^{75}Se-LABELLED BILE ACID ANALOGUES

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SUMMARY

The synthesis of several ^{75}Se-labelled bile acid analogues* is described in which selenium-75 replaces a carbon methylene group at different positions in the C-17 side-chain. Procedures for the synthesis of necessary non-radioactive intermediates are included. Some results for the organ distribution of the compounds in rats are given. 23-[^{75}Se]selena-25-homocholic acid and its glycine and taurine conjugates show particularly good properties for the study of the enterohepatic circulation of bile acids.

Key words: Selenium-75, Bile acids,
Tauro-23-[^{75}Se]selena-25-homocholic acid, SeHCAT.

INTRODUCTION AND DISCUSSION

The observation that ^{75}Se radioactivity accumulated in the gut following the administration of 19-methyl-[^{75}Se]selenocholesterol to humans led to the idea that a ^{75}Se-labelled bile acid analogue might be useful for studying the enterohepatic circulation. Cholic acid labelled with ^{14}C and ^{3}H and their taurine conjugates have been used to study bile acid malabsorption in the investigation of small bowel disease (1,2,3). A bile acid analogue showing similar physiological properties, but labelled with a gamma-emitting isotope, would obviate the need to collect and process faeces

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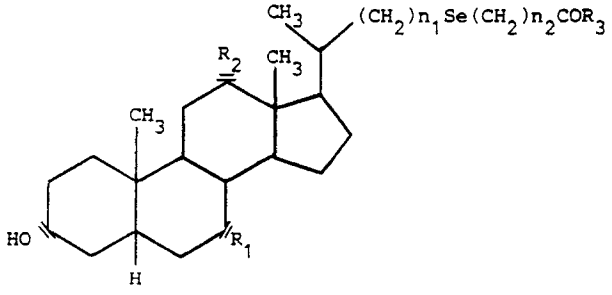
and thereby facilitate such investigations by allowing external body measurement (4,5). This paper describes the synthesis of ^{75}Se-labelled bile acid analogues in the search for a suitable labelled compound.

Selenium-75 ($t_{\frac{1}{2}}$, 120 days; principal gamma energies, 121, 136, 265, 280 and 401 keV) was incorporated into the C-17 side-chain of a number of bile acid analogues, with selenium replacing one of the methylene groups at various positions along the side-chain. Glycine and taurine conjugates of some of the acids were also synthesized (Figure 1). Table 1 lists the compounds synthesized. Compounds I, II and III were very probably mixtures of the 20R and 20S isomers.

Figure 1 - General formula for bile acid analogues



R_1: H or OH

R_2: H or OH

R_3: OH, $NHCH_2COOH$, or $NHCH_2CH_2SO_3H$

n_1: 0, 1, 2 or 3

n_2: 1 or 2

The general synthetic route consisted essentially of two successive nucleophilic displacement reactions. In Scheme 1 a selenocyanatocarboxylic acid, prepared by the reaction of potassium selenocyanate with either bromoacetate or β-propiolactone (6), was reduced to the selenol and reacted with a steroidal moiety carrying a terminal halogen atom in the C-17 side-chain. In Scheme 2 a steroidal halide and disodium diselenide were used to

Table 1 ^{75}Se-labelled Bile Acid Analogues

	Compound	"a" Nomenclature
I	3α,7α,12α-Trihydroxy-20ξ-(carboxymethyl-[^{75}Se]seleno)-5β-pregnane	22-[^{75}Se]Selenacholic acid
II	-	Glyco-22-[^{75}Se]selenacholic acid
III	-	Tauro-22-[^{75}Se]selenacholic acid
IV	3α,7α,12α-Trihydroxy-22-(carboxymethyl-[^{75}Se]seleno)-23,24-dinor-5β-cholane	23-[^{75}Se]Selena-25-homocholic acid
V	-	Glyco-23-[^{75}Se]selena-25-homocholic acid
VI	-	Tauro-23-[^{75}Se]selena-25-homocholic acid (SeHCAT)
VII	3α,12α-Dihydroxy-22-(carboxymethyl-[^{75}Se]seleno)-23,24-dinor-5β-cholane	23-[^{75}Se]Selena-25-homodeoxycholic acid
VIII	-	Tauro-23-[^{75}Se]selena-25-homodeoxycholic acid
IX	3α,7α-Dihydroxy-23-(β-carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane	-
X	3α,7α,12α-Trihydroxy-23-(β-carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane	-
XI	3α-Hydroxy-24-(carboxymethyl-[^{75}Se]seleno)-5β-cholane	-

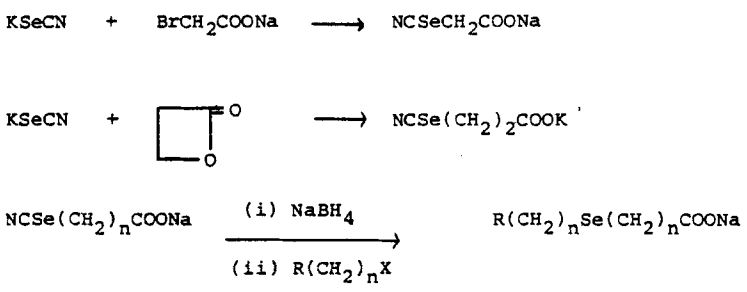
prepare a disteroidal diselenide, which was subsequently reduced and reacted with bromoacetate. The primary nucleophiles, potassium selenocyanate and disodium diselenide, were respectively prepared by dissolving red selenium in ethanolic potassium cyanide and by the reaction of red selenium with sodium borohydride in ethanol (7). Intermediate organo selenocyanates and diselenides were reduced to the selenol with sodium borohydride (8).

The steroidal precursors having a terminal halogen atom in the C-17 side-chain were prepared from bile acids in which the C-17 side-chain had been shortened or lengthened respectively by Barbier-Wieland degradation (9) or the Arndt-Eistert reaction (10). A particularly effective way of preparing 23,24-dinor-5β-cholanoic acids was by the oxidative decarboxylation of a cholanoic acid to a 24-nor-5β-chol-22-ene (11) followed by periodate/ permanganate oxidation of the 24-nor-5β-chol-22-ene (12). Replacement of the terminal carboxyl group in modified bile acids by either bromine or iodine was effected respectively by the Hunsdiecker (13) and the Barton (14) reaction. These reactions required the use of bile acids in which the hydroxyl groups were suitably protected with either formyl, acetyl or nitro groups.

