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STEROL GLYCOSIDES AND ACYLGLYCOSIDES

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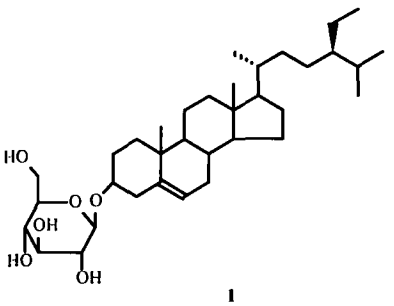
The structure, properties, distribution in nature, and biological activity of sterol glycosides and acylglycosides are reviewed.

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 the 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 comparision 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.



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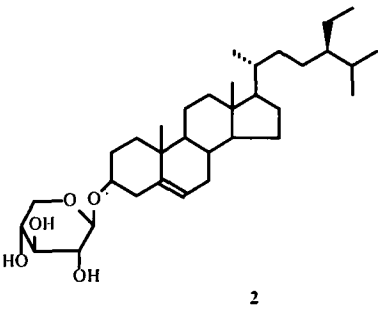

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A large number of plants yield β-sitosterol 3-O-β-*D*-glucopyranoside as the only sterol glycoside [38-106].

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

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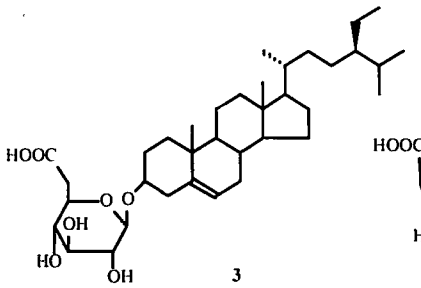
and *D*-glucose by reaction with 20% sulfuric acid with heating at 100 °C for 10 h in a mixture of $CHCl_3$ and CH_3OH has also been reported [49]. The reaction of compound **1** with acetic anhydride in pyridine gives the tetraacetate with mp 171 °C [41]. The analogous reaction with benzoyl chloride 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 [aglycone + $H]^+$, 397 [M - glucose + $H]^+$, 382, and 256 [55]. The ^1H NMR spectra (δ, here and further) of compound **1** in deuteropyridine have 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 ^{13}C 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 candicans* (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 $CDCl_3$ 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 anomeric proton H-1′ appears as a doublet at 4.56 ppm. The splitting constant of this doublet (J = 8 Hz) indicates that the 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′$_{eq}$, and H-5′$_{ax}$ (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 ^{13}C 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 ^{13}C NMR occuring 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.



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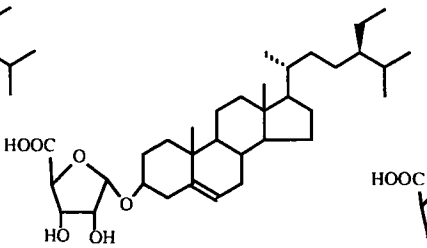

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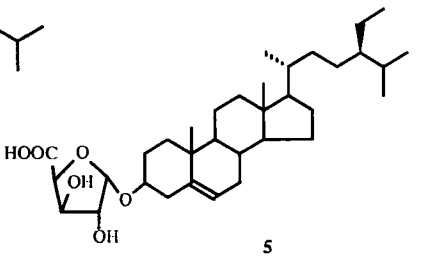

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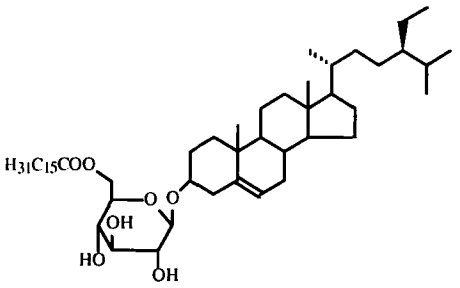
The β-sitosterol 3-O-β-*D*-glucuronopyranoside (**3**) was first found in the roots of *Senecio bonariensis* (Compositae) [128, 129]. The methanol extract of the roots contained glucuronide **3** as an inseparable mixture with glycoside **1** and stigmasterol 3-O-β-*D*-glucopyranoside (**26**). Compound **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 anomeric proton resonates at 4.38 ppm and that its signal appears as a doublet with a splitting constant J_{aa} = 7.5 Hz, which is characteristic of axial protons H-1′ and H-2′ and, consequently, the β-configuration. The ^{13}C NMR spectrum confirm that the configuration of C-24 is consistent with β-sitosterol. Furthermore, a comparison of the chemical shifts of the anomeric 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

ester. Thus, treatment of compound **3** with acetic anhydride in pyridine gives the acetate. Reduction of the methyl ester with $LiAlH_4$ in THF and subsequent hydrolysis under mild conditions gave β-sitosterol and glucose. The structure of the last two compounds was determined by TLC and chromatography-mass spectrometry. Glucuronide **3** was later observed also in the roots of *Vaccinium scopulorum* [130].

Analysis of the methanol extract of the subterranean part of *Bauhinia candicans* (Leguminosae) identified a fraction containing mainly glycoside **2** and a new compound β-sitosterol 3-O-α-*D*-riburonofuranoside (**4**) [131]. This fraction was methylated with diazomethane in order to separate it into its components. This produced the methyl ester of riburonofuranoside **4**, which was further acetylated to the acetate of the methyl ester in order to establish the structure. Reduction of this compound with $LiAlH_4$ in THF and subsequent hydrolysis with 6% HCl in aqueous methanol gave β-sitosterol and ribose. Their structures were proved by GLC.

The ^1H NMR spectrum of the acetylated methyl ester of glucoronide **4** in $CDCl_3$ contains signals characteristic of β-sitosterol at 68 (s), 0.82 (d), 0.84 (t), 0.92 (d), and 1.00 (s) ppm, which correspond to the 18-, 26/27-, 29-, 21-, and 19-methyl groups. The vinylic proton H-6 appears in the spectrum at 5.36 ppm; the methine proton H-3, at 3.50 ppm. The presence of 3-proton singlets at 2.09, 2.10, and 3.39 ppm is consistent with two acetoxy groups and a carboxymethyl group. The ^1H NMR spectrum of compound **4** confirms that the carbohydrate attached to the β-sitosterol is the pentafuranoside of uronic acid. Thus, the signal of H-1′ is a doublet (4.50 ppm) with $J_{1',2'} = 7.5$ Hz, which is characteristic for α-glycosides. Otherwise the constant would have a value less than 0.5 Hz, characteristic for β-glycosides. The signals of H-2′ and H-3′ appear as a doublet of doublets at 5.05 ppm ($J_{1',2'} = 7.5$ Hz, $J_{2',3'} = 10$ Hz) and 4.49 ppm ($J_{2',3'} = 10$ Hz, $J_{3',4'} = 5$ Hz), respectively. The doublet centered at 4.16 ppm is assigned to H-4′. The ^{13}C NMR spectrum of the methyl ester of glucoronide **4** confirms the structure of its sterol component. The resonance of C-24 at 45.7 ppm, which corresponds to the (24R)-configuration of sterol, is an important feature. Signals centered at 99.8, 71.9, 69.0, and 81.0 ppm correspond to the resonances of C-1′, C-2′, C-3′, and C-4′, respectively. The signal of C-5′ is situated in the carbonyl region.

Further investigation of the subterranean part of *Bauhinia candicans* identified yet another glycuronide, β-sitosterol 3-O-α-*D*-xyluronofuranoside (**5**) [132]. Compound **5** was isolated and identified after methylation with diazomethane and acetylation to the acetate of the methyl ester. The ^1H NMR of **5** in $CDCl_3$ contains signals for the protons of all methyl groups of β-sitosterol and a signal for the vinylic proton H-6 (5.32 ppm). Furthermore, the spectrum contains 3-proton singlets for the methoxy groups and two acetoxy groups at 3.48, 1.96, and 2.02 ppm. The spectrum contains a doublet for H-1′ (4.44 ppm), a doublets of doublets for H-2′ (5.04 ppm) and H-3′ (4.88 ppm), and a doublet for H-5′ (4.15 ppm) for the carbohydrate part of this compound. The ^{13}C NMR spectrum also confirms the structure of glucuronide **5**. In particular, the (24R)-configuration of the sterol can be confirmed using it. The structure of glucuronide **5** was ultimately confirmed by reduction of the acetate of the methyl ester with $LiAlH_4$ and subsequent hydrolysis by HCl in methanol. This gave β-sitosterol and xylose.



