NEW ADVANCES IN THE FIELD OF SYNTHESIS OF NATURAL POLYHYDROXYSTEROIDS

A. V. Kamernitskii, I. G. Reshetova, and E. I. Chernoburova

UDC 542.91:541.63

This review of the literature and of the authors' own work, devoted to a discussion of new methods for the synthesis of sections of steroid molecules responsible for their biological action, consists of two parts. The first is devoted to methods of constructing polyoxygenated side chains C_3-C_8 of steroids. In it are discussed the Grignard, Wittig, and Claisen reactions using Me-organic complexes including the C-20, C-21, C-22, C-23, and C-24 compositions, the C-22(23) double bond, the C-22, C-23, and C-24 centers, and the C-24 and C-25 centers, and other methods. In the second part methods of constructing the A/B rings of natural polyhydroxysteroid are discussed: the construction of the 2β,3β-vicinal diol grouping in the 5βH-Δ^7-6-keto fragment of the ecdysones, the construction of the 2α,3α-cis-diol grouping in the 7-oxa-6-keto-B-homo rings of the brass inolides, and methods of creating the Δ^5-7-oxygen-containing ring B of steroids of the antheridiol group.

Among the broad group of physiologically active steroids, an important position is occupied by compounds in which the side chain in ring D, with from 3 to 8 or 9 carbon atoms, contain one or more hydroxy groups. The appearance of C_3-C_8 oxygen-containing side chains is due to oxidative biotransformations of steroids belonging both to the vegetable and to the animal kingdoms. In the evolutionary development of organisms, the C_8-alkyl chains of steroids undergo mono- and polyhydroxylation. The specificity of the biological action of polyhydroxysteroids is determined, on the one hand, by such factors as the number of hydroxy groups in the side chain and their position and the stereochemistry and form of the side chain, and, on the other hand, by features of the structure of rings A/B. It is natural that the synthesis of compounds of this type sometimes acquire great practical importance.

The aim of the present review is an analysis of new methods of constructing polyhydroxy side chains of steroids and creating certain elements of the structure of rings A/B.

METHODS OF CONSTRUCTING POLYOXYGENATED SIDE CHAINS (C_3-C_8) OF STEROIDS

The isolation and characteristics of metabolites of cholesterol and of the sterols of man, plants, and animals, insect molting hormones, and the sex hormones of fungi and the biological significance of new natural compounds which, to a certain extent is determined by the structures of the modified side chains, have caused considerable interest. Intensive investigations in the field of the synthesis of the side chain have brought forward many new elegant methods which are of interest in general organic chemistry and have made a contribution to the possibility of the stereospecific construction of chiral carbon centers. Work up to 1978 has been discussed in sufficient detail in a number of reviews [1, 2]. We shall limit ourselves to a consideration of the literature that did not appear in these reviews and to later literature.

REACTIONS INCLUDING THE C-20 POSITION

The modified C_3-C_8 side chain of steroids contains up to four asymmetric centers (depending on the degree of substitution). The central position in any synthesis of mono- and polyoxygenated side chains is, above all, the stereospecific introduction of the asymmetric center at C-20. Investigations based on 17-ketones were discussed in detail in [1], and we therefore began our review of the literature with reactions at the C-20 center. The strategy of syntheses at C-20 includes direct nucleophilic reactions at a C-20 carbonyl: the Grignard reaction of organic metallic compounds and the Wittig reaction, with the subsequent formation

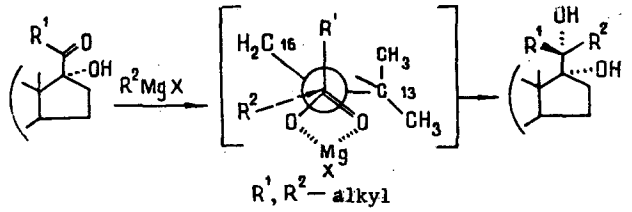
---

N. D. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR, Moscow.
Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 3-28, January-February, 1988. Original article submitted February 4, 1987.

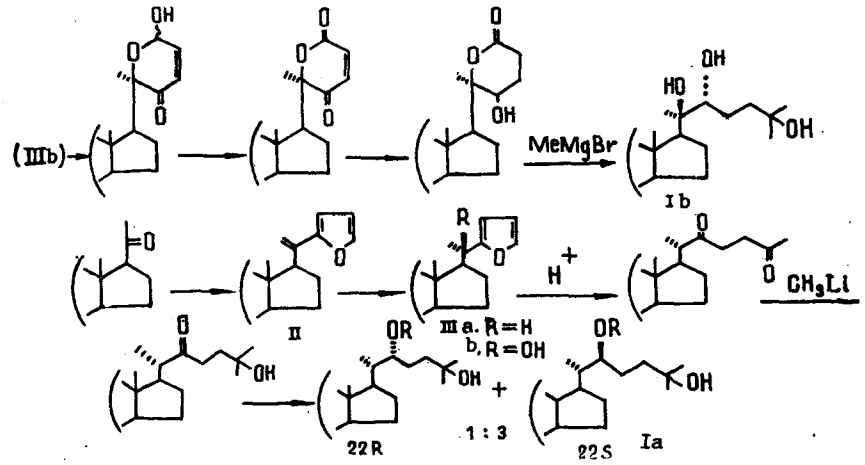
0009-3130/88/2401-0001 $12.50

of a $\Delta^{20(22)}$-bond and its hydrogenation, followed by functionalization in the necessary position (for example, at C-22) [3, 4].

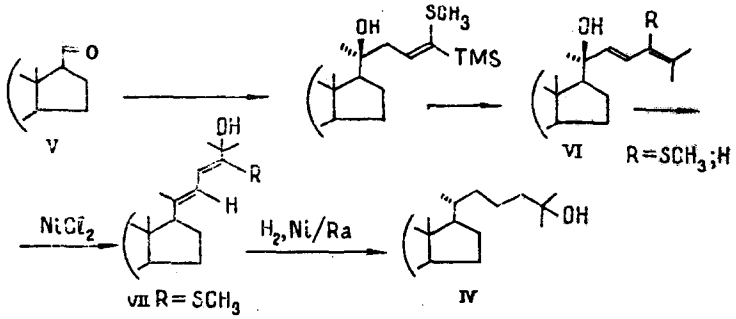
Addition at C-20 is the most studied reaction in connection with the construction of the steroid side chain [5]. Nucleophilic addition is highly selective, but its degree depends on the nature of the substituents at C-17 and C-16 in the skeleton. The reaction takes place through a cyclic model



Methods for the nonstereospecific construction of the side chain of ecdysterone from 20-ketosteroids with Grignard reagents have been discussed in detail in a review [6]. Kametani's group [4] has developed a simple and effective methodology for the transformation of pregnenolone via suitable furan derivatives (2-lithio-5-methyl-, 5-lithio-2-methoxy-) into (22R)-22,25-dihydrocholesterol and into compounds with the side chains of ecdysteroids and brassinosteroids. A noteworthy feature of the stereospecific synthesis of the side chain of ecdysone (Ia) is the stereospecific reduction of the methylene bond in (II), giving the 20S-compound (IIIa), in spite of the well-known fact of a disturbance of stereoselectivity on the hydrogenation of a double bond between C-20 and the following position [1]. The same authors used the 20-hydroxyfuranosteroid (IIIb) for the synthesis of the side chain of the 20R-hydroxyedysone (Ib) by Wiesner's scheme including a furan-pyrone rearrangement [7].

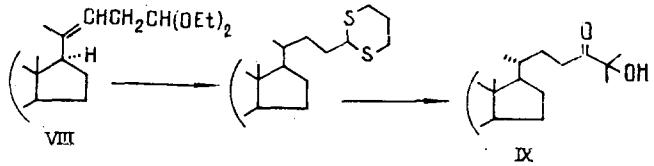


An elegant strictly stereodirected synthesis of 25-hydroxycholesterol (IV) was effected [3] by the condensation of the 20-ketones (V) in the presence of sec-butyllithium with 1-(arylthio) or 1-(alkylthio)-1-(trimethylsilyl)prop-2-ene. The TMS derivatives of homoallyl alcohols obtained were subjected to a second treatment with sec-butyllithium in acetone, which led to the simultaneous elimination of the TMA protection and to condensation to form the dienol (VI). The allyl rearrangement of the latter in the presence of $NiCl_2$ gave the di-



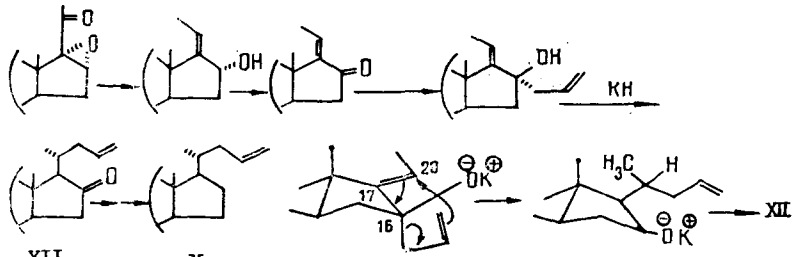
enol (VII), the reduction of the $\Delta^{20(22)}$ bond which led with a high degree of stereoselectivity to the required intermediate 20(22)-E compound.

The use of the Wittig condensation of pregnenolone with $(EtO)_2CHCH_2CH_2P(O)(OEt)_2$ in [8] enabled the propylidenepregnene (VIII) to be obtained which, after stereospecific hydrogenation and condensation with dithiopropane, gave a dithiane; the subsequent treatment of it with acetone and the hydrolysis of the thioketal led to 24-keto-25-hydroxycholesterol (IX).

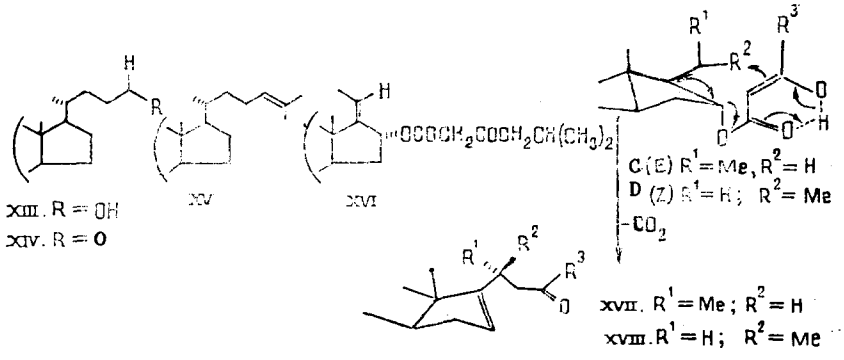


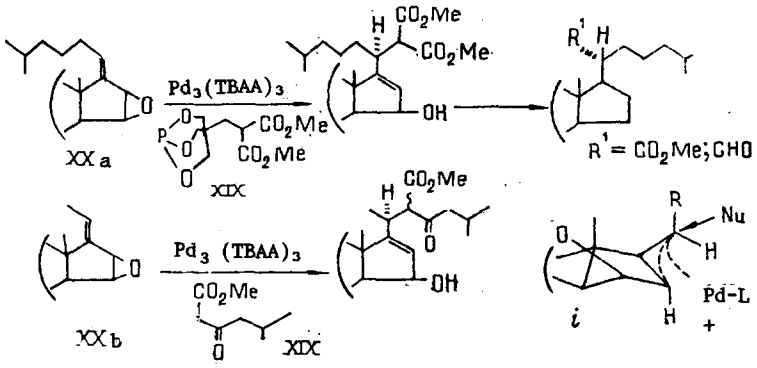
VIII IX

In addition to the direct creation of the chiral center by the introduction of the corresponding substituents, considerable use has been found by the Claisen and Cope rearrangements – [3,3]-sigmatropic rearrangements. One of the useful features of these reactions is their capacity for moving the chiral center along the carbon chain (three chiral centers may be included in the Claisen rearrangement). The principle of stereochemical transmission through the oxy-Cope rearrangement [9] starting from 16α,17α-epoxypregnenolone has opened up a new effective and completely stereocontrollable route to the synthesis of steroids with various 24,25-dihydroxy side chains from the intermediate olefin (X). Using 16α,17α-hydroxypregnenolone as an example, the Barton reaction with subsequent transformation by the scheme given led to the key allyl alcohol (XI), the treatment of which with potassium hydride caused rearrangement to form the 20R-ketoolefin (XII). The stereospecific generation of the 20R stereochemistry is due to the 6-membered transition state A in the chair form, which is involved in the rearrangement. The subsequent transformation of (XII) included its reduction to the 16β-alcohol, conversion into a phosphoramidate derivative, and reduction with lithium in liquid ammonia (X). The construction of the side chain was completed with hydroboration to the alcohol (XIII) and its oxidation in the aldehyde (XIV), after which the Wittig reaction with triphenylphosphonium isopropylide gave the desmosterol (XV), and then 24,25-dihydroxycholesterol.