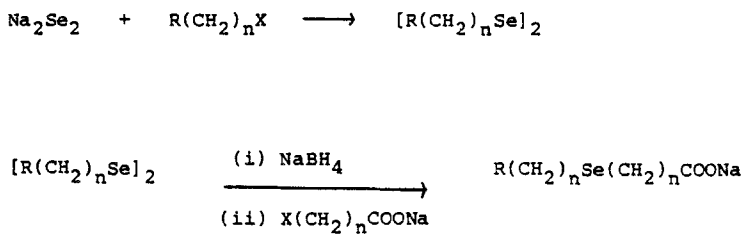
The bile acid analogues labelled with ^{75}Se in the C-17 side-chain were subsequently conjugated with either glycine or taurine by the mixed acid anhydride method (15) or by the method of Tserng et al using N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as the condensing agent (16).

Each ^{75}Se-labelled bile acid analogue was administered orally to rats and the organ distribution of the radioactivity determined 18 to 20 hours later (4). It has been estimated that the bile acids in a rat circulate in the enterohepatic system 10 to 12 times in 24 hours. Table 2 shows the percentage of radioactivity distributed in different body compartments. It is noteworthy that the 23-selena bile acids, compounds IV, V and VI, are characterized by a high retention in the enterohepatic circulation combined with a low concentration in other tissues. The 24-selena bile acids, compounds IX and X, which exhibited a high liver/small intestine concentration

Scheme 1



Scheme 2



R = steroidal moiety X = halogen

ratio also showed a high concentration in other tissues. The 22-selena bile acids, compounds I, II and III, whilst exhibiting a very low concentration in both liver and other tissues, were however excreted to a greater extent than the 23-selena bile acids.

TABLE 2. Percentage Distribution of ^{75}Se Radioactivity in Rats 18 to 20 hours after Oral Administration of ^{75}Se-labelled Bile Acid Analogues[a]

Compound administered	Excreted[b]	Enterohepatic circulation		Other tissues[c]
		Liver	Small intestine	
I	88.6	0.41	10.4	0.70
II	92.5	0.37	5.81	0.53
III	67.6	0.09	31.7	0.67
IV	35.0	1.70	61.9	1.50
V	35.0	1.44	62.8	0.79
VI	28.3	2.25	68.5	1.19
VII	48.3	2.12	45.8	4.27
IX	59.9	6.10	14.3	19.3
X	77.2	5.08	5.74	12.2
XI	77.3	2.56	11.9	8.72

[a] Two rats were employed for each compound and the data averaged.

[b] Sum of mean concentrations in caecum, colon, rectum, faeces and urine.

[c] Sum of mean concentrations in lung, spleen, stomach, kidneys, blood, muscle, bladder and carcass.

EXPERIMENTAL

Materials and Methods - Cholic and deoxycholic acids were obtained from BDH Chemical Co., lithocholic acid, taurine and glycine ethyl ester hydrochloride from Aldrich Chemical Co., nordeoxycholic acid diacetate from Steraloids Inc., and 3α,7α-dihydroxy-23-bromo-24-nor-5β-cholane-3,7-dinitrate from Amersham International plc. Diazomethane was generated from N-methyl-N-nitroso-p-toluenesulphonamide (Diazald$^{(R)}$, Aldrich Chemical Co.) using an Aldrich Diazald kit. Analytical TLC was carried out on 0.25 mm Merck Kieselgel 60 F_{254}; 1 mm Anachem silica gel was used for preparative TLC.

Plates were visualized by autoradiography (Kodak 'Kodirex' film) and by exposure to iodine vapour, and were scanned by an Amersham 100-channel analyser. Infra-red (IR) spectra were recorded on a Perkin-Elmer 457 spectrometer. ^{1}H NMR spectra were determined only on non-radioactive compounds using a Perkin-Elmer R34 220 MHz instrument. Non-radioactive seleno bile acids were used for identification and comparative purposes. Procedures for the synthesis of these have not been described as they followed closely the procedures for the corresponding radioactive compounds.

(i) Red [^{75}Se]selenium - Red [^{75}Se]selenium was precipitated by bubbling sulphur dioxide through a solution of sodium selenite (15.9 mg) in water (2 ml) and conc. HCl (4 ml) containing sodium [^{75}Se]selenite (11.7 mCi, 1.2 mg selenium). The precipitate was centrifuged off, washed thoroughly with de-ionised water, and dried over phosphorus pentoxide under vacuum.

(ii) Cholic acid triformate - Cholic acid (50 g) was treated with 100% formic acid (240 ml) and the whole was stirred at 70-80°C for 6 hours. The solution was cooled and most of the solvent was evaporated. The residue was triturated with ether (500 ml) giving a white solid which was filtered and dried (43 g). The crude product could be further purified by successive recrystallization from 60% aqueous ethanol and 1:1 60-80°C petrol, acetone. M.p of purified material 204-208°C.

(iii) 3α,7α,12α-Triformoxy-24-nor-5β-chol-22-ene - Cupric acetate dihydrate (1.0 g) and pyridine (0.7 ml) were added to benzene (170 ml) and the suspension was dried by azeotropic distillation using a Dean and Stark apparatus. After cooling somewhat, dry lead tetraacetate (20 g) and cholic acid triformate (10.5 g) were added and the reaction mixture was stirred and heated under reflux in an atmosphere of dry nitrogen for 1½ hours. It was allowed to cool and was filtered. The filtrate was washed successively with water, 1M sodium hydroxide solution and finally with water, and was dried over anhydrous sodium sulphate. Evaporation of the solvent and crystallization of the residue from ethanol gave 3α,7α,12α-triformoxy-24-nor-5β-chol-22-ene (4.0 g) m.p 188-190°. IR (KBr) $\bar{\nu}$ max: 3077, 2960, 2865, 1725, 1714, 1637, 1468, 1449, 1380, 1180 cm^{-1}. PMR ($CDCl_3$): τ 1.83, 1.91, 1.98 (3H, three singlets, 3-, 7- and 12- formate protons); τ 4.4 (1H,m,C_{22}-proton); τ 4.77 (1H,s,C_{12}-proton); τ 4.97 (1H,s,C_7-proton); τ 5.16 (1H,d,C_{23}-proton (cis)); τ 5.18 (1H,s,C_{23}-proton (trans); τ 5.30 (1H,m,C_3-proton); τ 9.07 (6H,s + d, C_{19}-protons + C_{21}-protons); τ 9.24 (3H,s,C_{18}-protons); τ 7.75 - 9.1 (22H, steroid nucleus).

