Review

Naturally occurring saponins: Chemistry and biology

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Naturally occurring saponins are glycosides of steroids, alkaloids and triterpenoids. They are widely distributed in nature and reported to be present in 500 genera of plants. A wide variety of plants belonging to family Liliaceae, Dioscoreaceae, Solanaceae, Sapindaceae and Agavaceae are the major source of saponins. They are amorphous substances having high molecular weight and are soluble in water and alcohol to produce foam but organic solvents inhibit their foaming property. Plants saponins are generally extracted into butanol through liquid-liquid partition and separated through column chromatography using silica gel as adsorbent and chloroform: methanol as mobile phase. HPLC, GC, Sephadex LH-20 Chromatography, DCCC, preparative paper chromatography and TLC were also used for the separation and isolation of saponins. The structures of saponins were determined by several spectroscopic techniques, viz., UV, IR, 1H NMR, 13C NMR and Mass spectroscopy. Saponins possess several biological activities such as antioxidant, immunostimulant, antihepatotoxic, antibacterial, anticarcinogenic, antiarrheal, antiulcerogenic, antioxoxygenic, antihypoglycemic, anticytotoxic and antimolluscicidal. Saponins are biologically synthesized by C5 isoprene units through cytosolic mevalonate pathway. 2,3-Oxidosqualene gives β-amyrin or triterpenoid skeletons on cyclization through isoprenoid pathway. The triterpenoid backbone then undergoes various modifications to form saponins.

Key words: Saponin, triterpenoid, isoprene, aglycone

INTRODUCTION

Saponins are recognized by their ability to produce a soapy lather when shaken with water. They are widely distributed in nature and reported to be present in 500 genera of plants. All saponins are polar in nature and are freely soluble in water but insoluble in non polar solvents. Saponins on hydrolysis yield an aglycone known as "sapogenin" and glycone known as sugar. A wide variety of plants belonging to family Liliaceae, Dioscoreaceae, Solanaceae, Sapindaceae and Agavaceae are the major source of saponins, however, a few neutral saponins have also been isolated and characterized from animal source. Steroidal saponins are widely distributed in nature and exhibit various biological activities. The aglycone of steroidal saponins is usually a spirostanol or its modification. They are found in oats, peppers, aubergine, tomato seed, alliums, asparagus, yam, fenugreek, yucca and ginseng. In Alkaloids saponins, aglycone carry N atom as a bridge between two rings e.g. solanidine (Ripperger and Schreiber, 1981) or ring F carrying –NH or –NCH3 e.g. hapepunine. Triterpenoid saponins are triterpene based glycosidic compounds, most of the triterpenoid compounds in adaptogenic plants are found as saponin glycosides which refers to the attachment of various sugar molecules to the triterpene unit. The sapogenins of these glycosides are tetracyclic or pentacyclic triterpenoids which on selenium dehydrogenation gives naphthalene and phenanthrene hydrocarbons, mainly sapotalene (1,2,7-teimethoxynaphthalene). Triterpenoids generally occur in family Leguminosae, Hippocastanaceae, Ranunculaceae, Symplocaceae, Euphorbiaceae, Verbenaceae and Araliaceae. Most of the triterpenoid sapogenins, with few exceptions, belong to β-amyrin group and are usually simple alcohol and acids. Occasionally sapogenin is encountered having aldehydic and lactone functional

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groups. Some examples of β-amyrin group triterpenoid aglycones are cincholic acid, oleanolic acid, gypsogenin, hederagenin, gummosogenin, erythrodiol and cochalic acid (Figure 1). The tetracyclic triterpenoid genins have also been reported, there are five examples namely panaxadiol, bryogenin, bacogenins, gratiogenin and cucurbitacins (Basu and Rastogi, 1967; Figure 2).