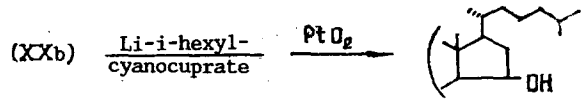


Using the same readily accessible 16,17-epoxypregnenolone and its conversion by the Wittig reaction into an allyl alcohol, other authors [10] converted it into the β-ketoacetate (XVI) – a key product in a stereocontrolled Claisen rearrangement at the C-20 center which took place as a concerted cyclic process with a high degree of selectivity. It follows from the structure of intermediates C and D that the E-isomer (C) gives the natural 20R-configuration at C-20 (XVII), while (D) gives the unnatural 20S configuration (XVIII).



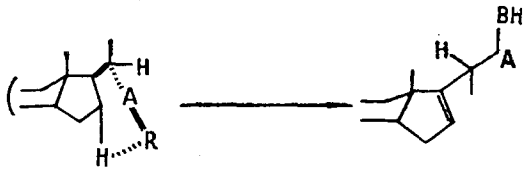


Beside the Claisen rearrangement, the 1,3- or 1,4-transfer of chirality can be effected via π-allylpalladium intermediates [1, 11]. Alkylation with the aid of $PdCl_2$ requires activation by phosphorus ligands [such as triphenylphosphine, HMPA, or bis(1,2-diphenylphosphino)-ethane]. This Pd-catalyzed syn-SN_2' reaction was performed with 15β,16β-epoxy-$\Delta^{17(20)}$-steroids in the presence of the phosphite ligand (XIX) and Pd(0) complex by the scheme shown above [12]. It is assumed that the initial attack of the Pd(0) takes place from the side opposite to the epoxide to form the π-allyl Pd complex (i), and the subsequent attack of the nucleophile from the side opposite to this complex takes place on the whole without isomerization at C-15 in the case of the 15,16-epoxides (XX). Thus, the 1,4-addition of nucleophiles to 15β,16β-epoxy-E-$\Delta^{17(20)}$-steroids is a new regio- and stereoselective method of introducing the side chain. The epoxides (XXa) and (XXb) have been used in a 1,4-chirality transfer reaction with organocuprates [13]. In this case, two asymmetric centers

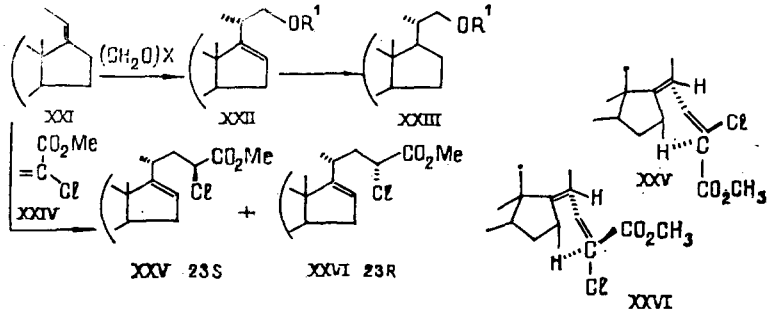


in the 1,4-positions are formed. The 1,4-trans-addition of alkylcyanocuprates to alkylideneoxiran derivatives of sterols is the only stereospecific method of introducing asymmetric centers when OH groups are present in the C-20 and 15β-positions. This strategy of synthesis can be applied to a wide range of functionalized side chains and to ring D of steroids (in particular for the synthesis of oogoniol [13]).

Uskokovits [14] and Dauben [15] have proposed to use as the key stage for the creation of the natural configuration at C-20 the ene reaction of a suitably substituted (17Z)-ethylidenesteroid. Stereochemical control is due to the attack of the enophile at C-20 exclusively from the less hindered α-region of the molecule. The performance of the ene reaction of compound (XXI) with formaldehyde as enophile led to the stereospecific introduction of a hydroxymethyl group and to the formation of compounds (XXII, XXIII) with the natural configuration at C-20. The catalyst used was $BF_3 \cdot Et_2O$.

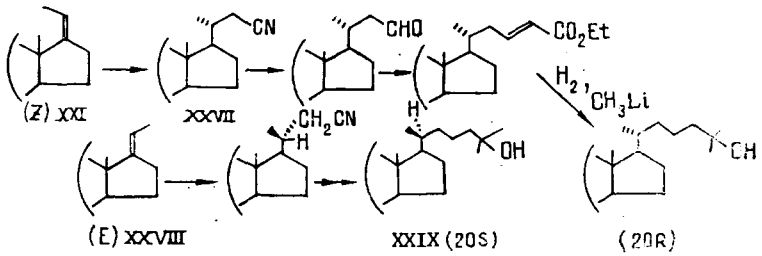


Other Lewis acids, such as $EtAlCl_2$, $AlCl_3$, and $AlBr_3$, or protonic acids caused rearrangements of the Wagner–Meerwein type. However, the use of $EtAlCl_2$ or Et_2AlCl in stoichiometric amounts in reactions with methyl acrylate or methyl propiolate [15] led to cholate esters. The use of $AlCl_3$ ($AlBr_3$) also proved effective when they were combined with such protons acceptors as pyridine and its derivatives (see scheme on following page). The introduction of α-substituted acrylate esters into the ene reaction permits the stereocontrolled synthesis of side chains with two chiral centers to be formed. Thus, the condensation of methyl 2-chloroacrylate (XXIV) with the E-pregnadiene (XXI) gave a mixture of the stereoisomers (XXV) and (XXVI) in a ratio of (23S) to (23R) of 6:1, with the natural 20R-configuration. The C-23 center is formed in the attack of the enophile, leading to methoxycarbonyl-endo and chloro-endo transition states, of which the former predominates. The chiral center at



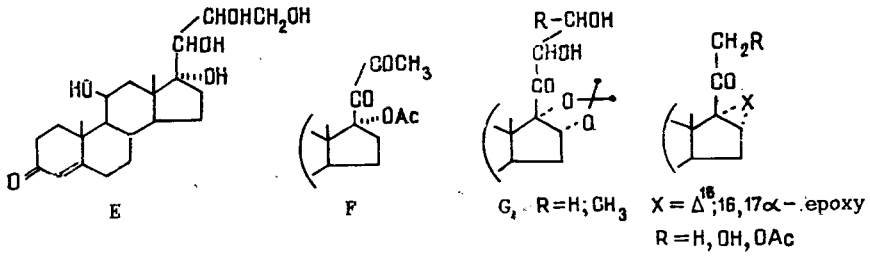
C-23 can be used for the growth of longer side chains of steroids, with the performance of stereocontrol, on the introduction of more remote groupings.

Studies in which the hydroboration of a $\Delta^{17(20)}$-bond is used have been widely developed [13-17]. In particular, strictly selective hydroboration from the α-region by 9-borobicyclononane and subsequent treatment with chloroacetonitrile in the presence of potassium 2,6-di-tert-butyl-4-methylphenolate have permitted the stereoselective formation from the Z-isomer (XXI) of the nitrile (XXVII) with the natural configuration at C-17 and C-20. The E-isomer (XXVIII) leads after similar transformations to the (20S)-25-hydroxycholesterol (XXIX).



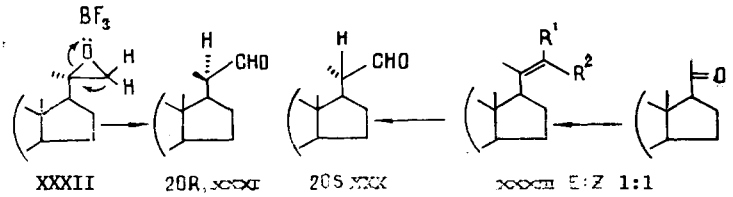
REACTIONS INVOLVING THE C-21 POSITION

Steroid hormones – corticosteroids – are the most deeply oxygenated analogs of sterols. Their C-17 side chains may be compared with the initial series of deoxysugars. The lengthening of these side chains has permitted an approach to homologs of steroid-substituted carbohydrates. Compounds of types (D, E, G) [18-33] are highly active antiinflammatory agents, while those of type G are also active in relation to the function of the adrenal cortex. Their synthesis is based mainly on two schemes, including aldol condensation reactions at the C-21 center with formaldehyde or with formic acid esters.



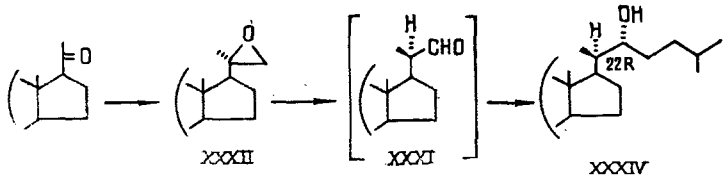
SYNTHESIS OF A C-22 ALDEHYDE AND STEREOCONTROLLED 22-HYDROXY GROUPS

To create a chiral center at C-22 (which is important in the synthesis of ecdysteroids and other phytosteroids) wide use is made of bisnorcholenaldehyde. Usually, the natural (20S)-aldehyde (XXX) is synthesized by the ozonization of 5,6-dibromostigmasterol followed



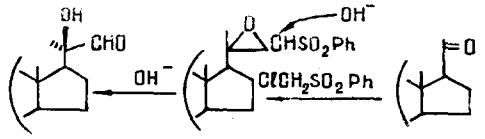
by debromination. The synthesis of the unnatural (20R)-aldehyde (XXXI) is carried out by the Corey–Chaykovsky method: interaction of pregnenolone with dimethylsulfoxonium methylide, the oxiran formed (XXXII) isomerizing on treatment with BF_3 into the individual 20R-aldehyde (XXXI) [34, 35].

Schönaur and Zbiral [36] have proposed a new method for synthesizing both the (20S) and the (20R) isomers. The reaction of pregnenolone with a new Wittig reagent – trimethylsilylethoxymethylenetriphenylphosphorane – gave the E- and Z-vinylidene derivatives (XXXIII), the latent silyl ether groups of the enols in which were hydrolyzed by treatment with 5% HF in acetonitrile, forming (XXX) and (XXXI), respectively. As had been observed previously [5], in the presence of isoamylmagnesium bromide the epoxide (XXXII) rearranged into the 20R-aldehyde (XXXI), which selectively added the reagent with the formation of the 22R-alcohol (XXXIV).



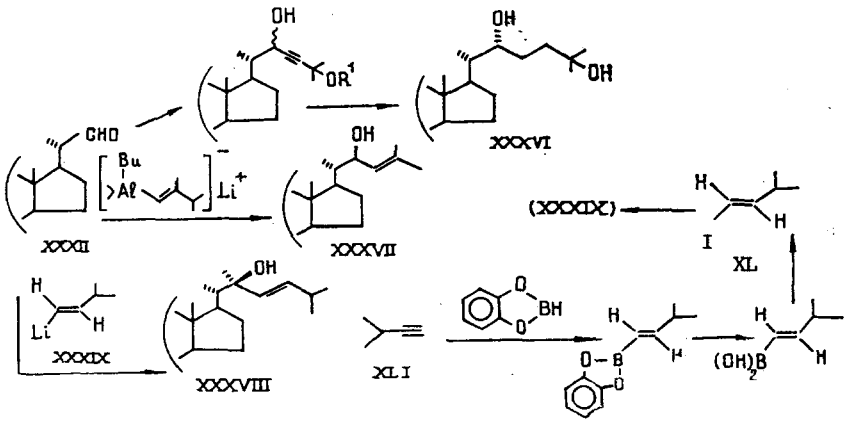
XXXII XXXI XXXIV

In connection with the partial synthesis of the side chain of ecdysone it would be desirable to start from the (20R)-20-hydroxypregnane-20-carboxaldehyde (XXXV). An extremely effective synthesis of this aldehyde with high yields and high stereoselectivity has been proposed [37]. The scheme of synthesis includes the Darzens reactions between prenenolone and chloromethyl phenyl sulfone. The authors assumed that the condensation must follow Cram's rule and lead to the desired 20R configuration of the α,β-epoxysulfone (XXXVI). Attack of the hydroxide ion (OH^-) on the α-position of (XXXVI) (at C-22) causes the formation of the desired 20R-product.



