SYNTHESIS OF A NEW ADRENAL CORTEX IMAGING AGENT 6β-^{131}I-IODOMETHYL-19-NOR CHOLEST-5(10)-EN-3β-OL (NP-59)

Garabed P. Basmadjian, Kenneth R. Hetzel, Rodney D. Ice and William H. Beierwaltes
College of Pharmacy and Nuclear Medicine Section, The University of Michigan Medical Center, Ann Arbor, Mi., U.S.A.
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SUMMARY

A new adrenal cortex imaging agent, 6β-^{131}I-iodomethyl-19-nor-cholest-5(10)-en-3β-ol (NP-59) [I] was synthesized by the homallylic rearrangement of 19-iodocholesterol or directly from cholest-5-ene-3β,19-diol-19-toluene-p-sulfonate via homoallylic rearrangement with the iodide ion as a nucleophile and subsequent exchange with Na^{131}I. NP-59 appears to concentrate 5 times better than 19-iodocholesterol in the rat adrenal and is currently being evaluated as a possible diagnostic agent in man.

INTRODUCTION

Radioiodinated 19-iodocholesterol was first synthesized by Counsell et al (1). The immediate most stable precursor of 19-iodocholesterol is the 3-acetate-19-toluene-p-sulfonate analogue of cholesterol. Selective hydrolysis of the acetate ester subsequently treated with sodium iodide in isopropanol yields 19-iodocholesterol. Radioiodination of 19-iodocholesterol has been accomplished by radionuclidic exchange with Na^{125}I or Na^{131}I in refluxing acetone.

^{131}I-19-Iodocholesterol has been demonstrated to be a useful adrenal cortical scanning agent in Cushing's disease, aldosterone producing tumors and localizing adrenal remnants associated with presistent or recurring Cushing's syndrome after "total" adrenalectomy (2,3,4).

During developmental research on 19-iodocholesterol, while this radiopharmaceutical was prepared for distribution, an "impurity" that was not iodide ion was noticed in the "cold" 19-iodocholesterol and also in the routine 250 mCi $Na^{131}I$ exchange runs. This "impurity" accounted for 10-25% of 19-iodocholesterol. While the major decomposition product of 19-iodocholesterol was the iodide ion, accelerated thermal decomposition studies indicated a two component curve not consistent with the iodide ion being the only impurity (5). The object of this study was to identify this "impurity", synthesize and evaluate it as a radiopharmaceutical.

EXPERIMENTAL

I. Cholest-5-ene-3β,19-diol-19-toluene-p-sulfonate [A]: Prepared according to Counsell et al (1).

II. 6β-Iodomethyl-19-nor-cholest-5(10)-en-3β-ol (NP-59):

A. From [A]:

A solution of 2.5g of [A] and 2.25g of NaI in 50 ml absolute alcohol was refluxed for 4 hrs. under nitrogen. The alcohol was evaporated under vacuo, the residue was extracted with ether (3 x 100 ml) and filtered. The combined ether extracts were washed with (3 x 50 ml) water, dried over anhydrous Na_2SO_4 and evaporated to dryness under vacuo at room temperature.

The solid residue was dissolved in absolute alcohol and water was added to cloudiness, refrigerated to precipitate a white, amorphous mass, which was filtered, washed with water and dried under vacuo at room temperature Yield 1.9g (83%).

Thin layer chromatography: Silica Gel G with chloroform as solvent, showed 90% NP-59 Rf 0.38-0.4 and 10% 19-iodocholesterol (1) Rf 0.3-0.32.

A portion of the above mixture in methanol was streaked on 2 mm thick Silica Gel G_{254} glass plates [a] and developed in 100% $CHCl_3$. The separated NP-59 was scraped, eluted with methanol and the solvent evaporated under vacuo, to give a colorless thick oil. This was dissolved in $CHCl_3$ and the solvent evaporated under high vacuum to give a white, fluffy powder.

m.p. 41-44°C; $[\alpha]_D^{20}$ + 35° (in cyclohexane).

Analysis: Calc. for $C_{27}H_{45}IO$; C = 63.27%, H = 8.85%

Found: C = 63.39%, H = 8.93%

IR: $\bar{\nu}$ max (neat): 3300, 1475, 1380, 1180, 1155, 1085, 1045, 875, 790 cm^{-1}.

NMR ($CDCl_3$:60MHZ): 42 cps (S,3,C_{18}-proton), 50 cps (S,6,C_{25}+C_{26}-proton), 55 cps (S,3,C_{21}-proton), 128 cps (OH), 194 cps (m,1,6-CH_2 I-proton), 214 cps (m,1,6-CH_2I-proton), 239 cps (1,m,3-H).

Mass Spectrum:

$[M-1]^+$ 511, P, $[M-1]^+$ 385, $[M-I-H_20]^+$ 367, $[HI]^+$ 128

Plus typical fragments corresponding to the cholestane skeleton: m/e 43,55,57,69,71,81,95,105,107,145,147,211,213.

