SYNTHESIS AND PURIFICATION OF RADIOACTIVE 6β-IODOMETHYL-19-NORCHOLEST-5(10)-EN-3β-OL

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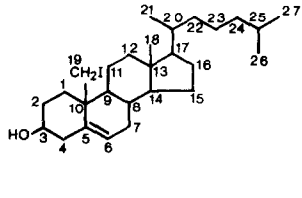
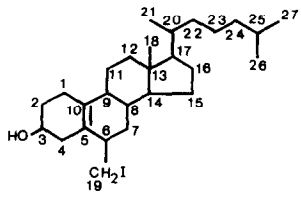
ABSTRACT

A method for the synthesis and purification of 6β-iodomethyl-19-norcholest-5(10)-ene-3β-ol-^{131}I of >98 mole% chemical purity and >99% radiochemical purity is presented. Carbon-13 and proton NMR were used to establish the identity and purity. Discrepancies in the characterization of this compound, previously published by two other research groups, are discussed.

Radioiodinated 19-iodocholest-5-en-3β-ol (I, Figure 1) was first synthesized by Counsell *et al.* in 1970 (1) and subsequently shown to be an effective adrenocortical imaging agent in humans(2). Kojima *et al.* (3,4) in 1975 showed by thin layer chromatography (TLC) that this compound contained an impurity which they identified as 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (II, Figure 1). This was subsequently confirmed by Basmadjian *et al.*(5) and Sarkar *et al.*(6). Characterization of II was by UV (3,4), IR (5), proton nuclear magnetic resonance (PMR) (3,4,5) and reduction to 6β-methyl-19-norcholest-5(10)-en-3β-ol (3). Both groups used preparative TLC to separate I and II.



I



II

Figure 1

The physical characteristics of II, as reported by the two groups, do not agree. Basmadjian et al. (5) obtained II as a low melting solid and Kojima et al. (3,4) as a glass. More importantly the PMR data disagree. Since there are discrepancies in the characterization of II by the two groups of investigators and since no evidence for the purity of II has been presented, we report here a procedure for the synthesis of II in gram amounts and of greater than 98 mole% chemical purity as proven by CMR. Since radioactive II accumulates to a greater extent (5-10 fold) in the adrenal gland than radioactive I (4,5,6), our synthetic method for the preparation of II is of radiopharmaceutical importance.

EXPERIMENTAL

Nuclear Magnetic Resonance Spectroscopy: Nuclear magnetic resonance spectra were obtained with a Bruker model HX-90 spectrometer equipped with a fast Fourier transform system using a Nicolet model 1083 computer. Proton spectra were obtained at 90.00 MHz and ^{13}C spectra at 22.63 MHz spectrometer operating frequencies. The samples were dissolved in a stock solution of deuteriochloroform which contained 5% by volume hexafluorobenzene (C_6F_6) and tetramethylsilane (TMS) in 1% by volume concentration for PMR spectra and 10% by volume concentration for CMR spectra. The fluorine signal from the C_6F_6 served as the field-frequency stabilization signal. Chemical shifts were measured relative to TMS. The CMR spectra shown in Figure 2 were obtained with simultaneous proton noise decoupling.

High Pressure Liquid Chromatography: A Waters Model 6000 Solvent Delivery System with Model U6K injector coupled to a LDC Model 1107 RefractoMonitor refractive index detector was employed for high pressure liquid chromatographic (HPLC) separations.

6β-Iodomethyl-19-norcholest-5(10)-en-3β-ol (II): Pure I (7) (1.88g) was refluxed under nitrogen in iso-propanol for 48 hours. The solvent was removed in vacuo to give an orange oil; TLC ($CHCl_3$, Eastman chromagram sheets with fluorescent indicator) R_f 0.34 and R_f 0.50; HPLC (5μ Lichrosorb, 25cm x 9.4mm ID, $CHCl_3$ at 3.0ml/min) disclosed one large (7.6min) and at least 6 smaller peaks. HPLC separation of this large peak gave 0.75g pale yellow glass; high resolution mass spectrum: Calcd. for M+, $C_{27}H_{45}IO$: m/e 512.2515. Found, 512.2521; TLC, $CHCl_3$ R_f 0.40; $CHCl_3$: acetone (20:1) R_f 0.44; $CHCl_3$: EtOH (1:1) R_f 0.62; benzene: ethyl acetate (1:1) R_f 0.54.

