

## ANNUAL RESEARCH REPORT

1. Name and address of reporting institution:

INSTITUTE OF HOP RESEARCH, Ž A L E C, YUGOSLAVIA

2. Name of principal investigator:

Dr. TONE WAGNER mr. pl. nut., dipl. ing. agr.

3. Project title:

AUTOCHTHONOUS HOP IN YUGOSLAVIA AND ITS USABILITY FOR  
BREEDING NEW VARIETIES IN COMPARISON WITH THE HOP VARIETIES  
GROWN AT PRESENT

4. Grant number: FG - YU-186, P-ZF-2

5. Dates of research period covered by report:

April 1, 1974 to March 31, 1975



A N N U A L   R E S E A R C H   R E P O R T

A. TITTLE PAGE

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## B. SUMMARY

1. Exploration of Wild Hop Habitats was done in two different regions, in Istria and in regions of Serbia and Macedonia. The plant wasn't spread in central and southern Istria. It is very frequent in the northern part of Istria. In regions of Serbia and Macedonia many habitats were developed according to different ecological condition in valleys, on banks of rivers and brooks and along the ditches. The frequency and development <sup>of</sup> plant grew smaller with altitude.

The morphological characters of plant were determined and where the cones had been formed they were collected and analyzed. The plants were mostly climbing with red bine and reddish petiola. The 3-5 lobed leaves prevailed. The cones were always light - green and downy mildew is very frequent but mould is rare. In collected cone samples the average was: total resins 12.58 %, soft resins 11.97 %, alpha acids 4,13 %, beta fraction 7,04 %, and the  $\alpha/\beta$  ratio was 0,59.

2. Wild Hop Collection Garden Pobrežje had 150 female and 90 male plants. The sex ratio of male to female plants was 1:1,67.

The female plants had mostly red or reddish bine. The plants were vigorous and had mostly wide columnar and capitate form. The yield of cones was estimated from 1 to 5 points and 52 % of plants were very productive. The majority of plants was middle late and late.

In the collection of male plants the plants with reddish bines prevailed. They had few leaves, they were less vigorous, than female ones but the columnar and capitate forms were mostly developed. Male plants were earlier than female ones.

Susceptibility to downy mildew is different. It was important to choose these plants which were resistant against downy mildew and those which had become infected but were so vigorous that the downy mildew had caused damage. Very and middle susceptible plants prevailed. In collection garden Motnik 6.6 % and in collection garden Pobrežje 11.4 % of plants were not damaged by disease. In garden Pobrežje the pressure of infection was not so very strong as in garden Motnik. Plants of with these level of resistance will be planted separately in next year.

The population of aphids was enormous and only some plants had small or middle population of aphids.

The average content of bitter resins of seeded cones in collection garden Pobrežje is : total resins - 10.78 %, soft resins - 9.62 %, alpha acids - 3.04 %, beta fraction - 6.58 %, hard resins - 1.15 % and ratio alpha/beta is 0.48. The variability of alpha acids is much higher as variability of beta fraction. The correlation between alpha acids and ratio alpha/beta is very high /  $r = 0.8213$  /.

The composition<sup>d</sup> of essential oil is represented by ratios of humulen to caryophylen /H/C /, farnesen to caryophylen /F/C/ and of posthumulen 1 to caryophylen / PH1/C / . The population of female wild plants is divided in two groups according to value of ratio H/C. All old european hop varieties which come from population of wild hop have the value H/C over 3. The wild plants with F/C ratio over 2 are rare; european hop varieties have the F/C ratio from 0 to 2. The presence of posthumulen 1 is characteristic of collection and the ratio PH1/C is mostly over 1.

3. Properties of Wild Hop Families in Year 1974.

831 plants of 16 families were planted and only 384 survived. 205 plants reached the generative phase: 74 males, 1 intersex and 130 females. The yield gave only 115 plants.

Because of a small number of seedlings which survived and reached the generative phase we came to a conclusion that the adaptability of plants was bad. We did not find the real reason why the plants were dying, however, we noticed that the main cause was downy mildew. Downy mildew was widely spread in the families containing luxuriant plants, especially in those with extremely long vegetation. We rarely noticed the signs of diseases on weak bad developed plants.

Powdery mildew was found only here and there in cones and did not cause any greater effect.

Our wild hop middle late, for the prevailing quantity of plants was ripe only in the middle of September. The families from the south-east of Yugoslavia were very late, they were ripe only in the middle of October.

The forms of late and very late plants were various. Late plants were luxuriant or weak, they were often capitate, but also narrow cylindric and fusiform. Very late plants were extremely luxuriant, capitate or columnar and they had long laterals. Violet bine prevailed, leaves were small therefore it looked like the plant had few leaves. The leaves often turned yellow and fell off.

Cones were in clusters, especially those of very late plants, they were small, light green, often reddish. They had red strigs, they were loose and had extremely gold-yellow lupulin. Aroma was typical, it was unpleasant and empty, however, it was not intrusive.



Very late hop had higher chemical value than the late, for it had the average of 4,8 % alpha acids, the late hop only 2,8 %. Ratio of alpha to beta acids was comparatively favourable, round 1, it was more than 1 for very late hop.

In some families the individual plants had a lot of alpha acids, almost to 8 %. We shall examine these individual plants closely and include them in the brewing programme.

4. Examining and introducing the methods of chemical analyses of hop included the analyses of bitter resins, determination of storage stability, essential oil and polyphenols in hop.

4.1 Wöllmer's analysis we used Rotavapor for steaming instead of standard distillation. So we analysed the double amount of samples and the explosion of ether was avoided.

The introducing of spectrophotometric method was done, the method was rapid determining of alpha-acids and beta - acids instead of beta - fraction. We examined also the method of 5 cones with Savinja Golding and A -varieties. The conventional spectrophotometric method gave better agreement of parallel results as the method of 5 cones which was more rapid and cheaper. The modified spectrophotometric method of 5 cones and Wöllmer's conductometric method showed very good agreement of results also for low values of alpha acids and also for the wild plants. The determination of alpha acids in fresh hop is very important for rapid determining of hop ripeness. The Likens's method could be used for common determining.

4.2 Storage stability is an important property of hop. The method of accelerated oxidation of resins is very efficient. The degree of oxidation for variety Apolon is rather greater than it is for another hybrid.

4.3 The gaschromatography of essential oil showed that myrcen is very variable substance, for its quantity varied greatly for the same variety when analyses were repeated.

Determination of the composition of essential oil of male flowers was done by injection of extract of lupulin through forecolumn filled with the Celit 545. We didn't notice myrcen in any male sample and some of them were without farnesen which is also characteristic of some male plants.

The GLC lupulin extract of 5 cones was tested and we got good results.

Quantity of polyphenols was determined in the samples of Savinia Golding, Saaz, and Yakima and it couldn't be taken as variety's characteristic according to the high deviation among individual samples.

### C. DETAILED REPORT

#### C/1/ Introduction

The work during the research period has been the continuation of the project E 30 CR 90. In this year the spreading and properties of wild hop in two different regions, the characters of collected plants and their families were studied and also some new chemical methods were included in the project.

#### C /2/ List of Scientists and the Technicians

and the percentage of their time devoted to the project during the annual report period

1. Dr. Tone Wagner, principal investigator, graduate in agronomy, master in plant nutrition, doctor in biology . . . . . 50 %
2. Mrs. Dragica Kralj, graduate in agronomy, master in genetics and plant breeding . . . . . 50 %
3. Milan Žolnir, graduate in agronomy . . . . . 16 %
4. Mrs. Marta Dolinar, graduate in biology . . . . . 16 %
5. Peter Grilanc, master in chemistry . . . . . 16 %
6. Mrs. Mira Hrovat, technician in chemistry . . . . . 100 %
7. Mrs. Nežika Medved, technician in agronomy . . . . . 100 %
8. Prof. dr. Franc Sušnik, professor of University of Ljubljana, doctor of biology - by contract
9. Milan Lovka, assistant of University of Ljubljana, graduate of biology - by contract.

C /3/e C/5/ Experimental Procedures, Results  
and Discussion

1 Habitats Explorations

1.1 Exploration of Extention of Wild Hop

In 1974 the extention of wild hop was explored particularly in some ecologically different regions: at west in Istria, at east and at south - east in regions of Serbia and Macedonia. In Istria the southern part of penninsula was explored particularly, where the influence of mediterranean climate is more expressive than in northern part. /Map I/ The exploration of extention of plant was done on the line : Buje - Pula - Rakalj - Žminj - Pazin - Cerovlje - Vranja - Opatija - Ilirska Bistrica - Vremski Britof. The presence of plant was determinited on each 10 km. We found two habitats only:

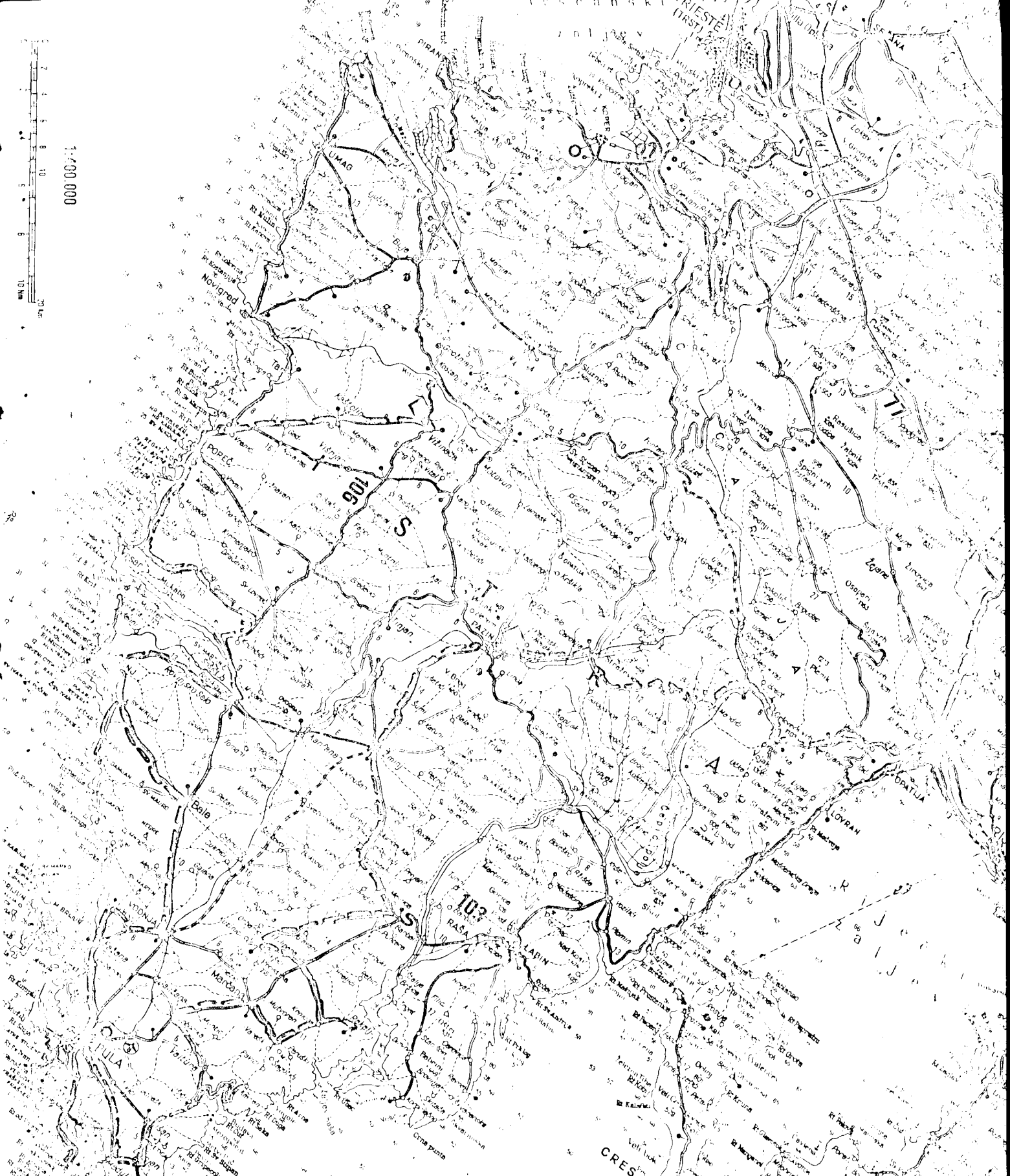
- Vižinada: the plant was climbing on hedge, had red bine and many spikes :
- Vremski Britof: in the valley of river Reka the big habitat of wild hop was found.

The plant wasn't spread in central and in southern Istria where it wasn't found in spite of detailed explorations. It was very frequent in the northern part of Istria which was known from the explorations of earlier years. The habitats were found in valleys of rivers Vremski Britof at river Reka.

In Serbia and Macedonia the particular explorations were done in geographycal regions: Šumadija, East Serbia, South - East Serbia, Kosovo, West Macedonia, Middle Macedonia, and East Macedonia /Map II/.

In all regions the habitats were spread quite well. The smaller habitats were found on total line of investigations: in some places the plant was spread more, but the vigorous and big habitats were rare. The exploration of plant began at Svetozarevo and at Bagradan the wild hop was found. From Bagradan to Kruševac the little habitats were spread along the local road . From Kruševac to Veliki Šiljigovac the plant was found but it wasn't below the mountain Veliki Jastrebac and around the Ribarska Banja. On the line Kaonik - Deligrad the vigorous habitats were spread. South of Aleksinac in place of Donji Krupac and in the vicinity of Vrela the smaller habitats were developed, but Popšica /from Vrela to Svrljig/ the big habitat of wild hop was found. The plant was present in Labinac and in Merdjelat at Svrljig. The bigger habitat was at the river Svrljigški Timok in place Okrugljica. Smaller habitats were found at Miranovac, along the road Svrljig - Bela Palanka, at Gornja Glama, and in the vicinity of Bela Palanka. The small habitats were at Maranovac, along the way Svrljig - Bela Palanka, Gornja Glama, in the vicinity of Bela Palanka. From Bela Palanka to Leskovac the individual plants were found /Hudurnik, Babušnica/ but their vitality and frequency were smaller with higher altitude. Some habitats were also found in villages Korbevac at Vranjska Banja, in Donja Lubata, and in the vicinity of Bosiljgrad. On line from Kumanovo to Sveti Nikola many habitats of wild hop were found. The plant was developed in Lukovica / in the vicinity of Kočani/. Some habitats were at rivers Raec and on the banks of Crna reka, south of Rosoman. In western Macedonia the plant was found in the vicinity of Prilep and on the north - western hills at village Debrešte along the road Prilep - Kičevo about 700 m high. The bigger habitats were at Makedonski Dvor and at Gostivar on the bank of river Vardar.

In the region of Kosovo the wild hop was very rare. A little habitat was found at village Dobrluka.

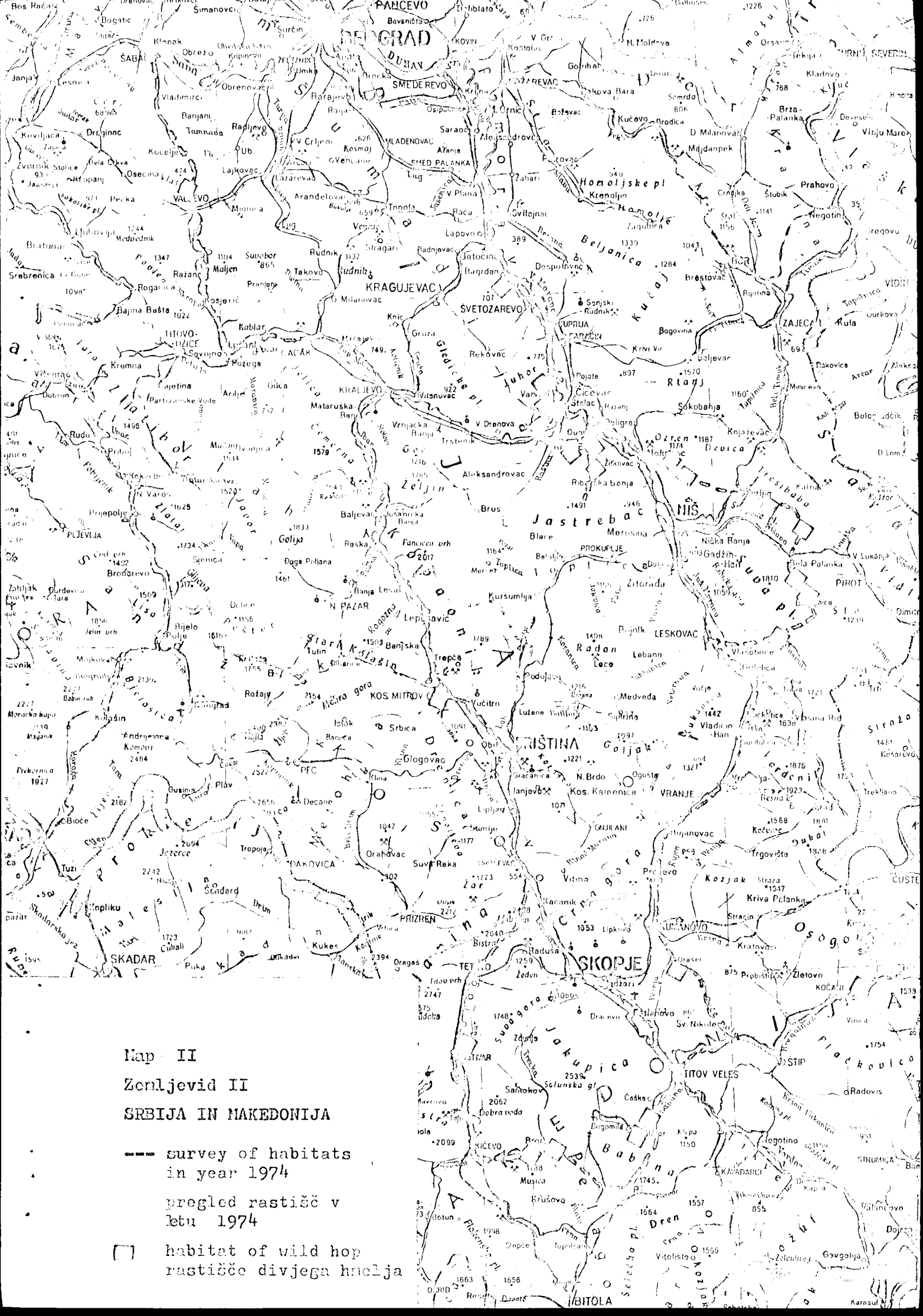


Map. I

Zemljevid I ISTRA

----- survey of habitats in year 1974  
 pregled rastišč v letu 1974

□ habitat of wild hop  
 rastišče divjega hmelja



Map II

Zemljevid II

SRBIJA IN MAKEDONIJA

--- survey of habitats  
in year 1974

pregled rastišč v  
letu 1974

□ habitat of wild hop  
rastišče divjega hmelja

The conclusion is: many habitats, which were developed differently according to the different ecological factors, are in valleys, on banks of rivers and brooks and along the ditches. The frequency and development of plants grew smaller with altitude.

Comparative explorations of spreading of wild hop show that the habitats were rare in middle Istria, in south Istria the plant was not found. In eastern geographical regions the plant was very frequent, many habitats were found - the ecological conditions are suitable in many locations for spreading of wild hop.

## 1.2 Properties of Wild Hop in Habitats

### 1.21 Morphological Characteristics

In habitats the morphological characteristics were determined and where the cones had been formed they were collected and analysed. We collected 17 samples of cones. 39 habitats were described: 4 in vegetative phase and 35 in phase of cone - forming or in physiological ripeness. We determined 22 female plants, 12 male plants and 5 plants where the sex was not able to determine.

The plants were mostly climbing /27/, some of them were in bush-form and one was creeping. The individual or bushy form is most frequent.

The bines were red /20/, some of them were green /12/ and only one plant had violet colour of bine. The pubescence is of middle density. The leaves were green /17/, some of plants had light green /9/ and dark green /8/ leaves, but only one plant had yellow - green leaves. The petiolar is reddish /22/.



The 3-5 lobed leaves prevailed /19/, some plants had 3 lobed leaves /5/ and one plant had 3 to 8 lobed leaves. Leaves were roughly or middle dentated with middle deep or shallow clefts. Leaf is of middle pubescence.

The laterals are simple or branched. The flowers are in bunches or stand individually. The plants have a lot of flowers. The cones were always light - green. The mould on cones is rare, but the downy mildew is more frequent. The mould is also rare on leaves but the downy mildew is quite frequent. On some plants the aphids and red spider were spread.

#### 1.22 Chemical Analysis of Cones

We collected cones from 17 habitats especially in regions of Serbia and Macedonia. The cones were seeded but they had quite a quantity of bitter resins. The average values are:

total resins 12.58 %, soft resins 11.17 %, alpha acids 4,13%, beta fraction 7.04 % and the  $\alpha/\beta$  ratio is 0.59. The particular data of chemical analysis are present in table 1.1.



ЗА ОКРУГЛИЦО  
ПОД КЛАНЦЕМ ♀

33A42,  
Okruglica



MIRANOVAC  
13 ♀

33A43, Miranovac



34A27, Korbeřad



34A29, Vodenice

## Chemical Analysis of Cones from Habitats in Year 1974

Habitat	Total resins	Soft resins	Alpha acids	Beta fraction	Hard resins	In total resins %			Bitter value	Antiseptical value	
						alpha	beta	hard resins			soft resins
20A17	10.18	9.36	2.92	6.44	0.82	28.68	63.26	8.06	91.94	3.63	50.07
31A05	11.31	10.46	3.57	6.89	0.85	31.56	60.92	7.52	92.48	4.33	5.87
32A22	14.47	12.49	3.73	8.76	1.98	25.78	60.34	13.68	86.32	4.70	6.65
33A41	17.27	15.83	5.58	10.25	1.44	32.41	59.35	8.34	91.66	6.72	9.00
33A42	15.94	14.16	5.85	8.31	1.78	36.70	52.13	11.17	88.83	6.77	8.62
33A43	11.90	9.57	3.32	6.25	2.33	27.90	52.52	19.58	79.42	4.01	5.40
33A44	12.86	11.81	4.97	6.84	1.05	38.65	53.19	8.16	91.84	5.73	7.25
34A18	10.95	8.98	3.74	5.24	1.97	34.16	47.85	17.99	82.01	4.33	5.49
34A20	8.92	8.33	3.40	4.93	0.59	38.12	55.27	6.61	93.39	3.95	5.04
34A21	8.08	7.26	3.06	4.20	0.82	37.87	51.98	10.15	89.85	3.53	4.46
34A26	15.05	13.99	5.75	8.24	1.06	38.20	54.75	7.05	92.95	6.66	8.50
34A29	13.87	11.95	4.46	7.49	1.92	32.15	54.00	13.85	86.15	5.29	6.96
35A03	11.91	10.84	4.57	6.27	1.07	38.37	52.64	8.99	91.01	5.27	6.66
36A06	11.33	10.24	2.56	7.68	1.09	22.59	67.78	9.63	90.37	3.41	5.12
40A24	13.91	12.26	4.65	7.61	1.65	33.43	54.71	11.86	88.14	5.49	7.19
40A27	12.35	9.99	3.64	6.35	2.36	29.47	51.42	19.11	80.89	4.34	5.76
41A44	13.49	12.39	4.39	8.00	1.30	32.54	59.30	8.16	91.84	5.28	7.06
Average	12.58	11.17	4.13	7.04	1.40	32.86	55.98	11.17	88.77	4.91	6.48

## 2. Wild Hop Collection Garden

### 2.1 Observations during the Vegetation

The collection of wild hop plants in garden Pobrežje consists of two parts: the first part was planted in spring 1972 and the second in spring 1973. The number of plants in garden according to sex is:

	female	male	total
the first part	72	38	110
the second part	78	52	130
total	150	90	240

The sex ratio of male to female plants is 1:1.67.

#### 2.11 Female plants

The plants with red bine prevailed. The total green bine was determined for plants: 19P03, 33P11, 37P03 and 40P02. The plant 40P02 had extremely dark violet colour of bine.

The plants had more leaves than cultivated varieties. Typical leaf - like form had plants: 07P17, 09P01, 09P07, 11P05, 12P20, 15P07, 20P03, 21P01, 21P03, 26P01, 32P01, 33P02, 33P10, 34P02, 41P26, and 41P27. The plant 11P04, and 40P06 had a little amount of leaves with deep clefts, but plants 16P02 and 35P02 had small of leaves.

In the second part of collection the differences in leaves development were recognized. Plants 17P02, 19P11, 24P08, 33P18, 33P32, 33P34 and 39P02 had a lot of leaves but on the contrary the plants 33P32 and 33P34 had a little amount of leaves. The differences in largeness of leaf was found: the plant 17P02 had big leaves but the plants 19P08, 19P11, 19P14, 19P19, 20P11, and 32P17 had small leaves.

The form of plant ins expressing its viability. So we found stafflike, fusiform, columnar, wide columnar, and capitate form of plant. The frequency of different plant forms is shown in table 2.1 .

