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Review

Effects of triterpenes on the immune system

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ABSTRACT

Ethnopharmacological relevance: Triterpenes, which comprise a broad chemical group of active principles, are implicated in the mechanisms of action and pharmacological effects of many medicinal plants used in folk medicine against diseases in which the immune system is implicated. They have been described as anti-inflammatory, antiviral, antimicrobial, and antitumoral agents, as well as being immunomodulator compounds. Several of them are implicated in the resolution of immune diseases, although their effects have not always been clearly correlated.

Aim of the review: The aim of this review is to compile relevant data on the mechanisms of action of triterpenes isolated from active ethnomedicinal plants and their role in the resolution of diseases in which the immune system is implicated to examine the mechanism by which they are useful as ethnopharmacological medicines.

Methods: The selection of papers was made using the most relevant databases for the biomedical sciences on the basis of their ethnopharmacological use. We principally chose those studies that examined the resolution of allergic responses *in vivo* and those that studied the effects of the more relevant mediators implicated in the immune response *in vitro*.

Results: The number of compounds actually studied is limited compared with the high number of principles that have been isolated and identified. Many studies focus on specific pathologies such cancer or inflammation, but in many cases they are clearly correlated with the immune response. Lanostanes, cucurbitanes, and oleananes are probably the most interesting groups; however, other compounds are also of potential importance.

Conclusions: Studies of specific mechanisms against mediators or transcription factors could be the objective for future research on ethnomedicinal plants used to combat immune diseases since the results obtained with cucurbitacins or derivatives of oleanolic acid support the use of different medicinal plants, thereby opening up a new frontier for future studies.

Contents

Abbreviations: AIDS, acquired immunodeficiency syndrome; APC, antigen-presenting cells; bZIP, basic leucine zipper; Con A, concanavalin A; COX, cyclooxygenase; DTH, delayed-type hypersensitivity; DNCB, dinitrochlorobenzene; DNFB, dinitrofluorobenzene; ERK, extracellular signal-regulated kinase; GM-CSF, granulocyte macrophage-colony stimulating factor; 5-HETE, 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid; HIV, human immunodeficiency virus; ICAM, intercellular adhesion molecule; IFN-γ, interferon-γ; Ig, immunoglobulin; IκB, inhibitor protein of NF-κB; IKK, IκB kinase; IL, interleukin; JNK, c-Jun N-terminal protein kinase; LOX, lipoxygenase; LPS, lipopolysaccharide; LT, leukotrienes; MHC, major histocompatibility complex; NF-AT, nuclear factor-AT; NF-κB, nuclear factor-κB; Nrf2, nuclear factor-erythroid 2-related factor 2; NK, natural killer; NO, nitric oxide; NOS, nitric oxide synthase; PG, prostaglandins; PHA, phytohemagglutinin A; SRBC, sheep red blood cells; STAT, signal transducers and activators of transcription; TAK1, transforming growth factor-β activated kinase-1; Tc, cytotoxic T cells; TCR, T cell receptor; TGF, transforming growth factor; TNF-α, tumor necrosis factor-α; VCAM, vascular cell adhesion molecule.

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1. Introduction: immunology and immune diseases

The objective of this review is to examine the role triterpenoids have in medicinal plants used against inflammatory diseases, especially those in which an immune response is implicated. In addition, a selection of several structurally related compounds has been compiled in order to analyze the possible structural characteristics and relationships between the different types of structures found in triterpenoids. The selection of active species was made on the basis of their immunomodulatory activity, as is the case with *Ganoderma lucidum*, *Panax ginseng*, *Tripterygium wilfordii*, *Boswellia serrata*, *Quillaja saponaria*, *Borassus flabellifer*, and *Cayaponia tayuya*, but other related species were selected for their potential and their relationship with the aforementioned species.

Immunity is the physiological defense of an organism against infectious diseases such as those caused by bacteria and viruses. Whereas specific cells and mediators are responsible for immunity itself, the collective and coordinated response against strange substances constitutes the immune response (Abbas and Lichtman, 2003). The most relevant cells involved in the immune response are lymphocytes, but other cells are also implicated, including monocytes/macrophages and polimorphonuclear granulocytes (Delves et al., 2006).

Macrophages and antigen-presenting cells (APC) are involved in all stages of non-specific immune responses and are responsible for phagocytosis; antigen processing and presentation; secretion of cytokines such as nitric oxide (NO) and tumor necrosis factor-α (TNF-α); and antibody-dependent cell-mediated cytotoxicity. Dendritic cells, which are immune accessory cells, have different functions in non-specific immune responses, including the activation not only of native T cells but also of both native and memory B-cells. At different stages of differentiation, dendritic cells can also regulate effectors of innate immunity such as natural killer (NK) cells, which can modulate natural and specific immune responses through of the production of interferon-γ (IFN-γ), TNF-α, and granulocyte macrophage-colony stimulating factor (GM-CSF) (Moradali et al., 2007). The third relevant element involved in innate immunity is the complement system. Among the physiological systems, the complement is the major effector of humoral immunity, which is involved in the host defense (Oh et al., 2000). Components of the complement system, including activated components C3a and C3b through C9, both mediate and amplify the immune reaction (Moradali et al., 2007).

In adaptive or specific immunity, the stimulation of APC is produced in a later interaction with the antigen-major histocompatibility complex (MHC) II. This leads to the production of various cytokines such as interleukin (IL)-4 and IL-12, which in turn activate a variety of implicated cells, including macrophages, B cells, and different types of T cells. Thus, the stimulation of Th1 cells pro-

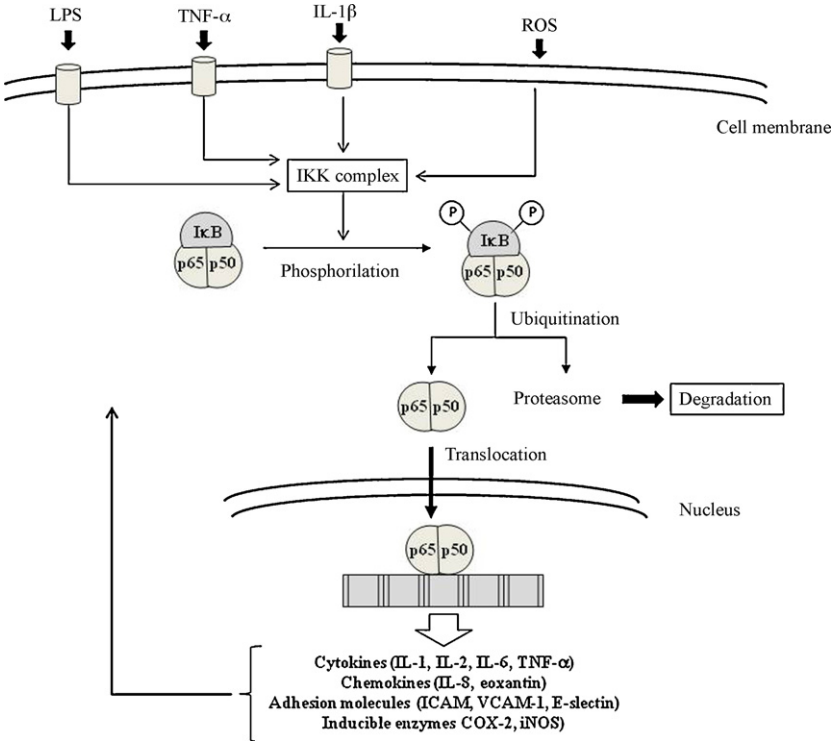


Fig. 1. Mechanism of activation of NF-κB.

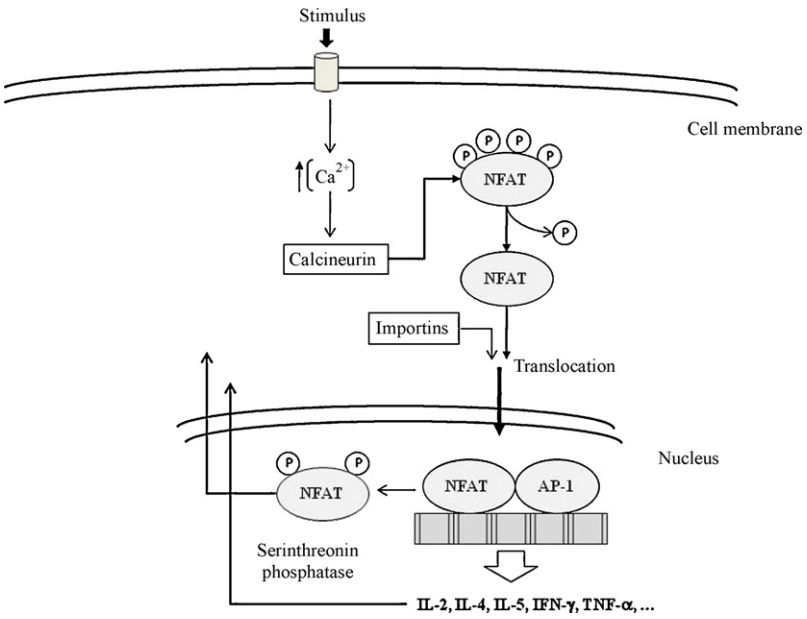


Fig. 2. Mechanism of activation of NF-AT.

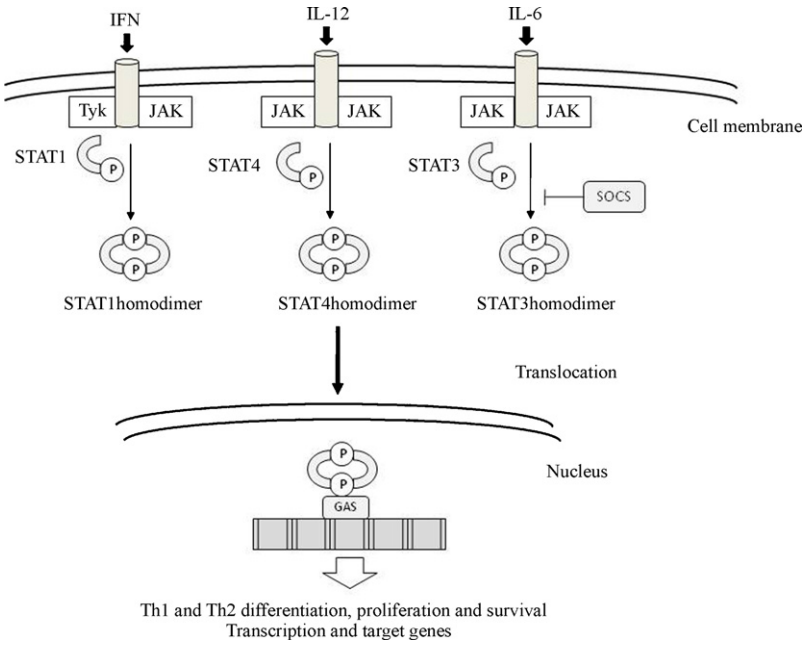
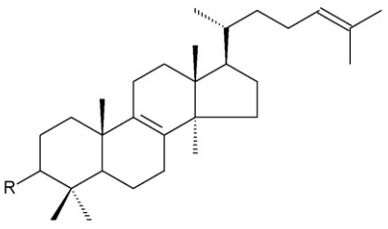


Fig. 3. Mechanism of activation of STATs.

duces IFN-γ, which subsequently activates various macrophages and cytotoxic T (Tc) cells (Moradali et al., 2007).

