C15 FUNCTIONALIZED DERIVATIVES OF *ent*-KAUR-16-EN-19-OIC ACID: ISOLATION FROM THE SUNFLOWER *HELIANTHUS ANNUUS* L. AND SYNTHESIS

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Abstract. The known diterpenic ester – 15α-angeloyl-*ent*-kaur-16-en-19-oic (angeloylgrandifloric) acid has been isolated from the dry wastes of *Helianthus annuus* L. The synthesis of 15α-hydroxy- and 15-oxo-*ent*-kaur-16-en-19-oic acids starting from *ent*-kaur-16-en-19-oic acid has been performed.

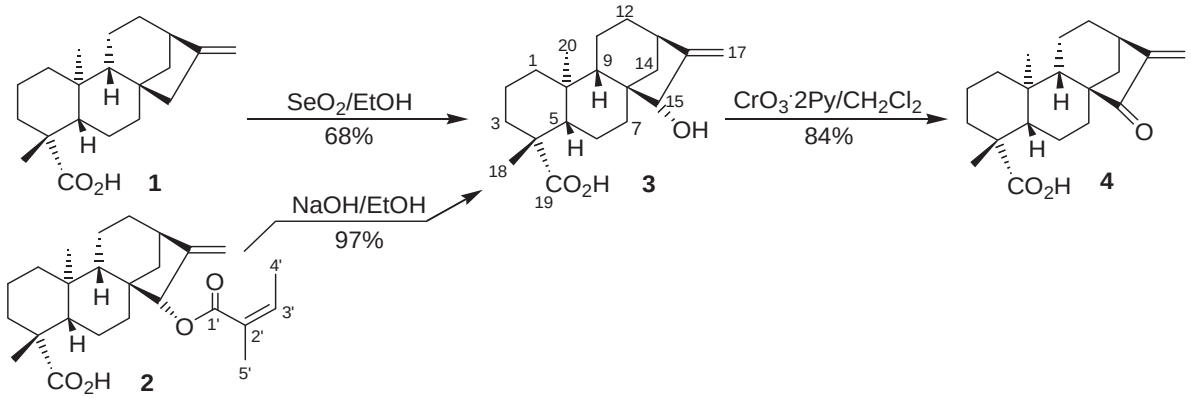
Keywords: diterpene, isolation, synthesis, 15α-angeloyl-*ent*-kaur-16-en-19-oic acid, 15α-hydroxy-*ent*-kaur-19-oic acid, 15-oxo-*ent*-kaur-16-en-19-oic acid.

1. Introduction

Kaurenic compounds possessing a broad spectrum of biological activities have been isolated from different natural sources [1, 2]. An encreasing interest towards this class of diterpenoids has been observed in the recent years [3, 4], due to potential applications, especially, in the field of pharmacological research. We have reported previously a new procedure for isolation of *ent*-kaur-16-en-19-oic acid (**1**) from the dry wastes of sunflower (*Helianthus annuus L.*) [5], that made the *ent*-kaurenic acid (**1**) readily available from renewable local raw material.

2. Results and discussion

In line with our continuous interest in the synthesis of functionalized terpenes, we present in this paper our results on the synthesis of some derivatives of *ent*-kaur-16-en-19-oic acid (**1**) which possess additional functional groups at C15 position. It is known, that a high degree of functionalization represents a prerequisite of a relevant biological activity. Following this idea, a similar C15 functionalized analog has been isolated from the same source, and its structure was proved both on the basis of spectral data, as well as of chemical transformations.



Following a simple procedure of extraction and chromatographical separation, a minor crystaline product was isolated along with previously reported *ent*-kaur-16-en-19-oic acid (**1**). It was identified on the basis of its spectral data (IR, ^{1}H and ^{13}C NMR) as 15α-angeloyl-*ent*-kaur-16-en-19-oic (**2**). This compound was indentical in all respects to the previously published data [6, 7].

The 15α-angeloyl-*ent*-kaur-16-en-19-oic acid (**2**) was isolated previously from the South American *Wedelia grandiflora* [8], from leaves of *Helianthus debilis* [6] and *Helianthus sp*. [9], from *Viguiera dentata* (Cav) [10], from *Montanoa tomentosa* ssp [11] and recently from leaves of *Helianthus annuus L*. [7].

On the saponification of the ester (**2**) with an ethanolic solution of potassium hydroxide, the 15α-hydroxy-*ent*-kaur-16-en-19-oic acid (**3**) was obtained in a 97% yield. It was identified on comparison of its spectral data with those published [12-15].

It is noteworthy mentioning that the hydroxyacid (**3**) is also a natural compound isolated previously from different plant material (*Helianthus niveus* subspecies *Canescens* [12], *Viguiera ladibractate* [16], *Viguiera potosina* [17], *Espeletia timotensis* [14]).

Due to the availability of the *ent*-kaur-16-en-19-oic acid (**1**), we have used it as starting material for the synthesis of other C-15 functionalized *ent*-kauranic derivatives. Accordingly, the oxidation of (**1**) with selenium dioxide in ethanol the corresponding allylic alcohol (**3**) was obtained in a good yield (68%). It was identical in all respects with the authentic sample of 15α-hydroxy-*ent*-kaur-16-en-19-oic acid (**3**), obtained from acid (**2**) as described above.

