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SYNTHESIS OF 3β-ACETOXY-24-NORCHOLA-5,20(22)-DIEN-23-AL. A NEW INTERMEDIATE FOR AN IMPROVED SYNTHESIS OF [24-^{14}C]-CHOLESTEROL

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SUMMARY

[24-^{14}C]-Cholesterol was synthesized by reaction between the phosphorane derivative of [1-^{14}C]-isobutyltriphenylphosphonium bromide and 3β-acetoxy-24-norchola-5,20(22)-dien-23-al (IX) followed by hydrogenation of the condensation product. The intermediate IX was prepared by two different procedures both starting from pregnenolone acetate.

IN THE course of our studies on the biosynthesis of bufadienolides by plants and animals, it was firstly established that plant bufadienolides were derived from pregnenolone[1, 2] as it has been found for plant cardenolides[3], but that animal bufadienolides were not derived from a pregnene-like intermediate[2].

It was later reported that in the case of animals, cholesterol or a closely related metabolite seems to be the true precursor in the biological synthesis of bufadienolides[4, 5]. This was established by finding that in toads[20-^{14}C]-cholesterol is a much better precursor of bufadienolides than mevalonic acid[5] whilst [20-^{14}C]-pregnenolone was not incorporated into the toad bufadienolides at all.

In order to confirm the postulated metabolic formation of animal bufadienolides, the next experiment to be done is to prove a side chain-labelled cholesterol, other than at C-20, as precursor of the mentioned cardiac genins. As it was claimed[6] that bile acids can provide the carbon-atoms skeleton of the bufadienolide, we planned to synthesize cholesterol labelled at C-24 to be used in biosynthetic experiments.

EXPERIMENTAL

General

Melting points were determined on a Fischer–Johns hot-plate and are uncorrected. Infrared spectra were registered with a Perkin–Elmer "Infracord" spectrophotometer. Ultraviolet spectra were taken in 95% ethanol solutions with a Beckman DK2 spectrophotometer. Nuclear magnetic resonance spectra were determined in deutero-chloroform solutions with a Varian A-60 spectrometer; tetramethylsilane was used as internal standard; chemical shifts are expressed in ppm (δ) (s: singlet, t: triplet, q: quartet, m: multiplet, and b.s.: broad signal).

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Radioactive samples were counted with a Packard model 93320 liquid scintillation spectrometer in the usual scintillation solutions. Thin-layer chromatography was performed on silica-gel or alumina (Merck): spots were detected either with iodine vapour (non-carbonylic compounds) or 2,4-dinitrophenylhydrazine (carbonylic compounds). [1-^{14}C]-Isobutyric acid sodium salt was purchased from the Comisión Nacional de Energía Atómica (Argentina). Microanalyses were performed by Drs. B. B. de Deferrari (F. C. EyN., UBA) and A. Bernhardt Laboratory (West Germany). Solvents were removed under reduced pressure below 50°.

3β-Acetoxy-20ξ-hydroxy-23-norchol-5-enic acid ethyl ester (II)

Pregnenolone acetate (I, 30 g) was dissolved in a mixture of benzene (10 ml): ethyl ether (10 ml) and the solution was treated with activated zinc powder (15 g) and a few crystals of iodine. To the stirred solution, ethyl bromoacetate (21 ml) was added dropwise maintaining a gentle reflux. When the addition was over, the mixture was stirred for 30 min and it was then poured onto ice-diluted hydrochloric acid. The organic layer was washed with saturated sodium hydrogen-carbonate solution, then with water and it was finally dried over magnesium sulphate. The residue obtained after evaporation of the solvent was treated with pyridine (50 ml) and acetic anhydride (50 ml) overnight at room temperature. The mixture was then poured onto ice-water and the solid was filtered off and dried. The product (29 g) was recrystallized from methanol giving pure II, m.p. 136–138°C; I.R. data: 3450, 1730 cm^{-1}; n.m.r. data: δ 0·86 (s, 3H, Me-18), 1·00 (s, 3H, Me-19), 1·25 (t, 3H, J 7 Hz, C<u>H</u>$_3$-CH$_2$O-), 1·28 (s, 3H, Me-21), 2·03 (s, 3H, CH$_3$-CO), 3·33 (b.s., 1H, OH), 4·20 (q, 2H, J 7Hz, CH$_3$-C<u>H</u>$_2$-O-), 4·61 (b.s., 1H, H-3), 5·25 (m, 1H, H-6).

