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ECDYSTEROIDS AND RELATED COMPOUNDS IN FUNGI

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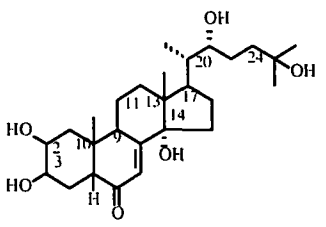
The structure and properties of ecdysteroids and related polyhydroxysterols isolated from fungi are reviewed.

Ecdysteroids are a large group of natural compounds with structures similar to α-ecdysone (**1**) [1, 2]. The structural features of these compounds are clearly evident from the structures of α-ecdysone and two other typical ecdysteroids, 20-hydroxyecdysone (**2**) and ponasterone A (**3**). At present about 300 ecdysteroids are known. These have been isolated mainly from invertebrates and plants. Ecdysteroids from insects and crustaceans are especially important. They act as hormones in these animals and control various vital functions, in particular, molting and metamorphosis [1, 3, 4].

Ecdysteroids in plants most probably play a protective role [5, 6]. It seems that these compounds elicit a number of hormonal disruptions when ingested with food by insects. These cause retarded development, birth of nonfertile offspring, and eventually a reduction in the damage caused to plants by insects. The simultaneous presence of ecdysteroids in plants and insects that feed on them is an interesting feature of the co-evolution of these large groups of living organisms [7, 8].

Fungi are currently considered to possess several characteristic features that differentiate them from plants and animals. For this reason they are sometimes classified as an independent kingdom. Therefore, the observation in the early 1990s that fungi contain compounds with structures similar to insect and plant ecdysteroids caused much excitement not only for the chemistry of natural compounds but also for the biological sciences, in particular, biochemical ecology. Fungi and insects are known in several instances to form a specific food chain. Therefore, the presence of ecdysteroids in fungi can hardly be considered chance. The compounds isolated from fungi can be called mycoecdysteroids, in analogy with those from animals (zooecdysteroids) and plants (phytoecdysteroids).



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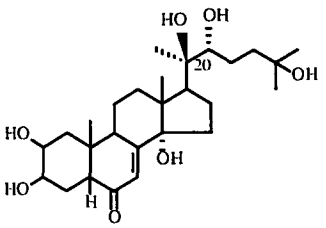

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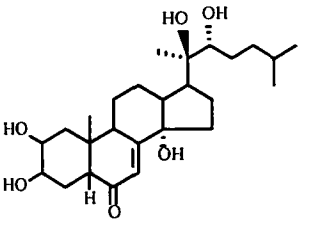

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Ohsawa *et al.* were among the first researchers of ecdysteroids in fungi [9]. They isolated seven ecdysteroids from the fruiting bodies of *Polyporus umbellatus* and named them polyporusterones A-G. This mushroom is used in Chinese and Japanese folk medicine for kidney ailments. The structure of the ecdysteroids from *P. umbellatus* was proved using spectra and chemical transformations. Thus, the ^{1}H and ^{13}C NMR (δ-scale) and IR spectra of polyporusterone A (**4**) suggest that the structure contains an α,β-unsaturated ketosteroid with four hydroxyls and is an ecdysteroid. Acetylation of compound **4** by acetic anhydride in pyridine for 1 h at room temperature gives the 2-monoacetate (**5**). Periodate oxidation of compound **5** produces poststerone 2-acetate (**6**) that is completely identical to the compound prepared analogously from 20-hydroxyecdysone (**2**). This confirms, first, the structure of the cyclic part of compound **4** and, second, the presence in it of a 20,22-diol. A second product of periodate oxidation of steroid **5** is 3,4-dimethylpentanal (**7**), which was identified as the 2,4-dinitrophenylhydrazone. The formation of this fragment is reliable proof of the structure of the side chain in compound **4**.

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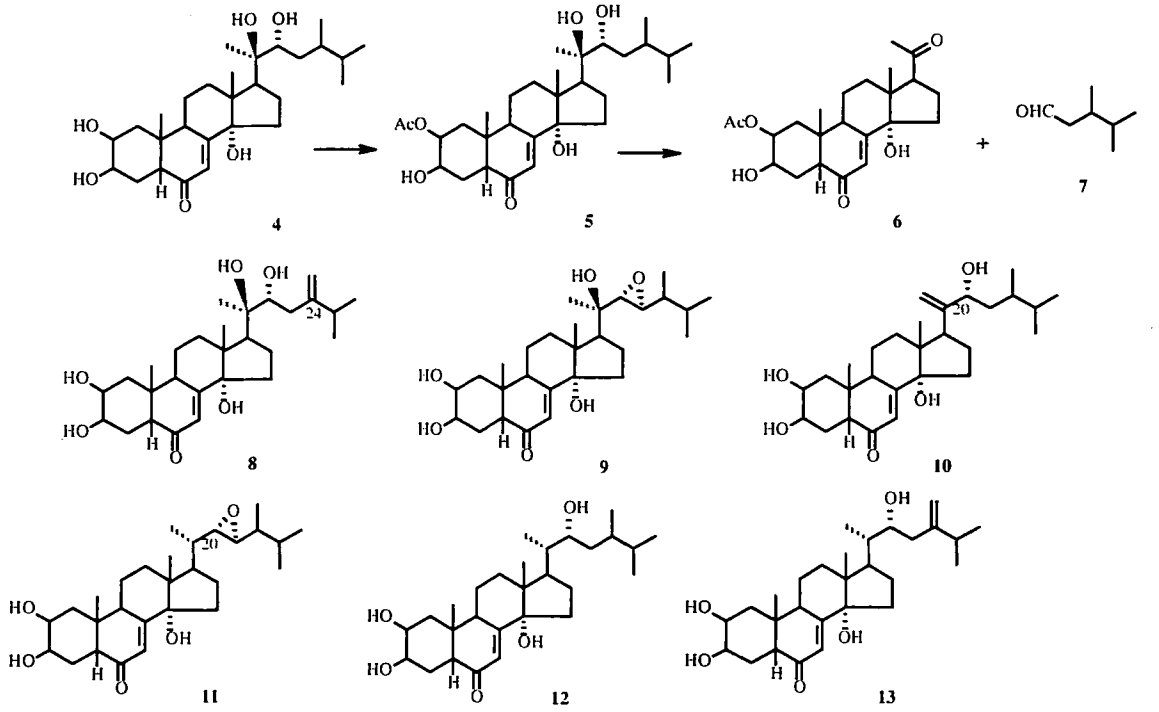
The ^{13}C NMR spectra, which contain a signal for a 28-methyl group (δ 21.2 ppm), lead to the same conclusion about the structure of compound **4**. Furthermore, the signal of C-22 in compound **4** shifts to upfield (74.3 ppm) compared with its position in the spectrum of 20-hydroxyecdysone (77.2 ppm). This shift is due to the γ-effect of the 28-methyl group. With the exception of this shift of C-22, the positions of the C-20 and C-21 atoms in the ^{13}C NMR spectra of compound **4** and 20-hydroxyecdysone are practically identical. This suggests the (20R,22R)-configuration for ecdysteroid **4**. It should be noted that the configuration of C-24 could not be determined.

The structure of another ecdysteroid from *P. umbellatus*, polyporusterone B (**8**), was solved analogously. In particular, mass, IR, and UV spectra indicate that compound **8** has a structure similar to that of ecdysteroid **4**. The ^{1}H NMR spectra of compound **8** contain signals of two vinylic protons H-28 at 5.10 and 4.96 ppm instead of a doublet for the 28-methyl group. The ^{13}C NMR spectra of compound **8** lack signals of the 28-methyl and 24-methine C atoms (21.2 and 36.0 ppm, respectively) that are characteristic of the spectra of ecdysteroid **4**. Instead the spectra exhibit signals for C-24 and C-28 (154.4 and 108.7 ppm, respectively) joined by a double bond.

