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MODIFIED STEROID HORMONES—XLIII[1]

THE STEREOCHEMISTRY OF THE EPIMERIC 17α-ACETOXY-6-HYDROXY-6-HYDROXYMETHYLPREGN-4-ENE-3,20-DIONES

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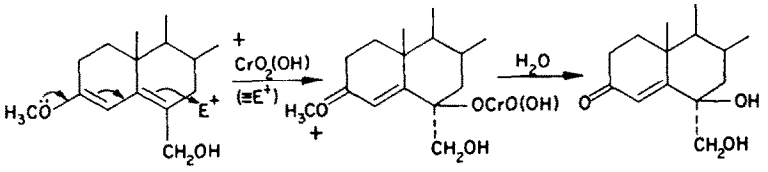
Abstract—The preparation of 17α-acetoxy-6β-hydroxy-6α-hydroxymethylpregn-4-ene-3,20-dione is described. The configurations of the C_6-substituents in this compound and in the epimeric 17α-acetoxy-6α-hydroxy-6β-hydroxymethylpregn-4-ene-3,20-dione[1] have been established by ORD, NMR and IR spectroscopy. The conformations adopted by the C_6-substituents are discussed.

THE preparation of a 17α-acetoxy-6-hydroxy-6-hydroxymethylpregn-4-ene-3,20-dione, conveniently referred to herein as "isomer A", was described in Part XLII[1] of this series. We now record the preparation of an epimeric compound, referred to herein as "isomer B", and present spectroscopic evidence for the assignment of configurations to the C_6-substituents present in these intermediates.

"Isomer A" was obtained by hydroxylation of 17α-acetoxy-6-methylenepregn-4-ene-3,20-dione[2] (I) with osmium tetroxide, and, on the assumption that attack of the reagent occurs on the α-face of the steroid molecule, the product would be expected to have the 6α-hydroxy-6β-hydroxymethyl stereochemical configuration (IIA). "Isomer B", in contrast, was prepared (30–35% yield) by oxidation of 17α-acetoxy-6-hydroxymethyl-3-methoxypregna-3,5-diene-3,20-dione[2] (III) with not more than 1·2 equivs of the Jones reagent.[3] Under these experimental conditions, the 6-methylen-4-en-3-one (I) was also obtained as a minor reaction product (10%), and was evidently formed by acid solvolysis of the starting material (III). The conversion of the

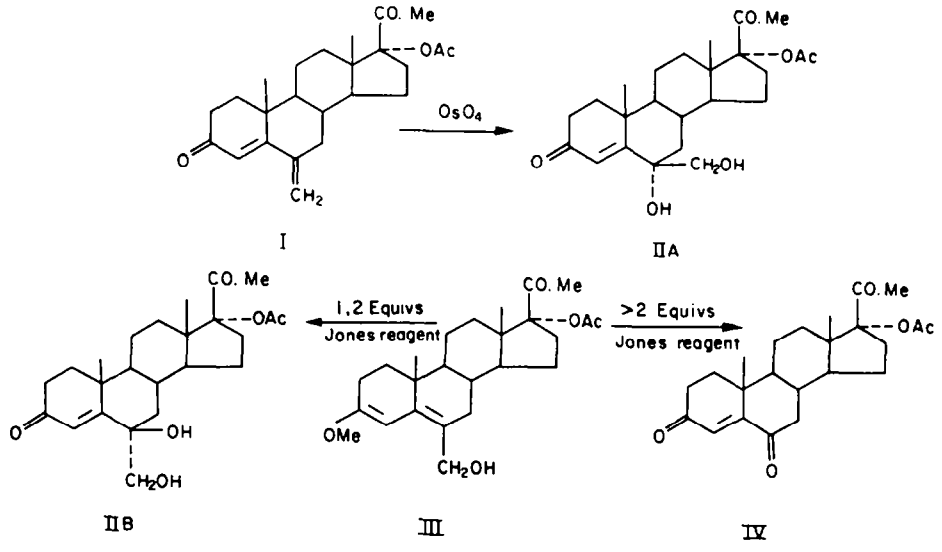


last compound into a 6-hydroxy-6-hydroxymethyl-4-en-3-one may be rationalized on the basis of the following mechanistic interpretation in which the β-approach of the electrophilic reagent is assumed.[4] It is seen that such a mechanism leads to the probability of a 6β-hydroxy-6α-hydroxymethyl stereochemical configuration (IIB) for "isomer B".

