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Structure–activity relationships of 1′*S*-1′-acetoxychavicol acetate for inhibitory effect on NO production in lipopolysaccharide-activated mouse peritoneal macrophages

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Abstract—1′*S*-1′-Acetoxychavicol acetate from the rhizomes of *Alpinia galanga* inhibited nitric oxide (NO) production in lipopolysaccharide-activated mouse peritoneal macrophages with an IC_{50} value of 2.3 μM. To clarify the structure–activity relationship of 1′*S*-1′-acetoxychavicol acetate, various natural and synthetic phenylpropanoids and synthetic phenylbutanoids were examined, and the following structural requirements were clarified. (1) The *para* or *ortho* substitution of the acetoxyl and 1-acetoxypropenyl groups at the benzene ring was essential. (2) The *S* configuration of the 1′-acetoxyl group was preferable. (3) The presence of the 3-methoxyl group and disappearance of the 2′–3′ double bond by hydrogenation reduced the activity. (4) The substitution of acetyl groups with propionyl or methyl groups reduced the activity. (5) Lengthening of the carbon chain between the 1′- and 2′-positions reduced the activity.

1. Introduction

The inorganic free radical nitric oxide (NO) has been implicated in physiological and pathological processes such as vasodilation, nonspecific host defense, ischemia reperfusion injury, and chronic or acute inflammation. NO is produced by the oxidation of L-arginine catalyzed by NO synthase (NOS). In the NOS family, inducible NOS is particularly well known to be involved in the pathological overproduction of NO.[1]

The Zingiberaceae plant *Alpinia* (*A.*) *galanga* SWARTZ (syn. *Languas galanga* STUNZ) is widely cultivated in South and Southeast Asian countries. The rhizomes of this plant are extensively used as a spice or ginger substitute for flavoring foods, and also as a stomathic in traditional Chinese medicine, or as a carminative, antiflatulent, antifungal, and anti-itching agents in traditional Thai medicine. We previously reported gastroprotective and antiallergic constituents from the rhizomes of *A. galanga* and their structure activity relationships as well as mechanism of action.[2,3] In our continuing studies on this natural medicine, we found that the aqueous acetone extract had a potent inhibitory effect on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in mouse peritoneal macrophages (IC_{50} = 7.3 μg/mL). From the aqueous acetone extract, five known phenylpropanoids [1′*S*-1′-acetoxychavicol acetate (**1*S***, 2.3 μM), 1′*S*-1′-acetoxyeugenol acetate (**3*S***, 11 μM), *trans*-*p*-hydroxycinnamaldehyde (**30**, 20 μM), *trans*-*p*-hydroxycinnamyl acetate (**31**, 72 μM), *trans*-*p*-coumaryl diacetate (**33**, 19 μM)], new three 8–9′ linked neolignans [galanganal (68 μM), galanganol B (88 μM)], and a new sesquineolignan [galanganol C (33 μM)] were isolated as active principles.[4] Recently, Ohata et al. reported that **1*S*** from *L. galanga* strongly inhibited the production of NO by preventing the activation of nuclear factor-κB (NF-κB), which regulates inducible NO synthase in LPS- or interferon (IFN)-γ-treated murine macrophage-like RAW264 cells.[5] However, the structure–activity relationship of **1*S*** was not clarified.

In the present study, we describe the structure–activity relationship of **1*S*** for the inhibition of NO production,

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making comparisons with the activities of various natural and synthetic related compounds.

