52. Isolation, Structure Determination and Synthesis of New Acetylenic Steroids from the Sponge *Calyx nicaaensis*

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Summary

Two acetylenic steroids, cholest-5-en-23-yn-3β-ol (**5**) and 26,27-dinorcholest-5-en-23-yn-3β-ol (**3**), and another unsaturated steroidalcohol, stigmasta-5,23-dien-3β-ol (**7**), were isolated from the sponge *Calyx nicaaensis*. The structures of these two acetylenic steroids were established by synthesis. Several attempts to synthesize the marine steroid alcohol calysterol (**1**)[1]), with a cyclopropene-containing side chain, starting from cholest-5-en-23-yn-3β-ol are also recorded. Addition of ethyl-diazoacetate to the triple bond was performed, but the reduction to the methyl derivative yielded decomposition products.

In a previous article [1] the isolation from the sponge *Calyx nicaaensis* of the first cyclopropene-containing sterol, calysterol (**1**)[1]), was reported. This paper deals with its attempted synthesis as well as the isolation and structure determination of the minor sterols contained in this sponge.

Isolation of minor sterols. The non-saponifiable material from the chloroform extract of *Calyx nicaaensis* was chromatographed on silica gel and the sterol fraction, after acetylation, was further fractionated on silica gel/silver nitrate to give a novel unsaturated sterol acetate (**6**, see below) and two more fractions **a** and **b**[2]). Fraction **a** was shown to be a mixture of cholesterol acetate, stigmasta-5,22-dien-3β-yl acetate and stigmast-5-en-3β-yl acetate, identified by combined GLC./MS. and co-GLC. with authentic samples. Fraction **b** was separated by TLC. into two compounds, whose structure elucidation forms the main subject of this paper.

The less polar acetate of fraction **b** has the empirical formula $C_{27}H_{40}O_2$ and the presence of a normal 3β-acetoxy-Δ^5-steroid system was deduced from the following

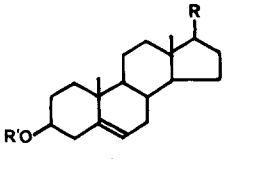
[1]) The IUPAC-name of calysterol is 23,28-cyclostigmasta-5,23(24)-dien-3β-ol.

[2]) On a $SiO_2/AgNO_3$ column calysterol, the major component, undergoes a fundamental structural modification [2].

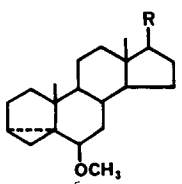
data: The NMR. spectrum included a broad signal corresponding to one H-atom at δ 4.54 (C*H*OAc), a signal for one olefinic proton at δ 5.25 and three singlets at δ 1.92 (CH_3CO), 1.01 ($H_3C(19)$) and 0.68 ($H_3C(18)$). Moreover it showed a signal for $H_3C(21)$ (δ 1.09, *d*, $J=6$ Hz), but no terminal dimethyl signals as in conventional sterols. The negative optical rotation was in the range of 3β-acetoxy-Δ^5-steroids. The empirical formula required two additional degrees of unsaturation which were shown to reside in the side chain. In the mass spectrum, the base peak (*m/e* 336) arose from the parent ion by loss of acetic acid, and the fragmentation pattern required that the unsaturation be located in the side chain between C(22) and C(25), because of the following characteristic peaks [3]: *m/e* 283 (loss of acetic acid and allylic fission of C(20)–C(22)), *m/e* 255 (loss of HOAc and the side chain), *m/e* 253 (loss of HOAc and the side chain with 2H), *m/e* 213 (loss of HOAc and typical ring D cleavage) [4]. The nature of this unsaturation was deduced from the NMR. spectrum, which lacked any olefinic signal except that of the proton on the endocyclic Δ^5-double bond, while exhibiting an additional singlet at δ 1.75 attributable to a methyl group linked to an acetylenic carbon atom. These results led to the tentative conclusion that we were dealing with the acetate of the first naturally occurring acetylenic sterol **3**, namely 26,27-dinorcholest-5-en-23-yn-3β-yl acetate (**2**). This structure was confirmed by comparison of its IR., NMR. and mass spectra, as well as chromatographic properties (GLC. and TLC. on $SiO_2/AgNO_3$) with those of a synthetic sample described below.

