THE STEROLS AND RELATED MINOR CONSTITUENTS OF VARIOUS RAPESEED OILS

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Introduction

Much effort has been made to analyse rapeseed oil, to isolate its constituents, and to elucidate their structures. LANZANI and JACINI (1971, 1973) were successful in research on components contained in the oil in low concentrations. Yet, rapeseed oil abounds of numerous minor constituents, and thus, new developments in analytical methods lead to the detection of hitherto unknown substances. Moreover, additional problems result from breeding of new varieties of rape with low content of erucic acid or glucosinolates.

In analysing the composition of sterol mixtures isolated from various seed oils we have found several so far unknown sterols (HOMBERG and SCHILLER, 1973). For instance, using a new method of analysis, we have detected ten sterols in sunflowerseed oil. We have isolated these compounds and elucidated their structures. The same combination of thin-layer chromatography and gas chromatography led to recognition of eight or nine sterols in rapeseed oil instead of the four sterols identified so far.

Experimental

We received the seeds from Prof. Röbbelen, Göttingen, and from the Norddeutsche Pflanzenzucht, Hohenlieth. To isolate the oils, seeds were ground and extracted with petroleum ether (b.p. 35 - 45° C) using the Soxhlet-method. The unsaponifiable matter has been isolated from the oils by the standard diethylether method. From the unsaponifiable matter the sterols were isolated by TLC in the way formerly described (HOMBERG and SEHER, 1972).

GLC analyses were carried out in all-glass system using columns, filled with 10 % OV-17 on Supelcoport (100/120 mesh). Runs were performed isothermally at 265° C.

Results and Discussion

Previously, we have used thin-layer chromatography on layers consisting of a mixture of magnesium oxide, aluminium oxide, and plaster of Paris (SEHER and HOMBERG, 1971, 1972). We have separated the 3,5-dinitrobenzoates of the sterols, because they are suitable for quantitative determination. When we applied this method to the quantitative analysis of sterols in rapeseed oil, we found surprisingly high contents of cholesterol.

Corresponding results were reported by KARTNIG and MIKURA (1970) who used a somewhat different method.

Complementary analysis by gas chromatography on OV-17 as stationary phase showed a low content of cholesterol in the oil. The results are compared in table 1.

Table 1: Analysis of sterols in rapeseed oil by different methods

Method	Sito-sterol	Campe-sterol	Chole-sterol	Brassica-sterol	Other sterols
TLC	51,0 %	26,0 %	13,0 %	8,0 %	2,0 %
GLC	52,1 %	38,7 %	0,3 %	8,1 %	0,9 %

It is obvious, that for sitosterol and brassicasterol nearly identical figures were obtained by TLC and GLC. Differences were observed in the content of campesterol and cholesterol. We have recently found that oxidation products of sitosterol may interfere with cholesterol in thin-layer chromatography and may lead to erroneous results. Perhaps, in GLC analysis similar interference occurs with campesterol. We are still studying this question.

A typical gas chromatogram of rapeseed sterols is shown in figure 1.

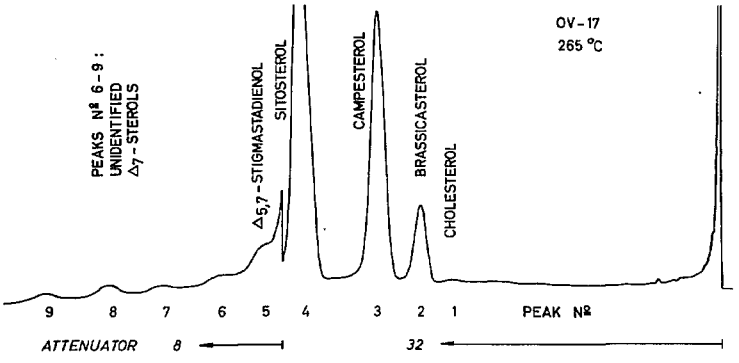

Figure 1: Gas chromatogram of the sterol mixture from rapeseed oil

The chromatogram shows the peaks from cholesterol, brassicasterol, campesterol, and sitosterol. After the latter can be recognized a number of peaks -- four or five depending upon the variety of rape. Figure 1 demonstrates the peaks of five new sterols and sterol derivates.

