Coumarin in Plants and Fruits: Implication in Perfumery

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Is there a risk for perfumers and flavorists to use coumarin in their formulations? The purpose of this article is to give users adequate information to answer to this question. Coumarin (1,2-benzopyrone, *cis-o*-coumarinic acid lactone) is a naturally occurring constituent of many plants and essential oils, including Tonka beans (*Diepteryx odorata*), sweet clover (*Melilotus alba*), sweet woodruff (*Asperula odorata*), galium (*Galium odoratum*) and lavender (*Lavandula*).

Through its natural occurrence in many consumable ingredients, the usage of coumarin as a food flavoring raises many controversies. Banned since 1954 by the FDA, the food limit currently authorized by the European commission is 2 mg/kg food.[1,2] This is a large restriction for a product that occurs naturally in various plants and essential oils, because its consumption in the human diet can easily reach 11 mg/day through natural food ingredients.

In this paper, the quantity of coumarin that can be daily absorbed will be demonstrated, coumarin metabolism will be reviewed to highlight the differences between humans and rodents, and the first results of a survey showing that high purity coumarin is not allergenic will be presented.

Voleg first isolated and purified coumarin from the Tonka bean in 1822. Perkin later synthesized it in 1868.[3] Haarman & Reimer first marketed synthetic coumarin in 1876. At the beginning of the 20th Century, over a dozen other firms commercially manufactured coumarin. Today, a few companies, including Rhodia, still produce 2000 tons/year for use as an additive in perfumes and consumer products, with use levels ranging from <0.5percent in fine fragrances to use in detergents at<0.01 percent.[4] The material appears in approximately 90 percent of fragrance compositions currently on the market. Moreover, coumarin derived by plant extraction is also used as a drug.

It is important to recognize that the accurate identification of coumarin and the specification of its purity are of critical importance; all studies we are referring to have been performed with pure coumarin (99.9 percent, without chlorinated derivatives). Coumarin is frequently confused with coumadin (di-coumarin), which is an anti-coagulant agent used both as a rodenticide and as an anticoagulant drug. Coumarin does not have anticoagulant properties and has a toxicological and health profile very different from coumadin. The purity of coumarin is important in understanding its toxicological profile and should be known prior to the interpretation of any study results. In addition, the purity of the material can influence coumarin's aromatic and functional performance. Figure 1 shows a comparison between two coumarin purity profiles: the Rhodiascent Extra Pure coumarin of Rhodia (made from phenol-based salicylaldehyde) and one example of Chinese synthetic coumarin (from orthocresol-based salicylaldehyde). The significant differences in quality must lead the users to pay attention to this point, particularly as orthocresol generates chlorinated impurities.

Figure 1. Comparative chromatograms of Rhodiascent Extra-Pure coumarin and coumarin from China (orthocresol route)

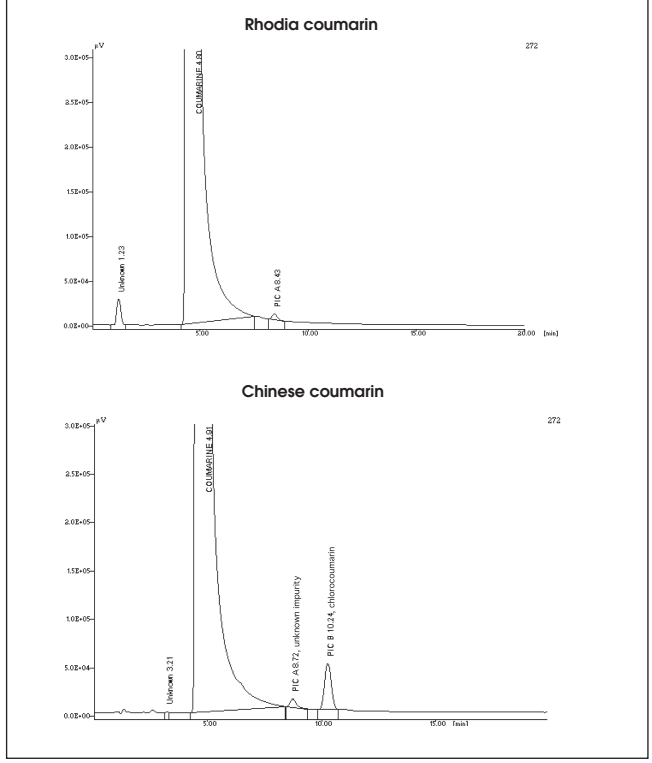

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Table 1. Coumarin ingestion in normal diet