The more polar acetate of fraction **b** had the molecular formula $C_{29}H_{44}O_2$ as determined by accurate mass measurement of the M–HOAc peak at *m/e* 364.3127. The NMR. data [δ 4.54 (*m*, br., 1H, CHOAc); 5.25 (*m*, 1H, H–C(6)); 1.93 (*s*, 3H, $H_3C(18)$)] again suggested the presence of a 3β-acetoxy-Δ^5-steroid system which was confirmed by catalytic hydrogenation at room temperature with a palladium catalyst to cholesterol acetate. The mass spectrum (peaks at *m/e* 283, 255, 253 and 213) indicated the presence of two degrees of unsaturation in the side chain, and the absence of an olefinic signal in the NMR. spectrum (apart from the H–C(6)) suggested the presence of a triple bond, which had to be located at C(23) on the basis of the prominent peak in the mass spectrum at *m/e* 283 (loss of HOAc and fission of the C(20)–C(22) bond). Therefore, we can attribute to this compound the structure of cholest-5-en-23-yn-3β-yl acetate (**4**) and to the corresponding alcohol the structure **5**. This was confirmed by comparison of the IR., NMR. and mass spectra as well as chromatographic properties of **4** with those of a synthetic sample.

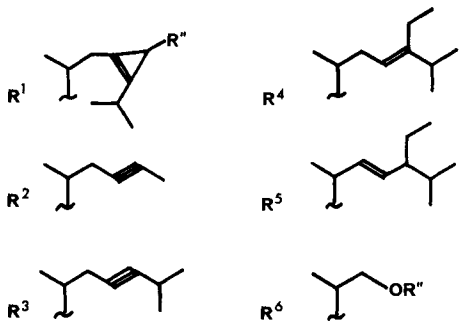
The sterol acetate, separated initially besides fraction **a** and **b**, possessed the molecular formula $C_{31}H_{50}O_2$ and therefore contained only two double bond equivalents. The NMR. [δ 5.30 (*m*, 1H, H–C(6)); 5.12 (*m*, 1H, H–C(23)); 4.50 (br., 1H, CHOAc); 1.92 (*s*, 3H, CH_3CO); 1.00 (*s*, 3H, H_3–C(19)); 0.68 (*s*, 3H, H_3–C(18))] and mass spectrum (*m/e* 283, 255, 253 and 213) were characteristic of a C_{29} 3β-acetoxy-Δ^5-steroid with one unsaturated linkage in the side chain. The skeletal structure was established by catalytic hydrogenation at 80° in ethanol to a tetrahydro-derivative which showed the same retention time as β-sitostanol acetate (stigmastan-3β-yl acetate) by GLC. The location of the double bond between C(23) and C(24) was deduced from the mass spectrum, which showed an intense peak at *m/e* 283, corresponding to allylic cleavage between C(20) and C(22) [3]. This was confirmed by permanganate/periodate oxydation of the sterol acetate, which gave ethyl isopropyl



1 R = R^1, R' = H, R" = CH_3
2 R = R^2, R' = Ac
3 R = R^2, R' = H
4 R = R^3, R' = Ac
5 R = R^3, R' = H
6 R = R^4, R' = Ac
7 R = R^4, R' = H
8 R = R^5, R' = Ts
17 R = R^1, R' = H, R" = CH_2OH
20 R = R^1, R' = Ac, R" = $COOC_2H_5$



9 R = R^5
10 R = R^6, R" = H
11 R = R^6, R" = Ts
12 R = R^3
13 R = R^2
14 R = R^1, R" = $COOC_2H_5$
15 R = R^1, R" = COOH
16 R = R^1, R" = CH_2OH
18 R = R^1, R" = CHO
19 R = R^1, R" = CH_3



ketone, identified by co-GLC. with an authentic sample. All these data are consistent with structure **6** for the acetate of the minor sponge sterol **7**.

Synthesis of the acetylenic sterols **5** *and* **3**. The isolation of acetylenic steroids from natural sources is unprecedented and therefore merited confirmation by synthesis.

Stigmasta-5,22-dien-3β-yl *p*-toluenesulfonate (**8**) was treated with pyridine in methanol to yield 6β-methoxy-3α,5-cyclo-5α-stigmast-22-ene (**9**). The ozonolysis of this compound followed by reduction with sodium bis(2-methoxy-ethoxy)aluminum-hydride (Red-Al) gave the alcohol **10**, which was transformed to its *p*-toluenesulfonate **11** by the usual procedure [5]. The reaction of **11** with the lithium salt of 3-methylbutyne in refluxing dioxane afforded the acetylenic compound **12**, while the same reaction with the lithium salt of propyne led to the lower homolog **13**. These compounds were transformed into the acetates **4** and **2**, respectively, and therefrom the free sterols **5** and **3** were obtained by reaction with zinc acetate in glacial acetic acid and reduction of the intermediate acetate with lithium aluminum hydride in ether.

