Chemistry of Natural Compounds, Bioorganic, and Biomolecular Chemistry

Synthesis and physiological activity of trialkyl phosphorothioates and trialkyl phosphorodithioates containing fragments of *N*-acylated amino acids

A. E. Shipov,[a]★ G. K. Genkina,[a] O. Yu. Eremina,[b] E. I. Bakanova,[b] A. V. Khrunin,[c] S. A. Roslavtseva,[b] and T. A. Mastryukova[a]

[a]*A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 ul. Vavilova, 117813 Moscow, Russian Federation. Fax: +7 (095) 135 5085*
[b]*Research Scientific Institute of Desinfectology, Ministry of Public Health of the Russian Federation, 18 Nauchnyi proezd, 117246 Moscow, Russian Federation*
[c]*Moscow State Pedagogical University, 1 ul. Malaya Pirogovskaya, 119435 Moscow, Russian Federation*

A series of *S*-[*N*-acyl-*N*-(alkoxycarbonylalkyl)aminomethyl] *O,O*-dialkyl phosphorothioates and -dithioates were prepared by the reactions of the corresponding alkali salts of dialkyl phosphorothioates or dialkyl phosphorodithioates with esters of *N*-acyl-*N*-(chloromethyl)glycine or *N*-acyl-*N*-(chloromethyl)-β-alanine and by the reactions of dialkylphosphorothioic or dialkylphosphorodithioic acids with *N*-acylated amino acids or their esters and paraformaldehyde in the presence of gaseous HCl. Some of the resulting compounds proved to be active permethrine synergists.

Key words: trialkyl phosphorothioates, trialkyl phosphorodithioates, thiophosphorylated derivatives of amino acids, dithiophosphorylated derivatives of amino acids, permethrine synergists.

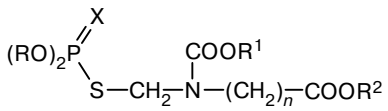
Previously, we have described the synthesis, physiological activity,[1] and the mechanism of action[2] of a series of *S*-[*N*-acyl-*N*-(alkoxycarbonylalkyl)aminomethyl] *O*-alkyl methylphosphonothioates and -dithioates among which highly active selective insectoacaricides were found. Some of these compounds also proved to be permethrine synergists,[3] which is indicative of their ability to inhibit enzymatic systems detoxifying pyrethroids. It is known[4—6] that detoxification of pyrethroids in insects occurs primarily under the action of monooxygenases (MO) and carboxyesterases (CE).

One would expect that phosphorodithioates analogous to the above-mentioned methylphosphonodithioates will be more active permethrine synergists because their ability to inhibit MO, which is typical of many phosphorothioates (for example, of *O,O*-diethyl *O*-phenyl phosphorothioate, *viz.*, SV-1[6]), is approximately identical to that of the phosphonates, whereas the abil-

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 1, pp. 141—145, January, 2001.

1066-5285/00/5001-0147 $25.00

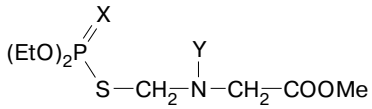
ity of the corresponding activation metabolites (phosphoromonothioates) to inhibit nonspecific esterases, including CE, is generally higher than that of phosphonates.[7] In this connection, a series of trialkyl phosphorothioates and trialkyl phosphorodithioates, which contain the glycine and β-alanine fragments with the variable alkyl substituents in the phosphate moiety of the molecule and in both alkoxycarbonyl groups (**1** and **2**), and analogous glycine derivatives with different *N*-acyl groups (**3** and **4**) were prepared and studied.



1a—r; 2a,f,g,j,k,o,q,r

1: X = S; **2**: X = O

	R	R^1	R^2	*n*		R	R^1	R^2	*n*
a	Et	Me	Me	1	**j**	Pr	Me	Me	1
b	Et	Me	Et	1	**k**	Pr^i	Me	Me	1
c	Et	Et	Me	1	**l**	Pr^i	Me	Bu^i	1
d	Et	Et	Et	1	**m**	Bu	Me	Me	1
e	Et	Me	Bu^i	1	***n***	Bu	Me	Bu^i	1
f	Et	Bu^i	Me	1	**o**	Et	Me	Me	2
g	Et	3-MeC_6H_4	Me	1	**p**	Et	Me	Et	2
h	Et	4-MeC_6H_4	Me	1	**q**	Et	Et	Et	2
i	Et	Me	H	1	**r**	Et	Bu^i	Me	2



