997. *Calciferol and its Relatives. Part V.*[1] *Epicalciferol.*[2]

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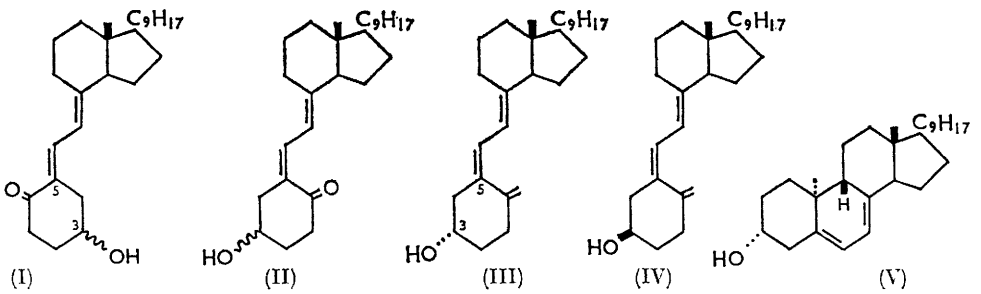
Epicalciferyl *p*-nitrobenzoate, identical with that previously obtained by synthesis,[3] has been prepared by irradiation of epilumisterol. Hydrolysis gave crystalline epicalciferol.

In our synthesis[3] of vitamin D_2 the bicyclic αβ-unsaturated aldehyde $C_{21}H_{34}O$[4] was converted into a mixture of the epimeric (3α- and 3β-) 5-*trans*-dienones (I),[5] then into the *cis*-isomers (II), and finally into a mixture of the epimeric 5-*cis*-trienols (III) and (IV) which were separated as crystalline nitrobenzoates. Calciferol (III) was isolated as the known 3,5-dinitrobenzoate. A second crystalline 3,5-dinitrobenzoate, m. p. 148°, was also isolated, and from it a new *p*-nitrobenzoate, m. p. 122—123°, was prepared; these new esters were regarded as derivatives of the hitherto unknown epicalciferol (IV). In order to provide authentic material for direct comparison with the synthetic samples, we have now prepared epicalciferol by a second unambiguous method.

The obvious way to prepare epicalciferol was by irradiation of epiergosterol, but since epiergosterol is not known we used epilumisterol (V) instead. A complex of lumisterol and epilumisterol is readily obtained[6] by epimerising lumisterol; it can be resolved by the



use of digitonin, or, as we now show, by crystallisation of the 3,5-dinitrobenzoates. Spectrometric observations showed that the course followed by the irradiation of both epimers is essentially the same, and in trial experiments with lumisterol conditions were found

[1] Part IV, Harrison and Lythgoe, *J.*, 1958, 843.
[2] A preliminary account has appeared; Harrison, Hurst, and Lythgoe, *Proc.*, 1959, 269.
[3] Harrison and Lythgoe, *Proc.*, 1957, 261; Part III, *J.*, 1958, 837.
[4] Heilbron, Jones, Samant, and Spring, *J.*, 1936, 905.
[5] Inhoffen, Brückner, and Gründel, *Chem. Ber.*, 1954, **87**, 1.
[6] Barnett, Heilbron, Jones, and Verill, *J.*, 1940, 1390.

which provided calciferol (as the 3,5-dinitrobenzoate) in a yield of 20%; although not optimal this was satisfactory for our purpose. Pure epilumisterol was irradiated under similar conditions, the solution was warmed to convert epiprecalciferol into epicalciferol, the products were converted into 3,5-dinitrobenzoates, and unchanged epilumisterol ester was removed by crystallisation. Chromatography of the residue gave a fraction of crude, non-crystalline epicalciferyl 3,5-dinitrobenzoate which was hydrolysed and re-esterified with *p*-nitrobenzoyl chloride. This gave epicalciferyl *p*-nitrobenzoate, identical with the synthetic material described in Part III.[3] We have also obtained this *p*-nitrobenzoate in yet a third way, by applying the synthetical methods of Part III to the isolated [7] α-epimer of the 5-*trans*-dienone (I). These experiments confirm the identity of the synthetic material and show that it is free from the β-epimer.