Attempted synthesis of calysterol (**1**). Calysterol (**1**) [1] is a particularly intriguing marine steroid alcohol since it is the first naturally occurring steroid possessing a cyclopropene ring. We felt, therefore, that the synthetic confirmation of the earlier structure assignment was a desirable goal. Since carbene addition to acetylenes is a well known route to cyclopropenes [6], we selected this approach, especially in view of the co-occurrence of calysterol with cholest-5-en-23-yn-3β-ol (**5**) in the sponge, but all attemps were unsuccessful.

Treatment at 130° of the acetylenic steroid intermediate **12** with ethyl diazoacetate in the presence of copper dust as a catalyst afforded in good yields the cyclopropenic ester **14** whose spectral properties (see exper. part) are fully consistent with the assigned structure. Saponification of the ester led to the free acid **15**, while lithium aluminum hydride reduction provided the alcohol **16**. None of these cyclopropene derivatives of 6β-methoxy-3α,5-cyclo-5α-steroids were crystalline, but removal of the now superfluous methyl ether protecting group with zinc acetate and treatment with lithium aluminum hydride gave crystalline 29-hydroxycalysterol (**17**). Several attempts were made to generate calysterol itself from the different precursors described above, but none were successful. Thus, treatment of the acetylenic sterol **12** with diazoethane under the conditions described for the diazoacetate addition produced no reaction, and neither did addition of the carbene derived from methylene chloride and methyl lithium [7]; the starting material was recovered in either case. Several reactions were tried on the acid chloride of **15** (prepared with oxalyl chloride): 1. In order to attempt

the reduction of the *p*-toluenesulfonyl hydrazide with diborane [8], the acid chloride was treated with tosylhydrazine, but a very complex reaction mixture was obtained, as shown by TLC.; reduction of this mixture with diborane was unsuccessful; 2. The reduction of the acid chloride to the aldehyde **18** by means of lithium tri(*t*-butoxy) aluminiumhydride in diglyme failed and **15** was recovered by TLC.; 3. The acid chloride of **15** treated with zinc chloride in methylene chloride [6] as well as the acid **15** treated with perchloric acid in acetic anhydride [9] gave after dilution with dry ether a purple powder which was expected to be the cyclopropenyl cation, but attempts to transform this product into the methyl derivative with methyl lithium in different conditions of temperature and atmosphere (room temp. to −80°, under air or nitrogen) failed to produce any of compound **19**, as shown by TLC. (no fast moving compound). Attempted conversion of the alcohol **16** to the *p*-toluenesulfonate with tosyl chloride at 0° in pyridine in order to try the reduction to **19** yielded a decomposition product. An attempt was also made to prepare the aldehyde **18** by oxidation of the alcohol **16** with the hope that the subsequent *Wolff-Kishner* reduction might yield compound **19**: the oxydation with a 6fold excess of CrO_3/pyridine complex (*Collins* reagent) [10] afforded a very complex mixture of 10 to 12 products which was not examined further. Finally, treatment of the *N,N,N',N'*-tetramethylphosphorodiamidate derivative of **16** with propylamine [11] failed to produce **19**.

Experimental part

General. – For the natural compounds and the comparison with the synthetic acetates the NMR. spectra in CCl_4 solutions were taken in Naples on a *Perkin-Elmer* R32 spectrometer. Chemical shifts are given in ppm relative to TMS as internal standard (0 ppm), coupling constants *J* in Hz. Mass spectra were performed on an *AEI* MS 902 spectrometer, mass numbers in *m/e*, relative intensities in parentheses. TLC. was performed on precoated plates of silica gel *Merck* F_{254}. GLC. of the sterols acetates was run using a *Perkin-Elmer* F30 instrument with FID, equipped with a glass column (2 m × 0.4 cm, 1% OV-1 on chromosorb W, N_2 flow 50 ml/min), at 245°. The gas chromatographic identification of methyl isopropyl ketone was carried out on the same instrument under the following conditions: steel column (2 m × 0.4 cm, packed with 10% DEGS on chromosorb W), oven temp. 45°, N_2 flow 75 ml/min. GLC./MS. was performed on an *AEI* MS 30 instrument: glass column (1.5 m × 0.5 cm, packed with 1% OV-1 on Gas Chrom P), oven temp. 230°, N_2 flow 50 ml/min, ionization beam 70 eV.

