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THE SYNTHESIS OF LABELED STEROLS

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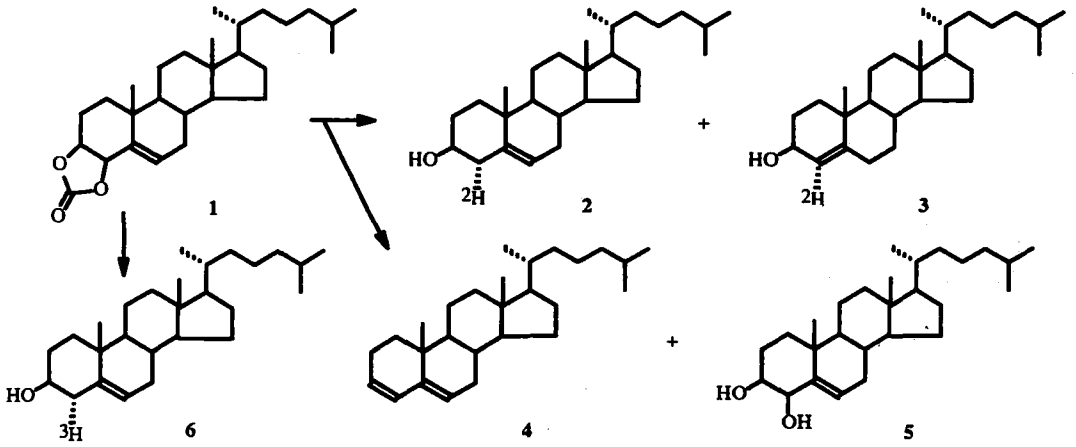
This review presents a discussion of methods for the chemical synthesis of sterols labeled with hydrogen and carbon isotopes.

Sterols are among the most intensively investigated natural compounds [1—3]. The continuing scientific interest in these substances is due to their wide distribution in the animal and vegetable kingdoms, to their important physiological functions, and to the great variety of the chemical structures of concrete representatives. Usually, sterols required for scientific investigations are isolated from natural raw material or are obtained specially by chemical synthesis. At the same time, sterols selectively labeled with ^{2}H, ^{3}H, ^{13}C, and ^{14}C atoms in strictly defined positions of the molecule are required for the solution of some scientific, especially biochemical, problems. The most diverse methods are being specially developed for obtaining such substances. The object of the present review is a discussion of the results obtained in this field.

Investigations for the development of methods of synthesizing sterols labeled with hydrogen and carbon isotopes were begun a fairly long time ago [1]. Thus, in 1943, for the introduction of deuterium into the cholesterol molecule Bloch and Rittenberg [4] proposed to heat cholesterol at 127°C in a mixture of deuterated water and acetic acid in the presence of platinum for three days. It was then possible to isolate from the reaction mixture about 44% of cholesterol with a deuterium content of 4.16%. It was established that the deuterium atoms were distributed in the molecule nonuniformly and were present both in the cyclic part and in the side-chain. This method of deuteration was later studied in more detail [5, 6]. It was also established [7] that the method under discussion can be used to obtain cholesterol labeled with tritium.

In another approach to obtaining deuterium-labeled cholesterol, the initial compound used for deuterium exchange was cholest-4-en-3-one [8]. When it was treated with a mixture of deuterated acetic acid and water in the presence of an Adams catalyst at 150°C for two days, deuterium-labeled cholest-4-en-3-one was formed. Interaction of the latter with acetic anhydride and acetyl chloride followed by reduction of the enol acetate with sodium tetrahydroborate gave deuterium-labeled cholesterol.

A common defect of these methods of obtaining labeled cholesterol is that the isotopic labels are introduced into the molecule extremely unselectively. This makes it difficult to interpret the scientific results obtained with the use of such compounds. Therefore, special methods ensuring the presence of a label in strictly defined positions of sterol molecules were subsequently developed [9]. Thus, the synthesis of a number of derivatives selectively labeled at C-4 with deuterium or tritium



Institute of Bioorganic Chemistry, Belarus National Academy of Sciences, 220141, Belarus', Minsk, Ul. Akad. Kuprevicha, 5/2. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 413—441, July-August, 1999. Original article submitted May 5, 1998.

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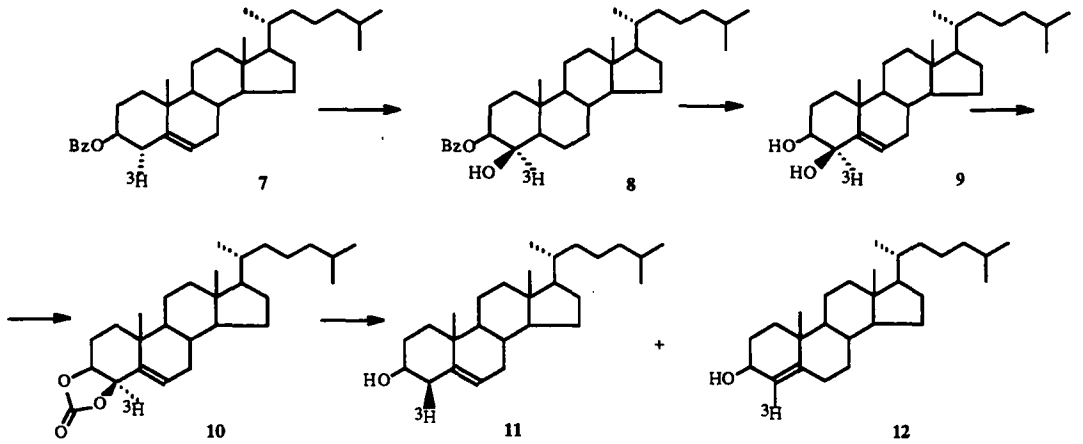
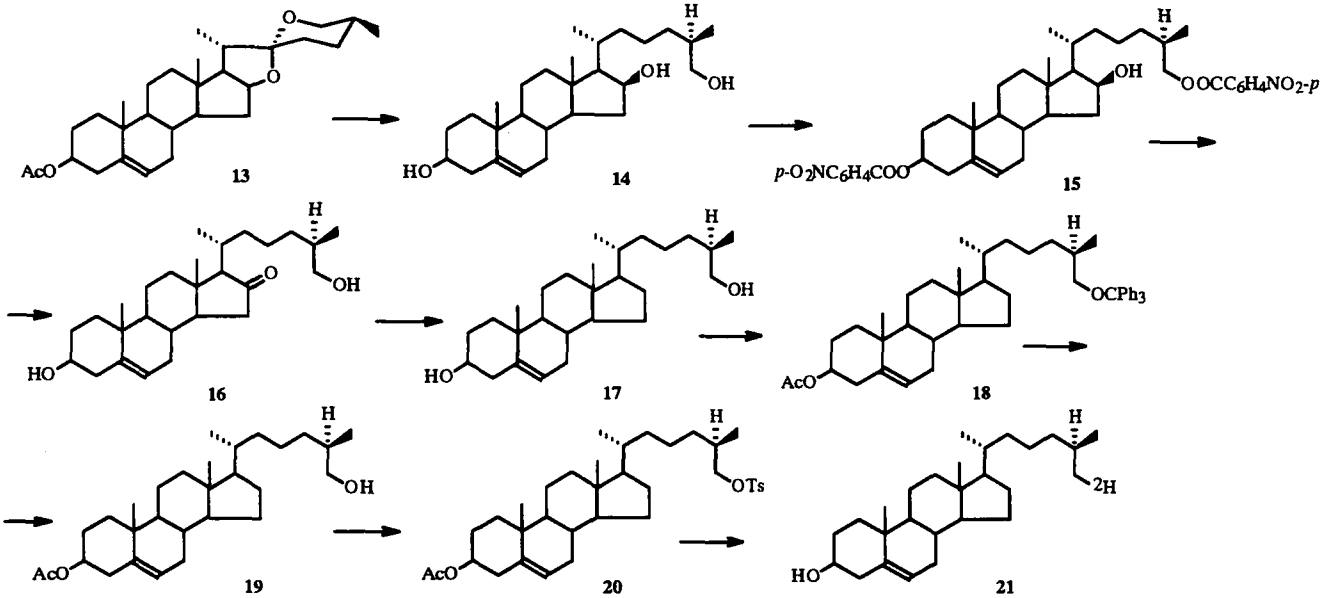
started from the cyclic carbonate of 4β-hydroxycholesterol (**1**) [10]. The reduction of compound (**1**) with sodium tetradeuterioborate in the presence of tetrakis(triphenylphosphine)platinum(0) and triphenylphosphine formed a mixture (9:1) of [4α-^{2}H]cholesterol (**2**) and [6α-^{2}H]-Δ^4-cholesterol (**3**) with an overall yield of 21%. In addition to these products, the diene (**4**) and the diol (**5**) were also formed in this reaction, being isolated with yields of 6.8 and 45%, respectively. The use for the reduction of steroid (**1**) with sodium tetradeuterioborate of a mixture of tetrakis(triphenylphosphine)platinum(0) and 1,2-bis(diphenylphosphino)ethane enabled a mixture (5.3:1) of the deuterated derivatives (**2**) and (**3**) to be obtained with an overall yield of 76%. At the same time, the yields of compounds (**4**) and (**5**) amounted to 8.2 and 16%, respectively. In its turn, when steroid (**1**) was reduced with sodium tetratritioborate under the conditions described above [4α-^{3}H]cholesterol (**6**) was obtained.

In [10], the synthesis of [4β-^{3}H]cholesterol (**11**) started from [4α-^{3}H]cholesterol benzoate (**7**). The allyl oxidation of compound (**7**) with selenium dioxide took place with the formation of the 4β-alcohol (**8**), the subsequent reduction of which with lithium tetrahydroaluminate gave the labeled 3β,4β-diol (**9**), and the interaction of this with carbonyldiimidazole led to the cyclic carbonate (**10**). As a result of the reduction of steroid (**10**) with sodium tetrahydroborate in the presence of tetrakis(triphenylphosphine)platinum(0) and triphenylphosphine, [4β-^{3}H]cholesterol (**11**) and the allyl alcohol (**12**) were formed in a ratio of 5:1 with an overall yield of 84%.



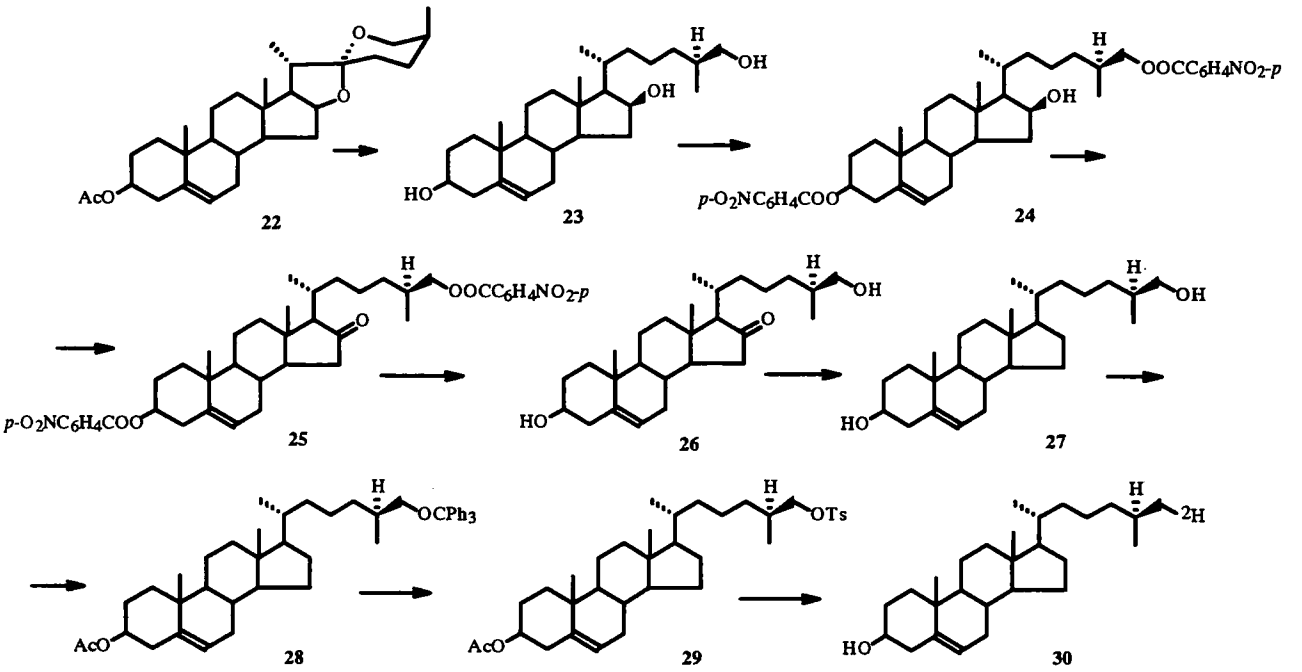
In the synthesis of (25S)-[26-^{2}H]cholesterol (**21**), diosgenin acetate (**13**) was used as the starting material [11]. Its Clemmensen reduction with zinc amalgam and hydrochloric acid in ethanol gave (25R)-16β,26-dihydroxycholesterol (**14**), which was transformed into the *p*-nitrobenzoyl derivative (**15**) by treatment with *p*-nitrobenzoyl chloride in a mixture of pyridine and ether. Subsequent Jones oxidation in acetone and hydrolysis with potassium hydroxide in methanol led to the 3,26-dihydroxy-16-ketone (**16**). Its Huang-Minlon reduction gave 26-hydroxycholesterol (**17**), which was then converted into the protected diol (**18**) by the successive action of trityl chloride in pyridine and of acetic anhydride. Hydrolysis of the trityl



protective grouping in compound (**18**) with hydrochloric acid in ethanol led to the 26-alcohol (**19**), the tosylation of which with *p*-toluenesulfonyl chloride in pyridine gave the 26-tosylate (**20**), and this was reduced with lithium tetradeuterioaluminate in ether to the required (25S)-[26-^{2}H]cholesterol (**21**).

(25R)-[26-^{2}H]Cholesterol has been synthesized from yamogenin acetate (**22**) [12]. The Clemmensen reduction of the sapogenin (**22**) with zinc amalgam and hydrochloric acid in ethanol led to the 3,16,26-triol (**23**), which was then converted into the 3,26-di-*p*-nitrobenzoate (**24**) by reaction with *p*-nitrobenzoyl chloride in a mixture of methylene chloride and pyridine. The Jones oxidation in acetone of the 16β-hydroxy group that had remained free in compound (**24**) led to the 16-ketone (**25**), and the hydrolysis of the protective groups in the latter with a methanolic solution of potassium hydroxide permitted the formation of the 3,26-dihydroxy-16-ketone (**26**).

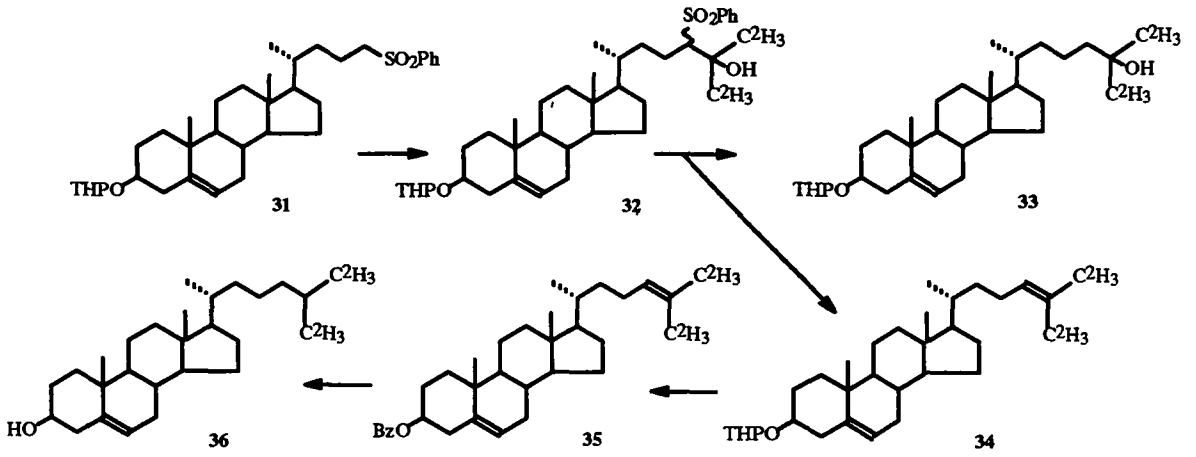
The Wolf-Kishner reduction of the keto group in compound (**26**) with hydrazine hydrate gave the 3,26-diol (**27**), and the selective protection of the primary 26-hydroxy group in this with trityl chloride in pyridine and of the secondary hydroxy group with acetic anhydride yielded the protected diol (**28**). Hydrolysis of the trityl grouping in the latter under the action of hydrochloric acid in ethanol followed by tosylation of the resulting 26-alcohol with *p*-toluenesulfonyl chloride in pyridine led to the 26-tosylate (**29**). The desired deuterated sterol (**30**) was obtained by reducing compound (**29**) with lithium tetradeuterioaluminate in ether.



