PREPARATION OF 2α,3α-DIHYDROXY-7-OXA-6-OXO-23,24-DINOR-B-HOMO-5α-CHOLANIC ACID, ITS ESTERS AND AMIDES AS BRASSINOLIDE ANALOGUES*

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Synthesis of brassinosteroids *IIb, d, g, i, k, m, o* and evaluation of their growth-promoting activity is reported. The acid *IIb* shows high activity in both the bean first and second internode assay.

Brassinolide (*I*) is a potent plant growth-promoting steroid[1]. The importance of its biological properties led several laboratories to the synthesis[2-7] of this substance and its analogues and to the investigation of relations between its structure and biological activity[8-18]. These studies revealed that both the structure of the A,B-ring part of the molecule and that of the chain are important for the biological function. Well established are the structural requirements for the A and B rings: The presence of a 2α,3α-diol moiety (which may be replaced by a 3α,4α-grouping) and of a 7-oxa-6-oxo-function (or 6-oxo group decreasing the activity only slightly). As concerns the side chain, the structure–activity relations seem to be controlled by more complex factors and are less straightforward. Removal of the entire side chain leads to almost complete loss of activity (as demonstrated with 2α,3α,17β-trihydroxy-7-oxa-B-homo-5α-androstan-6-one[17]) but shortening of the side chain by two terminal carbon atoms has no adverse effect[16]. The stereochemistry of 22,23-hydroxyls is important, high activity requiring the 22(*R*), 23(*R*)-configuration. However, the biological response to the changed stereochemistry of the 24-methyl, or to its replacement by an ethyl group shows marked dependence on the stereochemistry of the neighbouring hydroxyls and detailed relationships cannot be easily defined. Moreover, the exact evaluation is complicated by the fact that not always do various kinds of bioassays give parallel results. In general, many problems connected with the side chain remain open and more detailed investigation of structure–activity relations is justified and desirable.

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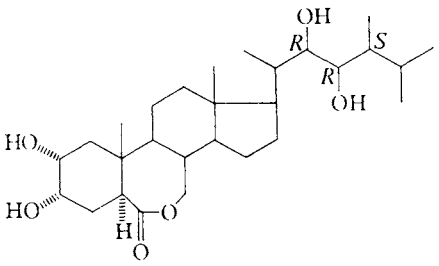
The aim of the present work is the preparation of the acid *IIb*, its alkyl esters and alkyl amides with various alkyl groups[19] (*IId, g, i, k, m, o*). Comparison of their activities should establish to what extent such analogues retain the brassinolide activity or how the magnitude of this activity depends on the similarity of this alkyl group to the corresponding part of the side chain in brassinolide.



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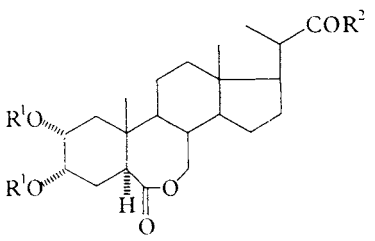
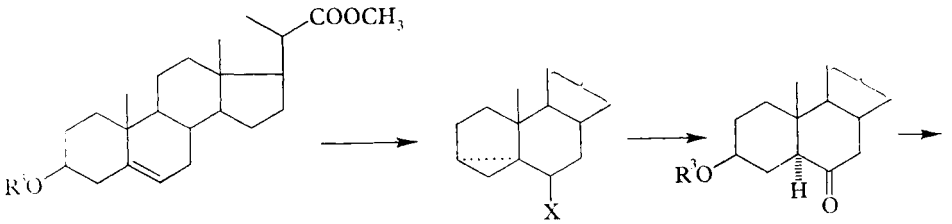


IIa–o

a, R^1 = Ac, R^2 = OCH_3
b, R^1 = H, R^2 = OH
c, R^1 = Ac, R^2 = OH
d, R^1 = H, R^2 = OCH_3
e, R^1 = Ac, R^2 = Cl
f, R^1 = Ac, R^2 = $OCH(CH_3)CH(CH_3)_2$
g, R^1 = H, R^2 = $OCH(CH_3)CH(CH_3)_2$
h, R^1 = Ac, R^2 = NH_2
i, R^1 = H, R^2 = NH_2
j, R^1 = Ac, R^2 = NHC_2H_5
k, R^1 = H, R^2 = NHC_2H_5
l, R^1 = Ac, R^2 = $NHCH(CH_3)_2$
m, R^1 = H, R^2 = $NHCH(CH_3)_2$
n, R^1 = Ac, R^2 = $NHCH_2CH(CH_3)_2$
o, R^1 = H, R^2 = $NHCH_2CH(CH_3)_2$

