Geographical Variation of the Furanocoumarin Composition of the Fruits of Icelandic *Angelica archangelica*

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Angelica archangelica fruits were collected from 64 locations around Iceland and analysed for furanocoumarins by high-performance liquid chromatography. The average furanocoumarin content was found to be 22.5 mg/g, ranging from 14.0 to 31.6 mg/g. Whereas imperatorin was the main compound in all samples, the order of other compounds was highly diverse. Considerable differences were observed between individuals from the same location and between neighbouring locations. However, strong geographical impact was observed on the composition, with isoimperatorin and bergapten being more pronounced in South Iceland, and oxypeucedanin and an unidentified compound being more pronounced in North Iceland and absent in many samples from South Iceland.

Key words: *Angelica archangelica*, Iceland, Imperatorin, HPLC

Introduction

Angelica archangelica has been long and widely used in folk medicine, and is one of the most respected medicinal herbs in Nordic countries, where it was cultivated during the Middle Ages and exported to other parts of Europe. *A. archangelica* growing in Iceland is of the subspecies *A. archangelica* ssp. *litoralis* (Löve, 1977). The most characteristic secondary metabolites of its fruits are essential oils and furanocoumarins (Newall *et al.*, 1996). Furanocoumarins have various pharmacological properties depending on their respective substituent groups. Xanthotoxin and bergapten have long been used in photochemotherapy with psoralen and UV-A (PUVA), due to their photosensitizing activities. The chemical structures of the furanocoumarins most relevant in this study are shown in Fig. 1.

Imperatorin is usually considered the most abundant furanocoumarin of *A. archangelica* fruit (Newall *et al.*, 1996). Its biological activity has been the subject of many studies, showing various effects on the inflammatory process (Ban *et al.*, 2003; Abad *et al.*, 2001; Wang *et al.*, 1999), antiproliferative activity on cancer cells (Kawaii *et al.*, 2001; Sigurdsson *et al.*, 2004), and induction of apoptosis (Pae *et al.*, 2002; Appendino *et al.*, 2004). Its effect on cytochromes P450 has been investigated in connection with drug and xenobi-otic metabolism (Kleiner *et al.*, 2008). It has been shown to induce vasodilation (Chiou *et al.*, 2001; He *et al.*, 2007). Imperatorin has been reported to inhibit the breakdown of the neurotransmitters acetylcholine (Kim *et al.*, 2002; Di Giovanni *et al.*, 2008) and γ-aminobutyric acid (GABA) (Choi *et al.*, 2005), to facilitate the release of the neurotransmitter glutamate (Wang *et al.*, 2008), and to inhibit phosphodiesterases 4A and 4B (Ivey *et al.*, 2008).

As the pharmacological properties of different furanocoumarins are diverse, knowledge of the furanocoumarin composition of raw material is highly relevant.

Material and Methods

Samples and extraction

Fruits of *A. archangelica* were collected from 64 locations between August 11 and August 21, 2008.

The fruits were usually from 20 individual plants in each location. The selected fruits had preferably reached full size but were not dry or drying. The fruits were dried at room temperature until their dry weight content had reached 90–93%. They were then ground. A portion of each sample was kept at 110 °C overnight to establish the dry weight. Thereafter the samples were extracted with 96% ethanol, approximately 70 mg in 7 ml, at room temperature for 7 d. They were subse-
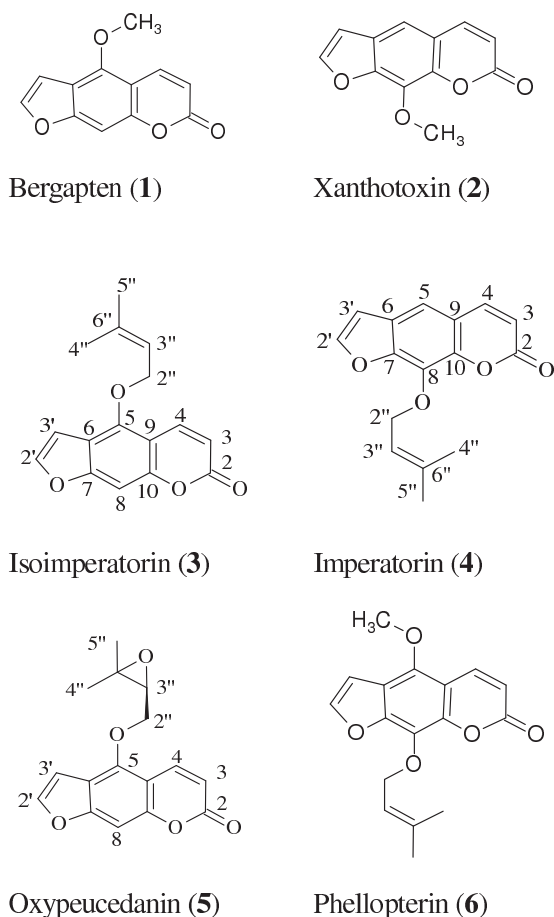
Fig. 1. Chemical structures of the analysed compounds.

