# BRASSINOSTEROIDS WITH A CHOLESTANE SIDE CHAIN*

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The synthesis of 2α,3α-dihydroxy-B-homo-7-oxa-cholestan-6-one (*VI*), 2β,3β-dihydroxy-B-homo-7-oxa-5α-cholestan-6-one (*VIII*) and 2α,3α-dihydroxy-B-homo-6-oxa-5α-cholestan-7-one (*XI*) is described. The activity of these brassinosteroids in the bean second internode bioassay is lower than the activity of 24-epibrassinolide (*XXI*).

Six years ago[1] a new plant hormone – brassinolide – was isolated, a natural steroidal lactone enhancing cell division, their elongation and thus the plant growth. The accessibility of brassinolide by isolation from plant material is rather difficult, because brassinolide occurs in plants only in very low concentrations. For example in rape pollen[1] its concentration is 0·1 ppm ($10^{-6}$), in insect galls of the chestnut tree[2] 0·5 ppb ($10^{-9}$), in fresh leaves of green tea[3] 0·02 ppb and in Chinese cabbage[4] 0·003 ppb. The accessibility of brassinolides by synthesis is also very difficult owing to the complexity of the synthetic procedures[5–15]. Therefore we approached the synthesis of analogues which retain the activity of the natural hormone and simultaneously would enable an easier preparation or even introduction into practice.

For the purpose of testing brassinoid activity we prepared the known[16–18] 2α,3α-dihydroxy-B-homo-7-oxa-5α-cholestan-6-one (*VI*). When synthetized by standard procedures some reactions do not take place under formation of individual products. Thus products were isolated which could be used for the synthesis of further brassino-analogues with a cholestane side chain: 2β,3β-dihydroxy-B-homo-7-oxa-5α-cholestan-6-one (*VIII*) and 2α,3α-dihydroxy-B-homo-6-oxa-cholestan-7-one (*XI*).

In the synthesis of analogue *VI* the known[19] 3β-bromo-5α-cholestan-6-one (*I*) can be used as starting material. Bromo ketone *I* was oxidized with 3-chloroperbenzoic acid or trifluoroperacetic acid. In both cases we obtained a practically identical mixture (even though the reaction with 3-chloroperbenzoic acid is substantially slower) which contained two lactones, the known[16] bromolactone *II* and the isomeric bromolactone *III*. The structure of the isomeric lactone *III* – 3β-bromo-B-homo-6-oxa-5α-cholestan-7-one – follows from its IR spectrum (the bands of the lactone

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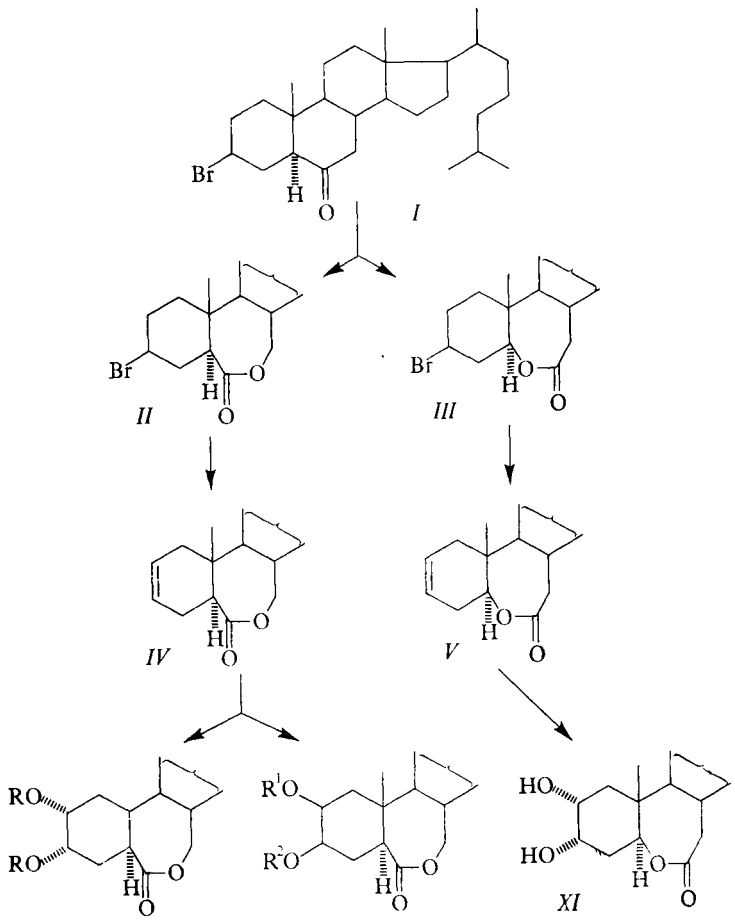
grouping at 1 741, 1 730 sh, 1 275 and 1 062 $cm^{-1}$) and $^1H$ NMR spectrum (5α-H as dd with $J$ = 5·5 Hz and $J'$ = 11 Hz). On elimination of hydrogen bromide bromolactone *II* afforded olefin *IV* which under the effect of osmium tetroxide in the presence of N-methylmorpholine N-oxide gave two products, *i.e.* the known[16] *VI* and the new *VIII*. From bromolactone *III* 2α,3α-dihydroxy-B-homo-6-oxa-5α-cholestan-7-one (*XI*) was prepared in a similar manner *via* olefin *V*.




