LIPIDS OF MULBERRY LEAVES AND OF MULBERRY SILKWORM EXCRETA

D. U. Uzakova, A. A. Kolesnik, Yu. L. Zherebin,
R. P. Evstigneeva, and I. K. Sarycheva

UDC 547.915.5

The composition and the amounts of various groups of liposoluble compounds in mulberry leaves and the excreta of the silkworm have been established by chromatographic and chemical methods. It has been shown that biologically valuable components – phytosterols, unsaturated fatty acids, phytol, carotenoids, and chlorophylls – are concentrated in silkworm excreta.

Silkworm excreta (SWE) can serve as a cheap source of a whole series of products in short supply: chlorophylls, metal complexes of porphyrins, intermediates for the synthesis of vitamins E and K, and protein–mineral additives to animal feeds [1]. The residue after the extraction of these compounds contains a complex of substances, especially of lipid nature, from which it is obviously possible to obtain several components presenting interest for medicine, the paint and varnish industry, and other branches of the national economy.

In view of this, we have investigated the chemical composition and have determined the amounts of lipids in SWE and mulberry leaves – the material consumed by the silkworm.

The lipids were isolated from mulberry leaves and SWE by a modified Bligh–Dyer method [2]. Extraction was continued until faintly colored extracts were obtained. The combined chloroform extracts were filtered and freed from nonlipid impurities by washing with 0.5% aqueous $CaCl_2$ solution. The total yields of liposoluble substances calculated to dry weight were 2.3% and 11.5%, respectively, for the mulberry leaves and SWE, the amount of liposoluble substances having increased by a factor of 5 in the SWE. Similar results were obtained previously for chlorophyll [3].

The combined lipids were fractionated by column chromatography on silica gel [4] with the isolation of the neutral lipids (NLs), glycolipids (GLs), and phospholipids (PLs). The predominant fraction proved to be the NLs, making up 62.3 and 89.9% of the total lipids, respectively, for the mulberry leaves and the SWE. The second fraction in the quantitative respect consisted of the GLs, which amounted to 30.2% for the leaves and 8.6% for the SWE. The smallest fraction consisted of the PLs, amounting to 7.5% for the mulberry leaves and 1.5% for the SWE.

The group composition of the NLs was determined by TLC in system 1, of the GLs in systems 1 and 2, and of the PLs in system 4. The groups of lipids were identified by chromatography in the presence of markers and by treating the chromatograms with color reagents [5]. Definitive conclusions on the affinity of chromatographically homogeneous zones to definite groups of chemical substances were made on the basis of the results of a study of the hydrolysis products of the lipids isolated from the TLC plates.

According to the experimental results (Table 1), the qualitative compositions of the groups of lipids of mulberry leaves and of the SWE were identical. Chlorophylls, fatty acids, sterols, carotenoids, fatty alcohols, and sterol glycosides predominated. The quantitative distributions of the lipid groups in the leaves and the SWE were similar on the whole, but in the SWE, as compared with the leaves, the amounts of carotenoids, chlorophylls, fatty acids, alcohols, and sterols were higher and, at the same time, the amounts of all the phospholipids and the glycolipids were lower, and so were those of the esterified sterols and fatty acids and of the triacylglycerols. To all appearance, in the process of its vital activity, the silkworm is capable of selectively utilizing certain groups of lipids from the leaves and of accumulating and excreting other lipid compounds.

A. V. Bogatskii Physicochemical Institute of the UkrSSR Academy of Sciences, Odessa.
Moscow Institute of Fine Chemical Technology. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 505-509, July-August, 1987. Original article submitted February 19, 1986; revision submitted February 16, 1987.

0009-3130/87/2304-0419$12.50

TABLE 1. Group Compositions of the Lipids (% of the total) from Mulberry Leaves and Silkworm Excreta

Group of lipids	Mulberry leaves	SWE
Carbohydrates	2,0	1,3
Carotenoids	5,6	9,3
Waxes	2,8	2,2
Fatty acid esters	3,1	0,7
Sterol esters	5,6	1,2
Triacylglycerols	1,2	0,4
Tocopherols	0,3	0,3
Fatty acids	12,4	15,6
Fatty alcohols	3,1	10,8
Diacylglycerols	0,6	2,8
Sterols	9,9	15,2
Chlorophylls and their derivatives	14,6	27,3
Naphthoquinones	0,7	1,8
Monoacylglycerols	0,5	1,3
Monogalactosyldiglycerides	6,0	0,8
Esterified sterol glycosides	2,7	0,4
Sterol glycosides	9,0	5,0
Cerebrosides	3,6	1,3
Digalactosyldiglycerides	6,9	0,8
Sulfoquinovosyldiglycerides	1,8	Tr.
Phosphatidylethanolamines	2,5	0,6
Phosphatidylcholines	2,0	0,1
Phosphatidylglycerols	1,4	0,2
Phospatidic acids	0,4	0,5
Phosphatidylinositols	1,0	0,1
Phosphatidylserines	0,3	Tr.

