Scavenger Properties of Synthetic Naphthenic Methyl Esters

Dubravka Štajner[a], Vera Ćirin-Novta[b], Aleksandra Pavlović[a]

[a] Faculty of Agriculture
[b] Institute of Chemistry, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Yugoslavia

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Oxygen Radical Scavengers, Synthetic Naphthenic Methyl Esters

To study the scavenger properties four naphthenic methyl esters were synthesized. The abilities of the compounds to act as radical scavengers were investigated in an experimental epinephrine/adrenochrome model using enzymic and non-enzymic systems. We found that our particular compounds exhibited radical scavenger activity and superoxide-dismutase activity inhibition. Investigated compounds also showed physiological activity. In the auxin test one compound induced elongation of the wheat coleoptile by 35%.

Introduction

Evidence has accumulated indicating that a disturbance of the balance between oxidative stress and antioxidant defense mechanisms could provoke a number of different diseases (Gey 1994; Matkovics *et al.*, 1997; Štajner *et al.*, 1997). Various kinds of natural and synthetic substances such as flavonoids, phenyl butenone polyamines and different plant extracts have received much attention as chain-breaking antioxidants which protect aerobic organisms from oxidative stress (Cotelle *et al.*, 1992; Varga *et al.*, 1993; Gebhardt, 1997).

The plant flavonoids, well-known antioxidants, catechin, quercetin, myricetin, rutin etc. exhibit the basic structure of 2-phenylbenzopyrane though numerous natural flavonoids are polyhydroxy flavones and are called flavones. Very reactive antioxidants are flavan -3-ols and their derivatives (Hsu *et al.*, 1993). Other synthetic non-flavonoids substances as styryl ketones also act as a good scavengers of oxygen radicals (Saldanha *et al.*, 1990). They are known to produce stable antioxidant radicals which are not toxic and break the reaction chain of lipid peroxidation. Oxygen radicals can disappear by several mechanisms including selfreaction of radicals or by chemical reduction to water, and to other non-toxic compounds. On the other hand chemical substances such as nitroxanthrone, triiodothyronine, lindane, etc. decreased antioxidant activity by producing superoxide and hydrogen peroxide (Escobar *et al.*, 1996).

Our previous results showed that naphthenic acids which are widely used in the industry of dyes and lacquer, leather processing, in fertilizer production exhibit high physiological activities as plant hormones both of gibberellin and auxin type (Ćirin-Novta *et al.*, 1995), but their scavenger activities are still under scrutiny.

Therefore the aim of this work was to develop new agents with scavenger properties and to study their structure-activity relationship. For that purpose 4 new naphthenic methyl esters were synthesized and their scavenger activity was studied.

Materials and Methods

Naphthenic methyl esters were synthesized as reported previously (Ćirin-Novta *et al.*, 1992). The structure and purity was tested by quantitative IR spectrometric analysis. IR spectra were recorded on a Perkin Elmer 457 IR spectrophotometer and mass spectra were obtained using Hewlett-Packard MS D HP 5790 system for GS-MS analysis. Low resolution mass spectra were obtained on Varian MAT-311A instrument under conditions of chemical ionization (Table I).

For testing oxygen radicals scavenger activities the compounds 1–4 were dissolved in dimethyl sulfoxide (DMSO) (Jacks and Hinojosa 1993) and applied in concentrations of 3×10^{-3}, 6×10^{-3} and 9×10^{-3} mol/l.

Reprint requests to Prof. D. Štajner.
Fax: 0038 12155662.
E-mail: stajnerd@polj.ns.ac.yu

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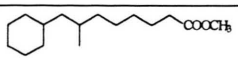
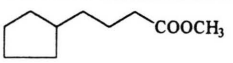
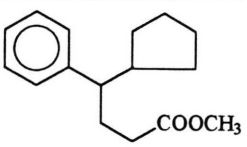
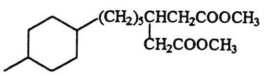
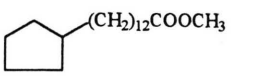
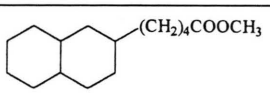
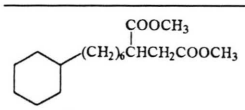
Table I. Structures of methyl esters of main components of some narrow fractions of synthetic naphthenic acids determined by GC-MS analysis.