Table 2.1

Frequency of different plant forms in %

Collection	Staff like	Fusiform	Columnar	Wide columnar	Capitate
First part	1	2	42	26	28
Second part	6	1	17	26	50

The plants were vigorous, the plants had mostly wide columnar and capitate form. These forms are depended on long laterals. The laterals were formed well and were long.

Plants with a lot of flowers were: 05P06, 11P06, 12P18, 15P06, 16P01, 33P02 and 19P07.

The yield was estimated from 1 to 5 points and in the first part of collection 59 % of plants were very productive, 31 % were productive and only 10 % were less productive plants: but in second part 45 % of plants were very productive, 32 % were productive and 23 % were less productive.

The duration of vegetation was determined by the period from cutting time to technical ripeness. The early plants were ripe in the middle of September, late plants were ripe at the end of September, but very late were ripe in the first half of October. The plants were distinguished by the time of flowering: early plants flowered in the begining of July, middle late in second half of July, late in August and very late in

the first half of September. The majority of plants was middle late and late. In the first part of collection 3 % of plants were early, 28 % middle late, 48 % late and 21 % were very late but in the second part 21 % of plants were middle early, 17 % middle late, 49 % late and 13 % of plants were very late.

#### 2.12 Male Plants

In collection of male plants the plants with reddish bines prevailed. The plants 21P02 and 19P09 had green bine but the plants 13P10, 17P01, 17P07, 24P02 and 33P19 had extremely violet bines. The plants had mostly a little amount of leaves and the distinctive big quantity of leaves was determined for plants: 13P06, 40P03, 26P04, 40P08 and 41P019. The male plants were less vigorous than females, but mostly the columnar and capitate forms were developed. In the collection of male plants 15 % were stafflike, 7 % fusiformed, 49 % columnar, 11 % wide columnar and 18 % capitate form of plant.

Male plants were mostly earlier than female. The early, middle late, late and very late plants were found in relation to the time of flowering. The early plants flowered before 15 th July, middle late in the first half of August, late in the second half of August but very late male plants flowered in September.

#### 2.2 Downy mildew

In year 1974 the downy mildew was studied in both wild hop collection gardens: Pobrežje and Motnik. The collection Pobrežje consisted of all plants but collection Motnik contained most plants which were collected till year 1972. In research program of downy mildew in years 1974 and 1975 it is to determine the susceptibility of wild plants and to choose these plants which were resistant against downy mildew and those which had become ill but were so vigorous that the downy mildew had no effect on them.

The collection Motnik was not treated by fungicides, but the collection Pobrežje was sprayed against downy mildew twice: after the pruning and after the training. The ecological conditions were favorable in both gardens. In June we had 226,9 mm of rain; it is 93 mm over the 20 years average. The pressure of infection was very strong and it was seen on the plants. The infection in collection Pobrežje was weaker probably because of sprayings with fungicides in spring.

In May the number of basal spikes was determined, in June the number of lateral spikes, infection of leaf and infection of all plant were estimated. On the basis of these observations and of the additional observations in August the plants were ranged in groups:

- I. Plants without signs of downy mildew or very little.  
The plant was not damaged by disease.
- II. Plants with middle infection
- III. Plants very infected and plants damaged by downy mildew.

The susceptibility of wild plants was different from plant to plant. Very and middle susceptible plants prevailed. The plants of first group which were infected but enough vigorous or vigour resistant that they were not damaged by disease were of interest.

The plants were ranged into groups according to resistance:

		Motnik		Pobrežje	
		Plants	%	Plants	%
Group of resistance	I	8	6,6	27	11,3
	II	88	72,7	190	79,2
	III	25	20,7	23	9,5
Total		121	100,0	240	100,0



The pressure of infection was very strong in collection Motnik especially in June when there were 21 infections. The plants that had shown resistance in previous years as 40M15, 40M19, 40M20, 40M21 were infected. In collection Pobrežje where the infection pressure was weaker, the collection was treated by fungicides in spring, these plants were less infected. Probably they are susceptible at the beginning of vegetative phase. The distribution in groups of resistance shows us also higher infection in collection Motnik than in collection Pobrežje.

The plants of the first group of resistance will be planted separately in next year according to the brief research work of downy mildew resistance.

### 2.3 Aphids

The population of aphids was observed in both gardens. The population was estimated from 1 to 3 prints. Point 3 presented the most frequent, point 1 the least population where some aphids or none were found.

The population of aphids in wild hop collection gardens was enormous and only some plants had small or middle population of aphids. These plants were: 05P05, 06P02, 12P16, 12P17, 12P19, 12M21, 12P21, 19P01, 21P01, 21P03, 24M01, 24P01, 24M02, 24P02, 33P04, 33M06, 34P04, 34M04, 40P01, and 41M19. In future the special study will be done on resistance against aphids of these plants.

### 2.4 Bitter resins

The samples of cones in collection garden Pobrežje were collected and the Wöllmer analysis was done. So we got the data of to-

tal resins, soft resins, alpha acids, beta fraction, and hard resins. We analysed 133 cone samples and the values of average and coefficient of variation were:

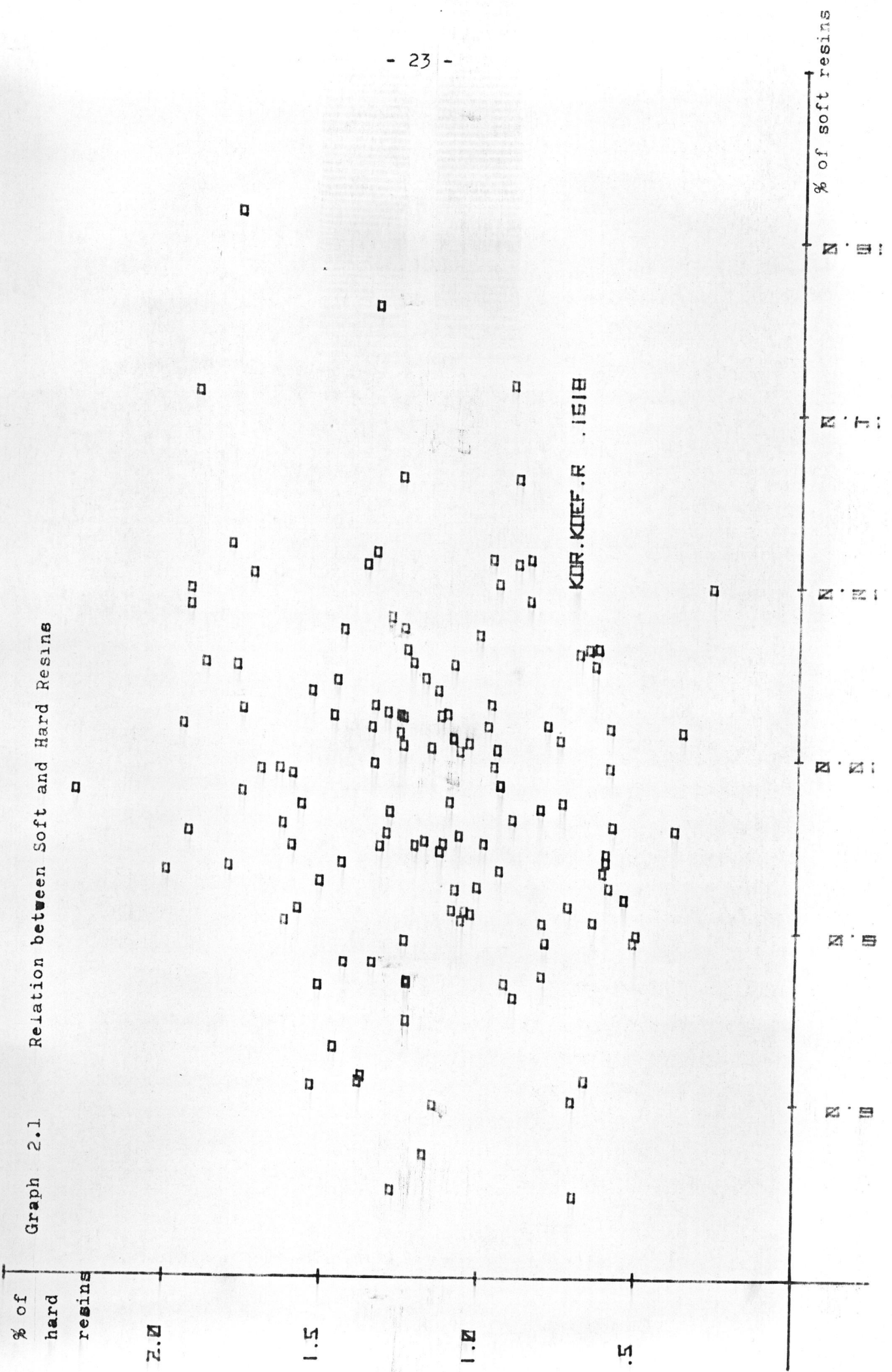
	average	coefficient of variation %
total resins	10,78 %	19,33 %
soft resins	9,62 %	20,50 %
alpha acids	3,04 %	38,00 %
beta fraction	6,58 %	21,62 %
hard resins	1,15 %	34,90 %
ratio alpha/beta	0,48 %	39,19 %

The average of chemical analyses and quantity of bitter resins were high in spite of seeded cones. The population of wild hop is a source of many european hop varieties. In collection the variability of those properties which are more influenced by environment is higher: such as alpha acids, beta fraction, hard resins, and ratio alpha/beta.

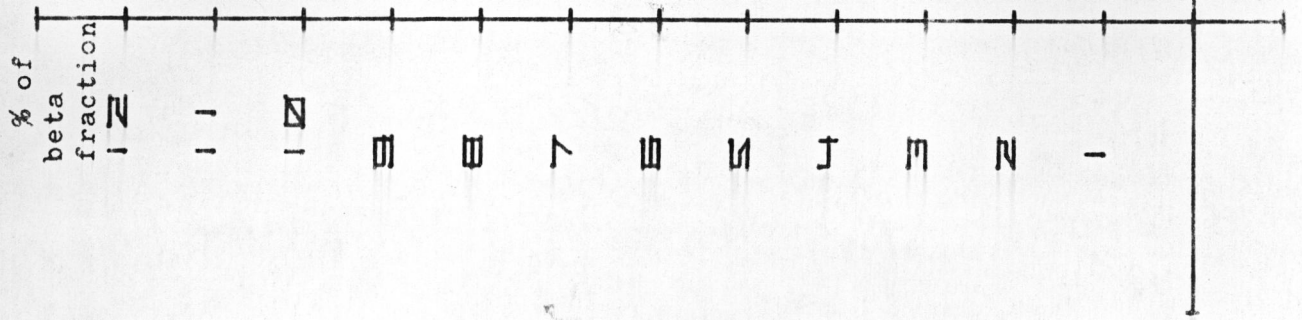
On the basis of chemical analyses the correlation coefficients were calculated among some data. We could not find the correlation of soft and hard resins /  $r = 0,1618$ / and relation is shown on graph 2.1. The correlation /graph 2.2 / is not found between alpha acids and beta fraction /  $r = 0,1629$ /. The variability of alpha acids is much higher as variability of beta fraction.

In graph 2.3 the relation between alpha acids and ratio alpha/beta is shown. The correlation is very high and positive /  $r = 0.8213$ /. On the basis of this relation it is possible to calculate beta fraction and soft resins from the data of alpha acids.

Graph 2.1 Relation between Soft and Hard Resins



Graph 2.2 Relation between Alpha Acids and Beta Fraction



KOR. KOFF. 1522

% of alpha acids

100

90

80

70

60

50

40

Graph 2.3 Relation between Alpha Acids and alpha/beta Ratio

alpha/beta ratio

.9

.8

.7

.6

.5

.4

.3

.2

.1

0

% alpha acids

6

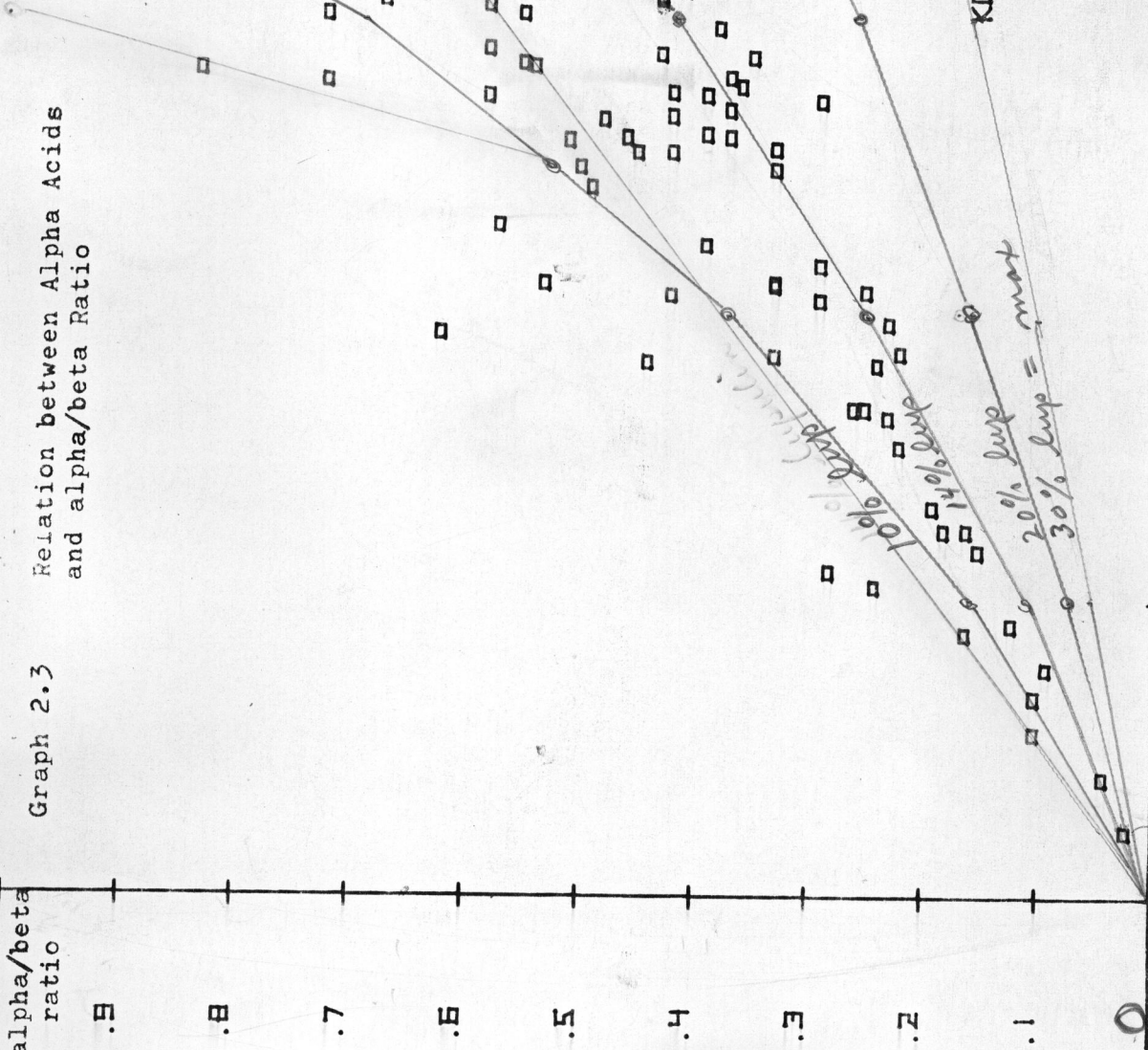
5

4

3

2

1



KOR. KOEF. R .8213

5.15%  $\alpha$ , 5.15%  $\beta$   
%L = 14%

5.9%  $\alpha$   
9.2%  $\beta$   
21% Lyp

3.75%  $\alpha$   
12.9%  $\beta$   
22.8% Lyp

100% (max) Lyp

20% Lyp = max  
30% Lyp = max

$\alpha = 25%$ ,  $\beta = 10%$   
%L = 14%

## 2.5 Essential oil

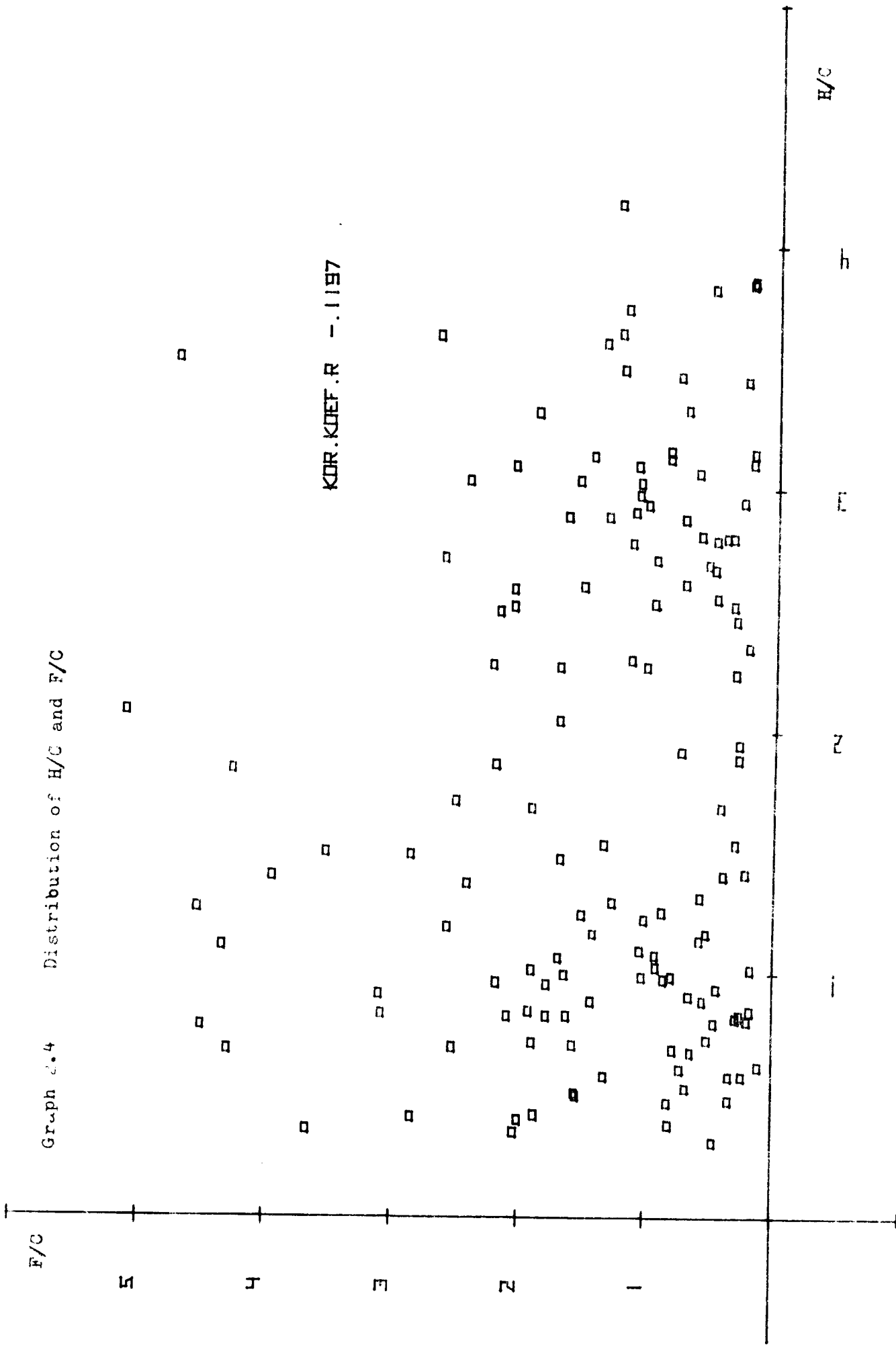
The composition of essential oil of female plants which is characteristic for genotypes is represented by ratios H/C, F/C and PHL/C. The distributions of ratio values are shown in graphs 2.4 and 2.5

The population of female wild plants is divided in two groups according to value of ratio H/C. These groups are in interaction; the group with low values /about 1/ and group with high values/about 3/. All old european hop varieties as local or clonal varieties which were rising from population of wild hop have the value H/C over 3.

The ratio F/C is also very variable, from 0 to 5. The plants with F/C ratio over 2 are rare. European hop varieties have the F/C ratio from 0 to 2. We could not find the correlation between ratios H/C and F/C / $r = -0.1197$ / as is shown in graph 2.4. Graph 2.5 shows the distribution of female plants on the basis of ratio PHL/C and correlation between PHL/C and H/C ratios. The presence of posthumulen 1 is characteristic of collection and the ratio PHL/C is mostly over 1. There is no correlation between ratios PHL/C and H/C /  $r = - 0.1047$  /.

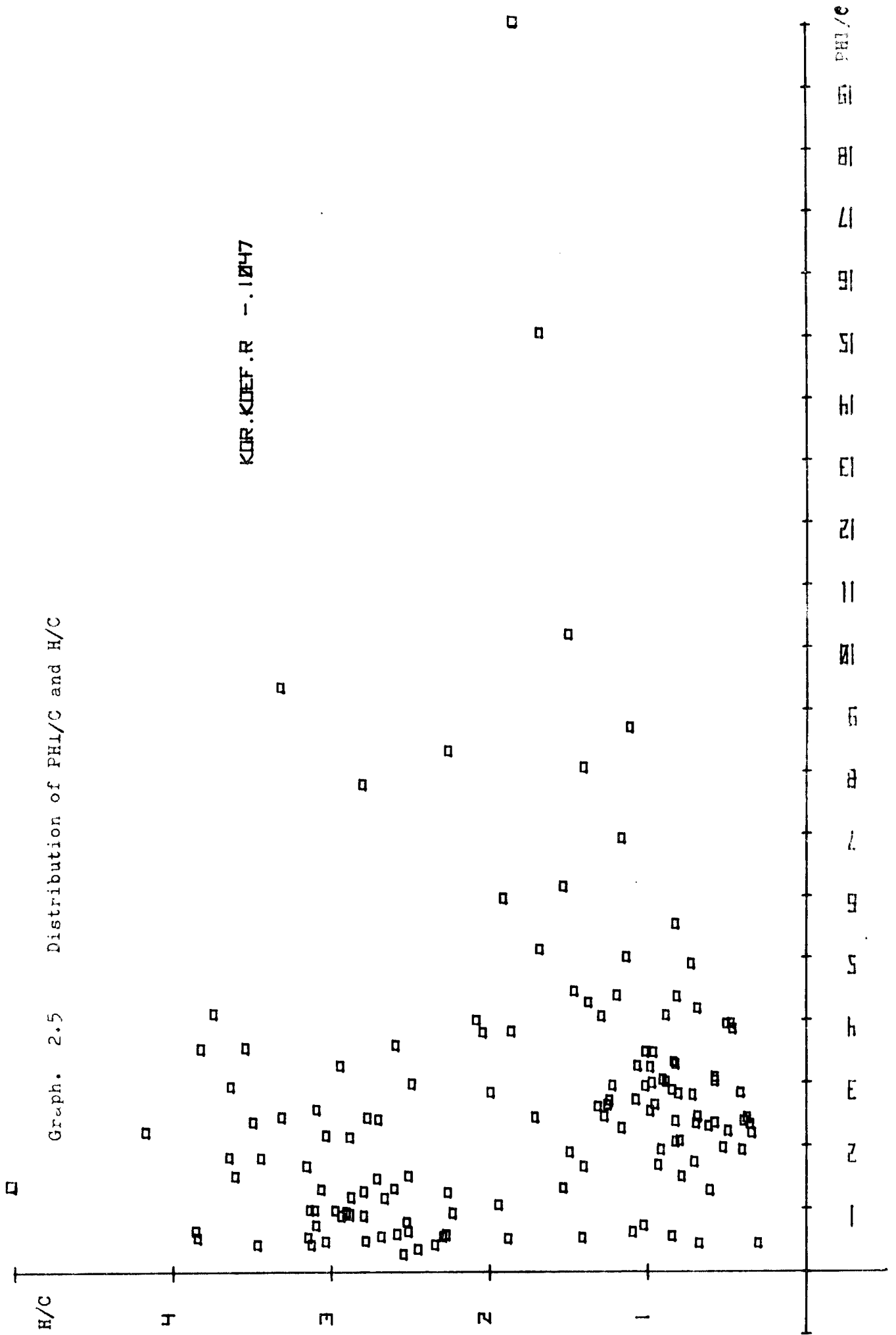
The chromatograms represent the composition of essential oil. They are grouped according to the value of ratio H/C :0-1, 1-2, 2-3 and 3-4. So the typical chemotypes are determined.

In tables 2.2, 2.3 and 2.4 the distribution of plants on the basis of values of ratios H/C, F/C and H/PHL is represented.



