154. *Attempted Preparation of a Hydroxylated Provitamin-D.*

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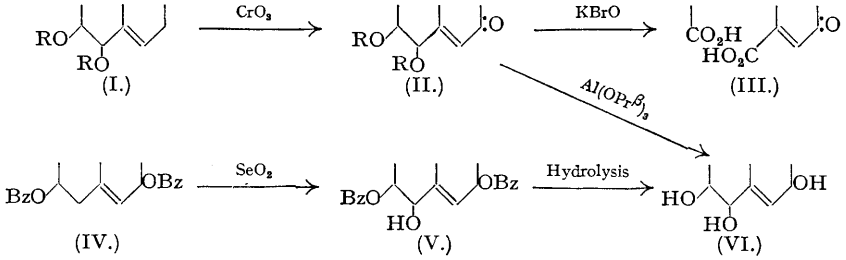
It was intended to prepare $\Delta^{5:7}$-cholestadien-3(β) : 4(β)-diol, a potential hydroxylated provitamin-D, in order to study the effect of nuclear substitution on the biological activity of the irradiated product. With this aim in view 3(β) : 4(β)-dibenzoyloxy-Δ^5-cholestene (I; R = Bz) has been oxidised with chromic acid to 3(β) : 4(β)-*dibenzoyloxy*-Δ^5-*cholesten*-7-*one* (II; R = Bz). The constitution of the unsaturated 7-keto-diol (II; R = H), obtained on hydrolysis, is established by its conversion into 7-keto- Diels's acid (III). Reduction with aluminium *iso*propoxide gave mainly the dextro-rotatory " α "-Δ^5-*cholesten*-3(β) : 4(β) : 7-*triol* * (VI) together with a small quantity of its C_7 epimeride, the lævo-rotatory " β "-*triol*. The dextro-rotatory " α "-Δ^5-cholesten-3(β) : 4(β) : 7-triol (VI) has been shown to belong to the same stereochemical series in respect of the C_7 hydroxyl-grouping as the dextro-rotatory " α "-Δ^5-cholesten-3(β) : 7-diol, since the dibenzoate (IV) of the latter compound afforded (VI) on selenium dioxide oxidation and subsequent hydrolysis. Attempts to prepare $\Delta^{5:7}$-cholestadien-3(β) : 4(β)-diol by pyrolytic cleavage of the C_7 hydroxyl grouping employing the *tribenzoates* of the two isomerides were not successful.

The only steroid nucleus which has so far been shown to undergo transformation into the vitamin-D type of structure under the action of ultra-violet light is that present in $\Delta^{5:7}$-cholestadien-3(β)-ol (7-dehydrocholesterol). Structural changes in the side chain, such as the presence of an additional methyl grouping (*i.e.*, 22-dihydroergosterol), or of a double bond in the latter (*i.e.*, ergosterol), do not affect the potential activation of these compounds by irradiation. But very slight changes in the positions of the nuclear double bonds apparently prevent activation. Thus $\Delta^{4:6}$-cholestadien-3(β)-ol was prepared by Petrow (*J.*, 1940, 66) for study as a possible precursor of vitamin-D_3, but no evidence of biological activity was detected on irradiation. $\Delta^{6:8}$-Cholestadien-3(β)-ol, a by-product formed during the thermal decomposition of " α "-3 : 7-dibenzoyloxy-Δ^5-cholestene * (Windaus, Linsert, and Eckhardt, *Annalen*, 1938, **534**, 22), was shown by Windaus and Zühlsdorff (*ibid.*, 1938, **536**, 204) to undergo a stereochemical transformation into $\Delta^{6:8}$-coprostadien-3(β)-ol under the action of ultra-violet light. A change in the configuration of the hydroxyl-grouping at C_3, on the other hand, does not prevent activation but leads to a ten-fold diminution in the biological activity of the irradiated product (Windaus and Naggatz, *Annalen*, 1939, **542**, 209).

