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Artículo Científico

Structure of *ent*-15α-hydroxy-kaur-16-en-19-oic acid

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Resumen:

El ácido *ent*-15α-hidroxi-kaur-16-en-19-oico, $C_{20}H_{30}O_3$, aislado de las hojas de *Coespeletia moritziana* se reporta en este trabajo. Este compuesto cristaliza en el sistema monoclínico de grupo espacial C2 y parámetros de celda ***a***=19.7509 (5), ***b***=10.5126 (3), ***c***=8.8020 (3) Å, β=93.722(2)° y V= 1823.73(9) Å^3. La estructura consiste en tres anillos de seis miembros etiquetados como A, B y C y un anillo de cinco miembros etiquetado como D. Adicionalmente, este compuesto muestra 7 átomos quirales cuya configuración, obtenida por dispersión anómala, es R-C4, S-C5, R-C8, S-C9, S-C10, R-C13 y R-C15.

Palabras clave: productos naturales; diterpenos *ent*-kaurenos; estructura cristalina.

Abstract

Ent-15α-hydroxy-kaur-16-en-19-oic acid, $C_{20}H_{30}O_3$, isolated from the leaves of *Coespeletia moritziana*, is reported in this work. This compound crystallizes in the monoclinic system, space group C2 and unit cell parameters ***a***=19.7509 (5), ***b***=10.5126 (3), ***c***=8.8020 (3) Å, β=93.722(2)° and V= 1823.73(9) Å^3. The structure consists of three six-membered rings, labeled A, B, and C and a five-membered ring, called ring D. Additionally, this compound shows 7 chiral atoms whose configuration, obtained by anomalous dispersion, is represented by R-C4, S-C5, R-C8, S-C9, S-C10, R-C13 and R-C15.

Keywords: natural products; *ent*-kaurene diterpenes; crystal structure.

Introducción

Espeletiinae (Asteraceae) are resinous plants, popularly known as *frailejón*, that grow in cold regions of the tropics, at the high Andean páramos of Venezuela, Colombia and Ecuador between 2500 and 4500 m above sea level[1]. *Coespeletia moritziana* is one of 75 species of Espeletiinae endemic to the Venezuela Andes. This plant, which grows above 4000 m, contains e*nt*-15α-hydroxy-kaur-16-en-19-oic acid, this compound is also known as grandiflorolic acid which was isolated for the first time from *Espeletia grandiflora*, a Colombian Espeletiinae[2]. It has also been isolated from *Espeletia schultzii*[3] and *Coespeletia timotensis*[4]. *Ent*-kaurene diterpenes are known to have interesting biological properties[5], some of these compounds are poly-hydroxylated and most of them have been found to be cytotoxic against several cancer cell lines. Nagashima *et al.*[6], have studied the biological properties of ent-11α-hydroxykaur-16-en-15-one in a human leukemia cell line and found evidence that this compound was able to induce apoptosis. More recently, Rundle *et al.*[7], described the ability of EOKA (*ent*-15-oxo-kaur-16-en-19-oic acid) to irreversibly prolong the mitotic arrest on human epithelial tumoral cell lines, a characteristic effect that sometimes precedes apoptosis[8,9]. Recently it has been reported that *ent*-15-oxo-kaur-16-en-19-oic acid, obtained by chromic acid oxidation of **I** has a proapoptotic effect on the human prostate carcinoma

epithelial cell line PC-3[10]. Although many of this type of compounds have been reported to have the capacity to induce apoptosis in different cell lines, their molecular targets differed significantly[11-13]. We report here the structure of 15α-hydroxy-(-)-kaur-16-en-19-oic acid, isolated from *Coespeletia moritziana* (Sch. Bip. Ex Wedd) Cuatr. a Venezuelan species[1].

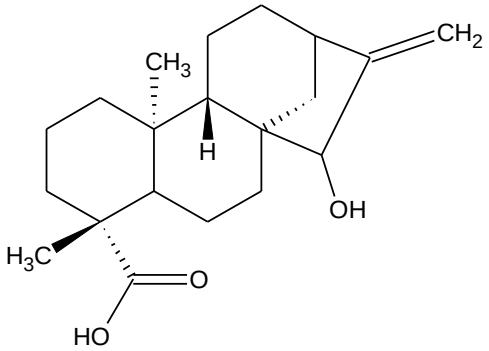

Figure 1: Molecular diagram of *ent*-15α-hydroxy-kaur-16-en-19-oic acid (**I**)

