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THE ASYMMETRIC SYNTHESIS OF LINEAR DIHYDROPYRANO-COUMARINS FOR ALZHEIMER'S DISEASE

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Abstract--Ca^{2+} antagonistic and acetylcholinesterase (AchE) inhibitory activity-guided c*is*-3'S, 4'S-disubstituted linear dihydropyranocoumarins were synthesized asymmetrically. The syntheses were performed with resorcinol or 2-methylresorcinol, enantioselectivity was chosen AD-mix-β to dihydroxylate asymmetrically. All products were screened against AchE *in vitro* and one showed potential inhibitory activity.

Alzheimer's Disease (AD) is a neurodegenerative illness characterized by a progressive decline in cognitive function. The deficit of cholinergic functions was one of hypotheses on AD pathogensis and an important therapeutic strategy has therefore been the prescription of AchE inhibitors for activating central chloinergic function,[1, 2] for example, tacrine, galanthamine, donepezil, rivastigmine, and huperzine A have been employed, which have all been available commercially. However, they don't provide radical cure to reverse the course of disease.[3] Lately, evidences has been presented that amyloid β-peptide (Aβ) deposition plays a key role in AD symptoms in connection with its damages to cell membrane, synapse, and axon.[4] The damages from ion fluxes across channels and AchE could accelerate the development of Aβ plaques and neurotoxicity.[4, 5] Nitrendipine and verapamil, the inhibitors of L-VDCCs, inhibited AchE expression and amended cognitive impairment of model animals of AD.[6, 7]

The Testa group [3, 8] discovered that some coumarin derivatives inhibiting MAO were also endowed with the same inhibitory activity towards AchE as ensaculin (Figure 1) *in vitro*. 7-(3-Chlorobenzyloxy)-3,4-dimethylcoumarin (Figure 1), one of the most active AchE inhibitors among them, could bind to the peripheral site of AchE as proposed for 3-chloro-7-hydroxy-4-methylcoumarin (CHMC) (Figure 1). Kang [9] and Kim [10] *et al.* reported coumarins, decursinol (Figure 1), isoimperatorin, imperatorin, etc., from *Angelica gigas* or *A. dahurica,* possessed inhibitory activity on AchE and Kang regarded that 3'OH is necessary to decursinol. During the course of phytochemical studies on *Angelica* plants as a part of our projects, some coumarins were also screened for AchE inhibition [11] and the structure-activity relationship showed that the configuration of position 3′ had a great influence than the substituent of -OH in position 3': the 3'β-substituted compounds endowed activity, while the 3'α-substituted compounds had no activity. Moreover, 3'β,4'β-disubstituted compounds, for example, (-)-anomalin [(-)-3'*R*,4'*R*-3',4'-diangeloly-3',4'-dihydroseselin] (Figure 1), also endowed inhibitory activity. c*is*-Disubstituted pyranocoumarins with the necessary configuration to antagonize Ca^{2+} influx [12] similar to verapamil could inhibit AchE. We therefore hypothesized that the possibility existed to discover new type of compounds from dihydropyranocoumarins with dual effects on inhibition of the influx of Ca^{2+} and AchE, and these compounds could promote discoveries of new medicines for AD. So we designed an asymmetrical synthesis method to obtain target compounds with *cis*-3'β,4'β-disubstituted linear dihydropyrano-coumarins for the first time.