In a continuation of earlier work on the hydroxy-derivatives of cholesterol (Petrow and Starling, *J.*, 1940, 60; Petrow, Rosenheim, and Starling, *J.*, 1943, 135), we have now succeeded in preparing " α "-Δ^5-*cholesten*-3(β) : 4(β) : 7-*triol* (VI) and the corresponding C_7 epimeride, the " β "-*triol*. Our object was to attempt the dehydration of these compounds to $\Delta^{5:7}$-cholestadien-3(β) : 4(β)-diol and hence to determine the effect of introducing a hydroxyl-grouping on the structural changes associated with the irradiation of ring systems of this type.

Oxidation of 3(β) : 4(β)-dibenzoyloxy-Δ^5-cholestene (I; R = Bz) (Rosenheim and Starling, *J.*, 1937, 377) with chromic acid led to the formation of 3(β) : 4(β)-*dibenzoyloxy*-Δ^5-*cholesten*-7-*one* (II; R = Bz) in 70% yield, hydrolysis of which furnished Δ^5-*cholesten*-3(β) : 4(β)-*diol*-7-*one* (II; R = H). The constitution assigned to this compound was based upon the following experimental evidence : (*a*) the presence of a carbonyl group was shown by the formation of an o-*tolylsemicarbazone*, (*b*) the ultra-violet absorption curve showed a maximum



at 2380 A., log ϵ = 3·96, characteristic of $\alpha\beta$-unsaturated ketones, (*c*) on hypobromite oxidation it yielded 7-keto-Diels's acid (7-keto-Δ^5-cholesten-3||4-diacid, Windaus, *Ber.*, 1908, **41**, 611) (III), further characterised by conversion into the monomethyl ester, and (*d*) the *cis*-configuration of the (β)-hydroxyl groups at C_3 and C_4 was confirmed by titration with lead tetra-acetate by Criegee's method. Oxidation of 3(β)-benzoyloxy-4(β)-acetoxy-Δ^5-cholestene gave likewise the 3(β)-*benzoyloxy*-4(β)-*acetoxy*-Δ^5-*cholesten*-7-*one*. The corresponding *diacetate* (II; R = Ac) was gelatinous and its manipulation difficult.

A re-examination of the reduction of 7-ketocholesterol by the Ponnsdorf–Meerwein method, first employed by Windaus, Lettré, and Schenk (*Annalen*, 1935, **520**, 98; U.S.P. 2,098,985) for the preparation of " 7-hydroxycholesterol," was carried out by Wintersteiner and Ruigh (*J. Amer. Chem. Soc.*, 1942, **64**, 2453). These authors showed the latter product to be a mixture of two isomeric Δ^5-cholesten-3(β) : 7-diols, which differed in the configuration of the hydroxyls at C_7. The main constituent of the mixture, the " α "-diol, was slightly dextro-

* In this communication the letters " α " and " β " are used as prefixes in order to distinguish the two C_7 isomeric Δ^5-cholesten-3(β) : 7-diols [as well as the newly described Δ^5-cholesten-3(β) : 4(β) : 7-triols]. It must be stressed that in these compounds the configuration of the C_7 hydroxyl group with reference to the methyl group at C_{10} is not known. The use in the recent literature for such compounds of the designations (α) and (β) after the position numeral implies a definite stereochemical relationship for which there is at present no evidence (cf. *Ann. Reports*, 1938, 283).

rotatory, and its dibenzoate underwent facile pyrolysis in distinction from the dibenzoate of the lævo-rotatory " β "-diol.

We have now found that the reduction of 3(β) : 4(β)-dibenzoyloxy-Δ⁵-cholesten-7-one (II; R = Bz) with aluminium *iso*propoxide also gave a mixture which yielded on fractionation and benzoylation the weakly dextro-rotatory " α "-Δ⁵-*cholesten*-3(β) : 4(β) : 7-*triol* (VI) as the main product. A small quantity of the isomeric lævo-rotatory " β "-*triol*, " β "-Δ⁵-*cholesten*-3(β) : 4(β) : 7-*triol* was obtained with difficulty from the mother liquors. Both compounds were characterised by conversion into the *tribenzoates*.

