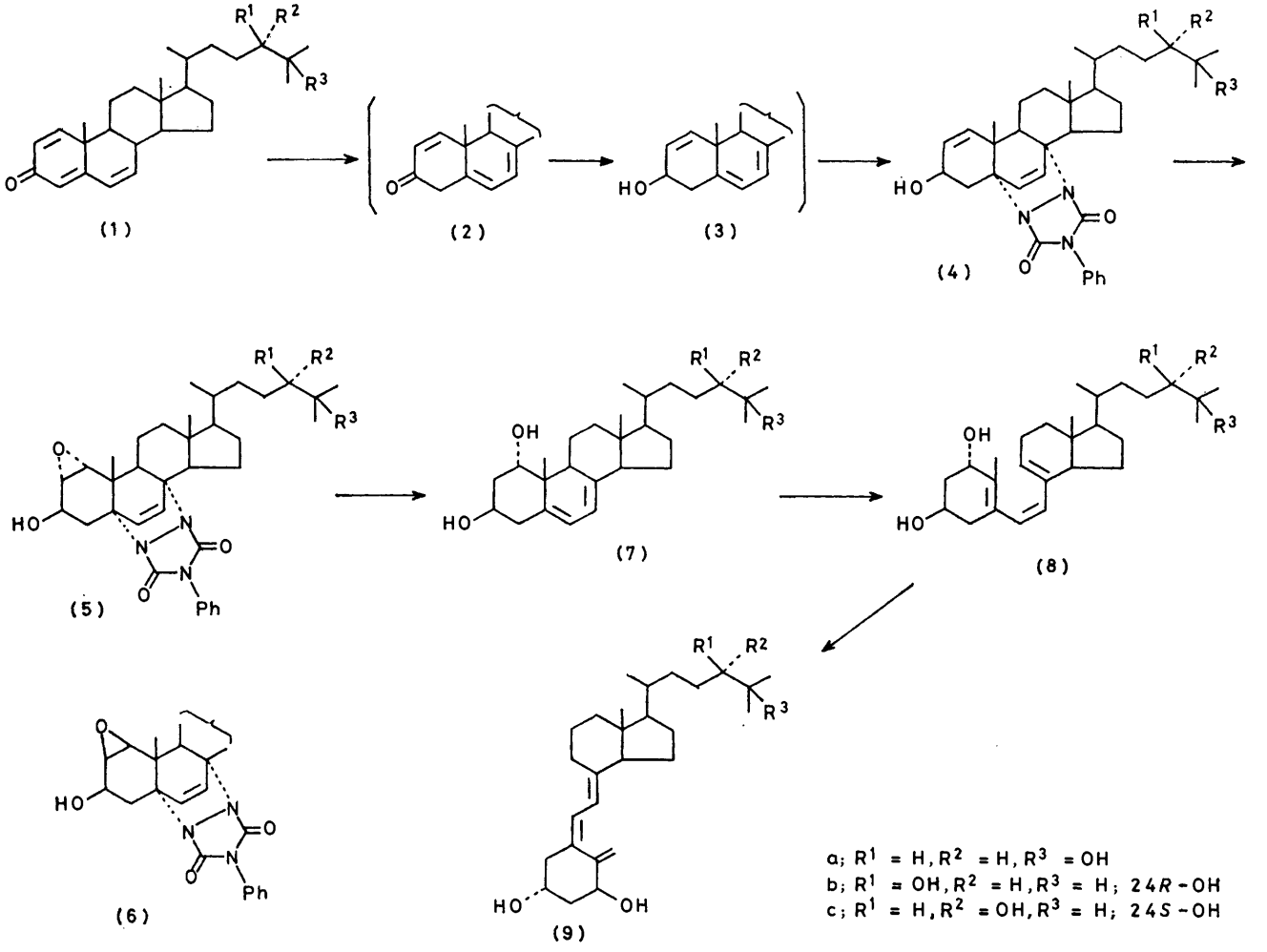
Synthetic Studies of Vitamin D_3 Analogues from Bile Acids. Part 3.[1] Syntheses of 1α,25-, 1α,24*R*-, and 1α,24*S*-Dihydroxycholecalciferols from Lithocholic Acid and their Biological Activities

By **Kiyoshige Ochi,* Isao Matsunaga, Hiroyuki Nagano, Masafumi Fukushima,** and **Minoru Shindo,** Research Laboratories, Chugai Pharmaceutical Co., Ltd., Takada 3–41–8, Toshima-ku, Tokyo 171, Japan
Chikara Kaneko, Faculty of Pharmaceutical Sciences, Kanazawa University, Takara-machi, Kanazawa 920, Japan
Masayuki Ishikawa, Research Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, Chiyoda-ku, Tokyo 101, Japan
Hector F. DeLuca, Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin–Madison, Madison, Wisconsin 53706, U.S.A.

