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Biological Activity of Total Lipids from Red and White Wine/Must

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Wine is an essential component of the Mediterranean diet, and it is thought to exert a protective effect against coronary heart disease. Although many efforts have been made to determine the protective compounds in wines, their exact nature and how they are involved in the protection mechanisms are still unclear. In this study, total lipids, total polar lipids, and total neutral lipids of five wines and three musts were tested in vitro for their ability to induce washed rabbit platelet aggregation and/or to inhibit platelet activating factor (PAF) induced aggregation. The results showed that the biological activity of wine/must total lipids can be attributed mainly to total polar lipids. In the red wine Cabernet Sauvignon, we fractionated total neutral lipids, total polar lipids, and pigments by HPLC. Each fraction was tested in vitro for its biological activity. Structural data of the most active fractions, based on biological, chemical, and spectral methods, are also presented.

Keywords: Wine; must; biologically active lipids; platelet-activating factor; PAF; coronary heart disease; arteriosclerosis; phenolic compounds

INTRODUCTION

Coronary heart disease is the most significant cause of death in the industrialized countries. The search for potential factors influencing the development and the course of the disease has therefore been of great interest. Moderate consumption of alcoholic beverages has been reported to reduce the risk of coronary heart disease (1, 2), while at higher alcohol intake, the risk of sudden cardiac death and the overall mortality increased because of an increased incidence of liver cirrhosis and cancer (2). It has been noted that the protective effects of wine might be more potent than those of other alcoholic beverages. St. Leger (1979) (3) was the first to describe a strong negative relationship between wine consumption and mortality from ischemic heart disease, based on data from 18 countries, with a weak relationship for beer or spirits. Other reports confirmed this inverse correlation (4–6). Supporting evidence came from the observation that despite a similar distribution of other coronary risk factors such as high blood pressure, serum cholesterol, body mass index, and cigarette smoking, the mortality of coronary heart disease in France was lower compared with the mortality in the other industrialized countries (7). This phenomenon was believed to be due to the frequent consumption of wine together with the Mediterranean-type diet rich in vegetables. A similar phenomenon was also noticed in Crete, where the population followed a traditional diet with consumption of wine (8).

There is evidence for several mechanisms by which the protective effects of wine, including alcohol, could be explained. Several reasons have been proposed for

this cardioprotective effect of alcohol, with the most important being the increase in the concentration of HDL, a well-known negative risk factor for coronary heart disease (9, 10). Other mechanisms such as reduced platelet aggregation and blood clotting may be attributed to ethanol. A large number of in vivo and in vitro experiments provide evidence for the antioxidant effects of wine phenolic components (2, 11–13). In most experiments red wine is a more potent antioxidant than white. However, many studies have noted no difference between red and white wines (14, 15). Moreover, phenolics seem to modify eicosanoid metabolism (16, 17) and to inhibit ADP (adenosine diphosphate) and thrombin-induced platelet aggregation (18, 19).

In contrast, the response to injury hypothesis explains atherosclerosis as a chronic inflammatory response to injury of the endothelium, which leads to complex cellular and molecular interactions among endothelial cells, smooth muscle cells, and several blood cell components (20, 21). We previously proposed a mechanism by which platelet-activating factor (PAF) (22) is implicated in atherogenesis (23) based on previous detection of PAF, which is produced during LDL oxidation (24) and causes in situ inflammation. Additional evidence for the implication of PAF in atherogenesis are the following: (1) PAF is the strongest inflammatory mediator; (2) PAF is essential for the activation of leukocytes and its binding in the endothelial cells; (3) studies show that PAF is a compound of atheromatic plaque (25); (4) PAF studies in animals indicate the protective action of PAF antagonist against atherosclerosis (26, 27); and (5) acetylhydrolase, the main enzyme responsible for the degradation of PAF, which exists in an active form in nature LDL, is converted to an inactive form in ox-LDL (oxidized low-density lipoprotein) (24).

