8 Commercial-Scale Tissue Culture for the Production of Plant Natural Products: Successes, Failures and Outlook

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Abbreviation

PNP Plant natural product

8.1 Introduction

Plant tissue culture can be broadly defined as the *in vitro* aseptic maintenance of cells, tissues or organs under defined conditions. The pioneering developments for sustaining isolated plant cells date back to the early 1900s [1]. Haberlandt already introduced the concept of totipotency, which refers to the unique genetic potential to regenerate a whole plant from a single somatic plant cell (validated in the 1960s [2]). During the decades following the initial discoveries, tissue cultures were established from seed embryos, cambial tissue, roots, and many additional plant parts (reviewed in [3]). Biotechnological applications of plant tissue culture for the production of medicinally relevant plant natural products (PNPs) emerged in the 1960s and 1970s [4]. Over the last forty years, several companies have had research and development programs aimed at optimizing plant tissue culture [5]. However, the large-scale market introduction of plant tissue culture products has mostly been confined to (i) early successes with isolated metabolites for the cosmetic (shikonin) [6] and pharmaceutical (paclitaxel) [7] (**see also Chap. 7 of this book**) industries and (ii) more recent uses of whole cell extracts as drinks, dietary supplements and food additives [5] (Table 8.1). The production of therapeutically relevant proteins in plant tissue cultures, which is also an area of very active research and development efforts, has been reviewed recently [8] and will not be covered here.

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Table 8.1 Commercial-scale production of PNPs by tissue culture technologies

Product	Production system	Manufacturer, trademark, and scale of production	Uses	Source
Berberine (alkaloid)	*Coptis japonica*	Mitsui Chemicals Inc. (Japan) (not commercialized) (750 l fermenter)	Pharmaceutical	[6]
Bilberry cells (polyphenol extract)	*Vaccinium* sp., cell suspension cultures	Diana Plant Sciences (USA), ActivBerry® (to be launched soon)	Dietary supplement	
Cocoa cells (polyphenolic extract)	*Theobroma cacao* L., cell suspension cells	Diana Plant Sciences (USA), Cocovanol® (launched in 2013)	Dietary supplement	www.diana-group.com/
Echinacea cells (polysaccharide extract)	*Echinacea* sp.	Diversa (Germany) (not commercialized) (75,000 l fermenters)	Pharmaceutical (immunostimulant)	[5]
Ginseng cells (ginsenoside extract)	*Panax ginseng* L., cell suspension cells	Nitto Denko Corp. (Japan), (since 1989; 25,000 l fermenters)	Food additive	www.nitto.com [15]
	Panax ginseng L., stem cell cultures	Unhwa Corp. (Korea), Ddobyul®, LifeAde (since 2011)	Energy drink, Cosmetics (creams)	www.unhwa.com [16]

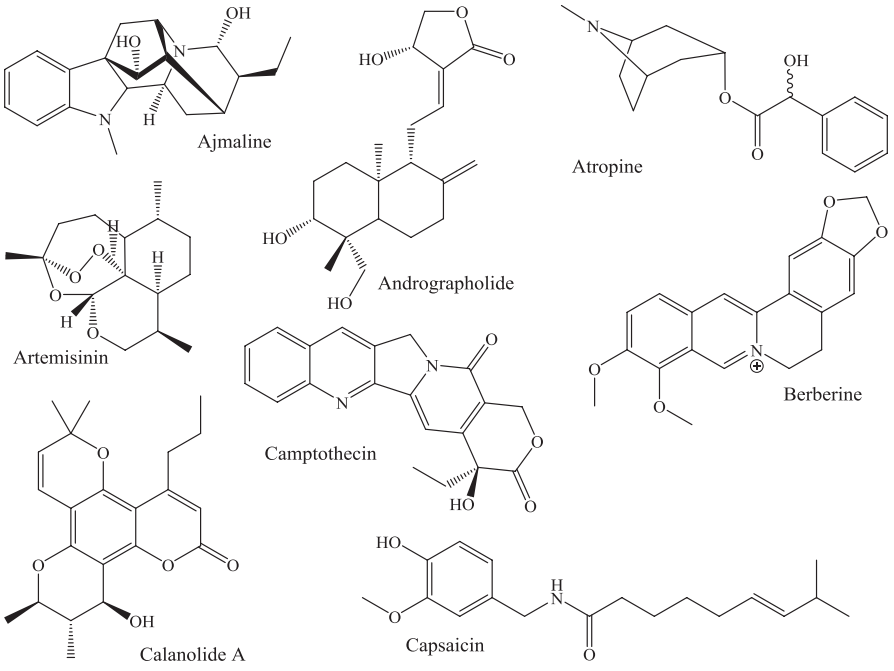
(continued)

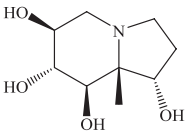
Paclitaxel (diterpene alkaloid)	*Taxus chinensis* L., cell suspension cultures	Phyton Biotech Inc. (USA/Germany) and Bristol-Myers Squibb (USA)	Pharmaceutical (several types of cancer)	www.phytonbiotech.com/ [7]
		Taxol® (since 2002)		
		(75,000 l fermenters at Phyton)		
		Samyang Biopharm (Korea)		www.samyangbiopharm.com
		Genexol® (since 2007)		[11]
Shikonin (naphthoquinone)	*Lithospermum erythrorhizon* Siebold & Zucc., cell suspension cultures	Mitsui Chemicals Inc. (Japan) and Kenebo Cosmetics Inc. (Japan)	Cosmetics	www.kanebo.com [6]
		Lady 80 BIO lipstick (started in 1983; 750 l fermenter)		