Oxidation of " α "-3(β) : 7-dibenzoyloxy-Δ⁵-cholestene (IV) (Windaus *et al.*, *Annalen*, 1935, **520**, 98) with selenium dioxide gave " α "-3(β) : 7-*dibenzoyloxy*-Δ⁵-*cholesten*-4(β)-*ol* (V). Acetylation of this furnished " α "-3(β) : 7-*dibenzoyloxy*-4(β)*acetoxy*-Δ⁵-*cholestene*, whilst benzoylation gave the tribenzoate of " α "-Δ⁵-cholesten-3(β) : 4(β) : 7-triol identical with the compound previously described. Hydrolysis of these esters yielded the " α "-triol. These observations thus furnish experimental proof that the unknown configuration of the C_7 hydroxyl grouping in " α "-Δ⁵-cholesten-3(β) : 4(β) : 7-triol is identical with that in " α "-Δ⁵-cholesten-3(β) : 7-diol. Since the dibenzoate of the latter easily undergoes pyrolysis to the provitamin Δ⁵:⁷-cholestadien-3(β)-ol, it is surprising that we were unable to convert the tribenzoates of the " α "- and " β "-triols into the corresponding doubly-unsaturated steroid under a variety of experimental conditions. This failure may be associated with the ease with which acyl migration occurs in a similar system (cf. Petrow, Rosenheim, and Starling, *J.*, 1943, 135). Under the experimental conditions the Δ⁵-unsaturated 3 : 4 : 7-triol may have passed into a Δ⁴-3 : 6 : 7-triol and undergone complex changes on pyrolysis. This possibility is being investigated further. An allylic change of the type Δ⁵-cholesten-3 : 7-diol ⟶ Δ⁶-cholesten-3 : 5-diol, which occurs readily in acetic acid solution (Bergström and Wintersteiner, *J. Biol. Chem.*, 1941, **141**, 600; 1942, **143**, 503), is excluded by the fact that the triols are not affected under these experimental conditions.

Experimental.

M.p.'s are uncorrected. Microanalyses are by Drs. Weiler and Strauss, Oxford. Optical rotations were measured in chloroform solution in a 4 dm. tube.

3(β) : 4(β)-*Dibenzoyloxy*-Δ⁵-*cholesten*-7-*one* (II; R = Bz).—To a suspension of finely powdered 3(β) : 4(β)-dibenzoyloxy-Δ⁵-cholestene (15 g.) in glacial acetic acid (300 ml., stabilised) at 55—60°, was added dropwise with mechanical stirring over a period of 2 hours a solution of chromic acid (12 g.) in water (9 ml.) and glacial acetic acid (45 ml.). After a further 2 hours at this temperature excess of chromic acid was removed by addition of alcohol (8 ml.), and crystallisation induced by dropwise addition of water (65 ml.). After 12 hours at 0° the crystalline product (8·2 g.) was collected, washed with 80% acetic acid, and recrystallised from spirit (180 ml.). 3(β) : 4(β)-*Dibenzoyloxy*-Δ⁵-*cholesten*-7-*one* formed plates, m. p. 145—146°; $[\alpha]_D^{20°}$ −45·5°; $[\alpha]_{5461}^{20°}$ −53·6° (*c*, 1·12); a_{5461}/a_D = 1·18 (Found: C, 78·9; H, 8·1. $C_{41}H_{52}O_5$ requires C, 78·8; H, 8·4%). Yield 7 g.

The o-*tolylsemicarbazone*, needles from alcohol, m. p. 211—212° (Found: N, 5·6. $C_{49}H_{61}O_5N_3$ requires N, 5·4%), was prepared by heating a mixture of the solutions of the dibenzoate (100 mg.) in alcohol (2 ml.), and of *o*-tolylsemicarbazide (50 mg.) in alcohol (3 ml.) containing 3 drops of acetic acid, for 1½ hours under reflux.

