ISOLATION OF *ENT*-KAUR-16-EN-19-OIC AND *ENT*-TRACHILOBAN-19-OIC ACIDS FROM THE SUNFLOWER *HELIANTHUS ANNUUS L.* DRY WASTE

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Abstract. A relatively simple method for isolation of the mixture of *ent*-kaur-16-en-19-oic (**1**) and *ent*-trachiloban-19-oic (**2**) acids from dry waste of sunflower processing has been elaborated, and it has been shown that the waste can serve as an accessible source of *ent*-kauranic and *ent*-trachilobanic diterpenoids.

Keywords: *ent*-kaur-16-en-19-oic acid, *ent*-trachiloban-19-oic acid, diterpene, isolation.

Introduction

Many representatives of tetracyclic ent-kauranic and pentacyclic *ent*-trachilobanic diterpenoids display a diverse biological activity [1, 2]. *ent*-Kauranic compounds are widely spread in plants, being biogenetical precursors of gibberillin plants growth hormones gibberillin [3]. Pentacyclic *ent*-trachylobanic compounds occur in natural sources more seldom. However, they are also of interest since they can serve as starting compounds for biomimetic synthesis of a number of tetracyclic diterpenoids [4]. Investigations of biologically active principls of medicinal plants, especially those used in non-traditional Chinese medicine, have shown that a large spectrum of biological activities, including the anti-microbial, anti-inflammatory, the cardio-vascular, diuretic, cytotoxic and ant-AIDS ones are conditioned by the presence in these plants of *ent*-kauranic and *ent*-trachilobanic diterpenoids [5].

Results and discussion

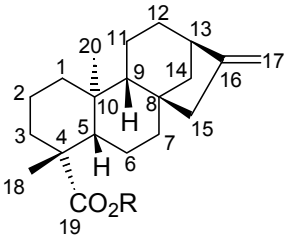
Kauranic and trachylobanic diterpenoids have a common biogenetic precursor [6]. However, according to available data, there are only two cases of common presence of kauranic and trachilobanic derivatives: in *Trahylobium verrucosum* [7] and *Helianthus annuus L*. (sunflower) [8]. *ent*-Kaur-16-en-19-oic (**1**) and *ent*-trachiloban-19-oic (**2**) acids have been isolated from sunflower inflorescence [8].

For finding accessible sources of *ent*-kauranic diterpenoids we carried out a literature search, its result being given in Table 1. It includes the name of plants that contain *ent*-kaurans, the kind of flora (wild, cultivated), the total content of *ent*-kaurans and the corresponding reference. From Table 1 data, one can see that the highest content of *ent*-kaurans in dry plants is to be found in sunflower. In the total extracts obtained from various parts of sunflower, ent-kaur-16-en-19-oic acid (**1**) is prevalent. In the Republic of Moldova, sunflower is one of the plants that is cultivated on industrial scale, thus making it attractive as accessible source of *ent*-kaur-16-en-19-oic acid (**1**).

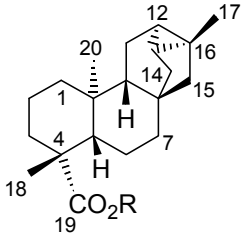
Table 1

The main sources of *ent*-kauran diterpenoids.

Plant	Natural habitat	Form habitat	Ent-kauran content (% from dry mass)	References
Euphorbia Wangii	China	Wild flora	0,003	[9]
Annona glabra	China, Taiwan	Wild flora	0,016	[10]
Annona squamosa	China, Taiwan	Wild flora	0,034	[11]
Aristolochia pubescens	Brazil	Wild flora	0,035	[12]
Annona senegalensis	Nigeria	Cultivated flora	0,074	[13]
Oyedaea verbesinoides	Central America	Wild flora	0,104	[14]
Paraschistochila pinnatifolia	New Zealand	Wild flora	0,120	[15]
Helianthus annuus	Worldwide	Cultivated flora	0,650	[8]



1 R = H
3 R = CH_3



2 R = H
4 R = CH_3

One of the most widely spread representatives of *ent*-kauranic diterpenoids is the *ent*-kaur-16-en-19-oic acid (**1**). First of all, it is of interest as a compound with a wide spectrum of biological activity. It displays activity against microbus *Bacillus sublitis*, [16, 17], *Staphylococcus aureus*, [17-19], *Micobacterium smegmatis* [17, 18], *Saccaromices cerevisiae* [17], *E. coli* and *Candida albicans* [19], as well as against very simple flagellates *Triponosoma cruzi* [20], which generate the Chagas disease. *ent*-Kaur-16-en-19-oic acid (**1**) also displays antifeedant activity against granaries vermin *Trilobium confusum Duv*., *Tragoderma granarium Ev*. and *Sitophilus granarius L*. [21]. Acid (**1**) has been tested on cytotoxicity [13, 22]. It displays a poor activity against leukemia, but a considerably selective activity against breast cancer cells [22], moderate inhibitory properties *in vitro* against lungs tumours, central nervous system's tumours, skin and vertebrae cancer [23].

