# Antiallodynic and Analgesic Effects of Maslinic Acid, a Pentacyclic Triterpenoid from Olea europaea

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# Antiallodynic and Analgesic Effects of Maslinic Acid, a Pentacyclic Triterpenoid from *Olea europaea*

Francisco R. Nieto,[†,‡] Enrique J. Cobos,[†,‡] José M. Entrena,[§] Andrés Parra,[⊥] Andrés García-Granados,[⊥] and José M. Baeyens*[,†,‡]

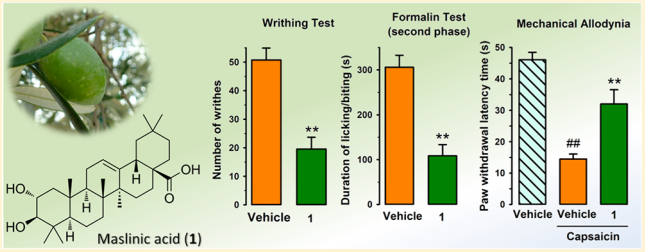
[†]Department of Pharmacology, School of Medicine, University of Granada, Avenida de Madrid 11, 18012 Granada, Spain

[‡]Institute of Neuroscience, Biomedical Research Center, University of Granada, Parque Tecnológico de Ciencias de la Salud, 18100 Armilla, Granada, Spain

[§]Animal Behavior Research Unit, Scientific Instrumentation Center, University of Granada, Parque Tecnológico de Ciencias de la Salud, 18100 Armilla, Granada, Spain

[⊥]Department of Organic Chemistry, Faculty of Sciences, University of Granada, Spain

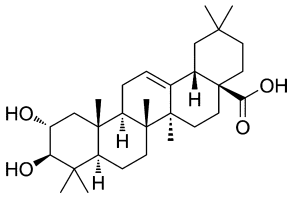
**ABSTRACT:** The effects of maslinic acid (**1**), a pentacyclic triterpenoid obtained from *Olea europaea,* were studied in several tests for nociception in mice. Systemic administration of **1** reduced acetic acid-induced writhing, the inflammatory phase of formalin-induced pain, and capsaicin-induced mechanical allodynia. However, it did not induce motor incoordination in the rotarod test. The topical administration of **1** also reduced the inflammatory phase of the formalin test, indicating that at least some of its effects are mediated peripherally. The present results demonstrate for the first time that maslinic acid induces antinociceptive and antiallodynic effects.




Pain is considered a global public health priority, and there is a need for new pain medications.[1] Natural products and/or natural product structures continue to play a considerable role in the discovery and development of analgesics.[2]

Pentacyclic triterpenoids are common secondary plant metabolites widespread in the fruits, leaves, and stem bark of many plants and represent different skeletal types such as ursanes, lupanes, and others.[3] The pharmacological properties of pentacyclic triterpenoids include antidiabetogenic, anti-inflammatory, antineoplastic, antioxidant, and hepatoprotective activities, although these vary widely according to the specific compound tested.[3,4] Interestingly, antinociceptive effects are induced by several of these compounds, including oleanolic acid (an oleanane), an isomeric mixture of $\alpha$-amyrin (an ursane) and $\beta$-amyrin (an oleanane), and ursolic acid (an ursane).[5−8]

Maslinic acid (2$\alpha$,3$\beta$-dihydroxyolean-12-en-28-oic acid, **1**) is a pentacyclic triterpenoid present in several plants and is found in particularly high concentrations in the fruits of the olive tree (*Olea europaea* L., Oleaceae).[9] This compound belongs to the oleanane type and is related structurally to oleanolic acid (3$\beta$-hydroxyolean-12-en-28-oic acid),[9] with differences in biological activity between the two compounds having been described.[10,11] Maslinic acid (**1**) possesses several of the above-mentioned properties of other pentacyclic triterpenes,[12−14] including antioxidant[15−17] and anti-inflammatory effects.[16,18,19] However, the possible antinociceptive properties of **1** have not been investigated. The aim of this study was to evaluate the effects of **1** in different experimental models indicative of several types of pain.




maslinic acid (**1**)

