



Secondary metabolites from *Argania spinosa* (L.) Skeels

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Key words: argan tree, *Argania spinosa*, fatty acid, flavonoid, pharmacology, saponin, terpene

Abstract

Argania spinosa (L.) Skeels is a tree that plays a crucial role in the rural and urban economy of Morocco. Not only is the tree used in traditional medicine but its fruits contain almonds used to prepare an edible oil, its leaves are utilized to feed cattle, and its wood is used as fuel. In addition the tree is particularly well-adapted to arid lands and could hence be used to limit the desert progression that is threatening subtropical African countries. Consequently a program aimed at a better understanding of all the aspects and uses of the argan tree is currently being carried out in Morocco. This review summarizes the results gathered so far on the phytochemical and pharmacological activity of *A. spinosa*.

Introduction

The argan tree (*Argania spinosa* (L.) Skeels; syn *A. sideroxylon* Rœm & Schult.) belongs to the Sapotaceae family and it is the only species of this tropical family remaining in the subtropical zone. The thorny evergreen tree grows endemically in Morocco, predominantly on the west side of the High Atlas, and it has also been propagated in Israeli deserts (Nerd et al., 1994). In Morocco, the argan tree has had an essential ecological function for centuries. The tree, which is shrubby, may, in some places reach 20 m. It protects the soil against erosion and its large canopy maintains soil fertility by shading domestic cultures that guarantee most of the dietary needs of small scale farmers (Morton and Voss, 1987). The argan tree also supports more directly the economy of the region (M'Hirit et al., 1998) since its fruits furnish an edible and marketable oil that accounts for a large part of the rural dwellers daily lipid-diet (Collier and Lemaire, 1974). However, despite its obvious usefulness the argan grove is steadily decreasing in terms of density and surface covered due to several factors including unprecedented arid years, over-use of the wood as fuel and of the aerial part as forage, as well as poor

germination capacity, not to mention some regretful shortsighted policies promoting the replacement of argan trees with exogenous species which bring in higher incomes for some years but induce disastrous soil damage over the long term. To limit the destruction of the argan grove and attempt to simultaneously stop the desert progression while preserving most of the native traditions, a reforestation program is being currently actively developed in Morocco with the support of several Moroccan and international organizations (Charrouf et al., 2002). The program includes a systematic investigation of the argan tree secondary metabolites in order to verify traditional medicinal uses and explore new opportunities (Charrouf, 1995), which would increase the commercial value of the argan tree and consequently strongly stimulate the reforestation program by getting the total adhesion of the would-be concerned rural populations.

Traditional uses of *Argania spinosa*

Traditionally, the argan tree is mainly used for the preparation of an oil that is extensively utilized for nutritional purposes but is also recommended to cure some

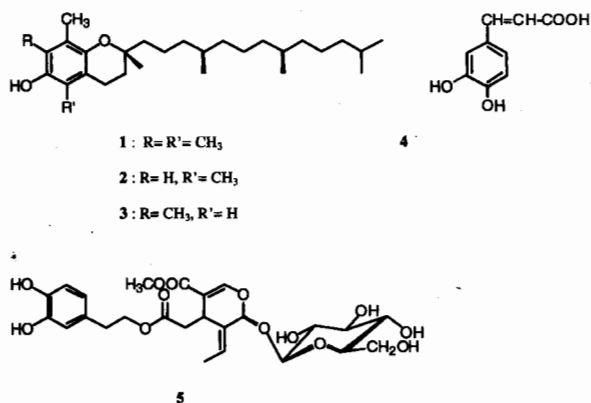


Figure 1. Structure of the main tocopherols and ortho-diphenols isolated from argan oil.

therapeutic disorders and can be used as a cosmetic (Bellakhdar, 1997).

The oil is prepared, exclusively by women, from argan fruits whose size is similar to that of an ordinary walnut. Following the ripened-fruit collection that occurs between May and August, the fruits are sun-dried for a few days. Then, using stones, women strip the pulp off the hard shelled argan seed (argan nut). The pulp is palatable to cattle and also provides a cheap protein-rich material for all the farm animals. Peeled argan nuts are then cracked with the stones used in the first step and roasted by mild heating in clay pans until brown (dried-argan shells are generally used as fuel). The roasted nuts are then crushed, using a millstone, into an oily dough to which small quantities of water are regularly added to obtain a smooth paste that is constantly kneaded until the separation of the oil which is finally bottled. When correctly prepared, more particularly when the added water is of good quality, argan oil preserves well for several months. In case of water shortage, low-purity water is sometimes used and the oil has to be consumed rapidly even if some salt can be added to improve its preservation. Starting from 34 kg of dried-fruits, about 20 h are generally necessary to obtain one liter of oil. The most time-consuming step is the shell-cracking process which is also unanimously considered as the most tedious by women.

