PARTIAL SYNTHESIS OF OSLADINE AGLYCONE FROM SOLASODINE*

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Osladine aglycone has been synthesized from solasodine (*I*) *via* (25*R*)-26-acetylamino-22-oxo-cholest-5-en-3β,16β-diol 16-acetate (*IIb*). Stereochemistry at $C_{(5)}$, $C_{(22)}$ and $C_{(25)}$ has been established and osladine aglycone formulated as (22*S*,25*R*,26*R*)-22,26-epoxy-6-oxo-5α-cholestan-3β,26-diol (*XVIa*).

Some time ago, two steroidal glycosides, osladine and polypodosaponin, were isolated at our Institute from the rhizomes of *Polypodium vulgare*. Osladine was shown[1-3] to be the sweet principle of the material and to have the structure of a bisdesmosidic glycoside of 22ξ,26-epoxy-6-oxo-5ξ,25ξ-cholestane-3β,26ξ-diol.

The aim of the present paper is to confirm the structure and to establish the stereochemistry at $C_{(5)}$, $C_{(22)}$, $C_{(25)}$ and $C_{(26)}$ of the osladine aglycone by its partial synthesis from the steroid alkaloid solasodine. This compound was chosen for this purpose since its structure permits easy modification of the side chain and since the center of chirality at $C_{(25)}$ has an unambiguously established configuration that can be preserved during the necessary series of transformations.

Solasodine (*I*) was first converted[4,5] into the 22-oxo derivative *IIa* *via* (25*R*)-3β,16β-diacetoxy-22,26-epimino-cholesta-5,22(N)-diene (pseudosolasodine-B). In the next step, selective reduction of the 22-oxo group was required but only partial success could be achieved both with sodium borohydride and lithium tri-tert-butoxyaluminium hydride. The 22-oxo group was found to react slowly and even when using a large excess of reagent the reduction of the 22-oxo group was incomplete. Under these conditions, the ester groups at $C_{(3)}$ and $C_{(16)}$ were also reduced so that a typical reaction mixture contained pairs of epimers *IIIa,b* and *IVa,b* as major products along with *IIb* and *Va,b*. Any attempt at completing the reduction led to an undesirable increase in the yield of the triols *Va,b* that could not be used in the subsequent procedure. The optimal approach proved to be a selective alkaline hydrolysis with potassium carbonate of *IIIa,b* to *IVa,b* yielding c. 65% of *IVa,b* from *IIa*. The pairs of 22-epimers of *III*–*V* could not be separated by thin-layer chromatography in a series of solvent systems. A chromatographic separation of 22-epimers

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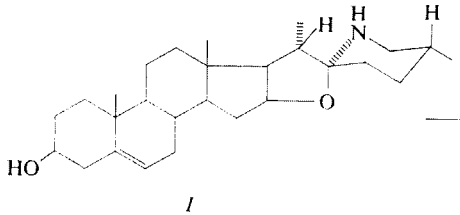
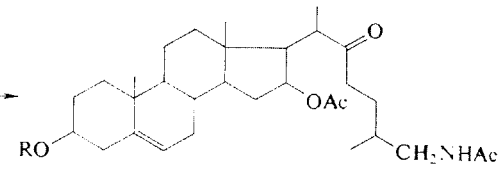
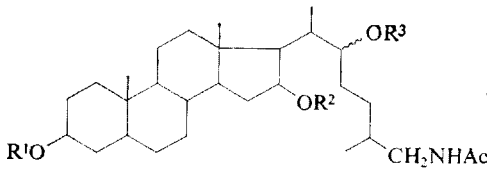


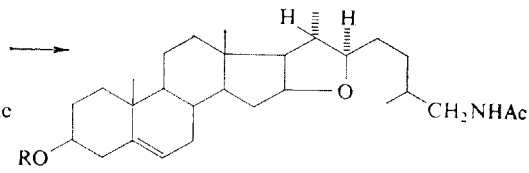
I



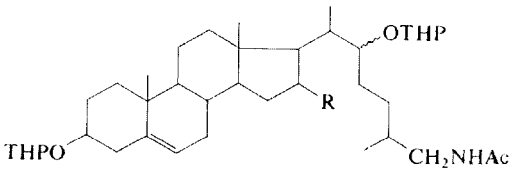
IIa, R = Ac
IIb, R = H



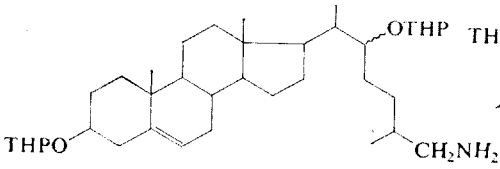
IIIa, $R^1 = R^2 = Ac$, $R^3 = H$, 22*S*
IIIb, $R^1 = R^2 = Ac$, $R^3 = H$, 22*R*
IVa, $R^1 = R^3 = H$, $R^2 = Ac$, 22*S*
IVb, $R^1 = R^3 = H$, $R^2 = Ac$, 22*R*
Va, $R^1 = R^2 = R^3 = H$, 22*S*
Vb, $R^1 = R^2 = R^3 = H$, 22*R*
VIa, $R^1 = R^3 = NO_2$, $R^2 = Ac$, 22*S*
VIb, $R^1 = R^3 = NO_2$, $R^2 = Ac$, 22*R*



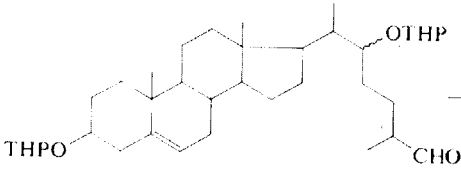
VIIa, $R = NO_2$
VIIb, R = H



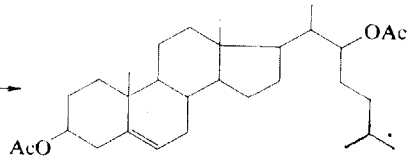
VIIIa, R = HOAc, 22*S*
VIIIb, R = HOAc, 22*R*
IXa, R = HOH, 22*S*
IXb, R = HOH, 22*R*
Xa, R = =O, 22*S*
Xb, R = =O, 22*R*
XIa, $R = H_2$, 22*S*
XIb, $R = H_2$, 22*R*



XIIa, 22*S*
XIIb, 22*R*



XIIIa, 22*S*
XIIIb, 22*R*



XVII

was achieved by using the nitrate esters *VIa* and *VIb* prepared in a conventional manner from the mixture *IVa,b*. The preponderant epimer (c. 70%). is more dextrorotatory than its 22-epimer; a series of compounds derived from this epimeric pair shows the same behavior (Table I). The known relationship[6,7] between the optical rotation and absolute configuration at $C_{(22)}$ permits the conclusion that the more dextrorotatory compounds *IVb*, *Vb*, *VIIIb*, *IXb*, *Xb* and *XIb* have the 22*R*-configuration.

The tempting idea of utilizing the nitrate group for protecting the hydroxyl in the subsequent step proved unfeasible. In the case of the (22*R*)-epimer *VIb*, an attempt at alkaline saponification of the 16-acetoxy group resulted in the formation of the furostane derivative *VIIa* owing to an intramolecular displacement at $C_{(22)}$. A similar result was obtained with the epimer *VIa*. For this reason, the nitrate ester *VIb* was converted to the diol *IVb* by treatment with zinc in acetic acid, an analogous procedure applied to *VIa* provided the pure *IVa*. Due to relative yields, the epimers of the (22*R*)-series were more accessible and the epimer *IVb* was therefore chosen for further synthesis. The following four steps were used for removal of the oxygen function from the position 16: preparation of tetrahydropyranyl derivative *VIIIb*, alkaline hydrolysis of the 16β-acetoxy group yielding the corresponding alcohol *IXb*, pyridine–chromium trioxide oxidation to *Xb* and the Huang-Minlon reduction of the latter providing the 16-deoxo derivative *XIIb*. All these steps were performed in the conventional manner without difficulties. Conversion of the 26-amino derivative *XIIb* into the 26-al *XIIIb* was achieved by application of the Ruschig method using diazabicycloundecene for dehydrohalogenation of the corresponding N-chloroamine; the reaction provided the aldehyde *XIIIb* in 65% yield.