3(β)-*Benzoyloxy*-4(β)-*acetoxy*-Δ⁵-*cholesten*-7-*one*.—Chromic acid (40 g.), in water (30 ml.) and glacial acetic acid (150 ml.), was added over 3 hours to the finely powdered 3(β)-benzoyloxy-4(β)-acetoxy-Δ⁵-cholestene (50 g.) (Rosenheim and Starling, *loc. cit.*), in glacial acetic acid (1 l.), at 60°. After a further hour excess of chromic acid was removed with alcohol, and, after 24 hours at room temperature, the product (m. p. 216°; 27 g.) was collected and recrystallised from acetone or dioxan–acetic acid (2 : 1). 3(β)-*Benzoyloxy*-4(β)*acetoxy*-Δ⁵-*cholesten*-7-*one* formed silky needles, m. p. 217—218°; $[\alpha]_D^{19°}$ −59·4°; $[\alpha]_{5461}^{19°}$ −68·6° (*c*, 1·364); a_{5461}/a_D = 1·14 (Found: C, 76·8; H, 8·8. $C_{36}H_{50}O_5$ requires C, 76·8; H, 8·9%).

Δ⁵-*Cholesten*-3(β) : 4(β)-*diol*-7-*one* (II; R = H).—To a suspension of finely powdered dibenzoate (5 g.) in boiling methanol (200 ml.), a solution of potassium hydrogen carbonate (5 g.) in water (20 ml.) was added. After 15 minutes under reflux, the yellow solution was treated with Norit, filtered, and poured into half-saturated salt solution (500 ml.). The cream-coloured product (3·8 g.) was extracted with ether and crystallised twice from acetone or methyl ethyl ketone, from which Δ⁵-*cholesten*-3(β) : 4(β)-*diol*-7-*one* slowly separated in colourless prismatic needles, m. p. 205—206°; $[\alpha]_D^{19°}$ −71·4°; $[\alpha]_{5461}^{19°}$ −86·8° (*c*, 1·088); a_{5461}/a_D = 1·2 (Found: C, 77·8; H, 10·7. $C_{27}H_{44}O_3$ requires C, 77·7; H, 10·6%). The compound separated on cooling its hot solutions in methanol, ethyl acetate, etc., as a transparent gel, which slowly changed into clusters of needles. The ultra-violet absorption spectrum showed a maximum at 2380 A., $\log \epsilon_m$ = 3·96. After 2 hours at room temperature, 0·1004 g. of the keto-diol consumed 4·94 ml. of N/10-lead tetra-acetate solution, corresponding to 1·02 atoms of oxygen, and after 24 hours 0·1026 g. consumed 4·74 ml. (0·96 atom of oxygen). The o-*tolylsemicarbazone* formed silky needles from alcohol, m. p. 235—236° (Found: N, 7·3. $C_{35}H_{53}O_3N_3$ requires N, 7·5%).

7-*Keto*-Δ⁵-*cholesten*-3‖4-*diacid* (7-Keto-Diels's acid) (III).—The finely-powdered keto-diol (500 mg.) was added to potassium hypobromite, prepared by adding bromine (0·3 ml.) to a 25% solution of potassium hydroxide (5 ml.) and ice (4 g.), and the suspension mechanically shaken for 2 hours; a clear solution was then obtained. Excess of bromine was removed by sulphur dioxide and the acidified solution extracted with ether. The ether residue was crystallised from 90% acetic acid, from which the crude acid (300 mg.) separated in prisms, m. p. 215—217°. After purification as the sodium salt, which crystallised from 90% alcohol in needles, 7-keto-Diels's acid was obtained from dilute acetic acid in stout prisms, m. p. 216—218° [Found: C, 73·0; H, 9·0. Calc. for $C_{27}H_{42}O_5$: C, 72·9; H, 9·1%), not depressed in admixture with an authentic specimen. On titration, 0·3074 g. required 13·58 ml. of N/10-sodium hydroxide, corresponding to *M*, 452 (dibasic). Calc. for $C_{27}H_{42}O_5$: *M*, 446]. The characteristic monomethyl ester, shiny long prisms from methanol, m. p. 136—138° (*lit.* 136—137°), was obtained by dissolving the acid (85 mg.) in 5% methyl alcoholic sulphuric acid (2 ml.), from which the ester crystallised on slow evaporation overnight.

