1 592 791

PATENT SPECIFICATION (11) 1 592 791

(21) Application No. 628/77 (22) Filed 7 Jan. 1977
(23) Complete Specification filed 9 Jan. 1978
(44) Complete Specification published 8 July 1981
(51) INT CL³ C07J 51/00//G01N 33/50
(52) Index at acceptance
C2U 2 3 4A1A 4A2A 4C10A 4C2 4C3 4CX 4N12 4N5 5 7A
C2C 20Y 300 30Y 366 367 368 38X 39X AA DA

(72) Inventors REGINALD MONKS and
ANTHONY LEONARD MARK RILEY

(19)

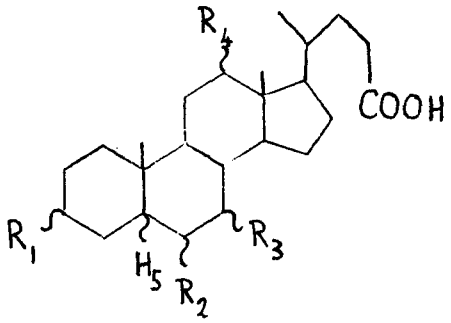
(54) SELENIUM- OR TELLURIUM-CONTAINING BILE ACIDS AND DERIVATIVES THEREOF

(71) We, THE RADIOCHEMICAL CENTRE LIMITED, a British Company of White Lion Road, Amersham, Buckinghamshire, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to selenium and tellurium derivatives, particularly γ-emitting radioactive derivatives of bile acids and bile salts. Such compounds are valuable in the examination of body function, especially small bowel function.

Bile salts are synthesized in the liver from cholesterol, pass via the hepatic and common bile ducts to the intestinal tract, are absorbed in the ileum and return to the liver via the portal venous system. During the enterohepatic circulation in a normal human more than 95 per cent of the bile salts entering the small intestine are reabsorbed, the remainder entering the large intestine and eventually appearing in the faeces. Malfunctioning of the ileum, which can be caused by a number of pathological conditions, can result in the deficient absorption of bile salts. A measurement of bile salt absorption by the intestine would therefor provide useful information enabling the distal small bowel to be recognised, or eliminated, as the source of gastrointestinal disorder.

Bile acids may be represented by the following formula:—



(1)

wherein R_1, R_2, R_3 and R_4 are each independently a hydrogen atom or an α- or β-hydroxyl group, and wherein H_5 is either in the α or β position.

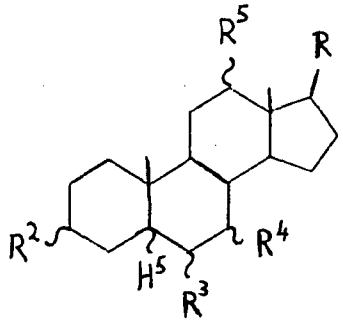
Bile salts are conjugates of the above bile acids with amino acids, in particular glycine and taurine.

Carboxyl-^{14}C-cholic acid (1, R_2=H, R_1=R_3=R_4=α—OH; H_5 is β) and its taurine conjugate have been used to study the absorption of bile salts in the intestine of both animals and man under a variety of pathological conditions, e.g. regional ileitis, ileal resection, and induced diarrhoea. The investigations have required the measurement of ^{14}C radioactivity in faeces, urine and bile. In the breath test as devised by Fromm and Hofmann glycine-1-[^{14}C] glycocholate is used to detect increased bacterial deconjugation of the bile salts. Upon deconjugation in the small bowel as a result of bacterial overgrowth or in the colon following bile salt malabsorption, the glycine liberated is metabolized, absorbed, and partly exhaled as $^{14}CO_2$. In the case of bile salt malabsorption some of the ^{14}C radioactivity will appear in the faeces. A faecal ^{14}C measurement is essential for complete

exploitation of the diagnostic scope of the breath test. In the diagnosis of bile acid malabsorption the Schilling test employing labelled cyanocobalamin with intrinsic factor is often helpful, but by itself it cannot discriminate between bacterial overgrowth and ileal dysfunction.

The measurement of bile acid adsorption as a routine test for small bowel function would be greatly facilitated if the bile acids could be labelled with a gamma emitting isotope. Counting of gamma emitters is in general easier and more economical than is counting of beta emitters: this particularly applies to biological samples such as bile of faeces, where for beta emitters it would be necessary to process the sample before counting could begin. Labelling with a gamma emitter would possess the additional advantage of allowing body counting and thus obviate the need to handle faecal samples; visualisation of the enterohepatic system would also be possible. The gamma emitting isotopes which could possibly be employed to label bile acids without changing their biological behaviour, and in which the label would remain attached throughout the enterohepatic cycle, are limited in number. This invention concerns the use of radioisotopes of selenium and tellurium, such as selenium-75 and tellurium-123m, to fulfil the required function. The incorporation of either selenium or tellurium into the structure of the bile acid molecule has so far not been described; this applies to both the radioactive and non-radioactive forms of these elements.

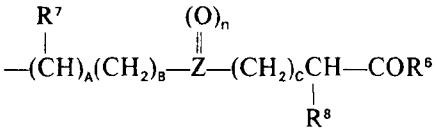
The invention provides labelled bile acids and their conjugates, as shown in formulae (2) below and which are substituted by Se or Te in the C-17 side chain of the molecule.



(2)

wherein

R is



and

A is 0 or 1,

B is 0 to 4,

C is 0 to 4, preferably 0 or 1,

Z is Se or Te,

R^6 is —OH or a radical derived by removal of a hydrogen atom from the amino group of an amino acid,

R^7 is hydrogen or saturated C_1 to C_4 alkyl group, preferably methyl, when A is 1,

R^8 is hydrogen or saturated C_1 to C_4 alkyl group, preferably hydrogen,

n is 0 or 1,

R^2, R^3, R^4 and R^5 are each independently hydrogen or an α- or β-hydroxyl group or an oxo group,

H^5 is an α- or β- H.