The immune system is involved in combating many types of infections and parasitoses, including those caused by bacteria, fungi, viruses, helminthes, protozoa, and others, but other diseases are actually caused or aggravated by the immune response. These include hypersensitivity, autoimmunity, immunodeficiency, and cancer (Delves et al., 2006). In some inflammatory pathologies, for example, a higher immune system response is observed, with a concomitant proliferation of immune cells and pro-inflammatory cytokines, which are produced by the activation of the corresponding genes via nuclear transcription. In this fashion, various IL, TNF, and IFN are produced, increasing the inflammatory response in which other mediators are also implicated, including prostaglandins (PG) and NO, which are produced after the



	R
Penasterol	OH (β)
Penasterone	=O
Acetylpenasterol	$OCOCH_3$ (β)

Fig. 4. Chemical structures of penasterol, penasterone and acetylpenasterol.

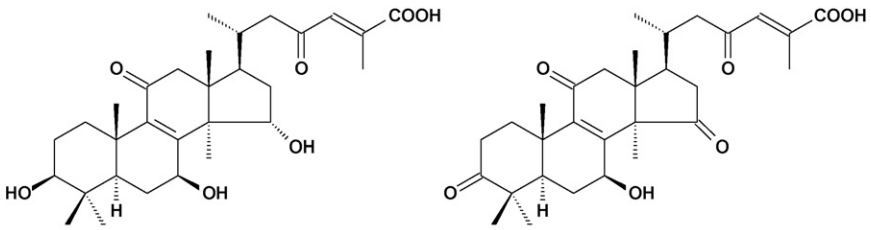


Fig. 5. Chemical structures of active ganoderic acids.

induction of the corresponding enzymes, e.g. cyclooxygenase-2 (COX-2), and nitric oxide synthase (iNOS). Likewise, both peptidic mediators and inducible enzymes are produced after the stimulation of transcription factors such as nuclear factor-κB (NF-κB), nuclear factor-AT (NF-AT), and signal transducers and activators of transcription (STATs). Among these transcription factors, NF-κB controls the expression of genes encoding the pro-inflammatory cytokines (IL-1, IL-2, IL-6, and TNF-α), as well as chemokines such as IL-8 and eotaxin. It also has an effect on the expression of the genes that encode adhesion molecules, including the intercellular adhesion molecule (ICAM), the vascular cell adhesion molecule (VCAM)-1, and E-selectin, along with inducible enzymes such as COX-2 and iNOS, growth factors, and immune receptors (Fig. 1) (Calixto et al., 2003; Ríos et al., 2009).

NF-AT plays a key role in controlling lymphokine gene expression in T cells in a Ca^{2+}-dependent manner. NF-AT itself is a cytoplasmic component that is translocated to the nucleus following T cell receptor (TCR) stimulation. NF-AT is also expressed in other immune cell types such as B cells, mast cells, and natural killer cells and it is essential for activating T cells and regulating the cell cycle (Fig. 2). In therapeutic practice, the activation of this transcription factor is a target of the immunosuppressive drugs cyclosporine and tacrolimus (Ríos et al., 2009).

Other nuclear transcription factors are implicated in both immunity and autoimmune diseases. STAT1 and STAT2, for example, are the major regulators of the innate immune response with additional functions in the stimulation of the cellular immune response via IFN signaling. In the case of STAT3, although it was previously described as an acute phase response factor activated by the pro-inflammatory cytokine IL-6, it is now recognized as the target of other pro- and anti-inflammatory cytokines. Finally, STAT4 is implicated in the regulation of both immunity and autoimmune disease as the critical mediator of IL-12-stimulated gene regulation (Fig. 3) (Pfitzner et al., 2004).

2. Effects of triterpenoids on hypersensitivity reactions

Adaptive immunity has the mission of defending an organism against microbial infections, but it can also produce damage and disease. Such is the case with hypersensitivity reactions, which are responses of the immune system against different agents or events, but in some case cause an excessive response that is not controlled by the organism. There are four types of hypersensitivity reactions, of these, the most relevant with regard to being treated with medicinal plants are type I or immediate hypersensitivity, which is due to IgE antibodies, and type IV, which is mediated by lymphocytes. In the former, allergy is the most relevant clinical manifestation, with anaphylaxis, asthma, rhinitis, or eczema being common, while in the latter, delayed-type hypersensitivity and contact dermatitis are the most common manifestations. Other diseases that can develop when lymphocyte-mediated hypersensitivity is implicated are rheumatoid arthritis, diabetes mellitus

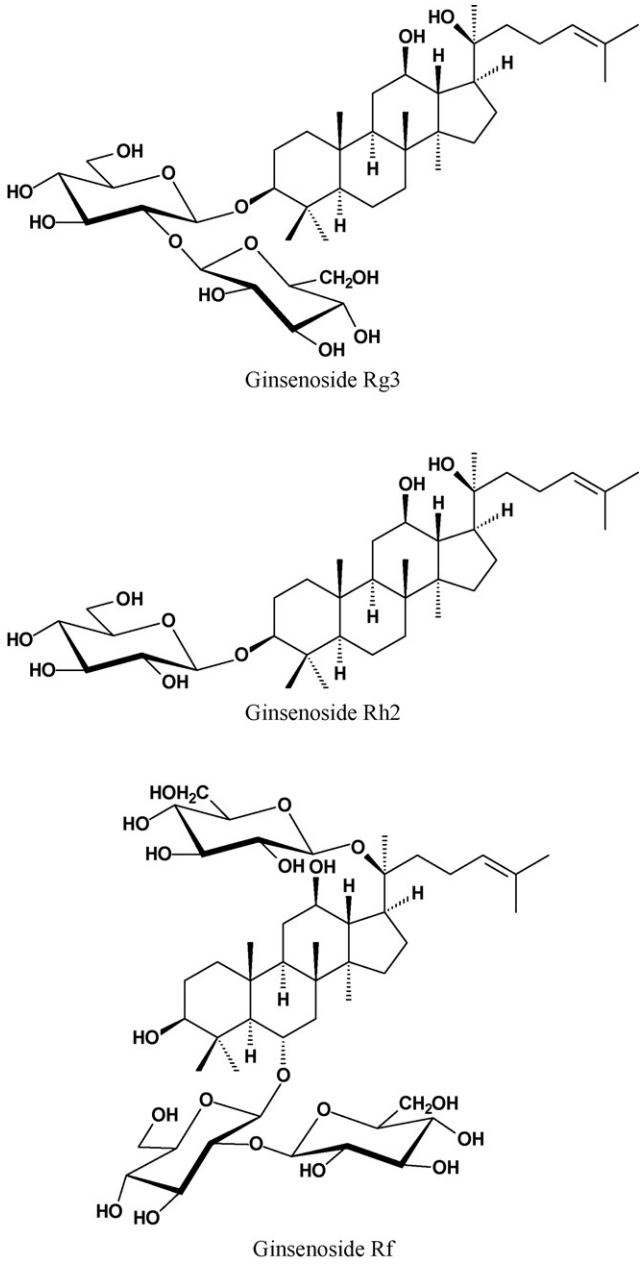


Fig. 6. Chemical structures of active ginsenosides.

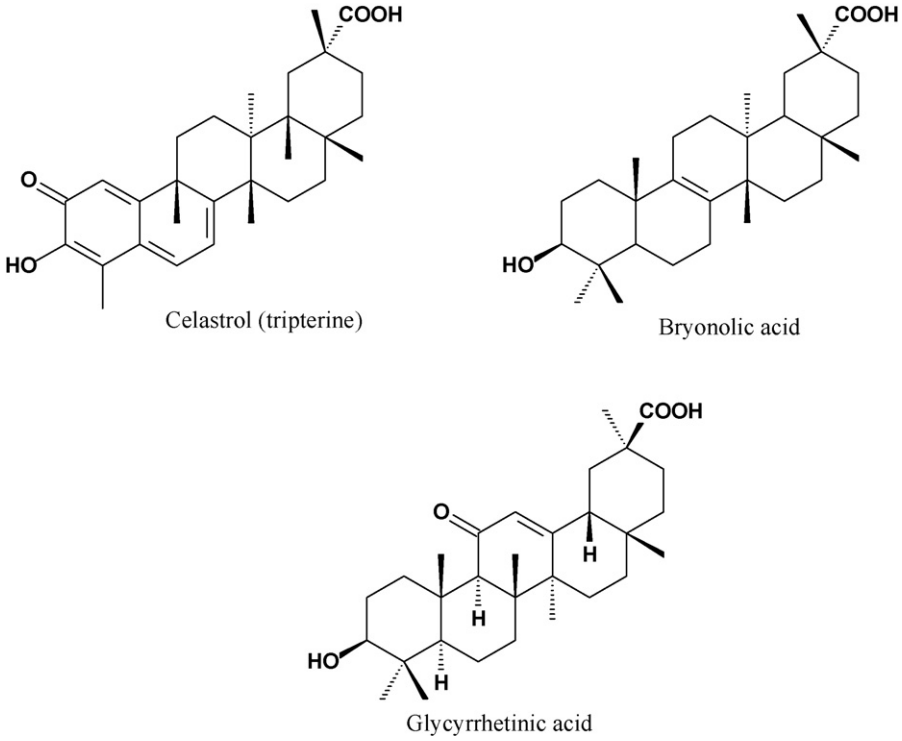


Fig. 7. Chemical structures of celastrol, bryonolic acid and glycyrrhetinic acid.

