Br. J. Pharmacol. (1989), **96**, 319–324

Structure-activity relationships of new analogues of arecaidine propargyl ester at muscarinic M_1 and M_2 receptor subtypes

[1]U. Moser, G. Lambrecht, M. Wagner, J. Wess, & E. Mutschler

Department of Pharmacology, University of Frankfurt/M, Theodor-Stern-Kai 7, Geb. 75 A, D-6000 Frankfurt/M, F.R.G.

1 The potency of arecaidine propargyl ester (APE) and of several analogues containing a modified ester side chain has been assessed at M_1 and M_2 muscarinic receptor subtypes. APE was shown to act as a potent agonist at ganglionic M_1 receptors in the pithed rat, at M_2 receptors in guinea-pig isolated atria ($-\log EC_{50} = 8.22$) and ileum ($-\log EC_{50} = 7.77$).

2 The arecaidine 2-butynyl and 2-pentynyl esters were approximately equipotent with APE at M_1 and M_2 receptors, whereas the 2-hexynyl derivative was found to be less potent than APE in atria ($-\log EC_{50} = 6.80$) and ileum ($-\log EC_{50} = 6.70$) by about one order of magnitude. The 2-heptynyl and 3-phenyl propargyl esters exhibited no agonist actions in atria and ileum.

3 Shifting the triple bond from the 2 to the 3 position and introducing a bulky group at position 1 of the ester side chain of APE and analogues resulted in competitive antagonists (pA_2 ranging from 4.9 to 7.3).

4 APE and its 2-butynyl analogue showed some agonistic selectivity for cardiac M_2 receptors (potency ratio, ileum/atria = 2.8 and 4.6 respectively). All antagonists in this series of compounds were not selective in terms of affinity since their pA_2 values at cardiac and ileal M_2 receptors were similar (potency ratios, ileum/atria = 0.4 to 1.2).

Introduction

Muscarinic receptors are composed of two major subpopulations which have been designated M_1 and M_2 (Hammer & Giachetti, 1982; Birdsall *et al.*, 1987; Lambrecht *et al.*, 1987). In addition, it is now well established that M_2 receptors can be further subdivided into an M_2 'cardiac' and an M_2 'smooth muscle/glandular type' (Mutschler & Lambrecht, 1984; Eglen & Whiting, 1986; Birdsall *et al.*, 1987; Doods *et al.*, 1987; Lambrecht *et al.*, 1987). This concept is mainly based on the availability of selective antagonists, such as pirenzepine, AF-DX 116, methoctramine, 4-DAMP or hexahydro-sila-difenidol (Birdsall *et al.*, 1987; Mutschler *et al.*, 1987). Although various agonists have also been reported to discriminate between the proposed muscarinic receptor subtypes (Mutschler *et al.*, 1987), the development of more selective potent muscarinic agonists still remains a highly important goal in muscarinic receptor research.

[1] Author for correspondence.

It has previously been reported that the muscarinic agonist arecaidine propargyl ester (APE, 1a; Figure 1) shows some selectivity for cardiac M_2 receptors as compared to those in the ileum (Mutschler & Hultzsch, 1973; Mutschler & Lambrecht, 1984). This selectivity profile of APE was confirmed by Barlow & Weston-Smith (1985). Additionally, APE also displayed considerable activity at ganglionic M_1 receptors in the pithed rat (Wess *et al.*, 1987).

In order to assess the role of the propargyl moiety for the observed activity/selectivity profile of APE, we have synthesized and tested several analogues of APE containing a modified ester side chain.

The following structural modifications were made (Figure 2): elongation of the ester side chain at C3; shift of the triple bond from C2/C3 to C3/C4; introduction of a methyl group at C1 or a phenyl group at C1 or C3.

All compounds (Table 2) were tested *in vitro* at ileal and atrial M_2 receptors of the guinea-pig. Based on the results of the *in vitro* experiments, several

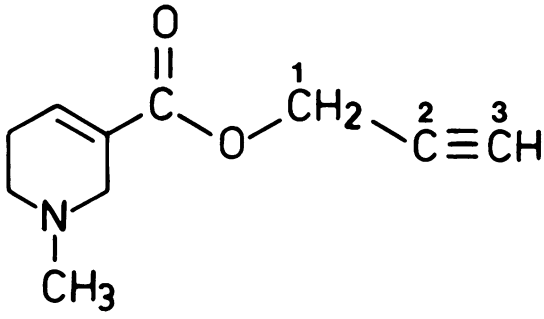


Figure 1 Chemical structure of arecaidine propargyl ester (APE, 1a)

agents were additionally tested on ganglionic M_1 receptors in the pithed rat by recording heart rate responses (Wess *et al.*, 1987).

