Review

Molecular mechanisms of toxicity of important food-borne phytotoxins

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At present, there is an increasing interest for plant ingredients and their use in drugs, for teas, or in food supplements. The present review describes the nature and mechanism of action of the phytochemicals presently receiving increased attention in the field of food toxicology. This relates to compounds including aristolochic acids, pyrrolizidine alkaloids, β-carotene, coumarin, the alkenylbenzenes safrole, methyleugenol and estragole, ephedrine alkaloids and synephrine, kavalactones, anisatin, St. John's wort ingredients, cyanogenic glycosides, solanine and chaconine, thujone, and glycyrrhizinic acid. It can be concluded that several of these phytotoxins cause concern, because of their bioactivation to reactive alkylating intermediates that are able to react with cellular macromolecules causing cellular toxicity, and, upon their reaction with DNA, genotoxicity resulting in tumors. Another group of the phytotoxins presented is active without the requirement for bioactivation and, in most cases, these compounds appear to act as neurotoxins interacting with one of the neurotransmitter systems. Altogether, the examples presented illustrate that natural does not equal safe and that in modern society adverse health effects, upon either acute or chronic exposure to phytochemicals, can occur as a result of use of plant- or herb-based foods, teas, or other extracts.

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Abbreviations: ADI, Acceptable Daily Intake; **EFSA**, European Food Safety Authority; **GABA**, γ-aminobutyric acid; **GSH**, glutathione; **MAO**, monoamine oxidase; **PA**, pyrrolizidine alkaloid; **SCF**, Scientific Committee on Food; **TDI**, tolerable daily intake; **VOD**, veno-occlusive disease

1 Introduction

Plants and their constituents have been used for ages as a source of bioactive ingredients for hunting, medical, warfare, and assassination purposes. The Ebers papyrus (*ca.* 1500 BC) already described a variety of poisons based on plant ingredients [1]. The ancient Greek and Roman literature gives several references to the use of poisons. Hemlock, which contains the nicotinic acid agonist alkaloids coniine and γ-conicine as its major toxic ingredients, was the official Greek state poison used for the execution of Socrates (470-399 BC) [1]. The use of plants and their ingredients for beneficial health purposes continues from these ancient times, through the Middle Ages, when botanical gardens were established and maintained as a source of medical plants, till modern time.

At present, there is an increasing interest for plant ingredients and their use in drugs, for teas or in food supplements. Many consumers equate "natural" with "safe" when considering plant-based food supplements or drug preparations. Unfortunately the assumption that natural products are safe is false. Scientific literature describes a wide variety of plant-derived toxins, known as phytotoxins, that can be present in the fruit and vegetable components of our diet. These bioactive ingredients are generally regarded as safe (GRAS) at the current levels of exposure. However, in spite of a long history of safe use, botanical or herb-based food items may contain individual ingredients known to be toxic and even genotoxic and carcinogenic, and they may become of concern upon increased exposure. The present review describes the nature and mechanism of action of the phytochemicals presently receiving increased attention in the field of plant- and herb-based food items. This relates to the mechanism of action and toxic effects of compounds including aristolochic acids, pyrrolizidine alkaloids, β-carotene, coumarin, the alkenylbenzenes safrole, methyleugenol and estragole, ephedrine alkaloids and synephrine, kavalactones, anisatin, St. John's wort ingredients, cyanogenic glycosides, solanine and chaconine, thujone and glycyrrhizinic acid.

2 Review

In the following sections the molecular mechanisms of toxicity of a series of phytotoxins of present interest in the field of food toxicology are summarized. Emphasis is on the molecular mechanisms underlying the toxicity. Compounds discussed have been selected because of their impact in the field of food toxicology during the past decade.

2.1 Aristolochic acids

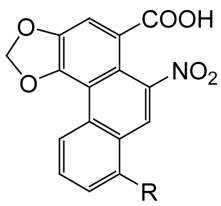
2.1.1 Major characteristics, occurrence, and intake

Aristolociaceae have been used since ancient times in herb-based medicine. In 1991, a unique form of nephropathy was reported in Belgium. Over 100 young women suffered from kidney damage, developing in several patients into cancer of the kidneys and the urinary tract [2, 3]. This adverse effect was associated with the prolonged intake of a Chinese herb-based weight loss preparation in which *Stephania tetranda* was accidentially replaced by *Aristolochia fanchi*, because both plants are used under the same name *'Fangji'* in Chinese folk medicine [2]. Aristolochic acids occur throughout the plant and appear in roots, stem, leaves, and

fruits of *Aristolochia fanchi*. Levels in the crude extract can range between 0.1% and 0.6 % dry weight [4, 5]. The clinical symptoms observed were named Chinese herb nephropathy (CHN) [3], and when it became clear that they were caused by aristolochic acids the disease was also named aristolochic acid nephropathy (AAN) [6]. The ingested dose of aristolochic acids by patients with CHN has been estimated to be in the range of a few microgram/kg bw/day [7].

2.1.2 Mechanism of toxic action

Aristolochic acids I and II (Fig. 1) are the major type of aristolochic acids and are know to be nephrotoxic, genotoxic, and carcinogenic [8 – 13]. The first symptoms of AAN are the excretion of low-molecular-weight proteins in urine,



R = OCH_3 = aristolochic acid I
R = H = aristolochic acid II

Figure 1. Structural formula of aristolochic acids [8 – 13]. With R=OCH_3 in aristolochic acid I (8-methoxy-6-nitro-phenanthro-(3,4-*d*)-1,3-dioxolo-5-carboxylic acid, and R=H in aristolochic acid II (6-nitro-phenanthro-(3,4-*d*)-1,3-dioxolo-5-carboxylic acid.

indicating a toxic effect on the proximal tubules [14]. Reductive metabolic activation of aristolochic acids by cytochromes P450 (CYP) 1A1 or 1A2, and/or by other enzymes including NADPH : P450 reductase, xanthine oxidase, NAD(P)H : quinone oxidoreductase (= DT-diaphorase), and peroxidases has been reported and results in formation of a cyclic reactive nitrenium ion (Fig. 2) able to form covalent DNA adducts at the exocyclic amino groups of guanine and adenosine [8, 9, 15 – 20]. The adduct detected with highest frequency is the adenine adduct leading to an AT to TA transversion. The human tumor suppressor gene *p53* was shown to be a hot spot for this type of mutation by aristolochic acid metabolites [9]. Furthermore, in rodents the activation of the Ha-*ras* gene by a specific AT to TA transversion mutation was reported [21, 22]. Studying 39 women with CHN, Nortier *et al.* [23] demonstrated aristolochic acid related DNA adducts in specimens of renal tissue and concluded that a cumulative dose of 201 g or more of a compound labelled as containing *Stephania tetrandia* but actually containing *Aristolochia fangchi* increases the risk for developing urothelial carcinomas.This was concluded based on the observation that among the 24 patients with CHN that consumed a total dose of 200 or less, 8 cases of urethelial cancer were detected, whereas among 15 patients who had ingested 201 g or more 10 cases were found, which was significantly higher ($P = 0.05$). Since the first reports of CHN in Belgium, similar cases have been described in several other countries including Spain, Japan, France, the UK, and China ([9] and references therein).

2.1.3 Polymorphisms of influence

To date, only a few percent of the patients treated with the slimming regimen are reported to have suffered from nephropathy [20]. A possible explanation for this observation may be differences in the enzymes involved in bioactivation/ detoxification of the aristolochic acids. Genetic polymorphisms in the enzymes catalyzing reductive activation, including CYP1A1 or CYP1A2, but also NADPH : P450 reductase, xanthine oxidase, NAD(P)H : quinone oxidoreductase (= DT-diaphorase), and peroxidases may be of influence. Thus, lifestyle factors like smoking which induces CYP1A, and genetic polymorphisms for CYP1A2 and for NAD(P)H : quinone oxidoreductase leading to poor metabolizer phenotypes, may influence the risk posed by aristolochic acid consumption.

2.1.4 Concluding remarks

Following the reports of Aristolochia-related nephrotoxicity, many countries have taken regulatory actions to protect the public by taking Aristolochia species from the supply chain. The European Agency for the Evaluation of Medicinal products even suggested to consider the prohibition of species at risk of being confused with Aristolochia species, unless appropriate quality control procedures are in place [7]. Such species include *Akebia quinata, Akebia trifoliata, Clematis armandii, Clematis montana, Cocculus orbiculatus, Cocculus laurifolius, Cocculus trilobus, and Stephania tetrandra* [7]. It can also be concluded that further identification of the enzymes principally involved in the bioactivation of aristolochic acids, and the screening of CHN patients for genetic polymorphisms in the major enzymes involved, seem important future steps to allow elucidation of possible relationships between genotypes and CHN, and to define the groups within the human population at increased risk.

2.2 Pyrrolizidine alkaloids (PAs)

2.2.1 Major characteristics, occurrence, and intake

Plants known to contain pyrrolizidine alkaloids (PAs) are widely used for medicinal purposes as home remedies all over the world, and some are even used as food. Human poisoning and even deaths from PAs have been reported in several countries including South Africa, Jamaica, Ecuador,

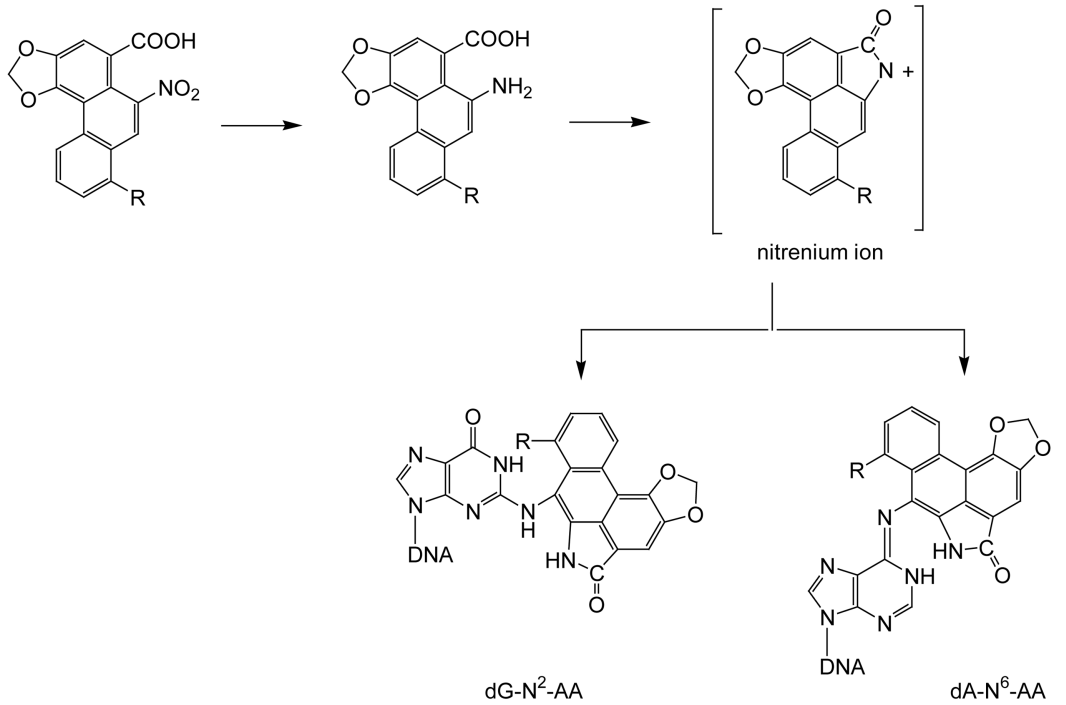


Figure 2. Metabolic activation of aristolochic acids to a cyclic reactive nitrenium ion and its subsequent covalent adduct formation with deoxyadenosine (dA-N^6-AA) and deoxyguanosine (dG-N^2-AA). Reductive activation of aristolochic acids in the first step can be catalyzed by CYP1A1 or CYP1A2, and/or by other enzymes including NADPH:P450 reductase, xanthine oxidase, NAD(P)H: quinone oxidoredutase (= DT-diaphorase), and peroxidases [8, 9, 15 – 20].

Hong Kong, India, the central Asian republics of the USSR, the UK, and the USA [24, 25]. PAs are present in plants of the families Boraginaceae (all genera), Compositae (*Senecionae* and *Eupatoriae),* and Leguminosae *(*genus *Crotalari*) [24]. PAs were known to be a hazard towards livestock for many decades. Human consumption of PAs occurs, for example, from consumption of *Symphytum* and *Senecio* species present in herbal preparations, such as "comfrey tea" or "groundsel tea". Over 200 PAs have been identified, about half of them estimated to be toxic. Figure 3 presents the structures of some important pyrrolizidine alkaloids including echimidine, the most toxic PA in *Symphytum officinale*, jacobine, the major toxic alkaloid in *Senecio jacobaea*, and retrorsine, the major toxic alkaloid in *Senecio vulgaris*. *S. officinale*, commonly called comfrey, contains in addition to echimidine also other alkaloids, including intermedine, lycopsamine, symphytine, and symglandine. The total content of PAs is nearly 0.5% in *S. caucasicum*, but lower in *S. officinale* (leaves: 0.02 – 0.18%; roots: 0.25 – 0.29%) and *S. peregrinum* was found to contain about 0.2% alkaloids in the tops [26]. Aside from ingesting the plants directly, PAs can be consumed by eating honey collected by bees that visit PA-containing plants (mainly species of *Senecio*) and by drinking milk or eating eggs produced by animals that have consumed PA-containing plants [26 – 29]. In honey originating from species of *Senecio*, the total concentration of PAs was 0.3 – 3.2 μg/kg. PAs could be detected in the concentration range of 30 – 70 μg/kg in honey from the Alpine foothills of Switzerland [26, 27]. The range of toxic doses in humans is about 0.1 – 10 mg/kg bw/day [26, 29]. However, the World Health Organization suggested that the lowest intake of PAs that caused adverse effects in a human was just 0.015 mg/kg bw/day, corresponding to 1 mg/day for a 70 kg adult, based on the use of comfrey [24, 26]. Exposure to PAs can vary since PA content of comfrey roots and leaves have been reported to vary between 450 – 8300 mg/kg for roots and between 15 – 55 mg/kg for leaves [26, 30].