The effects of the systemic administration of **1** were evaluated in two widely used tests of chemically induced tonic nociception: acetic acid-induced writhing and the formalin test. The intraperitoneal (ip) administration of acetic acid induces an inflammation of visceral (subdiaphragmatic organs) and somatic (muscle wall) tissues, inducing sustained pain-like responses (writhes).[20] Systemic (sc) treatment with **1** induced a 61% inhibition of writhing at the highest dose tested in comparison to vehicle-treated mice (Figure 1). The control drug ibuprofen also inhibited writhing (Figure 1), as previously reported.[21] The effects of **1** on visceral nociception are similar to those of some other pentacyclic triterpenoids.[5,22] This test is capable of detecting weak analgesic effects,[23] and **1** markedly inhibited writhing at the lowest dose tested. Nevertheless, this

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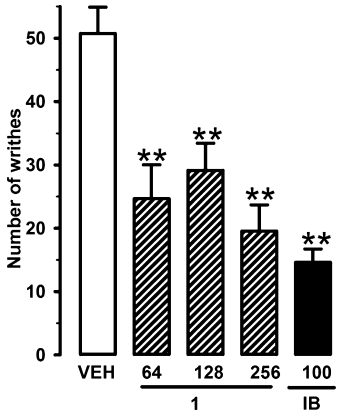


**Figure 1.** Effects of the systemic (sc) administration of maslinic acid (**1**) or its vehicle (VEH) on acetic acid-induced writhing. Ibuprofen (IB) was used as a control analgesic drug. Each bar and vertical line represents mean ± SEM of the values obtained in 8−12 mice. The doses (mg/kg) used in each treatment are indicated below their respective bars. Statistically significant differences between the values obtained in animals treated with **1** or ibuprofen and those treated with vehicle were $*p < 0.05$ and $**p < 0.01$ (one-way ANOVA followed by the Bonferroni test).

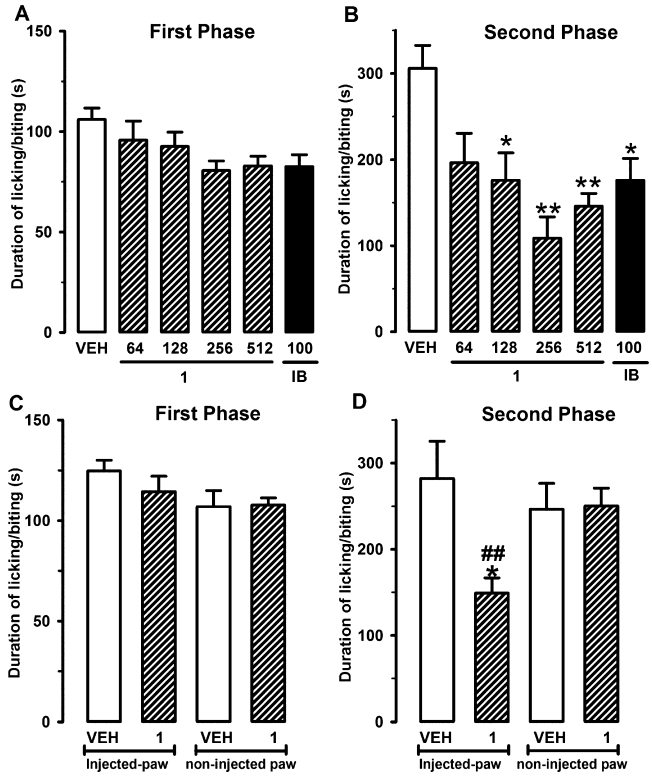


**Figure 2.** Effects of maslinic acid (**1**) systemically (sc) (A and B) or topically (1%) administered (C and D) in the first (A and C) and second (B and D) phase of the formalin test in comparison to the respective vehicles (VEH). Ibuprofen (IB) was used as a control analgesic drug in the experiments on the effects of sc administration of **1** (A and B). The doses (mg/kg) used in each systemic treatment are indicated below their respective bars. Each bar and vertical line represents mean ± SEM of the values obtained in 8−12 mice. Statistically significant differences between the values obtained in animals treated with **1** or IB and those treated with their vehicle were $*p < 0.05$ and $**p < 0.01$ (one-way ANOVA followed by the Bonferroni test). Statistically significant differences in values between mice topically treated with **1** on the formalin-injected paw and noninjected paw: $##p < 0.01$ (two-way ANOVA followed by the Bonferroni test).