A preliminary account of this investigation was presented at the 6th Camerino-Noordwijkerhout Symposium, Camerino, Italy, 1987.

Methods

Guinea-pig isolated ileum

Guinea-pigs (250–350 g) of either sex were killed by cervical dislocation. Strips of ileal longitudinal muscle (1.5–2 cm) were prepared according to the technique described by Paton & Zar (1968), transferred to 6 ml organ baths and loaded with a tension of 500 mg. Tyrode solution (pH = 7.4; temperature 32°C), bubbled with carbogen (95% O_2; 5% CO_2), was the bathing fluid. It had the following composition (mM): NaCl 137.0, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, glucose 5.6. The preparation was allowed to equilibrate for 30 min, during which time the bath fluid was changed every 10 min. Contractions were recorded isotonically with an electromechanical transducer connected to a Hellige amplifier and a Rikadenki recorder. Dose-response curves to APE were obtained by cumulative addition of the agonist (van Rossum, 1963). The concentration of agonist in the organ bath was increased approximately 3 fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Dose-response curves were repeatedly established until constant responses were obtained, allowing 30 min between each curve. Putative agonists were tested in the same manner as described for APE. Effects were expressed as percentages of the maximum effect induced by APE. $-\log EC_{50}$ values and intrinsic activities were determined graphically (van Rossum, 1963; Ariens & Simonis, 1964).

Antagonist affinities were determined by constructing concentration-response curves to APE at 3 different concentrations of antagonist (log conc. interval = 0.5), allowing 20–30 min equilibration time. EC_{50} values of APE were determined graphically for the control and the shifted concentration-response curves. Dose-ratios were calculated and Schild plots (Arunlakshana & Schild, 1959) were constructed from which pA_2 values were assessed according to Tallarida *et al.* (1979).

Guinea-pig isolated electrically paced left atria

Isolated left atria of guinea-pigs were suspended in Tyrode solution (composition and temperature as above) and oxygenated continuously with carbogen. The organ was electrically paced by supramaximal rectangular impulses of 3 ms duration (2 Hz, 4–10 V) using platinum electrodes. Preparations were preloaded with 500 mg and left to stabilize for 60 min. The effects of agonists were expressed as the percentage inhibition of the force of isometric contractions. Agonist and antagonist potencies were determined in essentially the same manner as described above for the ileum.

Pithed rat

Male normotensive White Wistar rats (200–300 g) were anaesthetized with pentobarbitone sodium (60 mg kg^{-1}, i.p.). The left jugular vein was cannulated for the administration of drugs. The heart rate was monitored continuously by means of a rate-meter (Hellige) which was triggered by the blood pressure pulse in the carotid artery. After catheterization of the trachea, heparin (300 iu kg^{-1}) was given i.v. to prevent blood coagulation. The rats were then pithed by introducing a steel rod into the spinal canal and artificial respiration with room air was provided (10 ml kg^{-1} body weight; 60 strokes min^{-1}). The body temperature was kept at 37 ± 1°C throughout the experiment by means of an overhead heating lamp.

All drugs were dissolved in saline (0.9% w/v) and injected i.v. in a volume of 1 ml kg^{-1}. To characterize agonist responses pharmacologically, pretreatment with pirenzepine (300 $\mu g\,kg^{-1}$ i.v.) was carried out 20 min before the administration of agonists. ED_{50} values were calculated graphically from log dose-response curves. The putative M_1 agonist McN-A-343 (Hammer & Giachetti, 1982; Wess *et al.*, 1984) served as a reference drug.

Statistics

The data are presented as means ± s.e.mean of n experiments. Differences between mean values were

tested for statistical significance ($P < 0.05$) by means of Student's t test.

