Steroid–Acid Colour Reactions. Part II.[1] Carbon-13 Nuclear Magnetic Resonance and Ultraviolet Spectra of Protonated Androst-4-ene-3,17-dione, Pregn-4-ene-3, 20-dione, and Androst-4-ene-3,11,17-trione

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The ^{13}C n.m.r. spectra of carbonium ions formed from the title steroids have been recorded. Basic sites on the same molecule are protonated independently but most charge is located on C-5. These spectra are compared with those of model compounds described in Part I. The u.v. spectra of these steroids in sulphuric acid are discussed in the light of data from the ^{13}C n.m.r. spectra.

COLOURS produced by dissolving steroids in concentrated sulphuric acid are used extensively in clinical analysis. In Part I [1] the ^{13}C n.m.r. spectra of carbonium ions from model compounds for steroids were described and the problem of solvation discussed in detail. In this report we shall describe the spectra of carbonium ions obtained from the title steroids. The three steroids selected are special in that they can be recovered from solution in sulphuric acid unchanged.[2] None of them gives any analytically significant colouration but the study is of value in that steroid carbonium ion formation must be an important step in all steroid–acid colour reactions. Most steroid carbonium ions undergo subsequent reactions of some complexity, such as angular methyl migration,[3] dehydration, and dienone–phenol rearrangement,[4] and the study of these ions will be difficult. A study of stable steroid carbonium ions is an obvious first step in this investigation. It is possible that the coloured materials obtained during clinical analysis arise, not from the steroid, but from some material found in association with the steroid. However, the fact that most patients recover after treatment based on the analyses suggests that this is not the case.

It is possible, by a variety of techniques, to assign all the resonance peaks in a steroid ^{13}C n.m.r. spectrum but this is a major task.[5] In this study the total spectrum of the steroid carbonium ion was recorded in all cases but only the low-field peaks, which include all the sites of protonation, have been reported. Assignment of these peaks can be made without difficulty. Complete peak assignments for a steroid carbonium ion will be reported in a future publication.

[1] Part I, preceding paper.
[2] H. A. Jones, Ph.D. Thesis, University of Western Australia, 1968.
[3] N. L. Wendler, 'Molecular Rearrangements,' ed. P. de Mayo, Interscience, New York, 1964, ch. 16.
[4] D. N. Kirk, D. K. Patel, and V. Petrow, *J. Chem. Soc.*, 1957, 1046.
[5] *e.g.* (*a*) H. J. Reich, M. Jautelat, M. T. Messe, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1969, **91**, 7445; (*b*) W. D. Smith and D. L. Deavenport, *J. Magnetic Resonance*, 1972, **6**, 256; G. Engelhardt, G. Schneider, I. Weisz-Vincoze, and A. Vass, *J. prakt. Chem.*, 1974, **316**, 391; G. Engelhardt, D. Zeigan, B. Schönecker, and K. Ponsold, *Z. Chem.*, 1975, 60; S. Lang, D. N. Lincoln, and V. Wray, *J.C.S. Perkin II*, 1975, 344.

RESULTS AND DISCUSSION

The first step was to determine if protonation of a steroid occurred at sites other than the carbonyl groups. Compound (II) was dissolved in concentrated deuteriosulphuric acid and precipitated by addition of deuterium oxide. The ^{13}C n.m.r. spectrum of this material was identical with that of untreated compound (II), *i.e.* no deuterium had been retained. Therefore, carbonium ion formation does not occur by protonation at positions apart from C-3, C-5, and C-20 or by hydride abstraction.

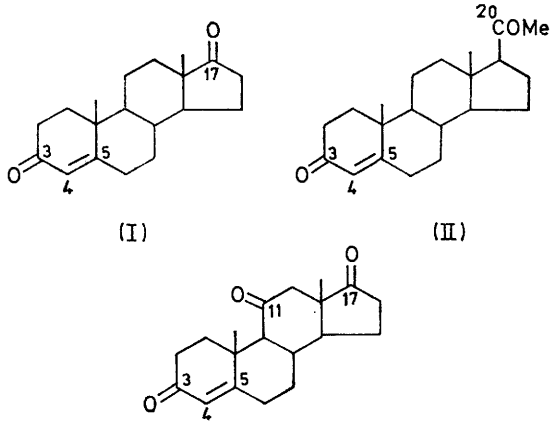


TABLE 1

Variation of chemical shift with acid concentration for androst-4-ene-3,17-dione (I)

