^{13}C Nuclear Magnetic Resonance Spectra of Some Ergosta-dienes and -trienes

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The natural abundance ^{13}C spectra of several ergosta-dienes and -trienes, measured at 25·2 MHz, have been completely assigned by using off-resonance decoupling, acetylation shifts, and lanthanide-induced shifts. The effect of functional groups on the chemical shifts is highly sensitive to the environments of the functional group and the carbon atom under consideration, and analogies from simpler systems (*e.g.* cyclohexanes) are not always reliable.

ASSIGNMENT of the ^{13}C spectra of steroids is a prerequisite for the development of any study utilising these spectra. Furthermore, the fact that the backbone of these compounds is essentially rigid suggests that they can be taken as model systems for the purpose of relating chemical shifts to molecular structure. However, since the paper by Roberts and his co-workers,[1] surprisingly few contributions [2-7] to this field have appeared, even though these reports clearly demonstrate the enormous sensitivity of the ^{13}C spectra of steroids towards chemical changes.

We now present the self-consistent and complete assignments of the ^{13}C spectra of ergosta-7,22-dien-3β-ol (1), ergosta-7,9,22-trien-3β-ol (2), ergosta-7,9,22-triene-3β,5α-diol (3), 9α,11α-epoxyergosta-7,22-dien-3β-ol (4), their respective acetates (1a)—(4a), and 3β-acetoxyergosta-7,22-dien-11-one (5a). These data together with those of the parent compounds cholestan-3β-ol (6) and ergosterol (7) illustrate the sensitivity of the ^{13}C chemical shifts to substituent effects.

EXPERIMENTAL

The steroids (1) and (2a)—(5a) were gifts from Glaxo Laboratories Ltd. Hydrolyses of the acetates (2a)—(4a) were carried out by refluxing for 1 h with methanolic potassium hydroxide [hydrolysis of (5a) leads to a rearrangement product], and acetylation of (1) was performed with acetic anhydride–pyridine. The products were recrystallised from methanol and characterised by m.p., i.r., and n.m.r. spectra, and elemental analysis.

For the ^{13}C spectra 0·1—0·5M-solutions (*ca.* 3 ml) in $CDCl_3$ were run in 12 mm sample tubes each carrying a concentric 5 mm tube containing D_2O as field frequency lock. Internal Me_4Si was used as reference. Spectra were obtained at 25·2 MHz with a Varian XL-100-15 spectrometer operating in the pulse-Fourier transform mode (Varian 620L computer, 8K core). Pulse widths <40 μs ($<30°$ flip angle) were employed with 0·4 s acquisition time to yield 2·5 Hz per point in the transformed spectrum (5 kHz spectrum width). Typically 50,000 transients were accumulated. The breakdown into quaternary, tertiary, secondary, and primary carbon atoms was achieved by the use of off-resonance noise-decoupling techniques. At least four different off-resonance spectra with offsets of 2500, −550, −1800, and −2500 Hz from the centre of the aliphatic proton resonances were taken for each compound, providing an unambiguous identification of the signals due to the various types of carbon atom. The lanthanide-induced shifts (LIS) considered later are defined as the changes in chemical shift ($\Delta\delta$) of a 0·01M-solution produced by the addition of 40 mg of $Eu(fod)_3$.†

RESULTS AND ASSIGNMENTS

The spectral assignments were made first by using the off-resonance spectra, then by division into the characteristic ^{13}C chemical shift regions,[8] and finally by using the assignments of ref. 1 and substituent effects. The shifts and assignments for compounds (1)—(5) and their acetates are presented in Table 1 together with the previous results for compounds (6) and (7) from ref. 1 [these were measured from external CS_2 and have been converted to the δ scale by taking $\delta(CS_2)$ as 192·3].

