SYNTHESIS OF LIPOPHILIC DIESTERS OF N-ACETYLMURAMOYL-L-ALANYL-D-GLUTAMIC ACID

V. V. Terekhov, A. E. Zemlyakov, and V. Ya. Chirva

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Lipophilic diesters of N-acetylmuramoyl-L-alanyl-D-glutamic acid have been synthesized from the dipentadecyl, didodecyl, and dioctyl esters of D-glutamic acid.

Analogues of N-acetylmuramoyl-L-alanyl-D-isoglutamine (I, MDP) (N-acetylmuramoyl-L-alanyl-D-glutamine (II) and N-acetylmuramoyl-L-alanyl-D-glutamic acid (III)) possess comparable adjuvant activities [1, 2]. The glutamine moieties in glycopeptides (I) and (II) have been used to obtain lipophilic monoesters [2, 3], while alkyl esters of (II) exhibit not only a high adjuvant activity but also apyrogenic properties [2]. For compound (III) only the dimethyl ester [4] and α-methyl-γ-alkyl diesters [3] are known.

We have previously synthesized a number of derivatives of MDP distinguished by the method of addition of the lipophilic component to the muramoyldipeptide molecule [5, 6]. The synthesis of such compounds is necessary for studying the influence of a change in the hydrophilic-lipophilic balance among MDP derivatives on their biological activity. Furthermore, lipophilic glycopeptides are excreted from the organism more slowly and can be used to obtain stable emulsions or liposomes containing adjuvants. In continuation of these investigations, we have performed the synthesis of lipophilic α,γ-dialkyl esters of N-acetylmuramoyl-L-alanyl-D-glutamic acid (VIII A-C) differing by the length of the aliphatic chain.

The synthesis of these compounds was performed by the following scheme. D-Glutamic acid was esterified with heptadecyl, dodecyl, and octyl alcohols, respectively. In the IR spectra of the diesters (IV A-C) intense absorption bands of methylene groups (2915, 2845 cm^{-1}) and of ester carbonyl groups (1740 cm^{-1}) were observed. Compounds (IV A-C) were condensed with the p-nitrophenyl ester of Boc-L-alanine, and the dipeptide derivatives (V A-C) were obtained with yields of about 80%. In the PMR spectrum of dipeptide (V A) (Table 1), the signals of the methyl groups of the Boc protection (singlet with CS 1.46 ppm), of an alanine residue (doublet of a methyl group with a CS of 1.37 ppm and quartet of a methine proton with a CS of 4.59 ppm), and of two heptadecyl fragments (triplet of the terminal methyl group with a CS of 0.89 ppm, multiplets of methine protons with CSs of 1.29 and 1.61 ppm, and two triplets of α-methylene groups with CSs of 4.07 and 4.13 ppm) were identified (see scheme on following page).

Glycopeptides (VI A-C) were synthesized by the N-hydroxysuccinimide method from 1α-O-benzyl-4,6-O-benzylidene-N-acetylmuramic acid and the α,γ-dialkyl esters of L-alanyl-D-glutamic acid. Compounds (VI A-C) had similar IR and PMR spectra. In the PMR spectra (Table 1) it was possible to see the signals of the lipophilic fragment (triplet of the two terminal methyls in the 0.85-0.89 ppm region, multiplet of methylene protons with CSs of 1.23-1.33 ppm, and triplets of $COOCH_2$ groups in the 3.97-4.14 ppm region). The structures of the carbohydrate moieties of the glycopeptides were confirmed by the signals of the protons of the benzylglycosidic and benzylidene protections (two doublet of the AB system of a $O\underline{CH}_2Ph$ group with CSs of 4.51-4.70 and 4.71-4.75 ppm, singlet of a methine proton with a CS of 5.58-5.71 ppm, and a multiplet of the phenyl protons with CSs of 7.37-7.39 ppm). The α configuration of the glycosidic centers was shown by the presence of one-proton doublets with CSs of 4.88-4.96 ppm and SSCCs of 3.0-3.5 Hz.

