Phytochemistry, 1980, Vol. 19, pp. 1375–1378. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0701-1375 $02.00/0

FATTY ACIDS OF LIPIDS FROM CULTURED SOYBEAN AND RAPE CELLS

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(*Revised received* 26 *November* 1979)

Key Word Index—*Glycine max*; Leguminosae; *Brassica napus*; Cruciferae; soybean; oil seed rape; cell cultures; fatty acids; neutral lipids; glycolipids; phospholipids.

Abstract—Lipids from cultured cells, leaves and seeds of two varieties each of soybean (*Glycine max*) and oil seed rape (*Brassica napus*) were separated into neutral lipids, glycolipids and phospholipids and their fatty acids were analysed. Usually, the fatty acid composition differed between corresponding fractions from cultured cells, leaves and seeds. Differences were least marked in (i) the phospholipids from cultured cells and leaves of soybean and (ii) the neutral lipids from cultured cells and seeds of rape. In the cultured cells, the fatty acid composition of the phospholipids differed from that of the glycolipids and neutral lipids, and fatty acids of chain length greater than C_{18} comprised a large proportion of the fatty acids of the glycolipids.

INTRODUCTION

Comparisons have been made of the lipids from cultured cells with those from tissues of whole plants [1–3] and there are reports of the fatty acid compositions of different lipids and lipid classes from cultured cells [1, 2, 4, 5]. However, there is no comparison of the fatty acid compositions of major lipid classes from cultured cells with those from whole plant tissues. Here we report the fatty acid (FA) compositions of whole fractions of neutral lipids (NL), glycolipids (GL) and phospholipids (PL) from seeds, leaves and cultured cells of two varieties each of soybean (*Glycine max*) and of oil seed rape (*Brassica napus*).

RESULTS AND DISCUSSION

Instead of replicating the experiments for one variety of each species, single experiments were done with two varieties of each species in the expectation that any common features of the two sets of results would be more likely to indicate a feature of the species as a whole. Conclusions about differences between replicate varieties were thus not possible. The results for replicate varieties were usually close (Tables 1 and 2) and these similarities form the basis of the main conclusions. With 4 replicate cultures of rape cv Rapora we have obtained standard errors of ±6% of the FA wt per g fr. wt and of $<\pm 4\%$ of the % FA compositions. Thus, there probably were varietal differences in the FA from the NL and GL of the cultured cells; the FA from PL were similar for replicate varieties.

It was expected (and found: Tables 1 and 2) (i) that seeds would have a very high content of FA from NL, (ii) that leaves would contain quite large amounts of FA from GL and that in these linolenic acid (18:3) would be very abundant. These would be characteristic, respectively, of structures specialized to store lipids and of photosynthetic tissues. Therefore, it was not expected that the cultured cells would be similar on these points. However, it was interesting to see if other components which might not have so well reflected specialization (e.g. the PL) were similar, and to see if the types (rather than amounts) of FA in NL were similar in the seeds and cultured cells.

The contents of FA from NL and PL (wt per unit fr. wt) varied from about the same or a little higher (soybean) to several times as much (rape) in the cultured cells, as compared with the leaves (Tables 1 and 2). There was less variation in the ratio of amounts of FA from NL and PL: in Bråvalla and Rapora they were *ca* the same in leaves and callus; in Fiskeby V the ratio was *ca* 20% higher in the leaves and in Maris Haplona it was *ca* 74% higher in the callus. The ratios of amounts of FA from PL and GL were between 3 and 4 for cultured cells, and *ca* 5.5 (rape) and nearly 8 (soybean) for seeds.

In soybean seeds the most abundant FA in all fractions was linoleic acid (18:2) and in soybean leaves the most abundant FA in all fractions was 18:3 (Table 1). In contrast, in the cultured cells from soybean different FA predominated in different fractions: 18:3 was the commonest in the PL and was a much lower proportion in the NL and GL (Table 1). The FA compositions of corresponding fractions from the different tissues usually did not correspond; the closest similarity was in the PL fractions form cultured cells and leaves (Table 1). Gregor [1] found very similar FA compositions for some PL from roots and from cultured cells of carrot.

