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MODIFIED STEROID HORMONES—XLIV[1]

THE PREPARATION AND STEREOCHEMICAL ANALYSIS OF STEROIDAL-2-ACETOXY-4,6-DIEN-3-KETONES

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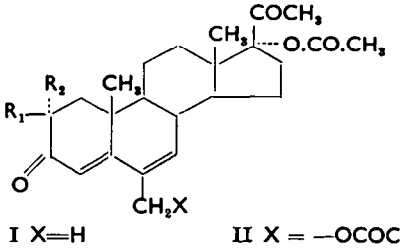
Abstract—The preparation of the 2α- and 2β-acetoxy derivatives of 17α-acetoxy-6-methylpregn-4,6-dien-3,20-dione is described. The configurations of the C_2-substituents in these compounds, and in the epimeric 2α- and 2β-acetoxy derivatives of 17α-acetoxy-6-acetoxymethylpregna-4,6-dien-3,20-dione have been elucidated by IR, UV and NMR spectroscopy. It is shown that 2β-acetoxylation of 4,6-dien-3-ketones has little distortive effect upon the semi-chair conformation adopted by ring A, this behaviour contrasting with that of the more flexible rings of either saturated 5α-3-ketones or 4-en-3-ketones.

DIRECT acetoxylation of steroidal-4-en-3-ketones with lead tetra-acetate leads in general to mixtures of the corresponding 2α- and 2β-acetoxy derivatives.[2] We have now found that acetoxylation of 17α-acetoxy-6-methyl-pregna-4,6-dien-3,20-dione[3] (megestrol acetate, Ia) under similar conditions also gives a mixture of the 2α- and 2β-isomers. These compounds were isolated by fractional crystallization and obtained in yields of approximately 20% and 15% respectively (no exhaustive examination for other products of the reaction was carried out at this stage). In contrast acetoxylation of the corresponding 6-acetoxymethyl analogue (IIa) gave an amorphous solid from which only the 2β-acetoxy derivative could be isolated, the 2α-isomer being obtained from it by an epimerization procedure.[4]



I X=H II X = —OCOCH$_3$

a. R_1 = R_2 = H
b. R_1 = H; R_2 = —OCOCH$_3$
c. R_1 = —OCOCH$_3$; R_2 = H

[1] Part XLIII, D. Burn, G. Cooley, M. T. Davies, B. Ellis, V. Petrow and J. P. Yardley, *Tetrahedron* **22**, 369 (1966).

[2] F. Sondheimer, St. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, *J. Amer. Chem. Soc.* **75**, 4712 (1953).

[3] B. Ellis, D. N. Kirk, V. Petrow, (Mrs.) B. E. Waterhouse and D. M. Williamson, *J. Chem. Soc.* 2828 (1960).

[4] G. Cooley, M. T. Davies, B. Ellis and V. Petrow, *Tetrahedron* **22**, 365 (1966).

The assignments of configurations to the C_2 substituents present in I(b, c) and II(b, c) are based primarily on IR and UV/ORD spectral data. These observations also give the first indication that ring A is conformationally less mobile, under the influence of C_2–C_{10} diaxial interaction, than the corresponding rings of saturated 5α- and 4-en-3-ketones. The NMR spectra confirm this view, by providing semi-quantitative data of the relative geometries of the C_1 and C_2 centres, which can be reconciled only with a semi-chair conformation of ring A, scarcely distorted by the introduction of a 2β-acetoxy substituent.

Comparison has also been made of the changes in molecular rotation ($[\phi]_D$) attending 2-acetoxy-substitution in 5α-3-ketones, 4-en-3-ketones and 4,6-dien-3-ketones. The results obtained show that the use of molecular rotation increments in configurational assignment, in these and similar circumstances, is invalid.