Acetylation of the C-3 hydroxy-group produces a characteristic downfield shift of the C-3 signal (*ca.* 3 p.p.m.), an upfield shift of the β-carbon (C-2 and C-4) signals (*ca.* 4 p.p.m.), and a smaller upfield shift of the γ-carbon (C-1 and C-5) signals (*ca.* 0·3 p.p.m.), as has been noted for cyclohexanols and other steroids.[1] All other resonances are unchanged to within 0·2 p.p.m. and in Table 1 the values for the acetates are given only when these differ significantly from those of the corresponding alcohols [except for (5a) since the corresponding alcohol was not studied]. These values are given in parentheses as shifts from the alcohol values, and are invaluable for the assignments of C-1 to C-5.

The side chain assignments (C-20 to C-28) follow precisely those of ergosterol (7).[1]

Compounds (1)—(3).—There are only two saturated quaternary carbon atoms in structures (1) and (2), yet their assignments presented some difficulties. It was difficult even to detect one of these signals: the C-13 signal was hidden under the C-12 signal in the spectra of (2) and (3). As both C-12 atoms are methylene carbon atoms their signal intensities under the experimental conditions used are far greater than those of the quaternary carbon atoms, and thus the total peak height is no

† Tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato)europium(III).

[1] H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1969, **91**, 7445.
[2] G. Lukacs, F. Khuong-Huu, C. R. Bennett, B. L. Buckwalter, and E. Wenkert, *Tetrahedron Letters*, 1972, 3515.
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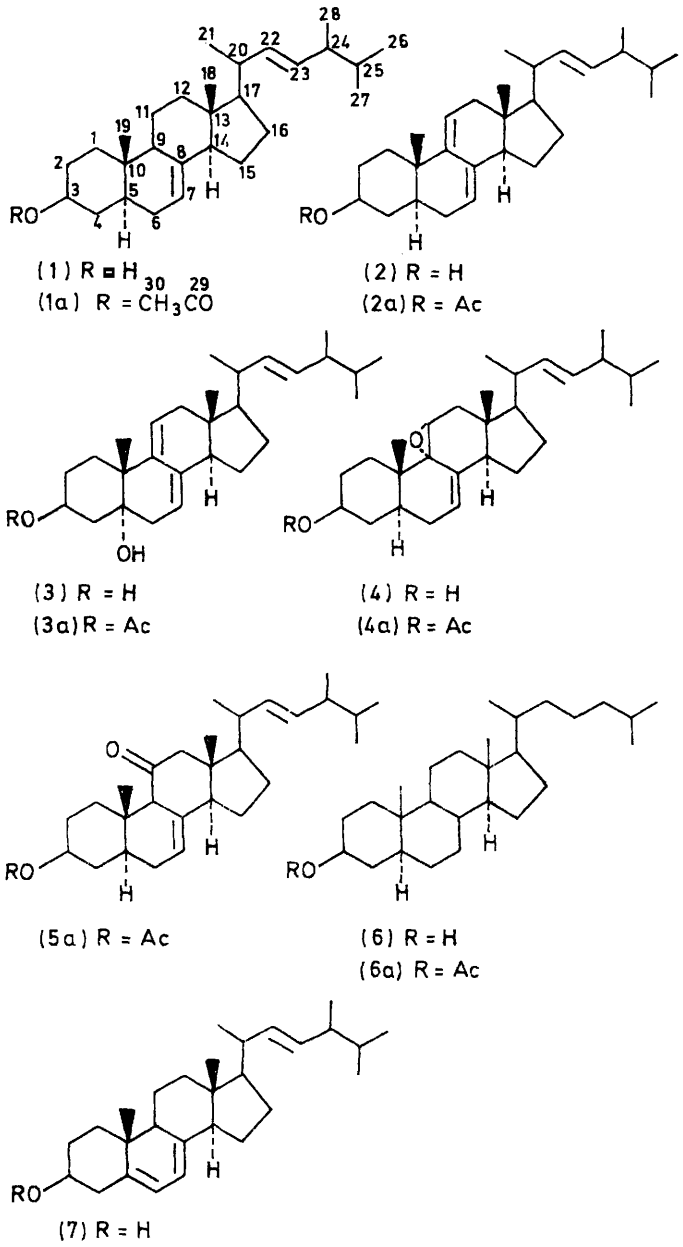
[5] T. A. Wittstruck and K. J. H. Williams, *J. Org. Chem.*, 1973, **38**, 1542.
[6] D. Leibfritz and J. D. Roberts, *J. Amer. Chem. Soc.*, 1973, **95**, 4996.
[7] D. E. Dorman, M. Jautelat, and J. D. Roberts, *J. Org. Chem.*, 1971, **36**, 2757.
[8] J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972.

