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CHEMICAL STUDIES OF MARINE INVERTEBRATES—XX[1]

THE STRUCTURES OF THE GENUINE AGLYCONES OF THELOTHURINS A AND B, DEFENSIVE SAPONINS OF THE INDO-PACIFIC SEA CUCUMBER *THELONOTA ANANAS* JAEGER (ECHINODERMATA)

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Abstract—Aqueous acetic acid hydrolysis of thelothurins A and B, defensive saponins isolated from the holothurian *Thelonota ananas*, yielded two aglycones: 23ξ-acetoxy-Δ^{8}-holostene-3β-ol (**2**) and 23ξ-acetoxy-$\Delta^{8,25}$-holostadiene-3β-ol (**3**). Their structure elucidation, chemical interconversion and correlation with the already known seychellogenin (**1**) are reported.

The presence in holothurians of large amounts of saponins known as holothurins[2] has been well documented.[2] Vigorous acid hydrolysis of these glycosides usually led to the obtention, in addition to carbohydrates, of mixtures of sapogenins which have been shown to be oxigenated lanostane derivatives.[3] Most of the described genins contain a $\Delta^{7,9(11)}$-heteroannular conjugated diene system[4] which is necessarily an artifact since the parent toxins do not exhibit any conjugated chromophore in UV. When enzymatic or milder acid hydrolysis conditions were used, it was possible to obtain from holothurin A 12-methoxylated compounds named "neoholothurinogenins".[5] These products also have to be artifacts since the starting saponin seems devoid of a methylether function in the aglycone moiety.[5] $\Delta^{9(11)}$-sapogenins were recently described,[6-8] and one of them (**29**) is believed to be the genuine aglycone of "holotoxin A", the most abundant toxin from *Stichopus japonicus*.[8]

In a previous communication[1] we have reported the presence of two new saponins, thelothurins A and B, in the Indo-pacific sea cucumber *Thelonota ananas* Jaeger. These compounds occur as a mixture that we could not separate and which exhibits potent cytotoxic and ichtyotoxic activities. The chemical and spectroscopic properties of the toxins were described.[1] We established the partial structure of the carbohydrate chain as 3 - O - methyl - β - D - glucosyl - (1→4) - β - D - xylosyl - (1→4) - β - D - glucosyl - (1→4) - 2 - O - X - 3 - O - methyl - β - D - glucosyl - (1→3′β - aglycones) where X is a yet unidentified substituent, different from a sulfate group. We wish to report here the structures of the genuine aglycones† of thelothurins A and B, and their chemical correlation with seychellogenin (**1**).[9]

Aqueous acetic acid hydrolysis[10] of the mixture of thelothurins A and B yields two sapogenins, 23ξ-acetoxy-Δ^{8}-holostene-3β-ol (**2**) and 23ξ-acetoxy-$\Delta^{8,25}$-holostadiene-3β-ol (**3**), separated by repetitive column chromatography on silica gel impregnated with 10% silver nitrate.

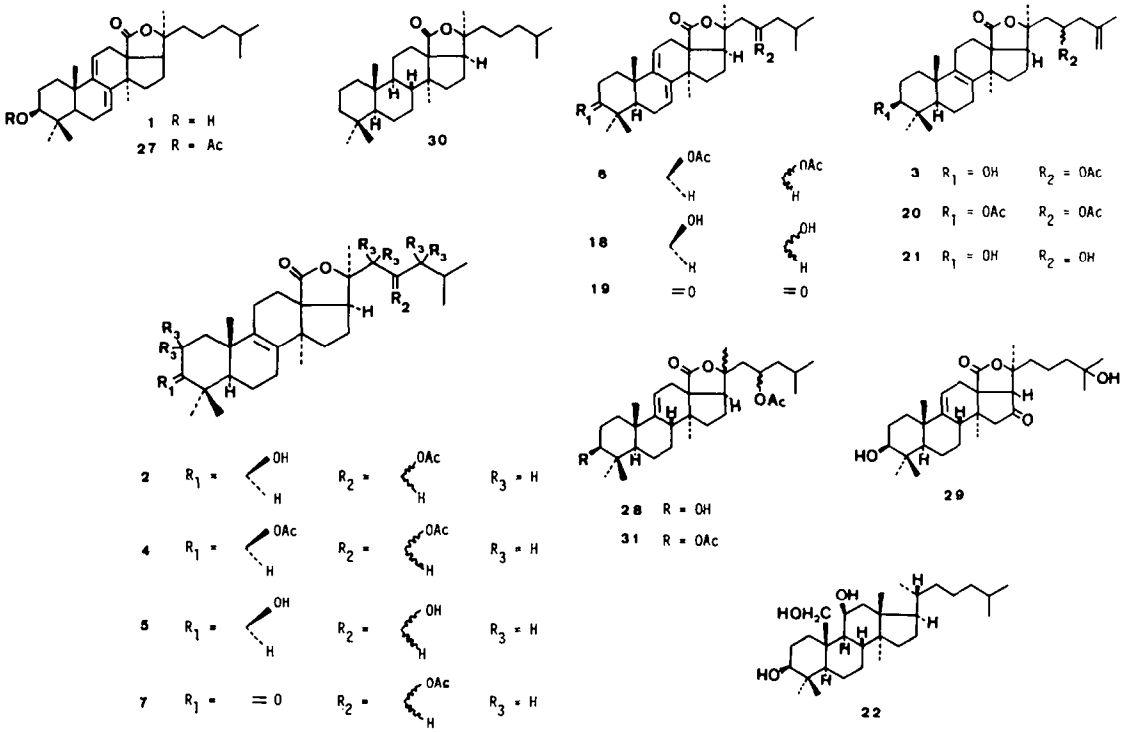
High resolution mass spectrometry establishes the empirical formula $C_{32}H_{50}O_5$ (514.3638, requires 514.3657) for 23ξ-acetoxy-Δ^{8}-holostene-3β-ol (**2**). The spectral data of **2** suggest the presence of a γ-lactone ($\nu_{C=O}$ 1765 cm^{-1}), an acetoxyl group ($\nu_{C=O}$ 1740 cm^{-1}, one 3H s at 2.05 ppm CH_3–CO, 1H m at 5.25 ppm H–C–OAc, loss of acetic acid in MS 514→454, corroborated by a metastable ion at 401) and a secondary OH function (ν_{OH} 3460 cm^{-1}, 1H m at 3.23 ppm H–C–OH) which could be acetylated under usual conditions to afford the diacetate **4** (no ν_{OH}) whose NMR spectrum (Table 1) displays a 6H s at 2.03 ppm CH_3–CO and a 1H m deshielded from 3.23 ppm in **2** to 4.50 ppm in **4**. The acetoxyl function of **2** is shown to be secondary by alkaline hydrolysis of **2** into diol **5** in which the 1H m at 5.25 ppm of **2** is shielded to 3.81 ppm (H–C–OH). The NMR of **2** (Table 1) shows striking similarities with the NMR spectra of other holothurinogenins[9] and suggests an holostane[4] (or 18→20 lanostanolide) skeleton (**30**). The 18→20 position of the γ-lactone is supported by the presence of a deshielded 3H singlet at 1.44 ppm (attributed to Me C-21), showing that the C-20 is quaternary and bears an O atom. Moreover, the C-18 Me group must be functionalized, as shown by the absence of its characteristic shielded signal at about 0.71 ppm in NMR. The holostane skeleton postulated here will be further substantiated by chemical and spectroscopic evidences and demonstrated by correlation of **2** with seychellogenin acetate (**27**)[9] (Scheme 2). From this preliminary spectral study, compound **2** seems to be closely related to the recently published 23ξ-acetoxy-$\Delta^{9(11)}$-holostene-3β-ol (**28**) isolated by hydrolysis of the saponin from *Stichopus chloronotus*.[6]

Seven of the eight unsaturations of **2** are associated with the acetate function and with the holostane nucleus. The lack of any vinylic proton (NMR) suggests the presence either of an additional ring or one tetrasubstituted double bond, necessarily located in Δ^{8}. The latter was established by selenium dioxide oxidation of **4** into the $\Delta^{7,9(11)}$-conjugated diene **6**.[11] Moreover, the UV spectrum of **6** (λ_{max} 236–244–252 nm, ϵ = 13000–13800–9900) characteristic of the lanostane series[12] is in agreement with the proposed skeleton. From these data it appears that **2** is a Δ^{8}-holostene having one secondary OH and one secondary acetoxyl functions.