The detection of lipid compounds (which inhibit PAF action) in Mediterranean foods could explain their protective effect against atherogenesis. Previous studies

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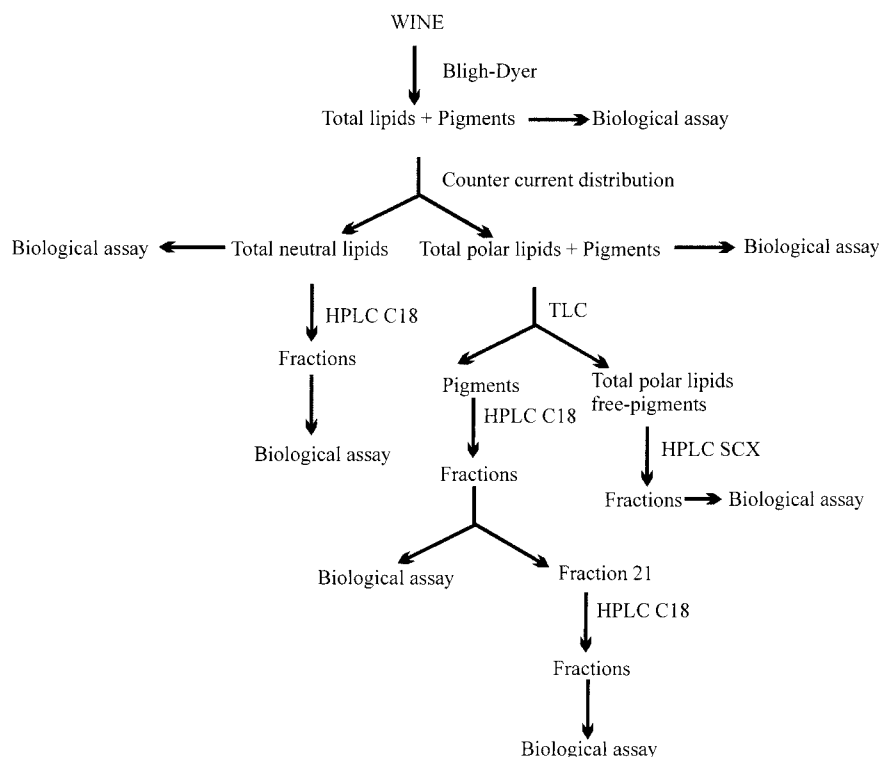


Figure 1. Schematic diagram of the extraction and the purification procedure of red wine Cabernet Sauvignon lipids.

in our laboratory demonstrated the existence of such compounds in olive oil (28), honey and wax (29), milk and yogurt (30), the *Scomber scombrus* fish (mackerel) (31), and more recently red wine (32) and cod (33). In this investigation we have examined the biological activity not only in red wine but also in white wine and must.

MATERIALS AND METHODS

Instrumentation. HPLC was performed on a Hewlett-Packard (Avondale, PA) series 1100, equipped with a 100 μ L loop Rheodyne (i 7725) injector. A 1100 HP UV spectrometer was used as detector. The spectrometer was connected to a Hewlett-Packard (Avondale, PA) model HP-3396A integrator-plotter.

Separation of lipids was carried out on a cation exchange column, SS 10 μ m Partisil 25 cm \times 4.6 mm i.d., PXS 10/25 SCX (Whatman; Clifton, NJ), and a 250 mm \times 4.6 mm i.d. reverse-phase C18 Nucleosil-300 column (Analysentechnik) at room temperature.

The PAF-induced aggregation was measured in a Crono-Log (Havertown, PA) aggregometer coupled to a Crono-Log recorder (Havertown, PA). A Varian Cary 3E UV-vis spectrometer was used.

Materials. All reagents and chemicals were of analytical grade supplied by Merck (Darmstadt, Germany). Resveratrol, quercetin, naringin, hesperidin, rutin, and gallic acid were purchased from Sigma (St. Louis, MO). High-performance liquid chromatography (HPLC) solvents were supplied from Rathburn (Walkerburn, Peebleshire, U.K.). Lipid standards were obtained from Sigma (St. Louis, MO). Semisynthetic PAF (80% C-16PAF and 20% C-18PAF) was synthesized in our laboratory as previously described (22). PAF acetylhydrolase from human serum was purified according to Stafforini et al. (34). Bovine serum albumin (BSA), creatine phosphate (CP)/creatine phosphate kinase (CPK), BN 52021, and indomethacin were obtained from Sigma (St. Louis, MO). Chromatographic material used for thin-layer chromatography (TLC) was silica gel H-60 (Merck). Domaine Hatzimichalis kindly supplied the following wines and musts: Ambelon (white wine with prin-

