Acta Poloniae Pharmaceutica - Drug Research, Vol. 57 No. 3 pp. 213–221, 2000

ISSN 0001-6837
Polish Pharmaceutical Society

PYRROLE ANALOGUES OF CHLORAMPHENICOL. I. SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF *DL–THREO*–1–(1–METHYL–4–NITRO–PYRROLE–2–YL)–2–DICHLOROACETAMIDOPROPANE–1,3–DIOL.

DOROTA KRAJEWSKA[1], MARTA DĄBROWSKA[1], PIOTR JAKONIUK[2], and ANDRZEJ RÓŻAŃSKI[1]

[1] Department of Organic Chemistry, Institute of Chemistry,
[2] Department of Microbiology, Medical Academy, 2A Mickiewicza Str., 15–230 Białystok, Poland

Abstract: The seven–stage synthesis of a pyrrole analogue of chloramphenicol [**IX**] was described. The compound exhibits a significant antibacterial activity, over the 12–50% range of the chloramphenicol activity.

Keywords: Chloramphenicol, synthetic analogues of antibiotics, pyrrole analogue of chloramphenicol.

Due to its relative structural simplicity, the antibiotic chloromycetin (chloramphenicol) has may times been subjected to various modifications. Moreover, approximately 2.000 compounds of a similar structure to this (1, 2, 3, 4) have been synthesized. Practical use has been found for two esters of chloramphenicol – hemisuccinate and palmitate (Figure 1). Both compounds are transformed *in vivo* into the parent antibiotic (1).

A lower activity was revealed by two analogues of chloramphenicol, for which the use in therapy was also found; these are: thiamphenicol and azidamphenicol (Figure 1) (5). Cetophenicol is similar to them in respect to the character of its activity, however, no its use was found in therapy (6, 7). One of the known compounds with a twofold larger activity than that of chloramphenicol was *D–threo*–1–(*p*–perchlorylphenyl)–2–dichloroacetamidopropane–1,3–diol (Figure 1). This compound, however, exhibits explosive properties, which makes its practical use impossible (8). Quite recently (1980), a fluorine analogue of thiamphenicol – Florphenicol – was introduced to the veterinary therapy. This analogue differs from the parent compound by the presence of a fluorine atom ($-CH_2OH \rightarrow -CH_2F$) (9, 10). The traces of this antibiotic can be found in some animal products (11).

Among numerous heterocyclic analogues of chloramphenicol synthesized (3, 12, 13, 14, 15), only one, i.e. its thiophenic analogue, is worthy to note, viz. *DL–threo*–1–(5–nitro–2–thienyl)–2–dichloroacetamidopropane–1,3–diol, with an antibacterial activity being approximately 50% of the racemic chloramphenicol (Figure 1) (12). Also this compound has found no usage. The derivatives containing some heteroaromatic systems in place of the benzene ring are inactive or exhibit a minimal antibacterial activity. Therefore, it can be stated, that nearly all the synthesized and examined analogues of chloramphenicol are devoid of the activity.

The negative characteristics of chloramphenicol is its remote toxicity, causing aplastic anaemia in some patients, even during many months after the end of the treatment (16). The phenomenon appears only for a very limited percentage of patients (approximately 0.005%) (16). However, the use of chloramphenicol in therapy was limited to the cases caused by *Salmonella* or *Shigella*, which do not respond to other antibiotics, as well as in the cases of severe sepsis (5). It is also used locally, particularly in ophthalmology, where it substitutes zidamphenicol. Until recently, there has been the common opinion that even a local use of chloramphenicol is burdened with the risk of delated aplastic anaemia. The most recent results define the risk to be minimal (17) or none (18).

The antibacterial activity as well as toxicity of chloramphenicol are closely connected with its structure. A large number of the synthesized analogues let one to find out which fragments of the parent molecule can be subjected to modification, and which modification causes inevitably the loss of activity.

The structure of the parent antibiotic is presented in Figure 2.

The remote toxicity of the parent antibiotic is associated with the influence of the aromatic fragment in the molecule corresponding to nitrobenzene, i.e. the nitro group connected with the benzene ring system. The specific metabolism of nitrobenzene derivatives causes the appearance of various products of its partial or total reduction in

