SYNTHESIS OF IODINE-131 LABELLED 6β-IODOMETHYL-19-NORCHOLEST-5(10)-EN-3α-OL AND 19-IODOCHOLEST-5-EN-3α-OL

H. Komatsu, M. Maeda, H. Morita, H. Shimoirisa and M. Kojima
Faculty of Pharmaceutical Sciences, Kyushu University 62,
Fukuoka 812, Japan

SUMMARY

6β-Iodomethyl-19-norcholest-5(10)-en-3α-ol (VI) was synthesized by the homoallylic rearrangement of 19-iodocholest-5-en-3α-ol (V), which was obtained by the hydrolysis of 19-iodocholest-5-en-3α-ol acetate derived from the displacement of cholest-5-ene-3α,19-diol 3-acetate 19-toluene-p-sulfonate with sodium iodide in isopropanol. The radioiodinated V and VI were prepared by isotope exchange with sodium iodide-I-131 in acetone.

Key Words: Adrenal Scanning Agent, 6β-Iodomethyl-19-norcholest-5(10)-en-3α-ol, 19-Iodocholest-5-en-3α-ol, Iodine-131

Considerable attention has been focused on 6β-substituted 19-norcholest-5(10)-en steroids after the finding that iodine-131 labelled 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (XII-^{131}I) shows an striking increased adrenal uptake, better adrenal-to-tissue ratios, and superior adrenal images as compared to iodine-131 labelled 19-iodocholest-5-en-3β-ol (X-^{131}I)(1-6). Further, tritium labelled 6β-methyl-19-norcholest-5(10)-en-3β-ol has been found to accumulate to a greater extent in adrenal than tritium labelled cholesterol, thus demonstrating that 6β-methyl analog has the basic structural feature having high affinity for adrenal (7). On the other hand, it has been shown that the substitution of bromine for iodine in XII results in the decrease in the selective accumulation in adrenal (8).

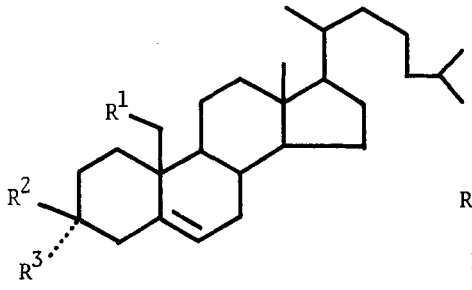
To obtain further information regarding the structure and adrenal localization of cholesterol analog is of potential value in designing radiopharmaceuticals

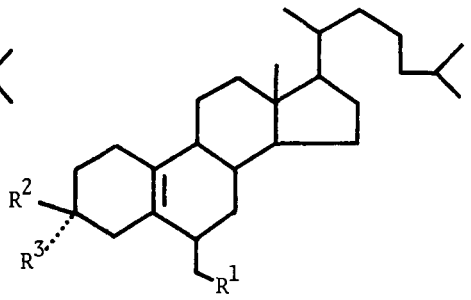
0362-4803/79/0216-0253$01.00

Received March 22, 1978

for specific diagnostic purposes. We have been faced with the need for the preparation of the corresponding 3α-ol epimers for X-^{131}I and XII-^{131}I in order to prove the stereochemical requirement of the hydroxy group at C-3 for adrenal accumulation. In this paper we wish to report the chemical synthetic part of the work and the results of the tissue distribution studies will be reported elsewhere.