(insulin-dependent), multiple sclerosis, and inflammatory bowel disease (Abbas and Lichtman, 2003).

2.1. The effects of triterpenoids on hypersensitivity type I reactions

Penasterone and acetylpenasterol (Fig. 4) isolated from the marine sponge *Penares incrustans* (Stellettidae) were found to inhibit the histamine release from rat peritoneal mast cells induced by anti-immunoglobulin (Ig) E in a dose-dependent manner. Penasterol (Fig. 4) isolated from the same source also inhibited the anti-IgE-induced histamine release, but only at higher concentration. Other structurally related lanostanes that have been described as inhibitors of histamine release from rat peritoneal mast cells are ganoderic acids C and D (Fig. 5) isolated from *Ganoderma lucidum*, species used against different immune implicated diseases, such as allergy, inflammation and cancer (Giner-Larza et al., 2000; Ríos, 2008).

The ginsenosides Rg3, Rf, and Rh2 (Fig. 6), isolated from the immunomodulator plant *Panax ginseng* (Araliaceae), inhibited the IgE-induced passive cutaneous anaphylaxis in mice, with ginsenoside Rh2 being the most potent inhibitor of the three. This compound was also the most potent inhibitor of degranulation of RBL-2H3 cells (Bae et al., 2006).

2.2. Triterpenes as inhibitors of delayed-type hypersensitivity (DTH)

Various triterpenes were assayed as inhibitors of induced DTH reactions, especially those isolated from the immunomodulator plants *Tripterygium wilfordii* (Li and Weir, 1990), *Cayaponia tayuya* (Ríos et al., 1990), *Astragalus membranaceus* (Ríos and Waterman, 1997) and *Glycyrrhiza glabra* (Kroes et al., 1997). Celastrol (tripterine) (Fig. 7), for example, was found to inhibit the DTH reaction induced by dinitrochlorobenzene (DNCB) in mouse skin. This effect was related to the compound's ability to inhibit the production of IL-1β and TNF-α in lipopolysaccharide (LPS)-stimulated human monocytes. Cytokine IL-1β is essential for the initiation of cutaneous immune responses and its upregulation by Langerhans cells is relevant in the early stages of skin sensitization, but this same cytokine, together with TNF-α, also plays a relevant role in Langerhans cell mobilization and the development of contact dermatitis (Ríos et al., 2005). In a comparative study between the anti-allergic activity of bryonolic acid and glycyrrhetinic acid (Fig. 7) on the contact hypersensitivity induced by picryl chloride in mice, bryonolic acid was found to suppress ear swelling in a dose-dependent fashion over time whereas glycyrrhetinic acid was so toxic that it could not be properly studied. Both compounds are triterpene derivatives, but while bryonolic acid is a friedoolean-type compound, glycyrrhetinic acid is of the oleanan-type. This fact marked a clear difference for both the toxicity and the effectiveness of bryonolic acid versus glycyrrhetinic acid in DTH reactions (Ríos et al., 2005).

Other triterpenes with a cucurbitacin structure, including dihydrocucurbitacin B and cucurbitacin R (Fig. 8) from *Cayaponia tayuya* (Cucurbitaceae), were found to inhibit the inflammatory reactions induced by oxazolone, dinitrofluorobenzene (DNFB), and sheep red blood cells (SRBC), reducing both the edema and cell infiltration. Dihydrocucurbitacin B reduced the presence of the most relevant cytokines implicated in these processes, including IL-1β, IL-4, and TNF-α, and was also found to inhibit the proliferation of phytohemagglutinin A (PHA)-stimulated human T lymphocytes, halting the cell cycle in the G_0 phase. In addition, this triterpene reduced the production of IL-2, IL-4, IL-10, and IFN-γ in human T lymphocytes and hampered the induction of the principal cyclins involved in the cell cycle. Finally, it selectively inhibited NF-AT in human lymphocytes without affecting calcium influx. The authors concluded that dihydrocucurbitacin B curbs DTH reactions by inhibiting NF-AT, which in turn suppresses the proliferation of the most relevant cells involved in DTH reactions, namely the T cells (Escandell et al., 2007b, 2010). This same triterpene, along with another structurally related compound, cucurbitacin R, exerted a clear inhibition in an experimental model of adjuvant-induced arthritis in rats, modify-

ing the evolution of the clinical symptoms while the histopathology of the paws demonstrated a reduction in the signs of arthritis. This experimental protocol is a model unique to rats that is widely used for studying the physiology, biochemistry, and pharmacology of inflammation; it is also used as a model of cell-mediated autoimmune disease. The *in vivo* study of the expression of pro-inflammatory enzymes (iNOS, COX-2) and mediators (TNF-α and PGE_2) demonstrated a clear decrease in these mediators in arthritic paws. In this case, the mechanism of action of cucurbitacin R was related to the inhibition of STAT3 activation in the T lymphocytes (Escandell et al., 2007a). In the case of dihydrocucurbitacin B, similar effects were reported, but no implication of transcription factors

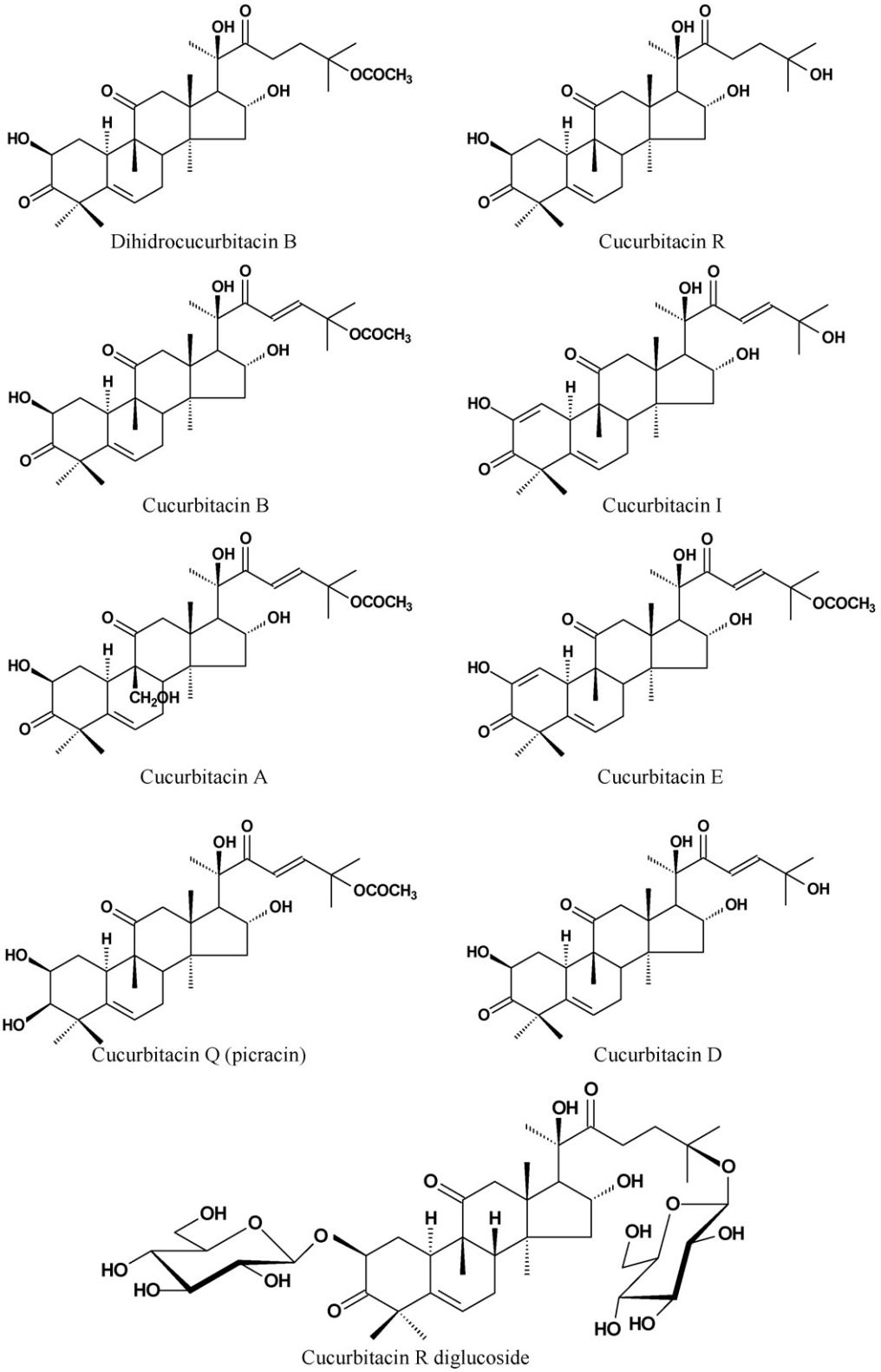


Fig. 8. Chemical structures of active cucurbitacins.

was studied. Instead, this compound reduced the synthesis, release, and activity of pro-inflammatory enzymes (elastase, COX-2, and iNOS) as well as other mediators (TNF-α and IL-1β) in the lymphocytes (Escandell et al., 2006).

The ginsenosides Rg3, Rf, and Rh2 were found to reduce oxazolone-induced contact dermatitis in mice. In fact, all three derivatives diminished the ear swelling and reduced the mRNA expression levels of COX-2, IL-1β, TNF-α, and IFN-γ, but only ginsenoside Rh2 reduced IL-4 expression. When these ginsenosides were assayed *in vitro* in LPS-stimulated murine macrophages, Rh2 inhibited the expression of both COX-2 and iNOS. These results justify the potential utility of ginseng and its constituents in the treatment of atopic and contact dermatitis through the regulation of pro-inflammatory enzymes and cytokine expression (Bae et al., 2006).

