

This article was downloaded by:

On: 30 January 2011

Access details: Access Details: Free Access

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Spectroscopy Letters

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597299>

A STUDY OF CHEMILUMINESCENCE FROM REACTIONS OF PEROXYOXALATE ESTERS, HYDROGEN PEROXIDE, AND 7-AMINO-4-TRIFLUOROMETHYLCUMARIN

Mojtaba Shamsipur^a; Mohammad Javad Chaichi^b

^a Department of Chemistry, Razi University, Kermanshah, Iran ^b Department of Chemistry, Tarbiat Moallem University, Tehran, Iran

Online publication date: 31 July 2001

To cite this Article Shamsipur, Mojtaba and Chaichi, Mohammad Javad(2001) 'A STUDY OF CHEMILUMINESCENCE FROM REACTIONS OF PEROXYOXALATE ESTERS, HYDROGEN PEROXIDE, AND 7-AMINO-4-TRIFLUOROMETHYLCUMARIN', *Spectroscopy Letters*, 34: 4, 459 — 468

To link to this Article: DOI: 10.1081/SL-100105093

URL: <http://dx.doi.org/10.1081/SL-100105093>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

**A STUDY OF CHEMILUMINESCENCE
FROM REACTIONS OF
PEROXYOXALATE ESTERS,
HYDROGEN PEROXIDE, AND 7-AMINO-
4-TRIFLUOROMETHYLCUMARIN**

Mojtaba Shamsipur^{1,*} and Mohammad Javad Chaichi²

¹Department of Chemistry, Razi University,
Kermanshah, Iran

²Department of Chemistry, Tarbiat Moallem
University, Tehran, Iran

ABSTRACT

The chemiluminescence arising from the reaction of bis(2,4,6-trichlorophenyl)oxalate (TCPO) and bis(2,4-dinitrophenyl)oxalate (DNPO) with hydrogen peroxide in the presence of 7-amino-4-trifluoromethylcumarin (ATFMC) have been studied. The influences of stirring solution, nature of peroxyoxalate and solvent properties on the resulting chemiluminescence were investigated. The relationship between the chemiluminescence intensity and concentrations of TCPO and ATFMC are reported.

*Corresponding author.

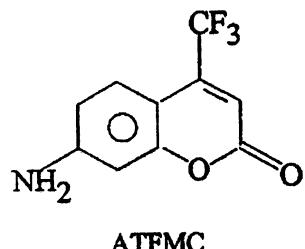
Key Words: Chemiluminescence; 7-Amino-4-trifluoromethyl-cumarin; Peroxyoxalate; H₂O₂

INTRODUCTION

The phenomenon of chemiluminescence is one of the most fascinating demonstrations of chemical energy¹. The brilliant emission resulting from the reaction of some oxalic acid derivatives is one of the most outstanding examples of chemiluminescence^{2,3}. This group of chemiluminescent reactions has been classified as the peroxyoxalate chemiluminescence (PO-CL) reactions. The PO-CL reactions have been reported as one of the most sensitive analytical tools to detect a wide range of fluorescent compounds^{4,5}. These are based on the reaction of hydrogen peroxide with an activated oxalate which result in the formation of one or more energy-rich intermediate(s). These intermediates are capable of exciting a large number of fluorophores^{1,4-7} through the chemically initiated electron exchange luminescence mechanism⁸.

The choice of activated oxalate for the PO-CL reactions has been the subject of numerous efforts⁹⁻¹⁴. On the basis of the results obtained, there are at least two characteristics necessary in the design of useful reagents for the PO-CL. First, the existence of electron-withdrawing groups around the central peroxyoxalate moiety which facilitate the generation of reactive intermediates responsible for the transfer of the excitation energy to a fluorophore compound^{13,14}. Second, the sufficient solubility of the leaving groups in the solvent for which the reagent was designed. Detailed discussion of the possible mechanisms of the PO-CL reactions are frequently reported and reviewed in the literature¹⁵⁻¹⁹. The results have led to a preferential use of bis(2,4,6-trichlorophenyl) oxalate (TCPO), bis(2,4-dinitrophenyl) oxalate (DNPO) and bis[2-(3,6,9-trioxa-decyloxycarbonyl)-4-nitrophenyl] oxalate (TDPO) in analytical detection systems.

