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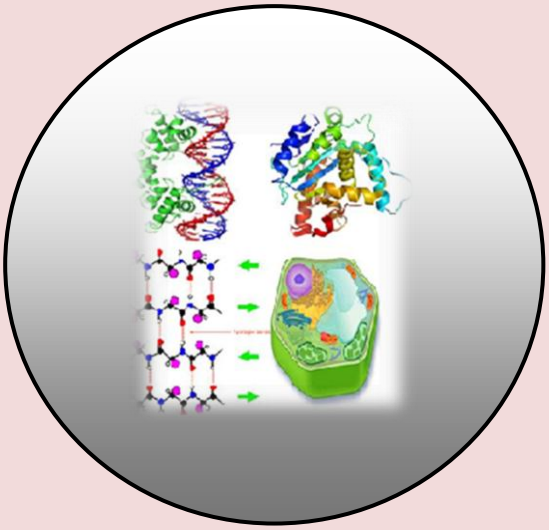

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Cardenolides and their Precursors: A Review

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ABSTRACT

Cardenolides are a fascinating series of steroids which are present in the plants of Asclepideaceae and Apocyanaceae family. They are used in vital blood pumping mechanism, is a medicine, they have C-23 carbon skeleton with usual perhydrocyclopentanophananthrene moiety with α, β-unsaturated-γ-lactone ring attached to it. They are found in plants as aglycone as well as their glycosides. The biogenetic precursor of these cardenolides are pregnane and nor pregnane. They are known for their anti-tumor, anti-inflammatory, cytotoxic and other activities. In the present review article, we have discussed the occurrence of cardenolides along with their precursors, pregnane and nor pregnane with their structure elucidation with the help of ^{1}H, ^{13}C NMR, 2D (COSY, TOCSY, HSQC and HMBC) and Mass spectrometry.

Keyword: Cardenolides, pregnanes, nor pregnane, ^{1}H, ^{13}C, 2D NMR and Mass spectrometry.

INTRODUCTION

Inspite of significant discoveries and use of synthetic drugs, natural products have continued to be an important segment of modern drugs in clinical use. The remarkable ability of plants to produce a wide array of diverse metabolites varying in chemical complexity and biological activity still continues to retain their biological significance as an important source of novel compounds useful directly as medicinal agents, as model compounds for synthetic and semi-synthetic structure modification and optimization and as biochemical and/or pharmacological probes. Therefore, in addition to search for synthetic drugs, medicinal plants which constitute an indispensible source of drugs, both preventive and curative, have been explored continuously. The chemical investigation and purification of extracts of plants purported (Handa et al., 2008) to have medicinal properties has produced numerous purified compounds like taxol (paclitaxel) derived from the relatively scarce Pacific and

Western Yew tree, *Taxus brevefolia* Nutt, used for the treatment of refractory ovarian cancer (Grogory et al., 1993). The Vinca alkaloids: Vinblastine and Vincristine isolated from *Catharanthus roseus* (L) are used as anticancer agents (Romano, 2013). *Digitalis purpurea* L. and *Digitalis lanata* have yielded the cardiotonic glycosides digoxin and digitoxin (Mbaveng et al., 2014). The Chinese antimalarial drug artimisnin has been isolated from the plant *Artemisia annua* L (Pamela et al., 2011). Physostigmine and pilocarpine isolated from *Physostigmine venenosum* (Bin Zhao et al., 2004) and *Pilocarpus jaborandi*s (Ilka et al., 2005) respectively act as parasympatomimetics. d-Tubocurarine derived from *Strychnos toxifera* (Bowman W C., 2006) is used as a skeletal muscle relaxant. These and many other examples (Balandrin et al., 1993) serve to illustrate the continuing value of plant derived secondary metabolites as viable compounds of modern drug product development. Some examples (Balandrin et al., 1993) emphasizing the continuing importance of plant derived secondary metabolites as model compounds include galegine an active principle of *Galega officinalis* L. which led to the development of metformin, a close relative of galegine which is used as an anti-diabetic drug. The study of synthetic analogs of khellin derived from the fruits of *Ammi visnaga* (L) *Lam* and formerly marketed as a bronchodialator and coronary medication led to the preparation and development of Cromolyn, now used as a major drug, used as a bronchodialator and for its antiallergenic properties. Related synthetic studies on amiodarone which was originally introduced as a coronary vasodilator for angina, was subsequently found to have a more useful application in the treatment of a specific type of arrhythmia, the Wolff-Parkinson-white Syndrome and for arrhythemias resistant to other drugs. Another class of medicinally efficacious compounds the steroidal compounds i.e. the cardiac glycosides (Deepak D. et al., 1996; (Deepak D. et al., 1997) and their precursors pregnanes (Deepak D. et al., 1989) and nor pregnanes (Pandey et al., 2022) which play important role in biological processes relating to cardiology, immunology, virology, cancer, antibiotic action, several other activities and a host of life threatening diseases (Petra. et al., 2020; Mai etal., 2020; Agrawal et al., 2021). These biologically active glycosides are abundantly found in plants of family Asclepeadiaceae and Apocyanaceae.

BIOSYNTHETIC PATHWAY OF CARDENOLIDES

The cardiac glythe cosides are present in more than ten dozen plant species belonging to ten different plant families and have been reported to be isolated, quite often simultaneously along with the pregnane glycosides which show similar biological activities as well as structural resemblence with them from plants like *Apocynamum cannabinum, Nerium oleander, Thevatia nerifolia, Asclepias fruticosa* belonging to the plant families like Asclepiadeaceae and Apocyanaceae. It is interesting to note that the nor derivatives of both the cardiac and pregnane glycosides have also been isolated from the same plant generas like Thevatia and Asclepias time and again. It was the co-existence of these glycosides that lead to a lot of speculation regarding the biogenesis of these compounds` and their corelation with each other in the biosynthetic pathway.

The biosynthetic pathway has now been well established (Herbert R. B., 1994) as under:

Acetic acid → mevalonic acid → isopentenylpyrophosphate → squalene-2,3-oxide → lanosterol-cholestrol → pregnenolone

This pregnenolone is known to be the biological precursor to the cardenolides like digitoxigenin and plant derived bufadienolides e.g. hellebrigenin (Tschesche et al., 1964; Tschesche et al., 1965).

The conversion of pregnenolone into digitoxigenin requires the inclusion of an acetate group (Caspi et al., 1968), whereas in the biogenesis of scilliroside, the α-pyrone ring is formed by the condensation of a pregnane derivative with one molecule of oxaloacetic acid Galagovsky et al., 1982). The pregnane route for the biosynthesis of cardenolides' (Deepak D. et al., 1996; Singh et al., 1970) has also been suggested by the isolation of a pregnane glycoside from *Mandevilla pentlandiana* (Cabrera et al., 1993) having a 21-O-methoxy-20-one C-17 side chain which is biogenetically related to 3β,14β,21-trihydroxy-5β-pregnane-20-one This 21-O- methylated compound is thought to be the storage form of 21-hydroxy-20-keto pregnane derivative. The position of the nor pregnanes in this biosynthetic pathway is still not clear and thus work is still being done to establish their status. However, workers like N. T. A. Chung, C. J. Nelson and T.R. Watson., 1989; have suggested that the nor compounds may be the artifacts of the normal compounds The extract of the plant *Hoya longifolia* belonging to family Asclepiadeaceae which was taken up by us for investigation has produced novel nor pregnanes along with pregnane and cardiac genins.

BIOLOGICAL ACTIVITY OF CARDIAC GLYCOSIDES AND THEIR PRECURSORS

Cardiac glycosides comprise of one of the most valuable group of therapeutic agents which are considered unique in being the only inotropic drugs clinically used to improve the myocardial contractility in the treatment of congestive heart failure. These increase 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 intercellular Na in exchange for extracellular K, resulting in an enhancement in the amount of free Ca^{2+} ions inside the cell. The Na^{+}' pump of animal cells generates and maintains the Na^+ and K^+ gradients across the cell membrane. These ion gradients are essential for the electrical excitability of the plasma membrane. In addition, the Na gradient serves as an energy source for the trans-membrane transport of specific substances, e.g. sugar and amino-acid import and Ca^{2+} transport out of the resting cell. Cardio active glycosides inhibit the Na^+ pump, thereby raising the intracellular Na concentration. The resulting decrease in the Na^+ gradient across the cell membrane of cardiac myocytes reduces the energy available for transport of Ca^{2+}out of the cell by Na^+/ Ca^{2+} exchange. This ultimately leads to the (medicinally used) positive inotropic effect of the cardiac glycosides (Hilton et al., 1996).

CARDENOLIDES

14-O-Acetyl acovenoside C obtained from *Acokenthera* spectabilis (fam Apocyanaceae) increased the intraventicular left pressure significantly and dp/dt about 15% (Pieri et al., 1992). It also increased the myocardial contractile force by 20% Three cardenolide glycosides, nerifolin, 3'-O-methyl evomonoside and 2'-acetyl nerifolin isolated from *Thevetia ahonia* (fam Apocyanaceae) exhibited a distinctive pattern of differential cytotoxicity in the National Cancer Institute's human disease oriented 60-cell line tumor screening panel (Decosterd et al., 1994). Three cardenolide, acovenosigenin A 3-O-α-L-ramnopyranoside, euonymoside A and euonymusoside A(I) isolated from the woods of *Euonymus alata* (fam Celastraceae) showed potent cytotoxicity against some neoplastic cell lines (Kitanaka et al., 1996). Two cardiac glycosides Asperoside I and Strebloside II obtained from stem bark of *Streblus asper* showed significant macrofilaricidal activity against *Litomosoides carin*ii and *Burgia malayi*in rodents (Chaterjee, 1992). The bark of *Euonymus sieboldianues* yielded Euonymoside A which was found to be cytotoxic (Back et al., 1994).

Three cardenolide glycosides isolated from wood of *Euonymus alata* were found to be antitumor agents. Five known cardenolides isolated from the stems of *Beaumontia brevitubals* were found to have cytotoxic activity against human and murine cancer cell lines (Kaneda et. al., 1992). The MeOH extract of the seeds of *Corchorus olitorius* L. (Morohoeiya) led to the isolation of Corchurososides A, B, C, D and E along with six known cardenolides which were found to show inhibitory effect against Na^+, K^+-ATPase and positive inotropic activity in the guinea pig isolated atria (Yoshikawa et. al., 1998). Erysimoside, Olitoriside, Corchoroside A and Helveticoside obtained from MeOH extract of *Corchous olitorius* seeds showed acute oral toxicity in male mice (Yoshikawa et al., 1998). Four cardenolides isolated from *Nerium oleander* isolated by bioactivity directed fractionation of the MeOH extract showed the central nervous system (CNS) depressent activity in mice (Siddiqui et. al., 1997). Cardenolides, Strophathidin, Erysimin, Korchoroside A, Olitoriside and Erysimoside isolated from top and seeds of *Syrenia sessiflora* and two glycosides isolated from *Erysimum* linifolium (Deepak et al., 1996) were found to possess high cardiotonic activity. Gluconerigoside obtained from leaves of *Nerium* was found to exhibit cardiotonic as well as diuretic activity. *Euonymus alata* yielded three cardiac glycosides of acovenosigenin which showed antitumor activity against mouse leukemia cells and human leukemia cells and human lung cancer cells. Strebloside and Mansonin isolated from *Streblus asper* (Chaterjee et al., 1992) and some glycosides from Asclepiadaceae including Calatropin have been reported to be cytotoxic (Coombi et. al., 1964; Kupchan et. al., 1964). Ghalakinoside from *Pergularia tomentosa* showed strong cytotoxic activity against KB cells 3-O-Methyl Evomonoside (Mansour et al., 1988; Jolad et al., 1981) a crystalline substance isolated from the ether extract of *Thevetia ahonia* exhibited strong cytotoxic activity against the human epidermoid carcinoma of nasopharynx (KB) test system (Geran et. al., 1972). Cymarin, Strophanthidin and a Strophanthidin glycoside from *Paraquentina nigrescences* were also found to be cytotoxic but inactive in the P-388 lymphocytic leukemia (3PS) system (Marks et al., 1975). Affinoside A, 4,5-dehydro-12-oxo-affinosideE and affinoside M obtained from Anodendron affine exhibited potent growth inhibitory activity against the second larval stage of *Bombyx mori* (Abe et al 1992).

BUFADIENOLIDES: *Kalanchoe* (syn: Bryophyllum) (fam: Crassulaceae) species are used in traditional medicine for the treatment of several ailments such as infections, rheumatism and inflammation and extracts of *Kalanchoe* have immunosuppressive effects (Costa et al., 1996). Daigremontianin and Bersaldegenin 1,3,5-ortho acetate isolated from *Kalanchoe daigremontiana* (fam: Crassulaceae) not only exhibited the expected strong positive inotropic effect, but also had a sedative effect at small doses and an effect on the central nervous system at higher doses Wagner et al., 1986). A cytotoxic compound, Kalanchoside was isolated from *K. Tomentosa* (Kasonaino et. al., 1993), an ornamental plant from Madagascar. The root bark of medicinal plant *Bersama abyssinica* (fam: Melianthaceae) was reported to yield four bufadienolides, abyssinin, abyssinol A, abyssinol B and abyssinol C which exhibited strong antifeedant effect on the cotton pest insect *Heliothis zea*, but also showed antibacterial activity against *Bacillus subtilis* (Kubo et al., 1984; Kubo et al., 1985; Tuniguchi et al., 1993). Three bufadienolides, 19- norbufalin (Lichtstein., 1993) ($C_{18}H_{32}O_4$), an isomer of Ouabin (Tymiak et al., 1993) and a dihydropyrone, substituted steroid, isolated from mammals were found to be inhibitors of Na^+/K^+-ATPase and were also found to be associated with hypertension and cataract formation (Hilton et al., 1996).

