Acid-catalysed Dehydration of Withanolide E, a 14α,17β,20α$_F$-Trihydroxysteroid; a Revision

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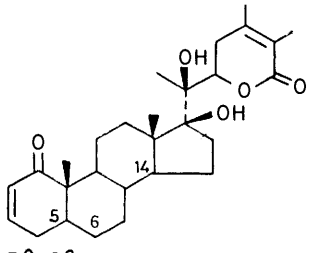
The structure of the products obtained by treatment of (17*S*,20*S*,22*R*)-5β,6β-epoxy-14α,17,20-trihydroxy-1-oxowitha-2,24-dienolide (withanolide E) with sulphuric acid in acetone solution has been reinvestigated. Whereas the major component is indeed (17*S*,20*S*,22*R*)-5α,6β,17,20-tetrahydroxy-1-oxowitha-2,14,24-trienolide, the minor component which was previously considered to be the $\Delta^{8(11)}$ isomer of the latter, has now been shown to be (17*S*,22*R*),14α,20ξ-epoxy-5α,6β,17-trihydroxy-1-oxowitha-2,24-dienclide. The configuration at C-20 is most probably 20*R*.

PREVIOUSLY [1] we reported the chemical work done for the characterization of (17*S*,20*S*,22*R*)-5β,6β-epoxy-14α,-17,20-trihydroxy-1-oxowitha-2,24-dienolide (withanolide E) (1). Treatment of the 2,3-dihydro-derivative of (1) with acetone containing a trace of 8N-aqueous sulphuric acid for 1 h at −10 °C induced the smooth elimination of the 14α-hydroxy-group to give (17*S*,-20*S*,22*R*)-5β,6β-epoxy-17,20-dihydroxy-1-oxowitha-14,24-dienolide (2) (83% yield) and a minor isomeric compound (3% yield) which was considered as the $\Delta^{8(14)}$ isomer of (2). Treatment of withanolide E (1) with acetone containing a larger amount of 8N-aqueous sulphuric acid for 4 h at room temperature [1] took place with concomitant opening of the epoxide ring to give a mixture of (17*S*,20*S*,22*R*)-5α,6β,17,20-tetrahydroxy-1-oxowitha-2,14,24-trienolide (3a) (75% yield) and an isomeric compound (20% yield) which was considered as the $\Delta^{8(14)}$ isomer of (3a). The ratio between these two compounds changed to 3 : 7 by performing the reaction with 98% sulphuric acid in acetone solution. The same results were obtained with withanolide S (4a).

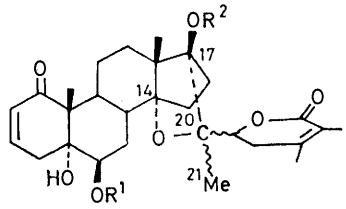
During a ^{13}C n.m.r. investigation of withanolides and related compounds [2] we became aware that in contrast to (3a), which possesses three carbon–carbon double bonds [in (3a) carbons 14 and 15 resonate at 153.2 and 113.8 p.p.m., respectively], the isomeric companion (5a) has only two such bonds (Δ^2 and Δ^{24}). We now present evidence that its structure is (17*S*,22*R*)-14α,20ε-epoxy-5α,6β,17-trihydroxy-1-oxowitha-2,24-dienolide (5a); the configuration at C-20 is most probably 20*R*.

Acetylation of (5a) with acetic anhydride in pyridine, overnight at room temperature, resulted in the 6-monoacetate (5b), whereas at higher temperature increasing amounts of the 6,17-diacetate (5c) were obtained. Treatment of the latter with thionyl chloride in pyridine afforded (17*S*,22*R*)-6β,17-diacetoxy-14α,20ξ-epoxy-1-oxowitha-2,4,24-trienolide (6), thus confirming that the 17-tertiary hydroxy-group was acetylated under forcing conditions. The presence of three tertiary hydroxy-groups in (3b) and of only two such groups in (5b) was confirmed by treatment (*in situ*, in the n.m.r. tube) with trichloroacetyl isocyanate: the former gave a tris(trichloroacetylcarbamate) (δ 8.37, 8.53 and 8.85 for NH), whereas the latter afforded a bis(trichloroacetylcarbamate) (δ 8.44 and 9.38 for NH). A monocarbamate derivative was obtained from the 6,17-diacetate (5c) (δ 8.53).