[1] Part XLII, G. Cooley, M. T. Davies, B. Ellis and V. Petrow, *Tetrahedron* **22**, 365 (1966).
[2] D. Burn, G. Cooley, M. T. Davies, J. W. Ducker, B. Ellis, P. Feather, A. K. Hiscock, D. N. Kirk, A. P. Leftwick, V. Petrow and D. M. Williamson, *Tetrahedron* **20**, 597 (1964).
[3] K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.* 39 (1946).
[4] L. H. Knox, E. Velarde, S. Berger, D. Cuadrillo, P. W. Landis and A. D. Cross, *J. Amer. Chem. Soc.* **85**, 1851 (1963).

It is of interest to record that when the amount of Jones reagent was increased to two or more equivs, the only isolable reaction product (20–25%) was 17α-acetoxypregn-4-ene-3,6,20-trione (IV), formed possibly by oxidative decarboxylation of a vinylogous β-keto-acid, or by acid-catalysed retro-aldol cleavage followed by oxidation at C_6.

The approach to C_6-configurational problems in 5-substituted-4-en-3-ketones by physical methods involving investigations of the spectroscopic properties of the



conjugated system (UV absorption or polarization spectroscopy), or of the magnetic shielding of the C_4, C_6 and C_{19} protons (NMR spectroscopy), is complicated when C_6 carries two substituents which mutually interact. Such interaction may be expected to result in some modification of the electrical properties of each substituent as determined from a study of each of the corresponding 6-mono-substituted-4-en-3-ketones taken as standards of reference. The 6-hydroxy-6-hydroxymethyl moiety is a 1,2-diol (primary and tertiary alcohols), and, as shown below, spectral observations indeed point to the existence of considerable interaction between these substituents in both isomers "A" and "B".

(a) *UV studies.* The effect of C_6 substituents upon the wavelengths of the K- and R-band maxima, and the rotatory dispersive characteristics of the latter region of the UV spectrum of the 4-en-3-ketone system, have been reviewed.[5] No data is available for C_6-disubstituted-4-en-3-ketones, or for the —CH_2OH substituent.

On the assumption that the effects of methyl and hydroxyl groups on the position of the K-band maximum at ca. 240 mμ are simply additive, and further, that hydroxymethyl and methyl groups have identical effects thereon, compounds represented by structures IIA and IIB may be calculated to show $\Delta\lambda$ (relative to the corresponding unsubstituted 4-en-3-ketone) of +1 to −2 mμ, and −2 to −6 mμ, respectively. We have now found experimentally that "isomer A" shows $\Delta\lambda = +2{\cdot}5$ mμ, whereas "isomer B" shows $\Delta\lambda = -4$ mμ. These results suggest that if the two compounds

[5] M. T. Davies and V. Petrow, *Tetrahedron* **19**, 1771 (1963).

are indeed represented by structures IIA and IIB respectively, the foregoing assumptions either annul each other or have some reasonable validity.

Comparison of the ORD spectra of isomers "A" and "B" (see Fig.) with those of 6-hydroxy- and 6-methyl-4-en-3-ketones[5,6] leads to the following considerations. When allowance is made for the greater positive background (due to the 17β-acetyl group), the curve of "isomer A" reveals a considerably more intense R-band Cotton effect, but of the same sign, than that of a 6β-methyl-4-en-3-ketone. The Cotton effect shown by "isomer B" has the same sign as that of "isomer A", but with an amplitude lower than that associated with a 6β-methyl substituent in 4-en-3-ketones.

It is now well established that reversal of the sign of the complex R-band Cotton effect observed on passing from a 6α- to a 6β-methyl-4-en-3-ketone system arises from the creation of an intense 1,3-diaxial interaction between the 6β- and 10-methyl groups, with consequent change in the chirality of the conjugated chromophore.[7] Interaction of this character is considerably less when the C_6-substituent is an hydroxyl group, the ORD curves of epimeric 6-hydroxy-4-en-3-ketones having the same sign.

