Facile access to oleuropein and hydroxytyrosol from Ligustrum vulgare – a plant material growing all over Eurasia

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Abstract: Leaves of Ligustrum vulgare are an alternative, sustainable source for oleuropein. Acidic hydrolysis of oleuropein furnishes hydroxytyrosol in good yield. This approach is one of the most convenient ways to access significant amounts of oleuropein as well as of hydroxytyrosol from plant material readily accessible all over the year and throughout Eurasia. Harvesting the leaves in winter doubles the amount of oleuropein that can be extracted from the plant material.

Keywords: Oleuropein; hydroxytyrosol; Ligustrum vulgare; extraction.

Introduction

In principle, any molecule can be synthesized using sufficient human and material resources. These syntheses are independent of the molecule’s size and stereochemical complexity. This has been impressively proven in the past with the synthesis of urea (Wöhler 1828), strychnine (Woodward 1954), vitamin B12 (Woodward and Eschenmoser 1973), and palytoxin (Kishi 1994). All of these syntheses greatly enriched our knowledge of methods and strategies. However, as the example of paclitaxel has shown, (partial)-syntheses are more efficient and - above all - more economical than total syntheses. A prerequisite for the latter in particular is the identification of a suitable precursor or of a target molecule to be isolated, for example from microorganisms or plants.

Results and Discussion

Hydroxytyrosol (1, Scheme 1) is subject to several clinical trials dealing with diseases to evaluate its potential to lower postprandial blood sugar levels in persons suffering from diabetes mellitus 1,2, or to investigate its influence on blood lipid levels to reduce the risk of several heart diseases 3-8. Also, it was found 9 that 1 increased the endogenous level of vitamin C, and 1 also influenced the progress of inflammation. Hydroxytyrosol also had some effect in the regulation of oxidative stress-related genes 10-12. It seems to be responsible for the nutraceutical properties and pharmacological effect of olive oil. Furthermore, adherence to the Mediterranean diet has been associated with a reduced incidence of neurodegenerative diseases, better cognitive performance and a lowered risk of cancer 13-15.

Scheme 1. Extraction of oleuropein (2) from plant material and isolation of hydroxytyrosol (1) by hydrolysis (0.1 M aq. hydrochloric acid, 25 °C, 48 h, 73%).

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DOI: http://dx.doi.org/10.13171/mjc7318101113-csuk

Received September 3, 2018
Accepted October 10, 2018
Published October 11, 2018
Several approaches for the synthesis of 1 have been reported. Many of them, however, are lengthy, and their over-all yield is low. One of the most rewarding approaches seems the hydrolysis of oleuropein (2). This compound is of its interest as a valuable and bio-renewable building block but also because of its cardio-

38-40, gastro-

25-37, neuro-

41, and hepatoprotective 42-47 properties. Its antioxidant activity is due to its orthodiphenolic structure. Furthermore, its anti-cancer 48-52, anti-

53, anti-diabetes 48-53, anti-

54, and anti-obesity activity 55 gave extended research dealing with this secondary natural product and derivatives thereof another impact.

Oleuropein can be obtained from fresh olives and their leaves. The access to the latter material, however, is limited, and its long-distance transport is expensive. Plants being non-endemic to Western Europe or the US have been suggested as sources for the extraction of 2. The cultivation, however, of these plants is often limited to green houses. As a consequence, we were looking for a source of oleuropein and its product of hydrolysis, hydroxytyrosol (1), from plant material that can be grown in large amounts all over Eurasia and the US. One of the most grown plants in Eurasia is Ligustrum vulgare, also known as Common Privet. Its leaves are well known in the Mediterranean historical medicine for their oropharyngeal anti-

56 inflammatory effects. Quite recently, extracts from this plant came again in the focus of scientific interest due its anti-inflammatory 57-60, anti-

51,62 and anti-lipoxigenase activity 63. Initial studies showed plants of the genus Ligustrum to contain oleuropein. Thus, we were interested in answering the questions whether this plant material is suited for obtaining oleuropein but also whether leaves harvested at different times (winter or summer) differ regarding the content of oleuropein.