Syntheses of 1α,25-, 1α,24*R*-, and 1α,24*S*-dihydroxycholecalciferols from lithocholic acid *via* the analogous 1,4,6-trien-3-ones by application of Kaneko's procedure are described. A new synthesis of 1α,25-dihydroxycholecalciferol from lithocholic acid *via* the 1,4-dien-3-one is also reported. The biological activities of the three cholecalciferols were tested.

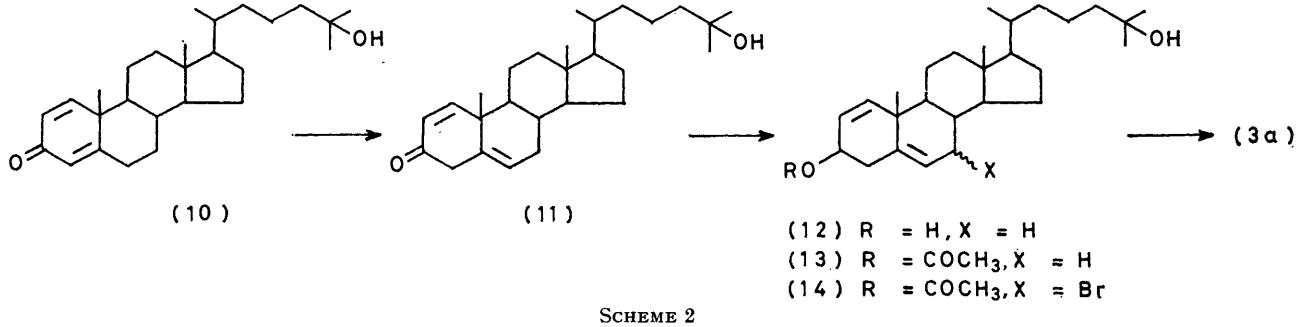
DeLuca,[2a] Norman *et al.*,[2b] and Kodicek [2c] have clearly shown that 1α,25-dihydroxycholecalciferol (9a) is the 'tissue-active' form of vitamin D_3 and has all the hormonal characteristics. Several other metabolites of vitamin D_3 are also known, such as 1α,24*R*,25-trihydroxy- and 24*R*,25-dihydroxy-cholecalciferol. However, these compounds are less active than (9a). The only other hydroxylated analogues with significant biological activity synthesized so far have been those in which hydroxy groups at the 1α-, 24*R*-, or 24*S*-positions, or some combination of these are present in the cholecalciferol frame work.[2]



Scheme 1

In this report we describe the syntheses of 1α,25-dihydroxycholecalciferol (9a) and two of its synthetic analogues, 1α,24*R*- and 1α,24*S*-dihydroxycholecalciferol (9b and c).[3] The starting materials used in this work are the cholesta-1,4,6-trien-3-ones (1a—c) and cholesta-1,4-dien-3-one (10) whose syntheses from lithocholic acid were reported in Part 2.[1]

Since these compounds already carry the desired hydroxylated side chains, the steps necessary for the syntheses of cholecalciferols (9a—c) are the introduction of 1α-hydroxy and 5,7-diene functions into compounds (1) and (10). The method employed in the present work is that used for the synthesis of 1α-hydroxy-7-dehydrocholesterol from cholesta-1,4,6-trien-3-one which requires only three purification steps and thus seems applicable to the synthesis of side chain oxygenated 7-dehydrocholesterol derivatives (7) from the corresponding trien-3-ones (1). As stated in Part 2,[1] since the yield of (1) from dien-3-one (10) is poor (*ca.* 25%), some alternative route from (10) to (7) is needed. Effort along this line has led us to find a new method whose application to the synthesis of (9a) is also described in this paper.



