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# Protective Effects of Polygodial and Related Compounds on Ethanol-Induced Gastric Mucosal Lesions in Rats: Structural Requirements and Mode of Action

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**Abstract**—The methanolic extract from the leaves of *Tasmannia lanceolata* was found to potently inhibit ethanol-induced gastric lesions in rats. Through bioassay-guided separation, three known sesquiterpenes, polygodial, polygodial 12α-acetal, and polygodial 12β-acetal, and a new sesquiterpene, methyl isodrimeninol, were isolated as the active constituents. Among them, polygodial showed very potent gastroprotective effects ($ED_{50} = 0.028$ mg/kg, po). From the gastroprotective effects of various reduction and oxidation derivatives of polygodial, the dialdehyde or diacetal structure was found to be essential for the strong activity. Since the gastroprotection of polygodial was attenuated by pretreatment with indomethacin, *N*-ethylmaleimide, $N^G$-nitro-L-arginine methyl ester and ruthenium red, endogenous prostaglandins, sulfhydryl compounds, nitric oxide and vanilloid receptors may be involved in the protective activity. 

## Introduction

In the course of our characterization studies on bioactive constituents from medicinal foodstuffs,[1] the methanolic extract from the leaves of *Tasmannia lanceolata* (Poir.) A. C. Smith (Winteraceae) (common name: mountain pepper) was found to show potent protective effect against ethanol-induced gastric lesions in rats. By bioassay-guided separation, three known sesquiterpenes, polygodial (**1**),[2] polygodial 12α-acetal (**2**),[3] and polygodial 12β-acetal (**3**),[3] and a new sesquiterpene, methyl isodrimeninol (**4**) (Chart 1), were isolated from the active fraction (AcOEt soluble portion). We found that **1** showed very potent gastroprotective effects ($ED_{50} = 0.028$ mg/kg, po).

Polygodial (**1**), a drimane-type sesquiterpene dialdehyde, was originally isolated from the Polygonaceae plant *Polygonum hydropiper*,[4] and subsequently from *Warburgia ugandensis*,[5] *Warburgia stuhlmannii* (Canellaceae),[5] and the Winteraceae plants *Tasmannia lanceolata*, *Drymis winteri*,[6] and *Pseudowintera colorata*.[7] Polygodial (**1**) has a sharp peppery taste, and it has been identified as an equivalent component in hot taste of peppery spices in traditional Japanese cuisine.[8] Polygodial (**1**) is known to exhibit various biological activities such as antihyperalgesia,[6] potent attachment–inhibitory activity,[9] insect antifeedant activity,[10] antinociception,[11] vasorelaxation action in vessels of rabbit and guinea-pig,[12] antiinflammatory activity,[13] antiallergic activity,[13] antifungal,[14] and antimicrobial activities.[14] However, the effects of **1** and its related compounds on gastrointestinal function have not been reported to date.

In this paper, we describe the protective effects of the principal drimane-type sesquiterpene constituents from the leaves of *T. lanceolata* against ethanol-induced gastric lesions in rats, as well as the structure elucidation of a new sesquiterpene (**4**). In addition, we discuss the structural requirements for the strong gastroprotective activity and mode of action of **1**, including the involvement of endogenous prostaglandins (PGs), nitric oxide (NO), sulfhydryl compounds (SHs) and vanilloid receptors, which play important roles in gastric defense mechanisms.

### Isolation of polygodial (1), polygodial 12α-acetal (2), polygodial 12β-acetal (3), and methyl isodrimeninol (4) from *T. lanceolata*

The dried leaves of *T. lanceolata* (500 g, cultivated in Tasmania, Australia) were extracted with methanol three times under reflux for 3 h. The methanolic extract (33.2% from this natural medicine) was partitioned into

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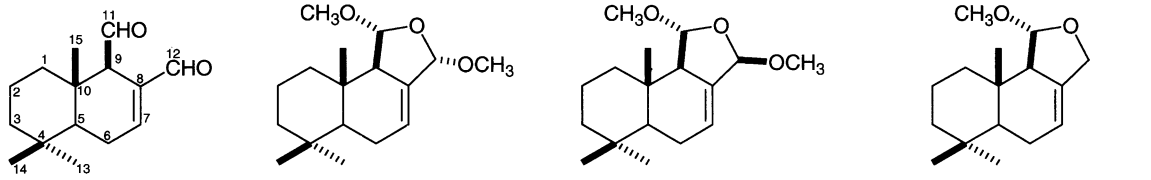