Argan oil has an hazelnut taste and it is prized for its organoleptic properties. Natives frequently consume it on toasts during breakfast or used it for cooking and as salad oil. Argan oil can easily replace olive or cottonseed oil in deep-fat frying (Yagmur et al., 2001). Mixed with almonds and honey, argan oil is also eaten as a spreading paste of high energy value

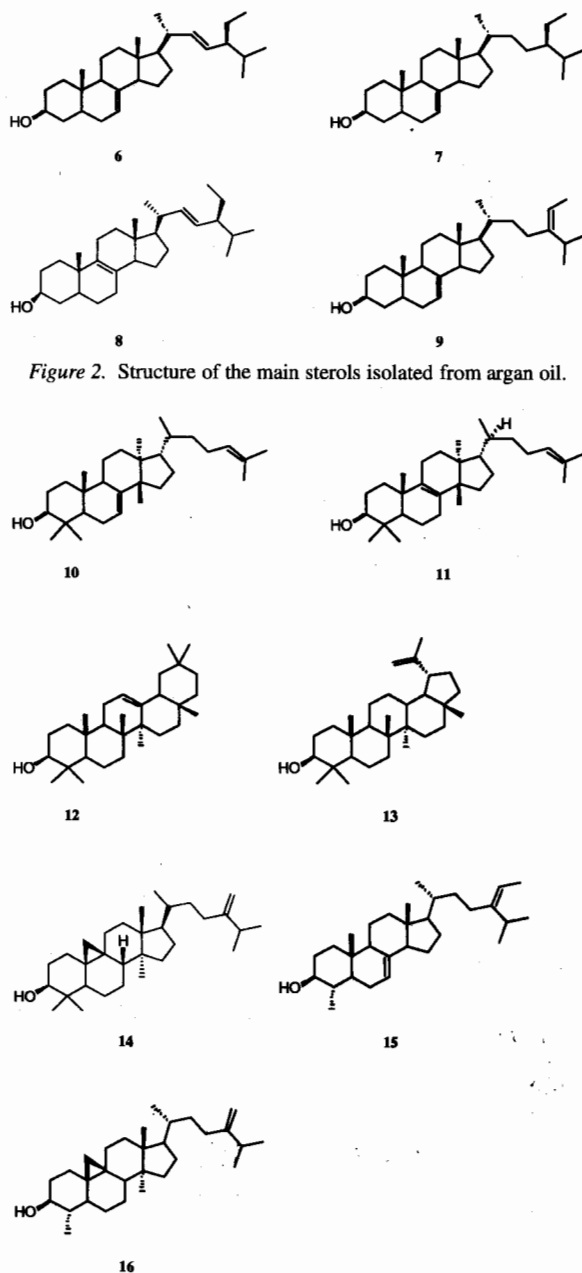


Figure 2. Structure of the main sterols isolated from argan oil.

Figure 3. Structure of the main triterpene alcohols isolated from argan oil.

named 'amlou'. As cosmetic, argan oil is traditionally used as a skin lotion indicated to cure all kinds of skin pimples, more particularly juvenile acne and chicken pox pustules. It is also recommended to reduce dry skin diseases and slow down the appearance rate of wrinkles as well as to increase the resistance of the hair shaft and preserve its integrity. Argan oil

is also used in rheumatology by application on the articulations to be cured. Orally used, argan oil is traditionally recommended as choleric, hepatoprotective agent and in the case of hypercholesterolemia and atherosclerosis. In obstetrics argan oil is counselled to prevent miscarriage. Amlou is said to be an efficient aphrodisiac.