Menu	Daily intake	Content[1] mg/100g	Amount
breakfast			
orange juice	250 mL	3.1	0.00775 mg
green tea	300 mL	30	0.09 mg
lavender honey	10 g	300	0.03 mg
lunch			
aperitive[2]	40 mL		0.4 mg
carrot	125 g	123.4	0.15425 mg
beer	250 mL	1	0.0025 mg
snack			
apple strudel pie[3]	200 g		
cinnamon	0.3 g	4.00E+06	10 mg
1 chewing gum[4]	5 g		0.025 mg
well known coca-based soda (caffeine free)	250 mL	5	0.0125 mg
dinner			
caramel candies[5]	20 g		0.2 mg
celery	200 g	57.8	0.1156 mg
wine	125 mL	3.6	0.0045 mg
			Total amount: 11.042 mg

[1]content of coumarin after hydrolysis (reaction)
[2]aperitive: coumarin limited at 10 ppm
[3]to make 800 g of apple strudel ("apfel strudel"), you need 4g of cinnamon
[4] chewing gum: coumarin limited at 50 ppm
assumption:10 percent is absorbed (10 percent is absorbed from the gum)
[5]caramel: coumarin limited at 10 ppm

Coumarin in Everyday Life

Until recently, there were no universal methods for the detection and measurement of coumarin in food, but methods had to be developed and adapted for the different food samples to be analyzed. F. Villeneuve and G. Abravanel proposed a general method for the quantitation of phenolics in plant extracts in 1982. F. Bourgaud et al. (1994) compared seven different methods for the extraction of coumarin and coumarin-glucoside from sweetclover, and D. Ehlers et al. (1995) described an HPLC method for the separation of coumarin, dihydrocoumarin and coumarinic acids. In 1998, P. B. Andrade devised a method for the detection of flavonoids, phenolic acids and coumarins in medicinal plants using HPLC/diode array instrumentation.

However, it remains difficult to determine the amount of coumarin present in plant-derived foods. The main reason for this is the difficulty of separating coumarin from the hundreds of other phenolic compounds present in plant tissues and to measure it accurately. This is especially true for food samples like leaves or fruits that are very rich in chlorophyll or other pigments, or for processed foods like tea or wine.

A new methodology using HPLC equipment elaborated by Francis Durst, gave great results in the identification of coumarin in many foods such as legumes, fruit juice, beverages.[8] Some of these results are summarized in Table 1, and clearly demonstrate that a food consumption of 11 mg/day can easily (and safely) be reached.

This quantity can easily increase if coumarin absorbed through medicinal uses is counted. Veinotonic medicines, still in use for more than 20 years, provide 12 mg/d of coumarin derivatives.

On the other hand, any human use of coumarin would not lead to environmental risk, as coumarin has a low ecotoxicity level (Daphnia EC50 > 30 mg/l), and has been shown to be non-persistent (readily biodegradable and no potential for bioaccumulation).

The amount of coumarin that can be daily absorbed from fruits, vegetables and other food ingredients is significant, and yet coumarin is subject to strict regulations. The Research Institute for Fragrance Materials (RIFM) launched a scientific program to highlight this contradiction. The goal of the coumarin research program is to understand the toxicological and carcinogenic mechanisms involved in the genesis of mouse lung tumors and rat liver tumors and to focus on species differences in coumarin metabolism and toxicity.

Review of Coumarin Metabolism

Results to date show that because of very large differences in the way rodents and humans metabolize coumarin, mice and rats should not be used to predict the effects of coumarin in humans and strongly suggest that low level coumarin exposure in cosmetic products will not have adverse effects in people (RIFM).

While 7-hydroxylation is the major metabolic pathway of coumarin biotransformation in humans and certain primates such as baboons (68-92 percent), it is only a minor pathway in rodent species.[5] The 3-hydroxylation, which can lead to *o*-hydroxyphenylacetic acid, is a minor biotransformation in humans as well as in rodents. The major metabolic route of coumarin biotransformation in the rat is by oxidation, which forms an unstable coumarin — 3,4 epoxide intermediate — which rearranges to form a toxic metabolite, *o*-hydroxyphenylacetaldehyde (*o*-HPA).[6] *o*-HPA can then be further metabolized to the non-toxic metabolites, *o*-hydroxyphenylacetic acid (*o*-HPAA) and *o*-hydroxyphenylethanol (*o*-HPE). Rats poorly detoxify

o-HPA in the liver, and consequently may develop liver tumors if exposed to coumarin at very high dose levels. Similarly, when dosed via stomach tube with very high levels of coumarin, mice may develop lung tumors because of poor detoxification pathways in specific cells in the lung.

