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Triterpene saponins from Polygalaceae

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Abstract

The aim of this review is to give updated results on the chemistry and biological activities of Polygalaceae saponins from literature data and from authors' studies. During the last decade the improvement of isolation and structure elucidation techniques allowed the characterization of about 150 complex triterpene saponins from Polygalaceae having mainly preatroxigenin, medicagenic acid and presenegenin as aglycone. The sequence 3-*O*-(*β*-D-glucopyranosyl)-presenegenin 28-[*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-fucopyranosyl] ester with additional acylations with *p*-methoxy-, di- or trimethoxycinnamoyl groups at the position 4 of the fucopyranosyl moiety often encountered in the genera *Polygala*, *Muraltia*, *Carpolobia*, and *Securidaca* may represent a chemotaxonomic marker for the Polygalaceae family. The biological and pharmacological properties of some Polygalaceae species such as *in vitro* activity on the central nervous system, the hypoglycemic, cytotoxic, immunomodulatory activities and *in vivo* immunoadjuvant properties are highlighted.

Introduction

The last review on the Polygalaceae family dated from 1992 (Delaude, 1992). At that time, only aglycones were characterized, and saponins were scarcely studied. Polygalaceae belonging to the order of Fabales represent a well individualized family with around 800 species spread over a limited number of 12 to 20 genera. The family is divided into three tribes: Xanthophyllae, Moutabae, and Polygalae. *Polygala* is the most important genus in number of species. It represents about the half of the members of the family. This genus has a cosmopolite geographic distribution except New Zeland, Polynesia and antartic zones whereas *Carpolobia*, *Atroxima*, *Muraltia*, and *Securidaca* belong to Africa.

From a therapeutic point of view, the main traditional uses of Polygalaceae are summarized as following: *P. senega* is used in western herbal medicine as expectorant to treat cough, bronchitis and asthma and *P. tenuifolia* is used in Traditional Chinese Medicine for the same purposes (Delaude, 1992). Furthermore, it has also been used in Traditional Korean medicine as sedative and as an antipsychotic agent (Chung et al. 1992). In Southern China, *P. fallax* is used as a tonic and antihepatitis agent whereas *P. japonica* as an expectorant, antiinflammatory and antibacterial agent (Zhang et al., 1995a). *P. myrtifolia* is used as poltice against gout in South Africa. *S. longepedunculata* is used in Africa to treat cough, bronchitis, cooling, fever, malaria, inflammation, rheumatism, snakebite, and leprosy. Furthermore, it is emetic, purgative, and diuretic (Delaude 1992).

Several species were reported to contain triterpene saponins. Most of them have presenegenin as aglycone and differ from each other in their glycosidic part. Consequently, this review will summarize updated phytochemical studies on the

Polygalaceae saponins from literature data and from authors' team. Then the biological and pharmacological properties of these compounds will be reported.

Status of the literature data on the structure of the saponins

Usually, a 70% aqueous ethanolic extract of the aerial parts of Polygalaceae species was passed through a porous polymer gel Mitsubishi Diaion HP-20 column. The absorbed materials were eluted with 50% aqueous MeOH and MeOH successively. The MeOH eluate was chromatographed on silica gel and octadecyl silica (ODS) followed by repeated semi-preparative HPLC on a reversed phase column (ODS, phenylalkyl silica (PhA)) to afford the isolated saponins (Zhang et al., 1995a,b; 1996a,b,c; 1998).

During the period 1992–2002, about 100 saponins were isolated and characterized on the basis of spectroscopic data (see refs in Figure 1). The genins of Polygalaceae saponins are strongly oxygenated pentacyclic triterpenoids whose structure are closely related. Among them, some fifty presenegenin glycosides have been characterized in many species such as *P. senega, P. tenuifolia, P. glomerata, P. fallax, P. reinii, P. japonica, P. arillata, Bredemeyera floribunda, Securidaca inappendiculata*, whereas saponins having bayogenin, polygalagenin, medicagenic acid and hederagenin as aglycones were found only in *P. japonica* (Figure 1). Most of the saponins based on the above aglycones are bidesmosidic glycosides having one or two glucoses at C-3, and one to six sugars at C-28, some of them being acylated by either *p*-, or di-, or trimethoxycinnamic acids and/or by acetic acid.

Phytochemical studies of the Polygalaceae in the authors' laboratory

Initially, we have isolated and characterized an unusual tri-*O*-acetyl-tri-*O*-benzoyl tetrasaccharide called amarelloside from the European species *P. amarella* (Dubois et al., 1989). Since the family of Polygalaceae represents a rich source of triterpene saponins as mentioned above, we decided to continue the study of the saponins of *Polygala amarella* and other Polygalaceae from West and South Africa, belonging to the genera *Atroxima, Polygala, Carpolobia, Muraltia*, and *Securidaca* in order to confirm chemotaxonomic significance and to find new biologically active compounds mainly in the field of immunology.