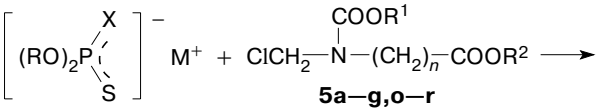
3a—c; 4a,b

3: X = S; **4**: X = O

Y = C(S)SMe (**a**), SO_2Me (**b**), SO_2NMe_2 (**c**)

Compounds **1—4**, like the analogous methylphosphonates,[1] were prepared according to two procedures, *viz.*, by the "salt" method and by the modified Mannich reaction.

According to the first procedure (procedure *A*; Eq. (1), Table 1), potassium or sodium *O,O*-dialkyl phosphorothioates (at 0—5 °C) or potassium *O,O*-dialkyl phosphorodithioates (at 20 °C) were introduced into the reactions with esters of *N*-alkoxycarbonyl-*N*-(chloromethyl)glycine or *N*-alkoxycarbonyl-*N*-(chloromethyl)-β-alanine (**5a—g,o—r**,[1] the notations of the substituents are analogous to those used for compounds **1** and **2**).

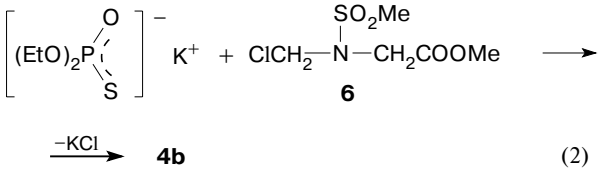


5a—g,o—r

1a—f,j—r; 2a,f,g,j,k,o,q,r (1)

R = Et, Pr, Pr^i, Bu;
X = S, O

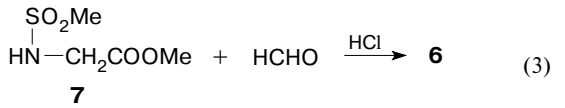
Compound **4b** was prepared analogously with the use of methyl *N*-(chloromethyl)-*N*-mesylglycinate (**6**) (Eq. (2), see Table 1).



6

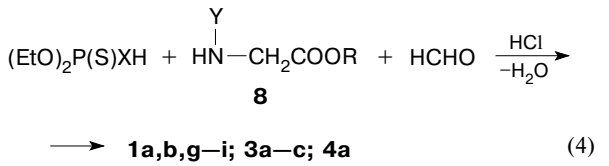
4b (2)

The corresponding chloromethyl derivative **6** was prepared by the reaction of methyl *N*-mesylglycinate (**7**)[1] with paraformaldehyde in the presence of gaseous HCl (Eq. (3), see the Experimental).



7 (3)

According to the second procedure (procedure *B*; Eq. (4), see Table 1), compounds **1**, **3**, and **4** were synthesized by the reactions of *O,O*-diethylphosphorothioic or *O,O*-diethylphosphorodithioic acids with *N*-acylglycine or its esters (**8**)[1] and paraformaldehyde in an inert solvent ($CHCl_3$ or CH_2Cl_2) in the presence of gaseous HCl.



8

1a,b,g—i; 3a—c; 4a (4)

X = S, O; R = H, Me, Et; Y = COOMe, COOEt, $COOC_6H_4Me$-3, $COOC_6H_4Me$-4, C(S)SMe, SO_2Me, SO_2NMe_2

The purities of the products were confirmed by TLC and elemental analysis. In most cases, additional purification was not needed; however, if required, the products were purified by chromatography on silica gel.

The structures of compounds **1—4** were confirmed by ^{31}P-{^1H} NMR spectroscopy. For compounds **1** and **3**, the signals are observed at δ 91—95. Compounds **2** and **4** give signals at δ 26—29. All compounds, except for **3b,c** and **4b**, are characterized by the presence of two singlets with Δδ 0.5—0.7 (for compounds **1** and **2**) or Δδ 2.5—2.8 (for compounds **3a** and **4a**), which is associated with the hindered rotation about the amide bonds in the carbamate and dithiocarbamate groups.