The spectra of the synthetic compounds were measured at Stanford University with the following instruments: Unless otherwise indicated, the NMR. spectra were taken on a *Varian* T-60 spectrometer (60 MHz); the 100 MHz spectra were measured by Dr. *L. Durham* on a *Varian* HA 100 spectrometer. The solvent was $CDCl_3$ and TMS was the internal reference. The IR. spectra were obtained on a *Perkin-Elmer* 700 IR spectrophotometer, absorptions in cm^{-1}. The low resolution mass spectra were measured on an *AEI* MS 9 mass spectrometer by Mr. *R. Ross*. The m.p.'s (uncorrected) were determined in a *Thomas Hoover* Uni-Melt capillary melting point apparatus. TLC. was performed on silica gel *Merck* HF_{254} using hexane/ethyl acetate 10:1 as eluent. The preparative plates were 20 × 20 cm with a 1.5 mm layer of silica gel. The optical rotations were measured in chloroform ($c \cong 10$ mg/ml). – Abbreviations: RT. = room temperature, i.V. = in vacuo.

Isolation of the natural compounds. – Sponges (*Calyx nicaaensis*) collected in the Bay of Taranto were supplied by the Stazione di Biologia Marina del Salento, Porto Cesareo. Fresh sponges (860 g dry material after the following) were extracted with 2 × 2.5 l of acetone and then with 2 × 2.5 l of chloroform. The combined extracts were concentrated under reduced pressure and the remaining aqueous residue extracted with ether. The organic layer was evaporated leaving an oily residue (4.5 g) which was heated for 2 h under reflux with a 10% KOH-solution in aqueous ethanol 85:15. The non-saponifiable material (3.1 g) was chromatographed on 300 g of SiO_2 using benzene/ethyl ether as eluent; fractions of 250 ml were collected. Fractions 5–10 afforded 2.6 g of an oil containing the crude mixture of sterols which was acetylated with Ac_2O in pyridine and fractionated on 250 g of $SiO_2/AgNO_3$ 3:1 using light petroleum (40–70°)/benzene as eluent. Fractions of 200 ml were collected, sterol acetate composition was determined as percent of total GLC. peak areas. Fractions 3–7 afforded 68 mg of a mixture of *cholesterol acetate* (7 mg), *stigmasta-5,22-dien-3β-yl acetate* (30 mg) and *stigmast-5-en-3β-yl acetate* (31 mg) identified by combined GLC./MS. and co-GLC. with authentic samples. Fractions 9–15 afforded 127 mg of *stigmasta-5,23-dien-3β-yl acetate* (**6**) which were recrystallized from ethanol. Fractions 18–25 gave 38 mg of a mixture of **4**

and **2** rechromatographed on SiO_2 by TLC. using light petroleum (60–80°)/benzene 65:35 as eluent. After two migrations, the Rf 0.35 and 0.45 bands (visualized by heating a strip sprayed with 5% ceric sulfate in 10% aqueous sulfuric acid) afforded 14 mg of **2** and 12 mg of **4** respectively.

26,27-Dinorcholest-5-en-23-yn-3β-yl acetate (**2**). M.p. 137–140°, $[\alpha]_D = -33.7°$ ($c = 3$ mg/ml). Accurate mass measurement of the peak at *m/e* 336 (M–CH_3COOH) gave 336.2819 ($C_{25}H_{36}$). – NMR.: 5.25 (1H, H–C(6)); 4.54 (br., H–C(3)); 1.92 (3H, CH_3CO); 1.01 (3H, 3H–C(19)); 0.68 (3H, 3H–C(18)). – MS.: 283, 255, 253, 213.

Cholest-5-en-23-yn-3β-yl acetate (**4**). M.p. 118–121°, $[\alpha]_D = -34.6°$ ($c = 4$ mg/ml). Accurate measurement of the peak at *m/e* 364 (M–CH_3COOH) gave 364.3127 ($C_{27}H_{40}$). – NMR.: 5.25 (*m*, 1H, H–C(6)); 4.54 (br., 1H, H–C(3)); 1.93 (*s*, 3H, CH_3CO); 1.02 (*s*, 3H, 3H–C(19)); 0.68 (*s*, 3H, 3H–C(18)). – MS.: 283, 255, 253, 213.