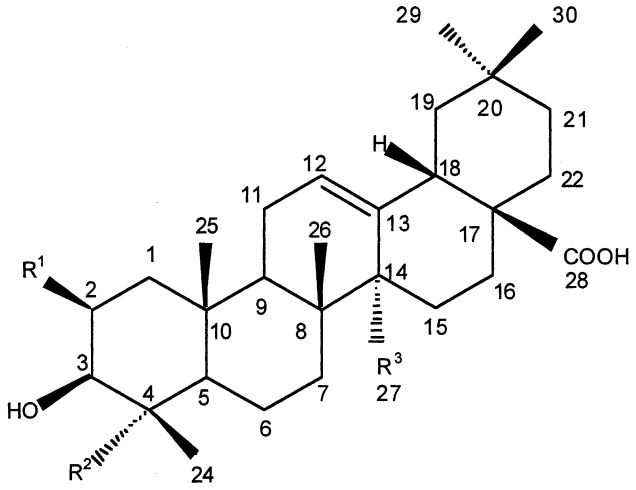
The procedure of saponin extraction was achieved according to a methodology described by Delaude, 1971. The maceration of the roots with 80% MeOH yielded after evaporation of the solvent to dryness an extract which was purified by precipitation in Et_2O, dialysis and decolorization with charcoal yielding the crude saponin mixture. The purification of this mixture was achieved by CC on Sephadex LH 20 (MeOH) and successive MPLC on Silica gel ($CHCl_3$/MeOH/H_2O 8:5:1 and 13:7:2, lower phase) and reversed phase Silica gel (MeOH–H_2O gradient). In the case of *A. congolana*, *P. arenaria*, *M. heisteria* and *M. satureioides*, the saponins were obtained as inseparable mixtures of *E*/*Z* cinnamoyl glycosides which were homogeneous by HPTLC but were separated into *E* and *Z* isomers by HPLC. All attempts to separate them by semi-preparative HPLC on a Vydac C-18 column (eluted in isocratic conditions by a mixture of 27% acetonitrile in water containing 0.06% trifluoroacetic acid) were unsuccessfull (Elbandy et al., 2003a, 2003b). This is due to the well known tautomeric – like comportment of these *E*/*Z* derivatives under light and in methanol solutions (Yoshikawa et al., 1995a).

All structures were elucidated mainly by extensive high field 1D and 2D NMR spectroscopic techniques and by mass spectrometry including FABMS and HRESIMS (Gaidi et al., 2002). By this way, around 60 saponins were characterized in the authors' laboratory from one European and six African species and they will be presented below according to the nature of their aglycone preatroxigenin, medicagenic acid and presenegenin.

Seven pairs of new preatroxigenin glycosides **1/2–13/14** called atroximasaponins A1/A2–G1/G2 were isolated from *Atroxima congolana* (Elbandy et al., 2003a, 2003b, Figure 2). All compounds possess the same aglycone preatroxigenin and one β-D-glucopyranose linked at C-3. They differ from each other by the nature of the glycosidic ester chain at C-28 which contains three to six sugars eventually acetylated. Compounds **3/4** are the acetylated derivatives of **1/2** whereas **11/12** are the

acetylated forms of **9/10**. Compounds **5/6** and **7/8** share the same sequence of 5 sugars at C-28 and differ by the 6^{th} sugar which is an acetyl-β-D-glucopyranosyl unit in **5/6** instead of a β-D-xylopyranosyl unit in **7/8**. Furthermore, the fucopyranosyl moiety located at C-28 is always acylated by a *E*/*Z*-4-methoxycinnamic acid.

The study of *Muraltia ononidifolia* (Delaude, 1990, 1991) led to the isolation and structural elucidation of seven saponin derivatives of medicagenic acid, five of them being new (**15–19**) (Elbandy et al., 2002a, Figure 3). This aglycone is relatively rare in this family and was only present in *P. japonica* (Zhang et al., 1996b). They contain one or two β-D-glucopyranosyl units at C-3 and two to four glycosidic units linked at C-28. Three compounds (**15–17**) contain a β-D-apiofuranosyl moiety, a relatively rare sugar in saponins. Compound **16** differs from **15** by the linkage position of the β-D-apiofuranosyl moiety whereas **17** differs from **16** only by the presence of an additional β-D-glucopyranosyl unit at C-3. With the exception of **19** which has a free C-28 position, all the saponins of *M. ononidifolia* share the same sequence at C-28