Anal. Calc. for $C_{27}H_{42}O_5$: C, 72·61; H, 9·48. Found: C, 72·28; H, 9·14.

3β-Acetoxy-23-norchola-5,20(22)-dienoic acid ethyl ester (III)

To a solution of compound II (25 g) in dry pyridine (300 ml) phosphorous oxychloride (50 ml) was slowly added and the reaction mixture was kept overnight at room temperature. It was then poured onto ice-diluted hydrochloric acid and the whole was extracted with ether (3 × 60 ml). The organic extract was washed successively with 6N hydrochloric acid, saturated sodium hydrogen-carbonate solution and water, and was then dried over magnesium sulphate. The residue obtained after evaporation of the solvent was chromatographed on alumina (Fluka, neutral, act. I); elution with benzene afforded compound III (20 g) which after recrystallization from ethanol has m.p. 140–144°C; U.V. data: λ_{max}^{EtOH} 225 nm (ε 6,700); I.R. data: 1735, 1700, 1625, 870 cm^{-1}; n.m.r. data: δ 0·63 (s, 3H, Me-18), 1·00 (s, 3H, Me-19), 1·46 (t, 3H, J 7 Hz, C<u>H</u>$_3$-CH$_2$-O-), 2·05 (s, 3H, CH$_3$-CO), 2·21 (d, 3H, J 1·5 Hz, Me-21), 4·18 (q, 2H, J 7 Hz, CH$_3$-C<u>H</u>$_2$-O-), 4·61 (b.s., 1H, H-3), 5·41 (b.s., 1H, H-6), 5·76 (b.s., 1H, H-22).

Anal. Calc. for $C_{27}H_{40}O_4$: C, 75·66; H, 9·41. Found: C, 75·38; H, 9·24.

3β,23-Dihydroxy-24-norchola-5,20(22)-diene (IV)

A solution of compound III (19·2 g) in dry ether (50 ml) was cooled to 0° and treated with lithium aluminum hydride (8 g) in small portions. The reaction mixture was kept overnight at room temperature. The surplus reagent was carefully destroyed by addition of moist ether followed by ice, and it was then

made acid with acetic acid. The organic layer was separated and the aqueous layer was extracted with ether (4 × 25 ml). The combined extracts were washed with saturated sodium hydrogen-carbonate solution and then with water. The residue obtained after evaporation of the dried (with magnesium sulphate) extract was recrystallized from ethyl acetate giving IV (14 g), m.p. 159–161°C; I.R. data: 3300 cm^{-1}; n.m.r. data: δ 0·60 (s, 3H, Me-18), 1·00 (s, 3H, Me-19), 1·60 (s, 3H, Me-21), 3·53 (b.s., 2H, both hydroxyl groups), 4·25 (d, 2H, J 7Hz, H-23), 4·50 (m, 2H, H-22 and H-6).

Anal. Calc. for $C_{23}H_{36}O_2$: C, 80·18; H, 10·53. Found: C, 79·99; H, 10·52.

