Microbial Transformation of Triterpenoids

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Abstract: Microbial transformation of triterpenoids has provided new derivatives that are potentially useful for pharmacological studies. In these biotransformation processes, several reactions that are difficult to achieve by chemical means have been accomplished, such as: introduction of hydroxyl groups into remote positions of the molecules; selective cleavage of the side chains of tetra-cyclic terpenoids to produce C_{19} steroids; regioselective glycosidic transfer reactions; selective ring cleavage through a Baeyer-Villiger-type oxidation to render *seco*-triterpenoids; and carbon skeleton rearrangements involving a methyl group migration. These biotransformations have also been used as *in vitro* models to mimic and predict the mammalian metabolism of biologically active triterpenoids.

Key Words: Microbial transformation, biotransformation, triterpenoid, triterpene, filamentous fungi, bacteria.

I. INTRODUCTION

Triterpenoids are a large and structurally diverse group of natural products [1] that display nearly 200 distinct skeletons. Most triterpenoids are 6-6-6-5 tetracycles, 6-6-6-6-5 pentacycles, or 6-6-6-6-6 pentacycles, but acyclic, monocyclic, bicyclic, tricyclic, and hexacyclic triterpenoids have also been isolated from natural sources. A recent review covering mechanisms of their formation from squalene, oxidosqualene or bisoxidosqualene, has been published [2].

Over the past decade, a large group of cyclic triterpenoid-like compounds, such as free triterpenoids, saponins (triterpenic glycosides), and phytosterols, have come to the foreground of interest due to their diverse biological effects. Triterpenoids are studied for their anti-neoplastic [3,4], anti-inflammatory [5,6], anti-ulcerogenic [7], antimicrobial [8], anti-plasmodial, antiviral (anti-HIV) [9-11], hepato- and cardio-protective [12-14], analgesic [15], anti-mycotic, immunomodulatory, and tonic effects. They are used for the prevention and treatment of hepatitis, parasitic and protozoal infections and above all, for their cytostatic properties [16]. From a biological standpoint, the most important triterpenoids structures are oleanane, ursane, lupane, and dammarane-euphane triterpenoids [17].

Microbial transformation is today considered to be a routine economically and ecologically competitive technology for synthetic organic chemists in search of new production routes for fine chemical, pharmaceutical, and agrochemical compounds [18-21]. Biotransformation is also an attractive approach to generate structural diversity in a chemical library, which can be used to synthesize chemical structures that are difficult to obtain by other means. A great difficulty for a specific biotransformation of a certain substrate is to find the appropriate microorganism, so that, traditionally, one of the most widely used techniques is screening with different microbial strains.

The microbial conversion of terpenoids has been investigated to produce new and useful metabolites, as an alternative to chemical synthesis for preparation of pharmacologically active compounds. Biotransformation has also been used to expand the chemical diversity of terpenoids, the largest group of natural products. Several reviews on microbial transformation of terpenoids have been published in recent years [22-24].

Microbial transformation of triterpenoids has been developed basically in the last 10 or 15 years. This technology is an important tool in the structural modification of these organic compounds, especially for natural products with complicated structures, due to their significant regio- and stereo-selectivity. Biotransformation has also been employed as an *in vitro* model to mimic and predict the mammalian metabolism of biologically active triterpenoids, and to obtain metabolites that are valuable for *in vivo* metabolism research. This review covers all the literature published in the field of the microbial transformation of triterpenoids. A review of biotransformation of pentacyclic triterpenes, written in Chinese, has been recently published [25].

II. ACYCLIC TRITERPENES

Most of the microbial transformations of triterpenoids have been carried out on tetra- and pentacyclic compounds, but there are a few studies on the biotransformation of acyclic triterpenes. Several microorganisms may be capable of degrading squalene (**1**) to intermediates useful in chemical syntheses. *Corynebacterium* sp. (S-401) which was isolated from soil, produced five metabolites from squalene (**1**), identified as mono-, di-, tri-, tetra-, and pentahydrated squalene derivatives, to form tertiary alcohols (**2**-**6**) [26]. Squalene (**1**) was also oxidized to squalenedioic acid (**7**) by *Nocardia* (BPM 1613) [27] and *Corynebacterium* sp. (SY-79) [28]. *Rhodococcus* sp., which could use squalene as sole carbon source, yielded a single major transformation product identified as squalen-12-one (**8**), an α,β-unsaturated ketone [29] (Fig. **1**).

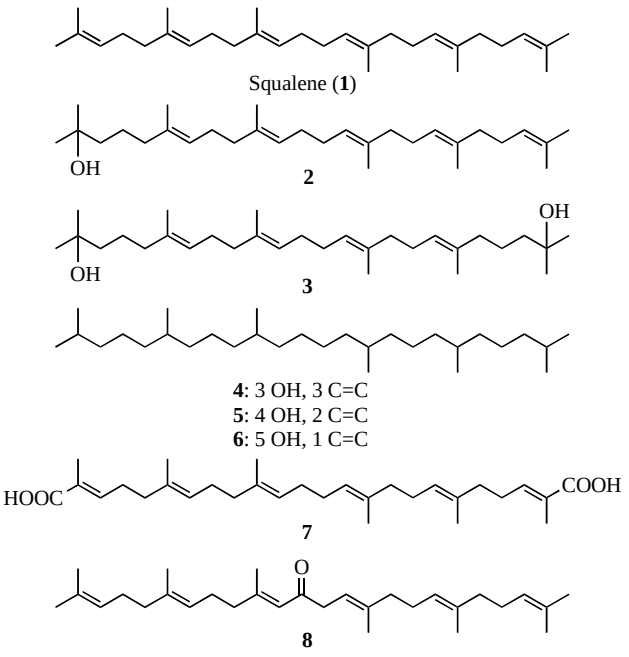

Fig. (1). Metabolites from biotransformations of Squalene (**1**).

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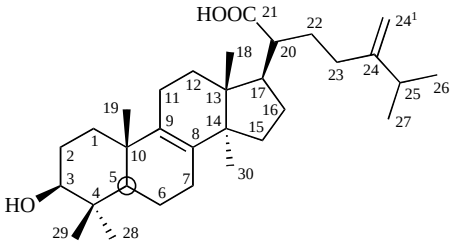
III. TETRACYCLIC TRITERPENOIDS

III.1. Eburicane Skeleton

Eburicoic acid (**9**) is a homo-triterpenoid compound with a skeleton closely related to lanostane-type triterpenoids. The biotransformation of this compound (**9**) by the fungus *Glomerella fusarioides* (ATCC 9552) produces 4-hydroxy-3,4-*seco*-eburica-8,24(28)-diene-3,21-dioic acid (**10**) [30] (Fig. **2**), via an oxidative cleavage of the A ring, analogous to a Baeyer-Villiger-type reaction.



Eburicoic acid (**9**)

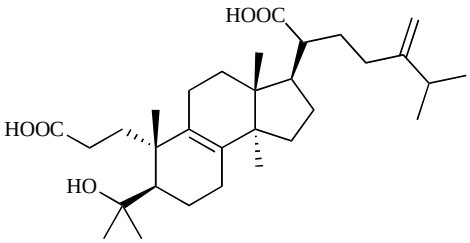


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Fig. (2). Metabolites from biotransformation of Eburicoic acid (**9**).

III.2. Lanostane Skeleton

It is well known that a wide range of microorganisms are able to utilize cholesterol and phytosterols for the selective cleavage of the side chains of these molecules, to give C_{19} and/or other useful steroid intermediates. It has been reported that the microbial transformation of lanosterol (**11**) yields androsta-4,8(14)-diene-3,17-dione (**12**) by *Mycobacterium* sp. (NRRL B-3805) as a major metabolite (30%) [31]. Also four additional minor metabolites (**13-16**) were isolated from this incubation [32] (Fig. **3**). During these transformations, a series of complex chemical changes occurred including demethylation at the 4α,4β- and 14α- positions, cleavage of the

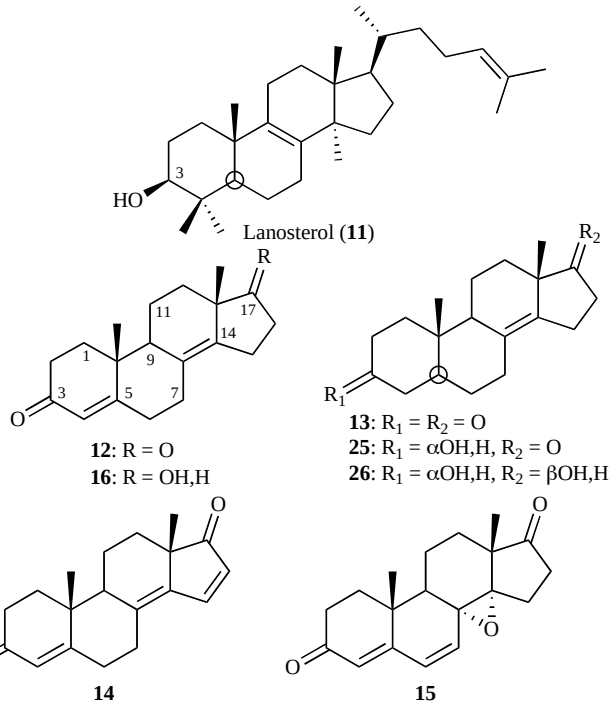


Fig. (3). C_{19} steroids from biotransformations of Lanosterol (**11**) and cycloartane triterpenes.

C-17 side-chain, and the disappearance of the $\Delta^{8(9)}$ double bond accompanied by the formation of other double bonds, mainly at $\Delta^{8(14)}$ position. This last change is of particular interest, and therefore several lanosterol derivatives, with substituent modification at C-8 and C-9, were prepared and subjected to transformation with this microorganism. Thus, microbial transformation of 8α,9α-epoxy-tetrahydrolanosterol (**17**) yielded 20(*S*)-hydroxymethyl-12α-hydroxypregn-4-en-3-one (**18**) and 20(*S*)-hydroxymethyl-4α,4β,14α-trimethylpregna-7,9(11)-dien-3β-ol (**19**) in relatively low yields of 5.0% and 1.6%, respectively. Also the substrate lanosta-7,9(11)-dien-3β-o1 (**20**) was incubated with the same microorganism, producing androsta-4,8(14)-diene-3,17-dione (**12**, 30%), and 5α-androst-8(14)-ene-3,17-dione (**13**, 7.1%), previously obtained in the biotransformation of lanosterol (**11**), together with lanosta-7,9(11)-dien-3-one (**21**, 4.2%), and methyl 12α-hydroxy-bisnorchola-4,17(20)-dien-22-oate (**22**, 0.7%) [33] (Fig. **4**).