This invention includes the inactive compounds and also, more particularly, the compounds labelled with radioactive isotopes of selenium and tellurium, e.g. selenium-75 and tellurium-123m. The inactive compounds are useful aids in determining the properties of the radioactive compounds. The labelled bile acids of the present invention and their amino acid conjugates may be prepared by the following routes:

The compounds may be prepared by the reaction of a suitable selenium or tellurium nucleophile with a modified bile acid having a terminal halogen atom, e.g. bromine or iodine, in the C_{17} side chain. These reactions are carried out in such solvents as ethanol, propanol, tetrahydrofuran or dimethylformamide, or mixtures of these solvents, generally at room temperature. The selenium or tellurium nucleophiles are produced by the reaction in the liquid ammonia of disodium diselenide or ditelluride with an ω-halogenated carboxylic acid or its ester, the resulting organic diselenide or ditelluride being dissolved in one of the above solvents and cleaved by reagents such as sodium borohydride or dithiothreitol; further reaction with the modified bile acid is effected in situ.

Alternatively, the halogenated bile acid may be reacted with disodium diselenide in a solvent such as propanol at elevated temperatures to provide a disteroidal diselenide. The disteroidal diselenide is dissolved in ethanol, cleaved with sodium borohydride, and the selenol reacted in situ with an ω-halogenated carboxylic acid ester.

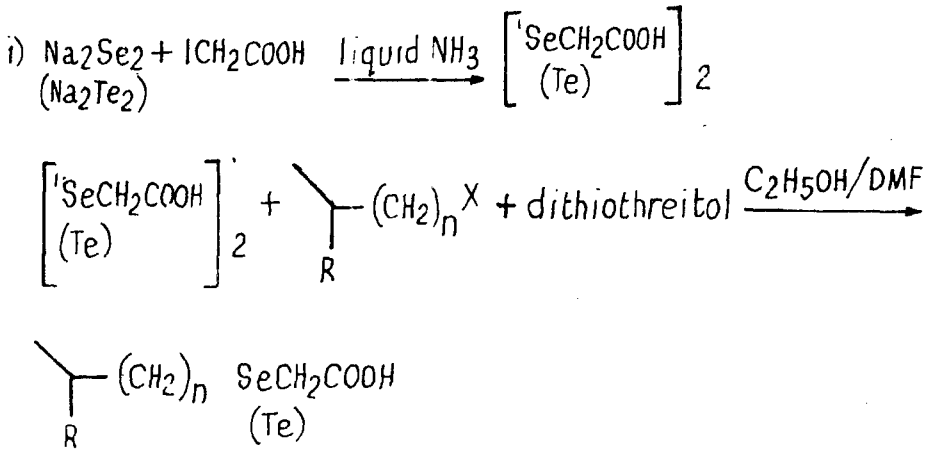
The use of potassium selenocyanate affords a useful route to the compounds of this group. Potassium selenocyanate, prepared by dissolving red selenium in ethanolic potassium cyanide, is reacted in ethanol at reduced temperatures with a ω-halogenocarboxylic acid ester. The resulting ω-selenocyanato-carboxylic acid ester is reduced with sodium borohydride and reacted in situ with the halogenated bile acid intermediate. These reactions are usually conducted at room temperature in ethanol or ethanol/tetrahydrofuran mixtures.

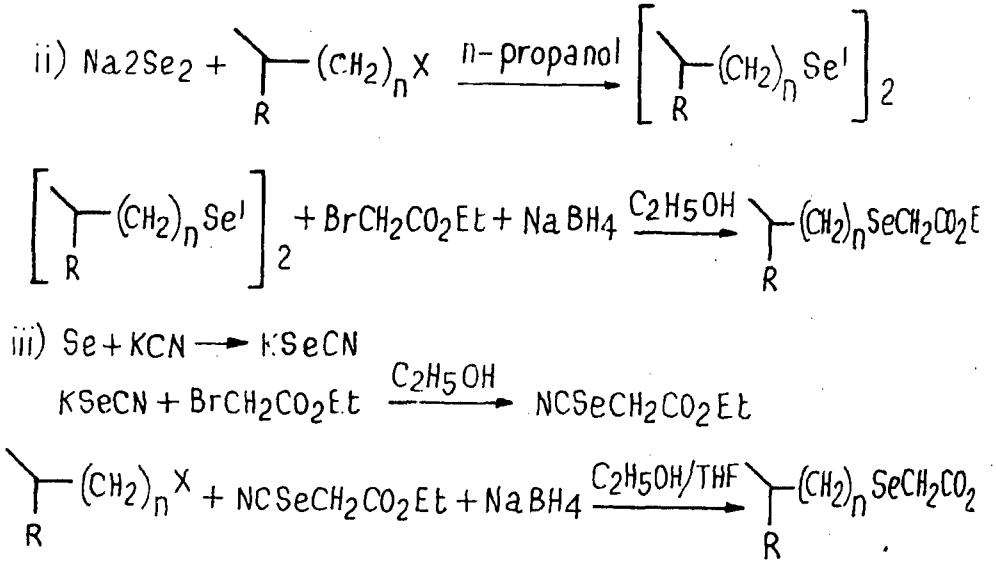
α-Halogenated carboxylic acid esters may be used in place of ω-halogenated compounds in the above reaction to provide products having a side chain in the α-position to the carboxyl group.

Where hydroxyl groups have been protected by acylation and carboxylic acid groups by esterification the protecting groups are removed by standard methods prior to final purification of the product by preparative layer chromatography on silica gel.

The bile acid analogues, containing either a selenium or a tellurium atom in the C_{17} side chain, may be conjugated via an amide linkage to amino acids such as glycine and taurine. The methods used to prepare the bile acid conjugates are well known in the art and depend on the condensation of the bile acid with the amino acid in a suitable solvent such as dimethylformamide and in the presence of a condensing agent such as a carbodiimide or N - ethoxy - carbonyl - 2 - ethoxy - dihydroquinoline (EEDQ).

The reaction schemes shown below illustrate the preparations broadly described above. Further detail is provided in the Examples. It is to be understood that these preparations may be carried out with either natural selenium or tellurium or with these elements enriched with their respective radioisotopes, e.g. ^{75}Se or ^{123m}Te.





