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VARIATIONS IN THE ESSENTIAL OILS OF *TANACETUM VULGARE*

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Key Word Index—*Tanacetum vulgare*; Compositae; essential oils; chemical variation; plant geography.

Abstract—The essential oil content of Hungarian samples of *Tanacetum vulgare* varies from 0·02 to 0·66% and exhibits a heterogeneous distribution indicating genetic variation. The rotatory power of the essential oil is also widely divergent and plants containing laevo-rotatory essential oils mainly occur in large numbers in the region east of the Tisza. The essential oil composition of wild plants remained constant during their cultivation. 26 different chemoforms have been found so far in Hungary. Individuals and populations containing artemisia ketone and umbellulone as their main components are the most frequent.

INTRODUCTION

The essential oil of *Tanacetum vulgare* L. [*Chrysanthemum vulgare* (L.) Bernh.] has long been used in medicine. Characteristically it contains about 50% thujone and is dextrorotatory [1]. However, Bittera [2] has reported a leavorotatory oil in a plant collected from the territory east of the river Tisza. More recently, detailed studies have been made of the chemical composition of essential oils from various populations of the species growing in West Germany and Finland [3–7]. Six chemical races were found in Germany, and 8 in Finland [8].

On the basis of these findings, it seemed worthwhile to study the *Tanacetum vulgare* populations found in Hungary, from the point of view of their essential oils.

RESULTS AND DISCUSSION

Essential oil content

As early as 1969, it was noticed that essential oil contents of individual samples collected from various sites in the country were very different. According to the literature, variations between 0·2 and 0·5% have been recorded. These differences were apparent during the following years, so that it seemed necessary to decide if these differences were due to the natural variation in essential oil content. Thus the data of the year 1972 were subjected to a distribution study (Fig. 1.). It is evident that the domestic material is significantly heterogeneous as regards essential oil content since the obtained χ^2 value significantly exceeds the expected value for the normal distribution. Thus the essential oil content of 450 samples examined changes from 0·02 to 0·66%, the mean $|\overline{X}|$ being 0·13136 and s.d. having a very high value, 0·0848. The distribution of values is abnormal because they do not coincide with the peak of the probability curve (Fig. 1.). The differ-

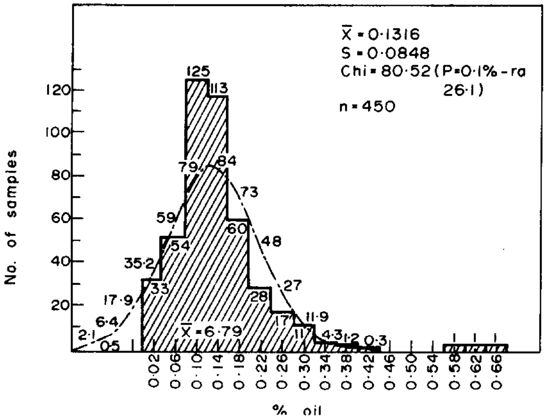

Fig. 1.

Table 1. Relationship between the site of origin of the sample and the optical rotation of the essential oil