2.2.2 Mechanism of toxic action

The toxic effects of PAs in humans are principally on the liver. Here, they can produce veno-occlusive disease (VOD), the major lesion being the occlusion of the central and sublobular hepatic veins [24]. The PAs are also known to cause liver damage in experimental and farm animals [24]. Furthermore, PAs are known to be mutagenic, carcinogenic, and teratogenic [24]. The mechanism of toxic action has been related to the formation of pyrrole-type metabolites [24, 31, 32]. These pyrrolizidine pyrroles are pyrrolic dehydro-alkaloids, formed by dehydrogenation of the pyrrolizidine alkaloids by hepatic monooxygenases, especially

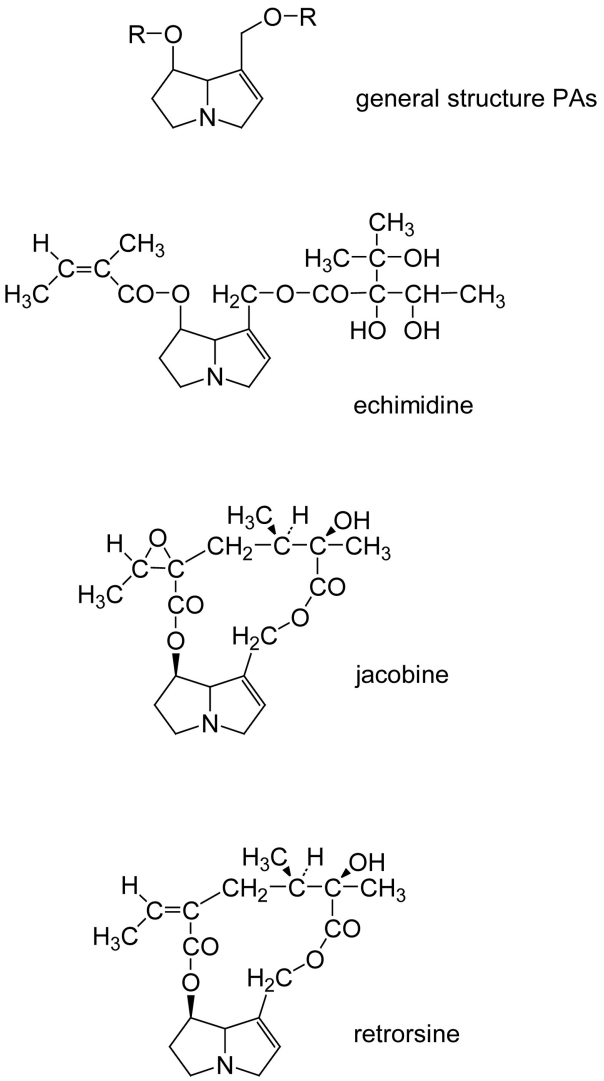


Figure 3. Schematic presentation of the general structure of PAs, and structural formula of some important PAs including echimidine, the most toxic PA in *Symphytum*, retrorsine, the major toxic alkaloid in *Senecio vulgaris* and jacobine, the major toxic alkaloid in *Senecio jacobaea*. The hepatotoxic alkaloids have a 1,2-double bond in the pyrrolizidine ring and branched side chains, in which the 9-hydroxyl and preferably also the 7-hydroxyl substituent are esterified [24].

CYPs (Fig. 4) [24, 32]. The formation of the pyrrolic metabolites results from an initial hydroxylation of the unsaturated pyrrolizidine ring adjacent to the nitrogen atom, leading to an unstable intermediate that decomposes to give the pyrrolic product [33]. The pyrrolic dehydro-alkaloid metabolites are reactive alkylating agents able to react with cellular nucleophiles (Fig. 4), thereby causing DNA alkylation, DNA cross-linking, and liver cell necrosis ([24] and references therein). The carcinogenic activity of PAs appears to parallel their mutagenic behavior but not their hepatotoxicity [24]. In addition, upon release from the liver the pyrroles may also affect the endothelium of blood vessels in the liver or lungs.

Hydrolysis of the ester groups of PAs by esterases and formation and excretion of water soluble *N*-oxides are detoxification mechanisms, although for some PAs *N*-oxidation may represent a bioactivation pathway [32 – 34]. Conjugation by cellular nucleophiles including especially glutathione is an important detoxification pathway for the pyrrolic dehydro-alkaloid metabolites ([32, 33] and references therein).

2.2.3 Polymorphisms of influence

Sheep, Guinea pigs, gerbils, rabbits, hamsters, and Japanese quail are highly resistant to pyrrolizidine alkaloid toxicity, whereas rats, cattle, horses, and chickens are highly susceptible [32]. These species dependent differences in sensitivity towards pyrrolizidine alkaloids have been related to species dependent differences in the conversion of the PAs to their pyrrole metabolites, although species dependent differences in detoxification mechanisms may also play a role [32, 34]. Metabolism of PAs to dehydropyrrolizidines is catalyzed by especially CYP3A and CYP2B6 isoenzymes [34]. Life style factors and genetic polymorphisms known to occur in the CYP3A family may play a role in interindividual differences in sensitivity towards PAs. This includes for example induction of CYP3A4, the most important drug-metabolising CYP in human liver, by drugs like rifampicin, dexamethasone, and phenobarbital.

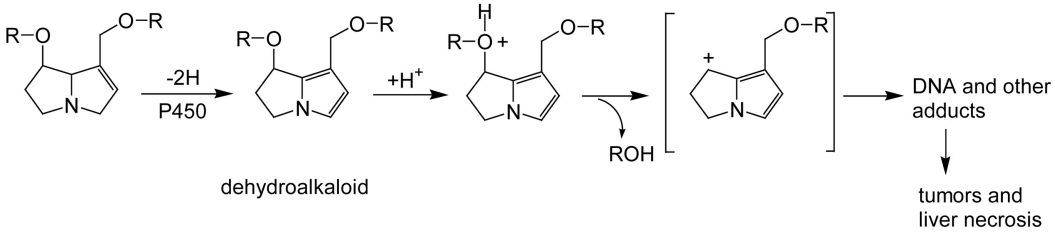


Figure 4. Metabolic activation of pyrrolizidine alkaloids to pyrrolic dehydro-alkaloids, proceeding by an initial hydroxylation of the unsaturated pyrrolizidine ring adjacent to the nitrogen atom by cytochromes P450, leading to an unstable intermediate that decomposes to give the pyrrolic dehydro-alkaloid product that reacts further to an alkylating intermediate [24].

Detoxification of the PAs to the corresponding *N*-oxides is catalyzed by CYPs, including CYP3A, and flavin containing monooxygenase [34], and detoxification of the pyrrolic metabolites by glutathione conjugation is catalyzed by glutathione *S*-transferases (GSTs). The human CYP and GST isoenzymes involved in these detoxification pathways largely remain to be elucidated. Elucidation of the CYPs and of the GST isoenzymes involved in the detoxification pathways of PAs is required to conclude what other life style factors and genetic polymorphisms are likely to influence the interindividual differences in sensitivity.

2.2.4 Concluding remarks

An intake of 1 mg PAs/day, the lowest dose reported to cause VOD in a human, would be reached upon intake of 0.7 g of a herb-based preparation that contains 1520 mg/kg. This is an amount that may be found in capsules on the market, indicating that such a product would be too toxic to consume on a regular basis. On the other hand products with no detectable PAs are also encountered. Together this indicates that regulatory actions are required until appropriate quality control procedures are in place.

2.3 β-Carotene

2.3.1 Major characteristics, occurrence, and intake

In industrialized countries, fruits and vegetables provide an estimated 1.7 – 3 mg/day of pro-vitamin A carotenoids, of which β-carotene is the principal component [35]. Other sources of β-carotene include food additives (1 – 2 mg/person/day) and supplements [35]. Carotenoids, including β-carotene and others, possess antioxidant and radical-scavenging ability [36 – 39]. However, experimental studies with β-carotene at present provide perhaps the best example of unexpected health risks related to increased intake levels of a bioactive plant ingredient. Observational epidemiologic studies indicate that diets high in carotenoid-rich fruits and vegetables as well as increased serum levels of β-carotene are associated with a decreased risk of lung cancer [40 – 42]. Based on these observations large human intervention trials with heavy smokers receiving β-carotene supplements were undertaken [43, 44]. The studies reported increased, instead of decreased, levels of lung cancer incidence in the population of heavy smokers receiving β-carotene supplements for several years. Similar to the effects of β-carotene on lung cancer risk in heavy smokers, an increased lung cancer risk due to β-carotene supplementation in asbestos-exposed workers was also reported [43]. More recently, Baron *et al*. [45] reported increased risk on colon cancer in cigarette smokers with a high intake of β-carotene.

2.3.2 Mechanism of toxic action

The mechanism by which β-carotene increases lung cancer risk in both heavy smokers and asbestos workers is at present unclear, although some hypotheses and initial results have been reported. One possible mechanism is a co-carcinogenic effect of β-carotene mediated through a stimulating effect of β-carotene on phase I bioactivating enzymes. Induction of CYP activity by β-carotene, in particular of CYP1A1 and CYP1A2 (activating aromatic amines, polychlorinated biphenyls, dioxins, and polyaromatic hydrocarbons (PAHs)), CYP2A (activating butadiene, hexamethyl phosphoramide, and nitrosamines), CYP2B1 (activating olefins and halogenated hydrocarbons), and CYP3A (activating aflatoxins, 1-nitropyrene, and PAHs), may result in increased formation of genotoxic metabolites of, amongst others, cigarette smoke constituents [35]. Another possible explanation suggests that high-dose β-carotene supplementation may enhance lung tumorigenesis in smokers by altering retinoid signalling. This may proceed through the formation of reactive oxidative cleavage products of β-carotene that are able to interfere with normal retinoid signalling. The high oxygen pressure in the lungs may favor this oxidative degradation of β-carotene [46]. In addition, the interaction between reactive oxygen species, derived from tobacco smoke or induced in the lung upon asbestos exposure, may result in β-carotene oxidation which could lead to these toxic β-carotene metabolites [47 – 50]. Reduction of retinoid signalling could also occur after induction of CYPs, CYP1A1, or CYP1A2 in particular, by cigarette smoke and high doses of β-carotene, resulting in enhanced retinoic acid catabolism in the lung [51]. A hypothesis explaining how disturbed retinoid signalling may result in the increased lung tumor risk is presented in Fig. 5. Alterations in retinoid signalling could result in reduced retinoid levels and suppression of RARβ gene expression, the latter representing a tumor suppressor gene [35, 48 – 51]. Furthermore, the whole process may induce increased expression of c-jun and c-fos genes resulting in higher levels of activator protein-1 (AP-1). Increased expression of c-Jun and c-Fos proteins has been reported for several mitogenic stimuli and tumor-promoting agents, and has been observed in tobacco-smoke exposed ferrets supplemented with high-dose β-carotene [50, 51].

Of importance to note is that these tumor-promoting effects of β-carotene are especially observed upon high-dose supplementation in heavy smokers. β-Carotene does not exert this tumor risk enhancing effect in former smokers [43]. In asbestos workers β-carotene oxidation may be stimulated by the inflammatory process known to be induced in asbestos-exposed lungs [47]. Inflammatory cells isolated from nonsmokers with asbestosis are known to release significantly increased amounts of reactive oxygen species compared to cells recovered from control individuals [52].

