



# Dietary Phytoecdysteroids

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**Abstract**

Phytoecdysteroids are polyhydroxylated steroids which are widely distributed in the plant world and are present in significant amounts in 5–6% of plant species. Their major role in the plant is probably to deter invertebrate predators, but ecdysteroids also have many beneficial effects in mammals and are attracting attention as therapeutic and nutraceutical agents. Four hundred analogues have been identified so far from plant sources, but 20-hydroxyecdysone is the most frequently encountered and is often the major analogue present. Here we consider the occurrence of phytoecdysteroids in food plants and the human diet and how this might change in the future against the backdrop of what we currently know about biosynthesis of these compounds in plants and their bioavailability, metabolism, and biological activities in mammals. Finally, we discuss the medical and pharmaceutical potential of these molecules, particularly in the area of muscle wasting diseases and diabetes, and indicate which areas of fundamental research require focused study.

**Keyword**

20-Hydroxyecdysone · Anabolic · Antidiabetic · Bioavailability · Biosynthesis · Dietary intake · Metabolism · Quinoa · Spinach · Steroid · Structure-activity relationship

**Abbreviations**

20E	20-hydroxyecdysone
2d20E	2-deoxy-20-hydroxyecdysone
4E-BP1	4E-binding protein 1
ADMET	adsorption, distribution, metabolism, excretion, toxicology
AjuC	ajugasterone C
CNS	central nervous system
Cyast	cyasterone
DHT	dihydrotestosterone
DMD	Duchenne muscular dystrophy
E	ecdysone
E2	estradiol
HPLC	high-performance liquid chromatography
i.p.	intraperitoneal
IGF-1	insulin-like growth factor 1
Ino	inokosterone
IntA	integristerone A
LC	liquid chromatography
LD <sub>50</sub>	dose bringing about 50% mortality
MakA	makisterone A
MS	mass spectrometry
mTORC1	mammalian (or mechanistic) target of rapamycin complex 1
NMR	nuclear magnetic resonance spectroscopy

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PI3K	phosphoinositide kinase-3
PoA	ponasterone A
PolB	polypodine B
Pter	pterosterone
RIA	radioimmunoassay
Rub	rubrosterone
SAR	structure-activity relationship
TLC	thin-layer chromatography
Turk	turkesterone
UV	ultraviolet

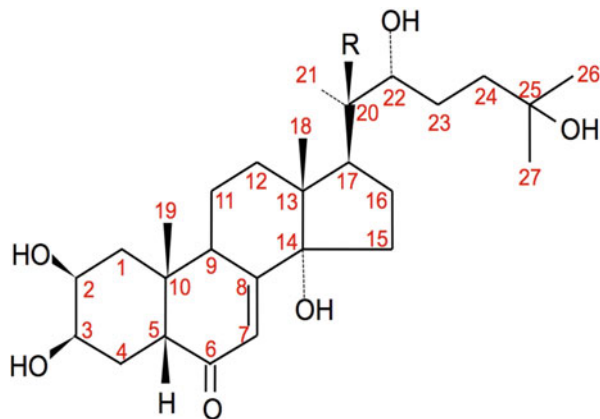
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## 1 Introduction

There are believed to be 50,000 edible plant species among the estimated 300,000 terrestrial plant species, but only a few hundred contribute significantly to the human diet. Further, fewer than 20 account for 90% of the plant food intake of humans, with just 3, maize, rice, and wheat, accounting for >50% of the calorific intake (<https://www.worldatlas.com/articles/most-important-staple-foods>). While this perhaps makes the plant secondary compounds of those staple foods particularly relevant, it should not be allowed to obscure the contribution that the secondary compounds present in the minor food species might contribute to the diet, since their phytochemical contents and geographical preponderance vary enormously, and relatively small intakes of particular secondary compounds can have a marked impact on the physiological status of the human body.

Ecdysteroids are a family of steroid hormones, which were initially identified in insects and crustacea as hormones regulating molting, development, and reproduction (Koolman 1989). These zooecdysteroids have since been found to be present in other arthropods and invertebrates, but their roles are not so clearly elucidated. Shortly after the characterization of the structures of ecdysone (previously known as  $\alpha$ -ecdysone) and 20-hydroxyecdysone (formerly known as  $\beta$ -ecdysone, ecdysterone, or crustecdysone) in the mid-1960s (Fig. 1), four research groups almost simultaneously discovered that ecdysteroids were also present in certain plant species (summarized in Nakanishi 2006), where they are referred to as phytoecdysteroids, in far higher concentrations than those found in invertebrates. To date, ca. 500 ecdysteroid analogues have been identified (Ecdybase; Lafont et al. 2002) from invertebrates, plants, and fungi (mycoecdysteroids), although the vast majority of these have been isolated from plants, reflecting the much higher concentrations found in ecdysteroid-containing plants (typically 0.1–1% of the dry weight but reaching 3.2% of the dry weight in the case of bark of *Diploclisia glaucescens*; Bandara et al. 1989). 20-Hydroxyecdysone is the most frequently encountered and major ecdysteroid analogue in arthropods and plants. Accumulating evidence supports the role of phytoecdysteroids in the reduction of invertebrate predation on the plants containing them either by endocrine disruption on ingestion of the plant material or by deterrence following contact or ingestion of a small

**Fig. 1** Structures of ecdysone (R = H) and 20-hydroxyecdysone (R = OH), showing the stereochemistry and the standard numbering system



sample of the plant. This has led to the suggestion that phytoecdysteroids might be used to enhance the protection of crop species against predation. Indeed, it appears that most, if not all, plant species retain the genetic capacity to produce ecdysteroids, but that differential regulation of the as yet not fully elucidated biosynthetic pathway determines whether a particular species will accumulate ecdysteroids, at what levels within different parts of the plant and what the mix and proportions of various ecdysteroid analogues (the ecdysteroid profile) will be (Dinan et al. 2001).

The attractiveness of this idea is considerably enhanced by the lack of toxicity of ecdysteroids to mammals and by a plethora of essentially beneficial pharmacological and physiological effects in mammals which have been ascribed to phytoecdysteroids on ingestion or injection (reviewed Dinan and Lafont 2006). Mammals do not have the capacity to synthesise ecdysteroids *de novo*, nor do they possess homologues of the arthropod ecdysteroid receptor proteins (EcR and USP/RXR). Also, ecdysteroids, which differ significantly from vertebrate steroid hormones in polarity, bulk and shape, do not appear to interact with the vertebrate steroid hormone nuclear receptors (Báthori et al. 2008). However, exogenous ecdysteroids, like other dietary xenobiotics, are absorbed, distributed, metabolized, and excreted by mammals. *In vivo* and *in vitro* studies indicate that ecdysteroids modulate mammalian cell activities rapidly (in seconds to minutes) by interaction with membrane receptors and/or ion channels, which are in distinct contrast to the direct genomic regulation via nuclear receptors in arthropod cells (over hours to days). In view of the diversity of potential pharmacological benefits of ecdysteroids in mammals, there is a rapidly growing interest in assessing these compounds for use in a variety of medical conditions, especially those which involve muscle wasting (sarcopenia, cachexia, Duchenne muscular dystrophy, etc.).

In this review we shall consider the occurrence and identity of ecdysteroids in major and minor dietary plants; summarize the currently available data on the bioavailability, metabolism, activities, and modes of action of ecdysteroids, mainly 20E, in mammalian systems; and discuss the current and potential impact of this class of compound on the human diet.

For those readers who are not already acquainted with it, the website “Ecdybase” (Lafont et al. 2002), which incorporates several updatable databases associated with various aspects of the ecdysteroid literature, provides a good companion reference to this chapter. Ecdybase grew out of *The Ecdysone Handbook* (Lafont and Wilson 1996). It provides structures, spectral data, and references for all the published phyto-, zoo-, and mycoecdysteroid analogues, biological activity data, and literature surveys of the occurrence of ecdysteroids in plant and non-arthropod species and the effects and uses of exogenous ecdysteroids in animals and plants. To save space in this chapter, rather than referencing many original articles in the primary literature, Ecdybase will be cited, and the original literature can be found there.

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## 2 Dietary Phytoecdysteroids

### 2.1 Bioactive Constituents

#### 2.1.1 Distribution of Ecdysteroids in the Plant World

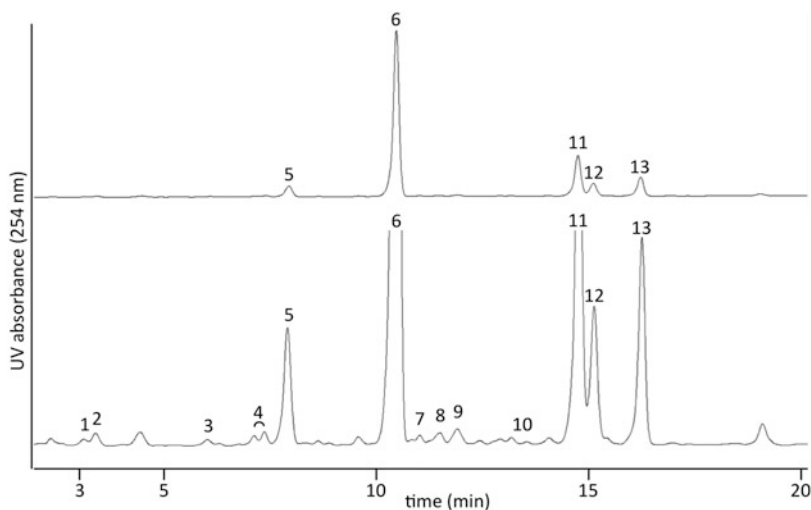
Somewhere in the region of 6000 species of plants have been assessed for the presence or absence of ecdysteroids, which accounts for about only 2% of the total number of terrestrial plant species. Additionally, one must bear in mind that studies have used different methods (ranging from bioassays detecting positive extracts to unambiguous spectroscopic identification of specific analogues by NMR or crystallography) differing in their selectivity and sensitivity. Also, different portions of the plants at different stages of development have been assessed. However, the most extensive study has been the Exeter Survey which assessed whether ecdysteroids are present in dormant seeds of ca. 5000 plant species by means of standardized micro-extraction methodology followed by an ecdysteroid-responsive cell-based bioassay (testing for 20E-like biological activity) and sensitive ecdysteroid-specific radioimmunoassays (testing for chemical similarity to E) (Dinan 1995b). Phytoecdysteroids have been detected in specific members of the ferns, gymnosperms, monocots, and dicots (Lafont 1998). Although phytoecdysteroid-containing species are more common in some genera than in others, where enough species in a genus have been examined, positive species have been found. Also, there is no correlation between species which contain high levels of ecdysteroids and the occurrence of ecdysteroids in other members of the same genus or family, as demonstrated by the rarity of ecdysteroid-containing species in the Asteraceae in general, but the presence of species like *Serratula coronata* and *Leuzea carthamoides*, which contain significantly high levels, in this family. However, if enough species within a family (e.g., the former Chenopodiaceae [now included in the Amaranthaceae]: Dinan et al. 1998) or large genus (e.g., *Silene*; Zibareva et al. 2003) are examined, a pattern in relation to taxonomic structure does begin to emerge, suggesting that the presence and profile of ecdysteroids have chemotaxonomic significance.

### 2.1.2 Distribution of Ecdysteroids Within Plants

Phytoecdysteroids are dynamic molecules within plants; they are not necessarily accumulated where they are biosynthesized; and they demonstrated seasonal variations in where they are located in the plant and how they are metabolized, sometimes resulting in different profiles in different organs at different stages of development. As would be expected of secondary compounds which represent a considerable investment of the plant's resources, phytoecdysteroids seem to be judiciously distributed to enhance the survival of the plant from one year to the next or from one generation to the next in the case of annual plants. Thus, annual cycling of ecdysteroids has been observed in herbaceous biennials or perennials (Lafont et al. 1991), and the association of high levels of ecdysteroids with new growth and/or flower- and seed-producing structures has been observed in several species. Thus, in spinach and quinoa, two annual species of dietary significance, the highest ecdysteroid levels are associated with young shoots, young leaves, flowers, and seeds (Dinan 1992a, b, 1995b).

### 2.1.3 Ecdysteroid Profiles

Ecdysteroid profiles in phytoecdysteroid-containing plants vary from simple, where one analogue prevails (Fig. 2), through moderate, where one to three major analogues are present alongside a "cocktail" of minor analogues, to complex where a mixture of analogues is present and none dominates or where the mixture varies



**Fig. 2** Chromatograms showing the ecdysteroid cocktail present in a crude extract of *Cyanotis* sp. roots. (1) Dihydrorubrosterone (17 $\beta$ -OH); (2) dihydrorubrosterone (17 $\alpha$ -OH); (3) turkesterone; (4) 20,26-dihydroxyecdysone (25R + 25S isomers); (5) rubrosterone; (6) 20E; (7) inokosterone; (8) 2-deoxy-20,26-dihydroxyecdysone; (9) poststerone; (10) stachysterone B; (11) 20E 3-acetate; (12) ajugasterone C; (13) 20E 2-acetate. The lower panel was recorded with a tenfold increase in sensitivity when compared to the upper panel

considerably in composition and total ecdysteroid content depending on the ecotype, growing conditions, or even the individual plant. One presumes that these various profiles are linked to the “strategy” that helps the plant to defend itself from invertebrate predation, but there is no conclusive proof for this. Whatever the distribution and profile of secondary compounds within a plant, it is relatively easy to speculate on a rationale for it, but obtaining substantiating evidence for it requires painstaking study of phytoecdysteroid levels, the generation of plant lines accumulating high and low levels of ecdysteroids, and observations of the effects on the appropriate predator species.