Experimental

General experimental procedures

Melting points were determined on Fisher Johns melting point apparatus and are uncorrected. The IR spectrum was recorded on a Perkin Elmer FT-IR instrument model 1720X, as KBr pellet. NMR spectra were recorded with a Bruker Avance 400 MHz instrument in $CDCl_3$ solution. The *ent*-15α-hydroxy-(-)-kaur-16-en-19-oic acid was characterized by acquisition of ^{1}HNMR, ^{13}CNMR, ^{1}H-COSY, HMQC, HMBC, and NOESY experiments and their absolute structure was solved by direct methods and refined to R= 0.050. Analytical thin-layer chromatography was performed on E. Merck aluminum-backed silica gel foils (F254). Flash chromatography was performed on silica gel E. Merck 60, 63-200 µm, by gradient elution with hexane and hexane-EtOAc mixtures. The single crystal X-ray diffraction analysis was carried out with an R-axis Diffractometer using Cu-Kα radiation (λ=1.54178 Å), and a graphite monochromator.

Compound I, *ent*-15α-hydroxy-(-)-kaur-16-en-19-oic acid, was solved by direct methods and refined by least-squared techniques, using the program SHELX-02[14]. The non-hydrogen atoms were refined anisotropic, while the hydrogen atoms were placed in calculated positions and refined using a *riding model* with their thermal parameters equal to 1.2 Uiso of the non-hydrogen atom to which they are attached. Given the poor diffracting power of the material, the refinement was carried out in blocks in order to maintain a proper data:parameter ratio.

Plant material

Compound I was obtained from the leaves of *Coespeletia moritziana* (3 Kg) collected at Collado del Condor (or Pico El Aguila), Mérida State, Venezuela, at 4.200m above sea level. A voucher specimen (Nº21) is deposited in the Merf Herbarium (Herbarium of the Faculty of Pharmacy, Universidad de Los Andes).

Isolation of ent-15α-hidroxy-(-)-kaur-16-en-19-oic acid (I).

The leaves were dried, ground, and extracted with 6.0 L of C_6H_{12}- EtOAc (7:3) mixture at room temperature. The hexane-acetate extract was concentrated to half its volume and shaken with an aqueous 0.5 N NaOH solution. The aqueous layer was acidified by addition of diluted HCl and shaken with a C_6H_{12}-EtOAc 7:3 mixture to yield 35g of acid fraction which was submitted to open column chromatography over silica gel (300g).The column was eluted first with n-hexane yielding *ent*-kaur-9(11)16-dien-19-oic acid, elution was continued with hexane-EtOAc mixtures. Fractions eluted with hexane-EtOAc (2:1) rendered 142mg of *ent*-15α-hydroxy-kaur-16-en-19-oic acid, which was further purified by flash chromatography over silica gel using hexane and hexane-EtOAc mixtures as solvent to yield 104mg of **I**.

Results and discussion

Compound I was crystallized from MeOH, mp 221-224ºC; IR (ν_{max}, cm^{-1}), 3420-2720 (broad band, COOH), 1695 (C=O), 1618 (C=C), 896 (=CH_2); ^{1}H NMR (CD_3Cl, 400 MHz): 5.07 (1H, s, H-17a), 5.21 (1H, s, H-17b), 3.81 (1H, s, H-15), 2.74 (1H, br s, H-13), 2.17 (1H, d, J = 11; 9 Hz, H-14a), 2.14 (1H, m, H-3a), 1.87 (1H, m, H-1b), 1.76 (1H, m, H-6b), 1.77 (1H, m, H-7a), 1.60 1H, m, H-11b), 1.59 (1H, m, H- 12b), 1.42 (1H, m, H-2a), 1.90 (1H, m, H-6a), 1.60 (1H, m, H-2b), 1.36 (1H, m, H-14b), 1.37 (1H, m, H-7b), 1.25 (3H, s, H-18), 1.03 (1H, m, H-9), 1.02 (1H, m, H-3b), 0.96 (3H, s, H-20), 0.60 (1H, dt, J = 4, 14 Hz, H-1a), ^{13}C NMR ($CDCl_3$, ppm): 183.6 (C-19), 160.3 (C-16), 108.4 (C-17), 82.8 (C-15), 53.5 (C-9), 47.8 (C-8), 57.1 (C-5), 43.7 (C-4), 39.9 (C-10), 40.8 (C-1), 42.4 (C-13), 37.9 (C- 3), 36.3 (C-14), 35.4 (C-7), 32.7 (C-12), 29.1 (C18), 21.1 (C-6), 19.2 (C-2), 18.4 (C-11), 16.0 (C-20).

