7576 J. Agric. Food Chem. 2003, 51, 7576−7582

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Antioxidative Properties of the Essential Oil from *Pinus mugo*

JOHANNA GRASSMANN,*,† SUSANNE HIPPELI,‡ RENATE VOLLMANN,§ AND ERICH F. ELSTNER||

Institute of Vegetable Science, Life Science Center Weihenstephan, TUM, Dürnast 2, 85350 Freising, Germany, Isarlab Systems, Birkenweg 9, 86441 Zusmarshausen, Germany, Allga Pharma Am Anger 8, 87538 Fischen, Germany, and Institute of Phytopathology, Life Science Center Weihenstephan, TUM, Am Hochanger 2, 85350 Freising, Germany

The essential oil from *Pinus mugo* (PMEO) was tested on its antioxidative capacity. For this purpose, several biochemical test sytems were chosen (e.g., the Fenton Systen, the xanthine oxidase assay, or the copper-induced oxidation of low-density lipoprotein (LDL)). The results show that there is moderate or weak antioxidative activity when tested in aqueous environments, like in the Fenton system, xanthine oxidase induced superoxide radical formation, or in the HOCl driven fragmentation of 1-aminocyclopropane-1-carboxylic acid (ACC). In contrast, when tested in more lipophilic environments (e.g., the ACC-cleavage by activated neutrophils in whole blood) the PMEO exhibits good antioxidative activity. PMEO does also show good antioxidative capacity in another lipophilic test system (i.e., the copper induced oxidation of LDL). Some components of PMEO (i.e., Δ^3-carene, camphene, α-pinene, (+)-limonene and terpinolene) were also tested. As the PMEO, they showed weak or no antioxidant activity in aqueous environments, but some of them were effective antioxidants regarding ACC-cleavage by activated neutrophils in whole blood or copper-induced LDL-oxidation. Terpinolene, a minor component of PMEO, exhibited remarkable protection against LDL-oxidation.

KEYWORDS: Antioxidants; *Pinus mugo*; essential oil; monoterpenes; LDL; inflammatory processes

INTRODUCTION

Essential oils derived from steam distillation of needles and bark of pinus- and picea-species and also from various other plant sources are widely in use as ointments, bathing oils or inhaling drugs for curing a wide range of bronchial-, skin-, and muscle-disorders of infectious, rheumatic or neuralgic origin (*1*−*8*). Those oils comprise various amounts of monoterpenes such as α- and β-pinene, Δ^3-carene, (+)-limonene, terpinolene, γ-terpinene, and myrcene as major components. In addition to these monoterpenes, sesquiterpenes and diterpenes are known (*8*, *9*).

Because many of the above-mentioned disorders are related to the formation of reactive oxygen species (ROS), the antioxidatve capacity of essential oils may provide an explanation for their efficacy in ameliorating these dysfunctions.

During inflammatory processes, ROS are formed by neutrophils to defend the organism against invading microorganisms or pathogens. Beside this intended effect, however, ROS can attack surrounding tissue and cause damage of the same (*10*, *11*). For this reason, protection against ROS must be provided in inflammatory regions. This protection by antioxidants is also important for low-density lipoproteins, whose oxidation plays a major role in atherogenesis (*10*, *12*). A good resistance of LDL against oxidation is therefore believed to help to prevent atherosclerosis, heart attack, or stroke (*13*). Therefore, antioxidants play a pivotal role in several pathological processes, and antioxidants may support the organism in getting through them without damage.

We recently reported on the antioxidative properties of essential oils from eucalyptus (*14*) and lemon (*15*) in test systems that simulate pathophysiological conditions such as inflammatory processes or LDL-oxidation. Because the oil from Pinus mugo is widely used for treatment of different disorders (see above), here experiments with PMEO in various physiological model systems are described. A wide variety of different test systems were chosen, which is a prerequisite to characterize the antixodative capacity of any test substances. The test sytems can be divided into two classes according to the environment as follows:

(a) Aqueous Environment. To this class belong the HOCl-driven ACC− fragmentation and the fragmentation of 1-keto-4-methylthiobutyric acid (KMB) in the Fenton-system, by 3-morpholino-sydnonimine (SIN-1), by NADH/diaphorase, or by xanthin/xanthine oxidase.

(b) Lipophilic Environment. This category contains the ACC fragmentation by activated neutrophils in whole blood or the copper induced LDL oxidation.

The purpose of the presented investigations was to characterize the antioxidative capacity of PMEO in aqueous environment

* To whom correspondence should be addressed. Tel.: +49−8161−713151. Fax: +49-8161-714491. E-mail: johanna.grassmann@wzw.tum.de.
† Institute of Vegetable Science.
‡ Isarlab Systems.
§ Allga Pharma Fischen/Allgäu.
|| Institute of Phytopathology.

10.1021/jf030496e CCC: $25.00 © 2003 American Chemical Society
Published on Web 11/14/2003

as well as in more lipophilic environment. The results may help to explain the antiinflammatory effects of PMEO.

MATERIALS AND METHODS

Chemicals. Authentic *Pinus mugo* essential oil was obtained from ALLGA-Pharma (Fischen/Allgäu).

ACC, $Fe(SO_4)$, HOCl, KMB, NADH, and xanthine were purchased from Sigma, Munich. $CuSO_4$*5 H_2O, EDTA, H_2O_2, KBr and saccharose were obtained from Merck, Darmstadt. Xanthine oxidase (XOD) and diaphorase were from Roche, Mannheim. SIN-1 was from Hoechst AG, Frankfurt. The gases for gas chromatography were purchased from Messer Griesheim, Darmstadt. The carrier gas was N_2, 25 mL/min; the burning gases were H_2, 25 mL/min and synthetic air, 250 mL/min; ethylene calibration gas (mixture of ethylene and synthetic air), 1 mL = 254.55 pmol, 1 bar.

