A Substance with Cytostatic and Apoptosis-Inducing Activity from Burdock Roots

R. S. BOEV

Institute of Petroleum Chemistry, Siberian Branch of the Russian Academy of Sciences, Prospekt Akademicheskiy 3, Tomsk 634021 (Russia)

E-mail: BRS-0@yandex.ru

(Received September 6, 2004; in revised form December 16, 2004)

Abstract

For the first time, b-asparagine was isolated from burdock roots and identified. It was established that b-asparagine extracted from roots exhibits cytostatic and apoptosis-inducing activity. Synthetic *L*-asparagine (manufactured in Japan) exhibits neither cytostatic nor apoptosis-inducing activity.

INTRODUCTION

Burdock root is included into the Russian pharmacopoeia and is widely used in folk medicine. Extracts from the roots are applied mainly as a diuretic, diaphoretic and blood-purifying remedy [1, 2], as antiulcer and adaptogenic agent [3, 4]. Antitumour action of the alcohol and dichloromethane extracts from burdock root towards experimental cancerous tumours in animals was proved by the authors of [5]. Burdock root was recommended as an antitumour remedy for prophylactics of malignant tumours [6].

We revealed that concentrated sap from roots exhibits not only cytostatic activity (stops growth and fission of tumour cells) but also induced apoptosis (genetically programmed cell death) [7].

Two burdock species grow over the territory of Russia: large burdock (*Arctium lappa* L.) and tomentose burdock (*Arctium tomentosum* Mil.). Burdock root contains inulin polysaccharide (up to 45 %), bardanic essential oil (up to 0.18 %), protein (about 12 %), vitamins: A, B complex, C, E, and P; mineral and tanning agents, flavonoids, polyacetylenic hydrocarbons, sitosterol, stigmasterol, alkaloids, 18 amino acids, 26 fatty acids [1, 2, 8]. No one of these compounds exhibits apoptosis-inducing or cytoststic activity.

The goal of the present work was to extract a substance from burdock root exhibiting cytostatic and apoptosis-inducing activity.

EXPERIMENTAL

Fresh spring roots of burdock of the second year of vegetation were used in the investigation. No species-based separation was carried out. Sap was squeezed from 10 kg of fresh root crushed in a kitchen unit; cellular tissue elements were filtered out. The volume of resulting sap was 5630 ml; it was a brown liquid with a sweetish specific taste and smell. Active fermentation process starts within an hour because of the high carbohydrate content, so the sap cannot be stored. In order to increase the possible storage time and concentration of biologically active compounds, the sap was evaporated under reduced pressure at a temperature not higher than 50 ^{o}C until the content of extractives reached about 70 %. After evaporation, 784 g of concentrated sap was obtained.

It was discovered that a substantial amount of crystal is formed in the concentrated sap within 10–20 days. In order to isolate the crystals from 30 g of concentrated sap, the viscous matter was washed with cold water (0–5 °C), the solid residue was collected on filters. After double recrystallization from water, 3 g (10 %) of colourless rhombic crystals were obtained. Found, %: C 35.20; H 6.48; N 22.60, $C_4H_8N_2O_3$ (HP-185 elemental analyzer for CHN). Calculated, %: C 36.36; H 6.06; N 21.21.

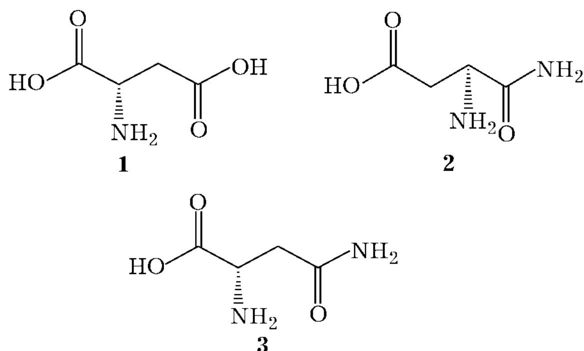
The IR spectra were recorded in KBr tablets with Bruker Vector 22 instrument (Germany). The NMR spectra were recorded with Bruker DRX 500 spectrometer, working frequency of the instrument: 500 MHz, external standard: HMDS; solvent: deuterated water.