(iv) 3α,7α,12α-Triformoxy-23,24-dinor-5β-cholan-22-oic acid - 3α,7α,12α-Triformoxy-24-nor-5β-chol-22-ene (2.4 g) was dissolved in 2-methylpropan-2-ol (800 ml) and potassium carbonate (1.41 g) in water (800 ml) was added. Sodium periodate (20.86 g) and potassium permanganate (0.395 g) were dissolved in water (1 litre) and an aliquot (435 ml) was added to the solution of the olefin. The solution was stirred at ambient temperature for 24 hours. Sufficient 40% sodium hydrogen sulphite solution was added to discharge the permanganate coloration and 5% sodium carbonate solution was added to pH 8. The solution was concentrated under reduced pressure to ca. 250 ml, extracted with chloroform (2 x 100 ml), treated with further 40% sodium hydrogen sulphite and acidified with conc. HCl. The mixture was extracted with chloroform (4 x 100 ml), and the combined extracts were washed successively with 5% sodium thiosulphate solution and water, and then dried. The solvent

was evaporated and 100% formic acid (30 ml) was added to the residue. The solution was stirred and heated at 70-80° for 6 hours, allowed to cool, poured into water and the precipitate was extracted into chloroform (3 x 50 ml). The combined organic extracts were washed with water, dried and solvent evaporated. The residue was recrystallized from ethanol to give 3α,7α,12α-triformoxy-23,24-dinor-5β-cholan-22-oic acid (0.8 g) m.p 165-170°. IR (KBr) $\bar{\nu}$ max: 3410, 2965, 2940, 2870,1722, 1450, 1385, 1178, 890 cm^{-1}. PMR ($CDCl_3$): τ 1.83, 1.91 and 1.98 (3H, 3 singlets, 3-, 7- and 12-formate protons); τ 4.78 (1H,s,C_{12}-proton); τ 4.93 (1H,s,C_7-proton); τ 5.30 (1H,m,C_3-proton); τ 6.29 (2H,q,$\underline{CH_2}$ of ethanol of crystallization); τ 7.64 (1H,q,C_{20}-proton); τ 8.77 (3H,t,$\underline{CH_3}$ of ethanol of crystallization); τ 8.88 (3H,d,C_{21}-protons); τ 9.05 (3H,s,C_{19}-protons); τ 9.22 (3H,s,C_{18}-protons); τ 7.75 -9.05 (19H, steroid nucleus).

(v) 3α,7α,12α-Triformoxy-20ξ-iodo-5β-pregnane - 3α,7α,12α-Triformoxy-23,24-dinor-5β-cholan-22-oic acid (0.2 g) in dry carbon tetrachloride (20 ml) was treated with dry, powdered lead tetraacetate (0.195g). The solution was heated to reflux under dry nitrogen whilst being irradiated with an Atlas 275 watt infra-red lamp. Iodine (0.105 g) in dry carbon tetrachloride (8 ml) was added in portions over a period of 10 minutes and the reaction mixture was irradiated and stirred for a further 1 hour. The reaction mixture was allowed to cool, filtered, and the filtrate washed successively with 5% sodium thiosulphate solution and water and then dried over anhydrous sodium sulphate. Evaporation of the solvent and crystallization of the residue from ethanol yielded 3α,7α,12α-triformoxy-20ξ-iodo-5β-pregnane (0.11 g), m.p 145 - 146.5°. TLC (Merck Kieselgel 60 F_{254}; chloroform): single UV absorbing component, Rf 0.61. IR (KBr) $\bar{\nu}$ max: 3405, 2950, 2860, 1713, 1445, 1377, 1180 cm^{-1}. PMR ($CDCl_3$): τ 1.81, 1.91 and 1.98 (3H, 3 singlets, 3-, 7- and 12-formate protons); τ 4.75 (1H,s,C_{12}- proton); τ 4.93 (1H,s,C_7-proton); τ 5.30 (1H,m,C_3-proton); τ 5.80 (1H,q,C_{20}-proton); τ 8.06 (3H,d,C_{21}-protons); τ 9.07 (3H,s,C_{19}-protons); τ 9.25 (3H,s,C_{18}-protons); τ 7.5 - 9.0 (19H, steroid nucleus).

Two further preparations of 3α,7α,12α-triformoxy-20ξ-iodo-5β-pregnane yielded products which, after repeated crystallization from ethanol, had melting points of 136 - 137° ($[\alpha]_D^{20}$ + 84.4°), and 149 - 151° ($[\alpha]_D^{20}$ + 74.8°). These two products gave virtually identical NMR ^1H spectra. It was concluded that mixtures of the 20R and 20S isomers were being obtained. Samples of 22-[^{75}Se]selenacholic acid prepared from the high and low melting point intermediates had identical chromatographic mobilities by TLC.

(vi) 22-[^{75}Se]Selenacholic acid (Compound I) - Red [^{75}Se]selenium (8.2 mg, 106 mCi/mA) was prepared as described in (i). It was suspended in ethanol (2 ml) and dry nitrogen was bubbled through the solution. The exit gases were passed through a trap containing 5% lead acetate solution. Sodium borohydride (2.7 mg) was added and the suspension was stirred at room temperature (~20°C) for 20 minutes. n-Propanol (5 ml) was added and the reaction mixture was heated on a boiling water bath for 20 minutes. 3α,7α,12α-Triformoxy-20ξ-iodo-5β-pregnane (35 mg; m.p 145 - 146.5°) in warm n-propanol (2 ml) was added to the solution of disodium di-[^{75}Se]selenide and the mixture was heated on a boiling water bath under dry nitrogen for 3½ hours. The reaction mixture was cooled, solvents removed under reduced pressure, and the residue was extracted with chloroform (5 ml). The solution was filtered and the chloroform evaporated to yield a residue of crude dipregnane di-[^{75}Se]selenide (4.2 mCi). Sodium borohydride (5 mg) and ethyl bromoacetate (20 µl) were dissolved in ethanol (1 ml) at 0°C. The dipregnane di-[^{75}Se]selenide (4.2 mCi) dissolved in ethanol (3 ml) was added dropwise over a period of 10 minutes. The reaction mixture was stirred for 2 hours, acetone (1 ml) was added, and solvents were then evaporated. The residue was extracted with chloroform (3 ml) and, after removal of solvent from the filtered solution, the product was hydrolysed by refluxing with sodium hydroxide (100 mg) in water (1 ml) for 3 hours. The solution was acidified with hydrochloric acid and lyophilized. The product was dissolved in acetic acid and purified by preparative TLC (Anachem Silica Gel GF, 1 mm;

dichloromethane, acetone, acetic acid, 7:2:1). The required component, located by autoradiography, was removed from the plate and extracted into acetic acid. Evaporation of the solvent yielded 22-[^{75}Se]selenacholic acid (0.8 mCi). TLC (Merck Kieselgel 60 F_{254}): dichloromethane, acetone, acetic acid, (7:2:1), major component Rf 0.22; chloroform, methanol, (5:1), major component Rf 0.11. IR (KBr) $\bar{\nu}$ max: 3400, 2925, 2780, 1715, 1440, 1375, 1265, 1073, 1040 cm^{-1}. (Note - Subsequent preparations of disodium di-[^{75}Se] selenide were carried out in ethanol alone instead of in a mixture of ethanol and n-propanol. The dipregnane di-[^{75}Se]selenide was isolated by preparative TLC using chloroform, methanol (12:1).