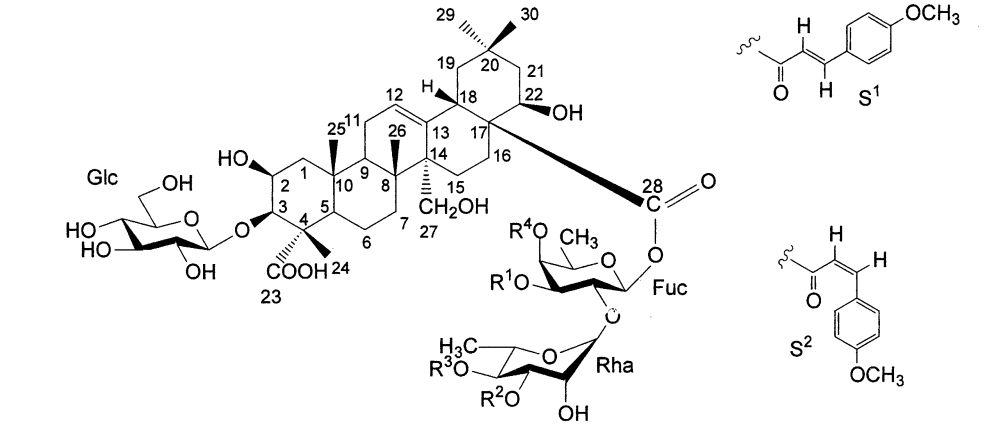


	R^1	R^2	R^3	Plants	References
Presenegenin	OH	COOH	CH_2OH	*P. senega, P. tenuifolia*	Pelletier et al., 1971,
				P. senega var. latifolia	Tsukitani et al., 1973a, 1973b; Yoshikawa et al., 1995 a, 1995b
				P. glomerata	Zhang et al. 1998
				P. fallax	Zhang et al., 1996a
				P. reinii	Miyase et al., 1995
				P. japonica	Zhang et al., 1996c
				P. arillata	Teng et al., 2002
				S. inappendiculata	Kuroda et al., 2001
				B. floribunda	Daros et al., 1996
Bayogenin	OH	CH_2OH	CH_3	*P. japonica*	Zhang et al., 1995a,b
Polygalagenin	OH	CHO	CH_3	*P. japonica*	Zhang et al., 1995b
Medicagenic acid	OH	COOH	CH_3	*P. japonica*	Zhang et al., 1996b
Hederagenin	H	CH_2OH	CH_3	*P. japonica*	Zhang et al., 1996b

Figure 1. Structure of the main aglycones of Polygalaceae saponins.

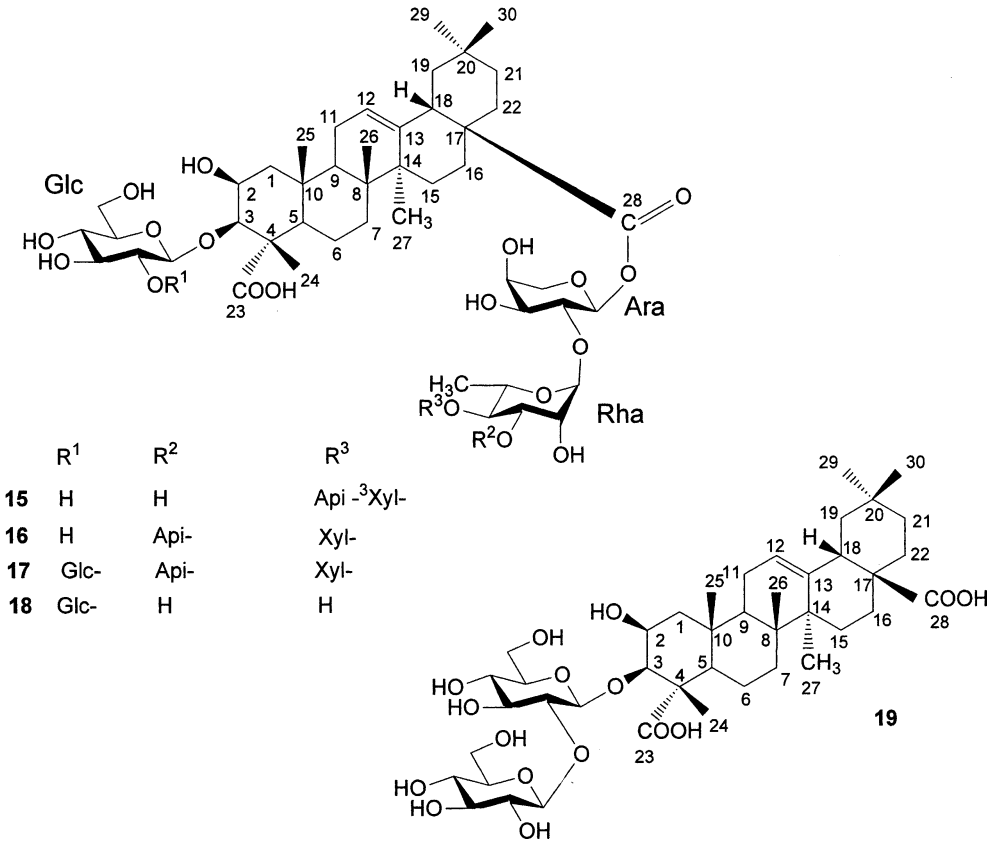
constituted by the disaccharidic unit α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside.