For phosphorothioates **1—4**, the toxicity and the synergistic activity in mixtures with permethrine (the compound : permethrine ratio was 10 : 1)[8] with respect to houseflies (*Musca domestica* L.) and males of German cockroaches (*Blattella germanica* L.) were determined. The synergistic activities were characterized by the calculated joint action coefficients (JAC)[8] (see Table 2). All compounds under study are virtually nontoxic to the above-mentioned insects (LD_{50} > 500 μg g^{-1}

Table 1. Yields and data of elemental analysis for compounds **1—4**

Compound[a]	Yield (%)	n_D^{20}	Found / Calculated (%)				Molecular formula
			C	H	P	S	
1a (*A*)[b]	92	1.5083	35.04 34.78	5.82 5.84	8.86 8.97	—	$C_{10}H_{20}NO_6PS_2$
1a (*B*)	95	1.5079	—	—	—	—	
1b (*A*)[b]	89	1.5025	—	—	8.52 8.62	18.38 17.84	$C_{11}H_{22}NO_6PS_2$
1b (*B*)	63	1.5020	—	—	—	—	
1c (*A*)	56	[c]	36.67 36.76	6.00 6.17	8.55 8.62	17.91 17.84	$C_{11}H_{22}NO_6PS_2$
1d (*A*)	94	1.4982	38.59 38.59	6.56 6.48	8.22 8.29	17.12 17.17	$C_{12}H_{24}NO_6PS_2$
1e (*A*)	37	1.4456	40.46 40.30	6.80 6.82	7.90 7.99	16.78 16.55	$C_{13}H_{26}NO_6PS_2$
1f (*A*)	58	[c]	40.50 40.30	7.20 6.82	7.39 7.99	—	$C_{13}H_{26}NO_6PS_2$
1g (*B*)	86	[c]	45.77 45.60	5.76 5.74	7.30 7.35	—	$C_{16}H_{24}NO_6PS_2$
1h (*B*)	82	[c]	45.79 45.60	5.74 5.74	7.32 7.35	—	$C_{16}H_{24}NO_6PS_2$
1i (*B*)	78	[d]	32.84 32.62	5.51 5.48	9.34 9.35	—	$C_9H_{18}NO_6PS_2$
1j (*A*)	58	1.5036	38.58 38.59	6.51 6.48	8.43 8.29	17.29 17.17	$C_{12}H_{24}NO_6PS_2$
1k (*A*)[b]	68	1.4995	38.59 38.59	6.71 6.48	8.54 8.29	17.18 17.17	$C_{12}H_{24}NO_6PS_2$
1l (*A*)	63	1.5240	43.50 43.36	7.46 7.80	7.38 7.45	15.00 15.43	$C_{15}H_{30}NO_6PS_2$
1m (*A*)	93	1.4982	41.42 41.88	7.20 7.03	7.67 7.71	—	$C_{14}H_{28}NO_6PS_2$
1n (*A*)	56	1.5301	45.36 46.03	7.79 7.73	7.09 6.98	14.47 14.46	$C_{17}H_{34}NO_6PS_2$
1o (*A*)	98	1.5094	36.68 36.76	6.06 6.17	8.69 8.62	—	$C_{11}H_{22}NO_6PS_2$
1p (*A*)	90	1.5041	—	—	8.14 8.29	—	$C_{12}H_{24}NO_6PS_2$
1q (*A*)	96	1.4994	—	—	8.02 7.99	—	$C_{13}H_{26}NO_6PS_2$
1r (*A*)	95	[e]	41.93 41.88	7.02 7.03	7.60 7.71	—	$C_{14}H_{28}NO_6PS_2$
2a (*A*)[b]	53	1.4783	—	—	9.40 9.41	—	$C_{10}H_{20}NO_7PS$
2f (*A*)	62	[e]	42.02 42.04	7.78 7.96	—	—	$C_{13}H_{26}NO_7PS$
2g (*A*)	95	[e]	47.68 47.40	5.97 5.97	7.73 7.67	—	$C_{16}H_{24}NO_7PS$
2j (*A*)	96	[e]	40.22 40.33	6.86 6.77	8.55 8.67	—	$C_{12}H_{24}NO_7PS$
2k (*A*)	95	[e]	40.58 40.33	6.78 6.77	8.52 8.67	—	$C_{12}H_{24}NO_7PS$
2o (*A*)	85	[e]	—	—	8.85 9.02	—	$C_{11}H_{22}NO_7PS$
2q (*A*)	86	[e]	—	—	8.05 8.34	—	$C_{13}H_{26}NO_7PS$
2r (*A*)[b]	70	[c]	44.02 43.63	7.50 7.32	7.61 8.04	—	$C_{14}H_{28}NO_7PS$
3a (*B*)[b]	64	[c]	32.03 31.82	5.35 5.34	8.19 8.20	—	$C_{10}H_{20}NO_4PS_4$
3b (*B*)[b]	59	[c]	—	—	—	26.04 26.32	$C_9H_{20}NO_6PS_3$

