Steroid Conformations in Solid and Solution: Stereoselective Synthesis of (20*S*)- and (20*R*)-[20-methyl-labelled]-20-methylpregn-5-ene-3β,17α,20-triol

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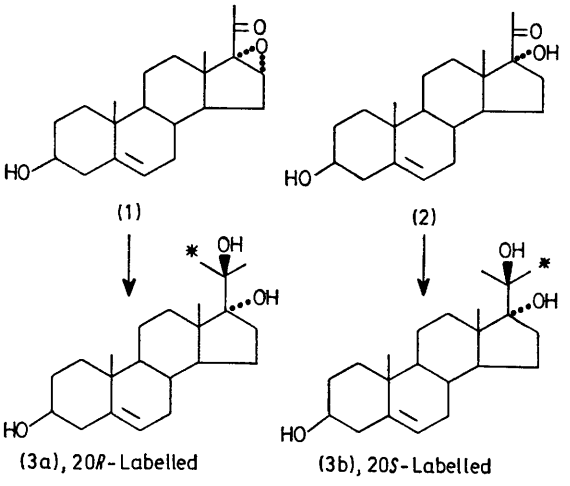
Summary Addition of isotopically labelled methylmagnesium bromide followed by reduction with lithium aluminium hydride of 17α-hydroxypregnenolone 3-acetate and 16α,17α-epoxypregnenolone acetate gave, respectively, (20*S*)- and (20*R*)-[20-$^{13}CH_3$]-, [20-C^3H_3]-, and [20-C^2H_3]-20-methylpregn-5-ene-3β,17α,20-triol (99% for *S*, 93% for *R*) owing to conformational localization of the 20-carbonyl group to positions of opposite chirality caused by neighbouring substitution.

AN efficient means of obtaining separate stereoisomers selectively labelled at the steroidal 21 and 22 positions was sought for our studies of the stereomechanism of steroid hormone biosynthesis.

Examination of the 20-carbonyl group in the solid state of 37 steroids having 17α-hydroxy or hydrogen substituents[1] shows that the oxygen, O(20), is located above the D-ring [∠C(13)–C(17)–C(20)–O(20) +75 to +115°] except in one structure having a 16β-bromo substituent [∠C(13)–C(17)–C(20)–O(20) −4·9°]. An unusual conformation in which the carbonyl group eclipses the C(13)–C(17) bond was found recently in 16α,17α-epoxypregnenolone (**1**) [∠C(13)–C(17)–C(20)–O(20) −0·4°].[2] The C- and D-ring regions of (**1**) and 17α-hydroxyprogesterone, as determined by *X*-ray crystallography, are shown in the Figure, (a) and (b), respectively,



to illustrate the stereoselective *re*- and *si*-face attack† by the reagent giving rise to the (20*R*) and (20*S*) introduction of the labelled group. The validity of the approach[3] of using carbonyl conformations observed in the solid state to predict the absolute configurations of reaction products was confirmed in the elucidation of the stereomechanism of oestrogen biosynthesis involving 19-oxygenated intermediates.[4]

