ISSN 2020-9021
ICRRD HIGH INDEX RESEARCH JOURNAL
(An Initiative of International Center for Research and Resources Development)
Reg. No: Printed: 1347468-T, Online: 201901038138, www.icrrd.com

SYNTHETIC TRANSFORMATIONS OF *ENT*-KAURENOIC ACID

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Received: April 24, 2019 **Accepted:** May 26, 2019 **Published:** Oct 16, 2019

Abstract. This paper presents a review on kaurane diterpenes, covering various aspects of chemical and microbiological transformations of native *ent*-kaurenoic acid, namely, its reactions *via* COOH groups, double bonds and rearrangements of the carbon skeleton that lead to a wide range of natural and synthetic derivatives with potential biologic activities and can present convenient synthons for the syntheses of other native *ent*-kauranes.

Keywords: diterpenes, *ent*-kaur-16-en-19-oic acid, synthesis, biological activity.

Introduction

Ent-kaur-16-en-19-oic acid **1** is a natural *ent*-kaurane-type diterpenoid that can be isolated in a good yield from many plants, such as *Wedelia* [1], *Mikania* [2], *Annona* [3], *Xylopia* [4] and *Helianthus* genera, especially from sunflower (*Helianthus annuus* sp) [5-16]. A wide spectrum of bioactivities of *ent*-kaur-16-en-19-oic acid **1** and its derivatives has been reported, including the following effects: trypanocidal [2], embryotoxic [17], cytotoxic [12, 17] anti-HIV, anti-inflammatory, anti-fertility, antibacterial, antifungal, molluscicidal [18], anti-feedant [19], anti-platelet aggregation [20], anti-cancer [21], anti-plasmodic and relaxant activities [22], anti-Alzheimer and antioxidant [23]; acid **1** was also used as remedy for the treatment of type 2 diabetes and obesity [24].

Ent-kaur-16-en-19-oic acid **1** is one of the intermediate compounds, which is involved in the biosynthesis of diverse *ent*-kaurane diterpenes, including gibberellins, a group of growth phyto-hormones. Therefore, it is not surprising that many *ent*-kauranes and their derivatives act as growth regulators in plants [25].

The broad spectrum of presented by the *ent*-kaurane diterpenes biological activities has motivated countless studies of structural modifications of the skeleton, aiming at obtaining the new potentially bioactive substances. These structural transformations have been achieved either chemically or microbiologically, by using microorganism cultures.

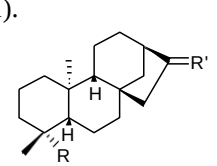
Chemical transformation of *ent*-kaur-16-en-19-oic acid

Chemical transformation of natural substances is an important and promising direction in medicinal chemistry. However, there are at least three factors that hinder investigations in this direction. The first factor is the afore-mentioned extremely low content of most of the diterpenoids in the natural sources. The second one is the presence of several reactive centers in these molecules, which complicates the course of chemo- and regioselective synthesis. The third factor is the susceptibility of *ent*-kauranes to skeletal rearrangements. Nevertheless, the huge structural variety in the class of the isolated *ent*-kaurane diterpenoids and relatively large content of some of these compounds in the available natural sources make them a very attractive basis for further chemical transformations.

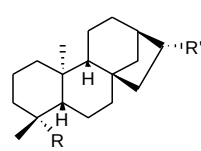
Functionalization of carboxy group in ent-kaur-16-en-19-oic acid

In *ent*-kauranes the carboxy group at C_4 is sterically screened by methyl groups of C_{18} and C_{20}, being, hence, less reactive than the analogous group in carboxylic acids. It should be noted that functionalization of a carboxyl group of *ent*-kauranes can significantly change the biological activity of the initial metabolite, but in some cases this appeared to be necessary for expression the biological activity.

In early investigations, the carboxyl group in *ent*-kaurane diterpenoids was esterified by diazomethane [26] and the resulting ester was reduced to a reactive primary hydroxyl group, which could then be readily modified. One of the first works [27] that employed this scheme for the transformation of *ent*-kaurane diterpenoids was reported in 1964, in which *ent*-kaur-16-en-19-oic acid **1**, 16α-*ent*-kauran-17,19-dioic acid **2** and other isolated from *Ricinocarpus stylosus* kauranes were subjected to sequential methylation, reduction, oxidation, and olefination, to yield a broad array of derivatives **3** - **9** etc. (Figure 1).



1 R = COOH; R' = CH_2
3 R = $COOCH_3$; R' = CH_2



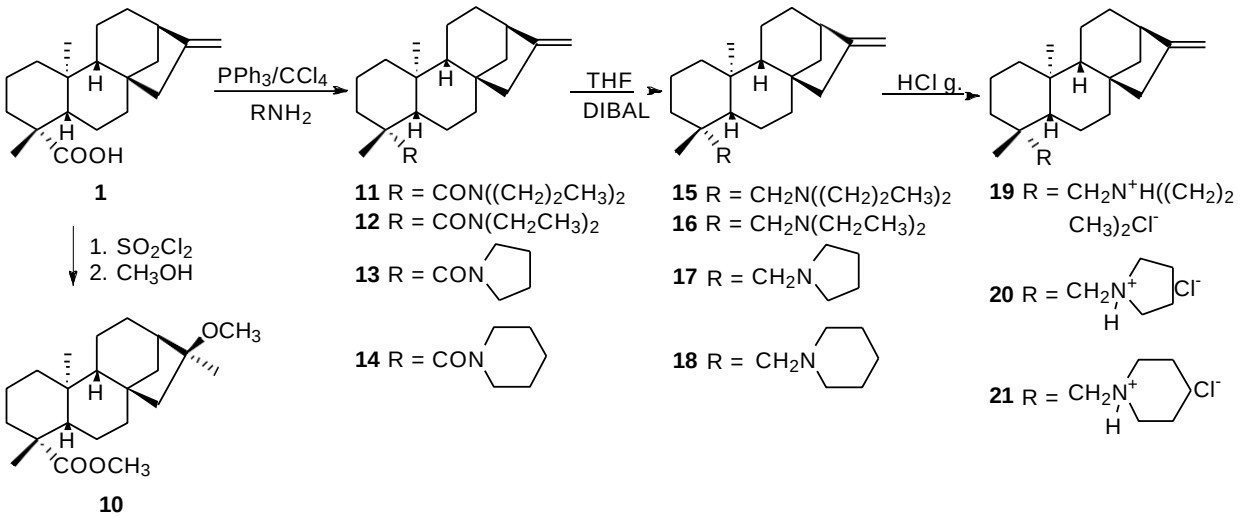
4 R = CH_2OH; R' = CH_2
5 R = COOH; R' = O