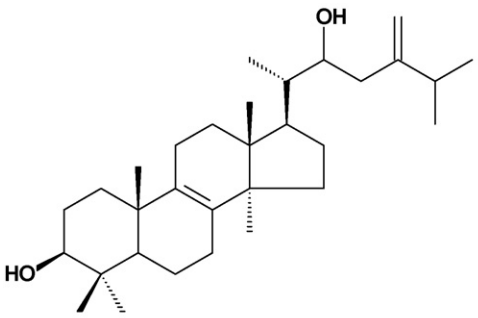
3. Immunosuppressive effects of triterpenoids

As mentioned above, dihydrocucurbitacin B is known to inhibit the proliferation of PHA-stimulated human T lymphocytes, halting the cell cycle in the G_0 phase (Escandell et al., 2007b). Similar effects have been described for different triterpenoids. Fujimoto et al. (1994) isolated different lanostanes with immunosuppressive activity from *Pisolithus tinctorius* (Sclerodermataceae) and evaluated their properties against the proliferation of mouse spleen lymphocytes stimulated with concanavalin A (Con A) and LPS. The authors established a relationship between the chemical structure of each compound and its suppressive effect, suggesting that the presence of a five-membered ring containing the 22,28-epoxy-28-hydroxyl moiety increases the suppressive potency, while the replacement of the hydroxyl at position 28 with a carbonyl decreases the potency of the compounds studied. The compound 24-methyllanosta-8,24(28)-diene-3β,22ξ-diol and a mixture of (22*S*,24*R*)-24-methyllanosta-8-en-22,28-epoxy-3β,28β-diol and (22*S*,24*S*)-24-methyllanosta-8-en-22,28-epoxy-3β,28β-diol (Fig. 9) inhibited both the Con A- and the LPS-induced proliferation of mouse spleen lymphocytes, respectively. For its part, pisolactone suppressed Con A-induced proliferation by only 31% while the LPS-induced proliferation was suppressed by a mere 20%, even at 25 μg/mL.

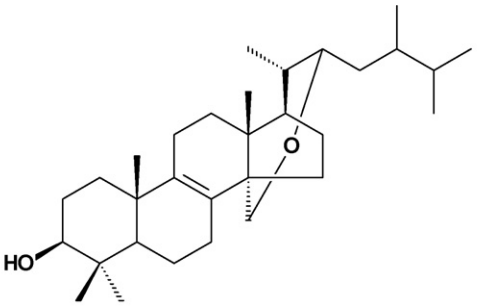
Scholz et al. (2004) identified the immunosuppressant principle of *Borassus flabellifer* (Arecaceae) as 17α-23-(*E*)-dammara-20,23-diene-3β,25-diol (Fig. 9). The researchers subsequently studied this compound in animal models of immunosuppression and inflammation using murine and porcine models of oxazolone-induced allergic contact dermatitis as the directing *in vivo* test system and demonstrated that biologically active compounds with the 17β configuration are thermodynamically more stable and much easier to synthesize.

Demethylzeylasteral (Fig. 10) isolated from *Tripterygium wilfordii* (Celastraceae) was shown to inhibit the mixed lymphocyte reaction and to prolong the survival time of rats with kidney transplants. It also inhibited proliferation of peripheral blood mononuclear cells with no cytotoxicity and suppressed levels of CD4 and other cell types involved in immune responses. It also suppressed levels of CD25, which is part of the IL-2 receptor, but did not inhibit IL-2 production. Other related congeners, such as 2,3-dihydroxy-1,3,5(10),7-tetraene-6α(1′-hydroxyethyl)-24-nor-D:A-friedooleane-29-oic acid and wilforic acid A (Fig. 10) were good inhibitors of the production of cytokines such as IL-1β, IL-2, and IFN-γ; the former also inhibited IL-8 and TNF-α (Brinker et al., 2007). In addition, demethylzeylasteral showed a potent immunosuppressive effect in the treatment of acute kidney transplant rejection reactions. In fact, the combination of demethylzeylasteral and prednisone was effective in suppressing the ongoing rejection of kidney grafts. These results demonstrate the strong immunosuppressive activity of demethylzeylasteral and suggest a possible clinical role for this compound as an immunosuppressive agent in the fields of organ transplantation and autoimmune disorders (Xu et al., 2009).

The immunosuppressive effects of *Tripterygium wilfordii* may also involve other triterpenoids, including celastrol (Fig. 7), pristimerin, and tripterygone (Fig. 10), as well as compounds such as oleanolic acid, 3-*epi*-katonic acid, triptotriterpenonic acid A, and



24-Methyllanosta-8,24(31)-diene-3β,22ζ-diol



(22*S*,24*R*)-24-Methyllanosta-8-en-22,28-epoxy-3β,28β-diol
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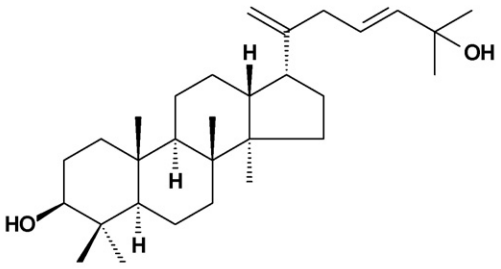


17α-23-(*E*)-Dammara-20,23-diene-3β,25-diol

Fig. 9. Chemical structures of active principles from *Pisolithus tinctorius* and *Borassus flabellifer*.

2α,3β-dihydroxy-olean-12-ene-22,29-lactone (Fig. 11). Celastrol, for example, has exhibited activity against different symptoms and mediators of autoimmune diseases. In asthmatic mice it reduced the airway inflammation and decreased the number of inflammatory cells in the lung tissue; in mice with lupus it lowered the production of serum auto-antibodies and the levels of IgG and NO, reduced albumin in the urine, and decreased both IL-10 production by peritoneal macrophages and the severity of glomerular lesions (Brinker et al., 2007). Some of these effects have to do with the inhibition of pro-inflammatory cytokines and enzymes, which is due to an inhibition of transcription factors. Thus, celastrol reduces the production of IL-1, IL-2, IL-6, IL-10, and TNF-α, lowers the production of PGE_2 and NO, inhibits the transfer of NF-κB to the nucleus, and decreases the levels of phosphorylated p38 in LPS-activated monocytes. Its related compounds, pristimerin and tripterygone, have similar properties, but different mechanisms of action (Brinker et al., 2007). For their part, oleanolic acid (Fig. 11) and its related compounds are known to be anti-inflammatory and immunosuppressive agents through their reduction of relevant cytokines such as IL-1β, IL-6, and TNF-α, as well as their effect on the classic pathway of complement activation though the inhibition of C3 convertase. Oleanolic acid also inhibits adenosine deaminase, an enzyme which is found at increased levels in various immune diseases (Brinker et al., 2007). Previously, no clear differences had been reported for the immune-inhibitory activity of oleanolic acid versus that of ursolic acid at various concentrations. However, new research shows that at high concentrations (>40 μg/mL), ursolic acid exerts more of an effect than oleanolic acid, for which only a moderate inhibition of the proliferation of human peripheral blood mononuclear cells and a strong enhancement of the secretion of IFN-γ at low concentrations was found (Chiang et al., 2003).

In various traditional medicines the oleogum resins from *Boswellia* species are used for the treatment of different diseases, including those involving the immune system. The anti-inflammatory principles of *Boswellia* resin are boswellic acids (Fig. 12), which have been shown to act by inhibiting different mediators and pro-inflammatory enzymes such as 5-lipoxygenase (LOX) and human elastase (Safayhi et al., 1997). However, their mechanism of action is also related to their inhibition of certain components of the immune system. Clinical pilot studies thus far suggest the efficacy of these acids in treating several autoimmune diseases including rheumatoid arthritis, Crohn's disease, ulcerative

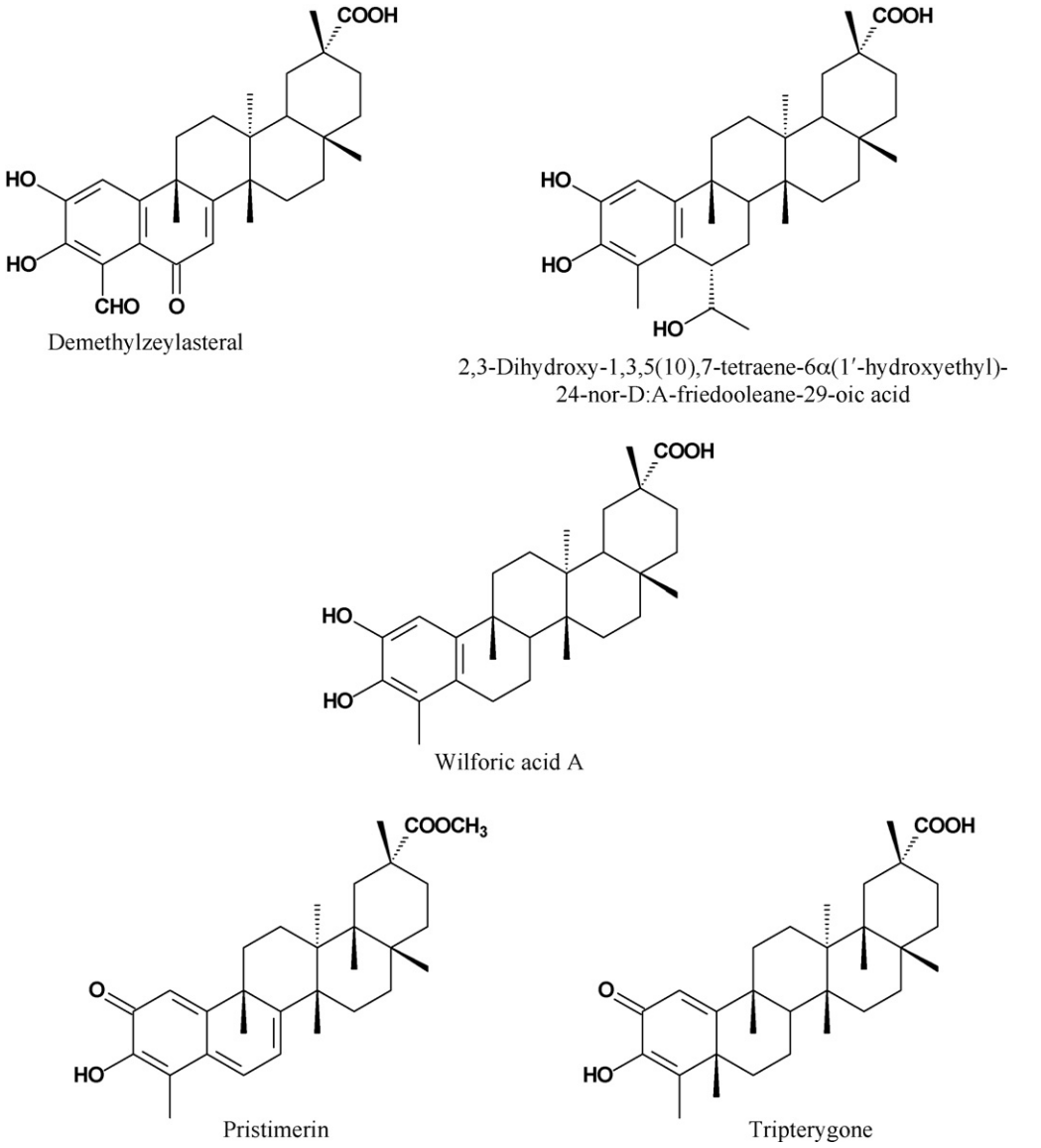


Fig. 10. Chemical structures of nor-friedolanes from *Tripterygium wilfordii*.

