SYNTHETIC TRANSFORMATIONS OF ENT-KAURENOIC ACID

Olga Morarescu

Institute of Chemistry of Academy of Sciences of Moldova, 3, Academiei str., Chisinau MD-2028, Republic of Moldova

E-mail: olea_chetraru@yahoo.com; phone: (+373-22) 73 97 75; fax: (+373-22) 73 97 75

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.

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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 C1 is sterically screened by methyl groups of C18 and C20, 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).

![Figure 1. Structures of ent-kaurenoic acid 1, ent-kaurandioic acid 2 and their derivatives [27].](image-url)
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 POCl₃, PCl₃ or SOCl₂. 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 CCl₄–PPh₃ 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].](image)

![Figure 2. Structures of the synthesized from ent-kaurenoic acid 1 isomeric oximes [31].](image)

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 C₁₉, (Scheme 1) and containing an oxime group at C₁₆ kaurane derivatives 22 – 25 (Figure 2). From all the mentioned compounds, only oxime 22 was more active than 1, presenting trypanosomacidal 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/PPh3 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/PPh3 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 PPh3 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:H2 = 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$_2$O$_2$ (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-ent-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 sulphydryl 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-ent-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).

![Scheme 9. Photooxygenation reactions of ent-kaurenoic acid 1 [46].](image)

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 (FSO₃H) 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].](image)

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

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

Punnnapayak 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.

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References