2 R = R' = COOH
6 R = R' = $COOCH_3$
7 R = COOH; R' = $COOCH_3$
8 R = CHO; R' = $COOCH_3$
9 R = COOH; R' = CH_2OH

Figure 1. Structures of *ent*-kaurenoic acid 1, *ent*-kaurandioic acid 2 and their derivatives [27].

The reaction of a carboxyl group of *ent*-kauranes with toxic and explosive diazomethane is by no means the best esterification method and this group is more frequently functionalized using the chloroanhydride pathway. According to this, the chloroanhydrides of carboxylic acids are typically obtained by using $POCl_3$, PCl_3 or $SOCl_2$. However, the interaction of these reactants with polyfunctional kaurenoids is frequently accompanied by undesired side reactions. For example, the preparation of chloroanhydride of *ent*-kaur-16-en-19-oic acid **1** may be accompanied by hydrochlorination of the double bond, resulting with the *ent*-kaur-16β-methoxy-19-oic acid **10** (Scheme 1) [28].

At the same time, the chloroanhydrides of carboxylic acids that contain labile in acidic media fragments can softly be obtained by using a CCl_4–PPh_3 mixture [29]. By means of this approach the chloroanhydride of *ent*-kaur-16-en-19-oic acid **1** was originally synthesized in 1974 in a good yield, being then used in obtaining of a series of esters [30].



Scheme 1. Synthesis of the *ent*-kaurane derivatives [28, 31].

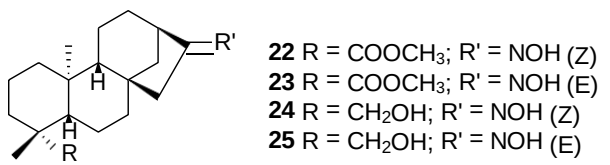


Figure 2. Structures of the synthesized from *ent*-kaurenoic acid 1 isomeric oximes [31].

In recent years, this method has also been successfully used to synthesize a large series of *ent*-kaurenoic derivatives. Intending to reduce or eliminate the lytic effect of *ent*-kaur-16-en-19-oic acid **1** on erythrocytes of the infected blood that are usually used in the *in vitro* assays against trypomastigote forms of *Trypanosoma cruzi*, Vieira et al. [31] succeeded in synthesis of a series of derivatives **11** – **21**, with amine or amide functions at C_{19}, (Scheme 1) and containing an oxime group at C_{16} kaurane derivatives **22 - 25** (Figure 2). From all the mentioned compounds, only oxime **22** was more active than **1**, presenting trypanosomicidal activity in all the tested concentrations (2.27 – 0.57 μM), along with a slight lysis of the red cells. Although hydrochloride **20** was the only product that did not produce haemolysis, its trypanosomicidal activity was comparable to that of the *ent*-kaur-16-en-19-oic acid **1**. Derivatives **23**, **24** and **25** were as active as **1**, presenting, however, a slight lysis of erythrocytes.

The same research centre, headed by Boaventura [32], reported on the preparation of novel monoamides **26** – **33** in good yields. They were obtained from the reaction of *ent*-kaur-16-en-19-oic acid **1** with monoamines and symmetrical diamines, by using a modified protocol for monoacylation (Figure 3). The activity of novel monoamides on seed germination and growth of radicle and shoot of *Lactuca sativa* (lettuce) has been tested. Amides from symmetrical diamines showed significant inhibitory activity at higher concentrations.

The abundance of *ent*-kaur-16-en-19-oic acid **1** in some plant species, along with the lack of a general method for the synthesis of alkyl kaurenoates, has motivated Boeck and collaborators [28] to carry out the chemical modification of this diterpene in order to synthesize new kaurane derivatives and to evaluate their potential pharmacological activities. As a result, a simple method was developed for preparing *ent*-kauranic esters **3, 34** – **39** (Figure 3), through the alkylation of the acid **1** with alkyl halides, in a KOH-acetone system, avoiding the use of anhydrous conditions and establishing a reproducible method for this reaction. Moreover, it was observed that only *ent*-kaur-16-en-19-oic acid **1** and its derivatives, containing a free carboxyl group, showed moderate antifungal activity against the assayed dermatophytes, suggesting that the presence of hydrophilic groups can be essential for the observed antifungal activity.