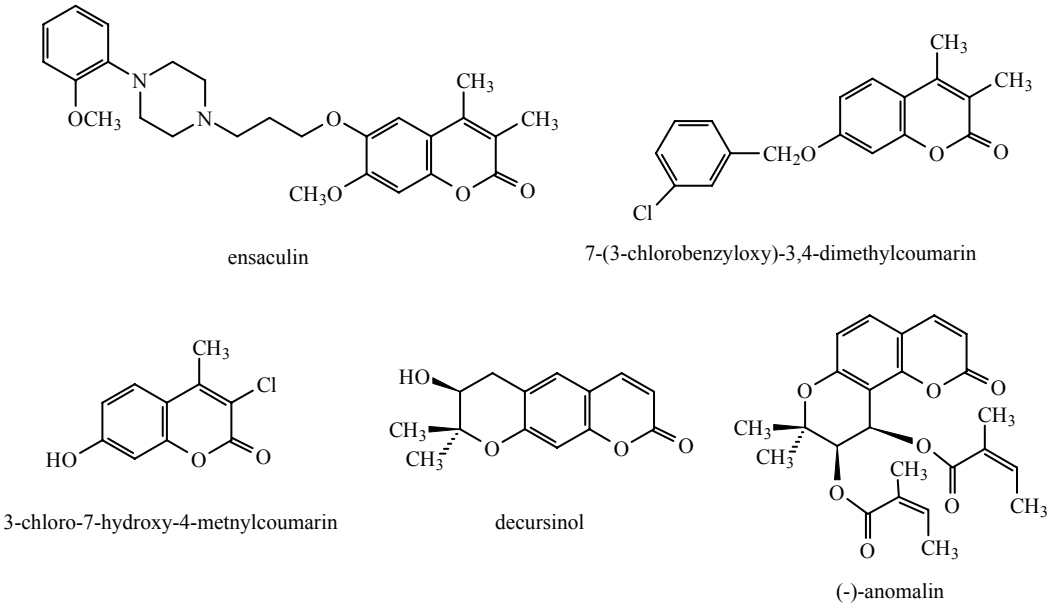
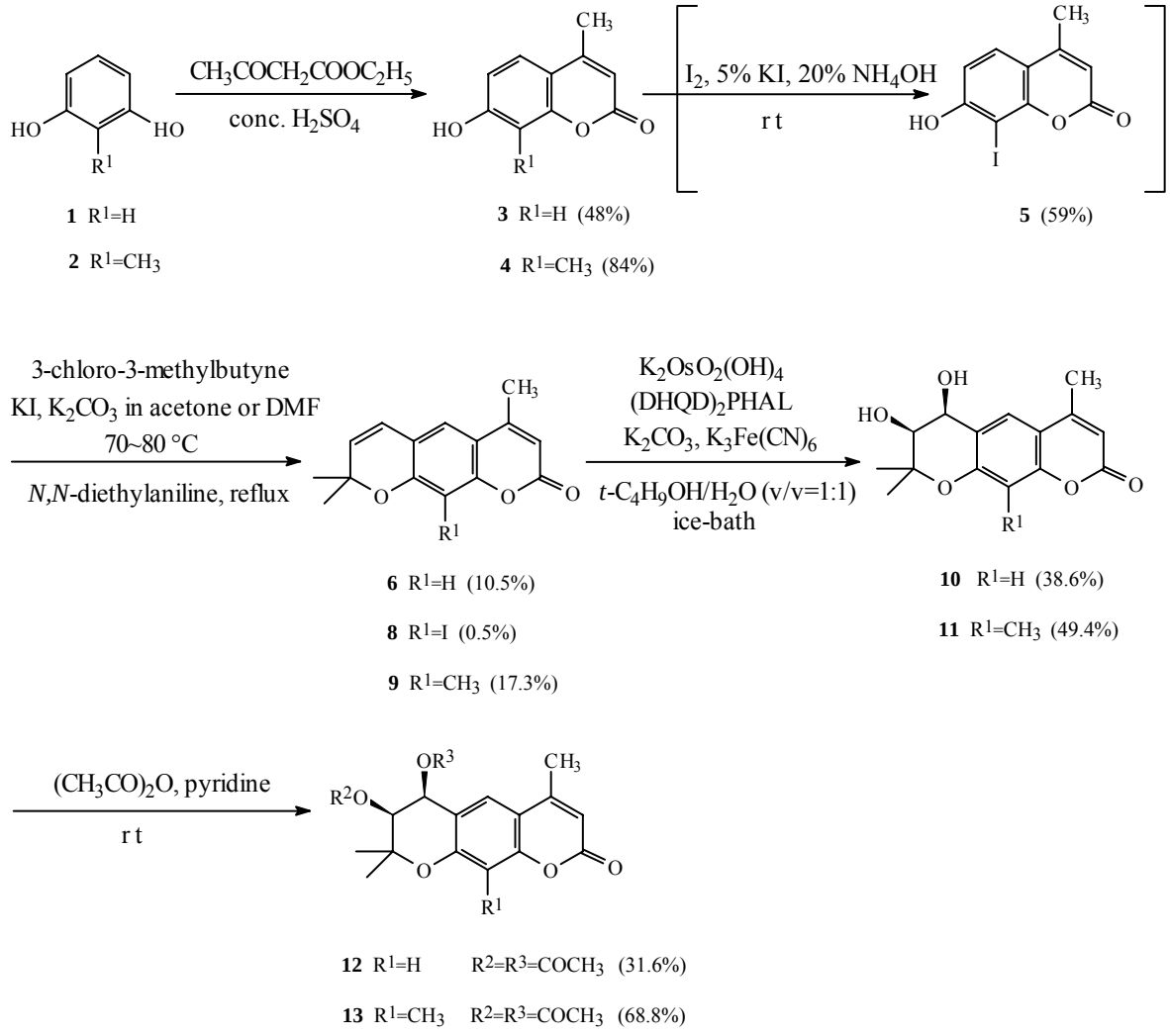


