# NEW OXO ACIDS OF THE SEED OIL OF *Galeopsis bifida*

S. D. Gusakova and T. V. Khomova

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Oxoacylglycerols have been isolated from the lipids of the seeds of *G. bifida* that contain in any of the three positions of the molecule a set of the 9(10)-oxo-18:0, 12-oxo-9-18:1, 9(10)-oxo-10(8)-18:1, 9-oxo-10,12-18:2, and 12(13)-oxo-9,13,15-(9,11,15)-18:3 acids.

From the lipids of the seed of *Galeopsis bifida* Boenn. (Lamiaceae), which are toxic for warm-blooded animals, we have isolated epoxy-, hydroxy-, and oxoacylglycerols. The structures of the fatty epoxy and hydroxy acids have been discussed previously [1]. In the present paper we give the results of an analysis of the oxoacylglycerols from *G. bifida* and their component fatty acids.

The oxoacylglycerol (oxo-AG) fraction was isolated from the combined lipids by chromatography on a column of silica gel. On a chromatographic plate (TLC), the fraction moved as a single spot with $R_f$ 0.51 that gave a positive reaction with 2,3-dinitrophenylhydrazine (2,4-DNPH). The free fatty acids (FFAs) usually present in the seed lipids of higher plants have the same chromatographic mobility. The presence of FFAs in the fraction studied was determined by comparing their composition before and after the treatment of the spot of the fraction on the plate with an ethereal solution of diazomethane. After methylation, the plate was chromatographed in system 1. The appearance in the sample that had been treated with diazomethane of the spot of methyl esters (MEs) with $R_f$ 0.90 and the simultaneous decrease in the area of the spot $R_f$ 0.51 served as a proof of the presence of FFAs.

The oxo-AGs were freed from FFAs by treating the fraction with diazomethane and separating the MEs formed by preparatory TLC in system 1. The amount of oxo-AGs was 0.3% of the mass of the lipids.

The UV spectrum of the oxo-AGs contained an absorption band at 227 nm assigned to a —CO—CH=CH— group and a band in the 270 nm region that is characteristic for an isolated carbonyl at an allyl dienic chromophore $-CO(CH{=}CH)_2-$ [2, p. 461]. The presence of allyl enone and dienone groups was confirmed in the IR spectrum of the oxo-AGs by a complex band of a carbonyl group and by the bands of conjugated double bonds at 1745, 1700, 1670, 1635, 990, and 955 $cm^{-1}$.

The mass spectrum of the oxo-AGs contained the peaks of molecular ions with m/z 890, 892, 894, 896, and 898, corresponding to monooxoacyldiacylglycerols with the general formula $C_{57}H_{94-102}O_7$. In this series of molecular ions, the peaks with m/z 892, 894, and 896 were the strongest. Weak $M^+$ ions with m/z 868, 870, and 872 present in the spectrum can be assigned to analogous oxo-AGs with the general formula $C_{55}H_{96-100}O_7$, where one of the acyls is the 16:0 species.

The set of fragments in the remainder of the mass spectrum was characteristic for unsaturated triacylglycerols [3]. The nature of the acyl residues in the oxo-AGs was determined from the mass numbers of the fragments $[R{-}CO]^+$ and the $[R{-}CO - 1]^+$. To them corresponded the peaks of ions with m/z 265 and 264 (18:1); 263 and 262 (18:2); 261 and 260 (18:3); and 239 (16:0). The acyl residues of the oxo acids were represented by the peaks of ions with m/z 281 (oxo-18:0); 279 and 278 (oxo-18:1); 277 and 276 (oxo-18:2); and 275 and 274 (oxo-18:3), among which the residues of the oxo-18:2 and oxo-18:1 acids predominated. Two series of homologous ions in the spectrum belonged to fragments of the type of $[M - RCOO]^+$, where the ions with m/z 619-611 were formed as the result of the elimination from the $M^+$ ions with m/z 896-892 of the radicals of unsubstituted unsaturated fatty acids and from those with m/z 605-595 of oxoacyl radicals. The high relative intensity of the latter (mainly the fragments with m/z 601 and 599) shows that the fatty oxoacyl residues were concentrated either in the sn-1 or the sn-3 positions of the oxo-AG molecule. The fragments observed in

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the mass spectrum simultaneously with m/z 355-349 ($[R-CO + 74]^+$) and m/z 409-403 ($[R-CO + 128]^+$) could be formed by the decomposition of oxo-AGs containing the oxoacyls in the sn-2 position.