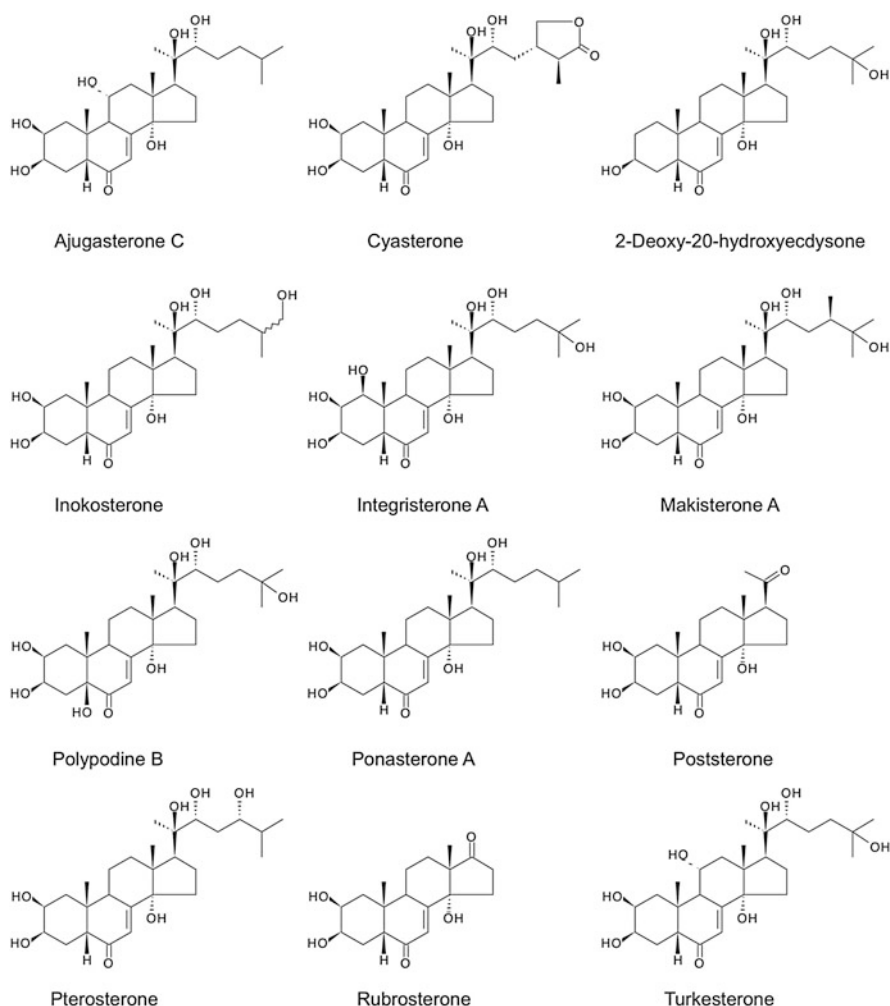
#### 2.1.4 Major Analogues

Ecdysteroids are polyhydroxylated steroids which can exist in the free steroid form or as polar or nonpolar conjugates. The structural limits of what constitutes an ecdysteroid are not fully defined, but the majority possesses a 14 $\alpha$ -hydroxy-6-keto-7-ene functional group (giving rise to a characteristic UV absorption at 242 nm [ $\epsilon = 12,400$  L/mol/cm] in methanol) with A/B-*cis* ring fusion and *trans* B/C- and C/D junctions. The number of C atoms in unconjugated ecdysteroids depends on which phytosterol they are derived from (C<sub>27</sub>, C<sub>28</sub>, or C<sub>29</sub>; see below) or the extent of side-chain cleavage during biosynthesis (C<sub>24</sub>, C<sub>21</sub>, C<sub>19</sub>). Hydroxyl groups may be located at various positions around the molecule and vary in their stereochemistry, but the most frequently encountered locations are 2 $\beta$ , 3 $\beta$ , 14 $\alpha$ , 20*R*, 22*R*, and 25, as found in 20E, the natural arthropod steroid hormone. 20E is the most commonly encountered phytoecdysteroid as it is present in the vast majority of phytoecdysteroid-containing species. 20E is normally also the most predominant ecdysteroid present in a species, frequently accounting for >50% of the total ecdysteroid. Other commonly encountered analogues are polypodine B, pterosterone, turkesterone, and makisterone A (Fig. 3).

#### 2.1.5 Range of Analogues and Potential Number

The structural features which categorize a molecule as an ecdysteroid have not been definitively defined, and the approach currently used by most workers in the field is that a molecule should possess several of the chemical features of the archetypal ecdysteroid 20E, i.e., steroid nucleus, polyhydroxylation, 7-en-6-one functional group, A/B-*cis*-ring junction, and C/D-*trans*-ring junction. However, it has been proposed that the spectrum of ecdysteroid analogues can be divided into “true ecdysteroids,” which possess a 5 $\beta$ -H, a 14 $\alpha$ -hydroxyl group, and a 7-en-6-one chromophore (regardless of whether they possess ecdysteroid biological activity), and “ecdysteroid-related compounds,” which do not fulfill all these criteria (Lafont and Horn 1989). A standardized system of abbreviations has been proposed for common ecdysteroids (Lafont et al. 1993).

As more analogues were identified, it became apparent that the range of analogues result from a combination of a number of biochemical changes (hydroxylations, oxidations, reductions, isomerizations, conjugation reactions, etc.) on a basic structure ([5 $\beta$ -H]14 $\alpha$ -hydroxy-6-oxo-7-ene cholestane). Over 500 ecdysteroid analogues have been identified, of which 400 have been identified in plants, and more



**Fig. 3** Structures of significant phytoecdysteroids. See Ecdybase for structures of all published ecdysteroids

are continuing to be isolated; considering the possible permutations of the possible biochemical changes involved allows a theoretical calculation that over 1000 analogues could ultimately be identified (Dinan 2001).

### 2.1.6 Biosynthesis in Plants

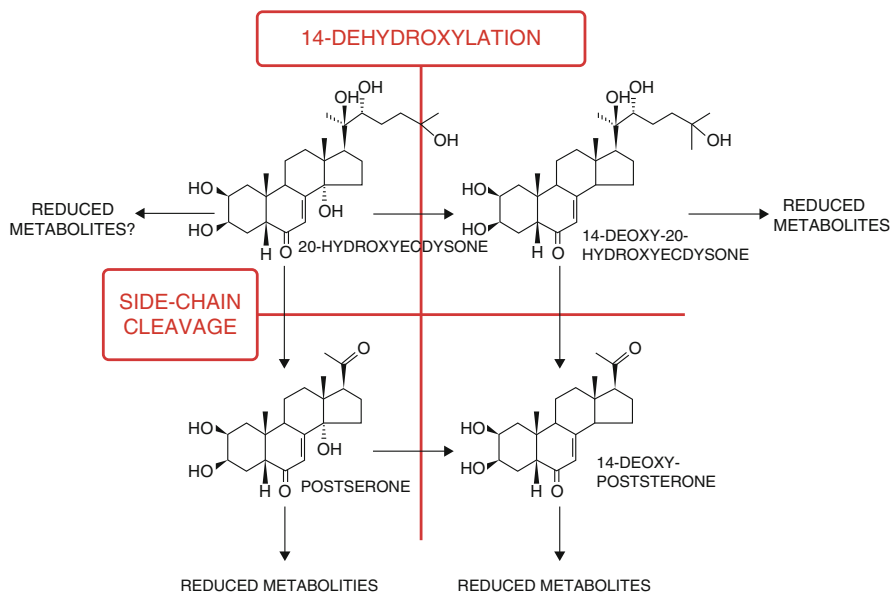
While considerable progress has recently been made in elucidating the later stages of ecdysteroid biosynthesis from dietary cholesterol or a related sterol in insects and other arthropods, understanding of the biosynthetic pathway(s) and the regulation in plants is still largely unknown. The current state of knowledge has been reviewed (Fujimoto et al. 2000, 2015; Dinan 2001). Very briefly, plants are capable of



generating sterols and steroids *de novo* by the cytosolic mevalonic acid (MVA) or plastidial methylerythritol phosphate (MEP) routes, although the mevalonate pathway seems to predominate in the biosynthesis of ecdysteroids. Synthesis from cholesterol gives rise to C<sub>27</sub>-ecdysteroids, while the presence of an alkyl (methyl, methenyl, ethyl, ethenyl) group at C-24 of the sterol gives rise to the corresponding C<sub>28</sub> and C<sub>29</sub> ecdysteroids. The wide structural diversity of phytoecdysteroids and the existence of complex cocktails of analogues in many species of phytoecdysteroid-containing plants suggest that there is a lower specificity of the terminal steps (hydroxylases, etc.) of the pathway (after a common intermediate which already contains the 14 $\alpha$ -hydroxy-6-oxo-7-ene group) which enables the production of multiple ecdysteroid analogues through permutations in the sequence of the various possible modifications. Thus, the later stages of phytoecdysteroid biosynthesis are probably not a linear pathway but rather a network of steps where differential regulation of the amounts and activities of particular enzymes determines which analogues are generated and their relative amounts in the profile. This would be advantageous for the plant in avoiding a lot of genetic redundancy associated with many specific enzymes and also providing a flexibility to redirect synthesis through differential regulation to new analogues should phytophagous predators become tolerant to previously predominant analogues. The wide taxonomic diversity of plants which accumulate significant amounts of ecdysteroids (see Ecdybase) and the presence of individual plants which accumulate albeit low levels of ecdysteroids in species which are regarded as ecdysteroid-negative (Dinan et al. 2001) imply that most, if not all, plants retain the genetic capacity to generate ecdysteroids, but that the activity of the biosynthetic pathway(s) is downregulated in ecdysteroid-negative plants.

## 2.2 Bioavailability and Metabolism in Mammals

Studies on the pharmacokinetics and metabolism of ecdysteroids in mammals have been sporadic, and our understanding of this important area is far from complete. The early studies (Hikino et al. 1972; Lafont et al. 1988) demonstrated that, in mice, 20E and E were excreted rapidly (within 24 h) and faster after *i.p.* injection than after ingestion, with the main excretion route being the feces. Ecdysteroids transiently increase in the plasma and liver after administration and then accumulate in the intestine within 1–2 h. Metabolism is initially very limited until the ecdysteroid reaches the large intestine, where gut bacteria dehydroxylate the molecule at C-14, reduce the 6-keto group and bring about C-20/C-22-side-chain cleavage and an enterohepatic cycle (as for bile acids) results in glucuronide conjugation in the liver, transport in the bile and deconjugation in the large intestine. The enterohepatic cycle results in an increasingly complex pattern of metabolites of the administered



**Fig. 4** Metabolism of 20E in mice. (Modified after Kumpun et al. 2011b)

ecdysteroid and also helps to maintain low but biologically significant levels of ecdysteroids in the plasma. The metabolism of E is simpler, since the absence of a C-20/C-22 diol precludes side-chain cleavage, such that 14-deoxyecdysone is the major metabolite (Girault et al. 1988). 20E is metabolized to 14-deoxy-20-hydroxyecdysone, 6 $\alpha$ -hydroxy-20E, 6 $\alpha$ -hydroxy-14-deoxy-20E, poststerone, 14-deoxy-poststerone and 20,26-dihydroxyecdysone as major metabolites (Foucault et al., in preparation). Further metabolites can occur by relocation of the B-ring double bond (Kumpun et al. 2011b) Fig. 4). It is currently not clear if observed biological effects are attributable only to the administered ecdysteroid or, in part, to the metabolites which are generated. Some of the metabolic transformations mentioned above have also been observed in calf urine after the oral administration of 20E (Destrez et al. 2009).

Studies on the pharmacokinetics and metabolism in humans are restricted as it is not possible to use radioactively labelled tracer molecules. Four pharmacokinetic studies have been carried out. Simon and Koolman (1989) used an ecdysteroid-specific immunoassay to monitor urinary excretion after oral ingestion of 15 mg 20E and found that significant immunoreactive material (peaking at ca. 0.5  $\mu$ M) was excreted in the first 8 h but continues to be excreted at low levels until 24 h (Simon 1988). Brandt (2003) used RIA and HPLC-MS to monitor the excretion of a single oral dose of 20 mg 20E which showed a urinary peak after 3–4 h with a total of 1–2 mg ecdysteroid being recovered in the urine. One of the metabolites was tentatively identified as 14-deoxy-20-hydroxyecdysone. Bolduc (2008) found that only 5 mg 20E was recovered in the urine after the ingestion of 434 mg 20E.

The bioavailability of 20E in mammals is low (<1%), while that of poststerone is somewhat higher (19%). This is in accord with the druggability scores (ADMET Traffic Light Scores; Lobell et al. 2006) for these compounds based on features dependent on their structures and polarities (MW, LogP, polar surface area, number of rotatable bonds, numbers of H-bond donors and acceptors), which gives 20E a score of 3 (“amber”; poor to moderate bioavailability), while Post has a score of 0 (“green”; good bioavailability). This is an aspect requiring attention since the consumption of relatively large amounts of 20E is required to bring about the necessary plasma concentrations required for the induction of biological responses in mammals.

## 2.3 Bioactivities of Ecdysteroids in Mammalian Systems

### 2.3.1 General

The literature up to 2009 has been extensively reviewed previously (Sláma and Lafont 1995; Lafont 1997; Lafont and Dinan 2003, 2009; Dinan and Lafont 2006; Báthori et al. 2008; Dinan 2009). Briefly, in the period after the initial identification of ecdysone and 20-hydroxyecdysone in the mid-1960s, it was considered that ecdysteroids might be used as insect pest control agents, and as a consequence studies were undertaken to obtain toxicological and safety data for mammals exposed to these agents, revealing that they are nontoxic and that they stimulated protein synthesis (summarized below). The next phase, up to about 1984, involved important studies indicating a wide diversity of essentially positive biological activities (anabolic, adaptogenic, wound healing, antidiabetic, hepatoprotective, anti-inflammatory activities, etc.), which were largely published in Russian and unfortunately mainly ignored in the USA and Europe. It was when it became apparent in the mid-1980s that ecdysteroids, in the form of the “Russian Secret,” were being used in the belief that they improved the performances of sportsmen and women by increasing muscle mass, stamina, recovery from injury, and mental attitude that the pharmaceutical and medical interest in these molecules started to rapidly increase, such that it is now fair to say that this is now the main focus of ecdysteroid research around the world. Another important factor has made a significant contribution to these advances, and that is the current availability of large amounts of 20E at reasonable cost; in the mid-1970s, 20E was available only in limited amounts and costs at least USD 2000/g, whereas it can now be purchased for USD 1000/kg, which has made clinical trials with this compound feasible.

Since 2010, investigations of the effects of ecdysteroids (essentially 20E) on mammals have concentrated on four main areas: increase of muscle mass, reduction of body fat, antidiabetic effect, and adaptogenic effects.

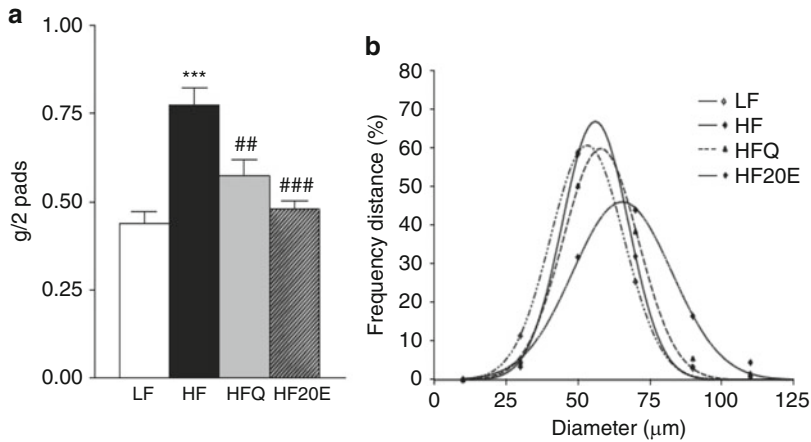
### 2.3.2 Diabetes

Several plant species (*Ajuga iva*, *A. turkestanica*, *Achyranthes bidentata*) used in traditional medicines for diabetes contain significant levels of ecdysteroids (Lafont 2012). It has been shown that pure 20E significantly reduces hyperglycemia in three

rodent models of diabetes at doses of 0.1–10 mg/kg and modifies enzyme levels in the liver such that less glucose would be released from glycogen and more glucose would be converted to lipids. 20E increases glucose consumption by insulin-resistant HepG2 (hepatocyte) cells in a dose- and time-dependent way with a maximal effect being reached at 5  $\mu$ M (Chen et al. 2006), which appears to be mediated by an increase in the Glut-4 glucose transporter activity (summarized in Lafont 2012). Kizelsztejn et al. (2009) showed, using rat H4IIE hepatoma cells, that 20E stimulates PI3K to phosphorylate/activate Akt, which in turn stimulates the translocation of Glut-4 to cell membranes and thereby facilitating glucose uptake. Further, 20E reduces transcription of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase, thus reducing the formation of glucose by release from glycogen and by gluconeogenesis, respectively. 20E also stimulates glucose use by peripheral tissues and increases glycogen content (Syrov et al. 1997), not only of the liver but also in the heart and skeletal muscle (Syrov et al. 1975).

