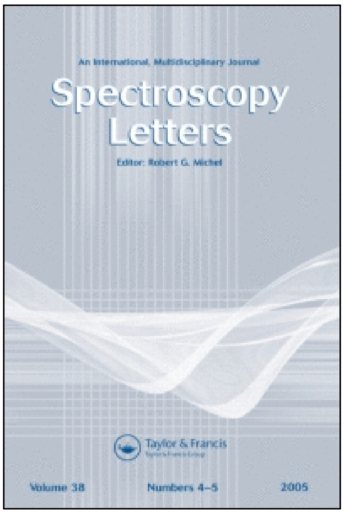

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A SINGLET OXYGEN QUENCHER IN PLANTS: VIRGATIC ACID FROM *SALVIA* SPECIES

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Abstract - Virgatic acid triterpene, extracted from *salvia* plants, is found to be quenched by singlet oxygen at chemical and photosensitized oxygenations. A charge-transfer energy transfer is attributed to the quenching of singlet oxygen, which may be accounted as a protective mechanism of *salvia* plants to photooxygenations.

INTRODUCTION

Singlet oxygen is known to add to olefinic bonds readily and the dioxetane, hydroperoxide adducts on decomposition could form the new olefinic structures with oxygen functional groups of hydroxy and/or keto[1] Destructive ability of singlet oxygen on organic structures has been balanced in living species by singlet oxygen quenchers, such as β-carotene. Singlet oxygen is converted to triplet oxygen by an energy transfer quenching mechanism of β-carotene. Foote[2] had shown that singlet oxygen produced from

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hypchloride-hydrogen peroxide in chlorophyll containing plants, is quenched by β-carotene, which results in *cis-trans* isomerization.

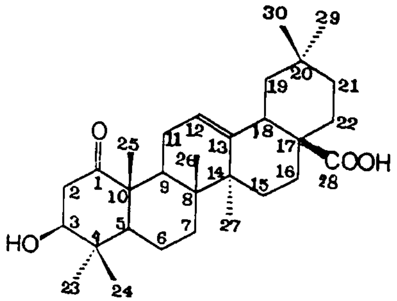
$${}^1O_2 + Q^{car} \longrightarrow {}^3O_2 + {}^3Q^{car}$$

A second mechanism is charge-transfer (C-T) quenching. Electron poor singlet oxygen forms a charge transfer complex with an electron donor. Intersystem crossings are relaxed in the complex, which then dissociates to donor and ground state oxygen[1].

$$D + {}^1O_2 \rightleftarrows [D^{\cdot}\cdots\cdots O_2^{\cdot}]^1 \rightleftarrows [D^{+}\cdots\cdots O_2^{\cdot}]^3 \rightleftarrows D + {}^3O_2$$

Amines, phenols, sulfides, some dyes and metal complexes are known to quench singlet oxygen by one of the above mechanisms. All the other unsaturated structures are known to give oxygenated products on photooxidations.

Olefinic groups in terpenoid structures react readily with singlet oxygen to form oxygenated products, such as the diterpenes of levopimaric[3], palustric[4] and abietic acids[5]. Singlet oxygen quenching is not been reported for these structures. The initial aim was to study the oxygenated products of virgatic acid extracted from *salvia virgata* and *salvia pinnata*[6]. Virgatic acid is a triterpene acid from *salvia* species. Thus it is considered that the oxygenated products on singlet oxygen reaction may give light to its presence as a major triterpene component in *Salvia* plants. The *Salvia* plants are used for tea consumption and are commercially used in Turkey.



One expects ene and/or dioxetane reactions of singlet oxygen on the double bond of above triterpene ring.

