Chapter 2

Cardiac Glycosides in Medicinal Plants

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Additional information is available at the end of the chapter

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Abstract

Plant active metabolites are under intensive examinations around the world to supplement the drugs with minimal side effects. Thus, there is vast potential to explore the possible medicine from the plant sources. Cardiac glycosides are a unique group of secondary metabolites that they are considered one of the most useful drugs in therapeutics. In this review, cardiac glycosides and their analogues are presented. The structure and distribution in plants, as well as structure elucidations, synthetic routes, and chemical analysis, are shown. In addition, the pharmacological activities, mode of action studies, and structureactivity relationships are discussed.

Keywords: cardiac glycosides, distribution in nature, structure features, structure elucidation, chemical analysis, pharmacological activities, structure-activity relationships

1. Introduction

Many research efforts have been done toward the proofs of the use of plant species in medicinal treatments in recent years. The effect of plants used has been examined traditionally to support treatment of various diseases. Cardiac glycosides are a group that comprises the most drug-like molecules subjected to several investigations and they were proved to be fruitful in developing potential drugs [1–5]. They are chemical compounds responsible for the poisoning of livestock and the treatment of congestive heart failure. Extracts or latexes of cardiac glycosides plants have been applied to poison arrows in Africa, Asia, and South America for use in hunting and fighting. It is expected to be evolved as a defense way in plants. Cardiac glycosides are steroids having the ability to exert specific powerful action on the cardiac muscle. A very small amount can exert a beneficial simulation on diseased heart. These compounds are primarily valuable in the treatment of congestive heart failure. They increase the force of heart contraction without a

concomitant increase on oxygen consumption. Consequently, the myocardium becomes more efficient pump and is able to meet the demands of circulatory system [6–8].

2. Structure diversity of cardiac glycosides

Cardiac glycosides are a group comprising two main classes of compounds that differ in the structure of their aglycone as shown in **Figure 1**. Cardiac glycosides are either C23 or C24 steroids with a basic nucleus of cyclopentanoperhydro phenanthrene substituted at C17. Cardenolides have a five-membered lactone group in the C17 with α, β-unsaturated γ-lactone ring (butenolide), whereas the other group, the bufadienolides, was first discovered as skin poisons in toads. The C17 substituent with a doubly unsaturated six-membered lactone ring (α-pyrone). Plants can produce both cardenolides and bufadienolides. Another group, isocardenolides, has the double bond of butenolid ring at position 21 or 22 instead of position 20 as shown in **Figure 1**. Most clinical attention was directed to the cardenolides owing to their therapeutic use. Digoxin and digitoxin are the two most widely used digitalis inotropes. There are two million patients receiving these cardenolides in the US. In general, some isocardenolides appeared to be devoid from any cardiac activity [9].

Cardiac glycosides, cardenolides, and bufadienolides, bear a structure resemblance to the steroid saponins and have the same solubility and foaming characteristics. They are also distinguished from other steroid glycosides by a 14-hydroxy group and some peculiar sugar incorporated in their skeleton. Other substituent groups may be present, for example, additional hydroxyl groups at C-1, 11, 12, 16, and 19. The sugars are always linked at C-3. Some members have an aldehyde group rather than methyl group at C-19 [10].

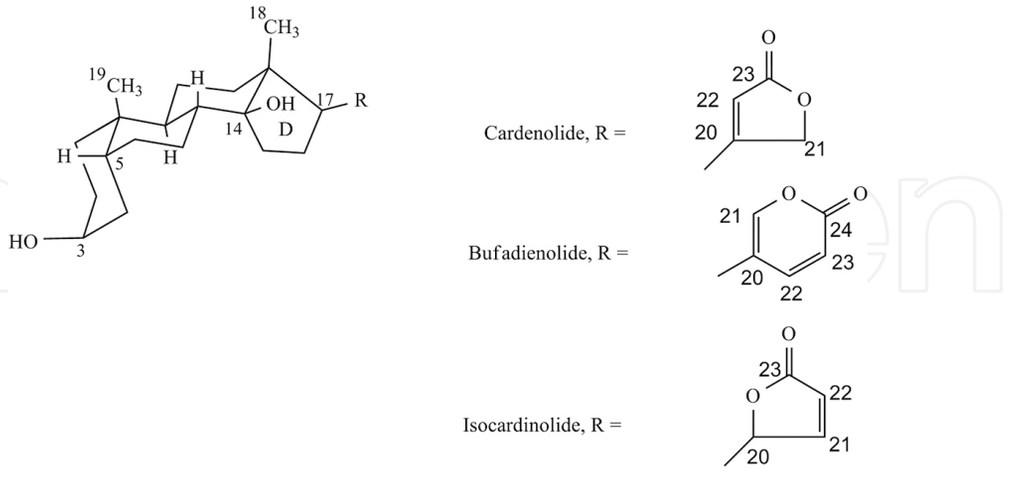

Figure 1. Structures of cardiac glycosides.

