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ARTICLE

# Acetylcholinesterase Inhibitory Activity of Volatile Oil from *Peltophorum dasyrachis* Kurz ex Bakar (Yellow Batai) and Bisabolane-Type Sesquiterpenoids

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In this study, the chemical compositions and acetylcholinesterase (AChE) inhibitory activitiy of the volatile oil from the bark of *Peltophorum dasyrachis* Kurz ex Bakar (yellow batai) were evaluated. As a result, 68 compounds, accounting for 88.0% of the total oil, were identified. The main characteristic constituent in *P. dasyrachis* was isolated by silica gel column chromatography and found to be a sesquiterpenoid, (+)-(*S*)-*ar*-turmerone (**1**). In the AChE inhibitory assay, the volatile oil showed potent inhibitory activity with the $IC_{50}$ value of 83.2 ± 2.8 μg/mL. Among the volatile oil components and characteristic sesquiterpenoids, (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**) were potent compounds, inhibiting AChE in a dose-dependent manner, with $IC_{50}$ values of 191.1 ± 0.3 and 81.5 ± 0.2 μM, respectively. (+)-(*S*)-Dihydro-*ar*-turmerone (**2**), in particular, was found to be the most potent AChE inhibitor. Also, bisabolane-type sesquiterpenoid derivatives, (+)-(7*S*,9*S*)-*ar*-turmerol (**3**), (+)-(7*S*,9*R*)-*ar*-turmerol (**4**), (+)-(7*S*,9*S*)-dihydro-*ar*-turmerol (**5**), (+)-(7*S*,9*R*)-dihydro-*ar*-turmerol (**6**), (+)-(*S*)-*ar*-curcumene (**7**), and (+)-(*S*)-dihydro-*ar*-curcumene (**8**), were synthesized and tested for their AChE inhibitory effect, and their structure−activity relationships were evaluated. All sesquiterpenoids exhibited AChE inhibitory activity. The order of AChE inhibitory potency by bisabolane-type sesquiterpenoids was as follows: ketones > alcohols > hydrocarbons. Furthermore, the inhibition kinetics analyzed by Dixon plots indicated that (+)-(*S*)-*ar*-turmerone (**1**) is a competitive inhibitor, with a $K_i$ value of 882.1 ± 2.1 μM, whereas (+)-(*S*)-dihydro-*ar*-turmerone (**2**) is a noncompetitive inhibitor.

**KEYWORDS: *Peltophorum dasyrachis*; yellow batai; volatile oil; sesquiterpens; acetylcholinesterase; (+)-(*S*)-*ar*-turmerone; (+)-(*S*)-dihydro-*ar*-turmerone**

## INTRODUCTION

Traditional herbs provide an interesting, largely unexplored source for the development of potential new drugs. The search for new traditional herbs for the development of new memory enhancement drugs, particularly edible herbs, which may cause lower side effects, has been emphasized and received a lot of attention recently. In addition, volatile constituents are likely to readily cross the blood−brain barrier because of their small molecular size and lipophilicity. Therefore, among these diverse natural compounds, volatile oils extracted from traditional herbs are attracting special attention. For example, it has been reported that volatile oils from some herbal medicines exhibit inhibitory activity against acetylcholinesterase (AChE) (*1*, *2*). Alzheimer's disease (AD) is a progressive brain disorder that gradually destroys a person's memory and ability to learn, reason, make judgements, communicate, and carry out daily activities. The greatly reduced presence of acetylcholine in the cerebral cortex is a significant factor in AD (*3*, *4*). The inhibition of AChE activity may be one of the most realistic approaches to the symptomatic treatment of AD. Therefore, volatile oils have gained importance because of their AChE inhibitory activity.

In our previous studies, some volatile oils and terpenoids from natural resources, *p*-menthane skeleton (*5*), essential oil of *Mentha* species (*6*), volatile α,β-unsaturated ketones (*7*), *Citrus paradisiaquatia* (*8*), bicyclic monoterpenoids (*9*), and tea tree oil (*10*), have demonstrated AChE inhibitory activity. In particular, *Mentha* species showed significant AChE inhibitory activity. Moreover, various volatile oils and their components from traditional herbs (*11*) and food crop (*12*) have also been investigated for their effect on AChE. It has been found that the majority of AChE inhibitors are monoterpenoids (*13*, *14*).

*Peltophorum dasyrachis* Kurz ex Bakar (Fabaceae) is a one of the Thai traditional herbs found throughout the tropics in moist area, Thailand, Cambodia, Malaysia, Laos, and Vietnam, where it grows as a large tree up to 30 m high. The leaves are bipinnate 15−40 cm long, pinnae 5−9 pairs, leaflets 6−16 pairs, oblong 5−10 mm wide, and 10−15 mm long. Stem barks have been used in traditional medicine for the treatment of antidysenteric,

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carminative, and antidiarrhea (*15*). In Thailand, its bark is drunk as a herbal tea. Furthermore, our previous paper reported that the $CHCl_3$ ext of *P. dasyrachis* showed an antimutagenic effect (*16*). However, no data were found on the chemical composition of volatile oil from *P. dasyrachis* and their biological property. Therefore, we investigated the bioactivity of the volatile oil from *P. dasyrachis* against AChE.

As a part our continuing programs to investigate AChE inhibitory activity by volatile components, we report here the chemical composition from volatile oil of *P. dasyrachis* and their AChE inhibitory activity to find a new source of a natural AD drug. Also, we report AChE inhibitory activities of main components to elucidate the responsible compounds for the activity of the volatile oil.

## MATERIALS AND METHODS

**Chemicals and Materials.** L-menthol, (+)-camphor, and eugenol (Nacalai Tesque, Inc., Kyoto, Japan), anisol and galanthamine (Wako Pure Chemical Industries, Osaka, Japan), (+)-borneol (Fluka Chemical Co. Inc., Japan), human erythrocyte acetylcholinesterase (AChE) (Sigma-Aldrich, Tokyo, Japan), 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and acetylthiocholine iodide (ATC) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) were purchased. Turmeric (*Curcuma longa* L.) oil was purchased from Synthite Industries Ltd. (+)-(*S*)-*ar*-Turmerone (**1**) was isolated from turmeric oil, and (+)-(*S*)-dihydro-*ar*-turmerone (**2**) was synthesized from compound **1** isolated from turmeric oil for quantitative analysis.