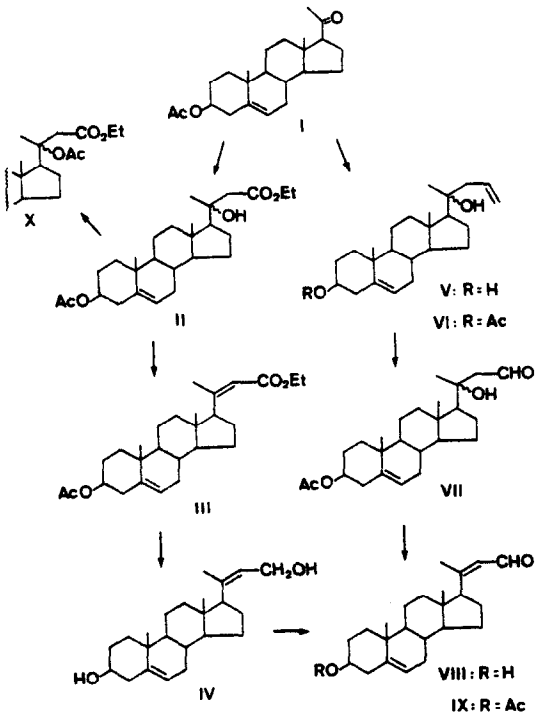

Fig. 1. Synthesis of 3β-acetoxy-24-norchola-5,20(22)-dien-23-al(IX) from pregnenolone acetate(I).

3β-*Acetoxy*-24-*norchola*-5,20(22)-*dien*-23-*al* (IX)

A solution of compound IV (13 g) in benzene:chloroform (4:1) (2 l) was treated with activated manganese dioxide (100 g) and the mixture was vigorously stirred for 12 h at room temperature. The solid was filtered off and the filtrate was evaporated to dryness. The solid residue was treated with pyridine (15 ml) and acetic anhydride (15 ml) overnight at room temperature. The mixture was poured onto ice-water and the solid was filtered off and dried. Recrystallization from cyclohexane afforded pure IX (8·5 g), m.p. 140–142°C; U.V. data: λ_{max}^{EtOH} 246 nm (ε 19,000); I.R. data: 1735, 1655, 1620 cm^{-1}; n.m.r. data: δ 0·60 (s, 3H, Me-18), 1·00 (s, 3H, Me-19), 2·01 (s, 3H, CH_3-CO), 2·20 (d, 3H, J 1Hz, Me-21), 4·50 (b.s., 1H, H-3), 5·50 (b.s., 1H, H-6), 6·06 (d, 1H, J 8 Hz, H-22), 10·13 (d, 1H, J 8 Hz, -CHO).

Anal. Calc. for $C_{25}H_{36}O_3$: C, 78·08; H, 9·44. Found: C, 77·88 H, 9·60.

Chola-5,23-dien-3β,20ξ-diol (V)

Pregnenolone acetate (1, 5·5g) was dissolved in benzene (50 ml) and the solution cooled to 0°. An ethereal solution of allylmagnesiumbromide (prepared from 8 g magnesium and 12 ml allylbromide in 110 ml dry ether) was added dropwise with constant stirring under nitrogen atmosphere. When the addition was over, the mixture was refluxed for 4 h. The Grignard reagent in excess was destroyed by careful addition of ice and diluted hydrochloric acid, and the whole was extracted with ether (3 × 100 ml) and ether : chloroform (3 : 1 v/v) (3 × 100 ml). The partially crystalline residue obtained after evaporation of the dried (with magnesium sulphate) extract was suspended in light petroleum and filtered. The crystalline solid (5·3 g) was recrystallized (twice) from methanol yielding 4·1 g of V, m.p. 130–131°C; I.R. data: 3200, 1655, 1625 cm^{-1}; n.m.r. data: δ 0·86 (s, 3H, Me-18), 1·00 (s, 3H, Me-19), 1·28 (s, 3H, Me-21), 3·45 (b.s., 1H, H-3), 4·90 (m, 1H, H-24), 5·30 (b.s., 1H, H-6), 5·75 (m, 1H, H-23). The product crystallized with half a molecule of solvent (MeOH) which accounts for a sharp singlet at δ 3·43 and also for the results of the elemental analysis.

Anal. Calc. for $C_{24}H_{38}O_2$. 1/2 CH_3OH: C, 78·56; H, 10·76. Found: C, 78·61; H, 10·39.

When dried at 80°C and 10^{-2} torr over P_2O_5 for 24 h, the O-methyl singlet disappears and a correct elemental analysis was obtained.

Anal. Calc. for $C_{24}H_{38}O_2$: C, 80·39; H, 10·68. Found: C, 80·53; H, 10·70.