2. Results and discussion

2.1. Compounds isolated from the rhizomes of *A. galanga*

1′*S*-1′-Acetoxychavicol acetate (**1*S***, 1.10% from the dried rhizome), 1′*S*-1′-hydroxychavicol acetate (**2*S***, 0.048%), 1′*S*-1′-acetoxyeugenol acetate (**3*S***, 0.038%), chavicol β-D-glucopyranoside (**17**, 0.023%), methyleugenol (**20**, 0.0006%), *trans*-*p*-hydroxycinnamaldehyde (**30**, 0.028%), *trans*-*p*-hydroxycinnamyl acetate (**31**, 0.021%), *trans*-*p*-coumaryl alcohol (**32**, 0.052%), and *trans*-*p*-coumaryl diacetate (**33**, 0.015%) were isolated from dried rhizomes of *A. galanga* as described previously.[2–4]

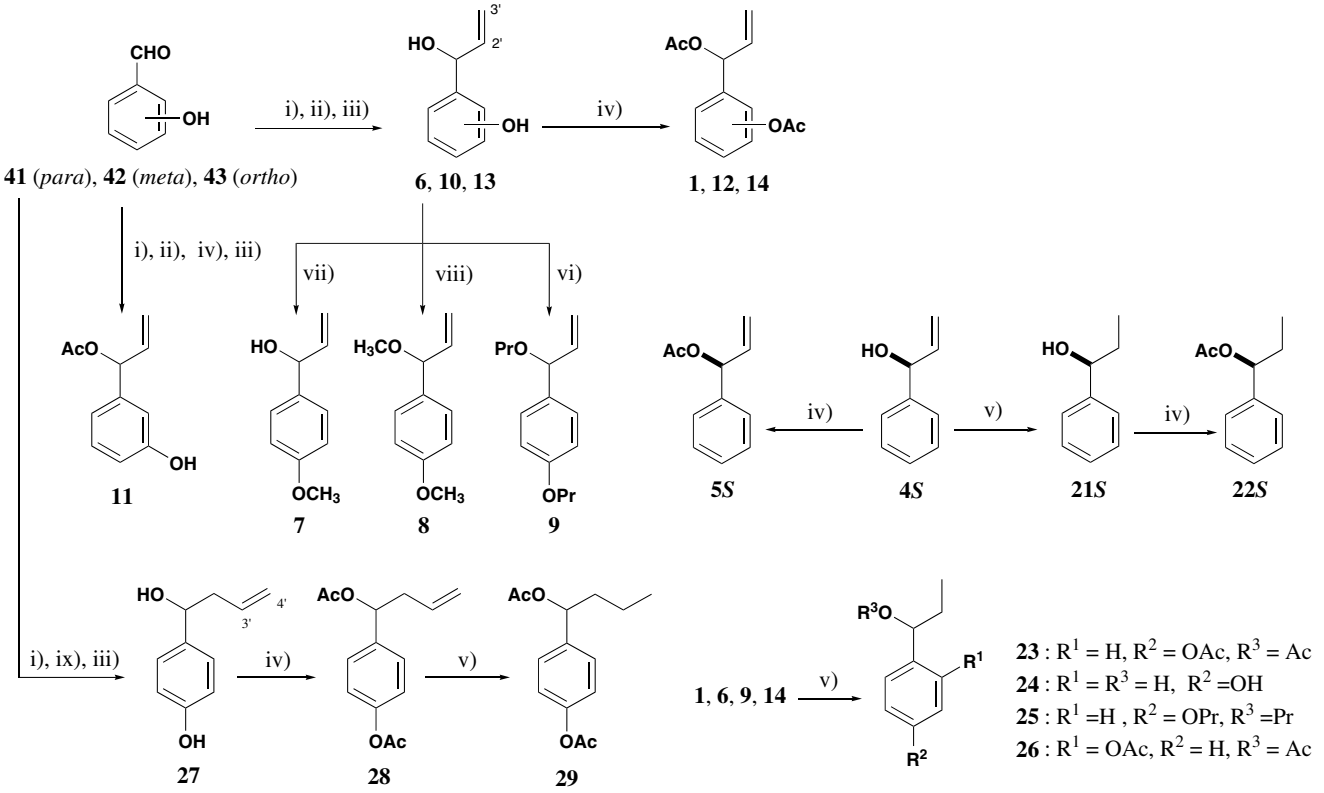
2.2. Related natural and synthetic compounds[6]