Figure 1. Coumarins with inhibitory activity on AchE

RESULTS AND DISCUSSION

Chemistry: Based on the nucleus of target compounds, xanthyletin or substituted xanthyletins are key intermediates, to date, their syntheses [13-16] can be outlined into two schemes: in the first one (Scheme 1) coumarin nucleus were cyclized by Peachmann reaction, then xanthyletin and its analogues were synthesized by direct cyclization in boiling *N*, *N*-diethylaniline of a propargyl or methylpropargyl ether of the appropriate methylhydroxycoumarins. In another scheme (Scheme 2), benzopyranone were firstly synthesized, then the carbonyl group was deoxidized and dehydrated to afford the moiety of pyrano ring with olefinic bond between position 3' and 4'. Upon Comparison of the two schemes, the former was chosen for the experiment.

Scheme 1



1 R^1=H

2 R^1=CH_3

3 R^1=H (48%)

4 R^1=CH_3 (84%)

5 (59%)

6 R^1=H (10.5%)

8 R^1=I (0.5%)

9 R^1=CH_3 (17.3%)

10 R^1=H (38.6%)

11 R^1=CH_3 (49.4%)

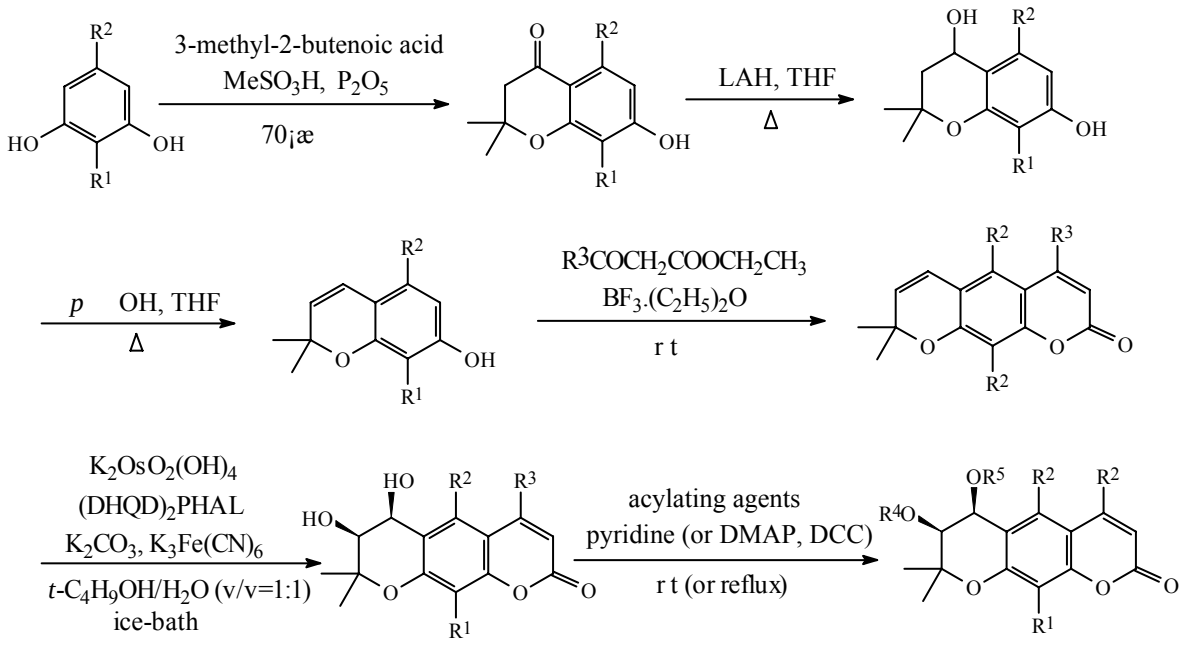
12 R^1=H R^2=R^3=$COCH_3$ (31.6%)

13 R^1=CH_3 R^2=R^3=$COCH_3$ (68.8%)

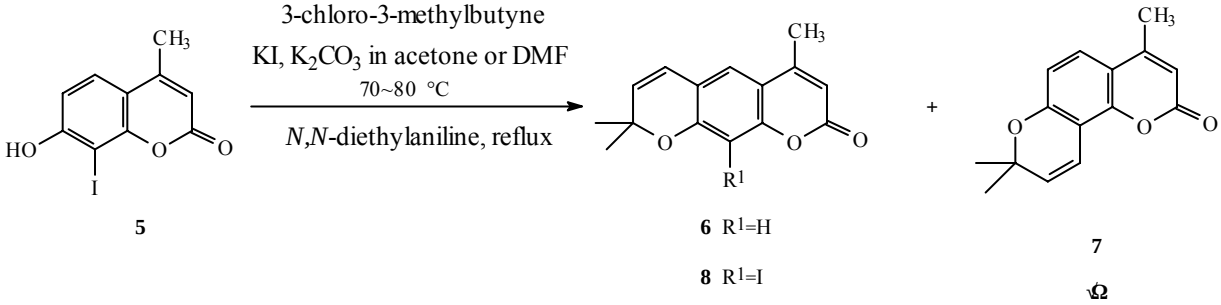
As shown above in Scheme 1, substituted 7-hydroxycoumarins were synthesized from resorcinol and 2-methylresorcinol by Pechamann reaction with a β-keto ester in the presence of sulfuric acid at rt. The

