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In Vivo Anti-inflammatory Activity of Some Naturally Occurring *O*- and *N*-Prenyl Secondary Metabolites

Francesco Epifano[a,*], Salvatore Genovese[a], Serena Fiorito[a], Roberto della Loggia[b], Aurelia Tubaro[b] and Silvio Sosa[b]

[a]*Department of Pharmacy, University "G. D'Annunzio" of Chieti-Pescara, Via dei Vestini 31, 66100 Chieti Scalo (CH), Italy*

[b]*Department of Life Sciences, University of Trieste, Via A. Valerio 6, 34127 Trieste, Italy*

fepifano@unich.it

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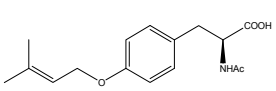
A series of *O*- and *N*-prenyl secondary metabolites of insect, fungal, and plant origin have been evaluated for their topical anti-inflammatory activity using the Croton oil ear test in mice as a model of acute inflammation. Some of the tested compounds revealed an effect (ID_{50} = 0.31 ÷0.56 μmol/cm^2) comparable with that of the reference non-steroidal anti-inflammatory drug indomethacin (ID_{50} = 0.23 μmol/cm^2).

Keywords: Anti-inflammatory activity, Azoprenylated secondary metabolites, Oxyprenylated secondary metabolites, Rutaceae.

Oxyprenylated and azoprenylated compounds, such as isopentenyl- (C5), geranyl- (C10), and farnesyl-(C15) derivatives, represent a class of secondary metabolites that have been recognized in the last two decades as interesting and valuable biologically-active phytochemicals. Some representative prenyloxyphenyl-propanoids have shown valuable anti-inflammatory properties [1]. Since inflammation is a universal and physiological response involved in different pathological conditions, the evaluation of anti-inflammatory properties of oxy- and azoprenylated secondary metabolites is a field of growing interest. Thus, we have evaluated the topical anti-inflammatory activity of 12 selected compounds (**1**-**12**) by the Croton oil ear test in mice [2].



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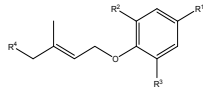
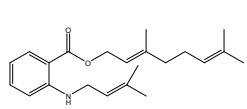


2 R^1 = CH_2CH_2OH, R^2 = R^3 = R^4 = H
3 R^1 = CH_2CH_2OAc, R^2 = R^3 = R^4 = H
4 R^1 = COOH, R^2 = R^3 = H, R^4 = isopentenyl
5 R^1 = CH_2CH_2OH, R^2 = R^3 = H, R^4 = isopentenyl
6 R^1 = CHO, R^2 = R^3 = R^4 = H
7 R^1 = CH_2OH, R^2 = R^3 = OMe, R^4 = H
8 R^1 = CHO, R^2 = R^3 = H, R^4 = isopentenyl
9 R^1 = $CH_2CH_2CH_2OH$, R^2 = R^3 = R^4 = H



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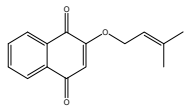

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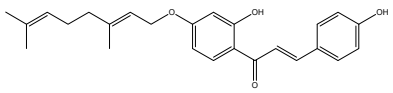

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N-acetyl-*O*-isopentenyl-L-tyrosine (**1**) has been previously obtained from the fungus *Pithomyces ellis*, etrogol (**2**) and its acetate (**3**) from *Citrus* spp. (Rutaceae), and geranyloxy-*p*-benzoic acid (**4**) from enzymatic extracts of *Piper crassinervium* Kunth. (Piperaceae). 3-(4-Geranyloxyphenyl)-1-ethanol (**5**) is a juvenile hormone of several insect species, *p*-isopentenyloxybenzaldehyde (**6**) has been isolated from the essential oil of *Clausena anisata* Hook f. (Rutaceae) leaves, 3,5-dimethoxy-4-isopentenyloxybenzyl alcohol (**7**), as its angelate ester, from the roots of *Erechtites hieracifolia* (L.) Raf ex DC. (Asteraceae), geranyloxyvanillin (**8**) from the apolar extracts of *Crithmum maritimum* L. (Apiaceae), 3-(4-isopentenyloxyphenyl)-1-propanol (**9**) from the roots of *Fagara zanthoxyloides* Lam. and *Zanthoxylum wutaiense* Chen (Rutaceae), *N*-isopentenylanthranilic acid geranyl ester (**10**) from *Esenbeckia yaaxhokob* Lundell (Rutaceae), 4'-geranyloxyisoliquiritigenin (**11**) from several *Glycyrrhiza* species, and finally lawsone isopentenyl ether (**12**) from the fungus *Streptocarpus dunnii* [3].

The anti-inflammatory activity of compounds **1**-**12**, administered at a dose of 0.3 μmol/cm^2, are reported in Table 1, in comparison to that of the same dose of the non-steroidal anti-inflammatory drug indomethacin, used as a reference. Compounds **1**, **2**, **4**, **5** and **7**-**11** induced significant reductions of edema, ranging from 14 to 52%, while compounds **3**, **6** and **12** were inactive. Among the active compounds, **4**, **8** and **11** exhibited only a weak anti-inflammatory effect, with edema reductions lower than 20%, whereas **2**, **5**, **7**, and **9** provoked about 30% reduction. The highest activity (about 50% edema reduction) was observed for the aminoacid derivatives *N*-acetyl-*O*-isopentenyl-L-tyrosine (**1**) and *N*-isopentenylanthranilic acid geranyl ester (**10**0. The equimolar dose of indomethacin induced a slightly higher effect (61% edema reduction).