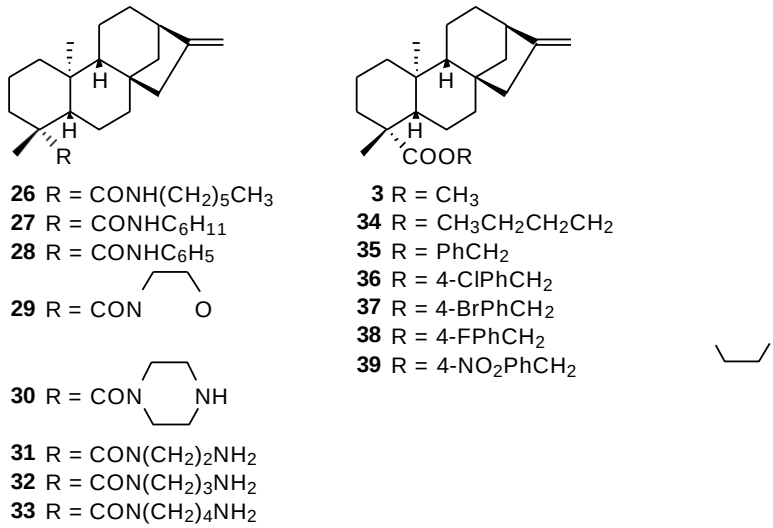
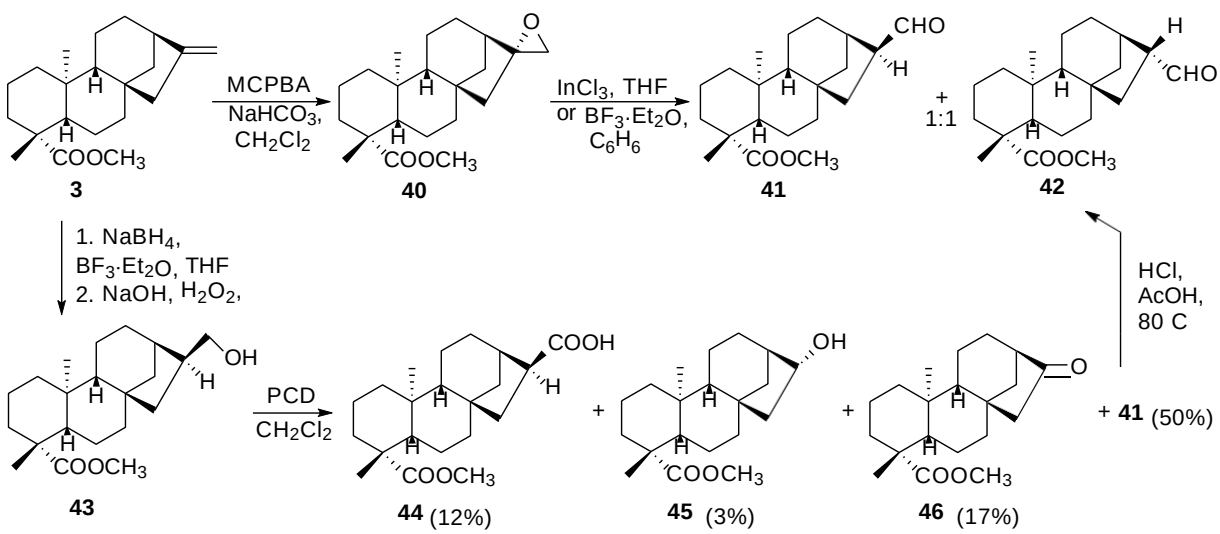


Figure 3. Monoamides and alkyl halide esters of *ent*-kaurenoic acid 1 [28, 32].

Functionalization of double bonds in ent-kaurenoic acid

One of the first reported chemical transformations of *ent*-kaurene diterpenoids was the reduction of C_{16}-C_{17} double bond by hydrogen, which was originally used in 1948 to reduce *ent*-kaurene to *ent*-kaurane [33]. In 1964, this method was applied to reduce *ent*-kaur-16-en-19-oic acid **1** [27]. It should be mentioned, that in *ent*-kaurenoids the double bond plays a significant role in manifestation of their biological activity.

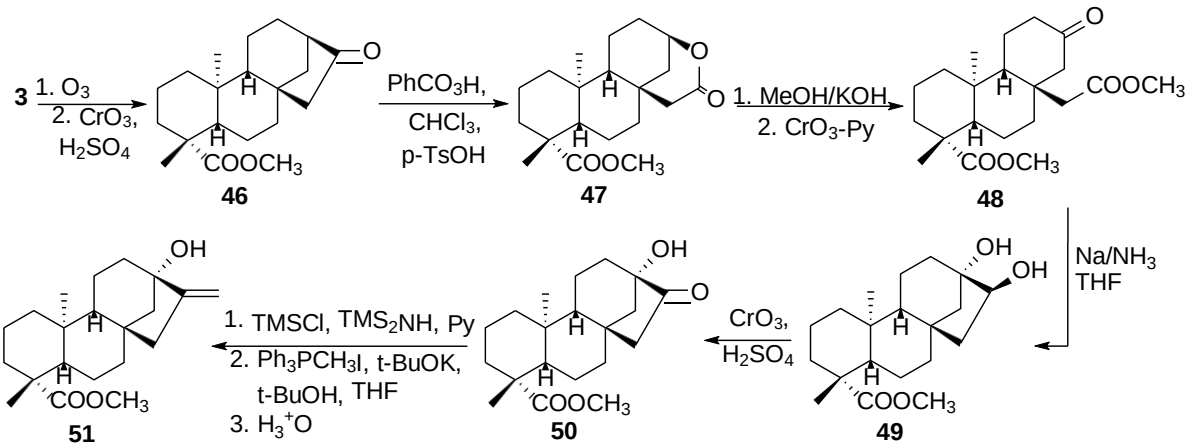
Oxidation of double bonds is an attractive direction in functionalization of *ent*-kaurenes for obtaining the new promising synthones. As a rule, this reaction is carried out by using *meta*-chloroperbenzoic acid (MCPBA) and this approach was used for the synthesis of many epoxy derivatives, including *ent*-kaur-16-en-19-oic acid epoxide **40** [34] (Scheme 2). Alternatively, the oxidation can be conducted by hydroxylation and ozonolysis, but the latter is frequently accompanied by undesired side reactions.



Scheme 2. Products of oxidation of *ent*-kaurenoic acid 1 [35].

