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Artículo científico

On the allylic hydroxylation of ent-kaurenic acid with SeO_2

Alexis Peña[1], Libia Alarcón[1], Rosa Aparicio[3], Julio Rojas[2], Alfredo Usubillaga[3*]

1) Departamento de Química. Grupo de investigación GIPRONA. Núcleo Universitario Rafael Rangel, Universidad de Los Andes, Trujillo, Venezuela.

2) Postgrado de Química de Medicamentos, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida, Venezuela.

3) Instituto de Investigaciones, Facultad de Farmacia y Bioanálisis, Universidad de Los Andes, Mérida, Venezuela.

(*) usubilla@ula.ve

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Resumen

La hidroxilación alílica del ácido *ent*-kaur-16-en-19-oico (**1a**) con SeO_2 se ensayó a temperatura ambiente utilizando dioxano o diclorometano como solventes así como diferentes concentraciones de reactivo y tiempo de reacción. Añadir H_2O_2 a la mezcla de reacción condujo a la formación de muchos subproductos además del ácido *ent*-15a-hidroxi-kaur-16-en-19-oico (**2a**), que es el principal producto de la oxidación. Al utilizar diclorometano como solvente, sin añadir H_2O_2, la reacción se hizo más lenta pero condujo principalmente a la formación de **2a** (70% a las 24 h, 53% a 48 h) y del ácido *ent*-17-oxo-kaur-15,16-en-19-oico (**3a**). El curso de la reacción se siguió mediante cromatografía de gases-masas, luego de metilar las mezclas de reacción.

Palabras clave: hidroxilación alílica; ácido *ent*-kaur-16-en-19-oico; ácido *ent*-15α-hidroxi-kaur-16-en-19-oico; ácido *ent*-15-oxo-kaur-16-en-19-oico

Abstract

Allylic hydroxylation of *ent*-kaur-16-en-19-oic acid (**1a**) with SeO_2 was tried using either dioxane or dichloromethane as solvents as well as different reagent and reaction time conditions at room temperature. Oxidation of **1a** in dioxane with H_2O_2 decreased reaction time but led to the formation of many by-products on addition to *ent*-15α-hydroxy-kaur-16-en-19-oic acid (**2a**), which was the main product. Using dichloromethane as solvent without addition of H_2O_2 made the reaction slower and yielded mainly **2a** (70% at 24 h, 53% at 48 h) and *ent*-17-oxo-kaur-15,16-en-19-oic acid (**3a**,18% at 24 h, 43% at 48 h). The course of the reaction was followed by GC-MS, after methylation of the reaction mixtures.

Keywords: Allylic hydroxylation; *ent*-kaur-16-en-19-oic acid; *ent*-15α-hydroxy-kaur-16-en-19-oic acid; *ent*-15-oxo-kaur-16-en-19-oic acid

Introduction

Several kaurenic diterpenes functionalized at ring D have biological activity. *Ent*-15α-hydroxy-kaur-16-en-19-oic acid (**2a**, Fig 1) has been found to be active *in vitro* against melanoma B16F1 in mice[1]. This compound has been isolated from several species of Espelletiinae[2] which are resinous plants that grow in the Andes above 2500 meters. On the other hand the proapoptotic effect of *ent*-15-oxo-kaur-16-en-19-oic acid (**5a**) on the human prostate carcinoma epithelial cell line PC-3 has been described[3]. This compound was first isolated by Ekong *et al* from *Xylopia aethiopica*[4] an African medicinal plant, but it is not readily available from natural sources. It has been obtained by chromic acid oxidation from (**2a**). Since **2a** is a rather scarce compound in Espelletiinae species investigated thus far, it was considered convenient to develop a hemi-synthetic method to obtain sufficient quantities of **2a** from *ent*-kaurenic acid to use it as starting compound to obtain **5a** and other derivatives to test their biological activity.

Allylic hydroxylation of *ent*-kaurenic acid with SeO_2/H_2O_2 produces **2a** as major product[5], but it also produces by-products. The molecular formulas of *ent*-kaurenic acid (**1a**) and its hydroxylation reaction products *ent*-15α-hydroxy-kaur-16-en-19-oic acid (**2a**), *ent*-17-oxo-kaur-15-en-19-oic acid (**3a**), *ent*-17-hydroxy-kaur-15-en-19-oic acid (**4a**), *ent*-15-oxo-kaurenic acid (**5a**), *ent*-15α-hydroxy- 16,17-epoxi-

kauran-19-oic acid (**6a**), *ent*-15,16-epoxi-17-hydroxy-kauran-19-oic acid (**7a**), *ent*-15-oxo-16,17,21-cyclopropyl-kauran-19-oic acid **(9)** and their methyl esters are shown on Figure1.

