610. *Nitro-steroids. Part I. The Reaction of Cholesteryl Acetate with Nitrosyl Chloride.*

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The reaction between cholesteryl acetate (I) and nitrosyl chloride [1] is shown to give the 5α-chloro-6β-nitro-compound (II), in agreement with the recent findings of Tanabe and Hayashi. With carefully purified nitrosyl chloride in ether the reaction is slow and probably involves addition of nitrosyl chloride followed by oxidation. In the presence of nitrogen dioxide the nitro-chloride (II) is formed rapidly, probably by a free-radical mechanism.

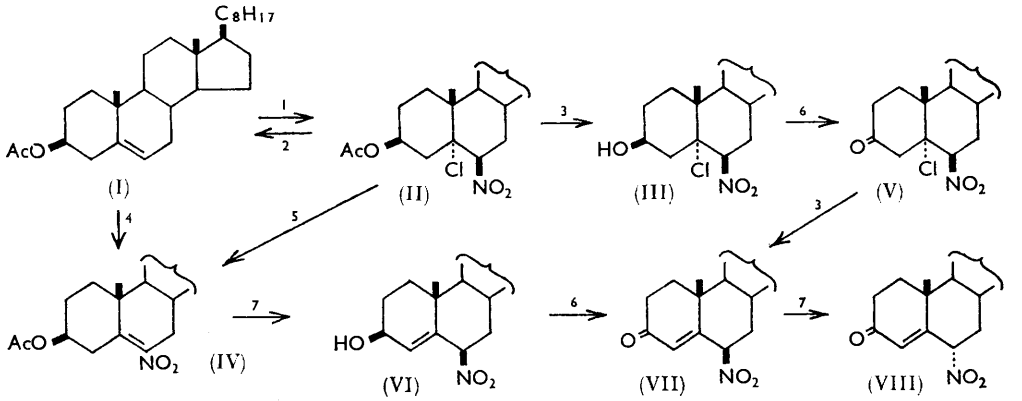
The 6β-configuration of the nitro-group was confirmed by carrying out the sequence (II) ⟶ (III) ⟶ (V) ⟶ 6β-nitrocholest-4-en-3-one (VII), which was also obtained by an alternative route starting from 6-nitro-cholesteryl acetate (IV).

This work, the first part of a general study of nitro-steroids and related compounds, is concerned with the reaction between cholesteryl acetate (I) and nitrosyl chloride. Our first investigation of this topic was carried out in 1938—1939, and at that time the reaction was thought to involve addition of nitrosyl chloride to the 5,6-double bond,[1] although explanation of the product's reactions on this basis presented insuperable difficulties. However, infrared spectroscopy disclosed the presence of a nitro-group, and in the work described below the 5α-chloro-6β-nitro-structure (II) was established. Since the same conclusion was reached recently by Tanabe and Hayashi [2] our results are presented only briefly where the investigations overlap; those aspects of the work not described by the Japanese workers are discussed more fully.

The nitro-compound (II) is obtained in good yield by the slow reaction (several days at 0°) of cholesteryl acetate with nitrosyl chloride in carbon tetrachloride,[2] or in ether in

[1] Wilkinson, Ph.D. Thesis, London, 1941.
[2] Tanabe and Hayashi, *Chem. and Pharm. Bull.* (*Japan*), 1962, **10**, 1177.

the presence of potassium acetate.[1] A *trans*-diaxial (5α,6β) orientation for the chloro- and nitro-groups was suggested by their ready reductive elimination to regenerate cholesteryl acetate. In the majority of ionic additions to the steroidal 5,6-double bond, the diaxial product formed has the more positive addendum in the 5α-configuration;[3] if the nitro-chloro-adduct involves ionic addition of nitrosyl chloride as the first stage a 6β-chloro-5α-nitro-structure would, therefore, be expected. However, there are exceptions