The third group of saponins is presented by around 40 presenegenin glycosides in some *Muraltia, Polygala* and *Carpolobia* species (Figure 4). From *M. heisteria*, five pairs of presenegenin glycosides **20/21–28/29** have been isolated. Three of them are new and are also found in *M. satureioides* (Elbandy et al., 2002b, 2004). All compounds contain one β-D-glucopyranosyl unit at C-3 and one glycosidic chain at C-28 of three to six sugars, eventually acylated. Once again, the acylation of fucose at C-4 by *E*/*Z*-3,4,5-trimethoxycinnamic acids explains that these compounds were obtained as inseparable mixtures. From the callus tissue culture of the European species *P. amarella*, the new saponin **30** without any acyl group was isolated (Desbène et al., 1999) together with the known polygalasaponin XXVIII (**31**) previously isolated from *P. japonica* (Zhang et al., 1996c).



	R^1	R^2	R^3	R^4
1	Glc-^{3}Glc-	H	Xyl-	S^1
2	Glc-^{3}Glc-	H	Xyl-	S^2
3	Glc Ac-^{3}Glc-	H	Xyl-	S^1
4	Glc Ac-^{3}Glc-	H	Xyl-	S^2
5	Glc Ac-^{3}Glc-	Api-	Xyl-	S^1
6	Glc Ac-^{3}Glc-	Api-	Xyl-	S^2
7	Xyl-^{3}Glc-	Api-	Xyl-	S^1
8	Xyl-^{3}Glc-	Api-	Xyl-	S^2
9	Glc-	H	Xyl-	S^1
10	Glc-	H	Xyl-	S^2
11	Glc Ac-	H	Xyl-	S^1
12	Glc Ac-	H	Xyl-	S^2
13	Api-	H	H	S^1
14	Api-	H	H	S^2

Figure 2. Preatroxigenin glycosides.

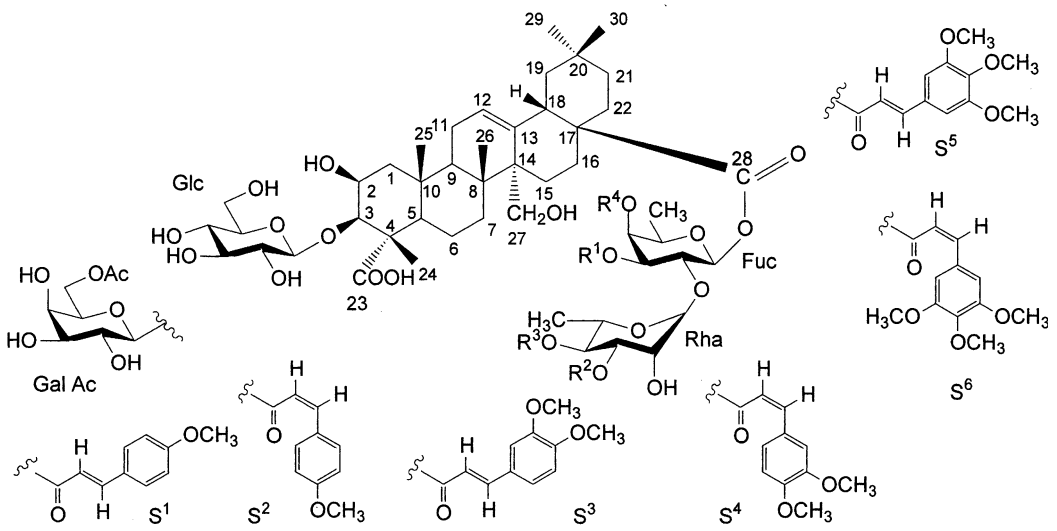


	R^1	R^2	R^3
15	H	H	Api -^{3}Xyl-
16	H	Api-	Xyl-
17	Glc-	Api-	Xyl-
18	Glc-	H	H

Figure 3. Medicagenic acid glycosides.

