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ADVANCES IN THE CHEMICAL TRANSFORMATION OF β-SITOSTEROL

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This review generalizes the most important results on the chemical synthesis of steroids from β-sitosterols.

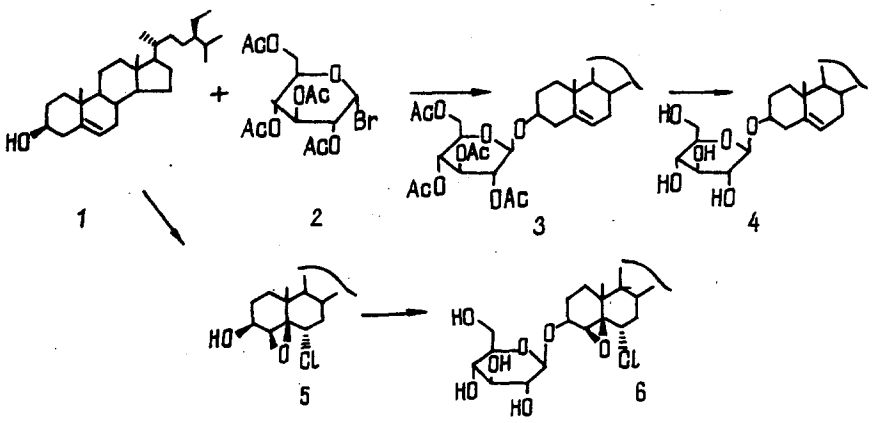
Advances in the production of biologically active steroids are determined to a considerable degree by the accessibility of the initial raw material. Recently, therefore, β-sitosterol (1) has been acquiring ever greater importance for the production of steroidal drugs. The importance of this sterol for the industrial production of steroidal hormones is determined by its wide distribution in natural sources, its comparatively low cost, and the simplicity of its technical isolation [1].

When β-sitosterol undergoes microbiological transformations under definite conditions, degradation of the side-chain takes placae with the formation of androstane derivatives — well-known intermediates in the industrial synthesis of steroids [2, 3]. In addition to microbiological methods, considerable interest is also presented by purely chemical methods of obtaining biologically active substances from β-sitosterol. Important results have been obtained by this route in recent years, and it is to these that the present paper is devoted.

It must be said that existing industrial methods of producing β-sitosterol generally lead to a technical product containing 60-70% of β-sitosterol. The main impurities in this technical β-sitosterol are campesterol, having a methyl group, rather than an ethyl group, at C-24, saturated sterols, and higher alcohols. In the majority of cases, technical β-sitosterol is completely suitable for chemical syntheses. However, if a high-purity preparation, including radioactively labeled material, is required for scientific investigations, special methods of synthesis have been proposed for its production [4-10].

The β-sitosterol molecule has several centers at which it is possible to perform chemical reactions. These include, first of all, the 3β-hydroxy group. Thus, Habib and Khalil [11] obtained from β-sitosterol the hemisuccinate, from which, by reaction with thionyl chloride, they then synthesized the acid chloride. Interaction of the latter with various thiols, amines, and phenols led to the formation of the corresponding heterocyclic derivatives. Among the steroids synthesized, substances were detected with antilipemic activities comparable with the activity of β-sitosterol itself.

Chemical reactions at the 3β-hydroxy group also include the glycosylation of β-sitosterol [12-14]. β-Sitosterol glycosides have been detected in plants and, in a number of cases, they possess interesting biological activity. Thus, two acylglycosides of β-sitosterol isolated from *Musa paradisiaca* and called sitoindosides I and II exhibit antiulcer activity [15]. Experiments on rats have shown that these compounds prevent the formation of the stomach ulcers caused by aspirin.



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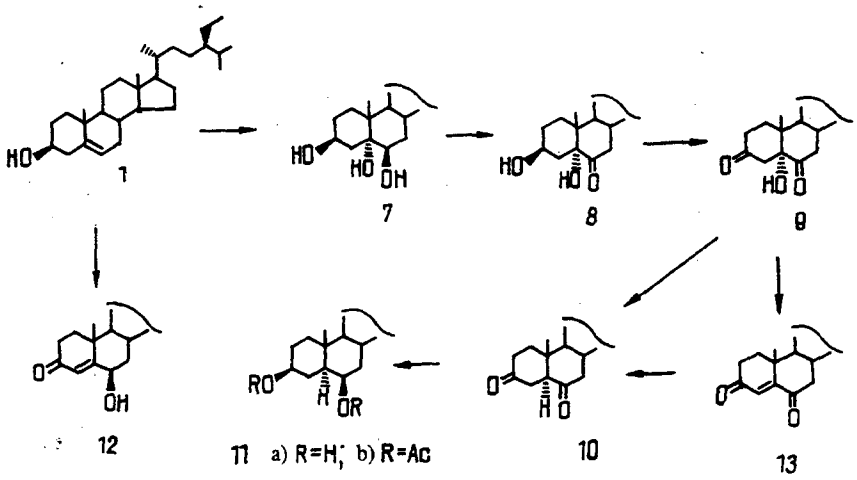
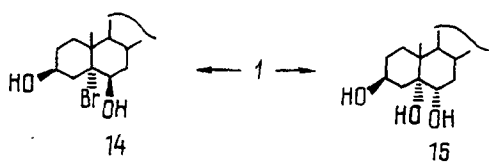
Examples of the synthesis of β-sitosterol tetra-O-acetyl-β-D-glucoside (3) and then β-sitosterol 3β-D-glucoside (4) are given in the literature [13, 14]. Anufriev and Evarova [13] give a comparative characterization of the known methods of the glycosylation for obtaining glucosides (3) and (4) from β-sitosterol. It must be mentioned that the yield of the final product amounted to not more than 30%. The same authors [13] improved the method of synthesizing the β-sitosterol derivative (3) and obtained it with yield of 46%. The productivity of the method developed depends substantially on the order of introducing the reactants. A solution of a mixture of β-sitosterol and tetra-O-acetyl-*D*-glucopyranosyl bromide (2) in toluene was added to a boiling suspension of cadmium carbonate in toluene with the simultaneous elimination of an azeotropic mixture of water with the solvent by distillation. After the mixture had been boiled for two hours, the tetraacetylglucoside (3) separated out, and this, by interaction with sodium methanolate in methanol, gave the glucoside (4).

The synthesis of blatellastanoside A (6), which is an aggregation pheromone of the common cockroach *Blattella germanica*, has been described [16]. In this, starting from β-sitosterol, the chloroepoxide (5) was obtained, and this was subjected to β-glycosylation with tetra-O-acetyl-*D*-glucopyranosyl bromide (2) in the presence of mercury cyanide. Subsequent hydrolysis of the acetoxy group under the action of sodium methanolate led to the desired compound (6), which, in its physicochemical characteristics and pheromonal activity, proved to be identical with the natural material.

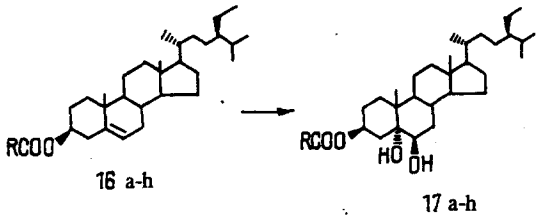


The presence in the β-sitosterol molecule of the 5(6)-double bond also permits the production of a number of natural steroid compounds and their analogues. The triol (7) has been obtained by the hydroxylation of the Δ^5 bond of β-sitosterol with 30% hydrogen peroxide in formic acid, followed by boiling with sodium hydroxide in methanol [17-19]. Oxidation of the alcohol (7) with N-bromosuccinimide in aqueous dioxane led to the hydroxyketone (8) [18, 19]. By the interaction of the latter with chromic acid, 5α-hydroxystigmasta-3,6-dione (9) was synthesized with a yield of 50% [18]. The reaction of β-sitosterol with chromium trioxide in acetic acid, followed by reduction with zinc dust, yielded the 3,6-diketone (10). The main product of hydrogenation over platinum oxide in acetic acid [20] or reduction with sodium tetrahydroborate in methanol [21] of the diketone (10) was the diol (11a), which, by interaction with acetic anhydride, gave the diacetate (11b) [20]. The ketoalcohol (12) was obtained by oxidizing β-sitosterol with chromic acid in acetic acid [22]. These substances (7-12) were synthesized in the investigations mentioned [17-21] in low overall yields for an unambiguous determination of the structures of the corresponding steroids isolated from natural sources.

