191. Synthesis of 24-Methylidene[24-^{14}C]- and 24-Methylidene[7-^{3}H]cholesterol

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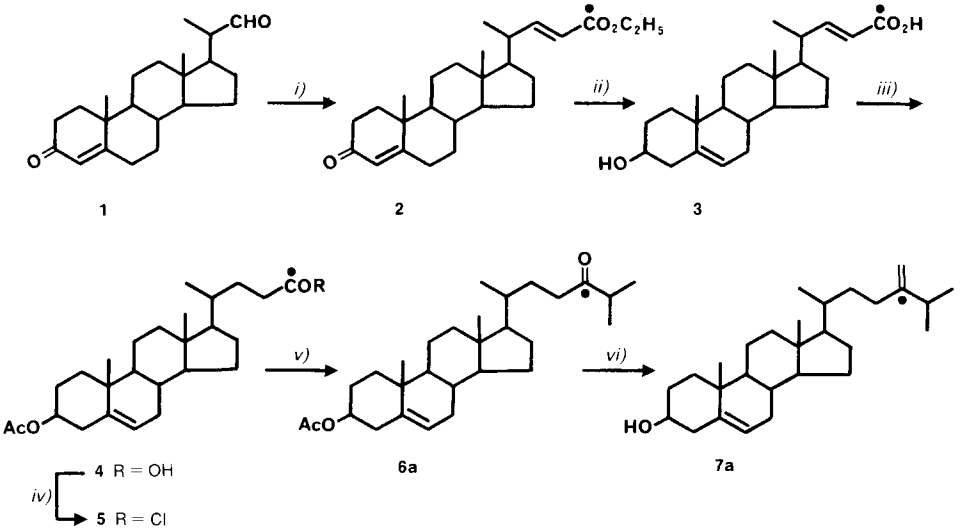
The syntheses of 24-methylidene[24-^{14}C]cholesterol (**7a**) and of 24-methylidene[7-^{3}H]cholesterol (**7b**) from commercially available (20*S*)-3-oxopregn-4-ene-20-carbaldehyde (**1**) are described. The method also provides simple preparations of 3β-acetoxy[24-^{14}C]chol-5-en-24-oic acid (**4**) and 24-oxocholest-5-en-3β-yl acetate (**6b**).

Introduction. – In connection with our studies on the biosynthesis of withanolides in *Acnistus breviflorus* [1], we required 24-methylidenecholesterol labelled with ^{14}C at C(24) (see **7a**) and with ^{3}H at C(7) (see **7b**) in order to carry out double-labelling experiments.

Previous syntheses of labelled 24-methylidenecholesterol afforded the sterol labelled with ^{14}C or ^{3}H at position 28 by a *Wittig* reaction between 24-oxocholest-5-en-3β-yl acetate (**6b**) and appropriately labelled methylidene(triphenyl)phosphorane [2]. We now describe a synthesis of the ^{14}C-labelled sterol from (20*S*)-3-oxopregn-4-ene-20-carbaldehyde (**1**) *via* 3β-acetoxy[24-^{14}C]chol-5-en-24-oic acid (**4**) and 24-oxo[24-^{14}C]cholest-5-en-3β-yl acetate (**6a**), the latter being converted into 24-methylidene[24-^{14}C]cholesterol (**7a**) by an improved procedure (for previous preparations of labelled **4**, see [3]). The ^{3}H-label was introduced on unlabelled 24-oxocholest-5-en-3β-yl acetate (**6b**) which was then converted to the ^{3}H-labelled 24-methylidenecholesterol **7b**.

