Chemistry of Natural Compounds, Vol. 32, No. 5, 1996

DETECTION OF DIOL LIPIDS IN PLANTS OF THE ELAEAGNACEAE FAMILY AND THE PROPERTIES OF THEIR SYNTHETIC ANALOGS

N. P. Goncharova and A. I. Glushenkova

UDC 547.915

The compositions of the alcohols of the triacylglycerol fraction of the fruit of Hippophae rhamnoides *and of the seeds of* Elaeagnus angustifolia *have been determined. The behavior under the action of pancreatic lipase of synthetic analogs of the diol compounds detected has been studied.*

In an investigation of the composition of the triacylglycerols (TAGs) of the lipids of the pericarp of sea buckthorn from the Central Asian region by stereospecific analysis, no unambiguous results were found in calculations of the distribution of fatty acids (FAs) between the three hydroxy groups in the glycerol molecule. This fact, and also the behavior of the TAGs in TLC, permitted the assumption of their inhomogeneity.

It is known [1] that glycerol is not the only polyhydric alcohol of the neutral lipids and phosphatides in the seeds of the majority of plants. The products of the alkaline methanolysis of the lipids contain lower diols, and the esters of these with FAs pass into the TAG fraction. An exception is coixenolide – the ester of *meso*-butane-2,3-diol with *cis*-palmitoleic and *trans*-vaccenic acids – which is satisfactorily separated from TAGs on silica gel.

In order to confirm the presence of these compounds in the TAG fraction of sea buckthorn pericarp, we have investigated the alcoholic component of this fraction.

Separation of the pericarp lipids by CC on silica gel gave a fraction enriched with triacylglycerols, which was then rechromatographed by PTLC. The fraction obtained was colored by the carotenoids present in it, and, to eliminate these, the initial TAGs were passed through a layer of diatomite.

The TAGs purified in this way were subjected to alkaline methanolysis with a 0.5% solution of Na methanolate. The reaction mixture was deposited on Al_2O_3 plates and was separated in system 1. The alcohols were eluted from the starting zone with methanol. After the solvent had been distilled off, the alcohols were acetylated with acetic anhydride in pyridine. TLC monitoring of the reaction products in system 2 revealed a number of acetates the migrational mobilities of which were close to those of acetylated glycerol, acetylated ethylene glycol, and acetylated propane- and butanediols.

Purified fractions of the TAGs from other organs of plants belonging to the Elaeagnaceae family – sea buckthorn and olive seeds – were analyzed by the same method. For identification we used model specimens of diols, having first acetylated each of them. A comparison of the relative retention volumes of the components of the natural mixtures and the model specimens was made by GLC on polyethyleneglycol succinate (Table 1).

Among the diol compounds, ethylene glycol and propane-1,3-diol in various amounts were identified in all the samples, and while, as stated in [1], the amounts of these substances in the seeds of the species studied previously were minor (from 0.5 to 2.5%), in the olive and sea buckthorn seeds their amounts were more considerable – from 5.0 to 9.1%. In the sea buckthorn pericarp we also identified butane-1,4-diol, and here the total weight of diols amounted to 19.4%.

In Nature, an elevated amount of diols may be observed under conditions of enhanced functional activity; for example, in ripening maize seeds 30-40% of them in relation to glycerol have been detected [3].

In addition to those mentioned above, on the GLC of the plant materials investigated three peaks issued with RRTs of 1.20, 1.56, and 1.75.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (3712) 89 14 75. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 697-700, September-October, 1996. Original article submitted February 12, 1996.

 0009-3130/96/3205-0686$15.00

TABLE 1. Compositions of the Alcoholic Components of the TAG Fractions from Various Organs of Plants of the Elaeagnaceae Family, % GLC

Acetate of	RRT to glycerol triacetate	TAG fractions		
		sea buckthorn pericarp	sea buckthorn seeds	olive seeds
1,2-Ethylene glycol*	0.15	6.5	7.8	3.7
Propane-1,3-diol*	0.60	9.1	1.3	1.3
Butane-1,4-diol*	0.75	3.8	–	–
Glycerol*	1.00	58.8	80.7	84.7
Unidentified 1	1.20	3.1	4.5	5.1
Unidentified 2	1.56	3.2	2.5	5.2
Unidentified 3	1.75	15.5	3.2	–

*Since the polyol acetates are not members of a single homologous series, the percentages of these di- and triacetates were calculated with the use of correction factors [2].

Of particular interest in this connection is sea buckthorn pericarp, which contained 22.2% of unidentified polyols in the TAG fraction.

The following stage of our work was the elucidation of how diol esters behave under pancreatic hydrolysis. The action of pancreatic lipase and of pancreatic juice on mono- and diesters of hexane-1,6-diol [4] and on full oleic acid esters of alcohols having from one to eight hydroxy groups – methanol, ethylene glycol, glycerol, erythritol, pentaerythritol, adonitol, sorbitol, and sucrose [5] – is known. It is also known that lipase is active at an oil-water phase-separation surface and specifically hydrolyzes esters of primary alcohols, while "in other respects it is considered that its action depends little on the nature of the diol" [1].