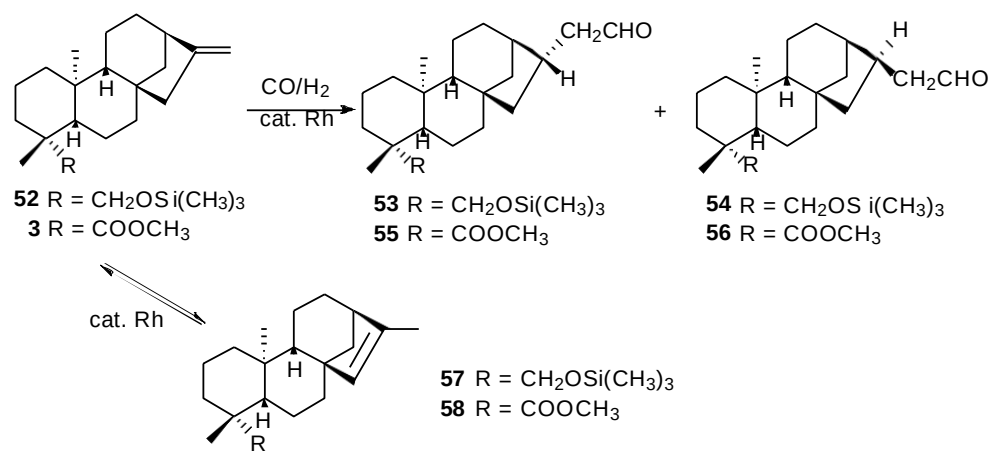
In the same line of research, Batista et al. [35] synthesized *ent*-kaurane aldehydes, methyl 16S,17-oxo-*ent*-kauran-19-oate **41** and methyl 16R,17-oxo-*ent*-kauran-19-oate **42**, important as semisynthetic coupling intermediates, starting from *ent*-kaur-16-en-19-oic acid **1** and its methyl ester **3**. Additionally they described, for the first time, synthesis of the *ent*-kaurane and *ent*-norkaurane derivatives **41, 44 - 46** under pyridinium dichromate (PDC) conditions. The initial oxidation of **43** afforded the expected aldehyde **41**, which in the presence of the chromate underwent further oxidation to the acid **44**. The latter can be considered the precursor of *ent*-norkauranes, methyl 16α-hydroxy-17-*ent*-norkauran-19-oate **45** and methyl 16-oxo-17-*ent*-norkauran-19-oate **46** (Scheme 2).

Synthesis of steviol by Cook and Knox [36-38] involves a series of oxidative transformations, starting with *ent*-kaur-16-en-19-oic acid **1** (Scheme 3). Conversion of methyl *ent*-kaur-16-en-19-oate **3** to nor-ketone **46** and subsequent Baeyer-Villiger oxidation afforded γ-lactone **47**, which was converted by hydrolysis, methylation and oxidation into the keto-diester **48**. Treatment of **48** with sodium-liquid ammonia gave the acyloin-like cyclization product, diol-acid **49**, which was oxidized to **50**. Silyl ether protection of **50** and subsequent Wittig reaction with methylenetriphenylphosphorane, followed by a dilute acid work-up, gave steviol **51**.



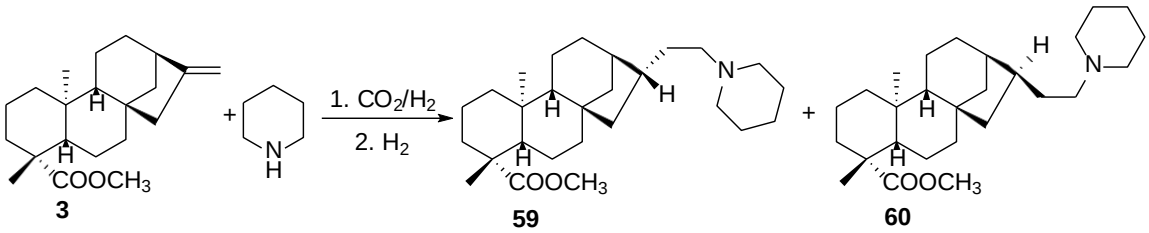
Scheme 3. Synthesis of steviol from *ent*-kaurenic acid 1 [36-38].

Recently, it has been reported that hydroformylation of the C_{16}= C_{17} bond in *ent*-kaur-16-en-19-oic acid **1** [39] and *ent*-kaur-16-en-19-ol **4** [40] was performed by the use of rhodium catalysts. Substrates, such as methyl *ent*-kaur-16-en-19-oate **3** and trimethylsilyl-*ent*-kaur-16-en-19-ol ether **52** have been hydroformylated by using unmodified Rh catalysts, as well as Rh/PPh_3 and Rh/*tris*-(*o*-*t*-butylphenyl) phosphite catalytic systems. It should be noted, that formation of the corresponding aldehydes **53**, **54** and **55**, **56,** respectively, was accompanied by the isomerization of substrates into derivatives **57** and **58** with endo-cyclic double bonds (Scheme 4).



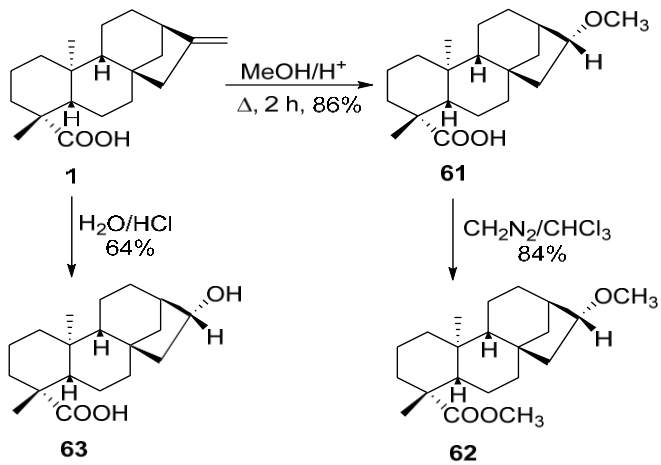
Scheme 4. Rh/PPh_3 hydroformylation of *ent*-kaurenic acid 1 [40].