EXPERIMENTAL

Details of instrumentation, solvents, etc used to determine the physical data reported herein have been described.[1,4] Samples of Ia, IIa, IIb and IIc were analytical[3,4] and the sample of testosterone was BPC grade, used without further purification. The ORD characteristics of Ia not hitherto reported, are as follows: 402 mμ (peak, +4660°); 397 mμ (trough, +4430°); 388 mμ (p, +5130°); 374 mμ (inflection, +1780°); 354 mμ (infl, −3800°); 340 mμ (tr, −6650°); 334 mμ (infl, −6180°); 316 mμ (infl, −2280°); 311 mμ (p, −1930°). (*c*, 0·02 g/100 ml, dioxan).

2α,17α-*Diacetoxy*-6-*methylpregna*-4,6-*dien*-3,20-*dione* (Ib). Compound Ia (5 g) in warm glacial acetic acid (75 ml) was treated with lead tetra-acetate (7·5 g) and the mixture heated at 100° for 2½ hr. Water was added to the point of turbidity and the mixture chilled thoroughly overnight. The precipitated crystalline solid was collected by filtration and purified from MeOH to give 2α,17α-*diacetoxy*-6-*methylpregna*-4,6-*dien*-3,20-*dione* (1·08 g), prisms, m.p. 278–283°, $[\alpha]_D^{27°}$ +3·1° (*c*, 0·73), $\nu^{CH_2Cl_2}$ 1736, 1716, 1674, 1625, 1581 cm^{-1}. ORD: 397 mμ (p, +2690°); 391 mμ (tr, +2370°); 385 mμ (p, +2470°); 344 mμ (tr, −1300°); 320 mμ (infl, +5020°); 309 mμ (p, +8490°). (*c*, 0·02 g/100 ml dioxan.) Found: C, 70·47; H, 7·66. $C_{26}H_{34}O_6$ requires: C, 70·56; H, 7·74%.

2β,17α-*Diacetoxy*-6-*methylpregna*-4,6-*dien*-3,20-*dione* (Ic). The aqueous filtrate from the last experiment was diluted with a further 800 ml water, the precipitated solid filtered off, washed thoroughly with water and dried. Purification from acetone–hexane gave 2β,17α-*diacetoxy*-6-*methylpregna*-4,6-*dien*-3,20-*dione* (0·75 g) as needles, m.p. 238°, $[\alpha]_D^{27°}$ −70·5° (*c*, 0·87); $\nu^{CH_2Cl_2}$ 1730, 1710, 1661, 1622, 1574 cm^{-1}. ORD: 414 mμ (p, +2800°); 408 mμ (tr, +2500°); 397 mμ (p, +3150°); 381 mμ (infl, −1250°); 363 mμ (infl, −8400°); 329 mμ (tr, −16,600°); 325 mμ (p, −16,400°). (*c*, 0·02 g/100 ml, dioxan). Found: C, 69·99; H, 7·64. $C_{26}H_{34}O_6$ requires: C, 70·56; H, 7·74%.

DISCUSSION

(a) *IR data.* A marked rise in the carbonyl stretching frequency with increasing coplanarity of the C=O and C-halogen bonds has been observed with saturated α-halocyclohexanones.[5] This effect is attributed to dipole–dipole interaction, resulting in decreased polarization of the C=O bond, with consequent rise in stretching frequency.

Similar effects are observed with the stretching frequency of the 12-keto-grouping in hecogenin acetate[6] (Table 1), consequent upon 11-acetoxylation. In this case, however, there is a noticeable increase in the keto-stretching frequency of the axial as well as the equatorial derivative. This presumably is due to the greater asymmetry of an acetoxy- relative to a halogeno-group. The situation is further complicated when conformational distortion occurs, as with steroidal-4-en-3-ketones, where β-substitution forces

[5] L. J. Bellamy and R. L. Williams, *J. Chem. Soc.* 4294 (1957) and Refs cited therein.
[6] J. Elks, G. H. Phillipps, T. Walker and L. J. Wyman, *J. Chem. Soc.* 4330 (1956).