Site of origin*		$\alpha_{\rm D}$(°)	Site of origin*		$\alpha_{\rm D}$(°)
NDB	Szendehely	−55·60		Szikszó	+6·30
TD	Rácegres	−55·40	ET	Kőrösszakál	+6·60
NDB	Szendehely	−47·40	ET	Mátészalka	+7·30
NDB	Szendehely	−40·60	TD	Daránypuszta	+8·20
NDB	Szendehely	−40·00		Tószeg	+8·50
ET	Tiszafüred	−27·75		Tószeg	+8·80
NDB	Szendehely	−24·80	TD	Dáránypuszta	+9·15
TD	Gödre	−18·80		Mátészalka	+9·70
NDB	Tésa	−16·00		Répáshuta	+10·10
TD	Daránypuszta	−15·60	ET	Szarvas	+10·50
NDB	Moson	−15·00	TD	Daránypuszta	+12·86
ET	Nagykereki	−13·40	ET	Sarkad	+13·60
NDB	Szendehely	−12·10	ET	Martfü	+13·70
ET	Csenger	−8·80	ET	Martfü	+14·30
TD	Nagyharsány	−8·20	TD	Gödre	+16·70
ET	Biharugra	−7·40	TD	Daránypuszta	+17·70
ET	Nagykereki	−7·30	TD	Magyarboly	+18·30
ET	Tiszakerecseny	−6·40	ET	Makó	+18·70
ET	Kunszentmárton	−6·00	TD	Szekszárd	+18·90
ET	Tiszakerecseny	−5·98		Sarkad	+19·00
NDB	Drégelypalánk	−5·41	ET	Csenger	+20·80
ET	Tiszaszederkény	−3·80		Sarkad	+21·60
ET	Pocsaj	−3·10	ET	Fehérgyarmat-Kisár	+21·80
	Kisterenye	−3·00		Dédestapolcsány	+22·30
TD	Magyarboly	−2·80	ET	Kunszentmárton	+23·20
ET	Fehérgyarmat	−2·70	TD	Pacsa	+23·40
ET	Mérk	−2·40		Szolnok	+24·60
NDB	Drégelypalánk	−1·80	TD	Kapuvár	+24·97
ET	Fehérgyarmat	−1·50	TD	Kapuvár	+26·50
ET	Fehérgyarmat	−1·50		Borsodbóta	+27·10
	Őrhalom	+0·15	TD	Tát	+28·70
TD	Kisdobsza	+1·22	TD	Rácegres	+29·00
NDB	Gönyü	+1·90	NDB	Verőce	+39·40
TD	Ivánc	+3·17	TD	Neszmély	+41·10
	Hollófészek	+3·30		Sarkad	+41·10
	Gibárt	+3·40	TD	Zalacséb	+46·50
ET	Tivador-Gulács	+4·50	ET	Gyulavári	+46·70
ET	Fehérgyarmat-Kisár	+4·50	TD	Viszák	+52·10
ET	Tivador-Gulács	+5·40			

* TD = territory west of the Danube; ET = terriroty east of the river Tisza; NDB = territory of the hilly area north of the Danube.

ence is highly significant at the 0·1% probability level. On the basis of these calculations, it appears that oil samples fall into at least two groups.

Optical rotation

The rotatory powers of our samples varied in 1969. According to the literature data, the optical rotation should vary from −4°50′ to +15°34′. According to our data (Table 1.), a relationship exists between the rotatory power and the site of the origin. It was found that samples collected from the Transdanubian territory furnished a dextrorotatory oil, while those from the region east of the Tisza were mainly laevorotatory (Table 1).

Composition of the essential oil

It was found as early as 1969 that the chemical compositions of the essential oils originating from various sites in Hungary are very different. On the basis of investigations carried out in 1970, it was found that "peak 7"—which was not known at that time—is characteristic of the essential oil of plants occurring in Hungary. Simultaneously, it was concluded that this component might be responsible for laevorotation. According to our present knowledge, this component is artemisia ketone; indeed, all our samples containing more than 50% of this component are laevorotatory.

Table 2. Samples containing more than 50% of the main component

Stock number	Site of origin	Main component (%)
1421/1/2	Gyulavári	66·2 (11)
M2/1	Rácegres	54·2 (11)
1424/2	Kőrösszakál	60·2 (11)
N/2	Dáránypuszta	56·2 (17)
1429/1	Tiszafüred	80·7 (16)
474/3	Baja	69·5 (7)
468/2	Márk	66·3 (7)
1445/4	Kisdobsza	59·1 (70)
494/4	Kerkakutas	91·4 (10)
497/4	Kapuvár	82·8 (10)
98/2	Miskolc	75·5 (10)
483/1	Tótszerdahely	66·1 (12)
480	Nagykanizsa	65·8 (12)
497/1	Kapuvár	58·0 (12)

In 1971, the essential oils of 120 samples were analyzed. The most interesting samples, containing the main component of the essential oil in amounts of more than 50%, are shown in Table 2.

Samples collected in the course of 1972 proved—in accordance with earlier years—that the compositions of essential oils are very varied. The extreme cases are summarized in Table 3; 26 chemical forms varying in essential oil composition have been found. Although the five samples collected from each of various areas were identical in several cases, we also sometimes found two chemoforms in the ratio 3:2. It was never observed that the five samples collected from any one site were all different. Of the 26 components present in the essential oil, 17 were identified, partly on the basis of the analysis carried out in Canada and Paris and partly from our own knowledge of components present.