PREGNANES AND NOR PREGNANES

Pregnane ester glycosides closely resemble the medicinally important class of compounds cardiac glycosides Pregnane glycosides isolated from different plant families activity have been found to posses varied biological activities like antitumor activity (Reichstein, T., 1967, Deepak et al., 1989; Yoshimura et al., 1983; Hayashi et al., 1969; Itokawa et al., 1988), against Ehrlich ascites carcinoma (Hayashi et al., 1980; Hayashi et al., 1981), anticarcinogenic activity, cytotoxic activity (Duh et al., 1987; Sethi et al., 2007; Luo et al., 1993), anticomplementary activity (Oshima et al., 1987), antifertility activity (Alicija et al., 2017; Yuan et al., 1991; Yuan et al., 1992) etc. Amongst the more recently isolated pregnanes, two pregnane glycosides auriculosides A and B isolated from the roots of *Cynanchum auriculatum* showed cytotoxic activities against PC, HCI-8693, Hela and PAA cell lines (Zhang et al, 2000). Four pregnane glycosides isolated from *Cynanchum wilfordi* (fam: Asclepiadeacae) enhanced activity of Vinblastine- cytotoxicity in multidrug resistant KB-V1 cells (Bang, et al., 1999). Hypoglaucin G and another known pregnane glycoside isolated by bioactivity-guided fractionation from EtOH extract of *Dioscorea collettii* var. *hypoglauca* induced morphological deformation of *Pyricularia oryzae mycelia*[79]. Further the other enroute precursor i.e., nor pregnanes resemble in their biological activities with the cardenolides. Nor pregnanes possess digitalis like effect as shown by the cardenolides and are found to bind strongly to the steroid recognition sites on the cardiac glycosides receptors[80] Na^+, K^+-ATPase (Templeton et al., 1992; Templeton et al., 1993). The C-3 glycosides apart from having cardiotonic activity also exert certain potentially useful effect on heart and kidney not shared by the digitalis drugs (Templeton et al., 1988). They are also involved in regulation of vascular tone and have shown significant anabolic potency (Melero et al., 2000). The 19-nor pregna-1,3,5(10),20- tetra-ene-3-O-a-fucopyranoside isolated from Thai Soft Coral, *Scleronephthya pallida* was seen to exhibit moderate antimalarial and cytotoxic activities (Woolfson et al., 1992).

BASIC SKELETON OF CARDIAC GLYCOSIDES AND THEIR PRECURSORS

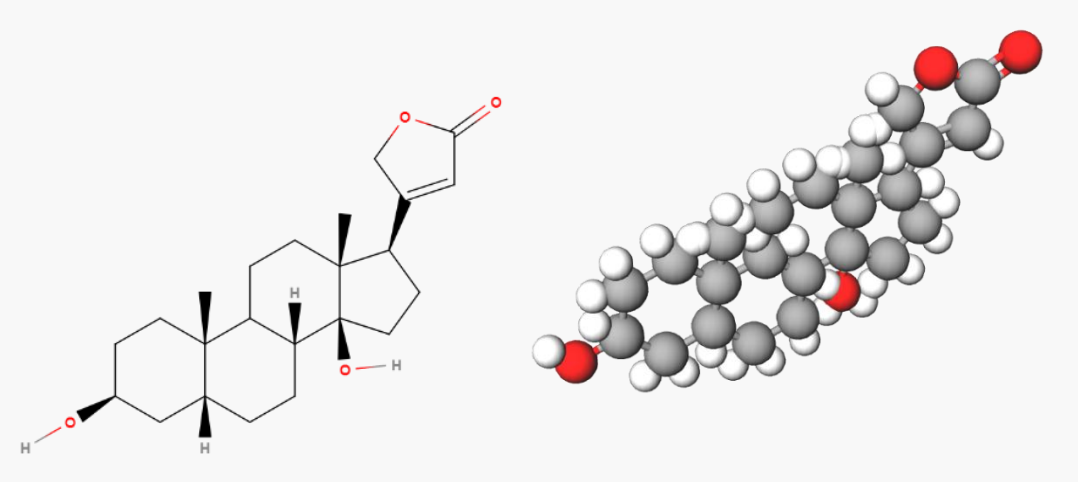
These are steroidal compounds containing a perhydrocyclopentanophenanthrene nucleus. Presence of a lactone ring at C-17 position differentiates a cardiac glycoside from a pregnane glycoside which possesses a two carbon side chain at C-17 position. Both these glycosides contain β-oriented angular methyl groups at C-10 and C-13 positions and a hydroxyl group at C-3 which in majority of the cases is β-oriented. A β-oriented hydroxyl group at C-14 position is always present in cardiac glycosides which may or may not be present in pregnane glycosides. These glycosides may occur on other secondary hydroxyl groups of pregnanes and cardenolides. The sugar moiety is linked to an alcoholic hydroxyl group of the steroidal moiety through an acetal linkage most frequently at C-3 in both the glycosides. The nature of the aglycone part in the cardiac glycosides leads to their classification into two different groups viz Cardenolides and Bufadienolides. The aglycone containing 23-C atoms with a five membered α, β unsaturated γ-lactone ring at C-17 is termed as cardenolide whereas an aglycone with 24-C atoms and a six membered doubly unsaturated lactone ring (α-pyrone) attached to C-17 is called a bufadienolide (Deepak et al., 1997).

STRUCTURAL FEATURES OF CARDIAC GLYCOSIDES

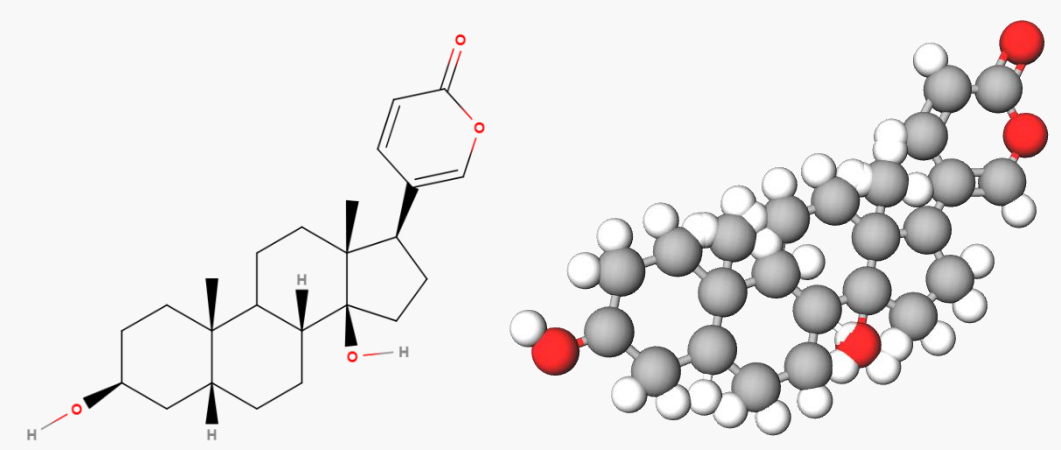
Some characteristic features of Cardenolides and Bufadienolidegenins are as follows-

- Substituent at C-3, C-5 and C-17 may have α or β orientation.
- Double bonds may be present at C-4, C-5 and C-16.
- Hydroxyl groups may be present in the 1β, 2α, 3β, 5β, 11α, 11β, 12β(α), 15β, 16βand 19positions. In certain cases, these hydroxyl groups may be esterified.
- Carbonyl functions are found at C-11, C-12 or C-19.
- 7-8β, 8-14β and 11-12β position may have epoxy groups.

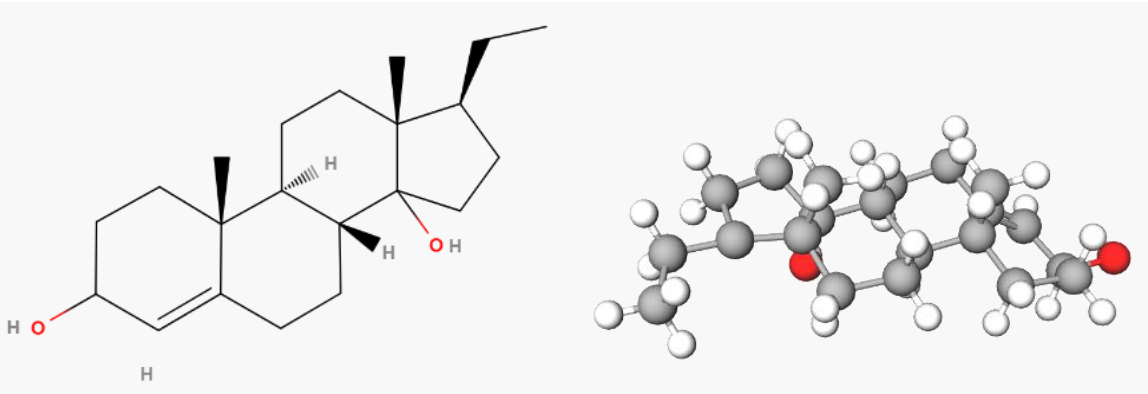
Oxidation of the angular methyl group at C-10 position to an aldehyde, a hydroxy methyl or a carboxylic acid group has been reported in certain cases. Sometimes oxygen functions at positions other than mentioned earlier have also been reported. Occasionally, epimerization may take place at C-3, C-5 and C-17 positions. In the presence of alkali, an internal rearrangement takes place leading to the formation of a C14-21 oxide called isogenin due to reaction between 14 β-hydroxyl group and the β-oriented lactone ring at C-17 position. In presence of acids, the labile hydroxyl group at C-14 yields the corresponding anhydrogenin viz: 14, 16 dianhydrogitoxigenin (Fig-1).



Cardnolides



Bufadienolides



Pregnane

Figure 1.

STRUCTURAL FEATURES AFFECTING THE BIOLOGICAL ACTIVITY OF CARDIAC GLYCOSIDES:

Cardiac glycosides constitute one of the most interesting medicinally efficacious groups of naturally occurring compounds that influence the vital blood pumping mechanism. The pharmacological qualities of these cardiac glycosides depend upon their structures. It is known that most of activity resides in the lactone ring and the sugar moieties enhance their activity by modifying the water and lipid solubility of the glycosides and thus affecting their potency and duration of action. When a chain of sugar is attached to the genin the sugar that is directly linked to the genin enhances the activity (Fig 2).

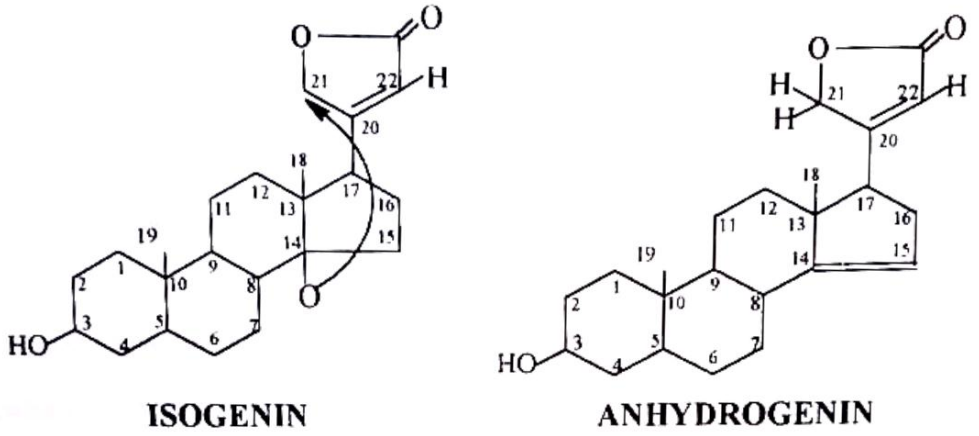


Figure 2.

STRUCTURAL FEATURES OF CARDENOLIDES PRECURSSORS

Factors necessary for optimal activity (Kittakoop et al., 1999)

- Presence of a β-oriented α,β-unsaturated lactone ring at C-17
- Cis fusion of ring A and B
- Trans fusion of ring B and C
- Cis fusion of ring C and D
- Presence of a 14 β-OH group
- Presence of a sugar at β-oriented OH group on C-3

Factors leading to decrease in activity (Stoll et al., 1941; Zelnik et al., 1957; Woolfson et al., 1992).

- Presence of extra OH group on aglycone moiety.
- Trans fusion of ring A/B leads to change in configuration at C-5 and hence reduce activity.
- Trans fusion of ring C/D leads to decrease in activity. 14α compoundss (14-epiditoxigenin) are inactive in contrast to their 14β- analoges.
- Reduction of the double bond between position 20 and 22 of the lactone ring leads to loss of activity.

PREGNANES

Some characteristic features of pregnane genins are as follows:

- Unsaturation at C-5 (Δ^5).
- Rings B and C always have a trans fusion.
- Presence of OH group at C-14 leads to Cis fusion of ring C and D whereas presence of H at C-14 leads to trans fusion between ring C and D.
- The two carbon side chain at C-17 may either be a keto methyl group or a hydroxy ethyl group which may have α or β-orientation.
- Presence of extra OH groups at 5α, 6β, 7α, 8β, 11α, 12α or 12β, 14β, 15α, 16α, 16α, 17β, 20 and 21 which in some cases may be esterified partially.
- Presence of carbonyl functions at C-1, C-12, C-15 and C-20
- The sugar moiety may be linked to OH group at C-3 or C-20 both (bisdesmosidic glycosides) through an acetal linkage (Zurcher et al., 1994; Palter et al., 1972; Abe et al., 1989).
- In some cases the sugar moiety is linked to hydroxyl functions at C-2, C-4 or C-21 (Abe et al., 1989; Nakanishi et al., 1988; Steyn et al., 1998).

NOR PREGNANES

The nor pregnanes are also steroidal compounds having the same basic perhydrocyclopentanophenanthrene nucleus along with substituent at C-17 position as in the pregnane. The only difference is the absence of one or more carbon atoms from the usual C-21 skeleton ie. when the angular methyl group at C-10 or C-13 is absent then the pregnane termed as a 19-nor or 18-nor pregnane respectively viz. 19-nor pregna-1,3,5 (10), 20-tetraen-3-O-α-fucopyranoside obtaines from the Thai Soft Coral, *Scleronephthya pallida* lacks the angular methyl group at C-10 (Woolfson et al., 1992). Neomarinoside is a pregnane glycoside with a B-nor (7)-6β-formyl-pregnane skeleton (Abe et al., 1992).

