SYNTHESIS OF IODINE-131 LABELED 6β-IODOMETHYL-19-NORSITOST-5(10)-EN-3β-OL AND 19-IODOSITOST-5-EN-3β-OL FOR ADRENAL IMAGING

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ABSTRACT

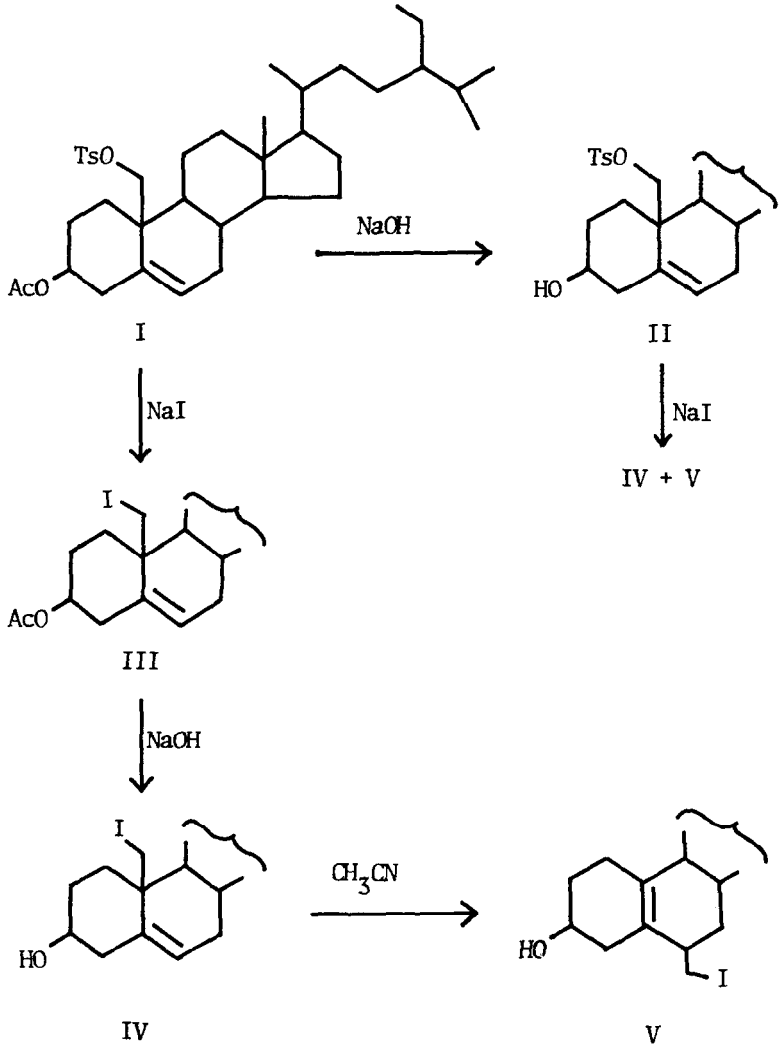
6β-Iodomethyl-19-norsitost-5(10)-en-3β-ol (V) was synthesized by homoallylic rearrangement of 19-iodositost-5-en-3β-ol (IV), which was obtained by the hydrolysis of 19-iodositost-5-en-3β-ol acetate (III) derived from the displacement of sitost-5-ene-3β,19-diol 3-acetate 19-p-toluenesulfonate (I) with sodium iodide in isopropanol. The radioiodinated IV and V were prepared by isotope exchange with sodium iodide-I-131.

It has recently been demonstrated that iodine-131 labeled 6β-iodomethyl-19-norcholest-5(10)-en-3β-ol (NCL-6-I-131) is a far superior radiopharmaceutical as an adrenal-scanning agent for human use than iodine-131 labeled 19-iodocholest-5-en-3β-ol (CL-19-I-131)(1-7). Our previous structure-distribution studies with 6β-substituted 19-nor steroids have shown that 6β-methyl analog which is the basic skeleton of NCL-6-I has the basic structural feature having high affinity for adrenal, and the β-configuration of the hydroxy group at C_3 is one of the important factors required for adrenal specificity (8,9). Fukushi et al.(10) have also investigated the relationship between adrenal affinity and plasma lipoprotein binding of various halogenated derivatives of cholesterol. In a continuing effort to obtain further information regarding the structural requirements necessary for adrenal localization, we have undertaken the synthesis of iodine-131 labeled 6β-iodomethyl-19-norsitost-5(10)-en-3β-ol (^{131}I-V) and 19-iodositost-

5-en-3β-ol (^{131}I-IV) to assess the influence of 17β-side chain for adrenal uptake.

