*IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)*
*ISSN: 2455-264X, Volume 2, Issue 6 Part: II (Sep. – Oct. 2016), PP 20-35*
*www.iosrjournals.org*

# Chemistry, spectroscopic characteristics and biological activity of natural occurring cardiac glycosides

Marzough Aziz DagerAlbalawi[1*]
[1] *Department of Chemistry, University college- Alwajh, University of Tabuk, Saudi Arabia*

***Abstract:****Cardiac glycosides are organic compounds containing two types namely Cardenolide and Bufadienolide. Cardiac glycosides are found in a diverse group of plants including Digitalis purpurea and Digitalis lanata (foxgloves), Nerium oleander (oleander),Thevetiaperuviana (yellow oleander), Convallariamajalis (lily of the valley), Urgineamaritima and Urgineaindica (squill), Strophanthusgratus (ouabain),Apocynumcannabinum (dogbane), and Cheiranthuscheiri (wallflower). In addition, the venom gland of cane toad (Bufomarinus) contains large quantities of a purported aphrodisiac substance that has resulted in cardiac glycoside poisoning.Therapeutic use of herbal cardiac glycosides continues to be a source of toxicity today. Recently, D.lanata was mistakenly substituted for plantain in herbal products marketed to cleanse the bowel; human toxicity resulted. Cardiac glycosides have been also found in Asian herbal products and have been a source of human toxicity.The most important use of Cardiac glycosides is its affects in treatment of cardiac failure and anticancer agent for several types of cancer. The therapeutic benefits of digitalis were first described by William Withering in 1785. Initially, digitalis was used to treat dropsy, which is an old term for edema. Subsequent investigations found that digitalis was most useful for edema that was caused by a weakened heart. Digitalis compounds have historically been used in the treatment of chronic heart failure owing to their cardiotonic effect. Although newer and more efficacious treatments for heart failure are available, Digitalis compounds have a small direct diuretic effect on the kidneys.In this study, A survey for Isolation, Identification and pharmacological action of cardiac glycosides*

***Keywords:****Heart failure, Cardenolides, Bufadienolide, Digitalis.*

## I. Introduction

Cardenolides and bufadienolides are described as cardiac glycosides owing to the similarity in their biological activity, viz. the increase in the contractile 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 the intercellular $Na^{+}$ in exchange for extracellular $K^{+}$[(1)].

**Distribution in Nature**

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 by natives of Africa and south America for preparation of arrow poisons for use in hunting and fighting[(2)]. In plants, cardenolides appear to be confined to the Angiosperms. It has more common abundance in families *Apocynaceae* and *Asclepiadaceae*. However, it could be also found in some plants belonging to *Liliceae*, *Ranunculaceae*, *Moraceae*, *Leguminosae*, *Scrophulariaceae*, *Cruciferae*, *Sterculiaceae*, *Euphorbiaceae*, *Tiliaceae* and *Celastraceae*[(3)]. Some of plants Genera containing natural cardenolides are illustrated in Table (1).

**Table (1)**

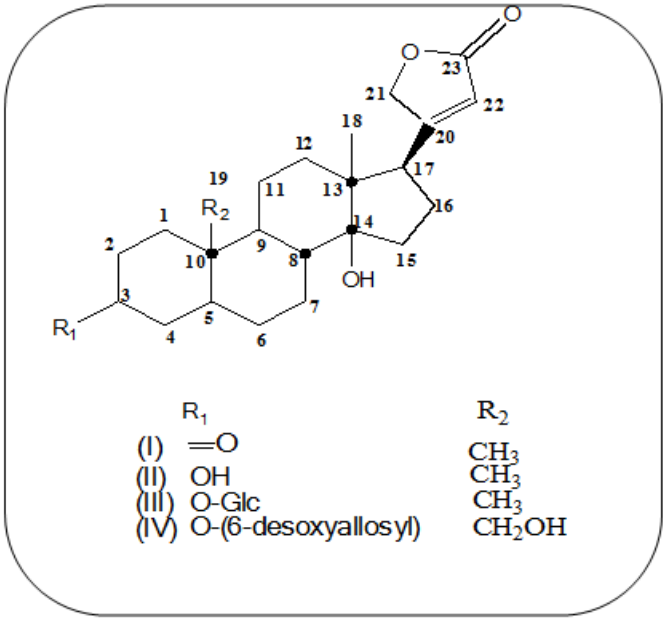
| **Family** | **Genera** |
|---|---|
| *Apocynaceae* | *Adenium, Acokanthera, Srophanthus, Apocynum, Cerbera, Thevetia, Nerium, Carissa, Urechites* |
| *Asclepiadaceae* | *Gomphocarpus, Calotropis, Pachycarpus, Asclepias, Xysmalobium, Cryptostegia, Menabea, Perploca* |
| *Moraceae* | *Antiaris, Antiaropsis,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

The bufadienolides occur in plants of families: Hyacinthaceae (Syn. Liliaceae),Crassulaceae, Iridiaceae, Melianthaceae, Ranunculaceae and Santalaceae. Two genera of the first family (Hyacinthaceae) are known to produce it as Urginea and Bowiea. A large proportion of the known bufadienolides has been isolated

from Urginea species and during the past decade many compounds were isolated from the genus Urginea maritime which commonly known as squill. It is worthy to mention that the genus Urginea is an aggregate of 6 species and it has been used in medicine since early times because of its powerful digitalis-like effect. There are various animal sources for bufadienolides e.g. Buffo (toad), Photinus (fireflies) and Rhabdophis (snakes) [(1)].
From Calotropisprocera latex Hesse,*et al.*[(4-6)]. separated the cardiac glycosides caltrophin, calactin, uscharidin, uscharincalotoxin and voruscharin. Phytochemical screening of *C. procera* grown in Egypt and its latex revealed that the latex contains calotrphin, calotoxin, uscharin and uscharidin[(7)]. The seeds of C. procera were shown by Reichstein's group, Brüschweiler,*et al.* [(8)] to contain frugoside, coroglancigenin and corotoxigenin. On the other hand, Brüschweiler,*et al.*[(8-9)] suggested a new formula for the previous cardiac glycosides isolated by Hesse, and isolated proceroside, uzarigenin and syriogenin.

Cheung and Waston[(10)] briefly studied the $^{1}H$ and $1^{3}C$ NMR of the compounds calactin, uscharidin, calotoxin, uscharin and voruscharin and established their stereochemistry. Akhtar et al.[(11)] isolated a new cardenolideproceragenin.

Four cardenolides [ uzarigenone (1), uzarigenine (II), deglucouzarin (III) and frugoside (IV)] were isolated from the stem of *C. procera*, a plant that grows wild in the Egyptian desert in Sinai [(12)].




Oleandringenin-ß-D-glucosy-ß-D-diginosideglucosylnerigoside and -ß- gentiobiosyl-α-L-oleandroside (gentiobiosyloleandrin) were isolated as the major cardiac glycosides of air-dried leaves of Neriumodorum (Apocynaceae) along with oleandrigein-ß-D-glucoside, digitoxigenin-ß-gentiobiosyl-ß-D-diginoside (gentiobiosylodoroside), 16-O-acetyl-digitalinum, $\Delta^{16}$-dehydroadynerigenin-D-glucosyl-ß-D-digitaloside and odoroside[(13)]. From the root bark of Neriumodorumuzarigenin, digitoxigenin, oleandrgenin[(14)], digitoxigenin -ß-gentiotriosy-(1→4) -ß-D-digitaloside, uzarigenin-ß- gentiotriosy-(1→4) -ß-D-digitaloside, and 5α-oleandrigenin glycosides were obtained along with 40 known cardenolide ingredients[(15)].
Polar glycosides from the air-dried leaves N.odorum were examined, and gentiobiosyl-nerigoside and gentiobiosylbeaumontoside isolated along the major trioside, gentiobiosyl-oleandrin. Minor triosides also include glycosidesof 8ß-hydroxy- and $\Delta^{16}$-8ß-hydroxy-digitoxigenin, and $\Delta^{16}$-neriagenin, along with glycosides of known cardenolides, oleandrigenin, digitoxigenin, adynerigenin, neriagenin and their $\Delta^{16-}$ derivatives[(16)].
Six cardiac glycosides having an auusual sugar linkage-elaeodendrosides B, C, F, G, K and L, were isolated from seeds of *Elaeodendronglaucum* (Celastraceae)[(17)].

The isolation and structures of 12 cardenolide glycosides from the leaves, stems and roots of *Strophanthusdivaricatus* (Apocynaceae) is described by Chen,*et al.* [(18)].

Gentiobiosyl-ß-D-cymaroside and gentiobiosyl-α-L-cymaroside of digitoxigenin were isolated from the seeds, unripe fruits, and leaves of *Beaumontiabrevituba*, and *B. murtonii* (Apoccynaceae). Oleandrigenin and/or $\Delta^{16}$-digitoxigenin glycosides having the same sugar moieties were not isolated from the leaves of *B. brevituba* but from the leaves of *B. murtonii* as well as the seeds of *B. brevituba*[(19)].