In contrast, humans produce little or no *o*-HPA and readily detoxify this metabolite to the non-toxic metabolites *o*-HPAA and *o*-HPE. This indicates that mouse and rat data on carcinogenicity are not relevant for humans. Metabolic pathways are illustrated in Figure 2.

Figure 3 illustrates the different amounts of toxic (*o*-HPA) and non-toxic (*o*-HPE and *o*-HPAA) coumarin metabolite produced by human, rat and mouse. It shows that neither rat nor mouse metabolism is representative of human metabolism of coumarin.

Given that human consumption of coumarin through natural food ingredients may be 11 mg/day or more, it is scientifically inconsistent that coumarin as a food additive is limited to 2 mg/day via naturals and is banned as a direct food additive (despite a purity for synthetic coumarin of 99.9 percent and without chlorine content). Today, coumarin is included in the 26 allergen list of Fragrance Allergens by the SCCNFP opinion of December 1999.

Figure 2. Metabolic pathways

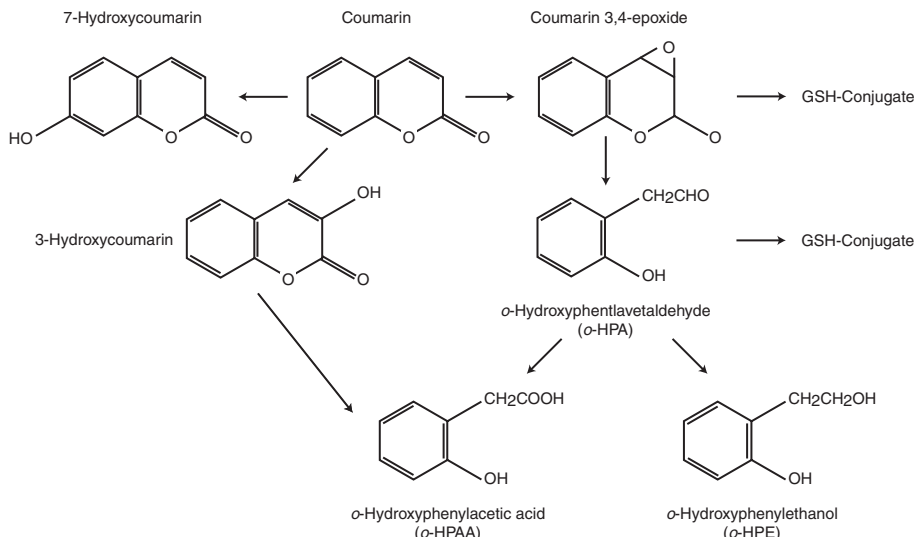
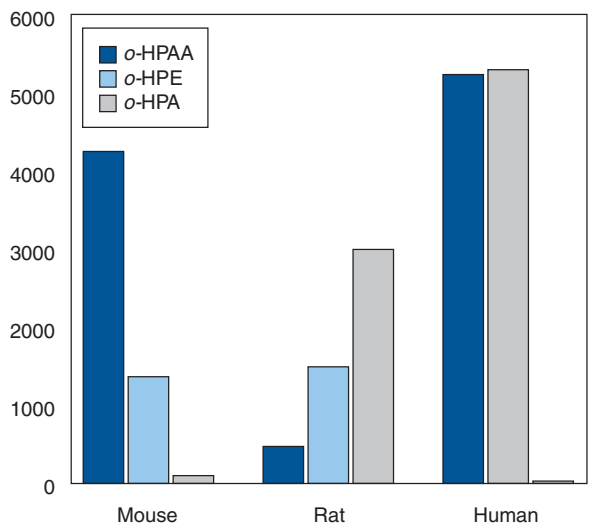


Figure 3. Different amounts of toxic (*o*-HPA) and non-toxic (*o*-HPE and *o*-HPAA) coumarin metabolite produced by human, rat and mouse



However, in our opinion, the validity of the research leading to this choice can be criticized considering the lack of rigor (reliability, details and so forth) in these studies. First, there is no analytical information about the coumarin used, and second, statistical results are non-significant.