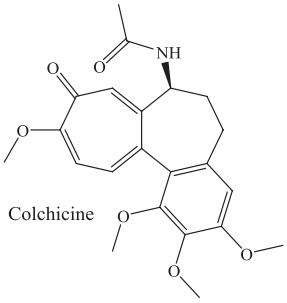
8.2 Commercial Products from Plant Tissue Culture

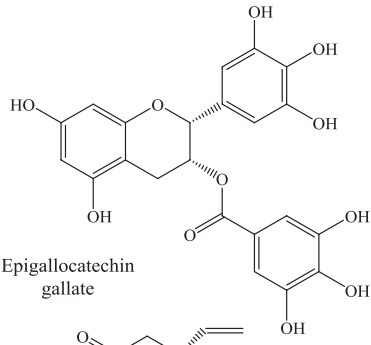
Shikonin The naphthoquinone pigment shikonin (Fig. 8.1) was produced by Mitsui Petrochemical Industries Ltd. (now Mitsui Chemicals Inc.) in *Lithospermum erythrorhizon* Siebold & Zucc. cell suspension cultures, formulated into the Lady 80 BIO lipstick by Kanebo Cosmetics Inc., and introduced to the market in 1983 (www.kanebo.com/aboutus/history/). High shikonin yields (up to 23% (w/w) of dry biomass) were achieved in 750 L bioreactors using batch processing [6]. It is difficult to assess how long Mitsui pursued the biotechnological production of shikonin, but it appears that a combination of unfavorable factors - delays in the regulatory approval of the product, a limited market size (150 kg per year), a decrease in product price ($ 4000 per kg in 1988), and high operating costs of fermenters - led to a termination of the project [9].

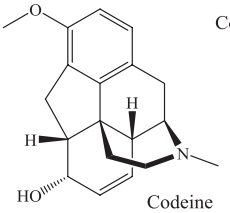
Paclitaxel Phyton Catalytic (now Phyton Biotech; owned by DFB Pharmaceuticals) began the scale-up of cell suspension cultures *Taxus chinensis* (Pilg.) Rehder in the early 1990s with the goal of a large-scale production of paclitaxel (taxol®) (see also Chap. 7 of this book) (Fig. 8.1), which had been in high demand for the treatment of