X-ray Crystal Structure Analysis of I.

The details of crystal data and refinement are given in Table 1. Figure 2 show the molecular structures with the atom numbering scheme. A search previous in the Cambribge Structural Database (CSD)[15] produced like result a compound (Refcode PTERKA) isomorph with the structure under study. The difference between compound PTERKA and compound under study is that the former is located hydroxyl substituent on carbon 9, whereas in the second there is any substituent.

Table 1: Crystal data and refinement for compound (I)

Crystal Data	
Formula	$C_{20}H_{30}O_3$
Formula Weight	318.44
Crystal System	Monoclinic
Space group	C2 (No.5)
a, b, c (Å)	19.7509(5)
	10.5126(3)
	8.8020(3)
α, β, γ (°)	90
	93.722(2)90
V(Å^3)	1823.73(9)
Z	4
D_{calc} (g/cm^3)	1.160
Mu(CuKα)	0.599
F(000)	696
Crystal Size(mm)	0.28 x 0.29 x 0.40
Data Collection	
Temperature (K)	292
Radiation (Å)	CuKα 1.54187
Theta Min-Max [Deg]	6.5, 63.7
Dataset	-22: 22; -8: 12; -10: 9
Tot., Uniq. Data, R(int)	5808, 2320, 0.039
Observed data [I >0.0sigma(I)]	2207
Refinement	
Nref, Npar	2320, 214
R, wR2, S	0.0358, 0.0990, 1.08
Max. And Av. Shift/Error	0.00,0.00
Flack x	0.1(3)
Min. and Max. Resd. Dens.[e/Å^3]	-0.12, 0.10

The molecular structure by compound I, presents three six-membered rings, labeled A, B, and C and a five-membered ring, called ring D.

Ring A exhibits a chair conformation with asymmetry parameters [ΔC2(2-3)min= 0.8(3), ΔC2(1-2)max= 1.3(3), ΔCS(2)min= 0.6(3), ΔCS(3)max= 1.3(3)]; Ring B and C exhibits a half chair conformation [$\Delta C_2(5\text{-}6)_{min}$= 5.8(2), $\Delta C_2(7\text{-}8)_{max}$= 16.8 (2), $\Delta C_S(6)_{min}$= 2.64(16), $\Delta C_S(7)_{max}$= 13.16(16)] for B ring [ΔC2(9-11)min= 6.9(3), ΔC2(8-9)max= 30.2(3), ΔCS(11)min= 7.7(2), ΔCS(8)max= 25.2(2)] for C ring . Ring D**,** which adopts an envelope conformation on C14 [ΔC2(8-14)min=16.5(2), ΔC2(13-16)max=74.6(2), ΔCS(14)min= 5.2(2), ΔCS(16)max= 56.8(2)].

The substitution pattern is *trans* for A/B rings and the substitution pattern is *cis* for B/C rings.

The analysis of the compound indicates that the methyl substituent on C10 (C20) is in axial position. On the other hand, C18 is in equatorial position on C4. Additionally, in C16, is a methylene substituent (C17) in equatorial position. This compound shows 7 chiral atoms whose configuration, obtained by anomalous dispersion, is represented by R-C4, S-C5, R-C8, S-C9, S-C10, R-C13 and R-C15[16].

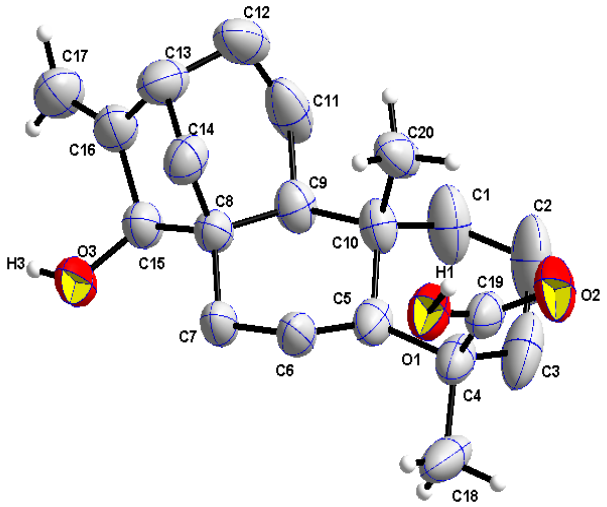

Figure 2: Molecular structure of ent-15αhydroxy-kaur-16-en-19-oic acid (I)[17].