STRUCTURE ELUCIDATION OF CARDIAC AND PREGNANE GLYCOSIDES

Conventional methods used for the structure elucidation of cardiac and pregnane glycosides involved acid hydrolysis followed by identification of aglycon and sugar separately (Srivastav et al., 1994), whereas UV technique was used to determine the site of glycosidation. But in recent years advent of non-destructive physiochemical techniques such NMR (^{1}H, ^{13}C and 2D) spectroscopy along with the mass spectrometry has provided new dimensions to the structure elucidation of these compounds which are described as under:

NMR SPECTROSCOPY

Nuclear magnetic resonance is the resonance absorption of electromagnetic radiation by a substance when it is placed in a strong magnetic field caused by the magnetic properties of the nuclei.

Thus nuclear magnetic resonance deals with the interaction between the magnetic moments of atomic nuclei and magnetic fields. NMR along with several other physical techniques is being applied for the conformation of structure and is common practice in studies related to the structures of natural products. Exact knowledge of the structure, stereochemistry, dynamics, and reactions of molecules provides the key to understand their functions and properties. NMR spectroscopy by the combination of 1D and 2D techniques has developed into the most important method for the investigation of these questions and especially the structures of steroids, particularly for their stereochemical problems.

ONE DIMENSIONAL NMR SPECTROSCOPY

^{1}H NMR SPECTROSCOPY

^{1}H NMR spectroscopy especially at high fields (400-600 MHz) provides useful information regarding the glycon as well as the aglycon moiety of cardiac as well as pregnane glycosides. In case of normal sugars, the anomeric proton appears as a double in the region of δ 4.6-5.2 J value (ie coupling constant) which depends upon the orientation of the H-1 and H-2 protons ie. when H-1 and H-2 are trans to each other than the coupling constant has a large value (8-10 Hz) whereas when H-1 and H-2 are cis to each other than the J value is small (2-4 Hz) (Srivastav et al., 1994; Shubiya et al., 1992). The protons at C-3, C-4, C-5 and protons of the CH_2OH group in normal hexoses appear as multiplets between δ 3.5-4.5. The 6-deoxy hexoses which differ from the normal hexoses in having a secondary methyl group in place of a CH_2OH consist of a doublet for 6'-CH_3 between δ 1-1.5 in their ^{1}H NMR. Rest of the spectra is similar to that of the normal hexoses. In the case of the 2- deoxy and 2,6-dideoxy sugars the anomeric proton appears as a double doublet's (Berger et al., 1988; Abe et al 1988) more or less the same region as that of the normal sugars and sometimes as a triplet (Deepak et al., 1985) if $J_{1,2(a)}=J_{1,2(b)}$ depending upon the nature of glycosidic linkage. Presence of these deoxy in sugars in the $^{4}C_1$ conformation having an axial anomeric proton and a β-glycosidic linkage is indicated by larger coupling constants in the region of 7-10 and 1-2 Hz (Allegeer et al., 1968), whereas presence of these sugars in the $^{1}C_4$ conformation with equatorial anomeric proton and α-glycosidic linkage is indicated by small J values viz:3-4 and 1Hz (Sethi et al., 1988). The equitorial and axial protons at C-2 in these 2-deoxy and 2,6-dideoxy sugars appear as two sets of multiplets in the upfield region ie at δ 2.0-2.5 and 1.5-2.0 respectively (Shubiya et al., 1992), whereas the secondary methyl group (6'-CH_3) of 2,6-dideoxy hexoses appears as a doublet between δ 1.0-1.5 with J-6Hz (Abe et al., 1989). The protons at C-3, C-4 and C-5 in the 2-deoxy and 2,6-dideoxy sugars appear in the same region as in the normal hexoses.

Characteristic signals of the perhydrocyclopentanophenantherene nucleus present in both the cardiac glycosides as well as in the pregnane precursors appear as singlets for three protons in the region of δ 0.7-1.2 for the two angular methyl groups present at C-10 and C-13 (Shubiya et al., 1992), while the methylene protons appear as multiplets in the region of δ1.5 -2.0 (Uzawa et al., 1979) and methine protons under a hydroxyl group appear as multiplets in the region of δ 3-4. The methine proton at C-11 under a hydroxyl function appears as a triplet in the region of δ 3.2-4.6 (Trivedi et al., 1989) or as a double doublet in the same region if a hydroxyl group is present at C-12, whereas methine proton at C-12 appears as a doublet or a quartet (Trivedi et al., 1990; Prakash et al., 1991) in the same region depending on the presence or absence of substituents on the neighbouring carbon.

The vinylic proton of a double bond appears as a multiplet in the region of δ 5.3-5.6. The only difference in these compounds lies in their substituents present at C-17. These differences are clearly evident in their ^{1}H NMR spectra, which helps in the differentiation of these compounds. The cardenolide which differ from the bufadienolide in having a five membered lactone ring rather than a six membered lactone ring gives characteristic signals of the γ-lactone ring as two double doublets or two doublets (Trujillo et al., 1990; Junior et al., 1980; Steyn et al., 1986) for H-21 between δ 4.7-5.1 and a single resonance between δ 5.8-6.0 for H-22 (Slothers J. B., 1972). Whereas in the case of bufadienolides H-21 appears as a doublet and H-22 as a double doublet between δ 7.2-8.0 and H-23 appears as a doublet in the region of δ 6.0-6.5 (Bose et al., 1975). The $CH(OH)CH_3$ or $COCH_3$ side chain present at C-17, which differentiates the pregnane glycosides from the cardiac glycosides, appears as a three proton doublet at 81.0-1.5 or a three proton singlet at δ 2.1 respectively for the C-21 protons, while the methine proton at C-20 appears in the region of δ 3.5-4.5 as a quartet if a OH group is present at C-17 or a multiplet if a H is present at C-17. However, in the case of nor compounds the signal for the group missing is absent i.e. in the case of 18-nor or 19-nor compounds one of the angular methyl group signal is absent. Esterification of the hydroxyl functions leads to a downfield shift in the signal of the corresponding methine proton by 0.6-1 ppm in comparison to its precursor. The number of acetyl group peaks in the region δ 2.1-2.3 of the acetylated compounds (Berger et al., 1988) establishes the number of primary and secondary hydroxyl groups on them. On the other hand, number of the tertiary hydroxyl groups can be deduced by D_2O exchange or by the trichloroacetyl isocyanate (TAI) reagent[116]. Protecting groups in these glycosides are commonly found to be present as esters of benzoic, cinnamic, isovaleric, tiglic, nicotinic, 2-methyl butanoic, β,β-dimethyl acrylic (ikemic) (Qiduan et al., 1988; Sunrmons et al., 1972; Singhal et al., 1980) or acetic acids which give characterstic signals in the 'H NMR spectra. The decoupling experiment (Agarwal P. K., 1992) provides an excellent mode for confirming the position of particular protons giving rise to a particular signal. Upon irradiation of the anomeric proton of a 2-deoxy or a 2,6-dideoxy sugar the double doublet signal for the anomeric proton is completely eliminated from the spectra and the multiplet for the methylene group at C-2 is simplified. Similarly, on irradiation of the secondary methyl group at C-6 the corresponding spectra is seen to be devoid of the doublet for the secondary methyl group and the proton at C-5 appears as a doublet instead of a multiplet in the region of δ 3.5- 4.5, thereby assisting in the distinguation of the methine proton at C-5 from the methine protons at C-3 and C- 4 which also appear as multiplets in the same region of δ 3.5-4.5. Likewise, the position of the secondary methyl signal in the -$CHOH$-CH_3 side chain may be ascertained by irradiating the methine proton at C-20 which results in the collapse of the secondary methyl doublet in the region of δ 1.0-1.5 to a singlet. This process is called decoupling experiments.

^{13}C NMR SPECTROSCOPY

The ^{13}C NMR of cardiac and pregnane glycosides is found to be considerably more useful, because of the enormous sensitivity of the ^{13}C chemical shifts to the structural changes and because each carbon atom of the skeleton and any attached groups can usually be examined individually. The ^{13}C NMR spectra which are complementary to ^{1}H NMR spectra due to greater chemical shift dispersion and lack of complexities arising from spin-spin coupling and overlap of resonance is considered to be more useful than ^{1}H NMR spectra in the structure elucidation of these steroidal glycosides.

^{13}C NMR spectrum not only helps to assign the number, sequence and linkage of the sugars but also helps to identify the sugar(s) on the basis of the chemical shift of the anomeric carbon(s). The anomeric carbons are found to appear between δ 89- 112 in the ^{13}C NMR spectra of cardiac and pregnane glycosides (Agarwal P. K., 1992; Zhang et al., 1988). By counting the number of signals in this region the number of monosaccharide units can be known. The nature of the glycosidic linkage can be established by the ^{13}C NMR spectra because the signals due to a β-linkage appear 2-6ppm downfield from their α-counterparts. The secondary methyl groups of the 6-deoxy hexoses appear in the region of δ 16-19 whereas the CH_3OH groups of normal and deoxy hexoses appear between δ 60-63.5.153(Agarwal P. K., 1992). The methoxy functions and the ring carbons appear in the region of δ 55-62 and δ 65- 85 respectively. ^{13}C NMR also helps to differentiate between furanoses and pyranoses because the C-1, C-2 and C-4 of furanoses generally appear 4-14 ppm downfield from the chemical shift exhibited by the pyranose isomer (Kitagawa et al., 1992). Important information regarding the nature of the cardiac and the pregnanegenins is also obtained by the ^{13}C NMR spectra. The diagnostically most important signals of the cardenolides appear in the region of δ 170-176 for C-23 and C-20 and between δ 116-118 for C-22 whereas the signals for the bufadienolides appear between δ 120-124 (C-20), δ 148-149 (C-21), δ 147-149(C-22), δ 115-115.5 (C-23) and δ 162-164 (C-24). The C-21 methyl group of the two carbon side chain in pregnane glycosides appears in the region of δ 15.0-24 if side chain is -$CH(OH)CH_3$ or in the region of δ 30- 35 if side chain is a -$COCH_3$ group. The C-20 of -$CH(OH)CH_3$ side chain appears in the region of δ 65-70 whereas C-20 of $COCH_3$ appears between δ 170-176. Some other resonances due to the aglycone moiety that are common to both the types of steroidal glycosides appear in the region of β 7-15.8 for the angular methyl group C-18, while the position of the angular methyl group C-19 varies between β 12-24.5 and depends upon the substituent and its configuration at C-5(Agarwal P. K., 1992; Bock et al., 1983). When the proton at C-5 is a then C- 19 appears at δ 10.8-17.0 and when proton at C-5 is β then C-19 appears at δ 23.7-24.0. If an OH group is present at C-5 then C-19 appears between δ15.0-17.0. However, if there is a double bond between C-5 and C-6 in the aglycone then C-19 appears at δ 15.5-20.0, while if there is a double bond between C-6 and C-7 then C-19 appears at δ14.4-14.7. The quaternary C-5 and vinylic C-6 (when double bond is between C-5 and C-6) resonate in the region of δ 140-144 and ò 117-123 respectively. The methylene and methine protons of the aglycon appear between δ 35-54, if no oxygen is present on these carbons. However, carbons bearing OH groups appear in the region of β 60-90. It has been found that carbons near hydroxyl functions can be readily identified from chemical shift changes attendant to acetylation. Esterification leads to a downfield shift in the ^{13}C NMR, of the corresponding carbon by 0.6-3.5ppm (Zucher et al., 1963). It also leads to an upfield shift of the adjecent carbons by 1.2-4.0 ppm. The carbonyl group of these ester moieties resonate between β 165-171 depending upon the presence or absence of unsaturation in the esters, while the methyl group of the acetates appear between β 20-22, the aromatic carbons of benzoyl group and cinnamoyl residues resonate between β 128-135the vinylic carbons of tigloyl and cinnamoyl groups appear between β 117-145 and the sp^2 hybridized carbon carrying the methyl group of ikemoyl appear in the region of β 160-164. As was the case with esterification, glycosidation also leads to a downfield shift of the corresponding carbon and an upfield shift of the adjacent carbons

there by providing useful information about the point of attachment of sugar chain to the aglycone.

The carbon involved in the glycosidation is seen to undergo a shift to lower field by 3-6 ppm while the adjacent carbons shift to higher field by 0.5-4 ppm (Agarwal P. K., 1992).

Partially Relaxed Fourier Transform (PRFT) measurements in the ^{13}C NMR spectra lead to differences in peak intensities of the inner and terminal sugars thereby providing useful information for the identification of the terminal sugar and the sugar sequence in pregnane and cardiac glycosides (Agarwal et al., 1983). The anomeric carbon of the terminal sugar (in case of diglycoside) appears 2-4 ppm downfield than that of the inner sugar. The sugar sequence in the glycoside can also be ascertained by measuring the spin lattice relaxation time (T_1), as the average NT_1, values for the sugar carbons in each unit increases with increasing distance from the aglycone moiety due to segmental motion in the oligosaccharide chain with the aglycone part exhibiting an anchoring effect. The long-range selective proton decoupling (LSPD) also helps to establish the location of ester functions within the aglycone. This technique has made it possible to correlate protons under ester groups with the corresponding carbonyl carbons, especially when the ester group is attached to C-11 and C-12 of the genin. This technique also helps to identify the chemical shift of the angular methyl carbons at C-10 and C-13 and the site of glycosidation. There is an increase in the intensity of C-19 and C-18 signals due to irradiation of the signals due to H-9 and H-12 respectively, while irradiation of the anomeric proton changes the splitting of the carbon to which it is glycosidically linked.