(*to be continued*)

Table 1 (*continued*)

Compound[a]	Yield (%)	n_D^{20}	Found / Calculated (%)				Molecular formula
			C	H	P	S	
3c (*B*)[b]	43	[c]	—	—	7.91 7.85	23.82 24.30	$C_{10}H_{23}N_2O_6PS_3$
4a (*B*)[b]	88	[e]	33.43 33.23	5.58 5.58	—	—	$C_{10}H_{20}NO_5PS_3$
4b (*A*)[b]	55	[e]	30.64 30.94	5.74 5.77	—	—	$C_9H_{20}NO_7PS_2$

[a] The procedure for the synthesis is given in parentheses.
[b] ^{31}P NMR, δ (CH_2Cl_2): **1a**, 93.76 and 94.49; **1b**, 93.73 and 94.44; **1k**, 90.80 and 91.53; **2a**, 26.95 and 7.57; **2r**, 27.26 and 27.79; **3a**, 92.33 and 95.12 ($CHCl_3$); **3b**, 95.07 (Me_2CO); **3c**, 96.03 (Me_2CO); **4a**, 26.25 and 28.90 ($CHCl_3$); **4b**, 27.02 (Me_2CO).
[c] A viscous oil purified by column chromatography.
[d] M.p. 65.5—66.5 °C (from an ether—hexane mixture).
[e] A viscous oil, without additional purification.

for flies and LD_{50} > 200 μg g^{-1} for cockroaches). However, most of these compounds exhibit pronounced synergistic activity with respect to permethrine. In some cases, this activity against flies is equal or somewhat higher than that of the standard, *viz.*, piperonyl butoxide (compounds **1a,d,o; 2j; 3a,b;** and **4a,b**; see Table 2). The activity against cockroaches is higher or even substantially higher than that of the standard (for example, compounds **1a,c,n; 2a,f;** and **4b**). In most cases, the synergistic activities of phosphoromonothioates **2** and **4** ("oxones" capable of inhibiting CE) against flies are lower and against cockroaches are higher than those of the corresponding phosphorodithioates **1** and **3** ("thione" compounds capable of inhibiting MO) (*cf.* the pairs of compounds **1a—2a**, **1f—2f**, **1r—2r**, and **3b—4b**). This fact confirms the suggestion made previously[8] that detoxification of pyrethroids in flies occurs primarily under the action of the enzymatic MO system, whereas detoxification in cockroaches occurs mainly under the action of CE. However, in the latter case, MOs also play a particular role in detoxification of pyrethroids because acid **1i**, which is a metabolite of hydrolytic detoxification of compound **1a**, cannot inhibit esterases even after oxidative desulfurization in insects, giving rise to the corresponding monothio analog ("oxone").[9] However, compound **1i** can synergize permethrine (JAC 1.3), probably, due to inhibition of MO.

Table 2. Characteristics of the synergistic activity of compounds **1—4** in a mixture with permethrine (10 : 1)

Compound	JAC*		Compound	JAC*	
	Houseflies	German cockroaches		Houseflies	German cockroaches
1a	2.5	4.2	**1o**	2.0	1.6
1b	1.2	1.8	**1r**	1.4	1.2
1c	1.7	2.1	**2a**	1.6	4.8
1d	1.8	1.8	**2f**	1.0	3.0
1e	1.2	1.3	**2j**	1.9	1.2
1f	1.5	1.5	**2o**	1.0	1.6
1h	0.9	1.6	**2r**	0.9	1.3
1k	1.0	1.6	**3a**	2.1	1.5
1l	1.0	1.8	**3b**	2.6	1.4
1m	1.4	1.5	**4a**	2.9	1.7
1n	0.8	2.6	**4b**	1.9	4.6
Piperonyl butoxide	2.1	1.0			

* The confidence intervals were ±0.1—0.4 (flies) and ±0.1—0.8 (cockroaches).