Following oxidation of the hydroxyacid (**3**) with the Collins reagent [18] in dichloromethane led to the formation of the 15-oxo-*ent*-kaur-16-en-19-oic acid (**4**) with a 84% yield. The obtained product was identified on the basis of its spectral data and comparison with the literature data [15, 19, 20]. We should mention, that the ketone (**4**) is a natural compound isolated from *Pteris longipes Don* [19] and *Espeletia grandiflora* and posess a relevant cytotoxic activity [21]. It was also shown to have a proapoptotic effect on the human prostate carcinoma epithelial cell line PC-3 [21].

3. Conclusion

The 15α-angeloyl-*ent*-kaur-16-en-19-oic acid (**2**) was isolated from the dry wastes of sunflower. A synthetic scheme for the synthesis of two C15-functionalized derivatives of *ent*-kaurenoic acid was elaborated, basing on readily available *ent*-kaur-16-en-19-oic acid (**1**).

4. Experimental

IR spectra were recorded on a Bio-Rad FTS 7 spectrophotometer.^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on Bruker 400 AVANCE III (400 MHz) spectrometer; chemical shifts are given in ppm and are referenced to chloroform ($CHCl_3$) as internal standard (δ = 7.26 ppm for proton and δ = 77.0 ppm for carbon). Optical rotations were measured in chloroform on a Jasco P 2000 polarimeter, using a 10 cm cell. Commercial Merck Si gel 60 (70–230 mesh ASTM) was used for flash chromatography, and Merck pre-coated SiO_2 gel plates were used for TLC. The chromatograms were sprayed with 0.1% solution of cerium (IV) sulfate in 2*N* sulfuric acid, and heated at 80 °C for 5 min to detect the spots. Treatment of reaction mixtures in organic solvents included the extraction by diethyl ether, washing of the extract with water up to neutral reaction, drying over anhydrous Na_2SO_4, filtering, and solvent removal in vacuum.

Obtaining of the extract from dry wastes of sunflower. Dry wastes of sunflower (500 g) have been preliminarily crumbled up and extracted in the soxhlet by diethyl ether during 2 hours. After evaporation of the solvent, the 65 g of extract was obtained in the form of a hard brown mass which, when heated at ~50°C, melts. The obtained extract is dissolved readily in diethyl ether, benzene, chloroform, but poorly in petrol ether. A part of the extract (7.2 g) has been dissolved in diethyl ether and treated with aqueous solution of alkali KOH (5% solution, 40 mL). The aqueous phase has been separated, acidified with 10% solution of H_2SO_4 (20 mL), extracted with ether, washed with brine up to the neutral reaction and concentrated in the vacuum, giving 6.3 g of yellow oil. A portion of the extract (4.1 g) has been separated on column with silica gel (120 g) by gradient elution with the mixture of petroleum ether and ethylacetate, affording 2.8 g of the mixture of *ent*-trachiloban-19-oic, *ent*-kaur-16-en-19-oic (**1**) and 15α-angeloyl-*ent*-kaur-16-en-19-oic (angeloylgrandifloric) (**2**) acids.

Chromatographic isolation of 15α-angeloyl-*ent*-kaur-16-en-19-oic (angeloylgrandifloric) acid (2). The mixture of acids (460 mg) has been separated on the column with $SiO_2/AgNO_3$ (25 g). Elution with ethylacetate - petroleum ether mixture (1:49) resulted in obtaining of 52 mg (11%) mixture of *ent*-trachyloban-19-oic and *ent*-kaur-16-en-19-oic acid (**1**), 262 mg (57%) of *ent*-kaur-16-en-19-oic acid (**1**) [5] and 123 mg (27%) 15α-angeloyl-*ent*-kaur-16-en-19-oic (angeloylgrandifloric) acid (**2**): colorless crystals, mp 195–197°C (from EtOAc). IR liquid film, (ν, cm^{-1}): 3206-2450, 1705, 1255, 1040, 1010, 890. ^{1}H NMR (400 MHz, δ_H): 0.95 (s, 3H), 1.25 (s, 3H), 1.88 (4, 3H, *J* = 1.0 Hz), 1.96 (dq, 1H, *J* = 8, 1.0 Hz), 2.78 (m, 1H), 5.42 (m, 1H), 5.09 (bs, 1H), 5.20 (bs, 1H) and 6.08 (qq, 1H, *J* = 8, 1.0 Hz). ^{13}C NMR (100 MHz, δ_C): 40.4 (t, C-1), 18.9 (t, C-2), 35.3 (t, C-3), 43.7 (s, C-4), 56.5 (d, C-5), 20.6 (t, C-6), 37.3 (t, C-7), 47.8 (s, C-8), 53.1 (d, C-9), 39.7 (s, C-10), 20.7 (t, C-11), 32.5 (t, C-12), 42.7 (d, C-13), 37.9 (t, C-14), 82.5 (d, C-15), 155.8 (s, C-16), 110.3 (t, C-17), 28.7 (q, C-18), 185.2 (s, C-19), 15.9 (q, C-20), 168.3 (s, C-1'), 128.6 (s, C-2'), 137.4 (d, C-3'), 15.9 (q, C-4'), 18.6 (q, C-5'). Compound (**2**) showed spectral data (IR, ^{1}H and ^{13}C NMR) identical with those of described [6, 7].

