The year of 2012 is the fiftieth anniversary of the discovery of the main biologically active compounds of ginseng (genus *Panax* L.)—ginsenosides, triterpene glycosides of the dammarane series. In the past half-century, a considerable progress in studying the structural diversity of these compounds in intact plants was made. For example, in a relatively short period (January 2000–September 2010), 123 individual ginsenosides were isolated from different ginseng species and characterized. In the last six months (October 2010–March 2011), another 12 new structures were discovered [1].

Studies of ginseng cell and tissue cultures in vitro were started almost simultaneously with the discovery of ginsenosides [2]; however, advances in the study of the diversity of these compounds in sterile cultures seem modest. The vast majority of studies in this direction boil down to considering various aspects of accumulation in cells in vitro of only seven major neutral ginsenosides (Rg1, Re, Rf, Rb1, Rc, Rb2, and Rd), commercial reference samples of which are readily available [3]. However, the cell culture of higher plants is a unique biological system—a population of somatic cells, secondary metabolism in which may differ significantly from that in intact plants [4]. In this regard, the possibility of formation in cells in vitro of a complex pattern of ginsenosides, characteristic of ginseng in planta, is of special interest.

This is the first paper to report the finding of the acylated ginsenoside malonyl-Rb1 in cell culture of *Panax japonicus* var. *repens*.

Malonyl-Ginsenoside Rb1 in Cell Suspension Culture of *Panax japonicus* var. *repens*

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The object of the study was the suspended cell culture derived from the root of an intact two-year-old plant (Primorsky Krai, Russia) in 1997 [5]. The strain was registered in the All-Russia Collection of Cell Cultures of Higher Plants (no. 62). The growth conditions of the culture were described previously [6]. The total fraction of triterpene glycosides (TFG) was isolated by the standard methods [1] from 500 g of air-dry biomass of the cell culture grown in the 500-L bioreactor (1T, Russia). The purification of TFG and the isolation of individual components were performed by successive use of low-pressure column chromatography and HPLC [7]. 1H and 13C NMR spectra of the obtained compounds in the pyridine–d5/D2O mixture were recorded on a Bruker Avance AV600 instrument (Germany) using tetramethylsilane as an internal standard. High-resolution mass spectra with electrospray ionization were obtained on a Bruker micrOTOF II instrument [8].

As a result, we isolated two major triterpene glycosides (1 and 2) with yields of 0.1 and 0.2% of the dry weight cell biomass, respectively. The comparison of 1H and 13C NMR spectra and the mass spectrum of compound 2 with the published data [9] showed that it is identical to ginsenoside *Rb1*.

The 1H NMR spectrum of compound 1 contained signals from four anomeric protons H1 of monosaccharides at 4.88, 5.05, 5.10, and 5.28 ppm (all doublets, *J*1,2 7.2 Hz) and eight tertiary methyl groups of the aglycone (all singlets) at 0.81 (H19), 0.95 (H18), 0.97 (H30), 0.98 (H29), 1.29 (H28), 1.59 (H26), and 1.65 (H21, H27) ppm. The signals in the 1H and 13C NMR spectra were assigned using two-dimensional NMR experiments (1H–1H COSY, TOCSY, ROESY, 1H–13C HSQC, and HMBC) [10].

The 13C NMR spectrum of compound 1 contained signals from four anemic protons H1 of monosaccharides at 4.88, 5.05, 5.10, and 5.28 ppm (all doublets, *J*1,2 7.2 Hz) and eight tertiary methyl groups of the aglycone (all singlets) at 0.81 (H19), 0.95 (H18), 0.97 (H30), 0.98 (H29), 1.29 (H28), 1.59 (H26), and 1.65 (H21, H27) ppm. The signals in the 1H and 13C NMR spectra were assigned using two-dimensional NMR experiments (1H–1H COSY, TOCSY, ROESY, 1H–13C HSQC, and HMBC) [10].

The comparison of the 13C NMR spectra of compound 1 and ginsenoside *Rb1* (table) shows that they both contained β-sophorose and β-gentiobiose residues linked through glycosidic bonds with hydroxyl groups of the aglycone 20(S)-protopanaxadiol at positions 3 and 20, respectively (ROESY and HMBC data, figure). In this case, the difference in the 13C NMR spectra of these two compounds was only in the magnitude of the chemical shifts of C5 and C6 of the terminal glucose residue in β-sophorose (table). In the spectrum of compound 1, the signal from C5 was

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shifted by 2.9 ppm towards the strong field and the signal from C6 was shifted by 2.3 ppm towards the weak field compared to their position in the spectrum of ginsenosides Rb1. Signals from H6 (HOCH2 group) of this glucose residue in compound 1 was also shifted downfield by 0.7 and 0.4 ppm. These data suggest that compound 1 is a derivative of ginsenosides Rb1, acylated at position 6 of the terminal glucose residue of β-sophorose. However, signals from any acyl substituent in NMR spectra were not detected.

Mass spectrum of negative ions of compound 1 showed the presence of basic ion \([M-\text{H}]^-\) with \(m/z\) 1193.60, which corresponded to the malonyl derivative of ginsenoside Rb1 (malonyl-Rb1) (calculated value for compound C57H94O26 with \(m/z\) 1193.60). The mass spectrum of positive ions contained the peaks of four ions of malonyl—Rb1 adducts with \(m/z\) 1195.61 (\([M+\text{H}]^+\)), 1212.64 (\([M+\text{NH}_4]^+\)), 1217.59 (\([M+\text{Na}]^+\)), and 1233.57 (\([M+\text{K}]^+\)) (all calculated values...