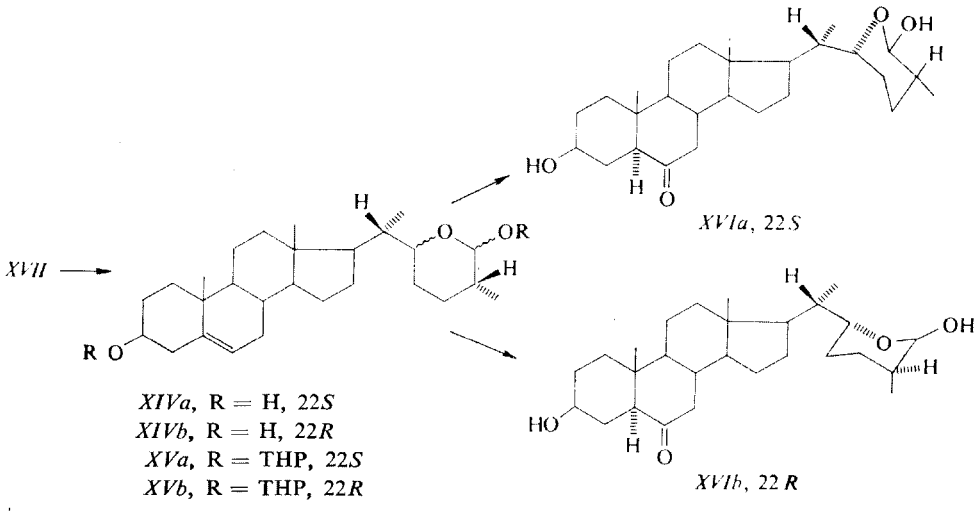


TABLE I

Specific Optical Rotations and Absolute Configurations of 22-Epimeric Alcohols and Their Derivatives

Compound	*VIa*	*VIb*	*IVa*	*IVb*	*VIIIa*	*VIIIb*
Absolute Configuration	*S*	*R*	*S*	*R*	*S*	*R*
$[\alpha]_D$	+10	+16	−5	+16	+8	+21
Compound	*IXa*	*IXb*	*Xa*	*Xb*	*XIa*	*XIb*
Absolute Configuration	*S*	*R*	*S*	*R*	*S*	*R*
$[\alpha]_D$	−46	−32	−148	−140	−23	−12

In order to check that no inversion at $C_{(22)}$ occurred in the course of these transformations, the aldehyde *XIIIb* was converted into the corresponding oxime, the latter reduced with lithium aluminum hydride and the product acetylated to give the starting acetamino derivative *XIb*.

Mild hydrolysis of *XIIIb* in dilute acetic acid recovered the 3-hydroxyl and led to formation of the hemiacetal *XIV*. Protection of both hydroxyls with tetrahydropyranyl groups, hydroboration of *XVb* followed by oxidation of the non-isolated 6-hydroxy derivative with chromium trioxide in pyridine followed by hydrolysis gave the compound *XVIb*. A comparison of this substance with the natural product showed, however, that the compounds were not identical. The same synthetic procedure was then applied to the (22*S*)-epimer *IVa* yielding the compound *XVIa*. Comparison of this substance with osladine aglycone showed identity in R_F-values, mixed m.p., IR, NMR and mass spectra.

The synthesis settles the stereochemistry at $C_{(5)}$, $C_{(22)}$, and $C_{(25)}$. The α-configuration at $C_{(5)}$ follows from the known steric course of 5,6-double bond·hydroboration[8]. This finding is also in agreement with the reported[2] correlation of the osladine aglycone with polypodosapogenin: osladine aglycone 26-O-methyl ether is formed under strong alkaline conditions that only the thermodynamically more stable 5α-isomer can survive. The (22*S*)-configuration follows from the assignments for the intermediates in Table I. Although based only on the rule of the levorotatory contribution of the hydroxyl in the (22*S*)-epimer, the reliability of the rule is well established not only for 22-hydroxycholesterol but also for its esters by recent work[6,7]. Similar rules are also valid for 23- and 24-hydroxycholesterols. In order to confirm this conclusion by independent evidence, the aldehyde *XIIIb* was subjected to Huang-

-Minlon reduction followed by acid hydrolysis and acetylation to give the known[6] (22*R*)-22-hydroxycholesterol 3,22-diacetate. The (25*R*)-configuration follows from the correlation with solasodine. Formulation of the configuration at the remaining chiral center in the side chain of *XVIa* as 26*R* assumes the more stable equatorial orientation of the 26-hydroxyl group in the hemiacetal cycle formed under equilibrating conditions.

Thus, evidence presented in this paper leads to the formulation of osladine aglycone as (22*S*,25*R*,26*R*)-22,26-epoxy-6-oxo-5α-cholestane-3β,26-diol (*XVIa*).

EXPERIMENTAL

Melting points were measured on a Kofler block and are uncorrected. Solutions were dried with magnesium sulfate. Unless stated otherwise, optica lrotation were measured in chloroform. The infrared spectra were measured in chloroform on a Zeiss UR 10 spectrophotometer. The NMR spectra were measured on a Varian HA-100 instrument in deuteriochloroform using tetramethylsilane as an internal reference. Chemical shifts are expressed in δ-scale.

