

PREPARATION AND SOME BIOLOGICAL PROPERTIES OF N-ACETYLMURAMYL-ALANYL-D-ISOGLUTAMINE (MDP) ANALOGUES*

Milan ZAORAL^a, Jan JEŽEK^a and Jiří ROTT^b

^a Institute of Organic Chemistry and Biochemistry,
Czechoslovak Academy of Sciences, 166 10 Prague 6 and

^b Institute of Hygiene and Epidemiology, 100 42 Prague 10

Received April 19th, 1982

The condensation of 1- α -O-benzyl-4,6-O-benzylidene-N-acetylgalactomuramic acid (I), 1- α -O-benzyl-4,6-O-benzylidene-N-acetylallomuramic acid (VIII), and 1- α -O-benzyl-4,6-O-benzylidene-N-acetylnorallomuramic acid (XI) with alanyl-D-isoglutamine benzyl ester afforded 1- α -O-benzyl-4,6-O-benzylidene-N-acetylgalactomuramyl-alanyl-D-isoglutamine benzyl ester (XII), 1- α -O-benzyl-4,6-O-benzylidene-N-acetylallomuramyl-alanyl-D-isoglutamine benzyl ester (XIII), and 1- α -O-benzyl-4,6-O-benzylidene-N-acetylnorallomuramyl-alanyl-D-isoglutamine benzyl ester (XIV). Protecting groups were removed from XII–XIV and N-acetylgalactomuramyl-alanyl-D-isoglutamine (XV), N-acetylallomuramyl-alanyl-D-isoglutamine (XVI), and N-acetylnorallomuramyl-alanyl-D-isoglutamine (XVII) were obtained. XV–XVII showed lower pyrogenic and immunoadjuvant effect than N-acetylmuramyl-alanyl-D-isoglutamine.

Almost all peptidoglycans studied so far contain N-acylmuramic acid (NAM)** — the only known exception is the peptidoglycan isolated from *Micrococcus lyso-deicticus* in which the presence of mannuramic acid as a minor component has been demonstrated^{1,2}. Therefore, it has been generally accepted that NAM is essential for the immunoadjuvant activity of the peptidoglycan. Quite recently, however, papers^{3–5} appeared announcing the synthesis of N-acetylmuramyl-alanyl-D-isoglutamine (MDP) analogs containing manno-, galacto-, allo-, xylo-, and L-idomuramic acid. These papers*** prompted us to publish our results on the synthesis of galacto- (XV) and allomuramyl-MDP (XVI) (carried out by routes different from those described in^{3–6}) and of norallomuramyl-MDP (XVII) and of some of the chemical and pharmacological properties of XV–XVII.

* Part III in the series Synthetic Glycopeptides; Part II: This Journal 45, 1424 (1980).

** Symbols and abbreviations usual in peptide and saccharide chemistry are used. Unless stated otherwise the chiral amino acids are of L- and sugars of D-configuration. Muramic acid, i.e. 2-amino-2-deoxy-3-O-[(R)-1-carboxyethyl]-D-glucose, norallo-Mur 2-amino-2-deoxy-3-O-carboxymethyl-D-allose.

*** During preparation of this paper for press the full communication was published⁶.

The compounds *XV–XVII* were obtained by condensation of 1- α -O-benzyl-4,6-O-benzylidene-N-acetylgalactomuramic acid⁷ (*I*), 1- α -O-benzyl-4,6-O-benzylidene-N-acetylallomuramic acid (*VIII*), and of 1- α -O-benzyl-4,6-O-benzylidene-N-acetylrorallomuramic acid (*XI*) with alanyl-D-isoglutamine benzyl ester and by simultaneous removal of the protecting groups by sodium in liquid ammonia⁸. The compound *I* was prepared from benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy- α -glucopyranoside⁹ (*II*) by acetylation¹⁰, removal of the benzylidene protecting group, mesylation, replacement of the mesyl residues by acetyls with simultaneous rearrangement in position 4, deacetylation, introduction of the benzylidene group (this reaction sequence was carried out essentially according to¹¹), and reaction with L-chloropropionic acid. To prepare *VIII* and *XI*, benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy- α -D-glucopyranoside (*II*) was mesylated at position 3, reacted with sodium acetate and hydrolyzed as described in¹². The product was condensed with L-chloropropionic acid methyl ester and chloroacetic acid ethyl ester, respectively. The resulting esters were saponified to *VIII* and *XI*.