Under conditions of free rotation of the substituents with respect to the steroid skeleton, an hydroxymethyl group would be expected to have a greater effective radius than a methyl group, and in the β-configuraton at C_6 should lead to even greater interference with the C_{10}-methyl group than is observed with 6β-methyl-4-en-3-ketones. An α-oriented C_6-hydroxymethyl group, in contrast, might be expected to interact with the semi-eclipsed C_4-hydrogen atom, and, if this interaction is significant, the resulting chirality change of the chromophore would be of the same sign as that resulting from the introduction of a β-oriented C_6-hydroxymethyl group although the effect would be less marked. The observed differences between the curves of isomers "A" and "B" and those of the 6,21-dimethylethisterones[5] are considerable and must arise from loss of conformational freedom of the C_6-substituents. This conclusion is supported by IR evidence presented below. Restriction of movement of the C_6-substituents with respect to the steroid skeleton is not expected to reduce the degree of interaction of a 6β-hydroxymethyl group with the C_{10}-methyl group, or of a 6α-hydroxymethyl group with the C_4-hydrogen atom; neither is it expected to lead to reversal in magnitude of these last effects upon the Cotton amplitudes of the corresponding ORD curves of 6-hydroxymethyl-6-hydroxy-4-en-3-ketones. The foregoing considerations lead to the assignment of structures IIA and IIB to isomers "A" and "B" respectively.

(b) *NMR studies.* Collins *et al.*[8] have discussed the proton resonance spectra of the epimeric 6-hydroxy- and 6-methylcholest-4-en-3-ones, and have noted characteristic changes in chemical shift of the C_4 and C_{19} protons, and in the allylic splitting pattern of the C_4 proton, depending upon the nature and configuration of the C_6-substituent. In the Table we have listed the chemical shifts of the major components of the spectra of isomers "A" and "B", of 17-acetoxyprogesterone, and of the relevant bands in the spectra of cholest-4-en-3-one and its 6-hydroxy and 6-methyl derivatives.[8]

Inspection of the data reveals that a 6α-hydroxy-substituent has a very marked deshielding effect upon the C_4 proton, whereas the effect of a 6β-hydroxyl group is negligible. The deshielding of the C_{19} protons is in the reverse order, as expected,

[6] C. Djerassi, R. Riniker and B. Riniker, *J. Amer. Chem. Soc.* **80**, 4001 (1958).
[7] W. B. Whalley, *Chem. and Ind.* 1024 (1962).
[8] D. J. Collins, J. J. Hobbs and S. Sternhell, *Tetrahedron Letters*, 197 (1963).

TABLE. CHEMICAL SHIFTS (TAU SCALE) AND COUPLING CONSTANTS (C/S) OF THE PRINCIPAL FEATURES IN THE NMR SPECTRA OF 17-ACETOXYPROGESTERONE, ISOMERS "A" AND "B", AND OF CHOLEST-4-EN-3-ONE[8] AND ITS 6-HYDROXY- AND METHYL-SUBSTITUTED DERIVATIVES

	Feature					
Compound	C_4H	C_6—CH_2O—	$C_{19}H$	17α-OCO-CH_3	17β-COC-H_3	$C_{18}H$
17α-Acetoxypregn-4-en-3,20-dione (17-acetoxy-progesterone)	4·24		8·795	7·95	7·89	9·310
"Isomer A" (identified as compound IIA)	3·64 (−0·60)	6·38 broad, unresolved	8·790 (−0·005)	7·95	7·89	9·318 (+0·008)
"Isomer B" (identified as compound IIB)	3·92 (−0·32)	6·19, 6·48 (AB type quartet J, 11)	8·595 (−0·200)	7·96	7·90	9·282 (−0·028)
Cholest-4-en-3-one[8]	4·45		8·84			
6α-methyl „	4·35 (−0·10)		8·81 (−0·02)			
6β-methyl „	4·36 (−0·09)		8·73 (−0·10)			
6α-hydroxy „	3·81 (−0·64)		8·81 (−0·02)			
6β-hydroxy „	4·38 (−0·07)		8·67 (−0·16)			

