

CHAPTER 2

Natural Products for Type II Diabetes Treatment

Amruta Bedekar, Karan Shah, and Mattheos Koffas¹

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Department of Chemical and Biological Engineering, University at Buffalo, SUNY, Buffalo, New York, USA

¹ Corresponding author: e-mail:mkoffas@eng.buffalo.edu

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Abstract

Natural products such as plant extracts and complex microbial secondary metabolites have recently attracted the attention of scientific world for their potential use as drugs for treating chronic diseases such as Type II diabetes. Non-Insulin-Dependent Diabetes Mellitus (NIDDM) or Type II diabetes has complicated basis and has various treatment options, each targeting different mechanism of action. One such option relies on digestive enzyme inhibition. Almost all of the currently used clinically digestive enzyme inhibitors are bacterial secondary metabolites. However in most cases understanding of their complete biosynthetic pathways remains a challenge.

The currently used digestive enzyme inhibitors have significant side effects that have restricted their usage. Hence, many active plant metabolites are being investigated as more effective treatment with fewer side effects. Flavonoids, terpenoids, glycosides are few to name in that class. Many of these are proven inhibitors of digestive enzymes but their large scale production remains a technical conundrum. Their successful heterologous production in simple host bacteria in scalable quantities gives a new dimension to the continuously active research for better treatment for type II diabetes. Looking at existing and new methods of mass level production of digestive inhibitors and latest efforts to effectively discover new potential drugs is the subject of this book chapter.

I. INTRODUCTION

Diabetes mellitus is a heterogeneous endocrine disorder in which hyperglycemia is the unifying feature. The number of patients with diabetes is rising by 4–5% every year (Wagman and Nuss, 2001), and through its long-term effects it is a cause of highest morbidity rate around the globe. Type I diabetes is an autoimmune disorder that results in an absolute insulin deficiency. Type II diabetes, however, has a more complex pathophysiologic basis which is not yet completely understood. Type II diabetes characteristically comprises three abnormalities: relative insulin deficiency, insulin resistance involving myocytes and adipocytes, and hepatic insulin resistance (resulting in increased gluconeogenesis and impaired glycogen synthesis). It is considered as one of the pathological manifestations of the so-called “metabolic syndrome.” Biochemical abnormalities of Type II diabetes may include hyperinsulinemia and high levels of serum triglycerides (TG). Microvascular and macrovascular diseases account for most of the morbidity and mortality associated with

Type II diabetes. The increased prevalence of macrovascular diseases in patients with diabetes is the result of numerous factors, including but not limited to obesity, lipid abnormalities, hypertension, hyperglycemia, hypercoagulation, platelet dysfunction and endothelial dysfunction. Diabetic microvascular diseases are responsible for diabetic retinopathy and blindness, diabetic neuropathy (potentially resulting in lower-limb amputation), and diabetic nephropathy (leading to end-stage renal disease and the need for renal dialysis or transplantation) (Lebovitz, 1992).

The pathogenesis of diabetes mellitus and its management by the oral administration of hypoglycemic agents have stimulated great interest in recent years. Control over hyperglycemia can be potentially achieved by different mechanisms: (1) An increase in insulin secretion; (2) A decrease in nutrient ingestion; (3) An increase in peripheral glucose uptake; (4) A decrease in hepatic glucose production. Various groups of oral antidiabetic agents are available for clinical use such as sulfonylureas (increase insulin secretion), biguanides (increase in glucose uptake), and digestive enzyme inhibitors (delay in complex carbohydrate digestion and absorption) (Lebovitz, 1992). In addition to these, various plant extracts are being used mainly in folklore medicine worldwide as therapeutics for diabetes, and many of these have proven hypoglycemic activity along with antiobesity and antioxidant properties which make them an attractive substitution for traditional antidiabetic drugs (Aslan *et al.*, 2007; Yan *et al.*, 2008).

Among various classes of antidiabetic drugs, digestive enzyme inhibitors are natural products usually derived from microorganisms. There is substantial evidence that inhibitors, such as α -glucosidase inhibitors, could be an effective treatment for prevention or at least delay in the development of disease in patients with impaired glucose tolerance (IGT) (Scheen, 2007; Scheen, 2003). To date, extensive studies have been conducted to analyze the mechanism of action of digestive enzyme inhibitors as well as their effects on hyperglycemia. Almost all the drugs from this class inhibit various digestive enzymes, including α -glucosidases and α -amylases. However, their administration is limited by the wide range of gastrointestinal side effects they have on the patients receiving the treatment, such as abdominal discomfort, diarrhea, and flatulence (Krentz and Bailey, 2005; Lebovitz, 1992; Scheen, 2003). Among the advantages of digestive enzyme inhibitors, however, is their side effects are not too severe- for example, they do not cause hypoglycemia. In fact, there have been reports stating that they result in some significant health benefits such as substantial weight loss (Vichayanrat *et al.*, 2002). Because of the lack of severe negative side effect, some of these inhibitors are also considered a better therapy for elderly patients for diabetes treatment (Johnston *et al.*, 1997).

Digestive enzyme inhibitors chiefly include acarbose, miglitol, and voglibose, which are currently commercially available for the treatment of Type II diabetes (Fig. 2.1). Among these, acarbose is the inhibitor most

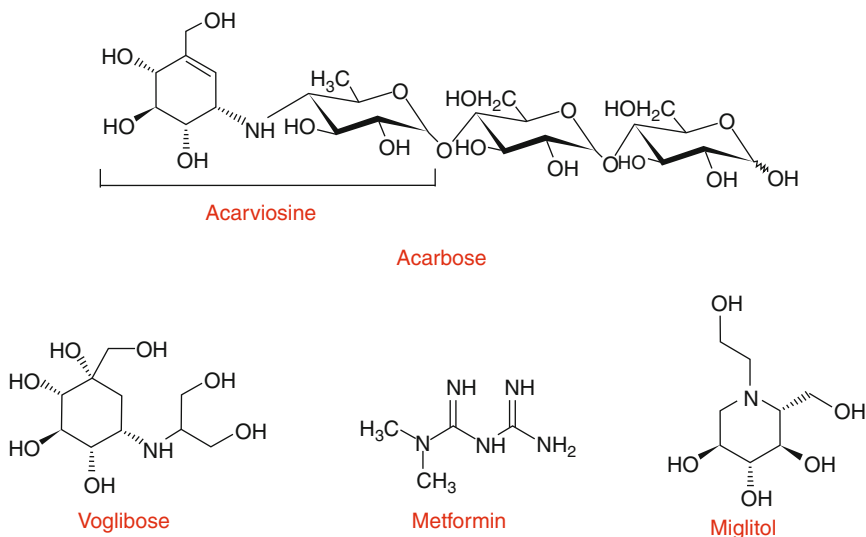


FIGURE 2.1 Structures of various digestive enzyme inhibitors and biguanides.

widely studied, but its complete biosynthetic route is not resolved; the biosynthetic routes of voglibose and miglitol are not fully understood either. Besides these three natural products, several other classes of compounds are currently of great interest as potential pharmacological agents for diabetes treatment: these are phytochemicals, with over 150 plant extracts currently being used in folklore medicine or currently under investigation for the treatment of diabetes. Some of their active compounds include flavonoids, tannins, alkaloids, glycosides, galactomannan, peptidoglycans, guanidine, terpenoids, inorganic ions, and glycopeptides. Most of the plant extracts that have the potential to treat Type II diabetes effectively have unfortunately not been studied in depth and very few have made it to the market as efficient drugs. In most cases, the biosynthetic pathways are not known completely while their chemical synthesis—if possible—tends to be cumbersome with low yields, which limits the potential progress to a successful drug. Considering the ongoing active research for novel solutions for the diabetes prevention and treatment, this large number of active compounds from plants can potentially provide better glycemic control with no or relatively fewer side effects. Here, an attempt has been made to summarize various natural compounds of either plant or microbial origin that possess proven or potential therapeutic properties for Type II diabetes treatment and their production methods.

II. ACARBOSE

Among the numerous antidiabetic drugs, acarbose is the most widely used digestive enzyme inhibitor for the treatment of Type-II diabetes. The story of acarbose begins with the screening by Bayer AG of various compounds isolated from a number of species of *Actinomycetes* as potent inhibitors of digestive enzymes such as α -amylase, sucrase, and maltase. It is now marketed by Bayer AG under the name Precose®. It is important to note that acarbose does not demonstrate any insulinotropic properties. This potent α -glucosidase inhibitor is a pseudotetrasacchride (Fig. 2.1) chemically known as *O*-4,6-dideoxy-4-[[*(1S,4R,5S,6S)*-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-*O*- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucose. It comprises acarviosine, which is made of a cyclitol moiety and an amino sugar with two glucose residues attached (Balfour and McTavish, 1993). Structural similarity of acarbose to oligosaccharides due to its glucose residues is considered to be responsible for the high-affinity binding to the sites of α -glucosidases. Acarbose inhibits various α -glucosidases in the following order: glucoamylase > sucrase > maltase > isomaltase (Tan, 1997); it also weakly inhibits α -amylases.

Acarbose is minimally absorbed (Scheen, 2007). Unlike other drugs for Type II diabetes treatment, acarbose does not have the risk of hypoglycemia (Donner, 2006), although it is important to note that acarbose and most other α -glucosidase inhibitors will be most effective when the diet is rich in starch and oligosaccharides. Because its action is directed against carbohydrate digestion, patients having diets rich in monosaccharides such as glucose will not see much help from acarbose therapy (Balfour and McTavish, 1993). Along with reducing blood glucose levels, acarbose also attenuates the levels of some other gastrointestinal and pancreatic hormones, such as decreasing plasma concentrations of gastrin and pacreozymmin and increasing concentration of somatostatin (Tan, 1997). Glucagon-like peptide-1 secretion is increased by acarbose, but the release of gastric inhibitory polypeptide is reduced (Krentz and Bailey, 2005). Such a combination of decrease in blood glucose levels and rise in the glucagon-like peptide-1 levels is being considered as the new approach in the treatment of Type II diabetes (Goke *et al.*, 1994).

Acarbose is slightly less effective for fasting plasma glucose levels than sulfonylureas or biguanides, but performs additively when used in combination to sulfonylureas or metformin, since its mechanism of action is different from that of these drugs (Balfour and McTavish, 1993). It has various side effects such as diarrhea, abdominal pain, borborygmus and flatulence, due to its effect of causing delayed carbohydrate absorption. The side effects can be minimized by starting the treatment with a small dose and then gradually increasing it to higher amounts (Lebovitz, 2004).

A. Mechanism of action and pharmacokinetics

Acarbose inhibits α -glucosidases located in the brush border of the enterocytes lining of intestine (Fig. 2.2) and pancreatic α -amylases located in the lumen of the intestine competitively. Pancreatic α -amylases help digest complex starches to oligosaccharides, whereas sucrases, maltases, isomaltases hydrolyze oligosaccharides, trisaccharides and disaccharides into simple sugars, such as glucose. Its high binding affinity to these enzymes prevents them from binding to oligosaccharides and disaccharides, thus avoiding their cleavage into simple monosaccharides and deferring their complete digestion further away in jejunum. This affects the insulin secretion as delayed glucose absorption alters secretion of intestinal hormones (Krentz and Bailey, 2005; Tan, 1997). Inability of the digestive enzymes to hydrolyze acarbose is due to the presence of an imino bridge which cannot be hydrolyzed by the digestive enzymes; it is hence considered to be the key element in the inhibitory action of the molecule (Wehmeier, 2003).

Various studies have been conducted on the competitive behavior of acarbose on various digestive enzymes. The K_i for competitive inhibition of Baker's yeast α -glucosidase and rat intestine α -glucosidase are 80 and 0.006 μM , respectively. Acarbose's K_i value for inhibition of porcine pancreatic α -amylase is 100 times higher than that for rat intestine α -glucosidase (Kim *et al.*, 1999). Acarbose is absorbed negligibly into the system of the patient (Clissold and Edwards, 1988); as a result, it is generally accepted that its main mechanism of action is within the intestine. No accumulation of acarbose was noted during administration at a standard dosage of 300 mg, three times daily for 3 months (Putter *et al.*, 1982).

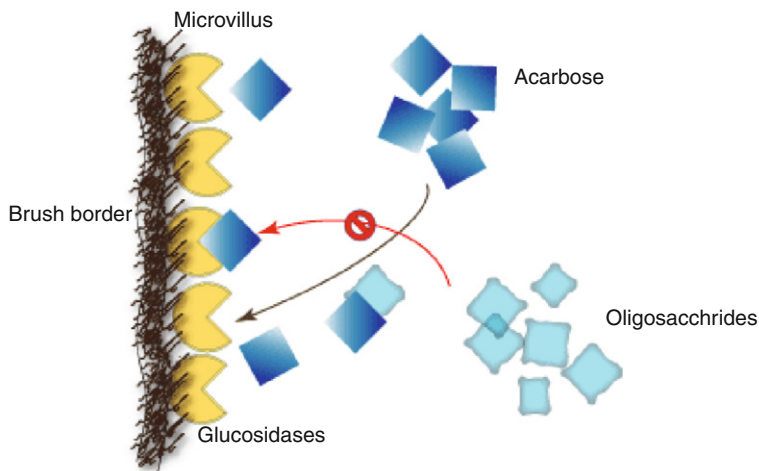


FIGURE 2.2 Mechanism of action of acarbose.

Acarbose degradation is observed to take place via two pathways: cleavage by intestinal digestive enzymes and biotransformation by intestinal microorganisms (Balfour and McTavish, 1993). Upon oral administration of the drug, 35% of acarbose and its metabolites are excreted by urinary and fecal routes whereas 94% is recovered in urinary form from an intravenous dose (Ahr *et al.*, 1989).

It is interesting to note the side effects acarbose has on patients, mainly due to its mechanism of action of delayed glucose absorption. The most common side effects are gastrointestinal symptoms that occurred to 83% of the subjects treated with acarbose versus only 60% treated with placebo in STOP-NIDDM study (Study To Prevent Non-Insulin-Dependent diabetes mellitus) (Chiasson *et al.*, 2002). Flatulence is the next most frequent side effect, with 68% of those treated with acarbose suffered from this as opposed to only 27% treated with placebo. Diarrhea, abdominal pain, nausea and constipation were observed in certain cases at comparable frequencies in both acarbose treated subjects as well as with placebo. It is remarkable that out of 714 subjects assigned for acarbose treatment for 3.3 years, 211 subjects withdrew from study within a year's time, mainly due to such side effects (Chiasson *et al.*, 2002). But as mentioned above, these effects can be alleviated by treating with small dosage initially and gradually increasing the dose strength.