3β-Acetoxy-chola-5,23-dien-20ξ-ol (VI)

Compound V (3·5 g) was dissolved in pyridine (40 ml) and the solution was treated with acetic anhydride (40 ml). After 20 h at room temperature the mixture was poured onto ice-water and the precipitate was filtered off. The dried product (3·6 g) m.p. 195°C, was recrystallized from ethanol giving pure VI (3·12 g) m.p. 196–197°C; I.R. data: 3500, 1720, 1660, 1640, 1250 cm^{-1}; n.m.r. data: δ 0·86 (s, 3H, Me-18), 1·01 (s, 3H, Me-19), 1·26 (s, 3H, Me-21), 2·00 (s, 3H, CH_3-CO), 4·55 (b.s., 1H, H-3), 4·91 (m, 1H, H-24), 5·32 (b.s., 1H, H-6), 5·75 (m, 1H, H-23).

Anal. Calc. for $C_{26}H_{40}O_3$: C, 77·95; H, 10·07. Found: C, 77·90; H, 10·01.

3β-Acetoxy-20ξ-hydroxy-24-norchol-5-en-23-al (VII). *Procedure* A

Typical experiment. Compound VI (1 g) was dissolved in freshly distilled dioxane (175 ml) and the solution was treated with osmium tetroxide (20 mg), with stirring at room temperature. When after 5 min the solution became yellow, water (25 ml) and sodium periodate (500 mg) were added. During the subsequent 30 min, stirring continuously and at room temperature, a total amount of 4·5 g of sodium periodate was added in small portions. After the addition was over, the reaction was maintained for 2 h, and during that period a precipitate was gradually formed. This was filtered off and washed with dioxane. The solid was identified as sodium iodate. The filtrate and washings were combined and evaporated to dryness. The residual solid was partitioned between chloroform and water, the water layer being extracted twice with chloroform. The chloroform extract (violet in colour) was washed with water and dried over magnesium sulphate. The organic extract was filtered through an alumina (Woelm, neutral, grade III) (25 g) column. Elution with chloroform was continued until no more residue was left after evaporation of the eluates. The white and crystalline residue (870 mg) was recrystallized (twice) from cyclohexane giving VII (579 mg) homogeneous on

t.l.c.; m.p. 169–172°C; I.R. data: 3450, 1720, 1700, 1660, 1225 cm^{-1}; n.m.r. data: δ 0·86 (s, 3H, Me-18), 1·01 (s, 3H, Me-19), 1·43 (s, 3H, Me-21), 2·00 (s, 3H, CH_3-CO), 4·55 (b.s., 1H, H-3), 5·33 (b.s., 1H, H-6), 9·81 (t, 1H, -CHO).

Anal. Calc. for $C_{25}H_{38}O_4$: C, 74·59; H, 9·52. Found: C, 74·82; H, 9·22.

Procedure B

Compound VI (200 mg) was dissolved in benzene (30 ml) and osmium tetroxide (10 mg) was added to the solution. The stirred solution, which became pale green almost immediately, was treated with water (10 ml) and sodium periodate (1 g). While the heterogeneous mixture was vigorously stirred, samples were taken and analyzed by means of t.l.c. The reaction was maintained for 7 days with daily additions of sodium periodate (200 mg each) until almost complete disappearance of the starting compound. The layers were separated, the aqueous phase was extracted with benzene and the combined organic extract was dried over magnesium sulphate. Filtration through alumina and elution of the column with chloroform until no more residue in the eluates afforded 153 mg of VII, m.p. 162–166°C which after recrystallization from cyclohexane was identical (m.p., I.R.) with the product prepared by Procedure A.

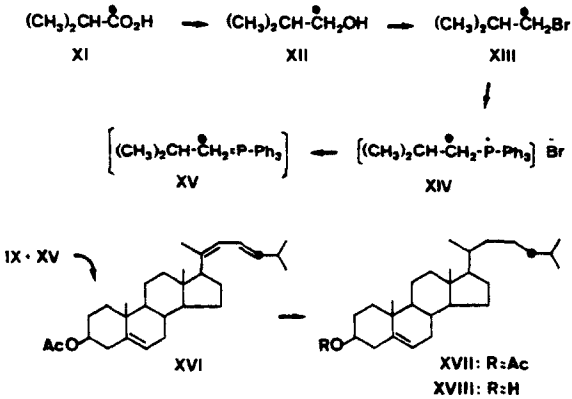

Fig. 2. Synthesis of [24-^{14}C] cholesterol trom [1-^{14}C] isobutyric acid (XI) and 3β-acetoxy-24-norchola-5,20(22)-dien-23-al (IX). Labelled carbon-atoms are indicated with heavy dots.