(vii) Glyco-22-[^{75}Se]selenacholic acid (Compound II) - 22-[^{75}Se] selenacholic acid (0.40 mCi; 1.9 mg) in acetic acid was evaporated to dryness. Dry ethyl acetate (450 µl) was added followed by N-ethoxycarbonyl-2-ethoxydihydroquinoline (14.2 mg). Ethyl glycinate hydrochloride (8.0 mg), suspended in dry ethyl acetate (0.6 ml), was treated with triethylamine (8.3 µl); the mixture was stirred for 30 minutes and was added to the solution of 22-[^{75}Se]selenacholic acid; a further quantity of ethyl acetate (0.4 ml) was used to complete the transfer. The reaction mixture was heated under reflux on a boiling water bath for 6 hours; it was then cooled and solvent was evaporated. Chloroform (4 ml) was added to the residue and insoluble material was removed by filtration. The ethyl 22-[^{75}Se]selena-glycocholate was purified by preparative layer chromatography (Anachem Silica Gel GF, 1 mm; chloroform, methanol 8:1). The major radioactive band was located by autoradiography, Rf 0.4; it was removed from the plate and extracted into methanol (3 x 4 ml). The solvent was evaporated, ethanol (4 ml) and 10% potassium carbonate solution (1 ml) were added and the solution was heated under reflux for 1 hour and allowed to stand at room temperature overnight. The solution was acidified with conc. HCl, evaporated to dryness and the product was extracted from the residue by dissolving in ethanol. The solution was filtered and solvent removed leaving glyco-22-[^{75}Se]

selenacholic acid (0.2 mCi). TLC (Merck Kieselgel 60 F_{254}; chloroform, methanol 3:1); major component (ca. 85%) Rf 0.04, (cf 22-Selenacholic acid, Rf 0.31 and glycocholic acid, Rf 0.02, in this system).

(viii) Tauro-22-[^{75}Se]selenacholic acid (Compound III) - Dry dimethylformamide (430 µl) and triethylamine (1.5 µl) were added to taurine (1.34 mg) and the suspension was stirred for 30 minutes. Dimethylformamide (250 µl) and isobutyl chloroformate (3.1 µl) were added to 22-[^{75}Se]selenacholic acid (492 µCi; 5.32 µmol; ex-iodopregnane intermediate, m.p 136 - 137°) at 0°C followed by triethylamine (3.0 µl) and dimethylformamide (250 µl). The solution was allowed to stand at 0°C for 30 minutes and was then added to the solution containing taurine, 200 µl of dimethylformamide being used to complete the transfer. The reaction mixture was stirred at 0°C for 2 hours and at room temperature overnight. The solvent was evaporated, water containing 2-3 drops of triethylamine was added and the clear solution was acidified with hydrochloric acid and lyophilized. The product was dissolved in methanol and subjected to preparative TLC (Anachem Silica Gel GF; chloroform, methanol - 2:1). Analytical TLCs were also developed in butanol, water, acetic acid - 60:25:15 and ethyl acetate, acetic acid - 2:1. The required component was located by autoradiography and extracted into methanol. Yield of tauro-22-[^{75}Se]selenacholic acid, 88 µCi. TLC (Merck Kieselgel 60 F_{254}; butanol, water, acetic acid - 60:25:15); major component, 92-93%, Rf 0.51, agreed identically with that of tauro-23-[^{75}Se] selena-25-homocholic acid.

(ix) 3α,7α,12α-Triacetoxy-22-iodo-23,24-dinor-5β-cholane - 3α,7α,12α-Triacetoxy-24-nor-5β-cholan-23-oic acid (4.1 g - prepared from methyl cholate by Barbier-Wieland degradation (9), in dry carbon tetrachoride (150 ml) was treated with dry, powdered, lead tetracetate (4.1 g) and was heated to reflux in an atmosphere of dry nitrogen. The solution was irradiated with an Atlas 275 watt infra-red lamp and a solution of iodine (2.25 g) in dry carbon tetrachloride (100 ml) was added portion-wise over a period of 15 minutes. The reaction mixture was irradiated and stirred for a further 1 hour and was

allowed to cool. The solution was filtered; the filtrate was washed successively with 5% sodium thiosulphate solution and water, and was dried over anhydrous sodium sulphate. Evaporation of the solvent left an oil which was dissolved in ethyl acetate/hexane (1/3). The solution was loaded on a column prepared from Merck Silica Gel 60 (70-230 mesh) (150 g) and the product was isolated by elution with ethyl acetate/hexane (1/3). The fractions containing the product were pooled and evaporated under reduced pressure giving 3α,7α,12α-triacetoxy-22-iodo-23,24-dinor-5β-cholane (1.9 g) as a solid foam which could not be crystallized. TLC (Merck Kieselgel 60 F_{254}; chloroform); single component Rf 0.80. IR (KBr) $\bar{\nu}$ max: 2940, 2870, 1737, 1450, 1380, 1368, 1245, 1023 cm^{-1}. PMR ($CDCl_3$): τ 4.93 (1H,s,C_{12}-proton); τ 5.07 (1H,s,C_7-proton); τ 5.40 (1H,m,C_3-proton); τ 6.73 (2H,m,C_{22}-protons); τ 7.87 - τ 7.94 (9H,3s,3-,7- and 12-acetate protons); τ 9.07 (6H,s(with minor splitting),C_{19}-protons + C_{21}-protons); τ 9.22 (3H,s,C_{18}-protons).

(x) 23-[^{75}Se]Selena-25-homocholic acid (Compound IV) - Red [^{75}Se] selenium (7.4 mg), 0.094 mA, 8.8 mCi) was suspended in ethanol (2 ml) and potassium cyanide (6.2 mg, 0.095 mmol) was added; the mixture was stirred at room temperature for 2 hours after which complete solution had occurred. Ethyl bromoacetate (10.5 µl) was added to the solution at 0°C and it was stirred for 1½ hours.

3α,7α,12α-Triacetoxy-22-iodo-23,24-dinor-5β-cholane (57 mg), 0.095 mmol) in ethanol (1 ml) was added to sodium borohydride (9 mg) in ethanol (1 ml). The reaction mixture was cooled in ice and the ethanolic solution of ethyl [^{75}Se]selenocyanatoacetate was added over a period of 10 minutes. Stirring was continued for a further 2 hours while the mixture attained room temperature. Acetone (1 ml) was added and the solvents were then evaporated under reduced pressure. Chloroform (2 ml) was added to the residue, insoluble material was removed by filtration and the solution was concentrated to a small bulk. The product was isolated by preparative layer chromatography (Anachem Silica Gel GF, 1 mm; ethyl acetate, hexane 1:2). The major

radioactive component, Rf 0.36, as located by autoradiography, was removed from the plate and extracted into ethyl acetate (3 x 4 ml). Yield of ethyl 3α,7α,12α-triacetoxy-23-[^{75}Se]selena-25-homo-5β-cholanoate, 5.1 mCi. IR (KBr) $\bar{\nu}$max: 2935, 2860, 1736, 1460, 1440, 1374, 1362, 1245, 1103, 1023 cm^{-1}