Homogeneity of *XV–XVII* was checked by usual methods. In addition to *XV–XVII*, MDP was investigated. It was found that MDP contains, besides the expected mixture of α and β anomers, two impurities (an anomeric mixture). These impurities were identified as arising from partially racemized lactyl residue (imperfect stereoselectivity of the coupling reaction between *II* and *rac*-2-bromopropionic acid methyl or ethyl ester). *XV* and *XVI* contained no such impurity. Details on the NMR and HPLC study of *XV–XVII* are presented in the following paper.

XV–XVII were assayed for pyrogenic and immunoadjuvant activity. The compounds increased markedly the body temperature of the experimental animal and the amount of circulating antibodies yet to a considerably smaller degree than MDP. The results obtained on the immunoadjuvant effect of *XV–XVII* differ from the results described in^{5,6} most probably due to differences in assay procedures. The biological properties of *XV–XVII* will be reported in a separate communication.

The sugar moiety is apparently not essential both for the pyrogenic and for the immunoadjuvant effect of peptidoglycan fragments. This follows both from our results^{13,14} and from results obtained in other laboratories¹⁵. However, the sugar moiety contributes deeply and in many ways (by evoking, stimulating, modulating *etc.*) to the effect.

EXPERIMENTAL

Melting points were determined on a Kofler block and are not corrected. The optical activity was measured in a Perkin-Elmer type 141 polarimeter, the infrared spectra in UR 20 Zeiss (Jena) spectrophotometer. The amino acid analyses were performed in type 6020 amino acid analyzer (Instrument Development Workshops, Czechoslovak Academy of Sciences, Prague) and the chromatographic check-up experiments on silica gel coated aluminium sheets (Kavalier, Vo-

tice). The detection was effected by chlorination^{16,17}. Unless stated otherwise the products were dried for analysis 15 h at 100°C and 10 Pa over phosphorus pentoxide. The values for galacto-, allo-, and norallomuramic acid are based on the color value of muramic acid¹⁴.

Benzyl 2-Acetamido-4,6-O-benzylidene-2-deoxy- α -D-glucopyranoside (*II*)

The treatment of D-glucosamine (obtained from its hydrochloride by the action of lithium methanolate¹⁸) with acetic anhydride in a mixture of dimethylformamide and methanol (17 : 3) afforded the N-acetyl derivative; from the latter the α -benzyl glycoside was obtained by the reaction with benzyl alcohol (2% HCl). The α -benzyl glycoside afforded compound *II* in a 52–64% yield by condensation with benzaldehyde in the presence of zinc chloride⁹. The m.p. of the once crystallized product was, in repeated syntheses, in the range 252–261°C, $[\alpha]_D^{22} + 104$ to $+110^\circ$ (c 1, pyridine). This product containing, according to the optical activity, c 92%–95% of the α -anomer was used in subsequent work. Recorded data¹⁰: α -anomer, m.p. 263–264°C, $[\alpha]_D^{20} + 120^\circ$ (c 1, pyridine), β -anomer¹⁰, m.p. 270–271°C, $[\alpha]_D^{25} - 89^\circ$ (c 0.8, pyridine).

Benzyl 2-Acetamido-3-O-acetyl-4,6-O-benzylidene-2-deoxy- α -D-glucopyranoside (*III*)

The product was prepared from 12.0 g (30 mmol) of *II* by treatment with acetic anhydride in pyridine according to paper¹⁰. At the end of the acetylation 5 ml of methanol was added to the mixture, the solution was taken to dryness under reduced pressure, the residue was mixed with 100 ml of toluene, and the mixture was evaporated under reduced pressure. The evaporation with toluene was repeated once more. The residue was crystallized from ethanol. Yield 11.9 g (90%), m.p. 203–205°C, $[\alpha]_D^{20} + 73.1^\circ$ (c 0.5, pyridine) (c 95% of α -anomer). Recorded data¹⁰: m.p. 198 to 200°C, $[\alpha]_D^{25} + 82^\circ$ (c 1, pyridine); β -anomer¹⁰, m.p. 273–274°C, $[\alpha]_D^{25} - 111^\circ$ (c 1, pyridine).

