Chemistry of Natural Compounds, Vol. 48, No. 3, July, 2012 [Russian original No. 3, May–June, 2012]

FATTY ACID COMPOSITION OF THE MONOGALACTOSYLDIACYLGLYCEROL FRACTION OBTAINED FROM *Rosa* sp. AND LITOZIN®

K. Grabowska* and Z. Janeczko

UDC 547.1:665.3

Rosa canina and *Rosa rugosa* are common shrubs of the genus *Rosa*, widely distributed all over Europe. Rose hip or pseudofruit (*Rosae fructus*) is reported to contain ascorbic acid, organic acids, and minerals. Phytochemical analysis has also revealed the presence of carotenoids, flavonoids, and phenolic acids in rose hips [1–6]. Rose seeds are rich in lipids [7, 8]. In traditional medicine rose hips are used as a tonic agent in the treatment of exhaustion, infections, and flu. Latest investigations indicated anti-inflammatory, antioxidant, antidiabetic, antimutagenic, and antimicrobial activity in rose hips [9–17].

Recently, in Danish Dog Rose seeds, monogalactosyldiacylglycerol, 3-β-D-galactopyranosyloxy-2-(octadeca-9*Z*,12*Z*,15*Z*-trienoyloxy)propanyl octadeca-9*Z*,12*Z*,15*Z*-trienoate (GOPO), was discovered. The compound exhibited *in vitro* anti-inflammatory activity by the inhibition of chemotaxis and chemiluminescence of human polymorphonuclear leukocytes (PMNs) [18, 19]. Dried powdered rose hips of a subspecies of *Rosa canina* (Litozin®, Dansk Droge, Denmark and Langelands-Hyben Vital International; ApS, Denmark) is used in the treatment of arthritis and osteoarthritis [20–22].

The aim of this study was to determine and compare the fatty acid (FA) composition of the monogalactosyldiacylglycerol (MGDG) fraction, obtained from rose pericarps and seeds, as well as Litozin® powder. In this work two *Rosa* species, growing wild in Poland, namely *Rosa canina* and *Rosa rugosa*, were investigated.

MGDG was eluted from the lipid extract using different solvents: ($CHCl_3$, $(CH_3)_2CO$, CH_3OH) and its presence was observed only in the acetone-soluble fraction. MGDG was isolated and purified by means of medium-pressure liquid chromatography (MPLC) and preparative TLC. It is worth noting that the MPLC method was used for the first time to fractionate and isolate the MGDG fraction from the lipid extract.

The carbohydrate part of MGDG was determined after acidic hydrolysis on a TLC plate. Only the spot with R_f value corresponding to galactose (R_f 0.33) was observed.

The FA composition of MGDG from the rose seeds, rose pericarps, and Litozin® was determined by gas-liquid chromatography (GLC). The results are presented in Table 1.

The FA composition of MGDG found in pericarps was similar for both rose species. Comparison of the FA composition of MGDG from the seeds of both species has also revealed no significant differences. However, there were some differences in the FA composition of MGDG between pericarps and seeds of the analyzed species. We have identified 13 FA in the pericarps and 10 FA in the seeds of both analyzed species. The FA composition of MGDG of the pericarps consisted mostly of fatty acids with chain length from 10 to 18 carbons. It is interesting that long-chain FA (24:0 and 24:1) have been observed only in the seeds of both species.

Our investigation has revealed that unsaturated FA were dominant in the pericarps of both species (93.745–94.856%). The major unsaturated FA of MGDG from the pericarps of *Rosa canina* and *Rosa rugosa* were those with chain length of 18 C (93.886% and 92.795%, respectively), while the content of unsaturated FA with chain length of 16 and 17 C was less than 1%. The dominant FA of MGDG from the pericarps of both species was linolenic acid (18:3 n-3), and the percentage of the compound was 74.486% of MGDG FA from *R. canina* and 86.057% of MGDG FA from *R. rugosa*. The analysis has also revealed high amounts of 18:2 n-6 FA, especially in MGDG from *R. canina* pericarps (17.330%).

Department of Pharmacognosy, Faculty of Pharmacy, Medical College, Jagiellonian University, 30-688, Cracow, Medyczna 9 Street, Poland, fax: +48 12 620 55 75, e-mail: kgrabowska@cm-uj.krakow.pl. Published in *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2012, pp. 416–418. Original article submitted February 28, 2011.

 0009-3130/12/4803-0462 ©2012 Springer Science+Business Media, Inc.