Hydride Reduction of *IIa*

a) Sodium borohydride (50 g) was added to a stirred and cooled solution of the 22-oxo derivative *IIa* (25 g) and the mixture allowed to stand at room temperature overnight, then diluted with water (3 l), the precipitate separated using a sintered glass filter, washed with water a dried. Chromatography of the product on silica gel (2 500 g) in chloroform–methanol (2·5%) gave the following fractions: *1*) 3β-hydroxy-22-oxo derivative *IIb* (5 g, 21%), m.p. 184–184·5°C (acetone–-hexane), $[\alpha]_D^{20}$ +19° (*c* 0·74). IR-spectrum: 1 251, 1 729 ($OCOCH_3$); 1 525, 1 667, 3 450 (NH. .$COCH_3$); 3 600 cm^{-1} (OH). NMR-spectrum: 0·875 s, 3 H (18-CH_3); 1·02 s, 3 H (19-CH_3); 0·89 d, J = 6·1 Hz, 3 H (27-CH_3); 1·13 d, J = 7·0 Hz, 3 H (21-CH_3); 1·98 s and 1·95 s (2 × $COCH_3$); 3·08 t, J = 5 Hz, 2 H (CH_2N); 3·57 br mt, 2 H (CHOH); 5·00 br mt, 1 H ($CHOCOCH_3$); 5·33 d, J = 5 Hz, 1 H (olefinic); 5·85 br mt, 1 H (NH). For $C_{31}H_{49}NO_5$ (515·7) calculated: 72·19% C, 9·58% H, 2·72% N; found: 72·52% C, 9·66% H, 2·55% N. *2*) Unseparable mixture of epimeric 22-diols *IIIa* and *IIIb* (8 g, 32%), m.p. 160–163°C, $[\alpha]_D^{20}$ +6° (*c* 0·74). IR-spectrum: 3 600 (OH); 1 255, 1 720 ($OCOCH_3$), 1 525, 1 663, 3 445 cm^{-1} ($NHCOCH_3$). NMR-spectrum: 0·91 s, 3 H and 1·02 s, 3 H (18- and 18-CH_3); 0·89 d, J = 6·4 Hz, 3 H (CH_3CH); 0·98 d, J = = 7 Hz, 3 H (CH_3CH); 1·97 s, 2·01 s, 2·03 s, (3 × $COCH_3$); 3·13 mt, 2 H (CH_2N), collapses to a 6 Hz doublet after CD_3COOD addition; 3·44 mt, 1 H (CHOH); 4·69 br mt, 1 H ($CHOCOCH_3$); 5·14 br mt, 1 H ($CHOCOCH_3$); 5·36 d, J = 5 Hz, 1 H (olefinic); 6·52 br t, J = 5 Hz, 1 H (exchangeable, NH). Mass spectrum: M^+ 559, *m/e* 499 (M – CH_3COOH), 439 (M – 2 × CH_3. .COOH), 424 (M – 2 × CH_3COOH–CH_3), 421 (M – 2 × CH_3COOH–H_2O), 253 (3,5,15- -androstatriene$^+$), 158 ($CH_3CONHCH_2(CH_3)CHCH_2CH_2CH{=}OH^+$). For $C_{33}H_{53}NO_6$ (559·8) calculated: 70·80% C, 9·54% H, 2·50% N; found: 70·85% C, 9·64% H, 2·22% N. *3*) Unseparable mixture of epimeric 22-diols *IVa* and *IVb* (8 g, 35%), m.p. 195–196°C, (acetone–ligroin), $[\alpha]_D^{21}$ +13° (*c* 0·76). IR-spectrum: 3 600 (OH); 1 259, 1 720 ($OCOCH_3$); 1 525, 1 663, 3 445 cm^{-1} ($NHCOCH_3$). Mass spectrum: M^+ not observed; *m/e* 457 (M – CH_3COOH), 442 (M – CH_3COOH–CH_3), 439 (M – CH_3COOH–H_2O), 424 (M – CH_3COOH–CH_3–H_2O) 158 ($CH_3CONHCH_2(CH_3)CHCH_2CH_2CH{=}OH^+$), 253 (3,5,15-androstatriene$^+$). NMR-spectrum (hexadeuteriodimethyl sulphoxide): partially overlapping pairs of signals in the methyl region, one component strongly predominant; further: 1·97 s and 2·04 s (2 × CH_3CO); 3·11 t,

2 H, $J = 6$ Hz (collapsing into a doublet after CH_3COOD addition, $J = 7$ Hz, CH_2N); 3·43 br mt, 2 H (CHOH); 5·10 br mt, 1 H ($CHOCOCH_3$); 5·33 br d, 1 H (olefinic), 6 29 t, $J = 6$ Hz, 1 H (exchangeable, NH). For $C_{31}H_{51}NO_5$ (517·7) calculated: 71·91% C, 9·93% H, 2·71% N; found: 72·15% C, 10·13% H, 2·43% N. *4)* A mixture of 22-epimeric triols *Va* and *Vb* (1·5 g, 7%), m.p. 123–124°C. IR-spectrum (nujol): 1010, 1025, 1030, 1060, 1072 (OH); 1580, 1589, 1622, 1653 ($NHCOCH_3$); 3440, 3400, 3300, 3280, 3250 cm^{-1} (NH···OH). NMR-spectrum: partially overlapping pairs of signals in the methyl region; further: 1·83 s, 3 H ($NCOCH_3$); 2·93 mt, 2 H (NCH_2); 3·27 br mt, 1 H (3α-H); 3·70 mt, 1 H and 4·12 mt, 1 H ($C_{(16)}$—H and $C_{(22)}$—H); 5·25 br d (olefinic); 7·73 br t 1 H (NH). For $C_{29}H_{49}NO_4$ (475·7) calculated: 73·22% C, 10·38% H, 2·94% N; found: 73·53% C, 10·66% H, 2·89% N.

b) Lithium tri-tert-butoxyaluminum hydride (37 g) was added to a stirred and cooled (0°C) solution of the 22-oxo derivative *IIa* (39 g) in tetrahydrofuran (300 ml) and the mixture allowed to stand at room temperature overnight. The excess hydride was decomposed by addition of water, 5% hydrochloric acid was added, the precipitate taken up in ether, the ethereal layer washed with dilute hydrochloric acid and worked up as usual. The residue (45 g) was dissolved in methanol (450 ml), an 11% aqueous solution of potassium carbonate was added and the mixture allowed to stand at room temperature for 5 hours. The bulk of methanol was removed under reduced pressure, the product taken up in chloroform, the extract washed with water, dried, and the solvent evaporated under reduced pressure. The residue (45 g) was chromatographed on silica gel (2000 g) in dichloromethane–methanol (19 : 1) to give the 22-oxo derivative *IIb* (3·5 g, 10%), a mixture of 3,22-dihydroxy derivatives *IVa* and *IVb* (24 g, 66%) and a mixture of the triols *Va* and *Vb* (6 g, 17%).

Mixture of 22-Epimeric (25*R*)-26-Acetylamino-5-cholestene-3β,16β,22-triol 16-Acetates *Va* and *Vb*

A solution of potassium carbonate (0·53 g) in water (5 ml) was added to a mixture of 22-epimeric 3β,16β-diacetoxy-22-hydroxy derivatives *IIIa* and *IIIb* (4·3 g) and the mixture allowed to stand at room temperature for 4·5 hours. After removing most of the solvent under reduced pressure, the residue was dissolved in chloroform, washed with water and dried. After evaporating the solvent under reduced pressure, the residue (4·1 g) was chromatographed on silica gel (200 g) in benzene–ether–acetone (1·2 : 1·2 : 1) to yield mixtures of diols *IVa* and *IVb* (2·8 g, 70%) and triols *Va* and *Vb* (0·4 g, 11%) with physico-chemical properties identical with those of the above preparations.

(22*R*,25*R*)-26-Acetylamino-5-cholestene-3β,16β,22-triol 16-Acetate 3,22-Dinitrate (*VIb*) and (22*S*,25*R*)-26-Acetylamino-5-cholestene-3β,16β,22-triol 16-Acetate 3,22-Dinitrate (*VIa*)

A solution of a mixture of 3,22-diols *IVa* and *IVb* (7·7 g) in chloroform (150 ml) was treated at −30 to −40°C with a reagent prepared from acetic anhydride (57 ml) and 65% nitric acid (14 ml) at −30°C. The reaction mixture was kept at −10 to −20°C for 30 minutes and an additional 30 minutes at −5 to −10°C. The solution was poured into ice, neutralized with aqueous ammonia and the yellow oil thus obtained was taken up in ether. After washing the ethereal solution with ammonia and water and drying, the solvent was evaporated under reduced pressure to yield a residue (9·9 g) that was submitted to chromatography on silica gel (850 g) in light petroleum–ether–acetone (5:3:2) to yield the less polar dinitrate *VIb* (6·6 g, 74%), after precipitation from ether–light petroleum at −80 °C m. p. 85–91 °C $[\alpha]_D^{21}$ + 16° (*c* 0·76). IR-spectrum: 1629, 1279, (ONO_2), 1729, 1249 ($OCOCH_3$), 3450, 1669, 1525 cm^{-1} ($NHCOCH_3$). NMR-spectrum: 0·92 s, 3 H and 1·03 s, 3 H (18-CH_3 and 19-CH_3); 0·92 d, $J = 6·4$ Hz, 3 H and 1·01 d,