This paper contains data concerning the elaboration of a convenient method for isolation of *ent*-kaur-16-en-19-oic (**1**) and *ent*-trachiloban-19-oicc (**2**) acids from dry waste of sunflower processing. The sunflower has proved to be a rich source of ent-kauranic compounds (Table 1).

Extraction of the vegetal raw material has been done by diethyl ether in a Soxhlet extractor. The solvent has been chosen by us with consideration of the fact that *ent*-kauranic and *ent*-trachilobanic diterpenoids are labile compounds that can be subjected to rearrangement of the carbon skeleton [24] during extraction at high temperature under the influence of accompanying substances of acidic character. The obtained extract has been treated with potassium hydroxide solutions of various concentrations. As a result of such treatment of the extract, the neutral compounds have been removed (control by TLC). According to experimental data, the best results for extraction of the acidic part have been realized using the 5% solution of KOH. Utilization of more concentrated alkali solutions (e.g. 10%) does not lead to the increase of output of the acidic part. When 3% and 5% of KOH solutions have been used, the obtained results practically do not differ. Utilization of sodium hydroxide solutions instead of potassium hydroxide has led to worse results. In this case, longer time has been necessary for separation of phases and, besides, some amount of neutral compounds got into the acid fraction.

The acidic part (1 g) has been subjected to separation on silica gel, in result of which the mixture of *ent*-kaur-16-en-19-oic (**1**) and *ent*-trachiloban-19-oic (**2**) acids has been obtained, their overall yield being 35%. Due to similar chromatographic properties, the mixture of acids **1** and **2** could not be resolved by flash chromatography on silica gel. The mixture of acids **1** and **2** has been separated by chromatography on columns with silica gel, impregnated with silver nitrate [25]. The ratio of *ent*-kaur-16-en-19-oic (**1**) and *ent*-trachiloban-19-oic (**2**) acids in the extract was ~7:2. Acids **1** and **2** have been identified by comparing their spectral and physico-chemical data and the data of their methyl esters (**3**) and (**4**), obtained by their methylation with diazomethane, with those available in the literature.

Conclusions

Thus, we have elaborated a simple method for isolating the mixture of *ent*-kaur-16-en-19-oic (**1**) and *ent*-trachiloban-19-oic (**2**) acids from dry waste of sunflower processing and their chromatographic separation. It has been shown that this waster can serve as an accessible source of *ent*-kaurenic and *ent*-trachilobanic diterpenoids. The novelty of this method consists in the direct isolation of the above mentioned diterpenic acids in their individual forms without having to methylate the acid mixture and chromatographic separation of their methyl esters as it has been done earlier [7,8].

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 WM 300 (300 MHz) and Bruker AC 80 (80 MHz) spectrometers; 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 precoated SiO_2 gel plates were used for TLC. The chromatograms were sprayed with 0.1% solution of cerium(iv) sulfate in 2N 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 (800 g) have been preliminarily crumbled up and extracted in the soxlet by diethyl ether during 2 hours. After evaporation of the solvent, the 85 g of extract was obtained in the form of a hard brown mass which, when heated at ~50°C, melts. The obtained extract dissolves readily in diethyl ether, benzene, chloroform, but badly in petrol ether. A part of the extract (9.3 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 7.5 g of yellow oil.

A portion of the extract (6.8 g) has been separated on column with silica gel (140 g) by gradient elution with the mixture of petrol ether and ethylacetate, affording the mixture of *ent*-kaur-16-en-19-oic (**1**) and *ent*-trachiloban-19-oic (**2**) acids (2.37 g, 35% yield).

Chromatographic separation of the mixture of ent-kaur-16-en-19-oic (1) and ent-trachiloban-19-oic (2) acids. The mixture of acids **1** and **2** (860 mg) has been separated on the column with $SiO_2/AgNO_3$ (38 g). Elution with 2% solution of ethylacetate in petrol ether resulted in obtaining of 192 mg of *ent*-trachiloban-19-oic acid (**2**) (22% yield) and 640 mg of *ent*-kaur-16-en-19-oic acid (**1**) (74% yield).