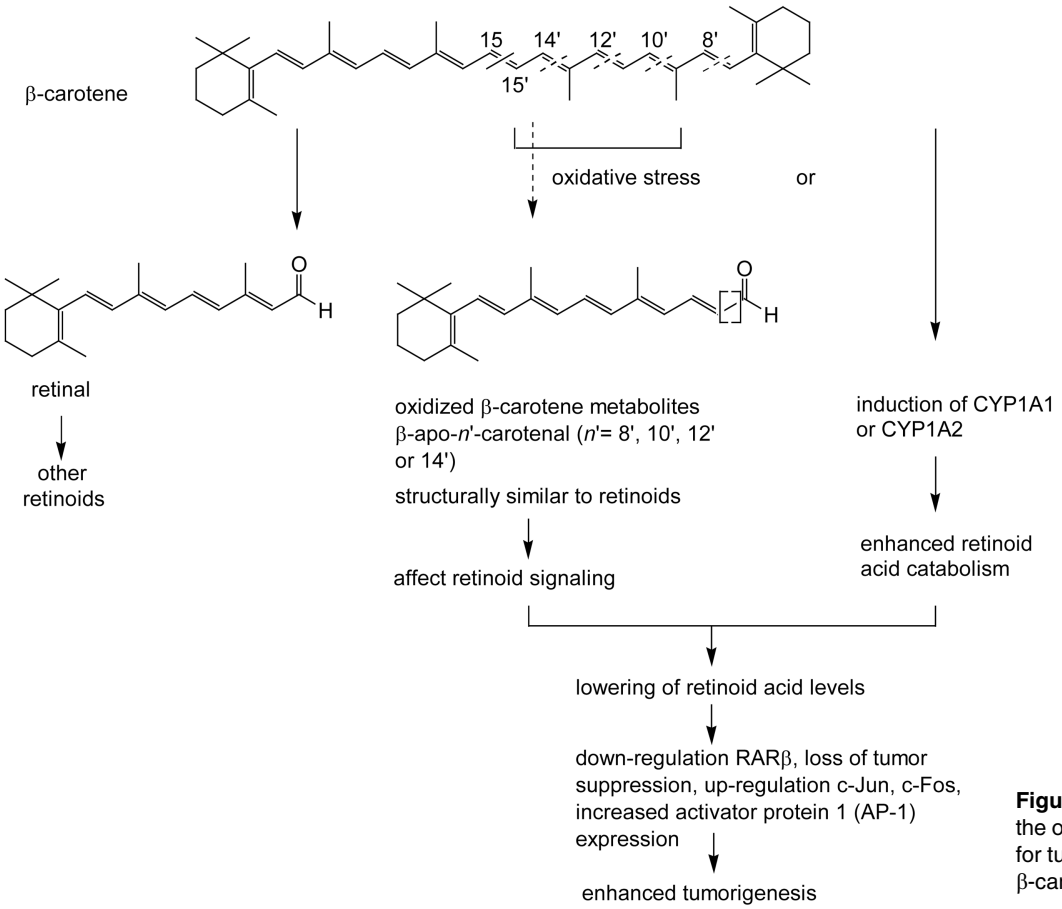


Figure 5. Hypotheses for the oxidative mechanism for tumor enhancement by β-carotene [46 – 51].

2.3.3 Polymorphisms of influence

Depending on the actual mechanism underlying the adverse effect of β-carotene in heavy smokers, different polymorphisms may be of influence. When the induction of CYPs involved in bioactivation of pro-carcinogens proves to be an important mechanism underlying the adverse effect in smokers, genetic polymorphisms in CYP1A1, CYP1A2, CYP2A, CYP2B1, and CYP3A may be of relevance. A role for increased retinoid acid catabolism has been related to the activity of especially CYP1A1 or CYP1A2. This implies that genetic polymorphisms modifying the activity of these two CYPs may influence the sensitivity of heavy smokers to the adverse effects of β-carotene.

2.3.4 Concluding remarks

Recently the Scientific Committee on Food (SCF) concluded that there may be a very small difference between the levels of β-carotene that may confer health benefits (up to 10 mg/day from especially natural sources), and those that may produce adverse effects in smokers (20 mg/day) [35]. Therefore, β-carotene suppletion should be regarded with caution. Furthermore, the role of other carotenoids in the reported association between reduced incidence of lung cancer and increased intake of vegetables and fruits rich in carotenoids remains to be elucidated [35]. The SCF also concluded that the possibility that some of the oxidative cleavage products of β-carotene could interfere with retinoic acid homeostasis requires further investigation [35].

The possible role of reduced retinoid acid levels in the mechanism of β-carotene-mediated enhancement of cigarette smoke-induced lung cancer has led some investigators to the conclusion that perhaps restoration of lung retinoic acid homeostasis by retinoic acid supplementation or by inhibition of CYP-enhanced retinoid acid catabolism can have chemopreventive effects against lung carcinogenesis [51]. This is an interesting hypothesis that needs further study.

2.4 Coumarin

2.4.1 Major characteristics, occurrence, and intake

Coumarin is a constituent of cinnamon, an important food flavor. Coumarin is naturally found at high levels in some essential oils, such as cinnamon bark oil (7000 mg/kg), cinnamon leaf oil (40 600 mg/kg), cassia leaf oil (up to 83 300

mg/kg), and lavender and peppermint oil (20 mg/kg) [53]. It is also found in some fruits (bilberry: 0.0005 mg/kg), green tea (1.2 – 1.7 mg/kg) [53], and other foods, such as chicory, honey cinnamon cake, and in "speculaas", a Dutch sweet spicey biscuit (35 mg/kg) [54]. The average human exposure to coumarin from the diet and from fragrance use in cosmetic products is about 0.06 (*i. e.*, 0.02 + 0.04) mg/kg bw/day [54, 55].

The use of coumarin as a food flavor was discontinued as a result of the finding of hepatotoxic effects in rats and dogs fed with coumarin in the diet [54 – 56]. Coumarin was banned in the USA in 1954 based on reports of hepatotoxicity in rats, prior to the existence of any carcinogenicity and mutagenicity data, and was recommended for withdrawal from use in the UK in 1965. In 1999 the EU Scientific Committee on Food (EU-SCF) listed coumarin as an "active principle" and set the maximum permitted concentration for coumarin in food and alcoholic beverages at 2 mg/kg [54]. Enforcement of this concentration means the withdrawal of many products from the market.

2.4.2 Mechanism of toxic action

Pharmacokinetic studies in humans have demonstrated that coumarin is completely absorbed from the gastrointestinal tract after oral administration and extensively converted by first pass metabolism in the liver, with only between 2 and 6% reaching the systemic circulation intact [55]. In the rat, a relatively large amount is excreted *via* the bile. Thus, an appreciable proportion of the dose is excreted in the feces. The urine appears to be the major route of excretion in humans. Based on animal data, the EU-SCF [54] concluded that coumarin is a carcinogen *via* the oral route, resulting in adenomas and carcinomas of the liver and bile ducts and adenomas of the kidney in rats, and in adenomas and carcinomas of the lung and adenomas of the liver in mice. The major characteristics of coumarin metabolism are presented in Fig. 6. The pathway leading to 7-hydroxylation is considered a detoxification pathway and the pathway leading to formation of a coumarin 3,4-epoxide intermediate is the toxic bioactivating route. A number of studies have demonstrated that both acute and chronic coumarin-induced liver injury in the rat appears to be due to the presence of the 3,4-double bond and that the first step in coumarin bioactivation involves the CYP-dependent formation of a 3,4-epoxide intermediate [54 – 56]. A key issue in the recent risk assessment of coumarin has been the question of whether or not coumarin is genotoxic. Recently, the European Food Safety Authority (EFSA) re-evaluated coumarin and concluded that data on the absence of DNA adduct formation in kidney and liver of coumarin-exposed rats indicate that coumarin does not bind covalently to DNA *in vivo*. These results suggest that coumarin induces tumors *via* a mechanism of action that is preceeded by toxicity in the target

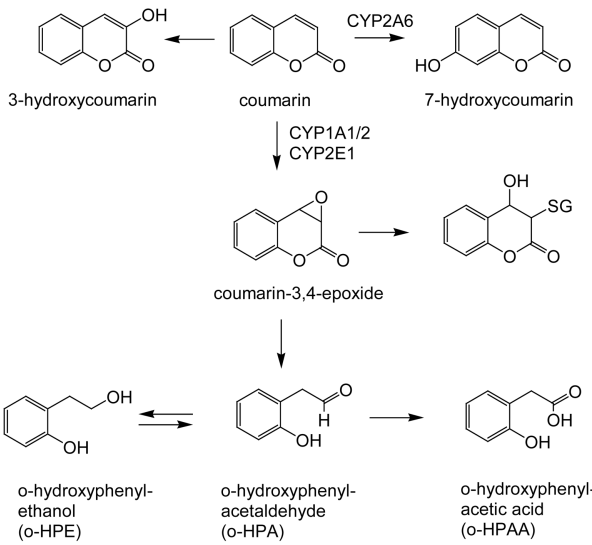


Figure 6. Biotransformation reactions of coumarin leading to detoxification (7-hydroxylation and 3-hydroxylation) or metabolic activation *via* the coumarin 3,4-epoxide pathway [55]. Formation of coumarin 3,4-epoxide is catalyzed by especially CYP2E1, and, to a lower extent, by CYP1A1 and CYP1A2 [62]. 7-Hydroxylation is catalyzed by CYP2A6 [55]. Coumarin 3,4-epoxide can either rearrange spontaneously to *o*-hydroxyphenylacetaldehyde (*o*-HPA) or conjugate with glutathione, the latter either chemically or catalyzed by GST-α or GST-μ, but not GST-π enzymes [57]. *o*-HPA is hepatotoxic [60] and is further converted by oxidation to *o*-hydroxyphenylacetic acid (*o*-HPAA) or by reduction to *o*-hydroxyphenylethanol (*o*-HPE).

organ and that this will be reflected in a dose-response curve with a threshold reflecting a no adverse effect level [53]. This conclusion was corroborated by the facts that coumarin does not induce unscheduled DNA synthesis in male SD rat hepatocytes [57] and that coumarin was negative in an *in vivo* micronucleus assay in mice [58]. Based on this threshold type dose-response curve for tumor formation, EFSA suggested a tolerable daily intake (TDI) for coumarin of 0 – 0.1 mg/kg bw/day [53]. The mechanism of this coumarin toxicity remains to be established, but metabolites down the 3,4-epoxidation pathway could play a role [53 – 56, 59]. Coumarin 3,4-epoxide can either rearrange spontaneously to *o*-hydroxyphenylacetaldehyde (*o*-HPA) or conjugate with glutathione, the latter either chemically or catalyzed by GST-α or GST-μ, but not GST-π enzymes (Fig. 6) [59]. *o*-HPA is hepatotoxic [60] and is further converted by oxidation to *o*-hydroxyphenylacetic acid (*o*-HPAA) or by reduction to *o*-hydroxyphenylethanol (*o*-HPE) (Fig. 6) [55, 59]. Oxidation to *o*-HPAA is considered a detoxification step coupled to urinary excretion, whereas reduction to *o*-HPE is followed by oxidation back to *o*-HPA thereby contributing to slower hepatic clearance of the hepatotoxic *o*-HPA [59]. Detoxification of *o*-HPA to *o*-HPAA may even be the major determinant of species differences in coumarin-induced hepatotoxicity [59].

2.4.3 Polymorphisms of influence

The extent of coumarin 7-hydroxylation appears to be species rather than dose-dependent [55]. The major pathway of coumarin metabolism in the rat is 3,4-epoxidation, 7-hydroxylation being a minor route. The 3,4-epoxidation is also the major route of coumarin metabolism in the mouse, although major sex and strain differences exist. It was demonstrated that coumarin was more toxic for C3H/HeJ than for DBA/2J mice [61]. This might be explained by the fact that DBA/2J strain mice have higher hepatic coumarin 7-hydroxylation than C3H/HeJ strain mice.

Unlike the rat and the mouse, where the 3,4-epoxidation pathway predominates, the major pathway of coumarin metabolism in humans is 7-hydroxylation. This might explain why there is little evidence of coumarin-induced toxicity in humans given therapeutic doses of coumarin that are up to 1900 times higher than those obtained from dietary sources and from fragrances used in cosmetic products. In the majority of human subjects studied, coumarin is extensively metabolized in the liver to 7-hydroxycoumarin by CYP2A6, although humans can also metabolize coumarin by 3,4-epoxidation [54 – 56, 62]. Studies using rat and human recombinant CYP enzymes have pointed at the formation of coumarin 3,4-epoxide by especially CYP2E1, and, to a lower extent, by CYP1A1 and CYP1A2 in both species [62].

There appears to be a marked interindividual variation in coumarin metabolism to 7-hydroxycoumarin in humans due to a genetic polymorphism which exists in human CYP2A6 [55, 63, 64]. The role of CYP2A6 polymorphism in human risk profiles for coumarin remains to be elucidated. At present it is still unknown which pathway takes over the metabolism of coumarin in individuals with the CYP2A6 deficiency. It cannot be ruled out that the pathway taking over is 3,4-epoxidation, also because CYPs other than CYP2A6, such as CYP2A13, have been reported to be able to catalyse both coumarin 7-hydroxylation but also the formation of metabolites representative for the 3,4-epoxide route, both to a comparable extent [65].

Recently, the CYP2A6 genotype and development of hepatotoxicity in patients who were dosed with 90 mg coumarin/day have been evaluated. From 231 patients 16 appeared to be defective for the CYP2A6 genotype, being heterozygous for the CYP2A6*2 allele that leads to an inactive protein. Of the nine patients showing evidence of hepatotoxicity only one had the variant allele, eight being wild-type homozygotes [66]. This result indicates that a single copy of a variant CYP2A6 allele does not confer susceptibility to liver dysfunction in patients treated with coumarin [66]. Since the conversion of *o*-HPA to *o*-HPAA is catalyzed by aldehyde dehydrogenase, polymorphisms known to occur in this enzyme could contribute to interindividual differences in sensitivity toward coumarin induced toxicity, although this remains to be demonstrated.

2.4.4 Concluding remarks

Recently, the EFSA re-evaluated coumarin and concluded that coumarin induces tumors *via* a mechanism of action that is preceeded by toxicity in the target organ [53]. Based on this, EFSA suggested a TDI for coumarin of 0.1 mg/kg bw/day [53]. The estimated theoretical maximum daily intake of coumarin *via* food is 4.085 mg/day (0.07 mg/kg bw/day) or lower (1.3 – 1.5 mg/day which equals 0.02 mg/kg bw/day), given a more realistic intake scenario [53, 55]. These intake scenarios are below the TDI, suggesting that withdrawal of products from the market would no longer be an issue.