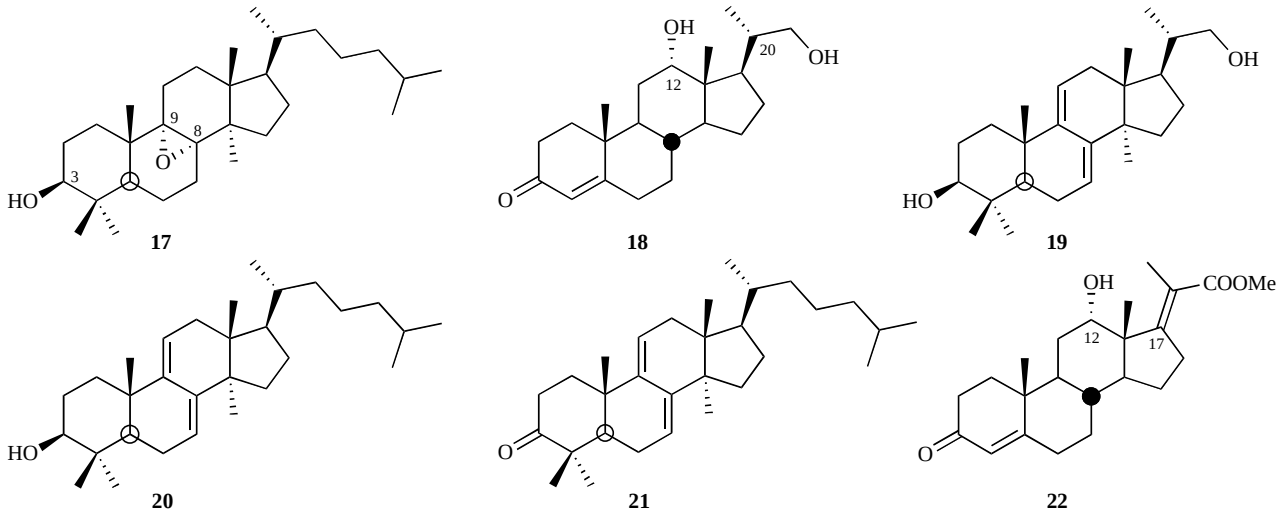
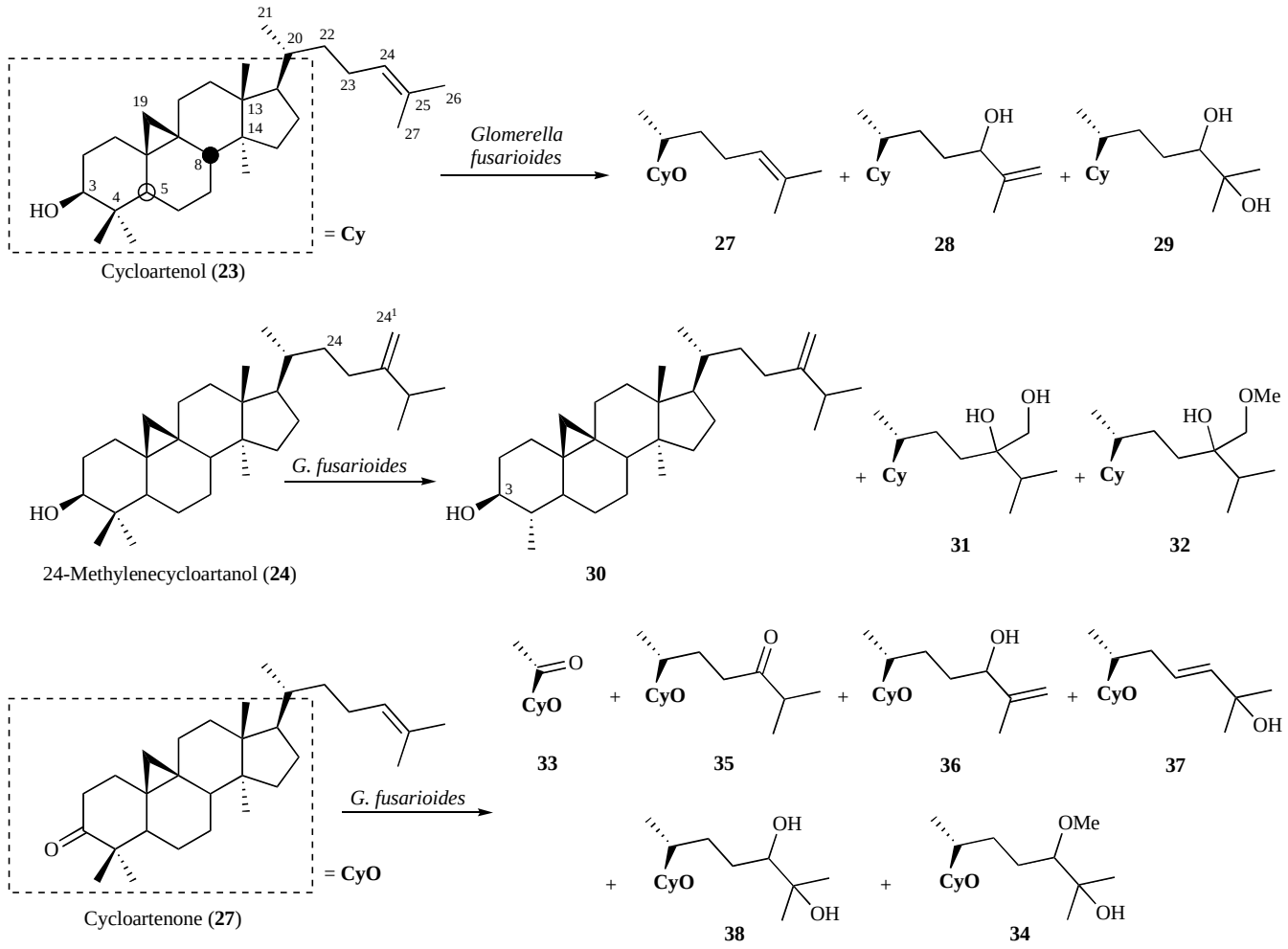


Fig. (4). Metabolites from biotransformation of lanosterol derivatives.



Scheme 1. Biotransformation of cycloartane triterpenes by *Glomerella fusarioides*.

III.3. Cycloartane Skeleton

Cycloartenol (**23**) and 24-methylenecycloartanol (**24**) are the major triterpene alcohols present in rice bran oil, and are considered to be good resources to develop a potent antitumor promoter. The microbial transformations of these compounds (**23** and **24**) by *Mycobacterium* sp. (NRRL B-3805) gave rise to the same C_{19}-steroids as in the biotransformation of lanosterol (**11**) by same microorganism [31,32]. Thus, metabolites **12**, **13**, **14** and **16** were again achieved. In a following study, two additional minor C_{19} steroids, 3α-hydroxy-5α-androst-8(14)-en-17-one (**25**) and 3α,17β-dihydroxy-5α-androst-8(14)-ene (**26**), were isolated [34] (Fig. **3**). Cycloartenol (**23**), 24-methylenecycloartanol (**24**), and cycloartenone (**27**) were incubated with the filamentous fungus *Glomerella fusarioides* (IFO 8831). Compound **23** was converted to **27** (2.2%), cycloart-25-ene-3β,24-diol (**28**, 0.8%), and cycloartane-3β,24,25-triol (**29**, 1.0%). Compound **24** was metabolized to cycloeucalenol (**30**, 1.9%) along with two new compounds, 24-methylcycloartane-3β,24,24[1]-triol (**31**, 0.7%) and 24[1]-methoxy-24-methylcycloartane-3β,24-diol (**32**, 2.6%). Whereas, compound **27** was converted into two new metabolites, 4α,4β,14α-trimethyl-9β,19-cyclopregnane-3,20-dione (**33**, 0.7%) and 25-hydroxy-24-methoxycycloartan-3-one (**34**, 0.8%), and four known compounds, viz., cycloartane-3,24-dione (**35**, 0.6%), 24-hydroxycycloart-25-en-3-one (**36**, 1.8%), (23*E*)-25-hydroxycycloart-23-en-3-one (**37**, 0.8%), and 24,25-dihydroxycycloartan-3-one (**38**, 2.0%) [35] (Scheme **1**). In these biotransformations, the fungus *G. fusarioides* yielded metabolites with C-3 hydroxyl group-oxidized, side-chain-oxygenated, C-4 demethylated, and side-chain-degraded structures, although in low transformation rates [35].

Argentatin A (**39**) and incanilin (**40**) are abundant tetracyclic triterpenes isolated from the rubber plant. The biotransformation of a mixture (1:4) of these compounds by *Gibberella saubinetti* (ATCC 20193) and *Septomyxa affinis* (ATCC 6737) gave one metabolite (**41**), that was found to be produced from incanilin substrate, formed by THF ring opening, reduction of the 3-oxo group and formation of new double bonds at 2 and 23-positions. Acetylation of this triterpenoid mixture led to a noteworthy biotransformation with *Septomyxa affinis* (ATCC 6737), yielding five metabolites. While argentatin A acetate was transformed to 3β,16β,30-trihydroxycycloarta-20,24-diene (**42**), methyl 16β-acetoxy-20*R*,24*R*-epoxy-25-hydroxy-3,4-*seco*-cycloart-4(28)-en-3-oate (**43**), and methyl 20*R*,24*R*-epoxy-16β,25-dihydroxy-3,4-*seco*-cycloart-4(28)-en-3-oate (**44**), incanilin acetate was converted to metabolite **41**, and methyl 16β-acetoxy-20*R*,24*R*-epoxy-25-hydroxy-3,4-*seco*-lanosta-1,4(28),8-trien-3-oate (**45**) (Fig. **5**). The oxidative ring A opening found in metabolites **43**, **44** and **45**, could be explained through a Bayer-Villiger-type oxidation by the insertion of an oxygen function between C-3 and C-4 to form an ester, then hydrolysis followed by a dehydration at 4(28)-position [36]. Argentatin B (**46**) is also a naturally occurring tetracyclic triterpene isolated from *Parthenium argentatum*, the rubber plant. For its biotransformation, 25 microorganisms were used for the screening purpose, selecting three of them. Thus, substrate **46** was microbiologically transformed to isoargentatin D (**47**), by *Nocardia corallina* var. *taoka* (ATCC 31338), *Mycobacterium* sp. (NRRL B3683) and *Septomyxa*

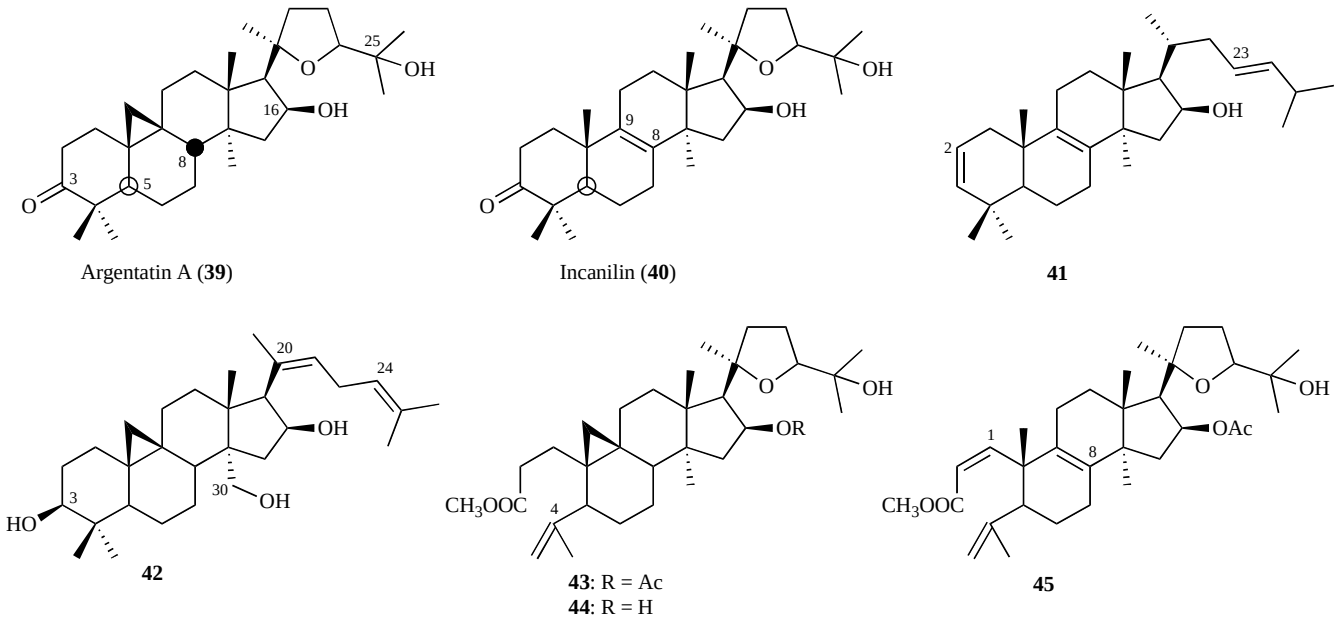


Fig. (5). Metabolites from biotransformations of Argentatin A (**39**) and Incanilin (**40**).