These compounds are also characterized by its unusual "U shape." This "U shape" has an A/B and C/D *cis* and B/C trans ring junctions. On the other hand, the adrenocortical steroids typically possess an A/B, B/C, and C/D all having trans conformation, while the bile salts

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characteristically have an A/B *cis* and B/C, trans orientation [10]. Although cardiac glycosides are more abundant than aglycones, some aglycones of cardiac glycosides are used for congestive heart failure and commercially available like digoxigenin, gitoxigenin, strophanthidin, and ouabagenin as shown in **Figure 2**. The most commercially important plant sources of cardiac glycosides are *digitalis purpurea, D. lanata, Strophanthus gratus*, and *Strophanthus kombé* [6]. **Figure 2** shows the structure of some common cardiac aglycones.

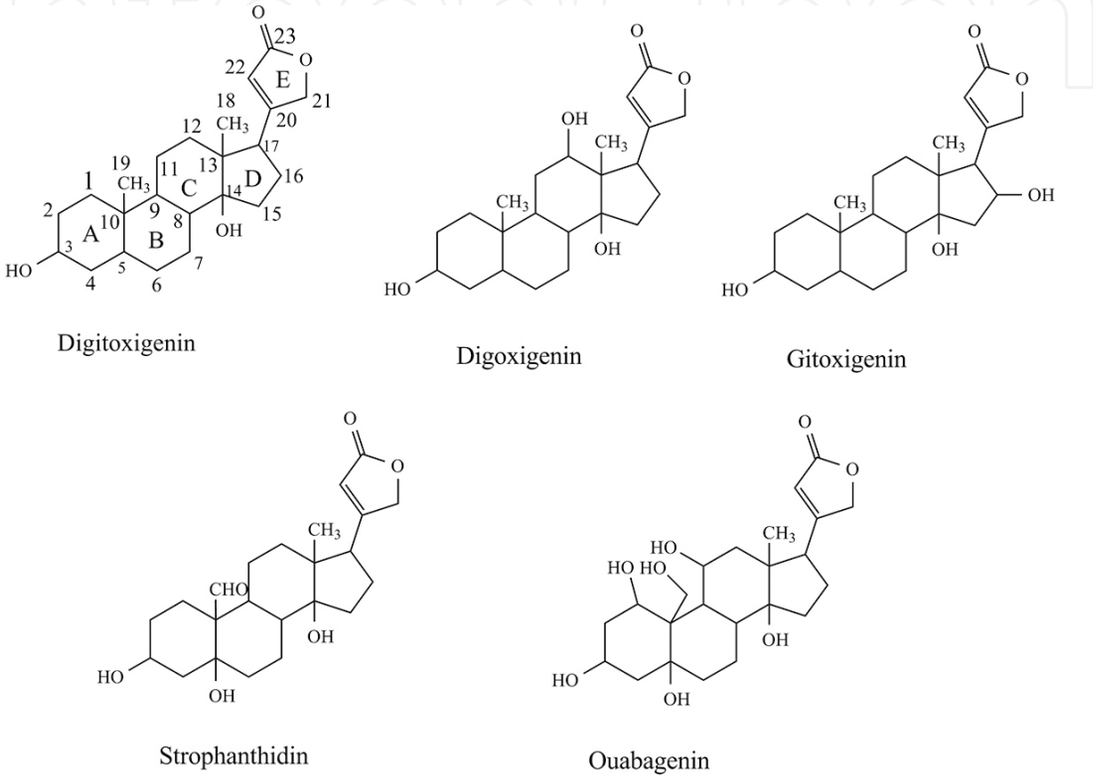

Figure 2. Structures of some common cardiac aglycones.