Experimental

General experimental procedures

Melting points were measured on a Fisatom D 430 hot stage and are uncorrected. IR spectra were measured on a Perkin Elmer FT Spectrum Two spectrometer, as KBr discs. ^{1}H and ^{13}C NMR measurements were performed on a Bruker Advance DRX-400. GC-MS was done on a Hewlett-Packard model 5973 spectrometer at 70 eV using an HP-5MS column (30 m long, 0.25 mm internal diameter and 0.25μm film), at an initial temp. of 200 ^{o}C, increasing the temp. at 10^{o}C /min up to 300 ^{o}C, and holding the final temp. for 5 min. Compounds **2b**, **3b**, **4b**, **5b**, **6b**, **7b**, and **8** were identified by comparison of their retention times and mass spectra with those of authentic samples. Flash chromatography was performed on silica gel Merck 60 (230-400 mesh); TLC was carried out on silica gel Merck 60 F254.

Isolation of ent-kaurenic acid (1a)

This compound was obtained from the aerial parts of *Espeletia nana*, collected at Páramo of Ortiz, vía Riecito, Trujillo State Venezuela, and compared with an authentic sample obtained from *E. semiglobulata* (mp, tlc, ^{1}H-NMR)[6]. Pure *ent*-kaurenic acid crystallized from hexane, mp 178-180 ^{o}C, M^{+} m/z 302 ($C_{20}H_{30}O_2$).

Reaction of ent-kaurenic acid with 0.48 mmol of SeO_2 and 0.4 mL 30%/H_2O_2

A dioxane soln. (5 mL) of 100 mg (0.33 mmol) of *ent*-kaurenic acid (**1a**) was mixed with 52 mg (0.48 mmol) SeO_2 and 0.4 mL of 30 %H_2O_2, and it was stirred at room temp. To analyze the reaction products aliquots (0.5 mL) were taken at 30 min and then at 1.0, 2.0, 4.0, 8.0, 24.0 and 48.0 hours. Water was added to each aliquot and the mixture was shaken with diethyl ether. The ether layer was dried with dry Na_2SO_4 and evaporated to dryness. Ethereal diazomethane soln. was added to each product mixture obtained and left to react till the following morning, when they were submitted to GC-MS analysis, as described in general experimental procedures. Aliquots taken at 1.0, 2.0, 4.0, 8.0, 24, and 48 hours of reaction time were treated and analyzed in the same way. The total ion chromatogram peaks of **2b** (Retention time 8.05 min), **3b** (8.24 min), **4b** (8.33 min), and **6b** (9.48 min) were completely separated and their relative masses calculated as shown on Table 1. Identification of compounds **2b**-**6b** was accomplished by comparison of the retention times and mass spectra of the methyl esters of compounds **2a**-**6a** which were obtained as follows. A dioxane soln. (50 mL) of 1.0 g of *ent*-kaurenic acid (**1a**, 3.3 mmol) was mixed with 5.2 mg (4.8 mmol) SeO_2 and 4.0 mL of 30% H_2O_2 and it was stirred at room temp. After 1.0 h of reaction water was added and the mixture was shaken with diethyl ether. The ether layer was dried over Na_2SO_4 and taken to dryness. The reaction product was submitted to flash chromatography over silica gel. Elution with hexane and hexane/EtOAc mixtures yielded **2a** 340 mg, mp 224-228 ^{o}C, identical to *ent*-15α-hydroxy-kaur-16-en-19-oic acid (mp, ^{1}H-RMN, TLC) isolated from *E. semiglobulata*[6]. Elution with hexane:EtOAc (5%) yielded **3a** (12 mg), which was methylated to yield **3b**, retention time 8.25 min, MW 330.2 g/mol, mp 68-70 ^{o}C, IR (ν_{max}, cm^{-1}): 2940, 2886, 2682 (CHO), 1726 (COOMe), 1690 (C=C-CHO), 1618, 1234; ^{1}H-RMN (Table5), ^{13}C-RMN (Table 6), it was identified as *ent*-17-oxo-kaur-15-en-19-oic acid methyl ester previously reported by Hueso *et al*.[7] Further elution yielded **4a** (43 mg), which was methylated to yield **4b**, retention time 8.33 min, MW 332 g/mol, mp 132-134^{o}C; IR cm^{-1}: 3315, 3032, 2965, 2962, 2844, 1728, 1667, 1232, 1158, ^{1}H-RMN (Table 5), ^{13}C-RMN (Table 6), which was identified as *ent*-17-hydroxy-kaur-15-en-19-oic acid methyl ester. Further elution with hexane:EtOAc (20%) yielded 58 mg of mixture which was methylated and acetylated. Flash chromatography of this mixture yielded 43 mg of a compound which showed a MW at 390.3 g/mol ($C_{23}H_{34}O_5$)**.** This compound was identified as *ent*-15α-acetoxy -16,17- epoxi- kauran-19-oic acid methyl ester (**6c**): mp 176-9^{o}C,the IR showed carbonyl vibrations at 1738 cm^{-1} and 1688 cm^{-1}, and a band at 1242 cm^{-1} (C-O-C).^{1}H-RMN (Table 5), ^{13}C-RMN (Table 6), Further elution with hexane:EtOAc 50% yielded a mixture that it was not possible to separate.