A change from glycine derivatives to β-alanine derivatives (*cf.* compounds **1a** and **1o**; see Table 2) has virtually no effect on the ability of compounds to synergize permethrine with respect to flies, but leads to a substantial decrease in this ability with respect to cockroaches. This is, apparently, associated with the fact that hydrolysis (detoxification) of these derivatives proceeds more readily than that of glycine derivatives under the action of CE,[2] which are more active in cockroaches than in flies. The replacement of the *N*-methoxycarbonyl group by the (methylthio)thiocarbonyl or mesyl group (a change from compound **1a** to compounds **3a,b**) has also virtually no effect on the synergistic activity of phosphorodithioates against flies, but sharply decreases this activity against cockroaches. In all cases, an increase in the size of the alkyl substituents in the phosphate moiety of the molecule or in any of the alkoxycarbonyl groups leads to a decrease in the synergistic activity of the compounds, apparently, due to the fact that their penetration through outward coats of insects proceeds more difficultly.

Experimental

The ^{31}P-{^1H} NMR spectra were recorded in solutions in CH_2Cl_2 on Bruker WP 200-SY and Bruker CXP-200 instruments operating at 81.02 MHz for ^{31}P MHz with 85% H_3PO_4 as the external standard.

Thin-layer chromatography was performed on anhydrous SiO_2 (Aldrich, 130—270 mesh) in the hexane—acetone system (4 : 1 or 3 : 2 ratio).

The compounds were purified on a column with the same sorbent (the compound : SiO_2 weight ratio was 1 : 15; gradient elution with a hexane—acetone mixture in the ratio range from 100 : 1 to 3 : 2).

Synthesis of *S*-[*N*-acyl-*N*-(alkoxycarbonylalkyl)aminomethyl] *O,O*-dialkyl phosphorothioates and -dithioates (1—4) (general procedure). ***A.*** *Compounds* **1a—f,j—r; 2a,f,g,j,k,o,q,r;** and **4b**. Chloromethyl derivative **5a—g,o—r** or **6** (25 mmol) in acetone (5 mL) was added dropwise with stirring to potassium *O,O*-dialkyl phosphorodithioate (27 mmol; at 20 °C) or sodium or potassium *O,O*-dialkyl phosphorothioate (27 mmol; at 0—5 °C) in dry acetone (20 mL). The mixture was stirred at 20 °C for 3 h and filtered. The precipitate was washed with acetone and the filtrate was concentrated *in vacuo*. The residue was dissolved in benzene (or in $CHCl_3$ in the case of phosphorothioates), washed successively with ice water, a cooled saturated $NaHCO_3$ solution, and water (10 ml each), and dried with Na_2SO_4. The solvent was removed *in vacuo* (at the end, at 75—80 °C, 1 Torr) and the corresponding phosphorothioates or -dithioates were isolated (see Table 1).

B. *Compounds* **1a,b, g—i; 3a—c;** and **4a**. A stream of dry HCl was rapidly passed with intense stirring and cooling (the initial temperature of the bath was maintained in the range from −20 to −30 °C) through *O,O*-diethylphosphorodithioic or -thioic acid (30 mmol), dry paraformaldehyde (30 mmol), and ester of *N*-acylglycine (or *N*-methoxycarbonylglycine) **8** (30 mmol) in dry $CHCl_3$ (or CH_2Cl_2) (30 mL) (the temperature of the mixture was maintained in the range from −5 to −10 °C) until the residue was completely dissolved and the mixture was saturated with HCl. Then the temperature was increased to −2—0 °C and the mixture was stirred under a weak stream of HCl at this temperature for 1.5 h. To remove excess HCl, the mixture was kept *in vacuo* without heating (the mixture was concentrated to three-fourths of the initial volume) and the residue was washed with ice water (2×10 mL), a cooled saturated $NaHCO_3$* solution (2×10 mL), and water (10 mL) and dried with Na_2SO_4. The solvent was removed *in vacuo* (at the final stage, at 75—80 °C, 1 Torr) and the corresponding phosphorodithioate or -thioate was isolated (see Table 1).