Cumarin derivatives are widely distributed in the plant kingdom, some of them being physiologically active²⁰. Antimicrobial properties of some cumarins have been investigated^{21,22}. Many cumarin derivatives are of great practical interest²⁰. They are widely used as optical brighteners and dyes for natural and synthetic materials as well as in lasers²³. 7-Amino-4-trifluoromethylcoumarin (ATFMC) is used in the synthesis of a substrate for the fluorometric assay of proteolytic enzymes in biological fluids²⁴ and for use as a laser dye²⁵ and as a marker for proteinase²⁶. In this paper we report the first study of chemiluminescence from reactions of peroxyoxalate esters, hydrogen peroxide and ATFMC.



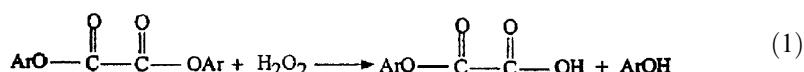
EXPERIMENTAL

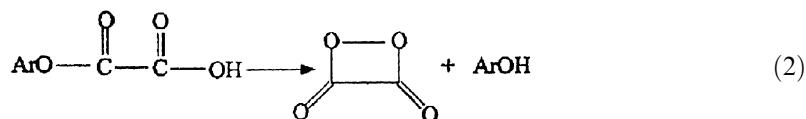
All chemicals were of the reagent-grade from Fluka chemical company and used as received. TCPO and DNPO were prepared from the reaction of 2,4,6-trichlorophenol and 2,4-dinitrophenol, respectively, with oxalyl chloride in the presence of triethylamine as described previously³. Hydrogen peroxide (30%) was concentrated via freeze drying (using a model FD-1 Fyela freeze dryer) up to 60%, mixed with dimethyl phthalate in a 1:1 v/v portions and shaked well on an electrical shaker. After 10 h, the organic phase was separated, dried on anhydrous Na₂SO₄ and the H₂O₂ concentration was determined by a standard potassium permanganate solution. Then a standard stock solution of hydrogen peroxide (1.5 M in 80:20 v:v dimethyl phthalate: tert-butyl alcohol containing 5.0 × 10⁻³ M sodium salicylate) was prepared from this solution. The stock solutions of TCPO, DNPO and ATFMC (0.01 M) were also prepared in ethyl acetate and methanol solutions.

Chemiluminescence detection was performed with a homemade apparatus equipped with a model BPY47 photocell (Leybold, Huerth, Germany). The apparatus was connected to a personal computer via a suitable interface (Micropars, Tehran, Iran). Experiments were carried out with magnetic stirring in flattened bottom glass cells of 15 mm diameter at room temperature.

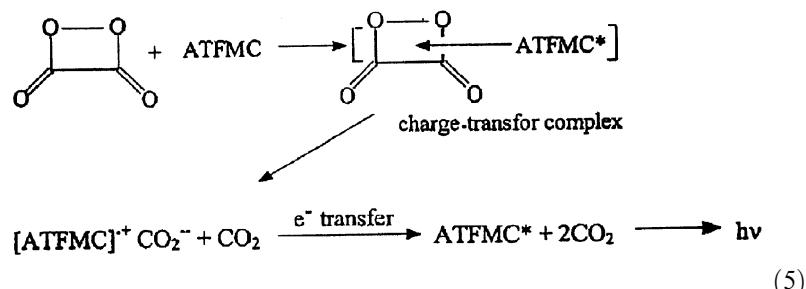
RESULTS AND DISCUSSION

Peroxyoxalate chemiluminescence (PO-CL) is one of the most efficient non-biological light producing systems. The most frequently proposed mechanism for the PO-CL process involves the following steps^{1,3,16,27}:





Excitation of the fluorescer ATFMC, step (3), most probably occurs via the chemically initiated electron exchange luminescence pathway (CLEEL) as follows^{27,28}:



In preliminary experiments, it was found that the addition of a few ml of the stock solution of hydrogen peroxide to a nearly colorless ethyl acetate solution containing 1.0×10^{-3} M ATFMC and 0.01 M TCPO results in a very intense blue light.