Composition of argan oil

Whereas nowadays most women still continue to extract argan oil in the traditional way, improvements have been made to the technology. These changes have been stimulated by the implementation of improved technology in several cooperatives in which women prepare argan oil and its derivatives (Charrouf et al., 2002). Particularly, mechanical presses have been introduced in these cooperatives making nut crushing and dough-water mixing unnecessary steps. This improved technology allows for the production in higher yield (50% vs. 30%) of an oil whose preservation properties are more predictable and reproducible (Charrouf et al., 1997; Charrouf, 2002). Infrared spectroscopy and thermogravimetric analysis have shown argan oil to be stable for months at ambient temperature. This long-term preservation capacity decreases if the storage is performed at a temperature above 64 °C (Fdili Alaoui et al., 2001). Oil can be obtained with a maximum yield by press-extraction when the crushed almonds have a granular size of 1 mm, and the pressure and the temperature are around 400 kg/cm² and 175 °C, respectively (Mountasser and El Hadek, 1999). However, for cosmetic or dietary uses, using such conditions is inconceivable for it would produce an oil with unsatisfactory organoleptic properties and hence only sub-optimal extraction conditions are applied. For industrial purposes, argan oil can also be obtained by solvent-extraction using a volatile lipophilic solvent (Hatinguais et al., 1983). Using hexane, the best extraction parameters have been found to be an extraction time of 8 h and the use of 3 ml of hexane per gram of crushed almonds. The extraction yield is then close to 55% and sometimes reaches 68% for some particularly oil-rich plants (Mountasser and El Hadek, 1999).

The physical and chemical properties of argan oil have been studied (Table 1). Similar data have been obtained with traditional extracted and solvent-extracted oil (Berrada, 1972; Charrouf, 1984) as well

as for oil prepared from trees grown in Morocco or Israel (Yaghmur et al., 1999).

Glycerides constitute 99% of the oil and the total-unsaturated-fatty-acids to total-saturated-fatty-acids ratio is above 4. Major unsaturated fatty acids are oleic and linoleic acids (linolenic acid is only present as traces), while major saturated fatty acids are palmitic and stearic acids (Table 2). Similar results have been reported for trees of diverse geographical origins or independently of the extraction method used (Charrouf, 1984; Farines et al., 1984a; Yaghmur et al., 1999).

Unsaponifiable matter contains carotenes, tocopherols, triterpene alcohols, polyphenols, sterols, and xanthophylls (Farines et al., 1984a). Interestingly, argan oil is almost twice as rich in tocopherol as olive oil (620 vs. 320 mg/kg). α -Tocopherol (**1**) as well as β - and γ -tocopherol (**2**, **3**) have been identified in argan oil (Charrouf, 1984). The presence of these tocopherols (Vitamin E) together with polyphenols (caffeic acid **4** and oleuropein **5**) (Chimi et al., 1988) probably plays a part in the good preservation qualities of argan oil. Four sterols have been isolated from argan oil (Farines et al., 1984b), spinasterol (**6**), schottenol (**7**), (3 β ,22E, 24S)-stigmasta-5,22-dien-3-ol (**8**), and (3 β ,24Z)-stigmasta-7,24-28-dien-3-ol (**9**). During the same study, several additional triterpene alcohols have also been isolated from the unsaponifiable matters. These are butyrospermol (**10**), tirucallol (**11**), β -amyrin (**12**), lupeol (**13**), 24-methylene cycloartanol (**14**), citrostadienol (**15**), and cycloeucalenol (**16**).

Experiments to confirm the relevance of the prescription of argan oil, in traditional medicine, in cases of hypercholesterolemia have been performed recently and found to be successful (Charrouf, 2002) as has been the confirmation of the oil antihypertensive properties (Berrada et al., 2000). These studies have been performed on two species of rodents. Chronic ingestion of 5 ml/kg/day of argan oil by spontaneous hypertensive rats restored normal blood pressure and induced hypocholesterolemia. Argan oil tocopherols and unsaturated fatty acids are suspected to be mainly responsible for these pharmacologic activities (Berrada et al., 2000).

Saponins and other secondary metabolites from *Argania spinosa*

The phytochemical analysis of different parts of the argan tree or of the press-cake (the by-product obtained from the preparation of argan oil) has also been in-