position 8 of coumarin nucleus is preferred in the cyclization and then was blocked by introducing into a suitable iodine or bromine atom for the preparation of xanthyletin or its analogues, while this position not already substituted. Here, 2-methylresorcinol (**2**) was chosen as one of the preliminary substrates. When resorcinol (**1**) was selected as another preliminary substrate to afford **3**, **3** was treated with iodine in ammonia gave 4-methyl-8-iodoumbellifron (**5**), as described in literature.[17] However, the formation of small amounts of 4-methylseseline (**7**) and 4-methyl-8-iodoxanthyletin (**8**) were obtained during the cyclization of propargylethers into xanthyletin series (Scheme 3).

Scheme 2



Scheme 3



5

6 R1=H

8 R1=I

7

The asymmetric dihydroxylation (AD) of substituted xanthyletin was catalyzed by $K_2OsO_2(OH)_4$ in the

presence of an enantioselected ligand: hydroquinidine 1,4-phthalazinediyl diether, $(DHQD)_2$-PHAL. In small-scale pre-tests, AD-mix-β was used and the products were obtained successfully. However, the AD reaction did not perform successfully, during large-scale tests that employed greater amounts of the same substrates, even after waiting for 48 hours. It was only reacted as the molar ration of $K_4OsO_2(OH)_4$: the olefinic bond was increased to 0.004 :1 from 0.002 : 1 (the ratio of reagent AD-mix-β).[18-20] The products were obtained and the absolute configuration of positions C-3', C-4' were all *S*-type determined by the enantioselected ligand $(DHQD)_2$-PHAL. When positions C-3', C-4' of linear pranocoumarins are β -disubsituted, their absolute configurations are all *s*-type. This may be also verified by the optical rotations and spectral characters of pyranocoumarins.[12, 21] The optical purities were determined by chiral separation with HPLC. The e.e. of **10** and **11** was respectively 96% and 95% determined by peak area normalization method.

The products of AD were acylated with acetic anhydride in the presence of pyridine. Here, only the acetylation of acetyl was necessary to produce pyranocoumarins capable of antagonizing Ca^{2+} influx,[12] other acyls will have to be studied at some future time.

Table 1. AchE inhibition data of coumarins (AchE from erythrocyte membrane of rat)

Samples	Concentration (mol/L)	Inhibitory rate (%)	Samples	Concentration (mol/L)	Inhibitory rate (%)
galanthamine	10^{-5}	73.94 ± 7.00	**8***	10^{-4}	39.06 ± 3.72
DMSO (solvent)	-	-	**9**	10^{-4}	25.12 ± 3.41
3	10^{-4}	17.94 ± 2.90	**10***	10^{-4}	45.47 ± 4.84
4	10^{-4}	14.20 ± 1.85	**11**	10^{-4}	14.80 ± 7.57
5*	10^{-4}	43.48 ± 6.52	**12***	10^{-4}	57.54 ± 9.32
6*	10^{-4}	33.51 ± 4.64	**13**	10^{-4}	-2.53 ± 8.09
7	10^{-4}	11.13 ± 2.14	decursinol	10^{-4}	28.90 ± 2.42

* The products were higher than decursinol towards AchE inhibition

Bioactivity: All the synthesized products that were screened showed AchE inhibition (Table 1), but their inhibitory rate were lower than the control galanthanmine. The rates for **5**, **6**, **8**, **10**, and **12** were higher than decursinol, and the inhibitory activity of **12,** with a rate of 57.54%, showed especially promising

potential. In our experiment, the inhibitory activity of decursinol was lower than values reported in literature (IC_{50}=2.8 x 10^{-5} M),[9] this difference may have been due to enzyme resources. However, one question that arises when considering the influences of coumarin nuclei on inhibitory factors is whether or not substitutions in positions 6 and 8 played vital roles in the inhibition of AchE. If positions 6 and 7 formed a pyran ring, the activity increased; when a supplied electron group, for example, methyl was introduced into position 8, the activity remarkably decreased; if a heavy atom, iodine, was introduced into position 8, the activity remarkably increased. The acylated compounds were higher in activity than the dihydroxylated ones, which was in contrast to the conclusion 9 that a hydroxy group essential to position 3′ to obtain inhibitory activity. This maybe results from 3′, 4′-disubsituted compound and suggests that acyl alternation could increase AchE inhibition.