B. Manufacturing of acarbose

As already mentioned, acarbose is manufactured by Bayer AG under various trade names in different countries, such as Precose® in North America, Prandose® in Canada, and originally Glucobay® in Europe. Acarbose is manufactured in a multistep batch fermentation process in its weakly basic form from *Actinoplanes* sp. SE50. Process volume is 30–100 m³ in a medium supplemented mainly with starch and maltose (as maltose supplementation increases yields; (Frommer *et al.*, 1977a)) and essential salts. The resulting product is a mixture of acarbose and many compounds having high similarities to acarbose (Wehmeier and Piepersberg, 2004). The presence of starch in the culture gives rise mainly to amino sugars having 4–8 hexose units which are suitable for further breakdown into acarbose-like compounds. On the other hand, starch-free nutrients with addition of maltose produce mixtures dominated by di- and trisacchrides (Frommer *et al.*, 1977b). Its downstream processing takes place in a stepwise manner (Rauenbusch and Schmidt, 1978), with various improvements incorporated over the years. The overall process is as follows:

- I. A strong acidic cation exchanger and a basic anion exchanger are simultaneously added to the culture broth, preferably without mycelium. Weakly basic acarbose binds to the cation exchanger strongly and about 80–90% acarbose is thus separated from the culture at this step.

- II. The resin mixture is separated from the broth along with mycelium (if any) via sieve screw centrifuge. After separation, washing by deionized water is carried out to free acarbose from adhering impurities.
- III. Elution of acarbose is then carried out by using dilute (~ 0.1 M) basic salt solutions in kettles with nozzle sieves.
 - a. Elution is pH and temperature sensitive. Having a pH in the 4.3–5.0 range is highly desirable. Also, low temperatures increase the adherence of acarbose to the column; hence room temperature or lower temperatures are maintained during the application of the substance, while for elution of acarbose raised temperatures of 40–70 °C are used. This results in rapid elution with good acarbose yields (Rauenbusch, 1987).
- IV. The eluant is subjected to further purification by passing it through a series of three columns; cation/anion/cation exchangers (strongly acidic/basic/strongly acidic) for removal of basic cations, to raise the pH above 3, and to bind acarbose and its analogs strongly, respectively. Most of the acarbose and other compounds are bound in the topmost part of the third column.
- V. This fraction is rinsed by deionized water and it is then eluted preferably by 0.025 N hydrochloric acid; the fractions containing acarbose are combined.
- VI. The pH of the fraction containing acarbose is increased to 6.0–6.5 by addition of anion exchanger.
- VII. The solution is then concentrated in vacuo, sterilized, and dried subsequently by lyophilization.

The schematic of the manufacturing process is given in Fig. 2.3. Various other improvements in the downstream processing and fermentation

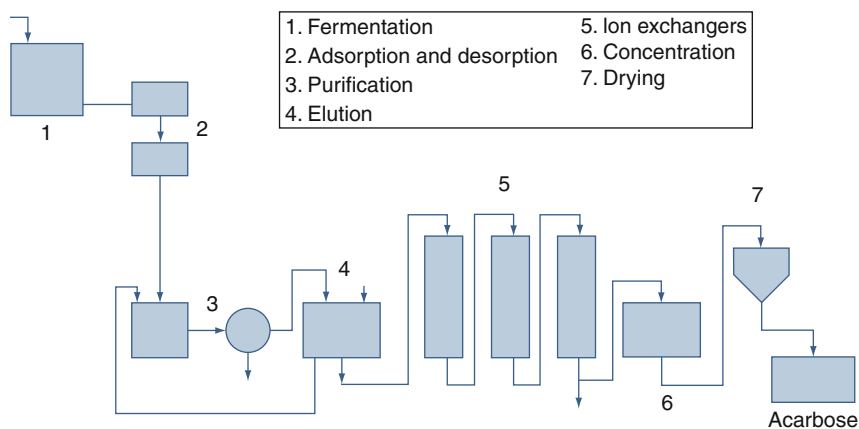


FIGURE 2.3 Manufacture of acarbose (Rauenbusch, 1987).

process have been published, such as use of special weakly acidic cation exchangers having carboxyl groups (Rauenbusch, 1987), controlling the osmolality of the culture at an optimum around 400 mosmol/kg (Beunink *et al.*, 1997), and the use of specifically developed cation exchangers made from macroporous, resistant polymers based on aromatic compounds (Lange and Rauenbusch, 1986). All these approaches have reported higher yields of acarbose and better separation.

The reported production of acarbose was 1 g/L for the *Actinoplanes* sp. SE50/110 under optimal culture conditions (Rauenbusch and Schmidt, 1978). The final concentration of acarbose is 98%.

C. Biosynthesis of acarbose in *Actinoplanes* sp. SE50/110

As already mentioned, acarbose is a secondary metabolite produced in bacterium species *Actinoplanes* sp. SE50. In the genome of *Actinoplanes* sp. SE50, 25 genes have been identified encoding various proteins necessary for biosynthesis of acarbose, its intra- and extracellular transport, and its metabolism (Wehmeier, 2003). A number of these genes have not been characterized and their function is speculated based on phylogenetic studies. A few crucial steps from the starting precursor 2-*epi*-5-*epi*-valiolone to acarviosine of acarbose are conjectural; in this chapter, the most recent developments in unraveling the acarbose biosynthetic pathway in *Actinoplanes* sp. SE50 are presented.

The entire *acb* gene cluster, consisting of 25 genes responsible for biosynthesis of the deoxyhexose and cyclitol moieties of acarbose, and its metabolism are shown in Fig. 2.4. The accession numbers of its genes are provided in Table 2.1.

Parallel to the formation of this cyclitol moiety of acarbose is the synthesis of the deoxysugar moiety, which starts with glucose-1-phosphate, activated by a nucleotidation step catalyzed by *acbA* (suggested dTDP-glucose synthase). The dTDP-D-glucose, thus formed, is further converted into dTDP-4-keto-6-deoxy-glucose by *acbB* (suggested dTDP-glucose-4-6-dehydratase) (Wehmeier and Piepersberg, 2004). The next reaction is catalyzed by *acbV*, an enzyme that belongs to the family of GabT-like aminotransferases, which is involved in primary metabolism (Piepersberg, 1997; Piepersberg and Distler, 1997; Piepersberg *et al.*, 2002). It is suggested that when *acbV* was heterologously expressed in *S. lividans* 66, it catalyzed the amination of dTDP-4-keto-6-deoxy-D-glucose probably resulting in dTDP-4-amino-4,6-dideoxy-D-glucose (Piepersberg *et al.*, 2002). This aminotransferase reaction is crucial in acarbose biosynthesis, as this amino group nitrogen bridges the valienamine moiety and the deoxysugar moiety. Another 12-step synthesis of dTDP-4-amino-4,6-dideoxy-D-glucose was suggested (Bowers *et al.*, 2002), wherein this

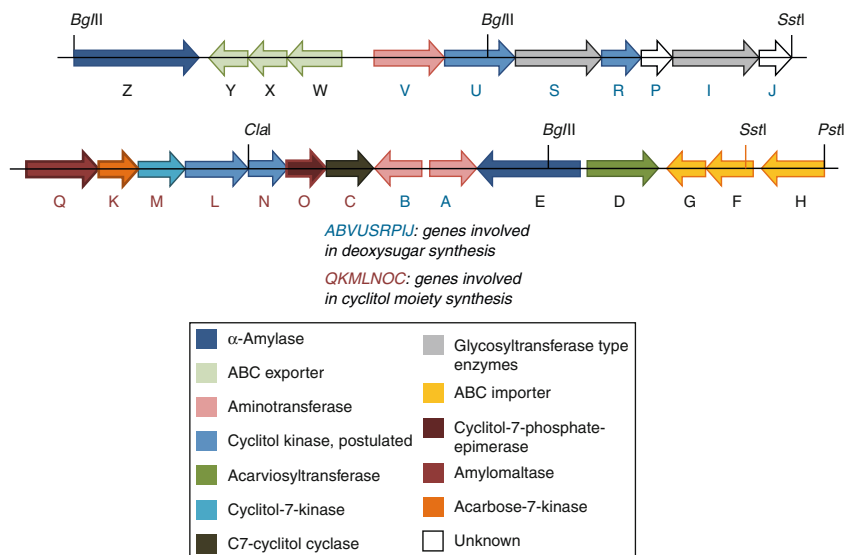


FIGURE 2.4 *Acb* gene cluster (Apeler *et al.*, 2001; Hemker *et al.*, 2001).

moiety is proposed to be synthesized from a galactoside which can be formed from D-galactose.

Table 2.1 lists the various digestive enzyme inhibitors and biguanides, along with their postulated names and function. These 25 genes have a very peculiar arrangement consisting of several transcription units, with three operons at the least. *AcbWXY* and *acbHFG* are two operons encoding three genes each. It is suggested that they are actually ABC-exporters and ABC-importers with *acbHFG* being membrane bound and extracytoplasmic enzymes, having highly conserved sequences to ABC-importers from *MsmEFG* of *Streptococcus mutans* (Wehmeier and Piepersberg, 2004). It is believed that *acbVUSRPIJQKMLNOC* forms a single transcription unit. The genes *acbKMLNOC* have been studied in detail and their functions are determined (Zhang *et al.*, 2002). More specifically, it is proposed that *acbC* (C7-cyclitol cyclase) is responsible for the synthesis of the 2-*epi*-5-*epi*-valiolone (Mahmud *et al.*, 1999). It was initially presumed that *acbK* (acarbose 7-kinase) is responsible for the phosphorylation of 2-*epi*-5-*epi*-valiolone, although in later experiments it was shown that *acbK* fails to phosphorylate 2-*epi*-5-*epi*-valiolone using an ATP-labeled phosphorylation assay. Instead, *acbM* showed positive results, suggesting that *acbM* was the first enzyme in the acarbose biosynthesis pathway resulting in the synthesis of 2-*epi*-5-*epi*-valiolone-7-phosphate. Further studies revealed that the next enzyme on the pathway was an epimerase encoded by *acbO*. The product of the enzymatic reaction of *acbO* was characterized

TABLE 2.1 Genes from *acb* cluster and their accession numbers

Gene	Enzyme	Accession number
<i>acbA</i>	dTDP-glucose synthase (i)	CAA77210
<i>acbB</i>	dTDP-4,6-dehydrogenase (i)	Q9ZAE8
<i>acbC</i>	C7-cyclitol cyclase (i)	CAA77208
<i>acbD</i>	Acarviosyltransferase (e)	CAJ81031
<i>acbE</i>	Acarbose resistant α -amylase (e)	CAJ81030
<i>acbF</i>	Carbohydrate ABC transporter (m)	CAJ81033
<i>acbG</i>	Carbohydrate ABC transporter (m)	CAJ81032
<i>acbH</i>	Carbohydrate ABC transporter(e-l)	CAJ81034
<i>acbI</i>	Glycosyl transferase (i)	CAJ81027
<i>acbJ</i>	Putative hydrolase (i)	CAJ81028
<i>acbK</i>	Acarbose-7-kinase (i)	CAD29481
<i>acbL</i>	Polyol dehydrogenase (i)	CAD29483
<i>acbM</i>	Cyclitol-7-kinase (i)	CAD29482
<i>acbN</i>	Oxidoreductase (i)	CAD29484
<i>acbO</i>	Cyclitol-7-phosphate-epimerase (i)	CAD29485
<i>acbP</i>	Unknown (i)	CAJ81026
<i>acbQ</i>	Amylomaltase (i)	CAJ81029
<i>acbR</i>	NDP-polyol synthase (i)	CAJ81025
<i>acbS</i>	Putative glycosyl transferase (i)	CAJ81024
<i>acbU</i>	Putative cyclitol kinase (i)	CAJ81023
<i>acbV</i>	Aminotransferase (i)	CAJ81022
<i>acbW</i>	ATP binding component of ABC exporter (i, m)	CAJ81021
<i>acbX</i>	ABC transporter (i, m)	CAJ81020
<i>acbY</i>	ABC exporter	CAJ81019
<i>acbZ</i>	α -Amylase (e)	CAJ81018

e, extracellular; *i*, intracellular (cytoplasmic); *e-l*, extracytoplasmic lipoprotein; *m*, membrane-integrated (Wehmeier and Piepersberg, 2004).

using mass spectrometry. *AcbO* functions independently without the need for cofactors or coenzymes indicating that it is a representative of a new class of epimerases (Zhang *et al.*, 2003a). *AcbN* and *acbL* showed distinct similarity to various members of oxidoreductase families such as zinc-dependent dehydrogenases and short chain alcohol dehydrogenases, respectively, and were shown to catalyze the formation of 1-*epi*-valienol-7-phosphate (Zhang *et al.*, 2002).

The cluster of *acbBAED* has been studied to some extent, with *acbD* having been expressed heterologously in *Streptomyces lividans* TK23, and

characterized. *AcbD*, which was initially postulated to be a glycosyltransferase, turned out to be acarviosyl transferase that modifies acarbose extracellularly (Hemker *et al.*, 2001). At the same time, *acbA* and *acbB* catalyze the first two reactions in the biosynthetic pathway for the formation of the deoxyhexose moiety (Liu and Thorson, 1994; Piepersberg, 1994).