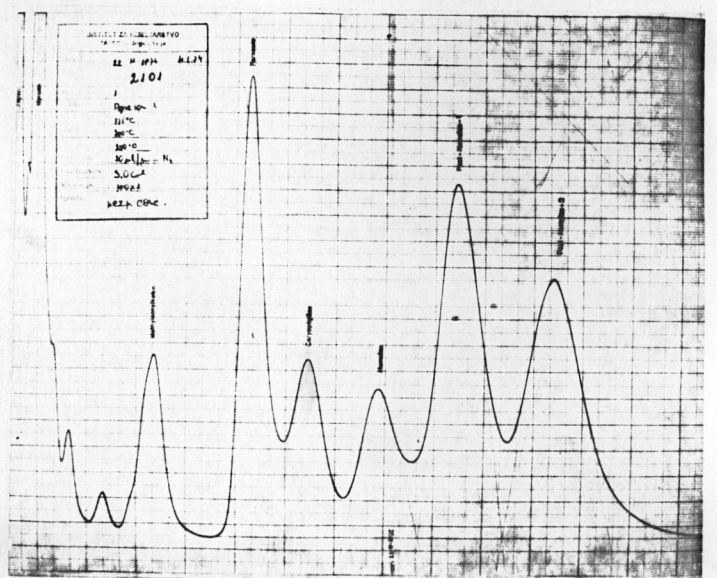
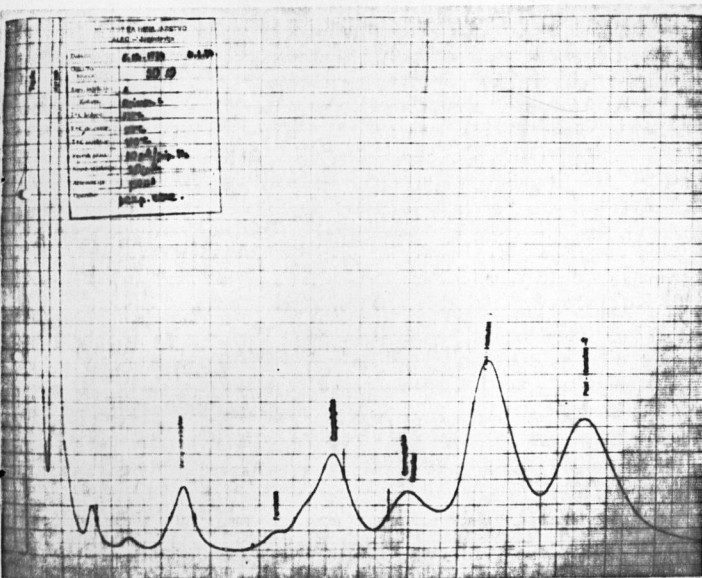
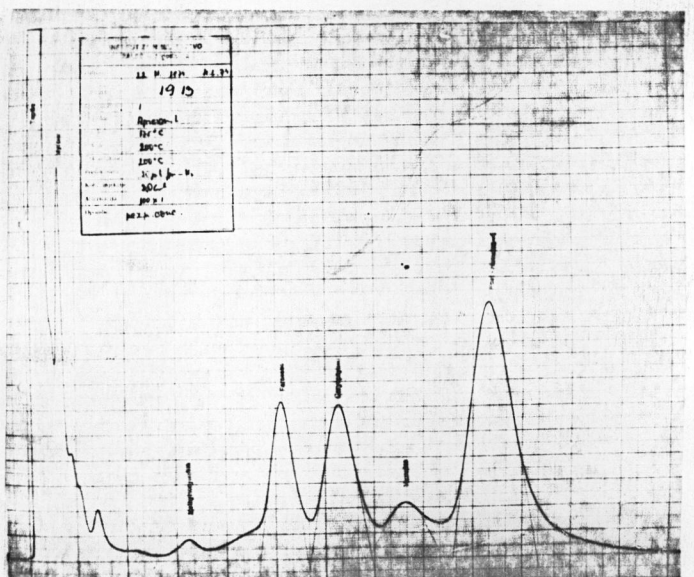
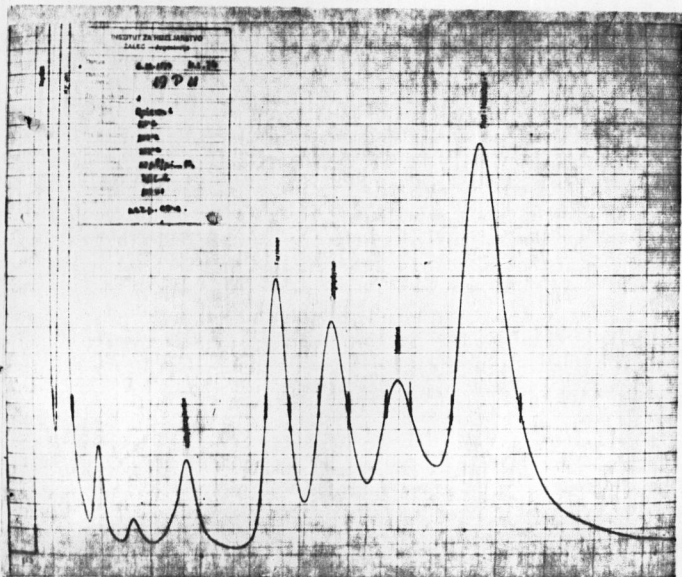
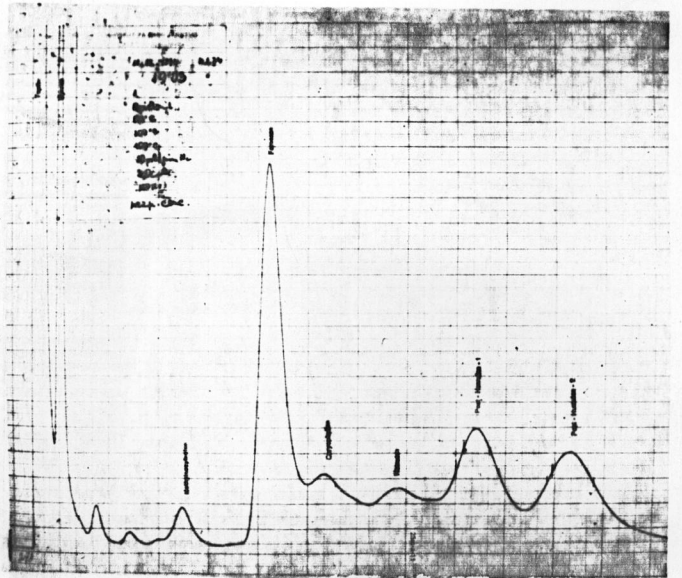
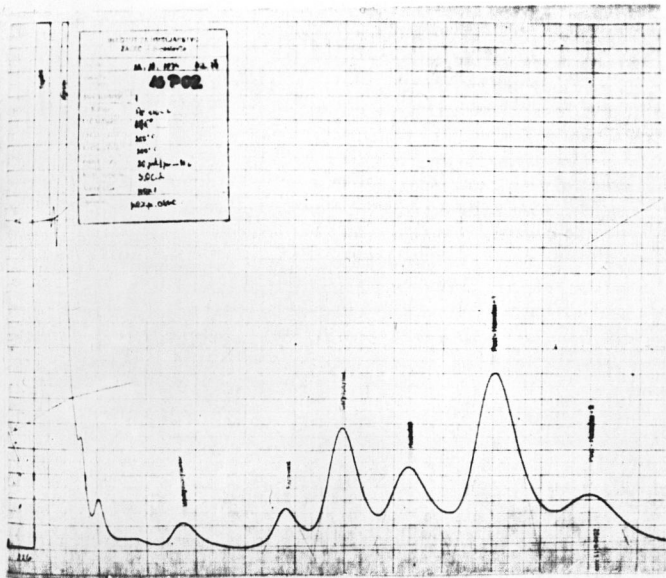
Graph 2.4 Distribution of H/C and F/C

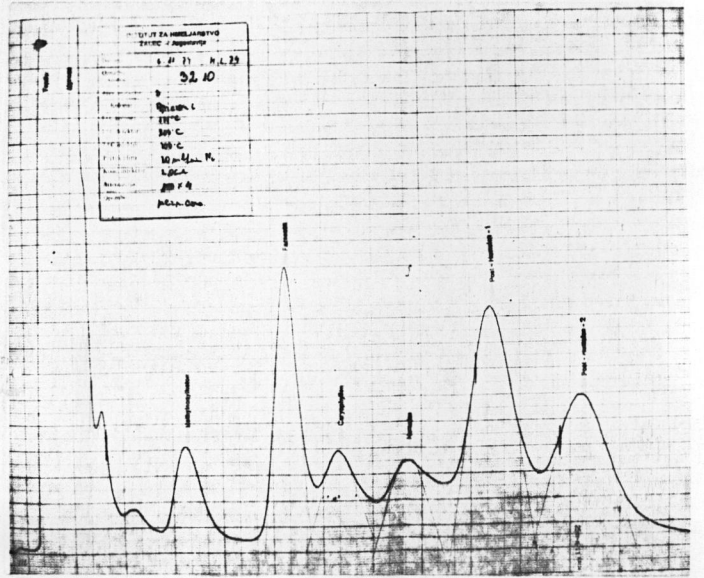
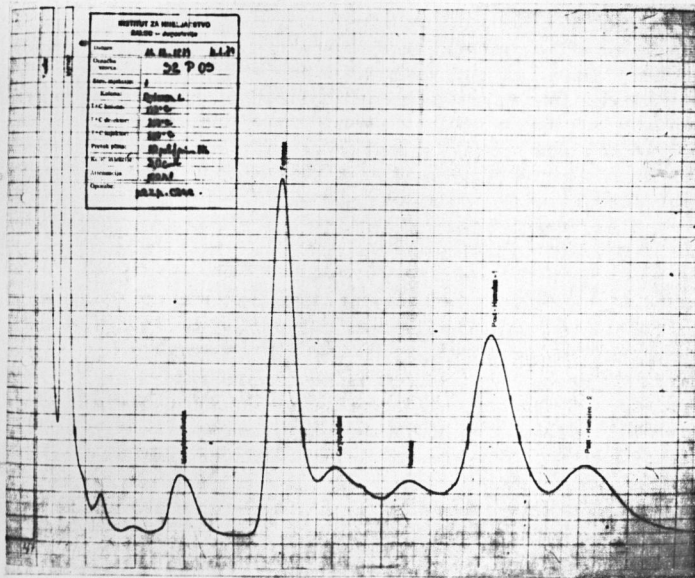
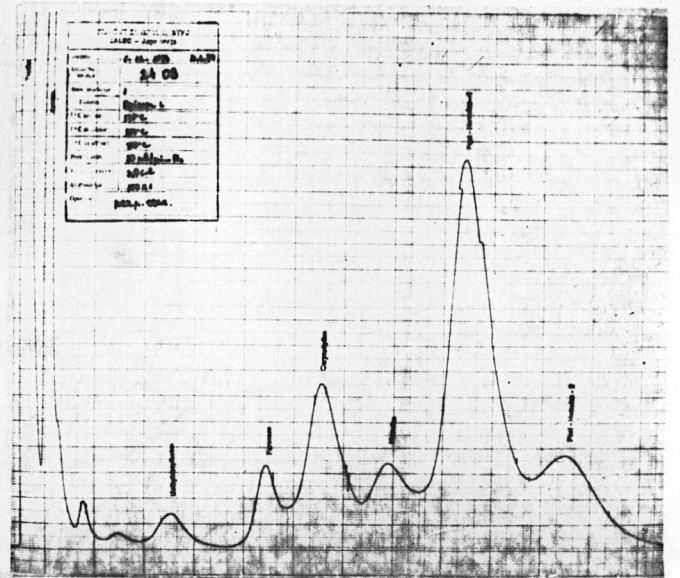
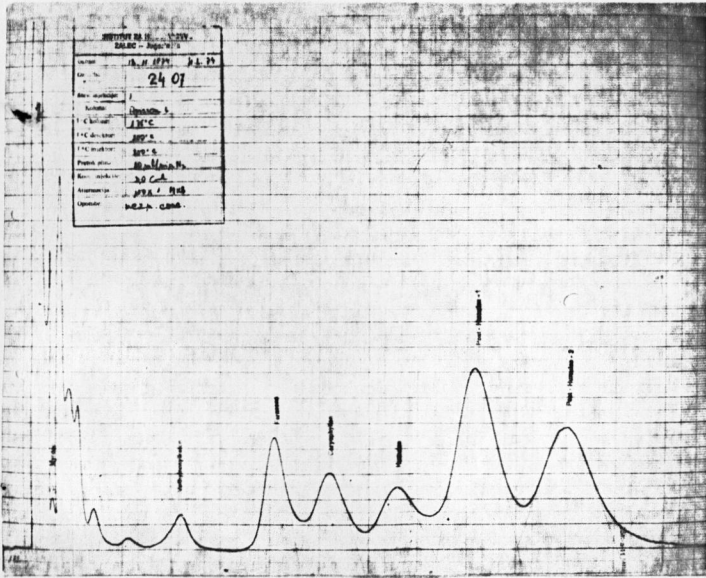
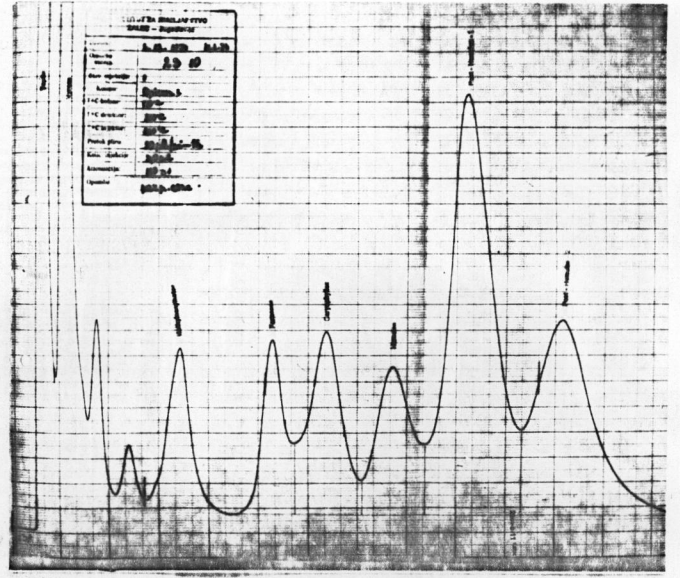
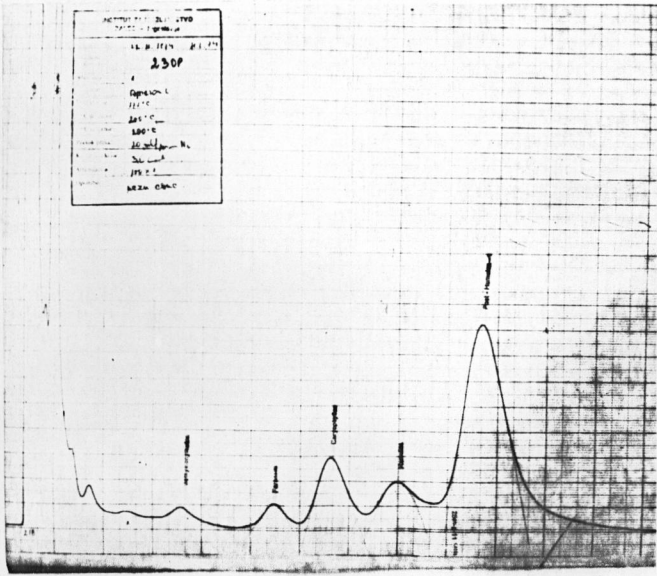
Graph. 2.5 Distribution of PHL/C and H/C

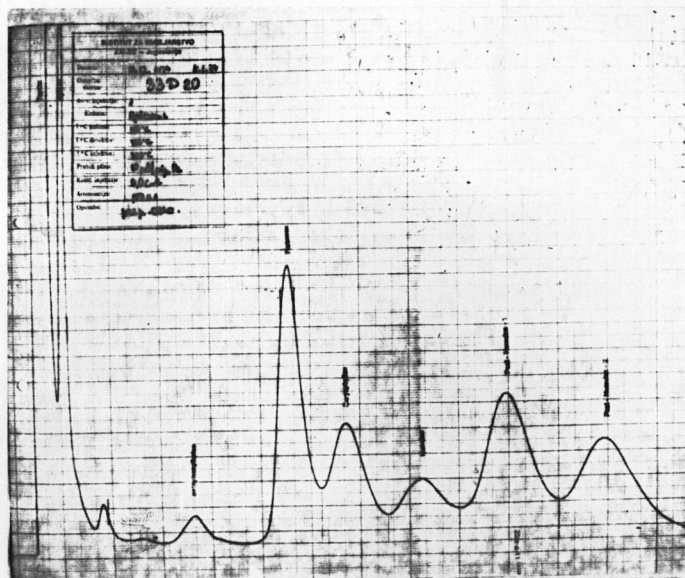
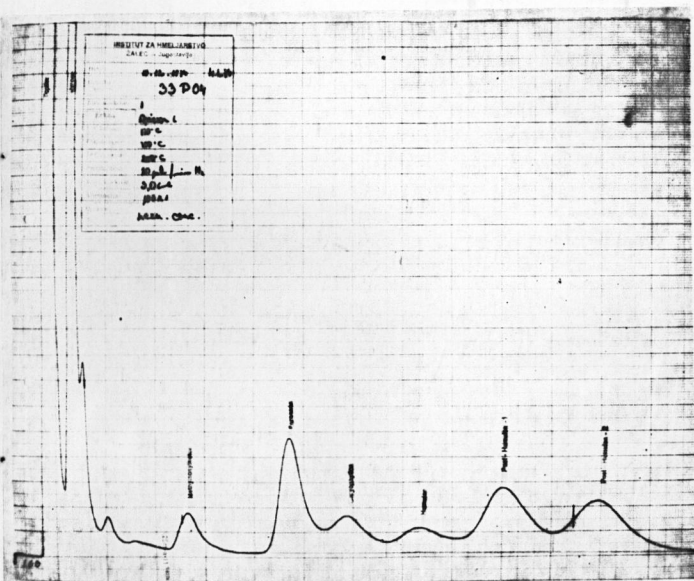


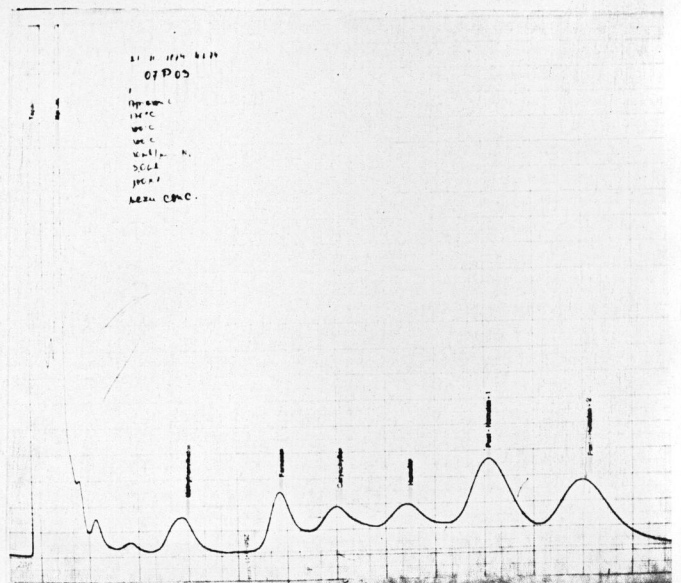
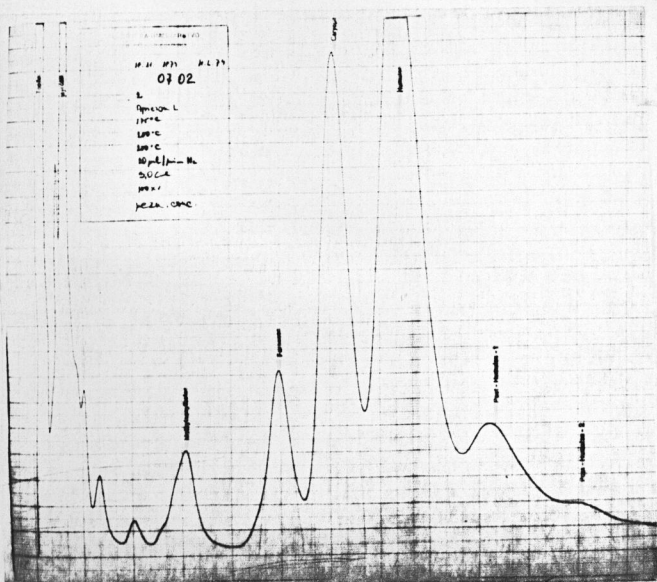
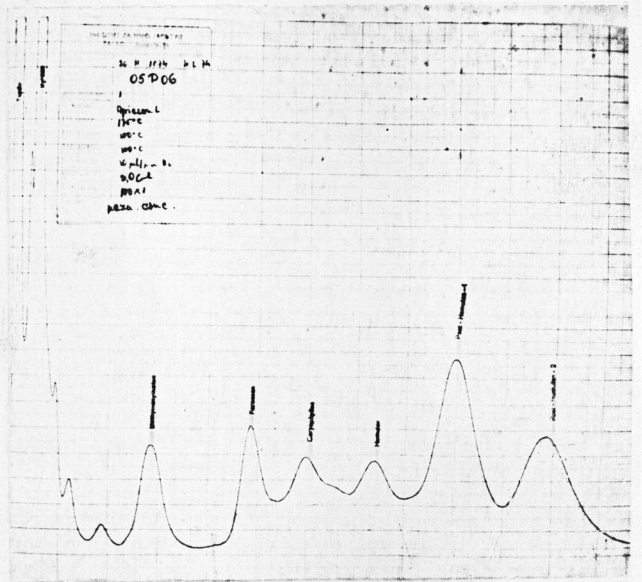
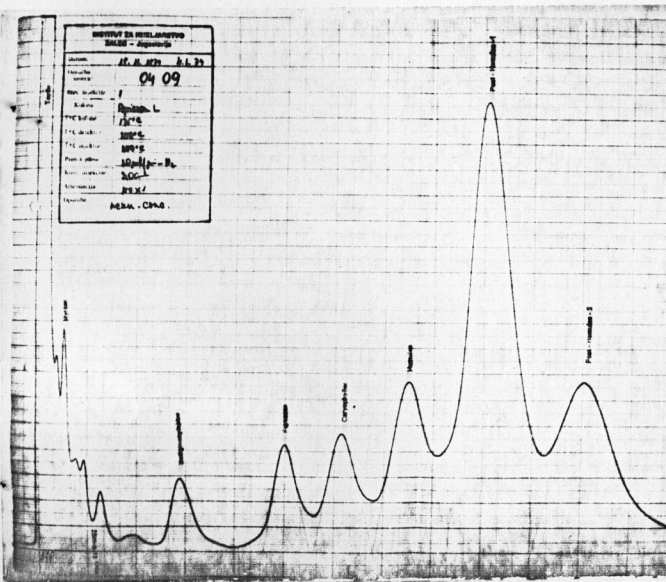
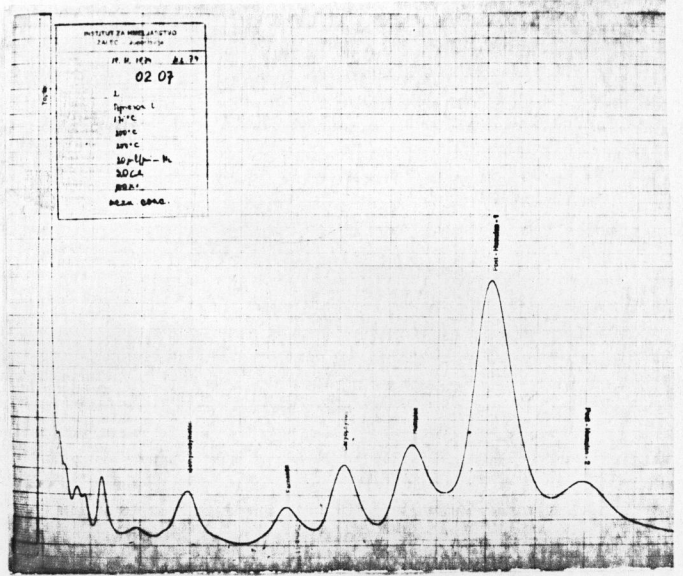
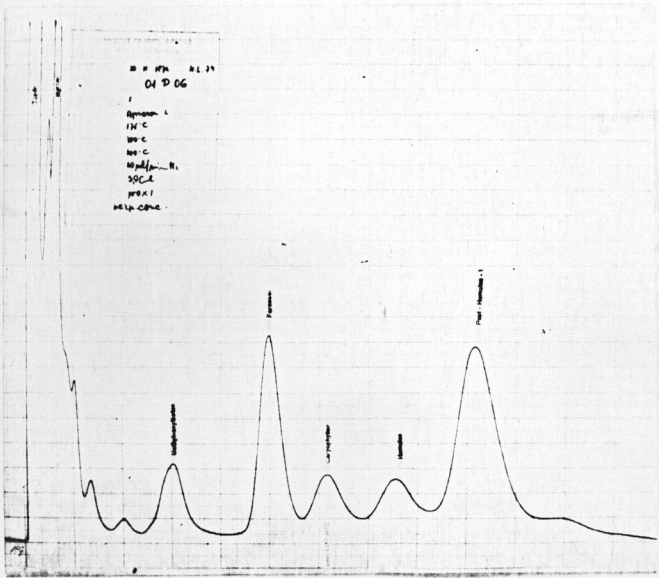


