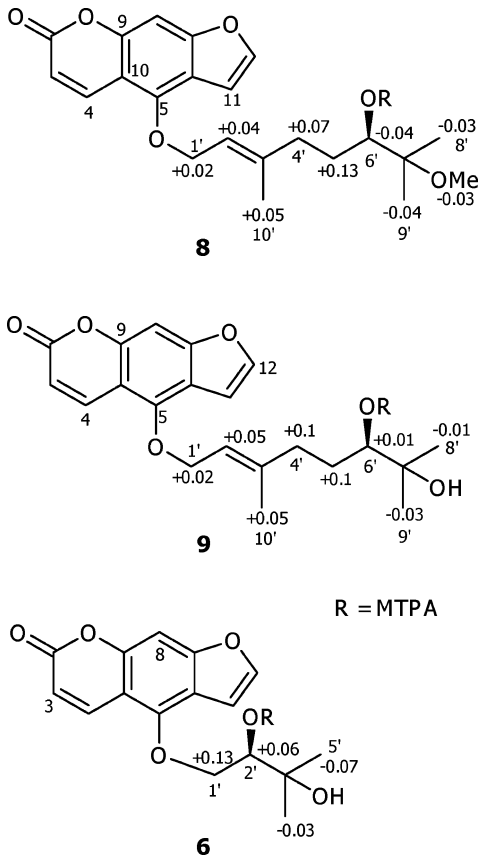

Figure 2. Distribution of $\Delta\delta_{S-R}$ values of the *S*- and *R*-MTPA esters of (+)-6′-hydroxy-7′-methoxybergamottin (**8**), (+)-6′,7′-dihydroxybergamottin (**9**),[20] and (+)-oxypeucedanin hydrate (**6**).[21]

Table 2. Cholinesterase Inhibitory Activity of Some Isolates

compound	IC_{50} (μM) ± SD
citrusoside A (**1**)	376 ± 2
citrusoside B (**2**)	339 ± 3
citrusoside D (**4**)	inactive[b]
oxypeucedanin (**5**)	64.0 ± 0.2
oxypeucedanin hydrate (**6**)	inactive[c]
isoimperatorin (**7**)	23.1 ± 0.2
galanthamine[a]	3.2 ± 0.2
6′-hydroxy-7′-methoxybergamottin (**8**)	11.2 ± 0.1
6′,7′-dihydroxybergamottin (**9**)	15.4 ± 0.3
bergomottin (**10**)	inactive[d]

[a] Positive control compound. [b] Inactive at 7.2 mM. [c] At 65.1 μM, % inhibition was found to be 24.6. [d] Inactive at 14.8 mM.

then reversed-phase C_{18}, MeOH−H_2O, 60:40 to 100:0) yielded (+)-4-*epi*-crytomeridiol (eudesmane-4β,11-diol, mp 120−122 °C, 35.1 mg), $[\alpha]^{28}_D$ +13.9 (*c* 0.24, $CHCl_3$).

The CH_2Cl_2 extract (155 g) was fractionated using silica gel CC with a gradient of CH_2Cl_2−MeOH (100:0 to 70:30) and gave 11 fractions. Fraction 3 (2.52 g) after CC (silica gel, CH_2Cl_2−MeOH, 97:3 to 85:15) yielded six subfractions (3.1−3.6). Subfraction 3.4 gave oxypeucedanin (**5**, 562.5 mg), $[\alpha]^{28}_D$ +13.2 (*c* 0.05, $CHCl_3$), after silica gel CC (CH_2Cl_2−MeOH, 97:3 to 90:10). Subfraction 3.2 was purified (silica gel, hexanes−EtOAc, 60:40 to 100:0, then reversed-phase C_{18}, MeOH−H_2O, 70:30 to 100:0) to yield dihydroxybergamottin (**9**, 32.4 mg). Fraction 5 (3.35 g) after CC (silica gel, hexanes−EtOAc, 40:60, to EtOAc−MeOH, 80:20) gave four subfractions (5.1−5.4). Subfraction 5.2 was further purified (reversed-phase C_{18}, MeOH−H_2O, 60:40 to 100:0, then silica gel, CH_2Cl_2−MeOH, 97:3 to 90:10) and yielded compound **1** (21.7 mg). Fraction 7 (8.45 g) was purified (silica gel, CH_2Cl_2−MeOH, 95:5 to 88:12) and gave six subfractions (7.1−7.6). Subfracion 7.3.3 was further purified (silica gel, hexanes−EtOAc, 97:3 to 70:30, then reversed-phase C_{18}, MeOH−H_2O, 70:30 to 100:0) and yielded an additional quantity of **2** (35.1 mg), **3** (18.4 mg), and **4** (13.1 mg). Subfraction 7.5 yielded β-sitosteryl glucopyranoside (65.9 mg). Subfraction 7.4 after purification (reversed-phase C_{18}, MeOH−H_2O, 60:40 to 100:0) gave three subfractions (7.4.1−7.4.3). Subfraction 7.4.2 (113.5 mg) was further purified using silica gel CC (CH_2Cl_2−MeOH, 97:3 to 95:5) to yield **3** (18.7 mg), and purification of subfraction 7.4.3 (mg) using silica gel CC (CH_2Cl_2−MeOH, 97:3 to 95:5) yielded **2** (11.8 mg). Fraction 9 (3.98 g) after CC (silica gel, CH_2Cl_2−MeOH, 95:5 to 80:20) gave three subfractions (9.1−9.3), and further purification of subfraction 9.2 using CC (reversed-phase C_{18}, MeOH−H_2O, 60:40 to 100:0, then silica gel, EtOAc−MeOH, 97:3 to 94:4) gave 1-*O*-isopropyl-β-D-glucopyranoside (10.1 mg).

