Tetrahedron Vol. 45, No. 1, pp. 155 to 164, 1989
Printed in Great Britain.

0040-4020/89 $3.00+.00

ALLENIC CHOLESTERYL DERIVATIVES AS INHIBITORS OF ECDYSONE BIOSYNTHESIS.

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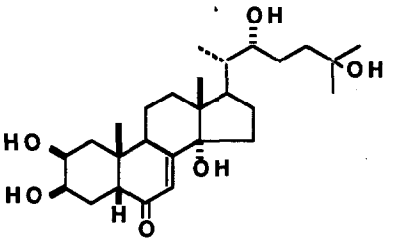
(*Received in Belgium* 28 *September* 1988)

Abstract. - A series of allenic derivatives of cholesterol was synthesized from pregnenolone. These compounds carry an allenic function at C-20 or C-22 and were devised with the aim to inhibit the C-22 hydroxylation of ecdysone biosynthesis by a suicide-substrate mechanism. The four compounds synthesized inhibit efficiently the synthesis of ecdysone in the prothoracic glands of Locusta.

The insect moulting hormone, ecdysone (1, (22R)-2β,3β,14,22,25-pentahydroxy-5β-cholest-7-en-6-one), is synthesized during larval and nymphal development in the so-called moulting glands or prothoracic glands (or homologous structures).



Ecdysone 1

In reproductively competent adult females, ecdysone is also synthesized in the follicular cells of the ovaries and is transferred into the eggs where it is believed to play a role during embryonic development (e.g. the control of embryonic cuticle deposition).[1)] For fundamental studies as well as applied research, we have set up a program for the synthesis of irreversible and selective inhibitors of ecdysone biosynthesis. Suicide substrate inhibitors would be the most appropriate for our studies. The design of this type of inhibitors must take into account both the molecular structure of the natural substrate and the enzymatic reaction one aims to block.

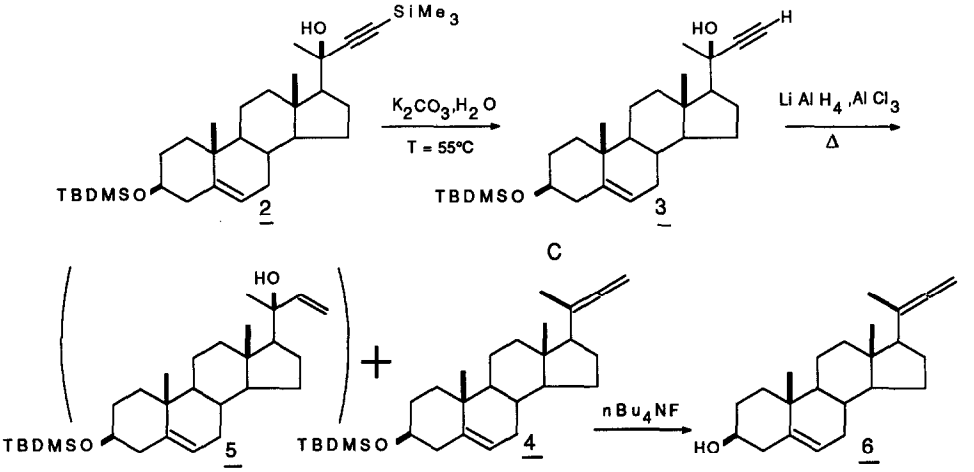
Our understanding of the biosynthetic pathway of ecdysone is still imperfect; however the sequence of the last three steps has been clearly established in several insect species. It is a sequence of hydroxylations from 2,22,25-trideoxyecdysone in the following order : at C-25, then at C-22 and finally at C-2.[2,3)]

In a first approach, we have chosen to inhibit the C-22 hydroxylation as this hydroxylation, which is catalysed by a cytochrome P-450 dependent monooxygenase,[4)] is requisite for the moulting effect.[5)] Such a hydroxylation has been reported to be inhibited by substances carrying an acetylenic, an allenic[6)] or an difluorinated function.[7)]

In a previous publication, we have reported the synthesis of sterol derivatives carrying an acetylenic function[8)] and the biological studies of the effect of these molecules on ecdysone biosynthesis were very promising.[9)]

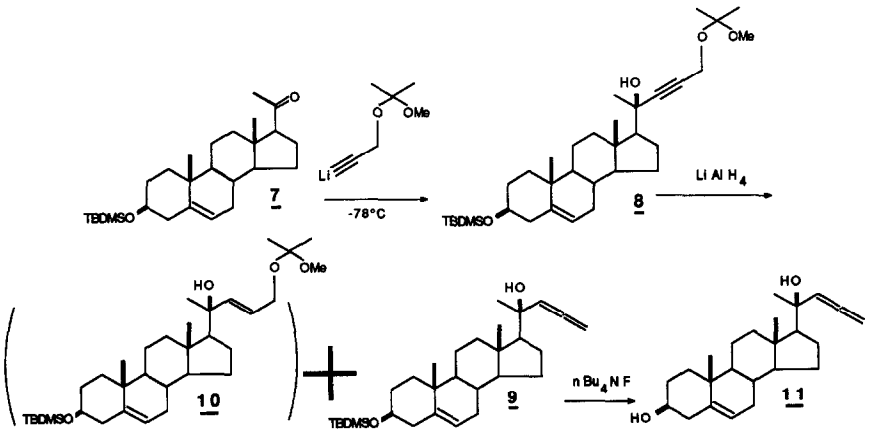
In the present paper, we describe the synthesis of another series of inhibitors which bear an allenic function, and the effect of these inhibitors is discussed.

CHEMICAL RESULTS



Scheme A

The synthesis of compound 6 was achieved using a classical pathway as shown in scheme A. Compound 2 has been obtained readily from TBDMS (t-butyl dimethylsilyl ether)-protected pregnenolone 7.[8] The hydrolysis of the trimethylsilyl group on the alkyne 2 was regiospecific and quantitative and gave the terminal alkyne 3. Reduction by the mixture LAH/$AlCl_3$ (3/1)[10,11] converted 3 to the expected allene 4 with a 55% yield. 4 was easily separated from the allylic alcohol 5 (35%) by silica gel chromatography. Fluoride ion n-Bu_4NF removed the t-butyl dimethylsilyl ether moiety in 4 to yield the sterol 6 quantitatively.



