^{2}H-LABELLED 3α-HYDROXY-5α-PREGNANE-11,20-DIONE AND 3α,21-DIHYDROXY-5α-PREGNANE-11,20-DIONE 21-ACETATE

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SUMMARY

3α-Hydroxy-5α-pregnane-11,20-dione-2,2,3,4,4-$^{2}H_5$ of 78% isotopic purity was prepared from 3α-hydroxy-5α-pregnane-11,20-dione. The major impurity (10%) was 3α-hydroxy-5α-pregnane-11,20-dione-2,2,4,4-$^{2}H_4$. 3α,21-Dihydroxy-5α-pregnane-11,20-dione-2,2,3,4,4-$^{2}H_5$ 21-acetate of 76% isotopic purity was prepared by reaction of the deuterated 3α-hydroxy compound with lead tetraacetate. The major impurity (10%) was 3α,21-dihydroxy-5α-pregnane-11,20-dione-2,2,4,4-$^{2}H_4$.

KEY WORDS. Deuterated 3α-hydroxy-5α-pregnane-11,20-dione, deuterated 3α-21-dihydroxy-5α-pregnane-11,20-dione 21-acetate, steroids, anaesthetics, drugs.

The steroids, 3α-hydroxy-5α-pregnane-11,20-dione (alphaxalone) (1) and 3α,21-dihydroxy-5α-pregnane-11,20-dione 21-acetate (alphadolone acetate) (6) are currently used for the rapid induction of short periods of anaesthesia. Studies with ^{14}C-labelled 3α-hydroxy-5α-pregnane-11,20-dione have shown that, after dosing, this compound disappears rapidly from plasma[1]. In addition, the two steroids are usually administered together in very low doses and, therefore, in order to study the pharmacokinetics of these drugs, a highly specific and sensitive assay was required. Gas chromatography-mass spectrometry (GCMS) was the method chosen to carry out this work and to optimise the sensitivity and precision of the assay, deuterium labelled 3α-hydroxy-5α-pregnane-11,20-dione and 3α,21-dihydroxy-5α-pregnane-11,20-dione 21-acetate were required as internal standards.

The preparation of deuterated keto-steroids may be readily achieved by

0362-4803/82/040597-08$01.00 Received October 20, 1981

acid or base catalysed exchange of enolizable hydrogens in deuterium oxide[2]. However, the deuterated steroids prepared in this way are usually unsuitable for use as internal standards in assays based on GCMS because back-exchange of deuterium can occur, either in the GCMS instrument or during extraction steps if acidic or basic conditions are used. This introduces a variable which may significantly reduce the precision of the assay. It was therefore essential that any introduced deuterium atoms be non-exchangeable under the proposed experimental conditions.