TABLE 2. Fatty Acid Compositions of the Acyl-Containing Lipids of Mulberry Leaves and the SWE

Sum of the lipids	Fatty acids, % of the sum										Sum of	
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	22:0	23:0	24:0	saturated	unsaturated
Mulberry leaves	1,6	11,2	4,1	3,9	14,6	24,1	38,4	0,9	0,4	0,8	18,8	81,2
SWE	3,5	23,1	4,7	6,6	16,4	14,8	26,0	2,2	0,6	2,1	38,1	61,9

In the SWE, chlorophylls *a* and b made up 63% of the sum of the green pigments. The remainder consisted of 10 magnesium- and phytol-free analogues of the initial chlorophylls (pheophytins, pheophorbides, chlorophyllides, and phylloerythrins) and oxidized structural analogues of the initial chlorophylls. In the mulberry leaves, the amount of derivative forms of chlorophylls *a* and b was low (<5% of the total pigments), which may indicate the occurrence of the processes involving the enzymatic cleavage of the chlorophylls in the digestive tract of the silkworm. Also in favor of this hypothesis is the fact that the increase in the amount of phytol-free forms of chlorophyll in the SWE correlates with the concentration of phytol (see Table 1).

Thus, it is obvious that on the formation of SWE not only is there a substantial accumulation of chlorophyll, but it simultaneously undergoes decomposition. However, in view of the considerable predominance of the first process over the second, the total amount of chlorophyll in the SWE, as compared with the leaves, rises.

The fatty alcohols were isolated by TLC and were acetylated with acetic anhydride in pyridine. The acetates so obtained were separated by preparative TLC/Ag^+ in the chloroform–carbon tetrachloride (1:1) solvent system. The components of each zone were identified from the results of GLC and IR and mass spectroscopy.

Among the alcohols in the SWE, phytol predominated quantitatively (87.5%). The ratio of methyl and methylene protons corresponded to a monounsaturated isoprenol structure. The presence of iso branching was confirmed by a doublet of the corresponding methyl groups superposed on a triplet of the other methyl groups. The remainder of the alcohols consisted of saturated and unsaturated alcohols of normal structure and of saturated isoprenols.

In view of the high amount of free and various esterified forms of sterols in the SWE and the leaves, we determined the composition of the sterol fragments of the sterol-containing

groups of lipids. In all cases, β-sitosterol predominated (70-90%). In addition, stigmasterol (3-20%), campesterol (1-8%), cholesterol (1-12%), and unidentified sterols (1-7%) were detected.

The fairly high amount of sterols in the SWE with a predominance of β-sitosterol permits the conclusion that the SWE may be a convenient source of phytosterols, which find use in medicine for the treatment of cardiovascular diseases.

In the glycolipids of the SWE and of mulberry leaves, the main monosaccharide fragments were galactose and glucose (~90%). Among the fatty acids of the acyl-containing lipids of the SWE and the leaves linolenic, linoleic, oleic, and palmitic acids predominated (Table 2). It must be mentioned that the degree of unsaturation of the SWE lipids was considerably lower than that of the lipids of the mulberry leaves. To all appearances, this is due to the oxidative degradation in the silkworm digestive tract of polyunsaturated fatty acids ($C_{18:2}$, $C_{18:3}$) the amount of which in the leaves exceeded 60%.

EXPERIMENTAL

The isolation of the lipids, and PC, TLC, and GLC has been described previously [4, 6]. Thin-layer chromatography was performed in the following solvent systems: 1) heptane–methyl ethyl ketone–acetic acid (42.5:7.5:0.5); 2) acetone–toluene–acetic acid–water (60:60:2:1); 3) chloroform–acetone–methanol–acetic acid–water (6:8:2:2:1); and 4) chloroform–methanol–7 N ammonia (65:30:4).

The groups of neutral lipids were determined quantitatively by a modification of Amenta's method based on the use of an oxidizing reagent [7]. The amount of glycolipids was estimated from the carbohydrate component using the procedure with orcinol [8]. The amount of phospholipids was determined from their phosphorus content [9]. The water-soluble products obtained after severe acid hydrolysis (2 N HCl, 125°C, 48 h) were separated and identified as described previously [6]. The sterol components were analyzed by GLC, as in [10]. The sugars in a hydrolysate of the GLs were determined by paper chromatography [11].

The total chlorophylls and their structural analogues were isolated from an extract of the total lipids by separation according to Krause [12] and by precipitation of the green pigments from pure petroleum ether [13]. They were separated into individual forms by the TLC method on silica gel by two runs in the heptane–methyl ethyl ketone (5:3) system [14]. The identification and quantitative determination of the pigments was carried out on the basis of the results of chromatography in the presence of markers and the spectral characteristics of homogeneous forms of the pigments in the visible region [15].

The fatty alcohols were analyzed by gas–liquid chromatography under conditions described previously [16]. The IR spectra of phytol contained bands at 3650-3590, 2926, 2853, 1465, and 1385-1375 cm^{-1}.