For further evaluation, other factors need investigating. It is possible that the differences are due to climatic factors. However, by testing the collection at Budakalász over the course of several years, this variable was eliminated. In the years 1970–1971 and 1971–1972, respectively, we examined identical plant material, i.e. genetically homogeneous clones. During these years, the weather varied widely and furnished an adequate basis for distinguishing genetic and climatic effects. The clones differed according to whether their main component was borneol, artemisia ketone, thujone or umbellulone. In the years 1970–1971, we studied nine components of five clones and in the years 1971–1972, six components of three other clones. For both groups under investigation, with a probability of more than 99·9%, the data were independent of the year of origin. Thus, the oil composition is genetically determined (Table 4).

In the Hungarian flora, we have not been able yet to determine all the occurrences of *Tanacetum vulgare* taxa with every different kind of chemical composition. According to our investigations, the taxa with the following main components have been located: pinene + camphene, chrysanthenyl acetate, cineole, γ-terpinene, artemisia ketone, thujone, camphor, borneol + bornyl acetate and umbellulone. In cooperation with Lawrence in Canada, we also succeeded in identifying a novel taxon with piperitone as the main component. The taxon containing thujyl alcohol has not been found so far and we have been unable to identify another six major chemoforms. Although the study is not yet terminated, it can be concluded that artemisia ketone and umbellulone are the most common main components. These components—either individually or together—furnish the bulk of the essential oil in nearly 40% of the samples and together with a third component they represent the bulk of the oil in a further 25% of the samples. This combined ratio of occurrence of 2:3 is characteristic of the Hungarian populations of *Tanacetum vulgare* just as are camphor-thujone components in Finnish plants, isothujone in Canada or the monoterpene hydrocarbons in plants of Western Europe.

EXPERIMENTAL

Plant material. Wild plant samples were collected during 4 seasons since 1969 from the whole area of Hungary (Fig. 2). In 1970, plants were brought into cultivation at our Institute in Budakalász and since 1971, suitable individuals were propagated by cloning when necessary. In 1969 essential oils were isolated from fresh flowering shoots with a stem of 10 cm, collected from 16 areas of the country. Since 1971, flowers from introduced stocks were sampled at the end of August or at the beginning of September, in all 120 samples being taken. Essential oils of wild plants under cultivation were examined again in 1972 and in addition 5 samples were collected from each of 88 sites in Hungary.

Analytical procedures. The essential oil was obtained by means of Clevenger steam distillation for 2 hr and then dried over Na_2SO_4. For dry wt determination 10 g samples were dried at 105° to constant weight. Rotation of the oil was

Table 3. Main components of

Serial no.	Percentage of the main components from the integrator values GLC analysis									
	1–2	3–4	5	6	7	8	10	11	12	13_{a1}
1	14·7	20·3	14·8							
2	23·8	30·6								
3			42·2		15·7	23·4				
4				33·3	46·2					
5					31·5	18·2				
6					82·2					
7					38·3					
8					56·5					
9							94·0			
10							29·3			
11								74·5	22·0	
12								16·8	70·7	
13								22·8		
14									56·4	
15									77·2	
16										42·2
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										

Peaks 1–2 = α- and β-pinene; 3 = camphene; 5 = 1,8-cineole; 6 = γ-terpinene; 7 = artemisia ketone; 8 = yomogi alkohol; 10 = thujone; 13_{a1} = camphor; 13_{a2} = chrysanthenyl acetate; 13_b = bornyl acetate; 15 = thujyl alkohol; 17_a = borneol; 17_b = umbellulone; 19 = piperitone; the other components are unknown.

Table 4. Investigation of genetically homogeneous clones in the years 1970–1971 and 1971–1972

Sample no.	Year	Percentage composition on GLC analysis								
		7	10	11	12	13_b	15	17_{ab}	19	26
N2	1970	23·0		31·0						
N2	1971	1·4		37·8						
M2/1	1970	5·1		19·3	56·0	7·7				
M2/1	1971	6·5		20·3	54·2	3·1				
L2/1	1970	21·6		5·8	12·6	8·9				
L2/1	1971	37·5		5·9	17·8					
1421/1	1970			16·7	54·1	7·0		2·0	3·0	7·0
1421/1	1971			15·4	66·2			5·0	4·1	
1457/2	1970		88·4							
1457/2	1971		81·3		13·0					
1461/1	1971	7·5		7·0	42·6		3·8			
1461/1	1972	13·6		16·6	52·7			12·8		
483/1	1971			27·5	35·0		7·0	4·5	11·6	
483/1	1972	1·9		23·9	58·4		2·8		4·8	1·9
1437/2	1971	15·4		24·5		15·8		18·7		9·7
1437/2	1972	22·8		22·8				48·0		

