7. Agnatasterone A and B, Unusual Pregnane Steroids Isolated from the North-East Atlantic Sponge *Axinella agnata*

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Two novel pregnatrienolones isolated in very small amounts from the North-East-Atlantic demosponge *Axinella agnata* (Tetractinomorpha, Axinellida) are unique in having C(2)=C(3) (or C(3)=C(4)), C(7)=C(8), and C(16)=C(17) bonds and a 12β-OH group which, being strongly H-bonded to a 20-keto group, resists acylation. ^{1}H- and ^{13}C-NMR spectroscopy of the steroids and of products of their selective epoxidation or reduction allow us to propose the structures (+)-12β-hydroxy-5α-pregna-2,7,16-trien-20-one (= agnatasterone A, (+)-**1**), and (+)-12β-hydroxy-5α-pregna-3,7,16-trien-20-one (= agnatasterone B, (+)-**5**), for the two steroids with minimal recourse to model compounds.

1. Introduction. – In the area of natural products marine sponges of the order Axinellida have aroused much interest for a wide variety of N-containing, unusual metabolites which include isonitrile [1] and mixed-biogenesis terpenoids [2], as well as guanidine-derived compounds [3]. In contrast, as regards novel steroids, of which the phylum Porifera is otherwise the most various producer [4], interest in the Axinellida has been so far concentrated on some A-ring contracted C_{27} sterols only [5].

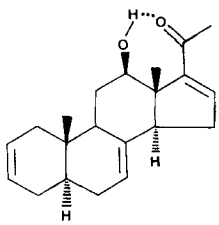
We expand here the interest towards the Axinellida steroids, and in general the sponge steroids, by reporting on two new pregnanes from a member of the Axinellida, the North-East-Atlantic sponge *Axinella agnata*. This has also an interest *per se* in the area of steroids as these novel pregnanes have unique structural features.

In fact, though pregnane steroids exert important hormonal functions in terrestrial organisms [6], only a few of them, not possessing any peculiar structural characteristic, have been so far isolated from marine organisms. Included are free pregnanes from the distant demosponge *Haliclona rubens* (Ceractinomorpha, Haposclerida) [7], the alcyonacean corals *Gersemia rubiformis* [8a] and *Capnella* sp. nov. and *Capnella erecta* [8b], and the gorgonian *Telesto riisei* [8c], other than aglycones of starfish saponins [9].

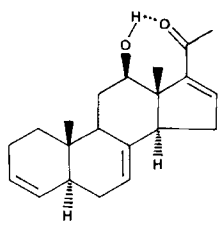
The structural elucidation of steroids is not easily accomplished by NMR techniques due to many closely lying signals, particularly in the ^{1}H-NMR spectrum even at high magnetic field. Two approaches are currently followed. One is to place much emphasis on ^{13}C-NMR data in comparison with reference steroids of known structure [10]. The other approach mainly relies on ^{1}H-NMR data [11], thus requiring much time on a latest generation, high-field NMR spectrometer in order to carry out two-dimensional experiments at high resolution. This [11], under favourable circumstances, allows to assign all ^{13}C and ^{1}H resonances, though large amounts of steroids are needed. Therefore, the second approach [11] has been used with known steroids as models.

As the new steroids have been isolated here in scarce amounts, while we intend to carry out a rigorous structural elucidation, we have followed an intermediate line of approach between the above two [10] [11]. We have placed much emphasis on both heteronuclear-decoupling techniques [12a] and decoupling differential spectroscopy (DDS) [12b]. As some ^{1}H resonances could not be assigned, we used literature ^{13}C-NMR data for reference steroids to assign the ^{13}C resonances.

This notwithstanding, some structural details could not be clarified. However, we could remove all structural ambiguities by carrying out independent NMR studies on the products of reactions of known mechanism with our novel steroids.



(+)-**1**



(+)-**2**

2. Results and Discussion. – High-field ^{1}H- and ^{13}C-NMR spectra of the two metabolites isolated here from *A. agnata,* agnatasterone A ((+)-**1**) and agnatasterone B ((+)-**2**) (*Tables 1* and *2*), immediately reveal two typical C(18) and C(19) steroidal CH_3 groups. The NMR and MS data *(Exper. Part)* indicate the molecular composition $C_{21}H_{28}O_2$ for both steroids. This suggests a pregnane skeleton possessing a keto group which can be placed at C(20) from the characteristic δ_C data for an α,β-unsaturated ketone *(Table 2)* [10a]. The NMR data also suggest one OH group and three olefinic bonds. Both the UV and the IR data *(Exper. Part)* further indicate the presence of the α,β-unsaturated keto group.

The NMR data of (+)-**1** and (+)-**2** in *Tables 1* and *2* further reveal that of the two remaining non-conjugated olefinic bonds one must be trisubstituted (and identically located in both steroids), whilst the other one must be disubstituted and its position must differ in the two steroids. As rings B and C do not offer two different arrangements for a disubstituted C=C bond, the latter must be in ring A in order to account for all the above data, and to find room for both C=C double bonds. Therefore, ruling out the C(1)=C(2) bond, which would demand a ^{13}C(19) H_3 resonance at *ca.* 4.5 ppm lower field than actually observed [10c, d] *(Table 2)*, only the C(2)=C(3) and C(3)=C(4) bonds are left to consideration. Clearly, here must lie the difference between the two steroids. Consequently, there must be either a C(7)=C(8) or a C(9)=C(11) trisubstituted bond. The first alternative is the one of choice, the latter one being ruled out for both steroids by the normal resonance of the C(19) H_3 group [10d].

The appearance of the deshielded proton at the OH-bearing C-atom as a *dd* at 3.76 ppm for coupling (J = 10.6 and 4.8 Hz) with a CH_2 group can be attributed to either H_α–C(1) or H_α–C(12) [13]. The latter situation has to be accepted, since it is the only one which leaves room for the required C(1)H_2 and C(11)H_2 groups with both steroids. In fact, the data in *Table 1* show that H–C(12) is ^3J-coupled with a CH_2-group. Should the OH group be at C(1), such a coupling pattern could not result with one of the two steroids. The 12β position for the OH group also accounts for the fact that neither one of the two steroids could be acetylated. This implies strong H-bonding of the OH with the CO group [14], as discussed later in more details.

The above conclusions are further supported by the coupling pattern among the protons at rings D, C, and B for either (+)-**1** or (+)-**2**, as deduced from decoupling differential spectroscopy experiments. In fact, from the data in *Table 1* it is seen that H–C(16) (assigned both by its typical ^{1}H-NMR chemical shift [15] and ^{13}C, ^{1}H shift-correlated 2D spectra [12]) is correlated with both H_α–C(15) and H_β–C(15). The latter two protons are correlated with H_α–C(14) by typical coupling constants of 6.8 and 11.7 Hz, respectively [16]. Moreover, H_α–C(14)

Table 1. *^{1}H-NMR Data*[a]) *in $CDCl_3$ for Agnatasterone A* ((+)-**1**) *and Agnatasterone B* ((+)-**2**)

