IL FARMACO, **48** (9), 1239-1248; 1993

SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF 4-CARBAMOYL-6-β-THIENYL-4,5-DIHYDROPYRIDAZIN-3-(2H)ONES (*)

GÉRARD AIMÈ PINNA - MARIA MICHELA CURZU
ISTITUTO DI CHIMICA FARMACEUTICA

ELISABETTA GAVINI - ANTONIO MULÈ - GEROLAMO PIRISINO (**)
ISTITUTO DI TECNICA FARMACEUTICA

MARGHERITA SATTA - ALESSANDRA PEANA
CATTEDRA DI FARMACOGNOSIA
UNIVERSITÀ DI SASSARI - VIA MURONI 23, 07100 SASSARI

SUMMARY — *A new series of 4-carbamoyl-6-β-thienyl-4,5-dihydropyridazin-3-(2H)ones* **4a-g** *have been synthesized and tested for their anti-inflammatory and analgesic properties. Among the tested compounds, only* **4f** *at 1 mmole/Kg showed antiinflammatory activity that was comparable with that of indomethacin (5 mg/Kg) though of shorter duration. Compounds* **4a, 4e** *and especially* **4g** *at 0.2 mmoles/Kg displayed relevant analgesic activity,* **4g** *being the most potent derivative in the writhing test. Compounds* **4c** *and* **4g** *were found to possess analgesic activity also in the hot plate test.*

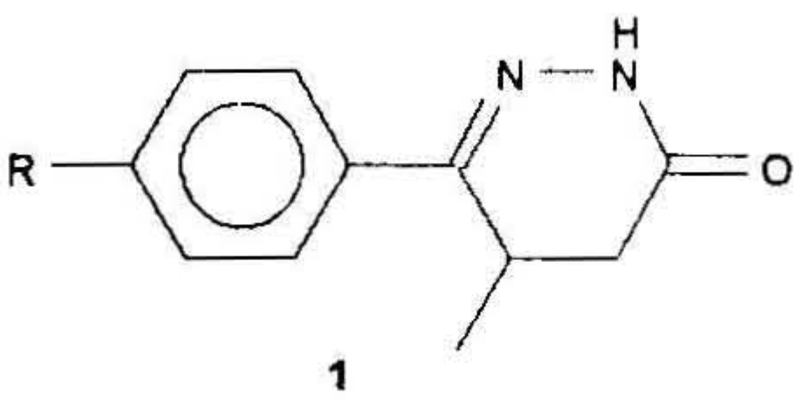
RIASSUNTO — *È stata sintetizzata e valutata l'attività antinfiammatoria e analgesica di una nuova serie di 4-carbamoil-6-β-tienil-4,5-diidropiridazin-3(2H)-oni. Fra i composti saggiati, solo* **4f** *alla dose di 1 mmole/Kg ha mostrato proprietà antinfiammatoria comparabile in intensità, ma di minore durata, rispetto a quella dell'indometacina assunta come farmaco di riferimento. D'altra parte alla dose di 0,2 mmoli/Kg* **4a** *e* **4e** *hanno manifestato una buona attività analgesica nel writhing test mentre il derivato* **4g** *ha dimostrato di essere più attivo dello stesso farmaco di riferimento. Infine i composti* **4c** *e* **4g** *hanno dimostrato di avere anche attività analgesica nel test della piastra calda.*

(*) An account of this work was communicated at the "3rd International Symposium on the chemistry and pharmacology of the pyridazines"; Como, 9-11 September 1992.

(**) To whom correspondence should be addressed.

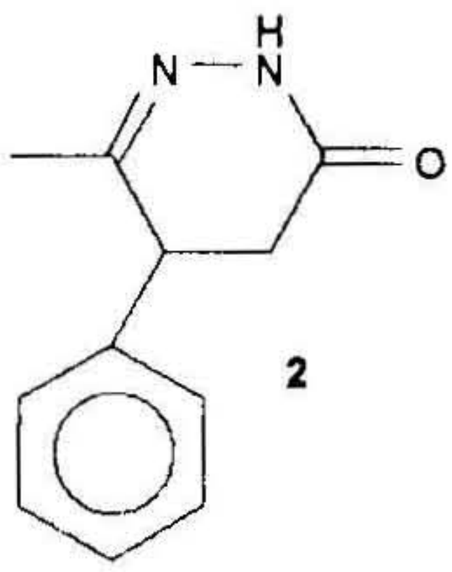
Introduction

6-Aryl-4,5-dihydropyridazinones **1** represent a well known class of PDE-III inhibitors with inotropic and vasodilator effects (inodilators) (1,2). Reversion of 5 and 6 substituents to give 5-aryl-6-methyl-4,5-dihydropyridazinones **2** caused loss of these properties while it induced in some of the tested compounds anti-inflammatory, antithrombotic and antiulcer activities (3). Moreover, the introduction of a variety of carbamoyl groups at C-4 of the dihydropyridazinonic ring of **2** gave compounds **3** provided with significant anti-inflammatory and good analgesic properties. Antinociceptive activity was especially displayed by carbamoyl derivatives bearing on the nitrogen a Cl or a CF_3 substituted aryl moiety (4).