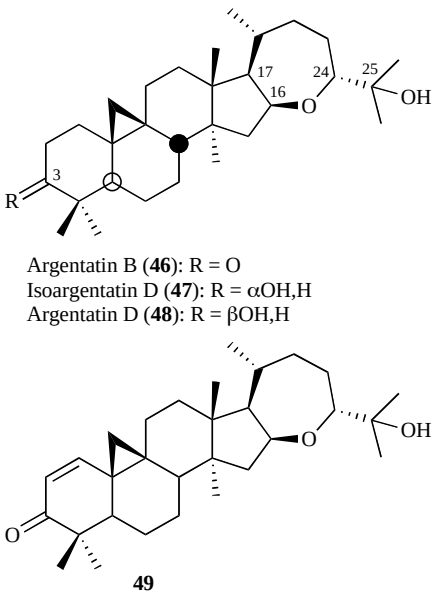


Fig. (6). Metabolites from biotransformations of Argentatin B (**46**).

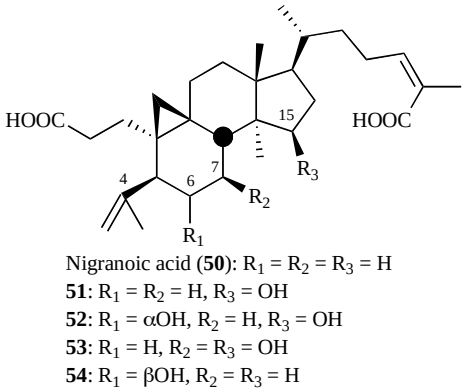


Fig. (7). Metabolites from biotransformations of substrate **50**.

affinis (ATCC 6737). The later microorganism also produced argentatin D (**48**) and 1,2-didehydroargentatin B (**49**) [37] (Fig. **6**).

Nigranoic acid (**50**) is an A ring-*seco*-cycloartene triterpenoid produced by plants belonging to the genera *Schisandra*, and has been reported to possess a variety of biological activities, including cytotoxic activity toward Leukemia and Hela cells, and inhibition of expression HIV reverse transcriptase and polymerase. Incubation of this substrate (**50**) with a culture of *Gliocladium roseum* (YMF 1.00133) led to the formation of three new more polar metabolites, in only very small quantities. The major metabolite was identified as 15β-hydroxynigranoic acid (**51**), and the minor metabolites as 6α,15β-dihydroxynigranoic acid (**52**), and 7β,15β-dihydroxynigranoic acid (**53**) [38]. Microbial transformation of the same substrate (**50**) by *Caryospora carllicarpa* (YMF 1.01026) afforded the new derivative 6β-hydroxynigranoic acid (**54**) [39] (Fig. **7**).

III.4. Cucurbitane Skeleton

Cucurbitacin E 2-*O*-β-D-glucopyranoside (**55**) is an abundant tetracyclic triterpenoid glucoside, isolated from *Citrullus colocyntbis* L. Schrad, as well as other plants in the Cucurbitaceae. The biotransformation of this substrate (**55**) by *Curvularia lunata* (NRRL 2178) gave cucurbitacin E (**56**), and three new metabolites, the isomeric (24*R*)- and (24*S*)-hydroxy-23,24-dihydrocucurbitacin E (**57** and **58**), and the 3-acetoxy-3-methylbutyl ester of (23-27)-penta-*nor*-cucurbitacin I 22-oic acid (**59**) (Fig. **8**). All of the metabolites lacked the glucose moiety, suggesting that the first step in the biotransformation sequence was glucolysis followed by secondary biotransformations only on the side-chain of the cucurbitacin skeleton [40].

III.5. Dammarane Skeleton

The ginseng saponins (ginsenosides) are one of the most important secondary metabolites in ginseng, and have various pharmacological activities such as tumor-suppressing, anti-carcinogenic, hepatoprotective, neuroprotective, etc. The main ginsenosides are glycosides that contain an aglycone with a dammarane skeleton. Biotransformation can be a useful tool to mimic mammalian metabolism *in vitro* and obtain metabolites that are valuable for *in vivo* metabolism research. Consequently, this microbiological technique has been used to study the metabolism of ginsenosides, and also to

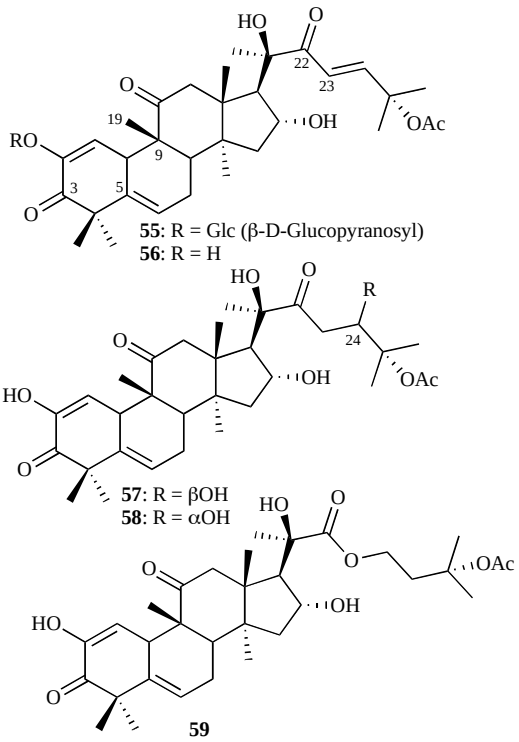
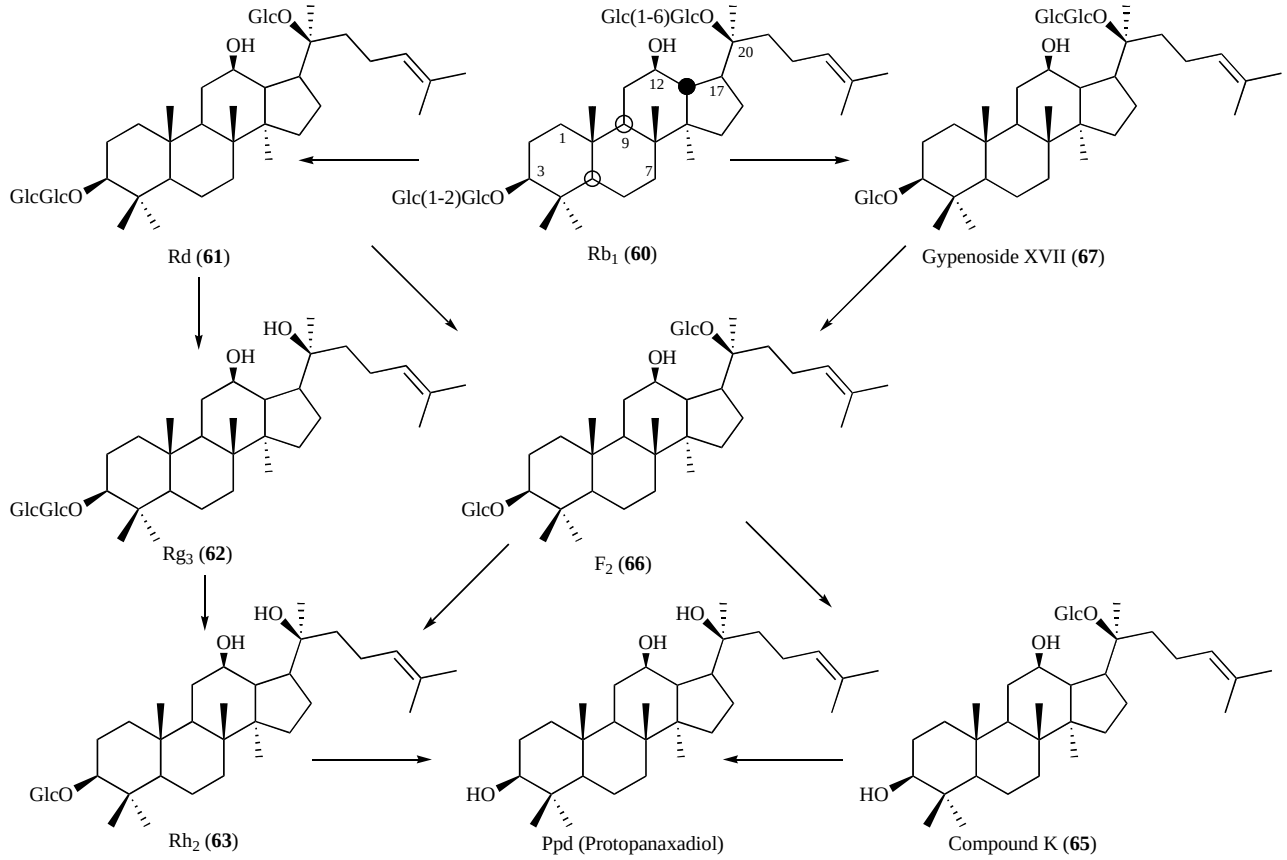


Fig. (8). Metabolites from biotransformation of Cucurbitacin glucopyranoside (**55**).

convert major ginsenosides into minor saponins, which may have more profound physiological properties.