Bergapten (**1**)   Xanthotoxin (**2**)

Isoimperatorin (**3**)   Imperatorin (**4**)

Oxypeucedanin (**5**)   Phellopterin (**6**)

quently filtered, and the furanocoumarin content was measured by high-performance liquid chromatography (HPLC). Each sample was extracted and analysed in triplicate.

Quantitative furanocoumarin analysis by HPLC

Samples were diluted to a concentration of $1-100\,\mu g/ml$ of individual compounds. A HP-1100 (Hewlett Packard, Palo Alto, CA, USA) instrument with a quaternary pump and a diode array detector was used. The stationary phase of the column (50 x 4.6 mm I.D.) was Zorbax SB-C18 with a particle size of $1.8\,\mu m$. Gradient elution was as follows: 0–7 min from 60–95% (v/v) aqueous methanol, detection at 305 and 309 nm at room temperature, with a flow rate of 1.0 ml/ min. Each sample was diluted appropriately (to a maximum of ca. $100\,\mu g/ml$ of individual furano-

coumarin) before measurement, and its signal at 305 and 309 nm was monitored and compared with standard curves of xanthotoxin with known concentrations. In the case of the other furanocoumarins, the same relationship of signal to concentration was assumed as for xanthotoxin and corrected using the respective extinction coefficients at their maxima at about 300 nm (Lee and Soine, 1969). In the cases of unidentified furanocoumarins (identified as such by their UV spectra), molecular weight and extinction coefficients were assumed as the average of those of the known compounds. A representative chromatogram is shown in Fig. 2.

Identification of furanocoumarins

In the case of xanthotoxin and bergapten, standards were purchased (Sigma, St. Louis, MO, USA). Imperatorin, isoimperatorin, oxypeucedanin, and prangenin were isolated from *A. archangelica* fruits, and identified using ^{1}H NMR spectroscopy and, in the case of imperatorin and isoimperatorin, ^{13}C{^{1}H} NMR spectroscopy. The NMR spectra were recorded in CDCl₃ at 297 K on a Bruker AC250 spectrometer (Karlsruhe, Germany) using as an internal standard the rest signal of CHCl₃ at 7.26 ppm for the ^{1}H NMR spectra and the signal of CDCl₃ at 77.00 ppm for the ^{13}C{^{1}H} NMR spectra. Identification of each substance was achieved by measuring the ^{1}H NMR and the ^{13}C{^{1}H} NMR spectra followed by the DEPT-135 experiment. For the exact identifi-
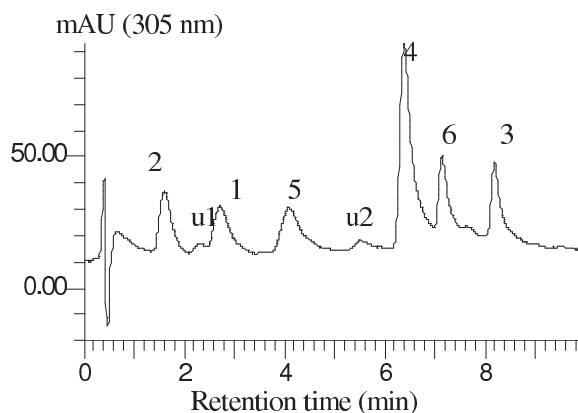

Fig. 2. Chromatogram of *A. archangelica* fruit extract: 2, xanthotoxin; u1, unidentified 1; 1, bergapten; 5, oxypeucedanin; u2, unidentified 2; 4, imperatorin; 6, assumed to be phellopterin; 3, isoimperatorin.

cation of the ^{1}H NMR signals, double resonance experiments were performed.