*VI*, R = H
*VII*, R = Ac

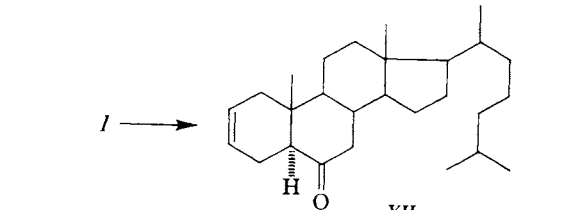
*VIII*, $R^1 = R^2 = H$
*IX*, $R^1$ = H, $R^2$ = Ac
*X*, $R^1 = R^2$ = Ac

*XI*

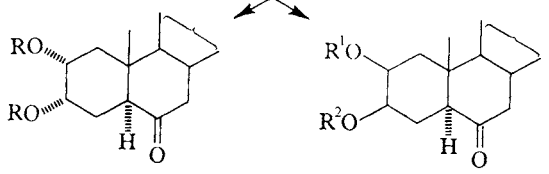
2α,3α-Dihydroxy-B-homo-7-oxa-5α-cholestan-6-one (*VI*) can also be prepared from 5α-cholest-2-en-6-one (*XII*) in the known manner[17,18] *via* dihydroxy ketone *XIII*, diacetoxy ketone *XIV* and diacetoxy lactone *VII*. The first step in this method

is the hydroxylation of the double bond in the position 2 with osmium tetroxide. From the literature[20] it is known that 2α,3α-diol is formed exclusively. However, when hydroxylating the double bond in the position 2 of the olefin *XII* with osmium tetroxide in the presence of N-methyl-morpholine N-oxide, we obtained a mixture from which pure diol *XIII* can be obtained by crystallization. After acetylation (at room temperature for 20 h) of the mother liquors diacetate *XVI* and monoacetate *XV* of the epimeric 2β,3β-diol (both these compounds are known from literature[21,22]) could be isolated in addition to diacetate *XIV* of 2α,3α-diol. From 2β,3β-diacetate *XV* diacetoxy lactone *X* was prepared by reaction with 3-chloroperbenzoic acid, and from it lactone *VIII* by saponification of the acetoxy group and acidification. Lactone *VIII* obtained in this manner was identical with the lactone described above.

From the comparison of both syntheses mentioned it follows that the synthesis *via* bromolactones *II* and *III* is more economical and that it leads to all three analogues.

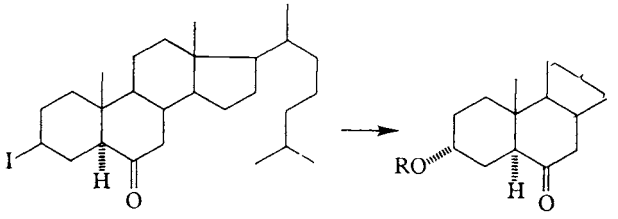



*XII*




*XIII*, R = H
*XIV*, R = Ac

*XV*, $R^1$ = H, $R^2$ = Ac
*XVI*, $R^1$ = $R^2$ = Ac




*XVII*

*XVIII*, R = H
*XIX*, R = (m-)$ClC_6H_4CO$
*XX*, R = $CF_3CO$

From the literature[23] it is known that the regioselectivity of the Baeyer–Villiger oxidation of 5α-cholestan-6-one derivatives is considerably affected by the nature of the substituent on $C_{(1)}$, $C_{(2)}$, or $C_{(3)}$. It interested us what effect the replacement of the bromine atom by iodine atom on $C_{(3)}$ would have on the ratio of 6-oxa-derivative : 7-oxa-derivative, which was in the case of 3β-bromo substituted compounds about 1 : 3. The oxidation of 3β-iodo-6-keto-5α-cholestane *XVII* was tried with 3-chloroperbenzoic acid, trifluoroperacetic acid, peracetic acid and a mixture of trifluoroacetic acid with hydrogen peroxide. In the first two cases we did indeed obtain a mixture with two main products, but none of them contained an iodine atom, while one of the products was identical in both instances. This identical product is the known[24] 3α-hydroxy-5α-cholestan-6-one (*XVIII*). The second product is the ester of alcohol *XVIII* with the acid corresponding to the peracid used, *i.e.* 3-chlorobenzoate *XIX*, or trifluoroacetate *XX*, respectively. No reaction took place with peracetic or trifluoroacetic acid alone. When trifluoroacetic acid was allowed to react simultaneously with hydrogen peroxide, the products *XVIII* and *XX* were obtained only in trace amounts. Hence it may be considered that in this reaction an oxidative elimination of the iodine atom from the molecule takes place.