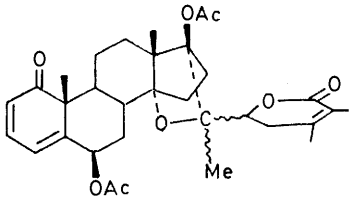
The assignment of the resonance signals of C-, CH-,



(1) 5β, 6β - epoxy, 14α - OH (Withanolide E)
(2) 5β, 6β - epoxy, Δ^{14}
(3a) 5α-OH, 6 β-OH, Δ^{14}
(3b) 5α-OH, 6 β-OAc, Δ^{14}
(4a) 5α-OH, 6 β-OH, 14 α-OH (Withanolide S),



(5) a; R^1 = R^2 = H
b; R^1 = Ac, R^2 = H
c; R^1 = R^2 = Ac



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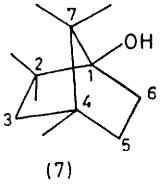


(7)

CH_2-, or CH_3-type carbons necessitated a full analysis of the ^{13}C n.m.r. spectra of compounds (5a—c) (Table 1) and could be accomplished, in part, on the basis of multiplicities in the single-frequency off-resonance

TABLE 1

^{13}C N.m.r. data

Carbon	(5a) [a]	(5b)	(5c)	Carbon	(5a) [a]	(5b)	(5c)
1	205.3	203.6	203.4	16	33.0 [c]	31.9 [c]	32.9 [c]
2	128.0	128.5	128.3	17	88.0 [b]	88.5 [b]	92.8
3	143.3	141.3	141.5	18	16.7	16.8	17.3
4	35.2	34.9	34.8	19	15.8	15.3	15.4
5	77.7	76.0	75.9	20	79.2	79.0	79.7
6	73.8	75.4	75.4	21	21.4	22.5	22.3
7	27.2	24.5	24.4 [b]	22	80.0	80.5	77.6
8	33.5	34.2	33.8	23	29.9	30.0	29.7
9	34.7	34.6	34.4	24	152.0	151.1	148.8
10	51.9	51.7	51.6	25	121.2	121.3	122.1
11	24.7	24.5	24.5 [b]	26	167.1	165.8	165.6
12	28.8	28.9	26.2	27	12.3	12.4	12.3
13	50.0	49.9	52.1	28	20.4	20.6	20.6
14	87.9 [b]	87.3 [b]	85.3	CH_3CO_2		21.4	21.4, 21.7
15	30.6 [c]	30.4 [c]	31.2 [c]	CH_3CO_2		169.9	169.7, 170.5

^{13}C N.m.r. spectra were recorded in $CDCl_3$ solutions at 22.63 MHz on a Bruker WH-90 spectrometer operating in the Fourier-transform mode; δ values related to internal tetramethylsilane.

[a] A small amount of MeOH was added to improve solubility. [b,c] Signals in the same column may be interchanged.

decoupled (s.f.o.r.d.) spectra. The magnitude of the residual couplings could also be related to the known ^{1}H shifts (Table 2), thus providing unambiguous assignments to the signals of the CH–O and CH_3 groups. Differences in relaxation rates were helpful in the analysis of crowded spectral regions. The magnitudes of the relaxation times of each carbon type ($C > CH_3 > CH > CH_2$) were compared by observing the linewidths in the noise-decoupled spectra of (5a) and (5b), and in the case of the less polar (5c) by an inversion–recovery experiment. The latter led also to the conclusion that C-23 relaxes slower than other methylene groups, whereas C-21 relaxes faster than other methyl groups. The slower relaxation of C-23 is probably due to the possibility of independent movement of the lactone side chain, while the faster relaxation of C-21 indicates its slower rotation, presumably due to steric hindrance.

Comparison of the ^{13}C chemical shifts of (5a—c) with those of withanolide S acetate (4b) and of the Δ^{14} derivative (3b),[2] shows that the only significant changes are for signals of carbon atoms in the region of ring D; more specifically, of the six signals in the 70—90 p.p.m. region (oxygenated carbons) three correspond by their chemical shifts and multiplicities to carbons 5, 6 and 22, while the other three belong to non-protonated carbons. Since compounds (3a) and (5a) are isomers, these data can only be accommodated by formation of an intramolecular ether bridge.

The structure assigned to compound (5a) is supported by the pyridine-induced shifts ($\Delta CDCl_3$–C_5D_5N) of