Instruments. Gas chromatograph: Varian Aerograph 3300 with Varian Integrator: column, 0.125 in. × 60 cm aluminum oxide; column and injector temperature, 80 °C; FID detector temperature, 225 °C. Spectrophotometer: Kontron Instruments Uvikon 922. Ultra centrifuge: Beckmann Optima LE−70, Swinging Bucket Rotor SW 40 Ti.

Test Systems and Methods. *Plasma Preparation*. The plasma used for the shown experiments was gained from blood of 10 healthy volunteers (♀ = 5, ♂ = 5) aged 17−38. The preparation of the plasma was as follows: To 100 mL of blood, 4 mL EDTA stock solution (25 mg/mL) was added immediately after receiving the blood. The blood was centrifuged at 10 °C for 20 min at 3000 rpm, plasma was withdrawn, and after addition of 1 mL of saccharose solution (60%) per 100 mL of plasma, it can be stored at −70 °C in N_2 atmosphere for a maximum of 6 months.

LDL Preparation. The preparation of LDL (d = 1.019−1.063 g/mL) by density gradient ultra-centrifugation was described by Giessauf and co-workers (*16*) and modified by Kögl and co-workers (*17*). Plasma (3 mL) was adjusted to a density of 1.41 g/mL with KBr in Beckmann Polyallomer Centrifuge tubes (No. 331372) and stratified with approximately 2.5 mL of density solution A (1.080 g/mL; 1 g of EDTA/l), B (1.050 g/mL; 1 g of EDTA/l) and C (1.000 g/mL; 1 g of EDTA/l) per layer. After 22 h centrifugation at 10 °C with 40 000 rpm (285 000*g*), three different layers appear above the plasma: lowest HDL, then LDL, and on the surface, VLDL and chylomicrones. After gaining the LDL layer, it was filtrated with a sterile filter (Nalgene 0.22 μm) and stored at 4 °C. Before use, the LDL was desalted with an EconoPac DG-10 column and its concentration defined by standard protein determination of BioRad.

The Hypochlorite/ACC System. HOCl is generated by polymorphonuclear leukocytes as a product of myeloperoxidase. It can be scavenged by addition onto double bonds. Ethylene formation from ACC with hypochlorite is a specific indicator and a decrease in the ethylene production indicates a protective ability of the test substance (*18*).

An assay contains the following in a 2-mL final volume: Phophate buffered saline (PBS) pH 7.2; ACC, 1 mM; HOCI, 25 μM; sample(s) (amounts indicated). After incubation of 30 min at 37 °C in sealed gastight reaction tubes, 1 mL of gas of the headspace has been retained with a gastight syringe and analyzed gas chromatographically.

The SIN-1 System. Peroxynitrite ($ONOO^-$), a strong oxidant, can cause nitration of proteins—especially at tyrosine residues—and is an indicator for inflammation. SIN-1 resolves rapidly into $O_2^{\bullet-}$ and NO, forming $ONOO^-$, which in turn releases ethylene from KMB. Ethylene can be quantified gas chromatographically, a decrease in ethylene production in the presence of the investigated substance is equivalent to detoxification of $ONOO^-$ (*19*, *20*).

An assay contains the following in a 2-mL final volume: phosphate buffer pH 7.4, 0.1 M; KMB, 1 mM; SIN-1, 10 μM; sample(s) (amounts indicated). Incubation and ethylene quantification were conducted as described above.

The Fenton System. The hydroxyl radical, $OH^\bullet$, is one of the most reactive ROS-inducing radical chain reactions with a multitude of organic molecules. It can be scavenged by hydrogen abstraction, addition and e^- transfer. In the Fenton system $OH^\bullet$-radicals are generated with hydrogen peroxide in the presence of Fe^{2+}- ions and as in the SIN-1-system the methionine derivative KMB is used as an indicator releasing ethylene. The inhibition of ethylene production in the presence of the test substance indicates its reaction with the $OH^\bullet$ radical (*18*, *20*).

An assay contains the following in a 2 mL final volume: phosphate buffer pH 7.4, 0.1 M; KMB, 1 mM; $Fe(SO_4)$, 10 μM; H_2O_2, 10 μM; sample(s) (amounts indicated). Incubation and ethylene quantification were conducted as described above.

The NADH/Diaphorase System. NAD(P)H-oxidases play a dominant role in various biomembranes (e.g., in leukocytes during the "respiratory burst"). At the expense of NAD(P)H, the flavin cofactor is reduced and in turn is able to reduce a wide range of autoxidable substances. In our test system, we use NADH-diaphorase instead of NADPH-oxidase, which catalyzes an analogous reaction. The autoxidation leads to ROS production, which is detectable as ethylene formation from KMB. A decrease of ethylene may indicate an inhibition of the enzyme or a scavenging activity of the substance investigated (*20*).

An assay contains the following in a 2 mL final volume: phosphate buffer pH 7.4, 0.1 M; KMB, 1 mM; NADH, 75 μM, diaphorase, 2.2 U; sample(s) (amounts indicated). Incubation and ethylene quantification were conducted as described above.

The Xanthine/Xanthine Oxidase System. Xanthine oxidase (XOD) produces $O_2^{\bullet-}$ and H_2O_2 using xanthine as substrate. This reaction is characteristic for reperfusion injury after ischemic events. Superoxide is detectable by oxidation of hydroxylamine to nitrite followed by generation of an azo-dye (Griess reaction), the hydroxyl radical formed from superoxide, and metal ions can be detected by ethylene production from KMB fragmentation. Again, a decrease in dye or ethylene formation points to scavengers or enzyme inhibitors in the investigated samples (*20*).