N.B. R=bile acid nucleus X=halogen

The insertion of either a selenium or tellurium atom into the C_{17} side chain of a bile acid according to formula (2) is dependent upon the availability of modified bile acid intermediates having a terminal halogen atom, e.g. bromine or iodine, in the C_{17} side chain. The provision of such intermediates has required the shortening or lengthening of the C_{17} side chain by methods known in the art, e.g. Barbier-Wieland degradation or the Arndt-Eistert reaction respectively. Replacement of the terminal carboxyl group by a halogen atom may be effected by the Hunsdiecker reaction. A particularly effective means of accomplishing this replacement is to treat the bile acid in refluxing carbon tetra-chloride with lead tetra-acetate/iodine reagent, the reaction mixture being irradiated with light meanwhile. The hydroxyl groups of the bile acid must be protected with suitable groups such as formyl, acetyl, nitro, etc. This reaction results in the replacement of the carboxyl group with an iodine atom. The degradation of the C_{17} side chain of cholic acid to provide a 20-iodo-5β-pregnane derivative may be effected by three consecutive reactions:

1. refluxing of the protected bile acid in dry benzene and under nitrogen with lead tetracetate in the presence of cupric acetate and pyridine provides the corresponding Δ^{22}-24-nor-5β-cholene, (A);

2. treatment of A with sodium periodate/potassium permanganate in aqueous 2-methylpropan-2-ol in presence of potassium carbonate causes oxidation of the Δ^{22} bond and provides the 3α,7α,12α - triformoxy - 23,24 - bisnor - 5β - cholanic acid, (B);

3. B is refluxed in carbon tetrachloride with lead tetra-acetate/iodine reagent under light irradiation to provide the 3α,7α,12α - triformoxy - 20 - iodo - 5β - pregnane derivative, (C). C is probably a mixture of R and S isomers but the proportions have not been determined.

The above reaction can be performed equally using steroids in the 5α- or the 5β-configuration. Available steroids in the 5α-configuration include 5α-cholanic acid-3β-ol and 22,23-bisnor-5α-cholanic acid-3β-ol.

The invention is illustrated by the following Examples.

Example 1

The Preparation of 3α,12α-dihydroxy-22-(carboxymethyl-[^{75}Se]-seleno)-23,24-bisnor-5β-cholane (23-Selena-25-homodeoxycholic Acid)

i) 3α,12α-Diacetoxy-22-iodo-23,24-bisnor-5β-cholane

3α,12α - Diacetoxy - 24 - nor - 5β - cholanic acid (0.3 g) in dry carbon tetrachloride (30 ml) was treated with dry, powdered, lead tetra-acetate (0.3 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 (0.16 g) in dry carbon

tetrachloride (12 ml) was added portionwise over a period of 10 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 then dried over anhydrous sodium sulphate. Evaporation of the solvent and crystallisation of the residue from ethanol gave 3α,12α - diacetoxy - 22 - iodo - 23,24 - bisnor - 5β - cholane (0.3 g, 85%) m.p. 172—174°. Atlas and Merck are Registered Trade Marks.

TLC (Merck Kieselgel 60 F_{254}; Chloroform)
Single component Rf 0.50.

IR Spectrum
$\bar{\nu}$ max: 2960, 2930, 2870, 1735, 1453, 1374, 1239, 1194, 1018 cm^{-1}.

NMR (220 MHz, $CDCl_3$)
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).

ii) 23-Selena-25-homodeoxycholic acid-^{75}Se

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

Red selenium-^{75}Se (8.4 mg, 0.11 mA, 109 mCi/mA) was suspended in ethanol (2 ml) and potassium cyanide (7 mg, 0.11 mmole) was added; the mixture was stirred at room temperature for two hours until complete solution had occurred. Redistilled ethyl bromoacetate (12 μl) was added to the solution at 0°C and it was stirred for 1½ hours. 3α,12α - Diacetoxy - 22 - iodo - 23,24 - bisnor - 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 selenocyanatoacetate-^{75}Se was added over a period of 10 minutes. Stirring was continued for a further 2 hours while the temperature rose to 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 aceate (3×4 ml). Yield of ethyl 3α,12α - diacetoxy - 23 - selena - 25 - homo - 5β - cholanate - ^{75}Se, 6.1 mCi.

IR Spectrum
$\bar{\nu}$ max: 2935, 2860, 1735, 1450, 1378, 1245, 1050, 750 cm^{-1}.

The solution was evaporated 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, 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 - selena - 25 - homodeoxycholic acid-^{75}Se (2.4 mCi). Bio-Rad is a Registered Trade Mark.

TLC (Merck Kieselgel 60 F_{254})
a) Chloroform, methanol-5:1; Major component (95%) Rf 0.36.
b) Iso octane, diisopropyl ether, acetic acid-2:1:1. Major component Rf 0.43.

IR Spectrum
$\bar{\nu}$ max: 3380, 2930, 2860, 1700, 1448, 1380, 1255, 1105, 1035 cm^{-1}.

iii) Tauro-23-selena-25-homodeoxycholic acid-^{75}Se

23 - Selena - 25 - homodeoxycholic acid-^{75}Se (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 acidifed by addition of concentrated hydrochloric acid and evaporated. 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 - selena - 25 - homodeoxycholic acid-^{75}Se (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 Spectrum

$\bar{\nu}$ max: 3400, 2940, 2870, 1698, 1650, 1545, 1390, 1208, 1180, 1070 cm^{-1}.

iv) Ethyl 3α,12α-Diacetoxy-23-selena-25-homo-5β-cholanate and 23-Selena-25-homodeoxycholic Acid

Non-radioactive ethyl 3α,12α - diacetoxy - 23 - selena - 25 - homo - 5β - cholanate and 23 - selena - 25 - homodeoxycholic acid were prepared by the method described in 1(ii). Quantities of reagents used:— ethyl selenocyanatoacetate, 35 mg in 0.7 ml ethanol; sodium borohydride, 12.6 mg; 3α,12α - diacetoxy - 22 - iodo - 23,24 - bisnor - 5β - cholane, 100 mg; ethanol, 5 ml; tetrahydrofuran, 1 ml. Yield of ethyl 3α,12α - diacetoxy - 23 - selena - 25 - homo - 5β - cholanate 64 mg.

IR Spectrum

$\bar{\nu}$ max: 2940, 2865, 1738, 1450, 1380, 1245, 1105, 1060 cm^{-1}.