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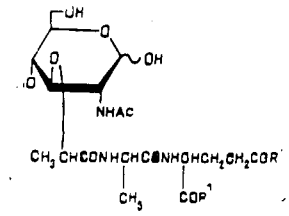
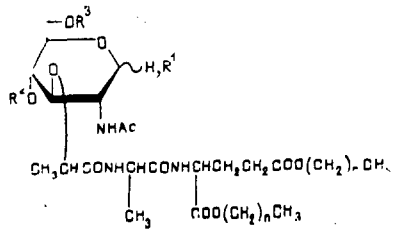


I. $R^1=NH_2$; $R^2=OH$
II. $R^1=OH$; $R^2=NH_2$
III. $R^1=R^2=OH$



VI. $R^1=\alpha OBzl$; R^2, $R^3=\geq CHPh$
VII. $R^1=\alpha OBzl$; $R^2=R^3=H$
VIII. $R^1=OH$; $R^2=R^3=H$

The temporary protections were eliminated by successive acid hydrolysis and catalytic hydrogenolysis. The desired compounds (VIII A-C) had identical IR spectra in which the absorption bands of phenyl groups were absent. The signals of the protons of the protective groups were not observed in the PMR spectra of glycopeptide (VII A), either (Table 1).

EXPERIMENTAL

Melting points were determined on a PTP instrument, and optical rotations at 20-22°C on a Polamat-A polarimeter (GDR). PMR spectra were obtained on Bruker WM-250 (250 MHz) and Bruker WM-500 (500 MHz) spectrometers with TMS as internal standard. IR spectra were recorded on Specord 75-IR spectrophotometer (GDR, KBr tablets), and TLC was conducted on Silufol UV-254 plates (Czechoslovakia), the zones being detected by carbonization at 300°C. The following solvent systems were used: 1) benzene–methanol (10:1); 2) chloroform–ethanol (10:1); and 3) chloroform–ethanol (5:1). Column chromatography was conducted on washed silica gel L 100-250 μm (Czechoslovakia). The results of the elementary analysis of all the compounds corresponded to the calculated values. D-Glutamic acid from the Peptide Institute Inc. (China) was used.

Diheptadecyl Ester of D-Glutamic ACid (IV A). A mixture of 0.50 g (3.4 mmole) of D-glutamic acid, 2.61 g (10.2 mmole) of heptadecan-1-ol, and 0.97 g (5.1 mmole) of p-toluenesulfonic acid in 50 ml of benzene was boiled with a Dean-Stark trap. The solution was neutralized with triethylamine and washed with water. The organic layer was dried with

TABLE 1. PMR Spectra of Compounds V A, VI A-C, and VIII A*

Compound	CH_3CH_2	$(CH_2)_n$	CH_2CH	NAc	$COOCH_2$
V A	0,89 t	1,29 m, 1,61 m	1,37 d		4,07 t, 4,13 t
VI A	0,89 t	1,33 m, 1,57 m	1,42 d, 1,49 d	1,96 s	4,07 t, 4,14 t
VI B	0,85 t	1,23 m, 1,62 m	1,23 m,	1,80 s	3,97 t, 4,00 t
VI C	0,89 t	1,28 m, 1,61 m	1,42 d, 1,43 d	1,96 s	4,04 t, 4,09 t
VIII A	0,89 t	1,28 m, 1,63 m	1,49 d, 1,42 d	2,01 s	4 05 t, 4,08 t

Compound	OCH_2Ph (J_{gem}, Hz)	H-1 ($J_{1,2}$, Hz)	CHPh	Ph	NH
V A					4,97 d, 6,83 d
VI A	4,70 d, 4,75 d (11)	4,96 d (3,5)	5,58 s	7,37 m	6,27 d, 6,92 d, 6,96 d
VI B	4,51 d, 4,71 d (12)	4,88 d (3,5)	5,71 s	7,39 m	7,57 d, 8,18 d, 8,39 d
VI C	4,51 d, 4,72 d (12)	4,96 d (3)	5,58 s	7.39 m	6,23 d, 6,90 d, 6,94 d
VIII A					7,18 d, 7,30 d, 7,38 d

*Working frequency 500 MHz, except for compound (VI B) for which it was 250 mHz. The solvent was $CDCl_3$, except for compound (VI B), for which it was DMSO-d_6.