20-Methylpregn-5-ene-3β,17α,20-triol (**3**) was synthesized from the acetate of (**1**) and 17α-hydroxypregnenolone 3-acetate [3-acetate of (**2**)] as previously described (Grignard addition followed by $LiAlH_4$ reduction)[5] and showed m.p. 184—188 °C (m.p. 195—200 °C in ref. 5a; m.p. 182—185 °C in ref. 5b); i.r. (KBr) 3400 and 1050 cm^{-1}; ^1H n.m.r. (60 MHz, $CDCl_3$–CD_3OD), δ 0·87 (3H, s, 13-Me), 1·03 (3H, s, 10-Me), 1·30 (3H, s, 20-*pro*-*R*-Me), 1·37 (3H, s, 20-*pro*-*S*-Me), and 5·40 (1H, m, 6-H); Δ (shift from resonance positions in $CDCl_3$–CD_3OD to those in pyridine) −0·33 (13-Me), −0·07 (10-Me), −0·33 (20-*pro*-*R*-Me), and −0·31 (20-*pro*-*S*-Me) p.p.m. The 3-acetate of (**3**) showed m.p. 177—179 °C; i.r. 3520, 3460, 1720, 1250, and 1030 cm^{-1}; ^1H n.m.r. (60 MHz, $CDCl_3$) δ 0·87 (3H, s, 13-Me), 1·03 (3H, s, 10-Me), 1·30 (3H, s, 20-*pro*-*R*-Me), 1·37 (3H, s, 20-*pro*-*S*-Me), 2·04 (3H, s, 3-OAc), and 5·40 (1H, m, 6-H); Δ −0·30 (13-Me), 0·00 (10-Me), −0·33 (20-*pro*-*R*-Me), −0·30 (20-*pro*-*S*-Me), and −0·01 (3-OAc). The n.m.r. assignments were made by the analysis of ^2H and ^{13}C-labelled isomers. Methylmagnesium iodide prepared from $^{13}CH_3I$ (90 atom %), C^3H_3I (100 mCi $mmol^{-1}$), or C^2H_3I (99·5 atom %) in ether was used for the synthesis of (20*R*) and (20*S*) 20-methyl labelled (**3a**) and (**3b**) from the

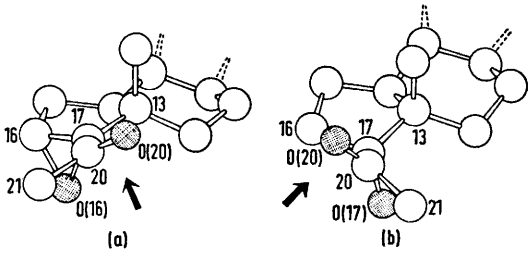


FIGURE. Conformation of 16α,17α-epoxypregnenolone (a) and 17α-hydroxyprogesterone (b) determined by *X*-ray crystallography. Preferred *re*-face and *si*-face attack from the less hindered α-side of the steroid by the reagent is indicated.

acetate of (**1**) and the 3-acetate of (**2**), respectively. [4-^{14}C]-(**3**) was prepared from [4-^{14}C]-(**2**) (60 mCi $mmol^{-1}$) by the same method. [4-^{14}C,20*S*-$^{13}CH_3$,20*S*-C^3H_3]-(**3**) was mixed with [20*R*-$^{13}CH_3$ + 20*R*-C^3H_3]-(**3**) and subjected to separation techniques including t.l.c., h.p.l.c., counter-current distribution and recrystallization. The results showed no observable sign of peak separation or change of the ^3H–^{14}C ratio. This was in contrast to the easy isolation of two rotamers of the 20-(2-hydroxyethyl) ether of (**3**) reported by Kohen *et al.*[6] The reinvestigation in the preceding communication[7] refuted the original claim.

Stereoselectivity of the Grignard addition was analysed by ^1H n.m.r. studies (60 MHz) of the isotopically labelled compounds. (20*S*)-Deuterium labelled 3-acetate of (**3b**) showed a single 20-Me signal at δ 1·30, whereas the (20*R*)-isomer showed the δ 1·30 and 1·37 peaks in a ratio of *ca.* 1 : 3.‡ Owing to the high background from the protons of the steroid skeleton in this region the ^{13}C labelled compounds were used for quantitative analysis. The (20*S*)-[20-$^{13}CH_3$] 3-acetate of (**3b**) showed the ^{13}C-methyl protons at δ 0·32 and 2·42 [J(H–^{13}C) 126 Hz], and the (20*R*)-labelled isomer showed the methyl protons at δ 0·25 and 2·35 [J(H–^{13}C) 126 Hz]. The relative intensities of the signals at δ 0·32 and 0·25, where there are no interfering background signals, were 99 : 1 in the (20*S*) 3-acetate of (**3b**) which was derived from the 17α-hydroxy-ketone (**2**), and 7 : 93 in the (20*R*) 3-acetate of (**3a**) derived from the 16α,17α-epoxy-ketone (**1**), respectively.