XXXV XXXVI (20R, 22R)

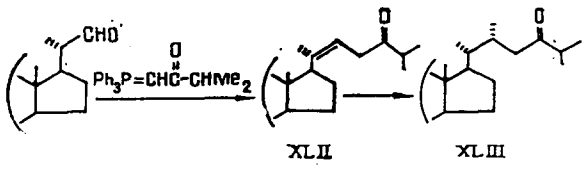
C-22-Aldehydes play an important role in the introduction of an asymmetric center at C-22 of such physiologically active steroids as the ecdysones, oogoniols, brassinolides, etc. It has been shown that the interaction of C-22-aldehydes with alkyl-substituted Grignard reagents permit the stereodirected introduction of a 22α-hydroxy side chain [38]. The addition of vinyl and of less hindered acetylene reagents leads to a lower stereoselectivity [2, 39, 40]. Reagents of this type are used for the synthesis of analogs of the ecdysones [6, 41, 42] and of hydroxylated metabolites of vitamin D (XXXVI) [4]. The introduction of an asymmetric center at C-22 is a very important for the synthesis of the side chain of brassinolides, oogoniols [43-46], and this all the more because C-22 controls the stereochemistry at C-23 and C-24.



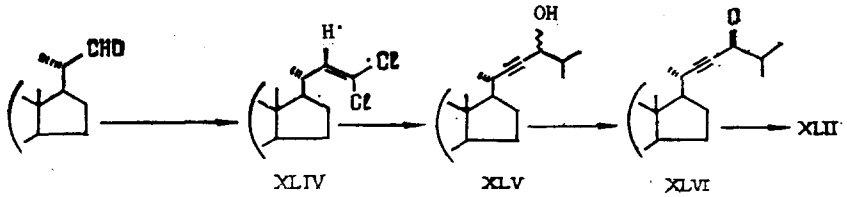
XXXVI XXXII XXXVII (XXXIX) I XL XXXIX XXXVIII XLI

An asymmetric center at C-22 has been created by the stereoselective alkylation of (XXXII) with lithium alanate to form compound (XXVII) [47, 48]. The condensation of the steroid aldehyde with lithium 3-methylbut-1(E)-ene (XXXIX) leads to the formation of the 24-nor analog of the 22S-allyl alcohol (XXXVIII) [49]. The scheme of synthesis includes lithium treatment of the derivative 1-iodo-3-methylbut-1(E)-ene (XL), this having been obtained by the authors concerned from the industrially available 3-methylbut-1-yne (XLI) by the reaction with catecholborane [50].

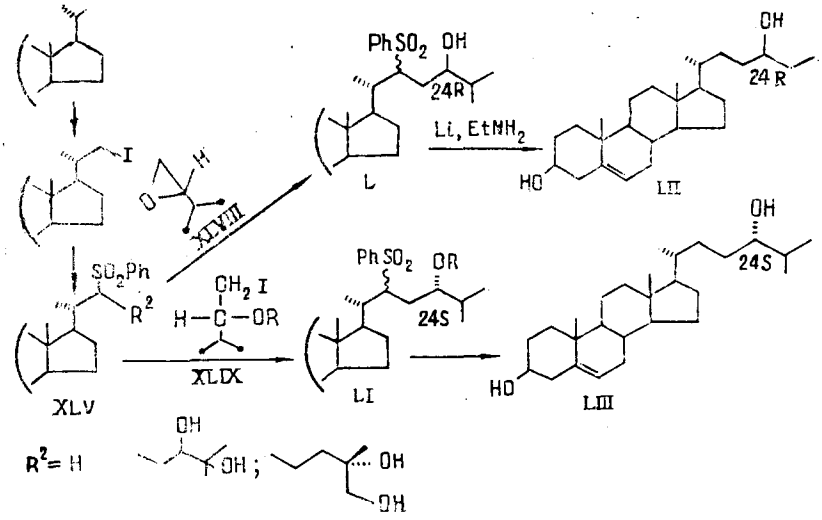
As a rule, the Wittig reaction with 22-aldehydes leads to the $\Delta^{22(23)}$-E-isomers. The $\Delta^{22(23)}$-24-ketone (XLII) has been synthesized in a number of investigations and has been widely used for subsequent transformations [51-54]. The $\Delta^{22(23)}$-bond is used for the stereocontrolled induction of a substituent at C-22 [for example, on interaction with lithium dimethylcuprate, the 22-methyl-24-ketone (XLIII) is formed] [53].



The use of a dichlorovinylidene reagent in the Wittig reaction enabled Ourisson, et al. [55] to achieve the synthesis of the dichlorovinylidene derivative (XLIV), the interaction of which with $(CH_3)_2CHCHO$ butyllithium led to a mixture of the isomeric acetylenic alcohols (XLV) (24S and 24R). Their oxidation gave the ketone (XLVI) and subsequent transformations the $\Delta^{22(23)}$-24-ketone (XLII); the latter, like its saturated analog, proved to be a highly effective cytotoxic agent in relation to hepatoma cells [55].

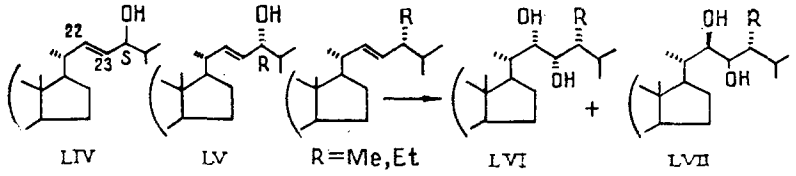


On the basis of the 22-aldehyde, the same authors have developed an original method for the functionalization of the C-24 side chain. The key stage of the synthesis is the interaction of the C-22 sulfone (XLVII), obtained by the scheme given above, with the chiral C_5-epoxide (XLVIII) or the iodide (XLIX) with the formation of the (24R)-alcohol (L) or the (24S)-alcohol (LI). The desulfonation of (L) and (LI) with the aid of lithium in ethylamine gave the corresponding (24R)- and (24S)-cholestane-3,24-diols (LII and LIII). As shown in [56], the sulfone (XLVII) is a steroid synthon activated in the C-22 position which can be combined with a wide range of electrophiles, and this makes it possible to introduce desired function into the C-23–C-37 part of the side chain.



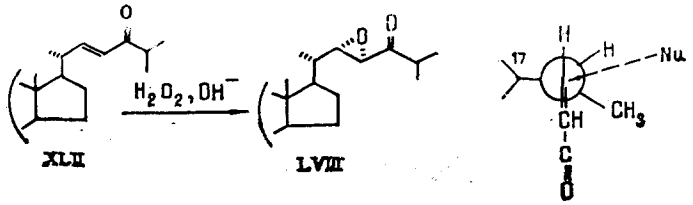
REACTIONS INVOLVING THE $\Delta^{22(23)}$-BOND

As a rule, the passage to steroid side chains containing the $\Delta^{22(23)}$-bond is effected by the hydrogenation of the corresponding acetylenic alcohols over Lindlar catalyst. The use of chiral aluminum complexes – $LiAlH_4$–[(–)-N-methylephedrine]–3,5-dimethylphenol (1:1:2) – for the reduction of steroid propargyl ketones [55, 57] has permited passage to the isomeric 24-allyl alcohols (LIV) and (LV).



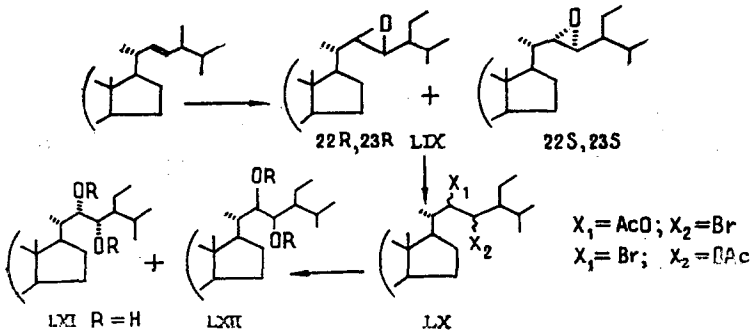
LIV LV R=Me,Et LVI LVII

The stereochemistry of addition reactions at the $\Delta^{22(23)}$-bond depends to a considerable degree on the nature of a substituent in the side chain. As has been shown by several groups of authors [58-63], cis-hydroxylation by OsO_4 to construct the 22,23-dihydroxy side chain of the brassinolides takes place nonstereospecifically, and the cis-22R,23R- and cis-22S, 23S-diols (LVI and LVII) are formed in ratios that depend on the substitution at C-24.



XLII LVIII

The stereochemistry of nucleophilic addition to the conjugated Δ-bond of the 22(E)-en-24-ones can be interpreted in a similar manner to the reaction with the 22-aldehyde (although the substrates are different) [5]. For example, the alkaline epoxidation of compound (XLII) led selectively to the 22S,23S-epoxide (LVIII) – an important intermediate in the synthesis of natural antheridiol. Epoxidation of an isolated $\Delta^{22(23)}$-bond (stigmasterol) gave a mixture of the (22R,23R)- and the (22S,23S)-epoxides (LIX) [63, 64], the opening of which with HBr in CH_3COOH led to a regio- and stereoisomeric mixture of the bromoacetates (LX). The replacement of the bromine by an acetoxy group and its subsequent saponification gave the two diols (LXI) and (LXII) in equal proportions. Thus, this method has no practical advantage over cis-hydroxylation in the specificity of the introduction of two vicinal hydroxyls at C-22 and C-23.

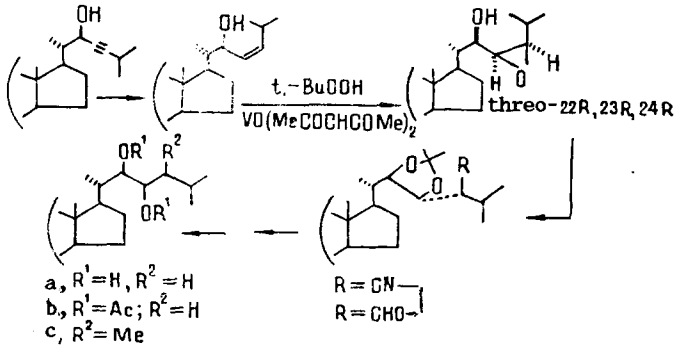


22R,23R LIX 22S,23S

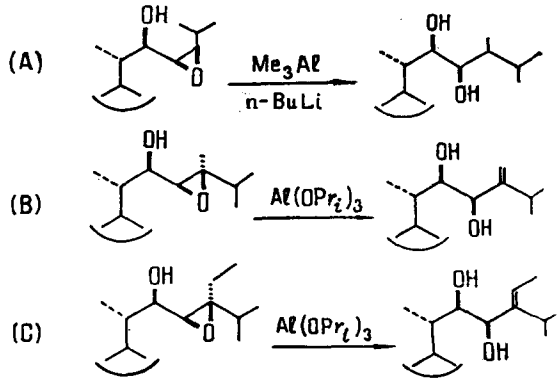
LXI R=H LXII LX

REACTIONS INVOLVING THE C-23 AND C-24 CENTERS

Since electrophilic reactions of direct cis-hydroxylation with the aid of OsO_4 provide no practical passage to the vicinal (22R,23R)-diols, an alternative approach to them has been developed [39, 40, 47]. An asymmetric center was first introduced at C-22, which was checked by stereochemical epoxidation according to Corey at C-23 and C-24 by the scheme given at the top of the following page. In this scheme for the construction of the side chain of the brassinosteroids, the key point is the stereoselective cleavage of the appropriate epoxides. Mori [48] has found suitable reagents: n-Bu:$LiMe_3Al$ in a ratio of 1:2.3, the use of which in tenfold excess in relation to the substrate permitted the regio- and stereoselective cleavage of 23,24-epoxides in accordance with the second scheme on p. 9.