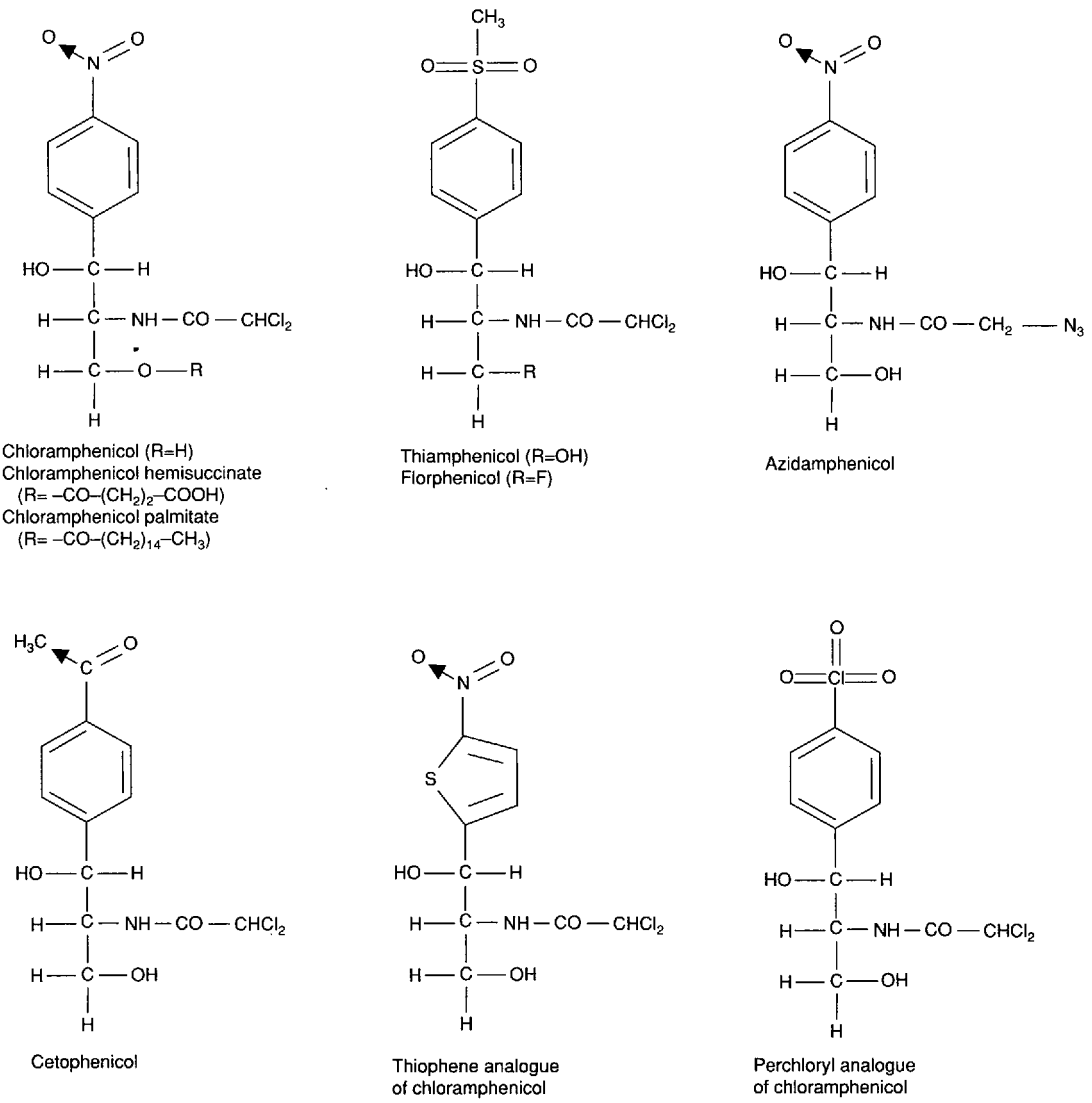


Figure 1. The structure of chloramphenicol and some of its analogues.

a human body. These are: nitroso compounds, hydroxylamine derivatives, azoxy compounds, azo compounds, or aromatic amines (16). These products are believed to influence the remote toxicity. A substitution of the nitro group by the methylsulfonyl group (in thiamphenicol) or the acetyl group (in cetophenicol) made it possible to obtain the derivatives of a reduced antibacterial activity. Now, only thiamphenicol is used in therapy. Despite of the fact that the remote toxicity of this compound is minimal, some reversible disorders in the function of bone marrow and kidneys were observed (5).

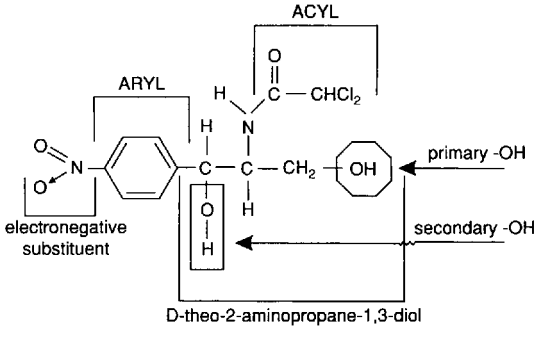
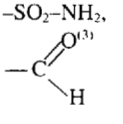
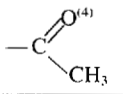


Figure 2. Structure of chloramphenicol.

Table 1. Transformations of chloramphenicol molecule not decreasing its antibacterial activity

Electronegative group	Aryl	Configuration of aminopropanediol	Secondary –OH	Primary –OH	Acyl
PATTERN ANTIBIOTIC					
$-NO_2$	1,4–phenylene–	*D–threo–*	–CHOH–	$-CH_2OH$	$Cl_2CH-CO-$
MODIFIED COMPOUNDS					
$-ClO_3$[1], $-SO_2-CH_3$[2], $-SO_2-NH_2$,  	2,5–thienylene–	*D–threo–*[5]		$-O-Acyl$[7] $-CH_2-F$[8]	$Br_2CH-CO-$ N_3-CH_2-CO-[9]

(1) – compound is a powerful explosive [8];
(2) – in thiamphenicol;
(3) – unstable compound [19];
(4) – in cetophenicol;
(5) – activity is displayed only in *D–threo–* isomers, while the others were inactive;
(6) – compound with antifungal activity;
(7) – esters are active *in vivo*;
(8) – in florphenicol;
(9) – in azidamphenicol

The aryl group can be either a fragment of a benzene molecule or a heteroaromatic system. Substituting benzene by thiophene leads to a slight reduction in antibacterial activity (12). Other data relating to the interdependence between the structure and antibacterial activity are presented in Table 1.

