Is DPPH Stable Free Radical a Good Scavenger for Oxygen Active Species?

P. IONITA

Institute of Physical Chemistry, Bucharest, Romania
University of York, Chemistry Department, YO10 5DD, UK
e-mail: pionita@chimfiz.icf.ro

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The reaction of 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) with active species of oxygen (hydrogen peroxide, *tert*-butyl peroxide, *tert*-butyl hydroperoxide, sodium peroxide, potassium superoxide, hydroxyl radical, tempo free radical, galvinoxyl free radical, Fremy's salt, *m*-chloroperbenzoic acid, potassium hydroxide, and sodium peroxynitrite) was reviewed and the new obtained results compared with previous literature data. It was shown that in all of these reactions the corresponding hydrazine DPPH-H (2,2-diphenyl-1-picrylhydrazine) is the major product, besides, in most of reactions, the nitro derivative of DPPH-H. The formation of the nitro derivative involves the previous formation of nitrogen dioxide, which can appear in the reaction mixture in two ways: *via* a radical attack on picryl moiety which releases the nitrogen dioxide or *via* a nucleophilic substitution of a nitro group, which releases the nitrite anion, which is subsequently oxidized to the nitrogen dioxide. Nitrogen dioxide reacts easily with DPPH to form the corresponding nitro derivative of DPPH-H. The scavenging activity of DPPH in terms of oxygen active species, which should yield oxy derivatives of DPPH, is very poor, only traces of these could be found.

In 1922, G*oldschmidt* and *Renn* [1] discovered the violet-coloured free stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), which now is used as ESR standard [2] and as colorimetric reagent [3, 4] for redox processes. Because DPPH can be kept indefinitely with little decomposition and because it neither dimerizes nor reacts with oxygen [5], it proved to be quite useful in a variety of investigations, such as polymerization inhibition or radical chemistry [6], the determination of antioxidant properties of amines, phenols or natural compounds (vitamins, plant extracts, medicinal drugs) and for inhibiting homolytic reactions [7—9]. DPPH is intensely violet like $KMnO_4$ and its reduced counterpart 2,2-diphenyl-1-picrylhydrazine (DPPH-H) is orange-yellow [6—9].

DPPH is insoluble in water; many other hydrophobic related hydrazyls were synthesized [10—19]. In order to confer hydrophilic properties, carboxy or sulfonic groups were attached to DPPH [20, 21]. These compounds could be employed for studying the species which are formed in water radiolysis [22].

Recent work demonstrated the usefulness of DPPH and its derivatives in studying interphasic processes assisted by transport agents such as crown ethers (CEs) or kryptands [23]. The species formed by these agents are supramolecular complexes and behave as stoichiometric compounds [24—26].

The stable free radical DPPH is well known as a good hydrogen abstractor yielding DPPH-H as by-product [27]. This is a redox type process and was first mentioned by *Goldschmidt* and *Renn* in DPPH oxidation of hydroquinone to benzoquinone [1]. More generally, the process was proved to be a homolytical breaking by the DPPH of a H-X bond (X = C, O, N, S, Cl, Br) like in hydrocarbons, alcohols, phenols, thiols, amines, enols, hydroxylamines, *N*-alkoxynitroanilines, hydracids [28—37].

The DPPH radical can participate in homolytic additions with other radicals species $R^{\bullet}$, yielding in many instances R-DPPH-H-substituted derivatives. It was also shown that DPPH-H anion ($DPPH^-$) can be oxidized to the DPPH radical. Some aspects of DPPH chemistry, such as its instability in alkaline media, the improvement of hydrogen abstractions from hydracids in the presence of salts $M^+ X^-$, and the reaction between DPPH and the *N*-methoxy-2,4,6-trinitroaniline anion yielding quantitatively the anion $DPPH^-$ and the aminyl radical of *N*-methoxy-2,4,6-trinitroaniline, suggested the electron-abstractor character of DPPH from some anionic species A^- [38, 39].

Active oxygen species such as hydrogen peroxide, hydroxyl radical, and superoxide anion radical, are readily generated in many cells by metabolic processes such as respiration, ischemia/reperfusion, and oxidation of fatty acids, and they are highly toxic to cells by damaging such components as DNA, lipids, and enzymes. Cells can be injured, and even killed under the most serious conditions, when the content of active

oxygen species exceeds the cellular antioxidant capacity. DPPH was used as indicator for measuring the antioxidant capacity in human plasma [40].