Benzyl 2-Acetamido-3-O-acetyl-2-deoxy- α -D-glucopyranoside (*IV*)

The removal of the benzylidene group and work up of the reaction mixture was carried out according to paper¹¹. The product was dried at 100 Pa over phosphorus pentoxide and recrystallized from ethyl acetate–light petroleum. From 47.7 g (108 mmol) of *III* a total of 32.6 g (85%) of *IV* was obtained. M.p. 110–118°C, $[\alpha]_D^{20} + 122.2^\circ$ (c 0.5, pyridine). Recorded data¹¹: m.p. 119–121°C, $[\alpha]_D^{25} + 132^\circ$ (c 1, pyridine).

Benzyl 2-Acetamido-3-O-acetyl-2-deoxy-4,6-di-O-methanesulfonyl- α -D-glucopyranoside (*V*)

The compound was obtained from *IV* according to¹¹. The quantity of *IV* used to start with was 28.2 g (82.5 mmol). Yield 33.8 g (80%). Recrystallization afforded 31.0 g (73.5%) of material of m.p. 138–144°C, $[\alpha]_D^{22} + 104^\circ$ (c 0.5, pyridine). Recorded data¹¹: m.p. 147–148°C, $[\alpha]_D^{25} + 107^\circ$ (c 1, pyridine).

Benzyl 2-Acetamido-2-deoxy- α -D-galactopyranoside (*VI*)

It was prepared according to paper¹¹, except that the saponification was effected by 1M-CH₃ONa. A quantity of 4.0 g (8 mmol) of *V* afforded 0.82 g (32.5%) of *VI*, m.p. 203–206°C, $[\alpha]_D^{22} + 228.5^\circ$ (c 0.4, water). Recorded data¹¹: yield 47%, m.p. 202–203°C, $[\alpha]_D^{25} + 211^\circ$ (c 1, water).

Benzyl 2-Acetamido-4,6-O-benzylidene-2-deoxy- α -D-galactopyranoside (VII)

The product was prepared according to¹¹, except that a double quantity of zinc chloride was employed in the reaction of VI with benzaldehyde. A quantity of 3.1 g (10 mmol) of VI afforded 3.34 g (84%) of VII m.p. 250–253°C, $[\alpha]_D^{20} + 223^\circ$ (c 0.5, pyridine) (after one crystallization from acetone–water). Recorded data^{11,19} m.p. 240–243°C, $[\alpha]_D^{25} + 186^\circ$ (c 1, pyridine); yield 40%, m.p. 246–247°C, $[\alpha]_D^{28} + 219^\circ$ (c 1, pyridine).

1- α -O-Benzyl-4,6-O-benzylidene-N-acetylgalactomuramic Acid (I)

The mixture of 2.75 g (6.9 mmol) of VII, 0.79 g (33 mmol) of sodium hydride in 190 ml of dioxane was stirred 1 h at 95°C. The temperature was decreased to 65° and 3.12 ml (36 mmol) of L-2-chloropropionic acid in 27 ml of dioxane, and after 1 h another portion of sodium hydride (3.15 g, 132 mmol) was added. The mixture was stirred 15 h at 65°C. Then it was cooled to 5°C, a mixture of 15 ml of dioxane and 3 ml water was added and the solution was evaporated *in vacuo*. The residue was dissolved in 100 ml of water, filtered and the filtrates were extracted twice with 30 ml of chloroform. The aqueous layer was cooled to 0°C and acidified to pH 3 with 6M-HCl. The product which had separated was filtered off, washed with water and dried. Yield 2.35 g (72.5%), m.p. 195–220°C. The product was dissolved in boiling ethyl acetate and filtered. The filtrates were set aside at +5°C overnight. The separated solid material was filtered off, washed with ethyl acetate and dried. Yield 1.15 g (35.4%), m.p. 219–221°C, $[\alpha]_D^{20} + 189.5^\circ$ (c 0.2, methanol), $[\alpha]_D^{20} + 179^\circ$ (c 0.2, pyridine). Recorded data⁷: m.p. 219–220°C, $[\alpha]_D^{20} + 188^\circ$ (c 0.4, methanol), $[\alpha]_D^{20} + 174^\circ$ (c 0.3, pyridine). L-Isomer⁷ m.p. 250–251°C, $[\alpha]_D^{20} + 166^\circ$ (c 0.2, methanol), $[\alpha]_D^{20} + 223^\circ$ (c 0.3, pyridine).