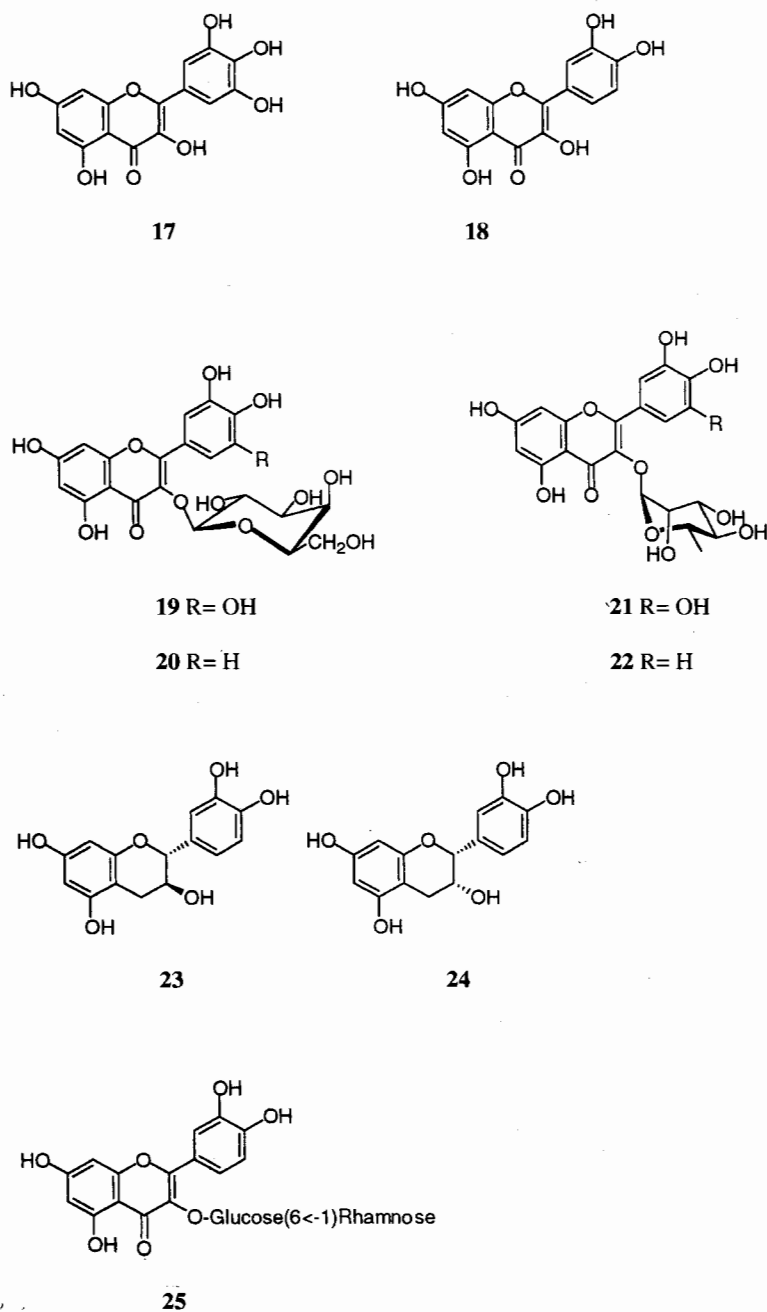


Figure 4. Structure of the main flavonoids isolated from *Argania spinosa*.

investigated in order to possibly identify new bioactive compounds or to increase the commercial value of the press-cake. A large diversity of secondary metabolites including triterpenes, sterols, flavonoids, volatile compounds, phenols and saponins have been isolated so far from various parts of the argan tree.

The total flavonoid content of the mixed leaves, stems and thorns of *A. spinosa* was found to be 17% of the extractable material (Tahrouch et al., 2000). The two major flavonols were already known compounds: myricetin (17) and quercetin (18) (Charrouf, 1995). Four flavonoid glycosides were identified as myricetin-3-O-galactoside (19),