(*S*)-α-Vinylbenzyl alcohol (**4*S***) was purchased from Fluka Co. Ltd. Eugenol (**19**), and *p*-methoxycinnamic acid (**38**) were from Wako Pure Chemical. *trans*-Cinnamic acid (**34**), *trans*-*o*-coumaric acid (**35**), and caffeic acid (**37**) were from Nacalai Tesque. *trans*-*m*-Coumaric acid (**36**) was from Tokyo Kasei Kogyo. 3,4-Dimethoxycinnamic acid (**39**) and 3,5-dimethoxy-4-hydroxycinnamic acid (**40**) were from Sigma. Demethyleugenol (**18**)[7] was isolated from the leaves of *Piper betle*. Chavicol (**15**) was obtained by hydrolysis of chavicol β-D-glucopyranoside (**17**) and acetylation of **15** afforded chavicol acetate (**16**). Compounds **1**,[8] **1*R***,[9] **5*S***,[10] **6**,[8] **7**–**14**,[11–18] **21*S***,[19] **22*S***,[20] **23**,[8] and **24**–**29**[21–26] were synthesized by Grignard reaction and then acylation, methylation or hydrogenation from benzaldehyde and *ortho*-, *meta*-, and *para*-hydroxybenzaldehydes as shown in Scheme 1.

2.3. Inhibitory effects of phenylpropanoids from *A. galanga* and related compounds on NO production in LPS-activated mouse peritoneal macrophages and the structural requirements for the activity

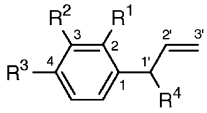
Among the phenylpropanoids from *A. galanga*, 1′*S*-1′-acetoxychavicol acetate (**1*S***) and 1′*S*-1′-acetoxyeugenol acetate (**3*S***) showed inhibitory activity with respective IC_{50} values of 2.3 and 11 μM and no cytotoxic effect (Table 1). The inhibitory activity of **1*S*** was stronger than those of two NOS inhibitors [N^G-monomethyl-L-arginine (L-NMMA, IC_{50} = 57 μM) and guanidinoethyldisulfide (GED, 7.4 μM)] and an inhibitor of NF-κB activation [caffeic acid phenethyl ester (CAPE, 15 μM)].[4]

To clarify the structure–activity relationships of the active phenylpropanoids **1*S*** and **3*S***, we examined the inhibitory effects of natural and synthetic phenylpropanoids (**1**–**26**, **30**–**40**) and synthetic phenylbutanoids (**27**–**29**) on the NO production. 1′*S*-1′-Hydroxychavicol acetate (**2*S***) lacking the 1′-acetyl group and chavicol acetate (**16**) lacking the 1′-acetoxyl group were less active than **1*S*** [**1*S*** (IC_{50} = 2.3 μM) > **16** (65 μM) > **2*S***

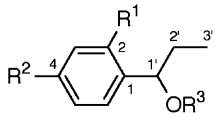


Scheme 1. Reagents and conditions: (i) *t*-butyldimethylchlorosilane, imidazole/pyridine, rt; (ii) vinylmagnesium bromide/THF, 0 °C; (iii) tetrabutylammonium fluoride/THF, rt; (iv) Ac_2O/pyridine, rt; (v) H_2, 5% Pd–C/MeOH, rt; (vi) propionylchloride/pyridine, rt; (vii) CH_3I, K_2CO_3/DMF, rt; (viii) CH_3I, NaH/DMF, rt; (ix) allylmagnesium bromide/THF, 0°C.