An assay contains the following in a 2-mL final volume: phosphate buffer pH 7.4, 0.1 M; KMB or hydroxylamine, 1 mM; xanthine, 0.5 mM; xanthine oxidase, 0.08 U; sample(s) (amounts indicated). Incubation and ethylene quantification were conducted as described above, in case of superoxide detection with hydroxylamine, the azo-dye was quantified spectrophotometrically at 540 nm.

Activated Leukocytes In Whole Blood. In inflammatory processes, polymorphonuclear leukocytes (PMN) are involved, thereby producing different reactive oxygen species. During the respiratory burst among other ROS, hydrogen peroxide is formed, which in turn is used in the course of the degranulation process by the myeloperoxidase to form HOCl. In the applied test system, neutrophile granulozytes are activated with zymosan in whole blood. HOCl is formed as described above, and liberates ethylene from ACC (*18*). The amount of released ethylene is proportional to the number and activity of the PMNs.

An assay contains the following in a total volume of 2 mL: 1 mL of blood (freshly withdrawn from healthy donors), 5 mg of zymosan, 1 mM ACC, and the indicated amounts of solubilized oils. All substances were solubilized in PBS-buffer. After incubation (60 min, 37 °C), ethylene was quantified as described in 3.3.3.

The Copper-Induced LDL Oxidation. LDL is the main lipoprotein fraction involved in atherogenesis, which in turn is the major cause of heart disease and stroke. One process of modification of LDL is its oxidation, which can be measured as an increase in absorption at 234 nm, reflecting lipid peroxidation associated diene conjugation. Thereby, a prolongation of the lag-phase (time until rapid extinction increase occurs) means higher antioxidative capacity of the substance investigated (*21*). To enrich LDL with the test substances, we incubated human blood plasma with PMEO or single monoterpenes for 1.5 h at 37 °C, and subsequently isolated the LDL.

For monitoring the diene conjugation as an indicator for the oxidation of LDL an assay contains in a final volume of 1 mL: PBS pH 7.4; $CuSO_4$, 1.67 μM; LDL, 25 μg; protein; sample(s) (amounts indicated). The change of the extinction was measured photometrically at 234 nm every 10 min for 1000 min at 37 °C.

In the isolated LDL, the terpenoids can be quantified by hexane extraction as follows: To 250 μl of LDL sample, 250 μl of ethanol and 500 μl of hexane are added. After vortexing for 1 min and centrifugation (4000*g*, 3 min), 1 μl of the hexane phase was used for the gaschromatographic analysis of the terpenoids, which was conducted on a Fisons DB-225 capillary column in a GC 86.10 (DANI, Mainz, Germany) with PTV injection and FID detection. The temperature program was as follows: 5 min isothermal at 65 °C // 5 °C/min → 70

Table 1. IC_{25} Values of PMEO and Its Components in Different Test Systems for Antioxidative Capacity

test system	PMEO	Δ^3-carene	α-pinene	(+)-limonene
xanthine/xanthinoxidase KMB	0.164 ± 0.03	0.098 ± 0.023	no reaction	no reaction
xanthine/xanthinoxidase hydroxylamine	0.06 ± 0.009	0.337 ± 0.016	no reaction	no reaction
NADH/diaphorase-KMB	0.397 ± 0.146	0.071 ± 0.03	no reaction	no reaction
Fenton-KMB	0.180 ± 0.025	0.029 ± 0.026	no reaction	no reaction
SIN-1-KMB	0.208 ± 0.023	0.259 ± 0.090	no reaction	no reaction
HOCl-ACC	no reaction	no reaction	no reaction	no reaction

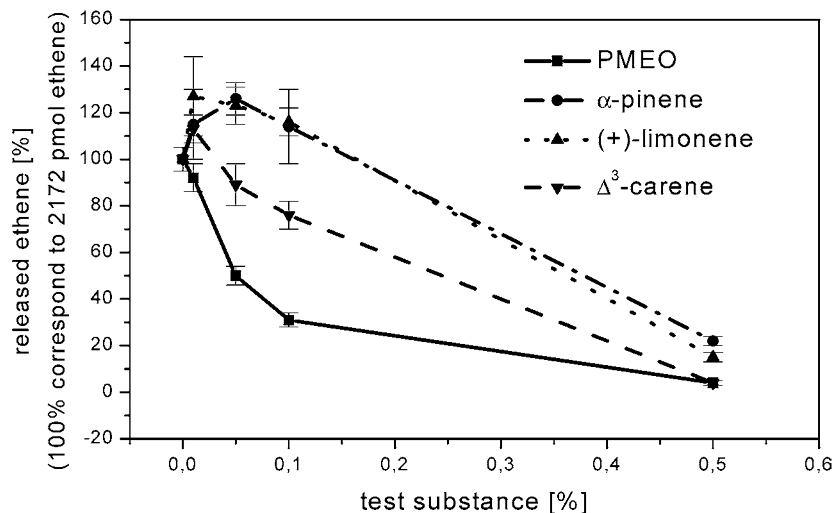

Figure 1. Influence of PMEO, α–pinene, (+)-limonene, and Δ^3-carene on ethene release by activiated neutrophils.