For the preparation of the above compounds we set out from the known[20] methyl 3β-hydroxy-23,24-dinor-5-cholenate(*IIIa*) which was converted into the tosylate *IIIb* and the latter refluxed in a buffered (pyridine, sodium acetate) aqueous acetone to yield the i-steroid *IVa*. Oxidation of this compound with pyridine–CrO_3 complex provided the 6-ketone *IVb* which was treated with a sulfuric and acetic acid mixture to give the 3β-hydroxy derivative *Va*. Tosylation and elimination of *p*-toluenesulfonic acid with LiBr in refluxing dimethylformamide gave the 2,3-unsaturated compound *VI* which on catalytic osmylation yielded the diol *VIIa*; acetylation, treatment with trifluoroperoxyacetic acid and alkaline hydrolysis provided successively the compounds *VIIb*, *IIa*, and *IIb*. The hydrolysis of the methoxycarbonyl group is difficult and, consequently, the methyl ester *IId* can be easily prepared by partial saponification. Acetylation of *IIb* provided the acid *IIc* which was converted to the chloride *IIe* from which all desired derivatives were prepared by treatment with the corresponding alcohols or amines followed by mild hydrolysis with potassium hydrogen carbonate. The structures of the above compounds are in agreement with spectroscopic properties; the 7-oxa-6-oxo structure of the lactone ring is proved by ^1H NMR spectrum of the compound *IIg*.

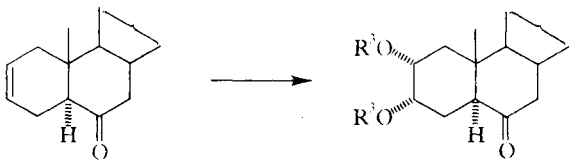
Using two different test systems based on curvature and elongation of the bean first[21,22] and second[1,23] internode, respectively, the biological activity of these analogues was compared with the activity of 24-epibrassinolide[8]. Apart from using 24-epibrassinolide as a standard, the compounds tested were also compared with the following known compounds: 2α,3α,17β-trihydroxy-7-oxa-B-homo-5α-androstan-6-one and 2α,3α,22(*R*),23(*R*)-tetrahydroxy-24(*R*)-methyl-5α-cholestan-6-one the activities of which have been reported[8,17]. We have found that the activities of these compounds parallel the published data and corroborate the suitability of the used plant material for the bioassay. In accord with the observation of other authors[16], the bioassays using the bean first and second internode do not give quantitatively corresponding results. Some of our compounds (*IId,o*) which in the first internode bioassay (Tab. I) are as active or more active than 24-epibrassinolide do not reach the activity of 24-epibrassinolide in the bean second internode bioassay. In our opinion, the bean second internode assay is more suitable for testing the brassinolide activity for its sensitivity and specificity[24]. It is pertinent to note that in both bioassays the substances required for the maximum physiological effect in relatively high quantities exhibit generally low maximum curvature and elongation of epicotyls.



IIIa, R^3 = H
IIIb, R^3 = Ts

IVa, X = OH
IVb, X = O

Va, R^3 = H
Vb, R^3 = Ts



VI

VIIa, R^3 = H
VIIb, R^3 = Ac

The bean second internode bioassay (Tab. II) showed markedly lower activity for esters and amides *IId, g, i, k, m, o*. The activities show differences but no signifi-

TABLE I

Biological activities of brassinosteroids in the bean first internode curvature bioassay

Compound	Quantity required for half of the maximum curvature (mol)	Quantity required for maximum curvature (mol)[a]
24-epibrassinolide	$1{\cdot}0 \cdot 10^{-10}$	$1{\cdot}0 \cdot 10^{-9}$ (–)
2α,3α,17β-trihydroxy-7-oxa-B-homo-5α-androstan-6-one	$1{\cdot}8 \cdot 10^{-9}$	$1{\cdot}0 \cdot 10^{-8}$ ($6{\cdot}2 \cdot 10^{-11}$)
2α,3α-22(*R*),23(*R*)-tetrahydroxy-24(*R*)-methyl-5α-cholestan-6-one	$1{\cdot}9 \cdot 10^{-10}$	$1{\cdot}0 \cdot 10^{-9}$ ($5{\cdot}6 \cdot 10^{-10}$)
IIb	$2{\cdot}4 \cdot 10^{-12}$	$1{\cdot}0 \cdot 10^{-10}$ ($7{\cdot}5 \cdot 10^{-10}$)
IId	$9{\cdot}0 \cdot 10^{-10}$	$1{\cdot}0 \cdot 10^{-8}$ ($7{\cdot}2 \cdot 10^{-10}$)
IIg	$3{\cdot}3 \cdot 10^{-9}$	$1{\cdot}0 \cdot 10^{-8}$ ($2{\cdot}5 \cdot 10^{-11}$)
IIi	—	—
IIk	$7{\cdot}9 \cdot 10^{-10}$	$1{\cdot}0 \cdot 10^{-7}$ ($1{\cdot}0 \cdot 10^{-9}$)
IIm	$7{\cdot}2 \cdot 10^{-10}$	$1{\cdot}0 \cdot 10^{-8}$ ($3{\cdot}8 \cdot 10^{-10}$)
IIo	$1{\cdot}0 \cdot 10^{-10}$	$1{\cdot}0 \cdot 10^{-9}$ ($3{\cdot}2 \cdot 10^{-10}$)

