Biotransformation of Turmerones by *Aspergillus niger*

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Biotransformation studies conducted on (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**) by the fungus *Aspergillus niger* have revealed that **1** was metabolized to give four oxidized metabolites, (+)-(7*S*)-hydroxydehydro-*ar*-todomatuic acid (**3**), (+)-(7*S*,10*E*)-12-hydroxydehydro-*ar*-todomatuic acid (**4**), (+)-(7*S*,10*E*)-7,12-dihydroxydehydro-*ar*-todomatuic acid (**5**), and (+)-(7*S*)-15-carboxy-9,13-epoxy-7-hydroxy-9,13-dehydro-*ar*-curcumene (**6**), and (+)-(*S*)-dihydro-*ar*-turmerone (**2**) was metabolized to (+)-7,11-dihydroxy-*ar*-todomatuic acid (**7**). Metabolites **3**−**7** were characterized using spectroscopic techniques. Metabolites **3**−**7** inhibited acetylcholinesterase (AChE) although less so than the parent substrates.

Bisabolane-type sesquiterpenoids occur naturally as (−)-α-bisabolol, (+)-(*S*)-*ar*-turmerone (**1**), and (+)-(*S*)-dihydro-*ar*-turmerone (**2**). These bisabolane-type sesquiterpenoids have a variety of biological activities.[1−5] We have reported potent acetylcholinesterase (AChE) inhibition by bisabolane-type terpenoids (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**).[6]

Biotransformation is today considered to be an economically competitive technology by synthetic organic chemists in search of new production routes for fine chemical, pharmaceutical, and agrochemical compounds.[7] In previous papers, we reported the biotransformation of some complex natural products and obtained a series of new products.[8−11] Therefore, it is envisioned that biotransformation of compounds **1** and **2** may provide analogues that could be tested for new and improved activities. The goal of the present work was the transformation of **1** and **2** by *A. niger*, and the evaluation of these compounds as AChE inhibitors (Figure 1).

Screening experiments showed that *Aspergillus niger* was capable of converting (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**) into more polar metabolites.

Incubation of (+)-(*S*)-*ar*-turmerone (**1**) with *A. niger* yielded the four metabolites **3**−**6**. Metabolite **3** was isolated as a colorless powder. The HRFABMS exhibited a [M + H]$^+$ peak at *m/z* 263.1284 (calcd 263.3105 for $C_{15}H_{19}O_4$), suggesting the formula $C_{15}H_{18}O_4$, 46 amu higher than the parent substrate **1**. The IR spectrum showed absorptions at 3440 and 1685 cm^{-1}, indicating the presence of hydroxyl and carbonyl groups. In comparison with the ^{1}H NMR spectrum of **1**, the spectra of **3** indicated the disappearance of methyl (C-15) and methine (C-7) signals. Comparison of the ^{13}C NMR spectroscopic data of **3** with **1** showed 13 similar chemical shifts. The remaining two ^{13}C chemical shifts included one oxyquaternary carbon (δ_C 73.7) and one carboxylic carbon (δ_C 170.4). The upfield shift of the aromatic C-4 quaternary carbon and downfield of the aromatic C-1 quaternary carbon coupled with the carboxylic carbon (C-15) in the ^{13}C NMR spectra showed the presence of an α,β-conjugated carboxylic group in **3**. The HMBC spectrum of **3** showed two-bond correlations between H-8 (δ_H 3.17 and 2.89)/C-7, H-14 (δ_H 1.52)/C-7 and three-bond correlations between H-2,6 (δ_H 7.55)/C-7. Thus, chemical structure of metabolite **3** was characterized as (+)-7-hydroxydehydro-*ar*-todomatuic acid, a new compound. The proton and carbon assignments were unambiguously made from the COSY, HSQC, and

HMBC spectra. The absolute configurations of **3** at the C-7 position was established to be *S* after conversion into tetrahydro-2-(4-carbomethoxyphenyl)-2,6,6-trimethyl-4*H*-pyran-4-one (**8**).[12] Compound **8** showed negative Cotton effects [262 nm (Δε −7.67), 290 nm (Δε −40.7), 355 nm (Δε −2.01)] in the CD spectrum; therefore compound **3** was determined to be (+)-(7*S*)-hydroxydehydro-*ar*-todomatuic acid.