" α "-Δ⁵-*Cholesten*-3(β) : 4(β) : 7-*triol* (VI).—A solution of aluminium *iso*propoxide (45 g.) in *iso*propanol (600 ml.) was added to a suspension of powdered 3(β) : 4(β)-dibenzoyloxy-Δ⁵-cholesten-7-one [or 3(β)-benzoyloxy-4(β)-acetoxy-Δ⁵-cholesten-7-one] (20 g.) in *iso*propanol (500 ml.). The mixture was kept gently boiling and distilling for 6 hours as described by Lund (*Ber.*, 1937, **70**, 1520). Ether (500 ml.) was added to the cooled concentrated solution followed by 2N-sodium hydroxide (500 ml.). The washed and dried ethereal extract was concentrated by distillation, and the remaining solvent allowed to evaporate at room temperature. The collected crystalline deposits from three such preparations (30 g.) melted unsharply from 150° to 185°. The optical rotation, $[\alpha]_D$ −28°, of this mixture of 7-epimeric " α "- and

" β "-triols decreased on fractional crystallisation from ethyl acetate to $[\alpha]_D$ −4° for the top fraction, m. p. 185—189°, and rose to $[\alpha]_D$ −47° for the combined recrystallised fractions recovered from the mother liquors. These values remained substantially unchanged on further crystallisation from different solvents. Complete separation was effected by benzoylation of the top fraction (10 g.) in pyridine (150 ml.) with benzoyl chloride (8 ml.) at room temperature. After 24 hours water was added to the solution, previously diluted with alcohol, until crystallisation began. The product, m. p. 188—190°, was collected and recrystallised twice from acetone, from which " α "-3(β) : 4(β) : 7-*tribenzoyloxy*-Δ⁵-*cholestene* separated in needles, m. p. 190—192°; $[\alpha]_D^{18°}$ +99·6°; $[\alpha]_{5461}^{18°}$ +122·0° (*c*, 1·00); a_{5461}/a_D = 1·2 (Found : C, 78·5; H, 7·9. $C_{48}H_{58}O_6$ requires C, 78·9; H, 8·0%). For hydrolysis, a solution of the tribenzoate (500 mg.) in *n*-propanol (10 ml.) was heated under reflux with a solution of potassium *n*-propoxide, prepared from potassium (500 mg.) and *n*-propanol (10 ml.) (cf. Picard, Dissertation, Munich, 1935). After cooling in ice the crystalline precipitate of potassium benzoate was removed (Found : K benzoate, 349·3 mg. Calc. for tribenzoate : 336·2 mg.; for dibenzoate : 224·1 mg.). The triol (350 mg.) was recovered from the filtrate by careful addition of water. After two recrystallisations from 90% acetone, " α "-Δ⁵-*cholesten*-3(β) : 4(β) : 7-*triol* formed large prisms, m. p. 188—190°, $[\alpha]_D^{20°}$ +5·0° (*c*, 1·10) (Found : C, 77·6; H, 10·9. $C_{27}H_{46}O_3$ requires C, 77·4; H, 11·1%).

" β "-3(β) : 4(β) : 7-*Tribenzoyloxy*-Δ⁵-*cholestene*.—The mother liquors obtained on fractionation of the mixture of " α "- and " β "-triols (above) were concentrated and the combined products fractionated from ethyl acetate. After removal of the top fractions, consisting mainly of the " α "-triol, the remaining fractions (10 g.) were recrystallised several times from 90% alcohol and gave a product, m. p. 168—170°, $[\alpha]_D$ −47°. As no further separation was effected either by chromatography or by recrystallisation from different solvents, the product (4 g.) was benzoylated in pyridine solution as above. The resulting benzoates yielded on recrystallisation from acetone a small, less-soluble, fraction of " α "-3(β) : 4(β) : 7-tribenzoate, m. p. 188—190° (see above). The main fraction (3·5 g.), recovered from the mother liquors at *ca.* 0°, was twice recrystallised from alcohol, from which " β "-3(β) : 4(β) : 7-*tribenzoyloxy*-Δ⁵-*cholestene* separated in prisms, m. p. 150—152°; $[\alpha]_D^{20°}$ −45·0°; $[\alpha]_{5461}^{20°}$ −52·0° (*c*, 1·11); a_{5461}/a_D = 1·2 (Found : C, 78·5; H, 7·9. $C_{48}H_{56}O_6$ requires C 78·9; H, 8·0%).