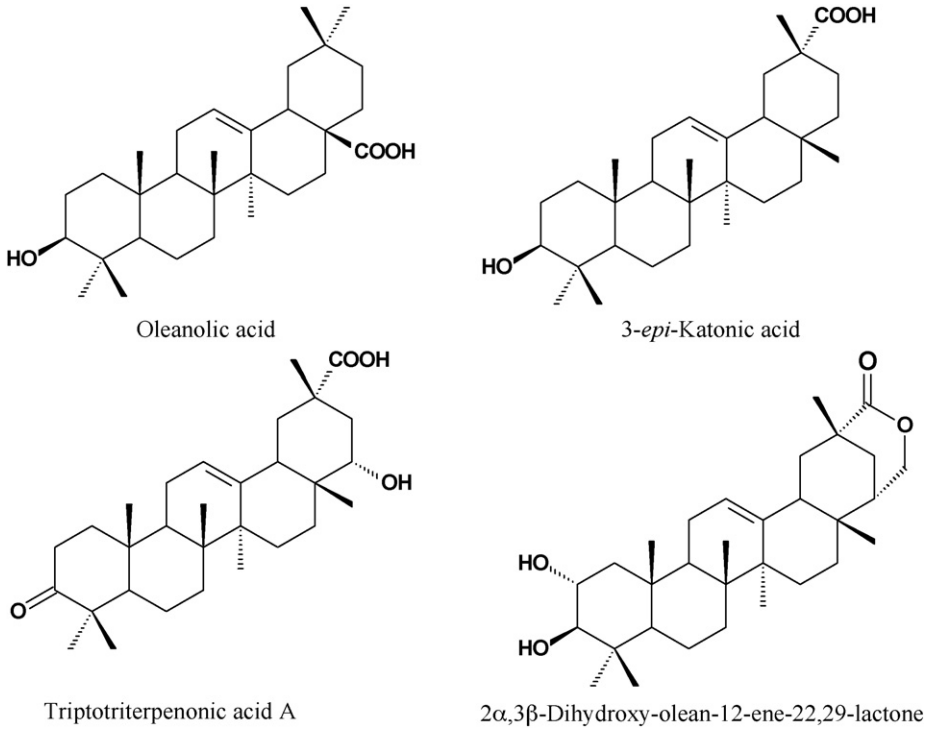


Fig. 11. Chemical structures of oleananes from *Tripterygium wilfordii*.

colitis, and bronchial asthma. As for their effect as an anticomplement system, a mixture of boswellic acids was found to inhibit the activity of C3 convertase in the classic complement pathway, which consequently suppressed the conversion of C3 into C3a and C3b. In the case of specific humoral defense, researchers have demonstrated that boswellic acids administered orally produce a dose-related reduction in the primary hemagglutinating antibody titers in serum four days after administration of SRBC to mice. In addition, boswellic acids produce a concentration-dependent inhibition of lymphocyte proliferation after stimulation of mice cells with different agents (Ammon, 2006).

Other triterpenoids with anticomplement properties include the saponins isolated from *Pueraria lobata* (Fabaceae). Of these, the diglycosidic derivatives are the most potent, followed by the mono- and triglycosidic compounds. In contrast, the corresponding aglycones actually enhance hemolysis in the presence of serum

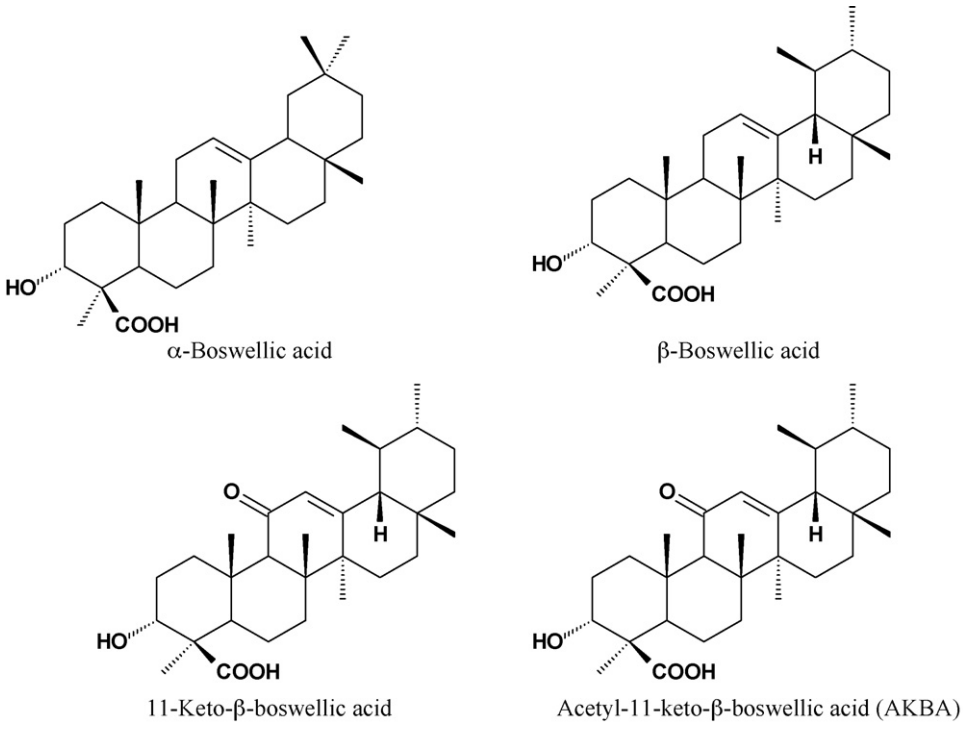
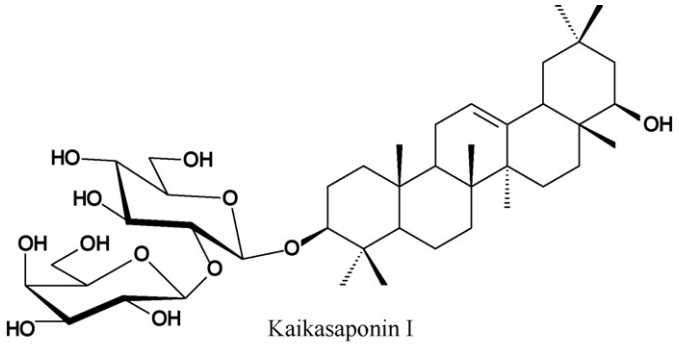
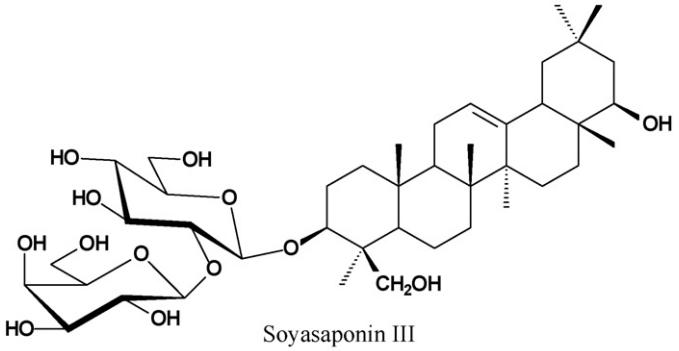


Fig. 12. Chemical structures of boswellic acids.





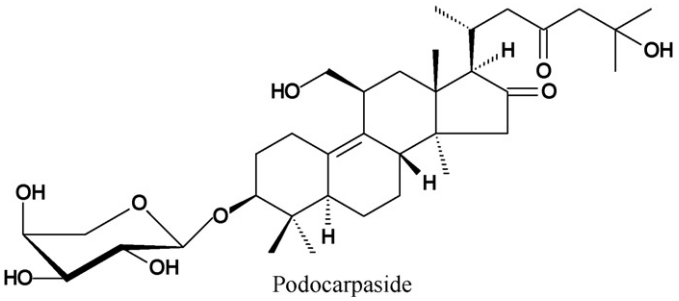
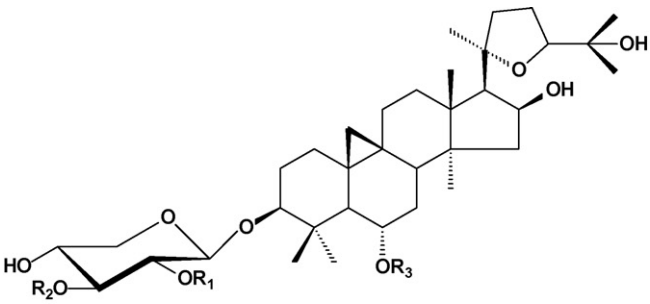
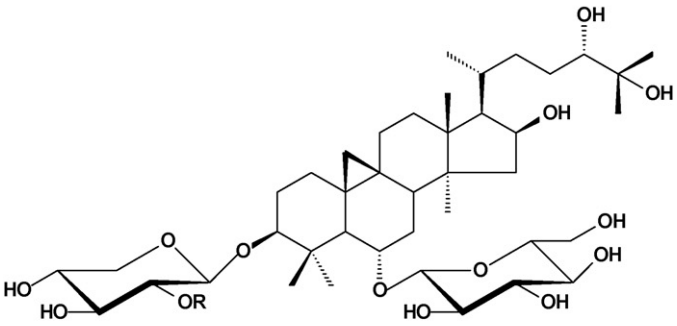


Fig. 13. Chemical structures of saponins from *Pueraria lobata* and *Actaea podocarpa*.