DPPH was used as scavenger for many other radicals, due to the easiness in following of this process – the violet colour of DPPH faints into the yellow colour of its reduced congener (DPPH-H), with a high λ-shift in the visible spectra (from 520 nm to 330 nm).

In this paper it is shown that although DPPH reacts with oxygen active species, these are not scavenged by it, the disappearance of DPPH follows a different way, mainly not the one in which DPPH couples with another radical (oxygen-centred).

EXPERIMENTAL

All the substances and the solvents used were purchased from Aldrich or Chimopar. Sodium peroxynitrite was synthesized as follows: an ice-cold solution of 10 cm^3 of 0.1 M-hydrogen peroxide in 0.3 M-sulfuric acid was rapidly mixed with 10 cm^3 of an ice-cold solution of 0.1 M-sodium nitrite and the resulting mixture was added as fast as possible to an ice-cold solution of 10 cm^3 of 0.1 M-sodium hydroxide; the yellow resulting solution can be kept several weeks at $-18\,°C$ with low decomposition. The concentration of the peroxynitrite anion can be measured by UV VIS, at λ_{max} = 300 nm ($\varepsilon = 1670$).

An UV VIS Specord M40 spectrophotometer was used for measuring the concentration, and an ESR Jeol-Jes FA 100 spectrometer for detecting the radicals using spin-trapping techniques.

The concentration of the compounds was evaluated in two ways: *a*) by UV VIS spectroscopy (DPPH λ_{max} = 520 nm; DPPH-H λ_{max} = 330 nm; NO_2-DPPH-H λ_{max} = 355 nm; HO-DPPH-H λ_{max} = 302 nm), and *b*) by isolation of the desired compound from the reaction mixture (by preparative TLC, stationary phase: silica gel, eluent: chloroform).

Reaction of DPPH with Hydroxyl Radical

To a solution of 10^{-2} M-DPPH in dichloromethane (DCM) an equal volume of 10^{-1} M-aqueous solution of hydrogen peroxide was added and the resulting biphasic system was stirred for about 24 h, adding in the first 6 h, from time to time, small amounts of ferrous sulfate, in order to produce the hydroxyl radical (see eqn (*A*)). The organic layer was separated, dried over anhydrous sulfate and the species formed were detected by UV VIS and TLC.

Reaction of DPPH with Fremy's Salt

To a solution of 10^{-2} M-DPPH in DCM an equal volume of 10^{-1} M-aqueous solution of Fremy's salt was added and the resulting biphasic system was stirred for about 24 h. The organic layer was separated, dried over anhydrous sulfate and the species formed were detected as previously.

Reaction of DPPH with Tempo or Galvinoxyl Free Radicals

To a solution of 10^{-2} M-DPPH in DCM a solution of tempo or galvinoxyl free radical in DCM was added and the mixture was stirred for about 24 h at room temperature. The mole ratios between DPPH and tempo or galvinoxyl were 1 : 1, 1: 5, and 1: 10, in all cases no reaction occurred.

Reaction of DPPH with Anions

The reactions with hydroxyl, peroxide, superoxide, and peroxynitrite anions were performed in a biphasic solid—liquid system, in the presence of 18-crown-6 as phase-transfer agent. The mixture was worked up by washing the organic layer with water and aqueous diluted hydrochloric acid, then dried over anhydrous sodium sulfate and the compounds formed were isolated by preparative TLC.

Reaction of DPPH with Peroxides

The reactions with hydrogen peroxide, *tert*-butyl peroxide, *tert*-butyl hydroperoxide, and *m*-chloroperbenzoic acid were performed in two ways: in monophasic system, using THF as solvent, or in a biphasic liquid—liquid system (DCM—water). The species formed were identified in the organic layer as described.

Reactions in the Presence of *tert*-Butylphenylnitrone

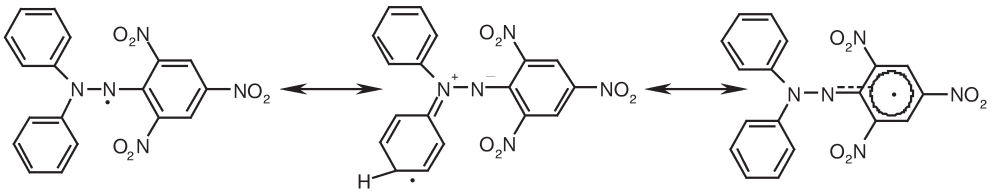
For the reaction systems studied in the presence of BPN as spin trapper, the only difference was the presence of BPN in a concentration of 10^{-2} mol dm^{-3}; after the reaction, the mixture was analyzed by ESR.