When we tried to isolate these sterols by preparative thin-layer chromato-

graphy, we found that they are rapidly oxidised. Thus, we encountered enormous losses in each purification step. Storing all extracts in a methanol slurry and excluding carefully UV-light we isolated some material for structural studies. The peak number 5 in figure 1 is due to a conjugated stigmastadienol. The behaviour of this compound corresponds with that of $\Delta^{5,7}$-stigmastadienol. So far, the identification of the other Δ^{7}-sterols, shown in the gas chromatogram, has not conclusively been finished.

It should be mentioned that ITOH et al. (1973) have found in rapeseed oil two 4-methyl-sterols, namely gramisterol and citrostadienol.

Both of these 4-methyl-sterols are derivates of lophenol. Gramisterol is the 24-methylen-derivative, and citrostadienol is the 24-ethyliden-derivative. Both compounds are also members of the Δ^{7}-series.

When we tried to extract the Δ^{7}-sterols from seeds that had been stored for six months, we found them only in reduced concentrations. But when we extracted freshly harvested seeds of the same variety the following year, the Δ^{7}-sterols could be found in the concentration as before. This fact demonstrates how instable these substances are, and this may be a reason why other investigators did not detect Δ^{7}-sterols in rapeseed oil. Also, quiet obviously, investigations of these sterols are most rewarding during a relatively short time intervall after the harvest.

The quantitative composition of the mixture of sterols seems to be correlated with the erucic acid content of analytical data from summer-rape.

The latter two columns of table 2 show the contents of erucic and oleic acids which are correlated. The Canadian varieties, ORO and SPAN, have a reduced content of brassicasterol. Thus, we were hoping to observe an interrelation of sterols similar to that of the fatty acids. Analogous observations have been reported by MORDRET and HELME (1974). But in the German strains, ORO 117 and ERGLU, a "normal" concentration of brassicasterol, and an enlarged area of the campesterol peak can be stated.

In table 3 the results for winter-rape are listed. This table is arranged in the same manner as the former. Similarily to the spring-type varieties there occurs a larger peak area of campesterol in the varieties having low contents of erucic acid.

The cross-breeds of these varieties are so complex in their composition, that genetic principles cannot be deduced from the few data we have available so far. But the pattern observed in the figures of both tables did not change for two or three generations of the varieties, which we have under analytical control.

These analyses which are part of our studies on rapeseeds are not final, but they may investigate further investigations.

Table 2: Composition of the sterol mixture in summer rape and the content of erucic acid

Variety	Chole-sterol	Brassica-sterol	Campe-sterol	Sito-sterol	other sterols	Erucic acid	Oleic acid
Echo	trace	11,2 %	27,7 %	59,4 %	1,6 %	23,4 %	33,1 %
Bronowski 109	0,3 %	11,4 %	38,0 %	48,4 %	1,9 %	18,9 %	33,9 %
Span (Canbra)	trace	8,6 %	34,8 %	55,1 %	1,5 %	4,2 %	56,1 %
Oro (Canbra)	trace	8,2 %	34,5 %	55,9 %	1,4 %	1,6 %	60,0 %
Oro 117	0,6 %	12,0 %	37,3 %	49,1 %	0,9 %	0,4 %	61,7 %
Erglu	0,5 %	10,3 %	42,6 %	44,6 %	2,0 %	0,3 %	57,1 %

Table 3: Composition of the sterol mixture in winter rape and the content of erucic acid

Variety	Chole-sterol	Brassica-sterol	Campe-sterol	Sito-sterol	other sterols	Erucic acid	Oleic acid
Rapol E 300	0,6 %	9,6 %	34,2 %	55,0 %	0,6 %	51,7 %	11,4 %
Lenora E 90/91	0,5 %	10,7 %	35,9 %	50,9 %	1,9 %	51,0 %	10,9 %
Diamant E 286	0,9 %	9,5 %	34,7 %	53,8 %	1,1 %	50,6 %	11,4 %
Rapol 120	0,5 %	9,3 %	33,1 %	56,0 %	1,1 %	48,7 %	11,0 %
Sinera (ind.)	trace	8,2 %	38,3 %	51,2 %	2,3 %	4,8 %	52,6 %
Sinera (lab.)	trace	9,1 %	39,8 %	50,1 %	1,1 %	1,6 %	58,3 %
Lesira	trace	7,2 %	38,6 %	53,5 %	0,7 %	0,8 %	59,1 %
Lesira (new)	0,3 %	8,1 %	38,7 %	52,1 %	0,9 %	0,4 %	60,4 %

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