H-Atom at	(+)-**1**	(+)-**2**
C(1)	2.07 (*m* H_β); 1.82 (*m*, H_α)	1.78 (*m*, H_β); 1.20 (*ddd*, J_{gem} = 13.4, $J(1\alpha,2\beta)$ = 10.6, $J(1\alpha,2\alpha)$ = 6.2, H_α)
C(2) } C(3) }	5.59, 5.57 (*AB*, *J* = 9.8[b]))	2.05 (*m*, H_α); 1.88 (*m*, H_β) 5.63 (*dd*, *J*(3,4) = 9.9, $J(3,2\alpha)$ = 2.9)
C(4)	2.07 (*m*, H_β); 1.82 (*m*, H_α)	5.33 (*dd*, *J*(4,3) = 9.9, $J(4,2\alpha)$ = 1.9)
C(5)	1.75 (*m*)	2.02 (*m*)
C(6)	2.02 (*m*, H_α); 1.78 (*m*, H_β)	1.85 (*m*, H_α); 1.79 (*m*, H_β)
C(7)	5.33 (*m*, $w_{1/2}$ 10.0)	5.39 (*m*, $w_{1/2}$ = 9.7)
C(9)	1.97 (*m*)	1.86 (br. *dd*, $J(9,11\beta)$ = 12.8, $J(9,11\alpha)$ = 6.5)
C(11)	1.94 (*ddd*, J_{gem} = 14.9, $J(11\alpha,9)$ = 6,6, $J(11\alpha,12\alpha)$ = 4.8, H_α); 1.57 (*ddd*, J_{gem} = $J(11\beta,9)$ = 14,9, $J(11\beta,12\alpha)$ = 10.6, H_β)	1.98 (*ddd*, J_{gem} = 12,8, *J* = (11α,9) = 6.5, $J(11\alpha, 12\alpha)$ = 4.8, H_α); 1.51 (*ddd*, J_{gem} = $J(11\beta,9)$ = 12.8, $J(11\beta,12\alpha)$ = 10.7, H_β)
C(12)	3.76 (*dd*, $J(12\alpha,11\beta)$ = 10.6, $J(12\alpha,11\alpha)$ = 4.8, H_α)	3.76 (*dd*, $J(12\alpha,11\beta)$ = 10.7, $J(12\alpha,11\alpha)$ = 4.8)
C(14)	2.20 (br. *dd*, $J(14,15\beta)$ = 11.7, $J(14,15\alpha)$ = 6.8)	2.23 (br. *dd*, $J(14,15\beta)$ = 11.8, $J(14,15\alpha)$ = 6.7)
C(15)	2.46 (*ddd*, J_{gem} = 17.7, $J(15\beta,14)$ = 11.7, $J(15\beta,16)$ = 1.9, H_β); 2.38 (*ddd*, J_{gem} = 17.7, $J(15\alpha,14)$ = 6.8, $J(15\alpha,16)$ = 3.3, H_α)	2.49 (*ddd*, J_{gem} = 17.6, $J(15\beta,14)$ = 11.8, $J(15\beta,16)$ = 2.1, H_β); 2.39 (*ddd*, J_{gem} = 17.6, $J(15\alpha,14)$ = 6.7, $J(15\alpha,16)$ = 3.3, H_α)
C(16)	7.00 (*dd*, $J(16,15\alpha)$ = 3.3, $J(16,15\beta)$ = 1.9)	7.00 (*dd*, $J(16,15\alpha)$ = 3.3, $J(16,15\beta)$ = 2.1)
C(18)	0.772 (*s*)	0.773 (*s*)
C(19)	0.846 (br. *s*)	0.797 (br. *s*)
C(21)	2.37 (*s*)	2.37 (*s*)
OH	5.94 (*s*)	5.94 (*s*)

[a]) The number of protons at each C-atom is defined by the respective structural formula.
[b]) With small coupling to 2H–C(1) or 2H–C(4).

is long-range coupled with H–C(7)[1]) which, in turn, is coupled both with H_α–C(6) (*J* = 5.7) and, though weakly, with H_β–C(6). From the other side, H_α–C(12), which has been assigned above, is coupled with both H_α–C(11) and (*trans* diaxial) H_β–C(11), and the latter, in turn, is *trans*-diaxially coupled with H_α–C(9).

As regards ring A, with compound (+)-**1**, the olefinic protons are coupled with both H_β–C(1) and H_β–C(4) and, more weakly, with H_α–C(1) and H_α–C(4). Here, the protons at the α face can be distinguished from those at the β face on the basis of ^{13}C, ^1H shift-correlated experiments. With compound (+)-**2**, the analysis is simpler as the olefinic protons at ring A have sufficiently different chemical shifts and coupling patterns to allow us a complete analysis of the proton system from C(1) to C(4). However, with neither (+)-**1** or (+)-**5** could couplings with H_α–C(5) be established.

With agnatasterone-A ((+)-**1**), the assignments of C(18)H_3 and C(19)H_3 unambiguously rest on the results of the influence of the shift reagent Eu(fod)$_3$ *(Table 3)*. It is seen that C(18)H_3 can be assigned on the basis of a dramatically larger relative shift. Consequently, the appearance of C(18)H_3 as a sharper *s* than C(19)H_3 *(Table 1)* can be used as a quicker criterion of position assignment and this can be safely used also with the other steroids of this series.

Full support to the above conclusions is obtained on subjecting (+)-**1** and (+)-**2** to reactions of known mechanisms. The products are then structurally investigated by NMR techniques according to the approach illustrated above, without any bias from previous knowledge about the structure of (+)-**1** and (+)-**2**. Thus, (+)-**1** undergoes

[1]) Selective heteronuclear decoupling by irradiation of H–C(14) brought about a sharpening of the resonance assigned to C(8). This further supports the C(7)=C(8) bond.

Table 2. *^{13}C-NMR Data in $CDCl_3$ for Agnatasterone A ((+)-**1**), its Derivatives (+)-**3**, (+)-**4**, (+)-**5**, and **10**, Agnatasterone B ((+)-**2**), its Derivatives (+)-**6**, **7**, **8**, and **9**, and the Common Derivatives **11a**, **11b**, **12a**, and **12b***

