A NEW METHOD FOR LABELLING 11β-HYDROXY STEROIDS: SYNTHESIS OF 18-^{2}H-CORTICOSTEROIDS

Michael J. Green*, Ho-Jane Shue, Peter Bartner, James B. Morton, Richard E. Youngstrom, and Jerrold Meinwald†
Natural Products Research and Physical Organic Chemistry, Research Division, Schering-Plough Corporation, Bloomfield, N.J. 07003 and †Department of Chemistry, Cornell University, Ithaca, N.Y. 14853

SUMMARY

A new method for labelling 11β-hydroxy steroids is described. The procedure involves specifically placing the label on the angular C_{13} methyl group *via* the photolysis of the 11β-nitrite ester (Barton reaction) and reaction of the resulting C_{18} radical with PhS-^{2}H *in situ*. Incorporations of ^{2}H were 30-40% for 9α-H, 12-21% for 9α-F and <5% for 9α-Cl corticosteroids. The extent of labelling was measured by mass spectrometry and the position of labelling was confirmed as C_{18} for two compounds by dmr, cmr and pmr spectroscopy.

Key Words: 18-^{2}H-Corticosteroids, Photolysis, Barton Reaction, Phenylthiol, Radical Trapping.

Current studies on the molecular biochemistry of corticosteroids and of corticosteroid receptor sites have created a need for the radiolabelled compounds of very high specific activity. At present radiolabelled corticosteroids are synthesized by a tritium reduction/dehydrogenation sequence on the 1,4-diene-3-one system in Ring A (1). Such methods are inefficient since the bulk of the tritium that is introduced by reduction is subsequently removed in the dehydrogenation step. Considerable chemical manipulation and purification is also required before the final product is attained - a tedious and time-consuming process when working with radioactive compounds. Furthermore the label is introduced in positions which are metabolically active (1a,2).

*Address correspondence to this author at Schering-Plough Corporation.

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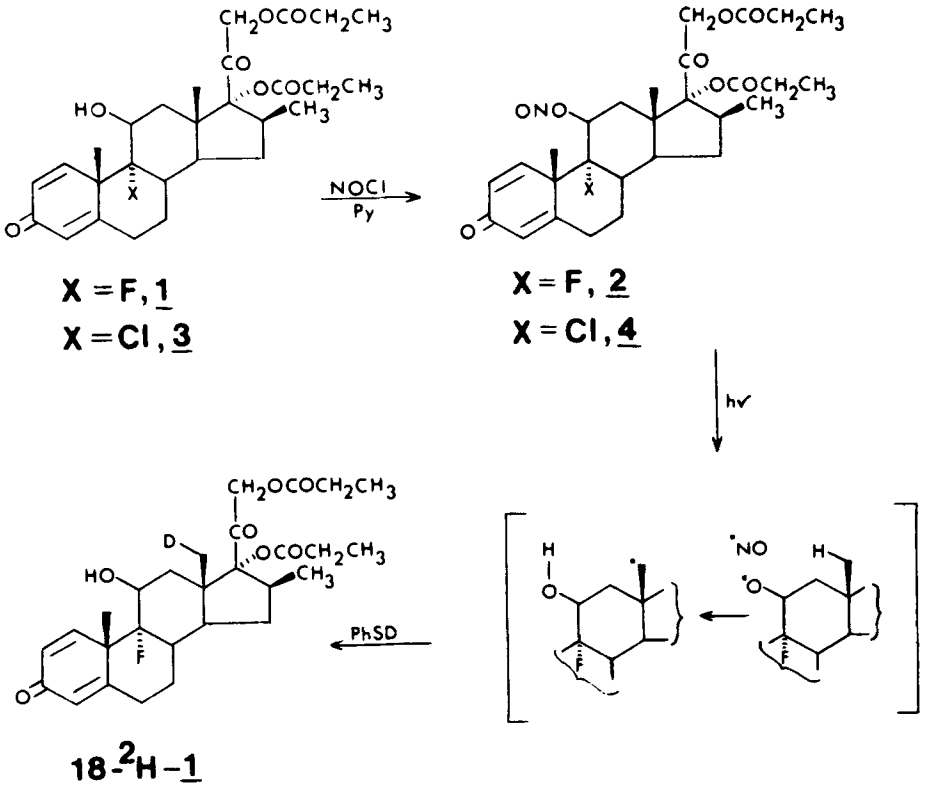
We would like to report here a new method of labelling corticosteroids which involves introduction of the label on the C_{13} methyl group - an unactivated and metabolically stable position. The method, based on the Barton nitrite photolysis, also has the advantage of introducing the label at the final step. This report details the work with the deuterium labelling for which incorporations of up to 40% have been obtained.