2.5 Alkenylbenzenes: safrole, methyleugenol, and estragole

The group of alkenylbenzenes includes compounds like safrole, methyleugenol, and estragole (Fig. 7), that are important constituents of herbs like nutmeg, cinnamon, anise star, tarragon, sweet basil, sweet fennel, and anise vert. The EU-SCF has launched scientific evaluations on these three alkenylbenzenes [67-69]. The EU-SCF concluded that safrole, methyleugenol, and estragole are genotoxic and carcinogenic, and indicated restrictions in use. Recently, however, an industrial expert panel from the Flavor and Extract Manufacturers Association (FEMA) published that exposure to methyleugenol and estragole, resulting from spice consumption, does not pose a significant cancer risk for humans [70]. The mechanistic argument underlying this conclusion relates to insight in the toxicokinetics and the mechanism of genotoxicity of the alkenylbenzenes.

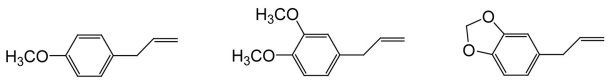


Figure 7. Structure of the alkenylbenzenes safrole, methyleugenol, and estragole.

2.5.1 Estragole

2.5.1.1 Major characteristics, occurrence, and intake

Estragole occurs naturally in a variety of foods including tarragon (60 – 70% of essential oil), sweet basil (20 – 43% of essential oil), sweet fennel (5 – 20% of essential oil), anis vert (1% of essential oil), and anis star (5 – 6% of essential oil [69]. There are several food categories to which estragole could be added. For alcoholic beverages, canned fish, and fats and oils estragol levels may amount to 100 mg/kg (approximately 4% of the market share), 50 mg/kg (approximately 30% of the market share), and 250 mg/kg (approximately 1 % of the market share), respectively [69].

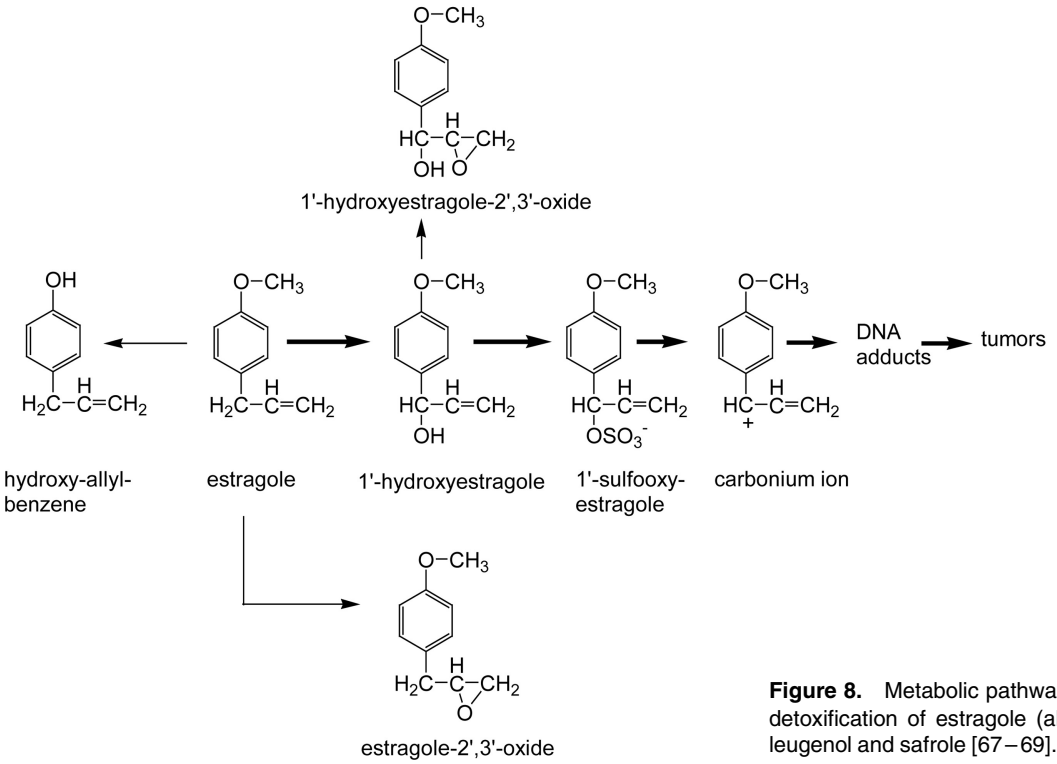


Figure 8. Metabolic pathways for bioactivation and detoxification of estragole (also relevant for methyleugenol and safrole [67 – 69].

Based on these assumptions, the average daily intake from food was estimated to amount to 4.3 mg/person/day and the 97th percentile to 8.7 mg/person/day [69].

2.5.1.2 Mechanism of toxic action

Figure 8 presents an overview of the relevant metabolic pathways of estragole, which is also representative for the metabolic profiles of methyleugenol and safrole. The CYP-derived metabolite 1′-hydroxyestragole is the putative proximate carcinogen of estragole. It has been found in the urine of men dosed with 1 μg estragole/kg bw [71]. The ultimate electrophilic and carcinogenic metabolite of estragole is formed as a result of sulfotransferases converting 1′-hydroxyestragole to 1′-sulfooxyestragole [72]. Another major metabolic pathway of estragole in rats, mice and humans includes *O*-demethylation. At higher doses the proportion of *O*-demethylation falls and the pathway leading to formation of 1′-hydroxyestragole increases (from 1.3 – 5.4% of the dose in the range of 0.05 – 50 mg/kg bw to 11.4 – 13.7% in the dose range of 500 – 1000 mg/kg bw for rats and mice) [73, 74]. Thus, it appears that the 1′-hydroxylation pathway is more prominent at higher levels of exposure. The FEMA USA Expert Panel even concluded that at low dose (100 μg/person which amounts to 1.5 μg/kg bw), human production of the 1′-hydroxy metabolite is expected to be very low given that urinary excretion of the 1′-hydroxy metabolite is below 0.5% of the dose administered. This relative decrease in conversion to the proximate carcinogenic metabolite at lower doses, was an important argument for the FEMA USA Expert Panel to conclude that exposure to methyleugenol and estragole resulting from spice consumption does not pose a significant cancer risk for humans [70].

In addition to formation of 1′-hydroxyestragole, formation of estragole-2′,3′-oxide and 1′-hydroxyestragole-2′,3′-oxide (Fig. 8) provide possible additional bioactivation pathways of estragole [75]. The estragole metabolites estragole-2′,3′-oxide and 1′-hydroxyestragole-2′,3′-oxide have been shown to be hepatocarcinogenic [72, 76] and to produce DNA adducts *in vitro* [77 – 79]. However, adducts of these 2′,3′-oxides were not among the major adducts found in mouse liver following *in vivo* administration of estragole [79]. Therefore, it is concluded that they do not contribute significantly to the genotoxicity of estragole. The apparent absence of a role for estragole epoxidation in the genotoxity of estragole has been ascribed to very rapid and efficient detoxification of the 2′,3′-oxides in the cell by a combination of epoxide hydrolases and GSTs [75].

2.5.1.3 Polymorphisms of influence

Human CYPs involved in the bioactivation of estragole to 1′-hydroxyestragole have not been identified. They may be in line with CYPs found to be involved in bioactivation of the related alkenylbenzenes methyleugenol and safrole, discussed below.

Glucuronidation of 1′-hydroxyestragole catalysed by uridine diphosphate glucuronosyltransferase (UGT) isoenzymes is a detoxification pathway and was recently shown to be catalyzed by human UGT2B7, UGT1A9, and UGT2B15 [80]. The UGT2B7 polymorphisms, leading to slow glucuronidators, may potentially lead to differences in toxicity of estragole. Life style factors, like concomitant chronic intake of therapeutic drugs and dietary components which increase the levels of UGT2B7 expression or which are substrates for UGT2B1 or UGT1A9, might also modify the relative risk [80]. Human CYPs of relevance for the detoxification pathways, and the type of sulfotransferases involved in the bioactivation to the ultimate electrophilic and carcinogenic metabolite 1′-sulfooxyestragole remain to be elucidated.

2.5.1.4 Concluding remarks

The EU-SCF concluded that estragole is genotoxic and carcinogenic, and indicated restrictions in use [74]. This will result in restrictions in use for the compound itself, but not for estragole containing herb extracts. Improved risk extrapolation from high-dose animal experiments to low-dose carcinogenic risks in man, taking into account the toxicokinetics of the CYPs responsible for the different biotransformation pathways, seems to be a prerequisite for future improvement of risk estimates for this alkenylbenzene.

2.5.2 Methyleugenol

2.5.2.1 Major characteristics, occurrence, and intake

Methyleugenol is a natural constituent of a number of plants, including nutmeg, pimento, lemongrass, tarragon, basil, star anise, and fennel [68]. The compound is also used as a flavoring agent in jellies, baked goods, nonalcoholic beverages, chewing gums, relish, and ice cream, and as a fragrance in several cosmetic products [68]. Intake estimates may vary widely because of lack of data about the concentration of the chemical in foodstuffs [68]. Intake estimates reported by the EU-SCF amount to an average intake for consumers of 13 mg/person/day and a 97th percentile of 36 mg/person/day [68].

2.5.2.2 Mechanism of toxic action

The metabolism and metabolic activation of methyleugenol proceeds similar to that depicted in Fig. 8 for estragole. Methyleugenol and its proximate carcinogenic metabolite 1′-hydroxymethyleugenol induce liver tumors in mice and rats [76, 81]. In addition, especially at higher doses, neuroendocrine tumors of the glandular stomach, as well as renal tube hyperplasia and adenomas were observed. *In vitro*, methyleugenol as well as its metabolites 1′-hydroxymethyleugenol and methyleugenol-2′,3′-oxide induce unscheduled DNA synthesis (UDS) in cultured rat hepatocytes [82]. Howes *et al.* [83] reported an excellent correlation between UDS induction in rat hepatocytes and results from rodent carcinogenicity studies for methyleugenol and also for related alkenylbenzenes like estragole and safrole.

In addition, methyleugenol has been shown to form adducts with DNA and protein in human fibroblasts V79 cells transfected with human genes expressing sulfotransferases and in the mouse liver *in vivo* [84 – 86] . The adduct formation with methyleugenol (72.7 pmol/mg DNA) was higher than that induced by estragole (30.0 pmol/mg DNA) or safrole (14.7 pmol/mg DNA) [86]. This order is in line with the relative differences in dose regimens required to induce tumors in animal studies, known to decrease in the order safrole > estragole > methyleugenol [67 – 69]. These observations, together with the fact that estimated daily intakes of methyleugenol (13 mg/person/day) [68] are higher than those estimated for estragole (4.3 mg/person/day) [69] and safrole (0.3 mg/person/day) [67], seem to put the priority for risk management on methyleugenol.

2.5.2.3 Polymorphisms of influence

Recent studies revealed human CYP1A2 and CYP2C9 to be important isoenzymes in the conversion of methyleugenol to 1′-hydroxymethyleugenol, with CYP2D6 and CYP2C19 perhaps also involved in methyleugenol 1′-hydroxylation [87]. The interindividual differences found in 15 human liver microsomes were larger (5-fold difference) than the interspecies and sex differences found in the incubations with microsomes prepared from pooled livers of male and female rats, mice, and humans (2-fold difference) [87]. Therefore, interindividual differences in methyleugenol 1′-hydroxylation seem to be at least as important as interspecies differences between rodents and humans. In particular people that smoke (induction of CYP1A2), use barbiturates (induction of 2C9), or have polymorphisms especially in the CYP2D6 gene leading to ultra rapid metabolizer phenotypes might have a higher methyleugenol 1′-hydroxylation rate. These groups of people might be at higher risk of the adverse effects of exposure to methyleugenol. Polymorphisms in CYP2C9 and CYP2D6 leading to poor metabolizer phenotypes may reduce the relative risk.

2.5.2.4 Concluding remarks

As for estragole, methyleugenol may be converted to a variety of metabolites with different toxicological impact. Identification of the biotransformation enzymes involved in the various bioactivation and detoxification pathways and the implementation of their toxicokinetics into risk assessment models seems to be required for improved methods for extrapolation from high-dose animal experiments to low-dose carcinogenic risks in man.

2.5.3 Safrole

2.5.3.1 Major characteristics, occurrence and intake

Similar to estragole and methyleugenol, safrole is a natural constituent of a number of spices, such as nutmeg, mace, cinnamon, anise, black pepper, and sweet basil. The most important dietary sources are nutmeg, mace, and their essential oils. Safrole is also present in cola drinks. Safrole was the first of the class of alkenylbenzenes shown to have carcinogenic properties [88]. An exposure assessment was made based on a selection of 28 food categories assuming a concentration of 0.5 mg safrole/kg for food in general, a concentration of 2 mg/kg for food containing cinnamon and of 5 mg/kg for food containing nutmeg. For beverages, canned fish and chewing gum the following concentrations were specified: beverages 5 mg/kg (4% of the market share), canned fish 20 mg/kg (30% of the market share), and chewing gum 10 mg/kg (2% of the market share). Using these assumptions the estimated average intake for consumers was calculated to amount to 0.3 mg/person/day and the 97th percentile to 0.5 mg/person/day [67].