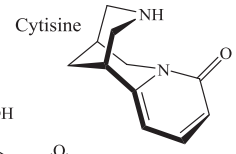


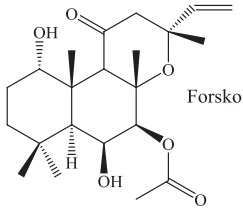

Castanospermine

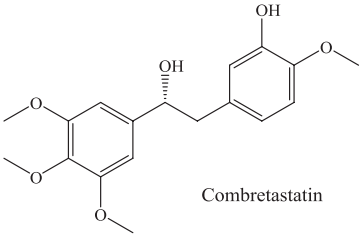

Colchicine

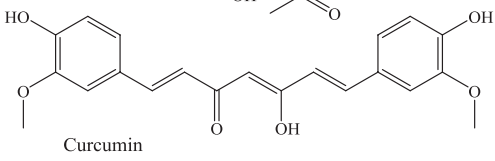

Epigallocatechin gallate

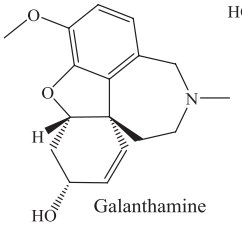

Codeine

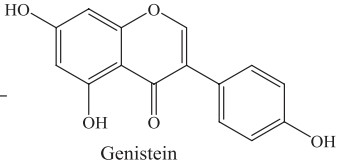

Cytisine

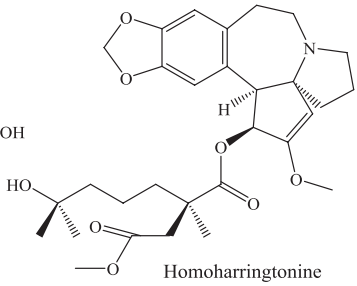

Forskolin

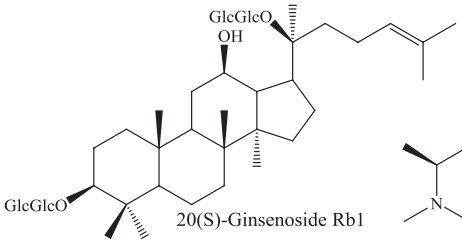

Combretastatin

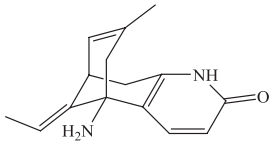

Curcumin

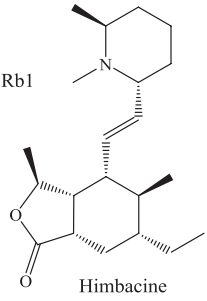

Galanthamine

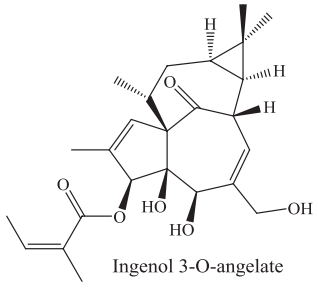

Genistein

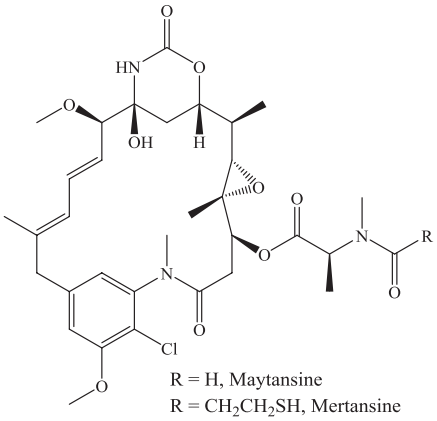

Homoharringtonine


20(S)-Ginsenoside Rb1

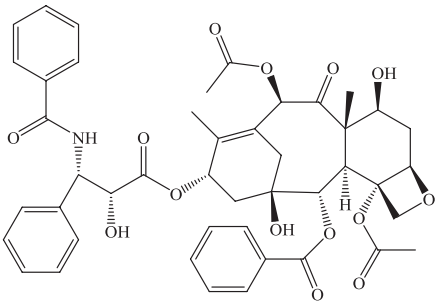

Huperzine A


Himbacine

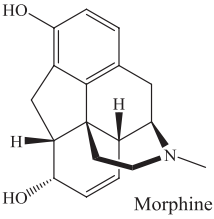

Ingenol 3-O-angelate



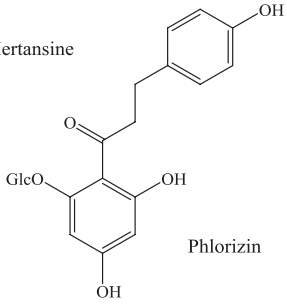
R = H, Maytansine
R = CH_2CH_2SH, Mertansine



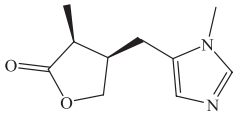
Paclitaxel (taxol™)



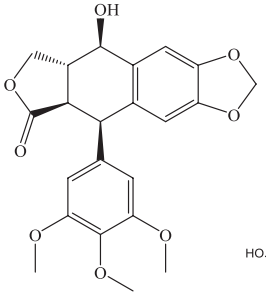
Morphine



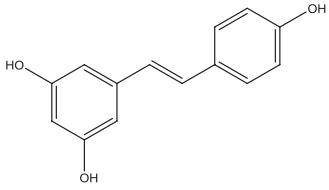
Phlorizin



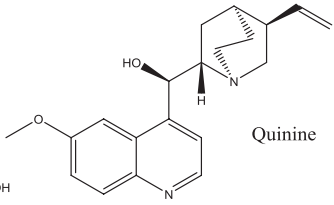
Pilocarpine



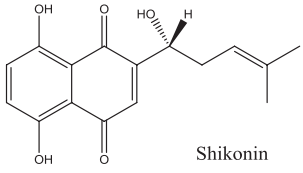
Podophyllotoxin



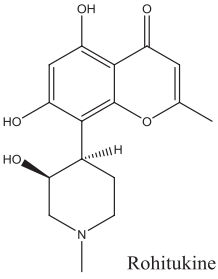
Resveratrol



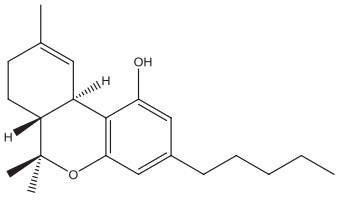
Quinine



Shikonin



Rohitukine



Tetrahydrocannabinol

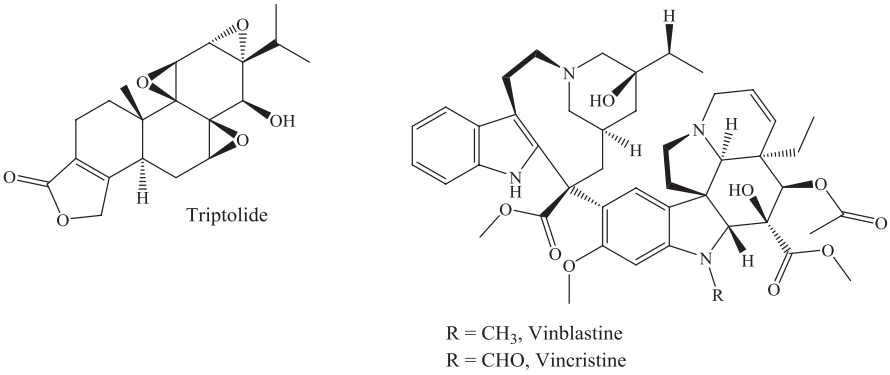


Fig. 8.1 Structures of PNPs discussed in this article (in alphabetical order). Abbreviations: *Glc* glucose

various forms of cancer (more details in). Following the acquisition of a manufacturing plant near Hamburg (Germany), the capacity increased to 75,000 L fermenters, which are still the largest vessels for plant tissue culture today. The plant cell fermentation technology was licensed to Bristol-Myers Squibb in 1995 to jointly commercialize paclitaxel production. In 2004, Bristol-Myers Squibb received the prestigious Presidential Green Chemistry Challenge Award of the American Chemical Society, following a transition, in 2002, to plant tissue culture as the sole source for its blockbuster anti-cancer treatment [10]. Patent protection for taxol® ended in 2000 and generic competition emerged, including a new tissue culture and formulation process for paclitaxel (and related taxanes) by Samyang Biopharm, which operates 35,000 L fermenters on its premises in Daejeon, South Korea [11].

