Chemistry of Natural Compounds, Vol. 30, No. 2, 1994

INFLUENCE OF THE CHEMICAL COMPOSITION OF PLANT OILS ON CYTOCHROME P-450

A. Sh. Isamukhamedov, N. P. Goncharova, and A. I. Glushetskova

UDC 547.915:664.34

The influence of sunflowerseed and cottonseed oils and also of fractions of normal and oxidized triacylglycerols isolated from them on cytochrome P-450 from rat liver microsomes has been investigated.

One of the components of some medicinal preparations is plant oil [1]. Edible plant oils consist to the extent of 95-97% of fatty acid glycerides, the remainder being substances accompanying the fats [2]. Minor components of edible oils are oxygenated triacylglycerols in which the hydroxyls of the glycerol are esterified with acids not only of the normal fatty series but also with oxygenated acids (o-TAGs). The physiological function of oxidized lipids as agents protecting plants from some pests and diseases has been reliably shown [3-5]. On the other hand, the action of oxidized compounds on the permeability of biomembranes is known. On the formation of lipid peroxides in the membranes of yeast cell mitochondria, the enzymes forming components of the membranes are inactivated [6, 7]. Oxidized lipids affect the transport of ions through a membrane [8], because of which its permeability rises [9-11].

The aim of the present work was to determine the influence on the living organism of ordinary oils and those containing oxygenated TAGs. The investigations were carried out on rat liver microsomes. As is known, a monooxygenase system containing cytochrome P-450 is localized in the membranes of the endoplasmic reticulum of the liver. Broad substrate specificity is a characteristic feature of it. Cytochrome-P-450-dependent systems are polyfunctional. They catalyze a whole set of enzyme reactions oxidizing substrates with the most diverse chemical structures [12, 13], thus promoting their removal from biological membranes and from the organism as a whole [14, 15].

Cytochrome P-450 is the terminal oxidase of the monooxygenase system, and its physicochemical properties are determined by the state of the lipid bilayer of the membranes [16, 17]. When a bilayer of cytochrome P-450 is damaged, the enzyme undergoes activation with its conversion into an inactive form — cytochrome P-420.

In the present case, as a quantitative characteristic of the inactivation of cytochrome P-450 we used the time of its half-inactivation, $t_{1/2}$, after which $\Delta A_{420\text{-}450}$ (Fig. 1) reaches half its initial value. It was determined by dividing the $\Delta A_{420\text{-}450}$ value of the initial point in two and finding the time corresponding to this magnitude on the axis of abscissas. The point on the axis of ordinates corresponds to the amount of cytochrome P-450 initially present in the microsome preparation.

As the object of study we selected three samples of commercial plant oils: sunflowerseed oil and refined and salad cottonseed oils. The presence of o-TAGs was detected qualitatively (TLC, UV and IR spectrometries) in the sunflowerseed oil and the refined cottonseed oils. For the oil sample studied we determined the total fatty acid composition of the normal fatty acids in the o-TAG fractions by GLC (Table 1).

The fractions enriched with hydroxyacids were separated from the normal TAGs by column chromatography on silica gel and were subjected to final purification by TLC. The purified o-TAG fractions were subjected to methanolysis and the products obtained were methylated and separated preparatively. Two zones of substances, corresponding to the methyl esters (MEs) of normal and of oxidized fatty acids were obtained, the weight ratio between them being 2:1, from which it follows that the oxidized acids esterified only one of the three positions of the glycerol molecule.

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent. Fax (3712) 62 73 48. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 187-191, March-April, 1994. Original article submitted June 23, 1993.