†We will demonstrate in the following paper that **2** and **3** are the genuine aglycones of thelothurins A and B.

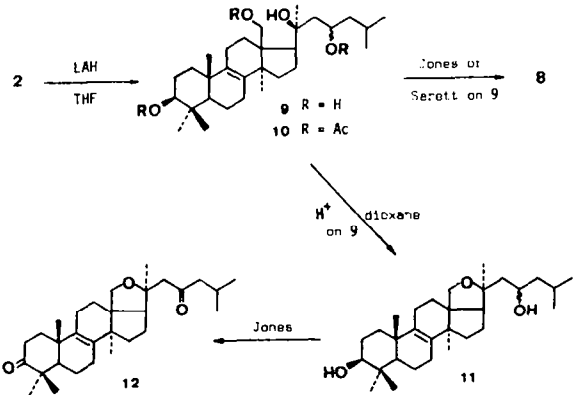
Table 1. (δ values) NMR data

	C-31	C-32	C-19	C-30	C-21	C-26,27	3αH	23ξH	Other signals
2	0.83 s	1.02 s	1.08 s	1.02 s	1.44 s	0.93 d J = 6	3.23 m	5.25 m	2.05 s (23ξ-OAc)
4	0.89 s	0.99 s	1.09 s	0.89 s	1.43 s	0.95 d J = 7	4.50 m	5.20 m	2.03 s (3β and 23ξ-OAc)
5	0.84 s	1.00 s	1.08 s	1.08 s	1.60 s	0.95 d J = 6	3.25 m	3.81 m	
7	1.09 s	1.09 s	1.22 s	1.03 s	1.44 s	0.93 d J = 7	—	5.25 m	2.04 s (23ξ-OAc)
3	0.84 s	1.00 s	1.05 s	1.00 s	1.44 s	4.80 m/1.77 bs	3.20 m	5.20 m	2.03 s (23ξ-OAc)
21	0.82 s	1.00 s	1.05 s	1.00 s	1.58 s	4.82 m/1.77 bs	3.22 m	3.81 m	



Location of the OH group at C-3 derives from Jones oxidation of **2** into monoketone **7**. The NMR spectrum of **7** (Table 1) shows a deshielding effect induced by the CO group on the C-31 and and C-32 Me groups from 0.83 and 1.02 ppm in **2** to 1.09 ppm in **7**. The β-configuration of the OH is indicated by the broad 1H signal at 3.23 ppm in the NMR spectrum of **2**. The 3αH in the lanostane series is known to occur between 3.18 and 3.30 ppm,[13] whereas the 3β proton absorbs more downfield.[14] $NaBH_4$ reduction of the monoketone **7** yields compound **2**, thus supporting the 3β-OH assignment.

The position of the acetoxyl group is established as follows. Diol **5** and tetrol **9** (*vide infra*), being unreactive to $NaIO_4$, are not α-glycols, hence the acetate in **2** cannot be at C-2. Oxidation of **5** with Jones reagent affords



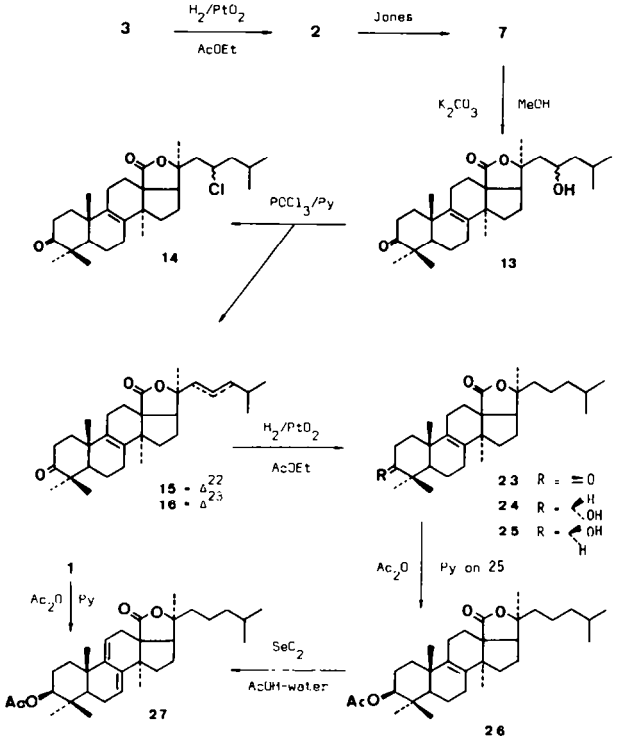
Scheme 1

diketone **8** whose IR spectrum shows it cannot be a 1,2-diketone or an α,β-unsaturated ketone. The C-2, C-7 and C-11 positions are therefore discarded. Moreover, the UV spectrum of **8** being devoid of the characteristic absorption of 1,3-diketones both in neutral and basic media,[15] excludes also C-1. From the IR spectrum of diketone **8** a cyclopentanone cannot be excluded since it has been shown quite recently[7] that the γ-lactone band at 1765 cm^{-1} overlaps completely the cyclopentanone absorption. Consequently, the C-15 and C-16 positions were eliminated following Scheme 1.

LAH reduction of **2** affords the tetrol **9** whose IR spectrum shows a strong OH absorption at 3300 cm^{-1} but no CO band. On acetylation under usual conditions, **9** gives a triacetate **10** (IR ν_{OH} 3550 cm^{-1}, $\nu_{C=O}$ 1740 cm^{-1}). The NMR of **10** shows two singlets, one of 6H at 2.03 ppm, the other of 3H at 2.16 ppm, respectively attributed to the 3β, 23ξ and 18 acetates and a downfield shift of the two 1H m from 3.20 and 4.08 ppm in **9** to 4.46 and 5.20 ppm; the $C^{18}\underline{H}_2AB$ system appears now at 3.92 and 4.43 ppm (two d $J = 11$ Hz). All these data support structure **9** for the tetrol. On Jones or Sarett oxidation of **9**, diketone **8** is recovered but in a poor yield. In order to avoid oxidation of the primary alcohol, **9** was transformed into the 18→20 ether **11** by refluxing in dioxane/0.1N H_2SO_4. Jones oxidation of this ether **11** affords a diketoether **12** whose IR spectrum, devoid of OH absorption, shows a CO band at 1710 cm^{-1} uncompatible with a cyclopentanone. Amongst the possible remaining positions for the acetoxyl group of **2**, position C-22 is also eliminated since tetrol **9** is unreactive to sodium periodate. Finally, if the acetate was located at C-6 or C-12, which are allylic in compound **6**, the proton on the carbon bearing the acetate would be deshielded by at least 0.3 ppm from its 5.25 ppm value in **2**. This is not observed and moreover, base hydrolysis of **6** into diol **18** followed by Jones oxidation affords diketone **19** devoid of α,β-unsaturated CO in IR.