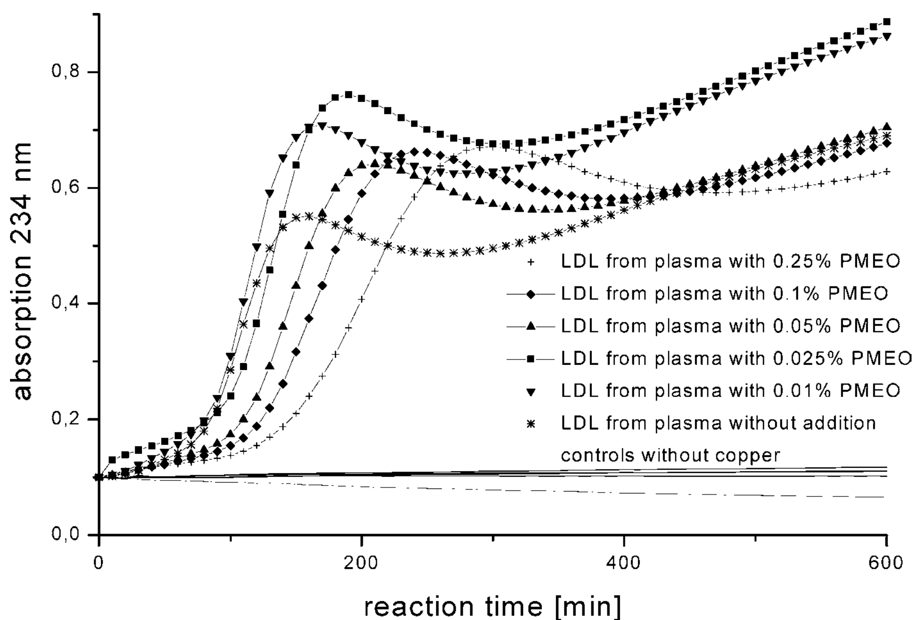

Figure 2. Influence of PMEO on copper-induced formation of conjugated dienes in LDL.

°C // 1 min isothermal at 70 °C // 10 °C/min → 200 °C // 3 min isothermal at 200 °C.

The presented results are means of four individual experiments undertaken on two different days ($n = 8$). Standard deviations are given as σ_{n-1}. In the case of LDL oxidation, plasma preincubation was conducted on two different days. From each plasma incubation, LDL was isolated and copper-induced oxidation was conducted twice. The presented results are from one representative experiment.

RESULTS

Antioxidative Activities of PMEO in Different Biochemical Model Reactions. The first section deals with hydrophilic test systems, which brings about the problem of limited water solubility of essential oils. However, to be able to draw conclusions about the activity of the essential oils in different environments and to compare the results with such of common antioxidants, it was our aim to investigate the essential oils in this kind of test sytem. Several emulsifiers or solvents for the essential oils were tested, but all showed interference with the test sytems. For this reason, essential oils and terpenoids were diluted in 10% ethanol and mixed thorougly before adding to the assay. By this procedure, an emulsion of oils in water was obtained. All samples again were mixed thorougly with a vortexer before incubation, so that during incubation a sufficient mixture of oil and water was assured.

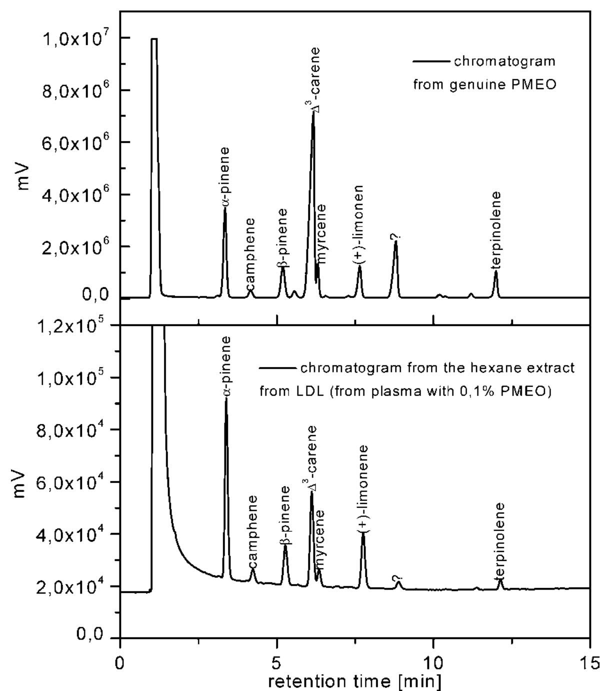

Figure 3. Chromatograms of PMEO and of a hexane extract from LDL (isolated from plasma, which was incubated with 0.1 PMEO).

In **Table 1**, the activities of the three major monoterpene moieties in the oil, namely Δ^3-carene, α-pinene, and (+)-limonene are compared with a genuine oil mixture, manufactured January 25, 1999. In the table, only the IC_{25} values can be given (i.e., the concentration of essential oil or terpene in the assay at which the reaction is reduced by 25%). IC_{50} values are not reached by reasonable concentrations, in part due to limited solubilities (see above). The results show that PMEO does not react with HOCl and reacts only slowly with the other tested ROS and also shows no inhibition of any of the used enzymes. It can also be seen that α-pinene and (+)-limonene are not reactive at all in six biochemical model systems. These tests were not conducted with terpinolene, because this is not a major component of PMEO.

Because Δ^3-carene in a similar manner as the genuine oil is reactive in five of the six test systems, most of the oil's activity seems to be due to the presence of this dominating compound.

Effect on Activated Neutrophils in Whole Blood. As shown in **Figure 1** zymosan-activated PMNs in whole blood release about 2000 pmol of ethylene from 1 mM ACC within 60 min.

PMEO tested in vitro exhibits very limited antioxidative capacities in aqueous environments (**Table 1**), and neither PMEO nor the individual monoterpenes tested (**Table 1**) react with HOCl. In contrast, PMEO as well as the monoterpenes α-pinene, (+)-limonene and Δ^3-carene inhibit ethylene formation from ACC by activated neutrophils in whole blood.