TABLE 2

^{1}H N.m.r. data

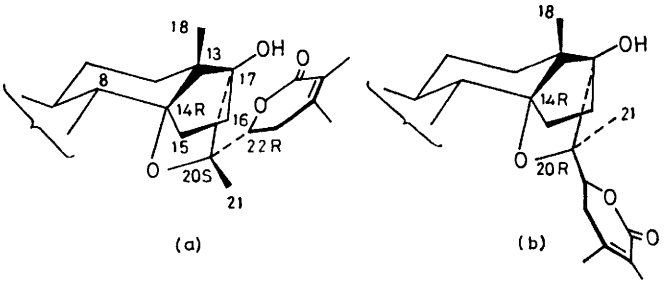
Proton	(3b)	(5b)	(5c)	(6)
2-H	5.89dd	5.84	5.85	6.05dd
	(10; 2.3)			(9.7; 0.9)
	[6.08]	[6.06]	[6.07]	
3-H	6.55ddd	6.55	6.54	6.94dd
	(10; 5; 2)			(9.7; 6.1)
	[6.55]	[6.57]	[6.56]	
4-H				6.32dd
				(6.1; 0.9)
6-H	4.90t	4.86	4.88	5.54t
	(2.5)			($W_{\frac{1}{2}}$ 5.5)
	[5.32]	[5.32]	[5.33]	
15-H	5.17t			
	($W_{\frac{1}{2}}$ 5.5)			
	[5.21]			
22-H	4.67dd	4.96	5.05dd	4.83dd
	(12; 4.1)		(12.7; 3.1)	(12.7; 3.1)
	[5.06]	[5.47]	[*ca.* 4.96]	
18-H	1.22s	1.07	1.11	1.14
	[1.50]	[1.22]	[1.15]	
19-H	1.33s	1.25	1.26	1.29
	[1.57]	[1.41]	[1.40]	
21-H	1.31s	1.33	1.54	1.51
	[1.65]	[1.45]	[1.51]	
27- and 28-H	1.88; 1.95	1.87; 1.94	1.88; 1.91	1.88; 1.86
	[1.75; 1.94]	[1.54; 1.77]	[1.71; 1.75]	
CH_3CO	2.12	2.11	2.102; 2.097	2.04
	[2.14]	[2.17]	[2.17; 2.19]	

Recorded at 270 MHz on a Bruker WH-270 spectrometer; solvent $CDCl_3$; δ values; data for solutions in C_5D_5N in square brackets; coupling constants (Hz) in parentheses.

several ^1H signals in the acetates (5b) and (5c) (Table 2). In the oxabicyclo[2.2.1]heptane partial structure, the 17β-OH bridgehead substituent [compound (5b)] is almost symmetrically situated between the 18- and 21-methyl groups (at nearly *gauche* dihedral angles) and therefore, both signals are moderately deshielded in pyridine solution (−0.15 and −0.12 p.p.m. respectively). The important downfield shift (−0.51 p.p.m.) of the 22-H signal can easily be explained by assuming that in the preferred conformation the 17-OH and 22-H bonds are almost *syn*-parallel (equivalent to a 1,3-diaxial relationship). As expected, the effect is cancelled in the diacetate (5c) in which the signal of 22-H is slightly shielded (+0.09 p.p.m.). In (3b) the 18-methyl group, which is almost eclipsed by the 17β-OH, is strongly deshielded in pyridine solution (−0.28 p.p.m.). (The solvent shift of the 21-methyl group is irrelevant to this discussion, since it is subject to the influence of both 17- and 20-OH.) It is noteworthy, however, that 22-H in (3b) is strongly deshielded (−0.39 p.p.m.), an indication that the preferred conformation about the C-20–C-22 bond is similar to that found in (5b).

Formation of compounds (2), (3a), and (5a) by treatment of withanolide E (1) with acid can be rationalized by assuming selective protonation of 14-OH with subsequent formation of a 14-carbonium ion, or alternatively non-selective protonation of either 14- or 20-OH to give a 14- or a 20-carbonium ion. According to the first alternative, elimination of a proton from C-15 leads to compounds (2) and (3a), whereas internal nucleophilic attack by 20-OH leads to closure to a 14,20-ether (5a). Based on steric considerations (the accurate stereochemistry of withanolide E was determined by crystallographic analysis [3]), such an attack can take place only with retention of configuration at C-14 and therefore the configuration of the bridgehead carbons should be 14*R*,20*S*. According to the second alternative, the C-14 carbonium ion should be responsible for formation of compounds (2) and (3a), whereas closure of the oxide bridge should be brought about by attack of the 14α-OH on a C-20 carbonium ion. In this case, retention of configuration at C-20 should lead to the 14*R*,20*S* stereochemistry, the same as above, whereas rotation about the C-17–C-20 bond should facilitate back-side attack at C-20 to give the 14*R*,20*R* stereoisomer in which the 21-methyl group is *endo* with respect to the oxabicyclo[2.2.1]heptane fragment.