[a] In parentheses: Quantity of 24-epibrassinolide causing the same maximum curvature as the tested substance.

TABLE II

Biological activities of brassinosteroids in the bean second internode elongation bioassay

Compound	Quantity required for half of the maximum elongation (mol)	Quantity required for maximum elongation (mol)[a]
24-epibrassinolide	$2{\cdot}5 \cdot 10^{-12}$	$1{\cdot}0 \cdot 10^{-10}$
2α,3α,17β-trihydroxy-7-oxa-B-homo-5α-androstan-6-one	$3{\cdot}4 \cdot 10^{-11}$	$1{\cdot}0 \cdot 10^{-9}$ ($1{\cdot}6 \cdot 10^{-13}$)
2α,3α,22(*R*),23(*R*)-tetrahydroxy-24(*R*)-methyl-5α-cholestan-6-one	$2{\cdot}5 \cdot 10^{-11}$	$1{\cdot}0 \cdot 10^{-9}$ ($8{\cdot}6 \cdot 10^{-11}$)
IIb	$3{\cdot}4 \cdot 10^{-12}$	$1{\cdot}0 \cdot 10^{-10}$ ($1{\cdot}4 \cdot 10^{-11}$)
IId	$2{\cdot}4 \cdot 10^{-11}$	$1{\cdot}0 \cdot 10^{-9}$ ($1{\cdot}4 \cdot 10^{-13}$)
IIg	$3{\cdot}9 \cdot 10^{-11}$	$1{\cdot}0 \cdot 10^{-9}$ ($1{\cdot}8 \cdot 10^{-13}$)
IIi	$3{\cdot}2 \cdot 10^{-10}$	$1{\cdot}0 \cdot 10^{-8}$ ($3{\cdot}4 \cdot 10^{-13}$)
IIk	$1{\cdot}3 \cdot 10^{-10}$	$1{\cdot}0 \cdot 10^{-9}$ ($1{\cdot}4 \cdot 10^{-12}$)
IIm	$3{\cdot}9 \cdot 10^{-12}$	$1{\cdot}0 \cdot 10^{-10}$ ($1{\cdot}0 \cdot 10^{-12}$)
IIo	$4{\cdot}6 \cdot 10^{-13}$	$1{\cdot}0 \cdot 10^{-11}$ ($7{\cdot}6 \cdot 10^{-14}$)

[a] In parentheses: Quantity of 24-epibrassinolide causing the same maximum elongation as the tested substance.

cant enhancement of activity is apparent when the alkyl group is modified so that the side chain of the respective analogue is more similar to the side chain in brassinolide. Up to this point, the results are in accord with the view that the side chain of brassinolide must retain its essential features.

On the other hand, the free acid *IIb* shows high activity in both assays. This observation demonstrates that even extensive modification of the side chain does not necessarily result in the loss of activity. It also indicates the possibility of finding structurally more simple analogues with high biological activity.

EXPERIMENTAL

Melting points were determined on a Kofler block. Optical rotation measurements were carried out in chloroform with an error of $\pm 3°$. The infrared spectra were recorded on a Zeiss UR 20 or on a Perkin–Elmer spectrometer. The ^{1}H NMR spectra were measured on a Varian XL 200 instrument, in deuteriochloroform with tetramethylsilane as internal reference.

Bean first internode curvature bioassay[21] sensitized by Strnad and Kamínek[22]: Bean seeds (*Phaseolus vulgaris* L., cv. HS-1906) were germinated for 2 days in moist paper wool at 25°C. Selected germinating seeds were transferred into vermiculite saturated with Knop's solution and placed into a light-controlled cultivation room (23°C, irradiance 6–8 W m^2, light/dark period 10/14 h). The first internodes (4–5 cm long) from partially etiolated 10 day old seedlings were collected and cut into 4 cm long sections. The tested compounds were dissolved in ethanol and different concentrations were applied on paper squares to the base of the internode sections prior to the application of 1 pmol of auxin (indole-3-acetic acid, IAA). The control plants were treated with auxin alone. The horizontal displacement of the upper end of the sections was measured 4 h after the application of auxin. The curvatures of controls were subtracted from the treated variants.