Upon the use of alcohol, instead of ethyl acetate, the light intensity was greatly reduced and the color shifted to green. It is worthy to note that, in the absence of sodium salicylate as a base, the emission of light was relatively long-lived but the time taken to reach maximum emission and the duration of the emission were not reproducible. However, in the presence of the base, reproducible emission intensity decay curves were obtained.

Typical intensity decay curves for the chemiluminescence of varying concentrations of ATFMC in the presence of fixed amounts of TCPO and H_2O_2 are shown in Fig. 1. As seen, the peak intensity increases sharply with increasing concentration of ATFMC as a suitable fluorescer. In all cases, the light intensity increases rapidly after mixing and reaches a maximum in $t_m = 8-10$ s. Whereas, the decay of light intensity from the maximum occurs at longer periods of time (up to 90 s) via an exponential process. The arbitrary

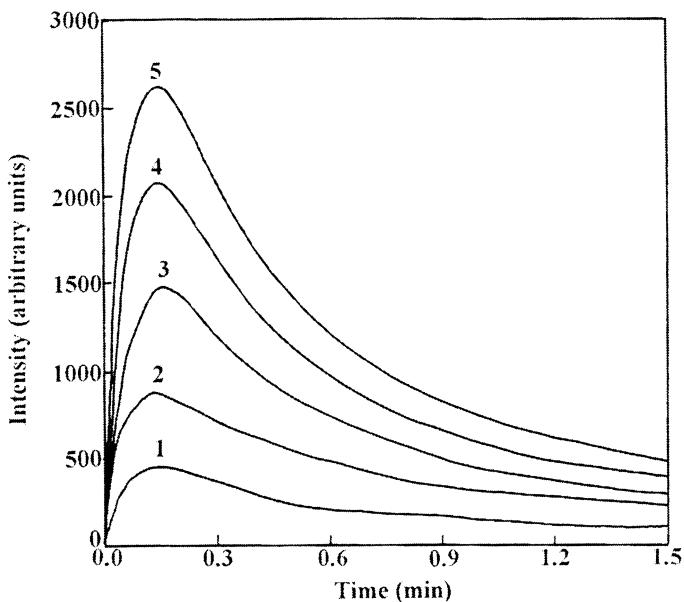


Figure 1. Emission intensity as a function of time for reaction of TCPO (5.0×10^{-3} M) with H_2O_2 (0.14 M) in ethyl acetate at various concentrations of ATFMC: (1) 5.0×10^{-4} M, (2) 1.0×10^{-3} M, (3) 2.0×10^{-3} M, (4) 5.0×10^{-3} M, (5) 1.0×10^{-2} M.

quantity $t_{1/2}$ (during which the luminescence light intensity decays to 1/2 of its maximum value) was chosen to permit the comparison of emission lifetimes (see Tables 1 and 2). It is interesting to note that the spectral distribution of chemiluminescence was found to essentially resemble the

Table 1. Effect of Stirring Sample Solution on t_m and I of Chemiluminescence of TCPO- H_2O_2 -ATFMC System in Ethyl Acetate

ATFMC (M)	Stirred		Unstirred	
	t_m (s)	I	t_m (s)	I
5.0×10^{-4}	8	493	29	275
1.0×10^{-3}	8	893	20	633
2.0×10^{-3}	9	1499	18	1223
5.0×10^{-3}	8	2084	17	1359
1.0×10^{-2}	9	2636	13	1791

Table 2. Effect of the Nature of Oxalate Ester on t_m , I and $t_{1/2}$ of Chemiluminescence of Various Concentrations of ATFMC

ATFMC Concentration (M)	TCPO			DNPO		
	t_m (s)	I	$t_{1/2}$ (s)	t_m (s)	I	$t_{1/2}$ (s)
5.0×10^{-4}	8	493	30	< 5	90	< 3
1.0×10^{-3}	8	893	38	< 5	93	5
2.0×10^{-3}	9	1499	36	< 5	118	6
5.0×10^{-3}	8	2084	31	< 5	120	< 5
1.0×10^{-2}	9	2636	32	< 5	123	< 5