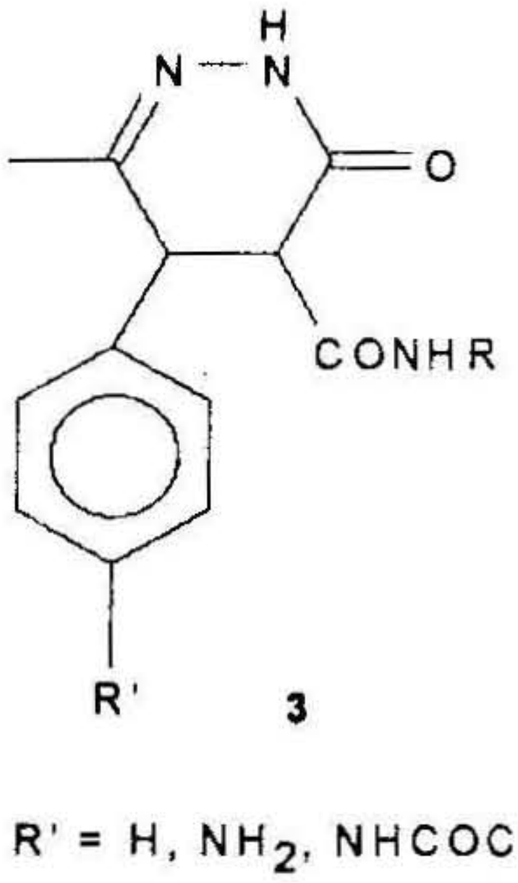
1

R = H, CN, NH_2, NHAc



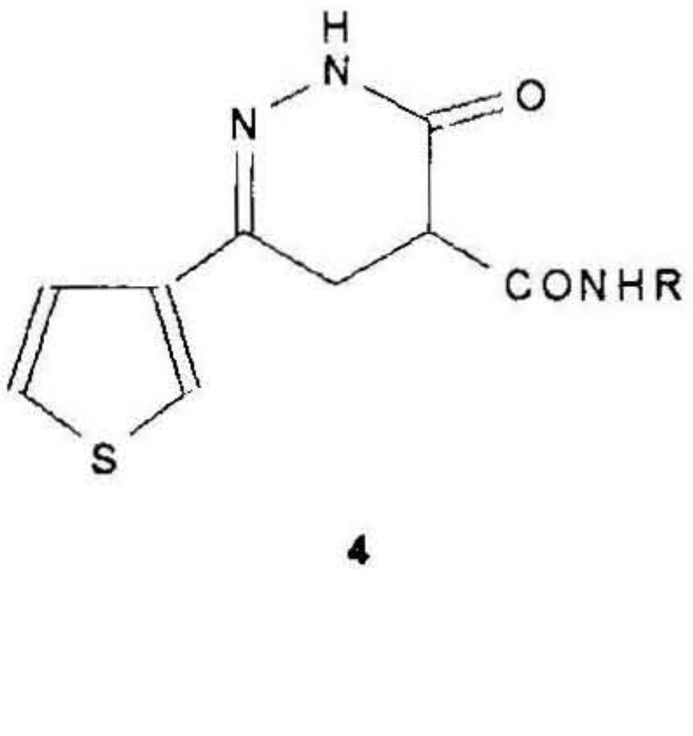
2

	R
a	pC_6H_4 Cl
b	pC_6H_4 F
c	pC_6H_4 CF_3
d	pC_6H_4 CH_3
e	pC_6H_4 OCH_3
f	pC_6H_4 OC_2H_5
g	C_6H_{11}