ciple grape Rompola, 1998); Chardonnay (white wine principle grape Chardonnay, 1997); Cuvee Maison (red wine with principle grapes Cabernet Sauvignon, Xinomauro, Limnio, 1997); Erythros (red wine with principle grapes Carignan, Grenache, Syrah, Cabernet Sauvignon, 1998); Cabernet Sauvignon (red wine with principle grape Cabernet Sauvignon, 1998); Rompola (white must); Rompola with extra yeast (white must); and Cabernet Sauvignon (red must).

Extraction of Total Lipids. Total lipids of the studied samples (wines and musts) were extracted according to the Bligh-Dyer method (35). The lipid fraction was separated into neutral and polar lipids by countercurrent distribution (36). The fractions, total lipids, total polar lipids, and total neutral lipids, were tested for biological activity.

Further Separation of Total Neutral and Total Polar Lipids of Cabernet Sauvignon. Total neutral lipids were further fractionated by HPLC (37). Briefly a Nucleosil-300 C18 column and a stepped gradient elution system were used. The solvents were the following: A, water/methanol 1:4 v/v; B, acetonitrile/methanol 7:5 v/v; C, acetonitrile/tetrahydrofuran 99.5:0.5 v/v; D, 2-propanol/acetonitrile 99:1 v/v; E, cyclohexane. The elution system consisted of a gradient from solvent A to solvent B in 10 min, a hold for 5 min, a gradient from solvent B to solvent C in 10 min, a second hold for 5 min, a gradient from solvent C to solvent D in 10 min, a third hold for 5 min, a gradient to solvent E in 10 min, and a finally hold in solvent E. The flow rate was 1 mL/min, and detection was at 208 nm.

Before the separation of total polar lipids with HPLC, a preparative TLC was done for the removal of coextracted pigments with the following system: acetone/methanol/water 40:20:10. Pigments migrated near the solvent front, whereas phospholipids migrated along the plate.

Total polar lipids, free of pigments, were further fractionation by HPLC (38), with a cation-exchange column and an isocratic elution system consisting of 60% acetonitrile/40% aqueous methanol 80%. The flow rate was 1 mL/min, and detection was at 208 nm.

The purified lipid fractions from the above separations were collected separately and tested for biological activity.

Separation of Coextracted Pigments from Total Polar Lipid Fraction of Cabernet Sauvignon. Coextracted polar

lipids pigments, after the TLC separation (Figure 1), were further fractionated by HPLC. A Nucleosil-300 C18 column was used, and a stepped gradient elution system with the following solvents was used: solvent A, methanol/water (20:80, v/v); solvent B, acetonitrile (100, v/v). The stepped gradient elution system was as follows: isocratic elution with solvent A for 30 min and flow of 1 mL/min; linear gradient to solvent B at 2 mL/min in 5 min; and finally a hold in solvent B at 2 mL/min for 15 min. The detection was at 208 nm. An amount from the fraction eluted in 31–40 min was kept and tested for biological activity, while the rest was rechromatographed with the same HPLC column and a stepped gradient elution system from 100% water to 100% methanol in 30 min. The flow was 1 mL/min, and the detection was at 280 nm.

Biological Assay. PAF, thrombin, and the aggregation induced by the purified compounds were tested with washed rabbit platelets according to the method of Demopoulos et al. (22). Briefly the examined samples or PAF samples were dissolved in 2.5 mg of bovine serum albumin (BSA)/mL of saline; thrombin was dissolved in saline. Desensitization experiments (39) with PAF and thrombin and experiments with specific inhibitors (0.7 mM creatine phosphate (CP)/creatin phosphate kinase (CPK), 10 μ M indomethacin, and 0.1 μ M BN 52021) were also performed. For the sample with inhibitory activity, the IC_{50} value was also calculated, which is the amount of samples (in mL) that inhibited 50% by PAF-induced (5×10^{-11} final concentration in the aggregometer cuvette) or thrombin-induced (0.025 units in the aggregometer cuvette) aggregation. The exact procedure of these experiments was previously described (30, 32).