NMR (220 MHz, $CDCl_3$)

τ 4.93 (1H, S, C_{12}-proton), τ 5.32 (1H, M, C_3-proton), τ 5.84 (2H, q, ethyl CH_2), τ 6.90 (2H, S, C_{24}-protons), τ 7.06 (1H, M, C_{22}-proton), τ 7.45 (1H, q, C_{22}-proton),

τ 7.90 (3H, S, 12-acetate protons), τ 7.96 (3H, S, 3-acetate protons),

τ 8.72 (3H, t, ethyl CH_3), τ 9.08 (3H, d, C_{21}-protons), τ 9.12 (3H, S, C_{19}-protons),

τ 9.25 (3H, S, C_{18}-protons), τ 8.0—9.25 (22H, steroid nucleus).

Ethyl 3α,12α - diacetoxy - 23 - selena - 25 - homo - 5β - cholanate (120 mg) was dissolved in ethanol (5 ml) and hydrolysed as described in 1(ii) giving 23-selena - 25 - homodeoxycholic acid (45 mg).

TLC (Merck Kieselgel 60 F_{254}; Chloroform, Methanol 5:1)

The product, visualised by exposure to iodine vapour, chromatographed as a single component (Rf 0.32) and coincided with the radioactive marker.

IR Spectrum

$\bar{\nu}$ max: 3430, 2920, 2855, 1700, 1448, 1375, 1255, 1038 cm^{-1}.

NMR (220 MHz, CD_3OD)

τ 5.12 (solvent peak), τ 6.05 (1H, S, C_{12}-proton), τ 6.50 (1H, m, C_3-proton), τ 6.7 (solvent peak), τ 6.93 (2H, S, C_{24}-protons), τ 7.07 (1H, m, C_{22}-proton), τ 7.54 (1H, q, C_{22}-proton), τ 7.85 (3H, S, CH_3CO_2H), τ 8.88 (3H, d, C_{21}-protons), τ 9.07 (3H, S, C_{19}-protons), τ 9.28 (3H, S, C_{18}-protons), τ 8.0—9.2 (22H, steroid nucleus).

v) 23-Selena-25-homodeoxycholic Acid Selenoxide-^{75}Se

23 - Selena - 25 - homodeoxycholic acid-^{75}Se [68.4 μCi, 1.1 μmole] in methanol (1.0 ml) was treated with an aqueous solution of hydrogen peroxide (5 μl, 4 μmole) and was allowed to stand at ambient temperature for 90 minutes.

TLC (Merck Kieselgel 60 F_{254}, Dichloromethane, Acetone, Acetic Acid-7/2/1)

Major component (greater than 90%) Rf 0.19 (cf 23 - selena - 25 - homodeoxycholic acid-^{75}Se Rf 0.84 in this system).

Example 2

Preparation of 3α,7α-Dihydroxy-23-(β-carboxyethylseleno)-24-nor-5β-cholane

i) 3α,7α-Dihydroxy-23-(β-carboxyethylseleno)-24-nor-5β-cholane-3,7-dinitrate-^{75}Se

Red selenium-^{75}Se (5.0 mg, 6.4 mCi) was prepared as described in Example 1 (ii) and was suspended in deionised 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 was precipitated) and evaporated. Ether (3 ml) was added to the residue and the solution of β-selenocyanatopropionic acid-^{75}Se was filtered to remove insoluble products and evaporated (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 β-selenocyanatopropionic acid-^{75}Se in ethanol (1.0 ml) was added in portions over 10 minutes. After a further 1 hour, acetone (1 ml) was added, the solution was acidified with concentrated hydrochloric acid and evaporated to dryness. The residue was extracted into ether and the solution was filtered from insoluble material. TLC (Merck Kieselgel 60 F_{254}; chloroform, methanol 10:1) demonstrated three major radioactive products Rf 0.97, 0.85 and 0.09. Component Rf 0.85 corresponded to inactive marker (Example 2 (iii)).

The product was isolated by preparative layer chromatography (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 (3x3 ml) giving 1.1 mCi of 3α,7α - dihydroxy - 23 - (β - carboxyethylseleno) - 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.

ii) 3α,7α-Dihydroxy-23-(β-carboxyethylseleno)-24-nor-5β-cholane-^{75}Se

The dinitrate (1.1 mCi — prepared as described above — 2 (i)) 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 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 - carboxyethylseleno) - 24 - nor - 5β - cholane (0.6 mCi).

TLC (Merck Kieselgel 60 F_{254})

a) chloroform methanol, 5:1; major component (97%) Rf 0.65.

b) chloroform, methanol; 10:1; major component Rf 0.22.

c) iso-octane, diisopropylether, acetic acid; 2:1:1; major component Rf 0.41.

In each case the product coincided with the non-radioactive standard.

iii) 3α,7α-Dihydroxy-23-(β-carboxyethylseleno)-24-nor-5β-cholane-3,7-dinitrate

Non-radioactive 3α,7α - dihydroxy - 23 - (β - carboxyethylseleno) - 24 - nor - 5β - cholane was prepared by the method described (2 (i)) using the quantities of reagents as follows:— 3α,7α - dihydroxy - 23 - bromo - 24 - nor - 5β - cholane - 3,7 - dinitrate (173.1 mg) in tetrahydrofuran (4 ml); sodium borohydride (45.8 mg) in ethanol (2.2 ml) and β-selenocyanatopropionic acid (61.4 mg) in ethanol (2.2 ml). the reaction mixture was treated with acetone (1 ml), it was poured into water (25 ml), acidified with concentrated hydrochloric acid and extracted with ether (2x20 ml). The combined ether extracts were washed with 5% sodium carbonate solution (2x20 ml) and the combined alkaline extracts were acidifed. The precipitate was isolated by ether, the extracts were dried and evaporated. The product was purified by preparative layer chromatography (Merck Kieselgel F_{254}, 2 mm — chloroform, methanol 10:1). The required band was located under u.v., it was removed from the plate and extracted into ether. Evaporation of the solvents left 3α,7α - dihydroxy - 23 - (β - carboxyethylseleno) - 24 - nor - 5β - cholane - 3,7 - dinitrate as a white solid (82 mg).

IR Spectrum
$\bar{\nu}$ max: 3450, 2940, 1710, 1620, 1278, 862 cm^{-1}.