The sugar moieties are mostly attached to the aglycone at C-3 by β-linkage and are composed of up to four sugar units. It may include glucose or rhamnose together with other deoxy sugars whose natural occurrence is, so far, known only in association with cardiac glycosides [11–15]. **Figure 3** shows the structures of some examples of sugar residues attached to cardenolides,

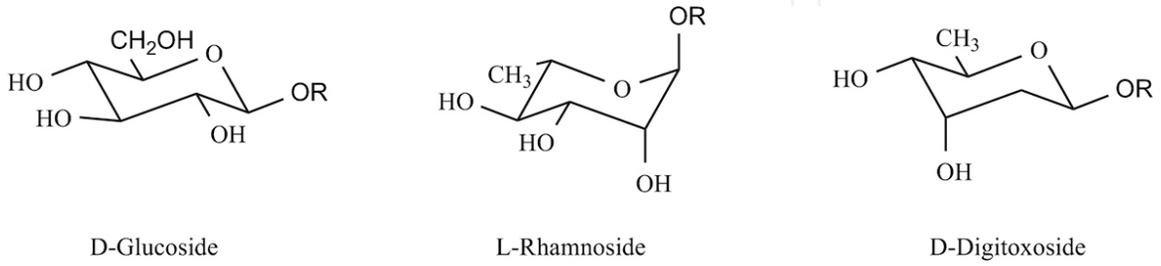

Figure 3. Examples of sugar residues attached to cardenolides.

which occur in the pyranoid form [11]. To differentiate between sugars with a hydroxyl group at C–2 and 2-deoxy-sugar chemically, hydrolysis is the first choice [16]. The latter are almost completely hydrolyzed by boiling in 0.05 N mineral acid in 50% aqueous methanol for 30 min, whereas the former sugars are not completely affected by this procedure.

3. Distribution in plant kingdom

Cardiac glycosides occur in small amounts in the seeds, leaves, stems, roots, and bark of plants of wide geographical distribution. Many species grow in tropical regions and have been employed, in the past, by natives of Africa, Asia, and South America for preparation of arrow poisons [17]. In plants, cardenolides appear to be confined to the angiosperms. They are more abundant in families Apocynaceae and Asclepiadaceae (now subsumed in Apocynaceae). However, it could be also found in some plants belonging to Liliaceae, Ranunculaceae, Moraceae, Leguminosae, Scrophulariaceae, Cruciferae, Sterculiaceae, Euphorbiaceae, Tiliaceae, and Celastraceae [18]. Some of the plants' genera containing natural cardenolides are illustrated in **Table 1**.

Family	Genera
Apocynaceae	*Adenium, Acokanthera, Strophanthus, Apocynum, Cerbera, Thevetia, Nerium, Carissa, Urechites*
Asclepiadaceae (subsumed in Apocynaceae)	*Gomphocarpus, Calotropis, Pachycarpus, Asclepias, Xysmalobium, Cryptostegia, Menabea, Periploca*
Moraceae	*Antiaris, Antiaropsis, Naucleopsis, Maquira, Castilla*
Leguminosae	*Coronilla*
Scrophulariaceae	*Digitalis, Isoplexis*
Cruciferae	*Erysimum, Cheiranthus*
Sterculiaceae	*Mansonia*
Tiliaceae	*Corchorus*
Celastraceae	*Euonymus, Lophopetalum*

Table 1. Some common plants containing natural cardenolides.

The bufadienolides occur in plants of families: Hyacinthaceae (Syn. Liliaceae), Crassulaceae, Iridaceae, Melianthaceae, Ranunculaceae, and Santalaceae. Two genera of Hyacinthaceae are known to produce them (Urginea and Bowiea). Several compounds of bufadienolides had been isolated from *Urginea maritima*, which is commonly known as Squill. It is worthy to mention that the genus *Urginea* is an aggregate of six species and it has been used in medicine since ancient times because of its powerful digitalis-like effect. There are various animal sources for bufadienolides, e.g., Buffo (toad), Photinus (fireflies), and Rhabdophis (snakes) [19].