Table 3. Effect of Solvent on I and k_L of TCPO-H₂O₂-ATFMC System at Various Concentrations of TCPO

TCPO (M)	Ethyl Acetate		Methanol	
	I	k_L (min)	I	k_L (min)
3.0×10^{-4}	38	0	—	—
5.0×10^{-4}	76	0.33	17	—
1.0×10^{-3}	257	0.86	21	—
3.0×10^{-3}	765	1.22	45	—
5.0×10^{-3}	1554	1.48	116	0.15
8.0×10^{-3}	2515	1.64	251	0.17

fluorescence spectrum of ATFMC, emphasizing that the first singlet excited state of the fluorescer is the chemiluminescent emitter.

The influence of stirring sample solution (at a rate of 500 rpm) on the time required to reach maximum intensity, t_m , and the chemiluminescence light intensity, I, for TCPO-H₂O₂-ATFMC system was investigated and the results are given in Table 1. As is obvious, stirring the sample solution resulted in a pronounced decrease in t_m value and, at the same time, a considerable enhancement in the chemiluminescence light intensity, at entire concentration range of the fluorescer studied.

In Table 2, the chemiluminescence characteristics of TCPO-H₂O₂-ATFMC and DNPO-H₂O₂-ATFMC systems in ethyl acetate solution are compared. It is well known that the nature of the leaving groups of the oxalic ester has a profound effect on the efficiency of the PO-CL systems. Diaryl esters of oxalic acid are reported to be among the most efficient substrates¹⁻¹⁹.

However, the nature of the substituents on the phenyl ring greatly affects the overall efficiency of the chemiluminescent systems^{1,2,6,10,12,29}.

As it is obvious from Table 2, the use of TCPO resulted in a great enhancement in chemiluminescence of the cumarin derivative used over that of DNPO. The observed trend could be due not only to the different electron-withdrawing effects of the chloro and nitro groups on TCPO and DNPO, respectively, but also to the influence of the amino group of ATFMC on the PO-CL system. It is noteworthy that such influence of the chemical structure of the fluorophores⁶ and the catalytic effects of nitrogen containing heterocyclic compounds^{30,31} on the peroxyoxalate chemiluminescence reactions are fully investigated.

The influence of solvent properties on the chemiluminescence of TCPO-H₂O₂-ATFMC system was investigated by using ethyl acetate and methanol as solvent and the results are summarized in Table 3. It should be noted that, in Table 3, k_L is the rate constant for exponential decay of the chemiluminescence intensity of the system. Obviously, the efficiency of the PO-CL system studied in ethyl acetate is much higher than that in methanol solution. It is well known that, in the CIEEL mechanism, the influence of

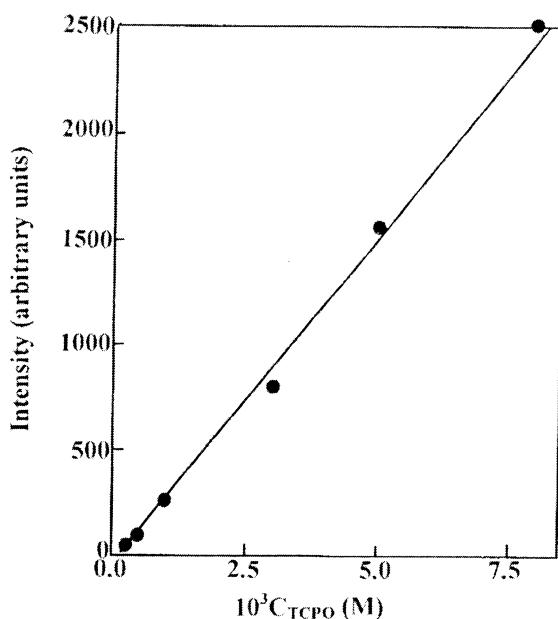


Figure 2. Effect of TCPO concentration on the chemiluminescence intensity of TCPO-H₂O₂-ATFMC system in ethyl acetate solution.