6β-^{131}Iodomethyl-19-norcholest-5(10)-en-3β-ol (II-^{131}I): Radioiodinated II was prepared by refluxing II with $Na^{131}I$ in acetone under N_2 for 4 hours. The acetone was then removed and the resulting oil was dissolved in ether, washed with water and dried over anhydrous Na_2SO_4. Removal of solvent gave a yellow oil which was dissolved in $CHCl_3$ and placed on a 2mm Silica Gel TLC plate with fluorescent indicator (Merck). Development with $CHCl_3$ gave 3 bands, R_f 0.79, 0.59, and 0.23. The R_f 0.23 band was scraped off and extracted with ether and $CHCl_3$. Removal of solvent gave a pale yellow oil shown to be 97% chemically pure II by CMR. TLC in the 4 solvent systems described above were identical with the non-radioactive II and showed the compound to be >99% radiochemically pure.

RESULTS

When we prepared II by refluxing I in iso-propanol for 48 hours, the resulting crude material was 80 to 100% II and the impurities were I and a number of other irreproducible steroidal compounds. The crude product was purified by HPLC, over 15 mg of >98% pure II being obtained from a single injection. Thus, HPLC provides a simple fast method for the purification of gram amounts of II. Insufficient quantities of II for toxicological studies were obtained by preparative TLC (6).

The purity of II was established by CMR. It has recently been shown in our laboratory that quantitation of mixtures of closely related compounds can be accomplished by CMR (8). By this method, components of 2 to 98 mole% can be quantitated with a standard deviation of ± 3% or less. A sufficient number of CMR scans were accumulated of solutions containing 100mg or more of II to yield spectra of such signal-to-noise ratio that carbon-containing components of 2 mole% or greater abundance could have been detected. Since in five different preparations of II, only the 27 expected carbon resonances of II were observed and no additional resonances indicating impurities were detected, the purity of II was shown to be consistently >98 mole%.

Using CMR we have unequivocally identified our product as 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol. This identification was based on detailed analysis of the CMR spectrum of II (Figure 2c) and comparison to the CMR spectra of seven related steroids (9): estr-5(10)-ene-3β,17β-diol 17-propionate (9,10), 17β-hydroxy-4-androsten-3-one (11), 17β-hydroxy-19-norandrost-4-ene-3-one (11), 17β-hydroxy-4-androsten-3-one 17-acetate (11), 5α-cholestane-3β,5-diol 3-acetate (12), 5α-cholestane-3β,5-diol-6β-methyl 3-acetate (12), and 3β-O-methyl-6β-iodomethyl-19-norcholest-5(10)-ene (9). Since the first published CMR spectra of steroids in 1969 (11), CMR spectroscopy of steroids has been thoroughly documented and is now well understood. Moreover, since the A and B ring structure of estr-5(10)-ene-3β,17β-diol 17-propionate

closely resembles that of II, the CMR spectra for the A and B rings of these two compounds are very similar. Thus there can be no ambiguity in our assignment of the CMR spectrum of II, and hence the identity of this compound.