We have developed preparative methods for synthesizing the 6-ketostigmastanes (8-10, 13) using technical β-sitosterol [23]. The *trans*-glycosylation of β-sitosterol with hydrogen peroxide in formic acid gave the triol (7), which was then subjected to the necessary reactions without purification. Its interaction with aqueous chromic acid in acetone by Jones's method for 10 min led with good yield to the ketone (8), which when the reaction time was increased to 1-2 days, was converted completely into the diketone (9). The dehydration of compound (9) with hydrochloric acid in chloroform formed the enedione (13). The reduction both of the hydroxydiketone (9) and the unsaturated ketone (13) with zinc dust in a mixture of acetic acid and chloroform formed the steroid (10) in high yield.



By the addition of hypobromous acid to the 5(6)-double bond of β-sitosterol and the *cis*-hydroxylation of this bond with osmium tetroxide we obtained the bromohydrin (14) and the 3β,5α,6α-triol (15) [23].



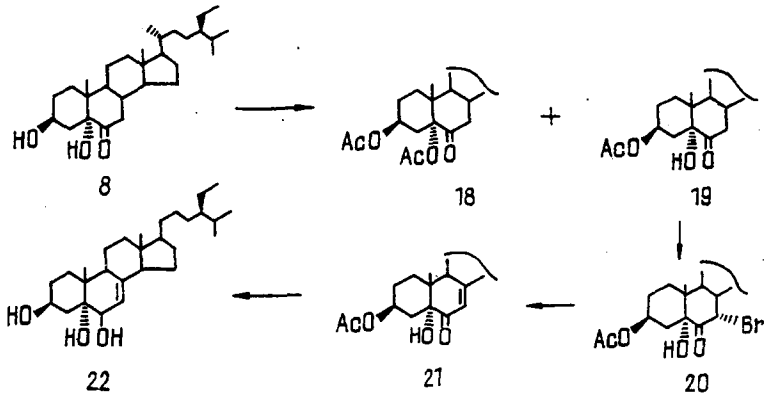
16 a-h 17 a-h

a) R=CH_3
b) R=H
c) R=C_3H_7
d) R=C_5H_{11}
e) R=C_8H_{17}
f) R=C_6H_5
g) R=$CH_3C_6H_4$
h) R=n-$NO_2C_6H_4$

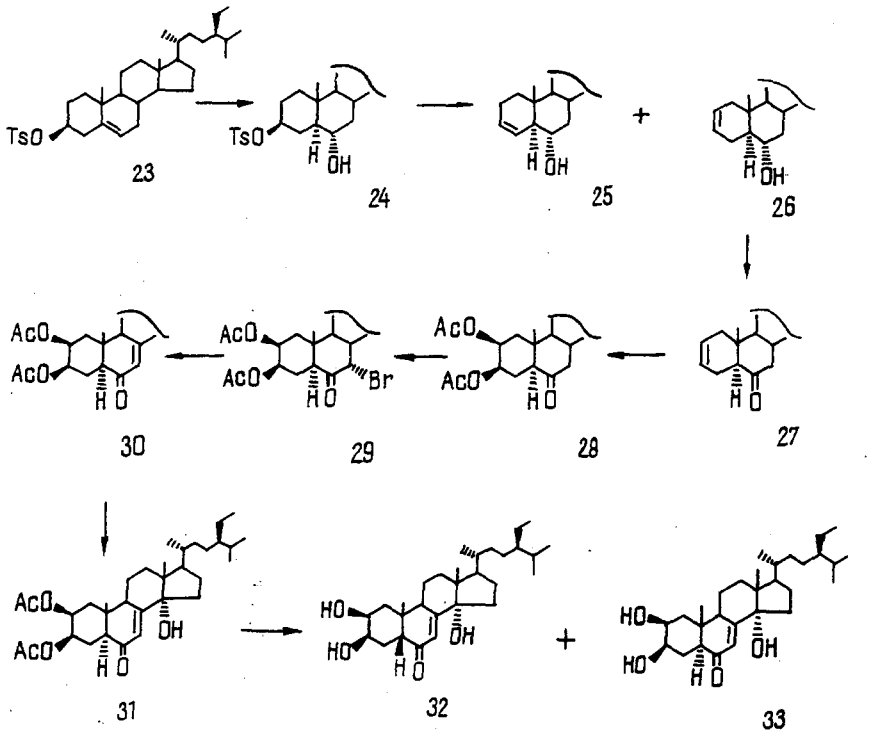
The possibility of using β-sitosterol as the initial compound for the synthesis of various biologically active steroids has also been demonstrated by Mukhina et al. [24, 25]. As a result of the interaction of β-sitosterol with aliphatic and aromatic carboxylic acids, esters of β-sitosterol (16a-h) were synthesized, and their reactions with hydrogen peroxide in dioxane permitted the *trans*-hydroxylation of the 5(6)-double bond with the formation of the *trans*-diols (17a-h) [25]. Steroids (17a-h), in their turn, are readily hydrolyzed to the triol (7).

As stated above, the isolation of pure β-sitosterol is a laborious process requiring additional expenditure, since its accompanying impurities (campesterol, etc.) behave almost identically in relation to organic solvents and chemical reagents. An attempt has been made to use without additional purification a phytosterol isolated from the by-products of the sulfate cellulose industry and containing from 61.0 to 77.1% of β-sitosterol as the initial compound for the synthesis of the β-sitosterol esters (16a-h) and also of the triol (7) [24]. β-Sitosterol acetate (16a), the formate (16b), the benzoate (16e), and the triol (7) have been obtained with yields of 70.0, 60.7, 78.1, and 45.0%, respectively. These substances are of interest as potential biologically active compounds lowering the cholesterol level in the blood.

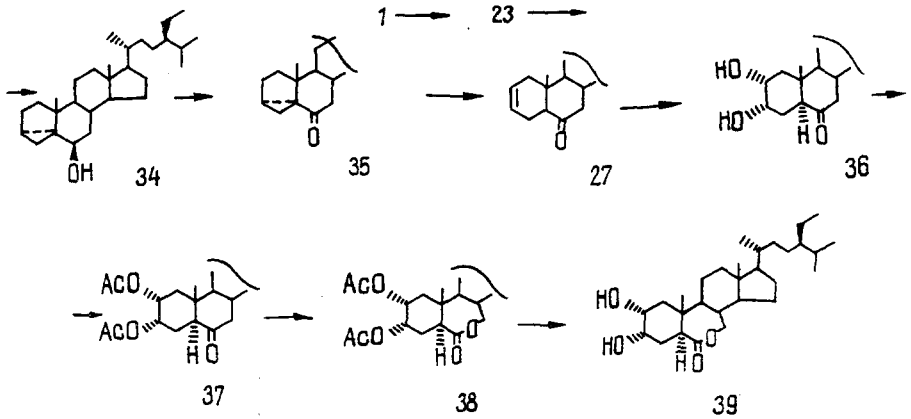
Another paper by Mukhina et al. [26] is devoted to a study of the conditions for the allyl bromination of β-sitosterol formate, acetate, benzoate, and *p*-nitrobenzoate with the aim of using the 7-bromo derivatives so formed in the synthesis of vitamin D_5. The best results were obtained in the bromination of β-sitosterol benzoate with 1,3-dibromo-5,5-dimethylhydantoin in the presence of azoisobutyronitrile.



Recently, reports have appeared on the isolation of sterol derivatives oxidized in rings *A* and *B* from marine organisms. The 3β,5α,6β-trihydroxysteroid (22), which we have synthesized from technical β-sitosterol, has been detected in the marine sponge *Spongionella gracilis* [27, 28]. The dihydroxyketone (8) was obtained by a procedure developed previously, and its acetylation with acetic anhydride in pyridine formed the diacetate (18) and the monoacetate (19) with yields of 35 and 39%, respectively. The 7-bromoketone (20) was synthesized by the bromination of compound (19) in a mixture of acetic anhydride and chloroform. When this was dehydrobrominated under the action of lithium carbonate and bromide in dimethylformamide, the unsaturated ketosteroid (21) was formed. The interaction of compound (21) with lithium tetrahydroaluminate in ether took place with the formation in a yield of 87% of the triol (22), which, by its physicochemical characteristics, was identical with the natural product.