3 H, $J = 6{\cdot}6$ Hz (27-CH_3 and 21-CH_3); 2·06 s, 3 H and 1·98 s, 3 H (2 × CH_3CO); 3·14 t, $J =$ = 6 Hz, 2 H (CH_2N); 4·9—5·6 overlapping multiplets $C_{(3)}$—H, $C_{(6)}$—H, $C_{(16)}$—H, $C_{(22)}$—H, NH. For $C_{31}H_{49}N_3O_9$ (607·7) calculated: 61·26% C, 8·13% H, 6·91% N; found: 61·54% C, 8·56% H, 6·46% N. Further elution with the same solvent mixture gave the more polar dinitrate *VIa* (1·35 g, 15%) as an amorphous substance, after precipitation three times from ether–light petroleum at −80°C m.p. 75—80°C, $[\alpha]_D^{27}$ +10° (*c* 0·74). IR-spectrum: 1630, 1280 (ONO_2), 1725, 1265 ($OCOCH_3$), 3445, 1669 cm^{-1} ($NHCOCH_3$). NMR-spectrum: 0·89 s, 3 H and 1·02 s, 3 H (18-CH_3 and 19-CH_3); 0·92 d, $J = 6{\cdot}2$ Hz, 3 H and 1·05, $J = 6{\cdot}8$ Hz, 3 H (27-CH_3, 21-CH_3); 1·97 s, 3 H and 2·02 s, 3 H (2 × $COCH_3$); 2·9—3·4 mt, 2 H (CH_2N); 4·6—5·6 overlapping mts, $C_{(3)}$—H, $C_{(6)}$—H, $C_{(16)}$—H, $C_{(22)}$—H, NH. Found: 61·34% C, 8·20% H, 6·79% N.

Attempts at Hydrolysis of the 16-Acetoxy Group in *VIb* and *VIa*

a) An aqueous solution of potassium carbonate (10 ml, 45%) was added to a solution of the dinitrate *VIb* (4·42 g) in methanol (80 ml) and the solution was refluxed in a nitrogen atmosphere for 20 hours. After concentrating the solution under reduced pressure, the residue was diluted with water, extracted with ether, the solution washed with water, dried, and the solvent removed under reduced pressure. The residue (3·9 g) was purified by chromatography on silica gel (200 g) in light petroleum–ether–acetone (5 : 3 : 2) to yield the compound *VIIa* (3·1 g, 87%), m.p. 125 to 128°C. The substance resisted oxidation with chromium trioxide under a variety of conditions and could not be acetylated with acetic anhydride in pyridine. M.p. 140—141°C (acetone–heptane), $[\alpha]_D^{22}$ −27° (*c* 0·74). IR-spectrum: 1628, 1277 (ONO_2), 3445, 1666, 1521 ($NHCOCH_3$). NMR-spectrum: 0·83 s, 3 H and 1·03 s, 3 H (18-CH_3, 19-CH_3); 0·86 d, $J = 6$ Hz, 3 H and 0·91 d, $J = 6{\cdot}5$ Hz, 3 H (2 × CH_3—CH); 1·98 s, 3 H ($COCH_3$); 3·14 mt, 2 H (CH_2N); 4·02 mt, 1 H 4·50 mt, 1 H and 4·80 mt, 1 H (3 × CHO—); 5·43 d, $J = 6$ Hz, 1 H (olefinic); 5·63 br s, 1 H (NH). Mass spectrum: M^+ 502, *m/e* 487 (M − CH_3), 439 (M − HNO_3), 424 (M − HNO_3—CH_3), 253 (3,5,15-androstatriene$^+$), 128 ($CH_3CONHCH_2(CH_3)CH.CH_2CH_2^+$). For $C_{29}H_{46}N_2O_5$ (502·7) calculated: 69·30% C, 9·22% H, 5·57% N; found: 69·14% C, 9·22% H, 5·59% N.

b) The attempt at hydrolysis of the (22*S*)-3,22-dinitrate *VIa* was performed in the same manner as under *a*) and 432 mg of *VIa* yielded 350 mg of a mixture of two compounds which could be separated by chromatography. The first fraction gave the furostene derivative m.p. and mixture m.p. with *VIIa* 140—141°C, $[\alpha]_D^{22}$ −30° (*c* 0·74). IR-spectrum and NMR-spectrum: identical with the spectra of *VIIa*. Further elution with the same solvent system gave an amorphours substance, m.p. 61—65°C, $[\alpha]_D^{20}$ +14°. IR-spectrum: 3600 cm^{-1} (OH), 1636, 1282 (ONO_2), 1669, 5151, 1530 cm^{-1} (NHAc). NMR-spectrum: 0·80 s, 3 H and 1·01 s, 3 H (18-CH_3, 19-CH_3); 0·89 d, 3 H and 0·98 d, 3 H (27-CH_3, 21-CH_3); 1·96 s, 3 H ($COCH_3$); 3·13 mt, 2 H (CH_2N); 5·33 mt, 1 H (olefinic); 5·65 mt, 1 H (NH). Mass spectrum: no molecular peak, *m/e* 457 (M − HNO_3). For $C_{29}H_{48}N_2O_6$ (520·7) calculated: 66·95% C, 9·30% H, 5·38% N; found: 66·79% C, 9·65% H, 5·80% N.

(25*R*)-26-Acetylamino-5-furosten-3β-ol (*VIIb*)

Zinc powder (3·5 g) was added to a solution of *VIIa* (800 mg) in acetic acid (50 ml) and the mixture was shaken at room temperature for 60 minutes. The inorganic material was separated by filtration, washed with methanol, the filtrate concentrated under reduced pressure and the residue chromatographed on silica gel (80 g). Elution with chloroform–methanol (19 : 1) gave the unreacted starting material (305 mg, 38%) followed by 3-hydroxyfurostene *VIIb* (380 mg, 52%). Analytical sample melted at 189—191°C (acetone–heptane), $[\alpha]_D^{22}$ −62° (*c* 0·78). IR-spectrum: 3600 (OH), 3450, 1666, 1523 ($NHCOCH_3$); 1047 cm^{-1} (C—O—C). Mass spectrum: M^+ 457.

For $C_{29}H_{47}NO_3$ (457·7) calculated: 76·10% C, 10·35% H, 3·06% N; found: 76·32% C, 10·48% H, 3·40% N.

(22*R*, 25*R*)-26-Acetylamino-5-cholestene-3β,22,16β-triol 16-Acetate (*IVb*)

A solution of the dinitrate *VIb* (300 mg) in acetic acid (30 ml) was shaken with zinc powder (2 g) for 45 min at room temperature. The inorganic material was removed by filtration, washed with methanol and ether, the filtrate concentrated under reduced pressure, the residue treated with water and taken up in ether. The extract was washed with water, ammonia and water, dried, the solvent removed *in vacuo* and the residue chromatographed on silica gel (15 g). Elution with chloroform–methanol (19 : 1) yielded the product (250 mg, 98%), m.p. 196°C. Analytical sample showed m.p. 196·5—197°C (acetone–heptane). Both the migration rates and the IR-spectra are identical. NMR-spectrum: 0·89 d, 3 H (27-CH_3); 0·90 s, 3 H (18-CH_3); 0·96 d, 3 H (21-CH_3); 1·00 s, 3 H (19-CH_3); 1·97 s, 3 H ($COCH_3$); 2·03 s, 3 H ($NHCOCH_3$); 3·13 mt, 2 H (CH_2N); 3·44 mt, 2 H (2 × CHOH); 5·08 mt, 1 H (CHOC); 5·34 d, 1 H (olefinic); 5·64 mt, 1 H (NH). For $C_{31}H_{51}NO_5$ (517·7) calculated: 71·91% C, 9·93% H, 2·71% N; found: 72·17% C, 10·14% H, 2·57% N.