Triterpene Saponin-Containing Cells In the mid-1980s, Nitto Denko Corp. (Ibaraki, Japan) began developing an industrial-scale process for the cultivation of ginseng cells (*Panax ginseng*). The product was approved for commercialization in Japan in 1988. The ginseng cells were shown to have contents of triterpene saponins (more specifically, ginsenosides (Fig. 8.1), which are considered to be the active principles of ginseng extracts [12]), that were very similar to those of field-cultivated ginseng [13]. The powder and extracts from the ginseng cell cultures are used to produce additives for foods, drinks and cosmetics [14]. Nitto Denko is employing bioreactors of up to 25,000 L in a two-stage production process, achieving a biomass productivity of 20 g dry weight per liter in 4 weeks of culture [15]. More recently, a collaborative team of scientists at Unhwa Corp. (Jeonju, South Korea) and the University of Edinburgh (United Kingdom) introduced cambial meristematic cells as a potentially cost-effective and reliable source of undifferentiated cells [16]. Based on information on the company website (www.unhwa.com), this technology has been adapted to generate wild ginseng cambial meristematic cells and a skincare product containing these cells (Ddobyul®) is now available commercially. More detailed information about the process (conditions and scale) is not available in the public domain at this time.

Polyphenol-Containing Cells DianaPlantSciences (Portland, OR; acquired by Symrise AG in 2014) has been developing processes for employing cell suspension cultures rich in polyphenols, which are formulated into cosmetics and dietary supplements. Cocovanol™ (launched in 2013) is a freeze-dried powder of cocoa suspension cells that, according to information on the company website (http://www.diana-group.com/), delivers high polyphenolic content without solvent extraction and contains only trace amounts of caffeine and theobromine (the bitter alkaloids of the cacao plant) (**see also Chap. 6 of this book**). Another polyphenol-containing product, ActivBerry™, which is based on bilberry suspension cells, will purportedly be launched in the near future (based on information on company website). Further information can be extracted from the patent literature but very little technical details are given in these documents. Unfortunately, no peer-reviewed publications are available on the topic.

8.3 Considerations for Commercial Targets

The commercial advances with plant tissue culture thus far have been confined to the production of (i) structurally complex pharmaceuticals with low natural abundance and (ii) cell biomass (without prior extraction) containing PNPs with presumed health-promoting properties. A combination of factors such as production cost and reliability, market scale, regulatory burden, and consumer perception determine the likelihood for a successful market introduction. In the following paragraphs, I will discuss challenges and opportunities for commercial-scale plant tissue culture.

Cost of Natural Material The prices of raw materials containing desirable PNPs vary greatly from a few U.S. dollars per kg (e.g., garlic bulbs (*Allium sativum*) (contain sulfoxides such as alliin)) to several thousand U.S. dollars per kg (e.g., saffron stigmas (*Crocus sativus*) (contain crocin and related apocarotenoid glycosides)) [17]. The extraction cost is also determined by the concentration and the accessibility of the PNP(s) of interest. For example, while Bulgarian rose (*Rosa damascena*) petals are readily available, the bulk oil price is several thousand U.S. dollars per kg [17]. The concentrations of some plant PNPs are exceedingly low (e.g., the alkaloid vincristine (Fig. 8.1) (**see also Chap. 5 of this book**) occurs at only 0.001% of dry biomass in the leaves of Madagaskar periwinkle (*Catharanthus roseus*)) and the cost of formulations containing them can be extraordinarily high [18]. Most plant tissue culture efforts have focused on such high-value products and the history for one commercial example, the production of paclitaxel (taxol®) by plant tissue culture, is discussed briefly above (**see also Chap. 7 of this book**).

Structural Complexity The extraction of salicylates from various plant sources to treat symptoms such as pain, fever and inflammation dates back to antiquity [19]. However, with the advent of facile chemical routes in the late 1800s, acetylsalicylic acid (better known as Aspirin®) went on to become the first blockbuster synthetic

drug [20]. A large number of commercial drugs, even those with a PNP scaffold, are obtained by chemical synthesis or semi-synthesis. In many cases, the biological material serves as source and additional chemical steps generate the desired end product. At present, a plant-based production of a particular PNP, including but not limited to the use of tissue culture, is only competitive when the target molecule is structurally complex with several chiral centers [21]. A high degree of functionalization in a PNP can also be an advantage when the extraction from a plant-based matrix competes with the production in engineered microbial hosts.