For the evaluation of the activity of synthetic brassinosteroids, consisting in the growth promoting activity of the synthetic brassinosteroids, a modified bioassay on the bean second internode was used[25]. The results are shown in Fig. 1. From this it follows that all the brassinosteroids prepared are less active than 24-epibrassinolide *XXI* ((22*R*,23*R*,24*R*)-2α,3α,22,23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one) of which it is known from the literature[17] that it is a very active analogue, the activity of which is close to that of natural brassinolide. The most active among

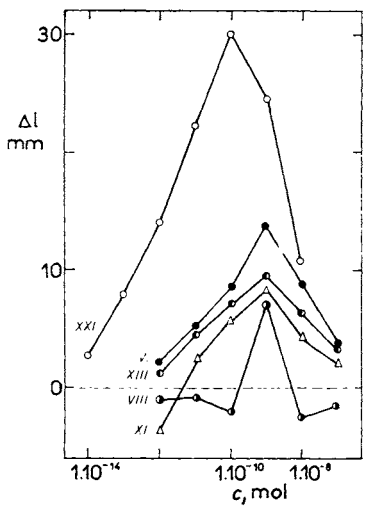



FIG. 1

Effect of brassinosteroids *VI*, *VIII*, *XI*, *XIII*, and *XXI* in the bean second internode bioassay (Δ*l* the difference between the elongation of the second internode of the tested plant and the control plant in mm; *c* the amount of the brassinosteroid applied in mol)

the synthetized compounds is the cholestane analogue *VI* with the brassinoid structure of the A and B rings, while its precursor, compound *XIII*, is less active. 6-Oxa--analogue *XI* is still less active. The least active among the substances compared is the analogue *VIII* the hydroxyl groups of which in the A ring have the opposite configuration than in the natural brassinolide. From these results it follows that the brassinoid side chain is not an indispensable prerequisite for the effect.

## EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. Optical rotation measurements were carried out in chloroform with a ± 3° accuracy. The infrared spectra were measured on a Zeiss UR 20 instrument in tetrachloromethane if not stated otherwise. The $^1$H NMR spectra were measured on a Tesla B 476 (60 MHz) spectrometer in deuteriochloroform with tetramethylsilane as internal reference, unless stated otherwise. The chemical shifts are given in $\delta$-scale. *W* means the width of the signal at half its height. The spectra were interpreted as 1st order spectra. The mass spectra were measured on an AEI MS 902 mass spectrometer. The identity of the samples prepared was checked by mixture melting point determinations, thin-layer chromatography (TLC), infrared spectra and $^1$H NMR spectra. For preparative TLC plates of 200 × × 200 mm dimensions were used, with a silica gel layer 0·7 mm thick. The term "conventional work-up" means that the solution was washed with 5% hydrochloric acid, water, 5% potassium hydrogen carbonate solution, and water, drying over sodium sulfate, filtration off of the sodium sulfate and evaporation of the solvent in a vacuum. If light petroleum was used, it was the fraction with b.p. 40–62°C.

### Bean Second Internode Bioassay

Seeds (56) of bean (*Phaseolus vulgaris*, var. *Pinto*) were planted in 7·0 cm clay pots containing a vermiculite with Hoagland's solution (half concentration, pH = 5·7). The plants were grown in growth room (temperature 23 – 27°C; light 6 000 lux for 16 h), and groups of eight 7-day-old bean seedlings with second internodes 2 mm long were treated with different concentrations (mol) of the tested compounds in 2 μl of lanolin. The control plants were treated with lanolin alone. The measurements were taken after 5 days. Differences in the length of the second internode of the treated and control plants ($\Delta l$ in mm) were compared. The differences were used as a measure of the activity of these compounds.

### 3β-Bromo-B-homo-7-oxa-5α-cholestan-6-one (II)

*a*) Bromo derivative *I* (ref.[19]) (0·20 g) and sodium hydrogen phosphate (1·2 g) in dichloromethane (12 ml) were added to a mixture of trifluoroacetic anhydride (1·4 ml), hydrogen peroxide (0·3 ml) (about 50%) and dichloromethane (2·0 ml), cooled with ice, and the mixture was allowed to stand at room temperature for 40 min. After pouring it into water the product was extracted with dichloromethane, the organic layer was separated, washed with 5% potassium hydrogen carbonate solution, saturated sodium chloride solution, dried over sodium sulfate and the solvent evaporated. Yield, 220 mg of an oil, containing two products according to TLC. Therefore the mixture was separated on a silica gel column (100 g, elution with light petroleum–benzene mixture, 1 : 1). Working up of the fractions containing the lipophilic product gave 130 mg of product which on crystallization from a mixture of dichloromethane and ethanol afforded 98 mg of bromolactone *II*, m.p. 180–183°C (refs[16,26] gives m.p. 171–183°C), $[\alpha]_D^{20}$ +54° (*c* 2). IR spectrum:

1 739, 1 329, 1 273, 1 182 (lactone) $cm^{-1}$. $^1H$ NMR spectrum: 0·68 (s, 18-H), 0·85 (d, $J$ = 5·6 Hz, 26-H and 27-H), 0·93 (s, 19-H), 3·7 (mt, $W$ = 20 Hz, 3α-H), 4·00 (mt, $W$ = 9·5 Hz, 7-H).

*b*) A mixture of bromo ketone *I* (160 mg), 3-chloroperbenzoic acid (160 mg) and dichloromethane (4 ml) was allowed to stand at room temperature for 8 days. After a similar working up as above under *a*) 170 mg of product were obtained, containing two compounds according to TLC. 100 mg of this mixture were separated by preparative TLC on 4 plates, using benzene for development. The fractions containing the lipophilic product were combined, the solvent distilled off and the bromolactone *II* (71 mg) crystallized from dichloromethane–ethanol, m.p. 179–182°C, $[\alpha]_D^{20}$ + 52° (*c* 1).

