Chemodiversity, as a new research concept, is highlighted by a discussion of selected publications of Professor Dr E. Wollenweber. Excretion phenomena of flavonoid aglycones are addressed, such as localization, chemosystematic, and applied aspects. Various classes of flavonoids have been reported from exudates; even flavonoid glycosides and biflavonoids were accumulated on the leaf surfaces of plants. The production of other exudate constituents outside the flavonoid pathway is briefly addressed. The connective role to biological disciplines is stressed, particularly as far as secretory structures are concerned.

**Keywords:** exudate flavonoids, chemodiversity, localization, systematic significance.

**Hey, bud(die), what’s your secret(ion)?**

This was one of the first key questions for Eckhard to ask at the beginning of his scientific career (bud excretions of *Populus* and *Betula* [1, 2]). “I am sticking to it” said young Eckhard (“Flavonoid aglycones from the bud exudates of three Betulaceae” [3]; “Series of novel flavanones identified by gas chromatography-mass spectrometry in bud exudates of *Populus fremontii* and *P. maximowiczii*” [4]). It seems that they got strong buddies after such a long research history. Eckhard always wanted to find out more about the diversity of excreted flavonoids, which are deposited on surfaces of plants instead of being stored somewhere hidden inside cells and tissues. The botanical world is sometimes sticky and stinky – appalling maybe to some people, but fascinating to Eckhard. Being a sticky subject, these plants would never let him go until their secret(ion)s could be lifted for everyone to see and understand. He became enamoured with the subject, and even worse, he convinced a series of followers to join in, making this a focal point in several symposia (e.g. “Exudate flavonoids in higher plants of arid regions” [5]; and “On the distribution of exudate flavonoids among Angiosperms” [6]). By 1981, some 462 flavonoids had been reported to occur as aglycones in/on various plant organs, with some of them assumed to be part of exudates [7].

One secret(ion) followed another – digging into exudate plant products became a habit of Eckhard, marking all his career. Indeed, it has been a productive effort, leading to somewhat close to 300 publications in outstanding international journals. In the present context, it would amount to a review the size of a book. Therefore, we keep the topic focused on selected publications that address major aspects of chemodiversity as now understood.

**What is chemodiversity?**

Before highlighting this topic by commenting on selected publications of Eckhard, we should shed some light on this term. **Chemodiversity** (“Chemical Biodiversity”) concerns the expression of chemical compounds in living organisms as a result of...
biosynthetic activities, which in turn is the expression of evolutionary and adaptational processes. Central is the study and interpretation of phenomena governing the accumulation of plant products. Ideally, aspects such as biosynthesis, storage, organ specificity, seasonal and ontogenetic variation, distribution studies, and functional aspects have to be covered to achieve a successful understanding of the mechanisms behind chemical product formation, and to make use of these compounds as chemical markers in systematic studies (chemosystematics, chemotaxonomy1).

At the beginning of Eckhard’s scientific career, chemotaxonomy or chemosystematics was the new topic in systematics, comparable to how molecular systematics is perceived now. In many cases, chemical compounds indicated plant relationships that were either obscure or had been wrongly interpreted. The development of chromatographic techniques such as TLC and various spectroscopic methods strongly aided the initial success of this new field. TLC is still a very reliable method, especially for flavonoid aglycones, in many cases still unmatched, particularly when combined with the long standing experience of Eckhard, who has isolated hundreds of flavonoids (many of them new) during his career.

We would like to focus on some topics that may be considered important in chemodiversity of exudate flavonoids. Recently, several of those have been considered briefly [8]; and a more in-depth review, addressing also historical, functional and systematic aspects, has been published by Eckhard already in 1989 [9]. Several other relevant aspects of chemodiversity research by other authors will also be presented.