Figures in parentheses are additional chemical shifts, relative to the corresponding feature in the spectrum of the appropriate reference compound, due to the presence of the 6-substituent(s). (The negative sign refers to shifts to lower field.) All signals are singlet, unless otherwise indicated, and refer to solutions in deuteriochloroform, tetramethylsilane as internal standard.

and is also smaller in magnitude. Similar, but much smaller effects are observed for 6-methyl substituents. In considering the effect of simultaneous substitution at C_6 by a methyl and a hydroxy group, it is assumed (as a first and over-simplified approximation), that the effect of each substituent is strictly additive. The following additional chemical shifts for C_4 and C_{19} protons, consequent upon simultaneous 6-hydroxy-6-methyl substitution may be calculated on this basis:

	C_4H	$C_{19}H$
6α-CH_3; 6β-OH	−0·17 ppm	−0·18 ppm
6β-CH_3; 6α-OH	−0·73 ppm	−0·12 ppm

These predictions are in good semi-quantitative agreement with the additional chemical shifts observed for the same features in the spectra of isomers "B" and "A" respectively, the major deviations resulting from (i) the assumptions involved in proposing strict additivity of the substituent deshielding effects (as measured in isolation from their co-substituents) and (ii) the fact that the deshielding power of a hydroxymethyl group must be greater and more anisotropic in effect than that of an unsubstituted methyl group. The differences between predicted and observed additional chemical shifts are discussed in the following section. Analysis of the NMR spectra of isomers "A" and "B" thus identifies these compounds with structures IIA and IIB respectively.

Conformational analysis of the 6-hydroxy-6-hydroxymethyl systems in IIA *and* IIB. The IR spectrum of IIA in dilute solution (ca. 0·01M) in CH_2Cl_2 exhibits a low intensity shoulder at ca. 3610 cm^{-1} (non-bonded OH stretching), a sharp peak at 3580 cm^{-1} (intramolecular hydrogen-bonded OH), and finally a broad weak band near 3450 cm^{-1} due to intermolecular hydrogen bonding common to sterically unhindered hydroxylic compounds. The corresponding spectrum of IIB shows not only all these bands but an additional peak at 3540 cm^{-1} of intensity almost equal to that at 3580 cm^{-1}.

The following tentative explanation is advanced to account for these bands. The weakness of the "free" hydroxyl bands near 3610 cm^{-1} indicates that both hydroxyl groups are perturbed, either by 1,2-hydrogen bonding or by interaction with the conjugated system. In IIA it is suggested that the proton-donating hydroxyl is the primary (i.e. CH_2OH—O), $\Delta\nu$ ("free" to bonded) $\approx$ 40 cm^{-1}.[9] Using the solvent shift data of reference,[9] and the non-bonded frequency of a typical 3° hydroxyl group (mean of axial and equatorial data) in CCl_4 solution[10] (the further assumption being made that solvent effects are identical for 1° and 3° hydroxyl in this case), the predicted non-bonded stretching frequency of a 3° —OH group in CH_2Cl_2 is 3595 cm^{-1}. The difference between this frequency and the observed band of 3580 cm^{-1}, displaying no intense feature on its high frequency flank, is possibly due to alignment of the OH dipole with that of the 4-en-3-keto-system, resulting in a slight lowering of the dipole moment and of the stretching frequency. Equally possible is the lowering of the 3° hydroxyl through the direct influence of the primary 1,2-OH—O bond already mentioned.

In IIB the same hydroxyl group would appear to be responsible for the intramolecular OH—O bond as in IIA, but the axial position of the 6β-hydroxyl group may permit the formation of a weak OH—π bond, the stretching frequency of this tertiary OH being lowered to 3540 cm^{-1} in consequence.

From these observations it is suggested that the OH group of the hydroxymethyl substituent is always orientated towards the oxygen atom of the tertiary hydroxyl, in a plane perpendicular to that of the steroid skeleton. The C_6-hydroxyl is itself probably aligned throughout virtually parallel with the dipole of the conjugated system, these orientations constituting the normal conformational arrangement for the C_6 centre in IIA and IIB.