Compound	Structure and name of ester	Fragment ion	Main series of fragment ions	% in Sample
1	 methyl-7-methyl-8-cyclohexyloctanoate (254)	$[C_nH_{2n-1}]^+$ $[C_nH_{2n+1}]^+$ $[C_nH_{2n}]^+$ $[(CH_2)_nCOOCH_3]^+$	41, 55, 69, 83, 97, 111, 125, 139 43, 57, 71, 85, 99, 113 70, 84, 98, 112 59, 74, 87, 101, 115, 129	80–82
2	 methyl-4-cyclopentyl-butanoate (170)	$[C_nH_{2n-1}]^+$ $[C_nH_{2n+1}]^+$ $[(CH_2)_nCOOCH_3]^+$	41, 55, 69, 83, 97, 111 43 32, 59, 74, 88, 101	60
	 methyl-4-phenyl-4-cyclo-pentylbutanoate (246)	$[C_nH_{2n-1}]^+$ $[(CH_2)_nCOOCH_3]^+$ $[M-C_5H_9]^+$	41, 55, 69 59, 74, 86 39, 49, 50, 52, 63, 65, 77, 91, 101, 101, 105, 118, 177	20
3	 methyl-3-methoxycarbonyl--8-(methyl)cyclohexyl-octanoate (326)	$[C_nH_{2n-1}]^+$ $[C_nH_{2n+1}]^+$ $[CH(CH_2)_2(COOCH_3)_2]^+$	41, 55, 69, 83, 97 71, 85 32, 59, 74, 87, 100	60
	 methyl-13-cyclopentyl-tridecanoate (294)	$[C_nH_{2n-1}]^+$ $[C_nH_{2n+1}]^+$ $[(CH_2)_nCOOCH_3]^+$	41, 55, 69, 83, 97, 111 43, 57, 71, 85, 127 59, 74, 87, 101, 115, 143, 157	30
4	 methyl-5-perhydronaphthyl-pentanoate (252)	$[C_nH_{2n-1}]^+$ $[C_nH_{2n+1}]^+$ $[C_nH_{2n+3}]^+$ $[(CH_2)_nCOOCH_3]^+$	43, 57 41, 55, 69 39, 67, 81, 95, 109 59, 74, 87, 101	75
	 3-methoxycarbonyl-9--cyclohexylnonanoate (366)	$[C_nH_{2n-1}]^+$ $[C_nH_{2n+1}]^+$ $[CHCH_2(COOCH_3)_2]^+$ $[CHCH_2COOCH_3]^+$	41, 55, 69, 83, 111 43, 57, 71 88, 114 59, 74, 87	25

Measurements concerning superoxide scavenger activity and adrenalin autoxidation were conducted in non-enzymic and enzymic systems. To obtain an enzymic system we used one g of soaked wheat seeds (after 48 hours). Seeds were ground with quartz sand in a cold mortar. The ground material was suspended in 5 ml 0.1 mol/l K_2HPO_4 at pH 7.0. After a 10 min long centrifugation at 4 °C and 15,000×*g* the supernatant was obtained. A Tsuchihashi solution ($CHCl_3$:ethanol 3:5 v/v) was added in the supernatant to obtain superoxide dismutase (SOD) containing wheat extract (Štajner *et al.*, 1995).

Superoxide radical scavenger activity

Superoxide radical scavenger activity was studied according to Greenwald (1985). Superoxide was generated by adrenalin and measured as quantities of generated adrenochrome and further conversion more stable products after 30 min. The reaction mixture consisted of 40 mmol/l sodium carbonate buffer (pH = 10.2) containing 0.1 mmol/l adrenalin and 0.1 mmol/l EDTA. Different amounts of test compounds dissolved in DMSO and 20 µl of SOD containing wheat extract (30 µmol protein/l) were added to a reaction mixture in order to investigate superoxide scavenger activity in a total volume of 3 ml. After incubation at 25 °C for 30 min the reaction was terminated by addition of 0.1 ml of 6 mmol/l $CuCl_2$ (Haraguchi *et al.*, 1988). The absorbance was recorded at 480 nm.

Adrenalin autoxidation

The conversion of epinephrine to adrenochrome was performed in sodium carbonate buffer 0.05 mol/l (pH 10.2) (Misra and Fridovics, 1972; Matkovics *et al.*, 1977). The reaction was initiated by adding 100 µl epinephrine solution concentration 0.01 mol/l, in order to obtain a rate of increase in absorbance of 0.025/min at 480 nm. The rate of absorption change due to adrenochrome generation was recorded during 5 min (Greenwald, 1985) in the presence of compounds 1–4 and SOD containing wheat extract.

For biological tests of auxin and gibberellin type 10^{-7} mol/l, solutions of K-salts of naphthenic methyl esters were used. Wheat seeds, after calcium hypochlorite sterilization, were cut transversely 3 mm from the distal end; the endosperm pieces were weighed in groups of 4 and incubated for 48 hours at 30° in 24×50 mm vials containing 1 ml of test solution and 500 µg streptomycin sulfate. Three replicates of each treatment were used (Bergner *et al.*, 1982).

