Cycloadducts of Ergosterol with Azo-type Dienophiles, and their Chemical Reactivities

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The 1,4-cycloadduct of ergosteryl acetate with pyridazine-3,6-dione was made and used in a synthesis of 3β-acetoxycholesta-5,7,22-triene. The cycloadducts of ergosteryl acetate with 4,5-dihydropyridazine-3,6-dione, phthalazine-1,4-dione, and 4-phenyl-1,2,4-triazoline-3,5-dione were also prepared. Selective hydrogenation of the 22,23-double bond was not achieved in any of these cycloadducts.

ERGOSTEROL was chosen as the starting compound for the attempted synthesis of [22,23-$^3H_2$]cholesta-5,7-dien-3β-ol and its 25-hydroxy-derivative. It was envisaged that the 22,23-double bond of ergosterol would provide a means for the modification of the side-chain.

Windaus [1] has shown that the side-chain of ergosteryl acetate can be selectively hydrogenated when the diene system of ring B is protected by formation of the 1,4-cycloadduct with maleic anhydride. Removal of the maleic anhydride involves a low-yield pyrolysis. DeLuca,[2] however, in a recent synthesis of [22,23-$^3H_2$]-ergosteryl acetate, has reported an improved procedure for this retro-cycloaddition. A recent re-examination [3] of the cycloaddition reaction showed that, in addition to the normal 1,4-cycloadduct, three other adducts were

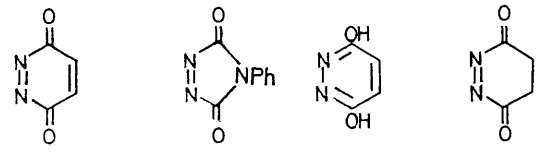
[1] A. Windaus and G. Trautmann, *Z. physiol. Chem.*, 1937, **247**, 185.

[2] H. F. DeLuca, M. Weller, J. Blunt, and P. F. Neville, *Arch. Biochem. Biophys.*, 1968, **124**, 122.

[3] D. N. Jones, P. F. Greenhalgh, and I. Thomas, *Tetrahedron*, 1968, **24**, 297.

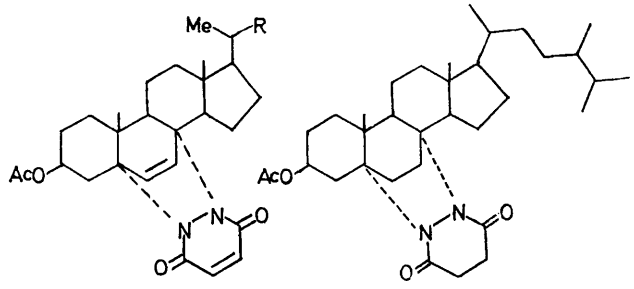
formed. This might account for the low yields observed in the retro-cycloaddition reaction.

We decided to examine the suitability of pyridazine-3,6-dione (I) [4] as dienophile. During this work, however, Barton [5] developed the use of 4-phenyl-1,2,4-triazoline-3,5-dione (II) as dienophile in reaction with ergosteryl acetate. This reagent proved more convenient than pyridazine-3,6-dione and subsequent investigations were carried out on the cycloadducts of the triazolinedione (II).



(I) (II) (III) (VIII)

Treatment of ergosteryl acetate with pyridazine-3,6-diol (III) and 1 equiv. of lead tetra-acetate, according to Clement's procedure [4] gave the cycloadduct (IV) in 50% yield. In the n.m.r. spectrum the olefinic proton signals appeared at δ 6·50 (ABq, ring B), 6·56 (s, pyridazinedione), and 5·26 p.p.m. (side-chain). Chemical proof that (IV) was indeed the 'normal' 1,4-cycloadduct was provided by catalytically reducing it to the hexahydroderivative (V).



(IV) R = CH=CH·CHMe·CHMe$_2$
(X) R = CH=CH·CH$_2$·CHMe$_2$
(XI) R = CH$_2$·CH$_2$·CH$_2$·CHMe$_2$
(IX) R = CHO

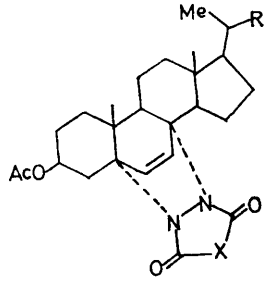
(V)

With 4-phenyl-1,2,4-triazoline-3,5-dione, the cycloadduct (VI) [5] was formed in 80% yield. The olefinic proton signals appeared at δ 6·33 (ABq, ring B) and 5·23 p.p.m. (side-chain).

Efficient retro-addition could be effected by heating the adduct (IV) with potassium hydroxide in ethanol (to give ergosterol in 80—90% yield) or by refluxing it with lithium aluminium hydride [5] in tetrahydrofuran. The adduct (IV) was also unstable to zinc in acetic acid and compound (VII) was formed. In this compound, the double bond of the pyridazinedione group was reduced, as evidenced by the disappearance of the singlet at δ 6·56 and the appearance of a broad four-proton peak at 2·40 p.p.m. Further treatment with the same reagent regenerated ergosteryl acetate, although in low yield. The adduct (VII) was also synthesised from ergosteryl acetate and 4,5-dihydropyridazine-3,6-dione (VIII).

