Additivity Relationships in the Carbon-13 Nuclear Magnetic Resonance Spectra of Polyhydroxy Steroids

Masaru Kobayashi
Faculty of Pharmaceutical Sciences, Hokkaido University, Kita-ku Sapporo 060, Japan

δ_c Values for the ^{13}C NMR spectra of polyhydroxy 5α,14α-steroids are, as a result of an additivity relationship, equivalent to the sum of the δ_c values of the individual 1,2- and 1,3-glycols present in the molecule. Differences in the experimental δ_c values ($\Delta\delta^g$), reported for a variety of glycols, from those derived from addition of monohydroxy substituent effects, were converted into dihydroxy steroid parameters. Ancillary data for 8β-hydroxy steroids have also been derived. The dihydroxy steroid parameters were applied to 14 different types of known polyhydroxy steroid (**6–19**) to provide calculated δ_c values. The results indicate that, within reasonable limits, this method generally predicts δ_c values for polyhydroxy steroids which are close to those observed, except where there is severe steric hindrance present (*e.g.* as found in the 4β,6β,8β,15β-tetrahydroxylated steroid **19**).

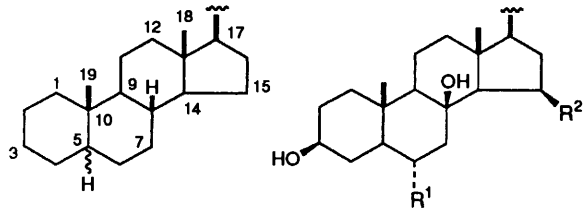
Eggert *et al.* who provided evidence for a number of ^{13}C NMR substituent effects in a variety of monohydroxy-substituted 5α,14α-steroids[1] showed that they reflected the characteristic environment for each OH group: namely, α-(OH substituted carbon), β-, γ- and δ_1-substituent effects. Subsequently VanAntwerp *et al.* examined the differences (shown as $\Delta\delta^g$ in this paper) in the observed δ_C values for a number of 1,2- and 1,3-glycols from those derived by addition of monohydroxy substituent effects.[2] The $\Delta\delta^g$s observed were rationalized in relation to the steric and electronic characteristics of each glycol type. Although the monohydroxy substituent effects were subsequently utilized for natural polyhydroxy steroids,[3] the additivity relationships in dihydroxy steroids have been little used. The $\Delta\delta^g$s derived from a limited number of glycols, which showed variations, were not parameterized for general application and simply provided δ_C values for the natural steroids carrying the same glycol group. The limited ^{13}C NMR results available for polyhydroxy 5α,14α-steroids when this earlier work was published made an extension of the additivity relationships to polyhydroxy steroids impossible.

Recently, a number of polyhydroxy 5α,14α-steroids have been isolated mainly from marine sources,[4] and in a preceding paper we tentatively applied the $\Delta\delta^g$ values to such a compound—the marine tetrahydroxy steroid **9**.[5] The $\Delta\delta^g$s due to 1α,5α-syndiaxial interaction were not recorded in the report by VanAntwerp *et al.* so that in this case the data for a 3α,5α-diol were used in place of the data for a 1α,5α-diol. The close similarity of the observed and calculated δ_C values for the α- and the β-carbon atoms suggested that the $\Delta\delta^g$ values can be utilized for general application in polyhydroxy 5α,14α-steroids. In a structural study, precise agreement of the predicted δ_C values would not be expected since introduction of a single OH group has effects even on remote carbon atoms, whilst configurational changes in the OH group cause changes in the torsional angles[6] which may affect the δ_C values. Other effects arising from the solvent used, concentration and temperature are also common. Thus, with this in mind, deviations of 2.5 ppm between the predicted and calculated δ_C values would be sufficient for adoption, or rejection, of a particular structure.

Results and Discussion

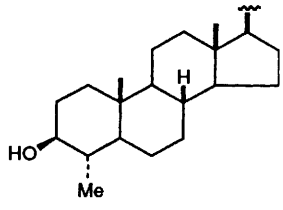
In steroidal alcohols the δ_Cs of the α-carbon and its β-carbon atoms would, upon hydroxy substitution of these positions, be expected to be affected by newly formed OH···OH and OH···C nonbonded interactions. If it is assumed that the increments due to each type of glycol involved in the molecule have linearly independent contributions, within certain limits, on the chemical shifts of the relevant carbon atoms, the δ_C values will, also within limits, reflect the sum of the additivity relationships found in individual 1,2- and 1,3-glycols. For consecutive triols, the contribution can be regarded as the sum of those of three glycol types (two 1,2-diols and one 1,3-diol). For example, C-5 of the 3β,5α,6β,7α-tetrahydroxy steroid **11** was assumed to be influenced by one 1,3-*trans* (C-3, C-5), one 1,2-*trans*-diaxial (C-5, C-6) and one 1,3-syndiaxial (C-5, C-7) interactions.

General Procedure.—The procedure involves initial addition of the monohydroxy substituent effects[1] to the δ_C values of 5α-cholestane **1**, followed by application of the dihydroxy steroid



1 5α-H
2 5β-H

3 $R^1 = R^2 = H$
4 $R^1 = R^2 = OH$



5

parameters described below. The androstane-type ring-D glycols were left for future study both because of differences in the nature of the steric interactions involved in ring D, and also because natural polyhydroxy 5α,14α-steroids are mostly 24-alkylated cholestane derivatives.[4]

Classification of Glycol Types.—In the original paper,[2] the glycols were classified into five types and are expressed in the present study as A (1,2-diequatorial), B (1,2-axial-equatorial), C

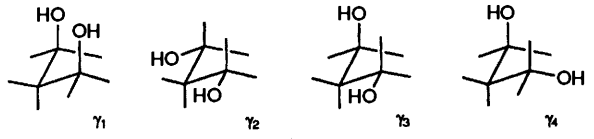
Table 1 Parameters of the δ_Cs of 1,2- and 1,3-glycols in ppm

