

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/358361667>

Deodorized distillate of sunflower oil as a source of kaurane compounds

Conference Paper · January 2022

DOI: 10.1063/5.0070161

CITATIONS

0

READS

19

5 authors, including:



Dmitry Shcherbakov

State Research Center of Virology and Biotechnology VECTOR

96 PUBLICATIONS 189 CITATIONS

[SEE PROFILE](#)



Tatyana Kukina

Novosibirsk Institute of Organic Chemistry

52 PUBLICATIONS 199 CITATIONS

[SEE PROFILE](#)



Petr Kolosov

Altai State University

9 PUBLICATIONS 18 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Using phage display technology to search for peptides specifically interacting with immune checkpoints [View project](#)



extractive substances [View project](#)

Deodorized distillate of sunflower oil as a source of kaurane compounds

Cite as: AIP Conference Proceedings **2390**, 030084 (2022); <https://doi.org/10.1063/5.0070161>
Published Online: 04 February 2022

D. N. Shcherbakov, T. P. Kukina, I. A. Elshin, et al.



[View Online](#)



[Export Citation](#)



Author Services

English Language Editing

High-quality assistance from subject specialists

[LEARN MORE](#)



Deodorized Distillate of Sunflower Oil as a Source of Kaurane Compounds

D. N. Shcherbakov ^{1, 2, a)}, T. P. Kukina ^{3, b)}, I. A. Elshin ^{3, c)}, O. I. Salnikova ^{3, d)}, and P. V. Kolosov ^{1, e)}

¹Altai State University, Barnaul 656049, Russia

²State Research Center of Virology and Biotechnology Vector, Koltsovo 630559, Novosibirsk oblast, Russia

³Vorozhtsov Novosibirsk Institute of Organic Chemistry, Siberian Branch,
Russian Academy of Sciences, Novosibirsk 630090, Russia

^{a)}Corresponding author: dnshcherbakov@gmail.com

^{b)}kukina@nioch.nsc.ru

^{c)}kumatoid@nioch.nsc.ru

^{d)}olga@nioch.nsc.ru

^{e)}petro.kolosov@gmail.com

Abstract. The composition of a large-capacity byproduct of sunflower oil refining – deodorization distillate (DD) – was studied. Acidic and neutral components were identified by gas chromatography-mass spectrometry. The analysis showed the presence of five kauranoic acids and seven neutral kauranoids, which constituted up to 6% of the total weight of the DD samples studied.

INTRODUCTION

Ent-kaurane diterpenoids, a unique category of the diterpenoid family, have a long-standing history of research and medical applications in traditional Eastern medicine and have garnered increasing interest since the last century due to their structural diversity and complexity together with extensive bioactivity profiles. The anticancer pharmacological profile of this class of natural compounds is of particular interest. Mechanistic investigations have revealed that ent-kauranoids possess versatile anticancer activities as regulators of a series of transcription factors, protein kinases, as well as pro- and/or anti-apoptotic proteins, including but not limited to telomerase inhibition, p53 activation, and NF- κ B inhibition. Last but not least, the clinical development of ent-kauranoids is usually hampered by their relatively moderate potency, limited aqueous solubility, and bioavailability [1-3]. Despite the wide distribution of kaurane diterpenoids in various plants, dependable sources of them are practically absent. In most plant species, the content of kaurane compounds is within 3–30 mg/100 g of raw materials [4-7]. An exception is the glycoside stevioside from the Asteraceae plant's *Stevia rebaudiana* Bertonii [1] and *Espeletia nana* Cuatrec. [8], where kaurane concentrations are significantly higher. The steviol glycoside stevioside is 300 times sweeter than sucrose [1]. In addition, it stimulates the production of insulin; therefore, *Stevia rebaudiana* (Asteraceae) and the related species *S. phlebophylla* are widely introduced for food and pharmacological purposes [1]. Steviol, the aglycone of stevioside, and its isomer have been patented as medications for heart and cerebral ischemia, arrhythmia, and heart failure [9]. Some kauranes demonstrate different types of activity, including cytotoxicity and *in vitro* anti-inflammatory effects. Ent-kaur-16(17)-en-19-oic acid (kauranoic acid, KA) is a tetracyclic diterpene prototype for natural anticavity agents. Six KA derivatives were prepared, and their antimicrobial activity against the main microorganisms involved in the cavity process was evaluated. The sodium salt of KA (KA-Na) was the most active, displaying very promising MIC values for most pathogens [10]. Ent-kauranoic acid derivatives were obtained by microbial transformation methods and tested against breast cancer cells (MCF-7) [11]. Ent-kaurane-2 α ,16 α -diol and ent-kaurane-