In order to confirm the fact that the combined oxo-AGs included species with a single oxoacyl radical in any of the three positions, part of the oxo-AGs was hydrolyzed with the aid of pancreatic lipase. The products of enzymatic hydrolysis were analyzed in system 2. The following were detected on the chromatogram: unsaturated fatty acids together with the residue of the uncleaved oxo-AGs ($R_f$ 0.77); sn-1,2(2,3)-triacylglycerols in combination with split-out fatty oxo-acids ($R_f$ 0.34); the sn-1,2(2,3)-oxoacylmonoacylglycerols ($R_f$ 0.20); the sn-2-monoacylglycerols ($R_f$ 0.05); and the sn-2-monooxoacylglycerols (at the starting point). The compounds mentioned were isolated by preparative GLC in the same system. The correctness of their identification was confirmed by the results obtained after the alkaline hydrolysis of the corresponding lipids, methylation of the fatty acids that had been split out, and comparative analysis of the behavior of the acid before and after methylation by the TLC method in system 1.

According to the analysis, taken together, the oxo-AGs were the monoxoacyldiacylglycerols in which the fatty oxo acids were represented by isologues of the $C_{18}$ series with 0-3 double bonds and one carbonyl group and were present in one of the three positions of the molecule, and the set of unsubstituted fatty acids bound to the other two positions were analogous to that of the ordinary triacylglycerols of *G. bifida* seeds [4].

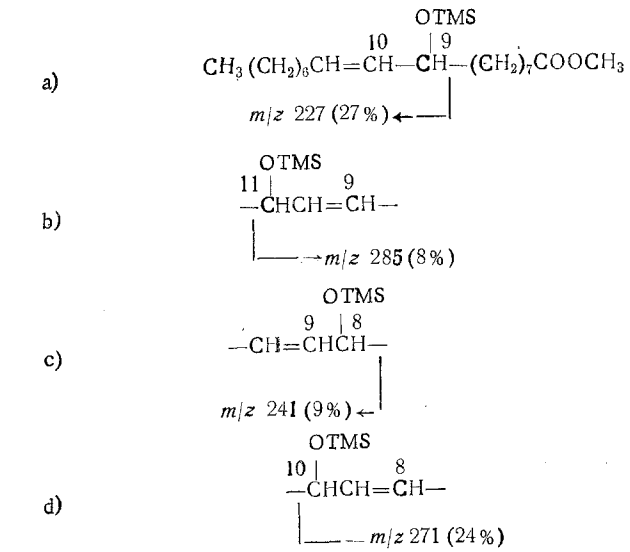
The quantitative composition of the unsubstituted fatty acids was determined by the GLC method using the corresponding MEs. For this purpose, part of the combined oxo-AGs was saponified with ethanolic caustic soda, the acids were methylated with diazomethane, and the MEs were separated by preparative TLC in system 1 ($R_f$ of the MEs of the oxo acids 0.53). The composition of the unsubstituted fatty acids of the oxo-AGs (GLC, %) was: 16:0 (3.9), 16:1 (0.2), 18:0 (0.9), 18:1 (22.8), 18:2 (49.4), 18:3 (22.8).