The choice between the two remaining positions C-23 and C-24 comes from the following results (Scheme 2). Alkaline hydrolysis of **7** leads to the keto-alcohol **13**. Treatment of **13** by phosphorus oxichloride yields, in addition to the chlorinated compound **14**, a mixture of the two expected dehydration isomers **15** and **16** whose UV spectrum is devoid of absorption above 210 nm. This is an additional indication that the acetoxyl group cannot be in rings B or C, since dehydration would lead to the homoannular diene $\Delta^{6,8}$ or $\Delta^{8,11}$, absorbing at 275 nm ($\epsilon = 5000$).[16] Moreover, the mixture of **15** and **16** shows no Me on a double bond in NMR, and position C-24 has then to be discarded. The acetoxyl function must therefore be in C-23. This is confirmed by the isotopic exchange of enolisable protons in diketone **8** to afford the expected *hexa*-deuteriated-diketone **17**. A final proof for the location of the CH_3COO group at C-23 comes from the reductive micro-ozonolysis[17] of the dehydration mixture **15** and **16** yielding isovaleraldehyde and isobutyraldehyde, both identified in GC by comparison with authentic samples.

High resolution MS of 23ξ-acetoxy-$\Delta^{8,25}$-holostadiene-3β-ol (**3**) establishes the empirical formula $C_{32}H_{48}O_5$ (512.3482 requires 512.3500). From the spectral data it appears that **3** has a γ-lactone ($\nu_{C=O}$ 1765 cm^{-1}), an OH group (ν_{OH} 3500 cm^{-1}, 1H m in NMR at 3.20 ppm $\underline{H}$–C–OH) which could be acetylated under usual conditions to afford diacetate **20** (no ν_{OH} in IR), an acetyl function ($\nu_{C=O}$ 1740 cm^{-1}, 3H s at 2.03 ppm $C\underline{H}_3$–COO, 1H m at 5.20 ppm $\underline{H}$–C–OAc, loss of acetic acid in MS from molecular ion corroborated by a metastable ion at 400), a vinylidene group ($\nu_{C=C}$ 1655 and $\delta_{C=CH_2}$ 890 cm^{-1}, 2H m at 4.80 ppm $C{=}C\underline{H}_2$) and one Me group on a double bond (broad s of 3H at 1.77 ppm $C{=}C{-}C\underline{H}_3$). The secondary nature of the acetyl function is shown by hydrolysis of **3** into diol **21** in which the 1H m at 5.20 ppm of **3**, is shielded to 3.81 ppm. The MS fragmentation



Scheme 2.

pattern of **3**, identical with that of **2**, with all ions shifted downwards by two mass units, and the striking similarities of the NMR (Table 1) and IR spectra of **2** and **3** strongly suggest **3** to be a dehydro derivative of **2**. This is proved by catalytic hydrogenation of **3** yielding a compound identical with **2** (R_f on TLC impregnated with silver nitrate, IR, MS and NMR). The disappearance on hydrogenation, in the NMR of **2**, of the 2H m at 4.80 ppm and of the Me at 1.77 ppm characteristic of **3** and the simultaneous appearance of an isopropyl group (6H d J = 6 Hz at 0.93 ppm) are indicative of an isopropenyl group in **3**. This correlation establishes structure **3** for 23ξ-acetoxy-$\Delta^{8,25}$-holostadiene-3β-ol, since there is only one position for an isopropenyl group in the holostane skeleton. This will be confirmed, in the next paper, through independent chemical methods.

The holostane skeleton postulated for **2** and **3** is in good agreement with the UV spectrum of **6** and with the striking similarities between the NMR spectra of **2** and already known holothurinogenins. Definite proof of the nature of the skeleton is obtained by chemical correlation of **2** with seychellogenin (**1**), a sapogenin that was correlated to lanosterol via lanostane-3β,11β,19-triol (**22**).[18] This is achieved following Scheme 2, by removal of the functionality at C-23 and introduction of the $\Delta^{7,9(11)}$-conjugated diene system in rings B and C.

Reactions leading to the obtention of the double bond isomers **15** and **16** have already been discussed (*vide supra*). Catalytic hydrogenation of the mixture **15** and **16** yields three products (**23**→**25**) separated by silica gel column chromatography. The less polar **23** results from hydrogenation of the side chain double bond only, whereas **24** and **25**, respectively the 3α- and 3β-hydroxy-Δ^8-holostene, result from simultaneous hydrogenation of the side chain double bond and reduction of the C-3 carbonyl. Acetylation of the 3β-OH epimer **25** and subsequent oxidation of the acetate **26** using selenium dioxide affords a compound identical to an authentic sample of seychellogenin acetate (**27**) by R_f, IR, MS, UV and NMR (270 MHz). The obtention of seychellogenin acetate (**27**) establishes the configuration at all centers, with the exception of C-23. We have represented all structures with the 20-S configuration since that absolute configuration has recently been established both for seychellogenin (**1**)[19] and stichopogenin A_4 (**29**).[7]

23ξ-acetoxy-Δ^8-holostene-3β-ol (**2**) is an isomer of the genin **28** isolated by hydrolysis of the saponin from *Stichopus chloronotus*[6] and is noteworthy for its Δ^8-double bond. 23ξ-acetoxy-$\Delta^{8,25}$-holostadiene-3β-ol (**3**) is unique in having, in addition to the rare Δ^8-double bond, an isopropenyl group hitherto unreported in the sea cucumber sapogenins and highly unusual in the lanostane series. Drastic hydrochloric acid hydrolysis of thelothurins A and B yields a complex mixture of rearranged genins whose formation, structure determination and intercorrelation will be discussed in the next paper.

EXPERIMENTAL

M.ps are determined on a A. Thomas apparatus and are uncorrected. Optical rotations are measured on a Perkin–Elmer 141 polarimeter at room temp. UV spectra are recorded in MeOH with a Unicam SP 800 spectrophotometer and IR spectra with a Unicam SP 1000 spectrophotometer. Low resolution MS are determined at 70 eV on a Hitachi-Perkin-Elmer RMU 6D or on a Finnigan 3005 apparatus; high resolution MS are carried out on a AEI MS 902 instrument. NMR spectra are recorded with a Varian A 60 or T60 spectrometer or at 270 MHz with a Brucker HFX 270 instrument; signal positions are given in ppm, δ scale, relative of internal TMS.† GC are carried out on a Hewlett-Packard 402 or on a Packard 421 instrument equipped with a FID system.

In the text, "the reaction mixture is worked up in the usual way" means: diluted with water, extracted with CH_2Cl_2, washed with a 10% Na_2CO_3 aq and then water, dried over $MgSO_4$, filtered off and evaporated to dryness under reduced pressure.