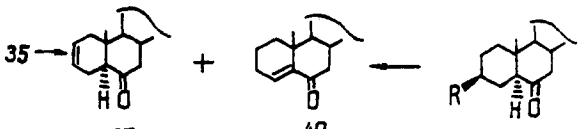
A preparative method for the chemical synthesis of the ecdysteroid analogues (32, 33) from β-sitosterol, obtained from stigmasterol by the method of Steele and Mosettig [4], has been developed [29]. The tosylation of β-sitosterol with *p*-toluenesulfonyl chloride in pyridine led to β-sitosterol tosylate (23), the hydroboration-oxidation of which gave a quantitative yield of the 6α-hydroxytosylate (24). Heating the tosylate (24) with lithium carbonate and bromide led to the formation of the required intermediate Δ^2- alcohol (26) as the main product, together with the Δ^3- alcohol (25). The Jones oxidation of the hydroxy derivative (26), hydroxylation of the Δ^2 bond of the resulting ketone (27) by Woodward's method, followed by acetylation, gave the 2β,3β-diacetate (28). Bromination of the latter formed the 7α-bromoketone (29), the dehydrobromination of which with lithium carbonate in dimethylformamide formed the enone (30). A 14α-hydroxy group was introduced by the interaction compound (30) with selenium dioxide. The saponification and isomerization of the resulting diacetoxyalcohol (31) with potassium carbonate in methanol led mainly to the required ecdysteroid analogue (32) and to the minor compound (33). The overall yield of the final product (32) was approximately 9%.



A new group of phytohormones — brassinosteroids, possessing a powerful growth-stimulating action — has recently been discovered [30, 31]. The chemical synthesis of compounds related to the brassinosteroids has recently been achieved. Thus, Mitra et al. [32] have proposed the following scheme for synthesizing 2α,3α-dihydroxy-5α-stigmast-6-one (36) and 2α,3α-dihydroxy-B-homo-7-oxa-5α-stigmast-6-one (39), which are analogues of (24S)-24-ethylbrassinone and of 28-homobrassinolide, respectively.

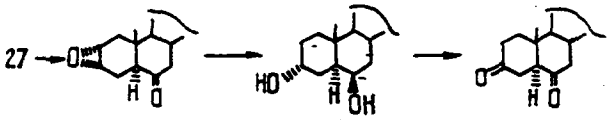
The solvolysis of β-sitosterol tosylate (23), obtained by treating β-sitosterol with *p*-toluenesulfonyl chloride in pyridine, in the presence of potassium acetate in aqueous acetone and oxidation of the resulting *i*-β-sitosterol (34) by the complex of chromium trioxide and pyridine in methylene chloride gave a 74% yield, calculated on the *i*-alcohol (34), of the *i*-ketone (35). A solution of the latter and *p*-toluenesulfonic acid in sulfolane was kept at 170°C for 90 min, which led to the formation of the enone (27), the Criegee hydroxylation of the Δ^2-bond of which, using a catalytic amount of osmium tetroxide in the presence of N-methylmorpholine N-oxide, formed the (24S)-24-ethylbrassinone analogue (36).

The Baeyer-Villiger oxidation with trifluoroperacetic acid in the presence of sodium hydrogen phosphate of the diacetate (37), formed as the result of the interaction of steroid (36) with acetic anhydride in pyridine, led to the diacetoxylactone (38) as the main product, the saponification of which with sodium carbonate in methanol gave the desired 22,23-dideoxy-28-homobrassinolide (39).



27 40 41 a) R=OTs; b) R=Cl

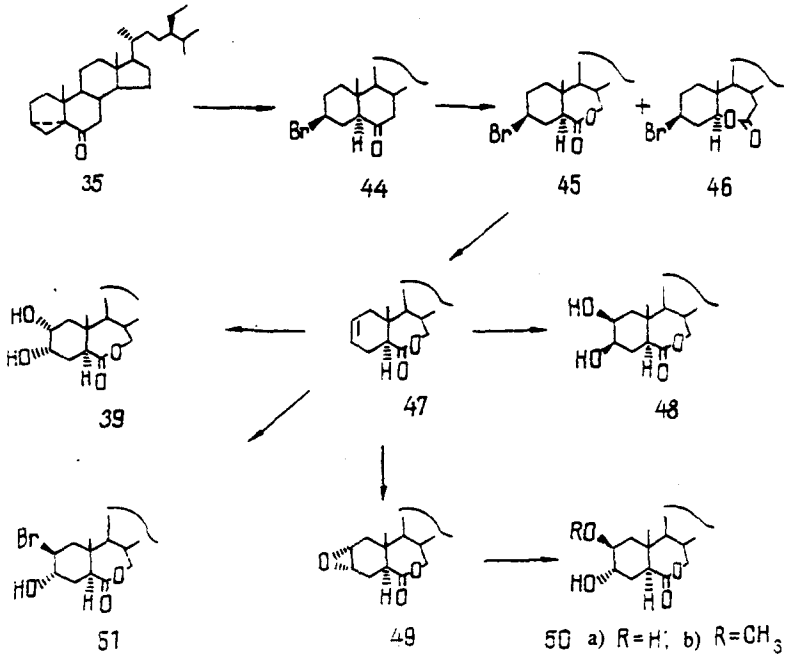
Biologically active ecdy- and brassinosteroids contain a *cis*-2,3-diol function, intermediate compounds in the formation of which are substances containing a Δ^2-bond. Above, we described methods of introducing an isolated 2(3)-double bond [29, 32]. A detailed investigation performed later has shown that on the interaction of the cyclopropane ketone (35) with *p*-toluenesulfonic acid in sulfolane, together with the main Δ^2-derivative (27), the Δ^4-steroid (40) is formed as a minor product [33]. Reactions of the tosylate (41a) and the chloroketone (41b) with sodium bromide in dimethylformamide take place analogously.



42 43 10

During the development of a new method of synthesizing 3α-hydroxy-6-ketobrassinosteroids, we have studied the lithium tetrahydroaluminate reduction of the 2α,3α-epoxy-6-ketone (42), obtained by the epoxidation of the Δ^2-6-ketone (27) [34]. It was established that the main reaction product is the 3α,6β-dihydroxysteroid (43). The structure followed from its spectra and was also confirmed by Jones oxidation to the 3,6-diketone (10).

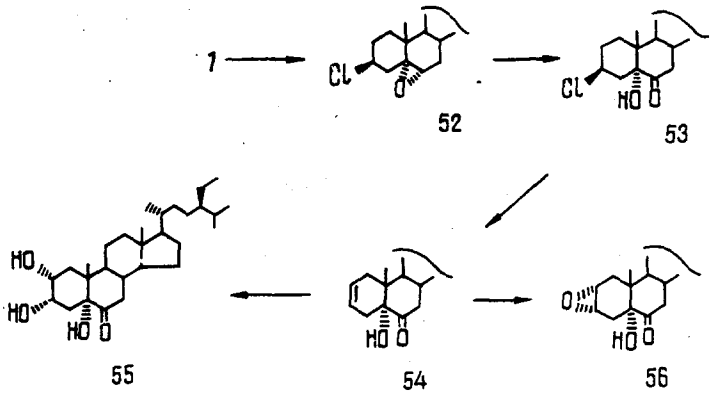
We have developed an effective route to the synthesis of the 22,23-dideoxy-28-homobrassinolide (39) [35]. It involves the reaction of the 3α,5-cyclo-6-ketone (35) with hypobromous acid, giving a high yield of the 3β-bromo-6-ketone (44) The Baeyer-Villiger oxidation of the bromoketone (44) with trifluoroperacetic acid led to the formation of the two isomeric lactones (45) and (46) with yields of 45 and 16%, respectively.



35 44 45 46 39 47 48 51 49 50 a) R=H; b) R=CH$_3$

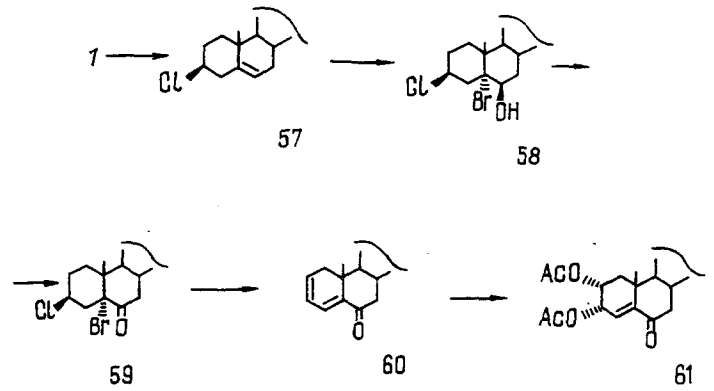
On the dehydrobromination of the main compound (45) with lithium carbonate and bromide in dimethylformamide, the enelactone (47) was formed with a yield of 65%. By means of various electrophilic addition reactions, the double bond of the latter permits the formation of various brassinosteroid analogues. Thus, the interaction of the enelactone (47) with N-methylmorpholine N-oxide and a catalytic amount of osmium tetroxide led with a yield of 78% to the 22,23-dideoxy-28-homobrassinolide (39). Hydroxylation of the Δ^2-bond in steroid (47) by the Woodward reaction with silver acetate and iodine in aqueous acetic acid followed by hydrolysis of the resulting hydroxyacetate with potassium carbonate in methanol gave the 2β,3β-dihydroxylactone (48) with a yield of 80%.