Solvent was removed and sodium hydroxide (200 mg) in ethanol (5 ml) and water (2 ml) was added. The solution was stirred and heated under reflux for 2½ hours and was allowed to stand at ambient temperature for 16 hours. Solvent was evaporated under reduced pressure and the residue was dissolved in water (2 ml), the solution was acidified with conc. HCl and then lyophilized. The residue was dissolved in a few drops of methanol and the product was isolated by preparative layer chromatography (Anachem Silica Gel GF, 1 mm; chloroform, methanol 3:1). The required band, Rf 0.33, was located by autoradiography; it was removed from the plate and the product was isolated by extraction into methanol. Evaporation of the solvent afforded 23-[^{75}Se]selena-25-homocholic acid (3.0 mCi, 34%). TLC Merck Kieselgel 60 F_{254}); chloroform, methanol - 3:1; major component (93%) Rf 0.42; dichloromethane, acetone, acetic acid - 7:2:1:5; major component (97%) Rf 0.76. IR (KBr) $\bar{\nu}$ max: 3400, 2920, 2860, 1708, 1380, 1263, 1104, 1025 cm^{-1}. PMR (C_5D_5N): τ 1.26, τ 2.40, τ 2.77 (solvent peaks); τ 5.76 (1H,s,C_{12}-proton); τ 5.90 (1H,s,C_7-proton); τ 6.23 (1H,m,C_3-proton); τ 6.49 (2H,s,C_{24}-protons); τ 8.58 (3H,d,C_{21}-protons); τ 9.00 (3H,s,C_{19}-protons); τ 9.19 (3H,s,C_{18}-protons).

(xi) Glyco-23-[^{75}Se]selena-25-homocholic acid (Compound V) - Ethyl glycinate hydrochloride (1.3 mg, 9.3 μmol) was suspended in dry ethyl acetate (70 μl) and triethylamine (1.35 μl); the suspension was stirred for 30 minutes. 23-[^{75}Se]selena-25-homocholic acid (1.06 mCi, 6.6 μmol) and N-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinoline (2.3 mg) were dissolved with stirring at room temperature in ethyl acetate (200 μl); the solution was then added to the ethyl glycinate hydrochloride using 0.5 ml of ethyl acetate to complete the transfer. The reaction mixture was stirred and heated at reflux

on a water bath for 6 hours and then left for 3 days before proceeding to the next stage. Ethyl acetate (5 ml) and water (5 ml) were added and the phases separated. The aqueous phase was extracted once with ethyl acetate (2 ml), and the combined ethyl acetate extracts were washed successively with 0.5M sodium hydroxide (4 ml), water (4 ml), 0.5M hydrochloric acid (2 x 4 ml) and finally with water (2 x 4 ml) to yield a solution of ethyl 23-[^{75}Se]selena-25-homocholylglycinate (426 µCi) in ethyl acetate. The ethyl acetate was removed and the residual product was hydrolysed by heating at reflux for 15 minutes with a mixture of 10% aqueous potassium carbonate (2 ml) and ethanol (2 ml). Solvents were evaporated and the residue was dissolved in water (2 ml); the aqueous solution was filtered, acidified with 0.5M hydrochloric acid, and then lyophilized. The residue was extracted with acetone (4 ml) and the solution was filtered from insoluble inorganic salts and concentrated to a small bulk. It was applied to a Merck Kieselgel 60 F_{254} 2 mm plate which was developed in dichloromethane, acetone, acetic acid - 7:5:5. The main radioactive band was located by autoradiography and the product was isolated by washing from the silica with acetone, acetic acid - 2:1. After evaporation of solvents the residue was dissolved in acetone (5 ml) and the solution filtered. Yield of glyco-23-[^{75}Se]selena-25-homocholic acid, 120 µCi. TLC (Merck Kieselgel 60 F_{254}): dichloromethane, acetone, acetic acid - 7:5:5 showed a single component, Rf 0.74, corresponding to the non-radioactive standard; cf glycocholic acid, Rf 0.47, and 23-selena-25-homocholic acid, Rf 0.89, in the same system; n-butanol, acetic acid, water - 60:15:25 showed a single component, Rf 0.78, corresponding to the non-radioactive standard; cf glycocholic acid, Rf 0.70, and 23-selena-25-homocholic acid, Rf 0.88, in the same system. PMR (D_2O containing NaOD):- τ 5.97 (1H,s,12βH); τ 6.09 (1H,s,7βH); τ 6.22 (2H,s, $NHC\underline{H}_2CO_2H$); τ 6.51 (1H, broad s, 3βH); τ 6.83 (1H,s,$C_{24}H$); τ 7.34 (2H,m,$C_{22}H$); τ 8.86 (3H,s,$C_{21}H$); τ9.09 (3H,s,$C_{19}H$); τ9.28 (3H,s,$C_{18}H$).

(xii) Tauro-23-[^{75}Se]selena-25-homocholic acid (Compound VI) - A solution of 23-[^{75}Se]selena-25-homocholic acid (0.97 mCi, 9.2 μmol) in methanol was evaporated to dryness. Dry dimethylformamide (200 μl) and N-ethoxycarbonyl-2-ethoxy dihydroquinoline (3.6 mg) were added and the solution was stirred at ambient temperature for 15 minutes. Taurine (1.3 mg) was treated with dimethylformamide (90 μl) containing dry triethylamine (1.8 μl) and stirred at ambient temperature for 15 minutes. The solution of the seleno bile acid was added to the taurine, 2 x 100 μl of dry dimethylformamide were used to complete the transfer and the reaction mixture was stirred at 90-95° for 30 minutes. The solvent was evaporated under reduced pressure and the residue was extracted with methanol; the solution was filtered, acidified with conc. HCl and evaporated to dryness. The product was purified by preparative layer chromatography (Anachem Silica Gel GF, 1 mm; chloroform, methanol 2:1). The required band was located by autoradiography (Rf 0.3); it was removed from the plate and the product was extracted into methanol. Yield of tauro-23-[^{75}Se]selena-25-homocholic acid, 0.64 mCi. TLC (Merck Kieselgel 60 F_{254}): n-butanol, water, acetic acid - 60:25:15; major component (96%) Rf 0.53 (cf. 23-selena-25-homocholic acid Rf 0.88); dichloromethane, acetone, acetic acid - 7:2:2; major component Rf 0.05 (cf. 23-selena-25-homocholic acid Rf 0.93). IR (KBr) $\bar{\nu}$ max: 3430, 2940, 2870, 1638, 1545, 1450, 1387, 1210, 1045 cm^{-1}. PMR (D_2O): τ 5.97 (1H,s,12β-H); τ 6.09 (1H,s,7βH); τ 6.38 (2H,t,-$\underline{CH_2}SO_3H$); τ 6.49 (1H, broad s, 3βH); τ6.74 (2H,s,$C_{24}H$); τ 6.87 (2H,t,-$\underline{CH_2}CH_2SO_3H$); τ 7.06 and 7.38 (2H,d+t,$C_{22}H$); τ 8.87 (3H,d,$C_{21}H$); τ 9.09 (3H,s,$C_{19}H$); τ 9.29 (3H,s,$C_{18}H$).