4. Extraction and purification of cardiac glycosides

The isolation and identification of pure cardiac glycosides from their crude mixture faced some difficulties in the past due to its low quantity or its presence as a complex mixture. Reichstein's group [16] suggested the defatting of dried and powdered seeds, and/or leaves with petroleum ether followed by digestion with water at 0°C to extract polysaccharides and hydrolytic enzymes. One of the most common methods of extraction of cardiac glycosides is the prior protection of plant material by its maceration in toluene and allowing it to stand for many days at 25–37°C to avoid the enzymatic hydrolysis. Then, it is followed by exhaustive extraction with water-alcohol mixture. The aqueous extract could be evaporated to a small volume under vacuum at 50°C. Fats could then be removed by extraction with petroleum ether and the aqueous syrup of glycosides is diluted with an equal volume of water. Tannic acid and other polyphenolic and acidic products are precipitated with freshly prepared lead hydroxide and the mixture is filtered through Hyflo-Super Gel. The clear filtrate is adjusted to pH 6, concentrated under vacuum and subjected to fractional extraction: first with ether, then chloroform, and finally with chloroform-alcohol, 2:1 and 3:2. For isolation of glycosides of high solubility in water, the residual aqueous phase is half saturated with sodium sulfate and then extracted with chloroform-alcohol [20, 21]. The less polar fractions are separated by chromatography on neutral alumina [22]. The more polar fractions are usually chromatographed after acetylation or benzoylation and the free glycosides recovered by hydrolysis with bicarbonate.

Reversed phase column chromatography are widely accepted in many fields including HPLC of cardiac glycosides with RP-8 or RP-18 column and acetonitrile/water or methanol/water as an eluent, followed by UV detector at 220 nm [23]. The employment of HPLC techniques also led to the isolation of large number of cardiac glycosides [24–27]. The technique of DCCC has seen rapid expansion over the past few years. It was used to isolate three new glycosides from digitalis lanata using the solvent systems $CHCl_3$-MeOH-H_2O (5:6:4) and CH_2Cl_2-MeOH-H_2O (5:6:4) [28]. Four strophanthidin glycosides, out of a total of eight isolated compounds, were separated from one another by DCCC. The solvent systems $CHCl_3$-MeOH-PrnOH-H_2O (5:6:1:4) and $CHCl_3$-MeOH-PrnOH-H_2O (45:70:5:40) were used [29]. Further application of DCCC has been reported for the isolation of affinosides from *Anodendron affine* [30, 31]. Recently, Kopp et al. [32] used the technique of DCCC in successful application to isolate 41 bufadienolides after fractionation by column chromatography. Moreover, radial centrifugal chromatography gives a good resolution and ease of operation to isolate cardiac glycosides [33].

5. Chemical analysis

The analytical methods for cardiac glycosides can be divided into two groups, which are classical and sensitive methods. The classical methods (μg range) including photometry and chromatography have an importance in the pharmacopoeias and are widely employed in control laboratories for quantitative determination of the content and purity of glycoside

preparations. Sensitive methods (ng range) include pharmacokinetic investigations, which require sophisticated apparatus. They comprise gas chromatography coupled to a mass spectrometer (GC-MS) and HPLC coupled to a sensitive detector (MS or fluorescence detector). Such method affords reliable measurements in the ng range [34], whereas the classical methods require preliminary purification, usually by chromatography [35].

In classical methods, direct measurement by UV led to the absorption maxima for cardenolides at 217 nm (εmol = 16,595) and for bufadienolides at 300 nm (εmol = 5250, εmol is the molar extinction coefficient). For qualitative and quantitative determination of the cardiac glycosides, it must therefore be converted into colored derivatives as shown in **Figure 4**. It can be converted into colored derivatives by reaction with polynitroaromatic derivatives in alkaline solution, with Keller-Kiliani or xanthydrolin acidic medium [34] or by treatment with strong acids and these can be measured by conventional photometers or fluorimeter [36, 37].