The MEs of the fatty oxo acids were analyzed for their degree of unsaturation by the $Ag^+$-TLC method in system 4 [5]. Two main spots were obtained, with $R_f$ 0.80 and 0.67, these being accompanied by weak spots with $R_f$ 0.92 and 0.85. In view of the very small amount of the initial MEs of the oxo acids, they were separated by preparative $Ag^+$-TLC in the same system into two fractions with $R_f$ 0.92-0.80 (I) and 0.67 (II). To determine the composition and structure of the oxo acids, each fraction was analyzed by UV and mass spectrometry, the mass-spectrometric analysis being carried out on the trimethylsilyl (TMS) derivatives of the hydroxy compounds corresponding to the oxo acids. The derivatives were obtained by reducing a part of fractions I and II with $NaBH_4$. The completeness of reduction was checked by UV spectrometry and by TLC in system 1, the mobilities of the MEs of the hydroxy derivatives being compared with a model sample of the MEs of ricinoleic acid. The oxo acid MEs obtained were converted into the TMS derivatives.

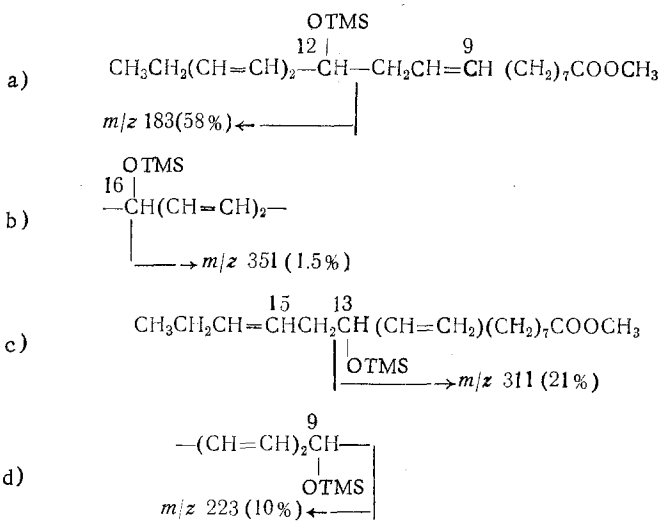
The UV spectrum of the MEs of oxo acids I was similar to that of the oxo-AGs. The UV spectrum of the reduced acids I lacked absorption in the 227 and 270 nm regions but a band was observed with its maximum at 233.5 nm, which is characteristic for cis, trans-conjugated dienes [2, p. 435].

According to the mass spectrum of their TMS derivatives, the esters (I) consisted of the sum of the MEs of the hydroxy-18:0 ($M^+$ 386), the hydroxy-18:1 ($M^+$ 384), and the hydroxy-18:2 ($M^+$ 382) acids. The greatest intensity in this spectrum was possessed by the peak of an ion with m/z 187 — the main diagnostically important ion observed for the fragmentation of the TMS derivative of the ME of ricinoleic (12-hydroxy-9-18:1) acid [6]. It was accompanied by other fragments known for this compound, with m/z 299 and 270. Ions with m/z 225 (20.3%) and 311 (9%) were assigned to the TMS derivatives of the isomeric 9(13)-hydroxy-10, 12(9,11)-18:2 acids, but the ratio of the intensities of these peaks permitted our preference to be given to the 9-hydroxy isomer [7]. In a similar manner, on the basis of the distribution of the relative intensities of the peaks of ions with m/z 227 and 285 formed in the breakdown of the TMS derivatives of the isomeric 9(11)-hydroxy-10(9)-18:1 acids (scheme 1), the presence of the 9-hydroxy-10-18:1 acid was more probable. From fragments with m/z 271 and 241 we identified the 10-hydroxy-8-18:1 acid [8] — yet another isomer of the hydroxy-18:1 acid with an allyl position of the hydroxy group.

The structures of the saturated 9- and 10-hydroxy-18:0 acids were established on the basis of the low-intensity peaks of ions with m/z 229, 259, and 273, 215, respectively.