RESULTS AND DISCUSSION

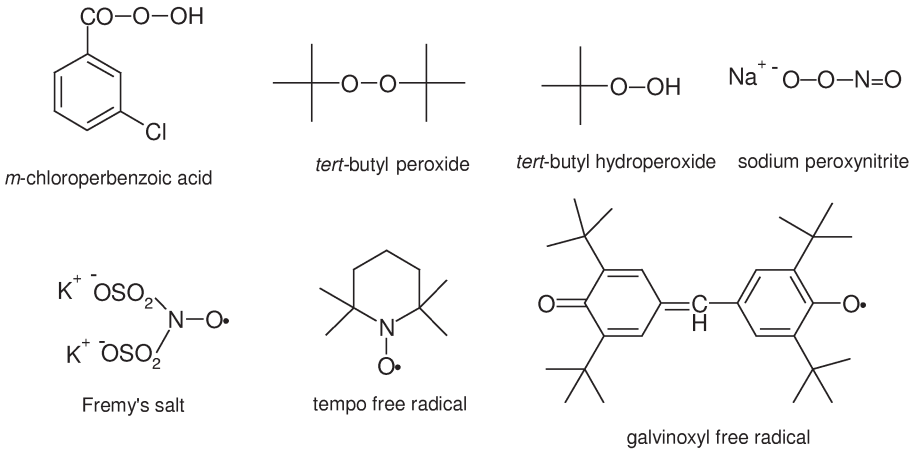
It is well known that the DPPH ability to capture free radicals is due to the delocalization of the unpaired electron all over the molecule. Scheme 1 shows the structure of DPPH.

Looking at this structure it is expected that DPPH can react with another free radical in several different ways: *i*) by coupling to the nitrogen-centred radical; *ii*) by coupling in the *para*-position on the phenyl ring, and *iii*) coupling somewhere on the picryl moiety. Experimental data available till now show that the main centre for a radical + radical type reaction is in the phenyl *para*-position [26, 38, 39].

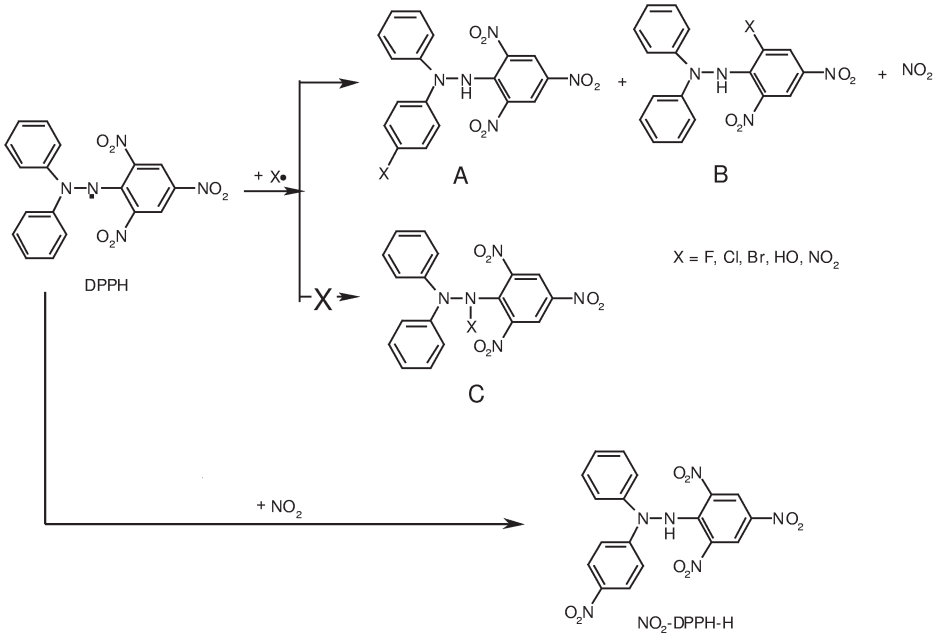
A glance on the literature has shown that some data regarding the reactions of DPPH [41—44] with



Scheme 1. The structure of the free stable radical DPPH.