Glycol type	α-Carbon Secondary[a]		α-Carbon Tertiary[a]		β-Carbon	
1,2-diequatorial	A	−4.1			A′	−1.2
1,2-axial–equatorial	B_{1a} (a)	−6.5				
	B_{1b}(e)	−5.5				
	B_{2a}(e)	−4.3	B_{2b}(a)	−5.0		
1,2-*trans*-diaxial	C_1	−3.5			C′	1.0
	C_{2a}	−2.0	C_{2b}	−2.0		
1,3-syndiaxial	D_1	8.0				
	D_{2a}	7.0	D_{2b}	10.0		
	D_{3a}	7.1 (C-15)	D_{3b}	4.0 (C-8)	D_3'	−4.3 (C-14)
1,3-*trans*	E_{1a}(a)	3.5			E′	0.7
	E_{1b}(e)	2.0				
	E_{2a}(e)	2.0	E_{2b} (a)	4.3		

[a] (a) = axial, (e) = equatorial.

(1,2-*trans* diaxial), D (1,3-syndiaxial) and E (1,3-*trans*) (see Table 1). The types B to E were divided into sub-type 1 (two secondary α-carbons) and sub-type 2 (secondary and tertiary α-carbons) since the glycols involving tertiary α-carbons are expected to be more resistant to skeletal deformation. The terms B_{2a} and B_{2b} in Table 1, for example, indicate 1,2-dihydroxy steroid parameters for a secondary α-carbon bearing an equatorial OH and a tertiary α-carbon bearing an axial OH, respectively (*e.g.* in a 4α,5α-diol).

For the 1,3-diols, there are 4 types (γ_1 to γ_4) of arrangement, including D- (γ_1) and E-type (γ_3) glycols. Fortunately, the γ_2



and γ_4 type glycols, which occur in the polyhydroxy steroids **7**, **8**, **16** and **17**, are found to have little influence on the additivity. The 8β,15α-glycol moiety in compound **17** resembles the γ_4 type. The predicted values (see below), using the parameters in Table 1 alone, showed sufficient agreement so that these two arrangements (γ_2 and γ_4) were decided to be unimportant for the additivity.

Dihydroxy Steroid Parameters.—The $\Delta\delta^g$s for the glycol types B_1 and C_1 have been reported for 4 glycol examples each.[2] For the A-, B_2-, and C_2-type glycols, the $\Delta\delta^g$s were from two glycols each. In contrast, for the D_1-, D_2-, E_1 and E_2-type glycols, the values derived are from only one example (D_1, 1α,3α-diol; D_2, 3α,5α-diol; E_1, 1α,3β-diol; E_2, 3β,5α-diol). For the C_2 type glycol, the $\Delta\delta^g$s were derived from the δ_Cs of 4β,5α- and 5α,6β-dihydroxy-cholestane.[7] Four $\Delta\delta^g$s (−3.7 to −4.3 ppm) have been reported for the α-carbon of A-type glycols. They were averaged (−4.1 ppm) and defined as parameter A. Similarly, the $\Delta\delta^g$s for the α-carbon of B-, C-, D- and E-type glycols were averaged and defined as the corresponding parameters (see Table 1).

The $\Delta\delta^g$s of β-carbons for various types of 1,2- and 1,3-glycols are less constant, but the magnitude is generally small except for that found for C-14 of a 8β,15β-diol (C-14, −4.3 ppm, see Table 1). They can be uniformly disregarded when the β carbon lacks hydrogen atoms which have a *gauche*-interaction with the vicinal OH group. After application to several examples, the deviations of the β-carbons were found to be significant only in the case of A-, C- and E-type glycols. The deviations at the β-carbons in A- and C-type glycols, presented as A′ and C′, were defined as −1.2 and 1.0 ppm, respectively. In the E-type 1,3-glycol, the outer and inner β-carbons, with a hydrogen atom *gauche* to the OH, showed increments of 0.6 to 1.2 ppm. The parameter E′ was defined, for simplicity and practical use, as 0.7 ppm (see Table 1).

δ_1-Hydroxy substituent effects, caused by 1,3-*syn* interaction of axial OH and angular methyl group, have been derived by Eggert *et al.*[1] A change in the γ-*trans* effect of 1α- and 5α-OH on C-19 was suggested for the 1,2-*trans* diaxial diols (1α,2β- and 5α,6β-diols) in which one hydroxy group is 1,3-syndiaxial with C-19.[2] This was also observed in the case of a 4β,5α-glycol.[7] Such deviations indicated that, for practical purposes, the complicated changes of substituent effects could be simplified in these cases (1α,2β-, 4β,5α- and 5α,6β-diols) by defining the δ_1-hydroxy substituent effect to C-19 as 1.0 ppm (see Table 2), instead of using the increments 3.8 (2β-OH), 2.4 (4β-OH) and 3.5 ppm (6β-OH), derived for monohydroxy steroids.[1]