C-Atom	(+)-**1**	(+)-**3**	(+)-**4**	(+)-**5**	**10**	(+)-**2**	(+)-**6**	**7**
C(1)	39.06 (*t*)	38.03 (*t*)	40.56 (*t*)	39.81 (*t*)	39.18 (*t*)	33.49 (*t*)	29.85 (*t*)	33.97 (*t*)
C(2)	125.40[a]) (*d*)	50.54 (*d*)	125.20[d]) (*d*)	50.18 (*d*)	125.09[g]) (*d*)	22.92 (*t*)	21.00 (*t*)	22.60 (*t*)
C(3)	125.05[a]) (*d*)	52.22 (*d*)	125.10[d]) (*d*)	51.68 (*d*)	125.34[g]) (*d*)	126.19 (*d*)	52.52 (*d*)	126.25 (*d*)
C(4)	30.04[b]) (*t*)	29.53[c]) (*t*)	30.55[e]) (*t*)	29.69[f]) (*t*)	30.47[h]) (*t*)	129.84 (*d*)	54.90 (*d*)	129.55 (*d*)
C(5)	36.76 (*d*)	32.35 (*d*)	33.55 (*d*)	32.62 (*d*)	36.62 (*d*)	41.51 (*d*)	41.63 (*d*)	34.98 (*d*)
C(6)	29.94[b]) (*t*)	29.96[c]) (*t*)	29.23[e]) (*t*)	27.00 (*t*)	29.91[h]) (*t*)	28.06 (*t*)	27.27 (*t*)	28.06 (*t*)
C(7)	118.68 (*d*)	118.60 (*d*)	59.24 (*d*)	58.83 (*d*)	118.69 (*d*)	119.73 (*d*)	119.06 (*d*)	59.83 (*d*)
C(8)	135.42 (*s*)	135.16 (*s*)	60.37 (*s*)	60.12 (*s*)	135.48 (*s*)	136.05 (*s*)	136.10 (*s*)	61.52 (*s*)
C(9)	47.90 (*d*)	48.05 (*d*)	45.48 (*d*)	45.64 (*d*)	47.90 (*d*)	47.29 (*d*)	46.65 (*d*)	44.22 (*d*)
C(10)	33.60 (*s*)	32.43 (*s*)	33.55 (*s*)	32.64 (*s*)	33.70 (*s*)	33.83 (*s*)	32.68 (*s*)	33.98 (*s*)
C(11)	28.94 (*t*)	28.69 (*t*)	29.63[e]) (*t*)	28.07[f]) (*t*)	29.86[h]) (*t*)	29.65 (*t*)	29.15 (*t*)	29.69 (*t*)
C(12)	73.25 (*d*)	73.16 (*d*)	72.70 (*d*)	72.59 (*d*)	73.69 (*d*)	73.22 (*d*)	73.03 (*d*)	72.67 (*d*)
C(13)	52.42 (*s*)	52.29 (*s*)	51.50 (*s*)	52.08 (*s*)	47.90 (*s*)	52.60 (*s*)	52.63 (*s*)	51.61 (*s*)
C(14)	52.80 (*d*)	52.70 (*d*)	51.84 (*d*)	51.63 (*d*)	43.07 (*d*)	53.04 (*d*)	52.90 (*d*)	52.03 (*d*)
C(15)	30.97 (*t*)	31.01 (*t*)	30.83 (*t*)	31.07 (*t*)	25.60 (*t*)	31.06 (*t*)	30.98 (*t*)	30.34 (*t*)
C(16)	149.83 (*d*)	149.71 (*d*)	149.28 (*d*)	149.17 (*d*)	60.30 (*d*)	149.86 (*d*)	149.70 (*d*)	149.24 (*d*)
C(17)	154.97 (*s*)	154.95 (*s*)	154.36 (*s*)	154.29 (*s*)	71.17 (*s*)	154.93 (*s*)	154.88 (*s*)	154.42 (*s*)
C(18)	11.65 (*q*)	11.63 (*q*)	11.55 (*q*)	11.52 (*q*)	11.62 (*q*)	11.68 (*q*)	11.73 (*q*)	11.58 (*q*)
C(19)	12.82 (*q*)	13.60 (*q*)	14.65 (*q*)	15.22 (*q*)	12.91 (*q*)	12.52 (*q*)	14.20 (*q*)	14.85 (*q*)
C(20)	199.20 (*s*)	199.0 (*s*)	199.0 (*s*)	198.97 (*s*)	208.00 (*s*)	199.21 (*s*)	199.20 (*s*)	198.98 (*s*)
C(21)	26.74 (*q*)	26.73 (*q*)	26.76 (*q*)	26.74 (*q*)	25.29 (*q*)	26.71 (*q*)	26.75 (*q*)	26.77 (*q*)

C-Atom	**9**	**11a**	**11b**[l])	**12a**	**12b**[r])	**8**
C(1)	33.58 (*t*)	38.86 (*t*)	38.81 (*t*)	38.24 (*t*)	38.19 (*t*)	29.57[u]) (*t*)
C(2)	22.89 (*t*)	22.09[j]) (*t*)	22.00 (*t*)	22.40 (*t*)	22.02 (*t*)	20.21 (*t*)
C(3)	126.19 (*d*)	26.42 (*t*)	26.83[m]) (*t*)	26.60[o]) (*t*)	26.60[s]) (*t*)	52.29 (*d*)
C(4)	129.77 (*d*)	28.67[k]) (*t*)	28.66[n]) (*t*)	29.05[p]) (*t*)	28.90[t]) (*t*)	54.61 (*d*)
C(5)	41.40 (*d*)	41.63 (*d*)	41.56 (*d*)	45.91 (*d*)	45.92 (*d*)	41.91 (*d*)
C(6)	28.06 (*t*)	29.59[k]) (*t*)	29.81[n]) (*t*)	29.50[p]) (*t*)	29.58[t]) (*t*)	27.41 (*t*)
C(7)	119.78 (*d*)	119.93 (*d*)	120.57 (*d*)	28.47 (*t*)	34.00 (*t*)	58.98 (*d*)
C(8)	135.49 (*s*)	136.26 (*s*)	135.76 (*s*)	[i])	125.51 (*s*)	[i])
C(9)	47.17 (*d*)	48.63 (*d*)	48.59 (*d*)	50.26 (*d*)	50.17 (*d*)	46.33 *d*)
C(10)	33.76 (*s*)	34.94 (*s*)	35.02 (*s*)	[i])	37.10 (*s*)	[i])
C(11)	30.04 (*t*)	29.92[k]) (*t*)	25.58 (*t*)	29.51[p]) (*t*)	25.80 (*t*)	29.58[u]) (*t*)
C(12)	71.11 (*d*)	77.57 (*d*)	80.58 (*d*)	75.83 (*d*)	79.55 (*d*)	72.50 (*d*)
C(13)	[i])	48.29 (*s*)	48.84 (*s*)	[i])	49.12 (*s*)	[i])
C(14)	43.23 (*d*)	52.37 (*d*)	53.49 (*d*)	[i])	[i])	52.29 (*d*)
C(15)	25.80 (*t*)	24.73 (*t*)	26.34[m]) (*t*)	25.34[o]) (*t*)	25.15[s]) (*t*)	30.40 (*t*)
C(16)	60.78 (*d*)	22.40[j]) (*t*)	22.95 (*t*)	25.77[o]) (*t*)	[i])	149.24 (*d*)
C(17)	71.52 (*s*)	63.79 (*d*)	61.25 (*d*)	64.10 (*d*)	62.12 (*t*)	[i])
C(18)	10.55 (*q*)	7.86 (*q*)	8.90 (*q*)	12.60 (*q*)	12.59 (*q*)	10.15 (*q*)
C(19)	12.59 (*q*)	12.87 (*q*)	12.83 (*q*)	[q]) (*q*)	15.15 (*q*)	14.13 (*q*)
C(20)	208.00 (*s*)	199.26 (*s*)	210.24 (*s*)	199.26 (*s*)	210.22 (*s*)	[i])
C(21)	25.29 (*q*)	30.27 (*q*)	31.56 (*q*)	30.40 (*q*)	31.34 (*q*)	26.55 (*q*)

[a]) to [h]), [j]), [k]), [m]) to [p]), [s]) to [u]) These assignments can be interchanged.

[i]) Not detected.

[l]) 170.98 (*s*, CH_3COO); 21.39 (*q*, CH_3COO).

[q]) Submerged by the signals of impurities.

[r]) 170.98 (*s*, CH_3COO); 21.34 (*q*, CH_3COO).