Catalytic hydrogenation of **4**. **4** (5 mg) dissolved in ethanol (3 ml) was hydrogenated overnight in the presence of Pd/C (2 mg) at RT. under 2 atm. After removal of the catalyst and solvent the residue was identified as cholesterol acetate by comparison of its gas chromatographic properties with those of an authentic sample.

Catalytic hydrogenation of **6**. **6** (10 mg) was hydrogenated overnight at 80° in ethanol with Pd/C under 2 atm. After removal of the catalyst by filtration, the residue was shown by GLC. to possess the same retention time as β-sitostanol acetate.

$KMnO_4$/$NaIO_4$ oxidation of **6**. To **6** (10 mg) in *t*-butyl alcohol (3 ml), 0.04M K_2CO_3 (0.5 ml) and an aqueous solution (3.5 ml) of 0.023M $KMnO_4$ and 0.039M $NaIO_4$ were added. The mixture was kept at RT. for 18 h. After acidification with 5N H_2SO_4 the solution was decolorized with $NaHSO_3$ and extracted with ether. The combined ether extracts were dried over $CaSO_4$, concentrated and the residue was shown to contain ethyl isopropyl ketone by co-GLC. with an authentic sample.

Synthetic studies. – *6β-Methoxy-3α,5-cyclo-5α-cholest-23-yne* (**12**). To a solution of about 5 g (73 mmol) of 3-methylbutyne in 250 ml of anhydrous dioxane at 0° were added slowly, with stirring, 25 ml of 2.45M *n*-butyl lithium in hexane (60 mmol) and the mixture was stirred for 2 h at 5° and 2 h at RT. To this solution were added 3 g of *p*-toluenesulfonate **11** prepared according to [5] and the mixture was heated to reflux. The reaction was almost complete after 48 h (TLC.). The cooled solution was poured into 1 l of water, the products were extracted with ethyl acetate, the organic layer was washed with water and saturated solution of sodium sulfate, dried over magnesium sulfate and evaporated. The yellow oily residue was chromatographed on a column of SiO_2 using hexane as eluent. After evaporation the colourless oily residue crystallized overnight and was recrystallized from ethyl acetate yielding 1.97 g of colorless needles, m.p. 97–99°, $[\alpha]_D = +57.08°$. – IR. (nujol): 1110. – NMR. (100 MHz): 3.32 (*s*, 3H, OCH_3); 2.77 (*t*, $J = 2.5$, H–C(6)); 1.15 (*d*, $J = 7$, 3H–C(26) and 3H–C(27)); 1.07 (*d*, $J = 4.5$, 3H–C(20)); 1.03 (*s*, 3H–C(19)); 0.74 (*s*, 3H–C(18)). – MS.: 396 (63, M^+), 381 (57, $M - CH_3$), 364 (65, $M - CH_3OH$), 355 (6, $M - C_3H_4$ (ring A cleavage)), 349 (10, $M - CH_3OH - CH_3$), 341 (100, $M - C_4H_7$ (ring A)), 338 (25, $M - C_2H_3OCH_3$ (ring B)), 283 (14, $M - CH_3OH$ and C(20)–C(22) cleavage), 255 (7, $M - CH_3OH$ – side chain), 253 ($M - CH_3OH$ – side chain – 2H), 227 (9, $M - CH_3OH$ – side chain – (C(16)–C(17) unit)), 215 and 213 (9 and 14, $M - CH_3OH$ and ring D cleavage).