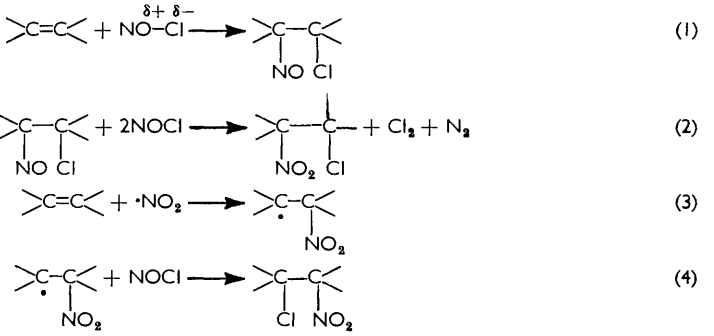


Reagents: 1, NOCl, etc. (see text); 2, Zn–AcOH; 3, HCl aq.–MeOH; 4, HNO_3; 5, NaOAc; 6, CrO_3–Me_2CO; 7, KOH.

to this generalisation,[4] and it is pertinent to note that the addition of nitrosyl halides to terpene systems can produce *cis*- rather than *trans*-products.[5] In the present case the 5α-configuration of the chlorine was established by conversion of the chloro-nitro-acetate (II) into 6-nitrocholest-5-en-3β-yl acetate (IV) under mild basic conditions,[1,2] and by the normal hydroxyl absorption of the 3β-hydroxyl group in the chloro-nitro-alcohol (III).[2] The 6β-configuration of the nitro-group is supported by molecular rotation data;[2] nuclear magnetic resonance examination of product (II) and the reactions described below confirm this by establishing the axial conformation of the nitro-group.

The formation of a nitro-chloride rather than a nitroso-chloride from cholesteryl acetate shows an interesting difference from the normal behaviour of terpenoid compounds. A significant observation is that cholesteryl acetate reacts very slowly with an excess of pure nitrosyl chloride in ether at −5°, the nitro-chloride (II) being isolated in only moderate yield (53%) from the mixture of products formed after 2 months. From Ogloblin's interpretation of the reaction between nitrosyl chloride and various butenes[6] it may be supposed that formation of the nitroso-chloride [stage (1)] is followed by a faster stage (2) in which the excess of nitrosyl chloride acts as an oxidising agent. The slowness of stage (1) with cholesteryl acetate allows other reactions to supervene under conditions in which reagents other than nitrosyl chloride are present. The nitro-chloride was formed more quickly when impure nitrosyl chloride was used, and this acceleration was traced to the presence of nitrogen dioxide, which is known to be one of the more persistent impurities in nitrosyl chloride. Thus, addition of nitrogen dioxide to a solution of cholesteryl acetate and nitrosyl chloride in ether at −15° gave the nitro-chloride (II) (74% yield) in 2 hours. This marked effect shows that the nitro-chloride is formed here by a different route, such as the rapid successive reactions (3) and (4) in which attack is initiated by nitrogen dioxide and nitrosyl chloride then acts as a source of chlorine atoms. (Reactions involving addition to ethylenic linkages of nitrogen dioxide and halogen, and of

[3] *E.g.*, Akhtar and Barton, *J. Amer. Chem. Soc.*, 1962, **84**, 1496.
[4] Bowers, Denot, and Becerra, *J. Amer. Chem. Soc.*, 1960, **82**, 4007.
[5] Meinwald, Meinwald and Baker, *J. Amer. Chem. Soc.*, 1963, **85**, 2513.
[6] Ogloblin, *J. Gen. Chem.* (*U.S.S.R.*), 1957, **27**, 2599; 1958, **28**, 3272.



nitryl halides have been studied extensively in the past decade [7] but there is no general agreement as to whether radical or ionic processes predominate.)

In the orginal work [1] the yield of nitro-chloride was improved by the presence of potassium acetate although the reaction still required a considerable time (8 days at 0°). Closer examination showed that with carefully dried potassium acetate the improvement was slight, the beneficial effect arising from the presence of a small amount of water in the "anhydrous" potassium acetate used. Under these conditions the nitro-chloride is probably formed by both routes. The nitrosyl chloride reacts with water at the surface of the potassium acetate to evolve gases which were shown to contain nitric oxide and nitrogen dioxide; reactions (3) and (4) then occur, while the slower sequence [(1) and (2)] continues to operate after the water is used up. A further function of the potassium acetate is to prevent the medium becoming acidic [1] by interaction of nitrosyl chloride and water. The Japanese authors consider that their reaction (92% of the nitro-chloride from cholesteryl acetate and nitrosyl chloride in carbon tetrachloride at 0° for 5 days) proceeds by addition of nitrosyl chloride and subsequent oxidation. This may well be so if the addition occurs more rapidly in the less polar solvent (carbon tetrachloride), but the alternative path cannot be excluded unless the nitrosyl chloride had been rigorously purified.