Table 3. *[Eu(fod)$_3$]-Induced ^{1}H-NMR Shifts for Agnatasterone A ((+)-1) in $CDCl_3$*[a])

	OH	H_α–C(12)	H_α–C(11)	H_β–C(11)	CH_3(18)	H_α–C(14)	CH_3CO	H–C(7)	CH_3(19)
Δ_X/Δ_{OH}[b])	1.00	0.33	0.31	0.30	0.18	0.11	0.06	0.05	0.04

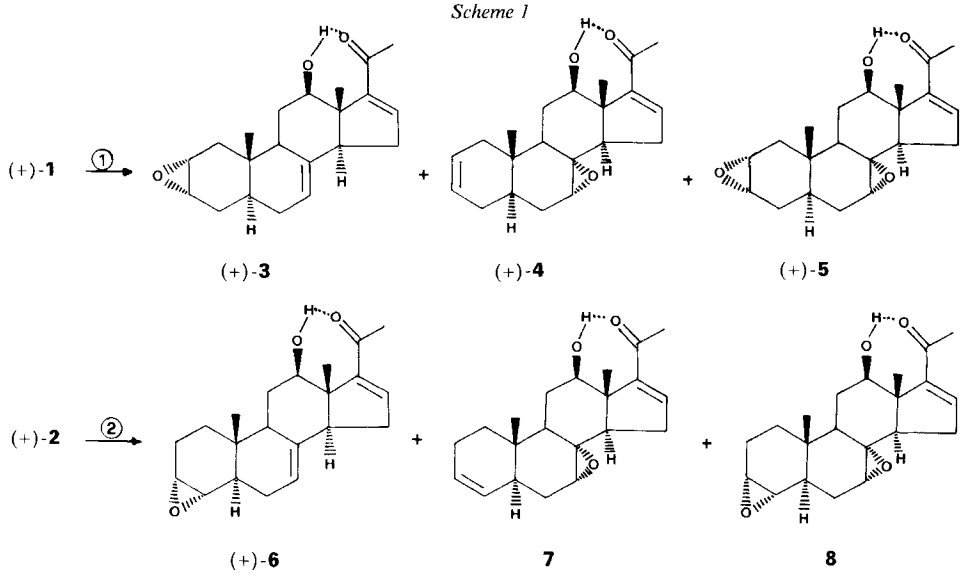
[a]) The shifts were observed for repeated additions of 0.01 ml of a 0.05M solution of [Eu(fod)$_3$] to 0.5 ml of a 0.016M solution of (+)-**1**.

[b]) Ratio of the shifts for the given proton X with respect to the shift of OH for the same amount of chemical-shift reagent.

peracid attack, for short reaction periods so as to minimize diepoxidation, to give the two monoepoxides (+)-**3** and (+)-**4** and the diepoxide (+)-**5** *(Scheme 1)*. It should be noticed that the ^{1}H-NMR spectra of the epoxysterones are simpler than those for the starting sterones owing to the absence of allylic and homoallylic couplings[2]).

Similarly, (+)-**2** undergoes peracid epoxidation to give the two monoepoxides (+)-**6** and **7**, and the diepoxide **8** *(Scheme 1)*[3]).

Scheme 1

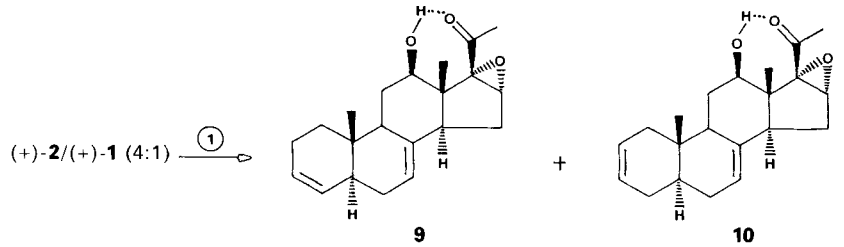


① *m*-Chloroperbenzoic acid, CH_2Cl_2, 0°, 2 h. ② *m*-Chloroperbenzoic acid, CH_2Cl_2, 0°, 1 h.

[2]) Though the small amount of the compounds at disposal did not allow us the use of heteronuclear correlation techniques, all ^{13}C-NMR resonances for (+)-**3** could be assigned *(Table 2)* by comparison with both (+)-**1** and some steroidal 2,3-epoxides of known configuration [17]. By extension of these empirical correlations, also the steroidal 7,8-epoxides (+)-**4** and (+)-**5** could be fully analyzed *(Table 2)*. In summary, taking (+)-**1** for comparison with (+)-**3** or (+)-**4**, changes in the ^{13}C-resonances only occur at rings A or B, respectively, whereas with (+)-**5** those of both ring A and B are affected *(Table 2)*. The same occurs also for the ^{1}H-resonances [18] *(Exper. Part)*. The fact that epoxidations from the α face largely predominate supports the *trans* ring junctions in (+)-**1**, the α face being sterically more accessible than the β face. As regards the regioselectivity of the epoxidation, the fact that (+)-**3** is formed in larger amounts than (+)-**4** reflects the dominance of steric over electronic factors, the C(2)=C(3) bond being less encumbered than the C(7)=C(8) bond by the angular CH_3 groups. As expected for an electrophilic reaction like a peracid epoxidation, epoxidation at the electron deficient C(16)=C(17) group was not observed.

[3]) Structural assignments and the rationalization of the stereo- (α-epoxides) and regioselectivity (predominance of (+)-**6** over **7**) for the epoxidation of (+)-**2** run in parallel with those for the reaction of (+)-**1** *(Scheme 1)*.

Scheme 2



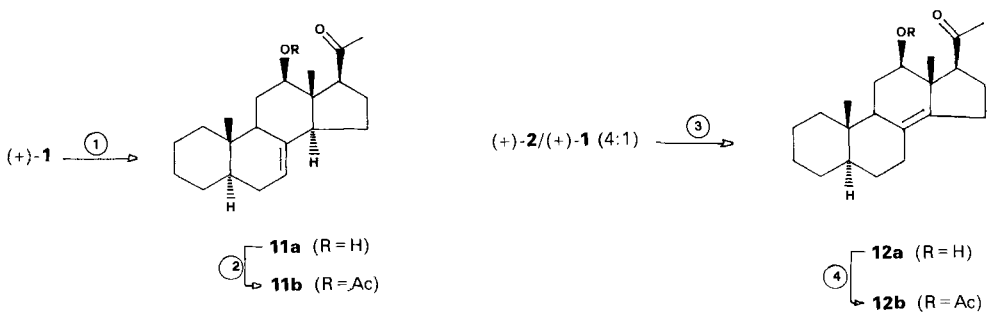
① aq. H_2O_2/NaOH, MeOH, 0°, 30 min.

Further authentication of the structures of steroids (+)-**1** and (+)-**2** can be obtained *via* epoxidation with alkaline H_2O_2. Thus, a mixture of (+)-**1** and (+)-**2** gives, as expected for a nucleophilic attack [19], only epoxidation at the C(16)=C(17) bond from the α face to give a mixture of **9** and **10** *(Scheme 2)*[4]).

It is also relevant that (+)-**1** can be selectively hydrogenated at the sterically less encumbered C(2)=C(3) and C(16)=C(17) bonds to give **11a** *(Scheme 3)*[5]). Catalytic hydrogenation of a mixture (+)-**1**/(+)-**2** for longer time intervals *(Scheme 3)* leads also to a shift of the trisubstituted C(7)=C(8) bond to the tetrasubstituted C(8)=C(14) position[6]).

The fact that neither agnatasterone A ((+)-**1**) nor B ((+)-**2**) could be acetylated is interesting and suggests strong H-bonding of the OH to the keto group [14]. This is in agreement with the appearance of the OH proton as a sharp singlet at 5.94 ppm in the ^{1}H-NMR spectrum *(Table 1)*. In accordance, molecular-mechanics calculations [20]

Scheme 3



① 5% Pt/C, AcOEt, r.t., 15 min. ② Ac_2O/pyridine, r.t., 48 h. ③ 5% Pt/C, AcOEt, r.t., 2 h. ④ Ac_2O/pyridine, r.t., 24 h.

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[4]) The facts supporting the gross structural assignments **9** and **10** are the disappearance of the enone chromophore and the modification of the ^{1}H- and ^{13}C-resonances of ring D of (+)-**1** and (+)-**2**. That epoxidations selectively occurred from the α face is evidenced by a marked shielding of the homoallylic [17] ^{13}C(14) resonance.

[5]) Structure **11a** is based on the disappearance of the enone chromophore and the preservation of the H–C(7)=C(8) ^{1}H- and ^{13}C-NMR signals of (+)-**1**. Preferential addition of H_2 at C(16)=C(17) from the α face, which is deduced from the characteristic ^{1}H-NMR pattern of H_α–C(17) [20], reflects a sterically less hindered approach to the α than the β face.