3

R' = H, NH_2, $NHCOCH_3$



4

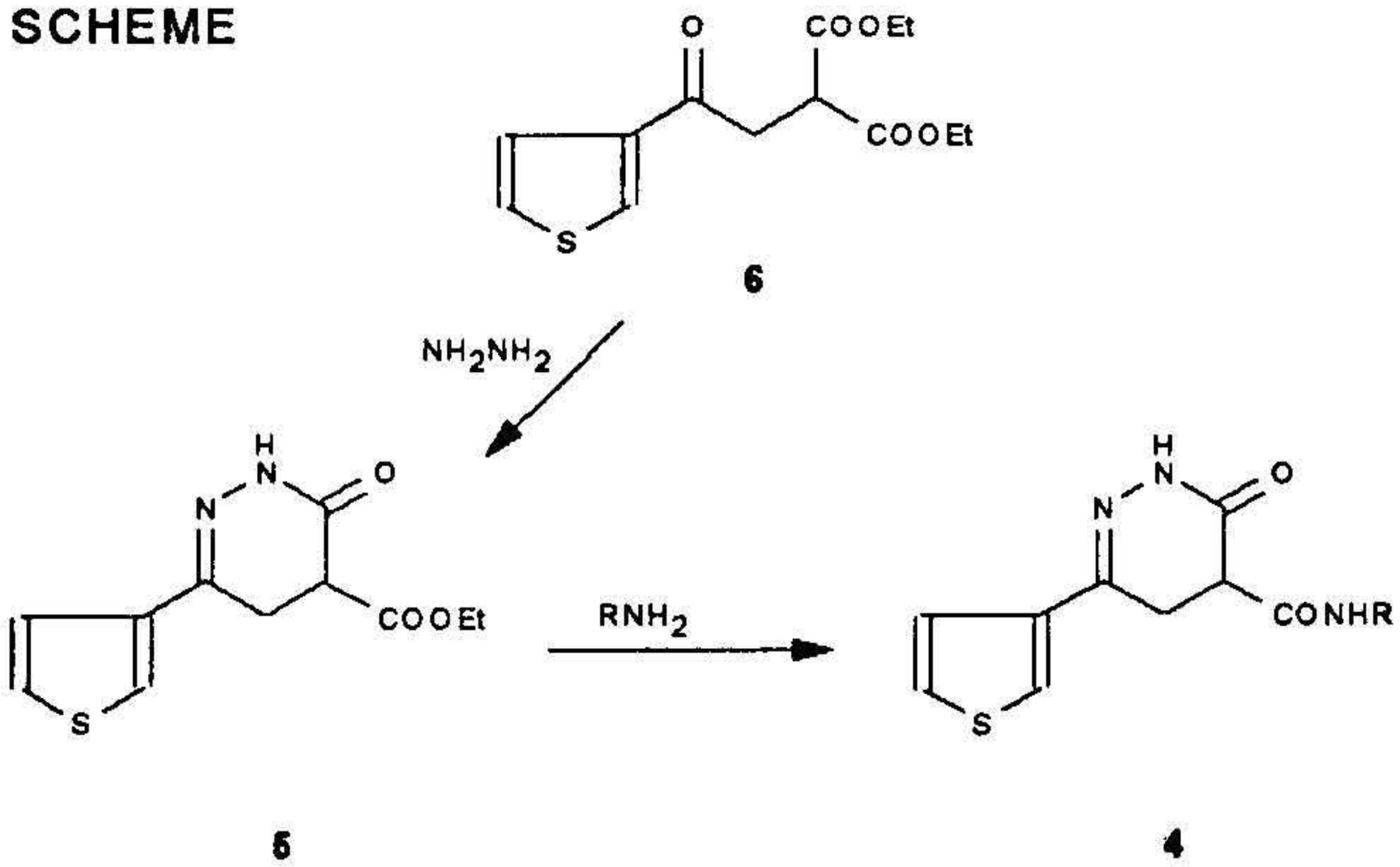
In this context, a new series of 6-β-thienyl-3(2H)-oxo-4,5-dihydropyridazine-4-carboxamides (**4a-g**), somewhat related with both sets of compounds **1** and **3**, have been prepared with the aim to study the eventual anti-inflammatory and analgesic properties.

Chemistry

Synthetic pathway for the title compounds is illustrated in the Scheme. The key intermediate 6-thienyl-4-ethoxycarbonyl-4,5-dihydropyridazin-3(2H)one (**5**) was prepared in 86% yield refluxing 2-β-thenoylmethylmalonate (**6**) (4) with hydrazine hydrate in 60% aqueous acetic acid.

Exchange reaction of ester **5** with appropriate amines in refluxing xylene in the presence of molecular sieves afforded the target compounds **4a-g**.