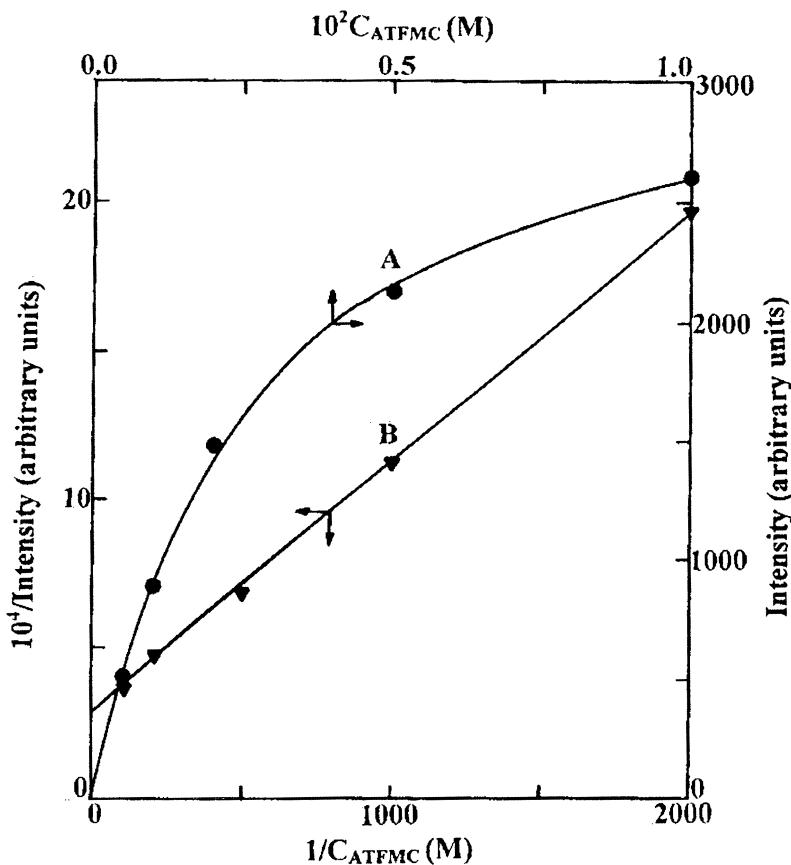


Figure 3. Effect of ATFMC concentration on the chemiluminescence intensity of TCPO-H₂O₂-ATFMC system in ethyl acetate solution: (A) I vs. C_{ATFMC} , (B) I^{-1} vs. C_{ATFMC}^{-1} .

different solvents on the chemiluminescence intensity depends on their ability to stabilize the intermediate charge-transfer complex, in equation (5), within a solvent cage^{27,32}. The viscous non-polar solvents such as ethyl acetate (as compared with methanol) are usually the best solvents for this purpose.

The influence of varying TCPO concentration in the presence of excess amounts of hydrogen peroxide was investigated and the results are given in Table 3 and Fig. 2. As it is seen from Fig. 2, there is a nice linear correlation between the chemiluminescence intensity and the TCPO concentration.

The basis for such linear correlation has already been discussed in detail in the literature¹⁵.

The effect of ATFMC concentration, at constant amount of TCPO, was studied and the results are shown in Table 2 and Fig. 3(A) and 3(B). As it has been clearly shown before³³, there is an exponential increase in chemiluminescence of the TCPO-H₂O₂-ATFMC system with increasing concentration of the fluorescer ATFMC (Fig. 3A). However, the reciprocal plots of chemiluminescence intensity against fluorescer concentration was found to result in a linear calibration plot, the slope and intercept of which were both dependent upon the initial TCPO concentration³³.