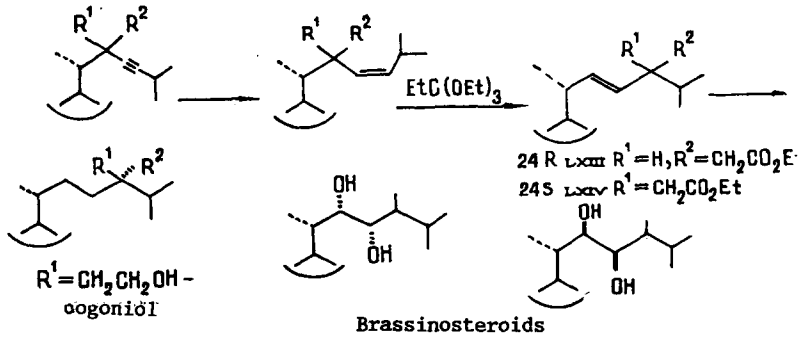


Using the scheme given below, the authors synthesized in high yields brassinolide castasterone, dolicholide, dolichosterone, homodolichoside, and other brassinosteroids [48]. However, they note that, in spite of the strict regio- and stereodirected cleavage of the 23,24-epoxides, this route to the synthesis of the side chains of the brassinosteroids is extremely lengthy, since it includes the cleavage of the stigmasterol side chain and its reconstruction. Investigations are being performed in the direction of the use of the native side chain of stigmasterol [65-67].

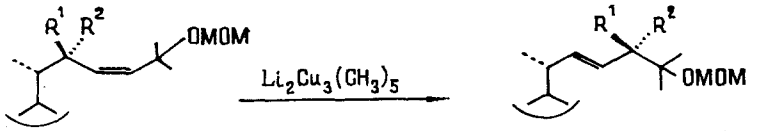


REACTIONS INVOLVING THE C-22, C-23, AND C-24 CENTERS

The asymmetric center at C-22 can control the configurations of the two newly formed neighboring C-23 and C-24 centers in the Claisen rearrangement. Thus, the Z-allyl alcohols obtained on the selective hydrogenation of the 22-acetylenic alcohols described above were subjected to rearrangement with triethyl orthoacetate and gave the $\Delta^{22(23)}$-unsaturated esters (LXIII and LXIV) [43-46, 57, 62, 68]. The configuration of the C-24 chiral center formed was controlled by the stereochemistry of the 22-hydroxy precursors, by the cis-geometry of the allyl double bond, and by the preferential chair geometry of the six-membered transition state during the rearrangement. It has been observed that the use of trimethyl orthoacetate gives a single reaction product, while the orthopropionate gives products homogeneous at C-24 but epimeric at the C-25 center. Subsequent osmylation leads to side chains of the brassinosteroid type [62]. On the other hand, the hydrogenation of the same $\Delta^{22(23)}$-bond and the reduction of the ethoxycarbonyl group provides the possibility of passing to the side chains of oogoniol [4, 44-46] and to marine sterols [68].

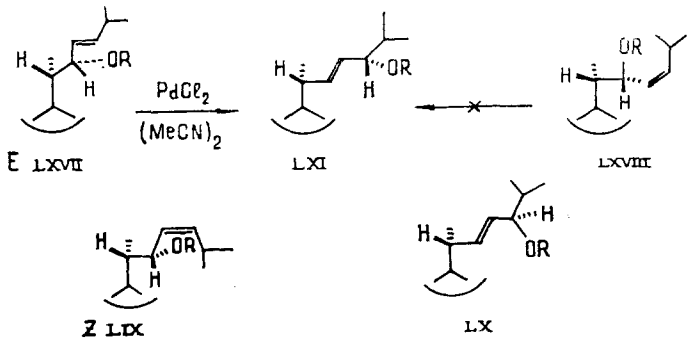


The stereospecific substitution of the allyl carbamate (LXV) by lithium cuprate [69] also permitted a 1,3-chiral transition to be performed [57], the assignment of the stereochemistry at C-24 being based by the authors on the presumed syn route of the substitution of carbonates by cuprates. The subsequent acid hydrolysis of the (LXVI) led to the first stereospecific synthesis of 25-hydroxy-7,8-dihydroergosterol.



LXV. (22S) R_1=OCONHPh LXVI R_1=H; R_2=CH_3(24S)

Japanese authors [45] have effected the stereocontrolled introduction of a 24-hydroxy group using a 1,3-chiral transition – the rearrangement of alkyl acetates catalyzed by Pd complexes. Using as examples the 22S- and 22R-isomeric allyl acetates investigated in the reaction, the influence of the conformational differences of the side chains of the E- and Z-isomers (LXVII and LXVIII) on their reactivities was demonstrated.

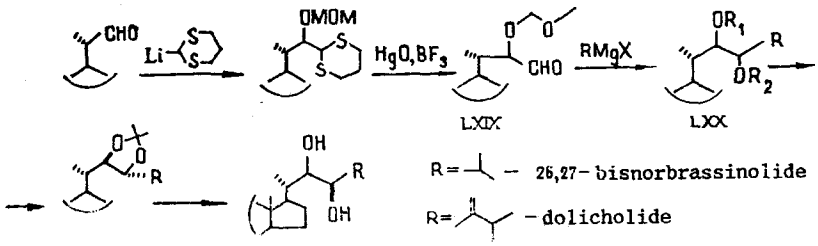


E LXVII LXI LXVIII

Z LIX LX

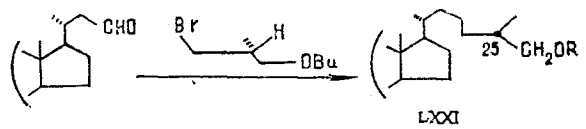
REACTIONS AT A C-23 ALDEHYDE GROUP

The stereospecific introduction of a (22R,23R)-vicinal diol grouping has been effected by the Grignard reaction with (22R)-23-aldehydes [70, 71]. The chelation-controlled reaction of the (22R)-23-aldehyde (LXIX) [71] with the Grignard reagent obtained from 2-bromo-3-methylbut-1-ene or from isobutyl (or isopropyl) bromide [70] led exclusively to the (22R, 23R)-22,23-vicinal diols (as the 22-methoxymethyl ethers) (LXX) – intermediates in the synthesis of brassinosteroids with modified side chains.



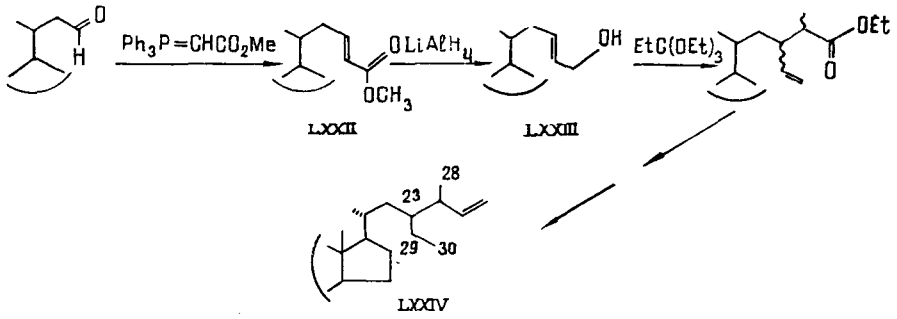
LXIX LXX

The condensation of the C-23 aldehyde with chiral synthons [72] – for example, with derivatives of (S)-1-bromo-(+)-3-hydroxy-2-methylpropane permitted the synthesis of the 26-hydroxycholesterols (LXXI) to be effected.



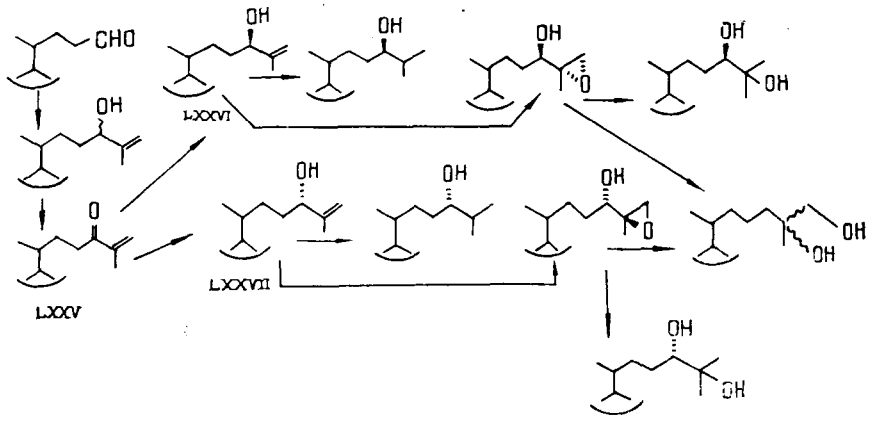
LXXI

The 23-aldehyde group has also been introduced in a Wittig reaction with $Ph_3P{=}CHCO_2Me$; the methoxycarbonyl group in compound (LXXII) was converted into a hydroxy group, and the use of the Claisen rearrangement of the steroid (LXXIII) permitted the synthesis of ficisterol as the first natural marine sterol with a 23-ethyl substituent (LXXIV) [73].

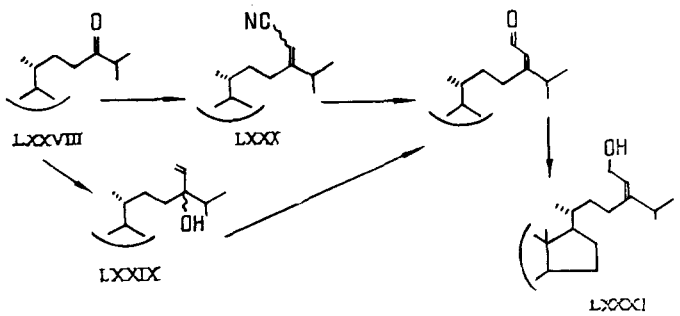


REACTIONS INVOLVING THE C-24 AND C-25 CENTERS

For the stereoselective introduction of hydroxy groups at C-24, C-25, and C-26, use is made of reactions with 24-aldehydes [74]. Their interaction with propenylmagnesium bromide followed by oxidation leads to the enols (LXXV). An important point in the subsequent transformations is the reduction of (LXXV) by a chiral complex consisting of $LiAlH_4$, ethanol, and 2,2'-dihydroxy-1,1'-binaphthyl. When the (R)(+)-dihydroxybinaphthyl is used, the main product of the reaction is the (24R)-alcohol (LXXVI), and when the (S)(−)-dihydroxybinaphthyl, is used it is the (24S)-isomer (LXXVII). The two isomers have been used for the introduction of hydroxy groups at C-25 and C-26 via the corresponding epoxides and their rearrangement by the following scheme:

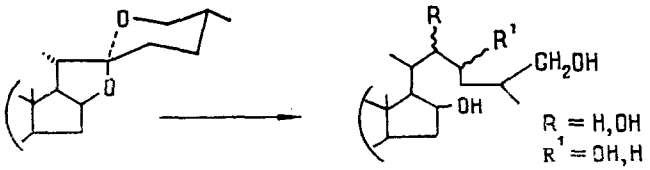


The reduction of the $\Delta^{24(28)}$-bond or its hydroboration [75] leads to the side chains of marine sterols or oogoniols. The latter have also been synthesized from the above-described $\Delta^{22(23)}$-24-ketone (XXII) [51, 53]. Its saturated analog (LXXVIII) has been used in the Wittig reaction with diethyl anomethylphosphonate or the Grignard reaction with vinyl magnesium bromide. The latter variant led to the known marine sterol saringosterol (LXXIX) [76]. Transformation of the nitrile (LXXX) – by subsequent reduction with diisobutylaluminum hydride – however, led to the side chain of dehydrooogoniol (LXXXI) [52].



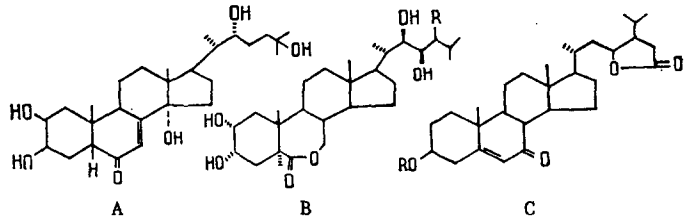
SYNTHESIS OF POLYHYDROXYSTEROIDS BY THE OPENING OF THE SPIROKETAL RINGS OF SAPOGENINS AND BY OTHER METHODS

Steroids with 23- and 26-hydroxy functionalized side chains have been obtained by the opening of the spiroketal rings of sapogenins in the presence of Lewis acids followed by $LiAlH_4$ reduction [74, 78], or by the remote functionalization of sterol side chains using photolysis [79].



METHODS OF CONSTRUCTING RINGS A/B OF NATURAL POLYHYDROXYSTEROIDS

The specificity of the biological action of such natural compounds as ecdysones, brassinolides, and steroids of the antheridiol group is determined, together with features of polyoxygenated side-chain derivatives, by the following structural elements of rings A/B: in the case of the ecdysones – a 2β,3β-cis-diol grouping, the cis-linkage of rings A/B, and a Δ^7-6-keto fragment (A) [6]; for the brassinolides – a 2α,3α-cis-diol grouping, ring B in the form of a seven-membered lactone ring, and the trans linkage of rings A/B (B) [80]; while for steroids of the antheridiol group (C) and a number of other physiologically active compounds oxygen substituents at C-7 are characteristic [81].