15 α ,16 α -epoxy-17 α -19-oic acid from the flower disc of *Helianthus annuus* L. showed cytotoxic activities on SF-268, MCF-7, and HepG2 cell lines [12]. Siegeskaurolic acid from *Siegesbeckia pubescens* roots inhibited the nuclear factor- κ B (NF- κ B) activation induced by LPS, and this was associated with the prevention of inhibitor κ B degradation (I κ B), and subsequently with lower nuclear p65 and p50 protein levels. These data indicate that the anti-inflammatory and antinociceptive properties of siegeskaurolic acid may be due to iNOS, COX-2, and TNF- α inhibition via the downregulation of the NF- κ B binding activity [13]. Screening of 26 diterpenes from natural sources or of synthetic/microbial transformations origin (mainly derivatives of kaurenoic acid) demonstrated considerable effects against trypomastigote forms of *Trypanosoma cruzi*. In vitro tests of kaurenoic acid, kaurenol, acutifloric acid, and stemodin showed complete elimination of parasites from blood [14]. Ent-kaurenoic acid derivatives are effective against human breast carcinoma cells in vitro [15]. Some kauranes demonstrate an antimalarial effect [16]. The composition of sunflower oil deodorization distillate (DD) has been investigated [17]. Thirteen aliphatic acidic components have been identified, more than 40% of the studied sample accruing to linoleic acid with F-vitamin activity. The acidic fraction of DD also contains five kaurenoic acids with similar mass spectra. More than thirty components of neutral nature found in DD are of interest as bioactive compounds. Of these, ten are phytosterols, fourteen are triterpene alcohols, and five are tocopherols [17].

MATERIALS AND METHODS

A rotary evaporator was used (Büchi, Switzerland). Thin-layer chromatography was performed on Sorbfil and Armsorb plates in the hexane – methyl *tert*-butyl ether system (6:1 and 1:1 for the analyses of the fraction of low-polarity compounds and all other fractions, respectively). The chromatogram was visualized by spraying the plates with a mixture of vanillin–sulfuric acid–ethanol at the ratio of 1:10:90 followed by heating of the plate. The target compounds were purified by column chromatography on silica gel from Sigma-Aldrich (Merck Grade 7734) 70-230 mesh. The gradient of 1 to 50% diethyl ether in hexane was used as the mobile phase. Mass spectra were recorded on a Hewlett Packard G 1800 A device consisting of an HP5890 series II gas chromatograph and an HP 5971 mass selective detector. Column 30 m \times 0.25 mm \times 0.25 μ m with HP-5MS sorbent (5% diphenyl, 95% dimethylsiloxane) was used. Helium was used as a carrier gas (1 mL/min). Column temperature mode: 2 min at 50 °C, then increase to 300 °C at the rate 4°/min, and 30 min at 300 °C. The evaporator temperature was 280 °C, the temperature of the ion source was 170 °C. Deodorization distillate samples were first divided into acidic and neutral components to facilitate the analysis. Free acids were isolated from the total extract by alkaline extraction with 2% aqueous sodium hydroxide. Also, an additional step of fractioning and saponification was required: fractions of total acids and unsaponifiable substances (USs) were obtained by alkaline hydrolysis as described earlier [17–18].