Supplemented Hydroxy Substituent Effects.—Many polyhydroxy steroids isolated from starfish (Echinoderms) bear an 8β-OH.[4] Prediction of the δ_C values of such compounds requires the substituent effects for an 8β-OH which were not recorded in the report of Eggert *et al.*[1] Ergost-7-en-3β-ol was converted into the 7α- and 7β-epoxides. Treatment of the minor 7β-epoxide with lithium aluminium hydride (LAH) afforded 3β,8β-dihydroxyergostane **3**, the δ_C signals for the side chain of which in its ^{13}C NMR spectrum were negated by subtracting the corresponding δ_C values for ergostane. The δ_C values assigned to the steroid nucleus were confirmed by HSQC (heteronuclear single quantum coherence) and HMBC (heteronuclear multiple bond correlation) spectroscopy which showed unequivocal correlations (4β-H, C-3, C-5; 12β-H, C-9, C-11, C-13, C-14, C-17; 15-H_2, C-13, C-14; 17-H, C-12, C-13, C-18). The derived α-, β-, γ- and δ_1-substituent effects of an 8β-OH are given in Table 2. The substituent effects of 15β- and 16β-OH on cholestane,[8,9] which are slightly different from those on androstane,[1] were added. Several substituent effects of 5β-steroids were taken from the literature 10 for the calculation of 3β,5β,6α-trihydroxycholestane **15**. The pseudo-1,3-syndiaxial interaction due to the 8β,15β-diol was determined and added independently as the D_3 parameters in Table 1. The δ_C values of an 8β,15β-diol are not available, so that the values are derived from the data of the 3β,6α,8β,15β-tetrahydroxy steroid **4** (C-8, δ_C 76.6 ppm; C-14, δ_C 61.7 ppm; C-15, δ_C 70.1 ppm).[11] Subtraction of the calculated δ_C values of **4** (C-8, δ_C 68.3 ppm; C-14, δ_C 66.0 ppm; C-15, δ_C 63.0 ppm) using the monohydroxy steroid substituent effect gave the parameters −4.3 ppm for C-14 (D_3') and 7.1 ppm for C-15 (D_{3a}). The deviation found for C-8 (8.3 ppm) was regarded as the sum of D_{3b} and E_{2b} (4.3 ppm) so that the parameter D_{3b} for C-8 was defined as 4.0 ppm. These

Table 2 Hydroxy substituent effects in ppm[a]

OH position	α-Carbon	β-Carbon	γ-Carbon	δ-Carbon
8β	38.0	7.7 (7); 2.2 (9) 3.5 (14)	−3.4 (6); 0.6 (10); −2.4 (11); 0.8 (13); −4.8 (15)	1.5 (18); 0.9 (19)
15β[8]	43.6	5.8 (14); 10.5 (16)	−4.0 (8); −0.5 (13); 0.1 (17)	
16β[9]	42.5	13.6 (15); 5.7 (17)	−1.8 (14); 0 (13); −5.5 (20)	
3β (5β-H)[10]	40.0	6.5 (2); 6.3 (4)	−7.7 (1); −7.2 (5)	
5β[7]	29.8	5.3 (4); 8.9 (6) 4.8 (10)	−6.2 (1); −5.3 (3); 2.1 (7); 2.7 (9); −7.2 (19)	
6α (5β-H)[27]	40.6	6.3 (5); 8.6 (7)	−6.2 (4); −0.8 (8); 1.3 (10)	
1α,2β; 4β,5α; 5α,6β				1.0 (19)

[a] Carbon atom numbers presented in parentheses.

D_3 values are quite different from those assigned for D_1 and D_2 type 1,3-glycols in the six-membered ring.

Application ot Polyhydroxy Steroids.—The parameters thus obtained were applied to 14 different polyhydroxy 5α,14α-steroids **6–19** (see Fig. 1). The calculated δ_C values of their α-, β- and γ-carbons are tabulated in Table 3. The first number in each item is the predicted δ_C value. The δ_C values derived from the addition of the monohydroxy steroid substituent effect and the dihydroxy steroid parameters are shown in the first set of parentheses. The Δδ ($\delta_{observed} - \delta_{calculated}$) values are shown in the next set of parentheses. The δ_C values of β- and γ-carbons which could be derived by monohydroxy substituent effects alone are presented only with Δδ in parentheses.

The derived δ_Cs are the predicted values in $CDCl_3$ which were used in the two original reports.[1,2] However, many of the spectra of the reference compounds **6–19** are recorded either in C_5D_5N or CD_3OD since polar steroids are insoluble in $CDCl_3$. Solvent molecules affect the δ_Cs through coordination with the OH group, or through conformational changes by hydrogen bonding. The rigidity of the steroid framework minimizes the conformational changes, compared with other types of compounds. In the differences found in the δ_C data of simple hydroxy steroids (3β-hydroxycholestane, 3α-hydroxycoprostane, 3β,6β-dihydroxycholestane, 3β,6α-dihydroxycholestane), the largest difference was observed between the δ_C data recorded in C_5D_5N and those in $CDCl_3$.[10] However, the differences are generally less than 1.0 ppm and fall within the expected permissible Δδ ±2.5 ppm. When most of the calculated values fall within this range, the prediction can be regarded as acceptable.

The δ_Cs of the synthetic 3β,4β,5α-trihydroxy steroid **6**[12] were calculated using the three glycol types, namely, one axial equatorial (C-3, C-4; B_1), one 1,2-*trans* diaxial (C-4, C-5; C_2) and one 1,3-*trans* glycol (C-3, C-5; E_2) interaction. The predicted value for C-1 (29.9 ppm) is the total of the δ_C value of compound **1** (38.8 ppm) plus the γ-effect of the 3β-OH (−1.7 ppm) and 5α-OH (−7.2 ppm).[1] On the other hand, the predicted value for C-4 (78.9 ppm, Δδ 0.3 ppm) is derived from such an addition [29.2 ppm plus α- and β-substituent effects (4α-OH, 43.2 ppm; 3β-OH, 9.1 ppm; 5α-OH, 5.2 ppm)] plus dihydroxy parameters (B_{1a}, −6.5 ppm; C_{2a}, −2.0 ppm; E′, 0.7 ppm). Other δ_C values were derived in the same way and afforded the predicted δ_C values which agreed well with actual values.