Leaves of Ligustrum vulgare were collected, air-dried, and extracted. Chromatographic work-up of the extract gave pure oleuropein (2). As a result, leaves harvested in June held only half of the amount of 2 as compared to leaves having been harvested in November. It can be assumed that the higher content of 2 during winter time might be part of a protection mechanism of the plant against herbivores. Although the content of 2 in L. vulgare (winter) is approximately half of the amount of 2 obtained from the leaves of olives (Olea europaea), L. vulgare is grown in huge amounts all over Eurasia and the US while growing of olives is limited to a relatively small geographical region. Furthermore, olives had a low content of 2 (0.1%), and almost no or an insignificant low amount of oleuropein can be found in the pomace.

Hydroxytyrosol (1) is the phenolic part in oleuropein. The cleavage of oleuropein has been described under a variety of different conditions, such as alkaline hydrolysis as well as hydrolysis by a broad variety of hydrolytic enzymes, viz. esterases and lipases. Reports describing acidic hydrolysis are scarcely found in literature. As a consequence, we studied the hydrolysis of 2 with diluted aqueous hydrochloric acid of 2 for 48 hours. Cleavage of 2 occurred nicely at ambient temperatures; usual work-up and column chromatography gave target compound 1 in 73% isolated yield.

Conclusion

To sum up, leaves of L. vulgare can be seen as an alternative, sustainable source for oleuropein. This plant material is more easily obtained than the leaves of olives, and they can be harvested all through the year, although harvesting the leaves in winter doubles the amount of 2 that can be extracted from the plant material. Acidic hydrolysis of 2 furnishes hydroxytyrosol (1) in good yield. Our approach is one of the most convenient ways to access significant amounts of oleuropein as well as of hydroxytyrosol from plant material readily accessible all over the year and throughout Eurasia.

Experimental

Melting points were determined with a Büchi melting point apparatus M565 and are uncorrected. NMR spectra were recorded on a Varian spectrometer Unity 500 (δ given in ppm, J in Hz), mass spectra were obtained on a Finnigan MAT LCQ 7000 (electrospray, voltage 4.1 kV, sheath gas nitrogen) instrument. The optical rotations were measured on a Perkin-Elmer polarimeter at 20 °C. Machery-Nagel ALUGRAM XtraSIL pre-coated silica gel 60 F254 plates were used for thin layer chromatography. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer Spectrum 1000 and wave numbers are expressed in cm⁻¹. The absorption spectra were measured on Perkin Elmer Lambda14 spectrometer. The solvents were dried according to usual procedures. A specimen of L. vulgare was deposited at the herbarium of the institute. An authentic sample of 2 was obtained from Cayman Chemical.

Oleuropein (2)

Fresh leaves of Ligustrum vulgare (2.0 kg, harvested in June) were air-dried, shredded and extracted with methanol (5 x 2 L) for one day each. The combined filtrates were evaporated under reduced pressure to a final volume of 250 mL and extracted with ethyl acetate (7 x 200 mL). The solvent was evaporated, and the residue was subjected to column chromatography (silica gel, CHCl3/MeOH, 9:1 → 8:2) to yield crude 2 (31.2 g) that was subjected to a second chromatographic purification (silica gel, CHCl3/MeOH, 10:0 → 9:1 → 8:2) to yield pure 2 (4. 1 g, 0.2% with respect to the plant material). Extraction of fresh leaves of L. vulgare (harvested in November, 2.0 kg) under the same conditions gave 2 (9.6 g, 0.5%) while the
extraction of leaves of olives (1 kg leaves; plant obtained from a local garden center, harvested in June) gave 2 (6.9 g, 0.7%). Extraction of olives (green, pitted, 1 kg, obtained from a local supermarket) furnished 2 (0.95 g, 0.1%) while the extraction of olive pomace (1 kg, obtained from Becker & Karsten UG, Andalusia, Spain) gave 2 (100 mg, 0.01%). Data for 2: off-white solid; m.p. 87-89 °C, m.m.p. 87-89 °C; Rf = 0.06 (CHCl3/MeOH, 95:5); 0.34 (CHCl3/MeOH, 4:1); [α]D = -160.34° (c = 0.315, CHCl3); IR (KBrs): ν = 3424s, 2935w, 1707m, 1630m, 1522w, 1442m, 1384m, 1285m, 1193m, 1161m, 1076s cm⁻¹; UV-vis (MeOH): λmax (log ε) = 256 (3.80), 303 (3.46) nm;