The bean second internode bioassay[1] was modified as follows: Seeds of bean (*Phaseolus vulgaris* L., cv. Pinto) were planted in pots containing vermiculite saturated with half strength Hoagland's solution and grown at 25–27°C (light 48 W/m^2, light/dark period 16/8 h). Test compounds in different concentrations (in 2 μL of lanoline) were applied to the base at the second internode (2–4 mm long) of 7-day-old seedlings. The control plants were treated with lanoline alone. Each treatment contained 7 plants. The length of the second internode was measured 5 days after application of the test compounds. Results are expressed in mm of elongation after subtraction of the length of the control.

Two different criteria were used for comparison of the biological activity of the tested substances: 1) their quantity which exhibits 50% of the maximum growth response and 2) the maximum of quantitative growth response at indicated quantity of the substance as compared with 24-epibrassinolide. When double-phasic growth response to brassinosteroids was obtained, the evaluation is based on the first peak of activity[22].

Methyl 6β-hydroxy-3α,5-cyclo-23,24-dinor-5α-cholanate (*IVa*)

Methyl ester *IIIa* (4 g) was treated with *p*-toluenesulfonyl chloride (6 g) in pyridine (50 ml) for three days at room temperature. The mixture was poured onto ice, diluted with water, the product collected with suction and taken up in ether. The solution was washed with water, $NaHCO_3$, dried with Na_2SO_4 and the solvent evaporated *in vacuo* to yield the crystalline tosylate *IIIb*, m.p. 141 °C. IR-spectrum (CCl_4): 1 738, (1 435), 1 162 cm^{-1} ($COOCH_3$), 1 368, 1 189, 1 178 cm^{-1}

($-SO_2$), 1 716 cm^{-1} (CO). For $C_{30}H_{42}O_5S$ (514·7) calculated: 6·28% S; found: 6·14% S. This material was added with stirring to a boiling solution of sodium acetate (3·2 g) and pyridine (20 ml) in acetone (1 L) containing water (250 ml). The mixture was boiled and stirred for an additional 2 h 15 min, then allowed to stand overnight at room temperature. Most of the acetone was removed at max. 45°C *in vacuo*, the mixture poured onto ice and extracted with ether. After washing with water (10×) the solvent was removed at max. 35°C under reduced pressure to give the crystalline product (4·2 g). A sample for analysis was dissolved in acetone at room temperature and kept at −20°C overnight; m.p. 127–128°C, $[\alpha]_D$ +36° (c = 1·3). For $C_{23}H_{36}O_3$ (360·5) calculated: 76·62% C, 10·07% H; found: 76·23% C, 10·04% H.

Methyl 6-oxo-3α,5-cyclo-23,24-dinor-5α-cholanate (*IVb*)

The crude *IVa* was dissolved in pyridine (40 ml) and the solution added with stirring to CrO_3 (5 g) –pyridine (40 ml) complex maintained at 0°C. Stirring was continued for additional 4 h at room temperature and the mixture was left to stand overnight. The mixture was diluted with ether and the undissolved portion separated by suction and washed well with ether. The filtrate was washed ten times with water, dried over Na_2SO_4 and the solvent distilled off. Chromatography of the residue on silica gel in benzene and benzene–ether (2%) yielded the desired product (2·2 g) which after crystallization from methanol gave the substance (*IVb*), 1·84 g), m.p. 112 to 113°C, $[\alpha]_D$ +31° (c = 1·3). IR-spectrum (CCl_4): 1 737, 1 433, 1 161 cm^{-1} ($COOCH_3$); 1 690, 3 080, 3 020, 3 005 cm^{-1} (carbonyl conj. with a cycloprop. ring). For $C_{23}H_{34}O_3$ (358·5) calculated: 77·05% C, 9·56% H; found: 77·28% C, 9·52% H.

Methyl 3β-hydroxy-6-oxo-23,24-dinor-5α-cholanate (*Va*)

The preceding *IVb* (500 mg) was refluxed with acetic acid (18 ml) and 1M-H_2SO_4 (3·6 ml) for 1 h, the mixture poured on ice, diluted with water, collected by suction and washed with water. The moist product was rinsed with methanol (50 ml) into a flask and after addition of a K_2CO_3 (400 mg) solution in water (3 ml) refluxed for 45 min, acidified with 10% HCl, poured on ice and collected by suction. Washing with water and crystallization from methanol yielded the product (330 mg), m.p. 177–178°C. IR-spectrum (CCl_4): 3 620 cm^{-1} (OH), 1 714 cm^{-1} (CO), 1 727, 1 161 cm^{-1} (COOR).