The biosynthetic pathway from 2-*epi*-5-*epi*-valiolone and glucose-1-phosphate to acarbose is depicted in Fig. 2.5. The first precursor responsible for the cyclitol moiety of acarbose is 2-*epi*-5-*epi*-valiolone, which was affirmed by NMR studies from its incorporation into acarbose pathway in *Actinoplanes* sp. (Mahmud *et al.*, 1999). Its precursor is sedoheptulose 7-phosphate, which is derived from the pentose phosphate pathway (Mahmud, 2003). Its phosphorylation at C7 takes place by a postulated enzyme, cyclitol-7-kinase, encoded by *acbM* to formulate 2-*epi*-5-*epi*-valiolone-7-phosphate (Zhang *et al.*, 2002). Its epimerization by *acbO* results in the formation of 5-*epi*-valiolone-7-phosphate, which has been characterized by mass spectroscopic and NMR spectroscopic methods (Zhang *et al.*, 2003a). Further reduction of the C-1 keto group takes place by NADH-dependent dehydrogenase, *acbL*, giving the next intermediate in the biosynthetic pathway, 5-*epi*-valiolol-7-phosphate. Further steps involve postulated enzymes based on phylogenetic studies and reaction intermediates which are not fully characterized yet. The *acbN* protein (suggested oxidoreductase) could catalyze synthesis of the next intermediate, 1-*epi*-valienol-7-phosphate. A new kinase activity was characterized in *Actinoplanes*, which could phosphorylate 1-*epi*-valienol to 1-*epi*-valienol-7-phosphate, although this kinase activity could not be attributed to any gene from the *acb* cluster (Thomas, 2001; Zhang *et al.*, 2003b). The enzyme necessary for catalyzing the conversion of 1-*epi*-valienol-7-phosphate to 1-7-diphospho-1-*epi*-valienol by performing a phosphorylation at the C-1 position is not characterized. The next enzyme, however, has been characterized: *acbR* (Tatusov *et al.*, 2001), an ADP-glucose synthase-like protein that catalyzes nucleotidation at C-1 position of 1-7-diphospho-1-*epi*-valienol, a proposed precursor. This reaction completes the synthesis of cyclitol moiety of acarbose which involves the majority of proteins encoded by genes in the *acbQKMLNOC* operon (Wehmeier and Piepersberg, 2004).

Parallel to the formation of this cyclitol moiety of acarbose is the synthesis of the deoxysugar moiety, which starts with glucose-1-phosphate, activated by a nucleotidation step catalyzed by *acbA* (suggested dTDP-glucose synthase). The dTDP-D-glucose, thus formed, is further converted into dTDP-4-keto-6-deoxy-glucose by *acbB* (suggested dTDP-glucose-4-6-dehydratase) (Wehmeier and Piepersberg, 2004). The next reaction is catalyzed by *acbV*, an enzyme that belongs to the family of GabT-like aminotransferases, which is involved in primary metabolism (Piepersberg, 1997; Piepersberg and Distler, 1997; Piepersberg *et al.*, 2002). It is suggested that when *acbV* was heterologously expressed in *S. lividans* 66, it catalyzed the amination of

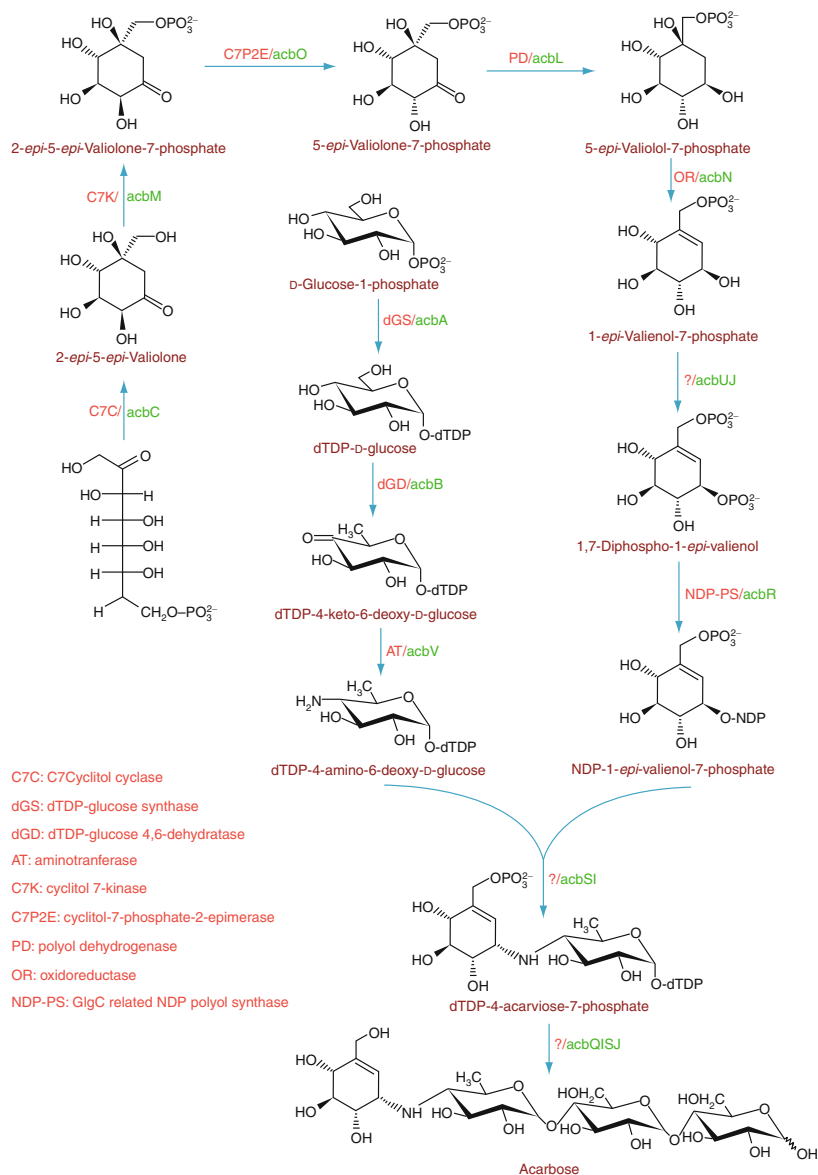


FIGURE 2.5 Biosynthesis pathway of acarbose (Mahmud *et al.*, 1999; Zhang *et al.*, 2002).

dTDP-4-keto-6-deoxy-D-glucose probably resulting in dTDP-4-amino-4,6-dideoxy-D-glucose (Piepersberg *et al.*, 2002). This aminotransferase reaction is crucial in acarbose biosynthesis, as this amino group nitrogen bridges the

valienamine moiety and the deoxysugar moiety. Another 12-step synthesis of dTDP-4-amino-4,6-dideoxy-D-glucose was suggested (Bowers *et al.*, 2002), wherein this moiety is proposed to be synthesized from a galactoside which can be formed from D-galactose.

AcbS and *acbl* are related to glycogen and sucrose synthases and they are thus speculated to catalyze the necessary glycosyltransferase-like reaction to formulate dTDP-acarvioside-7-phosphate. This is achieved by a combined reaction between the cyclitol precursor NDP-1-*epi*-valienol-7-phosphate and deoxysugar precursor dTDP-4-amino-4,6-dideoxy-D-glucose, thus synthesizing a speculated precursor of acarbose. However, the end product may be acarbose-7-phosphate or some other compound instead of acarbose itself (Wehmeier and Piepersberg, 2004); it might need more than just one step to convert dTDP-acarvioside-7-phosphate into acarbose. The biosynthetic pathway in Fig. 2.5 shows the postulated precursor and the postulated enzymes that catalyze its formation and its conversion to acarbose.

D. Effect on hyperglycemia

Various placebo-controlled dose comparison studies for the safety and efficacy of acarbose have been conducted and published, most of which demonstrated high efficiency of acarbose in correcting hyperglycemia. Comparison studies with other antidiabetic drugs indicate that acarbose can be used effectively as a monotherapy to reduce postprandial blood glucose levels or in combination with other drugs.

One such study checked the efficacy of acarbose as monotherapy, with metformin, with sulfonylureas, and with insulin on four different groups (50–200 mg twice daily (tid)) for 52 weeks (Chiasson *et al.*, 1994). Each group showed a remarkable decrease in glycosylated hemoglobin A (HbA_{1c} and HbA₁), with 0.9%, 0.8%, 0.9%, and 0.4% reduction, respectively, for each group against diet alone, only metformin, only sulfonylureas, and only insulin. Another study carried out indicates its enhanced antihyperglycemic effects when acarbose is applied in a combination therapy with metformin (Scheen *et al.*, 1993). The 24-week study for acarbose as monotherapy showed reduction in fasting blood glucose by 1.4 mM and postprandial glucose level by 2.2 mM and decreased HbA_{1c} by 1.1% as opposed to placebo. A comparison with glyburide (sulfonylureas) indicated similar decrease of 0.9% in HbA_{1c} on comparison with placebo (Hoffmann and Spengler, 1994). If acarbose is used as monotherapy in combination with adequate diet management, it has been found to decrease fasting glucose plasma level by 1 mmol/L and postprandial glucose levels by approximately 3 mmol/L. Postprandial insulin levels decreased by about 20–25%, while fasting insulin levels remained unchanged and HbA_{1c} values were decreased by 0.65 to 1.0% when compared with placebo (Coniff, 1991).

Recently, different comparative studies were carried out for different drugs for treatment of Type II diabetes, such as the Diabetes Prevention Program (DPP) in the United States, the Study TO Prevent Non-Insulin-Dependent diabetes mellitus (STOP-NIDDM) in various European countries and Canada, the Chinese Diabetes Prevention Study (CDPS), the Early Diabetes Intervention Trial (EDIT) in United Kingdom, and the Indian Diabetes Prevention Program (IDPP). Most of these studies are carried out in randomized, placebo-controlled and blind folded trials, for a prolonged period of 3 years to check the development of diabetes mellitus after treatments with drug or placebo in patients with IGT. The STOP-NIDDM study ([Chiasson *et al.*, 2002](#)) checked for delay in progress toward diabetes in patients having IGT, and their study showed reduction in risk of progression toward diabetes by 25% over 3.3 years. From a total of 1429 randomized patients (age ~ 55 years, body-mass index (BMI) 31 kg/m^2), 714 were treated with 100 mg (tid) acarbose and 715 were treated with placebo. Sixty-one patients out of the total pool were later excluded from the study since they did not meet IGT criteria nor had postrandomization data. Out of 682 analyzed for acarbose, 32% developed diabetes mellitus, whereas out of 686 treated with placebo, 42% developed diabetes. Administration of acarbose also increased the probability of IGT reverting to normal glucose tolerance over time ([Chiasson *et al.*, 2002](#)). In the CDPS study ([Yang *et al.*, 2001](#)), 88 patients having BMI 25 kg/m^2 and IGT were subjected to treatment with acarbose or metformin, while 85 received conventional education (control group). In this study, 50 mg of acarbose (tid) was administered to the patients for 6 years ([Scheen, 2007](#)). Of the control patients 34.9% progressed to diabetes, whereas only 6% of those treated with acarbose progressed to diabetes. This implies an 87.8% reduction in risk as compared with 25% from the previous study. However, the EDIT study did not confirm this high reduction in the progress to diabetes; however, it confirms that administration of acarbose decreased the risk of IGT patients progressing to diabetes ([Scheen, 2007](#)).

When checked for efficacy as a combination therapy with sulfonylureas, the effect is additive. However, acarbose is less effective than tolbutamide when both treatments are given independently ([Coniff *et al.*, 1995](#)). The mechanisms of action of sulfonylureas and α -glucosidase inhibitors are different, which perhaps can explain why the treatment with these compounds as monotherapy has different efficacies. A total of 290 subjects having Type II diabetes and fasting glucose levels no less than 140 mg/dL were treated with 200 mg of acarbose and 250–1000 mg (tid) of tolbutamide either alone or in combination for 24 weeks. The reduction in postprandial plasma glucose levels were reported as 85 mg/dL for acarbose-plus-tolbutamide, 71 mg/dL for tolbutamide alone, 56 mg/dL for acarbose alone, and only 13 mg/dL for placebo ([Coniff *et al.*, 1995](#)) ([Table 2.2](#)).

TABLE 2.2 Clinical trials of acarbose in Type II diabetes

Study (reference)	Drug(s)	Dose of acarbose (mg tid)	Duration of study	N	Results
(Chiasson <i>et al.</i> , 1994)	A,A/M,A/S,A/I	50–200	52 weeks	354	HbA _{1c} decreased by 0.9%, 0.8%, 0.9%, 0.4% resp.
(Hoffmann and Spengler, 1994)	A, G	100	24 weeks	96	HbA _{1c} decreased by 1.0%, 0.9% resp.
STOP-TYPE II DIABETES (Chiasson <i>et al.</i> , 2002)	A	100	3.2 years	1368	RR = 0.75, <i>P</i> = 0.0015
EDIT (Holman <i>et al.</i> , 2003; Scheen, 2007)	A	50	6 years	631	RR = 0.81, <i>P</i> = 0.81
	M	500	6 years	631	RR = 0.81, <i>P</i> = 0.94
CDPS (Yang <i>et al.</i> , 2001)	A	50	3 years	261	RR = 0.12, <i>P</i> = 0.00001
(Coniff <i>et al.</i> , 1995)	A, T, A/T	200	24 weeks	290	HbA _{1c} decreased by 0.54%, 0.93%, 1.32% resp.

A, acarbose; *M*, metformin; *S*, sulfonylureas; *I*, insulin; *G*, glyburide; *T*, tolbutamide; *RR*, relative risk of developing diabetes versus placebo.

Acarbose is expected to be useful in treatment of insulin-dependent diabetes mellitus (IDDM) as well, but there has not been as much data on IDDM published as there is for Type II diabetes. A few studies indicate positive results, proving that acarbose helps smoothing out postprandial glycemic fluctuations and preventing both hyperglycemia and hypoglycemia in insulin-treated patients having IDDM (Balfour and McTavish, 1993; Lebovitz, 1992). Reduction in the plasma glucose levels (0.1–1.1%) and insulin requirements to 35% were reported (Balfour and McTavish, 1993). Two hundred and thirty-six patients with IDDM were treated in a randomized double-blind study with acarbose 150–600 mg/day or placebo for 24 weeks along with usual insulin requirements. Such a treatment reduced postprandial plasma glucose levels at roughly 3 mmol/L. The study also showed that insulin requirements decreased by 3.5 IU/day (Hollander and Coniff, 1991).