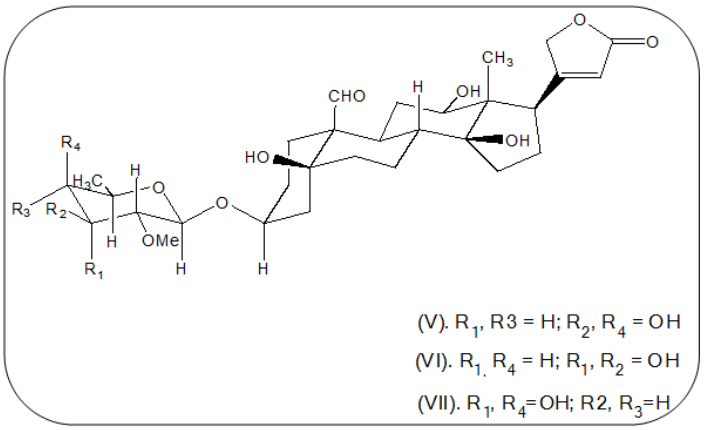
Four cardenolides were isolated for the first time the aerial parts of *Adonis aestivalis* (Ranunculaceac). Two new cardenolides were structurally elucidated: strophanthidin-3-O-ß-D-dioitoxosido-α-L-cymarosido-ß-D-glucoside and strophanthidin-3-O-ß-D-digitoxosido- ß-D-digitoxosido- ß-D-diginosido- ß-D-glucoside[20].

The principle cardiac glycosides present in *Maquira* (Moraceae) species are strophanthidin-based the main ones occurring in *Naucleopsis* (Moraceae) species are antiarigenin- as well as strophanthidin-based[21].

The investigation of the constituents from the leaves of *Thevetianeriifolia* (Apocynaccae) cultivated in Japan, the leaves of the same species collected on Singapore were examined, and C-nor-D-homocardenolide glycosides were obtained. α-L-Rhamnosides of digitoxigenin , cannogenin, thevetiogenin and glycosides of uzarigenin were isolated also from the leaves [22]. A new cardenolide, neriifoside, 3ß-O-(α-L-acofriosy)-14ß-hydroxy-5 α-carda-20:22-enolide has been isolated from the fresh , uncrushed leaves of *T.neriifolia* along with cardenolideperuvoside. These structures have been elucidated through detailed 1Dand 2D NMR studies [23].

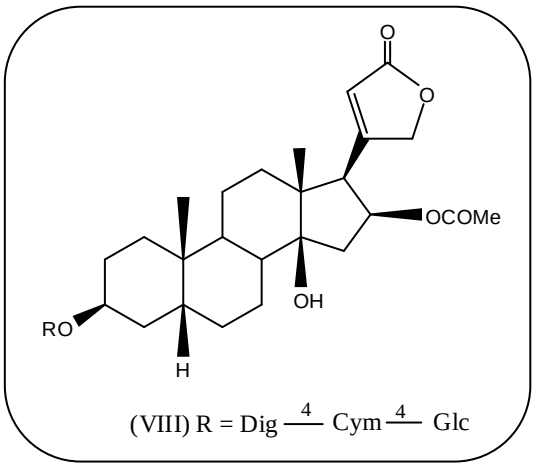
Forty one bufadienolides were isolated from the bulbs of *Urginea maritime* (Hyacynthaceae) from Egypt, 26 of them are new natural compounds. Structure elucidation was performed by comparison with authentic substances or by means of $^{1}H$, $^{13}C$ NMR and FAB mass spectroscopy[24].

Bioassay-guided fractionation of the chloroform/methanol extract of *Antiaristoxicaria* (Moraceae) latex, has led to the isolation of three new cardenolides, toxicarioside A(V) [25], toxicarioside B(VI) and toxicarioside C (VII)[26].

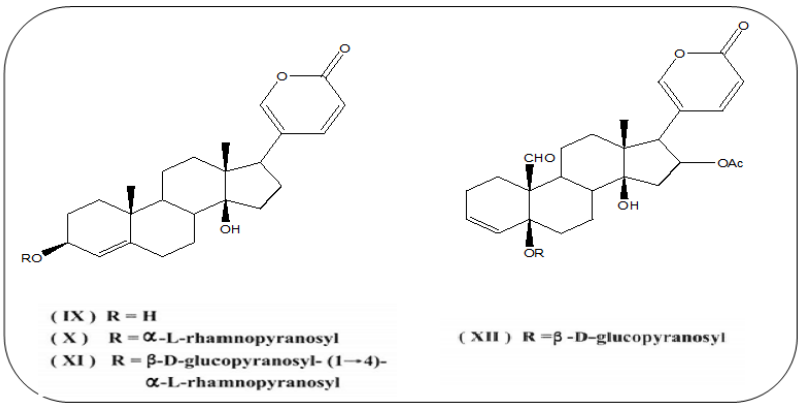



Two new cardiac glycosides were isolated from the seeds of *Erysimumcheiranthoides*(Cruciferae). Their structures were characterized as strophanthidin glycosides of 3-O-α-L rhamnopyranosyl-(1→4)-3-O-acetyl-ß-D-digitoxopyraosyl and 3-O-P-ß-D-glucpyranosyl-(1→4)- α-L-rhamnopyranosyl-(1→4)-3-O-acetyl-ß-D-digitoxopyranosyl[27].

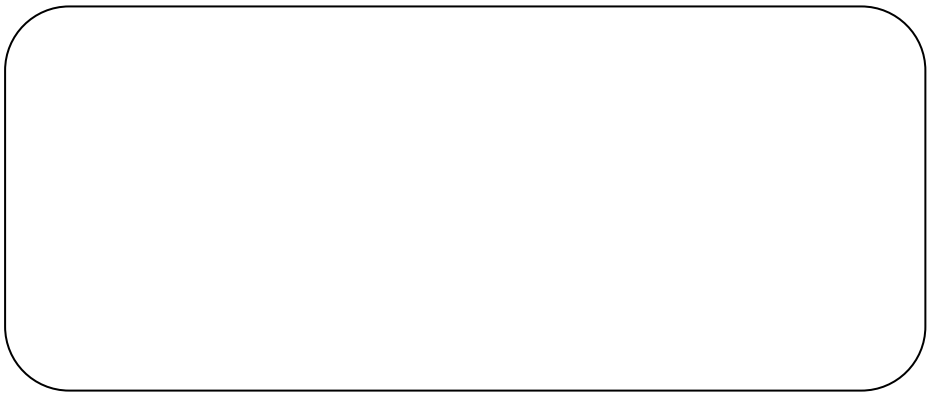
From the leaves of *Cryptostegiagrandiflora* (Asclepiadaceae), new cardiac glycoside oleandrigenin3-O-ß-glcopyranosyl-(1→4)-ß-cymaropyranosyi-(1→4)-ß-digitoxopyraoside, crytostigmin (VIII) have been isolated[28].




The bufadienolides produce from the genus Urginea are mostly plycosides and some of them are derived from scillarenin (IX), proscillaridine (X), scillaren A(XI) and scillicyanoside (XII)[1] .



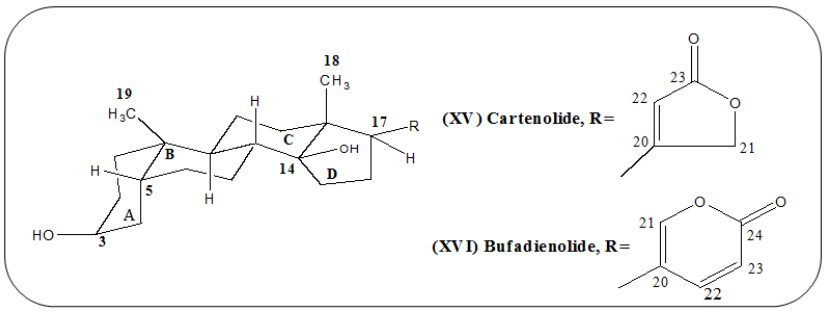

The bufadienolides found in *Buffo* (toad) occur not only in the unconjugates are also known: sulfates, dicarboxylic esters and amino acid-dicarboxylic esters. As examples of more polar conjugate is the bufotoxins (XIII) in which a suberoyl arginine ester is replaced by a succinyl arginine ester as in marinbofotoxin (XIV) [(1)].



## II. Structural Constitution

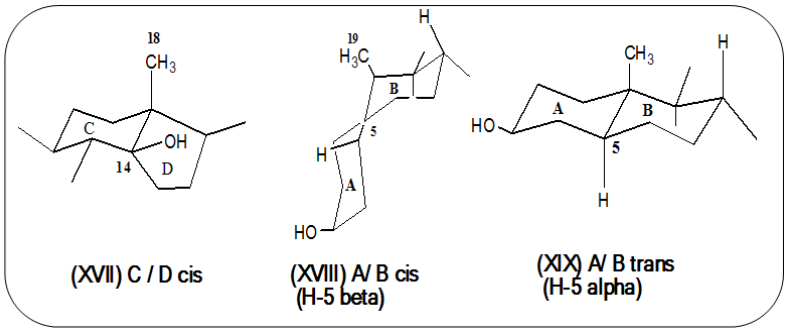
Cardiac glycosides consist from a cyclopentanoperhydro-phenanthrene nucleus substituted at C-17 with either butenolide (XV) or pentadienolde (XVI) rings to form cardinolides and bufadienolides, respectively. Most clinical attention was directed to the cardenolides owing to their therapeutic use. Digoxin and digitoxin are the two most widely used digitalis inotropes, there are an estimated two million patients receiving theses cardenolides in the U.S.A.

The cardiac glycosides, cardinolides and bufadienolides, bear a structure resembled 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 [(29)].Other substituent groups may be present, for example additional hydroxy 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 [(29)].




It also characterized by its unusual "U shape" (XV, XVI). This "U shape" is imparted by the A-B and C-D cis and B-C trans ring junctures. Structures (XVII) and (XVIII) show the effect of this unusual conformation on the orientation of the C-18 and C-19 angular methyl relative to the 14-OH and 5-H groups, respectively.

The A and B rings in natural cardiac glycosides generally show a cis configuration (XVIII). A change of configuration at C-5 normally yields less active products, trans configuration (XIX) [30, 31]. On other hand, the adrenocortical steroids typically possess an A-B, B-C, C-D all trans conformation, while the bile salts characteristically have an A-B cis and B-C, trans orientation [31].