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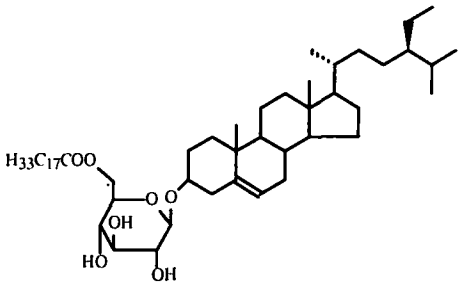

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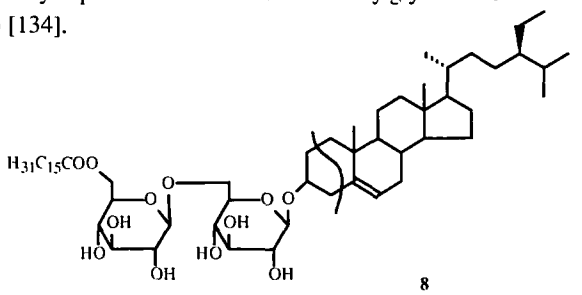
Fruits of the banana tree *Musa paradisiaca* (Musaceae) are used in Indian folk medicine as an anti-ulcer agent. In a search for the active principles, β-sitosterol acylglycosides called sitoindosides I (**6**) and II (**7**) were isolated [133]. The structure of the former was established as β-sitosterol 3-(6′-O-palmitoyl-β-*D*-glucopyranoside) from spectral data and chemical transformations. In particular, IR spectrum of compound **6** exhibits a band at 1730 cm^{-1}, which corresponds to stretching vibrations of an ester carbonyl. The ^1H NMR recorded in DMSO-D_6 contains a multiplet for the vinylic proton H-6 of the aglycone at 5.36 ppm. Signals for the anomeric proton H-1′ at 4.8 ppm and for the methylene protons H-6′ at 4.4 ppm are also consistent with the glycosidic structure of compound **6**. Hydrolysis of compound **6** by sodium methoxide in methanol produced a mixture of palmitic acid with methylpalmitate and β-sitosterol 3-O-β-*D*-glucoside (**1**). Further hydrolysis of the last by emulsin produced β-sitosterol and *D*-glucose. Methylation of compound **6** by methyl iodide in THF containing sodium hydride gave the

permethylate. The mass spectrum of this compound contains a peak for the molecular ion at *m/z* 856. Hydrolysis of the permethylate by aqueous sulfuric acid produces 2,3,4-tri-O-methylglucose in addition to palmitic acid and β-sitosterol, unambiguously indicating the attachment site of the palmitic acid.

The structure of sitoindoside II (7) as β-sitosterol 3-(6′-O-oleoyl-β-*D*-glucopyranoside) was established in an analogous manner [133]. Thus, the IR spectrum of this compound also exhibits a band at 1735 cm^{-1}, characteristic of an ester. The ^1H NMR in DMSO-D_6 of compound **7** contains signals at 5.35 ppm of three vinylic protons, H-6 of the β-sitosterol and two protons of the double bond in the fatty acid. Furthermore, the spectrum contains signals of the anomeric proton at 4.85 ppm and of the methylenic protons on C-6′ at 4.35 ppm. This confirms the glycosidic structure of compound **7**. Alkaline hydrolysis of compound **7** gives β-sitosterol 3-O-β-*D*-glucopyranoside (**1**) and oleic acid. Sitoindosides I and II are present in the plant in a 5:1 ratio. Oral administration of this mixture in doses of 50 mg/kg completely prevents the development of experimental ulcers caused by aspirin in white rats. Later acylglycoside **6** was also isolated from the roots of the nettle *Urtica dioica* (Urtikaceae) [134].



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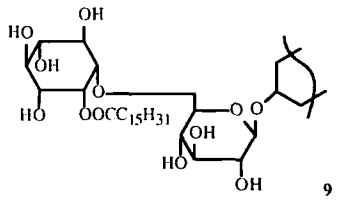

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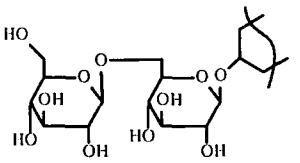

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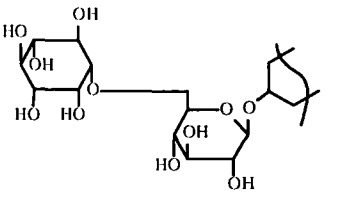

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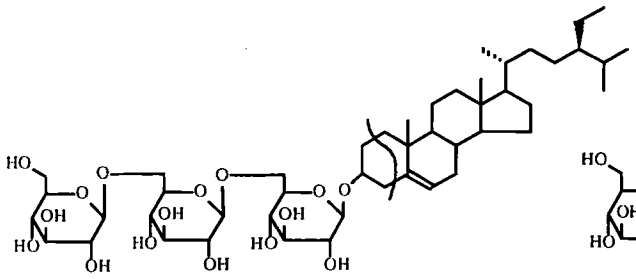
Further investigation of biologically active substances in *M. paradisiaca* yielded two new acylglycosides of β-sitosterol, which were named sitoindosides III (**8**) and IV (**9**), and two sterylglycosides SG-I (**10**) and SG-II (**11**) [135].

Analysis of spectral data and chemical transformations of compound **8** established the structure as β-sitosterol 3-[6″-O-palmitoyl-β-*D*-glucopyranosyl(1″→6′)-β-*D*-glucopyranoside]. Thus, field-desorption mass spectra of **8** contain the molecular ion at *m/z* 976. A band at 1730 cm^{-1} in the IR spectrum of **8** indicates the presence of an ester. The signal of the vinylic proton H-6 of β-sitosterol in the trimethylsilyl ester of **8** in $CDCl_3$ occurs at 5.3 ppm in the ^1H NMR spectrum. Furthermore, the NMR spectrum also contains a doublet at 4.95 ppm with splitting constant J = 7 Hz, characteristic of the anomeric proton. Alkaline hydrolysis of **8** by sodium methoxide in methanol produces β-sitosterol 3-O-gentiobioside (**10**) and methylpalmitate. Further enzymatic hydrolysis of compound **10** by emulsin gives β-sitosterol and *D*-glucose in a 1:2 ratio. Acid hydrolysis of **8** produces only *D*-glucose and no other carbohydrates. Successive treatment of **8** with NaH and methyl iodide gave the permethylate, acid hydrolysis of which by HCl in methanol formed palmitic acid, β-sitosterol, and 2,3,4-tri-O-methylglucose. The molar ratio of the last and β-sitosterol was 2:1.

The structure of sitoindoside IV (**9**) as β-sitosterol 3-[2″-O-palmitoyl-myoinosityl(1″→6′)-β-*D*-glucopyranoside] was proved mainly from chemical transformations. Thus, acetylation of compound **9** by acetic anhydride and triethylamine gave the heptaacetate. Alkaline hydrolysis of compound **9** yielded methylpalmitate and β-sitosterol myoinosityl-β-*D*-glucopyranoside (**11**). Further hydrolysis of glycoside **11** by HCl in methanol gave β-sitosterol, myoinositol, and *D*-glucose in a 1:1:1 ratio. In its turn, acid hydrolysis of sitoindoside IV permethylate, obtained as usual gave 3,4,5,6-tetra-O-methylmyoinositol, 2,3,4-tri-O-methylglucose and β-sitosterol in a 1:1:1 ratio. It was found that the 3,4,5,6-tetra-O-methylmyoinositol produced this way reacts with acetone and *p*-toluenesulfonic acid to give the monoacetonide.

The structures of SG-I (**10**) and SG-II (**11**) as β-sitosterol 3-O-gentiobioside and β-sitosterol myoinosityl-β-*D*-glucopyranoside, respectively, were rather easily proved since these compounds were identical to the products from alkaline hydrolysis of compounds **8** and **9**, respectively [135].