Compounds

The following were used: atropine sulphate (Merck), heparin sodium (Promonta), hexamethonium iodide (Fluka), McN-A-343 (4-[N-(3-chlorophenyl) carbamoyloxy]-2-butynyltrimethylammonium chloride, kindly provided by Dr R. Hammer (Boehringer Ingelheim Zentrale, F.R.G.), pentobarbitone sodium (Abott), pirenzepine dihydrochloride (Thomae).

Chemistry

The esters 1a–1h and 2a–2c (Table 2) were prepared by azeotropic esterification of 1-methyl-1,2,5,6-tetrahydropyridine -3-carboxylic acid (arecaidine) with the corresponding alcohols under acid conditions by the procedure of Mutschler & Hultzsch (1973). The alcohols were commercially available and used directly for synthesis. All compounds were obtained as tosylates (Table 1).

Melting points were determined in open glass capillaries with a Büchi-Tottoli melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1420 spectrophotometer. ^1H n.m.r. spectra were obtained on a Perkin Elmer R 23 spectrometer with Me_4Si as an internal standard. All spectral and analytical data for compounds 1a–1h and 2a–2c were consistent with the assigned structures. Microanalyses (C, H, N) were performed by the Department of Organic Chemistry, University of Frankfurt, F.R.G., and were correct within ± 0, 4% of the theoretical values. Thin layer chromatography was performed on Merck silica gel plates 60 F 245 in toluene/chloroform/acetone (9:4:14). Spots were visualized under 245 nm illumination or with Dragendorff spraying reagent.

Results

In vitro *studies*

The results obtained at atrial and ileal M_2 receptors are summarized in Table 2.

Lengthening of the ester side chain in APE (1a) from propargyl to 2-hexynyl (1b–1d) resulted in full agonists in both preparations. The effects of 1a–1d could be competitively blocked by atropine (30–300 nM), but were not affected by hexamethonium (30 μM) (data not shown). The arecaidine 2-butynyl (1b) and 2-pentynyl ester (1c) were approximately equipotent with APE in the atria ($P > 0.05$), whereas 1c was slightly (≈ 2 fold) more potent than 1a and 1b in the ileum ($P < 0.05$). In contrast, the arecaidine 2-hexynyl ester (1d) showed an about 10–30 fold lower potency than 1a–1c in both tissues (Table 2). While 1c and 1d displayed similar $-\log EC_{50}$ values at atrial and ileal M_2 receptors, compounds 1a and 1b were about 3–5 fold more potent in the atria than in the ileum (Table 2). Further elongation of the ester side chain led to the 2-heptynyl derivative 1e which behaved as a weak competitive muscarinic antagonist in both preparations.

Shift of the triple bond from 2- to 3-position in 1b–1d resulted in compounds 2a–2c which were devoid of agonist activity in both ileum and, except 2a (i.a. ≈ 0.2), in atria. In both preparations, 2a–2c proved to be competitive antagonists (Table 2).

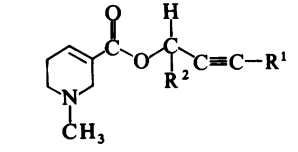
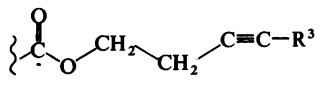
Introduction of a methyl group at C1 (Figure 1) in the ester side chain of APE led to a complete loss of efficacy in the ileum whereas weak partial agonist activity (i.a. = 0.2) was retained in the atria (1g). Introduction of a phenyl group at C1 or C3 in the ester side chain of APE resulted in competitive antagonists (1f, 1h). However, the 1-phenyl derivative (1h) showed an approximately 100 fold higher affinity than the 3-phenyl derivative (1f) in both preparations.

Table 1 Physical constants of arecaidine esters related to arecaidine propargyl ester (APE, 1a)

*Compound**	MP (°C)	Yield (%)	M_r	Formula
1b	128–129	23	365.45	$C_{18}H_{23}NO_5S$
1c	131–132	67	379.47	$C_{19}H_{25}NO_5S$
1d	122–123	26	393.50	$C_{20}H_{27}NO_5S$
1e	114–115	43	407.53	$C_{21}H_{29}NO_5S$
1f	135–137	44	427.52	$C_{23}H_{25}NO_5S$
1g	130–131	45	365.45	$C_8H_{23}NO_5S$
1h	177–178	10	427.52	$C_{23}H_{25}NO_5S$
2a	112–113	50	365.45	$C_{18}H_{23}NO_5S$
2b	104–105	39	379.48	$C_{19}H_{25}NO_5S$
2c	107–108	60	393.50	$C_{21}H_{29}NO_5S$

* Tosylates; colourless crystals; recrystn. solvent: acetone/ether.