Ginsenoside Rb_1 (**60**) is the most predominant protopanaxadiol-type ginsenoside in *Panax* species (ginseng). Several microbial transformations of this substrate (**60**) have been accomplished with an ample and varied group of microorganisms, all of these having β-glucosidase activities. Deglycosylation appears to be the major transformation pathway (Scheme **2**), and the intermediate and the final hydrolysis products of Rb_1 (**60**) depended on the microorganisms used. Thus, the fungus *Rhizopus stolonifer* (AS 3.822) produced ginsenoside Rd (**61**), ginsenoside Rg_3 (**62**), and ginsenoside Rh_2 (**63**) [41], whereas *Microbacterium* sp. (GS514) produced only Rd (**61**) and Rg_3 (**62**), but in a short period of 8 h [42]. A similar incubation procedure of Rb_1 (**60**) with *Curvularia lunata* (AS 3.1109) also yielded metabolite **61**, along with a new metabolite (**64**), as a result of a hydration reaction of the double bond of the substrate by the microorganism [41]. The same substrate (**60**) was transformed into ginsenoside compound K (**65**), via ginsenosides Rd (**61**) and F_2 (**66**), by *Bifidobacterium* sp. (Int57), *Bifidobacterium* sp. (SJ32), *Aspergillus niger* and *Aspergillus usamii* [43], and by *Caulobacter leidyia* (GP45) [44]. *Lactobacillus delbrueckii*, and *Leuconostoc paramesenteroides* transformed the same ginsenoside Rb_1 (**60**) into Rh_2 (**63**) via Rd (**61**) and F_2 (**66**) [43]. *Bifidobacterium* sp. (SH5) transformed ginsenoside Rb_1 (**60**) into F_2 (**66**) via Rd (**61**) [43], whereas *Intrasporangium* sp. (GS603) also produced ginsenoside F_2 (**66**), but through a different pathway via gypenoside XVII (**67**) [45] (Scheme **2**). Three strains, *Burkholderia pyrrocinia* (GP16), *Bacillus megaterium* (GP27) and *Sphingomonas echinoides* (GP50) convert the major ginsenoside Rb_1 (**60**), almost completely within 48 h, to the pharmaceutically active minor ginsenoside Rd (**61**) [46]. Fermentation of ginsenoside Rb_1 (**60**) with *Acremonium strictum* (AS 3.2058) gave three new compounds, 12β-hydroxydammar-3-one-20(*S*)-*O*-β-D-glucopyranoside (**68**), 12β,25-dihydroxydammar-(*E*)-20(22)-ene-3-*O*-β-D-glucopyranosyl-(1→2) -β-D-glucopyranoside (**69**), and 12β,20(*R*),25-trihydroxydammar-



Scheme 2. Biotransformation pathway of protopanaxadiol-type ginsenosides.

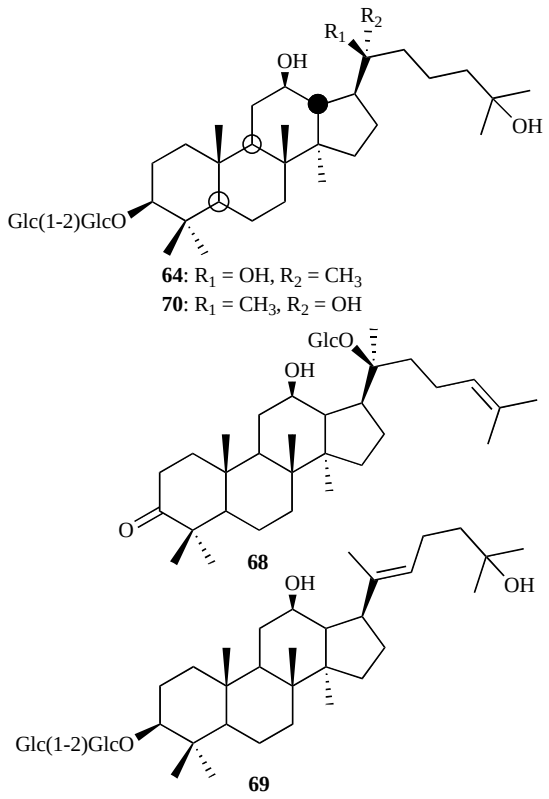
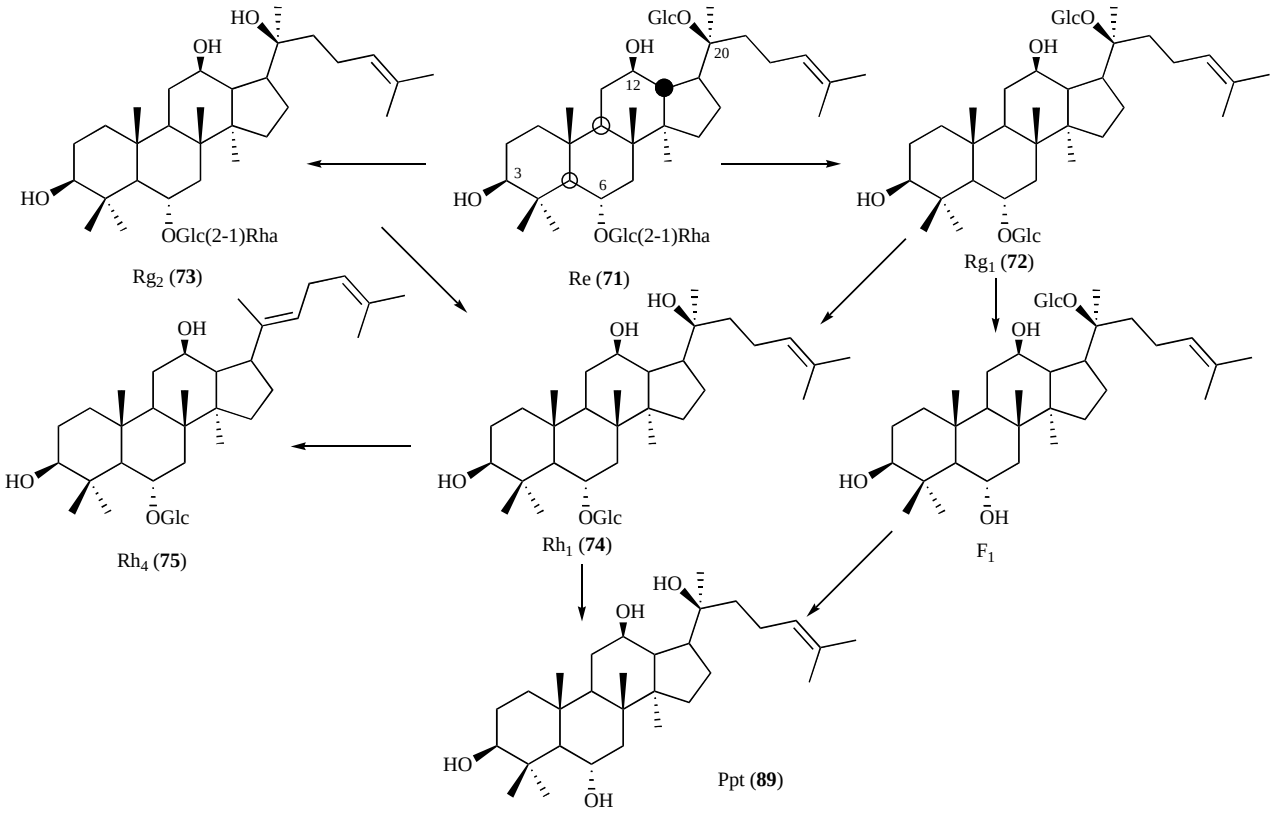


Fig. (9). Metabolites from biotransformations of Ginsenoside Rb_1 (**60**).

3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside (**70**) (Fig. **9**), along with five known compounds, ginsenosides Rd (**61**), Rg_3 (**62**), F_2 (**66**), and compound K (**65**), and gypenoside XVII (**67**) [47].

Ginsenosides Re (**71**) and Rg_1 (**72**), belong to the major protopanaxatriol-type saponins (Scheme **3**). In the transformation of ginsenoside Re (**71**), *Bifidobacterium* sp. (Int57) and *Bifidobacterium* sp. (SJ32) first hydrolyzed the β-glucosidic linkage bonded to the hydroxyl group at C-20 of substrate (**71**) to produce Rg_2 (**73**), and then further transformed Rg_2 (**73**) into Rh_1 (**74**). Meanwhile, *Aspergillus usamii* only transformed ginsenoside Re (**71**) into Rg_2 (**73**), but did not convert Rg_2 (**73**) thereafter [43]. The filamentous fungus *Aspergillus niger* also transformed substrate **71** into ginsenoside Rh_1 (**74**), but through a different pathway than *Bifidobacterium* sp., via Rg_1 (**72**) [43] (Scheme **3**). Ginsenoside Re (**71**), was transformed by the fungus *Absidia coerulea* (AS 3.3389) yielding ginsenoside Rh_4 (**75**), 3β,12β,25-trihydroxydammar-(*E*)-20(22)-ene-6-*O*-β-D-glucopyranoside (**76**), 20(*S*)-ginsenoside Rh_1 (**74**), 20(*R*)-ginsenoside Rh_1 (**77**), a mixture of 25-hydroxy-20(*S*)-ginsenoside Rh_1 and its C-20(*R*) epimer (**78**), 20(*S*)-ginsenoside Rg_2 (**73**), 20(*R*)-ginsenoside Rg_2 (**79**), 3β,12β,25-trihydroxydammar-(*E*)-20(22)-ene-6-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**80**), 3β,12β-dihydroxydammar-(*E*)-20(22),24-diene-6-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**81**), and 3β,12β,20,25-tetrahydroxydammaran-6-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**82**) [48] (Fig. **10**). *Aspergillus niger* (AS 3.1858) and *Absidia coerulea* (AS 3.3538) were found to convert ginsenoside Rg_1 (**72**) efficiently into the same metabolite, ginsenoside Rh_1 (**74**) [49] (Scheme **3**). *Aspergillus usamii* also brought about this transformation [43]. A more exhaustive study of biotransformation of ginsenoside Rg_1 (**72**) with *Absidia coerulea* (AS 3.3389) resulted in five metabolites (**74**-**78**), also isolated from the incubation of ginsenoside Re (**71**) with the same microorganism [48].

Notoginsenoside R_1 (**83**) is one the major 20(*S*)-protopanaxatriol-type saponins in *Panax notoginseng*. This substrate was converted into 10 metabolites by *Absidia coerulea* (AS 3.3389). Five of them (**74**-**78**), were also isolated in both incubations of gin-



Scheme 3. Biotransformation pathway of protopanaxatriol-type ginsenosides.

