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CHEMICAL TRANSFORMATION OF ECDYSTEROIDS: LATEST ADVANCES

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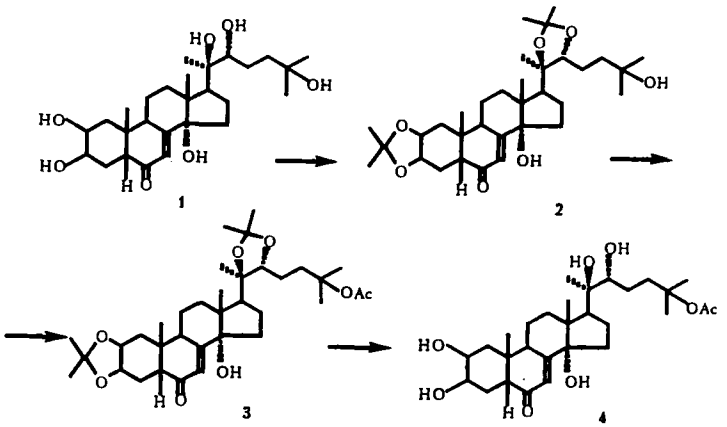
In this review the results of chemical transformations of ecdysteroids reported in the literature during 1986-1996 are discussed.

The large group of natural compounds called ecdysteroids after their first representative, α-ecdysone, is the object of intensive investigations [1-3]. The considerable scientific and practical interest in the ecdysteroids is due to the great diversity of the structures of concrete representatives of them, their wide distribution in the animal and plant world, and their extremely important biological functions for a number of invertebrates. In arthropods, the ecdysteroids are hormones of molting and metamorphosis. Ecdysteroids are frequently present in plants in extremely considerable amounts and are now commercially available even in kilogram amounts [4]. The latter circumstance presents considerable possibilities for the use of the most common natural phytoecdysteroids and, in the first place, 20-hydroxyecdysone (**1**) as the starting material in the chemical synthesis of other, less common, ecdysteroids and their derivatives, For a number of reasons, in some cases such chemical transformation of ecdysteroids proves to be the most preferred method and has substantial advantages over synthesis from traditional steroid raw material. In the present paper we consider the results in this direction that have been obtained in recent years (i.e., after the appearance of the monograph [1]).



In terms of their chemical structure, the ecdysteroids belong to the oxidized sterol derivatives and have a number of characteristic structural fragments in their molecule: a 2β,3β-diol grouping, a 14α-hydroxy-Δ^7-6-keto grouping, a *cis-A/B* linkage, and a sterol side-chain with several hydroxy groups [1]. It is just the presence of these structural units that determines the direction of the chemical transformations of ecdysteroid molecules. Here, naturally, the most important are the hydroxy groups, which, as became known even in the first stages of investigations of the ecdysteroids, differ greatly in reactivity. The different positions and steroid environments of the hydroxy groups enable the required derivatives to be obtained in a comparatively simple manner with a careful choice of reagents and conditions.

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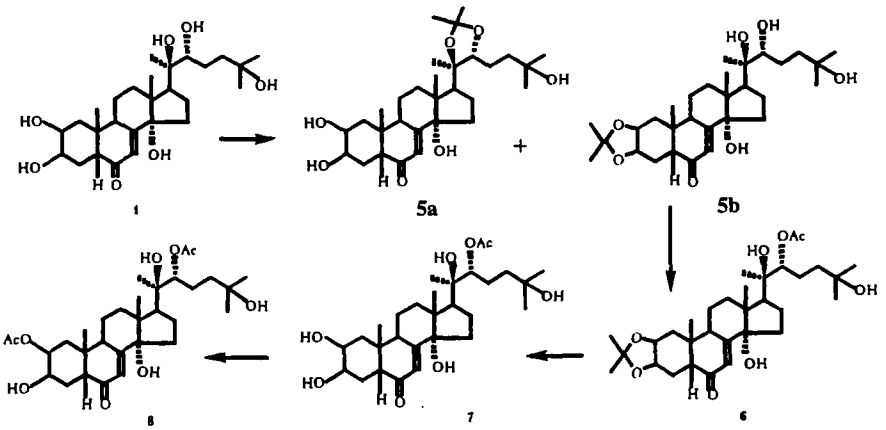
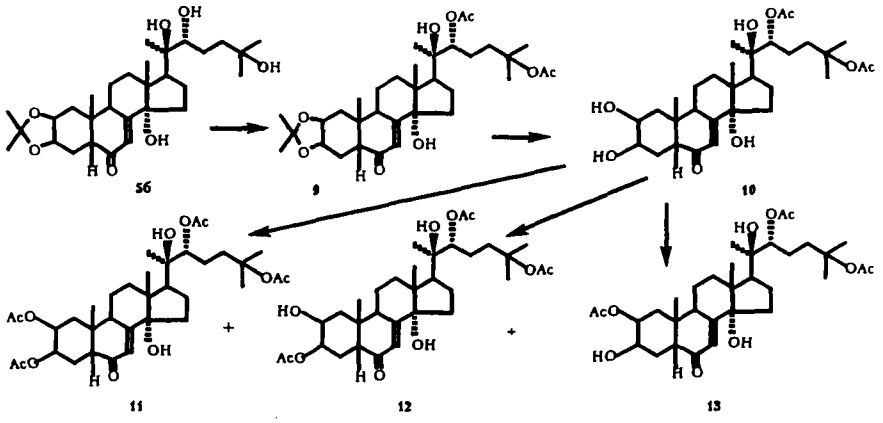
Thus, in a synthesis of the phytoecdysteroid viticosterone E (**4**) improved in comparison with that described previously [5], use was made of just these differences in the reactivities of the hydroxy groups in the molecule of 20-hydroxyecdysone (**1**) [6]. The interaction of (**1**) with acetone in the presence of molybdophosphoric acid gave a 65% yield of the 2,3:20,22-diacetonide (**2**). Prolonged acetylation of the tertiary 25-hydroxy group in compound (**2**) with acetic anhydride in pyridine for three weeks led in quantitative yield to the 2,3:20,22-diacetonide of viticosterone E (**3**). Hydrolysis of the acetonide groupings in the latter with 70% acetic acid in the presence of zinc chloride gave the required ecdysteroid (**4**) with a yield of 33%.

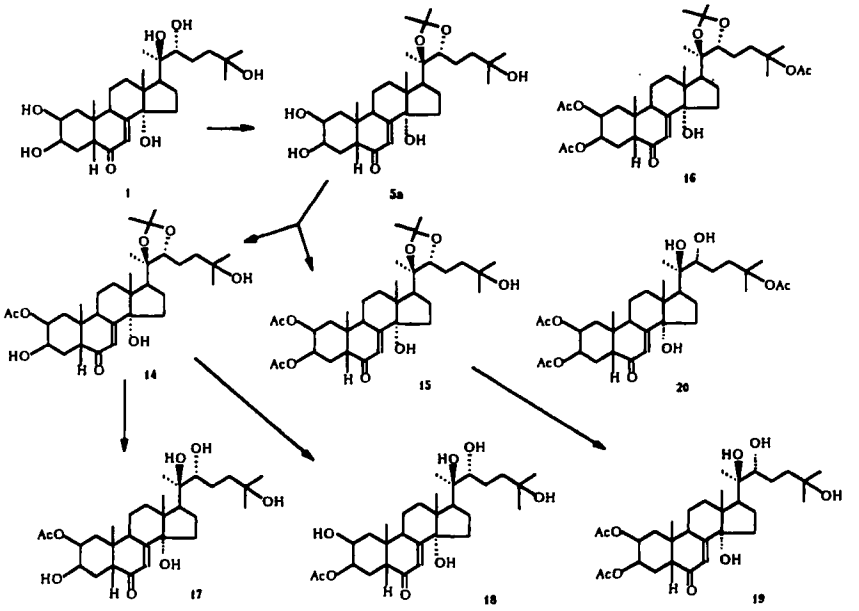


In a three-hour reaction of 20-hydroxyecdysone with acetone in the presence of pyridinium *p*-toluenesulfonate the 20,22-acetonide (**5a**) and the 2,3-acetonide (**5b**) were formed with yields of 38 and 23% [6]. The latter substance was used in the synthesis of 20-hydroxyecdysone acetates. The acetylation of compound (**5b**) with acetic anhydride in pyridine for 6 h led to the formation, in 91% yield, of the 22-acetoxy derivative (**6**) the hydrolysis of which with 70% acetic acid gave a 93% yield of 20-hydroxyecdysone 22-acetate (**7**). Subsequent acetylation of compound (**7**) with acetic anhydride in a mixture of pyridine and chloroform for 4 h permitted the synthesis of 20-hydroxyecdysone 2,22-diacetate (**8**) with a yield of 80% [6].