[6]) Structure **12a** rests on the absence of ^{1}H-NMR signals and on the fitting of the ^{13}C-NMR data *(Table 2)* for a C(8)=C(14) fragment of known steroids [10d].

indicate that for either (+)-**1** or (+)-**2** the lowest-energy conformation has hydroxy-to-keto group H-bonding. The strain energy has a calculated minimum for a dihedral angle C(13)–C(12)–O–H of *ca.* 38° towards the α face with a distance between the proton and the carbonyl O-atom of 1.94 Å. Such a short bond distance implies, in fact, strong H-bonding.

In contrast, when the C(16)=C(17) bond is hydrogenated (→**11a**), there is no more evidence for intramolecular H-bonding. Thus, **11a** can be acetylated to give **11b**, and molecular-mechanics calculations [20] fail to indicate any energy minimum on rotation of either the C(13)–O or the C(17)–C(20) bond.

This work is an example of thorough structural elucidation of novel, unusual steroids available in very limited amounts and without having to rely much on reference compounds. The result is the disclosure of two novel, unusual pregnanes[7]), a class of steroids which immediately suggests adrenal steroids. However, the pregnane steroids of *A. agnata* lack both hydroxylation at the 11β position and the 4-en-3-one moiety which is typical of all biologically active adrenal steroids [6]. The biogenesis and the role of these novel pregnane steroids awaits for elucidation.

We are grateful to Dr. *L. Cabioch* for the sponge identification, to Mr. *Alain Maron* for aid in collecting the sponge, to Mr. *Adriano Sterni* for recording the mass spectra, to the *Station Biologique de Roscoff* for laboratory facilities, and to both *M.P.I. (Progetti di Interesse Nazionale)* and *C.N.R.*, Roma, for financial support.

Experimental Part

1. *General.* All evaporations were carried out at reduced pressure at r.t. Yields are given on reacted starting compounds. TLC: *Merck Kieselgel 60 PF_{254}*. Flash chromatography (FC): *Merck Kieselgel 60*, 25–40 μm. Reverse-phase FC: *Merck LiChrosorb RP-18*, 15–25 μm. HPLC: *Merck LiChrosorb Si-60* (7 μm); reverse-phase HPLC: *Merck LiChrosorb RP-18* (7 μm; 25 × 1 cm column and 5 ml/min solvent flux in both cases). UV and IR spectra: *Perkin-Elmer Lambda-3* (λ_{max} in nm, ε in $dm^3mol^{-1}cm^{-1}$) and *Pye-Unicam SP3–100* (ν_{max} in cm^{-1}) spectrophotometers, resp. Polarimetric data: *JASCO-DIP-181* digital polarimeter. ^{1}H-NMR and ^{13}C-NMR spectra ($CDCl_3$): *Varian XL300* (300 or 75.43 MHz, resp.); δ (ppm) relative to internal Me_4Si (= 0 ppm) and *J* in Hz; coupling constants are derived from homonuclear differential decoupling; all chemical-shift assignments for both (+)-**1** and (+)-**2** are supported by either HETCOR [12] experiments or selective heterodecoupling. Carbon multiplicities: APT technique [22]. EI-MS (*m/z* (%)): home-built quadrupole mass spectrometer based on the *ELFS-4-162-8 Extranuclear* quadrupole [23].

2. *Isolations. A. agnata* (Topsent, 1896) was collected on September 22nd, 1984, from gill nets, where the sponge is occasionally entrapped, of professional fishermen. The nets were placed offshore Roscoff, Brittany, at 3–4 h motor-boat-sailing distance. A second collection was made in March 1986 by dredging in the Northern area of the Ile de Batz. The two collections proved identical under every respect. Fresh sponges were hand-pressed in order to remove as much H_2O as possible, then plunged in EtOH for some days, homogenized by a *Waring* blender, filtered (the residue was extracted with fresh EtOH to leave 35 g of dry sponge weight) and the filtrate evaporated. The residue from the evaporation was extracted with petroleum ether, the org. phase evaporated and the residue (0.9 g) subjected to FC (gradient elution with hexane/Et_2O) to give a mixture (+)-**1**/(+)-**2** together with fats and sterols. Further purification of this mixture of sterones from sterols was carried out by reverse-phase FC (gradient elution with MeOH/H_2O). Finally, repeated reverse-phase HPLC with CH_3CN/H_2O 72:28 lead to complete separation of the two sterones with t_R 16.3 and 17.0 min for (+)-**1** (0.009 g, 0.026%) and (+)-**2** (0.012 g, 0.035% of dry sponge weight, resp.).

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[7]) In particular, pregnane steroids have never been found before in Axinellida sponges where *A. agnata* is traditionally classified. However, it is interesting to this concern that in some quarters [21], according to spicule examination, *A. agnata* does not appear as a typical Axinellida. We thank Dr. *L. Cabioch* for bringing this point to our attention.

3. *Agnatasterone A* (= *(+)-12β-Hydroxy-5α-pregna-2,7,16-trien-20-one*; (+)-**1**). Crystals. M.p. 123–4° (hexane). $[\alpha]^{20}$ = +63.5°, +67.8°, and +122.5° at 589, 577, and 435 nm, resp. (*c* = 0.6, $CHCl_3$). UV ($CHCl_3$): 240.0 (5300). IR (nujol): 3450*s*, 1645*vs*, 1580*m*. MS: 312 (12, $M^{+\cdot}$), 297 (2, M^+ – Me), 294 (45, $M^{+\cdot}$–H_2O), 292 (44, 294 – 2H), 279 (100, 297 – H_2O), 277 (46, 279 – 2H), 105 (21), 91 (21), 79 (15).

4. *Epoxidation of (+)-***1**. To 0.005 g (0.016 mmol) of (+)-**1** in 1 ml of dry CH_2Cl_2 at 0° were added 0.0036 g of 85% *m*-chloroperbenzoic acid. Before that (+)-**1** had completely disappeared, in order to minimize diepoxidation, the mixture was quenched with 0.5 ml of sat. aq. $Na_2S_2O_4$ soln. and 0.5 ml of 5% aq. $NaHCO_3$ soln. The resulting heterogeneous mixture was passed through a *Whatman* phase-separation filter, the filtrate evaporated, and the residue subjected to HPLC with hexane/AcOEt 3:2 under a flow program from 5.0 to 7.0 ml/min. At t_R 6.0, 9.6, 14.3, and 23.5 min were obtained unreacted (+)-**1** (0.0005 g), (+)-**4** (0.001 g, 21%), (+)-**3** (0.002 g, 42%), and (+)-**5** (0.0014 g, 28%), resp. Presumable products of epoxidation from the β face were only detectable in trace amounts.