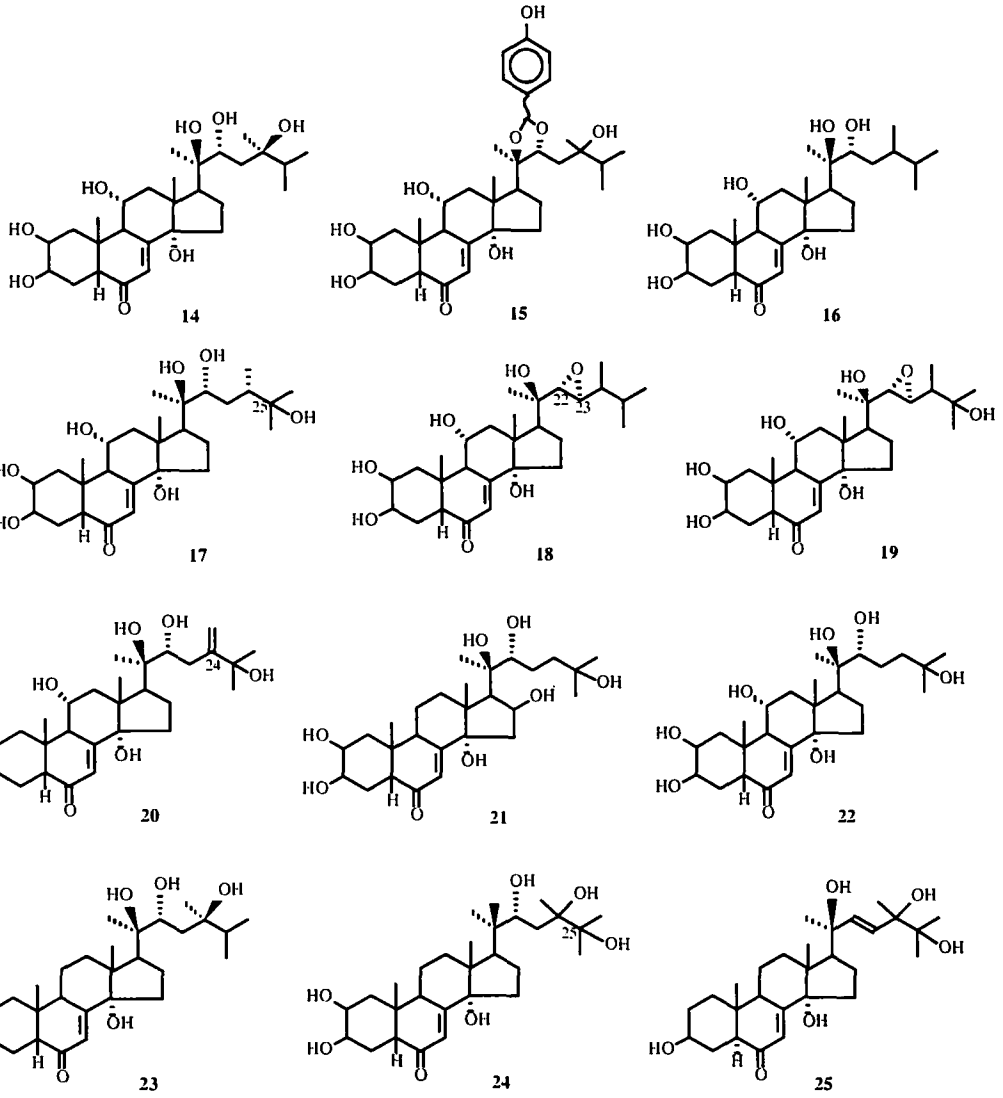
Polyporusterone C (**9**), which was also isolated from *P. umbellatus*, has a structure similar to that of ecdysteroid **4**. The ^{1}H and ^{13}C NMR spectra suggest that compound **9** contains a Δ^{7}-6-ketone, a 2β,3β-diol, and a 14α-hydroxyl. Furthermore, the *A* and *B* rings are *cis*-fused. Also, signals of two methine protons at 3.04 and 2.74 ppm in the ^{1}H NMR spectra of steroid **9** indicate the presence of a 22,23-epoxide. This is consistent with the ^{13}C NMR spectra, in which the C-22 and C-23 signals have the characteristic chemical shifts (66.2 and 58.1 ppm, respectively). The chemical shift of C-20 (72.0 ppm) suggests that steroid **9** contains a 20-hydroxyl. The ^{1}H and ^{13}C NMR spectra also suggest the presence of a 28-methyl group.



According to spectral data, polyporusterone D (**10**) is the 20(21)-dehydrated derivative of compound **4** [9]. The ^{1}H NMR spectrum of steroid **9** lacks a signal for a 21-methyl group and contains signals of two 21-vinylic protons (5.49 and 5.13 ppm). The chemical shifts of C-20 and C-21 (154.1 and 111.6 ppm, respectively) in the ^{13}C NMR spectrum are also consistent with vinylic atoms.

Polyporusterones E (**11**), F (**12**), and G (**13**) are 20-desoxy derivatives of compounds **9**, **4**, and **8**, respectively. The mass spectra of ecdysteroids **11-13** contain peaks of molecular ions that are 16 mass units less than those in the spectra of ecdysteroids **9**, **4**, and **8**, respectively. The ^{1}H NMR spectra of compounds **11-13** exhibit signals of the 21-methyl group as doublets at 0.94, 1.28, and 1.28 ppm, respectively. This is consistent with the lack of a 20-hydroxyl. The ^{13}C NMR spectra of compound **11-13** contain signals of C-20 at 36.0, 43.5, and 43.0 ppm, respectively. This also confirms that the 20-hydroxyl is absent. It should be noted that the spectra of ecdysteroids **11-13** are also consistent with the presence of the remaining structural fragments.

The cytotoxicity of polyporusterones A-G on L-1210 leukemia cells has been studied [9]. It was found that these compounds possess a marked dose-dependent cytotoxicity. However, considering their low content, ecdysteroids alone can hardly be responsible for the medicinal effect of *P. umbellatus*.



The velvet-footed pax *Paxillus atrotomentosus* (Paxillaceae) is widely distributed in Europe, including Belarus [10]. Recently it was shown that it contains ecdysteroids [11-14]. Eight ecdysteroids (**2**, **14-20**) were isolated from this mushroom. It is interesting that the principal ecdysteroid of plants (**2**) is only a minor component in *P. atrotomentosus* [14]. The principal ecdysteroid in this mushroom is paxillosterone (**14**). Spectroscopic and chemical methods were used to prove the structure of compound **14**. The empirical formula $C_{28}H_{46}O_8$ was determined from high-resolution mass spectra. Signals of all protons were assigned in the ^1H NMR spectrum at 500 MHz working frequency. All structure elements in compound **14** could be proved from ^1H and ^{13}C NMR spectra. Thus, a doublet of doublets for H-2α (4.00 ppm) and a quartet for H-3α (3.95 ppm) in the ^1H NMR spectrum indicates that ecdysteroid **14** contains a 2β,3β-diol.

The ^{13}C NMR spectra lead to the same conclusion. Signals of C-2 and C-3 have chemical shifts of 68.92 and 68.55 ppm. An absorption band with a maximum at 243 nm in the UV spectrum and stretching vibrations of a 6-ketone at 1650 cm^{-1} in the IR spectrum suggest that compound **14** is a Δ^7-6-ketone. The ^1H NMR spectrum contains a signal for the vinylic proton H-7 at 5.80 ppm for this structure fragment. The chemical shifts of C-6, C-7, and C-8 in the ^{13}C NMR spectrum are 206.58, 122.88, and 165.41 ppm, respectively. Such shifts are typical of ecdysteroids [1]. The ^1H NMR spectra clearly show that ecdysteroid

14 contains a 14α-hydroxyl. The doublet of doublets for the methine proton H-9 situated 1,3-diaxial to it shifts characteristically to downfield (3.13 ppm). The signal of C-14 in the ^{13}C NMR spectrum shifts analogously to downfield (85.4 ppm). This indicates that the hydroxyl is bonded to it. The appearance of a doublet of doublets for H-11α (4.09 ppm) in the ^{1}H NMR spectrum suggests that ecdysteroid **14** contains an 11α-hydroxyl. Furthermore, the shift to downfield for H-1α (2.58 ppm) compared with the position of the analogous signal in the spectrum of 20-hydroxyecdysone (1.79 ppm) is highly characteristic. Such a shift is caused by electrostatic interaction with the spatially close 11α-hydroxyl. The chemical shifts of the C atoms in the side chain unambiguously prove that compound **14** contains 20-, 22-, and 24-hydroxyls.

Reaction of compound **14** with acetone in the presence of *p*-toluenesulfonic acid and subsequent hydrolysis of the diacetonide in CH_3OH gives the 20,22-monoacetonide. Furthermore, reaction of compound **14** with phenylboronic acid gives a mixture of 20,22- and 22,24-phenylboronates. The configuration of the side chain in compound **14** was finally proved using the nuclear Overhauser effect in the ^{1}H NMR spectrum of this mixture.

The structures of the remaining new ecdysteroids **15-20** in *P. atrotomentosus* were established in principle by an analogous analysis of spectral data. For example, the ^{1}H NMR spectrum of compound **14** 20,22-*p*-hydroxybenzylideneacetal (**15**) contains signals of aromatic protons (6.77 and 7.29 ppm) in addition to others. Furthermore, the shift to downfield in the ^{1}H NMR spectrum of the signals for the 21- and 28-methyl groups (1.280 and 1.135 ppm, respectively) and for the methine proton H-22 (4.18 ppm) compared with the position of the analogous signals in the spectrum of compound **14** is very important for proving the structure of ecdysteroid **15**. The ^{13}C NMR spectrum of compound **15** contains additional signals for the acetal C atom (105.26 ppm) and the C atoms of the *p*-hydroxybenzene ring. The shifts to downfield for the C-20 (85.73 ppm) and C-22 (81.30 ppm) signals compared with their positions in the spectrum of compound **14** are due to the α-effect of the 20,22-benzylideneacetal.