Scheme B

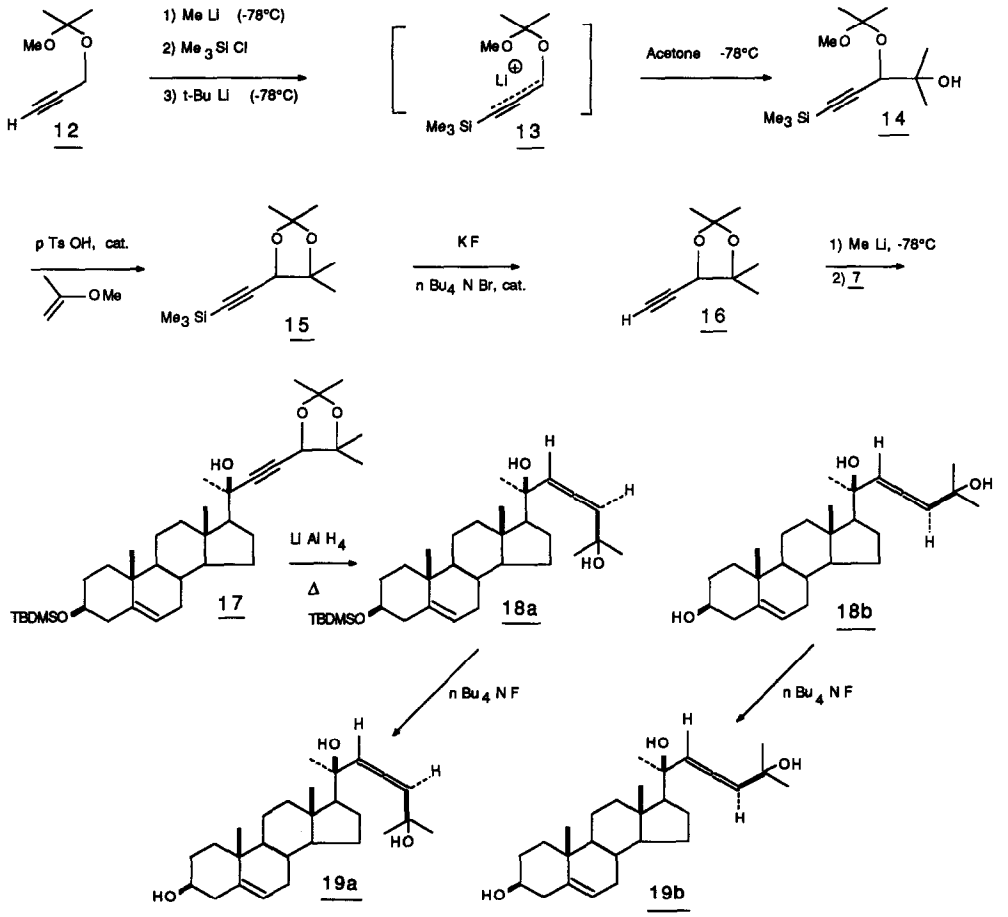
The α-allenic alcohol 11 was synthesized from 7[8] as shown in scheme B.

We have chosen to protect the propargylic alcohol in 12[12] with a 2-methoxy-2-propoxy protecting group rather than the tetrahydropyranyl

protection as it avoids the inconvenience of introducing a new asymetric center in the synthesis. 8 was obtained by reacting lithium acetylide of 12 (3 eq.) with the keto group of 7. The reaction was quantitative and stereoselective.[13] The Cram product with (20R)-configuration was obtained with only a trace of the more polar (20S) isomer (TLC). The NMR spectra of the (20R) and (20S) isomers are slightly different as the signal of the C-18 methyl appeared at δ = 0.97 for the (20R) and at δ = 0.89 for the (20S) isomer (in $CDCl_3$, 200 MHz).This chemical shift difference is typical for isomeric 20-OH-steroids.[13]

LAH in ether,[14-16] at room temperature reduced the primary protected propargylic alcohol 8 to the expected allene 9 (77%) and the allylic alcohol 10 (19%). We have never been able to avoid the formation of allylic alcohol 10 in the reduction of the precursor 8 with LAH because the chemical methods[17] which would strongly decrease the yield of this alcohol were not compatible with our compounds. When the reaction was carried out under reflux, some less polar products appeared.

The protecting group, 2-methoxy-2-propoxy ether, chosen for our synthesis of allene 9 was as suitable a leaving group as the THP ether,[14] in the LAH reduction; the yield of 9 was similar to those observed with THP ether derivatives of 1,4 diol alkynes in the synthesis of terminal allenes.[14] The NMR analysis of the olefinic protons at C-22 and at C-23 of compound 10 showed a coupling constant J_{AB} = 16 Hz which is indicative of a trans double bond.



Scheme C

Finally, compound 11 has been obtained from 9 with the same deprotecting agent as described for 6.

For the synthesis of allenic steroids with a side chain which presents some similarities with that of ecdysone (a hydroxyl group at C-25), we have first synthesized a six carbon chain and coupled it to the steroid as shown in scheme C. Compound 14 was synthesized from 12 in a one-pot synthesis.

The trimethylsilylalkyne prepared from 12 was directly treated with t-BuLi[18,19] and produced the ambident anion 13. This anion was finally condensed with acetone to give exclusively the β-acetylenic alcohol 14. The isopropylidene protection of the α,β-acetylenic diol was performed by intramolecular cyclisation under acid catalysis in the presence of 2-methoxy-propene which acts as a methanol trap.

This one-pot procedure produced 15 with an 85% yield.

KF, with a catalytic amount of a quaternary ammonium salt (nBu_4NBr), removed the trimethylsilyl moiety in 15 to yield the ethynyl-1,3- dioxolane 16 (84%).