Column chromatography (CC) was performed using 200−300 mesh silica gel 60 (Merck). Thin-layer chromatography (TLC) analysis was run on 60 $F_{254}$ precoated silica gel plates (Merck), and spots were visualized by heating after spraying with vanillic sulfate reagent.

**Plant Material.** Air-dried bark tips of *P. dasyrachis* brought from Four Minds Co., Ltd. (Bangkok, Thailand) was a gift from Picaso Co., Ltd. (Hyogo, Japan).

**General Experimental Procedures.** *Hydrodistillation Process.* The air-dried bark tips (5 kg) of *P. dasyrachis* were subjected to hydrodistillation for 2 h in a Linkens−Nickerson-type apparatus (Osaka Riko Seisakusyo Co. Ltd., Japan). The obtained essential oil was dried over anhydrous sodium sulfate and, after filtration, stored under refrigeration until analyzed and tested. The sample was isolated in yields of 0.01% (v/w).

*Isolation of Main Sesquiterpenenoids. P. dasyrachis* oil (510 mg) was chromatographed over silica gel using a mixture of *n*-hexane and $CH_2Cl_2$ as eluents to yield six fractions (fractions 1−6). Fraction 2 (150 mg) was rechromatographed over silica gel using a mixture of *n*-hexane and $Et_2O$ to yield (+)-(*S*)-*ar*-turmerone (**1**) (72 mg, 14% yield) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**) (2 mg, 0.39% yield), respectively (**Figure 1**).

*Quantification of the Characteristic Sesquiterpenoids.* (+)-(*S*)-*ar*-Turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**) (0.01−4 mg/mL) were injected to examine the linear relations, respectively. Then, calibration curves were made accordingly to yield the following regression equations and ranges for quantificative analysis: $y = 9E + 07x + 372\,280$, $r = 0.999\,98$, and 0.04−4 mg (compound **1**); $y = 2E + 08x + 992\,456$, $r = 0.999\,73$, and 0.01−1 mg (compound **2**).

*Preparation of* (+)-(7*S*,9*S*)-*ar-Turmerol* (**3**) *and* (+)-(7*S*,9*R*)-*ar-Turmerol* (**4**). $LiAlH_4$ (17.3 mg, 0.46 mmol) was slowly added to a solution of (+)-(*S*)-*ar*-turmerone (**1**) (100 mg, 0.46 mmol) in dry tetrahydrofuran (THF) (2 mL), and the mixture was stirred at room temperature for 30 min. The reaction was stopped by the addition of $H_2O$. Filtration over celite afforded a mixture of (+)-(7*S*,9*S*)-*ar*-turmerol (**3**) and (+)-(7*S*,9*R*)-*ar*-turmerol (**4**). After evapolation of the solvent, purification by column choromatography ($SiO_2$, 4:1 hexane/EtOAc) afforded the separation of compounds **3** (52 mg, 0.24 mmol) and **4** (37 mg, 0.17 mmol) identified by a comparison to described compounds (*17*, *18*).

*Preparation of* (+)-(*S*)-*ar-Curcumene* (*7*). A three-neck, round-bottled flask was purged by a gentle flow of liquid ammonia at −33 °C. Small pieces of lithium (0.75 equiv) were added slowly to the liquid ammonia. The resulting solution slowly turned blue over a period of 30 min. The acetate of compound **3** (100 mg, 0.36 mmol) in dry THF (3 mL) was slowly added. After 45 min, excess $NH_4Cl$ (0.75 g, 0.014 mmol) was added. After a further 1 h, excess of the resulting solution slowly turned white. The residue was repeatedly extracted into $Et_2O$, and purification by column choromatography ($SiO_2$, 9:1 hexane/EtOAc) afforded the compound **7** (53.1 mg, 0.26 mmol), identified by a comparison to described compounds (*19*, *20*).

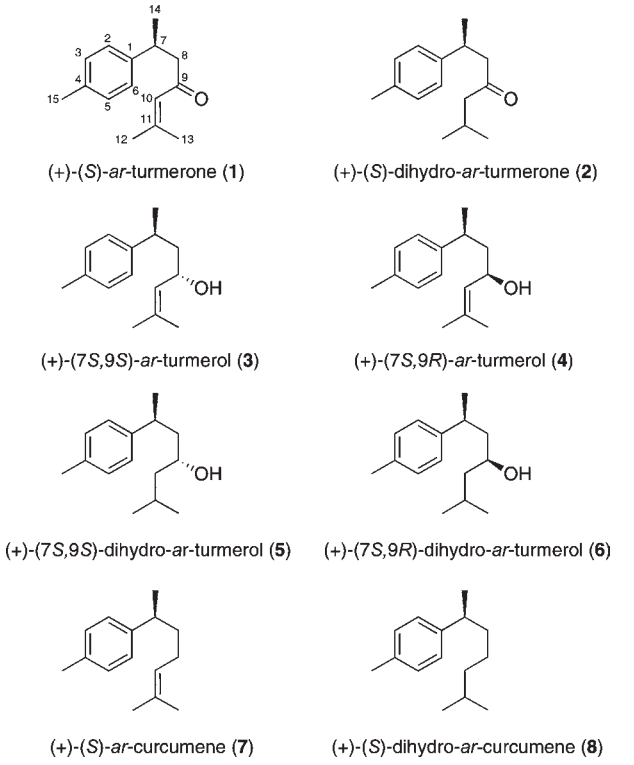


**Figure 1.** Structures of the isolated and synthetic sesquiterpenoid compounds **1**−**8**.

*Preparation of* (+)-(7*S*,9*S*)-*Dihydro-ar-turmerol* (**5**), (+)-(7*S*,9*R*)-*Dihydro-ar-turmerol* (**6**), *and* (+)-(*S*)-*Dihydro-ar-curcumene* (**8**). Compounds **3** (125 mg, 0.57 mmol), **4** (93 mg, 0.43 mmol), and **7** (76 mg, 0.38 mmol), and palladium on charcoal (0.2 equiv) in EtOH were added to a round-bottomed flask, respectively. The flask was connected to a rubber balloon filled with hydrogen. Stirring for the specified reaction time was then started. At the end of the reaction, palladium was immediately removed by filtration through celite. After evapolation of the solvent, purification by column choromatography afforded the compounds **5** (70 mg, 0.32), **6** (80 mg, 0.36 mmol), and **8** (55 mg, 0.25 mmol), respectively, identified by a comparison to described compounds (*21*, *22*).