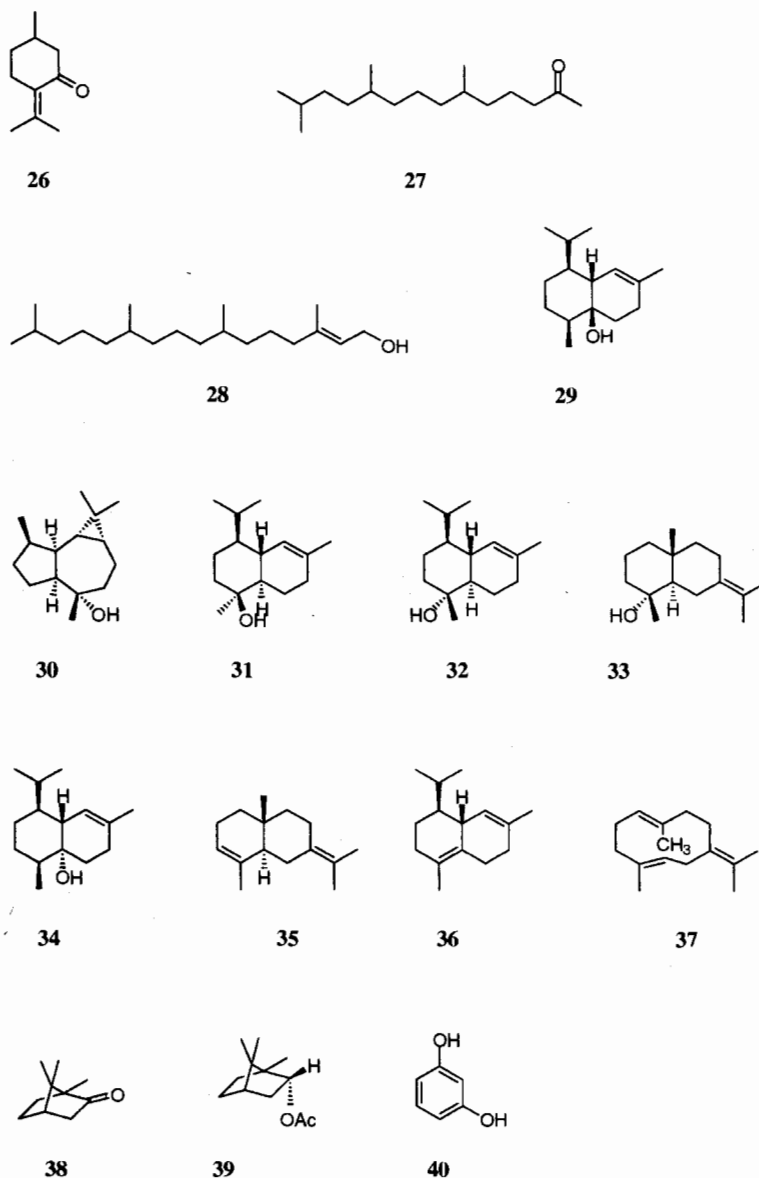


Figure 5. Structure of some of the volatile components isolated from *Argania spinosa*.

Table 2. Fatty acid composition of argan oil (%) (Charrouf, 1984; Farines et al., 1984a; Yaghmur et al., 1999).

Myristic acid	0.2	Linoleic acid	31–35
Palmitic acid	12–14	Linolenic acid	0–0.1
Palmitoleic acid	0–1	Arachidic acid	0–0.4
Stearic acid	5–6	Gadoleic acid	0
Oleic acid	42–47	Behenic acid	0

hyperoside (quercetin-3-O-galactoside) (**20**), myricitrin (myricetin-3-O-rhamnoside) (**21**), and quercitrin (quercetin-3-O-rhamnoside) (**22**) (Tahrouch et al., 2000; El Kabouss et al., 2001). From the fruit pulp, several other phenolic derivatives were isolated from which (+)-catechin (**23**), (-)-epicatechin (**24**), rutin (**25**), and *p*-hydroxybenzoic acid were unambiguously identified. Variations in the flavonoid content in relation with the form of the fruit and its maturity have also been studied (Chernane et al., 1999).

Table 1. Physical and chemical properties of argan oil (Berrada, 1972; Charrouf, 1984; Yaghmur et al., 1999).

Relative density 0.9	Iodine value 96.1–96.7
Refractive index 1.46	Saponification value 190.9–193.8
Acid value 1.3 (2.4±0.4 for Israeli oil)	Unsaponifiable (%) 1

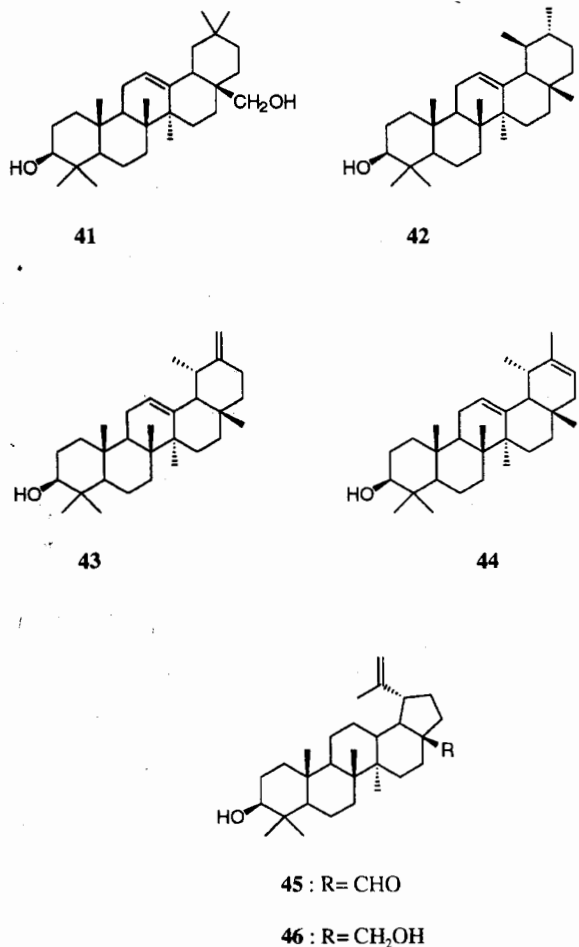
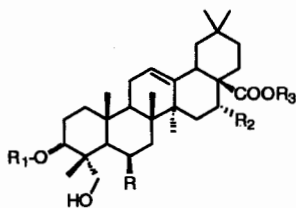


Figure 6. Structure of the triterpenes isolated from *Argania spinosa*.

