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Insights on Novel Biologically Active Natural Products: 7-Isopentenyloxycoumarin

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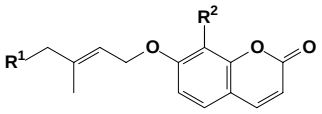
7-Isopentenyloxycoumarin is a prenyloxyphenylpropanoid derivative found in low concentration in a restricted number of plant families (Apiaceae, Asteraceae, and Rutaceae). Synthetic schemes were recently developed enabling sufficient quantities of the title coumarin to be obtained in order to evidence its valuable biological effects, mainly as an anti-cancer agent. The aim of this review is to examine the phytochemical and pharmacological properties of this compound.

Keywords: Anti-cancer activity, Apiaceae, Asteraceae, coumarins, prenyloxyphenylpropanoids, Rutaceae.

Coumarins represent a large class of natural compounds, mainly found in the families Rutaceae and Apiaceae. Although more than 1300 natural coumarins have been identified to date [1], most chemical and pharmacological studies were carried out on either coumarin itself or structurally simple derivatives. Coumarins can be divided into 3 groups: a) substituted coumarins, b) ring-fused coumarins and c) *C*- and *O*-prenylcoumarins. In particular, this third group comprises compounds in which a terpenyl side chain is attached to the benzopyrone ring either directly or through one or more phenoxy group, *via* an ether bond. While *C*-prenylcoumarins have been well studied both from a chemical and a pharmacological point of view, prenyloxycoumarins, considered for decades merely as biosynthetic intermediates of linear-, furano- and pyranocoumarins, have only in the last decade been characterized as secondary metabolites exerting valuable biological activities [1]. In this context, we have already reported the features of selected prenyloxycoumarins, like auraptene (**1**) [2], and collinin (**2**) [3]. The aim of this short review is to examine in detail from a phytochemical and pharmacological point of view, the properties of an additional coumarin derivative, 7-isopentenyloxycoumarin (**3**). This secondary metabolite was recently seen to exert promising and valuable anti-cancer, anti-inflammatory, anti-fungal, anti-microbial, and allelochemical effects.



1: R^1 = isopentenyl, R^2 = H
2: R^1 = isopentenyl, R^2 = OCH_3
3: R^1 = R^2 = H

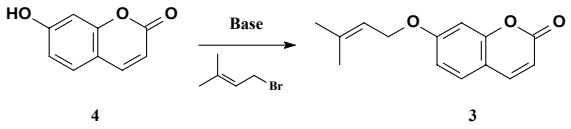
Natural and synthetic sources

Like many other coumarin derivatives, 7-isopentenyloxycoumarin has been found in a restricted number of families. In fact, to date, 7-isopentenyloxycoumarin has been obtained only from plants belonging to the Apiaceae, Asteraceae, and Rutaceae families. 7-Isopentenyloxycoumarin was first isolated in 1966 by Prokopenko from the fruits of *Libanotis intermedia* Rupr. (Apiaceae) [4]. This author provided chemical evidence for the structure of this novel secondary metabolite. A few years later, the structure of 7-isopentenyloxy-coumarin was unambiguously determined by UV, 1D and 2D NMR spectroscopy [5,6]. Compound **3** was isolated (Table 1) from several other species [7-39].

Table 1: Natural sources of 7-isopentenyloxycoumarin (**3**).

Family	Plant	Ref.
Apiaceae	*Angelica ursina* Regel	[7]
	Seseli libanotis W.D.J. Koch	[7]
	Heracleum dissectum Ledeb.	[8]
	Peucedanum stenocarpum Boiss & Reut ex Boiss	[9]
	Scandix pectens-veneris L.	[10]
	Heracleum lanatum Michx.	[10, 11]
	Ammi majus L.	[12]
	Tordylium apulum L.	[13]
	Lomatium nevadense (Watson) J. Coulter et Rose	[14]
Asteraceae	*Tagetes florida* Sweet	[15]
	Ophryosporus angustifolius B.L. Rob.	[16]
	Haplopappus tenuisectus (Greene) S.F. Blake in L.D. Benson	[17]
	Melampodium divaricatum DC.	[18]
	Haplopappus deserticola Phil.	[19]
	Baccharis pedunculata (Mill.) Cabrera	[20]
	Trichocline reptans Wedd.	[21]
	Heterotheca inuloides Cass.	[22]
	Haplopappus multifolius Phil. Ex Reiche	[23]
Rutaceae	*Euodia vitiflora* F. Muell.	[24]
	Ruta graveolens L.	[25]
	Coleonema album E. Mey	[26]
	Diosma acmaeophylla Eckl. & Zeyh.	[27]
	Coleonema aspalathoides A. Juss.	[28]
	Boenninghausenia albiflora (Hook) Meisn.	[29]
	Citrus limon (L.) Burm.f.	[30]
	Asterolasia phebalioides F. Muell.	[31]
	Ammi huntii H.C. Watson	[32]
	Haplophyllum patavinum (L.) Don. Fil.	[33]
	Melicope hayesii T.G. Hartley	[34]
	Melicope semecarpifolia (Merr.) T.G. Hartley	[35, 36]
	Phebalium brachycalyx Paul G. Wilson	[37]
	Zanthoxylum tingoassuiba A. St. Hil	[38]
	Melicope vitiflora (F. Muell.) T.G. Hartley	[39]