Usage of the same hydroformylation rhodium catalytic systems led to the development of processes for *tandem* sequential hydroxyaminomethylation (hydroformylation followed by hydrogenation *in situ*) [41]. Thus, the rhodium precursor [Rh(acac)$(CO)_2$] and a 15-fold excess of PPh_3 ligand were introduced into the reactor. The substrate, methyl *ent*-kaur-16-en-19-oate **3,** and dissolved in toluene piperidine were subsequently introduced into the reactor. The reaction was maintained at 100°C at a total pressure ($CO:H_2$ = 1:1) of 20 bar for 48 hours to ensure that any formed imine was transformed by hydroxyaminomethylation. As a result, the diastereoisomers **59** and **60** have been obtained, as potential biologically active compounds (Scheme 5). The GC-analysis after 48 hours showed the 84% conversion with a diastereomeric ratio **59** / **60** = 61:39.



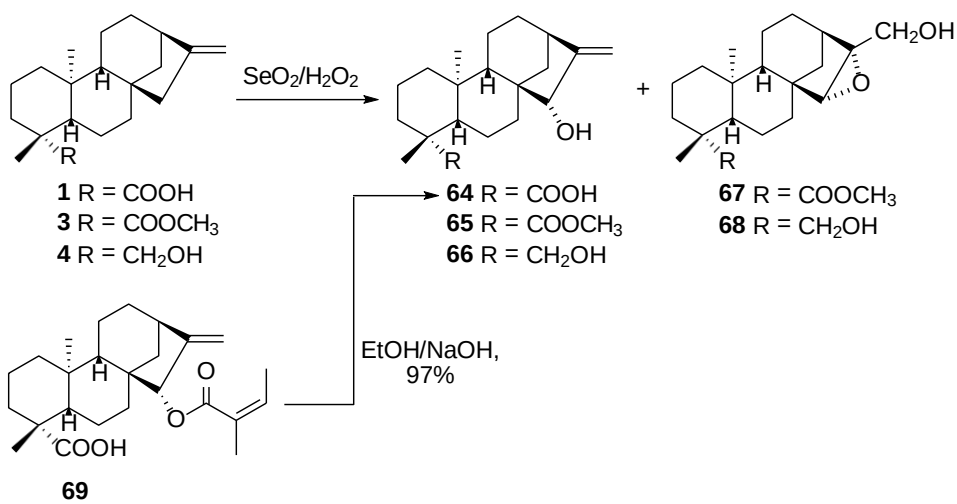
Scheme 5. Rhodium catalytic hydroxyaminomethylation of *ent*-kaurenoic acid 1 [41].

To study the cytotoxicity of *ent*-kauranes with respect to some human cancer cells, derivatives **61 - 63** were recently synthesized by electrophilic addition at the double bond of *ent*-kaur-16-en-19-oic acid **1** [44]. It was established, that this modification (Scheme 6) led to the complete disappearance of the anticancer effect.



Scheme 6. Electrophilic addition at a double bond of *ent*-kaurenoic acid 1 [44].

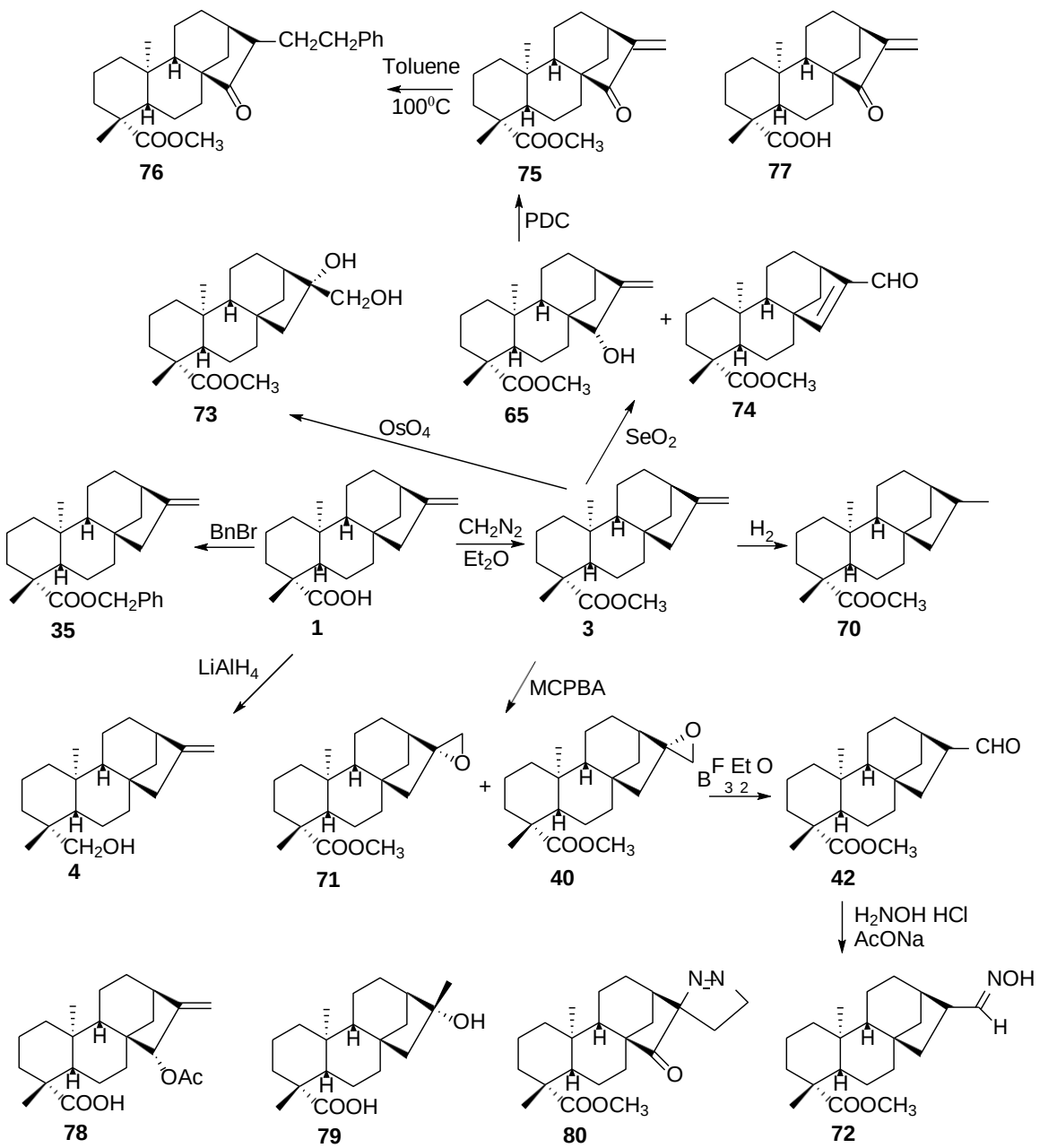
Aparicio et al. [42] described the allylic oxidation of *ent*-kaur-16-en-19-oic acid **1**, methyl *ent*-kaur-16-en-19-oate **3** and *ent*-kaur-16-en-19-ol **4** with SeO_2/H_2O_2 (Scheme 7). The reaction was run in a dioxan solution at room temperature at stirring for 4 hours. Treatment of acid **1** afforded 56% of 15α-hydroxy-*ent*-kaur-16-en-19-oic acid (grandifloric acid) **64**. However, treatment of methyl ester **3** furnished two products: methyl 15α-hydroxy-*ent*-kaur-16-en-19-oate **65** (34% yield) and methyl 15α,16α-epoxi-17-hydroxy-*ent*-kauran-19-oate **67** (59% yield). In a similar way, treatment of *ent*-kaur-16-en-19-ol **4** rendered two products: 15α,19-dihydroxy-*ent*-kaur-16-ene **66** (57% yield) and 15α,16α-epoxi-17,19-dihydroxy-*ent*-kaurane **68** (34% yield). In the same direction, 15α-hydroxy-*ent*-kaur-16-en-19-oic acid **64** was synthesized with a better yield by using SeO_2/EtOH system, starting with *ent*-kaur-16-en-19-oic acid **1** and by saponification of the 15α-angeloyl-*ent*-kaur-16-en-19-oic acid **69** [43].



