Chapter 6

Review on digitoxigenin-glucoside and related cardiac glycosides

6.1 Introduction

The virtues of foxglove (*Digitalis purpurea),* against a disease called dropsy, were already documented by Withering in 1785. Withering investigated this plant, and only after a decade of investigations published the results of his findings (Withering, 1785) showing that foxglove could be used to treat dropsy. Dropsy was known as an ailment characterised by swelling of the limbs and torso, which we know today is due to inadequate pumping action of the heart (Balick & Cox, 1996). The retention of fluid that swelled the dropsy patient's body was clearly alleviated by administration of foxglove, but the connection between dropsy and inadequate pumping action of the heart was not properly understood in Withering's day. Withering observed that foxglove "has the power over the motion of the heart, to a degree yet unobserved in any other medicine". Withering foresaw that "this power may be converted to salutary ends". He began prescribing foxglove for cases of dropsy, but gave it in doses much too large. He also discovered that standardising of the dosage was important. He found that the dose vary considerably in the plant during different seasons of the year, and found that the dose could be controlled by gathering and drying the leaves at late flowering. He soon began prescribing leaf infusions and later ground powdered leaves. His administration was astonishingly successful in the treatment of dropsy (Balick & Cox, 1996).

Powdered foxglove leaf is still prescribed in tablet or capsule form to treat congestive heart failure. *Digitalis* has been affixed to this crude drug as well as to the cardiac glycosides isolated from foxglove in the early twentieth century. These compounds are named after their powerful action on the heart. They increase the force of heart

contractions and allow the heart more time to rest between contractions. More than 30 cardiac glycosides have been isolated from dried foxglove leaves, including digitoxin and digoxin (Balick & Cox, 1996).

The compound isolated during this study from *E. croceum,* digitoxigenin-glucoside, was first isolated from *Digitalis lanata* (Humber *et al.,* 1983) and falls in the class of the cardiac glycosides with a structure that is very similar to digoxin and digitoxin. The compound shows however less toxicity than what is generally found with the cardiac glycosides. The medicinal uses of this compound are restricted to its cardiotonic activity (Humber *et al*., 1983). The *in vitro* inhibitory activity of this compound against HIV as described in this study could be a new application for the cardiac glycosides. This chapter highlights the important facts of the isolated compound, but also give some interesting information on the toxicity and medicinal uses of the group of cardiac glycosides.

The structural similar compounds that form the cardiac glycosides owe their name to their biological activity which is mainly the increase in the contractibility force of the heart by inhibiting the enzyme Na^+, K^+-ATPase. The enzyme is the only receptor for the cardiac glycosides and is responsible for the active extrusion of intercellular Na^+ in exchange for extracellular K^+. Digoxin and digitoxin are the two most widely used digitalis inotropes with an estimated two million patients receiving these cardiac glycosides in the USA. The acute toxicity of cardiac glycosides is due to their arrhythmogenic action causing cardiac arrest (Steyn & Van Heerden, 1998).

These cardiac glycosides have been mainly isolated from *Digitalis* spp. which is commonly named the foxgloves or purple foxgloves from the family Scrophulariaceae. The compounds from this species exhibit a therapeutic dose which is close to the toxic dose which causes anorexia, nausea, salivation, vomiting, diarrhoea, headache, drowsiness, disorientation, delirium, hallucinations and death may result. Due to their exceedingly narrow therapeutic index, digoxin and cardiac glycosides in general, are among the most hazardous drugs in routine use (Budavari *et al.,* 1989).

6.2 Digitoxigenin – 3-O-glucoside

The isolated compound (Figure 6.1) is a derivative of digitoxigenin and was isolated previously from *Digitalis lanata* (Humber *et al.,* 1983). The compound has a similar structure to the well-known compounds such as digoxin and digitoxin. Several derivatives and variants of these compounds are found, with varying biological uses and toxicity.

