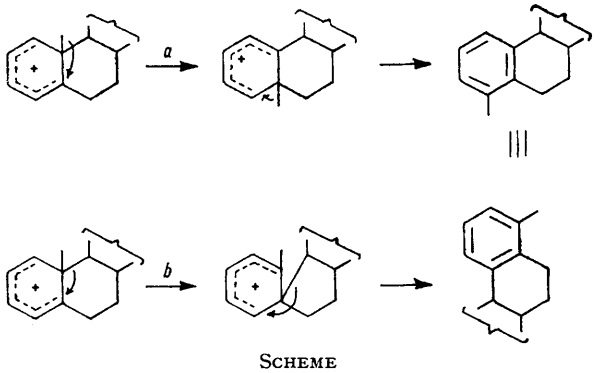
The ^{13}C Nuclear Magnetic Resonance Spectra of Some Methyloestratrienes; Application to the Mechanism of Their Formation

By **James R. Hanson** * and **Michael Siverns**, The School of Molecular Sciences, The University of Sussex, Brighton BN1 9QJ

The ^{13}C n.m.r. spectra of some methyloestratrienes have been assigned and the results have been used to locate the site of deuteriation in the products of an aromatization reaction, thus distinguishing between two reaction pathways.

^{13}C NUCLEAR magnetic resonance spectroscopy provides a useful method for locating the site of deuteriation in an organic molecule. Proton noise decoupling leads to enhanced signals from carbon atoms bearing hydrogen but not from those bearing deuterium. We have recently shown that a number of steroidal hydroxy-epoxides form 4-methyloestratrienes on treatment with



SCHEME

hydrogen bromide in glacial acetic acid.[1] Two general pathways may be envisaged for these reactions: one involving methyl migration as in the steroidal backbone rearrangement (pathway *a*) and the other skeletal rearrangement as in the dienol–benzene rearrangement (pathway *b*). These pathways have been distinguished in one case by the use of ^{14}C labelling and degradation of the aromatic steroid [2] or by using methyl-steroids and examining the methylation pattern of the resultant aromatic steroid.[3] The pathways may also be distinguished by examining the fate of a 3-deuterio-substrate. Methyl migration would afford a 3-deuterio-4-methyloestratriene whilst skeletal rearrangement would afford a 1-deuterio-4-methyloestratriene. The problem was therefore to assign the ^{13}C n.m.r. spectra of the 1- and 4-methyloestratrienes (1)—(8) and then to locate the deuterium in deuteriated aromatic products.

The ^{13}C n.m.r. spectra were obtained at 25·15 MHz by using a pulsed Fourier transform system with proton noise decoupling and off-resonance decoupling. The results obtained in deuteriochloroform solution for some 17β-acetoxy-1- and 4-methyloestratrienes and their related C-6 and C-17 ketones are tabulated.

Many of the resonances were assigned from their multiplicity in the off-resonance spectra. The quartets associated with C-18 and C-19 were readily distinguished.

[1] J. R. Hanson and H. J. Shapter, *J.C.S. Perkin I*, 1972, 1446; D. Baldwin and J. R. Hanson, *ibid.*, p. 1889; A. G. Ogilvie and J. R. Hanson, *ibid.*, p. 1981; D. Baldwin, J. R. Hanson, and A. M. Holtom, *J.C.S. Perkin I*, 1973, 1704, 2687; J. R. Hanson, and H. J. Wilkins, *ibid.*, 1974, 1388.

[2] E. Caspi, D. M. Piatak, and P. K. Grover, *J. Chem. Soc.* (*C*), 1966, 1034.

[3] J. R. Hanson and T. D. Organ, *J. Chem. Soc.* (*C*), 1971, 1313.