Dehydration of 3β-acetoxy-20ξ-hydroxy-24-norchol-5-en-23-al.

Compound VII (1 g) was dissolved in acetic acid (30 ml), the solution was refluxed for 15 min and evaporated to dryness. The residue (985 mg) showed three main spots on t.l.c., and was therefore chromatographed on alumina (Woelm, neutral, grade I) (50 g); elution was carried out with light petroleum: ethyl acetate (99:1 v/v) and (98:2 v/v). Fractions with the same mobility on t.l.c. were combined and evaporated. The first eluted product (350 mg) was recrystallized from methanol and identified as pregnenolone acetate (I) by means of m.p., I.R., and n.m.r. spectra. The second eluted compound (15 mg) was recrystallized from light petroleum and identified (m.p., I.R.) as the αβ-unsaturated aldehyde IX, while the third eluted product which showed a hydroxyl band in its I.R. spectrum was acetylated in the usual way giving 15 mg of IX, m.p. 138–140°C, identical to the second eluted compound and to the one prepared by the parallel method (see above).

[1-^{14}C]-*Isobutyl alcohol* (XII)

[1-^{14}C]-Sodium isobutyrate (1·25 g, $1{\cdot}80 \times 10^9$ d.p.m./mmol) was diluted with inactive material (5 g) and the mixture was treated at 0°C with 3N sulphuric acid (40 ml) until complete dissolution of the solid. The mixture was extracted with ether (4×20 ml) and the ethereal extract was washed with saturated sodium chloride solution (2×10 ml) and dried over magnesium sulphate. The ethereal solution of [1-^{14}C]-isobutyric acid (XI) was added dropwise into a stirred suspension of lithium aluminum hydride (6 g) in dry ether (120 ml) at such a rate as to maintain a gentle reflux. When the addition was over, the mixture was heated under reflux for 24 h. The surplus reagent was destroyed by a careful addition of ice and the whole was treated with enough 15% sulphuric acid to dissolve the precipitate. The aqueous layer was extracted with ether (3×25 ml) and the ethereal extracts were combined, washed with saturated sodium chloride solution (2×20 ml) and dried (magnesium sulphate). The ethereal solution was carefully evaporated through a fractionating column collecting the distilled ether at 35–36°C; when all the ether was evaporated, the [1-^{14}C]-isobutyl alcohol was distilled collecting the fraction between 102–109°C (3·2 g). The alcohol was treated with solid potassium hydroxide (120 mg), kept at room temperature for 40 h, and then distilled at atmospheric pressure. The fraction boiling between 104–108°C (2·8 g) was collected.

[1-^{14}C]-*Isobutyl bromide* (XIII)

The radioactive alcohol XII (2·8 g) cooled to −10°C was treated dropwise and with stirring, with phosphorous tribromide (1·6 ml) at such a rate as to keep the temperature of the reaction mixture below 0°C. When the addition was over the cooling bath was removed and the stirring continued at room temperature overnight. The crude [1-^{14}C]-isobutyl bromide was distilled under reduced pressure (about 200 torr) collecting the fraction boiling at 45–60°C; this fraction was treated with anhydrous potassium carbonate and carefully shaken until the smell of hydrobromic acid disappeared. It was then distilled at atmospheric pressure collecting the fraction boiling at 90–94°C (2·66 g).

[1-^{14}C]-*Isobutyltriphenylphosphonium bromide* (XIV)

The radioactive isobutyl bromide XIII (2·6 g) was dissolved in dry xylene (50 ml), the solution was treated with triphenylphosphine (5·5 g), and the mixture was stirred and heated under reflux for 3 days. The crystalline precipitate was filtered off and washed with xylene. The dried product weighed 1·05 g and had a specific activity of $3{\cdot}46 \times 10^8$ d.p.m./mmol.