Single Frequency off Resonance Decoupling (SFORD) helps to differentiate between the primary, secondary and tertiary carbon by reducing CH couplings to such an extent that the largest coupling constants [J (CH)] give rise to residual splittings, thus allowing determination of the number of attached hydrogens. The quaternary carbon gives rise to a singlet, the methine carbon to a doublet, the methylene carbon to a triplet and the methyl carbon to a quartet in the SFORD spectra. Recent techniques like Attached Proton Test (APT), Distortionless Enhancement by Polarization Transfer (DEPT) and Insensitive Nuclei Enhanced by Polarization Transfer (INEPT) also give information about the splitting pattern of carbons. The use of polarization transfer techniques in multiplicity determination is now much more widespread than their use for signal enhancement. This relies on the fact that different multiplicities lead to different dependences of signal enhancement on pulse sequence delays (INEPT) or flip angles (DEPT). Both the original INEPT experiment and the elegant DEPT sequence exploit the regularity of one bond coupling constants to provide "broad band" polarization transfer for all protonated carbons in a spin system; they can also be used for polarization transfer through long range couplings. Irradiation of the anomeric proton selectively enhances the carbon signal of the aglycone to which it is linked and vice-versa in the selective INEPT experiment helping to establish the connectivity between the anomeric proton and carbon atom of the aglycone. The multiplicities of carbon signals of the cardenolide B-anhydroepidigitoxigenin were determined by performing DEPT[102] revised the experiment using last pulse angles (Θ) 45°, 90° and 135°. Structure of pregnane condurangogenins A, B, C, D and E and their glycosides by using the technique of selective proton decoupling ingated decoupled ^{13}C NMR. The results indicated that the acetoxy group was attached to C-11 or 11α-OH and the cinnamate C- 12 which was the reverse of the originally proposed structure.

The terminal thevetopyranosyl signals were easily distinguished from other sugar signals of the pregnane genin Sinomarinoside A because in the ^{13}C NMR spectrum of the compound the thevetopyranosyl group had the longest dipole-dipole relaxation times by PR-FT measurements (Agarwal et al., 1983).

2D-NMR SPECTROSCOPY

Two dimensional magnetic resonance has become a fundamental need for the structure elucidation of natural products. The conventional one dimensional NMR spectra is generated on one frequency axis, thus very often severe problems are encountered in the 1D NMR spectra due to substantial overlap of multiplets which does not generally allow unambiguous assignments of all the signals. These difficulties however can be overcome by the use of two-dimensional techniques developed in recent years since such a 2D spectrum contains signals dispersed according to two characteristic frequencies rather than one and thus spreading apart overlapping signals to facilitate interpretation. The 2D spectra also provide us with important information regarding the inter-nuclear distances and the scalar coupling constants of the molecule. Thus the power of 2D NMR lies in its ability to resolve the overlapping signals to enhance the sensitivity and provides useful information not provided by the 1D spectra.

2D ^{1}H-^{1}H COSY (HOMO CORRELATED SPECTROSCOPY)

COSY is also referred to as homonuclear shift correlation through J coupling (Agarwal et al., 1989; Kessler et al., 1988; Wasylyk et al., 1989) and traces out scalar coupling networks by correlating a spectrum with itself. If two spins A and X are coupled, then as well as signals with frequency co-ordinates around the chemical shifts (δ_A,δ_x,) and (δ_x,8_x), the 2D COSY spectrum will show peaks around (δ_A,δ_x,) and (δ_x,δ_x). The first two signals belong to the class of diagonal peaks since they lie close to the diagonal line f_i=f_2, whereas the latter two are known as cross peaks and indicate the presence of significant scalar couplings between A and X. COSY spectra are now in widespread use, for the structure elucidation of cardiac glycosides and their precursors. Assignment of signals requires an initial point of identification within the individual spin system. In case of sugar moieties this starting point for assignment is provided by the anomeric proton which because of being attached to a carbon bearing two oxygen atoms appears most downfield as compared to all other protons. Therefore, within a typical aldohexopyranosyl ring where the coupling network is unidirectional H-1 is seen to couple with H-2 and H-2 to H-1 and H-3 and so on. In case of the aglycone H- 3 is generally taken as the starting point. The scalar (J) coupling pathways lead from H-3α to H-4α, H-4ß and to H-2α, H-2β and finally to H-1α, H-1β. Moreover, the COSY spectra provide useful information regarding the side chain attached to the perhydrocyclopentanophenanthrene nucleus in the cardiac glycosides and the pregnanes. In the γ-lactone ring of the cardenolides the two geminal protons at C-21are seen to couple with each other and with the C-22 proton in their respective chemical shift regions. In the bufadienolides, H-21 shows a long range coupling to H-22, which then further couples to H-23. The signal for the secondary methyl group of the $CH(OH)CH_3$, side chain in the pregnanes is seen to couple with the proton at C-20 which further couples to H-17 if present. The COSY spectra have been used as a vital tool to confirm the C-21 nor nature of the novel compounds.

The presence of a cross peak between the doublet of the secondary methyl group with a methine proton at C-17 in the up field region which further couples to a methylene proton at C-16 confirmed the presence of one carbon side chain in the compounds instead of the usual 2- carbon side chain. In the case of cardenolide 3'-epi-19nor afroside, the 'H-'H COSY spectra showed connectivity between the methylene protons at H-1 and a methine proton assignable to H-10, indicating that the compound was a 19-nor compound. This method also helps to assign the position of an ester function within the aglycone or the sugar moiety. However, one fundamental limitation of COSY is that couplings must be at least partially resolved before they can give rise to a cross peak.

COSY -45

COSY-45 has two advantages over basic COSY

- This technique makes it possible to identify correlations that would otherwise be hidden in the cluster of peaks close to the diagonal by reducing the intensity of transfer between parallel transitions as a result of reducing cross peaks within multiplets and thereby simplifying the appearance of the spectrum around the diagonal in a complete spectrum.
- This method allows determination of the relative sign of coupling constant in a system with three or more spins, by restricting multiplet transfers largely due to directly connected transitions.

The sugar of Caratuberside A from *Caralluma tuberculata* was identified by Ahmad et al. by making use of the spin couplings in the COSY-45 experiment. Information regarding the sequence of sugars in the glycoside was deduced from long-range (^{1}H-^{1}H) COSY-45 experiment (Adam et al., 1971).

DOUBLE QUANTUM FILTER CO-RELATED SPECTROSCOPY (DOF-COSY)

One of the most important derivatives of the COSY experiment is the use of multiple quantum filteration. The most widely used filteration is through double quantum coherence. The great advantage of double quantum filteration is that it suppresses the strong singlet signals, like from the solvents that can pose such dynamic range problems in COSY spectra. Normally, the only trace left of a solvent peak in a double quantum filtered COSY spectrum is a little t_1 noise. It also suppresses the strong singals emanating from tertiary methyls thereby allowing unambigous assignment of hidden multiplets which are isochronous to tertiary methyls. This method also helps in identifying particular sugar units, by providing characteristic multiplicity within the cross peak and also provides semi quantitative information on the coupling constants of protons involved in cross peaks. All H-H connectivity except those next to the angular methyl groups (Me-18-19) be in the aglycon part of the cardiac and pregnane glycosides can also be determined by DQF COSY.

NUCLEAR OVERHAUSER EFFECT SPECTROSCOPY (NOESY)

The 2D Nuclear Overhauser Effect provides information on the spatial proximity of nuclei, which complements the information on bond connectivities obtainable from the COSY spectra, decoupling experiments etc. The NOE effect depends on the distance between the cross-relaxing nuclei, and hence allows determination of intermolecular interatomic distances. NOE values therefore provide the most important parameter for the determination of three dimensional structures.

In general, 1,3-diaxial and equitorial- axial proton pairs in the pyranosyl rings produce intra NOESY cross peaks ie in case of sugars having a-glucopyranosyl configuration strong cross peak is observed between H- 1 and H-2, whereas in sugars with a B-glucopyranosyl configuration strong cross peaks are observed between H-1 and H-3(H-5). This technique is particularly useful in establishing the sequence of sugars and detemining the site of glycosidic linkages. In a glycoside (G-O-S_1-O-S_2), where the proton on C-1 of S_2 is close enough to proton on C-4 of S_1 (in case of a 1→4 linkage), a cross peak between H-1 of S_2 and H-4 of S_1 is observed making it possible to demonstrate a linkage between the two sugars. This technique also helps to determine the stereochemistry at C-3, C-17 and C-20 of the cardiac as well as the pregnane aglycons (Konda et al., 1992). The stereochemistry at C-3 in 3'-spiro-linked thiazolidinone was assigned as S- and R- in S-oxythiazolidinone derivatives of Δ^5 Calatropin by (DIF)NOE in the two cardenolides isolated by Abe et al., 2000. Selected NOE Difference experiments were used to confirm the configuration of the sugar residues in 19-nor-pregna-1,3,5(10),20- tetraen-3-O-α-fucopyranoside. By the use of NOESY Abe et. al. have suggested that ring D in Thevetioside A a C-nor-D- homo cardenolide exists in a half chair (sofa) conformation, raising C-17. With the help of a Dreiding stereo model, the stereochemistry of Stauntoside A, a pregnane glycoside having an unusual skeleton was determined from the ^{1}H NMR and NOESY spectra. The relative stereochemistry of Helbortin A a 19-nor bufadienolide was determined as having cis, trans,cis type of fusion between rings A/B, B/C and C/D, respectively with 18-methyl group, H-17, H-9 and H-10 hydroxyl groups held trans by the use of NOESY (Summers et al 1986). NOESY experiment on anaesthetic diastereomers (2β,3α,5α)-2-[(2R)-ethyl-4-morpholinyl]-3-hydroxy pregnane-11,20-dione and a (2β,3α,5α)-2-[(2S)-ethyl-4-morpholinyl]-3-hydroxy pregnane-11,20-dione showed that these molecules adopt specific conformations with no rotation about the C-2-N bond in either $CDCl_3$ or DMSO-d_6 (Fielding et al., 1997). In $CDCl_3$, ring A of the steroid is in a twist boat conformation and an intramolecular H-bond between the 3α-OH and the morpholine N which acts as a conformational lock preventing rotation of the morpholine ring. In DMSO-d_6, solutions, the ring A of the steroid is in a chair conformation and the van der Waals contacts with 19-methyl group prevent free rotation of the morpholine ring (Fielding et al., 1997). The point of attachment of the tetrasaccharide sequence to the pregnanegenin in Volubiloside A was confirmed with the help of Phase-Sensitive NOESY.

HOMONUCLEAR HARTMANN-HAHN SPECTROSCOPY (HOHAHA)

The isotropic mixing experiment in which the net magnetization is transferred under spin locking is the most useful method of relay in coherence along the chain of spins. A 'J-network' can be determined from a HOHAHA spectrum. A 'J-network' is defined as a group of protons that are serially linked via ^{1}H-^{1}H J (scalar) couplings i.e. all protons of a single saccharide unit belong to the same J-network. A complete spin system can be identified only if there is at least one resonance in the spin system, like the anomeric proton, which is well isolated and has a reasonably large coupling to its neighbouring spin. Thus a slice through a HOHAHA spectrum at each anomeric proton along the diagonal yields a ^{1}H sub spectrum containing all scalar-coupled protons within that sugar residue. However, the distribution of magnetization around the spin system can be impeded by small couplings (eg. H-4 and H-5 in the galactosyl residues) which lead to cross peaks upto H-4 but no further peak observed.

ROTATING FRAME OVERHAUSER ENHANCEMENT SPECTROSCOPY (ROESY)

The original name given to this technique was cross relaxation which was appropriate for minimolecules emulated by locked spin, but it is now more commonly termed as Rotating Frame over Hauser Enhancement Spectroscopy. In this experiment, the transverse cross relaxation is positive for all values of rotational correlation time, i.e., it is the same as that found for small molecules in the NOESY experiment. The original motivation for this experiment arose for a want to study the structure of intermediate size molecules, which have near zero longitudinal NOE effects. However, the positive sign of ROE effect also allows one to immediately distinguish cross relaxation from chemical exchange effects, which are always negative. This experiment has been developed to overcome the small to zero NOE, in some molecules where the NOESY spectrum may be definite. In this method a spin lock condition is made to arise which allows the two spins to reach a so called Hartmann and Hahn type of condition which allows the two spins to exchange their energy (and also magnatization). With the exchange of spins positive NOE's are produced and the resulting ROESY spectrum is complete. In pregnane glycosides a direct evidence for the sequence of sugars and their linkage sites can be obtained by this experiment, which shows correlated peaks between anomeric protons and the protons linked to the glycosylated carbon.

HOMONUCLEAR J-RESOLVED TWO-DIMENSIONAL SPECTROSCOPY (HOMO-2DJ)

Homonuclear J-resolved spectroscopy is used to extract multiplet structure from mildly overlapping spectra. It can provide unprecedented dispersion of the 'H NMR spectra but leaves unsolved assignments of individual resonances when strongly coupled nuclei are involved and/or multiplets originating from different spin systems overlap However, for weakly coupled spectra an apparently complete separation can be achieved by tilting the 2D spectrum through 45° in frequency space. The usefulness of the method declines with increasing number of sugar residues and becomes of limited value in the study of oligoglycosides structures due to overlapping of mutually coupled signals which causes distortions in multiplet pattern and prevents the use of cross sections for observing individual multiplets and for extraction of the desired ^{1}H-^{1}H couplings.

TOTAL CORRELATED SPECTROSCOPY (TOCSY)

This is a limiting case of multiple coherence transfers. In this experiment, a given proton shows cross peaks with all the spins which are a part of the same coupling network, regardless of whether it has direct couplings to them. Continuous strong proton irradiation is used which effectively scales all chemical shift differences to near zero. This causes the spins to become very strongly coupled so that if the irradiation continues for a time comparable to 1/J all coherences from a given spin system will mix. Although TOCSY, in principle, can be used in sorting out moderately crowded spectra, in practice it suffers from poor sensitivity.