Because this reaction is an indication for myeloperoxidase activity after degranulation, this process is interrupted by PMEO at a stage anterior to HOCl formation by activated PMNs.

Uptake of Terpenoids into LDL and Their Activities in Delaying Diene Conjugation. If human blood plasma is incubated with different amounts of PMEO (0.01% up to 0.25% v/v), retardation of diene conjugation is observed in LDL moieties stemming from incubations with 0.05% oil and higher (**Figure 2**). PMEO (0.01%) gave a lag time prolongation of 4 min, but 0.25% PMEO preincubated in human blood plasma already prolonged the lag time by 48 min.

As shown in **Figure 3**, hexane-extracts from LDL preparations obtained form PMEO-treated blood plasma contain the monoterpenes of PMEO in slight variation (see discussion) as compared to the genuine PMEO.

Furthermore, LDL was obtained from individual incubations with the characteristic monoterpenes, α-pinene, Δ^3-carene, camphene, and terpinolene. If these preparations are subject to copper-catalyzed oxidation, only weak effects are visible with the first three compounds, and a clear and strong delay of diene conjugation is only brought about by terpinolene, as shown in **Figure 4**. After incubation of human blood plasma with 0.25% terpinolene, the lag time is prolonged by 774 min. The inhibition of diene conjugation is clearly concentration dependent, a concentration of 0.025% terpinolene in the plasma already leads to a protection of LDL (**Figure 5**). The prolongation of lag time is in a linear correlation with the terpinolene concentration ($r = 0.9815$, $p < 0.0001$).

If terpinolene is compared with its isomer, γ-terpinene (preincubation of blood plasma with each 0.025, 0.05, and 0.25%) a much stronger effect is observed with γ-terpinene as compared to terpinolene (**Figure 6**). Terpinolene (0.025%) or γ-terpinene bring about a lag time prolongation of 112 and 177 min, respectively.

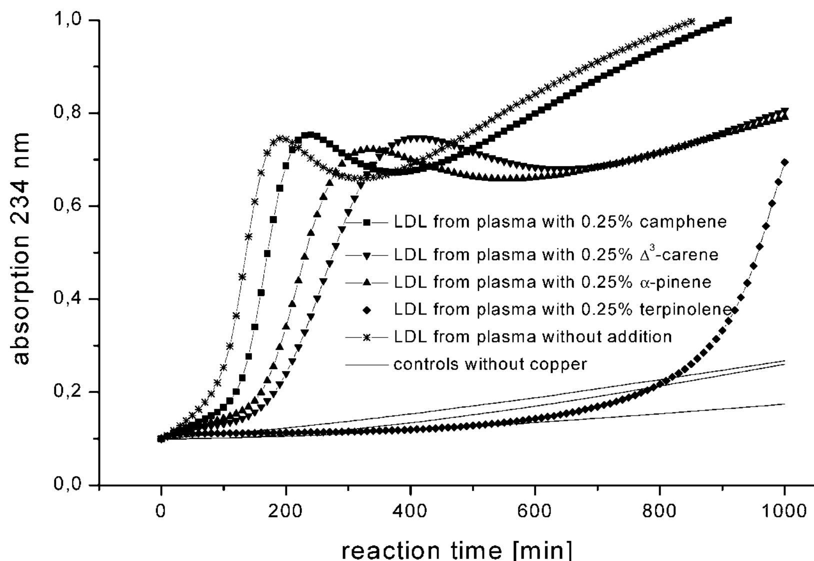

Figure 4. Influence of camphene, Δ^3-carene, α-pinene, and terpinolene on copper induced formation of conjugated dienes in LDL.

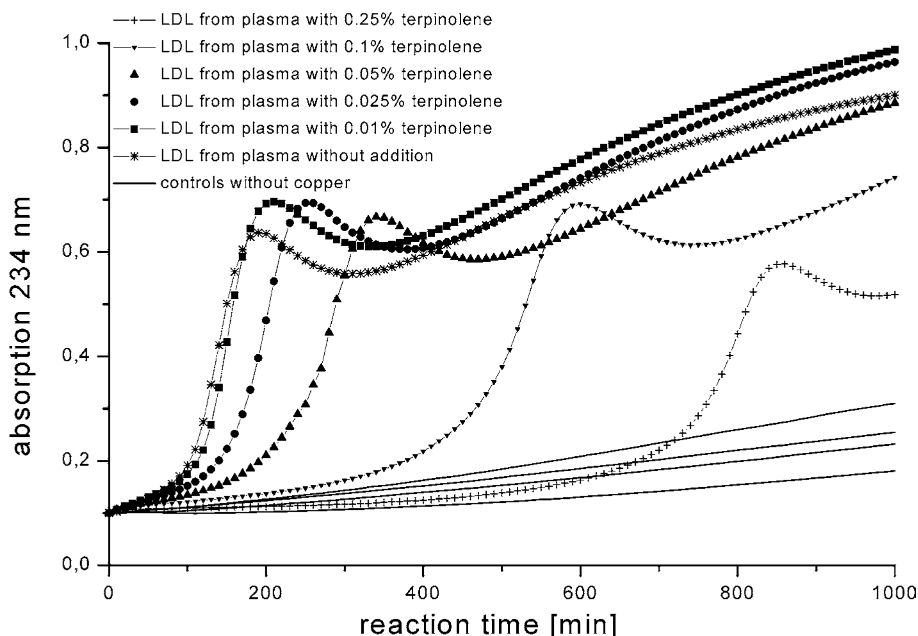

Figure 5. Influence of different concentrations of terpinolene on copper-induced formation of conjugated dienes in LDL.