	R_1	R_2	R_3
Astrasieversianin II	Ac	Ac	Xyl
Astrasieversianin X	Ac	Ac	Glu
Astragaloside I	Ac	H	Glu
Astragaloside II	H	H	Xyl
Astragaloside IV	H	H	Glu
Astragaloside VI	Glu	H	Glu



	R
Cyclocanthoside E	H
Cyclocanthoside G	Glucose

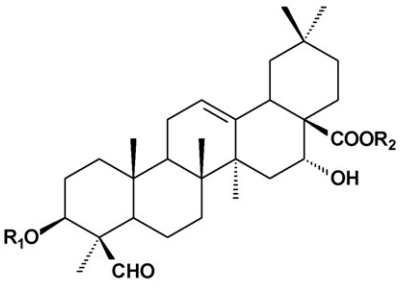


Fig. 14. Chemical structures of astrasieversianins, astragalosides and cyclocanthosides from *Astragalus melanophrurius* and *Quillaja* saponins.

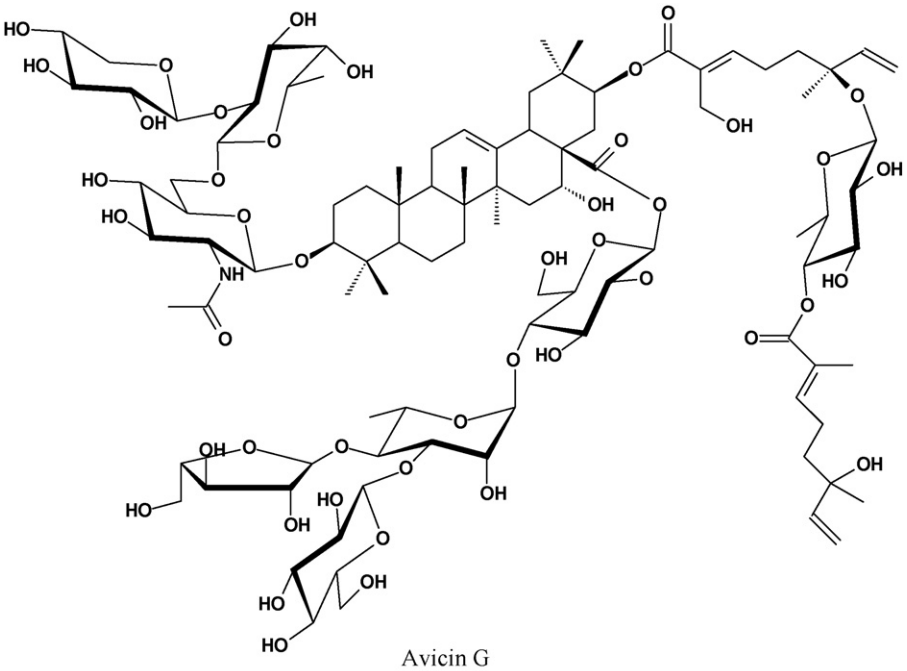
in the classic complement system pathway. None of them showed relevant effects on the alternative complement pathway. A study of the relationship between effects and activity showed that the presence of a free carboxyl in glucuronic acid increased the activity whereas the salts, methyl esters, and reduced form of this acid are less potent. Thus, the compounds which exhibited the highest potency as anticomplementary agents were kaikasaponin I and soyasaponin III (Fig. 13) (Oh et al., 2000).

Ali et al. (2006) isolated a new triterpenoid with anticomplement activity, namely podocarpaside (Fig. 13) from *Actaea podocarpa* (Ranunculaceae), which inhibited human complement activity. Still, it was less potent than compounds obtained from ursolic and oleanolic acids (Ali et al., 2006).

4. Immunostimulant effects of triterpenoids

Çalis et al. (1997) studied eight cycloartanes isolated from *Astragalus melanophrurius* (Fabaceae) and found that the compounds showed interesting immunomodulatory activity in an isolated human lymphocyte stimulation test. The researchers determined that astrasieversianins II and X; astragalosides I, II, IV, and VI; and cyclocanthosides E and G (Fig. 14) were all able to stimulate human lymphocyte proliferation in concentrations ranging from 0.01 to 10 μg/mL.

Behboudi et al. (1996, 1997) studied the effects of a mixture of triterpenes from *Quillaja saponaria* (Rosaceae) on the production of IL-1 and IL6, as well as their role in the activation of APC, a prerequisite for the development of immune responses. In such cell-mediated immune responses, IL-6 synergizes with IL-1 to promote T cell proliferation and both the differentiation of T helper cells and the development of T cell-mediated cytotoxicity by CD8+ cells. IL-1 constitutes a second signal for T cell activation, in this case provided by APC, which carries the first signal produced by the MHC class II molecules. After its release, IL-1 upregulates the activity of T helper cells. For its part, cytokine IL-6 is a key factor in cytolytic T lymphocyte generation, which is an important effector mechanism elicited by the immunostimulant complex (iscom)-borne antigens. Of the possible combinations of triterpenoids, including QH-A, QH-C, and spikoside, a semipurified *Quillaja* saponin product (Fig. 14),



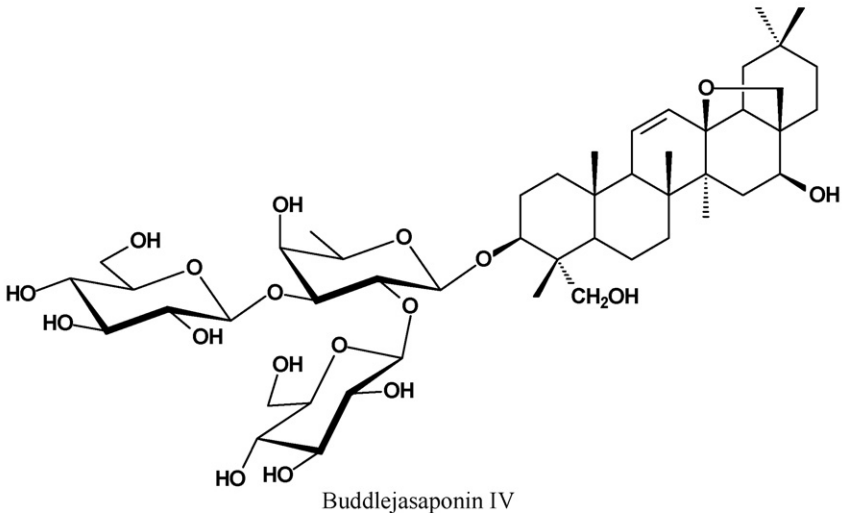


Fig. 15. Chemical structures of avicin G and buddlejasaponin IV.

all of which are mixtures of saponins, the researchers found that an increase in QH-A as opposed to QH-C increased the capacity to activate APC.

From *Luffa cylindrica* (Cucurbitaceae), Khajuria et al. (2007) isolated and studied the immunomodulatory activity of oleanolic acid and echinocystic acid. Both compounds increased the phagocytic index and showed stimulatory effects on macrophages, increasing both humoral and cell-mediated immune responses.

5. Triterpenoids with adaptogenic activity

Panossian et al. (1997) tested the adaptogenic activity of *Bryonia alba* roots in both preclinical and clinical trials, demonstrating that the compound responsible for this activity was cucurbitacin R-diglucoside (Fig. 8) (Panossian et al., 1999). This compound increases corticosteroid secretion by stimulating the adrenal cortex, modulating corticosteroid release until optimal levels are obtained, thereby protecting the adrenal cortex from hypotrophy. Moreover, cucurbitacin R-diglucoside not only modifies the metabolism of eicosanoids, thus increasing the production of PGE_2, but also inhibits the biosynthesis of the pro-inflammatory mediators from LOX such as LTB_4 and 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid (5-HETE), which activate the chemotaxis of neutrophils, lysosomal enzyme release, vascular permeability, and superoxide anion generation. Other cucurbutacins (B, D, and R) (Fig. 8) were assayed as immunomodulators, but their activity was of little interest. It should be noted, however, that cucurbitacin R gave IC_{50} values of 1.0 and 0.46 μg/mL against mitogen CoA-stimulated IL-2 dependent murine lymphoblasts and mitogen Con A-stimulated murine spleen cells, respectively (Frei et al., 1998).

6. Triterpenoids as potential immunomodulatory agents via the inhibition of transcription factors

6.1. Triterpenoids as inhibitors of NF-κB

Triterpenoids from different structural groups have been studied and described as anti-inflammatory and immunomodulatory

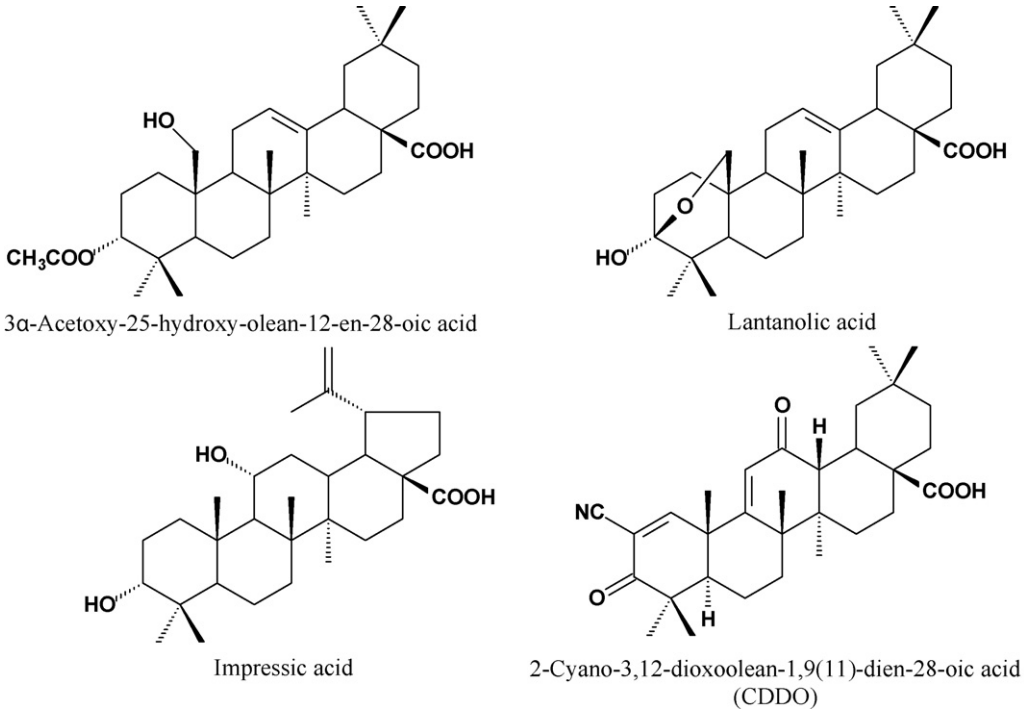


Fig. 16. Chemical structures of active principles from *Acanthopanax koreanum*, and *Liquidambar formosana*, and semisyntetic derivatives form oleanolic acid.