During attempts to obtain the nitro-chloride without prior preparation of nitrosyl chloride, aqueous sodium nitrite was added at 0° to a two-phase system of cholesteryl acetate in hexane, and concentrated hydrochloric acid. This afforded the nitro-chloride rapidly (1 hour) but in lower yield (36%) than the previous methods.

Acid hydrolysis of the nitro-chloride (II) to the 3-hydroxy-compound (III) and subsequent oxidation to the 3-ketone (V) duplicated the published work.[2] Base-induced elimination of hydrogen chloride from the 3-ketone (V) is reported [2] to give an amorphous product (thought to be 6α-nitrocholest-4-en-3-one) and cholest-4-ene-3,6-dione. By elimination under acidic conditions we obtained a crystalline 6-nitro-compound (VII), whose structure was established by an alternative preparation from 6-nitrocholesteryl acetate (IV).[8] Hydrolysis of the 3-acetate group of the latter with alkali was accompanied by double-bond migration; the structure (VI) of the product follows from spectroscopic examination and from analogy with similar reactions carried out by Bowers and his co-workers [9] with other 3β-acetoxy-6-nitro-Δ^5-steroids. Oxidation of the 3-hydroxy-Δ^4-compound (VI) afforded 6β-nitrocholest-4-en-3-one (VII), identical with the material obtained from the chloro-nitro-ketone (V). With alkali, under conditions of thermodynamic control, the 6β-nitro-compound (VII) was isomerised to the more stable 6α-epimer (VIII), the conversion involving the expected marked change in optical rotation.

[7] For leading references see Brand and Stevens, *J.*, 1958, 629; Stevens and Emmons, *J. Amer. Chem. Soc.*, 1958, **80**, 338; Backman, Logan, Hill, and Standish, *J. Org. Chem.*, 1960, **25**, 1312.
[8] Anagnostopoulos and Fieser, *J. Amer. Chem. Soc.*, 1954, **76**, 532.
[9] Bowers, Sanchez, and Ringold, *J. Amer. Chem. Soc.*, 1959, **81**, 3702.

Experimental

For general directions, see *J.*, 1958, 2156.

Nitrosyl Chloride.—This was generated from sodium nitrite and concentrated hydrochloric acid, and passed through tubes packed with sodium nitrite, potassium chloride, and calcium chloride.[10] A flask containing the product was attached to a Vigreux column which was connected through a tap to a vacuum manifold. The air inlet to the manifold contained efficient drying towers. The column was surrounded by a jacket, open at the top, which could be maintained at various temperatures (0° and below) by introducing appropriate cooling mixtures. With the jacket at about −30° and a large reservoir attached to the manifold the nitrosyl chloride was allowed to reflux for 40 min. at atmospheric pressure. At 10 min. intervals during this stage the tap between the column and the manifold was closed, the manifold and reservoir were evacuated and dry air was admitted, and the tap was opened and the refluxing continued. (The object of this operation was to remove oxides of nitrogen, especially nitrogen dioxide.) The nitrosyl chloride was then fractionated 3 times, middle cuts being collected and transferred back to the original flask by distillation. During these operations the temperatures of the jacket and of the receivers attached to the manifold were adjusted as appropriate to the direction of movement of nitrosyl chloride. The product, b. p. −7 to −5°, was distilled through the manifold into the flasks in which it was used.

5α-Chloro-6β-nitrocholestan-3β-yl Acetate (II).—(*a*) *Using nitrosyl chloride and potassium acetate.* Potassium acetate (3 g. of commercial anhydrous material) was added to a solution of cholesteryl acetate (10 g.) in dry ether (100 c.c.). The mixture was cooled to −5° and treated with nitrosyl chloride (5 g.) in ether (30 c.c.). The flask was securely sealed and kept at 0° for 8 days. After addition of water the product was isolated with ether and crystallised twice from acetone–methanol, to give 5α-chloro-6β-nitrocholestan-3β-yl acetate (II) as colourless plates (7·5 g.), m. p. 142—143·5°, $[\alpha]_D$ −86° (*c* 1·3) (Found: C, 68·4; H, 9·65; Cl, 7·2; N, 2·9. Calc. for $C_{29}H_{48}ClNO_4$: C, 68·3; H, 9·5; Cl, 6·95; N, 2·75%), $\nu_{max.}$ 1738 (acetate) and 1552 cm.$^{-1}$ (NO_2) (lit.,[2] m. p. 140—141°, $[\alpha]_D$ −82·6°). The nuclear magnetic resonance spectrum (determined in $CDCl_3$ on a Perkin-Elmer 60 Mc. instrument *) contained signals at 4·7 τ (width at half-height 20 c./sec.) (3α, axial H), 5·36 τ (distorted doublet, J = 3·2 c./sec.) (6α, equatorial H), and 8·96 τ (18-Me group).

