POTENTIAL TUMOR- OR ORGAN-IMAGING AGENTS. 28. RADIOIODINATED ESTERS OF CHOLESTEROL AND PREGNENOLONE

M. Van Dort, S. W. Schwendner, and R. E. Counsell

Department of Pharmacology, University of Michigan Medical School, Ann Arbor, MI 48109, USA

Received August 28, 1986
Revised September 8, 1987

ABSTRACT

Previous studies had shown radioiodinated esters of cholesterol and pregnenolone to accumulate in steroid-secreting tissues of the rat. This was particularly true for radioiodinated iopanoate esters. The present study was undertaken to examine the effect of the iopanoyl amino group on the tissue distribution of these esters. While the tissue distribution profiles for cholesteryl iopanoate and the desamino analog (III) were somewhat comparable, such was not the case for the corresponding esters of pregnenolone. Moreover, this subtle structural change of removing the amino group was observed to affect the in vivo stability of the esters to hydrolysis. This conclusion is in accordance with the observation that the tissue distribution profiles for the free acids I and II are not significantly different from each other. These studies serve to demonstrate that relatively minor modifications of the acyl moiety have a profound effect on both the uptake and distribution of these sterol esters in various tissues.

INTRODUCTION

Cholesterol and pregnenolone are two important precursors in corticosteroid hormone biosynthesis. In previous papers in this series (1-4) we reported that radioiodinated acyl derivatives of these sterols localized selectively in the liver and steroid-secreting tissues of the rat. Of special interest were the cholesterol and pregnenolone esters of iopanoic acid (ω-(3-amino-2,4,6-triiodophenyl)-α-ethyl) propionic acid, (I). For example, radioiodinated cholesteryl iopanoate demonstrated a sustained accumulation in the ovary, adrenal cortex and liver (2). Pregnen-

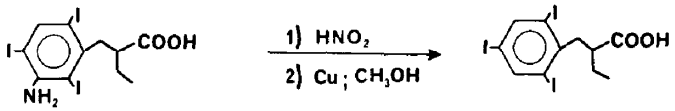
olone iopanoate, on the other hand, gave rise to unusually high levels of radioactivity in the adrenal cortex within 0.5 h of I.V. administration but declined to much lower levels by 24 h (2). We also found that the retention of these radioiodinated esters in specific tissues was related to their in vivo stability to hydrolysis (2,4).

The present study was undertaken to ascertain the importance of the 3-amino group in the iopanoyl portion of these esters for determining the tissue uptake and clearance of these compounds. Accordingly, the desamino derivatives III and IV were prepared (see Scheme I) and their tissue distribution properties analyzed.

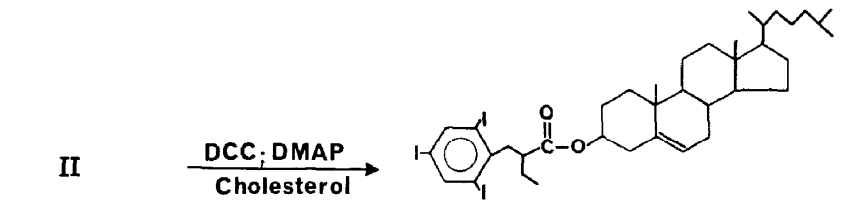
EXPERIMENTAL

Melting points were obtained in open capillary tubes with a Thomas-Hoover apparatus and are uncorrected. NMR spectra were obtained on a Varian EM360 A spectrometer with $CDCl_3$ as solvent. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane, which was used as the internal standard. IR spectra were obtained in the form of thin KBr wafers and recorded on a Perkin-Elmer 281 spectrophotometer. Elemental analyses were performed by Midwest Microlab Ltd., Indianapolis, IN. All analyses (C,H,N,I) are within $\pm 0.4\%$ of the calculated values. Thin-layer chromatography (TLC) was done with Analtech preadsorbent glass-backed silica gel plates with fluorescent indicator. TLC plates of radiolabeled compounds were scanned with a Vanguard 930 autoscanner. Column chromatography was done on silica gel from Grace Davison Chemical, Baltimore, MD. CH_2Cl_2 was freshly distilled over phosphorus pentoxide and dried with molecular sieves (4Å) prior to use. Dicyclohexylcarbodiimide, 4-dimethylaminopyridine, and other reagents were obtained from Aldrich Chemical Co., Milwaukee, WI. Iopanoic acid was purchased from CTC Organics, Atlanta, GA. The synthesis, radiolabeling, and tissue distribution studies of cholesteryl iopanoate and pregnenolone iopanoate have been previously described (2). Sodium iodide-[^{125}I] was obtained from New England Nuclear, Boston, MA. Tween® 20 was obtained from Sigma Chemical Co., St. Louis, MO.

