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Synthesis and anti-inflammatory activity of natural and semisynthetic geranyloxycoumarins

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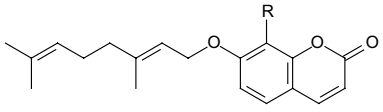
Abstract—Nine new 7-geranyloxycoumarin derivatives differently substituted at position 8 were semi-synthesised. Their topical anti-inflammatory activity was evaluated using the Croton oil ear test in mice as a model of acute inflammation. Auraptene (7-geranyloxycoumarin), its 8-methoxy (collinin, **1**) and 8-acetoxy derivatives (**5**) (1 μmol/cm^2) provoked 50% oedema reduction, similarly to 0.25 μmol/cm^2 of the reference drug indomethacin, a nonsteroidal anti-inflammatory drug.

Prenyloxycoumarins are secondary metabolites commonly present in plants belonging to the families of Rutaceae and Umbelliferae. Several of these coumarins were shown to possess valuable pharmacological properties. Among them, auraptene (7-geranyloxycoumarin, **1**) is a promising chemopreventive agent against skin, tongue, oesophagus and colon carcinogenesis in rodents.[1] Since inflammation is a universal and physiological response in the process of carcinogenesis, the in vivo and in vitro anti-inflammatory properties of this compound was also evaluated, giving contrasting results. Auraptene did not affect the oedematous response induced by TPA-dermatitis in mouse ears.[2] On the contrary, double pre-treatment of mouse skin with auraptene markedly suppressed oedema formation, hydrogen peroxide production and leukocyte infiltration induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[2] In addition, auraptene significantly attenuated the lipopolysaccharide induced protein expression of inducible isoforms of both nitric oxide synthase and cyclooxygenase, with decreased production of nitric anion and prostaglandin E_2 and yet suppressed the release of tumour necrosis factor α[2]. Furthermore, auraptene and collinin (50 and 100 μg/mL, respectively) were also reported to cause complete inhibition of platelet aggregation induced by arachidonic acid and PAF in vitro.[3] In order to clarify the in vivo anti-inflammatory effects of auraptene and collinin, the Croton oil induced dermatitis in mouse ear was used as an inflammatory model. The irritant principles of Croton oil are TPA and other phorbol esters. This in vivo inflammatory model possesses the advantage of using very small amount of pure compounds and, consequently, is particularly suitable in the biological screening of semisynthetic compounds.

During the last year we set up a short and good yielding synthetic method of collinin (7-geranyloxy-8-methoxycoumarin, **2**),[4] previously isolated in very small amounts only from *Zanthoxylum schinifolium*,[3,5,6] *Flindersia maculata*[7] and *Haplophyllum alberti-regelli*[8] (Rutaceae).



1 R = -H, **2** R = -OCH_3

Keywords: Geranyloxycoumarins; Collinin; Auraptene; Anti-inflammatory activity.
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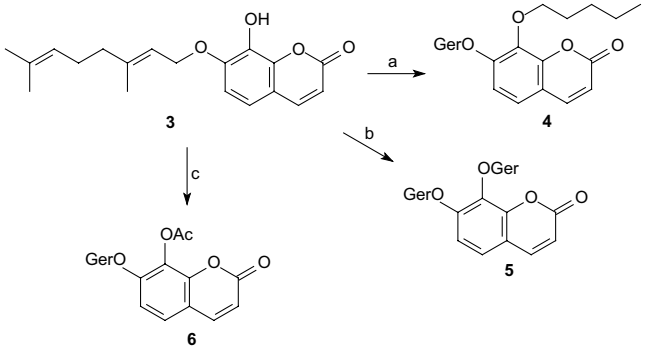
In this paper we describe the synthesis of several coumarin analogues (**3**–**7** and **9**–**12**) structurally related to auraptene and collinin and we evaluated their anti-inflammatory activity. Indomethacin, a nonsteroidal anti-inflammatory drug (NSAID) was used as a reference compound.

Ethers (**4**) and (**5**) were synthesised from 7-geranyloxy-8-hydroxycoumarin[4] (**3**) by alkylation with NaH and *n*-pentyl iodide and geranyl bromide in DMF (62% and 55% yield, respectively). 8-Acetoxy-7-geranyloxycoumarin (**6**) was synthesised in 93% yield from (**3**) by reaction with Ac_2O and Et_3N in dichloromethane catalysed by 4-pyrrolidinopyridine (Scheme 1).

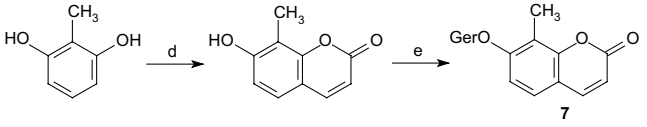
The synthesis of 8-methyl derivative (**7**) was performed starting from 2-methylresorcinol according to the pathway shown in Scheme 2.