Metabolite **4** had the molecular formula $C_{15}H_{18}O_4$, as deduced by HRFABMS and ^{1}H and ^{13}C NMR, 46 amu more than **1**. The IR spectrum of **4** showed absorptions at 3392 and 1689 cm^{-1}, indicating the presence of hydroxyl and carbonyl groups. In comparison with the ^{1}H NMR spectrum of **1**, the spectra of **4** indicated the appearance of a CH_2OH group and the disappearance of two methyl groups. This clearly indicated that compound **4** were produced by the hydroxylation of **1**. The ^{1}H and ^{13}C NMR spectroscopic data of **3** and **4** were similar. Their ^{1}H and ^{13}C NMR spectra indicated that compound **4** also possessed a carboxylic acid at C-15, as does **3**. In NOE experiments, compound **4** showed an NOE correlation between H-10 (δ_H 6.43) and H-12 (δ_H 4.04). Consequently, the structure of **4** was concluded to be (+)-(7*S*,10*E*)-12-hydroxydehydro-*ar*-todomatuic acid.

Metabolite **5** had the molecular formula $C_{15}H_{18}O_5$, as deduced by HRFABMS and ^{1}H and ^{13}C NMR, 62 amu higher than the parent compound **1**. The IR spectrum exhibited absorptions at 3395 and 1692 cm^{-1}, indicating the presence of hydroxyl and carbonyl groups. The ^{1}H NMR spectrum of compound **5** was nearly identical to that of compound **3**, except in the disappearance of a methyl signal and the appearance of an additional downfield shift of C-12 (δ_H 4.02), which indicated that the C-12 methyl group was selectively hydroxylated into a hydroxymethylene group. In NOE experiments, compound **5** showed a NOE correlation between H-10 (δ_H 6.43) and H-12 (δ_H 4.02). Consequently, the structure of **5** was concluded to be (+)-(10*E*)-7,12-dihydroxydehydro-*ar*-todomatuic acid.

Metabolite **6**, a colorless powder, was shown to have the molecular formula $C_{15}H_{16}O_4$ (HRFABMS). The IR spectrum exhibited absorption bands at 3562 and 1682 cm^{-1}, indicating the presence of hydroxyl and carbonyl groups, respectively. Comparison of the ^{1}H NMR spectrum of this compound with metabolite **3** revealed significant similarity, except that the C-13 methyl proton at δ_H 1.87 disappeared and an olefinic proton (δ_H 7.06) appeared in **6**. These suggested that metabolite **6** also had the same α,β-conjugated carboxylic group at C-15 and hydroxyl group at C-8. A comparison of the ^{13}C NMR spectrum of **3** and **6** showed the disappearance of the C-9 ketone signal. The HMBC spectrum showed correlations between (i) H-8/C-1, 7, 9, 10, and 14, (ii) H-12/C-10, 11, and 13, and (iii) H-13/C-9, 10, and 11, indicating the presence of a 4-methylfurano ring system in **6**. Thus the structure

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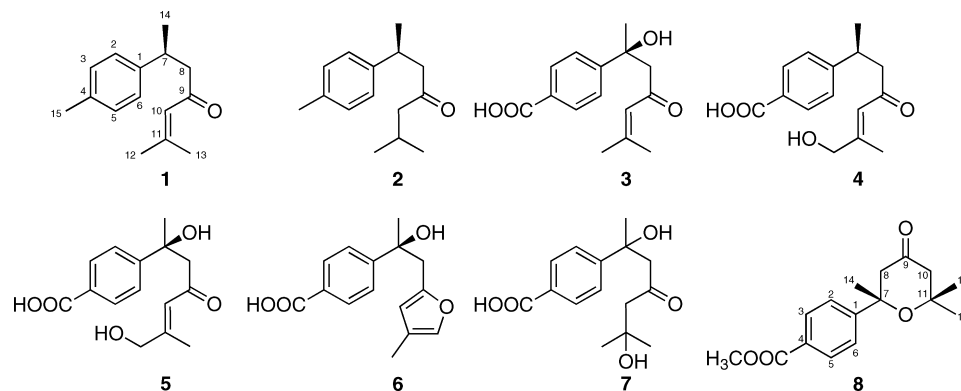 [‡] Picaso Cosmetic Laboratory Limited.