Table 2 Chemical structures of arecaidine propargyl ester (APE, 1a) and analogues (1b–1h, 2a–2c) and their muscarinic/antimuscarinic activity at atrial and ileal M_2 receptors: potency ratios (ileum/atria) are given as a measure of receptor selectivity

General formula	*Compound*	R^1	R^2	R^3	*Atria (force)* $-\log EC_{50}$	*Atria (force)* pA_2	*Ileum* $-\log EC_{50}$	*Ileum* pA_2	*Potency ratio*[a] (ileum/atria)
	1a	H	H		8.22 ± 0.04		7.77 ± 0.02		2.8
	1b	CH_3	H		8.33 ± 0.04		7.67 ± 0.04		4.6
	1c	C_2H_5	H		8.27 ± 0.08		8.04 ± 0.07		1.7
	1d	$n-C_3H_7$	H		6.80 ± 0.03		6.70 ± 0.04		1.3
	1e	$n-C_4H_9$	H			5.77 ± 0.06[b]		5.89 ± 0.04	0.8
	1f	C_6H_5	H			4.86 ± 0.02[b]		5.25 ± 0.03	0.4
	1g	H	CH_3			6.38 ± 0.04[c]		6.45 ± 0.03	0.8
	1h	H	C_6H_5			6.96 ± 0.04		7.26 ± 0.03	0.5
	2a			H		5.97 ± 0.04[c]		6.01 ± 0.03	0.9
	2b			CH_3		6.76 ± 0.06		6.69 ± 0.03	1.2
	2c			C_2H_5		6.74 ± 0.05		6.67 ± 0.04	1.2

Values are mean ± s.e.mean, n = 4–7 for agonists and 4–16 for antagonists.

[a] EC_{50} ileum/EC_{50} atria in case of agonists; K_D ileum/K_D atria for antagonists ($pA_2 = -\log K_D$).

[b] Only two concentrations were investigated. The pA_2 values were therefore determined from the individual dose-ratios according to Tallarida *et al.*, 1979.

[c] Partial agonists: intrinsic activity relative to APE ≈ 0.2.

In vivo *studies*

Compounds 1a–1c and 2a–2b were also tested for M_1 receptor activity in the pithed rat. The resting heart rate of the pithed rats before drug treatment was 275 ± 7 beats min^{-1} (n = 27).

1a–1c (0.2–6 μmol kg^{-1}, i.v.) caused a brief transient decrease in heart rate (max. decrease about 40 beats min^{-1}), followed by a dose-dependent tachycardia (ED_{50} values approx. 1 μmol kg^{-1}; Figure 2). These increases in heart rate proved to be highly sensitive to blockade by a low dose of pirenzepine (300 μg kg^{-1}, i.v.), indicating the involvement of ganglionic M_1 receptors. Compounds 1a–1c produced similar maximum responses as McN-A-343, but were about 2.5–5 times less potent than the reference drug (Figure 2). On the other hand, 2a and 2b (0.1–30 μmol kg^{-1}, i.v.) showed no agonist activity in the pithed rat.

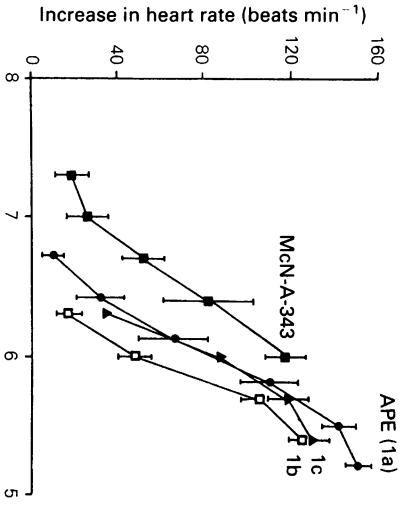


Figure 2 Dose-response curves for the increase in heart rate caused by McN-A-343 (■), 1a (●), 1b (□) and 1c (▲) in the pithed rat. Mean values are given (n = 3–10); s.e.means shown by vertical bars.