NMR (220 MHz, $CDCl_3$)
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).

iv) 3α,7α-Dihydroxy-23-(β-carboxyethylseleno)-24-nor-5β-cholane
3α,7α - Dihydroxy - 23 - (β - carboxyethylseleno) - 24 - nor - 5β - cholane (50 mg) was prepared from its dinitrate ester (80 mg) by the method described (2 (ii)).

IR Spectrum
$\bar{\nu}$ max: 3435, 2940, 2870, 1715, 1550, 1410, 1300, 1080, 960 cm^{-1}.

NMR (220 MHz, CD_3OD)
τ 5.16 (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).

Example 3
Preparation of 3α,7α,12α-Trihydroxy-23-(β-carboxyethylseleno)-24-nor-5β-cholane

i) 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 recrystallisation from 60% aqueous ethanol and 1:1 60—80° petrol, acetone, M.p. of purified material 204—208°C.

ii) 3α,7α,12α-Triformoxy-23-iodo-24-nor-5β-cholane
Cholic acid triformate (1.06 g) and lead tetracetate (0.97 g) were suspended in dry carbon tetrachloride (100 ml) and the suspension was stirred and heated to reflux in an atmosphere of dry nitrogen. Reflux was maintained by irradiation with an Atlas 275 watt infra-red lamp and a solution of iodine (0.52 g) in carbon tetrachloride (40 ml) was added in portions. Reflux was continued for a further 1 hour. The reaction mixture was allowed to cool and then filtered.
The filtrate was washed successively with 5% sodium thiosulphate solution and water, and was dried over anhydrous sodium sulphate. Evaporation of the solvent and recrystallisation of the residue from ethanol (twice) gave 3α,7α,12α - triformoxy - 23 - iodo - 24 - nor - 5β - cholane (0.65 g) as colourless crystals, m.p. 166—168°.

IR Spectrum
$\bar{\nu}$ max: 2960, 2938, 2862, 2712, 1721, 1518, 1360, 1160, 1060, 995, 600 cm^{-1}.

NMR (220 MHz, $CDCl_3$)
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-protons), 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).

iii) `α,7α,12α-Trihydroxy-23-(β-carboxyethylseleno)-24-nor-5β-cholane-^{75}Se
β-Selenocyanatopropionic acid-^{75}Se (4.42 mCi, 108 mCi/mmole) was prepared from red selenium-^{75}Se as described for Example 2 (i). 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-Selenocyanatopropionic acid-^{75}Se (4.42 mCi) in ethanol (0.8 ml) was added to the solution over a period of 10 minutes and stirring was allowed to continue for 1 hour. The reaction mixture was treated with acetone (1 ml), acidified with concentrated hydrochloric acid, and evaporated. 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 - carboxyethylseleno) - 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 - carboxyethylseleno) - 24 - nor - 5β - cholane - ^{75}Se (1.2 mCi).

TLC (Merck Kieselgel 60 F_{254})

a) chloroform, methanol 5:1 — major component (95%) Rf 0.57 corresponded to non-radioactive standard.

b) iso-octane, diisopropylether, acetic acid 2:1:1; Rf 0.21.

IR Spectrum

$\bar{\nu}$ max: 3520, 3416, 2930, 2870, 1740, 1718, 1440, 1380, 1322, 1170, 1080 cm^{-1}.

iv) 3α,7α,12α-Trihydroxy-23-(β-carboxyethylseleno)-24-nor-5β-cholane

Non-radioactive 3α,7α,12α - trihydroxy - 23 - (β - carboxyethylseleno) - 24 - nor - 5β - cholane was prepared by the method described in 3 (iii). The following quantities of reagents were used:— 3α,7α,12α - triformoxy - 23 - iodo - 24 - nor - 5β - cholane, 258.7 mg; sodium borohydride, 61.2 mg; β-selenocyanatopropionic acid, 79.6 mg. Following the final hydrolysis step the product was purified by partition between ether and 5% sodium carbonate solution. The acidic product was isolated and triturated with acetone to give 3α,7α,12α - trihydroxy - 23 - (β - carboxyethylseleno) - 24 - nor - 5β - cholane (70 mg) as a white powder, m.p. 198—200°.

IR Spectrum

$\bar{\nu}$ max: 3520, 3410, 2930, 2870, 1740, 1718, 1440, 1382, 1323, 1170, 1080 cm^{-1}.

NMR (220 MHz, 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).

Example 4

Preparation of 3α,7α,12α-trihydroxy-20-(carboxymethylseleno)-5β-pregnane-(22-selenacholic Acid)

i) 3α,7α,12α-Triformoxy-Δ^{22}-24-nor-5β-cholene

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 tetra-acetate (20 g) and cholic acid triformate (10.5 g, prepared as described in 3 (i) 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 crystallisation of the residue from ethanol gave 3α,7α,12α - triformoxy-Δ^{22} - 24 - nor - 5β - cholene (4.0 g) m.p. 188—190°.

IR Spectrum

$\bar{\nu}$ max: 3077, 2960, 2865, 1725, 1714, 1637, 1468, 1449, 1380, 1180 cm^{-1}.

NMR Spectrum

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—τ 9.1 (22H, steroid nucleus).

ii) 3α,7α,12α-Triformoxy-23,24-bisnor-5β-cholanic Acid

3α,7α,12α - Triformoxy - Δ^{22} - 24 - nor - 5β - cholene (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 colouration, 5% sodium carbonate solution was added to pH 8, and the solution was concentrated at reduced pressure to ca. 250 ml. It was extracted with chloroform (2x100 ml), treated with further 40% sodium hydrogen sulphite and acidified with concentrated hydrochloric acid. The mixture was extracted with chloroform (4x100 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 and was allowed to cool. It was poured into water and the precipitate was extracted into chloroform (3x50 ml). The combined organic extracts were washed with water, dried and evaporated. The residue was recrystallised from ethanol to give 3α,7α,12α - triformoxy - 23,24 - bisnor - 5β - cholanic acid (0.8 g) m.p. 165—170°.

IR Spectrum

$\bar{\nu}$ max: 3410, 2965, 2940, 2870, 1722, 1450, 1385, 1178, 890 cm^{-1}.