 0009-3130/94/3002-0170$12.50

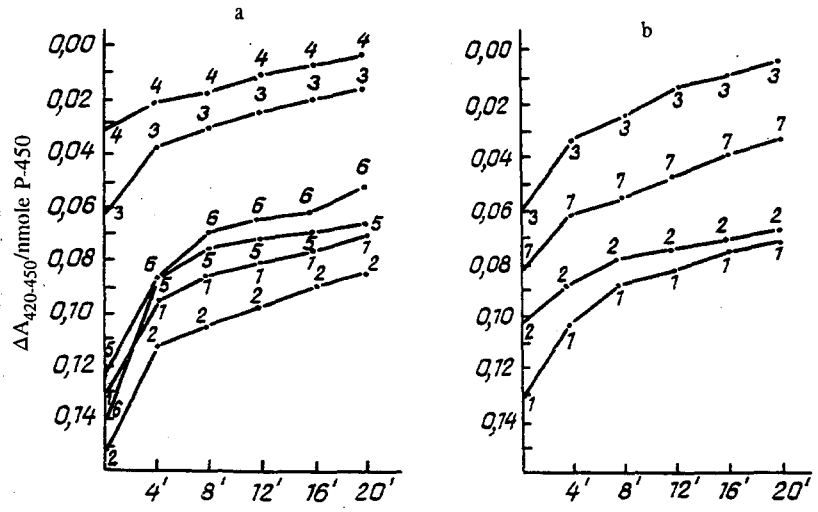

Fig. 1. Influence of sunflowerseed and cottonseed oils and also of normal and oxidized triacylglycerols isolated from sunflowerseed oil on the inactivation of the cytochrome P-450 of rat liver microsomes (a) and the influence of normal and oxidized triacylglycerols from sunflowerseed oil on the inactivation of the cytochrome P-450 of liver microsomes damaged by heliotrine (b). 1) Control microsomes (MCs); 2) MCs + TAGs; 3) MCs + o-TAGs; 4) MCs + sunflowerseed oil; 5) MCs + salad cottonseed oil; 6) MCs + refined cottonseed oil; 7) microsomes damaged by heliotrine (MCHs).

In the IR spectra of the hydroxyacids isolated a broad absorption band was observed at 3200-300 cm^{-1} (OH group) and bands at 950 and 990 cm^{-1} (*cis-trans*-CH=CH−CH=CH−). The UV spectra revealed a band at 233 nm which is characteristic for a conjuged dienic system.

The composition of the hydroxyacids was determined by the mass spectrometry of silyl derivatives of their MEs. The mass spectrum of the TMS derivatives of the MEs of the hydroxyacids of sunflowerseed oil showed two distinct peaks of the molecular ions M^+ 382 and M^+ 384, corresponding to hydroxydienic and hydroxymonoenic derivatives.

Characteristic fragments for the dienic hydroxyacids were M^+ 382, 367 $(M - 15)^+$, 351 $(M - 31)^+$, and 335 $(M - 47)^+$. The main peaks — those with m/z 311 and 225 — had practically identical intensities. The mass numbers and intensities of the other fragments corresponded to acids described in the literature [18, 19]. Two acids were detected with hydroxy groups in the Δ-9 position: 9-OH-12E,15E-18:2 and 9-OH-10Z,12E-18:2, and an isomer of the latter, 13-OH-9E,11Z-18:2 (coriolic acid). For the second acid, the intensity of a peak with m/z 225 was approximately equal to that of one with m/z 259. Peaks with m/z 185 and 299 (the peak with m/z 185 having a greater intensity than that of with m/z 299) enabled us to identify the 12-OH-9E,15E-18:2 acid (densipolic). In addition, we detected: an 11-OH-9,12-18:2 acid with the main fragments having m/z 199 and 285; a 16-OH-9,12-18:2 acid (m/z 131, 353); an 8-OH-octadecadienoic acid (m/z 239), and a 14-OH-octadecadienoic acid (m/z 325) with presumable positions of the double bonds at the Δ-9 and Δ-12-C atoms, starting from the predominating content of the normal 18:2 acid (61.8%, see Table 1) among the total fatty acids in the oil.

Of the monoenoic acids with m^+ 384 and common fragments with m/z 369 $(M - 15)^+$, 353 $(M - 31)^+$, and 337 $(M - 47)^+$, we found ricinoleic acid (m/z 187, 299); isoricinoleic acid (m/z 227 ≈ m/z 259; 230, 234); 8-OH-octadec-9-enoic acid (m/z 241, 271); and a 10-OH-octadecenic acid (m/z 215, 271).

The mass spectrum of the total TMS derivatives of the MEs of the hydroxyacids of cottonseed oil was similar to that described in the literature [20, 21].

In addition to the commercial oils mentioned, we investigated the bioactivity of pure substances; normal TAGs and o-TAGs isolated from sunflowerseed oil. It follows from Table 2 that the time of half-inactivation of cytochrome P-450 in control microsomes amounted to 20 min, and the same was observed on the incubation of the MCs with the TAGs and with salad cottonseed oil. When the MCs were incubated with refined cottonseed oil, sunflowerseed oil, and o-TAGs, the time of half-inactivation increased 2.5-fold in comparison with the control MCS.