The high-field signal from C-18 reflects the shielding by the adjacent C-17 substituents, and that from C-19 reflects changes at C-6. The methylene triplets were assigned in the following manner. 4-Methyloestratrien-17-one (3) was treated with MeO(^{2}H)–MeONa to exchange the C-16 protons with deuterium. The product was then reduced with lithium aluminium hydride and acetylated to afford 17β-acetoxy-4-methyl[16-$^2H_2$]oestratriene. This enabled the resonances at 27·4 p.p.m. from the acetate and at 35·7 p.p.m. from the 17-ketone to be assigned to C-16. 17β-Acetoxy-4-methyl[2,6,6-$^2H_3$]-oestratriene was prepared by base-catalysed deuteriation of 17β-hydroxyandrosta-1,4-dien-3-one, subsequent acetylation, reduction with sodium borohydride, and aromatization.[1] This enabled a resonance at 27·4 p.p.m. to be assigned also to C-6. The corresponding signal from 17β-acetoxy-1-methyloestratriene (6), which was absent from the spectrum of 17β-acetoxy-1-methyloestra-1,3,5(10),6-tetraene (7), appeared at 32·4 p.p.m., revealing a shielding effect on C-6 by the 4-methyl group. The resonance at 26·6 p.p.m. was assigned to C-7 since it had moved to 45·7 p.p.m. in the spectrum of 17β-acetoxy-4-methyloestratrien-6-one (2) and to 44·9 p.p.m. in that of 4-methyloestratriene-6,17-dione (4). When this diketone was treated with MeO(^{2}H)–MeONa, the resonances at 35·7 and 44·9 p.p.m. collapsed. The resonance at 37·0 p.p.m. in the spectrum of 17β-acetoxy-4-methyloestratriene (1) and at 31·3 p.p.m. in that of 4-methyloestratrien-17-one (3) was assigned to C-12, reflecting the known [4] effect of a 17-substituent on this centre. The highest field triplet was assigned to C-15 in view of the effect of changes at C-17; on the other hand the position of this resonance remained constant when changes occurred on ring A and at C-6.

Amongst the aliphatic doublets that due to C-17 appeared at a characteristic [5] position in the spectra of the 17β-acetates and that due to C-14 was relatively unaffected by substitution changes. The resonance at 37·7 p.p.m. was assigned to C-8 since it showed a small downfield shift on introduction of a C-6 oxo-group. The resonance at 44·5 p.p.m. was assigned to C-9, adjacent to the aromatic ring. The position of this resonance varied with the introduction of a C-1 methyl group and was moved upfield by the shielding cone of the C-6 oxo-group. The C-13 resonance appeared as a singlet, its position reflecting, as expected, the oxidation level of C-17.

The aromatic carbon resonances of 17β-acetoxy-4-methyloestratriene (1) fall into a group of three doublets at 123·2, 125·2, and 127·3 p.p.m. and three singlets at 135·0, 136·3, and 140 p.p.m. The resonance at 125·2 p.p.m. was assigned to C-2 since this resonance had collapsed in the spectrum of 17β-acetoxy-4-methyl[2,6,6-$^2H_3$]oestratriene. Although the dienol–benzene reaction is known to follow pathway *b*,[2] both pathways would lead to deuterium at this centre from a 2-deuteriated 3-hydroxyandrosta-1,4-diene. The resonance at 123·2 p.p.m. was absent in the spectrum of the 1-methyloestratriene (6) and was not affected by an oxo-group at C-6. Furthermore when 17β-acetoxy-3α-deuterioandrosta-1,4-dien-3-ol was aromatized under the conditions of the dienol–benzene rearrangement this resonance collapsed. It was therefore assigned to C-1. The singlet at 135·0 p.p.m. which was not present in the spectrum of the 1-methyloestratriene (6) and appeared further downfield in those of the C-6 ketones was assigned to C-4. The singlet at 136·3 p.p.m., also present (at 137·1 p.p.m.) in the spectrum of the 1-methyloestratriene was assigned to C-5. Calculations based on the substitution parameters of methyl, ethyl, and isopropyl [6] also gave the values of 123·3, 125·7, and 126·5 for C-1, -2, and -3 and, in poorer agreement but in the same order, 136·6, 142·8, and 148·0 p.p.m. for C-4, -5, and -10. The assignments for the 6-oxo-oestratrienes were made on the basis of similar calculations.

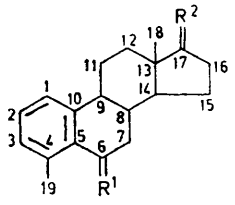
The mechanistic dichotomy elaborated in the Scheme was exemplified by a 4α,5α-epoxide, a 5α,6α-epoxide, and

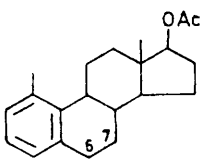
^{13}C N.m.r. spectra of some methyloestratrienes (in p.p.m. from Me_4Si)