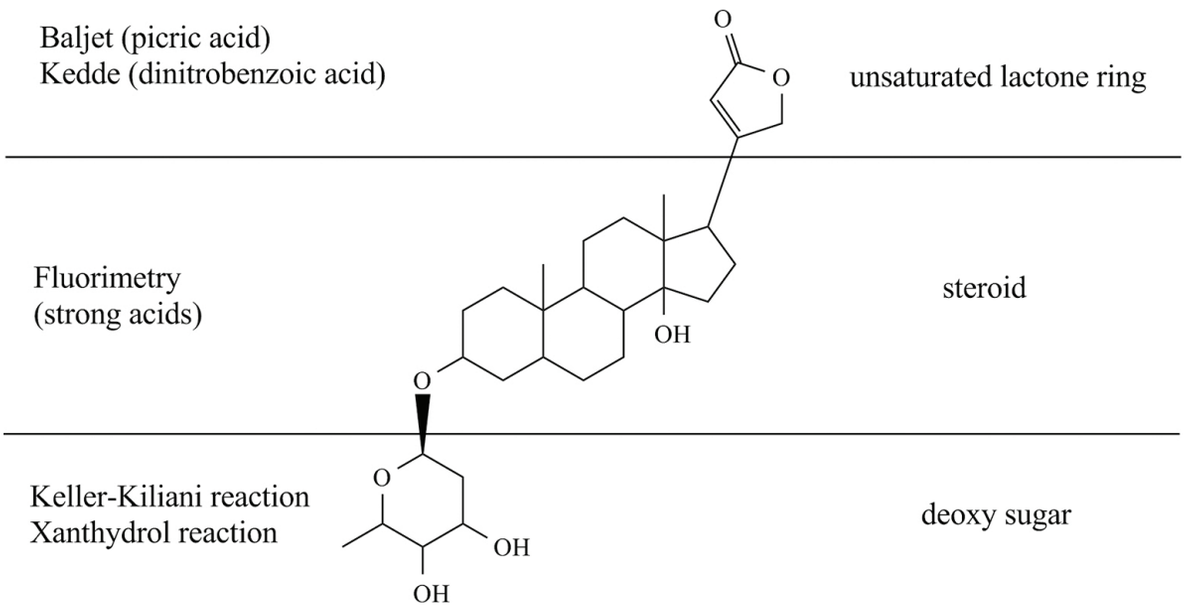

Figure 4. The chemical methods used for photometric and fluorimetric determination of cardenolides.

The reaction between cardenolides and polynitroaromatic derivatives in alkaline solution [38–41] are based on the C-C coupling of the unsaturated lactone ring with them to produce dye complexes which can be measured photometrically. The reagent may also be used as a spray reagent to visualize cardiac glycosides on TLC. The reagents that gained an established place are Baljet reagent (picric acid) [38], Kedde reagent (3,5-dinitrobenzoic acid) [40], and Rabitzsch reagent (tetranitrobiphenyl) [41]. However, the specificity of Baljet reagent is low because many other substances, e.g., ketones give intense color reaction with picric acid and alkali [34].

Various reaction mechanisms are suggested for the reaction of polynitroaromatic with cardiac glycosides [42]. According to the studies by Burns et al. [43] and Kovar et al. [42], splitting off of one proton at C21 produces a carbamine that consequently undergoes nucleophilic linkage to the polynitroaromatic molecule. The resulting complexes are cyclohexadienate type and known as Meisenheimer compounds [34], as shown in **Figure 5**.

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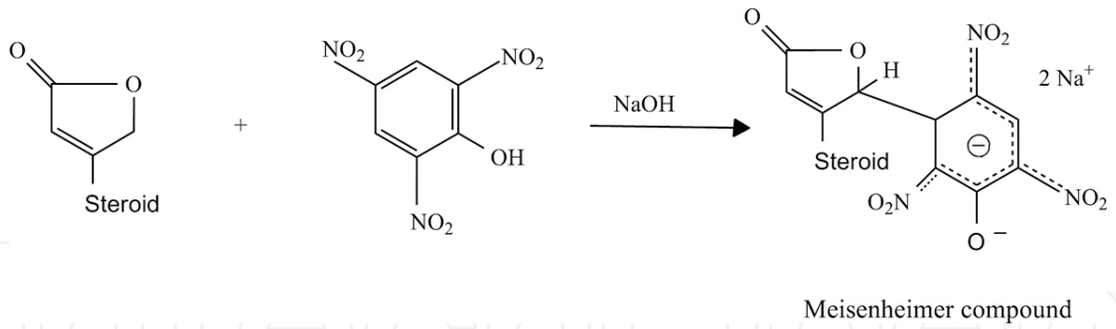

Figure 5. Meisenheimer complex formed of cardenolides with polynitroaromatic reagents.