Discussion

The data show that muscarinic potency of APE and its analogues at both M_1 and M_2 receptors is highly dependent on the position of the triple bond in the ester side chain. In general, agonist activity could only be observed with compounds where the triple bond is located between C2 and C3 (Figure 1); in these compounds, elongation of the ester side chain up to C_6 was tolerated without loss of full agonist activity.

The arecaidine 2-butynyl and 2-pentynyl ester (1b, 1c) were approximately equipotent with APE at atrial and ileal M_2 receptors in the *in vitro* experiments as well as at ganglionic M_1 receptors in the pithed rat. In contrast, the corresponding analogues 2a and 2b (triple bond shifted to C3/C4) displayed virtually no agonist activity at either M_1 and M_2 receptors. Thus, the activities of 1a–1c and 2a, 2b at M_2 receptors closely paralleled those observed at M_1 receptors and no selectivity for either M_1 or M_2 receptors was found.

Introduction of a methyl group at C1 of the propargyl chain in APE led to compound 1g which showed an almost complete loss of efficacy and proved to be a competitive antagonist of APE-induced responses ($pA_2 = 6.4$). This finding indicates that bulk tolerance with respect to agonist activity is low at the C1 position. Similar results were obtained in a series of methyl-substituted oxotremorine derivatives (Ringdahl & Jenden, 1983a,b; Amstutz *et al.*, 1985). These findings suggest that among APE analogues, agonists and antagonists bind to a common site on the receptor molecule, in contrast to other antimuscarinic agents (e.g. atropine type, benzilic acid ester type) which are believed to interact predominantly with accessory sites adjacent to the agonist binding site (Ariens & Simonis, 1967).

Whereas 1h (phenyl-substitution at C1) showed a pharmacological profile similar to that of 1g (methyl-substitution at C1), 1f (phenyl-substitution at C3) displayed an approximately 100 fold lower affinity for both atrial and ileal M_2 receptors, indicating that introduction of bulky groups at C3 is associated with a dramatic loss in affinity. Moreover, these data suggest that highly potent muscarinic antagonists may be obtained by introduction of even larger substituents at C1 in APE-related compounds. Although none of the compounds could clearly discriminate between M_1 and both M_2 receptor subtypes, APE and the arecaidine 2-butynyl ester (1b) showed some degree of cardio-selectivity (about 3–5 fold). Interestingly, structure-activity studies of APE analogues in which the N-methyl group had been replaced by larger N-alkyl substituents revealed that the N-ethyl derivative of APE (N-ethyl guvacine propargyl ester) shows an even more pronounced cardioselectivity. This compound proved to be a rather potent partial agonist at rat cardiac M_2 receptors ($-\log EC_{50} = 6.56$; i.a. = 0.8 relative to APE), but a competitive antagonist at rat ileal M_2 receptors ($pA_2 = 6.06$) (Mutschler *et al.*, 1987).

E.M. and G.L. thank the Fonds der Chemischen Industrie and ratiopharm (Ulm), F.R.G., for financial support.

References

AMSTUTZ, R., RINGDAHL, B., KARLEN, M., ROCH, M. & JENDEN, D.J. (1985). Stereoselectivity of muscarinic receptors in vivo and in vitro for oxotremorine analogues. N-[4-(tertiary amino)-2-butynyl]-5-methyl-2-pyrrolidones. *J. Med. Chem.*, **28**, 1760–1765.

ARIENS, E.J. & SIMONIS, A.M. (1964). A molecular basis for drug action. *J. Pharm. Pharmacol.*, **16**, 137–157.

ARIENS, E.J. & SIMONIS, A.M. (1967). Cholinergic and anticholinergic drugs, do they act on common receptors? *Ann. N.Y. Acad. Sci.*, **144**, 842–868.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.*, **14**, 48–58.

BARLOW, R.B. & WESTON-SMITH, P. (1985). The relative potencies of some agonists at M2 muscarinic receptors in guinea-pig ileum, atria and bronchi. *Br. J. Pharmacol.*, **85**, 437–440.