[24-^{14}C]-3β-*Acetoxycholesta*-5,20(22),23-*triene* (XVI)

[1-^{14}C]-Isobutyltriphenylphosphonium bromide (XIV, 1 g) was suspended in dry ether (10 ml) and the stirred mixture was treated, under nitrogen atmosphere, with 0·32N ethereal solution of butyllithium until complete dissolution of the solid with concomitant formation of the isobutylentriphenylphosphorane (XV). This solution was added dropwise to a solution of compound IX (930 mg) in dry ether (20 ml) under nitrogen atmosphere and with continuous stirring at room temperature. When the addition was completed, stirring was maintained for 10 min and the reaction was stopped by addition of moist ether followed by water. The ethereal layer was washed with water, dried over magnesium sulphate, and

evaporated. The residue was acetylated with pyridine (5 ml) and acetic anhydride (5 ml) for 20 h, and the mixture was then poured onto ice-water. The solid thus obtained was collected and dried. As it showed two spots on t.l.c., it was dissolved in benzene and chromatographed through an alumina (Woelm, neutral, grade I) column. Elution was with benzene. The homogeneous eluted compound (600 mg) was hydrogenated without further purification (see below). In a previous inactive preparation the compound eluted from the column was dissolved in hot methanol which have an amorphous solid on cooling. Compound XVI (inactive) had m.p. 60–65°C; I.R. data: 1735, 1670, 1640, 1600 cm^{-1}: n.m.r. data: δ 0·58 (s, 3H, Me-18), 0·97 (d, 6H, J 6 Hz, Me-26 and Me-27), 1·03 (s, 3H, Me-19), 1·77 (s, 3H, Me-21), 2·01 (s, 3H, CH_3-CO), 4·60 (b.s., 1H, H-3), 5·12 (q, 1H, J 9·5 and 5·5 Hz, H-23), 5·38 (b.s., 1H, H-6), 5·75 (q, 1H, J 9·5 and 11 Hz, H-24), 6·13 (d, 1H, J 5·5 Hz, H-22).

[24-^{14}C]-*Cholesterol acetate* (XVII)

Compound XVI (600 mg) was dissolved in dioxane (30 ml) and glacial acetic acid (1 ml), and hydrogenated over platinum oxide at room temperature and atmospheric pressure. After absorption of 2 moles of hydrogen, the catalyst was filtered off and the filtrate was evaporated to dryness. The crystalline residue (590 mg, $3·37 \times 10^8$ d.p.m./mmol) was recrystallized from methanol yielding 494 mg of XVII whose I.R. spectrum resulted identical to one obtained from authentic cholesterol acetate. Specific activity: $3·40 \times 10^8$ d.p.m./mmol.

[24-^{14}C]-*Cholesterol* (XVIII)

The labelled compound XVII (488 mg) was treated with ethanol (14 ml) and sodium hydroxide (1 g) in water (2 ml). The mixture was heated under reflux for 3 h. Addition of ice and water produced a precipitate which was filtered off and dried. The solid (442 mg) was recrystallized from methanol to pure XVIII (430 mg), m.p. 147–149°C, with an I.R. spectrum identical with that obtained from authentic cholesterol. Specific activity: $3·45 \times 10^8$ d.p.m./mmol.

RESULTS AND DISCUSSION

To obtain [24-^{14}C]-cholesterol using [1-^{14}C]-isobutyric acid (XI) it was necessary to prepare an appropriate steroidal moiety such that condensation between the labelled compound and the steroidal intermediate supplied the side chain of cholesterol with the radioactive carbon atom located at position 24. A related approach has been used for the formation of the side chain of cholesterol[7].