The composition of the volatile components and essential oils of the leaves of *A. spinosa* has been the subject of two independent studies (Tahrouch et al., 1998; El Kabouss et al., 2002). In their study, Tahrouch et al. found that the volatile components had a concentration of 98 mg/g of dried material. The structure of the volatile components was investigated using different techniques including gas chromatography/mass spectroscopy. Twenty five compounds were detected and nineteen were

fully identified. These are: nonane, 3-methylbutyric acid, octan-3-one, decane, methylbenzoate, undecane, pulegone (26), undecan-2-one, tridecane, *p*-hydroxyphenylethanol, pentadecane, heptadecane, hexahydrofarnesyl acetone (27), 14-methylidene-2,6,10-trimethylhexadecene, octanol, (*Z*)- and (*E*)-2,6,10-trimethyl hexadeca-1,3-diene, and phytol (28). The major component was by far, 14-methylidene-2,6,10-trimethylhexadecene (51.2%) followed by (*Z*)- and (*E*)-2,6,10-trimethyl hexadeca-1,3-diene (12.3 and 17%, respectively). In another study, El Kabouss et al. found that, in the leaves of *A. spinosa*, the essential oils had a concentration ranging from 0.03 to 0.05%. Thirty three compounds were isolated and twenty six identified. Oxygenated sesquiterpenoids were found to represent the most important fraction of the oil composition with 1, 10-di-epi-cubenol (29) being the major derivative (20.5%). Other sesquiterpene alcohols were identified as viridiflorol (30) (6.0%), τ -cadinol (31) (1.7%), α -cadinol (32) (1.5%), juniper camphor (33) (1.3%), and 1-epi-cubenol (34) (1.2%). Sesquiterpene hydrocarbons constituted another appreciable fraction of the oil composition, selina-3,7(11)-diene (35), δ -cadinene (36), and germacrene B (37) being particularly abundant (5.1%, 2.33%, and 1.1%, respectively). Three monoterpenes were isolated and two were identified as camphor (38) (2.6%) and bornyl acetate (39) (1.8%). Study of the volatile components contained in the pulp and kernel of argan fruits (Tahrouch et al., 1998) led to the isolation of seven compounds from the pulp: (*Z*)- and (*E*)-but-2-enol, *n*-octane, 3-methyl butyric acid, decane, resorcinol (40), and 14-methylidene-2,6,10-trimethylhexadecene. While this latter compound had been found in high concentration in the leaves (51.2%), the pulp was found to be resorcinol-rich (73.5%). Only 14-methylidene-2,6,10-trimethylhexadecene has been identified from the kernels (Tahrouch et al., 1998). Palmitic acid was also detected in the leaves and pulp but its concentration was reported to be subject to large variations (Tahrouch et al., 1998).