A highly publicized example is artemisinin (Fig. 8.1), a sesquiterpene lactone employed in antimalarial therapies. Amyris Inc. (Emeryville, CA, USA) and Sanofi (Paris, France) invested tens of millions of U.S. dollars in the development of an integrated synthetic biology/chemical synthesis platform for artemisinin; however, the commercial introduction in the market for malaria drugs has been very challenging [22]. One of the reasons is the cost of production, which still cannot compete with the extraction from artemisinin's natural source, sweet wormwood (*Artemisia annua*). Furthermore, while the production of terpenoid backbones (incl. that of amorphadiene, the precursor of artemisinin) has been achieved at fairly high titers in engineered microbes, the yields achieved for highly functionalized PNPs of plant origin have been substantially lower. Often, the pathways toward plant PNPs have only been partially elucidated, and not all genes required for transferring the pathway to microbes have been cloned yet (**see also Chaps. 7 and 5 of this book**). Moreover, the obtaining high activities from multiple plant enzymes produced recombinantly in microbial hosts can be quite challenging. In summary, while synthetic biology platform will continue to be improved and may eventually make a significant contribution to the production of plant PNPs, there are currently advantages for a plant-based production of high functionalized target molecules.

Market Size The substantial up-front investment in tissue culture facilities (fermenters) and maintenance (manpower, growth medium, and energy costs) has to be supported by cost savings (when compared with alternative methods for production) as well as an appropriate size of the market. While the development of high-yielding cell suspension cultures for the production of shikonin by Mitsui was a significant breakthrough at pilot scale, the product had only a fairly short period of success in the marketplace: (i) the price tag of cell culture-derived shikonin (approximately U.S. $ 4000 per kg) was only marginally below the cost for shikonin extracted from roots of *Lithospermum erythrorhizon* and (ii) the requirement of shikonin in 1988 was only about 150 kg per year, with a predicted annual market value of U.S. $ 600,000 [23]. As a comparison, the cost for the discovery and development efforts toward cell culture production of shikonin was estimated to have been in the tens of millions U.S. dollar range [23], and the product therefore did not have a longer term commercial future.

Regulatory Burden Most products from plant tissue culture require the same regulation as the corresponding products extracted from whole plants in the U.S. (assuming that these are not 'novel' foods or pharmaceutical ingredients). However,

the regulatory environment for registering plant tissue culture products differs significantly in various parts of the world. For example, plant tissue culture products would be treated as 'natural' in some markets, while in others they would be labeled as 'nature-identical' [24, 25]. Different federal agencies will be involved in reviewing commercialization efforts depending on if the product requires regulation as food ingredient or additive, or as active pharmaceutical ingredient [26]. Regulatory complications arise if genetic engineering is employed in plant tissue culture. While some concerns voiced by opponents of genetically engineered crops, for example the release of genetic material with potentially undesirable environmental effects, are irrelevant in plant tissue culture (which employs a closed production environment), the public perception in many countries has been that plant biotechnology in general poses undesirable risks [27]. Interestingly, some engineered organisms developed using newer gene editing technologies (including the revolutionary CRISPR/Cas9 system (acronym for Clustered Regularly Interspaced Short Palindromic Repeats and their associated nuclease (CRISPR associated protein 9)) are currently not regulated by the U.S. Department of Agriculture [28], and it remains to be seen if federal agencies in other countries will follow suit. The landscape for the commercialization of plant tissue culture-related products continues to evolve and future regulation in the area is therefore difficult to predict.

Processing into Products As discussed above, plant tissue cultures have been commercialized for the production and isolation of high-value PNPs. A very different, but equally viable, application of tissue culture technology is the direct formulation of suspension cells into cosmetics and dietary supplements. There are several potential advantages to the approach: (i) cost savings because no further processing is required, (ii) reduction of undesirable constituents because tissue culture is optimized for the accumulation of specific products in a fairly simple matrix, and (iii) high consistency of the product due to controlled growth conditions. Commercial examples include suspension cells of ginseng (www.unhwa.com) and cocoa (http://www.diana-group.com/). Several companies have purportedly experimented with additional plant tissue cultures at pilot scale (reviewed in [5]) but, while patent applications with limited technical detail are available, very few of these efforts have been described in the peer-reviewed literature. It is therefore not possible for an industry outsider to evaluate the commercial potential of formulated plant cells.

Consumer Acceptance In many countries, the demand for food additives, nutraceuticals and consumer care products carrying a 'natural' label has been increasing consistently over the last decade [29]. Plant tissue cultures can be a source of such 'natural' extracts, for which there is a marketing advantage over synthetic products. However, plant tissue culture products have not established a significant footprint in the marketplace, where plant parts are still the primary source of 'natural' extracts. Is synthetic biology, which includes the engineering of microbes for the production of metabolites originally sourced from plants, another challenge for the commercial development of plant tissue culture-derived products? Part of the answer to this

Table 8.2 Prospects for tissue culture in the production of PNPs

Disease area	Lead metabolite(s)	Source(s)	Comments on plant tissue culture opportunities	Generic name(s)	Development status
Actinic keratosis	Ingenol 3-O-angelate (or Ingenol mebutate)	*Euphorbia* spp.	Concentration in latex up to 0.2% [49]; tissue cultures have not been optimized for production of natural product	Picato	First FDA approval 2012
Alzheimer's disease	Galantamine (or Galanthamine)	*Galanthus* and other genera	Concentration in bulbs up to 0.3% of dry weight [50]; tissue cultures have not been optimized for production of natural product	Nivalin, Razdyne, Reminyl, Lycoremine	First FDA approval in 2001
	Huperzine	*Huperzia* and *Phlegmariurus* spp.	Concentration in above-ground tissues 0.04% ((52); up to 0.06% in *in vitro* propagated plants [51]; establishment of liquid cultures has not been successful	None	First approval by China FDA in 1990s; U.S. FDA lists no ongoing trials
Cancer	Camptothecin	*Camptotheca acuminata* (and other species of the same genus)	Concentration in leaves <0.4% [52]; approved drugs produced semi-synthetically; plant tissue culture yields of <0.1% [53] are not competitive	Irinotecan, Topotecan, Karenitecin, Gimatecan, CZ48	FDA approval 1996, FDA approval 2007, Phase III, Phase II, Phase I