Barton et al. (3) reported that while photolysis of steroidal 11β-nitrites gave both C_{19} and C_{18} oximes, when a 1,4-diene-3-one function was present in Ring A then functionalization only at C_{18} occurred (4). It was also shown (5) that the C_{18} radical generated in this reaction could be trapped by iodine or bromotrichloromethane to give unstable 18-iodo and 18-bromocorticosteroids respectively. We find that this radical can also be trapped efficiently by deuterated phenylthiol (PhSD) (6) to give $^{2}H\text{-}C_{18}$ corticosteroids.

The overall reaction scheme is shown in Fig. 1 for betamethasone 17,21-dipropionate (1). Reaction of 1 with nitrosyl chloride in pyridine gave the 11β -nitrite (2) in quantitative yield. After thorough drying, the nitrite was dissolved in toluene containing a five-fold excess of PhSD in a pyrex vessel and irradiated with 3500Å "black light." After the nitrite ester has been consumed (tlc), the solution was washed with water and chromatographed on thick layer silica gel plates to give a 30% yield of betamethasone 17,21-dipropionate containing a 21% incorporation of deuterium.

Our results for a variety of 11β-hydroxy steroids (Fig. 2) are shown in the Table. These results were obtained using a set of standard conditions (see Experimental) which were not fully optimized. However, it was determined that the percentage deuterium incorporated depended on the concentrations of the reactants, the steroid to PhSD ratio, and the surface area exposed to light: it was independent of the solvent used or the temperature.

FIGURE 1



The highest incorporations of ^{2}H occurred with the 9α-hydrogen compounds, fluocortolone 21-acetate (5, 40%) and desonide 21-acetate (6, 32%). Slightly lower incorporations were found with the 9α-fluoro compounds 1, betamethasone 17,21-dipropionate (21%) and 7, triamcinolone acetonide 21-acetate (20%). However, with the latter two compounds, it was noticed that the starting nitrites were somewhat unstable under the reaction conditions. The lower ^{2}H incorporations can then be attributed to partial hydrolysis in the photolysis cell presumably by trace amounts of water in the solvent and absorbed on the surface of the glassware. Beclomethasone 17,21-dipropionate 11β-nitrite (4),

which has a 9α-chlorine, was even more unstable. Complete hydrolysis occurred within 2 hours under the reaction conditions and only a 5% incorporation of deuterium could be detected. This method, therefore, cannot be used for labelling 9α-chloro corticosteroids using our experimental conditions.

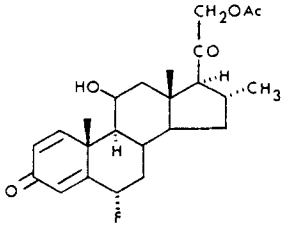
Compounds possessing free hydroxyl groups gave the lowest deuterium incorporations. Thus, dexamethasone 21-acetate (8) gave 12% and betamethasone 21-acetate (9) gave 17% of $^{2}H\text{-}C_{18}$ products. The lower incorporations can be explained by the exchange of the 17α-hydroxylic hydrogen with the PhSD--thus producing one mole equivalent of PhSH. The C_{18} radical is then trapped by the 4 equivalents of PhSD plus 1 equivalent of PhSH which on statistical grounds alone should result in a 5% lower yield of labelled 8 and 9. Thus, the 9% and 4% lower incorporation obtained reflects a small isotope effect which is consistent with the radical nature of the reaction. Presumably, one could eliminate this dilution of the label by exchanging the hydroxyl hydrogens for deuteriums prior to photolysis by washing the toluene solution with D_2O followed by thorough drying over molecular sieves.