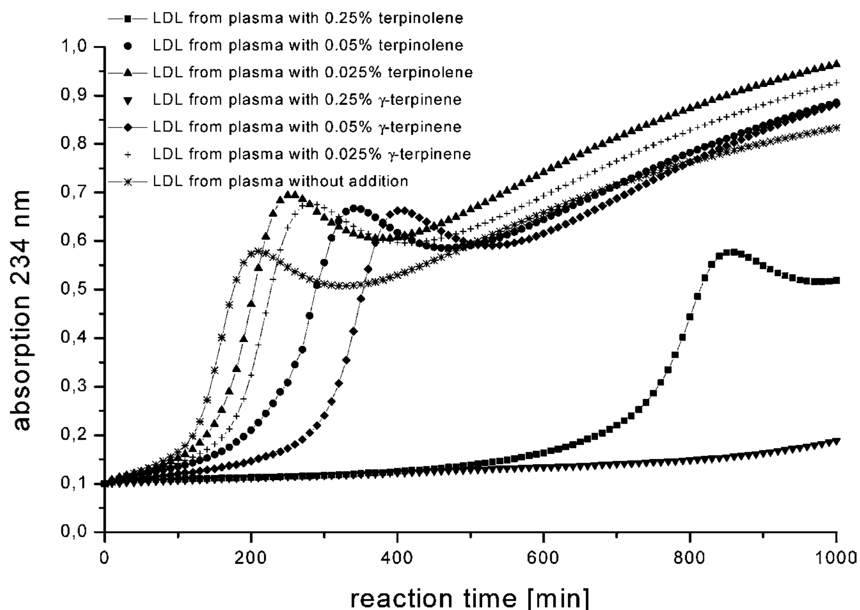

Figure 6. Influence of terpinolene and γ-terpinene on copper-induced formation of conjugated dienes in LDL.

DISCUSSION

ROS Formation in Vitro by Biochemical Model Reactions in Aqueous Systems. Biochemical model reactions have frequently been used to demonstrate potential antioxidant efficacies of drugs and food additives, for example in inflammatory processes (*22*). As shown in **Table 1,** the antioxidative activity of PMEO and its major components is not extremely strong, as compared to certain plant extracts such as bark and leaf extracts from Fraxinus and Populus (*20*) or the *Pinus maritima* bark extract containing the flavane pycnogenol (*23*). In contrast to these antioxidants, PMEO did not reach an IC_{50} at reasonable concentrations. This result is in agreement with an earlier report from our group where Grassmann and coworkers (*14*) have shown that essentil oil (EO) from Eucalyptus or the standardized EO, Myrtol, also exhibited only very weak or moderate effects at high concentrations in aqueous environments. It was also shown by other groups that essential oils are weak antioxidants in hydrophilic surrounding (*24*, *25*). However, several groups proved antioxidative capacity of different EO in retarding lipid peroxidation in soja and sunflower oil (i.e., in nonaqueous systems) (*26*, *27*). Therefore, the next aim was to investigate whether PMEO exhibits antioxidative properties in a more lipophilic environment. For this purpose, two model systems were chosen, namely activated neutrophils in whole blood and the copper-induced LDL oxidation. Activated neutrophils produce HOCl via myeloperoxidase. Because it has been shown that PMEO and its components stimulate HOCl formation by myeloperoxidase (MPO) in a cell-free system (*28*), it is surprising to see the inhibition of ethylene formation from ACC in whole blood.

In **Table 1**, it is documented that neither PMEO nor one of its major monoterpenes is reactive with HOCl. Nevertheless, PMEO as well as the monoterpenes α-pinene, (+)-limonene, and Δ^3-carene inhibit ethylene formation from ACC in whole blood as an indication for myeloperoxidase activity after degranulation.

There are good indications in the literature that monoterpenes in EOs such as Myrtol interact with mediators of inflammation and allergies (*29*). Monoterpenes such as (+)-limonene are known to interfere with signal transduction pathways, thus inhibiting cell proliferation and growth together with Ras-protein prenylation (*30*). The clear effect of inhibition of ethylene formation from ACC shown in **Figure 1** thus might be brought about by such an effect of PMEO or its individual components on the signal cascade between the initial zymosan recognition and the degranulation. The process of inhibition of ethylene formation from ACC by PMEO must be located at an earlier stage in the operational cascade of degranulation (i.e., before HOCl formation and respiratory burst initiation by the activated PMNs). The interactions of EO with cell membranes that are in part responsible for their antimicrobial action (*31*−*33*) might be important for this process. Investigation of this mechansim with other initiators of "burst" and degranulation, such as the leukotaktic tripeptid FMLP or phorbol esters (which are supposed to interact relatively late in this cascade), might be helpful, as shown for the modulatory function by adamantanes in this respect (*34*). These experiments have to be done with isolated PMNs and are currently underway in our laboratories.

Effects on LDL Oxidation. LDL oxidation is assumed to represent an early event in atherogenesis (*12*, *35*). The protective activities of EO from onion and garlic on atherosclerosis has been reported already a quarter of a decade ago by Bordia and co-workers (*36*, *37*). For practical reasons, copper-catalyzed diene conjugation has been used as a sensitive and relevant model system for testing LDL oxidation (*16*), and a biochemical mechanism for this reaction has been discussed (*38*). Our group recently reported on the effects of terpenoids on this reaction and found an outstanding retarding effect of γ-terpinene (*15*). In this communication, results are presented proving that incubation of blood plasma with PMEO or major components of it leads to an accumulation of terpenoids in LDL and to a more or less distincitve protection of LDL against copper-induced oxidation. Some components seem to preferentially be taken up by the lipoprotein particles, because their concentrations are reversed in the genuine oil and in LDL: this is the case for Δ^3-carene and α-pinene, whereas β-pinene and (+)-limonene are accumulated relative to their contents in PMEO.