**Chart 1.** Chemical structures of **1**–**4** from *Tasmannia lanceolata*.

ethyl acetate (AcOEt) and water mixture to give an AcOEt-soluble portion (18.2%) and a water-soluble portion (15.0%). The AcOEt portion was subjected to ordinary-phase silica-gel ($SiO_2$) [*n*-hexane–AcOEt (20:1→10:1→5:1→1:1)→AcOEt] and reversed-phase silica-gel (ODS) column chromatography (MeOH–$H_2O$), and finally HPLC (YMC-Pack ODS-5-A, 250×20 mm i.d., MeOH–$H_2O$ or $CH_3CN$–$H_2O$) to give polygodial (**1**,[1,15] 0.97%), polygodial 12α-acetal (**2**,[3] 0.038%), polygodial 12β-acetal (**3**,[3] 0.019%), and methyl isodrimeninol (**4**, 0.007%) together with five known constituents [eugenol[16] (0.010%), elemicin[17] (0.003%), myristicin[18] (0.019%), caryophyllene oxide[19] (0.002%), and (+)-cyclocolorenone[20] (0.004%)].

**Table 1.** Effects of methanolic extract, AcOEt- and $H_2O$-soluble portions from *T. lanceolata* on gastric lesions induced by ethanol in rats

| Treatment | Dose (mg/kg, po) | *N* | Gastric lesions | |
|---|---|---|---|---|
| | | | Lesion index (mm) | Inhibition (%) |
| Control | — | 9 | 133.1±11.8 | — |
| MeOH extract | 2.5 | 5 | 86.5±16.8 | 35.0 |
| | 5.0 | 5 | 39.8±14.0** | 70.1 |
| | 10 | 5 | 10.8±5.0** | 91.9 |
| AcOEt-soluble portion | 1.25 | 6 | 84.2±19.0 | 36.7 |
| | 2.5 | 6 | 19.0±15.3** | 85.7 |
| | 5.0 | 6 | 10.3±7.6** | 92.3 |
| $H_2O$-soluble portion | 5.0 | 5 | 107.5±15.8 | 19.2 |
| | 10 | 5 | 84.7±10.6 | 36.4 |
| | 20 | 5 | 84.6±18.6 | 36.4 |
| Control | — | 6 | 159.2±21.0 | — |
| Omeprazole | 10 | 6 | 90.6±21.2** | 43.1 |
| | 20 | 6 | 16.9±6.1** | 89.4 |

Ethanol (1.5 mL/rat) was orally administered to 24 h-fasted male Sprague–Dawley rats weighing about 250 g. One hour later, the animals were sacrificed by cervical dislocation under ether anesthesia, and the stomach was dissected out and distended by injection of 10 mL 1.5% formalin to fix the inner and outer layers of the gastric walls. Subsequently, the stomach was incised along the greater curvature and the lengths of the necrotizing lesions were examined. The total length (mm) was expressed as a lesion index. The methanolic extract, AcOEt- and $H_2O$-soluble portions were suspended in 5% acacia solution, while a reference drug, omeprazole, was suspended in 0.5% CMC-Na. The corresponding vehicles were used in the control groups. Test samples and vehicle were administered orally (po) at a dose of 5 mL/kg. Values were expressed as means±SEM. One-way analysis of variance following Dunnett's test was used for statistical analysis (**$p<0.01$).

## Chemical structure of methyl isodrimeninol (4)

Methyl isodrimeninol (**4**),[21] a colorless oil, $[\alpha]_D^{25}$ −28.4 ° ($c=1.3$, $CHCl_3$), $C_{16}H_{26}O_2$, showed quasimolecular ion peaks at *m*/*z* 251 $(M+H)^+$ and *m*/*z* 273 $(M+Na)^+$ in positive-ion fast bombardment (FAB)-MS. The IR spectrum showed absorption bands at 2957, 1460, and 1018 $cm^{-1}$ due to acetal function. The $^1H$ and $^{13}C$ NMR spectra of **4** indicated the presence of three methyls [δ 0.79, 0.87, 0.91 (3H each, all s, 15, 13, 14-$H_3$)], a methoxymethyl [δ 3.40 (3H, s, $-OCH_3$)], a methylene bearing the oxygen function [δ 4.15, 4.35 (ABq, $J=10.9$ Hz, 12-$H_2$)], an acetal [δ 4.80 (1H, d, $J=3.7$ Hz, 11-H)], and an olefin [δ 5.51 (1H, br s, 7-H)] together with four methylene (1, 2, 3, 6-$H_2$), two methine (5, 9-H), and three quaternary carbons (4, 8, 10-C). Various 2D NMR data, as shown in Figure 1, led us to designate the structure of **4** as a methyl ether derivative of isodrimeninol.[22]