NMR Spectrum (220 MHz, $CDCl_3$)

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, CH_2 of ethanol of crystallisation), τ 7.64 (1H, q, C_{20}-proton), τ 8.77 (3H, t, CH_3 of ethanol of crystallisation), τ 8.88 (3H, d, C_{21}-protons), τ 9.05 (3, S, C_{19}-protons), τ 9.22 (3H, S, C_{18}-protons), τ 7.75—9.05 (19H, steroid nucleus).

iii) 3α,7α,12α-Triformoxy-20-iodopregnane

3α,7α,12α - Triformoxy - 23,24 - bisnor - 5β - cholanic acid (0.2 g) was converted to 3α,7α,12α - triformoxy - 20 - iodopregnane (0.11 g) by the method described in 1 (i) m.p. 145—146.5° (decomp).

IR Spectrum

$\bar{\nu}$ max: 3405, 2950, 2860, 1713, 1445, 1377, 1180 cm^{-1} .

NMR Spectrum (220 MHz, $CDCl_3$)

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).

iv) 22-Selenacholic Acid-^{75}Se

Red selenium-^{75}Se (8.2 mg, 106 mCi/mA) was prepared as described in 1 (ii). 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 ambient temperature 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 - 20 - iodopregnane (35 mg) in warm n-propanol (2 ml) was added to the solution of disodium diselenide-^{75}Se and the whole was heated on a boiling water bath in an atmosphere of dry nitrogen for 3½ hours. It was allowed to cool; it was evaporated under reduced pressure and the residue was treated with chloroform (5 ml). The solution was filtered and evaporated to dryness leaving the impure dipregnane diselenide-^{75}Se (4.2 mCi).

Sodium borohydride (5 mg) was dissolved in ethanol (1 ml), the solution was cooled in ice and ethyl bromoacetate (20 μl) was added. The dipregnane diselenide-^{75}Se was dissolved in ethanol (3 ml) and was added dropwise over a period of 10 minutes. The reaction mixture was stirred for 2 hours, acetone (1 ml) was added and the solution was evaporated. Chloroform (3 ml) was added, inorganic salts were removed by filtration, and the solution was treated with sodium hydroxide (100 mg) in water (1 ml). The solution was heated under reflux for 3 hours, cooled and evaporated. The residue was dissolved in water (3 ml) and the solution was

acidified with concentrated hydrochloric acid and lyophilized. Acetic acid (3 ml) was added to the residue, the solution was filtered and concentrated to a small bulk. The product was purified by preparative layer chromatography, (Anachem Silica Gel GF, 1 mm; dichloromethane, acetone, acetic acid, 7:2:1). Its location was determined by autoradiography, the band was removed from the plate and the product was extracted into acetic acid and the solvent evaporated to give 22-selenacholic acid-^{75}Se (0.8 mCi).

TLC (Merck Kiesgel 60 F_{254})
a) Dichloromethane, acetone, acetic acid; (7:2:1) Major component Rf 0.22.
b) Chloroform, methanol; (5:1), major component Rf 0.11.

IR Spectrum
$\bar{\nu}$ max: 3400, 2925, 2780, 1715, 1440, 1375, 1265, 1073, 1040 cm^{-1}.

v) Glyco-22-selenacholic Acid-^{75}Se

22-Selenacholic-^{75}Se (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-selenacholic acid-^{75}Se, 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 evaporated. Chloroform (4 ml) was added to the residue and insoluble material was removed by filtration.

Ethyl 22-selenaglycocholate-^{75}Se 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 (3×4 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 concentrated hydrochloric acid, evaporated to dryness and the product was extracted from the residue by dissolving in ethanol. The solution was filtered and evaporated leaving glyco-22-selenacholic acid-^{75}Se (0.21 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).

Example 5

Preparation of 3α-Hydroxy-24-(carboxymethylseleno)-5β-cholane

i) 3α-Acetoxy-25-homo-5β-cholanic Acid

3α - Acetoxy - 25 - homo - 5β - cholanic acid was prepared from lithocholic acid using the Arndt-Eistert reaction for lengthening the C_{17} side chain.

ii) 3α-Acetoxy-24-iodo-5β-cholane

3α - Acetoxy - 25 - homo - 5β - cholanic acid was transformed to 3α - acetoxy - 24 - iodo - 5β - cholane by the method quoted in 3 (ii). The quantities of reagents used were as follows:— 3α - acetoxy - 25 - homo - 5β - cholanic acid (1.8 g) in dry carbon tetrachloride (120 ml), lead tetra-acetate (2.0 g) and iodine (1.04 g) in carbon tetrachloride (80 ml). The crude product was purified by preparative layer chromatography using five 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 Spectrum
$\bar{\nu}$ max: 2940, 2865, 1738, 1473, 1459, 1383, 1366, 1258, 1028 cm^{-1}.

NMR Spectrum (220 MHz, $CDCl_3$)
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).

iii) 3α-Hydroxy-24-(carboxymethylseleno)-5β-cholane-^{75}Se

Ethyl selenocyanatoacetate-^{75}Se (17 mg, 9.2 mCi) was prepared in the manner previously described (1 (ii)). 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 1 (ii). The intermediate 3α - acetoxy - 24 - (carboxymethylseleno) - 5β - cholane ethyl ester-^{75}Se 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 ethylacetate (3×4 ml). the solvent was evaporated, ethanol (5 ml) and potassium hydroxide (100 mg) in water (1 ml) were added and the solution was heated under reflux for 3 hours and allowed to cool. The solution was acidified with concentrated hydrochloric acid 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 - (carboxymethylseleno) - 5β - cholane - ^{75}Se (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 Spectrum

$\tilde{\nu}$ max: 3400, 2930, 2855, 1700, 1445, 1373, 1105, 1028 cm^{-1}.

iv) 3α-Acetoxy-24-(carboxymethylseleno)-5β-cholane Ethyl Ester

Non-radioactive 3α - acetoxy - 24 - (carboxymethylseleno) - 5β - cholane ethyl ester (160 mg) was prepared by the method given in 5 (iii) from 3α - acetoxy - 24 - iodo - 5β - cholane (200 mg), sodium borohydride (32 mg) and ethyl selenocyanatoacetate (74.7 mg).

IR Spectrum

$\tilde{\nu}$ max: 2925, 2855, 1733, 1445, 1375, 1360, 1238, 1100, 1023 cm^{-1}.