**Scheme 1. Main fragments of the TMS derivatives of the ME esters of the isomeric hydroxy-18:1 acids: a) 9-hydroxyoctadec-10-enoic; b) 11-hydroxyoctadec-9-enoic; c) 8-hydroxyoctadec-9-enoic; d) 10-hydroxyoctadec-8-enoic acids.**




**Scheme 2. The main fragments of the TMS derivatives of the ME esters of the isomeric hydroxy-18:3 acids: a) 12-hydroxyocta-9,13,15-trienoic; b) 16-hydroxyoctadeca-9,12,14-trienoic; c) 13-hydroxyoctadeca-9,11,15-trienoic; d) 9-hydroxyoctadeca-10,12,15-trienoic acids.**

Consequently, the MEs of the oxo acids I consisted of the MEs of the 12-oxo-9-18:1, 9-oxo-10-18:1, 10-oxo-8-18:1, 9-oxo-10,12-18:2, 9-oxo-18:0, and 10-oxo-18:0 acids.

The UV spectrum of the MEs of the oxo-18:3 acids (fraction II) showed an absorption band at 270 nm, which disappeared when the carbonyl group was reduced. The UV spectrum of the reduced product of (II) was similar to that of reduced fraction I, which gave grounds for assuming the presence of a $-CO(CH=CH)_2-$group in the oxo-18:3 acid.

The mass spectrum of the TMS derivatives of the esters of fraction II includes in addition to the $M^+$ peak with m/z 380, the peaks of ions with m/z 183, 351, and 223, 311, which have been assigned to the isomeric 12(16)-hydroxy-9,13,15(9,12,14)-18:3 and the 9(13)-hydroxy-10,12,15(9,11,15)-18:3 acids (scheme 2).

As in the case of the mass spectrum of the TMS derivatives of (I), the ratio of the relative intensities of the peaks of the ions formed from the acids isomeric with respect to the position of the hydroxydiene group permitted preference to be given to one of the isomers.

It was found from the results of analysis that the oxo-18:3 acids were represented by the 12-oxo-9,13,5- and the 13-oxo-9,11,15-18:3 acids. The presence of a small amount of the 9-oxo-10,12,15-18:3 acid was possible.

Thus, in the combined oxo fatty acids of the lipids of the seeds of *G. bifida* 9-oxooctadecanoic, 10-oxooctadecanoic, 12-oxooctadec-9-enoic, 9-oxooctadec-10-enoic, 10-oxooctadec-8-enoic, 9-oxooctadeca-10, 12-dienoic, 12-oxooctadeca-9,13,15-trienoic, and 13-oxooctadeca-9, 11,15-trienoic acids have been detected. The conjugated double bonds in the allyl dienic systems of these acids are present in the cis,trans figuration.

All the oxo fatty acids mentioned above with the exception of 9-oxooctadeca-10,12-dienoic acid, are new natural compounds, although some of them have been known previously as minor products of the autooxidation of unsaturated fatty acids. Thus, the isomeric 9(10)-oxooctadec-10(8)-enoic and 9(13)-oxooctadeca-10,12(9,11)-dienoic acids have been found among the products of the model autooxidation of the MEs of the 18:1 and 18:2 acids, respectively [8, 9]. At the same time, 9-oxooctadeca-10,12-dienoic acid is present in the seen lipids of one of the species of plants of the family Asteraceae, and the 13-oxooctadeca-9,11-dienoic acid has been detected in the oxidized lipids of seeds of a plant of the family Polygonaceae [10]. It must be mentioned that the 12(13)-oxooctadeca-9,13,15(9,11,15)-trienoic acids represent the oxo analogues of the corresponding fatty hydroxytrienic acids that are formed in the model oxidation of the 18:3-MEs [11]. 12-Oxooctadec-9-enoic acid is an oxo analogue of ricinoleic acid, which we have detected in the seed lipids of *G. bifida* [1].

## EXPERIMENTAL

IR spectra were taken of the substances in the form of films on a UR-10 instrument, UV spectra of solutions in cyclohexane and ethanol on a Hitachi spectrophotometer, and mass spectra on MKh 1310 and MKh 1303 instruments.