The *trans*-2,3-disubstituted brassinosteroid analogs (50a) and (50b) were obtained by the acid hydrolysis and methanolysis, respectively, of the epoxide ring in steroid (49) which was formed by the epoxidation of the Δ^2-bond of compound (47) with *m*-chloroperbenzoic acid. The addition of hypobromous acid to the Δ^2-bond of lactone (47) gave the bromohydrin (51). It was established by subsequent biotests that the 2α,3α-dihydroxylactone (39) possesses a high phytostimulating activity, which makes it useful in the germination of potatoes [36].



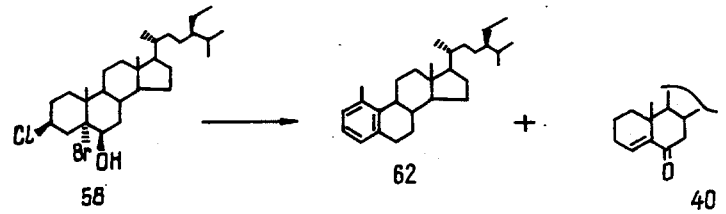
The synthesis of brassinosteroid analogues containing not only a 2α,3α-dihydroxy-6-oxo grouping but also an additional 5α-hydroxy group has been achieved, starting from β-sitosterol and stigmasterol [36]. The interaction of β-sitosterol with thionyl chloride followed by epoxidation with *m*-chloroperbenzoic acid of the 3β-chloro derivative formed gave the 5α,6α-epoxide (52). The Jones oxidation of epoxide (52) yielded the 5α-hydroxy-6-ketone (53), the dehydrohalogenation of which with lithium carbonate and bromide in dimethylformamide led to the Δ^2-5α-hydroxy-6-ketone (54).

The hydroxylation of steroid (54) with a catalytic amount of osmium tetroxide and N-methylmorpholine N-oxide enabled the required 2α,3α,5α-trihydroxy-6-ketone (55) to be obtained. In its turn, the epoxidation of the 2(3)-double bond in compound (54) with *m*-chloroperbenzoic acid led to the 2α,3α-epoxy-5α-hydroxy-6-ketone (56).



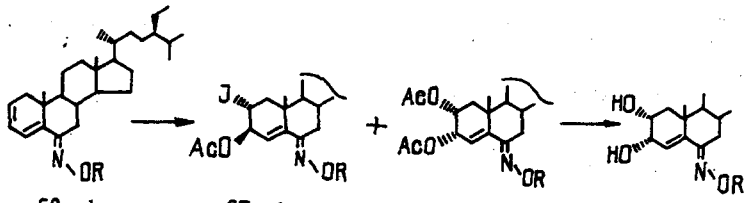
6-Ketosteroids the molecules of which contain a Δ^2-bond conjugated with a Δ^4-bond are of considerable interest for the synthesis of analogues of ecdy- and brassinosteroids. One of the most convenient methods of introducing the functional grouping mentioned consists in the dehydrohalogenation of the 3β-chloro-5α-bromostigmastan-6-one (59), synthesized by the addition of hypobromous acid to the Δ^5-bond of the chlorine derivative of β-sitosterol (57) and further Jones oxidation of the bromohydrin (58) formed, by boiling in the presence of lithium carbonate in dimethylformamide [37]. As a result of these transformations, the dienone (60) was obtained with a yield of 72%, calculated on the bromoketone (59).

It has been established [38, 39] that the hydroxylation of a Δ^2-bond conjugated with a Δ^4-6-keto grouping in the ketone (60) under the conditions of the Woodward reaction, followed by acetylation, takes place with a reversal of the stereodirectivity of this reaction. The formation of the 2α,3α-diacetate (61) was observed, in contrast to the hydroxylation under analogous conditions of an isolated Δ^2-2 bond, as a result of which 2β,3β-diols are formed.



58 62 40

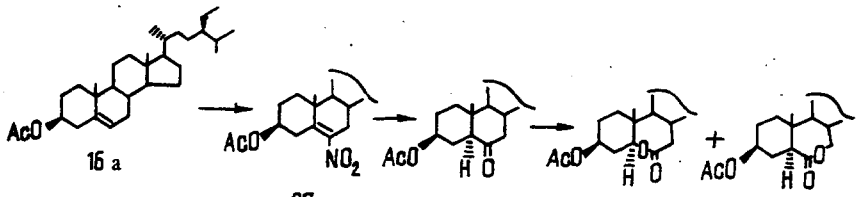
It has been found that the treatment of compound (58) with lithium carbonate and bromide in boiling dimethylformamide leads to the formation of, mainly, the *A*-aromatic steroid (62) and the enone (40), which were isolated with yields of 24 and 22%, respectively [40].



63 a,b 65 a,b 64 a,b 66 a,b

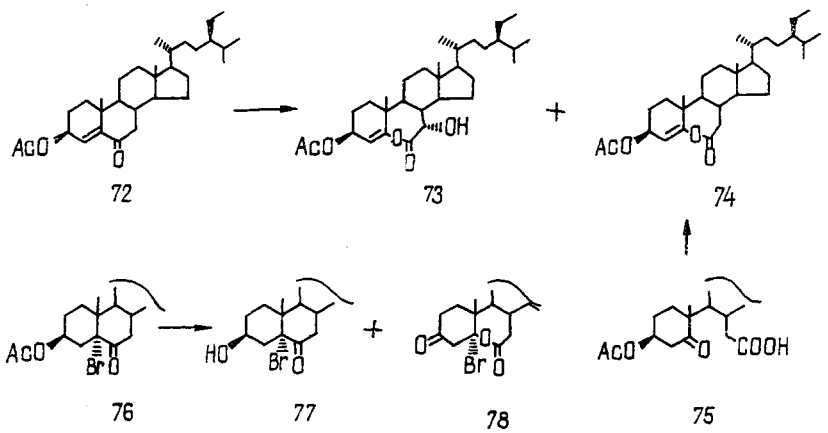
a) $R=CH_3$;
b) $R=C_2H_5$

It has been shown that, as in the case of the 2,4-diene-6-ketone (60) itself, its oximes (63a, b) are converted under the action of silver acetate and iodine in aqueous acetic acid mainly into 2α,3α-diols, these having been isolated in the form of the diacetates (64a, b) with yields of 45-48% [41]. Minor products were the iodoacetates (65a, b). The alkaline hydrolysis of the acetoxy groups in compounds (64a, b) formed the 2α,3α-diols (66a, b) with high yields.



16 a 67 69 70 71

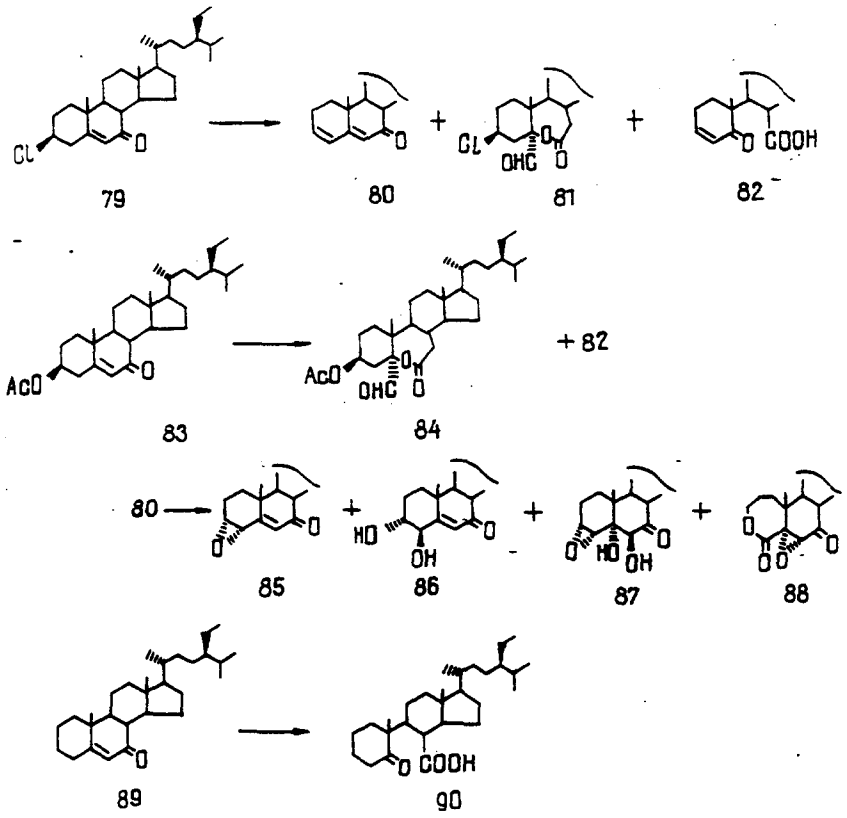
The interaction of various keto derivatives of β-sitosterol with perbenzoic acid in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate has been investigated [42-45]. It has been established [42] that as a result of this reaction, the acetoxyketone (69a) synthesized from β-sitosterol by acetylation, nitration of the acetate (16a), and reduction of the nitro derivative (67) so formed with zinc dust in acetic acid, is converted into a mixture of the two isomeric lactones (70) and (71).



