The Synthesis of Tritium-labeled 14α-Methyl-5α-cholest-7-en-3β-ol and Its Enzymatic Demethylation*

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SUMMARY

14α-Methyl-5α-cholest-7-en-3-one has been prepared and labeled with tritium by isotope exchange chromatography. Reduction with lithium aluminum hydride furnished pure, highly labeled 14α-methyl-5α-cholest-7-en-3β-ol. Incubation of this compound with homogenates of rat liver results in its conversion to cholesterol, which has been identified by a variety of chemical and chromatographic techniques. The results show that enzymatic removal of the 14α-methyl group from synthetic compounds is feasible, and offer a new approach to the study of the demethylation mechanism in steroid structures.

Ever since the isolation of 4,4-dimethyl-Δ5,14-5α-cholestadien-3β-ol (14α-demethyllanosterol) from rat liver and intestines by Schneider, Clayton, and Bloch (1) and Gautschi and Bloch (2, 3) it has been assumed that in the conversion of lanosterol to cholesterol by animal tissue the 14α-methyl group is invariably the first methyl group to be removed. The recent isolation of macdougallin (14α-methyl-Δ5-5α-cholestene-3β,6α-diol; Scheme 1, I) (4), from the cactus Peniocereus macdougallii, however, shows that in certain plants sterol biosynthesis may sometimes take an unusual course, with attack on the 4,4-dimethyl groups preceding loss of the 14α-methyl. Macdougallin may be only a side product which is not metabolized further by the cactus, but the possibility exists that it is the precursor of peniocerol (Δ7-5α-cholestadien-3β,6α-diol; Scheme 1, II) (4, 5) with which it is invariably found. This possibility would mean that loss of the 14α-methyl group could occur at a later stage than had been thought.

The analogy between plant and animal sterol biosynthesis suggested that a study of the metabolism of 14α-methylsterols by tissue homogenates would be of considerable interest. For the initial studies, 14α-methyl-Δ7-cholestenol was selected since the conversion of the corresponding Δ7-cholestenol to cholesterol has been well established (6-9). Isolation of the demethylated product as cholesterol by way of the dibromide, and as cholestanol following hydrogenation of the incubation product, indicated that loss of the 14α-methyl group is enzymatically feasible whether or not a 4,4-dimethyl group is present.

EXPERIMENTAL PROCEDURE AND RESULTS

The only method available for the introduction of a 14α-methyl group is that developed by Woodward et al. (10) for use in the first total synthesis of lanosterol (Scheme 2). For the present work this procedure was simplified by the use of the readily available m-chloroperoxybenzoic acid in place of peroxyphthalic acid for the oxidation of Δ7-5α-cholestenol benzoate (Scheme 2, III) to 15-oxo-Δ7,14-5α-cholestenol benzoate (IV). In addition, the yields of the subsequent methylation reaction and of the Wolff-Kishner reduction were considerably improved.

Synthesis of Tritium-labeled 14α-Methyl-Δ7-cholesterol

15-Oxo-Δ7(14),5α-cholesten-3β-ol Benzoate—Δ7,14-5α-Cholestadien-3β-ol benzoate (Scheme 2, III) (10.5 g; m.p. 150-152°C).

Melting points were observed on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were measured in chloroform with a Lippich manual polarimeter and a 1-dm cell. Infrared spectra were run on a Perkin-Elmer model 21 infrared spectrophotometer in chloroform solution. Microanalyses were carried out in the laboratory of Dr. A. Bernhardt, Munich, Germany. All compounds reported were purified until they gave a single spot on thin layer chromatography with Silica Gel HF254 (Brinkmann Instruments). Chromatographic plates were developed in hexane containing various amounts of ethyl acetate, and after drying were sprayed with ceric sulfate solution (2% in 2 N sulfuric acid) and heated in an oven to produce charring. Counting of chromatographic fractions was done in a Packard EX2 scintillation spectrometer. The scintillation fluid consisted of 4% 2,5-diphenyloxazole and 0.4% 1,4-bis-2-5'-(biphenyl-oxazolyl)benzene in toluene.