Imperatorin ($C_{16}H_{14}O_4$, M_r 270): ^{1}H NMR (CDCl$_3$): δ = 7.74 (d, J = 9.6 Hz, 1H, 4-H), 7.67 (d, J = 2.2 Hz, 1H, 2´-H), 7.33 (s, 1H, 5-H), 6.79 (d, J = 2.2 Hz, 1H, 3´-H), 6.33 (d, J = 9.6 Hz, 1H, 3-H), 5.58 (dd, J = 7.2 Hz, J = 1.4 Hz, 1H, 3''-H), 4.97 (d, J = 7.2 Hz, 2H, 2''-H), 1.71 (s, 3H, 4''-H), 1.69 (s, 3H, 5''-H). – ^{13}C{^{1}H} NMR: δ = 160.44 (C-2), 114.51 (C-3), 144.31 (C-4), 113.13 (C-5), 125.78 (C-6), 148.48 (C-7), 131.52 (C-8), 143.69 (C-9), 116.37 (C-10), 146.53 (C-2´), 106.68 (C-3´), 70.04 (C-2''), 119.70 (C-3''), 139.6 (C-6''), 25.71 (C-4''), 18.01 (C-5'').

Isoimperatorin ($C_{16}H_{14}O_4$, M_r 270): ^{1}H NMR (CDCl$_3$): δ = 8.13 [dd, J = 9.8 Hz, J = 0.7 Hz (long range), 1H, 4-H], 7.57 (d, J = 2.4 Hz, 1H, 2´-H), 7.11 (dd, J = 0.8 Hz, J = 0.9 Hz, 1H, 8-H), 6.94 (dd, J = 2.4 Hz, J = 0.9 Hz, 1H, 3´-H), 6.24 (d, J = 9.8 Hz, 1H, 3-H), 5.52 (dd, J = 7.2 Hz, J = 1.4 Hz, 1H, 3''-H), 4.90 (d, J = 7.0 Hz, 2H, 2''-H), 1.79 (d, J = 0.9 Hz, 3H, 4''-H), 1.69 (d, J = 0.9 Hz, 3H, 5''-H). – ^{13}C{^{1}H} NMR: δ = 161.28 (C-2), 105.08 (C-3), 139.63 (C-4), 139.76 (C-5), 158.04 (C-6), 114.04 (C-7), 94.10 (C-8), 152.54 (C-9), 107.35 (C-10), 144.88 (C-2´), 112.45 (C-3´), 69.62 (C-2''), 119.01 (C-3''), 139.76 (C-6''), 25.76 (C-4''), 18.17 (C-5'').

Oxypeucedanin ($C_{16}H_{14}O_5$, M_r 286): ^{1}H NMR (CDCl$_3$): δ = 8.21 [dd, J = 9.8 Hz, J = 0.6 Hz (long range), 1H, 4-H], 7.62 (d, J = 2.4 Hz, 1H, 2´-H), 7.20 (dd, J = 0.8 Hz, J = 0.9 Hz, 1H, 8-H), 6.95 (dd, J = 2.4 Hz, J = 0.9 Hz, 1H, 3´-H), 6.32 (d, J = 9.8 Hz, 1H, 3-H), 4.60 (dd, J = 10.8 Hz, J = 4.4 Hz, 1H, 2''-H), 4.45 (dd, J = 10.8 Hz, J = 4.4 Hz, 1H, 2''-H), 3.23 (dd, J = 4.4 Hz, J = 6.5 Hz, 1H, 3''-H), 1.41 (s, 3H, 4''-H) and 1.33 (s, 3H, 5''-H).

Prangenin ($C_{16}H_{14}O_5$, M_r 286): ^{1}H NMR (CDCl$_3$): δ = 7.77 (d, J = 9.6 Hz, 1H, 4-H), 7.70 (d, J = 2.2 Hz, 1H, 2´-H), 7.40 (s, 1H, 5-H), 6.83 (d, J = 2.2 Hz, 1H, 3´-H), 6.38 (d, J = 9.6 Hz, 1H, 3-H), 4.60 (dd, J = 14 Hz, J = 4.5 Hz, 1H, 2''-H), 4.58 (dd, J = 14 Hz, J = 4.5 Hz, 1H, 2''-H), 3.32 (dd, J = 4.4 Hz, J = 6.5 Hz, 1H, 3''-H), 1.35 (s, 3H, 4''-H) and 1.28 (s, 3H, 5''-H).