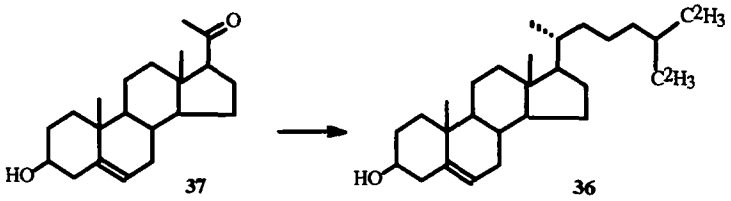
Two approaches have ben proposed for the synthesis of [26,27-$^2H_6$]cholesterol (**36**) from the sulfone (**31**) [13]. According to the first, the reaction of lithium diisopropylamide with the sulfone (**31**) gave an anion the addition of which to hexadeuteroacetone in tetrahydrofuran permitted the synthesis of the hydroxysulfone (**32**) in 81% yield. The reductive desulfuration of compound (**32**) with sodium amalgam in a mixture of tetrahydrofuran and methanol led to the formation of the 25-alcohol (**33**) and the 5,24-diene (**34**), which were isolated with yields of 28 and 48%, respectively.

The 5,24-diene (**35**) was obtained in an overall yield of 94% by hydrolysis of the tetrahydropyranyl protective grouping in compound (**34**) with hydrochloric acid in methanol followed by benzoylation with benzoyl chloride in pyridine. Selective reduction of the 24-double bond in compound (**35**) with the diimide generated in the thermal decomposition of tosylhydrazine gave with a yield of 46% the benzoate of a deuterated cholesterol, from which [26,27-$^2H_6$]cholesterol (**36**) itself was obtained with a yield of 82% by hydrolysis with potassium hydroxide in methanol.

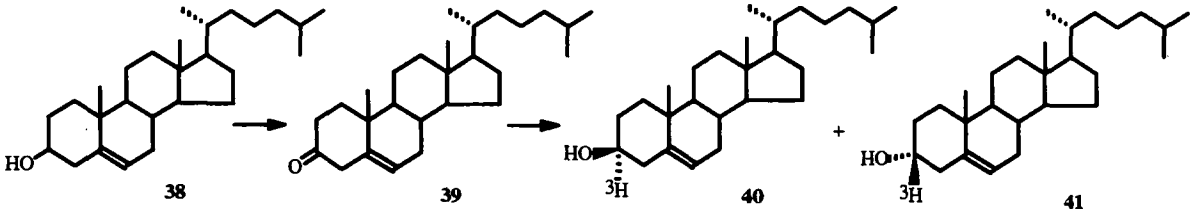
The second approach made use of the interaction of the 25-alcohol (**33**) with acetic anhydride in the presence of *p*-toluenesulfonic acid, forming with a yield of 80% the 3,25-diacetate, the reduction of which with lithium in ethylamine led with a yield of 72% to the required sterol (**36**) [13].



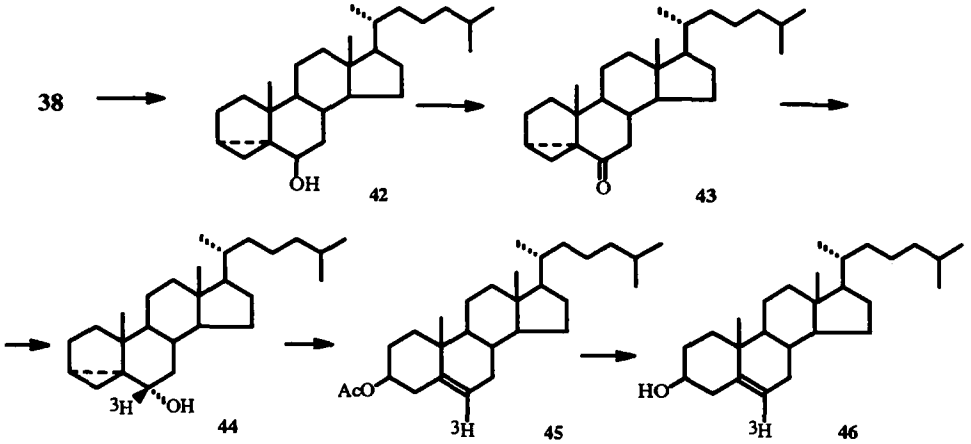
A nine-stage synthesis of [26,27-$^2H_6$]cholesterol (**36**) from pregnenolone (**37**) with an overall yield of 7% is described in [14]. In this synthesis the source of deuterium atoms was, again, hexadeuteroacetone.



To obtain [3α-^{3}H]cholesterol (**40**), use has been made of the oxidation of the initial cholesterol (**38**) with pyridinium chlorochromate in methylene chloride to the 3-ketone (**39**) [15]. The reduction of compound (**39**) with sodium tetratritioborate in ethanol took place with the formation of a mixture (86:14) of [3α-^{3}H]cholesterol (**40**) and [3β-^{3}H]-3-epicholesterol (**41**). The labeled sterols (**40**) and (**41**) were then separated by column chromatography on silica gel.

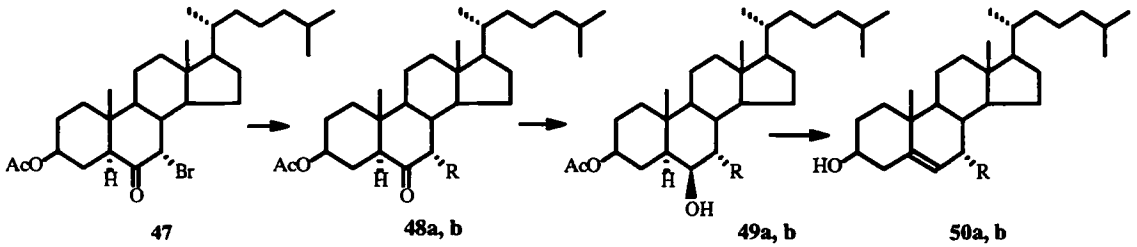


The introduction of a label into steroids deposited on a catalytic system on an adsorbent under the action of gaseous tritium activated by a microwave discharge at -196°C has been described in [16]. It was established that, under the given conditions, cholesterol deposited on a mixture of alumina and silica gel formed [^{3}H]cholesterol in quantitative radiochemical yield. Analogously, [^{3}H]-5α-cholestan-3β-ol was obtained from 5α-cholestan-3β-ol in a radiochemical yield of 71.4%.



The synthesis of a number of sterols labeled with tritium or deuterium in position 6 was undertaken in [17]. Thus, the 6β-alcohol (**42**) was obtained with a yield of 70% by the tosylation of cholesterol (**38**) with *p*-toluenesulfonyl chloride in pyridine, followed by a rearrangement of the resulting tosylate under the action of potassium acetate in aqueous acetone. The Jones oxidation of compound (**42**) with chromic acid in acetone led with a yield of 68% to the ketone (**43**). The result of the reduction of the keto group in compound (**43**) with a solution of sodium tetratritioborate in a mixture of ethanol and tetrahydrofuran (1:1) was the formation of the tritium derivative (**44**), the interaction of which with zinc acetate in acetic acid gave the labeled cholesterol acetate (**45**) in an overall yield of 70% from the ketone (**43**).

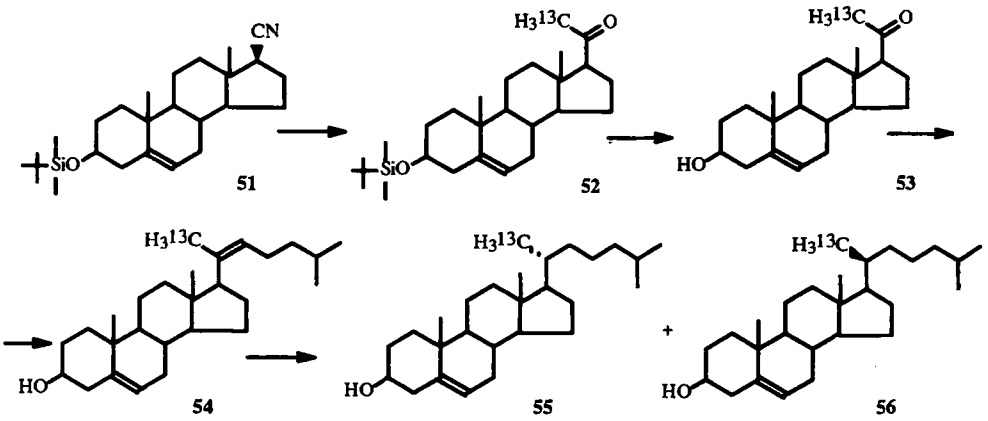
The required [6-^{3}H]cholesterol (**46**) was obtained with a radiochemical purity of 92% by the hydrolysis of the acetoxy group in compound (**45**) with a solution of potassium hydroxide in 80% methanol. In [17], it is reported that by means of the described reaction sequence it is also possible to obtain tritium-labeled fucosterol, 28-isofucosterol, β-sitosterol, and clerosterol. Moreover, the use of sodium tetradeuterioborate for the reduction of ketone (**43**) enables the corresponding deuterated derivatives to be obtained.



a. R =^{2}H, b. R =^{3}H

A method has been developed for the synthesis of the cholesterol derivatives (**50a,b**) labeled at C-7 with deuterium and tritium, respectively, that starts from the 7α-bromo-6-ketone (**47**) [18]. The reduction of steroid (**47**) with zinc dust in acetic acid labeled with deuterium or tritium forms the 6-ketones (**48a**) and (**48b**), respectively. The subsequent reduction of the keto group in compounds (**48a,b**) with sodium tetrahydroborate leads to the 6β-alcohols (**49a,b**) the dehydration of which with phosphorus oxychloride in pyridine followed by reduction of the acetoxy group with lithium tetrahydroaluminate enables the desired labeled sterols (**50a,b**) to be obtained. In their turn, [7β-^{3}H]- and [7β-^{2}H]cholesterols, have been obtained analogously from derivatives of the bromoketone (**47**) labeled at C-5 and C-7. In this case ordinary, unlabeled, acetic acid is used at the debromination stage.

[21-^{13}C]Cholesterol (**55**) has been synthesized from the nitrile (**51**) [19]. The reaction of compound (**51**) with carbon-13—labeled methylmagnesium iodide in a mixture of benzene and ether led with a yield of 65% to the substituted pregnenolone (**52**). Elimination of the silyl protection in the latter with the formation of the labeled pregnenolone (**53**) in a yield of 95% was achieved by the action of tetrabutylammonium fluoride in tetrahydrofuran.

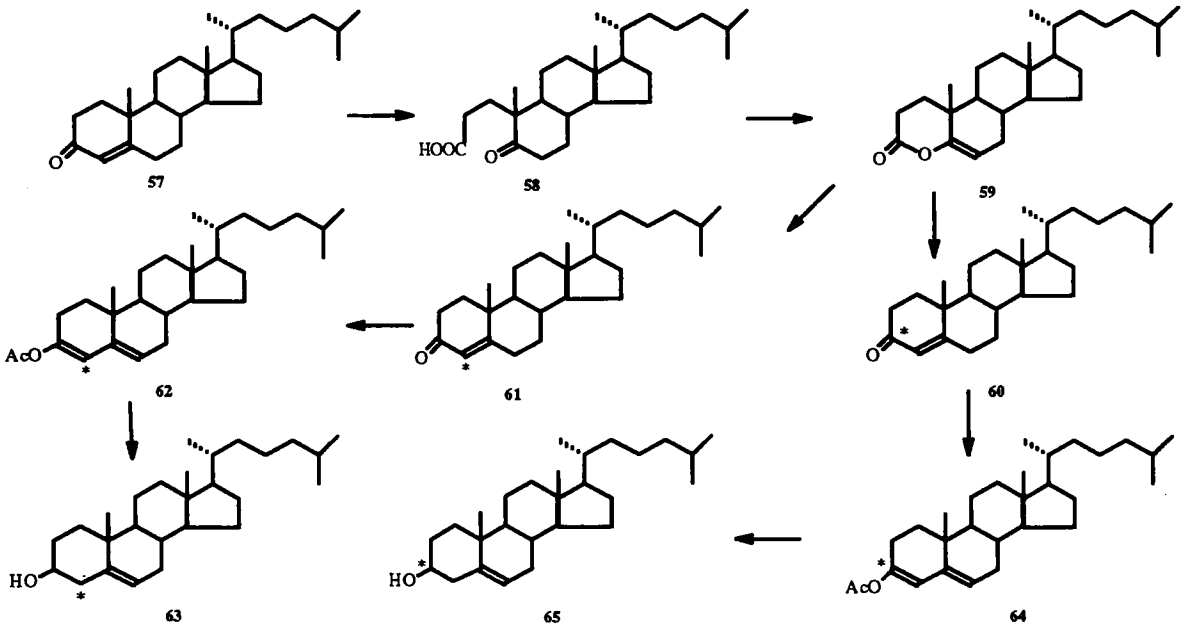


The $\Delta^{20(22)}$-derivative (**54**) has been synthesized by the Wittig condensation of the 20-ketone (**53**) with the corresponding phosphorus ylide in dioxane. Hydrogenation of the more accessible 20(22)-double bond in compound (**54**) over a platinum catalyst in a mixture of dioxane and acetic acid formed [21-^{13}C]cholesterol (**55**) and its (20S)- isomer (**56**), which were isolated from the reaction mixture by high-performance liquid chromatography with yields of 43 and 13%, respectively.

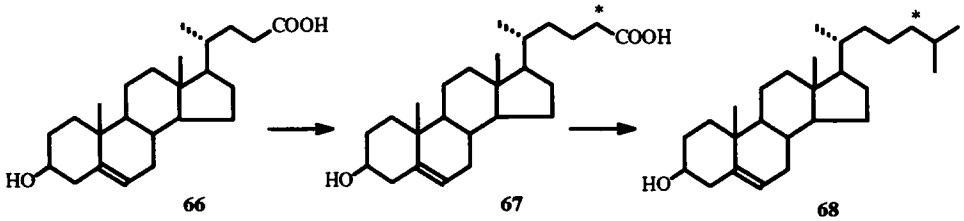
Methods for the specific introduction of ^{14}C atoms into positions 3 and 4 of natural sterols with the use of cholest-4-en-3-one (**57**) as the initial substance were first proposed in [20]. The ozonization of the double bond in (**57**) and oxidative cleavage of the ozonide forms the keto acid (**58**) the reaction of which with acetic anhydride and acetyl chloride leads to lactone (**59**). Condensation in benzene in the presence of sodium hydride of the lactone (**59**) with phenyl acetate labeled in the carboxyl moiety with ^{14}C, followed by decarboxylation and then cyclization by boiling with hydrochloric acid in acetic acid, gives [3-^{14}C]cholest-4-en-3-one (**60**).

In its turn, when phenyl acetate labeled with ^{14}C in the methyl group is used for the reaction with lactone (**59**), [4-^{14}C]cholest-4-en-3-one (**61**) is formed. It was later established [21, 22] that to obtain the [4-^{14}C]steroid (**61**) it is better to use condensation of the lactone with ^{14}C-labeled methylmagnesium iodide followed by cyclization of the addition product under the action of an acid and a base. The further transformation of the Δ^4-3-ketone (**61**) into the enol acetate (**62**) and reduction of the latter with lithium tetrahydroaluminate leads to [4-^{14}C]cholesterol (**63**) [23]. It has also been established [24, 25] that the use of sodium tetrahydroborate for the reduction of the enol acetate (**62**) leads to a considerable increase in the yield of the labeled cholesterol (**63**).

The enol acetate (**64**) has been obtained analogously from [3-^{14}C]cholest-4-en-3-one (**60**), and its reduction with sodium tetrahydroborate has given [3-^{14}C]cholesterol (**65**) with a yield of 60% [26].



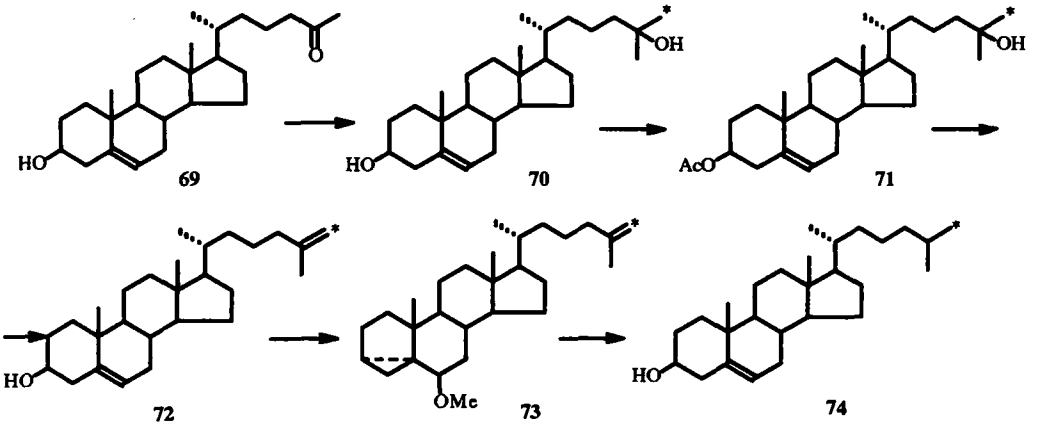
It has been reported [27] that [24-^{14}C]cholesterol (**68**) has been synthesized from cholic acid (**66**). By the Arndt-Eistert reaction with [^{14}C]diazomethane, the acid (**66**) was first converted into [24-^{14}C]homocholic acid (**67**), transformation of which led to the desired product (**68**). There are no details whatever of this synthesis in [27].