* This work was supported by the United States Atomic Energy Commission.
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1 The trivial names used are: 14a-methyl-Δ7-cholestenol, 14α-methyl-5α-cholest-7-en-3β-ol; Δ7-cholestenol, 5α-cholest-7-en-3β-ol; cholestanol, 5α-cholestan-3β-ol.
2 The constants given here refer to a crude fraction enriched in...
under reduced pressure, diluted with water, and extracted with large needles (2.66 g; m.p. 146-148°; [α]D +6° (c, 0.93); reported (10), m.p. 156-158°; [α]D +23° (c, 1.05)).

A solution of m-chloroperoxybenzoic acid (F. M. C. Corporation, Carteret, New Jersey) (85%; 8.74 g) in ether (600 ml) was then added, and the mixture was kept at 0° for 6 hours. The crystalline precipitate was filtered off and the filtrate was washed with 2.5 N sodium hydroxide solution and water, then evaporated under reduced pressure. The residual sticky solid was washed free of gum with ether, and the remaining crystals were combined with the precipitate and dissolved in a mixture of 600 ml of methanol and 200 ml of chloroform, to which 40 ml of concentrated hydrochloric acid were added. After 15 min at reflux, the pale yellow solution was concentrated to half its volume under reduced pressure, diluted with water, and extracted with ether. The ethereal layer was washed well with water, dilute hydrochloric acid (2 N), and dried (NagSO4), and evaporated. The residual colorless oil was acetylated by treatment with acetic anhydride and pyridine at 80° for 3 hours, and after isolation in the usual manner 14α-carboxy-15-oxo-Δ7-cholesterol benzoate (Scheme 2, IV) was crystallized from methanol as long needles (1.51 g; m.p. 96-97°; [α]D -150° (c, 1.04)) without the need for any further purification. (Reported (10), m.p. 95-96°; [α]D +4°.)

C20H32O2
Calculated: C 81.39, H 11.38
Found: C 81.34, H 11.36
C 81.37, H 11.18

14α-Methyl-Δ7-cholesterol-3-one—14α-Methyl-Δ7-cholesterol acetate (250 mg) (Scheme 2, VI) was saponified by refluxing with 5% methanolic potassium hydroxide for 1 hour. The free sterol 14α-methyl-Δ7-cholesterol (Scheme 2, VII), was isolated by ether extraction of the cooled and diluted solution, and crystallized from aqueous methanol as fine needles (200 mg; m.p. 128-130°; [α]D 0° (c, 0.63)).

This sterol (100 mg) was dissolved in 25 ml of acetone, and 8 N chromic acid in sulfuric acid (12) was added dropwise with stirring until an orange color persisted. Excess reagent was destroyed by the addition of a little methanol, and the solution was diluted with water and extracted with ether. Evaporation of the washed and dried ether layer gave 14α-methyl-Δ7-5α-cholesterol-3-one (Scheme 2, VII) as a white solid, which crystallized from methanol-chloroform as needles (70 mg; m.p. 147-148°; [α]D +23° (c, 1.05)).

C20H32O
Calculated: C 84.38, H 11.63
Found: C 84.38, H 11.53
C 84.23, H 11.53

Infrared maximum, 1705 cm⁻¹.

(2,4)-4H-14α-Methyl-Δ7-cholesterol⁴—14α-Methyl-Δ7-cholesterol-3-one (10 mg) was chromatographed on alumina (Merek, basic) previously deactivated by the addition of 3% by weight of tritiated water (13) (1 C per ml) in benzene-pentane (1:1, 1:4).

Infrared maximum, 1705 cm⁻¹.

(2,4)-4H-14α-Methyl-Δ7-cholesterol⁴—14α-Methyl-Δ7-cholesterol-3-one (10 mg) was chromatographed on alumina (Merek, basic) previously deactivated by the addition of 3% by weight of tritiated water (13) (1 C per ml) in benzene-pentane (1:1, 1:4).