indication of the presence of two signals. Furthermore, off-resonance decoupling does not clarify the position as the large signals are split into 1 : 2 : 1 triplets, with the



centre peak still obscuring the weak quaternary signal. These resonances were only finally identified, and also assigned, by observing the LIS for these molecules (Table 2). C-10 in both cases, but particularly in that of compound (2), shows a large shift, whereas, as might be expected, C-13 shows no effect in either case. This assignment is further supported by the substituent effect of the C-5 hydroxy-group [*viz.* (2) ⟶ (3)], which affects only C-10. Thus C-10 is assigned the high-field signal in the spectra of (2) and (3), in agreement with the other compounds in Table 1. However, these other assignments

TABLE 1

^{13}C Chemical shifts (δ) of steroids

Compd. Carbon	(1) [(1a)]	(2) [(2a)]	(3) [(3a)]	(4) [(4a)]	(5a)	(6) [a] [(6a)]	(7) [a]
1	37·1 [−0·4]	34·6 [−0·3]	29·7 [−0·3]	28·8 [−0·3]	31·7	37·1 [−0·4]	38·4
2	31·4 [−3·9]	31·6 [−4·0]	30·8 [−3·8]	30·9 [−4·1]	27·1	31·5 [−4·2]	32·0
3	70·8 [2·4]	70·3 [2·8]	67·0 [3·4]	70·0 [2·3]	72·9	70·2 [+2·8]	69·5
4	37·9 [−4·2]	37·7 [−4·1]	41·8 [−3·8]	37·5 [−4·1]	33·5	38·4 [−4·4]	40·5
5	40·2	39·1	73·6 [−0·3]	36·7	35·5	45·0 [−0·4]	140·5
6	29·6	29·8	37·4	29·7 [−0·3]	29·6	28·8 [c]	119·2
7	117·1	118·0 [d]	115·8 [d]	125·3 [−0·4]	122·3	32·1 [c]	116·5
8	139·0	135·7 [c]	136·3 [c]	134·5 [0·4]	134·9	35·6	140·4
9	49·4 [−0·3]	143·4 [c]	140·1 [c]	64·5 [−0·4]	57·9 [c]	54·6	46·3
10	34·1	35·6	41·1	34·6	33·9	35·4	37·0
11	21·5	118·8 [d]	122·8 [d]	55·2 [−0·4]	184·5	21·2	21·0
12	39·4	42·1	42·3	40·5	58·5	40·2 [b]	39·2 [b]
13	43·1	42·7	42·3	42·9	43·1	42·6	42·8
14	55·0	51·5	51·2	46·8	52·3 [c]	56·5	54·4
15	22·9	23·1	23·1	22·4	23·6	24·1	22·9
16	28·1	28·8	28·7	28·3	29·1	28·1 [b]	28·1 [b]
17	55·8	56·0	55·9	56·1	57·7	56·5	55·8
18	12·1 [c]	11·4	11·4	13·7	17·0	11·9	11·6
19	13·0 [c]	19·3	25·0 [−0·5]	15·6	20·4	11·8	15·8
20	40·4	40·3	40·2	40·1	40·1	35·7	40·3
21	19·6	19·6	19·6	19·7	19·8	18·4	19·2
22	131·4	131·5	131·7	131·7	132·3	36·2	132·0
23	135·2	135·0	134·8	134·7	134·0	23·9	135·8
24	42·7	42·7	42·7	42·7	42·7	39·5	42·8
25	33·0	32·9	32·8	32·8	32·9	27·8	33·0
26	19·9	19·8	19·8	19·8	19·8	22·2	19·5
27	21·1	20·6	20·6	20·8	21·1	22·4	20·8
28	17·5	17·6	17·6	17·6	17·6		17·2

[a] From ref. 1. [b] Reversed from ref. 1; see text. [c,d] Assignments may be interchanged. [e] The δ values for the acetoxy-carbons are 169·7 and 21·2 in all cases.