Table 1. Effects of phenylpropanoids from the rhizomes of *A. galanga* and related natural and synthetic compounds on NO production in LPS-activated mouse peritoneal macrophages-1



	R^1	R^2	R^3	R^4	IC_{50} (μM)
1′-Acetoxychavicol acetate (**1**)	H	H	OAc	OAc	4.9
1′*S*-1′-Acetoxychavicol acetate (**1*S***)	H	H	OAc	OAc (*S*)	2.3
1′*R*-1′-Acetoxychavicol acetate (**1*R***)	H	H	OAc	OAc (*R*)	8.2
1′*S*-1′-Hydroxychavicol acetate (**2*S***)	H	H	OAc	OH (*S*)	>100 (46%)
1′*S*-1′-Acetoxyeugenol acetate (**3*S***)	H	OCH_3	OAc	OAc (*S*)	11
4*S*	H	H	H	OH (*S*)	>100 (19%)
5*S*	H	H	H	OAc (*S*)	>100 (20%)
6	H	H	OH	OH	>100 (−1%)
7	H	H	OCH_3	OH	>30 (−3%)[a]
8	H	H	OCH_3	OCH_3	>100 (22%)
9	H	H	OPr	OPr	30
10	H	OH	H	OH	>100 (1%)
11	H	OH	H	OAc	>100 (25%)
12	H	OAc	H	OAc	>100 (20%)
13	OH	H	H	OH	>100 (0%)
14	OAc	H	H	OAc	3.2
Chavicol (**15**)	H	H	OH	H	>100 (43%)
Chavicol acetate (**16**)	H	H	OAc	H	65
Chavicol β-D-glucopyranoside (**17**)	H	H	*O*-Glc	H	>100 (6%)
Demethyleugenol (**18**)	H	OH	OH	H	15
Eugenol (**19**)	H	OCH_3	OH	H	>100 (15%)
Methyleugenol (**20**)	H	OCH_3	OCH_3	H	>100 (1%)



	R^1	R^2	R^3	IC_{50} (μM)
21*S*	H	H	H (*S*)	>100 (−4%)
22*S*	H	H	Ac (*S*)	>100 (25%)
23	H	OAc	Ac	10
24	H	OH	H	>100 (11%)
25	H	OPr	Pr	59
26	OAc	H	Ac	99

Values in parentheses represent the inhibition (%) at 100 μM ($N = 4$).

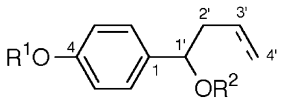
[a] Cytotoxic effect was observed at 100 μM. Bioassay methods for inhibitory activity in LPS-activated mouse peritoneal macrophages were described previously.[27]

(>100 μM)]. In addition, compound **5*S*** lacking the 4-acetoxyl group was inactive. Compound **14** (3.2 μM) with the 2 and 1′-acetoxyl groups showed equipotent activity to **1** (4.9 μM) with the 4 and 1′-acetoxyl groups, but compound **12** (>100 μM) with the 3 and 1′-acetoxyl groups lacked activity. Substitution of the acetyl groups of **1** with propionyl groups reduced the activity, and the substitution with methyl groups resulted in a complete loss of activity [**1** (4.9 μM) > **9** (30 μM) > **8** (>100 μM)]. On the other hand, 1′*S*-1′-acetoxyeugenol acetate (**3*S***, 11 μM) and demethyleugenol (**18**, 15 μM) showed substantial activity but were weaker than **1*S***, although **3*S*** has the 4 and 1′*S*-acetoxyl groups and **18** lacked the 4-acetyl and 1′-acetoxyl groups. These findings suggested that the 4- and 1′-acetoxyl groups of **1*S*** were essential for strong activity except for **18**, and the 3-methoxyl group reduced the activity. As to the relation between the absolute stereostructure of **1*S*** and the activity, **1*S*** showed stronger activity than **1*R*** and the racemate (**1**)[**1*S*** (2.3 μM) > **1** (4.9 μM) > **1*R*** (8.2 μM)]. This result suggested that the *S* configuration of the 1′-acetoxyl group was preferable for the activity. In addition, the substitution of acetyl groups with propionyl or methyl groups reduced the activity, and the substitution pattern of the acetoxyl and 1-acetoxylpropenyl groups at the benzene ring should be *ortho* or *para* for the activity (Table 2).