Compounds **7** and **8**, isolated from soft corals (*Sinularia numerosa* and *Sarcophyton glaucum*),[3a,13] are 1β,3β,5α-trihydroxysteroids with an additional 6β-OH in **8**. The calculated values agreed generally within ±2.0 ppm, except for the large value (Δδ −3.4 ppm) for C-5 of compound **7**. When the parameters were not applied, the Δδ became 5.2 ppm. For the 1α,3β,5α,6β-tetrahydroxy steroid **9**, isolated from *Sinularia* sp. soft corals,[9,13] the predicted δ_C values using the dihydroxy parameter (C-1 to C-7) and those using monohydroxy substituent effects alone (C-8 to C-10 and C-19) agreed well. Two E parameters applied for C-3 afforded an improved predicted δ_C value (Δδ 1.6 ppm). The 3β,5α,6β,7β-tetrahydroxy steroid **10** was isolated from a soft coral *Anthelia glauca*.[3d] In this report the 7β-monohydroxy substituent effect was applied to the known compound 3β,5α,6β-trihydroxycholestane and the δ_C values were derived for C-8, C-9 and C-14. When the dihydroxy steroid parameters are applied to compound **10**, they gave reasonably close δ_C values for α-carbons at C-5, C-6 and C-7 (Δδ −0.6, −1.0 and −0.3 ppm). For the 7α-OH isomer **11**, which was isolated from the starfish *Luidia maculata*,[14] the calculated values agreed well with those found except for C-6. The magnitude of the error (Δδ −3.4 ppm) is, however, similar to those generated by the monohydroxy substituent effect alone (C-1, 3.7 ppm; C-9, 3.6 ppm). In the 3β,5α-dihydroxy steroids (**6–11**), the δ_C values of C-1 show in common significantly large Δδ values. The predicted δ_C value for C-1 of 3β,5α-dihydroxycholestane is identical with that in compound **6** (29.9 ppm) but the actual value is 32.0 ppm.[15] Apparently, there is an unidentified change of steric and electric field effects when the 3β and 5α positions are simultaneously substituted. It seems proper in such compounds to calculate the δ_Cs from the δ_Cs of 3β,5α-dihydroxycholestane, or to adopt the temporary value 41.0 ppm, instead of 38.8 ppm, for the C-1 of cholestane.

Compound **12** is a 2β,3β,4β-trihydroxypregnane-type steroid isolated from a forest tree *Trichilia schomburgkii*.[16] The ^{13}C NMR data were reported only for the 3-monoacetate derivative so that the data for compound **12** (Fig. 1) were estimated by subtracting similar acetylation shifts observed in the 6β,7β-diol **10** and its 7-monoacetate (β-equatorial OAc, β-axial OH).[17] The δ_1-hydroxy substituent effects of 2β- and 4β-OH were applied using the original data of Eggert *et al.*[1]

For the C_2-type 4β,5α-diol and 5α,6β-diol in which one OH (5α) has a γ-*trans* effect on C-19 and another one (4β or 6β) is 1,3-syndiaxial to C-19, the δ_1-hydroxy substituent effect to C-19 was defined as 1.0 ppm. In the 1α,2β-diol the same change makes the Δδ for C-19 1.5 ppm, instead of 3.0 ppm.[2] With such alteration, the Δδs observed for C-19 are generally small in compounds **6–19**. In most cases they are less than ±1.5 ppm, except for compound **10** (Δδ −2.1 ppm).

Compound **13** is a 14β-steroid isolated from the marine sponge *Xestospongia bergguistia*.[18] The substituent effects were assumed to be similar to those of 14α-cholestane as regards C-1 to C-6, C-10 and C-19. The δ_C values predicted on this assumption show sufficient agreement with those observed.

For the 1α,3β,6β,11α-tetrahydroxy 4α-methyl steroid **14**, isolated from the sponge *Pseudopterogorgia acerosa*,[19] the calculation was based on the δ_C values of 3β-hydroxy-4α-methylcholestane **5**.[20] The δ_Cs of C-2 to C-5 of compound **5** include interaction of two equatorial substituents, the 3β-OH and the 4α-Me, a situation which is similar in nature to a 1,2-diequatorial type-A glycol. Application of 1α- and 6β-OH substituent effects alone gives the δ_C values 71.6 ppm for C-1 and

Table 3 ^{13}C NMR chemical shifts of compounds **1**, **2**, **3** and **5** in $CDCl_3$ and calculated chemical shifts and deviations of compounds **6**–**19** in ppm

Carbons	**1**[10]	**2**[10]	**3**	**5**[19]	**6**	**7**	**8**
1	38.8	37.7	38.6	36.9	29.9 (2.1)	72.0 (70.0 + E_{2a}) (1.2)	72.0 (70.0 + E_{2a}) (1.8)
2	22.3	21.4	32.2	31.2	27.0 (26.3 + E′) (−0.2)	44.0 (42.6 + E′E′) (−2.4)	44.0 (42.6 + E′E′) (0.1)
3	26.9	27.1[a]	70.9	76.6	68.6 (72.1 + $B_{1b}E_{2a}$) (0.6)	64.9 (62.9 + E_{2a}) (1.5)	64.9 (62.9 + E_{2a}) (0.4)
4	29.2	27.3[a]	39.2	39.4	78.9 (86.7 + $B_{1a}C_{2a}$E′) (0.3)	44.2 (43.5 + E′) (−2.0)	42.1 (40.4 + C′E′) (1.1)
5	47.1	43.8	46.3	51.1	76.2 (73.9 + $C_{2b}E_{2b}$) (0.3)	78.6 (70.0 + $E_{2b}E_{2b}$) (−3.4)	79.3 (72.7 + $C_{2b}E_{2b}E_{2b}$) (−2.3)
6	29.2	27.6[a]	25.8	24.2	33.2 (31.5 + C′E′) (−0.8)	35.3 (34.6 + E′) (−0.5)	76.6 (77.9 + C_{2a}E′) (0.3)
7	32.2	26.6	40.1	32.2	26.3 (0.5)	26.3 (1.4)	34.7 (33.7 + C′) (1.0)
8	35.6	36.0	73.8	36.1			30.3 (1.6)
9	54.9	40.6	56.9	54.7	46.2 (1.5)	46.5 (−1.5)	46.5 (0.6)
10	36.3	35.4	36.4	34.9	39.2 (−0.1)	45.5 (−1.5)	45.5 (−0.6)
11	20.9	20.9	19.2	21.1			
19	12.2	24.3	13.2	13.4	17.1 (−0.9)	10.6 (1.3)	11.6 (−0.8)