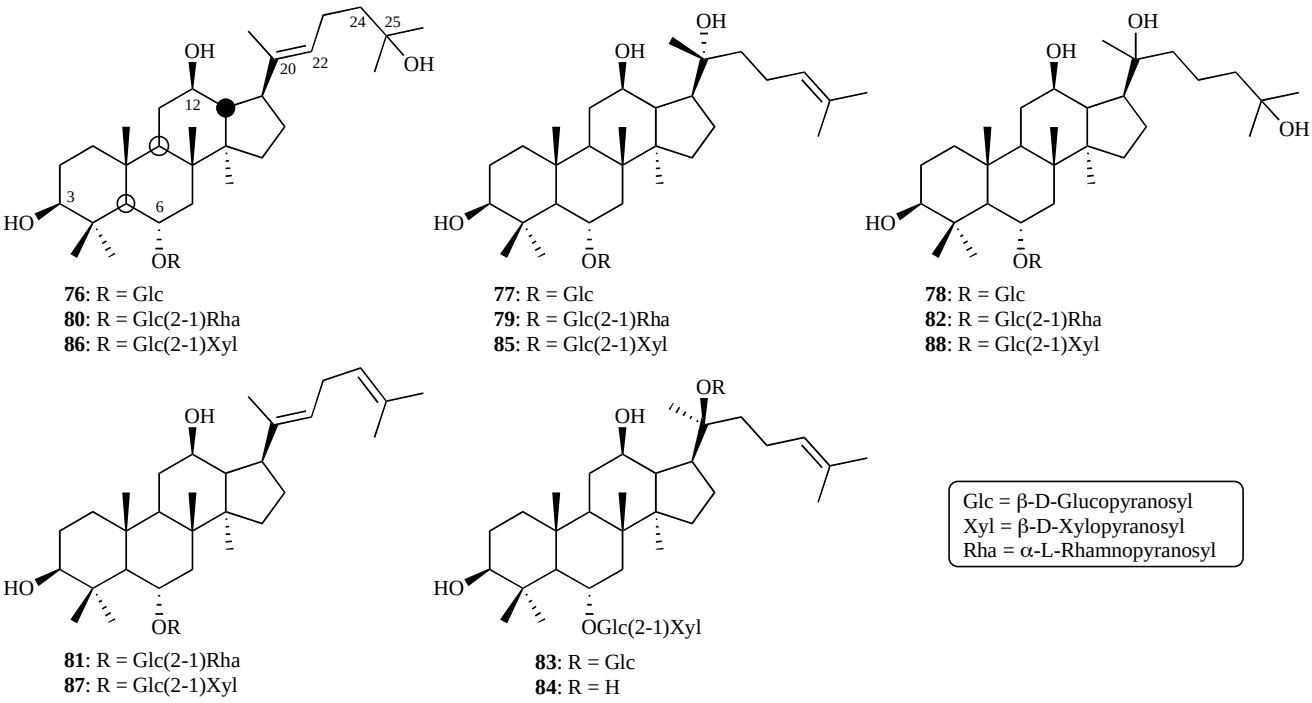


Fig. (10). Metabolites from biotransformations of Ginsenoside Re (**71**) and Notoginsenoside R_1 (**83**).

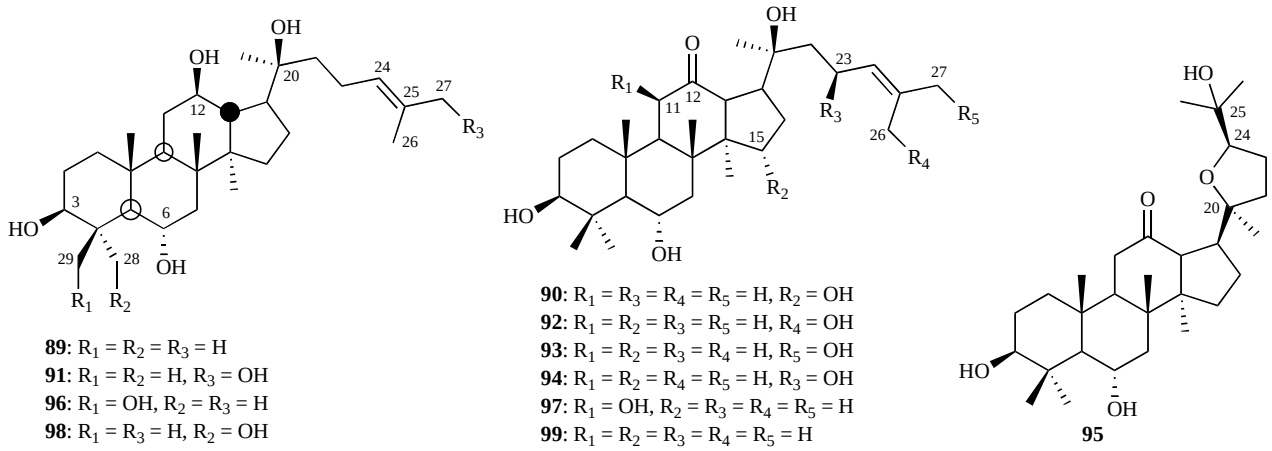
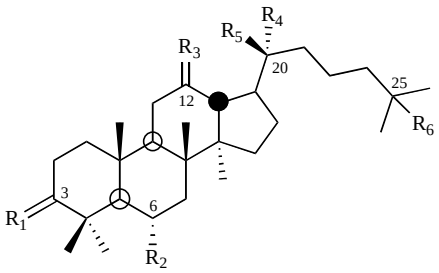


Fig. (11). Metabolites from biotransformation of 20(*S*)-Protopanaxatriol (**89**).

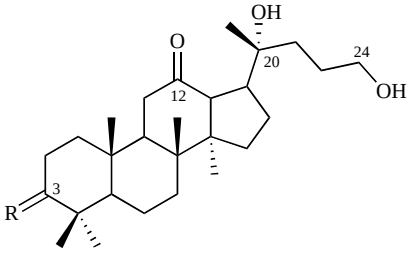
senosides Re (**71**) and Rg_1 (**72**) with the same microorganism. The other five were 20(*S*)-notoginsenoside R_2 (**84**), 20(*R*)-notoginsenoside R_2 (**85**), 3β,12β,25-trihydroxydammar-(*E*)-20(22)-ene-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (**86**), 3β,12β-dihydroxydammar-(*E*)-20(22),24-diene-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (**87**), and 3β,12β,20,25-tetrahydroxydammaran-6-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranoside (**88**) [48] (Fig. **10**).

Protopanaxatriol (Ppt) and protopanaxadiol (Ppd) are the corresponding aglycones of several ginsenosides with a dammarane skeleton, which is similar to lanostane except that Me-13 is replaced by Me-8. Microbial transformation of 20(*S*)-protopanaxatriol (**89**) by the fungus *Mucor spinosus* (AS 3.3450) yielded ten new metabolites, the structures of which were determined to be 12-oxo-15α-hydroxy-20(*S*)-protopanaxatriol (**90**), 27-hydroxy-20(*S*)-protopanaxatriol (**91**), 12-oxo-26-hydroxy-20(*S*)-protopanaxatriol (**92**), 12-oxo-27-hydroxy-20(*S*)-protopanaxatriol (**93**), 12-oxo-23β-hydroxy-20(*S*)-protopanaxatriol (**94**), 20*S*,24*R*-epoxy-dammaran-3β,6α,25-triol-12-one (**95**), 29-hydroxy-20(*S*)-protopanaxatriol (**96**), 12-oxo-11β-hydroxy-20(*S*)-protopanaxatriol (**97**), 28-hydroxy-20(*S*)-protopanaxatriol (**98**), and 12-oxo-20(*S*)-protopanaxatriol (**99**), respectively [50,51] (Fig. **11**). The *in vitro* cytotoxicities of these metabolites suggested that 12-carbonylation or hydroxylation at C-28 or C-29 would increase the cytotoxic activities, whereas introduction of a hydroxyl group at C-11β, C-15α, C-23β, C-26 or C-27 would reduce the activity of the substrate (**89**).

Microbial transformation of 20(*R*)-dihydroprotopanaxadiol (**100**), obtained from ginsenoside Rb_1 (**60**), by *Mycobacterium* sp. (NRRL B-3805) yielded the corresponding 3-oxo- (**101**, 24.7%) and 3-oxo-25-hydroxylated (**102**, 1.4%) derivatives. Biotransformation of the mixture of 20(*S*)- (**103**) and 20(*R*)-dihydroprotopanaxatriol (**104**), obtained from ginsenoside Rg_1 (**72**), with the same microorganism produced the corresponding 3-oxo- (**105**, 23.3%) and 3-oxo-25-hydroxylated (**106**, 20.9%) derivatives, respectively. Incubation of 20(*R*)-hydroxydammarane-3,12-dione (**107**), a oxidation product of **100**, with the same microorganism



100: $R_1 = R_3 = \beta OH,H$, $R_2 = R_6 = H$, $R_4 = OH$, $R_5 = CH_3$
101: $R_1 = O$, $R_2 = R_6 = H$, $R_3 = \beta OH,H$, $R_4 = OH$, $R_5 = CH_3$
102: $R_1 = O$, $R_2 = H$, $R_3 = \beta OH,H$, $R_4 = R_6 = OH$, $R_5 = CH_3$
103: $R_1 = R_3 = \beta OH,H$, $R_2 = R_5 = OH$, $R_4 = CH_3$, $R_6 = H$
104: $R_1 = R_3 = \beta OH,H$, $R_2 = R_4 = OH$, $R_5 = CH_3$, $R_6 = H$
105: $R_1 = O$, $R_2 = R_5 = OH$, $R_3 = \beta OH,H$, $R_4 = CH_3$, $R_6 = H$
106: $R_1 = O$, $R_2 = R_4 = R_6 = OH$, $R_3 = \beta OH,H$, $R_5 = CH_3$
107: $R_1 = R_3 = O$, $R_2 = R_6 = H$, $R_4 = OH$, $R_5 = CH_3$
108: $R_1 = \beta OH,H$, $R_2 = R_6 = H$, $R_3 = O$, $R_4 = OH$, $R_5 = CH_3$



109: R = O
110: R = $\beta OH,H$

Fig. (12). Metabolites from biotransformations of protopanaxadiol and protopanaxatriol derivatives.

yielded 3β,20(*R*)-dihydroxydammaran-12-one (**108**), 20(*R*),24-dihydroxypropakisnordammarane-3,12-dione (**109**), and 3β,20(*R*),24-trihydroxypropakisnordammaran-12-one (**110**) [52] (Fig. **12**). These latter metabolites (**109** and **110**) are the result of the partial selective cleavage of the side chain of the substrate, but no degradation occurred for the ring-bound methyl residues, as in the biotransformation of lanosterol (**11**) with the same microorganism, to give C_{19} sterols [31].

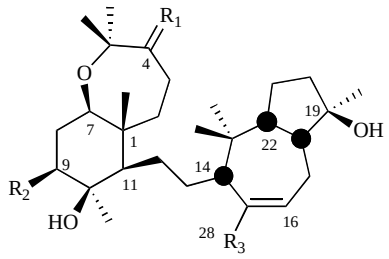
III.6. Sipholane Skeleton

Sipholenol A (**111**) and sipholenone A (**112**) are the major sipholane triterpenoids, and, isolated from the red sea sponge *Callyspongia siphonella*, exhibit varied biological activities. Biocatalysis of sipholenol A (**111**) by *Mucor ramannianus* (ATCC 9628) afforded four metabolites, 9β-hydroxysipholenol A (**113**), 16-oxosipholenol A (**114**), sipholenol G (**115**), and 28-hydroxysipholenol A (**116**). Sipholenone A (**112**) was biotransformed by *Cunninghamella elegans* (ATCC 7929) to 22-hydroxy-16-oxosipholenone A (**117**), and 15β,16β-epoxy-22-hydroxysipholenone A (**118**) [53] (Fig. **13**).