B. From 19-iodocholesterol:

Prepared according to Counsell et al (1), 19-iodocholesterol contains about 25-30% NP-59

When this mixture is refluxed under N_2 in isopropanol or absolute ethanol with a few crystals of NaI overnight, the proportion of NP-59 shifts to about 90% while about 10% is left as 19-iodocholesterol. No other products are detected on TLC.

Radioiodinated ^{131}I-NP-59 Isotope Exchange:

To 12 mg NP-59 in a 15 ml round bottom flask with a magnetic

[a] Brinkmann Instruments, Inc.
Westbury, N.Y. 11590

stirrer, was added 30 mCi $Na^{131}I$ (carrier free, high concentration[b]) using 2 x 2 ml absolute alcohol for transfer. This solution was stirred and refluxed for 1 hour. The solution was cooled to room temperature and passed through a 1.5 cm column of Cellex D (BioRad) pressed and washed several times with acetone and absolute alcohol in a 2.5 ml glaspak syringe. Sample eluate was collected in a 15 ml vacutainer. Column was rinsed with 2 x 2 ml absolute ethanol, and combined with eluate.

Radiochemical yield: 16 mCi (53% exchange)

Specific Activity: 1.3 mCi/mg

TLC on Silica Gel G_{254} plates in $CHCl_3$ or CH_2Cl_2 85%:EtoAc 15% showed one radioactive peak corresponding to "cold" NP-59 spot.

Formulation and Stability:

One mCi of ^{131}I-NP-59 (S.A. 1.3 mCi/mg) was formulated to 10 ml with 6.6% EtOH,%Polysorbate 80, q.s. bacteriostatic normal saline.

^{131}I-NP-59 in absolute alcohol appears to be stable from -20°C to 4°C for greater than a month. In formulation NP-59 is stable at 4°C for 2 weeks and at room temperature 20% deiodination occured in 4 days.

^{131}I-19-iodocholesterol in the above formulation at 4°C shows slow deiodination and formation of NP-59 with time such that the ratio of NP-59 to 19-iodocholesterol changes from 0.6 after 28 days to 3.0 after 84 days. This indicates that NP-59 is more stable in formulation than 19-iodocholesterol and that ^{131}I-19-iodocholesterol rearranges slowly at 4°C in an aqueous medium to NP-59.

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[b] New England Nuclear Corp
Boston, Mass

RESULTS AND CONCLUSIONS

In an effort to improve synthetic yields of 19-iodocholesterol the "impurity" was shown to be about 10% when methyl ethyl ketone or acetone was used instead of isopropanol as the solvent during the p-toluenesulfonate substitution with iodide ion. When absolute ethanol was used as the solvent, the "impurity" increased to 90%, leaving 10% 19-iodocholesterol! When 19-iodocholesterol was refluxed in isopropanol or absolute ethanol for 12 hours, 90% of the 19-iodocholesterol was converted to the "impurity". With these observations, the "impurity" was synthesized and ultimately characterized to be 6β-iodomethyl-19-nor-cholest-5(10)-en-3β-ol (NP-59) [1]. ^{131}I-NP-59 was prepared by exchange with high specific activity Na^{131}I in absolute ethanol.

Tissue distribution studies in rats show a 5 fold increase in accumulation of NP-59 compared to pure 19-iodocholesterol in the adrenal gland. Results of this study are reported elsewhere (6,7).

The difference in products produced by the treatment of 19-toluenesulfonate cholesterol in different solvents in the presence of a halide ion e.g. I^-, are summarized in Fig.1.

This difference can be explained by the formation of two discrete isomeric cations A & B (See Fig. 2) that are produced in the homoallylic rearrangement of 19-substituted steriods (8,9).

In ketonic solvents such as acetone, methyl ethyl ketone, cation A is formed to a greater extent than cation B; thus $k_1 >> k_2$ and the predominance of the 19-substituted steroid, while in alcoholic solvents like ethanol or isopropanol cation B is formed to a greater extent; thus $k_2 >> k_1$ and the predominance of the 6-β-methylhalo-5(10)-19 nor cholest-5(10)-en-3β-ol.