The second most abundant furanocoumarin, having a slightly higher retention time than imperatorin in the HPLC analysis, could not be identified with certainty (6 in Fig. 2). Its UV spectrum was identical with that published for isopimpinellin and phellopterin (Lee and Soine, 1969).

Comparison with a chromatogram in a published study using a similar HPLC system (Waksmundzka-Hajnos *et al.*, 2004) showed that the relative retention time makes it highly unlikely that the unidentified compound was isopimpinellin, the retention time of which would be expected between those of xanthotoxin and bergapten (as is the case for unidentified 1 in Fig. 2). Finally, as phellopterin is usually a prominent compound in *A. archangelica* fruit (Murray *et al.*, 1982), and not accounted for elsewhere in this analysis, the unidentified compound was assumed to be phellopterin.

Prangenin had been isolated and identified by NMR spectroscopy but could not be measured accurately by HPLC, as an unidentified furanocoumarin with a different UV spectrum had the same retention time. This unidentified compound was probably isopimpinellin, based on (i) its UV spectrum (results not shown), (ii) the fact that it has been detected by GC-MS in many samples of Icelandic *A. archangelica* fruit (results not shown), and (iii) its retention time relative to xanthotoxin and bergapten published in the previously mentioned study (Waksmundzka-Hajnos *et al.*, 2004). In the quantitative analysis, prangenin and this unidentified compound were taken together and called "unidentified 1".

A furanocoumarin with a retention time between oxypeucedanin and imperatorin could not be indentified, and was referred to as "unidentified 2" in this study. It is probably 8-substituted, with a UV spectrum similar to those of xanthotoxin and imperatorin (results not shown).

Results

The main results are taken together in Tables I and II. As seen in Table I, the average of the total furanocoumarin quantity was 22.5 mg/g ranging from 14.0 to 31.6 mg/g. In all cases, imperatorin was by far the most prominent compound of this class in the fruits, 33.9–58.2%. Apart from imperatorin, the order of the quantity was variable. The assumed phellopterin accounted for 6.1–23.8%, isoimperatorin for 2.0–25.0%, oxypeucedanin for 0–21.8%, bergapten for 3.3–13.3%, xanthotoxin for 2.9–14.7%, and unidentified 2 for 0–8.6%.

The main differences in composition were related to oxypeucedanin and unidentified 2 which could not be detected in 7 samples from South Iceland.

Table I. Furanocoumarin content (mg/g) in *A. arch-angelica* fruits from Iceland. The average content and the range (minimum and maximum) of each compound are given.

Furanocoumarin	Content (mg/g)		
	Average	Minimum	Maximum
Xanthotoxin	1.59	0.59	3.58
Unidentified 1	0.55	0.17	1.78
Bergapten	1.88	0.61	3.96
Oxypeucedanin	2.04	0.00	6.45
Unidentified 2	0.43	0.00	1.64
Imperatorin	10.35	5.82	15.55
Phellopterin	3.26	1.13	7.50
Isoimperatorin	2.36	0.34	6.17
Total	22.47	13.95	31.62

Table II. Furanocoumarin composition (%) in *A. arch-angelica* fruits from Iceland. The average ratio and the range (minimum and maximum) of each compound are given.

Furanocoumarin	Content (%)		
	Average	Minimum	Maximum
Xanthotoxin	7.2	2.9	14.7
Unidentified 1	2.5	0.7	8.9
Bergapten	8.3	3.3	13.3
Oxypeucedanin	9.0	0.0	21.8
Unidentified 2	2.1	0.0	8.6
Imperatorin	46.5	33.9	58.2
Phellopterin	14.3	6.1	23.8
Isoimperatorin	10.4	2.0	25.0

No generalizations could be made on the furano-coumarin compositions with regard to location, as considerable differences in composition were often found between samples from neighbouring locations. However, when the data was taken together, geographical impact was found on the furanocou-marin composition. Samples with high percentage of isoimperatorin and those with high percentage of bergapten were more likely to be found in South Iceland than in North Iceland (Figs. 3 and 4), whereas the opposite was true for oxypeucedanin and unidentified 2 (Figs. 5 and 6). Locations more abundant in xanthotoxin (Fig. 7) were distributed similarly to those high in bergapten, although xanthotoxin was more likely to be prominent in West Iceland whereas bergapten was more abundant in East Iceland. No geographical pattern was found in the distribution of locations with high abundance of imperatorin and assumed phellopterin.