The above experiment was repeated with potassium acetate which was fused immediately before use. The product was shown by infrared examination to contain about 35% of the nitro-chloride (II); fractional crystallisation afforded cholesteryl acetate (3·1 g.), m. p. 112—113°, and the nitro-chloride (2·6 g.), m. p. 138—140°.

(*b*) *Using nitrosyl chloride.* A solution of cholesteryl acetate (1 g.) and nitrosyl chloride (2 g.) in dry ether (25 c.c.) was kept at −5° for 2 months. After work-up, the product was crystallised twice from acetone–methanol, to give the nitro-chloride (II) (0·57 g.), m. p. 139—141°, identified by infrared examination.

(*c*) *Using nitrogen dioxide and nitrosyl chloride.* Nitrogen dioxide [prepared by thermal decomposition of lead nitrate (20 g.) and collected in a cooled U-tube] was passed slowly, in a stream of dry nitrogen, into a solution of cholesteryl acetate (2 g.) and nitrosyl chloride (2·5 g.) in dry ether (75 c.c.) at −15°. At the end of the addition (which took about 1·5 hr.) the solution was poured into aqueous sodium hydrogen carbonate. Isolation with ether and crystallisation afforded the nitro-chloride (1·7 g.), m. p. 141—142°.

(*d*) *Using sodium nitrite and hydrochloric acid.* Sodium nitrite (3·5 g.) in water (5 c.c.) was added in portions during 15 min. to a mixture of cholesteryl acetate (250 mg.), in hexane (30 c.c.) and concentrated hydrochloric acid (20 c.c.), and cooled to 0°. After 45 min. at 0° the product was isolated with hexane and recrystallised, to give the nitro-chloride (103 mg.), m. p. 138—140°.

Reduction of the Nitro-chloride (II).—A solution of the nitro-chloride (500 mg.) in acetic acid (25 c.c.) was heated at 100° with zinc dust (3 g.) for 2 hr. The product (295 mg. after two crystallisations from ethanol) had m. p. 112—114°, undepressed by admixture with cholesteryl acetate.

* This spectrum will be discussed in a later Paper dealing with the spectroscopic properties of the nitro-steroids.

[10] *Inorg. Synth.*, 1953, **4**, 48.

Dehydrochlorination of the Nitro-chloride (II).—A solution of the nitro-chloride (200 mg.) and sodium acetate (0·4 g.) in ethanol (35 c.c.) was refluxed for 1 hr. After crystallisation from methanol the product (130 mg., m. p. 104·5—105·5°) was identified as 6-nitrocholest-5-en-3β-yl acetate (IV) by mixed m. p. and comparison of spectra with an authentic specimen.[8]

5α-*Chloro*-6β-*nitrocholestan*-3β-*ol* (III).—The above nitro-chloride (400 mg.) in methanol (60 c.c.) was refluxed with concentrated hydrochloric acid (15 c.c.) for 2 hr. The product was crystallised twice from methanol, to give the nitro-alcohol (III) as needles (275 mg.) which melted between 95° and 105°, the value depending on the rate of heating. Examination on silica "chromatoplates" did not reveal the presence of impurity. Prolonged drying at 60°/0·1 mm. caused a 3·2% loss in weight, and the compound then had m. p. 96—100°, $[\alpha]_D$ −83° (*c* 1·0) (Found: C, 69·4; H, 10·1; N, 2·8. Calc. for $C_{27}H_{46}ClNO_3$: C, 69·3; H, 9·9; N, 3·0%), $\nu_{max.}$ (dilute CCl_4 solution, 1-cm. cell) 3623 cm.$^{-1}$ (ε 65) {lit.,[2] m. p. 98—104°, $[\alpha]_D$ −75·9°, $\nu_{max.}$ (dilute CCl_4 solution) 3629 cm.$^{-1}$}.