Reaction of ent-kaurenic acid with 0.25 mmol of SeO_2 and different amounts of 30%/H_2O_2.

Four different experiments were performed adding to a dioxane soln. of 50 mg (0.165 mmol) of *ent*-kaurenic acid (**1a**) mixed with 27.5 mg SeO_2 (0.25 mmol), 0.2; 0.1; 0.05; or 0.02 mL 30% H_2O_2. Each reaction mixture was shaken at room temp for 2 h. Water was added and each mixture was shaken with diethyl ether. The ether layer was treated with dry Na_2SO_4 and evaporated to dryness. The mixture was treated with diazomethane and the methylated product was analyzed by GC-MS as previously described. Results are shown on Table 2.

Reaction of ent-kaurenic acid with 0.25 mmol of SeO_2 without H_2O_2 at different reaction times

Ent-Kaurenic acid (100 mg, 0.33 mmol) in dioxane (5 mL) was mixed with SeO_2 (27.5 mg, 0.25 mmol). Instead of H_2O_2 a drop of water was added to improve solubility of SeO_2. Aliquots (0.5 mL) were taken at 30 min, as well as 1.0, 2.0, 4.0, 8.0, 24.0, and 48.0 h. To submit the samples

to GC-MS analysis they were treated as previously described. Results are shown on Table 3.

Reaction of ent-kaurenic acid with 0.53 mmol of SeO_2 without H_2O_2 using CH_2Cl_2 as solvent

Ent-kaurenic acid (100 mg, 0.33 mmol) in dichloromethane (30 mL) was mixed with SeO_2 (59.0 mg, 0.53 mmol) without adding any H_2O_2. The reaction was conducted at room temperature with constant stirring during 24 and 48 hours. Aliquots were methylated as previously described and submitted to GC-MS analysis which showed **2b** and **3b** as major products that made up about 90% of the reaction mixture, as shown on Table 4.

Oxidation of ent-15α-hydroxy-kaur-16-en-19-oic acid with Sarett reagent

Pyridine (3 mL) was treated under continued agitation with 0.3 g of CrO_3 to obtain Sarett reagent[8] which was mixed with 120 mg of **2a** and stirred 24 h. The reaction mixture was shaken with hexane/EtOAc 20%. The organic phase was dried, filtered, evaporated to dryness and purified on a silica gel column. Elution with hexane/EtOAc 10% yielded 87 mg of *ent*-15-oxo-kaur-16-en-19-oic acid (**5a**), mp 184-5 °C, IR (ν_{max}, cm^{-1}): 1724 (C=O), 1690 (COOH), 1644 (C=C), 906 (=C-H), data which agreed with those reported by Ruiz *et al.*[3]

Methylation of ent-15-oxo-kaur-16-en-19-oic acid with diazomethane

In order to have a compound suitable for gas chromatography **5a** (20 mg) was treated with freshly distilled CH_2N_2 in ether soln. Instead of obtaining the expected methyl ester **5b**, the exocyclic double bond formed a pyrazol ring yielding **8**, mp 108-11 °C IR (ν_{max}, cm^{-1}): 1742, 1720 (C=O) ^{1}H-RMN (Table 5), ^{13}C-RMN (Table 6) as described by Oliveira, Hanson, and Takahashi[9], Upon standing or when subjected to warming, this compound loses N_2 yielding **9**, yellow syrup, MW 344.5 g/mol, IR (ν_{max}, cm^{-1}): 1700 (C=O) ^{1}H-RMN (Table 5), ^{13}C-RMN (Table 6).

Results

In order to optimize the yield of **2a**, different reaction conditions were assayed varying reaction time as well as relative reagents concentration. As starting compound it was always used 100 mg (0.33 mmol) of *ent*-kaurenic acid (**1a**) dissolved in 5 mL of dioxane. All reactions were performed at room temperature. Aliquots of the reaction product (0.5 mL) were methylated and submitted to GC-MS analysis. Peak areas were calculated from TIC trace, but since all compounds had an *ent*-kaurenic nucleus, values observed were considered to be close to those obtainable by GC-FID. All peaks were well defined with 95-100% resolution.

In a first experiment 0.48 mmol of SeO_2 and 0.4 mL of 30% H_2O_2 were mixed with 100 mg (0.33 mmol) of *ent*-kaurenic acid and allowed to react at room temperature up to 48 h as shown on Table 1. The course of the reaction was followed by GC-MS and for that purpose aliquots of the reaction product (0.5 mL) were methylated and submitted to GC-MS analysis at 30 min, 1.0, 2.0, 4.0, 8.0, 24.0, and 48.0 h of reaction. Only peaks that were well defined were identified. Compounds were identified as methyl esters by comparison with substances isolated by flash chromatography as described in the experimental part. The main product after 30 min of reaction was *ent*-15α-hydroxy-kaur-16-en-19-oic acid (**2a**, 50.4%), the second most abundant product was *ent*-15α-hydroxy-16,17-epoxi–kauran-19-oic acid (**6a**, 14%). Two minor reaction products *ent*-17-oxo-kaur-15-en-19-oic acid (**3a**, 1.5%), *ent*-17-hydroxy-kaur-15-en-19-oic acid (**4a**, 7.4 %), were detected and isolated but other products, which represented 26.7%, were also formed but it was not possible to separate and analyze.