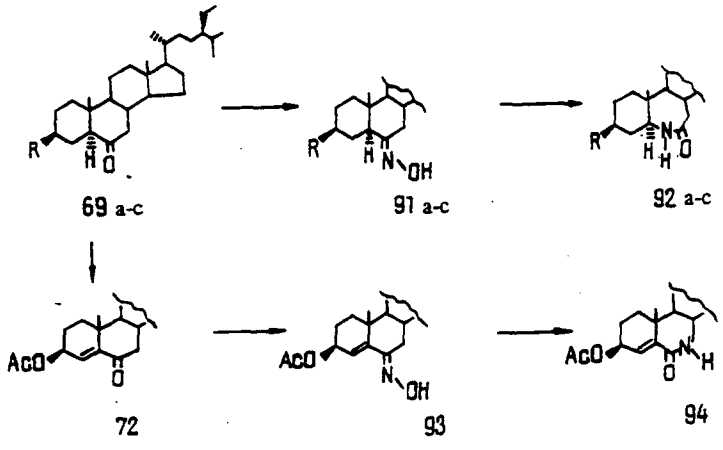
72 73 74

76 77 78 75

This result led to a further investigation of the Baeyer-Villiger oxidation of ketoximes. In this, the 6-oxa- derivatives (73) and (74) were obtained from the enone (72). Compound (74) has also been synthesized by the interaction of the seco-acid (75) with acetic anhydride [43]. In the case of the Baeyer-Villiger oxidation of the bromoacetoxyketone (76), the products were the alcohol (77), formed by the hydrolysis of the acetate (76), and the lactone (78), which is probably a product of the hydrolysis of the acetoxy group at C-3 of the steroid (76) and the subsequent interaction of the alcohol so formed (77) with perbenzoic acid [44].

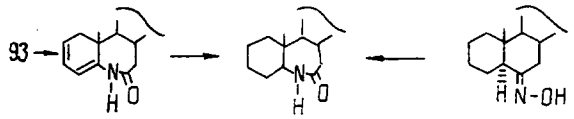


The oxidation of some unsaturated 7-ketones with various substituents at C-3 under analogous conditions has also been investigated [45]. The chloroketone (79) was converted into a mixture of the dienone (80), the lactone (81), and the seco-acid (82). The interaction of the acetoxyketone (83) with a peracid led to the formation of the aldehydolactone (84) and the seco-acid (82). Ahmad et al. [45] consider that the aldehydes (81) and (84) and the acid (82) are probably formed as the result of acid-catalyzed rearrangements. On the Baeyer-Villiger oxidation of the dienone (80), which is one of the products of the analogous reaction of the chloroketone (79) with perbenzoic acid, the α-epoxides (85, 87, and 88) and the ketoenediol (86) were identified. We may note that when the enone (89) was oxidized under analogous conditions a single product was isolated to which the structure represented by formula (90) was assigned.



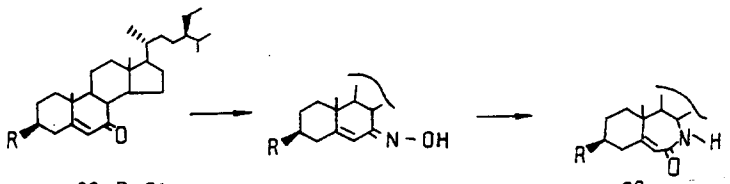
a) R=OAc; b) R=OH; c) R=Cl

The products of the Beckman rearrangement taking place on the interaction of the saturated and unsaturated 6- and 7-ketoximes obtained from β-sitosterol with thionyl chloride or *p*-toluenesulfonyl chloride are also of interest [46, 47]. The oximes (91a-c, 93, 98a-c) were obtained from the corresponding ketones by the usual method. Ketone (72) was synthesized as the result of the α-bromination of the steroid (69a), followed by dehydrobromination, while the 7-ketosteroid (83) is a product of the allyl oxidation with chromic acid of β-sitosterol acetate (16a). It has been established that the saturated 6-oximes (91a-c) are transformed into the corresponding 5a-azasteroids (92a-c) [46]. On the rearrangement of the unsaturated oxime (93), however, Indian workers observed the formation of the 6a-aza derivative (94).



95 96 97

Nevertheless, other authors [47] did not succeed in reproducing these results. Under analogous conditions the oxime (96) yielded the dienic lactam (95), which was reduced by hydrogenation over platinum oxide to the saturated lactam (96). The latter was also formed by the reaction of the oxime (97) with thionyl chloride.



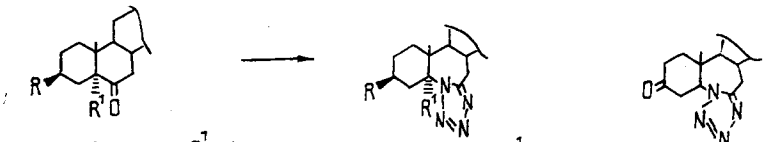
83 R=OAc
84 R=Cl

98 a,b

a) R=OAc; b) R=Cl; c) R=H

99 a-c

It follows from the results of Indian researchers [46] that the unsaturated 7-ketoximes (98a, b) rearrange into the corresponding 7a-aza-B-homosteroids (99a, b). To prove the structures of the chlorolactams, (99b) was reduced to the lactam (99c).

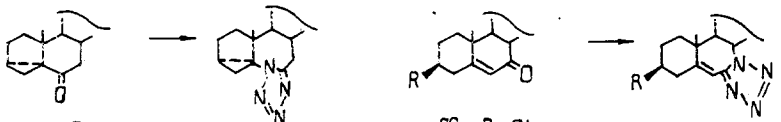


69 a) R=OAc; R^1=H
76 R=OAc; R^1=Br
100 a) R=R^1=H
b) R=OH; R^1=H
c) R=Cl; R^1=H

101 a) R=OAc; R^1=H
b) R=OAc; R^1=Br
c) R=R^1=H
d) R=OH; R^1=H
e) R=Cl; R^1=H

102

The interaction of keto derivatives of β-sitosterol containing various substituents at C-3 and C-5 with an excess of ammonia in benzene in the presence of boron trifluoride etherate has been studied [48]. In the case of the saturated compounds (69a), (76), and (100a-c), and also the cyclic ketone (35), the corresponding tetrazoles (100a-e, 103) were obtained.

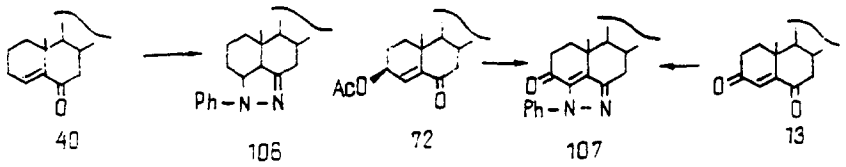


35 103

79 R=Cl
83 R=OAc
104 R=H

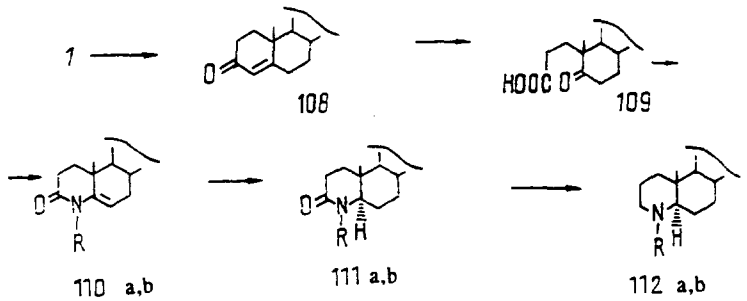
105 a) R=Cl; b) R=OAc;
c) R=H; d) R=OH

Under analogous conditions, the unsaturated keto derivatives (79), (83), and (104) were converted into the isomeric tetrazoles (105a-c), respectively. To confirm the structures of the substances obtained, compound (101e) was reduced to the steroid (101c), the bromoacetate (101b) to the tetrazole (101a), and the chloride (105a) to compound (105c). For the same purpose, the 3β-hydroxysteroid (101d) was oxidized to the ketone (102) and was also acetylated, with the formation of compound (101a). In addition, hydrolysis of the acetate (105b) gave the hydroxytetrazole (105d).