$[H_2SO_4]$/M	C-3	C-4	C-5	C-17
8.07	209.6	123.6	189.0	231.7
10.01	211.9	123.1	198.2	234.0
11.40	213.3	122.7	206.8	236.6
14.09	213.6	122.8	210.6	239.9
15.78	213.7	122.8	212.3	245.1
$CDCl_3$	198.9	124.2	170.0	219.9

The latter process has been reported for a number of paraffins.[6]

The three steroids were sufficiently soluble in sulphuric acid more concentrated than 8M for the ^{13}C n.m.r. spectrum to be obtained with 10 000 transients. In very concentrated acid considerable decomposition occurs, the solution goes dark brown, and extra peaks appear in the spectrum. Such spectra are difficult to interpret and have not been used in the present study. The low-field peaks of compound (I) in sulphuric acid are given in Table 1. Peak assignments in $CDCl_3$ were made on the basis of previously published work [5a] and those in sulphuric acid were confirmed by the off resonance spectrum in 11.40M-acid, in which the peak at 122.7 p.p.m. disappeared. This is the only one of the low-field peaks due to a carbon bearing a hydrogen and, on becoming a multiplet, the peak was lost in the general noise level. The spectra of a number of compounds, which are models for steroids, over the same acid range are given in Table 2. The acid concentrations at half-protonation are given for these compounds at the foot of each column.

The data in Table 1 indicate that *both* carbonyl groups of (I) are protonated over the complete range of acid concentrations, although one is a stronger base than the other. This means they must be completely insulated as any interaction between the two would prevent diprotonation. As values of the chemical shift for the completely protonated form of (I) could not be measured, it was impossible to determine the value of $[H_2SO_4]_{\frac{1}{2}}$. By comparing cyclopentanone (Table 2) and C-17 (they are the same in $CDCl_3$) it would appear that C-17 is less basic, if the changes on complete protonation for the two compounds are the same. This is, however, a matter of conjecture.

The shifts for C-3, C-4, and C-5 show interesting features. The change between 8.07 and 15.78M acid for C-3 is only 4.1 p.p.m. while that of cyclohex-2-enone is 8.4 p.p.m. However, for C-5 in (I) the change is 23.3 p.p.m., which is similar to that for C-3 in 2-cyclohexenone. This suggests greater transfer of charge to C-5 in (I) compared to the corresponding transfer in cyclohex-2-enone. As C-5 is a tertiary carbon atom its ability to accommodate positive charge is greater than the corresponding carbon atom in 2-cyclohexenone. The shift for C-3 in (I) reaches its final value in 11.40M acid and is, therefore, apparently more basic than the equivalent site in cyclohex-2-enone. On the other hand, the apparent basicity of C-5 in compound (I) appears to be similar to C-3 in cyclohex-2-enone. The reason for the different apparent basicities of these two interacting sites is differential solvation; the matter has been discussed in detail in Part I.[1] The chemical shift of C-4 in compound

TABLE 2

Variation of chemical shift with acid concentration for various ketones [a]

	Cyclopentanone	Et_2CO	Cyclohexanone		Cyclohex-2-enone	
$[H_2SO_4]$/M	C=O	C=O	C=O	C=O	C-2	C-3
8.07	234.0	225.1	225.7	210.7	129.7	163.3
10.01	235.4	227.1	228.0	213.6	129.0	168.8
11.40	239.7	229.7	232.2	216.4	128.1	176.1
14.09	245.5	235.2	238.5	218.6	127.8	182.8
15.78	249.9	242.2	246.3	219.1	127.3	185.3
$CDCl_3$	219.7	211.9	211.8	199.4	129.9	150.5
$[H_2SO_4]_{\frac{1}{2}}$/M	14.5	14.9	13.6	9.9		11.3

[a] Data from Part I, preceding paper.

[6] J. W. Otvos, D. P. Stevenson, C. D. Wagner, and O. Beeck, *J. Amer. Chem. Soc.*, 1951, **73**, 5741; R. L. Burwell and G. S. Gordon, *ibid.*, 1948, **70**, 3128.