Both the Keller-Kiliani and xanthydrol convert 2-deoxy-sugars into characteristic colored derivatives. In this way all digitoxose-containing glycosides can be qualitatively and quantitatively determined. All the acid reagents detect only those digitoxoses, which are easily hydrolyzed under the conditions of the test [34]. Keller-Kiliani reaction in acetic acid, ferric chloride, and sulfuric acid produces a blue coloration with absorption maxima at 470 and 590 nm. It is important to note that the color formation is dependent on time and it is affected by moisture content [37]. Xanthydrol reaction [44] in acetic acid/hydrochloric acid mixture produces red coloration with absorption maximum at 520 nm. However, the reagent is not very stable and decomposed products tend to interfere with the color reaction. Therefore, Pötter suggested the use or the more stable dixanthyl urea instead of xanthydrol [45].

Fluorescence spectroscopy is 10–100 times more sensitive than absorption photometry [46], so the reaction between cardiac glycosides and strong acids gives a restricted limit of detection in the ng range. For digoxin determinations, an activating wavelength of 340 nm is used and the emitted fluorescence is measured at 420 nm [47].

6. Structure elucidation

The earliest methods to determine the structure of cardiac glycosides depended on acid and/or enzymatic hydrolysis of the glycoside to the aglycone and sugar moieties followed by the identification of their nature. The method consumed a bigger quantity of the isolated glycoside, and consequently, it was only suitable for structure determination of the major constituents. The great development in the spectroscopic instruments and the analysis of the produced data in the last three decades was accompanied by a great jump in the study of structure and stereochemical behavior of the naturally occurring compounds. This development led to stabilize a clear relationship between the structure and the data obtained from the spectroscopic experiments.

Before developing the recent tools for chemical analysis of organic compounds, it was very difficult to elucidate the cardiac glycosides structures. In the past, it is important to perform acid hydrolysis [48–51] or enzymatic hydrolysis [52, 53] to obtain the sugar residues and the aglycone separately. Now, more sophisticated and accurate tools were used for identification

the structure of cardiac glycosides with the stereochemistry determination, which give a powerful way to understand the mechanism of action and facilitate the structure activity relationship studies. Examples of these tools are mass spectroscopy, and FTIR and NMR.

6.1. Nuclear magnetic resonance (NMR)

No doubt that NMR is the most powerful tool for the structure determination of cardiotonic compounds. The advantage of pulsed Fourier transformation and two-dimensional NMR spectroscopy is that they provide information related to the carbon skeleton of the molecule and the structure environment of each hydrogen and carbon. Tori et al. [54] reported the first ^{13}C-NMR analysis of 10 cardenolides by employing single-frequency off-resonance, noise off-resonance decoupling, and the comparison with spectra of structurally related compounds. Later on, he used ^{13}C-NMR spectroscopy to determine the structure of thevetin A and B [55]. Robien et al. [56] reviewed ^{13}C NMR data of 36 bufadienolides. Later on, Kopp et al. [32] used ^{13}C NMR for elucidation of the structure of bufadienolides compounds isolated from *Urginea maritima*.

Cheung and Watson [57] briefly studied the ^{1}H and ^{13}C NMR of the compounds calactin, uscharidin, calotoxin, uscharin, and voruscharin and established their stereochemistry. The ^{13}C-chemical shift of C-19 also gives valuable information on the stereochemistry of both cardenolides and bufadienolides at C-5. In 5β-series, C-19 have its signal at 21.7 ± 2.5 ppm, whereas in 5α-series at 12.2 ± 0.4 ppm. Moreover, 5α-series show the deshielding of C-7 and C-9 by ~5.5 and 13 ppm, respectively. The number of sugar moieties could be determined from the number of anomeric carbons at the region of 95–103 ppm in its ^{13}C-NMR spectrum. Moreover, α- and β-sugars could be distinguished from each other by measuring the coupling constant of the anomeric hydrogen at the region of 4.4–5.3 ppm in its ^{1}H-NMR spectrum. The anomeric hydrogen of α-sugar is coupled with the adjacent hydrogen at 2–3 Hz, while of β-sugar is coupled at 7–8 Hz [58].