A small-scale experiment was carried out to estimate the relative contributions of environmen-
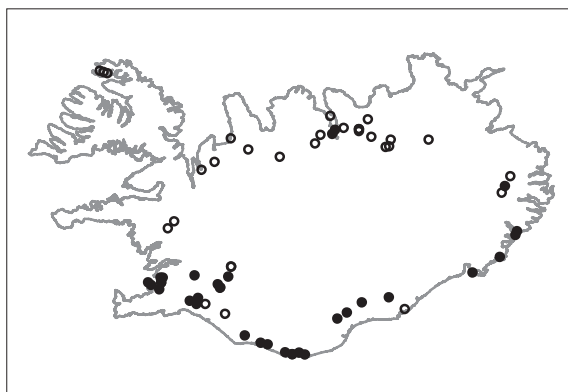

Fig. 3. Locations with isoimperatorin representing more than the average percentage (10.4%) of the total furanocoumarin content are shown with filled circles and locations with less than the average are shown with open circles.
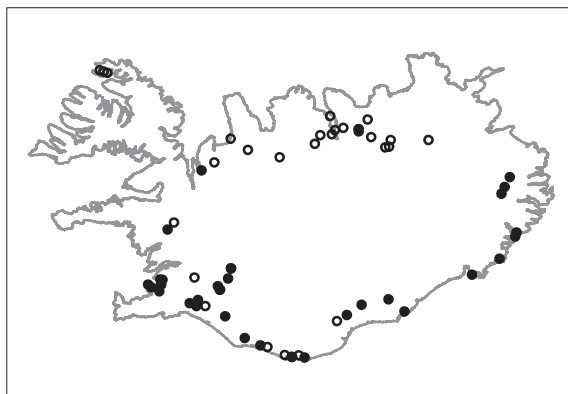

Fig. 4. Locations with bergapten representing more than the average percentage (8.3%) of the total furanocou-marin content are shown with filled circles and locations with less than the average are shown with open circles.

tal factors and individual genetic make-up. Fruits from 4 individual plants (A–D) growing within 5 m of each other in Southwest Iceland were collected, extracted, and analysed as described above, albeit in monoplicate. The results (Table III) reveal a highly diverse composition, individual A containing a lower percentage of xantho-toxin and assumed phellopterin than any pooled sample (1.6 and 4.6%, respectively), whereas the xanthotoxin percentage of individual D was at the opposite extreme with 14.5%. The impera-torin percentage of the individual plants ranged from 37.6% to 56.5%. And, finally, individual D
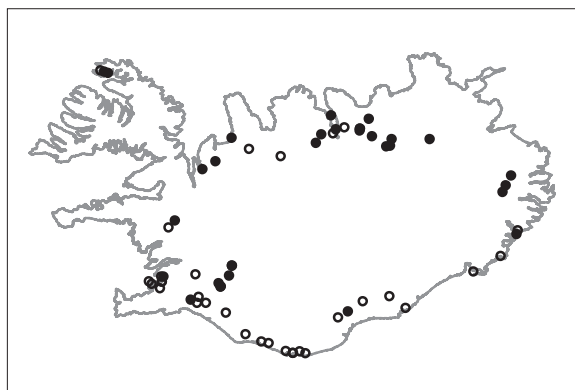
Fig. 5. Locations with oxypeucedanin representing more than the average percentage (9.0%) of the total furanocoumarin content are shown with filled circles and locations with less than the average are shown with open circles.
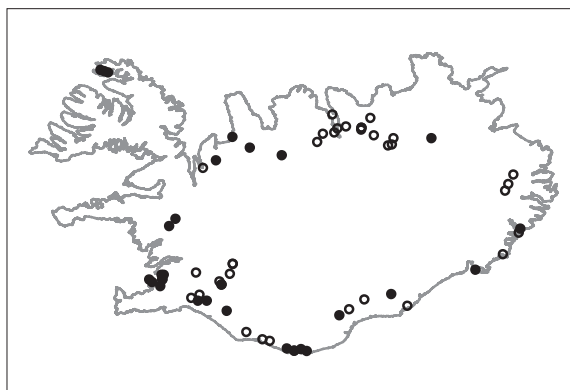