The syntheses of 19-iodocholest-5-en-3α-ol (V) and 6β-iodomethyl-19-nor-cholest-5(10)-en-3α-ol (VI) were carried out using the method similar to our previous work on the preparation of X and XII (2). The starting material for our present work was cholest-5-ene-3α,19-diol-3-acetate (I), which is readily accessible from 5α-bromo-6β,19-epoxy-cholestane-3β-ol (9). Treatment of the starting 19-hydroxy-3α-acetate (I) with p-toluenesulfonyl chloride in pyridine afforded the desired tosylate ester (II) in 56% yield. An attempt to obtain the 19-tosyloxy-3α-ol by hydrolysis of II led to a complex mixture which prevented isolation of the desired compound. The displacement of II with sodium iodide in isopropanol gave rise to a 95:5 mixture of the 19-iodo-3α-acetate (III) and the 6β-iodomethyl-3α-acetate (IV) together with another minor unidentified product, from which the desired compound (III) was readily separable by recrystallization. Subsequent conversion of III to IV was achieved by heating in acetonitrile in moderate yield. Although final hydrolysis of IV to VI was accomplished with sodium hydroxide in dioxane, this reaction gave rise to a side product and required repeated column chromatographys and recrystallizations of the crude reaction mixture to achieve the required chemical purity. Thus the route III → IV → VI for the synthesis of VI was somewhat disfavored because of the key low-yield (38% yield) step IV → VI. On the other hand, mild hydrolysis of III in THF gave the 19-iodo-3α-ol (V) as the sole product, and subsequent treatment with refluxing acetonitrile proved to be





I R^1=OH R^2=H R^3=OAc
II R^1=OTs R^2=H R^3=OAc
III R^1=I R^2=H R^3=OAc
V R^1=I R^2=H R^3=OH
VII R^1=OH R^2=OAc R^3=H
VIII R^1=OTs R^2=OAc R^3=H
IX R^1=I R^2=OAc R^3=H
X R^1=I R^2=OH R^3=H

IV R^1=I R^2=H R^3=OAc
VI R^1=I R^2=H R^3=OH
XI R^1=I R^2=OAc R^3=H
XII R^1=I R^2=OH R^3=H

a more reliable synthesis of VI, affording 52% overall yield from III. The nmr spectra confirmed the configurations at C-3 as judged from chemical shift data and line width of the protons on the OH or OAc-bearing carbon as shown in Table 1 (10-13).

The 19-iodo 3α-ol (V) and 6β-iodomethyl 3α-ol (VI) thus obtained were then subjected to isotope exchange with sodium iodide-I-131. As the 19-iodo 3α-ol (V) has a strong tendency to rearrange to 6β-iodomethyl 3α-ol (VI) upon heating in an appropriate solvent, a method for the preparation of the radioiodinated V (V-^{131}I) was required allowing to avoid the concomitant formation of the radioiodinated VI (VI-^{131}I). For this purpose, isotope exchange was carried out in acetone at 15°, though prolonged reaction time was required.

Table 1 Resonances for the C-3 Protons in the NMR Spectra[a]

Compds	Config. of 3-H	δ(ppm)	$W_{1/2}$[b]	W[c]	Ref.
I	β(eq)	5.00	9	16	(9)
II	β(eq)	4.92	7	16	
III	β(eq)	5.02	7	20	
IV	β(ax)[d]	4.86	20	32	
V	β(eq)	4.08	9	16	
VI	β(ax)[d]	3.86	18	36	
VII	α(ax)	4.65	20	36	(14)
VIII	α(ax)	4.58	20	34	(14)
IX	α(ax)	4.60	23	36	(15)
X	α(ax)	3.5	†	†	(2)
XI	α(eq)[d]	4.99	14	30	(2)
XII	α(eq)[d]	3.97	14	28	(2)

a) Determined with a JNM PS-100 spectrometer (100 MHz) in $CDCl_3$ with TMS as internal reference. b) Half-height width in Hertz. c) Base width in Hertz. d) A preferred conformation of ring A in 5(10)-unsaturated steroids is half-chair form, see refs. (11)-(13). † Unresolved by the overlapping of the C-19 CH_2I protons and the C-3 proton.

This isotope exchange, however, was accompanied by the formation of a radioactive by-product which was difficult to separate completely from V-^{131}I, and this impurity accounted for about 9% of V-^{131}I. This complication was not encountered with VI and 75% exchange was effected by heating in refluxing acetone for 7 hr. The radioiodinated VI (VI-^{131}I) was considerably stable and maintained its radiochemical purity of >95% after 8 days at 15°, whereas the radioiodinated V (V-^{131}I) was unstable and at the same temperature about 50% of deiodination occurred in 2 days.