Formula 1. Oxygen active compounds used.



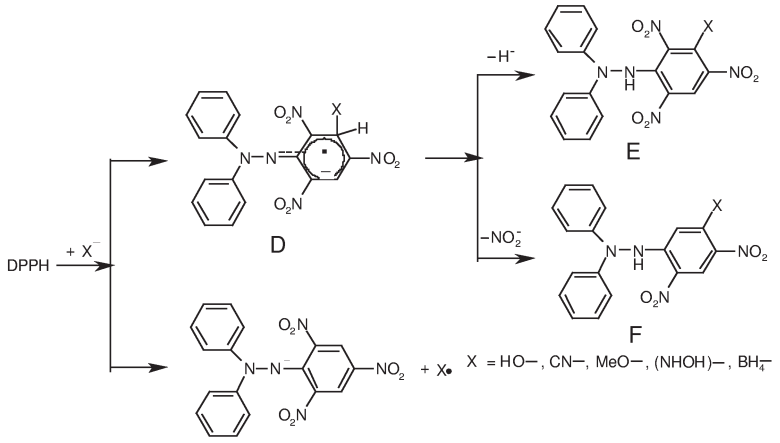
Scheme 2. The reaction of DPPH with a free radical X$^{\bullet}$ (the main reaction product is NO_2-DPPH-H).

potassium hydroxide, hydroxyl radical, and some peroxides are available, but there is very little information regarding the reaction products and/or the reaction with other free stable radicals, such as Fremy's salt (potassium nitrosodisulfonate), tempo or galvinoxyl free radical, and peroxynitrite anion (Formula 1).

Reactions of DPPH with oxygen-centred radicals (hydroxyl radical, tempo free radical, Fremy's salt, or galvinoxyl free radical) were studied. The reaction of DPPH with fluorine, chlorine, bromine, hydroxyl radical, and nitric dioxide is shown in the literature [44], but sometimes inconclusive (or wrong) results are presented. For example, it was claimed that *N*-hydroxy adduct (compound of type C, Scheme 2) is

Table 1. Product Isolated from the Reaction of DPPH with the Oxygen Active Species

Reactant	DPPH	DPPH-H	NO_2-DPPH-H	HO-DPPH-H
Hydroxyl radical	70	10	5	Traces
Tempo free radical	100	0	0	0
Fremy's salt	95	0	Traces	0
Galvinoxyl free radical	100	0	0	0
Sodium peroxide	5	55	33	0
Potassium superoxide	5	60	25	Traces
Potassium hydroxide	5	25	12	Traces
Sodium peroxynitrite	5	30	55	0
Hydrogen peroxide	0	100	0	0
tert-Butyl peroxide	85	0	9	0
tert-Butyl hydroperoxide	10	30	40	Traces
m-Chloroperbenzoic acid	20	40	17	Traces



Scheme 3. The reaction of DPPH with an anion X^-.

formed from the reaction of DPPH with NO_2 or with *tert*-butyl hydroperoxide, but later papers show that in fact the *p*-phenylhydroxy adduct or *p*-nitrophenyl adduct is formed (compounds of type A, Scheme 2). Our experiments show the same behaviour regarding the reaction of DPPH with hydroxyl radical (made *via* Fenton reaction, eqn (*A*)).

$$H_2O_2 + Fe^{2+} \longrightarrow {}^{\bullet}OH + HO^- + Fe^{3+} \qquad (A)$$

As it is shown in Table 1, the reaction of DPPH with $HO^{\bullet}$ is the only one which affords the hydroxy-derivative of DPPH (only in traces), as well as the nitroderivative (around 5 %). Although the formation of the *p*-hydroxyphenyl derivative is expected to be the main route of the reaction, the major by-product is in fact the NO_2-DPPH-H, which is formed *via* a radical *ipso*-substitution (formation of the compound of type B, Scheme 2) which releases NO_2 that reacts with the DPPH in the known way (radical + radical coupling reaction). The *ipso*-substitution on picryl ring was undoubtedly proved by ^{15}N-labeling of nitro group [45].

It can be concluded that DPPH is not a good scavenger for hydroxyl radical. Regarding the reaction of DPPH with Fremy's salt, tempo and galvinoxyl free radicals, no reaction occurs, as shown in Table 1.