Fig. 7. Locations with xanthotoxin representing more than the average percentage (7.2%) of the total furanocoumarin content are shown with filled circles and locations with less than the average are shown with open circles.
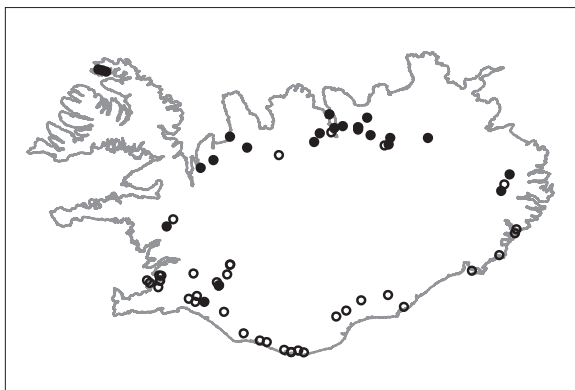

Fig. 6. Locations with "unidentified 2" representing more than the average percentage (2.1%) of the total furanocoumarin content are shown with filled circles and locations with less than the average are shown with open circles.

Table III. Furanocoumarin content (mg/g) in *A. archangelica* fruits from individual plants A, B, C, and D.

Furanocoumarin	Content (mg/g)			
	A	B	C	D
Xanthotoxin	0.4	1.0	1.1	2.0
Unidentified 1	0.4	1.1	0.4	0.1
Bergapten	3.9	1.3	2.1	1.5
Oxypeucedanin	2.6	1.5	1.2	0.0
Unidentified 2	0.8	0.1	0.8	0.0
Imperatorin	14.4	5.6	11.1	7.8
Phellopterin	1.2	2.5	2.5	1.2
Isoimperatorin	2.1	1.8	0.7	1.2
Total	25.8	14.9	19.9	13.8

had no detectable oxypeucedanin or unidentified 2, as was the case for 7 pooled samples from South Iceland.

Discussion

The furanocoumarin composition of Icelandic *A. archangelica* fruits is more or less similar, containing the same compounds in varying contents, and imperatorin being most abundant in all samples.

Besides xanthotoxin, bergapten, oxypeucedanin, imperatorin, and isoimperatorin, which could be identified with standards or purified compounds, the samples analysed by HPLC also contained furanocoumarins which could not be identified with certainty. The most abundant of those compounds was assumed to be phellopterin for reasons given above. The second most abundant of those compounds, unidentified 2, has a UV spectrum similar to those of xanthotoxin and imperatorin. Additionally, prangenin could not be quantified, due to an unidentified compound, possibly isopimpinellin, having the same retention time in the HPLC analysis.

The differences in composition relate mainly to oxypeucedanin and unidentified 2, which are totally absent in some locations in South Iceland but account for more than 8% each in other locations (in North Iceland).

Samples with a high proportion of isoimperatorin and bergapten were much more likely to be found in South Iceland than in North Iceland,

while the opposite was true for oxypeucedanin and unidentified 2. Samples with a high proportion of xanthotoxin were more likely to occur in the western half of Iceland.

When fruits from individual plants were analysed separately, more differences were seen. The four individuals from the same location, growing within meters of each other, showed great variation in furanocoumarin quantity and composition, respectively. One individual was devoid of oxypeucedanin and unidentified 2, like seven samples from South Iceland, one contained very little xanthotoxin and assumed phellopterin but much imperatorin. This indicates genetic control of quantity and composition of furanocoumarins and makes the idea of culturing or cloning individuals with desirable properties, such as high furanocoumarin content or high imperatorin content, feasible.

Taken together, the present results indicate some geographical impact on the furanocoumarin composition of Icelandic *A. archangelica* fruits, and that genetical factors may contribute significantly to the relative quantity of individual components. The results further demonstrate the importance of the analysis of plant material, as material from the same species can be highly diverse in its secondary metabolite composition.

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