Treatment of 2-methylresorcinol with propiolic acid in concentrated H_2SO_4 at 120 °C led to 8-methylumbelliferone in 37% yield involving a Pechmann condensation; alkylation of the latter with geranyl bromide and DBU in acetone gave (**7**) in 75% yield.

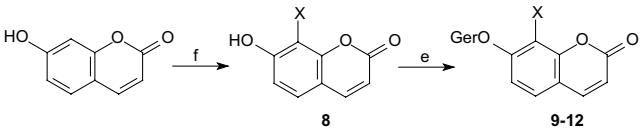
8-Halocoumarin derivatives were synthesised starting from umbelliferone (Scheme 3): 8-iodoumbelliferone (**8a**) was obtained in 42% yield by reaction with iodine and KI in 20% NH_4OH aqueous solution;[9] 8-bromoumbelliferone (**8b**) was synthesised in 35% yield by reaction with bromine and *t*-butyl amine in refluxing toluene;[9] 8-chloroumbelliferone (**8c**) was obtained in 32% yield by reaction with chloramine T in refluxing 1:1 water/dioxane mixture and finally 8-fluoroumbelliferone (**8d**) was synthesised by reaction with Selectfluor® in refluxing acetonitrile.



Scheme 1. Reagents and conditions: (a) NaH, *n*-pentyl iodide, DMF, rt, 2 h; (b) NaH, geranyl bromide, DMF, rt, 2 h; (c) Ac_2O, Et_3N, 4-pyrrolidinopyridine (cat.), CH_2Cl_2, rt, overnight.



Scheme 2. Reagents and conditions: (d) propiolic acid, H_2SO_4 concd, 120 °C, 30 min; (e) geranyl bromide, DBU, acetone, rt, 3 h.



Scheme 3. Reagents and conditions: (f) X = –I, I_2/KI, NH_4OH 20% rt, 1 h; X = –Br, Br_2, *t*-$BuNH_2$, toluene, reflux, 2 h; X = –Cl, Chloramine T, 1:1 dioxane/water, reflux, 1 h; X = –F, Selectfluor®, CH_3CN, reflux, 30 min; (e) geranyl bromide, DBU, acetone, rt, 3 h.

All these 8-halo derivatives were geranylated employing the same experimental conditions described above giving compounds **9**–**12** (**9**: X = –I, **10**: X = –Br, **11**: X = –Cl, **12**: X = –F) in 55%, 51%, 60% and 52% yield, respectively.[12]

The anti-inflammatory activity of the tested coumarins was evaluated as inhibition of the Croton oil ear oedema in mice (10 mice per treatment group).[10] Inflammation was induced on the right ear (surface: about 1 cm^2) of male CD-1 mice (28–32 g, Harlan-Italy, Udine, Italy) anaesthetised with ketamine hydrochloride (145 mg kg^{-1}, i.p., Virbac S.r.l., Milano, Italy) by application of 80 μg of Croton oil (Sigma Chemical Co., St. Louis, USA) dissolved in acetone. Control mice received only the irritant solution, whereas the others received both the irritant and the substances under test dissolved in acetone. Six hours later, mice were sacrificed and a plug (6 mm∅) was excised from both the treated and untreated ears: oedema was quantified by the difference in weight between the two plugs. The anti-inflammatory activity was expressed as percent reduction of the control mice using as reference, the NSAID indomethacin. Experiments complied with the Italian D.L. n. 116 of January 1992 and associated guidelines in the European Communities Council Directive of 24 November 1986. Oedema values, expressed as mean ± standard deviation of the mean, were analysed by one-way analysis of variance by Dunnett's test for multiple comparison of unpaired data. A probability level lower than 0.05 was considered as statistically significant.

The anti-inflammatory effects of auraptene (**1**), collinin (**2**) and compounds **3**–**7** and **9**–**12** (1 μmol/cm^2), are reported in Table 1.

At the tested doses auraptene, its 8-methoxy derivate (collinin, **2**) and its 8-acetoxy derivate (**6**) were the most active compounds inhibiting the oedematous response by about 50% (Table 1). The other compounds (**3**, **5**, **7**, **9**–**12**) showed an oedema reduction ranging from 27% to 43%. As expected, the reference NSAID indomethacin (0.25 μmol/cm^2), provoked 47% oedema reduction.

In our test system, aurapetene showed in vivo anti-inflammatory activity, while previously any effect was observed after acute application.[2] This apparent discrepancy with literature data can be ascribed to the severe dermatitis induced by Murakami et al.:[2] the oedematous response of controls was much more severe (11.2 mg/cm^2) than in the present study (7.0 mg/cm^2).