TABLE 1. Total Fatty-acid Compositions of the Oil, %, GLC

Oil samples	Acids							
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Refined cottonseed oil	0.3	0.9	20.9	1.3	3.2	17.8	55.6	—
Salad cottonseed oil	0.4	0.8	15.5	1.0	1.9	20.0	60.4	—
Sunflowerseed oil	0.3	0.4	10.0	0.6	3.0	22.9	61.8	1.0
TAGs of sunflowerseed oil	0.2	0.4	15.5	0.7	5.3	30.5	45.8	1.6
Unoxidized FAs of sunflowerseed oil in the o-TAG fraction	0.2	0.5	23.1	1.3	6.7	32.7	34.5	1.0

In microsomes damaged by heliotrine (MCHs), the time of half-inactivation of cytochrome P-450 was 14 min, which also showed a disturbance of the lipid matrix of the microsomal membranes. When the MCHs were incubated with the TAGs the time of half-inactivation increased 1.4-fold, while on incubation with the o-TAGs it decreased 3.5-fold in comparison with the MCHs and 5-fold in comparison with the MCs. At the same time, the content of cytochrome P-450 after incubation with sunflowerseed oil and o-TAGS fell by 20-80%, and a subsequent acceleration of the inactivation of the cytochrome P-450 in comparison with the control was observed.

When liver membranes were damaged with heliotrine, the specific content of cytochrome P-450 fell by 20% and the inactivation of the cytochrome P-450 increased sharply in comparison with the control.

Starting from the fact that the stabilization of cytochrome P-450 in the microsomal membranes is due to its lipid microenvironment, it may be suggested that TAGs do not enhance the activation of cytochrome P-450 but, conversely, protect the microsome from the action of the surrounding medium and do not possess the property of unbalancing the individual components of the microsomal membranes. This can be explained, again, from the idea that fatty microemulsions of TAGs are impermeable and therefore, probably, create an additional, new, artificial, membrane which opposes the penetration of a buffer solution within the microsomes.

In the cases of sunflowerseed oil and refined cottonseed oil, which contain o-TAGs in addition to the ordinary TAGs, we assume that the main inactivating agent consists of TAGs with acyls including OH groups. The o-TAGs are incorporated into the hydrophobic region of the bilayer of the membranes, increasing even further their permeability because of the OH groups. On the other hand, the o-TAGs possess a higher critical micelle concentration than TAGs with normal acyl radicals, i.e., these o-TAGs may play the role of a detergent, solubilizing proteins and lipids, which leads to a disturbance of the bilayer of the microsomal membranes.

On acting upon a damaged membrane, n-TAGs exert a stabilizing influence, i.e., they possess a protective action with respect to cytochrome P-450, while in the case of o-TAGs a pronounced toxic effect is observed.

Thus, it has been shown that o-TAGs suppress the monooxygenase system of rat liver microsomes, intensifying the inactivation of cytochrome P-450, one of the main components of the system, even though the amount of o-TAGs does not exceed 1% of the weight of the oil. Therefore, in order not to lower the bioactivity of pharmaceutical preparations, oils containing no oxidized forms of lipids must be used.

In addition to this, the results of the investigation have shown that commercial salad cottonseed oil is completely suitable as a base for the creation of certain medicinal forms.

EXPERIMENTAL

The work was carried out on random-bred white male rats weighing 100-120 g. As the experimental system we selected the model of acute hepatitis brought about by a single intraperitoneal injection of the hepatotropic poison heliotrine in a dose of 30 mg/100 g [22]. The control and the damaaged liposomes were isolated as described in [23].

The inactivation of cytochrome P-450 was recorded on a Hitachi-557 double-beam spectrometer every 4 min for 20 min at 37°C [16].

To prepare emulsions we used 100 mM Tris-HCl buffer, pH 7.4, containing 1 mg of oil in 1 ml that had been treated on a MSE Soniprer-150 ultrasonic disintegrator at medium power. The microsomes were incubated in the freshly prepared microemulsion at +4°C for 60 min. The incubation mixture contained 0.3 nmole of cytochrome P-450 in 1 ml of 100 mM tris-HCl buffer, pH 7.4.

TABLE 2

Time of half-inactivation ($t_{1/2}$) of cytochrome P-450, min									
MCs	Control MCs	MCs (TAG)	MCs (o-TAG)	Sunflower-seed oil MCs	Cotton-seed oil MCs	Refined cotton-seed oil MCs	MCHs	MCHs (TAGs)	MCs (o-TAGs)
$t_{1/2}$	20	20	8	8	20	8	14	20	4

Note. MCs — rat liver microsome; MCHs — rat liver microsomes treated with heliotrine.

The total fatty acid compositions of the oils and also the compositions of the unoxidized acyls of the o-TAGs were determined as described in [24].