Methyl 6-oxo-23,24-dinor-5α-chol-2-enate (*VI*)

The hydroxy derivative *Va* (500 mg) was treated with *p*-toluenesulfonyl chloride (750 mg) in pyridine (10 ml) for 48 h, ice was added and the mixture poured in ice water (150 ml). After several minutes, the product was isolated by suction, washed with water, extracted with ether, the solution washed with water, 5% HCl, $NaHCO_3$ and water. Evaporation *in vacuo* left the tosylate *Vb* (600 mg), m.p. 139–141°C, $[\alpha]_D$ −15° ($CHCl_3$, c = 1·6). IR-spectrum (CCl_4): 1 738, 1 162 cm^{-1} ($COOCH_3$), 1 368, 1 189, 1 178 cm^{-1} ($-SO_2-$), 1 716 cm^{-1} (CO). This product was refluxed for 45 min with LiBr (750 mg) in dimethylformamide (8 ml) under argon. The mixture was cooled, diluted with water, and extracted with ether, the extract was washed with water, 5% HCl, water, $NaHCO_3$ and water, 5% HCl, water, $NaHCO_3$ and water. Chromatography on silica gel (12 g) in benzene removed more polar impurities to give a pure crystalline fraction (333 mg) which was crystallized from methanol to yield the product *VI* (300 mg), m.p. 148–148·5°C, $[\alpha]_D$ +27° (c = 1·3). For $C_{23}H_{34}O_3$ (358·5) calculated: 77·05% C, 9·56% H; found: 77·18% C, 9·86% H.

Methyl 2α,3α-dihydroxy-6-oxo-23,24-dinor-5α-cholanate (*VIIa*)

To the unsaturated ester *VI* (1 g) dissolved in tetrahydrofuran (50 ml), was successively added: water (10 ml), N-methylmorpholin N-oxide (1·4 g) and osmium tetroxide (60 mg) dissolved in tert-butanol (c. 3 ml). The mixture was stirred under argon for 8 h at room temperature, let stand overnight, then $Na_2SO_3 . 7 H_2O$ (2 g) in water (10 ml) was added and the mixture stirred for 2 h. After dilution with water the product was taken up in dichloromethane, washed with 5% HCl, $NaHCO_3$ and water, the solvent evaporated and the residue crystallized from methanol at 5°C to yield the diol *VIIa* (630 mg), m.p. 196–197·5°C, $[\alpha]_D$ −6° (*c* = 2·8). IR-spectrum ($CHCl_3$): 3 620, 3 580, 1 045, 1 010 cm^{-1} (OH); 1 730, 1 438, 1 170 cm^{-1} ($COOCH_3$), 1 712 cm^{-1} (CO). Chromatography of the mother liquors on silica gel in benzene–methanol (0·75%) provided additional diol *VIIa* (400 mg). For $C_{23}H_{36}O_5$ (392·5) calculated: 70·37% C, 9·25% H; found: 70·77% C, 9·29% H.

Methyl 2α,3α-diacetoxy-6-oxo-23,24-dinor-5α-cholanate (*VIIb*)

The diol *VIIa* (500 mg) was acetylated in pyridine (5 ml) with acetic anhydride (3 ml) overnight. After pouring on ice, extraction with ether, washing of the solution with water, 5% HCl, $NaHCO_3$ and water, the product was crystallized from methanol to give the compound *VIIb* (530 mg), m.p. 206–208°C, $[\alpha]_D$ −14° (*c* = 1·8). IR-spectrum (CCl_4): 1 740, 1 250, 1 044 cm^{-1} (OAc), 1 745, 1 164 cm^{-1} ($COOCH_3$), 1 719 cm^{-1} (CO). For $C_{27}H_{40}O_7$ (476·6) calculated: 68·04% C, 8·46% H; found: 68·03% C, 8·25% H.