Four pairs of new presenegenin glycosides **32/33–38/39** were isolated from *P. arenaria* (Mitaine-Offer et al., 2003). The pairs **32/33** and **34/35** share the same sequence at position 4 of the α-L-rhamnopyranosyl unit which is a branched trisaccharide β-D-galactopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 3)]-β-D-xylopyranosyl- and differ only by the substitution at the position 4 of the β-D-fucopyranosyl unit, which is a *E*/*Z*-4-methoxycinnamoyl group in the case of **32/33** and a *E*/*Z*-3,4-dimethoxycinnamoyl group in the case of **34/35**. The third and fourth pairs **36/37** and **38/39** contain at the position 4 of the α-L-rhamnopyranosyl unit a disaccharide β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl- and is substituted by the same acyl residues as in **32/33** and **34/35**, respectively. The five pairs of presenegenin glycosides from *P. myrtifolia* (**40/41–48/49**) (Haddad et al., 2003) are substituted at the position 4 of the fucopyranosyl unit by a *E*/*Z*-4-methoxycinnamoyl group whereas the glycosides of *Carpolobia alba* (**50–52**) are substituted at the positions 3 and 4 by one acetyl group (Mitaine-Offer et al., 2002). Compounds **50** and **51** are common to both species C. alba and *C. lutea*.

Most of the saponins characterized in *Muraltia* (Elbandy et al., 2002b, 2004), *Polygala* (Sakuma and Shoji, 1981a, 1981b; Miyase et al., 1995; Yoshikawa et al., 1995a, b; Zhang et al., 1996a, 1996b, 1998; Desbène et al., 1999; Haddad et al., 2003; Mitaine-Offer et al., 2003), *Carpolobia* (Mitaine-Offer et al., 2002), and *Securidaca* (Kuroda et al., 2001), shared the same sequence 3-*O*-(β-D-glucopyranosyl)-presenegenin 28-[*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-fucopyranosyl] ester which may represent a chemotaxonomic marker for the Polygalaceae family. Furthermore, this sequence is completed by one additional glucosylation of the 3-*O*-(β-D-glucopyranosyl) part and acylations at positions 3 and 4 of the fucopyranosyl unit. Position 3 can be acylated by one acetyl group whereas the position 4 was acylated



	R^1	R^2	R^3	R^4
20	H	Gal-	Api-(1-3)[Xyl-(1-2)]Ara-	S^5
21	H	Gal-	Api-(1-3)[Xyl-(1-2)]Ara-	S^6
22	H	H	Ara-^{4}Gal-^{3}Xyl-	S^5
23	H	H	Ara-^{4}Gal-^{3}Xyl-	S^6
24	Gal Ac	Api-	Xyl-	S^5
25	Gal Ac	Api-	Xyl-	S^6
26	Gal Ac	Ac	H	S^5
27	Gal Ac	Ac	H	S^6
28	Xyl-	Ac	H	S^5
29	Xyl-	Ac	H	S^6
30	Glc-	H	Gal-^{3}Xyl-	H
31	H	H	Xyl-	H
32	H	H	Gal-(1-4)[Glc-(1-3)]Xyl-	S^1
33	H	H	Gal-(1-4)[Glc-(1-3)]Xyl-	S^2
34	H	H	Gal-(1-4)[Glc-(1-3)]Xyl-	S^3
35	H	H	Gal-(1-4)[Glc-(1-3)]Xyl-	S^4
36	H	H	Glc-^{3}Ara-	S^1
37	H	H	Glc-^{3}Ara-	S^2
38	H	H	Glc-^{3}Ara-	S^3
39	H	H	Glc-^{3}Ara-	S^4
40	Ara-	Api-	Gal-^{3}Xyl-	S^1
41	Ara-	Api-	Gal-^{3}Xyl-	S^2
42	H	Api-	Gal-^{3}Xyl-	S^1
43	H	Api-	Gal-^{3}Xyl-	S^2
44	H	H	Gal-^{3}Xyl-	S^1
45	H	H	Gal-^{3}Xyl-	S^2
46	H	Api-	H	S^1
47	H	Api-	H	S^2
48	H	Ara-	H	S^1
49	H	Ara-	H	S^2
50	Ac	H	Gal-(1-4)[Xyl-(1-3)]Xyl-	Ac
51	Ac	H	Gal-(1-4)[Ara-(1-3)]Xyl-	Ac
52	Ac	Api-	Xyl-	Ac

Figure 4. Presenegenin glycosides.

by either *E*/*Z*-4-methoxy-, or *E*/*Z*-3,4-dimethoxy-, or *E*/*Z*-3,4,5-trimethoxycinnamoyl groups, or by one acetyl group.