Table 1. Anti-inflammatory activity of auraptene (**1**), collinin (**2**) and compounds **3**–**7** and **9**–**12**.

Compound	Dose (μmol/cm^2)	Number of mice	Oedema (mg) m ± SE	Oedema reduction (%)
Controls	—	30	7.0 ± 0.1	—
1	1.00	10	3.4 ± 0.2*	51
2	1.00	10	3.7 ± 0.2*	47
3	1.00	10	4.3 ± 0.2*	39
4	1.00	10	6.4 ± 0.3	9
5	1.00	10	5.1 ± 0.3*	27
6	1.00	9	3.4 ± 0.2*	51
7	1.00	11	4.8 ± 0.3*	31
9	1.00	10	5.0 ± 0.3*	29
10	1.00	10	4.0 ± 0.3*	43
11	1.00	10	4.5 ± 0.2*	36
12	1.00	11	5.1 ± 0.3*	27
Indomethacin	0.25	10	3.7 ± 0.3*	47

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

We previously observed that severe inflammatory responses were unaffected or only slightly affected by NSAIDS, due to the damage induced by the irritant, histologically detected.[11]

From the obtained data, it can be concluded that auraptene inhibits the oedematous response induced by Croton oil. The substitution in position 8 affects the anti-inflammatory effect, that seems to be maximum when medium polarity substituents are present, such as methoxyl group (**2**) or acetyl group (**6**). Alogenate substituents in position 8 do not enhance the anti-inflammatory effect.

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References and notes

1. Muratami, A.; Nakamura, Y.; Tanaka, T.; Kawabata, K.; Takahashi, D.; Koishimizu, T.; Ohigashi, H. *Carcinogenesis* **2000**, 21.
2. Murakami, A.; Nakamura, Y.; Ohto, Y.; Yano, M.; Koshiba, T.; Koshimizu, K.; Tokuda, H.; Nishino, H.; Ohigashi, H. *BioFactor* **2000**, *12*, 187.
3. Chen, I. S.; Lin, Y. C.; Tsai, I. L.; Teng, C. M.; Ko, F. N.; Ishikawa, T.; Ishii, H. *Phytochemistry* **1995**, *39*, 1091.
4. Curini, M.; Epifano, F.; Maltese, F.; Marcotullio, M. C.; Prieto Gonzales, S.; Rodriguez, J. C. *Aust. J. Chem.* **2003**, *56*, 59.
5. Chang, C. T.; Doong, S. L.; Tsai, I. L.; Chen, I. S. *Phytochemistry* **1997**, *45*, 1419.
6. Tsai, I. L.; Lin, W. Y.; Teng, C. M.; Ishikawa, T.; Doong, S. L.; Huang, M. W.; Chen, Y. C.; Chen, I. S. *Planta Med.* **2000**, *66*, 618.
7. Brown, R. F. C.; Gilham, P. T.; Hughes, G. K.; Ritchie, E. *Aust. J. Chem.* **1954**, *7*, 181.
8. Tikhomirova, L. I.; Kuznetsova, G. A.; Pimenov, M. G. *Chem. Nat. Compd.* **1977**, *136*, 725.
9. Harayama, T.; Nishita, Y. *Chem. Pharm. Bull.* **1996**, *44*, 1986.
10. Tubaro, A.; Dri, P.; Delbello, G.; Zilli, C.; Della Loggia, R. *Agents Actions* **1985**, *17*, 347.
11. Tubaro, A.; Dri, P.; Melato, M.; Bianchi, P.; Mulas, G.; Del Negro, P.; Della Loggia, R. *Agents Actions* **1986**, *19*, 371, 373.
12. All new compounds were fully characterised by ^{1}H, ^{13}C NMR, IR and mass spectroscopy and gave satisfactory analytical data. Characteristics are given for a representative compound: **5**; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.62 (s, 3H), 1.70 (s, 3H), 1.73 (s, 3H), 2.02–2.11 (m, 4H), 2.37 (s, 3H), 4.79 (d, 1H, $J = 7.2$ Hz), 5.05–5.13 (m, 1H), 5.49–5.60 (m, 1H), 6.41 (d, 1H, $J = 9.6$ Hz), 7.02 (d, 1H, $J = 8.4$ Hz), 7.22 (d, 1H, $J = 8.4$ Hz), 7.71 (d, 1H, $J = 9.6$ Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 16.1, 17.5, 20.4, 24.0, 25.5, 26.0, 38.6, 69.9, 115.6, 118.0, 119.0, 122.2, 123.6, 131.5, 138.4, 142.7, 143.6, 146.5, 159.6, 168.5, 191.2; GC–MS m/z 356 (M^+); IR (cm^{-1}) 1726, 1710.