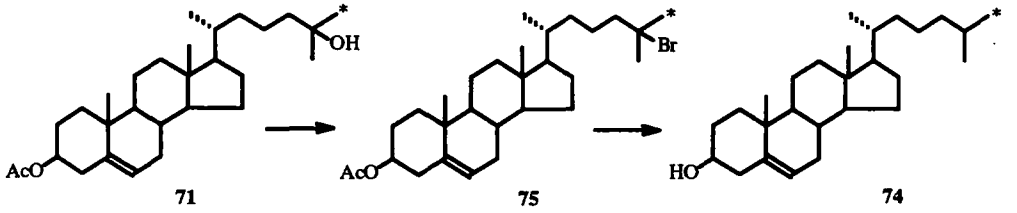
[26-^{14}C]Cholesterol (**74**) has been synthesized from the 25-ketone (**69**) [28]. As a result of the condensation of steroid (**69**) with [^{14}C]methylmagnesium iodide in benzene, the labeled 25-hydroxycholesterol (**70**) was obtained, and the acetylation of the secondary hydroxy group in its molecule with acetic anhydride in pyridine led to the 3-monoacetate (**71**) with a yield of 85.3%. By the dehydration of the latter under the action of phosphorus tribromide in benzene it was possible to synthesize the corresponding $\Delta^{25(26)}$- derivative, hydrolysis of the acetoxy group in which with potassium hydroxide in methanol gave the alcohol (**72**) with a yield of 91.5%.

Then, in order to protect the 5-double bond, the alcohol (**72**) was converted by reaction with *p*-toluenesulfonyl chloride

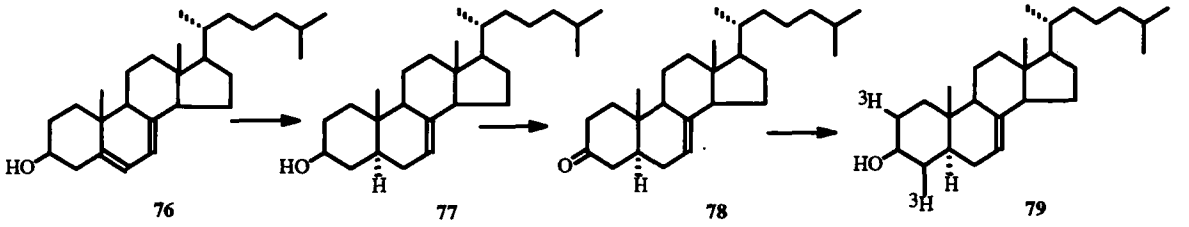
in pyridine into the tosylate, and the solvolysis of the latter in methanol in the presence of potassium acetate led to the methyl ether (**73**). The hydrogenation of this over a platinum catalyst in ethanol followed by regeneration of the 3β-hydroxy-Δ^5-grouping gave the desired compound (**74**). It must also be mentioned that an analogous synthesis of cholesterol from the acetate of compound (**69**) that can be used for the synthesis of labeled cholesterol has been published [29].



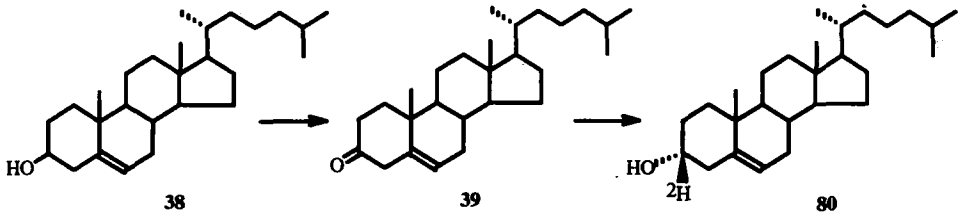
It has been shown [27] that [26-^{14}C]cholesterol (**74**) can be obtained from the 25-alcohol (**71**) by a simpler route. The interaction of compound (**71**) with phosphorus tribromide forms the 25-bromide (**75**). The successive hydrolysis of the 3β-acetoxy group in the latter under the action of caustic soda in methanol and hydrogenation over Raney nickel leads to the desired sterol (**74**).



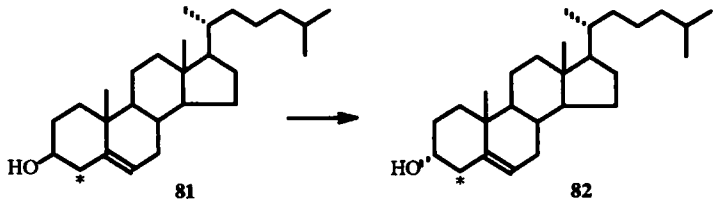
[2,4-$^3H_2$]Lathosterol (**79**) has been obtained from 7-dehydrocholesterol (**76**) [30]. 7-Dehydrocholesterol (**76**) was first hydrogenated over Raney nickel with the formation of lathosterol (**77**). Oxidation of the hydroxy group in the latter with chromium trioxide in pyridine led to the 3-ketone (**78**). When compound (**78**) was chromatographed on basic alumina that had first been treated with tritiated water, the corresponding [2,4-$^3H_2$]-3-ketone was formed, and the reduction of this with lithium tetrahydroaluminate in ether gave the labeled sterol (**79**).



A method of synthesizing [3β-^{2}H]-3-epicholesterol (**80**) has been proposed in [31]. The Jones oxidation of the initial cholesterol with chromium trioxide and sulfuric acid in acetone takes place with the formation of the Δ^5-3-ketone (**39**). The required deuterated sterol (**80**) is obtained from compound (**39**) by reduction with sodium tetradeuterioborate in ethanol.

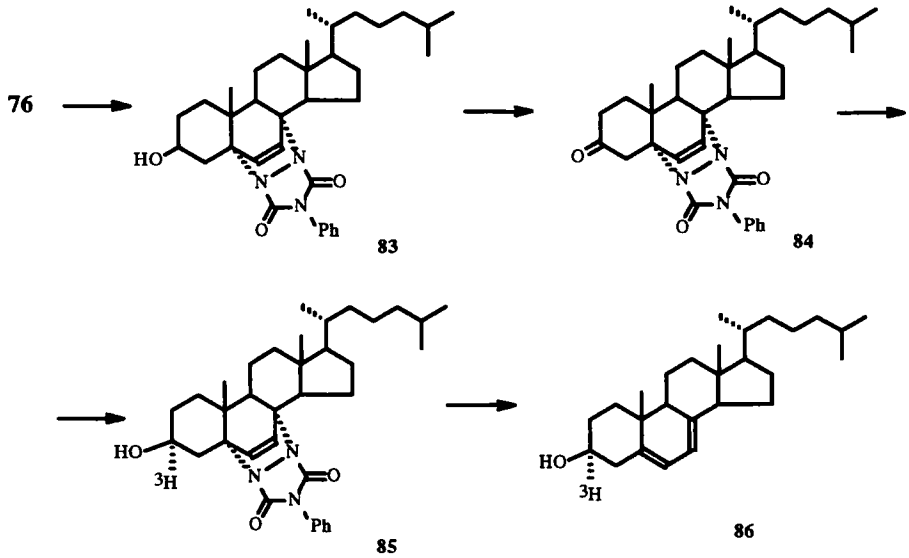


A method of converting [4-^{14}C]cholesterol (**81**) into [4-^{14}C]epicholesterol (**82**) with an overall yield of 40—53% has been described in [32]. The labeled sterol (**82**) is formed by the mesylation of the initial substance (**81**) with methanesulfonyl chloride in methylene chloride in the presence of triethylamine, the nucleophilic replacement of the mesyloxy group formed by an acetoxy group under the action of cesium acetate in toluene in the presence of 18-crown-6, and hydrolysis of the resulting 3α-acetate by a solution of potassium hydroxide in a mixture of methanol and tetrahydrofuran.



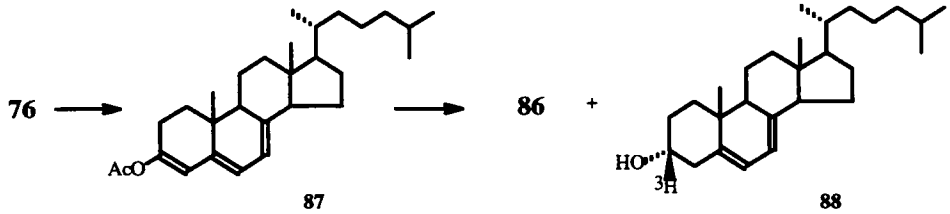
81 **82**

The synthesis of [3α-^{3}H]-7-dehydrocholesterol (**86**) from 7-dehydrocholesterol (**76**) is described in [33]. The interaction of sterol (**76**) with 4-phenyl-1,2,4-triazoline-3,5-dione in acetone gave the adduct (**83**) in 95% yield, and this was converted into the 3-ketone (**84**) by Jones oxidation in acetone. Reduction of ketone (**84**) with sodium tetratritioborate in methanol gave the labeled derivative (**85**), which was then reduced quantitatively with lithium tetrahydroaluminate in tetrahydrofuran to [3α-^{3}H]-7-dehydrocholesterol (**86**).



83 **84** **85** **86**

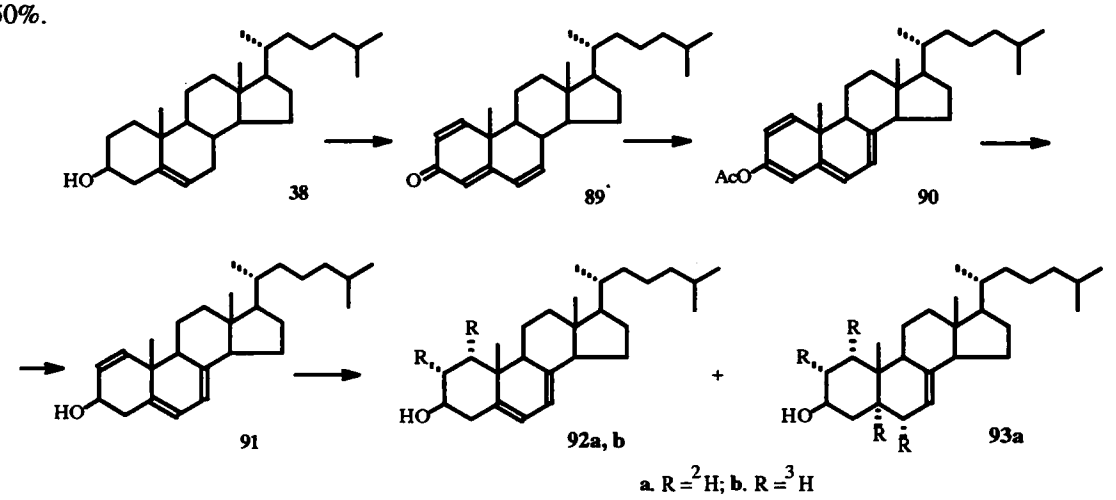
In the process of obtaining labeled vitamin D_3, Ray and Holick [34] also performed a synthesis of [3α-^{3}H]-7-dehydrocholesterol (**86**). First, the initial 7-dehydrocholesterol (**76**) was subjected to Oppenauer oxidation and the resulting 3-ketone was converted with acetic anhydride into the enol acetate (**87**). Reduction of the latter with sodium tetratritioborate led to the formation of the labeled sterol (**86**) and its [3β-^{3}H]-3α- isomer (**88**).



87 **88**

Several variants of the synthesis of 7-dehydrocholesterol labeled with deuterium and tritium in positions 1α and 2α (**92a** and **b**) have been investigated [35]. In the most successful of them, the first stage involved the interaction of cholesterol (**38**) with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone to give a 55% yield of the $\Delta^{1,4,6}$-trien-3-one (**89**), which was then converted by reaction with isopropenyl acetate in the presence of *p*-toluenesulfonic acid into the enol acetate (**90**) with a yield of 75%. Reduction of compound (**90**) with calcium tetrahydroborate took place with the exclusive formation of the 3β-alcohol (**91**) in a yield of 85%. The catalytic deuteration of the triene (**91**) with gaseous tritium in toluene in the presence of tris(triphenylphosphine)rhodium chloride gave [1α,2α-$^{2}H_2$]-7-dehydrocholesterol (**92a**) and [1α,2α,5α,6α-$^{2}H_4$]lathosterol (**93a**),

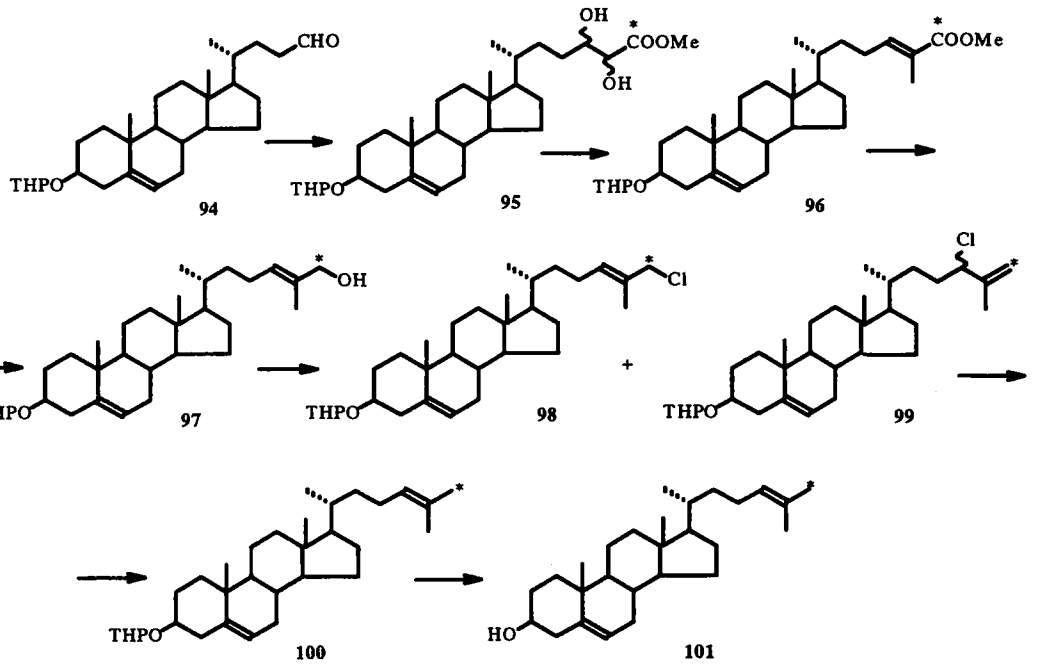
which were isolated from the reaction mixture with yields of 48 and 7%, respectively. In its turn, the tritiation of compound (**91**) with gaseous tritium under analogous conditions enabled [1α,2α-$^3H_2$]-7-dehydrocholesterol (**92b**) to be synthesized with a yield of 50%.



a. R = ^{2}H; b. R = ^{3}H

[26-^{13}C]Desmosterol (**101**) has been synthesized from the 24-aldehyde (**94**) [36]. First, the interaction of [1-^{13}C]propionic acid with lithium diisopropylamide in tetrahydrofuran was used to obtain a carbanion the addition of which at the carbonyl group of compound (**94**), followed by esterification with diazomethane, gave a mixture of diastereomeric hydroxyesters (**95**) with an overall yield of 78%. The mesylation of this mixture with methanesulfonyl chloride in methylene chloride in the presence of triethylamine and dimethylaminopyridine, with subsequent elimination of the resulting mesylate group under the action of 1,8-diazobicyclo[5.4.0]undec-7-ene in boiling benzene, led with an overall yield of 93% to the unsaturated ester (**96**).

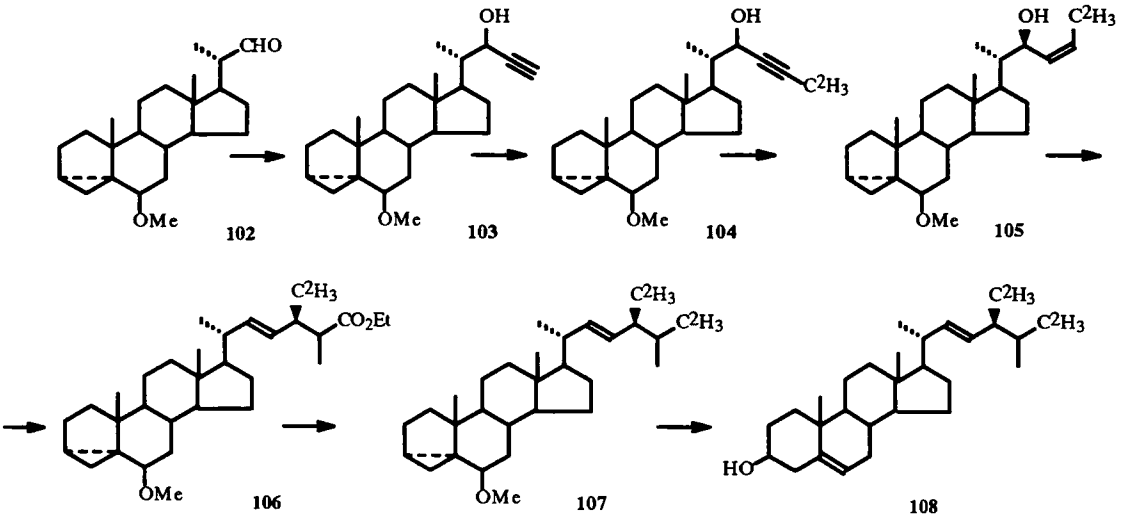
When the ester function in steroid (**96**) was reduced with diisobutylaluminum hydride in ether, the 26-alcohol (**97**) was obtained with a yield of 92%, and the interaction of this alcohol with thionyl chloride in pyridine—tetrahydrofuran gave a mixture (2:1) of the unsaturated chlorine derivatives (**98**) and (**99**). By reduction of the compounds (98) and (99) with lithium tetrahydroaluminate in ether the olefin (**100**) was synthesized with a yield of 51%, and the hydrolysis of its protective grouping with hydrochloric acid in a mixture of tetrahydrofuran and methanol led with a yield of 84% to the desired [26-^{13}C]desmosterol (**101**).