TABLE 2

LIS (Δδ) in steroid alcohols

Compound	(2)		(3a)	
Carbon †	δ [a]	Δδ [b]	δ [a]	Δδ [b]
1	34·6	0·8	29·4	0·6
2	31·6	1·9	27·0	0·7
3	70·3	8·8	70·4	2·2
4	37·7	2·4	38·0	1·2
5	39·1	1·3	73·3	1·5
6	29·8	0·4	37·2	0·5
7	118·0	0·3	115·8	0·3
8	135·7	0·3	136·3	0·3
9	143·4	0·3	140·1	0·2
10	35·6	1·1	41·1	0·4
11	118·8	0·3	122·8	0·5
19	19·3	0·9	24·5	0·8
29			169·5	−1·7
30			21·3	1·8

[a] δ of 0·10M-solution in $CDCl_3$. [b] Extra shift produced by the addition of 40 mg of $Eu(fod)_3$ (to 0·04M).

† Carbon atoms not listed show no change.

follow unambiguously from those of (6) and (7), for which there is substantial evidence,[1,2] as in the spectra of all these compounds the positions of the C-10 and C-13 signals are constant. The assignment of the new

quaternary carbon atom (C-5) in structure (3) is straightforward and follows from comparison of (3) and (3a) (Table 1).

The assignment of the quaternary olefinic signal in the spectrum of (1) to C-8 is unique, but those of the C-8 and C-9 signals in (2) and (3) are not unambiguous. They are based on the expected downfield shift due to β-carbon substitution [8] (C-9 has three β-carbon substituents, whereas C-8 has only two). In support of this, C-9 shows the larger shift when the C-5 hydroxy-group is introduced.

There are eleven methine carbon atoms in structure (1). Of their assignments those of C-3 and C-7 are immediately apparent and those of C-14, C-17, and the five side-chain carbon atoms follow from the data for (6) and (7), as also do those of C-5 and C-9 when the effect of introducing the double bond in ring B is considered (see later).

The shifts of C-17 and the side-chain carbon atoms remain virtually constant throughout the series (1) ⟶ (5) and their assignments are given immediately by analogy with (1), as also is that of C-3. Of the remaining four methine carbon atoms in (2), C-5 and C-14 can also be assigned by comparison with (1) on the basis of the effect of the 9,11-double bond. The assignment of the two olefinic methine carbon atoms is not unambiguous. Both are affected by the introduction of a hydroxy-group at C-5 [(2) ⟶ (3)], indicating a transmission of steric effects. However, the assignment given is more consistent with the other substituent effects of the axial C-5 hydroxy-group (see later).

There are eight methylene carbon atoms in structure (1), all within the steroid nucleus. Again we attempted to make assignments by comparison with data for (6) and (7) from ref. 1, but it soon became clear that these were leading to inconsistencies. In particular use of the original assignments [1] for C-12 and C-16 led to the conclusion that C-12 was unaffected by the introduction of the various substituents in compounds (1)—(5), but that C-16 was affected. This seemed unreasonable, and thus the previous assignments [1] of C-12 and C-16 have been interchanged. This error has been confirmed recently by Liebfritz and Roberts.[6,9] Table 1 gives the new assignments for (6) and (7) and those for compounds (1)—(5).

In compound (1) the shifts produced on acetylation permit assignments for C-1, C-2, and C-4, and the remaining signals are sufficiently close to those of (6) and (7) to allow immediate assignments. In particular the effect of the branched side-chain in compounds (7) and (1)—(5) moves the C-15 signal *ca.* 1 p.p.m. to high field as compared with compound (6). Detailed inspection of the effects of introducing the double bond [(6) ⟶ (1)] suggests that the assignments of C-6 and C-7 in (6) may be reversed; this observation would agree with the normal high-field shift of a carbon atom β to a double bond (see later).