RELAYED COHERENCE TRANSFER COSY (RCT 2D)

Relayed Coherence Transfer is a technique that establishes connectivity between two remote nuclei within a given spin system. Like in an AMX system where J_{AM} and J_{MX} represent vicinal couplings and J_{ax} equals zero (for a saturated compound), the COSY spectra would show peaks between A and M and M and X, but not between A and X.

However, RCT COSY establishes connectivity between these two remote protons i.e. between A and X. RCT COSY propagates the magnetization transfer from A to M through further couplings experienced by M. Recently, Hughes has used this technique for determining proton chemical shifts in steroids (Shaka et al., 1983; Eich et al 1982; Bax et al 1985).

HETERONUCLEAR 2D-NMR SPECTROSCOPY

Heteronuclear chemical shift correlation is one of the most powerful 2D experiments, combining the excellent resolving power of decoupled ^{13}C NMR with the ease of interpretation of proton chemical shifts. It offers very good chemical information and also allows the resolution of single sites in the most interactible spin systems. Only in very similar chemical environments it is possible that the two pairs of ^{1}H and ^{13}C shifts would be identical. In normal use an intensity map is produced by the 2D correlations in which each distinct -CH group in a molecule gives rise to one peak and the frequency coordinates are the 'H and 13C chemical shifts frequencies in the F_1 and F_2 dimensions.

^{13}C-^{1}H LONG RANGE COSY

Two dimensional heteronsuclear correlation via long-range coupling has been found to be useful in determining the connectivity of sugar to aglycone and also the sequence of sugars. The long-range coupling interactions are known to be highly stereospecific in rigid systems. J-couplings follow the empirical W-rule. Larger long range J-couplings observed for a path having a zig-zag or W like shape has been confirmed by double perturbation calculations. These coupling interactions can be used in configurational and conformational analysis if their stereospecificity is clearly demonstrated. The technique has been employed by Itokawa et al. for determining the sequence of six sugars in the glycosidic chain of the pregnane glycoside Periplocoside A.

HETERONUCLEAR MULTIPLE-QUANTUM COHERENCE (HMQC) Heteronuclear Multiple Quantum Coherence (HMQC) is a powerful method that leads to an unambigous assignment of ^{1}H and ^{13}C NMR spectra of cardiac and pregnane glycosides and the C-H correlation assignment. HMQC was used for geminal C-H correlations in deducing the structure of Verrucoside by Kashman et. al.

HETERONUCLEAR MULTIPLE BOND CORRELATION SPECTROSCOPY (HMBC)

The Heteronuclear Multiple Bond Correlation Spectroscopy is a modified version of the Heteronuclear Multiple Quantum Coherence (HMQC) which is suitable for determining long-range coupling ^{1}H-^{13}C connectivity. Since this is a long-range chemical shift correlation experiment, it provides almost the same information as the COLOC experiment. However, since this is also an inverse experiment it has higher sensitivity than the COLOC experiment. If more than one long-range ^{1}H-^{13}C connectivity is detected for one particular proton, the relative intensities of the corresponding resonance are directly related to the mangnitude of the coupling constants. Rashid et al assigned the oxymethine proton in the cardiac glycoside β-anhydro epi-digitoxigenin at C-3 with the help of the HMBC experiment. The isoveryl moiety in pregnane glycoside Penicilloside C was attached to C-20 based on the long range corelation between the carbonyl group and the H-20 in the HMBC experiment.

MASS SPECTROMETERY

Mass spectrometery is known to be an effective method for the structure elucidation of natural products which generally are avaiable only in limited amounts. It is well known that mass spectra of cardenolides and pregnanes provide useful information on the molecular weight as well as the stucture of the aglycone. In recent times the problem of low volatility has been overcome partially by new inlet techniques and partially by pioneering studies on specific volatile derivatives. With the advent of FAB and other recent mass spectroscopy techniques the problem of higher mass fragments has also been overcome. However, the electron impact mass spectroscopy (EIMS) often provides valuable structural information because fragments of lower mass value are more evident in it. EIMS also provides useful information about the substituent groups and their position within the aglycon. It has been demonstrated that the location of a hydroxy group strongly influences the fragmentation of the skeleton of cardiac and pregnane derivatives. The mass spectra have proved to be very useful in establishing the presence of hydroxyl groups at C-8, C-14 and other quartenary carbons, because at these positions the hydroxyl group becomes tertiary in nature and thus cannot be acetylated and consequently cannot be easily detected by NMR methods. The mass spectra also help in assigning the position of hydroxy functions at C-11, C-12, C-15 and C-16. The FAB mass spectra of these compounds invariably give a protonated or an alkali metal cationized molecular ion peak (when alkali metal salt is added to the liquid matrix) along with the fragments arising out of the fragmentation of the molecule thus helping in determining the molecular weight and structure of these biologically important class of compounds. Researchers have carried out extensive studies on the mass spectra of a number of cardenolides and bufadienolides and established a correlation between the structure of these compounds and their fragmentation pattern. Similar studies have also been carried out on the mass spectra of polyhydroxypregnanes by various workers. On the basis of the detailed studies on cardenolides and pregnanes it has be concluded that fragmentation pattern for the perhydrocyclopentanophenanthrene nucleus which is the basic frame of all these natural products follows the similar pathway for their fragmentation (Brown et al., 1972; Deepak et al., 1996; Deepak et al., 1997).

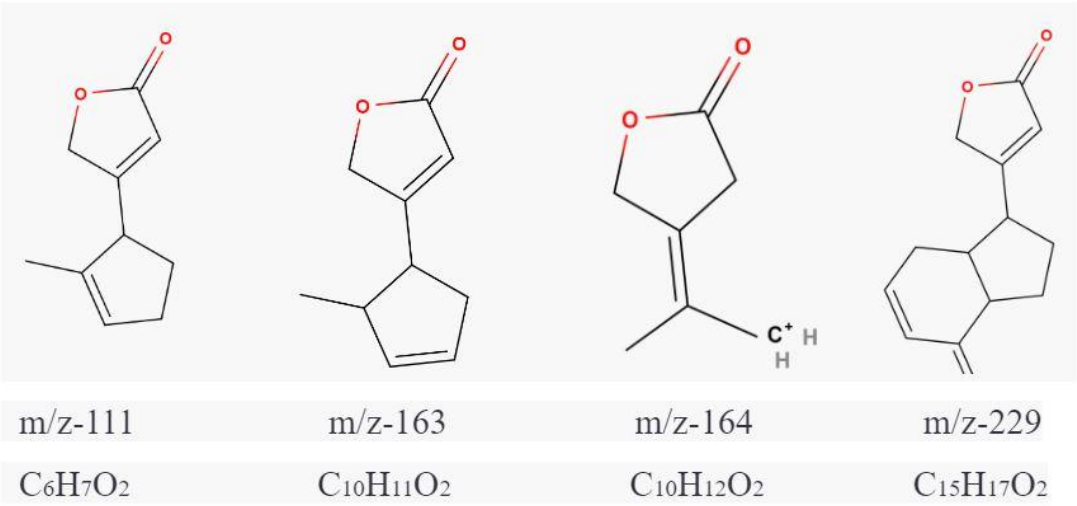


Figure 3.

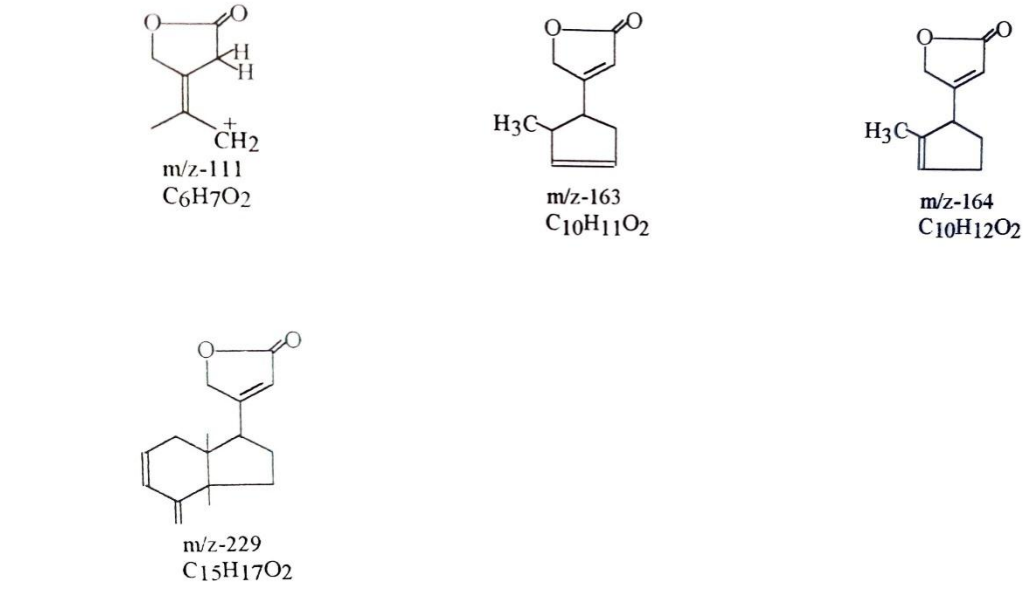


Figure 4.

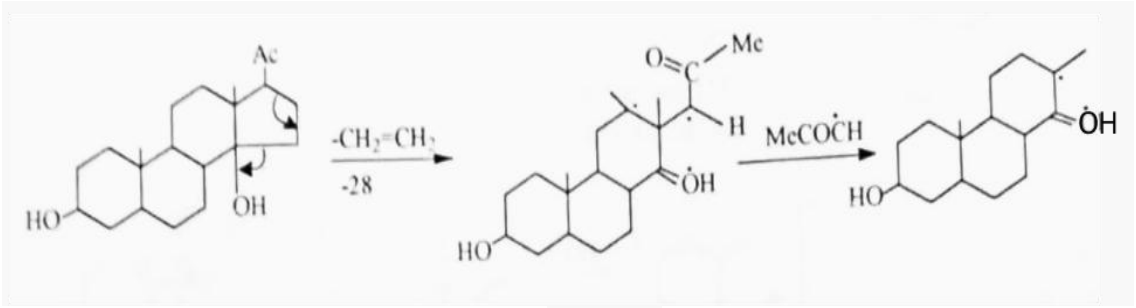


Figure 5.

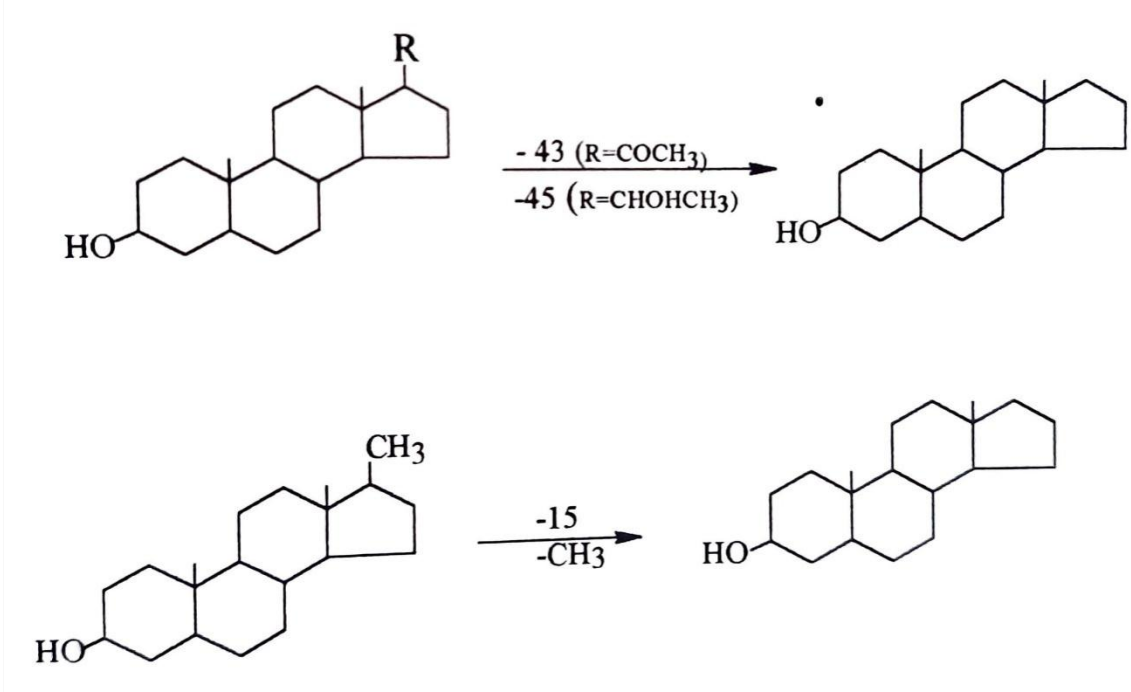
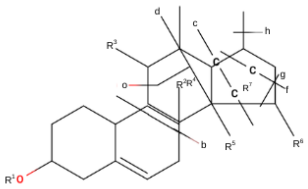
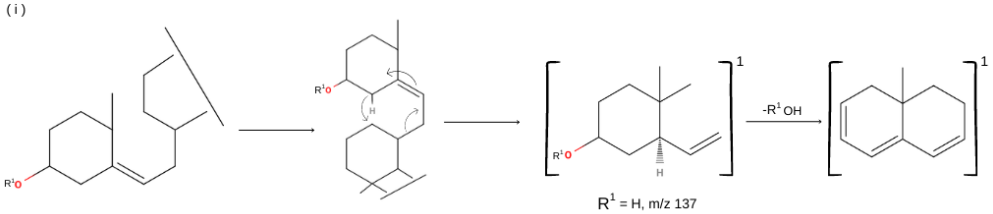


Figure 6.