EXPERIMENTAL

Melting points are uncorrected. The nmr spectra were obtained with a JNM PS-100 spectrometer (100 MHz) in $CDCl_3$ with TMS as internal reference and the ir spectra were taken on a JASCO DS-701G spectrometer. Optical rotations were measured for solutions in $CHCl_3$ with a JASCO DIP-SL automatic polarimeter. The thin-layer chromatography (TLC) was carried out on silica gel $60F_{254}$ (0.5 mm layer, Merck). Chromatograms of radioiodinated compounds were scanned with a Aloka TRM-1B radiochromatogram scanner and radioactivity was assayed in a Aloka IGC-2B digital curiemeter.

Cholest-5-ene-3α,19-diol 3-acetate 19-p-toluenesulfonate (II).-----A solution of 256 mg of cholest-5-ene-3α,19-diol 3-acetate (I)(9) in 4.2 ml of pyridine and 540 mg of p-toluenesulfonyl chloride was heated at 50° for 9.5 hr and then processed in the usual way through ether extraction. Recrystallization of the crude product from petroleum ether furnished 194 mg (56%) of the tosylate (II) as needles, m.p. 103°. $[\alpha]_D^{24}$ -25.2°(c 1.16); ν_{max}(KBr) 1735, 1600, 1190 and 1180 cm^{-1}; nmr δ 0.58(s, 3H, C-18 Me), 2.03(s, 3H, OAc), 2.48(s, 3H, Me), 3.98 and 4.10(dd, 2H, J=10 Hz, C-19 CH_2I), 4.92(m, 1H, C-3), 5.50(m, 1H, vinylic), and 7.35-7.80(A_2B_2m, 4H, aromatic). Anal. Calcd. for $C_{36}H_{54}O_5S$: C, 72.21; H, 9.09. Found: C, 72.19; H, 9.13.

19-Iodocholest-5-en-3α-ol acetate (III).-----The tosylate (II)(471 mg), dissolved in 48 ml of isopropanol, was heated under reflux for 1.5 hr with 298 mg of sodium iodide under nitrogen. The solution was concentrated to about 5 ml in vacuo and poured into ice-water. The resulting mixture was extracted with ether. Ther ether was successively washed with water, 1% sodium thiosulfate, and water, and dried over Na_2SO_4. The nmr spectrum of the product after removal of the solvent showed a 95:5 mixture of 19-iodocholest-5-en-3α-ol

acetate (III) and 6β-iodomethyl-19-norcholest-5(10)-en-3α-ol acetate (IV) together with another minor unidentified compound. Recrystallization from petroleum ether gave a pure sample of III (293 mg, 67%) as needles, m.p. 129-130°. $[\alpha]_D^{21}$ -32.2°(c 0.86); ν_{max}(KBr) 1730, 1250 and 1225 cm^{-1}; nmr δ 0.78(s, 3H, C-18 Me), 2.04(s, 3H, OAc), 3.31 and 3.57(dd, 2H, J=10 Hz, C-19 CH_2I), 5.02 (m, 1H, C-3), and 5.56(m, 1H, vinylic). Anal. Calcd. for $C_{29}H_{47}O_2I$: C, 62.79; H, 8.54. Found: C, 62.79; H, 8.55.

6β-Iodomethyl-19-norcholest-5(10)-en-3α-ol acetate (IV).-----A solution of the 19-iodo-3α-acetate (III)(293 mg) in acetonitrile (30 ml) was refluxed for 85 min. The solution was concentrated to about 1 ml under reduced pressure. The resulting mixture was extracted with ether and the ether was washed successively with water, 1% sodium thiosulfate, and water, and dried over Na_2SO_4. Removal of the solvent gave 165 mg (56%) of IV, m.p. 100-100.5°, after recrystallization from acetone and then from petroleum ether. $[\alpha]_D^{19}$ +72.9°(c 0.95); ν_{max}(KBr) 1735 and 1240 cm^{-1}; nmr δ 0.68(s, 3H, C-18 Me), 2.04(s, 3H, OAc), 3.02(t, 1H, J=10.5 Hz, C-6 CH_2I), 3.44(dd, 1H, J=10.5, 2.5 Hz, C-6 CH_2I), and 4.86(m, 1H, C-3). Anal. Calcd. for $C_{29}H_{47}O_2I$: C, 62.79; H, 8.54. Found: C, 62.76; H, 8.59.