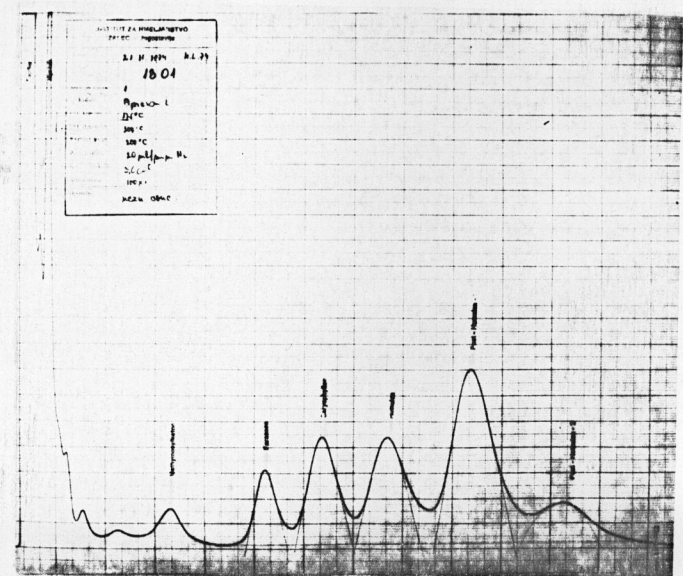
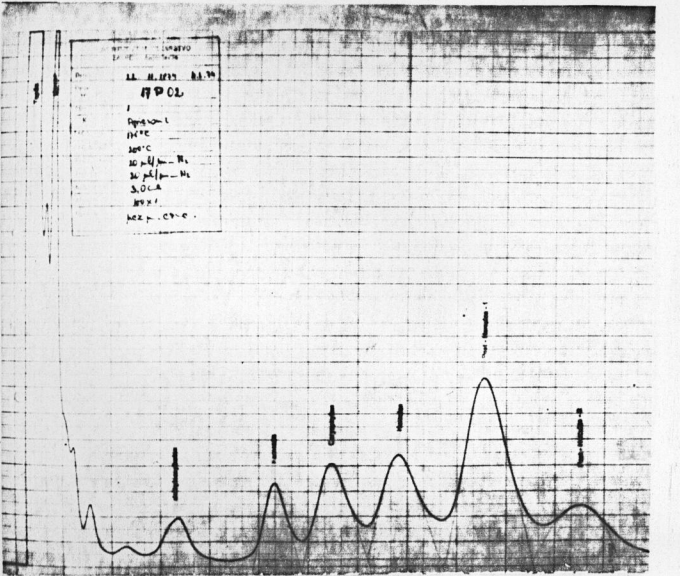
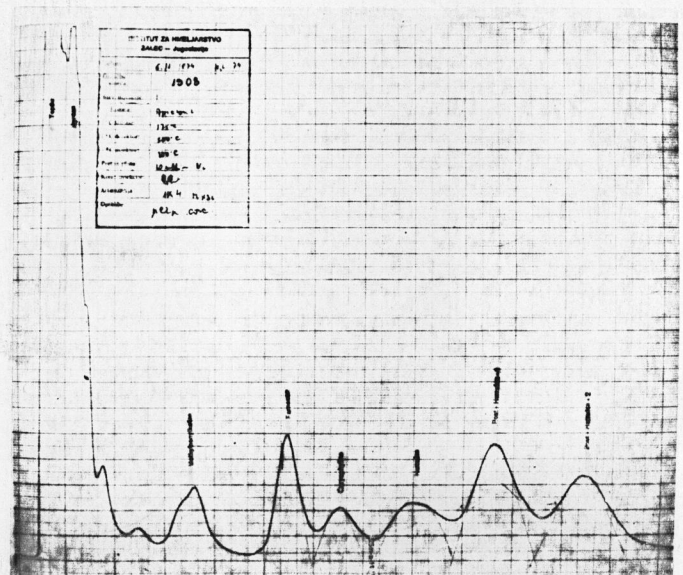
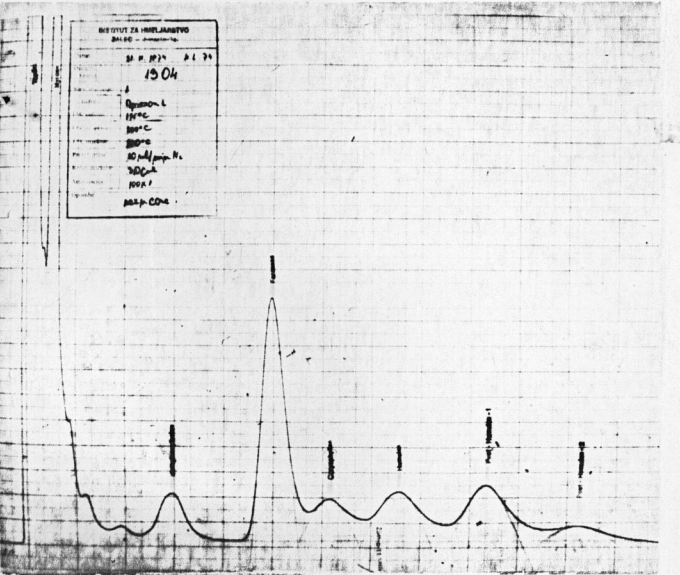
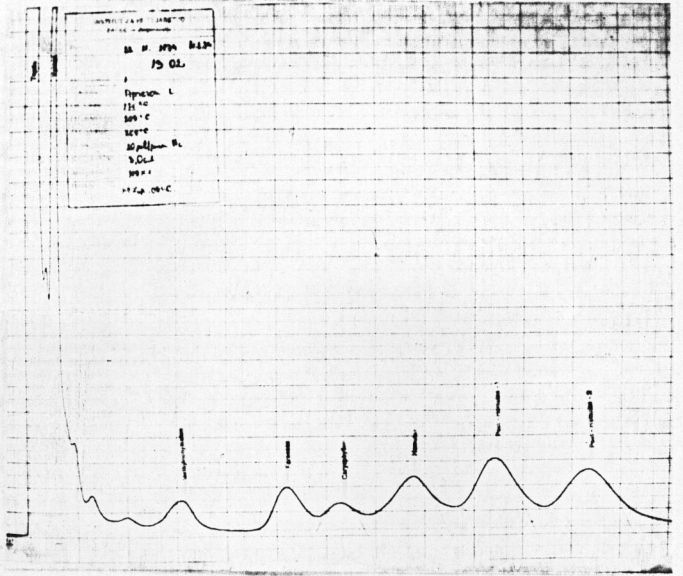
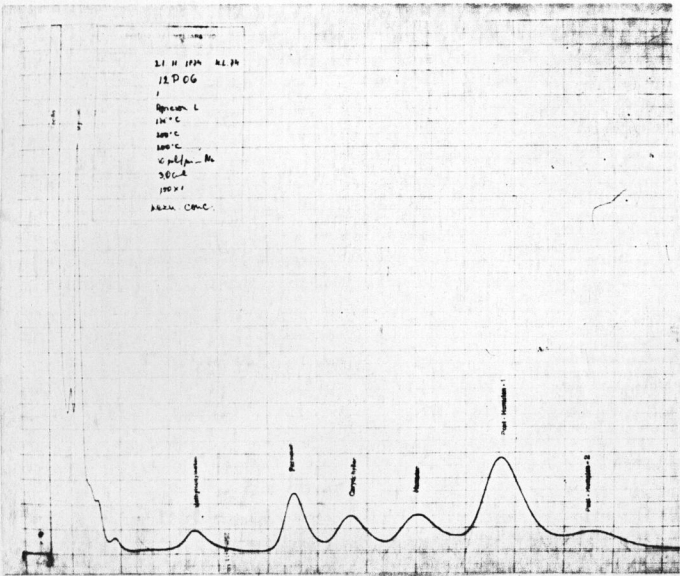


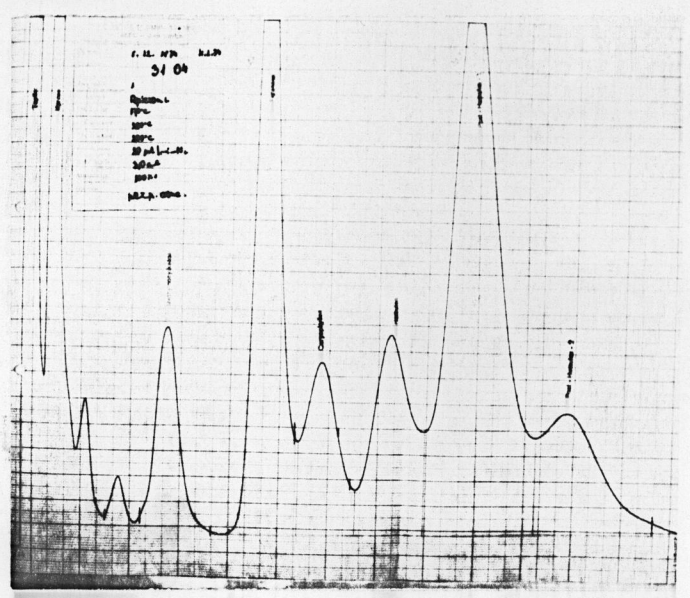
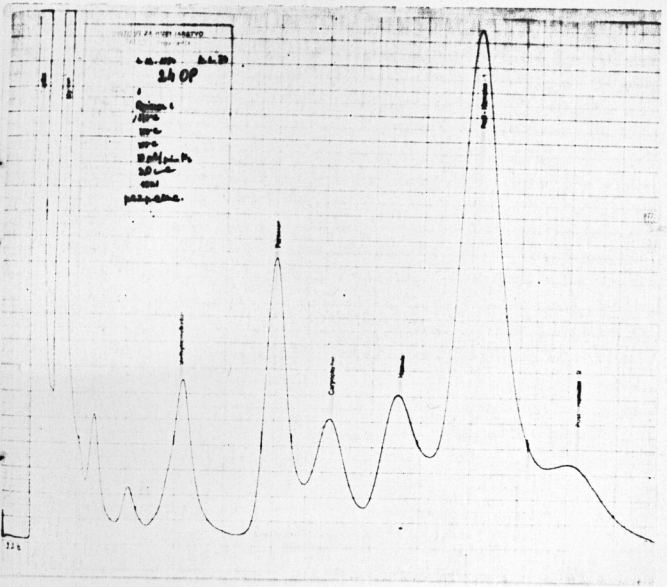
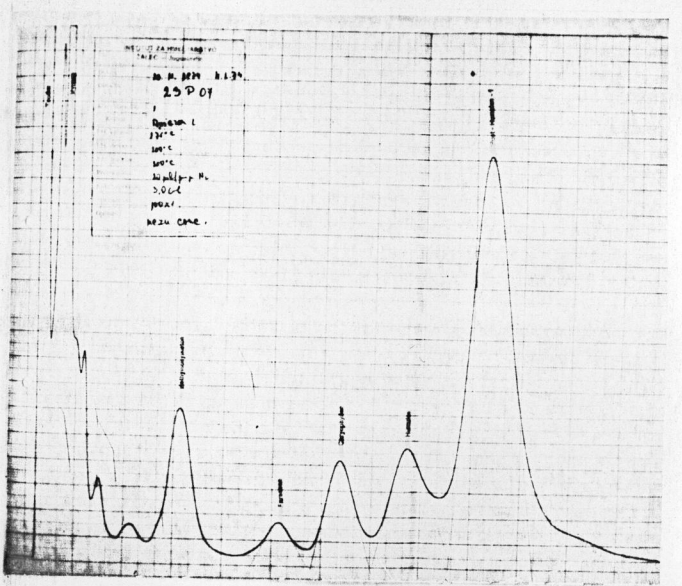
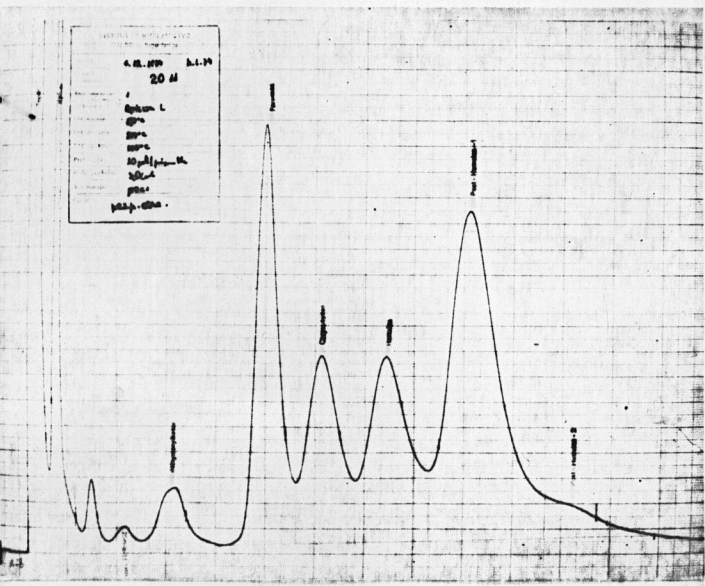
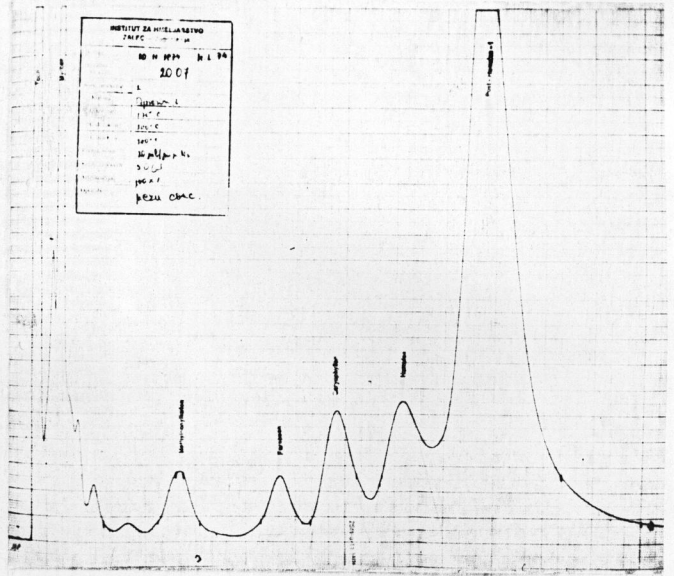
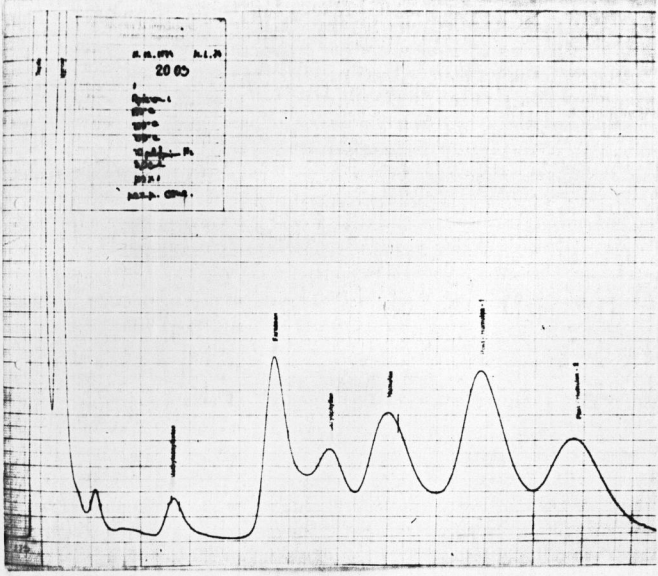


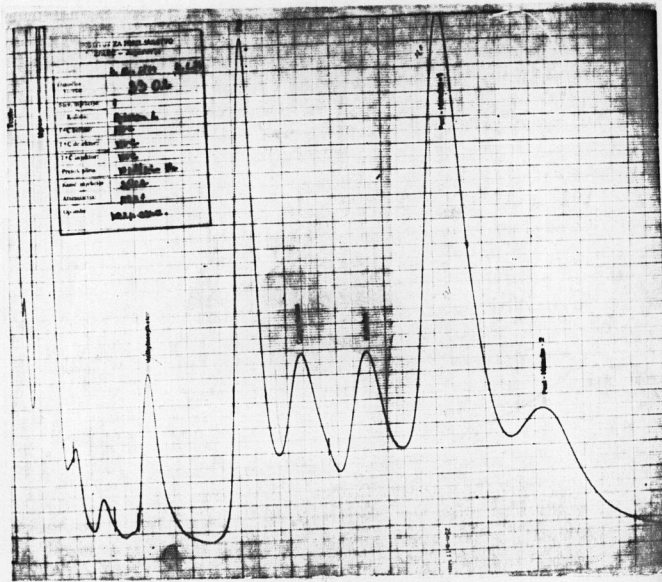
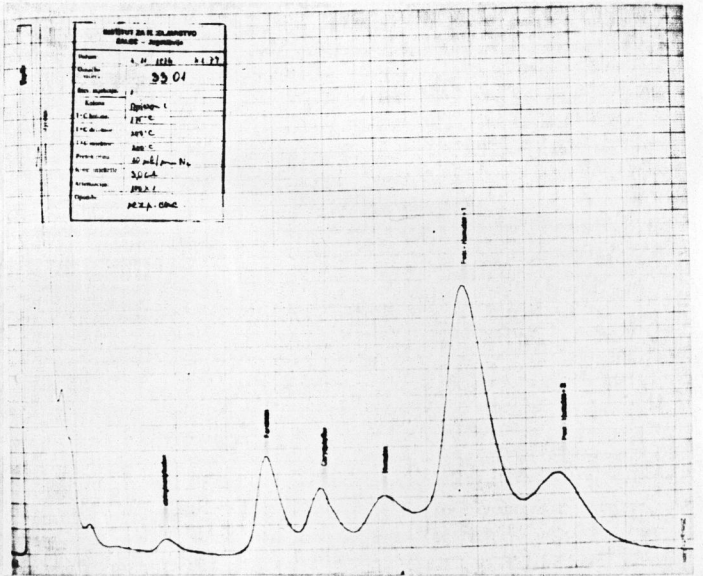
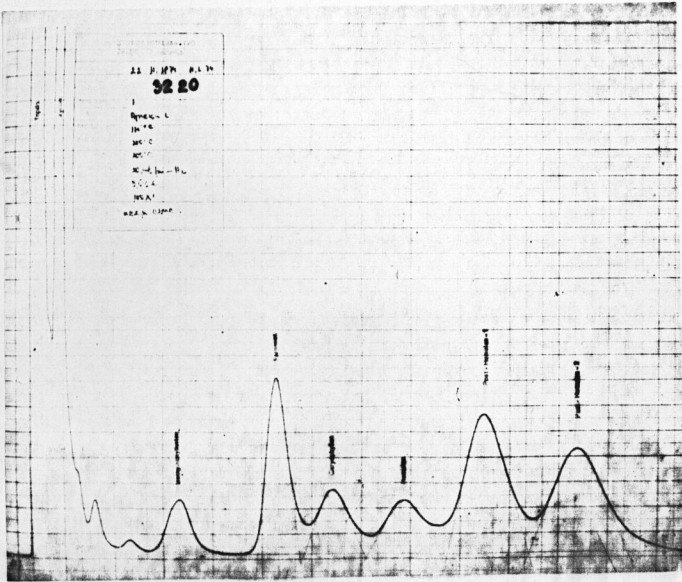




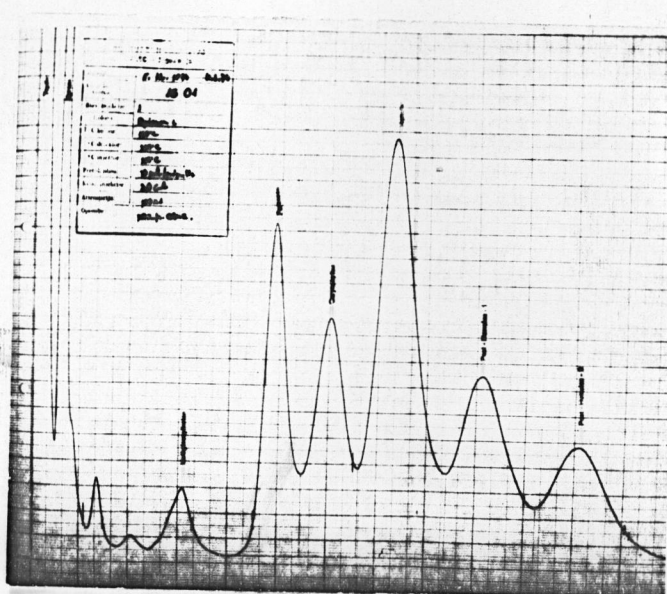
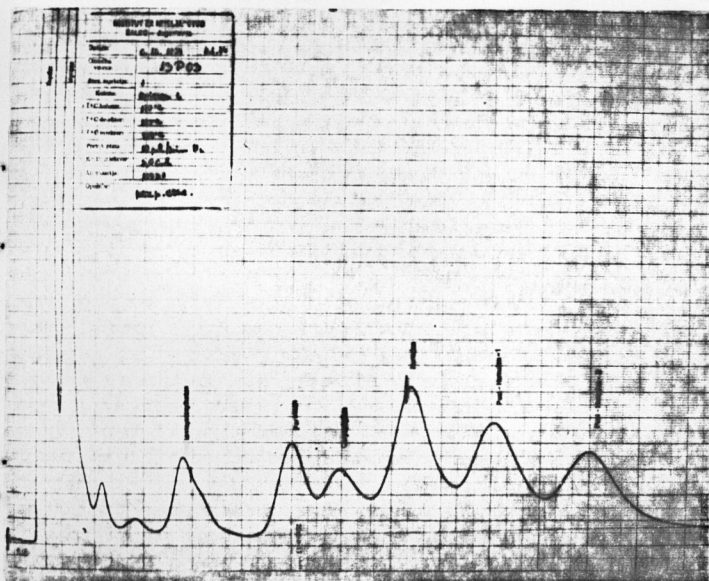
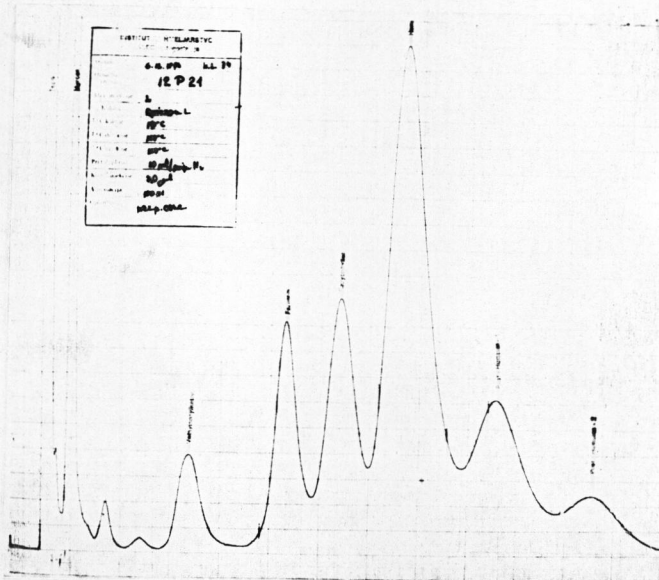
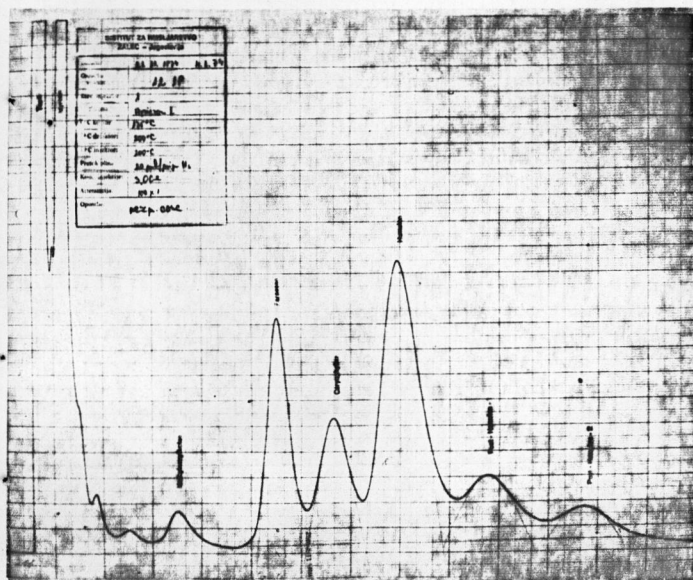
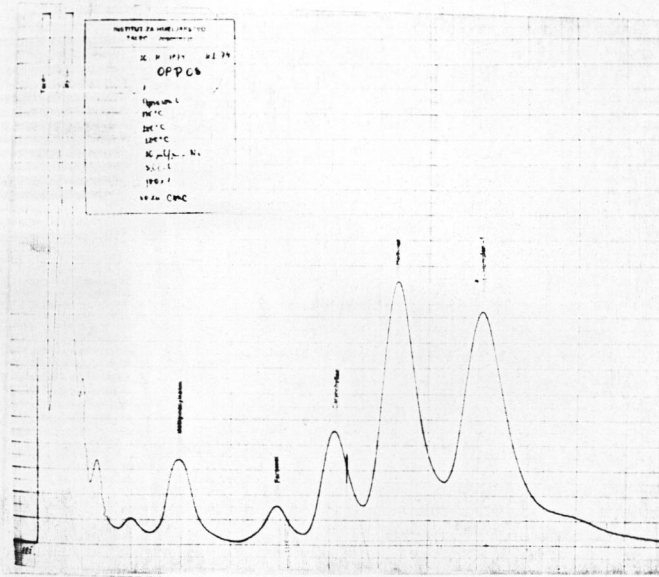
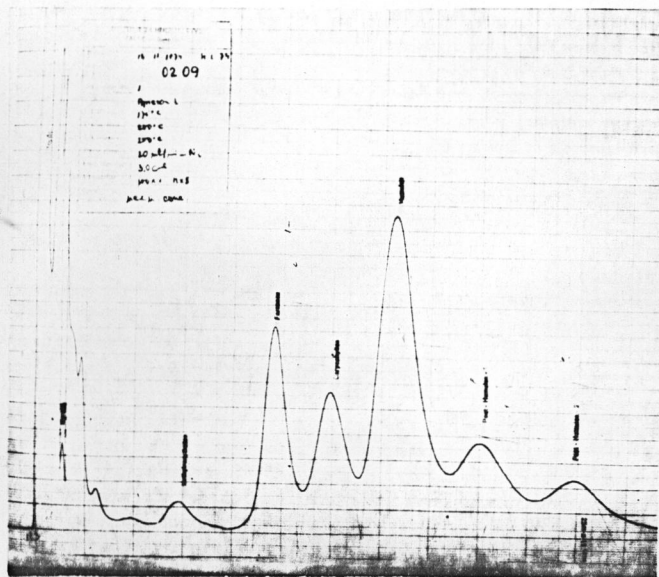