The low to modest chemical yields are difficult to explain. In all these reactions, a minor less polar product (5%) which was identified as the 11-ketone (mass spectrum), and a more polar product (10-20%) are formed. If the photolysis is performed without any added hydrogen (or deuterium) source, this polar product becomes the major product formed. Barton and co-workers (3,4) have shown that in the absence of any competing radical trap the C_{18} radical reacts with the NO radical to give a nitroso compound which rearranges first to the C_{18} oxime and then on heating, to the nitrone. Our polar materials appear to be the nitrones (e.g. partial structure 10). It seems, therefore, that even a five-fold excess of PhSD is not enough to completely overcome the preference of the C_{18} radical for the NO radical. This is probably due to the

TABLE: Results of the photolysis of the 11β-nitrite esters in the presence of PhSD.

Compound	Irradiation Time (hours)	Chemical Yield %	^2H Incorporation %	Ion (F^+) used in Mass Spec.
1	3.5	30	21	$[M-20]^+$
3	1.5	58	5	$[M-36]^+$
5	3.5	42	40	$[M-20]^+$
6	4.0	35	32	$[M]^+$
7	3.5	26	20	$[M]^+$
8	2.5	19	12	$[M-20]^+$
9	2.5	13	17	$[M-20]^+$

FIGURE 2



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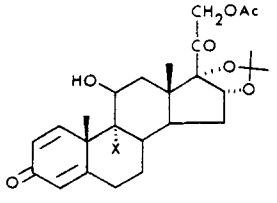
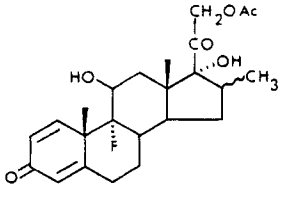


X=H, 6

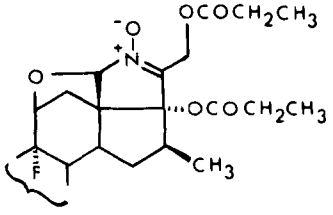
X=F, 7



16α-CH_3, 8

16β-CH_3, 9

fact that the latter reaction is simply a radical coupling process whereas the reaction with PhSD breaks an S-D bond and should therefore have a higher activation energy.



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The extent of ^{2}H-labelling was determined by mass spectrometry (7). The enrichment calculations were made on the molecular ion when it was of sufficient intensity. Otherwise, a fragment ion (F^{+}) was chosen that could be assigned unambiguously to a single specie. Intensity values used in calculating the ^{2}H incorporation were obtained by repetitively scanning the mass area of interest for both the unlabelled and labelled compounds and using the mean intensity values of eight scans as calculated by computer. Calculation of the intensities of the F+2, F+3 peaks for possible multiple ^{2}H incorporation gave values of 1% or less which is well within experimental error.

The position of deuterium incorporation was confirmed to be C_{18} from the proton decoupled deuterium magnetic resonance spectra of ^{2}H-labelled 1 and 5. Each spectrum showed a single resonance at 0.96 ppm which corresponds exactly with the pmr C_{13}-CH_3 resonances for these compounds. Also in their pmr spectra, 1 and 5, showed a 20% and 40% reduction, respectively, in the integrated intensity of their C_{13}-CH_3 resonance, which is consistent with both the extent of labelling as measured by mass spectrometry and with the position of ^{2}H incorporation. The cmr spectra of labelled 1 showed the expected upfield shift (8) for those C_{18} carbons carrying a ^{2}H of 0.27 + 0.04 ppm. This carbon resonance appeared as a triplet, $^{1}J_{C,D}$20 H_z at 16.06 ppm. A smaller upfield shift

(9) was also seen for the C_{13} resonance of labelled 5 (0.12 ± 0.04 ppm from 55.47 ppm). However, in the cmr spectrum of 1 the C resonance was obscured by one leg of the C_{10} doublet ($^2J_{C,F}$25H).