(10) (11)

(12) R = H, X = H
(13) R = $COCH_3$, X = H
(14) R = $COCH_3$, X = Br

SCHEME 2

Syntheses of 1α,25-, 1α,24R-, *and* 1α,24S-*Dihydroxy-7-dehydrocholesterols by Kaneko's Method.*[4]—The steps in this method are summarized in Scheme 1. The 1,4,6-trien-3-ones (1a—c) were deconjugated by treatment with potassium t-butoxide in dimethyl sulphoxide, and following addition to ice–water gave the corresponding 1,5,7-trien-3-ones (2). Ketones (2) were reduced with calcium borohydride to give cholesta-1,5,7-trien-3β-ols (3) which were isolated as the Diels–Alder adducts with 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD). Adducts (4a—c) showed satisfactory spectral data and the yields from (1) were *ca.* 30%, comparable with that reported by Kaneko *et al.*[4] in the case of cholesta-1,4,6-trien-3-one. Epoxidation of adducts (4) with *m*-chloroperbenzoic acid in dichloromethane gave a mixture of the α- and β-epoxides (5) and (6) in *ca.* 1 : 2 ratio. These were separated on silica gel column chromatography to give two crystalline products; the more polar are the α-epoxides (5) and the less polar the β-epoxides (6).

The two epoxides showed, irrespective of the side chain function, some characteristic n.m.r. and i.r. features in each series and thus could easily be classified as α- and β-derivatives. The chemical shift of the 10-methyl group in the α-epoxide always appeared at lower field (δ 1.04—1.06) than in the corresponding β-epoxide (δ 0.95—0.99).* Since this difference was not observed between the methyl signals of two isomeric 1,2-epoxy-3-methylcyclohexane derivatives,[6] it seems reasonable to assume that the downfield shift of the 10-methyl group in the α-epoxide occurs by interaction with the 3β-hydroxy group.[7] Inspection of a molecular model shows that the angular methyl group is very close to the hydroxy group in the α- but not in the β-epoxide. α- and β-Epoxides can also be distinguishable by examining the 700—800 and 1 000—1 100 cm^{-1} regions of the i.r. spectra (KBr pellets). Thus, in the former region, the α-epoxides showed the strongest band at *ca.* 745—750 (with a shoulder at *ca.* 740 cm^{-1}) and a less intense band at *ca.* 765 cm^{-1}, while in the β-epoxides the most intense band appeared at *ca.* 760—770 with two additional bands between 725 and 750 cm^{-1}. In the 1 000—1 100 cm^{-1} region, the α-epoxides showed three bands with comparable intensity while only a single broad band is observed at *ca.* 1 025 cm^{-1} in the β-epoxides.†

The α-epoxides (5) were then reduced with lithium aluminium hydride to give the triols with a 5,7-diene function as shown by the typical u.v. absorption of homoannular dienes. The overall yield of (7) from (1) is *ca.* 3%. The present results thus clearly indicate that Kaneko's method [4] can also be applied to side-chain oxygenated steroids.

Synthesis of 1α,25-*Dihydroxycholecalciferol by a New Method.*—This method is summarized in Scheme 2. It was originally applied successfully for the synthesis of 1α-hydroxy-7-dehydrocholesterol from cholesta-1,4-dien-3-one.

The dien-3-one (10) was deconjugated as in the above case to give the 1,5-dien-3-one (11). Reduction with calcium borohydride afforded cholesta-1,5-diene-3β,25-diol (12). The monoacetate (13) was brominated at C-7 and the 7-bromo derivative (14) was dehydrobrominated in refluxing xylene in the presence of collidine to give cholesta-1,5,7-triene-3β,25-diol (3a) which was isolated as the PTAD adduct (4a), identical with the sample obtained from (1). In this method, the overall yield of

* This difference was observed for 10-methyl groups of the isomeric two epoxides in the related 17-nor series.[5]

† For many steroid epoxides, Günthard *et al.*[8] found bands corresponding to the epoxy group between 1 050 and 1 035 cm^{-1} and tentatively assigned the former bands to ring vibrations and the latter to C–O stretching modes in the epoxy functions.

(4) from (10) is *ca.* 25%, comparable with the overall yield (27%) of (4) from (1). The new method is especially suitable for cases in which a trienone is not readily available [the yield of (1) from (10) is *ca.* 40%]. Thus, the overall yield of (4) from lithocholic acid in this method exceeds that attained from Kaneko's method.

Syntheses and Biological Activities of 1α,25-, 1α,24R-, *and* 1α,24S-*Dihydroxycholecalciferols* (9).—The 5,7-dienes (7) were irradiated in ether and the products were separated by chromatography on Sephadex LH-20 to give the previtamin D (8). Storage of (8) in ether under argon in the dark at room temperature for two weeks followed by chromatography on Sephadex LH-20 afforded pure vitamin D (9) in *ca.* 17% yield from (7).