In planning the synthetic route to the 6β-iodomethyl compound (V), the 19-iodo compound (IV) seemed to be an immediate and attractive precursor. The synthesis of this compound (IV), already reported by Counsell et al.(11), employed the reaction of sitost-5-ene-3β,19-diol 19-p-toluenesulfonate (II) with sodium iodide in refluxing isopropanol. Our interest in the reactivity of 19-substituted 5-ene steroids also induced us to reinvestigate this reaction. Thin layer chromatography (tlc) of the reaction mixture showed that the 6β-iodomethyl (V), with an Rf value slightly higher than that of the 19-iodo (IV), was formed along with IV, as was expected from the knowledge of our earlier works concerning the information of 6β-halogenomethyl 19-nor steroids (2,9,12). After evaporation of the solvent, the residue was immediately submitted to nmr analysis, and the ratio of V to IV was about 0.56. Furthermore, even when recrystallization of the crude product from acetone was performed, it was very difficult to obtain pure IV entirely free from V. Therefore, it is evident that the 19-iodo (IV) prepared as described by Counsell et al., if not purified further, contains the homoallylic isomer (V).

We have now adapted the synthetic route I → III → IV to give an improved method for the preparation of IV. The displacement of sitost-5-ene-3β,19-diol 3-acetate 19-p-toluenesulfonate (I) with sodium iodide in isopropanol gave the corresponding 19-iodo 3-acetate (III) without the detectable formation of other isomers and subsequent hydrolysis of the acetoxy group at room temperature gave pure IV as



determined by tlc and nmr analysis. Thus the conversion of IV to the requisite V was achieved by heating in acetonitrile in moderate yield. The nmr spectrum of V showed characteristic two-proton signals due to the methylene protons of the 6-iodomethyl group similar to those of NCL-6-I.

The 19-iodo (IV) and 6β-iodomethyl (V) thus obtained were then

subjected to isotope exchange with carrier-free $Na^{131}I$. In the case of V, 55% exchange was effected by heating in a mixture of acetone and acetonitrile at the reflux temperature for 4 hr. On the other hand, the exchange labeling of IV carried out in refluxing acetone, as expected, was accompanied in some runs by the formation of the radioiodinated V which was, however, separable by the tlc plates. The radioiodinated V, when stored in ethanol at room temperature, was indeed stable for 7 days, whereas the radioiodinated IV was very unstable and at the same temperature 20-60% of deiodination occurred in 3 days.

Preliminary tissue distribution study with the radioiodinated V in rats showed adrenal uptake almost similar to NCL-6-I-131, and the detailed results will be reported elsewhere.

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. The ir spectra were taken on a JASCO DS-701G and IRA-1 spectrometer. Optical rotations were determined for solutions in $CHCl_3$ with a JASCO DIP-SL automatic polarimeter. The mass spectrum was measured with a JEOL-JMS-OISG mass spectrometer. The thin-layer chromatography (tlc) was carried out on silica gel 60F 254 (0.5 and 0.25 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.

Reaction of sitost-5-ene-3β,19-diol 19-p-toluenesulfonate (II) with sodium iodide.---------- According to the method of Counsell et al., a solution of (II)(100 mg) and NaI (50 mg) in isopropanol (6.7 ml) was gently refluxed under nitrogen for 4 hr. A portion of the reaction mixture was analyzed at appropriate intervals by tlc. After 4 hr, tlc ($CHCl_3$) showed the presence of two products at Rf 0.32 and 0.23 with the absence of unchanged starting material. The solution was concentrated under vacuum to about 2 ml and poured into ice-water. The mixture was extracted with ether. The ether solution was washed successively with water, 1% sodium thiosulfate, and water, and dried over Na_2SO_4. Removal of the solvent left a colorless solid, part of which was immediately submitted to nmr analysis. The ratio of 6β-iodomethyl (V) to 19-iodo (IV) was 0.56 by the integration of 18-methyl protons. Furthermore, recrystallization from acetone gave colorless

needles, m.p. 113-114°(lit.(11), m.p. 124°). Inspection of this product by nmr analysis showed the presence of V in the ratio of 0.26.

The Rf values in various solvent systems are listed in Table 1.

TABLE 1. Rf values[a)]

Compounds	Chromatographic solvent systems[b)]			
	A	B	C	D
19-iodo (IV)	0.23	0.40	0.65	0.85
6-iodomethyl (V)	0.32	0.48	0.65	0.85

a) Spots were visualized by spraying with 10% H_2SO_4 and then heating the plates or detected with UV light.
b) A: $CHCl_3$ B: $CHCl_3$-acetone (95:5) C: $CHCl_3$-AcOEt (1:1) D: $CHCl_3$-EtOH (1:1)

19-Iodositost-5-en-3β-ol acetate (III).---------- Sitost-5-ene-3β,19-diol 3-acetate 19-p-toluenesulfonate (I)(11)(626 mg), dissolved in isopropanol (40 ml), was heated under reflux for 2 hr with NaI (300 mg) under nitrogen. The resulting mixture was extracted with ether. The ether was successively washed with water, 1% sodium thiosulfate, and water, and dried over Na_2SO_4. Removal of the solvent gave III (432 mg) as colorless needles, m.p. 99-101°, after recrystallization from acetone-methanol.