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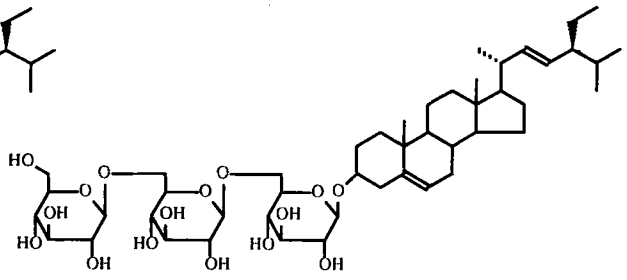

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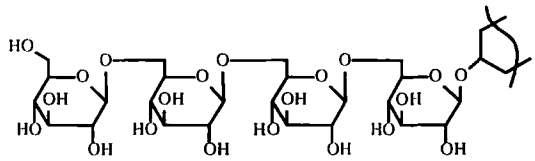

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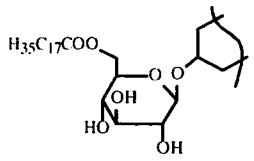

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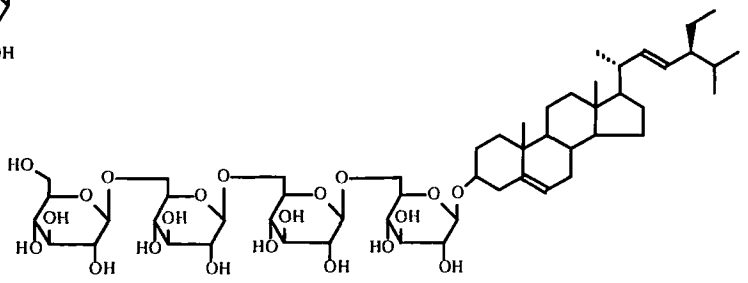

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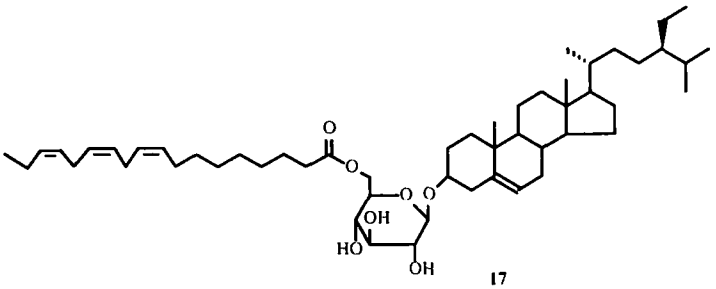
It is noteworthy that β-sitosterol 3-O-gentiobioside (**10**) was later isolated from the roots of *Gentiana macrophylla* [136]. Furthermore, glycoside **10** and sitoindoside I (**6**) together with analogous derivatives of stigmasterol are found in Adzuki beans of *Vigna angularis* (Fabaceae) [137]. In addition to these compounds, β-sitosterol 3-O-gentiotrioside (**12**), stigmasterol 3-O-gentiotrioside (**13**), β-sitosterol 3-O-gentiotetraoside (**14**), and stigmasterol 3-O-gentiotetraoside (**15**) were also identified in these beans. The structures of oligoglycosides **12-15** were proved using chemical and spectroscopic methods [137].

β-Sitosterol 3-O-6-stearoyl-β-*D*-glucopyranoside (**16**) was first isolated from the methyl extract of the rhizomes of *Alisma plantago-aquatica* (Alismataceae) [138]. The IR spectrum of compound **16** exhibits bands at 3400 and 1720 cm^{-1}, indicative of the presence of hydroxy groups and an ester. The ^1H NMR of **16** in $CDCl_3$ contains a doublet for the anomeric proton H-1′ (4.39 ppm, J = 7.7 Hz), doublets of doublets for the H-6′ protons (4.25 ppm, J_1 = 12.2 Hz, J_2 = 2.5 Hz and 4.52 ppm, J_1 = 12.2 Hz, J_2 = 4.0 Hz), and a multiplet for the vinylic proton H-6 (5.37 ppm). The structure of compound **16** as an acylglycoside of β-sitosterol was confirmed by ^{13}C NMR spectra. Methanolysis by sodium methoxide gave methylstearate and β-sitosterol 3-O-β-*D*-glucopyranoside (**1**). The structure of the latter was proved by conversion to the tetraacetate, which was identical to the authentic substance. Acetylation of acylglycoside **16** gives the triacetate. The signals of H-6′ in the ^1H NMR spectrum are not shifted to weak field compared to their position in the spectrum of **16**. Hence it was concluded that the stearic acid in compound **16** is bonded through the hydroxyl on C-6′ of the glucose.

We note also that β-sitosterol 3-O-β-*D*-glucopyranosyl-(1α→2)fructofuranoside was isolated from *Mentha arvensis* (Labiatae) [139].



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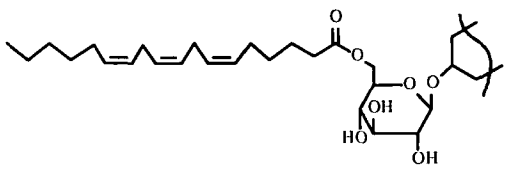

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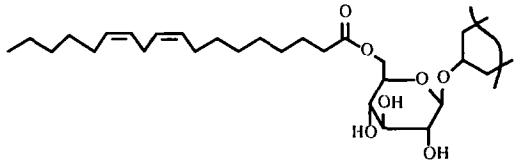
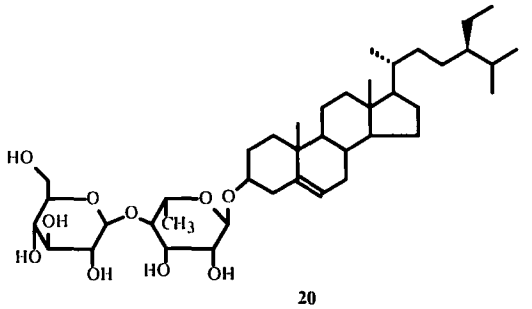


19

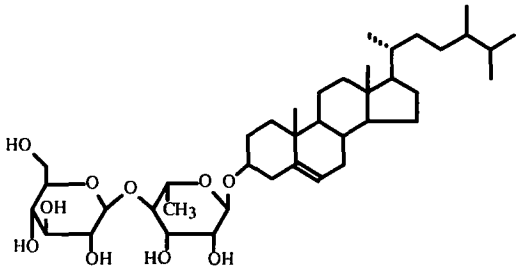
A phytochemical investigation of the cattail *Typha latifolia* (Typhaceae) yielded a mixture of four acylsterylglycosides consisting of three new compounds **17-19** and one previously known **16** [140]. The fact that the components of this mixture belong to the class of compounds under discussion was established by spectral analysis. In particular, IR spectra revealed the existence of hydroxy groups and esters. The ^{1}H NMR spectrum in $CDCl_3$ contains a doublet at 4.39 ppm with splitting constant J = 7.7 Hz for the annomeric proton H-1′. Furthermore, doublets of doublets at 4.39 ppm (J_1 = 12.1 Hz, J_2 = 2.2 Hz) and 4.52 ppm (J_1 = 12.1 Hz, J_2 = 5.3 Hz) for the H-6′ protons indicate that the acyl moieties are bonded to C-6′. The ^{13}C NMR spectrum of the mixture in $CDCl_3$ confirm the presence in the compounds **16-19** of β-sitosterol and β-*D*-glucose in addition to the attachment site of the acyl moieties.

Acetylation of the mixture of acylglycosides **16-19** by acetic anhydride in pyridine produced a mixture of triacetates, which was separated by preparative TLC into the pure components. The exact structures of acylglycosides **17-19** were elucidated by analyzing the spectra of the triacetates. Thus, the ^{1}H NMR spectrum of the triacetate of the principal compound **17** in $CDCl_3$ contains singlets for the 18- and 19-methyl groups (0.69 and 1.01 ppm, respectively), doublets for the 21-, 26-, and 27-methyl groups (0.92, 0.83, and 0.81 ppm, respectively), and a triplet for the 29-methyl group (0.85 ppm), all of which belong to β-sitosterol. The weak-field shift to 3.49 ppm of the signal of the H-3 proton indicates that the carbohydrate residue is bonded to C-3 of the sterol. The signals of H-1′-H-5′ of the acylated glucose are also well resolved in the spectrum. The ^{13}C NMR in $CDCl_3$ has signals that can be assigned to not only the β-sitosterol and the acylated glucose but also the triply unsaturated fatty acid. A comparison with the literature indicates that molecule of compound **17** contains residue of octadeca-(9Z,12Z,15Z)-trienoic acid, i.e., linolenic acid. Hydrolysis of the triacetate of acylglycoside **17** by NaOH in a mixture of $CHCl_3$ and CH_3OH confirms this. An acid identified by GLC as linolenic is formed after esterification with diazomethane. The structures of the triacetates of the compounds **18**, **19**, and **16** were established analogously. The fact that these compounds contain γ-linolenic, linoleic, and stearic acid, respectively, was proved not only by using spectra but also by performing alkaline hydrolysis and forming the corresponding methyl esters after treatment with diazomethane. We also note that a mixture of acylglycosides **6** and **19** was isolated from the methanol extract of a cell culture of ginseng *Panax ginseng* (Araliaceae) [141].