TABLE 1. IR AND UV ABSORPTION DATA FOR 2-ACETOXY-3-KETO-STEROIDS AND KETONES REFERRED TO IN THE TEXT. ($\Delta\lambda$ AND $\Delta\nu$ REFER TO CORRESPONDING BANDS IN THE PARENT KETONE) (*REFERS TO THE PRESENT WORK)

Compound	Reference	Conformation of acetoxy substituent	νC=O		$\Delta\nu$	K-band			$\Delta\lambda$
			(cm^{-1})	(solvent)	(cm^{-1})	λ_{max} (mμ)	ε	(solvent)	(mμ)
3β-Acetoxy-5α,25D-spirostan-12-one (hecogenin acetate)	6	—	1706	CS_2	—	—	—	—	—
3β,11α-Diacetoxy-5α,25D-spirostan-12-one	6	eq.	1729	CS_2	+23	—	—	—	—
3β,11α-Diacetoxy-5α,25D-spirostan-12-one	6	ax.	1720	CS_2	+14	—	—	—	—
17β-Hydroxyandrost-4-en-3-one (testosterone)	*	—	1656	$CHCl_3$	—	241	16200	EtOH	—
2α,17β-Diacetoxy androst-4-en-3-one	2	eq.	1686	$CHCl_3$	+30	240	17000	MeOH	−1
2β,17β-Diacetoxyandrost-4-en-3-one	2	(pseudo-eq.)	1682	$CHCl_3$	+26	242	17000	MeOH	+1
17α-Acetoxy-6-methylpregna-4,6-dien-3,20-dione (Ia)	*	—	1667	CCl_4	—	287·5	24340	EtOH	—
2α,17α-Diacetoxy-6-methylpregna-4,6-dien-3,20-dione (Ib)	*	eq.	1689	CCl_4	+22	289	23800	EtOH	+1·5
2β,17α-Diacetoxy-6-methylpregna-4,6-dien-3,20-dione (Ic)	*	ax.	1671	CCl_4	+4	295	23000	EtOH	+7·5
17α-Acetoxy-6-acetoxymethylpregna-4,6-dien-3,20-dione (IIa)	*	—	1666	CCl_4	—	280	23900	EtOH	—
2α,17α-Diacetoxy-6-acetoxymethyl-pregna-4,6-dien-3,20-dione (IIb)	*	eq.	1692	CCl_4	+26	280	21700	EtOH	0
2β,17α-Diacetoxy-6-acetoxymethyl-pregna-4,6-dien-3,20-dione (IIc)	*	ax.	1675	CCl_4	+9	286	22700	EtOH	+6

ring A into a skew conformation,[7] with the C_2 acetoxy grouping in pseudo-equatorial orientation. As a result, the 3-keto stretching frequencies of the isomeric 2-acetoxy-testosterone acetates[2] are both considerably raised relative to the parent ketone, and differ only by 4 cm^{-1}. (Table 1).

The C=O stretching frequencies of the C_3 keto-grouping in Ib, Ic and IIb, IIc enable configurations to be assigned to the C_2 substituents, the greater rise in frequency being attributed to an equatorial (2α) acetoxy group. The very considerable difference in magnitude of the shifts due to 2β- (+4 to +9 cm^{-1}) relative to 2α-acetoxylation (+22 to +26 cm^{-1}) is an indication that the conformation of ring A in these compounds is not greatly distorted by axial acetoxylation at C_2.