The coupling of 16 on the keto-group of compound 7 followed a procedure similar to that used in the synthesis of 8.

The two diastereomers 17 (a and b, a 1:1 mixture) obtained were not separable but could be distinguished by the chemical shift of the C-24 proton. One of the isomers had a singlet signal at $\delta = 5.60$, and the other a signal at $\delta = 5.59$ (C_6D_6, 200 MHz).

LAH reduction of the diastereomer mixture 17 (a and b) gave the two chiral allenes 18 (a and b), which could be separated by silica gel chromatography (42% yield for each isomer).

This reduction furnished 18 with a total yield similar to that of the synthesis of 9. In this case the acetonide of the acetylenic diol served as a good leaving group in the LAH reduction and proved to be advantageous as it gave directly the free C-25 alcohol. These two allenes have distinguishable physical properties, in particular their rotatory power. This has permitted an application of the Lowe-Brewster rule,[20,21] which has been useful in assigning the absolute configuration of many chiral synthetic and natural 1,3-disubstituted acyclic allenes.[22]

The $[\alpha]_D$ of the less polar allene 18 was -96 (c=0.5 in $CHCl_3$) while that of the more polar one was -11 (c=0.5 in $CHCl_3$). By first using the additivity rule of the chiral center in the allenes 18 in comparison to the allenic steroid 9 used as a reference ($[\alpha]_D = -43$, c = 0.5 in $CHCl_3$), and secondly with the use of the Lowe-Brewster rule, we were able to attribute the configurations of the allenes 18 as follows : The less polar, more laevorotatory, allenic steroid 18a should have the (23R)- configuration and the more polar one 18b ,the (23S)- configuration. The deprotected compounds 19a and 19b were obtained respectively from 18a and 18b as described in the synthesis of 6.

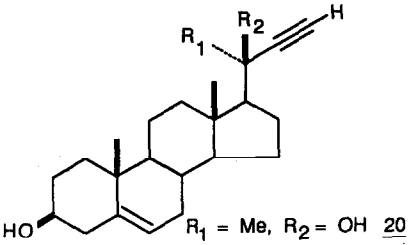
BIOLOGICAL RESULTS

The biological tests were performed on the migratory locust, Locusta migratoria. The inhibitory effects of the compounds 6, 11, 19a and 19b have been determined in vitro on ecdysone biosynthesis in larval prothoracic glands (for details see 8). All the allenic steroids induced a decrease in the synthesis of ecdysone. The depressory effect of these compounds, at a concentration of 10^{-4}M, on the ecdysone biosynthesis was about 30% in the presence of 6, 44% in the presence of 19a and 19b, and 70% in the presence of 11, and this effect was dose-dependent.

CONCLUSION

In a previous publication,[8] we had explored the activity of steroids with an acetylenic function on the side chain. Among the molecules synthesized, 20, presented a very advantageous activity : a clear and intense inhibitory effect (60% inhibition, in vitro, on the biosynthesis of ecdysone in prothoracic glands at 10^{-4}M) and a high specificity (only the C-22 hydroxylation was blocked).

In the present study, we have limited our synthesis to molecules presenting some homologies with the active acetylenic steroids. Among the allenic steroids prepared, that with the highest activity 11 was homologous to acetylenic 20. 20 has a (20R) stereochemistry, its (20S) isomer 21, which we had also synthesized was biologically completely inactive (unpublished results).



R_1 = Me, R_2 = OH 20

R_1 = OH, R_2 = Me 21

This result was indicative of an enzymatic stereospecificity near the C-22 position which we aim to block. In contrast, we have isolated and identified the 19a and 19b chiral allenic steroids, which presented the same biological activity. At present we do not know if there is an influence of structural modifications in the nucleus of the steroid. To answer this, we are now working on the synthesis of acetylenic and allenic molecules with the ecdysone polycycle system.

EXPERIMENTAL

Melting points were measured on a Reichert microscope and are uncorrected. [α]D values were measured on a Perkin-Elmer 141 polarimeter in $CHCl_3$. IR spectra were recorded in KBr on a Perkin-Elmer spectrophotometer or a Pye Unicam SP3-3000S infrared spectrophotometer (Philips). Absorptions are given in cm^{-1}. NMR spectra were recorded on a Bruker SY (200MHz) apparatus with $CHCl_3$ (δ= 7.27) or CH_2Cl_2 (δ = 5.35) as internal standard for ^1H NMR and $CDCl_3$ (δ = 76.9) or CD_2Cl_2 (δ = 53.6) as internal standard for ^{13}C NMR. The chemical shifts are reported in ppm downfield from TMS. MS were measured on a Thomson THN or a LKB 9000S apparatus by direct introduction using an ionization potential of 70 eV. Microanalyses were performed by the Strasbourg Division or by the Service Central de Vernaison de Microanalyses du CNRS.

THF and ether were freshly distilled from LAH before use. All air- or moisture-sensitive reactions were conducted in flame-dried glassware. Reactions were monitored by TLC on pre-coated plates of silica gel (60F254, Merck). The plates were dipped in a solution of vanillin (1g/l) in EtOH-H_2SO_4 (95-5) and heated on a hot plate to detect the compounds. Medium pressure chromatography (p = 0.3-0.4 b) was conducted on silica gel (40-63 µm, Merck) columns.