Figure 1. Chemical structures of (+)-(*S*)-*ar*-turemerone and (+)-(*S*)-dihydro-*ar*-turemerone derivatives.

Table 1. ^{13}C NMR Spectroscopic Data of Compounds **1**−**7**

^{13}C	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[b]	**6**[c]	**7**[b]
1	143.7	135.7	154.0	153.2	154.8	153.4	153.1
2, 6	126.7	126.6	124.7	127.9	125.8	125.0	124.6
3, 5	129.1	129.1	130.2	130.6	130.2	130.1	130.3
4	135.5	143.3	127.4	129.4	129.5	127.7	128.0
7	35.3	34.9	73.7	36.3	74.2	74.3	73.5
8	52.7	51.7	53.5	52.6	55.2	42.5	54.6
9	199.9	209.9	201.3	199.4	202.3	151.2	213.8
10	124.1	52.5	124.0	120.9	121.6	111.3	55.0
11	155.1	24.4	158.8	157.4	159.6	120.7	69.9
12	20.7	22.5	21.1	66.7	66.7	9.7	29.2[d]
13	27.6	22.5	27.9	16.0	16.2	138.7	29.3[d]
14	22.0	22.0	30.7	22.2	29.9	29.6	30.5
15	21.0	21.0	170.4	167.5	167.5	171.5	170.9

[a] Measured in CDCl$_3$ at 175 MHz. [b] Measured in acetone-d_6 at 175 MHz. [c] Measured in acetone-d_6 at 150 MHz. [d] Assignments may be interchanged.

of **6** was established as (+)-15-carboxy-9,13-epoxy-7-hydroxy-9,13-dehydro-*ar*-curcumene.

In the transformation of **1** the initial oxidation can occur at position 7 or 12, followed by the second oxidation to give **5**. Incubation of *A. niger* with compound **3** results in the formation of metabolites **5** and **6**, and incubation of compound **4** with the fungus results in the formation of metabolite **5**. Both products **5** showed the same optical rotation values as isolated compound **5** from **1**. As mentioned above, the absolute configuration of **5** and **6** at the C-7 position was established to be *S*; thus compounds **5** and **6** were determined to be (+)-(7*S*,10*E*)-7,12-dihydroxydehydro-*ar*-todomatuic acid (**5**) and (+)-(7*S*)-15-carboxy-9,13-epoxy-7-hydroxy-9,13-dehydro-*ar*-curcumene (**6**).

Compound **7** was assigned the molecular formula C$_{15}$H$_{20}$O$_5$ (HRFABMS). The ^{1}H NMR spectroscopic data of **7** were similar to those of **3**, except for H-10, 11 spectra which showed the disappearance of the C-10, 11 double bond. The upfield shift of the C-10, 11 carbons in the ^{13}C NMR spectra indicated the disappearance of the double bond and the presence of a hydroxyl group. The HMBC spectrum of **7** exhibited cross-correlations for C-11 (with H-10, H-12, and H-13), suggesting that the hydroxyl group was located at C-11. On the basis of these observations, the metabolite **7** was identified as (+)-7,11-dihydroxy-*ar*-todomatuic acid.

The metabolites **3**−**7** and substrates **1** and **2** were evaluated as AChE inhibitors. The results are given in Table 2. The biotransformation products **3**−**7** were less potent inhibitors of AChE than the corresponding precursors.

The results of this study established that (+)-(*S*)-*ar*-turmerone (**1**) was converted into two main oxidized metabolites, (+)-(7*S*,10*E*)-7,12-dihydroxydehydro-*ar*-todomatuic acid (**5**) and (+)-(7*S*)-15-carboxy-9,13-epoxy-7-hydroxy-9,13-dehydro-*ar*-curcumene (**6**), and minor two oxidized metabolites, (+)-(7*S*)-hydroxydehydro-*ar*-todomatuic acid (**3**) and (+)-(7*S*,10*E*)-12-hydroxydehydro-*ar*-todomatuic acid (**4**) by *A. niger*. (+)-(*S*)-

Dihydro-*ar*-turmerone (**2**) was converted into one oxidized metabolite, (+)-7,11-dihydroxy-*ar*-todomatuic acid (**7**). Metabolites **3**−**7** were new compounds. This evidence indicated that biocatalytic oxidation by *A. niger* is a feasible and effective approach to directly obtain new compounds **3**−**7**. These new metabolites will be useful reference standards for our continuing studies on structure−activity evaluation of bisabolane-type sesquiterpenoids.