(continued)

Table 8.2 (continued)

Disease area	Lead metabolite(s)	Source(s)	Comments on plant tissue culture opportunities	Generic name(s)	Development status
	Combretastatins A-1 and A4	*Combretum caffrum* (and other species of the same genus)	Sourcing from stems difficult; tissue cultures have not been optimized for production of natural product	OXi4503, Fosbretabulin	Phase II/III, Phase III
	Genistein	*Glycine max* (and other plants)	Concentration up to 0.5% in seeds [54]; being evaluated to reduce side effects of chemotherapy; hairy root cultures accumulate natural product at concentrations similar to natural source [55] and are not competitive	Genistein	Phase II
	Mertansine, Maytansine	*Maytenus* spp. (natural product might be of microbial origin)	Drug is a synthetic antibody conjugate; no plant source needed	Ado-trastuzumab emtansine, Kadcyla	First FDA approval 2013
	Homoharringtonine	*Cephalotaxus* spp.	Sourcing difficult; commercial product generated by semi-synthesis; root cultures accumulate natural product at <0.001% of dry weight [56] and are not competitive	Omacetaxine mepesuccinate	First FDA approval 2012

	Paclitaxel	*Taxus* spp.	Concentration in bark <0.05% of dry weight [57]; harvest from natural source unsustainable; successful commercial production in plant suspension cultures [7]	Taxol	FDA approval 1993 (ongoing clinical trials with analogs)
	Podophyllotoxin	*Podophyllum* spp.	Concentration in leaves up to 2.5% of dry weight [58]; cross-species co-cultures accumulate natural product at <0.01% of dry weight [59] and are not competitive; commercial drugs are produced semi-synthetically	Etoposide, Teniposide	FDA approval 1983, FDA approval 1992 (ongoing clinical trials with analogs)
	Triptolide	*Tripterygium wilfordii; Tripterygium regelii*	Extremely low concentration in roots (<0.01% of dry weight) [31]; unsustainable harvest of roots; root cultures excrete natural into medium at 5 mg/L [43]; continuous harvest possible; further investigation warranted	Minnelide	Phase I

(continued)

Table 8.2 (continued)

Disease area	Lead metabolite(s)	Source(s)	Comments on plant tissue culture opportunities	Generic name(s)	Development status
	Vinblastine	*Catharanthus roseus*	Concentration in leaves <0.01% of dry weight [60]; tissue cultures only produce biosynthetic intermediates; semi-synthetic approaches involving tissue cultures were developed in 1980s [61] but were not competitive; improvements have been made over the last few years but no step change has been achieved	Velban and others	First FDA approval 1961
	Vincristine	*Catharanthus roseus*	Concentration in leaves <0.001% of dry weight [60]; tissue cultures only produce biosynthetic intermediates; semi-synthetic approaches involving tissue cultures were developed in 1980s [61] but were not competitive; improvements have been made over the last few years but no step change has been achieved	Oncovin and others	First FDA approval 1963

Diabetes (type 2)	Phlorizin (Phloridzin)	*Malus* spp.	Concentration in fruit peel <0.1% of dry weight [62]; tissue cultures have not been optimized for production of natural product; commercial drugs produced by chemical synthesis	Dapagliflozin, Canagliflozin	FDA approval 2012, FDA approval 2013 (ongoing clinical trials with analogs)
Gout	Colchicine	*Colchicum* and *Gloriosa* spp.	Concentration in leaves and corms <0.2% of dry weight [63]; concentration in root cultures <0.03% [64] and therefore not competitive	Colcrys	FDA approval 2009
Heart arrhythmia	Ajmaline	*Rauwolfia* (or *Rauvolfia*) spp.	Concentration in roots >4% of dry weight [65]; concentration in hairy root cultures >0.5% [46], which could be competitive considering the challenge with procuring source material	Many	First FDA approval 1970s

(continued)

Table 8.2 (continued)

Disease area	Lead metabolite(s)	Source(s)	Comments on plant tissue culture opportunities	Generic name(s)	Development status
Hepatitis C virus	Castanospermine	*Castanospermum australe*	Concentration in various plant parts <0.1% [66]; cell suspension culture accumulate the natural product at low levels (<0.01%) [67] and are not competitive	Celgosivir	Phase II trials completed; current status unknown
HIV/AIDS	Calanolide A	*Calophyllum lanigerum* (and other species within the same genus)	Sourcing extremely difficult due to restrictions imposed by Malaysian government; no tissue culture data published in peer-reviewed literature	None	Phase I (completed but no follow-up)
Leukemia	Rohitukine	*Dysoxylum binectariferum* (possibly produced by endophytic fungus)	Drug produced by chemical synthesis; possibly not of plant origin and plant tissue culture therefore not applicable	Flavopiridol	Phase II