$C_{28}H_{44}O$ (396.66) Calc. C 84,79 H 11.18% Found C 84.69 H 11.27%

6β-Methoxy-3α,5-cyclo-26,27-dinorcholest-23-yne (**13**). **13** was prepared using the procedure described for the higher homolog **12**: 2 g of **11** treated with about 2 g of the lithium salt of propyne gave 0.937 g of crystalline product after chromatography on a column of alumina (*Merck*, neutral, activity II) with hexane as eluent. An analytical sample was prepared by recrystallization from abs. ethanol, m.p. 105–106°, $[\alpha]_D = +60.0°$. – IR. (nujol): 1105. – NMR.: 3.32 (*s*, OCH_3); 2.77 (*t*, $J = 5$, H–C(6)); 1.77 (*s*, 3H–C(25)); 1.06 (*d*, 3H–C(21)); 1.03 (*s*, 3H–C(19)); 0.74 (*s*, 3H–C(18)). – MS. (*cf.* **12**): 368 (77, M^+), 353 (64, $M - CH_3$), 336 (100, $M - CH_3OH$), 327 (5, $M - C_3H_4$ (ring A)), 321 (17, $M - CH_3OH - CH_3$), 313 (96, $M - C_4H_7$ (ring A [12])), 310 (31, $M - C_2H_3OCH_3$ (ring B)), 283 (12, $M - CH_3OH$ and C(20)–C(22) cleavage), 255 (8, $M - CH_3OH$ – side chain), 253 (10, $M - CH_3OH$ – side chain – 2H), 227 (9, $M - CH_3OH$ and ring D cleavage), 215, 213 (30 and 21, $M - CH_3OH$ and ring D cleavage).

$C_{27}H_{42}O$ (368.60) Calc. C 84.72 H 10.94% Found C 84.00 H 11.04%

Transformation to the acetates **2** *and* **4** *and the steroid alcohols* **3** *and* **5**. In a typical experiment the 6β-methoxy-3α,5-cyclo-5α-steroid **12** or **13** (50 mg) was dissolved in 5 ml of glacial acetic acid, 1 g of freshly fused zinc acetate was added and the mixture was heated under reflux for 2 h, then poured into water and extracted with chloroform. The organic layer was washed with water and saturated sodium sulfate, dried over magnesium sulfate and evaporated, leaving the crude acetate which was dried overnight i.V. (oil pump). For comparison with natural products, an analytical sample of both acetates was prepared by recrystallization from ethyl acetate.

2: m.p. 122–125°, $[\alpha]_D = -43.1°$. – IR. (nujol): 1710, 1225, 1010. – NMR.: 5.40 (1H, H–C(6)); 4.73 (br., 1H, C*H*OAc); 2.01 (*s*, 3H, CH_3CO); 1.77 (*s*, 3H–C(25)); 1.06 (*d*, $J=7$, 3H–C(21)); 1.03 (*s*, 3H–C(19)); 0.69 (*s*, 3H–C(18)). – MS.: 336 (100, $M-CH_3COOH=M'$), 321 (7, $M'-CH_3$), 296 (3, C(22)–C(23) cleavage), 283 (5, C(20)–C(22) cleavage), 282 (4, C(20)–C(22) cleavage − 1H), 267 (2, C(20)–C(22) cleavage − 1H − CH_3), 255 (3, M' − side chain), 253 (3, M' − side chain − 2H), 241 (2), 228 (4, ring D cleavage), 227 (3), 215 (6, ring D cleavage), 213 (12, ring D cleavage).

4: m.p. 151–153°, $[\alpha]_D = -40.4°$. – IR. (nujol): 1725, 1225, 1140. – NMR.: 5.40 (1H, H–C(6)); 4.60 (br., 1H, C*H*OAc); 2.00 (*s*, CH_3CO); 1.15 (*d*, $J=6$, 3H–C(26) and 3H–C(27)); 1.02 (*s*, 3H–C(19)); 0.78 (*s*, 3H–C(18)). – MS.: 364 (100, $M-CH_3COOH=M'$), 349 (6, $M'-CH_3$), 322 (1, $M'-C_3H_6$), 296 (2, C(22)–C(23) cleavage), 283 (6, C(20)–C(22) cleavage), 282 (5, C(20)–C(22) cleavage − 1H), 267 (2, C(20)–C(22) fission − 1H − CH_3), 255 (3, M' − side chain), 253 (3, M' − side chain − 2H), 244 (4), 242 (3), 228 (3, ring D cleavage), 215 (3, ring D cleavage), 213 (11, ring D cleavage).

The acetate **2** or **4** was taken up in 5 ml of ether and stirred for 30 min at RT. with a 4fold excess of lithium aluminum hydride. The excess of reagent was decomposed with saturated sodium sulfate and the ether solution was filtered and evaporated. The residue was recrystallized twice from methanol and dried overnight at 100° i.V. to give 35 mg of colorless needles.