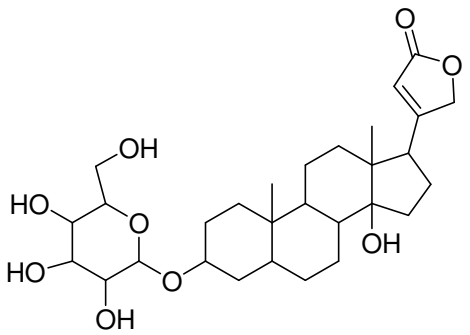

Figure 6.1 Digitoxigenin–3-O-glucoside.

Digitoxigenin-glucoside can be prepared from digitoxigenin with the Königs-Knorr condensation reaction, by partial hydrolysis of digitoxigenin. Digitoxigenin can be bought from Aldrich: D10320-9, Fluka: 37020 & Sigma: D5753. It has also been synthesised by cell cultures from *Digitalis purpurea* by feeding the cultures digitoxigenin (Kawaguchi *et al.,* 1989).

Another characteristic of the cardiac glycosides is that their cardiac activity and toxicity increase with an increase in the number of monosaccharides. The conformational distribution of the glycosidic moiety was postulated to be the major determinant of the biological activity of these cardenolides. The steroid aglycone provides the major part of the binding energy to the receptor, whereas the glycoside portion plays a secondary role in stabilising the cardenolide receptor complex (Steyn & Van Heerden, 1998).

Digitoxigenin-glucoside only contains one glucose molecule in contrast to three monosaccharides in digitoxin and digoxin. It is therefore expected to be less potent but lower in toxicity. It has also been found that the toxicity and lipophilicity are related in activity. When the hydrophilicity increases with the increasing number of monosaccharides, the toxicity will increase as well (Biagi *et al.,* 1991).

Digitalis and its preparations are known to have positive inotropic effects on the heart muscles. Digitoxigenin-glucoside were effective at $2x10^{-7}$M drug concentration (Takiura *et al.,*1974) and it was found that digoxin (0.112 µmol/kg) and digitoxigenin-glucosides (0.056 µmol/kg) produced similar increases in myocardial contractibility. Digitoxigenin-glucoside was also faster in onset of action, but had a shorter duration

of action. The shorter onset and duration of action may be due to more rapid drug-receptor association followed by rapid equilibrium of the drug with other tissues and fluid compartments. The results suggested that the rapid onset and short duration of the effect are a function of the glucose moiety. It was reported that the rapid onset and what appears to be a reduced tendency to accumulate may confer clinical potential for these analogues such as digitoxigenin-glucoside (Altman *et al.,* 1988).

Low acute toxicity (LD_{50}) in mice and high inotropic potency in guinea-pigs of digitoxigenin-glucoside prompted more studies on its cardiovascular effects. After a transient fall in contractibility during the infusion of digitoxigenin-glucoside, the contractibility increased relatively rapid to peak at 45 minutes, and declined to pre-treatment levels at 180 minutes. The hypertensive response was significantly greater than digoxin and was followed by a hypotensive response which reached a peak at 60 minutes. The heart rate was slightly reduced at time of infusion (Altman *et al*., 1988).

Radioactivity is used to determine the metabolism of the compounds after infusion. For digitoxigenin-glucoside the total plasma radioactivity was complete at 30 minutes and 60 minutes for digoxin. Digitoxigenin-glucoside appear to undergo fairly rapid metabolism when compared to digoxin (Altman *et al,*. 1988).

6.3 Related cardiac glycosides

6.3.1 3,14-Dihydroxycard-20(22)-enolide (digitoxigenin)

3,14-dihydroxycard-20(22)-enolide is the basic structure of a variety of compounds related to the cardiac glycosides. All the variants resemble the same structure as shown in Figure 6.2. Several variants of this compound are found with digitoxigenin one of these variants.

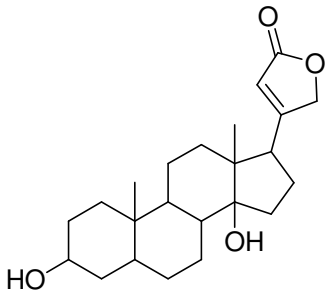

Figure 6.2 3,14-dihydroxycard-20(22)-enolide.