Experimental Section

Table 2. AChE Inhibitory Activity of Compounds **1**−**7**

compound	IC_{50} $(\mu M)^a$ or % inhibitory activity $(250 \ \mu M)^b$
1	191.1 ± 0.3^a
2	81.5 ± 0.2^a
3	$(41.9 \pm 1.3\%)^b$
4	$(31.0 \pm 0.7\%)^b$
5	$(34.3 \pm 0.5\%)^b$
6	$(33.6 \pm 0.9\%)^b$
7	$(37.6 \pm 1.5\%)^b$
galanthamine^c	2.6 ± 0.1^a

^{a} Concentration of compound required for 50% enzyme inhibition as calculated from the dose−response curve. ^{b} The percent AChE inhibition values (250 μM) were calculated as compared with control activities (assumed to be 100%). ^{c} Positive control.

and acetylthiocholine iodide (ATC) were from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan.

Culture Conditions. Spores of *A. niger* NBRC 4414 (purchased from NITE Biological Resource Center, Chiba Japan) maintained on nutrient agar slants at 4 °C were inoculated into the autoclaved culture medium (250 mL in a 500 mL conical flask) containing saccharose (15 g), glucose (15 g), polypeptone (5 g), potassium chloride (500 mg), magnesium sulfate (500 mg), potassium dihydrogen phosphate (1 g), and iron(II) sulfate (10 mg) in distilled water (1 L). The culture was grown for 2 days in a shaking incubator at 28 °C and 120 rpm. The mycelia were then transplanted to culture medium (30 mL in a 50 mL Petri dish) and incubated for 36−48 h (until mycelia occupied 60−80% of the surface area of the culture medium). A solution of substrate (3 mg) dissolved in dimethyl sulfoxide (DMSO) (200 μL) was added to the culture medium. The Petri dishes were incubated at 28 °C under static conditions, together with two controls, which contained either mycelia with medium or substrate dissolved in DMSO with medium. Culture media and mycelium from Petri dishes were separated by filtration. The mycelium was acidified to pH 4 and extracted with ethyl acetate (3 × 30 mL). The combined organic extract was dried over anhydrous Na_2SO_4, evaporated *in vacuo*, and analyzed by TLC and HPLC to confirm the presence of metabolic compounds. The mobile phase and detector used are described in the General Experimental Procedures section. The amount of these compounds was calculated by means of the absolute calibration curves. The time course of biotransformation is shown in the Supporting Information, Figures 1S and 2 S.

Isolation of Metabolic Compounds from (+)-(S)-ar-Turmerone. (+)-(S)-ar-Turmerone (**1**) (300 mg), dissolved in DMSO (20 mL), was fed to the culture as described above. After the incubation period (3 days), the culture medium and mycelia were separated by filtration. The broth was acidified to pH 4 and extracted with ethyl acetate (3 × 500 mL). The solvent was evaporated *in vacuo*, and a crude extract (400 mg) was obtained. The extract was distributed between 5% $NaHCO_3$(aq) and ethyl acetate, and the ethyl acetate phase was evaporated to give the neutral fraction (134 mg). No metabolic compound was detected from the neutral fraction by TLC and HPLC. The alkali phase was acidified to pH 3 with 2 N HCl and distributed between water and ethyl acetate. The ethyl acetate phase was evaporated, and the acid fraction (230 mg) was obtained. The acidic fraction was subjected to silica gel column chromatography eluted with a gradient of CH_2Cl_2/MeOH to afford two fractions. Fraction 2 was further subjected to semipreparative reversed-phase HPLC with CH_3CN−H_2O (60:40, v/v + 0.05% CH_3COOH) to give metabolites **3** (30 mg), **4** (25 mg), **5** (90 mg), and **6** (66 mg), respectively.