By far the best monoterpene of PMEO tested for protection of LDL oxidation was terpinolene (**Figure 4**). This process was clearly concentration dependent (**Figure 5**). If compared to γ-terpinene, however, the latter was clearly more protective by a factor close to two (**Figure 6**). Both terpenoids are enriched in LDL to similar amounts; incubation of human blood plasma with 0.5‰ terpinolene or γ-terpinene leads to an incorporation of 90 or 100 molecules of terpene per LDL particle. Therefore, the different extent by which this terpenoids protect LDL from oxidation cannot be attributed to the amount of terpenoid enriched in LDL. However, the effective enrichment of terpinolene or γ-terpinene again shows the outstanding properties of those terpenoids in a lipophilic environment.

The antioxidant activity is probably due to that, although both compounds are isomers with two double bonds each, terpinolene has only one bisallylic carbon, and γ-terpinene has two. Bisallylic carbons are known as potential electron donors for free radicals such as alkoxyl- and hydroperoxyl, thus terminating chain reactions. Foti and Ingold (*39*) proposed that γ-terpinene retards peroxidation of linoleic acid via chain-carrying HOO radicals, which react rapidly with linoelylperoxyl radicals. For this reaction, the bisallylic carbons are decisive, because they allow aromatization of γ-terpinene to p-cymene. It can be hypothesized that protection of lipids in skin and other organs may proceed via an analogous mechanism, thus providing an appropriate shield against various exogenous attacks.

ABBREVIATIONS USED

ACC, 1-aminocyclopropane-1-carboxylic acid; KMB, 1-keto-4-methylthiobutyric acid; LDL, low-density lipoprotein; MPO, myeloperoxidase; PMN, polymorphonuclear leukocytes; ROS, reactive oxygen species; SIN-1, 3-morpholino-sydnonimine; XOD, xanthine oxidase; EO, essential oil; PMEO, *Pinus mugo* essential oil; PBS, phosphate buffered saline

LITERATURE CITED

(1) Kohlert, C.; van Rensen, I.; März, R.; Schindler, G.; Graefe, E. U.; Veit, M. Bioavailability and pharmacokinetics of natural volatile terpenes in animals and humans. *Planta Med.* **2000**, *66*, 495−505.

(2) Bhattaram, V. A.; Graefe, U.; Kohlert, C.; Veit, M.; Derendorf, H. Pharmacokinetics and Bioavailability of Herbal Medicinal Products. *Phytomedicine* **2002**, *9*, 1−33.

(3) Hippeli, S.; Grassmann, J.; Dornisch, K.; Rohnert, U.; Elstner, E. F. Freie Radikale in Pathogenese und Therapie von entzündlichen Erkrankungen des Bronchialsystems. In *Entzündliche Erkrankungen des Bronchialsystems*; Meister, R., Ed.; Springer-Verlag: Berlin, Germany, 2000; pp 1−26.

(4) Craig, W. J. Health-promoting properties of common herbs. *Am. J. Clin. Nutr.* **1999**, *70*, 491S−499S.

(5) O'Hara, M.; Kiefer, D.; Farrell, K.; Kemper, K. A Review of 12 Commonly Used Medicinal Herbs. *Arch. Fam. Med.* **1998**, *7*, 523−536.

(6) Woolf, A. Essential Oil Poisoning. *Clin. Toxicol.* **1999**, *37*, 721−727.

(7) Saller, R.; Berger, T.; Reichling, J.; Harkenthal, M. Pharmaceutical and Medicinal Aspects of Australian Tea Tree Oil. *Phytomedicine* **1998**, *5*, 489−495.

(8) Bruneton, J. *Pharmocognosy, Phytochemistry, Medicinal Plants*, 2nd ed.; Lavoisier Publishing: Paris, France, 1999.

(9) Leung, A. Y.; Foster, S. *Encyclopedia of Common Natural Ingredients*, 2nd ed.; John Wiley and Sons: New York, 1996.

(10) Halliwell, B.; Gutteridge, J. M−C. *Free Radicals in Biology and Medicine*; Oxford University Press: Oxford, UK, 1999.

(11) Dröge, W. Free Radicals in the Physiological Control of Cell Function. *Physiol. Rev.* **2002**, *82*, 47−95.

(12) Chisolm, G. M.; Steinberg, D. The oxidative modification hypothesis of atherogenesis: An overview. *Free Rad. Biol. Med.* **2000**, *28*, 1815−1826.

(13) Niki, E.; Noguchi, N. Effects of antioxidants against atherosclerosis. *Mol. Cell Biochem.* **2002**, *234*−*235*, 19−25.

(14) Grassmann, J.; Hippeli, S.; Dornisch, K.; Rohnert, U.; Beuscher, N.; Elstner, E. F. Antioxidant properties of essential oils: possible explanation for their antiinflammatory effects. *Drug Res.* **2000**, *50*, 135−139.

(15) Grassmann, J.; Schneider, D.; Weiser, D.; Elstner, E. F. Antioxidative effects of lemon oil and its components on copper induced oxidation of low-density lipoprotein. *Drug Res.* **2001**, *51*, 799−805.

(16) Giessauf, A.; Steiner, E.; Esterbauer, H. Early destruction of tryptophane residues of apolipoprotein B is a vitamin E independent process during copper-mediated oxidation of LDL. *Biochim. Biophys. Acta* **1995**, *1256*, 221−232.