Treatment with Acetylhydrolase. The effect of PAF acetylhydrolase of human serum, an enzyme specific to short or intermediate length *sn*-2 chains, on the ability of fractions to induce platelet aggregation, was examined. Briefly Tris buffer (pH 7.4), human serum acetylhydrolase, and the examined sample in bovine serum albumin (BSA) at 2.5 mg/mL saline were added to a prewarmed (37 °C) test tube. The enzymatic system was incubated at 37 °C, and at different time intervals, aliquots were taken to test their ability to induce washed rabbit platelet aggregation.

Mild Alkaline Hydrolysis and Acetylation. This procedure was carried out according to the method of Demopoulos et al. (22). Briefly the sample was dissolved in 1 mL of chloroform/methanol (1:4 v/v), and then 0.1 mL of 1.2 N NaOH and 1 N 50% methanol was added and kept for 20 min at 60 °C. The mixture was neutralized with 0.15 mL of 1 N acetic acid and 2 mL of chloroform/methanol (9:1 v/v), and then 1 mL of methanol and 2 mL water were added. The two phases were separated, and chloroform was washed with 1 mL of methanol/water (1:2 v/v). Both phases were separately evaporated to dryness and subjected to reacylation by the addition of 1 mL of acetic anhydride and warming at 60 °C for 45 min. Each reaction mixture from the above phase was evaporated and extracted by the Bligh–Dyer method (35). All samples from this procedure were tested for their ability to induce washed rabbit platelet aggregation.

Chemical Determinations. Phosphorus determination was carried out according to the method of Bartlett (40).

Sugar determination was carried out according to the method of Galanos and Kapoulas (41).

Phenol Determination. Phenol content was determined using a modified method of Singleton and Rossi (42). Samples were dried under a stream of nitrogen and dissolved in 3.5 mL of water. An amount of 0.1 mL of Folin–Ciocalteu reagent was added, and after 3 min, 0.4 mL of 35% aqueous Na_2CO_3 was added. The reaction mixture was kept for 1 h, and the intensity of the blue color was measured at 725 nm. Standards of gallic acid were prepared similarly.

Ester determination was carried out according to the method of Renkonen (42).

RESULTS AND DISCUSSION

Extraction of Total Lipids. The extraction and the purification procedure of the lipids of wines and musts

Table 1. Biological Activities of Total Lipid Fractions

sample	IC_{50} (expressed as milliliters of wine or must)		
	total lipids	total polar lipids	total neutral lipids
Chardonnay (white wine)	0.47	0.42	239
Ambelon (white wine)	0.27	0.015	116
Cuvee Maison (white wine)	0.33	0.14	85
Erythros (red wine)	0.54	0.34	63
Cabernet Sauvignon (red wine)	0.24	0.091	45
Rompola (white must)	0.34	0.046	0.091
Rompola with extra yeast (white must)	0.35	0.042	0.37
Cabernet Sauvignon (red must)	0.34	0.38	0.60

are shown in Figure 1. A portion of the total lipids was tested for biological activity, while the rest was separated in neutral and polar lipids by countercurrent distribution. By this procedure, the total polar lipid fraction contains glyco- and phospholipids as well as pigments, which are coextracted.

Biological Activity of Total Lipid Fractions. The lipid fractions (total lipid, total polar lipid, total neutral lipid) of the five wines and the three musts were tested for their ability to induce washed rabbit platelet aggregation and/or to inhibit PAF-induced platelet aggregation. The results are presented in Table 1. All fractions inhibited PAF-induced platelet aggregation. The total neutral lipid fractions of all wines exhibited very low biological activity, 2–3 orders of magnitude lower (higher IC_{50} value), compared to total lipids and total polar lipids. On the other hand the total polar lipids were the most active fractions of lipids, suggesting that the biological activity of total lipids is attributed mainly to total polar lipids, while the contribution of total neutral lipids is rather small. White wine, Ambelon, and red wine, Cabernet Sauvignon, are found to contain the lipids with the most potent inhibitory activity against PAF-induced washed platelet aggregation because their IC_{50} values are lower than those of the other tested wines. Their superiority is more obvious when comparing the IC_{50} values of their polar lipids with ones of the rest wines (1 order of magnitude lower).