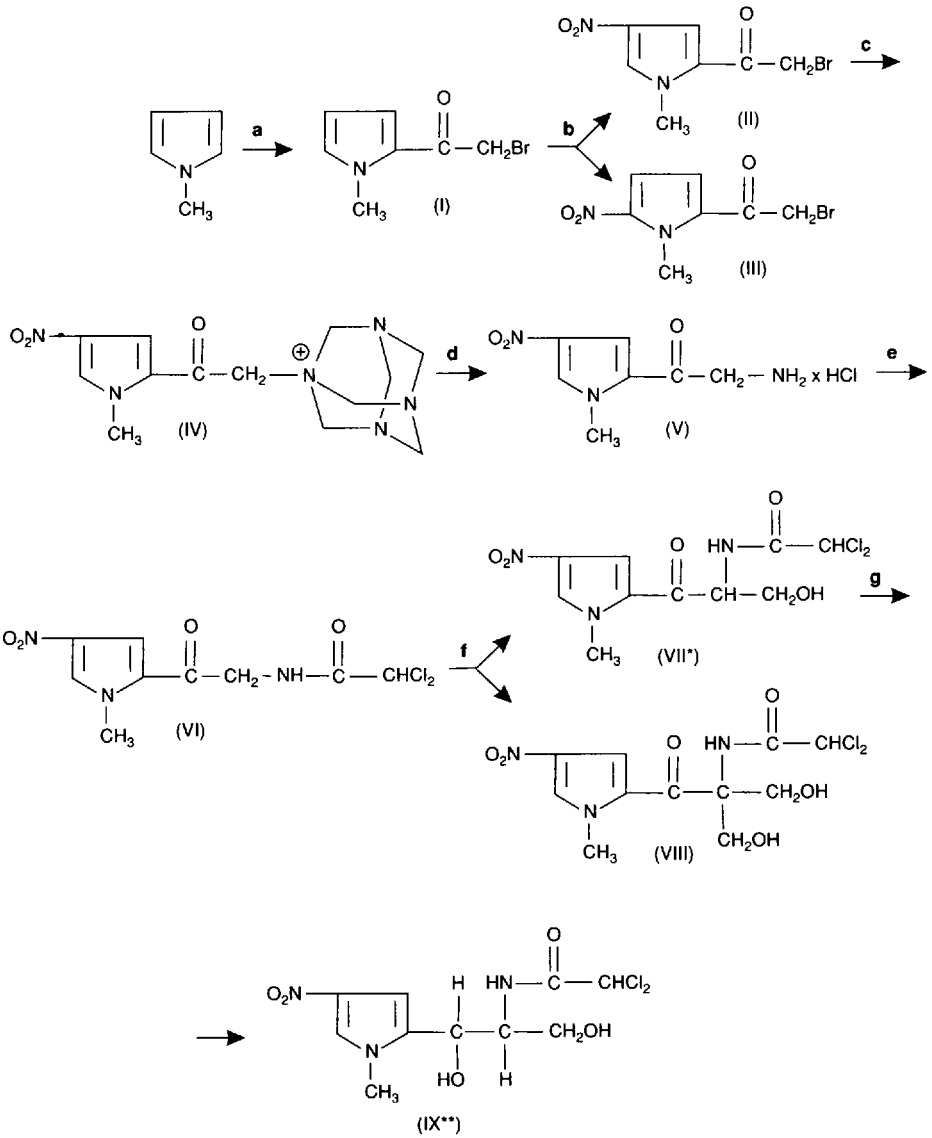
Professional literature at hand has given no information on the synthesis of pyrrole analogues of chloramphenicol (20). It can be expected that their activity would be similar to the activity of the tiophene analogues; thus, we decided to synthesize some novel pyrrole analogue. We have decided that it is inevitable to substitute the H atom in pyrrole by the methyl group (N–H → N–CH_3), due to the possibility of its participation in further consecutive steps of the synthesis in the undesired adverse reactions. The presence of the unsubstituted N–H group in the ring would also be the reason for the creation of a new system of hydrogen bondings, stabilizing the conformational option of a final molecule, different from the specific conformation of chloramphenicol and its active analogues (2, 21). Some reports regarding the nitro derivatives of benzene also suggest that the presence of the methyl groups has a significant effect on the reduction of the toxicity of nitro compounds, e.g. nitrotoluenes reveal a lesser toxicity than nitrobenzene; dinitrotoluenes are less toxic than dinitrobenzenes, and 2,4,6–trinitrotoluene (TNT) is less toxic than 1,3,5–trinitrobenzene (22). The data are supported by statistics of the cases of illnesses due to nitro compounds, observed in some production plants. Therefore, it may be expected that the presence of the *N*–methyl group would have a positive effect on the characteristics of the nitropyrrole analogue of chloramphenicol.

EXPERIMENTAL

Materials and methods

N–Methylpyrrole was the starting substrate, which was subjected to the reaction with bromoacetyl bromide. The crude 2–bromoacetyl–1–methylpyrrole **[I]** obtained was nitrated at the temperature of –55°C to afford a mixture of two isomeric nitroketones, viz. **[II]** and **[III]**, which were separated by column chromatography. The transformation of bromoketone **[II]** into the target compound **[IX]** was accomplished by means of the modified methods developed by Šorm (23) and Smoleński (26).

Bromoketone **[II]** reacts with hexamethylenetetraamine to yield a crystalline adduct **[IV]**, which, in turn, was transformed into aminoketone **[V]** as the result of hydrolysis. This compound was further transformed into amide **[VI]** by means of dichloroacetyl chloride, and this was hydroxymethylated (13, 24, 25), while the main product of the reaction **[VII]** was subjected to the Meer-



Reagents and conditions: (a) $BrCH_2COBr$, $AlCl_3$, Et_2O; (b) HNO_3, Ac_2O, CH_2Cl_2, (–) 55°C, then SiO_2; (c) $(CH_2)_6N_4$, $CHCl_3$; (d) HCl, H_2O/EtOH; (e) $Cl_2CHCOCl$, Et_3N, Me_2CO; (f) CH_2O, $NaHCO_3$, H_2O/EtOH; (g) i-PrOH, $(i\text{-}PrO)_3Al$

* – only formula of *D*-isomer is presented;

** – only formula of *D-threo*-isomer is presented.

Figure 3. Synthesis of compound **IX**.

wein–Panndorff reduction. This method enables to obtain mainly a compound of the desired configuration, i.e. *DL–threo* [**IX**]. The reaction scheme is outlined in Figure 3.