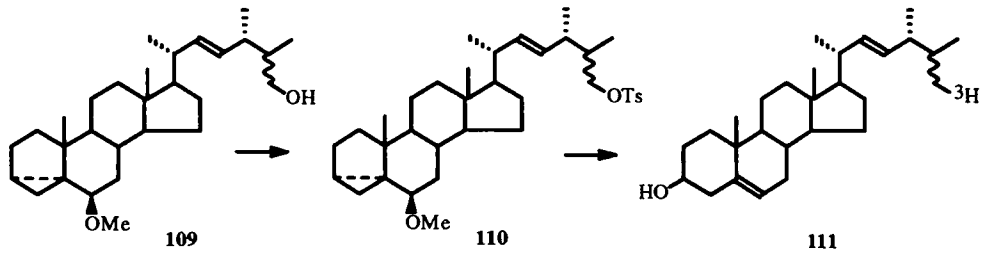
A stereoselective synthesis of [26,28-$^2H_6$]crinosterol (**108**) has been carried out with an overall yield of 50% from the 22-aldehyde (**102**) [37, 38]. The labeled crinosterol was then used for the synthesis of labeled brassinosteroids — castasterol

and brassinolide. As a result of the condensation of aldehyde (**102**) with lithium acetylide in tetrahydrofuran, the (22R)-22-alcohol (**103**) was obtained with a yield of 60.7%. By a reaction with triethylchlorosilane in pyridine, the 22-hydroxy group in compound (**103**) was protected in the form of a silyl ether, and then, by interaction with butyllithium in tetrahydrofuran, a lithium derivative was obtained the alkylation of which with [$^2H_3$]methyl iodide followed by elimination of the silyl protection under the action of tetrabutylammonium fluoride gave the (22R)-propargyl alcohol (**104**) with an overall yield of 95.6%.

Partial hydrogenation of the triple bond over Lindlar catalyst in ethyl acetate in the presence of quinoline led to the (22S,23S)-22-allyl alcohol (**105**) the orthoester Claisen rearrangement of which with triethyl orthopropionate in xylene in the presence of propionic acid enabled the ester (**106**) to be synthesized in an overall yield of 95.5% from the alcohol (**104**). On reduction of the ester function in compound (**106**) with lithium tetradeuterioaluminate in tetrahydrofuran, mesylation of the resulting 26-alcohol with methanesulfonyl chloride in pyridine, and reduction of the mesylate with lithium tetradeuterioaluminate in tetrahydrofuran, the hexadeutero derivative (**107**) was obtained in an overall yield of 96.2%. Hydrolysis of the protective groupings in rings *A* and *B* of the latter with aqueous dioxane in the presence of *p*-toluenesulfonic acid gave the desired labeled sterol (**108**).



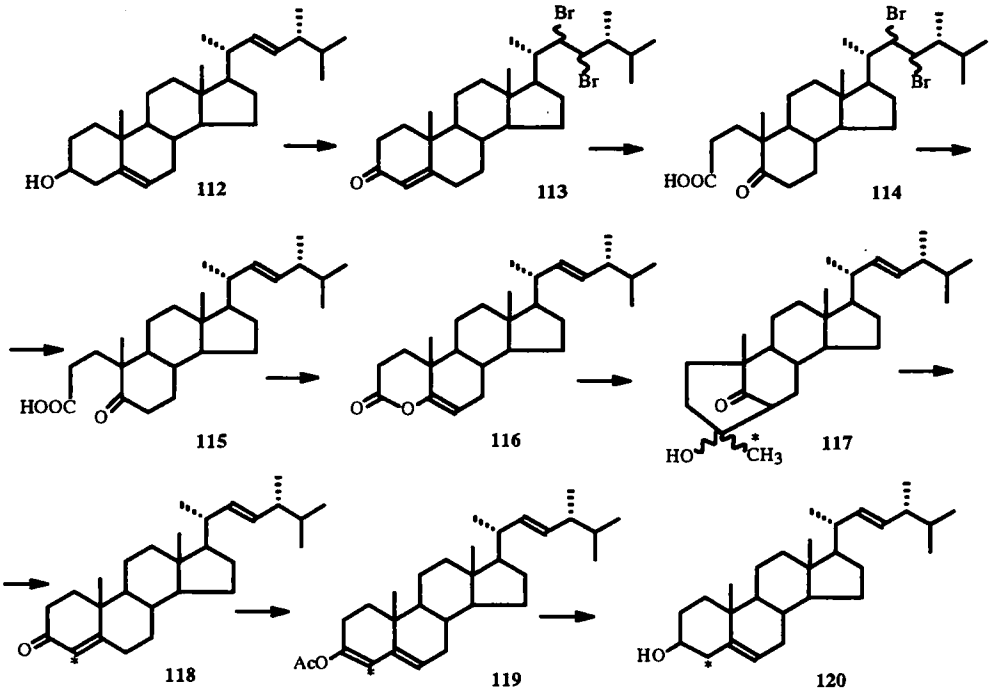
[26-^{3}H]Brassicasterol (**111**) has been obtained from the 26-alcohol (**109**) [39]. By the interaction of this alcohol with *p*-toluenesulfonyl chloride in pyridine the tosylate (**110**) was synthesized in the form of a mixture of isomers at C-25 with a yield of 76%. Reduction of the tosylate (**110**) with sodium tetratritioborate in dimethyl sulfoxide and subsequent hydrolysis of the 3α,5-cyclo-6β-methoxy group with aqueous dioxane in the presence of perchloric acid led to the desired labeled sterol (**111**). The synthesis of [26-^{3}H]crinosterol was performed analogously from the corresponding 26-alcohol.



The synthesis of a number of brassinosteroids labeled with the isotope ^{14}C has started from brassicasterol (**112**) [40]. Here, the first stage in the synthesis had the aim of introducing a radioactive label into the brassicasterol molecule to form [4-^{14}C]brassicasterol (**120**). The Oppenauer oxidation of brassicasterol (**112**) gave an 89% yield of a $\Delta^{4,22}$-3-ketone in which the 22-double bond was protected in the form of a dibromide with the production of steroid (**113**) in a yield of 67%. Ozonolysis of the 4-double bond in compound (**113**) and subsequent oxidative cleavage of the resulting ozonide with hydrogen peroxide led to the seco-acid (**114**), elimination of the dibromo grouping in which with zinc dust in acetic acid enabled the seco-acid (**115**) to be obtained with an overall yield of 49% from (**113**).

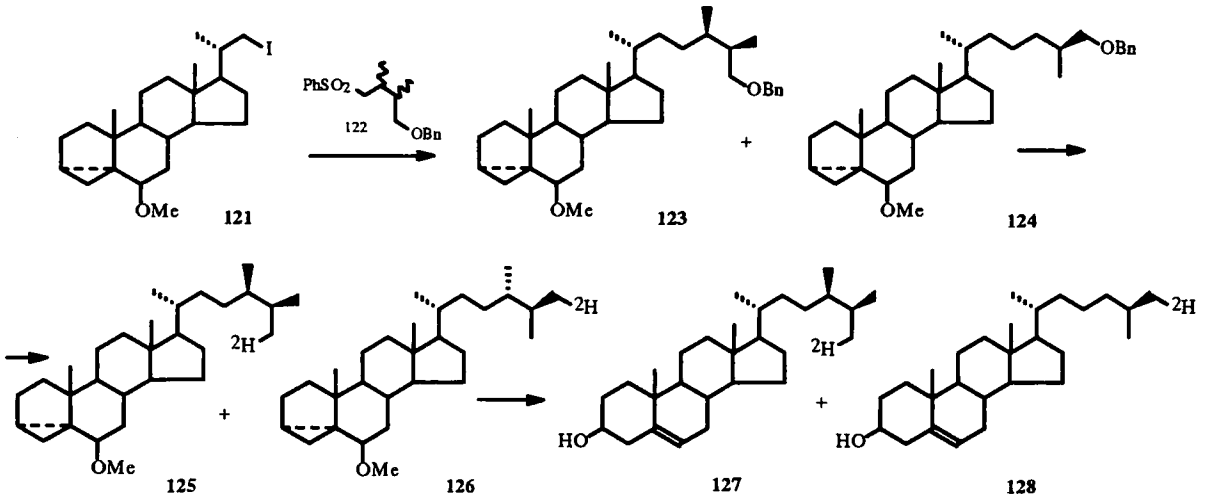
Interaction of the seco-acid (**115**) with acetic acid in the presence of sodium acetate gave an 80% yield of the enol lactone (**116**). Reaction of the lactone (**116**) with the Grignard reagent obtained from [^{14}C]methyl iodide and magnesium resulted in

the formation of the abeosterol (**117**) with a radiochemical yield of 76.1%. By the treatment of compound (**117**) with sodium hydroxide it was possible to obtain [4-^{14}C]brassicasterone (**118**) with a yield of about 90%. Then, by reaction with isopropenyl acetate in the presence of *p*-toluenesulfonic acid, the enone (**118**) was converted into the enol acetate (**119**), the reduction of which with sodium tetrahydroborate led to [4-^{14}C]brassicasterol (**120**).



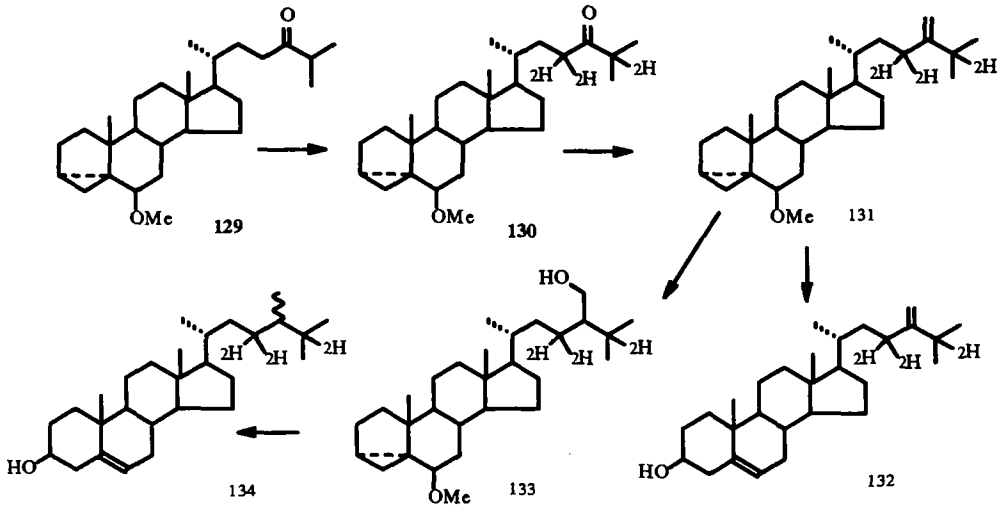
Colombo et al. [41] have achieved the synthesis of (25S)-[27-^{2}H]campesterol (**127**) and (25R)-[26-^{2}H]dihydrobrassicasterol (**128**). For this purpose, the sulfone (**122**) was first synthesized specially from meso-2,3-dimethylsuccinic acid. Reaction of the sulfone (**122**) with lithium diisopropylamide in tetrahydrofuran yielded an anion the alkylation with which of the 22-iodide (**121**) in a mixture of hexametapol and tetrahydrofuran, followed by elimination of the phenyl sulfone grouping with sodium amalgam in ethanol, led to the formation of a mixture of the ergostanes (**123** and (**124**).

Elimination of the benzyl protection in compounds (**123**) and (**124**) by hydrogenolysis over palladium on carbon, mesylation of the resulting 26-alcohols with methanesulfonyl chloride in pyridine, and reduction with lithium tetradeuterioaluminate in tetrahydrofuran led to the synthesis of a mixture of the deuterium derivatives (**125**) and (**126**). By hydrolysis with aqueous dioxane in the presence of *p*-toluenesulfonic acid, this mixture was converted into a mixture of the deuterated sterols (**127**) and (**128**).

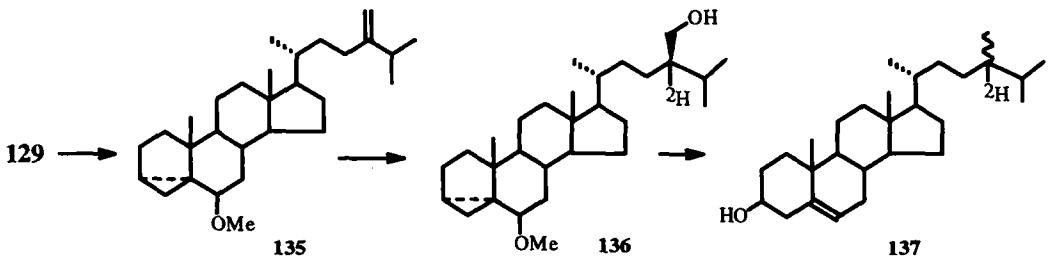


The synthesis of sterols (**132**), (**134**), and (**137**) labeled with deuterium in the side-chain started from the 24-ketone (**129**) [42]. On the interaction of steroid (**129**) with sodium in a mixture of heavy water and dioxane, deuterium exchange took place with the formation of the deuterated derivative (**130**). The Wittig condensation of the 24-ketone (**130**) with methylenetriphenylphosphorane led to the 24(28)-dehydrosteroid (**131**) the hydrolysis of which with aqueous dioxane in the presence of *p*-toluenesulfonic acid gave [23,23,25-$^2H_3$]-24-methylenecholesterol (**132**).

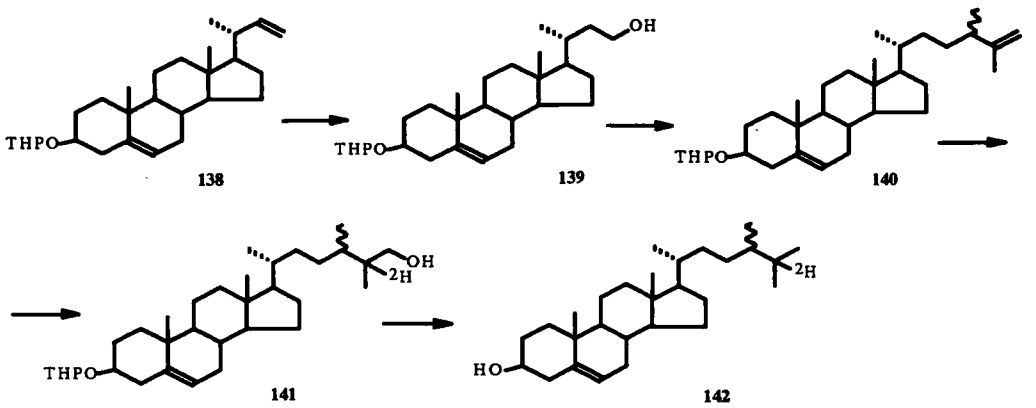
In its turn, the hydroboration—oxidation of the double bond in compound (**131**) with diborane, generated from sodium tetrahydroborate and boron trifluoride, gave the 28-alcohol (**133**). Subsequent mesylation of the alcohol (**133**) with methanesulfonyl chloride in pyridine, reduction of the mesylate with lithium tetrahydroaluminate in tetrahydrofuran, and hydrolysis of the protective groupings in rings *A* and *B* with *p*-toluenesulfonic acid in aqueous dioxane permitted the synthesis of [23,23,25-$^2H_3$]methylcholesterol (**134**).



As a result of the condensation of the 24-ketone (**129**) with methylenetriphenylphosphorane in tetrahydrofuran, the 24(28)-dehydrosteroid (**135**) was obtained with a yield of 45%. Hydroboration of the double bond in compound (**135**) with the diborane, generated by the reaction of sodium tetradeuterioborate with boron trifluoride, and subsequent oxidation with alkaline hydrogen peroxide led to the deuterated 28-alcohol (**136**) with a yield of 54%. Mesylation of the hydroxy group in the latter with methanesulfonyl chloride in pyridine, reduction of the resulting mesylate with lithium tetrahydroaluminate in tetrahydrofuran, and hydrolysis with *p*-toluenesulfonic acid in aqueous dioxane enabled [24-^{2}H]-24-methylcholesterol (**137**) to be synthesized with an overall yield of 30%.