(+)-(S)-ar-Turmerone (1):[6] pale yellow oil; $[\alpha]^{29}_D$ +63.7 (*c* 1.00, CHCl₃); IR (film) ν_{max} 1685, 1620 cm⁻¹; ¹H NMR (CDCl₃, 700 MHz) δ 7.10 (4H, brs, ArH), 6.02 (1H, m, H-10), 3.29 (1H, m, H-7), 2.71 (1H, dd, *J* = 15.5, 6.0 Hz, H-8), 2.61 (1H, dd, *J* = 15.5, 8.3 Hz, H-8), 2.31 (3H, s, H-15), 2.10 (3H, s, H-13), 1.85 (3H, d, *J* = 1.2, H-12), 1.24 (3H, d, *J* = 6.9 Hz, H-14); ¹³C NMR, see Table 1; EIMS *m/z* (relative intensity) 216 (M⁺ 30%), 201 (23%), 132 (22%), 119 (75%), 117 (14%), 91 (16%), 83 (100%), 77 (7%), 55 (18%); HREIMS 216.1602 (M⁺, $C_{15}H_{20}O$, calcd 216.1582).

(+)-(7S)-Hydroxydehydro-ar-todomatuic acid (3): colorless powder; $[\alpha]^{24.1}_D$ +5.93 (*c* 0.30, CHCl₃); IR (KBr) ν_{max} 3440, 1685 cm⁻¹; ¹H NMR (CDCl₃, 700 MHz) δ 8.05 (2H, dt, *J* = 8.8, 1.8 Hz, H-3, 5), 7.55 (2H, dt, *J* = 8.8, 1.8 Hz, H-2, 6), 5.99 (1H, m, H-10), 3.17 (1H,

d, *J* = 17.0 Hz, H-8), 2.89 (1H, d, *J* = 17.0 Hz, H-8), 2.06 (3H, d, *J* = 1.1 Hz, H-12), 1.87 (3H, d, *J* = 1.2 Hz, H-13), 1.52 (3H, s, H-14); ¹³C NMR, see Table 1; HRFABMS *m/z* 263.1284 [M + H]⁺ (calcd $C_{15}H_{19}O_4$, for 263.1283).

(+)-(7S,10E)-12-Hydroxydehydro-ar-todomatuic acid (4): colorless oil; $[\alpha]^{28.4}_D$ +48.6 (*c* 0.34, acetone); IR (film) ν_{max} 3392, 1689 cm⁻¹; ¹H NMR (CDCl₃, 700 MHz) δ 7.95 (2H, d, *J* = 8.2 Hz, H-3, 5), 7.40 (2H, d, *J* = 8.2 Hz, H-2, 6), 6.43 (1H, m, H-10), 4.04 (2H, brs, H-12), 3.40 (1H, m, H-7), 2.84 (1H, dd, *J* = 17.2, 6.7 Hz, H-8), 2.77 (1H, dd, *J* = 17.2, 7.6 Hz, H-8), 1.93 (3H, brs, H-13), 1.26 (3H, d, *J* = 7.0 Hz, H-14); ¹³C NMR, see Table 1; HRFABMS *m/z* 263.1287 [M + H]⁺ (calcd. $C_{15}H_{19}O_4$, for 263.1283).

(+)-(7S,10E)-7,12-Dihydroxydehydro-ar-todomatuic acid (5): colorless oil; $[\alpha]^{27.0}_D$ +2.04 (*c* 1.37, acetone); IR (film) ν_{max} 3395, 1692 cm⁻¹; ¹H NMR (acetone-d₆, 700 MHz) δ 7.96 (2H, dt, *J* = 8.2, 2.0 Hz, H-3, 5), 7.64 (2H, dt, *J* = 8.2, 2.0 Hz, H-2, 6), 6.43 (1H, m, H-10), 4.02 (2H, brs, H-12), 3.24 (1H, d, *J* = 16.2 Hz, H-8), 2.97 (1H, d, *J* = 16.2 Hz, H-8), 1.89 (3H, d, *J* = 1.0 Hz, H-13), 1.49 (3H, s, H-14); ¹³C NMR, see Table 1; HRFABMS *m/z* 279.1234 [M + H]⁺ (calcd $C_{15}H_{19}O_5$, for 279.1232).