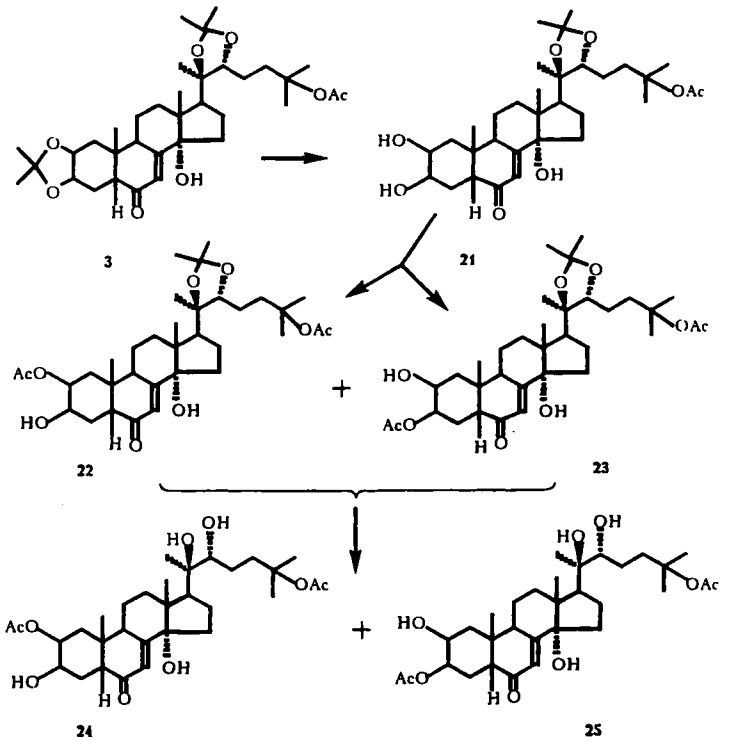


In its turn, the prolonged acetylation of steroid (**5b**) with acetic anhydride in pyridine for three weeks led with a yield of 86% to the 22,25-diacetoxy derivative (**9**) [6]. 20-Hydroxyecdysone 22,25-diacetate (**10**) was obtained with a yield of 90% by the hydrolysis of the acetonide grouping of compound (**9**) in 70% acetic acid. Acetylation of compound (**10**) with acetic anhydride in a mixture of pyridine and chloroform for 4 h took place with the formation of the 2,3,22,25-tetraacetate (**11**), the 3,22,25-triacetate (**12**), and the 2,22,25-triacetate (**13**), which were isolated after chromatographic purification with yields of 7, 10, and 48%, respectively.

It has been established [6] that a convenient method of obtaining the 20,22-acetonide (**5a**) is the reaction of 20-hydroxyecdysone with acetone in methanolic solution in the presence of *p*-toluenesulfonic acid for 1 h. The acetonide (**5a**) obtained in this way with a yield of 93% was converted on acetylation with acetic anhydride in a mixture of pyridine and chloroform into the 2-acetate (**14**) and the 2,3-diacetate (**15**), which were isolated with yields of 86 and 7%, respectively. On the other hand, the acetylation of steroid (**5a**) with acetic anhydride in pyridine for 6 h led to the 2,3-diacetate (**15**) with a yield of 83%.

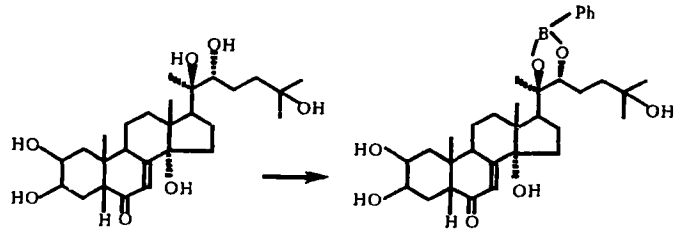


The even more prolonged acetylation of compound (**5a**) with acetic anhydride in pyridine (20 h at 30-32°C and then 2 days at 48-52°C) allowed the 2,3,25-triacetate (**16**) to be obtained with a yield of 60%. A mixture (3:1) of the 2-acetate (**17**) and the 3-acetate (**18**) was obtained from compound (**14**) with a total yield of 81% by hydrolyzing the acetonide grouping with 70% acetic acid in the presence of zinc chloride. The analogous hydrolysis of acetonide (**15**) led to the formation of the 2,3-diacetate (**19**) with a yield of 75%. In its turn, the 2,3,25-triacetate (**20**) was synthesized with a yield of 64% by hydrolyzing the acetonide grouping in compound (**16**) under the action of 70% acetic acid at 38-42°C for two days.



The mild hydrolysis of the 2,3:20,22-diacetonide of viticosterone E (**3**) under the action of 70% acetic acid for 2 h led to the formation of the monoacetonide (**21**) with a yield of 93% [6]. The acetylation of this compound in a mixture of pyridine and chloroform for 4 h gave the triacetate (**16**) (yield 3%) and a mixture (4:1) of the diacetates (**22**) and (**23**) (yield 83%). After hydrolysis of the acetonide groupings with 70% acetic acid in the presence of $ZnCl_2$, the mixture of compounds (**22**) and (**23**) was converted with an overall yield of 82% into a mixture (7:3) of the 2,25-diacetate (**24**) and the 3,25-diacetate (**25**).

It must be mentioned that in order to study inclusion in liposomes, Politova et al. [7] also undertook the synthesis from 20-hydroxyecdysone of the 2-acetate (**17**), the 2,3,22-triacetate (**33**), the 2,3,22,25-tetraacetate (**11**), and the 25-monoacetate (**4**).



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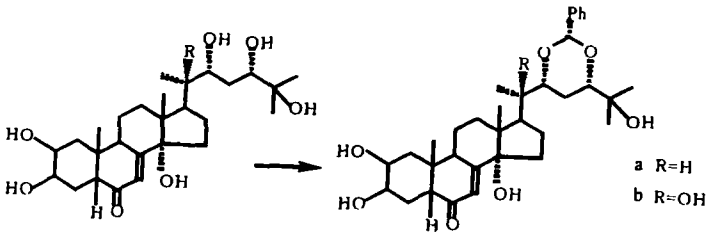
It had been shown previously [8] for the exemplary case of the ecdysteroid cyasterone that phenylboronic acid is an extremely suitable reagent for protecting the 20,22-diol grouping in the ecdysteroid side-chain. The reaction forms the 20,22-phenylboronate exclusively, and this can be reconverted into the initial 20,22-diol under the action of hydrogen peroxide. In these transformations, the 2,3-diol grouping remains free.

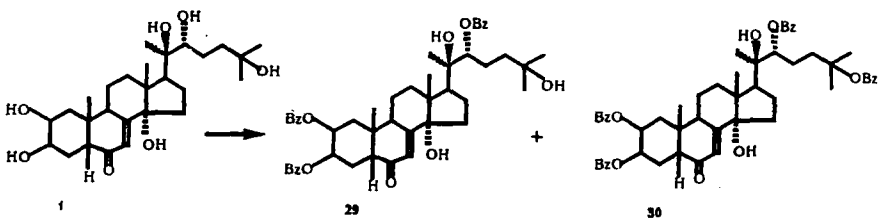
Recently, the reaction of ecdysteroids with phenylboronic acid has been investigated in more detail [9-13]. In particular, it has been established that the best method, ensuring the formation of 20-hydroxyecdysone 20,22-phenylboronate (**26**) with a yield of 93%, is the reaction of 20-hydroxyecdysone (**1**) with phenylboronic acid in methanol at room temperature for 10 min [10, 11]. The same method has been used to obtain the 20,22-phenylboronates of ponasterone A, of polypodin B, of 20-hydroxyecdysone 2,3-acetonide, and of makisterone A [10].

The same paper [10] reports a study of the use of various reagents (mainly diols and carboxylic acids) for eliminating the 20,22-phenylboronate grouping and regenerating the initial ecdysteroids. However, no significant results were obtained by the use of any of these reagents. At the present time, therefore, the best method of hydrolyzing ecdysteroid 20,22-phenylboronates is their reaction with hydrogen peroxide, as proposed in [8]. We may also note that Shim et al. [12] have studied the reaction of ecdysteroids not only with phenylboronic acid but also with methylboronic acid in methanol. It was found that although in all cases the corresponding 20,22-boronates are formed, the use of phenylboronic acid is preferable.



27a - b 28a - b

Coll et al. [14] have considered the interaction of the phytoecdysteroids 24-hydroxyecdysone (**27a**) and abutasterone (**27b**) with benzaldehyde in the presence of zinc chloride. This leads to the formation of the 22,24-(benzylidene acetal)s (**28a** and **b**), which serves as a reliable proof of the (24-S)-configuration of the initial compounds.