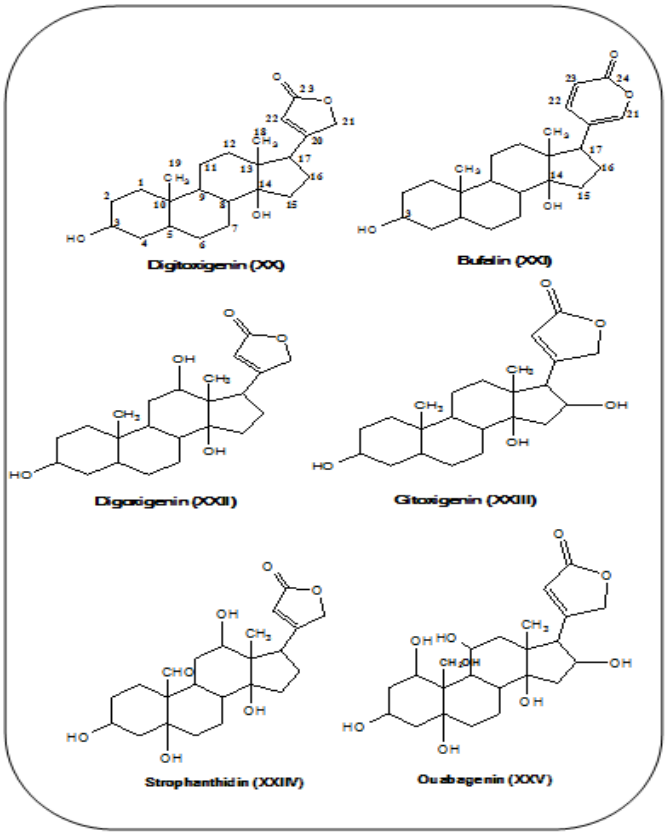

**II.1. Types of Genins (Aglycones)**

The naturally occurring cardiotonic steroids are classified into two broad groups, cardenolides and bufadienolides. The caredenolides, exemplified by digitoxigenin (XX), are the predominant group in nature and are $C_{23}$ steriods with an α: ß-unsaturated γ-lactone (butenolide)ring.

The bufadienolides, exemplified by bufalin (XXI), are $C_{24}$ steroids with a doubly un saturated 6-membered lactone (α-pyrone) ring. Both of them carry a hydroxyl group at C-14. Additional substitution by carbonyl, hydroxyl, aldehyde, epoxide and olefin groups at other positions give rise to various aglycones (genin) portions.

Although the current number of genin parts is relatively small, variation in the glycoside moiety, either α or ß at positions 3 has led to a considerable number of cardenolides. Some examples of aglycones of cardiac glycosides, already used for congestive heart failure and commercially available products, are digoxigenin (XXII), gitoxigenin (XXIII), strophanthidin (XXIV) and ouabagenin, Scheme 1.

The most commercially important sources of cardiac glycosides are Digiatis purpurea, D. lanata, Strophanthus gratus and Strophanthus kombe Table (2) [31-32].




**Schema 1**. Types of Genis (Aglycone)

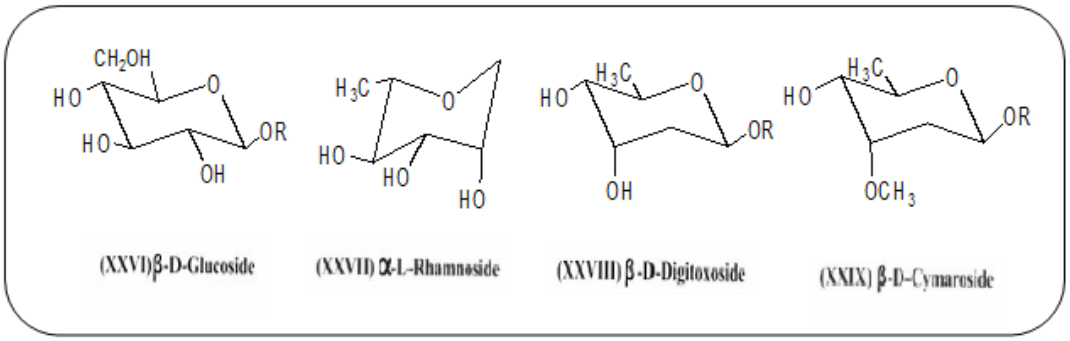
**Table (2):**

| Structure | Name |
|---|---|
| From Digitalis purpurea leaves | |
| Glucose-(digitoxose)$_3$-digitoxigenin | Pupurea glycoside A |
| (Digitoxose)$_3$-digitoxigenin | Digitoxin |
| Glucose-(digitoxose)$_3$-gitoxigenin | Purpurea glocoside B |
| (Digitoxose)$_3$-gitoxigenin | Gitoxin |
| From Digitalis lanata leaves | |
| Glucose-3-acetyldigitoxose-(digitoxose)$_2$- digitoxigenin | Lanatoside A |
| Glucose-( digitoxose)$_3$-digitoxigenin | Desacetyl lanatoside A |
| 3-Acetyldigitoxose-(digitoxose)$_2$-digitoxigenin | Acetyldigitoxin |
| (Digitoxose)$_3$- digitoxigenin | Digitoxin |
| Glucose-3-acetyldigitoxose- (dogitoxose)$_2$-gitoxigenin | Lanatoside |
| Glucose-(digitoxose)$_3$-gitoxigenin | Desacetyl Lanatoside B |
| 3-Acetyldigitoxose- (digitoxose)$_2$-gitoxigenin | Acetylgitoxin |
| (Digitoxose)$_3$- digitoxigenin | Gitoxin |
| Glucose-3-acetyldigitoxose-(digitoxose)$_2$-digoxigenin | Lanatoside C |
| Glucose-(digitoxose)$_3$-digoxigenin | Desacetyl lanatoside C |
| 3-Acetyldigitoxose-(digitoxose)$_2$-digoxigenin | Acetyldigoxin |
| (Digitoxose)$_3$-digoxigenin | Digoxin |
| From Strophanthus | |
| Rhamnose ouabagenin | Ouabain |
| From Strophanthus kombe seeds | |
| (Glucose)2-cymarose-strophanthidin | K-Strophanthoside |

## III. Sugar Types

The sugar moieties, are mostly, attached to the aglycone at C-3 by ß-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 [(3)]. Elderfield [(33)], Overend and Stacey [(34)], Reichstein and Weiss[(35)], Hanessian[(36)],Zorbach and Bhat [(37)], Reichstein [(38)] and Singhand Rastogi [(39)] have covered the sugars found in most of the natural occurring cardenolides.

The sugar residues attached to cardiac glycosides occur in the pyranoid form [(35)] (XXVI-XXIX).An empirical rule serves to differentiate between sugars with a hydroxyl group at C-2 (eg. XXVI, XXVII) and 2-deoxy sugar (eg. XXVIII,XXIX) [(40)] The latter are almost completely hydrolyzed by boiling in 0.05 N mineral acid in 50% aqueous methanol for 30 minutes whereas the former group is not completely affected by this procedure.




## IV. Isolation 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 [(40)] suggested the defatting of dried and powdered seeds, and/or leaves with petroleum ether followed by digestion with water at 0ºC to extract polysaccharides and hydrolytic enzymes.

The prior protection of plant material with toluene could be affected by allowing it to stand for many days at 25-37 ºCto avoid enzymatic hydrolysis. This could be followed by its exhaustive extraction with water-alcohol mixture of increasing alcohol ratio. The aqueous extract is evaporated to a small volume in vacuum at 50ºC. Fats are 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 then precipitated with freshly prepared lead hydroxide and the mixture is filtered through Hyflo-Super Cel. 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 solubility in water, the residual aqueous phase is half saturated with sodium sulfate and then extracted with C-M[(41-42)].

Chromatography on silica gel of controlled water content has been used advantageously by Stoll,*et al*. [43]. Ethyl acetate, either dry or saturated with water and containing 0.5-5% of methanol is used as the mobile phase. Cardiac glycosides containing an function group are separated most satisfactory by use of Girard's reagent [44].

Recently, methanol was used as an effective solvent to inhibit the enzymatic hydrolysis in the same time to extract cardiac glycosides. The grounded plant material exhaustively extracted at first with 70 to 80 % methanol at room temperature followed by distillation of the solvent under vacuum at a temperature not exceed 50ºC. Defatting was performed using petroleum ether and finally, the extract could be fractionated using chloroform /methanol mixture with different proportions [45].

The development of the chromatographic techniques used in isolation of cardiac glycosides began at 1950. First paper chromatography (PC) was introduced followed by thin layer chromatography (TLC) and liquid chromatography (LC). In the last few years' gas chromatography (GC), often in conjunction with mass spectrometry (MS) has been used specially for analytic purposes. Also, the high performance liquid chromatography (HPLC) has gained special prominence [46].

**IV.1. Paper and Thin Layer Chromatography (PC and TLC)**

Tschesche et al. [47] had been able to accomplish at leastpartial separation of a series of aglycones and their acetates by using simple solvent like ethyl and butyl acetate. Stahl and Kaltenbach[48] first used TLC on silica gel to separate cardioactive glycosides. This was followed by its successful application of TLC and PC [49,50].

Partition chromatography has generally proved rather more suitable for this large number of fairly polar compounds, as in Digitalis purpurea and D. lanata. Duncan[51] carried out partition chromatography on silica gel with benzene or chloroform and an addition of 5-50 % of methanol, propanol, isopropanol, butanol, acetone, butanone or ethyl acetate. Manzetti and Reichstein [52] have used butanone or butanone-benzene (90:10), saturated with water on silica gel layers impregnated with acetone-water (70:30) for chromatography of more water-soluble compounds. Cyclohexane-butanone (50:50) and (40:60) sufficed for the less polar digitoxigenin and oleandrigenin glycosides[49].