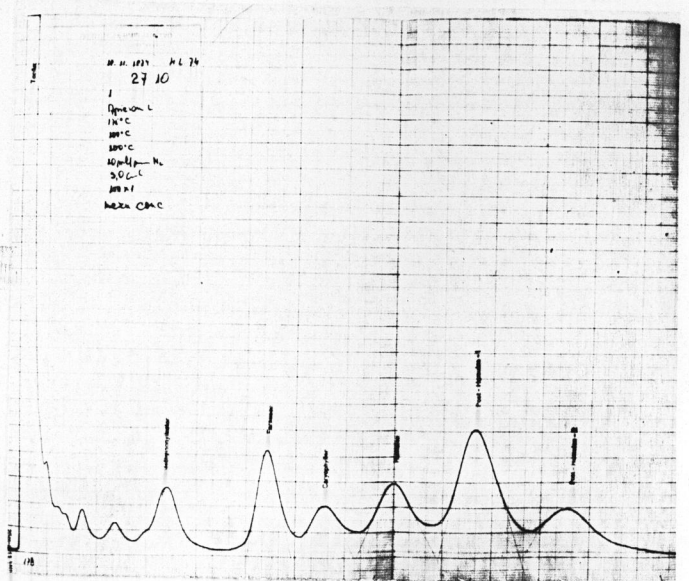
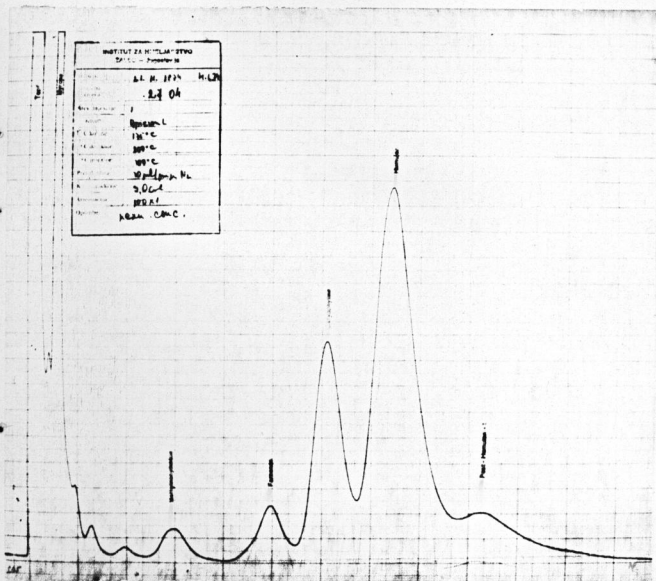
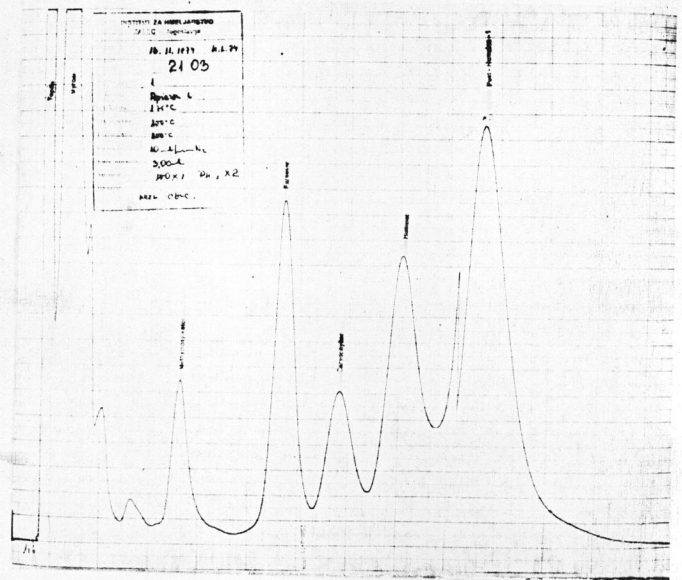
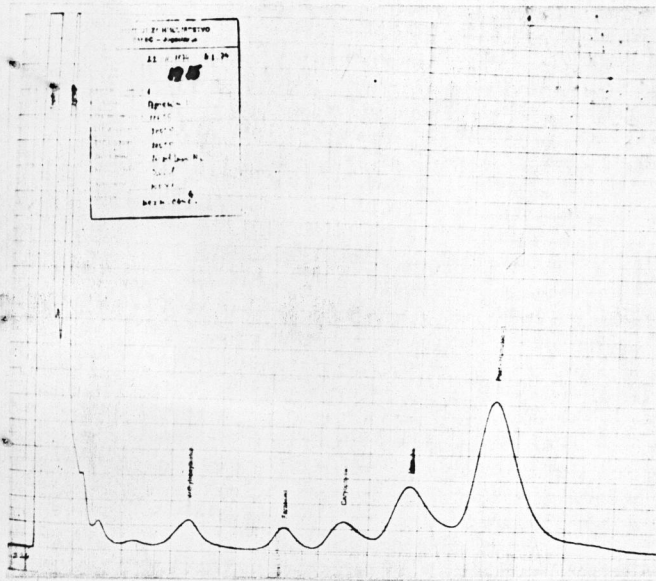
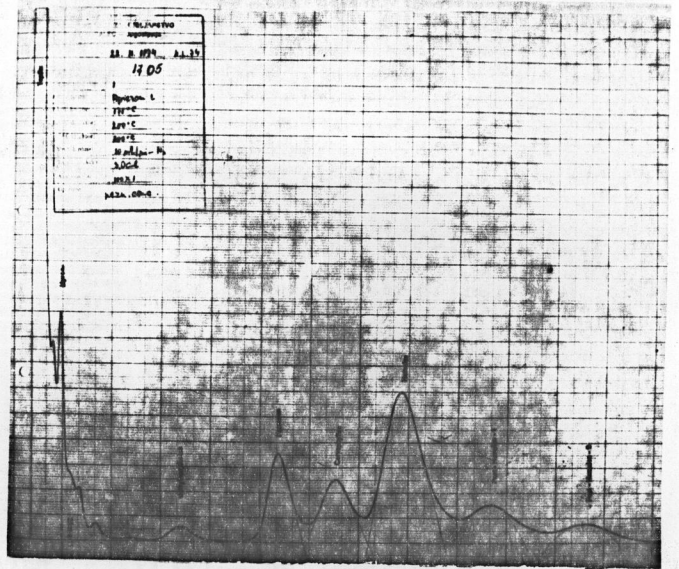
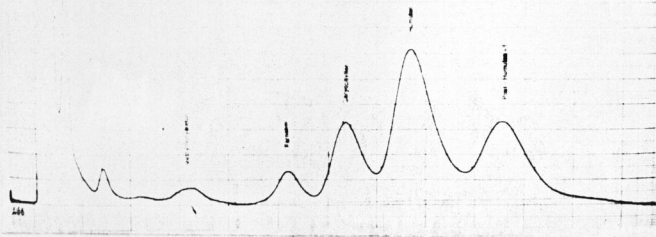


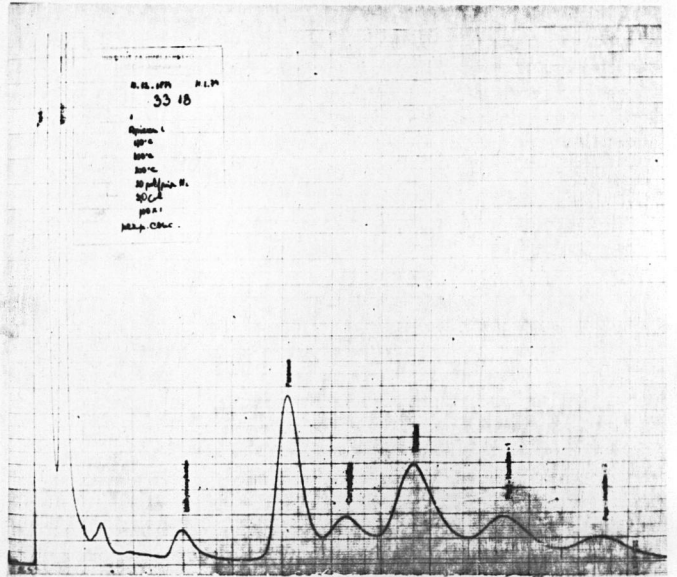
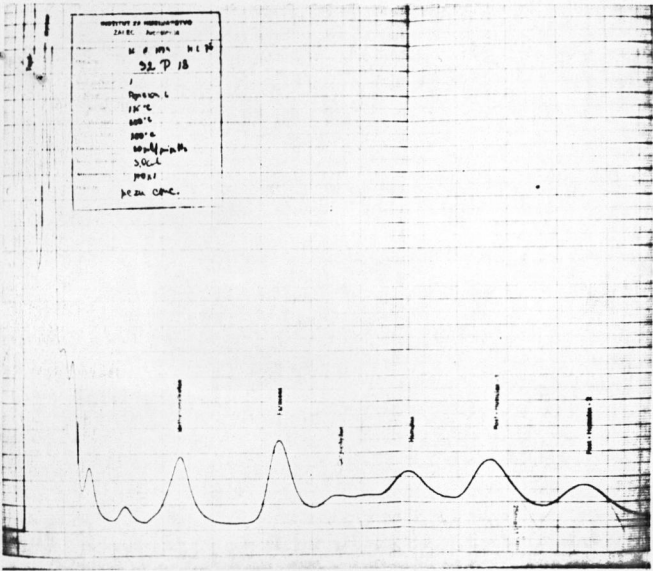
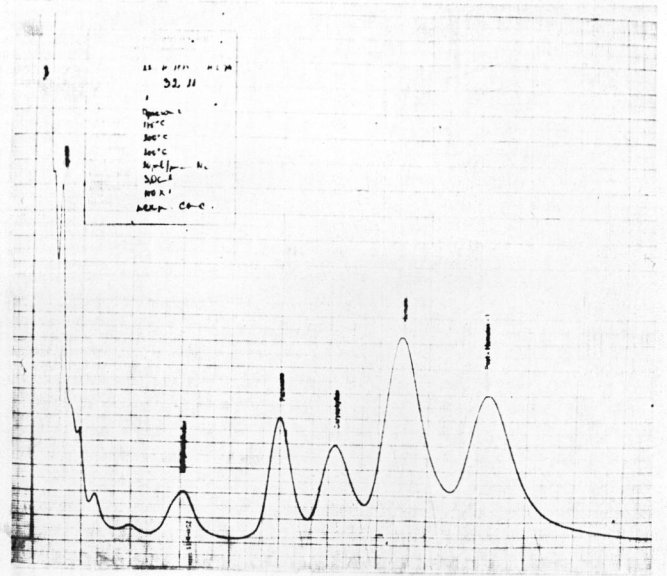
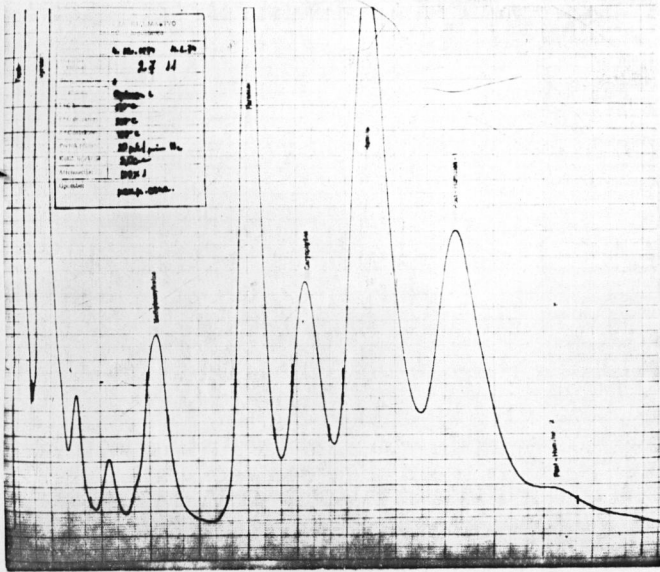




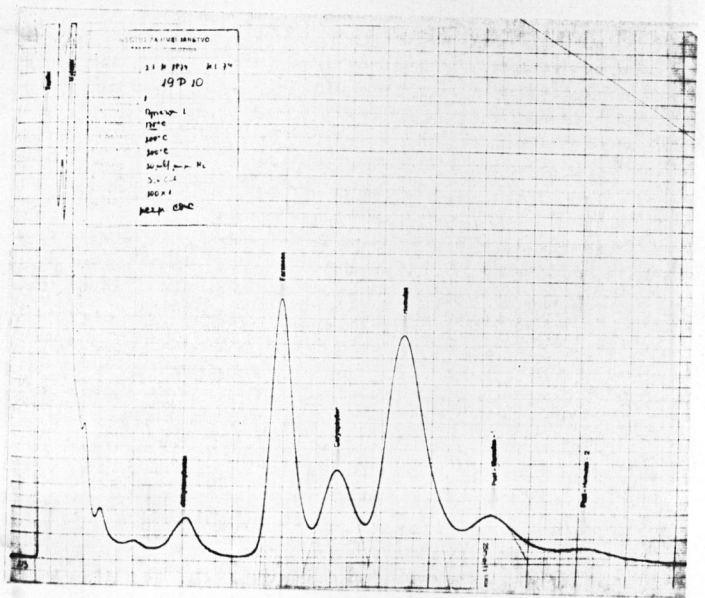
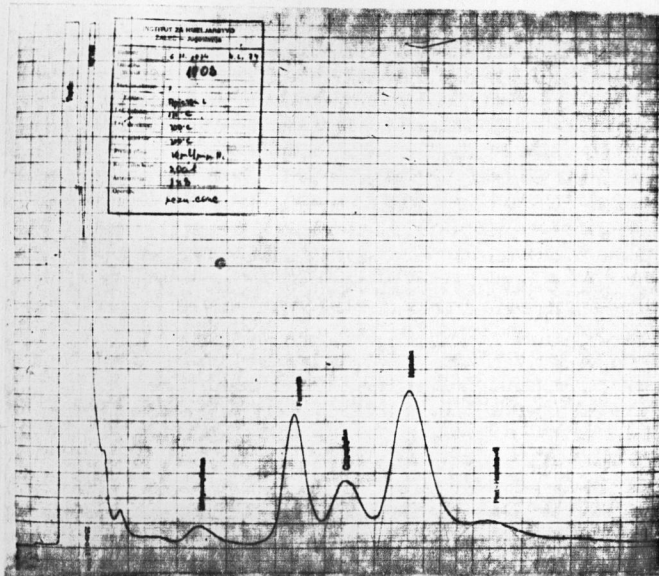
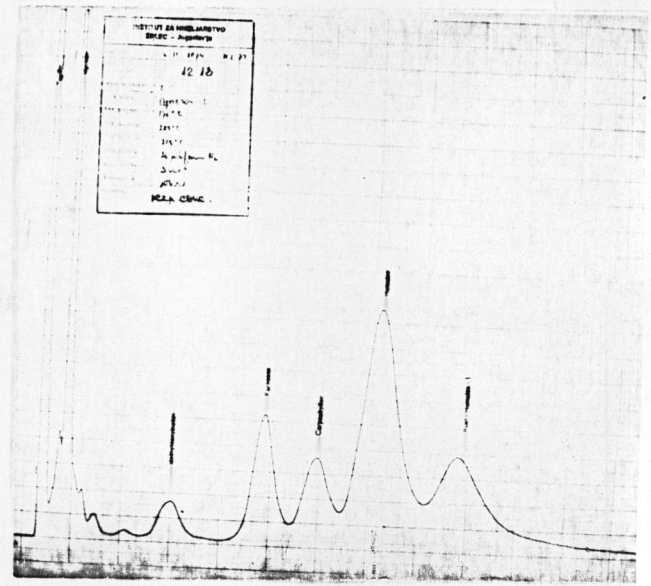
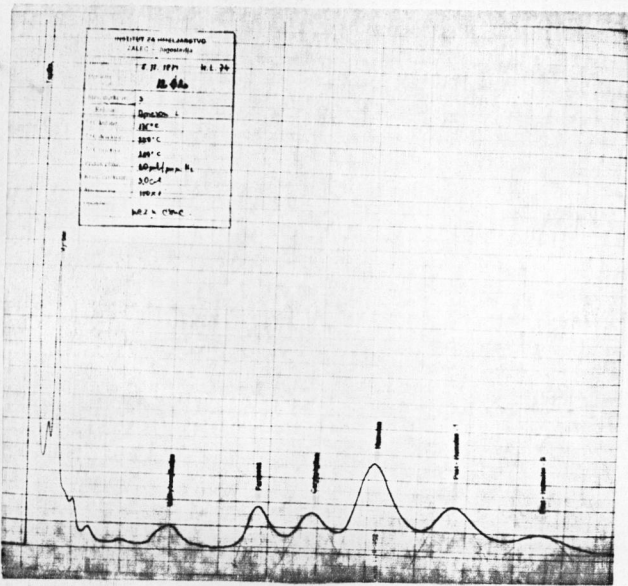
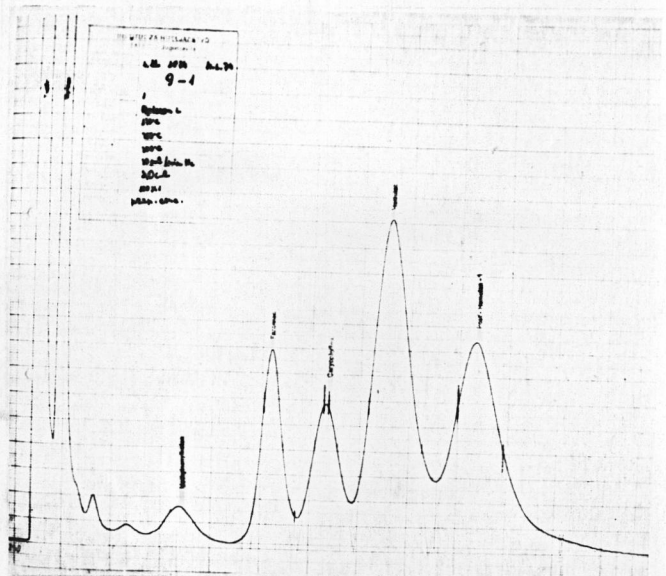
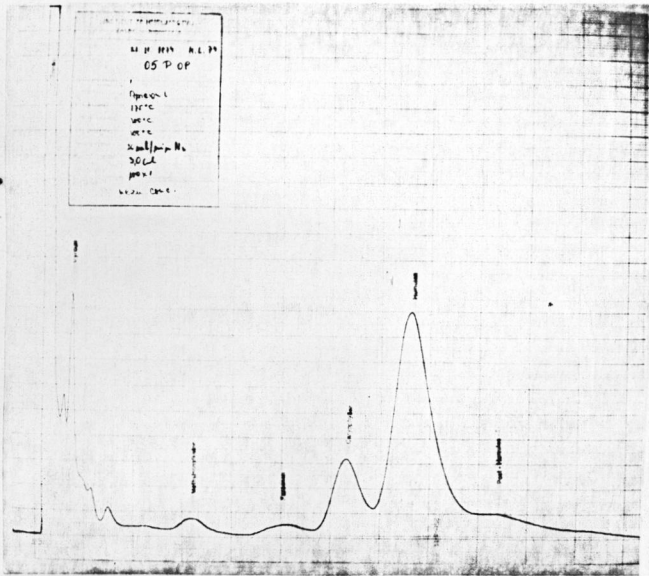


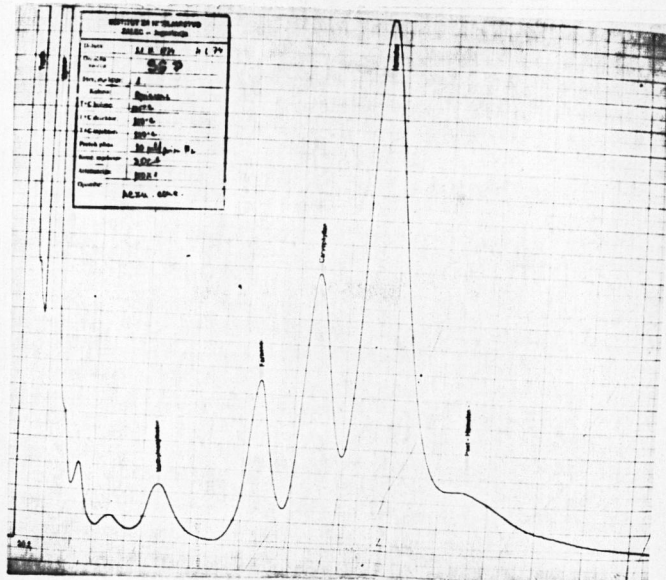
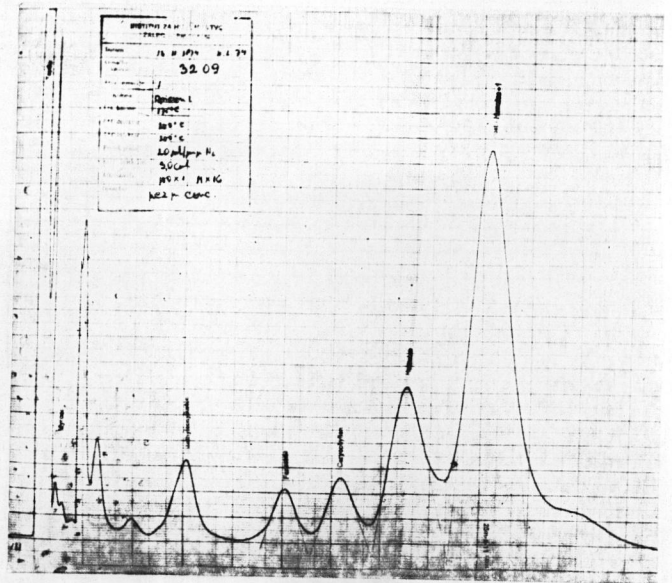
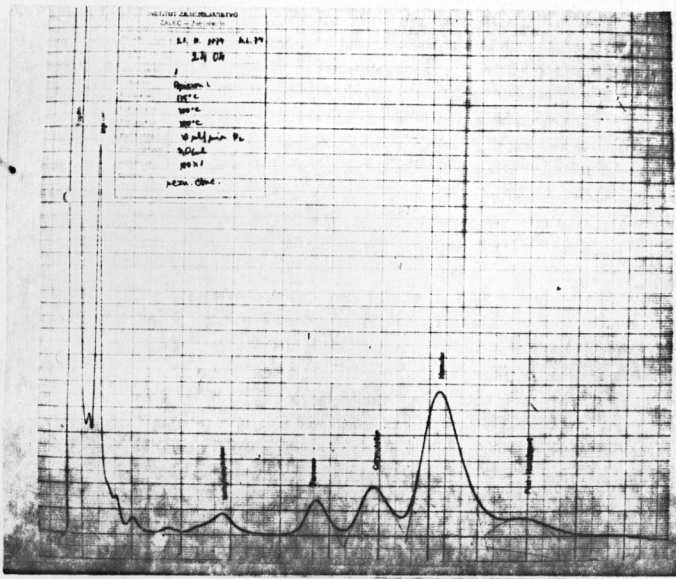
INSTITUTO DE INVESTIGACIONES  
 FARMACOLÓGICAS  
 No. 1079 A.L. 24  
 1704  
 Temperatura: 10°C  
 Humedad: 80%  
 Volumen: 200 ml  
 pH: 7.0  
 Marca: CMC