ent-*Kaur-16-en-19-oic acid (1)*, colourless crystals, m.p. 177-179°C (from hexane), $[\alpha]_D$ -105° (c 2.3; $CHCl_3$). IR liquid film, (ν, cm^{-1}): 2937, 1686, 1258, 874, 794, 635, 530. ^{1}H NMR (300 MHz, δ_H): 0.95 (3H, s, H_3-20), 1.00-1.16 (4H, m, $2CH_2$), 1.24 (3H, s, H_3-19), 1.46-2.18 (10H, m), 2.64 (1H, bd. s, H-13), 4.74 (1H, s, H_A-17), 4.80 (1H, s, H_B-17). ^{13}C NMR (75 MHz, δ_C): 15.6 (q, C-20), 18.5 (t, C-11), 19.1 (t, C-2), 21.9 (t, C-6), 29.0 (q, C-18), 33.2 (t, C-14), 37.8 (t, C-3), 39.7 (s, C-10), 39.7 (t, C-12), 40.7 (t, C-1), 41.3 (s, C-4), 41.3 (t, C-7), 43.8 (d, C-13), 44.3 (s, C-8), 49.0 (t, C-15), 55.1 (d, C-9), 57.1 (d, C-5), 103.4 (t, C-17), 155.9 (s, C-16), 184.8 (s, C-19). Literature data [26]: , m.p. 179-181°C, $[\alpha]_D$ -110° (c 3.0; $CHCl_3$).

ent-*Trachiloban-19-oic acid (2)*, colourless crystals, m.p. 124-126°C (from hexane), $[\alpha]_D$ -43° (c 1.5; $CHCl_3$). IR liquid film, (ν, cm^{-1}): 2846, 1691, 1443, 1261, 1178, 1022, 798, 630, 535. ^{1}H NMR (300 MHz, δ_H): 0.56 - 0.58 (2H, m, cyclopropane protons), 0.81-0.83 (2H, m), 0.87 (3H, s, 20-H3), 0.97-1.02 (3H, m), 1.13 (3H, s, 17-H_3), 1.21 (3H, s, 18-H_3), 1.23-2.14 (14H, m). ^{13}C NMR (75 MHz, δ_C): 12.5 (q, C-20), 18.7 (t, C-2), 19.8 (t, C-11), 20.6 (t, C-12), 20.7 (q, C-17), 21.8 (t, C-6), 22.4 (s, C-16) 24.3 (d, C-13), 28.9 (q, C-18), 33.2 (t, C-14), 37.9 (t, C-3), 38.9 (s, C-10), 39.3 (t, C-7), 39.4 (t, C-1), 40.8 (s, C-8), 43.7 (s, C-4), 50.4 (t, C-15), 52.8 (d, C-9), 57.0 (d, C-5), 184.5 (s, C-19). Literature data [27]: m.p. 125-127°C, $[\alpha]_D$ -52° (c 0.8, $CHCl_3$).

Methyl ester of ent-kaur-16-en-19-oic acid (3). *ent*-Kaur-16-en-19-oic acid (**1**) (70 mg) has been methylated by an excess of diethyl ether solution of diazomethane. The residue (71.6 mg), obtained after distillation of the solvent, was chromatographied on a column with silica gel (1.2 g). By the mixture of petrol ether and etylacetate (97:3), 68.2 mg (93% yield) of methyl ester of *ent*-kaur-16-en-19-oic acid (**3**) have been eluted, colourless crystals, m.p. 76-77.5°C (from CH_3OH), $[\alpha]_D$ -101° (c 1.8; $CHCl_3$). IR liquid film, (ν, cm^{-1}): 1720, 1670, 1240, 1225, 1190, 1145. ^{1}H NMR (80 MHz, δ_H): 0.84 (3H, s, H_3-20), 1.15 (3H, s, H_3-18), 2.62 (1H, bd.s, H-13), 3.62 (3H, s, CO_2Me), 4.72 (1H, s, H_A-17), 4.78 (1H, s, H_B-17). Literature data [8]: m.p. 73.5-74.5°C, $[\alpha]_D$ -104° ($CHCl_3$).

Methyl ester of ent-trachiloban-19-oic acid (4). *ent-Trachiloban-19-oic acid* (**2**) (40 mg) has been methylated by an excess of diethyl ether solution of diazomethane. After the solvent evaporation, the residue (39.8 mg), was chromatographied on the column with silica gel (0.7 g). By the mixture of petrol ether and etylacetate (97:3), (37.2 mg, 89% yield) of methyl ester of *ent*- trachiloban-19-oic acid (**4**) have been eluted, colourless crystals, m.p. 107-109°C (from CH_3OH), $[\alpha]_D$ -65.4° (c 1.2, $CHCl_3$). IR liquid film, (ν, cm^{-1}): 1720, 1261, 1230, 1200, 1162, 1150. ^{1}H NMR (80 MHz, δ_H): 0.55 - 0.60 (2H, m, cyclopropane protons), 0.85 (3H, s, 20-H_3), 1.08 (3H, s, 17-H_3), 1.13 (3H, s, 18-H_3), 3.60 (3H, s, CO_2Me). Literature data [7]: m.p. 110-112°C, $[\alpha]_D$ -41° ($CHCl_3$), [8]: m.p. 98-100°C, $[\alpha]_D$ -70.5° ($CHCl_3$).

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