For intestinal calcium transport and bone mineral mobilization measurements, male weanling rats of the Sprague–Dawley strain were fed for three weeks with a vitamin D deficient, low calcium (0.003%) diet.[9] At the end of the third week, rats received intrajugularly the appropriate dose of synthetic and biosynthetic (9a) [10] in 95% ethanol (0.05 ml). Other rats were orally dosed with (9b), (9c), and 1α-hydroxycholecalciferol (1α-OH-D_3) (2 × 0.25 μg) in O.D.O. (medium chain triglyceride). Eighteen hours later the rats were decapitated, and the blood and the duodena were collected. The everted duodenal sacs were prepared for measuring intestinal calcium transport activity.[11] For the assay of bone mineral mobilization activity, serum calcium concentrations in rats were determined with a Shimadzu atomic absorption spectrometer (AA-610S). The comparison of effects of synthetic and biosynthetic (9a) on intestinal calcium transport and bone mineral mobilization is summarized in Table 1.

TABLE 1

Comparison of effects of synthetic and biosynthetic (9a) on intestinal calcium transport and bone mineral mobilization in vitamin D deficient rats

	Intestinal calcium transport	Bone mineral mobilization
	Serosal : mucosal ^{45}Ca ratio	Serum calcium (mg dl^{-1})
Control	4.98 ± 0.45	4.69 ± 0.15
Synthetic (9a) 0.25 μg per rat	5.81 ± 0.62	6.86 ± 0.14
Synthetic (9a) 2.5 μg per rat	6.23 ± 0.43	7.35 ± 0.14
Biosynthetic (9a) 0.25 μg per rat	5.70 ± 0.17	6.73 ± 0.14

The dose of (9a) in 95% ethanol (0.05 ml) was administered intrajugularly. The rats were killed 18 h later and intestinal calcium transport and bone mineral mobilization activity were determined. Data show the mean values ± standard error.

Biological activities of synthetic (9a) were closely similar to those of (9a) prepared biologically from 25-hydroxycholecalciferol using an *in vitro* chick kidney incubation system.[10] The comparison of the effects of (9b), (9c), and 1α-OH-D_3 on intestinal calcium transport and bone mineral mobilization is shown in Table 2. Although each 0.25 μg of (9b and c) was able slightly to stimulate intestinal calcium transport, those activities were lower than the same dose of 1α-OH-D_3. Compound (9b) appeared to be less active in bone mineral mobilization than 1α-OH-D_3, and (9c) was unable to stimulate activity with the same amount of dose.

TABLE 2

Comparison of effects of (9b), (9c), and 1α-OH-D_3 on intestinal calcium transport and bone mineral mobilization in vitamin D deficient rats

	Intestinal calcium transport	Bone mineral mobilization
	Serosal : mucosal ^{45}Ca ratio	Serum calcium (mg dl^{-1})
Control	3.25 ± 0.16	5.02 ± 0.20
1α-OH-D_3	6.66 ± 0.46	8.11 ± 0.68
(9b)	3.81 ± 0.21	6.34 ± 0.41
(9c)	4.05 ± 0.31	4.96 ± 0.28

Rats were given drug (2 × 0.25 μg) orally in O.D.O. (medium chain triglyceride), and killed 18 h later. Data show the mean values ± standard error.

While more thorough investigations are needed on the biological activities of the two 24-epimers of 1α,24-dihydroxycholecalciferol, our preliminary results fit the conclusions by DeLuca *et al.*[12] that 24*R*-hydroxycholecalciferol analogues have higher biological activity than the 24*S*-congeners. However, they do not fit the recent observation made by Kawashima *et al.*[13] that the 24*R*-isomer if applied intrajugularly, is as potent at 1α-OH-D_3 in promoting intestinal calcium transport and in stimulating calcium mobilization from bone, though differences in administration might be a possible reason for this discrepancy.

EXPERIMENTAL

For general details, see Part 2.[1]