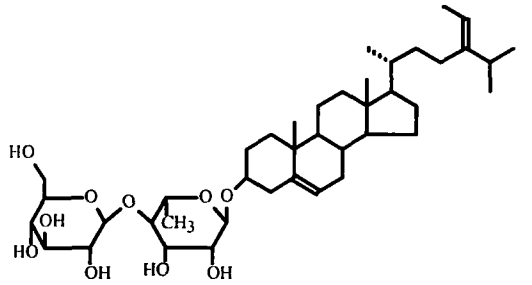
20

The composition of the stem bark of *Cardenia lucida* (Roxb.), the essential oil of which is used to treat skin conditions and repel insects, has been investigated [142]. The alcohol extract contains β-sitosterol[3-O-β-*D*-glucopyranosyl-(1→4)-O-α-*L*-rhamnopyranoside] (**20**). The structure of compound **20** was determined using spectral data and chemical reactions. Thus, hydrolysis of glycoside **20** by Kiliani solution gives β-sitosterol. The products from hydrolysis of **20** by sulfuric acid include *D*-glucose and *L*-rhamnose.



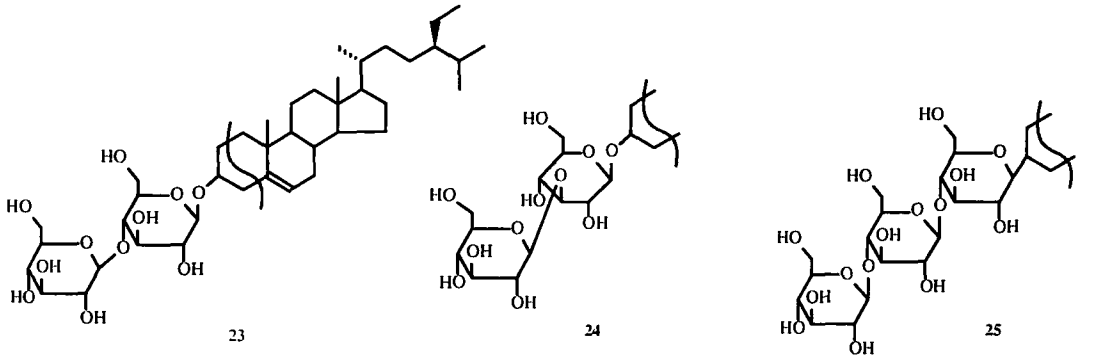
21

Isolation of ergost-5-en-3β-ol 3-O-[β-*D*-glucopyranosyl-(1→4)-O-α-*L*-rhamnopyranoside] (**21**) from the seeds of *Heterophragma quadriloculare* has also been reported [143]. The structure of this diglycoside was established by chemical transformations and spectral analysis.



22

Yet another new sterol diglycoside called stigmasta-5,24(28)-diene 3β-O-α-*D*-glucopyranosyl-α-*L*-rhamnopyranoside (**22**) was isolated from the stems of *Boswellia serrata* [144].

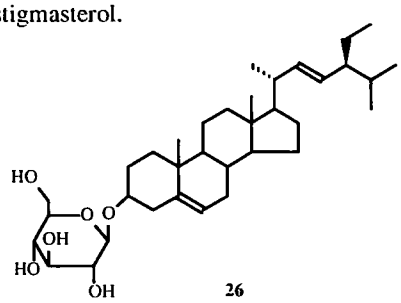


23 **24** **25**

Isolation from rice bran of diglycosides and triglycosides of β-sitosterol (**23-25**) has been described [145]. Their structures were proved using chemical and spectroscopic methods. Thus, hydrolysis of compounds **23-25** by HCl in methanol at 100 °C for 3 h produced sterol and only *D*-glucose. The ratios of sterol to glucose in these compounds are 1:2, 1:2, and 1:3, respectively. The predominance in compounds **23-25** of β-sitosterol (66.1-72.6%) was demonstrated by GLC. However, impurities of other sterols, mainly campesterol (10.7-17.2%) and stigmasterol (9.7-13.0%), are also present. The configurations of the anomeric centers in glycosides **23-25** were proved by oxidizing their acetates with CrO_3 in acetic acid, methanolysis, and GC analysis of the methylglycosides. The attachment site of the carbohydrates in glycosides **23-25** was determined as usual by methylation, methanolysis, and GC analysis of the resulting fragments. These results enable the assignment to glycoside **23** of the structure *D*-glucopyranosyl-(β1→4)-*D*-glucopyranosyl-(β1→3′)-β-sitosterol, i.e., β-sitosterol 3-O-cellobioside. Compound **24** has the structure *D*-glucopyranosyl-(β1→3)-*D*-glucopyranosyl-(β1→3′)-β-sitosterol. Finally, the triglycoside **25** is assigned

the structure *D*-glucopyranosyl-(β1→4)-*D*-glucopyranosyl-(β1→4)-*D*-glucopyranosyl-(β1→3′)-β-sitosterol, i.e., β-sitosterol 3-O-cellotrioside.

Stigmasterol 3-O-β-*D*-glucopyranoside (**26**) was first observed in soy oil [146]. This compound was found in a mixture with β-sitosterol 3-O-β-*D*-glucopyranoside (**1**). This mixture could not be separated into the pure components. The structure of glycoside **26** was established by hydrolysis of the mixture with sulfuric acid in ethanol. The hydrolysis products contained *D*-glucose and stigmasterol.



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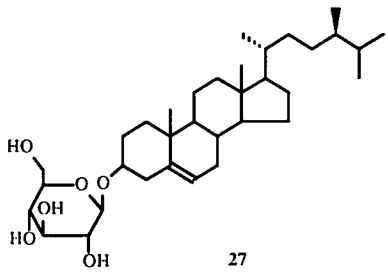
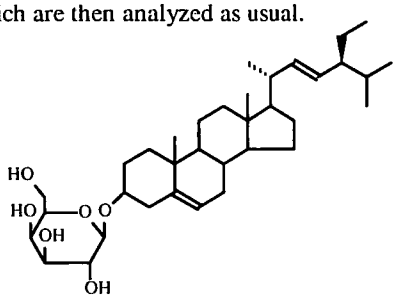


27

Glycoside **26** is rather widely distributed in higher plants [6, 34, 108, 110, 111, 113, 114, 116, 117, 119-125, 147-153]. This compound is usually found in a difficultly separated mixture with β-sitosterol 3-O-β-*D*-glucopyranoside (**1**) and campesterol 3-O-β-*D*-glucopyranoside (**27**). The compositions of these mixtures are usually determined after acid hydrolysis to form the free sterols, which are then analyzed as usual.



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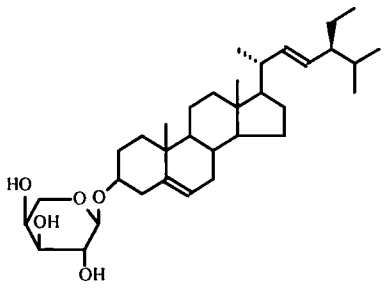
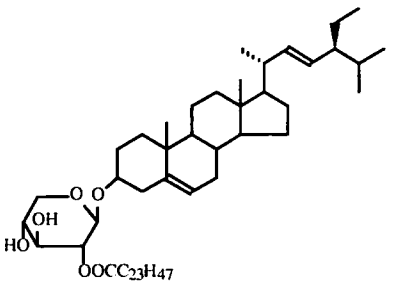


29

Stigmasterol 3-O-β-*D*-galactopyranoside (**28**) was first isolated from *Rhynchosia minima* [154]. The structure was proved by spectral analysis and chemical transformations.

Stigmasterol α-*L*-arabinopyranoside (**29**) was isolated from an aqueous-alcohol extract of the tropical plant *Walleria yunguensis* (Myrsinaceae) [155].

Two new glycosides were isolated from the methanol extract of the dried roots of *Asparagus adscendens* (Asparagaceae) [156]. One of these was assigned the structure 3-β-O-[β-*D*-2-tetracosylxylopyranosyl]-stigmasterol (**30**).