REFERENCES

1. Burr, J.G. *Chemi- and Bioluminescence*; Marcell Dekker Inc.: New York, 1985.
2. Rauhut, M.M. *Acc. Chem. Res.* **1969**, *2*, 80.
3. Mohan, A.G.; Turro, N.J. *J. Chem. Educ.* **1974**, *51*, 528.
4. Bowie, A.R.; Sanders, M.G.; Worsefold, P.J. *J. Biolumin. Chemilumin.* **1996**, *11*, 61.
5. Kwakman, P.J.M.; Brinkman, U.A.Th. *Anal. Chim. Acta* **1992**, *266*, 175.
6. Honda, K.; Miyaguchi, K.; Imai, K. *Anal. Chim. Acta* **1985**, *177*, 111.
7. Katayama, M.; Takeuchi, H.; Taniguchi, H. *Anal. Chim. Acta* **1993**, *281*, 111.
8. Schuster, G.B. *Acc. Chem. Res.* **1979**, *12*, 366.
9. Rauhut, M.M.; Bollyky, L.J.; Roberts, B.G.; Loy, M.; Whiteman, R.H.; Iannotta, A.V.; Semsel, A.M.; Clark, R.A. *J. Am. Chem. Soc.* **1967**, *89*, 6515.
10. Tseng, S.-S.; Mohan, A.G.; Haines, L.G.; Vizcarra, L.S.; Rauhut, M.M. *J. Org. Chem.* **1979**, *44*, 4413.
11. Honda, K.; Miyaguchi, K.; Imai, K. *Anal. Chim. Acta* **1985**, *177*, 103.
12. Nakashima, K.; Maki, K.; Akiyama, S.; Wang, W.H.; Tsukamoto, Y.; Imai, K. *Analyst* **1989**, *114*, 1413.
13. Stevani, C.V.; de Arruda Campos, I.P.; Baader, W.J. *J. Chem. Soc., Perkin Trans.* **1996**, *2*, 1645.
14. Hohman, J.R.; Givens, R.S.; Carlson, R.G.; Orosz, G. *Tetrahedron Lett.* **1996**, *37*, 8273.
15. Catherall, C.L.R.; Palmer, T.F.; Cundall, R.B. *J. Chem. Soc., Fraday Trans.* **1984**, *2* (80), 823.
16. Orlovic, M.; Schowen, R.L.; Givens, R.S.; Alvarez, F.; Matuszewski, B.; Parekh, N. *J. Org. Chem.* **1989**, *54*, 3606.

17. Neuvonen, H. J. Chem. Soc., Perkin Trans. **1994**, 2, 89.
18. Hadd, A.G.; Birks, J.W. In *Chemical Analysis Series, Selective Detectors*; Sievers, R.E., Ed.; Wiley: New York, 1995; Vol. 131, ch. 8.
19. Orosz, G.; Givens, R.S.; Schowen, R.L. Crit. Rev. Anal. Chem. **1996**, 26, 1.
20. Meuly, W.C. *Kirk-Othmer Encyclopedia of Chemical Technology*; Wiley-Interscience: New York, 1979; Vol. 7, 196–206.
21. Jurd, L.; King, A.D.; Mihara, K. Phytochem. **1971**, 10, 2965.
22. Jurd, J.; Corse, J.; King, A.D.; Bayne, H.; Mihara, K. Phytochem. **1971**, 10, 2971.
23. Krasovitskii, B.M.; Bolotin, B.M. *Organic Luminescent Materials*; VCH: New York, 1988.
24. Zimmermann, M. Anal. Biochem. **1976**, 58.
25. Fletcher, A. Appl. Phys. **1977**, 45, 295.
26. Bissel, E.R. J. Org. Chem. **1980**, 45, 2283.
27. Campbell, A.K. *Chemiluminescence, Principles and Applications in Biology and Medicine*; Ellis Horwood: Chichester, 1988.
28. Schuster, G.B. Acc. Chem. Res. **1979**, 12, 366.
29. Maulding, D.R.; Clarke, R.A.; Roberts, B.G.; Rauhut, M.M. J. Org. Chem. **1968**, 33, 250.
30. Hadd, A.G.; Birks, J.W. J. Org. Chem. **1996**, 61, 2657.
31. Jonsson, T.; Emteborg, M.; Irgum, K. Anal. Chim. Acta **1998**, 361, 295.
32. Berg-Brennan, C.A.; Yoon, D.I.; Slone, R.V.; Kazala, A.P.; Hupp, J.T. Inorg. Chem. **1996**, 35, 2032.
33. Catherall, C.C.R.; Palmer, T.F.; Cundall, R.B. J. Chem. Soc., Faraday Trans. **1984**, 2 (80), 837.

Received March 22, 2000

Accepted February 6, 2001