The isotope exchange procedure described replaces the enolic hydrogen atoms in positions 2 and 4 of the steroid nucleus with tritium. Since it is not known with certainty which of the available atoms is replaced in the chromatographic process, the designation (2,4)-4H— will be used to indicate compounds labeled by this procedure.
v/v) (Fig. 1). The radioactive ketone thus obtained (5.435 × 10^7 cpm) was diluted with 15 mg of inactive carrier, dissolved in 50 ml of ether, and refluxed for 1½ hours with 250 mg of lithium aluminum hydride. Excess reagent was decomposed by cautious addition of water, and dilute hydrochloric acid (2 N) was added to dissolve the precipitated aluminum salts. The mixture was extracted with ether, and the solution was washed with water, dried (NaSO₄), and evaporated, yielding a white solid. From this crude product, (2,3)-3H-14α-methyl-Δ⁷-cholestenol (Scheme 2, VII) was isolated by way of the digitonide in the usual manner (activity, 3.45 × 10^7 cpm). Thin layer chromatography showed only one spot with the same mobility as the unlabeled alcohol (Scheme 2, VII). As a further check on the purity of the labeled material, a portion was acetylated overnight by treatment with acetic anhydride and pyridine, and the acetate was chromatographed on silica gel in admixture with the corresponding 1-14C-acetate according to the method of Klein and Szczepanik (14). The identity of the two products is shown in Fig. 2, which also illustrates the effect of isotope fractionation, as shown by the slight rise in the isotope ratio across the peak. The effect was computed by the method of Klein, Simborg, and Szczepanik (15) with the results shown in Table I.

**Incubation Procedure**

Incubations were carried out with homogenates of rat liver (16). In a typical incubation, the livers of two male Sprague-Dawley rats were ground in 100 ml of a homogenization medium consisting of potassium dihydrogen phosphate buffer (0.1 M) containing 3.69 g per liter of nicotinamide (0.03 M) and 0.812 g per liter of magnesium chloride (0.004 M) and brought to pH 7.4 by addition of 10% potassium hydroxide solution. A glass homogenizer with a loosely fitting pestle was used, and, after centrifugation at 700 × g, the supernatant liquid was decanted, fortified with 50 mg of ATP and 25 mg of NAD, and brought to 37° in a constant temperature bath. The gas phase was oxygen saturated with water. The radioactive sterol (2 × 10^6 cpm) was added in a few drops of ether, which was removed by evaporation. After 2 hours of stirring at 37°, the mixture was diluted with 50 ml of chloroform-methanol (1:1, v/v) and extracted successively with chloroform and ether. The phospholipids were removed by precipitation with acetone and centrifugation and the supernatant liquid was evaporated to dryness, dissolved in acetone-methanol (1:1), and treated overnight with 1% solution of digitonin in 80% aqueous ethanol. Treatment of the digitonides in the usual manner gave the radioactive sterols, which were acetylated by treatment with acetic anhydride and pyridine at room temperature overnight. An aliquot was then chromatographed on silica (14).

The activity of homogenates prepared in this way was checked by carrying out a 2-hour trial incubation of (2,4)-3H-14α-methyl-Δ⁷-cholestenol. The sterol (2.56 × 10^6 cpm) was added to the homogenate, and from the resulting crude lipid (1.62 × 10^6 cpm) the digitonin-precipitable sterol acetates were obtained, with an activity of 1.48 × 10^6 cpm. An aliquot of this (101,000 cpm) in practice, recovery of activity added to the homogenate was not complete, but ranged from 60 to 80%.

**Table I**

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean ± S. E. M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobility of ¹⁴C-acetate</td>
<td>106.06 ± 0.091</td>
</tr>
<tr>
<td>Mobility of ³H-labeled compound</td>
<td>106.35 ± 0.082</td>
</tr>
<tr>
<td>Dispersion (o) of ¹⁴C</td>
<td>8.32 ± 0.005</td>
</tr>
<tr>
<td>Dispersion (o) of ³H</td>
<td>8.24 ± 0.085</td>
</tr>
<tr>
<td>Dispersion (μ) of ¹⁴C</td>
<td>8.32 ± 0.095</td>
</tr>
<tr>
<td>Dispersion (μ) of ³H</td>
<td>8.24 ± 0.085</td>
</tr>
<tr>
<td>Displacement (ΔM/%)</td>
<td>0.231 ± 0.115</td>
</tr>
<tr>
<td>By probit analysis</td>
<td>0.237 ± 0.075</td>
</tr>
<tr>
<td>From isotope ratio, correcting for differences in dispersion</td>
<td>0.273 ± 0.067</td>
</tr>
</tbody>
</table>