In seeds of rape, the most abundant FA was oleic acid (18:1), but there was more diversity between the different fractions than found for soybean seeds (Tables 1 and 2). In leaves of rape, 18:3 was again the

Table 1. Fatty acids of neutral lipids, glycolipids and phospholipids from seeds, leaves and cultured cells of two varieties of soybean (Bråvalla and Fiskeby V): weight of total FA per unit fr. wt and % by wt of different FA

	Total FA (mg/g)	Each FA as % by wt of total FA					
		16:0	16:1	18:1	18:2	18:3	>18
(i) Bråvalla							
(a) Seeds							
NL	102	11	—	19	57	13	—
GL	0.43	23	—	5	56	15	2
PL	3.23	15	—	4	71	11	—
Total	106	11	—	18	58	13	—
(b) Leaves							
NL	0.53	4	14	2	9	72	—
GL	0.61	8	1	3	5	83	—
PL	0.36	19	3	6	25	47	—
Total	1.50	9	6	3	11	70	—
(c) Cultured cells							
NL	0.58	19	1	22	13	23	22
GL	0.12	20	—	22	8	17	33
PL	0.40	27	—	10	15	42	5
Total	1.10	22	tr	18	13	30	17
(ii) Fiskeby V							
(a) Seeds							
NL	88	12	—	17	58	13	—
GL	0.41	24	—	15	46	12	3
PL	3.25	17	—	4	70	9	—
Total	92	12	—	16	58	13	—
(b) Leaves							
NL	0.78	5	13	2	7	74	—
GL	1.62	9	1	3	5	81	—
PL	0.42	20	1	6	22	51	—
Total	2.82	10	4	3	8	75	—
(c) Cultured cells							
NL	0.93	21	tr	39	20	14	6
GL	0.15	20	—	22	4	6	48
PL	0.60	25	—	13	16	42	4
Total	1.68	22	—	28	17	24	9

tr: FA detected and <1%.

most abundant FA in all fractions (Table 2). In cultured cells the pattern of FA again differed in different fractions (Table 2). The FA compositions of the corresponding fractions from the different tissues were usually different; in contrast to soybean, the closest similarity was in the neutral lipid fractions from the cultured cells and seeds of rape (Table 2). TLC showed triglycerides in cultured cells of var. Rapora (and in soybean var. Fiskeby V and not in the other varieties of rape and soybean; triglycerides were also present in leaves and, of course, in seeds; always, other neutral lipids were present [6]). Generally, triglycerides are found in cultured cells including rape [1, 5, 7, 8].

Gregor [1] found considerable similarities in % FA contents of different lipid classes from cultured cells of carrot. Radwan *et al.* [7] found significant differences between FA from triglycerides, on the one hand, and phosphatidyl serine and phosphatidyl glycerol on the other. Our results are consistent with the latter [7]; it appears that the FA composition of different lipid classes can differ considerably in cultured cells, and in our cases more so than in leaves and seeds (Tables 1 and 2). Song and Tattrie [5] and De Silva and Fowler [4] found large variations, during the growth cycle of cultured cells, in the FA compositions of some lipid classes; PL were relatively constant [4].

Previous reports have shown that in PL from cultured cells 18:2 is often present in largest amounts, followed by 16:0 and then, usually, by 18:3 [1, 4, 7]. Our cultures were different: in the PL from soybean the order was 18:3 > 16:0 > 18:2 and in rape 16:0, 18:1, 18:2 and 18:3 were all within 10% of each other (Tables 1 and 2). The GL contained large amounts of a FA of chain length $>C_{18}$ (Tables 1 and 2). So far as FA from the total lipids are concerned (Tables 1 and 2), published work shows a considerable variation in the predominant FA [2–5, 7–9].