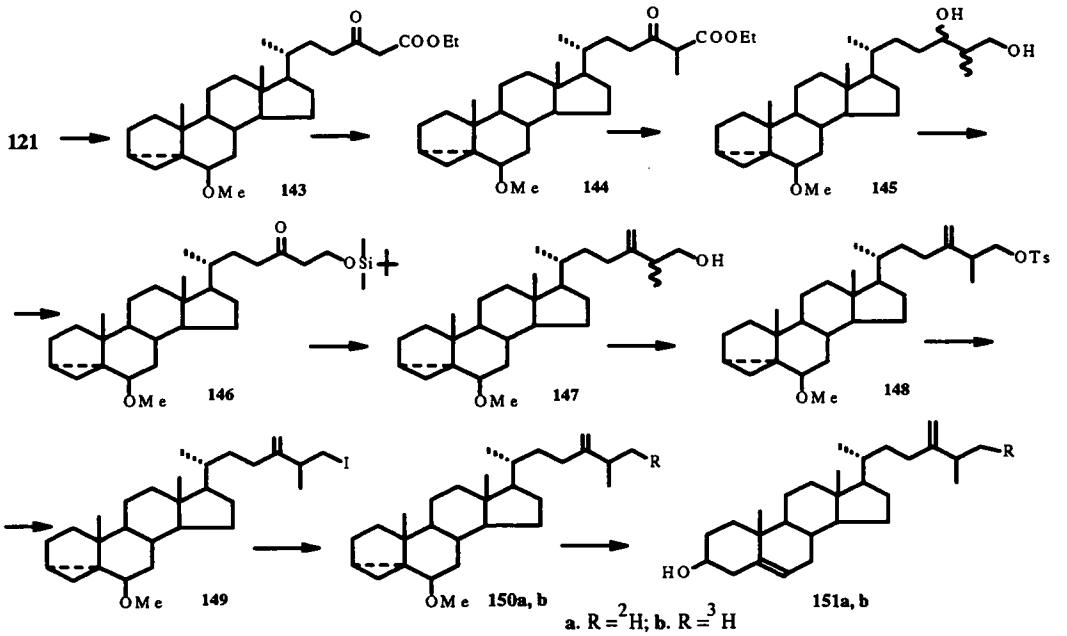
[25-^{2}H]-24ξ-Methylcholesterol (**142**) has been synthesized from 22(23)-dehydrosteroid (**138**) [42]. The hydroboration—oxidation of compound (**138**) with a complex of diborane and tetrahydrofuran led with yield of 91% to the 23-alcohol (**139**). On the tosylation of alcohol (**139**) with *p*-toluenesulfonyl chloride followed by reaction of the tosylate with 2-methylbut-2-enylmagnesium bromide in tetrahydrofuran, the $\Delta^{25(26)}$-ergostane derivative (**140**) was obtained with a yield of 23%. The hydroboration of the double bond in steroid (**140**) with deuterodiborane, obtained from sodium tetradeuterioborate and boron trifluoride etherate, in tetrahydrofuran and oxidation of the reaction product with alkaline hydrogen peroxide formed the 25-deuterated 26-alcohol (**141**) with a yield of 55%. Mesylation of the alcohol (**141**) with methanesulfonyl chloride, reduction of the mesylate with lithium tetrahydroaluminate, and hydrolysis of the protective tetrahydropyranyl grouping with *p*-toluenesulfonic acid in a mixture of methylene chloride and methanol led to the desired [25-^{2}H]-24ξ-methylcholesterol (**142**) with an overall yield of 73%.



[26-^{2}H]- and [26-^{3}H]-24-methylenecholesterol, (**151a**) and (**150b**), respectively, have been synthesized from the 22-iodide (**121**) [43]. When compound (**121**) was condensed with the dianion of acetoacetic ester in tetrahydrofuran, the ester (**143**) was obtained in a yield of 70%. The methylation of compound (**143**) with methyl iodide in tetrahydrofuran in the presence of potassium tert-butanolate led quantitatively to the ester (**144**). Reduction of the latter with lithium tetrahydroaluminate in tetrahydrofuran, silylation with tert-butyldimethylchlorosilane in dimethylformamide in the presence of imidazole of the primary 26-hydroxy group in the 24,26-diol (**145**) obtained, and oxidation of the still free 24-hydroxy group with pyridinium chlorochromate in dimethylformamide enabled the ketone (**146**) to be obtained with an overall yield of 62% from the ketoester (**144**).

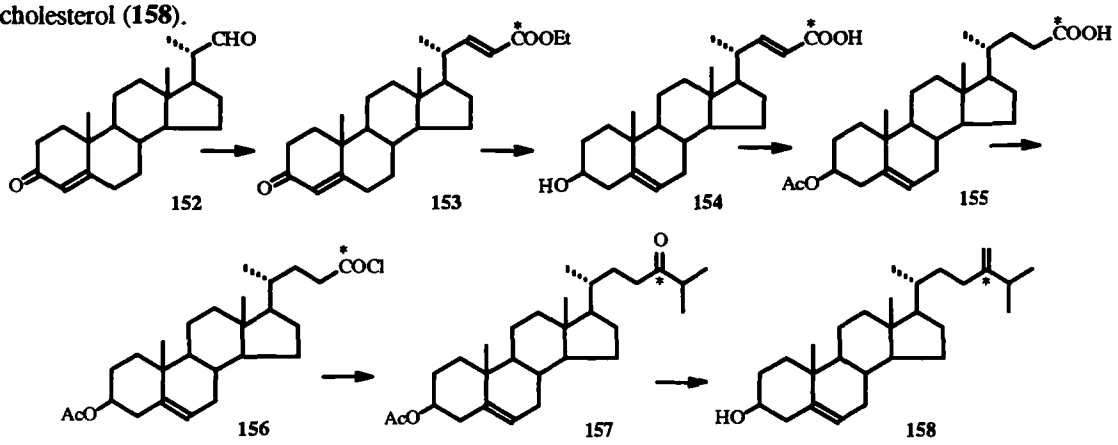
When ketone (**146**) was subjected to Wittig-Horner condensation with the phosphorus ylide obtained from methyltriphenylphosphonium iodide and butyllithium in tetrahydrofuran, followed by elimination of the silyl protection under the action of tetrabutylammonium fluoride in tetrahydrofuran, the unsaturated alcohol (**147**) was synthesized with an overall yield of 47%. Tosylation of the alcohol (**147**) with *p*-toluenesulfonyl chloride in pyridine led with a yield of 85% to the tosylate (**148**) the interaction of which with sodium iodide in acetone gave the 26-iodide quantitatively. A mixture of the initial substance and the deuterium derivative (**150a**) was obtained by reducing compound (**149**) with sodium tetradeuterioborate in dimethyl sulfoxide.

The further reduction of this mixture with lithium tetradeuterioaluminate in tetrahydrofuran led to the pure product (**150a**). The same substance was formed in a yield of 80% on the direct reduction of the tosylate (**148**) with lithium tetradeuterioaluminate in tetrahydrofuran. Isomerization of compound (**150a**) under the action of *p*-toluenesulfonic acid in aqueous dioxane led to the desired deuterated sterol (**151a**). In its turn, reduction of the iodide (**149**) with sodium tetratritioborate and then with sodium tetrahydroaluminate formed the tritium derivative (**150b**) the isomerization in rings *A* and *B* of which under conditions analogous to those described above enabled [26-^{3}H]-24-methylenecholesterol (**151b**) to be obtained.

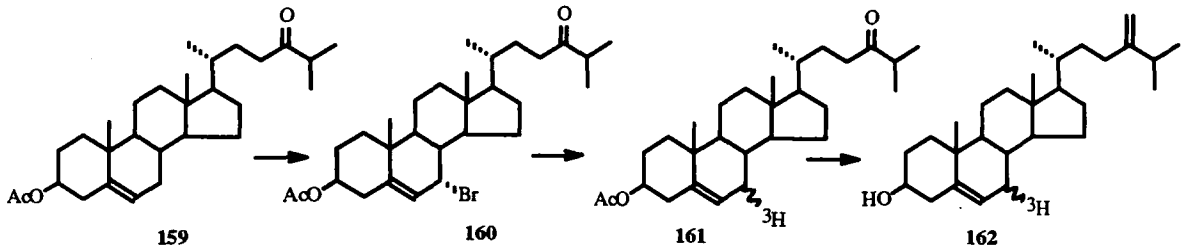


The synthesis of 24-methylenecholesterol specifically labeled with ^{14}C and ^{3}H described in [44] started from the 22-aldehyde (**152**). The reaction of compound (**152**) with ethoxy[^{14}C]carbonylmethylidenetriphenylphosphorane in acetonitrile gave a high yield of the (22E)- ester (**153**). Steroid (**153**) was then converted by reaction with acetyl chloride and acetic anhydride into an enol acetate the reduction of which with sodium tetrahydroborate in ethanol and hydrolysis of the ester grouping with sodium hydroxide gave the alcohol (**154**). Acetylation of the latter with acetic anhydride in pyridine and selective hydrogenation of the double bond in the side-chain over platinum in a mixture of dioxane and acetic acid led to the acid (**155**).

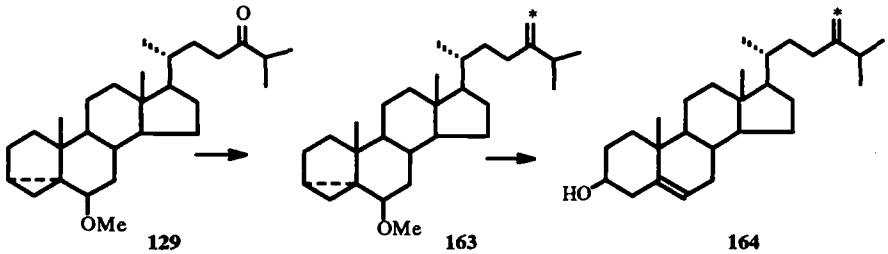
By its interaction with oxalyl chloride, the acid (**155**) was converted into the acid chloride (**156**), the reaction of which with isopropylmagnesium bromide in ether in the presence of cadmium chloride gave the labeled 24-ketone (**157**). Treatment of the latter with the complex of methylene bromide and titanium tetrachloride and zinc in methylene chloride, followed by hydrolysis of the acetate function with a methanolic solution of sodium hydroxide led to the desired [22-^{14}C]-24-methylenecholesterol (**158**).



The steroid (**159**) has been used to obtain [7-^{3}H]-24-methylenecholesterol (**162**) [44]. The allyl bromination of steroid (**159**) with N-bromosuccinimide in carbon tetrachloride led to the 7-bromide (**160**), which was then reduced with sodium cyanotritritioborate in ether in the presence of zinc chloride. In this process, partial reduction of the 24-keto group occurred. In order to regenerate it, therefore, the product was subjected to Jones oxidation, with the formation of the tritium derivative (**161**). The subsequent treatment of compound (**161**) with methylene bromide, titanium tetrachloride, and zinc in methylene chloride and hydrolysis of the acetoxy function with a methanolic solution of sodium hydroxide led to the desired labeled sterol (**162**).



[28-^{14}C]-24-Methylenecholesterol(**164**) has been obtained from the 24-ketone (**129**) [45]. The olefin (**163**) was formed by the Wittig reaction from the 24-ketone (**129**) and [^{13}C]methylenetriphenylphosphorane. The hydrolysis of this olefin with *p*-toluenesulfonic acid in aqueous dioxane led to the desired sterol (**164**) with a yield of 45%.

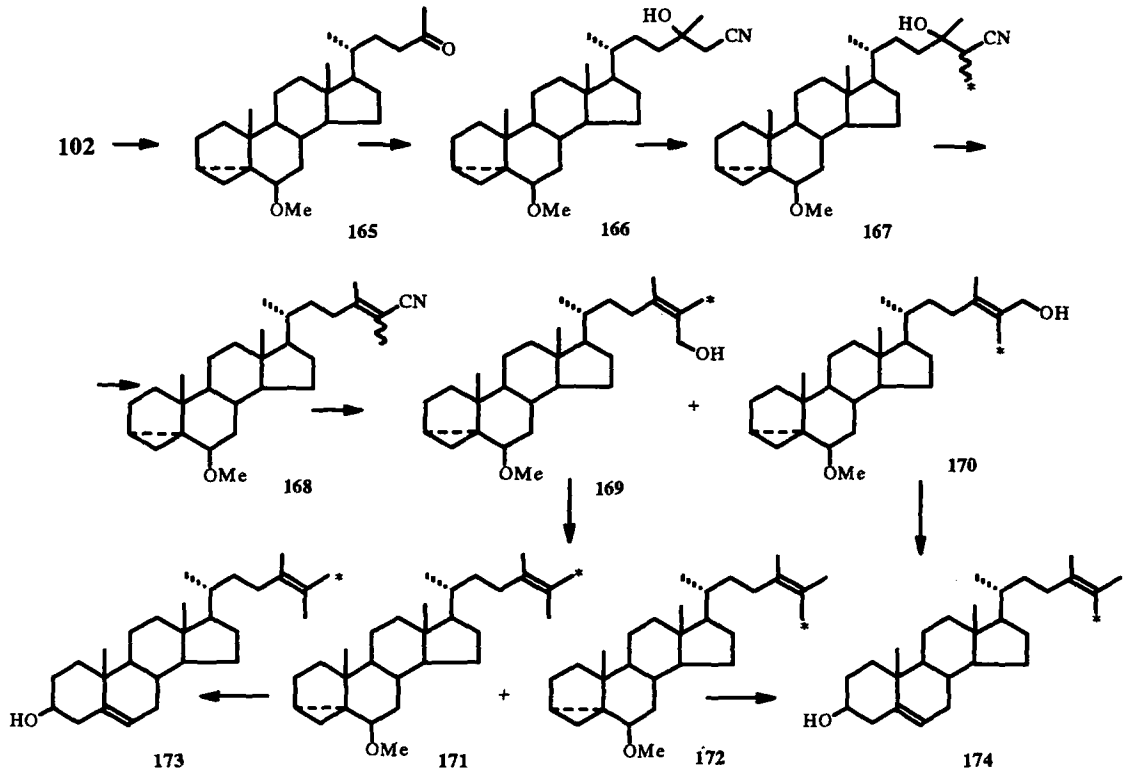


The synthesis of ^{13}C-labeled derivatives of 24-methyldesmosterol, (**173**) and (**174**), from the 22-aldehyde (**102**) has been described in [46].

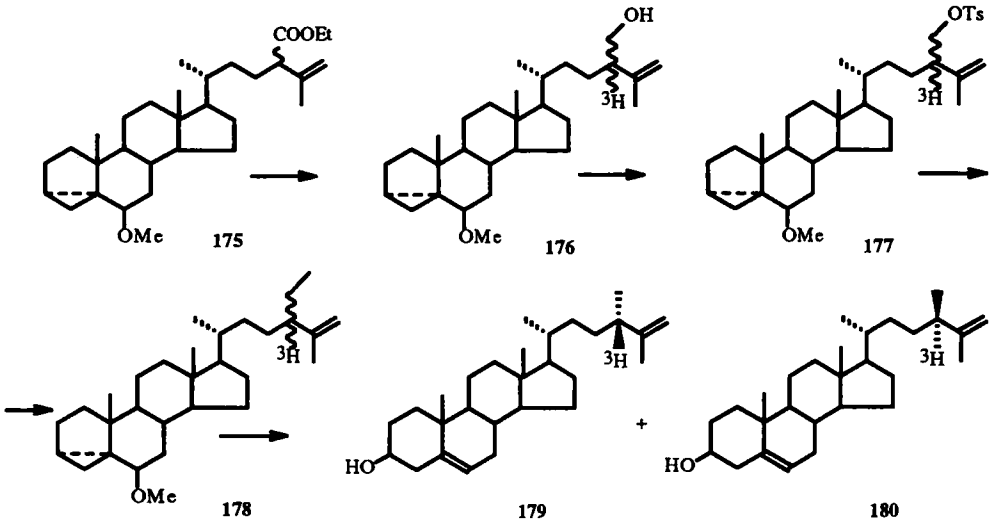
The reaction of acetone with lithium diisopropylamide produced an anion the addition of which to the carbonyl group of compound (**102**), followed by the mesylation of the resulting 22-alcohol with methanesulfonyl chloride in the presence of triethylamine, elimination of the mesylate function under the action of 1,5-diazobicyclo[5.4.0]undec-5-ene, and hydrogenation of the 22-double bond over palladium on carbon enabled the 24-ketone (**165**) to be obtained in an overall yield of 50%. The subsequent interaction of compound (**165**) with acetonitrile in the presence of lithium diisopropylamide led with a yield of 91% to the hydroxynitrile (**166**).

The reaction of steroid (**166**) with lithium diisopropylamide produced an anion the alkylation of which with [^{13}C]methyl iodide gave the labeled derivative (**167**) with a yield of 41%. The dehydration of alcohol (**167**) under the action of thionyl chloride formed the unsaturated nitrile (**168**) quantitatively, and this was then reduced with diisobutylaluminum hydride to the alcohols (**169**) and (**170**), isolated with yields of 19 and 25%, respectively.

By reaction with methanesulfonyl chloride, the alcohols (**169**) and (**170**) formed mesylates the reduction of which with lithium tetrahydroaluminate led with a yield of 70% to the deoxy derivatives (**171**) and (**172**), respectively. Hydrolysis of the protective groupings in rings *A* and *B* under the action of *p*-toluenesulfonic acid enabled the corresponding labeled compounds (**173**) and (**174**) to be obtained with yield of 80%.