1. Fragmentation 'b'



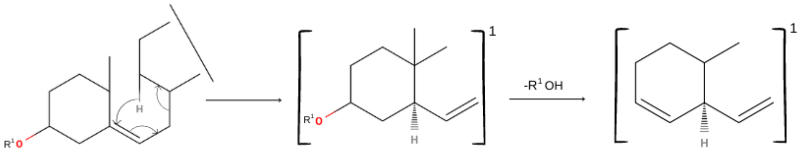
(i)



R^1 = H, m/z 137

R^1 = Ac, m/z 179

(ii)

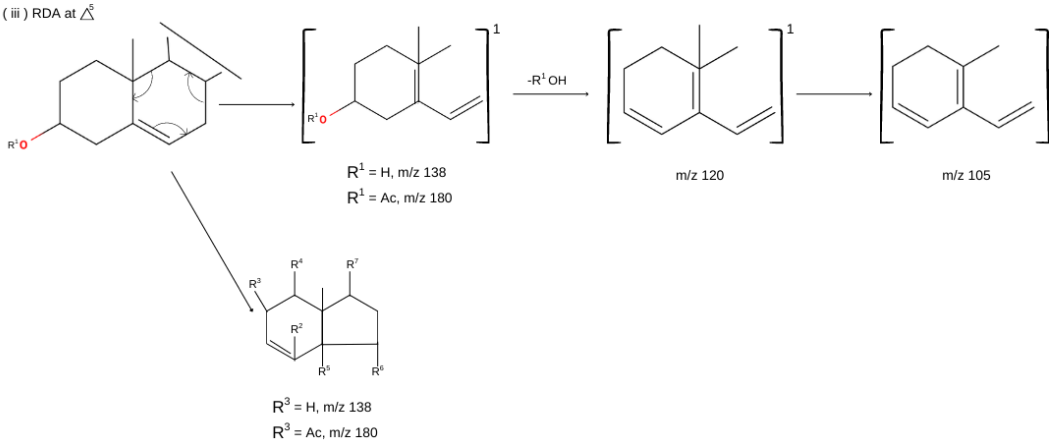


R^1 = H, m/z 139

R^1 = Ac, m/z 181

m/z 121

(iii) RDA at $\overset{5}{\triangle}$



R^1 = H, m/z 138

R^1 = Ac, m/z 180

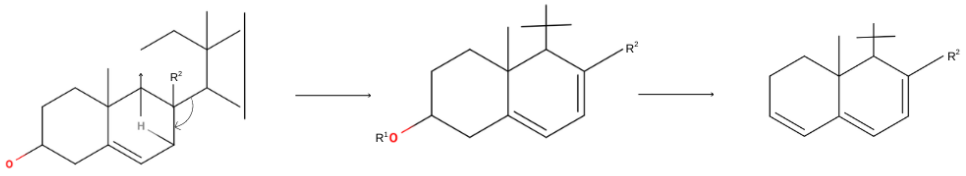
m/z 120

m/z 105

R^3 = H, m/z 138

R^3 = Ac, m/z 180

2. Fragmentation 'c'



R^1 = R^2 = H, m/z 163

R^1 = Ac, R^2 = H, m/z 205

R^1 = Ac, R^2 = OH, m/z 179

R^1 = Ac, R^2 = OH, m/z 221

R^2 = H, m/z 145

R^2 = OH, m/z 161

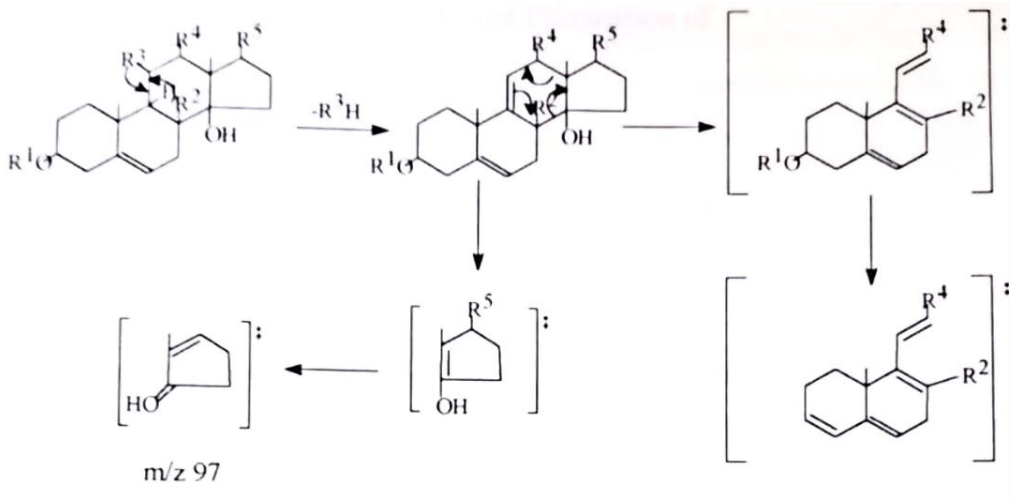


Figure 7.

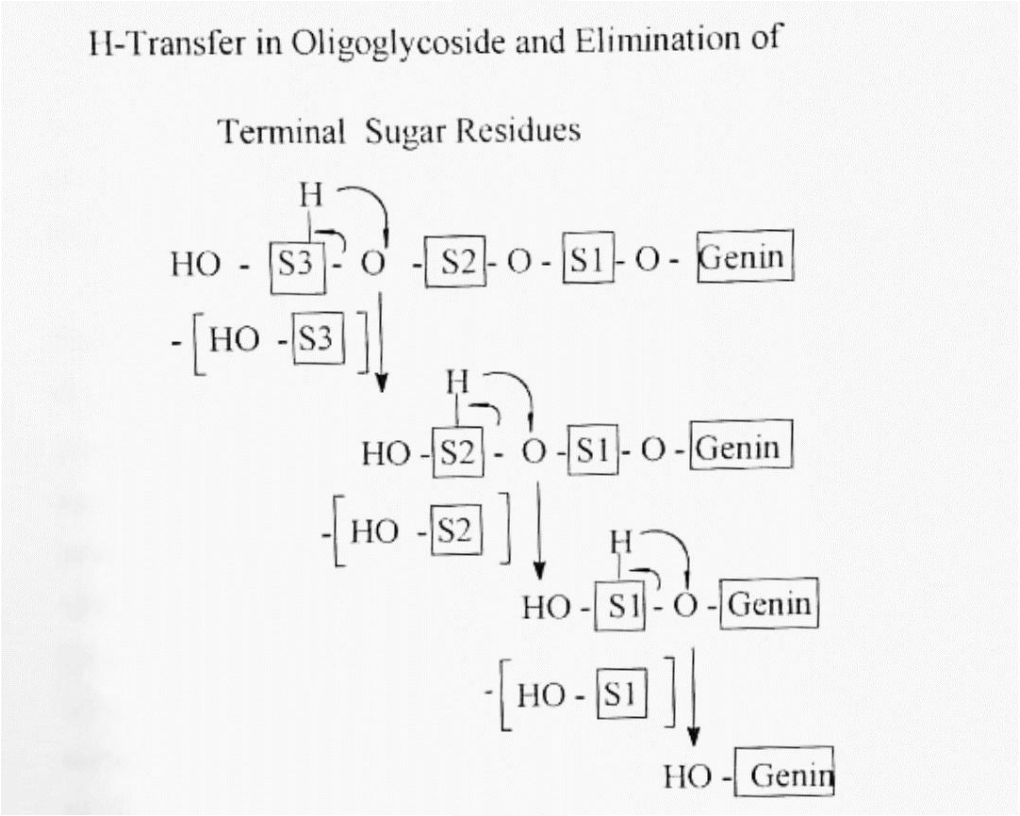


Figure 8.

Characterstic Fragments for the Cardenolides and Bufadienolides

- Characteristic mass fragments for cardenolides and bufadienolides shows the most important fragments at m/z 111 and m/z 123 respectively (Fig 3 and 4) which arise by the fragmentation of the five membered and six membered lactone ring respectively.
- The other important fragments for cardiac genins are those involving the D ring with five and six membered lactone ring and giving fragments at m/z 163/164 and 191 respectively (Fig 3 and 4).
- The cardenolides also show another characteristic fragment at 229 which encorporates the ring C and D along with the five membered lactone ring (Fig 3).

Characterstic Fragments for the Pregnanes and Nor Pregnanes

- Pregnanes having 3-OR-Δ^5 (R=H or acyl group) undergo retero Diels Alder fission followed by elimination of -ROH molecule and Methyl radical to give prominent peaks. Similarly, retero Diels Alder fission cleavage is observed in 11-OR pregnanes (R=H or acyl group) after elimination of oxygen function as -ROH involving C-9 H.
- 14βhydroxypregnanes having 20-keto, 17β side chain undergo D-ring cleavage with the loss of ethylene molecule, the fragments thus reported are M^+-28, M^+-46, M^+-74, M^+-85 and M^+-89. This fragmentation is highly stereoselective and is independent of the presence or absence of the functional groups in the molecule, and compounds with 17α- side chain do not show this fragmentation, instead they show fragment M^+-51, M^+-69, M^+-88, M^+-18, and M+nH2O-$COCH_3$ (Fig 5).
- The only difference between the fragmentation of pregnanes and C-21 nor pregnanes is the loss of the side chain. In the case of the pregnanes loss of ions of m/z 45 or m/z 43 from M is seen which is caused by the fragmentation of CH(OH)CH, or COCH, side chains respectively while in the case of C-21 nor pregnanes the loss is of m/z 15 only (Fig:6).

All the other fragmentations in the cardenolides, bufadienolides and the pregnanes resemble very much with each other because of the fact that they arise from the perhydrocyclopentanophenantherene nucleus (Fig 7).

The stereochemistry (α or β orientation) of the C-17 side chain can also be determined by Mass spectrometery. Mass spectrometery also provides useful information regarding the sequence of monosaccharides in the oligosaccharide chain linked to the genin moiety in the cardiac as well as the pregnane glycosides. The individual monosaccharide units get detached from the molecular ion peak at the point of linkage along with hydroxyl group displacement. The stepwise elimination of the monosaccharide units starting from the terminal end leads to the formation of the genin fragment. At the same time the entire oligosaccharide unit (ie. M-Genin) is obtained, which further fragments by repeated H-transfers accompanied by terminal sugar less water giving rise to an ion of the same minimal mass as the molecular ion of the corresponding oligosaccharide with one less monosaccharide residue and so on until only the monosaccharide remains (Fig 8).

ULTRA VIOLET SPECTROSCOPY (U.V.)

The presence of conjugated systems in cardiac glycosides makes U.V. spectroscopy a useful tool in the structure elucidation of these compounds. The cardenolides consist of a single maximum at 220nm whereas the U.V. spectra of bufadienolides are characterized by a single maximum at 298 nm (ε5000) and a second band between 220nm and 200$nm^2$of similar intensity. However, this technique is not very useful for the structure elucidation of pregnane glycosides because of the absence of conjugated systems in pregnanes. The technique may be useful when pregnane esters containing α,β- unsaturated and/or aromatic acids are encountered (Deepak et al 1996; Deepak et al., 1997).

INFRA-RED SPECTROSCOPY (I.R.)

IR Spectroscopy plays a vital role in the structure elucidation of cardiac and pregnane glycosides. This method is employed to establish the presence of functional groups like carbonyl functions, hydroxyl groups, double bonds as well as methyl groups. The lactone ring in cardiac glycosides is characterized by the presence of peaks in the region of 1625-1790 cm for the double bond and the carbonyl group of the lactone ring. The deformation bands for methyl group appear at 1360cm^{-1},

while the stretching bands for hydroxy groups are visible at 3350cm^{-1} (3546cm^{-1} for C-14 OH and 3472-3436 cm^{-1} for C-3 OH group) in case of cardiac as well as pregnane glycosides. I R spectroscopy helps to differentiate between carbonyl functions and hydroxy ethyl and acetyl side chain on C-17 by giving peaks in the region of 1740-1715 cm^{-1} for the carbonyl functions. This method is also used to establish the presence of ester functions, the presence of associated and free hydroxyl groups (3400cm^{-1}) as well as the presence of unsaturation in these glycosides (Deepak et al 1996; Deepak et al., 1997).

REFERENCES

Sukhdev Swami Handa, Suman Preet Singh Khanuja, Gennaro Longo and Dev Dutt Rakesh (2008). Extraction Technologies for Medicinal and Aromatic Plants, International Centre for Science and High Technology ICS-UNIDO, AREA Science Park Padriciano 99, 34012 Trieste, Italy.

Gregory, R.E. and DeLisa, A.F. (1993). Paclitaxel: a new antineoplastic agent for refractory ovarian cancer, *Clinical Pharmacy*, 12(6): 401-415.

Romano Silvestri (2013). New prospects for vinblastine analogues as anticancer agents, *J. Med. Chem*. 56, 3, 625–627.

Mbaveng Armelle, T., Hamm Rebecca and Kuete Victor (2014). Toxicological Survey of African Medicinal Plants, 557-576.

Pamela J. Weathers, Patrick R. Arsenault, Patrick S. Covello, Anthony McMickle, Keat H. Teoh, and Darwin W. Reed (2011). Artemisinin production in Artemisia annua: studies in planta and results of a novel delivery method for treating malaria and other neglected diseases, *Phytochem Rev. Jun*; 10(2): 173–183.

Bin Zhao, Shabbir M. Moochhala and Su-Yin Tham (2004). Biologically active components of *Physostigma venenosum*, *J Chromatogr B*, 812 (1-2), 183-192.

Ilka Nacif De Abreu, Alexandra Christine H. F. Sawaya, Marcos Nogueira Eberlin and Paulo Mazzafera (2005). Production of pilocarpine in callus of jaborandi (*Pilocarpus microphyllus* stapf), In Vitro Cellular and Developmental Biology - Plant, Volume 41, pages 806–811.

Bowman, W.C. (2006). Neuromuscular block, *Br J Pharmacol.*; 147 (Suppl 1): S277–S286. Published online 2006 Jan 9. doi: 10.1038/sj.bjp.0706404

Balandrin, M.F., Kinghorn, A.D. and Farnsworth, N.R. (1993). "Human Medicinal Agents from Plants": *American Chemical Society*, 534.