19-Iodocholest-5-en-3α-ol (V).----- A solution of NaOH (180 mg) in 20% aqueous methanol (14 ml) was added dropwise to a solution of the 19-iodo-3α-acetate (III)(390 mg) in THF (23 ml) in an ice-water bath. The solution was then stirred for 140 min. at room temperature and poured into ice-water. The resulting mixture was extracted with ether and the ether extracts washed with water and dried over Na_2SO_4. Removal of the ether gave a solid, which was recrystallized from n-hexane to afford V (249 mg, 69%) as a powder, m.p. 128-129°. $[\alpha]_D^{19}$ -103.3°(c 0.80); ν_{max}(KBr) 3320 cm^{-1}; nmr δ 0.80(s, 3H, C-18 Me), 1.58(s, 1H, OH), 3.26 and 3.56(dd, 2H, J=11 Hz, C-19 CH_2I), 4.08(m, 1H, C-3),

and 5.70(m, 1H, vinylic); TLC: chloroform, Rf 0.36. Anal. Calcd. for $C_{27}H_{45}OI$: C, 63.27; H, 8.85. Found: C, 63.83; H, 8.94.

6β-Iodomethyl-19-norcholest-5(10)-en-3α-ol (VI).-----a) A solution of V (181 mg) in acetonitrile (13 ml) was refluxed for 130 min. The solution was concentrated to about 1 ml under reduced pressure. The resulting mixture was extracted with ether and the ether was successively washed with water, 1% sodium thiosulfate, and water, and dried (Na_2SO_4). Removal of the solvent left a solid, which was recrystallized from cyclohexane or acetonitrile to give VI (138 mg, 76%) as needles, m.p.(decomp) 122° $[\alpha]_D^{19}$ +65.6°(c 0.80); ν_{max}(KBr) 3280 cm^{-1}; nmr δ 0.72(s, 3H, C-18 Me), 1.62(s, 1H, OH), 3.07(t, 1H, J=10 Hz, C-6 CH_2I), 3.49(dd, 1H, J=10, 2 Hz, C-6 CH_2I), and 3.86(m, 1H, C-3); TLC: chloroform, Rf 0.18; chloroform:acetone (95:5), Rf 0.52; n-hexane:ethyl acetate (3:1), Rf 0.44. Anal. Calcd. for $C_{27}H_{45}OI$: C, 63.27; H, 8.85. Found: C, 63.37; H, 8.90.

b) A solution of NaOH (72 mg) in 20% aqueous methanol (6 ml) was added dropwise to a solution of IV (142 mg) in dioxane (5 ml) in an ice-water bath. The solution was then stirred for 80 min. at room temperature. The mixture was poured into ice-water and extraction with ether gave a solid on removal of the solvent in vacuo. Recrystallization from petroleum ether yielded 88 mg of amorphous residue, m.p. 118°(decomp). Examination by tlc using n-hexane:ethyl acetate (3:1) as eluent showed the presence of two components, Rf 0.49(minor) and Rf 0.44(major). The mixture was then purified by repeated column chromatographys on silica gel (silicic acid, 100 mesh, Mallinckrodt) using n-hexane:ethyl acetate (6:1) and recrystallization from petroleum ether to give 50 mg (38%) of VI, m.p.(decomp) 122°, identical in all respects with the product described above.