(17) Kögl, C.; Schneider, W.; Elstner, E. F. The influence of magnesium-pyridoxal-5′-phosphate-glutamate in comparison with probucol, α-tocopherol, and trolox on copper-induced oxidation of human low-density lipoprotein. *Biochem. Pharmacol.* **1994**, *47*, 2201−2206.

(18) Von Kruedener, S.; Schempp, H.; Elstner, E. F. Gas chromatographic differentiation between myeloperoxidase activity and Fenton-type oxidants. *Free Rad. Biol. Med.* **1995**, *19*, 141−146.

(19) Hippeli, S.; Elstner, E. F. OH-radical type reactive oxygen species: A short review on the mechanisms of OH-radical- and peroxynitrite toxicity. *Z. Naturforsch.* **1997**, *52c*, 555−563.

(20) Schempp, H.; Weiser, D.; Elstner, E. F. Biochemical model reactions indicative of inflammatory processes − Activities of extracts from Fraxinus excelsior and Populus tremula. *Drug Res.* **2000**, *50*, 362−372.

(21) Esterbauer, H.; Striegl, G.; Puhl, H.; Rotheneder, M. Continuous monitoring of in vitro oxidation of human low-density lipoprotein. *Free Rad. Res. Comm.* **1989**, *6*, 67−75.

(22) Hippeli, S.; Elstner, E. F. Inhibition of Biochemical Model Reactions for Inflammatory Processes by Plant Extracts: A Review on Recent Developments. *Free Rad. Res.* **1999**, *31*, 81−87.

(23) Janisch, K.; Hippeli, S.; Dornisch, K.; Kern, S.; Elstner, E. F. Determination of the antioxidative potential of human plasma after supplementation with pycnogenol and whey. *Food Res. Int.* **2002**, *35*, 257−266.

(24) Mantle, D.; Anderton, J. G.; Falkous, G.; Barnes, M.; Jones, P.; Perry, E. K. Comparison of method for determination of total antioxidant status: application to analysis of medicinal plant essential oils. *Comp. Biochem. Physiol. B*. **1998**, *121*, 385−391.

(25) Fadel, H.; Marx, F.; El-Sawy, A.; El-Ghorab, A. Effect of extraction techniques on the chemical composition and antioxidant activity of *Eucalyptus camaldulensis* var. *brevirostris* leaf oils. *Z. Lebensm. Unters. Forsch. A.* **1999**, *208*, 212−216.

(26) Abdalla, A. E.; Roozen, J. P. Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. *Food Chem.* **1999**, *64*, 323−329.

(27) Zygadlo, P. J. A.; Lamarque, A. L.; Maestri, D. M.; Grosso, N. R. Use of essentail oils as natural antioxidants. *Grasas Acet.* **1995**, *46*, 285−288.

(28) Hippeli, S.; Grassmann, J.; Vollmann, R.; Elstner, E. F. Essential Oil from *Pinus mugo* − Formation and possible function of peroxides, in preparation.

(29) Beuscher, N.; Kietzmann, M.; Bien, E.; Champeroux, P. Interference of Myrtol Standardized with Inflammatory and Allergic Mediators. *Drug Res*. **1998**, *48*, 985−989.

(30) Harrewijn, P.; van Osten, A. M.; Piron, P. G. M. *Natural terpenoids as Messengers. A multidisciplinary study of their production*, *biological functions*, *and practical applications*; Kluwer Academic Publishers: Dordrecht, Boston, London, 2001.

(31) Hammer, K. A.; Carson, C. F.; Riley, T. V. Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Microbiol.* **1999**, *86*, 985−990.

(32) Lis-Balchin, M.; Deans, S. G.; Eaglesham, E. Relationship between bioactivity and chemical composition of commercial essential oils. *Flav. Frag. J.* **1998**, *13*, 98−104.

(33) Smith-Palmer, A.; Stewart, J.; Fyfe, L. Antimicrobial properties of plant essential oils and essences against five important foodborne pathogens. *Lett. Appl. Micorbiol.* **1998**, *26*, 118−122.

(34) Albrecht-Goepfert, E.; Schempp, H.; Elstner, E. F. Modulation of the Production of Reactive Oxygen Species by Preactivated Neutrophils by Aminoadamantane Derivatives. *Biochem. Pharmacol.* **1998**, *56*, 141−152.

(35) Parthasarathy, S.; Santanam, N.; Ramachandran, S.; Meilhac, O. Oxidants and antioxidants: an appraisal. *J. Lipid Res.* **1999**, *40*, 2143−2157.

(36) Bordia, A.; Arora, S. K.; Kothari, L. K.; Jain, K. C.; Rathore, B. S.; Rathore, A. S.; Dube, M. K.; Bhu, N. The protective action of essential oils of onion and garlic in cholesterol-fed rabbits. *Atherosclerosis* **1975**, *22*, 103−109.

(37) Bordia, A.; Verma, S. K.; Vyas, A. K.; Khabya, B. L.; Rathore, A. S.; Bhu, N.; Bedi, N. K. Effect of essential oils of onion and garlic on experimental atherosclerosis in rabbits. *Atherosclerosis* **1977**, *26*, 379−386.

(38) Patel, R. P.; Darley-Usmar, V. M. Molecular mechanisms of the copper dependent oxidation of low-density lipoprotein. *Free Rad. Res.* **1999**, *30*, 1−9.

(39) Foti, M. C.; Ingold, K. U. Mechanism of Inhibition of Lipid Peroxidation by gamma-Terpinene, an Unusual and Potentially Useful Hydrocarbon Antioxidant. *J. Agric. Food Chem.* **2003**, *51*, 2758−65.

Received for review July 11, 2003. Revised manuscript received September 12, 2003. Accepted September 14, 2003.

JF030496E