Yet another ecdysteroid of *P. atrotomentosus*, atrotosterone A (**16**), is a 24-desoxy derivative of compound **14**. Its empirical formula $C_{28}H_{46}O_7$ was established from the $(M + H)^+$ peak in the fast-atom bombardment high-resolution mass spectrum. The ^{1}H and ^{13}C NMR spectra of substance **16** contain signals of all atoms and prove the structure. The lack of a 24-hydroxyl in compound **16** lead, first, to the appearance in the ^{1}H NMR spectrum of a signal for the methine proton H-24 (1.67 ppm) and, second, to a doublet for the 28-methyl group that is shifted to upfield (0.851 ppm) compared with its position in the spectrum of compound **14** (1.081 ppm). Analogous shifts are observed in the ^{13}C NMR spectrum. The signal for C-24 shifts to upfield (36.68 ppm) (76.25 ppm in the spectrum of compound **14**). The signals for C-23 (37.50 ppm) and C-25 (30.37 ppm) in the spectrum of substance **16** are also shifted to upfield compared with their positions in the spectrum of compound **14** (41.20 and 37.32 ppm, respectively) owing to the absence of a 24-hydroxyl. It should be noted that the stereochemistry of C-24 in compound **16** could not be determined.

The structure of 25-hydroxyatrotosterone A (**17**) is similar to those of the ecdysteroids listed above. The configuration of C-24 in compound **17** was determined by comparing the chemical shifts of the protons in the side chain in the ^{1}H NMR spectrum with those in the spectra of makisterone A and 24-epimakisterone A. As it turned out the spectral parameters of compound **17** and 24-epimakisterone A are practically identical. This suggests that ecdysteroid **17** has the (24S)-configuration.

The presence of a 22(23)-epoxide in atrotosterone B (**18**) was proved using ^{1}H and ^{13}C NMR spectra. The ^{1}H NMR spectrum of compound **18** contains doublets for the methine protons H-22 (2.85 ppm) and H-23 (2.72 ppm), which are geminal to the epoxide, in addition to other signals. The vicinal coupling constant of these protons (J = 2.4 Hz) indicates that they are oriented *trans* to each other. The signals for C-22 and C-23 in the ^{13}C NMR spectrum occur at values (66.75 and 59.93 ppm, respectively) typical of epoxide C atoms.

Analogous trends are observed in the ^{1}H and ^{13}C NMR spectra of 25-hydroxyatrotosterone B (**19**). This unambiguously indicates the presence of a 22,23-epoxide in it.

According to spectral data, atrotosterone C (**20**) has a structure similar to that of compound **17**. Insignificant differences occur because molecule of compound **20** contains not a 28-methyl but a 24(28)-methylene group. This leads to the appearance in the ^{1}H NMR spectrum of signals for exomethylene protons at 5.15 and 4.96 ppm. The signals for C-24 and C-28 in the ^{13}C NMR spectrum have chemical shifts of 155.34 and 110.42 ppm, respectively. All remaining spectral parameters indicate that molecule of compound **20** contains the corresponding functional groups.

It was recently found that *Tapinella panuoides* (Paxillaceae) contains ecdysteroids [12, 15]. Eight ecdysteroids were isolated from this mushroom. Six of these, 20-hydroxyecdysone (**2**), ponasterone A (**3**), malacosterone (**21**), turkesterone (**22**), and paxillosterone (**14**), were known. They were isolated previously from various natural sources [1, 2]. Therefore, the structures of these ecdysteroids from *T. panuoides* were established by comparing their spectra with those of authentic

compounds. Furthermore, the authenticity of ecdysteroids **2**, **3**, **22**, and **14** was confirmed by direct comparison with known samples. Next panuosterone (**23**) and 25-hydroxypanuosterone (**24**) were isolated for the first time from *T. panuoides*. Their structures were established by analyzing spectra. In particular, the ^{1}H NMR spectra suggest that panuosterone is the 11-desoxy derivative of paxillosterone (**14**).

The spectrum of ecdysteroid **23** contains a signal for H-11β at 1.79 ppm; for H-11α at 1.71 ppm. This is consistent with the lack of an 11α-hydroxyl in substance **23**. Furthermore, the signal for H-1α, which is shifted to downfield (2.58 ppm) in compound **14** owing to interaction with the 11α-hydroxyl, appears at 1.79 ppm in the spectrum of ecdysteroid **23**. Such a position for the H-1α signal is typical for the spectra of ecdysteroids that have no 11α-hydroxyl, e.g., 20-hydroxyecdysone.

The remaining ^{1}H NMR spectral parameters of panuosterone and paxillosterone that were recorded on an instrument with 500 MHz working frequency are very similar. A comparison of the ^{13}C NMR spectra of both compounds also suggests that panuosterone lacks an 11α-hydroxyl. Thus, the signal of C-11 is observed at 21.54 ppm. This is in complete agreement with the analogous value for 20-hydroxyecdysone (21.50 ppm) but not for paxillosterone (69.46 ppm).

The structure of 25-hydroxypanuosterone (**24**) was established by analyzing IR, mass, and ^{1}H and ^{13}C NMR spectra. Comparison of the ^{1}H and ^{13}C NMR spectra of ecdysteroids **23** and **24** is especially useful in this regard. In particular, the principal features of the ^{1}H NMR spectra of these compounds coincide. The exceptions are, first, the lack of a signal for the methine proton H-25 in the spectrum of 25-hydroxypanuosterone and, second, a downfield shift of the signals for the 26- and 27-methyl groups (1.257 and 1.242 ppm, respectively) compared with their positions in the spectrum of panuosterone (0.980 and 0.911 ppm, respectively). These effects are undoubtedly due to the presence in ecdysteroid **24** of an additional 25-hydroxyl.

The signal for C-25 in the ^{13}C NMR spectrum of 25-hydroxypanuosterone is shifted to downfield (77.51 ppm) compared with its position in the spectrum of panuosterone (37.28 ppm). This shift is due to the α-effect of the 25-hydroxyl. Furthermore, the β-effect of the 25-hydroxyl shifts the signals for C-26 and C-27 to downfield (both at 25.25 ppm) in the spectrum of ecdysteroid **24** compared with their positions in the spectrum of compound **23** (18.82 and 17.30 ppm, respectively).

It is interesting that the chemical shifts of C-24 in the spectrum of both compounds are practically identical (76.26 ppm for panuosterone and 76.27 ppm for 25-hydroxypanuosterone). This suggests that the 25-hydroxyl in compound **24** does not exert a β-effect on the chemical shift of C-24.

The biological activity of certain mycoecdysteroids of *P. atrotomentosus* and *T. panuoides* was tested *in vitro* using B_{II} cells of *Drosophila melanogaster* [16, 17]. This test would show if the compounds are antagonists or agonists of the ecdysteroid receptor [18, 19]. It was found that none of the studied compounds acts as an antagonist. However, these ecdysteroids are clearly capable of binding to the ecdysteroid receptor. The most active compound in this test was 20-hydroxyecdysone.

The polyhydroxysteroid **25** that was isolated from *Lasiosphaera nipponica* (Gasteromycetes) has a structure similar to those of the ecdysteroids discussed above [20]. The structure was determined using spectral data. In particular, the empirical formula $C_{28}H_{44}O_7$ was found by high-resolution mass spectrometry. The IR and UV spectra suggest that compound **25** contains an α,β-unsaturated ketone and several hydroxyls. Acetylation of compound **25** forms only a monoacetate. Thus it can be concluded that only one of the six hydroxyls is primary or secondary whereas the remainder are tertiary.

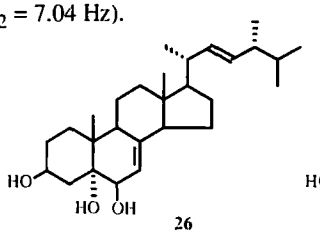
The structure of compound **25** as a Δ^7-6-ketosteroid was confirmed by the presence in the ^{1}H NMR spectrum of a doublet for the vinylic proton H-7 (6.18 ppm). Atoms C-6, C-7, and C-8 have chemical shifts in the ^{13}C NMR spectrum (202.2, 121.6, and 164.9 ppm, respectively) that are typical of Δ^7-6-ketosteroids. A triplet of triplets in the ^{1}H NMR spectrum for the methine proton H-3 (3.86 ppm) is consistent with the presence of a 3β-hydroxyl in this steroid. A nuclear Overhauser effect is observed between the signals for H-3 and H-5 (2.31 ppm). This indicates that they are situated 1,3-diaxial to each other, which is possible only for *trans-A/B* fusion.

The downfield shift of the signals for H-9α (3.64 ppm), H-12α (2.90 ppm), and H-15α (2.92 ppm) is consistent with the presence in molecule of substance **25** of 14α- and 17α-hydroxyls. The presence of a 22(23) double bond can be inferred from the signals for vinylic protons H-22 (6.86 ppm) and H-23 (6.63 ppm). Both signals are doublets (J = 16.1 Hz). This indicates, first, that the double bond has *E*-geometry and, second, that C-20 and C-24 have no protons. This also proves that hydroxyls are present at the 20 and 24 positions.

Compounds that are structurally similar to ecdysteroids are found in fungi in addition to typical ecdysteroids. As a rule, these are metabolites of ergosterol. The most common of these steroids is cerevisterol (**26**) [21].