(22*S*,25*R*)-26-Acetylamino-5-cholestene-3β,22,16β-triol 16-Acetate (*IVa*)

A solution of (22*S*)-dinitrate *VIa* (608 mg) in acetic acid (60 ml) was treated with zinc powder (2·5 g) and shaken for 1·5 hours; then an additional 2·5 g of zinc was added and shaking was continued for 90 minutes. The inorganic material was filtered off, washed with methanol, the filtrate concentrated under reduced pressure, the residue taken up in ether–ethyl acetate, the extract washed with water, ammonia, water, and dried. The solvent was evaporated under reduced pressure, the residue dissolved in chloroform–methanol (19 : 1) and passed through a column of silica gel (10 g) to give the (22*S*)-diol *IVa* (510 mg, 98%). Recrystallization from acetone–heptane provided an analytical sample, m.p. 173·5—175·5°C, $[\alpha]_D^{22}$ −5° (*c* 0·74). IR-spectrum: 3 600 (OH); 1 723, 1 260 (OAc); 1 665, 1 528, 3 450 cm^{-1} ($NHCOCH_3$); the spectrum is not identical with that of the (22*R*)-epimer; the migration rates in a variety of solvent systems are identical. NMR-spectrum: 0·89 d, 3 H (27-CH_3); 0·88 s, 3 H (18-CH_3); 0·93 d, 3 H (21-CH_3); 1·00 s, 3 H (19-CH_3); 1·95 s, 3 H ($OCOCH_3$); 1·97 s, 3 H ($NHCOCH_3$); 3·12 br t, 2 H (CH_2N); 3·92 mt, 2 H (2 × CHOH); 5·24 mt, 1 H ($CHOCOCH_3$); 5·32 mt, 1 H (olefinic), 5·85 mt, 1 H (NH). For $C_{31}H_{51}NO_5$ (517·7) calculated: 71·91% C, 9·93% H, 2·71% N; found: 71·57% C, 9·98% H, 2·35% N.

(22*R*,25*R*)-26-Acetylamino-3β,22-bis(tetrahydropyranyloxy)-5-cholesten-16β-ol 16-Acetate (*VIIIb*)

A mixture of (22*R*)-diol *IVb*) (2·2 g), dihydropyran (11 ml) and 6 drops of hydrochloric acid in chloroform (77 ml) was shaken for 1 hour and after 2 hours of standing at room temperature was neutralized with a solution of potassium hydrogen carbonate. The mixture was diluted with ether, the organic layer washed with potassium hydrogen carbonate, water, and dried. After evaporating the solvent under reduced pressure the residue (3·5 g) was chromatographed on silica gel (350 g) in light petroleum–ether–acetone (6 : 3 : 1) to yield an amorphous product (2·3 g) which, after precipitation from ether–light petroleum at low temperature, melted at 75—78°C, $[\alpha_D^{20}]$ +21° (*c* 0·75). IR-spectrum: 1735, 1261 ($OCOCH_3$); 3450, 1678, 1535 (NH. .$COCH_3$); 1079, 1135 cm^{-1} (O—C—O). NMR-spectrum: 0·89 s, 3 H (18-CH_3); 1·00 s, 3 H (19-CH_3); 0·90 d, 3 H (27-CH_3); 1·01 d, 3 H (21-CH_3); 1·98 s, 3 H ($OCOCH_3$); 2·04 s, 3 H

($NHCOCH_3$); 3·00—3·25 mt, 2 H (CH_2N); 5·33 mt, 1 H (olef.); 5·50 mt, 1 H (NH). For $C_{41}H_{67}NO_7$ (686·0) calculated: 71·80% C, 9·84% H, 2·04% N; found: 71·69% C, 10·04% H, 1·78% N.

(22*S*,25*R*)-26-Acetylamino-3β,22-bis(tetrahydropyranyloxy)-5-cholesten-16β-ol 16-Acetate (*VIIIa*)

The same procedure as in the case of *IVb* was applied to 520 mg of the (22*S*)-diol *IVa*. Chromatography on silica gel (135 g) in light petroleum–ether–acetone (2 : 1 : 1) gave the (22*S*)-derivative *VIIIa* (605 mg, 88%, amorphous, precipitated), m.p. 61—65°C, $[\alpha]_D^{20}$ +8° (*c* 0·79). IR-spectrum: 1724, 1260 ($OCOCH_3$); 3450, 1669, 1522 ($NHCOCH_3$), 1030, 1076, 1134 cm^{-1} (O—C—O). NMR-spectrum: 0·96 s, 3 H (18-CH_3); 1·10 s, 3 H (19-CH_3); 0·99 d, 3 H (27-CH_3); 1·06 d, 3 H (21-CH_3); 1·96 s, 6 H ($OCOCH_3$ and $NHCOCH_3$); 2·95—3·25 mt, 2 H (CH_2N); 5·60 to 5·90 mt, 1 H (NH); 5·33 mt, 1 H (olefinic). For $C_{41}H_{67}NO_7$ (686·0) calculated: 71·80% C, 9·84% H, 2·04% N; found: 71·93% C, 9·89% H, 1·62% N.

(22*R*,25*R*)-26-Acetylamino-3β,22-bis(tetrahydropyranyloxy)-5-cholesten-16β-ol (*IXb*)

A solution of 16-acetate *VIIIb* (7 g) in methanol (240 ml) and aqueous potassium carbonate (60 ml, 80%) was refluxed for 48 hours, the solution concentrated under reduced pressure and the product isolated with ether–ethyl acetate. The extract was washed with water, dried and the solvent removed under reduced pressure. Chromatography on silica gel (200 g) and elution with light petroleum–ether–acetone (2 : 1 : 1) gave the 16-hydroxy derivative (5·6 g, 85%], amorphous, precipitated from ether–light petroleum at low temperature, m.p. 78—85°C, $[\alpha]_D^{22}$ −32° (*c* 0·80). IR-spectrum: 3610 (OH); 3450, 1666, 1523 ($NHCOCH_3$); 1075, 1133 cm^{-1} (O—C—O). NMR-spectrum: 0·90 d, 6 H (21-CH_3 and 27-CH_3); 0·91 s, 3 H (18-CH_3); 1·01 s, 3 H (19-CH_3); 2·00 s, 3 H ($NHCOCH_3$); 3·00—3·25 mt, 2 H (CH_2N); 5·35 mt, 1 H (olefinic); 5·55 mt, 1 H (NH). For $C_{39}H_{65}NO_6$ (643·9) calculated: 72·74% C, 10·19% H, 2·18% N; found: 72·65% C, 10·37%H, 2·65% N.

(22*S*,25*R*)-26-Acetylamino-3β,22-bis(tetrahydropyranyloxy)-5-cholesten-16β-ol (*IXa*)

The 16-acetoxy derivative *VIIIa* (3·43 g) was saponified in the same manner as the 22-epimer *VIIIb*. Chromatography on silica gel (100 g) gave the amorphous product (900 mg, 28%), precipitated from ether–light petroleum at low temperature. m.p. 88—95°C, $[\alpha]_D^{22}$ −46° (*c* 0·74). IR-spectrum: 3610 (OH); 3450, 1668, 1525 ($NHCOCH_3$); 1030, 1085, 1134 cm^{-1} (O—C—O), not identical with the spectrum of IXb. NMR-spectrum: 0·93 s, 3 H (18-CH_3); 1·01 s, 3 H (19-CH_3); 0·92 d, 3 H and 0·99 d, 3 H (27-CH_3 and 21-CH_3); 1·97 s, 3 H ($NHCOCH_3$); 5·35 mt 1 H (olefinic), 5·67 mt, 1 H (NH). For $C_{39}H_{65}NO_6$ (643·9) calculated: 72·74% C, 10·18% H, 2·18% N; found: 72·47% C, 9·96% H, 1·85% N.