According to the data presented formerly, one cannot distinguish between the 20*S* and 20*R* configurations. The latter is, however, supported by the chemical shifts of the carbon atoms involved in the oxabicyclo-[2.2.1]heptane fragment, and in particular those of C-15 and -16, whose resonances are in the 30.5—33 p.p.m. region. For comparison, chemical shifts were calculated according to additivity rules [4,5] for the model compound (7). The values obtained for C-5 and -6 in this model (35.8 and 26.0 p.p.m., respectively) are significantly different from those found for C-15 and -16, the equivalent carbons in compounds (5a–c). While C-15 would be expected to be more shielded than the corresponding carbon in (7) due to a γ-interaction with C-7, the only way to explain the strong deshielding of C-16 (relative to C-6 in the model) is to assume that the strong γ-effect of the *endo*-substituent [4] is substantially



Partial structure of compound (5a); (a) 14*R*,20*S*-configuration; (b) 14*R*,20*R*-configuration

decreased. Since γ-effects are transmitted through C–H bonds, an *endo*-lactone substituent at C-20 (20*S*) [Figure (a)] would produce a γ-interaction only if the 22-H bond is pointing towards C-16. This would, however, be in disagreement with the strong negative pyridine-induced shift of the 22-H signal (see above). Conversely, an *endo*-methyl substituent at C-20 (20*R*) [Figure (b)] cannot avoid such an interaction. The small difference between the chemical shifts of C-15 and -16 [30.4 and 31.9 p.p.m. in (5b)] suggests therefore the 20*R*-configuration. This assumption is confirmed by comparing the chemical shifts of C-21 and -22 in compounds (4b) [2] and (5b); while the C-22 signal remains virtually unchanged (81.1 and 80.5 p.p.m.) due to the same number of γ interactions in both compounds, the C-21 signal in (5b) (20*R*) is deshielded by *ca.* 3 p.p.m. as compared to (4b), due to loss of a γ-interaction with C-16.

In view of the above data, the isomer of (2) [compound (7) in ref. 1] should have the same oxabicyclo[2.2.1]-heptane partial structure as compound (5a). Unfortunately, this compound was not available in sufficient amount in order to determine its ^{13}C n.m.r. spectrum.

The final proof for the configuration at C-20 will be based on a crystallographic analysis of compound (5c) which is now being performed.

EXPERIMENTAL

General details have been reported.[1]

Dehydration of Withanolide E (1) *with* 98% *Sulphuric Acid.*—98% Sulphuric acid was slowly added to a stirred solution of (1) (950 mg) in acetone. After 4 h at room temperature the solution was worked up as already described,[1] to yield (3a) (250 mg) and (5a) (685 mg). Physical constants and spectral data are as reported.[1]

Acetylation of (5a) *to the Monoacetate* (5b) *and the Diacetate* (5c).—Compound (5a) (100 mg) was acetylated with acetic anhydride (1.5 ml) and pyridine (1.5 ml) overnight at room temperature to yield (5b) only [compound (15b) in ref. 1]. When the acetylation was performed at 50 °C for 5 h, a mixture of (5b) and (5c) was obtained in the ratio 4 : 1. After 18 h at 95 °C, the *diacetate* (5c) was obtained, 80%

yield, m.p. 290—293° (decomp) (from ethanol), $[\alpha]_D$ +19.6° (*c* 0.3); *m/e* 510.3 (0.4%, $M - CH_3CO_2H$), 445.3 (12.5, $M - 125$), 385.1 (15.9, $M - 125 - CH_3CO_2H$), 367.1 (3.9, $M - 125 - CH_3CO_2H - H_2O$), 325.2 (7, $M - 125 - 2CH_3CO_2H$), 307.1 (20, $M - 125 - 2CH_3CO_2H - H_2O$), and 125.0 (22, δ-lactone ring) (Found: C, 66.9; H, 7.5. $C_{32}H_{42}O_9$ requires C, 67.3; H, 7.4%).

Dehydration of (5c) *to the Dienone* (6).—To a solution of the diacetate (5c) (50 mg) in dry pyridine (5 ml) at −12 °C (ice–salt mixture), cold freshly distilled thionyl chloride (1 ml) was slowly added. After 5 min the mixture was poured onto ice and the yellow solid which separated was filtered off, washed, and dried. Preparative t.l.c. (ethyl acetate–benzene, 4 : 1) gave the *dienone* (6) (40 mg), m.p. 270—274° (decomp.) (from ethanol), $[\alpha]_D$ −13.4° (*c* 0.3); $\lambda_{max.}$ 307 and 225 nm (ε 5 000 and 8 900);[6] $\nu_{max.}$ 1 625, 1 660, 1 708, and 1 740 cm^{-1}; *m/e* 492.2 (2.3%, $M - CH_3CO_2H$), 367.2 (6.9, $M - CH_3CO_2H - 125$), 307.1 (7, $M - 2\ CH_3CO_2H - 125$), and 125.0 (6.76). Found: C, 69.0; H, 7.5. $C_{32}H_{40}O_8$ requires C, 69.5; H, 7.3%).

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