3β-Bromo-B-homo-6-oxa-5α-cholestan-7-one (*III*)

*a*) Working up of the fractions containing the polar product from chromatography of bromolactone *II* and using procedure *a*) gave 50 mg of a product which was crystallized from a mixture of dichloromethane and ethanol to afford 29 mg of bromolactone *III*, m.p. 189–190·5°C (ref.[26] gives m.p. 183°C), $[\alpha]_D^{20}$ +40° (*c* 1·4). IR spectrum: 1 741, 1 730 sh, 1 275, 1 062 (lactone) $cm^{-1}$. $^1H$ NMR spectrum: 0·69 (s, 18-H), 0·87 (d, $J$ = 5·6 Hz, 26-H and 27-H), 0·95 (s, 19-H), 2·42 (mt, $W$ = 11·5 Hz, 7-H), 3·72 (mt, $W$ = 20 Hz, 3α-H), 4·17 (dd, $J$ = 5·5 Hz, $J'$ = 11 Hz, 5α-H).

*b*) On working up the zones with the more polar product from preparative TLC after the preparation of bromolactone *II* and applying procedure *b*) 28 mg of bromolactone *III* were obtained, m.p. 187–190°C (dichloromethane–ethanol), $[\alpha]_D^{20}$ +41° (*c* 1).

B-Homo-7-oxa-5α-cholest-2-en-6-one (*IV*)

A solution of bromolactone *II* (5·4 g) in N,N-dimethylformamide (100 ml) and lithium bromide (2·7 g) and lithium carbonate (2·7 g) was heated in a bath at 140–150°C for 4 h. After cooling the mixture was poured into water and allowed to stand overnight. The precipitated crystals were filtered off under suction, washed with water and dried. On prepurification on a silica gel column (500 g, elution with light petroleum–ether 19 : 1) 3·1 g of a product were obtained, which when crystallized from ethanol gave 2·7 g of olefin *IV*, m.p. 139–142°C (ref.[16] gives m.p. 140 to 142°C), $[\alpha]_D^{20}$ −34° (*c* 1). IR spectrum: 3 035, 1 681, 670 (double bond), 1 736, 1 729, 1 317, 1 212, 1 060 (lactone) $cm^{-1}$. $^1H$ NMR spectrum: 0·70 (s, 18-H), 0·86 (d, $J$ = 5·3 Hz, 26-H and 27-H), 0·90 (s, 19-H), 0·91 (d, $J$ = 5 Hz, 21-H), 4·46 (asymmetric mt, $W$ = 7·5 Hz, 7a-H), 5·63 (br s, $W$ = 4·5 Hz, 2-H and 3-H).

B-Homo-6-oxa-5α-cholest-2-en-7-one (*V*)

Bromolactone *III* (1·95 g) in N,N-dimethylformamide (50 ml) was mixed with lithium iodide (1 g) and lithium carbonate (1 g) and heated in a bath at 140–150°C for 4 h. After cooling the mixture was poured into water, the precipitated product was washed with water and dissolved in ether and the ethereal solution dried. After distilling off the solvent 1·19 g of product were obtained, which was purified by column chromatography on silica gel (200 g) using a light petroleum–ether mixture (19 : 1) for elution. Yield, 1·28 g of a material which was crystallized from ethanol to afford 295 mg of pure olefin *V*, m.p. 157–162°C, $[\alpha]_D^{20}$ +41° (*c* 1). IR spectrum: 3 035, 1 673 (double bond), 1 740, 1 729 sh, 1 275, 1 043 (lactone) $cm^{-1}$. Mass spectrum: *m/z* 400 (M), 385 (M − $CH_3$), 382 (M − $H_2O$), 372 (M − CO), 346 (M − $C_4H_6$), 318 (M − CO − $C_4H_6$). $^1H$ NMR spectrum: 0·70 (s, 18-H), 0·86 (d, $J$ = 5·9 Hz, 26-H and 27-H), 0·90 (d, $J$ = = 5·3 Hz, 21-H), 0·91 (s, 19-H), 4·46 (dd, $J$ = 8·5 Hz, $J'$ = 8·5 Hz, 5α-H), 5·55 (unsymmetrical doublet, $J$ = 2·5 Hz, 2-H and 3-H). For $C_{27}H_{44}O_2$ (400·6) calculated: 80·94% C, 11·07% H; found: 80·33% C, 10·58% H.

2α,3α-Dihydroxy-B-homo-7-oxa-5α-cholestan-6-one (*VI*)

*a*) Osmium tetroxide (155 mg) in tert-butanol (1·55 ml) was added to a solution of olefin *IV* (3·1 g) in acetone (155 ml). Additional 3·1 ml of tert-butanol and 3·1 g of N-methylmorpholine N-oxide were added to this solution and the mixture was stirred under nitrogen for 2 h. A 10% sodium thiosulfate solution (31 ml) was added and the mixture stirred at room temperature for 1 h. It was then poured into water and worked up as usual by extraction with chloroform. Thus 3·4 g of an oil were obtained, which was chromatographed on a silica gel column (400 g) using light petroleum–acetone (4 : 1) for elution. The fractions containing the lipophilic product were combined, the solvent distilled off and the residue crystallized from aqueous ethanol. Yield, 2·9 g of diol *VI*, m.p. 136–139°C, $[\alpha]_D^{20}$ +55° (*c* 1·2) [+53° (ref.[17]) or +30° (ref.[18])]. IR spectrum (KBr): 3 450, 1 069, 1 029 (hydroxy groups), 1 730, 1 711 sh, 1 183 (lactone) $cm^{-1}$. Mass spectrum: *m/z* 434 (M), 416 (M − $H_2O$), 398 (M − 2 × $H_2O$). $^1H$ NMR spectrum: 0·68 (s, 18-H), 0·85 (d, $J$ = 5·5 Hz, 26-H and 27-H), 0·90 (s, 19-H), 2·90 to 4·18 (mts, 2-H, 3-H and 7a-H).