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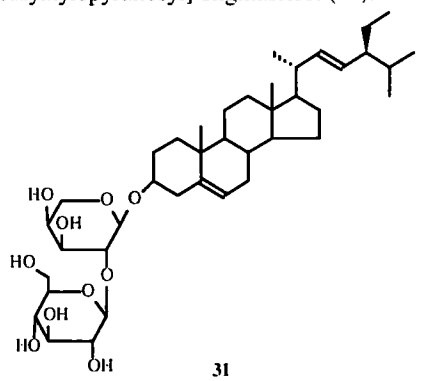


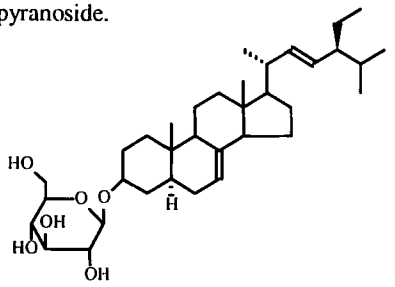
31

Acid hydrolysis of compound **30** by HCl in methanol produced stigmasterol and xylose. Their structures were established by comparison with authentic samples. Alkaline hydrolysis of glycoside **30** by methanolic KOH gave stigmasterol 3β-*D*-xylopyranoside and tetracosanic acid. Further acid hydrolysis of stigmasterol 3β-*D*-xylopyranoside gave stigmasterol and *D*-xylose. The ^{1}H NMR spectra of compound **30** and stigmasterol 3β-*D*-xylopyranoside in $CDCl_3$ contain signals of the

anomeric proton of xylose at 4.38 ppm with a characteristic splitting constant J = 8 Hz. The attachment of the carbohydrate to C-3 of stigmasterol was confirmed by the weak-field shift to 79.0 ppm of the C-3 signal in the ^{13}C NMR spectrum of glycoside **30** compared with the analogous signal in the spectrum of stigmasterol (71.8 ppm). Absorptions at 1740 and 725 cm^{-1} in the IR spectrum of glucoside **30** are consistent with the presence of esters. This is also confirmed by the ^{1}H and ^{13}C NMR spectra. Thus, triplets at 0.90 and 2.25 ppm with splitting constants J = 6 Hz and a broad singlet at 1.25 ppm were assigned to protons of the terminal methyl group, the $-(COCH_2)-$ group, and the methylene protons of tetracosanic acid. The mass spectrum of compound **30** contains peaks with *m/z* 412, 368, and 150, which correspond to stigmasterol, tetracosanic acid, and xylose, respectively. The location of the ester was proved by the ^{13}C NMR spectrum of acylglycoside **30** and the products from alkaline hydrolysis of stigmasterol 3β-*D*-xylopyranoside. Thus, signals of carbohydrate atoms C-1′ and C-3′ undergo a strong-field shift from 104.0 and 78.1 ppm to 101.0 and 74.8 ppm, respectively, in the spectrum of the new glycoside **30**. The signal of C-2′ undergoes a weak-field shift from 76.4 to 78.0 ppm. Therefore, tetracosanic acid is bonded to the hydroxy group of the xylose C-2′ atom.

The second glycoside isolated from *A. adscendens* was assigned the structure 3-β-O-[β-*D*-glucopyranosyl-(1→2)-α-*L*-arabinopyranosyl]-stigmasterol (**31**) [156]. With the exception of the absence of a characteristic ester absorption, its IR spectrum is analogous to that of glycoside **30**. Total acid hydrolysis of compound **31** by HCl in methanol for 8 h gave glucose, arabinose, and stigmasterol. Their structures were determined by comparision with authentic samples. Partial acid hydrolysis of compound **31** by HCl for 10 min produced glucose and stigmasterol α-*L*-arabinopyranoside (**29**), the properties of which agree with those in the literature [155]. Therefore, it was concluded that the terminal carbohydrate is glucose. The ^{1}H NMR spectra of glycoside **31** in deuteropyridine contain doublets for the anomeric protons of arabinose at 4.95 ppm (J = 5 Hz) and glucose at 4.86 ppm (J = 8 Hz). The attachment site of the glucose was found using ^{13}C NMR spectra.

Recently a mixture of the 3-O-β-*D*-glucopyranosides (24R)-24-ethyl-5α-cholest-7-en-3β-ol, stigmasta-7,E-24(28)-dien-3β-ol, and 4α-methylstigmasta-7,E-24(28)-dien-3β-ol was isolated from the roots of *Bryonia melanocarpa* (Cucurbitaceae) [157]. Although pure compounds could not be isolated from this mixture, their structures were determined by Smith cleavage to produce the corresponding sterols. It was determined that more than half of the mixture is (24R)-24-ethyl-5α-cholest-7-en-3β-ol 3-O-β-*D*-glucopyranoside.



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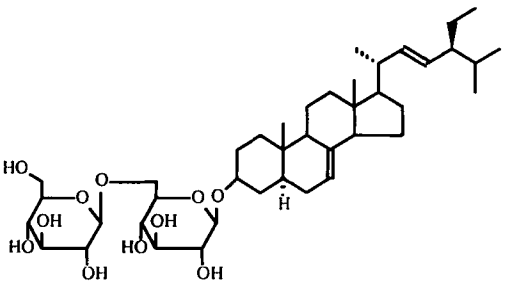


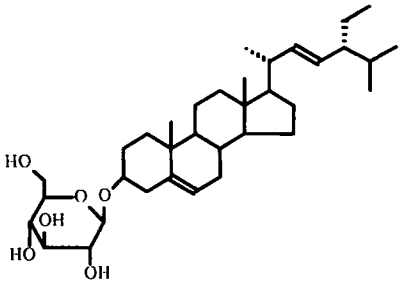
33

α-Spinasterol 3-O-β-*D*-glucopyranoside (**32**) was isolated pure as 3β-O-β-*D*-glucopyranosylspinasterol from an alcohol extract of the Brazilian plant *Amarthospermum schomburghinana* (Miq.) Baehni (Sapotaceae) [158]. Its structure was determined from spectral data and chemical transformations. Acetylation of glycoside **32** by acetic anhydride in pyridine gave the tetraacetate. Hydrolysis of compound **32** by 6% HCl in aqueous methanol gave α-spinasterol. Glycoside **32** was also isolated from the oil of *Acacia arabica* [118], from the roots of *Gypsophila struthium* [159] and *Camellia sinensis* [160], and from *Inga edulis* var. parviflora [161], *Stellaria dichotoma* var. lanceolata [162], and *Gynostemma longipens* [163]. It should be noted that glycoside **32** occurs in the roots of *S. dichotoma* var. lanceolata together with ergost-7-en-3β-ol and stigmast-7-en-3β-ol [162]. Furthermore, glycoside **32** occurs in the leaves and stems of *Prunella vulgaris* (Prunellidae) together with the corresponding monoglucosides of β-sitosterol, stigmasterol, and stigmast-7-en-3β-ol [115].

The new diglycoside α-spinasterol 3-O-gentiobioside (**33**) was first isolated from the methanol extract of the green tea *Thea sinensis* (Theaceae) [164]. It was assigned the structure β-*D*-glucopyranosyl-(1″→6′)-β-*D*-glucopyranosyl-3-O-α-spinasterol. Compound **33** gave positive tests in the Liebermann-Burchard and Molisch tests. Acid hydrolysis of glycoside **33** for 3 h at 80°C gave α-spinasterol and *D*-glucose. The structure of the latter was proved by comparison with an authentic sample. Acetylation of compound **33** by acetic anhydride in pyridine gave the heptaacetate. The ^{1}H NMR spectrum of the heptaacetate contains signals for protons of the six methyl groups of α-spinasterol and for the three protons of the double bonds as a multiplet centered at 5.15 ppm in addition to other signals. Furthermore, signals for protons of the seven acetoxy groups

of the carbohydrate occur in the range 1.99-2.18 ppm. The ratio of the aglycone and carbohydrate determined from the ^{1}H NMR spectra is 1:2. The attachment site of the carbohydrates of compound **33** was determined exactly by exhaustive methylation in the presence of NaH and subsequent acid hydrolysis. This gave α-spinasterol and two carbohydrates that were identified as 2,3,4,6-tetra-O-methyl-*D*-glucose and 2,3,4-tri-O-methyl-*D*-glucose by comparison with authentic samples. Their formation confirms that the second glucose in compound **33** is attached at the 6′-position of the first, which, in turn, is attached to α-spinasterol. Klyne's rule was used to prove the β-attachment of the carbohydrate to α-spinasterol.

Poriferasterol 3-O-β-*D*-glucopyranoside (**34**) was first isolated as stigmasta-5,22-dien-3β-O-β-*D*-glucopyranoside from the roots of *Sapium insigne* [165]. Chemical and spectroscopic methods were used to prove the structure of glycoside **34**.

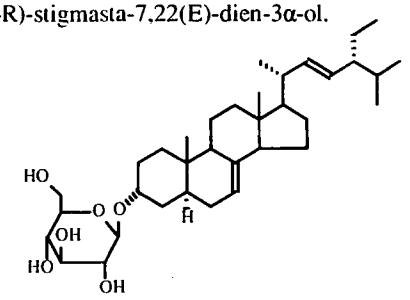


34

Thus, hydrolysis of compound **34** by sulfuric acid gave *D*-glucose and poriferasterol. Hakomori methylation of glycoside **34** and subsequent hydrolysis gave poriferasterol and 2,3,4,6-tetra-O-methyl-*D*-glucose.