	9	**10**	**11**	**12**
1	73.1 (62.6 + $D_{2a}E_{1a}$) (2.5)	29.9 (3.4)	29.9 (3.7)	43.5 (−0.1)
2	39.6 (38.2 + E′E′) (0.8)	32.3 (31.6 + E′) (0.1)	32.3 (31.6 + E′) (−0.6)	73.5 (72.0 + $B_{1a}D_1$) (−1.1)
3	62.4 (58.4 + $E_{1b}E_{2a}$) (1.6)	67.1 (65.1 + E_{2a}) (−0.1)	67.1 (65.1 + E_{2a}) (0.6)	74.3 (85.3 + $B_{1b}B_{1b}$) (−3.0)
4	42.8 (40.4 + C′E′E′) (0.3)	42.8 (40.4 + C′E′E′) (0.1)	42.1 (40.4 + C′E′) (−0.6)	77.7 (76.2 + $B_{1a}D_1$) (−1.1)
5	77.8 (65.5 + $C_{2b}D_{2b}E_{2b}$) (0.6)	77.2 (70.6 + $C_{2b}E_{2b}E_{2b}$) (−0.6)	78.0 (65.7 + $C_{2b}D_{2b}E_{2b}$) (0.2)	47.9 (1.6)
6	76.6 (77.9 + C_{2a}E′) (−0.8)	80.3 (87.4 + $B_{1a}C_{2a}$E′E′) (−1.0)	80.7 (85.5 + C_1C_{2a}E′) (−3.4)	26.1 (−0.7)
7	34.7 (33.7 + C′) (1.0)	73.2 (76.7 + $B_{1b}E_{2a}$) (−0.3)	74.2 (69.7 + $C_1C'D_{2a}$) (0.6)	
8	30.3 (0.8)	39.0 (38.3 + E′) (0.4)	33.9 (2.2)	
9	38.5 (2.5)	44.4 (0.2)	37.7 (3.6)	
10	43.0 (−1.3)	39.3 (−0.8)	39.3 (0.6)	35.9 (−0.7)
11				
19	17.8 (−0.1)	17.1 (0.3)	17.1 (−2.1)	17.1 (0)

Table 3 (*contd*)

	13	**14**	**15**	**16**
1	75.3 (78.8 + $B_{1b}E_{1b}$) (1.7)	75.1 (71.6 + E_{1a}) (−0.4)	23.8 (2.6)	37.1 (2.6)
2	76.5 (85.8 + $B_{1a}C_1E'$) (−0.3)	38.5 (37.8 + E′) (−1.4)	27.9 (0.2)	26.3 (−0.1)
3	71.3 (71.3 + C_1E_{1a}) (−0.7)	71.9 (69.9 + E_{1b}) (−0.4)	68.8 (61.8 + D_{2a}) (−1.7)	72.8 (78.3 + B_{1b}) (0.9)
4	25.9 (24.2 + C′E′) (0.4)	37.0 (36.3 + E′) (−0.8)	32.7 (−1.9)	68.6 (75.1 + B_{1a}) (0.5)
5	41.7 (42.9 + A′) (0.3)	45.7 (1.0)	78.6 (72.7 + AD_{2b}) (−0.1)	55.3 (54.6 + E′) (2.1)
6	75.4 (79.5 + A) (−0.4)	67.5 (−0.7)	73.0 (77.1 + A) (−1.3)	65.5 (63.5 + E_{2a}) (−0.7)
7		39.6 (0.4)	36.1 (37.3 + A′) (−0.8)	50.1 (49.4 + E′) (—)[b]
8		30.2 (−1.2)	35.2 (−1.0)	76.5 (68.2 + $D_{3b}E_{2b}$) (0.7)
9		53.1 (0.2)	43.3 (0)	57.1 (1.4)
10	42.8 (0.7)	40.6 (0.8)	41.5 (0.1)	37.4 (0.8)
11		69.4 (−2.0)		18.5 (0.4)
19	9.2 (0.8)	17.6 (−1.0)	17.1 (−0.2)	15.5 (1.4)
		51.7 (−1.0, C-12)		

	17	**18**	**19**
1	37.1 (2.8)	37.1 (2.5)	37.1 (3.6)
2	26.3 (0)	31.6 (−0.1)	26.3 (0.1)
3	72.8 (78.3 + B_{1b}) (0.8)	71.3 (1.0)	72.8 (78.3 + B_{1b}) (−0.1)
4	68.6 (75.1 + B_{1a}) (0.6)	31.9 (0.4)	79.9 (78.4 + $B_{1a}D_1$) (−2.5)
5	55.3 (54.6 + E′) (1.9)	44.4 (43.7 + E′) (0.1)	42.7 (2.1)
6	65.5 (63.5 + E_{2a}) (−0.8)	70.7 (74.2 + $B_{1b}E_{2a}$) (−1.8)	86.1 (73.6 + $C_1C'D_1D_{2a}$) (−5.2)
7	50.1 (49.4 + E′) (0.4)	77.6 (85.4 + $B_{1a}C_{2a}E'$) (−1.1)	77.8 (83.3 + C_1C_{2a}) (−5.1)
8	76.1 (71.8 + E_{2b}) (−0.1)	78.2 (75.9 + $C_{2b}E_{2b}$) (−0.5)	80.3 (68.3 + $C_{2b}D_{2b}D_{3b}$) (−2.5)
9	57.1 (0.3)	48.6 (2.6)	48.6 (2.0)
10	37.4 (0.6)	37.5 (0.3)	36.8 (0.1)
11	18.5 (0.4)	18.5 (0.8)	18.5 (−0.3)
19	15.5 (1.5)	13.1 (0.8)	19.0 (−0.1)

[a] Assignments may be interchanged. [b] δ_C Value not reported.