### 2.3.3 Adaptogens

Adaptogens are metabolic regulators present in extracts of certain plants (e.g., *Bryonia alba*, *Eleutherococcus senticosus*, *Rhodiola rosea*, *Schisandra chinensis*) which increase the ability of an organism to adapt to physical, chemical, or biological environmental factors (“stressors”) and to avoid damage from such factors by favoring maintenance of homeostasis. Thus, adaptogens help an organism to survive and cope with situations of intense or prolonged stress and to enhance work capacity/mental performance over a longer period, in contrast to stimulants which provide a short-term increase in capacity followed by a rapid decline in performance to below that of the pre-stimulated state (Panossian et al. 1999). The difference seems to derive from adaptogens providing a more general metabolic stimulation of the cells and organs of the organism (involving stimulation of RNA and protein synthesis and ATP production) involving non-specific and specific mechanisms, whereas stimulants act on more specific sites in the CNS which, after a short time, results in exhaustion of the supply of stimulatory neurotransmitter substances. Extracts of the active plants are more effective than pure compounds isolated from the same species, which is in accord with general stimulation through multiple mechanisms, rather than a mode of action involving a single biochemical target. Progress is starting to be made in the elucidation of the complex biochemical modes of action of adaptogens (Panossian 2017), but much remains to be clarified. Some of the plants proposed as adaptogens (*Leuzea carthamoides*, *Tinospora cordifolia*; Panossian and Wikman 2005) are known to be ecdysteroid-containing. *L. carthamoides* is known as maral root, after the maral deer, which has been observed to dig out and consume the rhizomes (containing ca. 1% of the dry weight as 20E) of the plant at the beginning of spring, which improves their overwintered condition remarkably. Consequently, in Eastern Europe, the roots are harvested, dried, and powdered to prepare an adaptogenic tonic for humans.



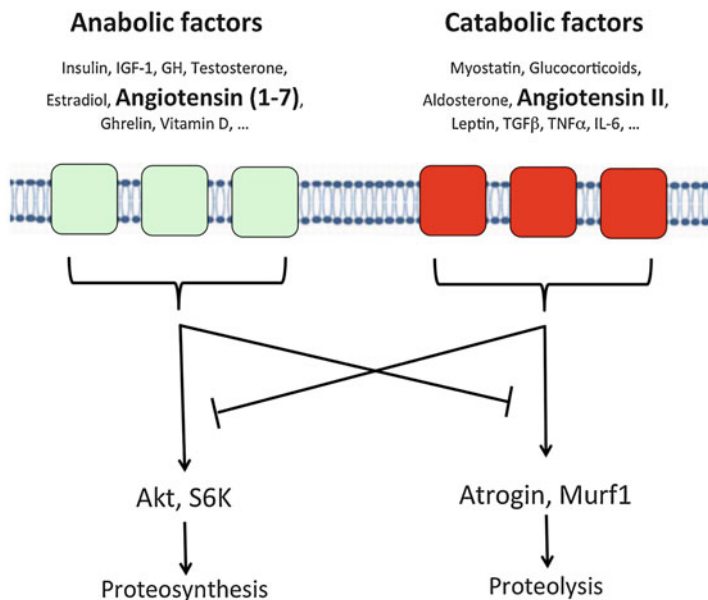
**Fig. 5** Body growth and adipose tissue cellularity. Data are expressed as mean  $\pm$  s.e.m. ( $n = 12$  per group). (a) Epididymal adipose tissue weight; (b) distribution of epididymal adipose cell diameters. \*\*\* $P < 0.001$ , when compared to LF group; # $P < 0.05$ , ## $P < 0.01$  and ### $P < 0.001$ , when compared to HF group. 20E 20-hydroxyecdysone, HF high-fat diet, LF low-fat diet, Q quinoa extract enriched in 20E. (After Foucault et al. 2012, reproduced with permission)

### 2.3.4 Body Fat

Foucault et al. (2012, 2014) conducted experiments using a diet-induced mouse obesity model, where feeding a high-fat diet rapidly results in the mice becoming obese, but when simultaneously fed 20E at 5 or 10 mg/kg, there was a much lower fat increase which was not a consequence of lower food intake. Adipocytes from treated mice were reduced in size, but not in number and tissue inflammation, and the levels of cytokines involved in adipocyte growth and differentiation were reduced (Fig. 5). Adiposity was also reduced in ovariectomized rats treated with 20E (Seidlova-Wuttke et al. 2010a). The mechanism of action of this response has not yet been investigated.

### 2.3.5 Muscle Mass

The mouse C<sub>2</sub>C<sub>12</sub> myocyte cell line, when stimulated with 20E or turkesterone, increases the incorporation of [<sup>3</sup>H]leucine into protein rapidly (maximum incorporation after 2 h) and in a dose-dependent manner. The mechanism of action involves the PI3K/Akt system and an influx of Ca<sup>2+</sup> ions. The effects of 20E were suppressed by inhibitors of GPCRs, phospholipase C, and PI3K. In parallel in vivo experiments, it was shown that treatment of rats with 20E for 28 days significantly increased their grip strength (Gorelick-Feldman et al. 2008; 2010). Male Wistar rats receiving 20E (5 mg/kg body weight) by subcutaneous injection into the left thigh for 8 days showed a significant increase in body mass, and the *soleus* and *extensor digitorum longus* muscles were significantly enlarged on both sides with increases in the number of muscle fiber nuclei, implying activation of the satellite cells (Tóth et al. 2008). In conjunction with the effect on adipocytes described above, 20E enhances lean body mass.



**Fig. 6** Major actors and signalling pathways coordinating muscle protein balance. Red and green square represent various membrane receptors

Previous studies had shown that insulin and IGF-1 stimulation of muscle protein is mediated by PI3K/Akt activation of mTORC1 assembly and signal transduction (Fig. 6). Anthony et al. (2015) could find no evidence for enhanced phosphorylation of Akt, mTOR, or 4E-BP1 in rat muscle or liver after oral administration of 20E. Bioavailability of the 20E was poor in the rats, and even using an excipient (Labrasol) to enhance 20E bioavailability did not result in increased phosphorylation of the signalling components. These data appear to be in conflict with those of Gorelick-Feldman et al. (2008, 2010), but the route of 20E application was different. Unfortunately, it is not known if the rats used by Anthony et al. (2015) would have demonstrated muscular hypertrophy, as the animals were sacrificed after 4 h or less after gavage with 20E.

### 2.3.6 The Nutritional and Medical Applications of Phytoecdysteroids

In view of the broad spectrum of positive biological activities of ecdysteroids in mammals and their low toxicity, ecdysteroids have nutritional and pharmaceutical potential as food supplements and medicines. While these two areas possess some aspects in common, they do differ fundamentally in several important ways:

*Food supplements:* Preparations sold as food supplements normally consist of an extract enriched in the active component(s) in a largely undefined matrix which should be nontoxic; the registration procedure is considerably facilitated if the concentrated extract derives from a recognized food plant. The daily intake of the active substances is low (<100 mg/day), although the amount of the plant extract

may be considerably higher (1–2 g), depending on the concentration of the active compound in the plant matrix. The activity may be a result of the active components alone, or derive from additive or synergistic interactions between the identified active substances and components of the matrix. The claimed activities of food supplements should be supported by scientific evidence, but it is not necessary that the mode of action should be fully understood. Food supplements are made available to the general public (i.e., to a more or less healthy population), to improve/maintain general well-being, improve diet and digestion, and enhance the ability to cope with stress. Nutritional clinical trials may be performed on potential food supplements based on dietary plants, but there is no legal requirement for this.

Quinoa, an extract of *Chenopodium quinoa* seed enriched in ecdysteroids, was investigated as a food supplement to promote muscle mass and fat loss. It is perhaps instructive to briefly describe the developments in this area as a fairly typical example of its kind. Initially, certain ecdysteroid-containing food plant species (*Ajuga iva*, *Chenopodium quinoa*, *Spinacia oleracea*; Bakrim et al. 2008, 2014; Kumpun et al. 2011a) were assessed for their ecdysteroid distributions, levels, and profiles, which led to quinoa seed being selected for further development. The initial studies had investigated the ecdysteroid contents of seeds from various sources and also the ecdysteroid contents in bran and flour after various milling techniques, which identified quinoa bran as a more concentrated source of 20E (Kumpun et al. 2011a). The next stage was to develop a simple and cost-effective extraction procedure which was amenable to scale-up. Quinoa bran (450 kg; ca. 0.08% w/w 20E) was mixed with water (4500 L) and shaken for 2 h at room temperature and then filtered. The filtrate was heated to coagulate protein, filtered again, and then concentrated tenfold, before being applied to an Amberlite FPX66 resin column, to which the ecdysteroids adhere and were eluted with ethanol. The eluate was spray-dried to yield a preparation which contained 2.4% (w/w) 20E (Foucault 2012). An alternative approach based on leaching ecdysteroids from quinoa seeds has also been described (Graf et al. 2014).

The 20E-enriched extract (quinoa) when fed to mice prevented significantly the development of obesity in the same way as an equivalent amount of 20E, which could be attributed to effects on energy metabolism such that more glucose was oxidized, lipogenesis decreased, and less dietary lipid uptake was observed (Fig. 5: Foucault et al. 2012, 2014). On the basis of these very encouraging results, a clinical trial of quinoa, involving 118 overweight and obese adult humans, compared the effects of 1.6 g quinoa/day (containing 40 mg 20E) to 1.6 g of placebo in a 12-week controlled double-blind trial separated into two 6-week periods, a weight-loss phase (restricted calorific intake) followed by a weight-loss maintenance phase. Patients were monitored for body weight, obesity parameters, dietary intake, and various relevant biochemical parameters at 0, 6, and 12 weeks. Although the data indicated a tendency to prevent body weight and fat mass regain when compared to placebo, the differences were not statistically significant to the required degree, which may be attributable to the relatively low dosage of 20E in the quinoa and reflect one of the

difficulties in preparing food supplements: the balance between the amount of active ingredient (20E) and the daily amount of the preparation (quinoa) that the patient can be expected/is willing to take. However, a statistically significant improvement of insulin sensitivity could be observed in this study.

*Medications:* The regulatory regime is much stricter for compounds intended as medicines. These are aimed for use by a smaller population suffering from a recognized and defined medical condition. The preparation consists of an essentially pure active compound in a fully defined formulation, and the dosage is normally considerably higher than those encountered in food supplements, and this dosage has to be defined and shown to be effective and safe in relation to the age and gender of the target group. With regard to plant-derived active compounds, the source of the compound, its purification, its formulation, and its route of application must be fully defined. The drug will also have to successfully undergo preclinical and clinical trials before it receives authorization from the national regulatory agency:

*Preclinical:* In vitro and in vivo (nonhuman animal) studies to obtain information about efficacy, toxicity, pharmacokinetics, and mode of action.

*Phase 0:* Studies performed on a small number [10–15] people to obtain preliminary information on the bioavailability and half-life of the drug in humans.

*Phase I:* The drug is tested on healthy volunteers (20–100) with ascending doses to assess safety. Pharmacokinetics and pharmacodynamics are also determined.

*Phase II:* Tested on patients (100–300) suffering from the specific disease at the therapeutic dose to assess efficacy and identify side effects.

*Phase III:* The therapeutic dose is tested in a large-scale, multicenter trial (300–3000 patients) to assess efficacy, effectiveness, and safety.

The sequence of clinical trials are increasingly costly, and only a relatively low percentage of drugs entering Phase I trials successfully complete Phase III and obtain regulatory approval. There is also a Phase IV trial which monitors the performance of the drug once it becomes available for prescription. It used to be the case that large pharmaceutical companies funded the necessary research and development from initial lead compound to marketed medicine, but increasingly the identification of leads and initial trials are being undertaken by smaller innovative companies specializing in particular therapeutic areas or approaches, with the larger companies becoming involved, through licensing or purchase, to guide and finance a promising drug through the Phase III trial.

Although many patents attest to the pharmaceutical potential of ecdysteroids in human medicine, no drugs based on ecdysteroids have yet received regulatory approval.

### **2.3.7 Mode(s) of Action**

The modes of action of 20E at responding mammalian cells have not been fully clarified, and several models are being pursued. The models are not mutually



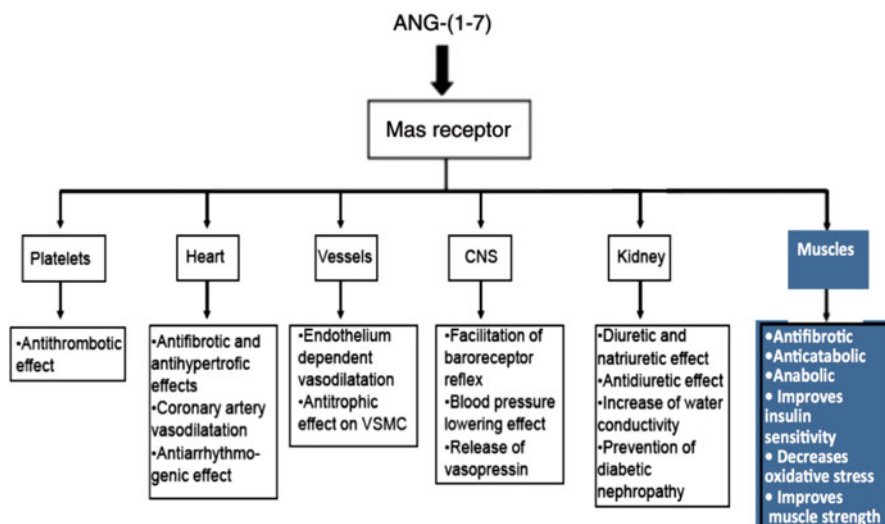
exclusive, and it is possible that they could operate simultaneously or in different tissues. Given the pleiotropic effects described for 20E on mammalian cells, it seems possible that it operates through several signalling pathways, which could be more or less tissue-specific.

Gorelick-Feldman et al. (2010) clearly established that 20E acts through a membrane GPCR receptor, and this has been confirmed using albumin-bound 20E which cannot cross cell membranes (Raynal et al. 2015).