This compound was isolated first from yeast [22-24]. The structure of the naturally occurring compound was proven by comparison with a sample synthesized chemically from ergosterol [25]. Cerevisterol has been isolated several times from various fungi [20, 26-37]. The structure of newly isolated cerevisterol is usually proved either by direct comparison with an

authentic sample or from spectral data. Data from ^{1}H [33, 34, 36] and ^{13}C [35, 36] NMR spectra are especially significant for establishing the structure of triol **26**. In particular, the ^{1}H NMR spectrum of compound **26** in $CDCl_3$ contains a multiplet for H-3 (4.07 ppm) and a doublet for H-6 (3.62 ppm, J = 4.81 Hz) [35]. The spectrum also contains a doublet of doublets for the vinylic protons H-7 (5.35 ppm, J_1 = 15.39 Hz, J_2 = 8.09 Hz), H-22 (5.16 ppm, J_1 = 15.39 Hz, J_2 = 8.09 Hz), and H-23 (5.23 ppm, J_1 = 15.39 Hz, J_2 = 7.04 Hz).



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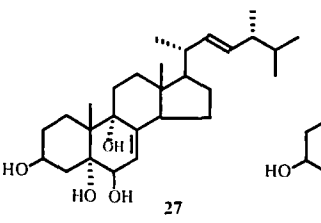

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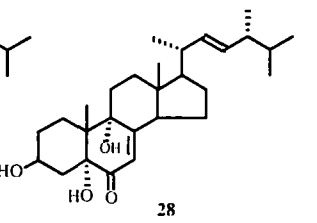

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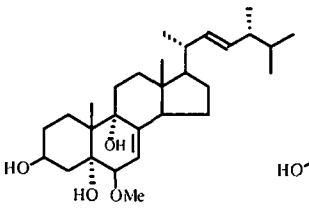

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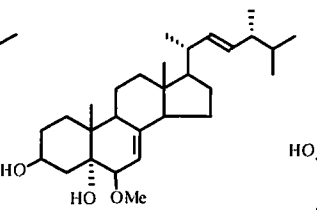

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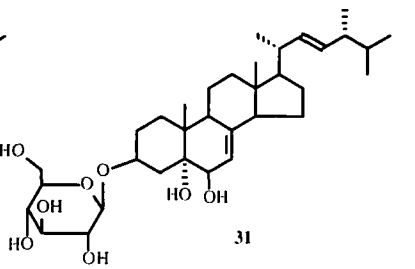

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It should be noted that using $CDCl_3$ to record the spectrum significantly changes the position of the signals for H-3 (4.86 ppm), H-6 (4.34 ppm), and H-7 (5.76 ppm) [36]. The ^{13}C NMR spectrum of compound **26** exhibits among others three signals for C atoms with hydroxyls attached, C-3 (67.6 ppm), C-5 (76.1 ppm), and C-6 (74.2 ppm) [35]. The signals for the vinylic C atoms C-7 (117.33 ppm), C-8 (143.22 ppm), C-22 (135.30 ppm), and C-23 (131.88 ppm) are also important for proving the structure [35].

It was found [37] that the pendulous-disc polypore *Polyporus versicolor* contains polyhydroxysteroids **26-29**, which are related to ecdysteroids. The structure of compound **26** was proved by analyzing spectra of the 3,6-diacetate acetylation product. In particular, the ^{1}H NMR spectrum of the diacetate contains signals characteristic of H-6α (4.92 ppm), H-3α (5.14 ppm), and H-7 (5.38 ppm).

Yet another compound isolated from polypores is 3β,5α,6β,9α-tetrahydroxyergosta-7,22-diene (**27**). The structure of tetraol **27** was established by converting it to the 3,6-diacetate via acetylation with acetic anhydride in pyridine. The ^{1}H NMR spectrum of this diacetate exhibits signals for methine protons geminal to acetoxy groups at 4.95 and 5.14 ppm. These signals, which are assigned to H-6 and H-3, and the signal for the vinylic proton H-7 at 5.28 ppm are identical to those for cerevisterol diacetate. Hence steroid **27**, like cerevisterol, has the Δ^7-3β,5α,6β-trihydroxy grouping.

The position of the fourth hydroxyl, which is tertiary according to the acetylation results, was found by comparing the ^{1}H NMR spectra of the diacetates of steroids **26** and **27**. Judging from the chemical shifts and the multiplicity of the signals for the side-chain methyl groups, positions 17, 20, 24, and 25 can be excluded. Of the two remaining positions, namely 9 and 14, the former is favored based on silylation results. Tetraol **27** forms only the tris(trimethylsilyl) ether. A 14α-hydroxyl, which occurs in ecdysteroids, forms a silyl ether under these conditions [1]. This suggests that the fourth hydroxyl in compound **27** is located at the 9α-position.

A third compound isolated from *P. versicolor*, (**28**), contains an α,β-unsaturated ketone according to IR (bands at 1670 and 1620 cm^{-1}) and UV (band at 237 nm) spectral data. Signals for axial protons H-3 (4.06 ppm), H-7 (5.65 ppm), and H-22 and H-23 (5.20 ppm) could be identified in the ^{1}H NMR spectrum of compound **28**. Because the signal for H-7 appears as a doublet (J = 1.9 Hz), it can be concluded, first, that compound **28** contains a 6-ketone and, second, that a hydroxyl occurs in one of the allyl positions (i.e., 9 or 14). On the other hand, the ^{13}C NMR spectrum of compound **28** contains a signal for C-3 (67.2 ppm) and two signals (74.7 and 79.7 ppm) for tertiary C atoms with hydroxyls attached. The ^{1}H NMR spectrum suggests that these hydroxyls are most likely located on C-5 and C-9 or C-14.

The structure of steroid **28** was finally confirmed as 3β,5α,9α-trihydroxyergosta-7,22-dien-6-one by comparison with the specially synthesized compounds 3α,5β-dihydroxycholest-7-en-6-one and 3β,5α,14α-trihydroxyergosta-7,22-dien-6-one [37].

The formation of compound **28** via allylic oxidation of tetraol **27** by activated MnO_2 provides further confirmation of its structure.

The structure of the fourth polyhydroxysteroid from *P. versicolor* was established as 3β,5α,9α-trihydroxy-6β-methoxyergosta-7,22-diene (**29**) by comparing its spectra with those of compounds **26-28**. In particular, the ^{1}H NMR spectra demonstrate that substance **29** contains an intact side chain of ergosterol with a 22(23)-double bond. The spectrum also exhibits a multiplet for axial proton H-3α (3.66 ppm), a doublet of doublets for H-6α (3.23 ppm), and a doublet of doublets for vinylic proton H-7 (5.48 ppm). A 3-proton singlet for the methoxy group (3.41 ppm) could also be identified in the spectrum.

The ^{13}C NMR spectrum of steroid **29** contains a signal for C-3 (67.5 ppm) and two signals (75.2 and 78.1 ppm) that belong to tertiary C atoms with hydroxyls. There is another signal at 85.2 ppm that is consistent with a secondary C atom with an attached methoxy group. The spectroscopic data for compound **29** were verified by results from acetylation, which forms only a monoacetate. Furthermore, silylation of steroid **29** produces a bis(trimethylsilyl) ether, which excludes the 14α-position for one of the tertiary hydroxyls. Thus, only formula **29** remains possible for this compound.

The cytotoxicity of the *P. versicolor* polyhydroxysteroids to HTC rat hepatoma cells has been studied [37]. It was found that the Δ^7-6-ketone **28** and the methyl ether **29** are very active in this test. However, cerevisterol (**26**) and the tetraol (**27**) were inactive.

It was demonstrated [35] that the basidiomycete *Agaricus blazei* (Agaricaceae) contains four polyhydroxysteroids. The structures of these are analogous to compounds isolated earlier from *P. versicolor*. Thus, steroids **26**, **27**, **28**, and a new compound, 3β,5α-hydroxy-6β-methoxyergosta-7,22-diene (**30**) were isolated from *A. blazei*. The structures of steroids **26-28** were established by comparing their spectra with those of the corresponding compounds isolated earlier form *P. versicolor*. The structure of the new steroid **30** was determined by spectral analysis. Thus, the empirical formula $C_{29}H_{48}O_3$ was found from high-resolution mass spectra. The ^{1}H NMR spectrum of compound **30** is very similar to that of steroid **26**. The exceptions are, first, the presence in the spectrum of steroid **30** of an addition methoxy signal (3.38 ppm) and, second, a upfield shift (3.16 ppm) for the doublet of the methine proton H-6 compared with its position in the spectrum of compound **26** (3.62 ppm).

Acetylation of steroid **30** gives a monoacetate. The ^{1}H NMR spectrum of the monoacetate is interesting because of the downfield shift (5.13 ppm) of the signal for H-3 compared with its position in the spectrum of the starting alcohol (4.03 ppm). Also, the chemical shifts of the signals for H-6α in the spectrum of diol **30** and the monoacetate are practically identical. These data enable the position of the methoxy group in compound **30** to be assigned to C-6. An analysis of the ^{13}C NMR spectra in $CDCl_3$ leads to the same conclusion.

The chemical shifts for most of the corresponding C atoms in spectra of compound **26** and methoxydiol **30** are practically identical. The exception is the additional signal for the methoxy group of steroid **30** (58.25 ppm). The downfield shift (82.43 ppm) of the signal for C-6 compared with its position in the spectrum of compound **26** (73.06 ppm) is interesting. The shift is surely caused by the α-effect of the 6-methoxy group.