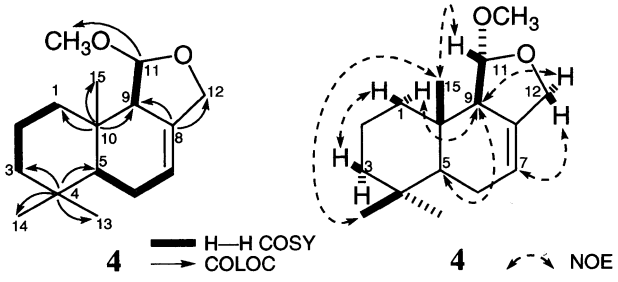


**Figure 1.** H–H COSY, COLOC, and NOE correlations of **4**.

## Gastroprotective activities of sesquiterpene constituents from *T. lanceolata*

The methanolic extract (10 mg/kg, po) and AcOEt-soluble fraction (5.0 mg/kg, po) from the leaves of *T. lanceolata* almost completely inhibited ethanol-induced gastric mucosal lesions (Table 1). First, sesquiterpene constituents (**1**–**4**) from the active fraction were examined. As shown in Table 2, oral administration of polygodial (**1**), having two aldehyde groups, showed potent protective effects on ethanol-induced gastric lesions in rats ($ED_{50}=0.028$ mg/kg). Compounds **2** and **3**, having a diacetal structure, also showed strong activity with $ED_{50}$ values of 0.17 and 0.15 mg/kg, but their activities were weaker than that of **1**. Methyl isodrimeninol (**4**), having a monoacetal structure, showed significant inhibition at a dose of 1.0 mg/kg, but weaker than **2** and **3**.

### Structural requirements of the active constituents for gastroprotective effects

Next, to clarify the structural requirements of **1** for strong activity, reduction and oxidation derivatives (**5**–**8**) of **1** were examined as shown in Figure 2. It was found that none of the dialcohol derivative drimendiol (**5**),[15] the diacetate (**6**),[15] the dicarboxyl derivative (**7**),[23] and its dimethyl ester (**8**)[15] showed strong protection even at a dose of 1.0 mg/kg.

Among the bicyclic sesquiterpenes, polygodial (**1**), having two aldehyde groups, showed very potent inhibition of the lesions, whereas derivatives (**5**–**8**) of the aldehyde groups of **1** showed weak activity at a dose above 1.0 mg/kg. Therefore, it is possible that the observed lack of gastroprotective activity was caused by a loss of the aldehyde groups. In the tricyclic compounds, both **2** and **3**, having a diacetal structure, showed stronger activity than methyl isodrimeninol (**4**), which has a monoacetal structure. Additionally, the hydrofuran derivative **9** lacking an acetal structure did not show significant effects at 1.0 mg/kg. These findings suggest that the aldehyde or acetal group is important for the strong protective activity. To best our knowledge, with the exception of PGs, **1** is the most potent protective compound against ethanol-induced gastric lesions.

### Mode of action of polygodial (1)

Finally, we investigated the involvement of PGs, NO, SHs and vanilloid receptors in the protective effects of polygodial (**1**). PGs are known to be involved in cytoprotection. It was reported that exogenous PGs protect

**Table 2.** Effects of the sesquiterpene constituents (**1**–**4**) from *T. lanceolata* and related compounds (**5**–**9**) on gastric lesions induced by ethanol in rats