Digitoxigenin has been isolated from several plant families including Scrophulariaceae, Asclepiadaceae, Apocynaceae and Periplocaceae. The compound has been used as a cardiotonic agent with a LD_{50} (mus, oral) of 26.2 mg/kg. Other variants of this compound include urizeginen, 3-epiuzarigenin, 3-epidigitoxigenin, allouzarigenin and uzarigenin that is also a cardiotonic agent (Dictionary of Natural Products, 2005).

The LD_{50} of the compound has been determined for digitoxigenin at 36 nmole/10 g weight. The LD_{50} values are at least several times as high as those of the digitose series (Takiura *et al.,*1974).

Guinea pig atrial studies showed that the configuration of the A/B ring junction did not influence the cardiotonic activity of digitoxigenin significantly. The substitute groups showed a significant influence on the cardiotonic activity. Glucosidation decreased the potency of uzarigenin a variant of digitoxigenin by 63%. Conjunction with rhamnose increased the potency of both (Brown & Thomas 1984).

It was found that the sugar residue which is found in digitoxigenin-glucoside has a profound influence on inotropic potency on isolated left atrium of the guinea pig. Digitoxigenin-glucoside was 2.8 times more potent than digitoxigenin. Digoxin with three sugar residues was 2.1 times as effective as digitoxigenin. Digitoxin was however 8.8 times as effective as digitoxigenin (Brown *et al.*, 1981).

The derivatives of digitoxigenin are given in Table 6.1 with the toxicity and medicinal uses indicated for those that have been studied. The isolated digitoxigenin-glucoside is one of these derivatives and is also listed in the table.

Table 6.1 Derivatives of digitoxigenin, their uses and toxicity. Gpg = guinea pig, orl = orally and ivn = intravenous (Dictionary of Natural Products, 2005).

Name	Toxicity	Uses
Beaumontoside	It is very toxic intravenous (no values provided)	Cardiotonic agent
Cerberin	LD_{50} (cat, ivn) of 0.147 mg/kg	Cardiotonic agent
Digitoxigenin digitaloside	LD_{50} (cat, ivn) of 0.2 mg/kg	-
Digitoxigenin-glucoside	LD_{50} > 310 nm/10g body weight	Cardiotonic agent
Echujin	Highly toxic: LD_{50} (cat) of 0.3 mg/kg	It has been used as arrow poison
Evomoside	LD_{50} (cat, ivn) of 0.278mg/kg	-
Glucodigifucoside	It is very toxic intravenous	-
Lanatoside	Toxicity of LD_{50}(gpg, orl) 100 mg/kg and LD_{50} (rat, ivn) of 16 mg/kg	Cadriotonic agent
Neriifolin	LD_{50} (cat, ivn) of 0.2 mg/kg	-
Purpureaglycoside	Toxicity of LD_{50} (cat, ivn) 0.33 mg/kg	Cardiotonic agent
Ramnodigin	-	Cardiotonic agent
Solanoside	It is very toxic intravenous	-
Somalin	-	Antineoplastic agent
Vallaroside	Very toxic by intravenous route	Cardiotonic agent
Wallichoside	-	Cardiotonic agent

6.3.2 Digoxin

The compound digoxin (Figure 6.3) was extracted from *Digitalis lanata*, and the biological uses include inotropic activity and it is also a cardiotonic agent. This compound is well known and currently used as a drug to increase adequate pumping of the heart. Some of the commercial names used for this drug include Cordioxil, Davoxin, Digacin, Dilanacin, Dixina, Dokim, Dynamos, Lanacordin, Lanicor, Lanoxin, LenoxiCaps, Lenoxin, Longdigox, Neo-Dioxanin, Rougoxin, Stillacor and Vanoxin (Budavari *et al.,* 1989).

