The structure, properties, distribution in nature, and biological activity of sterol glycosides and acylglycosides are reviewed.

Sterols are one of the most widely distributed natural substances [1-3]. They exist in animals and plants in the free state and as derivatives. Glycoside and acylglycoside derivatives of sterols have been known for a long time. In the plant world, these compounds are found in higher plants [2, 4-6], algae [7], fungi [8-10], and bacteria [11-16]. In the animal kingdom, these substances have been identified in soft coral [17], holothuriae [18-20], amphibians [21], snakes [21, 22], and birds [21, 23]. Such a wide distribution in nature suggests that sterol glycosides and acylglycosides possess important physiological functions. Therefore, these compounds are constantly under intense scientific scrutiny. Sterol glycosides and acylglycosides are usually isolated from natural sources as very complicated mixtures that cannot always be separated into pure components. However, several physicochemical methods have recently been proposed for separating, analyzing, and identifying them [23-34]. Use of these methods greatly facilitates the investigation of glycosides and acylglycosides.

Owing to the wide distribution in nature and the variety of physiological functions for glycosides and acylglycosides, research articles about them are published in a wide range of scientific literature. However, this subject has not yet been reviewed. The present work is intended to fill that gap. It should be noted that we pay the most attention to discreet natural compounds that can be isolated pure and for which the structures are reliably proved since this is where our interest lies. Substances that have been isolated as mixtures and are insufficiently characterized are examined in less detail.

Some of the most widely distributed sterol glycosides are β-sitosterol 3-O-β-D-glucopyranoside (1). This compound was isolated from higher plants early in the 20th century under various names: ipuranol, citrullol, trifolianol, etc. [35, 36]. Later glycoside 1 was prepared by chemical synthesis via glycosylation of β-sitosterol [37]. Direct comparison with this synthesized compound provided final structural proof for various natural samples of 1 [36]. Glycoside 1 is the most typical sterol glycoside of higher plants and is usually observed in preliminary phytochemical analysis of them.

A large number of plants yield β-sitosterol 3-O-β-D-glucopyranoside as the only sterol glycoside [38-106]. Furthermore, compound 1 in several studies was isolated or observed in plants in a mixture with other sterol glycosides [6, 34, 81, 107-125].

Hydrolysis of glycoside 1 by sulfuric acid in ethanol with boiling for 22 h yields β-sitosterol and D-glucose [41]. The same result is obtained by hydrolysis in 6% HCl with heating for 50 min [46]. Hydrolysis of compound 1 to give β-sitosterol
and D-glucose by reaction with 20% sulfuric acid with heating at 100 °C for 10 h in a mixture of CHCl₃ and CH₃OH has also been reported [49]. The reaction of compound 1 with acetic anhydride in pyridine gives the tetraacetate with mp 171 °C [41]. The analogous reaction with benzoyl chloride in pyridine forms the tetrabenzoate with mp 201 °C [41].

The structure of glycoside 1 observed by phytochemical analysis is usually proved by comparison with an authentic sample isolated previously or obtained synthetically. Spectral data can also be a great aid to proving the structure of glycoside 1. Thus, field-desorption mass spectra of glycoside 1 contain peaks with $m/z$ 599 [M + Na]⁺, 577 [M + H]⁺, 415 [glycone + H]⁺, 397 [M - glucose + H]⁺, 382, and 256 [55]. The ¹H NMR spectra (δ, here and further) of compound 1 contain signals characteristic of six methyl groups of the β-sitosterol (δ 0.59, 0.77-0.85, and 0.92 ppm), of the D-glucose protons geminal to the hydroxy groups (3.8-4.5 ppm), and of the anomeric proton H-1' (4.93 ppm) and the vinylic proton H-6 (5.23 ppm) [49]. The signal of H-1' appears as a doublet. The magnitude of the splitting constant (J = 7.5 Hz) suggests that the glycoside exists in the β-configuration [49]. In other studies the signal of H-1' appears as a doublet at 4.72 ppm with J = 6.5 Hz [55]. Signals of all 35 C atoms can be assigned in the ¹³C NMR of 1 in deuteropyridine [49]. The signals of C-3 (78.7 ppm), C-5 (141.1 ppm), C-6 (121.9 ppm), and C-1' (102.8 ppm) are very important for the structure proof.

Another β-sitosterol monoglycoside, namely β-sitosterol 3-O-β-D-xylopyranoside (2), was isolated first from Maytenus senegalensis (Celastraceae) [126]. The structure of compound 2 was proved [126] mainly by acid hydrolysis to give β-sitosterol and D-xylose. Later [127] glycoside 2 was observed in the Argentinian plant Bauhinia candidans (Leguminosae), which is used in folk medicine. The compound was converted into the triacetate, the structure of which was proved through spectral analysis, in order to purify it. In particular, the ¹H NMR spectrum of the triacetate in CDCl₃ contains signals for the protons of the 18-, 19-, 26/27-, 29-, and 21-methyl groups at 0.68, 0.99, 0.83, 0.84, and 0.92 ppm, respectively, which is characteristic of β-sitosterol. A multiplet at 5.35 ppm for the vinylic proton H-6 also confirms that compound 2 contains the β-sitosterol moiety. The signal for the anomic proton H-1' appears as a doublet at 4.56 ppm. The splitting constant of this doublet (J = 8 Hz) indicates that this glycoside has the β-configuration. The spectrum of the triacetate also contains signals for the carbohydrate protons H-2', H-3', H-4', H-5' α and H-5' α (4.94, 5.12, 4.85-5.23, 4.19, and 3.71 ppm, respectively). The signals of all C atoms can be assigned in the ¹³C NMR spectrum of compound 2. The magnitude of the chemical shift of C-1" (99.5 ppm) indicates that it has the β-configuration. Furthermore, the chemical shifts of C-3" (71.4 ppm), C-4" (68.6 ppm), and C-5" (61.9 ppm) suggest that the xylose in glucoside triacetate 2 is the pyranose isomer. The aglycone in compound 2 has the (24R)-configuration, i.e., is a sitosterol residue, which is consistent with the signal of C-24 in the ¹³C NMR occurring at 45.7 ppm. Hydrolysis of compound triacetate 2 by HCl in methanol provides additional confirmation of its structure. The β-sitosterol and xylose are formed.

The β-sitosterol 3-O-β-D-glucuronopyranoside (3) was first found in the roots of Senecio bonariensis (Compositae) [128, 129]. The methanol extract of the roots contained glucuronide 3 as an inseparable mixture with glycoside 1 and stigmasterol 3-O-β-D-glucoside (26). Compound 3 was isolated as the methyl ester from this mixture after methylation by diazomethane. The ¹H NMR of 3 contains signals characteristic of the β-sitosterol methyl groups and a signal for the vinylic proton H-6 (5.34 ppm). The protons on the carbohydrate atoms are observed at 3.0-4.0 ppm. It should be noted that the anemic proton resonates at 4.38 ppm and that its signal appears as a doublet with a splitting constant $J_{ax} = 7.5$ Hz, which is characteristic of axial protons H-1' and H-2' and, consequently, the β-configuration. The ¹³C NMR spectrum confirm that the configuration of C-24 is consistent with β-sitosterol. Furthermore, a comparison of the chemical shifts of the anemic C atom (101.6 ppm) and the other C atoms of the carbohydrate with the analogous shifts of known compounds confirms that 3 has the β-configuration and the pyranose form. The structure of glycoside 3 was also confirmed by chemical conversions of its methyl