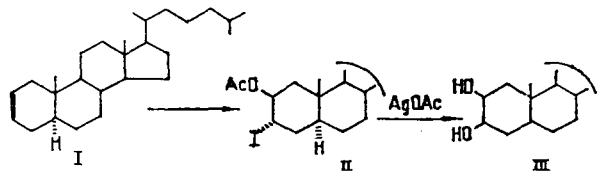
Thus, the task of synthesizing these compounds and their analogs includes the necessity of introducing functional groups and imparting a definite stereochemistry to the linkage of rings A/B. Such systems are created, as a rule, before or after the construction of the corresponding side chains with the use as the initial natural steroid material of sterols (Δ^5-steroids with various side-chain structures), ergosterol, or other $\Delta^{5,7}$-steroid dienes.

CREATION OF THE STRUCTURE OF RINGS A/B OF THE ECDYSONES

The construction of the 2β,3β-cis-diol-5β-H-Δ^7-6-keto grouping of the ecdysones includes the stagewise introduction of 2β,3β-cis-diol and Δ^7-6-keto functions with the cis-linkage of A/B. Here the sequence of introduction of the groupings mentioned is connected with the chemical nature of the initial compounds. Thus, the initial introduction of a 2β,3β-cis-diol grouping is, as a rule, based on the use of Δ^2-, 3-keto-5αH-, 3-keto-Δ^5-, and 3,6-diketosteroids. The use of $\Delta^{5,7}$- and 3,5α-cyclosteroids enables the Δ^7-6-keto fragment to be introduced first and creates the creation of the prerequisites for the construction of the cis-diol grouping.

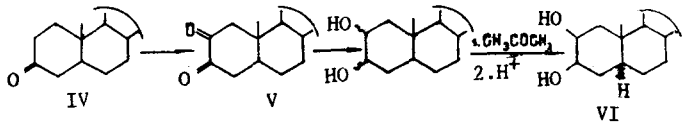
INTRODUCTION OF A VICINAL 2β,3β-DIOL GROUPING

An effective method for the construction of a 2β,3β-cis-diol grouping in the chemical synthesis of ecdysones is the cis-hydroxylation of Δ^2-steroids [82, 83]. In its turn, the synthesis of the Δ^2-compounds is carried out via the tosylates [6, 84-86] or the mesylates [6, 87] of the corresponding 3-hydroxy-Δ^5-steroids. The elimination of the 3-tosyloxy or the 3-mesyloxy group is done either by heating with lithium bromide [85] or with lithium bromide and carbonate in dimethylformamide [6], depending on the nature of the actual steroid. The reaction of the Δ^2-olefins so formed with iodine and silver acetate in aqueous acetic acid (the Woodward reaction) leads to 2,3-cis-diols with the β-configurations of the hydroxy groups [82]. Using this method, Δ^2-cholestene (I), on being heated with potassium iodide in AcOH, gave 86% of 2β-acetoxy-3α-iodo-5α-cholestane (II), the subsequent boiling

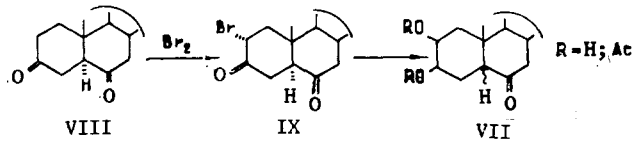


of which with silver acetate in aqueous AcOH and hydrolysis with alkali led to 84% of 5α-cholestane-2β,3β-diol (III) [83]. The expensive silver salt can be replaced by the equivalent amount of copper acetate or potassium acetate; even the simple boiling of the trans-iodohydrin (II) in aqueous AcOH led to 76% of the diol (III) [88]. The use of thallium acetate and iodine in the reaction with alkenes gave the corresponding α-iodoacetate which, on being boiled in aqueous AcOH, yielded the corresponding cis-hydroxy acetates and then, on solvolysis in glacial AcOH in the presence of sodium acetate, the trans-diacetates [89-91]. The presence of a 5α-hydroxy group in the molecule of a Δ^2-steroid favors the Woodward hydroxylation reaction with silver acetate or thallium triacetate and leads mainly to the 2β,3β-diols (ecdysteroids) [90].

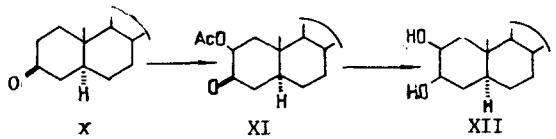
Other methods of synthesizing 2β,3β-dihydroxysteroids are based on the transformation of the corresponding 3-ketosteroids. Thus, Mori has developed a new method of introducing a 2β,3β-diol grouping, with 4,4-dimethylandrostanes as examples [6]. The introduction of a 2β,3β-diol fragment into a 2-unsubstituted 5α-cholestan-3-ol (IV) was effected by its preliminary autooxidation by Barton's method to the 2,3-diketone (V). The mixture of two of its enolic forms arising was reduced with sodium tetrahydroborate in methanol to a mixture of diols, and the 2β,3β-diol (VI) was isolated via the corresponding acetonide. We may note that the reduction of the 2,3-diketo derivatives of the (4-demethyl) 5α-series takes place less stereospecifically than the reduction of the 2-hydroxy-3-keto-4,4-dimethyl-Δ^5-steroids [6].



Passage to the 2β,3β-dihydroxysteroids (VII) can also be brought about by the bromination of the 3,6-diketo-5α- analogs (VIII) followed by the selective reduction of the 3-keto group in the resulting 2α-bromo-3-keto derivatives (IX) and the replacement of the bromine by an acetoxy group [6, 87].

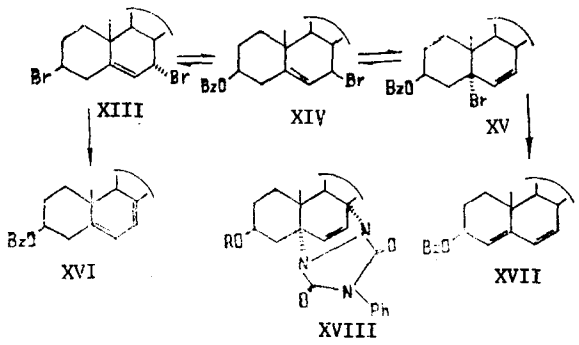


A 2β-acetoxy group can be introduced directly into the 3-keto-5α-steroid (X) by its reaction with lead tetraacetate. The 2β-acetoxy-3-keto derivative (XI) so obtained is reduced with lithium tetrahydroaluminate to the 2β,3β-diol (XII) [92].

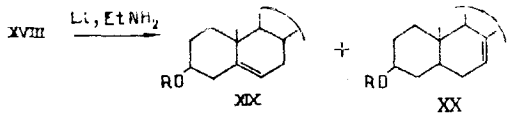


CONSTRUCTION OF A 5β-H-Δ^7-6-KETO FRAGMENT

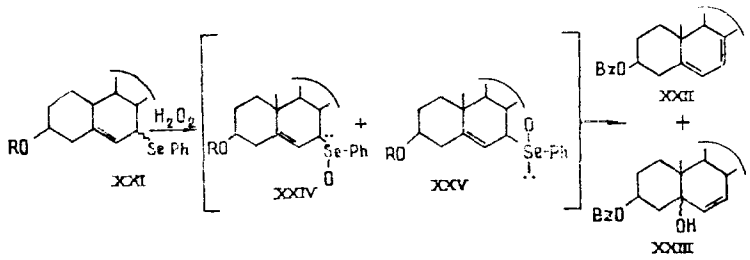
As a rule, the Δ^7-6-keto function of an ecdysone is created on the basis of $\Delta^{5,7}$-olefinic steroids – ergosterol or steroids obtained synthetically [6]. The development of methods for synthesizing such steroids has been the subject of great attention. One of the methods of obtaining $\Delta^{5,7}$-steroid derivatives is the thermal decomposition of the lithium salts of the corresponding Δ^5-7-tosylhydrazones [93, 94]. A widespread method of introducing a Δ^7-bond into Δ^5-steroids is allyl bromination followed by dehydrobromination [6]. The allyl bromination of cholesterol benzoate gives an equilibrium mixture of the 7α- and 7β-epimers.



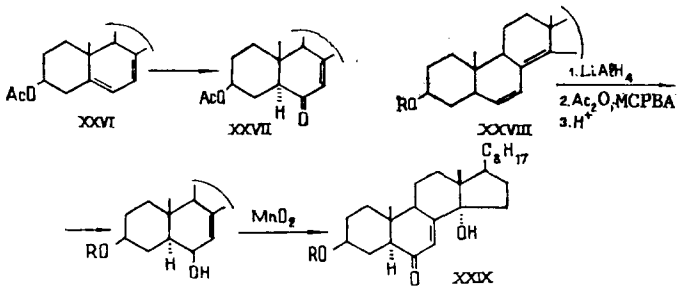
It has been shown that thanks to the allyl rearrangement which the 7β-epimer undergoes, three substances exist in the equilibrium – the 7α- and 7β-bromocholesterol esters (XIII and XIV) and 5α-bromocholest-6-en-3β-ol (XV) [95]. Their structures were confirmed by dehydrobromination to the corresponding 5,7- and 4,6-dienes (XVI and XVII). It was established that the rate of rearrangement of the 7-bromo derivative and, consequently, the nature of the dienes formed are affected by such factors as the solvent, the temperature, and to a considerable degree, the base used for dehydrobromination [95]. Organic and inorganic bases possess no selective action, which leads to a mixture of isomers. When hydroxyalkylpyridines were used as catalysts, an important role of the position of the hydroxy group in the catalyst for the selective formation of 5,7- or 4,6-dienes was observed. Dehydrobromination in the presence of 2-(hydroxyalkyl)pyridines led to the 5,7-diene, while catalysts by 3-(hydroxyalkyl)pyridines led mainly to the 4,6-diene [95]. On the dehydrobromination of 7-bromocholesterol benzoate with sodium bicarbonate in the presence of α-picoline, the yield of the 5,7-diene amounted to 66% and that of the 4,6-diene to 25%; in the presence of sym-collidine, the 5,7-diene was obtained with a yield of 39% and the 4,6-diene with one of 36% [95]. However, up to the present time it has not been possible to devise conditions for shifting the equilibrium of the epimeric mixture of bromides in the direction of the formation of the 7α-epimer and to obtain the 5,7-diene in high yield. To separate the 5,7- and 4,6-dienes, the reaction mixture after dehydrobromination was treated with 4-phenyl-1,2,4-triazoline-3,5-diol in order to convert the 5,7-diene into an adduct the treatment of which with sodium dihydrobis(2-methoxyethoxy)aluminate or lithium tetrahydroaluminate in THF led to the isolation of the individual 5,7-diene [95]. Anastasia [61] has shown that on the reduction of the adduct (XVIII) obtained from ergosterol with lithium in ethylamine, a mixture of the Δ^5- and Δ^7-olefins (XIX and XX, respectively) is formed.



A more effective method of synthesizing and isolating the desired 5,7-diene consists in the use of the allyl 7ξ-selenides (XXI), the oxidation of which with 90% H_2O_2 leads to a readily separable mixture of (XXII) and (XXIII) [96]. The authors explain the formation of these products by different directions of the reactions of the intermediate selenoxides (XXIV and XXV).

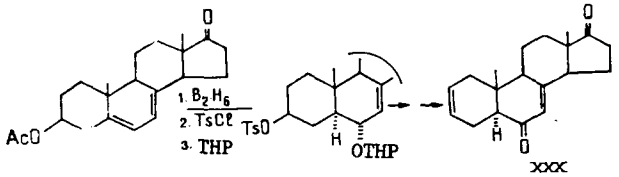


The 5,7-dienic systems synthesized by the methods described above have been used to introduce a 6-keto group. Thus, the oxidation of ergosterol acetate with chromium trioxide in AcOH followed by reduction by zinc dust in AcOH gave the enone (XXVII) [6, 95]. The reaction of cholesterol-5,7-diene with sulfur dioxide in pyridine [97] led to the isomeric 6,8(14)-diene (XXVIII), the subsequent treatment of which with lithium tetrahydroaluminate,

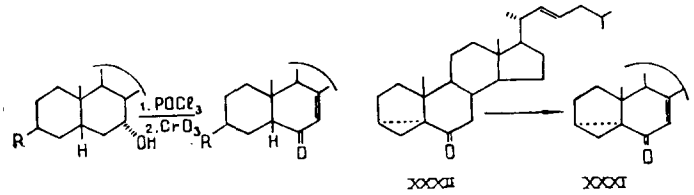


acetylation, epoxidation, hydrolysis and oxidation with manganese dioxide permitted the isolation of the Δ^7-14α-hydroxy-6-keto analog of cholesterol (XXIX).