40 106 72 107 13

The interaction with phenylhydrazine in acetic acid of a number of compounds obtained from β-sitosterol and containing a Δ^4-6-keto grouping has been studied [49]. Under the given conditions the enone (40) was converted into the pyrazole (106), while the 3β-acetate (72) and the diketone (13) were transforamed into one and the same steroid (107).



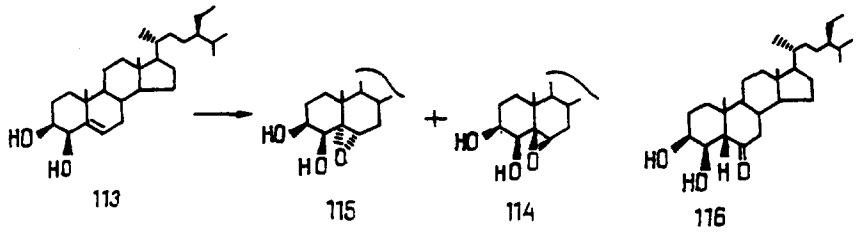
108 109

110 a,b 111 a,b 112 a,b

a) R=H; b) $R=CH_3$

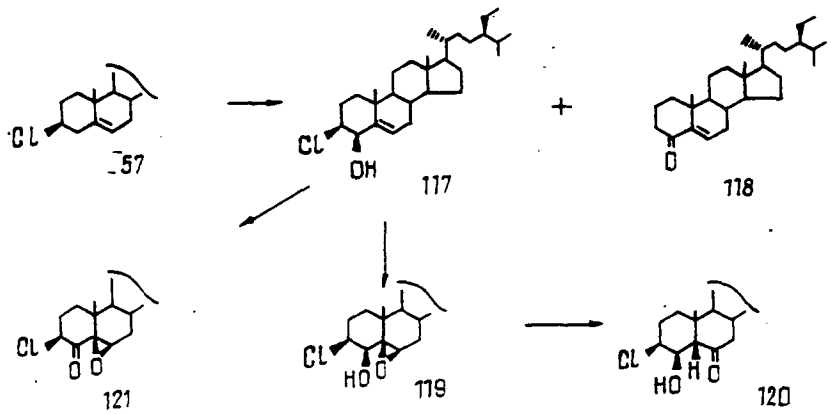
A transformation of β-sitosterol accompanied by the replacement of the C-4 atom by a nitrogen atom has been effected [50]. The Oppenauer oxidation of β-sitosterol led to the synthesis of the enone (108), the ozonolysis of which formed the seco-acid (109) in moderate yield. Interaction of the latter with ammonia and methylamine gave the ketones (110a, b), respectively, and their hydrogenation in glacial acetic acid over a platinum catalyst led to the corresponding steroids (111a, b).

The final products (112a, b) were obtained by reducing the lactams (111a, b) with lithium tetrohydroaluminate. In a test with Gram-negative bacteria, the substances obtained proved to be less effective antimicrobial agents than the corresponding cholesterol derivative ND-502 but they showed activity against other microorganisms [50]. It must be mentioned that stigmast-4-en-3-one (108) is an inhibitor of testosterone 5α-reductase, testosterone 17β-dehydrogenase, and estradiol 17β-dehydrogenase. This steroid is recommended as an agent for stimulating the growth of hair [51].



113 115 114 116

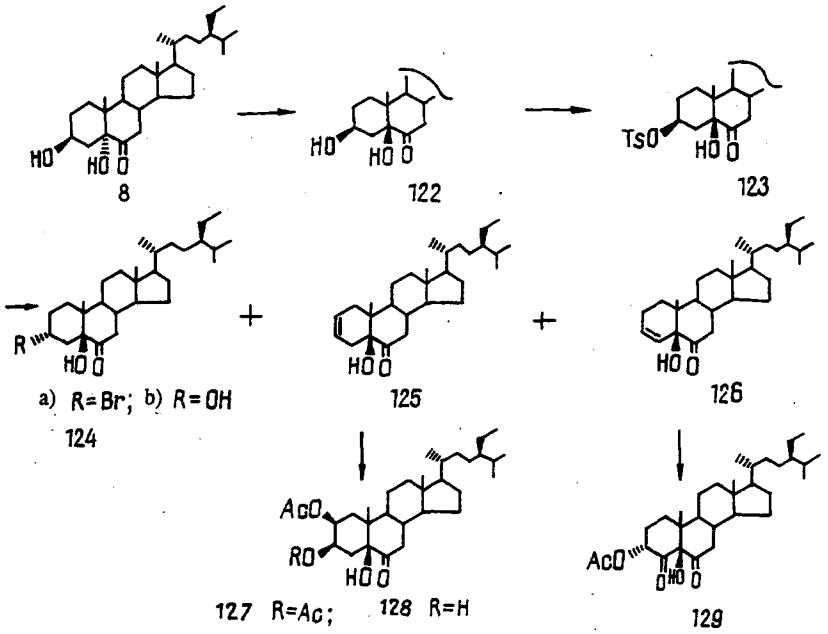
The allyl hydroxylation of β-sitosterol by selenium dioxide gave a moderate yield of the 3β,4β-diol (113) [52]. The interaction of the latter with *m*-chloroperbenzoic acid in chloroform led to the formation of two epoxides (114) and (115) with yields of 44 and 28%, respectively. The rearrangement of the 5β,6β-epoxide (114) under the action of trifluoroacetic acid gave the required steroid (116) with a yield of 74%. Repeated attempts to synthesize this ketone by isomerizing the 5α,6α-oxide (115) under analogous conditions proved unsuccessful.



57 117 118

121 119 120

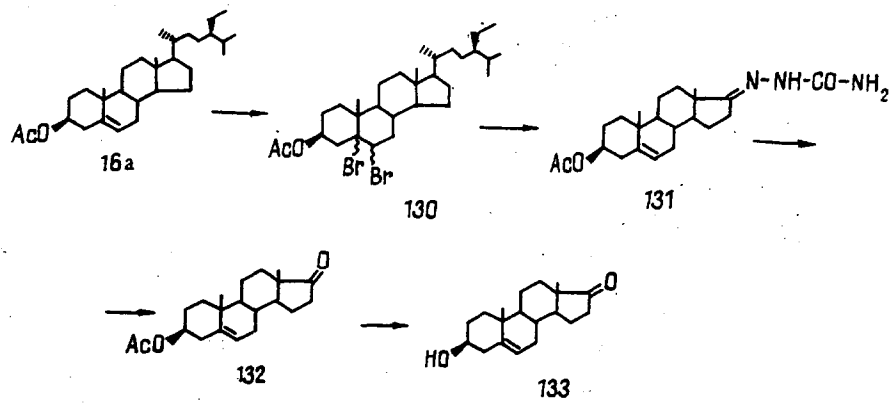
The oxidation of the 3β-chloro derivative (57) with selenium dioxide in dioxane led mainly to the 4β-hydroxysteroid (117) [53, 54]. The unsaturated 4-ketone (118), probably formed as the result of the dehydrochlorination of the steroid (117), was identified as a minor product of this reaction. We have used compound (117) for the synthesis of the 4β-hydroxy-6-ketone (120), which has a high activity as a plant-growth stimulator [55]. The interaction of the Δ^5-steroid (117) with trifluoroperacetic acid formed the 5β,6β-epoxide (119) with a yield of 90%. The acid isomerization of the latter under the action of trifluoroacetic acid gave a 75% yield of the plant-growth stimulator (120). It is interesting to note that the Jones oxidation of the 4β-hydroxysteroid (117) led to the formation of the 5β,6β-epoxy-4-ketone (121).