EXPERIMENTAL

CHEMISTRY

Melting points were determined on an X4 micromelting point apparatus and were uncorrected. Optical rotations were recorded with a PE-241MC polarimeter at 25 ºC at the sodium D-line. ^{1}HNMR spectra were recorded on a Bruker ACF-300 instrument using TMS as an internal standard. IR spectra were recorded with Nicolet Impact-410 spectrophotometer. EIMS and ESIMS spectra were recorded with a HP5989A and an Agilent 1100 Series LC/MSD Trap instrument. Element analysis was performed at Carlo Erba 1106. Chiral separation was achieved with Angilent HP 1100 on a ODS column (4.6 x 150, Dikma, USA). Unless otherwise stated, all reagent chemicals and solvents were purchased from Nanking No. 1 Chemical Reagent Factory and Shanghai General Factory of Chemical Reagent. AD-mix-β, $K_2OsO_2(OH)_4$, $(DHQD)_2PHAL$ were the products of Aldrich Chemical Co., 2-methyl-3-butyn-2-ol, methanesulfonamide of Fisher Scientific, 2-methylresorcinol of ICN Biomedicals, TLC Si-gel 60GF_{254} of Yantai Chemical Reagent Factory, Si-gel 100-200 mesh and 200-300 mesh of Qingdao Ocean Chemical Reagent Factory. All reagents were analytical grade.

General procedure for synthesizing substituted 7-Hydroxycoumarins (3, 4)

H_2SO_4 (98%, 2 mL) was slowly added to a mixture of an resorcinol (1.1 g, 10 mmol) or 2-methylresorcinol (1.24 g, 10 mmol) and acetyl acetoacetate (1.95 g, 15 mmol) in a 1:1.5 molar ratio with stirring at 0 ºC over the course of 2 h. Then, the mixture was stirred at r t for 12 h, and was poured into ice-water while was stirred strongly, and allowed to stand overnight. The precipitated solid was

filtered, washed with water until neutral, and dried *in vacuo* to afford the residues.

7-Hydroxy-4-methylcoumarin (3): The residue was purified by the flash column chromatography with an eluant of petroleum ether:EtOAc=7:3 to yield the product, and recrystallized with EtOH as colorless needles, mp 187.0-188.0 ºC (lit.,[20] 186-188 ºC). Yield: 4.22 g (48%) (starting with 5.55 g of **1**). IR ν (KBr) 3160, 1678, 1598, 1389, 1273, 1238, 1212, 1158, 1133, 1067, 981, 845, 746 cm^{-1}; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.37 (3H, d, *J*=1.0 Hz, 4-CH_3), 6.13 (3H, d, *J*=1.0 Hz, H-3), 6.70 (1H, d, *J*=2.4 Hz, H-8), 6.81 (1H, dd, *J*=8.4, 2.4 Hz, H-6), 7.60 (1H, d, *J*=8.4 Hz, H-5), 10.51 (1H, s, -OH); ESIMS *m/z*: 177 $[M+H]^+$, 199 $[M+Na]^+$. Anal. Calcd for $C_{10}H_8O_3$: C, 68.18; H, 4.58. Found: C, 68.03; H, 4.48.

4, 8-Dimethyl-7-hydroxycoumarin (4): The residue was refluxed with acetone, filtered to afford white powder, mp 186.0-187.0 ºC (EtOH). Yield: 7.9 g (84%) (starting with 6.21 g of **2**). IR ν (KBr) 3221, 1696, 1681, 1611, 1572, 1384, 1365, 1317, 1089, 859, 810, 759 cm^{-1}; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.15 (3H, s, 8-CH_3), 2.36 (3H, s, 4-CH_3), 6.13 (1H, s, 3-H), 6.86 (1H, d, *J*=8.4 Hz, H-6), 7.65 (1H, d, *J*=8.4 Hz, H-5); ESIMS *m/z*: 191 $[M+H]^+$, 213 $[M+Na]^+$. Anal. Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30. Found: C, 69.58; H, 5.10.