test is prone to false positive results (i.e., some agents that inhibit writhing have no analgesic action).[23] For this reason, the effects of **1** were also evaluated in the formalin test, a much less sensitive model of chemical nociception.[23]

The intraplantar (ipl) injection of formalin induces biphasic nociceptive responses (licking of the hindpaw). The first phase largely results from the direct stimulation of nociceptors, whereas the mechanisms of the second phase are more complex and involve an acute inflammatory process accompanied by spontaneous activity of primary afferent neurons together with central alterations of pain processing.[23] Systemic treatment with **1** did not significantly alter the first phase of acute pain (Figure 2A) but notably reduced nociceptive responses during the second (inflammatory) phase (Figure 2B). These results agree with the previously reported differential effects of amyrin octanoate in the first and second phase of the formalin test.[22] Likewise, ibuprofen decreased the behavioral responses in the inflammatory phase without affecting the responses in the first phase (Figure 2A and B), as previously reported for this and other nonsteroidal anti-inflammatory drugs (NSAIDs).[23,24]

To test whether this effect of **1** could be produced locally, a **1**-enriched gel (1%) was administered topically on the hindpaw before formalin injection. Gel application on the formalin-injected paw significantly decreased the inflammatory phase (Figure 2D) without affecting the first phase (Figure 2C). Importantly, when **1** was applied on the noninjected hindpaw (contralateral to the formalin injection), it did not alter the pain-like responses during either the first (Figure 2C) or second phase (Figure 2D). These results indicate that **1** has local antinociceptive effects that are consistent with the local (ipl) antihyperalgesic activity of the isomeric mixture of $\alpha$- and $\beta$-amyrin during chronic inflammation.[8]

The effects of **1** on capsaicin-induced secondary mechanical allodynia were also tested. This mechanical hypersensitivity results from central sensitization, and the antiallodynic activity of compounds in this test is thought to be predictive of their antineuropathic effects in both humans and rodents.[25] Non-sensitized animals treated with the solvent of **1** showed a latency to paw withdrawal close to the predetermined cutoff time (46.10 ± 2.37 s) when an innocuous mechanical stimulus (0.5 g force) was applied (Figure 3). However, after the ipl administration of capsaicin, the paw withdrawal latency markedly decreased in solvent-treated mice (14.46 ± 1.61 s) in response to this normally innocuous mechanical stimulation; that is, the animals exhibited mechanical allodynia. Systemic treatment with **1** induced a dose-dependent antiallodynic effect in capsaicin-treated mice, and the paw withdrawal latencies observed with the highest doses of **1** tested did not significantly differ from those found in naive mice (Figure 3). Ibuprofen was inactive in this test (Figure 3), as documented for this and other NSAIDs,[24,25] whereas the antineuropathic drug pregabalin was effective (Figure 3), as previously reported.[25]

It might be thought that the similar effects of **1** and ibuprofen in the writhing and formalin tests, which show a clear inflammatory component,[20,23] could be mediated by their shared inhibitory action on cyclooxygenase activity.[26] However, the differential effects of **1** and ibuprofen in capsaicin-induced mechanical hypersensitivity indicate that additional mechanisms participate in the antiallodynic effect of **1**. Reactive oxygen species play a major role in the generation of central


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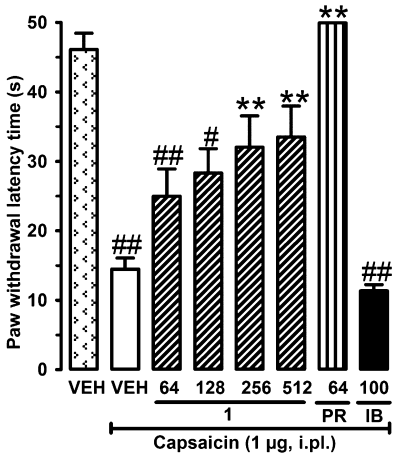