| Treatment | Dose (mg/kg, po) | *N* | Gastric lesions | | $ED_{50}$ (mg/kg) |
|---|---|---|---|---|---|
| | | | Lesion index (mm) | Inhibition (%) | |
| Control | — | 6 | 169.5±14.6 | — | |
| Polygodial (**1**) | 0.0125 | 5 | 129.6±16.4 | 23.5 | 0.028 |
| | 0.025 | 5 | 84.8±25.8** | 50.0 | |
| | 0.05 | 5 | 48.8±5.9** | 71.2 | |
| | 0.1 | 5 | 29.1±6.3** | 82.8 | |
| | 0.2 | 5 | 12.6±3.7** | 92.6 | |
| | 0.5 | 7 | 0.0±0.0** | 100.0 | |
| Control | — | 12 | 135.6±11.1 | — | |
| Polygodial 12α-acetal (**2**) | 0.05 | 7 | 120.0±23.1 | 11.5 | 0.17 |
| | 0.1 | 7 | 90.3±20.6 | 33.4 | |
| | 0.2 | 7 | 62.7±16.4** | 53.8 | |
| | 0.5 | 7 | 17.1±6.2** | 87.4 | |
| | 1.0 | 6 | 8.5±5.5** | 93.7 | |
| Control | — | 12 | 131.9±8.7 | — | |
| Polygodial 12β-acetal (**3**) | 0.1 | 7 | 95.0±20.0 | 28.0 | 0.15 |
| | 0.2 | 7 | 50.0±20.6** | 62.1 | |
| | 0.5 | 7 | 4.3±1.9** | 96.7 | |
| | 1.0 | 7 | 0.9±0.5** | 99.3 | |
| Control | — | 6 | 151.2±16.3 | — | |
| Methyl isodrimeninol (**4**) | 0.2 | 5 | 91.3±18.6 | 39.6 | |
| | 1.0 | 5 | 68.7±20.8* | 54.6 | |
| Control | — | 16 | 138.8±11.1 | — | |
| Drimendiol (**5**) | 1.0 | 9 | 100.5±9.5 | 27.6 | |
| **6** | 1.0 | 9 | 88.1±13.9** | 36.5 | |
| Control | — | 7 | 146.2±14.6 | — | |
| **7** | 1.0 | 6 | 76.4±14.3* | 47.7 | |
| **8** | 1.0 | 6 | 91.0±17.7* | 37.8 | |
| **9** | 1.0 | 6 | 103.7±18.4 | 29.1 | |
| Control | — | 16 | 138.8±11.1 | — | |
| 16,16-dmPGE$_2$ | 0.0005 | 10 | 49.2±13.9** | 64.5 | |
| | 0.001 | 9 | 12.1±5.9** | 91.3 | |

Compounds **1**–**9** and 16,16-dimethyl prostaglandin $E_2$ (16,16-dmPGE$_2$) were initially dissolved in DMSO and diluted with distilled water to desirable dose [the final concentration of DMSO was 0.2% (v/v)]. Values were expressed as means±SEM. Significantly different from the control at *$p<0.05$, **$p<0.01$.

gastric mucosa against necrotizing agents, and mild irritants protect the gastric mucosa against damage via induction of endogenous PGs as well.[24] In this study, pretreatment with indomethacin (10 mg/kg, sc) attenuated the protection afforded by **1** (0.2 mg/kg, po) (Fig. 3A). This finding suggests that PGs participate in the protective effect of **1**.

Ethanol-induced gastric damage has been shown to be associated with a depletion of endogenous SHs such as glutathione, and pretreatment with SH-blockers prevented the gastroprotection of SH-containing substances.[25] In this study, pretreatment with *N*-ethylmaleimide (10 mg/kg, sc), an SH-blocker, reduced the protection afforded by polygodial (**1**, 0.2 mg/kg, po) (Fig. 3B). This finding indicates that endogenous SHs may be involved in the protection of **1**.

Since vascular changes in gastric mucosa appear to be the most pronounced feature of absolute ethanol-induced injury, maintenance of mucosal vasculature and normal blood flow may be the major mechanism of cytoprotection. It has been demonstrated that the gastric mucosa produces endogenous NO derived from L-arginine, and that NO participates in gastric defense mechanisms by regulating the gastric mucosal blood flow and gastric mucus secretion.[26] As shown in Figure 3C, an NO synthase inhibitor, $N^G$-nitro-L-arginine methyl ester (L-NAME, 70 mg/kg, ip) significantly attenuate the gastroprotection of polygodial (**1**, 0.05 and 0.1 mg/kg, po). This finding suggests that endogenous NO partly participate in the protective effect of **1**.