III. MIGLITOL

One of the widely used α -glucosidase inhibitors for treatment of Type II diabetes is miglitol ($C_8H_{17}NO_5$; IUPAC name (2R,3R,4R,5S)-1-(2-hydroxyethyl)-2-(hydroxymethyl)piperidine-3,4,5-triol; molecular weight 207.2). Miglitol is a second-generation α -glucosidase inhibitor derived from 1-deoxynojirimycin, which is yet another α -glucosidase inhibitor and is structurally similar to glucose (Fig. 2.1) (Tan, 1997). It is a white to pale yellow powder and is soluble in water (Campbell *et al.*, 2000). Miglitol was approved by the U.S. Food and Drug Administration (FDA) in 1996 as an additional therapy to diet alone therapy or diet plus sulfonylurea therapy in patients with Type II diabetes. Miglitol's story begins with the successful attempts for identifying new compounds with inhibitory properties, which initially resulted in the discovery of nojirimycin, deoxynojirimycin, and their derivatives from various *Bacillus* and *Streptomyces* strains (Schmidt *et al.*, 1979). During initial attempts, 1-deoxynojirimycin was successfully obtained (Schmidt *et al.*, 1979); however, its *N*-hydroxyethyl analog (miglitol) later proved to possess better inhibitory activities. It is currently being manufactured by Bayer AG under the trade name of Glyset® in USA and as Diastabol® in Europe. Miglitol is considered to be a good choice for the therapy of patients who have the relative risk of developing hypoglycemia, weight gain, or lactic acidosis (Campbell *et al.*, 2000). It is observed to have the same efficacy as acarbose at lesser dosages (50 and 100 mg tid). Miglitol therapy provides better reduction on fasting and postprandial plasma glucose levels in patients in comparison with sulfonylureas (Scott and Spencer, 2000), whereas voglibose, another α -glucosidase inhibitor, could achieve reduction only for postprandial glucose levels.

A. Mechanism of action and pharmacokinetics

The mechanism of action of miglitol is very similar to that of acarbose; it has strong binding affinity to digestive enzymes and, as a result, prevents these enzymes from binding to complex carbohydrates thereby delaying glucose absorption and resulting in reduction in postprandial plasma levels. The difference to note is that miglitol is a competitive inhibitor of digestive enzymes as a substitute for glucose, whereas acarbose functions as a substitute for the starch and oligosaccharides. Miglitol shows inhibitory action toward almost all the digestive enzymes present in the brush border of small intestine with the following ranking order: sucrase > glucoamylase > isomaltase > lactase > trehalase, and some inhibitory activity toward α -amylase (Lembcke *et al.*, 1985; Scott and Spencer, 2000).

Both acarbose and voglibose are not absorbed in the upper section of upper intestine. Miglitol, however, is almost completely absorbed in the small intestine (Scott and Spencer, 2000; Tan, 1997). The absorption of miglitol is dose dependent, with 25 mg of miglitol rapidly and completely absorbed. However, higher doses of up to 100 mg do not get fully absorbed, and 95% of miglitol is excreted out of the system via urine and feces almost unchanged. The amount excreted depends upon the systemic absorption, and therefore on the dose administered. With the lowest dose of 25 mg, almost 95% excretion is achieved, but with higher dosages this amount drops. The half-life of miglitol in healthy volunteers is 2–3 h for a less potent dose of 50 mg (Ahr *et al.*, 1997; Campbell *et al.*, 2000; Scott and Spencer, 2000).

B. Biosynthesis and large-scale production of miglitol

Several efforts were put into achieving large-scale production of miglitol. Unlike acarbose, large-scale production of miglitol involves a combination of biochemical and chemical syntheses.

As mentioned above, miglitol is an N-derivative of 1-deoxynojirimycin which is obtained from D-glucose as a starting material. The formation of miglitol from D-glucose is made possible by the ability of *Glucanobactor oxydans* to regio- and stereoselectively oxidize polyol substrates (Schedel, 2000). This conversion from glucose to miglitol is a simple three-step reaction using a highly selective enzyme, polyol dehydrogenase, which essentially rules out the necessity of any protection group chemistry. The first step carries out amination to obtain 1-amino-D-sorbitol through a reduction reaction. 1-Amino-D-sorbitol thus obtained is then oxidized at C5 by polyol dehydrogenase from *Glucanobactor oxydans*. Finally, ring closure is achieved by reduction (Deppenmeier *et al.*, 2002; Schedel, 2000) (Fig. 2.6).

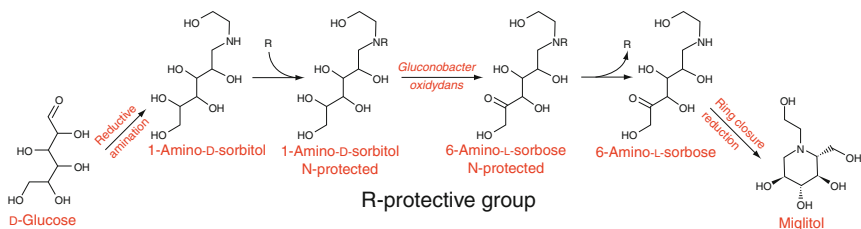


FIGURE 2.6 Combined approach of chemical and biotechnological synthesis of miglitol (Deppenmeier *et al.*, 2002; Schedel, 2000).

Miglitol requires 1-deoxynojirimycin as a precursor which can be obtained via three different routes: extraction from plants such as the mulberry tree, fermentation using various bacterial strains, and a complete chemical synthesis. Industrially feasible production of miglitol was, however, restricted by expensive purification steps or low yields. Hence, a new approach was adopted in which a combination of biochemical and chemical synthesis was employed (Schedel, 2000). In this approach, D-glucose is converted to 1-amino-D-sorbitol by reduction with suitable amines and hydrogen, with nickel as catalyst, and then further reaction of products with appropriate acid esters (Kinast and Schedel, 1979). The oxidation of 1-amino-D-sorbitol to 6-amino-L-sorbose is then carried out in a fermenter using *Gluconobacter oxydans* grown at temperatures between 20 and 45 °C, preferably at room temperature and maintaining the pH between 2.0 and 9.0. At this pH, 6-amino-L-sorbose is present in the medium as piperidinoose, which is reduced to 1-desoxy-nojirimycin in the presence of inert solvents and by choosing the appropriate pH. After this, miglitol is obtained by first centrifuging the biomass followed by clarification using active charcoal. Next, separation of catalyst, evaporation of solvents, and isolation of any remaining salts in the medium is carried out (Kinast and Schedel, 1979). An important modification necessary in this process is the addition of protection groups before feeding 1-amino-D-sorbitol to the *G. oxydans* cultures and their subsequent removal before the ring-closure reaction. This is necessary since 1-amino-D-sorbitol is readily oxidized by *G. oxydans* strains to form 3-hydroxy-2-hydroxymethyl-pyridine at near-neutral pH as a result of spontaneous ring closure (Schedel, 2000).

C. Effect on hyperglycemia

As mentioned earlier, miglitol was approved as a monotherapy or as a combination therapy with sulfonylureas in patients with Type II diabetes along with dietary control. Various double-blind, randomized studies for efficacy and safety of miglitol have been carried out on healthy volunteers as well as on patients with Type II diabetes. When studied as

monotherapy in patients for its efficacy and tolerability in comparison with sulfonylureas, it was observed that small doses of miglitol are not as effective as sulfonylureas. In a 1-year long, double-blind, randomized, placebo-controlled trial, 411 elderly patients (age 60 or more) were split into four groups wherein the first group received placebo, the second group received miglitol at 25 mg (tid), the third received 50 mg miglitol (tid), and the last received glyburide based on fasting glucose levels (once daily). After a year, HbA_{1c} levels were dropped by 0.49%, 0.4%, and 0.92% for 25 mg miglitol, 50 mg miglitol, and glyburide groups, respectively versus placebo ($P < 0.05$ – 0.01 vs. placebo) (Johnston *et al.*, 1997). Similar results were obtained when 100 patients were treated with either glyburide or miglitol (47 and 49, respectively) for a total of 24 weeks, where the administration of drug was 50 mg (tid) for miglitol for the first 6 weeks which was then raised to 100 mg (tid) for 18 weeks while for glyburide the administered dose was 2.5 mg (tid) for 6 weeks raised to 5 mg (tid) for the rest of the study. After 24 weeks, HbA_{1c} was reduced from baseline by 0.78% for the miglitol group whereas it was reduced by 1.18% for glyburide, indicating it is more potent than miglitol in reducing plasma glucose levels. When compared for postprandial glucose reduction, miglitol was observed to achieve similar decrease as glyburide after breakfast; however, after lunch the effect was more pronounced for miglitol with 57.6 mg/dL ($P < 0.001$ vs. placebo) as compared to only 36 mg/dL for glyburide ($P < 0.001$ vs. placebo) (Pagano *et al.*, 1995).

Another multicenter, double-blind, randomized, and placebo-controlled study to determine the effect of miglitol treatment involved 192 patients who had been receiving sulfonylureas for at least 6 months prior to the trial and the results were recorded after 8, 14, and 20 weeks from the start of the study (Campbell *et al.*, 2000; Johnston *et al.*, 1994). After a placebo treatment for 6 weeks, the subjects were randomized into three groups receiving 50 mg miglitol (tid), 100 mg (tid) miglitol, or placebo. The resulting reduction in HbA_{1c} was significant ($P = 0.0001$) with 50 and 100 mg of miglitol treatment (0.49% and 0.41% decrease, respectively) as compared to placebo (treatment with sulfonylurea only), indicating an additive effect due to combinational therapy. Weight increase was observed by 0.13 kg in placebo, 0.55 kg with miglitol treatment of 50 mg, and 0.08 kg with 100 mg (NS) miglitol treatment (Johnston *et al.*, 1994). Similar rise in lowering HbA_{1c} was also observed in a study on patients receiving insulin therapy (Escobar-Jimenez *et al.*, 1995); it was also reported that requirement of insulin in such patients dropped (Dimitriadis *et al.*, 1991).

Miglitol has similar side effects as acarbose, such as diarrhea, flatulence, and abdominal pain (Campbell *et al.*, 2000; Johnston *et al.*, 1994; Scott and Spencer, 2000). All these side effects were dose dependent and were subdued on continued therapy and resolved completely upon stopping the treatment (Johnston *et al.*, 1994).

IV. VGLIBOSE

Voglibose, whose IUPAC name is 5-(1,3-dihydroxypropan-2-ylamino)-1-(hydroxymethyl)cyclohexane-1,2,3,4-tetrol ($C_{10}H_{21}NO_7$), is mainly used as the antidiabetic drug in Asia and is sold under different trade names in various countries. In India, it is sold by Ranbaxy Labs under the trade name Volix[®], whereas it is marketed under the name of Basen[®] in Japan. It is synthesized from valioline, which is isolated from fermentation broth of *Streptomyces hydroscopicus* subsp. *limoneus* (Matsuo *et al.*, 1992). Just like acarbose and miglitol, it falls in the category of α -glucosidase inhibitors and inhibits competitively and reversibly glucoamylase, sucrase, and isomaltase. While acarbose also weakly inhibits α -amylase, voglibose has no inhibitory action toward α -amylases (Goke *et al.*, 1995; Horii *et al.*, 1986). Various studies have proven that voglibose successfully reduces plasma glucose levels, insulin, and C peptide postprandially in a dose-dependent manner (Chen *et al.*, 2006; Goke *et al.*, 1995; Matsuo *et al.*, 1992).

A. Mechanism of action and pharmacokinetics

As observed in the case of acarbose, voglibose inhibits α -glucosidases in a reversible and competitive manner and delays the complex carbohydrate absorption resulting in decrease in hyperglycemia and hyperinsulinemia (Chen *et al.*, 2006; Vichayanrat *et al.*, 2002). Its structure is similar to oligosaccharides and other derivatives of starch and involves a valioline moiety connected to propanediol moiety with a nitrogen bridge which plays an important role in the drug's activity. Voglibose binds to the digestive enzymes and as a result they fail to break it down due to this nitrogen bridge. Its IC_{50} values toward porcine maltase and sucrase have been evaluated and reported as 1.5×10^{-2} and 4.6×10^{-3} μ M, respectively (Chen *et al.*, 2006). Valienamine derivatives were observed to have lesser inhibition of porcine maltase and sucrase as compared to valioline derivatives. Although its chemical synthesis from glucose has been described (Chen *et al.*, 2006), its exact biosynthesis from valioline (obtained from *S. hydroscopicus* subsp. *limoneus*) is not completely understood.

B. Effect on hyperglycemia

Voglibose (AO-128) is not as widely studied as acarbose, the latter being the first representative α -glucosidase inhibitor. Despite that, several groups have studied its effects on postprandial glucose levels and on insulin and C-peptide content in patients with Type II diabetes. In general,

voglibose has been found to significantly decrease the rapid rise in postprandial glucose. One of the early studies on voglibose efficiency and its effects on digestive enzymes indicated up to 33-fold increased inhibition of semipurified porcine small intestine disaccharidases than acarbose (Matsuo *et al.*, 1992). It reduced blood glucose levels after administration of maltose, sucrose, and starch but not upon administration of glucose, fructose, and lactose, something that is in accordance with its mechanism of action wherein it binds to enzymes which break down complex starch and disaccharides into simple sugars. It also showed successful reduction in plasma insulin and plasma glucose levels (Matsuo *et al.*, 1992). Although α -glucosidase inhibitors work based on the same mechanism of delaying carbohydrate absorption and break down into simple sugars, they have differences in their potency, probably due to differences in their structure and affinity to digestive enzymes. While comparing the efficacy and safety of acarbose and voglibose in 30 subjects, both were observed to decrease 1-h postprandial blood glucose levels, from 224.9 ± 42.8 to 206 ± 38.9 mg/dL for voglibose, whereas the decrease was 228.3 ± 37.4 to 186.6 ± 36.1 mg/dL for acarbose (Vichayanrat *et al.*, 2002). The quantity of the drug administered was different, with acarbose treatment being 100 mg (tid) whereas for voglibose it was 0.2 mg (tid). Abdominal discomfort and increased flatulence were observed in both therapies; however, the effects were more pronounced in acarbose therapy. Interestingly, while both therapies showed decrease in 1-h postprandial blood glucose levels and rise in insulin levels, only acarbose showed significant decrease in 2-h postprandial blood glucose levels (Vichayanrat *et al.*, 2002).