2.5.3.2 Mechanism of toxic action

Chronic administration of safrole in the diet at 0.5 – 1.0% for a year or more caused liver tumors in adult mice and rats [72, 76, 89, 90]. The carcinogenicity of safrole metabolites, namely 1′-hydroxysafrole, safrole-2′,3′-oxide and 1′-hydroxysafrole-2′,3′-oxide was also clearly demonstrated [72, 76]. The main metabolic pathways are allylic hydroxylation to 1′-hydroxysafrole and oxidation and *O*-dealkylation, the latter leading to 4-allylcatechol that is easily oxidised to 4-allyl-*o*-quinone (Fig. 9), and epoxidation of the allylic side chain or the aromatic ring. 1′-Hydroxysafrole and 4-allylcatechol represent the main metabolites. 1′-Hydroxysafrole can be conjugated by sulfotransferases giving rise to a conjugate that can easily split producing the ultimate carcinogenic electrophilic carbonium ion (Fig. 8). In addition oxidation of 4-allylcatechol to 4-allyl-*o*-quinone may also generate an electrophilic toxic metabolite [91] (Fig. 9).

Safrole-DNA adducts were identified in livers of mice given cola beverages instead of drinking water [92]. Inhibition of both DNA adduct formation and carcinogenicity of

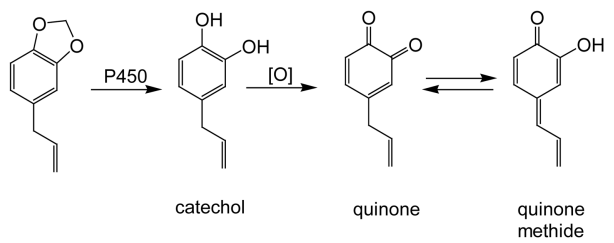


Figure 9. Alternative bioactivation pathway for safrol leading to formation of catechol and quinone-type metabolites [91].

1′-hydroxysafrole was shown in the liver of mice deficient in synthesis of 3′-phosphoadenosine 5′-phosphosulfide (PAPS) the cofactor required for sulfotransferase reactions, or mice treated with pentachlorophenol, a strong inhibitor of sulfotransferases [93].

2.5.3.3 Polymorphisms of influence

The CYP enzymes involved in the bioactivation of safrole to its proximate carcinogen 1′-hydroxysafrole in man were identified to be CYP2C9, CYP2A6, CYP2D6, and CYP2E1 [94, 95]. Data from Gentest microsomes in which the activities towards enzyme-selective substrates are considered to be in the same order as the mean activities found in human liver microsomes, reveal CYP2A6 to contribute about two times more than the other CYPs that are active in safrole 1′-hydroxylation [94]. Because CYP2C9, CYP2A6 and CYP2D6 are polymorphic [96], the bioactivation of safrole to 1′-hydroxysafrole in man is expected to be influenced by polymorphisms in these CYPs. Polymorphisms in CYP2C9, CYP2A6, and CYP2D6, leading to poor metabolizer phenotypes, may reduce the relative risk on the harmful effects of safrole, whereas life style factors like the use of alcohol, an inducer of CYP2E1 and barbiturates, inducers of CYP2C9 and polymorphisms in CYP2D6 and CYP2A6 leading to ultrarapid metabolizer phenotypes may increase the relative risk.

2.5.3.4 Concluding remarks

As for estragole and methyleugenol, safrole may be converted to a variety of metabolites with different toxicological impact. Identification of the biotransformation enzymes involved in the various bioactivation and detoxification pathways and the implementation of their toxicokinetics into risk assessment models seem to be required for improved methods for extrapolation from high-dose animal experiments to low-dose carcinogenic risks in man.

2.6 Ephedrine alkaloids

2.6.1 Major characteristics, occurrence, and intake

Herbs like *Ephedra sinica*, *Ephedra intermedia,* and *Ephedra equisatine*, also known by their Chinese name "Ma Huang", contain so-called ephedrine alkaloids, among which ephedrine (Fig. 10) is the dominant one. Other ephedrine alkaloids present include pseudo-ephedrine, norephedrine, methylephedrine, methylpseudo-ephedrine, and norpseudo-ephedrine. Certain dietary supplements also include ephedra, with the most popular uses being for improvement of weight loss and athletic performance [97]. Ephedrine, pseudo-ephedrine, and related alkaloids are found in the herb Ma Huang at levels usually up to around 0.5 – 2.5% in total [98]. In Ma Huang sold as a powdered

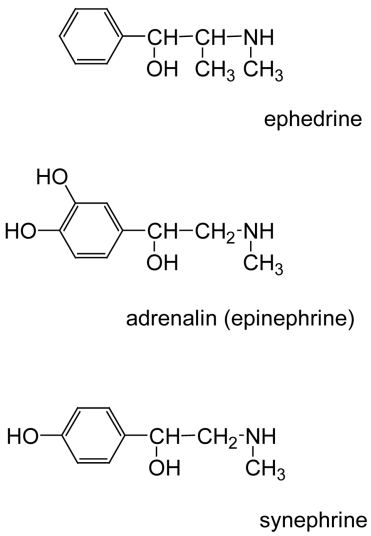


Figure 10. Structure of ephedrine, the major ephedrine alkaloid in ephedra, of adrenalin, the neurotransmitter towards which ephedrine acts an an agonist, and of synephrine another adrenalin agonist.

herb as well as in standardized extracts, total alkaloid levels may range from 6 – 8%, with one product even containing 12% [99]. Of the products tested by FDA, the mean contained 21.4 mg per dose [99].

2.6.2 Mechanism of toxic action

The chemical structure of ephedrine resembles that of the neurotransmitter adrenalin (= epinephrine) (Fig. 10). The mechanism of action of ephedrine alkaloids is based on this structural similarity. As adrenalin agonists, these alkaloids produce a sympathomimetic response, characterized by increased heart rhythm, hypertension (elevated blood pressure), and central nervous system stimulation [100 – 102]. Doses higher than 50 mg/day/adult may cause heart palpitations, nausea, dizziness, headache, sweating, neuropathy (nerve damage), and tremors. The stimulating effect on the central nervous system can also result in loss of appetite, insomnia, nervousness, seizures, and euphoria. At doses above 500 – 1000 mg ephedrine the effects observed are nausea, vomiting, fever, phsychoses, spasms, convulsions, respiratory disorders, coma, heart attack, and death. A dose of 2000 mg/adult is considered lethal. Chronic exposure to ephedrines may cause behavioral disturbances and psychoses. Several case reports on the toxicity of ephedrine-containing herb preparations have been described, some of them with fatal outcome [97].

Exposure to ephedrine, which is an adrenaline agonist, in combination with drugs known to inhibit monoamine oxidase (MAO) can cause an increased risk on adverse effects. This because the MAO inhibitors block the degradation of adrenalin by MAO, thereby increasing the adrenalin concentration and stimulating the adrenergic neurotransmitter system. Likewise coexposure with caffeine also has a synergistic effect on the action of ephedrine. Caffeine inhibits phosphodiesterase, the enzyme that hydrolyses cAMP, the second messenger of adrenalin-mediated signal transduction. This also stimulates cholinergic neurotransmission.

2.6.3 Polymorphisms of influence

Biodegradation of ephedrine alkaloids may proceed similar to that of adrenalin, by oxidative deamination catalyzed by MAO. Genetic polymorphisms of influence on ephedrine alkaloid toxicity may thus be found at the level of this biodegradation enzyme and/or at the level of the adrenergic receptors for which polymorphisms have been described [103].

2.6.4 Concluding remarks

Recently most countries installed a ban on the use of ephedrine alkaloids in food supplements or other foods. This rule seems to reflect the scientific evidence showing that ephedra poses an unacceptable risk to consumers health.

2.7 Synephrine

2.7.1 Major characteristics, occurrence, and intake

Synephrine is the main active principle found in the fruit of several Citrus species including *Citrus aurantum* and *Citrus reticulata* [104]. In traditional Chinese medicine the fruit is also known as Chih-shih. Synephrine occurs in all citrus products in very low concentrations (0.1 – 2.0%) with 0.25% representing an average value [105]. It is consumed by humans through a citrus fruit-containing diet.

2.7.2 Mechanism of toxic action

Synephrine is chemically very similar to ephedrine (Fig. 10). Both compounds act on the nervous system in a similar way [106, 107]. Synephrine is a drug in Europe (oxedrine; Sympatol) produced for use as a sympathomimeticum. It acts as a cardiac performance enhancer [108].

2.7.3 Polymorphisms of influence

See remarks made for ephedrine alkaloids.

2.7.4 Concluding remarks

After the FDA banned ephedra, diet-pill companies tried to find a possibly safer alternative turning to synephrine. Whether synephrine may act with potentially fewer side effects, like high blood pressure and increased heart rate, remains to be established. Consumers having high blood

pressure or other heart problems should better not use any of these substances.

2.8 Kavalactones

2.8.1 Major characteristics, occurrence, and intake

The rootstock of the kava (*Piper methysticum*) plant contains a mixture of lipid soluble α-pyrones, also called kava-pyrones or kavalactones. Pharmacologic activity of kava-kava has been related to six important kavalactones; kawain, 7,8-dihydrokawain, 5,6-dehydrokawain, methysticin, dihydromethysticin, and yangonin (Fig. 11). High-quality kava rhizomes contain 5.5 – 8.3% kavalactones. Medicinal extracts used in Europe contain 30 – 70% kavalactones.

2.8.2 Mechanism of toxic action

Extracts containing high concentrations of kavalactones, at three times 100 mg doses of kava extract standardized to 70% kavalactone content a day, are used for the treatment of anxiety and depression and are claimed to have a calming effect and induce a state of happiness [109, 110]. Through their action on the nervous system they exert sedative, analgesic, anticonvulsant, and muscle relaxant effects. The exact mechanism of action for this beneficial effect is not exactly known. Results from animal experiments suggest mechanisms that include inhibition of MAO activity, inhibition of noradrenalin reuptake in the presynaptic neuron, and/or action as a dopamine antagonist [111, 112]. The major toxic side effects of kava-kava are dermopathy [113 – 115] and liver toxicity [116 – 123]. Since 1999, cases of severe hepatic toxicity in people using kava-containing herbal products have been reported in Europe and the United States [120, 124], including several cases in which patients required liver transplantation following the use of kava-containing products [124]. Liver damage has been reported at intake levels of 60 – 120 mg kavalactones/day for as short as 14 days. Potential mechanisms underlying the liver toxicity have been related to glutathione depletion or quinone formation [125, 126]. Glutathione (**GSH**) was reported to bind with kavalactones by a Michael-type addition, resulting in opening of the lactone ring (Fig. 12 A) [125], and high doses of kavalactones may lead to rapid depletion of GSH followed by toxicity of the lactones to the GSH-depleted liver cells [125]. For kavalactones containing a methylenedioxyphenyl moiety, such as methysticin and dehydromethysticin, another mechanism may become relevant. CYP catalyzed *O*-dealkylation of the methylenedioxyphenyl moiety may generate a catechol moiety that may subsequently be oxidised to the corresponding electrophilic *o*-quinone (Fig. 12 B) [126]. The CYP isoenzyme(s) catalyzing this bioactivation have not been identified. Alternatively, it has been suggested that kava alkaloids, rather than

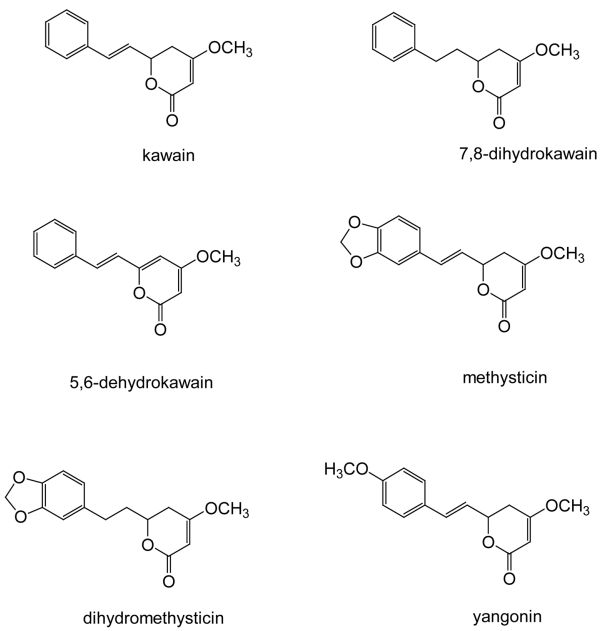


Figure 11. Structures of the major kavalactones from kava-kava.

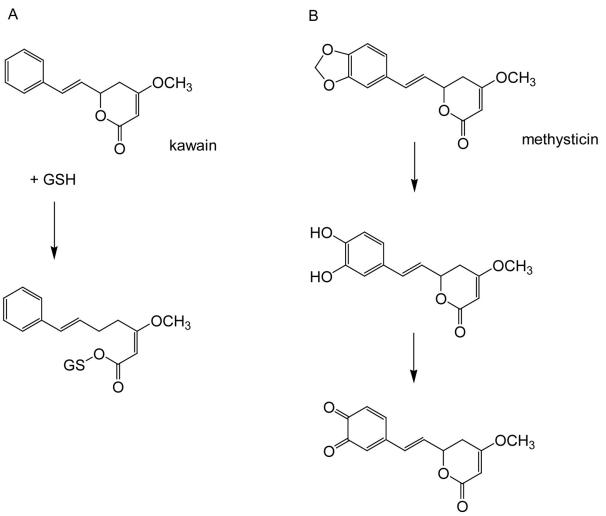


Figure 12. Reactions underlying possible mechanisms for kavalactone induced liver toxicity, including (A) glutathione depletion and (B) electrophilic quinone formation [125, 126].

kavalactones may be responsible for the hepatotoxicty [127].