*Analysis of Isolation Compounds.* Optical rotations were measured on a Japan Spectroscopic Co. LTDDIP-1000. IR spectra were determined with a FT/IR-470 Plus Fourier transform infrared spectrometer (JASCO Co., Ltd., Japan). High-resolution electron impact−mass spectrometry (HREI−MS) and EI−MS were measured on the JEOL tandem MS station JMS-700 (Japan Electron Optics Laboratory Co., Ltd., Tokyo Japan). $^1H$ and $^{13}C$ nuclear magnetic resonance (NMR), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond coherence (HMBC) spectra were measured on JEOL ECA spectrometers operating at 500 and 700 MHz for $^1H$ NMR and 125 and 175 MHz for $^{13}C$ NMR respectively, using tetramethylsilane (TMS) as an internal standard.

(+)-(*S*)-*ar-Turmerone* (*1*). Pale yellow oil. $[\alpha]^{29}_D$ +63.7 (*c* 1.00, $CHCl_3$). HREI−MS: 216.1602 ($M^+$, $C_{15}H_{20}O$, Calcd 216.1582). EI−MS *m*/*z* (relative intensity): 216 ($M^+$ 30%), 201 (23%), 132 (22%), 119 (75%), 117 (14%), 91 (16%), 83 (100%), 77 (7%), 55 (18%). IR (film) $cm^{-1}$: 1685, 1620. $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$: 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). $^{13}C$ NMR ($CDCl_3$, 125 MHz) $\delta$: 199.9 (C-9), 155.1 (C-11), 143.7 (C-1), 135.5 (C-4), 129.1 (C-3, C-5), 126.7 (C-2, C-6), 124.1 (C-10), 52.7 (C-8), 35.3 (C-7), 27.6 (C-13), 22.0 (C-14), 21.0 (C-15), 20.7 (C-12).

(+)-(*S*)-*Dihydro-ar-turmerone* (**2**). Colorless oil. $[\alpha]^{29}_{D}$ +32.1 (*c* 1.01, $CHCl_3$). HREI−MS: 218.1777 ($M^+$, $C_{15}H_{22}O$, Calcd 218.1741). EI−MS *m*/*z* (relative intensity): 218 ($M^+$ 26%), 203 (25%), 161 (19%), 119 (100%), 105 (13%), 91 (13%), 85 (13%), 57 (18%). IR (film) $cm^{-1}$: 1712, 1514. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 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, H-13). $^{13}$C NMR ($CDCl_3$, 125 MHz) $\delta$: 209.9 (C-9), 143.3 (C-4), 135.7 (C-1), 129.1 (C-3, C-5), 126.6 (C-2, C-6), 52.5 (C-10), 51.7 (C-8), 34.9 (C-7), 24.4 (C-11), 22.5 (C-12, C-13), 22.0 (C-14), 21.0 (C-15).

(+)-(*7S,9S*)-*ar-Turmerol* (**3**). Colorless oil. $[\alpha]^{22}_{D}$ +22.4 (*c* 1.00, $CHCl_3$). HREI−MS: 218.1800 ($M^+$, $C_{15}H_{22}O$, Calcd 218.1741). EI−MS *m*/*z* (relative intensity): 218 ($M^+$ 5%), 200 (9%), 157 (9%), 120 (17%), 119 (100%), 105 (14%), 91 (14%), 85 (42%). IR (film) $cm^{-1}$: 3373, 2965, 2925, 1515. $^1$H NMR ($CDCl_3$, 700 MHz) $\delta$: 7.09 (2H, dd, *J* = 8.8, 2.2 Hz, H-3, H-5), 7.06 (2H, dd, *J* = 8.8, 2.2 Hz, H-2, H-6), 5.15 (1H, dqq, *J* = 8.6, 1.2, 1.2 Hz, H-10), 4.20 (1H, m, H-9), 2.72 (1H, m, H-7), 2.31 (1H, s, H-15), 1.93 (1H, ddd, *J* = 13.4, 8.8, 6.7 Hz, H-8), 1.73 (3H, d, *J* = 1.2 Hz, H-12), 1.64 (1H, ddd, *J* = 13.4, 7.2, 6.2 Hz, H-8), 1.54 (1H, d, *J* = 1.2 Hz, H-13), 1.24 (3H, d, *J* = 7.0 Hz, H-14). $^{13}$C NMR ($CDCl_3$, 175 MHz) $\delta$: 144.1 (C-1), 135.6 (C-4), 135.4 (C-11), 129.1 (C-3, C-5), 128.0 (C-10), 126.8 (C-2, C-6), 67.0 (C-9), 46.1 (C-8), 36.1 (C-7), 25.8 (C-13), 22.9 (C-14), 21.0 (C-15), 18.3 (C-12).