(b) *UV data.* The UV characteristics of I(a, b and c) and II(a, b and c) are tabulated above. In the high intensity K-band spectrum, introduction of a 2α-acetoxy substituent has virtually no effect on either wavelength or absorption intensity, whereas a 2β-acetoxy substituent is seen to cause a bathochromic shift of between 6 and 7·5 mμ, again with no appreciable effect upon intensity. Such a wavelength shift is not given by the epimeric 2-acetoxytestosterone acetates,[2] suggesting that the effect is due to some form of interaction between a truly axially orientated 2-acetoxy group and the conjugated ketone system. In this connection, it is noteworthy that *both* isomers of *6*-acetoxy-4-en-3-ketones (where significant distortion of the acetoxy-bearing ring B does not occur) show shifts in λ_{max} of approximately −5 mμ.[8]

The position of maximum absorption (ca. 280 mμ) of the n–π^* transition of saturated ketones carrying an acetoxy grouping on an adjacent carbon atom has been shown[9,10] to be related to the conformation of the acetoxy substituent. During the present investigation, the R-band spectrum of the model compound 6-methylcholesta-4,6-dien-3-one was required as a standard of reference. In contrast to the well-resolved spectrum of cholest-4-en-3-one,[8] however, the spectrum of the 6-methyl-4,6-dien-3-one showed only three, ill-defined, inflections, at 349, 332 and 324 mμ in cyclohexane solution, and no resolvable features in dioxan solution. In view of the sparing solubility of I(a, b and c) and II(a, b and c) in solvents of low polarity, the direct use of R-band spectral shifts in the determination of configuration of C_2 acetoxy substituents in 4,6-dien-3-ketones proved impractical.

Nevertheless, we were able to determine the R-band shifts indirectly by comparison of the rotatory dispersion spectra of these compounds, in dioxan, in which solvent adequate resolution was obtained. (Fig. 1)

The average wavelength shifts of the dispersion features over the range 420–320 mμ for Ib and Ic, relative to II, were found to be −3 and +9·5 mμ respectively. These results are in fair agreement with the average shifts of $\Delta\lambda_{equat} = -5$ mμ and $\Delta\lambda_{axial} =$ +7 to +11 mμ, found for λ_{max} of the n–π^* transition of saturated keto-groups in rings B and C of steroids[9,10] when acetoxy groups are introduced into adjacent carbon atoms. The forms of the ORD spectra of the R-band region above 350 mμ, given by I(a, b and c) are closely similar to each other. This observation suggests that ring A is largely undistorted by a 2β (axial) acetoxy substituent in the 4,6-dien-3-ketone series.

[7] K. Kuriyama, E. Kondo and K. Tori, *Tetrahedron Letters* 1485 (1963).
[8] C. W. Bird, R. C. Cookson and S. H. Dandegaonker, *J. Chem. Soc.* 3675 (1956); H. J. Ringold and A. Bowers, *Experientia* **17**, 65 (1961).
[9] O. Schindler and T. Reichstein, *Helv. Chim. Acta* **37**, 667 (1954).
[10] R. C. Cookson and S. H. Dandegaonker, *J. Chem. Soc.* 352 (1955).

A similar substitution pattern in 4-en-3-ketones[7] leads to inversion of the dispersion curve as a result of 2β, but not of 2α-acetoxylation, due to change in chirality of the conjugated system occasioned by skewing of ring A. Thus, the UV and ORD data provide additional evidence for the assignment of configurations at C_2 in I(b, c) and II(b, c) and lend further support to the view that ring A in 4,6-dien-3-ketones possesses low conformational mobility.

(c) *NMR studies.* Kuriyama *et al.*[7] have examined the NMR spectra of a number