* M denotes mean elution volume.
sodium acetate, which was added drop by drop until an orange color persisted. The suspension of dibromide, which began to crystallize rapidly, was cooled in an ice bath, centrifuged free from mother liquor, and washed with acetic acid. This procedure was sufficient to give a pure sample of cholesterol, and the dibromides were isolated as described above and crystallized twice from chloroform-methanol to give fine needles (42.91 mg) with a specific activity of 2354 cpm per mg. This material was suspended in 9 ml of methanol containing 3 drops of acetic acid and 250 mg of zinc dust and refluxed for 15 min. After cooling, the solution was diluted with water and extracted with ether. The washed and dried extract was evaporated to give 27.43 mg of cholesterol with a specific activity of 3496 cpm per mg. One crystallization from methanol gave 22.21 mg of material with essentially the same specific activity (3237 cpm per mg). This was acetylated with acetic anhydride and pyridine overnight at room temperature and chromatographed with cholesterol 1$^-$$^13$C-acetate on silica (14). The resultant chromatogram clearly indicates the identity of the material thus isolated with cholesterol (Fig. 4). The amount of conversion was found to be 7.1%.

**Isolation of Cholesterol from Hydrogenated Incubation Product**

An alternative procedure was also used in dealing with the incubation product, in order to provide independent evidence of the production of cholesterol. An incubation of the 14α-methyl compound (Scheme 2, VII) (2.27 x 10$^6$ cpm) was carried out with a fresh homogenate, and the sterols were isolated as described, acetylated, and chromatographed on silica with a cholesterol 1$^-$$^13$C-acetate standard (Fig. 5).

It was evident from the plot of the $^3$H-$^13$C ratio shown in Fig. 5 that the large peak due to unchanged 14α-methyl-Δ$^7$-cholestenol acetate also contained a small component with an elution volume comparable to that of cholesterol. However, since the peaks were too close to permit resolution, a method had to be found to render this component more visible. Hydrogenation of the incubation product under mild conditions followed by oxidation to remove unchanged starting material seemed to be an attractive possibility, since cholesterol acetate was known to precipitate sterols (33.6 mg). Upon purification this gave cholesterol with a specific activity of 3393 cpm per mg, corresponding to a conversion of 7.8% of the recovered activity or 4.2% of the total activity.

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**Isolation of Cholesterol dibromide from incubation of (2,4)-$^3$H-14α-methyl-Δ$^7$-cholestenol**

<table>
<thead>
<tr>
<th>Product</th>
<th>Control</th>
<th>2-Hour incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amount</td>
<td>Activity</td>
</tr>
<tr>
<td>Crude sterols</td>
<td>mg</td>
<td>cpm/mg</td>
</tr>
<tr>
<td>Sterols after dilution in</td>
<td>4.43</td>
<td>180,586</td>
</tr>
<tr>
<td>active cholesterol</td>
<td></td>
<td>2.95</td>
</tr>
<tr>
<td>Washed dibromides</td>
<td>15.58</td>
<td>50,347</td>
</tr>
<tr>
<td>First recrystallization</td>
<td>11.09</td>
<td>521</td>
</tr>
<tr>
<td></td>
<td>6.65</td>
<td>962</td>
</tr>
<tr>
<td>Second recrystallization</td>
<td>4.48</td>
<td>1,013</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>28.35</td>
<td>280,550</td>
</tr>
</tbody>
</table>

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Since it had been shown that the dibromides could be readily separated from unchanged starting material, the isolation of sufficient pure cholesterol for a double label chromatogram was undertaken. Eight separate incubations were carried out, each prepared with the liver of one rat and 5.0 x 10$^6$ cpm of (2,4)-$^3$H-14α-methyl-Δ$^7$-cholestenol. The sterols from each incubation were pooled (total, 45.11 mg, diluted to 59.21 mg with pure cholesterol), and the dibromides were isolated as described above and crystallized twice from chloroform-methanol to give fine needles (42.91 mg) with a specific activity of 2354 cpm per mg. This material was suspended in 9 ml of methanol containing 3 drops of acetic acid and 250 mg of zinc dust and refluxed for 15 min. After cooling, the solution was diluted with water and extracted with ether. The washed and dried extract was evaporated to give 27.43 mg of cholesterol with a specific activity of 3496 cpm per mg. One crystallization from methanol gave 22.21 mg of material with essentially the same specific activity (3237 cpm per mg). This was acetylated with acetic anhydride and pyridine overnight at room temperature and chromatographed with cholesterol 1$^-$$^13$C-acetate on silica (14). The resultant chromatogram clearly indicates the identity of the material thus isolated with cholesterol (Fig. 4). The amount of conversion was found to be 7.1%.