2.8.3 Polymorphisms of influence

Phenotyping of CYP2D6 activity with debrisoquine in two patients who developed clinical symptoms upon kavalactone ingestion revealed that both were poor metabolizers of debrisoquine. Since the local prevalence of CYP2D6 deficiency was 9%, the probability that two consecutive

patients would be deficient was reported to be less than 0.01% and it was concluded that these data suggest that CYP2D6 deficiency is a risk factor for hepatotoxicity due to kavalactones [128].

2.8.4 Concluding remarks

Several case studies linking kava-kava to liver damage prompted several governments to remove kava-kava extracts from the market. Although it seems generally accepted that kava-kava can be an effective symptomatic treatment option for anxiety [110, 129], the present advices are that these herbal preparations should not be used until the mechanism for hepatotoxicity is clearly ascertained [129].

2.9 Anisatin

2.9.1 Major characteristics, occurrence, and intake

The spice Chinese star anise (*Illicium verum*) is used in many cultures, mostly for preparing tea. In Southern European countries (France, Spain) star anise is also used against intestinal complaints in children. Recently, health problems have been observed due to the use of herb tea containing star anise [130]. In September 2001, in the Netherlands, more than 60 persons showed nausea and vomiting, after drinking a herbal tea called starmix tea, 22 persons were hospitalized due to tonic-clonic insults [131]. EEGs showed epileptiform abnormalities indicating a diffuse cerebral disease [132]. The complaints were ascribed to a toxic star anise species comparable to Japanese star anise (*Illicium anisatum*) which was accidentally exchanged for the nontoxic Chinese staranise (*Illicium verum*) [131]. NMR analysis of this star anise species revealed the presence of anisatin. Anisatin is a sesquiterpenoid (Fig. 13) causing

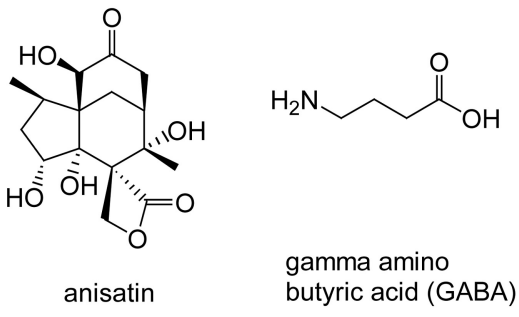


Figure 13. Structure of anisatin and of the neurotransmitter GABA for which anisatin is a competitive antagonist.

numerous other symptoms like lower heart beat and hallucinations. Because of the latter the Japanese star anise is also mentioned *Illicium religiosum*.

2.9.2 Mechanism of toxic action

Anisatin acts as a noncompetitive γ-amino butyric acid (GABA)-antagonist that can cause tonic-clonic insults [133]. GABA (Fig. 13) is the most common message-altering neurotransmitter in the brain and produces stop signals. It is known that abnormal levels of GABA unbalance the brain's message delivery system causing a seizure or epileptic attack. Most of the new developments of epilepsy drugs stem from the discovery of GABA.

2.9.3 Polymorphisms of influence

There is no information available.

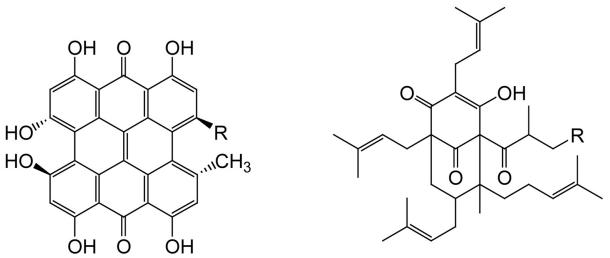
2.9.4 Concluding remarks

Illicium verum has been considered safe for consumption but it contains veranisatins in very low concentrations [134]. Taken the fact that relatively small quantities in infants may be sufficient to produce adverse neurologic reactions and the chances on possible adulteration of *Illicium verum* with *Illicium anisatum*, one could recommend against administering star anise to children [135].

2.10 St. John's wort

2.10.1 Major characteristics, occurrence, and intake

The herbaceous plant St. John's wort (*Hypericum perforatum)* is a member of the Hypericaceae family. The fresh plant contains up to 0.3% naphthodianthrones, including up to 0.09 % hypericin and 0.23% pseudohypericin (Fig. 14). The content of the phloroglucinol compound hyperforin (Fig. 14) ranges from 2.0–4.5% of the fresh plant. Adhyperforin (Fig. 14), another phloroglucinol compound, comprises 0.2–1.8% of the plant. Phloroglucinols are structurally related to the bitter substances in hops. The phloroglucinols and naphthodianthrones are mainly localised in the flowers and buds. Other constituents include flavonols



R = CH_3 = hypericin
R = CH_2OH = pseudohypericin
R = H = hyperforin
R = CH_3 = adhyperforin

Figure 14. Structures of the major active ingredients from St. John's wort.

(*e. g.*, kaempferol, quercetin), flavonoid glycosides (hyperoside, rutin), and biflavonoids (biapigenin) [136 – 139].

St. John's wort is widely used as a treatment for depression. In the United States most St. John's wort is used without medical supervision. Results of a meta-analysis study in 1996 concluded that hydroalcoholic extracts of St. John's wort were superior to placebo for treatment of mild to moderate depression [140]. Subsequent studies concluded that St. John's wort is comparable to amitryptiline, imipramine, and fluoxetine. However, questions have been raised about methodological limitations of these studies. A double-blind, randomized, placebo-controlled trial revealed no efficacy of St. John's wort in moderately severe major depression. In this 8-week parallel-group study 340 patients received daily doses of 900 – 1500 mg of the standardized St. John's wort extract LI-160, 50 – 100 mg of sertraline or placebo. Neither agent was superior to placebo on the two primary outcome measures for which the HAM-D total score and a combination of the HAM-D total score and the CGI score were used. Sertraline performed better than placebo on the secondary measure CGI-improvement scale [141]. Several studies indicate that St. John's wort extracts inhibit synaptosomal uptake of serotonin, dopamine, and noradrenalin. Results of an *in vitro* study indicated that hyperforin is responsible for the inhibition of serotonin uptake [142].

2.10.2 Mechanism of toxic action

Side effects of treatment with St. John's wort tend to be mild and include swelling, anorgasmia, and frequent urination [141]. Several cases of increased sensitivity to sunlight following use of St. John's wort were reported. These reactions, however, are rare and may be encountered following prolonged exposure at high or very high doses [139]. In a study on the antiviral effects of hypericin, in patients with chronic hepatitis C virus infections, 5 of 12 subjects receiving 0.05 mg/kg bw/day orally for 8 weeks and 6 of 7 subjects receiving 0.10 mg/kg bw/day developed phototoxic reactions. In both groups paresthesias were the most common reactions followed by dermatitis. In the high-dose group darkened coloration and pruritic nodules were also reported. Apart from these effects no other serious adverse events were reported [143]. Similar phototoxic reactions were found in a study when intravenous hypericin was given to human immunodeficiency virus (HIV)-infected patients [144]. One could hypothesize that indivuals with certain virus infections are more prone to develop phototoxic reactions to hypericin.

Of concern is the capability of St. John's wort to interact with certain drugs. In 1999, a review of available literature published in the Lancet [145] raised questions regarding the safety of St. John's wort when used concomitantly with various prescribed drugs. This review discussed eight cases of interactions between St. John's wort and concomitant medications. The drugs for which interactions of St. John's wort are of concern are drugs that are metabolized by hepatic CYP enzymes. This is because ingredients in St. John's wort induce specific CYP activities involved in drug inactivation. Because of CYP induction plasma concentrations of certain drugs are subtherapeutic [145]. Subsequently the European Agency for the Evaluation of Medicinal products issued a warning for this potential effect of St. John's wort [146].

2.10.3 Polymorphisms of influence

Recently the effect of St. John's wort on the activity of different CYP2C19 genotypes was studied by investigating mephenytoin pharmacokinetics in 12 healthy subjects by administering a single dose of the drug after two weeks of St. John's wort treatment [147]. Mephenytoin is almost exclusively metabolized by CYP2C19. Treatment with St. John's wort significantly increased mephenytoin metabolism in six wild-genotype CYP2C19*1/*1 subjects. The other six subjects which were homozygous for CYP2C19*2 or heterozygous for CYP2C19*2/*3 were found to be poor metabolizers of mephenytoin. In the poor metabolizers St. John's wort exerted no significant effect on mephenytoin metabolism. St. John's wort administration did not significantly alter CYP1A2 activity determined by measuring caffeine metabolism [147]. When the effect of St. John's wort on the metabolism of amitriptyline and its metabolites was studied no correlation between CYP2D6, 2C9, or 2C19 genotype and amitriptyline pharmacokinetics could be observed [148].

In humans one case of reduced plasma concentrations of theophylline, a drug metabolized by CYP1A2 and combined use of St. John's wort was reported. However, a study of 12 healthy subjects taking a single dose of theophylline after two weeks of St. John's wort use failed to show significant changes in theophylline plasma concentrations compared to theophylline plasma concentrations without treatment with St. John's wort [149].

CYP3A4 and CYP3A5, members of the CYP3A subfamily, are the most abundantly expressed CYP enzymes in the liver and gastrointestinal tract of humans. They metabolize more than 120 frequently prescribed drugs [150]. A study in mice showed that St. John's wort induced the CYP3A subfamily and that hyperforin played an important role in this effect [151]. When human hepatocytes were treated *in vitro* with hyperforin and St. John's wort, expression of CYP3A4 was markedly increased by either treatment. Hyperforin accounted for much of the effect of St. John's wort in this study [152]. By contrast, treatment of rats for 10 days with St. John's wort failed to show an increased expression of hepatic CYP3A. Amounts of CYP1A2, multi-

Table 1. Examples of interactions of St. John's wort with drugs

Drug	Drug type	Result of interaction	Possible mechanism	Ref.
Cyclosporine	Immunosupressant	Reduced plasma concentration	Induction of CYP3A4 or P-glycoprotein	[157, 158, 161]
Tacrolimus	Immunosupressant	Decreased tacrolimus AUC	Induction of CYP3A or P-glycoprotein	[162]
Digoxin	Cardiovascular drug	Reduction of digoxin AUC	Induction of CYP3A or P-glycoprotein	[160]
Ethinyl oestradiol/dienogesterol, and other contraceptives	Oral contraceptive	unexpected pregnancy, intermenstrual Bleadings	induction of CYP3A4	[159, 163]
Indinavir	HIV-1 protease inhibitor	Reduced plasma concentration	Induction of CYP3A4	[156]
Irinotecan	Antineoplastic agent	Reduced plasma concentration	Induction of CYP3A4	[155]
Mephenytoin	Anticonvulsant	Increased urine clearance of metabolite	Induction of CYP2C19	[147]
Warfarin	Anticoagulant	Reduced anticoagulant effect	Induction of CYP2C9	[159]
Amitriptyline, nortriptyline	Tricyclic antidepressants	Reduced plasma concentration	Iduction of cytochrome P-450, mainly CYP3A4 or P-glycoprotein	[148]
Buspirone	Antianxiety drug	Serotonin syndrome	Overstimulation of 5-HT receptors	[164]
Nefazodon, sertraline	Selective serotonin reuptake inhibitor antidepressants	Manic episodes, central serotonin excess	Overstimulation of 5-HT receptors	[145, 165]

drug resistance protein 2 (MRP2), and GST-π, however, were increased up to respectively 357%, 304%, and 252% of controls in the liver of rats exposed for 10 days to 400 mg St. John's wort suspension/kg bw/day estimated to be an antidepressant effective dose in rats [153].

A systemic review of clinical trials concerning interactions of St. John's wort with prescribed drugs revealed that of 19 trials for which drug plasma data were available, 17 found a decrease in the systemic bioavailability of the drug upon coadministration with St. John's wort [154]. Cotreatment with St. John's wort was shown to reduce plasma concentrations of the antineoplastic agent irinotecan by 42% [155]. In healthy volunteers St. John's wort reduced the area under the curve in plasma of the HIV-1 protease inhibitor indinavir by 57% [156]. Two cases of acute rejection in heart transplant patients were reported where St. John's wort was used concomitantly with the immunosuppressive agent cyclosporin [157]. Other drugs affected by St. John's wort are the tricyclic antidepressants amitriptyline and its metabolites nortriptyline, digoxin, ethinylestradiol, and warfarin [145, 148, 158–160]. Table 1 presents an overview of examples of interactions of St. John's wort with drugs [145, 147,148, 155–165]. There have been reports on pharmacodynamic interactions of St. John's wort with drugs. Combined use of St. John's wort with serotonine reuptake inhibitors, antidepressant drugs, by five elderly patients resulted in symptoms characteristic of central serotonin excess. These symptoms include changes in mental status, tremor, gastrointestinal upset, headache, myalgia, and restlessness [145].