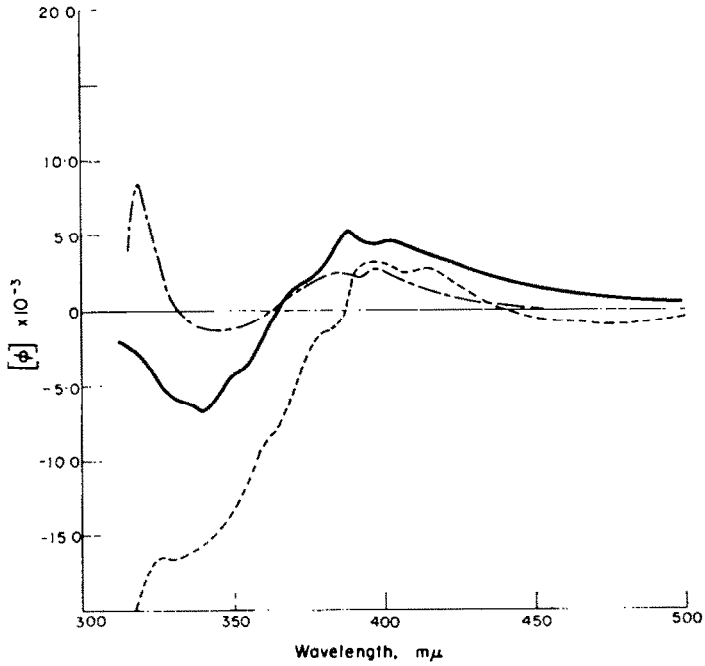


FIG. 1. ORD Spectra of 17α-acetoxy-6-methylpregna-4,6-dien-3,20-dione (Ia, ———); 2α,17α-diacetoxy-6-methylpregna-4,6-dien-3,20-dione (Ib, – . – . – . –) and 2β, 17α-diacetoxy-6-methylpregna-4,6-dien-3,20-dione (Ic, - - - - - -).

of epimeric pairs of steroidal 2-acetoxy and 2-hydroxy-4-en-3-ketones, and by a necessarily first order analysis of the C_2 proton signal (the A-portion of an AXY spin-coupled system, where the XY resonances are obscured) were able to obtain values of Karplus constants[11] relating C_{1-2} coupling constants and the dihedral angles between the C_1 and C_2 C—H bonds.

These constants are identical with those obtained by Abraham and Holker[12] for the C_1–C_2 proton resonances of the 2α- and 2β-bromolanost-8-3n-3-ones, where full AXY analyses are possible. Kuriyama *et al.* found that their data could be accommodated only by skew ring A conformations in the cases of 2β-acetoxylation.

The NMR spectra obtained for compounds of series I and II (Table 2) bear a formal

[11] M. Karplus, *J. Chem. Phys.* **30**, 11 (1959); *J. Amer. Chem. Soc.* **85**, 2870 (1963).

[12] R. J. Abraham and J. S. E. Holker *J. Chem. Soc.* 806 (1963); The Karplus equations found by Abraham and Holker to apply to 2-bromo-5α-3-keto-steroids are as follows: $J_1 = k_1 \cos^2 \phi$ ($0° < \phi < 90°$) and $J_2 = k_2 \cos^2 \phi$ ($90° < \phi < 180°$), where $k_1 = 12{\cdot}4$ ($\pm 0{\cdot}5$) and $k_2 = 14{\cdot}3$ ($\pm 0{\cdot}5$).

Table 2. NMR data. Determinations were made using 3–8% solutions in $CDCl_3$, with tetramethylsilane as internal reference. Changes in the chemical shifts of bands of the parent ketone, occasioned by 2-acetoxylation, are shown in parentheses. Half-intensity band widths of unresolved multiplets ($w_{\frac{1}{2}}$), and all coupling constants, are expressed in c/s.