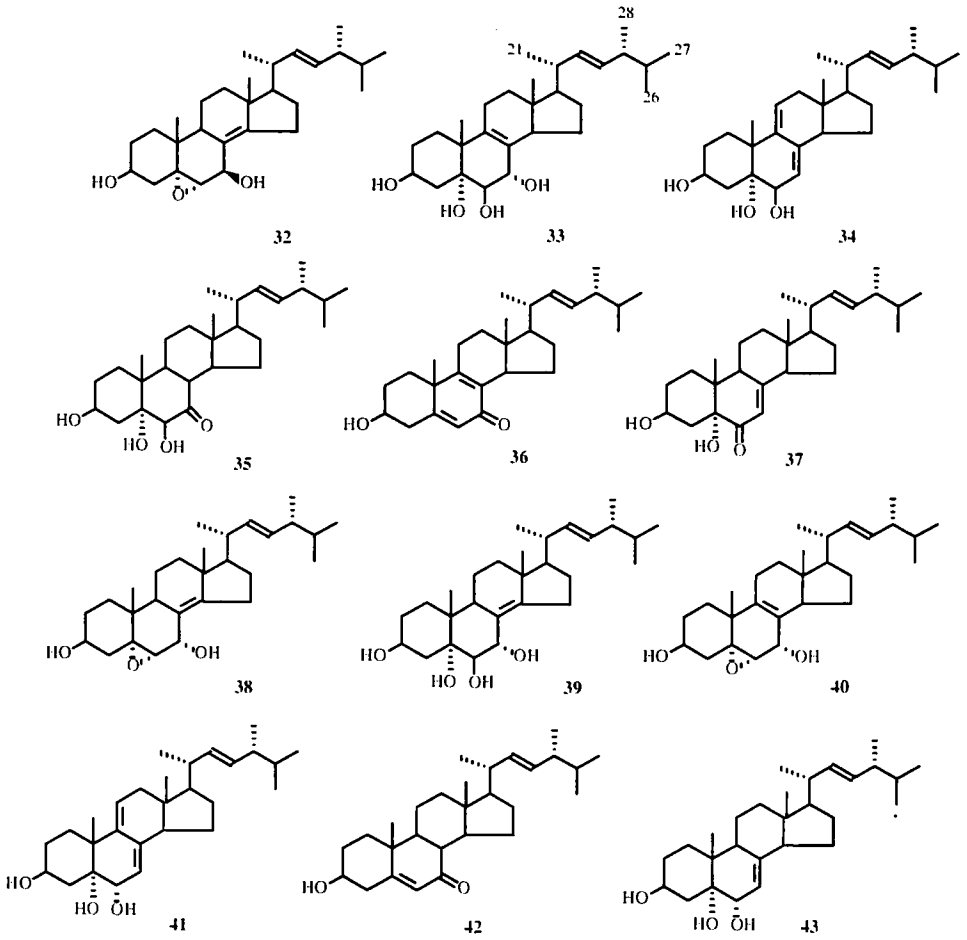
Steroids **27**, **28**, and **30** have been reported [35] to possess cytotoxicity toward HeLa cells. The minimal concentration that completely inhibits cell growth is 63 μg/ml for tetraol **27**; 16, for compound **28**; and 16, for methoxydiol **30**. It was also found that cerevisterol is inactive in this test.

Polyhydroxysteroids have been found in the basidiomycete *Hericum erinacens*, an edible mushroom that is used in Chinese folk medicine to cure ulcers and chronic gastritis [36]. A new compound, 3β-glucopyranosyl-5α,6β-dihydroxyergosta-7,22-diene (**31**), in addition to compounds **26** and **28**, were found during the study. Enzymatic hydrolysis of compound **31** by hesperiginase produces glucose and cerevisterol (**26**). The ^{1}H NMR spectrum of the glycoside **31** contains signals characteristic of **26** and of glucose protons: H-1′ (4.99 ppm), H-2′ (4.08 ppm), H-3′ (4.21 ppm), H-4′ (4.32 ppm), H-5′ (3.55 ppm), and H-6′ (4.41 and 4.50 ppm).

The splitting constant (J = 7.8 Hz) of H-1′ suggest that the anomeric center has the β-configuration. The ^{13}C NMR spectra unambiguously indicate the point of attachment of the hydrocarbon and aglycone. The signal of C-3 is shifted to downfield (76.8 ppm) by glycosylation compared with its position in the spectrum of compound **26** (69.9 ppm). The signals for C-2 (30.6 ppm) and C-4 (38.0 ppm) of the aglycone are similarly shifted to upfield compared with their position in the spectrum of compound **26** (33.0 and 42.0 ppm, respectively) for the same reason.

The mushroom *Grifola frondosa* (Polyporaceae) yielded the polyhydroxysteroids **26-28** and **32-43** [38]. Compounds **32-35** were new and found in nature for the first time. Steroids **26-28** and **36-43** were previously known. Their structures were determined by comparing spectral characteristics with those described in the literature for similar compounds. For example, (22E,24R)-5α,6α-epoxyergosta-8(14),22-dien-3β,7α-diol (**38**) and (22E,24R)-5α,6α-epoxyergosta-8,22-dien-3β,7α-diol (**40**) were

entirely identical to compounds that were isolated earlier [39]. In one's turn the compounds (22E,24R)-ergosta-7,9(11),22-trien-3β,5α,6α-triol (**41**) and (22E,24R)-ergosta-7,22-dien-3β5α,6α-triol (**43**) were isolated earlier [40]. The compound (22E,24R)-3β-hydroxyergosta-5,22-dien-7-one (**42**) was also found earlier in nature [41,42].



Spectral analysis showed that the structure of the new steroid **32** was (22E,24R)-5α,6α-epoxyergosta-8(14),22-dien-3β,7β-diol. Thus, high-resolution mass spectra gave the empirical formula $C_{28}H_{44}O_3$. The IR spectrum contains bands characteristic of hydroxy stretching vibrations at 3611 cm^{-1}. Judging from the presence in the ^1H NMR spectrum of characteristic doublets for the 21-, 26-, 27-, and 28-methyl groups, this compound has an ergostane side chain. Signals for only H-22 and H-23 (5.20 ppm) are observed for the vinylic protons. This is consistent with a 22(23)-double bond. Furthermore, the spectrum contains signals for H-3 (3.89 ppm) and H-7 (4.52 ppm), which are geminal to hydroxyls. The chemical shift and multiplicity of the doublet for H-6 (3.00 ppm, J = 2.5 Hz) are due to the 5α,6α-epoxide.

The chemical shifts of C-8 and C-9 (126.8 and 151.6 ppm, respectively) in the ^{13}C NMR spectrum confirm the presence of an 8(14)-double bond. The ^{13}C NMR spectra of the isomeric steroids **32** and **38** are similar except for the signal for C-7. The configuration of the 7β-hydroxyl in steroid **32** was established on the basis of the significant downfield shift by 0.26 ppm of the signal for the 19-methyl group for the spectrum recorded in deuteropyridine compared with the position of this signal for the spectrum recorded in $CDCl_3$. This shift is due to solvation of the 7β-hydroxyl.

It should be noted that the structure of compound **32** is very close to that of ergokonine isolated from the mycelium of *Trichoderma koningii* [43].

Like for the first instance, high-resolution mass spectra gave the empirical formula $C_{28}H_{46}O_4$ of yet another new compound from *G. frondosa*, (22E,24R)-ergosta-8,22-dien-3β,5α,6β,7α-tetraol (**33**). The IR spectrum of compound **33** contains stretching vibrations of hydroxyls at 3612 and 3398 cm^{-1}. According to ^1H and ^{13}C NMR spectra, steroid **33** contains an ergosterol side chain, which has a 22(23)-double bond. Furthermore, the ^1H NMR spectrum resolves signals for three methine

protons geminal to hydroxyls, H-3 (4.16 ppm), H-6 (3.75 ppm), and H-7 (3.96 ppm).

The chemical shifts in the ^{13}C NMR spectrum of C-3 (67.0 ppm), C-5 (75.3 ppm), C-6 (79.3 ppm), and C-7 (72.7 ppm) enable the structure of compound **33** to be assigned as the 3β,5α,6β,7α-tetraol. The chemical shifts of C-8 and C-9 (127.8 and 137.6 ppm, respectively) suggest that compound **33** contains an 8(9)-double bond. The configuration of C-7 in compound **33** was determined by comparing its ^1H NMR spectrum with that of 3β,5α,6β,7α-tetrahydroxycholest-8-en-11-one. It should be noted that the related to tetraol **33** (24S)-24-ethylcholest-8-en-3β,5α,6β,7α-tetraol was isolated from the marine sponge *Neofibularia nolitangere* [44].

The empirical formula $C_{28}H_{44}O_3$ of (22E,24R)-ergosta-7,9(11),22-trien-3β,5α,6β-triol (**34**) was determined from high-resolution mass spectra. The IR spectrum of substance **34** contains vibrations of hydroxyls at 3612 and 3414 cm^{-1}. The UV spectrum has a band characteristic of conjugated dienes with a maximum at 244 nm. The ^1H NMR spectrum suggests that compound **34** contains an ergost-22-ene side chain. Furthermore, the ^1H NMR spectrum confirms that molecule **34** contains a 7,9(11)-diene (signals for H-7 and H-11 at 5.45 and 5.75 ppm, respectively) and 3β- and 6α-hydroxyls (signals for H-3 and H-6 at 4.12 and 3.82 ppm, respectively). The stereochemistry of the 6β-hydroxyl was proved, first, by comparison with the isomeric 6α-alcohol **41** and, second, by the downfield shift of the signal for the 19-methyl group in deuteropyridine compared with its position in $CDCl_3$.