As reaction time increased, the yield of **2a** diminished, while the yield of **6a** and other not identified products increased. After 48 h of reaction yield of **2a** had diminished to 18.4%, while **6a** yield was 25.1%

Table 1: Allylic hydroxylation of *ent*-kaurenic acid with 0.48 mmol SeO_2 and 0.4 mL of H_2O_2. Reaction time runs from 0.5 to 48 h.

Time	[M$^+$]	316	332	330	332	344	348	
	C(RT)	**1b**	**2b**	**3b**	**4b**	**9**	**6b**	**OP**
(h)	min..	(%)	(%)	(%)	(%)	(%)	(%)	(%)
0.5	6.40	-	50.4	1.5	7.4	-	14.0	26.7
1.0	8.05	-	42.3	1.2	6.4	-	17.3	32.8
2.0	8.24	-	43.5	-	-	-	26.8	28.9
4.0	8.33	-	42.8	-	-	-	25.1	32.1
8.0	8.71	-	43.5	-	-	-	25.1	31.4
24	9.48	-	27.6	-	-	-	-	72.4
48	------	-	18.4	-	-	-	-	81.6

C = compounds; [M$^+$]=molecular ion; TR = retention time (min$_)$);
OP = Other products.

Since it was observed that concentration of **2a** decreased with reaction time a new experiment was performed using only SeO_2 0.25 mmol and different volumes of 30% H_2O_2 (0.4, 0.1, 0.05, and 0.02 mL). The reaction time was 2 h. Results are shown on Table 2.

Table 2: Allylic hydroxylation of *ent*-kaurenic acid with 0.25 mmol SeO_2 and different concentrations of 30% H_2O_2. Reaction time 2 h.

H_2O_2	**1b**	**2b**	**3b**	**4b**	**9**	**6b**	**Other**
(mL)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
0.2	0	43.5	‘	-	-	26.8	28.9
0.1	0	68.4	2.0	7.4	2.1	8.8	8.8
0.05	0	63.9	5.2	10.5	2.6	6.5	6.5
0.02	3.8	45.8	11.2	15.0	5.8	-	-

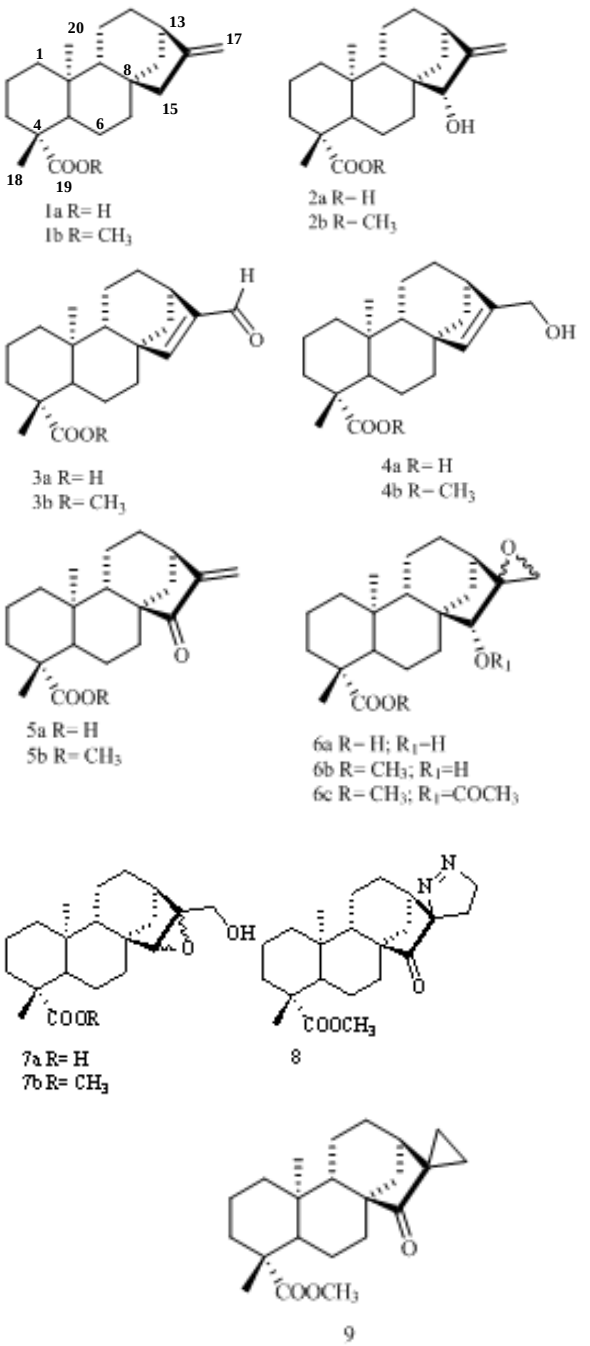

Fig. 1: Molecular formulas of *ent*-kaurenic acid and its allylic hydroxylation reaction products.