The renin-angiotensin system (RAS) is strongly implicated in maintaining muscle function, and one of the peptide products of this system, angiotensin II, targets skeletal muscle cells via the AT1R receptor and has been implicated in the development of sarcopenia (Yoshida et al. 2013), both directly through AT1R which results in increased resistance to insulin and IGF-1 and indirectly by increased production of myostatin, glucocorticoids, TNF- $\alpha$ , and IL-6. Consequently, ACE inhibitors (which inhibit the production of angiotensin II) have found some application in the treatment of sarcopenia. Another actor of the RAS is angiotensin 1-7 which has been identified as the natural ligand for another GPCR, Mas, activation of which has been hypothesized to enhance protein synthesis in muscle cells. Thus, RAS would have a “harmful arm” acting through AT1R where activation of the receptor enhances proteolysis, but this can be counteracted by a “protective arm,” acting through Mas, where activation of the receptor enhances protein synthesis. Thus, according to this hypothesis, protection against muscle wasting can be achieved by reducing activation of AT1R, or activation of Mas, or a combination of the two. There are reasons to believe that activation of Mas would be more effective at stimulating muscle anabolism and reducing adipose tissue than an AT1R antagonist, and has fewer side effects than ACE inhibitors.

Mas receptor is expressed in many tissues, and its activation in different tissues (e. g., heart, kidney, CNS) may evoke protective effects (Höcht et al. 2009) (Fig. 7). Activation of Mas by 20E could explain the anabolic effects of 20E on muscle cells (Raynal et al. 2015).

Parr et al. (2014) have put forward an alternative model for the mode of action of 20E in bringing about the hypertrophy of mammalian skeletal muscle cells which involves interaction of the steroid with nuclear estrogen receptor- $\beta$  (ER $\beta$ ). Using C<sub>2</sub>C<sub>12</sub> cells, they observed that both 20E (1  $\mu$ M) and E2 (1nM) enhanced myotube diameter. Co-treatment with the antiestrogen ZK antagonized the hypertrophy brought about not only by E2 but also by 20E, indicating that E2 and 20E share a common mode of action. The authors next showed that a reporter gene under the control of an estrogen response element could be activated dose dependently by E2 or 20E interacting with ER $\alpha$  or ER $\beta$  and that this activation could be prevented if ZK was also present. Use of the estrogen receptor-specific agonists, ALPHA (for ER $\alpha$ ) and BETA (for ER $\beta$ ), indicated that ER $\beta$  mediated the hypertrophy of the C<sub>2</sub>C<sub>12</sub> myotubes, but a note of caution is required because these agonists are only selective at low concentrations and the beta dose-response curve was bell-shaped. Finally, the selective ER $\beta$ -antagonist (ANTIBETA) antagonized the effects of E2 and 20E in the C<sub>2</sub>C<sub>12</sub> cells. The authors suggest that, as it had previously been shown that ER $\beta$  can modulate Akt phosphorylation, this could be the link by which 20E brings about



**Fig. 7** The multiple targets of Mas receptor. (Adapted from Höcht et al. 2009)

hypertrophy in the  $C_2C_{12}$  cells. *In silico* docking studies suggest that 20E can interact with the ligand-binding domains of  $ER\alpha$  and  $ER\beta$  but has the potential for stronger interaction with  $ER\beta$  (Parr et al. 2015). However, we may question the exact nature of the receptor involved in the observed effects, because all binding studies performed with nuclear receptors  $ER\alpha$  and  $ER\beta$  were negative (Báthori et al. 2008), and truncated membrane-bound forms of ER receptors have been described that may display different ligand specificity (Schreihöfer et al. 2018). Finally, we wish to mention the recent article by Sobrino et al. (2017). These authors provide evidence that in primary human umbilical vein endothelial cells (HUVEC) cultures, E2 acts via membrane-bound ER to bring about NO-dependent vasodilation and they show that this effect also requires Mas activation, as it is abolished by a Mas inhibitor. Although this a different system to the muscle cells above, it might perhaps give an indication of how both 20E and E2 can bring about muscle hypertrophy.

### 2.3.8 Potency and Structure-Activity Relationships

The availability of so many ecdysteroid analogues, which often differ by just one biochemical change from other known analogues, is a considerable resource for SAR studies and has been used to advantage to characterize the ecdysteroid nuclear receptor in insects (Dinan and Hormann 2005). However, no such comparable extensive study has yet been performed for an ecdysteroid-induced effect in a mammalian system. There are several reasons for this, but most are related to the fact that the biological systems are not yet well characterized enough. The potency of a molecule depends on its affinity for its target, rates of metabolism and excretion, sequestration, and the nature of the cellular response. Most responses to ecdysteroids in mammalian systems appear to require concentrations of 20E in the 0.1–10  $\mu\text{M}$

range, but this does not mean very much unless one has a good understanding of the mode of action and metabolism, both of which might vary from system to system and are only now starting to be elucidated.

The earliest SAR for ecdysteroids in a mammalian system was for the stimulation of protein synthesis in mouse liver (Uchiyama and Otaka 1974), where 11 ecdysteroids were compared at 50 µg/100 g body weight. Nine of the ecdysteroids (20E, inokosterone, cyasterone, ponasterone A, pterosterone, shidasterone, lemmasterone, ponasteroside A, and polypodine B), all possessing a C-20/C-22-diol, were active in a time-dependent manner, as was rubrosterone, which is a C19 ecdysteroid lacking the side chain. Ecdysone (E), however, showed no activity. When four of the ecdysteroids (20E, Ino, Cyast, and Rub) were tested at 5 µg/100 g body weight, only cyasterone remained active. Rubrosterone may have a different mode of action to the other ecdysteroids, as it has been suggested that its structure is close to the androgenic compound androsta-4-en-3,17-dione (Báthori et al. 2008).

Gorelick-Feldman et al. (2008) compared four ecdysteroids (20E, Turk, PolB, and PoA) for their ability to stimulate the incorporation of [<sup>3</sup>H]leucine into protein in mouse C<sub>2</sub>C<sub>12</sub> cells in culture which had been differentiated into muscle myotubes. All four were active in a dose-dependent manner, with 20E and Turk appearing to be more active (EC<sub>50</sub> = ca. 0.04 µM) than PolB and PoA (EC<sub>50</sub> = ca. 0.1 µM).

Báthori et al. (2008) laudably collated the diverse findings in the early literature (published in Russian) on the anabolic activities of 23 ecdysteroid analogues in 6 types of rodent-based assays (not all analogues had been assessed in all of the assays). This brought out the importance of the hydroxyls at 2β, 3β, 11α, and 20R for the stimulation of amino acid incorporation into mouse liver proteins, such that turkesterone and its tetra-acetylated derivative had the highest activity. However, the relative potencies of 20E and Turk depend on the biological system used (cf. Gorelick-Feldman et al. 2008), indicating that factors other than target sensitivity (e.g., bioavailability, metabolism, sequestration, clearance) also affect overall potency.

An initial investigation of the relationship between ecdysteroid structure and the promotion of stamina has been assessed for swimming duration until exhaustion in mice (Mamadalieva et al. 2008; Table 1). Notably, all ten pure ecdysteroid analogues tested promoted the duration of swimming by 10–40% after receiving 5 mg/kg body weight per os 1 h before the test, while siverinol (a mixture of ecdysteroids extracted from aerial portions of *Silene viridiflora*), also at 5 mg/kg body weight, enhanced swimming duration by just under 50%, implying synergistic interaction between the ecdysteroids in the mixture. The most active pure analogues were 20E, Turk, silenoside A, and silenoside D, but not enough analogues were tested to develop a meaningful SAR. The ecdysteroids were more effective than a couple of adaptogenic plant extracts and some synthetic compounds (an androgenic compound and a psychotropic substance) used in higher amounts. Only Fenamin (an anti-inflammatory agent) prolonged swimming duration more than the ecdysteroids (Table 1).

**Table 1** Influence of ecdysteroids (and other substances) on swimming duration until exhaustion of mice bearing a load (5% of body weight). Animals were treated 1 h before starting the swimming period. Male mice (18–20 g) received treatments per os; pure ecdysteroids or siverinol (5 mg/kg b. w.), natural product mixtures as *Eleutherococcus* (Siberian ginseng) extract containing a complex mixture of phenolic compounds (0.2 mL/20 g b.w) or Saparal (a mixture of triterpene glycosides extracted from the roots of *Aralia manchurica*; 20 mg/kg b.w.), and synthetic compounds as Nerobol (methandrostenolone, a non-aromatizable anabolic androgen, 10 mg/kg b.w.), Bemithyl (2-ethylthiobenzimidazole hydrobromide, a psychotropic substance, 50 mg/kg b.w.) and Fenamin (mefenamic acid, a non-steroidal anti-inflammatory analgesic, 10 mg/kg b.w.). Data from Mamadalieva et al. (2008)

Experimental conditions	Duration of swimming (min)	% relative to control
Control	34.0 ± 1.2	100
Ecdysone	38.7 ± 0.7	114
2-Deoxyecdysone	37.0 ± 1.2	109
Integristerone A	39.3 ± 0.4	116
Polypodine B	41.0 ± 0.6	121
20-Hydroxyecdysone	45.6 ± 0.9	134
Turkesterone	46.5 ± 0.6	137
Silenoside A	48.8 ± 1.0	143
Silenoside C	43.0 ± 0.8	126
Silenoside D	46.7 ± 0.9	137
Silenoside E	41.3 ± 0.8	121
Siverinol <sup>a</sup>	50.7 ± 0.8	149
<i>Eleutherococcus</i> extract	39.3 ± 1.0	116
Saparal	41.2 ± 1.3	121
Bemithyl	44.7 ± 1.8	131
Nerobol	40.0 ± 0.9	118
Fenamin	64.8 ± 2.5	191

<sup>a</sup>A partially purified ecdysteroid-containing methanol extract of *Silene viridiflora* (Caryophyllaceae)

While these studies provide amenable assays and demonstrate the fundamental characteristics of time and dose dependency, they are too preliminary in terms of validation, reproducibility of the response, and range of tested analogues to provide anything other than a glimpse of what the SAR might be.

## 2.4 Potential Benefits

### 2.4.1 Aging

Loss of muscle mass and strength is a normal aspect of aging, starting from about the age of 40 and with a loss of ca. 1% per annum, rising to 2% beyond the age of 70. Although inevitable for the vast majority of people, individuals can slow down the process by regular exercise and a good, balanced diet. Unfortunately, the decline can become a vicious circle, since declining muscle strength results in ever less inclination to remain active, with many only recognizing the problem once it has become established, and it is perhaps too late to pursue a more active lifestyle.

Biochemically, the rate of skeletal muscle protein synthesis declines with aging, owing largely to decreased activation of the PI3K/Akt/mTORC1 signalling pathway (Zwetsloot et al. 2014). Hence, the potential attractiveness of mild anabolic agents, such as the ecdysteroids, which activate this signalling pathway through a G-protein-coupled receptor, and, unlike the androgenic anabolic steroids, does not require a concomitant exercise regime for effect and does not produce unpleasant physical and psychological side effects. However promising ecdysteroids may seem as “exercise mimetics,” they need yet to be thoroughly tested by means of double-blind, placebo-controlled clinical trials to assess for efficacy and safety, especially as it is a medication which will need to be taken for the rest of life. Also, it cannot be expected that agents like ecdysteroids would be able to reverse age-related muscle loss, but they could slow the progress of the decline.

### **2.4.2 Sarcopenia**

Under certain circumstances in a proportion of the aging population, the rate of muscle mass and function loss is greater and causes a general deterioration of physical state. Muscle fibers atrophy and the muscles become fibrotic (contain regions of collagen). This process is accompanied by motoneuron death. Obesity can aggravate sarcopenia, by decreasing functional capacities further. Sarcopenia enhances the risk of falls and fractures and is associated with greater incidence of dementia. Once muscle mass/strength is lost past the age of ca. 60, it is increasingly difficult to reverse the process, and beyond the age of 70, it is virtually impossible to do so, hence the importance of a healthy, active life from at least middle age and preferably throughout life. It is estimated that 30% of the US population between 65 and 79 is affected by sarcopenia, rising to 50% among the over 80s. This translates as 14.8 million sufferers among a population of 308 million (Baumgartner et al. 1998).

### **2.4.3 Cachexia**

Cachexia is the rapid muscle and adipose tissue wasting associated with loss of weight, fatigue, weakness, and loss of appetite occurring in conjunction with certain conditions, e.g., cancer, multiple sclerosis, rheumatoid arthritis, tuberculosis, or anorexia nervosa. Loss of >5% of body weight over 6 months indicates that cachexia may be present. Ca. 50% of cancer patients suffer from cachexia, and it significantly increases the risk of mortality. The mechanism(s) by which the above diseases cause cachexia are not well understood, and control of the condition is currently poor. It is plausible that ecdysteroids might help to slow loss of body mass associated with cachexia, but there is no current proof of this. A recent study has implicated activation of the Mas receptor (see Sect. 2.3.7 above) in the attenuation of muscle wasting in cachexia (Murphy et al. 2018).

### **2.4.4 Duchenne Muscular Dystrophy**

DMD is a rare recessive X-linked inherited genetic disorder which affects about 1 in 3,500 boys (ca. 30,000 afflicted in Europe). It involves mutations of various degrees of severity which affect the expression of the dystrophin gene, the product of which is a major component of the cytoskeleton in muscle cells and is important in

maintaining muscle cell structure and providing resistance to damage. Those affected undergo a gradual physical decline from about the age of 5, becoming increasingly weaker and less independently mobile, with death normally ensuing by the age of 30–40. A number of therapeutic strategies are currently being assessed for DMD, but no cure is presently available. It is envisaged that ecdysteroids might slow down the muscular decline or support the maintenance of muscle mass after gene therapy.

#### **2.4.5 Type 2 Diabetes (Metabolic Syndrome)**

The inexorable rise of the incidence of type 2 diabetes around the world (rising from 108 million sufferers in 1980 to 422 million in 2014, corresponding to 8.5% of the adult population; [www.who.int/news-room/fact-sheets/detail/diabetes](http://www.who.int/news-room/fact-sheets/detail/diabetes)) is a major health and social problem. The characteristic symptom is elevated and imperfectly regulated blood glucose levels, but this can arise by a number of mechanisms (reduced/inadequate insulin production, reduced sensitivity to insulin, etc.), so that in reality there is a syndrome of metabolic diseases of differing severity (Ahlqvist et al. 2018). Although genetic predisposition plays a role, it is the modern lifestyle which combines poor diet (excessive calories, processed foods, low amounts of fresh fruit and vegetables) with limited regular exercise, resulting in large sections of the population becoming overweight or even obese, which is strongly associated with the development of type 2 diabetes, which normally begins with lethargy or the need to drink larger volumes of water, but, if not adequately treated, can go on, after two to three decades, to result in blindness, loss of sensory feeling and poor blood flow in extremities (especially the feet), amputations, and early death. The onset used to be in late middle age, but in addition to the rapidly increasing incidence, the age of onset is dropping, such that the incidence among children is now very concerning. Early detection, before nerve and blood vessel damage has become too established, coupled with lifestyle/dietary changes and medication (if appropriate) is paramount to controlling blood glucose levels and prolonging the patient's active life. The effects of ecdysteroids on carbohydrate, protein, and lipid metabolism in mammals are particularly interesting in this respect. Many studies have now shown that ecdysteroids, while not affecting blood glucose levels in healthy subjects (Uchiyama and Yoshida 1974), reduce hyperglycemia. Again, these promising preclinical studies need to be supported by thorough double-blind clinical studies.