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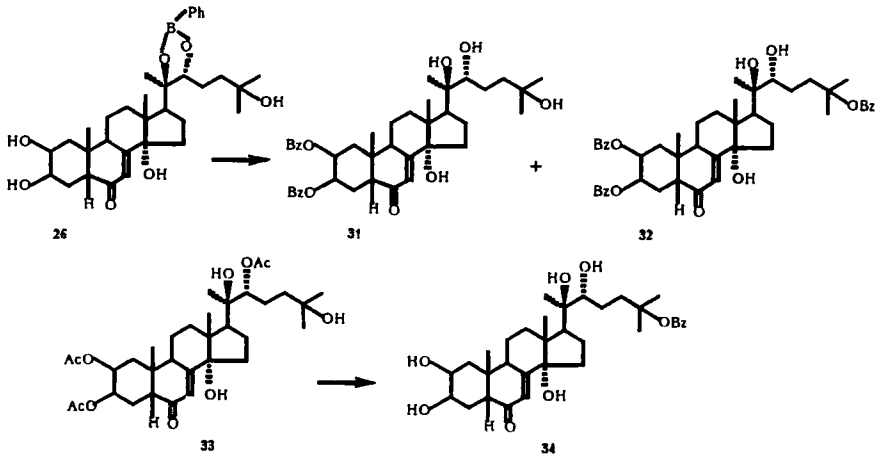
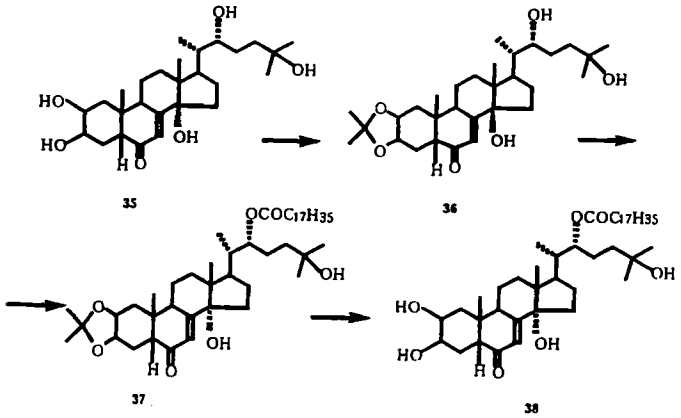
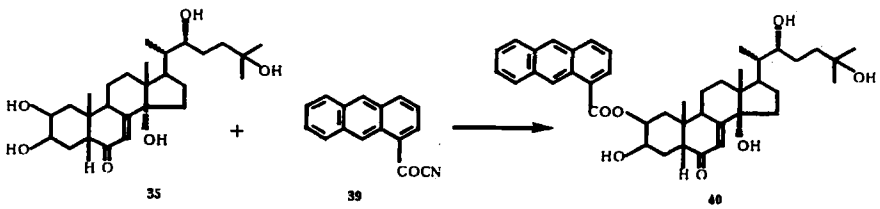


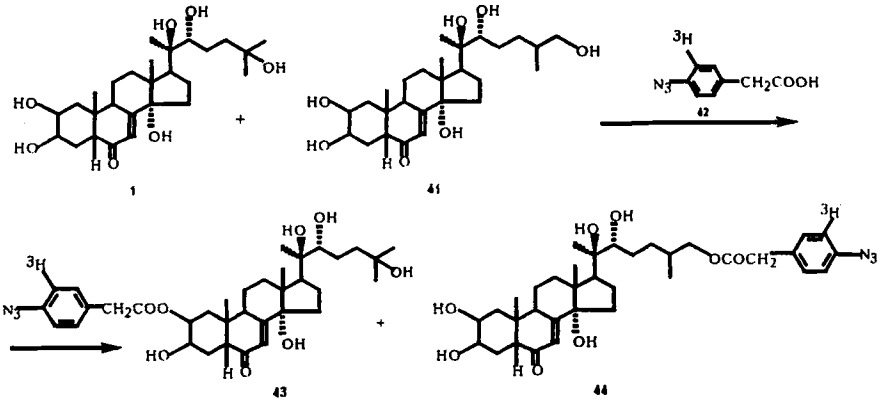
It has been shown [9] that the direct benzoylation of 20-hydroxyecdysone (**1**) with benzoyl chloride in pyridine forms the 2,3,22-tribenzoate (**29**). At the same time, benzoylation of the phenylboronate (**26**) followed by the removal of the protective grouping in the steroid side-chain permitted the synthesis of the 2,3-dibenzoate (**31**) and the 2,3,25-tribenzoate (**32**). To obtain the 25-monobenzoate (**34**), use was made of the benzoylation of 20-hydroxyecdysone 2,3,22-triacetate (**33**), followed by the selective hydrolysis of the acetoxy groups with potassium cyanide in methanol.



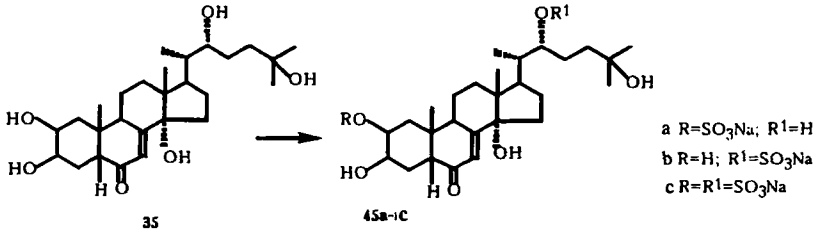
The synthesis of esters of higher fatty acids and α-ecdysone (**35**) at C-22 has been described by Dinan [15]. The 2,3-acetonide (**36**) was obtained in a yield of about 90% by the reaction of α-ecdysone with acetone in the presence of *p*-toluenesulfonic acid at room temperature for 4 h. It was then possible to obtain α-ecdysone 2,3-acetonide 22-stearate (**37**) by the reaction of the acetonide (**36**) with stearic anhydride in a mixture of pyridine and benzene at 50°C for 20 h. Hydrolysis of the acetonide grouping in compound (**37**) in dioxane in the presence of a solution of hydrochloric acid at room temperature for 5 h formed the required 22-ester (**38**) in high yield. The same author [15] has described the analogous preparation of other α-ecdysone esters: α-ecdysone 22-laurate, 22-myristate, 22-palmitate, 22-arachidate, 22-oleate, 22-linoleate, and 22-linolenate. It must also be mentioned that Dinan's method [15] has been used with slight modifications [16] for the synthesis of α-ecdysone 22-palmitate, 22-linoleate, 22-oleate, and 22-stearate.



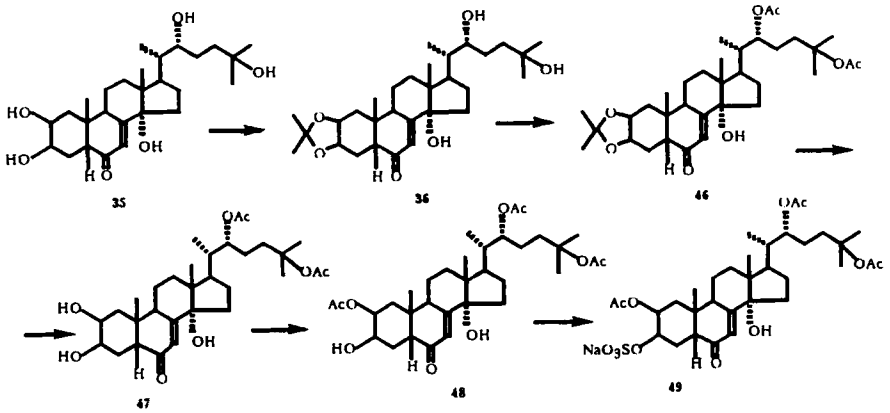
Anthroyl derivatives of the type of (**40**) have been proposed for the analysis of ecdysteroids by fluorescent methods [17, 18]. The procedure for synthesizing these compounds consists in the interaction of α-ecdysone (**35**) with 1-anthroyl nitrile (**39**) in acetonitrile in the presence of quinuclidine at 60°C for 30 min.



In [19], the synthesis of new radioactive photoaffinity ecdysteroid derivatives was effected with the use as starting materials of a mixture (1.3:1) of 20-hydroxyecdysone (**1**) and inokosterone (**41**). The interaction of this mixture from the roots of *Achyranthes fauriei* with *p*-azido-*m*-[^{3}H]-phenylacetic acid (**42**), dicyclohexylcarbodiimide, and dimethylaminopyridine in tetrahydrofuran for 30 h gave 2-O-(*p*-azido-*m*-[^{3}H]-phenylacetyl)-20-hydroxyecdysone (**43**) and 26-O-(*p*-azido-*m*-[^{3}H]-phenylacetyl)inokosterone (**44**), which were then separated by high-performance liquid chromatography.

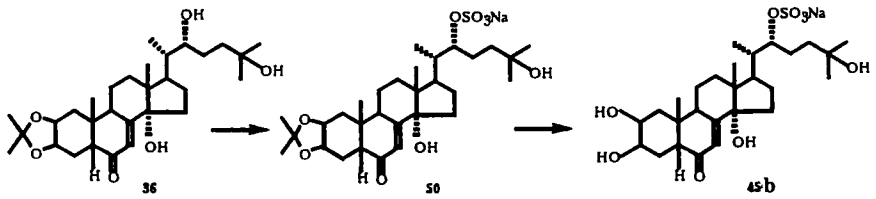


The synthesis of a number of α-ecdysone sulfates has been reported in [20]. The direct sulfation of α-ecdysone with a complex of sulfur trioxide and triethylamine in dimethylformamide at room temperature for 1 h 45 min gave, together with unchanged starting material, the 2-sulfate (**45a**), the 22-sulfate (**45b**), and the 2,22-disulfate (**45c**), which were isolated with yields of 22, 20.5, and 14%, respectively. The best results were obtained when some of the hydroxy groups in the α-ecdysone molecule were first protected and those remaining free were then sulfated.