For most topical corticosteroids the method presented here has the advantage that the label is introduced at the final step of the synthesis. However, with one further step, i.e. ester hydrolysis, labelled C_{18} corticosteroids with free dihydroxyacetone side chains become available. Also, after homogeneous catalytic reduction (1d,10) of the 1,2-double bond C_{18} labelled cortisol and corticosterone derivatives can be prepared.

EXPERIMENTAL

Silica gel thick (1000μ) and thin (250μ) layer chromatography plates GF were from Analtech. Inc., toluene (Mallinckrodt Analar grade) and thiophenol (Aldrich, 97%) were used as received. Nitrosyl chloride was supplied by Matheson Gas Products, Inc. Melting points were taken on a Fisher-Johns hot-stage melting point apparatus. The pmr spectra were obtained on a Varian T-60A or a CFT-20 spectrometer, and the cmr spectra on a Varian XL-100-15 spectrometer (operating in the FT mode with a Varian 620L100 disc accessory) for $CDCl_3$ solutions with TMS as internal standard. The dmr spectra (Department of Chemistry, McMaster University) were obtained on a Brucker WH90 spectrometer at 13.81 MHZ with broad band decoupling and an external deuterium lock for $CHCl_3$ solutions. Mass spectra were taken on a Varian Mat CH5 spectrometer using a 70eV source.

PREPARATION OF PhSD:

Thiophenol (5 mL) and toluene (5 mL) were vigorously stirred with 99.98% D_2O (Merck, 10 mL) for 5 minutes. The phases were allowed to separate and the aqueous layer was discarded. Two repetitions of this procedure followed by overnight drying over molecular sieves 4A (Linde) gave a toluene solution of thiophenol which was better than 93% deuterated (nmr integration).

PREPARATION OF 11β-NITRITE ESTERS (11):

The steroid was dissolved in pyridine (1 mmole in 15 mL) and cooled to 0°. Nitrosyl chloride was bubbled into the solution until a brown color persisted. Dilution with water, filtration and thorough washing with water gave the 11β-nitrite ester. Drying first in air and then under high vacuum at room temperature gave material suitable for photolysis. In the case of beclomethasone 17,21-dipropionate (3), the 11β-nitrite was crystallized from EtOAc/hexane to give needles m.p. 187-193°;

$[\alpha]D^{26}$ +146.6 (dimethylformamide c, 0.2%); microanalysis

$C_{28}H_{36}O_8ClN$, calculated C, 61.14; H, 6.59; N, 2.54; Cl, 6.44%

found C, 61.13; H, 6.66; N, 2.30; Cl, 6.43%.

PHOTOLYSIS OF 11β-NITRITE ESTERS:

The photolysis cell consisted of a Pyrex test tube (internal diameter 16 mm) into which a glass rod (diameter 12 mm) had been placed. The steroid (100 mg) in toluene (2.5 mL) when placed in this cell gave a surface area of approximately 15.5 cm^2. After adding the toluene solution of PhSD (5 molar equivalents), the solution was purged with argon by means of a Teflon tube (1 mm diameter) inserted to the bottom of the test tube. The cell was then placed in the center of Rayonet Photochemical Reactor equipped with 3500Å photochemical reactor lamps (Cat. No. R.P.R.-3500A) and with continuous argon purging, was irradiated until a sample showed the absence of starting nitrite ester (tlc). The solution was diluted with EtOAc and washed three times with water, dried over anydrous $MgSO_4$ and evaporated to a solid residue. Chromatography on 4 silica gel thick layer plates (20 x 20 cm) gave the ^2H-C_{18} steroid. Trituration with ether/hexane (1:1) and then crystallization gave the deuterated steroid suitable for mass spectrometric analysis.

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