A recent double-blind study (Goke *et al.*, 1995) treated 72 healthy volunteers around 30 years of age and body mass of 75 kg with varying dosage of voglibose (0.5, 1.0, 2.0, or 5.0 mg) or placebo. At the end of 7 days, blood glucose levels were at 10 mg/dL for the most potent dose of voglibose. As for insulin, voglibose reduced the rise in its levels in a dose-dependent manner: the highest reduction of 75% as compared to placebo was achieved when 5 mg doses were administered to the patients. It was also found to reduce gastric inhibitory polypeptide (GIP) plasma concentrations up to 50%. Similar phenomena were observed in the case of acarbose administration (Folsch *et al.*, 1987); thus, it is suggested that glucose absorption is necessary for GIP secretion, the delay of which results in a decrease in GIP rise. At the same time, it increased incretin hormone glucagon-like peptide 1 (GLP-1) plasma concentrations in dose-dependent manner wherein a rise of 5 pmol/L was observed in the 5.0 mg dose on the 7th day (Goke *et al.*, 1995). In a study conducted in Japan for combination therapy of voglibose with a thiazolidinedione (pioglitazone), 31 patients were separated into two groups, of which 16 received 30 mg of thiazolidinedione and

0.9 mg voglibose treatment whereas the rest were administered only voglibose for 12 weeks. The results indicated increased reduction in the plasma glucose levels in patients on the combination therapy of both the drugs (Abe *et al.*, 2007).

V. ANTHOCYANINS

Various polyphenols such as flavonoids have demonstrated numerous health benefits, especially in the treatment of obesity and diabetes. It is interesting that natural compounds can be addressing the issue of obesity and diabetes in more than one way, such as digestive enzyme inhibition, induction of apoptosis in adipose tissue, etc. (Nelson-Dooley *et al.*, 2005). Anthocyanins, a subgroup of flavonoids, are water-soluble plant pigments responsible for the blue, purple, and red color of many plant tissues. In plants they provide some important functions such as UV protection, signaling, antimicrobial activities, etc. In the past two decades, they have received great attention worldwide due to their potential and proven health benefits in humans, such as anti-inflammatory, anticancer, antiobesity and antidiabetic properties (Yan *et al.*, 2008). They occur primarily as glycosides of their respective aglycon anthocyanidin chromophores (Prior and Wu, 2006). The sugar moiety is mainly attached at the 3-position on the C-ring or the 5, 7-positions on the A-ring. Glucose (glc), galactose (gal), arabinose (arab), rhamnose (rham), and xylose (xyl) are the most common sugars that are bonded to anthocyanidins in the form of mono-, di-, or trisaccharides except for the 3-deoxyanthocyanidins such as luteolinidin and apigeninidin in sorghum (Wu and Prior, 2005).

About 17 anthocyanidins, the aglycon forms of anthocyanins, are found in nature, whereas only 6 of them, cyanidin (Cy), delphinidin (Dp), petunidin (Pt), peonidin (Pn), pelargonidin (Pg), and malvidin (Mv), are ubiquitously distributed (Fig. 2.7). The differences in chemical structure of these six common anthocyanidins occur at the 3' and 5' positions of the B-ring. The sugar moieties may also be acylated by a range of aromatic or aliphatic acids. Over 600 naturally occurring anthocyanins are known to be present (Anderson, 2002) and they vary in (1) the number and position of hydroxyl and methoxyl groups on the basic anthocyanidin skeleton; (2) The identity, number, and positions at which the sugars are attached; and (3) The extent of sugar acylation and the identity of the acylating agent.

A. Anthocyanin metabolism

Anthocyanidin glycosides are hydrolyzed by the intestinal microflora within 20 min to 2 h after consumption, depending on the sugar moiety (Keppler and Humpf, 2005). Due to the high instability of the released

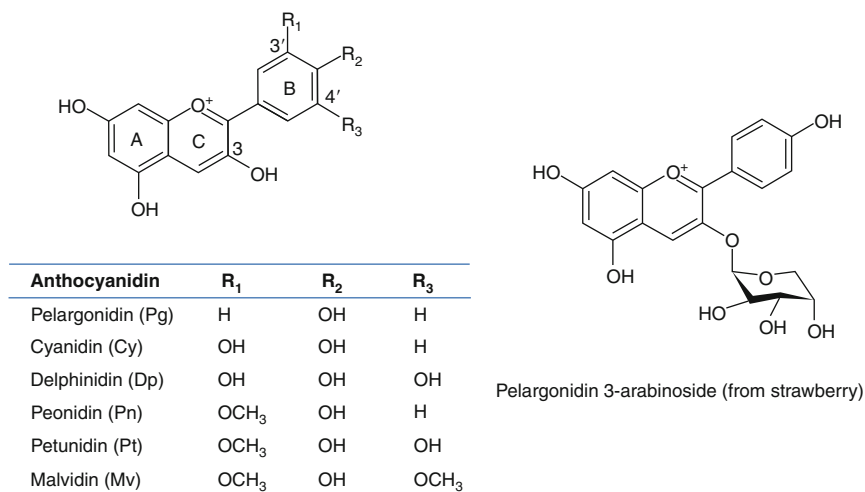


FIGURE 2.7 Structure of anthocyanidins and anthocyanins.

anthocyanidin aglycones at neutral pH, primary phenolic degradation products are detected within 20 min of incubation. Further metabolism of the phenolic acids is accompanied by demethylation. Such anthocyanin metabolites, derived from anthocyanin metabolism, may be responsible for the observed antioxidant activities and other physiological effects *in vivo*. Moreover, anthocyanins have low bioavailability and therefore are unlikely to provide protection at the cellular level. For example, a large proportion of the ingested polyphenols taken from berries is not taken up in to the circulation but instead passes through the upper gastrointestinal tract (GIT) to the large intestine where polyphenols may be biotransformed or broken down by the indigenous microflora (McDougall and Stewart, 2005). Due to the complexity of phenolic composition, it is hard to determine the exact nature of the compounds that are actually generated from anthocyanins. Labeled anthocyanins are thus necessary to determine the degraded compounds that are generated from anthocyanins (Prior and Wu, 2006).

Biotransformation enzymes involved in the pathway may include UDP-glucuronosyl transferase, UDP-glucose dehydrogenase, or catechol-O-methyltransferase (COMT), which are located in the small intestine, liver, or kidney. Depending on the chemical structure, anthocyanins could exist mainly in their native forms or as metabolites in blood and urine, whereas most other flavonoids are generally recovered as metabolites (Prior and Wu, 2006). Glucuronidation has been demonstrated to be a major chemical modification of anthocyanins. However, the extent of glucuronidation is significantly affected by the type of aglycone, substitution pattern, and amount of anthocyanins consumed (Wu *et al.*, 2005).

Anthocyanins have shown significant antiobesity and antidiabetic effects. More specifically, anthocyanin-rich foods have been shown to lead to a 24% decrease in weight gain in mice and decreased lipid accumulation in the liver, including a significant decrease in liver triacylglycerol concentration (Prior and Wu, 2006). Moreover, it was shown that anthocyanins enhance adipocytokine secretion and upregulate adipocyte-specific gene expression through AMP activated protein kinase activation (Ghosh, 2005). Anthocyanins from Cornelian cherries (*Cornus mas*) such as Cy-3-glc, Dp-3-glc, Cy-3-gal, and Pg-3-gal stimulate insulin secretion from rodent pancreatic beta-cells. Pg-3-gal, and its aglycone, Pg, caused a 1.4-fold increase in insulin secretion. The rest of the anthocyanins tested had only marginal effects on insulin (Jayaprakasam *et al.*, 2005).

B. Novel production technique

The variety of their biological roles has drawn much attention to anthocyanins, something that necessitates the development of methodologies for their efficient production. Anthocyanins are most commonly extracted as mixtures from plants or plant waste. However, due to the low anthocyanin concentration *in planta*, abundant natural resources are required for large-scale production. To resolve this problem, certain plants have been genetically engineered by increasing the activity of anthocyanin biosynthetic enzymes. However, the existence of competing pathways in plants complicates the substantial increase of content of specific anthocyanin compounds (Liu *et al.*, 2002). For that reason, blocking of competing pathways had to be implemented in order to further increase its content (Yu *et al.*, 2003). Plant cultivation also depends heavily on environmental, seasonal, and geological conditions. Therefore, consistent quality and quantity of plant resources could present a rate-limiting step to large-scale production. In the downstream processing line, anthocyanin extraction and purification is also inefficient due to the contamination of numerous plant small molecules and the loss of products due to processing conditions (Wang and Murphy, 1996).

In addition to extraction from plants, anthocyanins have already been known to be produced by plant cell cultures like *Vitis hybrids*, *Haplopappus gracilis*, and *Daucus carota*. However, plant cell cultures are not always a straightforward approach for meeting market needs due to several problems involved with the fermentation process. For example, plant cells tend to form aggregates that influence anthocyanin culture productivity since cells within aggregates are not adequately exposed to lighting required to induce anthocyanin biosynthesis. For example, formation of phenylalanine ammonia lyase (PAL), a key enzyme in the biosynthetic pathway, is promoted primarily by UV, particularly those of the UV-B region (Wellmann, 1975). Other enzymes in the pathway, particularly

those of the anthocyanin biosynthetic branch, appear to be regulated in part by UV and in part by phytochrome-activating wavelengths (700–800 nm) (Meyer *et al.*, 2002). In that respect, irradiance becomes a limiting factor to productivity (Hall and Yeoman, 1986). Also, the average light dosage is reduced or insufficient within a dense cell culture since the cell wall composition selectively restricts certain wavelengths (Smith and Spomer, 1995).

As a result of some of the limitations mentioned with the current production methodologies, anthocyanins are becoming attractive targets for fermentation production from well-characterized microbial hosts such as *Escherichia coli* (Yan *et al.*, 2005). In general, flavonoid production in recombinant microorganisms is advantageous because the cloned pathway(s) are under microbial promoters and therefore the production is independent of light or other regulatory elements (such as the MYB transcription factors) required by plants. In addition, *E. coli* and *S. cerevisiae* cultures can achieve higher yields than plant cell cultures because of their better duplication times. In addition, no plant peroxidases are present in bacteria and yeast and therefore the “browning effect” problem is significantly reduced. Browning effect refers to the formation of a brown color in plant anthocyanin extracts as a result of a two-step process. First, anthocyanins are oxidized by plant polyphenol oxidases present in the plant extract. Second, the oxidized anthocyanins undergo condensation and form brown pigments, which are usually undesired by the food industry. A simplified extraction procedure is another advantage of using microbial production platforms over plant crops or cultures. Since anthocyanins are not naturally produced in microbial hosts, a much less complicated matrix of products is generated through the heterologous expression of pathways that lead to specific product targets. This minimizes the downstream processing required for purification of the target molecules.

Engineering microorganisms for the production of anthocyanins has been facilitated by the discovery of the core metabolic pathway leading to their production. This biosynthetic pathway begins with chalcones leading to flavanone, dihydroflavonol, anthocyanidin, and finally anthocyanin (Fig. 2.8). Recently, *E. coli* was engineered to produce anthocyanins (Yan *et al.*, 2005). To achieve this goal, the flavanone pathway was bypassed by supplemental feeding of flavanones into *E. coli* JM109 strain carrying a gene assembly which consisted of *M. domestica* F3H (flavanone 3'-hydroxylase), *A. andraeanum* DFR (dihydroflavonol 4-reductase), ANS (anthocyanin synthase) from *M. domestica*, and a PGT8 from *P. hybrida*. Upon heterologous expression of these genes in *E. coli* and feeding glucose supplemented cultures with the flavanones naringenin or eriodictyol, their corresponding anthocyanins pelargonidin 3-O-glucoside and cyanidin 3-O-glucoside were obtained in the

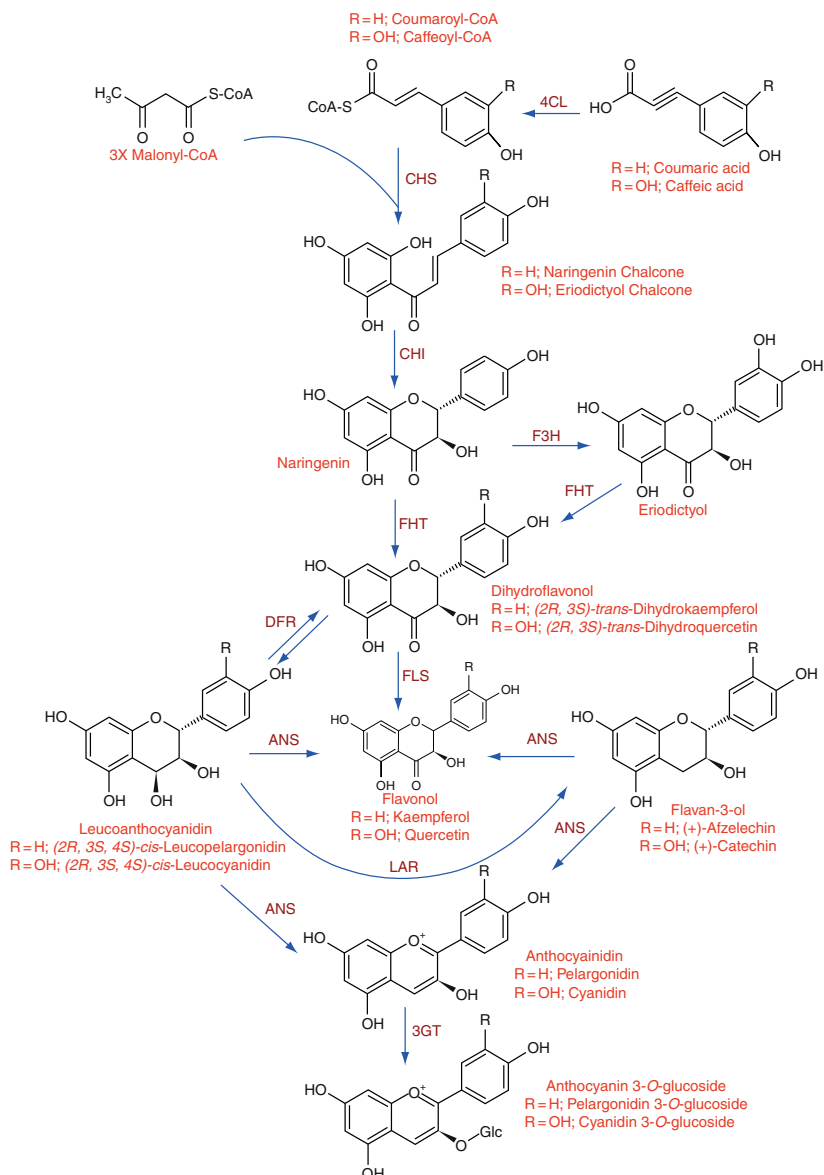


FIGURE 2.8 Biosynthesis of anthocyanins in *E. coli* (Yan *et al.*, 2008; Yan *et al.*, 2005).