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have a sufficiently low relative elution volume (0.86) to permit its resolution from either 14α-methyl-Δ7-cholestenol acetate (relative elution volume, 1.11) or cholesterol acetate.

Before this approach could be used, however, it was necessary to show that 14α-methyl-Δ7-cholestenol would not be affected by the hydrogenation conditions. (2,4)-3H-14α-Methyl-Δ7-cholestenol acetate (300,000 cpm) was therefore dissolved in 20 ml of ethyl acetate containing a few drops of acetic acid, and stirred with hydrogen over a platinum catalyst (20 mg) for 24 hours. The product obtained by evaporation of the filtered solvent was mixed with cholestanol 1-14C acetate (40,000 cpm), dissolved in 1 ml of benzene and 2 ml of acetic acid, and treated with a solution of 25 mg of chromium trioxide in 2 ml of acetic acid containing a few drops of water for 21 min at room temperature. The mixture was then diluted with water and extracted with ether. Chromatography of the product revealed only the 14C-labeled component, thus showing that the 14α-methyl-Δ7-cholestenol was unaffected by the hydrogenation and was readily oxidized by the chromium trioxide. If, on the other hand, the incubation merely effected a shift of the double bond of 14α-methyl-Δ7-cholestenol (Scheme 2, VII) without removing the 14α-methyl group, hydrogenation of the intermediate could conceivably give rise to 14α-methylcholestanol, which would survive the oxidation step. (2,3)-3H-14α-Methylcholestanol acetate and cholestanol 1-14C acetate were therefore chromatographed together, and it was found that the difference between their relative elution volumes (0.86 against 0.94) was sufficient to impart a very steep slope to the plot of the 3H:14C ratio, which made it easy to distinguish between the two.

The sterol acetates (80,000 cpm) from an incubation of the alcohol (Scheme 2, VII) together with 40,000 cpm of cholesterol 1-14C acetate were then hydrogenated under the conditions

Later experiments confirmed the difficulty of hydrogenation of a Δ5 double bond in the presence of a 14α-methyl group. Under the more severe conditions of Chanley and Mezzetti (17), i.e. in acetic acid solvent containing perchloric acid and platinum catalyst for a period of 1 week, less than 1.0% reduction to the 14α-methylcholestanol (relative elution volume, 0.91) occurred. The main effect was to cause partial isomerization to the Δ3-compound. The reaction mixture was analyzed by gas chromatography with an Aerograph A600 (Wilkens Instrument and Research Company). The column was SE 30 (9%) on silanized Chromosorb W (80 to 100 mesh) at 265°.
Cholestanol derived from enzymatically produced cholesterol, found to be coincident within the limits of isotope effects (Fig. 7), chromatographed on silica, the tritium and 14C peaks were 3H : 14C ratio across this peak indicated the identity of the tritium-

Description of tritium-labeled cholestanol acetate remaining after chromium trioxide oxidation of the hydrogenated and acetylated sterols from the incubation of (2,4)-3H-14a-methyl-Δ9-cholestenol (Scheme 2, VII) with a rat liver homogenate. Chromatographed with a cholestanol 1-14C-acetate standard. ——, 14C; ⋄, 3H; ⋄, 3H : 14C.

**TABLE III**

<table>
<thead>
<tr>
<th></th>
<th>Synthetic</th>
<th>From incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mobility of 14C-acetate (reference)</strong></td>
<td>92.37 ± 0.04</td>
<td>94.44 ± 0.07</td>
</tr>
<tr>
<td><strong>Mobility of (2,4)-3H-acetate</strong></td>
<td>92.94 ± 0.04</td>
<td>94.61 ± 0.07</td>
</tr>
<tr>
<td><strong>Dispersion (σ) of 14C</strong></td>
<td>4.93 ± 0.04</td>
<td>4.93 ± 0.07</td>
</tr>
<tr>
<td><strong>Dispersion (σ) of 3H</strong></td>
<td>5.02 ± 0.04</td>
<td>4.95 ± 0.07</td>
</tr>
<tr>
<td><strong>Displacement (ΔM, %)</strong></td>
<td>0.295 ± 0.061</td>
<td>0.181 ± 0.106</td>
</tr>
<tr>
<td><strong>By probit analysis</strong></td>
<td>0.295 ± 0.047</td>
<td>0.177 ± 0.081</td>
</tr>
<tr>
<td><strong>From isotope ratio, correcting for differences in dispersion</strong></td>
<td>0.265 ± 0.037</td>
<td>0.178 ± 0.081</td>
</tr>
</tbody>
</table>

* Standard error of the mean.