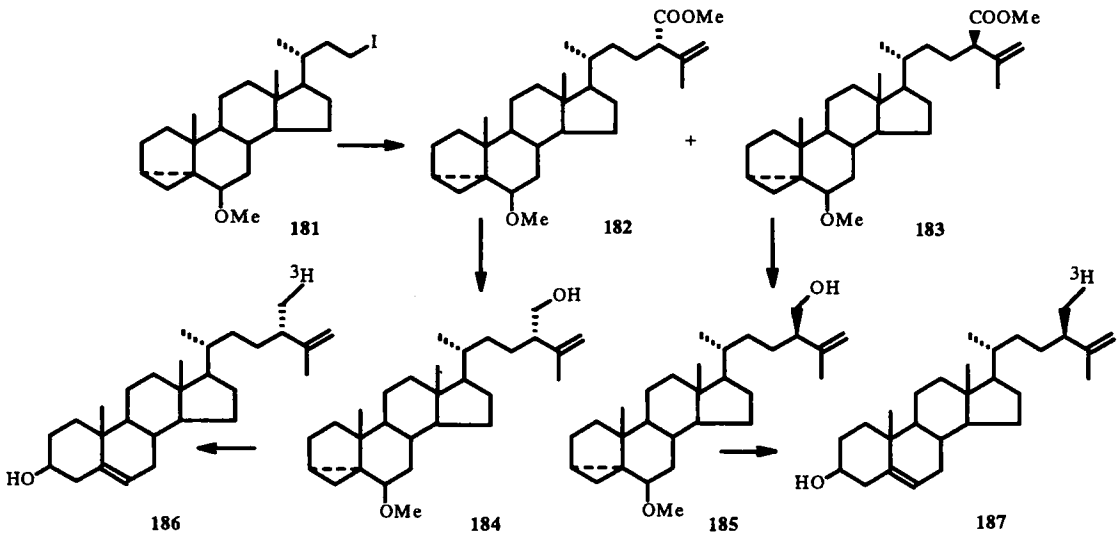
The tritium-labeled [24-^3H]codisterol (**179**) and [24-^3H]epicodisterol (**180**) have been synthesized from the ester (**175**) [47]. The reaction of compound (**175**) with lithium diisopropylamide in tetrahydrofuran followed by treatment of the resulting anion with tritiated water and reduction of the ester function with lithium tetrahydroaluminate led with an overall yield of 92% to the [24-^3H]-28-alcohol (**176**). Tosylation of the alcohol (**176**) with *p*-toluenesulfonyl chloride in pyridine gave with a yield of 80% the tosylate (**177**), by the reduction of which with lithium tetrahydroaluminate in ether an 87% yield of the tritiated derivative (**178**) was obtained. Hydrolysis of the latter by the action of *p*-toluenesulfonic acid in aqueous dioxane led to the formation in 81% overall yield of a mixture (1:1) of codisterol (**179**) and epicodisterol (**180**).



Silva and Djerassi [48] undertook the synthesis of [28-^{3}H]codisterol (**186**) and [28-^{3}H]epicodisterol (**187**) from the 23-iodide (**181**). The interaction methyl 3-methylbut-2-enoate with lithium diisopropylamide in a mixture of tetrahydrofuran and hexametapol formed a carbanion the alkylation of which with the iodide (**181**) led to the formation of the esters (**182**) and (**183**), isolated with yields of 37 and 55%, respectively. The reduction of esters (**182**) and (**183**) with lithium tetrahydroaluminate in tetrahydrofuran enabled the synthesis of the alcohols (**184**) and (**185**), respectively, with yields of 95%.

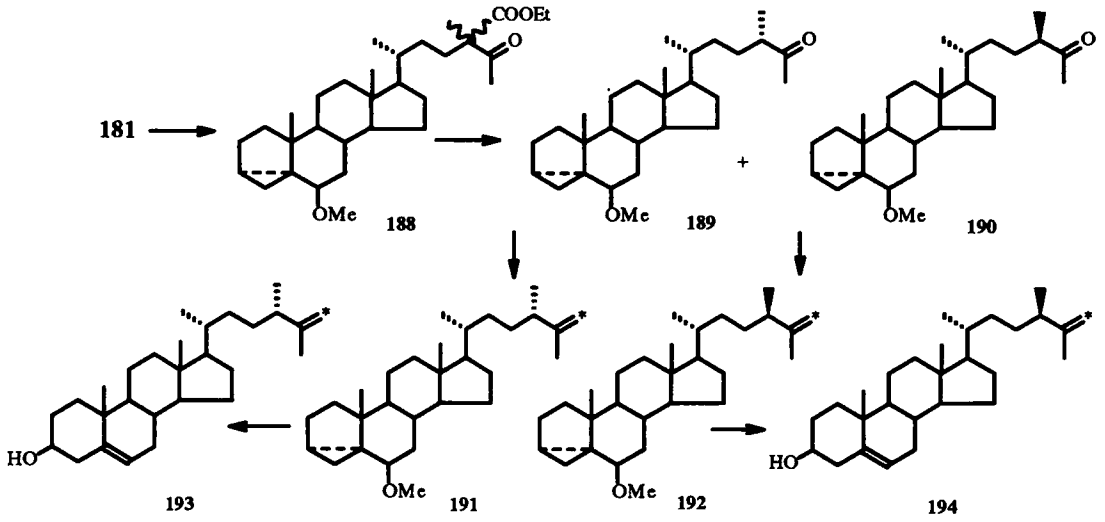
Oxidation of the hydroxy group in compound (**184**) with pyridinium chlorochromate in methylene chloride, reduction of the resulting aldehyde with sodium tetratritioborate in ethanol, mesylation of the alcohol with methanesulfonyl chloride in methylene chloride, reduction with lithium tetrahydroaluminate, and hydrolysis of the protective grouping in rings *A* and *B* with *p*-toluenesulfonic acid in aqueous dioxane led with an overall yield of 32% to [28-^{3}H]codisterol (**186**).

[28-^{3}H]Epicodisterol (**187**) was obtained analogously from the alcohol (**185**). These labeled derivatives are useful for the study of the metabolism of codisterol, which has been isolated from the green alga *Codium fragile* [49].

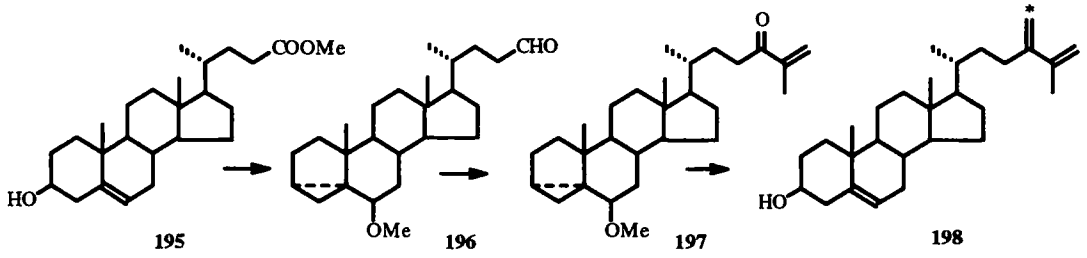


[26-^{14}C]Codisterol (**193**) and [26-^{14}C]epicodisterol (**194**) have been synthesized from the 23-iodide (**181**) [45]. When this iodide was condensed with ethyl 2-methylacetylacetate, the ketoester (**188**) was obtained in a yield of 71%. Hydrolysis of the ester function followed by decarboxylation, taking place on the interaction of compound (**188**) with potassium hydroxide in ethanol, led with an overall yield of 88% to a mixture (1:1) of the 25-keto derivatives (**189**) and (**190**).

Compounds (**189**) and (**190**) were then subjected to the Wittig-Horner reaction with [^{14}C]methyltriphenylphosphonium iodide and *n*-butyllithium. The labeled steroids (**191**) and (**192**) were obtained quantitatively and were separated by high-performance liquid chromatography. The required sterols (**193**) and (**194**) were synthesized by the hydrolysis of compounds (**191**) and (**192**) with aqueous dioxane in the presence of *p*-toluenesulfonic acid.



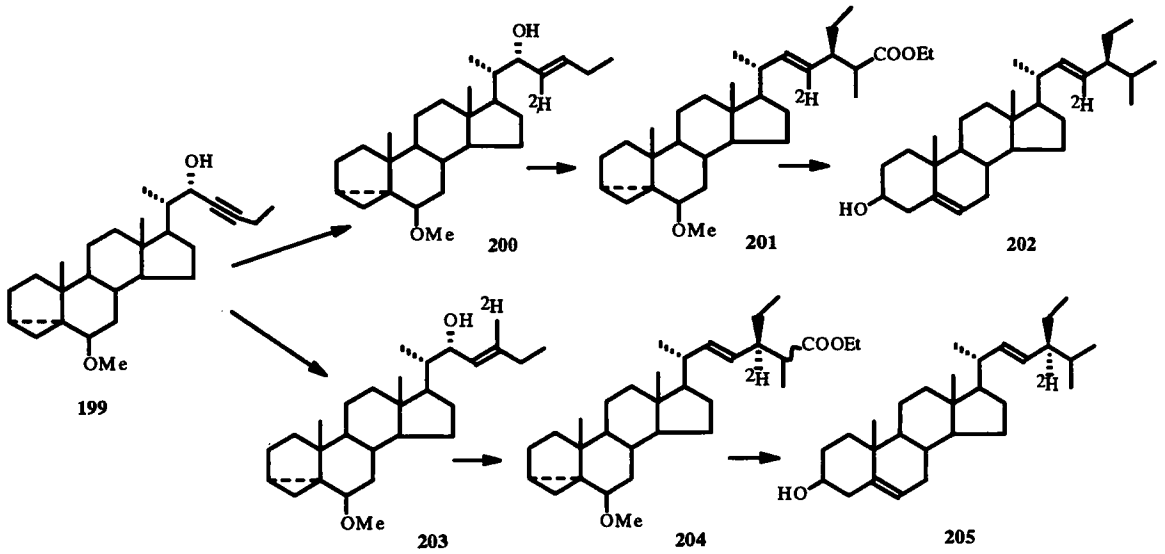
[28-^{14}C]Ergosta-5,24(28),25-trien-3β-ol (**198**) has been synthesized from methyl cholenate (**195**), which was first converted by a known method into the 24-aldehyde (**196**) [50]. Then the Grignard reagent obtained by the reaction of 2-bromopropene with magnesium in ether was added to the keto group of steroid (**196**) and subsequent oxidation with the Collins reagent led to the 24-ketone (**197**) with an overall yield of 34%. The Wittig-Horner condensation of compound (**197**) with [^{14}C H_3]methyltriphenylphosphonium iodide in ether in the presence of *n*-butyllithium followed by hydrolysis of the protective grouping in rings *A* and *B* under the action of *p*-toluenesulfonic acid in aqueous dioxane gave the required sterol (**198**).



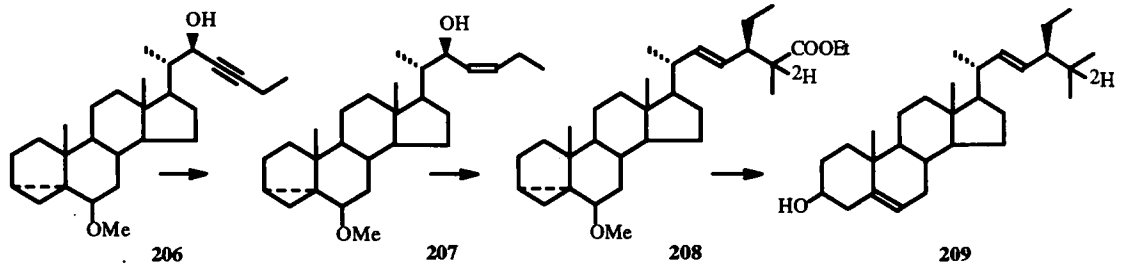
A number of selectively deuterated stigmasterol derivatives have been obtained by Fujimoto et al. [51]. Thus, the [23-^{2}H]-(22R)-allyl alcohol (**200**) was obtained in 64% yield by the reduction of the (22S)-propargyl alcohol (**199**) with lithium tetradeuterioaluminate in the presence of sodium ethanolate followed by treatment of the reaction mixture with water. As a result of an orthoester Claisen rearrangement taking place on the interaction of the alcohol (**200**) with triethyl propionate in the presence of propionic acid, the [23-^{2}H]-(24R)- ester (**201**) was obtained quantitatively in the form of a mixture of isomers at C-25.

The successive reduction of the ester (**201**) with lithium tetrahydroaluminate, tosylation of the resulting 26-alcohol, reduction of the tosylate with lithium tetrahydroaluminate, and hydrolysis of the *i*-steroid grouping in rings *A* and *B* in the presence of *p*-toluenesulfonic acid gave [23-^{2}H]stigmasterol (**202**) with an overall yield of 54%. Reduction of alcohol (**199**) with lithium tetrahydroaluminate in the presence of potassium methanolate followed by treatment of the reaction mixture with deuterated water enabled the [24-^{2}H]-(22R)-allyl alcohol (**203**) to be obtained with a yield of 54%.

The ester (**204**) was synthesized from the alcohol (**203**) by means of a Claisen rearrangement, and it was converted into [24-^{2}H]stigmasterol (**205**) by the sequence of reactions described above.



When the (22R)-propargyl alcohol (**206**) was hydrogenated over a Lindlar catalyst in the presence of quinoline the (22S)-allyl alcohol was formed quantitatively. By means of a Claisen rearrangement, compound (**207**) gave a 70% yield of the corresponding ester, the interaction of which with lithium diisopropylamide followed by treatment with deuterated water led to the 25-deuterated derivative (**208**). [25-^{2}H]Stigmasterol (**209**) was obtained from compound (**208**) by the method described above.

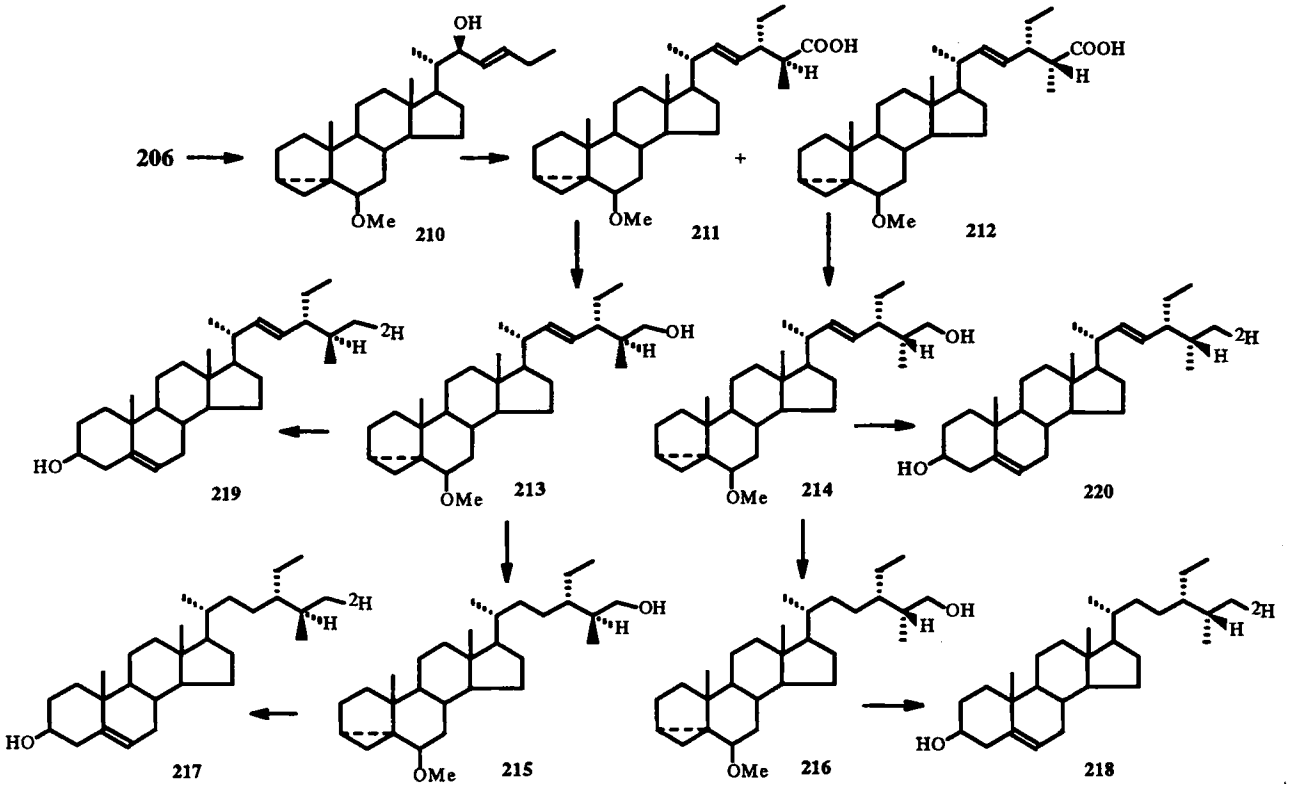


A synthesis of a number of 24-ethylsterols selectively labeled at C-6 has been described in [52]. The initial substance in this synthesis was stigmasterol, from which the (22R)-22-propargyl alcohol (**206**) and its (22S)- isomer (**199**) were obtained. The triple bond in compound (**206**) was reduced with lithium in a mixture of liquid ammonia and tetrahydrofuran with a yield of 84% to the (22S,23E)-allyl alcohol (**210**). The interaction of alcohol (**210**) with propionic anhydride in pyridine in the presence of 4-dimethylaminopyridine gave a quantitative yield of the 22-propionate, and the reaction of this with lithium diisopropylamide in tetrahydrofuran led to a mixture of the E- and Z-enolates with the former predominating, and this was then converted by reaction with tert-butyldimethylchlorosilane in a mixture of tetrahydrofuran and hexametapol into silyl enol ethers. Elimination of the silyl protection with tetrabutylammonium fluoride in tetrahydrofuran was accompanied by an allyl Claisen rearrangement with the formation of a mixture (9:1) of the acids (**211**) and (**212**) in an overall yield of 92%. By recrystallizing this mixture, the pure acid (**211**) was isolated in a yield of 58%. In its turn, a reaction of the propionate of alcohol (**210**) with lithium diisopropylamide in a mixture of hexametapol and tetrahydrofuran took place with the formation of E- and Z-enolates in which the latter predominated. From this mixture, via the silyl ethers, a mixture (1:4) of the acids (**211**) and (**212**) was obtained with an overall yield of 90%. Methylation of the acid (**211**) with diazomethane in ether took place with the quantitative formation of the methyl ester, the reduction of which with lithium tetrahydroaluminate in a mixture of ether and tetrahydrofuran gave the 26-alcohol (**213**) quantitatively.