Later compound **34** was isolated as a poriferasterol monoglucoside during a study of the composition of the cell membranes of the slime mold *Physarum polycephalum* by its differentiation from a haploid mycoameba into a diploid plasmodium [166]. The structure of glycoside **34** was determined on the basis of physicochemical analysis. Thus, fast-atom bombardment mass spectra of the negative ions from compound **34** showed a peak for $[M - H]^-$ with *m/z* 573, which corresponds to a molecular mass of 574. The hydrolysis products (HCl in methanol) of **34** as the O-trimethylsilyl esters and standards were compared by GLC and mass spectrometry. It was found that compound **34** contains glucose and sterol. The sterol could be stigmasterol or poriferasterol. The latter is favored according to ^{1}H NMR spectra of glycoside **34** and standard stigmasterol and poriferasterol acetates. The proton signals for the side chains of compound **34** and poriferasterol have exactly the same chemical shifts, which differ from those of the analogous protons in the spectrum of stigmasterol. The presence in the ^{1}H NMR spectrum of compound **34** of a doublet for the anomeric proton H-1′ at 4.2 ppm is important for proving the structure. The splitting constant of this signal (J = 6.6 Hz) suggests the β-configuration for the anomeric center. This is also confirmed by the fact that glycoside **34** is hydrolyzed by β-glucosidase and is stable toward α-glucosidase. The ratio of the glucose and poriferasterol was determined as 1:1 in molecule of glycoside **34** by comparing the integrated intensities of the H-6 (5.32 ppm) and H-1′ (4.2 ppm) signals in the ^{1}H NMR. Finally, it was demonstrated that heat shock produces glycoside **34** in the cell membranes of *Physarum polycephalum* [167].

Two new steryl glycosides, (24R)-stigmast-7,22(E)-dien-3α-ol 3-O-β-*D*-glucopyranoside (**35**) and (24R)-stigmast-7,22(E)-dien-3α-ol 3-O-β-*D*-galactopyranoside (**36**), were isolated from the methanol extract of *Mimusops elengi* (Sapotaceae) [168]. Spectal data and chemical conversions were used to establish that both compounds are glycosides of the previously unknown sterol (24R)-stigmasta-7,22(E)-dien-3α-ol.



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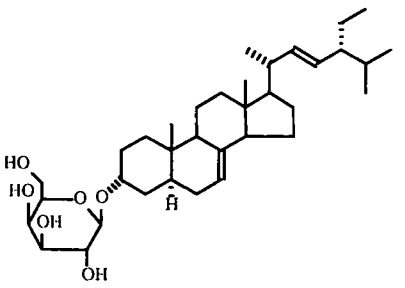
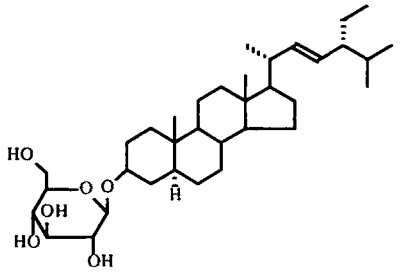


36

Stigmast-22-en-3β-ol 3-O-β-*D*-glucopyranoside (**37**) was isolated from the roots of the Mediterranean plant *Gypsophila struhium* (Caryophyllaceae) [159]. This compound forms a tetraacetate. The structure of glycoside **37** was established by

spectral analysis of the tetraacetate. Thus, although the mass spectrum does not contain a parent peak, there are peaks due to successive loss from it of the carbohydrate, methyl, and water with *m/z* 414, 399, and 381, respectively. Furthermore, the spectrum has peaks characteristic of tetra-O-acetylglucose with *m/z* 331, 271, 257, and 169. The ^{1}H NMR spectrum of the tetraacetate in $CDCl_3$ has signals for the methine proton H-3α (3.59 ppm) and the vinylic protons H-22 (5.16 ppm) and H-23 (5.03 ppm). The signal of the 29-methyl group occurs as a triplet at 0.82 ppm with J = 7.5 Hz. The anomeric proton H-1′ appears as a doublet at 4.59 ppm with J = 7.8 Hz, which is characteristic of β-glucosides. Furthermore, the spectrum contains signals for the remaining protons of tetra-O-acetylglucose.



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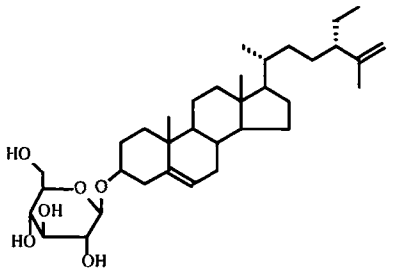
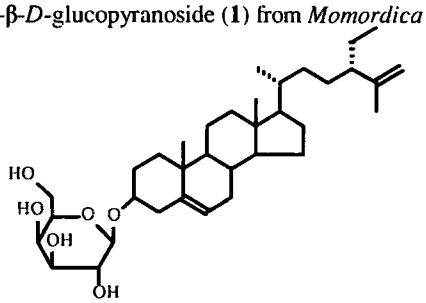


38

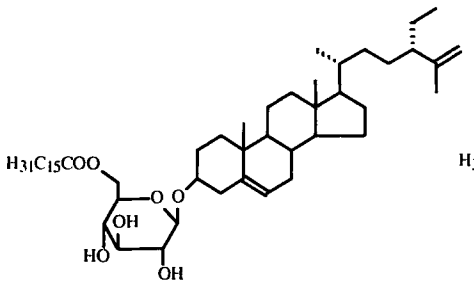
On the basis of these data, structure **37** was assigned to the glycoside. The main argument in favor of the (24R)-configuration of the aglycone residue is the fact that (24R)-stigmast-22-en-3β-ol was isolated simultaneously from this plant.

Clerosterol 3-O-β-*D*-glucopyranoside (**38**) was isolated from the leaves of *Clerodendron colebrookianum* [169]. The structure was established using spectral data. In particular, the configuration of C-24 was proved by comparison with the spectrum of clerosterol. Glycoside **38** is probably identical to $\Delta^{5,25}$-stigmastadienol-(3β)-β-*D*-glucoside, which was isolated earlier in a mixture with β-sitosterol 3-O-β-*D*-glucopyranoside (**1**) from *Momordica charantia* L. (Cucurbitaceae) [107].



39

Clerosterol 3-O-β-*D*-galactopyranoside (**39**) was isolated from the green alga *Codium decorticatum* [170].



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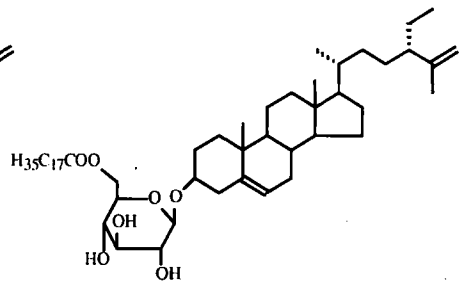
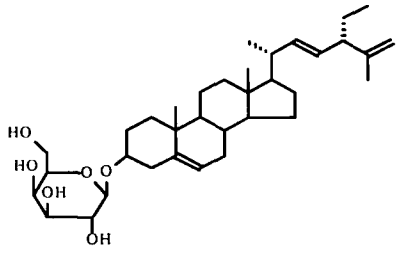


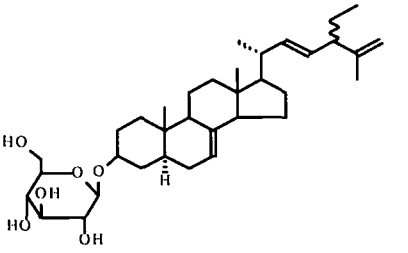
41

Stigmasta-5,25(27)-dien-3β-ol 3-O-[6′-O-palmitoyl-β-*D*-glucopyranoside] (**40**) and stigmasta-5,25(27)-dien-3β-ol 3-O-[6′-O-stearoyl-β-*D*-glucopyranoside] (**41**) were first found in the ethanol extract of the green fruits of *Momordica charantia* (Cucurbitaceae) [171]. The acylglycosides **40** and **41** were isolated as a mixture. The principal component is the palmitate **40**. The structures of these compounds were established by analyzing the physicochemical properties of the mixture. The ^{1}H NMR spectrum of a mixture of the triacetates of acylglycosides **40** and **41** contains proton signals characteristic of stigmasta-5,25(27)-dien-3β-ol. Thus, the 21-, 29-, and 26-methyl groups resonate at 0.92, 0.82, and 1.54 ppm as a doublet (J = 6.5 Hz), a triplet (J = 7 Hz), and a broad singlet, respectively. The 18- and 19-methyl groups give singlets at 0.68 and 1.01 ppm, respectively. Signals of the geminal vinylic protons on C-27 occur at 4.73 and 4.56 ppm. The vinylic proton H-6 gives a broad doublet