Results and Discussion. – Reaction of (20*S*)-3-oxopregn-4-ene-20-carbaldehyde (**1**) with [(ethoxy[^{14}C]carbonyl)methylidene]triphenylphosphorane in dry MeCN afforded the (*E*)-ester **2** in high yield. The latter was converted to the 3-enol acetate by treatment with AcCl/Ac_2O and reduced to the 3β-hydroxy-5-ene steroid with $NaBH_4$ in 70% EtOH. Saponification of the ester group (→**3**), acetylation, and hydrogenation afforded 3β-acetoxy[24-^{14}C]chol-5-en-24-oic acid (**4**) in 81% overall yield (61% radiochemical yield from ethyl bromo[1-^{14}C]acetate; *Scheme 1*). Conversion of cholenoic acid **4** to ketone **6a** was carried out by a modification of the procedure described by *Riegel* and *Kaye* [4]. Reaction of acyl chloride **5** (obtained from **4** and oxalyl chloride) with $(i\text{-Pr})_2Cd$ gave poor yields of the desired ketone probably due to the known instability of secondary-alkyl cadmium reagents. Similar results were obtained upon treatment of the acyl chloride with i-PrMgBr in the presence of $ZnBr_2$ [5]. However, when solid $CdCl_2$ was added to 1 equiv. of i-PrMgBr in Et_2O at 0° and the resulting mixture treated with 1 equiv. of **5** in benzene, 24-oxo[24-^{14}C]cholest-5-en-3β-yl acetate (**6a**) was obtained in 95% yield. This reaction may involve direct reaction of the acyl chloride with an 'ate complex' formed between $CdCl_2$ and the *Grignard* reagent. Alternatively, an isopropylcadmium halide may be produced by the slow decomposition of the initially formed complex which would then

Scheme 1



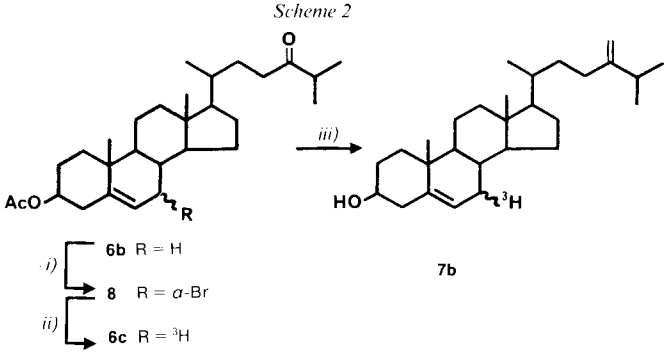
i) $Ph_3P\overset{*}{C}HCO_2Et$, MeCN. *ii*) 1) AcCl/$Ac_2O$; 2) $NaBH_4$, 70% EtOH; 3) NaOH, EtOH, reflux. *iii*) 1) Ac_2O/py; 2) H_2, PtO_2, dioxane/AcOH 30:1. *iv*) $(COCl)_2$. *v*) i-PrMgBr, $CdCl_2$, Et_2O. *vi*) 1) $CH_2Br_2/TiCl_4/Zn$, CH_2Cl_2; 2) NaOH/MeOH. * = ^{14}C.

react with the acyl chloride; in any case, avoiding the formation of the unstable $(i\text{-Pr})_2Cd$ greatly enhanced the yield of the transformation.

Finally, the introduction of the methylidene group at C(24) was attained in high yield by reaction of **6a** with the $Zn/CH_2Br_2/TiCl_4$ reagent [6], this being a significant improvement over the analogous reaction with methylidenetriphenylphosphorane. Saponification of the acetate group at C(3) afforded 24-methylidene[24-^{14}C]cholesterol (**7a**) in 69% overall yield from labelled acid **4**.

The introduction of the ^{3}H-label was carried out by conversion of 24-oxocholest-5-en-3β-yl acetate (**6b**) to the 7-bromo derivative **8** which was reduced to the tritiated ketone **6c** with 'zinc-modified' sodium [^{3}H]cyanoborohydride in Et_2O [7] (*Scheme 2*). The

Scheme 2



i) NBS/CCl_4. *ii*) 1) $Na[B^3H_3CN]$, $ZnCl_2$, Et_2O; 2) CrO_3, H_2SO_4, acetone. *iii*) 1) $CH_2Br_2/TiCl_4/Zn$, CH_2Cl_2; 2) NaOH/MeOH.