RESULTS AND DISCUSSION

The content of free acids was about 70% of the total sample weight. The neutral part was thus the sum of aliphatic hydrocarbons and aliphatic and triterpene alcohols in free form and in the form of esters with aliphatic acids. The total acid content was 80–82% of the total sample weight. The major total acid constituents are presented in [17]. The US amounted about 20%. Unsaponifiable fractions are the sum of aliphatic and terpene hydrocarbons; aliphatic and diterpene alcohols, including ent-kaurane substances; and sterols and triterpenols described in [17]. The groups of substances were obtained by column chromatography on silica gel. About 30% of the USs mass are nonpolar substances such as hydrocarbons, aliphatic and terpene ketones and aldehydes. More polar fractions such as tocopherols, aliphatic alcohols, sterols and triterpenols were investigated earlier [17].

A more detailed study of the unsaponifiable nonpolar DD fraction identified 49 neutral compounds, including kaurenes. The diterpene hydrocarbons, alcohols, and acids of the kaurane structure found in DD indicate that the original sunflower oil was native, since it is known that kaurane derivatives are a chemotaxonomic marker of many plants of the Compositae family to which sunflower belongs [19]. In addition to the above compounds, hydrocarbons (more than one-quarter of the unsaponifiable matter), both aliphatic and diterpenic, were found in the studied sample. The aliphatic components are predominantly unsaturated, which may point to their artefact origin; i.e. they are products of the dehydration of aliphatic alcohols. Phytadiene isomers, products of phytol dehydration and isomerization, were found. The data obtained indicate that sunflower oil DD is a promising source of bioactive substances, cosmetic ingredients, and pharmaceutical components.

TABLE 1. Distribution of components in the fraction of aliphatic hydrocarbons, mg/100g of the DD sample weight.

Component	Content in DD (mg/100g)	Component	Content in DD (mg/100g)
Pentadecadiene	43.2	Tetracosadiene	19.8
Pentadecene	23.7	Tetracosene	10.7
Octadecatriene	39.6	<i>n</i> -Pentacosane	27.2
Octadecadiene	43.1	Hexacosatetraene	143.3
Octadec-1-ene	11.2	Hexacosatriene	135.1
Neophytodiene 1	283.3	Hexacosadiene	40.1
Neophytodiene 2	15.5	<i>n</i> -Heptacosane	120.1
Neophytodiene 3	169.8	<i>n</i> -Octacosane	21.2
(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	427.5	Squalene	2048.8
Heneicosene	24.4	<i>n</i> -Nonacosane	331.9
Tricosatriene	175.2	<i>n</i> -triacontane	56.1
Tricosadiene	89.6	<i>n</i> -Hentriacontane	361.6
1-Tricosene	14.9		

Table 1 presents the qualitative and quantitative compositions of aliphatic constituents of USs that were obtained from GC-MS analyses normalized to the masses of fractions obtained from the chromatographic separation. The main constituent is bioactive squalene. Its content is more than 2 g per 100 g of DD sample.

TABLE 2. Distribution of components in the fraction of terpene hydrocarbons in mg/100g of the sample weight.

Component	Content in DD (mg/100g)	Component	Content in DD (mg/100g)
Isoatiserene	64.3	Palustradiene	0.9
Isokaurene (Kaur-15-ene)	208.0	Stigmastan-3,5,22-triene	39.1
Atiserene	44.5	Stigmastan-3,5-diene	131.1
Ent-kaurene	937.6	Ergosta-4,6,22-triene	16.6

Table 2 shows the qualitative and quantitative compositions of terpene hydrocarbons, including ent-kaurenes. The nonpolar kaurene diterpene content exceeds 1 g per 100 g DD sample. The main component is ent-kaurene (kaur-16-ene). Its content is more than 0.9 g per 100 g of DD sample. The triterpenoid components are probably products of the dehydration of sterols such as β -sitosterol and stigmasterol.

TABLE 3. Distribution of components in the fraction of aliphatic and triterpenoid ketones in mg/100g of sample weight.