The resultant chromatogram showed a distinct tritium-containing peak with the same relative elution volume as that of the 14C-labeled material (Fig. 6). The constant 3H : 14C ratio across this peak indicated the identity of the tritium-labeled component as cholestanol acetate.

When the excess starting material was destroyed by chromium trioxide oxidation as described above and the product was chromatographed on silica, the tritium and 14C peaks were found to be coincident within the limits of isotope effects (Fig. 7), indicating that all of the product could be accounted for as cholestanol derived from enzymatically produced cholesterol, and amounting to about 7% of the starting material. The displacement between the means of the two isotope peaks was computed (15) and found to be identical, to within 0.1%, with that determined for cholestanol 1-14C-acetate and (2,4)-3H-cholestanol acetate (Table III), the latter prepared from cholestanone by isotope exchange chromatography and reduction (13).

**DISCUSSION**

A great deal of emphasis has been given to the search for partially demethylated metabolites of lanosterol. This has resulted in the isolation and identification of several 4a-methyl- and 4,4-dimethyl sterols, including 4a-methyl-Δ9-5α-cholestenol (methostenol, lophenol) from rat skin and feces (18); 4a-methyl-Δ9-5α-cholestenol from preputial gland tumor of the mouse (6, 7); and 4,4-dimethyl-Δ9,14-α-cholestadienol from rat liver and intestines (1-3). The presence of 4,4-dimethyl-Δ9,5α-cholestenol and its Δ8 isomer in rat skin has also been reported (19). Prior to the isolation of macdougallin, no sterols had been found with a 4,14-dimethyl or a 14α-methyl system, a fact which gave support to the hypothesis that removal of the 14α-methyl group was invariably an early event.

Parallel work on other aspects of the conversion of lanosterol to cholesterol has shown, however, that there is no set order in which these steps must take place. For instance, saturation of the side chain double bond may occur as the first (8, 9, 20, 21) or the last (22) step. Furthermore, since the rates at which the various postulated intermediates are utilized by tissue homogenates can vary considerably (7, 20), it is quite possible that different routes are followed in different organs.

No Δ8-cholestenol (relative elution volume, 1.40) was found in the incubation product, although if present it would have been readily visible and separable from cholesterol or 14α-methyl-Δ9-cholestenol (Fig. 3). This is not entirely unexpected, since its rate of conversion to cholesterol by the homogenate was faster (25% in 2 hours) than the rate of utilization of the 14α-methyl compound and hence none would be expected to accumulate.

The mechanism by which the demethylation occurs is not clear at present, but is under further study. If it occurs by way of a decarboxylation as is commonly accepted, then Δ14α-cholesterol may be an intermediate, since decarboxylation of β,γ-un-saturated acids is normally accompanied by rearrangement of the double bond to the α,β-position (23).

Since there is no nearby hydroxyl group which can facilitate such a decarboxylation as by undergoing temporary conversion to a carbonyl, the mechanism of demethylation at C-14 is undoubtedly different from that by which the 4,4-dimethyl groups are lost. The demonstration that enzymatic demethylation of synthetic 14α-methylsterols is feasible offers a new approach to the study of this mechanism without the complications ensuing from competing demethylations at C-4.

**REFERENCES**


* The percentage of conversion in the case of the hydrogenation and oxidation experiments, calculated from the isotope ratios before oxidation and after chromatography, gave values of 6.3, 7.6, and 7.5% (recovered activity) or 4.5, 5.4, and 5.3% (total activity). These methods, which were all obtained with aliquots from the same incubation, agree very well with the results from the debromination experiments.
The Synthesis of Tritium-labeled 14α-Methyl-5α-cholest-7-en-3β-ol and Its Enzymatic Demethylation
John C. Knight, Peter D. Klein and Patricia A. Szczepanik