Methyl 2α,3α-diacetoxy-23,24-dinor-B-homo-7-oxa-6-oxo-5α-cholanate (*IIa*)

To a solution of *VIIb* (446 mg) in dichloromethane (6 ml) was added a solution of trifluoroperoxyacetic acid prepared from $(CF_3CO)_2O$ (1 ml) and H_2O_2 (70%, 0·15 ml) in dichloromethane (15 ml) at 0°C. The mixture was allowed to stand at room temperature for 5 h, then washed with water, $NaHCO_3$ and water, dried and evaporated. Crystallization from methanol yielded the compound *IIa* (383 mg), m.p. 265–267°C, $[\alpha]_D$ +34 (*c* = 1·3). IR-spectrum ($CHCl_3$): ν(CO): 1 732 cm^{-1} ($COOCH_3$, OAc, lactone); 1 435, 1 170 cm^{-1} ($COOCH_3$), 1 252, 1 045 cm^{-1} (OAc), 1 183 cm^{-1} (lactone). For $C_{27}H_{40}O_8$ (492·6) calculated: 65·83% C, 8·19% H; found: 66·37% C, 8·15% H.

Methyl 2α,3α-dihydroxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanate (*IId*)

A solution of *IIa* (150 mg), $KHCO_3$ (120 mg) in methanol (30 ml) and water (1 ml) was refluxed for 30 min, acidified with 5% HCl, concentrated *in vacuo* to a small volume, water was added and the product taken up in ether. Washing with water and evaporation of the solvent under reduced pressure gave a product (110 mg) which after crystallization from heptane–methanol amounted to 81 mg, m.p. 185–186°C, $[\alpha]_D$ +32° (*c* = 1·4). IR-spectrum ($CHCl_3$): 3 610, 1 050 cm^{-1} (OH), 1 723, 1 435, 1 170 cm^{-1} (lactone). For $C_{23}H_{36}O_6$ (408·5) calculated: 67·62% C, 8·88% H; found: 67·86% C, 8·90% H.

2α,3α-Dihydroxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanic Acid (*IIb*)

A solution of *IIa* (500 mg) and NaOH (800 mg) in ethanol (26 ml) and water (9·5 ml) was refluxed under argon for 37 h. The solution was then acidified with HCl, concentrated to 1/3 of the original volume, diluted with 5 vol. of water and allowed to stand overnight, separated crystals (400 mg) m.p. 305–310°C were collected by suction and crystallized from aqueous ethanol

to yield the acid (303 mg), m.p. 317–319°C. For $C_{22}H_{34}O_6$ (394·5) calculated: 66·98% C 8·69% H; found: 67·05% C, 8·83% H.

2α,3α-Diacetoxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanic Acid (*IIc*)

The dihydroxy acid *IIb* (300 mg) was acetylated in pyridine (12 ml) with acetic acid anhydride (5 ml) at room temperature overnight. Water (5 ml) was then added and heated at 90–100°C for 1 h. Dilution with water and acidification with HCl led to crystallization of the product which was collected after 5 min by suction. The yield of this substance was 338 mg, m.p. 266 to 268°C. Crystallization from aqueous acetic acid provided pure acid (320 mg, m.p. 271–273·5°C, $[\alpha]_D$ +26° (c = 1·5). IR-spectrum ($CHCl_3$): 3 500–2 500, 1 711 cm^{-1} (COOH), 1 740, 1 256 cm^{-1} (OAc), 1 730, 1 186 cm^{-1} (lactone). For $C_{26}H_{38}O_8$ (478·6) calculated: 65·25% C, 8·00% H; found: 65·42% C, 8·13% H.

(*S*)-1,2-Dimethylpropyl 2α,3α-Diacetoxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanate (*IIf*)

The acid *IIc* (290 mg) was dissolved in benzene (40 ml) and this solution was concentrated to 15 ml; oxalyl chloride (1·2 ml) was then added, the mixture was allowed to stand for 3 h, one drop of pyridine was added and after 2 h the mixture was evaporated (at max. 35°C). Traces of oxalyl chloride were removed by repeated addition of benzene and its removal *in vacuo* (max. 35°C). The chloride *IIe* was dissolved in dry benzene (3 ml), (*S*)-3-methylbutan-2-ol (0·2 ml) was added, the mixture allowed to stand overnight, diluted with ether, the solution washed with water and then with NaHCO. Evaporation of the solvent and chromatography on silica gel (20 g) in benzene–ether (10%) separated the crystalline ester *IIf* (246 mg) from a small amount of less polar impurity. Crystallization from ethanol yielded the pure *IIf* (196 mg), m.p. 240 to 241°C, $[\alpha]_D$ +35° (c = 1·3). IR-spectrum ($CHCl_3$): 1 736, 1 256, 1 050 cm^{-1} (OAc); 1 726, 1 184 cm^{-1} (COO). Mass spectrum: M^+ = 548. For $C_{31}H_{48}O_8$ (548·7) calculated: 67·85% C, 8·82% H; found: 68·16% C, 8·80% H.