#### **2.4.6 Anabolic Activity**

Ecdysteroids (mainly 20E, but also turkesterone) are already extensively used by certain sportsmen, bodybuilders, bouncers, etc. to increase muscle mass and stamina. 11 $\alpha$ -Hydroxy ecdysteroids, such as turkesterone, are claimed to have higher anabolic activity than 20E (Syrov 2000). The absence of the side effects (long-term muscle degeneration, virilization, roid (as it is a contraction of steroid) rage, etc.) characteristic of androgenic-anabolic steroids is an attractive feature of ecdysteroids for these user groups. Further, the anti-inflammatory effects may aid recovery from injuries. Many unregulated products are sold largely through the Internet, and they contain purified ecdysteroids or partially purified plant extracts (mainly from *Pfaffia*

*glomerata* or *Cyanotis arachnoidea* [for 20E] and *Ajuga turkestanica* [for Turk]), often combined with other supposedly anabolic substances (protein, essential amino acids, creatine, etc.), but the composition and batch-to-batch reproducibility are uncertain. Their consumption is not supervised and does not follow a scientifically verified regimen; rather the users tend to follow the empirical advice of other users. As with all other unregulated pharmaceuticals, the safety of such preparations can be questioned. Regulation and standardization would benefit all.

#### **2.4.7 Osteoporosis**

Menopause in women is often accompanied by osteoporosis, where bone strength is lost and the incidence of fractures increases. Ecdysteroid-containing plants (*Achyranthes bidentata*, *Tinospora cordifolia*) have been traditionally used in Chinese and Indian medicines for the treatment of osteoporosis. Ecdysteroid-containing plant extracts or pure 20E have an osteoprotective effect in ovariectomized rats without any effect on the uterus or mammary glands (Seidlova-Wuttke et al. 2010b). 20E stimulates the proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells (Lafont 2012). Ecdysteroids also show promise to counteract secondary osteoporosis brought about when glucocorticoids are used in medication (Dai et al. 2017).

#### **2.4.8 Anti-stress**

The adaptogenic properties of ecdysteroids have been recognized in Eastern Europe and Russia for several decades (Panossian and Wikman 2005), and there is scope to expand this knowledge to increase resilience to the modern lifestyle by reducing the impacts of physical and mental stress, increase stamina, and increase resistance to infections. In this sense preparations of the water-soluble ecdysteroid might be consumed on an occasional basis as tonics and as components of teas or infusions. It seems that adaptogens cease to be effective if they are consumed on a daily basis for too long a period (a week to a few months, depending on the adaptogen and the performance required; Panossian and Wikman 2005), so it is to be expected that the ecdysteroid preparations would be consumed when fatigued and stressed, rather than as a “daily tonic.” Thus, such preparations might help in people with seasonal affective disorder (SAD), loss of libido, loss of appetite, chronic fatigue, age-associated lethargy, sport-associated weariness, poor sleep patterns, and alcohol or narcotic withdrawal (Panossian et al. 1999)

#### **2.4.9 Alzheimer’s Disease**

There are numerous reports indicating that ecdysteroids have potential as neuroprotective agents, in the treatment of neurological diseases, and the improvement of memory and learning. Thus, it is perhaps not surprising that their effects in model systems for Alzheimer’s disease are beginning to be assessed. Alzheimer’s disease is a neurodegenerative disease and is believed to account for 60–70% of cases of dementia. As humans are living longer, the incidence of Alzheimer’s is increasing, such that there were 30 million sufferers worldwide in 2015. In the USA, in 2000, the incidence was 1.6% in the 64–74 age group, 19% in the 75–84 age group, and 42% in the >84 year olds, at a cost of \$100 billion per year (Wikipedia:



Alzheimer's disease). The risk factors are predominantly (70%) genetic, but environmental factors also play a role (e.g., smoking, pollution, poor diet, lack of physical or mental exercise). There is currently no cure, but certain treatments and lifestyle choices may delay onset or the development of symptoms. Progress in the search for effective medication has been hampered by the first symptoms (usually short-term memory loss) becoming apparent many years (perhaps decades) after the biochemical defects have been initiated, with the result that clinical trials of possible treatments have been performed on patients at a too advanced stage of the disease. Several hypotheses have been proposed for the underlying cause of the disease, of which the two major ones are the amyloid and tau hypotheses, which are, respectively, associated with the deposition and accumulation of insoluble extracellular amyloid- $\beta$  plaques and the hyperphosphorylation of tau protein, which normally stabilizes microtubules, resulting in neurofibrillary tangles in nerve cell bodies, bringing about cell death. Studies involving ecdysteroids have concerned the amyloid hypothesis. The N-terminal fragment of amyloid precursor protein (N-APP) and the adjacent amyloid- $\beta$  proteins ( $A\beta$ ; 39–42 amino acids in length) are aberrantly cleaved from the large amyloid precursor proteins (APP; several isoforms) by  $\beta$ -secretase 1 and  $\gamma$ -secretase (Chakraborty and Basu 2017). According to one version of the hypothesis,  $A\beta_{1-42}$  initiates the plaques seen in the brains of Alzheimer's patients, and soluble forms bind to neuron cell-surface receptors and disrupt neuronal communication, while, according to a more recent version of the hypothesis, N-APP binds to neuronal receptor DR6 inducing self-destruction of the cell by apoptosis. An initial study (Yang et al. 2003) considered the ability of 20E to prevent aggregation of  $A\beta_{1-42}$  into fibrils (precursors of the plaques) and found that 100  $\mu\text{M}$  20E prevented fibril formation in PBS and that neuronal cell viability consequent on treatment with  $A\beta_{1-42}$  was increased in a dose-dependent manner by 20–100  $\mu\text{M}$  20E. In an independent study (Yang et al. 2010), involving six ecdysteroid analogues isolated from *Klaseopsis chinensis*, five of the analogues were found to enhance or decrease fibril formation, but, oddly, 20E was without effect at 50  $\mu\text{M}$ . In a more recent study (Chakraborty and Basu 2017), 20E (20  $\mu\text{M}$ ) was found to prevent  $A\beta_{25-35}$  (an analogue of  $A\beta_{1-42}$ , which also forms fibrils, but perhaps not with the same kinetics or affinity) aggregation in vitro. This study also looked at the effect of 20E on  $\beta$ -secretase 1 (BACE 1) and provides experimental evidence that 20E both binds to BACE 1 with a relatively high affinity ( $K_d = 1.75 \mu\text{M}$ ) and prevents its catalytical activity, which were supported by in silico molecular docking studies which indicated that 20E binds to the active cavity of the enzyme, but does not interact directly with the amino acid residues responsible for catalysis. 20E also prevents  $A\beta$ -induced mitochondrial-dependent apoptosis in cultured human neuroblastoma cells (Xu et al. 2018). Thus, ecdysteroids appear to have potential to target several possible sites in the early development of Alzheimer's disease. However, studies on rats after i.p. injections of ecdysteroids show that although 20E diacetoneide will cross the blood-brain barrier, 20E itself does not (Kalász et al. 2017).



## 2.5 Application in Food

### 2.5.1 Dietary Intake

Findeisen (2004) undertook a survey of 115 food items for the presence of ecdysteroids as detected by enzyme immunoassay, using the ecdysteroid-specific DBL2 antiserum. In addition to various fruits, vegetables, and nuts, she also tested a selection of meats, fish, ready meals, herbs and spices, dairy products, and drinks. Although low levels of immune-positive material were detected in most food samples, only fresh spinach (*Spinacia oleracea*; Amaranthaceae) contained a significant level (2210  $\mu\text{g}$  20E eq./g dw [0.22%]; 185  $\mu\text{g}$ /g fw), with the next highest concentration being found in a sample of common mushrooms (*Agaricus bisporus*) at only 95  $\mu\text{g}$  20E eq./g dw; 8.5  $\mu\text{g}$ /g fw. On the basis of the data obtained, it was possible to estimate the intake of ecdysteroids associated with four main courses with meat or fish, or fast food, or vegetarian, all of which were calculated to contain between 10 and 66  $\mu\text{g}$  20E equivalents/meal, although none of these meals contained spinach and any losses during preparation were not allowed for. However, the data clearly indicate that the average dietary intake of ecdysteroids, at least in the Western European diet, is rather low, probably only very occasionally exceeding 100  $\mu\text{g}$ /day. Also, these approximate calculations do not allow for the effects of cooking. Thus, Simon (1988) found that cooked spinach contained low levels of immunoreactive ecdysteroids (0.7 nmol ecdysone eq./kg = 0.3 ng E eq./g wet weight), indicating that most is leached out or destroyed during cooking of the leaves and stalks.

Around the world, the diversity of food plants is considerably greater than the range customarily consumed in the current Western diet. It is estimated that in total 20,000 species of plants are consumed to a greater or lesser extent by humans (<https://pfaf.org/user/edibleuses.aspx>). Most of these correspond to wild plants, and not to cultivars which have been optimized for yield and uniformity, but may also have reduced resistance to predators and diseases owing to lower levels of secondary compounds. However, 90% of the calorific intake from plant sources across the world is accounted for by just 33 crops (Table 2). Where these plants have been assessed for ecdysteroid content (not necessarily the consumed part), these major crop plants appear to contain little or no detectable ecdysteroids.

In certain parts of the world, the so-called pseudocereals have contributed significantly to the diets of the local populations over many centuries, and several of these have recently been “discovered” by Western societies and valued for their nutritional properties and even regarded as “super foods.” Significantly, a high proportion of these crops are members of the Amaranthaceae (Table 3) and contain levels of ecdysteroids similar to those found in spinach; most of the other pseudocereals have not yet been adequately assessed to be able to say whether or not they contain ecdysteroids. A 100 g portion of quinoa would contain ca. 50 mg ecdysteroids (mainly 20E) before preparation and cooking. The extent of retention of ecdysteroids in quinoa grain during the usual preparation and cooking procedures has not been extensively studied, but it is known that immersion in water even at 25 °C results in leaching of about 20% of the ecdysteroid in 24 h (Graf et al. 2014). However, Kumpun et al. (2011a) found that, under normal cooking conditions (boiling for 20 min), >70% of the 20E was retained within the grain after cooking.

**Table 2** The 33 major world plant crops. (Modified from <http://oregonstate.edu/instruct/ces330/two/Unit4Notes.htm>) and their content of ecdysteroids: (a) sourced from Ecdybase (+ relevant reference); (b) sourced from Exeter Survey

Plant	Latin binomial	Family <sup>a</sup>	Plant parts eaten	Area harvested (Ha × 10 <sup>6</sup> )*	Production (tonne × 10 <sup>6</sup> )*	Ecdysteroids <sup>b</sup>
Wheat	<i>Triticum aestivum</i>	Poaceae	Grain	211	568	<sup>a</sup> —(seeds: Dinan 1995a) <sup>b</sup> Grain: —
Rice	<i>Oryza sativa</i>	Poaceae	Grain	146	579	<sup>a</sup> —(plant: Takemoto et al. 1967)/(+[0.09\$] (leaf: Blackford et al. 1996) <sup>b</sup> Grain: —
Maize	<i>Zea mays</i>	Poaceae	Cobs	139	602	<sup>a</sup> —(plant: Devarenne et al. 1995; leaf: Blackford et al. 1996) <sup>b</sup> Kernels: +[0–4\$]
Soybean	<i>Glycine max</i>	Fabaceae	Seeds	79	180	<sup>a</sup> —(plant: Takemoto et al. 1967; leaf: Blackford et al. 1996) <sup>b</sup> Beans: —
Barley	<i>Hordeum vulgare</i>	Poaceae	Grain	54	132	<sup>a</sup> —(aerial parts: Clément and Dinan 1991) <sup>b</sup> Grain: —
Sorghum	<i>Sorghum bicolor</i>	Poaceae	Grain	42	55	<sup>a</sup> —(leaf: Blackford et al. 1996) <sup>b</sup> Grain: —
Finger millet	<i>Eleusine coracana</i>	Poaceae	Grain	37	26	nt <sup>b</sup> Grain: —
Proso millet	<i>Panicum miliaceum</i>					<sup>a</sup> —(leaf: Blackford et al. 1996) <sup>b</sup> Grain: —
Pearl millet	<i>Pennisetum glaucum</i>					nt
Foxtail millet	<i>Setaria italica</i>					<sup>a</sup> —(leaf, stem, root, flower: Dinan et al. 2001) <sup>b</sup> Grain: —
Yellow bristle grass	<i>Setaria pumila</i>					nt

Groundnut (peanut)	<i>Arachis hypogaea</i>	Fabaceae	Seeds	26	34	a <sup>a</sup> -[0.1§] (leaf: Blackford et al. 1996) bNuts: —
Mung bean	<i>Phaseolus aureus</i>	Fabaceae	Seeds	25	18	a <sup>a</sup> -(leaf: Blackford et al. 1996) bBeans: —
Runner bean	<i>Phaseolus coccineus</i>					
Lima/butter bean	<i>Phaseolus lunatus</i>					a <sup>a</sup> -(leaf: Blackford et al. 1996) bBeans: +[0.2§]
French bean	<i>Phaseolus vulgaris</i>					nt bBeans: —
Rapeseed	<i>Brassica napus</i>	Brassicaceae	Seed oil	23	33	nt bBeans: — a <sup>a</sup> -(leaf: Blackford et al. 1996) bSeed: +[0.4§]
Sugarcane	<i>Saccharum officinarum</i>	Poaceae	Cane juice, stems	20	1288	nt bSeed: —
Sunflower	<i>Helianthus annuus</i>	Asteraceae	Seeds	20	23	a <sup>a</sup> -(leaf: Blackford et al. 1996) bSeeds: —
Potato	<i>Solanum tuberosum</i>	Solanaceae	Tuber	19	308	a <sup>a</sup> -(leaf: Blackford and Dinan 1997a, b)
Cassava	<i>Manihot esculenta</i>	Euphorbiaceae	Root	17	180	a <sup>a</sup> -(leaf: Blackford et al. 1996) bSeeds: —
Oats	<i>Avena sativa</i>	Poaceae	Grain	13	28	a <sup>a</sup> -(Dinan 1995a) bGrain: —
Coconut	<i>Cocos nucifera</i>	Areaceae	Flesh, water	11	49	a <sup>a</sup> -(inflorescence: Sreejit 2014)

(continued)

Table 2 (continued)