EXPERIMENTAL

a. Chemical Oxidation[7]

Virgatic acid (47 mg, 0.10 mmole) was dissolved in 10 ml 0.05 % sodium hydroxide and 30 ml of methanol was added. 243 mg of sodium molybdate (1.0 mmole) was dissolved in the same mixture. The pH of the solution was adjusted to be 9.0-9.5. Hydrogen peroxide (35 %) was added in 0.5 ml portions. Following each addition a dark brown colour appeared and the colour was turned into pale yellow after 10 minutes of stirring. The addition was repeated eight times and the reaction was followed on tlc. No product formation was observed. The solvents were evaporated under vacuum, after the final addition. The mass, proton and carbon-13 nmr analysis have revealed the existence of only the reactant, virgatic acid. The reaction was repeated and the solution was stirred for 2 hours with addition of 50 mg of thiourea prior to evaporation of solvents, in order to reduce the peroxides to alcohol, if formed. The spectral analysis had shown again only the presence of virgatic acid.

The abietic acid has been completely converted to oxygenated products after three times addition of hydrogen peroxide under similar conditions, as followed on tlc and on mass spectrometry analysis and in comparison to earlier studies on abietic acid[5b].

b. Photochemical Oxidations

with perylene 3,4,9,10-tetracarboxylic acid-bis- N,N'-phenyl diimide photosensitizer[5b]; 47 mg (0.10 mmole) of virgatic acid and 1 mg (0.003 mmole) perylene bisphenyldiimide was dissolved in 30 ml acetonitrile. The mixture was photolized for 12 hours in a pyrex vessel with an oxygen inlet and inner water cooling system, in a cylindirical photoreactor equipped with 120 W Phillips 254 nm mercury lamps. The solvent was distilled under vacuum. The ir, mass, proton and carbon-13 nmr spectral analysis have shown the existence of only the reactant. ^{1}H nmr ($CDCl_3$), δ ppm: 0.68 (3H, s, Me-26), 0.70 (3H, s, Me-25), 0.84 (6H, s, Me-24 and Me-29), 0.86 (3H, s, Me-30), 0.91 (3H, s, Me-23), 1.06 (3H, s, Me-27), 2.75 (1H, dd, J=4.0 Hz and J=10.2 Hz, H-18), 3.15 (1H, dd, J=5.8 Hz and 9.5 Hz, H-3), 5.21 (1H, t, J=3.1 Hz, H-12). ^{13}C-APT nmr, see table 1. The proton and carbon-13 nmr assignments were completed with the analysis of HETCOR spectrum (Fig. 1). Ir ν_{max} cm^{-1}: 3440 (O-H), 2940 and 2870 (C-H), 1700 (C=O). [M^+] at m/e 470, $C_{30}H_{46}O_4$ (required [M^+] m/e 470.693).

The abietic diterpene acid is being converted to photooxidized products in quantitave yield under similar conditions, as reported[5b].

with eosin photosensitizer; The above reaction was repeated in methanol with eosin photosensitizer for 18 hours under irradiation with 96 W Philips black light lamps at radiation maxima of 365 nm. The spectral analysis has shown the presence of only unreacted virgatic acid.

Thiazolidinethiones are reported to be photooxidized in high yield under similar conditions[8].

with triphenylporhine (TPP) photosensitizer[9]; 410 mg (5 mmole) cis,trans-2,4-hexadiene and 2 mg tetraphenylporhirine (TPP) was dissolved in 50 ml carbontetrachloride. The oxygen purged solution was photolized with Tesla 400 W sodium lamp for 0.5 h. The proton and carbon-13 nmr analysis have shown a complete conversion to *cis* and *trans* 3-hexene-2,5-endoperoxides at a ratio of 1/3.5, respectively. ^{1}H nmr ($CDCl_3$), δ ppm: 1.18 (6H, d, J=6.5 Hz, Me-*cis*), 1.28 (6H, d, J=6.5 Hz, Me-*trans*), 4.59 (2H, q, J=6.4 Hz, CH-O-*trans*), 4.74 (2H, q, J=6.4 Hz, CH-O-*cis*), 5.79 (2H, s, =CH-*cis*), 5.83 (2H, s, =CH-*trans*). ^{13}C nmr ($CDCl_3$), δ ppm: 17.58 (Me-*cis*), 18.50 (Me-*trans*), 73.82 (C-O-*cis*), 74.18 (C-O-*trans*), 128.68 (=C-*trans*), 128.89 (=C-*cis*). Virgatic acid, 24 mg (0.05 mmole) and 1mg TPP was dissolved in 10 ml $CDCl_3$. Mixture was irradiated under similar conditions. The solution was immediately analysed at proton and carbon-13 nmr. Spectral analysis has shown the presence of only virgatic acid. No product or intermediate formations were detected at tlc and nmr analysis.