*b*) A potassium carbonate solution (1·8 g) in water (9 ml) was added to a solution of diacetate *VII* (900 mg) in methanol (10 ml) followed by 2 drops of Aliquate (tricaprylylmethylammonium chloride; a mixture of $C_8$ and $C_{10}$ chains with $C_8$ predominating; phase-transfer catalyst) and the mixture was refluxed for 1 h. Tetrahydrofuran (45 ml) was then added and the solution acidified with 37% hydrochloric acid and refluxed for another hour. After cooling the solution was concentrated to about 1/5 of its original volume, the residue poured into water and the product extracted with ethyl acetate. The ethyl acetate extract was separated, washed with a 5% potassium hydrogen carbonate solution and water, dried and evaporated by distillation. Yield, 720 mg of product which was purified by column chromatography on silica gel (250 g, elution with light petroleum–acetone 9 : 1). The material obtained (670 mg) was crystallized from methanol to give 345 mg of diol *VI*, m.p. 136–139°C, $[\alpha]_D^{20}$ +57° (*c* 1).

2α,3α-Diacetoxy-B-homo-7-oxa-5α-cholestan-6-one (*VII*)

*a*) Ketone *XIV* (0·15 g) in dichloromethane (9 ml) was added to an ice-cooled solution prepared by addition of 50% hydrogen peroxide (0·23 ml) to trifluoroperacetic anhydride (1·05 ml) in dichloromethane (1·5 ml) and the mixture was allowed to stand at room temperature for 35 min. It was poured into water and the product extracted with dichloromethane. The extract was washed with 5% potassium hydrogen carbonate, saturated sodium chloride solution, dried and the solvent evaporated. Yield, 106 mg of a product which was crystallized from ethanol to give 65 mg of lactone *VII*, m.p. 187–190°C, $[\alpha]_D^{20}$ +44° (*c* 1·6); refs[16–18] give m.p. in the 185·5–191°C interval and $[\alpha]_D^{20}$ from +49 to +51°. IR spectrum: 1 746, 1 247, 1 225 (acetates), 1 746, 1 225, 1 182 (lactone) $cm^{-1}$. Mass spectrum: *m/z* 518 (M), 503 (M − $CH_3$), 488 (M − $CH_3$ − $CH_3$), 458 (M − HOAc), 398 (M − HOAc − HOAc). $^1H$ NMR spectrum: 0·70 (s, 18-H), 0·86 (d, $J$ = 5·6 Hz, 26-H and 27-H), 0·90 (d, $J$ = 5 Hz, 21-H), 0·98 (s, 19-H), 1·98 and 2·09 (2 s, 2α-OAc and 3α-OAc), 2·99 (dd, $J$ = 6 Hz, $J'$ = 11·5 Hz, 5α-H), 4·05 (mt, $W$ = 9 Hz, 7-H), 4·83 (mt, $W$ = 9 Hz, 7-H), 4·83 (mt, $W$ = 22 Hz, 2β-H), 5·33 (mt, $W$ = 9 Hz, 3β-H).

*b*) 3-Chloroperbenzoic acid (150 mg) was added to a solution of ketone *XIV* (150 mg) in dichloromethane (3 ml) and the mixture was allowed to stand at room temperature for 15 days. After pouring it into water it was extracted with dichloromethane and worked up as in the preceding section *a*). The product was purified by preparative TLC (chloroform–acetone 9 : 1 as developing solvent), yielding 86 mg of lactone *VII*, which after crystallization from methanol gave 59 mg of pure product with m.p. 186–189°C, $[\alpha]_D^{20}$ +45° (*c* 1).

2β,3β-Dihydroxy-B-homo-7-oxa-5α-cholestan-6-one (*VIII*)

Processing of the fractions with the polar product from chromatography as in the preparation of lactone *VI* (under *a*)) gave 178 mg of a product which when crystallized from aqueous ethanol gave 115 mg of lactone *VIII*, m.p. 238—241°C, $[\alpha]_D^{20}$ −93° (*c* 1). IR spectrum (chloroform): 3 615, 3 565 (hydroxyl groups), 1 722 (lactone) $cm^{-1}$. Mass spectrum: *m/z* 434 (M), 416 (M − $H_2O$). $^1H$ NMR spectrum: 0·70 (s, 18-H), 0·86 (d, $J$ = 5·8 Hz, 26-H and 27-H), 0·89 (d, $J$ = 5 Hz, 21-H), 1·07 (s, 19-H), 3·37 to 4·16 (mts, 2-H, 3-H and 6-H). For $C_{27}H_{46}O_4$ (434·6) calculated: 74·61% C, 10·67% H; found: 73·25% C, 10·47% H.