In the spectra of compounds (2) and (3), and in those of the remaining compounds, the C-15 and C-16 signals are unchanged in position from those of (7) and are in consequence easily assigned; so also are the C-1, C-2, and C-4 signals, as previously. This only leaves the C-6 and C-12 signals which are well separated and therefore unlikely to have the reverse assignments. We note the large effect of the C-5 hydroxy-group on C-6 (*ca.* 9 p.p.m.), consistent with β-substitution.

There are six methyl carbon atoms in structure (1); of these four are in the side chain and their assignments are identical with those of (7). The assignment of the remaining resonances to C-18 and C-19 is not unambiguous in (1) (they are only 0·9 p.p.m. apart) but was confirmed in (3a) by the LIS (Table 2), those of C-19 being considerable but those of C-18 negligible, as expected.

Compounds (4) *and* (5a).—The assignment of the spectra of these compounds was made by comparison with that for compound (1).

Introduction of the 9,11-epoxide system in (4) and of the C-11 oxo-group in (5) has no effect on the quaternary carbon atoms C-10 and C-13, and the remaining quarternary carbon atoms (C-8 and C-9) in (4) are also readily assigned.

Of the tertiary carbon resonances, the signals for C-17 and the side-chain carbon atoms are almost unchanged and therefore assigned immediately. Of the remainder, the assignments of C-3 and C-7 in (4) are unambiguous. This leaves C-11 and C-14, which have been assigned on the basis of the much larger residual splitting in the off-resonance decoupled spectrum of the δ 55·2 peak, which is thus assigned to C-11 [^1J(C–H) of oxiran is 176 Hz; that of cyclohexane is 125 Hz [8]].

Similar reasoning provides the assignment for (5a) except that in this case there is the possibility of reversing the assignments of C-9 and C-14.

There are seven methylene carbon atoms in structure (4). Of these C-2, C-4, C-6, C-15, and C-16 may be assigned from the shifts produced by acetylation (Table 1) and by analogy with (1). This leaves C-1 and C-12. If the acetylation shift of −0·3 p.p.m. is regarded as significant, C-1 may be assigned as shown, but this leaves unanswered the reason for the large upfield shift (8·3 p.p.m.) in going from (1) to (4). Reversing these assignments means that the C-12 signal moves upfield by *ca.* 10 p.p.m. on epoxide formation, which would appear equally probable.

This problem is by-passed in (5a). Similar considerations allow assignment of C-2, C-4, C-6, C-15, and C-16, the large downfield effect at C-12 due to the carbonyl group makes this assignment clear, and the C-1 signal is assigned by elimination. Again, however, we note the significant effect of the carbonyl group at C-1.

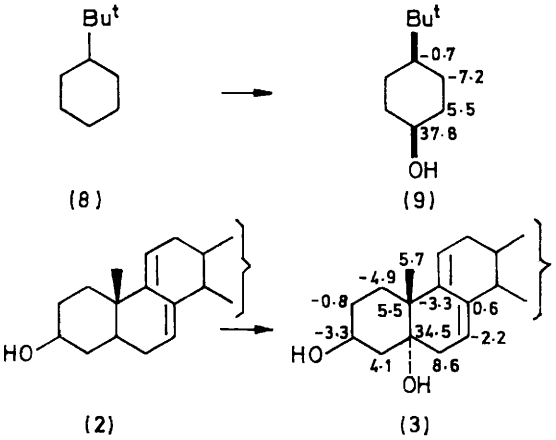
The two ring methyl carbon atoms (C-18 and C-19) are also assigned by analogy with (1) but again these assignments are not unambiguous since the difference in their chemical shifts in (1) is small compared with the size of the shifts due to the epoxy- and carbonyl groups.

[9] H. Eggert and C. Djerassi, *J. Amer. Chem. Soc.*, 1973, **95**, 3710.

DISCUSSION

A number of the assignments have been made by considering the substituent chemical shifts in the steroid nucleus and it is pertinent to summarise these here.