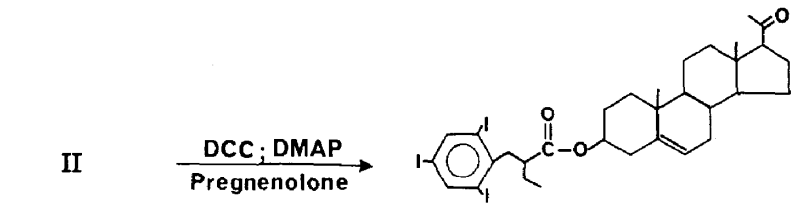
SCHEME I



I II



III



IV

Rats were obtained from Harlan Sprague-Dawley, Inc., Haslett, MI. TLC of tissue extracts was performed on plastic-backed silica gel plates with fluorescent indicator from Eastman Kodak Co.

ω-(2,4,6-Triiodophenyl)-α-ethyl-propionic acid (II). A suspension of iopanoic acid (I, 5.0 g, 8.8 mmol) in glacial acetic acid (50 mL) was mechanically stirred and treated dropwise over a period of 45 min at 12°C with a solution of $NaNO_2$ (0.68 g, 9.8 mmol) in conc H_2SO_4 (6.8 mL). The yellow suspension was stirred for a further 30 min at 20°C and treated with a suspension of copper powder (2.8 g, 44 mmol) in methanol (50 mL). The solution was stirred an additional 30 min, filtered, and the residue washed with hot methanol (5 mL). The mixture of combined filtrates was heated on the steam bath for 10 min, evaporated *in vacuo* to turbidity, and chilled in an ice-water bath. The crude product weighing 5.0 g was recrystallized successively from acetone and chloroform to afford 2.15 g (44%) of analytically pure material: mp 144.0-145.5°C (d); IR (KBr) 1695 cm^{-1} (C=O); ^1H NMR ($CDCl_3$):δ 8.28 (s, 2, Ar-H); 3.35 (d, 2, β-CH_2); 2.78 (m, 1, C<u>H</u>); 1.68 (m, 2, ethyl C<u>H</u>$_2$); 0.99 (t, 3, C<u>H</u>$_3$). Anal. Calcd for $C_{11}H_{11}I_3O_2$: <u>C</u> 23.77, <u>H</u> 1.99, <u>N</u> 68.48. Found: <u>C</u> 23.91, <u>H</u> 2.02, <u>N</u> 68.56.

General Procedure for Esterification. A solution of the sterol (1.0 mmol) and II (1.0 mmol) in dry CH_2Cl_2 (5.0 mL) was treated with dicyclohexylcarbodiimide (1.1 mmol) followed by 4-dimethylaminopyridine (0.1 mmol). The reaction mixture was stirred for 24 h at room temperature under nitrogen. Dilution of the suspension with CH_2Cl_2 (40 mL), followed by filtration removed the precipitated dicyclohexylurea. The filtrate was extracted with 0.5 N HCl (2x40 mL), saturated aqueous $NaHCO_3$ (1x40 mL), saturated brine (1x40 mL) and dried ($MgSO_4$). The residue obtained following removal of the solvent under reduced pressure was chromatographed on a silica gel column with hexane:ethyl acetate (4:1) as solvent system. Analytically pure material was obtained following recrystallization from a suitable solvent system.

Cholesteryl[ω-(2,4,6-triiodophenyl)-α-ethyl]propionate (III). 345 mg (75%); mp 143.5-144.0°C (1:1 CH_2Cl_2:acetone); IR (KBr): 1728 cm (C=O); ^1H NMR ($CDCl_3$): 8.16 (s, 2, Ar-H); 5.38 (br m, 1, H-6); 4.57 (m, 1, H-31); 3.37 (d, 2, β-C<u>H</u>$_2$). Anal. Calcd for $C_{38}H_{55}I_3O_2$: <u>C</u> 49.37, <u>H</u> 5.99, <u>I</u> 41.18. Found: <u>C</u> 49.26, <u>H</u> 6.10, <u>I</u> 40.97.