We have investigated the possibility of introducing into the β-sterol molecule the 2β,3β,5β-trihydroxy-6-keto grouping that is present in some phytoecdysteroids possessing a high insect-hormonal activity [56]. In the first stage, the dihydroxyketone (8) was synthesized from β-sitosterol by a method that we had developed. The reversal of the configuration of the 5α-hydroxy group of the latter under the action of potassium hydroxide in methanol and tosylation of the diol (122) formed led to the 3-tosylate (123).

After the interaction of compound (123) with lithium carbonate and bromide in dimethylformamide, products of nucleophilic substitution with reversal of the configuration (124a, b) were isolated and so also was a mixture of the products of an elimination reaction: the Δ^2-steroid (125) and the Δ^3-steroid (126), with the latter predominating.

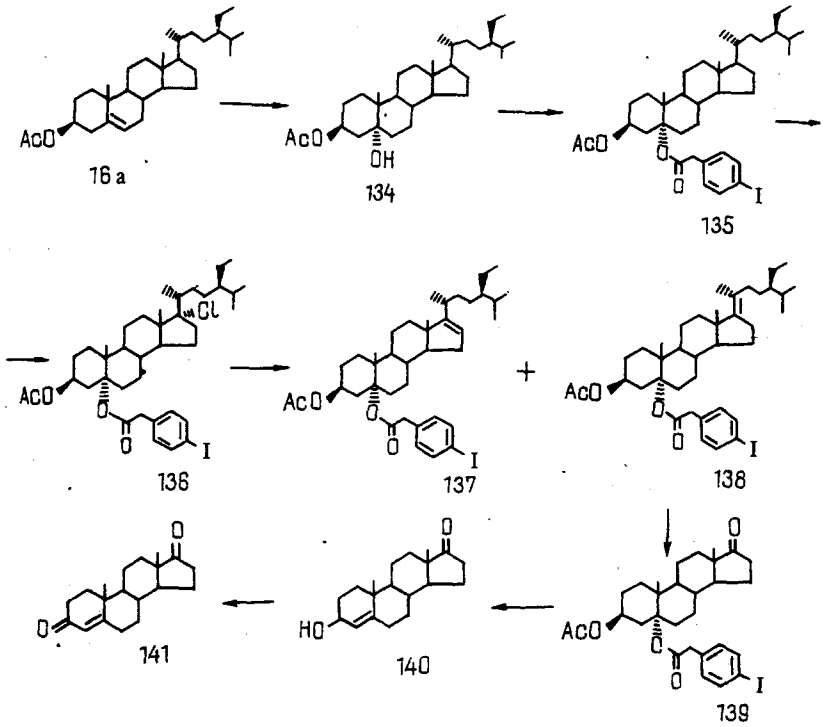
The Criegee *cis*-hydroxylation of the Δ^2-bond in compound (125) under the action of a catalytic amount of osmium tetroxide and N-methylmorpholine N-oxide, followed by acetylation, took place with the production of the 2β,3β,5β-trihydroxy-6-ketone, which was isolated in the form of the 2,3-diacetate (127) and the 2-monoacetate (128). In its turn, under the given conditions the Δ^3-steroid (126) formed the 3α-acetoxy-5β-hydroxy-4,6-diketone (129).



Chemical methods of degrading the side chain are also possible. Investigations have shown that β-sitosterol obtained from sulfate liquor can be used as a raw material for the production of androgenic hormones. The method of obtaining them

from β-sitosterol and phytosterol (containing about 65% of the main substance) consists in the oxidation of β-sitosterol acetate (16a) and also its 5,6-dibromo derivatives (130), with chromium trioxide [57]. The desired product is isolated in the form of the semicarbazone of androstenolone 3-acetate (131). We may note that the yield of the latter is substantially affected by the amounts of chromium trioxide and catalyst, and also by the temperature.

The highest yield of the semicarbazone (131) (12%) was observed when the mixture of dibromides (130) was oxidized in the presence of manganese sulfate or vanadium pentoxide [58]. By interaction with formalin in acetic acid, it was then possible to convert the semicarbazone (131) with a yield of 78% into androstenolone 3-acetate (132), which, on heating in an alcoholic solution of sodium hydroxide, gave androstenolone (133) [59].

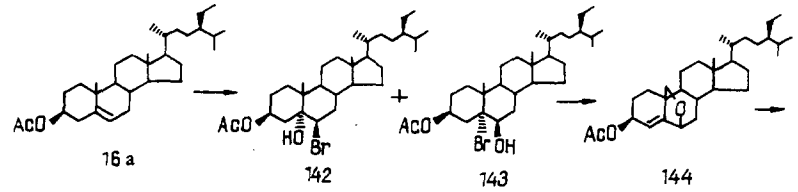


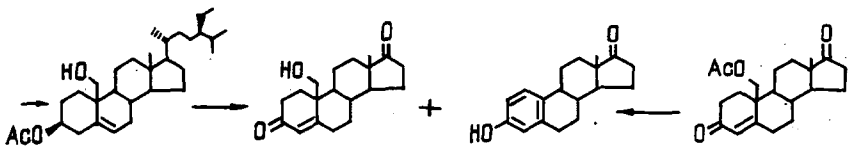
An effective chemical method of degrading the side-chain of β-sitosterol, the key stage of which is remote halogenation has been proposed by Welzel et al. [60, 61]. As the initial material, a commercial sample consisting of a mixture of β-sitosterol and campesterol (6:4) was used. The β-sitosterol was acetylated, the acetate formed (16a) was irradiated in methylene chloride in an atmosphere of oxygen in the presence of pyridine, and the irradiation products were then converted by hydrogenation over platinum in ethyl acetate into the 5α-hydroxy derivative (134). The latter was boiled with *p*-iodophenylacetyl chloride in toluene in the presence of calcium hydride and a catalytic amount of tetrabutylammonium iodide, to give a good yield of the 5-*p*-iodophenylacetate (135)

Compound 135 is a very important intermediate in this method. In view of the fact that the *p*-iodophenyl acetate group is attached not to C-3 but to C-5, a direction of attack at the C-17 hydrogen becomes possible. Furthermore, the functional group at C-5 acts as a protective group for the Δ^5-bond. The chloro derivative (136) was obtained by the UV irradiation of steroid (135) in carbon tetrachloride in the presence of thionyl chloride and azoisobutyronitrile (yield 59%) or by irradiation in chloroform in the presence of iodobenzene dichloride (yield 58%).

The subsequent heating of compound (136) with pyridine led to the formation of the Δ^{16}-dehydro derivative (137) with a yield of 87%, while heating with diazabicyloundecene gave a mixture of $\Delta^{17(20)}$-dehydro derivatives (137 and 138), the ozonolysis of which led to the 5-*p*-iodophenylacetate (139) with quantitative yield.

Boiling the latter with lithium hydroxide in aqueous tetrahydrofuran formed the allyl alcohol (140). The oxidation of compound (140) with pyridinium chlorochromate in methylene chloride in the presence of sodium acetate followed by a further treatment with lithium hydroxide led quantitatively to androst-4-ene-3,17-dione (141).





145 146 147 148

A method of obtaining 17-ketosteroids from β-sitosterol in which chemical and microbiological transformations are used deserves attention [62]. Thus, the addition of hypobromous acid to the double bond of β-sitosterol acetate (16a) gave as the main product the bromohydrin (143), and also its isomer (142). Oxidation of steroid (143) with lead tetraacetate in the presence of iodine led to the formation of the 6β,19-epoxide (144) with a yield of 90%.

The reductive cleavage of the oxide ring of compound (114) in boiling isopropanol under the action of zinc dust in the presence of acetic acid gave a 79% yield of the 19-hydroxy derivative (145). We may note that the modification of β-sitosterol to form the steroid (145) was carried out in order to inhibit the cleavage by microorganisms of the steroid skeleton competing with the oxidation of the side-chain. Then, as the result of the transformation of compound (145) by bacteria, the 17-ketosteroids (146) and (147) were obtained.

It has been established that, depending on the conditions of performing this reaction, it is possible to obtain predominantly 19-hydroxyandrostenedione (146) or the product of its further transformation, estrone (147). It has also been shown that under analogous conditions the acetate (148) is transformed into compound (147).

Thus, β-sitosterol is a promising raw material for the chemical synthesis of various biologically active steroids.