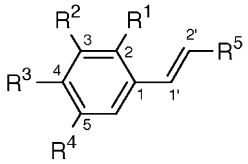
Next, contribution of the 2′–3′ double bond of **1** to the activity was investigated. The dihydroderivatives of **1**, **9**, and **14** were less active than **1**, **9**, and **14** [**1** (4.9 μM) > **23** (10 μM); **9** (30 μM) > **25** (59 μM); **14** (3.2 μM) > **26** (99 μM)].

On the other hand, phenylpropanoids (**32**, **34**–**40**) with the 1′–2′ double bond did not inhibit the production of NO either. However, **30** (20 μM) with the 4-hydroxyl and 2′-aldehyde groups, **31** (72 μM) with the 4-hydroxyl

Table 2. Effects of phenylpropanoids from the rhizomes of *A. galanga* and related natural and synthetic compounds on NO production in LPS-activated mouse peritoneal macrophages-2



	3′–4′	R^1	R^2	IC_{50} (μM)
27	C=C	H	H	>100 (13%)
28	C=C	Ac	Ac	9.2
29	C—C	Ac	Ac	47



	R^1	R^2	R^3	R^4	R^5	IC_{50} (μM)
trans-p-Hydroxycinnamaldehyde (**30**)	H	H	OH	H	CHO	20
trans-p-Hydroxycinnamyl acetate (**31**)	H	H	OH	H	CH_2OAc	72
trans-p-Coumaryl alcohol (**32**)	H	H	OH	H	CH_2OH	>100 (24%)
trans-p-Coumaryl diacetate (**33**)	H	H	OAc	H	CH_2OAc	19
trans-Cinnamic acid (**34**)	H	H	H	H	COOH	>100 (15%)
trans-o-Coumaric acid (**35**)	OH	H	H	H	COOH	>100 (7%)
trans-m-Coumaric acid (**36**)	H	OH	H	H	COOH	>100 (3%)
Caffeic acid (**37**)	H	OH	OH	H	COOH	>100 (15%)
p-Methoxycinnamic acid (**38**)	H	H	OCH_3	H	COOH	>100 (27%)
3,4-Dimethoxycinnamic acid (**39**)	H	OCH_3	OCH_3	H	COOH	>100 (1%)
3,5-Dimethoxy-4-hydroxycinnamic acid (**40**)	H	OCH_3	OH	OCH_3	COOH	>100 (27%)

Refer to footnotes of Table 1.

and 3′-acetoxyl groups, and **33** (19 μM) with the 4- and 3′-acetoxyl groups showed moderate levels of activity. Phenylbutanoid **28** with the 4- and 1′-acetoxyl groups and the 3′–4′ double bond was also active, but less active than **1**, and the dihydroderivative of **29** was less active than **28** [**1** (4.9 μM) > **28** (9.2 μM) > **29** (47 μM)]. These results suggested that the hydrogenation of the 2′–3′ double bond of **1** and the 3′–4′ double bond of **28** and lengthening of the carbon chain between the 1′- and 2′-positions in **1** reduced the activity.

In conclusion, the structural requirements of 1′*S*-1′-acetoxychavicol acetate (**1*S***) and 1′*S*-1′-acetoxyeugenol acetate (**3*S***) from the rhizomes of *A. galanga* for activity to inhibit production of NO were clarified as follows. (1) The *para* or *ortho* substitution of the acetoxyl and 1-acetoxylpropenyl group at the benzene ring was essential. (2) The *S* configuration of the 1′-acetoxyl group was preferable. (3) The presence of the 3-methoxyl group and disappearance of the 2′–3′ double bond by hydrogenation reduced the activity. (4) The substitution of acetyl groups with propionyl or methyl groups reduced the activity. (5) Lengthening of the carbon chain between the 1′- and 2′-positions reduced the activity.