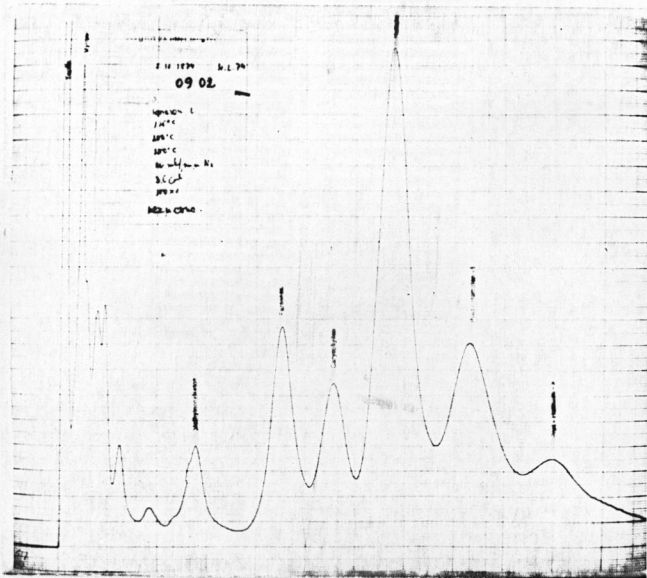


Table 2.2

R a t i o H/C

to 1	1-2	2-3	3-4	over4
05P12	01P06	02P09	02P03	09P02
05P13	02P07	09P08	03P03	
07P17		12P19	04P11	
11P05	04P09	12P21	05P03	
12P02	05P06		05P05	
12P16	07P02	13P03	05P08	
12P20	07P03	16P01	05P09	
13P01	11P04	17P04	07P15	
15P06	11P06	17P05	09P01	
15P07	12P06	19P07	12P12	
16P02	13P02	19P15	12P18	
17P08	13P04	20P14	19P08	
19P02	13P08	21P03	19P10	
19P03	17P02	26P05	24P04	
19P11	18P01	27P04	26P03	
19P13	20P03	27P09	32P02	
20P01	20P07	27P10	32P09	
20P10	20P08	27P11	32P17	
21P01	20P11	32P04	33P15	
23P09	23P07	32P11	40P02	
23P10	24P09	32P18	40P16	
24P07	26P02	33P18	41P24	
24P08	26P05	33P32		
24P10	31P04	34P16		
32P03	32P01	37P03	Savinia Golding	
32P07	32P20	40P17	Hallertau	
32P10	33P01	41P26	Sanz	
32P19	33P02	41P42		
33P04	33P33			
33P10	33P34			
33P13	34P02			
33P20	37P04			
33P30	40P19			
33P36	41P27			
35P02	41P37			
37P01				
38P01				
40P01				
40P20				
41P28				
41P32				



Table 2.3

R a t i o F/C

0-0,25	0,25-0,50	0,50-1	1-2	2 - 3	over 3
05P08	02P07	04P09	02P03	01P06	13P04
05P09	07P02	05P03	02P09	13P08	19P03
13P01	09P08	05P06	03P03	19P10	26P05
16P02	11P05	07P03	04P11	26P02	32P01
20P10	11P06	07P15	05P05	31P04	32P03
24P08	13P02	07P17	05P12	32P04	33P13
24P10	17P04	09P01	05P13	32P10	33P34
26P03	17P08	11P06	09P01	32P17	34P02
27P04	19P07	12P12	09P02	32P18	34P07
32P02	20P07	12P21	11P04	33P04	40P16
33P10	20P14	15P06	12P02	33P18	41P32
40P02	23P07	15P07	12P06	33P30	
41P37	23P09	16P01	12P16	33P33	
	24P04	17P02	12P18	33P36	
	24P08	18P01	12P19	37P01	
	27P09	19P02	12P20	41P24	
	34P16	19P11	13P03		
	40P17	19P13	17P05		
	41P28	19P15	19P08		
	41P42	20P01	20P03		
		23P10	20P11		
		32P09	21P01		
		32P11	21P03		
		33P15	24P07		
		37P03	24P09		
		37P04	27P10		
		41P26	27P11		
		41P27	32P19		
			32P20		
		Savinia	33P01		
		Golding	33P02		
			33P20		
			33P32		
			35P02		
			38P01		
			40P01		
			40P19		
			40P20		
			Saaz		

Table 2.4

R a t i o H/PH1

to 1	to 1	1 - 2	2 - 3	3 - 4	4
01P06	27P10	02P03	03P03	02P09	05P08
02P07	31P04	03P03	04P11	05P05	05P09
04P09	32P01	09P01	05P03	12P19	07P02
05P06	32P03	09P02	12P18	17P05	07P15
05P12	32P07	12P12	12P21	40P02	19P07
05P13	32P09	13P02	20P14	40P17	19P08
07P03	32P10	13P03	34P16	41P26	19P10
07P17	32P18	16P01	41P37		27P04
09P08	32P19	17P04	41P42		27P09
11P04	33P01	27P11			32P02
11P05	33P02	32P11			32P04
11P06	33P04	32P17			33P15
12P02	33P13	33P10			33P32
12P06	33P20	33P18			37E03
12P16	33P30	40P16			
12P20	33P33	40P19			
13P01	33P34	41P24			
13P04	33P36	41P27			
13P08	34P02	26P03			
15P06	35P02				
15P07	37P01				
16P02	37P04				
17P02	38P01				
17P08	40P01				
18P01	40P20				
19P02	41P28				
19P03	41P32				
19P11	32P20				
19P13					
19P15					
20P01					
20P03					
20P07					
20P08					
20P10					
20P11					
21P01					
21P03					
23P07					
23P09					
23P10					
24P04					
24P07					
24P08					
24P09					
24P10					
26P02					
26P05					

### 3. Properties of Wild Hop Families in Year 1974

We have sowed the seed gained by the wild pollination of plants: 05P08, 05P13, 11P04, 12P16, 21P01, 27P09 and the seed of seeded plants which was gathered in habitats: 19A10, 32A11, 32A18, 33A15, 33A16, 33A22, 37A01, 41A32, 41A37 and 41A39.

The seedlings were bred in the glass-house and planted in the wire-work in the same way as commercial hop is, only they were not sprayed with fungicides. In the vegetation phase we observed the spreading of diseases, growth, development and the shape of the plant. Cones were not seeded. When ripe, green cones were estimated organoleptically. Alpha and beta acids were determined spectrophotometrically.

#### 3.1 Properties of Families / Table 3.1 and 3.2 /

Family 05P08 contained 6 plants from which one was male, the others died in the vegetation phase because they were easily affected by the down mildew.

Family 05P13 contained 82 seedlings, 55 % of plants died. In the generative phase we had 1 intersex, 5 male and 18 female plants. In the vegetation phase the plants were bushy, luxuriant and had only few leaves. One half of the plants was weak with thin bine, the other half of luxuriant plants had thick bine of violet colour prevailing. The leaves often turned yellow, dry and fell off.

The plants which survived were middle infected by the down mildew it occurred also in cones. The plants were ripe on September 13, the yield was middle, cones were small of fine appearance and loose. They had little lupulin of gold-yellow colour and unpleasant empty aroma.

Cones had the average of 2,7 % alpha acids, 2,4 % beta acids. alpha/beta was 1.1. Maximum was 4,72 % of alpha acids.

Family 11PO4: we planted 61 seedlings, 53 % of plants died. In the generative phase we determined 6 male and 12 female plants. In the vegetation phase some plants were weak with short laterals, the others which were better developed were of capitate form, to two thirds of the plant were without leaves because they turned dry. Generally the plants of this family had few leaves and violet bine. On the survived plants the down mildew was middle spread, the cones were here and there affected by the powdery mildew.

Cones were ripe on September 13, the yield was fairly great. Cones were thin, light and reddish, of favourable appearance, loose. They had little lupulin of gold-yellow colour and unpleasant aroma. There were 2,4 % of alpha acids, 2,8 % of beta acids, alpha/beta is 0,9. The maximum quantity of alpha acids was 4,26 %.

Family 12P16: we planted 83 seedlings, 58 % of plants died. In the generative phase we determined 15 male and 10 female plants.

In the vegetation these plants were weaker than the plants of other families, they had narrow-cylindric form, some plants had capitate form. The bine was green and violet. The leaves of these plants also turned yellow but they did not fall off during the vegetation. Some plants looked like cultivated hop.

Down mildew was little spread and affected also cones. Cones were ripe up to September 13, the yield was middle great. Cones were small, loose, looked bad, had some more lupulin of gold-yellow colour and also of darker yellow, aroma was unpleasant. Cones had little alpha acids /1,9 %/, some more beta

acids /2,8 %/, alpha/beta was 0,7. Cone had the maximum of 3,78 % alpha acids.

Family 21P01 had only 8 seedlings, all died in the vegetation.

Family 27P09 contained 66 seedlings, 55 % of them died. In the generative phase we determined 8 male and 17 female plants. In the vegetation the plants were comparatively weak of cylindrical and staff-like form. Some plants were extremely bad developed. Bine was green to violet, plants had few leaves of small size.

Down mildew was little spread, but affected also cones. here and there was also powdery mildew in cones. Plants were ripe on September 13, yield was bad to middle. Cones looked fine, rather dense, they had little lupulin of gold-yellow colour. Aroma was unpleasant, there was 2,3 % alpha acids and 2,2 % beta acids, alpha /beta was 1,1. 4,05 % alpha acids were the maximum.

Family 19A10 had 120 seedlings, 63 % of plants died during the vegetation. We determined 11 male and 22 female plants in the generative phase.

Plants had cylindrical form very similar to cultivated hop, only some individuals developed capitate form. Leaves were small, 3 lobed and did not turn yellow. Bine was of dark violet colour.

Down mildew was not much spread but it affected also cones. Plants were ripe up to September 13, yield was middle great, cones were light green, some of them bad developed and of different sizes. They were small but also very big. Red strig was frequent. The samples got had organoleptic mark for appearance, growth and quantity of lupulin. Lupulin was gold-yellow. Aroma was unpleasant, little alpha acids /2% / and little beta acids /2,3 %/.

Ratio alpha/b eta was 0,9. 3,54 % of alpha acids were the maximum.

Family 32A11: we planted 105 seedlings, 80 % of them died. In the generative phase we determined 3 male and 3 female plants. Family was not adaptable. Some of the survived plants were luxuriantly developed and formed capitate form, had thick violet bine and few leaves.

These plants were little affected by down mildew, they were ripe up to September 13, Yield was middle good. Cones were small, rather dense, they had little lupulin of dark yellow colour. Aroma was extremely unfavourable, 5,3 % of alpha acids, 3,8 % of beta acids, alpha/beta was 1,4. The maximum was 6,35 % of alpha acids. Family had extremely few individuals which had similar chemical values as cultivated hop.

Family 32A18: we planted 32 seedlings, 59 % of plants died. In the generative phase we determined 4 male and 3 female plants. The plants were of weak growth, fussyform, had few leaves and thin violet bine. They were middle affected by down mildew, it affected also cones. Plants were ripe up to September 13. Yield was bad, cones had red strigs and were loose. Lupulin was gold-yellow. 3,4 % of alpha acids, 4,3 % of beta acids, alpha/beta was 0,8.

Family 33A15: we planted 16 seedlings, 63 % of plants died, only two female plants bloomed, only one developed normally. We picked it on September 13. It had cones which looked bad with some gold-yellow lupulin and unpleasant aroma. 3,6 % of alpha acids, 28 % of beta acids, ratio alpha/beta was 1,3.

Family 33A16: we planted 21 seedlings, 81 % of plants died. In the generative phase we determined 3 male and 1 female plant, which was luxuriant, however badly affected by down mildew.

The plant was picked on September 16, yield was middle good, cones looked bad, loose with little lupulin of gold-yellow colour and of extremely bad aroma.

The chemical value of cones was low, they had only 1,3 % of alpha acids and 3,1 % of beta acids, ratio alpha/beta was 0,4 .

Family 33A22 had 33 plants, 64 % of them died. In the generative phase we determined 1 male and 5 female plants.

Plants were weak, some of them of capitate form. The bine was violet, there was a lot of down mildew on plants. They were ripe up to September 16, The yield variet, the average was middle good. Cones had red strigs and developed late. They were dense and had a lot of alpha acids /5,1 %/ and 2,9 % of beta acids, ratio alpha /beta was 1,8.

Family 37A01: we planted 62 plants, 53 % of them died. In the generative phase we determined 4 male and 14 female plants.

The family had very wild, luxuriantly grown plants which formed capitate form. The lower part of the plant was without leaves because they turned brown and fell, off. The bine was violet, down mildew was middle spread .

Cones were ripe on September 13. The yield was rich, cones were little reddish, loose, bad developed, had little lupulin of gold-yellow colour and unpleasant aroma. 2,9 % of alpha acids, 3,1 % of beta acids, ratio alpha/beta was 0,9. The maximum was 4,32 % of alpha acids.

Family 41A32 had 58 seedlings, 47 % for them died. We determined 4 male and 16 female plants in the generative phase.

The family had luxuriant plants which were of capitate form. Leaves turned dry and fell off. plants had few leaves. The bine was strong, thick with long laterals. Down mildew was rather spread. We noticed 3 laterals in the node of the plant 144/18.

The plants were late, they were ripe on 14 October, the yield was rather good. Cones were loose with little lupulin of yellow colour and of extremely unpleasant aroma. 4,4 % of alpha acids, 2,2 % of beta acids, ratio alpha/beta was 2.0. The maximum was 8,3 % alpha acids which corresponds to cultivated hop.

Family 41A37: we planted 18 plants, 47 % of them died. In the generative phase we determined 2 male and 3 female plants.

The plants looked like wild hop with extremely long laterals and formed a wide cylinder. They had few leaves, they turned yellow. The bine was violet. There was not much down mildew. The plants were late, they were ripe in October /up to 14 October /. The yield was good, cones were small, rather dense, they were developed late and had darker lupulin. Aroma is unpleasant, 4,6 % of alpha acids, 3,9 % of beta acids, ratio alpha/beta was 1,2. The maximum was 6,65 alpha acids.

Family 41A39 had 60 seedlings, 65 % of them died. In the generative phase we determined 5 male and 4 female plants. The development of plants was unequal, only 4 plants were luxuriant and were of capitata form, the others remained undeveloped. The bine was violet, leaves dried. Down mildew was rather spread. The plants were very late, they were ripe only in October / up to 14 October /. The yield was rather good. Cones were loose, small, light green and empty. They had gold-yellow lupulin and extremely unpleasant aroma, however, they had a lot of alpha acids /7,2 %/ and 2,8 % beta acids, ratio alpha/beta was 2,6. The maximum of alpha acids was 7,65 %.

831 plants of 16 families were planted and only 384 survived. 205 plants reached the generative phase: 74 males, 1 intersex and 130 females. The yield gave only 115 plants.

Because of a small number of seedlings which survived and reached the generative phase we came to a conclusion that the adaptability of plants was bad. We did not find the real reason why the



plants were dying, however, we noticed that the main cause was down mildew. Down mildew was widely spread in the families containing luxuriant plants, especially in those with extremely long vegetation. We rarely noticed the signs of diseases on weak bad developed plants.

Powdery mildew was found only here and there in cones and did not cause any greater effect.

Our wild hop <sup>was</sup> middle late, for the prevailing quantity of plants was ripe only in the middle of September. The families from the south-east of Yugoslavia were very late, they were ripe only in the middle of October.

The forms of late and very late plants were various. Late plants were luxuriant or weak, they were often capitate, but also narrow cylindrical and fusiform. Very late plants were extremely luxuriant, capitate or columnar and they had long laterals.

Violet bine prevailed, leaves were small therefore it looked like the plant had few leaves. The leaves often turned yellow and fell off.

Cones were in clusters, especially those of very late plants, they were small, light green, often reddish. They had red strigs, they were loose and had extremely gold-yellow lupulin. Aroma was typical, it was unpleasant and empty, however, it was not intrusive.

Very late hop had higher chemical value than the late, for it had the average of 4,8 % alpha acids, the late hop only 2,8 %, Ratio of alpha to beta acids was comparatively favourable, round 1, it was more than 1 for very late hop.

In some families the individual plants had a lot of alpha acids, almost to 8 %. We shall examine these individual plants closely and include them in the brewing programme.

Table 3.1

Properties of Wild Hop Families

Family	Ripeness	Plants Planted	Died plants		Yielded Plants		Yield		Downy mildew	
			Number	%	Number	%	$\bar{x}$	V %	$\bar{x}$	V %
05P08		6	6	100	0	0	-	-	-	-
05P13		82	44	55	15	18	2.9	38	2.0	80
11P04		61	32	53	9	15	3.4	12	1.9	90
12P16		83	48	58	9	11	2.8	36	0.8	163
21P01		8	8	100	0	0	-	-	-	-
27P09		66	30	55	15	23	2.4	50	1.1	146
19A10		120	75	63	21	18	2.9	41	0.8	175
32A11		105	28	80	3	3	2.7		1.0	170
32A18		32	19	59	2	6	2.0		1.6	-
33A15		16	10	63	1	6	5.0		1.0	-
33A16		21	17	81	1	5	4.0		2.3	-
33A22		33	21	64	4	12	3.0		2.4	-
33A01		62	33	53	13	21	3.6	25	1.6	100
41A32		58	27	47	16	28	3.3	36	2.1	76
41A37		18	10	56	3	17	3.7		2.2	100
41A39		60	39	65	3	5	3.0		2.3	52

$\bar{x}$  = average  
 V% = coefficient of variation

Table 3.2

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## Properties of Cones

Family	Appearance Points 1-5		Density Points 1-5		Lupulin Points 1-5		Colour of lupulin Points 1-5		Aroma Points 1-5		Acids %		Acids %		ratio	
	$\bar{x}$	V%	$\bar{x}$	V%	$\bar{x}$	V%	$\bar{x}$	V%	$\bar{x}$	V%	$\bar{x}$	V%	$\bar{x}$	V%	$\bar{x}$	V%
05P08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
05P13	2.5	16	2.1	19	1.7	24	5.0	-	1.9	-	2.7	-	2.4	32	2.0	31
11P04	2.3	17	2.2	18	1.8	22	5.0	-	2.2	-	2.4	-	2.8	46	2.0	59
12P16	1.8	55	2.0	40	2.2	46	4.9	-	1.6	-	1.9	-	2.8	63	2.0	63
21P01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27P09	2.3	17	2.3	30	2.1	14	5.0	-	1.7	-	2.3	-	2.2	44	2.0	62
19A10	2.1	33	1.8	33	1.9	26	5.0	-	1.7	-	2.0	-	2.3	82	2.0	85
32A11	2.3	-	2.3	-	1.7	-	4.7	-	1.0	-	5.3	-	3.8	-	2.0	-
32A18	2.0	-	2.0	-	2.0	-	5.0	-	2.5	-	3.4	-	4.3	-	2.0	-
33A15	1.0	-	2.0	-	3.0	-	5.0	-	2.0	-	3.6	-	2.8	-	2.0	-
33A16	1.0	-	1.0	-	2.0	-	5.0	-	1.0	-	1.3	-	3.1	-	2.0	-
33A22	2.8	-	2.3	-	2.0	-	5.0	-	5.0	-	5.1	-	2.9	-	2.0	-
37A01	1.5	40	1.8	27	1.8	39	5.0	-	1.5	-	2.9	-	3.1	29	2.0	39
41A32	2.0	35	1.8	22	1.6	-	4.9	-	1.9	-	4.4	-	2.2	37	2.0	66
41A37	2.3	-	2.0	-	2.0	-	4.7	-	2.0	-	4.6	-	3.9	-	2.0	-
41A39	2.7	-	2.0	-	2.0	-	5.0	-	1.0	-	7.2	-	2.8	-	2.0	-

 $\bar{x}$  = average

V% = coefficient of variation

Table 3.3

Sex Ratio of Families

Family	Male	Female	Intersex	Ratio of Female to Male
05P08	1	-	-	-
05P13	5	18	1	3,6 : 1
11P04	6	12		2,0 : 1
12F16	15	10		0,7 : 1
19A10	12	21		1,75: 1
21P01	0	0		
27P09	8	17		2,10:1
32A11	3	3		1 : 1
32A18	4	3		0,75: 1
33A15	0	2		-
33A16	3	1		0,3 : 1
33A22	1	5		5 : 1
37A01	4	14		3,5 : 1
41A32	4	16		4 : 1
41A37	2	3		1,5 : 1
41A39	5	4		0,8 : 1

#### 4. Examining and Introducing the Methods of Chemical Analyses of Hop

Beside common analyses we have tested some new methods of determining the composition of hop resins and essential oil so as to find out the way of analysing where the greater amount of samples can be analysed and less time spent. We have also examined the method of rapid determination of storage stability and the chromatographic method of determining essential oil composition and polyphenols in hop.

##### 4.1 Bitter Resins

##### 4.1.1 Wöllmer's analysis

Wöllmer's analysis of hop is still widely used in Europe. 300 Wöllmer's analyses were made each year in our lab.

The distillation of solvents took place on Liebig Condensor without vacuum. The great quantity of ether had to be evaporated, therefore the work was health hazardous. We used rotary evaporator for evaporation instead of standard distillation and compared the results obtained by both methods. It is seen from table 4.1 that the quick and safe way of distillation can be used instead of time consuming conventional method.

##### 4.1.2 Spectrophotometric analysis

We tried to introduce the spectrophotometric method of more rapid determining of alpha acids; the spectrophotometric method shows the quantity of beta-acids instead of beta fraction. Lower values were obtained by experiment with 5 months old hop, the variety of Bullion which was analysed by both methods when the spectrophotometric method was used. It can be seen from table 4.2.

Table 4.1

	Sample 1997				Sample 1998			
	Rotavapor dist.		Conventional dist.		Rotavapor dist.		Conventional dist.	
	Run. 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
Total resins	11.26	11.20	11.53	11.11	13.08	13.21	12.98	12.53
Soft resins	10.33	10.26	10.60	10.31	11.38	11.47	11.24	11.30
Alpha acids	5.23	5.11	5.17	5.13	3.94	3.87	4.00	3.89
Beta acids	5.10	5.15	5.43	5.18	7.44	7.60	7.24	7.41
Hard resins	0.93	0.94	0.93	0.80	1.70	1.74	1.74	1.23

Table 4.2

	Spectrophotometric		Conductometric
Alpha acids	4,4	4,6	6,6
Beta acids	4,9	4,9	10,9 /beta fraction/

Table 4.3

Analysis of 5 cones /Savinia Golding/

Sample	Repetitions		
	1	2	3
Alpha	5,8	4,2	5,3
Beta	4,4	5,0	5,2
Alpha cond. from toluen extract.	6,5	6,3	

Table 4.4

Spectrophotometric analysis of 5 cones  
/Savinia Golding/

Sample	Alpha	Beta
1	3.66	2.00
2	4.14	1.91
3	4.35	1.88
4	3.25	1.66
5	5.12	2.00

Table 4.5

Spectrophoto 5 cones analysis and conventional  
spectrophot. analysis /Aurora /

Sample	5 cones		conventional	
	alpha	beta	alpha	beta
1	12.40	3.48	12.18	3.42
2	12.21	3.63	12.66	2.88
3	13.66	4.04	11.99	3.06
4	10.98	2.84		
5	15.55	2.57		

Table 4.6

Spectrophotometric analysis of 5 cones from  
2 plants of Aurora

Sample	First plant		second plant	
	alpha	beta	alpha	beta
1	13.48	1.35	14.23	4.72
2	12.19	1.11	9.63	3.94
3	10.21	1.43	9.45	3.78
4	11.06	1.44	9.32	1.86
5	11.17	1.76	11.29	2.11

The same sample has been analysed spectrophotometric in 3 repetitions also by the method of 5 cones / table 4.3/.

The spectrophotometric method gives lower values than the conductometric one which is characteristic of old hop: so the experiment was repeated and the hop of 1974 crop which was just picked was used. According to literature the spectrophotometric values are higher than the conductometric ones.

On 9th August we analysed Savinija Golding and Aurora by the method of 5 cones which is very suitable because picking and storage is cheaper, for the amount of samples is smaller. The results received on shown in table 4.4 and 4.5.

Then we picked 5 samples each containing 5 cones from the two neighbouring plants of Aurora variety. The results are given in table 4.6. Alpha acid was 12.70 % by the conductometric method.