5α-*Chloro*-6β-*nitrocholestan*-3-*one* (V).—Oxidation of the above alcohol (III) (250 mg.) in acetone with 8N-chromic acid afforded the nitro-ketone (140 mg. after crystallisation from acetone–methanol), m. p. 153—155° (decomp.), $[\alpha]_D$ −86° (*c* 0·9), $\nu_{max.}$ 1726 (CO) and 1550 cm.$^{-1}$ (NO_2) {lit.,[2] m. p. 150—151°, $[\alpha]_D$ −85·5, $\nu_{max.}$ (Nujol) 1724 and 1548 cm.$^{-1}$}.

6β-*Nitrocholest*-4-*en*-3-*one* (VII).—(*a*) *From* 5α-*chloro*-6β-*nitrocholestan*-3-*one* (V). A solution of the nitro-ketone (250 mg.) in methanol (200 c.c.) and concentrated hydrochloric acid (10 c.c.) was kept at 40—50° for 4 hr. After the addition of ice and dilution with water the insoluble material was collected and dried, to give 6β-nitrocholest-4-en-3-one (85 mg.), m. p. 141—143° (from hexane), identified by mixed m. p. and comparison of infrared spectra with authentic material described below.

(*b*) *From* 6-*nitrocholest*-5-*en*-3β-*yl acetate* (IV). 5% Methanolic potassium hydroxide (200 c.c.) was added to a solution of 6-nitrocholesteryl acetate (2·5 g.) [8] in methanol (200 c.c.). After 6 days at 20° the solution was acidified with acetic acid and neutralised with aqueous sodium hydrogen carbonate. The volume was reduced to about 100 c.c. by evaporation at 30°/20 mm., water (200 c.c.) was added, and the insoluble material collected. Crystallisation from methanol afforded 6β-*nitrocholest*-4-*en*-3β-*ol* as needles (1·5 g.), m. p. 154—156°, $[\alpha]_D$ −111° (*c* 1·5) (Found: C, 75·0; H, 10·3. $C_{27}H_{45}NO_3$ requires C, 75·1; H, 10·5%), $\nu_{max.}$ 1550 cm.$^{-1}$ (non-conjugated NO_2), absence of the ultraviolet absorption at 2580 Å (ε 1940) associated with the Δ^5-6-nitro-chromophore.[8]

Oxidation of this 3β-alcohol (500 mg.) in acetone with 8N-chromic acid, and two crystallisations of the product from hexane, gave 6β-*nitrocholest*-4-*en*-3-*one* (365 mg.), m. p. 141—143°, with considerable melting and change in crystalline form from about 125°, $[\alpha]_D$ −102° (*c* 1·0°) (Found: C, 75·6; H, 9·9; N, 3·3. $C_{27}H_{43}NO_3$ requires C, 75·5; H, 10·1; N, 3·3%), $\nu_{max.}$ 1688 (conjugated CO) and 1550 cm.$^{-1}$ (NO_2), $\lambda_{max.}$ 2300 Å (ε 12,300).

6α-*Nitrocholest*-4-*en*-3-*one* (VIII).—A solution of potassium hydroxide (80 mg.) in methanol (5 c.c.) was added at 0° to the above 6β-nitro-ketone (220 mg.) in methanol (10 c.c.). After 5 min., water (200 c.c.) containing acetic acid (0·5 c.c.) was added, and the mixture extracted with ether. The product was crystallised twice from hexane, to give 6α-*nitrocholest*-4-*en*-3-*one* (65 mg.) as plates, m. p. 136—138°, $[\alpha]_D$ +78° (*c* 0·5) (Found: C, 75·7; H, 9·9; N, 2·9%), $\nu_{max.}$ 1688 and 1551 cm.$^{-1}$, $\lambda_{max.}$ 2300 Å (ε 15,100). 6α-Nitrocholest-4-en-3-one is described [2] as an amorphous powder, $\nu_{max.}$ (Nujol) 1692, 1626, and 1558 cm.$^{-1}$, $\lambda_{max.}$ 2320 Å (ε 13,900).

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