Adduct (4a) *of Cholesta*-1,5,7-*triene*-3β,25-*diol with* 4-*Phenyl*-1,2,4-*triazoline*-3,5-*dione*.—To the solution of the 1,4,6-trienone (1a) (2.56 g) in dry dimethyl sulphoxide (125 ml) and ether (10 ml), finely powdered potassium t-butoxide [freshly prepared from potassium (1.25 g) and t-butyl alcohol] was added and the mixture was stirred for 30 min at 20 °C under argon. The mixture was poured into ice–water saturated with dry ice and extracted with ether. The extract was washed with water, dried, and evaporated to give the unstable 1,5,7-trienone (2a) as an oily residue. A solution of (2a) in ether (50 ml) was added dropwise to a solution of calcium borohydride, prepared from sodium borohydride (2.5 g) in methanol (100 ml) and calcium chloride (4.9 g) in methanol (110 ml) at −10 °C or below, and the mixture was stirred for 1 h at −10 °C. After decomposition of the excess of reagent with aqueous acetic acid the mixture was evaporated and extracted with dichloromethane. The extract was washed with water, dried, and evaporated to give the crude 1,5,7-trienol (3a) as an oily residue. The residue was dissolved in dichloromethane (50 ml) and 4-phenyl-1,2,4-triazoline-3,5-dione (*ca.* 0.8 g) was added in small portions until discharge of the red colour terminated. After stirring for 1 h at room temperature, the solvent was evaporated and the residue was chromatographed on alumina. Elution with 1% methanol in chloroform afforded the *adduct* (4a) (1.066 g), m.p. 159—160° (from methanol), $[\alpha]_D^{25}$ −26.7° (*c* 2, chloroform), $\lambda_{max.}$ 257 nm (log ε 3.65), $\nu_{max.}$ 3 440, 1 750, and 1 688 cm^{-1}, δ($CDCl_3$) 0.80, 1.07 (each 3 H, s), 1.19 (6 H, s), 5.02 (1 H, m, 3-H), 5.76 (2 H, s, 1- and 2-H), 6.27, 6.50 (each

1 H, d, *J* 8 Hz, 6- and 7-H), and 7.41br (5 H, s, Ph) (Found: C, 72.05; H, 8.15; N, 7.45. $C_{35}H_{47}N_3O_4,1/2H_2O$ requires C, 72.15; H, 8.3; N, 7.2%).

Adducts of Cholesta-1,5,7-triene-3β,24R-diol (4b) *and -3β,24S-diol* (4c) *with 4-Phenyl-1,2,4-triazoline-3,5-dione* (4c).—To the solution of the 1,4,6-trienone (1b) (889.5 mg) in dimethyl sulphoxide (30 ml), potassium t-butoxide [from potassium (0.5 g)] was added and the mixture was stirred for 30 min at 20 °C under argon. The same treatment and work-up as for (1a) gave a crude oily residue which was chromatographed on silica gel. Elution with 1% methanol in chloroform afforded *adduct* (4b) (400.35 mg), m.p. 150—156° (from ether), $[\alpha]_D^{25}$ −25.3° (*c* 1, chloroform), $\nu_{max.}$ 3 430, 1 752, and 1 700 cm^{-1}, δ($CDCl_3$) 0.82, 1.07 (each 3 H, s), 0.88 (6 H, d, *J* 7 Hz), 5.00 (1 H, m, 3-H), 5.71 (2 H, s, 1- and 2-H), 6.24, 6.45 (each 1 H, d, *J* 8 Hz, 6- and 7-H), and 7.38br (5 H, s, Ph) (Found: C, 73.15; H, 8.2; N, 7.15. $C_{35}H_{47}N_3O_4$ requires C, 73.25; H, 8.25; N, 7.3%).

The same treatment of the 1,4,6-trienone (1c) (866.5 mg) afforded *adduct* (4c) (469.2 mg), m.p. 170—180° (from ether), $[\alpha]_D^{25}$ −29.0° (*c* 0.3, chloroform) (Found: C, 73.15; H, 8.35; N, 7.2%). Spectral properties (i.r. and n.m.r.) were almost identical with those of adduct (4b).

Epoxidation of Adduct (4).—Adduct (4a) (197.3 mg) was dissolved in chloroform (10 ml) and *m*-chloroperbenzoic acid (237 mg) was added. The mixture was stirred for 40 h at 25 °C. The mixture was washed with aqueous potassium carbonate and then water, dried, and evaporated. The residue was chromatographed on silica gel. Elution with 1% methanol in chloroform afforded the 1β,2β-*epoxide* (6a) (114.6 mg), m.p. 159—160.5° (from methanol), $[\alpha]_D^{25}$ −68.3° (*c* 1, chloroform), $\nu_{max.}$ 3 450, 1 758, and 1 709 cm^{-1}, δ($CDCl_3$) 0.78, 0.99 (each 3 H, s), 1.20 (6 H, s), 3.17, 3.47 (each 1 H, d, *J* 5 Hz, 1- and 2-H), 4.98 (1 H, m, 3-H), 6.19, 6.40 (each 1 H, d, *J* 8 Hz, 6- and 7-H), and 7.40br (5 H, s, Ph) (Found: C, 71.3; H, 8.15; N, 7.0. $C_{35}H_{47}N_3O_5$ requires C, 71.3; H, 8.05; N, 7.1%).