**PROPERTIES**

The structural complexity of saponins results in a number of physical, chemical and biological properties. Saponins are usually amorphous substances having high molecular weight. They are soluble in water and produce foam but organic solvent like chloroform, acetone and ether inhibit their foaming property. Solubility of saponins is also affected by the properties of the solvent (as affected by temperature, composition, and pH), while water and alcohols (methanol, ethanol) are the most common extraction solvents for saponins. Due to the presence of a lipid-soluble aglycone and water soluble sugar chain in their structure (amphiphilic nature), saponins are surface active compounds. In aqueous solutions
surfactants form micelles above a critical concentration called critical micelle concentration (cmc). The micelle forming property is affected by temperature, salt concentration and pH of the aqueous phase (Mitra and Dangan, 1997). Saponins possess a variety of biological activities such as antioxidant, immunostimulant, antihepatotoxic, anticarcinogenic, antidiarrheal, antiulcerogenic, antiyoxytoxic, hypocholesterolemic, anticoagulant, hepatoprotective, hypoglycemic, neuroprotective, antiinflammatory, inhibition of dental caries and platelet aggregation (Guclu-ustundag and Mazza, 2007; Rao and Gurfinkel, 2000) and also useful in diabetic retinopathy and reproduction. Many saponins are known to be antimicrobial to inhibit mould and to protect plants from insects. They may be considered as defense system and have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants.

EXTRACTION AND CHARACTERIZATION

Traditionally, saponins are extracted into water/ethanol mixtures, after which the ethanol is removed by distillation and the saponins extracted from the water phase into 1-butanol through liquid-liquid partition. Supercritical CO₂ extraction in combination with modifiers such as methanol, ethanol or aqueous methanol has proven successful (Guclu-ustundag and Mazza, 2007). High performance liquid chromatography is the most important method of choice for the separation of saponins. Both normal phase and reverse phase columns have been used. However, RP-HPLC with C₁₈ columns and gradient elution seems to be the most preferred
method. There are several strategies available for the isolation of saponins. They are separated by column chromatography on silica gel with mobile phase composed of CHCl₃-MeOH with increasing polarity. Sephadex LH-20 has successfully been used for the separation of steroidal saponins. The carbonyl group in steroidal saponins absorbs UV light in the range of 280-300 nm and ethylenic double bond appears at 195-198 nm. Due to lack of strong chromophore in some sapogenins they do not absorb UV light. IR spectra of saponins and sapogenins provided valuable information about the various functional groups and also stereochemistry of molecules to some extent. Spirostan derivatives showed absorptions between 1350-875 cm⁻¹. The relative intensities of absorptions around 920-950 cm⁻¹ and 900-884 cm⁻¹ permit a choice of 25 R or 25 S compounds. In 25 S the former is more intense than the latter whereas it is vice versa in 25 R configuration. In Hopane triterpenoids, IR spectra is useful for the determination of the substitution patterns of hopane 6α-, 15α-, 22α-, 7β-, 22, 24-triol. Peak at 1700-1702 cm⁻¹ indicates the presence of C-12 carbonyl group whereas at 1660-1680 cm⁻¹ suggest the conjugated carbonyl group. ¹H NMR spectrum of saponin peracetate or permethylate is helpful in determination of mode of sugar linkages. The signals of anomeric proton in the spectrum are assignable to that of a D-glucopyranose (or L-arabinopyranose) unit, the sugar may be regarded as having β-configuration. The J value suggests trans-dixial relationship of the proton at C1 and C2 of the pyranose residues. When anomeric proton signal appears with J= 1-3 Hz suggesting the equatorial-equatorial or axial-equatorial orientation of C1 and C2 proton. However, the anomeric proton signal of α-D-glucoside, α-D-mannoside, α-L-rhamnoside and β-L-arabinoside appears generally at lower field (δ 5.0-6.0) than those of corresponding β and α anomers respectively (δ 4.5-5.0). This difference is also helpful in the differentiation of the anomeric structure (Mahato et al., 1981). In both 25 R and 25 S series, the 27-methyl protons resonated upfield than the 21-methyl protons. Moreover, 27-methyl signal in 25 R appears upfield than in 25 S, hence these two isomers can be distinguished (Kutne 1963).