Lowering the amount of H_2O_2 to 0.1 mL made the reaction slower and increased the relative yield of **2a** (68.4%), but using only 0.05 mL of H_2O_2 yielded only 63.9% of **2a**, 0.02 mL of H_2O_2 produced even less **2a** (43.8%) but the yield of **3a**, **4a**. and **5a** increased at the expense of other products.

The reaction was also performed not using any H_2O_2 at all but adding one drop of H_2O instead. Results are presented on Table 3. Reaction time was varied from 1 h to 48 h

Table 3: Allylic hydroxylation of *ent*-kaurenic acid with 0.25 mmol SeO_2 and one drop of H_2O. Reaction time runs from 1h to 48 h.

Time (h)	1b (%)	2b (%)	3b (%)	4b (%)	5b (%)	Other (%)
0.5	32.1	37.9	1.7	2.5	3.6	22.2
1	11.5	40.5	2.4	3.0	3.6	39.0
2	3.1	49.8	3.6	2.6	2.8	38.1
4	2.1	73.9	3.5	2.5	3.8	14.2
8	1.4	64.4	4.9	2.5	3.7	23.1
24	-	74.0	8.3	-	4.2	13.5
48	-	68.9	5.6	-	3.8	21.7

As shown on Table 3, the absence of H_2O_2 makes allylic hydroxylation of (**1a**) even slower (thermodynamic control), on the other hand there was a progressive increase in the selectivity of the reaction increasing the yield of **2a**. Best results were obtained at 4 h. Longer time of reaction did not improve the yield of **2a**.

Finally, it was decided to perform the reaction using dichloromethane as solvent, as done by Hueso and colleagues[7], however, in this case an inert atmosphere was not used and the reaction was carried out during 24 h or 48 h, as shown on Table 4. Using CH_2Cl_2 the yield of **2a** was 70.4% and the yield of **3a** 17.8 % but no other major products were formed. Increasing the time of reaction to 48 h decreased the yield of **2a** to 53.1 % and increased the amount of **3a** to 43 %.The reaction was cleaner and since only two major compounds were produced separating them by flash chromatography was easier.

Table 4: Allylic hydroxylation of *ent*-kaurenic acid with 0.53 mmol SeO_2, 30 mL diclorometane solvent and one drop of H_2O. Reaction time runs from 24h and 48h.

Time (h)	1b (%)	2b (%)	3b (%)
24	-	70.4	17.8
48	-	53.1	43.0

Discussion

To obtain a good gas chromatogram of carboxylic acid derivatives on non polar columns it is necessary to inject them as their methyl esters. Treatment of *ent*-15-oxo-kaur-16-en-19-oic acid produces, on addition to methylation of the carboxylic acid moiety, reaction of the exocyclic double bond to yield a pyrazol ring as described by Oliveira, Hanson, and Takahashi.[9] This means that methylation of the reaction product of hydroxylation did not show on the TIC trace the methylated derivative (**5b**) (MW 330 g/mol) but the derivative **9** (344 g/mol), which is produced when the pyrazol derivative **8** is heated in the gas chromatographic column.

Table 5: ^{1}H-NMR chemical shifts of *ent*-15α-hydroxy-kaur-16-en-19-oic acid methyl ester (**2b**), *ent*-17-oxo-kaur-15-en-19-oic acid methyl ester (**3b**), *ent*-17-hydroxy-kaur-15-en-19-oic acid methyl ester (**4b**), *ent*-15-oxo-kaur-16-en-19-oic acid methyl ester (**5b**), *ent*-15α-acetoxy-16,17-epoxi-kauran-19-oic acid methyl ester (**6c**), *ent*-15,16-epoxi-17-hydroxy-kauran-19-oic acid methyl ester (**7b**), *ent*-15-oxo-16,17-pyrazolyl-kauran-19-oic acid methyl ester (**8**), *ent*-15-oxo-16,17,21-cyclopropyl-kauran-19-oic acid methyl ester (**9**).

