# ISOLATION OF TRILOBOLIDE FROM STEMS AND LEAVES OF THE HORSE CARAWAY

**Martin Grbavčic**[a], **Michal Jurášek**[b], **and Pavel Drašar**[b]

[a] *Seva Flora s.r.o., Mikulovská 366, 691 42 Valtice, Czech Republic;* [b]*Department of Chemistry of Natural Substances, University of Chemistry and Technology, Technická 5, 166 28 Prague 6, Czech Republic*
*lecivky1@seznam.cz, jurasekm@vscht.cz, drasarp@vscht.cz*

Isolation of trilobolide from the stems and leaves of horse caraway is simple and, despite the lower content of the substance, appears to be economical due to the quantity of material that can be available and thanks to a relatively simple procedure.

Keywords: horse caraway cultivation, trilobolide, supercritical extraction

Fig. 1. **Horse caraway**[1]

Horse caraway (Fig. 1) (gladich, baltracan, Kefe cumin, *Laser trilobum* (L.) Borkh.; Kingdom: Plantae, Order: Apiales, Family: Apiaceae) is a perennial white-flowering herb of the family Apiaceae with a short underground rhizome, thick roots and a strong straight stem that is 60–120 cm long, which grows wild in the Czech Republic only in the forest in the Kukle National Natural Monument, in the Divácký les between the villages of Diváky and Kurdějov near Hustopeče in southern Moravia[2], and therefore belongs to the critically endangered plants.

For the project, which dealt with the constituent of this herb, trilobolide[3] (Fig. 2), and another structurally close guaianolide, thapsigargin[4] (Fig. 3) (originally obtained from deadly carrots *Thapsia garganica* L.), to which trilobolide can be preparatively converted[5], horse caraway was grown on an area of ca 0.5 hectare and gradually exploited for the preparation of larger amounts of trilobolide, which was used for further investigation and synthesis of a series of derivatives[6–10] including the clinically tested prodrug mipsagargin[11] (G-202), Fig. 4.

As a by-product of this cultivating anabasis, there were established stable growths of horse caraway, the breeding of verified genotypes is underway and the registration of the variety is being prepared, including the delivery of seeds to the Central Institute for Supervising and Testing in Agriculture.

The growths were gradually established from available sources and individual genotypes propagated in spatial isolation. Two approximately 0.25-hectare stands were established from the offspring of the genotypes. One semi-operational to obtain cuttings, seeds and roots and gain growing experience. The second stand is a breeding section to verify agrotechnical measures and especially breeding the genotype – variety – with the best content of active substances. The establishment of stands is very specific. After mastering this stage, growing horse caraway is a joy. It very willingly tolerates growing in a thicket, thanks to its very fast spring growth and massive habit (Fig. 5); it can outcompete other types of plants. It begins to flower at the end of May and ends its vegetation

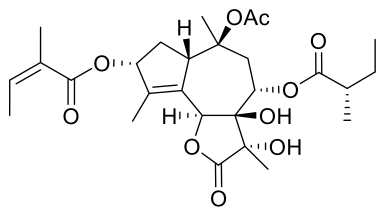

Fig. 2. **Trilobolide**

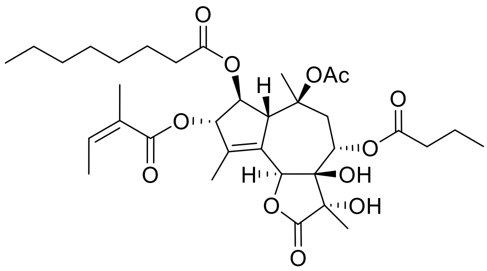

Fig. 3. **Thapsigargin**

 https://doi.org/10.54779/chl20240448

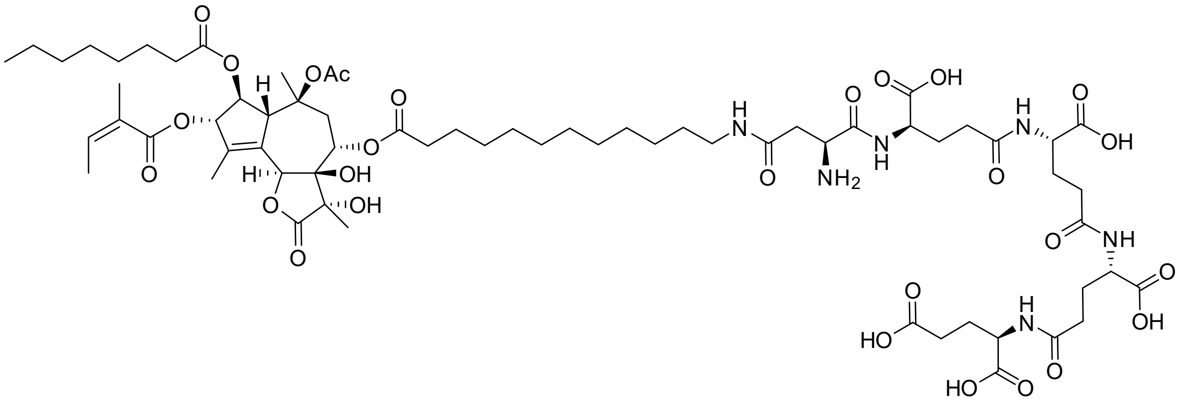


Fig. 4. **Mipsagargin**

in mid-August. It is very suitable for cultivation in "bio" conditions.