NMR Spectrum (220 MHz, $CDCl_3$)

τ 5.29 (1H, m, C_3-proton), τ 5.83 (2H, q, ethyl CH_2), τ 6.86 (2H, S, C_{26}-protons), τ 7.98 (3H, S, acetate protons), τ 8.72 (3H, q, ethyl CH_3), τ 9.0 (6H, 1s+1d, C_{19}-protons+C_{21}-protons), τ 9.36 (3H, S, C_{18}-protons).

v) 3α-Hydroxy-24-(carboxymethylseleno)-5β-cholane

Hydrolysis of 3α - acetoxy - 24 - (carboxymethylseleno) - 5β - cholane ethyl ester according to the method in 5 (iii) gave 3α - hydroxy - 24 - (carboxymethylseleno) - 5β - cholane m.p. 117—121°C.

IR Spectrum

$\tilde{\nu}$ max: 3440, 2920, 2855, 1705, 1443, 1372, 1270, 1165, 1105, 1026 cm^{-1}.

Example 6

Preparation of 23-(Carboxymethylseleno)-24-nor-5β-cholane-3,7,12-trione-^{75}Se

i) 23-Iodo-24-nor-5β-cholane-3,7,12-trione

5β-Cholanic acid-3,7,12-trione was converted to 23 - iodo - 24 - nor - 5β - cholane - 3,7,12 - trione by the method described in 3 (ii). The quantities of reagents used were as follows:— 5β-cholanic acid-3,7,12-trione (2 g) in carbon tetrachloride (200 ml), lead tetra-acetate (2.3 g), iodine (1.2 g) in carbon tetrachloride (100 ml). The product was recrystallised successively from ethanol and petrol (60—80°)-ethyl acetate, m.p. 256—257°C.

TLC (Merck Kieselgel 60 F_{254}, Chloroform)

Major component Rf 0.36 (if 5β-cholanic acid-3,7,12-trione, Rf 008, in this system).

IR Spectrum

$\tilde{\nu}$ max: 2960, 2930, 1727, 1708, 1472, 1438, 1392, 1382, 1304, 1280, 1226 cm^{-1}.

ii) 23-(Carboxymethylseleno)-24-nor-5β-cholane-3,7,12-trione-^{75}Se

An ethanolic solution of ethyl selenocyanatoacetate-^{75}Se (15.3 mg, 8.8 mCi) was prepared by the method described in 1 (ii); it was added to a solution of sodium borohydride (6.6 mg) in ethanol (1 ml) at 0°. After stirring at 0° for 20 minutes, acetone (1 ml was added followed by 23 - iodo - 24 - nor - 5β - cholane - 3,7,12 - trione (39 mg) in tetrahydrofuran (1 ml). The reaction mixture was stirred at ambient temperature for 16 hours. The solution was evaporated, chloroform (2 ml) was added and, after filtration, the solution was concentrated and applied to an Anachem 1 mm silica plate which was developed in chloroform, methanol 20:1. Three main radioactive bands were located by autoradiography (Rfs 0.33, 0.49, 0.69); they were removed separately from the plate and the radioactive component was isolated from each by extraction with ether, ethanol (10:1). An examination of the separated components by thin layer chromatography (Merck Kieselgel 60 F_{254}; chloroform) and by infra-red spectroscopy indicated that component Rf 0.49 was the required 23 - (carboxymethylseleno) - 24 - nor - 5β - cholane - 3,7,12 - trione ethyl ester-^{75}Se, component Rf 0.33 was a mixture of two unidentified compounds and component Rf 0.69 was non-steroidal.

23 - (Carboxymethylseleno) - 24 - nor - 5β - cholane - 3,7,12 - trione ethyl ester-^{75}Se (2.05 mCi) was dissolved in ethanol (5 ml) and 10% potassium carbonate solution (1 ml) was added. The solution was heated under reflux for 2 hours, cooled and evaporated under reduced pressure. Water (4 ml) was added, some insoluble material was removed by filtration and the solution was acidified with concentrated hydrochloric acid and lyophilized. Chloroform (0.5 ml) was added to the residue and the product was isolated by preparative layer chromatography (Anachem Silica Gel GF, 1mm; chloroform, methanol-10:1). The main radioactive band was located by autoradiography (Rf 0.32); it was removed from the plate and the product was isolated by extraction with methanol. Evaporation of the solvent gave 23 - (carboxymethylseleno) - 24 - nor - 5β - cholane - 3,7,12 - trione - ^{75}Se (1.3 mCi).

TLC (Merck Kieselgel 60 F_{254}; Chloroform, Methanol 20:1)
Major component — greater than 95% Rf 0.31.

IR Spectrum
$\bar{\nu}$ max: 2965, 2895, 1717, 1475, 1428, 1395, 1275, 1118 cm^{-1}.

Example 7

Preparation of 3α,12α-Dihydroxy-23-(carboxymethyltelluro)-24-nor-5β-cholane

i) 3α,12α-Diformoxy-23-Iodo-24-nor-5β-cholane

3α,12α-Diformoxy-5β-cholanic acid was prepared from deoxycholic acid (25 g) and 100% formic acid (100 ml) by the method described in 3 (i). The product was recrystallised from ethanol giving colourless crystals (17.5 g), m.p. 197—199°C.

3α,12α-Diformoxy-5β-cholanic acid (4 g) was converted to 3α,12α - diformoxy - 23 - iodo - 24 - nor - 5β - cholane by the method previously described (3 (ii)) using lead tetra-acetate (4.0 g) and iodine (1.9 g). The crude product was crystallised from ethanol giving colourless crystals m.p. 123—125°C (3.1 g).

IR Spectrum
$\bar{\nu}$ max: 2940, 2865, 1723, 1447, 1383, 1205, 1190, 1180 cm^{-1}.