^{131}I-19-Iodocholest-5-en-3α-ol (V-^{131}I).-----A solution of V (1.76 mg) and

$Na^{131}I$ (1.90 mCi) in dry acetone (1 ml) was stirred at 15° for 22 hr. The acetone was removed in vacuo and the residue was dissolved in ether. The mixture was streaked on 0.5 mm thick silica gel glass plate and developed in chloroform. The separated radioiodinated V was scraped off and eluted with a mixture of ether and ethyl acetate. Removal of the solvent gave the radioiodinated V (V-^{131}I)(1 mg) as colorless crystals with a specific activity of 823 μCi/mg. V-^{131}I thus obtained was contaminated by an unidentified impurity with an Rf value (0.22) slightly lower than that (0.36) of V-^{131}I (chloroform as eluent). This impurity accounted for about 9% of V-^{131}I. Further purification was resisted owing to the instability of V-^{131}I.

6β-^{131}I-Iodomethyl-19-norcholest-5(10)-en-3α-ol (VI-^{131}I).-----A solution of VI (4.88 mg) and $Na^{131}I$ (1.86 mCi) in dry acetone (1 ml) was refluxed for 7 hr under an atmosphere of nitrogen. The acetone was evaporated in vacuo and the residue was dissolved in ether. The ether solution was washed (1% sodium thiosulfate and water) and dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was dissolved in chloroform and the mixture was streaked on 0.5 mm thick silica gel glass plate and developed in chloroform. The separated VI-^{131}I was scraped off and eluted with a mixture of chloroform and ether. Removal of the solvent afforded VI-^{131}I (4 mg) as colorless needles with a specific activity of 342 μCi/mg. TLC using the two solvent systems used for non-radioactive VI showed all of the radioactivity coincident with the spot corresponding to cold VI.

Acknowledgment

Financial support from the Ministry of Education of Japan is gratefully acknowledged.

References

1. Kojima M., Maeda M., Ogawa H., Nitta K., and Ito T. -J. nucl. Med., 16: 666 (1975); idem., Chem. Pharm. Bull., 24: 2322 (1976).
2. Maeda M., Kojima M., Ogawa H., Nitta K., and Ito T. -Steroids, 26: 241 (1975).
3. Basmadjian G.P., Hetzel K.R., Ice R.D., and Beierwaltes W.H. -J. Labelled Compounds, 11: 427 (1975).
4. Sarkar S.D., Beierwaltes W.H., Ice R.D., Basmadjian G.P., Hetzel K.R., Kennedy W.P., and Mason M.M. -J. nucl. Med., 16: 1038 (1975).
5. Scott K.N., Couch M.W., Mareci T.H., and Williams C.M. -Steroids, 28: 295 (1976).
6. Couch M.W. and Williams C.M. -J. nucl. Med., 18: 724 (1977).
7. Kojima M., Maeda M., Ogawa H., Nitta K., Ito T., and Umeda F. -Radioisotopes, 25: 222 (1976).
8. Kojima M., Maeda M., Komatsu H., Shimoirisa H., Ogawa H., Nitta K., and Ito T. -Steroids, 29: 443 (1977).
9. Watanabe Y., Mizuhara Y., and Shiota M. -J. Org. Chem., 33: 468 (1968). The authors have isolated the acetate (I) as an oil. The acetate obtained in our studies, however, was a crystalline substance (m.p. 100°) which showed the same nmr spectral data as reported.
10. Jackman L.M. and Sternhell S., "Application of Nuclear Magnetic Spectroscopy in Organic Chemistry", 2nd ed., Pergamon Press, Oxford, 1969, p 228.
11. Levine S.G., Eudy N.H., and Leffler C.F. -J. Org. Chem., 31: 3995 (1966).
12. Borgna J.-L. and Mousseron-Canet M. -Bull. Soc. chim. France, 2210 (1970).
13. Borgna J.-L. and Mousseron-Canet M. -Bull. Soc. chim. France, 2218 (1970).
14. Akhtar M. and Barton D.H.R. -J. Am. Chem. Soc., 86: 1524 (1964).
15. Counsell R.E., Ranade V.V., Blair R.J., Beierwaltes W.H., and Weinhold P.V. -Steroids, 16: 317 (1970).