The structure of the new steroid **35** was determined in principle in an analogous manner as (22E,24R)-3β,5α,6β-trihydroxyergost-22-en-7-one. Thus, high-resolution mass spectra gave the empirical formula $C_{28}H_{46}O_4$. The IR spectrum exhibits hydroxy stretches at 3614 and 3410 cm^{-1} and ketone vibrations at 1708 cm^{-1}. The presence of an ergosterol side chain is consistent with signals for the 21-, 26-, 27-, and 28-methyl groups and the H-22 and H-23 vinylic protons that have the appropriate chemical shifts and multiplicities in the ^1H NMR spectrum. Proton H-8 (2.93 ppm) could be identified by the downfield shift caused by the neighboring 7-ketone. The presence of 3β,5α,6β-trihydroxyls is confirmed by the signals for H-3 (4.85 ppm) and H-6 (4.31 ppm). Furthermore, the significant downfield shift of the signals for H-3, H-4, H-8, and the 19-methyl group in the spectra recorded in deuteropyridine compared with their positions in spectra recorded in $CDCl_3$ are characteristic of this fragment.

Yet another steroid (**36**) isolated from *G. frondosa* has the structure (22E,24R)-3β-hydroxyergosta-5,8,22-trien-7-one. The empirical formula $C_{28}H_{42}O_2$ was determined from high-resolution mass spectra. The IR spectra of substance **36** contain bands of hydroxyls at 3457 cm^{-1} and bands characteristic of a cross-conjugated dienone at 1662, 1626, and 1593 cm^{-1}. An analogous conclusion about the presence of a cross-conjugated diene in compound **36** is suggested by the UV spectrum, which contains an absorption maximum at 246 nm. The structure of steroid **36** was finally proved using ^1H and ^{13}C NMR spectra. It should be noted that the $\Delta^{5,8(9)}$-7-ketone **36** was earlier prepared by chemical synthesis [45]. However, its occurrence in nature was first demonstrated later [38].

The structure of (22E,24R)-3β-5α-dihydroxyergosta-7,22-dien-6-one (**37**) was determined using spectral data and finally proved by synthesis as ergosterol acetate. The chemical synthesis includes oxidation of ergosterol acetate by sodium dichromate in a mixture of acetic acid and benzene to give the corresponding 3β-acetoxy-5α-hydroxy-6-ketone and its subsequent saponification by sodium carbonate in CH_3OH.

It should be mentioned that 5α,8α-peroxides of ergosterol, 9(11)-dehydroergosterol, and 22,23-dihydroergosterol were isolated in addition to steroids **26-28** and **32-43** from *G. frondosa* [38].

The edible mushroom *Lentinus edodes* has a very complicated array of polyhydroxysteroids [46]. In addition to the previously known substances **26-28**, **41**, and **43**, the new compounds **44-47** and **50** were isolated from it. The structures of these compounds were determined by comparing their spectra with those of similar compounds. For example, the trihydroxyketosteroid **44** has a structure similar to trihydroxyketone **28**. The empirical formula $C_{29}H_{46}O_4$ of steroid **44** was established from high-resolution mass spectra and the ^{13}C NMR spectrum. The presence of an absorption maximum at 237 nm in the UV spectrum is consistent with an α,β-unsaturated ketone. This is also consistent with the IR spectrum, which contains stretching vibrations of a ketone at 1676 cm^{-1} and a double bond conjugated to it at 1626 cm^{-1}. Furthermore, bands at 3588 and 3413 cm^{-1} in the IR spectrum suggest that compound **44** contains hydroxyls. The structure of the cyclic portion of steroid **44** was proved by comparing its ^1H and ^{13}C NMR spectra with the corresponding spectra of trihydroxyketone **28**. The presence of the side chain in compound **44** was established by comparing its mass and ^1H NMR spectra with those of (22E,24R)-23,24-dimethylcholesta-5,22-dien-3β-ol. Thus, the structure (22E,24R)-3β,5α,9α-trihydroxy-23-methylergosta-7,22-dien-6-one was assigned to steroid **44**.

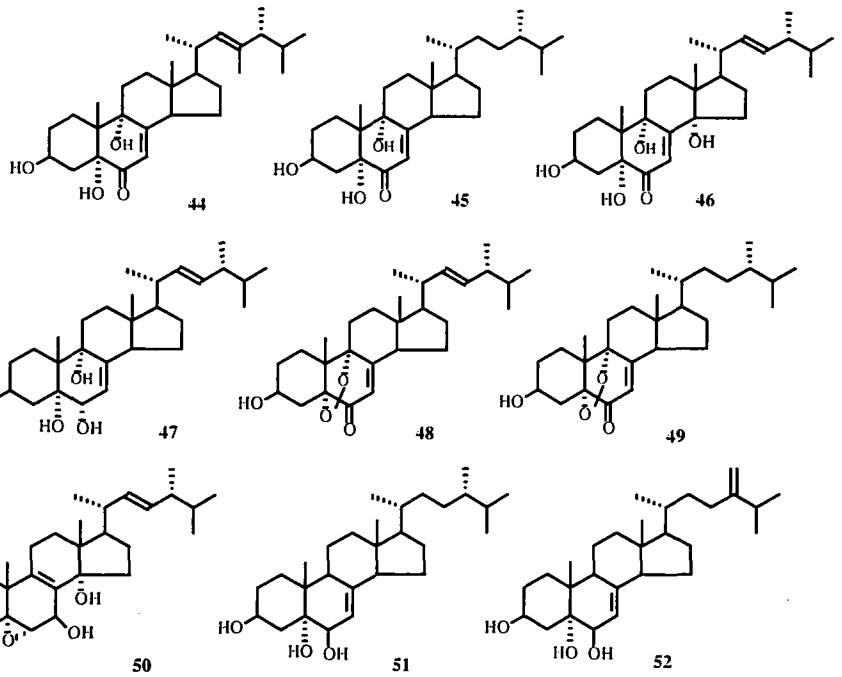
The structure of (24S)-3β,5α,9α-trihydroxyergost-7-en-6-one (**45**) was established in an analogous manner. The ^1H and

^{13}C NMR spectra were compared with those for steroid **44** in order to prove the presence of the functional groups and their stereochemistry. The spectral parameters of both compounds are very similar. The exceptions are the chemical shifts of the H and C atoms in the side chains. For example, the chemical shifts of the signals for C-22 and C-23 in the ^{13}C NMR spectrum of steroid **45** are 33.8 and 31.0 ppm, respectively. This greatly differs from the shifts of C-22 and C-23 in the spectrum of compound **44** (131.6 and 135.6 ppm, respectively). These differences indicate that compound **45** does not contain a 22(23)-double bond. The mass spectra lead to an analogous conclusion. A fragment with *m/z* 301 corresponds to the loss of the side chain from $[M^+ - H_2O]$ (*m/z* 428). The stereochemistry of the side chain in steroid **45** was finally proved by comparing ^1H and ^{13}C NMR spectra with those of the dihydrobrassicasterol.

The empirical formula $C_{28}H_{44}O_5$ for (22E,24R)-3β,5α,9α,14α-tetrahydroxyergosta-7,22-dien-6-one (**46**) was found from high-resolution mass spectra ($M^+ - H_2O$ with *m/z* 442) and ^{13} C NMR [46]. The IR spectrum suggests that this compound has hydroxyls (band at 3354 cm^{-1}) and an α,β-unsaturated ketone (band at 1687 cm^{-1}). The UV spectrum also suggests that this structural fragment is present. An absorption maximum is observed at 225 nm. The ^1H NMR spectrum in deuteropyridine contains signals for H-3 (4.61 ppm), H-7 (6.25 ppm), H-22 (5.26 ppm), and H-23 (5.31 ppm). The appearance of a singlet for H-7 indicates that allylic H atoms are absent in the 9- and 14-positions, interaction with which usually produces splitting.

The presence of 9α- and 14α-hydroxyls in compound **46** was proved more convincingly using the ^{13}C NMR spectrum. Signals for C-9 and C-14 appear at 77.2 and 86.2 ppm, respectively. A upfield shift of the signal for C-17 (50.5 ppm) compared with its position in the spectrum of trihydroxyketone **28** (56.1 ppm) is also characteristic. This shift is undoubtedly due to the γ,*gauche*-effect of the 14-hydroxyl, which should therefore have the α-configuration.

Yet another new compound isolated from *L. edodes* is (22E,24R)-ergosta-7,22-dien-3β,5α,6α,9α-tetraol (**47**) [46]. The emipircal formula $C_{28}H_{46}O_4$ was established using high-resolution mass spectrometry. The IR spectrum of steroid **47** contains stretches of hydroxyls at 3608 and 3443 cm^{-1}. Peaks in the electron-impact mass spectrum at *m/z* 374 ($M^+ - 4H_2O$), 249 ($M^+ - 4H_2O$ - side chain), and 207 ($M^+ - 4H_2O$ - *D* ring) suggest that this compound contains four hydroxyls. The presence of a 22(23)-double bond in the ergosterol side chain was proved using ^1H and ^{13}C NMR spectra. The ^1H NMR spectrum indicates that compound **47** contains two secondary and two tertiary hydroxyls. This follows from the positions of the signals for H-3 (4.03 ppm) and H-6 (3.96 ppm). Furthermore, the spectrum contains a signal for the vinylic proton H-7 (5.06 ppm). The ^{13}C NMR spectra of steroids **47** and **28** are exceedingly similar with the exception of the positions for the C-6 signals. The α-orientation of the 6-hydroxyl in tetraol **47** is consistent with a nuclear Overhauser effect between the protons of the 19-methyl group and the methine proton H-6.