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9. Compound **1*R*** was purified from **1**[8] using HPLC [column: CHIRALCEL OF (250 × 4.6 mm i.d., Daicel Chemical Ind., Ltd); detection: UV (254 nm); mobile phase: isopropanol/hexane (1:90, v/v); flow rate 1.0 mL/min; t_R **1*R***: 33.6 min $\{[\alpha]_D^{21}$ +57.1 (*c* 0.28, EtOH)}, **1*S***: 36.6 min $\{[\alpha]_D^{22}$ −56.5 (*c* 1.00, EtOH)} (ca. 1:1)].
10. Compound **5*S***: colorless oil. High resolution EI-MS: calcd for $C_{11}H_{12}O_2$ (M^+), 176.0837. Found: 176.0830. ^1H NMR (270 MHz, $CDCl_3$): δ 2.04 (3H, s), 5.18 (2H, m), 6.02 (1H,

m), 6.19 (1H, d, J = 6.0 Hz), 7.17–7.28 (5H, m). EI-MS m/z (%): 176 (M^+, 4), 83 (100).

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12. Compound **8**: colorless oil. High resolution EI-MS: calcd for $C_{11}H_{14}O_2$ (M^+), 178.0994. Found: 178.0998. ^{1}H NMR (270 MHz, $CDCl_3$): δ 3.30, 3.80 (3H each, both s), 4.57 (1H, d, J = 6.6 Hz), 5.18 (1H, br d, J = ca. 10 Hz), 5.25 (1H, br d, J = ca. 17 Hz), 5.93 (1H, ddd, J = 6.6, 10.4, 17.0 Hz), 6.88, 7.24 (2H, d, J = 8.6 Hz). EI-MS m/z (%): 178 (M^+, 3), 83 (100).
13. Compound **9**: colorless oil. High resolution EI-MS: calcd for $C_{15}H_{18}O_4$ (M^+), 262.1205. Found: 262.1196. ^{1}H NMR (270 MHz, $CDCl_3$): δ 1.15 (3H, t, J = 7.6 Hz), 1.26 (3H, t, J = 7.4 Hz), 2.38 (2H, q, J = 7.4 Hz), 2.58 (2H, q, J = 7.6 Hz), 5.24 (1H, br d, J = ca. 10 Hz), 5.29 (1H, br d, J = ca. 17 Hz), 5.98 (1H, ddd, J = 5.8, 10.4, 16.7 Hz), 6.28 (1H, d, J = 5.8 Hz), 7.07, 7.36 (2H each, both d, J = 8.6 Hz). EI-MS m/z (%): 262 (M^+, 4), 206 (50), 150 (72), 132 (100).
14. Compound **10**: colorless oil. High resolution EI-MS: calcd for $C_9H_{10}O_2$ (M^+), 150.0681. Found: 150.0687. ^{1}H NMR (270 MHz, $CDCl_3$): δ 5.03 (1H, d, J = 6.1 Hz), 5.11 (1H, br d, J = ca. 10 Hz), 5.26 (1H, br d, J = ca. 17 Hz), 5.99 (1H, ddd, J = 6.1, 10.4, 17.1 Hz), 6.66 (1H, ddd, J = 2.1, 2.5, 8.1 Hz), 6.81 (2H, m), 7.13 (1H, dd, J = 8.1, 8.1 Hz). EI-MS m/z (%): 150 (M^+, 34), 132 (100).
15. Compound **11**: colorless oil. High resolution EI-MS: calcd for $C_{11}H_{12}O_3$ (M^+), 192.0786. Found: 192.0791. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.12 (3H, s), 5.24 (1H, br d, J = ca. 10 Hz), 5.29 (1H, br d, J = ca. 17 Hz), 5.98 (1H, ddd, J = 5.9, 10.4, 17.1 Hz), 6.19 (1H, d, J = 5.9 Hz), 6.77 (1H, ddd, J = 2.0, 2.6, 8.1 Hz), 6.83 (1H, dd, J = 2.0, 2.0 Hz), 6.91 (1H, br d, J = ca. 8 Hz), 7.21 (1H, dd, J = 7.7, 8.1 Hz). EI-MS m/z (%): 192 (M^+, 31), 132 (100).