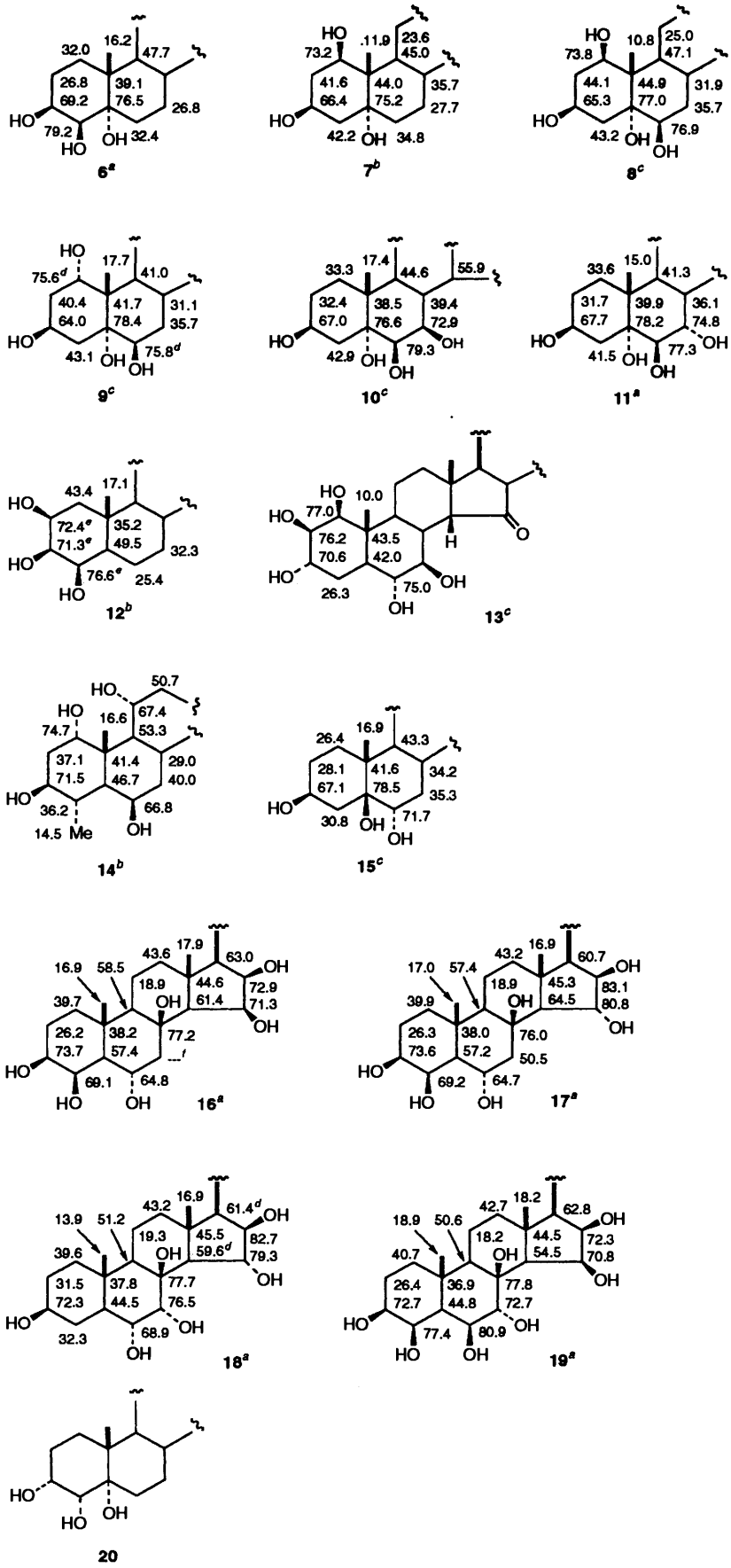


Fig. 1 Partial structures of compounds **6**–**20** and observed δ_Cs (ppm) for compounds **6**–**19**. [a] In CD_3OD. [b] In $CDCl_3$. [c] In C_5D_5N. [d] Assignments interchangeable. [e] Estimated from the δ_C values of the 3-monoacetate by subtraction of the acetylation shift. [f] Not reported.

69.9 ppm for C-3. When the E_1 parameter was applied, it gave the close δ_C values, 75.1 ppm for C-1 and 71.9 ppm for C-3 ($\Delta\delta$ each −0.4 ppm). The 4α-methyl and 6β-OH represent the arrangement which is similar to a γ_4-type 1,3-*trans* glycol. This causes little effect and the δ_C value of C-6 (66.8 ppm) is close to that derived by 6β-monohydroxy substituent effect alone (67.5 ppm).

The $\Delta\delta$ value becomes larger when the E′ parameter was applied to C-2 and C-4 in compound **14** and for C-2, C-4, C-6 of compound **7** and C-2 of compound **11**. With the exception of these cases, incorporation of E′ parameters improved the predicted δ_C values.

The 3β,5β,6α-trihydroxy steroid **15**, isolated from a *Sclerophytum* sp. soft coral,[21] was included as a 5β-steroid example. Addition of the monohydroxy substituent effects (3β-, 5β- and 6α-OH,[10] Table 2) on the δ_C values of 5β-cholestane **2**, followed by application of the dihydroxy steroid parameters afforded close δ_C values, except for C-1 ($\Delta\delta$ 2.6 ppm).