2.10.4 Concluding remarks

Evidence of interactions of St. John's wort with an increasing number of commonly used medicines is growing and more attention is required to prevent adverse effects of concomitant use of St. John's wort and drugs. When used properly this drug can be used safely. Medical staff should routinely ask patients for self medication with St. John's wort and other herbs. Governmental bodies need to focus on prevention measures including communication of risks of interactions of certain food supplements with medicines to the general public.

2.11 Cyanogenic glycosides

2.11.1 Major characteristics, occurrence, and intake

Cyanogenic glycosides are present in a number of food plants and seeds and include compounds like amygdalin, dhurrin, linamarin, linustatin, lotaustralin, neolinustatin, prunasin, sambunigrin, and toxiphyllin. Figure 15 presents

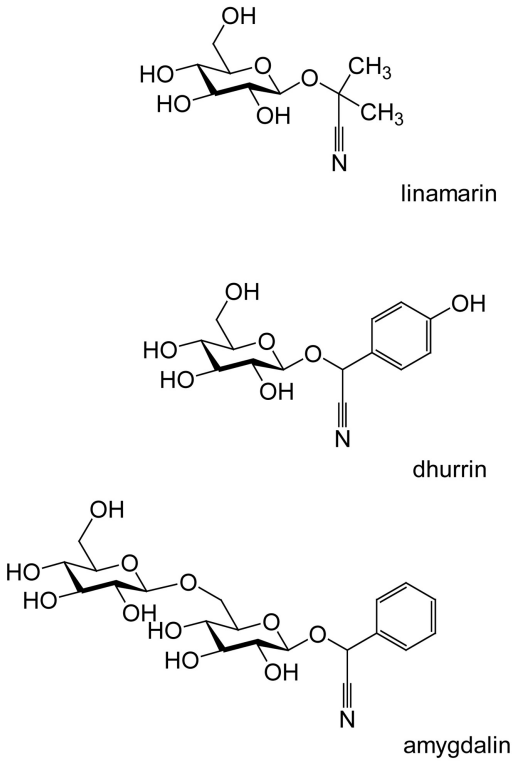


Figure 15. Examples of three important cyanogenic glycosides, linamarin from cassava, dhurrin from sorghum and amygdalin from, for example, abricots kernels and apple kernels.

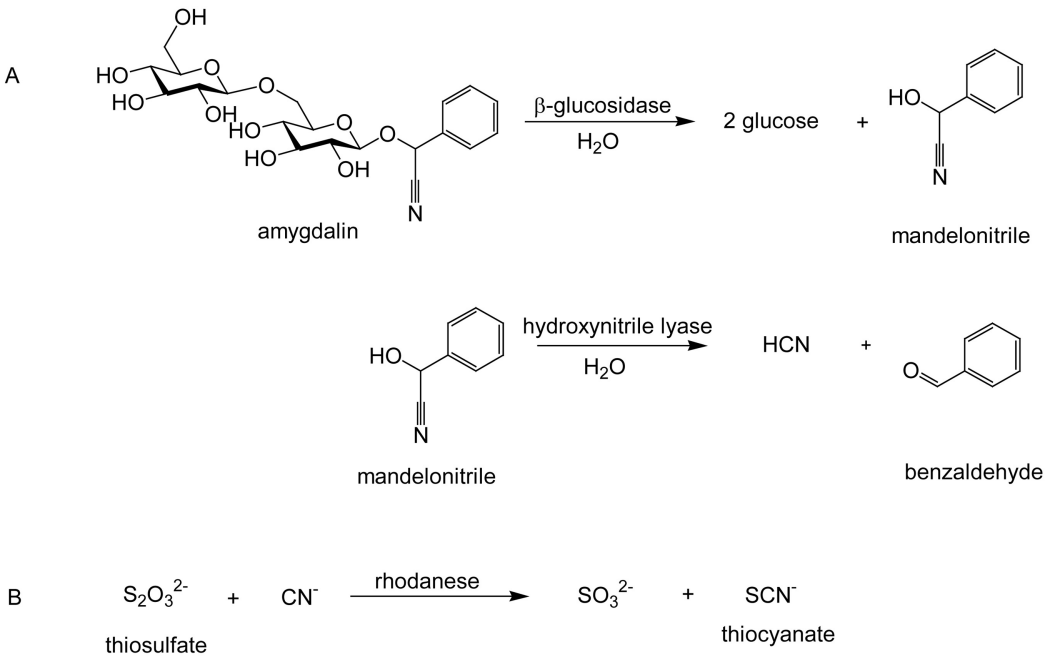


Figure 16. (A) Enzymatic conversion of amygdalin to cyanide, and (B) conversion of cyanide by rhodanese generating thiocyanate.

some relevant cyanogenic glycosides including linamarin, present in the roots of cassava (*Manihot esculenta*) at levels of 10 – 1120 mg hydrogen cyanide (HCN; free and bound)/kg, as well as in Lima bean seeds (*Phaseolus lunatus*) at levels of 100 – 3000 mg HCN (free and bound)/kg, dhurrin from sorghum, and amygdalin, a natural substance found in seeds of apples and pears, as well as in the leaves, fruit and seeds of black cherry, almond, cherry, plum, peach, and apricot trees at levels that may be as high as 300 – 4000 mg HCN (free and bound)/kg [166]. Holzbecher *et al*. [167] report that apricot seeds contain 2.92 mg/g HCN and peach seeds contain 2.60 mg/g HCN, while apple seeds contain only 0.61 mg/g HCN. The mean and 97th percentile overall daily intake of HCN have been estimated to be about 46 – 95 and 214 – 372 μg/person which corresponds to 0.7 – 1.4 and 3.3 – 5.4 μg/kg bw/day [166]. Food products containing relatively high levels of HCN (free and bound) are almonds and/or marzipan-containing confectionery and baked goods, that may contain levels up to 40 mg/kg, with raw marzipan paste containing the highest level of 50 mg HCN (free and bound)/kg [166].

2.11.2 Mechanism of toxic action

Cyanogenic glycosides are a cause of concern, because once ingested they are metabolized to HCN. HCN is released from the cyanogenic glycosides by plant β-glucosidases, that come into contact with the cyanogenic glycosides when fresh plant material is macerated as in chewing, or by β-glucosidases present in the gut flora. Figure 16A presents the hydrolysis of amygdalin to HCN in the gastrointestinal tract. It is a two-step process catalyzed by the enzymes β-glucosidase (produced by intestinal bacteria) and hydroxynitrile lyase [168]. The cyanogenic glycosides, linamarin and lotaustralin from cassava are converted to HCN in the presence of linamarase, a naturally occurring enzyme in cassava. Linamarase acts on the glycosides when the cells are ruptured. Cassava is an important source of carbohydrate for people in Africa and South America. The toxic ingredients are detoxified by hydrolysis through chopping and grinding in running water prior to preparation [169].

Cyanide causes toxic effects by binding to cytochrome oxidase the terminal enzyme in the mitochondrial electron transport chain. By hampering the generation of ATP and oxygen utilization, a histotoxic anoxia is produced. In small doses, the body can detoxify cyanide. In man, cyanide (CN^-) is detoxified by conversion in the liver to thiocyanate by rhodanese (Fig. 16B), by direct chemical combination with sulfur-containing amino acids or by combination with hydroxycobalamine. Methemoglobin effectively competes with cytochrome oxidase for cyanide and the formation of methemoglobin from hemoglobin by therapeutically added nitrite or amylnitrite is used in the treatment of cyanide poisoning. If untreated, large doses of cyanide are fatal [170 – 173]. The acute lethal oral dose of cyanide for humans is reported to vary between 0.5 to 3.5 mg CN^-/kg bw [171 – 173] and the sensitivity to cyanide may be highly variable

depending on age, body mass, and health status of the individual [174]. Holzbecher *et al*. [167] report that apricot seeds contain 2.92 mg/g HCN and peach seeds contain 2.60 mg/g HCN, while apple seeds contain only 0.61 mg/g HCN. Accordingly, these authors conclude that a person can easily consume a lethal quantity of apricot or peach seeds, but is unlikely to eat a lethal amount of apple seeds. The consumption of 60 bitter almonds, containing an average cyanide content of 6.2 mg HCN/bitter almond [175] (leading to an intake of 6.2 mg HCN/kg bw which is above the acute lethal oral dose of 0.5 – 3.5 mg/kg bw), has been reported to be deadly for an adult [176]. Several case studies with fatal outcome upon ingestion of high levels of amygdalin have been reported [175, 177 – 179]. Acute cyanide toxicity at small doses can cause headache, tightness in throat and chest, and muscle weakness.

The effects of chronic (long-term) exposure to cyanide are less well-known. Chronic exposure to linamarin from cassavas has been reported to cause malnutrition, diabetes, congenital malformations, neurological disorder, and myelopathy [180, 181]. It has been proposed as the cause of epidemics of Konzo, a form of tropical myelinopathy with sudden onset of spastic paralysis [182, 183]. Degeneration of the corticospinal motor pathway in affected individuals may be the result of the production of thiocyanate from linamarin and the stimulation of neuronal glutamate receptors by thiocyanate [25, 184]. Thiocyanate is formed from HCN by the mitochondrial enzyme rhodanese (Fig. 16 B), a reaction that is generally considered a detoxification pathway of cyanide. The less toxic thiocyanate is excreted in the urine [176, 185]. In countries with low iodine uptake, thiocyanate exposure after cassava consumption might be a risk factor for goitre [186]. Goitre is thought to occur when cyanogenic glycosides are present at a level of 10 – 50 mg/kg in food. This thyrotoxic effect of cyanide results from the action of thiocyanate as a iodine antagonist.

In the 1970s and early 1980s, amygdalin was proposed as an anticancer drug (also named laetrile and vitamin B17).. However, the dangers and ineffectiveness of laetrile were soon recognized. The American Cancer Society has since then indicated that laetrile is a "toxic drug that is not effective as a cancer treatment". And in recent years products containing amygdalin, including apricot kernels, were banned as free-over-the-counter products in many countries.

2.11.3 Polymorphisms of influence

There is no information available.

2.11.4 Concluding remarks

Several regulatory agencies have evaluated the toxicological and epidemiological data in order to establish a safe level of intake of cyanogenic glycosides. A numerical value for safe intake levels, *i. e.*, a TDI, has not been derived, and it is generally concluded that application of limits for the presence of HCN in foods and beverages should be continued.

2.12 Solanine and chaconine

2.12.1 Major characteristics, occurrence, and intake

α-Solanine and α-chaconine (Fig. 17) are toxic saponin-like alkaloids. They exist as β-D-glycosides, and are present in potatoes (*Solanum tuberosum*). The total glycoalkaloid content in potatoes normally varies between 2 – 10 mg/100 g and in chips between 2 and 60 mg/100 g, the latter due to the fact that processing of the potatoe in order to make chips may increase the amounts of glycoalkaloids [187]. FDA regulations limit the solanine content in potatoes to no more than 20 mg/100 g.

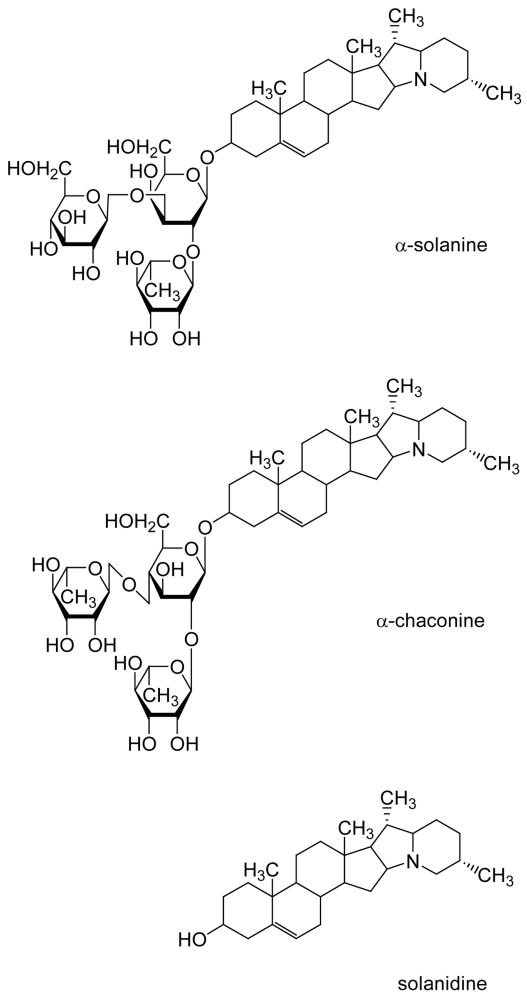


Figure 17. Structures of α-solanine, α-chaconine and solanidine from potatoes.

2.12.2 Mechanism of toxic action

The compounds inhibit cholinesterase enzymes: butyrylcholinesterase (BuChE), that is concentrated in the liver and lungs, and acetylcholinesterase (AChE), that is required to hydrolyse and inactivate the neurotransmitter acetylcholine [188, 189]. The mechanism of this inhibition was shown to be reversible and at a concentration of 2.88×10^{-5} M α-chaconine and α-solanine were shown to inhibit BuChE by about 70% and 50%, respectively [190]. Changes in the glycoalkaloid content of potatoes may occur during storage, under the influence of light and radiation, following mechanical damage and as a result of food processing. Solanine is heat-stable and insoluble in water, therefore cooking does not remove the toxicant. Human toxicity from ingestion of green potatoes with a high solanum glycoalkaloid content is associated with gastric pain, weakness, nausea, and vomiting.