Citrusoside A (1): colorless, sticky liquid; $[\alpha]^{30}_D$ −20.6 (*c* 0.66, MeOH); FT-IR (KBr) ν_{max} 3407, 2969, 2918, 1723, 1652, 1454, 1380, 1316, 1270, 1183, 1087, 1038, 927, 832, 643 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ_H 6.95 (1H, dt, *J* = 15.3, 7.5 Hz, H-3″), 5.82 (1H, dt, *J* = 15.3, 1.4 Hz, H-3″), 5.05 (1H, q, *J* = 7.1, 1.2 Hz, H-8″), 4.38 (1H, br d, *J* = 3.6 Hz, H-6), 4.31 (1H, d, *J* = 7.7 Hz, H-1), 3.95 (1H, br quint, *J* = 6.2 Hz, H-1′), 3.53 (1H, br t, *J* = 9.0 Hz, H-3), 3.45 (1H, dd, *J* = 9.7, 3.9 Hz, H-5), 3.37 (1H, br t, *J* = 9.7 Hz, H-4), 3.33 (1H, m, H-2), 2.20 (1H, m, H-4″a), 2.00 (1H, m, H-4″b), 1.93 (1H, m, H-7″a), 1.65 (3H, s, H-10″), 1.61 (1H, m, H-5″), 1.59 (1H, m, H-7″b), 1.57 (3H, s, H-11″), 1.31 (1H, m, H-6″a), 1.22 (1H, d, *J* = 6.2 Hz, H-2′), 1.17 (1H, d, *J* = 6.2 Hz, H-3′), 1.14 (1H, m, H-6″b), 0.87 (3H, d, *J* = 6.7 H, H-12″); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 167.1 (C, C-1″), 149.5 (CH, C-3″), 131.5 (C, C-9″), 124.4 (CH, C-8″), 121.7 (CH, C-2″), 101.2 (CH, C-1), 76.1 (CH, C-3), 73.9 (CH, C-5), 73.4 (CH, C-2), 72.5 (CH, C-1′), 70.3 (CH, C-4), 63.5 (CH_2, C-6), 39.7 (CH_2, C-4″), 36.7 (CH_2, C-6″), 32.0 (CH, C-5″), 25.7 (CH_3, C-10″), 25.5 (CH_2, C-7″), 23.4 (CH_3, C-2′), 22.1 (CH_3, C-3′), 19.5 (CH_3, C-12″), 17.6 (CH_3, C-11″); HRESIMS $[M + Na]^+$ ion *m/z* 423.2361 (calcd for $C_{21}H_{36}O_7Na$, 423.2349).

Citrusoside B (2): colorless, sticky liquid; $[\alpha]^{30}_D$ −16.2 (*c* 0.40, MeOH); FT-IR (KBr) ν_{max} 3368, 2921, 1731, 1626, 1579, 1455, 1382, 1205, 1131, 1074, 825, 749 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; HRESIMS *m/z* 743.2885 $[M + Na]^+$ (calcd for $C_{36}H_{48}O_{15}Na$, 743.2877).

Citrusoside C (3): colorless, sticky liquid; $[\alpha]^{30}_D$ −2.8 (*c* 0.45, MeOH); FT-IR (KBr) ν_{max} 3401, 2975, 2925, 1731, 1623, 1579, 1455, 1355, 1285, 1204, 1134, 1081,87, 751 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; HRESIMS *m/z* 675.2266 $[M + Na]^+$ (calcd for $C_{31}H_{40}O_{15}Na$, 675.2253).

Citrusoside D (4): colorless, sticky liquid; $[\alpha]^{30}_D$ −4.7 (*c* 0.40, MeOH); FT-IR (KBr) ν_{max} 3401, 2924, 2852, 1731, 1623, 1579, 1456, 1382, 1354, 1285, 1257, 1206, 1157, 1135, 1080, 1039, 826, 751 cm^{-1};^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; HRESIMS *m/z* 675.2259 $[M + Na]^+$ (calcd for $C_{31}H_{40}O_{15}Na$, 675.2253).

Bioassays. Butyrylcholinesterase inhibitory activity of the isolates was evaluated according to Ellman and co-workers[23] with some modification using acetylthiocholine iodide (as substrate), cholinesterase (from horse serum, EC 3.1.1.8), and galantamine hydrobromide from *Lycoris* sp. Absorbance was read at 405 nm every 5 s for 2 min. The IC_{50} is the concentration of inhibitor required to decrease enzyme activity by 50% and was determined in triplicate. The data are shown as mean ± standard deviation values (Table 2).

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Supporting Information Available: ^1H and ^{13}C NMR spectra of **1**−**4**, Scheme S1 Syntheses of **1a**, HMBC correlations of **2**−**4**, tabulated ^1H and ^{13}C NMR data of *R*-(−)-**1a**, *S*-(+)-**1a**, and acetate derivatives of **1**, **2**, and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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