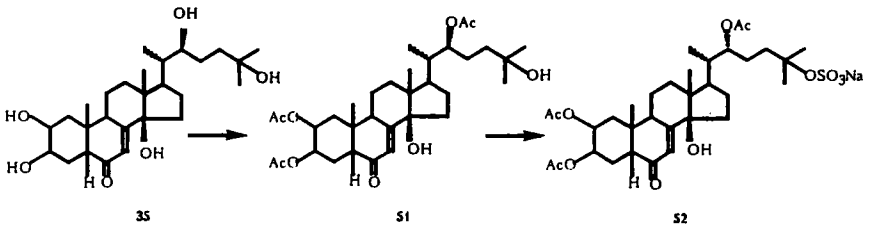


With this aim, the 2,3-acetonide (**36**) was synthesized in 85% yield by the reaction of α-ecdysone (**35**) with acetone and 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid. Acetylation of this acetonide with acetic anhydride in pyridine in the presence of dimethylaminopyridine enabled α-ecdysone 2,3-acetonide 22,25-diacetate (**46**) to be obtained with a yield of 68%. Hydrolysis of the acetonide grouping in compound (**46**) with aqueous methanol in the presence of trifluoroacetic acid led to the formation of the 22,25-diacetate (**47**) with a yield of 87%. By further acetylation of the diacetate (**47**) with acetic anhydride in pyridine at room temperature for 2 h it was possible to obtain a 44% yield of the 2,22,25-triacetate (**48**), in which the 3β-hydroxy group remained free. The 3-sulfate (**49**) was synthesized quantitatively by the sulfation of the steroid (**48**) with

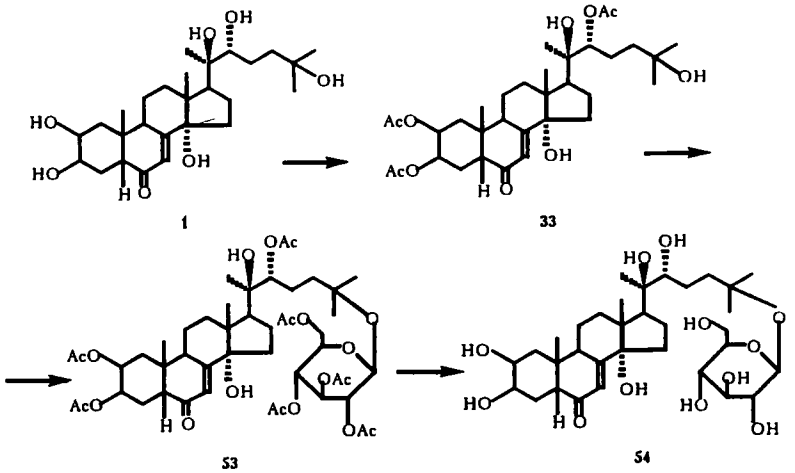
the complex of sulfur trioxide and triethylamine. It has been reported, however, that all attempts to achieve selective hydrolysis of the acetoxy groups in compound (**49**) with the aim of synthesizing α-ecdysone 3-sulfate have proved unsuccessful.



It has been established [20] that the reaction of α-ecdysone 2,3-acetonide with the complex of sulfur trioxide and triethylamine in dimethylformamide at room temperature for 2 h proceeds with the formation in 68% yield of α-ecdysone 2,3-acetonide 22-sulfate (**50**). Hydrolysis of the acetonide grouping in steroid (**50**) with aqueous trifluoroacetic acid for 10 min led to α-ecdysone 22-sulfate (**45b**) with a yield of 96%.

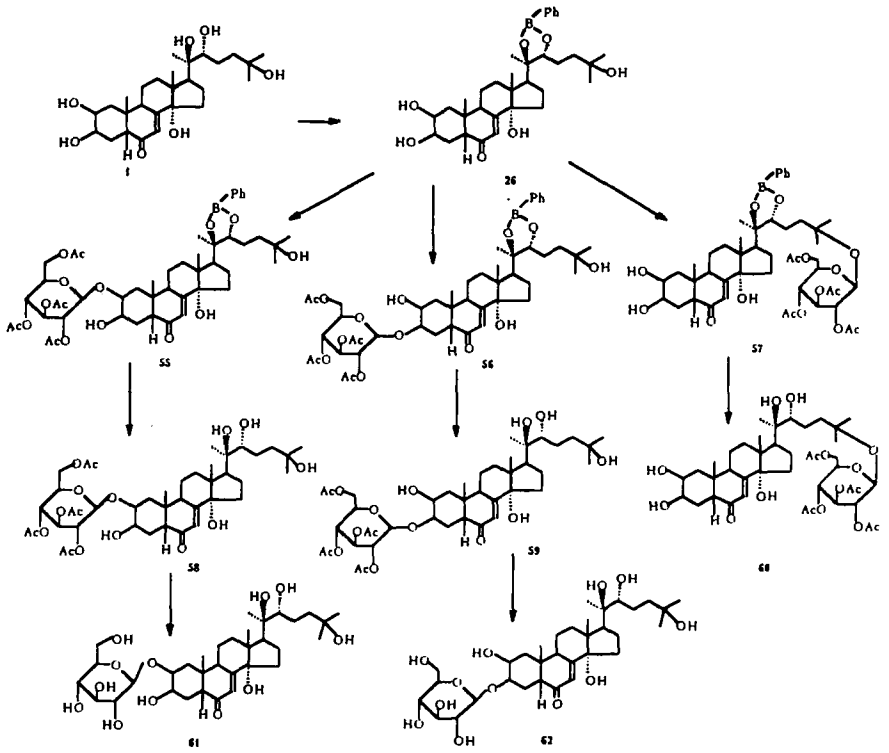


The acetylation of α-ecdysone (**35**) with acetic anhydride in the presence of dimethylaminopyridine at room temperature for 2 h gave the 2,3,22-triacetate (**51**) (yield 65%) and the 2,3,22,25-tetraacetate (yield 30%) [20]. By sulfating the triacetate (**51**) with the complex of sulfur trioxide and triethylamine it was possible to obtain an 85% yield of the 25-sulfate (**52**), which, however, proved to be extremely unstable.

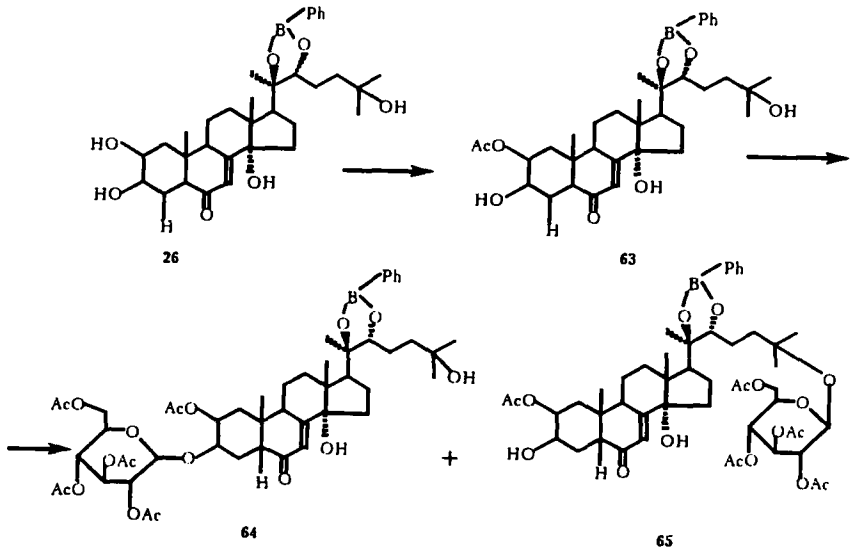


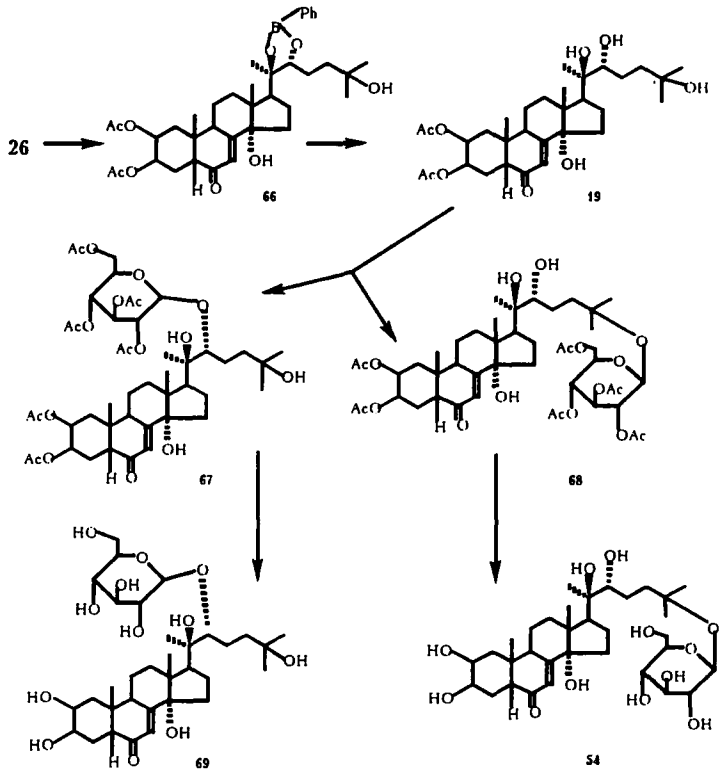
For the synthesis of β-*D*-glucosides of 20-hydroxyecdysone it was first necessary to obtain its derivatives with selectively protected hydroxy groups in the form of acetates and 20,22-phenylboronates [9, 21]. The acetylation of 20-hydroxyecdysone with acetic anhydride in pyridine in the presence of dimethylaminopyridine at room temperature for 3 h gave a 68% yield of the 2,3,22-triacetate (**33**). The glycosylation of this substance with tetra-O-acetylglucopyranosyl bromide in the presence of silver silicate led to the formation of the acetylated 25-glucoside (**53**) with a yield of 69%.