Further elution with the same solvent afforded the 1α,2α-*epoxide* (5a) (68.3 mg), m.p. 162—163.5° (from methanol), $[\alpha]_D^{25}$ −64.9° (*c* 1, chloroform), $\nu_{max.}$ 3 440, 1 750, and 1 685 cm^{-1}, δ($CDCl_3$) 0.81, 1.04 (each 3 H, s), 1.19 (6 H, s), 3.20br (2 H, s, 1- and 2-H), 4.98 (1 H, m, 3-H), 6.19, 6.45 (each 1 H, d, *J* 8 Hz, 6- and 7-H), and 7.42br (5 H, s, Ph) (Found: C, 70.0; H, 7.85; N, 7.05. $C_{35}H_{47}N_3O_5,1/2H_2O$ requires C, 70.2; H, 8.05; N, 7.0%).

The same treatment of adduct (4b) (400.35 mg) gave the corresponding epoxides. 1β,2β-*Epoxide* (6b) (170.6 mg) had m.p. 161.5—163° (from ether–methanol), $[\alpha]_D^{25}$ −43.8° (*c* 1, chloroform), δ($CDCl_3$) 0.78, 0.98 (each 3 H, s), 0.88 (6 H, d, *J* 7 Hz), 3.16, 3.47 (1 H, d, *J* 5 Hz, 1- and 2-H), 4.91 (1 H, m, 3-H), 6.18, 6.38 (each 1 H, d, *J* 8 Hz, 6- and 7-H), and 7.38br (5 H, s, Ph) (Found: C, 71.1; H, 7.9; N, 7.25. $C_{35}H_{47}N_3O_5$ requires C, 71.3; H, 8.05; N, 7.1%). 1α,2α-*Epoxide* (5b) (83.6 mg) had m.p. 206—206.5° (from ether–methanol), $[\alpha]_D^{25}$ −46.8° (*c* 0.5, chloroform), δ($CDCl_3$) 0.84, 1.06 (each 3 H, s), 0.89 (6 H, d, *J* 7 Hz), 3.20br (2 H, s, 1- and 2-H), 4.95 (1 H, m, 3-H), 6.45, 6.18 (each 1 H, d, *J* 8 Hz, 6- and 7-H), and 7.42br (5 H, s, Ph) (Found: C, 70.55; H, 7.9; N, 6.95. $C_{35}H_{47}N_3O_5,1/2H_2O$ requires C, 70.2; H, 8.05; N, 7.0%).

Similarly, 1β,2β-*epoxide* (6c), m.p. 161.5—162.5° (from ether–methanol), $[\alpha]_D^{25}$ −67.4° (*c* 1, chloroform) (Found: C, 71.2; H, 7.9; N, 7.15. $C_{35}H_{47}N_3O_5$ requires C, 71.3; H, 8.05; N, 7.1%), and 1α,2α-*epoxide* (5c), m.p. 217—218° (from ether–methanol), $[\alpha]_D^{25}$ −54.6° (*c* 0.5, chloroform) (Found: C, 70.45; H, 8.0; N, 6.95. $C_{35}H_{47}N_3O_5,-1/2H_2O$ requires C, 70.2; H, 8.05; N, 7.0%), were obtained from adduct (4c). The i.r. and n.m.r. spectra of (5c) and (6c) were almost identical with those of (5b) and (6b) respectively.

25-Hydroxycholesta-1,5-dien-3-one (11).—To the solution of the 1,4-dienone (10) (276 mg) in dry dimethyl sulphoxide (20 ml), potassium t-butoxide [from potassium (0.1 g)] was added and the mixture was stirred for 1 h at 20 °C under argon. The mixture was poured into ice–water containing acetic acid (1 ml) and extracted with ethyl acetate. The extract was washed with water, dried, and evaporated. The residue was chromatographed on silica gel. Elution with chloroform afforded the 1,5-*dienone* (11) (132.1 mg), m.p. 144—148° (from ether–hexane), $[\alpha]_D^{25}$ +65.6° (*c* 0.2, chloroform), δ($CDCl_3$) 0.73, 0.98 (each 3 H, s), 1.21 (6 H, s), 5.46 (1 H, m, 5-H), 5.89 and 7.12 (each 1 H, d, *J* 10 Hz, 1- and 2-H) (Found: C, 81.2; H, 10.5. $C_{27}H_{42}O_2$ requires C, 81.35; H, 10.6%).