Plant	Latin binomial	Family§	Plant parts eaten	Area harvested (Ha × 10 <sup>6</sup> )*	Production (tonne × 10 <sup>6</sup> )*	Ecdysteroids§
Oil palm	<i>Elaeis guineensis</i>	Arecaceae	Fruit oil	11	136	nt
Chickpea	<i>Cicer arietinum</i>	Fabaceae	Seeds	11	8	nt
Coffee	<i>Coffea arabica</i>	Rubiaceae	Beans	11	8	nt bSeeds: —
	<i>Coffea conephora</i>					nt
Rye	<i>Secale cereale</i>	Poaceae	Grain	10	21	nt bGrain: —
Sweet potato	<i>Ipomoea batatas</i>	Convolvulaceae	Tuber	9	141	a—(leaf: Blackford et al. 1996)
Cowpea	<i>Vigna unguiculata</i>	Fabaceae	Seeds	9	3	nt
Olives	<i>Olea europaea</i>	Oleaceae	Fruit	8	15	nt bSeeds: —
Grape	<i>Vitis vinifera</i>	Vitaceae	Fruit	7	62	a—(leaf: Blackford and Dinan 1997b) bSeeds: —
Sesame	<i>Sesamum indicum</i>	Pedaliaceae	Seeds	7	3	nt bSeeds: —
Cocoa	<i>Theobroma cacao</i>	Sterculiaceae	Beans	7	3	a+[0.08§] (leaf: Blackford et al. 1996)
Sugar beet	<i>Beta vulgaris</i> var. <i>vulgaris</i>	Amaranthaceae	Root	6	252	a—(aerial parts: Clément and Dinan 1991)/ +(?; Bathory et al. 1984) bSeeds: —

Pea	<i>Pisum sativum</i>	Fabaceae	Seed	6	10	nt b <sup>Seeds</sup> : —
Apple	<i>Malus pumila</i>	Rosaceae	Fruit	6	58	nt b <sup>Seeds</sup> : —
Plantain	<i>Musa sapientum</i>	Musaceae	Fruit	5	29	nt b <sup>Seeds</sup> : —

++, significant amounts detected; +, low levels detected; —, not detected; *nt*, not tested

\$Family name according to APGIV (2016)

§Where values are given they correspond to µg ecdysone equivalents/g dw as determined with the antiserum DBL1 in an ecdysteroid-specific RIA

\*Data for 2002

**Table 3** Pseudocereals and the content of ecdysteroids in the grain/seed

Common name	Latin binomial	Family <sup>a</sup>	Plant part assessed	Amount ecdysteroid <sup>b</sup>	Method	References
Purple amaranth	<i>Amaranthus cruentus</i>	Amaranthaceae	Plant in flower	0.015% dw as 20E	HPLC	Bespayeva et al. (2012)
			Seeds	0.2 µg E eq/g	RIA	Exeter Survey
Common amaranth	<i>Amaranthus retroflexus</i>	Amaranthaceae		nt		Ecdybase
			Seeds	–	RIA	Exeter Survey
Prickly amaranth	<i>Amaranthus spinosus</i>	Amaranthaceae	Whole plant	+	Bioassay	Takemoto et al. (1967)
Amaranth	<i>Amaranthus tricolor</i>	Amaranthaceae		nt		Ecdybase
			Seeds	–	RIA	Exeter Survey
Slender amaranth	<i>Amaranthus viridis</i>	Amaranthaceae	Whole plant	+	Bioassay	Takemoto et al. (1967)
			Leaf			Saeng-ngam et al. (2004)
			Stem	0.7mg20E% dw 0.2mg20E% dw	TLC TLC	
Pitseed goosefoot	<i>Chenopodium berlandieri</i>	Amaranthaceae		+		Ecdybase
			Seeds	145 µg E eq/g	RIA	Dinan et al. (1998)
Kaniwa	<i>Chenopodium pallidicaule</i>	Amaranthaceae	Seeds	15 µg 20E/g	Isolation	Rastrelli et al. (1996)
Quinoa	<i>Chenopodium quinoa</i>	Amaranthaceae	Seeds	612–1292 µg E eq/g	RIA	Dinan et al. (1998)
			Seeds	316–421 µg 20E/g	HPLC	Kumpun et al. (2011a)
			Seeds	419 µg 20E/g	LC/UV/MS	Graf et al. (2014)
Hanza, aizen, boscia	<i>Boscia senegalensis</i>	Capparaceae		nt nt		Ecdybase Exeter Survey
Chia	<i>Salvia hispanica</i>	Lamiaceae		nt		Ecdybase
			Seeds	–	HPLC/MS	Biophytis (unpublished)
Flax, linseed	<i>Linum usitatissimum</i>	Linaceae		nt		Ecdybase
			Seeds	–	HPLC/MS	Biophytis (unpublished)
Breadnut	<i>Brosimum alicastrum</i>	Moraceae		nt nt		Ecdybase Exeter Survey

(continued)

**Table 3** (continued)

Common name	Latin binomial	Family <sup>a</sup>	Plant part assessed	Amount ecdysteroid <sup>b</sup>	Method	References
Sesame	<i>Sesamum indicum</i>	Pedaliaceae		nt		Ecdybase
			Seeds	–	RIA	Exeter Survey
			Seeds	–	HPLC/MS	Biophytis (unpublished)
Buckwheat	<i>Fagopyrum esculentum</i>	Polygonaceae	Whole plant	–	Bioassay	Takemoto et al. (1967)

<sup>a</sup>Family name according to APGIV (2016)

<sup>b</sup>Levels given are for intact plant parts and do not allow for changes in content during preparations and/or cooking

These two findings together imply that ecdysteroids are present in quinoa grain in two forms, a readily leachable form and a more strongly retained form.

As part of the preparation for this chapter, a database was compiled of the major and minor food plants and the parts of the plant consumed, combined with information on whether any part of the plant had yet been examined for the presence of ecdysteroids and the levels detected (derived from the literature/Ecdybase and the Exeter Survey of ecdysteroid levels in plant seeds). Of the 746 edible plants included, 294 have been in any way examined for the presence of ecdysteroids, and only 17 (5.8%) contained more than 10 µg/g dw (>0.001%): *Amaranthus* spp. (Saeng-ngam et al. 2004; Bespayeva et al. 2012), *Atriplex* spp. (Dinan et al. 1998), *Chenopodium* spp. (Rastrelli et al. 1996; Dinan et al. 1998), *Dioscorea dumetorum* (Sautour et al. 2008), *Halimione portulacoides* (Dinan et al. 1998), *Lamium* spp. (Savchenko et al. 2001), *Silene acaulis* (Zibareva et al. 2003), *Spinacia oleracea* (Dinan et al. 1998), and *Vitex donania* (Ochieng et al. 2013). Further, where plants have been assessed, it is often not the portions of the plant which are consumed which have been assessed for ecdysteroids. The database will be added to the Ecdybase website to provide access to the information and in a format which can be updated as further information becomes available.

In view of the limited nature of the data concerning the presence and quantification of ecdysteroids in food plants, it seemed worthwhile to assess a range of staple foods and a wide variety of fruits and vegetables with a specific HPLC-MS/MS method for the quantification of 20E (the most frequently encountered phytoecdysteroid in the plant world) and targeting those parts of the plants which are typically consumed. Of the plant food sources assessed, only spinach leaves/petioles and quinoa grain contained significant amounts of 20E, while virtually all other samples (Table 4) contained no detectable 20E. The spinach sample was found to contain 236 µg 20E/g dw, while a sample of commercial quinoa grain contained 138 µg 20E/g dw, which was reduced to 98 µg/g dw after heating at 100 °C in 10 volumes of water for 15 min (i.e., 32% of the 20E was lost to, and recovered in, the cooking water). The only other sample found to be positive was lemon

**Table 4** Plant foods (vegetables, fruits, herbs, and spices) tested for the presence of 20-hydroxyecdysone by means of a sensitive and specific HPLC-MS/MS method. Plant materials were purchased from supermarkets, freeze-dried, and powdered, and samples (ca. 100 mg) were extracted with 50% aq. ethanol (1.5 mL) at 75 °C for 2 h. An aliquot (1 mL) was diluted with water (4 mL) and partially purified on a pre-activated RP-SPE (SEPAK; Waters) cartridge, washed with 10% aq. methanol (5 mL), and eluted with 100% methanol (5 mL). Aliquots (5 µL) were subjected to RP-HPLC-MS for quantification of 20E (limit of detection = 50 ng/mL). See the text for a summary of the results

Latin binomial	Family <sup>a</sup>	Common name	Plant parts assessed
<i>Abelmoschus esculentum</i>	Malvaceae	Okra	Fruit
<i>Actinidia arguta</i>	Actinidiaceae	Negri, mini kiwi	Fruit
<i>Actinidia deliciosa</i>	Actinidiaceae	Kiwi fruit	Fruit pulp
<i>Agaricus bisporus</i>	Agaricaceae	Common mushroom	Fruiting bodies
<i>Allium cepa ascalonicum</i>	Amaryllidaceae	Shallot	Bulb
<i>Allium cepa</i>	Amaryllidaceae	Onion	Bulb
<i>Allium porrum</i>	Amaryllidaceae	Leek	Leaf sheath
<i>Allium sativum</i>	Amaryllidaceae	Garlic	Cloves
<i>Ananas comosus</i>	Bromeliaceae	Pineapple	Fruit
<i>Annona cherimola</i>	Annonaceae	Cherimoya, custard apple	Fruit
<i>Apium graveolens</i>	Apiaceae	Celeriac	Swollen hypocotyl
<i>Arachis hypogaea</i>	Fabaceae	Peanut, groundnut, monkey nut	Nut (not shell)
<i>Avena sativa</i>	Poaceae	Oats	Rolled oats
<i>Averrhoa carambola</i>	Oxalidaceae	Starfruit	Fruit
<i>Beta vulgaris vulgaris</i>	Amaranthaceae	Beetroot	Swollen root
<i>Brassica oleracea botrytis</i>	Brassicaceae	Cauliflower	Florets
<i>Brassica oleracea capitata</i>	Brassicaceae	Savoy cabbage	Leaves
<i>Brassica oleracea italica</i>	Brassicaceae	Broccoli	Florets
<i>Brassica rapa var. rapa</i>	Brassicaceae	Turnip	Swollen taproot
<i>Capsicum annuum</i>	Solanaceae	Sweet pepper	Fruit
<i>Carica papaya</i>	Caricaceae	Papaya	Fruit flesh
<i>Carya illinoensis</i>	Juglandaceae	Pecan	Nuts
<i>Castanea sativa</i>	Fagaceae	Sweet chestnut	Seed (not shell)
<i>Chenopodium quinoa</i> <sup>c</sup>	Amaranthaceae	Quinoa	Grain
<i>Chenopodium quinoa</i> <sup>c</sup>	Amaranthaceae	Quinoa	Cooking water
<i>Chenopodium quinoa</i> <sup>c</sup>	Amaranthaceae	Quinoa	Cooked grain
<i>Cicer arietinum</i>	Fabaceae	Chickpea	Dry seed
<i>Citrus x clementina</i>	Rutaceae	Clementine	Fruit flesh
<i>Citrus hystrix</i>	Rutaceae	Kaffir lime	Whole fruit
<i>Citrus japonica</i>	Rutaceae	Kumquat	Whole fruit

(continued)



**Table 4** (continued)

Latin binomial	Family <sup>a</sup>	Common name	Plant parts assessed
<i>Citrus limon</i> <sup>b</sup>	Rutaceae	Lemon	Fruit
<i>Citrus maxima</i> ( <i>C. grandis</i> )	Rutaceae	Pomelo	Fruit flesh
<i>Citrus medica</i> var <i>sarcodactylis</i>	Rutaceae	Citron “hand of Buddha”	Fruit
<i>Cocos nucifera</i>	Arecaceae	Coconut	Coconut water
<i>Cocos nucifera</i>	Arecaceae	Coconut	Coconut flesh
<i>Coriandrum sativum</i>	Apiaceae	Coriander	Seeds
<i>Corylus avellana</i>	Betulaceae	Hazelnut, cobnut, filbert	Nut (no shell)
<i>Cucumis metuliferus</i>	Cucurbitaceae	Kiwano, horned melon, melano	Fruit flesh and seeds
<i>Cucurbita moschata</i>	Cucurbitaceae	Butternut squash	Fruit
<i>Cucurbita pepo</i>	Cucurbitaceae	Courgette, zucchini	Fruit
<i>Cuminum cyminum</i>	Apiaceae	Cumin	Seeds
<i>Cydonia oblonga</i>	Rosaceae	Quince	Fruit flesh
<i>Cyphomandria betacea</i> ( <i>Solanum betaceum</i> )	Solanaceae	Tamarillo	Whole fruit
<i>Daucus carota</i>	Apiaceae	Carrot	Root
<i>Diospyros kaki</i>	Ebenaceae	Asian persimmon	Fruit
<i>Elettaria cardamomum</i>	Zingiberaceae	Green cardamom	Pods
<i>Ficus carica</i>	Moraceae	Fig	Fruit
<i>Foeniculum vulgare</i>	Apiaceae	Florence fennel	Bulb and stem
<i>Fragaria x ananassa</i>	Rosaceae	Strawberry	Fruit
<i>Garcinia mangostana</i>	Clusiaceae	Purple mangosteen	Fruit pulp and seed
<i>Helianthus tuberosus</i>	Asteraceae	Jerusalem artichoke	Tuber
<i>Hylocereus megalanthus</i>	Cactaceae	Yellow pitaya	Fruit flesh
<i>Ipomoea batatas</i>	Convolvulaceae	Sweet potato	Tuber
<i>Juglans regia</i>	Juglandaceae	Walnut	Kernels
<i>Linum usitatissimum</i>	Linaceae	Common flax, linseed	Seeds
<i>Lycopersicon esculentum</i>	Solanaceae	Tomato	Fruit
<i>Malus domestica</i>	Rosaceae	Apple	Flesh
<i>Mangifera indica</i>	Anacardiaceae	Mango	Fruit flesh
<i>Musa x paradisiaca</i>	Musaceae	Banana	Flesh
<i>Myristica fragrans</i>	Myristicaceae	Nutmeg	Ground seeds
<i>Nephelium lappaceum</i>	Sapindaceae	Rambutan	Fruit flesh
<i>Nephelium lappaceum</i>	Sapindaceae	Rambutan	Fruit coat
<i>Nephelium lappaceum</i>	Sapindaceae	Rambutan	Seeds
<i>Nigella sativa</i>	Ranunculaceae	Love-in-a-mist, black cumin	Seeds
<i>Opuntia ficus-indica</i>	Cactaceae	Prickly pear, Barbary fig	Fruit
<i>Oryza sativa</i>	Poaceae	Brown rice	Grain

(continued)