NMR Spectrum (220 MHz, $CDCl_3$)
τ 1.89 and 1.98 (2H, two singlets, 3- and 12-formate protons), τ 4.75 (1H, S, C_{12}-proton), τ 5.19 (1H, m, C_3-proton), τ 6.71 (1H, m, C_{23}-proton), τ 6.96 (1H, q, C_{23}-proton), τ 9.06 (3H, S, C_{19}-protons), τ 9.16 (3H, d, C_{21}-protons), τ 9.22 (3H, S, C_{18}-protons), τ 7.95—9.15 (24H, steroid nucleus).

ii) 3α,12α-Dihydroxy-23-(carboxymethyltelluro)-24-nor-5β-cholane-^{123m}Te

^{123m}-Tellurium (6 mg, 5 mCi) was dissolved in concentrated hydrochloric acid (2 ml) and hydrogen peroxide (100 vol. 2 drops). Tellurium oxide (23 mg inactive) was added and the resulting solution was diluted with water (32 ml). Tellurium metal was precipitated using sulphur dioxide gas, was washed twice with water and then with ethanol, and was finally dried in vacuum.

To tellurium metal (24.6 mg, 5 mCi) in a reaction vessel containing 15 ml of

liquid ammonia was added sodium (4.4 mg), the vessel being connected to a vacuum manifold and vented to the atmosphere via a carbosorb/charcoal trap. The reaction mixture was stirred for 5 minutes to obtain disodium ditelluride-^{123m}Te and then iodoacetic acid (35.8 mg) was added. The ammonia was allowed to evaporate, and traces of volatile matter were removed under reduced pressure.

The residue was redissolved in ethanol (20 ml) and dimethylformamide (10 ml) and stirred under an atmosphere of nitrogen. Sodium hydroxide (0.1 g) in water (3 ml) and dithiothreitol (50 mg) in water (2 ml) were added. After 20 minutes 3α,12α - diformoxy - 23 - iodo - 24 - nor - 5β - cholane in dimethyl formamide (2 ml), was added. The reaction mixture was stirred at 60° for 1 hour and at room temperature overnight. The solvents were evaporated in vacuo, and the residue dissolved in chloroform (2 ml) and then purified by preparative layer chromatography on cellulose (Avicel F Butanol, water, acetic acid, 60:25:15). The active band, Rf 0.9—0.96 as observed by autoradiography, was removed from the plate, and extracted into chloroform. Evaporation of the chloroform yielded a residue of 350 μCi (7%). Avicel is a Registered Trade Mark.

TLC

Cellulose; (butanol, water, acetic acid 60:25:15), major component (>95%) — Rf 0.95.

IR Spectrum

$\bar{\nu}$ max: 2950, 2920, 2860, 1725, 1450, 1385, 1125, 1070, 1035, 875, 790, 740 cm^{-1}.

iii) 3α,12α-Dihydroxy-23-(carboxymethyltelluro)-24-nor-5β-cholane

This was prepared as in 7 (ii). Tellurium (59 mg), sodium (11.5 mg), iodoacetic acid (84 mg), sodium hydroxide (0.2 g), dithiothreitol (100 mg) 3α,12α - diformoxy - 23 - iodo - 24 - nor - 5β - cholane (190 mg) were used.

Yield 30 mg (16%).

IR Spectrum

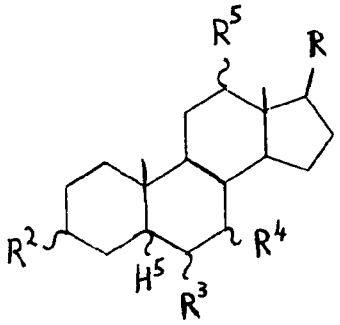
$\bar{\nu}$ max: 2940, 2860, 1725, 1450, 1385, 1130, 1070, 875, 790 cm^{-1}.

NMR Spectrum (CD_3OD) (220 MHz)

τ 6.05 (1H, S, C_{12}-proton), τ 8.97 (3H, d, C_{21}-protons), τ 9.08 (3H, S, C_{19}-protons), τ 9.28 (3H, S, C_{18}-protons).

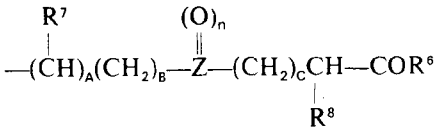
WHAT WE CLAIM IS:—

1. Compounds having the general formula



wherein

R is



and

A is 0 or 1,

B is 0 to 4,

C is 0 to 4,

Z is Se or Te,

R^6 is —OH or a radical derived by removal of a hydrogen atom from the amino group of an amino acid,

R^7 is hydrogen or a saturated C_1 to C_4 alkyl group, when A is 1,

R^8 is hydrogen or saturated C_1 to C_4 alkyl group,

n is 0 or 1,

R^2, R^3, R^4 and R^5 are each independently hydrogen or an α- or β-hydroxyl group, or an oxo group,

H^5 is an α- or β-H.

2. Compounds according to Claim 1, wherein Z is selenium-75 or tellurium-123m.

3. Compounds according to Claim 1 or Claim 2, wherein R^6 is a residue of glycine or taurine.

4. Compounds according to any one of Claims 1 to 3, wherein

C is 0 or 1,

R^7 is methyl,

R^8 is hydrogen,

n is 0,

H^5 is β-H.

5. $3\alpha,12\alpha$ - Dihydroxy - 22 - (carboxymethyl - [^{75}Se]seleno) - 23,24 - bisnor - 5β - cholane.

6. $3\alpha,7\alpha$ - Dihydroxy - 23 - (β - carboxyethyl - [^{75}Se]seleno) - 24 - nor - 5β - cholane.

7. $3\alpha,7\alpha,12\alpha$ - Trihydroxy - 23 - (β - carboxyethyl-[^{75}Se]seleno) - 24 - nor - 5β - cholane.

8. $3\alpha,7\alpha,12\alpha$ - Trihydroxy - 20 - (carboxymethyl-[^{75}Se]seleno) - 5β - pregnane.

9. Glyco - 22 - [^{75}Se]selenacholic acid.

10. 3α - Hydroxy - 24 - (carboxymethyl[^{75}Se]seleno) - 5β - cholane.

11. $3\alpha,12\alpha$ - Dihydroxy - 23 - (carboxymethyl-[^{123m}Te]telluro) - 24 - nor - 5β - cholane.

12. Tauro - 23 - [^{75}Se]selena - 25 - homodeoxycholic acid.

13. 3,7,12 - Trioxo - 23 - (carboxymethyl-[^{75}Se]seleno) - 24 - nor - 5β - cholane.

STEVENS, HEWLETT & PERKINS,
Chartered Patent Agents,
5, Quality Court,
Chancery Lane,
London, WC2.

Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1981
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.