Compound	Methyl and methylene singlet proton resonances (τ)				C_2—H Resonance		Olefinic protons ppm	
	C_{19}	C_{18}	C_6	CH_3CO (all)	τ	J_{1-2}	C_4 (singlet)	C_7 (multiplet)
17α-Acetoxy-6-methylpregna-4,6-dien-3,20-dione (Ia)	8·902	9·270	8·15	7·92, 7·95	—	—	4·10	4·00 ($w_{\frac{1}{2}} = 7$)
2α,17α-Diacetoxy-6-methyl pregna-4,6-dien-3,20-dione (Ib)	8·745 (−0·157)	9·265 (−0·005)	8·13 (−0·02)	7·80, 7·90, 7·93	4·37	6, 12·6 (quartet)	4·07 (−0·03)	4·07 +0·07)
2β,17α-Diacetoxy-6-methyl-pregna-4,6-dien-3,20-dione (Ic)	8·795 (−0·107)	9·280 (+0·010)	8·10 (−0·05)	7·90, 7·95	4·65	2·5, 5·5 (quartet)	3·95 (−0·15)	3·95 (−0·05)
17α-Acetoxy-6-acetoxymethyl pregna-4,6-dien-3,20-dione (IIa)	8·892	9·266	5·28 ($w_{\frac{1}{2}} = 3·5$)	7·90, 7·94	—	—	4·08	3·70 ($w_{\frac{1}{2}} = 7$)
2α,17α-Diacetoxy-6-acetoxy methylpregna-4,6-dien-3,20-dione (IIb)	8·745 (−0·147)	9·262 (−0·004)	5·28 ($w_{\frac{1}{2}} = 3·5$)	7·80, 7·90, 7·93	4·35	6·4, 12·9 (quartet)	4·04 (−0·04)	3·70 (0)
2β,17α-Diacetoxy-6-acetoxy-methylpregna-4,6-dien-3,20-dione (IIc)	8·790	9·270	5·23	7·90–7·92 (multiplet)	4·60	not calcd (unresolved multiplet) $w_{\frac{1}{2}} = 9$	3·93 (−0·15)	3·67 (−0·03)

resemblance to those of the corresponding 4-en-3-ketones, in that the C_2 and C_{19} protons of the 2β-acetoxy-compounds resonate at higher fields than those of the 2α-epimers. A first order analysis of the C_2 proton multiplet in the spectrum of Ic gives approximate coupling constants (C_1–C_2) of 2·5 and 5·5 c/s.

Substitution of these J-values into the Karplus equations of Abraham and Holker provides angles $\phi_{2-1\alpha} = 63°$ and $\phi_{2-1\beta} = 48°$,which can be accommodated only by a 2β-acetoxy substitution pattern with ring A in a slightly distorted chair conformation almost identical with that revealed by Dreiding stereo-models. A similar first order analysis of the C_2 proton resonances of Ib and IIb gave mean C_{2-1} coupling constants of 6·2 and 12·8 c/s, which by use of the same Karplus equations yield dihedral angles of $\phi_{2-1\alpha} = 160°$ and $\phi_{2-1\beta} = 46°$. The differences between these angles, and those reported by Kuriyama *et al.* for their 2α-acetoxy-4-en-3-ketones (168° and 48° respectively) is insignificant.

The foregoing NMR spectral data establish that ring A of 4,6-dien-3-ketones assumes the semi-chair conformation, and that the introduction of a $C_{2\beta}$-acetoxy substituent fails to lead to distortion of the apparently rigid ring structure.

(d) *Molecular rotational effects at* 589 mμ. The following Table gives molecular rotation differences obtained for epimeric pairs of 2-acetoxylated 5α-H, 4-ene- and 4,6-diene-3-ketosteroids.

	$\Delta[\phi]_D(2\alpha \rightarrow 2\beta)$
(i) 2-acetoxy-5α-cholestan-3-one(s)[13]	+158°
(ii) 2-acetoxytestosterone acetate(s)[2]	−528°
(iii) Compounds Ib, Ic.	−326°
Compounds IIb, IIc.	−215°

Inspection of these results reveals a gross dissimilarity in magnitude between the conformationally similar species (i) and (ii). In contrast, the results obtained for species (ii) and (iii) are less divergent although the conformations of ring A in these species have been shown *vide supra* to be entirely different.

These results therefore emphasize the need for caution in the use of molecular rotation increments in configurational assignments.

[13] K. L. Williamson and W. S. Johnson, *J. Org. Chem.* **26**, 4563 (1961).