Scheme 7. Allylic oxidation of *ent*-kaurenoic acid 1 [42, 43].

Due to a continuing interest in the evaluation of biological potential of natural diterpenes, Hueso-Falcónin et al. [44] reported a study on the preparation of *ent*-kaurane derivatives from the natural *ent*-kaur-16-en-19-oic acid **1** (Scheme 8). These compounds were tested for their ability to induce apoptosis of signaling pathway in mouse and human cancer cells and some conclusions about structure–activity relationships have been made. The most active compounds were investigated and they were able to induce apoptosis with methyl 15-oxo-e*nt*-kaur-16-en-19-oate **75** being the best inducer.

Presence of the α-oxo methylene moiety seems to play an important role in expressing the bio-activity and this fragment can act as Michael acceptor for nucleophilic residues, especially cysteine sulfhydryl groups [45]. Replacement of the oxo group or double bond in compounds **75** leads to the loss of the cytotoxic activity. Thus, such natural compounds as: acetate **78**, alcohol **79** or in the case of 15-oxo-*ent*-kaurene derivatives, α-phenylethylketone **76** and α-oxopyrazoline **80** exhibit no such activity. Moreover, the cytotoxic activity of methyl 15-oxo-e*nt*-kaur-16-en-19-oate **75** is 35 times greater, than that of initial acid **77** [44].

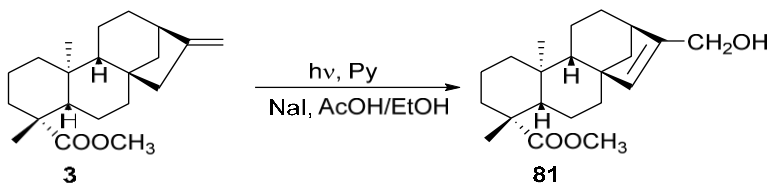


Scheme 8. Preparation of *ent*-kaurane derivatives from the *ent*-kaurenoic acid 1 [44].

Carbon skeleton rearrangements in ent-kaur-16-en-19-oic acid

Many isoprenoids, particularly diterpenoids, are characterized by susceptibility to skeletal rearrangement - a process accompanied by changes in the carbocyclic framework. These reactions play a special role in the functionalization of *ent*-kauranes, making possible the synthesis of compounds with rather unusual structures that cannot be obtained by other methods.

Photooxygenation is one of the first rearrangement reactions of *ent*-kaur-16-en-19-oic acid **1** [46]. The oxygenation of olefins containing allylic hydrogen atoms in the presence of a suitable sensitizer and visible light gives allylic hydroperoxides, the process being invariably accompanied by a shift of the double bond. The dissolved in pyridine methyl *ent*- kaur-16-en-19-oate **3** was irradiated with fluorescent tubes and hematoporphyrin was employed as a sensitizer. The resulting hydroperoxide was not isolated, being directly reduced in an ethanol solution with sodium iodide and acetic acid. The chromatography of the product over silica gel afforded the allylic alcohol **81** in a 30% yield (Scheme 9).