Benzyl 2-Acetamido-4,6-O-benzylidene-2-deoxy-3-O-methanesulphonyl- α -D-glucopyranoside (IX)

The compound was prepared according to¹². M.p. 198–200°C, $[\alpha]_D^{22} + 69^\circ$ (c 0.5, dimethyl sulphoxide), $[\alpha]_D^{22} + 69.4^\circ$ (c 0.5, pyridine). Literature^{12,10}: m.p. 198–199°C, $[\alpha]_D^{20} + 60.5^\circ$ (c 1, dimethyl sulphoxide); m.p. 198–199°C, $[\alpha]_D^{25} + 76^\circ$ (c 1, pyridine).

Benzyl 2-Acetamido-4,6-O-benzylidene-2-deoxy- α -D-allopyranoside (X)

X was obtained as described in¹². M.p. 203–206°C, $[\alpha]_D^{22} + 119^\circ$ (c 0.5, dimethyl sulphoxide). Literature¹²: m.p. 203–206°C, $[\alpha]_D^{20} + 118.5^\circ$ (c 1, dimethyl sulphoxide).

1- α -O-Benzyl-4,6-O-benzylidene-N-acetylallomuramic Acid (VIII)

The mixture of benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy- α -D-allopyranoside (2.76 g, 6.9 mmol), 0.24 g (10 mmol) sodium hydride and 50 ml of dimethylformamide was heated with vigorous stirring 1 h at 40°C. L-2-Chloropropionic acid methyl ester (2.15 ml, 20 mmol) was added to the mixture in the course of 15 min. Heating (70°C) and stirring was continued for 5 h. The solvent was evaporated *in vacuo*, 100 ml of water (0°C) was added to the residue and pH of the mixture was adjusted to 7 with HCl. The product was extracted into ethyl acetate (4 \times 40 ml). The combined extracts were dried (Na₂SO₄) and evaporated *in vacuo*. The residue was heated (80°C) 1 h with 50 ml methanol, 30 ml H₂O and 1.4 g NaOH. The mixture was concentrated to c. 1/3 *in vacuo*, 30 ml of water was added and the separated material was filtered off. The filtrates were acidified with 6M-HCl to pH 3. The separated product was filtered off, washed with

water and dried. Yield 1.9 g (60%), m.p. in the range 85–95°C. The product was converted to dicyclohexylammonium salt (prepared in ethyl acetate). Yield 2.1 g (78%). After two crystallizations from ethyl acetate–petroleum ether m.p. of the salt was 92–93°C, $[\alpha]_D^{23} + 79.3^\circ$ (*c* 0.4, methanol). For $C_{37}H_{52}N_2O_8$ (652.8) calculated: 68.07% C, 8.03% H, 4.29% N; found: 68.13% C, 8.25% H, 4.07% N. Homogeneous in TLC, benzene–ethyl acetate–methanol (5 : 2 : 5) and methanol–chloroform (3 : 1).

1- α -O-Benzyl-4,6-O-benzylidene-N-acetylneurallomuramic Acid (XI)

This product was synthesized according to the same procedure as VIII. From 2.75 g (6.9 mmol) of X, 0.24 g (10 mmol) of NaH and 2.12 ml (20 mmol) ethyl chloroacetate was obtained 1.55 g (50%) of crude and, after recrystallization from methanol–water, 1.14 g (36%) of the purified product m.p. 189–200°C. This material was used for further synthesis. Another two crystallizations from the same solvent system afforded analytically pure material. M.p. 200–201°C, $[\alpha]_D^{22} 94^\circ$ (*c* 0.2, methanol). For $C_{24}H_{27}NO_8$ (457.5) calculated: 63.01% C, 5.97% H, 3.06% N; found: 62.89% C, 5.92% H, 2.94% N. Homogeneous in TLC, benzene–methanol (7 : 3) and chloroform–methanol (47 : 3).

1- α -O-Benzyl-4,6-O-benzylidene-N-acetylgalactomuramyl-alanyl-D-isoglutamine Benzyl Ester (XII)