TABLE 1. Fatty Acid Composition of MGDG from Two Polish Roses and Litozin®, %, GLC

Fatty acid	*Rosa canina*		*Rosa rugosa*		Litozin®
	pericarp	semen	pericarp	semen	
10:0	0.295	0.0	0.659	0.0	0.290
12:0	1.226	11.228	1.611	6.019	2.366
14:0	0.878	12.145	0.977	8.055	2.270
15:0	0.034	0.0	0.055	0.0	0.174
16:0	0.976	9.096	1.119	5.829	3.306
16:1 n-7	0.635	0.0	0.321	0.0	1.413
17:0	0.469	0.0	0.216	0.0	0.233
17:1	0.335	0.0	0.629	0.0	1.017
18:0	0.256	7.033	0.450	4.964	0.880
18:1 n-9	1.736	4.004	0.632	3.389	5.105
18:1 n-7	0.334	0.307	0.039	0.261	0.334
18:2 n-6	17.330	11.965	6.067	19.615	8.362
18:3 n-3	74.486	27.545	86.057	37.510	71.751
24:0	0.0	1.052	0.0	0.989	0.0
24:1	0.0	0.486	0.0	0.369	0.0
$\Sigma_{sat.}$	4.134	40.554	5.087	25.856	9.519
$\Sigma_{unsat.}$	94.856	44.307	93.745	61.144	87.982
$\Sigma_{unsat.\ 18C\ FA}$	93.886	43.821	92.795	60.775	85.552
$\Sigma_{unidentified}$	1.011	15.139	1.168	13.00	2.499
$\Sigma_{identified}$	98.989	84.861	98.832	87.00	97.501

As opposed to MGDG from the pericarps, the content of the saturated FA of MGDG from the seeds of both species was 6–8 times higher (25.856 to 40.554%). The saturated FA compositon of MGDG found in the seeds consisted of the following FA: 12:0, 14:0, 16:0, 18:0, 24:0. In the MGDG composition from the seeds of both species we have also observed high amounts of unsaturated FA. The dominant unsaturated FA was linolenic acid, and its percentage was 27.545% in the seeds of *R. canina* and 37.510% in *R. rugosa* seeds. We have also noticed high amounts of 18:2 n-6 and 18:1 n-9 FA. Interestingly, the content of 18:1 n-9 was much higher in the seeds than in the pericarps.

A comparison of the MGDG FA composition of pericarps and seeds of the two analyzed rose species with the MGDG fraction isolated from Litozin® has revealed some similarities. The MGDG fraction of Litozin® contained slightly less than 90% of unsaturated FA and the predominant FA was, as in the analyzed pericarps and seeds, linolenic acid. Our study has also revealed high contents of 18:2 n-6 and 18:1 n-9 FA that were characteristic for MGDG FA from the seeds. A comparison of the unsaturated FA content of the MGDG fraction obtained from Litozin® and from pericarps and seeds of the analyzed species has shown some differences. The MGDG FA from Litozin® contained higher amounts of saturated FA than MGDG FA from pericarps. However, the amount of saturated FA of MGDG from the seeds was greater than from Litozin®.

In the MGDG FA from Litozin®, our investigation has revealed no FA with chain length of 24 C, which were observed in the MGDG FA from the seeds.

Plant Material. The fruits of *Rosa canina* and *Rosa rugosa* were collected in September 2009 from the Garden of Medicinal Plants, Jagiellonian University, Cracow, Poland. The seeds were separated from the pericarps and milled. The samples of plant material have been stored in –20°C at the Department of Pharmacognosy, Faculty of Pharmacy, Medical College, Jagiellonian University, Cracow.

Extraction and Isolation. The fresh plant material was immersed in hot methanol to deactivate enzymes. Both fresh plant material and Litozin® Forte powder (Dansk Droge, Denmark, Lot No. 178709) were exhaustively extracted with methanol. The obtained extracts were evaporated in a rotary evaporator under reduced pressure.

The glycolipid fraction (GL) was separated from the total lipid extract by column chromatography (silica gel 60, 2 cm × 3 cm). Elution with solvents of increasing polarity ($CHCl_3$, $(CH_3)_2CO$, CH_3OH) was used.

The collected fractions were examined by TLC together with the MGDG standard (Lipid Products, Nutfield Nurseries, UK) in the solvent system $CHCl_3$–CH_3OH–CH_3COOH (40:10:1 v/v). Chromatograms were visualized by spraying the TLC plates with orcinol reagent and heating (105°C) [23]. MGDG was identified by comparison of R_f values.

The acetone-soluble fraction was subjected to medium-pressure chromatography (MPLC) using the following solvent systems: $CHCl_3$–CH_3OH–CH_3COOH (40:10:1 v/v) and $CHCl_3$–CH_3OH–NH_4OH (40:10:1 v/v). In this work a Buchi Sepacore MPLC apparatus equipped with two pump modules C-601 and C-605 connected with pump manager C-610 and fraction collector C-660 was used. The MPLC conditions were as follows: silica gel (Lichroprep Si 60, Merck), column (12 mm × 150 mm), flow rate 2.5 mL/min. A major MGDG fraction was obtained and purified by means of preparative TLC (Analtech, Silica Gel G, 20 × 20 cm, 500 μm). The plates were developed in $CHCl_3$–CH_3OH–NH_4OH (40:10:1 v/v). Elution of MGDG was done using $CHCl_3$–CH_3OH mixture (2:1 v/v).