Recently, Szallasi et al. reported that various sesquiterpene dialdehydes and related terpenoids including

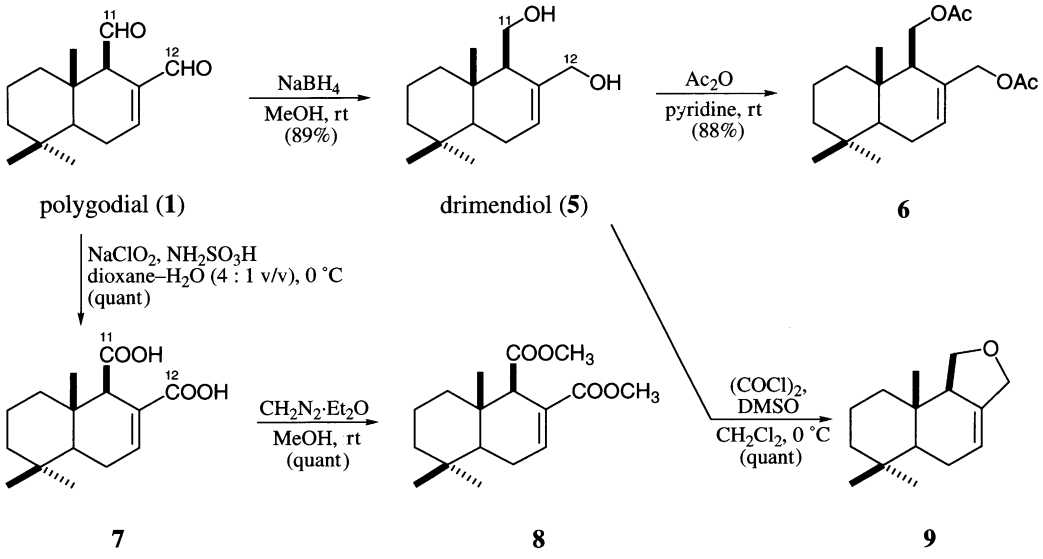


**Figure 2.** Syntheses of **5**–**9**.

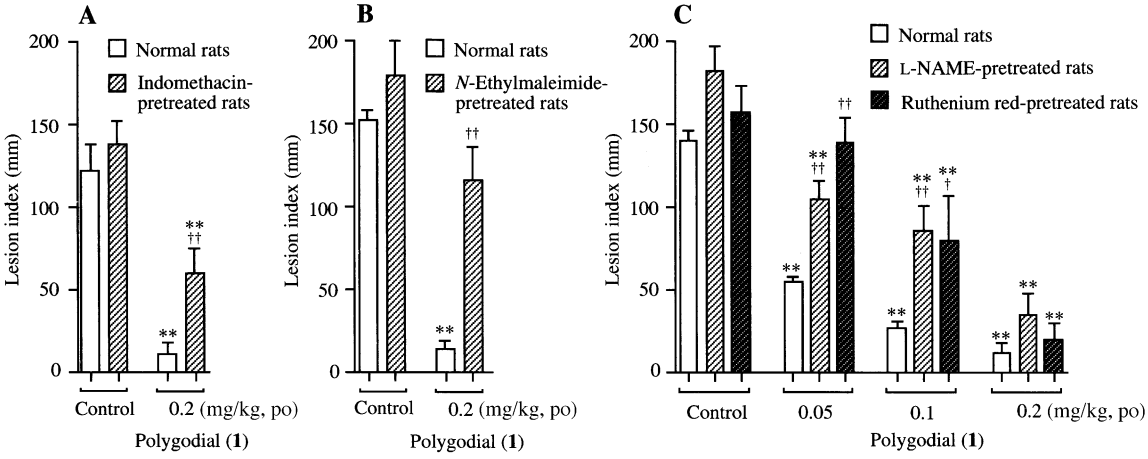


**Figure 3.** Effects of polygodial (**1**) on gastric lesions induced by ethanol in indomethacin (A)-, *N*-ethylmaleimide (B)- L-NAME (C)-, and ruthenium red (C)-pretreated rats. Indomethacin (10 mg/kg, dissolved in 5% $NaHCO_3$ solution, and diluted in distilled water, sc), *N*-ethylmaleimide (10 mg/kg, dissolved in saline, sc), $N^G$-nitro-L-arginine methyl ester (L-NAME, 70 mg/kg, dissolved in saline, ip), or ruthenium red (3.5 mg/kg, dissolved in saline, sc) was injected 30 min before the administration of the sample. Ethanol was administered to rats 1 h after administration of test sample. Each value represents the mean with SEM. Significantly different from the control at $^{**}p<0.01$, and from the polygodial (**1**)-treated group in normal rats at $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ ($N=5$–7).