Hydrolysis of the *p*-nitrobenzoate gave free epicalciferol, m. p. 85—86°, $[\alpha]_D$ −12° (in benzene), now obtained crystalline for the first time. It showed $\lambda_{max.}$ 265—266 mμ (ε 17,900); the β-epimer has $\lambda_{max.}$ 265—266 mμ (ε 18,300).

The crude epicalciferyl 3,5-dinitrobenzoate mentioned above failed to crystallise even when seeded with our synthetic 3,5-dinitrobenzoate, m. p. 148°; this casts doubt on the latter's identity. When solutions of the non-crystalline ester and solutions of calciferyl 3,5-dinitrobenzoate were mixed they readily gave crystalline material identical with the synthetic ester, m. p. 148°. This material therefore contains both epimeric 3,5-dinitrobenzoates.[8] It is less soluble than either of its components, its physical constants are reproducible, and this, together with the rotation data, indicates that it is a 1 : 1 complex of the epimeric esters. In contrast to the 3,5-dinitrobenzoates, the epimeric *p*-nitrobenzoates do not form a complex. When the complex, m. p. 148°, was hydrolysed and re-esterified with *p*-nitrobenzoyl chloride, crystallisation gave, as the less-soluble component, epicalciferyl *p*-nitrobenzoate, m. p. 122—123°; the more-soluble calciferyl *p*-nitrobenzoate, m. p. 92—93°, was then recovered from the mother-liquors. These solubility relations form the basis for the original isolation [3] of epicalciferyl *p*-nitrobenzoate from the mixture of the epimers (III) and (IV) obtained by synthesis.

Whilst the above work was in progress Inhoffen and his associates [7] described a synthesis of calciferol and epicalciferol. They had earlier [9] shown that 5-*trans*-calciferol, obtained by the chemical isomerisation of calciferol,[10] could be reconverted into the vitamin by a special irradiation technique of which the essential feature was the use of glass as a filter for the ultraviolet light. It having been shown [3,11] that the Wittig [12] reaction can be applied to carbonyl compounds containing unprotected hydroxyl groups, they were able to convert the separated epimers of the *trans*-dienone (I) into the epimers of 5-*trans*-calciferol (I; $=CH_2$ instead of $=O$); this synthesis of the β-epimer completed a synthesis of the vitamin. Irradiation by the Inhoffen technique of the synthetic 3α-5-*trans*-calciferol gave epicalciferol as an oil, $\varepsilon_{max.}$ 14,800 (pure material has $\varepsilon_{max.}$ 17,900), characterised as an amorphous 3,5-dinitrobenzoate, m. p. 100—110°.

The German workers adversely criticised several aspects of our synthesis; [3] they also claimed that epicalciferol was unknown prior to their work.[7] The general validity of our synthesis and the authenticity of our synthetic calciferyl *p*-nitrobenzoate are, we think, sufficiently confirmed by the present paper. We wish, moreover, to correct the statement [7] that our dienone isomerisation was effected by the Inhoffen glass-filtered irradiation technique. The effective chromophore in our experiment being a dienone, not a triene

[7] Inhoffen, Irmscher, Hirschfeld, Stache, and Kreutzer, *Chem. Ber.*, 1958, **91**, 2309. An abbreviated account is available: *idem*, *J.*, 1959, 385.
[8] Its composite nature was first suggested in ref. 7
[9] Inhoffen, Quinkert, and Hess, *Naturwiss.*, 1957, **44**, 11; (with Hirschfeld) *Chem. Ber.*, 1957, **90**, 2544.
[10] Koevoet, Verloop, and Havinga, *Rec. Trav. chim.*, 1955, **74**, 788, 1125; Inhoffen, Quinkert, Hess, and Erdmann, *Chem. Ber.*, 1956, **89**, 2273.
[11] Sondheimer and Mechoulam, *J. Amer. Chem. Soc.*, 1957, **79**, 5029.
[12] Wittig and Schöllkopf, *Chem. Ber.*, 1954, **87**, 1318.

system, different conditions are required, namely, low temperature and the removal of light of wavelength less than 320 mμ. If these are fulfilled, the irradiation can be conducted in either quartz or glass; we used glass for its cheapness. The Inhoffen technique, which removes only light of wavelength shorter than 290 mμ,[9] is not recommended for our isomerisation.