2β-Hydroxy-3β-acetoxy-B-homo-7-oxa-5α-cholestan-6-one (*IX*)

Working up of the fractions containing the polar product from the chromatography in the preparation of diacetate *X* (according to procedure *a*)) gave 28 mg of a product which on crystallization from aqueous methanol gave 14 mg of monoacetate *IX*, m.p. 168—170°C, $[\alpha]_D^{20}$ +73° (*c* 0·4). IR spectrum: 3 610 (hydroxyl), 1 741, 1 729 sh, 1 244 (acetate), 1 741, 1 729 sh, 1 190 (lactone) $cm^{-1}$. $^1H$ NMR spectrum: 0·69 (s, 18-H), 0·86 (d, $J$ = 5·6 Hz, 26-H and 27-H), 0·89 (d, $J$ = 5 Hz, 21-H), 0·99 (s, 19-H), 4·04 (mts, 2α-H, 3α-H and 7a-H). For $C_{29}H_{48}O_5$ (476·7) calculated: 73·07% C, 10·15% H; found: 72·96% C, 9·96% H.

2β,3β-Diacetoxy-B-homo-7-oxa-5α-cholestan-6-one (*X*)

*a*) Diol *VIII* (52 mg) in pyridine (1 ml) and acetic anhydride (0·5 ml) was allowed to stand at room temperature for 25 h. The mixture was poured into water and processed as usual with ether. Yield, 55 mg of a mixture which was separated by preparative chromatography on TLC (elution with light petroleum–acetone 4 : 1). The working up of the fractions containing the lipophilic product (18 mg) and crystallization from methanol gave 6 mg of diacetate *X*, m.p. 235—236°C, $[\alpha]_D^{20}$ +90° (*c* 0·7). IR spectrum: 1 742, 1 730 sh, 1 249, 1 233 (acetyls and lactone) $cm^{-1}$. $^1H$ NMR spectrum: 0·69 (s, 18-H), 0·86 (d, $J$ = 5·5 Hz, 26-H and 27-H), 0·89 (d, $J$ = 5 Hz, 21-H), 1·04 (s, 19-H), 2·00 and 2·06 (2 s, 2-acetate and 3-acetate), 3·90 to 4·18 (asymmetric mt, 7a-H), 4·76 (mt, $W$ = 17 Hz, 3α-H), 5·21 (mt, $W$ = 12 Hz, 2α-H). For $C_{31}H_{50}O_6$ (518·7) calculated: 71·78% C, 9·72% H; found: 71·49% C, 9·58% H.

*b*) Ketone *XVI* (19 mg) was dissolved in dichloromethane (1 ml) and 3-chloroperbenzoic acid (50 mg) was added to it and the mixture was allowed to stand at room temperature for 18 days. After pouring into water the mixture was extracted with dichloromethane and the extract was separated, washed with 5% potassium hydrogen carbonate and water, dried and the solvent evaporated. The 22 mg of oil obtained were purified by preparative TLC on one plate, using light petroleum–acetone 4 : 1 for development. Yield, 15 mg of lactone *X*, m.p. 233—235°C, $[\alpha]_D^{20}$ +86° (*c* 0·5).

2α,3α-Dihydroxy-B-homo-6-oxa-5α-cholestan-7-one (*XI*)

Osmium tetroxide (11·5 mg) in tert-butanol (0·12 ml), tert-butanol (0·24 ml), and N-methylmorpholine N-oxide (231 mg) were added to a solution of olefin *V* (231 mg) in acetone (11·5 ml) and the mixture was stirred at room temperature and under nitrogen for 2 h. Sodium thiosulfate (1 ml of 10% solution) was then added and the stirring continued for another hour. After pouring into water the product was extracted with chloroform. The extract was worked up in the conventional manner. On filtration through a small column of silica gel (5 g), and evaporation of the solvent 132 mg of a product were obtained which was crystallized from methanol to give 29 mg of the first crop and 62 mg of the second crop of crystals (from mother liquors), *i.e.* a total of

91 mg of lactone *XI*, m.p. 221–224°C, $[\alpha]_D^{20}$ +49° (*c* 0·8). Mass spectrum: *m/z* 434 (M), 416 (M − $H_2O$), 398 (M − 2 × $H_2O$). IR spectrum (KBr): 1 720, 1 730 sh, 1 693 sh, 1 281 (lactone), 1 082, 1 040 (hydroxyls) $cm^{-1}$. $^1H$ NMR spectrum: 0·67 (s, 18-H), 0·85 (d, *J* = 5·5 Hz, 26-H and 27-H), 0·92 (s, 19-H), 3·67 to 4·78 (mts, 2-H, 3-H and 5-H). For $C_{27}H_{46}O_5$ (434·64) calculated: 74·61% C, 10·67% H; found: 74·35% C, 10·42% H.

5α-Cholest-2-en-6-one (*XII*)

*a*) Lithium iodide (1 g) and lithium carbonate (1 g) were added to a solution of iodoketone *XVII* (1 g) and N,N-dimethylformamide (30 ml) and the mixture was heated at 140°C and under nitrogen for 4 h. After pouring into water a crystalline precipitate was formed which was suction-dried and crystallized from acetone. Yield, 520 mg of olefin *XII*, m.p. 106–109°C, $[\alpha]_D^{20}$ +33° (*c* 1) (in agreement with literature[17,18]). IR spectrum: 3 030, 1 659, 671 (double bond), 1 712 (carbonyl) $cm^{-1}$. $^1H$ NMR spectrum: 0·70 (s, 18-H), 0·67 (s, 19-H), 0·86 (d, *J* = 5·5 Hz, 26-H and 27-H), 5·31 to 5·72 (mt, 2-H and 3-H).