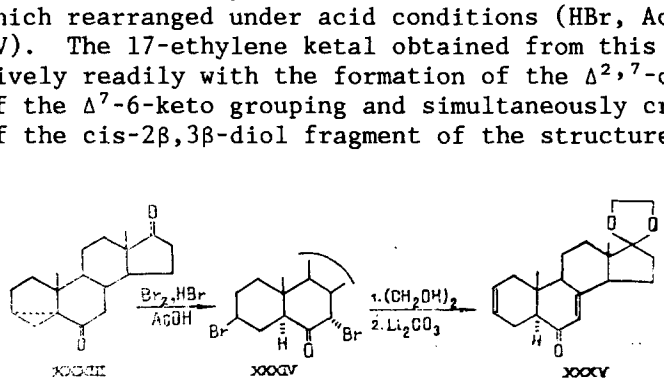
The hydroboration–oxidation reaction [84] has been used for the conversion of the $\Delta^{5,7}$-diene system of 17-ketones of the androstane series, as is shown in the scheme given below. The presence of a Δ^2-bond in compound (XXX) permits its use for the introduction of the cis-diol fragment, as has been shown in the literature [39, 40, 47, 59, 63, 66, 67, 82, 83, 88-91, 98-103].



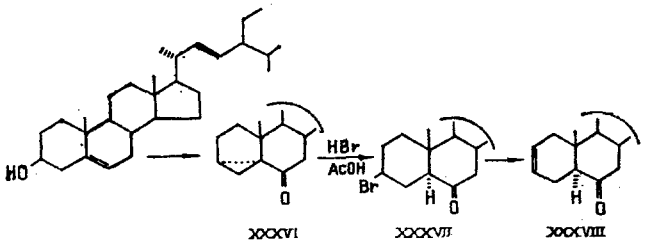
Other starting compounds for creating a Δ^7-6-keto grouping may be either a 7-hydroxy compound (for example, a cholic acid derivative) or saturated 6-ketones [104, 105, 107, 108].



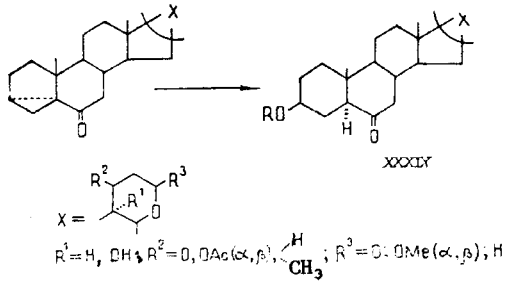
For the initial introduction of a Δ^7-6-keto fragment, which is characteristic for the structure of rings A/B of ecdysones, synthesis via 3,5a-cyclosteroids has found wide use [6]. A method of synthesizing the Δ^7-6-keto-3,5α-cyclosteroid (XXXI) from (XXXII) [66] proved to be unusual, and the authors concerned explained it by the oxidation of the enolic form of the ketone (XXXII) under the action of trifluoroperacetic acid (TFPAA) followed by the conversion of the epoxyalcohol formed into the 7-hydroxy-6-ketone and the dehydration of the latter. The bromination of a cyclosteroid of the androstane series (XXXIII) led to the 3β,5α-dibromide, which rearranged under acid conditions (HBr, AcOH) into the 3β,7α-dibromo derivative (XXXIV). The 17-ethylene ketal obtained from this compound split off two HBr molecules comparatively readily with the formation of the $\Delta^{2,7}$-diene (XXXV). This confirmed the formation of the Δ^7-6-keto grouping and simultaneously created the conditions for the introduction of the cis-2β,3β-diol fragment of the structure of rings A/B of the ecdysones [6].



On applying the same sequence of reactions to stigmasterol [66], the cyclic ketone (XXXVI) was obtained, the regioselective opening of the three-membered ring of which led to the desired 3β-bromo-6-ketone (XXXVII). Subsequent dehydrobromination enabled the Δ^2-derivative of stigmasterol (XXXVIII) to be obtained.



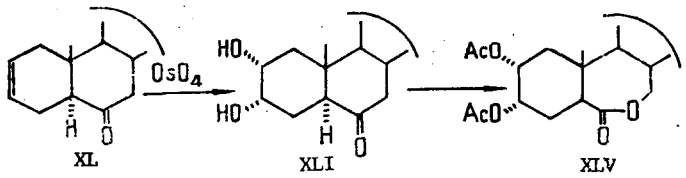
3,5α-Cyclosteroids have served as convenient intermediates for the synthesis of 5αH-6-ketosteroids (XXXIX) with an additional pyran ring E, which possess an antiecdysone action [109-115].



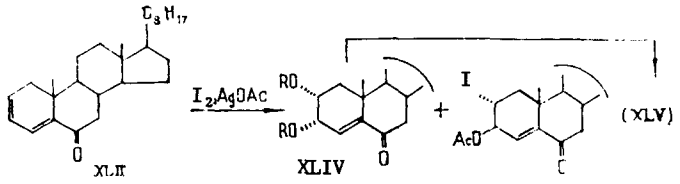
As a rule, natural ecdysteroids have the A/B-cis-linkage of the ring, which is achieved by epimerization at the C-5 center under the conditions of alkaline hydrolysis [84, 85, 107].

CREATION OF THE STRUCTURE OF RINGS A/B OF THE BRASSINOLIDES

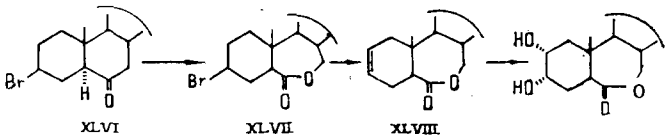
Characteristic structural elements of rings A/B of the brassinolides are: a 2α,3α-cis-diol grouping (in contrast to the 2β,3β-cis-diol group in the ecdysones), and ring B in the form of a seven-membered lactone [80]. To introduce a B-homo-7-oxa-6-ketone grouping into the steroid molecule, wide use is made of the Baeyer–Villiger oxidation of 6-ketosteroids. It is most effective to use TFPAA for these purposes [39, 40, 47, 63, 66, 99-103, 116-118], which is due to its comparative stability and high oxidizing capacity, the mild conditions of performing the reaction, and the predominant formation of one (7-oxa-6-keto) of the two possible regioisomeric lactones. Electron-donating substituents in the 1α-, 2α-, 2β-, 3α-, and 3β-positions and a 2α,3α-dihydroxy or -diacetoxy grouping lead to an increase in the yield of the 7-oxa compounds [71]. In a number of studies on the synthesis of brassinolides [39, 40, 47, 63, 66, 99-103, 116-118], one and the same sequence of reactions has been used to construct the A/B rings, namely: Δ^2-6-ketosteroids (XL), obtained by one or other of the known methods [6, 84, 119] are subjected to hydroxylation with osmium tetroxide [39, 40, 47, 59, 63, 66, 99-103] to the 2α,3α-dihydroxy-6-ketones (XLI), in contrast to Woodward hydroxylation, which leads to the 2β,3β-diols.



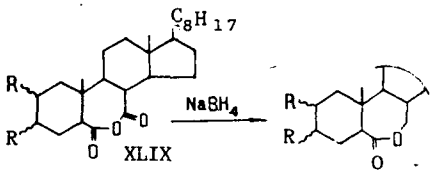
It has been shown that the stereochemistry of the cis-hydroxylation reaction is affected not only by the nature of the hydroxylating agent but also by the structure of the initial steroid [98]. Thus, the presence of a second double bond at Δ^4 in the Δ^2-ketosteroid (XLII) leads to a change in the spatial direction of Woodward's cis-hydroxylation reaction, as a result of which the 2α,3α-dihydroxy-6-ketone derivatives (XLIV) are formed, and this permits ring A of the brassinolides to be created by this method. The 2α,3α-dihydroxy-6-ketones obtained, in the form of the diacetates are oxidized by the Baeyer–Villiger method to the 7-oxa-6-ketones (XLV).



A different sequence of reactions in the construction of rings A/B of the brassinolides has been proposed by Akhrem, et al. [67]. A lactone grouping is first created in ring B. The oxidation of 3β-bromo-5α-cholestan-6-one (XLVI) by the Baeyer–Villiger method gives the 7-oxa-6-ketone (XLVII). The introduction of a Δ^2-bond and the formation of the ketolactone (XLVIII) is achieved by the dehydrobromination reaction. The performance of hydroxylation in the concluding stage avoids the necessity for protecting the 2α,3α-dihydroxy group and for the subsequent removal of the protection and shortens the total number of stages for obtaining the desired product.

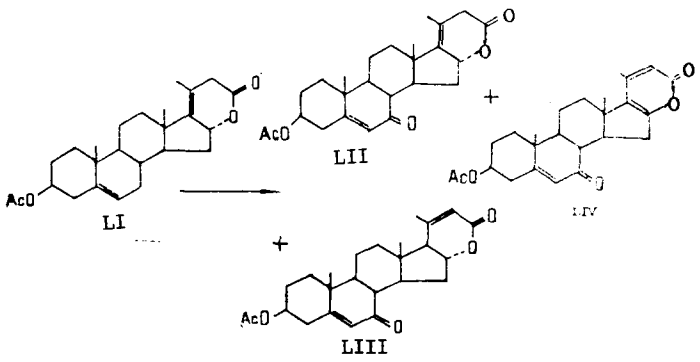


The construction of the B-homo-7-oxa-6-keto grouping of ring B of a brassinolide can be done on the basis of the cyclic anhydrides of the corresponding acids, which are obtained through the 6-keto- and 7α-bromo-6-ketosteroids. Anhydrides of 6,7-seco-5α-cholestane-6,7-dicarboxylic acids (XLIX) and of their analogs (3β-Cl, 3β-OMe, 3β-OAc, 3β-OCOPh, and 2α,3α-di-OAc) are reduced regioselectively with the aid of sodium tetrahydroborate to the corresponding B-homo-7-oxa-6-keto-5-α-cholestanes (L) [120].



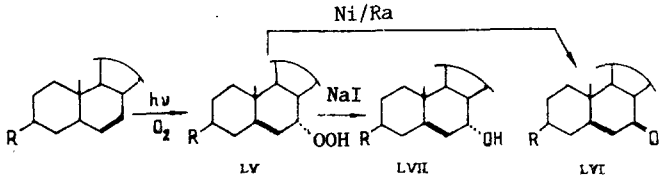
METHODS OF CREATING THE STRUCTURE OF RINGS A/B OF STEROIDS OF THE ANTHERIDIOL GROUP

Characteristic for steroids of the antheridiol group is the presence of 7-keto or 7-hydroxy functions in ring B [81]. Such groupings are created by the allyl oxidation of Δ^5-olefinic steroids with various side chains. The oxidizing agents used are Cr^{VI} in the form of the oxide in various solvents [121-125], in the form of complexes with pyridine [126-128], and in the form of a complex with 3,5-dimethylpyrazole (DMP) [129, 130], or in the form of sodium or potassium chromate [131-134]. Reactions with tert-butyl chromate [132, 134-136] take place at an elevated temperature (60-80°C) in 10-70 h, the yields of final products varying within wide limits. The use of the complex $CrO_3 \cdot 2Py$ in methylene chloride leads, unlike oxidation bv tert-butyl chromate, to a selectivity of the process in connection with



a decrease in the formation of peroxide products, which has been shown for the cases of the oxidation of androstenediol and cholesterol acetates [127, 137]. The allyl oxidation of Δ^5-steroids with δ-lactone rings E (LI) by sodium chlorate or the complex of chromium trioxide with DMP has led to the synthesis of the 7-keto derivatives (LII, LIII, and LIV), differing by the number and positions of the double bonds in the lactone ring E and possessing cytostatic activity [138].