Sjoholm [35] recognized that separation of complex mixtures of cardioactive glycosides could not be achieved by conventional TLC and obtained improved resolution by using two-dimensional TLC.

Lewbart,*etal*.[50] and Bennett and Heftmann [54] improved TLC resolution by using of a continuous development technique whereby a trough filled with adsortbent material was fixed to the top edge of the TLC plate to take up the mobile phase. Lisboa and co-workers [55,56] used a similartechnique for TLC of steroids, including cardenolides and found that continuous development gave better resolution of some closely related steroids than multiple developments in the same mobile phase. Clarke and Cobb [57] describe the use of continuous development TLC in two directions to obtain resolution of highly complex mixtures of Digitalis cardenolides, including extract of *D.lanata* leaves.

The thin-layer chromatography behavior of cardenolides and their derivatives was investigated in the presence of boric acid, which forms cyclic derivatives with cis-1,2- and -1,3-diols. Boric acid reduces the mobility of cardenolide glycosides containing diol units in their carbohydrate moiety whereas with cardonlides or cardenolide glycosides possessing 1,3-diol units in the genin part it increases the mobility. The formation of boric acid derivatives resulted in an improved separation of certain cardiac steroids and afforded the possibility of detecting cardenolides containing reactive diol units [58].

An analytical method for the determination of cardiac glycosides in D. lanata leaves by reserved-phase thin-layer chromatography (RP-TLC) was developed[59]. The procedure consisted of extraction of dry leaf powder with 50 % methanol and clean-up by Sep-Pak cartridges prior to RP-TLC analysis. RP-TLC was performed on an octadecylsilyl bonded silica gel plate, using a developing solvent of acetonitnle-methanol-0.5 M NaCl(1:1:1) for primary glycosides and acetonitrile-methonal-0.5 M NaCl(12:7:9) for secondary glycosides. The plate was scanned with a reflectance densitometer at 225 nm. The quantitation was carried out by the internal standard method. The present method is reliable and relatively simple for the determination of cardiac glycosides in *Digitalislanata* leaves[59]. Approximately 100 seed samples of the Apocynaceae family, chiefly the genus Strophanthus, were examined by paper chromatography. Cardiac glycosides and aglycones were identified as $Ag(NH_3)_2^+$ -reducing spots of characteristics mobility in various solvent systems [60].

**IV.2. Column Chromatography**

Kaiser [61] used column chromatography with silica gel or aluminum oxide as an adsorbent for preparative partition of plant extracts. Latter, sephadex G-200 [62], sephadex LH-20 [63], and ion exchange [64] were used as column filling materials.

The behavior of six cardenolides and eight cardiac glycosides related to digitoxigenin during column chromatography on Sephadex LH-20 gel has been investigated[65]. Complete resolution was obtained for

mixtures of digitoxigenin, gitoxigenin and digoxigenin, but not for those of the 3-epimeric cardenolides. It was possible to achieve a group separation of cardenolides and their glycosides of the digitoxigenin series from those of the digoxigenin or gitoxigenin series. The solvents employed for elution were methanol or water saturated with ethyl acetate, chloroform, or cholorform-ethanol. Also, the flash chromatography technique was applied for isolation and purification of cardiacglycosides[66].

Reversed phase column chromatography are giving wide acceptance in many fields including HPLC of cardiac glycosides with RP-8or RP-18 column and acetonitrile/water or methanol/water as an eluent, followed by UV detector at220 nm[67].

Recently, several procedures had been developed for extraction, partition and/or isolation of cardinolides from various plant parts. Warashine and Noro achieved the extraction of the dried whole plant with methanol[68]. The residue resulted from extraction was suspended between water/$Et_2O$ mixture. The aqueous phase was fractionated in Mitsubshi Diation MP-20 column with 50% MeOH in $H_2O$ and MeOH. The eluate MeOH residue was chromatographed on silica gel column. Other groups from Japan and China fractionated the MeOH extract of plant seeds between n-hexane and water. The aqueous layer was subjected to CC over MCl gel CHP-20P and elution sequentially with $H_2O$, 40%, 60%,80% and 100% MeOH[69]. A collaborative Germany/Egyption group [70] performed the fractionation of the $CHCl_3$ extract of the plant on vacuum liquid chromatography (VLC) column on silica gel-H(Merck).

**IV.3. High- Performance liquid chromatography (HPLC)**

The employment of HPLC techniques, also, led to the isolation of large number of cardiac glycosides[70-75].

The separation of mixtures of cardiac glycosides by reserved-phase high- performance liquid chromatography on silica gel with chemically grafted diphenylsily groups using water-ethanol as the eluent was carried out. It is shown that the configuration and confirmation of the glycoside molecules and the hydrophilic properties of their aglycones and glycones, influence the separation. The hydrophilic properties of the aglycones are more important that those of the glycones. The glycosides with more hydrophilic aglycones have higher biological activity[76].

A quantitative micro high-performance liquid chromatographic (MHPLC) procedure for the determination of gitoxin and digitoxin in Digitalis purpurea leaves has been developed. The extract of dry leaf powder was submitted to a solvent-partition sequence followed by preparative thin-layer chromatography before MHPLC amalysis. MHPLC was performed on an ODS micro column, using a mobile phase of acetonitrile-methanol-water (15:15:19) and an ultraviolet detector(at 220 nm). The proposed method using MHPLC has proved to be precise and reproducible [77].

Another analytic method for the determination of secondary cardiac glycosides in Digitalis purpurea leaves by high-performance liquid chromatography (HPLC) is described. The procedure consisted of extraction of dry leaf powder with ethanol-chloroform(2:1) and clean-up by Sep-Pak cartridges prior to HPLC analysis. HPLC was performed on an octylsily bonded silica column, using acetonitrile-methanol- water(4:4:5) for trisdigitoxosides and acetonitrile-methanol-water (8:30-43) for strospeside, the effluent was monitored by ultraviolet detection (at 220 nm). This method is sufficiently sensitive and reproducible to assay secondary glycosides in D. purpurea leaves [78].

Recent procedures using high-performance liquid chromatography radioimmunoassay (HPLC-RIA)and HPLC following derivatization show appreciable improvements in accuracy and specificity for quantitating digoxin and/or its metabolites in the low nanogram range [79].

An original method based upon high-performance liquid chromatography coupled to ionspray mass spectrometry (HPLC-ISP-MS) has been developed for the identification and quantification of several cardiac glycosides, namely digoxin, digitoxin, lanatoside and acetyldigitoxin[80].

**IV.4. Droplet Counter Current Chromatographt (DCCC)**

The technique of DCCC has been a rapid expansion over the past few years. It was used to isolate three new glycosides from Digitalis lanata using the solvent systems CHCL3-MeOH-$H_2O$ (5:6:4) and $CH_2CL_2$-MeOH-$H_2O$ (5:6:4)[81]. Four strophanthidin glycosides, out of a total of eight compounds isolated, were separated from one another by DCCC. The solvent systems $CHCL_3$MeOH-$Pr^nOH$-$H_2O$ (5:6:1:4) and $CHCL_3$-MeOH-$Pr^nOH$-$H_2O$ (45:70:5:40) were used [82]. Further application of DCCC have been reported for the isolation of affinosides from Anodendron affine [83,84]. Recently Kopp et al[75] were used the technique of DCCC in successful application to isolate 41 bufadienolides after fractionation by column chromatography.

**IV.5. Micellar electrokinetic chromatograph (MEKC)**

The interest of micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC) for the resolution of four cardiac glycosides(digoxin, acetyldigoxin, acetyldigitoxin and deslanoside) is demonstrated. First, the influence of some parameters on the resolution of the solutes in MEKC such as the concentration of the surfactant, ph, addition of organic modifiers and urea is discussed. Then, results are compared with those obtained in MEEKC using different microemulsim composition. Results indicate that MEEKC possesses several advantages over MEKC for the separation of relatively hydrophobic, compounds such as digitalic compounds. Moreover, efficiency is improved with shorter analysis time [(85)].

**IV.6. Radial Centrifugal Chromatography:**

It is the preparative, centrifugal accelerated, radial thin layer chromatography, which gives good resolution and ease of operation. Martin et al [(66)] used this technique to isolate cardiac glycosides with 2- or 4-mm silica gel 60 $PF_{254}$ plates.

## V. Hydrolysis

**V.1. Acid Hydrolysis**

The acid hydrolysis of a 2-hydroxy sugar linked to a genin required vigorous conditions that the product is usually a mono-, di, trianhydro- genin[(1)].Voss and Wachs[(86)] studied model glycosides and found that alcoholysis sometimes proceeds as much as one hundred times as fast as hydrolysis and introduced this procedure for effecting cleavage under less destructive conditions[(87)]. Thus convallamarin, a glucoside-dirhamnoside, was successfully cleaved to convallamaretin without dehydration by allowing the glycoside to stand for several days at 35 ºC in 2% methanolic hydrogen chloride, the sugar was split quantitatively as methylglucoside. Also, when ouabain, which is sparingly soluble in acetone, is suspended in acetone containing hydrogen chlorid and shaken in the cold the solid soon dissolved in the form of a monoacetonide of ouabagenin (80% yield), since the genin contains a 1,3-glycol group. This feature is by no means essential to the success of the method. The acetonide is hydrolyzed with 0.6 % sulfuric acid(80 -90 % yield) or by the action of refluxing with dilute alcohol (quantitative yield)[(2)]. Another methods of effecting cleavage under sparing conditions were also reported [(88-90)].