SCHEME



Chemical experimental section

Melting points were determined with a Kofler apparatus and are uncorrected. IR spectra were registered in nujol mull on a Perkin Elmer Infrared 781 spectrometer. UV spectra were recorded with a Perkin Elmer Lambda 5 spectrometer. ^{1}H-NMR spectra were recorded on a Varian XL-200 spectrometer; chemical shits are reported as ppm relative to tetramethylsilane as internal standard. Silica gel 60 (Merck; 230-400 mesh) was used for flash chromatography. TLC on silica gel plates was used to control the purity of the products. The elemental analyses (C,H,N, Br,Cl,F,S) were within $\pm 0.4\%$ of the theoretical values.

4-Ethoxycarbonyl-6-β-thienyl-4,5-dihydropyridazin-3(2H)one (5)

A mixture of diethyl 2-β-thenoilmethylmalonate (**6**) (5) (5 g; 0.018 mol) and hydrazine hydrate (1.80 g; 0.036 mol) in 60% aqueous acetic acid (17.5 ml) was refluxed for 1.5 h. After cooling, the solution was poured onto 5% solution of $NaHCO_3$ (~80 ml). The white solid which precipitated was collected on filter and washed with water to give 3.89 g (86%) of **6**, m.p. 156°-158°C. TLC (benzene-acetone, 7:3): Rf 0.61.

The analytical sample was crystallized from ethanol or toluene, m.p. 160°-162°C.

IR cm^{-1}: 3200 (NH); 1720 (ester CO); 1660 (hydrazide CO);

UV (EtOH) λmax nm (log ϵ): 204.2 (4.96); 286.0 (4.19);

^{1}H-NMR ($CDCl_3$) δ: 1.2 (t, 3H, J = 7.12 Hz); 3.02-3.64 (m, 3H), 4.26 (q, 2H, J = 7.12 Hz); 7.26-7.58 (m, 3H); 8.79 (br s, 1H, exchanges with D_2O). ^{13}C-NMR 50 MHz ($CDCl_3$) δ: 14.00 (q, CH_3), 26.49 (t, C_5-H_2); 43.27 (d, C_4-H); 62.23 (t, CH_2 of the ester); 125.22 (d, C_2'-H and C_4'-H); 126.76 (d, C_5'-H); 137.96 (s, C_3'); 147.06 (s, C_6 = N); 163.07 (s, CO); 167.93 (s, CO).

*General procedure for 4-N-substituted-carbamoyl-6-β-thienyl-4,5 dihydropyridazin-3-ones (**4a-g**)*

A solution of the pyridazinonic ester **5** (0.02 mol) and the required amine (0.04 mol) in anhydrous toluene (300 ml) was refluxed for 20-24 hrs in the presence of molecular sieves (4A). The mixture was filtered still hot and evaporated *in vacuo* to give a solid residue which was purified by crystallization (see Table I and II).

TABLE I

Physical properties of compounds 4a-g

COMPD	YIELD %	M.P. °C	FORMULA	ANALYSES
4a	90	280-282[a]	$C_{15}H_{12}N_3O_2SCl$	C,H,N,S,Cl
4b	84	248-250[b]	$C_{15}H_{12}N_3O_2SF$	C,H,N,S,F
4c	83	273-276[c]	$C_{16}H_{12}N_3O_2SF_3$	C,H,N,S,F
4d	79	260-262[d]	$C_{16}H_{15}N_3O_2S$	C,H,N,S
4e	90	301[b]	$C_{16}H_{15}N_3O_3S$	C,H,N,S
4f	88	308[b]	$C_{17}H_{17}N_3O_3S$	C,H,N,S
4g	86	213-216[b]	$C_{15}H_{19}N_3O_2S$	C,H,N,S

Compound were crystallized from ethyl acetate (a), ethanol (b), methanol (c), acetone (d).