Tests of the anti-neoplastic (anti-tumour) action were carried out with the suspension cultures of tumour cells of Ehrlich's carcinoma (2.5 10^5 cells/ml) towards inclusion of ^{3}H-thymidine followed by analysis with a Mark-III beta-counter and towards the reaction of blast transformation of human lymphocytes stimulated by phytohemagglutinin (PHA) after the action of b-asparagine within the concentration range (0.04–75) 10^{-3} M. Estimation of the effect of b-asparagine on the proliferative activity of lymphocytes in the induced PHA test was carried out according to the recommendations of the pharmacological committee of the Ministry of Health of RF [9]. In parallel with b-asparagine extracted from burdock root, we examined the biological activity of synthetic *L*-asparagine (Japan, lot 20010626).

RESULTS AND DISCUSSION

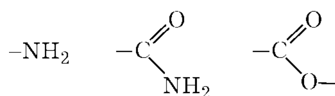
The crystal compounds isolated from the concentrated sap of burdock root are insoluble in organic solvents (hydrocarbons, alcohols, acetone, dimethylsulphoxide) and soluble in water and in the solutions of acids and alkalis.

The substance melts at a temperature above 200 °C with decomposition; we failed to establish the exact melting point. The physical characteristics revealed in the investigation are characteristic of the amino acids and their derivatives [10]. The ninhydrin reaction for amino acid gave a positive result. Ammonia is released during heating of the alkaline aqueous solutions of the crystals, which is characteristic of amides. The molecular formula calculated on the basis of elemental analysis corresponds to the formula of asparagine, or monoamide of aspartic acid (**1**). Depending on the positions of amino groups with respect to the amide group, a- and b-asparagine (**2**, **3**) is distinguished [11].



Since a-asparagine is obtained by means of synthesis, while the tissues of animals and plants contains b-asparagine mainly in the *L* form, it may be assumed that b-asparagine was isolated from burdock root sap. Its structure was confirmed with the help of spectroscopic methods.

The IR spectrum contains absorption bands confirming that the compound under investigation contains functional groups



Within the region of stretching N–H vibrations of primary amines, the bands at 3455 and 3382 cm^{-1} are observed. The amide group manifests itself in the spectrum as a band of stretching N–H vibrations at 3111 cm^{-1} and bending N–H vibrations at 1644 cm^{-1} (the amide II band), which is characteristic of primary amides. The second region in which these vibrations are manifested lied at lower frequencies: at 1429 cm^{-1} (the amide III band). The stretching vibrations of carbonyl group (C=O) of amides were discovered at 1682 cm^{-1} (the amide I band). The region 1500–1700 cm^{-1} contains a number of intensive bands attributed to the amide I, II, III bands as indicated above; the band at 1578 cm^{-1} corresponds to stretching