Isolation of thelothurins A and B. Whole specimens of sun-dried *Thelonota ananas* (1400 g) were defatted with CH_2Cl_2 (soluble residue: 40 g) and extracted in a Sohxlet apparatus with MeOH. Water was added and the MeOH removed under reduced pressure at 40°. The aqueous soln was extracted several times with n-BuOH. The organic layer was evaporated to dryness under high vacuum (t° = 45°). The solid residue was crystallized several times from EtOH to afford 49 g of thelothurins A and B as white thin needles (3.5% on the basis of dry starting material). This mixture contained some 75% of thelothurin A and 25% of the B compound. The sample contained 0.28% of S and 2.56% of Na (marine salt contamination). $(\alpha)_D^{25} - 42.2°$ in MeOH ($c = 0.50$); UV: end absorption; IR_{KBr}: ν_{OH} 3450 cm^{-1}, $\nu_{C=O}$ 1765 and 1740 cm^{-1}, ν_{C-O} 1250 cm^{-1}; MS: no molecular ion, fragmentation pattern corresponding to the aglycones A and B: 514 (32)/512 (9); 499(4)/497 (2); 496 (2)/494 (1); 481 (2)/479 (1); 472 (2)/470 (1); 454 (7)/452 (4); 439 (8)/437 (3); 421 (9)/419 (3); 409 (3)/407 (1); 395 (12)/393 (5) with metastable ions at 484.5/484.5 514/512→499/497 loss of Me and 401.0/400.0 514/512→454/452 loss of acetic acid.

Hydrolysis of thelothurins A and B. Thelothurins A and B (3 g) were treated with 80% aqueous AcOH (100 ml) at 100° during 4 hr. The reaction medium was evaporated to dryness and partitioned between water and CH_2Cl_2. The organic layer was worked up in the usual way. The crude sapogenin mixture was chromatographed on silica gel, using hexane–acetone 20% as elution solvents, to afford 140 mg of a mixture of **2** and **3**.

Isolation of pure **2** *and* **3**. The mixture of **2** and **3** (140 mg) was chromatographed several times on columns of silica gel impregnated with 10% $AgNO_3$. Elution occurred with a mixture of hexane–acetone 10%. This yielded 100 mg of pure **2** and 35 mg of pure **3**.

Compound **2**. m.p. 198–201°; $(\alpha)_D = +13.8°$ ($c = 0.5$ in MeOH): UV: end absorption; IR_{film}: ν_{OH} 3460 cm^{-1}, $\nu_{C=O}$ 1765 and 1740 cm^{-1}, ν_{C-O} 1250 cm^{-1}; MS: M^+ 514.3638 ($C_{32}H_{50}O_4$ requires: 514.3657, 19), 499 (3, M^+-CH_3, metastable at 484.5: 514→499), 481 (1, M^+-CH_3, H_2O), 454.3445 (5, $C_{30}H_{46}O_3$ requires 454.3446, loss of AcOH, metastable at 401.0), 439 (3, M^+-CH_3, CH_3COOH), 421.1310 (9, $C_{29}H_{41}O_2$ requires: 421.3105 loss of CH_3, CH_3COOH, H_2O), 409.3458 (3, $C_{29}H_{45}O$ requires: 409.3469 loss of CH_3COOH, CO_2, H), 395 (27, M^+-CH_3COOH, CH_3, CO_2), 353.2491 (1, $C_{24}H_{33}O_2$ requires: 353.2480 loss of side chain and water), 325.2530 (4, $C_{23}H_{33}O$ requires: 325.2530 loss of side chain. CO_2, H_2), 325.2170 (2, $C_{22}H_{29}O_2$ requires: 325.2166 loss of side chain, $C^{21}H_3$, C^{20}, H_2O, H^{17}); NMR ($CDCl_3$): Table 1.

Compound **3**. m.p. 196–198°; UV: end absorption; IR_{KBr}: ν_{OH} 3500 cm^{-1}, $\nu_{C=O}$ 1765 and 1740 cm^{-1}, $\nu_{C=C}$ 1655 cm^{-1}, ν_{C-O} 1240 cm^{-1} and $\delta_{C=CH_2}$ 890 cm^{-1}; MS: M^+ 512.3482 (8, $C_{32}H_{48}O_4$ requires: 512.3500), 497 (1, M^+-CH_3), 479 (1, M^+-CH_3, H_2O), 452 (3, M^+-CH_3COOH, metastable ion at 399.5), 437 (3, M^+-CH_3, CH_3COOH), 419 (2, M^+-CH_3COOH, CH_3, H_2O), 393 (5, M^+-CH_3, CO_2, CH_3COOH), 325 (4); NMR ($CDCl_3$): Table 1.

Acetylation of **2** *into diacetate* **4**. Compound **2** (160 mg) was treated with Ac_2O (2 ml) in pyridine (2 ml) at room temp. for 15 hr. The reaction medium was treated in the usual way to afford 165 mg of pure **4**: UV: end absorption; IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1765 and 1740 cm^{-1}, ν_{C-O} 1250 cm^{-1}; MS: M^+ 556 (32, $C_{34}H_{52}O_6$), 541 (2, M^+-CH_3), 496 (10, M^+-CH_3COOH, metastable at 443), 481 (11, M^+-CH_3COOH, CH_3), 437 (40, M^+-CH_3COOH, CH_3, CO_2), 421 (26, M^+-CH_3COOH, CH_3, CH_3COOH), 377 (4, M^+-CH_3COOH, CH_3 CH_3COOH, CO_2), 367 (5, M^+-CH_3COOH, ring A); NMR ($CDCl_3$): Table 1.

Alkaline hydrolysis of **2**. Compound **2** (40 mg) was treated with sat. methanolic K_2CO_3 (15 ml) at room temp. during 7 hr. The reaction medium was neutralized with dil. HCl, water was

†The following abreviations are used: b, broad; d, doublet; m, multiplet; s, singlet.

added and usual work up afforded 34 mg of pure **5**: IR_{KBr}: ν_{OH} 3400 cm^{-1}, $\nu_{C=O}$ 1765 cm^{-1}; MS: M^+ 472 (15, $C_{30}H_{48}O_4$), 457 (2, M^+-CH_3, metastable at 442.5), 454 (1, M^+-H_2O), 439 (3, M^+-CH_3, H_2O), 421 (3, M^+-CH_3, H_2O, H_2O, metastable ion at 403.8: 439→421), 395 (5, M^+-CH_3, H_2O, CO_2), 386 (2), 371 (5), 352 (11), 332 (2), 325 (5); NMR ($CDCl_3$–CD_3OD): Table 1.