3: 104–105°, $[\alpha]_D = -42.2°$. – IR. (nujol): 3250, 1025. – NMR.: 5.34 (1H, H–C(6)); 3.50 (1H, H–C(3)); 1.97 (*s*, 3H–C(25)); 1.07 (*d*, 3H–C(21)); 1.00 (*s*, 3H–C(19)); 0.68 (*s*, 3H–C(18)). – MS.: 354 (100, M^+), 339 (10, $M-CH_3$), 336 (17, $M-H_2O$), 321 (11, $M-H_2O-CH_3$), 301 (5, C(20)–C(22) cleavage), 283 (8, 301 − H_2O), 269 (16, $M-C_5H_8OH$), 243 (16, ring B cleavage), 255 (3, $M-H_2O$ − side chain), 231 (7, ring D cleavage), 215 (10), 213 (15, $M-H_2O$ and ring D cleavage).

$C_{25}H_{38}O$ (354.58) Calc. C 84.69 H 10.80% Found C 84.29 H 10.80%

5: m.p. 119–120°, $[\alpha]_D = -38.8°$. – IR. (nujol): 3270, 1075. – NMR. (100 MHz): 5.35 (1H, H–C(6)); 3.47 (1H, H–C(3)); 1.14 (*d*, $J=7$, 3H–C(26) and 3H–C(27)); 1.08 (*d*, $J=4.5$, 3H–C(21)); 1.01 (*s*, 3H–C(19)); 0.70 (*s*, 3H–C(18)). – MS.: 382 (100, M^+), 367 (19, $M-CH_3$), 364 (24, $M-H_2O$), 349 (15, $M-H_2O-CH_3$), 339 (14, C(24)–C(25) cleavage), 322 (6, $M-H_2O-C_3H_6$), 321 (6, C(24)–C(25) cleavage − H_2O), 301 (11, C(20)–C(22) cleavage), 283 (20, 301 − H_2O), 297 (21, $M-C_5H_8OH$), 271 (50, ring B cleavage), 255 (9, $M-H_2O$ − side chain), 231 (12, ring D cleavage), 215 (20), 213 (28, $M-H_2O$ and ring D cleavage).

$C_{27}H_{42}O$ (382.63) Calc. C 84.75 H 11.06% Found C 83.99 H 11.12%

Ethyl 6β-methoxy-(3α,5),(23,28)-bicyclo-5α-stygmast-23-en-29-oate (**14**). To a mixture of 500 mg of alkyne **12** and 50 mg of copper dust stirred at 130° under nitrogen (the stream of nitrogen was stopped before the reaction) were added over 6 h (2 drops every 10 min) 2 ml of ethyl diazoacetate by means of a syringe. A strong evolution of nitrogen occured after each addition of reagent. The mixture was stirred another 30 min, then allowed to cool and taken up in chloroform/methanol 1:1. The copper catalyst was filtered off and the product chromatographed on silica gel plates (hexane/ethyl acetate 10:1) yielding 370 mg of colorless oil, $[\alpha]_D = +47.8°$. – IR. (neat): 2950, 1900 (cyclopropene), 1725,1470, 1390, 1180, 1105. – NMR.: 4.06 (*q*, $J=7$, OCH_2CH_3); 3.27 (*s*, OCH_3); 1.99 (*s*, 1H, H–C(28)); 1.30 (*t*, $J=7$, OCH_2CH_3); 1.09 (*d*, $J=7$, 3H–C(26) and 3H–C(27)); 0.98 (*s*, 3H–C(19)); 0.70 (*s*, 3H–C(18)). – MS.: 482 (6, M^+), 467 (3, $M-CH_3$), 450 (1, $M-CH_3OH$), 427 (3, ring A cleavage), 409 (57, $M-COOC_2H_5$), 283 (4, $M-CH_3OH$ and C(20)–C(22) cleavage), 253 (13, $M-CH_3OH$ − side chain − 2H), 213 (3, $M-CH_3OH$ and ring D cleavage), 167 (100, C(20)–C(22) cleavage).