New Assessments of Cutaneous Allergic Potential

We have examined the Scientific Committee on Cosmetic Products and Non-Food Products intended for consumers (SCCNFP) position paper for Fragrance Allergy in Consumers (SCCNFP/0017/98 final December 1999) as well as the European Cosmetic, Toiletry and Perfumery Association (COLIPA) and RIFM proposals for this particular substance.[9] None of these papers indicate a clear purity of the material used. This is an important point as indicated in Hausen (1989), because some derivatives in the coumarin family could have weak-to-moderate sensitizing properties.[10]

What are the main questions in some of these papers:

- Malten (1984) reported on 182 patients in four clinics with no reaction to coumarin, even at 8 percent, which could be demonstrated to be an irritant in other studies
- De Groot (1988) reported on 119 patients in seven clinics, and indicate 1/119 positive when exposed to 5 percent in petrolatum.
- Larsen (1996) reported on 177 patients in seven clinics with a final 1.2 percent positive reaction rate with indication of dermal irritation and cross reactivity (eliciting agent).
- Van Joost only reported statistics indicating concomitant reactions with eugenol and isoeugenol.
- Bruinzel indicated in a poster presentation, 18

patients out of 14,000 patients examined were reactive only to coumarin at 5 percent in petrolatum, while 56 patients were reactive to coumarin as well as other fragrance compounds.

Further to purity, one can raise questions on concentration and irritation (homogeneity and stability of suspension in petrolatum), and cases of cross-reaction to allergens for which coumarin may be only a detector reagent. In order to answer these questions for pure coumarin (Rhodiascent Extra-Pure), Rhodia has run several tests confirming the thesis that coumarin is not a dermal allergen.

First, an animal study [Local Lymph Node Assay (LLNA) at 25-10 percent and 5 percent in acetone/oil] was run and results were negative, showing no allergenic induction when the animals were treated with high purity coumarin. A second study was conducted on a panel of 100 people who were coming to their physician for pre-existing dermal allergies (already known or unknown). One critical aspect of this study was to produce a stable and homogeneous suspension of coumarin in petrolatum. The difficulty in obtaining a homogeneous mixture was highlighted by several assays, starting with grinding crystals in a mortar, which does not lead to homogeneous preparation into petrolatum. There were also difficulties using solvents like ethyl, and in mixing coumarin in liquid petrolatum at 45°C and using a blender mixer to get homogeneous suspensions. UV dosages indicate a good homogeneity at 10 percent, 8 percent, 5 percent, 3 percent and 1 percent.

Stability was controlled, and at the end of the clinical study, it gave the same range of homogeneity. The study was then designed to test for irritation and/or allergy on a panel of 100 patients visiting the hospital for dermatitis, and who need patch tests to identify their allergens or determine further allergic response when some allergens had already been determined.

It was agreed that the study would begin with 10 percent and 1 percent applications for 24 h in Finn chambers (the usual device allowing the physician to apply a substance for the patch test to the skin), with readings immediately and 48 h or 72 h after removal. If five to 10 successive patients showed dermal irritation, then the study would continue with 8 percent or less, and 1 percent up to no irritation.

In fact, the dermatologists tested the 100 patients with 10 percent and 1 percent without noting any allergic reaction in these patients with known or unknown allergic dermatitis. It can be concluded from this study that a homogenous suspension of pure coumarin in petrolatum does not lead to irritation or allergic reaction. These results confirm what was found with animal studies and particularly the LLNA run with the same material.

Conclusion

We have learned that coumarin from natural sources is well represented in our daily food. Coumarin (without chlorine derivatives) is not carcinogenic for humans due to non-relevance of the results seen in rats or mice. These species have different metabolic pathways for coumarin than humans, and, more importantly, a different ability to detoxify coumarin in various tissues, leading in those species to toxicity and subsequent appearance of related tumors. This may prompt a review of the ban of coumarin in food.

We have also seen that pure coumarin does not lead to irritant reactions or sensitization (induction) in animals (mouse LLNA) or in humans (elicitation), provided an homogeneous suspension is used. This also indicates the importance of the purity and quality of substances as well of methodologies to assess their hazard potential.

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