Oxidation of **4** *into* **6**. Diacetate **4** (150 mg) was refluxed with SeO_2 (300 mg) in 90% AcOH–H_2O (5 ml) for 17 hr. The mixture was worked up in the usual way. Solid selenium was eliminated from the crude orange residue by filtration on a 5 cm silica gel column (elution: hexane–acetone 20%). This yielded a mixture of **31** (the $\Delta^{9(11)}$ isomer of **4**—see following paper) and of the heteroannular diene **6**. Repetitive column chromatographies on silica gel (elution: hexane–AcOEt 4.5%) gave, in addition to 27 mg of **31**, 35 mg of pure **6**: UV: 236–244–252 nm (ϵ = 13000–13800–9900); IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1770 and 1740 cm^{-1}, $\nu_{C=C}$ 1610 cm^{-1}, ν_{C-O} 1245 cm^{-1}, $\delta_{C=CH}$ 940 cm^{-1}; MS: M^+ 554 (80, $C_{34}H_{50}O_6$), 494 (3, M^+-CH_3COOH), 493 (4), 479 (30, M^+-CH_3COOH, CH_3), 451 (9), 435 (17, M^+-CH_3COOH, CH_3, CO_2), 425 (22, M^+-ring A), 419 (24, M^+-CH_3COOH, CH_3, CH_3COOH), 375 (16, M^+-CH_3COOH, CH_3, CH_3COOH, CO_2), 365 (38, M^+-ring A, CH_3COOH), 351 (7), 340 (7), 339 (8), 325 (18, M^+-CH_3, CO_2, $C_{10}H_{18}O_2$ cleavage at C_{17}/C_{20} after the loss of CO_2); NMR ($CDCl_3$): 0.88(s, 3H, C-31); 0.92 (d J = 6.5 Hz, 6H, C-26, 27); 0.97 (s, 3H, C-32); 1.00 (s, 3H, C-30); 1.11 (s, 3H, C-19); 1.40 (s, 3H, C-21); 2.03 (s, 6H, 3β and 23ξ–CH_3–COO); 2.53 (bd J = 4.5 Hz, 2H, $C^{12}H_2$?); 4.52 (m, 1H), 3αH); 5.23 (m, 2H, 11-H and H–C–OAc) and 5.55 ppm (m, 1H, 7-H).

Oxidation of **2** *into monoketone* **7**. Compound **2** (105 mg was dissolved in acetone (3 ml) and oxidized with Jones reagent (1 ml; 13.4 g CrO_3 in conc. H_2SO_4 diluted with water to a volume of 50 ml) at room temp. during 4 min. The mixture was worked up in the usual way to afford 97 mg (yield: 94%) of pure **7**: UV: end absorption; IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1765, 1740 and 1710 cm^{-1}, $\nu_{C=C}$ 1670 cm^{-1}, ν_{C-O} 1245 cm^{-1}; MS: M⁺ 512 (10, $C_{32}H_{48}O_3$), 497 (1, M^+-CH_3), 466 (3), 452 (5, M^+-CH_3COOH), 437 (4, M^+-CH_3COOH, CH_3), 419 (1), 407 (4, M^+-CH_3COOH, CO_2, H), 393 (25, M^+-CH_3COOH, CH_3, CO_2); NMR ($CDCl_3$): Table 1.

Reduction of **7** *into* **2**. Monoketone **7** (33 mg) in dioxane (20 ml) and water (1.5 ml) was reduced with $NaBH_4$ (55 mg) at room temp. for 3½ hr. The excess reagent was destroyed by addition of acetone and the mixture worked up in the usual way. Chromatography of the crude extract on a silica gel column (elution: gradient of acetone in hexane from 5 to 20%) yielded 12 mg of a compound identical by UV, IR, MS and NMR with **2**.

Treatment of diol **5** *with* $NaIO_4$. Diol **5** (2 mg) in MeOH–H_2O (10 ml; 1/1, v/v) was allowed to react with $NaIO_4$ (10 mg). The mixture was stirred at room temp., and followed by TLC each 30 min. After 4.5 hr, $NaIO_4$ (10 mg) was added and the oxidation pursued for another 3 hr period. Treatment of the mixture in the usual way gave unchanged diol **5**.

Oxidation of diol **5** *into diketone* **8**. Diol **5** (2 mg) dissolved in acetone (2 ml) was treated with Jones reagent (*vide supra*) at room temp. for 2 min. The mixture was treated in the usual way. The crude extract was filtered on a 7 cm silica gel column (elution: hexane–acetone 30%) and yielded 1.5 mg of **8**: UV: end absorption both in neutral MeOH and in MeOH made 0.01 M in NaOH; IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1765 and 1710 cm^{-1}, $\nu_{C=C}$ 1665 cm^{-1}, ν_{C-O} 1280 cm^{-1}; MS: M^+ 468 (71, $C_{30}H_{44}O_4$), 453 (13, M^+-CH_3), 435 (9), 422 (16), 407 (86, M^+-CH_3, CO_2, H_2), 389 (12), 383 (13, $M^+-C_4H_9O$ cleavage at C-22/C-23), 369 (25, $M^+-C_6H_{11}O$ side chain), 351 (21), 337 (46), 325 (90, $M^+-C_6H_{11}O$, CO_2), 323 (100, $M^+-C_6H_{11}O$, CO_2, H_2), 309 (41), 307 (49).

Reduction of **2** *into tetrol* **9**. Compound **2** (34 mg) dissolved in anhydrous THF (5 ml) was refluxed during 5 hr with LAH (50 mg) in anhydrous THF (2 ml). The excess reagent was destroyed by careful addition of AcOEt. The mixture was treated by a sat. aqueous of $MgSO_4$ aq and then worked up in the usual way. The crude extract was purified by column chromatography on silica gel using a gradient elution of AcOEt (from 10 to 30%) in benzene. 30 mg of tetrol **9** was obtained: UV: end absorption; IR_{KBr}: ν_{OH} 3300 cm^{-1}, no $\nu_{C=O}$, ν_{C-O} 1260 cm^{-1}; MS: M^+ at 476 ($C_{30}H_{52}O_4$, not observed), 458 (26, M^+-H_2O), 443 (9, M^+-H_2O, CH_3), 440 (8, M^+-H_2O, H_2O), 428 (8, M^+-H_2O, HCOH), 425 (11, M^+-H_2O, CH_3, H_2O), 413 (27, M^+-H_2O, CH_3, HCOH), 401 (3), 395 (7, M^+-H_2O, CH_3, H_2O, HCOH), 389 (3, $M^+-C_5H_{11}O$ cleavage at C-22/C-23), 384 (4), 371 (9, $M^+-C_5H_{11}O$, H_2O), 357 (100, M^+-H_2O, $C_6H_{13}O$ cleavage at C_{20}/C_{22}), 339 (74, $M^+-C_6H_{13}O$, $2H_2O$ metastable ion at 322: 357→339), 315 (12), 299 (69); NMR ($CDCl_3CD_3OD$): 0.82 (s, 3H, C-31); 0.93 (s, 3H, C-32); 0.94 (d J = 6.5 Hz, 6H, C-26, 27); 0.99 (s, 3H, C-30); 1.01 (s, 3H, C-19); 1.46 (s, 3H, C-21); 3.20 (m, 1H, 3αH); 3.52 (s, 2H, OH) and 4.08 ppm (m, 1H, 23–H–C–OH).