Na_2SO_4 and evaporated. The residue was purified by column chromatography [eluent: benzene–ethanol (100:1)], giving 1.89 g (89%) of the diester (IV A), mp 52-53°C, $[\alpha]_{546}$ +2° (c 1.0; $CHCl_3$), R_f 0.40 (system 1); ν_{max}^{KBr}, cm^{-1}: 3455-3340 (NH_2), 2915, 2845 (CH_2), 1740 (C=O).

The amorphous didodecyl ester (IV B) was obtained similarly with a yield of 82%, $[\alpha]_{546}$ +2° (c 1.0; $CHCl_3$), R_f 0.35 (system 1); ν_{max}^{KBr}, cm^{-1}: 3430-3260 (NH_2), 2915, 2845 (CH_2), 1740 (C=O), and so was the dioctyl ester (IV C) with a yield of 86%, $[\alpha]_{546}$ −5° (c 1.0; $CHCl_3$), R_f 0.32 (system 1); ν_{max}^{KBr}, cm^{-1}: 3395-3340 (NH_2), 2915, 2845 (CH_2), 1740 (C=O).

Diheptadecyl Ester of tert-Butoxycarbonyl-L-Alanyl-D-Glutamic Acid (V A). A solution of 1.70 g (2.7 mmole) of compound (IV A) in 7 ml of dry dioxane was treated with 0.84 g (2.7 mmole) of the p-nitrophenyl ester of tert-butoxycarbonyl-L-alanine [7] and 0.47 ml (3.4 mmole) of triethylamine. The reaction mixture was stirred at 40°C for 48 h and was then evaporated. The residue was dissolved in 100 ml of ethyl acetate, and the p-nitrophenol was washed out with 0.1 N aqueous ammonia. The organic layer was dried with Na_2SO_4 and evaporated. The residue was recrystallized from acetone. The yield of dipeptide (V A) was 1.67 g (78%), mp 55-56°C, $[\alpha]_{546}$ −4° (c 1.0; $CHCl_3$), R_f 0.76 (system 1); ν_{max}^{KBr}, cm^{-1}: 3325 (NH), 2915, 2845 (CH_2), 1740, 1700 (C=O), 1675, 1530 (amide).

Compound (V B) was isolated similarly after additional purification by column chromatography [with benzene–ethanol (100:1) as the eluent]; yield 78%, mp 48-50°C, $[\alpha]_{546}$ −14° (c 1.0; $CHCl_3$), R_f 0.71 (system 1); ν_{max}^{KBr}, cm^{-1}: 3315 (NH), 2915, 2845 (CH_2), 1740, 1690 (C=O), 1660, 1525 (amide); and so was compound (V C), yield 76%, $[\alpha]_{546}$ −22° (c 1.7; $CHCl_3$), R_f 0.64 (system 1); ν_{max}^{KBr}, cm^{-1}: 3330 (NH), 2915, 2845 (CH_2), 1740, 1700 (C=O), 1660, 1530 (amide).

Diheptadecyl Ester of (2-Acetamido-1-O-benzyl-4,6-O-benzylidene-2-deoxy-α-D-glucopyranos-3-yl)-D-lactoyl-L-alanyl-D-glutamid Acid (VI A). Benzyl 2-acetamido-4,6-O-benzylidene-3-O-(D,L-carboxyethyl)-2-deoxy-α-D-glucopyranoside [8] (240 mg, 0.5 mmole) was dissolved in 5 ml of THF and, with stirring, 63 mg (0.55 mmole) of N-hydroxysuccinimide and 113 mg (0.55 mmole) of dicyclohexylcarbodiimide were added. After 3 h, the precipitate of dicyclohexylurea was filtered off and it was washed with 2 ml of THF. The dipeptide (obtained by treating 400 mg (0.5 mmole) of compound (V A) with 3 ml of trifluoroacetic acid, followed by evaporation to dryness) and 0.11 ml of N-methylmorpholine were added. The precipitate was filtered off after 24 h and was purified by column chromatography [with benzene–ethanol (100:1) as eluent]. This gave 448 mg (68%) of the amorphous compound (VI A), $[\alpha]_{546}$ +30° (c 1.0; $CHCl_3$), R_f 0.73 (system 2); ν_{max}^{KBr}, cm^{-1}: 3275 (NH), 2915, 2845 (CH_2), 1735 (C=O), 1660, 1550 (amide), 735, 700 (Ph).