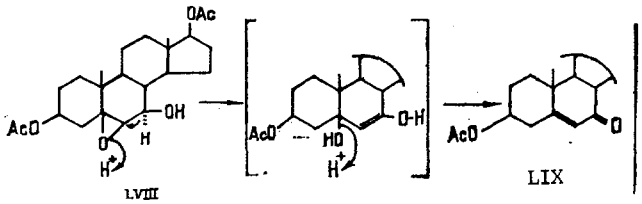
Pearson [139, 140] has shown that on the oxidation of Δ^5- and Δ^6-steroids with tert-butyl hydroperoxide in the presence of hexacarbonylchromium the corresponding 7-keto derivatives are formed. The photooxidation of Δ^5-precursors is widely used for the introduction of a 7-keto group [141], the direction of the reaction being greatly affected by the chemical nature of the solvent used [122, 142-144]. The photooxidation of Δ^6-steroids takes place stereospecifically and leads exclusively to the 7α-hydroperoxides (LV), the decomposition of which to the enones (LVI) is performed by treatment with Raney Ni in pyridine, while reduction to the alcohol (LVII) is carried out with the aid of sodium iodide.



LV LVII LVI

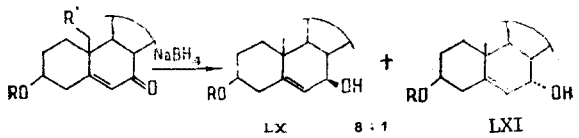
The formation of C-7-oxidized products shows the cis stereochemistry of the photooxidation of Δ^6-steroids [122, 141].

Δ^5-7-Ketosteroids can be formed as the result of various rearrangements. Thus, in the acid-catalyzed reaction of a 7β-hydroxy-5β,6β-epoxide, the initial stage is the opening of the epoxide ring, and the enediol formed rearranges into the corresponding Δ^5-7-ketone [145, 146]. This has been shown, as an example, for the interaction (−)-3β,17β-diacetoxy-5β,6β-epoxyandrostan-7β-ol (LVIII) with phosphorus oxychloride, as a result of which the 7-ketone (LIX) was isolated.



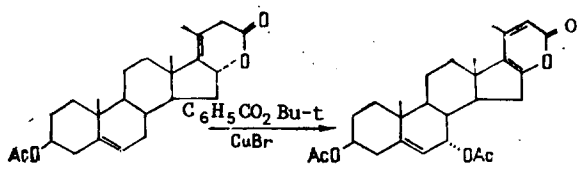
LVIII LIX

7-Hydroxysteroids are obtained in the reduction of the corresponding 7-keto derivatives, and the stereochemistry of the substituent C-7 depends on the nature of the reducing agent. Thus, the Meerwein–Ponndorf reduction of 7-ketones with aluminum isopropanolate leads to a mixture of 7α- and 7β-hydroxy derivatives [147]. In the case of reduction with $NiAlH_4$, the main product is the 7β-hydroxy derivative (LX) [148]. To obtain its 7α-epimer (LXI), the reaction is performed in two stages: the replacement of the hydroxy group by chlorine with retention of the configuration, and the subsequent acetolysis of the 7β-chloro derivative formed. On the reduction of 7-ketones with sodium tetrahydroborate, the reaction takes place less stereospecifically with the formation of the 7β- and 7α-epimers in a ratio of 8:1 [138, 149].



LX 8 : 1 LXI

The use of $t\text{-}Bu_3LiAlH$ as reducing agent led, in the case of the Δ^5-7-ketones, to the corresponding Δ^5-7β-alcohols, while in the case of saturated steroid 7-ketones of Δ^4-7-ketones it led to the 7α-hydroxy derivatives [146]. The radical oxidation of $\Delta^{5,17(20)}$-lactone with tert-butyl perbenzoate in acetic acid in the presence of cuprous bromide gave the 7α-acetoxysteroid $\Delta^{16,20(22)}$-lactone (LXII) [138].



LXII

It is not difficult to see from the material that has been considered that the high biological significance of natural steroids has led to the development of numerous methods for introducing oxygen-containing substituents both in the side chain and into rings A/B, and some of these methods have become traditional and are being used in the synthesis not only of the natural steroids but also of their transformed analogs. Here the choice of the method of modifying the given sections of the molecule depends in each case on the chemical nature of substituents in the side chain and in the D region of the steroid molecule.

LITERATURE CITED

1. D. M. Piatak and J. Wicha, Chem. Rev., 78, 199 (1978).
2. J. Redpath and F. J. Zeelen, J. Chem. Soc. Rev., 12, 75 (1983).
3. K. S. Kyler, A. Bashir-Hashemi, and D. S. Watt, J. Org. Chem., 49, 1084 (1984).
4. T. Kametani, K. Katoh, M. Tsubuki, and T. Honda, J. Am. Chem. Soc., 108, 7055 (1986).
5. P. A. Bartlett, Tetrahedron, 36, 2 (1980).
6. A. A. Akhrem, I. S. Levina, and Yu. A. Titov, Ecdysones — Steroid Hormones of Insects [in Russian], Minsk (1973).
7. T. Kametani, M. Tsubuki, H. Furuyama, and T. Honda, J. Chem. Soc. Chem. Commun., 375 (1984).
8. Y. Mazur, D. Freeman, and H. Cheves, Israeli Patent IL55409 (1982); Chem. Abstr., 98, 89739s (1983).
9. M. Koreeda, Y. Tanaka, and A. Schwartz, J. Org. Chem., 45, 1172 (1980).
10. M. Tanabe and K. Hayashi, J. Am. Chem. Soc., 102, 862 (1980).
11. J. Tsuji, Tetrahedron, 42, 4301 (1986).
12. T. Takahashi, A. Ootake, and J. Tsuji, Tetrahedron Lett., 25, 1921 (1984).
13. J. P. Marino and H. Abe, J. Am. Chem. Soc., 103, 2907 (1981).
14. M. Wovkulich, A. D. Batcho, and M. R. Uskokovic, Helv. Chim. Acta, 67, 612 (1984).
15. W. G. Dauben and T. Brookhart, J. Am. Chem. Soc., 103, 237 (1981).
16. M. M. Midland and Y. C. Kwon, J. Org. Chem., 46, 229 (1981).
17. M. M. Midland and Y. C. Kwon, Tetrahedron Lett., 23, 2077 (1982).
18. S. Noguchi and M. Obayashi, Japanese Patent No. 71.25.375; Chem. Abstr., 75, 141064j (1971).
19. S. Noguchi, Chem. Pharm. Bull., 27, 1352 (1979).
20. S. Noguchi and K. Morita, Chem. Pharm Bull., 11, 1235 (1963).
21. S. Noguchi, H. Otsaka, M. Obayashi, M. Imanishi, and T. Takahashi, Steroids, 11, 9 (1968).
22. M. Obayashi, E. Mizuta, and S. Noguchi, Chem. Pharm. Bull., 27, 1679 (1979).
23. E. J. Agnello and S. K. Figdor, J. Org. Chem., 28, 1531 (1963).
24. A. F. Hirsch and G. I. Fujimoto, J. Org. Chem., 35, 495 (1970).
25. E. J. Agnello, Experientia, 16, 357 (1960).
26. E. J. Agnello and G. D. Laubach, US Patent No. 3234095 (1966); Chem. Abstr., 64, 12766h (1966).
27. A. A. Akhrem, A. V. Kamernitskii, and I. G. Reshetova, Izv. Akad. Nauk SSSR, Ser. Khim., 1364 (1972).
28. A. A. Akhrem, A. V. Kamernitskii, A. A. Obynochnyi, and I. G. Reshetova, Izv. Akad. Nauk SSSR, Ser. Khim., 1622 (1972).
29. I. A. Akhrem, A. V. Kamernitskii, I. G. Reshetova, and I. I. Voznesenskaya, Izv. Akad. Nauk SSSR, Ser. Khim., 2573 (1972).
30. A. A. Akhrem, A. V. Kamernitskii, and I. G. Reshetova, Izv. Akad. Nauk SSSR, Ser. Khim., 1128 (1973).
31. P. P. Kolikov, A. V. Kamernitskii, I. G. Reshetova, and K. Yu. Chernyuk, Problemy Éndokrinologii, 100 (1977).
32. P. P. Golikov, A. S. Bobkova, A. V. Kamernitskii, and I. G. Reshetova, Farmakol. Toksikol., 89 (1982).
33. P. P. Golikov, A. S. Bobkova, A. V. Kamernitzky (Kamernitskii), and I. G. Reshetova, Endokrinologie, 80, 18 (1982).

34. I. V. Torgov, Izv. Akad. Nauk SSSR, Ser. Khim., 299 (1982).
35. W. Sukrow and M. Van Nooy, Ann. Chem., 1987 (1982).
36. K. Schönauer and E. Zbiral, Tetrahedron Lett., 24, 573 (1983).
37. M. Adamczyk and E. K. Dolence, J. Org. Chem., 49, 1378 (1984).
38. J. M. Midley and R. M. Upton, J. Chem. Res., 2513 (1983).
39. S. Takatsuto, N. Yazawa, M. Ishiguro, M. Morisaki, and N. Ikekawa, J. Chem. Soc., Perkin Trans. I, 139 (1984).
40. M. Ishiguro, S. Takatsuto, M. Morisaki, and N. Ikekawa, J. Chem. Soc., Chem. Commun., 962 (1980).
41. M. N. Galbraith, D. H. S. Horn, and B. A. Kelly, Aust. J. Chem., 34, 2607 (1981).
42. C. Hetru and Y. Nakatani, Nouv. J. Chem., 7, 587 (1983).
43. M. W. Preus and T. C. McMorris, J. Am. Chem. Soc., 101, 3066 (1979).
44. M. Anastasia, A. Fiecchi, and A. Scala, J. Chem. Soc., Chem. Commun., 858 (1979).
45. S. Takatsuto, M. Ishiguro, and N. Ikekawa, J. Chem. Soc., Chem. Commun., 258 (1982).
46. T. Yokota, Agric. Biol. Chem., 47, 659, 1409, 2149 (1983).
47. S. Fung and J. B. Siddal, J. Am. Chem. Soc., 102, 6580 (1984).
48. K. Mori, M. Sakakibara, and K. Okada, Tetrahedron, 40, 1767 (1984).
49. A. Bagchi, P. Neogi, P. Sahai, and A. B. Ray, Phytochemistry, 26, 353 (1984).
50. J. R. Wiersig, N. Waespe-Sercevic, and C. Djerassi, J. Org. Chem., 44, 3374 (1979).
51. T. C. McMorris, J. Org. Chem., 48, 3370 (1983).
52. P. H. Le, M. W. Preus, and T. C. McMorris, J. Org. Chem., 47, 2163 (1984).
53. T. Gebreyesus and C. Djerassi, J. Org. Chem., 49, 987 (1984).
54. R. D. Walkup, C. D. Anderson, and C. Djerassi, Tetrahedron Lett. 767 (1979).
55. P. Koch, Y. Nakatani, B. Lui, and G. Ourisson, Bull. Soc. Chim., 189 (1983).
56. M. Ohmori, S. Yamada, and H. Takayama, Tetrahedron Lett., 23, 4709 (1982).
57. F. J. Sardina, A. Mourino, and L. Castedo, Tetrahedron Lett., 24, 4477 (1983).
58. S. Takatsuto, N. Yazawa, and N. Ikekawa, Phytochemistry, 22, 13 (1983).
59. M. J. Tompson and N. Mandava, J. Org. Chem., 44, 5002 (1979).
60. M. J. Tompson, Steroids, 3, 567 (1981).
61. M. Anastasia, P. Giuffreda, and A. Fiecchi, J. Chem. Soc., Perkin Trans. I, 379 (1983).
62. M. Anastasia, J. Chem. Soc., Perkin Trans. I, 383 (1983).
63. S. Takatsuto and N. Ikekawa, Chem. Pharm. Bull., 30, 4181 (1982).
64. S. Takatsuto and N. Ikekawa, J. Chem. Soc., Perkin Trans. I, 439 (1984).
65. M. Sakakibara and K. Mori, Agric. Biol. Chem., 47, 1405, 1409 (1983).
66. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, V. N. Zhabinskii, and N. V. Kovganko, Dokl. Akad. Nauk SSSR, 275, 1089 (1984).
67. A. A. Akhrem, F. A. Lakhvich, V. A. Khripach, and N. V. Kovganko, Dokl. Akad. Nauk SSSR, 269, 366 (1983).
68. Y. Hirano and C. Djerassi, J. Org. Chem., 47, 2420 (1982).
69. H. L. Goering, S. S. Kanther, and Ch. Ch. Tseng, J. Org. Chem., 48, 715 (1983).
70. S. Takatsuto and N. Ikekawa, Phytochemistry, 23, 525 (1984).
71. S. Takatsuto and N. Ikekawa, Tetrahedron Lett., 24, 917 (1983).
72. C. Y. Byon, M. Gut, and V. Toome, J. Org. Chem., 46, 3901 (1981).
73. M. Karpf and C. Djerassi, Tetrahedron Lett., 21, 1603 (1980).
74. M. Ishiguro, N. Koizumi, M. Yasuda, and N. Ikekawa, J. Chem. Soc., Chem. Commun., 115 (1981).
75. Y. Fujimoto and N. Ikekawa, J. Org. Chem., 44, 1011 (1979).
76. A. N. Catalan, W. C. M. Kokke, C. Duque, and C. Djerassi, J. Org. Chem., 48, 5207 (1983).
77. A. G. Gonzalez, Tetrahedron Lett., 4289 (1974).
78. G. R. Pettit, J. Am. Chem. Soc., 100, 7781 (1978).
79. R. Breslow, U. Maitra, and D. Heyer, Tetrahedron Lett., 25, 1123 (1984).
80. G. Adam and V. Marquardt, Phytochemistry, 8, 787 (1986).
81. J. E. Cook, J. G. Lloyd-Jones, H. H. Rees, and T. W. Goodwin, J. Biochem., 136, 135 (1973).
82. F. D. Gunstone, in: Advances in Organic Chemistry, R. A. Raphael, E. C. Taylor, and H. Wynbenrg (eds.), Vol. 1, Interscience Publ., New York (1960), p. 103.
83. J. M. Cornforth and D. T. Green, J. Chem. Soc. (c), 846 (1970).
84. R. Capito, L. Mangoni, P. Monaco, G. Palumbo, and L. Previtera, Gazz. Chim. Ital., 109, 651 (1979).
85. E. Lee, Y. T. Liu, P. H. Solomon, and K. Nakanishi, J. Am. Chem. Soc., 98, 1634 (1976).
86. K. T. Alston, P. M. Bebbington, S. E. Green, E. D. Morgan, and C. F. Pole, Steroids, 27, 609 (1976).