For the three typical 8β-hydroxy steroids **16**,[22] **17**[23] and **18**,[24] isolated from starfish, the additivity relationships afforded values close to those observed, except for C-1, which showed in common smaller values (2.5–2.8 ppm). The $\Delta\delta$s for C-6, C-7 and C-8 of compounds **16–18** indicate that incorporation of B_1, C_2, E_2 and D_3 parameters is appropriate in such compounds having a hydroxylated C-ring. However, it caused exceptionally large $\Delta\delta$s (C-6, −5.2 ppm; C-7, −5.1 ppm) for the heptaol **19**.[25] In this case the C-19 and 8β-OH each undergo three 1,3-syndiaxial interactions, together with an additional one between the 8β-OH and the 15β-OH. Probably the steroid nucleus and the glycol moieties involved are deformed excessively, resulting in various unidentified steric and electric field effects. In such exceptional cases, the dihydroxy steroid parameters cannot be linearly applied.

When the δ_C data are available for compounds which lack one OH group of the polyhydroxy steroid in question, application of the monohydroxy substituent effect and the dihydroxy steroid parameters corresponding to the newly introduced interaction gives the predicted δ_C values in the same way. This method sometimes gives better results, *e.g.* for C-1 of compounds **6–11** and compounds **16–19**, but in most cases the improvements are modest, as compared with those starting from the δ_Cs of 5α-cholestane **1**.

The $\Delta\delta$ values observed for the 14 polyhydroxy steroid indicate that the dihydroxy steroid parameters, derived from the $\Delta\delta^g$s reported by VanAntwerp *et al.*,[2] are generally applicable. In most cases, the $\Delta\delta$s fall well within a range of ± 2.5 ppm and they are useful for prediction, and interpretation, of the δ_C values of polyhydroxy 5α,14α-steroids if there is no severe steric hindrance as found in compound **19**. For the synthetic compound 3α,4α,5α-trihydroxycholestane **20**[26] they give the predicted δ_C values 72.7 ppm (82.5 + $B_{1b}B_{2a}$) for the C-4 and 77.4 ppm (72.4 + $B_{2b}D_{2b}$) for C-5, indicating that the assignments (C-4, 77.0; C-5, 71.1 ppm) reported by addition of the monohydroxy substituent effects should be reversed. The results observed for the 4α-methyl steroid **14** and the 5β-hydroxy steroid **15** suggest that these sets of parameters are applicable to other types of steroids, if their monohydroxy substituent effects are available.

Experimental

General Details.—M.p. is determined on a Kofler hot stage and are uncorrected. The optical rotation was determined on a JASCO DIP-370 digital polarimeter and is recorded in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded on a JEOL JNM GX 400 spectrometer at 400 MHz (^{1}H) and at 100 MHz (^{13}C) with $CHCl_3$ (δ_H 7.26 ppm) and C_5D_5N (δ_C 135.5 ppm) as internal standards. *J* Values are given in Hz. Mass spectra were determined on a JEOL JMS D 300 mass spectrometer. Flash column chromatography was performed on silica gel (Wako gel C-300, 200–300 mesh, Wako Pure Chemical industries).

24-*Methylcholestane*-3β,8β-*diol* (*or Ergostane*-3β,8β-*diol*) **3**.—A mixture of ergost-7-en-3β-ol acetate (700 mg) in CH_2Cl_2 (14 cm^3) and $NaHCO_3$ (400 mg) was treated with *m*-chloroperbenzoic acid (393 mg) and stirred at room temp. for 2 h. The mixture was diluted with CH_2Cl_2 and washed with 5% aqueous NaOH, water, and saturated aqueous NaCl. Preliminary silica gel column chromatography of the mixture with hexane–$CHCl_3$ (1:2) showed that the major 7α-epoxide had partly decomposed, probably due to the residual HCl in the solvent. It was chromatographed with ethyl acetate–hexane (1:19) giving a mixture (39 mg) containing the less polar 7β-epoxide. The mixture in THF (1.5 cm^3) was treated with LAH (21 mg) under reflux for 5 h. The mixture was diluted with ethyl acetate and filtered through cotton and then washed with 5% aqueous HCl, water, and saturated aqueous NaCl. The mixture was chromatographed over silica gel eluting with ethyl acetate–benzene (1:9) giving the less polar 3β,8β-diol **3** (19.8 mg, colourless needles, m.p. 110–112 °C (from MeOH), $[\alpha]_D$ +19 (*c* 1.98 in $CHCl_3$); δ_H(C_5D_5N) 0.82, 0.83, 0.88 (each 3 H, d, *J* 7.0, 26-, 27- and 28-CH_3) 1.01 (3 H, d, *J* 6.5, 21-CH_3), 1.23 (3 H, s, 18-CH_3), 1.31 (3 H, s, 19-CH_3) and 3.94 (1 H, m, 3α-H); δ_H($CDCl_3$) 0.77 (3 H, d, *J* 7.0, 28-CH_3), 0.78 (3 H, d, *J* 7.0, 27-CH_3), 0.85 (3 H, d, *J* 7.0, 26-CH_3), 0.88 (3 H, d, *J* 6.5, 21-CH_3), 0.92 (3 H, s, 18-CH_3), 0.97 (3 H, s, 19-CH_3) and 3.59 (1 H, m, 3α-H); δ_C(C_5D_5N) 38.6 (C-1), 32.2 (C-2), 70.9 (C-3), 39.2 (C-4), 46.3 (C-5), 25.8 (C-6), 40.1 (C-7), 73.8 (C-8), 56.9 (C-9), 36.4 (C-10), 19.2 (C-11), 41.9 (C-12), 43.7 (C-13), 60.2 (C-14), 19.6 (C-15), 28.3 (C-16), 57.1 (C-17), 14.0 (C-18), 13.2 (C-19), 36.0 (C-20), 19.0 (C-21), 33.9 (C-22), 31.0 (C-23), 39.4 (C-24), 31.8 (C-25), 17.8 (C-26), 20.7 (C-27) and 15.7 (C-28); *m/z* (EI) 418 (M^+), 403, 401, 400, 385, 305, 289, 273, 271, 255 and 235; HRMS/EI (Found: M^+, 418.3803. Calc. for $C_{28}H_{50}O_2$: *M*, 418.3810).