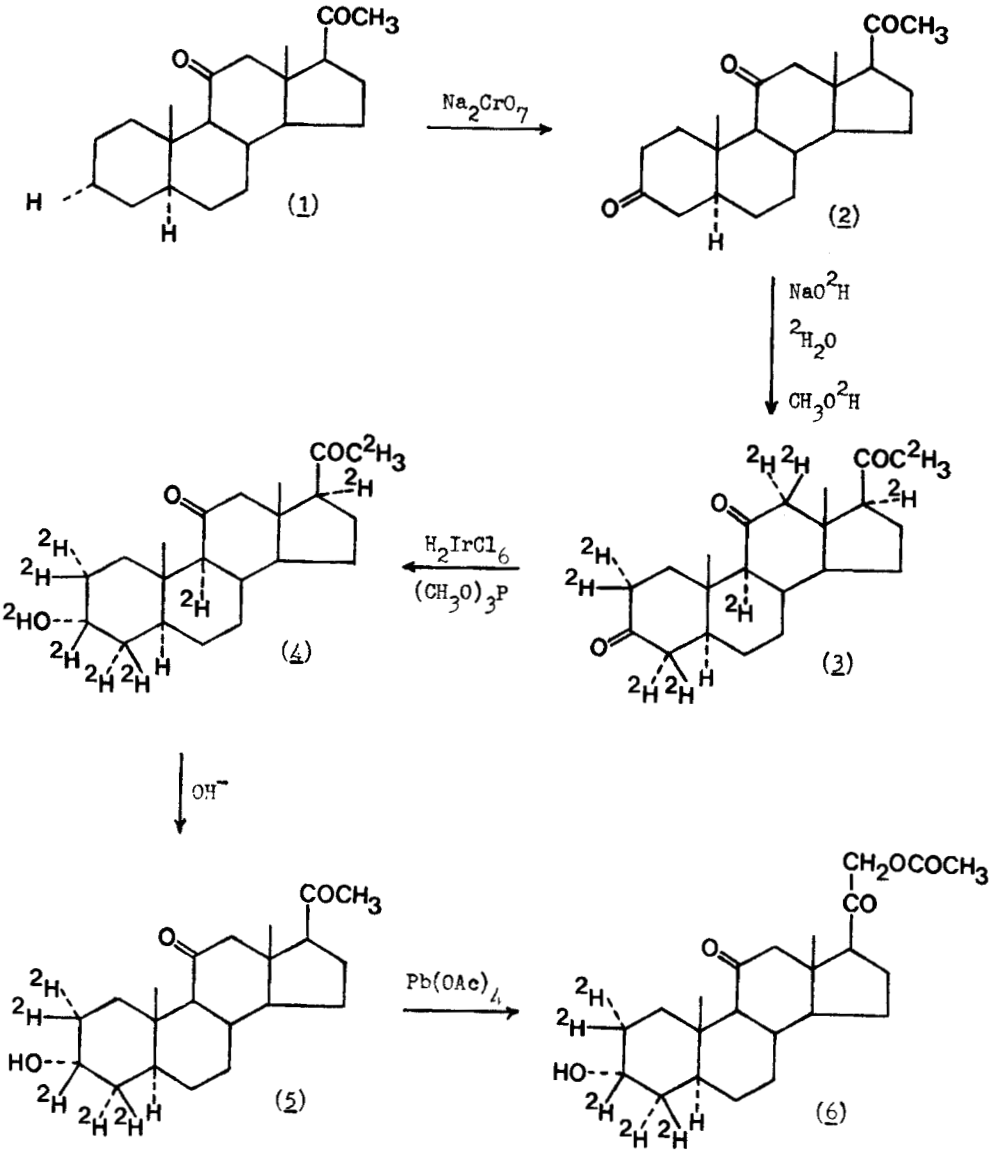
The deuterated steroids were prepared by the reaction sequence shown in Scheme 1. As epimerisation at C-17 can occur in the two base catalysed exchange reactions, and has also been reported to occur during reduction with chloroiridic acid[3] to give the equilibrium mixture of C-17α (20-25%) and C-17β (75-80%) epimers, no attempt was made to separate these epimers until the final products had been obtained.

Base catalysed exchange of the 3-keto steroid (2) in $^{2}H_{2}O$ gave the deuterated 3-keto steroid (3) which was shown, by mass spectrometry, to contain an average of 10.4 deuterium atoms per molecule (theoretical value = 11).

Stereospecific reduction of the 3-keto group with chloroiridic acid, trimethyl phosphite, propan-2-(^{2}H)-ol and $^{2}H_{2}O$ gave deuterated 3α-hydroxy-5α-pregnane-11,20-dione (4) in somewhat lower yield (53%) than the 63% yield reported by Browne and Kirk for the unlabelled steroid[3]. It has been suggested that transfer of 'hydride ion' from the reagent to the carbonyl group is involved in determining the reaction rate[3,4] and it might be expected that this transfer would occur more slowly from deuterated reagents. Propan-2-(^{2}H)-ol was used in this reaction because it has been reported to lead to the introduction of a deuterium atom at C-3[4]. GCMS analysis of the crude reaction product indicated that this had occurred. Back exchange of enolizable ^{2}H atoms in $H_{2}O$ and purification by t.l.c. then gave pure 3α-hydroxy-5α-pregnane-11,20-dione-$^{2}H_{5}$ (5).

Deuterated 3α,21-dihydroxy-5α-pregnane-11,20-dione 21-acetate (6) was obtained by reaction of crude deuterated 3α-hydroxy-5α-pregnane-11,20-dione (5) with lead tetraacetate and purification of the product by t.l.c.