(20R)-3β-t-Butyldimethylsilyloxy-24-nor-chol-5-en-22-yn-20-ol (3)

A stirred suspension of 2 (502mg, 0.95mmol) and K_2CO_3 (407mg, 2.95mmol) in THF-EtOH-H_2O (11.5ml, 5-4-2.5) was heated to 55-60°C. After 7h the solution was cooled to R.T. and the reaction neutralized by cautious addition of an Aq. solution of HCl (1N, 4ml). The mixture was refluxed and EtOH was added to obtain a homogenous solution. Then stirring and heating were stopped. After one night at R.T. and an additional 4h at 4°C, the cristalline precipitate was filtered, washed twice with water and dried under vacuum (70°C, 0.5mm Hg) to yield compound 3 (390mg, 90%).

mp = 175-177°C. $[\alpha]_D$ = -28 (c = 1.3 $CHCl_3$). IR : 3500 (m), 3340 (m), 2120 (m), 1260 (m), 1100 (3). ^1H NMR (CD_2Cl_2) : 0.05 (6H, s, $Si(Me)_2$), 0.89 (9H, s, $SiC(Me)_3$), 0.97 (3H, s, CH_3-18), 1.02 (3H, s, CH_3-19), 1.48 (3H, s, CH_3-21), 2.55 (1H, s, H-23), 3.50 (1H, m, wl/2 = 25Hz, H-3). ^{13}C NMR in table 1. MS m/z : 456 (M^+, 1), 401 (2), 399 (100), 381 (9), 373 (7), 331 (8). Found : C, 76.39 ; H, 10.56. Calc. for $C_{29}H_{48}O_2Si$ (456.76) : C, 76.25 ; H, 10.59.

3β-t-Butyldimethylsilyloxy-24-nor-chola-5,20(22),23-triene (4)

A solution of compound 3 (457mg, 1mmol) in dry THF (6ml) was added to a stirred suspension of LAH (76mg, 2mmol) and $AlCl_3$ (88mg, 0.66mmol) in dry THF (7ml) under Ar. After 2h at reflux, the temperature of the reaction mixture was raised to 0°C and Na_2SO_4, $10H_2O$ (1g) was gradually added. The ice bath was then removed, and stirring was continued for an additional hour. The clear solution was filtered from the precipitate over celite, the solvent was removed, and the crude extract was chromatographed (hexane) to yield 4 (260mg, 56%).

mp = 126-128°C (EtOH). $[\alpha]_D$ = +14 (c = 0.5 in $CHCl_3$). IR : 1950 (w), 1245 (m), 1080 (s). ^1H NMR ($CDCl_3$) : 0.06 (6H, s, $Si(Me)_2$), 0.63 (3H, s, CH_3-18), 0.90 (9H, s, $SiC(Me)_3$), 1.01 (3H, s, CH_3-19), 1.72 (3H, t : J = 3Hz, CH_3-21), 3.49 (1H, m: wl/2 = 25Hz, H-3), 4.61 (2H, m : wl/2 = 12Hz, H-23), 5.33 (1H), bd : J = 5Hz, H-6). ^{13}C NMR in table 1. MS m/z : 440 (M^+, 2), 425 (2), 402 (50), 383 (100), 307 (6). Found : C, 78.98 ; H, 10.97. Calc. for $C_{29}H_{48}OSi$ (440.76) : C, 79.02 ; H, 10.98.

(20S)-3β-t-Butyldimethylsilyloxy-24-nor-chola-5,22-dien-20-ol (5)

Further chromatography (hexane-Et_2O 5%) furnished 5 (160mg, 35%)

^1H NMR ($CDCl_3$) : 0.05 (6H, s, $Si(Me)_2$), 0.83 (3H, s, CH_3-18), 0.89 (9H, s, $SiC(Me)_3$), 1.00 (3H, s, CH_3-19), 1.34 (3H, s, CH_3-21), 3.48 (1H, m : wl/2 = 25Hz, H-3), 4.97 (1H, d.d. : J_1 = 10.5Hz, J_2 = 1.5Hz, H-23cis), 5.15 (1H, d.d. : J_1 = 17Hz, J_2 = 1.5Hz, H-23 trans), 5.35 (1H, bd : J = 5Hz, H-6), 5.99 (1H, d.d. : J_1 = 17Hz, J_2 = 10.5Hz, H-22). ^{13}C NMR in table 1. MS m/z : 458 (M^+, 1), 443 (4), 401 (100), 383 (9), 331 (39).

General procedure for the deprotection of silylethers 4, 9, 18a and 18b

n-Bu_4NF (1.5eq, 1M in THF) was added to a stirred solution of the respective silylether in dry THF (0.5M). After 24h, Na_2SO_4, $10H_2O$ was added into the solution. The mixture was filtered over celite and evaporated to dryness.

24-Nor-chola-5,20(22)-trien-3β-ol (6)

6 was prepared from 4 as described in general procedure. Short column chromatography (hexane-Et_2O 20%) furnished the pure compound (97%).

mp = 146-148°C (EtOH 95%). $[\alpha]D$ = +12 (c = 0.5 in $CHCl_3$). IR : 3440 (bs), 1950 (m), 1155 (s), 1140 (s). ^1H NMR ($CDCl_3$) : 0.63 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-19), 1.72 (3H, t : J = 3Hz, CH_3-21), 3.54 (1H, m : wl/2 = 25Hz, H-3), 4.61 (2H, m : wl/2 = 12Hz, H-23), 5.35 (1H, bd : J = 5Hz, H-6). ^{13}C NMR in table 1. MS m/z (15ev) : 326 (M^+, 100), 311 (8), 308 (50), 293 (13), 258 (9), 241 (5), 215 (3). Found : C, 82.17 ; H, 10.45. Calc. for $C_{23}H_{34}O_1$, $0.5H_2O$ (335.51) : C, 82.33 ; H, 10.21.

(20R)-3β-t-Butyldimethylsilyloxy-24(2-methoxy-2-propoxy)-chol-5-en-22-yn-20-ol (8)

n-BuLi (1.3ml, 3mmol) was added dropwise to a stirred cooled solution (-78°C) of protected propargyllic alcohol 12[12] (385mg, 3mmol) in dry THF (10ml) under Ar. After 1h, a solution of 7 (431mg, 1mmol) in THF (10ml) was introduced dropwise. The stirring was continued for 1h at -78°C, then the dry ice bath was removed and Na_2SO_4, $10H_2O$ was added. The mixture was filtered over celite and evaporated to dryness. Chromatography over silica gel pre-treated with Et_3N (1%) (Hexane-Et_2O-Et_3N, 80-19-1) gave the acid unstable compound 8 (502mg, 90%).