The effect of acetylating the C-3 hydroxy-group, used to assign C-1, C-2, C-3, and C-4 in these compounds, is extremely useful. However inspection of Table 1 shows some further effects. In some cases other nearby carbon atoms are affected, *e.g.* C-5 in (6) and (3) and C-9 in (1), but the shifts are small and relatively insignificant. More interestingly, C-19 in (3) shows a sizeable effect (0·5 p.p.m.) but not C-19 in (1) or (2); this has no obvious explanation. Also of interest is the effect of acetylation on the epoxy-carbon atoms of (4), both of which show a sizeable shift; this however is not much larger than the experimental error (which for $\Delta\delta$ values becomes $\pm$0·2 p.p.m.), and a general statement would require confirmation from other systems. Our observation does indicate, however, that acetate shifts may operate over quite a long range.



Another important substituent effect is that of the axial hydroxy-group at C-5 [*viz.* (2) ⟶ (3)]. Comparison of these shifts with those in the corresponding cyclohexane derivatives (8) and (9) [8,10] shows a consistent picture of low-field α and β shifts and a high-field γ effect. However, the surprising effect on the axial C-19 methyl carbon atom is not consistent with the behaviour of simpler compounds, *e.g.* propane C-1 15·4, propanol C-1 10·5, though the fact that the gauche conformer is significantly populated in propanol may explain the difference.

There is also a sizeable LIS on C-19 in both compounds (2) and (3a) (*cf.* Table 2). However, although the position of attachment of the Eu(fod)$_3$ in (2) appears normal from the shifts, this is *not* the case for (3a). The spread of effects on the two oxygen carbons C-3 and C-5 and the unusual high-field shifts of the acetoxy carbonyl carbon atom all suggest that the Eu(fod)$_3$ is complexing with the acetoxy-group in competition with the hydroxy-group in this molecule.

Finally, the results in Table 1 give some indication of the substituent effects of a double bond in these systems. Reich *et al.*[1] have noted that the introduction of a double bond in cyclohexane causes the α-carbon signals to shift 1·8 p.p.m. to high field and the β-carbon signals 4·5 p.p.m. to high field. This simple pattern is modified in these molecules. For example, the introduction of the 7,8-double bond in ring B [(6) ⟶ (1)] produces the following shifts: α-carbons C-6 and C-9, −2·5 * and −5·2; β-carbons C-5 and C-10, −4·8 and −1·3. Similarly, the introduction of an exocyclic double bond in the cyclohexane series (methylcyclohexane to methylenecyclohexane) gives shifts ($\Delta\delta$ values) of 0·0, 2·0, and −1·9 for the α-, β-, and γ-carbons of the ring.[8,11] In ring C the corresponding values for the conversion (6) ⟶ (1) are, for the α- (C-9, C-14), β- (C-11, C-13), and γ- (C-12) carbons, −5·2, −1·5; 0·3, 0·5; and −0·8 p.p.m. In both cases the effects are similar but the actual values differ considerably in the two systems.

The effects of the introduction of the second double bond [*viz.* (1) ⟶ (2)] are also different from predictions on the basis of these simple considerations. In particular, large changes are observed in ring C. For C-12, C-13, and C-14 the shifts are 2·7, −1·0, and −3·5 p.p.m. The major effects are shifts to high field, but even so the C-12 signal moves 2·5 p.p.m. to low field.

Some of these ambiguities may be due to problems of assignment but it is clear also that analogies based on the simple cyclohexane system cannot be transferred easily to the steroid system. This is probably because of the presence of long-range effects in the latter; in particular a major effect to consider is the relief of steric strain in the saturated system, notably with reference to the 1,3-diaxial interactions between C-18 and C-10.[1] Long-range effects are also clearly seen in the effects of introducing the 9,11-epoxy- and 11-oxo-groups [(1) ⟶ (4) and (1) ⟶ (5)]. In both (4) and (5), the C-1, C-5, and C-7 signals, and even those of C-18 and C-19 in (5), are considerably shifted from their values in (1), showing the long-range effects of these functional groups in these particular molecules.

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* With the assignments of C-6 and C-7 of (6) reversed from Table 1.

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[11] M. Jautelat, D. E. Dorman, and J. D. Roberts, *J. Org. Chem.*, 1971, **36**, 2757.