H	2b	3b	4b	5b	6c	7b	8	9
H-1a	0.85 m	0.76 m	0.78dt; J=3;12Hz	0.83dt; J=3;9 Hz	0.86dt; J=4;14Hz	0.85m	0.81dt	0.85m
H-1b	1,80 m	1,88 m	1,83 m	1,87 m	1,87 m	1,84m	1,87m	1,84 m
H-2a	1,40 m	1,57 m	1,57m	1,68 m	1,72 m	1,57m	1,73m	1,70 m
H-2b	1,45 m	1,44 m	1,42 m	1,43 m	1,50 m	1,57m	1,47 m	1,48m
H-3a	2,14 m	2,24 d; J=3 Hz	2,13 d; J=12Hz	2,18d; J=13,4Hz	2,18d; J=12 Hz	2,15d; J=23Hz	2,16	2,16d; J=12z
H-3b	1,02 m	1,03 m	1,00 t; J=8Hz	1,05 dd; J=7;15Hz	1,04 m	1,00 m	1,03 m	1,02 m
H-5	1,00 m	1,05 m	1,03 m	1,02 m	1,06 m	1,04d; J=2;12Hz	1,08 m	1,13 m
H-6a	1,44 m	1,70 m	1,63 m	1, 95 m	1,99 m	1,86m	na	1,75 m
H-6b	1,80 m	1,87 m	1,81 m	1,87 m	1,94 m	1,80m	1,90 m	1,92 m
H-7a	1,78 m	1,73 m	1,52 m	1,47 m	1,62 m	1,55m	1,50 m	1,35 m
H-7b	1,32 m	2,20 m	1,57 m	1,85 m	1,80 m	1,10m	1,82 m	1,78 m
H-9	1,06 m	1,06 m	0,96 m	1,15 m	1,22 m	1,15m	1,20 m	1,09 m
H-11a	1,44 m	1,45 m	1,42 m	1,50 m	1,47 m	1,53m	1,44 m	1,43 m
H-11b	1,65 m	1,63 m	1,50 m	1,72 m	1,52 m	1,57m	1,70 m	1,55 m
H-12a	1,64 m	1,50 m	1,43 m	1,69 m	1,65d; J=16 Hz	1,60m	1,60 m	1,65 m
H-12b	1,70 m	1,55 m	1,48 m	1,85 m	1,86 m	1,52m	1,50 m	152 m
H-13	2,78 sa	3,02 bs	2,53 bs	3,05 sa	1,82 sa	2,28sa	2,38sa	2,43 d; J=12Hz
H-14a	2,18 d; J=12Hz	2,18 d; J=12Hz	2,06 d; J=12Hz	2,40 d; J=12 Hz	2,61 sa	1,70m	2,57 m	1,84 m
H-14b	1,52 m	1,50 m	1,39 m	1,42 m	2,61 sa	1,44m	na	na
H-15	3,80 s	6,57s	5,35 s	-----	4,70m	2,94s	----	----
H-17a	5,09 s	9,71 s	4,17 s	5,25 s	3,03 d	4,03d; J=13Hz	1,40 sa	0,82 m
H-17b	5,24 s	---	4,17 s	5,94 s	2,76 d	3,78d; J=13Hz	1,43m	1,20 m
H-18	1,22 s	1,18 s	1,15 s	1,26 s	1,27 s	1,19 s	1,21 s	1,17 s
H-20	0,98 s	0,89 s	0,83 s	1,00 s	0,86 s	0,82 s	0,84 s	0,84 s
H-21a	----	-----	-----	-----	-----	-----	4,60 s	0,87m
H-22	----	-----	-----	-----	2,07 s	-----	------	------
OCH_3	3,70 s	3,63 s	3,63 s	3,65 s	3,64 s	3,63 s	3,64s	3,64 s1

Table 6: ^{13}C-NMR chemical shifts of *ent*-15α-hydroxy-kaur-16-en-19-oic acid methyl ester (**2b**), *ent*-17-oxo-kaur-15-en-19-oic acid methyl ester (**3b**), *ent*-17-hydroxy-kaur-15-en-19-oic acid methyl ester (**4b**), *ent*-15-oxo-kaur-16-en-19-oic acid methyl ester (**5b**), *ent*-15α-acetoxy-16,17-epoxi-kauran-19-oic acid methyl ester (**6c**), *ent*-15,16-epoxi-17-hydroxy-kauran-19-oic acid methyl ester (**7b**), *ent*-15-oxo-16,17-pyrazolyl-kauran-19-oic acid methyl ester (**8**), *ent*-15-oxo-16,17,21-cyclopropyl-kauran-19-oic acid methyl ester (**9**).