The antibacterial activities of compounds **VI, VII, VIII,** and **IX** were determined using the method presented by Sahm (27).

^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 200F spectrometer, using TMS as an internal standard. IR spectra were taken on a Specord M80 infrared spectrometer. All the melting point data were measured with a Büchi 535 apparatus and are uncorrected. Analytical TLC was performed using Merck silica gel 60 F_{254} plates. The solvent systems used were (v:v): A = heptane/AcOEt (1:2); B = benzene/CH_2Cl_2 (1:1); C = AcOEt/EtOH/AcOH/H_2O (3:2:1:1); D = EtOH/benzene/CH_2Cl_2/AcOEt/AcOH/H_2O (18:7:7:6:2:2); E – benzene/CH_2Cl_2/EtOH/AcOEt (5:5:2:1); F = $CHCl_3$/MeOH (85:15).

A column chromatography was performed with silica gel 60 (Merck) as the adsorbent.

RESULTS

2–BROMOACETYL–1–METHYLPYRROLE [I]

To a solution of bromoacetyl bromide (73.1 g, 0.35 mole) in diethyl ether (200 ml) 44 g (0.33 mole) of anh. $AlCl_3$ was added portionwise at 0–5°C. A solution of *N*–methylpyrrole (25.6 g, 0.3 mole) in diethyl ether (100 ml) was added within 0.5 h, and the mixture was stirred for 4 h, and then poured into crushed ice (500 g). This was stirred for 1 h at r.t. The ethereal phase was separated, and the aqueous phase was extracte dwith benzene (3×150 ml). The combined organic layers were washed with brine (2×150 ml), dried (Na_2SO_4), and evaporated to give the crude product [**I**] (39.62 g, 62.1%).

2–BROMOACETYL–1–METHYL–4–NITROPYRROLE [II] AND 2–BROMOACETYL–1–METHYL–5–NITROPYRROLE [III]

The crude 2–bromoacetyl–1–methylpyrrole [**I**] (39.6 g, approx. 0.19 mole) was dissolved in CH_2Cl_2 (130 ml) and acetic anhydride (64 ml). The mixture was cooled to –55°C, and a solution of fuming HNO_3 (17.6 ml) in acetic anhydride (64 ml) was added dropwise within 25 min. The cooling bath was removed, and the mixture was stirred for 4 h at r.t., poured into crushed ice (300 g), stirred until the ice had melted, and neutralized with solid K_2CO_3.

The organic layer was separated, the aqueous layer was extracted with benzene (4×150 ml), and the combined organic phase was dried (Na_2SO_4), filtered, and concentrated to give a yellowish solid (27.0 g), containing compounds [**II**] and [**III**]. The two products were separated by column chromatography (system B), and rechromatographed to afford 21.7 g (45%) of **II** and 1.93 g (4%) of **III**.

N–[(1–METHYL–4–NITROPYRROLE–2–YL)–CARBONYLMETHYL]–HEXAMETHYLENETETRAMINIUM BROMIDE [IV]

Nitroketone **II** (9.87 g, 0.04 mole) was dissolved in anhydrous $CHCl_3$ (150 ml) and the solution was warmed up to 50°C. Hexamethylenetetraamine, synthesized according to (28), (6.14 g, 0.043 mole) was dissolved in anhydrous $CHCl_3$ (70 ml), warmed up to 50°C, and added to the solution of compound **II**. The mixture was stirred at 50°C for 4 h, then left overnight at 4°C. The product [**IV**] was collected by filtation. From the mother liquors a second crop of **IV** was obtained; total yield 15 g (97%).

2–AMINOACETYL–1–METHYL–4–NITROPYRROLE HYDROCHLORIDE [V]

A mixture of conc. aq. HCl and 95% EtOH (75 ml + 15 ml) was added to 6.53 g (16.8 mmol) of compound **IV**, and the resulted suspension was stirred at 30°C for 1 h, then left overnight at 5°C. The crude compound **V**, containing NH_4Cl, was collected by filtration, and to the filter cake a mixture of conc. aq. HCl and H_2O (0.1 ml + 8.5 ml) was added. The suspension was stirred at 25°C for 15 min.; next, it was cooled to 10°C and left at this temp. for 15 min. The pure product **V** (3.15 g; 85%) was collected by filtration.

N–[(1–METHYL–4–NITROPYRROLE–2–YL)–CARBONYLMETHYL]–2,2–DICHLOROACETAMIDE [VI]

To a well–stirred mixture of aminoketone **V** (8.2 g, 37 mmol) and anhydr. acetone (130 ml) at 0°C dichloroacetyl chloride (13.9 g, 94 mmol) was slowly added, then anhydr. triethylamine (15.4 g, 152 mmol) was added dropwise. The reaction mixture was stirred for 3 h at 0°C, then left overnight at 10°C. The precipitate was filtered off, and the filtrate was evaporated under reduced pressure to give a sirup, from which a product (3.87 g) crystallized within a few days. To the mother liquors, containing triethylamine hydrochloride, H_2O (40 ml) was added, and the mixture was extracted with AcOEt (4×40 ml). The combined organic layers were dried (Na_2SO_4) and concentrated to give a second crop of the product **VI** (0.49 g). The combined crops were recrystallized from 65 ml of methanol–acetone (2:1) to afford 4.2 g (31.9%) of pure **VI**.