(+)-(7S)-15-Carboxy-9,13-epoxy-7-hydroxy-9,13-dehydro-ar-curcumene (6): colorless powder; $[\alpha]^{24.2}_D$ +78.1 (*c* 0.53, CHCl₃); IR (KBr) ν_{max} 3562, 1682 cm⁻¹; ¹H NMR (acetone-d₆, 600 MHz) δ 8.07 (2H, d, *J* = 8.5 Hz, H-3, 5), 7.55 (2H, d, *J* = 8.2 Hz, H-2, 6), 7.06 (1H, m, H-13), 5.85 (1H, s, H-10), 3.13 (1H, d, *J* = 15.1 Hz, H-8), 3.07 (1H, d, *J* = 15.0 Hz, H-8), 1.94 (3H, d, *J* = 1.2 Hz, H-12), 1.57 (3H, s, H-14); ¹³C NMR, see Table 1; HRFABMS *m/z* 261.1154 [M + H]⁺ (calcd. $C_{15}H_{17}O_4$, for 261.1126).

Tetrahydro-2-(4-carbomethoxyphenyl)-2,6,6-trimetyl-4H-pyran-4-one (8). (+)-(7S)-Hydroxydehydro-ar-todomatuic acid (**3**) (4 mg) was dissolved in CH_2Cl_2 (1 mL), and CH_2N_2 (1 mL) was added to the solution. The solution was evaporated, and methylation fraction was obtained. The methylation fraction was subjected to column chromatography (200−300 mesh silica gel 60, Merck) with hexane−ethyl acetate (1:1) to yield the methyl ester derivative of **3** (3 mg, 75%). $Al(OCl_4)\cdot 9H_2O$ (catal.) was slowly added to a solution of a methyl ester derivative of **3** (3 mg, 0.46 mmol) in dry CH_3CN (2 mL), and the mixture was stirred at room temperature for 24 h. The mixture was extracted with ethyl acetate. After extraction, purification by column chromatography with hexane−acetone (7:1) gave **8** (1.2 mg, 4.3 mmol), identified by spectroscopic data. Colorless oil: $[\alpha]^{28.0}_D$ −76.7 (*c* 0.13, CHCl₃); IR (film) ν_{max} 1725, 1280 cm⁻¹; UV λ_{max} {MeOH, nm (log ε)} 278.1 (2.91), 238.5 (4.19); ¹H NMR (CD₂Cl₂, 500 MHz) δ 7.96 (2H, dt, *J* = 8.8, 2.0 Hz, H-3, 5), 7.54 (2H, dt, *J* = 8.8, 2.0 Hz, H-2, 6), 3.88 (3H, s, COO$\underline{CH_3}$), 3.13 (1H, dd, *J* = 16.0, 0.6 Hz, H-10α), 2.78 (1H, dd, *J* = 16.0, 0.7 Hz, H-10β), 2.35 (1H, d, *J* = 15.8 Hz, H-8β), 2.21 (1H, d, *J* = 15.8 Hz, H-8α), 1.52 (3H, s, H-14), 1.34 (3H, s, H-12), 1.11 (3H, s, H-13); ¹³C NMR (CD₂Cl₂, 125 MHz) δ 206.5 (C-9), 166.3 ($\underline{C}$OOCH₃), 152.6 (C-1), 129.1 (C-3, 5), 128.7 (C-4), 125.0 (C-2, 6), 76.9 (C-7), 74.9 (C-11), 51.6 (COO$\underline{CH_3}$), 50.6 (C-10), 48.6 (C-8), 33.6 (C-14), 31.2 (C-12), 29.9 (C-13); HRFABMS *m/z* 277.3296 [M + H]⁺ (calcd $C_{16}H_{21}O_4$, for 277.3292).