All measurements were made in triplicate. Statistical evaluation was performed with two-tailed Student's t-test.

Results and Discussion

Results obtained from the study of effect of naphthenic methyl esters are presented in Tables II, III and IV. For easier comparison the results are expressed as percentage of inhibition compared to the control values.

As indicated in Tables II and III, the spontaneous adrenalin autooxidation was inhibited in the presence of naphthenic methyl esters except in the presence of 9×10^{-3} mol/l compound No. 1 and 3×10^{-3} mol/l compound No. 2 (Table II), when inhibition decreased compared to the control value. The inhibition was not proportional to the applied concentrations of naphthenic methyl esters and was highest at a concentration of 6×10^{-3} mol/l of the investigated substances.

Table II. Effect of naphthenic methyl esters on spontaneous adrenalin autooxidation.

Compound No.	Concentration [mol/l]	Inhibition rate (%)
1	3×10^{-3}	31.4±10
	6×10^{-3}	11.4±7
	9×10^{-3}	−25.7±9
2	3×10^{-3}	−2.7±0.5
	6×10^{-3}	27.0±5
	9×10^{-3}	32.4±8
3	3×10^{-3}	8.1±2
	6×10^{-3}	29.7±11
	9×10^{-3}	5.4±2
4	3×10^{-3}	8.3±2
	6×10^{-3}	71.5±11
	9×10^{-3}	45.0±10

Control: 0%.

Compounds 1–4 in the presence of wheat SOD exhibited a marked inhibitory effect (Table III). Compounds 1 and 2 were active and inhibited autooxidation between 35.5% and 64.3%. They had

inhibitory effect even in concentration of 9×10^{-3} mol/l (compound 1) and 3×10^{-3} mol/l (compound 2). Compound 1 was most active at concentration of 6×10^{-3} mol/l. Therefore it must be envisaged that inhibition of adrenalin autooxidation in the concentrations mentioned was influenced by the action of plant SOD. Compound 3 was more active as a scavenger in the presence of plant SOD though compound 4 was active only in a concentration of 3×10^{-3} mol/l. In other concentrations it was less effective than in the non-enzymic system.

Superoxide scavenging activities of investigated compounds are presented in Table IV. These compounds showed appreciable scavenging activity which increased (compounds 1 and 2) or decreased (compounds 2 and 3) in the presence of plant SOD. Compound 1 is the most potent superoxide scavenger in the presence of plant SOD although scavenger activity of compounds 3 and 4 decreased in the of presence of plant SOD. Scavenger activity of compound 2 was smaller than of compound 1.

Table III. Effect of petroleum methyl esters on adrenalin autooxidation in the presence of wheat SOD.

Compound No.	Concentration [mol/l]	Inhibition rate (%)
1	3×10^{-3}	35.5±5
	6×10^{-3}	50.0±7
	9×10^{-3}	47.6±5
2	3×10^{-3}	45.2±6
	6×10^{-3}	45.2±6
	9×10^{-3}	64.2±8
3	3×10^{-3}	40.0±7
	6×10^{-3}	37.5±4
	9×10^{-3}	17.5±3
4	3×10^{-3}	25.0±3
	6×10^{-3}	15.0±2
	9×10^{-3}	25.0±4

Control: 0%.

Our results showed that the investigated synthetic naphthenic methyl esters act as radical scavengers. We propose that they act by a mechanism similar to that of vitamin E. They donate a hydrogen atom to a peroxy or alkoxy radical interfering with the propagation of lipid peroxidation. The formation of a stable radical insufficiently reactive to abstract further hydrogen atom breaks the reaction chain of lipid peroxidation.

Table IV. Superoxide scavenger activities of synthetic naphthenic methyl esters.

Compound [6×10^{-3} mol/l]	Scavenger activity (%)	Scavenger activity in the presence of wheat SOD (%)
1	70.5±11	93.4±15
2	34.4±14	68. ±12
3	97.0±11	82.0±7
4	60.7±8	13.4±2

Control: 0% Control rate (= 100%) was 18.3 μmol/mg Protein×min.

Compound 1 was a potent radical scavenger also in the presence of plant SOD which induces superoxide generation. During the scavenger process compound 1 forms secondary stable radical by abstracting an H atom from the tertiary carbon atom.

Physiological investigations showed that compound 1 in the auxin test induced elongation of wheat coleoptile by 35% compared to the control what proves it as highly-active substance.

The findings listed above provide a piece of evidence of high antioxidant and physiological activity of our synthetic compounds especially of compound 1. Nevertheless, the significance of these results must be verified by toxicity and *in vivo* tests to establish whether these new synthetic compounds are useful scavengers of toxic oxygen radicals.

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