BIRDSALL, N.J.M., CURTIS, C.A.M., EVELEIGH, P., HULME, E.C., PEDDER, E.K., POYNER, D., STOCKTON, J.M. & WHEATLEY, M. (1987). Muscarinic receptor subclasses. In *Cellular and Molecular Basis of Cholinergic Function.* ed. Dowdall, M.J. & Hawthorne, J.N. pp. 46–55. Weinheim, F.R.G.: VCH Verlagsgesellschaft mbH.

DOODS, H.N., MATHY, M.J., DAVIDESKO, D., VAN CHARLDORP, K.J., DE JONGE, A. & VAN ZWIETEN, P.A. (1987). Selectivity of muscarinic antagonists in radioligand and in vivo experiments for the putative M1, M2 and M3 receptors. *J. Pharmacol. Exp. Ther.*, **242**, 257–262.

EGLEN, R.M. & WHITING, R.L. (1986). Muscarinic receptor subtypes: a critique of the current classification and a proposal for a working nomenclature. *J. Auton. Pharmacol.*, **5**, 323–346.

HAMMER, R. & GIACHETTI, A. (1982). Muscarinic receptor subtypes: M1 and M2. Biochemical and functional characterization. *Life Sci.*, **31**, 2991–2998.

LAMBRECHT, G., MOSER, U., RIOTTE, J., WAGNER, M., WESS, J., GMELIN, G., TACKE, R., ZILCH, H. & MUTSCHLER, E. (1987). Heterogeneity in muscarinic receptors: evidence from pharmacological and electrophysiological studies with selective antagonists. In *International Symposium on Muscarinic Cholinergic Mechanisms.* ed. Cohen, S. & Sokolovsky, M. pp. 245–253. London: Freund Publishing House Ltd.

MUTSCHLER, E. & HULTZSCH, K. (1973). Über Struktur-Wirkungs-Beziehungen von ungesättigten Estern des Arecaidins und Dihydroarecaidins. *Arzneim.-Forsch.*, **23**, 732–737.

MUTSCHLER, E. & LAMBRECHT, G. (1984). Selective muscarinic agonists and antagonists in functional tests. *Trends Pharmacol. Sci.*, **5** (Suppl.), 39–44.

MUTSCHLER, E., GMELIN, G., MOSER, U., WESS, J. & LAMBRECHT, G. (1987). Structure-activity relationships of drugs acting selectively at different muscarinic receptors. In *Pharmacology. International Congress Series No. 750.* ed. Rand, M.J. & Raper, C. pp. 67–75. Amsterdam: Excerpta Medica (Elsevier).

PATON, W.D.M. & ZAR, M.A. (1968). The origin of acetylcholine release from guinea-pig intestine and longitudinal muscle strips. *J. Physiol.*, **194**, 13–33.

RINGDAHL, B. & JENDEN, D.J. (1983a). Pharmacological

properties of oxotremorine and its analogs. *Life Sci.*, **32**, 2401–2413.

RINGDAHL, B. & JENDEN, D.J. (1983b). Affinity, efficacy, and stereoselectivity of oxotremorine analogues for muscarinic receptors in the isolated guinea pig ileum. *Mol. Pharmacol.*, **23**, 17–25.

TALLARIDA, R.J., COWAN, A. & ADLER, M.W. (1979). pA_2 and receptor differentiation: a statistical analysis of competitive antagonism. *Life Sci.*, **25**, 637–654.

VAN ROSSUM, J.M. (1963). Cumulative dose-response curves. *Arch. Int. Pharmacodyn. Ther.*, **143**, 299–330.

WESS, J., LAMBRECHT, G., MOSER, U. & MUTSCHLER, E. (1984). A comparison of the antimuscarinic effects of pirenzepine and N-methylatropine on ganglionic and vascular muscarinic receptors in the rat. *Life Sci.*, **35**, 553–560.

WESS, J., LAMBRECHT, G., MOSER, U. & MUTSCHLER, E. (1987). Stimulation of ganglionic muscarinic M1 receptors by a series of tertiary arecaidine and isoarecaidine esters in the pithed rat. *Eur. J. Pharmacol.*, **134**, 61–67.

(Received May 11, 1988
Revised September 2, 1988
Accepted September 27, 1988)