$[\alpha]_D$ = -16 (c = 1,2 in $CHCl_3$). IR : 3480 (m), 1260 (m), 1100 (s). ^1H NMR (CD_2Cl_2) : 0.07 (6H, s, $Si(Me)_2$), 0.91 (9H, s, $SiC(Me)_3$, 0.97 (3H, s, CH_3-18), 1.04 (3H, s, CH_3-19), 1.35 (6H, s, $OC(Me)_2O$), 1.48 (3H, s, CH_3-21), 3.20 (3H, s, OMe), 3.50 (1H, m : wl/2 = 25Hz, H-3), 4.13 (2H, s, H-24). ^{13}C NMR in table 1. MS m/z : 558 (M^+, 2), 543 (3), 527 (2) 501 (85), 469 (17), 429 (25), 413 (18), 411 (24), 373 (100).

(20S)-3β-t-Butyldimethylsilyloxy-chola-5,22,23-trien-20-ol (9)

Compound 8 (279mg, 0.5mmol) in dry Et_2O (8ml) was slowly added dropwise to a stirred suspension of LAH (30mg, 0.79mmol) in dry Et_2O (8ml) under Ar. After 0.5h, the mixture was cooled (0°C) and Na_2SO_4, $10H_2O$ (360mg) was gradually added. Stirring was continued for an additional 1h. The clear solution was then filtered from the white precipitate with celite and evaporated to dryness. Chromatography (hexane-Et_2O 15%) over silica gel pretreated with Et_3N (1%) gave the α-allenic alcohol 9 (182mg, 77%).

mp = 137-139°C (EtOH). $[\alpha]_D$ = -43 (c = 0.5 in $CHCl_3$). IR : 3600 (w), 3040 (w), 1950 (m), 1260 (m), 1090 (s). ^1H NMR ($CDCl_3$) : 0.06 (6H, s, $Si(Me)_2$), 0.84 (3H, s, CH_3-18), 0.89 (9H, s, $SiC(Me)_3$), 1.01 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-21), 3.52 (1H, m : wl/2 = 25Hz, H-3), 4.89 (2H, d : J = 6.7Hz, H-24), 5.35 (1H, t : J = 6.7Hz, H-22), 5.35 (1H, bs, H-6). ^{13}C NMR in table 1. MS m/z: 470 (M^+, 2), 455 (4), 452 (1), 431 (4), 413 (100), 395 (12), 373 (13), 331 (28). Found : C, 76.64 ; H, 10.61. Calc. for $C_{30}H_{50}O_2Si$ (470.80) : C, 76.53 ; H, 10.71.

(20S)-3β-t-Butyldimethylsiloxy-24-(2-methoxy-2-propoxy)-chola-5,22(E)-dien-20-ol (10)

Further chromatography (hexane-Et_2O, 20%) gave mainly the deprotected C-24 alcohol of the acid unstable compound 10 (47mg, 19%).

^1H NMR (CD_2Cl_2) : 0.07 (6H, s, $Si(Me)_2$), 0.84 (3H, s, CH_3-18), 0.90 (9H, s, $SiC(Me)_3$), 1.02 (3H, s, CH_3-19), 1.34 (3H, s, CH_3-21), 3.50 (1H, m : wl/2 : 25Hz, H-3), 4.12 (2H, d : J = 4.5Hz, H-24), 5.35 (1H, bd : J = 5Hz, H-6), 5.74 (1H, ABX_2 : J_{AB} = 16Hz, J_{AX2} = 4.5Hz, H-23), 5.85 (1H, ABX_2 : J_{AB} = 16Hz, $J_{BX2} \simeq$ 0Hz, H-22). ^{13}C NMR in table 1.

(20S)-Chola-5,22,23-triene 3β,20-diol (11)

Chromatography (hexane-Et_2O 30%) gave compound 11 (96%) prepared from 9 according to the general procedure.

mp = 173-174°C (AcOEt). $[\alpha]_D$ = -58 (c = 1 in $CHCl_3$). IR = 3580 (w), 3300 (bs), 3040 (w), 1950 (m), 1060 (s). ^1H NMR ($CDCl_3$) : 0.85 (3H, s, CH_3-18), 1.02 (3H, s, CH_3-19), 1.39 (3H, s, CH_3-21), 3.50 (1H, m : wl/2 = 25Hz, H-3), 4.90 (2H, d : J = 6.7Hz, H-24), 5.35 (1H, t : J = 6.7Hz, H-22), 5.35 (1H, bs, H-6). ^{13}C NMR in table 1. MS m/z : 356 (M^+, 8), 341 (2), 338 (4), 323 (2), 317 (18), 299 (9), 274 (36), 256 (100). Found : C, 80.79 ; H, 10.32. Calc. for $C_{24}H_{36}O_2$ (356.53) : C, 80.85 ; H, 10.18.

5-(Trimethylsilyl)ethynyl-2,2,4,4-tetramethyl-1,3-dioxalane (15)

MeLi in ether (20ml, 30mmol) was added dropwise to a stirred and cooled solution (-78°C) of compound 12 (3.84g, 30mmol) in dry THF (75ml) under Ar. The reaction temperature was raised to -30°C during a 30 min period afterwards trimethylchlorosilane was added dropwise. The stirring was continued for 1h at -30°C, and the reaction was then cooled to -78°C. t-BuLi in pentane (19.5ml, 33mmol) was then added dropwise during a 30 min period. After an additional 1h at -78°C, freshly distilled acetone (4.4ml, 60mmol) in dry THF (10ml) was added dropwise to the resulting orange solution of the anion 13. After 30min, the dry-ice bath was removed and the reaction temperature was raised to 0°C. A solution of HCl (30ml, 1N) was added to the pale yellow solution. The organic phase was separated and the aq. phase was extracted with ether. The combined extracts were washed with water and brine, dried with Na_2SO_4, and filtered. The combined etheral layer was concentrated in vacuo to give the crude hydroxy-ether 14, which was directly dissolved in dry CH_2Cl_2 (10ml). A catalytic amount of pTsOH, H_2O (57mg, 0.3mmol) was added to the cooled solution (0°C) under Ar followed by 2-methoxy-propen (3ml, 30mmol). After 2h, K_2CO_3 (1g) was added, and the mixture was filtered over silica gel. The filtrate was washed with hexane-Et_2O (5%). The combined organic phase was concentrated in vacuo. The resulting pale yellow crude extract was distilled under reduced pressure to give 15 (5.75g, 85%) as a colorless liquid.