In addition, the results of musts, on comparison with the respective wines that are the fermentation products, showed that the total neutral lipids of musts exerted a stronger inhibitory activity than the total neutral lipids of wines, attaining approximately the same order of magnitude with the total lipids and total polar lipids. This dramatic decrease in biological activity of total neutral lipids from must to wine may be attributed to chemical modifications during fermentation. Also, the changes in biological activity of total polar lipids show significant differences in white and red musts during fermentation. Specifically, in the white wine Ambelon, the biological activity remained practically unchanged before and after fermentation, while in red wine Cabernet Sauvignon the biological activity increased. The above results indicate that although the polar lipids of white grape must Rompola are more potent inhibitors than the lipids of red grape must Cabernet Sauvignon, after fermentation red wine corresponding lipids possess the same or even higher biological activity than those of the white wine lipids.

Separation and Biological Activity of Total Neutral Lipids of Cabernet Sauvignon. Although the total neutral lipids seem to have much less biological activity than total polar lipids, they were further

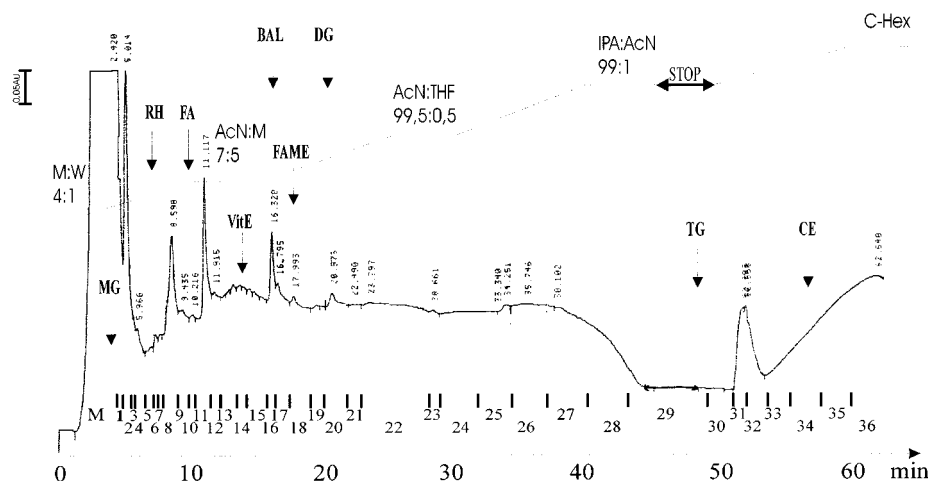


Figure 2. Reverse-phase HPLC separation, with a gradient elution, of total neutral lipids from wine Cabernet Sauvignon: MG, monoglyceride; RH, hydrocarbons; FA, fatty acids; VitE, vitamine E; BAL, batylic alcohol; FAME, methylesters of FA; DG, diglycerides; TG, triglycerides; CE, cholesterol esters.

Table 2. Biological Activities of Most Potent Neutral Lipid Fraction of Cabernet Sauvignon after Separation with HPLC

fraction ^b	biological action	IC ₅₀ ^a expressed as milliliters of red wine	
		PAF	thrombin
M	inhibition	3.1	
NL2	inhibition	59	40
NL4	inhibition	138	38
NL22	inhibition	255	93
NL23	inhibition	141	76
NL24	inhibition	155	15
NL25	inhibition	163	53
NL26	inhibition	45	27
NL27	inhibition	83	
NL28	inhibition	71	

^a IC₅₀ value is expressed as milliliters of wine/must that inhibit 50% of the PAF- or thrombin-induced aggregation. ^b Other fractions have IC₅₀ values for both PAF and thrombin greater than 100.

fractionated on a reverse-phase HPLC (37). A typical profile of neutral lipids separated from red wine Cabernet Sauvignon is shown in Figure 2. Thirty-six fractions were collected and tested for their biological activity.

All fractions of total neutral lipids were tested for their ability to induce aggregation and/or to inhibit PAF-induced and/or thrombin-induced aggregation. All fractions exhibited inhibitory effects against the aggregating agents, as shown in Table 2. The most potent inhibitors against PAF and thrombin were fractions NL2, NL26. Additionally potent inhibitors only against PAF were NL27 and NL28, while potent inhibitors only against thrombin were NL4, NL24, and NL25. The solvent front (M), which exerted the strongest inhibitory activity of all the others neutral lipids fractions, was kept for further study.