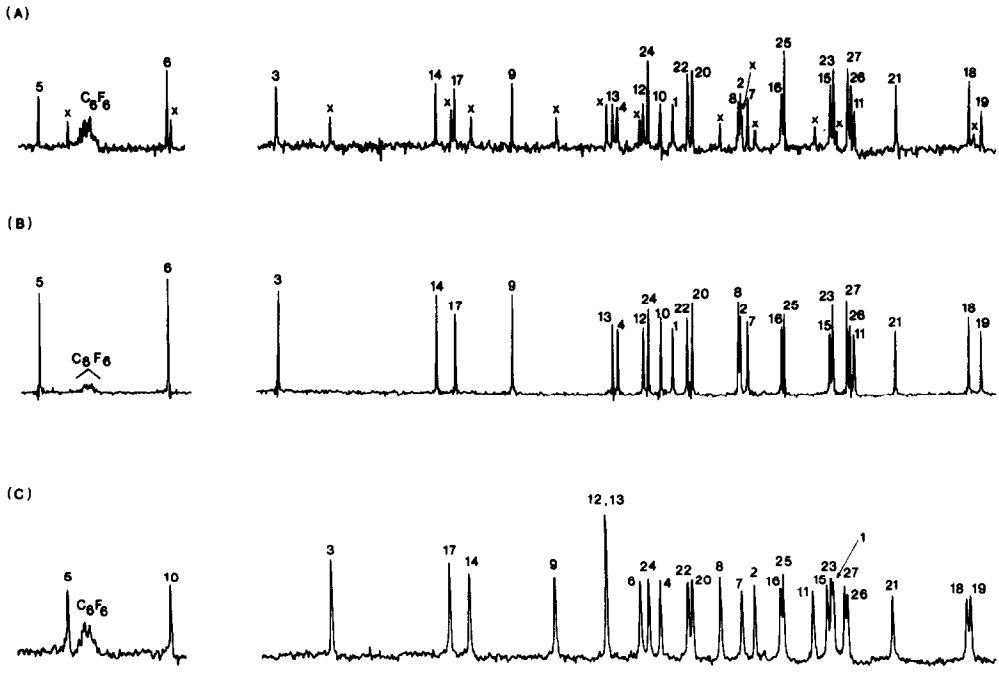


Figure 2. Carbon-13 nuclear magnetic resonance spectra. The spectra are displayed in two segments: the left segment shows the unsaturated carbons and the up-field half of the hexafluorobenzene reference signal; the right segment shows the saturated carbons. (A) Compound I, prepared by the method of reference (1). The impurity peaks are designated by x. (B) Compound I, prepared by the method of reference (7). (C) Compound II. Spectra (A) and (B) have been published previously (7), but are included to show the relationship of the three spectra.

Comparison of Figure 2a and 2c clearly shows that the impurity peaks observed in 2a are the peaks corresponding

to carbons 1 to 13, 17 to 19 of II in 2c. Thus CMR unequivocally shows that the impurity in 19-iodocholesterol obtained by the literature method (1) is indeed compound II.

Our PMR data for II (Figure 3c) agree to within 0.02 ppm with those obtained by Kojima et al.(3). The most upfield methyl signal at 0.70 ppm has been assigned by us and Kojima et al. to the C-18 methyl protons. Clearly, it is the same singlet as that observed in impure I (Figure 3a) and formerly (1) erroneously assigned to the C-18 methyl protons of I. Since PMR data for pure I have not been published previously, we include these data in Figure 3b.