TABLE II

UV, IR, ^{1}H-NMR spectral data of 4a-g

COMPD	UV λmax (logε)[a]	IR ν (cm^{-1})[b]	^{1}H-NMR (δ ppm)[c]
4a	203(4.25) 258(4.13) sh 282(4.05)	3210,3080 1670,1640	3.15-3.27(dd, 2H); 3.67(t, 1H); 7.52(ABq, 4H); 7.37-7.42(m, 1H, J=2.82Hz, $C_{4'}$-H); 7.50(d, 1H, J=5.06Hz $C_{5'}$-H); 7.71(d, 1H, J=1.66Hz $C_{2'}$-H); 10.26(br. s, 1H, exch with D_2O); 11.00(br. s, 1H, exch with D_2O).
4b	201.8(4.22) 232.0(4.03) 238.6(4.05) 282.4(4.07)	3220,3080 1670,1640	3.23(d, 2H); 3.64(t, 1H); 6.98-7.78(m, 7H), 10.23(br. s, 1H, exch with D_2O); 11.04(br. s, 1H, exch with D_2O).
4c	203.8(4.40) 255.2(4.29) 284.0(4.19)	3220,3100 1690,1650	3.21-3.31(m, 2H); 3.70(dd, 1H); 7.35-7.81(m, 7H); 10.39(br. s, 1H, exch with D_2O); 10.88(br. s, 1H, exch with D_2O).
4d	203.8(4.54) 254.0(4.36) 241.0(4.35) 281.2(4.34)	3210,3100 1680,1650	2.30(s, 3H); 3.10-3.38(m, 2H); 3.65(dd, 1H); 7.07-7.69(m, 7H); 9.90(br. s, 1H, exch with D_2O); 10.83(br. s, 1H, exch. with D_2O).
4e	202.2(4.29) 262.0(4.25) 230.0(4.08) 359.0(3.57)	3220,3100 1680,1650	3.20(s, 3H); 3.22-3.29(m, 2H); 3.63-3.81(m, 1H); 6.80-7-75(m, 7H); 9.90(br. s, 1H exch with D_2O); 10.87(br. s, 1H, exch with D_2O).
4f	202.8(4.33) inf234.0(4.06) inf244.0(4.13) 262.2(4.25)	3240,3100 1680,1650	1.37(t, 3H), 3.22-3.33(m, 2H); 3.94-4.04(m, 1H); 6.78-7.94(m, 7H); 9.97(br. s, 1H, exch with D_2O); 10.97(br. s, 1H, exch with D_2O).
4g	203.4(4.33) 283.8(4.31)	3340,3250 3100,1680 1650	1.25-1.89(m, 10H); 3.10-3.40(m, 3H); 3.60-3.78(m, 1H); 7.34-7.62(m, 4H); 10.67(br. s, 1H, exch with D_2O).

Spectra were recorded in (a) EtOH for UV, in (b) nujol mulls for IR and in (c) $CDCl_3$ with a few drops of $(CD_3)_2SO$ for ^{1}H-NMR

Pharmacology

Anti-inflammatory activity was evaluated on male albino Wistar rats (150 g). For the detection of antinociceptive properties, male Swiss mice (18-22 g) were used. In all cases the animals were fed with Morini feed, in pellets, and aqua fontis.

Pharmacological experimental section

Anti-inflammatory activity: it was evaluated by inhibition of the carrageenin-induced hind paw oedema in rats (6).

One hour before the injection of carrageenin (0.05 ml of 1% solution) the test compounds were administered *per os* at the dose of 1 mmol/Kg suspended in 10 ml/Kg of 1% solution of methylcellulose. Activity was evaluated by oedema inhibition at 1,3 and 5 hours after treatment with carrageenin and results expressed as mean percent of oedema volume compared to that in control animals.

Peripheral analgesia: it was evaluated through the inhbition of writhings induced by the i.p. injection of formic acid (25 mg/Kg) as 0.25% solution in distilled water (7).

The test compounds were administered *per os* at the dosage of 1/5 mmol/Kg suspended in 10 ml/Kg of 1% solution of methylcellulose, one hour before the formic acid injection. The control animals received only the formic acid i.p. and 1% solution of methylcellulose *per os*.

After injection of formic acid writhings were recorded for 20 mins. The results are expressed as the percent variation in number of writhings compared to that of the control animals. Indomethacin at the dose of 5 mg/Kg was used for comparison.

Central analgesia: to evaluate this kind of analgesia, typically overspinal, we used the hot-plate test (8). The plate was kept at 55°C and the reaction time (in seconds) was determined on the basis of licking, raising of hind paws, jumping and escape reaction. The reaction time was measured before the administration of the test compounds and 60, 120 and 180 minutes after the dosing. The test compounds were administered *per os* at the dose of 1/5 mmol/Kg, suspended in a 1% solution of methylcellulose. Morphine at the dose of 10 mg/Kg s.c. was used for comparison.

Results and discussion

Anti-inflammatory activity

As shown in Table III, only **4f** at the dose of 1 mmol/Kg was found to have an anti-inflammatory activity comparable with that of indometacin, though of shorter duration. On the contrary compounds **4c** and **4d** produced an increase of the flogistic process. All the other derivatives did not display a significant anti-inflammatory property.