$[\alpha]_D^{21}$ -54.6° (c 0.69); ν_{max}(Nujol) 1739 and 1250 cm^{-1}($OCOCH_3$); nmr δ 0.78(s, 3H, 18-CH_3), 1.96(s, 3H, $OCOCH_3$), 3.23 and 3.53(dd, 2H, J 11 Hz, 19-CH_2I), 4.48(broad, 1H, 3-H), and 5.64 ppm(m, 1H, vinylic).

Anal. Calcd. for $C_{31}H_{51}O_2I$: C, 63.91; H, 8.82. Found: C, 63.58; H, 8.83.

19-Iodositost-5-en-3β-ol (IV).---------- A solution of NaOH (400 mg) in 20% aqueous methanol (40 ml) was added dropwise to a solution of III (800 mg) in dioxane (27 ml) in an ice-water bath. The solution was then stirred for 30 min. at room temperature and poured into ice-water. The resulting mixture was extracted with ether and the ether was washed with water, and dried over Na_2SO_4. Removal of the ether gave a solid, which was recrystallized from petroleum ether to afford pure IV (690 mg) as colorless needles, m.p. 116-119°.

$[\alpha]_D^{21}$ -40.7° (c 0.5); ν_{max}(Nujol) 3350 cm^{-1}(OH); nmr δ 0.77(s, 3H, 18-CH_3), 1.57(OH, D_2O exchangeable), 3.22 and 3.53(dd, 2H, J 11 Hz, 19-CH_2I), ca.3.4(broad, 1H, 3-H), and 5.58 ppm(m, 1H, vinylic).

Anal. Calcd. for $C_{29}H_{49}OI$: C, 64.43; H, 9.14. Found: C, 64.57; H, 9.24.

6β-Iodomethyl-19-norsitost-5(10)-en-3β-ol (V).---------- A solution of IV (50 mg) in acetonitrile (6 ml) was refluxed for 2 hr. Tlc showed

the complete disappearance of the starting material. The solvent was evaporated in vacuo and the residue was dissolved in $CHCl_3$. The mixture was streaked on silica gel glass plates and developed with $CHCl_3$-acetone (95:5) or $CHCl_3$. The separated V was scraped off, eluted with a mixture of ether and $CHCl_3$, and filtered. Removal of the solvent gave V (37 mg) as colorless glass.

Mass: 540 (M^+); $[\alpha]_D^{21}$ +105.3°(c 0.3); ν_{max}(KBr) 3360 cm^{-1}(OH); nmr δ 0.68(s, 3H, 18-CH_3), 1.74(OH, D_2O exchangeable), 3.05(t, 1H, J 10 Hz, 6-CH_2I), 3.46(dd, 1H, J 10, 2.5 Hz, 6-CH_2I), and 3.98 ppm(m, 1H, 3-H).

Anal. Calcd. for $C_{29}H_{49}OI$; C, 64.43; H, 9.14. Found: C, 64.46; H, 9.21.

^{131}I-19-Iodositost-5-en-3β-ol (^{131}I-IV).--------- A solution of IV (1.3 mg) and Na^{131}I (1.89 mCi) in dry acetone (2 ml) was refluxed for 3 hr under an atmosphere of nitrogen. Tlc showed 51% ^{131}I-IV and 36% ^{131}I-V together with 13% Na^{131}I at the origin. The solvent was evaporated in vacuo and the residue was dissolved in $CHCl_3$, placed on tlc plates, and developed with $CHCl_3$ or $CHCl_3$-acetone (95:5). The separated ^{131}I-IV was scraped off, extracted with a mixture of $CHCl_3$ and ether, and filtered. Removal of the solvent gave ^{131}I-IV (525 μCi), which was contaminated by about 5% free iodide as determined by tlc. Further purification was resisted owing to the instability of ^{131}I-IV.

^{131}I-6β-Iodomethyl-19-norsitost-5(10)-en-3β-ol (^{131}I-V).---------- A solution of V (2.4 mg) and Na^{131}I (2.8 mCi) in dry acetone-acetonitrile (1:1)(3 ml) was refluxed for 4 hr. The solvent was evaporated in vacuo and the residue was dissolved in $CHCl_3$, placed on tlc plates, and developed with $CHCl_3$-acetone (95:5). The Rf 0.48 band was scraped off, extracted with a mixture of $CHCl_3$ and ether, and filtered. Removal of the solvent afforded ^{131}I-V with a specific activity of 570 μCi/mg. The radiochemical yield was 41%. Tlc showed all of the radioactivity coincident with the spot corresponding to nonradioactive V.

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