(+)-(*7S,9R*)-*ar-Turmerol* (**4**). Colorless oil. $[\alpha]^{22}_{D}$ +13.9 (*c* 0.86, $CHCl_3$). HREI−MS: 216.1798 ($M^+$, $C_{15}H_{22}O$, Calcd 218.1741). EI−MS *m*/*z* (relative intensity): 218 ($M^+$ 5%), 200 (8%), 157 (8%), 120 (19%), 119 (100%), 105 (16%), 91 (14%), 85 (46%). IR (film) $cm^{-1}$: 3347, 2960, 2924, 1513. $^1$H NMR ($CDCl_3$, 700 MHz) $\delta$: 7.11 (4H, s, ArH), 5.15 (1H, dqq, *J* = 8.6, 1.2, 1.2 Hz, H-10), 4.17 (1H, m, H-9), 2.86 (1H, m, H-7), 2.32 (1H, s, H-15), 1.80 (1H, ddd, *J* = 13.4, 8.8, 5.8 Hz, H-8), 1.67 (3H, m, H-8), 1.67 (1H, d, *J* = 1.2 Hz, H-12), 1.53 (1H, d, *J* = 1.2 Hz, H-13), 1.23 (3H, d, *J* = 7.0 Hz, H-14). $^{13}$C NMR ($CDCl_3$, 175 MHz) $\delta$: 143.9 (C-1), 135.4 (C-4), 134.6 (C-11), 129.1 (C-3, C-5), 128.4 (C-10), 126.9 (C-2, C-6), 66.9 (C-9), 45.9 (C-8), 35.8 (C-7), 25.7 (C-13), 23.0 (C-14), 21.0 (C-15), 18.1 (C-12).

(+)-(*7S,9S*)-*Dihydro-ar-turmerol* (**5**). Colorless oil. $[\alpha]^{29}_{D}$ +13.7 (*c* 1.03, $CHCl_3$). HREI−MS: 220.1897 ($M^+$, $C_{15}H_{24}O$, Calcd 220.1900). EI−MS *m*/*z* (relative intensity): 220 ($M^+$ 6%), 202 (13%),145 (35%),132 (46%), 131 (25%), 120 (38%), 119 (100%), 105 (28%), 91 (18%). IR (film) $cm^{-1}$: 3365, 2956, 2926, 1515. $^1$H NMR ($CDCl_3$, 700 MHz) $\delta$: 7.11 (4H, s, ArH), 3.66−3.70 (1H, m, H-9), 2.84−2.89 (1H, m, H-7), 2.31 (3H, s, H-15), 1.72−1.77 (1H, m, H-11), 1.69−1.73 (1H, m, H-8), 1.64 (1H, ddd, *J* = 14.0, 7.6, 4.4 Hz, H-8), 1.36 (1H, ddd, *J* = 14.0, 8.8, 5.2 Hz, H-10), 1.25 (3H, d, *J* = 4.6 Hz, H-14), 1.23−1.27 (1H, m, H-10), 0.91 (3H, d, *J* = 6.7 Hz, H-12 or H-13), 0.86 (3H, d, *J* = 6.7 Hz, H-12 or H-13). $^{13}$C NMR ($CDCl_3$, 175 MHz) $\delta$: 144.4 (C-1), 135.6 (C-4), 129.2 (C-3, C-5), 126.7 (C-2, C-6), 68.4 (C-9), 47.1 (C-10), 46.9 (C-8), 36.4 (C-7), 24.5 (C-11), 23.5 (C-12 or C-13), 22.1 (C-14), 22.0 (C-12 or C-13), 21.0 (C-15).

(+)-(*7S,9R*)-*Dihydro-ar-turmerol* (**6**). Colorless oil. $[\alpha]^{29}_{D}$ +24.2 (*c* 1.03, $CHCl_3$). HREI−MS: 220.1999 ($M^+$, $C_{15}H_{24}O$, Calcd 220.1900). EI−MS *m*/*z* (relative intensity): 220 ($M^+$ 6%), 202 (13%), 145 (31%), 132 (41%), 131 (23%), 120 (43%), 119 (100%), 105 (28%), 91 (18%). IR (film) $cm^{-1}$: 3354, 2956, 2926, 1515. $^1$H NMR ($CDCl_3$, 700 MHz) $\delta$: 7.10 (4H, s, ArH), 3.41−3.44 (1H, m, H-9), 2.93−2.98 (1H, m, H-7), 2.32 (3H, s, H-15), 1.66−1.72 (4H, m, H-8, H-11), 1.60−1.65 (1H, m, H-8), 1.33 (1H, ddd, *J* = 13.7, 8.4, 6.0 Hz, H-10), 1.24 (3H, d, *J* = 6.8 Hz, H-14), 1.19 (1H, d, *J* = 13.7, 8.4, 4.8 Hz, H-10), 0.82 (3H, d, *J* = 3.4 Hz, H-12 or H-13), 0.81 (3H, d, *J* = 3.4 Hz, H-12 or H-13). $^{13}$C NMR ($CDCl_3$, 175 MHz) $\delta$: 143.7 (C-1), 135.4 (C-4), 129.1 (C-3, C-5), 126.9 (C-2, C-6), 67.9 (C-9), 47.3 (C-10), 46.1 (C-8), 36.0 (C-7), 24.6 (C-11), 23.4 (C-14), 23.2 (C-12 or C-13), 22.3 (C-12 or C-13), 21.0 (C-15).

(+)-(*S*)-*Curcumene* (**7**). Colorless oil. $[\alpha]^{25.4}_{D}$ +48.4 (*c* 1.02, $CHCl_3$). HREI−MS: 202.3333 ($M^+$, $C_{15}H_{22}$, Calcd 202.3367). EI−MS *m*/*z* (relative intensity): 202 ($M^+$ 29%), 145 (31%), 132 (98%), 131 (35%), 119 (100%), 105 (50%), 91 (30%), 55 (16%), 41 (33%). IR (film) $cm^{-1}$: 2962, 2921, 1514. $^1$H NMR ($CDCl_3$, 700 MHz) $\delta$: 7.09 (2H, dt, *J* = 8.2, 1.8 Hz, H-3, H-5), 7.07 (2H, dt, *J* = 8.2, 1.8 Hz, H-2, H-6), 5.09 (1H, m, H-10), 2.65 (1H, m, H-7), 2.31 (3H, s, H-15), 1.89 (2H, m, H-9), 1.67 (3H, *J* = 0.98 Hz, H-12), 1.59 (2H, m, H-8), 1.52 (3H, s, H-13), 1.21 (3H, d, *J* = 6.8 Hz, H-14). $^{13}$C NMR ($CDCl_3$, 175 MHz) $\delta$: 144.7 (C-1), 135.1 (C-4), 131.4 (C-11), 128.9 (C-3, C-5), 126.9 (C-2, C-6), 124.6 (C-10), 39.0 (C-7), 38.5 (C-8), 26.2 (C-9), 25.7 (C-12), 22.5 (C-14), 21.0 (C-15), 17.7 (C-13).