agents through their ability to inhibit both the transcription factors implicated in these processes as well as the mediators produced by their activation. Celastrol (Fig. 7), a nor-triterpenoid from *Celastrus orbiculatus*, and its analogous derivative pristimerin (Fig. 11), have been studied as inhibitors of the transcription factor NF-κB (Ríos et al., 2009). Celastrol was found to block the NF-κB activation induced by different stimuli and cell types without affecting the DNA-binding activity of AP-1. It also completely blocked the LPS-, TNF-α-, and TPA-induced degradation and phosphorylation of the inhibitor protein of NF-κB (IκB)α, (Lee et al., 2006). The experimental data suggest an interference with one or more of the common steps of NF-κB activation, such as the IκB phosphorylation at Ser-32 and Ser-36 carried out by IκB kinase (IKK). Moreover, celastrol prevented not only the expression of iNOS and TNF-α, but also the production of NO and TNF-α in LPS-stimulated RAW 264.7 cells. It also inhibited the NF-κB activation induced by transforming growth factor (TGF)-β activated kinase-1 (TAK1) (Sethi et al., 2007). Its analogous compound pristimerin was also reported to be a potent inhibitor of NF-κB, reducing both the induction of iNOS and NO synthesis in LPS-stimulated RAW 264.7 cells in a dose-dependent fashion. However, this activity may actually be due to cytotoxic effects (Dirsch et al., 1997).

Another group of triterpenoids that act as immunomodulators are the saponins. The avicins, for example, have been studied as potential inhibitors of NF-κB activation. Of the compounds studied, avicin G from *Acacia victoriae* (Fig. 15) was able to impair the TNFα-induced binding of NF-κB to DNA in Jurkat cells, but this effect was reversed by dithiothreitol, which suggests that avicins may modify a sulfidryl group critical for NF-κB activation. On the other hand, they may also target the cysteine residues in the NF-κB molecule. While avicins did not affect the degradation of IκB, they were able to decrease the nuclear localization of the p65 subunit of NF-κB (Haridas et al., 2001).

Buddlejasaponin IV from *Pleurospermum kamtschatidum* (Fig. 15) also inhibited NF-κB with parallel reductions in IκBα degradation and phosphorylation as well as in the nuclear translocation of the p65 subunit. The inhibitory activity was due to the prevention of IκBα degradation and the consequent down-regulation of the expression of the enzymes iNOS and COX-2 (Won et al., 2006).

Different ginsenosides from *Panax ginseng* (Araliaceae) have been demonstrated to be NF-κB inhibitors, with consequent inhibition of the mediators implicated in both inflammation and immune diseases. For example, ginsenoside Rg3 inhibited the NF-κB activity in colon cancer cells and enhanced the response to docetaxel and other chemotherapeutics (Kim et al., 2009a) while ginsenoside Rp1 inhibited the production of IL-1β in murine macrophages through the down-regulation of both IKK/IκBα phosphorylation and the consequent activation of NF-κB without affecting the activation of its upstream signaling enzymes (Kim et al., 2009b). Ginsenoside Rb1 also inhibited the TNF-α-induced over-expression of VCAM-1 in human umbilical cells (HUVECs and HMVECs-L) as well as the activation of p38, c-Jun N-terminal protein kinase (JNK), extracellular signal-regulated kinase (ERK)-1/2, and IκBα (Chai et al., 2008).

6.2. Triterpenoids as inhibitors of NF-AT

Impressic acid from *Acanthopanax koreanum*, and 3α-acetoxy-25-hydroxy-olean-12-en-28-oic acid, and lantanolic acid from *Liquidambar formosana* (Fig. 16) all inhibited NF-AT; however, other structurally related compounds did not. These data indicate a relationship between chemical structure and pharmacological activity. In this case, it was established that the presence of an oxy methylene group at C_{25} along with an acetoxyl group at C_3 generally increases the inhibitory activity against NF-AT (Ríos et al., 2009).

As mentioned above, dihydrocucurbitacin B (Fig. 8) inhibited the DTH reaction by suppressing lymphocyte proliferation through a direct inhibition of NF-AT activity. This, in turn, led to a reduction in the expression of cyclins and cytokines, especially those implicated in this type of reaction, such as IL-2 and IFN-γ (Escandell et al., 2007b).

6.3. Triterpenoids as inhibitors of STATs

Cucurbitacins have been reported to be potential immunomodulatory agents, especially through the modification of the non-specific immune response (Panossian et al., 1997, 1999) or selectively via the reduction of T cell proliferation (Escandell et al., 2007b). Some of these effects are due to the ability of these compounds to inhibit constitutively activated STAT3. Indeed, cucurbitacins were found to be highly selective for JAK/STAT3 and did not inhibit other pathways. Thus, cucurbitacin I (Fig. 8) reduced the levels of phosphotyrosine of constitutively activated STAT3 in different human cancer cell lines (Blaskovich et al., 2003). This suppression resulted in the blockade of STAT3 DNA-binding activity and STAT3-mediated gene transcription with a high selectivity in its disruption of STAT3 signaling over other pivotal pathways. Sun et al. (2005) later carried out a structure-activity relationship study with five structural analogues and found that while cucurbitacin Q inhibited the activation of STAT3 but not that of JAK2, cucurbitacin A inhibited the activation of JAK2 but not that of STAT3. For their part, cucurbitacins B, E, and I inhibited the activation of both.

Escandell et al. (2007a) demonstrated that cucurbitacin R (Fig. 8) inhibited STAT3 activation in peripheral human blood lymphocytes stimulated with IL-6. It seems likely that in diseases in which the activation of the JAK/STAT system is implicated, treatment should include the use of new agents that inhibit these transcription pathways.

Oleanolic acid is currently undergoing clinical trials for its anticancer and anti-inflammatory effects through its potential ability to inhibit transcription factors such as STAT3 and STAT5. Its status of development is phase I/II (Butler, 2008).

6.4. Triterpenoids as inhibitors of other transcription factors

Nuclear factor-erythroid 2-related factor 2 (Nrf2) is a basic leucine zipper (bZIP) domain oxidant-responsive transcription factor responsible for regulating a battery of cytoprotective genes, including antioxidants, while maintaining cellular redox homeostasis. It has a critical role in improving survival during sepsis, which is characterized by an inappropriate host immune-inflammatory response and sustained oxidative damage. Pretreatment of peripheral blood mononuclear cells with cyano-derivatives of oleanolic acid (CDDO-Im and CDDO-Me) (Fig. 16) has been shown to attenuate LPS-induced cytokine expression and to increase levels of antioxidant genes (Thimmulappa et al., 2007).

7. Triterpenes as potential anti-acquired immunodeficiency syndrome (AIDS) agents

Yu et al. (2007) reviewed the potential of natural products as anti-AIDS compounds. While their review included many triterpenoids, the pharmacological effects of these compounds did not necessary include an increase in the immune response. Rather, they were described as antiviral agents that could inhibit the replication of the human immunodeficiency virus (HIV) in lymphocytes or increase cellular cytotoxicity or apoptosis. This review is of great interest for natural products researchers, specifically those specializing in triterpenoids, as many compounds and their mechanisms of action are reviewed.

8. Future perspectives

Triterpenes comprise one of the most interesting groups of natural products due to their high potential as pharmacological agents. Usually they are present in plants used as ethnomedicines, such as *Astragalus membranaceus*, *Boswellia serrata*, *Cayaponia tayuya*, *Ganoderma lucidum*, *Panax gisneng* or *Tripterygium wilfordii*. Many such compounds can either be used directly as active compounds or modified to increase their selectivity and potency. Although they have generally been examined for their anti-inflammatory and antiviral properties, their possible use as immunosuppressant drugs should be considered for future research. In addition, new paths of investigation should be pursued, including studies on their effects on transcriptional pathways other than that of NF-κB, as well as their implication in immune responses. Several structural groups of triterpenes have demonstrated specificity against transcriptional factors such as NF-AT or STAT3; these could be of particular interest in treating inflammation, cancer, and immune diseases.