$C_{32}H_{50}O_3$ (482.75) Calc. C 79.62 H 10.44% Found C 79.28 H 10.45%

6β-Methoxy-(3α,5),(23,28)-bicyclo-5α-stigmast-23-en-29-oic acid (**15**). The ester **14** (609 mg) was heated with a solution of 2M KOH in methanol/water 10:1 (10 ml) under reflux for 1 h. The mixture was allowed to cool, acidified with 1N HCl and the product extracted with ether. The crude acid was purified by TLC. (hexane/ethyl acetate 5:1). The oily residue (533 mg) dried i.V. solidified into glass that afforded a white, non crystalline powder on scratching, $[\alpha]_D = +51.9°$. – IR. (nujol): 3000, 1900 (cyclopropene), 1685, 1100. – NMR.: 3.32 (*s*, 3H, OCH_3); 2.78 (1H, H–C(6)); 2.03 (*s*, 1H, H–C(28)); 1.14 (*d*, $J=7$, 3H–C(26) and 3H–C(27)); 1.02 (*s*, 3H–C(19)); 0.74 (*s*, 3H–C(18)). – MS.: 454 (20, M^+), 449 (19, $M-CH_3$), 422 (20, $M-CH_3OH$), 409 (16, $M-COOH$), 399 (39, ring A cleavage), 284 (12), 283 (15, $M-CH_3$ and C(20)–C(22) cleavage), 255 (16, $M-CH_3OH-$side chain), 253 (28, $M-CH_3OH-$side chain$-2H$), 213 (12, $M-CH_3OH$ and ring D cleavage, 139 (100, C(20)–C(22) cleavage).

$C_{30}H_{46}O_3$ (454.69) Calc. C 79.25 H 10.20% Found C 78.97 H 10.44%

6β-Methoxy-(3α,5),(23,28)-bicyclo-5α-stigmast-23-en-29-ol (**16**). Lithium aluminum hydride (15 mg) was stirred at RT. for 10 min in 5 ml of ether, then 100 mg of ester **14** in 1 ml of ether were added and the mixture stirred for 30 min. The excess of reagent was decomposed with ethyl acetate, then water was added and the product extracted with ethyl acetate. The crude alcohol was purified by TLC. (hexane/ethyl acetate 5:1): colorless oil, $[\alpha]_D = +50.0°$. – IR. (neat): 3360 br., 1860 (cyclopropene), 1100, 1025. – NMR.: 3.54 (*d*, $J=4$, 2H–C(29)); 3.30 (*s*, OCH_3); 1.14 (*d*, $J=6$, 3H–C(26) and 3H–C(27)); 1.03 (*s*, 3H–C(19)); 0.75 (*s*, 3H–C(18)). – MS.: 440 (<1, M^+), 425 (4, $M-CH_3$), 409 (20, $M-CH_2OH$), 408 (6, $M-CH_3OH$), 385 (6, ring A cleavage), 299 (4, ring B cleavage), 285 (6, $M-$side chain$-2H$), 283 ($M-CH_3OH$ and C(20)–C(22) cleavage), 282 (10, $283-1H$), 267 (4, $M-CH_3OH$ and ring B cleavage), 255 (10, $M-CH_3OH-$side chain), 253 (20, $M-CH_3OH-$side chain$-2H$), 227 (8), 215 (7) and 213 (12, $M-CH_3OH$ and ring D cleavage).

$C_{30}H_{48}O_2$ (440.71) Calc. C 81.76 H 10.98% Found C 80.98 H 11.05%

23,28-Cyclostigmasta-5(6),23(24)-dien-3β,29-diol (**17**). Ester **14** (100 mg) was treated as previously described (see **2** and **4**) yielding the acetate **20** (IR.: 1910 (cyclopropene); MS.: 510 (M^+)). The crude acetate was reduced in the usual manner by lithium aluminum hydride to the diol **17**. The crude product (90 mg) was purified by TLC. (hexane/ethyl acetate 10:3) and the semicrystalline residue was recrystallized from ethyl acetate: cubes of m.p. 136°, $[\alpha]_D = -29.9°$. – IR. (nujol): 3200, 1860, 1050, 1010. – NMR.: 5.33 (1H, H–C(6)); 3.53 (*d*, $J=5$, 2H–C(29)); 1.11 (*d*, $J=7$, 3H–C(26) and 3H–C(27)); 0.99 (*s*, 3H–C(19)); 0.70 (*s*, 3H–C(18)). – MS.: 426 (1, M^+), 411 (1, $M-CH_3$), 408 (1, $M-H_2O$), 395 (100, $M-CH_2OH$), 300 (4, C(20)–C(22) cleavage$-1H$), 282 (3, $M-H_2O$ and C(20)–C(22) cleavage$-1H$), 271 (11, $M-$side chain$-2H$), 215 (3) and 213 (3, $M-H_2O$ and ring D cleavage).

$C_{29}H_{46}O_2$ (426.68) Calc. C 81.63 H 10.87% Found C 81.46 H 10.93%

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