TABLE III

Compoud	Anti-inflammatory activity (rat)[a] % inhibition[b]		
	1h	3h	5h
Indomethacin[c]	−38	−38	−47
4a	+ 6	− 3	+ 6
4b	−12	−10	−19
4c	− 6	+90	+53
4d	+50	+45	+37
4e	+ 6	−34	−16
4f	−31	−31	−16
4g	0	− 3	+ 6

a) Carrageenin-induced rat paw oedema: %of inhibition of the oral dose of 1 mmole/kg suspended in 10 ml/kg of a solution of methyl cellule (1%). Groups of six animals were used.
b) Inibition (−) or increase (+) compared to the oedema volume of control animals.
c) Indomethacin was administered orally at the dose of 5 mg/kg

TABLE IV

Compoud	Analgesic activity: writhings test (mouse) % inhibition[a]
Indomethacin[b]	−65
4a	−53
4b	−39
4c	−42
4d	−48
4e	−62
4f	−47
4g	−88

a) % inhibition at the oral dose of 0.2 mmoles/kg. Groups of eight animnals were used.
b) Indomethacin was administered at the oral dose of 5 mg/kg

Analgesic activity

In the writhing test all the compounds (see Table IV and Fig. 1) showed at the dose of 0.2 mmol/Kg a good peripheral analgesic activity, particularly pronounced for **4a,4e** and **4g**, the latter being the most potent one respect to indomethacin. Therefore, the presence of a cycloalkyl group on the carbamoyl function is clearly responsible for the relevant analgesic effect. The substitution of cycloalkyl with a p-substituted aryl group, determines a decrease of activity that however, still remains good. The effect of the substituents on the phenyl moiety is not rationalizable by the data obtained in the writhing test.