" β "-Δ,⁵-*Cholesten*-3(β) : 4(β) : 7-*triol*, obtained by hydrolysis of the tribenzoate with potassium *n*-propoxide in *n*-propanol (see above), formed prisms from aqueous alcohol, m. p. 169—170°, forming liquid crystals at 130° (Koffler), $[\alpha]_D^{20°}$ −96·9° (*c*, 1·58) (Found : C, 77·8; H, 10·9. $C_{27}H_{46}O_3$ requires C, 77·4; H, 11·1%).

" α "-3(β) : 7-*Dibenzoyloxy*-Δ⁵-*cholesten*-4(β)-*ol* (V).—A solution of selenium dioxide (2 g.) in water (1 ml.) and glacial acetic acid (25 ml.) was added to a solution of " α "-3(β) : 7-dibenzoyloxy-Δ⁵-cholestene (5 g.) (Windaus *et al.*, *loc. cit.*) in dioxan (25 ml.) and the mixture heated on the water-bath. Separation of black selenium commenced within 3 minutes. After 5 hours' heating and the addition of sodium acetate (crystals, 1 g.), the solution was treated with Norit (500 mg.) and filtered. Water (5 ml.) was added dropwise to the filtrate; crystallisation then began, and, after 24 hours the product (3·5 g.) was collected. Selenium was removed from the yellowish combined products of three such preparations (10·5 g.) by pouring the solution in dioxan (30 ml.), under mechanical stirring, into 10% potassium cyanide solution (500 ml.). The resulting product, m. p. 185—190°, was fractionated from acetone–methanol (2 : 1). After removal of the top fraction (250 mg.; m. p. 174—176°), consisting of unchanged starting material, the fractions m. p. 192—195° were combined and repeatedly crystallised from chloroform–methanol (1 : 2), from which " α "-3(β) : 7-*dibenzoyloxy*-Δ⁵-*cholesten*-4(β)-*ol* separated in needles, m. p. 196—198°; $[\alpha]_D^{20°}$ +88·2°; $[\alpha]_{5461}^{20°}$ +103·1° (*c*, 1·04); a_{5461}/a_D = 1·17 (Found : C 78·6; H, 8·7. $C_{41}H_{54}O_5$ requires C, 78·6; H, 8·9%). Benzoylation by the usual technique yielded " α "-3(β) : 4(β) : 7-tribenzoyloxy-Δ⁵-cholestene, m. p. 188—190°, $[\alpha]_D^{15}$ +100·6° (*c*, 0·97), not depressed in admixture with the product described above. Hydrolysis of the tribenzoate with potassium *n*-propoxide in *n*-propanol (see above) gave " α "-Δ⁵-cholesten-3(β) : 4(β) : 7-triol, m. p. 188—190° alone or in admixture with the " α "-triol obtained by reduction of 3(β) : 4(β)-dibenzoyloxy-Δ⁵-cholesten-7-one.

" α "-3(β) : 7-*Dibenzoyloxy*-4(β)-*acetoxy*-Δ⁵-*cholestene*.—A solution of " α "-3(β) : 7-dibenzoyloxy-Δ⁵-cholesten-4(β)-ol (270 mg.) in pyridine (2 ml.) was heated with acetic anhydride (1 ml.) for 40 minutes on the water-bath. The product (290 mg.) was twice crystallised from acetone, from which " α "-3(β) : 7-*dibenzoyloxy*-4(β)-*acetoxy*-Δ⁵-*cholestene* separated in prismatic needles, m. p. 231—233°; $[\alpha]_D^{19°}$ +68·1°; $[\alpha]_{5461}^{19°}$ +84·2° (*c*, 1·09); a_{5461}/a_D = 1·2 (Found : C, 77·2; H, 8·4. $C_{43}H_{56}O_6$ requires C, 77·2; H, 8·4%).

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