Cholesta-1,5-diene-3β,25-diol (12) *and 3β-Acetoxycholesta-1,5-dien-25-ol* (13).—A solution of the 1,5-dienone (11) (103 mg) in methanol (10 ml) was added dropwise to the solution of calcium borohydride, prepared from sodium borohydride (123 mg) and calcium chloride (235 mg) at −10 °C or below, and the whole was stirred for 1 h. The excess of reagent was decomposed with aqueous acetic acid and the mixture was evaporated. The residue was extracted with ether. The extract was washed with water, dried, and evaporated to give a solid. Recrystallization from ethanol afforded the 1,5-*dienol* (12), m.p. 156—157°, δ($CDCl_3$) 0.71, 1.09 (each 3 H, s), 1.21 (6 H, s), 4.20 (1 H, m, 3-H), 5.43 (1 H, m, 5-H), 5.52, and 5.80 (each 1 H, d, *J* 10 Hz, 1- and 2-H).

Acetylation with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature afforded the *acetate* (13) (92.9 mg), m.p. 117—120° (from methanol), $[\alpha]_D^{25}$ +79.3° (*c* 0.2, chloroform), δ($CDCl_3$) 2.03 (3 H, s, $COCH_3$), 3.55 (1 H, m, 3-H), 5.40 (1 H, m, 5-H), 5.47, and 5.87 (each 1 H, d, *J* 10 Hz, 1- and 2-H) (Found: C, 78.6; H, 10.35. $C_{29}H_{46}O_3$ requires C, 78.7; H, 10.45%).

Preparation of Adduct (4a) *from Acetate* (13).—To the solution of the acetate (13) (92.9 mg) in carbon tetrachloride (3 ml), finely powdered *N*-bromosuccinimide (41.1 mg) was added and the mixture was refluxed for 6 min, irradiating with visible light (300 W; Toshiba). To the mixture, collidine (0.1 ml) was added and after removal of the precipitate by filtration the filtrate was evaporated to give the 7-bromo compound (14) as an oil. The residue was dissolved in xylene (2 ml) and the solution was refluxed for 15 min. The mixture was evaporated and the residue was extracted with ether. The extract was washed with diluted hydrochloric acid and then water, dried, and evaporated. Treatment of the residue with 2% potassium hydroxide in methanol (5 ml) gave the crude 1,5,7-trienol (3a), which was treated with 4-phenyl-1,2,4-triazoline-3,5-dione to give the 1,4-adduct. Purification by chromatography on silica gel afforded adduct (4a) (38.7 mg) as crystals, identical (i.r., n.m.r., and mixed m.p.) with the foregoing sample.

Lithium Aluminium Hydride Reduction of the 1α,2α-*Epoxide* (5).—The 1α,2α-epoxide (5a) (141.5 mg) was dissolved in dry tetrahydrofuran (30 ml) and lithium aluminium hydride (141.5 mg) was added in small portions. The mixture was refluxed gently for 1 h. After decomposition of the excess of reagent with saturated sodium sulphate

solution, the organic layer was separated, dried, and evaporated. The residue was purified by chromatography on Sephadex LH-20. Elution with chloroform–hexane (65 : 35 v/v) afforded *cholesta*-5,7-*diene*-1α,3β,25-*triol* (7a) (31.36 mg), m.p. 149—152° (from ether–hexane), $\lambda_{max.}$ 263, 273, and 294 nm, *m/e* 416 (M^+), 398, 380, 362, and 59 (Found: M^+, 416.328 0. $C_{27}H_{44}O_3$ requires M, 416.329 4).

Similarly, *cholesta*-5,7-*diene*-1α,3β,24R-*triol* (7b) (21.3 mg), m.p. 96—99° (from ether) (Found: M^+, 416.327 2), was obtained from the 1α,2α-epoxide (5b) (83.6 mg), and *cholesta*-5,7-*diene*-1α,3β,24S-*triol* (7c) (30.52 mg), m.p. 121—124° (from ether) (Found: M^+, 416.325 2), from the 1α,2α-epoxide (5c) (137.3 mg) respectively, *m/e* 416 (M^+), 398, 380, and 365.

Irradiation of the 5,7-*Diene* (7).—The solution of the 5,7-diene (7a) (31.36 mg) in ether (400 ml) was irradiated with a 400 W Toshiba high pressure immersion mercury lamp with a Vycor filter. During irradiation (1 min), the solution was agitated with a stream of argon. Evaporation of the mixture gave a residue, which was chromatographed on Sephadex LH-20 with chloroform–hexane (65 : 35 v/v) as eluant. The fractions corresponding to the previtamin (8a) were combined. Storage of (8a) in ether (100 ml) completed the thermal 1,7-antarafacial hydrogen migration to give 1α,25-dihydroxycholecalciferol (9a). The residue obtained by evaporation was again chromatographed on Sephadex LH-20 with the same solvent as above and pure (9a) (5.01 mg) was obtained, m.p. 95—99° (from chloroform) (lit.,[14] 106—112°), $\lambda_{max.}$ 264 nm (Found: M^+, 416.321 1. Calc. for $C_{27}H_{44}O_3$: M^+, 416.329 4), *m/e* 416 (M^+), 398, 380, 362, 269, 251, 152, 134, and 59.