**Figure 3.** Antiallodynic effect of the systemic (sc) administration of maslinic acid (**1**) or its vehicle (VEH) in capsaicin-induced mechanical allodynia. Ibuprofen (IB) and pregabalin (PR) were used as control drugs. Each bar and vertical line represents mean ± SEM of the values obtained in 8−12 mice. The doses (mg/kg) used in each treatment are indicated below their respective bars. Statistically significant differences between the values obtained in capsaicin-sensitized mice treated with **1** or a control drug and those treated with vehicle were $*p < 0.05$ and $**p < 0.01$ and those between the values obtained in capsaicin-sensitized mice treated with **1**, ibuprofen, or their vehicle and those receiving vehicle but not capsaicin were $\#p < 0.05$ and $\#\#p < 0.01$ (two-way ANOVA followed by the Bonferroni test).

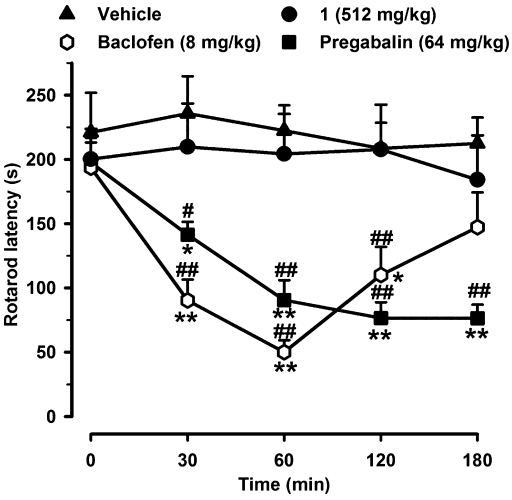


**Figure 4.** Time-course of the effects on the rotarod test of the sc administration of maslinic acid (**1**), pregabalin, baclofen, and their vehicle (2% Tween 80). The latency to fall down from the rotarod was recorded in each mouse immediately before (time 0) and at 30, 60, 120, and 180 min after the sc injection. Each point and vertical line represents mean ± SEM of values obtained in 8−12 mice. Statistically significant differences between the drug-treated and solvent-treated groups at the same postinjection time point were $*p < 0.05$ and $**p < 0.01$ and those between the values at time 0 and postinjection time points were $\#p < 0.05$ and $\#\#p < 0.01$ (two-way repeated measures ANOVA followed by the Bonferroni test).

sensitization, and antioxidant drugs inhibit capsaicin-induced secondary hypersensitivity.[27] The known antioxidant effect of **1**[14−17] might contribute to inhibiting central sensitization, thereby decreasing capsaicin-induced mechanical hypersensitivity. Hence, multiple mechanisms (anti-inflammatory and antioxidant actions) may be responsible for the wide antinociceptive profile of **1**.

Finally, animals treated with the highest systemic dose of **1** showed no change in latency to fall down from the rotarod versus the baseline value (time 0) at any measurement time point during the 3 h evaluation period (Figure 4). Hence, the results of the nociception tests would not be influenced by **1**-induced motor impairment. In contrast, mice treated with either the antineuropathic drug pregabalin or the muscle relaxant baclofen showed significantly reduced latencies to fall down from the rotarod (Figure 4), as previously reported.[28,29] These results agree with previous reports that pregabalin is effective in several pain conditions but at doses that induce motor incoordination.[29,30] Therefore, although the antiallodynic activity of **1** was limited in comparison to pregabalin, it might offer a better tolerability profile.

In summary, this study demonstrates for the first time that maslinic acid (**1**) exhibits antinociceptive and antiallodynic activities in different pain models in mice. These effects are broader than those of the common analgesic ibuprofen. This pentacyclic triterpenoid is active against chemically induced inflammatory nociception (visceral or somatic) and in a surrogate model of neuropathic pain. In addition, at least part of its analgesic effect on chemically induced somatic inflammatory nociception is produced locally. This compound induced no apparent toxicity or motor performance alteration. These results suggest that **1** may be useful in the treatment of several types of pain. Therefore, further research is warranted to elucidate the possible mechanisms involved in this antinociceptive/antiallodynic activity.