It was possible to obtain 20-hydroxyecdysone 25-O-β-*D*-glucopyranoside (**54**) with a yield of 85% by the hydrolysis of compound (**53**) with methanol in the presence of potassium cyanide. It has been reported [21] that the use of potassium cyanide in this stage avoids epimerization at C-5 in rings *A* and *B* of the ecdysteroid aglycon.



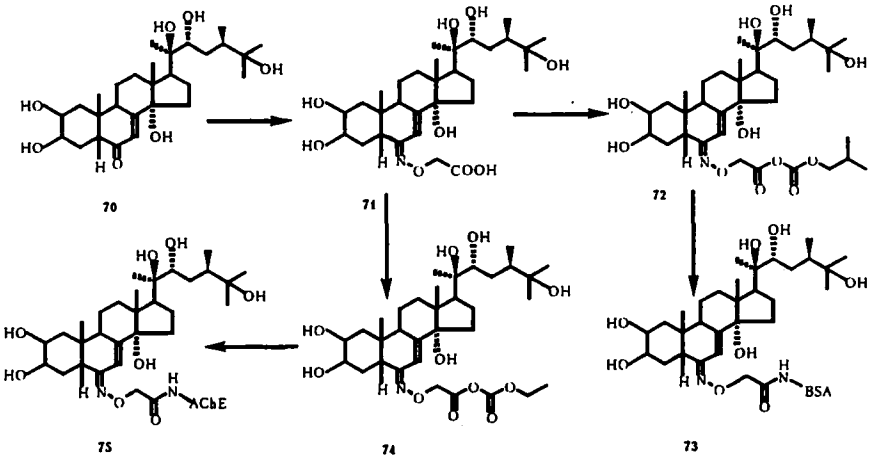
By .reaction with phenylboronic acid, 20-hydroxyecdysone (**1**) was converted in 93% yield into the 20,22-phenylboronate (**26**), the glycosylation of which under the conditions described above took place with the formation of the acetylated glycosides (**55**), (**56**), and (**57**), isolated with yields of 42, 21, and 7%, respectively. Elimination of the protective phenylboronate grouping in compound (**55**) with hydrogen peroxide in methanol enabled the acetylated glycoside (**58**) to be obtained with a yield of 91%. In the same way, glycosides (**59**) and (**60**) were synthesized from phenylboronates (**56**) and (**57**) with yields of 75 and 91%, respectively. Elimination of the acetoxy groups in compound (**58**) under the action of potassium cyanide in methanol led with a yield of 72% to 20-hydroxyecdysone 2-O-*β*-*D*-glucopyranoside (**61**). In its turn, 20-hydroxyecdysone 3-O-*β*-*D*-glucopyranoside (**62**) was synthesized similarly from the acetylated glycoside (**59**) with a yield of 69%.





In order to raise the yield of the 3-glucoside (**62**), the free 2-hydroxy group in steroid (**26**) was first protected by reaction with acetic anhydride in pyridine for 1 h. This formed a 77% yield of the 2-monoacetate (**63**). However, it was found that when this compound was subjected to glycosylation, not only the 3-glycoside (**64**) but also the 25-glycoside (**65**) were formed, being isolated in yields of 27 and 20%, respectively.

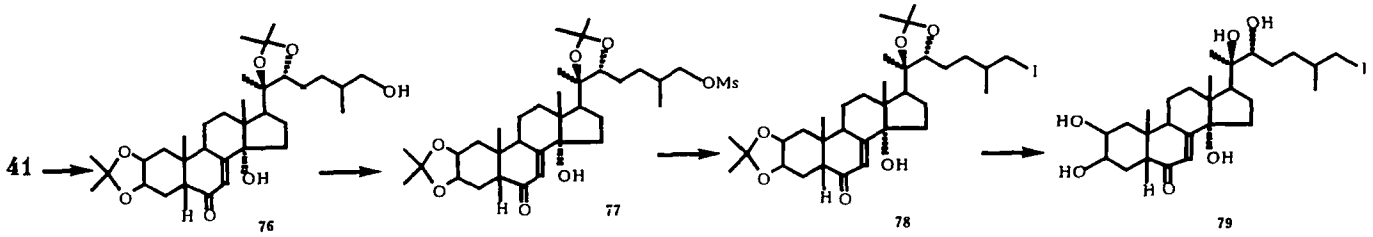
The acetylation of 20-hydroxyecdysone 20,22-phenylboronate (**26**) in the presence of dimethylaminopyridine for 3 h gave a 78% yield of the 2,3-diacetate (**66**). Elimination of the boronate grouping in compound (**66**) with hydrogen peroxide in methanol formed the 2,3-diacetate (**19**) with a yield of 89%. By the glycosylation of the latter, the acetylated glycosides (**67**) and (**68**) were synthesized with yields of 9 and 32%, respectively. The hydrolysis of compound (**67**) with methanol in the presence of potassium cyanide enabled 20-hydroxyecdysone 22-O-*β*-*D*-glucopyranoside (**69**) to be obtained with a yield of 78%. The 25-glycoside (**54**) was obtained analogously from the acetyl derivative (**68**).



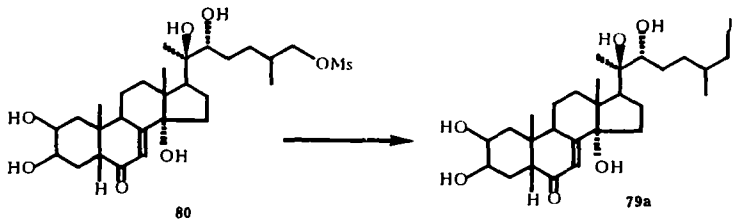
In the development of a method for the immunoenzyme assay of makisterone A (**70**) it was necessary to obtain its conjugate with bovine serum albumin (BSA) and acetylcholinesterase (AChE) [22, 23]. For this purpose the oxime derivative (**71**) was obtained by the reaction of makisterone A (**70**) with carboxymethoxyamine. The interaction of compound (**71**) with

ethyl chloroformate in dimethylformamide in the presence of tri-*n*-butylamine led to the mixed anhydride (**72**), which was then bound to bovine serum albumin with the formation of the immunogen (**73**). In its turn, the reaction of compound (**71**) with ethyl chloroformate in dimethylformamide in the presence of triethylamine led to the mixed anhydride (**74**) the binding of which with the globular tetrameric form of acetylcholinesterase gave the enzyme label (**75**) required for immunoenzyme assay.

Starting from inokosterone (**41**), Lee et al. [24] have achieved the synthesis of 26-iodoponasterone (**79**). The reaction of inokosterone (**41**) with acetone in the presence of *p*-toluenesulfonic acid gave an 87% yield of the 2,3:20,22-diacetonide (**76**). The primary 26-hydroxy group in the diacetonide (**76**) was esterified with methanesulfonyl chloride in pyridine with the formation of the 26-mesylate (**77**).

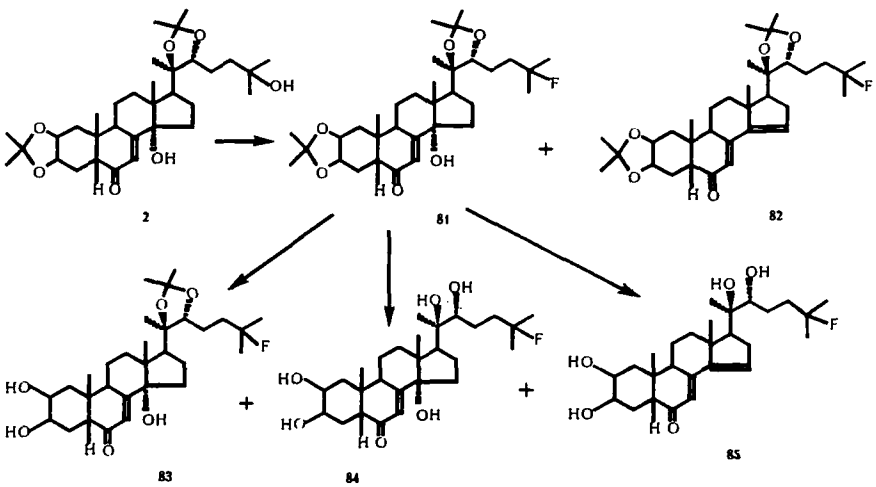


Replacement of the mesyl group by an iodine atom under the action of tetrabutylammonium iodide led to the synthesis of the 26-iodo derivative (**78**), hydrolysis of the acetonide groupings in which with 10% $HClO_4$–MeOH (1:1) led with a yield of 70% to the required ecdysteroid (**79**). It may be mentioned that in a test with drosophila Kc cells, 26-iodoponasterone A was the most active of known ecdysteroids, in this respect exceeding 20-hydroxyecdysone 160-fold.