Figure 3 show two intermolecular hydrogen bonds O_1-H_1...O_3 [1.84A, 176º] (blue bond) and O_3-H_3…O_2 [2.04A, 157º] (green bond). The O_1-H_1…O_3 hydrogen bond produces a infinite chains along *b* axes, and can be described by the graph set symbol $C^2_1(8)$. The intermolecular hydrogen bonds O_3-H_3...O_2 produce a helical chain along 101 direction. This bond can be described by the graph set symbol C(10).

The intramolecular hydrogen bonds C6-H6A…O1 can be described by the graph set symbol S(6), while the intramolecular hydrogen bond C18-H18B...O1 is described by the graph set symbol S(5).

The hydrogen bonds are produced by the interaction of hydroxyl group from carboxyl substituent with the hydroxyl substituent from other molecule, O1-H1...O3, and the interaction of the carbonil group from one molecule with the hydroxyl substituent from other molecule related by binary axes along *b* axes O3-H3...O2 forming a ring of twelve-membered ring which can be described by the higher-order graph set symbol $R^4_4(12)$. The hydrogen bonds described for this molecule are summarized in table 2.

Supporting Information Available:

X-ray crystallo-graphic data for this structure has been deposited at the Cambridge Crystallographic Data Center under code CCDC 820553.

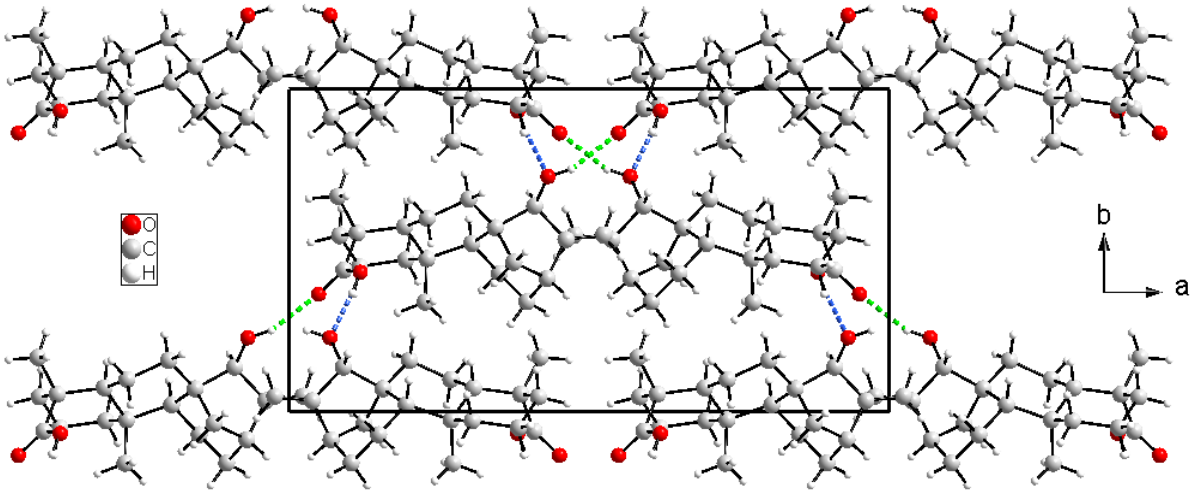

Figure 3: View of the hydrogen bonds pattern of the structure of ent-15α-hydroxy-kaur-16-en-19-oic acid (I) along the ***c*** axes. [17]

Table 2. Hydrogen bonds for compound I.

Bond	D-H	H-A	D-A	D-H-A	Symmetry	Graph	
O1- H1...O3	0.8200	1.8400	2.655(2)	176.00	3/2-x,-1/2+y,1-z	$C^2_1(8)$	$R^4_4(12)$
O3- H3...O2	0.8200	2.0400	2.812(2)	157.00	-1/2+x,1/2+y,z	C(10)	
C6- H6A... O1	0.9700	2.5200	2.938(3)	106.00		S(6)	
C18- H18B... O1	0.9600	2.5500	2.883(4)	100.00		S(5)	

Conclusions

In design of drug, natural products have been a major source of drug prototypes. One of the more ambitious goals of modern chemistry is to find the relationship between the molecular structure of organic and biological function that they serve. Best method to determine exactly the molecular structure of any product, whether a drug, a mineral, a protein or even a virus is the X-ray diffraction. Through this technique one can know the structure of any substance, depending on how they are organized atoms and molecules, which are the smallest units that make up a material. For this reason and considering that the several kaurene have demonstrated interesting pharmacological activities, we report the analysis of *ent*-15α-hydroxy-kaur-16-en-19-oic acid (I), which was isolated from the leaves of *Coespeletia moritziana*, was established by single crystal X-ray diffraction, and this is the first X-ray report of this compound.