Separation and Biological Activity of Pigment-Free Total Polar Lipids of Cabernet Sauvignon. After the removal of pigments with TLC, as described previously, the pigment-free total polar lipids were further separated on HPLC (38). Twenty fractions were collected and tested for their biological activity. In this system according to standards, fraction 3, 7, 12, and 15 eluted in the same retention time with lyso-phospha-

Table 3. Biological Activities of Each Polar Lipid Fraction of Cabernet Sauvignon after Separation with HPLC

fractions	biological action	IC ₅₀ ^a expressed in milliliters of red wine		aggregation	
		PAF	thrombin	[PAF] ^b × 10 ⁻¹¹ M	milliter of wine
PL2	inhibition	250	90		
PL3	inhibition	209	234		
PL4	inhibition	124	no		
PL5	inhibition	247	no		
PL6	inhibition	320	no		
PL7	none				
PL8	aggregation			0.51	270
PL9	inhibition	175	162		
PL10	aggregation			0.85	90
PL11	inhibition	323	561		
PL12	inhibition	27	155		
PL13	inhibition	306	314		
PL14	aggregation			0.86	225
PL15	aggregation			0.83	135
PL16	inhibition	117			
PL17	inhibition	50			
PL18	aggregation			0.71	90
PL19	inhibition	243	-		
PL20	inhibition	449	-		

^a IC₅₀ value is expressed as milliliters of wine/must that inhibit 50% of the PAF- or thrombin-induced aggregation. ^b [PAF]: equivalent concentration of PAF that exerts in platelets the same biological activity as the amount (milliliters of wine) of fraction.

tidylethanolamine, with phosphatidylcholine, with sphingomuelin, and with lyso- phosphatidylcholine, respectively.

Five of the peaks (peaks 8, 10, 14, 15, 18) induced rabbit platelet aggregation with a dose-dependent aggregation pattern similar to that of PAF with the exception of fraction 10, in which the aggregation curve showed a slight slope and was not reversed even in lower doses. Experiments with specific inhibitors were carried out on these fractions, as well as cross desensitization experiments, and the results are summarized in Table 4. In accord with the experimental data fraction 8 acts mostly through PAF receptors. Fraction 10 does not seem to act through the PAF receptor or arachidonic pathway or through secretion of ADP, since the specific inhibitors do not influence its activity, while fractions 14, 15, 18 seem to act in a nonspecific way. Thirteen of them exerted inhibitory activity (Table 3), and fractions