(xiii) 3α,12α-Diacetoxy-22-iodo-23,24-dinor-5β-cholane - 3α,12α-Diacetoxy-24-nor-5β-cholan-23-oic acid (0.3 g) was converted to 3α,12α-diacetoxy-22-iodo-23,24-dinor-5β-cholane by the method described in (v) using carbon tetrachloride (30 ml), lead tetracetate (0.3 g), and iodine (0.16 g) in carbon tetrachloride (12 ml). Crystallization of the crude product from ethanol yielded 3α,12α-diacetoxy-22-iodo-23,24-dinor-5β-cholane (0.3 g, 85%) m.p

172 - 174°. TLC (Merck Kieselgel 60 F_{254}; chloroform: single component Rf 0.50. IR (KBr) $\bar{\nu}$ max: 2960, 2930, 2870, 1735, 1453, 1374, 1239, 1194, 1018 cm^{-1}. PMR ($CDCl_3$): τ 4.95 (1H,s,C_{12}-proton); τ 5.32 (1H,m,C_3-proton); τ 6.76 (2H,m,C_{22}-H); τ 7.86 (3H,s,12-Acetate protons); τ 7.98 (3H,s,3-acetate protons); τ 8.00 - 9.05 (22H, steroid nucleus); τ 9.10 (6H,s (with minor splitting), C_{19}-H+C_{21}-H); τ 9.23 (3H,s,C_{18}-H).

(xiv) 23-[^{75}Se]Selena-25-homodeoxycholic acid (Compound VII) - Red [^{75}Se]selenium (8.4 mg, 0.11 mA, 109 mCi/mA) was suspended in ethanol (2 ml) and potassium cyanide (7 mg), 0.11 mmol) was added; the mixture was stirred at room temperature for 2 hours until complete solution had occurred. Redistilled ethyl bromoacetate (12 µl) was added to the solution at 0°C and it was stirred for 1 1/2 hours. 3α,12α-Diacetoxy-22-iodo-23,24-dinor-5β-cholane (60 mg) in dry tetrahydrofuran (1 ml) was added to sodium borohydride (9 mg) in ethanol (1 ml). The reaction mixture was cooled in ice and the ethanolic solution of ethyl [^{75}Se]selenocyanatoacetate was added over a period of 10 minutes. Stirring was continued for a further 2 hours while the mixture attained room temperature. Acetone (1 ml) was added and the solution was evaporated under reduced pressure. Chloroform (2 ml) was added to the residue, insoluble material was removed by filtration and the solution was concentrated to a small bulk. The required product was isolated by preparative layer chromatography (Anachem Silica Gel GF, 1 mm; chloroform, methanol 20:1). The major component, Rf 0.85, as observed by autoradiography, was removed from the plate and extracted into ethyl acetate (3 x 4 ml). Yield of ethyl 3α,12α-diacetoxy-23-[^{75}Se]selena-25-homo-5β-cholanoate, 6.1 mCi. IR (KBr) $\bar{\nu}$ max: 2935, 2860, 1735, 1450, 1378, 1245, 1050, 750 cm^{-1}.

Solvent was removed and sodium hydroxide (100 mg) in ethanol (5 ml) and water (1 ml) was added. The solution was stirred and heated under reflux for 2 hours; it was then cooled and evaporated. Water (3 ml) was added and the solution was filtered from some insoluble material and acidified by the addition of Bio-Rad AG 50W-X12 cation exchange resin in the H+ form. The resin was removed by filtration, it was washed with methanol (3 ml) and the

combined filtrate was evaporated. The residue was dissolved in the minimum of methanol and the product was isolated by preparative layer chromatography (Anachem Silica Gel GF, 1 mm; chloroform, methanol 6:1). The required band, Rf 0.42, was located by autoradiography; it was removed from the plate and isolated by extraction into methanol. Evaporation of the solvent afforded 23-[^{75}Se]selena-25-homodeoxycholic acid (2.4 mCi). TLC (Merck Kieselgel 60 F_{254}): chloroform, methanol 5:1, major component (95%) Rf 0.36; iso-octane, diisopropyl ether, acetic acid 2:1:1, major component Rf 0.43. IR (KBr) $\bar{\nu}$ max: 3380, 2930, 2860, 1700, 1448, 1380, 1255, 1105, 1035 cm^{-1}.

(xv) Tauro-23-[^{75}Se]selena-25-homodeoxycholic acid (Compound VIII) - 23-[^{75}Se]Selena-25-homodeoxycholic acid (0.27 mCi, 2.0 mg) was treated with a solution of N-ethoxycarbonyl-2-ethoxy-dihydroquinoline (3 mg) in dry dimethylformamide (620 µl) and stirred for 30 minutes. The solution was added to a mixture of taurine (1.55 mg) in dimethylformamide (350 µl) containing triethylamine (3.3 µl) and the reaction mixture was heated at ca 90° for 30 minutes. After standing at ambient temperature overnight, water (1 ml) was added; the solution was acidified with conc. HCl and evaporated to dryness. Ethanol (0.5 ml) was added to the residue and the product was isolated by preparative layer chromatography (Anachem Silica Gel GF, 1 mm; chloroform, methanol 5:2). The product band, Rf 0.32, was removed from the plate and the product was isolated by extraction with methanol. Evaporation of the solvent gave tauro-23-[^{75}Se]selena-25-homodeoxycholic acid (0.14 mCi). TLC (Merck Kieselgel 60 F_{254}; chloroform, methanol 3:1): major component (94%) Rf 0.34 (cf 23-selena-25-homodeoxycholic acid Rf 0.65 in the same system). IR (KBr) $\bar{\nu}$ max: 3400, 2940, 2870, 1698, 1650, 1545, 1390, 1208, 1180, 1070 cm^{-1}.