CONCLUSION

The composition and amounts of the lipid substances in mulberry leaves and silkworm excreta (SWE) have been investigated. Their qualitative compositions have been shown to be identical and quantitative differences between the lipids of the leaves and the SWE have been determined. It has been found that the main components of the total lipids are chlorophylls, sterols, fatty acids, carotenoids, sterol glycosides, and phytol (in the SWE).

A fivefold increase in the amount of lipids in the SWE as compared with mulberry leaves has been detected, which gives grounds for considering the wastes from sericulture to be a promising raw material for the isolation of a number of valuable liposoluble compounds on the industrial scale.

LITERATURE CITED

1. K. A. Askarov and I. P. Pavlova, The Present State and Prospects of the Development of Work on Porphyrins in Uzbekestan. 3rd All-Union Conference on Porphyrin Chemistry and Biochemistry [in Russian], Samarkand (1983), p. 13.
2. A. V. Bogatskii, Yu. L. Zherebin, and A. A. Kolesnik, Vinod. Vinograd. SSSR, No. 8, 26 (1978).
3. B. D. Berezin, K. A. Askarov, C. T. Rashidova, N. L. Golubchikova, O. I. Koifman, and N. S. Enikolopov, Izv. Vyssh. Ucheb. Zaved. Khimiya Tekhnol., 26, 874 (1983).

4. Yu. L. Zherebin, A. A. Kolesnik, and A. V. Bogatskii, Prikl. Biokhim. Mikrobiol., 17, No. 4, 614 (1981).
5. M. Kates, Techniques of Lipidology, American Elsevier, New York (1972) [Russian translation (1975), p. 157]; J. G. Kirchner, Thin Layer Chromatography (2nd edn.), Wiley-Interscience, New York (1978) [Russian translation, Moscow, Vol. 2 (1981), p. 257].
6. Yu. L. Zherebin and A. A. Kolesnik, Khim, Prir. Soedin., 165 (1984); Yu. L. Zherebin and A. A. Kolesnik, Khim. Prir. Soedin., 196 (1984).
7. A. V. Bogatskii, Yu. L. Zherebin, and A. A. Kolesnik, Vinod. Vinograd., SSSR, No. 6, 21 (1980).
8. Methods of Biochemical Investigations [in Russian], Leningrad (1982), p. 85.
9. B. P. Pleshkov, Practical Handbook of Plant Biochemistry [in Russian], Moscow (1985), p. 187.
10. G. G. Alkhazashvili, V. G. Leont'eva, D. N. Kalatozishvili, T. I. Mayakova, L. A. Mudzhiri, A. A. Semenov, and V. Yu. Vitkovskii, Khim. Prir. Soedin., No. 4, 536 (1983).
11. G. N. Zaitsev and T. P. Afanas'eva, Biokhimiya, 22, 1035 (1957).
12. K. N. Vekirchik, Plant Physiology (Practical Handbook) [in Russian], Kiev (1984), p. 58.
13. Pigments of the Plastids of Green Plants and Methods for Their Investigation [in Russian], Moscow (1964), p. 77.
14. Yu. L. Zherebin and A. A. Kolesnik, Khim. Prir. Soedin., 663 (1983).
15. H. H. Strain and W. A. Svec, The Chlorophylls, Interscience, New York (1966), p. 63.
16. Yu. L. Zherebin and A. A. Kolesnik, Prikl. Biokhim. Mikrobiol., 20, No. 3, 407 (1984).

STEREOSPECIFIC ANALYSIS OF THE TRIACYLGLYCEROLS OF A COTTONSEED OIL HYDROGENATE AND ITS FRACTIONS

G. U. Tullaeva, N. T. Ul'chenko, I. P. Nazarova, and Kh. I. Isaev

UDC (665.335.2+665.353.4)094.1

A cottonseed oil hydrogenate has been fractionated into three fractions: high-melting, medium-melting, and liquid. It has been established that the fat of the second fraction, because of its physicochemical indices, consistency, and the distribution of the saturated fatty acyl radicals in the extreme positions of the TAG molecules, can be regarded as a cocoa butter substitute.

The hydrogenation and fractionation of oils and fats is discussed in a number of publications [1, 2], but the structure of the triacylglycerols (TAGs) in the products obtained has been studied inadequately.

Since the quality and digestibility of a fat depends on the distribution of the acyl radicals in the TAGs, we have studied the distribution of the fatty acid radicals in the TAG molecules of individual fractions of a fractionated cottonseed oil hydrogenate obtained from the Tashkent oils and fats combine. Fractionation was performed with the aim of obtaining an additional raw material for the confectionery industry. The hydrogenates were fractionated by a method described previously [3].

The characteristics of the hydrogenate and of the fractions obtained are given in Table 1. The fat of fraction I was characterized by a high hardness, a high melting point, and a considerable content of trans-acids. Fraction II, with a high hardness, had a lower melting point. Fraction III was liquid, with a considerably smaller amount of trans-acids and

Institute of the Chemistry of Plant Substances of the Uzbek SSR Academy of Sciences, Tashkent. Institute of Bioorganic Chemistry of the Uzbek SSR Academy of Sciences, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 509-513, July-August, 1987. Original article submitted December 10, 1986.

 0009-3130/87/2304-0422$12.50