Component	Content in DD (mg/100g)	Component	Content in DD (mg/100g)
13-Epi-manoyl oxide	20.1	2-Heneicosanone	45.4
2-Heneicosanone 3.1	3.1	2-Tricosanone	4.4
Tetracos-2,6,10,14,18-pentaen-22-one,2,6,10,15,19,23-hexamethyl-, all (E)-	4.1	2-Pentacosanone	1.1
15-Nonacosanone	23.8	2-Heptacosanone	3.7
2-Heptadecanone	6.5	Cholesta-3,5-dien-7-one	4.6
2-Nonadecanone	55.5	Stigmasta-3,5-dien-7-one	4.0
2-Eicosanone	1.2		

Table 3 presents the qualitative and quantitative compositions of aliphatic and triterpenoid ketones of DD USs that were determined from GC-MS analyses of fractions isolated by chromatographic separation. These fractions also contain 13-epi-manoyl oxide. The major components are 2-nonadecanone and 2-heneicosanone. More polar fractions contained also 16-hydroxykaurenal (0.9), kaurane-16-ol (36.5), and 17-norkaur-15-en-18-ol (18.3). Numerals in parentheses indicate contents, mg per 100 g DD.

The acidic fraction of DD consists of twenty-two components, including five kaurenoic acids with similar mass-spectra. Five aliphatic acids were identified in addition to the thirteen discovered earlier. The major components are bioactive linoleic and oleic acids. We found also palmitoleic, vaccenic, and gadoleic acids, known to be active constituents of sea buckthorn [20-23].

TABLE 4. Distribution of components in the fraction of aliphatic free and total acids,mg/100g of sample weight.

Acid	Content in DD (mg/100g)		
	Free	Total	Free /total ratio
Lauric	54.5	82.3	1:1.5
Myristic	95.1	169.1	1:1.8
Palmitic	5064.5	9496.0	1:1.9
Palmitoleic	109.9	188.3	1:1.7
Margaric	7.2	11.7	1:1.6
Stearic	2371.6	4873.4	1:2.1
Oleic	12532.3	14560.2	1:1.2
Vaccenic	112.3	138.3	1:1.2
Linoleic	35235.2	43775.5	1:1.2
Kaur-16-en-18-oic	788.4	887.4	1:1.1
Kaur-16-en-18-oic (isomer)	1383.9	1671.4	1:1.2
13-Methyl-17-norkaur-15-en-18-oic	1486.1	1749.7	1:1.2
Octadecadienoic	82.3	108.9	1:1.3
Kaur-16-en-18-oic (isomer)	128.9	164.1	1:1.2
Kaur-16-en-18-oic (isomer)	143.8	175.8	1:1.2
Gadoleic	86.5	141.3	1:1.6
Arachidic	211.4	601.6	1:2.8
Cis-11-eicosenic	183.4	232.5	1:1.3
Cis-13-eicosenic	225.8	345.8	1:1.5
Behenic	536.9	1437.3	1:2.7
Tricosanoic	78.6	120.8	1:1.5
Lignoceric	168.0	534.9	1:3.2

CONCLUSIONS

The chemical composition of sunflower oil deodorization distillate (DD) was investigated by gas chromatography-mass spectrometry.

Forty-nine neutral and five acid constituents were identified. The free /total ratio was counted for each identified acid. Seven neutral and five acidic kaurane constituents were found in DD.

High percentages of these compounds, as well as phytosterols, triterpenols, and tocopherols, increase the prospects of DD as a bioactive substance.