A solution of 1.41 g (3 mmol) of I and 0.33 ml (3 mmol) of N-methylmorpholine in 15 ml of dimethylformamide was cooled down to –15°C and treated with 0.286 ml (3 mmol) of ethyl chloroformate. The mixture was allowed to stand 5 min at 0°C, cooled down to –15°C and a solution was added of 1.26 g (3 mmol) of H-Ala-D-iGln(OBzl) trifluoroacetate and of 0.33 ml (3 mmol) of N-methylmorpholine in 20 ml of dimethylformamide, precooled to –15°C. The mixture was set aside for 1 h at +5°C and then poured into 160 ml of 1% HCl cooled down to 0°C. The product which had separated was filtered off, triturated (3 \times) with 1% HCl, a solution of sodium hydrogen carbonate (3 \times), water (2 \times), filtered off, and dried. Yield 2.11 g (92.5%), m.p. 188–197°C. Recrystallization from dimethylformamide–water afforded 1.84 g (81%) of a product of m.p. 256–257°C, $[\alpha]_D^{25} + 136.7^\circ$ (*c* 0.3, dimethylformamide). For $C_{40}H_{48}N_4O_{11}$ (760.8) calculated: 63.15% C, 6.36% H, 7.36% N; found: 62.86% C, 6.26% H, 7.50% N. The infrared spectrum was in accordance with the structure of the compound. It differed markedly from the corresponding derivative of N-acetylmuramic acid. When subjected to the thin-layer chromatography in systems 1-butanol–tert-butanol–acetic acid–water (2 : 2 : 1 : 1), chloroform–methanol (9 : 1) the product behaved as a homogeneous substance. Amino acid analysis: galactomuramic acid 1.3, Ala 1.0, Glu 0.97.

1- α -O-Benzyl-4,6-O-benzylidene-N-acetylallomuramyl-alanyl-D-isoglutamine Benzyl Ester (XIII)

XIII was prepared in the same way as XII. From 0.96 g (2.04 mmol) of VIII, 0.86 g (2.04 mmol) H-Ala-D-iGln(OBzl).CF₃COOH was obtained 1.53 g (84.5%) of crude and 1.42 g (78%) of purified (reprecipitation from tetrachloromethane–light petroleum) XIII. M.p. 85–95°C (unchanged after further reprecipitation from the same solvents). $[\alpha]_D^{23} + 34.2^\circ$ (*c* 0.5, dimethylformamide). For $C_{40}H_{48}N_4O_{11}$ (760.9) calculated: 63.15% C, 6.36% H, 7.36% N; found: 62.92% C, 6.43% H, 7.49% N. Homogeneous in TLC 1-butanol–acetic acid–water (4 : 1 : 1) and methanol–chloroform (1 : 10).

1- α -O-Benzyl-4,6-O-benzylidene-N-acetyl norallomuramylalanyl-D-isoglutamine Benzyl Ester (XIV)

XIV was prepared in the same manner as XIII. From 1.13 g (2.48 mmol) of XI and 1.04 g (2.48 mmol) of alanyl-D-isoglutamine benzyl ester trifluoroacetate was obtained 1.8 g (98%) of crude and 1.67 g (90%) of purified (reprecipitation from benzene–light petroleum) XIV. M.p. 86–88°C, $[\alpha]_D^{22} + 46.9^\circ$ (c 0.5, dimethylformamide). For $C_{39}H_{46}N_4O_{11}$ (746.9) calculated: 62.72% C, 6.21% H, 7.50% N; found: 62.60% C, 6.42% H, 7.49% N. Amino acid analysis: norallomur 0.51, Ala 1.0, Glu 1.01. Homogeneous in TLC 1-butanol–acetic acid–water (4 : 1 : 1), and chloroform–ethanol (47 : 3).

N-Acetylgalactomuramyl-alanyl-D-isoglutamine (XV)

The preparation of XV from XII was effected in the same manner as the preparation of the corresponding muramic acid derivative⁸. From 760 mg (1 mmol) of XII was obtained 474 mg of desalted product (lyophilisate). The product was purified on a Zerolite FF column (50 \times 1.5 cm acetate form). The fractions containing the product were pooled and freeze-dried. The lyophilisate was further dried 24 h *in vacuo* over P_2O_5 . 310 mg (60%). $[\alpha]_D^{22} + 58.2^\circ$ (c 0.5, H_2O); + 56.5° (after 24 h). For $C_{19}H_{32}N_4O_{11} \cdot H_2O$ (510.5) calculated: 44.70% C, 6.71% H, 10.98% N; found: 44.68% C, 6.5% H, 10.72% N. Amino acid analysis: galactomuramic acid 1.46, Ala 1.0, Glu 0.98, NH_3 1.08. Homogeneous in TLC (1-butanol, acetic acid, water 4 : 1 : 1 and in 1-butanol, acetic acid, water, pyridine 15 : 3 : 10 : 12). Electrophoretically homogeneous in pyridine–acetate buffer pH 5.7.