The potential for teratogenic effects has been a significant public concern in populations consuming large amounts of potatoes. The concern arises from studies with Syrian hamsters. Animals treated orally with potato sprouts containing solanidine (Fig. 17), the common aglycon of α-solanine and α-chaconine, had offspring with craniofacial malformations [191]. A 1972 report of Renwick [192] suggesting certain birth (neural tube) defects in humans in areas with higher consumption of potatoes infested with Phytophthora infestans, an infectious potatoe disease inducing the amount of solanine, could not be supported in animal experimental and human studies [193, 194]. The 2-week and 90-day NOAEL's (no observed adverse effect levels) for solanine are 35 and 22.5 mg/kg bw/day, respectively.

2.12.3 Polymorphisms of influence

There is no information available.

2.12.4 Concluding remarks

There is a long history of human consumption of plants containing glycoalkaloids, and the consumption of potatoes with normal glycoalkaloid levels found in properly grown and handled tubers are not of concern.

2.13 Thujone

2.13.1 Major characteristics, occurrence, and intake

The terpenoids α- and β-thujone (Fig. 18) occur together in the essential oils and parts of the plants of *Artemesia absinthum* (wormwood), *Salvia officinalis* (sage), *Salvia scarea* (clary), *Tanacetum vulgaris* (tansy) and in *Juniperus* and *Cedris* spp. The ratio of α- to β-thujone varies with the

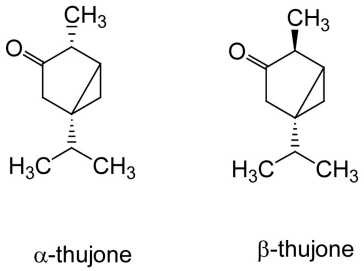


Figure 18. Active principles from absinthe; α- and β-thujone.

source [195]. Synthetic α-thujone is also available commercially.

Thujone is present in food ingredients with flavoring properties. Estimates of intakes of thujone have been made in France and the United Kingdom. In France, the mean and the 97.5th percentile daily intakes were estimated to be 15.6 and 44.3 μg/kg bw/day, respectively. The intakes in the United Kingdom were estimated to be somewhat lower at 3.9 and 14.2 μg/kg bw/day, respectively. The major dietary contribution to intake appeared to derive from sage and sage-flavored products and alcoholic beverages including absinthe [195].

Absinthe is an emerald-green liquor that was very popular at the end of the 19th century. It was associated with the Bohemian lifestyle and was coupled to the inspiration of famous artists and poets. Because of its widespread abuse and the associated toxicity of its content of oil of wormwood, absinthe was made illegal in most countries in the 1910s.

2.13.2 Mechanism of toxic action

The most likely ingredient responsible for the toxicity is believed to be α-thujone. The content of β-thujone often exceeds that of α-thujone, but the β-stereoisomer is generally considered to be of lower toxicity than α-thujone [195 – 197]. The thujone content of old absinthe was about 260 ppm. Already by the end of the 19th century it was recognized that absinthe could cause convulsions, hyperactivity, excitability, hallucinations, and psychotic behavior, including suicide [196] . Other reported side effects of thujone and wormwood are nausea, vomiting, insomnia, restlessness, vertigo, tremors, and seizures. Large doses of thujone have been found to cause delirium, convulsions, seizures, paralysis, brain damage, renal failure, and death.

The mechanism of neurotoxicity of α-thujone has been ascribed to the fact that it blocks the receptors for γ-aminobutyric acid (GABA) in the brain [196]. Without access to GABA, a natural inhibitor of nerve impulses, neurons fire too easily and their signaling goes out of control. Thus, the

mechanism underlying the adverse effect of α-thujone is comparable to that of anisatin (Section 2.9).

2.13.3 Polymorphisms of influence

There is no information available.

2.13.4 Concluding remarks

In the 1990s absinthe has become popular again. Its newly fashionable image, combined with possibilities for global purchase through the internet has helped initiate its revival. The currently available versions of absinthe have levels of thujone of about 10 ppm. This is below the current upper limit set in Annnex II of Directive 88/388 EEC of 35 mg/kg (ppm) in bitters. The EU-SCF considered the available data inadequate to establish a TDI but noted that some of the deficiencies in the database were being addressed in ongoing studies and they recommended that the results of these should be reviewed when available [195]. Current levels of α- and β-thujone in absinthe are, however, judged to be of less toxicological concern than its ethanol content [198].

2.14 Glycyrrhizinic acid

2.14.1 Major characteristics, occurrence, and intake

Glycyrrhizinic acid (Fig. 19) is a food-flavoring substance extracted from the roots and rhizomes of the liquorice plant (*Glycyrrhiza glabra*). Because of its sweetness this triterpenoid saponin compound (33 – 200 times as sweet as sucrose) is an important component of a range of foodstuffs such as liquorice confectionery, tooth paste, cough drops, herbal teas, chewing gum, chewing tobacco, and several alcoholic beverages (*e.g.*, pastis). Herbal liquorice teas can contribute significantly to the intake of glycyrrhizinic acid. A Dutch study found for prepared herbal liquorice teas a mean concentration of 149 mg/L glycyrrhizinic acid (range 25 – 450 mg/L). The mean concentration of glycyrrhizinic acid in liquorice was 0.15% [199].

In the Netherlands liquorice confectionery is consumed in relatively high quantities. In 1998, 0.4% of the Dutch population consumed 50 g liquorice per day and 0.1% consumed more than 100 g per day leading to intakes of glycyrrhizinic acid of more than 75 mg and 150 mg per day, respectively [200]. The average daily consumption among regular consumers was 11.5 g liquorice/person [201].

In herbal medicine the root is used as Liquiritiae radix for treating cough, inflammation of the upper respiratory tract, gastritis, and gastric ulcers. Glycyrrhizinic acid is present in the liquorice root as ammonium and calcium salts. When hydrolyzed, glycyrrhizinic acid yields di-glucuronic acid

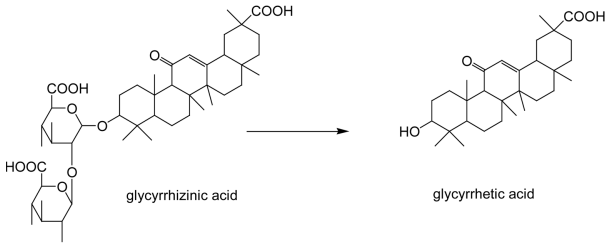


Figure 19. Structural formula of glycyrrhizinic acid and its conversion to the biologically active metabolite glycyrrhetic acid.

and glycyrrhetic acid (Fig. 19). Other constituents of liquorice root are triterpenoid saponins (*e.g.*, 24-hydroxyglycyrrhizin, glabranin A and B), flavonoids (*e.g.*, the isoflavan glabridin), and coumarins [202].

2.14.2 Mechanism of toxic action

An essential step for absorption in the gastro-intestinal tract is the hydrolysis of glycyrrhizinic acid by intestinal bacteria into glycyrrhetic acid, which is the ultimate biologically active molecule (Fig. 19). Absorption of glycyrrhyzinic acid, in the form of glycyrrhetic acid, from solutions or from liquorice is comparable and virtually complete. Due to its lipophilic nature excretion of glycyrrhetic acid by the kidney is very low. After a slow uptake by the liver the substance is subject to entero-hepatic circulation. Glycyrrhetic acid is the biologically active metabolite, which inhibits the enzyme 11-β-hydroxysteroid dehydrogenase-2 (11-BOHD-2). This enzyme is found in the distal kidney tubules and converts the steroid hormone cortisol to cortisone. Cortisol binds to the mineralocorticoid receptor but cortisone does not. A decreased activity of 11-BOHD-2 leads to an excess of cortisol and an overstimulation of this mineralocorticoid receptor. This causes water and sodium retention and an increased excretion of potassium. When exposed to large doses of glycyrrhizinic acid over a prolonged period, these electrolyte imbalance and water retention can cause hypokalaemia, hypernatraemia, oedema, hypertension, and cardiac disorders [201, 203].

2.14.3 Polymorphisms of influence

An *in vitro* study showed that liquorice root extract and its major flavonoid glabridin inhibited human CYP3A4. Furthermore, glabridin inhibited CYP2B6 which is responsible for the metabolism of roughly 3% of prescribed drugs, such as ketamine, phenobarbital, and rifampin [204]. Glycyrrhizinic acid was found to increase plasma concentrations of prednisolone in humans [205]. In a repeated dose-response study in human volunteers a NOAEL of 2 mg/kg bw/day glycyrrhizinic acid was derived.

Table 2. Overview of the food-borne phytochemicals of present concern in food toxicology and the mechanism of their toxic effect as discussed in the present paper

Compound(s)	Requires bioactivation	Mechanism of action
Aristolochic acids	+	Formation of reactive nitrenium ion causing Chinese herb nephropathy and urothelial cancers
Pyrrolizidine alkaloids	+	Formation of pyrrolic dehydro-alkaloid metabolites alkylating DNA and other macromolcules, causing liver cell necrosis and liver cancer
β-Carotene	+	Oxidation products resemble retinal, disturbing retinal homeostasis leading, in combination with cigarette smoke, to (lung) tumor promotion
Coumarin	+	Formation of toxic tumor inducing metabolites in the coumarin-3,4-oxide pathway
Alkenylbenzenes: safrole, methyleugenol, estragole	+	Formation of genotoxic, carcinogenic 1′-sulfooxymetabolites
Ephedrine alkaloids	–	Adrenalin agonist
Synephrine	–	Adrenalin agonist
Kavalactones	+/–	Glutathione depletion and/or quinone formation
Anisatin	–	GABA antagonist
St. John's wort ingredients	–	Interfere with CYP-mediated biotransformation resulting in drug interactions
Cyanogenic glycosides	+	Release of cyanide, inhibits cytochrome *c* oxidase
Solanine	–	Cholinesterase inhibition
Thujone	–	GABA antagonist
Glycyrrhizinic acid	+	Hydrolysis generates glycyrrhetic acid that inhibits 11-β-hydroxysteroid dehydrogenase-2 (11-BOHD-2), leading to an excess cortisol and overstimulation of the mineralocorticoid receptor, causing water and sodium retention and increased potassium excretion

2.14.4 Concluding remarks

Because of limited data the EU-SCF [201] could not derive an Acceptable Daily Intake (ADI) for glycyrrhizinic acid. The Committee was however of the opinion that ingestion of glycyrrhizinic acid should not exceed 100 mg/day which should protect the majority of the population. Yet certain subgroups with a decreased 11-BOHD-2 activity or hypertension might not be sufficiently protected [201].

3 Conclusions

The present review describes the nature and mechanism of action of the phytochemicals nowadays receiving increased attention in the field of plant and herb-based food items, and Table 2 gives an overview of the data presented. From this it can be concluded that a variety of phytotoxins may cause concern, because of their bioactivation to reactive alkylating intermediates that are able to react with cellular macromolcules causing cellular toxicity, and, upon their reaction with DNA, genotoxicity resulting in tumors. Another group of phytotoxins of present concern is active without the requirement for bioactivation and, in most cases, appear to act as neurotoxins interacting with one of the neurotransmitter systems.

For most compounds regulatory agents are aware of the problems encountered and have taken or are considering appropriate regulatory actions to protect the public. These regulatory actions may vary from setting TDIs (such as, for example, for coumarin), application of limits for the presence of a compound in foods and beverages (such as for HCN, thujone, and glycoalkaloids), trying to define safe upper limits (such as for β-carotene), advising on a strategy aiming at restrictions in use (such as for estragole, methyleugenol, and safrole), informing the public to be cautious and aware of possible adverse side effects (as for St. John's worth, glycyrrhizinic acid and kava-kava), or taking specific plant varieties and/or their ingredients from the market (such as for aristolochic acids, pyrrolizidine alkaloids, and kava-kava).

In spite of this regulatory awareness, and previous and recent regulatory actions taken, it can not be excluded that specific developments may still result in problems. This includes (i) phenomena such as overconsumption by particular groups, sometimes stimulated by companies making illegal claims on their websites or in their literature; (ii) the fact that many consumers equate "natural" with "safe" when considering plant-based food supplements or drug preparations; (iii) the over-the-counter selling of food supplements through internet sites from countries where regulations are not in place; and (iv) the fact that there is yet no system in place to guarantee the safety and quality of botanical supplements. The latter is especially worrying as products on the market are known be of variable quality with high variation in the content of the active but also of the toxic principles, and the fact that already several examples of replacement of a harmless variety with a toxic alternative have occurred, either intentionally or accidentially. Misidentification of plants harvested from the wild may add to the problem. The growing volume of products and sales call for a more formal pre-marketing assessment and better and stricter controls than presently available.

Altogether, the examples above illustrate that "natural" does not equal "safe" and that in modern society adverse health effects, upon either acute or chronic exposure to phytochemicals, can occur as a result of (mis)use of plant- or herb-based foods, botanicals or botanical preparations intended for human consumption as food supplements, teas or other extracts. At present regulatory bodies have become

more aware of the problem and are increasing their efforts to ensure the safety of botanical supplements [206, 207].

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