Compd.	Carbon atom 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
(1) [a]	123·2	125·2	127·3	135·0	136·3	27·4	27·4	37·7	44·5	140·0	26·3	37·0	42·8	50·0	23·3	27·4	82·8	12·0	19·8 [e]
(2) [b]	123·0	132·3	130·6	141·3	131·2	199·5	45·7	38·6	43·4	147·8	25·7	36·5	42·6	50·1	23·0	27·4	82·1	11·9	23·4 [e]
(3) [c]	123·0	125·5	127·5	134·9	136·3	27·0	26·6	37·5	44·7	139·6	26·0	31·7	47·8	50·6	21·6	35·9	220·5	13·8	19·8
(4) [b]	122·9	132·4	130·7	141·4	131·0	199·1	44·9	38·4	43·6	147·3	25·5	31·3	47·5	50·7	21·4	35·7	219·6	13·6	23·4
(5) [b]	123·2	125·2	127·2	135·2	136·3	27·3	26·9	38·3	44·8	140·7	25·4	40·5	40·9	53·8	20·6	28·3	38·9	17·5	19·8
(6) [d]	138·4	125·2	127·2	129·0	137·1	32·4	27·8	41·4	47·1	138·4	25·5	37·8	43·8	50·3	23·4	27·4	82·8	12·9	22·2 [e]
(7) [d]	125·3	126·0	129·2	135·1	135·9	131·2	132·5	38·6	46·0	136·3	26·8	37·4	42·4	48·5	23·7	27·3	82·6	11·8	25·0 [e]
(8) [e]	136·8	133·9	126·1	125·3	137·1	198·4	41·4	38·5	46·4	144·2	26·4	37·4	43·3	49·0	23·1	27·5	82·2	12·6	22·4 [e]

[e] CH_3·CO 21·1—21·2; CH_3·CO 171·0—171·1.

[a] J. Schmitt, J. J. Panousse, P. J. Cornu, A. Hallot, H. Pluchet, and P. Comoy, *Bull. Soc. chim. France*, 1965, 1934. [b] E. Caspi, D. M. Piatak, and P. K. Grover, *J. Chem. Soc.* (*C*), 1966, 1034. [c] E. Caspi, P. K. Grover, N. Grover, and E. J. Lynde, *J. Chem. Soc.*, 1962, 1710. [d] H. Dannenberg and H. G. Neumann, *Annalen*, 1961, **646**, 184. [e] J. R. Hanson and H. J. Shapter, *J.C.S. Perkin I*, 1972, 1445.





(1) $R^1 = H_2$, $R^2 = \beta - OAc$, $\alpha - H$
(2) $R^1 = O$, $R^2 = \beta - OAc$, $\alpha - H$
(3) $R^1 = H_2$, $R^2 = O$
(4) $R^1 = R^2 = O$
(5) $R^1 = R^2 = H_2$
(6)
(7) $\Delta^{6,7}$
(8) 6-ketone

[1] H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1969, **91**, 7445.

[2] T. A. Wittstruck and K. I. H. Williams, *J. Org. Chem.*, 1973, **38**, 1542.

[4] J. T. Clerc, E. Pretsch, and S. Sternhell, '^{13}C-Kernresonanzspektroskopie,' Akademische Verlagsgesellschaft, Frankfurt, 1973, p. 101.

a 4-ene. Each of these might react by a methyl migration for which there are known precedents [7] and for which they are sterically suited, or they might follow the dienol–benzene pathway. 17β-Acetoxy-3α-deuterio-5α,6α-epoxy-3β-methylsulphonyloxyandrostane was prepared by hydrolysis of 17β-acetoxy-3-trichloroacetoxyandrosta-3,5-diene [8] with methanol containing a few drops of triethylamine followed by immediate reduction with sodium borodeuteride to afford 17β-acetoxy-3α-deuterio-3β-hydroxyandrost-5-ene. This was then converted into its methanesulphonate and epoxidized with *m*-chloroperbenzoic acid.[1] 3β,17β-Diacetoxy-3α-deuterio-4α,5α-epoxyandrostane was prepared by reduction of testosterone acetate with sodium borodeuteride followed by acetylation and epoxidation.[1] 6β,17β-Diacetoxy-3α-deuterioandrost-4-en-3β-ol was prepared by reduction of 6β,17β-diacetoxyandrost-4-en-3-one with sodium borodeuteride in methanol.[1] Although the aromatic protons of 17β-acetoxy-4-methyloestratriene underwent partial exchange on refluxing in 48% deuterium bromide in deuterioacetic acid for 20 min, relatively little exchange took place when the solution was heated to reflux and then immediately cooled. The aromatization reactions were therefore carried out with only 1—2 min reflux. In each case a deuteriated 17β-acetoxy-4-methyloestratriene was isolated and in each case the resonance at 123·2 p.p.m., associated with C-1, had collapsed. Hence despite the presence of 5α-oriented substituents, these reactions had followed pathway *b* in which the 9,10-bond migrates to C-5 and this pathway appears to be established as a general rearrangement route.