7-Isopentenyloxycoumarin is biosynthesized from umbelliferone (**4**) and dimethylallyl diphosphate, in turn deriving from the 1-deoxy-xylulose-5-phosphate (DOXP) pathway [40]. The coupling step is catalyzed by the enzyme dimethylallyl diphosphate umbelliferone transferase (DDU-7 transferase, E.C. 2.5.1), [41]. This enzyme is located in the membrane of the endoplasmic reticulum and is able to catalyze both the *C*-6 and the *O*-prenylation of umbelliferone. Hamerski and coworkers demonstrated that the *O*-prenylation occurred more favourably in the presence of Mn^{+2} ions as an enzymatic cofactor [41]. However, until now, 7-isopentenyloxycoumarin was obtained from natural sources in very small amounts. So, valuable, high yield, and environmentally friendly synthetic schemes were developed in order to handle this natural compound in sufficient quantities to perform tests aimed at depicting its pharmacological profile. 7-Isopentenyloxycoumarin was obtained by a Williamson etherification reaction starting from umbelliferone (**4**) and 3,3-dimethylallyl bromide as alkylating agent, and either tetrabutyl ammonium



4 **3**

Scheme 1

hydroxide [42] or dry K_2CO_3 as the base [40] (Scheme 1). In the last case, 7-isopentenyloxy-coumarin was obtained in very good yield simply by crystallization from either *n*-hexane or aqueous methanol, without the need of any chromatographic procedure [5,43].

Nowadays several efficient analytical methods (e.g HPLC, HPTLC, high speed countercurrent chromatography) for the selective separation, detection and quantification of 7-isopentenyloxy-coumarin in plant extracts are also available [30,39, 44-48].

Biological activity

The first set of data about biological activity of 7-isopenetenyloxycoumarin was reported in 1971 by Jurd and coworkers [49]. They screened 7-isopentenyloxycoumarin, at a concentration of 500 ppm, against selected bacterial (*Bacillus cereus*, *Sarcina lutea*, *Staphylococcus aureus*, *Streptococcus lactis*, *Alcaligenes faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella tiphymurium*, *Serratia marcescens*), and fungal strains (*Zygosaccharomyces japoni*, *Candida tropicalis*, *Pichia codati* var. *fermentans*, *Hansenula anomala*, *Saccharomyces cerevisiae* var. *ellipsoideus*, *Torula utilis*, *Aspergillus flavus*, *Aspergillus niger*, *Byssochlamis fluva*, *Penicillium chrysogenum*, *Rhizopus senti*), showing no activity in any case. Further investigations of the anti-fungal properties of 7-isopentenyloxycoumarin were reported in 1995 by Rahalison and coworkers. First, they tested 7-isopentenyloxycoumarin, by the agar dilution method, as an inhibitory agent of the growth of the phytopathogenic fungus *Cladosporium cucumerinum* [50]. 7-Isopentenyloxycoumarin revealed a complete inhibitory effect at a concentration of 100 μg/mL. On the basis of this result, Rahalison and coworkers tested 7-isopentenyloxycoumarin against a panel of human pathogenic fungi, such as *Candida albicans*, *Phytosporum ovale*, *Aspergillus fumigatus*, *Epidermophyton floccosum*, *Microsporum canis*, *Microsporum gypseum*, and *Trycophyton mentagrophytes*. The compound showed activity only against *E. floccosum* at a concentration of 10 μg/mL, the same value recorded for myconazole, used as the

reference drug. Considering that this fungus is among the most common causes of dermatomycoses, this effect was interesting when compared with the cytotoxic effect of 7-isopentenyloxycoumarin on human epidermal keratinocytes (LD_{50} = 15 μg/mL) [50]. Finally, 7-isopentenyloxycoumarin was not active against the phytopathogenic fungus *Botrytis cinerea* [51].

When tested as a myorelaxant, at high dosage (100 μM), 7-isopentenyloxycoumarin showed a good inhibitory effect (80 %) on the KCl (60 mM)-induced contraction of the rat thoracic aorta [29].