The epoxytriol **50**, which was first isolated from *L. edodes*, has a structure slightly different from the remaining polyhydroxysteroids [46]. The empirical formula $C_{28}H_{44}O_4$ for compound **50** was determined from high-resolution mass spectra, which produce an ion $M^+ - H_2O$ with *m/z* 426. The IR spectrum of compound **50** has hydroxy stretching bands at 3506 cm^{-1}. The presence in the structure of steroid **50** of the ergosterol side chain was established using ^1H NMR spectra. The presence in the ^1H NMR spectrum of signals for H-3 (3.93 ppm), H-6 (3.22 ppm), and H-7 (4.76 ppm) is very important. The magnitude of the chemical shift of the signal for H-6 and its multiplicity (doublet with J = 2.9 Hz) are consistent with the presence of a 5α,6α-epoxide in steroid **50**.

The signals of C-8 and C-9 in the ^{13}C NMR spectrum have chemical shifts at 128.9 and 137.3 ppm, respectively. This is consistent with the presence of an 8(9)-double bond. The presence of a 14-hydroxyl is also evident from the ^{13}C NMR spectrum, in which the signal of C-14 is observed at 85.3 ppm. A upfield shift of the signal for C-17 (to 49.0 ppm) compared with its position in the spectrum of (22E,24R)-5α,6α-epoxyergosta-8,22-dien-3β,7α-diol (**40**) (53.7 ppm) is undoubtedly caused by the γ-*gauche*-effect of the 14-hydroxyl, which is possible only if it has the α-orientation.

The composition of polyhydroxysteroids from the velvet-foot *Flammulina velutipes* has been studied [46]. Cerevisterol (**26**), its 22(23)-dihydroxy derivative (**51**), and the tetraol **47** were isolated from this mushroom. The structure of (24S)-ergost-7-en-3β,5α,6β-triol (**51**) was proved by comparing its spectra with those in the literature [47, 48] for a compound of the same structure isolated from marine sponges.

The polyhydroxysteroids **26**, **28**, **45-49**, **51**, and **52** were isolated from the mushroom *Hypsizygus marmoreus* [46]. The unstable 5α,9α-epidioxides **48** and **49** were isolated first. Their structures were proved using spectra. In particular, high-resolution mass spectra of steroid **48** established the empirical formula $C_{28}H_{42}O_4$. The IR spectrum of compound **48** contains vibrations of hydroxyls at 3606 and 3386 cm^{-1}, of a carbonyl at 1685 cm^{-1}, and of a double bond conjugated to it at 1643 cm^{-1}. The chemical shifts of the signals for the methyl groups and the 22- and 23-vinylic protons in the ^1H NMR spectrum suggested the presence of an ergosterol side chain in structure of compound **48**.

The spectrum also contains a signal for the vinylic proton H-7 (5.94 ppm). In addition, signals for H-3 (3.91 ppm) and H-14 (2.50 ppm) are observed. The ^{13}C NMR spectrum, in which the signals for C-5 and C-9 have characteristic chemical shifts (85.6 and 91.0 ppm, respectively) is consistent with a 5α,9α-epidioxide in steroid **48**. These data enable the structure (22E,24R)-5α,9α-epidioxy-3β-hydroxyergosta-7,22-dien-6-one to be assigned to steroid **48**.

Yet another new compound (**49**) from *H. marmoreus* has the empirical formula $C_{28}H_{44}O_4$ according to high-resolution mass spectrometry. The spectral parameters of compound **49** are very similar to those of epidioxide **48**. The exceptions are the chemical shifts of the side-chain atoms in the ^1H and ^{13}C NMR spectra of both compounds. The ^1H NMR spectrum of steroid **49** lacks signals for the vinylic protons H-22 and H-23. It is assigned the structure of the 22,23-dihydro derivative of compound **48**. The stereochemistry of the side chain was determined by comparing the ^1H NMR spectra of steroid **49** and dihydrobrassikasterol. This made it possible to establish the structure (24S)-5α,9α-epidioxy-3β-hydroxyergosta-7-en-6-one to substance **49**.

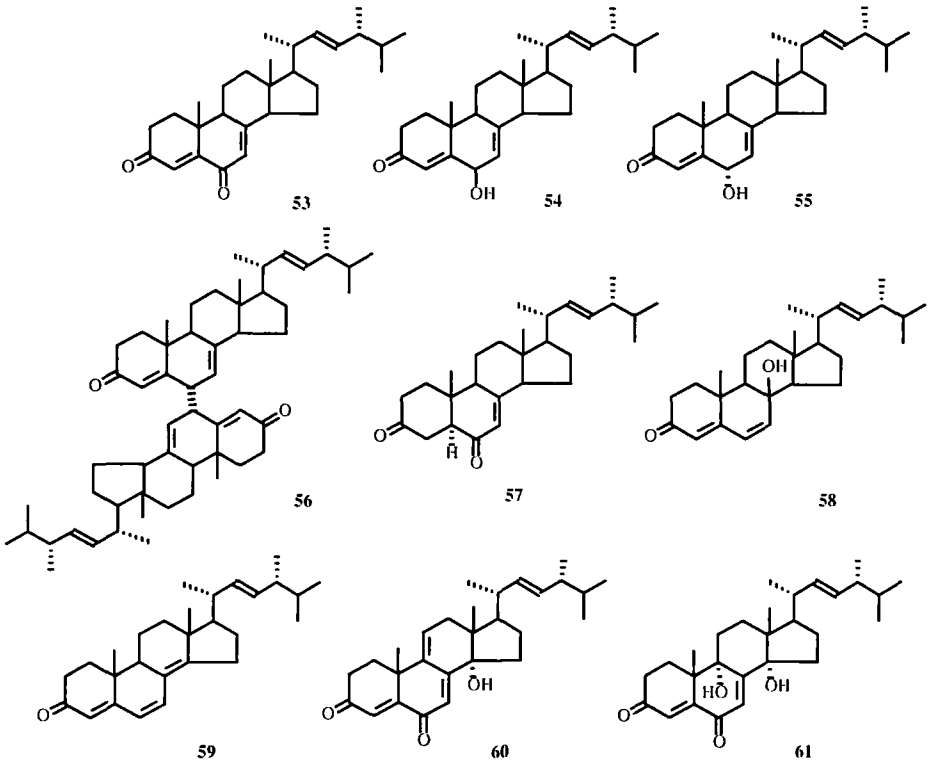
The structure of ergosta-7,24(28)-dien-3β,5α,6β-triol (**52**) was proved by comparing its spectra with the analogous spectra of the compound isolated previously from marine sponges [47-50].

It has been demonstrated [46] that oyster mushrooms *Pleurotus ostreatus* also contain polyhydroxysteroids. The trihydroxyketones **28** and **45**, tetrahydroxyketone **46**, tetraol **47**, epidioxide **48**, cerevisterol **26**, and triol **51** were isolated from this mushroom. A continuation of these studies isolated steroids **28**, **45-47**, and **51** from the edible mushroom *Pholiota nameko* [46].

Oxidized derivatives of ergosterol **53-55** were isolated from the basidiomycete *Ganoderma lucidum* [51, 52]. The structure of ergosta-4,7,22-trien-3,6-dione **53** was established from spectra [51]. The empirical formula $C_{28}H_{40}O_2$ was determined from high-resolution mass spectra. The presence in the UV spectrum of a band at 275 nm indicates that the structure of steroid **53** is an α,β,α′,β′-unsaturated ketone. The IR spectrum, in which vibrations of carboxyls at 1665 and 1640 cm^{-1} and double bonds conjugated to them at 1620 and 1600 cm^{-1} are observed, lead to an analogous conclusion.

The ^1H NMR spectrum of compound **53** suggests the presence of an intact ergosterol side chain with a 22(23)-double bond. Furthermore, the spectrum contains signals for vinylic protons H-4 (singlet, 6.47 ppm) and H-7 (triplet, 5.98 ppm, J = 1.8 Hz). The ^{13}C NMR spectrum of substance **53** typically contains signals for four vinylic tertiary (123.9, 126.0, 132.8, and 134.8 ppm), two quaternary (158.2 and 167.7 ppm), and two carbonyl (187.0 and 199.3 ppm) C atoms. It should be noted that steroid **53** was first isolated from the marine sponge *Raphidostila incisa* [53]. We note that several cholestane derivatives related to **53** are used in the biosynthesis of ecdysteroids [54, 55].