The hydrolytic properties of the pancreatic lipase from the porcine pancreatic gland have been studied on the esters of FAs with diols that we synthesized. The diols were acylated with previously synthesized FA chlorides. For comparison we used a synthetic triacylglycerol. Since the acylation process is a stepwise one, the reaction products were transferred to an Al_2O_3 column and were eluted with chloroform, which separated the complete and incomplete products of the esterification of the diols.

Table 2 shows the dependence of the degree of hydrolysis on the chain length of the alcohol component. Considering only derivatives diacylated at primary hydroxy groups, we can detect the following tendency: the proportion of acid liberated after hydrolysis for 40 min falls with an increase in the length of the chain of the diol component.

All diol esters are hydrolyzed somewhat more slowly than TAGs [4]. In a study of the hydrolysis of mono- and diesters of hexane-1,6-diol, Mattson and Volpenhein [4] also found that TAGs are hydrolyzed faster.

To determine the influence of the nature of the esterifying acid, experiments were performed on diesters of ethylene glycol with various acids, both saturated and unsaturated (Table 3).

As can be seen from Table 3, hydrolysis by pancreatic lipase took place somewhat faster for the ester of the unsaturated acid.

Thus, it has been established that, of the synthetic compounds studied, diol esters containing primary hydroxy groups, and also TAGs, undergo hydrolysis by pancreatic lipase, but the 1- and 3-positions of TAGs are more sensitive to attack by this enzyme.

The minor amounts of diol esters usually encountered in plant seeds cannot appreciably affect the results of the fine chemical investigation of TAG fractions. However, the presence of appreciable amounts of them in plant materials must be taken into account in performing certain analyses of TAGs.

EXPERIMENTAL

The oils were obtained from the plant seeds and pericarp by extracting the ground raw material with hexane, using the method of repeated steeping at room temperature.

The TAG fractions of the total lipids were obtained by CC on silica gel, the column being eluted by hexane with the gradual addition of diethyl ether. The enriched TAG fractions were eluted by solvent system 3.

TABLE 2. Influence of the Alcohol Components on the Degree of Hydrolysis of Diol Esters by Pancreatic Lipase

Esters of the 16:0 acid	Proportion of acid liberated, %	
	15 min	40 min
1,2-Ethylene glycol	27.5	63.5
Propane-1,2-diol	32.3	48.6
Propane-1,3-diol	35.6	57.5
Butane-1,3-diol	29.6	47.5
Butane-1,4-diol	30.3	49.9
Glycerol	58.4	70.3

TABLE 3. Influence of the Acyl Component on the Degree of Hydrolysis of Diol Esters by Pancreatic Lipase

Diesters of ethylene glycol with the FAs	Proportion of acid liberated after hydrolysis for 40 min, %
16:0	57.2
18:0	56.4
18:1	60.5

The PTLC of the enriched TAG fractions was conducted in solvent system 3.

Solvent systems: 1) hexane–diethyl ether (95:5); 2) hexane–diethyl ether (1:1); 3) hexane–diethyl ether (4:1).

Methanolysis of the TAGs. A freshly prepared 0.5% solution of Na methanolate was used [6]. The calculated amounts of Na methanolate solution were added to identical weights of chromatographically pure isolated TAG fractions, and, with stirring of the reaction mixture by a magnetic stirrer, hydrolysis was conducted for 4 h, monitoring being performed by TLC in system 3 using methyl esters of FAs as markers.

FA chlorides were synthesized from the 16:0, 18:0, and 18:1 acids and thionyl chloride [7].

Synthesis of the esters of FAs and diols was carried out with the chlorides of the 16:0, 18:0, and 18:1 acids and the diols ethylene glycol, propane-1,2- and -1,3-diols, and butane-1,3- and -1,4-diols [8].

Synthesis of Glycerol Tripalmitate. A mixture of the initial acid and glycerol was heated at 236°C under reduced pressure for 1 h.

GLC of the polyol acetates: steel column filled with 17% of PEGS on Chromosorb; column temperature 200°C.

Enzymatic hydrolysis of the TAGs by pancreatic lipase was conducted as in [9].

REFERENCES

1. V. A. Vaver, A. N. Ushakov, and L. D. Bergel'son, Usp. Biol. Khim., **14**, 227 (1973).
2. V. A. Vaver, A. N. Ushakov, and L. D. Bergel'son, Izv. Akad. Nauk SSSR, Ser. Khim., No. 6, 1187 (1967).
3. V. A. Vaver, N. V. Prokazova, G. D. Strakhova, and L. D. Bergel'son, Dokl. Akad. Nauk SSSR, **188**, No. 1, 227 (1969).
4. F. H. Mattson and R. A. Volpenheim, J. Lipid Res., **13**, No. 2, 256 (1972).
5. F. H. Mattson and R. A. Volpenheim, J. Lipid Res., **13**, No. 3, 325 (1972).
6. L. Fieser and M. Fieser, Reagents for Organic Synthesis, Wiley, New York (1967).
7. Preparative Organic Chemistry [Russian translation], Mir, Moscow (1979), pp. 422 and 643.
8. L. A. Chechulina, S. M. Puchkova, A. P. Novikova, Z. M. Baskakova, V. I. Vysokov, and G. B. Afanas'eva, Khim.-farm. Zh., **3**, 138 (1985).
9. A. L. Markman, T. V. Chernenko, and A. U. Umarov, Prikl. Biokhim. Mikrobiol., **5**, 616 (1969).