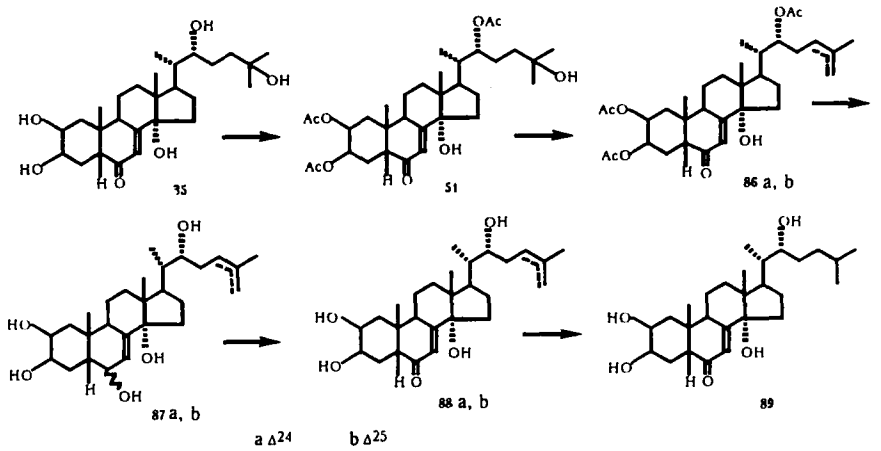


The synthesis of ^{125}I-labeled 26-iodoponasterone A (**79a**) was effected directly from inokosterone 26-mesylate (**80**) [25]. The reaction of compound (**80**) with $Na^{125}I$ in acetone at 80°C for 8-12 h enabled the required steroid (**79a**) to be obtained with a yield of 12-15%.

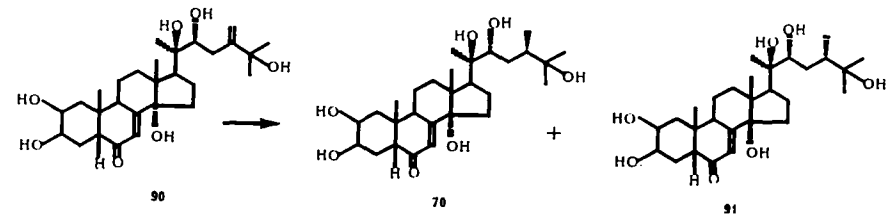
The synthesis of 25-fluoroponasterone A (**84**) from 20-hydroxyecdysone is described in [26]. First, the 2,3:20,22-diacetonide (**2**) was obtained in quantitative yield by the interaction of 20-hydroxyecdysone with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid. Then the authors showed that the reaction of the diacetonide (**2**) with diethylamine sulfur trifluoride formed the 25-fluoro derivative (**81**) and its dehydration product (**82**). By careful choice of temperature and reaction time it was possible to obtain compound (**81**) with a yield of about 70%. It was observed that attempts to hydrolyze the protective acetonide groups in steroid (**81**) in methanolic solution in the presence of *p*-toluenesulfonic acid or perchloric acid led mainly to the 25-fluoro-14-dehydrosteroid (**85**).



Satisfactory results have been obtained on the use of the ion-exchange resin Amberlite XAD-15 as an acid catalyst. Under these conditions, the required 25-fluoroponasterone A (**84**) and the 20,22-monoacetonide (**83**) were obtained from the diacetonide (**81**) with yields of 30 and 42%, respectively. The authors concerned [26] also established that it is best to use Amberlite XAD-15 in methanol for the hydrolysis of diacetonide (**2**) with the aim of obtaining 20-hydroxyecdysone (**1**).



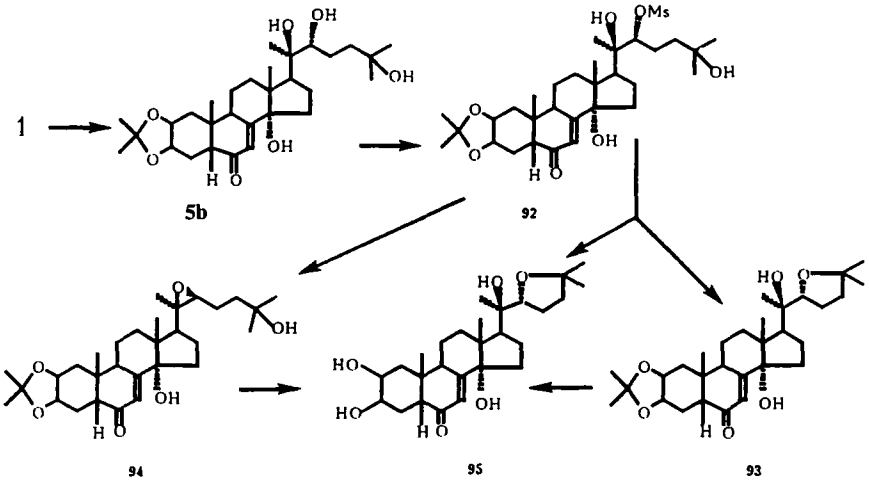
Syntheses of 25-deoxyecdysone (**89**) and 2,25-dideoxyecdysone have been effected from α-ecdysone and 2-deoxyecdysone, respectively [27]. According to the scheme of synthesis developed, in the first stage α-ecdysone (**35**), for example, was converted by reaction with acetic anhydride in pyridine in the presence of dimethylaminopyridine into the 2,3,22-triacetate (**51**) with a yield of about 70%. Subsequent dehydration of the 25-hydroxy group in compound (**51**) under the action of phosphorus oxychloride in pyridine took place with the formation of a mixture of the Δ^{24} and Δ^{25} derivatives (**86a** and **b**), obtained with a total yield of 76%. Reduction with diisobutylaluminum hydride was used to remove the protective acetate groups from the hydroxy groups. Under these conditions, of course, the 6-keto group was also reduced, and as a result of this reaction the pentaols (**87a** and **b**) were synthesized from the sterols (**86a** and **b**) with a yield of more than 85%. Selective oxidation of the 6-hydroxy groups in compounds (**87a** and **b**) under the action of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in dioxane led with a yield of 61% to a mixture of the 6-ketones (**88a** and **b**), hydrogenation of the double bonds in the side chains of which over a palladium catalyst gave the required 25-deoxyecdysone (**89**) quantitatively. The synthesis of 2,25-dideoxyecdysone was achieved by the same scheme, starting from 2-deoxyecdysone, with approximately the same yields in the individual stages.



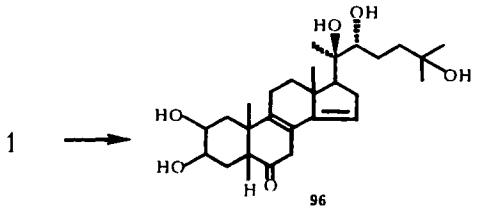
Makisterone A (**70**) and 24-epimakisterone (**91**) have been obtained by the hydrogenation of 24(28)-dehydromakisterone A (**90**) over 5% palladium on carbon [28]. Sodium nitrite was added to the reaction mixture in order to prevent epimerization during the hydrogenation of the 25-hydroxy group.

Starting from 20-hydroxyecdysone (**1**), Roussel et al. [29] synthesized the 22-mesylate (**92**) and studied some of its chemical reactions. Initially, the reaction of 20-hydroxyecdysone with phenylboronic acid in dimethylformamide gave the 20,22-phenylboronate, which was then converted by reaction with 2,2-dimethoxypropane and acetone in the presence of *p*-toluenesulfonic acid into its 2,3-acetonide. Selective hydrolysis of the protective grouping in the side-chain of the latter under the action of alkaline hydrogen peroxide enabled the 2,3-monoacetonide (**5b**) to be obtained with an overall yield of 65%.

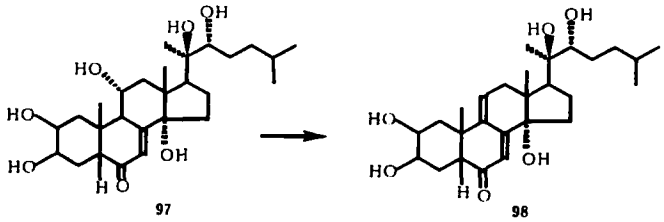
Mesylation of the secondary 22-hydroxy group in compound (**5b**) under the action of methanesulfonyl chloride in methylene chloride in the presence of diisopropylethylamine led to the formation of the 22-mesylate (**92**) and the 2,3-acetonide of the phytoecdysteroid shidasterone (**93**).