(22*R*,25*R*)-26-Acetylamino-3β,22-bis(tetrahydropyranyloxy)-5-cholesten-16-one (*Xb*)

A solution of the 16-hydroxy derivative (*IXb*, 1·59 g) in pyridine (30 ml) was added with stirring to chromium trioxide (3 g) in pyridine (50 ml) at 0 °C. The mixture was stirred at room temperature overnight, diluted with water, extracted with ether and the ethereal solution washed with water five times, dried and the solvent removed under reduced pressure. The product (1·5 g, 95%) was recrystallized three times from acetone–ether–heptane, m.p. 157—159°C, $[\alpha]_D^{22}$ −140° (*c* 0·78). IR-spectrum: 1741 (CO); 3450, 1677, 1535 ($NHCOCH_3$); 1137, 1079 cm^{-1} (O—C—O). NMR-spectrum: 0·86 s, 3 H (18-CH_3); 1·03 s, 3 H (19-CH_3); 0·88 d, 3 H (27-CH_3); 0·99 d, 3 H

(21-CH_3); 1·97 s, 3 H ($NHCOCH_3$); 3·0—3·2 mt, 2 H (CH_2N); 5·35 mt, 1 H (olefinic); 5·60 mt, 1 H (NH). For $C_{39}H_{63}NO_6$ (641·9) calculated: 72·97% C, 9·89% H, 2·18% N; found: 72·95% C, 9·88% H, 2·18 N.

(22*S*,25*R*)-26-Acetylamino-3β,22-bis(tetrahydropyranyloxy)-5-cholesten-16-one (*Xa*)

A solution of the 16-hydroxy derivative *IXa* (350 mg) in pyridine (5 ml) was added to a stirred preparation of chromium trioxide (600 mg) in pyridine (20 ml) at 0°C. Stirring was continued for an additional 24 hours at room temperature and the mixture worked up as in the case of *Xb*. The product (320 mg, 91%) was crystallized from acetone–ether–light petroleum, m.p. 110—114°C, $[\alpha]_D^{22}$ −148° (*c* 0·73). IR-spectrum: 1740 (CO), 3450, 1675, 1532 ($NHCOCH_3$); 1030, 1075, 1135 cm^{-1} (O—C—O). For $C_{39}H_{63}NO_6$ (641·9) calculated: 72·97% C, 9·89% H, 2·18% N; found: 72·69% C, 10·04% H, 1·96% N.

(22*R*,25*R*)-26-Acetylamino-3β,22-bis(tetrahydropyranyloxy)-5-cholestene (*XIb*)

a) *From* (22*R*,25*R*)-26-*amino*-3β,22-*bistetrahydropyranyloxy*-5-*cholestene* (XIIb); A solution of the base *XIIb* (110 mg) in pyridine (3 ml) and acetic anhydride (1 ml) was allowed to stand at room temperature overnight, excess acetic anhydride was decomposed with ice and ammonia. The mixture was extracted with ether, the solution washed with dilute ammonia and water (five times), dried and the solvent removed under reduced pressure. The residue (120 mg) was purified by column chromatography over silica gel (10 g) in light petroleum–acetone–ether (3 : 1 : 1). Crystallization (aceton–heptane) gave the product, m.p. 159—161°C, $[\alpha]_D^{24}$ −12° (*c* 0·74). IR-spectrum: 1132, 1075, 1031, 1022 cm^{-1} (O—C—O); 3·450, 1664, 1525 cm^{-1} ($NHCOCH_3$). NMR-spectrum: 0·75 s, 3 H (18-CH_3); 0·90 d, J = 6·5 Hz, 3 H (21-CH_3 or 27-CH_3); 0·975 d, J = 6·5 Hz, 3 H (27-Me or 21-Me); 1·00 s, 3 H (19-Me); 1·975 s, 3 H ($NCOCH_3$); 2·95—4·80 m. 10 H (2 × CH_2—O—, 2 × CH—O, 2 × O—CH—O—, CH_2N—); 5·34, broad signal, 1 H ($C_{(6)}$—H); 7·05 broad signal, 1 H (NH). For $C_{39}H_{65}NO_5$ (627·9) calculated: 75·59% C, 10·43% H, 2·23% N; found: 74·87% C, 10·48% H, 2·21% N.

b) *From* (22*R*,25*R*)-3β,22-*bis*(*tetrahydropyranyloxy*)-5-*cholesten*-26-*al* (XIIIb): Hydroxylamine (100 mg) in ethanol was added to a solution of the aldehyde *XIIIb* (150 mg) in ethanol (3 ml). After standing overnight the mixture was heated on a water bath for two hours, then cooled and the solvent evaporated under reduced pressure. The residue was reduced for 8 hours with lithium aluminum hydride (150 mg) in boiling ether (30 ml) with stirring. After decomposition of the unreacted hydride with a few drops of water, the precipitate was filtered off, washed with ether five times and the combined ethereal solutions were evaporated under reduced pressure. The product (110 mg) was dissolved in pyridine (5 ml) and acetylated with acetic anhydride (3 ml) at room temperature overnight. Excess acetic anhydride was decomposed with ice, the mixture extracted with ether, the solution washed with dilute aqueous ammonia, then with water five times, dried with magnesium sulfate, the solvent evaporated under reduced pressure and the residue was filtered through a short column of silica gel (10 g) in light petroleum–acetone–ether (2 : 1 : 1) to give a product (90 mg), m.p. 152—155°C, showing the same migratory rate as the acetylated base *XIIb*. The analytical sample showed the m.p. and mixture m.p. with *XIb* (158 to 160°C (acetone–heptane). The IR-spectra of the compounds from both procedures were identical.

(22*S*,25*R*)-26-Acetylamino-3β,22-bis(tetrahydropyranyloxy)-5-cholestene (*XIa*)

The same acetylation procedure as in the case of the base *XIIb* was applied to 70 mg of the base *XIIa*. Chromatography over silica gel (20 g) in light petroleum–ether–acetone (3 : 1 : 1) gave the

amorphous (22*S*)-derivative *XIa* (64 mg) showing a distinctly different rate in thin-layer chromatography than the compound *XIb*. After reprecipitation from ether–light petroleum at low temperature (dry ice) the product melted at 145–148°C, $[\alpha]_D^{22}$ −23° (*c* 0·74). IR-spectrum: 3450, 1667, 1521 cm^{-1} ($NHCOCH_3$); 1134, 1076, 1030 cm^{-1} (O—C—O). NMR-spectrum: 0·89 s, 3 H (18-CH_3); 0·89 d, $J \sim 6$, 3 H (21-CH_3 or 27-CH_3); 0·95 d, $J \sim 6$, 3 H (27-CH_3 or 21-CH_3); 1·00 s, 3 H (19-CH_3); 1·96 s, 3 H ($NCOCH_3$); 2·95–4·80, 10 H (2 × CH_2O—, 2 × C—H—O—, 2 × O—CH—O, CH_2N); 5·325 broad signal, 1 H ($C_{(6)}$—H); 7·01 broad signal, 1 H (NH). For $C_{39}H_{65}NO_5$ (627·9) calculated: 74·59% C, 10·43% H, 2·23% N; found: 74·82% C, 10·25% H, 2·42% N.