Carbon	2b	3b	4b	5b	6c	7b	8	9
C-1	40.8	40.3	40.1	40.1	40.9	40.9	40.1	40.4
C-2	19.2	19.4	19.0	19.5	19.0	19.0	19.5	19,3
C-3	37.9	38.3	38.4	37.8	38.0	38.1	38.2	38.3
C-4	43.7	44.2	44.2	43.9	44.0	44.0	44.4	44.1
C-5	57.1	56.9	57.1	56.3	57.1	56.8	56.5	56.5
C-6	21.0	20.7	21.1	20.5	21.1	20.8	20.6	20.6
C-7	35.4	38.5	39.6	33.9	29.2	35.6	34.7	34,3
C-8	47.8	51.3	49.3	52.6	44.0	43.3	53.1	53.8
C-9	53.4	46.2	47.8	51.8	53.2	49.4	52.1	51.5
C-10	39.9	40.2	40.0	40.5	40.2	39.4	40.4	37.4
C-11	18.4	19.0	19.2	18.5	20.1	18.3	19.1	19,3
C-12	32.7	25.4	25.8	32.4	35.3	26.6	27.4	28.7
C-13	42.4	38.2	41.4	38.3	41.5	35.7	39.7	37.4
C-14	36.3	43.3	44.2	36.8	36.6	37.0	36.1	40.4
C-15	82.8	161.9	135.8	210.8	82.6	65.3	217.2	210.2
C-16	160.3	149.0	146.5	149.7	66.6	65.4	105.8	33.9
C-17	108.4	189.8	61.7	114.6	50.0	59.1	22.4	20,0
C-18	29.1	29.1	29.1	29.1	29.3	28.8	29.0	29.0
C-19	178.4	178.1	178.4	178.3	178.3	178.0	178.1	178.2
C-20	15.9	15.7	15.6	15.7	20.1	15.3	16.1	15.7
C-21	----	---	---	----	171,5	---	78.2	12.3
C-22	----	---	---	----	21.1	---	---	---
OCH_3	51.1	51.5	51.5	51.4	51.4	51.3	51.5	51.5

Diazomethane is widely used to form methyl esters, however, there are some authors that report that in case of compounds containing conjugated double bonds (electron deficient alkenes) these compounds addition the diazo moiety in a similar fashion to the Diels-Alder reaction to yield a pyrazol ring[10]. The compound observed in the TIC trace with MW 344 is formed in the chromatographic column when it is heated leading to the formation of compound **9** which contains a cyclopropyl ring upon release of N_2, as shown on Figure 2. Compound **9** has not been previously reported in the literature. To obtain **5b** the methyl ester of *ent*-15-oxo-kaur-16-en-19-oic acid (**5a**) it is necessary to treat *ent*-15α-hydroxy-kaur-16-en-19-oic acid methyl ester (**2b**) with CrO_3-pyridine complex to obtain de desired 15-oxo derivative without affecting the Δ16 exocyclic double bond.

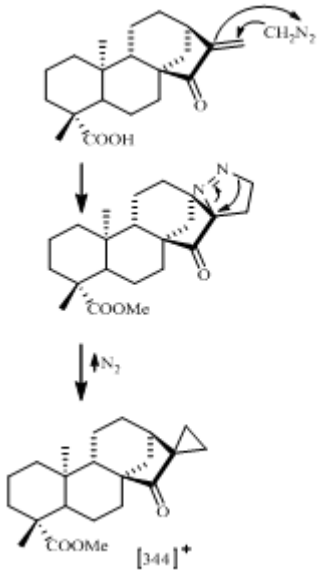

Fig. 2: Reaction of *ent*-15-oxo-kaur-16-en-19-oic acid (**5a)**with CH_2N_2 to yield *ent*-15-oxo-16,17-pyrazolyl-kauran-19-oic acid methyl ester (**8**) and its loss of N_2 upon heating to yield **9**.

Formation of compound (**2a**), the main product of this reaction, could be explained by a type N electrophylic attack of O=Se=O to the exocyclic double bond causing the formation of a Δ15 double bond. This process propitiates a [2,3] sigmatropic movement that re-establishes the original exocyclic double bond producing the formation of a bond between C-15 and the oxygen atom of the O=Se-OH moeity. As depicted in Figure 3 hydrolysis of a silanol molecule generates the formation of the C-15 allylic alcohol.

The possible mechanism of formation of compound (**4a**), could be explained as a consequence of displacement of the exocyclic double bond to carbons 15 and 16. In such event the C-17 methyl would be allylic to the double bond would become an epoxide, as proposed by Aparicio *et al*.[5]

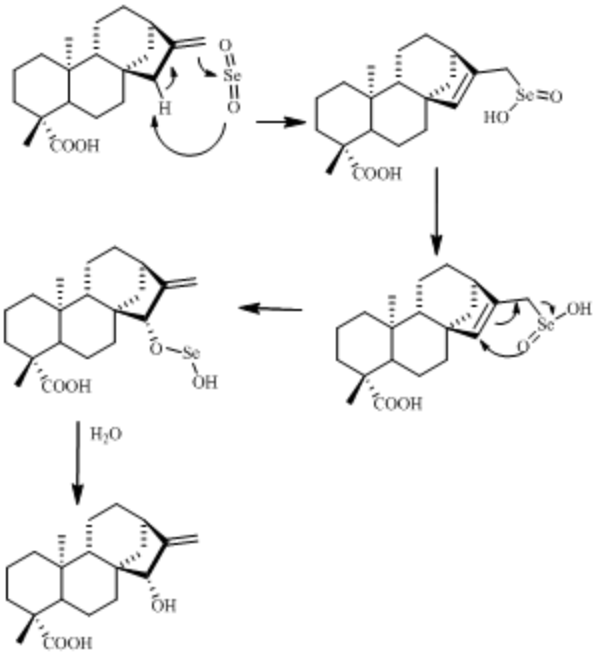

Fig. 3: Proposed mechanism of reaction of *ent*-kaurenic acid with SeO_2 to yield *ent*-15α-hydroxy-kaur-16-en-19-oic acid (**2a**).