Elgamal et al. isolated and studied the structure of several cardiac glycosides [58–60]. Some of these compounds are shown in **Figure 6**. The structure elucidation of some example compounds, **A, B**, and **C**, was presented based on NMR spectral assignments [60], which were confirmed by DEPT, gs-COSY, TOCSY, *gs*-HMQC, ^{13}*C*-coupled *gs*-HMQC, ROESY, *gs*-HMBC, and 2D INAPT experiments. The ^{1}H and ^{13}C assignments are shown in **Table 2**. The stereochemistry of the steroid ring system and all substituents could be determined beyond doubt from the ^{1}H; ^{1}H coupling constants, as far as identifiable, and the ROESY cross-peaks. The ROE measurements are very informative to perform signal assignment and to determine the ring junction forms. In compound **B**, some ROESY cross-peaks gave evidence of the trans form of ring junctions A/B and B/C which revealed by H-2b/H-19, H-19/H-8b, and H-8b/H-18 cross-peaks, as shown in **Figure 7**. In addition, the ring junction C/D should be *cis*, as follows from the cross-peaks H-9/H-15a and H-12a/H-15a. The orientation of aglycone and glucose moiety in compound **C** was obtained from spatial proximities obtained from ROESY as shown in **Figure 8**.

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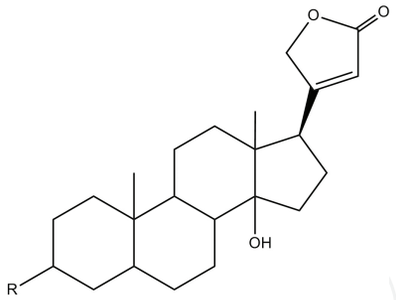

	R
A	=O
B	OH
C	O Glc

Figure 6. Structure of some cardenolides isolated from *Calotropis procera*.

		A		**B**		**C**	
		^{1}H	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**
1	α	1.29	38.3	1.10	38.3	1.10	38.3
	β	1.96		1.84		1.85	
2	α	2.23	37.8	1.85	32.1	1.97	30.4
	β	2.32		1.47		1.62	
3	α	–	211.4	3.60	71.8	3.80	79.2
4	α	2.04	44.3	1.64	38.8	1.82	35.3
	β	2.21		1.36		1.41	
5	α	1.45	46.1	1.21	45.8	1.20	45.6
6	α	1.37	28.6	1.44	30.0	1.47	30.0
	β	1.23		1.35		1.38	
7	α	1.03	27.0	1.20	28.7	1.20	28.8
	β	1.96		2.13		2.13	
8	β	1.52	41.2	1.68	42.6	1.68	42.6
9	α	0.95	49.1	1.07	51.1	1.07	51.1
10		–	35.7	–	36.9	–	37.0
11	α	1.48	21.2	1.63	22.3	1.62	22.3
	β	1.30		1.38		1.38	
12	α	1.32	39.4	1.56	40.9	1.56	40.9
	β	1.45		1.56		1.56	
13		–	49.5	–	51.0	–	51.0

		A		B		C	
		^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
14		–	84.7	–	86.3	–	86.3
15	α	1.98	32.7	2.20	33.4	2.20	33.4
	β	1.63		1.79		1.80	
16	α	2.07	26.7	2.23	28.0	2.23	28.0
	β	1.79		1.95		1.95	
17	α	2.72	50.7	2.91	52.1	2.90	52.1
18		0.84	15.7	0.97	16.4	0.97	16.4
19	a	0.94	11.2	0.91	12.6	0.92	12.6
	b	–		–		–	
20		–	174.8	–	178.4	–	178.4
21	a	4.76	73.3	5.01	75.3	5.00	75.4
	b	4.95		5.11		5.12	
22		5.81	117.4	5.98	117.8	5.98	117.8
23		–	174.4	–	177.2	–	177.3
1′						4.47 (7.8)	102.3
2′						3.22 (8.8)	75.2
3′						3.43 (8.8)	78.1
4′						3.35 (9.6)	71.7
5′						3.35 (5.1;1.5)	77.9
6′	a					3.73 (11.9;5.1)	62.8
	b					3.93 (11.9,1.5)	

Table 2. ^{1}H and ^{13}C NMR chemical shifts and characteristic *J* (H,H) couplings of three cardenolides **A–C**.

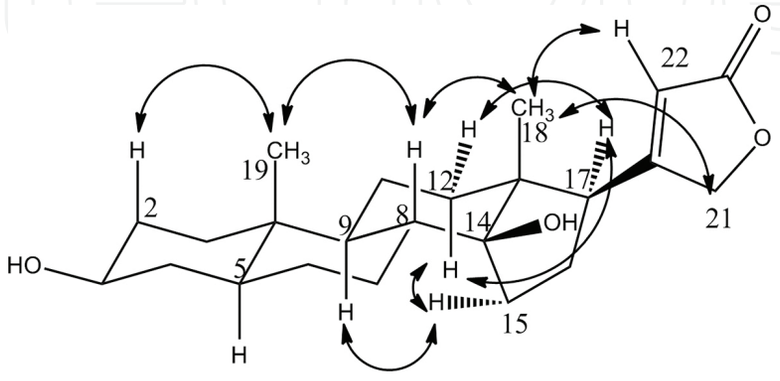

Figure 7. Stereostructure of **B**. The arrows indicate steric proximities (from ROESY experiment).

