The amount of lappaconitine in the total alkaloids was determined by a gravimetric method at the stage of treating the total ether-soluble alkaloids with methanol and on column chromatography of the mother solution on alumina [diethyl ether–hexane (1:1)]. Lappaconitine made up about 25% of the total alkaloids, i.e., 0.12-0.15% on the weight of the raw material.

Thus, for the first time, six alkaloids have been isolated from the epigeal part of Caucasian monkshood collected in the Northern Caucasus in the incipient vegetation phase, five of which were diterpene alkaloids (lappaconitine, lappaconine, N-deacetyllappaconitine, lycoctonine, and gigactonine), while one belonged to the isoquinoline group – corydine. Lappaconine and corydine have not previously been isolated from this species.

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SYNTHESIS OF THE DIBENZYL ESTER OF tert-BUTOXYCARBONYL-L-ALANYL-D-GLUTAMIC ACID FROM RACEMIC GLUTAMIC ACID

A. E. Zemlyakov, D. A. Poteev, and V. Ya. Chirva

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The synthesis has been effected of the dibenzyl ester of Boc-L-alanyl-D-glutamic acid by the esterification of D,L-glutamic acid, condensation of the racemic diester with the p-nitrophenyl ester of Boc-L-alanine, and separation of the mixture of diastereomers.

The dipeptide L-alanyl-D-glutamic acid (L-Ala-D-Glu) is a component part of a number of immunoactive glycopeptides [1-3]. Methods are known for obtaining the dibenzyl ester (DBE) of Z-Ala-D-Glu [2], the DBE of Boc-L-Ala-D-Glu (I) [1, 4, 5], and the dimethyl ester of Boc-L-Ala-D-Glu [6] in which D-glutamic acid was used. We have developed a synthesis of (I) from the cheap and readily available racemic glutamic acid which includes the stage of the azeotropic esterification of D,L-glutamic acid with benzyl alcohol and the condensation of the racemic diester with the p-nitrophenyl ester of Boc-L-alanine [7], with the subsequent separation of the mixture of diasteromers by crystallization from ether.

A mixture of 3.0 g (20.4 mmoles) of D,L-glutamic acid, 4.26 g (24.8 mmoles) of TsOH, 25 ml of benzyl alcohol, and 40 ml of benzene was boiled with a Dean–Stark trap until the evolution of water ceased. The solution was evaporated, and the DBE of D,L-Glu was precipitated with ether in an amount of 5.56 g (90%). A solution of 3.0 g (9.2 mmoles) of the diester in 5 ml of DMFA was treated with 3.0 g (9.7 mmoles) of the p-nitrophenyl ester of Boc-L-Ala and 1.3 ml of Et_3N, and the mixture was stirred at 40°C for 2 days. The resulting solution was evaporated and the residue was dissolved in EtOAc and was washed free from p-nitrophenol with 1 N aqueous ammonia. The organic layer was dried and evaporated. The residue (3.84 g; 84%) was crystallized from ether, to give 1.77 g (39%) of dipeptide (I) with mp 105-107°C and $[\alpha]_{546}^{20}$ −2.0° (c 2.0; MeOH). The mother solution was evaporated, giving a product crystallizing with difficulty from hexane (the DBE of Boc-L-Ala-L-Glu, II) with $[\alpha]_{546}^{20}$ −33° (c 2.0; MeOH).

M. V. Frunze Simferopol' State University.

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The additional crystallization of the dipeptide (I) from n-butanol gave 1.64 g (36%) of crystals with mp 106-107°C and $[\alpha]_{546}^{20}$ +1.4° (c 4.0; MeOH). PMR (500 MHz, $CDCl_3$): 1.34 (3H, d, J_{CH_3CH} = 7 Hz, CH_3CH), 1.45 (9H, s, Me_3C), 4.65 (1H, q, $CHCH_3$), 5.0 s, 5.14 d, 5.17 d (4H, $2COOCH_2$), 4.94 (1H, d, NH-Ala), 6.89 (1H, d, NH-Glu), 7.35 (10H, m, 2Ph).

From the DBEs of D-Glu and L-Glu we obtained authentic samples of dipeptides (I) and (II), respectively: (I) with mp 106-108°C, $[\alpha]_{546}^{20}$ +1.6° (c 4.0; MeOH), +7.4° (c 4.0; DMFA); the literature gave bp 99-101°, $[\alpha]_D^{25}$ +7° (DMFA) [1], mp 106.5-107°, $[\alpha]_D^{20}$ −8.2° (EtAc) [4], mp 98-100°, $[\alpha]_D^{20}$ −37.6° (c 4.0; MeOH); the literature gave bp 59-61°, $[\alpha]_D^{25}$ −34.8° (MeOH) [1].

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ANTIBIOTICS FROM STRAINS OF Bacillus pumilus ISOLATED FROM A MARINE SPONGE Dendrilla sp.

Sh. Sh. Afiyatullov, N. I. Kalinovskaya, T. A. Kuznetsova, E. P. Ivanova, and V. V. Mikhailov

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Continuing a study of the secondary metabolites of microorganisms associated with marine invertebrates [1], we have investigated two strains (D-7 and D-12) from the sponge Dendrilla sp. collected in November, 1986, off the coast of Madagascar. We have shown that during their development the strains D-7 and D-12 synthesize substances with an antimicrobial activity. From their morphological characteristics and physiological tests, the microorganisms were identified as Bacillus pumilus [2].

Bacillus pumilus D-7 was grown on Yoshimitsu–Kimura medium in a thermostat at 30°C for 120 h. The culture liquid (20 liters) was chromatographed on a column of Polikhrom-1 with elution by 8, 20, and 40% ethyl alcohols successively. The fractions isolated were tested for antimicrobial activity by the agar diffusion method and by bioautography, using Staphylococcus aureus as the test culture.

The fraction eluted by 20% alcohol was separated in the $CHCl_3$–CH_3OH (3:1, 2:1, and 1:1) systems on a column of silica gel that had been treated beforehand with a mixture of 0.2 M solutions of KH_2PO_4 and $Na_2HPO_4 \cdot 12H_2O$ (1:1, v/v). Evaporation of the $CHCl_3$–CH_3OH (1:1) fraction yielded 200 mg of compound (I); mass spectrum: 425 (M + H); UV spectrum, λ_{max} (methanol): 246, 314 nm, while the ^1H and ^{13}C NMR spectra of (I) coincided with the corresponding spectra of antibiotic B isolated previously by Japanese workers from a soil strain of B. pumilus, A1-77 [3].

Strain D-12 was grown on Yoshimitsu–Kimura medium on a shaking machine at 25°C for 48 h. The culture liquid (20 liters) was chromatographed on a column of Polikhrom-1. The

Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Branch, Russian Academy of Sciences, Vladivostok.

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