partial reduction of the 24-oxo group observed in some cases could be easily circumvented by *Jones* oxidation of the crude reaction product. The [7-^{3}H]ketone **6c** was converted to 24-methylidene[7-^{3}H]cholesterol (**7b**) as above, by treatment with the Zn/CH_2Br_2/$TiCl_4$ reagent and saponification. The method described for the introduction of ^{3}H at C(7) provides an easy alternative to catalytic hydrogenolysis of the allylic bromide **8** with gaseous $^3H_2$ [8]; in our case, the labelling may be carried out without any special precautions, except the usual ones when handling labelled materials. ^{3}H-Labelled sodium cyanoborohydride may be conveniently prepared by acid-catalysed exchange of Na[BH_3CN] in [$^3H_2$]O [9].

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Experimental Part

General. The Zn/CH_2Br_2/$TiCl_4$ reagent was prepared according to [6] and kept at –20°. Prep. HPLC: *Micromeritics* liquid chromatograph equipped with a refractive-index detector; *Altex Ultrasphere C-18* 5 µm column (250 × 10 mm), MeCN/AcOEt/MeOH 2:2:1, 3 ml/min. Radioactivity measurements: *Packard TriCarb 3003* liquid scintillation spectrometer. M.p.: uncorrected. ^{1}H-NMR spectra: at 100.1 MHz in $CDCl_3$; *Varian-XL-100-15* spectrometer operating in the FT mode; δ in ppm downfield from internal TMS (= 0 ppm). MS: direct inlet at 70 eV; *Varian-Mat-CH7-A* spectrometer.

[(Ethoxy[^{14}C]carbonyl)methylidene]triphenylphosphorane. To a soln. of PPh_3 (629 mg) in benzene (3.5 ml) was added a soln. of ethyl bromo-[1-^{14}C]acetate (400 mg, 1 mCi/mmol) in benzene (3.5 ml), and the mixture was stirred for 16 h at r.t. The precipitated phosphonium salt was filtered, washed with toluene and pentane, dissolved in H_2O (23 ml), and cooled to 0°. Then 0.1N NaOH was slowly added to pH 9, and the precipitate filtered off, washed with cold H_2O, and dried: title product (720 mg, 0.94 mCi/mmol). M.p. 124–125° ([10]: 124–125°).

(22E)-Ethyl-3-oxo[24-^{14}C]chola-4,22-dien-24-oate (**2**). A soln. of **1** (576 mg) and [(ethoxy[^{14}C]carbonyl)-methylidene]triphenylphosphorane (720 mg) in dry MeCN (38 ml) was stirred for 72 h at r.t. The residue obtained after evaporation of the solvent was purified by flash chromatography (silica gel, hexane/AcOEt 3:2): pure **2** (655 mg, 0.94 mCi/mmol). M.p. 159–160° ([11]: 159–161°). ^{1}H-NMR: 0.76 (*s*, CH_3(18)); 1.10 (*d*, *J* = 7, CH_3(21)); 1.20 (*s*, CH_3(19)); 1.30 (*t*, *J* = 7, CH_3CH_2O); 4.19 (*q*, *J* = 7, CH_3CH_2O); 5.74 (*s*, H–C(4)); 5.75 (*d*, *J* = 16, H–C(23)); 6.84 (*dd*, *J* = 16, 9, H–C(22)). MS: 398 (98, M^+), 356 (66), 271 (76), 229 (69), 149 (68), 147 (84), 145 (75), 133 (78), 128 (100), 124 (89), 109 (68), 93 (68).