(+)-2α,3α-Epoxy-12β-hydroxy-5α-pregna-7,16-dien-20-one ((+)-**3**). $[\alpha]_D^{20}$ = +66.6° (*c* = 0.05, $CHCl_3$). IR (film): 3450*s*, 1650*s*, 1585*m*. ^{1}H-NMR: 2.05 (*m*, H_β–C(1)); 1.60 (*m*, H_α–C(1)); 3.12 (*dd*, $J(2\beta,3\beta)$ = 3.9, $J(2\beta,1\beta)$ = 5.5, H_β–C(2)); 3.17 (*dd*, $J(3\beta,2\beta)$ = 3.9, $J(3\beta,4\alpha)$ = 2.0, H_β–C(3)); 2.05 (*m*, H_α–C(4)); 1.60 (*m*, H_β–C(4)); 1.58 (*m*, H–C(5)); 1.85 (*m*, H_α–C(6)); 1.70 (*m*, H_β–C(6)); 5.29 (*m*, $w_{½}$ = 9.6, H–C(7)); 1.90 (*m*, H–C(9)); 1.92 (*ddd*, J_{gem} = 12.9, $J(11\alpha,9)$ = 6.1, $J(11\alpha,12\alpha)$ = 4.0, H_α–C(11)); 1.51 (*ddd*, J_{gem} = $J(11\beta,9)$ = 12.9, $J(11\beta,12\alpha)$ = 10.3, H_β–C(11)); 3.76 (*dd*, $J(12\alpha,11\beta)$ = 10.3, $J(12\alpha,11\alpha)$ = 4.0, H_α–C(12)); 2.18 (br. *dd*, $J(14,15\beta)$ = 11.6, $J(14,15\alpha)$ = 6.8, H–C(14)); 2.37 (*ddd*, J_{gem} = 17.8, $J(15\alpha,14)$ = 6.8, $J(15\alpha,16)$ = 3.0, H_α–C(15)); 2.45 (*ddd*, J_{gem} = 17.8, $J(15\beta,14)$ = 11.6, $J(15\beta,16)$ = 2.1, H_β–C(15)); 6.98 (*dd*, $J(16,15\alpha)$ = 3.0, $J(16,15\beta)$ = 2.1, H–C(16)); 0.740 (*s*, 3H–C(18)); 0.805 (br. *s*, 3H–C(19)); 2.37 (*s*, 3H–C(21)); 5.97 (*s*, OH). MS: 328 (38, $M^{+\cdot}$), 313 (14, M^+ – Me), 310 (41, M^+ – H_2O), 295 (100, 313 – H_2O), 157 (15), 91 (26), 43 (61).

(+)-7α,8α-Epoxy-12β-hydroxy-5α-pregna-2,16-dien-20-one ((+)-**4**). $[\alpha]_D^{20}$ = +15.0° (*c* = 0.14, $CHCl_3$). ^{1}H-NMR: 5.58 (*m*, H–C(2), H–C(3)); 3.39 (*m*, $w_{½}$ = 5, H–C(7)); 2.07 (*ddd*, J_{gem} = 12.6, $J(11\alpha,9)$ = $J(11\alpha,12\alpha)$ = 5.7, H_α–C(11)); 1.78 (*ddd*, J_{gem} = $J(11\beta,9)$ = 12.6, $J(11\beta,12\alpha)$ = 10.2, H_β–C(11)); 3.83 (*dd*, $J(12\alpha,11\beta)$ = 10.2, $J(12\alpha,11\alpha)$ = 5.7, H_α–C(12)); 2.27 (br. *dd*, $J(14,15\beta)$ = 10.2, $J(14,15\alpha)$ = 5.0, H–C(14)); 2.36 (*ddd*, J_{gem} = 14.5, $J(15\alpha,14)$ = 5.0, $J(15\alpha,16)$ = 2.9, H_α–C(15)); 2.14 (*ddd*, J_{gem} = 14.5, $J(15\beta,14)$ = 10.2, $J(15\beta,16)$ = 2.2, H_β–C(15)); 6.95 (*dd*, $J(16,15\alpha)$ = 2.9, $J(16,15\beta)$ = 2.2, H–C(16)); 0.974 (*s*, 3H–C(18)); 0.839 (br. *s*, 3H–C(19)); 2.37 (*s*, 3H–C(21)); 5.98 (*s*, OH). MS: 328 (3, $M^{+\cdot}$), 310 (64, $M^{+\cdot}$ – H_2O), 295 (71, 310 – Me), 277 (24), 267 (36), 216 (90), 201 (18), 43 (100).

(+)-2α,3α:7α,8α-Diepoxy-12β-hydroxy-5α-pregna-16-en-20-one ((+)-**5**). $[\alpha]_D^{20}$ = +19.5° (*c* = 0.15, $CHCl_3$). ^{1}H-NMR: 2.03 (*m*, H_β–C(1)); 1.57 (*m*, H_α–C(1)); 3.05 (*dd*, $J(2\beta,1\beta)$ = 5.5, $J(2\beta,3\beta)$ = 4.0, H_β–C(2)); 3.14 (br. *d*, $J(3\beta,2\beta)$ = 4.0, H_β–C(3)); 1.95 (br. *dd*, J_{gem} = 15.3, $J(4\alpha,3\beta)$ = 5.5, H_α–C(4)); 1.55 (br. *dd*, J_{gem} = 15.3, $J(4\beta,5\alpha)$ = 11.2, H_β–C(4)); 1.83 (*ddd*, J_{gem} = 15.1, $J(6\alpha,7)$ = 4.0, $J(6\alpha,5\alpha)$ = 3.5, H_α–C(6)); 1.52 (*m*, H_β–C(6)); 3.34 (*m*, $w_{½}$ = 4.9, H–C(7)); 2.05 (*ddd*, J_{gem} = 12.2, $J(11\alpha,9)$ = 5.8, $J(11\alpha,12\alpha)$ = 4.7, H_α–C(11)); 1.71 (*ddd*, J_{gem} = $J(11\beta,9)$ = 12.2, $J(11\beta,12\alpha)$ = 10.6, H_β–C(11)); 3.81 (*dd*, $J(12\alpha,11\beta)$ = 10.6, $J(12\alpha,11\alpha)$ = 4.7, H_α–C(12)); 2.26 (*dd*, $J(14,15\alpha)$ = 5.7, $J(14,15\beta)$ = 10.6, H–C(14)); 2.34 (*ddd*, J_{gem} = 15.2, $J(15\alpha,14)$ = 5.7, $J(15\alpha,16)$ = 3.2, H_α–C(15)); 2.15 (*ddd*, J_{gem} = 15.2, $J(15\beta,14)$ = 10.6, $J(15\beta,16)$ = 2.0, H_β–C(15)); 6.93 (*dd*, $J(16,15\alpha)$ = 3.2, $J(16,15\beta)$ = 2.0, H–C(16)); 0.931 (*s*, 3H–C(18)); 0.839 (br. *s*, 3H–C(19)); 2.36 (*s*, 3H–C(21)); 5.99 (*s*, OH). MS: 344 (2, $M^{+\cdot}$), 326 (39, $M^{+\cdot}$ – H_2O), 310 (32), 308 (18), 295 (10), 293 (10), 283 (22), 216 (100), 201 (23), 43 (62).

5. *Agnatasterone B* (= *(+)-12β-Hydroxy-5α-pregna-3,7,16-trien-20-one* ((+)-**2**). Crystals. M.p. 104–5° (hexane). $[\alpha]^{20}$ = +17.6°, +18.8°, and +17.0° at 589, 577, and 435 nm, resp. (*c* = 0.45, $CHCl_3$). UV ($CHCl_3$): 240.0 (5100). IR (nujol): 3450*s*, 1650*vs*, 1580*m*. MS: 312 (9, $M^{+\cdot}$), 297 (1, M^+ – Me), 294 (52, $M^{+\cdot}$ – H_2O), 292 (41, 294 – 2H), 279 (100, 297 – H_2O), 277 (53), 171 (29), 128 (20), 79 (20).

6. *Peracid Epoxidation of (+)-***2**. Following the procedure for (+)-**1** *(Exper. 4)* with 0.008 g (0.026 mmol) of (+)-**2**, the mixture was quenched after 1 h leading to unreacted (+)-**2** (0.002 g), **7** (0.0018 g, 30%), (+)-**6** (0.0028 g, 45%), and **8** (0.0007 g, 11%) at t_R 6.8, 9.2, 11.1, and 21.5 min, resp.