The synthesis of the steroidal portion was accomplished in two ways both starting from pregnenolone acetate (I). Refortmasky reaction between I and ethyl bromoacetate followed by acetylation of the condensation product led to 3β-acetoxy-20ξ-hydroxy-23-norchol-5-enic acid ethyl ester (II). A similar reaction but using methyl bromoacetate instead of the ethyl ester had been reported in the literature[8]. Compound II was dehydrated by reaction with phosphorous oxychloride in pyridine (9) giving 3β-acetoxy-23-norchola-5,20(22)-dienoic acid ethyl ester (III). In our case treatment of compound II with boiling acetic anhydride[8] did not produce the unsaturated compound III as the sole reaction product. Dehydration was attempted by refluxing a solution of compound II in acetic anhydride but instead of the α, β-unsaturated carboxylic ester of compound III a mixture was obtained. N.m.r. and U.V. analyses suggested that the

mixture was composed of the dehydrated compound III and of 3β, 20ξ-diacetoxy-23-norchol-5-enoic acid ethyl ester (X). If this assumption is correct, the not very common acetylation of a tertiary hydroxyl group took place.

Compound III was reduced with lithium aluminum hydride in anhydrous ethyl ether yielding 24-norchola-5,20(22)-dien-3β,23-diol (IV). The allylic alcohol IV gave with manganese dioxide (10) the expected 3β-hydroxy-24-norchola-5,20(22)-dien-23-al (VIII) which on acetylation gave the 3β-acetoxy derivative IX (title product).

In the second approach, a Grignard reaction between pregnenolone acetate (I) and allylmagnesiumbromide (11) produced chola-5,23-diene-3β,20ξ-diol (V) which on acetylation gave, the 3β-acetoxy derivative VI. A solution of compound VI in dioxane-water was treated with osmium tetroxide-sodium periodate giving 3β-acetoxy-20ξ-hydroxy-24-norchol-5-en-23-al (VII). It is noteworthy that the C_5–C_6 double bond of steroids is not attacked by osmium tetroxide when the hydroxyl group at C-3 is esterified[12]. The same compound VII was also obtained by a similar treatment using a heterogeneous solvent mixture (benzene-water) instead of the homogeneous one (dioxane-water). Compound VII was dehydrated on acid treatment to 3β-acetoxy-24-norchola-5,20(22)-dien-23-al (IX) in very poor yield. This last step, although theoretically easy to accomplish, presented many difficulties. The main reaction product was identified as pregnenolone acetate (I). The yield of compound IX could not be increased after trying several dehydration procedures.

Our initial explanation for the formation of pregnenolone was that in the reaction of compound VI with osmium tetroxide-sodium periodate some of the formed β-hydroxy-aldehyde VII suffered dehydration *in situ* giving the α,β-unsaturated aldehyde VIII which subsequently reacted with the reagent (OsO_4–$NaIO_4$) producing pregnenolone. Pregnenolone would be therefore present before the dehydration step when the last reaction was conducted on a not very pure compound VII. To check this hypothesis compound VII was isolated, thoroughly purified, and submitted to the dehydration step. The main product was again pregnenolone whilst the expected unsaturated aldehyde VIII was only formed in small amounts. At this point we can just assume that pregnenolone could have been produced from compound VII by a retro-aldol reaction.

For the preparation of the labelled isobutyl derivative XV, [1-^{14}C]-isobutyric acid (XI), prepared from [1-^{14}C]-sodium isobutyrate, was reduced to [1-^{14}C]-isobutyl alcohol (XII) in 80% yield by reaction with lithium aluminum hydride [13]. The purified alcohol XII on reaction with phosphorous tribromide[14] was converted into [1-^{14}C]-isobutyl bromide (XIII) which reacted with triphenylphosphine in boiling xylene giving [1-^{14}C]-isobutyl triphenylphosphonium bromide (XIV).

The bromide XIV was converted, by treatment with butyllithium[15], into [1-^{14}C]-isobutyltriphenylphosphorane (XV) which was condensed *in situ* with the intermediate IX giving 3β-acetoxycholesta-5,20(22),23-triene (XVI) radioactive. Catalytic reduction of compound XVI gave [24-^{14}C]-cholesterol acetate compound (XVII) which on saponification with sodium hydroxide gave [24-^{14}C]-cholesterol (XVIII). The labelled compound XVIII was identical (I.R., t.l.c.) with an authentic standard.

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