(5.35 ppm, J = 5 Hz). The structure of the aglycone was confirmed using mass spectra of the free sterol obtained by acid hydrolysis of the compounds **40** and **41** mixture. The appearance of a molecular peak with *m/z* 412 and peaks with *m/z* 397 [M - Me]$^+$, 394 [M - H_2O], and 271 [M - $C_{10}H_{19}$ - 2H]$^+$ proves that the aglycone is a C_{29}-sterol with two double bonds located in the cyclic part and the side chain. Peaks with *m/z* 328 and 314 are characteristic of C-25-unsaturated sterols. Furthermore, the structure of the aglycone was confirmed by comparing the ^{1}H and ^{13}C NMR spectra of the acylglycosides **40** and **41** mixture with the known characteristics of related compounds. Spectral data are consistent with the presence of fatty acid residues in molecules of compounds **40** and **41**. Thus, a broad singlet at 1.26 ppm in the ^{1}H NMR spectrum is characteristic of methylene protons. A 2-proton triplet at 2.36 ppm (J = 6.5 Hz) is due to an α-methylene group. Signals for a carbonyl carbon at 174.6 ppm and a large number of methylene C atoms in the ^{13}C NMR spectrum belong to fatty acid residues. This is also confirmed by a band at 1734 cm^{-1} in the IR spectrum of acylglycosides **40** and **41**, which is characteristic of an ester. Furthermore, saponification of compounds **40** and **41** and subsequent methylation of the products produced methyl esters of palmitic and stearic acids in the ratio 2.3:1 according to GC-MS. The ^{1}H NMR spectrum of a mixture of the triacetates of acylglycosides **40** and **41** contains signals due to protons of β-*D*-glucose. The splitting constants of the doublet for the anomeric proton (4.58 ppm, J = 8 Hz) is consistent with β-attachment of the carbohydrate. Two doublets of doublets centered at 4.22 ppm (J_1 = 12 Hz, J_2 = 5 Hz) and 4.12 ppm (J_1 = 12 Hz, J_2 = 2.5 Hz) are assigned to the two geminal protons of C-6′. A comparison of the ^{13}C NMR spectrum for the mixture of compounds **40** and **41** with known data for methylglucopyranoses confirmed that the fatty acid is attached through the hydroxy group of the glucose C-6′ atom. Comparison of the glucose proton signals in the ^{1}H NMR spectrum of acylglycosides **40** and **41** and the glycosylsterol resulting from their saponification leads to the same conclusion. Only the signals for the C-6′ protons of the latter are shifted to strong field. On the other hand, the formation of triacetates by acetylation of compounds **40** and **41** indicates that only one fatty acid is present in them. The presence of only *D*-glucose was confirmed by GLC of the trimethylsilyl derivative prepared by acid hydrolysis of the mixture. The mixture of compounds **40** and **41** at a dose of 0.0125 mg/g in mice exhibits high antimutagenic activity toward the well known mutagen mitomycin C [171].



42

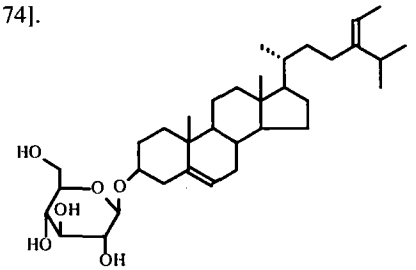


43

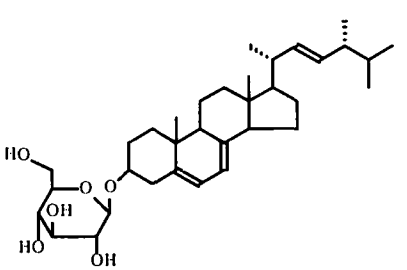
The isolation from *Clerodendron inerme* of (24β)-ethylcholesta-5,22,25-trien-3β-ol 3-O-β-*D*-galactopyranoside (**42**) as 3-O-β-*D*-galactopyranosyl-(24β)-ethylcholesta-5,22,25-triene has been reported [172].

We also note that three acylglycoside sterols were isolated from the bark of *Aegiphila thotzkyana* and *A. obducta* [173]. These compounds were assigned the structures 3-O-[6′-O-*n*-acyl-β-*D*-glucosyl]-22-dehydroclerosterol, 3-O-[6′-O-*n*-acyl-β-*D*-glucosyl]-clerosterol, and 3-O-[6′-O-*n*-acyl-β-*D*-glucosyl]-stigmasterol.

Stigmasta-7,22,25-trien-3β-ol 3-O-β-*D*-glucopyranoside (**43**) was isolated as glycoside A from the methanol extract of the peel of cucumber *Cucumis sativus* (Cucurbitaceae) [174]. The structure of glycoside **43** was determined mainly by hydrolysis by 10% sulfuric acid for 10 h. This produced stigmasta-7,22,25-trien-3β-ol and glucose. Methylation of the glycoside and subsequent methanolysis gave methyl-2,3,4,6-tetra-O-methyl-*D*-glucose. It should be noted that the exact stereochemistry of C-24 was not determined [174]. Glycoside **43** occurs mainly in cucumber peel whereas the seeds contain mainly free sterols and their esters [174].



44

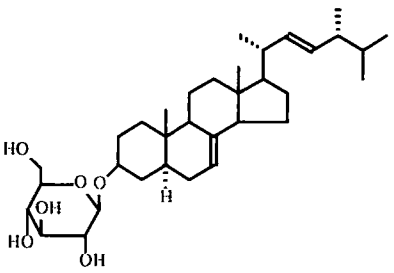


45

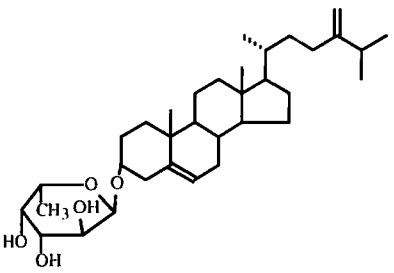
Isofucosterol 3-O-β-*D*-glucopyranoside (**44**) was identified as β-*D*-glucosyl isofucosterol in a cell culture of the tomato *Lycopersicon esculentum* (Solanaceae) [175]. Both chemical and spectroscopic data were used to prove the structure of compound **44**. It should be noted that it was demonstrated earlier [7] that glycoside **44** is the main component (~85%) in a mixture of glycosides of sterols from the green alga *Ulva gigantea*. However, in this instance the pure compound was not isolated from the mixture. Glycosides and acylglycosides of fucosterol, 28-isofucosterol, cholesterol, clionasterol, 24-methylenecholesterol, and desmosterol in green, brown, and red alga occur mainly as monoglycosides and their palmitates [7]. The low content of these compounds in the lipid fraction that is obtained by extracting alga with a $CHCl_3$–CH_3OH (2:1) mixture and contains numerous compounds makes it difficult to isolate them pure in quantities sufficient for research.

Ergosterol β-*D*-glucopyranoside (**45**) was isolated from the methanol extract of fruiting bodies of *Hericum erinaceus*, which is used in China for treatment of chronic gastritis [9]. The IR spectrum of compound **45** contains stretching vibrations of hydroxy groups at 3400 cm^{-1}. The ^1H NMR spectra of compound **45** show signals characteristic of ergosterol for the protons of the two 18- and 19-methyl groups at 0.66 and 0.93 ppm and for the four secondary 21-, 26-, 27-, and 28-methyls (doublets) at 1.09, 0.88, 0.89, and 0.99 ppm, respectively. In addition, typical signals for the glucose protons occur in the spectrum of compound **45** at 3.99-5.05 ppm. The signal for the anomeric proton H-1′ appears as a doublet centered at 5.05 ppm with J = 7.3 Hz. This is consistent with β-attachment of the glucose to ergosterol. Of the 34 C signals observed in the ^{13}C NMR, 6 are assigned to the carbohydrate, the remainder to ergosterol. Attachment of glucose to C-3 causes the signals of C-1 (38.0 ppm), C-2 (30.6 ppm), and C-4 (38.0 ppm) to shift to strong field; of C-3 (76.8 ppm), to weak field compared with their positions in the spectrum of ergosterol.

The mass spectrum of compound **45** contains a weak peak for the molecular ion $[M]^+$ with *m/z* 558 and peaks characteristic of fragmentation products with *m/z* 378 [M - glucose]$^+$, 363 [378 - CH_3]$^+$, 337, 253 [M - glucose - side chain]$^+$, 159, 145, and 69.