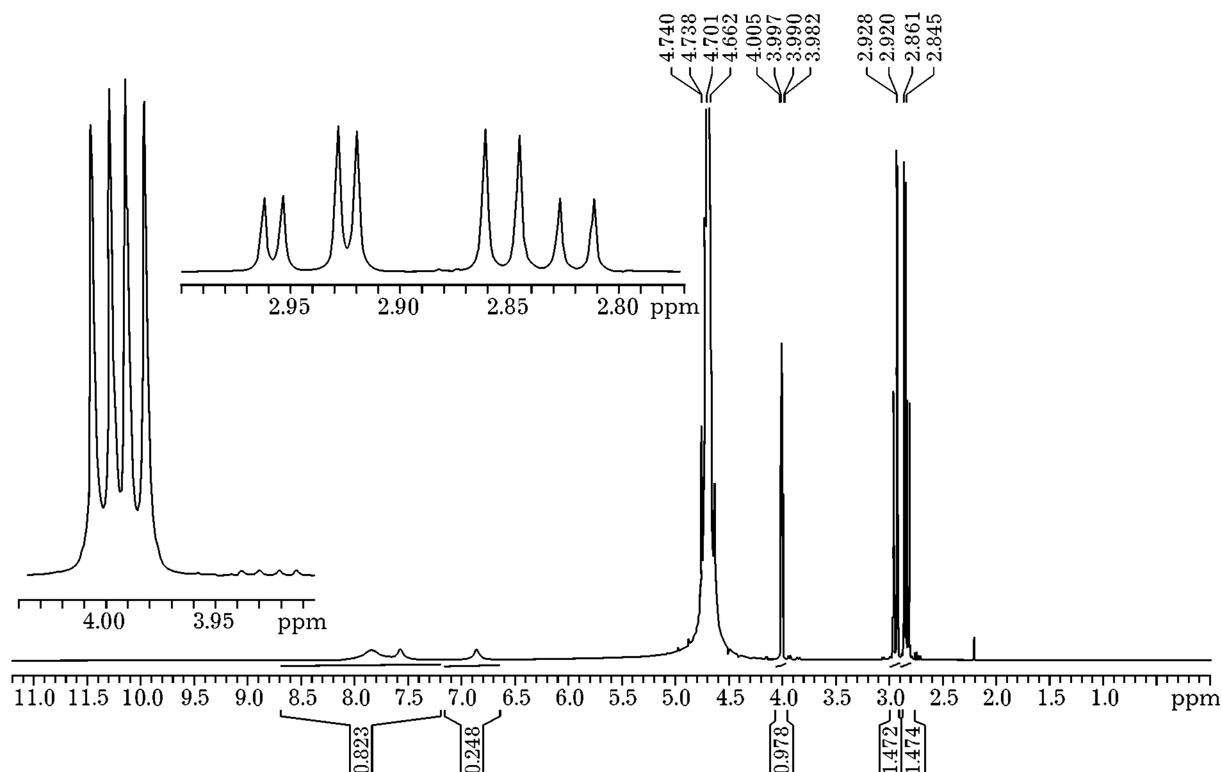


Fig. 1. PMR spectrum of b-asparagine isolated from the sap of burdock root.

vibrations of C=O in the ionized carboxyl group of the amino acid, which points to the zwitter-ion structure of asparagine. The band of bending N–H vibrations in the $^{+}NH_3$ group at 1528 cm^{-1} also confirms the zwitter ion structure. A broadened absorption band at 669 cm^{-1} corresponds to the bending vibrations of N–H in amines.

The IR spectrum of asparagine chlorohydrate provides evidence of the disappearance of zwitter ion structure; the bands of stretching C=O vibrations of non-ionized carboxyl of amino acid at 1730 cm^{-1} are present in the spectrum [12].

The PMR spectrum of the compound (Fig. 1) contains resonance signals from the protons of amino group at 7.85 ppm, protons of the amide group at 7.60 and 6.86 ppm. A doublet of doublets with the chemical shift of 4.0 ppm (J_1 = 4 Hz, J_2 = 8 Hz) corresponds to the methyne proton. Its multiplet character is the evidence of non-equivalence of the neighbouring methylene protons. Simulation of the part of the spectrum related to the mathylene protons gave the following parameters: the signal of one of the methylene protons has a chemical shift of 2.94 ppm (J_1 = 4 Hz), another methylene proton has 2.84 ppm (J_2 = 8 Hz), the absolute value of the spin-spin interaction between methylene protons is 17 Hz.

Investigation of the anti-blastic action showed that b-asparagine crystals isolated from burdock root suppress the PHA-induced proliferation activity of lymphocytes (Fig. 2). It was established that the synthetic preparation did not exhibit cytostatic activity, though the

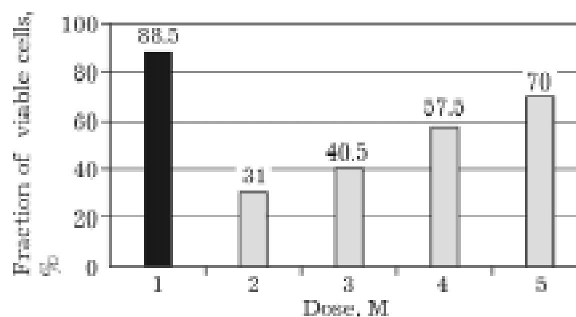


Fig. 2. Influence of b-asparagine from burdock root on the PHA-induced proliferation of donor lymphocytes (cytostatic effect): 1 – reference sample (cells + PHA), 2–5 – b-asparagine (2 – 75 10^{-3} M, 3 – 7.5 10^{-3} M, 4 – 0.75 10^{-3} M, 5 – 0.075 10^{-3} M).