The same method was carried out for the 5,7-dienes (7b and c). 1α,24*R*-Dihydroxycholecalciferol (9b) (3.64 mg) (Found: M^+, 416.322 0) and 1α,24*S*-dihydroxycholecalciferol (9c) (5.54 mg) (Found: M^+, 416.326 5) were obtained from the 5,7-diene (7b) (21.3 mg) and (7c) (30.52 mg) as oils. The spectra (u.v. and mass) of the isomers (9b and c) were identical, *m/e* 416 (M^+), 398, 380, 362, 287, 269, 251, 152, 134, and 43.

[7/2017 *Received*, 16*th November*, 1977]

REFERENCES

[1] Part 2, K. Ochi, I. Matsunaga, M. Shindo, and C. Kaneko, preceding paper.

[2] For recent reviews of physiological studies of this and related compounds, see (*a*) H. F. DeLuca, *J. Lab. Clin. Medicin.*, 1976, **87**, 7; (*b*) A. W. Norman, D. A. Procsal, W. A. Okamura, and R. M. Wing, *J. Steroid Biochem.*, 1975, **6**, 461; (*c*) E. Kodicek, *Lancet*, 1974, 325.

[3] (*a*) M. Morisaki, N. Koizumi, N. Ikekawa, T. Takeshita, and S. Ishimoto, *J.C.S. Perkin I*, 1975, 1421; (*b*) N. Koizumi, M. Morisaki, N. Ikekawa, A. Suzuki, and T. Takeshita, *Tetrahedron Letters*, 1975, 2203.

[4] C. Kaneko, A. Sugimoto, Y. Eguchi, S. Yamada, M. Ishikawa, S. Sasaki, and T. Suda, *Tetrahedron*, 1974, **30**, 2701.

[5] H. Sakamoto, A. Sugimoto, and C. Kaneko, *Chem. and Pharm. Bull.* (*Japan*), 1974, **22**, 2903.

[6] M. L. Lunnon and J. Macmillan, *J.C.S. Perkin I*, 1977, 2317.

[7] Y. Kawazoe, Y. Sato, M. Natsume, H. Hasegawa, T. Okamoto, and K. Tsuda, *Chem. and Pharm. Bull.* (*Japan*), 1962, **10**, 338.

[8] H. H. Günthard, H. Heusser, and A. Fürst, *Helv. Chim. Acta*, 1953, **36**, 1900. See also L. J. Bellamy, 'The Infrared Spectra of Complex Molecules', Wiley, New York, 1958, p. 118.

[9] T. Suda, H. F. DeLuca, and Y. Tanaka, *J. Nutrition*, 1970, **100**, 1049.

[10] I. T. Boyle, L. Miravet, R. W. Holick, and H. F. DeLuca, *Endocrinology*, 1972, **90**, 605.

[11] (*a*) D. L. Martin and H. F. DeLuca, *Amer. J. Physiol.*, 1969, **217**, 1351; (*b*) C. Kaneko, S. Yamada, A. Sugimoto, M. Ishikawa, T. Suda, M. Suzuki, and S. Sasaki, *J.C.S. Perkin I*, 1975, 1104.

[12] (*a*) Y. Tanaka, H. F. DeLuca, N. Koizumi, and N. Ikekawa, *Biochemistry*, 1975, **14**, 3293; (*b*) Y. Tanaka, H. F. DeLuca, N. Ikekawa, M. Morisaki, and N. Koizumi, *Arch. Biochem. Biophys.*, 1975, **170**, 620.

[13] H. Kawashima, K. Hoshina, S. Ishizuka, Y. Hashimoto, T. Takeshita, S. Ishimoto, T. Noguchi, N. Ikekawa, M. Morisaki, and H. Orimo, Third Workshop on Vitamin D, Abstracts, pp. 143—145, Asilomar, California, 1977.

[14] D. H. R. Barton, R. H. Hesse, M. M. Pecket, and E. Rizzardo, *J.C.S. Chem. Comm.* 1974, 203.