(*S*)-1,2-Dimethylpropyl 2α,3α-dihydroxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanate (*IIg*)

The diacetoxy derivative *IIf* (150 mg) was refluxed with aqueous (1 ml of water) methanolic (30 ml) solution of $KHCO_3$ (120 mg) for 30 min. The mixture was acidified with HCl, concentrated to a small volume, diluted with water and the product taken up in ether. The solution was washed with water and $NaHCO_3$, the solvent evaporated *in vacuo* and the residue (128 mg) crystallized from benzene–heptane and methanol–heptane to give the ester *IIg* (101 mg), m.p. 215–215·5°C, $[\alpha]_D$ +38° (c = 1·6). IR-spectrum ($CHCl_3$): 3 610, 3 585, 1 027, 1 040, 1 067 cm^{-1} (OH), 1 721, 1 183 cm^{-1} (COO). ^{1}H NMR spectrum: 3·72 (1 H, mt, $\Sigma J \doteq 22$ Hz, $J_{1\alpha,2\alpha}$ = = 11·4 Hz, 2β-H), 4·00 (1 H, mt, $\Sigma J \doteq 9$ Hz, $J_{3\beta,4\alpha}$ = 4·3 Hz, $J_{3\beta,4\beta}$ = 2·4 Hz, 3β-H), 1·95 and 2·15 (2 H, ddd, J_{gem} = −15·5 Hz, 4α-H and 4β-H), 3·12 (1 H, dd, $J_{4\beta,5\alpha}$ = 12·1 Hz, $J_{4\beta,5\alpha}$ = 4·8 Hz, 5α-H), 4·08 (2 H, d, J = 5·5 Hz, 7aα-H, 7aβ-H), 0·72 (3 H, s, 18-H), 0·92 (3 H, s, 19-H), 0·91 (6 H, d, J = 6·6 Hz, protons of the terminal methyls in the ester group), 1·14 (3 H, d, J = 6·4 Hz, protons of the remaining methyl in the ester group), 1·18 (3 H, d, J = = 6·8 Hz, 21-H). For $C_{27}H_{44}O_6$ (464·6) calculated: 69·79% C, 9·55% H; found: 70·03% C, 9·52% H.

2α,3α-Diacetoxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanamide (*IIh*)

Following the procedure used in the preparation of *IIf*, the acid *IIc* (157 mg) was converted to its chloride which was dissolved in benzene (15 ml) and NH_3 was passed through this solution for 5 min. After standing overnight the mixture was diluted with benzene containing ca 5%

of chloroform and ethyl acetate. Washing with water, HCl and $NaHCO_3$, drying and evaporation provided *IIh* (110 mg), m.p. 308–310°C. IR-spectrum ($CHCl_3$): 3 530, 3 415, 1 684, 1 591 cm^{-1} ($CONH_2$); 1 739, 1 255 cm^{-1} (OAc); 1 729, 1 189 cm^{-1} (lactone).

2α,3α-Dihydroxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanamide (*IIi*)

$KHCO_3$ (80 mg) was dissolved in water (1 ml), added to the amide *IIh* (100 mg) and methanol (25 ml) and the mixture was refluxed for 30 min. Concentration of the mixture and acidification with HCl yielded a product (76 mg) which after crystallization from ethanol gave *IIi* (57 mg), m.p. 341–343°C (decomp.). IR-spectrum (KBr): 1 679, 1 634, 1 621 cm^{-1} ($CONH_2$), 1 056, 1 029 cm^{-1} (OH), 1 722, 1 184 (lactone). For $C_{22}H_{35}NO_5$ (393·5) calculated: 67·14% C, 8·96% H, 3·56% N; found: 67·12% C, 8·73% H, 3·59% N.

N-Ethyl-2α,3α-diacetoxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanamide (*IIj*)

Following the procedure used in the preparation of *IIf*, the acid *IIc* (157 mg) was converted to its chloride, the latter dissolved in benzene (15 ml) and a benzene solution of ethylamine (50%, 1 ml) was added. After standing overnight, the mixture was diluted with benzene, washed with water, 5% HCl and $NaHCO_3$. Evaporation *in vacuo* gave *IIj* (146 mg), m. p. 289–290°C. IR-spectrum ($CHCl_3$): 1 740, 1 252 cm^{-1} (OAc); 1 740, 1 189 cm^{-1} (lactone); 3 455, 1 763, 1 521 cm^{-1} (CONH).