(I) remains essentially unaffected by protonation, as was the case with cyclohex-2-enone. The detection of extensive delocalization of positive charge in ring A of compound (I), as shown by the changes in chemical shift of C-5, confirms the results of the work on the proton n.m.r. of steroid carbonium ions by Jones.[7]

Similar data for pregn-4-ene-3,20-dione (II) are given in Table 3. Here there is an even smaller change on C-3

TABLE 3

Variation of chemical shift with acid concentration for pregn-4-ene-3,20-dione (II)

$[H_2SO_4]$/M	C-3	C-4	C-5	C-20
8.07	210.2	123.6	191.1	221.3
10.01	210.3	123.3	196.5	224.1
11.40	211.2	122.9	202.6	227.4
14.09	213.4	122.6	211.8	235.1
15.78	213.8	122.7	213.5	242.0
$CDCl_3$	199.1	124.0	170.7	209.0

and a larger one on C-5 than with (I). There is no obvious reason for this as ring A is essentially the same in compounds (I) and (II). C-4 Remains essentially unaffected by protonation. The change of shift for C-20 (20.7 p.p.m.) is slightly larger than the comparable change for the model compounds, diethyl ketone (17.1 p.p.m.) and there is no indication that the change in chemical shift is complete in 15.78M-acid.

The results for androst-4-ene-3,11,17-trione (III) are given in Table 4. Here there is a slight problem of peak

TABLE 4

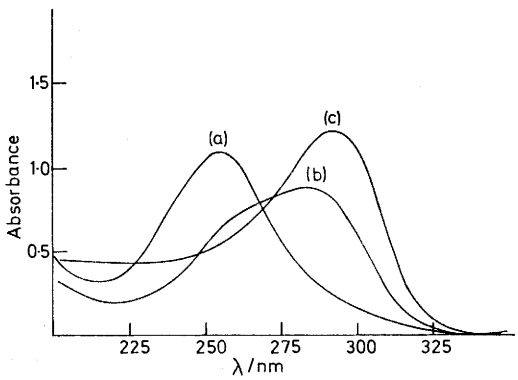
Variation of chemical shift with acid concentration for androst-4-ene-3,11,17-trione (III)

$[H_2SO_4]$/M	C-3	C-4	C-5	C-11	C-17
8.07	210.3	124.4	185.5	215.3	227.6
10.01	212.5	124.0	195.1	216.2	228.9
11.40	213.8	123.6	200.0	217.0	229.9
14.09	214.6	123.7	205.0	218.9	232.0
15.78	214.7	123.7	206.4	220.3	233.7

assignment as the shifts for C-11 and C-17 do not differ very much. The values listed for C-17 are similar to those of C-17 in compound (I). The assumed values for C-11 are at higher field and this is consistent with the sterically perturbed position of C-11.[8] The order also corresponds to that of the model compounds (Table 2). The values for C-3, C-4, and C-5 show the same pattern as in compounds (I) and (II); there is only a small change in C-3, C-4 undergoes a slight *upfield* movement, and the largest change is in C-5. It is clear that cyclohex-2-enone is a good model for the A ring of these steroids, but in steroid carbonium ions a greater proportion of the charge is located on C-5 than on the equivalent atom in cyclohex-2-enone.

The chemical shifts of C-11 and C-17 in compound (III) show a new feature. Both carbonyl groups undergo protonation but the changes in chemical shift (5.0 and 6.1 p.p.m. respectively) over an equal change in acid concentration are smaller than for cyclopentanone (15.9 p.p.m.), cyclohexanone (13.9 p.p.m.), and C-17 of compound (I) (13.6 p.p.m.). This suggests that the steroid may protonate on one site or the other. It is probably not valid to establish the apparent basicity of the carbonyl group at C-11 by comparison with cyclohexanone as cyclohexanone is not a good model for the C ring of compound (III) fused, as it is, to two other rings.

This study has shown that the steroids (I), (II), and (III) protonate on carbonyl oxygens to give carbonium ions with ^{13}C n.m.r. spectra which can be resolved, that remote sites protonate independently but proximity prevents diprotonation, that extensive delocalization of charge occurs, and that cyclopentanone, cyclohexanone, and cyclohex-2-enone are fairly good models for such a study of steroid carbonium ions.