Another developed procedure, which is useful when the objective is to isolate a rare sugar, or if the aglycone has no acid-sensitive groups, consists in refluxing the glycoside with a mixture of 35 ml acetic acid, 55ml water and 10 ml concentrated hydrochloric acid[(91-92)]. Rangaswami and Reichstein, using 50% methanol, 0.5 N in sulfuric acid for the same purpose, were able to isolate crystalline oleandrose from oleandrin[(93)].

**V.2.Enzymatic Hydrolysis**

The use of specific enzyme preparations aids in elucidating the structure of the sugars present in these glycosides. For example, an enzyme prepared from Aspergillus oryzae will split off D-glucose if it is directly bound to the aglycone. Other important enzymes are those from the intestinal tract of snails, seeds of Medicago species and from species of Aspergillus and Penicillium. The latter will split hexamethyl sugars and their methyl ethers from the aglycones. The latter will split hexamethyl sugars and their mrthyl ethers from the aglycones. Especially important are the enzymes that split the terminal glucose from the primary glycosides. These include the digilandiase in the leaves of the laniferous Digitalis, digipurpidase from the leaves of Digitalis , the strophanthobiose from the seeds of Strophanthus species, the scillarenase from species of Scilla and an enzyme from the seeds of Adenium multiforum is especially active. These enzymes are active in thr fresh drugs, special precautions must be taken in order to be able to isolate the glycosides intact. Also, any a-glycoside linkage can be split by enzymes from yeast [(94)].

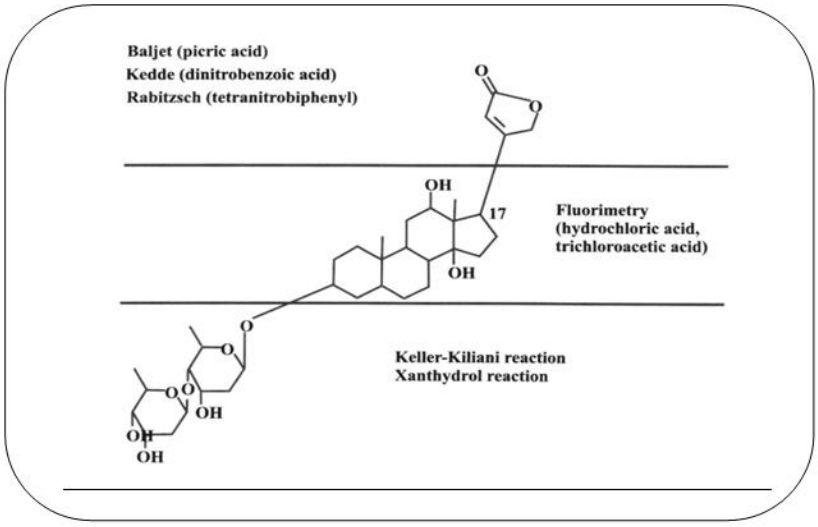
Stoll,*et al*.[(95)] obtained an enzyme preparation from Coronilla glauca seeds capable of cleaving a D-glucose unit joined directly to the genin. A commercial enzyme preparation known as "luizym"[(96)] has been used bto affect hydrolysis of uzarin (aglycone-D-glucose-D-glucose) to uzarigenin in high yield while acid hydrolysis had given only two anhydro derivatives of the genin. Reichstein and co-workers[(97)] used an enzyme preparation from Helix pomatia (snail) for the same purpose. The enzyme mixture from a Japanese snail splits a D-fucose from Digitalis purpurea in acetate buffer, while in distilled water only glucose residues and hydrolyzed[(98)]. An interesting case of hydrolysis of a rhamnoside (proscillaridin= scillaren-L-rhamnose) with an adaptive enzyme is reported by Stoll,*et al.*[(99)]. The enzyme was obtained from a Penicillium species grown in a medium containing rhamnose as the only carbon source.

## VI. Qualitative and Quantitative Estimation

The analytic methods for cardiac glycosides can be divided into two groups. In the µg range the classical methods of photometry and chromatography have an establish place in the pharmacopoeias and very widely employed in control laboratories for quantitative determination of the content and purity of glycoside preparations. For pharmacokinetic investigations in the µg range the available methods require greater

expenditure on apparatus. They comprise: 1. The isotope technique, 2. Gas chromatography coupled to a mass spectrometer (GC-MS) and 3. A well developed "Cold Analytical Method" by coupling the chromatographic unit, GC or HPLC to sensitive detector (MS or Fluoresence detector). Such method affords a reliable measurements in µg range [(44).] Whereas the classical methods required preliminary purification, usually by chromatography, with the isotope technique or the assays it is possible to measure the glycosides directly in the presence of other substance, e.g. in biological materials [(46)]. Direct measuremet by UV led to the absorption maxima for cardenolides at 217 nm ($\varepsilon_{mol}$ = 16,595) and for bufadienolides at 300 nm ($\varepsilon_{mol}$= 5,250), where is the extinction coefficient.

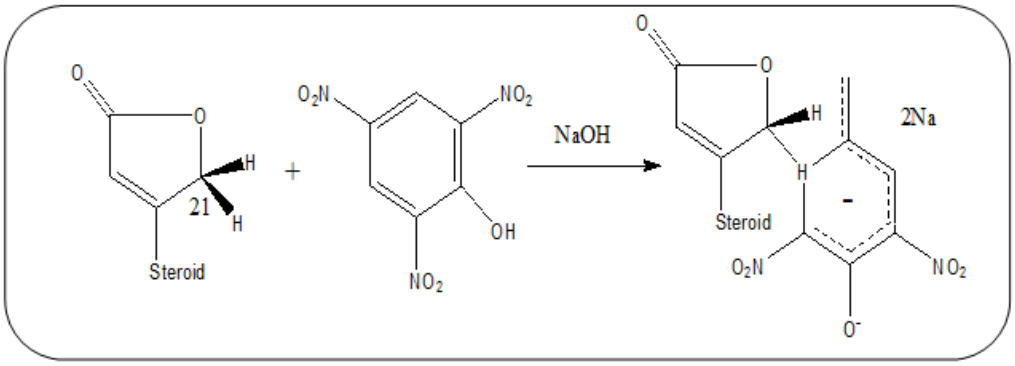
For qualitative and quantitative determination the cardiac glycosides must therefore be converted into coloured derivatives. It can be converted into coloured derivatives by reaction with polynitroaromatic derivatives in alkaline solution or with Keller- Kiliani and xanthydrol in acidic medium[(46).]




**VI.1. Alkaline Reagent**

The reaction between cardenolides and polynitroaromatic derivatives in alkaline solution [(100-101)]are based on the C-C coupling of the unsaturated lactone ring with them to produce dye complexes which can be measured photometrically or used as a spray reagent to visualize cardiac glycosides on TLC. The reagents that gained an established place are Baljet reagent (picric acid)[(100)], Kedde reagent (3,5-dinitrobenzoic acid)[(102)] and Rabitzsch reagent (tetranitrobiphenyl)[(103)]. The specificity of Baljet reagent is low because many other substances e.g. ketones give intense colour reaction with picric acid and alkali [(46)].

Various reaction mechanisms are suggested for the reaction of polynitroaromatic compounds with cardiac glycosides[(104)] , splitting off one proton at C-21 produces a carbamine which undergoes nucleophilic linkage to the polynitroaromatic molecule. The resulting dye complexes are known as Meisenheimer compounds (XXX) of the cyclohexadienate type[(46)].




It is worthy to mention that Baljet and Kedde reagents have established place in the pharmacopoeias and very widely employed tell the present time. As examples are the assay of digioxin in its preparations[(106)].

**VI.2. Acidic Reagent**

Both the Keller-Kiliani and xanthydrol convert 2-deoxysugars into characteristics coloured 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[(46)].

Thus Keller-Kiliani reaction in acetic acid, ferric chloride and sulfuric acid produces a blue colouration with absorption maxima at 470 and 590 nm. It is important to note that colour formation is dependent on time and also affected by moisture content[(107,108).]

The xanthydrol reaction[(109)] in acetic/hydrochloric acid mixture produces red colouration with absorption maximum at 520 nm. However, the reagent is not very stable and decomposition products tend to interfere with the colour reaction. Pötter [(110)], therefore, suggested the use of the more stable dixanthylurea instead of xanthydrol.

The most recently introduced reagent is thiobarbituric acid was also introduced to detect 2-deoxy sugars. According to Mesnard and Devant [(111)] deoxy sugars are oxidized by $HIO_4$ to dialdehhydes, the reaction being accompanied by opening of the ring. With 2-thiobarbituric acid these dialdehydes form coloured complexes, which can be measured by spectrophotometry at 532 nm.

## VII. Methods of Structure Determination

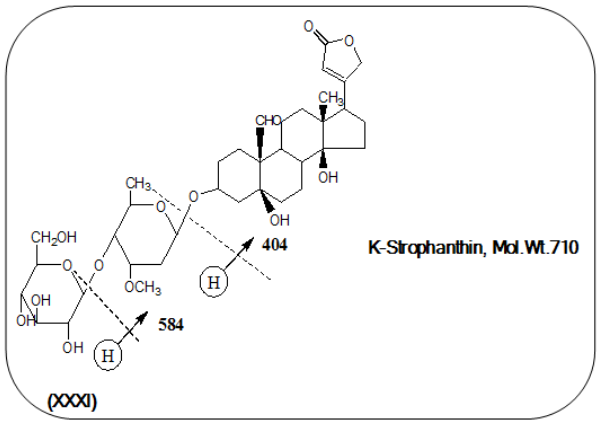
The great development in the spectroscopic instruments and mass spectrometers and the analysis of the produced data in the last three decades was accompanied by a great jump in the study of structure and stereochemical behavior of the naturally occurring compounds. This development led to stabilize a clear relationship between the structure and the data obtained from the spectroscopic experiments.