General procedure for synthesizing 4-methyl-7-hydroxy-8-iodocoumarin (5)

Iodine 6.44 g (25.4 mmol) previously dissolved in 200 mL (60 mmol) of 5% aqueous KI was added dropwise to 4.0 g (22.7 mmol) of **3** in 100 mL of 20% aqueous NH_4OH solution during 30 min while under agitation. Agitation was maintained for an additional 2 h, and the necessary amount of 1.0 mol/L H_2SO_4 (285 mmol) was added to adjust to a slightly acidic pH, producing the precipitation. Filtration and recrystallization from EtOH/H_2O produced white granulated crystals, mp 223.5-225.0 ºC (lit.,[17] 219-221 ºC). Yield: 3.76 g (59%) (starting with 4.00 g of **3**). IR ν (KBr) 3166, 1682, 1598, 1538, 1381, 1308, 1181, 1078, 1043, 808, 755, 447 cm^{-1}; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.38 (3H, s, 4-CH_3), 6.18 (1H, s, 3-H), 6.92 (1H, d, *J*=8.9 Hz, H-6), 7.61 (1H, d, *J*=8.9 Hz, H-5), 11.35 (1H, s, -OH); ESIMS *m/z*: 303 $[M+H]^+$, 325 $[M+Na]^+$; EIMS *m/z* (%): 302 (M^+, 100), 275 (12), 274 (89), 147(11), 91 (19), 89 (11), 65 (11). Anal. Calcd for $C_{10}H_7O_3I$: C, 39.76; H, 2.34; I, 42.01. Found: C, 39.55; H, 2.52; I, 42.22%.

General procedure for synthesizing substituted xanthyletin (6-9)

A solution of 1 mmol of **4** or **5** in 2 mL of DMF (or 60 mL acetone) was reacted with 0.10 g (1.2 mmol) of 3-chloro-3-methylbutyne in the presence of anhydrous 0.345 g (2.5 mmol) of K_2CO_3 and 0.166 g (1 mmol) of KI by refluxing the mixture at 70~80 ºC for 4 h, monitored by TLC. After cooling, the mixture

was filtered and the solvent was concentrated *in vacuo*. The residue, without purification, was directly heated to reflux in 2 mL of *N,N*-diethylaniline for 4-6 h. The reaction mixture was cooled to rt, diluted with EtOAc, washed 2 times respectively with 10% aqueous HCl and water, and brine to neutral, and the organic layer was separated and solvent was removed *in vacuo*. The residue was purified by flash column chromatography with an eluent of petroleum ether:EtOAc=7:3 to afford substituted xanthyletins as **7**, **6**, **8** successively from **5**, as **9** from **4**.

4-Methyxanthyletin (6): Colorless prism, mp 181.0-182.0 ºC (petroleum ether:EtOAc) (lit.,[17] 176-177 ºC). Yield: 310 mg (10.5%, started with 3.7 g of **5**). IR ν (KBr): 3414, 2965, 1716, 1619, 1557, 1385, 1361, 1335, 1281, 1146, 1110, 1062, 850, 786, 764 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.46 (6H, s, 2'-CH_3 x 2), 2.44 (1H, d, *J*=1.0 Hz, 4-CH_3), 5.85 (1H, d, *J*=9.8 Hz, H-3'), 6.14 (1H, d, *J*=1.0 Hz, H-4'), 6.67 (1H, s, H-8), 7.48 (1H, s, H-5); ESIMS *m/z*: 243 $[M+H]^+$. Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.49; H, 5.57.

4-Methylseselin (7): Colorless needle, mp 145.0-147.0 ºC (petroleum ether:EtOAc) (lit.,[19] 141-143 ºC). Yield: 195 mg (6.6%, started with 3.7 g of **5**). IR ν (KBr): 3424, 1728, 1590, 1380, 1286, 1115, 1071, 740 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.47 (6H, s, 2'-CH_3 x 2), 2.38 (3H, d, *J*=1.5 Hz, 4-CH_3), 5.72 (1H, d, *J*=10.3 Hz, H-3'), 6.12 (1H , d, *J*=1.5 Hz, H-3), 6.74 (1H, d, *J*=8.3 Hz, H-6), 6.90 (1H, d, *J*=10.3 Hz, H-4'), 7.35 (1H, d, *J*=8.3 Hz, H-6); ESIMS *m/z*: 243 $[M+H]^+$. Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.21; H, 5.89.

4-Methy-8-iodoxanthyletin (8): Lightly yellow powder, mp 228.5-231.0 ºC (petroleum ether:EtOAc). Yield: 23 mg (0.5%, started with 3.7 g of **5**). IR ν (KBr): 3435, 2971, 2926, 1711, 1608, 1385, 1362, 1160, 1075, 876 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.46 (6H, s, 2'- CH_3 x 2), 2.40 (3H, s, 4-CH_3), 5.89 (1H, d, *J*=9.8 Hz, H-3'), 6.25 (1H, s, H-3), 6.51 (1H, d, *J*=9.8 Hz, H-4'), 7.54 (1H, s, H-5); ESIMS *m/z*: 369 $[M+H]^+$, 391 $[M+Na]^+$, Anal. Calcd for: $C_{15}H_{13}O_3I$: C, 48.93; H, 3.56; I, 34.47. Found: C, 48.63; H, 3.76; I, 34.34.