(xvi) 3α,7α-Dihydroxy-23-(β-carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane (Compound IX) - Red [^{75}Se]selenium (5.0 mg, 6.4 mCi) was prepared as described in (i) and was suspended in de-ionised water (0.55 ml). Potassium cyanide (4 mg) was added and the mixture was stirred until all the selenium had dissolved. β-Propiolactone (5 µl) was added and after stirring for 15 minutes the solution was acidified by the dropwise addition of concentrated

hydrochloric acid (some red selenium precipitated but not removed) and then lyophilized. Ether (3 ml) was added to the residue and the solution was filtered. Evaporation of solvent yielded β-[^{75}Se]selenocyanatopropionic acid (5.4 mCi). 3α,7α-Dihydroxy-23-bromo-24-nor-5β-cholane-3,7-dinitrate (30.8 mg) was dissolved in tetrahydrofuran (1.0 ml) and was added to sodium borohydride (8.3 mg) in ethanol (0.7 ml). The solution was cooled in ice and the β-[^{75}Se]selenocyanatopropionic acid in ethanol (1.0 ml) was added in portions over 10 minutes. After a further 1 hour, acetone (1 ml) was added and the solution was acidified with conc. HCl and evaporated to dryness. The residue was extracted into ether and the solution was filtered. TLC (Merck Kieselgel 60 F_{254}; chloroform, methanol 10:1) demonstrated 3 major radioactive products Rf 0.97, 0.85 and 0.09. Component Rf 0.85 corresponded to inactive marker. The product was isolated by preparative layer chromotography (Anachem Silica Gel GF, 1 mm; chloroform, methanol 10:1). It was located by autoradiography (Rf 0.41), removed from the plate and extracted into ether (3 x 3 ml) giving 1.1 mCi of 3α,7α-dihydroxy-23-(β-carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane-3,7-dinitrate. TLC (Merck Kieselgel 60 F_{254}; chloroform, methanol 10:1): major component (95%) Rf 0.54 corresponds to non-radioactive standard. IR (KBr) $\bar{\nu}$ max: 3450, 2940, 1710, 1630, 1278, 862 cm^{-1}. PMR ($CDCl_3$): τ 4.95 (1H,s,C_7-proton); τ 5.22 (1H,m,C_3 proton); τ 7.23 (4H,s,C_{25} and C_{26}-protons); τ 7.6 (2H,m,C_{23}-protons); τ 9.05 (6H,s + d, C_{19}-protons and C_{21}-protons); τ 9.32 (3H,s,C_{18}-protons); τ 7.85-9.10 (24H, steroid nucleus).

The dinitrate (1.1 mCi) was dissolved in glacial acetic acid (1 ml) and zinc dust (60 mg) was added in portions. The reaction mixture was stirred at ambient temperature for 1 hour and stored at -20°C overnight. After warming to room temperature the solution was filtered and the filtrate was lyophilized. The product was isolated by preparative layer chromatography (Anachem Silica Gel GF, 1 mm; chloroform, methanol (7:1). It was located by autoradiography (Rf 0.30), removed from the plate and extracted into

methanol to give 3α,7α-dihydroxy-23-(β-carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane (0.6 mCi). TLC (Merck Kieselgel 60 F_{254}): chloroform, methanol 5:1, major component (97%) Rf 0.65; chloroform, methanol 10:1, major component Rf 0.22; isooctane, diisopropyl ether, acetic acid 2:1:1, major component Rf 0.41. In each case the product coincided with the non-radioactive standard. IR (KBr) $\bar{\nu}$ max: 3435, 2940, 2870, 1715, 1550, 1410, 1300, 1080, 960 cm^{-1}. PMR (CD_3OD): τ5.16 (solvent peak); τ 6.20 (1H,s,C_7-proton); τ 6.94 (1H,m,C_3-proton); τ 6.99 (solvent peak); τ 7.25 (4H,s,C_{25} and C_{26}-protons); τ 7.45 (2H,m,C_{23}-protons); τ 9.02 (3H,d,C_{21}-protons); τ 9.07 (3H,s,C_{19}-protons); τ 9.29 (3H,s,C_{18}-protons).

(xvii) 3α,7α,12α-Triformoxy-23-iodo-24-nor-5β-cholane - Cholic acid triformate (1.06 g) was converted to 3α,7α,12α-triformoxy-23-iodo-24-nor-5β-cholane by the method described in (v) using carbon tetrachloride (100 ml), lead tetraacetate (0.97 g), and iodine (0.52 g) in carbon tetrachloride (40 ml). The crude product was twice recrystallized from ethanol to give 3α,7α,12α-triformoxy-23-iodo-24-nor-5β-cholane (0.65 g) as colourless crystals, m.p 166-168°. IR (KBr) $\bar{\nu}$ max: 2960, 2938, 2862, 2712, 1721, 1518, 1360, 1160, 1060, 995, 600 cm^{-1}. PMR ($CDCl_3$): τ 1.85, 1.90, 1.98 (3H, 3 singlets, 3-,7- and 12-formate protons); τ 4.74 (1H,s,C_{12}-proton); τ 4.94 (1H,s,C_7-proton); τ 5.30 (1H,m,C_3-proton); τ 6.72 + 6.95 (2H,m, C_{23}-protons); τ 9.06 (3H,s,C_{19}-protons); τ 9.15 (3H,d,C_{21}-protons); τ 9.22 (3H,s,C_{18}-protons); τ 7.8 - 9.05 (22H, steroid nucleus).

(xviii) 3α,7α,12α-Trihydroxy-23-(β-carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane (Compound X) - β-[^{75}Se]Selenocyanatopropionic acid (4.42 mCi, 108 mCi/mmol) was prepared as described in (xvii). 3α,7α,12α-Triformoxy-23-iodo-24-nor-5β-cholane (23 mg) in tetrahydrofuran (0.5 ml) was added to sodium borohydride (5.5 mg) in ethanol (0.5 ml) and the solution was cooled in ice. β-[^{75}Se]Selenocyanatopropionic acid (4.42 mCi) in ethanol (0.8 ml) was added to the solution over a period of 10 minutes and stirring continued for 1 hour.

The reaction mixture was treated with acetone (1 ml), acidified with conc. HCl, and evaporated to dryness. The residue was partitioned between ether and water and the ethereal phase was separated and extracted with 5% aqueous sodium carbonate solution. The alkaline extract was acidified and the precipitate was isolated by ether extraction.

Ethanol (2 ml), water (0.75 ml) and potassium hydroxide (100 mg) was added to the crude sample of 3α,7α,12α-triformoxy-23-(β-carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane. The solution was stirred at ambient temperature for 2 hours; it was then acidified and evaporated. Methanol (2 ml) was added to the residue, the solution was filtered from insoluble material and concentrated to small bulk. The product was purified by preparative layer chromatography (Merck Kieselgel 60 F_{254} 1 mm; chloroform, methanol 5:1). The required band was located by autoradiography (Rf 0.35); it was removed from the plate and extracted into methanol to give 3α,7α,12α-trihydroxy-23-(β-carboxyethyl-[^{75}Se]seleno)-24-nor-5β-cholane (1.2 mCi). TLC (Merck Kieselgel 60 F_{254}): chloroform, methanol 5:1 - major component (95%) Rf 0.57 corresponded to non-radioactive standard; isooctane, diisopropyl ether, acetic acid 2:1:1, Rf 0.21. IR (KBr) $\bar{\nu}$ max: 3520, 3416, 2930, 2870, 1740, 1718, 1440, 1380, 1322, 1170, 1080 cm^{-1}. PMR (CD_3OD): τ 5.11 (solvent peak); τ 6.06 (1H,s,C_{12}-proton); τ 6.23 (1H,s,C_7-proton); τ 6.67 (1H,m,C_3-proton); τ 6.71 (solvent peak); τ 7.30 (4H,s,C_{25} + C_{26}-protons); τ 7.47 and τ 7.78 (2H,m,C_{23}-protons); τ 8.97 (3H,d,C_{21}-protons); τ 9.10 (3H,s,C_{19}-protons); τ 9.30 (3H,s,C_{18}-protons); τ 9.70 (unidentified).