bp = 74-75°C (2mm Hg). IR (film) : 2160 (w), 1250 (s), 850 (s). ^1H NMR ($CDCl_3$) : 0.18 (9H, s, $Si(Me)_3$), 1.34 (3H, s), 1.35 (3H, s), 1.36 (3H, s), 1.50 (3H, s), 4.44 (1H, s, H-5). ^{13}C NMR ($CDCl_3$) : 0.7 ($Si(Me)_3$), 25.2 (Me), 26.9 (Me), 28.0 (Me), 29.1 (Me), 75.5 (C-5), 81.9 (C-4), 93.8 (C≡C-Si), 101.3 (C≡C-Si), 109.7 (C-2). MS m/z : 211 (M^+-15, 90), 168 (100), 153 (76), 110 (66), 95 (48). Found : C, 63.39 ; H, 9.81. Calc. for $C_{12}H_{22}O_2Si$ (226.39) : C, 63.66 ; H, 9.80.

5-Ethynyl-2,2,4,4-tetramethyl-1,3-dioxolane (16)

KF (1.9g, 33mmol) and n-Bu_4NBr (71mg, 0.22mmol) were added to a stirred solution of compound 15 (5g, 22mmol) in THF-CH_3CN-H_2O (13.2ml : 10-2-1.2). After 20h H_2O was added, the organic phase was separated and the aqueous phase was extracted with ether. The combined layers were washed with brine, dried with Na_2SO_4, filtered and concentrated in vacuo (15°C). The resulting orange crude extract was distilled under reduced pressure to give 16 (2.85g, 84%) as a colorless liquid.

bp = 53-54°C (14mm Hg). IR (film) : 3300 (bm), 2120 (w). ^1H NMR ($CDCl_3$) : 1.36 (6H, s), 1.37 (3H, s), 1.50 (3H, s), 2.55 (1H, d : J = 2.2Hz, C≡C-H) 4.45 (1H, d : J = 2.2Hz, H-5). ^{13}C NMR ($CDCl_3$) : 24.3 (Me), 26.0 (Me), 27.1 (Me), 28.2 (Me), 74.2 (C-5), 75.7 (C≡CH), 78.9 (C≡CH), 81.0 (C-4), 109.0 (C-2). MS m/z : 155 (M^+ +1, 1,5), 139 (14), 111 (8), 84 (14), 43 (100). Found : C, 70.43 ; H, 9.11. Calc. for $C_9H_{14}O_2$ (154.2) : C, 70.62 ; H, 9.15.

(20S,24RS)-3β-t-Butyldimethylsilyloxy-24,25-isopropylidenedioxy-cholest-5-en-22-yne-20-ol (17)

Compound 17 was prepared as described for 8, using ethynyl dioxolane 16 (925mg, 6mmol) and protected pregnenolone 7 (862mg, 2mmol). Chromatography (hexane-Et_2O 15%) gave the pure compound 17 (1.09g, 93%).

$[\alpha]_D$ = -7.5 (c = 0.5 in $CHCl_3$). IR : 3500 (m), 3460 (m), 2240 (w), 1260 (m), 1100 (s), 1030 (m), 840 (s). ^1H NMR ($CDCl_3$) : 0.06 (6H, s, $Si(Me)_2$), 0.89 (9H, s, Si $C(Me)_3$), 0.97 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-19), 1.33 (6H, s), 1.37 (3H, s), 1.49 (3H, s, CH_3-21), 1.52 (3H, s), 3.49 (1H, m : wl/2 = 25Hz, H-3), 4.49 (1H, s, H-24), 5.35 (1H, bd : J = 5Hz, H-6). ^{13}C NMR in table 1. MS m/z : 569 (M^+-15, 2), 527 (22), 509 (2), 469 (5), 451 (3), 415 (9), 373 (100). Found : C, 73.92 ; H, 10.35. Calc. for $C_{36}H_{60}O_4Si$ (584.93) : C, 73.92 ; H, 10.26.

(20S, 22,23R)-3β-t-Butyldimethylsilyloxy-cholesta-5,22,23-triene-20, 25-diol (18a)

Compound 17 (1.02g, 1.75mmol) in dry Et_2O (20ml) was slowly added dropwise to a stirred suspension of LAH (200mg, 5.25mmol) in dry Et_2O (20ml) under Ar. After 3h at reflux, the mixture was cooled in an ice bath and Na_2SO_4, $10H_2O$ (2.4g) was fractionally added. Work-up as described for 9 and chromatography (hexane-Et_2O 25 to 30%) gave the less polar allene 18a (389mg, 42%).

mp = 164-166°C (EtOH).$[\alpha]_D$ = -96 (c = 0.5 in $CHCl_3$). IR : 3420 (bm), 1960 (w), 1250 (m), 1090 (s), 840 (m), 780 (m). ^1H NMR ($CDCl_3$) : 0.06 (6H, s, $Si(Me)_2$), 0.85 (3H, s, CH_3-18), 0.90 (9H, s, $Si(Me)_3$), 1.01 (3H, s, CH_3-19), 1.38 (6H, s, CH_3-26 and CH_3-27), 1.41 (3H, s, CH_3-21), 3.49 (1H, m : wl/2 = 25Hz, H-3), 5.33 (1H, bd : J = 5Hz, H-6), 5.56 and 5.58 (2H, AB : J_{AB} = 6.3Hz, H-22 and H-24). ^{13}C NMR in table 1. MS m/z : 528 (M^+, 8), 513 (12), 510 (16), 471 (80), 453 (43), 431 (23), 373 (100), 256 (75). Found: C, 74.75 ; H, 10.42. Calc. for $C_{33}H_{56}O_3Si$ (528.87) : C, 74.97 ; H, 10.67.