The glycopeptide (VI B) was synthesized similarly in a yield of 75%, $[\alpha]_{546}$ +40° (c 1.0; $CHCl_3$), R_f 0.71 (system 2); ν_{max}^{KBr}, cm^{-1}: 3275 (NH), 2915, 2845 (CH_2), 1735 (C=O), 1655, 1555 (amide), 735, 700 (Ph), and so was glycopeptide (VI C), in yield of 78%, $[\alpha]_{546}$ +44° (c 1.0; $CHCl_3$), R_f 0.68 (system 2); ν_{max}^{KBr}, cm^{-1}: 3275 (NH), 2915, 2845 (CH_2), 1740 (C=O), 1665, 1550 (amide), 735, 700 (Ph).

Diheptadecyl Ester of (2-Acetamido-1-O-benzyl-2-deoxy-α-D-glucopyranos-3-yl)-D-lactoyl-L-alanyl-D-glutamic Acid (VII A). Glycopeptide (VI A) (400 mg, 0.35 mmole) was dissolved with heating on the boiling water bath in 6 ml of 80% acetic acid, and the solution was heated for 30 min and was then evaporated to dryness. Column chromatography [with chloroform–ethanol (50:1) as eluent] led to the isolation of 267 mg (72%) of the diol (VII A), $[\alpha]_{546}$ +52° (c 0.5; $CHCl_3$), R_f 0.69 (system 3); ν_{max}^{KBr}, cm^{-1}: 3420-3260 (OH, NH), 2900, 2835 (CH_2), 1735 (C=O), 1645, 1545 (amide), 740, 705 (Ph).

Diol (VII B) was obtained similarly in a yield of 70%, $[\alpha]_{546}$ +56° (c 0.5; $CHCl_3$), R_f 0.68 (system 3); ν_{max}^{KBr}, cm^{-1}: 3420-3275 (OH, NH), 2915, 2845 (CH_2), 1735 (C=O), 1645, 1555 (amide), 740, 705 (Ph), and so was diol (VII C) in a yield of 73%, $[\alpha]_{546}$ +58° (c 0.5; $CHCl_3$), R_f 0.67 (system 3); ν_{max}^{KBr}, cm^{-1}: 3420-3275 (OH, NH), 2915, 2845 (CH_2), 1735 (C=O), 1645, 1550 (amide), 735, 700 (Ph).

Diheptadecyl Ester of O-(2-Acetamido-2-deoxy-D-glucopyranos-3-yl)-D-lactoyl-L-alanyl-D-glutamic Acid (VIII A). The benzyl glycoside (VII A) (230 mg, 0.22 mmole) was dissolved in 10 ml of THF–water (4:1) and was subjected to hydrogenolysis over 200 mg of 10% Pd/C at room temperature. After 48 h, the catalyst was filtered off and was washed with 3 ml of ethanol. The filtrate was evaporated and the residue was triturated in ether. This gave 166 mg (78%) of compound (VIII A), $[\alpha]_{546}$ +4° (c 1.0; $CHCl_3$), R_f 0.56 (system 3); ν_{max}^{KBr}, cm^{-1}: 3415-3275 (OH, NH), 2905, 2835 (CH_2), 1740 (C=O), 1660, 1545 (amide).