(+)-(*S*)-*Dihydro-ar-curcumene* (**8**). Colorless oil. $[\alpha]^{25.4}_{D}$ +29.2 (*c* 1.025, $CHCl_3$). HREI−MS: 204.3500 ($M^+$, $C_{15}H_{22}$, Calcd 204.3526). EI−MS *m*/*z* (relative intensity): 204 ($M^+$ 11%), 120 (11%), 119 (100%), 118 (3%), 117 (6%), 115 (3%), 105 (10%), 91 (7%), 41 (3%). IR (film) $cm^{-1}$: 2955, 2925,1514. $^1$H NMR ($CDCl_3$, 500 MHz) $\delta$: 7.10 (2H, dt, *J* = 8.0, 2.0 Hz, H-3, H-5), 7.07 (2H, dt, *J* = 8.0, 2.0 Hz, H-2, H-6), 2.64 (1H, m, H-10), 2.32 (3H, s, H-15), 1.58−1.43 (1H, m, H-11), 1.58−1.43 (2H, m, H-9), 1.21 (3H, d, *J* = 7.2 Hz, H-14), 1.28−1.10 (2H, m, H-10), 1.28−1.10 (2H, m, H-8), 0.83 (3H, d, *J* = 6.8 Hz, H-12 or H-13), 0.82 (3H, d, *J* = 6.8 Hz, H-2 or H-13). $^{13}$C NMR ($CDCl_3$, 175 MHz) $\delta$: 145.0 (C-1), 135.1 (C-4), 128.9 (C-3, C-5), 126.8 (C-2, C-6), 39.5 (C-7), 39.0 (C-8), 38.7 (C-9), 27.8 (C-11), 22.7 (C-12 or C-13), 22.6 (C-12 or C-13), 22.4 (C-14), 21.0 (C-15). These spectral data were compared to the published data.

*Gas Chromatography−Mass Spectrometry (GC−MS) Conditions.* GC−MS was used with a Hewlett-Packard 6890-5973 system (Agilent, Tokyo, Japan) using either of two capillary columns (DB-WAX, 15 m × 0.25 mm, film thickness of 0.25 $\mu$m, or HP-5MS, 30 m × 0.25 mm, film thickness of 0.25 $\mu$m). On DB-WAX, the column temperature was programmed from 40 to 240 °C at 4 °C/min and held at 240 °C for 5 min. On HP-5MS, the column temperature was programmed from 40 to 260 °C at 4 °C/min and held at 260 °C for 5 min. The injector and detector temperatures were 270 and 280 °C, respectively. The flow rate of carrier gas (He) was 1.4 mL/min, and the split ratio was 1:10. The detector interface temperature was set at 280 °C. The initial column temperature was kept at 40 °C for 5 min and programmed to 280 °C, with the actual temperature in the MS source reaching approximately 230 °C at a rate of 4 °C/min and then kept constant at 260 °C for 5 min. The ionization voltage was 70 eV, and acquisition mass range was 39−450 amu. Identification of the volatile oil components were carried out by a comparison of their relative retention times to those of authentic samples or by a comparison of their retention index (RI) relative to the series of *n*-hydrocarbons. Computer matching against commercial (NIST 98 and MassFinder 3.1) (*23*, *24*) and homemade library mass spectra made of pure substances and components of known oils and MS literature data was also used for the identification.

**AChE Inhibitory Assay.** The 96-well microplate method previously reported by Brühmann et al. (*25*) was modified. Briefly, the wells contained 20 $\mu$L of human erythrocyte AChE solution (0.037 unit/mL in 0.01 M phosphate buffer at pH 7.4), 200 $\mu$L of DTNB (0.15 mM in 0.1 M phosphate buffer at pH 7.4), 30 $\mu$L of ATC in $H_2O$ (final concentration of 0.25 mM), and 20 $\mu$L of a MeOH solution of the inhibitor. Control wells had MeOH added instead of the inhibitor. The mixture was incubated at room temperature (RT) for 15 min. The time at which the substrate addition was performed was considered as time zero. After 15 min of incubation, the absorbance of the mixture was measured at 405 nm using a microplate reader. The inhibition percentage of the AChE activity was calculated using the equation

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

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

**Determination of $IC_{50}$ Values and Kinetic Analysis.** The sample concentration showing 50% inhibition ($IC_{50}$) was calculated by plotting the inhibition percentages against the corresponding sample solution concentrations. Dissociation constants ($K_i$ values) were determined by interpretation of Dixon plots (*26*).

## RESULTS AND DISCUSSION

Pale yellowish colored volatile oil was obtained by hydrodistillation from the bark of *P. dasyrachis*. The identity of the chemical components of volatile oil was assigned by a comparison of their retention indices, relative to a series of *n*-hydrocarbon indices on the two capillary columns (DB-WAX and HP-5MS) and GC−MS spectra from the NIST 98 and MassFinder 3.1 MS data (see **Table 1**).