Returning to the NMR spectral analysis, and especially to the deviations between the loosely made predictions for the additional chemical shifts of the C_4 and C_{19} protons of cholest-4-en-3-one consequent upon disubstitution at C_6 by hydroxyl plus methyl, and those observed for the corresponding groups in compounds IIA and IIB, CH_3 being identified with CH_2OH, it is now possible to see why such deviations arise. As an example, the conformation adopted by the CH_2OH group in IIB will necessarily bring the oxygen atom of this group to within 2·5 Å of the centre of the C_4 hydrogen, the nucleus of which would be expected to suffer greater deshielding than that produced by a 6α-methyl substituent; in fact, a shift of 0·32 ppm downfield, as opposed to the predicted shift of 0·17 ppm, based on 6α-CH_3—6β-OH. Similarly, in the case of IIA, hydrogen bonding onto the oxygen of the 6α-hydroxyl will lower

[9] A. Allerhand and P. von R. Schleyer, *J. Amer. Chem. Soc.* **85**, 371 (1963).
[10] A. R. H. Cole, G. T. A. Müller, D. W. Thornton and R. L. S. Willix, *J. Chem. Soc.* 1218 (1959).

the dipole moment of this group, and hence its deshielding action upon the C_4 proton, which should now exhibit a lower chemical shift than that predicted for 6β-CH_2OH—6α-OH, i.e. 0·73 ppm. The observed additional chemical shift for the C_4 proton of

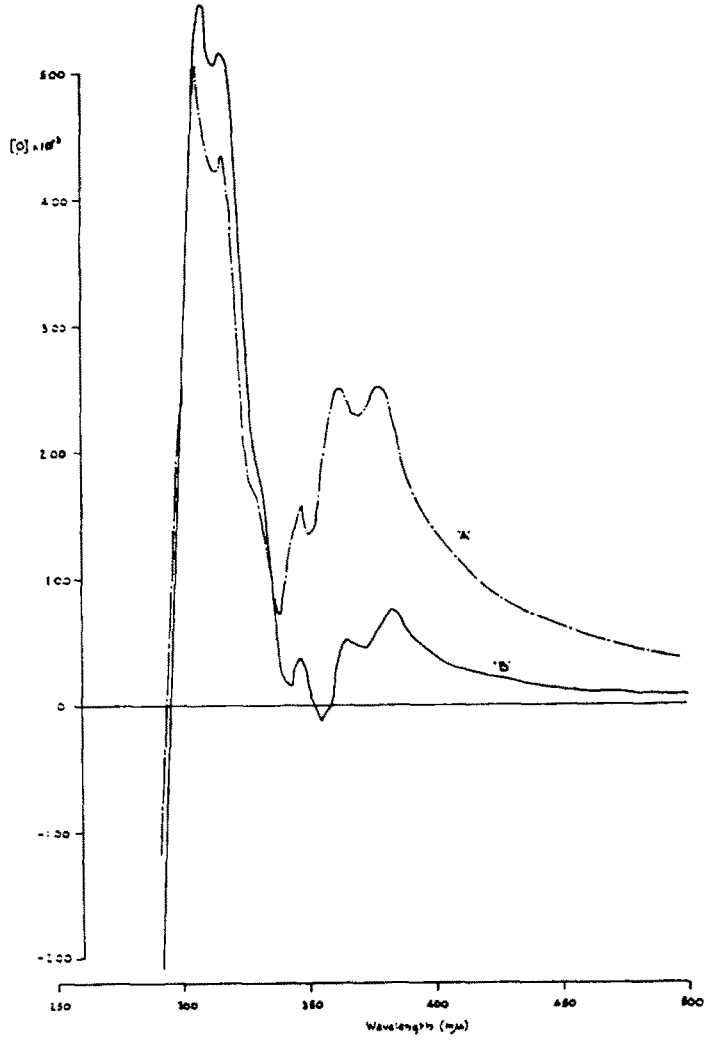


FIG. 1. ORD spectra of "isomer A" (17α-acetoxy-6α-hydroxy-6β-hydroxymethylpregn-4-ene-3,20-dione, IIa) (–·—·–) and "isomer B" (17α-acetoxy-6β-hydroxy-6α-hydroxymethylpregn-4-ene-3,20-dione (————).