Deepak D., Srivastava, S., Khare, N.K. and Khare, A. (1996). Fortschritte der Chemie Organischer Naturstoffe, 69, 71-155 3.

Deepak, D., Srivastav, S. and Khare, A. (1997). Fortschritte der Chemie Organischer Naturstoffe, 71, 169-325 1.

Desh Deepak, Anakshi Khare and M.P. Khare (1989). Plant Pregnanes: Review, *Phytochemistry*, 28 (12), 3255-3263

Satish C. Pandey, Kuldeep Kumar Shashi Bala and Desh Deepak (2022). Isolation of Novel C-21 Nor Precursors of Pregnane from *Hoya longifolia*, *J. Biol. Chem. Research*, Vol. 39 (2) 158-171.

Petra J. Pederson, Shengxin Cai, Chase Carver, Douglas R. Powell, April L. Risinger, Tanja Grkovic, Barry R. O'Keefe, Susan L. Mooberry and Robert H. Cichewicz (2020). Triple-Negative Breast Cancer Cells Exhibit Differential Sensitivity to Cardenolides from *Calotropis gigantea*, *J. Nat. Prod*, 83, 7, 2269–2280.

Mai T.T. Nguyen, Khang D.H. Nguyen, Phu H. Dang, Hai X. Nguyen, Suresh Awale and Nhan T. Nguyen and Calosides A.F. (2020). Cardenolides from *Calotropis gigantea* and Their Cytotoxic Activity, *J. Nat. Prod.* 83, 2, 385–391.

Agrawal Anurag A., Katalin Böröczky, Meena Haribal and Christophe Duplais (2021). Cardenolides, toxicity, and the costs of sequestration in the co-evolutionary interaction between monarchs and milkweeds, *BIOLOGICAL SCIENCES*, 13, 118 (16) e2024463118.

Herbert, R.B. (1994). "The Biosynthesis of Secondary Metabolites". Chapman and Hall, London, 63-95.

Tschesche, R. and Linienweiss, G.Z. (1964). Cardenolid-Biosynthese aus Pregnenolonglucosid, Naturforsch Teil B, 19, 265.

Tschesche, R. and Brassat, B.Z. (1965). Zur Biosynthese von Steroid-Derivaten im Pflanzenreich, *Naturforsch Teil B*, 20, 707.

Caspi, E. and Hornby, G.M. (1968). Biosynthesis of plant sterols-III. Mechanism of saturation of ring B in pregnenolone during its conversion to digitoxigenin in *Digitalis lanata*, *Phytochem*. 7, 423.

Galagovsky, L.R., Porto, A.M., Burton, G. and Gros, E.G. (1983). Bufadienolides of plant and animal origin, *Z. Naturforsch Teil C*, 39, 38.

Cabrera, G., Seldes, A.M. and Gros, E.G. (1992). A pregnane glycoside from the roots of *Mandevilla pentlandiana*, *Phytochem*. 32, 171.

Singh, B. and Rastogi, R.P. (1970). Cardenolides—glycosides and genins, *Phytochem*, 9, 315.

Chung, N.T.A., Nelson, C.J. and Watson, T.R. (1989). Cardenolide glycosides with 5, 6-unsaturation from *Asclepias vestita*, *J. Chem. soc. Perkin Trans.* 1, 1563.

Pieri, F., Arnould, G.M.C. and Sefraui, E.H. (1992). Cardiotonic glycosides from *Acokanthera spectabilis*, *Fitoterapia*, 63, 333.

Decosterd, L., Gustafson, K.R., Cardellina, J., John, H. II., Cragg, G.M. and Boyd, M.R. (1994). The differential cytotoxicity of cardenolides from *Thevetia ahouia*, *Phytother. Res* 8(2), 74-77.

Kitanaka, S., Takido, M., Mizaie, K., Nakaiki, S. (1996). Cytotoxic cardenolides from woods of *Euonymus alata*, *Chem. Pharm. Bull*, 44(3), 615-17.

Chaterjee, R.K., Fatma, N., Murthy, P.K; Sinha, P., Kulshrestha, D.K. and Dhawan, B.N. (1992). Macrofilaricidal activity of the stembark of *Streblus asper* and its major active constituents, *Drug Dev. Res.* 26 (1), 67-78.

Back, N.I., Lee, Y.H., Park, J.D., Kim, S.II., Ahm, B. and Euonymoside, A. (1994). A new cytotoxic cardenolide glycoside from the bark of *Euonymus sieboldianus*, *J. Planta Med.* 60 (1), 26- 9.

Kaneda, N., Chai, H., Pezzuto, J.M., Kinghom, A.D., Farnsworth, N.R., Tuchinada, P., Udachachon, J., Santisuk, T. and Reutrakul, V. (1992). Cytotoxic Activity of Cardenolides from *Beaumontia brevituba* Stems, *Planta Med.* 58(5). 429-31.

Yoshikawa, M. Murkami, T. Shimada, H. Fukada, N. Matsuda, H. Sashida, Y. and Yamaharaj (1998). Corchorusosides A, B, C, D, and E, new cardiotonic oligoglycosides from the seeds of *Corchorus olitorius* L. (Moroheiya), *Heterocycles*. 48(5), 869-873.

Siddiqui, B.S. Sultana, R. Begum, S. Zia and A. Seira (1997). Cardenolides from the Methanolic Extract of Nerium oleander Leaves Possessing Central Nervous System Depressant Activity in Mice, *J. Nat. Prod.* 60(6), 540- 544.

Polania, J., Aranjo, M.H. and Polania, M.A. (1966). Cardiac glycosides, *An. Fac. Farm. Porto.* 26, 83.

Coombi, R.G. and Watson, T.R. (1964). The cardiac glycosides of *Gomphocarpus fruticosus* R. Br. III. Gomphoside, *Aust. J. Chem.,* 17, 92.

Kupchan, S.M., Kuox, J.R. and Kelsey, J.E. (1964). Renauld, Calotropin, a Cytotoxic Principle Isolated from *Asclepias curassavica L.*, J.A.S. Science, 146, 3652, 1685-1686.

Mansour, S., Hifnawy, M.S., Mc. Phail, A.T., Mc. Phail and D.R. Ghalakinoside (1988). A cytotoxic cardiac glycoside from *Pergularia tomentosa*, *Phytochem*. 27, 3245.

Jolad, S.D., Hofmann, J.J., Cole, J.R., Tempesta, M.S. and Bates, R.B. (1981). 3'-O-Methylevomonoside: a new cytotoxic cardiac glycoside from *Thevetia ahouia* A. DC (Apocynaceae), *J.Org. Chem.*, 46, 1946.

Geran, R.I., Greenberg, R.H. MacDonald, M.N., Schumache, A.M. and Abbott, B.J. (1972). Protocols for screening chemical agents and natural products against animal tumors and other biological systems, *J .Cancer Chemother. Rep.* 3(3), 17.

Marks, W.H., Fong, H.H.S., Tin, Wa-M. and Farnsworth, R. (1975). Cytotoxic principles of *Parquetina nigrescens* (Afzel.) bullock (asclepiadaceae), *J. Pharm. Sci.,* 64, 1674.

Abe, F., Yamauchi, T. and Nohara, T. (1992). C-nor-d-Homo-cardenolide glycosides from *Thevetia neriifolia*, *Phytochem*, 31, 251.

Costa, S.S., Jossang, A. and Bodo, B. (1996). 4''''-Acetylsagittatin A, a Kaempferol Triglycoside from *Kalanchoe streptantha*, *J. Nat. Prod.* 59,327.

Wagner, H., Fischer, M. and Lotter, H. (1986). Die toxischen und sedierend wirkenden Bufadienolide von *Kalanchoe daigremontiana* HAMET et PERR, *Helv. Chim Acta,* 69, 359.

Kasoanaino, P., Galeffi, C., Multari, G., Nicolette, M. and Capolongo, L. (1993). Research on African Medicinal Plants. Part 31. Kalanchoside, a Cytotoxic Bufadienolidic Glycoside from *Kalanchoe tomentosa* Baker, *Gazz. Chim. Ital,* 123, 539.

Kubo, I. and Matsumoto, T. (1984). Abyssinin, a potent insect anti-feedant from an African medicinal plant, *Bersama abyssinica*, *Tetrahedron Lett.* 25, 4601.

Kubo, L. and Matsumoto, T. (1985). Potent Insect Antifeedants from the African Medicinal Plant *Bersama abyssinica*, ACS Symp Ser. 276, 183.

Tuniguchi, M. and Kubo, I. (1993). Ethnobotanical Drug Discovery Based on Medicine Men's Trials in the African Savanna: Screening of East African Plants for Antimicrobial Activity II, *J. Nat. Prod.* 56, 1539.

Lichtstein, D., Gati, L., Samuelov, S., Berson, D., Rozenman, Y., Landau, L. and Deitsch, J. (1993). Identification of digitalis-like compounds in human cataractous lenses, *Eur. J. Biochem*, 216, 261.

Tymiak, A.A., Norman, J.A. Bolgar, M., Do Donato, G.C., Lee, H., Parker, N.L., Lo, l. C., Berova, N., Nakanish, K., Haber, E. and Haupert, G.T. (1993). Proc. Structural studies on bioactive natural products: Cardiac glycosides and polyene macrolides, Natl. Acad. Sci. 90, 8189.

Hilton, P.J., White, W.R. W., Forni, G.A. Mckinnon, N., Gordon, F.M.D., Hilton, M.J. Forni, L.G., Mckinnon, W., Ismail, F.M.D., Keenan, M., Jones, K. and Mordin, W.E. (1996). An inhibitor of the sodium pump obtained from human placenta, *The Lancet*, 348, 303.

Reichstein, T. (1967). Cardenolid-und pregnanglykoside, *Naturwissenschaften*, 54, 53.

Deepak, D., Khare, A. and Khare, M.P. (1989). Plant Pregnanes, *Phytochem*. 28, 3255.

Yoshimura, S.I., Narita, H., Hayashi K. and Mitsuhashi, H. (1983). Studies on the constituents of asclepiadaceae plants. LVI. Isolation of new antitumor-active glycosides from *Dregea volubilis* (L.) BENTH., *Chem. Pharm. Bull.* 31, 3971

Hayashi, K. Nakao, A. and Mitsuhashi, K. (1969). Studies on the Constituents of Asclepiadaceae Plants. XXVI. Isolation of a New Glycoside from *Dreges volubilia* (L.) BENTH., *Chem. Pharm. Bull.* 17, 2629.

Yoshimura, S.I., Narita, H. Hayashi, K. and Mitsuhashi, H. (1985). Studies on the constituents of Asclepiadaceae plants. LIX. The structures of five new glycosides from *Dregea volubilis* (L.) BENTH, *Chem. Pharm. Bull.* 33, 2287.

Itokawa, H., Xu, J. and Takeya, K. (1988). Studies on chemical constituents of antitumor fraction from *Periploca sepium*. V. Structures of new pregnane glycosides, periplocosides J, K, F and O, *Chem. Pharm. Bull.* 36, 4441.

Itokawa, H., Xu, J. and Takeya, K. (1988). Studies on chemical constituents of antitumor fraction from *Periploca sepium*. IV.: structures of new pregnane glycosides, periplocosides D, E, L, and M, *Chem. Pharm. Bull.* 36, 2084.

Itokawa, H., Xu, J., Takeya, K., Watanabe, K. and Shoji (1988). Studies on chemical constituents of antitumor fraction from *Periploca sepium*. II.: Structures of new pregnane glycosides, periplocosides A, B and C, *J. Chem. Pharm. Bull.* 36, 982.

Itokawa, H., Xu, J. and Takeya, K. (1988). Pregnane glycosides from an antitumour fraction of *Periploca sepium*, *Phytochem*. 27, 1173.

Hayashi, K., Wada, K., Mitsuhashi, H., Takase, M., Terada, S. Koidi, Y., Mizuno, D., Bando, H., Aiba, T. and Narita, T. (1980). Antitumor active glycosides from Condurango Cortex, *Chem. Pharm. Bull.* 28.

Hayashi, K., Wada, K., Mitsuhashi, H., Bando, H., Takase, M., Terada, S., Koidi, Y., Aiba, T., Narita, T. and Mizuno, D. (1981). Further investigation of antitumor condurangoglycosides with C-18 oxygenated aglycone, *Chem. Pharm. Bull.* 29, 2725.

Duh, C.Y., Pezzuta, J.M., Kinghorn, A.D., Leung, S.L. and Farnsworth, N.R. (1987). Plant anticancer agents, XLIV. Cytotoxic constituents from *Stizophyllum riparium*, *J. Nat. Prod.* 50, 63.

Sethi, A., Maurya, A. Tewari, V. Srivastava, S. Faridi, S., Bhatia G., Khan, M.M., Khanna, A. K. and Saxena, J.K. (2007). Expeditious and convenient synthesis of pregnanes and its glycosides as potential anti-dyslipidemic and anti-oxidant agents, *Bioorganic & Medicinal Chemistry*, 15, 4520–4527.

Luo, S.Q., Lin, L.Z., Cortdell, G.A. Xui, L. and Johnson, M.E. (1993). Polyoxypregnanes from *Marsdenia tenacissima*, *Phytochem*. 34, 1615.

Oshima, Y., Hirota, T. and Hikin, H. (1987). Periplosides A, B and C, steroidal glycosides of *Periploca sepium* root-barks, *Heterocycles*, 26, 2093.

Alicja Kozera-Kępniak, Karol Jastrzębski, Jakub Walenczak, Andrzej Klimek and Andrzej Głąbiński (1986). 66034T/C polymorphism of the human pregnane X receptor (hPXR) as potential risk factor for drug resistance in epilepsy – Preliminary study, Neurol Neurochir Pol, 51(1): 19-23.