(22*R*, 25*R*)-26-Amino-3β,22-bis(tetrahydropyranyloxy)-5-cholestene (*XIIb*)

A solution of 16-oxo derivative *Xb* (25 g), potassium hydroxide (140 g), hydrazine hydrate (200 ml, 80%) in triethylene glycol was heated at 100°C for 5 minutes, at 140°C for 30 minutes and at 200°C for 3 hours. After cooling to room temperature the solution was poured on ice, the precipitate filtered off, washed with water and dried. This material was chromatographed on silica gel (1500 g). Elution with chloroform (saturated with ammonia) gave a small amount of less polar compounds that were not identified. Subsequent fractions provided the base *XIIb* (15·6 g, 49%), m.p. 97–102°C (aqueous methanol). IR-spectrum: 1022, 1031, 1176, 1132 (O—C—O); 3310 cm^{-1} (NH_2). Mass spectrum: M^+ 585. For $C_{37}H_{63}NO_4$ (585·9) calculated: 75·85% C, 10·84% H, 2·39% N; found: 76·11% C, 11·05% H, 2·14% N.

(22*S*, 25*R*)-26-Amino-3β,22-bis(tetrahydropyranyloxy)-5-cholestene (*XIIa*)

The 16-oxo derivative *Xa* (260 mg) was submitted to Huang–Minlon reduction as described in the case of *Xb*. Chromatography of the product on silica gel (15 g) in chloroform (saturated with ammonia) gave the base *XIa* (140 mg, 58%), m.p. 83–85°C (aqueous methanol). IR-spectrum: 1022, 1030, 1065, 1076, 1108, 1131 (O—C—O); 3440 cm^{-1} (NH_2 assoc.). For $C_{37}H_{63}NO_4$ (585·9) calculated: 75·85% C, 10·84% H, 2·39% N; found: 75·98% C, 10·57% H, 2·62% N.

(22*R*,25*R*)-3β,22-Bis(tetrahydropyranyloxy)-5-cholesten-26-al (*XIIIb*)

N-Chlorosuccinimide (1·6 g) was added to a stirred and cooled (0°C) solution of the base *XIIb* (6 g) in dichloromethane (60 ml). After the addition had been completed, the solution was allowed to stand at 0°C for 60 minutes, then diluted with ether, the solution washed repeatedly with water, dried and the solvent evaporated under reduced pressure. The residue was dried azeotropically with benzene, then dissolved in benzene (40 ml) and treated with diazabicycloundecene (10 ml) at 30°C overnight. Water was then added and the organic material taken up in ether, the solution washed thoroughly with water and dried. The solvent was removed *in vacuo* and the residue chromatographed on silica gel (600 g). Elution with light petroleum–ether (successively 5–15% of ether) provided the product *XIIIb* (3·9 g, 65%), m.p. 138–139°C (ligroin), $[\alpha]_D^{20}$ −110° (*c* 0·78). IR-spectrum: 1720 (CHO): 1023, 1032, 1077, 1133 (O—C—O); 1670 cm^{-1} (C=C). NMR-spectrum: 0·69 s, 3 H and 1·01 s, 3 H (19-CH_3, 18-CH_3); 0·87 d, 3 H and 1·10 d, 3 H, J = 7 Hz (21-CH_3, 27-CH_3); 3·48 br mt, 4 H (2 × —CH_2O—); 3·88 br mt, 2 H ($C_{(3)}$—H, $C_{(22)}$—H); 4·60 br mt and 4·72 br mt, 2 H, 2 × (O—CH—O); 5·34 br d, 1 H (olefinic); 9·63 d, 1 H, $J_{25,26}$ = 2·0 Hz (CH=O). For $C_{37}H_{60}O_5$ (584·8) calculated: 75·98% C, 10·34% H; found. 76·16% C, 10·37% H.

(22*S*,25*R*)-3β,22-Bis(tetrahydropyranyloxy)-5-cholesten-26-al (*XIIIa*)

The base *XIIa* (0·9 g) in dichloromethane (10 ml) was treated with N-chlorosuccinimide (240 mg) at 0°C for 1 hour. The reaction mixture was worked up in the case of *XIIIb*. Chromatography on silica gel (120 g) in light petroleum–ether (15%) yielded the aldehyde *XIIIa* (242 mg, 27%), m.p. 141—142°C, $[\alpha]_D^{22}$ −116° (*c* 0·75). IR-spectrum: 1720 (CHO); 1025, 1032, 1134 (O—C—O); 1669 cm^{-1} (C=C). The migration rate of both epimers *XIIIa* and *XIIIb* in a series of systems is identical. For $C_{37}H_{60}O_5$ (584·8) calculated: 75·98% C, 10·34% H; found: 76·11% C, 10·22% H.

(22*R*)-5-Cholesten-3β,22-diol Diacetate (*XVII*)

A solution of the aldehyde *XIIIb* (120 mg), potassium hydroxide (500 mg), and hydrazine hydrate (1 ml, 80%) in triethylene glycol (10 ml) was heated to 100°C for 10 minutes, to 140°C for 30 minutes and to 195—200°C for 3 hours. After cooling, the mixture was poured on ice, the precipitate separated by filtration, washed with water and dried to give 95 mg of material which was dissolved in light petroleum–acetone (3%) and the solution filtered through a column of silica gel (10 g). The product (68 mg) was dissolved in warm 80% acetic acid (5 ml), the solution heated to 100°C for five minutes, cooled and diluted with ether. The organic layer was washed with water two times, then with aqueous ammonia, water, dried and the solvent evaporated under reduced pressure. The residue (65 mg) was chromatographed over silica gel (15 g) in light petroleum–acetone (5 : 1). The product (50 mg) m.p. 175—178°C was acetylated in pyridine at room temperature. The usual workup gave the product that was recrystallized from heptane three times and from methanol to give the pure compound *XVII* (22 mg, 22%), m.p. 100—102°C, $[\alpha]_D^{20}$ −32° (*c* 0·74). Literature[6] reports m.p. 102°C, $[\alpha]_D$ −37·5° for (22*R*)-22-hydroxycholesterol diacetate. The compound exhibited no m.p. depression on admixture with the authentic sample. IR-spectrum (KBr) of the compound *XVII* is identical with that of the authentic (22*R*)-22-hydroxycholesterol diacetate; the spectrum of the (22*S*)-epimer is distinctly different.

(22*R*,25*R*)-22,26-Epoxy-5-cholesten-3β,26-diol (*XIVb*)

A solution of the aldehyde *XIIIb* (2·93 g) in 80% acetic acid (50 ml) was heated under nitrogen atmosphere at 100°C for 15 minutes. After cooling, the mixture was neutralized with aqueous ammonia and the product was isolated with ether. The organic layer was washed with water, dried with magnesium sulfate and the solvent evaporated under reduced pressure. The residue (2·0 g) was chromatographed on silica gel (150 g) in ligroin–acetone (4 : 1) to furnish the product *XIVb* (1·65 g, 80%), m.p. 163—168°C (acetone–heptane), $[\alpha]_D$ −12° (*c* 0·74). IR-spectrum: 3615 cm^{-1} (OH); 1665 cm^{-1} (C=C); 1050, 1022 cm^{-1} (O—C—OH). NMR-spectrum: 0·67 s, 3 H and 0·96 s, 3 H (18-CH_3 and 19-CH_3); 0·92 br d, 3 H and 1·25 d, 3 H, J = 7 Hz (21-CH_3 and 27-CH_3); 3·06—3·61 br mt, 2 H (2 × CH—O); 5·25 br mt, 1 H (olefinic). For $C_{27}H_{44}O_3$ (416·6) calculated: 77·83% C, 10·65% H; found: 77·50% C, 10·81% H.