The structures of the isomeric 6-hydroxy-$\Delta^{4,22}$-3-ketosteroids **54** and **55** were established using chemical and spectroscopic methods [52]. In particular, their empirical formulas $C_{28}H_{42}O_2$ were established on the basis of high-resolution mass spectra. The ^1H NMR spectra suggest that both compounds have ergosterol side chains, Δ^4-3-ketones, and 6-hydroxyls. This is also consistent with results from the oxidation of steroids **54** and **55** by pyridinium dichromate. Both compounds form the same product, the diketone **53**. The configuration of the hydroxyl in compound **54** was determined by comparing its spectrum with that of a synthetic compound of the same structure that was previously prepared [53]. Because compounds **54** and **55** are isomers, the α-configuration of the 6-hydroxyl should be assigned to the latter.



Polyhydroxysteroids of the puffball *Calvatia cyathiformis* (Lycoperdaceae) have structures similar to ecdysteroids [56-58]. The dimeric steroid calvasterone (**56**) [56], ergosta-4,7,22-trien-3,6-dione (**53**), cyathisterone (**57**), cyathisterol (**58**), ergosta-4,6,8(14),22-tetraen-3-one (**59**) [57], and calvasterols A (**60**) and B (**61**) [58] were isolated from it. The structures of the known compounds **53** and **59** were established by comparing their spectra with those reported in the literature.

Calvasterone (**56**) is a dimeric steroid according to spectra data [56]. Thus, fast-atom bombardment negative-ion mass spectra exhibit a peak for the quasimolecular ion $(M - H)^-$ with *m/z* 785. This corresponds to the empirical formula $C_{56}H_{82}O_2$, which was confirmed by elemental analysis in addition to mass spectra. The IR spectrum of the bissteroid contains vibrations at 1680 cm^{-1} for a ketone conjugated to double bonds. The structure of compound **56** as an α,β-unsaturated ketone is also proved by the presence in the UV spectrum of an absorption maximum at 238 nm. The presence in the ^1H NMR spectrum of signals for the appropriate methyl groups indicates that compound **56** contains the ergostane side chain. Furthermore, the ^1H NMR spectra typically contain signals for vinylic protons H-4 and H-4′ (5.85 ppm), H-7 and H-7′ (4.74 ppm), H-22 and H-22′ (5.15 ppm), and H-23 and H-23′ (5.22 ppm). The signals for H-6 and H-6′ appear as a broad singlet at 3.56 ppm. The configurations at the 6- and 6′-positions of molecule **56** were proved by the presence of a nuclear Overhauser effect of these protons and the 19- and 19′-methyl groups, respectively. It was found that photo-oxidation of compound **56** gives the tetraenone **59**, which also confirms the structure of **56**.

The structure of cyathisterone (**57**) was found using spectral data [57]. Thus, the empirical formula $C_{28}H_{42}O_2$ was established using high-resolution mass spectrometry and the molecular-ion peak with *m/z* 410. The UV spectrum of compound **57** contains an absorption maximum at 242 nm, which suggests that the structure is an α,β-unsaturated ketone. This is confirmed by the IR spectrum, which contains vibrations of a ketone at 1660 cm^{-1}. Furthermore, a band is observed at

1720 cm^{-1}, which is consistent with yet another ketone in structure of compound **57**. The ^1H NMR spectra prove the presence in molecule **57** of an ergosterol side chain, including a 22(23)-double bond (signals for H-22 and H-23 at 5.17 and 5.27 ppm, respectively). Comparison of the ^1H NMR spectra of compound **57** and ergosta-4,7,22-trien-3,6-dione (**53**) establishes that structure of compound **57** contains a 7(8)-double bond (signal for H-7 at 5.78 ppm) and lacks a 3(4)-double bond. Comparison of the ^{13}C NMR spectra of both compounds leads to the same conclusion.

The empirical formula $C_{28}H_{42}O_2$ of cyathisterol **58** was established from the molecular-ion peak (*m/z* 410) in the high-resolution mass spectrum [57]. The UV spectrum of compound **58** contains an absorption band at 278 nm, which indicates that **58** contains a ketone conjugated to two double bonds. The IR spectrum, which contains vibrations of a ketone at 1650 cm^{-1}, is also consistent with this. A band at 3350 cm^{-1} in the IR spectrum suggests that the second O atom in molecule **58** exists as a hydroxyl. The ^1H and ^{13}C NMR spectra establish unambiguously that compound **58** contains an ergosterol side chain. Furthermore, the positions of the signals for the vinylic protons in the ^1H NMR are very characteristic: H-4 (singlet, 5.76 ppm), and H-6 (doublet, 6.27 ppm, J = 9.9 Hz), and H-7 (doublet, 6.52 ppm, J = 9.9 Hz).

The positions and multiplicities of these signals correspond completely to the analogous values in the spectrum of steroid **59**. This indicates that compound **58** contains a $\Delta^{4,6}$-3-ketone. The hydroxyl in molecule **58** should be tertiary because the ^1H NMR spectrum lacks a signal for a proton geminal to it. Its position on C-8 was established using the ^{13}C NMR spectrum and confirmed from results of dehydration by fluorescent irradiation to give the tetraenone **59**.

The structures of calvasterols A and B were elucidated from their spectra [58]. Thus, the empirical formula $C_{28}H_{38}O_3$ of compound **60** was determined from high-resolution mass spectra. The UV spectrum of compound **60** contains strong bands with maxima at 250 and 340 nm. The IR spectrum exhibits vibrations at 3450 and 1660 cm^{-1}. These data are consistent with hydroxyl and an α,β-unsaturated ketone in molecule **60**.

The ^1H NMR spectra of compound **60** indicate the presence of an ergosterol side chain with a 22(23)-double bond. The ^1H NMR spectrum of compound **60** has characteristic singlets for the vinylic protons H-4 (6.45 ppm) and H-7 (6.17 ppm). The signal of vinylic proton H-11 appears as a doublet of doublets (6.22 ppm, J_1 = 1.8 Hz, J_2 = 6.6 Hz). The appearance of the signal for H-7 as a singlet proves that C-9 and C-14 of structure of compound **60** do not have H atoms on them. Comparison of the ^{13}C NMR spectra of steroids **60** and **53** was very useful in establishing the structure of the former. The coincidental chemical shifts of corresponding C atoms suggested the presence in molecule **60** of an ergost-22-en side chain in the $\Delta^{4,7}$-3,6-diketone. The downfield shift of the signal for C-14 (to 84.7 ppm) in compound **60** compared with its position in the spectrum of steroid **53** (56.3 ppm) unambiguously indicates that molecule **60** contains a 14α-hydroxyl. The signals for C-9 and C-11 in the spectrum of compound **60** have chemical shifts of 138.5 and 132.9 ppm, respectively. This convincingly confirms the presence of a 9(11)-double bond.

The empirical formula $C_{28}H_{40}O_4$ of calvasterol B (**61**) was found from high-resolution mass spectra with a molecular-ion peak with *m/z* 440 [58]. The IR spectrum of compound **61** contains vibrations of hydroxyl (3450 cm^{-1}) and a carbonyl conjugated to a double bond (1660 cm^{-1}). The presence of the latter feature is also confirmed by a strong absorption band with a maximum at 266 nm in the UV spectrum. The ^1H and ^{13}C NMR spectra suggest that molecule **61** has an ergosterol side chain. The ^1H NMR of steroid **61** exhibits singlets for the vinylic protons H-4 (6.60 ppm) and H-7 (6.19 ppm). The ^{13}C NMR parameters of calvasterols A and B are very similar. However, the signal for C-9 in calvasterol B appears at 74.4 ppm. This confirms that it contains an additional 9α-hydroxyl. It was also found that steroid **61** is formed, although in small quantities, during auto-oxidation of ergosta-4,7,22-trien-3,6-dione (**53**).

Discussions of the structure of polyhydroxysteroids from mushrooms often draw attention to the fact that they are similar or identical to polyhydroxysteroids isolated from marine sponges. Several examples of this can be cited in addition to the ones listed above. Thus, 5α-cholest-7-en-3β,6α-diol, 5α-cholest-7,24-dien-3β,6α-diol, and 24-methylen-5α-cholest-7-en-3β,6α-diol were isolated from the marine sponge *Spongionella gracilis* [59]. Six 3β,5α,6β,9α-tetrahydroxysteroids with structures very similar to those of analogous compounds from mushrooms, in particular tetraol **27**, were detected in the marine sponge *Spongia officinalis* [60]. Several 3β,5α,6β-triols and their 6-methoxy derivatives, including steroids **26** and **30**, were identified in the marine sponge *Dictyonella incisa* [62]. Also, the Mediterranean sponge *Clathrina clathrus* contains $\Delta^{5,8(9)}$-7-ketosteroids, one of which has the structure of compound **36** or its 24-epimer [62]. Epoxydiols with structures close to those of steroids **38** and **40** were observed in the marine sponge *Melithaea ocracea* [63]. The sponge *Oscarella lobularis* yielded 5α,6α-epoxy-7-ketosteroids [64]. We also note that several 3β,5α,6β-Δ^7-trihydroxysteroids, including cerevisterol, were identified in the Mediterranean Bryozoa *Myriapora truncata* [65].

Such structural similarity of polyhydroxysteroids from fungi and sponges is not fully understood. The biological

principles for this phenomenon may be unraveled during further research. In conclusion, the author thanks Rene Lafont and Juraj Harmatha for help in selecting the references for this work.

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