4, 8-Dimethylxanthyletin (9): White powder, mp175.0-177.0 ºC (petroleum ether:EtOAc) (lit.,[17] 176-179 ºC). Yield: 1.15 g (17.3%, started with 4.9 g of **4**). IR ν (KBr): 3450, 2972, 1703, 1616, 1397, 1123, 1108, 873, 752 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.47 (6H, s, 2'-CH_3 x 2), 2.27 (1H, s, 8- CH_3), 2.38 (3H, d, *J*=1.0 Hz, 4-CH_3), 5.69 (1H, d, *J*=9.8 Hz, H-3'), 6.11 (1H, d, *J*=1.0 Hz, H-3), 6.36 (1H, d, *J*=9.8 Hz, H-4'), 7.05 (1H, s, H-5); ESIMS *m/z*: 257 $[M+H]^+$, 279 $[M+Na]^+$. Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98; H, 6.29. Found: C, 74.72; H, 6.37.

General procedure of asymmetric dihydroxylation for synthesizing substituted (-)-*cis*-3', 4'-dihydroxy-3', 4'-dihydroxanthyletin (10, 11), chiral separation of 10 and 11 by HPLC

A mixture of 1.4 g of AD-mix-β and 0.0007 g (0.002 mmol) of $K_2OsO_2(OH)_4$, was solubilized in 20 mL of *t*-BuOH/H_2O (v/v, 1:1) at rt, then the solution was cooled to 0 ºC and 0.095 g (1 mmol) of methanesulfonamide added under stirring. When the solution turned from a light yellow to an orange color, the substituted xanthyletin compounds (1 mmol of **6** or **9**) were added. The mixture was stirred at 0 ºC until the reaction was complete as monitored by TLC. Excess $Na_2S_2O_3$, water, and EtOAc were added. After stirring for 30 min at rt, the mixture was extracted with EtOAc three times. The combined organic layer was dried with $MgSO_4$, then solvent was removed. The residue was separated by flash column chromatography with an eluent of petroleum ether:acetone=3:1 to afford appropriately substituted (-)-*cis*-dihydroxanthyletin, or not separated for directed acylation.

Chiral separation was achieved with Angilent HP 1100 on a ODS column (4.6 x 150, Dikma, USA), the mixture of methanol:acetonitrle:5% hydroxypropyl-β-cyclodextrin (β-HCD) (3:1:6) as mobile phase, flow rate 1 mL/min, UV 320 nm as detected wave length. The e.e. (%) were determined by peak area normalization method.

(-)-3'S,4'S-3',4'-Dihydroxy-4-methyl-3',4'-dihydroxanthyletin (10): White powder, mp 205-208 ºC (EtOH), e.e. 96%, $[\alpha]_D$ –113.7º (c 1.25 x 10^{-3}, $CHCl_3$). Yield: 132 mg (38.6 %, started with 300mg of **6**). IR ν (KBr): 3418, 1700, 1625, 1562, 1382, 1362, 1289, 1158, 1132, 1107, 847 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ1.25, 1.40 (each 3H, s, 2'-CH_3 x 2), 2.39 (3H, s, 4-CH_3), 3.17 (1H, d, *J*=5.4 Hz, H-3'), 5.07 (1H, d, *J*=5.4 Hz, H-4'), 6.18 (1H, d, *J*=1.0 Hz, H-3), 6.68 (1H, s, H-8), 7.77 (1H, s, H-5); ESIMS *m/z*: 277 $[M+H]^+$, 299 $[M+Na]^+$. Anal. Calcd for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found: C, 65.37; H, 5.67.

(-)-3'S,4'S-3',4'-Dihydroxy-4,8-dimethyl-3',4'-dihydroxanthyletin (11): White powder, mp 195.0- 198.0 ºC (petroleum ether:acetone), e.e. 95% , $[\alpha]_D$ -108.3º (c 8.4 x 10^{-3}, $CHCl_3$). Yield: 645 mg (49.4%, started with 1161 mg of **9**). IR ν (KBr): 3416, 2918, 1714, 1618, 1574, 1390, 1367, 1136, 1112, 943, 845 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.32, 1.57 (each 3H, s, 2'-CH_3 x 2), 2.28 (3H, s, 8-CH_3), 2.41 (3H, s, 4-CH_3), 3.80 (1H, d, *J*=4.4 Hz, H-3'), 4.89 (1H, d, *J*=4.4 Hz, H-4'), 6.11 (1H, s, H-3), 7.67 (1H, s, H-5); ESIMS *m/z*: 291 $[M+H]^+$, 313 $[M+Na]^+$. Anal. Calcd for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found: C, 66.39; H, 6.08.