Reaction of DPPH with hydroxide anion, peroxide anion, superoxide anion, and peroxynitrite anion is another one studied. From the beginning it is worth to resume some previous results obtained in such reactions: DPPH reacts with potassium hydroxide in a biphasic system leading mainly to the corresponding hydrazine DPPH-H and the nitro derivative NO_2-DPPH-H; the reaction with superoxide anion led to almost the same results, while the reactions with peroxide anion and peroxynitrite anion have not been studied till now [26, 41]. Peroxynitrite anion is an important oxygen active compound which can be formed *in vivo* by the reaction of nitric oxide with superoxide anion (eqn (*B*)).

$$NO + O_2^{-\bullet} \longrightarrow O{=}N{-}O{-}O^{-\bullet} \qquad (B)$$

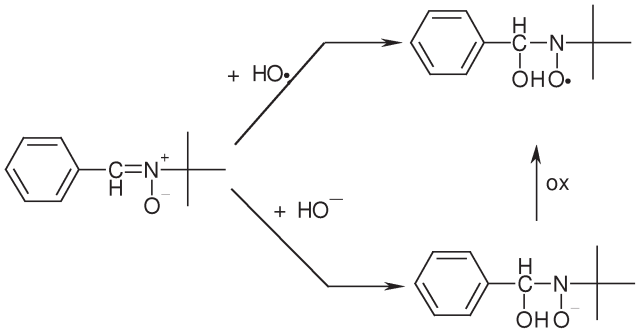
There are two ways in which DPPH can react with an anion (Scheme 3): *i*) the anion acts as a nucleophile and makes a Meinseinhaimer complex (compound of type D), which decomposes after that by losing a hydride anion or a nitrite anion, leading to the final compounds (E and F), or *ii*) the DPPH radical is strong enough to abstract one electron from the anion and

to oxidize it to the short-lived radical X$^{\bullet}$, which reacts with DPPH in the same way shown in Scheme 2, yielding also finally the nitro derivative of DPPH [46—50].

The reaction of DPPH with sodium peroxide or peroxynitrite follows the same trend: the major by-product is the nitro derivative, while the hydroxyl derivative appears only in traces or not at all (see Table 1).

DPPH reacts with hydrogen peroxide, *tert*-butyl hydroperoxide, *tert*-butyl peroxide, and *m*-chloroperbenzoic acid. At the reaction with hydrogen peroxide only the corresponding reduced hydrazine DPPH-H is formed in a quantitative yield; no other compound was found. If the reaction is conducted in an aqueous basic solution (by adding potassium hydroxide in order to form the peroxide anion), the same result was obtained. The route of the formation of DPPH-H is not clear at this stage: if it is a radical reaction, the hydroperoxy radical should be formed (HOO$^{\bullet}$), and for this it is expected that an *ipso*-radical attack on picryl ring occurs, but experimentally no derivative of DPPH was observed; if it is a nucleophilic attack, also it is expected to yield a derivative of DPPH, again it was not found. In the reaction with *tert*-butyl peroxide or hydroperoxide the major by-product is the nitro derivative NO_2-DPPH-H and traces of the hydroxylated compound HO-DPPH-H. *tert*-Butyl hydroperoxide is more active, forming 40 % of the nitro derivative and only 10 % of the starting material was recovered (Table 1). Approximately the same result was obtained in the reaction with *m*-chloroperbenzoic acid, yielding 17 % of the nitro derivative and traces of HO-DPPH-H. It can be assumed that for these reactions a radical process occurs (Scheme 2).

In order to find the short-lived radicals involved in these processes, the reactions were conducted in the presence of a spin-trapping agent, namely *N*-*tert*-butylphenylnitrone (BPN). This compound is extensively used as a scavenger for unstable radicals, including those ones which contain the radical centre at the oxygen atom. In all the processes in which the nitro derivative NO_2-DPPH-H was found, using the ESR spin-trapping technique, two kinds of spin-adducts were found: the hydroxy adduct of BPN (Ph—CHOH—N(O$^{\bullet}$)—CMe_3) and the oxidized derivative of it (Ph—CO—N(O$^{\bullet}$)—CMe_3), which is well known to appear by oxidation of the former or by nitrogen dioxide capture. Regarding the formation of the hydroxy spin-adduct of BPN, this is not an exclusive tool to demonstrate the formation of hydroxyl radical, under certain conditions it can be obtained without any implication of a short-lived radical (Scheme 4). The EPR parameters a_N = 1.33 mT and $a_{H(\beta)}$ = 0.19 mT measured on the ESR spectra confirm a nitroxide radical and are the same as those from literature [51] and as the parameters obtained in the reaction between Fenton reagent (the common source



Scheme 4. Formation of the hydroxy spin-adduct of BPN *via* an authentic capture of HO$^{\bullet}$ (top) or *via* a nucleophilic addition followed by an oxidative process (bottom).

for hydroxyl radicals) and BPN. Using the same scavenger as for the identification of hydroxyl radical, NO_2 has been trapped. The EPR parameter (a_N = 0.79 mT) of this species is identical to those mentioned in literature [51].