Only one report took into consideration 7-isopentenyloxycoumarin as an anti-inflammatory agent. However, the compound exerted a slight effect on the inhibition of LPS/IFN-γ induced NO generation in RAW 264.7 cells (30% inhibition at a concentration of 50 μM) [52].

When assayed for its capacity for anti-mutagenicity and anti-carcinogenicity against mutations induced by benzo(*a*)pyrene and hydrogen peroxide in *S. typhimurium* strains TA100 and TA102 (modified Ames test), 7-isopentenyloxycoumarin revealed only a moderate activity. At concentrations of 2, 4, and 8 μg/plate, 19, 29, and 36% protection was achieved against benzo(*a*)pyrene-induced mutations, respectively. In the case of hydrogen peroxide mutagenicity, the percentages of protection were far less (9%, 14%, and 17% at 1, 2, and 4 μg/plate, respectively) [53].

In the last decade, several studies describing the anti-cancer effects of 7-isopentenyloxycoumarin were reported. In 1998, Kofinas and coworkers assayed 7-isopentenyloxycoumarin on KB (human rhinopharinx cancer) and NSCLC-N6 (human bronchial epidermoid carcinoma) cell lines, recording ID_{50} values of 10.6 and 9.9 μg/mL, respectively [13]. In 2001, Kawaii and coworkers determined the activity of 7-isopentenyloxycoumarin on four other cancer cell lines, but virtually no activity was revealed. In fact, 7-isopentenyloxycoumarin showed IC_{50} values of 94 μM, 84 μM, 73 μM, and 57 μM against A549 (human lung carcinoma), B16 melanoma 4A5 (melanin pigment producing mouse melanoma), CCRF-HSB2 (human T-cell leukemia), and TGBC11TKB (metastasized lymphoma) cell lines, respectively [54]. The best results for 7-isopentenyloxycoumarin as an anti-cancer agent were obtained in 2002 by Baba and coworkers [55]. These authors first examined the inhibitory activity of 7-isopentenyloxycoumarin on 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-induced Epstein-Barr virus early antigen expression (EBV-EA). At a dose of 32 μM 7-isopentenyloxycoumarin exerted a 100% inhibition. It was also able to prevent the TPA-induced-$^{32}P_i$-incorporation into phospolipids in HeLa cells (89% inhibition at 1.62 mM). Encouraged by these results, Baba and coworkers studied also the effect of 7-isopentenyloxycoumarin against TPA-induced skin tumor formation in 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated mice, after topical administration. It was found that 7-isopentenyloxycoumarin strongly suppressed tumor formation in a dose dependent manner, with an inhibition degree of about 75% at a dose of 3.24 μM. Moreover, no toxicity for animals was recorded at the doses used to perform these *in vivo* tests. Finally, they examined the effects on TPA-induced murine epidermal ornithine decarboxylase (ODC) activity. Over-induction of ODC is known to be strongly associated with tumor progression. 7-Isopentenyloxy-coumarin exhibited an inhibitory effect also in this test, although not in a so evident manner as that shown in the previous assays. Taken together, the data reported by Baba and coworkers may indicate that 7-isopentenyloxycoumarin is a potent chemopreventive agent of skin tumors. The last report on anti-cancer properties of 7-isopentenyloxy-coumarin was given in 2005 by Chou and coworkers. These authors showed that 7-isopentenyloxy-coumarin exerted a mild inhibitory effect on HT-29 cell line (human colon adenocarcinoma, ED_{50} = 4.85 μg/mL), while it was virtually inactive on P-388 cells (murine leukemia, ED_{50} > 50.0 μg/mL) [36].

Finally, Epifano and coworkers found that 7-isopentenyloxycoumarin could be regarded as a novel neuroprotective agent [56]. In a preliminary *in vitro* screening, these authors showed that 7-isopentenyloxycoumarin is able to protect neuronal cells (astrocytes and neurons) from death induced by *N*-methyl-D-aspartate (50.5 % protection at 100 μM).

Conclusions

From the data reported in this short review, it can be seen that 7-isopentenyloxycoumarin may be regarded nowadays as a potential drug for the chemoprevention of some types of cancer, in particular those affecting skin. This coumarin has now become widely and easily available in huge

amounts by chemical synthesis, which is accomplished by isopentenylation of commercially available umbelliferone with 3,3-dimethylallyl bromide. The title prenyloxycoumarin and its structurally related natural and semi-synthetic compounds have become a topic of current and growing interest and it is hoped that in the near future other studies aimed at the further characterization of their pharmacological properties, not only in cancer therapy, but also in other fields, will be reported.

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