culture and their biosynthesis confirmed by high-performance liquid chromatography (HPLC) and mass spectrometry (MS) analyses (Yan *et al.*, 2005). The product levels obtained from such system were low,

however, due to various systemic constraints. Some of the limitations in this production system were found to include the instability of the final anthocyanin molecules at normal pH, the intracellular availability of UDP-glucose, and the substrate specificity of ANS. Hence, several strategies were employed to overcome these barriers: (1) The culture medium pH was adjusted to pH 5.0 for enhanced stability of anthocyanins; (2) A translational fusion protein between ANS and 3GT (flavonoid 3-O-glucosyltransferase) was created in order to duplicate the possible multienzyme system in the plant cell; (3) The native *E. coli* metabolic network was manipulated by overexpressions and deletions in order to improve the intracellular UDP-glucose pool along with other cofactors necessary for ANS activity; (4) Catechin, rather than flavanones were employed as the precursor anthocyanin metabolites (Yan *et al.*, 2008). As a result of these optimization efforts, the volumetric production of pelargonidin 3-O-glucoside and cyanidin 3-O-glucoside was increased several fold. More specifically, the development of a two-step fermentation strategy in which first high biomass of *E. coli* was obtained in rich medium which was next transferred into minimal media at pH 5.0 during which period anthocyanins were produced resulted in a volumetric production of 38.9 mg/L of cyanidin 3-O-glucoside. Creation of the fusion protein 3GT-ANS boosted the production even further by 16% to approximately 45 mg/L. Combined implementation of coexpression of the two enzymes involved in the conversion of glucose 6 phosphate to UDP-glucose, deletion of UDP-glucose-consuming metabolic reactions, and fermentation media optimization resulted in a further increase in anthocyanin production to a final volumetric yield of approximately 75 mg/L (Yan *et al.*, 2008). Recently, the metabolism of *E. coli* was successfully manipulated toward the production of anthocyanins through the introduction of novel carbon assimilation pathways and the attenuation of several gene targets in order to increase the availability of UDP-glucose. With these genetic and metabolic engineering strategies, the recombinant production of anthocyanins has reached up to 113 mg/L (Leonard *et al.*, 2008).

C. Mechanism of action

Recent studies on classes of polyphenols such as anthocyanins and ellagitannins have shown that they have pathophysiological properties such as antioxidant and antihypertensive activities and have also demonstrated the inhibition of lipid oxidation (Matsui *et al.*, 2001a). More specifically, anthocyanins inhibit α -glucosidase activity and can reduce blood glucose levels after starch-rich meals. In general, polyphenolic extracts from a number of plants have been shown to be effective inhibitors of intestinal α -glucosidase/maltase activity (Matsui *et al.*, 2001b) with K_i values in the same range as other inhibitors such as the previously

described acarbose and voglibose (Toeller, 1994). From the various polyphenols, it has been shown that α -glucosidase activity *in vitro* is significantly inhibited by anthocyanin-rich extracts of blueberry and blackcurrant which contain a small proportion of acylated anthocyanins (McDougall *et al.*, 2005). The inhibitory effects of anthocyanins depend on their structure. Acylated anthocyanins are more potent α -glucosidase inhibitors than deacylated anthocyanins. Acylated anthocyanins from *Clitoria ternatea* flowers (Terahara *et al.*, 1996) and acylated anthocyanins from storage roots of purple sweet potato, *Ipomoea batatas* (Terahara *et al.*, 1996, 1999), have shown inhibitory effects on α -glucosidase activity thus lowering the glucose absorption. Three deacylated anthocyanins isolated from the red flowers of the morning glory, *Pharbitis nil* cv. Scarlett O'Hara (SOA), and the storage roots of the purple sweet potato, *I. batatas* cv. Ayamurasaki (YGM), with different aglycons of pelargonidin (Pg), cyanidin (Cy), and peonidin (Pn) have been shown experimentally to have a lower inhibitory effect than acylated extracts. Inhibitory activities of these deacylated anthocyanins are decreased by a factor of 1/70–1/90 compared to their acylated forms. The enhanced inhibition exhibited by acylated anthocyanins over their deacylated forms (Matsui *et al.*, 2001a) perhaps reflects the enhanced stability of the acylated anthocyanins at intestinal pH but such differences in effectiveness may not be particularly relevant when 200 mg of anthocyanins are available from a single portion of berries (Clifford, 2000). Ellagitannins also inhibit α -amylase activity and there is potential for synergistic effects on starch degradation after ingestion of berries such as raspberries and strawberries, which contain substantial amounts of ellagitannins and anthocyanins (McDougall and Stewart, 2005). Finally, anthocyanins have been shown to directly induce secretion of insulin from pancreatic cells in *ex vivo* assays (Jayaprakasam *et al.*, 2005), but this effect may be marginal *in vivo* because of the low serum bioavailability of anthocyanins. The mechanism of α -glucosidase inhibition action by anthocyanins is not fully understood but one can assume that the inhibition, like that of acarbose, is competitive and results from the structural similarity between the normal substrate maltose and the glucosyl groups β -linked to the anthocyanin which bind to the active site but are not hydrolyzed. Structure–activity relationships with respect to the aglycone and the attached sugars are still not well understood. Various other anthocyanin-rich plant extracts have been studied and they have shown different degree of α -glucosidase inhibition. Table 2.3 summarizes the different anthocyanin-rich plant extracts that have been tested for their potential therapeutic properties against Type II diabetes through inhibition of α -glucosidase. The order of α -glucosidase inhibition is SOA > BOC > YGM > ternatin, with SOA extract showing the strongest α -glucosidase inhibitory activity with an IC_{50} value of 0.22 mg/mL (Matsui *et al.*, 2001b) (Table 2.4).

TABLE 2.3 Anthocyanin extracts from various plants (Keppler and Humpf, 2005)

Scientific name	English name	Abbreviation
<i>Clitoria ternatea</i> L. flower	Butterfly pea	Ternatin
<i>Ipomoea batatas</i> L. root	Sweet potato	YGM
<i>Brassica oleracea</i> L. leaf	Cabbage	BOC
<i>Papianus sativus</i> L. root	Radish	RPS
<i>Dioscorea alata</i> L. tuber	Yam	DOA
<i>Pisum sativum</i> L. pod	Pea	PSP
<i>Sambucus nigra</i> L. berry	Elderberry	SNB
<i>Fatsia japonica</i> L. berry skin	–	FJB
<i>Rubus loganbaccus</i> berry	Boysenberry	RLB
<i>Pharbitis nil</i> cv. Scarlett O' Hara flower	Morning glory	SOA
<i>Houttuynia cordata</i> Thunb. Leaf	–	HCT
<i>Zea mays</i> L. seed coat	Corn	ZML

TABLE 2.4 α -Glucosidase inhibitions by two active anthocyanins extract (Matsui *et al.*, 2001b)

Extract	IC ₅₀ (mg/mL)		
	Free α -glucosidase	Immobilized α -glucosidase	α -Amylase
SOA	0.35	0.17	0.43
YGM	0.36	0.26	0.61
Green tea	0.23	0.22	–

These anthocyanin extracts have also been shown to inhibit both free and immobilized α -glucosidase. Among the extracts, SOA (IC₅₀ = 0.35 mg/mL) and YGM (IC₅₀ = 0.36 mg/mL) are sufficient α -glucosidase inhibitors, being comparable to green tea extract (IC₅₀ = 0.23 mg/mL) (Table 2.4). In addition, these extracts were tested for their inhibitory potential against sucrase: none of them inhibited sucrase whereas green tea extract did inhibit sucrase activity (Matsui *et al.*, 2001b). α -glucosidase inhibitory activities of all the isolated anthocyanins are 5 times higher than that of the natural inhibitor, D-xylose (IC₅₀ = 1190 μ M). Among them, SOA-4 possessed the most potent activity as shown by an IC₅₀ value of 60 μ M, and their inhibitory behavior is in the order of SOA-4 > SOA-6 > YGM-3 = YGM-6 (Table 2.5). The inhibitory effects when compared with therapeutic α -glucosidase inhibitors like Acarbose and Voglibose, is very less. But a daily intake of the extracts as food helps to keep a moderate plasma blood glucose control. Thus anthocyanins have a great potential as therapeutic drugs for diabetes.

TABLE 2.5 iAGH inhibitory activities of isolated acylated anthocyanins, D-Xylose and therapeutic drugs voglibose and acarbose

Compound	IC ₅₀ value	
	μM	mg/mL
SOA-4	60	0.081
SOA-6	107	0.128
YGM-3	193	0.239
YGM-6	200	0.245
D-Xylose	1190	0.1785 mg/mL
Voglibose	0.0055	0.0015×10 ⁻³ mg/mL
Acarbose	0.426	0.275×10 ⁻³ mg/mL

VI. PINE BARK EXTRACT

Pycnogenol is the patented trade name for the water extract of French maritime Pine bark (*Pinus pinaster*) extract that is rich in polyphenols (Markham and Porter, 1973), and it has recently been demonstrated to have various biological and health beneficial effects (Sanbongi *et al.*, 1997). Pycnogenol contains oligomeric proanthocyanidins as well as other bioflavonoids like catechin, epicatechin, phenolic fruit acids (such as ferulic acid and caffeic acid), and taxifolin. Procyanidins are oligomeric catechins found at high concentrations in red wine, grapes, cocoa, cranberries, and apples.

Among others, pycnogenol has been reported to display antidiabetic effects (Liu *et al.*, 2004a). Supplementation with 100 mg Pycnogenol for 3 months significantly lowered blood glucose levels compared to placebo, and improved endothelial function was observed in patients with Type II diabetes. The glucose lowering effect was dose dependent and not accompanied by an increase in insulin secretion (Liu *et al.*, 2004b).

Among others, it is believed that the antidiabetic effect of Pycnogenol is due to its digestive enzyme inhibitory activity. The digestive enzyme inhibitory activity of Pycnogenol is comparable to that of green tea catechin extracts and to acarbose (Schafer and Hogger, 2007). Its IC₅₀ (5.34 μg/mL) is about four times lower than that of green tea extract, which has an IC₅₀ of 19.74 μg/mL. Acarbose had a 190 times lower ability to inhibit α-glucosidase than the pine bark extract (Table 2.6).

Experiments were carried out to determine which of the monomeric polyphenolic compounds in the extract fractions of Pycnogenol were responsible for the inhibitory activity toward α-glucosidase (Schafer and

TABLE 2.6 Comparison of inhibitory effects of pycnogenol (Schafer and Hogger, 2007)

Compound	IC ₅₀ (μg/mL)
Acarbose	1010 ± 210
(+) Catechin	52.14 ± 20.3
Pycnogenol	5.34 ± 0.91
Green tea extract	19.74 ± 1.8

Hogger, 2007). Only (+)-catechin and procyanidins were shown to inhibit α -glucosidase, whereas the other compounds such as ferulic acid, (–)-epicatechin, gallic acid, 4-hydroxybenzoic acid, caffeic acid, protocatechuic acid, sinapic acid, and (±) taxifolin did not demonstrate any inhibitory activity. The activity of Pycnogenol toward α -glucosidase increases with the degree of polymerization of procyanidins (Schafer and Hogger, 2007). In addition to alpha-glucosidase, pine bark extract has shown competitive inhibition against human salivary and porcine pancreas α -amylases as well (Kim *et al.*, 2005).

Pine bark extract is found to be very stable under thermal conditions and comparatively stable under acidic conditions. Thus, pine bark extract is easier to handle during processing or manufacturing steps and remains stable when exposed to the human digestive tract.

VII. OTHER EXTRACTS OF PLANT ORIGIN

As mentioned, various plant extracts harbor the promise of potential Type II diabetes treatment through different mechanisms, with inhibition of digestive enzymes being the most prominent. However, in most cases, the active substance(s) of many of these extracts have not been found. In addition, lots of information related to their therapeutic potential is anecdotal or indirect or still limited to animal studies. Broadly, we have classified plant extracts into two groups, one composed of plant extracts with known active compounds, and the other where the active compounds are unknown, although positive inhibitory effects on digestive enzymes have been reported. A number of such plants and their active component (when known) are provided in Table 2.7.

A. Known active compounds

Momordica charantia: *Momordica charantia* known as bittermelon or bittergourd belongs to the family of *Cucurbitaceae*. Cultivated fruits of *Momordica charantia* are widely used to treat diabetes in Asia and Australia.