The cardenolides and bufadienolides are basically distinguished by an unsaturated lactone ring attached at C-17 (mostly ß-oriented) and an mostly ß-oriented C-14 hydroxyl group in addition to a hydroxyl group at C-3 (mostly ß-oriented).The structure is frequently complexed by additional oxygen functions of various types located at various sites of the steroid nucleus and by different sugar moieties attached at C-3. The application of the modern spectroscopic techniques as mass spectra and nuclear magnetic resonance have gained an important role for structure determination in various classes of naturally occurring cardioactive compounds.

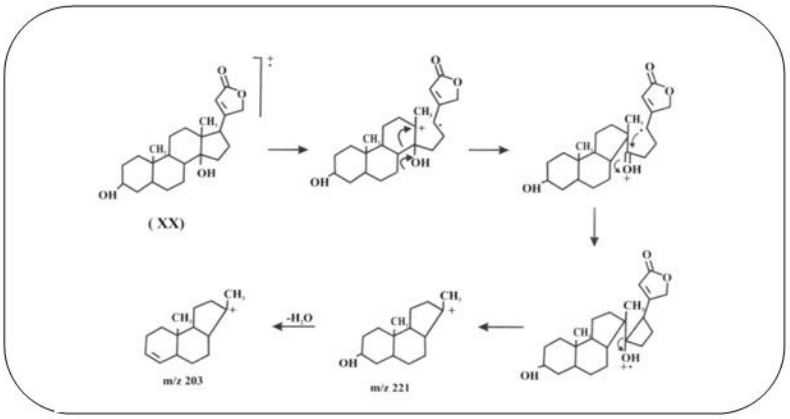
### VII.1. Mass Spectra (MS)

Brown,*et al*.[(112)] reported the first MS comparisons of underivatised cardenolides under conditions of electron impact (EI) and field ionization (FI).Two systems of cardenolides were investigated, one was based on digitoxigenin(XX) and the other on strophanthidin(XXIV). Preliminary, 70 ev. EI spectra of digitoxigenin (XX) and strophanthidin (XXIV) indicated much fragmentation.However, in the FI mode, fragmentation was greatly reduced and intensity of the molecular ion was much greater.

Comparable results were seen with other steroidal glycosides and the general FI mode fragmentation pathways have been delineated [(112)]. The most important feature was a hydrogen ion transfer and subsequent elimination of the terminal sugar unit with loss of water. Sequence fragmentation of $M^+$ ion gave rise to an ion, which corresponded in mass and constitution of the cardenolide with one less monoside unit and finally the genin is produced (XXXI). Therefore, the molecular weight of the expelled monosaccharide can be deduced by addition of 18 mass units to the difference between adjacent members or the unique set of peaks.

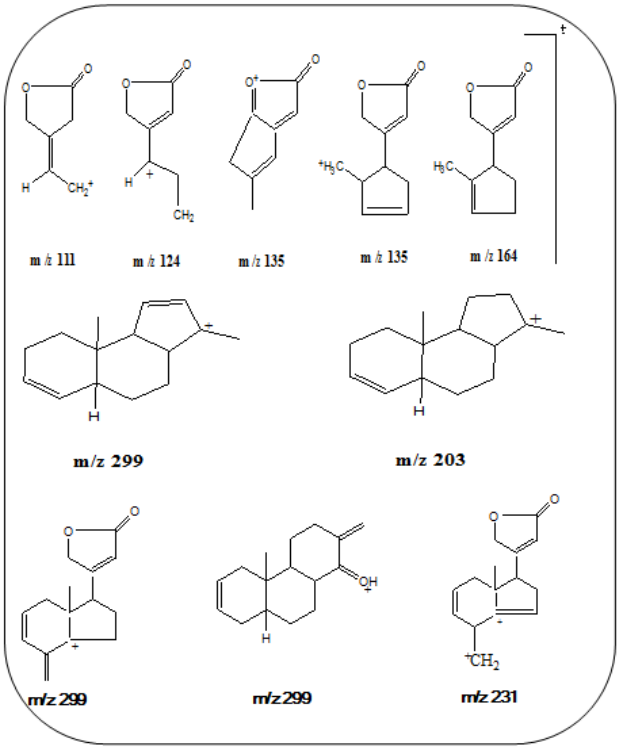



The mass spectra of several cardenolides aglycones and the principal modes of fragmentation were also outlined by Brown et al [(112)]. As an example is the MS fragmentation of digitoxigenin (XX).




Fayez and Negm [113] reported that in addition to conventional expulsion of functional groups, the spectra exhibit $C_{15}$, $C_{16}$ and $C_{17}$ fragment ions. It resulted by elimination of ring D and the side chain as well as ionscomprising the latter group with remnants of ring D. The structure of some fragment ions comprising ring D or devoid from it were also suggested[112,113].

Flaskamp and Budzikiewicz [114] reported on the fragmentation sequences for digitoxigenin (XX) and its acetate by the use of metastable transitions, exact mass measurements and labeling. Isobe,*et al.*[115] were investigated the fielddesorption mass (FD/MS) and the fast atom bombardment mass spectra (FAB/MS) of cardiac glycosides. They suggested that FD/MS is a satisfactory method for the identification and structural evaluation of the glycosides because of simple fragmentation pattern and the presence of prominent molecular ions and fragments in the high mass region. Evaluation of positive and negative ion in FAB/MS for structural investigations was also studied on 30 cardiac glycosides in comparison with their field FD/MS[116].




**VII.2. Nuclear Magnetic Resonance (NMR)**

NMR, in recent years, remains the most valuable tool for the structure determination of cardiotonic compounds. The advantage of pulsed fourier transformation and two dimensional (homo- and hetero-nuclear) spectroscopy provide information related to the carbon skeleton of the molecule and the structure environment of each hydrogen. The most commonly usded Ld $^{1}$H- and $^{13}$C-NMR techniques are the routine pulsed, $^{1}$H spin decoupling, long-range selective proton decoupling $^{13}$C-NMR, nuclear Overhauser effect in $^{1}$H(NOE) and distorsionless enhancement by polarization transfer in $^{13}$C (DEPT). In 2D(homo- and hetro-nuclear) NMR is

used the J-resolved and chemical shift correlation (COSY), two dimensional Overhauser effect(NOESY), $^{1}$H-detected hetero-nuclear multiple-quantum coherence (HMQC) and $^{1}$H- detected hetero-nuclear multiple bond connectivity (HMBC).

Tori,*et al.*[117] reported the first $^{13}$C-NMR analysis of ten cardenolides by employing single-frequency off-resonance, noise off-resonance decoupling and the comparison with spectra of structurally related compounds. Latter, he used $^{13}$C-NMR spectroscopy to determine the structure of thevetin A and B [118]. Robien,*et al.*[119] were reviewed $^{13}$C-NMRdata of 36 budfadienolides . Latter on, Kopp,*et al.*[75] used $^{13}$C-NMR for elucidation the structure of bufadienolides compounds isolated from Urginea maritime.

Bufadienolides are characterized by the signals of the pyrone ring; $\delta_H$ ([$^2H_6$] acetone): 7.85 (dd, J =9.8 and 2.6 Hz, H-22), 7.40 (dd, J =2.7 and 0.9 Hz, H-21) and 6.17 (dd,J =9.8 and 6.8 Hz, H-23); $\delta_c$ : 122.1 (C-20), 149.0 (C-21),147.0 (C-22),114.3 (C-23) and 161.2 (C-24)[1]

Cardenolides are characterized by the signals of the lactone ring; $\delta_H$: 5.2± 0.5(dd,H-21α), 5.0± 0.25 (dd,H-21ß) and 6.25± 0.5 (s.br, H-22); $\delta_C$: 176 ± 5 (C-20), 74.8 ±2 (C-21), 116.5±5 (C-22) and 175.0 ± 2(C-23) [66,73,74,118,120-122].

The $^{13}$C chemical shift of C-19 also gives valuable information on the stereochemistry ofboth cardenolides and bufadienolides at C-5. In 5ß-series, C-19 have its signal at 21.7±2.5 ppm whereas in 5α-series at 12.2 ±0.4ppm.

The number of sugar moieties could be determined from the number of anormeric carbons at the region of 95-103 ppm in its $^{13}$C-NMR spectrum [66,73,74,118,120,122]. Moreover, α- and ß-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 coupled with the adjacent hydrogen by 2 to 3 Hz, while of ß-sugar coupled by 7 to 8 Hz[123].

## VIII. Pharmacological Action

The pharmacological effectiveness of the cardio-active glycosides is dependent on both the aglycone and the sugar attachements. The inherent activity resides in the aglycone, but the sugars render the compounds more soluble and increase the power of fixation of the glycosides to the heart muscle. It appears that the key grouping for the attachment of the molecule through a hydrogen bond to the phosphorylated receptor enzyme (ATPase) is the α,ß-unsaturated carbonyl function of the ring. All the active aglycones features hydroxyls at C-3 and C-14 and the presence of a third hydroxyl at C12 or C16 will modify the activity and toxicity of the compound. The stereochemical requirements for activity include the cis- configuration between rings C and D. The ß-orientation of the unsaturated lactone ring at C-17 and the 3-ß-orientation of the glycosidic linkage [3].

The most important use of the cardiac glycosides is its affects in treatment of cardiac failure[124,125]. In cardiac failure, or congestive heart failure, heart cannot pump sufficient blood to maintain body needs. During each heart contraction, there is 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 plycosides inhibit $Na^+/K^+$-ATPase, and consequently increase the force of myocardial contraction[32,126].