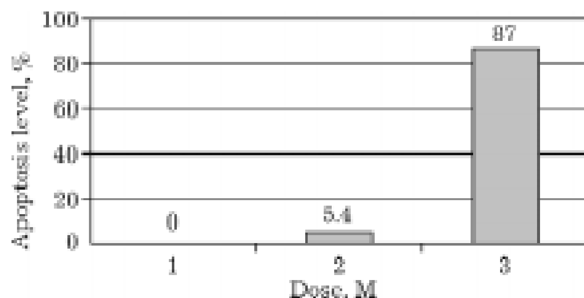


Fig. 3. Apoptosis-inducing action of b-asparagine from burdock root on tumour cells of Ehrlich's carcinoma: 1 – reference (cells without the preparation), 2, 3 – b-asparagine (2 – 0.4 10^{-3} M , 3 – 4 10^{-3} M b-asparagine).

IR and PMR spectra of *L*-asparagine (Japan) are almost identical with the spectra of asparagine extracted from burdock root.

Tests of the apoptosis-inducing action of the claimed preparation were carried out with the tumour cells of Ehrlich's carcinoma according to the procedure described in [13].

The data obtained provide evidence that b-asparagine isolated from burdock roots possesses dose-dependent apoptosis-inducing effect on tumour cells, which is most clearly exhibited for the concentration of about 4 10^{-3} M (apoptosis induction up to 87 % with respect to the reference sample containing tumour cells without the action), at the concentration of 0.4 10^{-3} M the induction of apoptosis is 5.4 % (Fig. 3). No apoptosis-inducing activity was detected in synthetic *L*-asparagine.

CONCLUSION

So, b-asparagine from burdock root was isolated and identified for the first time. Its apoptosis-inducing and cytostatic activity was proved.

REFERENCES

1 G. V. Krylov, Travy zhizni i ikh iskateli, Zap.-Sib. kn. izd-vo, Novosibirsk, 1972.

2 V. G. Minaeva, Lekarstvennye rasteniya Sibiri, Nauka, Novosibirsk, 1991.

3 T. A. Kanakina, Protivoyazvennaya aktivnost' izvlecheniy iz lopukha voylochnogo: Biological Sciences Candidate's Dissertation (Author's abstract), izd. Institute of Pharmacology TSC SB RAMS, Tomsk, 1997, 22 p.

4 V. N. Zhdanov, Vliyaniye izvlecheniy iz lopukha voylochnogo na toksicheskiye effekty etanola i gipoksiyu: Biological Sciences Candidate's Dissertation (Author's abstract), izd. Institute of Pharmacology TSC SB RAMS, Tomsk, 1997, 20 p.

5 S. Foldeak, G. A. Dombradi, *Acta Phys. Chem.*, 10(1964) 93.

6 Su-Mi Kim and Jong-Sang Kim, *FASEB J.*, 3 (1997) 369.

7 R. C. Boev, V. M. Plotnikov, Apoptozindutsiruyushchaya i tsitostaticheskaya aktivnost' kontsentrirovannogo soka kornya lopukha, Materialy konf. "Aktual'nye voprosy razrabotki i primeneniya immunobiologvicheskikh I farmatsevticheskikh preparatov", Tomsk, 2004, p. 276.

8 D. A. Shmatkov, Ispol'zovaniye fiziko-khimicheskikh metodov analiza dlya izucheniya khimicheskogo sostava, otsenki kachestva i standartizatsii korney lopukha: Phamacological Sciences Candidate's Dissertation, Moscow, 2000, p. 122.

9 *Vedomosti Farmakol. Komiteta*, 1 (1999) 31.

10 P. Karrer, Kurs organicheskoy khimii, Izd-vo khim. lit., Leningrad, 1962.

11 Khimicheskaya entsiklopediya, vol. 1, Izd-vo SE, Moscow, 1988.

12 K. Nakanisi, Infrakrasnye spektry i stroyeniye organicheskikh soyedineniy, Mir, Moscow, 1967.

13 S. N. Orlov., T. V. Dam, J. Trembly, *Biochem. Biophys. Res. Commun.*, 221 (1996) 708.