TABLE 2.7 Traditional antidiabetic plants with active substance

Plants	Active part of plants	Active substance	Activity demonstrated in	References
<i>Momordica Charantia</i>	Fruits and seeds	Charantin, alkaloids	Alloxan-induced diabetic rats	Fernandes et al. (2007)
<i>Phyllanthus Reticulatus</i>	Leaves	Terpenoids glycosides	Aloxan-induced diabetic mice	Kumar et al. (2008)
<i>Panax Ginseng</i>	Roots, berry, leaves	Ginsenosides	Diabetic mice	Xie et al. (2005)
<i>Zea mays</i> (purple corn)	Kernel	Anthocyanins	Rats	Jones (2005)
<i>Trigonella foenumgraecum</i>	Seeds	Trigonelline (alkaloid)	Alloxan-induced diabetic mice	Vijayakumar et al. (2005)
<i>Ipomoea batatas</i>	Purple fleshed sweet potato	Acylated anthocyanins, caffeoylsophorose	Sprague Dawley Rats	Matsui et al. (2004) , Suda et al. (2003)
<i>Lens esculenta</i>	Seeds	Condensed tannins (polyphenols)	<i>In vitro</i>	Quesada et al. (1996)
<i>Theobroma cacao</i>	Seeds	Condensed tannins (polyphenols)	<i>In vitro</i>	Quesada et al. (1996)
<i>Cephalotaxus sinensis</i>	Leaves	Epigenin, epigenin glycoside	Streptozotocin-induced diabetic rats	Li et al. (2007)
<i>Feculae bombycis</i>	Unknown	1-Deoxynojirimycin (DNJ)	Wistar rats	Zhu et al. (2005)

(continued)

TABLE 2.7 (continued)

Plants	Active part of plants	Active substance	Activity demonstrated in	References
<i>Curcuma longa</i>	Unknown	Natural curcumin, demethoxycurcumin	<i>In vitro</i>	Du <i>et al.</i> (2006)
<i>Bauhinia forficata</i>	Leaves	Kaempferitin	Alloxan-induced diabetic rats	de Sousa <i>et al.</i> (2004)
<i>Syzygium zeylanicum</i>	Leaves	Polyphenols	<i>In vitro</i>	Mai <i>et al.</i> (2007)
<i>Cleistocalyx operculatus</i>	Leaves	Polyphenols	<i>In vitro</i>	Mai <i>et al.</i> (2007)
<i>Careya arborea</i>	Leaves	Polyphenols	<i>In vitro</i>	Mai <i>et al.</i> (2007)
<i>Horsfieldia amygdalina</i>	Leaves	Polyphenols	<i>In vitro</i>	Mai <i>et al.</i> (2007)
<i>Aconitum carmichaeli</i>	Root	Aconitan A (polysaccharide)	Diabetic mice	Konno <i>et al.</i> (1985a)
<i>Allium cepa</i>	Bulb	Alkyldisulfides	Diabetic rabbits	Augusti (1974)
<i>Allium sativum</i>	Bulb	Alkyldisulfides	Healthy rabbits	Jain <i>et al.</i> (1973)
<i>Blighia sapida</i>	Fruit	Hypoglycins	IDDM and Type II diabetes patients	Bressler <i>et al.</i> (1969)
<i>Catharanthus roseus</i>	Leaf	Hypoglycins	IDDM and Type II diabetes patients	Bressler <i>et al.</i> (1969)
<i>Cyamopsis tetragonolobus</i>	Seed and pod	Alkaloids	Healthy rats	Farnsworth and Segelman (1971), Peters (1957)

<i>Vaccinium myrtillus</i>	Leaf	Neomyrtillin	Diabetic humans	Allen (1927)
<i>Tecoma stans</i>	Leaf	Saccharan C, characterized alkaloid	Diabetic mice, diabetic rabbit	Hammouda and Amer (1966), Hammouda and Khalafallah (1971)
<i>Saccharum officinarum</i>	Stalk	Quiquefolans	Diabetic mice	Takahashi <i>et al.</i> (1985)
<i>Momordica foetida</i>	Ariel	Uncharacterized glycosides and alkaloids	Type II diabetes patients	Olaniyi (1975)
<i>Galega officinalis</i>	Leaf	Guanidine	Diabetic mice	Hermann (1973)
<i>Ficus bengalensis</i>	Stem bark	Bengalenoside(glycoside)	Diabetic mice	Brahmachari and Augusti (1964)
<i>Ephedra distachya</i>	Aerial	Eleutherans	Diabetic mice	Konno <i>et al.</i> (1985b)
<i>Ganoderma lucidum</i>	Fruit body	Uncharacterized glycoside	Healthy rodents	Hikino <i>et al.</i> (1985)
<i>Lupines termis</i>	Seed	Quinolizidine alkaloids	Alloxan-induced diabetic mice	Mishkinsky <i>et al.</i> (1974)
<i>Cinnamomum cassia</i>	Bark of tree	Procyanidin type-A polymers	Clinical trials	Chase and McQueen (2007)

Despite their bitter taste, they are often included in regular diet due to their prophylactic properties. The fruit and its extract have been introduced in Europe as alternatives to conventional treatments for non-insulin-dependent diabetes mellitus. Charantin, a mixture of glycosides, mainly β -sitosterol-D-glucoside and stigmadine glucoside, is the active substance responsible for the fruit's hypoglycemic action (Fernandes *et al.*, 2007). It has been shown that the hypoglycemic effect is mediated through (1) suppression of the key hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase, and (2) an accelerated rate of glucose metabolism through the pentose phosphate pathway (Jain *et al.*, 1973). Fifty milliliters of the aqueous extract of the plant reduced glucose concentration of diabetic patients by 20% within 1 h. The above results have been shown in healthy and alloxan-induced diabetic animals. Ethanolic extracts of *M. charantia* showed an antihyperglycemic as well as hypoglycemic effect in normal and streptozotocin (STZ) induced diabetic rats by decreasing blood sugar by 23% and 27%, respectively (Shibib *et al.*, 1993).

Allium sativum: Garlic, scientifically known as *Allium sativum*, is a perennial herb cultivated throughout India and is commonly used as a food ingredient. It has long been used as dietary supplement for traditional treatment of diabetes in Asia, Europe, and the Middle East. Concentrated plant extracts exerted a weak hypoglycemic effect in healthy and alloxan-induced diabetic animals and healthy humans. Fasting glucose levels were lowered and oral glucose tolerance was improved by 7–18% within 1–2 h after oral administration of aqueous and ethanolic extracts of garlic at doses of 10 g extract/kg body weight. This effect has been attributed to the volatile oils allyl propyl disulfide and diallyl disulfide oxide (Jain *et al.*, 1973). S-Allyl cysteine (SACS), precursor of allicin and garlic oil, is a sulfur-containing amino acid. It is isolated from garlic extract and pharmacokinetic studies show its rapid absorption in the gastrointestinal tract and high bioavailability after oral administration (Wagman and Nuss, 2001). Oral administration of SACS to alloxan-diabetic rats for 1 month ameliorated glucose intolerance and resulted in weight loss and depletion of liver glycogen in diabetic rats in comparison to glibenclamide and insulin-treated animals. SACS also stimulated *in vitro* insulin secretion from β cells isolated from rats (Jain *et al.*, 1973).

Panax Ginseng: Ginseng, often described as the “king herb,” holds an important position in traditional Oriental medicine in many countries. This highly valued plant is currently cultivated in China, Korea, Japan, Russia, the United States, and Canada. Of the several species of ginseng, the root of the Asian and American varieties (*Panax ginseng*) is a popular dietary supplement in the United States.

Historical records on traditional medicinal systems reveal that ginseng root was used to treat diabetes. Research on the effects of treatment with ginseng root on blood sugar levels started early last century. Between

1921 and 1932, Japanese scientists reported that ginseng root decreased baseline blood glucose and reduced hyperglycemia caused by adrenaline or high concentration glucose administration. Ginseng root has since been used to treat patients with diabetes. Results of *in vitro* studies, animal experiments, and clinical trials strongly support the claim that ginseng root possesses antidiabetic properties (Xie *et al.*, 2005). The root of the ginseng plant is constituted of organic (80–90%) and inorganic substances (approximately 10%) and consists of a number of active constituents, such as saponins or ginsenosides, carbohydrates (including polysaccharides), nitrogenous substances, amino acids, peptides, phytosterol, essential oils, organic acids, vitamins, and minerals. Of these, the extract fractions containing ginsenosides and polysaccharides have demonstrated hypoglycemic activity, with ginsenosides being the principal bioactive constituents of ginseng (Xie *et al.*, 2005). Ginsenoside content is in the order leaf > berry > root. Ginsenosides are classified as protopanaxatriol (Rg1, Rg2, Rg3, Re, and Rf) and protopanaxadiol (Rb1, Rb2, Rc, and Rd). Among these, ginsenoside Re has demonstrated significant antihyperglycemic effect as well as reduction of serum insulin levels in fed or fasting mice (Atte *et al.*, 2002). Ginseng berry extract exhibited significantly more potent antidiabetic effects than the root extract. The difference in ginsenoside Re content between root and berry extracts may account for the difference in their pharmacological effects. Ginseng might mediate its antidiabetic actions through a variety of mechanisms, including actions on the insulin-secreting pancreatic β -cells and the target tissues that take up glucose. Ginseng treatment increased insulin release from pancreatic β -cells, which is probably caused by increased β -cell stimulation and increased insulin synthesis. Furthermore, the effect of ginsenoside Re in reducing expression of enzymes involved in lipid metabolism could be beneficial in diabetes (Xie *et al.*, 2005).

Moreover, Asian ginseng root extracts administered to alloxan-treated diabetic mouse models decreased blood glucose levels significantly. Complex components in the carbohydrate fraction of ginseng root extract, including different panaxans A, B, C, D, E, panaxans I, J, K, L, and panaxans Q, R, S, T, U also demonstrated hypoglycemic properties in normal and alloxan-induced hyperglycemic mice. Similar to Asian ginseng, three constituents obtained from the water extracts of American ginseng root, viz. quinquefolans A, B, and C, displayed hypoglycemic actions in normal and hyperglycemic mice. Clinical trials have also reported antihyperglycemic activity when a single dose of 3 g American ginseng root was administered in both nondiabetic and Type II diabetic individuals.

Trigonella foenumgraecum (fenugreek): The seeds of *T. foenumgraecum* are widely recommended for patients with diabetes. More specifically, fenugreek seed extracts (FSE) have been shown to significantly improve

glucose homeostasis in alloxan-induced diabetic mice by effectively lowering blood glucose levels (Jain *et al.*, 1973; Vijayakumar *et al.*, 2005). The hypoglycemic activity has been attributed to an uncharacterized alkaloid termed trigonelline, although other possible hypoglycemic agents such as nicotinic acid have been isolated from the seeds (Bailey and Day, 1989).

Glucose uptake, facilitated by translocation of glucose transporters from an intracellular site to the plasma membrane, is the rate-limiting step in hyperglycemic conditions. FSE induces a rapid, dose-dependent stimulatory effect on cellular glucose uptake by activating cellular responses that lead to glucose transporter's translocation to the cell surface (Jain *et al.*, 1973). Moreover, a novel amino acid (4-hydroxyisoleucine) extracted and purified from fenugreek seeds has shown an increase in glucose-induced insulin release in both rats and humans (Grover *et al.*, 2002; Vijayakumar *et al.*, 2005).

Ipomoea batatas: This is a trialing herb cultivated for its succulent tuberous roots. Oral administration of *I. batatas* reduces hyperinsulinemia in Zucker fatty rats by 23%, 26%, 60%, and 50% after 3, 4, 6, and 8 weeks, respectively. In addition, inhibition of blood glucose level after glucose loading was observed after 7 weeks of treatment along with reggranulation of pancreatic beta cells and reduction in insulin resistance (Kusano and Abe, 2000; Matsui *et al.*, 2004; Suda *et al.*, 2003). Hypolipidemic activity has also been described (Kusano and Abe, 2000). Acylated anthocyanins are the active substances isolated from *I. batatas* and are responsible for its hypoglycemic action.

Cinnamomum cassia (cinnamon): Cinnamon is a common spice that has recently gained attention as a possible treatment for diabetes. Historically, cinnamon has been used medicinally for loss of appetite and dyspeptic complaints. The two major types of cinnamon are from the bark of different but related trees (Chase and McQueen, 2007): (1) *Cinnamomum verum*, also known as *Cinnamomum zeylanicum*, is true cinnamon, (2) *C. cassia*, also known as *Cinnamomum aromaticum*, Chinese cinnamon, cassia cinnamon, and bastard cinnamon, is commonly used as a spice. This more easily available species is also the type used medicinally. Little is known about the mechanism of action and activities of cinnamon. *C. cassia* is thought to have more insulin-stimulating properties than true cinnamon (Verspohl *et al.*, 2005). The active compounds responsible for the insulin-like activity of cinnamon are believed to be procyanidin type-A polymers (Anderson *et al.*, 2004); these compounds have been shown to promote insulin receptor autophosphorylation, increasing insulin sensitivity (Anderson *et al.*, 2004; Imparl-Radosevich *et al.*, 1998). It has been shown that a quarter of a teaspoonful of cinnamon taken twice a day by patients with diabetes lowers blood sugar by an average of 18–29%, triglycerides by 23–30%, LDL cholesterol by 7–27%, and total cholesterol by 12–26% (Khan *et al.*, 2003).

Cinnamon may have the potential to become a common adjunct to diabetes treatment. Relative safety, low cost, and potential efficacy make cinnamon a viable option for patients interested in using more natural therapies.

B. Unknown active compounds

Agaricus bisporus (common edible mushroom): It is considered a useful dietary adjunct for diabetes in Europe, and a hypoglycemic effect has been shown in streptozocin (STZ) induced diabetic mice. More specifically, *A. bisporus* consumption improved insulin sensitivity in diabetic mice and a lectin from this mushroom stimulated insulin release by isolated islets of healthy rats (Swanston-Flatt *et al.*, 1989).

Aloe vera: It is a native of North Africa and is also cultivated in Turkey and drier parts of India. *Aloe* species have been commonly used for centuries as laxatives and for their anti-inflammatory and antitumor activities. Moreover *Aloe vera* extracts from leaf pulp and leaf gel have shown significant antidiabetic activity (Okyar *et al.*, 2001). Extracts of aloe gum effectively increased glucose tolerance in both normal and diabetic rats (Ghannam *et al.*, 1986). Chronic but not single administration of the exudate of the leaves of *Aloe barbadensis* (500 mg/kg PO) showed significant hypoglycemic effect in alloxan-diabetic mice. However, single as well as chronic administration of the bitter principle (5 mg/kg IP) showed significant hypoglycemic effect in the same model. The hypoglycemic effect of single dose of the bitter principle was extended over a period of 24 h with maximum hypoglycemia observed at 8 h, while chronic administration (exudate twice daily and the bitter principle once a day for 4 days) showed maximum reduction in plasma glucose level on the 5th day (Grover *et al.*, 2002). Hypoglycemic effect of aloe and its bitter principle is mediated through stimulation of synthesis and/or release of insulin from the β -cells of langerhans. Additionally, both *Aloe vera* and *Aloe gibberellins* (over a dose range of 2–100 mg/kg) inhibit inflammation in a dose-response manner and improve wound healing in STZ diabetic mice. The dried sap of the plant (half a teaspoonful daily for 4–14 weeks) has shown significant hypoglycemic effect both clinically as well as experimentally.