Scheme 1


COCH3
O
H
(1)
Na2CrO7
(2)
NaO2H
2H2O
CH3O2H
COC2H3
2H
(3)
H2IrCl6
(CH3O)3P
(4)
OH⁻
(5)
Pb(OAc)4
CH2OCOCH3
CO
(6)
HO

The content and distribution of deuterium in the final products were determined by chemical ionisation (CI) and electron impact (EI) mass spectrometry and by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

The ammonia CI mass spectra of the deuterated steroids (5) and (6) contained only isotopic peaks corresponding to the adduct ion $M+NH_4^+$. By measurement of the relative abundances of these peaks and correction for contributions from ^{13}C, ^{15}N and ^{18}O, (5) was shown to have the composition: d_2 0.53%, d_3 1.8%, d_4 16.6%, d_5 77.6%, d_6 3.0% and d_7 0.47%; and (6) was shown to have the composition: d_2 1.3%, d_3 1.9%, d_4 17.6%, d_5 76.2%, d_6 2.8% and d_7 0.1%. In both cases these compositions correspond to an average of 4.8 deuterium atoms per molecule.

The changes in chemical shift observed when the proton-decoupled ^{13}C NMR spectra of (1) and its 3-acetate were compared enabled the peaks corresponding to C-1 and C-5, C-2 and C-4 and C-3 in each spectrum to be identified. In the spectrum of (1) the following assignments were made: 65.6 ppm C-3, 38.4 and 30.5 ppm C-1 and C-5, 34.9 and 28.4 ppm C-2 and C-4.

The ^{13}C NMR spectrum of the corresponding deuterated steroid (5) was identical to that of (1) except that the singlet peak at 65.6, was replaced by a three line pattern of very low intensity and the singlets at 34.9 and 28.4 ppm were replaced by multiplets of such low intensity that they were barely distinguishable from baseline noise. It was therefore apparent that, in this deuterated steroid, there was one ^{2}H atom on C-3 and two ^{2}H atoms on both C-2 and C-4. Owing to the high level of deuterium incorporation it was not possible to estimate the proportion of ^{2}H to ^{1}H at C-2,-3 and -4.

The ^{1}H atoms on C-1,C-2, C-4 and C-5 could not be unambiguously located in the ^{1}H NMR spectrum of (1) owing to the complexity of the region between δ1.0 and δ2.0. However, the relative proportions of ^{2}H and ^{1}H at C-3 could be estimated by ^{1}H NMR spectroscopy. By comparison of integrals of the signal from C-3 ^{1}H in the spectra of (1) and (5) it was found that the proportion of ^{2}H to ^{1}H on C-3 in (5) was approximately 10:1.

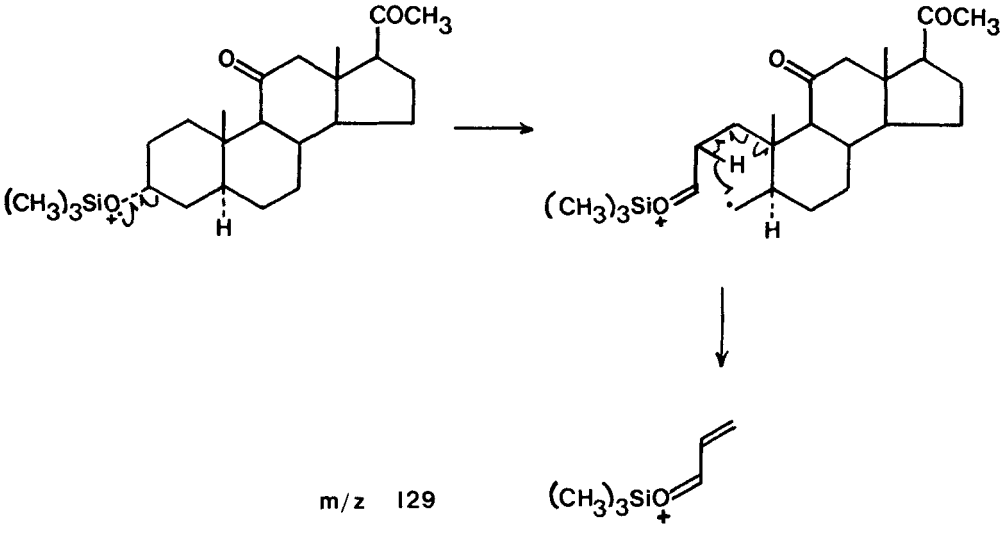
Therefore, it was concluded that the deuterated steroid (5) consisted