IIA is 0·60 ppm. These correlations, although tentative, appear to be reasonably accurate, and therefore lend considerable weight to the validity of both the IR interpretation, and to the basic NMR analysis.

EXPERIMENTAL

Physical measurements. Experimental conditions relating to the measurement of the IR, UV and NMR spectra were as described previously.[1] Optical rotatory dispersion spectra were determined

with a Polarmatic 62 Spectropolarimeter,[11] at ca. 28°, using solutions of concentration ca. 0·1% in AR dioxan.

ORD data (recorded as molecular rotations, $[\phi]_\lambda$) (p = peak, tr = trough).

(i) 17α-*Acetoxy*-6α-*hydroxy*-6β-*hydroxymethylpregn*-4-*en*-3,20-dione (*IIA*, "*Isomer A*"). 377 mμ (p, +2520°); 370 mμ (tr, +2300°); 363 mμ (p, +2510°); 350 mμ (tr, +1360°); 347 mμ (p, +1580°); 338 mμ (tr, +730°); 316 mμ (p, +4350°); 313 mμ (tr, +4230°); 305 mμ (p, +5050°).

(ii) 17α-*Acetoxy*-6β-*hydroxy*-6α-*hydroxymethylpregn*-4-*en*-3,20-*dione* (IIB, "Isomer B"). 383 mμ (p, +758°); 371 mμ (tr, +464°); 365 mμ (p, +522°); 355 mμ (tr, −112°); 347 mμ (p, +380°); 343 mμ (tr, +159°); 315 mμ (p, +5170°); 312 mμ (tr, +5070°); 308 mμ (p, +5580°).

Materials used

(i) 17α-*Acetoxy-progesterone*. The sample used in this work was an analytical sample from the reference collection of these Laboratories, and possessed the following physical constants: m.p. 243–245°, $[\alpha]_D^{20}$ +57° (1·0%, dioxan) [Lit.[12] 243–244·5°, +56° (dioxan at 20°)], λ_{max}^{EtOH} 239 mμ (ε, 16,200).

(ii) 17α-*Acetoxy*-6β-*hydroxy*-6α-*hydroxymethylpregn*-4-*en*-3,20-*dione* (IIB). To a stirred solution of III (4 g) in acetone (100 ml) at 0° was added, in one portion, 2·8 ml CrO_3–H_2SO_4 reagent.[8] After 2 min, 4 ml isopropanol was added, followed by 500 ml water, and the precipitated solid (ca. 0·4–0·5 g) was collected. After crystallization from CH_2Cl_2–MeOH this material melted at 240–242°, not depressed in admixture with authentic (I). Its IR spectrum was also identical with that of I. Extraction of the mother liquors with $CHCl_3$ afforded a gummy solid which was chromatographed on silica gel (50 g). Elution with acetone–CH_2Cl_2 (1:5) gave 17α-*acetoxy*-6β-*hydroxy*-6α-*hydroxymethylpregn*-4-*en*-3,20-*dione*, needles, m.p. 215–218° (dec), $[\alpha]_D$ −16·4°, λ_{max}^{EtOH} 237 mμ (e, 13,220); νCH_2Cl_2. 3700–3200 cm^{-1} (complex absorption, see text), 1729, 1713 cm^{-1} (17α-acetoxy-20-ketone C=O stretching bands), 1671, 1606 (C=O, C=C stretching bands of 4-en-3-ketone system). (Found: C, 68·45; H, 8·4. $C_{24}H_{34}O_6$ requires C, 68·9; H, 8·2%.)

[11] Manufactured jointly by Bellingham & Stanley Ltd., London, and Bendix Electronics Ltd., Nottingham.

[12] R. B. Turner, *J. Amer. Chem. Soc.* **75**, 3489 (1953).