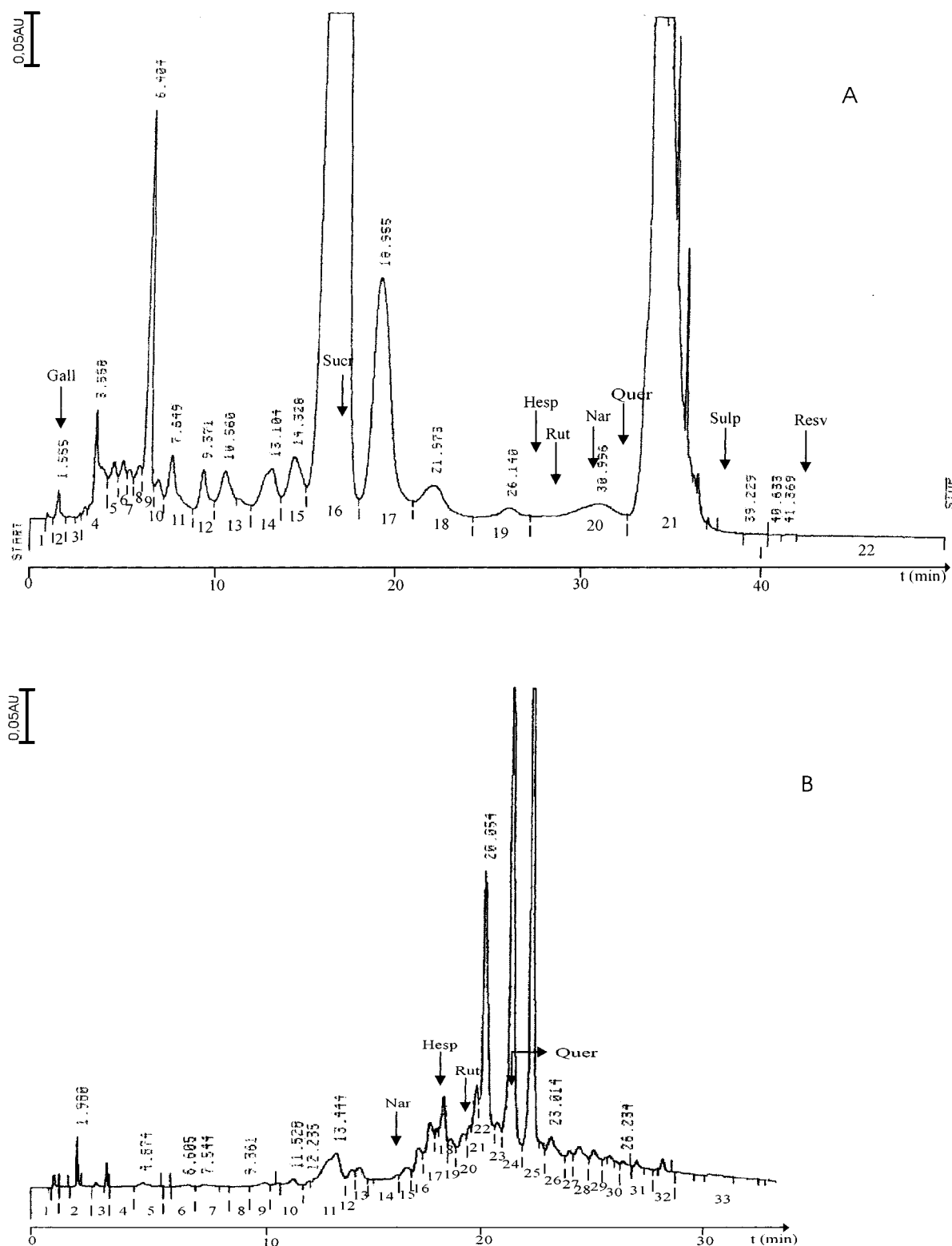


Figure 3. (A) Typical reverse-phase HPLC separation of pigments resulting from total polar lipids of wine Cabernet Sauvignon and (B) further separation of fraction 21 on a reverse-phase HPLC: Gall, gallic acid; Sucr, sucrose; Hesp, hesperidin; Rut, rutin; Nar, narigin; Querc, quercetin; sulp, sulfatides; Resv, resveratrol.

12, 16, 17 seem to be the most potent inhibitors, while only one, fraction 7, did not exert any biological activity.

Fraction PL10. The UV spectrum of this fraction shows two peaks at 210 and 281 nm and a shoulder at 231 nm, suggesting the presence of a phenolic component. This was also confirmed with phenolic determination ($0.646 \mu\text{mol}$ of gallic acid/total fraction), while

determinations of fatty acid ester and sugar were negative. Moreover, ^{31}P NMR excluded the existence of phosphorus. Treatment with acetylhydrolase did not affect the biological activity of this fraction, indicating that no acetic group(s) or other small esterified acid(s) is present in this molecule. Alkaline hydrolysis rendered it inactive, while reacylation of chloroform and water

phases from the hydrolysis mentioned above did not reinstate the lost activity. These data suggest the presence of an ester bond(s), but the molecule has no esterified fatty acid(s). The ester bond(s) seems to be important for the expression of the biological activity of the molecule. The hydrolysis of this ester bond seems to break the main skeleton of the molecule. Acetylation of the intact fraction shows a dramatic decrease of the biological activity, suggesting the existence of a free hydroxyl group(s) in the molecule that contributes to the biological activity of this molecule.