3α,4α-Epoxy-12β-hydroxy-5α-pregna-7,16-dien-20-one ((+)-**6**). $[\alpha]_D^{20}$ = +23.5° (*c* = 0.10, $CHCl_3$). ^{1}H-NMR: 1.47 (*ddd*, J_{gem} = 12.9, $J(1\beta,2\alpha) \approx J(1\beta,2\beta)$ = 5.9, H_β–C(1)); 1.04 (*ddd*, J_{gem} = $J(1\alpha,2\beta)$ = 12.9, $J(1\alpha,2\alpha)$ = 5.7, H_α–C(1)); 2.04 (*m*, H_α–C(2)); 1.80 (*dddd*, J_{gem} = 15.2, $J(2\beta,1\alpha)$ = 12.9, $J(2\beta,1\beta)$ = 5.9, $J(2\beta,3\beta)$ = 2.3, H_β–C(2)); 3.19 (*m*, $w_{1/2}$ = 7.6, H_β–C(3)); 2.73 (*d*, $J(4\beta,3\beta)$ = 3.8, H_β–C(4)); 1.62 (*dd*, $J(5\alpha,6\beta)$ = 12.1, $J(5\alpha,6\alpha)$ = 5.2, H_α–C(5)); 2.12 (br. *dd*, J_{gem} = 17.0, $J(6\alpha,5\alpha)$ = 5.5, H_α–C(6)); 2.00 (*m*, H_β–C(6)); 5.39 (*m*, $w_{1/2}$ = 9.7, H–C(7)); 1.93 (*m*, H–C(9)); 1.94 (*ddd*, J_{gem} = 12.9, $J(11\alpha,9)$ = 5.9, $J(11\alpha,12\alpha)$ = 5.1, H_α–C(11)); 1.38 (*ddd*, J_{gem} = 12.9, $J(11\beta,9)$ = 12.7, $J(11\beta,12\alpha)$ = 10.7, H_β–C(11)); 3.75 (*dd*, $J(12\alpha,11\alpha)$ = 5.1, $J(12\alpha,11\beta)$ = 10.7, H_α–C(12)); 2.22 (br. *dd*, $J(14,15\beta)$ = 11.8, $J(14,15\alpha)$ = 6.5, H–C(14)); 2.46 (*ddd*, J_{gem} = 17.6, $J(15\beta,14)$ = 11.8, $J(15\beta,16)$ = 2.2,

H_β–C(15)); 2.38 (*ddd*, J_{gem} = 17.6, J(15α,14) = 6.5, J(15α,16) = 3.0, H_α–C(15)); 6.99 (*dd*, J(16,15α) = 3.0, J(16,15β) = 2.2, H–C(16)); 0.749 (*s*, 3 H–C(18)); 0.776 (br. *s*, 3 H–C(19)); 2.37 (*s*, 3 H–C(21)); 5.90 (*s*, OH). MS: 328 (100, $M^{+\cdot}$), 313 (10, $M^{+\cdot}$ – Me), 310 (65, $M^{+\cdot} - H_2O$), 295 (77, 310 – Me), 91 (21), 84 (22), 43 (84).

7α,8α-Epoxy-12β-hydroxy-5α-pregna-3,16-dien-20-one (**7**). ^{1}H-NMR: 1.70 (*m*, H_β–C(1)); 1.20 (partially submerged by Et_2O as an impurity, H_α–C(1)); 1.96 (*m*, H_α–C(2)); 1.71 (*m*, H_β–C(2)); 5.60 (br. *d*, J(3,4) = 10.0, H–C(3)); 5.27 (*dd*, J(4,3) = 10.0, J(4,2α) = 1.6, H–C(4)); 2.12 (*m*, H–C(5)); 1.86 (*ddd*, J_{gem} = 14.8, J(6α,7β) ≈ J(6α,5α) = 3.6, H_α–C(6)); 1.60 (*m*, H_β–C(6)); 3.44 (*m*, $w_{1/2}$ = 5.0, H_β–C(7)); 1.55 (*m*, H–C(9)); 2.18 (*ddd*, J_{gem} = 14.0, J(11α,12α) ≈ J(11α,9) = 5.6, H_α–C(11)); 1.70 (*ddd*, J_{gem} = 14.0, J(11β,9) = 13.5, J(11β,12α) = 10.5, H_β–C(11)); 3.82 (*dd*, J(12α,11β) = 10.5, J(12α,11α) = 5.6, H_α–C(12)); 2.28 (*m*, H–C(14)); 2.36 (*ddd*, J_{gem} = 14.5, J(15α,14) = 5.0, J(15α,16) = 2.9, H_α–C(15)); 2.17 (*ddd*, J_{gem} = 14.5, J(15β,14) = 10.2, J(15β,16) = 2.4, H_β–C(15)); 6.95 (*dd*, J(16,15α) = 2.9, J(16,15β) = 2.4, H–C(16)); 0.989 (*s*, 3 H–C(18)); 0.853 (br. *s*, 3 H–C(19)); 2.36 (*s*, 3 H–C(21)); 5.97 (*s*, OH). MS: 328 (25, $M^{+\cdot}$), 313 (2, $M^{+\cdot}$ – Me), 310 (50, $M^{+\cdot} - H_2O$), 295 (30, 310 – Me), 292 (18), 277 (31), 267 (28), 251 (17), 216 (62), 201 (15), 123 (27), 108 (34), 91 (31), 43 (100).

3α,4α:7α,8α-Diepoxy-12β-hydroxy-5α-pregna-16-en-20-one (**8**). ^{1}H-NMR: 1.38 (*m*, H_β–C(1)); 1.02 (*ddd*, J_{gem} = J(1α,2β) = 13.0, J(1α,2α) = 5.6, H_α–C(1)); 1.98 (*dd*, J_{gem} = 14.0, J(2α,1β) = 5.6, H_α–C(2)); 1.38 (*m*, H_β–C(2)); 3.15 (*m*, $w_{1/2}$ = 8.5, H_β–C(3)); 2.70 (br. *d*, J(4β,3β) = 3.9, H_β–C(4)); 2.11 (*ddd*, J_{gem} = 13.8, J(6α,7β) = 4.4, J(6α,5α) = 4.0, H_α–C(6)); 1.78 (*m*, H_β–C(6)); 3.43 (*m*, $w_{1/2}$ = 4.9, H_β–C(7)); 2.09 (br. *dd*, J_{gem} = 13.4, J(11α,12α) = 5.8, H_α–C(11)); 1.54 (*ddd*, J_{gem} = J(11β,9) = 13.4, J(11β,12α) = 10.6, H_β–C(11)); 3.80 (*dd*, J(12α,11β) = 10.6, J(12α,11α) = 5.8, H_α–C(12)); 2.27 (*dd*, J(14,15β) = 10.8, J(14,15α) = 5.3, H–C(14)); 2.37 (*ddd*, J_{gem} = 15.1, J(15α,14) = 5.3, J(15α,16) = 2.9, H_α–C(15)); 2.17 (*ddd*, J_{gem} = 15.1, J(15β,14) = 10.8, J(15β,16) = 2.0, H_β–C(15)); 6.94 (*dd*, J(16,15α) = 2.9, J(16,15β) = 2.0, H–C(16)); 0.959 (*s*, 3 H–C(18)); 0.826 (br. *s*, 3 H–C(19)); 2.364 (*s*, 3 H–C(21)); 5.99 (*s*, OH).