DL–2–DICHLOROACETAMIDO–3–HYDROXY–1–(1–METHYL–4–NITROPYRROLE–2–YL)–PROPAN–1–ONE [VII] and 2–DICHLOROACETAMIDO–3–HYDROXY–2–HYDROXYMETHYL–1–(1–METHYL–4–NITROPYRROLE–2–YL)–PROPAN–1–ONE [VIII]

A mixture of dichloracetamide **VI** 1.31 g, 4.46 mmol), 36% formaldehyde aq. solution (1.38 ml, 16.5 mmol), and $NaHCO_3$ (67 mg) in 96% EtOH (6.7 ml) was stirred for 4.5 h at r.t., then was concentrated in vacuo. TLC (system E) showed 2 products: **VII** and **VIII**, which were separated by column chromatography (system E), yielding, respectively, 0.46 g (31.9%) of compound **VII**, and 0.25 g (15.8%) of compound **VIII**.

DL–THREO–2–DICHLOROACETAMIDO–1–(1–METHYL–4–NITROPYRROLE–2–YL)–PROPANE–1,3–DIOL [IX]

Table 2. Physical and spectral data of compounds I – IX

Compd.	Formula (Molecular mass)	R_f(system)	M.p. [°C]	Yield [%]	IR, cm^{-1} solvent	^1H NMR, δ (ppm) solvent	^{13}C NMR, δ (ppm) solvent
I	C_7H_8BrNO (202)	A=0.59 B=0.32	–	62.1			
II	$C_7H_7BrN_2O_3$ (247)	B=0.28	140.3	45	3150, 1568 and 1528 (C-H, Ar), 1680 (C=O), 1412 and 1328 (NO_2), 848(C-N). $CHCl_3$	4.03 (s,3H, NCH_3), 4.61 (s, 2H, CH_2Br), 7.75 and 8.09 (2×d, 2H, C_3H+C_5H, Ar, $J_{3,5}$=1.79Hz). $(CD_3)_2CO$	32.69 ($\underline{C}O$-$\underline{C}H_2Br$), 38.77 (NCH_3), 113.9 and 128.27 (C_2+C_4, Ar), 114.88 and 131.15 (C_3+C_5, Ar), 183.63 (C=O). $(CD_3)_2CO$
III	$C_7H_7BrN_2O_3$ (247)	B=0.45	121.7	4	3120, 1590 and 1500 (C-H, Ar), 1680 (C=O), 1366 and 1296 (NO_2). $CHCl_3$	4.22 (s,3H, NCH_3), 4.62 (s, 2H, CH_2Br), 7.17 and 7.27 (2×d, 2H, C_3H+C_4H, Ar, $J_{3,4}$=4.72Hz). $(CD_3)_2CO$	33.57 (CH_2Br), 35.79 (N-CH_3), 112.16 and 118.29 (C_5+C_4, Ar), 121.0 and 131.45 (C_2+C_5, Ar), 184.91 (C=O). $(CD_3)_2CO$
IV	$C_{13}H_{19}BrN_6O_3$ (387)	–	189 (R)	97	3150, 1578 and 1520 (C-H, Ar), 1680 (C=O), 1498 (NCH_2) 1428 and 1328 (NO_2). nujol	3.95 (s,3H, NCH_3), 4.41 (s, 2H, -CO-CH_2-), 4.52 and 4.63 (2×d, 6H, 3×CH_2-N-, $J_{Ha,He}$= 12.57Hz) 5.37 (s, 6H, 3×CH^2-N^+), 7.99 and 8.46 (2×d, 2H, C_3H+C_5H, Ar, $J_{3,5}$=1.61Hz). DMSO-d_6	32.81)NCH_3), 57.85 (CH_2, 70.00 and 79.24 (6×CH_2-, urotropin), 114.19 and 131.73 (C_3×C_5, Ar), 128.10 and 134.42 (C_2+C_4, Ar), 181.21)C=O). DSMO-d_6
V	$C_7H_{10}ClN_3O_3$ 219.5	C=0.62 D=0.44	253 (R)	85.8	3400 (N-H—, 3170, 1590 (C-H, Ar, 1696 (C=O), 1574 and 1472 (NO_2) nujol	4.02 (s, 3H, NCH_3), 4.42 (s, 2H, CH_2), 7.75 and 8.08 (2×d, 2H, C_3H+C_5H, Ar, $J_{3,5}$=1.64Hzt). CD_3OD	38.38 (NCH_3), 45.16 (CH_2, 114.88 and 131.34 (C_3+C_5, Ar), 127.93 and 136.39 (C_2+C_4, Ar), 183.57 (C=O). CD_3OD
VI	$C_{93}H_{99}Cl_2N_3O_4$ (294)	C=0.91 D=0.88 E=-0.73	151.5	40	1700, 1550 and 1248 (CONH), 1696)C=O), 1600 and 1510 (C-H,Ar), 1460 and 1360 (NO_2). nujol	4.02 (s, 3H, NCH_3), 4.68 (t, 2h, CH_2), 6.5 (s, 1H, $CHCl_2$, 7.76 and 8.07 (2×d, 2H, C_3H+ C_5H, $J_{3,5}$=1.63Hz), 8.08 (s, 1H, -NH-). $(CD_3)_2CO$	38.46 (NCH_3), 46.96 (CH_2), 67.29 ($CHCl_2$), 113.64 and 130.59 (C_3+C_5, Ar), 128.80 and 135.97 (C_2+C_4), 164.92 (NH-CO), 185.99 (CO-CH_2). $(CD_3)_2CO$
VII	$C_{10}H_{11}Cl_2N_3O_5$ (324)	E=0.62 F=0.64	151.1	31.9	3200 and 1060 (OH), 3120, 1600 and 1480 (C-H, Ar), 1680 (C=O, 1468 and 1348 (NO_2). nujol	4.03 (m, 5H, NCH_3+CH_2OH), 4.29 (t, 1H, OH), 5.30 (dt, 1H, CH), 6.54 (s, 1H, $CHCl_2$), 7.84 and 8.09 (2×d, 2H, C_3H+C_5H, Ar, $J_{3,5}$=1.77Hz), 8.08 (s, 1F, NH). $(CD_3)_2CO$	38.74 (NCH_3), 58.98 (CH-NH), 63.51 (-CH_2OH), 67.29 ($CHCl_2$), 114.49 and 130.93 (C_3C_5, Ar), 129.12 and 135.92 (C_2+C_4, Ar), 164.39 (NH-CO), 188.12 ($\underline{C}O$-CH). $(CD_3)_2C$
VIII	$C_{11}H_{13}Cl_2N_3O_6$ (354)	E=0.53	180.9	15.8	3400 and 1050 (OH), 1668 (C=O), 1584 and 1550 (C-H, Ar), 1458 and 1296 (NO_2). nujol	3.93 (s, 3H, NCH^3), 4.19 (t, 4H, 2×CH_2OH, J=5.67Hz), 4.37 (t, 2H, 2×CH_2OH, J=6.01Hz) 6.41 (s, 1H, $CHCl_2$), 7.73 and 7.97 (2×d, 2H, C_3H+C_5H, Ar, $J_{3,5}$=1.65Hz), 8.33 (s, 1H, NH-CO). $(CD_{32}CO$	38.86 (NCH_3), 62.53 (2×CH_2OH), 67.56 ($CHCl_2$), 69.55 (CO-C-NH), 112.77 and 129.51 (C_3+C_5), 128.74 and 135.17, (C_2+C_4), 164.06 (CO-NH), 191.65 ($\underline{C}O$-C). $(CD_3)_2CO$
IX	$C_{10}H_{13}Cl_2N_3O_5$ (326)	F=0.43	110	22.9	3400, 1100 and 1050 (OH), 2900, 1600 (C-H,Ar), 1470 and 1376 (NO_2). nujol	3.87 (s+m, 5H, CH_2OH+NCH_3), 4.28 (m, 1H, CH-NH), 4.40 (t, 1H, CH_2OH), 5.01 (d, 1H, CH-OH), 5.20 (dd, 1H, Ar-CH-OH), 6.46 (s, 1H, $CHCl_2$), 6.63 i 7.67 (2×d, 2H, C_3H+C_5H, Ar, $J_{3,5}$=1.98Hz), 7.68 (d, 1H, NH-CO). $(CD_3)_2CO$ 3.67 (m, 2H, CH_2OH), 3.79 (s, 3H, NCH_3), 4.23 (m, 1H, CH-NH), 5.18 (d, 1H, Ar-CH-OH, J=2.84Hz), 6.46 (s, 1H, $CHCl_2$), 6.62 and 7.69 (2×d, 2H, C_3H+C_5H, Ar, $J_{3,5}$=1.94Hz). D_2O→$(CD_3)_2CO$	35.30 (NCH_3), 55.29 (CH-NH), 61.85)CH_2OH), 64.55 ($CHCl_2$), 67.41 (Ar-CH), 103.98 and 124.69 (C_3+C_5, Ar), 135.13 and 135.89 (C_2+C_4, Ar), 164.87 (CO-NH). $(CD_3)_2CO$