The question which arises now is how we can distinguish between a radical process or a nucleophilic substitution in which the DPPH reactant is consumed. One way is to compare the redox potential of the hydrazyl radical with the redox potential of the substrate: if the oxidative potential of DPPH is higher, it can be expected that DPPH will oxidize the substrate *via* one electron abstraction yielding the corresponding short-lived radical, which usually undergoes the reactions shown in Scheme 2. The main problem of this test is the lack of the data regarding these redox potentials (which should be measured in the same experimental conditions). If the oxidation potential of DPPH cannot pass the redox potential of the substrate, there are two possibilities: first, no reaction occurs, and second, a nucleoplilic attack on the picryl ring can be involved, which leads to the reaction routes shown in Scheme 3. In all the cases in which a reaction occurs the nitro derivative of DPPH appears as reaction product, so the presence or absence of this compound does not bring any supplementary information, as well as the use of a spin-trap to evaluate the formation of short-lived radicals in these processes.

CONCLUSION

DPPH free stable radical is not a good scavenger for oxygen active species.

REFERENCES

1. Goldschmidt, S. and Renn, K., *Ber. 55,* 628 (1922).
2. Chen, M. M., Sane, K. V., Walter, R. I., and Weil, J. A., *J. Phys. Chem. 65*, 713 (1961).
3. Papariello, G. J. and Janish, M. A. N., *Anal. Chem. 37*, 899 (1965).