(22E)-3β-Hydroxy[24-^{14}C]chola-5,22-dien-24-oic Acid (**3**). Ester **2** (655 mg) was dissolved in Ac_2O (33 ml) and AcCl (40 ml) added. The soln. was heated under reflux under N_2 for 16 h and then evaporated. The resulting 3-enol acetate (651 mg) was dissolved in 95% EtOH (340 ml) and cooled to 5°. This soln. was added to a cold soln. of $NaBH_4$ (1.23 g) in 70% EtOH (31 ml). After 2.5 h at 5°, the mixture was treated with 5% aq. NaOH soln. (31 ml) and heated under reflux for 15 h. The EtOH was evaporated and the resulting aq. soln. washed with Et_2O. The aq. layer was acidified to pH 2 with 2N HCl and extracted exhaustively with Et_2O. The Et_2O extracts were dried (Na_2SO_4) and evaporated: **3** (536 mg). ^{1}H-NMR: 0.73 (*s*, CH_3(18)); 1.02 (*s*, CH_3(19)); 1.11 (*d*, *J* = 7, CH_3(21)); 3.60 (br. *s*, H–C(3)); 5.36 (*m*, H–C(6)); 5.75 (*d*, *J* = 16, H–C(23)); 6.96 (*dd*, *J* = 16, 9, H–C(22)).

3β-Acetoxy[24-^{14}C]chol-5-en-24-oic Acid (**4**). Acid **3** (536 mg) was dissolved in Ac_2O (13.5 ml) and pyridine (13.5 ml) and allowed to stand for 16 h at r.t. The mixture was diluted with H_2O, acidified to pH 3 (aq. HCl soln.), stirred for 1 h at r.t., and extracted with CH_2Cl_2. The org. layer was washed with H_2O, dried (Na_2SO_4), and evaporated. To the residue (579 mg) in dioxane/AcOH 30:1 (77 ml), PtO_2 (57 mg) was added and the mixture hydrogenated (1 atm) for 24 h at r.t. Filtration and evaporation gave **4** (580 mg, 0.93 mCi/mmol). M.p. 181–183° ([4]: 183–185°). ^{1}H-NMR: 0.69 (*s*, CH_3(18)); 0.94 (*d*, *J* = 7, CH_3(21)); 1.02 (*s*, CH_3(19)); 2.03 (*s*, AcO); 4.62 (br. *s*, H–C(3)); 5.40 (*m*, H–C(6)). MS: 356 (100, [M – AcOH]$^+$), 341 (26), 255 (17), 249 (10), 248 (23), 235 (38), 213 (15).

24-Oxo[24-^{14}C]cholest-5-en-3β-yl Acetate (**6a**). To a stirred slurry of **4** (100 mg) in dry benzene (2 ml), oxalyl chloride was added and the mixture stirred for 5 h at r.t. The resulting soln. was evaporated: **5** (104 mg) which was used immediately in the following step. To an Et_2O soln. of i-PrMgBr (from Mg (70 mg), i-PrBr (0.28 ml), and Et_2O (2 ml)) cooled to 0° was added anh. $CdCl_2$ (625 mg). The suspension was stirred for 20 min under N_2. To the

resulting slurry, **5** (104 mg) in anh. benzene (6.5 ml) was added and the mixture warmed to r.t. and stirred for 10 h. The reaction was stopped by addition of 1N HCl/THF 1:1 followed by dilution with 0.2N HCl. Extractive workup with CH_2Cl_2 and CC of the residue on silica gel afforded **6a** (100 mg). M.p. 127–129° ([4]: 127–128°). ^{1}H-NMR: 0.69 (*s*, CH_3(18)); 0.93 (*d*, $J = 7$, CH_3(21)); 1.03 (*s*, CH_3(19)); 1.09 (*d*, $J = 7$, CH_3(26), CH_3(27)); 2.04 (*s*, AcO); 2.58 (*sept.*, $J = 7$, H–C(25)); 4.60 (br. *s*, H–C(3)); 5.38 (*m*, H–C(6)). MS: 442 (4, M^+), 382 (100, $[M - AcOH]^+$), 367 (16), 315 (4), 296 (19), 281 (12), 255 (25), 253 (13), 228 (9).