Table 3 illustrates some glycosides and dosages that used in treatment of congestive heart failure, or of slow ventricular rate in patients with atrial flutter or fibrillation[31].

**Table (3)**

| **Name** | **Dosage Forms** |
|---|---|
| Digitoxin | Tablets:0.05,0.1,0.15,0.2 mg<br>Injection:1-ml ampoules; 0.2 mg/ml |
| Digitalis(powdered digitalis leaf) | Tablets: 32.5, 48.75,65,100 mg Capsules: 100mg |
| Gitalin | Tablets:0.5 mg |
| Lanatoside C | Tablets:0.5 mg |
| Deslanatoside Cdesacetyl lanatoside C | Injection: 2ml ampoules, 0.2 mg/ml |
| Digoxin | Tablets:0.125, 0.5 mg<br>Elixir, pediatric:0.05 mg/ml<br>Injection: 2ml ampoules, 0.25 mg/ml injection<br>Pediatric: 1ml ampoules; 0.1 mg/ml |
| Ouabain | Injection: 2 ml ampoules; 0.25 mg/ml |

On other hand, some cardiac glycosides were investigated for their antitumor activity and show positive response towards: KB. And KB., P-388[127]. The following compounds were assayed for such tests:

a- Acokanthera sp.: Acolongiflorisede M, Acoschimperoside P, Acospectoside A and Acovenoside A
b- Calotropis sp. Calotropin.
c- Digitalis sp.: Lanatoside-A and Lanatoside B

Digitoxin used as a potential anticancer agent for several types of cancer[(128)]. Anvirzel is an extract of Nerium oleander used as a potential treatment for cancer. Two of the active components of Anvirzel are the cariac glycosides oleandrin and oleandrigenin[(129,130)]. Cardiac glycosides such as digitoxin and ouabain have been shown to be selectively cytotoic to tumor as opposed to normal cells. Moreover, this class of agents has also been shown to act as potent radiosensitizers. Huang et al., examined ouabain –included anticancer effect in human androgen- independent prostate cancer PC-3 cells[(131)].

## References


[1]. Fieser, L. and Fieser, M., Steroids, Reinhold Publishing Corporation, New York, Chapman & Hali, LTD., London 1959.
[2]. Evans, W.C., Pharmacognosy, 13th edition , Baillière Tindall, London, 1989.
[3]. Steyn, P.S. and van Heerden, F.R., Natural Products Reports 397, 1998.
[4]. Hesse, G. and Reicheneder, F., Ann. Chem 526, 1936.
[5]. Hesse, G.Heuser, L.J., Hütz, E. and Reicheneder, F., Ann. Chem. 566, 130, 1950.
[6]. Hesse, G. and Lettenbauer, G., Angew Chem. 69,392,1957
[7]. Mahran, G., Rizakallah,M.M. and Saber, A.H., Bull. Fac. Pharm. Cairo Univ. 10, 1, 1971.
[8]. Brüschweiler, F., Stöcklin, W., Atöckel, K. and Reichstein, T., Helv.Chem. Acta 52, 2086,1969
[9]. Brüschweiler, F., Stöcklin, W., Atöckel, W. and Reichstein, T., Helv Chem. Acta 52,2276, 1969.
[10]. Cheung, H.T.A. and Waston, T.R.,J Chem. Soc. Perkin Trans. 1, 2162, 1980.
[11]. Akhtar, N.,Malik, A., AliS.n. and Kazmi, S.U., Phytochemistry 31, 2821,1980
[12]. Elgamal, H.M.A., Hanna, A. G., Morsy, N.A.M., Duddeck, H.,Simon, A., Gati, T. and Toth, G., J. Molecular Structure 477, 201, 1999
[13]. Yamauchi, T.,Takata, N. and Mimura, T., Phytochemistery 14(5-6), 1379,1975.
[14]. Yamauchi, T., Takahashi, M. and Abe, F., Photochemistery 15 (8), 1275, 1976.
[15]. Hanada,R., Abe, F. and Yamauchi,T., Phytochemistry 31 (9), 3183, 1992.
[16]. Abe, F.and Yamauchi, T., Phytochemistry 31 (7), 2459, 1992.
[17]. Shimade, K., Kyuno, T., Nambara, T. and Uchida, I., Phytochemistry 24(6), 1345,1985
[18]. Chen, R., Abe, F., Yamauchi, Tt. And Taki M. Phytochemistry 26(8), 2351, 1987.
[19]. Yamauchi, T., Abe, F. and Santisuk, T., Phytochemistry 29 (6), 1961, 1990.
[20]. Kopp, B.,Krenn, L., Kubelkaqa, E. and Kubelka, W., Phytochemistery 31(9), 3195,1992.
[21]. Shrestha, T., Kopp, B. and Bisset, NG., J. of Ethanopharmacology 37(2),129,1992.
[22]. Ebe, F., Yamauchi, T. and Wan, A,S,C, Phytochemistry 31 (9),3189,1992.
[23]. Siddiqui, S., Siddiqui, B.S., Adil, Q. and Begum, S., Phytochemistry 31 (10), 3541,1992
[24]. Kopp, B., Krenn, L., Draxler, M., Hoyer, A., Terkola, R., Vallaster, P. and Robien, W., Phytochemistry 42 (2), 513, 1996.
[25]. Carter, C.A., Forney, R.W., Gray, E.A., Gehring, A.M., Schneider, T.L., Young, D.B., Lovett, C.M.,Jr., Scott, L., Messer, A.C. and Richardson, D.P., Tetrahedron, 53 (40), 13557, 1997.
[26]. Carter,C,A.,Gray,E.A.,Schneider, T.L.,Lovett, C.M. Ja., Scott,L., Messer, A.C. and Rechardson, D.P., Tetrahedron 53v(50), 16959, 1997.
[27]. Lei, Z.H, Jin, Z.X., Ma,Y.L., Tai, B.S., Kong, Q., Yahara, S. and Nohara, T., Phytochemistry 49 (6), 1801,1998.
[28]. Kamel, M.S., Assaf, M.H., Abe, Y., Ohtani, K., Kasai, R., and Yamasaki, K., Phytochemistry 58 (4), 537, 2001.
[29]. Robinson, T., The Organic Constituents of Higher Plants; Their Chemistry and Interrelationship, 2nd ed. Burgess Publishing Co., Minneapolis, Minnesota, p.319, 1967.
[30]. Okada, M. and Anjyo, T., Chem. Pharm. Bull 23, 2039, 1975.
[31]. Foye, W.O., Principles of Medicinal Chemistry.3rd Ed., Mangold, J.B. and Langner, R.D., Lea& Febiger/ Philadephia, London, p. 359, 1989.
[32]. Fullerton, D.s., Text book of organic Medicinal and Pharmaceutical Chemistry. (ED, Delgado, J.N. and Remers, W.A.) J.B. Lippincott Company, Philadelphia, USA, 1991.
[33]. Elderfield, R.C., Advan. Carbohyd. Chem. 1, 147, 1945.
[34]. Overend, W.G. and Stacey, M., Advan, Carbohyd. Chem. 8, 45, 1953.
[35]. Reichstein, T. and Weiss, E., Advan. Carbohyd. Chem. 17, 65, 1962.
[36]. Hanessian, S., Advan. Carbohyd. Chem. 21, 143, 1966.
[37]. Zorbach, W. W. and Bhat, K. V., Advan. Carbohyd. Chem. 21, 273, 1966.
[38]. Reichestein, T., Naturwissenschaften 54, 53, 1967.
[39]. Singh, B, and Rastogi, R.P., Phtochemistry 9, 315, 1970.
[40]. Von Euw, J., Hess, H., Speiser P. and Reichstein, T., Hlv. Chim. Acta 34, 1821, 1951.
[41]. Bally, P.R.O., Mohr, K. and T., Helv. Chim. Acta 35, 45, 1952.
[42]. Muhr, H., Hunger, A. and Reichstein, T., Helv. Chim. Acta 37, 403, 1954.
[43]. Stoll, A., Angliker, E., Barfuss, F., Kussmaul, W. and Renz, J., Helv. Chim. Acta 34, 1460, 1951.
[44]. Schindler, O., and Reichstein, T., Helv. Chim. Acta 34, 521, 1951.
[45]. Deepak, D., Srivastava S. and Khare, N.K., Prog. Chem. Org. Nat, Prod. 69, 71, 1996.
[46]. Geeff, K., Cardiac glycosides, Experimental Pharmacology. Part 1, Springer- Verlag. Berlin, Heidelberg, 1981.
[47]. Tschesche, R., Freytag, Wand Snatzke, U.C., Chem. Ber. 92, 3053, 1959.
[48]. Stahl, E. and Kaltenbach, U., J. Chromatogr. 5, 458, 1961.
[49]. Kaufmann, h., Wehrli, W. and Reichstein, T., Helv. Chem. Acta 48, 65, 1965.
[50]. Lewbart, M.L., WEHRLI, W. and Reichstein, T., Helv. Chim. Acta 46, 505, 1963.
[51]. Duncan, G.R., J. Chromatogar.8,37, 1962.
[52]. Manzetti, A.R and Reichstein, T., Helv. Chim. Acta 47, 2303, 1964.
[53]. Sjoholm, Si., Farm. Tids. 66, 321, 1962: C.A.57:13888J, 1962.
[54]. Bennett, R.d. and Heftmann, E., J. Chromatogr. 12, 245, 1963
[55]. Zullich, G., Braun, W. and Lisboa, B.P., J. Chromatogr. 103, 396, 1975.
[56]. Lisboa, B.P. and Hoffmann, U., J. Chromatogr. 115, 117, 1975.
[57]. Clarke, C.J. and Cobb, P.H., J. Chromatogr. 168, 541, 1979.