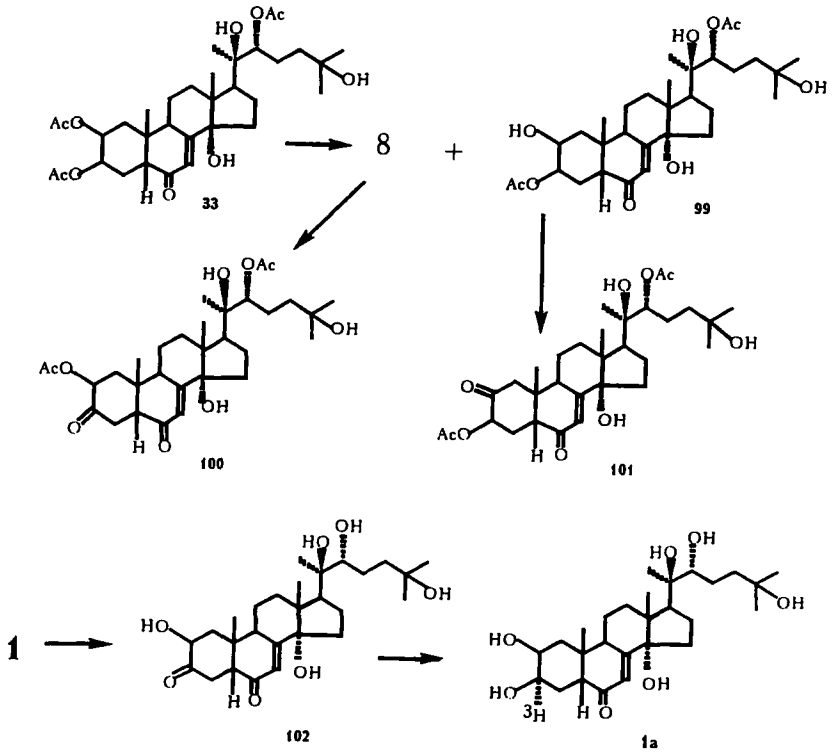
When the mesylate (**92**) was treated with potassium fluoride in acetonitrile or with tetrabutylammonium fluoride in anhydrous tetrahydrofuran, the 20,22-epoxide (**94**) was formed with yields of 76 and 57%. The interaction of steroid (**93**) with the complex of hydrogen fluoride and triethylamine, followed by acid hydrolysis of the acetonide grouping led with a yield of 48% to shidasterone (**95**). Analogously, shidasterone was formed with a yield of more than 90% by the acid hydrolysis of both the acetonide (**93**) and the epoxyacetonide (**94**).



The phytoecdysteroid podecdysone B (**96**) was obtained with a yield of 37% by the treatment of 20-hydroxyecdysone (**1**) with dilute hydrochloric acid in methanol at room temperature for 45 min [30].

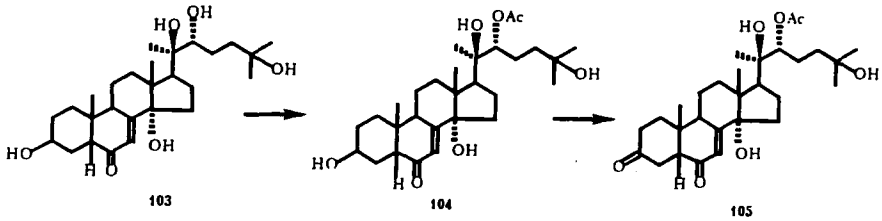


In its turn, the dehydration of ajugasterone C (**97**) under the action of a 5% solution of sodium hydroxide in methanol at room temperature led with a yield of more than 80% to the phytoecdysteroid dacrihainansterone (**98**) [31]. An analogous transformation has been observed by Szendrei et al. [32] on the passage of a solution of ajugasterone C through a column of alumina. From this fact they drew the conclusion that dacrihainansterone (synonym of 5-deoxykaladasterone) (**98**) is not a natural substance but is formed from ajugasterone C during its isolation. The partial hydrolysis of 20-hydroxyecdysone 2,3,22-triacetate (**33**) with a solution of potassium carbonate in aqueous methanol led to the formation of the 2,22-diacetate (**8**) and the 3,22-diacetate (**99**) [33]. The oxidation of these compounds with dimethyl sulfoxide in the presence of trifluoroacetic anhydride by the method of [34] gave the 3-ketone (**100**) and the 2-ketone (**101**), respectively. Attempts to obtain 2-dehydro-20-hydroxyecdysone from the latter by hydrolyzing the acetoxy group with potassium carbonate proved unsuccessful. It was established that in this process epimerization at C-5 also took place, with the formation of the 5α-epimer.

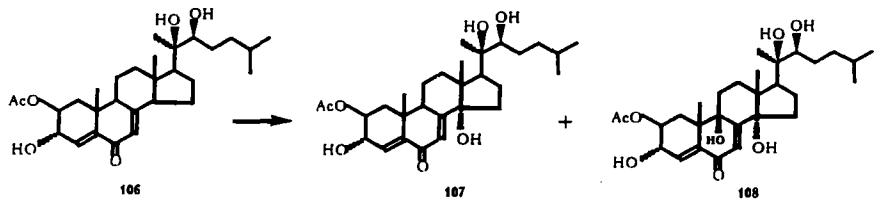


To obtain 3-dehydro-20-hydroxyecdysone (**102**), Girault et al. [33] used the oxidation of 20-hydroxyecdysone (**1**) with oxygen in hot distilled water in the presence of a freshly reduced platinum catalyst. Under these conditions the yield of the ecdysteroid (**102**) reached 50%. At the same time, the oxidation of α-ecdysone (**35**) under these conditions led to 3-dehydroecdysone with a yield of only 20%. It was reported that the decrease in yield was due to the formation of considerable amounts of the corresponding 3,22-dioxo derivative.

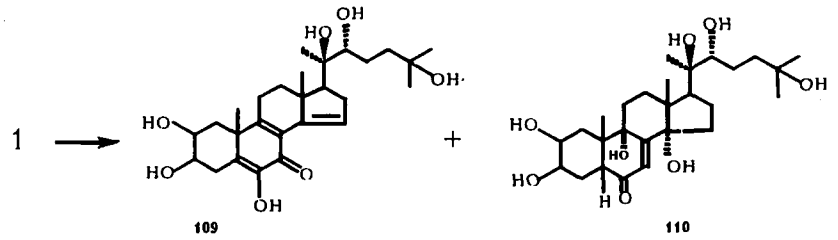
A method of obtaining the radioactively-labeled [3-^{3}H]-20-hydroxyecdysone (**1a**) starting from 3-dehydro-20-hydroxyecdysone (**102**) has been proposed [35]. The required compound (**1a**) is formed on the reduction of 3-dehydro-20-hydroxyecdysone (**102**) with sodium tetratritioborate in a mixture of anhydrous alcohol and tetrahydrofuran (1:1) for 10 min, followed by oxidation of the reaction product with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in anhydrous dioxane for 16 h.



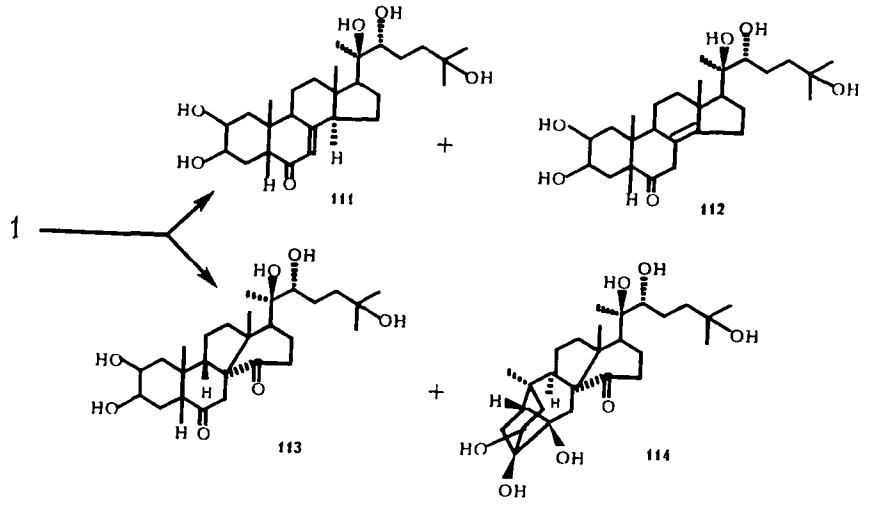
The 22-monoacetate (**104**) has been obtained in a yield of 25% by the partial acetylation of 2-deoxy-20-hydroxyecdysone (**103**) with acetic anhydride in pyridine for 1.5 h [33]. The Jones oxidation of this permitted the synthesis of the 3-keto derivative (**105**) with a yield of about 40%.