Sclerocarya birrea: Popularly known as a Cider tree, it is a South African medicinal plant. The stem bark, roots, and leaves of the plant have been reported to possess medicinal and other properties in addition to the nutritional values of the fruits and seeds of the plant. In southern and some other parts of Africa, the stem bark, roots, and leaves of *S. birrea* are used for the treatment of diabetes mellitus. The plant's aqueous extract like chlorpropamide (a sulfonylurea antidiabetic agent) produced significant reductions in the blood glucose levels of the fasted normal and fasted

STZ-treated diabetic rats. The hypoglycemic effects of *S. birrea* stem bark aqueous extract therefore appear to be mediated via a mechanism that is similar to that of chlorpropamide, a sulfonylurea antidiabetic drug (Ojewole, 2004). *S. birrea* has been widely reported to contain many chemical compounds, including coumarins, terpenoids, flavonoids, tannins, β -sitosterol, oils, organic acids, and inorganic substances. At present, the exact chemical constituents of the plant's stem bark aqueous extract that is specifically responsible for the antidiabetic activity of the plant remain speculative.

Tinospora cordifolia: It is found in Asia and is widely used in Ayurveda as tonic, vitalizer, and as a remedy for diabetes mellitus and metabolic disorders. Diabetic rats with mild (plasma sugar = 180 mg/dL), moderate (plasma sugar = 280 mg/dL), and severe (plasma sugar = 400 mg/dL) diabetes, respectively, showed maximum hypoglycemia after being administered the aqueous extract of *T. cordifolia* for 15 weeks. The functional status of the pancreatic β -cells influences the hypoglycemic effect, and a significant reduction in blood glucose, increase in body weight, total hemoglobin, and hepatic hexokinase in alloxanized diabetic rats is observed by its oral administration (Stanely *et al.*, 2000). Thus, the plant has a potential to be used as an antidiabetic drug, but further research is required in order to identify its active substance and the mechanism of action.

Eucalyptus globules: It is a lofty tree of about 90 m in height and is grown in various parts of India. Aqueous extract (0.5 gm/L) of eucalyptus increases peripheral glucose utilization in the mouse abdominal muscle and stepwise enhancement of insulin secretion from the clonal pancreatic β -cell line by 70–160% (Gray and Flatt, 1998). Administration of *E. globulus* leaves in the diet of normal rats (6.25%, w/w) for 12 days did not result in hypoglycemia. In addition, STZ administration to these pretreated rats did not produce hyperglycemia. Pretreated rats have also shown less polydypsia and body weight loss. (Swanston-Flatt *et al.*, 1989).

The various plants having antidiabetic properties but where the active components are yet unknown are summarized in Table 2.8.

VIII. METFORMIN

The history of diabetes mellitus is replete with many therapies, nearly all, including insulin, first given without any knowledge of a mechanism of action. In medieval times, a prescription of *Galega officinalis*, also known as Goat's rue, the French lilac, or Italian fitch, was given to cure diabetes mellitus. It has also been used as galactagogue in cows. The active ingredient in the French lilac that produced the lowering of blood glucose was shown to be galegine or isoamylene guanidine (Cusi and DeFronzo, 1998;

TABLE 2.8 Traditional antidiabetic plants with hypoglycemic effects

Plants	Active part of the plant	Activity demonstrated in	Reference
<i>Sclerocarya birrea</i>	Stem bark	Diabetic mice	Ojewole (2004)
<i>Aconitum carmichaeli</i>	Root	Streptozotocin-induced diabetic mice	Liou <i>et al.</i> (2006)
Pine bark	Bark	Rats	Kim <i>et al.</i> (2005)
<i>Clitoria ternatea</i>	Flower	<i>In vitro</i>	Matsui <i>et al.</i> (2001b)
<i>Brassica oleracea</i>	Leaf	<i>In vitro</i>	Matsui <i>et al.</i> (2001b)
<i>Pisum sativum</i>	Pod	<i>In vitro</i>	Matsui <i>et al.</i> (2001b)
<i>Dioscorea alata</i>	Tuber	<i>In vitro</i>	Matsui <i>et al.</i> (2001b)
<i>Pharbitis nil</i> cv.	Flower	<i>In vitro</i>	Matsui <i>et al.</i> (2001b)
<i>Enicostemma littorale</i>	Aqueous extract (whole plant)	Alloxan-induced diabetic mice	Maroo <i>et al.</i> (2002)
<i>Commelina communis</i>	Dried leaves, stem	Male ICR mice	Youn <i>et al.</i> (2004)
<i>Potentilla fulgens</i>	Tap roots	Alloxan-induced diabetic mice	Syiem <i>et al.</i> (2002)
<i>Helichrysum graveolens</i>	Capitulum	Streptozotocin-induced diabetic rat	Aslan <i>et al.</i> (2007)
<i>Agaricus bisporas</i>	Mushrooms	Streptozocin-induced diabetic rats	Swanston-Flatt <i>et al.</i> (1989)
<i>Coriandrum sativum</i>	Seeds	Streptozocin-induced diabetic mice	Sharaf <i>et al.</i> (1963)
<i>Juniperus communis</i>	Berries	Streptozocin-induced diabetic mice	Swanston-Flatt <i>et al.</i> (1990)

(continued)

TABLE 2.8 (continued)

Plants	Active part of the plant	Activity demonstrated in	Reference
<i>Opuntia streptacantha</i>	Stems	Type II diabetes patients, alloxan-induced diabetic rabbits	Ibanez-Camacho <i>et al.</i> (1983)
<i>Tinospora cordifolia</i>	Aqueous extract	Alloxan-induced diabetic rats	Swanston-Flatt <i>et al.</i> (1990)
<i>Phaseolus vulgaris</i>	Pods	Alloxan-induced diabetic rats	Sharaf <i>et al.</i> (1963)
<i>Aloe vera</i>	Ariel	Type II diabetes patients, alloxan-induced diabetic mice	Ghannam <i>et al.</i> (1986)
<i>Coprinus comatus</i>	Fruit body	Healthy rats and mice	Lelley (1983)
<i>Eucalyptus globules</i>	Leaf	Diabetic mice	Swanston-Flatt <i>et al.</i> (1990)
<i>Salvia lavandulifolia</i>	Flower	Diabetic rabbits	Jimenez <i>et al.</i> (1986)
<i>Teucrium oliverianum</i>	Aerial	Diabetic mice	Ajabnoor <i>et al.</i> (1984)
<i>Lythrum salicaria</i>	Aerial	Healthy rabbits	Torres and Suarez (1980)
<i>Cuminum nigram</i>	Seeds	Diabetic rabbits	Akhtar and Ali (1985)

Witters, 2001). The guanidine agent's hypoglycemic activity for lowering of blood glucose levels was demonstrated from the mistaken notion that the tetany of hypoparathyroidism was due to the production of increased guanidine following parathyroidectomy (Watanabe, 1918). Though guanidine and certain derivatives are too toxic for the treatment of diabetes mellitus, the biguanides (two linked guanidine rings) have proved useful and became commercially available for diabetes therapy in the 1950s.

Phenformin and buformin, both belonging to the biguanide class, were withdrawn from the pharmacopoeia in the early 1970s due to the emergence of frequent lactic acidosis and increased cardiac mortality. Metformin, a less lipophilic biguanide, proved safer and has been in use in Europe since the 1950s. The U.S. FDA approved the drug Metformin on December 30, 1994. It is indicated for the treatment of Type II diabetes as an adjunct to diet and exercise, either as a single oral agent or in combination with sulfonylureas, α -glucosidase inhibitors, or insulin. An extended-release (XR) formulation was approved in October 2000, and combination products containing metformin and glyburide, rosiglitazone, or glipizide have since been approved (Goodarzi and Bryer-Ash, 2005).

Metformin has been marketed under different trade names such as Glucophage[®], Riomet[®], Fortamet[®], Glumetza[®], and Diabex[®]. In the past few years, it has become the most popular anti diabetic drug in USA and is one of the two oral antidiabetics in the World Health Organization Model list of Essential Medicines. The chemical composition of metformin is *N,N*-dimethyl imido dicarbonimidic diamide (C₄H₁₁N₅). Due to its structure, metformin becomes polar and less soluble in lipids. The structure of metformin is shown in Fig. 2.1.

A. Mechanism of action

The blood-glucose-normalizing component of this drug is its biguanide structure; however, the exact mechanism of metformin's action is not completely understood. Its main blood-glucose-lowering activity appears to be primarily through suppression of hepatic glucose output (Einhorn *et al.*, 2000; Jones *et al.*, 2003). Metformin reduces gluconeogenesis by 0.6 mg/kg per min, in effect leading to a 75% reduction in hepatic glucose output.

Metformin does not induce hypoglycemia when used by nondiabetic patients (Karam *et al.*, 1975). It has little or no effect on gastrointestinal glucose absorption. Glucoregulatory actions of metformin occur at the liver to reduce glucose output and at the peripheral tissues to augment glucose uptake, chiefly in muscle (McIntyre *et al.*, 1991) although this has not been confirmed by all investigators (Fendri *et al.*, 1993). Clinical trials show that metformin monotherapy in patients with Type II diabetes mellitus reduces fasting plasma glucose by 3–4 mmol/L and hemoglobin

A1c (HbA_{1c}) by 1.5–2% while the failure to metformin therapy occurs with approximately the same frequency (about 5–10% per year) as with sulfonylureas (U.K. prospective diabetes study 16, 1995).

A variety of possible mechanisms elucidating metformin's inhibition of glucogenesis, which includes phosphorylation of the insulin receptor and insulin receptor substrate-2 have been proposed; inhibition of key enzymes like phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase; and activation of pyruvate kinase (Gunton *et al.*, 2003). Moreover, depolarization of the hepatocyte membrane and inhibition of mitochondrial respiration by metformin have been speculated as other possible mechanisms (Lutz *et al.*, 2001; Detaille *et al.*, 2002). Recent studies performed in metformin-treated rats showed adenosine monophosphate activated protein kinase (AMPK) activation, accompanied by an inhibition of lipogenesis, and a modest stimulation of skeletal muscle glucose uptake (Gunton *et al.*, 2003). However, metformin does not lead to AMPK activation *in vitro*, indicating that it activates the kinase indirectly (Witters, 2001).

B. Pharmacokinetics and other effects

In addition to its actions on glucose metabolism, several other metabolic effects of metformin have been known, with beneficial effect on patients with typical Type II diabetic. Administration of metformin has resulted in either modest weight reduction or stability of weight, while patients taking sulfonylurea, thiazolidinedione (TZD), or insulin therapy have observed an increase in weight. A number of beneficial effects relating to the cardiovascular profile of the patients have been reported, which include lowering of total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides (DeFronzo and Goodman, 1995). An additional, possibly beneficial, effect of metformin supplementation is the improvement of endothelium-dependent vasodilation, reduction of fibrinogen levels, and increase activity of the fibrinolytic system. Reduced levels of C-reactive protein have also been associated with metformin therapy (Goodarzi and Bryer-Ash, 2005).

C. Adverse effects

The most frequently encountered adverse effects of metformin therapy are gastrointestinal in nature. Abdominal discomfort, anorexia, or diarrhea initially affect about one-fifth of patients. Fortunately, these effects are minimized when metformin is administered with meals and with gradual dosage titration, and generally necessitate discontinuation of the drug in less than 5% of patients (Goodarzi and Bryer-Ash, 2005; Krentz *et al.*, 1994). Other adverse effects are few, including a metallic taste, reduction in serum B12 levels, and cholestatic jaundice (Goodarzi

and Bryer-Ash, 2005). Moreover, hypoglycemia has been reported when metformin is used in combination therapy. Combination therapy of the α -glucosidase inhibitor acarbose and metformin causes a significant reduction in bioavailability and peak plasma levels of metformin, though the HbA_{1c} levels are improved (Scheen *et al.*, 1994).

Lactic acidosis is another adverse effect associated with biguanide treatment, which leads to respiratory distress and nonspecific abdominal pain. Action of biguanides on nonoxidative glucose metabolism results in accelerated conversion of pyruvate to both lactate and acetyl CoA, leading to the above effect. However, the incidence of lactic acidosis with metformin is very low and has been estimated at 1 case per 30,000 patient per year (Goodarzi and Bryer-Ash, 2005).

D. Metformin in combination therapy

In recent years, there has been a lot of focus on developing new antidiabetic drugs in which metformin has been combined with other antidiabetic agents to give an overall improved effect in diabetic patients. Much data have been published regarding the combination therapy of metformin and there are drugs available in the market based on this therapy.

Metformin with sulfonylureas: Combination of metformin with sulfonylureas has been frequently used and does not generally result in weight gain (DeFronzo and Goodman, 1995). Conversely, sulfonylureas may be added when glycemic control is suboptimal with metformin alone. Most patients remain on maximal dosage of sulfonylurea when metformin is added or vice versa. Dosage titration should be gradual, because the tendency of sulfonylureas to cause hypoglycemia may resurface when metformin is added.

Metformin with Repaglinide: Combined metformin and repaglinide therapy exhibits enhanced glycemic control over monotherapy with metformin or repaglinide in subjects with poorly controlled Type II diabetes mellitus. Repaglinide plus metformin therapy showed greater reductions in HbA_{1c} and fasting glucose levels than observed in nateglinide plus metformin.

Metformin with Pioglitazone: Studies have been conducted where the combined effect of pioglitazone with metformin has been observed. Patients attained greater reduction in fasting plasma glucose and HbA_{1c} levels as well as greater improvements in triglycerides and HDL cholesterol (Einhorn *et al.*, 2000). The dosage given was 30 mg daily, which indicates a very useful combination therapy.

Metformin with Rosiglitazone: Combination of rosiglitazone and metformin has consistently shown improved glycemic control over metformin monotherapy along with better insulin sensitivity and β -cell function (Jones *et al.*, 2003). The adverse effects associated with combination therapy are low and comparable to the metformin monotherapy, thereby increasing its therapeutic importance (Table 2.9).

TABLE 2.9 Currently available formulations containing metformin

Formulation	Brand names	Available doses (mg)
Metformin	GLUCOPHAGE®	500, 850, 1000
Metformin XR	GLUCOPHAGE® XR	500, 750
Glyburide/metformin	GLUCOVANCE®	1.25/500, 2.5/500, 5/500
Glipizide/metformin	METAGLIP®	2.5/500, 5/500
Rosiglitazone/ metformin	AVADAMENT®	1/500, 2/500, 4/500, 2/1000, 4/1000

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