¹³C NMR spectroscopy is very useful tool for the structure elucidation of saponins. The chemical shift values for sugar moieties and for few steroidal sapogenins have been reported. The points of linkages are confirmed by the glycosylation shift rules (Kasai et al., 1979), according to which α- and β- carbon of the aglycones as well as sugar moieties undergo characteristic shifts on glycosylation. The α-C is shifted 6-9 ppm downfield whereas the β-C signals move slightly upfield. If 27-CH₃ is axial a small α effect is observed in C-25 signal. This signal appears 3-4 ppm upfield in 25 S neoyonogenins than that of 25 R yonogen. The difference of deoxytigogenin and tigogenin is reflected in the C-3 signal appearing 45 ppm downfield due to the electronegativity of oxygen in tigogenin. The presence of double bond at C-5 has remarkable effect on the chemical shift of C-5 and C-6 in diosgenin and tigogenin. The signal intensity of the carbonyl carbon is always very low and it is recorded in the range of 200-220 ppm. This can be explained by comparing hecogenin with tigogenin. The C-12 signal is recorded downfield at δ 213 ppm in hecogenin due to the influence of the doubly bonded oxygen. The C-11 and C-13 signals are also recorded downfield by 16.4 and 14.4 ppm, respectively, in hecogenin. Acetylated C-3 OH group causes a downfield shift of about 2.2 ppm for C-3 (α-effect) and upfield shift of 4 ppm for C-2 and C-4 signals (β-effect). Electron ionization mass spectroscopy (EI-MS) has been shown to be a very useful method for identification and structure elucidation of saponins (Kasai et al., 1977). Saponins containing more than four sugars do not give molecular ions, even when derivatized. However, MS has limited application in the field of underivatized oligosaccharides because it required volatilization and ionization of the sample. Ionization and volatilization are coupled in one process in field desorption mass spectrometry (FD-MS). FD-MS of underivatized steroidal and triterpenoidal saponins have been reported. The spectra showed the intense ions formed by attachment of alkali cation to the neutral molecule. A new technique of plasma desorption mass spectrometry (PD-MS) has also been used for molecular weight determination of underivatized steroidal saponins (Hostettmann et al., 1978). The positions of various substituents in the sapogenin are determined by estimating the shifts in the masses of the characteristic fragments with relation of the peaks of the standard.

BIOSYNTHESES

The sequence of enzyme catalyzes reactions by which complex molecules in living cells are formed from nutrients with relatively simple structures are known as biosynthesis. Triterpenes belong to a large group of compounds arranged in four or five ring configurations of 30 carbons attached with several oxygens. These are formed by assembly of C5 isoprene units through the cytosolic mevalonate pathway to make C30 compounds. They are synthesized via the isoprenoid pathway by cyclization of 2,3-oxidosqualene to give primarily oleanane (β-amyrin) or dammarane triterpenoid skeletons. The triterpenoid backbone then undergoes various modifications (oxidation, substitution and glycosylation), mediated by glycosyltransferases and other enzymes. The cyclization of 2,3-oxidosqualene to lanosterol and cycloartenol skeleton is initiated by participation of a neighbouring π-bond via protosteryl C-20 cation. This cation then undergoes a series of 1,2-methyl and hydride shifts with proton elimination to yield either lanosterol or cycloartenol skeleton. The cyclisation of 2,3-oxidosqualene to sterols and triterpenoids
Figure 3. Biosynthesis of C_{27} sapogenins.

represents a bridge point between primary and secondary metabolism (Henry et al., 1992).

In lanosterol biosynthesis two 1,2-hydride shifts take place from protosteryl cation from C-17 and then two 1,2-methyl shifts occur from 14β to 13β and from 8α to 14α accompanied by elimination of C-9β proton. The mechanism of cyclization of oxidosqualene into cycloartenol is the same as lanosterol except the final 9β,19-cyclopropane ring closure instead of C-9 hydrogen migration. The steroidal sapogenins are spiroketals having same configuration at C-22 but stereoisomerism at C-25 as in cholesterol. Hydroxycholesterol was shown to be the first intermediate which was converted to diosgenin (Figure 3). And diosgenin on reduction is converted to tigogenin (Tschesche et al., 1968; Tschesc et al., 1970) but not to yamogenin, however, yamogenin obtained from (25S)-hydroxycholesterol. Yamogenin converted into neotigogenin on reduction and (25S)-hydroxycholesterol was shown to be the key intermediate in the formation of neotigogenin (Ronchetti et al., 1975).

CONCLUSION

Saponins possesses a variety of biological activities such as antioxidant, immunostimulant, antihypertoxic, antibacterial, anticarcinogenic, antidiarrheal, antilucreogenic, antioxytocic, antihypoglycemic, anticytotoxic and antimolluscicidal. Saponins are biologically synthesized by C5 isoprene units through cytosolic mevalonate pathway. They may be considered as defense system and have been included in a large group of protective molecules named phytoanticipins or phytoprotectants.

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