Table 3. M.I.C.'s (μg/ml) of chloramphenicol and its analogues

Microorganisms	Derivative			
	Chloramphenicol *(D–thereo)*	Thiamphenicol *(D–threo)**	Pyrrole analogue	
			VII *(DL)*	**IX** *(DL–threo)*
*Sarcina lutea***	1		8	4
Staphylococcus aureus ATCC 12600	0.5	25***	8	4
Bacillus subtilis ATCC 6051	0.5		0.8	8
Pseudomonas aeruginosa CCM 1960	16	100***	256	64
Pasteurella multocida Harvard No. 1		2.5		
Proteus vulgaris		100		
*Proteus mirabilis***	8		128	64
Escherichia coli ATCC 11775	2	50***	32	16
*Salmonella typhi***	2		32	16

* – literature data [30];
** – clinical isolates;
*** – microorganisms: *S. aureus* 209; *Ps. aeruginosa* 211; *E. Coli* 198 [30].

From aluminium isopropoxide, synthesized according to (29), its ca. 1M solution in anhydrous propan–2–ol was prepared.

A mixture of **VII** 1 g, 3.1 mol), 1M aluminium isopropoxide (7.13 ml, 7.0 mmol), and anhyd. propan–2–ol (10 ml) was heated for 10 h at 65°C, passing through the reaction pot a slow stream of dry argon. Afterwards, TLC showed no remaining starting material (system F). Then, to the reaction mixture EtOH and H_2O (4 ml + 1.4 ml) were added, and the solvent was evaporated under reduced pressure. Next, silica gel (2 g), EtOH (5 ml) and AcMe (10 ml) were added, and the solvent was removed under reduced pressure. The residue was poured over a column of silica gel (10 cm × 1.5 cm diam.). Elution with MeOH/AcMe/AcOEt (2:1:1) gave afforded crude **IX**, which was purified by column chromatography (42 cm × 1.5 cm) (system F) to give compound **IX** (0.23 g, 22.9%) as chromatographically homogenous glassy solid, which crystallized on standing.

Physical and spectral data of compounds **I–IX** are presented in Table 2.