47 : R= OH, R₁= Glucose(1->6)Glucose, R₂= OH,

R₃= Rhamnose(1->3)Xylose(1->3)Rhamnose(1->2)Arabinose

48 : R= OH, R₁= Glucose(1->6)Glucose, R₂= OH,

R₃= Apiose(1->3)Xylose(1->3)Rhamnose(1->2)Arabinose

49 : R= OH, R₁= Glucose, R₂= OH,

R₃= Rhamnose(1->3)Xylose(1->3)Rhamnose(1->2)Arabinose

50 : R= OH, R₁= Glucose(1->6)Glucose, R₂= H,

R₃= Rhamnose(1->3)Xylose(1->3)Rhamnose(1->2)Arabinose

51 : R= OH, R₁= Glucose(1->6)Glucose, R₂= H,

R₃= Apiose(1->3)Xylose(1->3)Rhamnose(1->2)Arabinose

52 : R= OH, R₁= Glucose, R₂= H,

R₃= Apiose(1->3)Xylose(1->3)Rhamnose(1->2)Arabinose

53 : R= OH, R₁= Glucose, R₂= H,

R₃= Rhamnose(1->3)Xylose(1->3)Rhamnose(1->2)Arabinose

54 : R= OH, R₁= R₂= R₃=H

55 : R= OH, R₁= R₃= H, R₂= OH

56 : R= H, R₁= Apiose(1->4)Glucose, R₂= H, R₃= Glucose

57 : R= H, R₁= Apiose(1->4)Glucose, R₂= H, R₃= Arabinose

58 : R= H, R₁= Apiose(1->4)Glucose, R₂= H,

R₃= Apiose(1->3)Xylose(1->4)Rhamnose(1->2)Arabinose

59 : R= R₁= R₂= R₃= H

60 : R= OH, R₁= Glucose(1->3)Glucose, R₂= H,

R₃= Rhamnose(1->3)Xylose(1->4)Rhamnose(1->2)Arabinose

61 : R= H, R₁= Glucose, R₂= OH,

R₃= Rhamnose(1->3)Xylose(1->4)Rhamnose(1->2)Arabinose

62 : R= OH, R₁= Glucose(1->3)Glucose, R₂= OH,

R₃= Rhamnose(1->3)Xylose(1->4)Rhamnose(1->2)Arabinose

63 : R= OH, R₁= Glucose(1->3)Glucose, R₂= H,

R₃= Rhamnose(1->3)Xylose(1->4)[Rhamnose(1->3)]Rhamnose(1->2)Arabinose

64 : R= OH, R₁= Glucose(1->3)Glucose, R₂= OH,

R₃= Rhamnose(1->3)Xylose(1->4)[Rhamnose(1->3)]Rhamnose(1->2)Arabinose

Figure 7. Structure of the aglycones and saponins isolated from *Argania spinosa*.

The higher terpene fraction of the leaves of *A. spinosa* has also been studied (Chahboun, 1993). This has led to the identification of mono and dihydroxylated terpenes such as erythrodiol (41), lupeol (13), α - and β -amyrin (42, 12), taraxasterol (43), and ψ -taraxasterol (44). In addition, sterols [spinassterol (6), schottenol (7)], and tocopherols (1-3) were also isolated. From the pulp of argan fruits, the same terpenes were also identified (Charrouf et al., 1990, 1991b) together with betunaldehyde (45) and betulin (46) (Charrouf, 1991a). From the latex of the fruit, *cis*- and *trans*-polyisoprene were isolated (Fellat-Zarrouk et al., 1987).

In addition to these sometimes ubiquitous secondary metabolites, the argan tree has been shown to be a source of saponins. Since the tree is fungus-resistant, the idea of a possible link between this resistance and the saponins has triggered the investigation of the saponin-content of various parts of the tree and of the press cake. Research in this area was further stimulated by the numerous biological and pharmacological activities attributed to these secondary metabolites (Hostettmann and Marston, 1995) and hence the possibility to isolate new pharmacological leads from *A. spinosa*.

Saponins in the argan-tree kernel were the first to be investigated (Charrouf et al., 1992). This study led to the isolation of seven saponins, five of which were new compounds (arganine A, B, D, E, and F (47, 48, 50-52). Arganine C (49) corresponded to a known but unnamed saponin (Gariboldi et al., 1990) and the seventh saponin was already named misaponin A (53) (Kitagawa et al., 1975). The seven saponins turned out to be bidesmosidic derivatives having 5 or 6 sugar residues and whose genin was an hydroxylated triterpene belonging to the Δ -12 oleanan family. The aglycone of the saponins was identified as either protobassic acid (54) or 16 α -OH-protobassic acid (55) and, combining degradative and non-degradative spectroscopic methods, the sugar side-chains were located at position 3 and 28 of the aglycone. The sugar chain substituting position-3, and bound to the aglycone by an ether linkage, was composed of a one or two glucose residue(s) and the second sugar side-chain, bound to the aglycone by an ester linkage, was composed of four sugar residues in linear form. Consequent to this study, the structure of saponins contained in the trunk of argan tree was investigated (Oulad-Ali et al., 1996). Three novel bidesmosidic saponins [arganine G, H, J (56-58)] were identified using non degradative methods exclusively. Interestingly, the three saponins had a