References

1 H. Eggert, C. L. VanAntwerp, N. S. Bhacca and C. Djerassi, *J. Org. Chem.*, 1976, **41**, 71; *J. Org. Chem.*, 1976, **41**, 4051.
2 C. L. VanAntwerp, H. Eggert, G. D. Meakins, J. O. Miners and C. Djerassi, *J. Org. Chem.*, 1977, **42**, 789.
3 (*a*) M. Kobayashi, T. Hayashi, F. Nakajima and H. Mitsuhashi, *Steroids*, 1979, **34**, 285; (*b*) Y. Yamada, S. Suzuki, K. Iguchi, H. Kikuchi, Y. Tsukitani, H. Horiai and H. Nakanishi, *Chem. Pharm. Bull.*, 1980, **28**, 473; (*c*) N. Fusetani, S. Matsunaga and S. Konosu, *Tetrahedron Lett.*, 1981, **22**, 1985; (*d*) U. Sjostrand, L. Bohlin, L. Fisher, M. Colin and C. Djerassi, *Steroids*, 1981, **38**, 347.
4 M. V. D'Auria, L. Minale and R. Riccio, *Chem. Rev.*, 1993, **93**, 1839 and references cited therein.
5 M. Kobayashi, K. M. C. A. Rao, M. M. Krishna and V. Anjaneyulu, *J. Chem. Res.*, 1994, (S), 180.
6 G. M. Schwenzer, *J. Org. Chem.*, 1978, **43**, 1079.
7 J. M. Coxon and J. R. Gibson, *Aust. J. Chem.*, 1981, **34**, 1451.
8 M. Kobayashi, M. M. Krishna, B. Haribabu and V. Anjaneyulu, *Chem. Pharm. Bull.*, 1993, **41**, 87.
9 M. Kobayashi, M. M. Krishna and V. Anjaneyulu, *Chem. Pharm. Bull.*, 1992, **40**, 2845.
10 J. W. Blunt and J. B. Stothers, *Org. Magn. Reson.*, 1977, **9**, 439.
11 L. Minale, C. Pizza, F. Zollo and R. Riccio, *J. Nat. Prod.*, 1983, **46**, 736.
12 M. V. D'Auria, R. Riccio, E. Uriarte, L. Minale, J. Tanaka and T. Higa, *J. Org. Chem.*, 1989, **54**, 234.
13 J. Su, X. Yu, L. Zeng and T. C. W. Mak, *J. Nat. Prod.*, 1989, **52**, 934.
14 L. Minale, C. Pizza, R. Riccio, O. S. Greco, F. Zollo, J. Pusset and J. L. Menou, *J. Nat. Prod.*, 1984, **47**, 784.
15 C. Konno and H. Hikino, *Tetrahedron*, 1976, **32**, 325.
16 W. F. Tinto, P. K. Jagessar, P. Ketwaru, W. F. Reynolds and S. McLean, *J. Nat. Prod.*, 1991, **54**, 972.
17 I. Kitagawa, M. Kobayashi, Z. Cui, Y. Kiyota and M. Ohnishi, *Chem. Pharm. Bull.*, 1986, **34**, 4590.

18 N. Shoji, A. Umeyama, K. Shin, K. Takeda, S. Arihara, J. Kobayashi and M. Takei, *J. Org. Chem.*, 1992, **57**, 299.
19 L. M. D. John, W. F. Tinto, S. McLean and W. F. Reynolds, *J. Nat. Prod.*, 1993, **55**, 144.
20 T. Iida, M. Kikuchi, T. Ishikawa, T. Tamura and T. Matsumoto, *Org. Magn. Reson.*, 1982, **19**, 228.
21 M. Kobayashi, F. Kanda, C. V. L. Rao, S. M. D. Kumar, G. Trimurtulu and C. B. Rao, *Chem. Pharm. Bull.*, 1990, **38**, 1724.
22 A. Casapullo, E. Finamore, L. Minale, F. Zollo, J. B. Carre, C. Debitus, D. Laurent, A. Folgore and F. Galdiero, *J. Nat. Prod.*, 1993, **56**, 105.
23 L. Minale, C. Pizza, R. Riccio, C. Sorrentino, F. Zollo, J. Pusset and G. Bargibant, *J. Nat. Prod.*, 1984, **47**, 790.
24 R. Riccio and J. Pusset, *Tetrahedron*, 1982, **38**, 3615.
25 M. Iorizzi, L. Minale, R. Riccio and T. Yasumoto, *J. Nat. Prod.*, 1992, **55**, 866.
26 Z. Boncza-Tomaszewski, *Can. J. Chem.*, 1987, **65**, 656.
27 D. Leibfritz and J. D. Roberts, *J. Am. Chem. Soc.*, 1973, **95**, 4996; H. Beierbeck, J. K. Saunders and J. W. ApSimon, *Can. J. Chem.*, 1977, **55**, 2813. Assignment of C-4 and C-7 of 5β-cholane-3α,6α-diol was reversed in this report. The β- and γ-effects of 6α-OH for C-7 and C-4 are therefore 8.6 and −6.2 ppm, respectively.

Paper 4/04112G
Received 5*th July* 1994
Accepted 12*th September* 1994