Analogously a mixture (22:78) of the alcohols (**213**) and (**214**) was obtained from the mixture of acids (**211**) and (**212**) in which the latter predominated. The pure alcohol (**214**) was obtained from this mixture in an overall yield of 50% by preparative high-performance liquid chromatography.

When compound (**213**) was hydrogenated over 10% palladium on carbon in ethyl acetate the 22(23)-dihydro derivative (**215**) was obtained with a yield of 95%. In its turn, the analogous hydrogenation of the double bond in compound (**214**) took place with the formation of the saturated derivative (**216**) in 91% yield. The tosylation of alcohol (**215**) with *p*-toluenesulfonyl chloride in pyridine gave a 95% yield of the corresponding 26-tosylate, and the quantitative reduction of this with lithium

tetradeuterioaluminate in ether, followed by regeneration with an 87% yield of the 3β-hydroxy-Δ^5- grouping by heating in aqueous dioxane in the presence of *p*-toluenesulfonic acid gave [26-^{2}H]-(25R)-clionasterol (**217**).

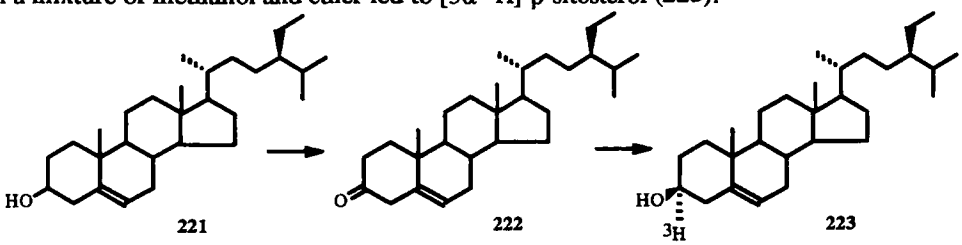


[26-^{2}H]-(25S)-clionasterol (**218**) was synthesized from the alcohol (**216**) analogously. In addition, [26-^{2}H]-(25R)-poriferasterol (**219**) and [26-^{2}H]-(25S)-poriferasterol (**220**) were synthesized from alcohols (**213**) and (**214**) by the same route. It must also be mentioned that synthesis of [26-^{2}H]-(25S)-sitosterol, [26-^{2}H]-(25R)-sitosterol, [26-^{2}H]-(25S)-stigmasterol, and [26-^{2}H]-(25R)-stigmasterol have also been achieved from the (22S)-alcohol (**199**).

There is also a report [53] of syntheses of β-sitosterol, stigmasterol, clionasterol, and poriferasterol labeled with deuterium at C-25. The starting material selected for these syntheses was the 22-aldehyde (**102**), and the formation of the corresponding side-chains was achieved by condensing this aldehyde with the appropriate deuterated sulfones.

A method has been proposed [54] for obtaining a number of sterols — stigmasterol, β-sitosterol, campesterol, and fucosterol — labeled with tritium in positions 2 and 4. The scheme of synthesis includes oxidation of the sterols to Δ^4-3-ketones, isotope exchange reactions of the resulting compounds in a chromatographic column with alumina containing tritiated water, and conversion of the labeled compounds so obtained into the corresponding enol acetates, followed by reduction with sodium tetrahydroborate.

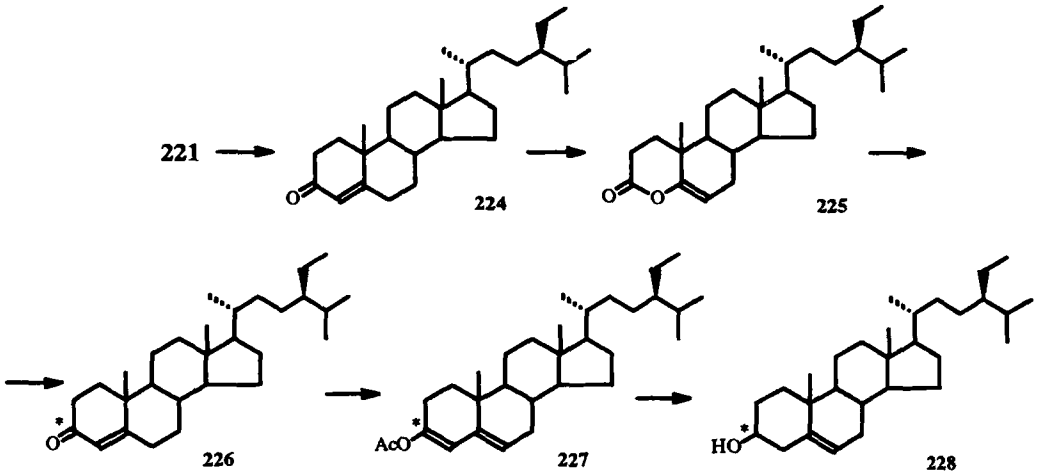
A method has been developed for obtaining [3α-^{3}H]-β-sitosterol (**223**) [55]. The oxidation of β-sitosterol (**221**) with the Jones reagent in acetone for 4 min gave the Δ^4-3-ketone (**222**). The reduction of this compound with sodium tetratritioborate in a mixture of methanol and ether led to [3α-^{3}H]-β-sitosterol (**223**).



Otto et al. [56] have carried out the synthesis of [3-^{14}C]-β-sitosterol (**228**) with a radiochemical purity of more than 98%. Oppenauer oxidation of the initial β-sitosterol (**221**) gave a 71% yield of the Δ^4-3-ketone (**224**). The ozonization of compound (**224**) in a mixture of ethyl acetate and acetic acid, followed by oxidation of the ozonization product with hydrogen peroxide and

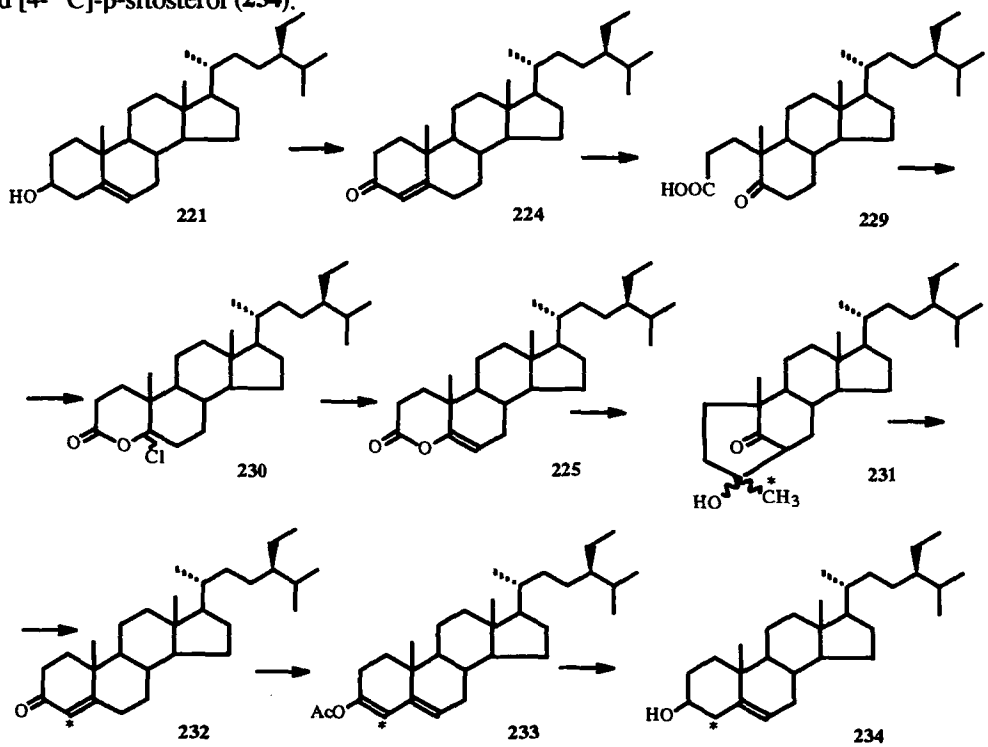
cyclization of the resulting ketoacid by the successive action of oxalyl chloride and acetic anhydride, led to the enol lactone (225) with an overall yield of 55%.

The Δ^4-3-ketone labeled at C-3 (226) has been synthesized by condensing steroid (225) with phenyl [1-^{14}C]acetate in benzene in the presence of sodium hydride. This ketone (226) was converted by reaction with isopropenyl acetate into the enol acetate (227), the reduction of which with sodium tetrahydroborate in aqueous methanol led to the labeled β-sitosterol (228).



The synthesis of [4-^{14}C]-β-sitosterol (234) was first described in [57]. The Δ^4-3-ketone (224) was obtained by the Oppenauer oxidation of the initial β-sitosterol as a result of boiling it in acetone in the presence of aluminum isopropanolate for ten days. By ozonization of the double bond in steroid (224) in a mixture of acetic acid and ethyl acetate followed by the oxidative cleavage with hydrogen peroxide of the ozonide formed it was possible to synthesize the ketoacid (229) with an overall yield of 46%. The cyclization of compound (229) under the action of oxalyl chloride in benzene gave a 33% yield of the chlorolactone (230), and this was boiled with acetic anhydride to give the enol lactone (225).

A reaction of the enol lactone (225) with [^{14}C]methylmagnesium iodide in ether led to the quantitative formation of the labeled hydroxyketone (231) the subsequent interaction of which with an aqueous-methanolic solution of potassium hydroxide gave a 78% yield of the [4-^{14}C]-Δ^4-3-ketone (232). By reaction with isopropenyl acetate in the presence of *p*-toluenesulfonic acid, compound (232) was converted into the enol acetate (233) the reduction of which with sodium tetrahydroborate in ethanol gave the required [4-^{14}C]-β-sitosterol (234).

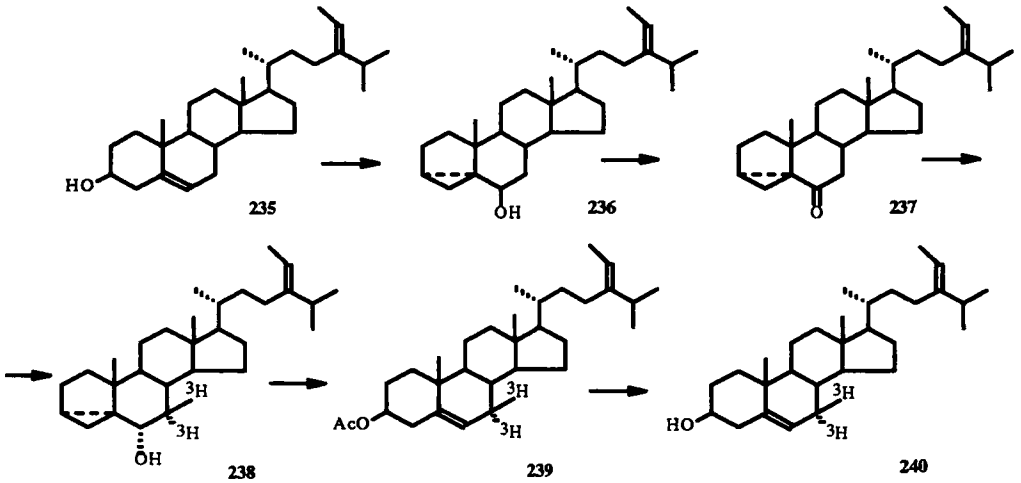


Later, another variant of the synthesis of [4-^{14}C]-β-sitosterol (**234**) was proposed [58]. According to this, the initial β-sitosterol was first oxidized by the Oppenauer method with a yield of 70% to the Δ^4-3-ketone (**224**) the further oxidation of which with periodate and potassium permanganate in aqueous tert-butanol led with a yield of 53% to the ketoacid (**229**). Successive reactions of the ketoacid (**229**), first with oxalyl chloride and then with acetic anhydride, gave an 80% yield of the enol lactone (**225**).

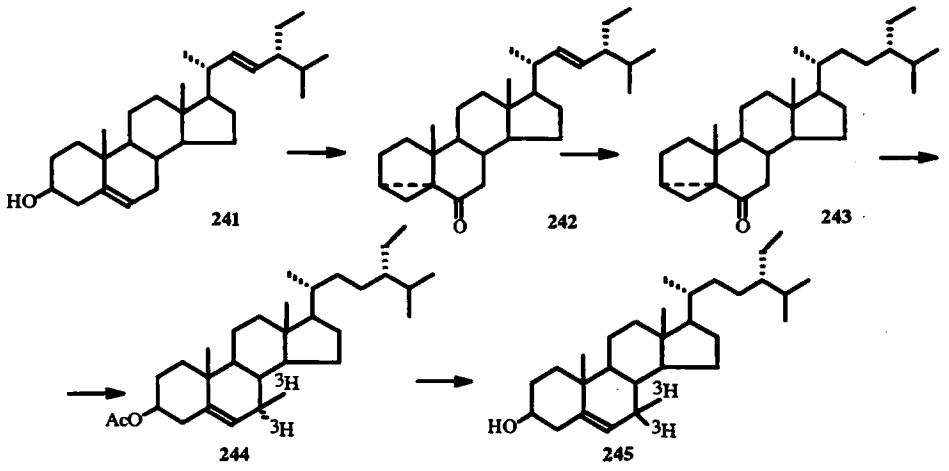
The interaction of compound (**225**) with [^{14}C]methylmagnesium iodide in ether took place with the formation of [4-^{14}C]stigmast-4-en-3-one (**232**), which, without additional purification, was converted by reaction with acetyl chloride and acetic anhydride into the enol acetate (**233**). Reduction of compound (**233**) with sodium tetrahydroborate in aqueous acetone gave the required labeled β-sitosterol (**234**), which was purified via the digitonide with the formation of a product having a specific activity of 302 MBq/g (8.2 mCi/g). The overall radiochemical yield of sterol (**234**) amounted to 10.2%.

A scheme for the synthesis of [7,7-$^3H_2$]fucosterol (**240**) and [7,7-$^3H_2$]isofucosterol has been proposed that uses the corresponding unlabeled sterols as the starting compounds [59, 60]. Thus, for example, fucosterol (**235**), isolated from *Fucus spiralis*, was first converted into the tosylate, and the subsequent solvolysis of this under the action of potassium acetate in aqueous acetone led to the 3α,5-cyclo-6β-alcohol (**236**). The Jones oxidation of the latter gave the 6-ketone (**237**) the further treatment of which with tritiated water and reduction with lithium tetrahydroaluminate enabled the labeled 6α-alcohol (**238**) to be obtained.

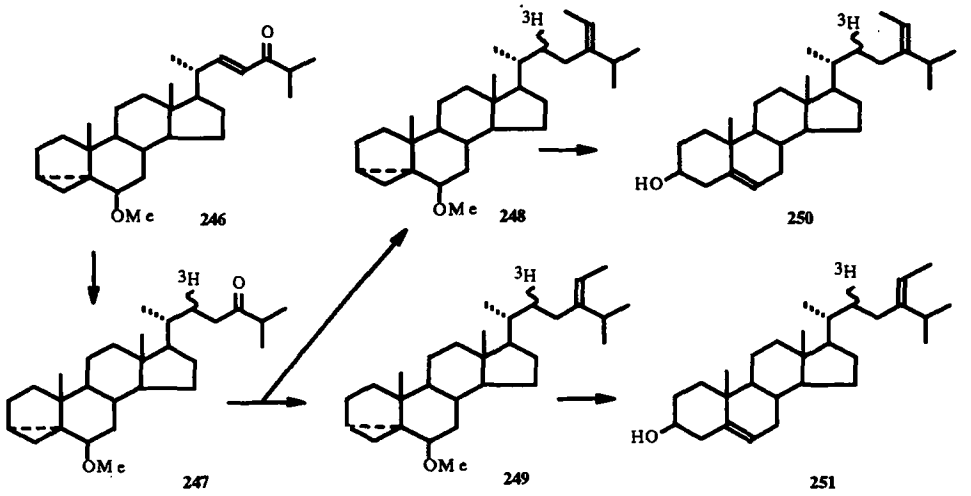
The isomerization of compound (**238**) under the action of zinc acetate in acetic acid took place with the formation of the 3β-acetate (**239**), the saponification which gave the required [7,7-$^3H_2$]isofucosterol (**240**) analogously.