(xix) 3α-acetoxy-25-homo-5β-cholan-25-oic acid - 3α-acetoxy-25-homo-5β-cholan-25-oic acid was prepared from lithocholic acid using the Arndt-Eistert reaction for lengthening the C-17 side-chain. Lithocholic acid acetate was refluxed for 3 hours with thionyl chloride to provide 3α-acetoxy-5β-cholanyl chloride, which was subsequently reacted with diazomethane to yield 3α-acetoxy-24-keto-25-diazo-25-homocholane (17). The diazoketone was converted

to 3α-acetoxy-25-homo-5β-cholan-25-oic acid essentially by the method described by Pearlman for 25-homocholic acid (10).

(xx) 3α-Acetoxy-24-iodo-5β-cholane - 3α-Acetoxy-25-homo-5β-cholan-25-oic acid was converted to 3α-acetoxy-24-iodo-5β-cholane by the method quoted in (v). The quantities of reagents used were as follows:- 3α-acetoxy-25-homo-5β-cholan-25-oic acid (1.8 g) in dry carbon tetrachloride (120 ml), lead tetraacetate (2.0 g) and iodine (1.04 g) in carbon tetrachloride (80 ml). The crude product was purified by preparative layer chromatography using 5 Merck Kieselgel 60 F_{254}, 2 mm plates developed in chloroform. The required UV absorbing band was removed from each plate and the product was isolated by extraction with ether. Evaporation of the solvent and trituration of the residue with ethanol gave 3α-acetoxy-24-iodo-5β-cholane (0.43 g); m.p 140 - 146°) as a white powder. IR (KBr) $\bar{\nu}$ max: 2940, 2865, 1738, 1473, 1459, 1383, 1366, 1258, 1028 cm^{-1}. PMR ($CDCl_3$): τ 5.19 (1H,m,C_3-proton); τ 6.83 (2H,m,C_{24}-protons); τ 7.98 (3H,s, acetate protons); τ 9.07 (6H,1s + 1d, C_{19} + C_{21}-protons); τ 9.36 (3H,s,C_{18}-protons); τ 8.0 - 9.1 (28H, steroid nucleus).

(xxi) 3α-Hydroxy-24-(carboxymethyl-[^{75}Se]seleno)-5β-cholane - Ethyl [^{75}Se]selenocyanatoacetate (17 mg, 9.2 mCi) was prepared in the manner previously described in (xiv). It was reacted with sodium borohydride (8.2 mg) in ethanol (2 ml) and 3α-acetoxy-24-iodo-5β-cholane (50 mg) in tetrahydrofuran (3 ml) as described in (xiv). The intermediate 3α-acetoxy-24-(carboxymethyl-[^{75}Se]seleno)-5β-cholane ethyl ester was isolated by preparative layer chromatography (Anachem Silica Gel GF; chloroform). The main radioactive band was located by autoradiography (Rf 0.55); it was removed from the plate and the product was isolated by extraction with ethyl acetate (3 x 4 ml). The solvent was evaporated, ethanol (5 ml) and potassium hydroxide (100 mg) in water (1 ml) were added, the solution was heated under reflux for 3 hours and allowed to cool. The solution was acidified with conc. HCl and evaporated under reduced pressure. Ethanol

(1 ml) was added to the residue; the solution was filtered and the product isolated by preparative layer chromatography (Anachem Silica Gel GF); chloroform, methanol 12:1). The required band (Rf 0.20) was located by autoradiography; it was removed from the plate and the product was isolated by extraction with ethanol. Evaporation of the solvent gave 3α-hydroxy-24-(carboxymethyl-[^{75}Se]seleno-5β-cholane (0.8 mCi). TLC (Merck Kieselgel 60 F_{254}; dichloromethane, methanol 15:1): major component (94%) Rf 0.25, coincided with the non-radioactive standard. IR (KBr) $\bar{\nu}$ max: 3400, 2930, 2855, 1700, 1445, 1373, 1105, 1028 cm^{-1}.

* Patents on the ^{75}Se-labelled bile acid analogues are held by Amersham International plc. See Reference 4.

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REFERENCES

1. Stanley M.M. and Nemchausky B. - J. Lab. Clin. Med. 70: 627 (1967).
2. Fromm H., Thomas P.J. and Hofmann A.F. - Gastroenterology 64: 1077 (1973).
3. Van Blankenstein M., Hoyset T., Horchner P., Frenkel M. and Wilson J.H.P. - Neth. J. Med. 20: 248 (1977).
4. Boyd G.S., Merrick M.V., Monks R. and Thomas I.L. - J.Nucl. Med. 22: 720 (1981).

5. Merrick M.V., Eastwood M.A., Anderson J.R. and Ross H.McL. - J. Nucl. Med. 23: 126 (1982).

6. Agenas L.-B. and Persson B. - Acta Chem. Scand. 21: 837 (1967).

7. Klayman D.L. and Scott Griffin T. - J. Amer. Chem. Soc. 95: 197 (1973).

8. Gunther W.H.H. and Mautner H.G. - J. Med. Chem. 7: 229 (1964).

9. Riegel B., Moffett R.B. and McIntosh A.V. - p.38 in Organic Syntheses, Vol.24, John Wiley and Sons, Inc., New York, (1944).

10. Pearlman W.H. - J. Amer. Chem. Soc. 69: 1475 (1947).

11. Vaidya A.S., Dixit S.M. and Rao A.S. - Tetrahedron Letters No. 50: 5173 (1968).

12. Von Rudloff E. - Can. J. Chem. 34: 1413 (1956); 43: 2660 (1965).

13. Brink N.G., Clark D.M. and Wallis E.S. - J. Biol. Chem. 162: 695 (1946).

14. Barton D.H.R., Faro H.P., Serebryakov E.P. and Woolsey N.F. - J. Chem. Soc. 2438 (1965).

15. Norman A. - Ark. Kemi. 8: 331 (1955).

16. Tserng K-Y., Hachey D.L. and Klein P.D.- J. Lipid Res. 18: 404 (1977).

17. Ruzicka L., Plattner P.A. and Heusser H. - Helv. Chim. Acta, 27: 186 (1944).