The most active compounds (**1** and **10**), as well as compounds inducing about 30% edema reduction (**2**, **5**, **7** and **9**), were then further investigated for their dose–activity relationship, in comparison with indomethacin. The obtained results are summarized in Table 2. At doses ranging from 0.1 to 1.0 μmol/cm^2, all the selected compounds exerted a dose-dependent anti-inflammatory activity, with edema inhibition reaching about 70% (compound **9**) or 80 % (compounds **1**, **2**, **5**, **7**, **10**) at the highest administered dose (1.0 μmol/cm^2), comparable with that induced by indomethacin at the same dose (82% reduction). The anti-inflammatory potency of these compounds was evaluated by the dose inducing 50% edema inhibition (ID_{50}). The nitrogen-containing secondary metabolites **1** and **10** (ID_{50} = 0.31 and 0.36 μmol/cm^2, respectively) were the most active, with an

Table 1: Anti-inflammatory activity of compounds **1-12**.

Entry	No. animals	Dose (μmol/cm^2)	Edema (mg) Mean ± SD	Red. (%)
Controls	10	--	8.3 ± 0.3	
1	10	0.3	4.0 ± 0.5*	52
2	10	0.3	5.7 ± 0.4*	32
3	9	0.3	7.4 ± 0.5	11
4	9	0.3	6.7 ± 0.4*	19
5	10	0.3	5.6 ± 0.3*	33
6	10	0.3	7.8 ± 0.4	6
7	10	0.3	5.7 ± 0.4*	31
8	9	0.3	6.9 ± 0.5*	17
9	8	0.3	6.0 ± 0.2*	28
10	8	0.3	4.1 ± 0.3*	51
11	10	0.3	7.1 ± 0.4*	14
12	9	0.3	7.5 ± 0.4	10
Indomethacin	10	0.3	3.2 ± 0.3*	61

*p <0.05 at the analysis of variance, compared with controls.

Table 2: Dose-dependent anti-inflammatory activity of compounds **1**, **2**, **5**, **7**, **9**, and **10**.

Entry	No. animals	Dose (μmol/cm^2)	Edema (mg) Mean ± SD	Red. (%)	ID_{50} (μmol/cm^2)
Controls	10	--	7.4 ± 0.4	--	--
1	9	0.1	6.0 ± 0.3*	19	0.31
	9	0.3	3.6 ± 0.4*	51	
	9	1.0	1.4 ± 0.2*	81	
2	8	0.1	6.7 ± 0.4	9	0.44
	10	0.3	5.1 ± 0.3*	31	
	10	1.0	1.6 ± 0.2*	78	
5	9	0.1	6.8 ± 0.3	8	0.45
	10	0.3	5.0 ± 0.2*	32	
	9	1.0	1.7 ± 0.2*	77	
7	8	0.1	6.8 ± 0.1	8	0.45
	10	0.3	5.1 ± 0.2*	31	
	10	1.0	1.7 ± 0.2*	77	
9	9	0.1	7.0 ± 0.2	5	0.56
	9	0.3	5.3 ± 0.3*	28	
	9	1.0	2.4 ± 0.3*	68	
10	10	0.1	6.7 ± 0.3	9	0.36
	10	0.3	3.7 ± 0.3*	50	
	10	1.0	1.6 ± 0.1*	78	
Indomethacin	10	0.1	6.0 ± 0.3*	19	0.26
	10	0.3	2.8 ± 0.3*	62	
	10	1.0	1.3 ± 0.2*	82	

*p <0.05 at the analysis of variance, compared with controls.

inflammatory potency comparable with that of indomethacin (ID_{50} = 0.26 μmol/cm^2). Anyway, the anti-inflammatory potency of the other compounds (ID_{50} = 0.44-0.56 μmol/cm^2) was only two or less times lower than that of indomethacin. The results obtained herein represent in part a parallelism with already reported data indicating how tyrosine and *N*-substituted anthranilic acid derivatives act as effective anti-inflammatory agents [4].

In conclusion, the findings described herein indicate that some oxy- and azoprenylated naturally-occurring secondary metabolites could be regarded as potential novel and effective anti-inflammatory agents, with a potency comparable with that of the NSAID indomethacin. Two of these derivatives showed a high topical anti-inflammatory activity in the Croton oil-induced ear dermatitis test in mice. For these reasons, the results of the present study could be considered as a topic for future studies aimed at better defining the pharmacological profile of these and other oxy- and azoprenylated secondary metabolites, and to develop a novel series of lead compounds.

Experimental

Compounds: The synthesis of compounds (**1–12**), was accomplished according to the procedures described previously [3]. All products were obtained in a high degree of purity (> 97%) estimated by GC-MS.

Biological testing: Experiments complied with the Italian Decree n. 116 of January 1992 and associated guidelines in the EU Directive 2010/63/EU, and the European Convention ETS 123. Male CD-1 mice (28–32 g; Harlan Laboratories; Udine, Italy) were anesthetized with ketamine hydrochloride (145 mg/kg, intraperitoneally; Virbac, Milan, Italy). Inflammation was induced on the right ear (surface: about 1 cm^2) by application of 80 μg of croton oil (Sigma-Aldrich; Milan, Italy) dissolved in acetone. Control mice received only the irritant solution, whereas the others received both the irritant and the compounds under test dissolved in acetone. Six h later, mice were sacrificed and a plug (6 mm ∅) was excised from both the treated and untreated ears to quantify edema as weight difference between the two plugs. The anti-inflammatory activity was expressed as % edema reduction in mice treated with the compounds under test with regard to control mice. Edema values, expressed as means ± standard error (S.E.) of the mean, were analysed by one way analysis of variance followed by Dunnett's test for multiple comparison of unpaired data. A probability level lower than 0.05 was considered as significant.

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