Acknowledgements

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References

Abbas, A.K., Lichtman, A.H., 2003. Cellular and Molecular Immunology. Elsevier, Amsterdam.
Ali, Z., Khan, S.I., Ferreira, D., Khan, I.A., 2006. Podocarpaside, a triterpenoid possessing a new backbone from *Actaea podocarpa*. Organic Letters 8, 5529–5532.
Ammon, H.P., 2006. Boswellic acids in chronic inflammatory diseases. Planta Medica 72, 1100–1116.
Bae, E.A., Han, M.J., Shin, Y.W., Kim, D.H., 2006. Inhibitory effects of Korean red ginseng and its genuine constituents ginsenosides Rg3, Rf, and Rh2 in mouse passive cutaneous anaphylaxis reaction and contact dermatitis models. Biological and Pharmaceutical Bulletin 29, 1862–1867.
Behboudi, S., Morein, B., Villacres-Eriksson, M., 1996. In vitro activation of antigen-presenting cells (APC) by defined composition of *Quillaja saponaria* Molina triterpenoids. Clinical and Experimental Immunology 105, 26–30.
Behboudi, S., Morein, B., Villacres-Eriksson, M., 1997. *In vivo* and *in vitro* induction of IL-6 by *Quillaja saponaria* Molina triterpenoid formulations. Cytokine 9, 682–687.
Blaskovich, M.A., Sun, J., Cantor, A., Turkson, J., Jove, R., Sebti, S.M., 2003. Discovery of JSI-124 (cucurbitacin I), a selective Janus kinase/signal transducer and activator of transcription 3 signaling pathway inhibitor with potent antitumor activity against human and murine cancer cells in mice. Cancer Research 63, 1270–1279.
Brinker, A.M., Ma, J., Lipsky, P.E., Raskin, I., 2007. Medicinal chemistry and pharmacology of genus *Tripterygium* (Celastraceae). Phytochemistry 68, 732–766.
Butler, M.S., 2008. Natural products to drugs: natural products-derived compounds in clinical trials. Natural Product Reports 25, 475–516.
Çalis, I., Yürüker, A., Taşdemir, D., Wright, A.D., Sticher, O., Luo, Y.D., Pezzuto, J.M., 1997. Cycloartane triterpene glycosides from the roots of *Astragalus melanophrurius*. Planta Medica 63, 183–186.
Calixto, J.B., Otuki, M.F., Santos, A.R.S., 2003. Anti-inflammatory compounds of plant origin. Part I. Action on arachidonic acid pathway, nitric oxide and nuclear factor κB (NF-κB). Planta Medica 69, 973–983.
Chai, H., Wang, Q., Huang, L., Xie, T., Fu, Y., 2008. Ginsenoside Rb1 inhibits tumor necrosis factor-α-induced vascular cell adhesion molecule-1 expression in human endothelial cells. Biological and Pharmaceutical Bulletin 31, 2050–2056.
Chiang, L.C., Ng, L.T., Chiang, W., Chang, M.Y., Lin, C.C., 2003. Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of *Plantago* species. Planta Medica 69, 600–604.
Delves, P., Martin, S., Burton, D., Roitt, I., 2006. Roitt's Essential Immunology, 11th ed. Wiley-Blackwell, Hoboken, NJ, USA.
Dirsch, V.M., Kiemer, A.K., Wagner, H., Vollmar, A.M., 1997. The triterpenoid quinonemethide pristimerin inhibits induction of inducible nitric oxide synthase in murine macrophages. European Journal of Pharmacology 336, 211–217.
Escandell, J.M., Recio, M.C., Máñez, S., Giner, R.M., Cerdá-Nicolás, M., Ríos, J.L., 2006. Dihydrocucurbitacin B, isolated from *Cayaponia tayuya*, reduces damage in adjuvant-induced arthritis. European Journal of Pharmacology 532, 145–154.
Escandell, J.M., Recio, M.C., Máñez, S., Giner, R.M., Cerdá-Nicolás, M., Ríos, J.L., 2007a. Cucurbitacin R reduces the inflammation and bone damage associated with adjuvant arthritis in Lewis rats by suppression of TNF-α in T lymphocytes and macrophages. Journal of Pharmacology and Experimental Therapeutics 320, 581–590.
Escandell, J.M., Recio, M.C., Máñez, S., Giner, R.M., Cerdá-Nicolás, M., Gil-Benso, R., Ríos, J.L., 2007b. Dihydrocucurbitacin B inhibits delayed-type hypersensitivity reaction by suppressing lymphocyte proliferation. Journal of Pharmacology and Experimental Therapeutics 322, 1261–1268.
Escandell, J.M., Recio, M.C., Giner, R.M., Máñez, S., Cerdá-Nicolás, M., Merfort, I., Ríos, J.L., 2010. Inhibition of delayed-type hypersensitivity by cucurbitacin R through the curving of lymphocyte proliferation and cytokine expression by means of NF-AT translocation to the nucleus. Journal of Pharmacology and Experimental Therapeutics 332, 352–363.

Frei, B., Heinrich, M., Herrmann, D., Orjala, J.E., Schmitt, J., Sticher, O., 1998. Phytochemical and biological investigation of *Begonia heracleifolia*. Planta Medica 64, 385–386.

Fujimoto, H., Nakayama, M., Nakayama, Y., Yamazaki, M., 1994. Isolation and characterization of immunosuppressive components of three mushrooms, *Pisolithus tinctorius*, *Microporus flabelliformis* and *Lenzites betulina*. Chemical and Pharmaceutical Bulletin 42, 694–697.

Giner-Larza, E.M., Máñez, S., Recio, M.C., Giner, R.M., Ríos, J.L., 2000. A review on the pharmacology of lanostanes and related tetracyclic triterpenes. In: Mohan, R.M. (Ed.), Research Advances in Phytochemistry. Global Research Network, Trivandrum (India), pp. 65–82.

Haridas, V., Arntzen, C.J., Gutterman, J.U., 2001. Avicins, a family of triterpenoid saponins from *Acacia victoriae* (Bentham), inhibit activation of nuclear factor-κB by inhibiting both its nuclear localization and ability to bind DNA. Proceeding of the Natural Academy of Sciences USA 98, 11557–11562.

Khajuria, A., Gupta, A., Garai, S., Wakhloo, B.P., 2007. Immunomodulatory effects of two sapogenins 1 and 2 isolated from *Luffa cylindrica* in Balb/C mice. Bioorganic and Medicinal Chemistry Letters 17, 1608–1612.

Kim, S.M., Lee, S.Y., Yuk, D.Y., Moon, D.C., Choi, S.S., Kim, Y., Han, S.B., Oh, K.W., Hong, J.T., 2009a. Inhibition of NF-κB by ginsenoside Rg3 enhances the susceptibility of colon cancer cells to docetaxel. Archives of Pharmacal Research 32, 755–765.

Kim, B.H., Lee, Y.G., Park, T.Y., Kim, H.B., Rhee, M.H., Cho, J.Y., 2009b. Ginsenoside Rp1, a ginsenoside derivative, blocks lipopolysaccharide-induced interleukin-1β production via suppression of the NF-κB pathway. Planta Medica 75, 321–326.

Kroes, B.H., Beukelman, C.J., van den Berg, A.J., Wolbink, G.J., van Dijk, H., Labadie, R.P., 1997. Inhibition of human complement by β-glycyrrhetinic acid. Immunology 90, 115–120.

Lee, J.H., Koo, T.H., Yoon, H., Jung, H.S., Jin, H.Z., Lee, K., Hong, Y.S., Lee, J.J., 2006. Inhibition of NF-κB activation through targeting IκB kinase by celastrol, a quinone methide triterpenoid. Biochemical Pharmacology 72, 1311–1321.

Li, X.W., Weir, M.R., 1990. Radix T*ripterygium wilfordii*—a Chinese herbal medicine with potent immunosuppressive properties. Transplantation 50, 82–86.

Moradali, M.-F., Mostafavi, H., Ghods, S., Hedjaroude, G.-A., 2007. Immunomodulating and anticancer agents in the realm of macromycetes fungi (macrofungi). International Immunopharmacology 7, 701–724.

Oh, S.R., Kinjo, J., Shii, Y., Ikeda, T., Nohara, T., Ahn, K.S., Kim, J.H., Lee, H.K., 2000. Effects of triterpenoids from *Pueraria lobata* on immunohemolysis: β-D-glucuronic acid plays an active role in anticomplementary activity *in vitro*. Planta Medica 66, 506–510.

Panossian, A., Gabrielian, E., Wagner, H., 1997. Plant adaptogens II. Bryonia as an adaptogen. Phytomedicine 4, 83–97.

Panossian, A., Gabrielian, E., Wagner, H., 1999. On the mechanism of action of plant adaptogens with particular reference to cucurbitacin R diglucoside. Phytomedicine 6, 147–155.

Pfitzner, E., Kliem, S., Baus, D., Littnerst, C.M., 2004. The role of STATs in inflammation and inflammatory diseases. Current Pharmaceutical Design 10, 2839–2850.

Ríos, J.L., 2008. *Ganoderma lucidum*, un hongo con potenciales propiedades inmunoestimulantes. Revista de Fitoterapia 8, 135–146.

Ríos, J.L., Bas, E., Recio, M.C., 2005. Effects of natural products on contact dermatitis. Current Medicinal Chemistry. Anti-inflammatory and Anti-allergic Agents 4, 65–80.

Ríos, J.L., Giner, R.M., Jiménez, M.J., Hancke, J.L., Wickman, G., 1990. Studies on the anti-inflammatory activity of *Cayaponia tayuya* root. Fitoterapia 61, 275–278.

Ríos, J.L., Recio, M.C., Escandell, J.M., Andújar, I., 2009. Inhibition of transcription factors by plant-derived compounds and their implications in inflammation and cancer. Current Pharmaceutical Design 15, 1212–1237.

Ríos, J.L., Waterman, P.G., 1997. A review on the pharmacology and toxicology of *Astragalus*. Phytotherapy Research 11, 411–418.

Safayhi, H., Rall, B., Sailer, E.R., Ammon, H.P., 1997. Inhibition by boswellic acids of human leukocyte elastase. Journal of Pharmacology and Experimental Therapeutics 281, 460–463.

Scholz, D., Baumann, K., Grassberger, M., Wolff-Winiski, B., Rihs, G., Walter, H., Meingassner, J.G., 2004. Synthesis of dammarane-type triterpenoids with anti-inflammatory activity in vivo. Bioorganic and Medicinal Chemistry Letters 14, 2983–2986.

Sethi, G., Ahn, K.S., Pandey, M.K., Aggarwal, B.B., 2007. Celastrol, a novel triterpene, potentiates TNF-induced apoptosis and suppresses invasion of tumor cells by inhibiting NF-κB-regulated gene products and TAK1-mediated NF-κB activation. Blood 109, 2727–2735.

Sun, J., Blaskovich, M.A., Jove, R., Livingston, S.K., Coppola, D., Sebti, M., 2005. Cucurbitacin Q: a selective STAT3 activation inhibitor with potent antitumor activity. Oncogene 24, 3236–3245.

Thimmulappa, R.K., Fuchs, R.J., Malhotra, D., Scollick, C., Traore, K., Bream, J.H., Trush, M.A., Liby, K.T., Sporn, M.B., Kensler, T.W., Biswal, S., 2007. Preclinical evaluation of targeting the Nrf2 pathway by triterpenoids (CDDO-Im and CDDO-Me) for protection from LPS-induced inflammatory response and reactive oxygen species in human peripheral blood mononuclear cells and neutrophils. Antioxidant and Redox Signaling 9, 1963–1970.

Won, J.H., Im, H.T., Kim, Y.H., Yun, K.J., Park, H.J., Choi, J.W., Lee, K.T., 2006. Anti-inflammatory effect of buddlejasaponin IV through the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages via the NF-κB inactivation. British Journal of Pharmacology 148, 216–225.

Xu, W., Lin, Z., Yang, C., Zhang, Y., Wang, G., Xu, X., Lv, Q., Ren, Y., Dong, Y., 2009. Immunosuppressive effects of demethylzeylasteral in a rat kidney transplantation model. International Immunopharmacology 9, 996–1001.

Yu, D., Morris-Natschke, S.L., Lee, K.H., 2007. New developments in natural products-based anti-AIDS research. Medicinal Research Reviews 27, 108–132.