Yuan, J.L., Lu, Z.Z., Chen, G.L.X., Ding, W.P. Zhou, B.N. Erdelmeier, C.A.J., Hamberger, M.O. Fong, H.H.S. and Cordell, G.A. (1992). The pregnane glycoside marsdekoiside A from Marsdenia koi, *Phytochem*. 31, 1058.

Yuan, J.L., Ding, W.P. Shi, J.P. Lu, Z.Z. Zhou, B.N. Erdelmeier, C.A.J. Cordell, G.A.; Fong, H.H.S. and Farnsworth, N.R. (1991). Studies on the antifertility components from Marsdenia koi, *J.Tong-Ji. Med Univ.* 11, 165.

Zhang, R.S., Ping, Y.Y., Mao, Y. and Ling, L.H. (2000). Two new cytotoxic C-21 steroidal glycosides from the root of *Cynanchum auriculatum*, *Tetrahedron* 56(24), 3875-3879.

Bang, Y.H., Si, E.K. Kim, Y.H. Kim, H.S., Hong, Y.S., Ro, J.S., Lee, K.S. and Lee, J. (1999). Pregnane Glycoside Multidrug-Resistance Modulators from *Cynanchum wilfordii*, *J. Nat. Prod.* 62, 640-643.

Templeton, J.F., Ling, Y., Zeglam, T.M., Marat, K. and LaBella, F.S. (1992). Endogenous digitalis-like factors, *J. Chem. Soc. Perkinl.* 2503-2517.

Templeton, J.F., Ling, Y., Zeglam, T.M., Marat, K. and LaBella, F.S. (1993). Synthesis of 20-hydroxy-, 20-amino-, and 20-nitro-14-hydroxy-21-nor-5.beta., 14.beta.-pregnane C-3 glycosides and related derivatives: structure-activity relationships of pregnanes that bind to the digitalis receptor, *J. Med. Chem.* 36, 42-45.

Templeton, J.F., Kumar, V.P.S., Bose, D., Smyth, D.D., Kim R.S. and LaBella, F.S. (1988). Pregnanes that bind to the digitalis receptor: synthesis of 14-hydroxy-5. beta., 14. beta.-pregnane glycosides from digitoxin and digitoxigenin, *J. Physiol. Pharmacol.* 66, 1420-1424.

Melero, C.P., Medarde M. and Feliciano, A.S. (2000). A Short Review on Cardiotonic Steroids and Their Aminoguanidine Analogues, *Molecules*, *5*(1), 51-81.

Woolfson, R.G., Graves. J.G, LaBella, F.S., Templeton, J.F. and Posten, L. (1992). Effect of bufalin and pregnanes on vasoreactivity of human resistance arteries, *Biochem. Biophy s. Res. Commun.,* 186, 1-7.

Kittakoop, P., Suttisri R. Chaichantiyuth, C., Vetchagarun, S. Suwanborirux and K. Norpregnane (1999). Glycosides from a Thai Soft Coral, *Scleronephthya pallida*, *J. Nat. Prod.* 62, 318-320.

Stoll, A. and Renz, A. (1941). Die Überführung von Scillaren A in Epi-allo-lithocholsäure (3 β-Oxy-allo-cholansäure). Mitteilung über Herzglykoside, *Helv. Chim. Acta.* 24, 1380,

Zelnik, R.U. and Shindler, O. (1957). Die Glykoside der Samen von Strophanthus Eminii Asch et Pax. 3. Mitteilung. Glykoside und Aglykone, 184. Mitteilung, *Helv. Chim Acta.* 40, 2110.

Zurcher, W., Berg, E.W. and Tamm, C.H. (1969). 14-Epi-Digitoxigenin und 3-Desoxydigitoxigenin. 19. Mitteilung über Reaktionen mit Mikroorganismen [1], *Helv. Chim. Acta.* 52, 2449.

Chandra, R., Deepak, D. and Khare, A. (1994). Pregnane glycosides from *Hemidesmus indicus*, *Phytochem*. 35, 1545.

Palter, R., Haddon, W.F. and Lundin, R.E. (1972). Structure of a safflower steroid cellobioside, *Phytochem.* 11, 2327.

Abe, F. and Yamauchi, T. (1989). Pregnane and Prenane Glycosides from *Trachelospermum liukiuense*, *Chem. Pharm. Bull.* 37, 33.

Nakanishi, T., Kobayashi, M., Murata, H. and Inada, A. (1988). Phytochemical Studies on Meliaceous Plants. IV: Structure of a New Pregnane Glycoside, Toosendanoside, from Leaves of *Melia toosendan* SIEB. et Z UC, *Chem. Pharm. Bull.* 36, 4148.

Steyn, P.S. and Van Heerden, F.R. (1998). Bufadienolides of plant and animal origin, *Nat. Prod. Rep.* 397-413.

Abe, F. and Yamauchi, T. (1992). Two pregnanes from oleander leaves, *Phytochem*. 31, 2819.

Srivastav, S., Deepak, D. and Khare, A. (1994). Structural studies of trisaccharide of leptaculatin, *J. Carbohydr. Chem.* 13, 75.

Martson, A. and Hostelmann, K. (1991). Modern separation methods, *Nat. Prod. Rep.* 8, 391.

Srivastav, S., Deepak, D. and Khare, A. (1994). Three novel pregnane glycosides from *Leptadenia reticulata* wight and arn, *Tetrahedron*. 50, 789.

Shibuya, H., Zhang, R., Park, J.D., Baek, N.I., Takeda, Y., Yoshikawa, M. and Kitagawa, I. (1992). Indonesian Medicinal Plants. V. Chemical Structures of Calotroposides C, D, E, F, and G, Five Additional New Oxypregnane-Oligoglycosides from the Root of *Calotropis gigantea* (Asclepiadaceae), *Chem. Pharm. Bull.* 40, 2647.

Berger, S., Junior, P. and Kopanski, L. (1988). Structural revision of pregnane ester glycosides from condurango cortex and new compounds, *Phytochem*. 27, 1451.

Abe, F. and Yamauchi, T. (1988). Pregnane Glycosides of Teikasides B and C Series, from *Trachelospermum asiaticumn*, *Chem. Pharm. Bull.* 36, 4330.

Deepak, D., Khare, M.P. and Khare, A. (1985). A pregnane ester diglycoside from *Periploca calophylla*, *Phytochem*. 24, 3015.

Allegeer, H. (1968). Struktur der Drebyssobiose, Lilacinobiose und Viminose: Desoxyzucker, 45. Mitteilung, *Helv. Chim. Acta.* 51, 311.

Sethi, A., Deepak, D., Khare, M.P. and Khare, A. (1988). A novel pregnane glycoside from *Periploca calophylla*, *J. Nat. Prod.* 51, 787.

Trivedi, R., Khare, A. and Khare, M.P. (1989). A pregnane ester oligoglycoside from *Oxystelma esculentum*, *Phytochem*. 28, 1211.

Trivedi, R., Khare, A., Khare, M.P. (1990). A pregnane triglycoside from *Oxystelma esculentum*, *Phytochem*. 29, 3967.

Prakash, K., Sethi, A., Deepak, D., Khare, A. and Khare, M.P. (1991). Two pregnane glycosides from *Hemidesmus indicus*, *Phytochem*. 30, 297.

Tru jillo, J.M., Hernandez, O. and Navarro, E. (1990). Monoglycosides from *Isoplexis chalcantha*, *J. Nat. Prod.* 53, 167.

Junior, P. and Wichtl, M. (1980). 3-*epi*-periplogenin: ein neues cardenolid aus *Adonis vernalis*, *Phytochem*. Volume 19, (10), 2193-2197

Steyn, P.S., Van., H., Fanei, R., Vleggaar, R. and Anderson, L.A.P. (1986). Bufadienolide glycosides of the Crassulaceae. Structure and stereochemistry of orbicusides A—C, novel toxic metabolites of *Cotyledon orbiculata*, *J. Chem. Soc.Perkin Trans.* 1. 1633.

Stothers, J.B. (1972). Carbon-13 NMR Spectroscopy, New York Academic Press, 1972, 38.

Bose, A.K. and Srinivasan, P.R. (1975). NMR spectral studies—XII: Trichloroacetyl isocyanate as an in situ derivatizing reagent for 13C NMR spectroscopy of alcohols, phenols and amines, *Tetrahedron*. 31, 3025.

Qiduan, J., Qianlan, Z. and Quanzhang, M. (1988). Structure of dregeoside a from *Dregea sinensis* var. corrugata, *Plant Diversity*, 10 (4), 1-3

Aue, W.P., Karhan, J. and Ernst, R.P. (1976). Homonuclear broad band decoupling and two-dimensional J-resolved NMR spectroscopy, *J. Chem. Phys.* 64, 4226.

Sunrmons, R.E., Ellis, J. and Gellert, E. (1972). Steroidal alkaloids of *Marsdenia rostrata*, *Phytochem*. 11, 3335.

Singhal, S., Khare, M.P. and Khare, A. (1980). Tenasogenin, a pregnane ester from *Marsdenia tenacissima*, *Phytochem*. 19, 2431.

Hayashi, K., Iida, I., Nakao, Y. and Kaneko, Y. (1988). Four pregnane glycosides, boucerosides AI, AII, BI and BII, from *Boucerosia aucheriana*, *Phytochem*. 27, 3919.

Agarwal, P.K. (1992). NMR Spectroscopy in the structural elucidation of oligosaccharides and glycosides, *Phytochem*. 31(10), 3307.

Zhang, Z.X., Zhou, J., Hayashi, K. and Kaneko, K. (1988). Atratosides A, B, C and D, steroid glycosides from the root of *Cynanchum atratum*, *Phytochem*. 27(9), 2835.

Kitagawa, I., Zhang, R. Park, J.D., Baek, N.I., Takeda, Y., Yoshikawa, M. and Shibuya, H. (1992). Indonesian medicinal plants. I. Chemical structures of calotroposides A and B, two new oxypregnane-oligoglycosides from the root of *Calotropis gigantea*, *Chem. Pharm. Bull.* 40.

Bock, K. and Pedersen, C. (1983). Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Monosaccharides, *Advances in Carbohydrate Chem. and Biochem*., 41, 27.

Zucher, R.F. (1963). Protonenresonanzspektrosopie und Steroidstruktur. II. Die Lage der C-18-und C-19-Methylsignale in Abhängigkeit von den Substituenten am Steroidgerüs, *Helv. Chim. Acta,* 46, 2054.

Agarwal, P.K., Schneider, H.J., Malik, M.S. and Rastogi, S.N. (1983). 13C NMR shifts and conformations of substituted indans, *Org. Magn. Reson.* 21(2), 146.

Agarwal, P.K. and Bansal, M.C. (1989). Studies in Organic Chemistry, 39, 42, Elsevier Publication, Amsterdam.

Kessler, H., Gehrke, M. and Griesinger, C. (1988). Two-Dimensional NMR Spectroscopy: Background and Overview of the Experiments [New Analytical Methods (36)], *Angew. Chem.* 27, 490.

Wasylyk, J.M., Martin, G.E., Weinheimer, A.J. and Alam, M. (1989). Isolation and Identification of a New Pregnene Glycoside from the Gorgonian *Pseudoplexaura wagenaari*, *J. Nat. Prod.* 52, 391.

Adam Allerhand, David Doddrell and Richard A. Komoroski (1971). Natural Abundance Carbon-13 Partially Relaxed Fourier Transform Nuclear Magnetic Resonance Spectra of Complex Molecules, *The Journal of Chemical Physics* 55(1):189-198.

Konda, Y., Toda, Y., Harigaya, Y., Lou, H., Li, X. and Onda, M. (1992). Two new glycosides, hancoside and neohancoside A, from *Cynanchum hancockianum*, *J. Nat. Prod.* 55, 1447.

Abe, F. and Yamauchi, T. (2000). An androstane bioside and 3'-thiazolidinone derivatives of doubly-linked cardenolide glycosides from the roots of *Asclepias tuberosa*, *Chem. Pharm. Bull.* 48(7), 991-993.

Fielding, L., Hamilton, M. and McGuire, R. (1997). Conformations of Anaesthetic Steroids: a 1H and 13C NMR Study of (2β,3α,5α)-2-[(2R)-Ethyl-4- morpholinyl]-3-hydroxypregnane-11,20-dione and (2β,3α,5α)-2-[(2S)-Ethyl-4-morpholinyl]- 3-hydroxypregnane-11,20-dione, *Magn. Reson. Chem.* 35, 184-190.

Aquino, R., Peluso, G., Tommasi, N.D., Simoni, F.D. and Pizza, C. (1996). New Polyoxypregnane Ester Derivatives from *Leptadenia hastata*, *J. Nat. Prod.* 59, 555.

Warashina, T. and Noro, T. (2000). Steroidal glycosides from the aerial part of *Asclepias incarnata* L. II, *Chem. Pharm. Bull.* 48(1), 99.

Summers, M.F., Marzilli, L.G. and Bax, A. (1986). Complete proton and carbon-13 assignments of coenzyme B12 through the use of new two-dimensional NMR experiments, *J. Am. Chem. Soc.* 108, 4285.

Criesinger, C. and Ernst, R.R. (1987). Frequency offset effects and their elimination in NMR rotating-frame cross-relaxation spectroscopy, *J. Magn. Res.* 75, 261.

Brown, P., Kamano, Y. and Pettit, G.R. (1972). High resolution mass spectrometry. Bufadienolides, *Org. Mass. Spec.* 6, 47-74.

Shaka, A.J. and Freeman, R. (1983). Simplification of NMR spectra by filtration through multiple-quantum coherence, *J. Magn. Reson.* 51, 169.

Eich, G., Bodenhausen, G. and Ernst, R.R. (1982). Exploring nuclear spin systems by relayed magnetization transfer, *J. Am. Chem. Soc* 104, 3731.

Bax, A. and Drobny, G. (1985). Optimization of two-dimensional homonuclear relayed coherence transfer NMR spectroscopy, *J. Magn. Reson.* 61, 306.

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