(22*S*,25*R*)-22,26-Epoxy-5-cholestene-3β,26-diol (*XIVa*)

The same procedure was applied to the aldehyde *XIIIa* (600 mg). Chromatography over silica gel in light petroleum–acetone (4 : 1) gave the (25*S*)-derivative *XIVe* (400 mg), m.p. 179—180°C. Analytical sample melted at 182—183°C (acetone–heptane), $[\alpha]_D^{22}$ +3° (*c* 0·74). IR-spectrum: 3610 cm^{-1} (OH); 1670 cm^{-1} (C=C); 1005, 1020, 1047 cm^{-1} (O—C—OH). NMR-spectrum: 0·66 s, 3 H and 0·95 s, 3 H (18-CH_3 and 19-CH_3); 0·86 d, 3 H and 1·23 d, 3 H, J = 7 Hz (21-Me, 27-CH_3); 3·00—3·50 br mt, 2 H (2 × CH—O); 5·24 br d, 1 H (olefinic). For $C_{27}H_{44}O_3$ (416·6) calculated: 77·83% C, 10·65% H; found: 77·61% C, 10·85% H.

(22*R*,25*R*)-3β,26-Bis(tetrahydropyranyloxy)-22,26-epoxy-5-cholestene (*XVb*)

Dihydropyran (6 ml) and 6 drops of hydrochloric acid were added to a solution of the hemiacetal *XIVb* (1·25 g) in chloroform (30 ml) and the mixture was shaken for 1 hour. After standing an additional 2 hours at room temperature, the mixture was poured on ice, alkalized with ammonia and the product isolated with ether. The ethereal extract was washed with water, dried and the solvent removed under reduced pressure. The residue was chromatographed over silica gel (300 g) in light petroleum–ether–acetone (3 : 1 : 1) to furnish the amorphous product *XVb* (1·20 g, 68%), after repeated precipitation from ether–light petroleum at low temperature (dry ice–acetone) m.p. 83–85°C, $[\alpha]_D^{20}$ −16° (*c* 0·74). IR-spectrum: 1621 cm^{-1} (C=C), 1129, 1075, 1036, 1025, 970 cm^{-1} (O—C—O). NMR-spectrum: 0·69 s, 3 H and 1·01 s, 3 H (18-CH_3 and 19-CH_3); 0·95 d, 3 H and 0·99 d, 3 H, J = 7 Hz; 3·50 mt, 4 H (2 × O—CH_2); 3·92 mt, 2 H ($C_{(3)}$—H and $C_{(26)}$—H); 4·71 mt, 1 H ($C_{(22)}$—H); 5·34 br d, 1 H (olefinic). For $C_{37}H_{60}O_5$ (584·8) calculated: 75·98% C, 10·63% H; found: 76·09% C, 10·58% H.

(22*S*,25*R*)-3β,26-Bis(tetrahydropyranyloxy)-22,26-epoxy-5-cholestene (*XVa*)

The same procedure as in the case of *XVb* was applied starting from *XIVa* (350 mg). Chromatography of the crude product over silica gel (90 g) in light petroleum–ether–acetone (3 : 1 : 1) gave the (22*S*)-derivative *XVa* (300 mg, 49%), m.p. 87–89°C, $[\alpha]_D^{22}$ −5° (*c* 0·74). IR-spectrum: 1668 and 1621 cm^{-1} (C=C); 1130, 1024, 1033, 1076 cm^{-1} (O—C—O). NMR-spectrum: 0·69 s, 3 H and 1·01 s, 3 H (18-CH_3 and 19-CH_3); 0·97 d, 3 H, J = 7 Hz and 1·32 d, 3 H, J = 7 Hz (21-CH_3 and 27-CH_3); 3·50 mt, 5 H (2 × OCH_2 and $C_{(22)}$—H); 3·90 mt, 2 H ($C_{(3)}$—H and $C_{(26)}$—H); 4·07 mt, 1 H (—O—CH—O); 4·22 mt 1H (—O—CH—O—); 5·34 br d, 1 H (olefinic). For $C_{37}H_{60}O_5$ (584·8) calculated: 75·98% C, 10·63% H; found: 75·70% C, 10·34% H.

(22*R*,25*R*,26*S*)-3β,26-Dihydroxy-22,26-epoxy-5α-cholestan-6-one (*XVIb*)

Sodium borohydride (520 mg) was added to a solution of the compound *XVb* in diglyme (30 ml), the mixture was cooled with ice and boron trifluoride etherate (1·4 ml) was added dropwise with stirring under a nitrogen atmosphere. Stirring was continued for one hour at 0°C and for three hours at room temperature. Excess diborane was decomposed by the addition of a few drops of water followed by 3M-NaOH (5 ml) and hydrogen peroxide (30%), 5 ml). The mixture was stirred at 30–35°C for two hours, cooled, extracted with ether, the ethereal extract washed with water five times, dried, and the solvent evaporated under reduced pressure. The residue (1·5 g) was oxidized with chromium trioxide (800 mg) in pyridine (20 ml) at room temperature with stirring overnight. The mixture was diluted with ether, washed with water five times, dried and the solvent evaporated under reduced pressure. The residue (1·2 g) was dissolved in warm acetic acid (80%, 25 ml), the solution heated at 100°C for 5 minutes, cooled, diluted with ether, the organic layer washed repeatedly with water, then with ammonia and water, dried, and the solvent removed under reduced pressure. The crude product *XVIb* (1·05 g) was purified by chromatography over silica gel (150 g in chloroform–methanol, 2·5%) to obtain the compound *XVIb* (710 mg, 60%), m.p. 176–179°C. Analytical sample melted at 181–183°C (methanol), the mixture m.p. with *XVIa* (ref.[3]) showed a depression of 7°C. The migration rates (silica gel, chloroform–8% methanol) of *XVIa* and *XVIb* were distinctly different. IR-spectrum (nujol): 3400 cm^{-1}; 1704 cm^{-1} (CO); 1061, 1029, 1024, 1068 cm^{-1} (O—C—OH). For $C_{27}H_{44}O_4$ (432·6) calculated: 74·95% C, 10·25% H; found: 75·18% C, 10·39% H.

(22*S*,25*R*,26*R*)-3β,26-Dihydroxy-22,26-epoxy-5α-cholestan-6-one (*XVIa*)

The foregoing procedure was also used for the preparation of the compound *XVIa* starting from *XVa* (150 mg). The crude ketone (76 mg) was chromatographed over silica gel (15 g) in chloroform–8% methanol to yield the (22*S*)-derivative *XVIa* (46 mg, 41%), m.p. 190—192°C. Crystallization from methanol raised the m.p. to 191·5—192°C, mixture m.p. with the authentic sample[3] showed no depression. Both compounds exhibited the same migratory rate in thin-layer chromatography (chloroform–methanol 92 : 8). Their IR- and mass spectra are identical. IR-spectrum (nujol): 3400 cm^{-1} (OH), 1705 cm^{-1} (CO); 1060, 1030, 1015, 968 cm^{-1} (O—C—OH).

The analyses were carried out in the Analytical Laboratory of this Institute by Mr V. Štěrba, Mrs V. Rusová and Mrs E. Sýkorová under the direction of Dr J. Horáček. The infrared spectra were recorded by Mr P. Formánek under the direction of Dr J. Smolíková. The NMR-spectra were recorded and interpreted by Dr M. Buděšínský. The mass spectra were recorded and interpreted by Dr L. Dolejš.

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