First plant had more alpha and less beta acids than the second one. We find out that the analysis is influenced by the rapidity of maceration. It is also important how long the homogenate was left after maceration. After maceration some samples were turbid or the hard parts were not precipitated. Toluene is toxic but we got in touch it in maceration and filtration. Instead of that we shook the samples of five cones in plastic equipped bottles with fiberglass filters. After 4 hours of shaking we got by the spectrophotometric method the following results for Savinia Golding and A-varieties shown in table 4,7 and 4,8.

The method of 5 cones doesn't show such a great agreement of values between parallel results as it is found in the conventional method; however it enables more rapid analysis of greater amount of samples.

For hop samples of 1974 crop we compared also the results of the conventional spectrophotometric method, the method of 5 cones and the conductometric method /Table 4.9 /. We got lower values by the conventional spectrophotometric method than by the method of 5 cones, however, the conventional method showed



better agreement of parallel results. Using plastic bottles and fiberglass filters we analysed by the method of 5 cones the samples of Aurora, Atlas and Apolon which have been kept in a storehouse at normal temperature for 5 months. The lowest degree of oxidation was found for Aurora, the highest for Atlas /Table 4.10/.

#### 4.13 Conductometric method of 5-cones

We wanted to find out whether the conductometric method is suitable also for the samples containing only 5 cones. We determined by conductometric and the spectrophotometric alpha acids of 2 samples in the toluen extract of 5 cones /Table 4.11.

We tried to find the applicability of both methods for samples of wild hops.

1,4 alpha acids was the smallest value that could be determined by the conductometric method of 5 cones samples. Working at high sensitivity of conductometric it was impossible to read out the correct values of conductivity. The spectrophotometric values of the samples examined varied between 0,4 to 2 %. Yet the modified spectrophotometric method of 5 cones and conventional conductometric method showed very good agreement of results also for low values of alpha acids as indicated in table 4.12. The samples of 10 grams were extracted with ether for conductometric method. So we established that the results of the method of 5 cones are reliable and could be used for rapid characterization of greater amount of samples.

#### 4.14 Determination of alpha-acids in fresh hop

By determination of alpha acids in fresh hop we examined Likens's method for the conductometric determining of alpha acids in fresh hop. Likens's method enables more rapid evaluation of hop ripeness in individual hop gardens. By that method we find 11,20 % and 11,40 % of alpha acids in dried substance for 2

parallel samples of fresh hop containing 81 % moisture, and 11,15 % of alpha acids in dried substance of dried hop. This method could be used for common determining but top drive macerator and an apparatus for quick determination of moisture would be needed.

#### 4.2 Storage stability

The method of accelerated oxidation of alpha and beta acids examined on 2 samples is very efficient in determining of storage stability. Storage stability depends on membrane permeability of lupulin glands and on the composition of substances in lupulin glands which can hasten or slow the oxidation.

By breaking of lupulin glands with stirring rod we eliminated air permeability of membrane and determined the stability in regard to the composition of glands. As it is seen from the table 4.13 the degree of oxidation for Apolon variety is already at the beginning of the experiment with the accelerated oxidation rather greater than it is for another hybrid.

#### 4.3 Essential oil

##### 4.31 Determination of myrcen

The gaschromatography of essential oil showed that myrcen is very variable substance, for its quantity varied greatly for the same variety when analyses were repeated. The method of gaining of essential oil from the light petroleum layer is a main reason for great deviation in results. We found that the composition of light petroleum layer /given in percents/ before distillation of solvent is essentially different from the composition of oil /chromatograms 4.1 and 4.2 / while the ratio of cariophylen to humulen doesn't change. Because the quantity of myrcen reduces greatly while the samples are stored we didn't calculate the percent of individual substances and determined only relations of areas of individual substances in comparison to the areas of cariophylen.

Table 4.7

Savinia Golding

Repetition	alpha	beta
1	7.70	2.95
2	8.34	2.90
3	7.66	2.98
4	7.97	2.75
5	6.06	2.28

Table 4.8

A-varieties

Variety	Repetition	alpha	beta
Atlas	1	11.22	2.82
	2	11.00	2.62
Ahil	1	12.81	4.31
	2	12.63	5.06
Aurora	1	11.72	4.80
	2	10.03	4.41
Apolon	1	13.30	3.81
	2	14.22	2.93

Table 4.9

Variety	Repetition	5 cones		5 cones $\frac{A\ 275}{A\ 325}$	convention.		conv. $\frac{A\ 275}{A\ 325}$	conductometric alpha
		alpha	beta		alpha	beta		
Aurora	1	10,9	6,1	0,33	9,6	7,7	0,26	12,0
	2	9,8	7,1	0,33	10,4	6,3	0,26	
Atlas	1	9,0	3,8	0,34	8,2	3,5	0,36	11,1
	2	7,7	3,8	0,47	7,7	3,6	0,37	
Apolon	1	10,6	4,8	0,27	9,2	4,1	0,27	13,5
	2	10,5	5,7	0,26	9,3	4,1	0,34	

Table 4.10

Spectrophotometric analysis of hop kept in a storehouse for 5 months

Variety	Repetition	alpha %	beta %	$\frac{A\ 275}{A\ 325}$
Aurora	1	12,36	3,66	0,33
	2	11,99	3,91	0,33
Atlas	1	5,5	2,2	0,57
	2	6,6	1,8	0,50
	3	5,8	2,7	0,50
	4	6,3	2,4	0,49
	5	5,5	2,0	0,54
Apolon	1	8,8	3,4	0,36
	2	8,1	3,4	0,34
	3	6,8	4,1	0,36
	4	1,1	3,1	0,35
	5	10,4	4,8	0,36

Table 4.11

Comparison between spectrophotometric and conductometric values for alpha acids by 5 cones samples

Sample	alpha spectrophotometric	alpha conductometric
1	3,6	5,5
2	4,9	4,4

Table 4.12

Comparison of chemical analyses of wild hop

Sample	alpha spectrophot. 5 cones	alpha Wöllmer conductom.	beta spectrophotom.
1	2.4	2.9	4.3
2	1.5	1.7	2.7
3	2.1	2.5	3.2
4	3.3	2.9	2.0
5	2.8	2.8	1.6

#### 4.32 Determination of the composition of essential oil<sup>of</sup> male flowers

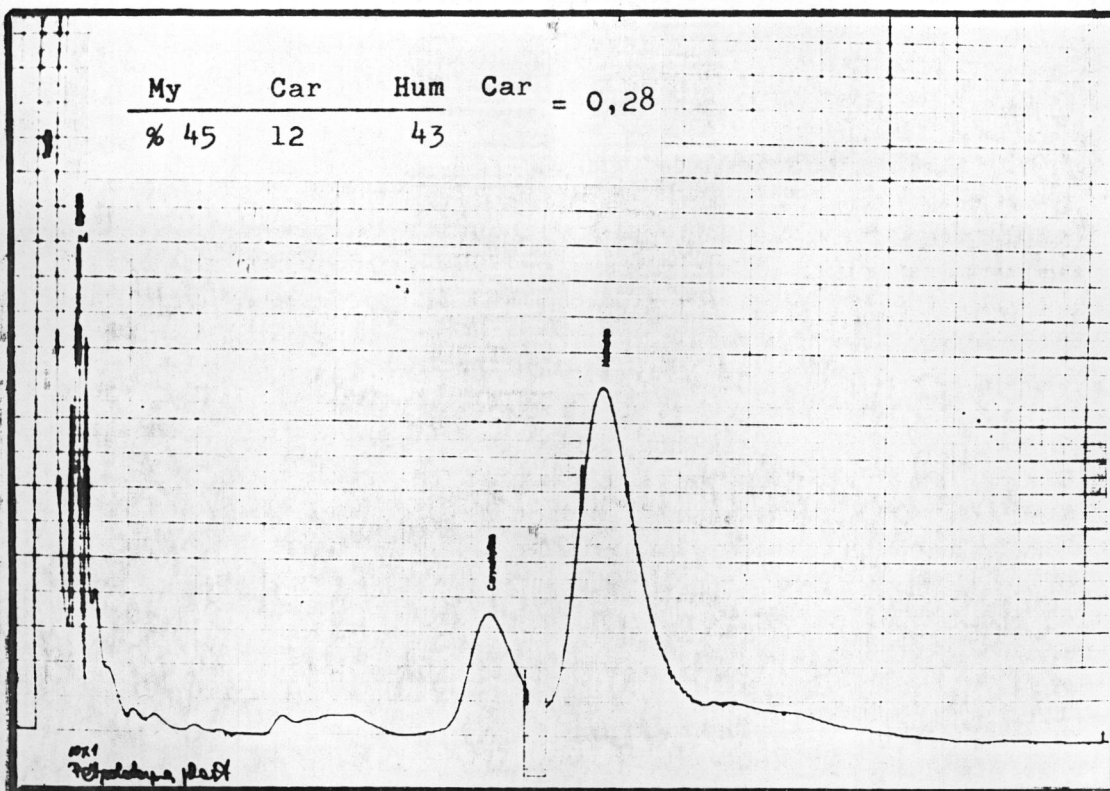
Although the male plant has no brewing value it has a great role in transferring of genetical characteristics such as storage stability and flavour. Very large samples are required for the gas chromatographic analyses of essential oil of male flowers if essential oil is gained by the steam distillation method.

Table 4.13

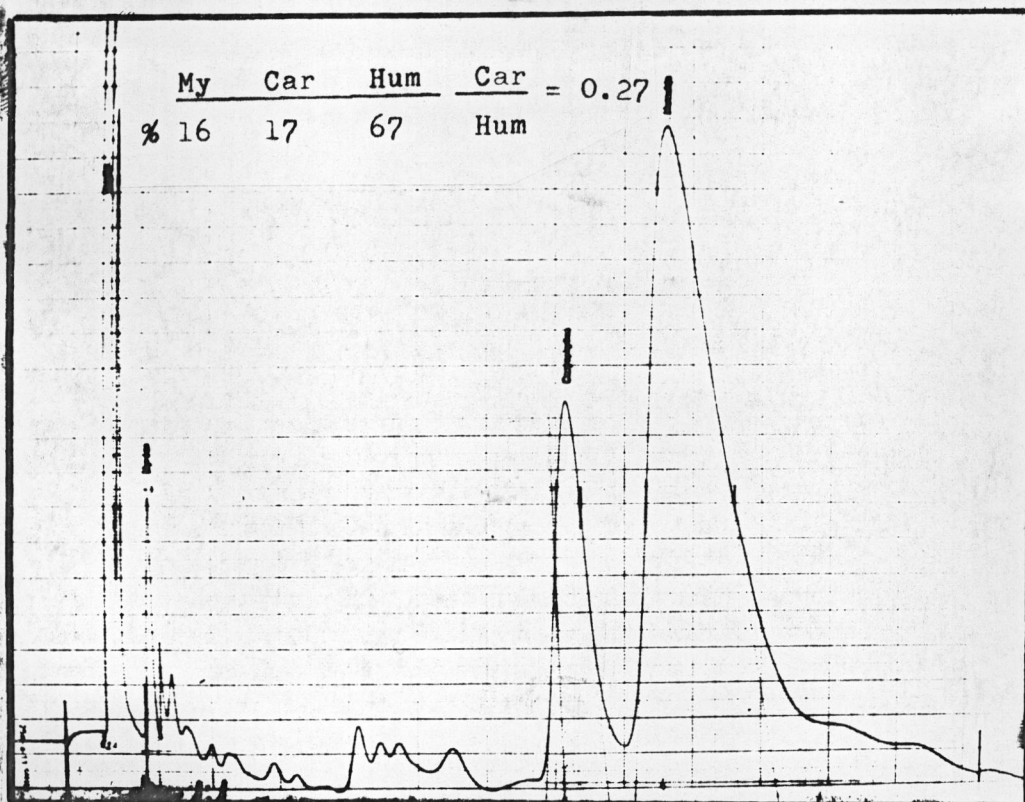
Determination of storage stability

Genitype	Time /hours/	5 mg of lupulin Temperat. of deterioration 85 °C		
		A <sub>275</sub>	A <sub>325</sub>	A <sub>275</sub> /A <sub>325</sub>
Hybrid	1	0.455	1.220	0.373
	2	0.485	1.170	0.414
	3	0.625	0.980	0.637
	4	0.685	0.735	0.932
	5	0.905	0.635	1.425
	6	0.790	0.385	2.052
	7	0.900	0.445	2.022
	8	1.200	0.531	2.260
Apolon	1	0.525	0.490	1.07
	2	0.680	0.565	1.20
	3	0.875	0.530	1.48
	4	0.690	0.415	1.66
	5	0.750	0.353	2.12
	6	0.665	0.310	2.14
	7	0.640	0.321	2.00
	8	0.840	0.380	2.21

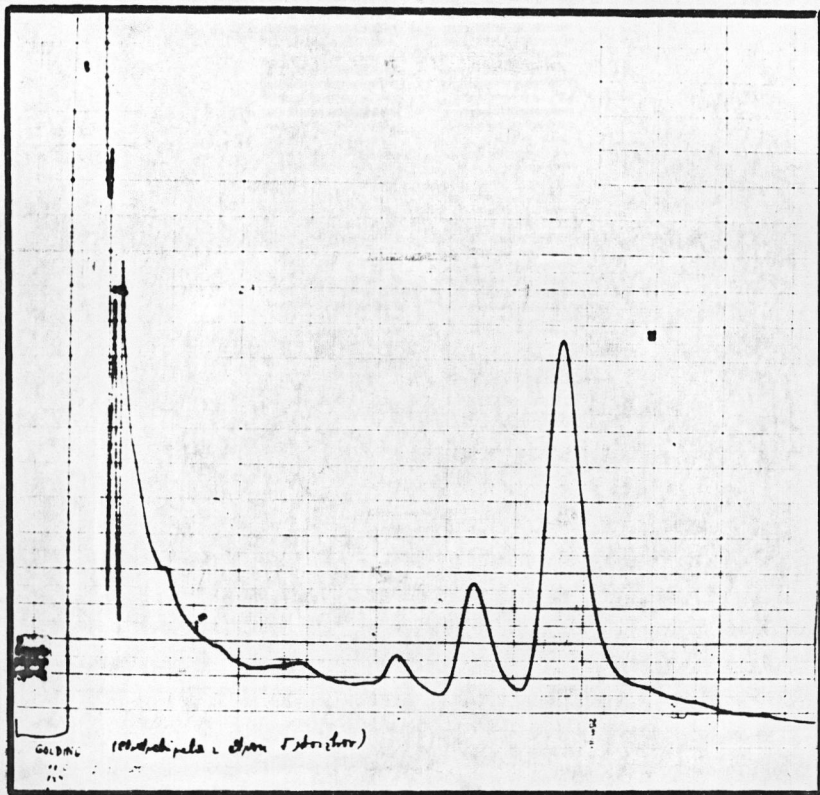
It is because the male flowers have thousand times less lupulin glands than the female ones. The method is simpler if we inject the extract of lupulin. In order to retain the components of low volatility injected ether extract through forecolumn packed with Celit 545. To test this method 5 cones of Savinia Golding were extracted by ether. The chromatogram 4.3 corresponds to chromatogram of essential oil, only substances such as limonen, linalool and pinen are not present.



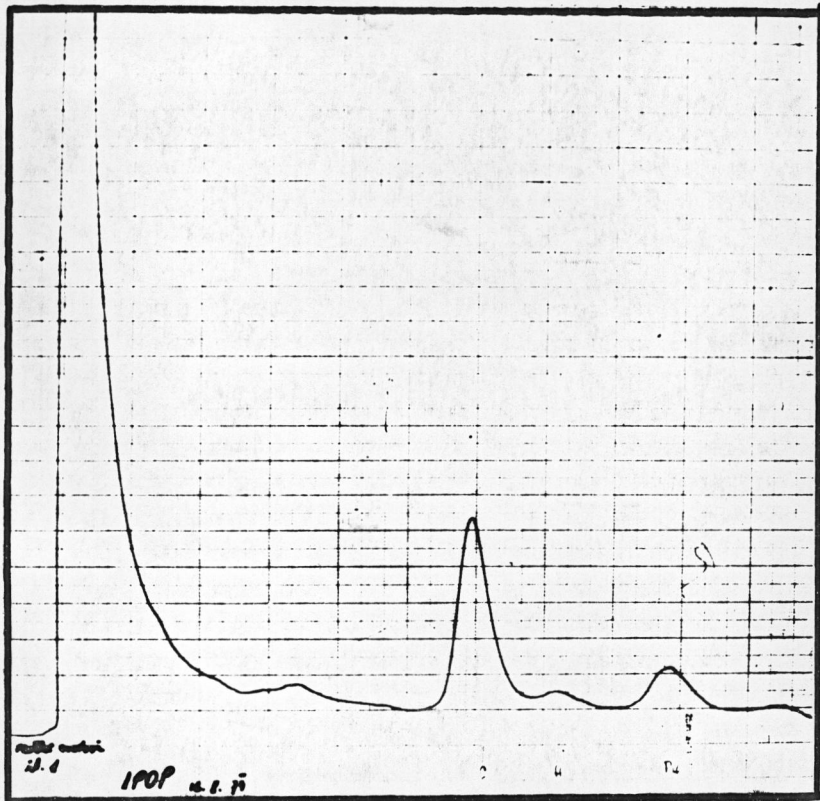
Graph 4.1 : Chromatogram of essential oil from the light-petroleum layer



Graph 4.2: Chromatogram of essential oil after the evaporation of solvent

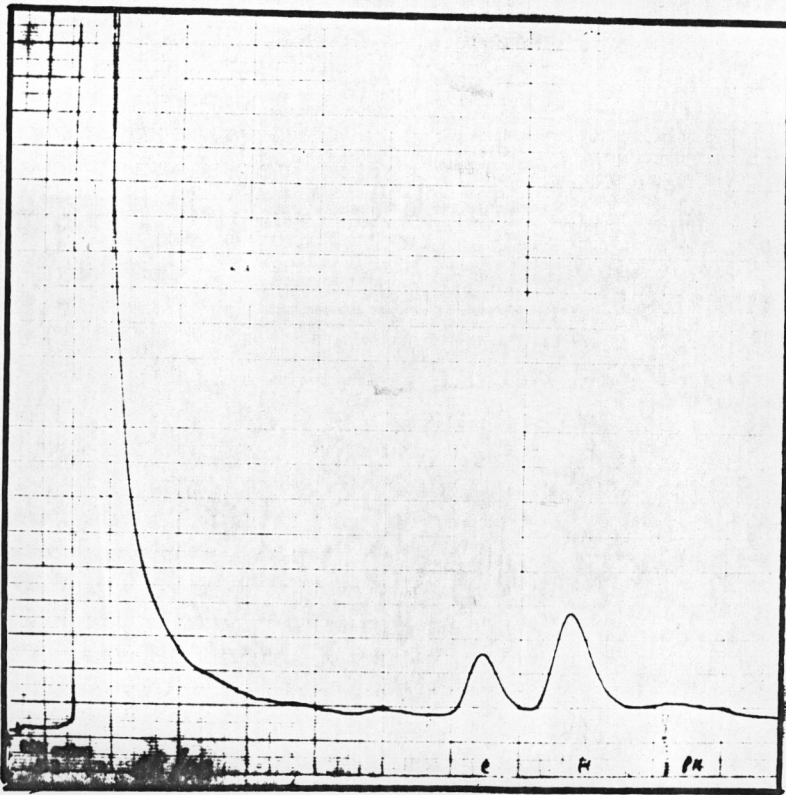


Graph 4.3: Essential oil from ether lupulin extract  
5-cones, Savinia Golding

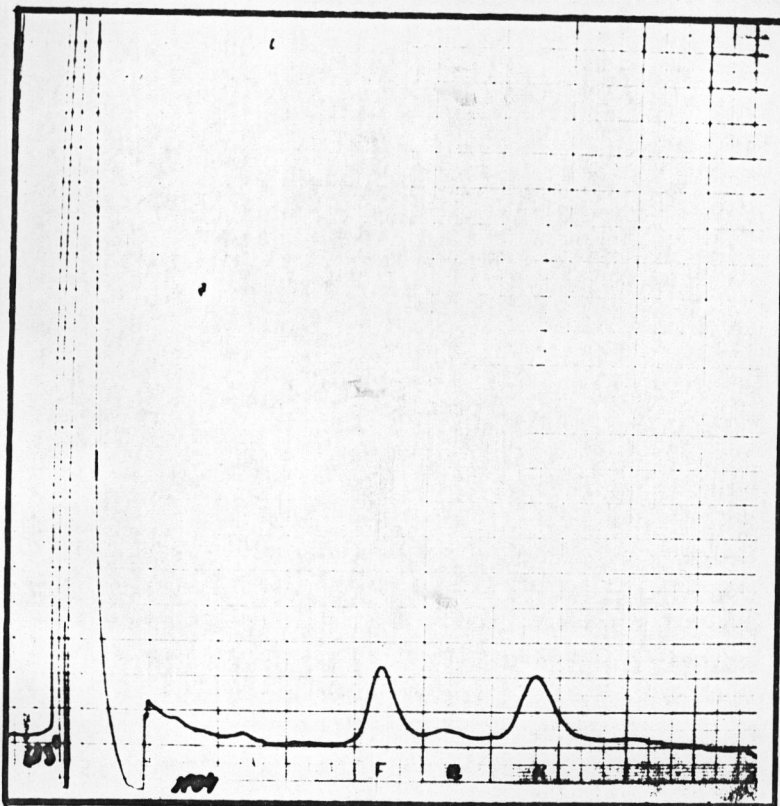


Graph 4.4: Essential oil wild male plant 19P09

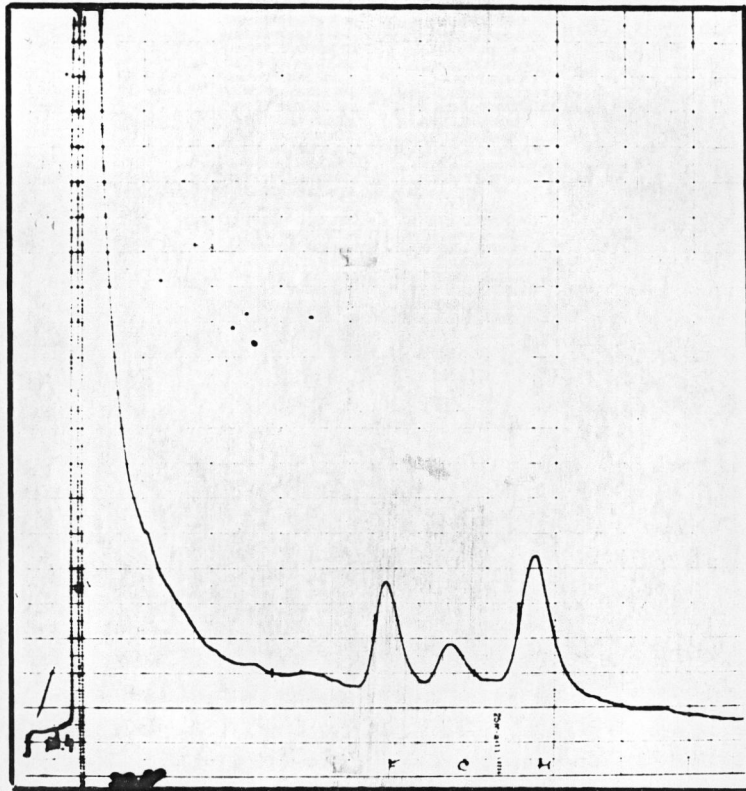




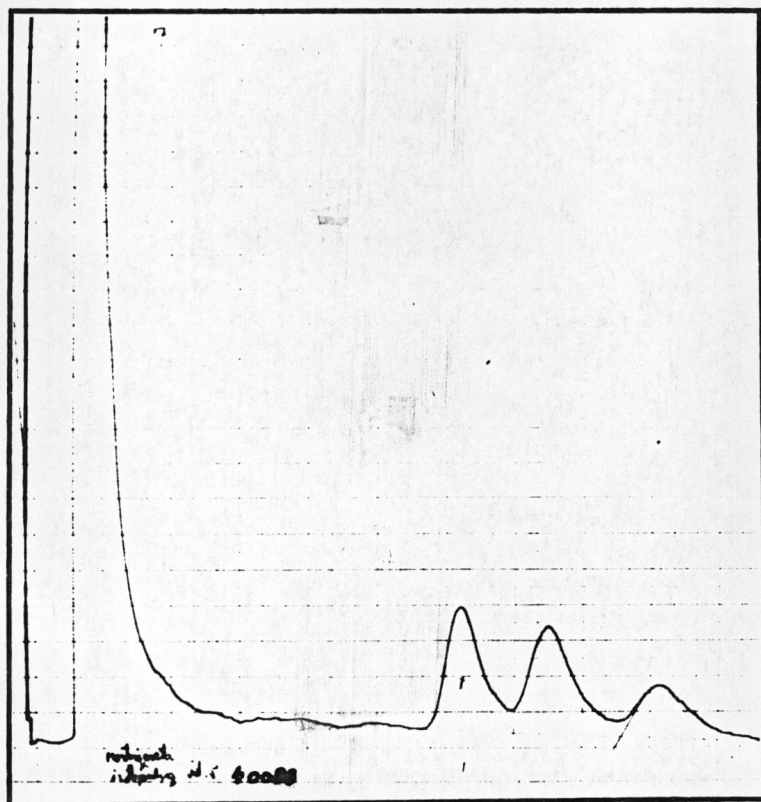
Graph 4.5: Essential oil of hybrid, male 65/46