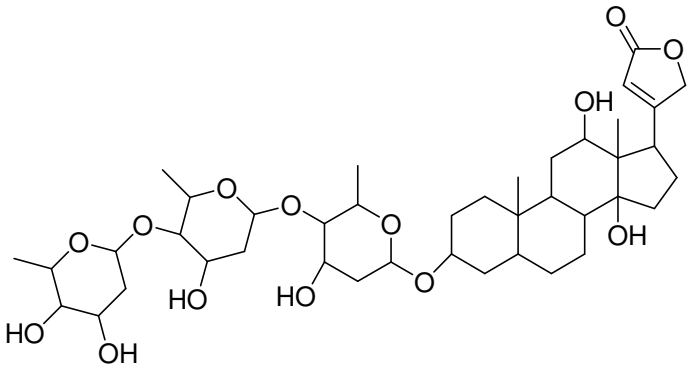

Figure 6.3 Digoxin.

6.3.3 Digitoxin

Digitoxin (Figure 6.4) is a structural derivative of digoxin and the compound was extracted from several plants including *Digitalis purpurea, D. lanata* and other *Digitalis* species (Humber *et al.,* 1983). The biological uses for the plant include inotropic activity and it is also a cardiotonic agent. The compound is currently used as a drug to improve the pumping action of the heart and some of the commercial names include Digitalin, Asthenthilo, Cardigin, Carditoxin, Coramedan, Digicor, Digilong, Digimed, Digipural, Ditaven, Digisidin, Lanatoxin, Myodigin, Purodigin, Tardigal and Unidigin (Budavari *et al.,* 1989).

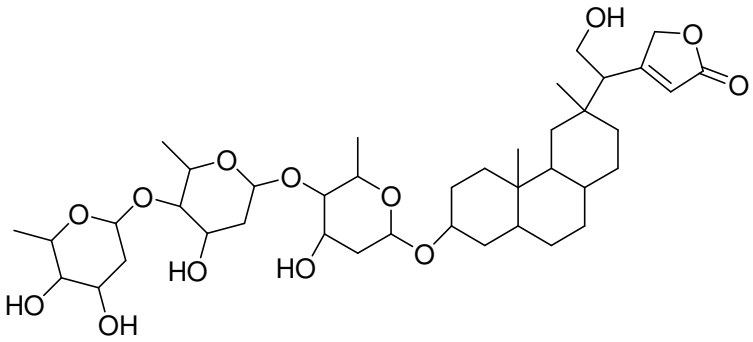

Figure 6.4 Digitoxin.

6.3.4 Actodigin

Actodigin (Figure 6.5) is a synthetic isomer of digitoxigenin-glucoside with a modified lactone ring (Brown *et al.*, 1981). It seems as if the biological properties of this molecule arise because the isomeric lactone alters the drug-receptor interaction in such a way that the sugar portion is directed away from the sugar-binding site on the receptor (Brown *et al.*, 1981).

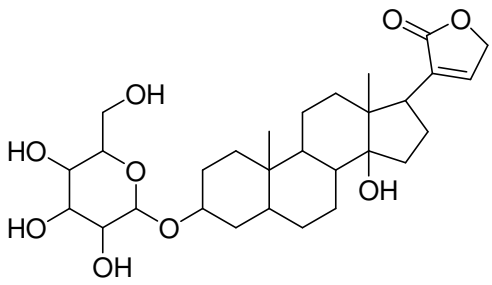

Figure 6.5 Actodigin.

Studies performed on actodigin's genin shows that the genin's ability to inhibit Na^+/K^+-ATPase can be largely explained by its lactone carbonyl oxygen position and molecular conformation (Cheung *et al.,* 1981). The D ring of actodigin was found to be a half chair; unlike the natural digitalis D rings like digitoxigenin-glucoside which exist in an envelope. The glucose make however an unexpectedly large contribution to the activity. Table 6.2 shows the I_{50} values of the Na^+/K^+-ATPase activity of actogdigin, its genin, digitoxigenin and its glycoside.