similar aglycone that was, however, different from the aglycone of arganine A-F (47-52). The aglycone of arganine G, H, J was identified as bayogenin (59), also a member of the Δ -12 oleanan family corresponding to the 6-deoxy-analogue of protobassic acid. The second main difference came from the sugar side-chain substituting the aglycone 3-position. In the saponins isolated from the trunk, a disugar unit composed of an apiose and a glucose residue was consistently found. Position 28 of the aglycone was substituted with either a single sugar residue: a glucose residue for arganine G, an arabinose residue for arganine H or a linear four-sugar-residue chain for arganine H. Another study concentrated on the saponins of the pulp of argan fruits (Alaoui et al., 2001). Three saponins were identified, two of them (60, 61) being already known derivatives (Lavaud et al., 1996; Kitagawa et al., 1975). The previously unknown saponin was named arganine K (62) and its structure was reminiscent of the structure of the saponins isolated from the kernel. Its aglycone was found to be 16α -OH protobassic acid substituted on its 3-position with a 1 \rightarrow 3 diglucose residue and whose 28-position was substituted with a linear four-sugar-residue side chain. From the shell protecting argan nuts, four saponins were isolated (Alaoui et al., 2002). Among these the two known saponins 60 and 61 previously also isolated from the pulp of argan fruits were isolated together with two novel saponins (63, 64) having the same sugar pattern: a diglucose unit on the aglycone C3 position and a branched pentaglycosidic unit on C28 and whose aglycone was either protobassic acid or 16α -OH protobassic acid, respectively. These studies, although still in progress, have already indicated a major and constant difference in the structure of the arganine aglycones even if all belong to the Δ -12 oleanan series. Indeed, the aglycone of the fruit saponins (pulp, shell, or kernel) are consistently more oxidized than the trunk aglycones. Furthermore this oxidation occurs first at the Δ -12 oleanan skeleton 6-position then at the 16-position.

Biological properties of *Argania spinosa*

The main biological properties of the argan tree seem to be associated with the oil, although biological evaluation of other secondary metabolites has been performed. Considering the numerous pharmacological activities reported for secondary metabolites also isolated from argan tree (Uchida et al., 1983; Villasenor et al., 1996; Villasenor and Domingo, 2000;

Rajic et al., 2000; Wada et al., 2001; Saleem et al., 2001), several biological areas could be envisaged. As an example, this has led to the evaluation of the anti-microbial activity of the leaves of *A. spinosa* (El Kabouss, 2002). However, considering the large quantity of saponins isolated from *A. spinosa* and their structure peculiarity, pharmacological studies have been particularly concentrated on these metabolites. As a preliminary test, the molluscicide activity of a crude mixture of kernel saponins was evaluated against *Biomphalaria glabrata* the temporary host of *Schistosoma mansoni*. An inhibitory activity was found at concentration of 400 μ g/ml (Charrouf, 1991b). The anti-fungus activity of the same saponin mixture was evaluated and the minimal inhibitory concentration was obtained at 12.5 and 50 μ g/ml against *Cladosporium cucumerinum* and *Polysticus versicolor*, respectively (Charrouf, 1991b).

The analgesic and anti-inflammatory properties of the argan tree saponins were also evaluated on rats and mice. No central analgesic activity was found but an anti-inflammatory activity was detected with oral doses around 500 mg/kg. Interaction with leucotrienes in the arachidonic acid cascade has been proposed to explain this activity (Alaoui et al., 1998a). Acute and chronic toxicity of the saponins of the argan tree have also been determined following oral and intraperitoneal administration in mice and rats. Concerning the acute toxicity, for the oral route the LD₅₀ was 1.3 g/kg. Concerning the chronic toxicity, at doses of 100 and 200 mg/kg, a decrease in blood sugar level was reported after the third month together with a possible renal pathology (Alaoui et al., 1998b). Finally, very recently, the efficiency of argan press-cake saponins on the activation of lipolysis has been evidenced on human adipocytes by measurement of the released glycerol (Henry et al., 2002). Additional activities, such as glutathione biosynthesis stimulation and DNA protection against UV-B also seem to indicate that saponins of high therapeutic index are contained in the argan tree and their inclusion in new cosmetic preparations can be reasonably considered.

Conclusion

The full success of the argan tree reforestation program is undoubtedly subordinated to the discovery of new markets allowing, through the use of its secondary metabolites, an increase in the argan tree commercial value. For this, two axes are currently being invest-

igated. One concerns the use of argan derivatives as cosmetics and the second their use as therapeutic agents. The former use is already developed and revitalizing emulsions containing argan oil are found on Western markets. Additional products can also be expected in a near future (Pauly et al., 2002). Concerning the therapeutic use, despite the large pharmacological activities shown by the ubiquitous secondary metabolites isolated from the argan tree, the uniqueness of most of the saponins isolated from the different parts of *A. spinosa* studied so far makes this class of compounds the most promising, at the present time, for the discovery of new therapeutically or phytochemically active derivatives if their pharmacological selectivity can be demonstrated.

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