The oldest field is 11 years old, and the plants and roots do not degrade. It is an example of its longevity and durability.

Indicative harvests were carried out continuously between the third and tenth year of cultivation. On average, 0.5 to 2 kg of fruits/seeds was harvested from one are, and before flowering, approx. 10 kg of stems and leaves were harvested from are – that is, however, relatively reluctant to desiccate. The roots were dug up, cleaned, and dried very easily, yielding approx. 3.5 kg from one are.

Methods for isolating a preparative amount of trilobolide from dried fruits were gradually developed, where an isolable trilobolide yield from the dried fruits of up to 0.7% was found, as well as up to 0.1% from dried rhizomes and roots.

In this communication, we will describe the preparation of trilobolide from dried stems and leaves of horse caraway. Horse caraway was grown, as mentioned above, in a field in the Czech Republic (South Moravian Region). The voucher specimen was deposited under the code 03013KBFR in the herbarium of the Department of Botany and Plant Physiology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague and the University of Chemical Technology, Prague.

Fig. 5. **Field with the horse caraway**

The dried stems and leaves (120 kg) were crushed and extracted with supercritical $CO_2$ at 63 °C for 25 min at a flow rate of 600 kg $CO_2$ per hour, at a pressure of 230 bar. For the extraction of plant material with supercritical $CO_2$, a NATEX extractor (Ternitz, Austria) was used at TRUMF International s.r.o. company (No. 157, 751 23 Dolní Újezd, CZ). There was obtained 361 g of extract in the form of a gray powder. The powder (100 g) was mixed in 1 L of methanol and the mixture was stirred at room temperature for 3 days and filtered through a paper filter and the filtrate evaporated on a vacuum rotary evaporator. The residue (17.5 g) was applied to a silica gel column (1 L) and extracted with cyclohexane to which 50 mL of ethyl acetate was added with each 500 mL portion. The combined fractions containing trilobolide were evaporated and recrystallized from ethyl acetate/cyclohexane to yield 1.3 g of trilobolide (0.004% of the original stems and leaves weight) obtained in the form of yellowish crystals with properties identical to those reported in the literature[13].

Thus, it seems disadvantageous to isolate trilobolide from the horse caraway stems and leaves. The disadvantage is not so obvious if we consider several factors. Even though it is a renewable resource, its harvest can be incomparably greater than the harvest of fruits, rhizomes, and roots. In addition, isolation is relatively simple compared to the processing of material obtained by supercritical $CO_2$ extraction from roots, rhizomes, and fruits, where the extract contains large amounts of oils and waxes.

REFERENCES


1. Sturm J.: *Flora von Deutschland*, K. G. Lutz, Stuttgart, 1900–1906.
2. Portál Informačního systému ochrany přírody (Portal of the Nature Protection Information System, ISOP); https://portal.nature.cz/cs/w/druh-37853?p_l_back_url=%2Fhledej%3Fq%3Dtimoj#/, downloaded 17. 4. 2024.
3. PubChem: https://pubchem.ncbi.nlm.nih.gov/compound/Trilobolide, downloaded 16. 4. 2024.
4. PubChem: https://pubchem.ncbi.nlm.nih.gov/compound/446378, downloaded 16. 4. 2024.
5. Zimmermann T., Drašar P., Rimpelová S., Christensen S. B., Khripach V. A., Jurášek M.: Biomolecules *10*, 1640 (2020).
6. Jurášek M., Rimpelová S., Kmoníčková E., Drašar P., Ruml T.: J. Med. Chem. *57*, 7947 (2014).
7. Tomanová P., Rimpelová S., Jurášek M., Buděšínský M., Vejvodová L., Ruml T., Kmoníčková E., Drašar P. B.: Steroids *97*, 8 (2015).
8. Škorpilová L., Rimpelová S., Jurášek M., Buděšínský M., Lokajová J., Effenberg R., Slepička P., Ruml T., Kmoníčková E., Drašar P. B., Wimmer Z.: Beilstein J. Org. Chem. *13*, 1316 (2017).
9. Huml L., Jurášek M., Mikšátková P., Zimmermann T., Tomanová P., Buděšínský M., Rottnerová Z., Šimková M., Harmatha J., Kmoníčková E., Lapčík O., Drašar P. B.: Steroids *117*, 105 (2017).
10. Jurášek M. and 13 co-authors: Steroids *117*, 97 (2017).
11. Mahalingam D., Peguero J., Cen P., Arora S. P., Sarantopoulos J., Rowe J., Allgood V., Tubb B., Campos L.: Cancers (Basel) *11*, 833 (2019).
12. Isaacs J. T., Brennen W. N., Christensen S. B., Denmeade S. R.: Molecules *26*, 7469 (2021).
13. Harmatha J., Buděšínský M., Jurášek M., Zimmermann T., Drašar P., Zídek Z., Kmoníčková E., Vejvodová L.: Fitoterapia *134*, 88 (2019).