Table 6.2 I_{50} of selected cardiac glycosides on Na^+/K^+-ATPase activity.

Steroid	**I_{50} without preincubation (M)**	**I_{50} with 10 minutes preincubation (M)**
Actodigin	1.0×10^{-6}	1.0×10^{-6}
Actodigin genin	7.0×10^{-5}	7.0×10^{-5}
Digitoxigenin	3.5×10^{-7}	3.5×10^{-7}
Digitoxigenin-glucoside	3.1×10^{-7}	1.3×10^{-7}

The data show the remarkable effect of glucose on actodigin's Na^+/K^+-ATPase inhibiting potency. Actodigin is 70 times more active than its genin. In contrast, digitoxigenin-glucoside is only slightly more active than its genin digitoxigenin. The glucose of actodigin is having a much greater effect on its Na^+/ K^+-ATPase inhibitory activity than the glucose of digitoxigenin-glucoside. This confirms that binding of a glycoside to the Na^+/K^+-ATPase proceeds in two steps: first binding of the genin to the enzyme, and second binding of the sugar to the enzyme (Cheung *et al.,* 1981).

6.3.5 Glycyrrhizic acid

The compound glycyrrhizic acid (Figure 6.6) is not part of the group of cardiac glycosides, but is included in the review because of its anti-HIV activity, and its general structure shows correlation with the cardiac glycosides. The compound has been tested as a long-term treatment and as a combination therapy with AZT or DDI on HIV-1 carriers (Ikegami *et al.,* 1996). The efficacy of this compound as an oral monotherapy (150-225 mg/day) for 5 to 10 years and as a combination therapy with AZT or DDI on HIV-1 carriers was evaluated on the viral RNA levels in plasma samples. Patients who started the monotherapy at an early stage had low or undetectable levels.

Other biological uses include its anti-inflammatory activity and its antihaemorrhagic activity. It has adverse effects of hypermineralocorticoidism that result from intake. The toxicity for the compound is LD_{50} (rat, orl) 3000 mg/kg and LD_{50} (rat, ipr) 2000 mg/kg.

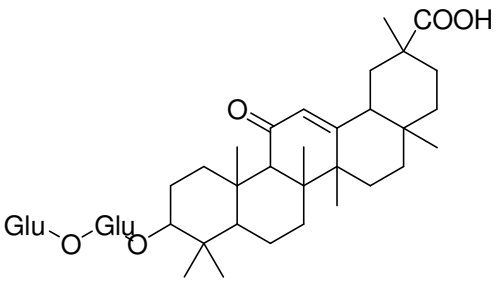

Figure 6.6 Glycyrrhizic acid (Glycyrrhizin).

6.4 Discussion

Cardiac glycosides are known for their cardiac activity with more than 2 million users in USA. Digitoxigenin-glucoside is part of that group of compounds and has also been tested for its cardiac activity. If the structure of digitoxigenin-glucoside is compared to the cardiac glycosides, it is strikingly similar in major parts of the compound. The major difference between these compounds is the number and type of sugar moieties attached to the terpenoid structure.

Its low toxicity could be ascribed to its one glucoside compared to other cardiac glycosides such as digoxin. It was also postulated that the potency depends on the number and type of sugar moieties. Digitoxigenin-glucoside has other effects such as hypertension, and it is also known as a fast onset compound that does not have a lasting effect on the heart. This in itself is not always negative, as certain applications need a fast reaction without long-term effects.

The compound was synthesised by several methods, and makes it an easy option to prepare the compound synthetically instead of isolating the compound from the plant

material. Digitoxigenin is easily obtainable from chemical suppliers, and digitoxigenin-glucoside can therefore be easily synthesised in the laboratory.

In 1974 Takiura *et al.,* stated that derivatives such as digitoxigenin-glucoside (LD_{50} > 310 nm/10 g body weight) are much safer compounds to be used than digitoxigenin (LD_{50} = 36 nm/10 g body weight) because of its lower toxicity and decrease in potent effects.