Pregnenolone[ω-(2,4,6-triiodophenyl)-α-ethyl]propionate (IV). 336 mg (79%); mp 154-156°C (acetone); IR (KBr): 1727 cm^{-1} (ester C=O), 1695 cm^{1} (keto C=O): ^1H NMR ($CDCl_3$): 8.18 (s, 2, Ar-H); 5.38 (br m, 1, H-6); 4.61 (m, 1, H-3); 3.32 (d, 2, β-C<u>H</u>$_2$); 2.14 (s, 3, 21-C<u>H</u>$_3$). Anal. Calcd for $C_{32}H_{41}I_3O_3$: <u>C</u> 44.98, <u>H</u> 4.84, <u>I</u> 44.56. Found: <u>C</u> 45.15, <u>H</u> 4.94, <u>I</u> 44.43.

Radioiosotope Exchange in Pivalic Acid. The isotope exchange with $Na^{125}I$ in pivalic acid was performed as previously reported

(5). Purification of the radioiodinated compounds was achieved by column chromatography on silica gel with hexanes:EtOAc (5:2) for elution of the sterol esters, and benzene:THF (2:1) for elution of the free acid II. Radiochemical purity was established by chromatographic comparison with the unlabeled material. The specific activities for compounds II, III, and IV were 0.27, 0.63, and 0.18 Ci/mmol, respectively. These values were in the same approximate range of iopanoic acid (0.09), cholesteryl iopanoate (0.17), and pregnenolone iopanoate (0.39).

Tissue Distribution Studies. The radiolabeled compounds were dissolved in benzene and Tween® 20 was added. The benzene was evaporated under a stream of nitrogen and physiological saline was added. Any remaining benzene was removed by a stream of nitrogen until a clear solution (2-3% in Tween® 20) resulted. The radiolabeled compound, thus solubilized, was administered intravenously to adult female Sprague-Dawley rats weighing 190-300 g. Three to four rats were used for each compound at each time period, and the dose ranged between 10 and 30 μCi per animal. The rats were killed by exsanguination under ether anesthesia at 0.5 h and 24 h and the major organs were removed and blotted free of excess blood. Large organs were minced with scissors. Weighed samples of tissue were placed in cellulose acetate capsules and counted (81-85% efficiency) in a well scintillation counter (Searle 1185). The results are summarized in Tables 1 and 2. The concentration of radioactivity in each tissue was expressed as the percentage of administered dose/g of tissue and was calculated as follows:

$$\frac{(\text{CPM - BACKGROUND/MG OF TISSUE}) \times 1000 \times 100}{(\text{EFFICIENCY} \times 2.2 \times 10^6\ \text{DPM}/\mu\text{Ci}) \times \mu\text{Ci DOSE}}$$

Plasma and Tissue Extraction. Radioactivity was extracted from plasma, adrenal cortex, and liver by the procedure described previously (3). A system of benzene:ethyl acetate (9:1) was employed for TLC analysis of the lipid extracts. The plates were then developed with the appropriate solvent system for 14.5 cm and air-dried. The plates were cut into 1-cm strips starting 0.5 cm below the origin and continuing to the solvent front. Each strip was placed in a counting tube and assayed for radioactivity. Each unlabeled ester was cochromatographed with the radioactive samples and visualized with iodine vapor to serve as a reference standard. The results are summarized in Table 3.

RESULTS AND DISCUSSION

ω-(2,4,6-Triiodophenyl)-α-ethyl-propionic acid (II) was synthesized from iopanoic acid (I) by a reductive deamination sequence utilizing nitrous acid followed by the addition of

copper powder in methanol (6). Dicyclohexylcarbodiimide (DCC) promoted esterification of cholesterol and pregnenolone with II, afforded the corresponding sterol esters III and IV in good yield. Subsequent radioiodination of these esters as well as the free acid (II) was conducted as described previously, by isotope exchange in pivalic acid (4,6). The radiochemical yields in this exchange procedure were in excess of 75% in each case.

Adult female Sprague-Dawley rats were employed for the in vivo tissue distribution studies. Tables 1 and 2 compare the tissue distribution profiles of free acid II and esters III and IV with results previously obtained for iopanoic acid (I), cholesteryl iopanoate, and pregnenolone iopanoate. Groups of animals were sacrificed at 0.5-h and 24.0-h intervals and tissues analyzed in a gamma-counter for uptake of radioactivity. Although a total of twelve tissues were analyzed, only those tissues displaying high concentrations of radioactivity are reported.