Acetylation of **9** *into triacetate* **10**. Compound **9** (30 mg) was treated with Ac_2O (1.5 ml) in pyridine (1.5 ml) at room temp. for 24 hr. The reaction medium, worked up in the usual way, gave 30 mg of pure **10**: UV: end absorption; IR_{film}: ν_{OH} 3550 cm^{-1}, $\nu_{C=O}$ 1740 cm^{-1}, ν_{C-O} 1245 cm^{-1}; MS: M^+ 602 (1, $C_{36}H_{58}O_7$), 584 (13, M^+-H_2O), 569 (2, M^+-H_2O, CH_3), 556 (1), 538 (2), 524 (47, M^+-H_2O, CH_3COOH), 509 (7, M^+-H_2O, CH_3, CH_3COOH), 497 (4), 482 (4), 467 (13), 464 (27, M^+-H_2O, CH_3COOH, CH_3COOH), 456 (11), 451 (61, M^+-H_2O, CH_3COOH, $C_3H_5O_2$ cleavage at C_{13}/C_{18}), 449 (61, M^+-H_2O, CH_3COOH, CH_3, CH_3COOH), 442 (6), 428 (8), 422 (6), 413 (15), 407 (9, M^+-H_2O, CH_3COOH, CH_3COOH, CH_3, CH_2–CO), 399 (10), 396 (10), 391 (12, M^+-H_2O, CH_3COOH, $C_3H_5O_2$, CH_3COOH), 389 (18, M^+-H_2O, CH_3COOH, CH_3COOH, CH_3, CH_3COOH); NMR ($CDCl_3$): 0.88 (s, 6H, C-30, 31); 0.93 (d J = 6 Hz, 6H, C-26, 27); 0.97 (s, 3H, C-32); 1.02 (s, 3H, C-19); 1.36 (s, 3H, C-21); 2.03 (bs, 6H, 3β and 23ξ CH_3–COO); 2.16 (s, 3H, 18–CH_3–COO); AB system at 3.92 and 4.43 each 1H d J = 11 Hz, C^{18}–H_2); 4.46 (m, 1H, 3α H) and 5.20 ppm (m, 1H, 23 H–C–OAc).

Jones oxidation of **9**. Compound **9** (3 mg) dissolved in acetone (2 ml) was oxidized with Jones reagent at room temp. during 2 min. The reaction medium was worked up in the usual way. The crude extract was purified by silica gel column chromatography (elution: hexane–acetone 10%) to yield 1 mg of pure **8**: IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1765 and 1715 cm^{-1}; MS: M⁺ 468 (100, $C_{30}H_{44}O_4$), 453 (10, M^+-CH_3), 435 (4), 422 (9), 407 (39, M^+-CO_2, H_2), 383 (7, $M^+-C_4H_9O$ cleavage at C_{22}/C_{23}), 369 (13, $M^+-C_6H_{11}O$ side chain), 351 (7), 337 (14), 325 (22, $M^+-C_6H_{11}O$, CO_2), 323 (34, $M^+-C_6H_{11}O$, CO_2, H_2).

Ratcliffe oxidation of **9** *into* **8**. Tetrol **9** (2 mg) was oxidized with $(Py)_2CrO_3$ complex (0.4 ml) in anhyd. CH_2Cl_2 at room temp. during 1 hr. Treatment of the reaction medium in the usual way afforded a mixture of at least 3 products among which the most abundant was identical with **8** (TLC).

Acid treatment of **9**. Compound **9** (17 mg) was refluxed during 1.25 hr in dioxane acidified by addition of 10 droplets of conc. H_2SO_4. The crude extract was chromatographed on a silica gel column using a gradient elution of AcOEt (from 5 to 40%) in benzene. Pure ether **11** (12 mg) was isolated (yield 70%): UV: end absorption: IR_{film}: ν_{OH} 3440 cm^{-1}, no $\nu_{C=O}$, ν_{C-O} 1260 cm^{-1}; MS: M^+ 458 (27, $C_{30}H_{50}O_3$), 443 (13, M^+-CH_3), 425 (23, M^+-CH_3, H_2O), 413 (21), 407 (12, M^+-CH_3, H_2O, H_2O), 395 (17), 371 (20, $M^+-C_4H_{11}O$ cleavage at C_{22}/C_{23}), 357 (90, $M^+-C_6H_{13}O$ side chain), 339 (62, $M^+-C_6H_{13}O$, H_2O), 299 (60, M^+–HCOH, $C_8H_{16}O$ cleavage at C_{17}/C_{20}, H), 281 (48, M^+–HCOH, $C_8H_{16}O$, H, H_2O); NMR ($CDCl_3$): 0.81 (s, 3H, C-31); 0.92 (d J = 7 Hz, 6H, C-26, 27); 0.93 (s, 3H, C-30); 0.98 (s, 6H, C-19, 30); 1.21 (s, 3H, C-21); 3.20 (m, 1H, 3αH); 3.34 (dd, 2H, $C^{18}H_2$) and 3.83 ppm (m, 1H, 23 H–C–OH).

Oxidation of **11** *into diketone-ether* **12**. Ether **11** (5 mg) dissolved in acetone (2 ml) was oxidized with Jones reagent at room temp. during 3 min. The reaction medium was treated in the usual way. The crude extract was purified by silica gel column chromatography using a gradient elution of acetone (from 0 to 10%) in hexane. Pure **12** (3 mg) was obtained: UV: end absorption; IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1710 cm^{-1}, ν_{C-O} 1260 cm^{-1}; MS: M^+ 454 (16, $C_{30}H_{46}O_3$), 439 (22, M^+-CH_3), 423 (7), 409 (11, M^+-CH_3, HCOH), 391 (9), 385 (10), 383 (11), 369 (31, $M^+-C_5H_9O$ cleavage at C_{22}/C_{23}), 355 (95, $M^+-C_6H_{11}O$ side chain), 354 (100, $M^+-C_6H_{11}O$, H), 339 (38, $M^+-C_5H_9O$, HCOH), 337 (38), 325 (36, $M^+-C_6H_{11}O$, HCOH), 311 (46), 297 (57, $M^+-C_8H_{14}O$ cleavage at C_{17}/C_{20}, HCOH, H), 287 (31), 269 (28).

Treatment of **9** *with* $NaIO_4$. Tetrol **9** (1 mg) dissolved in MeOH–H_2O (5 ml; 1/1, v/v), was stirred at room temp. with $NaIO_4$ (10 mg). After 20 hr, **9** was recovered unchanged (TLC).

Hydrolysis of ketone **7** *into ketone-alcohol* **13**. Monoketone **7** (97 mg) was stirred at room temp. for 15 hr in sat. methanolic K_2CO_3 (50 ml). The mixture was neutralized by dropwise

addition of 10% HCl aq and worked up in the usual way to give 95 mg (yield 99%) of pure **13**: UV: end absorption; IR_{film}: ν_{OH} 3500 cm^{-1}, $\nu_{C=O}$ 1770 and 1720 cm^{-1}, $\nu_{C=C}$ 1670 cm^{-1}; MS: M^+ 470 (28, $C_{30}H_{46}O_4$), 455 (1, M^+-CH_3), 452 (1, M^+-H_2O), 437 (2, M^+-CH_3, H_2O), 407 (2, M^+-H_2O, CO_2, H), 393 (15, M^+-CH_3, H_2O, CO_2), 384 (8), 369 (16), 325 (11), 323 (20); NMR ($CDCl_3$): 0.93 (d J = 7 Hz, 6H, C-26, 27); 1.03(s, 3H, C-30); 1.09 (s, 6H, C-31, 32); 1.22 (s, 3H, C-19); 1.56 (s, 3H, C-21) and 3.95 (m, 1H, H–C–OH).