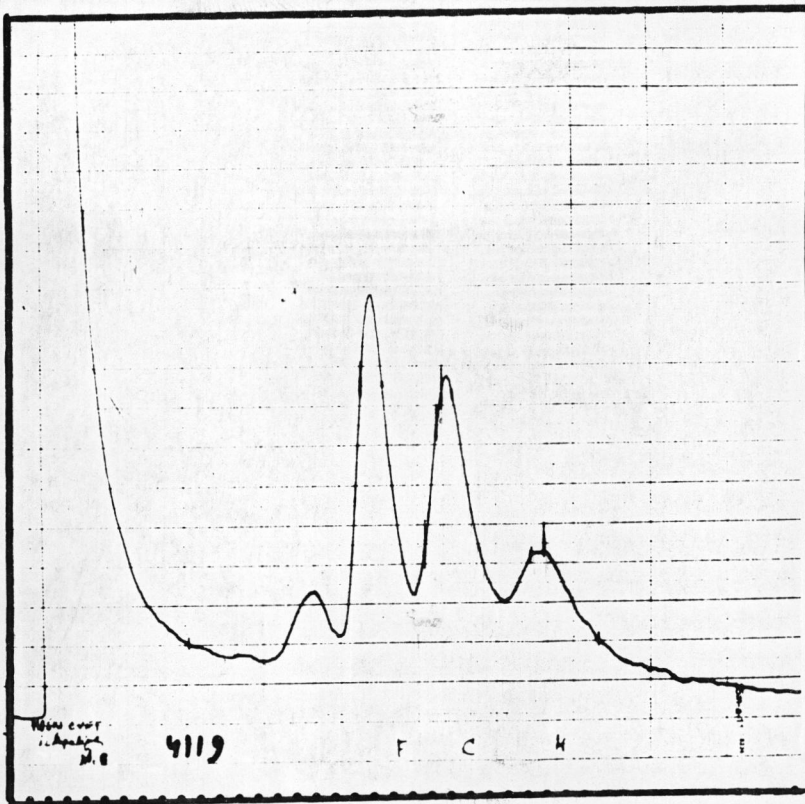
Graph 4.6: Essential oil, wild male 19P04



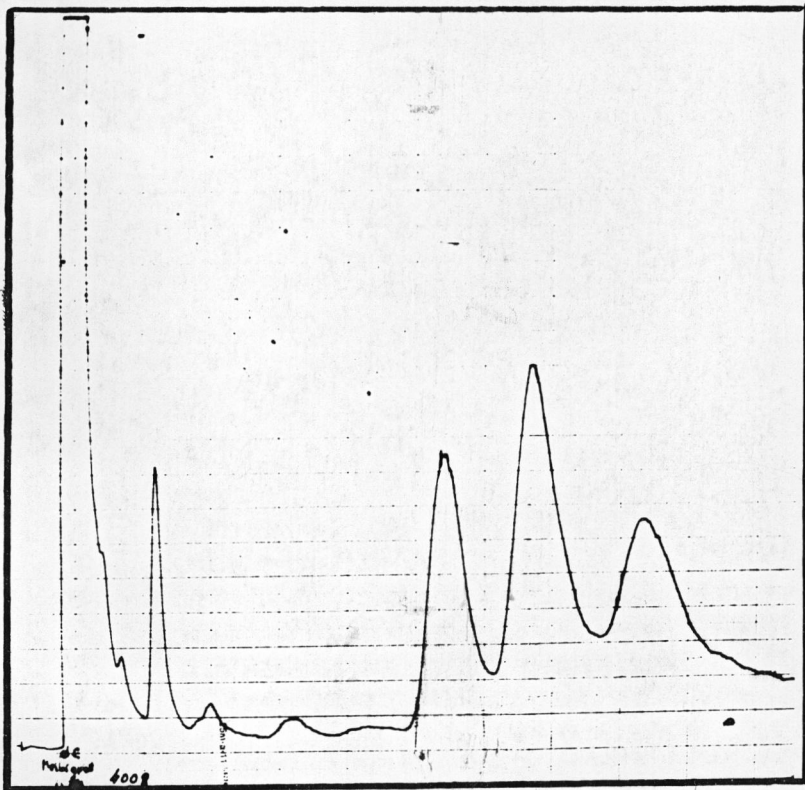
Graph 4.7: Essential oil, wild male 33P19



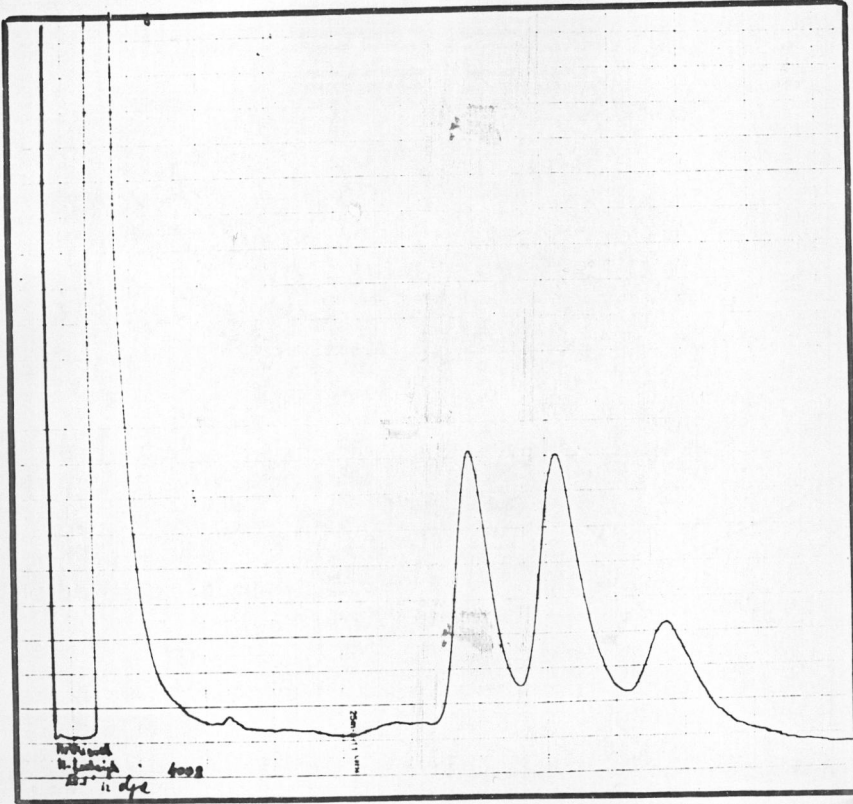
Graph 4.8: Essential oil, wild male 40P08



Graph 4.9 : Essential oil, wild male 41P19



Graph 4.10: Essential oil by distillation, wild male 40P08



Graph 4.11: Chromatogram of H-fraction, wild male 40P08

Extracted lupulin of male flowers was analysed by this method /Graph. 4.4 to 4.9/. No corresponding samples available, identification of substances except cariophylen wasn't possible. We suspect that substances such as farnesen, humulen and selinen are the main components. We didn't notice myrcen in any sample, what corresponds to literature. Sample 1 and 2 /Graph. 4.4 and 4.5 / are without farnesen which is also a characteristic of some male plants.

We gained essential oil from greater amounts of flowers of sample 5 by distillation and obtained a chromatogram very similar to those of ether extract /graph. 4.10 / except that also a substance with the same retention time like myrcen is present. We found that it doesn't belong to H-fraction /graph. 4.11 / and so it isn't myrcen.

#### 4.33 GLC Lupulin Extract of 5 Cones.

Gaining of essential oil by distillation with steam takes a long time and it is accompanied by changes in the composition of essential oil: so we wanted to find a more rapid method of determining substances vapouring easily. By headspace injection used by Hautke and Petriček we didn't achieved satisfactory results because the identification of individual components is not possible. The method of injection of light petroleum extract of 5 cones through forecolumn fuled with Celit 545 was examined on A-varieties, Ratios F/C and H/C were calculated and given in table 4.14.

Table 4.14

Ratio of F/C and H/C

Variety	Sample	F/C	H/C	Variety	Sample	F/C	H/C
Atlas	1	3.88	1.96		1	1.20	2.80
	2	3.60	1.62		2	1.28	3.41
	3	5.53	1.53	Aurora	3	2.18	4.13
	oil	2.98	2.08		oil	1.36	3.20
Ahil	1	3.55	1.95		1	3.42	1.96
	2	3.32	2.04		2	3.82	1.79
	3	3.64	2.09	Apolon	3	4.21	1.98
	oil	2.94	1.86		oil	3.36	2.06

#### 4.4 Determination of polyphenols in hop

We have determined polyphenols in the samples of Savinia Golding, Saaz and Yakima by the spectrophotometric method of Jernmais. According to literature the quantity of polyphenols in individual samples varies greatly; particularly in samples of different crops. Results and data from the literature shows table 4.15

According to literature the lowest value of quantity of polyphenols is 5 %, the highest 10 %, the deviation amount individual samples is more than 3 %, therefore the quantity of polyphenols in hop couldn't be taken as a characteristic of individual variety. According to the literature, only some varieties could be ascribed a characteristic of a low or high value of quantity of polyphenols.

To identify individual varieties we examined also the qualitative determination of phenols in hop leaves and used the paper chromatography method after Melouk, however, we haven't

received usable chromatograms because of improper method of detection. After spraying with  $\text{FeCl}_3$  and  $\text{K}_3/\text{Fe}/\text{CN}_6/$  the whole chromatogram turned blue and we didn't succeed in identifying of phenols.

Table 4.15

Quantity of polyphenols

Sample	Repe- tition	% polyphenols	Average	Data from literature		
				Minimum	Maximum	Average
Savinia Golding	1	8,34				
	2	9,41	8,10	8,12	8,91	8,81
	3	7,86				
Saaz	1	10,06				
	2	8,53	8,70	9,01	10,4	9,53
	3	7,60				
Yakima	1	9,12				
	2	8,87	8,99	7,81	8,28	8,04

### C /6/ Conclusions

Further investigations of wild hop in Yugoslavia in habitats as well as in collection gardens show that there is a great variability of population in physiological and chemical properties. The resistance against downy mildew is various. The duration of vegetation and the productiveness of the plant are also various. Especially interesting are the results about the amount and the composition of bitter resins and of essential oil. Positive correlation between the amount of alpha resins and the ratio of alpha to beta has been found. In regard to values of ratios of more important sesquiterpens one plant differs greatly from the other. The study of families of wild hop gave also usable results and it will become the main part of the project. Examining and introducing new method of chemical analyses of hop has methodically enriched the project.

### C /7/ Plan for Future Work

The examination of habitats and determination of characteristics of plants in collection garden will be equally important in future. In next period the brief explorations of Dinaric and Mediterranean macroregions will be done.

In wild hop collection gardens the observations of phenotypic characteristics will be continued and also the study of downy mildew resistant plants in separate garden will begin. We shall begin with genetical analysis of superior wild plants.

In research work the new chemical methods will be included, as analyses of 5-cones, determination of index of deterioration and storage stability, determination of ratios among important sesquiterpens etc.



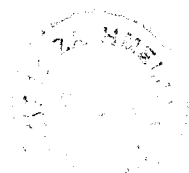
C /8/ Publications

In reporting period the doctor`s thesis "Wild Hop - Humulus lupulus L. in Yugoslavia" was prepared. The thesis was defended by the principal investigator Tone Wagner, mgr.agr. at Biotechnical Faculty, University of Ljubljana on May 12, 1975. It will be printed in autumn 1975 but the summary is included in this report.

C//9/ Graduate Degrees

The principal investigator Tone Wagner, mgr.agr. defended the doctor`s thesis on May 12, 1975. The annual report is finished with delay of 3 month therefore we ask for exuse.

Žalec, June 1975 .



Principal Investigator

Dr. Tone Wagner, mr.pl.nut,  
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Official authorized  
to sign for the grantee:

Zvone Pelikan, dipl.ing.agr.  
director of Institute of  
Hop Research

S U M M A R Y

of

Anton WAGNER

WILD HOP - HUMULUS LUPULUS L. IN YUGOSLAVIA

/Dissertation/

Ljubljana, 1975

## S U M M A R Y

I have investigated the population of wild hop in Yugoslavia for several years. I examined the ~~th~~ extension of wild hop habitats, hop microassociation, its floristical composition, and ecological, morphological, physiological, and chemical properties of wild hop in natural conditions. I collected planting material from habitats and formed a collection garden. There I could observe the properties of plants under the same ecological conditions, which were collected all over Yugoslavia. In collection garden I observed morphological, physiological, and chemical properties of plants and tried to determine the characteristics of wild hop population in Yugoslavia in comparison to hop varieties.

### A/ Investigation of Wild Hop Habitats

Wild hop is spread all over Yugoslavia, however The frequency of habitats in particular macroregions and in geographical regions is different, depending on ecological conditions and on the spreading of cultivated area. I described 398 habitats. The highest frequency of habitats is in the Carpatho-Balkanian macroregion. Habitats of Wild hop are spread everywhere where favourable soil conditions are found. Smaller, but various habitats are in the Dinaric, Mediterranean and Pindo-Rhodopian macroregion, while habitats in northern macroregions are bigger and more alike. Wild hop is a plant of humid microclimate and alluvial humus soil, therefore it is not found in mountainous area, in the dinaric karst country and in typical mediterranean land.

Hop microassociation is various. It consists of plants which are characteristical of individual plant associations; hop is not a characteristical ~~plant of a~~ species of individual plant association. Wild hop is a characteristical plant of hop micro-association having climbing, bush like or creeping appearance

which depends on accompanying plants. The growth form of wild hop depends among others factors on accompanying plants.

The average overgrowth of wild hop habitats in Yugoslavia is 80%, hop covering 18% of habitat, other lianas 15%, trees 20%, shrubs 14% and perennial herbs 13%. Ecologically typical plant species are characteristic of individual habitats.

Depending on ecological conditions in microassociation wild hop has different abundance, associability, form, and growth.

There is middle to great abundance of wild hop in majority of habitats. It is greater in northern macroregions, where better ~~and~~ soil conditions are found and also the influence of cultivated hop which is spread in the Alpine and Pannonian macroregion is possible. In the Dinaric macroregion the plant is more often growing alone while it is more luxuriant and appears in bushes because of more favourable soil conditions in other macroregions. Different forms of growth of wild hop~~x~~ are found over all investigated area. The climbing form of plant ~~is~~ prevails.

Morphological properties of wild hop in habitats are the result of genetical and ecological conditions. The colour of bine is red, reddish, rarely green. Green bines are more frequent in northern and central macroregions. The thickness of bine is various, considerably depending on ecological factors.

The colour of petiole is in positive correlation to the colour of bine. Red and reddish petioles prevail, they are rarely green. Petiole is the longest in the Alpine macroregion, where bush like and creeping forms of the plant prevail. The shortest are those petioles from the Carpatho-Balkanian macroregion, where the climbing form of wild hop is widely spread.

There is variability in the size of leaf blade. Leaf blade is to 14 cm long, rarely longer. The plants of the Alpine, Pannonian and Carpatho-Balkanian macroregion have narrow leaf blade. The

widest leaf blade is found in the Dinaric macroregion. The shape of the leaf blade is expressed by relation between the length and the width of leaf blade; it is 0,67 in the Mediterranean macroregion where leaves are the widest; and 1,11 in the Alpine macroregion where leaves are long and narrow. Leaves are three and five-lobbed. Leaves with seven lobes are rare. The smallest lobation is found in the Carpatho-Balkanian macroregion where three lobbed leaves prevail.

Main lobe is a characteristical part of leaf blade varying in length and dentation. Main lobe is short in the Alpine and Pannonian, however long in the Pindo-Rhodopian and Mediterranean macroregion. Main lobe apex is longer in the Dinaric and Mediterranean macroregion. Sharpened leaves with long apex exist in habitats of the Mediterranean macroregion.

Relation between length of main lobe and length of leaf denotes the indentation of leaf and it is higher in the Pindo-Rhodopian and Mediterranean macroregion, although the great variability is present in every macroregion.

Sinus cleft of leaf is narrow or middle wide. Wide sinus cleft is rare and can be found mostly in the Dinaric and Mediterranean macroregion where greater variability of sinuses is found.

The pubescence of leaf is determined by the type of pubescence, density and length of hair. Type of pubescence III, where hair are spread all over leaf blade is frequent in the Pindo-Rhodopian macroregion, however in the Carpatho-Balkanian macroregion the pubescence type I, where hair is spread only on the main leaf veins is widely spread. Denser pubescence and long hair are found mainly in the southern part of the Pindo-Rhodopian macroregion.

Cone has oval shape, it is rarely round. An average cone is 28,60 mm long, 18,02 mm wide; The weight of 100 cones is 11,19 g. Various cone shape is frequent in the habitats of the Pindo-Rhodopian and Mediterranean macroregion.

Cone bract is oblong; the ratio of length to width is 1,8 to 2,1.

It has short, obtuse or sharp tip. Mostly it has seven veins.

The seed in cones is well developed; The average length 2,66 and the average width 2,24 mm. It has more or less reddish colour. Dark violet seed is frequent in the Mediterranean macroregion.

The physiological characteristics of wild hop in habitats are different. Plants are not equally resistant against diseases and insects. Downy mildew is frequently found on leaves, rarely on shoots. It is equally spread over all area, some plants are more resistant. Powdery mildew is rare and only found in habitats of macroregions with warm climate. Red spider and aphids are very rare and I could not find any sign of damage.

From chemical characteristics I have uninvestigated mainly the content and composition of bitter resins and composition of hop oil in female plants. Content of bitter resins in hop cone varies greatly from habitat to habitat. The content of total resins is 13,99%, the average of soft resins is 12,67%, of alfa acids 4,07%. Cones from some habitats have over 6% alpha acids although they are seeded. The relation between alpha acids and beta fraction is 0,49.

The composition of hop oil in cones which were gathered in different habitats is various. The quantities of sesquiterpens vary greatly, and ratios of characteristical sesquiterpens are frequently different. I found following characteristics interesting: the ratio of humulen to caryophylen, the ratio of farnesen to caryophylen, and the ratio of humulen to posthumulen I. The values of ratio of humulen to caryophylen are less than 1 to 8,93 and are for many samples different from values characteristic of european hop varieties. Different values of relation between humulen and caryophylen are found over all investigated area and I could not find out the correlation to geographical regionalization.

#### B/ Investigation of Plants in Collection Garden

Many collected plants are in collection garden under the same ecological conditions and under the same technology. Collection

representing the population of wild hop in Yugoslavia consists of 72 female and 37 male plants and I have been determining their morphological, physiological, and chemical properties.

I have investigated morphological characteristics of leaf for female and male plants separately. There are not any significant differences between female and male plants. Leaf blade of female plants is  $124,32 \pm 1,56$  mm long and  $151,71 \pm 2,60$  mm wide with coefficient of variation 10,67 respectively 14,55%. Average leaf blade of male plants is  $122,89 \pm 2,65$  mm long and  $147,97 \pm 3,20$  mm wide with coefficient of variation 12,75% resp. 12,77%. Male and female plants have also other morphological properties similar, coefficient of variation is low. Female plants have  $78,71 \pm 1,15$  mm long main lobe, male plants  $76,66 \pm 1,71$  mm. Five lobbed leaves prevail, some plants have three lobbed leaves, rare are those with seven lobes: 09P01 - Skradin, 13P06 - Gračac, 15P02 - Slunj, and 16P02 - Mala Gorica. Plant 09P01 had seven lobbed leaves also in habitat 09A01 - Skradin.

The dentation of main lobe is middle, from 14 to 26 dents and similar to european hop varieties. Leaf pubescence of type I and II prevail. Plants with leaf pubescence of type III are rare, there are 17% of them among female plants and 23% among male ones. Pubescence of type III is found at plants which come from the Mediterranean and Pindo-Rhodopian macroregion. It occurs on male plants chiefly.

The ratio of the length to the width of leaf blade of female plants is  $0,83 \pm 0,01$ , of male plants  $0,82 \pm 0,02$ . The ratio of main lobe length to the length of leaf blade is  $0,63 \pm 0,01$  for female plants and  $0,62 \pm 0,01$  for male plants. The variability of mentioned ratios is little and the values of coefficient of variation are 12% or less.

The colour of bine is red or reddish, also violet; plants with green bines are rare /14 or 16%.

Morphological analysis of leaf and bine showed that morphological complex of wild hop population is very homogenous and except of

type of pubescence it is not possible to denote geographical regionalization according to morphological characters.

Cones are middle heavy / in 1973 average weight of 100 cones was 13,30 g; 12,86 g in 1974/. The shape of cone is oval in relation length to width 1,69 /year 1973/, and 1.63 /year 1974/. There is also small variability ; coefficient of variability is 11,77% and 11,45%.

Aroma of wild hop is not ho like; the majority of samples have strange, hop unlike aroma.

The bract of cone is oblong, narrow, ratio of length to width is 2,15. It has six to ten veins and the pubescence is middle.

Physiological properties of plants are more variable than morphological.

Viability of plant is expressed by plant form. In collection of female plants there were 3% of weak and 54% of very luxuriant ones. In collection of male plants we had 8% of weak and 46% of very luxuriant plants.

The duration of vegetation in collection was very various. Female plants were mostly late and very late /69%/, male plants early /73%/. Female plants were middle productive / average of 1,19 kg of green hop per plant/ with great variation /coefficient of variation = 59,97%/. In female collection there are plants which are rather productive.

During the vegetation I observed downy mildew infection. Systemic infection has not spread, while in vegetative phase and even more in generative phase downy mildew considerably developed. In 1974 at the end of vegetative phase plants which were not protected were infected as follows:

female collection: 25% of plants were very much infected, 30% of plants middle infected, 45% little infected. The plant 37P03 - Zumski put showed no signs of infection. 44897  
male collection: 29% of plants were very infected, 31% middle,



and 40% little. Rather resistant plants to downy mildew were: 33P06 - Majdanpek, 34P04 - Kuršumljija, and 40P21 - Ohrid.

Bitter resins were determined in female and in male plants. There is considerable variability in the content of alpha acids of female plants: in 1973 from 0,32% to 5,63%, in 1974 from 0,53% to 4,95%. Ratio of alpha acids to beta fraction has considerable variability from 0,09 to 0,79. There is the positive correlation between percent of alpha acids and alpha/beta ratio  $r = +0,8919$  respectively  $-0,8497/$ . Male flowers contain average 0,64% of alpha acids.

Composition of hop oil is very various. There ~~is~~ are pretty different values for ratios of sesquiterpens for female and male plants. European varieties have ratio of humulen to caryophylen from 3 to 4. In collection of wild hop female plants ratio H/C for 38% of plants is less than 1, for 20% of plants it is from 2 to 3 and only for 17% of plants the ratio H/C is from 3 to 4. Plants with different ratio H/C are from different habitats all over Yugoslavia. Ratio of farnesen to caryophylen is from 0 to 2 for european varieties. It is characteristic of wild hop collection that only 11% of plants have ratio F/C over 2.

The hop oil of wild hop from Yugoslavia contains posthumulens. The majority of samples contains more posthumulen 1 than humulen/64%/, there are only 25% of plants which contain more posthumulen 2 than humulen. The majority of plants has low ratios H/C and H/PH1 which is expressed also in value of correlation coefficient  $r = +0,6784/$ .

For majority of male plants ratio H/C is more than 1, ratio F/C is mainly lower than 1. There is small quantity of posthumulens.

I analysed cytologically 4 female and 7 male plants, which are all diploid with chromosome number  $2n = 20$ .

The investigation of wild hop in habitats and in collection garden gives an idea where in Yugoslavia wild hop is spread and what are the properties of wild hop population. Morphological and

physiological properties of the plant are influenced by heterogeneous ecological factors. We can get the real picture of the plant by growing it in collection garden. There are not any important differences in properties between hop plants in habitats and those in collection garden, however I have not compared morphological and physiological properties of the plant in habitat and the one in collection garden because of different ecological conditions of habitat.

To describe the population of wild hop in Yugoslavia in relation to european hop varieties there are properties of wild individual plants in collection garden that are important. Morphological properties show little variability, they are similar to european hop varieties. So we can say that wild hop in Yugoslavia belongs to european morphological complex of wild hop. Physiological and chemical properties are much more changeable, individual plants representing the population of wild hop show considerable variability. Among them ~~are~~ there are plants which are similar to cultivated hop and also those which have rather different physiological and chemical properties from european hop varieties. However chemical properties, content and composition of bitter resins and hop oil composition reach in some plants values characteristic of european hop varieties. The population of wild hop in Yugoslavia is therefore a treasure for genetic variability of european hop and may serve for breeding new hop varieties in future.