As anticipated from our previous studies, the sterol esters III and IV showed a tissue distribution pattern significantly different from that of the parent free acid (II). Furthermore, the nature of the sterol also played a significant role in the overall distribution of radioactivity. For example, cholesteryl desaminoiopanoate (III) showed a selective time-dependent accumulation in the liver and steroid-secreting tissues. This accumu-

Table 1

Distribution of Radioactivity at 0.5 h after Intravenous Administration of ^{125}I-Labeled Sterol Esters

Tissue	Desamino Iopanoic Acid (II)	Cholesteryl Desaminoiopanoate (III)	Pregnenolone Desaminoiopanoate (IV)
Adrenal Cortex	0.42 ± 0.02[a] (0.37 ± 0.02)[b]	3.22 ± 0.32 (4.90 ± 0.72)[c]	4.23 ± 0.22 (23.08 ± 1.58)[d]
Blood	1.23 ± 0.05 (1.20 ± 0.08)	6.03 ± 0.21 (5.19 ± 0.44)	3.50 ± 0.07 (1.73 ± 0.10)
Liver	1.57 ± 0.16 (1.46 ± 0.07)	2.26 ± 0.09 (4.03 ± 0.543)	5.40 ± 0.37 (5.21 ± 0.50)
Ovary	0.57 ± 0.05 (0.63 ± 0.05)	4.02 ± 0.39 (5.16 ± 0.72)	1.66 ± 0.23 (2.13 ± 0.20)
Thyroid	21.06 ± 5.24 (6.91 ± 0.57)	2.26 ± 0.10 (0.87 ± 0.10)	14.25 ± 2.38 (0.94 ± 0.07)

[a] Values expressed as % administered dose per gram of tissue (n = 3-4).
[b,c,d] Values in parentheses are those obtained for iopanoic acid (I), cholesteryl iopanoate, and pregnenolone iopanoate, respectively, under similar experimental conditions (2).

Table 2

Distribution of Radioactivity at 24.0 h after Intravenous Administration of ^{125}I-Labeled Sterol Esters

Tissue	Desamino Iopanoic Acid (II)	Cholesteryl Desaminoiopanoate (III)	Pregnenolone Desaminoiopanoate (IV)
Adrenal Cortex	0.07 ± 0.01[a] (0.03 ± 0.01)[b]	5.48 ± 0.30 (11.03 ± 2.17)[c]	1.09 ± 0.08 (4.26 ± 0.74)[d]
Blood	0.14 ± 0.03 (0.05 ± 0.01)	1.15 ± 0.07 (0.70 ± 0.05)	0.09 ± 0.01 (0.04 ± 0.00)
Liver	0.21 ± 0.04 (0.12 ± 0.01)	4.43 ± 0.34 (6.59 ± 0.32)	0.46 ± 0.03 (0.39 ± 0.02)
Ovary	0.13 ± 0.01 (0.04 ± 0.00)	15.80 ± 1.95 (24.54 ± 2.96)	0.37 ± 0.02 (1.08 ± 0.12)
Thyroid	455.97 ± 68.14 (265.14 ± 33.62)	23.91 ± 3.27 (3.57 ± 0.22)	471.31 ± 64.47 (156.70 ± 24.94

[a] Values expressed as % administered dose per gram of tissue (n = 3-4).
[b,c,d] Values in parentheses are those obtained for iopanoic acid (I), cholesteryl iopanoate, and pregnenolone iopanoate, respectively, under similar experimental conditions (2).