Acknowledgements

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References

1. J Cuatrecasas. A new subtribe in the Heliantheae (Compositae):Espeletiinae. **Phytología, 35**, 43-61 (1976).
2. F Piozzi, V Sprio, S Passannanti, R Mondelli, R. Struttura dellacido grandiflorico. **Gazz. Chim. Ital**., 907-910 (1968).
3. C Brieskorn, E Pöhlmann. Diterpene vom kaurantyp aus der composite espeletia schultzii (wedd) **Tetrahedron Letters**, **9**, 5661-5664 (1968).
4. N Pérez-Rodriguez. Estudio de los componentes de la *Espeletia timotensis.* Doctoral thesis, Faculty of Pharmacy, University of Los Andes (1972).
5. E Ghisalberti, The biological activity of naturally occurring kaurane diterpenes. **Fitoterapia, 68,** 303-325 (1997).
6. F Nagashima, M Kondoh, M Kawase, S Simizu, H Osada, M Fujii, Y Watanabe, M Sato, Y Asakawa. Apoptosis-inducing properties of ent-Kaurene-type Diterpenoids from the Liverwort Jungermannia truncata. **Planta Med., 69**, 377-379 (2003).
7. N Rundle, J Nelson, M Flory, M; J Joseph, J Th'ng, R Aebersold, M Dasso, R Andersen, M Roberge. An ent-kaurene that inhibits mitotic chromosome movement and binds the kinetochore protein ran-binding protein 2. **ACS Chem. Biol., 1**, 443-450 (2006).

8. J Sasaki, R Ramesh, S Chada, Y Gomyo, J Roth, T Mukhopadhyay. The anthelmintic drug mebendazole induces mitotic arrest and apoptosis by depolymerizing tubulin in non-small cell lung cancer cells. **Mol Cancer Ther., 1**, 1201-1209 (2002).

9. Y Ling, J Jiang, J Holland, R Perez-Soler. Arsenic trioxide produces polymerization of microtubules and mitotic arrest before apoptosis in human tumor cell lines. **Mol Pharmacol., 62**, 529-538 (2002)..

10. Y Ruiz, J Rodrigues, F Arvelo, A Usubillaga, M Monsalve, N Diez, I Galindo-Castro, I. Cytotoxic and apoptosis-inducing effect of *ent*-15-oxo-kaur-16-en-19-oic acid, a derivative of grandiflorolic acid from *Espeletia schultzii***. Phytochemistry**, **69**, 2, 432-438 (2008).

11. A Morales, P Pérez, R Mendoza, R Compagnone, A Suárez, F Arvelo, J Ramirez, I Galindo-Castro. Cytotoxic and proapototic activity of ent-16β-17α-dihydrocaurane on Human Mammary Carcinoma Cell Line MCF-7. **Cancer Lett.**, **221,** 1, 109-116 (2005).

12. A Castrillo, B de Las Heras, S Hortelano, B Rodriguez, A Villar, L Bosca. Inhibition of the nuclear factor κB(NF-κB) pathway by tetracyclic kaurene diterpenes in macrophages. Specific affects on NF-κB-inducing kinase activity and on the coordinate activation of ERK and p38 MAPK. **J. Biol. Chem., 276**, 15854-15860 (2001).

13. J Lee, T Koo, B Hwang, J Lee. Kaurane Diterpene, Kamebakaurin, Inhibits NF-κB by Directly Targeting the DNA-binding Activity of p50 and Blocks the Expression of Antiapoptotic NF-κB Target Genes **J. Biol. Chem., 277**, 18411-18420 (2002).

14. G Sheldrick. A short history of SHELX. **Acta Cryst., A64**, 112-122 (2007).

15. F Allen. The Cambridge Structural Database: a quarter of a million crystal structures and rising. **Acta Cryst., B58**, 380-388 (2002).

16. H Flack, G Bernardinelli. Reporting and evaluating absolute-structure and absolute-configuration determinations. **J. Appl. Cryst., 33**, 1143-1148 (2000**)**.

17. K Brandenburg. DIAMOND. Release 2.1e. Crystal Impact GbR, Bonn, Germany, (2001).