Malaria	Artemisinin	*Artemisia annua*	Concentration up to 1.3% of dry weight in leaves [68]; plant grows vigorously; cost of $ 250/kg [22]; concentration in hairy root cultures (<0.1% of cell biomass or 26 mg/l) [69]) is not competitive	Many	First China FDA approval in 1985; first U.S. FDA approval in 2009
Malaria	Quinine	*Cinchona* spp.	Concentration of <1% in bark [70]; concentrations of <0.1% were reported for hairy root cultures [71]; tissue culture not competitive	Many	Used widely until 1940s; rarely recommended now
Multiple sclerosis	Andrographolide	*Andrographis paniculata*	Concentration of >2% of dry weight in leaves [72]; concentration in cell suspension cultures <0.1% [73] and therefore not competitive	None	Phase I/II

(continued)

Table 8.2 (continued)

Disease area	Lead metabolite(s)	Source(s)	Comments on plant tissue culture opportunities	Generic name(s)	Development status
Myocardial infarction	Himbacine	*Galmulimima belgraveana*	Structure of commercial product significantly different from lead; produced by chemical synthesis	Vorapaxar	First FDA approval 2014
Nicotine dependence	Cytisine	*Laburnum anagyroides* (or *Cytisus laburnum*) (and other plants)	Little quantitative information available in peer-reviewed literature; commercial drug obtained by chemical synthesis [74]	Tabex, Varenicline, Chantix	Marketed in Bulgaria since 1960s, first FDA approval 2006
Obesity	Forskolin	*Coleus forskohlii* (or *Plectranthus barbatus*)	Concentration in roots <0.1% [72]; hairy root cultures accumulate natural product in slightly higher concentrations than roots [46] but are not competitive	None	Phase III (extract)
Pain	Capsaisin	*Capsicum annuum*	Concentration varies tremendously across cultivars; up to 1.5% of fruit dry weight [75]; cell suspension cultures produce <0.1% [76] and are not competitive	Qutenza, Theragen, Rezil	First FDA approval 2009

	Codeine	*Papaver somniferum*	Concentration of up to 10% in opium (dried latex of unripe seed pods) [77]; hairy root cultures produce up to 0.3% [78] and are not competitive	Many	Approved in many countries; often regulated under narcotic control laws
Pain	Morphine	*Papaver somniferum*	Concentration of up to 9% in opium (dried latex of unripe seed pods) [77]; hairy root cultures produce up to 0.3% [78] and are not competitive	Many	Approved in many countries; often regulated under narcotic control laws
	Tetrahydrocannabinol	*Cannabis sativa*	Concentration of up to 20% of dry weight have been reported [79]; many studies on various types of tissue cultures, but quantities are not competitive [79]; only synthetic product approved by U.S. FDA	Dronabinol	First FDA approval 1992 (other products at various stages of clinical development)

(continued)

Table 8.2 (continued)

Disease area	Lead metabolite(s)	Source(s)	Comments on plant tissue culture opportunities	Generic name(s)	Development status
Diverse ailments	Atropine, Hyoscyamine, scopolamine	Many members of Solanaceae	Concentrations vary substantially across plant species and organs but can reach 0.5% of dry weight [80]; many studies with tissue cultures have been published; however, the fairly low cost and availability of natural sources are a critical deterrent for commercial development of tissue cultures [81]	Many	Several products on market and in clinical development
	Berberine	*Berberis* spp., *Coptis* spp., *Eschscholtzia* spp., and others	Concentration up to 2.4% of dry weight in roots [82]; Berberis cannot be grown commercially in U.S. (alternate host for wheat rust fungus) [47], and other species have poor agronomic characteristics; cell cultures accumulate natural product at >2.4% [48]; further evaluation warranted	None	37 clinical trials listed by clinicaltrials.gov (most advanced: Phase II)

	Curcumin	*Curcuma longa* (and other plants)	Concentration in rhizome 0.4–2.2% of dry weight [83]; FDA issued warning letters against marketers of products claiming that cucurmin provides anti-disease effects or overall health benefits; tissue culture protocols for micropropagation have been developed but no high cucurmin producers have been published	Marketed as supplement under various names	Phase I

(continued)

Table 8.2 (continued)

Disease area	Lead metabolite(s)	Source(s)	Comments on plant tissue culture opportunities	Generic name(s)	Development status
	Epigallocatechin 3-gallate (EGCG)	*Camellia sinensis*	Concentration in green tea leaves up to 4% of dry weight [84]; FDA issued warning letters against marketers of products claiming that EGCG provides anti-disease effects or overall health benefits; it should be noted that green tea extract is an FDA-approved drug for treating certain kinds of warts; EGCG contents in tissue cultures (e.g., 0.2% in adventitious roots [85]) are not competitive	None	Phase III
	Pilocarpine	*Pilocarpus* spp.	Concentration in leaves <0.6% of dry weight [86]; production levels in tissue culture are extremely low [87] and not competitive	Many	FDA-approved for ailments affecting the eye and mouth

	Resveratrol	*Vitis vinifera* (and other plants)	Concentration in fruit skin <0.001% of dry weight [88]; large body of food science and nutrition literature; clinical evidence matter of significant dispute [89]; price too low to warrant further development of plant tissue cultures	Resveratrol	Mostly pre-clinical

question likely depends on labeling requirements for ingredients derived from genetically modified organisms. The regulatory landscape is highly complex and often differs across countries [30] (**see also Chap. 11 of this book**). The current trend toward the establishment of labeling standards would appear to be favorable for plant tissue culture that does not rely on genetic engineering. However, public concern about genetically modified organisms does not appear to be directed toward pharmaceuticals in the same way as they affect food and consumer care products. In May 2012, Protalix Biotherapeutics (www.protalix.com) was approved by the U.S. Food and Drug Administration to employ plant tissue cultures for the production of recombinant taliglucerase alpha (Elelyso®), and more products using the same platform are in advanced stages of development. It is not unlikely that plant tissue culture for producing PNPs with pharmaceutical applications would be acceptable for consumers, even if genetic engineering technologies should have been brought to bear.