Generally, the compounds prepared according to procedures A and B do not require additional purification, but if necessary, they can be purified by column chromatography (see above).

Methyl *N*-(chloromethyl)-*N*-mesylglycinate (6). A stream of dry HCl was rapidly passed with intense stirring through a mixture of ester **7** (1 g, 6 mmol) and dry paraformaldehyde (0.18 g, 6 mmol) in dry $CHCl_3$ (10 mL) at −10 °C until the precipitate was completely dissolved and the solution was saturated. The temperature was increased to 0 °C, Na_2SO_4 was added, and the mixture was stirred under a weak stream of HCl for 1 h. Then the mixture was warmed to 20 °C, the solution was decanted from the precipitate, and the precipitate was washed with $CHCl_3$. The solvent was removed *in vacuo* (at the end, at 2 Torr). Crystalline compound **6** was isolated in a yield of 0.66 g (51%), m.p. 78—81 °C. Found (%): C, 27.84; H, 4.75; S, 15.30. $C_5H_{10}ClNO_4S$. Calculated (%): C, 27.85; H, 4.67; S, 14.87.

The toxicity with respect to the insects and the joint action coefficients (JAC) with permethrine were calculated as described previously.[8]

* Glycine derivative **1i** was not washed with a solution of $NaHCO_3$.

References

1. A. E. Shipov, G. K. Genkina, G. V. Zhdanova, P. V. Petrovskii, S. A. Roslavtseva, I. N. Sazonova, O. V. Sundukov, L. S. Golovkina, R. I. Volkova, Yu. S. Kagan, E. A. Ershova, T. A. Mastryukova, and M. I. Kabachnik, *Izv. Akad. Nauk, Ser. Khim.*, 1994, 1294 [*Russ. Chem. Bull.*, 1994, **43**, 1231 (Engl. Transl.)].
2. A. E. Shipov, G. K. Genkina, G. V. Zhdanova, G. F. Makhaeva, R. I. Volkova, E. B. Maizel′, O. V. Sundukov, T. A. Mastryukova, and M. I. Kabachnik, *Bioorg. Khim.*, 1995, **21**, 240 [*Russ. J. Bioorg. Chem.*, 1995, **21** (Engl. Transl.)].
3. O. Yu. Eremina, S. A. Roslavtseva, E. I. Bakanova, A. E. Shipov, G. K. Genkina, G. V. Zhdanova, T. A. Mastryukova, and M. I. Kabachnik, *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1955, 178 [*Bull. Acad. Sci. USSR, Biology*, 1955 (Engl. Transl.)].
4. D. Kasida, *Agrokhimiya* [*Agricultural Chemistry*], 1983, 102 (in Russian).
5. Yu. B. Filippovich, S. A. Roslavtseva, N. M. Kutuzova, M. N. Barybkina, T. A. Pereguda, and G. B. Ivanova, in *Itogi nauki i tekhniki. Entomologiya* [*Advances in Science and Technology. Entomology*], VINITI, Moscow, 1988, **8**, 193 (in Russian).
6. I. Ishaaya, *Arch. Insect Biochem. Physiol.*, 1993, **22**, 263.
7. R. I. Volkova and E. V. Titova, *Biokhimiya*, 1985, **50**, 475 [*Biochemistry (USSR)*, 1985, **50** (Engl. Transl.)].
8. O. Yu. Eremina, E. I. Bakanova, S. A. Roslavtseva, A. E. Shipov, G. K. Genkina, O. I. Artyushin, Z. O. Mndzhoyan, R. I. Volkova, T. A. Mastryukova, and M. I. Kabachnik, *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1996, 664 [*Bull. Russ. Acad. Sci., Biology*, 1996 (Engl. Transl.)].
9. V. I. Rozengart and O. E. Sherstobitov, *Izbiratel′naya toksichnost′ fosfororganicheskikh insektoakaritsidov* [*Selective Toxicity of Organophosphorus Insectoacaricides*], Nauka, Leningrad, 1978, 95 pp. (in Russian).

Received July 6, 2000;
in revised form September 22, 2000