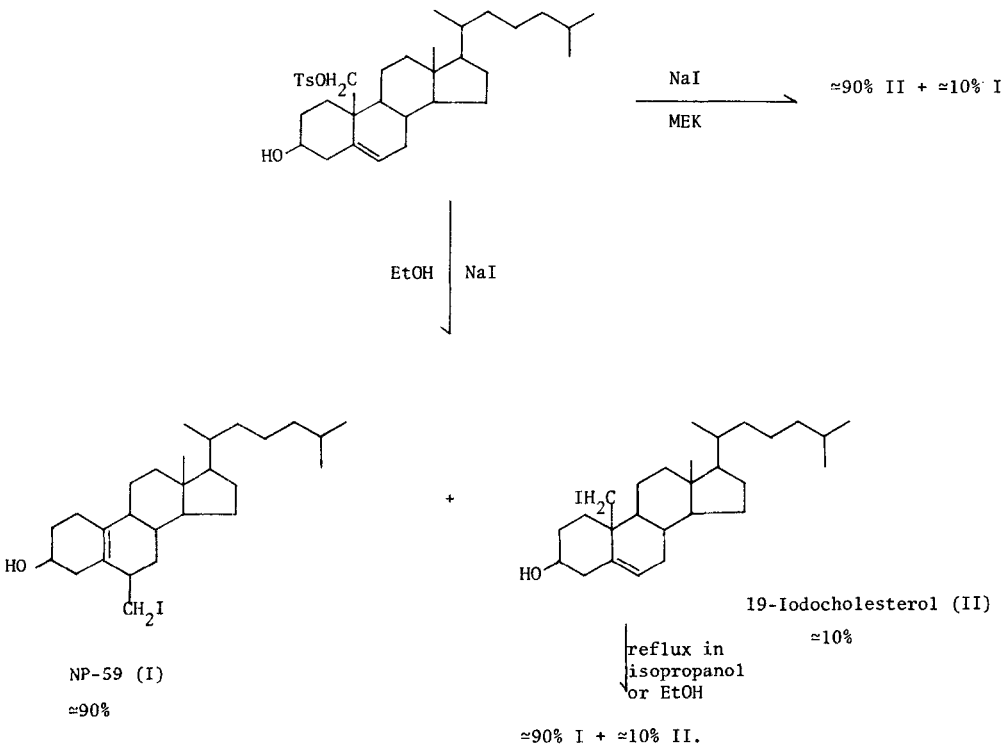

FIGURE 1

Synthetic scheme for a preparation of NP-59 or 19-iodocholesterol.

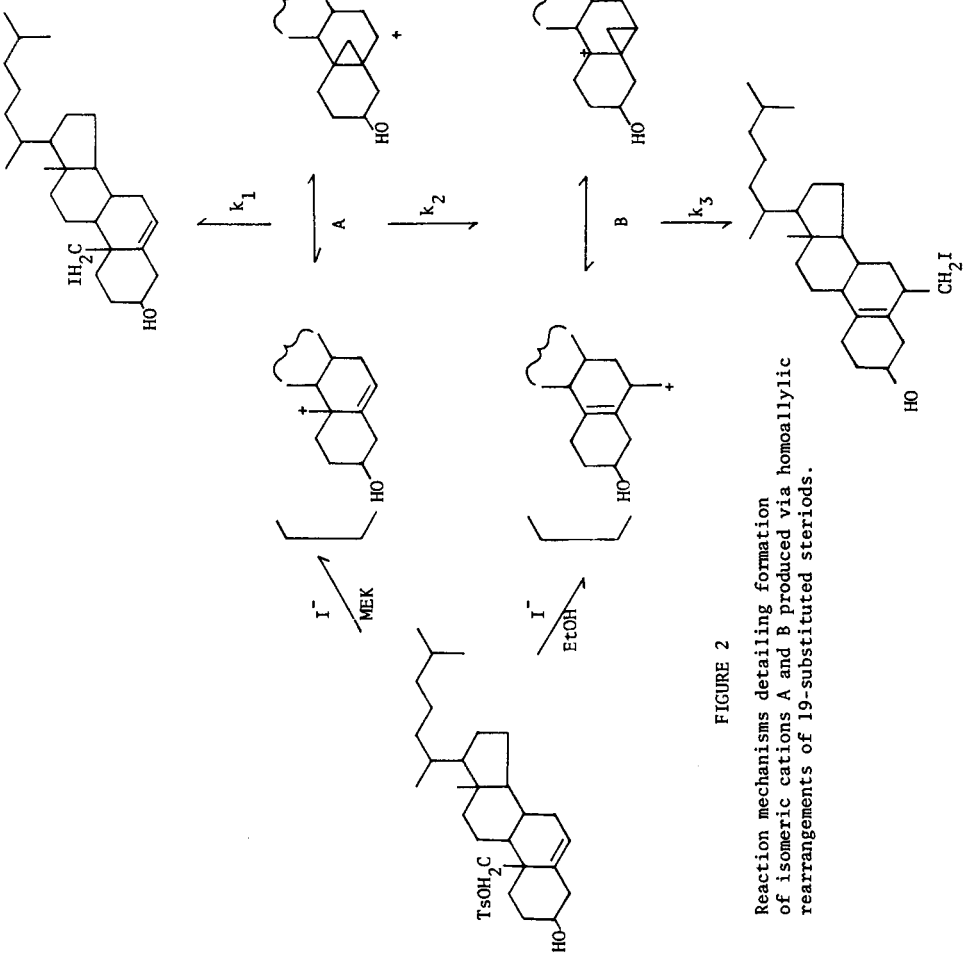

FIGURE 2

Reaction mechanisms detailing formation of isomeric cations A and B produced via homoallylic rearrangements of 19-substituted steriods.

NP-59 can be synthesized either from 19-iodocholesterol by refluxing in an alcoholic solvent due to the elimination of I^- leaving the carbonium ion A which rearranges much quicker to the carbonium ion B followed by the nucleophilic attack of I^- to give NP-59. The same holds true of 19-toluenesulfonate cholesterol in alcoholic solvents. Tissue distribution studies (6) shows that ^{131}I-NP-59 administered intravenously to rats demonstrated adrenal uptake of 10% [kgm] D/gm at 24 hrs or 5 times that of ^{131}I-19-iodocholesterol.

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