Thus, (i) the different major lipid fractions can differ markedly from each other in their % FA composition, and (ii) fractions, both in whole plant tissues and in cultures of other species, can differ in their % FA composition from the corresponding fraction in cultured cells. It is possible (at least in different cultures) that the functional role of some of these lipid classes would be similar, so more similarity in FA composi-

Table 2. Fatty acids of neutral lipids, glycolipids and phospholipids from seeds, leaves and cultured cells of two varieties of rape (Maris Haplona and Rapora): wt of total FA per unit fr. wt and % by wt of different FA

	Total FA (mg/g)	Each FA as % by wt of total FA					
		16:0	16:1	18:1	18:2	18:3	>18
(i) Maris Haplona							
(a) Seeds							
NL	274	7	—	58	18	6	12
GL	0.46	14	1	32	41	9	3
PL	2.58	9	—	57	32	3	—
Total	278	7	—	58	18	6	11
(b) Leaves							
NL	0.30	3	32	1	4	60	—
GL	0.33	17	9	3	13	58	—
PL	0.29	25	tr	3	18	55	—
Total	0.92	15	14	2	11	59	—
(c) Cultured cells							
NL	1.50	16	—	48	17	8	11
GL	0.28	15	1	39	2	5	38
PL	0.84	18	—	28	24	20	9
Total	2.62	17	tr	41	18	12	13
(ii) Rapora							
(a) Seeds							
NL	196	4	—	41	12	6	38
GL	0.46	12	1	39	35	11	2
PL	2.50	9	—	53	33	5	—
Total	199	4	—	41	13	6	37
(b) Leaves							
NL	0.32	3	32	1	6	58	—
GL	0.46	14	7	1	7	74	—
PL	0.33	23	tr	2	22	53	—
Total	1.11	13	12	1	11	62	—
(c) Cultured cells							
NL	0.85	24	—	31	18	14	14
GL	0.24	20	tr	26	5	7	43
PL	0.81	20	—	26	28	20	6
Total	1.90	21	—	28	21	16	14

tr: FA detected and <1%.

tion might have been expected. The degree of variation in cultured cells encourages the speculation that, for many acyl lipids, variation in type of FA, at least within limits, does not greatly alter their role.

EXPERIMENTAL

Two varieties each of soybean (*Glycine max* cv Bråvalla and cv Fiskeby V) and rape (*Brassica napus* cv Maris Haplona and cv Rapora) were used. The seeds were obtained from Algot Holmberg & Soner A. B., Sweden (soybean), Norddeutsche pflanzenzucht Hans-Georg Lembke KG-2331 Hohenlieth, West Germany (rape cv Rapora) and the Plant Breeding Institute, Cambridge (rape cv Maris Haplona) and the analyses of seeds were made on those originally supplied. Green leaves for analysis were collected in late summer 1977 from plants grown from these seeds, for several months in the field near Newcastle. Cell cultures were obtained from seedlings from the same seeds, placed on the medium of [10] (solidified with agar). Suspension cultures were obtained by transferring pieces (3–5 g fr. wt) of cultured tissue (after several transfers to fresh solidified medium) to 50 ml of the same medium [10], without agar, in 150 ml Erlenmeyer flasks and placing on a horizontal gyratory shaker at 160 rpm with 1 cm throw [6]. The suspension cultures were transferred to fresh medium every 21 days by adding 10 ml of old culture to 50 ml of fresh medium. Several transfers were made before the cultures were used in the expts. Cells were analysed for lipids at the end of the growth cycle (as judged by fr. and dry wt measurements). Culture was in the dark at 25°. All solvents contained butylated hydroxytoluene and, where possible, a N_2 environment was used. The tissues were extracted with hot *iso*-PrOH followed by $CHCl_3$–MeOH. Non-lipid contaminants were removed using Sephadex. The different lipid classes were obtained using a column of acid-washed Florisil and the solvent series: $CHCl_3$(EtOH-free), $CHCl_3$–Me_2CO (1:1), Me_2CO, $CHCl_3$–MeOH (9:1 and 1:1), MeOH. The acyl lipids were hydrolysed and the fatty acids methylated by refluxing with MeOH–toluene–conc H_2SO_4 (150:75:7.5 by vol.). The Me esters were analysed by GLC–FID using 5% PEGA at 160° (N_2 carrier, 50 ml per min).

Acknowledgements—K.S.E. thanks the Republic of Iraq for financial support.

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