Compound (VIII B) was obtained similarly after additional purification by column chromatography [with chloroform–ethanol (25:1) as eluent] with a yield of 76%, $[\alpha]_{546}$ +4° (c 1.0; $CHCl_3$), R_f 0.49 (system 3); ν_{max}^{KBr}, cm^{-1}: 3415-3275 (OH, NH), 2905, 2835 (CH_2), 1745 (C=O), 1660, 1545 (amide), and so was compound (VIII C), with a yield of 74%, $[\alpha]_{546}$ −2° (c 1.0; $CHCl_3$), R_f 0.40 (system 3); ν_{max}^{KBr}, cm^{-1}: 3405-3275 (OH, NH), 2905, 2835 (CH_2), 1735 (C=O), 1660, 1545 (amide).

LITERATURE CITED

1. E. Lederer, J. Med. Chem., 23, No. 8, 819 (1980).
2. P. Lefrancier, M. Derrien, X. Jamet, J. Choay, E. Lederer, F. Audibert, M. Parant, F. Parant, and L. Chedid, J. Med. Chem., 25, No. 1, 87 (1982).
3. P. Lefrancier, M. Petitou, M. Level, M. Derrien, J. Choay, and E. Lederer, Int. J. Peptide Protein Res., 14, 437 (1979).
4. P. Lefrancier, J. Choay, M. Derrien, and I. Lederman, Int. J. Peptide Protein Res., 9, 249 (1977).
5. A. E. Zemlyakov and V. Ya. Chirva, Khim. Prir. Soedin., No. 5, 714 (1987).
6. A. E. Zemlyakov and V. Ya. Chirva, Khim. Prir. Soedin., No. 6, 892 (1988); A. E. Zemlyakov and V. Ya. Chirva, Bioorg. Khim., 14, No. 9, 1271 (1989); A. E. Zemlyakov, V. V. Terekhov, and V. Ya. Chirva, Khim. Prir. Soedin., No. 2, 249 (1990).
7. A. E. Lanzilotti, E. Benz, and L. Goldman, J. Am. Chem. Soc., 86, No. 9, 1980 (1964).
8. T. Osawa and R. W. Jeanloz, J. Org. Chem., 30, No. 2, 448 (1965).

HYDROPHOBIC PROPERTIES OF MAIZE ZEIN

I. M. Savich

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The proteins of the zein complex from maize endosperm have been studied with the aid of hydrophobic chromatography. Their best separation was achieved on a column with TSK gel HW-65f. By comparing the results of fractionation by hydrophobic chromatography with those of electrophoresis, it was found that electrophoresis in PAAG under denaturing conditions separates the zein protein into groups according to their surface hydrophobicity. The most hydrophobic is the high-molecular-mass group of zein polypeptides.

It is known that prolamines, the main reserve proteins of cereal grain, are hydrophobic compounds. They can exist in dissolved form only in the presence of polar solvents (ethanol, propanol, isopropanol, dimethyl sulfoxide, dimethylformamide) or in a buffer containing detergents (Na dodecyl sulfate, cetyltrimethylammonium bromide, Na deoxycholate, Triton X-100), urea, or guanidine-HCl. Their hydrophobicity is due, above all, to their peculiar amino acid composition: large amounts of isoleucine, leucine, proline, alanine, and amidated amino acids [1].

Using the principles of hydrophobic interaction, the inhomogeneity of individual groups of wheat gliadin has been demonstrated [2]. The separation of wheat gliadins and glutelins by hydrophobic chromatography on phenyl-Sepharose CL-4B showed that the unreduced aggregated polypeptides of these proteins were present in several fractions, which indicated their different surface hydrophobicities [3]. The surface hydrophobicities of α-, β-, and γ-gliadins depend on the presence of aromatic and aliphatic amino acid residues, while that of the ω-gliadins depends mainly on aromatic side chains [4]. This type of hydrophobicity (surface hydrophobicity) has been well studied mainly on wheat gliadins, and therefore an investigation of the zein proteins from maize endosperm with the aid of hydrophobic chromatography

V. R. Vil'yams Kazakh Scientific-Research Agricultural Institute, Alma-Ata Province.

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