## EXPERIMENTAL SECTION

**General Experimental Procedures.** All experiments were performed in female CD-1 mice (Charles River, Barcelona, Spain) weighing 25−30 g each. Animals were housed in rooms at 22 ± 1 °C, under a 12 h light−dark cycle, with food and water freely available. All procedures followed the guidelines of the European Council Directive 86/609/EEC and were approved by the University of Granada Ethics Committee.

**Test Compounds.** Maslinic acid (**1**) was obtained from solid wastes resulting from olive-oil production employing a previously described method.[31] The purity of **1** was more than 98% (determined by HPLC) in all batches used in this study. The anti-inflammatory drug ibuprofen sodium salt, the antineuropathic/antiepileptic pregabalin, and the muscle relaxant baclofen were used as comparison drugs, all from Sigma-Aldrich (Madrid, Spain).

For the systemic administration of these compounds, they were dissolved in 2% Tween 80 (Sigma-Aldrich) in ultrapure water and administered sc in the interscapular area (5 mL/kg). Maslinic acid was administered in a wide range of doses (64−512 mg/kg) that are known to be in its safety dosage range.[32] Selection of the doses of ibuprofen (100 mg/kg), pregabalin (64 mg/kg), and baclofen (8 mg/kg) was based on previous studies.[24,25,28] Drug effects were tested 30 min after their administration except for the assessment of the impact on motor coordination, which was done at multiple time points postinjection. To test the local effects of **1**, a preparation enriched in this compound (1%) was prepared in a Carbopol-based gel (1%) with triethanolamine (2%) in propylene glycol (5%). The antinociceptive effects of this topical treatment were tested in the formalin test. Briefly, unanesthetized mice were gently restrained, and 0.1 g of the **1**-enriched gel or its vehicle was applied with a spatula onto the plantar surface of either the right or the left hindpaw 15 min before receiving the formalin injection.

**Behavioral Studies.** Two different tests of chemically induced nociception were used: the writhing and formalin tests. The writhing


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test was assessed as previously described.[33] Mice were injected ip with 0.6% acetic acid (10 mL/kg), and the number of writhes (lengthwise stretches of the torso with a concomitant concave arching of the back and extension of the legs) was counted cumulatively over a period of 30 min. The formalin test was performed as previously described,[34] with slight modifications. Briefly, mice were injected intraplantarly with 20 $\mu$L of 5% formalin solution, and the time spent licking or biting the injected hindpaw was measured. Drug effects were assessed in both the first (0−5 min) and second (10−50 min) phase of this test.

To evaluate capsaicin-induced mechanical allodynia, a previously described experimental procedure was used.[25] Briefly, at 15 min after the ipl administration of 20 $\mu$L of capsaicin (1 $\mu$g) in 1% DMSO, a mechanical stimulation (0.5 g force) was applied with an electronic von Frey device (Ugo Basile, Comerio, Italy) at least 5 mm from the site of injection toward the toes (area of secondary mechanical hypersensitivity), and the paw withdrawal latency time was automatically recorded. Each mouse was tested in three trials at 30 s intervals. A cutoff time of 50 s was used in each trial.

Motor coordination was assessed with an accelerating rotarod (Cibertec, Madrid, Spain), as previously described.[28] Mice were required to walk against the motion of an elevated rotating drum at increasing speed (4 to 40 rpm over 5 min), and the latency to fall down was recorded with a cutoff of 300 s. Mice were given three training sessions 24 h before drug testing.

**Data Analysis.** Differences between the values in the behavioral tests were examined by one-way or two-way analysis of variance (ANOVA), as indicated in the figure legends. A Bonferroni posthoc test was performed in all cases. Differences between means were considered statistically significant when the $p$ value was below 0.05.

## AUTHOR INFORMATION


### Corresponding Author

*Tel: +34-958-243538. Fax: +34-958-243537. E-mail: jbaeyens@ugr.es.



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### Notes

The authors declare no competing financial interest.


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