General procedure of acylation for synthesizing 12, 13

0.08 g (0.29 mmol) of **10** or 0.08 g (0.28 mmol) of **11** was dissolved in 3.25 g (3 mL) of acetic anhydride,

1.47 g (1.5 mL) of anhydrous pyridine was added, under stirring for 2~4 h until the reaction was complete as monitored by TLC. EtOAc was added and the mixture was washed with 5% aqueous HCl, water respectively 2 times, and brine to neutral. The organic layer was dried with anhydrous $MgSO_4$, then solvent was removed *in vacuo*. The residue was purified by flash column chromatography with an eluent of petroleum ether:EtOAc=7:2 to afford appropriately substituted products.

(+)-3'S,4'S-3',4'-Diacetyloxy-4-methyl-3',4'-dihydroxanthyletin (12): Colorless jelly, $[\alpha]_D$ +37.3° (c 1.544 x 10^{-3}, $CHCl_3$). Yield: 33 mg (31.6%, started with 80 mg of **10**). IR ν (KBr): 2926, 1736, 1712, 1628, 1372, 1233, 1162, 1142, 1061 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.45 (6H, s, 2'-CH_3 x 2), 2.08, 2.17 (each 3H, s, 2"and 2"'-CH_3), 2.40 (3H, s, 4-CH_3), 5.36 (1H, d, *J*=4.8 Hz, H-3′), 6.15 (1H, s, H-3), 6.22 (1H, d, *J*=4.8 Hz, H-4′), 6.80 (1H, s, H-8), 7.42 (1H, s, H-5); ESIMS *m/z*: 361 $[M+H]^+$, 721 $[2M+H]^+$. Anal. Calcd for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59. Found: C, 63.04; H, 5.67.

(+)-3'S,4'S-3',4'-Diacetyloxy-4, 8-dimethyl-3',4'-dihydroxanthyletin (13): Colorless granulated crystals, mp 113.0-117.0 °C (EtOH), $[\alpha]_D$ +22.8° (c 1.643 x 10^{-3}, $CHCl_3$). Yield: 71 mg (68.8%, started with 80 mg of **11**). IR ν (KBr): 2928, 2850, 1754, 1729, 1574, 1376, 1240, 1090, 910, 876 cm^{-1}; ^{1}HNMR (300 MHz, $CDCl_3$) δ 1.45, 1.43 (each 3H, s, 2'-CH_3 x 2), 2.07, 2.15 (each 3H, s, 2", 2"'-CH_3), 2.30 (3H, s, 8-CH_3), 2.38 (3H, s, 4-CH_3), 5.35 (1H, d, *J*=4.5 Hz, H-3′), 6.14 (1H, s, H-3), 6.23 (1H, d, *J*=4.5 Hz, H-4′), 7.27(1H, s, H-5); ESIMS *m/z*: 375 $[M+H]^+$, 749 $[2M+H]^+$. Anal. Calcd for $C_{20}H_{22}O_7$: C, 64.16; H, 5.92. Found: C, 64.39; H, 5.66.

BIOACTIVITY

AchE inhibitory activity was determined by general method of Ellman *et al*.[22] using acetylthiocholine bromide as a substrate. For the enzyme source, 5-8 mL of blood from aorta of SD rat (350-400 g) belly was added 0.2 mL of heparin sodium, centrifuged at 1000 g for 15 min. Then hematocyte was obtained and washed with 0.15 mol/L of NaCl and 5 mmol/L of pH 7.4 phosphate-buffered isoosmotic solution (v/v=1:8) 3 times to afford red blood cell (rbc). 8.45 mmol/L of pH 8.0 phosphate-buffered hypoosmotic solution previously cooled was added to rbc and hemolysized in ice-water for 2 h, centrifuged at 9000 g at 4 °C to afford membrane of rbc, then was washed with hypoosmotic solution 3 times, centrifuged at 9000 g at 4 °C in buffer 10 mmol/L pH7.4 Tris-HCl, washed 3 times to afford milk white mash membrane of rbc, and preserved in suspension of Tris-HCl at -20 °C. The AchE activity was determined in 4 mL of Ellmans reaction mixture containing 0.1 mL of acetylthiocholine bromide (0.3 mmol/L), 1 mL of phosphate buffer (0.1 mol/L, pH 7.4), and 0.1 mL of sample, incubated at 37 °C for 5 min. Then added

0.1~0.2 mL enzyme solution and added water to 4 mL, incubated for 8 min. 1mL 3% SDS was added to stop reaction and 1 mL of 0.2% DTNB was added, absorbance (OD) at 440 nm was read at 752c UV spectroscopy.

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