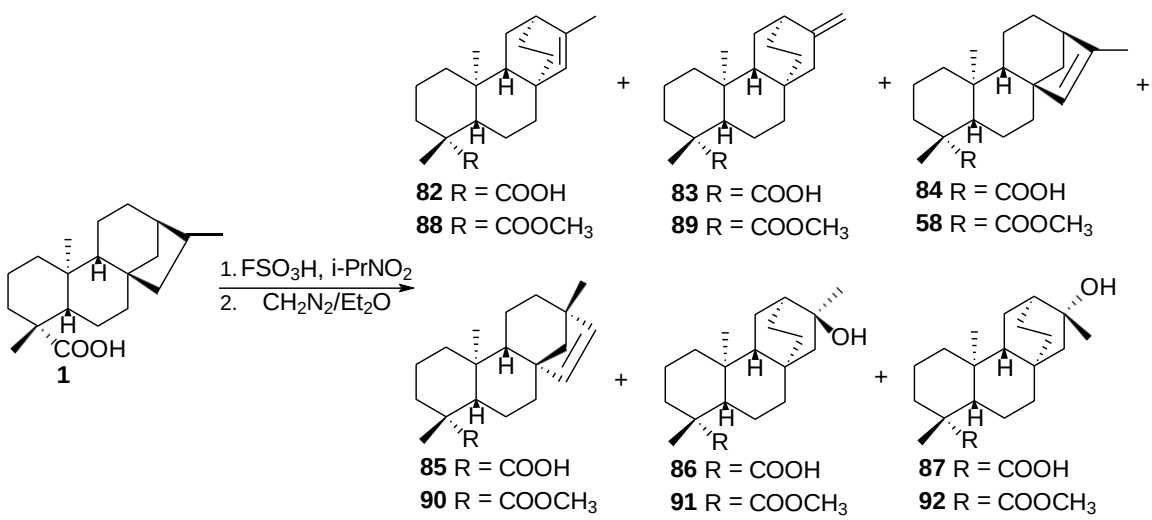


3 81

Scheme 9. **Photooxygenation reactions of *ent*-kaurenoic acid 1 [46].**

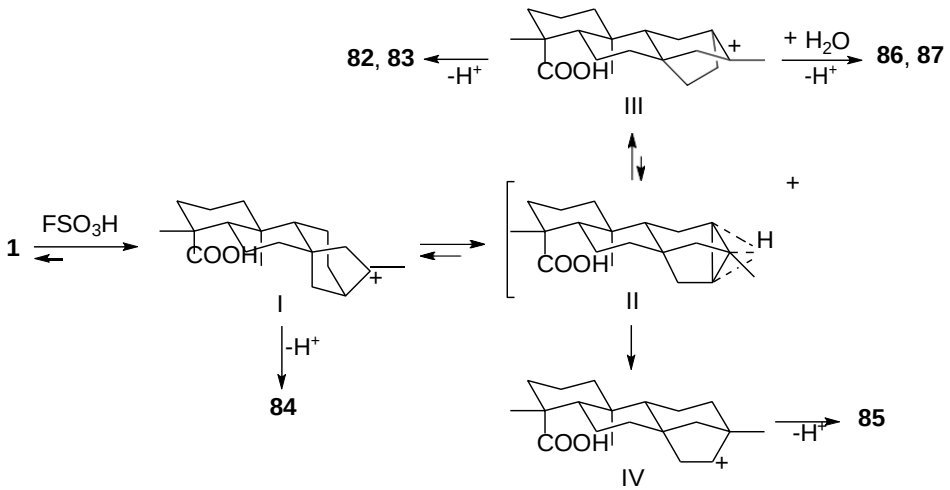
The rearrangements of *ent*-kaurane diterpenes under the action of different reagents have been reported [47]. Most of the examples report on the reactions that involve formation of the non-classical carbocation. It is well-known from the work of Olah et al. [48], that superacids are very convenient generators of these species, in particular, fluorosulfonic acid (FSO_3H) is known as an efficient promoter of cyclizations and rearrangements of terpenoids.

Recently [49] an efficient one-step, retro-biomimetic procedure for the synthesis of natural products having the atisane structure has been reported (Scheme 10), which are natural components of medicinal plants and possess a relevant biological activity.



Scheme 10. Rearrangements of *ent*-kaurenoic acid 1 [49].

Treatment of *ent*-kaur-16-en-19-oic acid **1** with fluorosulfonic acid under mild reaction conditions allowed carbonium ion generation and skeletal rearrangement to occur. The reaction product included: *ent*-atis-15-en-19-oic acid **82** (8%), *ent*-atis-16-en-19-oic acid **83** (22%), recovered starting material **1** (18%), *ent*-kaur-15-en-19-oic acid **84** (17%), *ent*-beyer-15-en-19-oic acid **85** (7%), 16β-hydroxy-*ent*-atisan-19-oic acid **86** (5%) and 16α-hydroxy-*ent*-atisan-19-oic acid **87** (4%). Treatment of the individual acids **82** – **87** with an Et_2O solution of diazomethane led to the corresponding esters: methyl *ent*-atis-15-en-19-oate **88**, methyl *ent*-atis-16-en-19-oate **89**, methyl *ent*-kaur-15-en-19-oate **58**, methyl *ent*-beyer-15-en-19-oate **90**, methyl 16β-hydroxy-*ent*-atisan-19-oate **91** and methyl 16α-hydroxy-*ent*-atisan 19-oate **92**.



Scheme 11. The proposed reaction course for superacid-promoted isomerization of *ent*-kaurenoic acid 1 [49].

Thus, the superacid-promoted rearrangement of *ent*-kaur-16-en-19-oic acid **1** led predominantly to tetracyclic *ent*-atisane diterpenoids (see: **82**, **83**, **86** and **87**), with an overall yield of 39%. Taking into account the recovered starting material **1**, the combined yield of atisane-type compounds amounted to ca. 62%.

The transformation of *ent*-kaur-16-en-19-oic acid **1** into *ent*-atisane-type compounds takes place by the formation of carbonium ion **I**, which rearranges *via* the nonclassical pentacyclic ion **II** to the *ent*-atisane carbonium ion **III** (Scheme 11). The latter undergoes a H-atom loss either from C_{15} or C_{17}, to form the double bond isomeric *ent*-atis-15-en-19-oic acid **82** and *ent*-atis-16-en-19-oic acid **83**. The hydroxylated *ent*-atisanoic acids **86** and **87** are formed by quenching the cabocation **III** with a water molecule. On loss of one H-atom from C_{15} of *ent*-kauranoic acid carbonium ion **I**, the *ent*-kaur-15-en-19-oic acid **84** is obtained. The *ent*-beyer-15-en-19-oic acid **85** is formed after transformation of the nonclassical carbonium ion **II** to the *ent*-beyeranoic acid cation **IV** and subsequent H-atom loss from C_{16}.