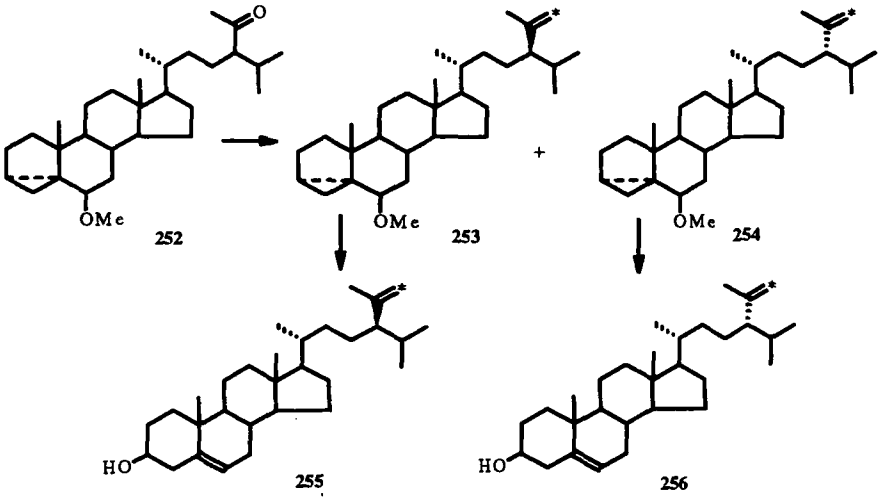
This method [59, 60] was used subsequently [61] for the synthesis of [7,7-$^3H_2$]clionasterol (**245**). According to the scheme of synthesis, poriferasterol (**241**) was first converted into the Δ^{22}-6-ketone (**242**), hydrogenation of the 22-double bond in which over palladium on carbon gave the 6-ketone (**243**). By the introduction of tritium atoms in the α-position to the 6-keto group, its subsequent reduction, and the isomerization of the resulting 6-alcohol under the action of zinc acetate in acetic acid it was possible to obtain the acetate (**244**). Hydrolysis of the acetoxy group in compound (**244**) led to the desired labeled clionasterol (**245**).



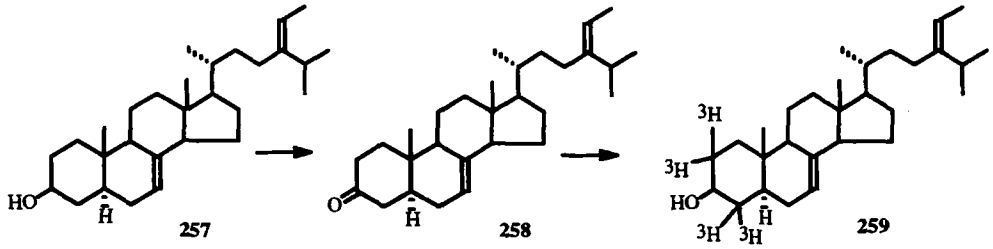
For the synthesis of the labeled [22-^{3}H]fucosterol (**250**) and [22-^{3}H]isofucosterol the initial compound used was the Δ^{22}-24-ketone (**246**) [62]. Its reduction with sodium tetratritioborate in pyridine gave a 28.1% yield, calculated on the initial steroid, or 8.12% calculated on the sodium tetratritioborate, of the tritium derivative (**247**). The Wittig-Horner interaction of the ketone (**247**) with ethyltriphenylphosphonium iodide and *n*-butyllithium led to the formation with a total yield of 47% of the steroids (**248**) and (**249**) in a ratio of 24:76. Without separation, this mixture was hydrolyzed with aqueous dioxane in the presence of *p*-toluenesulfonic acid, and, after chromatographic separation, the required labeled sterols (**250**) and (**251**) were obtained.



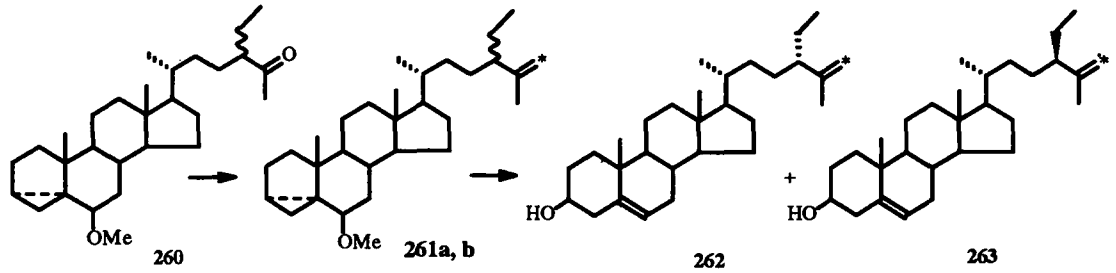
In the same paper [62], in addition, the synthesis of [30-^{14}C]-(24S)-isopropenylcholesterol (**256**) and its (24R)-stereoisomer (**255**) is described. The introduction of the radioactive label at C-30 was achieved by the Wittig reaction of ketone (**252**) with [^{13}C]methyltriphenylphosphonium iodide. The resulting *i*-methyl ethers (**253**) and (**254**) were separated by high-performance liquid chromatography and then each, separately, was subjected to hydrolysis with aqueous dioxane in the presence of *p*-toluenesulfonic acid. The yields of the sterols (**255**) and (**256**) amounted to approximately 44—46%.



In [59, 60], 5α-stigma-7Z,24(28)-dien-3β-ol (**257**) was converted into the tritium derivative (**259**). For this, sterol (**257**) was first oxidized by the Jones reaction to the ketone (**258**), the treatment of which with tritiated water in a dioxane solution of caustic potash followed by reduction with sodium tetrahydroborate led to the desired compound (**259**).

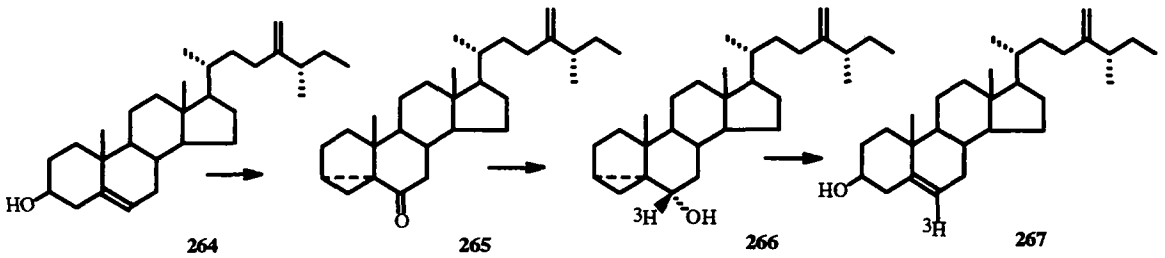


The labeled [26-^{14}C]clerosterol (**262**) and its 24-epimer (**263**) have been synthesized from the 25-ketone (**260**) [47]. Wittig condensation of ketone (**260**) with the appropriate labeled phosphorane led to the formation of the olefins (**261a** and **b**) epimeric at C-24. Acid hydrolysis of compounds (**261a** and **b**) gave the required sterols (**262**) and (**263**).



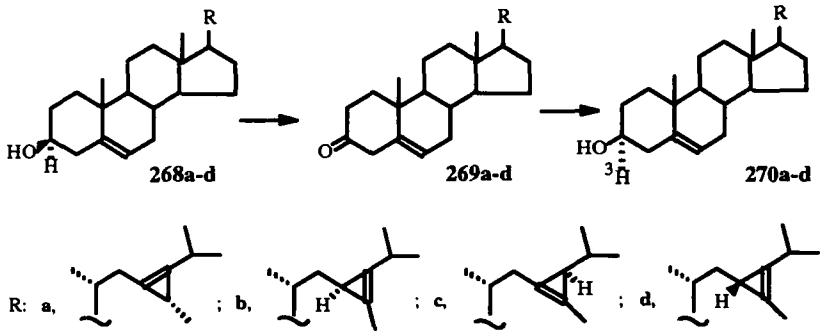
260 **261a, b** **262** **263**

In the investigation described in [47], 24(28)-dehydroaplysterol (**264**) was converted into the tritium derivative (**267**). The interaction of sterol (**264**) with *p*-toluenesulfonyl chloride in pyridine yielded a tosylate the rearrangement of which under the action of potassium acetate in aqueous dioxane followed by the Jones oxidation of the resulting 3α,5-cyclo-6β-alcohol in acetone gave the 3α,5-cyclo-6-ketone (**265**). Reduction of the keto group in compound (**265**) with sodium tetratritioborate in a mixture of ethanol and tetrahydrofuran led to [6β-^{3}H]-3α,5-cyclo-6α-alcohol (**266**) the hydrolysis of which under the action of *p*-toluenesulfonic acid in aqueous dioxane gave the desired [6-^{3}H]-24(28)-dehydroaplysterol (**267**). By this method, the yield of compound (**267**) from sterol (**264**) amounted to 25%. The corresponding [6-^{3}H]- tritium derivative was obtained analogously from (25R)-24(28)-dehydroaplysterol.



264 **265** **266** **267**

The Pfitzer-Moffatt oxidation of calisterol (**268a**), (23R)-23H-isocalisterol (**268b**), 24H-isocalisterol (**268c**) and (23S)-23H-isocalisterol (**268d**) gave the corresponding 3-ketones (**269a—d**) [63]. The reduction of compounds (**269a—d**) with sodium tetratritioborate led to the formation of the labeled sterols (**270a—d**).



268a-d **269a-d** **270a-d**

It has been reported [64] that desmosterol, 24-methylenecholesterol, fucosterol, isofucosterol, and 24-vinylcholesterol labeled at C-3 with tritium have been synthesized by a method proposed previously [55, 63]. However, no experimental details on the synthesis of these substances are given in [64].

REFERENCES

1. L. Fieser and M. Fieser, *Steroids*, Reinhold, New York (1959) [Russian translation, Mir, Miscow (1964), p. 452].
2. G. B. Elyakov and V. A. Stonik, *The Steroids of Marine Organisms* [in Russian], Nauka, Moscow (1988).

3. N. V. Kovganko and A. A. Akhrem, *Steroids: Ecological Functions* [in Russian], Nauka i Tekhnika, Minsk (1990).
4. K. Bloch and D. Rittenberg, *J. Biol. Chem.*, **149**, 505 (1943).
5. H. S. Anker and K. Bloch, *J. Am. Chem. Soc.*, **66**, 1752 (1944).
6. D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, **198**, 861 (1952).
7. S. O. Byers and M. W. Biggs, *Arch. Biochem.*, **39**, 301 (1952).
8. D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, **198**, 871 (1952).
9. R. Hampi and L. Starek, *Radioisotopy*, **19**, 337 (1978).
10. M. H. Rabinowitz, *Tetrahedron Lett.*, **32**, 6081 (1992).
11. S. Seo, Y. Yoshimura, T. Satoh, A. Uomori, and K. Takeda, *J. Chem. Soc., Perkin Trans. I*, 411 (1986).
12. A. Uomori, S. Seo, T. Sato, Y. Yoshimura, and K. Takeda, *J. Chem. Soc., Perkin Trans. I*, 1713 (1987).
13. D. N. Kirk, M. J. Varley, H. L. J. Makin, and D. J. H. Trafford, *J. Chem. Soc., Perkin Trans. I*, 2563 (1983).
14. T. Holm and I. Crossland, *J. Labelled Comp. Radiopharm.*, **38**, 803 (1996).
15. S. Malik, M. Kenny, and S. Ahmad, *J. Labelled Comp. Radiopharm.*, **31**, 861 (1992).
16. G. Z. Tang and C. T. Peng, *J. Labelled Comp. Radiopharm.*, **25**, 585 (1988).
17. M. A. Palmer, J. L. Goad, and T. N. Goodwin, *Nouv. J. Chim.*, **2**, 401 (1978).
18. S. Bergstrom, S. Lindstedt, B. Samuelson, E. J. Corey, and G. S. Gregoriou, *J. Am. Chem. Soc.*, **80**, 2337 (1958).
19. G. M. Caballero and E. G. Gros, *J. Labelled Comp. Radiopharm.*, **34**, 127 (1944).
20. R. B. Turner, *J. Am. Chem. Soc.*,, **72**, 579 (1950).
21. G. I. Fujimoto, *J. Am. Chem. Soc.*, **73**, 1856 (1951).
22. R. D. H. Herd and P. Ziegler, *J. Am. Chem. Soc.*, **73**,,4036 (1951).
23. W. G. Dauben and J. F. Eastham, *J. Am. Chem. Soc.*, **73**, 3260 (1951).
24. W. G. Dauben and J. F. Eastham, *J. Am. Chem. Soc.*, **73**, 4463 (1951).
25. B. Belleau and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 4458 (1951).
26. E. Schwenk, M. Gut, and J. Belisle, *Arch. Biochem.*, **31**, 456 (1951).
27. W. G. Dauben and P. H. Payot, *J. Org. Chem.*, **21**, 1299 (1956).
28. W..G. Dauben and H. L. Bradlow, *J. Am. Chem. Soc.*, **72**, 4248 (1950).
29. A. I. Ryder, W. H. Gebert, and N. M. Murrill, *J. Am. Chem. Soc.*, **72**, 4247 (1950).
30. P. D. Klein and J. C. Knight, *J. Am. Chem. Soc.*, **87**, 2657 (1965).
31. R. Murari, M. P. Murari, and W J. Baumann, *Biochemistry*, **25**, 1062 (1986).
32. J. Yan and R. Bittman, *J. Lipid Res.*, **31**, 160 (1990).
33. A. K. Batta, G. Salen, G. S. Tint, A. Honda, and S. Shefer, *Steroids*, **62**, 700 (1997).
34. R. Ray and M. F. Holick, *Methods Enzymol.*, **282**, 157 (1997).
35. F. Dolle, C. Kappler, C. Hetru, B. Rousseau, M. Coppo, B. Luu, and J. A. Hoffmann, *Tetrahedron*, **46**, 5305 (1990).
36. Y. Ikuina, Y. Kanzawa, Y. Fujimoto, and K. Kakinuma, *Chem. Pharm. Bull.*, **37**, 1755 (1989).
37. S. Takatsuto and N. Ikekawa, *Chem. Pharm. Bull.*, **34**, 1415 (1986).
38. S. Takatsuto and N. Ikekawa, *J. Chem. Soc., Perkin Trans. I*, 591 (1986).
39. M. H. Rabinowitz and C. Djerassi, *J. Am. Chem. Soc.*, **114**, 304 (1992).
40. S. Seo, T. Nagasaki, Y. Katsuyama, P. Matsubara, T. Sakata, M. Yoshioka, and Y. Makisumi, *J. Labelled Comp. Radiopharm.*, **27**, 1383 (1989).
41. D. Colombo, F. Ronchetti, G. Russo, and L. Toma, *J. Chem. Soc., Chem. Commun.*, 263 (1990).
42. S. Maruyama, Y. Fujimoto, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, **23**, 1701 (1982).
43. L. Stoilov, T. G. Back, J. E. Thompson, and C. Djerassi, *Tetrahedron*, **42**, 4156 (1986).
44. E. S. Monteagudo, C. Burton, and E. G. Gros, *Helv. Chim. Acta*, **73**, 2097 (1990).
45. C. A. N. Catalan, J. E. Thompson, W. C. M. C. Kokke, and C. Djerassi, *Tetrahedron*, **41**, 1073 (1985).
46. Y. Fujimoto, N. Sato, K. Iwai, H. Hamada, J. Yamada, and M. J. Morisaki, *J. Chem. Soc., Chem. Commun.*, 681 (1997).
47. I. L. Stoilov, J. E. Thompson, J.-H. Cho, and C. Djerassi, *J. Am. Chem. Soc.*, **108**, 8235 (1986).
48. C. J. Silva and C. Djerassi, *Coll. Czech. Chem. Commun.*, **56**, 1093 (1991).

49. I. Rubinstein and L. J. Goad, *Phytochemistry*, **13**, 481 (1974).
50. T. Gebreyesus, I. Stoilov, F.-T. Luo, and C. Djerassi, *Steroids*, **45**, 447 (1985).
51. N. Fujimoto, M. Kimura, A. Takasu, F. A. M. Khalifa, M. Morisaki, and N. Ikekawa, *Tetrahedron Lett.*, **25**, 1501 (1984).
52. I. Horibe, H. Nakai, T. Sato, S. Seo, K. Takeda, and S. Takatsuto, *J. Chem. Soc., Perkin Trans. I*, 1957 (1989).
53. M.-P. Marsan, W. Warnock, I. Muller, Y. Nakatani, G. Ourisson, and A. Milon, *J. Org. Chem.*, **61**, 4252 (1996).
54. M. J. Thompson, O. W. Berngruber, and P. D. Klein, *Lipids*, **6**, 233 (1971).
55. B. Dayal, G. Salen, G. S. Tint, and C. Biswas, *Steroids*, **42**, 635 (1983).
56. P. P. H. L. Otto, A. C. Besemer, and W. H. J. M. Wientjens, *J. Labelled Comp. Radiopharm.*, **6**, 111 (1970).
57. G. I. Fujimoto and A. E. Jacobson, *J. Org. Chem.*, **29**, 3377 (1964).
58. B. Z. Askenazi, L. N. Kivokurtseva, N. S. Bobrova, and N. Ya. Kazarinskaya, *Khim.-farm. Zh.*, **19**, 1221 (1985).
59. F. F. Knapp, J. B. Greig, L. J. Goad, and T. W. Goodwin, *J. Chem. Soc., Chem. Commun.*, 707 (1971).
60. F. F. Knapp, L. J. Goad, and T. W. Goodwin, *Phytochemistry*, **16**, 1683 (1997).
61. F. Nicotra, F. Ronchetti, G. Russo, and L. Toma, *Experientia*, **41**, 65 (1985).
62. I. L. Stoilov, J. E. Thompson, and C. Djerassi, *Tetrahedron*, **42**, 4147 (1986).
63. G. A. Dosa, C. Margot, G. Sodano, and C. Djerassi, *Tetrahedron Lett.*, **29**, 6051 (1988).
64. R. G. Kerr, S. L. Kerr, S. Malik, and C. Djerassi, *J. Am. Chem. Soc.*, **114**, 299 (1992).