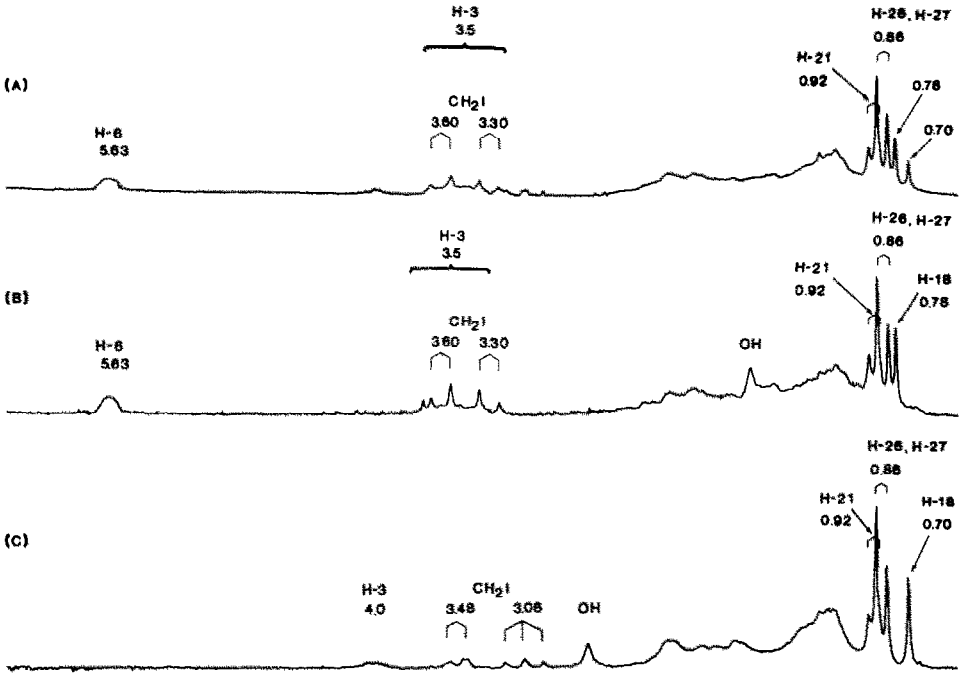


Figure 3. Proton nuclear magnetic resonance spectra. Chemical shifts are given in ppm from TMS. (A) Compound I, prepared by the method of reference (1). (B) Compound I, prepared by the method of reference (7). (C) Compound II.

Our PMR data for II, and hence our assignments of the peaks, do not agree with those reported by Basmadjian et al. (5). These authors, like Kojima et al. and we, observed the C-18 methyl proton singlet at 0.70 ppm corresponding in area to 3 protons. However, Basmadjian et al. report a large singlet, corresponding in area to 6 protons, at 50 cps (0.83 ppm) and a smaller singlet at 55 cps (0.92 ppm) corresponding in area to 3 protons. Thus, although we all observe peaks in the same spot in the spectrum (the three peaks assigned to H-18, H-26 and H-27 in Figure 3c), the intensities reported by Basmadjian et al. are totally different from those shown in Figure 3c. Moreover, the peak positions and the intensity pattern reported by Basmadjian et al. have not been observed for H-18, H-26 and H-27 in any of the previously reported PMR spectra of steroids which contain the C-20 cholestane side chain (13,14). The intensity pattern observed has consistently been that shown in Figure 3c.

Basmadjian et al., like Kojima et al. and we, observe a complex multiplet at 4.0 ppm for the C-3 proton. However, for the CH_2I protons they do not observe the well-defined triplet at 3.06 ppm and doublet-doublet at 3.48 ppm shown in Figure 3c and also reported by Kojima et al. Basmadjian et al. report two "multiplets" at 194 cps (3.23 ppm) and 214 cps (3.56 ppm). These values are surprisingly similar to the CH_2I chemical shifts of 3.25 and 3.51 ppm reported for impure I (1).

Since Basmadjian et al. do not show a PMR spectrum of their product, we cannot explain the anomalous data reported by them. A possible explanation would be that although they probably have obtained II, it is contaminated by I and other unidentified impurities.

DISCUSSION

Our results emphasize the inadvisability of relying heavily on the PMR spectrum to establish the identity and purity of newly synthesized steroids. The PMR of both I (1) and II (5) have been incorrectly assigned and impurities in these supposedly pure compounds were not recognized. Thus, the radiopharmaceutical properties of neither I nor II can be evaluated from the results reported in (1,2,5,6) because of the variable and unknown concentration of these two components. Our results emphasize the importance of CMR spectroscopy in establishing the identity and purity of steroids. From the CMR spectra we could readily observe that I (1) contained impurities. Furthermore we could characterize this impurity and then could establish the identity and purity of II. Now that we have presented methods for the preparation of I (7) and II of >98 mole% chemical purity and >99% radiochemical purity and in gram amounts, the toxicity and radiopharmaceutical properties of these compounds can be evaluated.

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