Stereoselectivity in the synthesis of (20*R*) and (20*S*) 20-methyl labelled (**3a**) and (**3b**) from the acetate of (**1**) and the 3-acetate of (**2**) reflect not only the probability of

† The designation of *re* and *si* refers to the direction that the sequence rule around the trigonal C-20 [(O-(20) > C-(17) > C-(21)] is right- and left-handed, respectively ('Stereochemistry and Its Application in Biochemistry,' W. L. Alworth, Wiley-Interscience, London, 1972, p. 142).

‡ Chaudhuri *et al.* (ref. 8) reported that reaction of (**1**) with ethylmagnesium bromide followed by reduction with $LiAlH_4$ resulted in two 20-methyl peaks at δ 1·22 and 1·28 in a 3 : 2 ratio.

localization§ of the carbonyl group but also the degree of steric hindrance of each steroid conformation to the Grignard reagent. The highly stereoselective ratios actually observed show that the conformation found in the solid state also predominates in the solution under the reaction conditions. The assignment of the absolute configuration made in this study is opposite to that made by Chaudhuri *et al.*[8] to the products of addition of ethyl and isohexyl Grignard reagents to (**2**).

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§ W. R. Nes and T. E. Varkey (*J. Org. Chem.*, 1976, **41**, 1652) discussed the *R*/*S* ratio of the products as being directly correlated with the equilibrium of the rotamers of the carbonyl group of 17α-hydrogen 20-ketones in reactions involving different Grignard reagents. They concluded that the results of poor selectivity (1·7 : 1·0) of product formation is due to the conformational equilibrium between the '*cis*' and '*trans*' conformers of pregnenolone. However, difference in the size of reagents and the steric environment among rotamers also influence the α-side/β-side attack ratio, making such an approach inconclusive.

[1] W. L. Duax, C. M. Weeks, D. C. Rohrer, Y. Osawa, and M. E. Wolff, *J. Steroid Biochem.*, 1975, **6**, 195; W. L. Duax and D. A. Norton, eds., 'Atlas of Steroid Structure,' Vol. 1, Plenum Press, New York, 1975, p. 35.

[2] J. P. Hazel, C. M. Weeks, and Y. Osawa, *Cryst. Struct. Comm.*, 1976, **5**, 103.

[3] Y. Osawa, in 'Endocrinology,' Proceedings of 4th International Congress of Endocrinology, Washington, D.C. (1972); R. O. Scow, ed., Excerpta Medica, Amsterdam, 1973, p. 814.

[4] K. Shibata, W. L. Duax, and Y. Osawa, *J. Steroid Biochem.*, 1974, **5**, 301; Y. Osawa, K. Shibata, D. Rohrer, C. M. Weeks, and W. L. Duax, *J. Amer. Chem. Soc.*, 1975, **97**, 4400; D. Arigoni, R. Battaglia, M. Akhtar, and T. Smith, *J.C.S. Chem. Comm.*, 1975, 185.

[5] (a) P. L. Julian, J. W. Cole, E. W. Meyer, and M. J. Karpel, U.S.P. 2,887,478 (1959); (b) M. Uskoković, M. Gut, and R. I. Dorfman, *J. Amer. Chem. Soc.*, 1959, **81**, 4561.

[6] F. Kohen, R. A. Mallory, and I. Scheer, *Chem. Comm.*, 1969, 580.

[7] Y. Osawa, T. Makino, and C. M. Weeks, preceding communication.

[8] N. K. Chaudhuri, J. G. Williams, R. Nickolson, and M. Gut, *J. Org. Chem.*, 1969, **34**, 3759.