Treatment of **13** *with* $POCl_3$ *in pyridine.* Ketone–alcohol **13** (95 mg) in pyridine (4 ml) reacted with $POCl_3$ (0.4 ml). The mixture was stirred at room temp. during 2.25 hr and worked up in the usual way. The crude residue was chromatographed on a silica gel column using gradient elution with hexane–acetone (from 2 to 10%). The fractions 15–17 contained 8 mg (yield 9%) of pure chlorinated **14**: UV: end absorption; IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1765 and 1710 cm^{-1}, ν_{C-O} 1255 cm^{-1}; MS: M^+ 490/488 (24/61, $C_{30}H_{45}O_3Cl$), 475/473 (16/32, M^+-CH_3), 466 (11), 452 (48, M^+–HCl), 445/443 (12/16, M^+-CO_2, H), 437 (17, M^+–HCl, CH_3), 429/427 (29/24, M^+-CH_3, CO_2, H_2), 419 (8), 407 (12, M^+–HCl, CO_2, H), 393 (29, M^+–HCl, CH_3, CO_2), 384 (16, $M^+-C_5H_9Cl$ cleavage at C_{22}/C_{23}), 369 (17, $M^+-C_6H_{12}Cl$ side chain), 341 (40), 325 (19, $M^+-C_6H_{12}Cl$, CO_2), 323 (25, $M^+-C_6H_{12}Cl$, CO_2, H_2). The fractions 10–14 contained 33 mg (yield 39%) of the mixture of the expected dehydrated compounds **15** and **16**. Spectral data are recorded on the mixture of **15** and **16**: UV: end absorption; IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1765 and 1710 cm^{-1}, $\delta_{C=CH}$ 975 cm^{-1}; MS: M^+ 452 (8, $C_{30}H_{44}O_3$), 437 (1, M^+-CH_3), 393 (8, M^+-CH_3, CO_2), 341 (2), 315 (1); NMR ($CDCl_3$): 0.91 (d J = 6 Hz, 6H, C-26, 27); 1.13 (s, 9H, C-30, 31, 32); 1.23 (s, 3H, C-19); 1.46 (s, 3H, C-21) and a complex signal centered at 5.40 ppm (m, 2H, H–C=C).

Ozonolysis of the dehydrated mixture **15** *and* **16**. The mixture **15** and **16** (2 mg) in anhyd. AcOEt was treated at −70° with excess O_3. Triphenylphosphine was added and the ozonolysis medium analysed by GC. Isobutyraldehyde was detected on a 10% carbowax (60°, isotherm) and on a 20% 1,2,3-tris-(2-cyanoethoxy)-propane column (program of temp.: 5°/min from room temp. until 130°). Isovaleraldehyde was detected on the latter column only.

Perdeuteriation of diketone **8**. Diketone **8** (2 mg) in anhyd. dioxane (3 ml) was refluxed with D_2O (0.2 ml) and K_2CO_3 during 1 day. The mixture was worked up in the usual way and the residue further treated, under the same conditions, until a constant pattern in the MS of **17** was observed: M^+ 474 (20, $C_{30}H_{40}D_6O_4$), 473 (22, $C_{30}H_{41}D_5O_4$), 459 (4, M^+-CH_3), 441 (4), 439 (5), 413 (15), 387 (5, $M^+-C_5H_7D_2O$ cleavage at C_{22}/C_{23}), 327 (14, $M^+-C_6H_7D_4O$ side chain, CO_2).

Alkaline hydrolysis of **6** *into* **18**. Compound **6** (35 mg) was treated as described for **7**. The crude extract, purified by silica gel column chromatography (elution: hexane–acetone 10%), gave pure diol **18** (29 mg): $(\alpha)_D = -9.8°$ (c = 0.93 in $CHCl_3$); UV: 237–244–253 (ϵ = 12200–13000–9400); IR_{KBr}: ν_{OH} 3350 cm^{-1}, $\nu_{C=O}$ 1770 cm^{-1}, $\delta_{C=CH}$ 945 cm^{-1}; MS: M^+ 470 (64, $C_{30}H_{46}O_4$), 455 (2, M^+-CH_3), 452 (3, M^+-H_2O), 437 (26, M^+-CH_3, H_2O), 419 (6, M^+-H_2O, CH_3, H_2O), 413 (6), 393 (9, M^+-CH_3, H_2O, CO_2), 383 (25, M^+-ring A), 375 (5), 365 (10, M^+-ring A, H_2O), 351 (9), 325 (22, $M^+-C_6H_{13}O$ side chain, CO_2), 297 (22); NMR ($CDCl_3$–CD_3OD): 0.89 (s, 3H, C-31); 0.94 (d J = 7 Hz, 6H, C-26, 27); 1.00(s, 3H, C-32); 1.05 (s, 3H, C-30); 1.09 (s, 3H, C-19); 1.58 (s, 3H, C-21); 2.61 (d J = 4.5 Hz, 2H, C^{12}–H_2); 3.21 (m, 1H, 3α H); 3.80 (m, 1H, H–C–OH); 5.27 (m, 1H, 11–H–C=C) and 5.62 ppm (m, 1H, 7–H–C=C).

Oxidation of **18** *into* **19**. Diol **18** (27 mg) dissolved in acetone (5 ml) was oxidized with Jones reagent at room temp. during 4 min. The mixture was treated in the usual way. The crude extract was chromatographed on a silica gel column using hexane–acetone 5%. Diketone **19** (24 mg) was obtained: UV: 236–244–252 (ϵ = 11800–12600–9200); IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1760 and 1715 cm^{-1}, $\delta_{C=CH}$ 940 cm^{-1}; MS: M^+ 466 (63, $C_{30}H_{42}O_4$), 451 (2, M^+-CH_3), 420 (7, M^+-CO_2, H_2), 407 (15, M^+-CH_3, CO_2), 405 (9, M^+-CH_3, CO_2, H_2), 381 (11, M^+-ring A), 367 (3, $M^+-C_6H_{11}O$ side chain), 335 (8), 323 (14, M^+-CO_2, $C_6H_{11}O$), 321 (14, $M^+-C_6H_{11}O$, CO_2, H_2), 307 (7); NMR ($CDCl_3$): 0.93 (d J = 7 Hz, 6H, C-26, 27); 1.01 (s, 3H, C-30); 1.09 (s, 3H, C-31); 1.15 (s, 3H, C-32); 1.33 (s, 3H, C-19); 1.50 (s, 3H, C-21); 2.59 (d J = 4.5 Hz, 2H, C^{12}–H_2); 2.97 (s, 2H, $C^{22}H_2$); 5.31 (m, 1H, H–C=C) and 5.60 ppm (m, 1H, 7–C=C–H).

Acetylation of **3**. Compound **3** (4 mg) was treated with Ac_2O (1 ml) and pyridine (1 ml) at room temp. for 16 hr. The mixture was worked up in the usual way to afford crude **20** purified by column chromatography on silica gel (elution: hexane–acetone 10%): UV: end absorption; IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1765 and 1745 cm^{-1}, ν_{C-O} 1250 cm^{-1} and $\delta_{C=CH_2}$ 890 cm^{-1}; MS 554 (4, $C_{34}H_{50}O_6$), 539 (1, M^+-CH_3), 521 (1), 512 (1, M^+-CH_2CO), 494 (3, M^+-CH_3COOH), 479 (2, M^+-CH_3COOH, CH_3), 435 (5, M^+-CH_3COOH, CH_3, CO_2), 419 (5, M^+-CH_3COOH, CH_3, CH_3COOH), 375 (4, M^+-CH_3, CH_3COOH, CH_3COOH, CO_2), 365 (3, M^+-CH_3COOH, ring A).