DETERMINATION OF ANTIBACTERIAL ACTIVITY

The antibacterial activities of compounds **VI, VII, VIII** and **IX** were determined by establishing their minimal inhibitory concentrations (M.I.C.) against strains of microorganisms, as described by Sahm (27).

Simultaneously, the M.I.C.'s of chloramphenicol were determined.

Results of the antibacterial spectrum assay are presented in Table 3.

The growth of microorganisms was inhibited by compounds **VII** and **IX**. Compounds **VI** and **VIII** were inactive.

DISCUSSION

The seven–stage synthesis of compound **IX** was performed by means of the classical method used then, when the derivatives of pyrrole are obtained as well as by means of some selected methods used for the forming of the three–carbon

aliphatic fragment of chloramphenicol. There is no information in the literature concerning the compounds which we have synthesized, i.e. **I–XI**.

Compound **II** can be obtained either by the consecutive nitration and acylation reactions of *N*–methylpyrrole, or the order of the two reactions can be reserved. The most recent studies (31) have revealed the possibility of explosion of the reaction mixture during the acylation of the nitrocompounds in the presence of $AlCl_3$. It is why the acylation of *N*–methylpyrrole by bromoacetyl bromide was performed at first. The pyrrole ring is subjected to the acylation only in its position 2, according to the literature data (32, 33, 34), and the obtained 2–bromoacetyl–1–methylpyrrole [**I**] was next nitrated (33, 34, 35, 36, 37). In the ^{1}H NMR spectra of compounds **II** and **III**, the presence of pyrrole proton couplings constants was observed, 1.79 Hz and 4.72 Hz, respectively (38), which allowed to assign them structurally to 2–bromoacetyl–1–methyl–4–nitropyrrole [**II**] and 2–bromoacetyl–1–methyl–5–nitropyrrole [**III**], respectively.

Compound **II** during its reaction with urotropine (39, 40) was subjected to the transformation into a quaternary ammonium salt **IV**. The hydrolysis (37, 39, 40) of this salt gave hydrochloride of aminoketone **V**, easily subjected to the decomposition in alkaline or neutral media, even during a chromatogrpahy on silica gel. The transformation of aminoketone **V** into amide **VI** (41, 42) was carried out with excess dichloroacetyl chloride in order to avoid the decomposition of the substrate in the presence of triethylamine. The aldol condensation of compound **VI** with formaldehyde (24, 25, 43) in the presence of $NaHCO_3$ has resulted, according to the expectations, in the mixture of two compounds, being the products of the addition of 1 or 2 moles of HCHO, i. e. **VII** and **VIII**. The structure of these compounds was confirmed by means of the NMR spectrometry.

The reduction of the oxo grup in compound **VII** can, depending on the used reducing reagents, result in a product having either a *threo*– or *erythro*– configuration. It is possible to obtain the desired compound **IX** with the *threo*– configuration, due to the reduction with aluminium isopropoxide according to the Meerwein–Ponndoff method (26). The hardly filtrated aluminium hydroxide was eliminated by its precipitation on silica gel, while compound **IX** was extracted and purified by means of column chromatography.

^{1}H NMR and ^{13}C NMR spectra of the final product were compared with the spectra of chloramphenicol as well as with the data presented in the literature (2), regarding the spectra of *erythro*–chloramphenicol isomer. The data thus obtained clearly indicate that compound **IX** has a *threo*–configuration; its *erythro*– configuration was excluded. The $J_{1',2'}$ coupling constants are practically identical for compound **IX** and for chloramphenicol (2.84 Hz and 2.4 Hz). A great similarity of signals of the aliphatic fragment for compound **IX** and chloramphenicol also confirms the same configuration of these fragments.

The data presented by Jardetzky (2) have suggested that the distribution of electron density in the aromatic ring also has an influence on the $J_{1',2'}$ coupling constants. Therefore, it can be assumed that the heteroaromatic fragment of the compound obtained is characterized by its electronic structure, which is very close to that of the benzenoid fragment in chloramphenicol.

The results of the efforts to establish the minimal concentration inhibiting the growth of microorganisms have indicated that compound **IX**, being the racemate (*DL*), is 4–16 times less active than chloramphenicol.

A great number of data presented in the professional literature regarding the activity of *D–threo*– enantiomers of various analogues of chloramphenicol, and their proper racemates (*DL–threo*–) indicate that the antibacterial activity of a racemate is always twofold less as the activity of the correspondng *D–threo*– compound. When calculating it per *D–threo*–enantiomer, present in the racemate **IX**, its activity is 2–8 times smaller than that of a *D–threo*–chloramphenicol, i. e. it is closer to the activity of thiamphenicol (3) or cetophenicol (7).

Compound **VII** reveals a fairly significant activity, similarly to other analogues of chloramphenicol containing the oxo group instead of the –CHOH– group (3). The lack of biological activity in compounds **VI** and **VIII** is in agreement with the expectations, and the respective literature data regarding the comounds of analogous structure (3).

The presence of biological activity in compound **IX** can suggest that other derivatives of chloromycetin which contain pyrrole rings and other electronegative groups linked to them, can indicate greater activity than that of chloromycetin, but with a lesser toxicity at the same time. It can be suspected that the biotransformation of the nitrophenyl group is carried out through a different path than that of the differently substituted nitropyrrole group.