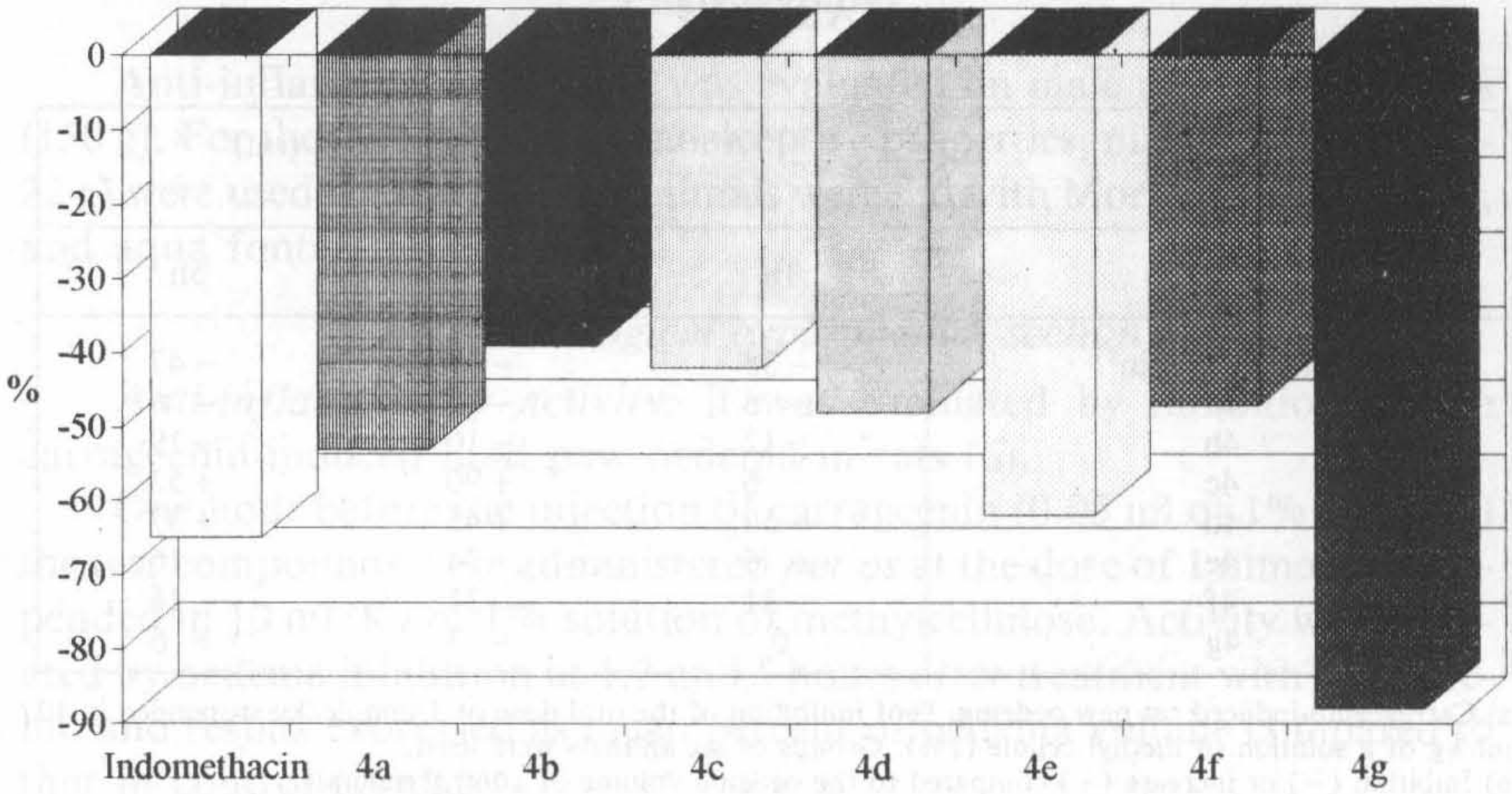


Fig. 1

Analgesic activity: Writhings test (mouse)

TABLE V

Compund[a]	Analgesic activity: hot plate test (mouse) (In parenthesis % change relative to 0 value) Mean reaction time (sec ± S.E.) at the following times (hours) after treatment			
	0	1	2	3
Control	6.56 ± 0.94	7.14 ± 0.97 (**9**)	7.9 ± 1.07 (**20**)	7.06 ± 1.42 (**8**)
Morphine[b]	7.84 ± 0.23	16.15 ± 0.81 (**106**)	11.78 ± 1.04 (**50**)	9.94 ± 0.08 (**27**)
4a	7.34 ± 0.48	9.33 ± 0.83 (**27**)	10.37 ± 1.01 (**41**)	10.51 ± 0.79 (**43**)
4b	7.82 ± 0.44	10.44 ± 1.28 (**33**)	9.26 ± 0.64 (**18**)	9.33 ± 0.75 (**19**)
4c	7.15 ± 0.70	7.98 ± 0.97 (**12**)	11.99 ± 1.61 (**68**)	9.57 ± 1.13 (**34**)
4d	7.75 ± 0.38	7.77 ± 0.92 (**0**)	8.90 ± 0.60 (**15**)	9.45 ± 1.07 (**22**)
4e	7.79 ± 0.33	10.74 ± 0.75 (**38**)	9.70 ± 0.62 (**24**)	10.31 ± 0.59 (**32**)
4f	8.20 ± 0.34	11.24 ± 0.32 (**37**)	10.13 ± 0.53 (**23**)	11.20 ± 0.94 (**37**)
4g	6.72 ± 0.26	8.62 ± 0.54 (**28**)	9.90 ± 1.15 (**47**)	10.41 ± 0.92 (**55**)

a) Compounds were given orally at the dose of 0.2 mmoles/kg. Groups of ten animals were used.
b) Morphine was administrated at the dose of 10 mg/Kg s.c.

Results from the hot plate test are indicated in Table V and Fig. 2. Compounds **4c** and **4g** showed a certain central analgesic activity, **4g** being the most potent one at the second and third hour after the medication.

In conclusion, the data currently available seem to indicate that the simultaneous substitution of the aryl ring of the model compound with a thiophenic one and its shifting to position 6, have furnished derivatives **4a-g** which were devoid of or endowed with a scarce anti-inflammatory effect. Central analgesic activity was occasionally present in compounds **4c** and **4g**, while all compounds **4a-g** displayed good peripheral antinociceptive activity.