Experimental

Ultraviolet absorption data refer to solutions in alcohol.

Epilumisterol.—The crude epimer mixture obtained by epimerising [6] lumisterol (12·7 g.) was esterified in pyridine (20 c.c.) with 3,5-dinitrobenzoyl chloride (15 g.) in benzene (20 c.c.). The reaction mixture was diluted with ether (300 c.c.) and washed thoroughly with dilute hydrochloric acid and then with water; a yellow solid was precipitated at the interface. The ether phase was kept at −40° for 30 min. to complete the precipitation; the solid (7·1 g.) was collected; it had m. p. 137—139° and was almost pure lumisteryl 3,5-dinitrobenzoate. The ethereal filtrate was evaporated and the residue crystallised from acetone–alcohol giving *epilumisteryl 3,5-dinitrobenzoate* (4·8 g.), m. p. 184—185°, $[\alpha]_D^{18}$ +134° (*c* 1·4, in benzene) (Found: C, 71·35; H, 7·7; N, 4·7. $C_{35}H_{46}N_2O_6$ requires C, 71·2; H, 7·8; N, 4·7%). Hydrolysis with aqueous methanolic potassium hydroxide gave epilumisterol (3·0 g.), m. p. 112—113°, $[\alpha]_D^{18}$ +227° *c* 1·2, in chloroform); lit.,[13] m. p. 113°, $[\alpha]_D$ +224·6° (in chloroform).

Irradiation of Epilumisterol.—A solution of the sterol (2·29 g.) in alcohol (1100 c.c.) and benzene (120 c.c.) was divided into two portions; each was stirred under nitrogen in a silica flask and irradiated (Thermal Syndicate lamp T/M5/401) for 45 min. at 20°, and then heated under reflux for 3½ hr. The combined solutions were evaporated under reduced pressure and the residue was esterified with 3,5-dinitrobenzoyl chloride (2·1 g.) in benzene (10 c.c.) and pyridine (10 c.c.) at 18° for 16 hr. The product, isolated in the usual manner, crystallised from acetone–alcohol giving epilumisteryl 3,5-dinitrobenzoate (1·15 g.); the mother-liquor material was transferred to benzene and chromatographed on neutral alumina (10 g.). Elution with benzene gave crude epicalciferyl 3,5-dinitrobenzoate (630 mg.) which separated from acetone–alcohol as a gel. Its solution in benzene (5 c.c.) was mixed with a solution of sodium hydroxide (600 mg.) in water (0·5 c.c.) and alcohol (20 c.c.), and after 10 min. the mixture was diluted with water and light petroleum (b. p. 40—60°). The washed and dried petroleum phase was evaporated, giving crude epicalciferol as a gum (400 mg.). This was esterified with *p*-nitrobenzoyl chloride in benzene and pyridine, and the crude *p*-nitrobenzoate, isolated in the usual manner, was purified by chromatography on alumina. Crystallisation from acetone–alcohol gave epicalciferyl *p*-nitrobenzoate (200 mg.) as fine needles or prisms, m. p. 122—123°, $[\alpha]_D^{22}$ +7° (*c* 2·1, in benzene) (Found: C, 76·95; H, 8·55. Calc. for $C_{35}H_{47}NO_4$: C, 77·0; H, 8·7%). It gave no depression on admixture with the synthetic specimen described in Part III, which had m. p. 122—123°, $[\alpha]_D^{18}$ +7° (*c* 3·8, in benzene). The *p*-nitrobenzoate had $\lambda_{max.}$ 262—263 mμ (ε 31,500); calciferyl *p*-nitrobenzoate has $\lambda_{max.}$ 262—263 mμ (ε 31,500). In some experiments a second crystalline form, needles, m. p. 120·5—121°, was obtained; it had the same optical rotation as that described above, and the two forms were interconvertible by seeding solutions with the appropriate crystals.