The conditions for gas–liquid, column, and thin-layer chromatography have been described previously. The following solvent systems were used for TLC: 1) and 2) hexane–diethyl ether (7:3) and (8:7); 3) benzene; 4) benzene–chloroform–diethyl ether (50:50:15).

The substance were revealed in $I_2$ vapor. An 8% ethanolic solution of 2,4-dinitrophenylhydrazine prepared by a known method [12] was used to detect the oxo-AGs and the fatty oxo acids.

*G. bifida* seeds were collected in the forest-steppe zone of the Bashkir Predural'e on gray forest soil in August, 1980, in the stage of complete ripeness.

The oil was obtained by five extractions with petroleum ether (40-60°C), the comminuted seeds being steeped at room temperature. The oxoacylglycerols were isolated from 28 g of lipids.

The alkaline hydrolysis of the oxo-AGs was carried out with a 10% aqueous methanolic [$H_2O$–MeOH (1:9, v/v)] solution of KOH at room temperature, and enzymatic hydrolysis as described in [13]. The products of the enzymatic hydrolysis of the oxo-AGs were identified by TLC with reference to the products of the analogous hydrolysis of normal TAGs and taking into account coloration of the spots of the compounds after the plate had been sprayed with 2,4-DNPH solution.

To reduce the MEs of the oxo acids, a weighed sample of the substance in tetrahydrofuran was treated with $NaBH_4$ which had previously been ground in the presence of a small amount of the same solvent (ratio of substance of solvent to $NaBH_4$: 1:0.1:2). After the mixture had been allowed to stand for half an hour during which the evolution of gas slowed down, it was boiled in a flask with a reflux condenser on the water bath for 2 h and was then left for 12 h. The solution of the reduced MEs was diluted with water, acidified with 50% HCl, and extracted five times with diethyl ether. The combined ethereal extracts were washed to neutrality and dried with anhydrous $Na_2SO_4$.

Ricinoleic acid was isolated from castor oil and, in the form of its MEs, was freed from the MEs of unsubstituted fatty acids by TLC in system 1.

The silyl derivatives were obtained as described by Gunstone and Shuler [14].

## CONCLUSION

1) 9-Oxo- and 10-oxooctadecanoic, 12-oxooctadec-9-enoic, 9-oxooctadeca-10,12-dienoic, 9(10)-oxooctadec-10(8)-enoic, and 12(13)-oxooctadeca-9,13,15(9,11,15)-trienoic acids, which, with the exception of the 9-oxooctadeca-10,12-dienoic acid, are new natural compounds, have been isolated from the seed lipids of *Galeopsis bifida*.

2) These acids occupy any of the three positions of the oxoacyldiacylglycerols.

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# THE CHEMICAL STUDY OF THE MAIN PHOSPHOLIPIDS OF THE SEED KERNELS OF COTTON PLANTS OF THE SPECIES *Gossypium barbadense*


A. Sh. Isamukhamedov and S. T. Akramov



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The qualitative and quantitative compositions and also the general and position distributions of the fatty acids of the seeds of the thin-fibered varieties of the cotton plant S-6022, S-6015, S-6034, S-6037, and 9123-I have been studied.


The pathogenic fungus *Fusarium* develops in the soil and damages cotton plants through their root system. Its toxins cause irreversible changes in the vascular system of the plant, which may lead to wilting.

An infected plant that has passed through the complete cycle of its development (the formation of full-value seeds) develops immunity to the action of the fungus for the new generation. By successive selection, new wilt-resistant varieties of the cotton plant are being created [1].

The formation of immunity in living organisms is largely determined by the state of their lipid metabolism, and the phospholipid fraction of the membranes determines their resistance to and their capacity for being penetrated by bacterial toxins [2, 3]. Thus, any changes in the qualitative and quantitative ratio of the phospholipids (PLs) in the seeds will play a definite role in the development of the cotton plant, i.e., these changes prob-


Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR, Tashkent.


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