[58]. Szeleczky, Z., J. Chromatogr 178 (2), 453, 1979.
[59]. Ikeda, Y., Fujii, Y., Umemura, M., Hatakeyama, T., Morita, M. and Yamazaki, M., J. Chromatogr. 746 (2), 255, 1996.
[60]. Heftmann, E., Berner, P., Hayden, A. L., Miller, H.K. and Mosettig, E,m Arch. Biochem. Biophys. 51 (2), 329, 1954.
[61]. Kaiser, F., Arch. Pharm. 299, 263, 1966.
[62]. Grade, K. and Forster, W., Biochem. Pharmacol. 16, 1299, 1967.
[63]. Gault, M.H., Ahmed, M. Symes, A.,L. and Vance, J., Clin, Biochem. 9, 46, 1976.
[64]. Evans, R.I., J. Chromatogr. 88, 411, 1974.
[65]. Zullich, G., Braun, W. and Lisboa, B.P., J. Chromatogr. 115 (1), 117,975.
[66]. Martin, R.A., Lynch, S.P., F.J., Pordesimo, E.O., Toth, S. and Horten, R.Y., Phytochemistry 30, 3935, 1991.
[67]. Castle, M., C., J. Chromatogr.115, 437, 1975.
[68]. Warashina, T. and Noro, T., Phytochemistry 37, 801, 1994.
[69]. Lei, Z.-H., Yahara, S., Nohara, T., Shan, T.-B and Xiang, J.Z., Phytochemistry 34, 1399,1993; 38 (4), 943, 1995.
[70]. El-Askary, H., Hozl, J., Hilal, S. and Al-Kashoury, E., Phytochemistry 34, 1399, 1993; 38 (4), 943, 1995.
[71]. Nachtmann, F., Spitzy, H. and Frei, R. W., Anal. Chem. 48, 1576, 1976.
[72]. Linder, W. and Frei, R.W., J. Chromatogr.117, 81, 1976.
[73]. Abdel-Azim, N.S., Hammouda, F.M., Hunkler, D. and Rimpler, H., Phytochemistry 42, 523, 1996.
[74]. Abe, F, Yamauchi, T., Yahara, S. and Nohara, T., Phytochemistry 37, 1429, 1994.
[75]. Kopp, B. Krenn, L., Draxler, M., Hoyer, A., Terkola, R., Vallaster, P. and Robin, W., Phytochemistry 42, 513, 1996.
[76]. Davydov, V. Ya., Gonzalez, M. E. and Kiselev, A., V., J. Chromatogr. 248 (1), 49, 1982.
[77]. Fujii, Y., Fujii, H. and Yamazaki, M., J. Chromatogr. 258, 147, 1983.
[78]. Fujii, Y., Ikeda Y and Yamazaki, M., J. Chromatogr. 479, 319, 1989.
[79]. Vetticaden, S. J., J. Chromatogr. 531, 215, 1990.
[80]. Tracqui, A., Kintz, P., Ludes, B. and Mangin, P., J. Chromatogr. 692 (1), 101, 1997.
[81]. Krüger, D., Junior, P. and Wichtl, M., Planta Med. 49, 74,1983.
[82]. Wagner, H., Habermeier, H. and Schulten, H.R., Helv. Chim. Acta 67, 54, 1984.
[83]. Abe, F. and Yamauchi, T., Chem. Pharm. Bull. 30,1183,1982.
[84]. Abe, F. and Yamauchi, T., Chem. Pharm. Bull. 31, 1199. 1983.
[85]. Debusschere, L., Demesmay, C., Rocca, J.L., Lachatre, G. and Lofti, H., J. Chromatogr. 779(1-2), 227, 1997.
[86]. Voss, W. and Wachs, W., Ann. 522, 240, 1936.
[87]. Voss, W. and Vogt, G., Ber. 69, 2333, 1936.
[88]. Mannich, C. and Siewert, G., Ber, 737, 1942.
[89]. Von Euw, J. and Reichstein, T., Helv. Chim. Ata 33, 687, 1952.
[90]. Rosselet, J.P. and Hunger, A., Helv. Chim. Achta 34, 1036, 1951.
[91]. Kiliani, H., Ber. 63. 2866, 1930.
[92]. Rheiner, A. .hunger, A. and Reichstein, T., Helv. Chim. Acta 35, 687, 1952,
[93]. Rangaswami, S. and Reichstien, T. Helv.Chim. Acta32, 939, 1949
[94]. Miller, L.P., Phytochemistry, The process and products of Photosynthesis 1, Van Nstrand Reinhold Company, USA, 1973.
[95]. Stoll, A., Pereira, A. and Renz, J., Helv. Chem. Acta 32, 293, 1949.
[96]. Tschesche, R., Sellhom, K. and Brathge, K.H., Ber. 84, 576, 1951.
[97]. Huber, H., Blindebacher, F, Mohr, K., Speiser, P. and Reichstein, T., Helv. Chim. Acta 34, 46, 1951.
[98]. Miyatake, K., Okano, A., Hoji, K. and Miki, T., Pharm. Bull. (Tokyo) 5,157,1957
[99]. Stoll, A. Renz, J. and Brack, A., Helv. Chim. Acta 34, 2301, 1951.
[100]. Baljet, H., Pharm. Weekbl. 55, 457, 1918.
[101]. Raymond, W.P., Analyst. 63, 457, 1918.
[102]. Kedde, D.L., Pharm. Weekbl.82.741.1947.
[103]. Rabitzsch, G. and Tambbor, U., Pharmazie 24, 262, 1969.
[104]. Kovar, K.A., Frankas, G. and Seidel, R., Arch. Pharm. 310, 40, 1977.
[105]. Bums, L.b., Stedmann, R.J. and Tuckerman, M.H., J. Pharm. Sci. 66, 753, 1977.
[106]. British, Pharmacopoeia Vol. 1, 1998.
[107]. Stoll, A. and Junker, E., Phytosterin. Steroidsaponine and Herzglykoside. In: Methoden der Pflanzenanalyse III. (Ed: Peach and Tracey), Springer S. 216, 1955.
[108]. Rowson, J.M. and Dyer, F.J., J. Pharm. Pharmacol. 4, 831, 1952.
[109]. Pesez, M., Ann. Pharm. Fr. 10, 104, 1952.
[110]. Pötter, H., Pharmazie 20, 737, 1965.
[111]. Mesnard, P. and Devant, G., Compt. Rend. 253, 497, 1961.
[112]. Brown, P., Brüschweiler, F. and Pettit, G.R., Helv. Chim. ACTA 55, 531, 1972.
[113]. Fayez, M.B.E. and Negm, S.A.R., J. Pharm. Sci. 61, 765, 1972.
[114]. Flaskamp, E. and Budzikiewicz, H., Biomed. Mass Spectrom. 4, 354, 1977.
[115]. Isobe, R., Maetani, I., Komori, T., Kawasaki, T., Abe, F. and Yamauchi, T., Koenshu- Iyo Masu Kenkyukai 8, 219, 1983; C.A. 100: 109020r, 1984.
[116]. Isobe, R., komori, T., Abe, F. and Yamauchi, T., Biomed. Enviroa. MassSpectrom.13,585, 1986; C.A. 107: 59351W,1987.
[117]. Tori, K., Ishii, H., Wolkowski, Z.W., Chachaty, C., Sangarè, M., Piriou, F. and Lukacs, G., Tetrahedron Letter 13,1077,1973.
[118]. Tori, K., Thang, T.T., Sangare, M.and Lukacs, G., Tetrahedron Letter 8,717,1966.
[119]. Robien, W., Kopp, B., Schabl, D. and Schwartz, H. Prog. Nucl. Mag. Reson, Spectrosc. 19,131,1987; C.A. 107: 217906n, 1987.
[120]. Siddiqui, S., Hafeez, F., Begum, S. and Siddiqui, B.S., Phytochemistry 26,237, 1987.
[121]. Hanada, R., Abe, F. and Yamauchi, T., Phytochemistry 31, 3183, 1992.
[122]. Krenn, L., Kopp, B., Deim, A., Robien , W. and Kubelka, W., Planata Med. 60,63, 1994.
[123]. Massiot, G. and Lavoud, C., Structural Elucidation of Saponin. Attaur-Rahman (Ed), " Studies in Ntural products Chemistry", 15, p187, 1995.
[124]. YAO, M., Zhang, H., Chong, S., Zhu, M. and Morrison, R.A., J. Pharm. Biomed. Analysis 32 (6), 1189, 2003.
[125]. El-Armouche, A., Jaeckel, E., Boheler, K.R., Boknik, P., Hertle, B., Neumann, J. and Eschenhagen, T., Biochem. Biophys. Res. Communi. 318 (1), 219, 2004.
[126]. Verdonck, F., Volders, P.G.A., VOS, M.A. and Sipido, K.R., J. Molecular and Cellular Cardiology 35 (1), 5, 2003.
[127]. Aszalos, A., Antiitumor Compounds of Natural Origin: Chemistry and Biochemistry 1,CRC Press, Inc. Boca Raton, Florida, 1981.

[128]. Haux, J., Med. Hypotheses 53 (6), 543, 1999.
[129]. Smith, J.A., Madden, T., Vijjeswarapu, M. and Newman, R.A., Bichem. Pharm. 62 (4), 469, 2001.
[130]. Nasu, S., Milas, L., Kawabe, S., Raju, U. and Newman, R.A., Cancer Letter 185 (2), 145, 2002.
[131]. Huang, Y.T., Chueh, S.C., Teng, C.M. and Guh, J.H., Biochem. Pharm. 67 (4), 727, 2004.