**Table 1.** Composition of the Volatile Oil of *P. dasyrachis*

| RI[a] | | | | |
|---|---|---|---|---|
| DB-WAX | HP-5MS | compounds[b] | relative content (%) | identification[c] |
| 1157 | | 1,4-cineole | 0.12 | MS, RI |
| 1183 | 1010 | 1,8-cineole | 0.43 | MS, RI |
| 1242 | 1004 | *p*-cymene | 0.12 | MS, RI |
| 1328 | | roseoxide | 0.16 | MS, RI |
| 1340 | | hexanol | 0.10 | MS, RI |
| 1403 | | *p*-cymenene | 0.40 | MS, RI |
| 1419 | 1052 | *trans*-linalool oxide | 1.25 | MS, RI |
| 1423 | 1070 | acetic acid | 0.38 | MS, RI |
| 1428 | | furfural | 0.25 | MS, RI |
| 1447 | 1068 | *cis*-linalool oxide | 0.67 | MS, RI |
| 1471 | 1125 | (+)-camphor | 5.95 | MS, RI, co-GC |
| 1478 | | benzaldehyde | 0.38 | MS, RI |
| 1503 | | 2-nonenal | 0.04 | |
| 1530 | | lonalool | 0.32 | MS, RI |
| 1533 | | 5-methyl furfural | 0.21 | MS, RI |
| 1540 | | isopulegol | 0.95 | MS, RI |
| 1557 | | 6-methyl-3,5-heptadiene-2-one | tr[d] | MS, RI |
| 1573 | | terpinen-4-ol | 1.63 | MS, RI |
| 1598 | 1175 | butyric acid | 0.13 | MS, RI |
| 1602 | | acetophenone | 0.18 | MS, RI |
| 1615 | | L-menthol | 2.40 | MS, RI, co-GC |
| 1634 | 1170 | isoborneol | 0.52 | MS, RI |
| 1640 | | isovaleric acid | 0.43 | MS, RI |
| 1668 | 1146 | (+)-borneol | 2.94 | MS, RI, co-GC |
| 1691 | 1162 | $\beta$-bisabolene | 0.41 | MS, RI |
| 1694 | | 1,4-dimethoxybenzene | 0.19 | MS, RI |
| 1722 | | 3-methyl-acetophenone | 1.09 | MS, RI |
| 1730 | | 2-methyl-3-phenylpropanal | 0.58 | MS, RI |
| 1735 | 1463 | (+)-(*S*)-*ar*-curcumene | 1.55 | MS, RI |
| 1744 | 1470 | 3,7-dimethyl-6-octen-1-ol | 0.29 | MS, RI |
| 1779 | | anisole | 1.38 | MS, RI, co-GC |
| 1811 | | caproic acid | 4.67 | MS, RI |
| 1821 | | safrole | 0.78 | MS, RI |
| 1833 | 1360 | benzyl alcohol | 0.46 | MS, RI |
| 1895 | | unknown | 1.72 | |
| 1917 | | enanthic acid | 0.55 | MS, RI |
| 1959 | | 2-methyl phenol | 0.26 | MS, RI |
| 1963 | 1384 | *p*-anisaldehyde | 0.44 | MS, RI |
| 1971 | | methyl eugenol | 0.67 | MS, RI |
| 1977 | 1363 | 3-phenyl-2-propanal | 0.17 | MS, RI |
| 2017 | 1175 | methyl cinnamate | 0.70 | MS, RI |
| 2023 | 1573 | caprylic acid | 16.50 | MS, RI |
| 2028 | 1445 | (+)-(*S*)-dihydro-*ar*-turmerone | 1.35 | MS, RI, NMR |
| 2072 | | ethyl cinnamate | 0.95 | MS, RI |
| 2080 | 1585 | $\alpha$-elemene | 0.29 | MS, RI |
| 2117 | | unknown | 1.01 | |
| 2126 | | unknown | 0.69 | |
| 2133 | 1350 | pelargonic acid | 0.69 | MS, RI |
| 2141 | 1367 | eugenol | 2.18 | MS, RI, co-GC |
| 2146 | | unknown | 0.19 | |
| 2150 | | 4-propanoyl anisole | 0.47 | MS, RI |
| 2159 | 1271 | 1,6-dimethyl-4-(1-methylethyl)-naphtalene | 0.43 | MS, RI |
| 2172 | 1590 | thymol | 0.61 | MS, RI |
| 2186 | 1646 | iso-$\alpha$-curcumene | 1.42 | MS, RI |
| 2201 | 1663 | (+)-(*S*)-*ar*-turmerone | 22.30 | MS, RI, NMR |
| 2234 | | capric acid | 0.81 | MS, RI |
| 2269 | | unknown | 0.46 | |
| 2282 | | 4-(2-propenyl)-phenol | 0.17 | MS, RI |
| 2400 | | (*Z*)-ethyl *p*-methoxy cinnamate | 0.32 | MS, RI |
| 2415 | | unknown | 1.91 | |
| 2450 | | lauric acid | 1.10 | MS, RI |
| 2566 | | (*E*)-ethyl *p*-methoxy cinnamate | 0.57 | MS, RI |
| 2658 | | myristic acid | 1.10 | MS, RI |
| 2753 | | pentadecylic acid | 0.37 | MS, RI |
| 2778 | | 13-methyl-oxacyclotetradecane-2,11-dione | 0.31 | MS, RI |
| 2847 | | palmitic acid | 6.58 | MS, RI |
| 3044 | | oleic acid | 0.92 | MS, RI |
| 3085 | | linoleic acid | 0.37 | MS, RI |
| | | total identified | 88.02 | |
| | | total | 100.00 | |

[a] RI = retention index on the DB-WAX or HP-5MS column. [b] Listed in order of elution from a DB-WAX column. [c] co-GC = co-injection with an authentic sample. [d] tr = trace (<0.05%).

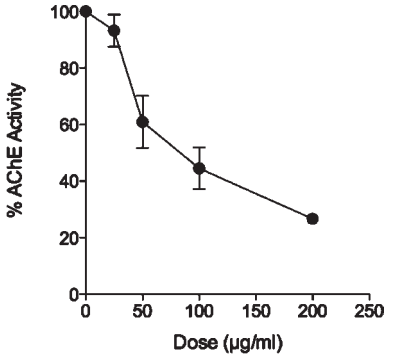


**Figure 2.** Inhibition of AChE activity by the volatile oil from *P. dashrachis*.

A total of 68 compounds were characterized and represented 88.0% of the total oil. This oil was characterized by a high content of (+)-(*S*)-*ar*-turmerone (**1**) (22.3%), caprylic acid (16.5%), and palmitic acid (6.6%). Hydrocarbons, such as alkenes, classificated as "others" constituented 3.9% of the volatile oil, followed by oxygenated sesquiterpenoids and oxygenated monoterpenenoids with 22.9 and 17.6%, respectively (**Table 1**). *P. dasyrachis* oil also contained a low amount of hydrocarbon sesquiterpenoids and hydrocarbon monoterpenoids, 3.1 and 0.5%, respectively. To the best of our knowledge, this is the first report of the volatile oil composition from *P. dasyrachis*.