15α-Hydroxy-*ent*-kaur-19-oic acid (3). a) 15α-Angeloyl-*ent*-kaur-16-en-19-oic (**2**) (115 mg, 0.287 mmol) was dissolved in 2.0 mL of EtOH and 1.4 mL of 10% KOH/EtOH solution were added. The reaction mixture was refluxed for 2 h. After the usual workup, 98 mg of a crude residue was obtained which was purified on a silica gel column (2 g) (petroleum ether–ethylacetate, 4:1) to give 89.2 mg (97%) of 15α-hydroxy-*ent*-kaur-19-oic acid (**3**), a white solid which was crystallized from MeOH yielding 48.2 mg of colorless needles, mp 219-221 °C, $[\alpha]_D^{25}$ -103° (c, 0.50, $CHCl_3$). IR liquid film, (ν, cm^{-1}): 3415-2725, 1690, 1620, 895. ^{1}H NMR (400 MHz, δ_H): 0.96 (3H, s, H_3-20), 1.25 (3H, s, H_3-18), 2.75 (1H, bd. s, H-13), 3.80 (1H, bd. s, H-15), 5.08 (1H, s, H_A-17), 5.22 (1H, s, H_B-17). ^{13}C NMR (100 MHz, δ_C): 40.6 (t, C-1), 19.1 (t, C-2), 37.8 (t, C-3), 43.5 (s, C-4), 57.3 (d, C-5), 21.0 (t, C-6), 35.5 (t, C-7), 47.7 (s, C-8), 53.4 (d, C-9), 40.1 (s, C-10), 18.4 (t, C-11), 32.8 (t, C-12), 42.3 (d, C-13), 36.2 (t, C-14), 82.7 (d, C-15), 160.4 (s, C-16), 108.3 (t, C-17), 29.1 (q, C-18), 183.7 (s, C-19), 15.9 (q, C-20). Compound (**3**) showed spectral data (IR, ^{1}H and ^{13}C NMR) identical with those of described [6, 12, 14, 15].

b) To a solution of selenium dioxide 23 mg (0.207 mmol) in ethanol (1.5 mL), was added the solution of *ent*-kaur-

16-en-19-oic acid (**1**), (125 mg, 0.414 mmol) in ethanol (1.5 mL) and the mixture was refluxed for 4 h. After the mixture was cooled to 0°C and added $NaBH_4$ (7.8 mg, 0.207 mmol) and the mixture were stirred for 0.5 h at r.t. After the usual workup, 92 mg of a crude residue was obtained and purified on a silica gel (2 g) column (petroleum ether–ethylacetate, 4:1) to give 89.2 mg (68%) of 15α-hydroxy-*ent*-kaur-16-en-19-oic acid (**3**), identical with authentic sample.

15-Oxo-*ent*-kaur-16-en-19-oic acid (**4**). Complex $CrO_3 2C_5H_5N$ (85 mg, 0.33 mmol) was added to a solution of alcohol (**3**) (35 mg, 0.11 mmol) in dry CH_2Cl_2 (4 mL). The reaction mixture was stirred at room temperature for 24 h, and then filtered on silica gel to give 31.5 mg of a crude product, which was chromatographed on a silica gel (0.5 g) column (petroleum ether-EtOAc, 17:3) to give 29.2 mg (84%) of a crystalline 15-oxo-*ent*-kaur-16-en-19-oic acid (**4**): mp 196-198°C (from EtOAc), $[\alpha]_D$ -176° (c 0.15, $CHCl_3$). IR liquid film, (ν, cm^{-1}): 1718, 1680, 1637, 896. ^{1}H NMR (400 MHz, δ_H): 0.97 (3H, s, H_3-20), 1.25 (3H, s, H_3-18), 2.93 (1H, bd. s, H-13), 5.22 (1H, s, H_A-17), 5.88 (1H, s, H_B-17). ^{13}C NMR (100 MHz, δ_C): 39.8 (t, C-1), 18.9 (t, C-2), 37.9 (t, C-3), 43.5 (s, C-4), 57.0 (d, C-5), 20.9 (t, C-6), 32.4 (t, C-7), 52.3 (s, C-8), 51.4 (d, C-9), 40.3 (s, C-10), 18.5 (t, C-11), 33.6 (t, C-12), 38.4 (d, C-13), 36.7 (t, C-14), 210.8 (s, C-15), 149.8 (s, C-16), 114.7 (t, C-17), 29.1 (q, C-18), 192.5 (s, C-19), 15.7 (q, C-20). Compound (**4**) showed spectral data (IR, ^{1}H and ^{13}C NMR) identical with those of described [15, 18, 20].

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6. References

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