8.4 Future Opportunities

Based on the considerations presented above, it would seem that plant tissue cultures have commercial potential when harvested cells are directly formulated into a nutraceutical product (simplified plant matrix and uncomplicated processing) or a PNP of particularly high pharmaceutical value is accumulated in high concentrations. Generally speaking, high value PNPs will be structurally complex (with multiple chiral centers and elaborate functionalization), accumulate at low levels in the source plant, and/or occur in species where access is limited (endangered or poor agronomic characteristics). In this paragraph, I will discuss examples of individual PNPs (not extracts) that might be produced at commercial scale by plant tissue culture technology. PNPs that have fallen out of favor among clinicians (e.g., digitoxin, ouabain, sanguinarine, tubocurarine, and yohimbine) will not be covered here (**see also Chap. 5 of this book**). I will also not discuss in the narrative PNPs whose clinical efficacy has not been demonstrated conclusively (e.g., cucurmin, epigallocatechin 3-gallate, and resveratrol) (Fig. 8.1; Table 8.2) (**see also Chap. 3 of this book**).

For some PNPs that meet the high value criteria listed above, the development of optimized tissue cultures has not been attempted or was not published; among these are ingenol 3-angelate, galanthamine, huperzine, combretastatins, and calanolide A (Fig. 8.1, Table 8.2). In other cases, the pharmaceutically relevant product is structurally distinct from the PNP lead and is obtained by chemical synthesis (which means that no plant source is needed); examples include ado-trastuzumab emtansine (synthetic antibody conjugate linked to the benzoansamacrolide, maytansine), dapagliflozin (employed for treatment of type 2 diabetes; based on the dihydrochalcone glucoside, phlorizin), vorapaxar (used for treatment of patients with a history of myocardial infarction; based on the alkaloid, himbacine), and tabex (employed to

aid with smoking cessation; chemical synthesis more efficient than extraction of PNP, cytisine) (Fig. 8.1, Table 8.2).

Tissue cultures have been developed for many plants that contain pharmaceutically relevant metabolites, but the concentration of the PNP of interest has mostly been equal to or below that reported for the natural source (Table 8.2). A few tissue culture resources, however, would seem to be worth further consideration.

The diterpene epoxide, **triptolide** (Fig. 8.1, Table 8.2), accumulates to only very low concentrations (<0.01% of dry weight in various organs) in members of the genus *Tripterygium* [31]. The current harvesting of roots from mature plants requires significant agronomic inputs and suffers from low efficiency [32]. Chemical derivatives of triptolide have been evaluated in phase I clinical trials [33, 34], and minnelide, a water soluble pro-drug analogue of triptolide, has shown particularly promising activity in multiple animal models of pancreatic cancer [35]. Various types of tissue cultures of *Tripterygium* producing different PNPs were developed in the 1980s and 1990s [36–39]. However, it was recognized only recently that triptolide concentrations produced by tissue cultures (up to 0.15%) [40–42] far exceed those reported for roots. In one *Tripterygium* root culture, more than 70% of the metabolites extracted from the culture medium with an organic solvent were characterized as diterpenoids (with triptolide accounting for 16% of all detected metabolites) [43]. Such an unprecedented enrichment of the target PNP, which is very difficult to obtain in sufficient quantities from natural sources, makes tissue culture an attractive alternative to the unsustainable harvest from *Tripterygium* roots.

Ajmaline (Fig. 8.1, Table 8.2) is an alkaloid that has been used since the 1970s as a treatment of heart arrhythmias (more recently, semi-synthetic derivatives have been introduced) [44]. The commercial cultivation of the medicinal plant *Rauwolfia serpentina*, which accumulates ajmaline in stem bark and roots, has met several challenges [45]. Despite decent yields from extracting the natural producer, the scarcity of the source materials has led to the high cost of treatments involving ajmaline. A tissue culture source, such as hairy roots with yields of >0.5% [46], would be a desirable alternative.

A larger number of clinical trials (>30) have been conducted with **berberine** (Fig. 8.1, Table 8.2), in particular as a treatment for type 2 diabetes (more information at www.clinicaltrials.gov) (**see also Chap. 5 of this book**). The extraction of berberine from members of the genus *Berberis* is reasonably straightforward but the plant cannot be grown commercially (by law) in several countries due to the fact that is serves as an alternate host for the wheat rust fungus [47]. Other natural producers, such as *Coptis* spp. or *Eschscholtzia* spp., have very poor agronomic performance and are not viable alternatives. Tissue cultures had been developed to produce berberine at fairly high yields (>3%) in the 1980s [48], and a reevaluation of their commercial potential would therefore be warranted.

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