c. Instruments

Proton and carbon-13 nmr spectra were recorded with a Bruker AC 200 L 200 MHz NMR spectrometer, mass spectra were taken with a VG Zabspec GC-MS system. A Perkin Elmer 983 IR spectrophotometer was used for ir spectroscopy, samples in KBr.

d. Materials

Virgatic acid was extracted from *Salvia* plants in Turkey and purified by preparative tlc[6]. Perylene 3,4,9,10-tetracarboxylic acid bis N,N'-phenyldiimide was synthesized as described. TPP was obtained from the laboratory of Professor W. Adam. *Cis,trans*-2,4-hexadiene was supplied from Fluka at purum grade. Solvents, methanol and CCl_4 were double distilled and gc analysis were carried out for purity. Acetonitrile and $CDCl_3$ were supplied from Fluka at purum grade.

RESULTS AND DISCUSSION

Virgatic acid is extracted from *Salvia* plants in Turkey, as one of the major triterpene structure[6]. The presence of a double bond in the structure could cause the formation of oxygenated products on reaction with singlet oxygen. But no product formation was detected in spite of the vigorous reactions with singlet oxygen as described below, with three different photosensitizers of perylene bis-N,N'-phenyl diimide, eosin and TPP on photooxygenations and on chemical oxygenations[7]. The oxygenated products were detected easily with abietic diterpene acid[5], thiazolidinethione[8] and hexadiene under these conditions, through ene and/or dioxetane reactions. Hexadiene, in presence of TPP photosensitizer, has formed the analogous *cis* and *trans* endoperoxide mixture readily, but virgatic acid has not yielded any products (Fig. 1 and Table 1). Linker[9] has shown ene reaction to hydroperoxide formation in 1,4-cyclohexadiene with a carboxyl moiety at 3-position on photooxygenation. Chemical method for production of singlet oxygen yielded also the unperturbed virgatic acid, where under similar conditions abietic acid has been shown to oxidize quantitatively to numerous products as followed on tlc. Unreactivity of virgatic acid with singlet oxygen, proves the stability under atmospheric conditions and may explain the existence of virgatic acid as a dominant triterpene in plants.

Above results may point that the singlet oxygen has been quenched prior to addition reaction with virgatic acid. One may expect an energy transfer process and quenching of singlet oxygen to triplet oxygen as in β-Carotenes. The energy transfer mechanism, such as in carotenes, should result in products through a triplet virgatic acid quencher. It is evident that this mechanism is unlikely to occur. But a charge-transfer complex formation between electron poor singlet oxygen and unsaturated groups of virgatic acid is a more possible approach. Singlet oxygen is expected to lie between two carbonyl groups and the olefinic bond, in the C-T complex. Energy transfer in the complex, could result in formation of triplet oxygen and the unreacted virgatic acid. The presence of a keto group at 1-position is attributed to the formation of this favorable C-T complex.