16. Compound **12**: colorless oil. High resolution EI-MS: calcd for $C_{13}H_{14}O_4$ (M^+), 234.0892. Found: 234.0888. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.11, 2.29 (3H each, both s), 5.26 (1H br d, J = ca. 10 Hz), 5.31 (1H, br d, J = ca. 17 Hz), 5.97 (1H, ddd, J = 5.9, 10.2, 16.8 Hz), 6.26 (1H, d, J = 5.9 Hz), 7.04 (1H, br d, J = ca. 8 Hz), 7.09 (1H, br s), 7.21 (1H, br d, J = ca. 8 Hz), 7.35 (1H, dd, J = 7.7, 7.9 Hz). EI-MS m/z (%): 234 (M^+, 9), 150 (100).
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18. Compound **14**: colorless oil. High resolution EI-MS: calcd for $C_{13}H_{14}O_4$ (M^+), 234.0892. Found: 234.0888. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.07, 2.30 (3H each, both s), 5.25 (1H, br d, J = ca. 10 Hz), 5.27 (1H, br d, J = ca. 17 Hz), 6.00 (1H, ddd, J = 5.6, 10.6, 17.2 Hz), 6.44 (1H, d, J = 5.6 Hz), 7.07 (1H, br d, J = ca. 8 Hz), 7.23 (1H, br dd, J = ca. 8, 8 Hz), 7.33 (1H, br dd, J = ca. 8, 8 Hz), 7.43 (1H, br d, J = ca. 8 Hz). EI-MS m/z (%): 234 (M^+, 3), 132 (100).
19. Compound **21*S*** was identified by comparison of its physical data with that of commercially obtained sample (Fluka Co. Ltd).
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22. Compound **25**: colorless oil. High resolution EI-MS: calcd for $C_{15}H_{20}O_4$ (M^+), 264.1361. Found: 264.1357. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.88 (3H, t, J = 7.3 Hz), 1.13 (3H, t, J = 7.7 Hz), 1.26 (3H, t, J = 7.3 Hz), 1.85 (2H, m), 2.35 (2H, q, J = 7.3 Hz), 2.58 (2H, q, J = 7.7 Hz), 5.68 (1H, t, J = 7.0 Hz), 7.05, 7.32 (2H each, both d, J = 8.4 Hz). EI-MS m/z (%): 264 (M^+, 4), 208 (17), 134 (100).
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25. Compound **28**: colorless oil. High resolution EI-MS: calcd for $C_{14}H_{16}O_4$ (M^+), 248.1049. Found: 248.1040. ^{1}H NMR (270 MHz, $CDCl_3$): δ 2.05, 2.27 (3H each, both s), 2.58 (2H, m), 5.05, 5.08 (1H each, both d-like), 5.67 (1H, m), 5.80 (1H, t, J = 6.3 Hz), 7.06, 7.34 (2H, d, J = 8.7 Hz). EI-MS m/z (%): 248 (M^+, 1), 146 (100).
26. Compound **29**: colorless oil. High resolution EI-MS: calcd for $C_{14}H_{18}O_4$ (M^+), 250.1205. Found: 250.1214. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.91 (3H, t, J = 7.3 Hz), 1.29, 1.80 (2H each, both m), 2.05, 2.28 (3H each, both s), 5.74 (1H, t, J = 6.3 Hz), 7.05, 7.33 (2H, d, J = 8.6 Hz). EI-MS m/z (%): 250 (M^+, 5), 123 (100).
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