(20S, 22,23R)-3β-t-Butyldimethylsilyloxy-cholesta-5,22,23-triene-20, 25-diol (18b)

Further chromatography gave the more polar allene 18b (392mg, 42%).

mp = 155-157°C (EtOH).$[\alpha]_D$ = -11 (c = 0.5 in $CHCl_3$). IR : 3400 (bm), 1960 (w), 1250 (m), 1090 (s), 840 (m), 780 (m). ^1H NMR ($CDCl_3$) : 0.06 (6H, s, $Si(Me)_2$), 0.86 (3H, s, CH_3-18), 0.90 (9H, s, $Si(Me)_3$), 1.01 (3H, s, CH_3-19), 1.38 (6H, s, CH_3-26 and CH_3-27), 1.40 (3H, s, CH_3-21), 3.49 (1H, m : wl/2 = 25Hz, H-3), 5.32 (1H, bd : J = 5Hz, H-6), 5.54 and 5.57 (2H, AB : J_{AB} = 6.3Hz, H-22 and H-24). ^{13}C NMR in table 1. MS m/z : 528 (M^+, 1), 513 (3), 510 (2), 471 (16), 453 (13), 395 (32), 373 (100). Found : 74.99 ; H, 10.65. Calc. for $C_{33}H_{56}O_3Si$ (528.87) : C, 74.97 ; H, 10.67.

(20S, 22,23R)-Cholesta-5,22,23-triene-3β,20,25-triol (19a)

Chromatography (hexane-AcOEt 30%) gave compound 19a (96%) prepared from 18a according to the general procedure.

mp = 175-176°C (EtOH 95%).$[\alpha]_D$ = -127 (c = 0.5 in $CHCl_3$). IR : 3340 (bs), 1960 (w), 1150 (m), 1060 (m). ^1H NMR ($CDCl_3$) : 0.86 (3H, s, CH_3-18), 1.02 (3H, s, CH_3-19), 1.37 (6H, s, CH_3-26 and CH_3-27), 1.41 (3H, s, CH_3-21), 3.52 (1H, m : wl/2 = 25Hz, H-3), 5.36 (1H, bd : J = 5Hz), 5.56 and 5.58 (2H, AB : J_{AB} = 6.3Hz, H-22 and H-24). ^{13}C NMR in table 1. MS m/z : 414 (M^+, 6), 396 (24), 381 (17), 378 (10), 317 (89), 299 (60), 256 (100). Found : C, 76.68 ; H, 10.08. Calc. for $C_{27}H_{42}O_3$, $0.5H_2O$ (423.62) : C, 76.65 ; H, 10.23.

(20S, 22,23S)-Cholesta-5,22,23-triene-3β,20,25-triol (19b)

Chromatography (hexane-AcOEt 30%) gave compound 19b (97%) prepared from 18b according to the general procedure.

mp = 183-185°C (EtOH 95%).$[\alpha]_D$ = -20 (c = 0.25 in $CHCl_3$). IR : 3320 (bs), 1960 (w), 1150 (m), 1040 (m). ^1H NMR ($CDCl_3$) : 0.87 (3H, s, CH_3-18), 1.02 (3H, s, CH_3-19), 1.38 (6H, s, CH_3-26 and CH_3-27), 1.40 (3H, s, CH_3-21), 3.51 (1H, m : wl/2 = 25Hz, H-3), 5.35 (1H, bd : J = 5Hz, H-6), 5.57 and 5.54 (2H, AB : J_{AB} = 6.3Hz, H-22 and H-24). ^{13}C NMR in table 1. MS m/z : 414 (M^+, 15), 396 (21), 381 (16), 378 (6), 317 (77), 299 (43), 256 (100). Found : C, 78.10 ; H, 10.08. Calc. for $C_{27}H_{42}O_3$ (414.61) : C, 78.21; H, 10.21.

N°C	3[1]	4[1]	5[1]	6[1]	8[2]	9[1]	10[2]	11[2]	17[1]	18a[1]	18b[1]	19a[3]	19b[4]
1	37.3	37.4	37.3	37.3	37.8	37.4	37.7	37.6	37.4	37.2	37.3	36.8	37.0
2	31.8'	31.8'	31.7'	31.9'	32.3'	31.9'	32.1'	32.0'	31.8'	31.7'	31.7'	31.2'	31.2'
3	72.5	72.5	72.5	71.9	73.0	72.8	73.0	71.9	72.6	72.5	72.5	70.7	71.2
4	42.7	42.8	42.7	42.4	43.3	42.9	43.2	43.2	42.8	42.7	42.7	41.7	42.6
5	141.6	141.5	141.5	140.8	142.1	141.5	142.0	141.3	141.7	141.5	141.5	140.6	140.7
6	120.9	120.9	120.9	121.6	121.3	121.4	121.5	121.6	120.8	120.9	120.9	120.6	121.1
7	32.0'	32.0'	32.0'	31.7'	32.5'	32.1'	32.4'	32.0'	32.1'	32.0'	32.0'	31.0'	31.5'
8	31.3	32.2	31.3	32.4	31.9	31.4	31.7	31.7	31.4	31.3	31.3	30.8	31.1
9	50.0	50.3	50.1	50.4	50.8	50.3	50.7	50.5	50.2	50.1	50.0	49.6	49.9
10	36.5	36.6	36.5	36.6	37.0	36.5	36.9	36.8	36.6	36.5	36.5	36.0	36.3
11	20.7	21.1	20.8	21.2	21.2	20.9	21.1	21.2	20.8	20.8	20.8	20.3	20.7
12	40.2	39.0	40.1	39.1	40.7	40.2	40.6	40.5	40.2	40.1	40.0	39.6	39.9
13	43.2	43.7	42.7	43.8	43.6	43.0	43.2	43.2	43.3	42.9	42.8	42.4	42.6
14	56.2	56.4	56.8	56.5	56.9	56.9	57.2	57.1	56.3	56.7	56.7	56.3	56.6
15	24.1+	24.2+	23.1+	24.3+	25.5	23.4+	23.4+	23.6+	24.2+	23.3+	23.4+	22.8+	23.2+
16	25.0+	26.6+	23.7+	26.7+		23.9+	24.0+	24.1+	25.2+	23.7+	23.8+	23.3+	23.6+
17	59.9	53.3	59.4	53.4	60.8	60.2	60.3	60.5	60.1	60.0	59.8	59.7	60.0
18	13.3	12.7	13.7	12.8	13.5	13.6	13.8	13.7	13.5	13.6	13.5	13.0	13.3
19	19.7	19.4	19.3	19.4	19.5	19.4	19.4	19.4	19.3	19.3	19.3	18.7	19.0
20	71.2	99.4	75.6	99.4	71.5	73.4	75.3	73.6	71.3	73.7	73.8	72.9	73.8
21	32.7	20.0	28.7	20.1	32.7	29.3	29.1	29.3	32.7	29.3"	29.1"		28.1"
22	87.6	207.2	146.0	207.3	89.0	100.9	140.3	100.9	91.6	105.5§	105.6§	104.7§	104.5§
23	73.6	74.4	110.1	74.5	83.0	205.5	125.8	205.4	80.7§	196.7	196.6	196.5	197.2
24					49.5	78.5	63.5	78.3	74.7	105.1§	105.4§	104.1§	104.3§
25									81.2§	69.7	69.7	68.8	69.3
26									26.1"	29.9"	30.0"	28.7"	29.6"
27									24.6"	29.9"	30.0"	28.7"	29.3"
1'	-4.7	-4.6	-4.7		-4.5	-4.7	-4.8		-4.6	-4.7	-4.7		
2'	18.1	18.1	18.1		18.3				18.1	18.2	18.1		
3'	25.9	25.9	25.8		26.0	25.8	25.9		25.9	25.9	25.9		
1"					101.0				108.7				
2"					24.5				28.3&				
3"					24.5				27.1&				
1"					48.7								