4. Hazell, J. E. and Russel, K. E., *Can. J. Chem. 36*, 1729 (1958).
5. Poirier, R. H., Kahler, E. J., and Benington, F., *J. Org. Chem. 17*, 1437 (1952).
6. Fargere, T., Abdennadher, M., Delmas, M., and Boutevin, B., *Eur. Polym. J. 31*, 489 (1995).
7. Glavind, J. and Halmer, G., *J. Am. Oil Chem. Soc. 44*, 539 (1967).
8. Emaru, K., Askal, H., and Saleh, G., *Talanta 38*, 1219 (1991).
9. Akitame, M. and Hajime, H., *Neurosciences 16*, 83 (1990).
10. Chen, M. M., D'Adamo, A. F., and Walter, R. I., *J. Org. Chem. 26*, 2721 (1961).
11. Balaban, A. T., Frangopol, P. T., Marculescu, M., and Bally, I., *Tetrahedron 13*, 258 (1961).
12. Balaban, A. T., Marculescu, M., Pascaru, I., Rotaru, M., Valerin, A., and Weiner, M., *Z. Phys. Chem.* (Leipzig) *219*, 285 (1964).
13. Balaban, A. T. and Istratoiu, I., *Tetrahedron Lett. 1973*, 1879.
14. Negoita, N., Baican, R., and Balaban, A. T., *Tetrahedron 30*, 73 (1974).
15. Balaban, A. T., Caproiu, M. T., Negoita, N., and Baican, R., *Tetrahedron Lett. 1974*, 4091.
16. Balaban, A. T., Caproiu, M. T., Negoita, N., and Baican, R., *Tetrahedron 33*, 2249 (1977).
17. Baican, R., Negoita, N., and Balaban, A. T., *Rev. Roum. Phys. 21*, 213 (1976).
18. Balaban, A. T., Caproiu, M. T., and Negoita, N., *Rev. Roum. Chim. 25*, 1327 (1980).
19. Caproiu, M. T., Elian, M., Grecu, N., Negoita, N., and Balaban, A. T., *J. Chem. Soc., Perkin Trans. 2 1983,* 591.
20. Matevosyan, R. O. and Ikrina, M. A., *Zh. Obshch. Khim. 33*, 499 (1963).
21. Putirszkaja, G. V. and Siladi, T., *Acta Chim. Hung. 72*, 329 (1972).
22. Putirszkaja, G. V., Chernova, A. I., and Matus, L., *Acta Chim. Acad. Sci. Hung. 21*, 289 (1959).
23. Luca, C., Ionita, P., and Constantinescu, T., *Rev. Roum. Chim. 42*, 985 (1997).
24. Webe, E., Toner, J. L., Goldberg, I., Vogtle, F., Laider, D. A., Stoddart, J. F., Bartsch, R. A., and Liotta, C. L., *Crown Ethers and Analogs.* (Patai, S. and Rappoport, Z., Editors.) Wiley, New York, 1989.
25. Ionita, G., Constantinescu, T., and Ionita, P., *J. Planar Chromatogr. Modern TLC 11*, 142 (1998).
26. Luca, C., Ionita, P., Caproiu, M. T., Caldararu, H., and Constantinescu, T., *Rev. Roum. Chim. 43*, 129 (1998).
27. Forester, A. R., Hay, J. M., and Thomson, R. H., *Organic Chemistry of Free Radicals.* Academic Press, London, 1968.
28. Bergstrom, C. and Lagercrantz, L., *Acta Chem. Scand. 18*, 560 (1964).
29. Rao, A. M. and Sing, C. V., *Biochim. Biophys. Acta 71*, 134 (1982).
30. Hazell, J. E. and Russel, K. E., *Can. J. Chem. 36*, 1729 (1958).
31. McGovan, J. C., Powell, T., and Raw, R., *J. Chem. Soc. 1959*, 3103.
32. Russel, K. E., *J. Phys. Chem. 58*, 437 (1954).
33. Flood, J. and Russel, K. E., *Can. J. Chem. 53*, 1123 (1975).
34. Glavind, J., *Acta Chem. Scand. 17*, 1635 (1963).
35. Brook, A. G., Anderson, R. J., and Palot, J. T., *Can. J. Chem. 36*, 159 (1958).
36. Stanciuc, G., Caproiu, M. T., Caldararu, H., Caragheorgheopol, A., Constantinescu, T., and Balaban, A. T., *Z. Naturforsch. 446*, 1459 (1989).
37. Solomon, D. H. and Swift, J. D., *J. Polym. Sci.(A) 3*, 3107 (1965).
38. Covaci, I. C., Constantinescu, T., Caproiu, M. T., Caldararu, H., Ionita, P., and Balaban, A. T., *Pol. J. Chem. 75*, 1427 (2001).
39. Ionita, P., Caproiu, M. T., Meghea, A., Maior, O., Rovinaru, M., and Ionita, G., *Pol. J. Chem. 73*, 1177 (1999).
40. Yukowa, O. and Nakajima, T., *Int. J. Radiation Biol. 75*, 1189 (1999).
41. Hristea, E., Hillebrand, M., Caproiu, M. T., Caldararu, H., Constantinescu, T., and Balaban, A. T., *Arkivoc (ii)* 123 (2002).
42. Ionita, P., *South. Braz. J. Chem. 6*, 101 (1998).
43. Ionita, P., Rovinaru, M., and Maior, O., *South. Braz. J. Chem. 6*, 59 (1998).
44. Gille, L., Prosch, U., and Stoesser, R., *Radiat. Phys. Chem. 40*, 461 (1992).
45. Caproiu, M. T., Covaci, I. C., Ionita, P., Luca, C., Constantinescu, T., and Balaban, A. T., *J. Nucl. Radioanal. Chem. 242*, 773 (1999).
46. Ionita, P., Gilbert, B. C., and Whitwood, A. C., *J. Chem. Soc., Perkin Trans. 2 2001*, 1453.
47. Ionita, P., Gilbert, B. C., and Whitwood, A. C., *J. Chem. Soc., Perkin Trans. 2 2000*, 2436.
48. Ionita, P., Constantinescu, T., Luca, C., Caldararu, H., Caproiu, M. T., and Balaban, A. T., *New J. Chem. 21*, 511 (1997).
49. Hristea, E., Radutiu, C., Constantinescu, T., Ionita, P., Caproiu, M. T., Hillebrand, M., and Balaban, A. T., *Rev. Roum. Chim. 45*, 1089 (2000).
50. Ionita, P., Caproiu, M. T., and Draghici, C., *Rev. Roum. Chim. 46*, 803 (2001).
51. Buettner, G. R., *Free Radical Biol. Med. 3*, 259 (1987).