N-Acetylallomuramyl-alanyl-D-isoglutamine (XVI)

XVI was prepared in the same manner as XV. From 760 mg (1 mmol) of XIII was obtained 520 mg of crude and 380 mg (72%) of purified product $[\alpha]_D^{22} - 61.7^\circ$ (c 0.3, H_2O); $- 67.8^\circ$ (after 24 h). For $C_{19}H_{32}N_4O_{11} \cdot 1 \frac{1}{2} H_2O$ (519.5) calculated: 43.93% C, 6.79% H, 10.78% N; found: 43.80% C, 6.49% H, 10.56% N. Amino acid analysis: allomuramic acid 0.32, Ala 1.0, Glu 0.98. Homogeneous in TLC (1-butanol, acetic acid, water 4 : 1 : 1 and in 1-butanol, acetic acid, water, pyridine 15 : 3 : 10 : 12). Electrophoretically homogeneous in pyridine–acetate buffer pH 5.7.

N-Acetyl norallomuramyl-alanyl-D-isoglutamine (XVII)

The same procedure as described in the preceding experiment was used for the preparation of XVII from XIV. From 747 mg (1 mmol) of XIV was obtained 520 mg of crude and 360 mg (70%) of purified XVII. $[\alpha]_D^{22} - 48.3^\circ$ (c 0.24, H_2O); $- 49.7^\circ$ (after 24 h). For $C_{18}H_{30}N_4O_{11} \cdot 2 H_2O$ (514.5) calculated: 42.02% C, 6.66% H, 10.89% N; found: 42.13% C, 6.69% H, 10.92% N. Amino acid analysis: norallomuramic acid 0.52, Ala 1.0, Glu 1.0, Homogeneous in TLC 1-butanol–acetic acid–water (4 : 1 : 1) 1-butanol–acetic acid–water–pyridine (15 : 3 : 10 : 12) and electrophoretically homogeneous in pyridine–acetate buffer pH 5.7.

REFERENCES

1. Hoshino O., Zehavi U., Sinaÿ P., Jeanloz R. W.: J. Biol. Chem. 247, 381 (1972).
2. Sinaÿ P., Halford M. D. A., Choudhary M. S., Gross P. H., Jeanloz R. W.: J. Biol. Chem. 247, 391 (1972).

3. Hasegawa A., Okomura H., Kiso M., Azuma I., Yamamura Y.: *Carbohydr. Res.* **79**, c 20 (1980).
4. Hasegawa A., Kaneda Y., Amano M., Kiso M., Azuma I.: *Agric. Biol. Chem.* **42**, 2187 (1978).
5. Kiso M., Kaneda Y., Goh Y., Hasegawa A., Azuma I.: *Agric. Biol. Chem.* **44**, 1971 (1980).
6. Hasegawa A., Kaneda Y., Goh Y., Nishibori K., Kiso M., Azuma I.: *Carbohydr. Res.* **94**, 143 (1981).
7. Sinaý P., Jeanloz R. W.: *Carbohydr. Res.* **10**, 189 (1969).
8. Zaoral M., Ježek J., Straka R., Mašek K.: *This Journal* **43**, 1797 (1978).
9. Kuhn R., Baer H., Seeliger H.: *Justus Liebigs Ann. Chem.* **611**, 236 (1958).
10. Gross P. H., Jeanloz R. W.: *J. Org. Chem.* **32**, 2759 (1967).
11. Gross P. H., Du Bois F., Jeanloz R. W.: *Carbohydr. Res.* **4**, 244 (1967).
12. zu Reckerndorf W. M.: *Chem. Ber.* **102**, 4207 (1969).
13. Mašek K., Zaoral M., Ježek J., Krchňák V.: *Experientia* **35**, 1397 (1979).
14. Zaoral M., Ježek J., Krchňák V., Straka R.: *This Journal* **45**, 1424 (1980).
15. Migliore-Samour D., Bouchaudon J., Floch F., Zerial A., Ninet L., Werner G. H., Jollés P.: *C. R. Acad. Sci., Sér. D* **289**, 473 (1979).
16. Reindel F., Hoppe W.: *Chem. Ber.* **87**, 1103 (1954).
17. Brenner M., Zimmermann J. P., Wehrmüller J., Quitt P., Hartmann A., Schneider W., Beglinger U.: *Helv. Chim. Acta* **40**, 1497 (1957).
18. Arendt A., Kolodziejczyk A., Sokolowska T.: *Rocz. Chem.* **48**, 1707 (1974).
19. Flowers H. M., Shapiro D.: *J. Org. Chem.* **30**, 2041 (1965).