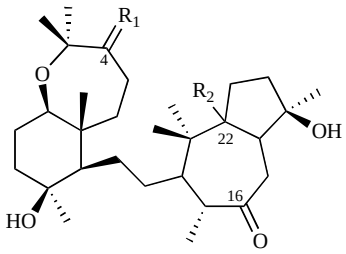
IV. PENTACYCLIC TRITERPENOIDS

IV.1. Oleanane Skeleton

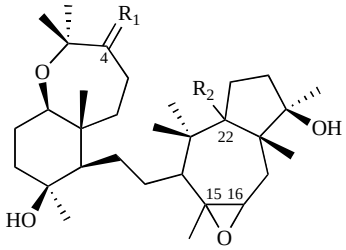
Glycyrrhetinic acid (GA, **119**), also known as glycyrrhetic acid or 18β-glycyrrhetinic acid, is the aglycone of the saponins isolated from *Glycyrrhiza glabra* and others *Glycyrrhiza* species. This triterpenoid (**119**) and its derivatives have several pharmacological activities, such as anti-inflammatory, anti-ulcer and anti-tumorpromoting activities. The major product of the microbial transformation of GA (**119**) by *Curvularia lunata* (ATCC 13432)



111: $R_1 = \alpha OH,H$, $R_2 = H$, $R_3 = CH_3$
112: $R_1 = O$, $R_2 = H$, $R_3 = CH_3$
113: $R_1 = \alpha OH,H$, $R_2 = OH$, $R_3 = CH_3$
116: $R_1 = \alpha OH,H$, $R_2 = H$, $R_3 = CH_2OH$



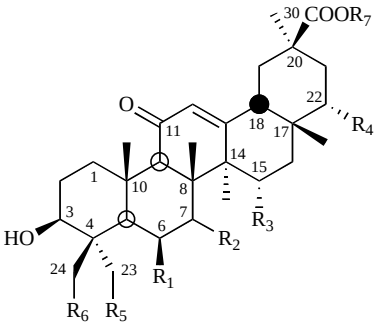
114: $R_1 = \alpha OH,H$, $R_2 = \beta H$
117: $R_1 = O$, $R_2 = OH$



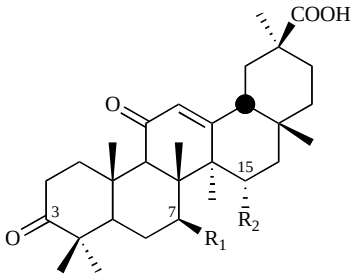
115: $R_1 = \alpha OH,H$, $R_2 = \beta H$ (15α,16α-epoxy)
118: $R_1 = O$, $R_2 = OH$ (15β,16β-epoxy)

Fig. (13). Metabolites from biotransformations of Sipholenol A (**111**) and Sipholenone A (**112**).

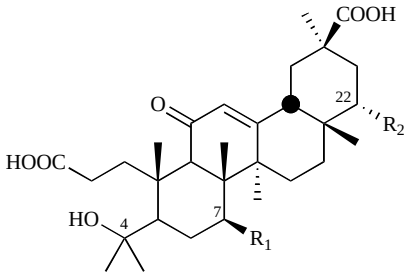
[54] and by *Mucor Spinosus* (AS 3.3450) [55] was identified as 7β-hydroxyglycyrrhetinic acid (**120**) (Fig. **14**). *Mucor polymorphosporus* also produced 7β-hydroxyglycyrrhetinic acid (**120**) as a major metabolite (26.8%) from GA (**119**), along with 15α-hydroxyglycyrrhetinic acid (**121**,18.2%), and the minor metabolites: 24-hydroxyglycyrrhetinic acid (**122**, 2.7%), 6β-hydroxyglycyrrhetinic acid (**123**, 1.3%), 7α-hydroxyglycyrrhetinic acid (**124**, 1.4%), 3-oxo-7β-hydroxyglycyrrhetinic acid (**125**, 1.1%), and 3-oxo-15α-hydroxyglycyrrhetinic acid (**126**, 0.9%) [56]. The main action of *Trichothecium roseum* (ATCC 8685) on GA (**119**) was also directed to C-7 and C-15, yielding 7β- (**127**), 15α-hydroxy (**128**), and 7β,15α-dihydroxy (**129**) derivatives, which were also methylated in the carboxylic acid group by the microorganism [57] (Fig. **14**). The formation of a methyl ester was also detected in the incubation of GA (**119**) with *Nocardia* sp. (NRRL 5646), which was converted to its methyl ester (methyl glycyrrhetinate, **130**) [58]. GA (**119**) was also incubated with the mycelium of *Streptomyces* sp. (G-20) to give three conversion products. The major metabolite is a 22α-hydroxy derivative (**131**), and the minor, a 22α,23- (**132**) and a 22α,24-dihydroxy (**133**) derivatives [59]. *Chainia antibiotica* (IFO 12,246) converted GA (**119**) into two new 3,4-*seco*-oleanane-type compounds (**134** and **135**). The same microorganism also produced a 3,4-*seco*-oleanane-type derivative (**136**) from 22α-hydroxyglycyrrhetinic acid (**131**). The proposed mechanism pathway involved the transformation of GA (**119**) and 22α-hydroxy GA (**131**) into 3-oxo-intermediates, and then to the



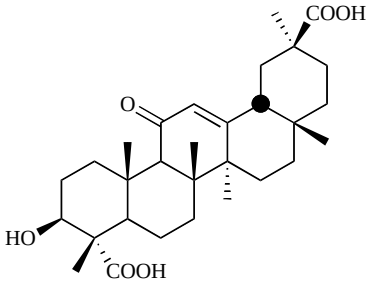
GA (**119**): $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = H$
120: $R_1 = R_3 = R_4 = R_5 = R_6 = R_7 = H$, $R_2 = \beta OH$
121: $R_1 = R_2 = R_4 = R_5 = R_6 = R_7 = H$, $R_3 = OH$
122: $R_1 = R_2 = R_3 = R_4 = R_5 = R_7 = H$, $R_6 = OH$
123: $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = R_6 = R_7 = H$
124: $R_1 = R_3 = R_4 = R_5 = R_6 = R_7 = H$, $R_2 = \alpha OH$
127: $R_1 = R_3 = R_4 = R_5 = R_6 = H$, $R_2 = \beta OH$, $R_7 = CH_3$
128: $R_1 = R_2 = R_4 = R_5 = R_6 = H$, $R_3 = OH$, $R_7 = CH_3$
129: $R_1 = R_4 = R_5 = R_6 = H$, $R_2 = \beta OH$, $R_3 = OH$, $R_7 = CH_3$
130: $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H$, $R_7 = CH_3$
131: $R_1 = R_2 = R_3 = R_5 = R_6 = R_7 = H$, $R_4 = OH$
132: $R_1 = R_2 = R_3 = R_6 = R_7 = H$, $R_4 = R_5 = OH$
133: $R_1 = R_2 = R_3 = R_5 = R_7 = H$, $R_4 = R_6 = OH$



125: $R_1 = OH$, $R_2 = H$
126: $R_1 = H$, $R_2 = OH$
153: $R_1 = R_2 = H$



134: $R_1 = R_2 = H$
135: $R_1 = OH$, $R_2 = H$
136: $R_1 = H$, $R_2 = OH$



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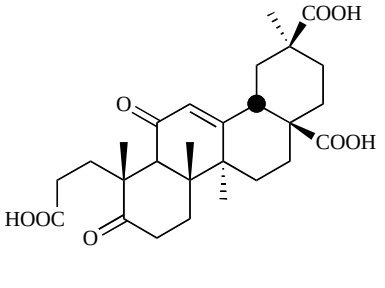

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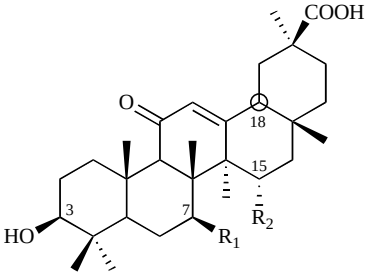
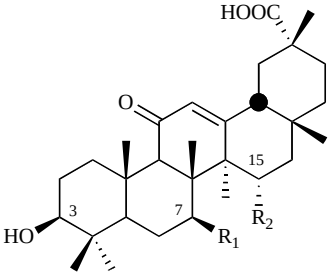
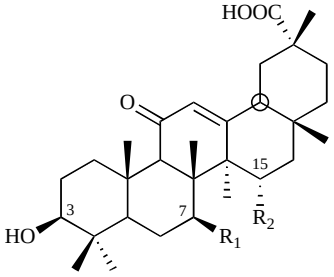


18α-GA (**139**): $R_1 = R_2 = H$
142: $R_1 = OH$, $R_2 = H$
144: $R_1 = H$, $R_2 = OH$
146: $R_1 = R_2 = OH$



LA (**140**): $R_1 = R_2 = H$
143: $R_1 = OH$, $R_2 = H$
145: $R_1 = H$, $R_2 = OH$
147: $R_1 = R_2 = OH$



18α-LA (**141**): $R_1 = R_2 = H$
148: $R_1 = OH$, $R_2 = H$
149: $R_1 = R_2 = OH$

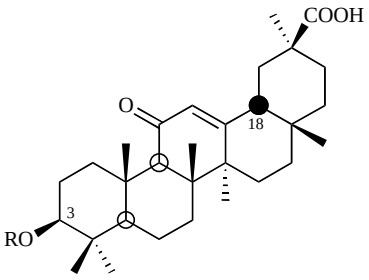
Fig. (14). Metabolites from biotransformations of GA (**119**) and other derivatives.

3,4-*seco*-type compounds (**134**-**136**), through a Baeyer-Villiger-type oxidation of 3-oxo-intermediates, followed by hydrolysis of the resulting seven-membered ring lactones [60]. The microbial transformation of GA (**119**) by *Sphingomonas paucimobilis* (strain G5), with the addition of oxygenase inhibitors, produced a new metabolite identified as 3β-hydroxy-11-oxoolean-12-ene-23,30-dioic acid (**137**) [61]. The same biotransformation, under normal conditions, yielded 3,4-*seco*-4,23,24-*trinor*-11-oxoolean-12-ene-3,28,30-trioic acid (**138**) [62], proposing a metabolic pathway in which **137** is subsequently oxidized and further metabolized to **138** [61] (Fig. **14**).

18α-glycyrrhetinic acid (18α-GA, **139**), liquiritic acid (LA, **140**), and 18α-liquiritic acid (18α-LA, **141**) are C-18 or (and) C-20 epimers of GA (**119**). 18α-GA (**139**) and LA (**140**) were partially converted by *Curvularia lunata* (ATCC 13432), *Trichothecium roseum* (ATCC 8685), *Cunninghamella* (ATCC 3229), *Mucor griseo-cyanus* (ATCC 1207-a), and *Helicostylum piriforme* (ATCC 8992) into their 7β-hydroxy derivatives (**142** and **143**, respectively), as well as into their 15α-hydroxy (**144** and **145**, respectively) and (or) 7β,15α-dihydroxy derivatives (**146** and **147**, respectively) [63]. 18α-LA (**141**) was converted into its 7β-hydroxy derivative (**148**) by *H. piriforme* and *Cunninghamella*, and into its 7β,15α-dihydroxy derivative (**149**) by *T. roseum* [63] (Fig. **14**).

Glycyrrhizin (**150**), a triterpenoid saponin, is the major active component obtained from *Glycyrrhiza glabra*. *Cryptococcus magnus* (MG-27) selectively hydrolyzed the terminal β-glucuronyl linkage of glycyrrhizin (**150**) to yield glycyrrhetinic acid 3-*O*-mono-β-D-glucuronide (**151**, 95%), a potent sweetener [64]. Immobilized *Aspergillus terreus*, selectively hydrolyzed the β-glucuronyl linkage of ammonium glycyrrhizinate (**152**) to yield GA (**119**, 21.72%) and 3-oxo-glycyrrhetinic acid (**153**, 4.06%) [65] (Fig. **15**).