Isolation of Metabolic Compounds from (+)-(S)-Dihydro-ar-turmerone. (+)-(S)-Dihydro-ar-turmerone (**2**) (270 mg), dissolved in DMSO (18 mL), was fed to the culture as described above. After three days, the culture medium and mycelia were separated by filtration. The broth was acidified to pH 4 and extracted with ethyl acetate (3 × 500 mL). The solvent was evaporated *in vacuo*, and a crude extract (350 mg) was obtained. The extract was distributed between 5% $NaHCO_3$(aq) and ethyl acetate, and the ethyl acetate phase was evaporated to give the neutral fraction (123 mg). No metabolic compound was detected from the neutral fraction by TLC and HPLC. The alkali phase was acidified to pH 3 with 2 N HCl and distributed between water and ethyl acetate. The ethyl acetate phase was evaporated, and the acid fraction (190 mg) was obtained. The acidic fraction was subjected to silica gel column chromatography eluted with a gradient of CH_2Cl_2−MeOH to afford two fractions. Fraction 2 was further subjected to semipreparative reversed-phase HPLC with CH_3CN−H_2O (60/40, v/v) to give metabolite **7** (173 mg).

(+)-(S)-Dihydro-ar-turmerone (2):[6] colorless oil; $[\alpha]^{29}_D$ +32.1 (*c* 1.01, CHCl₃); IR (film) ν_{max} 1712, 1514 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 7.10 (4H, brs, ArH), 3.28 (1H, ddd, *J* = 8.0, 7.2, 6.3 Hz, H-7), 2.68 (1H, dd, *J* = 16.1, 6.3 Hz, H-8), 2.58 (1H, dd, *J* = 16.1, 8.0 Hz, H-8), 2.31 (3H, s, H-15), 2.19 (2H, m, H-10), 2.08 (1H, m,

H-11), 1.23 (3H, d, J = 7.2 Hz, H-14), 0.85 (6H, d, J = 4.6 Hz, H-12,13); ^{13}C NMR, see Table 1; EIMS m/z (relative intensity) 218 (M$^+$ 26%), 203 (25%), 161 (19%), 119 (100%), 105 (13%), 91 (13%), 85 (13%), 57 (18%); HREIMS 218.1777 (M$^+$, C$_{15}$H$_{22}$O, calcd 218.1741).

(+)-7,11-Dihydroxy-*ar*-todomatuic acid (7): colorless oil; [α]$^{25.2}_D$ −7.11 (c 1.62, CHCl$_3$); IR (film) ν$_{max}$ 3431, 1695 cm^{-1}; ^{1}H NMR (acetone-d_6, 700 MHz) δ 8.07 (2H, dt, J = 8.7, 1.8 Hz, H-3, 5), 7.53 (2H, dt, J = 8.7, 1.8 Hz, H-2, 6), 3.24 (1H, d, J = 17.2 Hz, H-8), 2.92 (1H, d, J = 17.2 Hz, H-8), 2.60 (1H, d, J = 16.8 Hz, H-10), 2.52 (1H, d, J = 16.8 Hz, H-10), 1.54 (3H, s, H-14), 1.19 (3H, s, H-12 or 13), 1.14 (3H, s, H-12 or 13); ^{13}C NMR, see Table 1; HRFABMS m/z 281.1400 [M + H]$^+$ (calcd C$_{15}$H$_{21}$O$_5$, for 281.1389).

Acetylcholinesterase Inhibitory Assay. The assay was carried out according to a published method.[6] Briefly, the wells contained 20 μL of human erythrocyte AChE solution (0.037 unit/mL in 0.01 M phosphate buffer, pH 7.4), 200 μL of DTNB (0.15 mM in 0.1 M phosphate buffer, pH 7.4), 30 μL of ATC in H$_2$O (final concentrations 0.25 mM), and 20 μL of a MeOH solution of the inhibitor. Control wells had MeOH added instead of inhibitor. The mixture was incubated at 28 °C for 15 min. The time at which the substrate addition was performed was considered as time zero. After the 15 min incubation, the absorbance of the mixture was measured at 405 nm using a microplate reader. The inhibition percentage of the AChE activity was calculated by using the equation

$$I(\%) = \{(A_{control} - A_{sample})/A_{control}\} \times 100\%$$

where A_{sample} is the absorbance of the sample-containing reaction mixture and $A_{control}$ is the absorbance of the reaction-control mixture. Galanthamine was used as positive control. All assays were run in triplicate.

References and Notes

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