24-Methylidene[24-^{14}C]cholesterol (**7a**). Ketone **6a** (50 mg) was dissolved in anh. CH_2Cl_2 (5 ml) and $Zn/CH_2Br_2/TiCl_4$ reagent (1.2 ml) added at 0°. After 45 min, a slurry of $NaHCO_3$ and H_2O was added and the mixture stirred until a clear soln. was obtained. The soln. was filtered and dried (Na_2SO_4), the solvent evaporated, and the solid purified by flash chromatography (silica gel): 3-*O*-acetyl-24-methylidene[24-^{14}C]cholesterol (48 mg). The latter in MeOH (7 ml) was stirred with 10% aq. NaOH soln. (0.7 ml) for 45 min at r.t. under N_2. After dilution with H_2O, the mixture was neutralized with 1N HCl and extracted with CH_2Cl_2, the org. phase evaporated, and the residue purified by prep. reversed-phase HPLC: **7a** (33 mg, 1.07 mCi/mmol), identical to an authentic sample (HPLC, ^{1}H-NMR, MS). M.p. 140–142° ([2a]: 143°).

Sodium [^{3}H]Cyanoborohydride. A soln. of $Na[BH_3CN]$ (50 mg) in [$^3H_2$]O (0.25 ml, 8.4 mCi/mmol) containing a trace of methyl orange was adjusted to pH 3 (red-orange colour) by addition of 0.1% HCl soln., and the mixture was stirred for 30 min at r.t., maintaining the pH by periodic additions of 0.025% HCl soln. After neutralization with solid Na_2CO_3, the soln. was evaporated, the residue stirred for 16 h with anh. THF (4 ml), filtered, and the resulting soln. evaporated affording sodium [^{3}H]cyanoborohydride (49 mg, 0.22 mCi/mmol).

7α-Bromo-24-oxocholest-5-en-3β-yl Acetate (**8**). To a soln. of unlabelled **6b** (40 mg; obtained as above (see **6a**) but using unlabelled phosphorane) in freshly distilled CCl_4 (3.5 ml), *N*-bromosuccinimide (NBS; 22 mg) was added and heated under reflux for 25 min. The mixture was allowed to cool to r.t., the precipitated succinimide filtered off, and the filtrate washed with aq. $Na_2S_2O_3$ soln., dried (Na_2SO_4), and evaporated: **8** (47 mg) which was used immediately in the following step. ^{1}H-NMR: 0.72 (*s*, CH_3(18)); 0.93 (*d*, $J = 7$, CH_3(21)); 1.06 (*s*, CH_3(19)); 1.10 (*d*, $J = 7$, CH_3(26), CH_3(27)); 2.04 (*s*, AcO); 2.58 (*sept.*, $J = 7$, H–C(25)); 4.60 (br. *s*, H–C(3)); 4.68 (br. *s*, H_β–C(7)); 5.76 (*d*, $J = 6$, H–C(6)).

24-Oxo[7-^{3}H]cholest-5-en-3β-yl Acetate (**6c**). To a stirred soln. of anh. $ZnCl_2$ (6.5 mg) in dry Et_2O (1 ml) was added sodium [^{3}H]cyanoborohydride (5.9 mg, 0.22 mCi/mmol) under N_2. After 20 min at r.t., a soln. of **8** (47 mg) in dry Et_2O (1 ml) was added and stirring continued for 30 min. The mixture was diluted with 10% aq. $NaHCO_3$ soln. and extracted with Et_2O and the org. layer washed with H_2O and sat. aq. NaCl soln., dried (Na_2SO_4), and evaporated. The residue was dissolved in acetone (2 ml) and *Jones* reagent added at 0° until persistent orange colour. Dilution with H_2O and extraction with Et_2O afforded **6c** (39 mg, 0.21 mCi/mmol), identical (TLC, m.p., ^{1}H-NMR, MS) to **6a** obtained above.

24-Methylidene[7-^{3}H]cholesterol (**7b**). Labelled **6c** (39 mg) in dry CH_2Cl_2 (3.9 ml) was treated with $Zn/CH_2Br_2/TiCl_4$ reagent (0.94 ml) as above, yielding 3-*O*-acetyl-24-methylidene[7-^{3}H]cholesterol (37 mg) which was saponified and purified by HPLC as described above: **7b** (25 mg, 0.22 mCi/mmol), identical (HPLC, m.p., ^{1}H-NMR, MS) to **7a** obtained above.

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