Oleanolic acid (**154**) is a triterpenoid compound that exists widely in natural plants in the form of free acid or aglycone for triterpenoid saponins. This triterpene (**154**) has diverse pharmacological effects such as hepatoprotective, hypoglycemic, hypolipidemic, anti-inflammatory, antiviral, anti-oxidative, anti-



Glycyrrhizin (**150**): R = Glcr(1-2)Glcr
151: R = Glcr (β-D-Glucuronyl)
152: R = Glcr(1-2)Glcr ammonium derivative

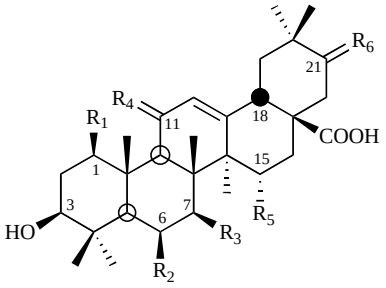
Fig. (15). Metabolites from biotransformations of Glycyrrhizin (**150**) and other derivatives.

carcinogenic, etc. Biotransformation of oleanolic acid (**154**) by *Cunninghamella blakesleeana* gave the following six metabolites: 3β-hydroxyoleana-11,13(18)-dien-28-oic acid (**155**), 3β,7β-dihydroxyolean-12-en-28-oic acid (**156**), 3β-hydroxy-11-oxoolean-12-en-28-oic acid (**157**), 3β,7β-dihydroxyoleana-11,13(18)-dien-28-oic acid (**158**), 3β,7β,13β-trihydroxyolean-11-ene-28,13-lactone (**159**), and 1β,3β-dihydroxyolean-12-en-28-oic acid (**160**) [66] (Fig. **16**). The microbial transformation of the same substrate (**154**) with *Fusarium lini* also produced metabolites **155** and **157**, together with a 15α-hydroxy derivative (**161**) and a lactone (oleanderolide, **162**) [67]. Oleanolic acid (**154**) was also incubated with *Penicillium chrysogenum* yielding the corresponding 21β-hydroxy (**163**), 21-oxo (**164**), 3-oxo-21β-hydroxy (**165**), and 3,21-dioxo (**166**) derivatives [68]. The 21-oxo derivative (**164**) of oleanolic acid was also isolated in the incubation of this substrate (**154**) with *Colletotrichum phomoides*, along with a 6β-hydroxy (**167**) and a 6β,21β-dihydroxy (**168**) derivative [69]. *Aspergillus ochraceus* (NG1203) produced only an 11α-hydroxy derivative (**169**, 10.12%) from oleanolic acid (**154**) [70]. A C-3 oxidized derivative of oleanolic acid was also incubated with a filamentous fungus, so 3-oxoolean-12-en-28-oic acid (**170**) was transformed by the *Chaetomium longirostre* (RF-1095) into 4-hydroxy-3,4-*seco*-olean-12-ene-3,28-dioic acid (**171**) and the corresponding 21β-hydroxylated derivative (**172**) [71] (Fig. **16**).

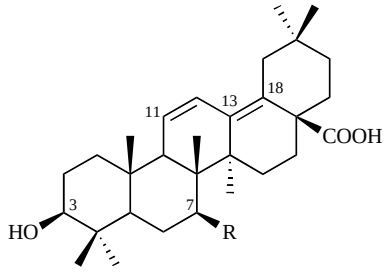
Senegenin (**173**) is an artificial aglycone from the vigorous ethanolic hydrochloride hydrolysis of the saponins from *Polygala senega*. This compound (**173**) was converted to a *nor*-triterpene, senegenic acid 28-methyl ester (**174**), by *Nocardia* sp. (NRRL 5646), via an unprecedented C–C bond cleavage and a selective methyl esterfication at C-28 [58] (Fig. **16**).

IV.2. Ursane Skeleton

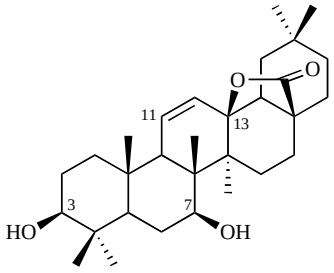
Ursolic acid (**175**), a triterpenoid compound widely distributed in several plant species, has been shown to possess a wide range of



Oleanolic acid (**154**): $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = R_6 = H,H$
156: $R_1 = R_2 = R_5 = H$, $R_3 = OH$, $R_4 = R_6 = H,H$
157: $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = O$, $R_6 = H,H$
160: $R_1 = OH$, $R_2 = R_3 = R_5 = H$, $R_4 = R_6 = H,H$
161: $R_1 = R_2 = R_3 = H$, $R_5 = OH$, $R_4 = R_6 = H,H$
163: $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = H,H$, $R_6 = \beta OH,H$
164: $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = H,H$, $R_6 = O$
167: $R_1 = R_3 = R_5 = H$, $R_2 = OH$, $R_4 = R_6 = H,H$
168: $R_1 = R_3 = R_5 = H$, $R_2 = OH$, $R_4 = H,H$, $R_6 = \beta OH,H$
169: $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = \alpha OH,H$, $R_6 = H,H$
176: $R_1 = R_2 = R_3 = R_5 = H$, $R_4 = R_6 = H,H$ methyl ester



155: R = H
158: R = OH



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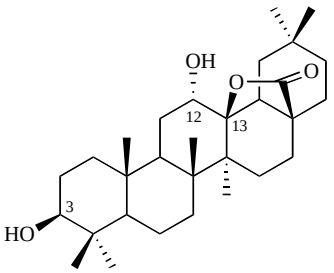
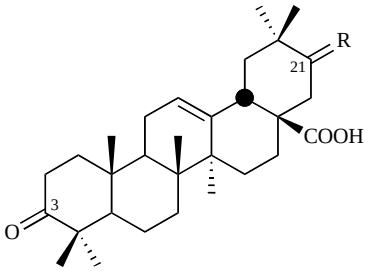
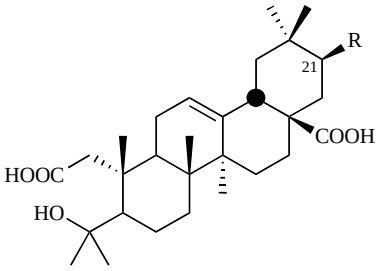


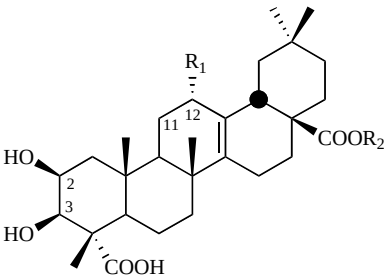
Oleanderolide (**162**)



165: R = βOH,H
166: R = O
170: R = H,H

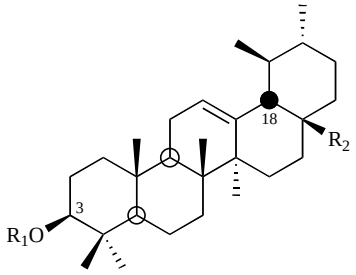


171: R = H
172: R = OH

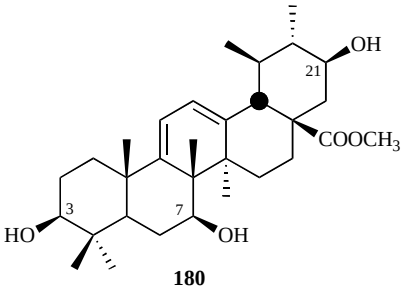


Senegenin (**173**): $R_1 = CH_2Cl$, $R_2 = H$
174: $R_1 = H$, $R_2 = CH_3$

Fig. (16). Metabolites from biotransformations of Oleanolic acid (**154**) and a derivative (**170**).

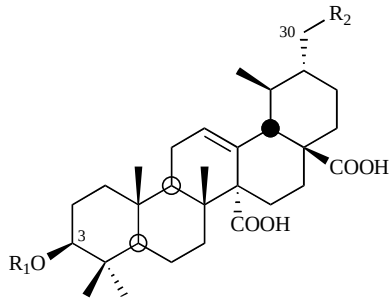


Ursolic acid (**175**): R_1 = H, R_2 = COOH
177: R_1 = H, R_2 = $COOCH_3$
Uvaol (**178**): R_1 = H, R_2 = CH_2OH
179: R_1 = $CON(CH_3)_2$, R_2 = $CH_2OCON(CH_3)_2$

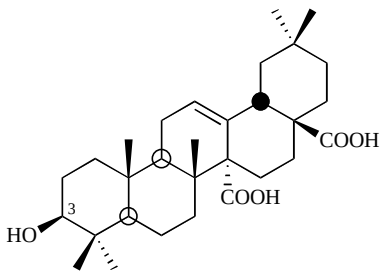


180

Fig. (17). Metabolites from biotransformations of Ursolic acid (**175**) and a derivative (**177**).



181: R_1 = 6-deoxy-Glc, R_2 = H
Quinovic acid (**182**): R_1 = R_2 = H
184: R_1 = 6-deoxy-Glc, R_2 = OH



Cincholic acid (**183**)

Fig. (18). Metabolites from biotransformations of Quinovic acid glucopyranoside (**181**).

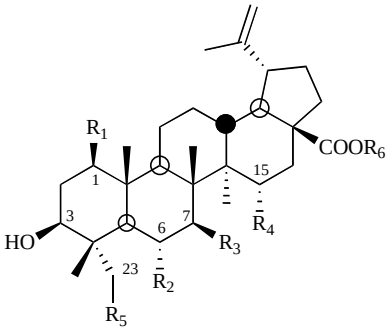
biological properties, such as anti-cancer and anti-HIV, among others activities. *Nocardia* sp. (NRRL 5646) converted ursolic acid (**175**) to oleanolic acid methyl ester (**176**) via two intermediates, oleanolic acid (**154**) and ursolic acid methyl ester (**177**) (Fig. **17**). This methylation reaction at the C-28 carboxylic acid occurred through catalysis by the enzyme system of *Nocardia* sp., as in biotransformation of senegenin (**173**) with the same microorganism. The two oleanane-type metabolites (**154** and **176**) were formed by participation of a "retro-biosynthetic" methyl migration from C-19 to C-20 [58]. Ursolic acid methyl ester (**177**), 3β,28-dihydroxyurs-12-ene (uvaol, **178**) and 3β,28-bis(dimethylcarbamoxy)urs-12-ene (**179**) were also incubated with *Mucor plumbeus* (ATCC 4740). While substrates **178** and **179** were recovered untransformed from the fermentation broth, **177** underwent hydroxylation to give methyl 3β,7β,21β-trihydroxyursa-9(11),12-dien-28-oate (**180**) as the sole product (Fig. **17**). The double bond between C-9 and C-11 of this metabolite (**180**) was probably formed through an 11-hydroxy intermediate that was readily eliminated to form the conjugated diene [72].

Quinovic acid 3-*O*-β-6-deoxy-D-glucopyranoside (**181**) is the main constituent from the bark of *Mitragyna inermis*, a famous folk medicine in West Africa traditionally used for treating stomach, intestinal disorders, and hepatic diseases. This substrate (**181**) was deglycosylated by *Nocardia* sp. (NRRL 5646) to their aglycone quinovic acid (**182**, 40%) and its biogenetic counterpart, cincholic acid (**183**, 20%) [73], via a carbon skeleton rearrangement involving a methyl group migration, similar to that observed in the conversion of ursolic acid (**175**) to oleanolic acid (**154**) by the same microorganism [58]. Microbial transformation of the same substrate (**181**) by *Streptomyces griseus* (ATCC 13273) afforded a new oxidized metabolite (**184**), as a result of the hydroxylation of the methyl group at C-30 [74] (Fig. **18**).