Microbiological transformation of ent-kaur-16-en-19-oic acid

Microorganisms are able to transform a huge variety of organic compounds, such as hydrocarbons, terpenoids, steroids, alkaloids, antibiotics and amino-acids. Hydroxylation of inactivated carbons remains the most explored area in the microbial transformation of organic compounds. This reaction makes possible the preparation of countless novel diterpenoid derivatives that are inaccessible by chemical means.

Microbiological transformations of *ent*-kaur-16-en-19-oic acid **1** with *Calonectria decora*, *Rhizopus nigricans* ans *Aspergillus ochraceous* have been investigated by Ghisalberti et al. affording the following hidroxy derivatives: 7α-hydroxy-*ent*-kaur-16-en-19-oic acid **93**, 7β-hydroxy-*ent*-kaur-16-en-19-oic acid **94** and 16α,17-dihydroxy-*ent*-kauran-19-oic acid **95** [50] (Figure 4).

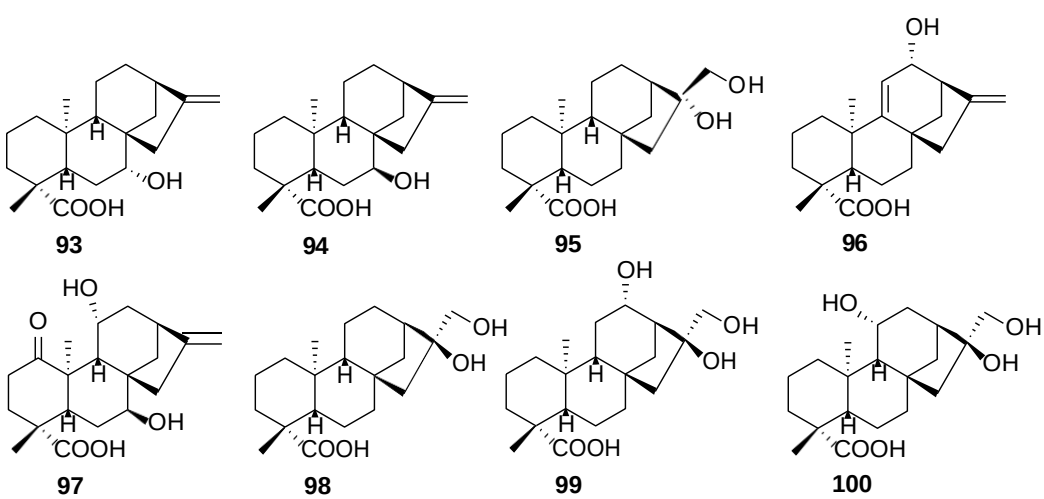


Figure 4. The obtained by microbiological transformation derivatives of *ent*-kaurenoic acid 1 [50-53].

As a part of the program of transformation the diterpenoids by microorganisms, Silva et al. [51] carried out the transformation of *ent*-kaur-16-en-19-oic acid **1**, by using *Rhizopus stolonifer*. Results of the incubations indicate that the obtained in the microbial transformation products were formed by hydroxylation in the B, C and D rings.

The incubation of **1** with *R. stolonifer* for seven days yielded compounds: 7β-hydroxy-*ent*-kaur-16-en-19-oic acid **94** and 12α-hydroxy-*ent*-kaur-9(11),16-dien-19-oic acid **96**, the former being the major product (~ 5%). The incubation of **1** with *R. stolonifer*, under the same conditions, for a longer period (for 15 days), led to the formation of a third metabolite, 16α,17-dihydroxy-*ent*-kauran-19-oic acid **95** (Figure 4).

Punnapayak et al. [52] reported generation of an interesting compound, 7β,11α-dihydroxy-l-oxo-*ent*-kaur-16-en-19-oic acid **97** that was obtained by fermentation of *ent*-kaur-16-en-19-oic acid **1** with *Aspergillus niger* for 7 days (Figure 4).

J. Pechwang et al. [53] described the biotransformation by *Psilocybe cubensis* of *ent*-kaur-16-en-19-oic acid **1** to produce its derivatives, along with *in vitro* evaluation of cytotoxic activity of all metabolites against human tumor cells. After two days of incubation the *ent*-kaur-16-en-19-oic acid **1**, 16β,17-dihydroxy-*ent*-kauran-19-oic acid **98** was isolated. After a further incubation for nine days, two novel metabolites, 12α,16β,17-trihydroxy-*ent*-kauran-19-oic acid **99** and 11α,16β,17-trihydroxy-*ent*-kauran-19-oic acid **100**, were obtained (Figure 4).

Conclusions

This paper reviewed the occurrence and biological activities of *ent*-kaur-16-en-19-oic acid, but especially the synthetic and semisynthetic methods that offer a wide range of natural and synthetic *ent*-kaurane derivatives. The accumulation of *ent*-kaur-16-en-19-oic acid and other naturally occurring kaurane diterpenes in some plant species make them important sources of these compounds, which are thus available as starting materials in the synthesis of new derivatives for biomedical and industrial research. Indeed, in the last few years, a growing number of publications have reported the use of *ent*-kaurane diterpenes for the synthesis of novel sweetening, antimicrobial, cytotoxic and trypanocidal agents, and this synthetic approach is still far from being fully exploited by the natural products chemistry community.

Acknowledgments

The author is very grateful to Dr. hab. Nicon Ungur and Dr. Veaceslav Kulciţki for assistance in the preparation of the manuscript.

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