7. *Epoxidation of a 4:1 Mixture (+)-***2**/(+)-**1** *with H_2O_2 in Alkali.* To 0.007 g (0.2 mmol) of (+)-**2**/(+)-**1** (4:1) in MeOH at 0° were added 50 µl of 30% H_2O_2 soln. and 15 µl of 10% aq. NaOH soln. Starting sterones disappeared after 30 min, and the mixture was quenched with sat. aq. $NaHCO_3$ soln. and passed through a phase-separation filter. Evaporation and TLC of the residue with hexane/AcOEt 7:3 led to **9**/**10** (4:1; 0.0044 g, 60%) which was separated by reverse-phase HPLC with CH_3CN/H_2O 75:25 (**10**, 0.0007 g, t_R 11.0 min; **9**, 0.003 g, t_R 11.7 min). A less polar product (0.001 g) was also obtained from the above TLC. NMR data are consistent with either a 2- or 3-unsaturated acetal derived from intramolecular addition of the OH O-atom to the C=O group.

16α,17α-Epoxy-12β-hydroxy-5α-pregna-3,7-dien-20-one (**9**). ^{1}H-NMR: 5.63 (*dd*, J(3,4) = 9.9, J(3,2α) = 2.5, H–C(3)); 5.32 (*dd*, J(4,3) = 9.9, J(4,2α) = 1.8, H–C(4)); 5.24 (*m*, $w_{1/2}$ = 8.0, H–C(7)); 2.00 (*ddd*, J_{gem} = 12.8, J(11α,12α) ≈ J(11α,9) = 5.1, H_α–C(11)); 1.43 (*ddd*, J_{gem} = J(11β,9) = 12.8, J(11β,12α) = 10.9, H_β–C(11)); 3.82 (*dd*, J(12α,11β) = 10.9, J(12α,11α) = 5.1, H_α–C(12)); 3.80 (*m*, $w_{1/2}$ = 3.0, H_β–C(16)); 0.835 (*s*, 3 H–C(18)); 0.766 (br. *s*, 3 H–C(19)); 2.12 (*s*, 3 H–C(21)); 4.50 (*s*, OH).

16α,17α-Epoxy-12β-hydroxy-5α-pregna-2,7-dien-20-one (**10**). ^{1}H-NMR: 5.58 (*m*, H–C(2), H–C(3)); 5.12 (*m*, $w_{1/2}$ = 9.5, H–C(7)); 3.84 (*dd*, J(12α,11β) = 10.6, J(12α,11α) = 4.9, H_α–C(12)); 3.80 (*m*, $w_{1/2}$ = 3.0, H_β–C(16)); 4.50 (*s*, OH); 0.814 (*s*, 3 H–C(18)); 0.835 (br. *s*, 3 H–C(19)); 2.11 (*s*, 3 H–C(21)); 4.50 (*s*, OH).

Mixture **9**/**10** *(4:1).* MS: 310 (41, $M^{+\cdot} - H_2O$), 295 (61, 310 – Me), 285 (28, $M^{+\cdot}$ – COMe), 279 (34), 277 (5), 267 (91), 107 (13), 105 (14), 91 (29), 84 (25), 43 (64).

8. *Catalytic Reduction of (+)-***1**. Compound (+)-**1** (0.004 g, 0.013 mmol) in 1 ml of AcOEt and 5% Pt/C were hydrogenated at r.t. and 1 atm for 15 min. On standard workup, a 4:1 mixture **11a**/**12a** was isolated in 93% yield and treated with Ac_2O in pyridine at r.t. for 48 h. Standard workup led to a residue which was subjected to HPLC with hexane/i-PrOH 97:3. Only the major isomer, **11b**, could thus be isolated at t_R 8.5 min (0.0023 g, 55%).

12β-Hydroxy-5α-pregna-7-en-20-one (**11a**; from the 4:1 mixture with **12a**). ^{1}H-NMR: 5.23 (*m*, $w_{1/2}$ = 8.0, H–C(7)); 3.54 (*dd*, J(12α,11β) = 11.4, J(12α,11α) = 4.4, H_α–C(12)); 2.55 (*dd*, J(17α,16α) = J(17α,16β) = 8.8, H_α–C(17)); 0.574 (br. *s*, 3 H–C(18)); 0.763 (br. *s*, 3 H–C(19)); 2.21 (*s*, 3 H–C(21)); the OH signal was submerged. MS: 298 (7, $M^{+\cdot} - H_2O$), 283 (57, 298 – Me), 255 (56, 298 – COMe), 109 (56), 97 (97), 95 (79), 91 (46), 85 (74), 83 (66), 81 (58), 71 (91), 69 (84), 67 (47), 57 (100), 55 (66), 43 (94), 41 (73).

12β-Acetoxy-5α-pregna-7-en-20-one (**11b**). ^{1}H-NMR: 5.25 (*m*, $w_{1/2}$ = 10.0, H–C(7)); 1.85 (*ddd*, J_{gem} = 14.8, J(11α,12α) = 4.8, J(11α,9) = 5.8, H_α–C(11)); 1.41 (*ddd*, J_{gem} = J(11β,9) = 14.8, J(11β,12α) = 11.5, H_β–C(11)); 4.84 (*dd*, J(12α,11β) = 11.5, J(12α,11α) = 4.8, H_α–C(12)); 2.79 (*dd*, J(17α,16β) = J(17α,16α) = 9.3, H_α–C(17)); 0.700 (br. *s*, 3 H–C(18)); 0.763 (br. *s*, 3 H–C(19)); 2.08 (*s*, 3 H–C(21)); 2.00 (*s*, Ac). MS: 298 (30, $M^{+\cdot}$ – AcOH), 283 (52, 298 – Me), 255 (100, 298 – COMe), 159 (29), 145 (22), 109 (21), 95 (19), 91 (21), 55 (33), 43 (51).

9. *Catalytic Reduction of a 4:1 Mixture (+)-***2**/(+)-**1**. The title mixture (0.003 g, 0.0096 mmol) was hydrogenated, quenched after 2 h, and worked up as in *Exper. 8* whereby **12a** (0.0026 g, 86%) of purity over 95% was

isolated. Acetylation for 24 h and similar workup as in *Exper. 8* above led to **12b** which was purified by reverse-phase HPLC with CH_3CN/H_2O 75:25, t_R 13.5 min (0.0015 g, 51%).

12β-Hydroxy-5α-pregna-8(14)-en-20-one (**12a**). ^{1}H-NMR: 1.78 (br. *dd*, J_{gem} = 12.4, $J(11\alpha,12\alpha)$ = 4.5, H_α–C(11)); 1.40 (*ddd*, $J_{gem} = J(11\beta,9)$ = 12.4, $J(11\beta,12\alpha)$ = 12.1, H_β–C(11)); 3.47 (*dd*, $J(12\alpha,11\beta)$ = 12.2, $J(12\alpha,11\alpha)$ = 3.4, H_α–C(12)); 0.646, 0.873 (2 br. *s*, 3 H–C(18), 3 H–C(19)); 2.24 (*s*, 3 H–C(21)); 4.93 (br. *s*, OH).

12β-Acetoxy-5α-pregna-8(14)-en-20-one (**12b**). ^{1}H-NMR: 4.76 (*dd*, $J(12\alpha,11\beta)$ = 12.1, $J(12\alpha,11\alpha)$ = 4.5, H_α–C(12)); 2.59 (*dd*, $J(17\alpha,16\beta)$ = 11.8, $J(17\alpha,16\alpha)$ = 7.1, H_α–C(17)); 0.667, 0.848 (2 br. *s*, 3 H–C(18), 3 H–C(19)); 2.083 (*s*, 3 H–C(21)); 2.005 (*s*, Ac). MS: 315 (100, M^+ – COMe), 283 (2), 255 (3).

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