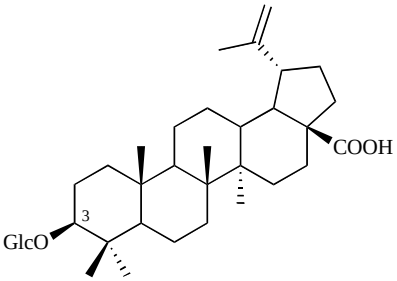
IV.3. Lupane Skeleton

Betulinic acid (**185**), a lupane-type triterpene, is widely distributed in the plant kingdom; the birch tree being one of the most widely reported sources. Betulinic acid (**185**) has been reported to have anti-melanoma, anti-neuroblastoma, anti-leukemia, anti-HIV, and anti-malaria properties. Microbial transformation of this substrate (**185**) with *Cunninghamella* sp. (NRRL 5695) resulted in the production of a new metabolite (**186**, 0.77%), in which the fungus had introduced a β-D-glucopyranosyl at the carboxylic acid group [75]. Incubation of **185** with *Bacillus megaterium* (ATCC 14581) yielded the known 3-oxo derivative (betulonic acid, **187**), along with the new 7β-hydroxy (**188**), and 6α,7β-dihydroxy derivatives (**189**). *Mucor mucedo* (UI-4605) also produced the same 7β-hydroxy derivative (**188**), whereas *Cunninghamella elegans* (ATCC 9244) gave the 1β,7β-dihydroxy derivative (**190**) [76] (Fig. **19**). Another strain of *Bacillus megaterium* (ATCC 13368) was used for a preparative-scale biotransformation of betulinic acid (**185**), yielding betulonic acid (**187**), together with 11α-hydroxy (**191**) and 1β-hydroxy-betulonic acids (**192**), and 3β,7β,15α-trihydroxylup-20(29)-en-28-oic acid (**193**) [77]. Betulonic acid (**187**) was also produced in the biotransformation of betulinic acid (**185**) by *Chaetophoma* (DPB125) and *Dematium* (DPB157), whereas *Colletotrichum* (DPB136) converted **185** into 15α-hydroxybetulonic acid (**194**) [78]. Incubation of **185** and 23-hydroxybetulinic acid (**195**) with *Nocardia* sp. (NRRL 5646) afforded their corresponding methyl esters (**196** and **197**), respectively [58] (Fig. **19**).

Betulonic acid (**187**) is also a naturally occurring triterpene found in many plants, which can be prepared by chemical oxidation from betulinic acid (**185**), and presents anti-inflammatory, anti-melanoma, and anti-viral properties. Microbial transformation of betulonic acid (**187**) by the fungus *Chaetomium longirostre* (IFO 9873) yielded 4-hydroxy-3,4-*seco*-lup-20(29)-ene-3,28-dioic acid (**198**, 6%), 4,7β,17-trihydroxy-3,4-*seco*-28-*nor*-lup-20(29)-en-3-oic acid (**199**, 12%), and 7β,15α-dihydroxybetulonic acid (**200**, 4%). This microorganism produced several transformations such as, oxidative ring cleavage, hydroxylation, and decarboxylation of substrate (**187**) [79]. Biotransformation of betulonic acid (**187**) with *Arthrobotrys* (DPB134) and *Colletotrichum* (DPB136) also produced the previously isolated 7β,15α-dihydroxy derivative (**200**), along with 7β-hydroxybetulonic acid (**201**) and 7β,30-dihydroxybetulonic acid (**202**), by *Arthrobotrys* (DPB134), or 15α-



Betulinic acid (**185**): $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H$
188: $R_1 = R_2 = R_4 = R_5 = R_6 = H$, $R_3 = OH$
189: $R_1 = R_4 = R_5 = R_6 = H$, $R_2 = R_3 = OH$
190: $R_1 = R_3 = OH$, $R_2 = R_4 = R_5 = R_6 = H$
193: $R_1 = R_2 = R_5 = R_6 = H$, $R_3 = R_4 = OH$
195: $R_1 = R_2 = R_3 = R_4 = R_6 = H$, $R_5 = OH$
196: $R_1 = R_2 = R_3 = R_4 = R_5 = H$, $R_6 = CH_3$
197: $R_1 = R_2 = R_3 = R_4 = H$, $R_5 = OH$, $R_6 = CH_3$



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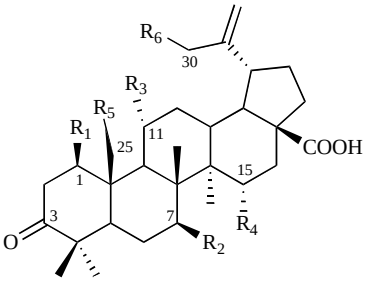
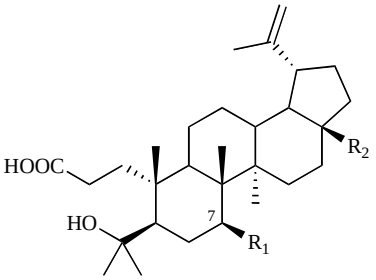
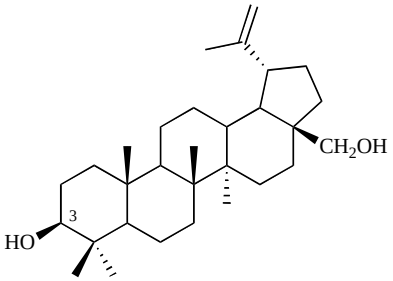


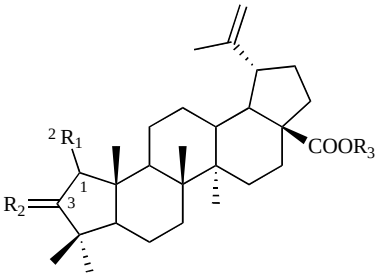
Betulonic acid (**187**): $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = H$
191: $R_1 = R_2 = R_4 = R_5 = R_6 = H$, $R_3 = OH$
192: $R_1 = OH$, $R_2 = R_3 = R_4 = R_5 = R_6 = H$
194: $R_1 = R_2 = R_3 = R_5 = R_6 = H$, $R_4 = OH$
200: $R_1 = R_3 = R_5 = R_6 = H$, $R_2 = R_4 = OH$
201: $R_1 = R_3 = R_4 = R_5 = R_6 = H$, $R_2 = OH$
202: $R_1 = R_3 = R_4 = R_5 = H$, $R_2 = R_6 = OH$
203: $R_1 = R_2 = R_3 = R_4 = R_6 = H$, $R_5 = OH$



198: $R_1 = H$, $R_2 = COOH$
199: $R_1 = R_2 = OH$
205: $R_1 = H$, $R_2 = CH_2OH$



Betulin (**204**)



Ceanothic acid (**206**): $R_1 = \alpha COOH$, $R_2 = \beta OH,H$, $R_3 = H$
207: $R_1 = \alpha COOCH_3$, $R_2 = \beta OH,H$, $R_3 = CH_3$
208: $R_1 = \alpha COOCH_3$, $R_2 = O$, $R_3 = CH_3$
209: $R_1 = \beta COOCH_3$, $R_2 = O$, $R_3 = CH_3$

Fig. (19). Metabolites from biotransformations of lupanane and ceanothane triterpenes.

hydroxybetulonic acid (**194**) by *Colletotrichum* (DPB136) [78]. *Chaetophoma* (DPB125) converted the same substrate (**187**) into 25-hydroxybetulonic acid (**203**) [78] (Fig. **19**).

Betulin (**204**) is a natural triterpene, abundantly available also from birch bark. Incubation of this substrate (**204**) with *Chaetomium longirostre* (IFO 9873) on a preparative scale resulted in formation of 4,28-dihydroxy-3,4-*seco*-lup-20(29)-en-3-oic acid (**205**, 3%) [79]. In other study of biocatalysis, no transformation was found for betulin (**204**) or methyl betulinate (**196**) by *Rhizopus oryzae* (ATCC 11145) [80] (Fig. **19**).

IV.4. Ceanothane Skeleton

Ceanothic acid (**206**), the major component from *Paliurus ramosissimus*, was simply converted to the dimethyl ester derivative (**207**) by *Mycobacterium* sp. (NRRL B-3805). Nevertheless, in the incubation of this dimethyl ceanothate (**207**) with the same microorganism, no detectable products were observed and the starting material was recovered unchanged. Microbial transformation of 3-dehydroceanothic acid dimethyl ester (**208**), with the same microorganism, gave a metabolite (**209**) with a structure of 3-dehydro-1-*epi*-ceanothic acid 2,28-dimethyl ester, as a result of a C-1 epimerization [81] (Fig. **19**).

V. CONCLUSIONS

Several conclusions can be drawn from the biotransformation studies reviewed:

- The microbial transformation of triterpenoids is an important tool, in search of new biologically active compounds, and to mimic and predict the mammalian metabolism of biologically active compounds. Most of these biotransformations have been performed onto tetra- and pentacyclic triterpenoids.

- In 3β-hydroxylated tetracyclic or pentacyclic triterpenes, diverse microorganisms have oxidized this hydroxyl group. Several 3-oxo triterpenes have been converted to 3,4-*seco*-type compounds, through a Baeyer-Villiger-type oxidation of these 3-oxo derivatives, followed by hydrolysis of the resulting seven-membered ring lactones.

- *Mycobacterium* sp. (NRRL B-3805) has produced a series of complex chemical changes in tetracyclic terpenoids, such as lanosterol (**11**), cycloartenol (**24**), and 24-methylenecycloartanol (**25**), including the selective cleavage of the side chain of these triterpenoids, and the demethylation at the 4α,4β- and 14α- positions, to produce C_{19} steroids.

- Diverse microorganisms have been used to investigate the biotransformation pathways of ginsenosides, and also to convert major ginsenosides into more active minor saponins. These microorganisms have produced regioselective deglycosylation reactions to produce specific ginsenosides using an appropriate combination of ginsenoside substrates and specific microorganism.

- A good number of microorganisms have demonstrated their capacity for hydroxylation at non-activated positions of the triterpene skeleton. In the 3β-hydroxylated pentacyclic triterpenes: gly-

cyrrhetinic (GA, **119**), oleanolic (**154**), ursolic (**175**), and betulinic (**185**) acids, the main action of the different microorganisms used was directed to C-7, producing 7β-hydroxylated metabolites. In addition, in most of these substrates, diverse microorganisms introduced a 15α-hydroxyl group. Other positions of the molecules as C-1, C-6, C-11, C-21, C-22, C-23, C-24, C-25, and C-30, have also been hydroxylated, obtaining a wide variety of new hydroxyl derivatives which are difficult to achieve by classical chemical means.

- *Nocardia* sp. (NRRL 5646) has converted the ursane triterpenoids: ursolic acid (**175**) and quinovic acid (**182**), to the corresponding oleanane compounds: oleanolic acid (**154**) and cincholic acid (**183**), respectively, via a carbon skeleton rearrangement involving a methyl group migration from C-19 to C-20.

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