As part of our effort to investigate the AChE inhibitory activity of the volatile oil from *P. dasyrachis*, the volatile oil revealed to inhibit this enzyme and an $IC_{50}$ value of 83.2 ± 2.8 $\mu$g/mL (**Figure 2**). To clarify the cause of the AChE inhibitory effect of the volatile oil, main monoterpenoids, (+)-camphor, L-menthol, and (+)-borneol; isolated sesquiterpenoids, (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**); and aromatic compounds, anisol and eugenol, were also examined for their AChE inhibitory activities. The results are expressed as graphs (**Figure 3**), and the $IC_{50}$ values are summarized in **Table 2**. These results suggest that (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**) more strongly inhibited AChE activity ($IC_{50}$ values of 191.1 ± 0.3 and 81.5 ± 0.2 $\mu$M, respectively) than that of the volatile oil (**Table 2**). On the other hand, monoterpenoids and aromatic compounds did not inhibit the enzyme at the maximum concentration tested, 250 $\mu$M. The same effect has been reported previously for terpinen-4-ol, $\gamma$-terpinene, $\alpha$-terpinene, *p*-cymene, and 1,8-cineol (*5*, *10*, *14*, *27*). It was suggested that the AChE inhibitory effect of the volatile oil may be performed in the presence of bisabolane-type sesquiterpenoids, (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**). Furthermore, active compounds, (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**), were quantitatively analyzed

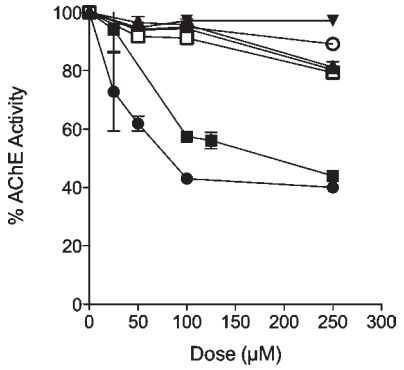


**Figure 3.** Inhibition of AChE activity by the main constituents found in the volatile oil from *P. dashrachis*: (■) (+)-(*S*)-*ar*-turmerone (**1**), (●) (+)-(*S*)-dihydro-*ar*-turmerone (**2**), (▲) (+)-camphor, (□) L-menthol, (◆) (+)-borneol, (○) anisol, and (▼) eugenol.

**Table 2.** $IC_{50}$ Values of Studied Compounds Affecting AChE Activity

| compounds | $IC_{50}$ ($\mu$M)[a] or percent inhibitory activity (250 $\mu$M)[b] |
|---|---|
| sesquiterpenoids | |
| (+)-(*S*)-*ar*-turmerone (**1**) | 191.1 ± 0.3[a] |
| (+)-(*S*)-dihydro-*ar*-turmerone (**2**) | 81.5 ± 0.2[a] |
| (+)-(7*S*,9*S*)-*ar*-turmerol (**3**) | (35.6 ± 0.9%)[b] |
| (+)-(7*S*,9*R*)-*ar*-turmerol (**4**) | (42.5 ± 3.3%)[b] |
| (+)-(7*S*,9*S*)-dihydro-*ar*-turmerol (**5**) | (48.6 ± 0.6%)[b] |
| (+)-(7*S*,9*R*)-dihydro-*ar*-turmerol (**6**) | 229.4 ± 2.6[a] |
| (+)-(*S*)-*ar*-curcumene (**7**) | (14.6 ± 0.9%)[b] |
| (+)-(*S*)-dihydro-*ar*-curcumene (**8**) | (35.6 ± 1.2%)[b] |
| monoterpenoids | |
| (+)-camphor | (18.7 ± 2.2%)[b] |
| (+)-borneol | (19.7 ± 4.3%)[b] |
| L-methol | (20.7 ± 3.1%)[b] |
| aromatic compounds | |
| anisol | (10.9 ± 1.1%)[b] |
| eugenol | (1.1 ± 0.6%)[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 $\mu$M). [c] Positive control.

as 13.2 and 0.32% (v/v), respectively, by means of the respective calibration curves in the volatile oil. If the oil, which gave 73 ± 1.2% inhibition of human AChE at a final concentration of 200 $\mu$g/mL (**Figure 2**), is taken as a mixture of 100%, then a final concentration of 13.2% (+)-(*S*)-*ar*-turmerone (**1**) and 0.32% (+)-(*S*)-dihydro-*ar*-turmerone (**2**) in the oil would be 26 $\mu$g/mL (0.12 mM) and 0.64 $\mu$g/mL (0.029 mM), respectively. As calculated from a mean dose−response curve of (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**), a final concentration of 26 and 0.64 $\mu$g/mL would give 44 and 29% inhibition of human AChE.

To the best of our knowledge, no report has been described thus far for bisabolane-type sesquiterpenoids with an AChE inhibitory effect. To search for a more effective AChE inhibitor from bisabolane-type sesquiterpenoids and explore structure−activity relationships, various derivative compounds (+)-(7*S*,9*S*)-*ar*-turmerol (**3**), (+)-(7*S*,9*R*)-*ar*-turmerol (**4**), (+)-(7*S*,9*S*)-dihydro-*ar*-turmerol (**5**), (+)-(7*S*,9*R*)-dihydro-*ar*-turmerol (**6**), (+)-(*S*)-*ar*-curcumene (**7**), and (+)-(*S*)-dihydro-*ar*-curcumene (**8**) were tested for AChE inhibitory activity. The dose-dependent manner is shown in **Figure 4**. All sesquiterpenoids **1**−**8** exhibited AChE inhibitory activities. The $IC_{50}$ values of these sesquiterpenoids **1**−**8**, which indicated their AChE inhibitory activity, are presented in **Table 2**.