*b*) Proceeding similarly as under *a*) 7 g of bromoketone *I* were worked up. Crystallization from ethanol gave 4·9 g of olefin *XII*, m.p. 108–109°C, $[\alpha]_D^{20}$ +35° (*c* 1).

2α,3α-Dihydroxy-5α-cholestan-6-one (*XIII*)

Similarly as in the preparation of diol *XI*, olefin *XII* (3·26 g) reacted with osmium tetroxide (163 mg) and N-methylmorpholine N-oxide (3·26 g) to give 2·92 g of a product which was crystallized from methanol, affording 1·44 g of diol *XIII*, m.p. 210–211·5°C, $[\alpha]_D^{20}$ +11° (*c* 0·8) (in agreement with literature[17]). IR spectrum (chloroform): 3 620, 3 580, 3 450, 1 054 (hydroxyls), 1 707 (carbonyl) $cm^{-1}$. $^1H$ NMR spectrum: 0·65 (s, 18-H), 0·75 (s, 19-H), 0·86 (d, *J* = 5·6 Hz, 26-H and 27-H), 0·91 (d, *J* = 5 Hz, 21-H), 3·49 to 4·14 (two unseparated mts, 2β-H and 3β-H).

2α,3α-Diacetoxy-5α-cholestan-6-one (*XIV*)

The dry residue (1·4 g) of the mother liquors after crystallization of compound *XIII* was dissolved in pyridine (10 ml), acetic anhydride (5 ml) was added to it and the mixture allowed to stand at room temperature for 24 h. After the conventional work-up (extraction with ether) the residue (1·7 g) was analysed by TLC. It contained three products and therefore it was chromatographed on a silica gel column (500 g, elution with light petroleum–acetone 19 : 1). Two fractions were obtained, the first containing 2 lipophilic products and the second one more polar product. The first fraction (1·45 g) was crystallized from ethanol, affording 1·04 g of pure diacetate *XIV*, m.p. 156–158°C (after a change of crystal form at 150–151°C) (literature gives m.p. 150·5 to 152·5, ref.[18], and 156–157°C, ref.[17]), $[\alpha]_D^{20}$ 0° (*c* 0·94). IR spectrum: 1 749, 1 250, 1 233 sh (acetate), 1 718 (carbonyl) $cm^{-1}$. $^1H$ NMR spectrum: 0·66 (s, 18-H), 0·82 (s, 19-H), 0·86 (d, *J* = 5·2 Hz, 26-H and 27-H), 0·91 (d, *J* = 5 Hz, 21-H), 1·97 and 2·06 (2 s, 2α-acetate and 3α-acetate), 4·96 (mt, *W* = 24 Hz, 2β-H), 5·37 (mt, *W* = 5·5 Hz, 3β-H).

2β-Hydroxy-3β-acetoxy-5α-cholestan-6-one (*XV*)

The fraction with the polar product from the chromatography carried out in the preparation of diacetate *XIV* was evaporated (128 mg) and crystallized from methanol. Yield, 85 mg of monoacetate *XV*, m.p. 180–184°C (methanol) (ref.[22] gives m.p. 178–179°C), $[\alpha]_D^{20}$ −2° (*c* 0·7). IR spectrum: 3 610 (hydroxyl), 1 743, 1 249, 1 235, 1 042 (acetate), 1 721 (carbonyl) $cm^{-1}$. $^1H$ NMR spectrum: 0·66 (s, 18-H), 0·85 (d, *J* = 5·5 Hz, 26-H and 27-H), 0·90 (d, *J* = 5 Hz, 21-H), 1·00 (s, 19-H), 2·06 (s, 3-acetate), 3·45 (s, 2-OH), 4·07 (mt, *W* = 5·5 Hz, 2α-H), 4·77 (mt, *W* = 17 Hz, 3α-H).

2β,3β-Diacetoxy-5α-cholestan-5-one (*XVI*)

*a*) The mother liquors after the crystallization of diacetate *XIV* were evaporated and the residue (410 mg) separated by prepartive TLC on 20 plates with light petroleum–acetone 4 : 1. Thus, in addition to 370 mg of diacetate *XIV* 22 mg of a residue were also obtained which was crystallized from methanol to give 12 mg of diacetate *XVI*, m.p. 188–190°C, $[\alpha]_D^{20}$ +4° (*c* 0·7) (both data in agreement with the literature[21,22]). IR spectrum: 1 741, 1 248, 1 235 (acetates), 1 718 (carbonyl) $cm^{-1}$. $^1H$ NMR spectrum: 0·66 (s, 18-H), 0·85 (d, $J$ = 5·5 Hz, 26-H and 27-H), 0·90 (d, $J$ = 5 Hz, 21-H), 0·90 (s, 19-H), 1·98 and 2·06 (2 s, 2β-acetate and 3β-acetate), 4·76 (mt, $W$ = 21-H, 3α-H), 5·27 (mt, $W$ = 6 Hz, 3β-H).

*b*) Acetic anhydride (0·5 ml) was added to a solution of monoacetate *XV* (20 mg) in pyridine (1 ml) and the mixture was allowed to stand at room temperature for 5 days. After pouring it into water the mixture was worked up in the conventional manner (extraction with ether). Yield, 22 mg of diacetate *XVI*, m.p. 185–187°C, $[\alpha]_D^{20}$ +3° (*c* 1).