REFERENCES

1. Bitny–Szlachto S.: Wiad. Chem. 7, 391 (1953).
2. Jardetzky O.: J. Biol. Chem. 238, 2498 (1963).

3. Kolossov M.N., Shemyakin M.M., Khokhlov A.S., Gurevitch A.I.: in Khimiya Antibiotikov, Vol. 1, Shemyakin M.M., Khokhlov A.S., Kolossov M.N., Bergelson L.D., Antonov W.K. Eds., p. 337, Academy of Sciences, U.S.S.R., Moscow 1961 (in Russian).
4. Korzybski T., Kowszyk–Gindifer Z., Kuryłowicz W.: in Antybiotyki. Pochodzenie, rodzaje i właściwości, Vol. 1, p. 403, PZWL, Warszawa 1977.
5. Podlewski J.K., Chwalibogowska–Podlewska A.: in Leki współczesnej terapii, p. 47, PZWL, Warszawa 1985.
6. Shavel J., Jr., Bobowski G.: Belg. Pat. 669, 982, C. A. 66, 18596w (1967).
7. von Standtmann M., Bobowski G., Shavel J., Jr.: J. Med. Chem. 10, 888 (1967).
8. Ziebell G., Gross H., Bradler G.: Pharmazie 38, 587 (1983).
9. The Merck Index: Twelfth Edition. Merck & Co., Whitehouse Station, NJ 1996.
10. Hoar B.R., Jelinski M.D., Ribble C.S., Janzen E.D., Johnson J.C.: Can. Vet. J. 39 (3), 161 (1998).
11. Pfenning A.P., Madson M.R., Roybal J.E., Turnipseed S.B., Gonzales S.A., Hurlbut J.A.: J. AOAC Int. 81 (4), 714 (1998).
12. (a) Hermann E.C., Kreuchunas A.: J. Am. Chem. Soc. 74, 5168 (1952).
(b) Carrara G., Weitnauer G.: Gazz. Chim. Ital. 81, 142 (1951). Cit. after [3]
13. (a) Long M.L., Jensen N.D.: US Pat. 2, 547, 712, Apr 3, 1951, C. A. 45, 9564b (1951).
(b) Long M.L., Jenesel N.D.: US Pat. 2, 547, 713, Apr 3, 1951, C. A. 45, 9565d (1951).
14. Nielsen P.A., Leick V., Buchardt O.: Acta Chem. Scand. B 29, 662 (1975).
15. Rebstock M.C., Stratton C.D.,: J. Am. Chem. Soc. 77, 3082 (1955).
16. Knolle P.: Dtsch. Apoth. Ztg. 113, 39 (1973).
17. Laporte J.R., Vidal X., Ballarin E., Ibanez L.: Br. J. Clin. Pharmacol. 46 (2), 181 (1998).
18. Walker S., Diaper C.J., Bowman R., Sweeney G., Kirkness C.M.: Eye 12 (Pt 5), 875 (1998).
19. Shemyakin M.M., Lurie M.J.: Zhurn. Obshch. Khim. 29, 2531 (1959).
20. Chemical Base Beilstein (Cross Fire plus Reactions plus Abstracts) (1999).
21. Jones R.A.: Adv. in Heterocycl. Chem. 11, 383 (1970).
22. Urbański T.: in Chemia i technologia materiałów wybuchowych, Vol. 1, pp. 113, 118, 137, Wyd. MON, Warszawa 1954.
23. Šorm F., Gut J., Suchý M., Reichl D.: Collect. Czech. Chem. Commun. 15, 501 (1950).
24. Evans D.D., Douglas S.M., Smith S.D., Tivey D.J.: J. Chem. Soc. 1954, 1687 (1954).
25. Long M.L., Troutman H.D.: J. Am. Chem. Soc. 71, 2469 (1949).
26. Smoleński J.: Przem. Chem. 9, 228 (1953).
27. Sahm D.F., Washington J.A. II: in Manual of Clinical Microbiology, Balows A. et al. Eds., p. 1105, American Society for Microbiology, Washington DC 1991.
28. Bobrański B.: in Preparatyka organicznych środków leczniczych, p. 134, PZWL, Warszawa 1971.
29. Vogel A.I.: in Preparatyka organiczna, p. 231, WNT, Warszawa 1984.
30. Diana G.D., Cutler R.A.: J. Med. Chem. 11, 1100 (1968).
31. Eisch J.J., Qian Y.: Chem. & Eng. News, Feb 9, p. 2 (1998).
32. Baird E.E., Dervan P.B.: J. Am. Chem. Soc. 118, 6141 (1996).
33. Treibs A., Jacob K.: Liebigs Ann. 733, 27 (1970).
34. Vecchietti V., Della Torre A., Lauria F., Castellino S., Monti G., Trane F., de Carneri I.: Eur. J. Med. Chem. 9, 76 (1974).
35. Chen Y.H., Lown J.W.: J. Am. Chem. Soc. 116, 6996 (1994).
36. Fournari P., Tirouflet J.: Bull. Soc. Chim. France 1963, 484 (1963).
37. Long M.L., Troutman H.D.: J. Am. Chem. Soc. 71, 2473 (1949).
38. Breitmeier E.: in Structure elucidation by NMR in organic chemistry, p. 24, 192, J. Wiley & Sons, Chichester – New York – Brisbane – Toronto – Singapore 1993.
39. Franklin C.S., Morris D.S., Smith S.D.: J. Chem. Soc. 1954, 1683 (1954).
40. Houben–Weyl Methoden der organischen Chemie. Band XI/1. Stickstoffverbindungen II. Amine, p. 105, Georg Thieme Verlag, Stuttgart 1957.
41. Kimoto S., Okamoto M., Yamaguchi S.: J. Pharm. Soc. Japan 72, 496 (1953).
42. Rebstock M.C., Pfeiffer E.L.: J. Am. Chem. Soc. 74, 3207 (1952).
43. Long M.L., Troutman H.D.: US Pa 2, 545, 092, Mar. 13, 1951, C. A. 45: 7149h (1951).

Received: 23.07.1999