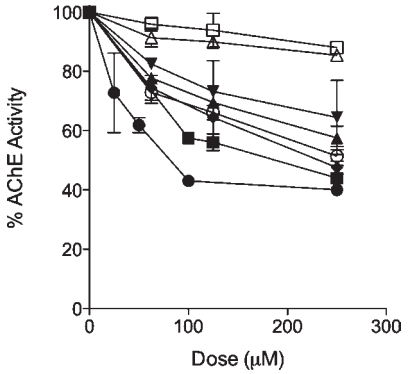


**Figure 4.** AChE inhibitory activities of the bisabolane-type sesquiterpenoids: (■) (+)-(*S*)-*ar*-turmerone (**1**), (●) (+)-(*S*)-dihydro-*ar*-turmerone (**2**), (▲) (+)-(7*S*,9*R*)-*ar*-turmerol (**3**), (▼) (+)-(7*S*,9*S*)-*ar*-turmerol (**4**), (◆) (+)-(7*S*,9*R*)-dihydro-*ar*-turmerol (**5**), (○) (+)-(7*S*,9*S*)-dihydro-*ar*-turmerol (**6**), (□) (+)-*ar*-curcumene (**7**), and (△) (+)-dihydro-*ar*-curcumene (**8**).

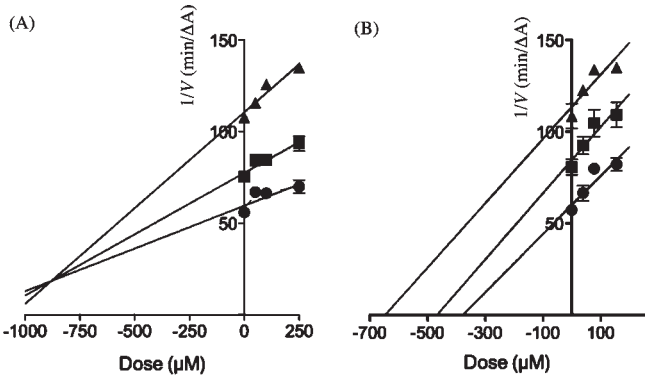


**Figure 5.** Dixon plots of the inhibition of AChE activities by (A) (+)-(*S*)-*ar*-turmerone and (B) (+)-(*S*)-dihydro-*ar*-turmerone. Each concentration of ATC is (▲) 50 $\mu$M, (■) 125 $\mu$M, and (●) 250 $\mu$M.

As seen from **Table 2**, compound **2** had the strongest inhibition (60.9 ± 0.2%) of AChE at a concentration of 250 $\mu$M, followed by compounds **1** (56.2 ± 0.2%), **6** (51.3 ± 2.6%), **5** (48.6 ± 0.6%), **4** (42.5 ± 3.3%), **3** (35.6 ± 0.9%), **8** (35.6 ± 1.2%), and **7** (14.6 ± 0.9%). This result showed that oxdation sesquiterpenoids have a higher AChE inhibitory activity compared to that of other hydrocarbon sesquiterpenoids. The order of AChE inhibitory potency by bisabolane-type sesquiterpenoids was as follows: ketones > alcohols > hydrocarbons (**Figure 4**). Additionally, the degree to which sesquiterpenoids inhibited AChE differed depending upon the presence of the C10−C11 double bond. While C10−C11 single-bond compounds **2**, **5**, **6**, and **7** were potent inhibitor of AChE, C10−C11 double-bond compounds **1**, **3**, **4**, and **7** were less potent inhibitors of AChE. From these results, we suggested that the bisabolane-type sesquiterpenoid skeleton containing an oxidation functional group at C9 and the single-bond moiety at C10−C11 plays an important role in the inhibitory activity against AChE.

Moreover, we investigated the Dixon plots of these isolated compounds on the inhibition of AChE (**Figure 5**). The (+)-(*S*)-*ar*-turmerone (**1**) result showed a competitive inhibitor, as indicated by increasing the substrate concentration and the interactions on the Dixon plot. The moderate active component, (+)-(*S*)-*ar*-turmerone (**1**), competes with the substrate for its active site on the enzyme. On the other hand, (+)-(*S*)-dihydro-*ar*-turmerone (**2**) was an uncompetitive inhibitor, as indicated by the decreasing inhibition associated with decreasing substrate concentrations

and parallelism of the Dixon plot. The most active component, (+)-(*S*)-dihydro-*ar*-turmerone (**2**), bound not to the enzyme but to the enzyme−substrate complex, thus preventing product formation.

This is the first study to screen the composition and inhibition of AChE activity of volatile oil from *P. dasyrachis*. The volatile oil showed a valuable AChE inhibitor, and isolated bisabolane-type sesquiterpenoids exhibited excellent AChE inhibitory activities. Especially, (+)-(*S*)-dihydro-*ar*-turmerone (**2**) was found to be the most potent inhibitor of AChE in an uncompetitive manner. Therefore, we have undertaken an investigation to the structure−activity relationship of bisabolane-type sesquiterpenoids. Interestingly, bisabolane-type sesquiterpenoid derivatives showed weak-to-high AChE inhibitory activity, depending upon their structural features. The highest activity was displayed by the oxidation functional group at C9 and the single-bond moiety at the C10−C11 structure, (+)-(*S*)-dihydro-*ar*-turmerone. The potent sesquiterpenoids, such as (+)-(*S*)-*ar*-turmerone (**1**) and (+)-(*S*)-dihydro-*ar*-turmerone (**2**), were fairly stable and volatile in nature. From our studies, the *P. dasyrachis* oil and bisabolane-type sesquiterpenoids are potential candidates for natural AChE inhibitors.

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