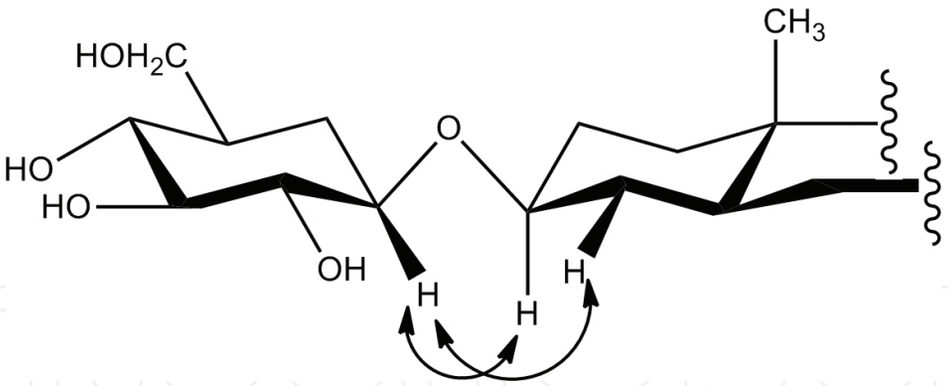

Figure 8. Relative orientation of the aglycone and the sugar moiety in **C**. The arrows refer to spatial proximities obtained from ROESY.

7. Pharmacological action of cardiac glycosides

The most important use of the cardiac glycosides is its effects in treatment of cardiac failure. In cardiac failure, or congestive heart failure, heart cannot pump sufficient blood to maintain body needs. During each heart contraction, there is an influx of Na^+ and an outflow of K^+. Before the next contraction, Na^+, K^+-ATPase must reestablish the concentration gradient pumping Na^+ into the cell against a concentration gradient. This process requires energy, which is obtained from hydrolysis of ATP to ADP by Na^+, K^+-ATPase. Cardiac glycosides inhibit Na^+, K^+-ATPase, and consequently increase the force of myocardial contraction [8]. On the other hand, some cardiac glycosides were investigated for their antitumor activity [61]. In addition, it has been reported that some cardiac glycosides display an inhibitory activity against rhinovirus [62].

8. Structure-activity relationship

In cardenolides, the steroidal part is considered the pharmacophoric moiety, responsible for the activity of these compounds [63]. Specifically, the 5β,14β-androstane-3β,14-diol skeleton has shown the same binding properties to the enzyme as digitalis compounds.

Furthermore, the bending in the structure as shown in *cis* junctions between A/B and C/D rings is very important to get the highest interaction energy. Any modification of A and/or B rings related to B-C plane, reduces the interaction energy [64]. In general, OH groups at any position of steroidal skeleton reduce the interaction energy, which depends on the position on the skeleton and the spatial location. This fact may be explained by the steric hindrance and the decreasing of steroidal positive potential field. Moreover, the OH group at position C14β is not an essential feature for inotropic activity, although when it is replaced by hydrogen atom, potency decreases considerably [65]. The change of the A/B junction does not mean a decrease of activity of aglycones but it decreases the activity of the corresponding glycosides. Thus, the

main effect of A/B junction is revealed from its ability to put the sugar into its suitable position [66]. The lactone ring at C17β has been considered to be responsible for inotropic activity, bringing about conformational changes on the enzyme that would give rise to its inhibition [67]. Indeed, that is the most differentiating feature from steroid hormones, and its contribution to the interaction [68]. Sugar attachment to the steroid part modifies both pharmacokinetics as well as pharmacodynamics of digitalis glycosides. Free aglycones are absorbed faster than glycosides and they are easily metabolized to less active 3α-OH epimer. Thus, the action of free aglycone is fast and short lasting. The sugar moiety significance for digitalis activity is well established but sugar parts themselves do not show any activity [10].

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