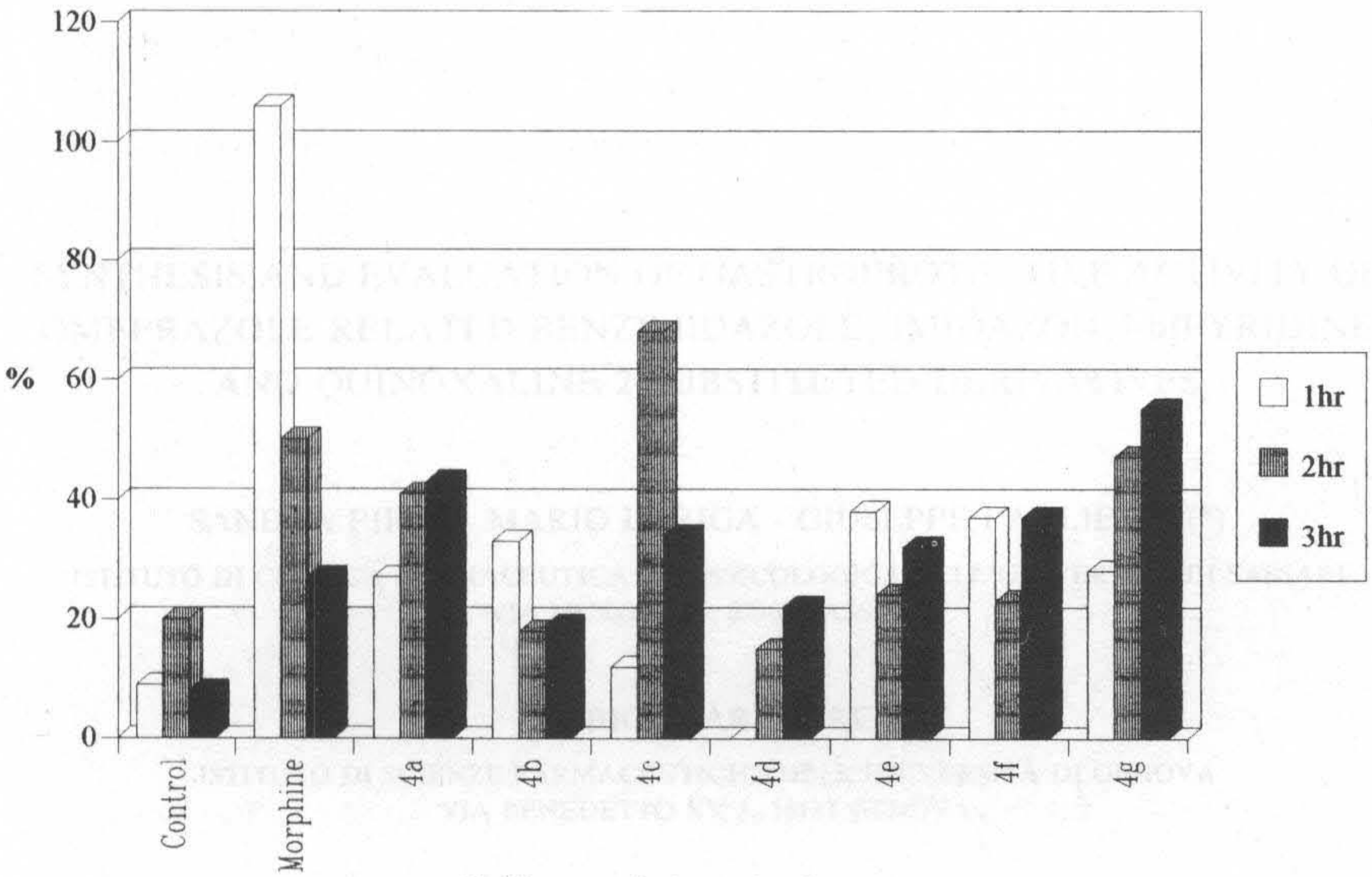


Fig. 2

Analgesic activity: hot Plate test (mouse)

Acknowledgement

This work was supported by a grant of M.U.R.S.T. (60%).

REFERENCES

1) MOOS W.H., HUMBLET C.C., SIRCAR I., RITHNER C., WEISHAAR R.E., BRISTOL J.A., McPHAIL A.T., *J. Med. Chem.*, **30**, 1963; 1987.
2) COATES W.J., PRAIN H.D., REEVES M.L., WARRINGTON B.H., *J. Med. Chem.*, **33** 1735; 1990.
3) PINNA G.A., CURZU M.M., BARLOCCO D., CIGNARELLA G., CAVALLETTI E., GERMINI M., BERGER K., *Il Farmaco "Ed. Sc."*, **43**, 539; 1988.
4) PINNA G.A., SARDU F., CURZU M.M., SATTA M., PEANA A., CIGNARELLA G., BARLOCCO D., *Il Farmaco*, **47**, 449; 1992.
5) McDOWELL D.W.H., GREENWOOD T.D., *J. Heterocyclic Chem.*, **2**, 44; 1965.
6) WINTER C.A., RISLEY E.A., NUSS G.W., *Proc. Soc. Exp. Biol. Med.*, **111**, 544; 1962.
7) SIEGMUND E., CADMUS R., *Proc. Soc. Exp. Biol. Med.*, **95**, 729; 1957.
8) WOOLFE G., McDONALD A.O., *J. Pharmacol. Exp. Ther.*, **80**, 300; 1944.

Received April 30, 1993; accepted June 28, 1993.