Fraction PL16. The UV spectrum of this fraction shows two peaks at 204 and 281 nm and a shoulder at 231 nm, suggesting the presence of a phenolic component. This was also confirmed with phenolic determination ($0.829 \mu\text{mol}$ of gallic acid/total fraction). Determinations of sugar ($0.617 \mu\text{mol}$ of glucose/total fraction) and fatty acid ester ($2.29 \mu\text{mol}$ /total fraction) were also positive, while the phosphorus determination was negative. This fraction inhibits PAF-induced platelet aggregation. After alkaline hydrolysis of the amount that inhibits 100% of the action of PAF, both chloroform and water phases continue to inhibit 100% PAF-induced platelet aggregation. In contrast, acetylation of the chloroform and water phases from the above hydrolysis rendered them to induce platelet aggregation. These data suggest that the molecule probably consists of two parts, one rather polar and one nonpolar that are linked to an ester bond. Acetylation of hydrolysis products leads to new molecules, which induce platelet aggregation.

Fraction PL8. This fraction is from our previous work with the same wine Cabernet Sauvignon. Briefly we followed the same extraction procedure except that we removed the pigment with two TLC runnings before the countercurrent distribution (32). This fraction resulted from the cation exchange HPLC separation of polar lipids and was eluted at 32–36 min. The UV spectrum of this fraction shows two peaks at 204 and 281 nm and a shoulder at 231 nm. ^{31}P NMR excluded the occurrence of phosphorus in this molecule. Sugar determination ($0.198 \mu\text{mol}$ of glucose/total fraction) was positive, while the amount of this fraction was not enough for other chemical determinations. Acetylation of the intact fraction resulted in loss of the activity, suggesting that there is a free hydroxyl group(s) that contributes to the biological activity of the molecule. Alkaline hydrolysis rendered it inactive, while acetylation of the chloroform phase of the above hydrolysis did not reinstate the lost activity. These data indicate the existence of an ester bond(s) such that its hydrolysis breaks the main skeleton of the molecule and the resulting products are not biologically active. The ester bond(s) is not an acetyl group; acetylation does not reinstate the initial activity. Treatment with acetylhydrolase did not affect its ability to induce aggregation, confirming the absence of acetic or other small esterified fatty acid groups.

Separation and Biological Activity of Pigments Resulting from Total Polar Lipids of Cabernet Sauvignon. The pigments resulting from the preparative TLC of total polar lipids of Cabernet Sauvignon was further fractionated on HPLC. All fractions were tested for their biological activity. Fraction 21 was further separated on HPLC, and the resulting fractions were also tested for their biological activity. Typical separation profiles are shown in Figure 3. All fractions of pigments exerted biological activity. Some of them

Table 4. Biological Characterization of Fractions from Total Polar Lipids and Pigments of Cabernet Sauvignon That Induced Rabbit Platelet Aggregation

fraction that induced aggregation	% desensitized			% inhibition	
	PAF	thrombin	BN	indomethacin	CP/CPK
PL8	100	32			
PL10	0		0	0	0
PL14	74	45	100		65
PL15	55	82	100	62	70
PL18	42		100	100	100
Pg2		62	100	57	50
Pg3		100	100	36	33
Pg8		100	100	36	
Pg21/10	7		24	18	23
dPg21/15	82		100	97	
Pg21/25			100	84	95

inhibited PAF-induced platelet aggregation, and some induced rabbit platelet aggregation in a dose-dependent manner. Experiments with specific inhibitors were carried out for a number of these fractions (Table 4). Fraction Pg2 seems to act mostly through PAF receptors and secondarily through an arachidonic way and through secretion of ADP. Fraction Pg21/10 seems to act, in a small percentage, through all the ways.

In this work, biological activity of total, neutral, and polar lipids of several wines and musts were compared. The results showed that the biological activity is attributed mainly to total polar lipids, while the contribution of total neutral lipids is rather small. Two wines were the most potent, including one red wine, Cabernet Sauvignon, and one white wine, Ambelon (principal grape: Rompolo). In Cabernet Sauvignon the existence of a variety of PAF and thrombin inhibitors was demonstrated along with weak PAF-like agonist action, which minimized the biological effects of PAF in several cells and/or tissues.

The findings support the beneficial effects of moderate wine consumption because wines and musts contain a significant number of lipid-like components with anti-thrombotic and antiatherogenic action in vitro. It is of interest to trace the origin and source (i.e., grape, yeast, or fermentation products) of the biologically active compounds, which will be the subject of future study. These data in correlation with our proposed mechanism of atherogenesis may offer a biochemical insight into the well-known "French paradox".

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