87. H. Lettre, J. Greiner, K. Rutz, L. Hoffman, A. Egle, and W. Beiger, Ann. Chem., 758, 89 (1972).
88. L. Mangoni, M. Adinolfi, G. Barone, and M. Parrilli, Tetrahedron Lett., No. 45, 4485 (1973).
89. R. C. Cambie, R. C. Hayward, J. L. Roberts, and P. S. Rutledge, J. Chem. Soc., Chem. Commun., 359 (1973).
90. E. Glotter and A. Schwartz, J. Chem. Soc., Perkin Trans. I, 1660 (1976).
91. R. B. Woodward and F. V. Brutcher, J. Am. Chem. Soc., 80, 209 (1958).
92. J. Herran, G. Rozenkranz, and F. Sondheimer, J. Am. Chem. Soc., 76, 5531 (1954).
93. E. V. Yablonskaya and G. M. Segal', Khim. Prir. Soedin., 739 (1973).
94. J. Jaczczynski, R. R. Sicinsky, and W. J. Rodewald, Polish J. Chem., 58, 711 (1984).
95. R. I. Yakhimovich, The Chemistry of the Vitamins D [in Russian], Naukova Dumka, Kiev (1978).
96. W. G. Salmond, M. A. Barta, A. M. Cain, and M. Sobala, Tetrahedron Lett., 1683 (1977).
97. K. Wada, Agric. Biol. Chem., 39, 1679 (1975).
98. A. A. Akhrem, F. A. Lakhvin, V. A. Khripach, and N. V. Kovganko, Dokl. Akad. Nauk SSSR, 257, 1133 (1981).
99. S. Takatsuto and N. Ikekawa, Chem. Pharm. Bull., 32, 2001 (1984).
100. Wei-Shan Zhou and Wei-Sheng Tian, Acta Chim. Sin., 42, 1173 (1984).
101. M. Sakakibara, K. Okada, Y. Ichikawa, and K. Mori, Heterocycles, 17, 301 (1982).
102. K. Mori, M. Sakakibara, Y. Ichikawa, H. Ueda, K. Okada, T. Umemura, et al., Tetrahedron, No. 38, 2099 (1982).
103. S. Takatsuto and N. Ikekawa, J. Chem. Soc., Perkin Trans. I, 2133 (1983).
104. M. N. Galbraith, D. H. S. Horn, B. A. Kelly, J. F. Kinnear, M. D. Martin, E. J. Middleton, and C. T. F. Virgona, Aust. J. Chem., 34, 2607 (1981).
105. J. R. Dias, J. Chem. Eng. Data, 22, 445 (1977).
106. J. F. Kinear, M. D. Martin, D. H. S. Horn, E. J. Middleton, J. S. Wilkie, M. N. Galbraith, R. I. Willing, Aust. J. Chem., 29, 1815 (1976).
107. Y. M. Lee, E. Lee, and K. Nakanishi, Tetrahedron Lett., 21, 4323 (1980).
108. W. B. Smith, G. P. Newsoroff, and N. Y. Wu, Steroids, 21, 609 (1973).
109. A. V. Kamernitskii, V. G. Levi, and I. G. Reshetova, Izv. Akad. Nauk SSSR, Ser. Khim., 1156 (1982).
110. A. V. Kamernitskii, V. G. Levi, I. G. Reshetova, and E. I. Chernoburova, Khim. Prir. Soedin., 732 (1983).
111. A. V. Kamernitskii, V. G. Levi, I. G. Reshetova, V. S. Bogdanov, and E. G. Cherepanova, Izv. Akad. Nauk SSSR, Ser. Khim., 1153 (1984).
112. V. E. Shklover, S. V. Lindeman, Yu. T. Struchkov, A. V. Kamernitzky (Kamernitskii), V. G. Levy, and I. G. Reshetova, Cryst. Struct. Comm., 11, 1873 (1982).
113. A. V. Kamernitskii, E. I. Chernoburova, and I. G. Reshetova, Khim. Prir. Soedin., 190 (1983).
114. A. V. Kamernitskii, V. A. Krivoruchko, I. G. Reshetova, and E. I. Chernoburova, Khim. Prir. Soedin., 184 (1981).
115. A. V. Kamernitskii, V. A. Krivoruchko, I. G. Reshetova, and B. M. Zolotarev, Izv. Akad. Nauk SSSR, Ser. Khim., 1394 (1980).
116. K. Mori, Agric. Biol. Chem., 44, 1211 (1980).
117. S. Takatsuto, B. Ying, M. Morisaki, and M. Ikekawa, Chem. Pharm. Bull., 29, 903 (1981).
118. M. J. Tompson, W. J. Meudt, N. B. Mandava, S. R. Dutky, W. R. Lusby, and D. W. Spaulding, Steroids, 39, 89 (1982).
119. R. B. Mitra, B. G. Hazza, and V. M. Kapoor, Indian J. Chem., 23B, 106 (1984).
120. C. J. W. Brooks and I. V. Ekhato, J. Chem. Soc., Chem. Commun., 943 (1982).
121. K. B. Wiberg and S. D. Nielsen, Org. Chem., 29, 3353 (1964).
122. W. A. Noyes and A. S. Hammond, Adv. Photochem., 6, 2280 (1968).
123. H. Velgova, J. Smolikova, A. Tzka, and A. Vitek, Collect. Czech. Chem. Commun., 47, 2280 (1982).
124. K. P. Cheng, H. Nagano, L. Bang, and G. Ourisson, J. Chem. Res., (M), 2501 (1977).
125. J. Fajkos and J. Joska, Collect. Czech. Chem. Commun., 43, 1142 (1978).
126. J. H. Dygos and B. N. Desai, J. Org. Chem., 44, 1590 (1979).
127. W. G. Dauben, M. Lorber, and D. S. Fullerton, J. Org. Chem., 34, 3587 (1969).
128. Y. Itiro and K. Takeatsu, Chem. Pharm. Bull., 13, 1430 (1965).
129. W. G. Salmond, M. A. Barta, and J. L. Havens, J. Org. Chem., 43, 2057 (1978).
130. T. C. McMorris, P. H. Le, M. W. Preus, S. R. Schow, and G. R. Weihe, J. Org. Chem., 48, 3370 (1983).

131. S. Bory, D. J. Lin, and M. Fetizon, Bull. Soc. Chim., 1298 (1971).
132. C. W. Marshall, R. E. Ray, I. Laos, and B. Riegel, J. Am. Chem. Soc., 79, 6308 (1957).
133. H. Hiroshi, S. Yoshioko, O. Tomoko, K. Kaeko, M. Shinichi, and N. Toshio, Chem. Pharm. Bull., 26, 2210 (1978).
134. H. Nagano, J. P. Poyser, K. P. Cheng, L. Bang, G. Ourisson, and J. P. Beck, J. Chem. Res. (S), 217 (1977).
135. K. Heusler and A. Wettstein, Helv. Chim. Acta, 35, 284 (1952).
136. W. Klyne, J. Chem. Soc., 3449 (1951).
137. E. J. Corey and R. H. Sneen, J. Am. Chem. Soc., 78, 6269 (1956).
138. A. V. Kamernitskii, I. G. Reshetova, E. I. Chernoburova, and N. E. Voishvillo, Khim.-farm. Zh., 1437 (1985).
139. J. Pearson, Y. S. Chen, S. Y. Hsu, and T. Ray, Tetrahedron Lett., 25, 1235 (1984).
140. A. J. Pearson, Y. S. Chen, G. R. Han, S. Y. Hsu, and T. Ray, J. Chem. Soc., Perkin Trans. I, 267 (1985).
141. A. Nichon and J. F. Bagli, J. Am. Chem. Soc., 83, 1498 (1961).
142. J. A. Edwards and J. S. Mills, J. Am. Chem. Soc., 91, 1248 (1973).
143. C. R. Popplestone and A. M. Unrau, Can. J. Chem., 51, 1223 (1973).
144. P. M. Green and J. A. Edwards, Tetrahedron, 27, 1199 (1971).
145. J. R. Hanson, A. W. Johnson, and M. A. C. Kaplan, J. Chem. Soc., Perkin Trans. I, 263 (1978).
146. D. Baldwin and J. R. Hanson, J. Chem. Soc., Perkin Trans. I, 1941 (1975).
147. A. Windaus, H. Lettre, and F. Schenk, Ann. Chem., 520, 98 (1935).
148. L. E. Fieser, M. Fieser,and R. N. Chakravarti, J. Am. Chem. Soc., 71, 2226 (1949).
149. I. Stary and P. Kocovsky, Coll. Czech. Chem. Commun., 50, 1227 (1985).

USE OF CARBOCYANINE DYES IN ANALYSIS OF BACTERIAL LIPOLYSACCHARIDES (ENDOTOXINS).

III. LIPOPOLYSACCHARIDES OF Yersinia enterocolitica

M. N. Mikheeva, R. P. Gorshkova, and L. I. Brutko

UDC 543.42.062.8

On the basis of a study of the conditions for the formation of associates of a carbocyanine dye with lipopolysaccharides, a new verification of the quantitative determination of these substances by a spectrophotometric method has been proposed.

A study of the possibility of replacing biological methods of estimating bacterial endotoxins – lipopolysaccharides (PLSs) – by suitable physicochemical methods is an extremely complex and important task. Its realization will permit a passage to the solution of the problem of determining ultramicro amounts of impurities of pyrogenic substances in parenteral medicinal forms.

Among the physicochemical methods of determining LPSs mention must be made of a colorimetric method proposed by Janda and Work [1] and studied in detail by Zey and Jackson [2]. This is based on the capacity of a carbocyanine dye for forming colored associates with LPSs the spectral maxima of which are shifted in the short-wave direction as compared with the maximum of the dye itself.

Analysis of literature information has shown that solutions of a dye in an acid medium – pH 4.05 – are usually used for the determination of LPSs. However, to obtain stable associates in this case it is necessary to add an antioxidant (ascorbic acid) to the reaction

All-Union Scientific-Research Institute of Pharmacy, Moscow. Pacific Ocean Institute of Bioorganic Chemistry, Far Eastern Scientific Center, Academy of Sciences of the USSR, Vladivostok. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 29-32, January-February, 1988. Original article submitted April 13, 1987; revision submitted July 10, 1987.

 0009-3130/88/2401-0022 $12.50