Biological and pharmacological activities of Polygalaceae saponins

Plant saponins are a group of naturally occuring glycosides including a large number of biologically and pharmacologically active compounds (Lacaille-Dubois and Wagner, 1996, 2000; Lacaille-Dubois, 1999, 2000). We will summarize below some of the recent advances concerning activity on the central nervous system (CNS), hypoglycemic, cytotoxic and immunoadjuvant properties of Polygalaceae saponins. Then, results of the authors' laboratory on the immunomodulating activity of saponins of *P. amarella* and *P. arenaria* will be presented.

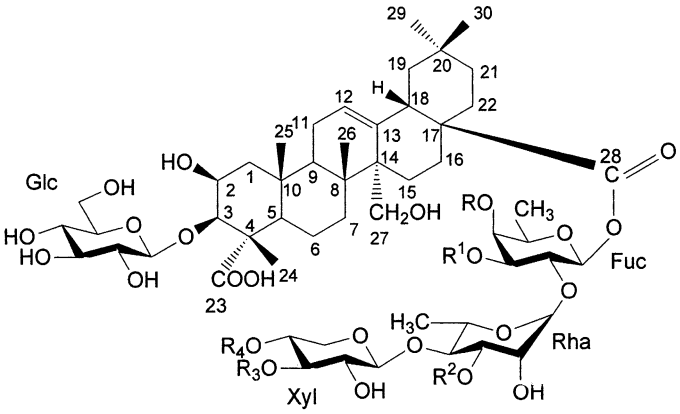
P. tenufolia used as an expectorant in Korean traditional medicine, has also been used as a sedative and an antipsychotic agent (Chung et al., 1992). Several *in vivo* experiments in mice have shown that Polygalasaponin (25–500 mg/kg) from *P. tenuifolia* (Korean patent No. 0262072) produced a dose-related inhibition of apomorphine (2.5 mg/kg sc)-induced climbing behavior, reduced the behaviors produced by 5-hydroxytryptamine (5-HTP) (50 mg/kg sc) and inhibited hyperactivity induced by MK-801 (0.3 mg/kg ip), both in a dose-dependent manner. Furthermore, Polygalasaponin produced a partial inhibition of the hyperactivity induced by cocaine (40 mg/kg ip) in rats (Chung et al., 2002). These results showed that Polygalasaponin from *P. tenuifolia* can prevent the behaviors that are mediated by both dopamine-2 and serotonin-2 receptors in the CNS in a dose-related manner. Since the monoamine receptors are presumed to be involved in mental disorders, this *in vivo* activity may suggest the therapeutic potential of this saponin in the treatment of psychotic disturbances (Chung et al., 2002).

Onjisaponins A-B, E-G bidesmosidic presenegenin glycosides acylated respectively with *E*/*Z*-4-methoxycinnamoyl (A-B) and *E*/*Z*-3,4,5-trimethoxycinnamoyl groups (E-G) (Figure 5) , as major saponins from the roots of *P. tenuifolia* were found to be active on the CNS. After 24 h incubation with each saponin (0.1–10 μg/ml), each medium was collected and the neurotrophin, such as the nerve growth factor (NGF) content was determined by ELISA. Onjisaponins were shown to significantly increase NGF secretion from cultured astrocytes. Furthermore, onjisaponin F (1 or 10 μg/ml) was shown to induce choline acetyl transferase (ChAT) mRNA expression of the rat basal forebrain cells by using the RT-PCR method. These results indicate the possibility that onjisaponins may have potential therapeutic effects for the treatment of Alzheimer Disease (AD) patients (Yabe et al., 2003).

Two acylated presenegenin glycosides from the rhizomes of *P. senega* var. *latifolia*, senegins II and III (Figure 5) at a dose of 1 and 5 mg/kg were shown to reduce the blood glucose level 4 h after intraperitoneal administration to normal mice and KK-Ay mice, an animal model of obese non-insulin-dependent diabetes mellitus (NID-DM) with hyperinsulinemia (Kako et al., 1997). This activity was dose-dependent and superior to that of the positive control tolbutamide. It is interesting to note that these saponins produce a significant hypoglycemic effect, indicating that such compounds may be useful for treating NID-DM.