GLC conditions: Chrom-41 instrument with a flame-ionization detector, 4 mm × 2 m column filled with 17% of poly(ethylene glycol succinate) on ChromW at 200°C. Column chromatography was conducted on silica gel L 100/160, and TLC on silica gel L 5/40, using the solvent system hexane-diethyl ether with increasing concentrations of the latter.

The silyl derivatives of the hydroxy acids were obtained by treating the hydroxyacid MEs with a mixture of hexamethyldisilazane and trimethylchlorosilane in absolute pyridine.

IR spectra were taken on a UR-10 instrument in a film, UV spectra on a Hitachi spectrophotometer, and mass spectra on a MKh-1310 instrument.

REFERENCES

1. M. D. Mashkovskii, Drugs [in Russian], Meditsina, Moscow, Ch. 1 and 2 (1984).
2. B. N. Tyutyunnikov. The Chemistry of Fats [in Russian], Pishchevaya Prom-st', Moscow (1974).
3. A. V. Rama Rao and E. Rajarathnam Reddy, Tetrahedron Lett., **27**, 2279 (1986).
4. A. V. Rama Rao and E. Rajarathnam Reddy, Tetrahedron, **43**, 4385 (1987).
5. S. A. Avdyushko, A. I. Chalova, O. L. Ozeretskovkaya, G. I. Chalenko, A. B. Imbe, N. A. Lytyshev, S. G. Bezuglov, and L. D. Bergel'son, Dokl. Akad. Nauk SSSR, **296**, 1012 (1987).
6. I. K. Kolomiitseva and A. M. Kuzin, The Role of Peroxides and Oxygen in the Initial Stages of the Radiobiological Effect [in Russian], Izd-vo AN SSSR (1960).
7. A. Ottolenghi, F. Bernheim, and K. M. Wilbur, Arch. Biochem. Biophys., **56**, 157 (1955).
8. R. T. Sonders and A. S. J. Giese, J. Gen. Physiol., **42**, 589 (1959).
9. D. I. Roshchunin, A. I. Mirzaev, and Yu. A. Vladimirov, Biofizika, Moscow (1972).
10. J. S. Cook and H. F. Blum, J. Cell Comp. Physiol., **53**, 44 (1959).
11. M. Erecinska, Acta Biochim. Polon., No. 14, 21 (1967).
12. A. I. Archakov, Microsomal Oxidation [in Russian], Nauka, Moscow (1975), p. 327.
13. G. I. Mannering, in: Concepts in Drug Metabolism, Part B, (eds. P. Jenner and B. Testa), Marcel Dekker, New York (1981), p. 53.
14. A. I. Archakov, Oxygenases of Biological Membranes [in Russian], Nauka, Moscow (1983), p. 54.
15. D. I. Metelina, The Modeling of Redox Enzymes [in Russian], Nauka Tekhnika, Minsk (1984), p. 54.
16. A. I. Archakov and G. I. Bachmanova [Bakhmanova], in: Microsomes, Drug Oxidation, and Drug Toxicity (eds. R. Sato and R. Cato), Scientific Press, Tokyo, (1982), p. 67.
17. A. I. Archakov, G. I. Bachmanova [Bakhmanova], and V. Yu. Uvarov, in: Biochemistry, Biophysics, and Regulation of Cytochrome P-450 (ed. J.-Å. Gustafson), Elsevier/North Holland, Amsterdam (1980), p. 551.
18. R. Kleiman and G. F. Spencer, J. Am. Oil Chem. Soc., **50**, No. 2, 31 (1973).
19. C. H. Brieskorn and L. Kabelitz, Phytochemistry, **10**, 3195 (1971).
20. K. Kadyrov, É. I. Gigienova, and A. U. Umarov, Khim. Prir. Soedin., 710 (1976).
21. G. G. Yunusova, S. D. Gusakova, and Ya. V. Rashkes, Khim. Prir. Soedin., 436 (1981).
22. N. Kh. Abdullaev and R. K. Azimov, Alimentary-Toxic Affections of the Liver [in Russian], Meditsina, Tashkent (1978), p. 153.

23. M. A. Abdugafurova, A Comparative Investigation of the Effectiveness of the Reparative Action of Phosphoramide Preparations on Damaged Membranes of Liver Cells [in Russian], Dissertation ... Candidate of Biological Sciences, Moscow (1990).
24. N. T. Ul'chenko, É. I. Gigienova, K. L. Seitanidi, and A. U. Umarov, Khim. Prir. Soedin., 699 (1978).