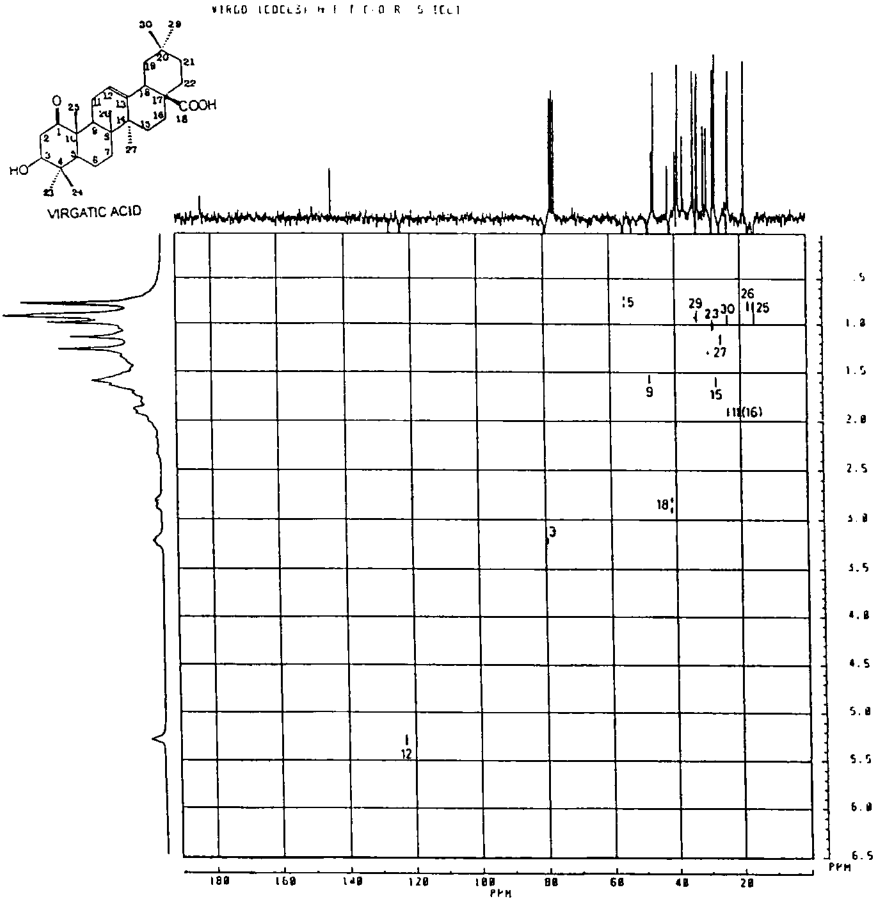
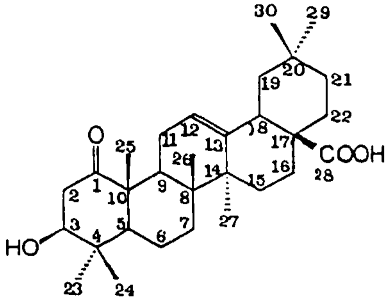


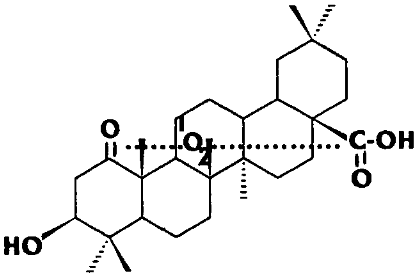
Figure 1. HETCOR (two dimentional) nmr spectrum of virgatic acid in $CDCl_3$.

Table 1. Carbon-13 nmr (APT) data of virgatic acid in $CDCl_3$, δ in ppm.



Carbon	δ	Carbon	δ
1	198.8	16	23.4*
2	27.1	17	46.5
3	79.1	18	41.0
4	37.1	19	45.9
5	55.2	20	30.7
6	18.3	21	33.8
7	32.4	22	32.9
8	38.7	23	28.1
9	47.6	24	15.3
10	41.6	25	15.6
11	22.9*	26	17.0
12	122.6	27	25.9
13	143.6	28	183.9
14	39.3	29	33.1
15	27.6	30	23.6

*Assignments are interchangeable.



This protective mechanism may explain the unusual photostability of virgatic acid and its dominance in *salvia* plants under natural conditions.

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