Securiosides A and B, acylated bidesmosidic presenegenin glycosides isolated from *Securidaca inappendiculata* roots (Figure 5) showed potent selective cytotoxic activity against M-CSF-stimulated macrophages from CH 3/He male mice with $IC_{50} < 0.25$ μM. They were suggested to have potential as new agents for the treatment of pathological states in which macrophage proliferation occurs such as tumor, inflammation and atherosclerosis (Kuroda et al., 2001, Yui et al., 2001). The corresponding deacyl derivatives were not cytotoxic themselves underlining the importance of the 3,4-dimethoxycinnamoyl group at the position 4 of the fucopyranosyl residue. The selective macrophage death by these saponins appeared not to be mediated by a membrane effect, but through induction of apoptosis. Namely, they did not hemolyze sheep erythrocytes even at the sample concentration of 10 μg/ml but induced change in macrophage morphology and DNA fragmentation.

Formulation of effective vaccines generally requires an appropriate adjuvant to optimize protective humoral and cell mediated immune responses (Lacaille-Dubois, 1999). If the saponins from *Quillaja saponaria* (Rosaceae) which are quillaic acid derivatives acylated by a dimeric fatty acid and their analogs have been extensively



	R	R^1	R^2	R^3	R^4
Onjisaponin A	4-methoxycinnamoyl-	Rha-	Api-	H	Gal-
Onjisaponin B =Senegin III	4-methoxycinnamoyl-	Rha-	H	H	Gal-
Onjisaponin E	3,4,5-trimethoxycinnamoyl-	H	H	H	Gal-
Onjisaponin F	3,4,5-trimethoxycinnamoyl-	H	Api-	Ara-	H
Onjisaponin G	3,4,5-trimethoxycinnamoyl-	H	Api-	H	H
Senegin II	3,4-dimethoxycinnamoyl-	H	H	H	Gal-
Securioside A	3,4-dimethoxycinnamoyl-	Glc^6-Ac	H	H	H
Securioside B	3,4-dimethoxycinnamoyl-	H	H	Ara-	Gal-

Figure 5. Structure of bioactive saponins.

studied (Kensil et al., 1991) and used as immunoadjuvants in veterinary vaccines, few saponins from other sources have been reported to possess this property except some families including the Polygalaceae. Two saponin fractions PS-1 and PS-2 of *P. senega* were shown to increase specific antibody level to the antigens, both in mice immunized with OVA and in hens immunized with rotavirus in a manner comparable to Quil A (a mixture of partially purified saponins from *Q. saponaria*) (Estrada et al., 2000). In mice, there was a preferential increase of the lgG2a subclass. The subsequent *in vitro* production of IL-2 and IFN-γ by lymphocytes in spleen cell culture supernatants in response to a secondary *in vitro* OVA antigen stimulation was also enhanced. Since the saponins were found less toxic at the same dose than Quil A saponins, well known as potent adjuvants, these saponin fractions might represent a promising source of vaccine adjuvants and needed further structural requirements.

Onjisaponins A and E-G (Figure 5) from *P. tenuifolia* (10 μg each) were shown to have mucosal adjuvant activities when administered intranasally in mice with influenza HA and diphteria-pertussis-tetanus (DPT) vaccines (Nagai et al., 2001). After primary administration of influenza vaccine with onjisaponins, re-inoculation with influenza vaccine alone three weeks latter, enhanced both nasal anti-influenza virus IgA and serum hemagglutination-inhibiting (HI) antibody (Ab) titers over those mice initially administered influenza vaccine alone (Nagai et al., 2001). Similar results were obtained by intranasal inoculation of onjisaponins with DPT vaccine which showed significant increases in serum IgG and nasal IgA antibody titers after two weeks following secondary vaccination over mice inoculated with DPT vaccine alone (Nagai et al., 2001). All onjisaponins were not hemolytic up to 100 μg/ml and onjisaponins E and F were not hemolytic at even 200 μg/ml suggesting that they might provide safe and potent adjuvants for intranasal inoculation of influenza HA and DPT vaccines. Since Quil A, a potent adjuvant was not used in human vaccines of its high toxicity, and QS 21, a purified saponin from Quil A was shown to be a potent adjuvant and hemolytic at 25 μg/ml, onjisaponins may be

considered safer than both Quil A and QS 21 when used as vaccine adjuvants. The presence of a carboxyl group at C-23 instead of a formyl group as in *Quillaja* saponins can be just as effective for inducing adjuvant activity. A correlation between adjuvant activity and amphipathic structure of saponin was established by comparison of the antibody response against chicken ovalbumin (OVA) in mice and hydrophile–lipophile balance (HLB, ratio of hydrophilic sugar chain to lipophilic aglycone) of structurally analogs of soyasaponins (Oda et al., 2003). The saponins bearing sugar chains showed adjuvanticity stimulating anti-OVA total IgG and IgG1 antibody responses, while their corresponding aglycones did not. This finding supporting a relationship between increasing adjuvant activity and HLB value was observed not only for soyasaponins but also for many other saponins such as *Gypsophila* saponins (HLB 39.4), QS 21 from *Q. saponaria* (HLB 36.3) and onjisaponin A from *P. tenuifolia* (HLB 20.3) (Oda et al., 2003).