polygodial (**1**) might function as vanilloid receptor agonists, although they are structurally unrelated to either capsaicin or resiniferatoxin.[27] These compounds have been found to stimulate capsaicin-sensitive neurons in a vanilloid receptor-mediated fashion.[28] Capsaicin sensory nerves play an important role in gastric defense mechanisms, which counteract the effects of ulcerogens.[29] Vanilloid receptors on the gastrointestinal mucosa are involved in gastroenteric motility regulation, acid secretion, increases in gastric blood flow through the action of calcitonin gene-related peptide (CGRP) and stimulation of bicarbonate and gastric mucus secretion or maintenance of mucosal integrity in the presence of noxious substances.[30] In this study, pretreatment with ruthenium red (3.5 mg/kg, sc), a vanilloid receptor antagonist,[31] reduced the protection afforded by **1** (0.05 and 0.1 mg/kg, po) (Fig. 3C). This finding and previous studies suggest that the gastroprotective action of **1** is partly mediated by interactions with capsaicin-sensitive sensory neurons via vanilloid receptors.

In conclusion, polygodial (**1**), polygodial 12α-acetal (**2**), and polygodial 12β-acetal (**3**) from *T. lanceolata*, but particulary **1**, markedly inhibited the gastric mucosal lesions induced by ethanol in rats. Regarding the structural requirements of the active constituents, the dialdehyde group or the acetal structure was essential for the strong activity. In addition, since the gastroprotection of **1** was attenuated by pretreatment with indomethacin, *N*-ethylmaleimide, L-NAME and ruthenium red, endogenous PGs, SHs, NO and vanilloid receptors may be involved in the protective activity of **1**.

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21. **4**: High-resolution FAB-MS: calcd for $C_{16}H_{26}O_2Na$ $(M+Na)^+$: 273.1830. Found: 273.1820. $^1H$ NMR (500 MHz, $CDCl_3$) δ 0.79, 0.87, 0.91 (3H each, all s, 15, 13, 14-$H_3$), 1.23 (1H, m, 3β-H), 1.26 (1H, m, 1α-H), 1.30 (1H, m, 5-H), 1.45 (1H, m, 3α-H), 1.47, 1.56 (1H each, both m, 2-$H_2$), 1.68 (1H, m, 1β-H), 1.91, 2.18 (1H each, both m, 6-$H_2$), 2.23 (1H, d, $J=3.7$ Hz, 9-H), 3.40 (3H, s, –$OCH_3$), 4.15, 4.35 (ABq, $J=10.9$ Hz, 12-$H_2$), 4.80 (1H, d, $J=3.7$ Hz, 11-H), 5.51 (1H, br s, 7-H). $^{13}C$ NMR (125 MHz, $CDCl_3$) δc 13.9 (15-C), 18.5 (2-C), 21.4 (14-C), 23.7 (6-C), 32.9 (4-C), 33.0 (13-C), 33.3 (10-C), 39.8 (1-C), 42.4 (3-C), 49.8 (5-C), 55.5 (–$OCH_3$), 60.4 (9-C), 69.0 (12-C), 106.1 (11-C), 116.9 (7-C), 136.5 (8-C).
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23. **7**: A white powder, $[\alpha]_D^{25}$ −22.6 ° ($c=1.0$, $CHCl_3$). High-resolution FAB-MS: calcd for $C_{15}H_{23}O_4$ $(M+H)^+$: 267.1596. Found: 267.1600. UV [MeOH, nm (log ε)]: 214 (3.93). IR (KBr): 2926, 1709, 1655 $cm^{-1}$. $^1H$ NMR (500 MHz, $CDCl_3$) δ 0.91, 0.94, 0.94 (3H each, all s, 13, 14, 15-$H_3$), 1.24 (1H, m, 5-H), 1.24, 1.49 (1H each, both m, 3-$H_2$), [1.33 (1H, br dd, $J=$ca. 4, 13 Hz), 1.99 (1H, br s, $J=$ca. 13 Hz), 1-$H_2$], 1.49 (2H, m, 2-$H_2$), [2.10 (1H, dd-like), 2.30 (1H, d-like), 6-$H_2$], 3.15 (1H, s, 9-H), 7.17 (1H, dd, $J=2.4$, 3.7 Hz, 7-H). $^{13}C$ NMR (125 MHz, $CDCl_3$) δc 15.2 (15-C), 18.5 (2-C), 22.0 (14-C), 24.2 (6-C), 33.0 (10-C), 33.3 (13-C), 35.8 (4-C), 40.1 (1-C), 41.9 (3-C), 48.7 (5-C), 57.2 (9-C), 128.1 (8-C), 142.7 (7-C), 172.1 (12-C), 178.7 (11-C). Positive-ion FAB-MS $m/z$ 267 $(M+H)^+$.
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