N-Ethyl-2α,3α-dihydroxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanamide (*IIk*)

Following the procedure applied in the preparation of *IIg*, the diacetate *IIj* (138 mg) was hydrolyzed to give crude *IIk* (105 mg) which after crystallization from methanol–ether gave the pure *IIk* (70 mg), m.p. 301–303°C, $[\alpha]_D$ +45° ($CHCl_3$–C_2H_5OH, $c = 1.3$). IR-spectrum ($CHCl_3$): 1 720 cm^{-1} (lactone); 3 450, 1 668, 1 658, 1 520 cm^{-1} (CONH); 3 625, 3 585 cm^{-1} (OH). For $C_{24}H_{39}NO_5$ (421·6) calculated: 68·37% C, 9·32% H, 3·32% N; found: 68·49% C, 9·26% H, 3·28% N.

N-Isopropyl-2α,3α-diacetoxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanamide (*IIl*)

The chloride *IIe*, prepared from the acid *IIc* (227 mg) as given in the preparation of *IIf*, was dissolved in benzene (10 ml) and treated with 2-aminopropane (0·5 ml) for 15 h. The mixture was diluted with benzene, washed with water, HCl (5%), $NaHCO_3$ and water, concentrated to a small volume and ether was added which led to crystallization of the amide (155 mg), m.p. 321–322°C, $[\alpha]_D$ +25° ($c = 1.7$). Analogous treatment of the mother liquors furnished an additional amount (65 mg) with the same m.p. IR-spectrum ($CHCl_3$): 3 440, 1 661, 1 514 cm^{-1} (CONH); 1 736, 1 252, 1 052 cm^{-1} (OAc); 1 736, 1 189 cm^{-1} (lactone). For $C_{29}H_{45}NO_7$ (519·7) calculated: 67·02% C, 8·73% H, 2·69% N; found: 66·93% C, 8·96% H, 2·67% N.

N-Isopropyl-2α,3α-dihydroxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanamide (*IIm*)

The diacetoxy derivative *IIl* (135 mg) was hydrolyzed as in the preparation of *IIg*. Repeated concentration of the reaction mixture, dilution with water and concentration yielded the product (96 mg) which was crystallized from methanol–ether to give pure *IIm* (91 mg), m.p. 325–326°C, $[\alpha]_D$ +34° ($c = 1.3$). IR-spectrum ($CHCl_3$): 3 440, 1 660, 1 517 cm^{-1} (NHCO), 1 722, 1 186 cm^{-1} (lactone), 3 620, 3 585, 1 027 cm^{-1} (OH). For $C_{25}H_{41}NO_5$ (435·6) calculated: 68·92% C, 9·49% H, 3·22% N; found: 69·15% C, 9·58% H, 3·48% N.

N-Isobutyl-2α,3α-diacetoxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanamide (*IIn*)

The chloride *IIe* prepared from the acid *IIc* (245 mg) was treated with isobutylamine analogously to the above conversion of *IIe* into *III*. Concentration of the benzene solution and addition of ether gave *IIn* (187 mg) m.p. 298–300°C; an analytical sample was obtained by crystallization from benzene–ether, m.p. 300–301·5°C, $[\alpha]_D$ + 34° (c = 1·3). IR-spectrum ($CHCl_3$): 1 740, 1 253 (OAc), 3 455, 1 666, 1 523 cm^{-1} (CONH), 1 730 infl., 1 188 cm^{-1} (lactone). For $C_{30}H_{47}NO_7$ (533·7) calculated: 67·51% C, 8·89% H, 2·62% N; found: 67·12% C, 9·11% H, 2·48% N.

N-Isobutyl-2α,3α-dihydroxy-7-oxa-6-oxo-23,24-dinor-B-homo-5α-cholanamide (*IIo*)

The diacetoxy derivative *IIn* (170 mg) was hydrolyzed with $KHCO_3$ as described for *IIg*, the reaction mixture was then concentrated to 1/4 of the original volume, four volumes of water were added and the mixtures was allowed to stand overnight. This procedure and analogous treatment of the mother liquors gave a product (115 mg), m.p. 291–293°C which after crystallization from methanol–ether yielded the amide *IIo* (83 mg), m.p. 297–298·5°C, $[\alpha]_D$ +19° (c = 1·3). IR-spectrum ($CHCl_3$): 3 455, 1 661, 1 526 cm^{-1} (CONH), 1 721, 1 185, cm^{-1} (COO), 3 620, 3 570, 3 370, 1 067 cm^{-1} (OH). For $C_{26}H_{43}NO_5$ (449·6) calculated: 69·45% C, 9·64% H, 3·12% N; found: 69·34% C, 9·82% H, 2·91% N.

The elemental analyses were carried out in the Analytical Laboratory of this Institute under the direction of Dr J. Horáček. The infrared spectra were recorded by Mrs K. Matoušková and interpreted by Dr J. Smolíková. The ^{1}H NMR *spectra were measured and interpreted by Dr J. Zajíček.*

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