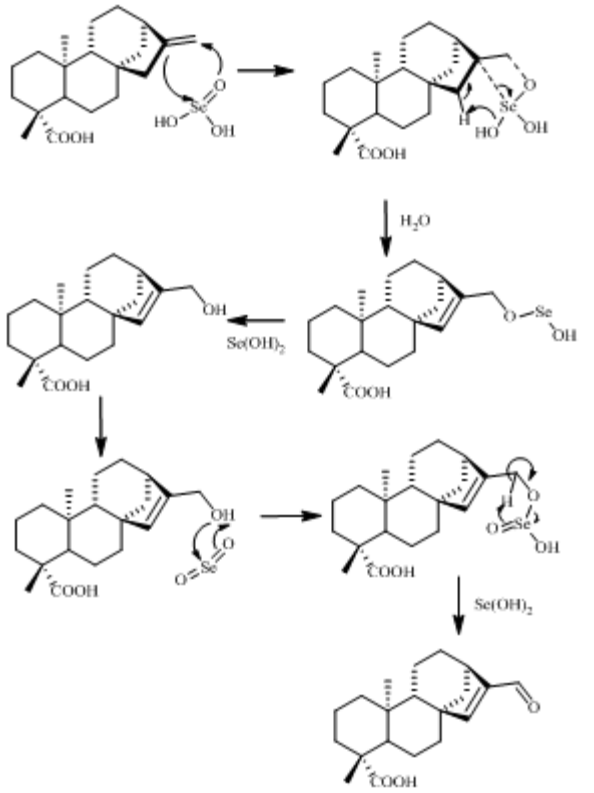

Fig. 4: Proposed mechanism of reaction of *ent*-kaurenic acid with SeO_2 to yield *ent*-17-hydroxy-kaur-15-en-19-oic acid (**4a**), and *ent*-17-oxo-kaur-15-en-19-oic acid (**3a**)

As shown in Figure 4, attack to the C-17 hydroxyl group by another SeO_2 molecule would yield the *ent*-17-oxo-kaur-15,16-en-19-oic acid (**3a**).

Finally, as shown on Figure 5, attack to the C-15 hydroxyl of compound (**2a**) by another SeO_2 molecule would yield compound (**5a**).

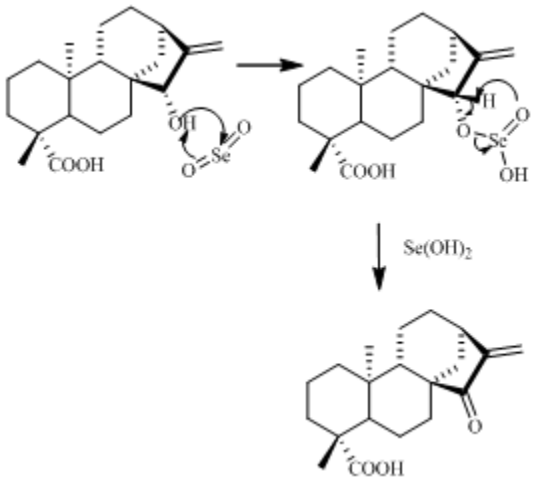

Fig. 5: Proposed mechanism of reaction of *ent*-kaurenic acid with SeO_2 to yield *ent*-15-oxo-kaur-16-en-19-oic acid (**5a)** from *ent*-15α-hydroxy-kaur-16-en-19-oic acid (**2a**).

Conclusions

This study indicates that allylic oxidation of *ent*-kaurenic acid (**1a**) with selenium dioxide gives a better yield of *ent*-15α-hydroxy-kaur-16-en-19-oic acid (**2a**) if the reaction is performed without H_2O_2 and using CH_2Cl_2 as solvent instead of dioxane. As a byproduct *ent*-17-oxo-kaur-15,16-en-19-oic acid (**3a**), a derivative of *iso-ent*-kaurenic acid is obtained in good yield (43%) after 48 h of reaction. Probably the yield of this substance will increase with longer reaction time, opening a path to obtain a family of *iso-ent*-kaurenic acid derivatives. Only about 10% of other products are formed which makes purification easier.

It was found that reaction in dioxane solution without addition of H_2O_2 gives a good yield of **2a**, 74% after 24 h. On the other hand addition of H_2O_2 leads to the formation of epoxide derivatives **6a** and **7a**. It would be interesting to find out if addition of H_2O_2 has the same effect in CH_2Cl_2.

Finally properties of *ent*-15-oxo-16,17-pyrazolyl-kauran-19-oic acid (**8**) and *ent*-15-oxo-16,17,21-cyclopropyl-kauran-19-oic acid (**9**) are reported.

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