Table 1.
Chemical shift, δC [1] in $CDCl_3$, [2] in CD_2Cl_2, [3] in $CDCl_3$ + $(CD_3)_2CO$ or [4] in $CDCl_3$ + CD_3OD; ', +, §, ", or & interchangeable assignments. The assignments were based upon : 1) Shielding data[23,24]; 2) off resonance decoupled spectra; 3) selective proton decoupled spectra (distortionless enhancement by polarization transfer : DEPT-technique); 4) by comparison with the spectrum of cholesterol.[25]

ACKNOWLEDGMENTS

The authors wish to thank Mrs Elisabeth Krempp for NMR spectra. We express our gratitude to Pr. G. Ourisson for valuable discussions and for critical reading of this manuscript.

REFERENCES

(1) M. Lagueux, J.A. Hoffmann, F. Goltzené, C. Kappler, G. Tsoupras, C. Hetru and B. Luu, "Biosynthesis, Metabolism and Mode of Action of Invertebrates Hormones", (edited by J.A. Hoffmann and M. Porchet), Springer-Verlag, Berlin-Heidelberg, 168 (1984).

(2) C. Hetru, C. Kappler, J.A. Hoffmann, R. Nearn, B. Luu and D.H.S. Horn, Mol. Cell. Endocrinol., 26, 51 (1982).

(3) T. Haag, M.F. Meister, C. Hetru, C. Kappler, Y. Nakatani, J.P. Beaucourt, B. Rousseau and B. Luu, Insect Biochem., 17, 291 (1987).

(4) C. Kappler, M. Kabbouh, C. Hetru, F. Durst and J.A. Hoffmann, Steroid Biochemistry in press.

(5) M.N. Galbraith, D.H.S. Horn, E.J. Middleton, and R.J. Hackney, Aust. J. Chem., 22, 1517 (1969).

(6) P.R. Ortiz de Montellano and K.L. Kunze, Biochem. Biophys. Res. Commun., 94, 443 (1980).

(7) C. Walsh, Tetrahedron, 38, 871 (1982).

(8) A. Burger, F. Colobert, C. Hetru and B. Luu, Tetrahedron, 44, 1141 (1988).

(9) A. Burger, J.P. Roussel, F. Colobert, C. Hetru, B. Luu and J.A. Hoffmann, Pest. Biochem. Physiol., 29, 197 (1987).

(10) W.T. Borden and E.J. Corey, Tetrahedron Lett., 313 (1969).

(11) L.A. van Dijck, B.J. Lankwerden, J.G.C.M. Vermeer and A.J.M. Weber, Rec. Trav. Chim. Pays-Bas, 90, 801 (1971).

(12) H. Gerlach and P. Küntzler, Helv. Chim. Acta, 61,2503 (1978).

(13) D.M. Piatak and J. Wicha, Chem. Rev., 78, 200 (1978).

(14) J.S. Cowie, P.D. Landor and S.R. Landor, J. Chem. Soc. Perkin I, 720 (1973).

(15) A. Claesson, L.-I. Olsson and C. Bogentoft, Acta Chem. Scand., 27, 2941 (1973).

(16) L.-I. Olsson and A. Claesson, Acta Chem. Scand., B31, 614 (1977) and references cited therein.

(17) A. Claesson and C. Bogentoft, Acta Chem. Scand., 26, 2540 (1972).

(18) M. Ishiguro, N. Ikeda and H. Yamamoto, J. Org. Chem., 47, 2225 (1982).

(19) C. Huynh and G. Linstrumelle, J. Chem. Soc. Chem. Commun., 1133 (1983).

(20) G. Lowe, J. Chem. Soc. Chem. Commun., 411 (1965).

(21) J.H. Brewster, Topics in Stereochemistry, 2, 1 (1967).

(22) R. Rossi and P. Diversi, Synthesis, 25 (1973).

(23) R.H.A.M. Janssen, R.J.J.Ch. Lowsberg and M.J.A. de Bie, J. R. Neth. Chem. Soc., 100, 85 (1981)

(24) P.L. Beaulieu, V.M. Morisset and D.G. Garratt, Can. J. Chem., 58, 928 (1980).

(25) K.N. Scott, T.H. Mareci, Can. J. Chem., 57, 27 (1979).