primarily of 3α-hydroxy-5α-pregnane-11,20-dione-2,2,3,4,4-$^2H_5$ (78%) together with 3α-hydroxy-5α-pregnane-11,20-dione-2,2,4,4-$^2H_4$ (10%). The remainder, 12% consisted of a number of isotopic species, none of which was present in sufficient quantity to be characterised.

Similarly, the deuterated steroid (6) was shown to consist of 3α-hydroxy-5α-pregnane-11,20-dione-2,2,3,4,4-$^2H_5$ 21-acetate (76%), 3α-hydroxy--5α-pregnane-11,20-dione-2,2,4,4-$^2H_4$ 21-acetate (10%) and other minor isotopic species (14%).

Further evidence in support of the NMR data was obtained by inspection of the mass spectra of both the deuterium labelled and unlabelled steroids. The E.I. mass spectra of the unlabelled steroids (1) and (6) contained a significant ion peak at m/z 147. It has been shown that this ion is formed by cleavage of the C-9, C-11 and C-8, C-14 bonds and concomitant elimination of a molecule of water[6]. The ionic product, m/z 147, therefore contains the carbon skeleton and hydrogen atoms of rings A and B. In the E.I. mass spectrum of the deuterated steroids (5) and (6) this ion was shifted to m/z 152 which indicated that rings A and B contained five ^2H atoms.

Scheme 2



The EI mass spectrum of the trimethylsilyl ether formed from (1) (Scheme 2) contained a prominent and characteristic fragment ion at m/z 129. The mechanism by which this ion is formed is shown in Scheme 2[7]. The spectrum of the deuterated steroid (5) did not contain m/z 129 but instead exhibited a prominent ion at m/z 131. Since the mechanism by which this ion was formed involves transfer of ^{1}H (or ^{2}H) from C-2 to C-4, the deuterated steroid (5) must have two ^{2}H atoms on C-2. If this were not so, and C-2 carried only one ^{2}H atom, m/z 130 would also have been observed. In fact, m/z 130 would be more abundant than m/z 131 as transfer of ^{1}H is more favourable than transfer of ^{2}H because the C-^{1}H bond is weaker than the C-^{2}H bond.

Experimental

Mass spectra were obtained using a Finnigan 3200E gas chromatograph-mass spectrometer coupled to a Finnigan 6110 data system. Samples were introduced into the mass spectrometer by means of the direct insertion probe or by injection into the gas chromatograph. The glass column (0.6 m x 0.2 mm I.D.) was packed with 3% OVI on Gas Chrom Q (100-120 mesh) and was operated at 240°. ^{1}H NMR spectra were measured at 100 and 400 MHz in chloroform-^{2}H containing tetramethylsilane as internal standard. ^{13}C NMR were measured at 90 MHz in chloroform-^{2}H which also served as internal standard. Chemical shifts are expressed as ppm downfield from tetramethylsilane. Authentic samples of 3α-hydroxy-5α-pregnane-11,20-dione and 3α,21-dihydroxy-5α-pregnane-11,20-dione 21-acetate were obtained from Glaxo Laboratories Ltd. (Greenford, Middlesex, U.K.).

5α-Pregnane-3,11,20-trione (2) 3α-Hydroxy-5α-pregnane-11,20-dione (1) (500 mg) in acetone (30 ml) was titrated with Jones reagent. The reaction mixture was poured into water (100 ml) and extracted with chloroform (2 x 20 ml). The organic layer was washed twice with water, dried (Na_2SO_4) and evaporated to dryness. Recrystallisation of the residue from acetone/hexane gave 5α-pregnane-3,11,20-trione (2) (95%), mp 214-217°; m/z (methane CI) 331 (MH^+, base peak), 359 ($M+C_2H_5^+$) and 371 ($M+C_3H_5^+$).