**Table 4** (continued)

Latin binomial	Family <sup>a</sup>	Common name	Plant parts assessed
<i>Papaver</i> sp.	Papaveraceae	Poppy	Seeds
<i>Passiflora edulis</i>	Passifloraceae	Passion fruit	Fruit pulp and seeds
<i>Pastinaca sativa</i>	Apiaceae	Parsnip	Root
<i>Persea americana</i>	Lauraceae	Avocado	Flesh
<i>Persea americana</i>	Lauraceae	Avocado	Kernel
<i>Phaseolus vulgaris</i>	Fabaceae	French bean	Immature pods
<i>Pimpinella anisum</i>	Apiaceae	Anise	Seeds
<i>Pinus pinea</i>	Pinaceae	Pine nuts	Kernels
<i>Prunus dulcis</i>	Rosaceae	Almond	Shelled nut
<i>Punica granatum</i>	Lythraceae	Pomegranate	Fruit
<i>Pyrus communis</i>	Rosaceae	European pear	Fruit flesh
<i>Raphanus raphanistrum sativus</i>	Brassicaceae	Radish	Swollen root
<i>Ribes rubrum</i>	Grossulariaceae	Redcurrant	Fruit
<i>Robinia pseudoacacia</i>	Fabaceae	Black locust	Seed
<i>Rubus fruticosus</i>	Rosaceae	Blackberry	Fruit
<i>Rubus idaeus</i>	Rosaceae	Raspberry	Fruit
<i>Sechium edule</i>	Cucurbitaceae	Chayote, mirliton squash	Fruit
<i>Sesamum indicum</i>	Pedaliaceae	Sesame	Seeds
<i>Sinapis alba</i>	Brassicaceae	White mustard	Seeds
<i>Solanum melongena</i>	Solanaceae	Aubergine, eggplant	Fruit
<i>Solanum tuberosum</i>	Solanaceae	Potato	Tuber
<i>Spinacia oleracea</i> <sup>c</sup>	Amaranthaceae	Spinach	Leaves and stalks
<i>Tamarindus indica</i>	Fabaceae	Tamarind	Fruit pulp
<i>Tamarindus indica</i>	Fabaceae	Tamarind	Seeds
<i>Tamarindus indica</i>	Fabaceae	Tamarind	Shell
<i>Trigonella foenum-graecum</i>	Fabaceae	Fenugreek	Seeds
<i>Triticum aestivum</i>	Poaceae	Wheat	Flour (wholemeal)
<i>Vaccinium macrocarpon</i>	Ericaceae	Cranberry	Fruit
<i>Vaccinium myrtillus</i>	Ericaceae	Blueberry	Fruit
<i>Vigna radiata</i>	Fabaceae	Mung bean	Seeds
<i>Vitis vinifera</i>	Lamiaceae	Grape	Fruit
<i>Zingiber officinale</i>	Zingiberaceae	Ginger	Rhizome

<sup>a</sup>Family name according to APGIV (2016)

<sup>b</sup>Positive for 20E at the LOD (see text)

<sup>c</sup>Positive for 20E (see text)

(*Citrus limon*) fruit at 1.04  $\mu\text{g/g}$  dw, which was at the limit of detection. However, this is interesting in the light of the report of the presence of ecdysteroids in *Citrus medica* (citron; Yin et al. 2015), which is a wild progenitor from which modern lemon and some lime hybrid cultivars are derived (summarized in Velasco and Licciardello 2014). Assessment of fruits of *C. medica* var. *sarcodactylis* (hand of Buddha), *C. maxima* (pomelo), *C. x clementina* (clementine), *C. hystrix* (kaffir lime), or *C. japonica* (kumquat) by HPLC-MS/MS did not detect 20E at or above the limit of detection (Table 4).

A further database has been prepared for the occurrence of ecdysteroids and ecdysteroid-related molecules in edible fungi. The world production of edible mushrooms is not insignificant, being 7,700,000 tonnes in 2011, of which 65% were produced in China followed by 10% in Italy and 5% in the USA (Wikipedia: edible mushrooms). Mycoecdysteroids (reviewed in Kovganko 1999) have been much less extensively investigated than phytoecdysteroids, but it appears from the data currently available that the levels found in fruiting bodies are generally low in comparison to those in phytoecdysteroid-containing plants and that a different range of structural modifications are found in fungi than in plants, such that 20E is often not the predominant ecdysteroid in a mycoecdysteroid profile and the ergostane-type ecdysteroid analogues are often related to cerevisterol ([22*E*,24*R*]-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -trihydroxyergosta-7,22-diene). Since mushroom fruiting bodies are susceptible to attack by invertebrate pests (predominantly, but not exclusively, soil nematodes and dipteran sciarid [fungus gnats], phorid [humpbacked flies], and cecid [midges] flies; Singh and Sharma 2016), it is possible that mycoecdysteroids also serve a defensive function. No studies have been performed on the biosynthesis of mycoecdysteroids, so it is currently not possible to say if they are endogenously synthesized and taken up from the substrate or a precursor is taken up and modified by the fungus. An extract of the database, focusing on the edible mushrooms which have been investigated for ecdysteroids and ecdysteroid-related compounds, is presented in Table 5. It is clear that currently very few species of edible mushrooms have been examined for the presence of ecdysteroids and ecdysteroid-related compounds. The most extensively studied species is *Polyporus umbellatus*, which is not only edible but it is used extensively in traditional Chinese, Japanese and Indian medicines, primarily to treat oedema and as a diuretic, but also for other conditions, and without toxicity or side-effects (Zhao 2013).

### 2.5.2 Dietary Supplements

At the present time, 20E or extracts of ecdysteroid-containing plants (particularly *Pfaffia glomerata*; Brazilian ginseng) are incorporated into protein supplements aimed at body builders and sportsmen with the stated claim of enhancing the anabolic effect. With the growing interest in the potential of ecdysteroids to delay or reduce the impacts of age-related or genetically determined muscle wasting conditions or, more generally, to counteract various stresses as adaptogens, it is to be expected that range of ecdysteroid-supplemented foods will increase significantly in the near future and that they will be of interest to wider sections of society.

**Table 5** Edible fungal species for which the fruiting bodies have been assessed for the presence or absence of ecdysteroids or ecdysteroid-related compounds. Where possible, the quantities determined and the methods used are also given. This table summarizes and expands the information provided in the reviews by Kovganko (1999) and Mamadaliyeva (2013)

Latin name	Family	Common name	Category	Ecdysteroids and ecdysteroid-related compounds	Reference & Method
<i>Agaricus blazei</i> ( <i>A. subrufescens</i> )	Agaricaceae	Almond mushroom, himematsutake	Commercially cultivated	Cerevissterol (2.3 mg from 5 kg fw), 3 $\beta$ ,5 $\alpha$ -dihydroxy-6 $\beta$ -methoxyergosta-7,22-diene (3.4 mg), 3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,9 $\alpha$ -tetrahydroxyergosta-7,22-diene (6.3 mg), 3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -trihydroxyergosta-7,22-diene-6-one (5.7 mg)	Kawagishi et al. (1988); isolation and spectroscopic identification
<i>Agaricus bisporus</i>	Agaricaceae	Common mushroom	Commercially cultivated	Chestnut: 0.32 $\mu$ g 20E eq./g dw White 1: 20.1 20E eq. $\mu$ g/g dw White 2: 95.2 20E eq. $\mu$ g/g dw	Findeisen (2004); ecdysteroid EIA (DBL2 antiserum)
<i>Amanita muscaria</i>	Amanitaceae	Fly agaric	Edible only after cooking	–	Biophytis (unpublished); HPLC-MS/MS for 20E
<i>Amanita rubescens</i>	Amanitaceae	Blusher	Edible only after cooking	–	Dinan (unpublished); RIA for ecdysteroids (DBL1 antiserum)
<i>Boletus edulis</i>	Boletaceae	Edible boletus	Commercially harvested from the wild	–	Dinan (unpublished); RIA for ecdysteroids (DBL1 antiserum)
					Dinan (unpublished); RIA for ecdysteroids (DBL1 antiserum)

<i>Calvatia cyathiformis</i>	Lycoperdaceae	Purple-spored puffball	Edible wild species	Calvasterone [(6,6'R)-biergosta-4,4',7,7',22,22'-hexaene-3,3'-dione]	Kawahara et al. (1993); isolation and spectroscopic identification
				Cyathisterone (ergosta-7,22-diene-3,6-dione; 30 mg from 4 kg fw), cyathisterol (8 $\beta$ -hydroxyergost-4,6,22-trien-3-one; 80 mg), ergosta-4,7,22-triene-3,6-dione (300 mg), ergosta-4,6,8(14),22-tetraen-3-one (200 mg)	Kawahara et al. (1994); isolation and spectroscopic identification
<i>Flammulina velutipes</i>	Physalacriaceae	Enoki mushroom, velvet shank, winter fungus	Commercially cultivated	Calvasterol A (14 $\alpha$ -hydroxyergosta-4,7,9,22-tetraen-3,6-dione; 10 mg from 4 kg fw), calvasterol B (9 $\alpha$ ,14 $\alpha$ -dihydroxyergosta-4,7,22-trien-3,6-dione; 15 mg from 4 kg fw),	Kawahara et al. (1995); isolation and spectroscopic identification
				5 $\alpha$ ,8 $\alpha$ -Epidioxy-(24S)-ergost-6-en-3 $\beta$ -ol (0.9 mg from 4.1 kg fw), (22E,24R)-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\alpha$ ,9 $\alpha$ -tetrol (0.2 mg), (22E,24R)-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (0.5 mg), (24S)-ergost-7-ene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (0.2 mg)	Yaoita et al. (1998); isolation and spectroscopic identification

(continued)

Table 5 (continued)

Latin name	Family	Common name	Category	Ecdysteroids and ecdysteroid-related compounds	Reference & Method
<i>Ganoderma lucidum</i>	Ganodermataceae	Lingzhi	Commercially exploited for nutritional and dietary products	Ergosta-4,7,22-triene-3,6-dione (314 mg from 5.32 kg fw)  6 $\alpha$ -Hydroxyergosta-4,7,22-trien-3-one, 6 $\beta$ -hydroxyergosta-4,7,22-trien-3-one	Hirotsu et al. (1987); spectroscopic identification  Nishitoba et al. (1988); isolation and spectroscopic identification
<i>Grifola frondosa</i>	Meripilaceae	Maitake, hen-of-the-woods	Commercially harvested from the wild	(22E,24R)-Ergosta-7,9(11),22-triene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (2.7 mg from 20 kg fw), (22E,24R)-ergosta-7,9(11),22-triene-3 $\beta$ ,5 $\alpha$ ,6 $\alpha$ -triol (1.5 mg), 3 $\beta$ ,5 $\alpha$ -dihydroxy-(22E,24R)-ergosta-7,22-dien-6-one (1.1 mg), 3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -trihydroxy-(22E,24R)-ergosta-7,22-dien-6-one (4.3 mg), (22E,24R)-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\alpha$ -triol (1.0 mg), (22E,24R)-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (8.2 mg), (22E,24R)-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,9 $\alpha$ -tetrol (4.7 mg)	Ishizuka et al. (1997); isolation and spectroscopic identification
<i>Hericium erinaceus</i>	Hericaceae	Lion's mane, monkey head	Commercially cultivated/commercially harvested from the wild/used as food additive	Cerevissterol (73 mg from 3.8 kg dw), cerevissterol-3-glucoside (29 mg), 3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -trihydroxyergosta-7,22-dien-6-one (32 mg)	Takaishi et al. (1991); isolation and spectroscopic identification

<i>Hypsizygus tessellatus</i> ( <i>H. marmoratus</i> )	Tricholomataceae	Beech mushroom	Commercially cultivated/edible only after cooking	<p>5<math>\alpha</math>,9<math>\alpha</math>-Epidioxo-3<math>\beta</math>-hydroxy-(22<i>E</i>,24<i>R</i>)-ergosta-7,22-dien-6-one (8.4 mg from 4.3 kg fw), 5<math>\alpha</math>,9<math>\alpha</math>-epidioxo-3<math>\beta</math>-hydroxy-(24<i>S</i>)-ergost-7-en-6-one (4.2 mg), 3<math>\beta</math>,5<math>\alpha</math>,9<math>\alpha</math>-trihydroxy-(24<i>S</i>)-ergost-7-en-6-one (1.0 mg), 3<math>\beta</math>,5<math>\alpha</math>,9<math>\alpha</math>,14<math>\alpha</math>-tetrahydroxy-(22<i>E</i>,24<i>R</i>)-ergosta-7,22-dien-6-one (1.4 mg), 3<math>\beta</math>,5<math>\alpha</math>,9<math>\alpha</math>-trihydroxy-(22<i>E</i>,24<i>R</i>)-ergosta-7,22-dien-6-one (3.6 mg), (24<i>S</i>)-ergost-7-ene-3<math>\beta</math>,5<math>\alpha</math>,6<math>\beta</math>-triol (12.8 mg), (22<i>E</i>,24<i>R</i>)-ergosta-7,22-diene-3<math>\beta</math>,5<math>\alpha</math>,6<math>\beta</math>-triol (38 mg), (22<i>E</i>,24<i>R</i>)-ergosta-7,22-diene-3<math>\beta</math>,5<math>\alpha</math>,6<math>\alpha</math>,9<math>\alpha</math>-tetrol (1.4 mg), ergosta-7,24(28)-diene-3<math>\beta</math>,5<math>\alpha</math>,6<math>\beta</math>-triol (0.3 mg)</p>	Yaoita et al. (1998); isolation and spectroscopic identification
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(continued)

Table 5 (continued)

Latin name	Family	Common name	Category	Ecdysteroids and ecdysteroid-related compounds	Reference & Method
<i>Lentinula edodes</i> ( <i>Lentinus edodes</i> )	Marasmiaceae	Shiitake mushroom	Commercially cultivated	3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -Trihydroxy-(22 <i>E</i> ,24 <i>R</i> )-2,2-methylergosta-7,22-dien-6-one (0.5 mg from 4.7 kg fw), 3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -trihydroxy-(24 <i>S</i> )-ergost-7-en-6-one (0.9 mg), 3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ ,14 $\alpha$ -tetrahydroxy-22 <i>E</i> ,24 <i>R</i> )-ergosta-7,22-dien-6-one (1.0 mg), 3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -trihydroxy-(22 <i>E</i> ,24 <i>R</i> )-ergosta-7,22-dien-6-one (17.1 mg), (22 <i>E</i> ,24 <i>R</i> )-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\alpha$ ,9 $\alpha$ -tetrol (1.3 mg), (22 <i>E</i> ,24 <i>R</i> )-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\alpha$ -triol (0.6 mg), (22 <i>E</i> ,24 <i>R</i> )-ergosta-7,9(11),22-triene-3 $\beta$ ,5 $\alpha$ ,6 $\alpha$ -triol (0.6 mg), (22 <i>E</i> ,24 <i>R</i> )-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (3.3 mg), (22 <i>E</i> ,24 <i>R</i> )-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,9 $\alpha$ -tetrol (6.2 mg)	Yaoita et al. (1998); isolation and spectroscopic identification
<i>Pholiota microspora</i> ( <i>P. nameko</i> )	Strophariaceae	Nameko, butterscotch mushroom	Usually eaten cooked	3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -Trihydroxy-(24 <i>S</i> )-ergost-7-en-6-one (1.0 mg from 3.0 kg fw), 4 (0.7 mg), 3 $\beta$ ,5 $\alpha$ ,9 $\alpha$ -trihydroxy-(22 <i>E</i> ,24 <i>R</i> )-ergosta-7,22-dien-6-one (3.0 mg), (22 <i>E</i> ,24 <i>R</i> )-ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\alpha$ ,9 $\alpha$ -tetrol (0.6 mg), (24 <i>S</i> )-ergost-7-ene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (0.2 mg)	Yaoita et al. (1998); isolation and spectroscopic identification