Spectra of 6.78×10^{-5}M pregn-4-ene-3,20-dione in sulphuric acid: (a) 5.83M, (b) 10.01M, and (c) 17.22M

A further aim in this study was to correlate the u.v. spectra of steroids in sulphuric acid with the pattern of protonation determined by ^{13}C n.m.r. spectroscopy. Spectra of steroids in nonpolar solvents, where there is little interaction between solvent and steroid, have been recorded by Dorfman [9] and by Dusza *et al.*[10] Zaffaroni [11] examined the spectra of several steroids in 98% sulphuric acid but the solutions were set aside for 2 h before the spectra were recorded and it is possible that reaction had occurred in some cases. This was extended to a comprehensive study by Bernstein and Lenhard [12] but the fullest investigation is that of Jones.[2,13]

With the steroids under consideration in this study the variation of spectrum with acidity was deceptively simple. The general effect was a change in the absorption maximum from *ca.* 240 to 290 nm as the acid concentration was changed from 1.81 to 17.22M. A selection of the spectra for (II) is shown in the Figure. The positions of the maxima for all three steroids are given in

[7] H. A. Jones, *J. Chem. Soc.* (*B*), 1971, 99.
[8] D. M. Grant and B. V. Cheney, *J. Amer. Chem. Soc.*, 1967, **89**, 5315.
[9] L. Dorfman, *Chem. Rev.*, 1953, **53**, 47.
[10] J. P. Dusza, M. Heller, and S. Bernstein, 'Physical Properties of the Steroid Hormones,' ed. L. L. Engel, Pergamon Press, Oxford, 1963, pp. 69—287.
[11] A. Zaffaroni, *J. Amer. Chem. Soc.*, 1950, **72**, 3828.
[12] S. Bernstein and R. H. Lenhard, *J. Org. Chem.*, 1953, **18**, 1146; 1954, **19**, 1269; 1960, **25**, 1405.
[13] H. A. Jones, *Canad. J. Spectroscopy*, 1971, **16**, 1.

Table 5. The absorbance was hardly affected by protonation. From the Figure it is seen that there are no isosbestic points so that the results in Table 5 cannot be due to a mixture of two absorbing species unless the extinction coefficients vary with the acidity. In any event the data cannot be analysed by the treatment of Coleman *et al.*[14] to give further information on the equilibrium constants.

TABLE 5

Variation of absorption maxima (in nm) with acid concentration for steroids (I), (II), and (III)

$[H_2SO_4]$/M	(I)	(II)	(III)
1.81	248	249	245
5.83	254	255	248
10.01	282	283	260
11.40	287	288	278
14.09	288	290	282
17.22	292	293	284

The similarity of the spectral changes for three different steroids suggests that only ring A, which is the same in all three, is involved in u.v. absorption. The delocalization of charge over C-3, C-4, and C-5 may produce the observed bathochromic shift. In view of what has been said about the protonation of cyclohex-2-enone and differential solvation, it is not surprising that spectral changes in steroids do not follow the H_0 acidity function.[15] The u.v. spectra of stable steroid carbonium ions are obviously not simple but more detailed study is not of great interest. Those steroids which give intense chromophores on protonation are a different matter and will be considered in a future publication.

EXPERIMENTAL

Materials.—The steroids were commercial samples dried before use. Deuteriosulphuric was prepared by addition of sulphur trioxide to D_2O. Standard sulphuric acid was prepared as described previously.[1]

Spectral Measurements.—The ^{13}C n.m.r. technique has been described previously.[1] The samples were 0.5M in steroid; extensive decomposition occurred in the time required for weaker solutions. The solutions for u.v. study were prepared by adding a concentrated solution of steroid in ethanol (0.1 ml) to standard sulphuric acid (10 ml). The spectra were recorded on a Unicam SP 800 spectrophotometer.

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[14] J. S. Coleman, L. P. Barga, and S. H. Mastin, *Inorg. Chem.*, 1970, **9**, 1015.
[15] R. I. Zalewski, Ph.D. Thesis, University of Poznań, 1967.