After these literature data on the cytotoxic and immunoadjuvant activities of Polygalaceae saponins, the following deals with results obtained in the authors' laboratory. Presenegenin glycosides **30** and **31** (Figure 4) isolated from the callus tissue cutures from the seeds of *Polygala amarella* showed significant immunological properties based on the enhancement of granulocyte phagocytosis *in vitro* (Desbene et al., 1999). The saponin fraction containing a mixture of **32/33** and **34/35**, acylated presenegenin glycosides from. *P. arenaria* (Figure 4) was tested in an *in vitro* lymphocyte proliferation assay on Jurkat T-leukemia cells (Mitaine-Offer et al., 2003). The cellular proliferation was measured by [^{3}H]thymidine incorporation in the DNA of the cells (Gaidi et al., 2002). The test compounds, in the concentration range 10^{-4}–10μM showed a concentration-dependent immunomodulatory effect. They stimulated weakly Jurkat cell proliferation in the concentration range 10^{-4}–1 μM with a maximal stimulation index (SI) of 1.25 and in the range 5–10 μM inhibition of the proliferation was observed with a SI of 0.14. Their prosapogenin, tenuifolin (3-*O*-β-D-g1ucopyranosyl-presenegenin) displayed a proliferative activity with a SI of 1.37, but was not cytotoxic up to a concentration of 10 μM. These results underlined the importance of the oligosaccharidic ester chain at C-28. In order to see which part of the glycosidic ester chain was important for the activity, we tested other saponins. Those of *Muraltia ononidifolia* (**15–19**) without 4-methoxycinnamoyl groups (Figure 3) were not cytotoxic at all tested concentrations, whereas the saponins **22/23** acylated by a trimethoxycinnamic acid from *M. heisteria* (Figure 4) showed a cytotoxic effect from 10μM which was abrogated after deacylation (Unpublished results, 2004). This result corroborated previous results obtained with jenisseensosides from *Silene jenisseensis* (Gaidi et al., 2002) showing the importance of the acyl group linked at the position 4 of fucose at C-28. This ester moiety can increase the lipophilicity of the molecule and the membrane permeabilisation effect which might be responsible of the saponins' toxicity of Jurkat cells.

Conclusion

Polygalaceae is a rich source of triterpene saponins particularly in the genus *Polygala*. This review gives an important contribution of chemical and biological literature data during 1992–2002 and updated reports on newly isolated saponins in the authors' laboratory having preatroxigenin, medicagenic acid and presenegenin as aglycone in *Atroxima, Muraltia, Polygala* and *Carpolobia* species.

Most saponins from these species shared the same sequence 3-*O*-(β-D-glucopyranosyl)-presenegenin 28-[O-α-L-rhamnopyranosyl-(1 $\rightarrow$ 2)-β-D-fucopyranosyl] ester which may represent a chemotaxonomic marker for the Polygalaceae family. Furthermore, this sequence is completed by one additional glucosylation of the 3-*O*-(β-D-glucopyranosyl) unit and acylations at the positions 3 and 4 of the fucopyranosyl residue. Position 3 can be acylated by one acetyl group whereas position 4 was acylated by either *E*/*Z*-4-methoxy-, or *E*/*Z*-3,4-dimethoxy-, or *E*/*Z*-3,4,5-trimethoxycinnamoyl groups, or by one acetyl group. The reported bioactivities of Polygalaceae family concern CNS activity, hypoglycemic, cytotoxic, immunoadjuvant and lymphoproliferative effects. These latter were demonstrated for the saponins of *P. arenaria* (**32–35**) in Jurkat cells at low concentrations whereas cytotoxic properties were observed from 1μM. After alkaline hydrolysis, the cytotoxic effect was abrogated and the prosapogenin tenuifolin (3-*O*-β-D-glucopyranosyl-presenegenin) showed an immunostimulant activity at all

tested concentrations, underlining the importance of the oligosaccharidic ester chain at C-28 in the cytotoxicity of Jurkat cells.

It is hoped that this review might contribute to bring a rational basis to the medicinal uses of some Polygalaceae species.

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