**Glandular trichomes and further localization secrets**

Plants are often covered with trichomes, some of which are glandular; these are very productive. They contribute mostly to the aromatic smell of many plants of Lamiaceae, Asteraceae and of other families, by excreting essential oils and/or other lipophilic material. These glands were shown to be biosynthetically active, and what Eckhard liked most, they were also proven to excrete flavonoid aglycones, as was reported from *Mentha* [10] and *Origanum* [11]. Similar results became evident from analysis of trichome constituents of *Scalesia* (Asteraceae) [12]. Eckhard earlier was enlightened when he analyzed the glands of *Empetrum*, proving their phenolic contents (“Lipophilic phenolics from the leaves of *Empetrum nigrum* – chemical structures and exudate localization” [13], Figure 1). Deposits of flavonoid aglycones may be found on surfaces, as was nicely demonstrated by special microscopic methods applied to the cuticles [14]. The biological role can be well explained by studies on the influence of excess solar radiation, indicating *in situ* production of flavonoids in glandular trichomes of *Phillyrea* [15]. All these points were not addressed in a recent review on surface flavonoids [16].

**Flavonol glycosides** as hydrophilic compounds looked somewhat misplaced on leaf surfaces, and Eckhard looked worried. Earlier, it had been demonstrated for *Nicotiana* species that there were two different kinds of glandular trichomes producing different biosynthetic products (hydrophilic versus lipophilic). Different transportation and excreting mechanisms have been postulated for the hydrophilic compounds (for detailed discussion see [17]). Plants may often be a source of surprise.

Surprises never stop: at some point Eckhard saw double. What are Gymnosperms doing with their exudates? They excrete their **biflavonoids**, which they also accumulate in their cells [18]. Studies on the micromorphology of the so-called waxes indicate that tubular fine structures, sometimes restricted to stomata lines, are vectors for excretion of these compounds. Eckhard showed that the same compounds were also present in the tissues, which suggests some kind of transport mechanism. As this also concerns the phytoremedy *Ginkgo biloba*, this might be of practical relevance for rapid isolation of active compounds.

There appear to be still some unresolved secrets. Lipophilic deposits on leaf surfaces may also occur even if no secretory structures are found. Discussions on this topic can be found in [19] as well as in [5]. Later, a successful study was carried out to mimic the epicuticular wax crystal formation *in vitro* [20]. It has been shown experimentally that the cuticular water current takes those compounds along in a process similar to steam distillation. There is a need for much more research on this aspect, as the authors stress correctly. These experiments again show the
importance to include biological thinking into phytochemistry research, to obtain a better understanding of the mechanisms and processes that govern production and accumulation of phytochemicals.

Figure 1: “Enlightened” Eckhard with Empetrum glands [13].

Farina is not a flour brand, but may even be found on a flower

The genus Primula is well known for the production of a mealy farina, coating its leaves and inflorescences. Interestingly, this meal contains mainly the unsubstituted flavone, which had been known for some 90 years. In 1970, Eckhard demonstrated that other flavonoid compounds were also present in varying amounts [21]. Studies did not stop at that point [22-25], leading even to applied fields such as “Biological effects of epicuticular flavonoids from Primula denticulata on human leukaemia cells” [26], which bridged the gap between phytochemistry and medicine. Interest in Primulaceae has not lessened, and some work is still in progress on the related genus Dionysia.

Seeking more farina in the plant kingdom, some taxa of Pteridaceae were found to produce a conspicuous white or yellow coating on the lower leaf surfaces, a phenomenon described as early as 1877. Already in 1906 the first compounds were isolated (see [27]). Ferns have a very complex and diverse chemistry, and many interesting structures from practically all classes of flavonoids, including new classes and esters have been described [27, 28]. In comparative (chemosystematic) studies, one will always find new compounds, which lead to an increase in the numbers of collaborators working on structure elucidation (e.g. [29], diterpenoids).

Farina constituents of ferns are excellent characters in systematics, leading to reconsideration of the taxonomy of Pityrogramma [30]. Eckhard paved the way for such an approach by analyzing chemotypes of P. triangularis [31], and by commenting on the use of exudate flavonoids as chemotaxonomic markers in Mexican ferns [32]. The checking of possible chemotypes is an essential step in chemodiversity research, which means analyzing variability on the populational and individual level. This not only applies to ferns, but to all biological subjects.