REFERENCES

1. A. A. Semenov, V.G. Kartsev, *Fundamentals of chemistry of natural compound* (ISCPF, Moscow, 2009, Vol. 1) pp. 252–255 (In Russian).
2. A.I. Suárez, K. Chavez, E., Mateu, R.S. Compagnone, A. Muñoz, F. Sojo, F. Arvelo, M. Mijares, and J.B. De Sanctis, *Nat. Prod. Commun.* **4**(11), 1547–1550 (2009).
3. C. Ding, Y. Ding, H. Chen, and J. Zhou, *Studies in Natural Products Chemistry* **54**, 141–197 (2017). doi.org/10.1016/B978-0-444-63929-5.00005-X.
4. T. Kilic, *Molecules* **11**(4), 257–262 (2006). doi.org/10.3390/11040257.
5. W. Herz and M. Bruno, *Phytochemistry* **25** (8). 1913–1916 (1986).
6. W. Herz and P. Kulanthaiavel, *Phytochemistry* **23** (7). 1453–1459 (1984).
7. W. Herz, P. Kulanthaiavel. *Phytochemistry*. **22** (11). 2543–2546 (1983).
8. A. Peña, L. Alarcón, J. Baptista, R. Aparicio, T. Villasmil, A. Usubillaga, *Avances en Química* **7** (3), 187–192 (2012).
9. U. Tan. Patent RU 2345761.
10. B. B. de Andrade, M. R. Moreira, S. R. Ambrosio, N. A. Furtado, W. R. Cunha, V. C. Heleno, A. N. Silva, M. R. Simão, E. M. da Rocha, C. H. Martins, and R. C. Veneziani. *Nat. Prod. Commun.* **6** (6), 777–780.

11. F. de S. Vargas, P. D. O. de Almeida, E. S. P. Aranha, A. P. de A. Boleti, P. Newton, M. C. de Vasconcellos, V. F. Veiga Junior, and E. S. Lima, [Molecules](#) **20**, 6194–6210 (2015).
12. M. R. Suo, Z. Tian, J.S. Yang, Y. Lu, L. Wu, W. Li, Yao Xue Xue Bao. **42** (2), 166–170 (2007).
13. H. J. Park, I. T. Kim, J. H. Won, S. H. Jeong, E.Y. Park, J.H. Nam, J. Choi, and K. T. Lee, [Eur. J. Pharmacol.](#) **558** (1–3), 185–193 (2007).
14. J. A. Takahashi, H. S. Vieira, E. A. Silva, M. A. D. Boaventura, A. B. de Oliveira, E. Chiari, [Revista Brasileira de Farmacognosia](#) **12**, 118–120 (2002).
15. R. M. da Costa, J. K. Bastos, M. C. A. Costa, M. M. C. Ferreira, C. S. Mizuno, G. F. Caramori, G. R. Nagurniak, M. R. Simão, R. A. Dos Santos, R. C. S. Veneziani, S. R. Ambrósio, R. L. T. Parreira, [Phytochemistry](#) **156**, 214–223 (2018). doi: 10.1016/j.phytochem.2018.10.005.
16. T. Villasmil, J. Rojas, R. Aparicioa, N. Gamboa, M. E. Acosta, J. Rodrigues, and A. Usubillaga, *Nat. Prod. Commun.* **12** (2), 217–220 (2017).
17. D. N. Shcherbakov, T. P. Kukina, N.V. Panteleeva, O. I. Salnikova, and P. V. Kolosov, [Khimiya rastitel'nogo syr'ya](#), **1**, 199–206 (2020); doi: 10.14258/jcprm.2020015909, (In Russian).
18. T. P. Kukina, T. S. Frolova, and O. I. Salnikova, [Chemistry of Nat. Comps.](#) **50**, (2), 233–236 (2014), doi:10.1007/s10600-014-0920-1.
19. F. Seaman, F. Bohlmann, C. Zdero, T. J. Marby. *Diterpenes of Flowering Plants: Compositae (Asteraceae)* (Springer Science and Business Media, 2012).
20. H. Ito, K. Kasama, S. Nasure, and K. Shimura, [Cancer letter](#) **17** (2), 197–203 (1982).
21. B. Yang, H. P. Kallio. [J. Agric. Food Chem.](#) **49**, (4), 1939–1947 (2001).
22. B. Yang, H. P. Kallio. [Trends Food Sci. Technol.](#) **13**, 160–167 (2002).
23. T. P. Kukina, D. N. Shcherbakov, K. V. Gensh, E. A. Tulysheva, O. I. Salnikova, A. E. Grazhdannikov, E. A. Kolosova, [Russian Journal of Bioorganic Chemistry](#). **43** (7), 747–751 (2017).
24. A. Zielińska and I. Nowak. [Lipids in Health and Disease](#) **16**, 95–106 (2017); doi: 10.1186/s12944-017-0469-7.