At the level $P = 0{\cdot}1\%$ *the tabular* χ value is higher; $r = 0{\cdot}09$ or $r = 0{\cdot}13$, respectively; the composition of the active agent is independent of the year of origin.

selected *Tanacetum* samples

13_{a2}	13_b	14	15	16	17_a	17_b	18	19	23	α_D
										+
										+
										–
										–1·8
				33·3						
						22·5				–13·5
										+
					42·6					+
										+
										+41·8
							48·0			
									36·2	
										+41·4
					23·6					–
53·0										+10·5
	41·2				30·2					
		95·0								
		21·6	63·9							
			84·0							
				77·5						+
					54·4					
					31·4			23·1		
						81·5				–
							94·0			
								64·2		

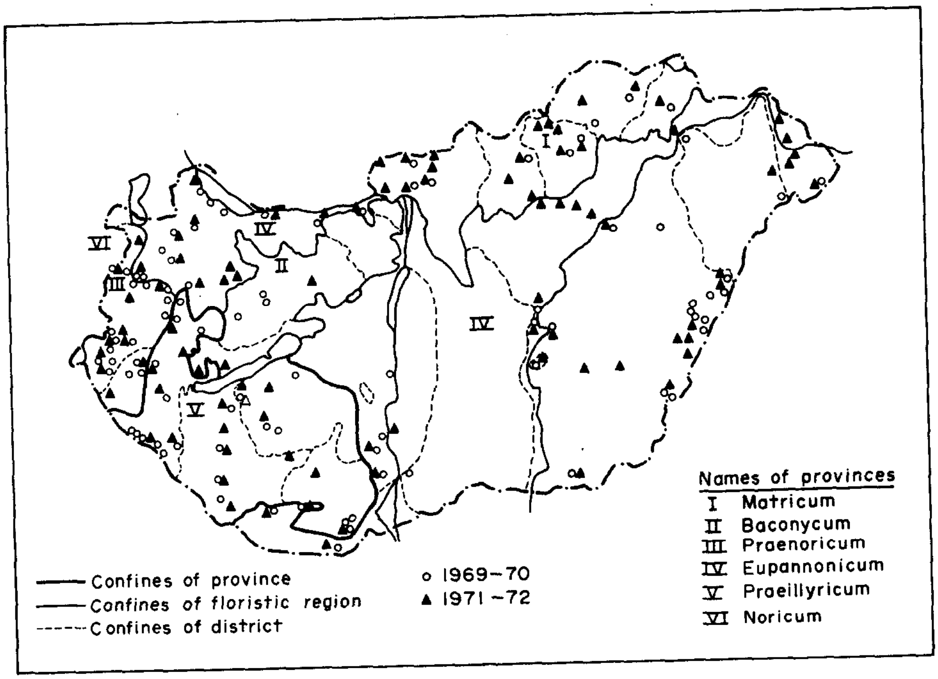

Fig. 2.

measured with an Opton photoelectric polarimeter in a 10 mm cell at 2 wavelengths and extrapolated to the Na_D line using the given formula $\lambda = 589{\cdot}2$ nm. When the amount of essential oil was only enough for determining the direction of rotation, this was measured in benzene. GLC analyses were carried out at 200° with N_2, 15 ml/min; using ϕ 3 mm × 1·75 m with 10% OV 17 on Chromosorb G AW and 10% DEGS on Chromosorb W sil. Each sample was run simultaneously on both columns. Analysis values from the more selective DEGS column were recorded with an electronic integrator. Quantitative determination was carried out using internal standards. Qualitative evaluation was carried out using as standard the essential oil of the sample from Nagykereki which gave 26 peaks on the DEGS packing. Our standard oil was analyzed in the Paris centre of the Jeol Corp. with the cooperation of T. Okuda, by means of GC/MS in 1970. In 1972, Brain M. Lawrence and coworkers carried out the analysis of 5 of our samples in Canada. In addition to the quantitative and qualitative tests by GLC the rotation of the essential oils was measured on an Opton photoelectric polarimeter.

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