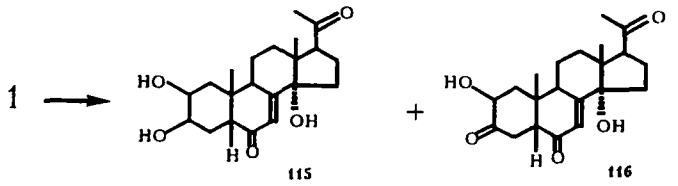
It has been established [36] that the oxidation of the ecdysteroid acetylpinnasterol (**106**) with selenium dioxide in aqueous dioxane at 60°C for a day forms the 14α-hydroxy derivative (**107**) and the 9α,14α-dihydroxy derivative (**108**), isolated from the reaction mixture with yields of 20 and 10%, respectively.



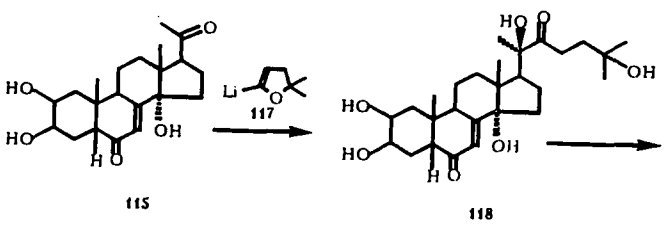
The autooxidation of 20-hydroxyecdysone (**1**) under alkaline conditions has been studied by Suksamrarn et al. [37]. These authors established that keeping 20-hydroxyecdysone in a 2% aqueous methanolic solution of sodium hydroxide at 30-32°C for 2 h led to the formation of the phytoecdysteroid calonysterone (**109**) and 9α,20-dihydroxyecdysone (**110**), isolated with yields of 35 and 29%, respectively.

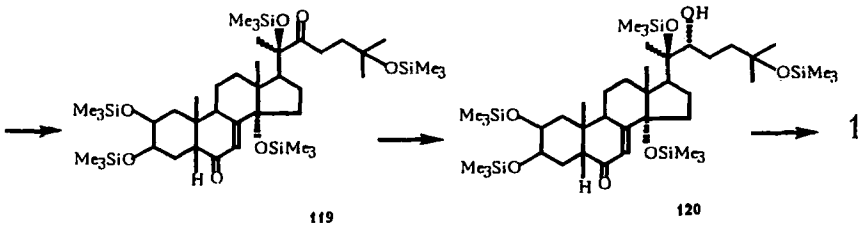


It has been established [38] that the ultraviolet irradiation of an aqueous solution of 20-hydroxyecdysone with a high-pressure mercury lamp giving radiation with a wavelength of more than 290 nm forms steroids (**111-114**) with yields of 15, 35, 23, and 18%, respectively.

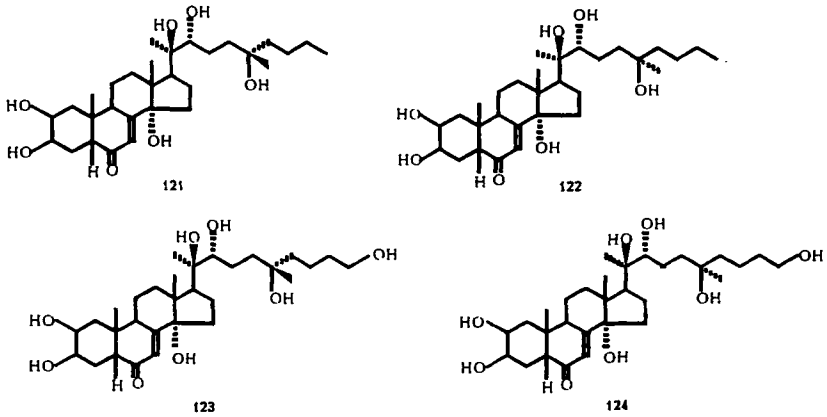


A convenient method for oxidizing 20-hydroxyecdysone (**1**) to poststerone (**115**) has been proposed in [39]. The Jones oxidation of 20-hydroxyecdysone with 1.2 equiv. of chromic acid takes place with the formation, as the main product, of a mixture (2:1) of poststerone (**115**) and 3-dehydropoststerone (**116**). The poststerone can be obtained with a yield of 62%.

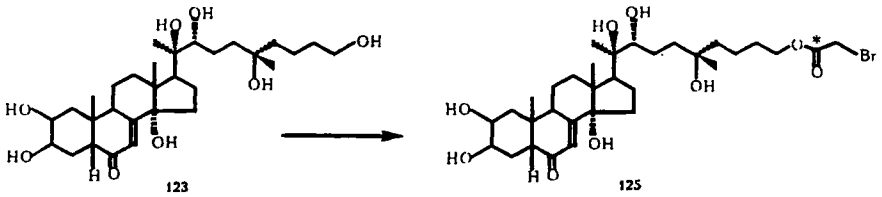




In its turn, poststerone has been converted into 20-hydroxyecdysone (**1**) [40, 41]. The alkylation of the 20-keto group in the poststerone molecule with the lithium derivative of dihydrofuran (**117**) followed by the reaction of the addition products so formed with hydrochloric acid in tetrahydrofuran gave the pentahydroxy-6,22-ketone (**118**) with an overall yield of 79%. Silylation of the hydroxy group in compound (**118**) with trimethylsilyl triflate in the presence of 2,6-lutidine led to the formation in 74% yield of the fully silylated derivative (**119**), the reaction of which with diisobutylaluminum hydride led to the reduction of both keto groups. However, the subsequent oxidation with activated manganese dioxide of the allyl 6-hydroxy group formed in the reduction process permitted the synthesis with an overall yield of 83% of the (22R)22-alcohol (**120**) containing the (22S)-isomer as an impurity in only trace amounts. Hydrolysis of the trimethylsilyl groups in steroid (**120**) with tetrabutylammonium fluoride led to 20-hydroxyecdysone with a yield of 80%.



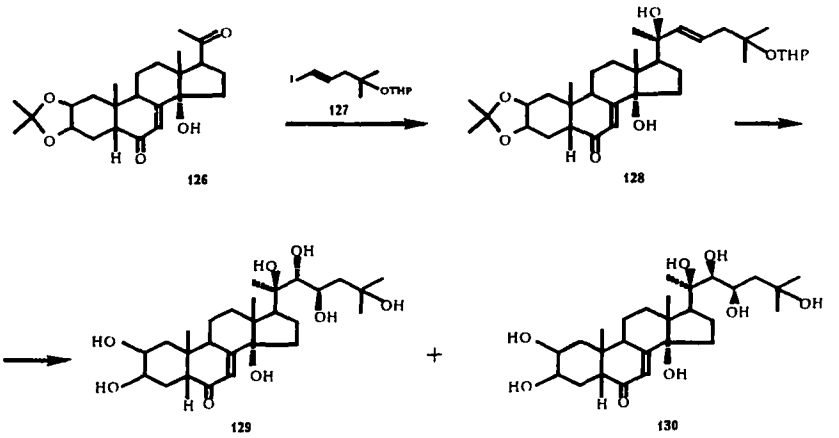
Of course, since poststerone itself is obtained from 20-hydroxyecdysone, this synthesis is of no practical significance. However, the scheme developed permits other ecdysteroids to be obtained, as well. Thus, later, the ecdysteroids (**121-124**) were synthesized by this scheme, using the appropriate 5-lithium derivatives of 3,3-disubstituted 2,3-dihydrofurans for alkylating poststerone (**115**) and subsequent transformations analogous to those described above in the synthesis of 20-hydroxyecdysone [40, 41].



The bromoacetylation of the primary hydroxy group in steroid (**123**) under the action of bromo-[1-^{14}C]-acetic acid has given the bromoacetyl derivative (**125**) [42]. It has been reported that this substance binds rapidly, quantitatively, and irreversibly to the ecdysteroid receptor and is of interest as an affinity label for it.

With the aim of showing the stereochemistry of the side-chain, the phytoecdysteroids abutasterone and gerardiasterone have been synthesized from poststerone [43, 44]. Thus, the alkylation of poststerone acetonide (**126**) with the anion obtained from the tetrahydropyranyl ether of (E)-4-iodo-2-methylpent-4-en-2-ol (**127**) and *tert*-butyllithium led to the formation of the allyl alcohol (**128**) with a yield of 70%. The *cis*-hydroxylation of the Δ^{22} bond in compound (**128**) with osmium tetroxide in pyridine, followed by hydrolysis of the protective groupings in the presence of camphorsulfonic acid, formed gerardiasterone and its (22S,23R)-isomer (**130**) in a ratio of 79:21. The use of dihydroquinine *p*-chlorobenzoate as a chiral ligand in the

osmium tetroxide hydroxylation reaction enabled ecdysteroids (**129**) and (**130**) to be obtained in a ratio of 95:5. On the other hand, the presence of a different chiral ligand — dihydroquinidine *p*-chlorobenzoate — in the reaction mixture led to the formation of compounds (**129**) and (**130**) in a ratio of 21:79.



Thus, the examples given above permit the confident assumption that, since in many cases the starting materials are fully accessible, the chemical transformation of ecdysteroids is a promising method of obtaining various derivatives having both scientific and practical interest.

The author expresses his deep gratitude to Prof. René Lafonte (École Normale Supérieure, Paris, France) for assistance in the choice of original literature for this paper and a fruitful discussion of some of its aspects.

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