<p><i>Pleurotus ostreatus</i></p>	<p>Pleurotaceae</p>	<p>Oyster mushroom</p>	<p>Commercially cultivated/ commercially harvested from the wild</p>	<p>5<math>\alpha</math>,9<math>\alpha</math>-Epidioxo-3<math>\beta</math>-hydroxy-(22<i>E</i>,24<i>R</i>)-ergosta-7,22-dien-6-one (1.9 mg from 2.7 kg fw), 3<math>\beta</math>,5<math>\alpha</math>,9<math>\alpha</math>-trihydroxy-(24<i>S</i>)-ergost-7-en-6-one (0.6 mg), 3<math>\beta</math>,5<math>\alpha</math>,9<math>\alpha</math>,14<math>\alpha</math>-tetrahydroxy-(22<i>E</i>,24<i>R</i>)-ergosta-7,22-dien-6-one (0.4 mg), 3<math>\beta</math>,5<math>\alpha</math>,9<math>\alpha</math>-trihydroxy-(22<i>E</i>,24<i>R</i>)-ergosta-7,22-dien-6-one (3.5 mg), (22<i>E</i>,24<i>R</i>)-ergosta-7,22-diene-3<math>\beta</math>,5<math>\alpha</math>,6<math>\alpha</math>,9<math>\alpha</math>-tetrol (0.4 mg), 3<math>\beta</math>,5<math>\alpha</math>-dihydroxy-(22<i>E</i>,24<i>R</i>)-ergosta-7,22-dien-6-one (0.2 mg), (22<i>E</i>,24<i>R</i>)-ergosta-7,22-diene-3<math>\beta</math>,5<math>\alpha</math>,6<math>\beta</math>-triol (13.4 mg), (24<i>S</i>)-ergost-7-ene-3<math>\beta</math>,5<math>\alpha</math>,6<math>\beta</math>-triol (0.2 mg)</p>	<p>Yaoita et al. (1998); isolation and spectroscopic identification</p>
<p>0.07 <math>\mu</math>g 20E eq./g dw</p>					

(continued)

Table 5 (continued)

Latin name	Family	Common name	Category	Ecdysteroids and ecdysteroid-related compounds	Reference & Method
<i>Polyporus umbellatus</i>	Polyporaceae	Lumpy bracket, umbrella polypore	Only young fruiting bodies are edible	Polyporsterones A (300 mg from 100 kg dw), B (300 mg), C (26 mg), D (8 mg), E (22 mg), F (10 mg) and G (10 mg) (20S,22R,24R)-16,22-Epoxy-3 $\beta$ ,14 $\alpha$ ,23 $\beta$ ,25-tetrahydroergost-7-en-6-one (4 mg from 39.2 kg dw), (23R,24R,25R)-23,26-epoxy-3 $\beta$ ,14 $\alpha$ ,21 $\alpha$ ,22 $\alpha$ -Tetrahydroergost-7-en-6-one (8 mg)	Findeisen (2004); EIA of ecdysteroids (DBL2 antiserum) Ohsawa et al. (1992); isolation and spectroscopic identification Zhou et al. (2007); isolation and spectroscopic identification
				Polyporoids A (2 mg from 1 kg dw), B (7 mg) and C (3 mg), polyporsterones A (10 mg), B (14 mg), C (18 mg) and G (3 mg), ergosta-7,22-diene-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (3 mg) (22E,24R)-Ergosta-6-en-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (0–0.040 mg/g dw), (22E,24R)-ergosta-6,22-dien-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol	Sun and Yasukawa (2008); isolation and spectroscopic identification Zhao et al. (2009); HPLC-MS

<i>Tapinella atrotomentosa</i> ( <i>Paxillus atrotomentosus</i> )	Tapinellaceae	Velvet roll-rim, velvet-footed pax	Only young fruiting bodies are edible	(0–0.073 mg/g dw), polyporusterone F (trace – 0.087 mg/g dw) Paxillosterone (356 mg from 6.65 kg fw), paxillosterone 20,22-p-hydroxybenzylidene acetal (5.5 mg), atrotosterones A (41 mg), B (3.9 mg) & C (5.5 mg), 25-hydroxyatrotosterones A (11.9 mg) & B (4.2 mg), 20E (41 mg)	Vokáč et al. (1998); isolation and spectroscopic identification
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## 2.6 Safety: Toxicity and Side Effects

### 2.6.1 Data on Low Toxicity and Absence of Negative Side Effects

Ogawa et al. (1974) determined LD<sub>50</sub>s for ingested 20E or inokosterone in mice of >9 g/kg and an LD<sub>50</sub>s of 6.4 g/kg and 7.8 g/kg for i.p.-injected 20E and inokosterone, respectively. Thus, ecdysteroids are regarded as nontoxic to mammals. Also, no effects were seen after the administration of these two ecdysteroids to bullfrogs or rabbits. The two ecdysteroids did not have sex hormonal or, interestingly in the context of more recent studies, anti-inflammatory or anabolic effects in rats.

### 2.6.2 Ecdysteroids and Cancer

El Mofty et al. (1987, 1994) have claimed that E is carcinogenic to toads and mice, but the certainty of these findings has been questioned (Sláma and Lafont 1995; Lafont and Dinan 2009) on the basis of the low doses used, relative to the amounts of ecdysteroids expected in the animals' normal diets. Neves et al. (2016) believe that 20-hydroxyecdysone is genotoxic in a rat bone marrow micronuclei assay and an *Allium cepa* (onion) cell abnormality assay. However, other reports have claimed that ecdysteroids have no effects on tumor cell growth or even possess antitumor activity either alone (Hirono et al. 1969; Lagova and Valueva 1981; Takasaki et al. 1999) or in conjunction with known anticancer agents (Konovalova et al. 2002; Martins et al. 2012, 2015). Studies of this sort should be conducted with ecdysteroids of the highest degree of purity, which may not always have been the case.

### 2.6.3 Ecdysone and Effects on the Kidney

Recently (Lu et al. 2018a, b), it has been suggested that ecdysone (E, which is rarely a major phytoecdysteroid) has detrimental effects on renal function in mice at a dose of 6 µg/g/d, bringing about glomerular injury and proteinuria. The authors propose that E acts as a mimic of aldosterone, activating the mineralocorticoid nuclear receptor (MR), and that the detrimental effects can largely be blocked by spironolactone, an antagonist of MR. As yet, it is not known if these effects are common to other ecdysteroids, e.g., 20E.

### 2.6.4 Potential for Environmental Endocrine Disruption

As indicated above, the toxicity and safety data for 20E and the few other ecdysteroid analogues which have been in any way assessed do not indicate that dietary or environmental contact would have negative impacts on mammals. However, significant releases of ecdysteroids into the environment, either as a by-product of the isolation of large amounts of 20E for pharmaceutical/nutraceutical purposes or as the consequence of excretion of 20E or its metabolites by those taking doses (probably 0.5–5 g/person/day) for medical, health, or aesthetic reasons, might have serious detrimental effects on arthropods and other invertebrates, with aquatic insects and crustaceans being particularly susceptible if waste water and sewage are not appropriately treated before it enters natural water courses (Lafont and Dinan 2009). It will be necessary to determine the extent to which ecdysteroids are subjected to microbial degradation in passing through a sewage works and whether

they can be readily removed from purified water by adsorption onto charcoal or reverse-phase materials. One can also envisage that signal invertebrate species (e.g., Asada et al. 2014) or cell-based bioassays (Dinan 1995a; Pounds et al. 2002) could be used to detect and monitor for the release of ecdysteroid agonists into the environment and thereby avoid serious environmental impacts.

## 2.7 Marketed Products

The Verde Vital series of commercial supplements ([www.verdevital.de](http://www.verdevital.de)) contain spinach powder in preparations with other plant extracts, vitamins, minerals, fatty acids, creatine, resveratrol, etc. with designations for osteoporosis, muscle, joint, heart, and circulation problems and for improved stamina. The website indicates that the daily dose for most of the supplements in the series contains 400–900 mg of “ecdysone,” but this seems questionable since E is a minor ecdysteroid in spinach, and it may be that the amounts refer to the mass of spinach powder, in which case the amount of 20E would be ca.0.5–1 mg/day. Additionally, it has been questioned whether the ecdysteroid profile of these products is compatible with spinach alone, and evidence has been provided that ecdysteroids from another plant species (*Cyanotis arachnoidea*) have been added; quantification of 20E revealed 2–24 mg in daily doses of the various preparations (Hunyadi et al. 2016).

## 2.8 Relevant Patents

Over 275 separate patents concerning phytoecdysteroid preparations and their pharmaceutical, medical, and nutraceutical applications have been published over the last 50 years, with the number per annum increasing exponentially over the period. It is not possible to review all these patents in the context of this chapter, so we shall add the list of relevant patents to Ecdybase. Also, it is possible to access most patents on Google Patents, searching by criteria (key terms, year, inventor, etc.), and often the patent can be read in several languages.

## 2.9 Perspectives

Present evidence indicates that ecdysteroids are currently rare, nontoxic components of the human diet, with only those relatively small population groups (Andean Indians etc.) which consume a significant proportion of amaranth crops (quinoa, etc.) potentially receiving significant amounts of ecdysteroids, mainly 20E. It should be determined how much ecdysteroid those for whom quinoa or other amaranths are staple foods really do consume once it has been cooked, whether the pharmacokinetics of ecdysteroids are different in these people through genetic selection or induction of enzymes or other relevant biological processes and whether they are

less susceptible to conditions which the consumption of ecdysteroids is believed to counteract (e.g., sarcopenia, type 2 diabetes).

One can expect the Western diet to undergo major changes over the next few decades, driven by two major factors: climate change and the need to reverse the spread of metabolic syndrome. Reduction in the consumption of meat, together with greater proportion and diversity of vegetables in the diet, the medicinal use of ecdysteroids, social health uses as adaptogens, anabolics, etc., can all be expected to increase the intake of phytoecdysteroids. Enhanced levels of ecdysteroid in crop plants could arise from increasing the levels of these compounds by GM or non-GM means for crop protection purposes or for health/nutraceutical reasons (e.g., using the diet to help counteract the effects of aging and/or inactivity).

Clearly, for this to become feasible, we need fundamental data on the elucidation and characterization of the biosynthetic pathway(s) for ecdysteroids in plants and a thorough understanding of how their synthesis and metabolism are regulated. Only then will it be possible to devise rational strategies for enhancing ecdysteroid levels in other crop species and also for controlling the regulation so that the desired analogues are produced at the desired concentrations, at the right time, and in the correct parts of the plant to optimize crop protection or nutraceutical value.

On the other side of the equation is our currently incomplete understanding of the mode(s) of action, pharmacokinetics, and metabolism of ecdysteroids in mammals and particularly in humans. The low toxicity and plethora of beneficial effects which have been ascribed to ecdysteroids are encouraging for their future development, but the low bioavailability means that ingested doses of 20E have to be relatively large, which increases cost and raises supply problems (especially as 20E can only be realistically obtained by isolation from a rich plant source; 20E is chemically too complicated for efficient commercial chemical synthesis) and the potential release of an invertebrate endocrine disruptor into the environment.

In theory, bioavailability might be increased by improved formulation, modified routes of application (e.g., sublingual or dermal implants; Dittrich et al. 2000), or the use of new analogues with greater potency (improved bioavailability, reduced metabolism and/or rate of excretion, enhanced biological activity), but since 20E is currently the only ecdysteroid which can be isolated economically in adequate amounts and semisynthetic routes from this analogue would have to be high yield, the options are presently restricted.

The mode(s) of action of ecdysteroids in mammalian systems needs to be fully established. It is clear that it is very different from the main modes of action of ecdysteroids in insects or crustaceans and that it involves signalling from the plasma membrane, but the precise nature and whether the same or different signalling systems operate in different mammalian target tissues need to be determined. As for vertebrate steroid hormones, membrane-, as well as nuclear-, receptors for ecdysteroids have relatively recently been detected in invertebrate systems (e.g., Elmogy et al. 2004; Srivastava et al. 2005), and thus research on ecdysteroid membrane receptors in mammals and invertebrates can be expected to cross-fertilize each other.

Only after concerted effort to understand the bioavailability, mode of action, metabolism, and excretion of ecdysteroids in specific mammalian systems will it be possible to fully exploit the extensive range of analogues which already exist to identify more active analogues and more potent analogues which possess better bioavailability or resistance to metabolism and excretion.

The historical and continuing traditional medicinal uses of ecdysteroid-containing plants and the large number of scientific publications attesting to a plethora of essentially beneficial pharmacological effects in mammals, including humans, attest to the great promise and strong potential of ecdysteroids for the development of a new class of drug for the treatment or at least slowing the progress of several currently intractable muscle wasting diseases and modern societal syndromes, such as obesity or type 2 diabetes. The way to reach this goal is long and demanding, as it requires companies to invest much time, effort, and money into the identification of a good plant source of 20E, secure a reliable supply of the compound, develop an economically viable industrial-scale procedure for the preparation of pharmaceutical grade drug; obtain thorough evidence for the effectiveness, efficacy, pharmacokinetics, and mode of action in animals and humans; and successfully complete all the necessary clinical trials. Preclinical and clinical trials of 20E have recently been initiated for a number of designated disease conditions (sarcopenia, Duchenne muscular dystrophy) and can be expected to be completed within the next year. This is reassuring because although the first data on the effects of ecdysteroids on mammals goes back almost 50 years, the area had been very deficient in thorough clinical trials because enough pure 20E simply was not available.

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### 3 Conclusions

Phytoecdysteroids accumulate in a wide range of plant species where they serve to deter invertebrate predation. In this context, ecdysteroids are currently natural components of a limited number of natural food plants, with quinoa and spinach being responsible for the largest amounts of ecdysteroids in the human diet. 20E, at least, is nontoxic and appears to have many positive biological effects in mammalian systems. Thus, one could envisage the amount of dietary ecdysteroids significantly increasing in the near future, if (i) ecdysteroid levels are elevated in other crop species either to enhance their resistance to predation or to take advantage of the promising biological activities of the ecdysteroids, (ii) phytoecdysteroids become established as therapeutic agents for muscle wasting diseases or type 2 diabetes, and (iii) phytoecdysteroid-containing preparations become more popular as anabolic and adaptogenic dietary supplements among specific groups (sportsmen, body builders, etc.) or as tonics for the general public. There remains a strong need to elucidate biosynthesis and regulation of phytoecdysteroid biosynthesis and to clarify the pharmacokinetics and mode of action in responding mammalian cells, but ecdysteroids are showing considerable promise and potential as therapeutic agents of the future.

## 4 Cross-References

- ▶ [Antioxidants in Diets and Food](#)
- ▶ [Triterpenoids](#)

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