Deuterated 5α-pregnane-3,11,20-trione (3). 5α-Pregnane-3,11,20-trione (2) (400 mg), CH_3O^2H (40 ml) and 5% NaO^2H in ^2H_2O (21 ml) were heated under reflux for 42 h. After cooling the reaction mixture was diluted with ^2H_2O and extracted with ether (3 x 20 ml). The combined ether extracts were washed twice with water, dried (Na_2SO_4) and evaporated to dryness. The residue was recrystallised from acetone/hexane to give deuterated 5α-pregnane-3,11,20-trione (3) (90%). Ammonia CI mass spectrometry showed the deuterium content of the product was d_8 2.7%, d_9 10.0%, d_{10} 31.0%, d_{11} 55.4%, d_{12} 0.7%, d_{13} 0.2%.

Deuterated 3α-hydroxy-5α-pregnane-11,20-dione (4). Deuterated 5α-pregnane-3,11,20-trione (3) (250 mg) was treated with chloroiridic acid, trimethyl phosphite, ^2H_2O and propan-2-(^2H)-ol using the method of Browne and Kirk[3]. The reaction mixture was cooled and poured into ^2H_2O and extracted with ether. The ether extract was washed with water, dilute sodium hydrogen carbonate solution, dried (Na_2SO_4) and evaporated to give a mixture of deuterated products. Analysis by GCMS showed that the composition of this mixture was 24% 5α-pregnane-3,11,20-trione, 53% (17β) and 15% (17α) 3α-hydroxy-5α-pregnane-11,20-dione and 8% unidentified compounds.

3α-Hydroxy-5α-pregnane-11,20-dione-$^2H_5$ (5). Crude deuterated 3α-hydroxy-5α-pregnane-11,20-dione (4) (150 mg), methanol (20 ml) and 5% sodium hydroxide solution (10 ml) were heated under reflux for 48 h. The cooled reaction mixture was diluted with water and extracted with ether (3 x 10 ml). The combined ether extracts were washed with water, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by preparative t.l.c. (silica gel) using benzene-ethyl acetate-chloroform (4/3/3) for development. The band with R_f 0.2 was removed, extracted with chloroform, dried (Na_2SO_4) and evaporated to dryness to give 3α-hydroxy-5α-pregnane-11,20-dione-$^2H_5$ (5) (71 mg) of 97% purity (by GCMS).

3α,21-Dihydroxy-5α-pregnane-11,20-dione-$^2H_5$ 21-acetate (6). 3α-Hydroxy-5 -pregnane-11,20-dione-$^2H_5$ (60 mg) and lead tetraacetate (90 mg) were stirred in a mixture of benzene (2.6 ml) and CH_3O^2H (0.14 ml) and treated with boron trifluoride etherate (0.35 ml). The reaction mixture was stirred at room temperature for 4 h and then poured into ice-cold ^2H_2O. The product was isolated with chloroform and

purified by t.l.c. (silica gel) using benzene-ethyl acetate-chloroform (4/3/3) for development. The band with R_f 0.25 was removed, extracted with chloroform, dried (Na_2SO_4) and evaporated to dryness to give 3α,21-dihydroxy-5α-pregnane-11, 20-dione-$^2H_5$ (6) (28 mg) of 98% purity (by GCMS).

Trimethylsilyl derivatives. Trimethylsilylation was carried out by dissolving the steroid (1 mg) in 0.1 ml Trisil (Pierce Chemical Company, Rockford, IL., U.S.A.) in a 1.0 ml capacity reaction vial sealed with a teflon-faced septum. The reaction mixture was made up to 1.0 ml by the addition of dry pyridine. Aliquots of this solution were used for GCMS analyses.

ACKNOWLEDGEMENTS

The authors wish to thank Dr J.L.E. Nemorin for 100 and 400 MHz ^1H NMR spectra and Mr B. Tattam for ^{13}C NMR spectra.

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