What do bees do with exudates?

A “poplar” question this may sound. Propolis (bee glue) consists of exudates of plants, with a strong connection to poplar bud exudates [33, 34]. Most of the propolis constituents largely correspond to compounds found in poplar bud excretions, indicating them to be a major source. In view of propolis being a phytoremedy since ancient times, analysis of its constituents has been of great interest. Being allergic to propolis can be a serious problem, and Eckhard and his collaborators have clarified the active principles involved in contact dermatitis: caffeic acid derivatives – thank goodness, not the flavonoids [35-38]! This was nicely shown in several papers that inevitably also deal with newly identified compounds [39]. Things may be different across the Atlantic. Although poplars are less common in the Sonoran desert, being found mainly along creeks, feral honey bees incorporated their material among other plant sources. It has also been shown that Ambrosia and Encelia provide some of the right material, on the basis of identical flavonoid compounds [40].

Excreted flavonoids and plant relationships

Chemosystematics or chemotaxonomy was quite modern some decades ago, and results from exudate flavonoids have been very helpful on occasions. Their efficacy with ferns was discussed above, and the excretions of poplars, betulas and Nothofagus species can also be mentioned [41]. The personal interest of the first author of this presentation has been with the genus Achillea, and Eckhard became involved with its exudate flavonoids. Due to previous work on vacuolar flavonoid glycoside diversification in this genus, it allowed comparison of the
substitution trends of both types of compounds, since they have different biosynthetic histories (vacuoles versus glandular hairs). Indeed, they showed different substitution patterns indicative of different enzymes at work (for aspects of biosynthesis see also [42]). Some accumulation tendencies proved to be correlated to taxonomically recognized units of *Achillea*, but this was not consistently observed in all parts of the genus [43].

Since Asteraceae has always been an interesting subject, Eckhard could not resist analyzing many of them (e.g. [44-48]). Who ever thought there would be only flavonoids in exudates was certainly wrong (terpenoids, benzofurans, oxyacetylenicoumarins [49-51]). The genus *Artemisia* provides a further example of exuding not only flavonoids, but also coumarins and phloroactophenones [52]. Thus, exudates may be quite complex and provide a good example of chemodiversity. Although only 30 species of *Artemisia* have been studied so far out of some 500, accumulation trends appear specific for some of its subtaxa. In fact, this is one of the most interesting genera of Asteraceae, as a model for extended chemodiversity studies.

The first author of this presentation has always enjoyed correlating flavonoid data with chemosystematics in Asteraceae [53] and chemodiversity in Lamiaceae [54]. The term “chemodiversity” appears to be more appropriate, as it includes many other functional and ecological aspects apart from chemosystematics, which have been addressed [52-54]. Exudate flavonoids have also been reported from many other families [6, 7] amongst them Scrophulariaceae [55] and Solanaceae [17]. In many cases, the accumulation was linked to ecological factors such as xeric habitats and/or stronger UV-radiation. Recently, proof of UV-photoprotective activity came from a study on flavonoid gene expression in *Petunia* leaves [56]. Knowing about the background of formation and accumulation of ecologically important compounds is essential to their interpretation as possible chemical characters, allowing relation of structure to function.

**Conclusion**

We are all much indebted to Eckhard for the wealth of scientific information he has made available to us on exudate flavonoids and on many other exudate compounds of the non-flavonoid pathway. These data form the basis for much further research and for development of scientific hypotheses. Interdisciplinary aspects of chemodiversity research can now be addressed more effectively, and we find more and more connections with general biological fields and those of applied research (pharmacy, medicine, agriculture, ecology; see [26, 57]). Many secrets still remain, but I am pretty sure Eckhard would want us to continue on the path that he has opened for us. Thanks, Eckhard, for all your efforts, and we will do our best not to disappoint you with our future research.

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**References**


Chemodiversity of exudate flavonoids