Hydrolysis. Acid hydrolysis of MGDG was performed using HCl in statu nascendi for 30 min at 60°C. The plates were developed twice in the following solvent system: $CHCl_3$–CH_3OH–H_2O (23:12:2 v/v). Identification of the carbohydrate component was achieved by spraying the TLC plate with aniline phthalate and heating [24].

Fatty Acid Analysis. MGDG was converted to methyl esters according to AOAC Official Method 991.39 and then subjected to GLC analysis [25]. The conditions were as follows: capillary column (30 m × 0.25 mm); Supelcowax 10; column temperatures 160°C (3 min), 3°C/min to 220°C, 220°C 35 min; carrier gas He, flame-ionization detector (TRACE GC Ultra, Thermo Electron Corporation).

The FA were identified by comparing retention times (R_t) with the retention time of PUFA standards.

REFERENCES

1. S. Nojavan, F. Khalilian, F. M. Kiaie, A. Rahimi, A. Arabanian, and S. Chalavi, *J. Food. Compos. Anal.*, **21** (4), 300 (2008).
2. S. Ercisli, *Food. Chem.*, **104** (4), 1379 (2007).
3. Y. Hashidoko, *Phytochemistry*, **43** (3), 535 (1996).
4. R. Nowak, *Acta. Pol. Pharm.*, **63** (4), 281 (2006).
5. R. Nowak, *JPC-J. Planar Chromatogr.*, **20** (1), 43 (2007).
6. T. Hodisan, C. Socaciu, I. Ropan, and G. Neamtu, *J. Pharm. Biomed.*, **16** (3), 521 (1997).
7. K. Szentmihalyi, P. Vinkler, B. Lakatos, V. Illes, and M. Then, *Bioresource Technol.*, **82** (2), 195 (2002).
8. R. Nowak, *Acta. Soc. Bot. Pol.*, **74** (3), 229 (2005).
9. C. Chrubasik, R. K. Duke, and S. Chrubasik, *Phytother. Res.*, **20** (1), 1 (2006).
10. C. Chrubasik, B. D. Roufogalis, U. Muller-Ladner, and S. Chrubasik, *Phytother. Res.*, **22** (6), 725 (2008).
11. D. A. Daels-Rakotoarison, B. Gressier, F. Trotin, C. Brunet, M. Luyckx, T. Dine, F. Bailleul, M. Cazin, and J. C. Cazin, *Phytother. Res.*, **16** (2), 157 (2002).
12. X. Gao, L. Bork, V. Trajkovski, and M. Uggla, *J. Sci. Food. Agr.*, **80** (14), 2021 (2000).
13. D. Deliorman Orhan, A. Hartevioglu, E. Kupeli, and E. Yesilada, *J. Ethnopharmacol.*, **112** (2), 394 (2007).
14. A. K. Jager, K. N. Petersen, G. Thomasen, and S. B. Christensen, *Phytother. Res.*, **22** (7), 982 (2008).
15. N. Orhan, M. Aslan, S. Hosbas, and O. D. Deliorman, *Pharmacogn. Mag.*, **5** (20), 309 (2009).
16. K. Winther, E. Rein, and A. Kharazmi, *Inflammopharmacology*, **7** (1), 63 (1999).
17. A. Kharazmi and K. Winther, *Inflammopharmacology*, **7** (4), 377 (1999).
18. E. Larsen, A. Kharazmi, L. P. Christensen, and S. B. Christensen, *J. Nat. Prod.*, **66** (7), 994 (2003).
19. A. Kharazmi, *Osteoarthr. Cartilage*, **16**, S5 (2008).
20. S. N. Willich, K. Rossnagel, S. Roll, A. Wagner, O. Mune, J. Erlendson, A. Kharazmi, H. Sorensen, and K. Winther, *Phytomedicine,* **17**, 87 (2010).
21. C. Chrubasik, R. K. Duke, and S. Chrubasik, *Phytother. Res.*, **20** (1), 1 (2006).
22. R. Christensen, E. M. Bartels, R. D. Altman, A. Astrup, and H. Bliddal, *Osteoarthr. Cartilage*, **16**, 965 (2008).
23. M. Pasciak, O. Holst, B. Lindner, M. Mierzchaia, A. Grzegorzewicz, H. Mordarska, and A. Gamian, *BBA-Gen. Subjects,* **1675**, 54 (2004).
24. Z. Janeczko, J. Sendra, K. Kmiec, and C. H. Brieskorn, *Phytochemistry*, **29** (12), 3385 (1990).
25. P. Cunniff, *Official Methods of Analysis of AOAC International*, 16th Ed., AOAC International, Arlington, VA, 1995.