REFERENCES

1. V. V. Sokirka, V. V. Panina, B. V. Shemeryankin, V. B. Nekrasova, and V. G. Smirnova, Khim.-Farm Zh., **21**, 1192 (1987).
2. D. Onken and D. Onken, Pharmazie, **35**, 193 (1980).
3. F. J. Zeelen, Medicinal Chemistry of Steroids, Elsevier, Amsterdam (1990).
4. J. A. Steele and E. Mosettig, J. Org. Chem., **28**, 571 (1963).
5. P. P. H. L. Otto, A. C. Besemer, and W. H. J. M. Wientyens, J. Labelled Compounds, **6**, 111 (1970).
6. M. A. Palmer, L. J. Goad, and T. W. Goodwin, Nouv. J. Chim., **2**, 401 (1978).
7. B. Dayal, G. Salen, G. S. Tint, and C. Biswas, Steroids, **42**, 635 (1983).
8. B. Z. Askinazi, L. H. Kibokurtseva, N. S. Bobrova, and N. Ya. Kozarinskaya, Khim.-Farm. Zh., **19**, 1221 (1985).
9. S. J. Stohs and M. M. El-Olemy, Phytochemistry, **10**, 2987 (1971).
10. R. Ikan, A. Markus, and E. D. Bergmann, J. Org. Chem., **36**, 3944 (1971).
11. N. S. Habib and M. A. Khalil, Arch. Pharm. (Weinheim), **323**, 401 (1990).
12 N. I. Uvarova, G. I. Oshitok, V. V. Isakov, A. K. Dzizenko, and G. V. Elyakov, Khim. Prir. Soedin., 842 (1971).
13. V. F. Anufriev and N. I. Uvarova, Khim. Prir. Soedin., 119 (1984).
14. F. A. del Villar, P. M. Paya, and M. R. Zaragoza, Ars. Pharm., **27**, 103 (1986).
15. S. Ghosal and K. S. Saini, J. Chem. Res. Synop., No. 4, 110 (1984).
16. K. Mori and K. Fukamatsu, Proc. Jpn. Acad., Ser. B, **69**, 61 (1993).
17. S. S. Deshmane and S. Dev, Tetrahedron, **27**, 1109 (1971).
18. T. Murakami, H. Wada, N. Tanaka, T. Yamagishi, Y. Saiki, and G.-M. Chen., Chem. Pharm. Bull., **28**, 3137 (1980).
19. J. P. Campello, S. F. Fonseca, C.-J. Chang, and E. Wenkert, Phytochemistry, **14**, 243 (1975).
20. R. E. Marker, H. M. Crooks, Jr., E. M. Jones, and E. L. Wittbecker, J. Am. Chem. Soc., **64**, 219 (1942).
21. B. Talapatra, A. K. Mallick, and S. K. Talapatra, J. Indian Chem. Soc., **58**, 815 (1981).
22. W. H. Hui, M. M. Li, and K. K. Ng, Phytochemistry, **14**, 816 (1975).
23. N. V. Kovganko and Z. N. Kashkan, Khim. Prir. Soedin., 771 (1990).
24. M. V. Mukhina, S. S. Geras'kina, T. F. Ionova, V. E. Kovalev, and V. B. Nekrasova, Izv. Vyssh. Uchebn. Zaved., Lesn. Zh., No. 1, 91 (1981).
25. V. G. Atabekyan, M. V. Mukhina, and A. S. Sopova, Zh. Organ. Khim., **12**, 1231 (1976).

26. M. V. Mukhina, S. S. Geras'kina, and A. S. Sopova, Khim.-Farm. Zh., **11**, 74 (1977).
27. A. A. Akhrem, Zh. N. Kashkan, and N. V. Kovganko, Dokl. Akad. Nauk SSSR, **305**, 618 (1989).
28. A. Akhrem, Zh. Kaskan, and N. Kovganko, in: IXth Soviet-Indian Symposium on the Chemistry of Natural Products; Abstracts of Papers, Riga (1989), p. 110.
29. M. J. Thompson, W. E. Robbins, J. N. Kaplanis, C. F. Cohen, and S. M. Lancaster, Steroids, **16**, 85 (1970).
30. N. V. Kovganko, Khim. Prir. Soedin, 159 (1991).
31. N. V. Kovranko, Zh. Vses. Khim. Obshch. im. D. I. Mendeleeva, **35**, 440 (1991).
32. R. B. Mitra, B. G. Hazra, and V. M. Karpoor, Indian J. Chem., **23B**, 106 (1984).
33. R. B. Mitra and V. M. Kapoor, Synth. Commun., **15**, 1087 (1985).
34. N. V. Kovganko, O. P. Kananovich, and S. K. Ananich, Khim. Prir. Soedin., 667 (1992).
35. N. V. Kovganko and Zh. N. Kaskkan, Zh. Organ. Khim., **26**, 2545 (1990).
36. N. V. Kovganko and S. K. Ananich, Zh. Organ Khim., **27**, 103 (1991).
37. A. A. Akhram, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, Vesti Akad. Navuk BSSR, Ser. Khim. Navuk., No. 2, 65 (1983).
38. V. A. Khripach, V. N. Zhabinskii, N. V. Kovanko, and A. A. Akhrem, in: Abstracts of Lectures at an All-Union Conference on the Chemistry of Unsaturated Compounds, Devoted to the Memory of A. M. Butlerov (1828-1886) [in Russian], Kazan, Vol. 3 (1986), p. 70.
39. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, N. V. Kovganko, and V. N. Zhabinskii, Zh. Org. Khim., **25**, 1661 (1989).
40. A. A. Akhrem, V. A. Khripach, Zh. N. Kashkan, and N. V. Kovganko, Zh. Org. Khim., **25**, 441 (1989).
41. A. A. Akhrem, V. A. Khripach, Zh. N. Kashkan, and N. V. Kovganko, Dokl. Akad. Nauk SSSR, **301**, 878 (1988).
42. M. S. Ahmad and G. Moinuddin, J. Org. Chem., **43**, 163 (1978).
43. M. S. Ahmad and M. S. Moinuddin, Indian J. Chem., **20B**, 811 (1981).
44. M. S. Ahmad, G. Moinuddin, I. A. Ansari, and S. A. Ansari, Indian J. Chem., **23B**, 220 (1984).
45. M. S. Ahmad, I. A. Ansari, K. Saleem, and G. Moinuddin, Indian J. Chem., **23B**, 1110 (1984).
46. M. S. Ahmad, I. A. Ansari, and G. Moinuddin, Indian J. Chem., **20B**, No. 7 (1981).
47. M. Anastasia, P. Allevi, P. Ciuffreda, A. Fiecchi, and A. J. Scala, J. Chem. Soc., Perkin Trans. I, 2133 (1986).
48. M. S. Ahmad, I. A. Ansari, C. A. Ansari, and G. Moinuddin, Indian J. Chem., **24B**, 664 (1985).
49. M. S. Ahmad, Z. Alam, and S. M. Ali, Indian J. Chem., **27B**, 959 (1988).
50. H. Y. Aboul-Enein and N. J. Doorenbos, J. Heterocycl. Chem., **11**, 557 (1974).
51. Japanese Patent No. 61.148.196, Chem. Abstr., **106**, 23080x (1987).
52. N. V. Kovganko and Zh. N. Kashkan, Zh. Org. Khim., **27**, 1896 (1991).
53. V. A. Khripach and N. V. Kovganko, USSR Inventors' Certificate 1356428.
54. A. A. Akhrem, Zh. N. Kashkan, and N. V. Kovganko, USSR Inventors' Certificate 1536783.
55. V. A. Khripach, N. V. Kovganko, A. I. Bykhovets, M. P. Borisova, and V. A. Strel'tsova. USSR Inventors' Certificate 1371003.
56. N. V. Kovganko and Zh. N. Kashkan, Khim. Prir. Soedin., 718 (1993).
57. M. V. Mukhina, E. V. Ksenofontova, and A. M. Khaletskii, Zh. Obshch. Khim., **37**, 1767 (1967).
58. M. V. Vasil'eva and A. M. Khaletskii, Zh. Obshch. Khim., **33**, 3831 (1963).
59. A. M. Khaletskii, E. V. Ksenofontova, M. V. Mukhina, P. I. Bel'kevich, F. L. Kaganovich, and E. A. Yurkevich, Vesti Akad. Navuk BSSR, Ser. Khim. Navuk., No. 2, 72 (1968).
60. P. Welzel, K. Hobert, A. Ponty, and T. Milkova, Tetrahedron Lett., **24**, 3199 (1983).
61. P. Welzel, K. Hobert, A. Ponty, D. Neunert, and H. Klein, Tetrahedron, **41**, 4509 (1985).
62. T. S. Kolyvanova, Khim.-Farm. Zh., **16**, 78, 334 (1982).