Epicalciferol.—(a) *From the* p-*nitrobenzoate.* Solutions of the *p*-nitrobenzoate (48 mg.; m. p. 122—123°) in benzene (0·5 c.c.) and sodium hydroxide (100 mg.) in 95% alcohol (2·1 c.c.) were mixed, kept for 35 min., and then diluted with water and light petroleum (b. p. 40—60°). The petroleum phase was washed, dried, and evaporated, and the residue crystallised from dry acetone at −25°, giving *epicalciferol* (27 mg.) as needles, m. p. 85—86°, $[\alpha]_D^{22}$ −12° (*c* 2·4, in benzene), $\lambda_{max.}$ 265—266 mμ (ε 17,900) (Found: C, 84·5; H, 11·3. $C_{28}H_{44}O$ requires C, 84·8; H, 11·2%).

(b) *From irradiated epilumisterol.* Epilumisterol (2·63 g.) was irradiated, as described above, giving crude epicalciferol (565 mg.) which crystallised from dry acetone at −25° giving epicalciferol (195 mg.), m. p. 85—86°.

The Complex of Calciferyl and Epicalciferyl 3,5-Dinitrobenzoates.—Crude epicalciferyl 3,5-dinitrobenzoate (490 mg.), obtained by irradiation of epilumisterol (1 g.) as described above, was mixed with calciferyl 3,5-dinitrobenzoate (150 mg.). Crystallisation from acetone–alcohol

[13] Heilbron, Kennedy, Spring, and Swain, *J.*, 1938, 869.

gave the complex (260 mg.) as prisms, m. p. 148·5°, $[\alpha]_D^{17}$ +35° (*c* 1·4, in benzene), identical (mixed m. p.) with the synthetic material described in Part III.

Hydrolysis of a portion (130 mg.) of the complex, and conversion into the *p*-nitrobenzoates followed by crystallisation from acetone–alcohol gave, as the first crop, crude epicalciferyl *p*-nitrobenzoate (33 mg.), m. p. 115—120°; recrystallisation gave pure material (15 mg.), m. p. 122—123°, $[\alpha]_D^{17}$ +8° (*c* 2·1, in benzene). The mother-liquors from the first crystallisation gave crude calciferyl *p*-nitrobenzoate (31 mg.) as prisms, m. p. 75—81°; recrystallisation gave pure material, m. p. and mixed m. p. 92—93°, $[\alpha]_D^{18}$ +51° (*c* 1·1, in benzene).

Conversion of the trans-*Dienone* (I; α-OH) *into Epicalciferyl* p-*Nitrobenzoate.*—Pure α-epimer (800 mg.), m. p. 137—137·5°, $[\alpha]_D^{17}$ +112° (*c* 1·4, in benzene) (lit.,[7] m. p. 132—133·5°, $[\alpha]_D$ +108°), dissolved in carbon tetrachloride (540 c.c.), was irradiated with light of 365 mμ wavelength until approximately 35% had been converted into the *cis*-isomer. The residue obtained by removal of the solvent was dissolved in a little light petroleum (b. p. 40—60°), and some unchanged *trans*-dienone (260 mg.) which separated was removed. The residual material (540 mg.) was brought into reaction with methylenetriphenylphosphorane (from 1·2 g. of methyltriphenylphosphonium bromide) in tetrahydrofuran (35 c.c.) as described in Part III. The crude product was chromatographed on neutral alumina (25 g.; Grade III). The first fractions (170 mg.), eluted with benzene (50 c.c.), crystallised from acetone–alcohol giving 3α-5-*trans*-calciferol (80 mg.), m. p. 129—131°. The second fractions (200 mg.), eluted with benzene (120 c.c.), gave crude epicalciferol (100 mg.), m. p. 81°, on crystallisation from acetone at −30°. Reaction with *p*-nitrobenzoyl chloride and crystallisation from acetone–alcohol gave epicalciferyl *p*-nitrobenzoate (77 mg.), m. p. and mixed m. p. 121°, $[\alpha]_D^{17}$ +6° (*c* 3·9, in benzene).

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