46



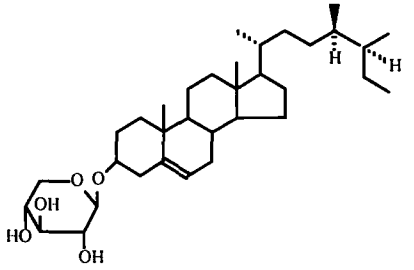
47

Ergosta-7,22-dien-3β-ol 3-O-β-*D*-glucopyranoside (**46**) was first isolated from a methanol extract of the fruiting bodies of *Tylopilus neofeleus* (Boletaceae) [10]. Its IR spectrum exhibits vibrations of hydroxy groups at 3500 cm^{-1}. The ^1H NMR spectrum in deuteropyridine contains signals for protons of two tertiary and four secondary methyl groups in addition to those of the carbohydrate. The ^{13}C NMR spectrum in deuteropyridine contains signals for 34 C atoms, 6 of which are assigned to glucose. The chemical shifts of the remaining signals correspond identically to those of the C atoms of ergsta-7,22-dien-3β-ol, except for a triplet at 30.1 ppm, a doublet at 77.5 ppm, and a triplet at 34.9 ppm, which are due to C-2, C-3, and C-4, respectively. These same atoms in the spectrum of ergosta-7,22-dien-3β-ol have shifts of 32.3, 70.3, and 38.9 ppm, respectively. Therefore, the glucose in compound **46** is attached through C-3 of ergosta-7,22-dien-3β-ol. The mass spectrum of compound **46** has a peak for the molecular ion with *m/z* 560.4060, which corresponds to $C_{34}H_{56}O_6$, in addition to peaks with *m/z* 381 [M - glucose + H], 273 [M - glucose - C_9H_{17} + H], and 125 [C_9H_{17}]. The products from acid hydrolysis of compound **46** are glucose and ergosta-7,22-dien-3β-ol. The splitting constant of the doublet for the anomeric proton H-1′ (4.92 ppm, J = 7.6 Hz) in the ^1H NMR is consistent with β-attachment of the glucose.

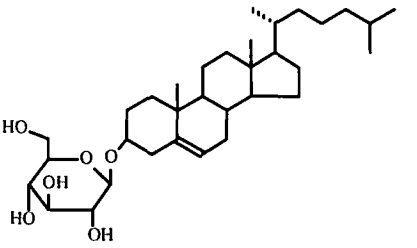
A new sterol glycoside was isolated from the soft coral *Sinularia hirta*. Spectral data suggested the structure 24-methylenecholesterol 3-O-α-*L*-fucopyranoside (**47**) [17]. Thus, the ^1H NMR spectrum in deuteropyridine contains signals for the angular 18- and 19-methyl groups (0.65 and 0.97 ppm, respectively) and for the secondary 21-, 26-, and 27-methyl groups (0.98, 1.06, and 1.07 ppm, respectively). The vinylic protons H-28 give two broad singlets (4.86 and 4.87 ppm). The signal for H-6 appears at 5.33 ppm. The resonances of the carbohydrate of compound **47** appear as a doublet of doublets for H-2′ (4.61 ppm, J_1 = 10 Hz, J_2 = 3.5 Hz), a doublet of doublets for H-3′ (4.50 ppm, J_1 = 10 Hz, J_2 = 3 Hz), and a broad doublet for H-4′ (4.22 ppm). Furthermore, a quartet for H-5′ (4.46 ppm) and a doublet for the 6-methyl group (1.59 ppm, J = 6.8 Hz) can be

found in the spectrum. The doublet of the anomeric proton H-1′ at 5.48 ppm has a splitting constant (J = 3.9 Hz) characteristic of α-attachment. The electron-impact mass spectrum of glycoside **47** contains no peak for the molecular ion. However, a peak is observed at *m/z* 380, which is due to loss of the carbohydrate. The structure of glycoside **47** was proved by comparing its ^{13}C NMR spectrum with that of 24-methylenecholesterol.

Aplysterol 3-O-β-*D*-xylopyranoside (**48**) was isolated as 24,26-dimethylcholest-5-en-3β-O-xylopyranoside from the methanol extract of *Holothuria scabra* (Holothurioidea) [176].



48



49

The empirical formula of compound **48** was found by elemental analysis and mass spectrometry, which gave a peak for the molecular ion with *m/z* 546. The mass spectrum also contains peaks with *m/z* 517 $[M - 29]^+$, 461 $[M - C_6H_{13}]^+$, 405 [M - side chain]$^+$, and 413 $[M - \text{xylose}]^+$. The triacetate was prepared by acetylation of glycoside **48** with acetic anhydride in pyridine for a day at room temperature. The ^1H NMR spectrum of the triacetate has peaks characteristic of Δ^5-sterols with singlets for the two tertiary methyl groups (0.75 and 1.12 ppm) and the three secondary methyl groups (0.96 ppm), a triplet for the primary methyl group (1.02 ppm, J = 6 Hz), a multiplet for H-3 (3.75 ppm, W/2 = 16 Hz), and a signal for H-6 (5.3 ppm). Furthermore, the spectrum of the triacetate has a doublet for the anomeric proton H-1′ (4.9 ppm). The splitting constant for this signal (J = 7 Hz) suggests that H-1′ and H-2′ are positioned *trans*-diaxial to each other. Therefore, the anomeric center has the β-configuration.

Acid hydrolysis of glycoside **48** by HCl in methanol yields aplysterol and xylose.

Cholesterol 3-O-β-*D*-glucopyranoside (**49**) was first prepared by chemical synthesis by glucosylation of cholesterol [37]. Subsequently this glycoside was identified in bacteria [11, 13, 15, 16], yeast [8], and chick [21, 23] and snake epidermis [22]. The presence of glycoside **49** in these sources was usually proven either by direct comparison with a synthetic sample or by acid hydrolysis. For example, acid hydrolysis by HCl in methanol at 50°C of glycoside **49** isolated from the cells of the yeast *Candida bogoriensis* was demonstrated [8] to form cholesterol. Acetylation of compound **49** by acetic anhydride in pyridine at 40°C for 2 h gave the tetraacetate, the structure of which was unambiguously proven by ^1H NMR spectra [22]. It was noted [21, 23] that glycoside **49** occurs in chick epidermis as a mixture with 5α-cholestanol 3-O-β-*D*-glucopyranoside and could not be isolated pure from the mixture.

Several natural sources have yielded 6-acyl derivatives of glycoside **49** [12, 21-23]. However, judging from spectral data and hydrolysis results, these substances are mixtures with a very complicated array of fatty acids.

Cholesterol 3-O-α-*D*-glucopyranoside was identified as one of the lipids of the bacteria *Helicobacter felis*, *H. muridarum*, *H. mustelae*, and *H. fenneliae* [177]. In addition to this compound, cholesterol [6-O-tetradecanoyl-α-*D*-glucopyranoside] was found in *H. felis*; cholesterol [6-O-dodecanoyl-α-*D*-glucopyranoside], in *H. muridarum*; and cholesterol [6-O-phosphatidyl-α-*D*-glucopyranoside], in *H. fenneliae*.

Cholesterol diglycoside that is acylated with fatty acids was isolated from the bacterium *Acholeplasma axanthum* [14]. The structure α-*D*-glucopyranosyl-(1→3)-(O-acyl)-α-*D*-glucopyranosyl-(1→3)-cholesterol was proposed for these compounds. The structures of the fatty acids and their attachment site to the carbohydrate were not determined.

Judging from the literature [18-20], a rich source of sterol glycosides of widely varying structure are holothuriae. However, these compounds from holothuriae have been studied mainly as mixtures owing to difficulties with their separation. Thus, *Isostichopus badionotus* (Stichopodidae) contains a mixture of the 3-O-β-*D*-xylopyranosides Δ^0-, Δ^7-, $\Delta^{7,22}$-C_{27}-, C_{28}-, and C_{29} -sterols. A fraction of 3-O-β-*D*-xylopyranosides in which the aglycones are 18 sterols was isolated from *Stichopus japonicum* [19]. The glycosides of *Cucumaria japonica* are 3-O-β-*D*-xylopyranosides and arabinopyranosides of 7 sterols [20].

In conclusion, it should be noted that glycosides and acylglycosides of sterols are, judging from the literature, a class of biologically active substances with a wide spectrum of action. For example, sterol glycosides possess hypocholesterinemic

[178-180], anti-inflammatory [181], hemolytic [182], and anti-ulcer [133] activity. They are also inhibitors of prostaglandin synthetase [183] and antioxidants [184]. Sterol acylglycosides have been demonstrated to be repellents for earthworms [185]. Furthermore, sterol glycosides can donate glucose for biosynthesis of ceramides [186]. Stigmasterol 3-O-β-*D*-glucopyranoside has been found to enhance the plant-growth stimulating activity of brassionolide [187]. However a mixture of β-sitosterol and campesterol 3-O-β-*D*-glucopyranosides do not possess such activity. Liposomes based on sterol glycosides are definitely of practical interest for producing medicinal preparations [188-190]. Furthermore, β-sitosterol 3-O-β-*D*-glucopyranoside has been proposed for use as a food emulsifier and preservative [191].

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