EXPERIMENTAL

General experimental details have been described previously.[9]

The ^{13}C n.m.r. spectra were determined on a JEOL PFT-100 Fourier transform spectrometer operating at 25·15 MHz. The spectral width was 250 p.p.m.; 8192 data points were used for 5—10,000 accumulations. The pulse length was 7 μs at a pulse interval of 1·0 s. The samples (80—150 mg) were dissolved in deuteriochloroform (0·5 ml). Tetramethylsilane was used as internal standard. The shifts are estimated to be accurate to within ±0·1 p.p.m.

Deuteriation Reactions.—(*a*) Sodium (100 mg) was dissolved in methan[^{2}H]ol (5 ml) and 4-methyloestra-1,3,5(10)-trien-17-one (500 mg) was added. The solution was heated under reflux for 1 h, cooled, and acidified with acetic [^{2}H]-acid, and the [16-$^2H_2$]steroid, m.p. 176—178°, *m/e* 270, crystallized. The steroid (150 mg) in ether (5 ml) was treated with lithium aluminium hydride (50 mg) for 2 h at room temperature. The suspension was diluted with moist ether, washed with dilute hydrochloric acid, dried, and evaporated. The residue was acetylated with acetic anhydride (1 ml) in pyridine (2 ml) overnight. The solution was poured into dilute hydrochloric acid and the product recovered in chloroform. 17β-Acetoxy-4-methyl[16-$^2H_2$]oestratriene crystallized from light petroleum as needles, m.p. 184—186°.

(*b*) 4-Methyl[7,16-$^2H_4$]oestra-1,3,5(10)-triene-6,17-dione, *m/e* 286, was prepared in a similar manner.

(*c*) Sodium (100 mg) was dissolved in methan[^{2}H]ol (15 ml). 17β-Hydroxyandrosta-1,4-dien-3-one (500 mg) was added and the solution was heated under reflux for 2 h, then acidified with acetic acid. The steroid [*m/e* 290, τ 2·95 (1H, s, 1-H)] was recovered in chloroform, crystallized, and dissolved in methanol. Sodium borohydride (130 mg) was added and the solution was stirred for 3 h. Acetic acid was added, the solution was poured into water, and the steroid was recovered in chloroform. It was heated to reflux in acetic acid (3 ml) containing 48% hydrobromic acid (1 ml) for 2 min and the solution was then poured into aqueous sodium hydrogen carbonate. The steroid was recovered in chloroform and acetylated with acetic anhydride–pyridine to afford 17β-acetoxy-4-methyl[2,6-$^2H_3$]oestratriene, m.p. 183—185°, τ 2·95br and 3·15br (each 1H, s, 1- and 3-H).

(*d*) A solution of 17β-acetoxy-3-trichloroacetoxyandrosta-3,5-diene (490 mg) [8] in spectroscopic grade methanol (25 ml) containing triethylamine (5 drops) was heated on a boiling water-bath for 10 min. The solution was cooled in ice and sodium borodeuteride (250 mg) was added. The solution was set aside at room temperature for 1 h. Dilute hydrochloric acid was added and the steroid was recovered in chloroform. 17β-Acetoxy-3α-deuterioandrost-5-en-3-ol (210 mg) crystallized from acetone–light petroleum as needles, m.p. 135—137°, *m/e* 333. Treatment with methanesulphonyl chloride in pyridine afforded the 3β-methanesulphonate,[10] which was converted into the epoxide as described previously.

Aromatization Reaction: General Procedure.—The steroid (*ca.* 150 mg) in 48% hydrobromic acid (1 ml) and glacial acetic acid (3 ml) was rapidly heated to reflux and maintained at that temperature for 1—2 min. The blue solution was rapidly cooled and poured into aqueous sodium hydrogen carbonate, and the steroid was recovered in chloroform. The steroid was chromatographed on alumina. 17β-Acetoxy-4-methyloestratriene was isolated in the fractions eluted with light petroleum and light petroleum–2·5% ether.

[4/2731 *Received*, 31*st December*, 1974]

[7] D. N. Kirk and M. P. Hartshorn, 'Steroid Reaction Mechanisms,' Elsevier, Amsterdam, 1968, pp. 292, 363.
[8] D. Amar, V. Permutti, and Y. Mazur, *Tetrahedron*, 1969, **25**, 1717.
[9] J. R. Hanson and T. D. Organ, *J. Chem. Soc.* (*C*), 1970, 1065.
[10] J. S. Cochrane and J. R. Hanson, *J. Chem. Soc.* (*C*), 1971, 3730.