Hydrolysis of **3** *into* **21**. Compound **3** (25 mg) was treated as described for **2**. The mixture, worked up in the usual way, gave pure **21** (22 mg): UV: end absorption: IR_{KBr}: ν_{OH} 3500 cm^{-1}, $\nu_{C=O}$ 1765 cm^{-1}, $\nu_{C=C}$ 1650 cm^{-1} and $\delta_{C=CH_2}$ 890 cm^{-1}; MS: M^+ 470 (12, $C_{30}H_{46}O_4$), 455 (2, M^+-CH_3), 452 (1, M^+-H_2O), 437 (3, M^+-CH_3, H_2O), 419 (2, M^+-CH_3, H_2O, H_2O), 415 (6), 399 (2), 393 (3, M^+-CH_3, H_2O, CO_2), 386 (2), 381 (4), 371 (4), 353 (9), 325 (6); NMR ($CDCl_3$–CD_3OD): Table 1.

Hydrogenation of **3** *into* **2**. Compound **3** (30 mg) in AcOEt (10 ml) was hydrogenated on PtO_2 at room temp. during 6.5 hr under atmospheric pressure of H_2. The catalyst was eliminated by filtration on a 7 cm silica gel column using AcOEt as eluent. Evaporation under reduced pressure of the solvent yielded 28 mg (yield 95%) of a compound identical with **2** by R_f on silica gel plates impregnated with $AgNO_3$, UV IR (disparition of the absorption band at 890 cm^{-1}), MS (M^+, fragmentation pattern and ions intensities) and NMR (no methyle on a double bond, disparition of the 2H m at 4.82 ppm and apparition of an isopropyl signal at 0.93 ppm d J = 7 Hz).

Hydrogenation of the dehydrated mixture **15** *and* **16**. Hydrogenation of the dehydrated mixture **15** and **16** (30 mg) occurred as described for **3**. This yielded 27 mg of a crude mixture of three compounds separated by column chromatography on silica gel using gradient elution with hexane–acetone (from 2 to 10%). The fractions 8–10 contain 9 mg (yield 30%) of **23**: UV: end absorption; IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1760 and 1710 cm^{-1}, no $\delta_{C=CH}$ at 975 cm^{-1}; MS: M^+ 454 (78, $C_{30}H_{46}O_3$), 439 (34, M^+-CH_3), 421 (2, M^+-CH_3, H_2O), 409 (21, M^+-CO_2, H), 395 (80, M^+-CH_3, CO_2), 393 (85, M^+-CH_3, CO_2, H_2), 341 (29), 321 (71); NMR ($CDCl_3$): 0.89 (d J = 6 Hz, 6H, C-26, 27); 1.10 (s, 9H, C-30, 31, 32); 1.24 (s, 3H, C-19) and 1.43 ppm (s, 3H, C-21).

The fractions 12–14 contain 0.7 mg (yield 2.5%) of **24**: UV: end absorption; IR_{film}: ν_{OH} 3520 cm^{-1}, $\nu_{C=O}$ 1760 cm^{-1}, ν_{C-O} 1260 cm^{-1}, no $\delta_{C=CH}$ at 975 cm^{-1}; MS: M^+ 456 (11, $C_{30}H_{48}O_3$), 441 (29, M^+-CH_3), 423 (15, M^+-CH_3, H_2O), 395 (8, M^+-CH_3, CO_2, H_2), 379 (4, M^+-CH_3, H_2O, CO_2), 371 (3), 367 (2), 325 (4), 316 (20).

The fractions 17–25 contain 15.5 mg (yield 54%) of **25**: UV: end absorption; IR_{film}: ν_{OH} 3340 cm^{-1}, $\nu_{C=O}$ 1755 cm^{-1}, ν_{C-O} 1260 cm^{-1}, no $\delta_{C=CH}$ at 975 cm^{-1}; MS: M^+ 456 (37, $C_{30}H_{48}O_3$), 441 (19, M^+-CH_3), 423 (22, M^+-CH_3, H_2O), 395 (19, M^+-CH_3, CO_2, H_2), 379 (12, M^+-CH_3, H_2O CO_2), 371 (1), 367 (1), 325 (4), 316 (29); NMR ($CDCl_3$): 0.88 (d, J = 6 Hz, 6H, C-26, 27); 0.92 (s, 3H, C-31); 1.00 (s, 6H, C-30, 32); 1.05 (s, 3H, C-19); 1.41 (s, 3H, C-21) and 3.25 ppm (m, 1H, 3α H).

Acetylation of **25** *into* **26**. Compound **25** (15 mg) was treated with Ac_2O (1.5 ml) and pyridine (1.5 ml) at room temp. for 14 hr. The mixture was worked up in the usual way to give 14 mg (yield 91%) of **26**: UV: end absorption; IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1760 and 1735 cm^{-1}, $\nu_{C=C}$ 1650 cm^{-1}, ν_{C-O} 1265 cm^{-1}; MS: M^+ 498 (13, $C_{32}H_{50}O_4$), 483 (3, M^+-CH_3), 455 (2, M^+-CH_3CO), 440 (7, M^+-CH_3CO, CH_3), 439 (8, M^+-CH_3COO), 438 (7, M^+-CH_3COOH), 423 (25, M^+-CH_3COOH, CH_3), 395 (4, M^+-CH_3COO, CO_2), 385 (4), 379 (10, M^+-CH_3, CH_3COOH, CO_2), 377 (4), 343 (10), 325 (9), 316 (20); NMR ($CDCl_3$): 0.88 (d J = 6 Hz, 6H, C-26, 27); 0.89 (s, 3H, C-31); 0.93 (s, 3H, C-32); 0.98 (s, 3H, C-30); 1.02 (s, 3H, C-19); 1.42 (s, 3H, C-21); 2.06 (s, 3H, CH_3–COO) and 4.46 (m, 1H, 3α H).

Oxidation of **26** *into seychellogenin acetate* **27**. Monoacetate **26** (14 mg) dissolved in AcOH (1 ml) was refluxed with SeO_2 (20 mg) in 90% AcOH-water (2 ml) during 4 hr. The mixture was worked up in the usual way. Fine solid Se was eliminated from the crude orange residue by filtration on a 5 cm silica gel column (elution: hexane–acetone 30%). This treatment afforded 11 mg of a mixture from which 4 mg of seychellogenin acetate **27** (yield 29%)

was isolated by column chromatography on silica gel using a gradient elution of acetone (from 2 to 4%) in hexane. **27** proved to be seychellogenin acetate by comparison with an authentic sample: UV: 236–243–252 (ϵ = 12200–12500–8800); IR_{film}: no ν_{OH}, $\nu_{C=O}$ 1770 and 1720 cm^{-1}, $\nu_{C=C}$ 1655 cm^{-1}, ν_{C-O} 1270 cm^{-1}; MS: M^+ 496 (3, $C_{32}H_{48}O_4$), 481 (1, M^+–CH_3), 453 (1, M^+–CH_3CO), 437 (5, M^+–CH_3COO), 436 (1, M^+–CH_3COOH), 421 (6, M^+–CH_3COOH, CH_3), 393 (1, M^+–CH_3COO, CO_2), 377 (3, M^+–CH_3COOH, CH_3, CO_2), 367 (11), 354 (1), 339 (2), 325 (3); NMR ($CDCl_3$, 270 MHz): 0.88 (d J = 7 Hz, 6H, C-26, 27); 0.89 (s, 3H, C-31); 0.97 (s, 3H, C-32); 1.00 (s, 3H, C-30); 1.13 (s, 3H, C-19); 1.40 (s, 3H, C-21); 2.06 (s, 3H, CH_3–COO); 4.52 (dd J = 9 and 7 Hz, 1H, 3αH); 5.23 (m, 1H, $C=C^{11}$–H) and 5.54 ppm (m, 1H, $C=C^7$H).

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