Table 3

Analysis of Lipid-Soluble Radioactivity Extracted from Tissue Samples

Tissue	% $CHCl_3/CH_3OH$ Extractable Compound		% Parent Compound as Determined by TLC	
	0.5 h	24 h	0.5 h	24 h
Cholesteryl desaminoiopanoate (III)				
Adrenal cortex	88.1 ± 1.6 (83.1 ± 1.7)	88.4 ± 1.0 (92.1 ± 0.4)	85.4 ± 1.5 (96.9 ± 0.3)	85.8 ± 1.1 (96.9 ± 0.2)
Liver	89.6 ± 0.9 (87.2 ± 2.0)	90.1 ± 0.9 (91.2 ± 1.9)	89.6 ± 0.2 (97.8 ± 0.7)	88.0 ± 0.3 (97.8 ± 0.2)
Plasma	82.4 ± 3.3 (83.4 ± 1.8)	90.2 ± 1.6 (85.8 ± 1.2)	89.7 ± 0.1 (96.0 ± 0.7)	76.2 ± 5.8 (96.1 ± 0.2)
Pregnenolone desaminoiopanoate (IV)				
Adrenal cortex	72.1 ± 2.7 (89.5 ± 0.7)	84.6 ± 2.0 (87.3 ± 0.4)	64.3 ± 6.0 (92.1 ± 0.7)	61.9 ± 4.9 (68.2 ± 1.7)
Liver	70.9 ± 4.3 (87.6 ± 0.2)	88.0 ± 2.1 (76.1 ± 1.1)	61.1 ± 6.2 (89.2 ± 0.6)	34.1 ± 2.3 (69.1 ± 2.2)
Plasma	71.4 ± 2.0 (86.7 ± 1.0)	58.0 ± 16.5 (60.0 ± 8.4)	58.8 ± 6.2 (91.9 ± 0.3)	12.4 ± 0.9 (44.6 ± 7.7)

[a] Values expressed as % administered dose per gram of tissue (n = 3-4).
[b,c,d] Values in parentheses are those obtained for iopanoic acid (I), cholesteryl iopanoate, and pregnenolone iopanoate, respectively, under similar experimental conditions (2).

lation was significant with respect to the ovaries, with an uptake of approximately 16% of administered dose per gram of tissue at 24 h. Lipid extraction of the adrenal cortex, liver, and plasma followed by subsequent TLC analysis of the extracts, revealed that over 76% of the radioactivity associated with these tissues at 24 h was still present as the original ester (Table 3). In contrast, pregnenolone desaminoiopanoate (IV) was found to clear rapidly from these tissues, as indicated by the tissue distribution of radioactivity at 24 h after intravenous administration. Moreover, Folch extraction (7) of tissues at this time interval revealed that this ester was more susceptible than pregnenolone iopanoate to in vivo hydrolysis (Table 3). Further evidence as to the in vivo instability of IV was provided by polyacrylamide gel electrophoresis (PAGE) of plasma samples. Significant amounts of radioactivity (41% of total) were found to be associated with the serum albumin fraction at early time periods. Acids such as iopanoic acid are known to bind avidly to serum albumin (8).

A somewhat surprising observation was the effect of the removal of the amino functionality on the tissue uptake and disposition of the sterol esters. This effect was most dramatic for the adrenal uptake in the pregnenolone ester series (23% of administered dose per gram for pregnenolone iopanoate versus 4% for IV at 0.5 h). Moreover, Folch lipid analysis of the 24-h plasma samples revealed that, whereas 45% of the radioactivity

was still in the form of pregnenolone iopanoate, only 12% of IV was present as the intact ester. These findings suggest that the presence of the amino functionality imparts some stability to the sterol esters towards hydrolysis when they are administered parenterally to rats.

ACKNOWLEDGMENTS

This investigation was supported by USPHS grant CA-08349 awarded by the National Cancer Institute. The pregnenolone used in this study was generously provided by G.D. Searle and Co., Skokie, IL. The authors wish to thank Edie Quenby and Chris Bigelow for their technical assistance and Linda Harbison for typing the manuscript.

REFERENCES

1. Counsell RE, Seevers RH, Korn N, and Schwendner SW (1980). J MED CHEM 24:5.
2. Seevers RH, Groziak MP, Weichert JP, Schwendner SW, Szabo SM, Longino MA, and Counsell RE (1982). J MED CHEM 25:1500.
3. Seevers RH, Schwendner SW, Swayze SL, and Counsell RE (1982). J MED CHEM 25:618.
4. Van Dort M, Schwendner SW, Skinner RWS, Gross MD, and Counsell RE (1984). STEROIDS 44:85.
5. Goldberg AA, Jefferies HS, Turner HS, and Besly DM (1946). Q J PHARM PHARMACOL **19**:483.
6. Weichert JP, Van Dort ME, Groziak MP, and Counsell RE (1986). INT J RAD APPL INSTRUM [A] **37**:907.
7. Folch J, Lees M, and Sloane-Stanley GH (1957). J BIOL CHEM 226:497.
8. Kragh-Hansen U (1981). PHARMACOL REV 337.

NOTE

*To whom correspondence should be addressed.