3α-Hydroxy-5α-cholestan-6-one (XVIII)

*a*) The fractions with the polar product from chromatography in the preparation of ester *XX* were chromatographed on 6 preparative silica gel plates with chloroform–ether mixture 1 : 1. Yield, 78 mg of alcohol *XVIII*, m.p. 156–158°C (in agreement with literature[24]), $[\alpha]_D^{20}$ + 2° (*c* 1·24). Mass spectrum: *m/z* 402 (M), 384 (M – $H_2O$), 387 (M – $CH_3$), 369 (M – $CH_3$ – $H_2O$). IR spectrum: 3 625 (hydroxyl), 1 711 (carbonyl) $cm^{-1}$. $^1H$ NMR spectrum: 0·66 (s, 18-H), 0·72 (s, 19-H), 0·86 (d, $J$ = 5·5 Hz, 26-H and 27-H), 0·90 (d, $J$ = 5 Hz, 21-H), 4·16 (mt, $W$ = 6 Hz, 3β-H).

*b*) Working up of the fraction with the polar product from the chromatography in the preparation of ester *XIX* gave 508 mg of a residue which was crystallized from methanol, giving 366 mg of alcohol *XVIII*, m.p. 160–163°C, $[\alpha]_D^{20}$ 0° (*c* 0·72).

3α-*m*-Chlorobenzoyloxy-5α-cholestan-6-one (*XIX*)

3-Chloroperbenzoic acid (1 g) was added to a solution of iodo ketone *XVII* (1 g) in dichloromethane (20 ml) and the mixture was allowed to stand at room temperature for 6 days. After pouring into water the mixture was extracted with dichloromethane and the separated extract washed with a 10% potassium hydrogen carbonate solution, saturated sodium chloride solution and water, dried and evaporated to dryness. The residue (920 mg) was chromatographed on a silica gel column (400 g) with light petroleum–ether mixtures 9 : 1, 3 : 1, 2 : 1, and 1 : 1, and then with chloroform–ether mixture 1 : 1. The fractions containing the lipophilic product were worked up to give 125 mg of a residue which was crystallized from ethyl acetate–methanol to yield 85 mg of ester *XIX*, m.p. 142–145°C, $[\alpha]_D^{20}$ –13° (*c* 1·6). IR spectrum: 1 721 (carbonyl), 1 711, 1 597, 1 577, 1 290, 1 280 (benzoate) $cm^{-1}$. Mass spectrum: *m/z* 540 (M), 384 (M – $ClC_6H_4COOH$), high resolution: 384·3396 ($C_{27}H_{44}O$, M – $ClC_6H_4COOH$). $^1H$ NMR spectrum: 0·68 (s, 18-H), 0·79 (s, 19-H), 0·86 (d, $J$ = 6 Hz, 26-H and 27-H), 0·91 (d, $J$ = 5 Hz, 21-H), 5·37 (mt, $W$ = 8·5 Hz, 3β-H), 7·34 to 7·57 and 7·80 to 8·05 (2 mt, phenyl protons). For $C_{34}H_{49}ClO_3$ (541·2) calculated: 75·46% C, 9·13% H, 6·55% Cl; found: 75·27% C, 8·94% H, 6·31% Cl.

3α-Trifluoroacetoxy-5α-cholestan-6-one (*XX*)

Hydrogen peroxide (50%; 0·4 ml) was added to a cooled solution of trifluoroacetic anhydride (1·9 ml) in dichloromethane (2·7 ml) and the obtained solution of trifluoroperacetic acid was additioned under cooling with ice with 1·6 g of sodium hydrogen phosphate and 0·27 g of 3β-iodo-

-5α-cholestan-6-one (*XVII*, ref.[19]) in dichloromethane (16 ml). The mixture started to turn pink immediately and eventually became violet. After two days' standing at room temperature the mixture was poured into dichloromethane and the solution obtained was washed with a saturated potassium hydrogen carbonate solution, 10% sodium thiosulfate solution, saturated sodium chloride solution, dried and the solvent distilled off in a vacuum. Yield, 0·3 g of a product which according to TLC contains two main products. This mixture was chromatographed on a silica gel column (50 g) with light petroleum–ether mixtures, beginning with a 19 : 1 ratio, increasing the proportion of ether gradually and finally with pure ether. Two main fractions were obtained. The fraction with the lipophilic product (70 mg) was purified by preparative TLC on 4 silica gel plates using light petroleum–ether (9 : 1) for development. Yield, 35 mg of non-crystallizing ester *XX*, $[\alpha]_D^{20}$ +16° (*c* 0·6). IR spectrum: 1 782, 1 225, 1 173, 1 147 (trifluoroacetate), 1 713 (carbonyl) $cm^{-1}$. Mass spectrum: *m/z* 489 (M), 483 (M − $CH_3$), 385 (M − $CF_3COO^{\bullet}$), 384 (M − $CF_3$.COOH). $^1H$ NMR spectrum: 0·67 (s, 18-H), 0·76 (s, 19-H), 0·86 (d, $J$ = 5·6 Hz, 26-H and 27-H), 0·91 (d, $J$ = 5 Hz, 21-H), 5·32 (mt, $W$ = 7·5 Hz, 3β-H). For $C_{29}H_{45}F_3O_3$ (498·7) calculated: 68·85% C, 9·10% H, 11·43% F; found: 69·60% C, 9·05% H, 10·19% F.

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