1H NMR (500 MHz, DMSO-d6): δ = 8.71 (d, J = 20.3 Hz, 2H, OH (23,24)), 7.53 (s, 1H, 11-H), 6.65 (d, J = 8.0 Hz, 1H, 12-H), 6.62 (d, J = 2.1 Hz, 1H, 22-H), 6.49 (dd, J = 8.0, 2.1 Hz, 1H, 21-H), 5.98 (qd, J = 6.8, 1.3 Hz, 1H, 12-H), 5.88 (t, J = 1.6 Hz, 1H, 7-H), 4.66 (d, J = 7.8 Hz, 1H, 6-H), 4.08 (dt, J = 20.7, 9.9, 7.2 Hz, 2H, 18-H), 3.87 (dd, J = 9.1, 4.3 Hz, 1H, 9-H), 3.69 (dd, J = 11.6, 6.2, 1.9 Hz, 1H, 5b-H), 3.66 (s, 3H, OCH3), 3.47 (dd, J = 11.2, 6.2, 4.2 Hz, 1H, 5a-H), 3.24 – 3.20 (m, 2H, 4-H), 3.20 – 3.17 (m, 2H, 4-H), 3.13 – 3.10 (m, 1H, 11-H), 3.10 – 3.08 (m, 1H, 3-H), 2.69 (t, J = 7.2 Hz, 2H, 19-H), 2.64 (dd, J = 14.4, 4.3 Hz, 1H, 16b-H), 2.41 (dd, J = 14.5, 9.2 Hz, 1H, 16a-H), 1.66 (dd, J = 7.1, 1.4 Hz, 3H, 13-CH3);

13C NMR (125 MHz, DMSO-d6): δ = 171.1 (C-17), 166.6 (C-14), 153.8 (C-11), 145.5 (C-24), 144.2 (C-2323), 129.6 (C-8), 128.8 (C-20), 123.5 (C-12), 120.0 (C-21), 116.6 (C-22), 116.0 (C-25), 108.1 (C-10), 99.5 (C-6), 93.4 (C-7), 77.8 (C-4), 77.0 (C-2), 73.7 (C-1), 70.4 (C-3), 65.5 (C-18), 61.6 (C-5), 51.7 (OCH3, C-15), 40.5 (C-16), 34.2 (C-19), 30.6 (C-9), 13.4 (C-13) ppm; 1H NMR (500 MHz, D2O): δ = 7.55 (s, 1H, 11-H), 6.90 (d, J = 8.1 Hz, 1H, 25-H), 6.85 (d, J = 2.0 Hz, 1H, 22-H), 6.76 (dd, J = 8.1, 2.1 Hz, 1H, 21-H), 3.80 (t, J = 6.7 Hz, 2H, OCH2), 2.76 (t, J = 6.7 Hz, 2H, CH2) ppm;

13C NMR (125 MHz, D2O): δ = 143.8 (C-24), 142.2 (C-23), 131.5 (C-20), 121.2 (C-21), 116.7 (C-22), 116.2 (C-25), 62.6 (OCH3), 37.0 (CH3) ppm; ESI-MS (MeOH): m/z = 153 (100%, [M-H]), 199 (3.2%, [M+HCO2]); 307 (11.1%, [2M-H]), 385 (44.4%, [2M-H]), 407 (14.3%, [2M+2H+Na]); Analysis caleed for C19H13O (154.16) C 62.33, H 6.54; found: C 62.17, H 6.71.

Acknowledgments

Many thanks are due to M.Sc. S. Tschullik for experimental help, to Dr. D. Ströhl and his team for the NMR spectra and to Dr. R. Kluge for the MS-measurements. IR- and UV-vis spectra and optical rotations were recorded by Ms V. Simon. The authors like to thank Becker & Karsten UG (Andalucia, Spain) for the generous donation of olive pomace. Financial support by the “ScienceCampus Halle, WCH” (W13004216 to R.C.) is gratefully acknowledged. The authors declare no conflict of interests.


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