Ozonolysis of the adduct (IV) with 1 equiv. of ozone and subsequent reduction with hexamethylphosphorous triamide [6] gave the chromatographically pure hexanor-aldehyde (IX) in 70% yield. The n.m.r. spectrum of the aldehyde (IX) showed a doublet centred at δ 10·16 p.p.m. (J 3 Hz) due to the aldehydic proton of the 20S-compound. After chromatography on alumina (grade III), the spectrum showed a new doublet, centred at 10·08 p.p.m. (J 5 Hz) due to the 20R-epimer. This is in agreement with the findings of Barton.[5] A Wittig reaction of the hexanor-aldehyde with the phosphorane derived from isopentyltriphenylphosphonium bromide [7] gave the pyridazinedione adduct of 3β-acetoxycholesta-5,7,22-triene (X). It was expected that selective catalytic hydrogenation of this compound would lead to the pyridazinedione adduct of cholesta-5,7-dien-3β-yl acetate (XI) and (by use of tritium) to its tritiated derivatives.

However, attempts at selective reduction of the side-chain double bond of the adduct (X), and also of the adducts (IV), (VI), (VII), and (XII), under conditions which had reduced the side-chain of the ergosterol–maleic anhydride adduct,[1] failed. In fact, the double bond in the protecting group of the adducts (X) and (IV) was reduced more quickly than the ring B double bond, and the ring B double bond was reduced much more quickly than the side-chain in all cases. The same observation was made when the reductions were carried out with chlorotris(triphenylphosphine)rhodium,[8] diborane,[9] or di-imide.[10]



(VI) R = CH=CH·CHMe·CHMe$_2$, X = NPh
(VII) R = CH=CH·CHMe·CHMe$_2$, X = [CH$_2$]$_2$
(XIII) R = CHO, X = NPh
(XIV) R = CH$_2$H, X = NPh
(XII) R = CH=CH·CHMe·CHMe$_2$, X = o-C$_6$H$_4$)

Since selective reduction of the side-chain was not achieved, we attempted to introduce the side-chain by reactions of Grignard reagents or lithium alkyls on the aldehyde (XIII).[5] Reactions of lithium alkyls and dialkyl-lithium cuprates on the mesylate, iodide, and tosylate derived from the alcohol (XIV) were also

[4] R. A. Clement, *J. Org. Chem.*, 1962, **27**, 1115.
[5] D. H. R. Barton, T. Shioiri, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1971, 1968.
[6] A. Furlenmeir, A. Furst, A. Langemann, G. Waldvogel, P. Hocks, U. Kerb, and R. Wiechert, *Helv. Chim. Acta*, 1967, **50**, 2387.
[7] H. H. Inhoffen, K. Irmscher, G. Friedrich, D. Kampe, and O. Berges, *Chem. Ber.*, 1959, **92**, 1772.
[8] C. Djerassi and J. Gutzwiller, *J. Amer. Chem. Soc.*, 1966, **88**, 4537.
[9] H. C. Brown and K. Murray, *J. Amer. Chem. Soc.*, 1959, **81**, 4108.
[10] J. M. Hoffman and R. H. Schlessinger, *Chem. Comm.*, 1971, 1245.

studied. In all cases, the nucleophiles added in a direct or Michael-type manner to the protecting group, giving mixtures from which very little, if any, 5,7-diene could be regenerated.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. Optical rotations were determined for solutions in chloroform. I.r. spectra were taken for solutions in carbon tetrachloride solution and u.v. spectra for solutions in ethanol. N.m.r. spectra were recorded with a Varian T60 or HA 100 spectrometer with carbon tetrachloride or deuteriochloroform as solvent and tetramethylsilane as internal reference. Mass spectra were run on an A.E.I. MS902 spectrometer. G.l.c. retention times were determined by use of 4% SE-30 on Chromosorb G (100—120 mesh) at 296°. Woelm aluminium oxide was used for column chromatography and Merck silica gel $HF_{254+366}$ for preparative layer chromatography (p.l.c.) (20 × 20 cm plates, 0·75 mm thick). Solutions were dried over anhydrous magnesium sulphate. Elemental analyses were carried out by Dr. C. Daessle, Montreal, and Beller Mikroanalytisches Laboratorium, Germany.

The 1,4-*Cycloadduct* (IV) *from Ergosteryl Acetate and Pyridazine*-3,6-*dione*.—Ergosteryl acetate [11] (41·0 g) and pyridazine-3,6-diol [4] (1·12 g) in dry methylene chloride (1·5 l) under dry nitrogen, were treated with a solution of lead tetra-acetate (30·0 g) in dry methylene chloride (250 ml) containing acetic acid (8 ml), dropwise, with stirring and cooling. After 2 h the addition was complete and the mixture was allowed to warm to room temperature. Termination of the reaction was checked by u.v. spectroscopy [disappearance of the diene absorption at $\lambda_{max.}$ (EtOH) 282 nm]. The mixture was set aside overnight, filtered, and mixed with 10% potassium iodide solution (200 ml), and the resulting heavy emulsion was washed with 10% sodium thiosulphate solution (400 ml). The organic layer was dried, filtered, and evaporated. The yellow residue was crystallized to give the *cycloadduct* (IV) as yellow crystals (29·0 g, 51%), m.p. 184—185° (from ether–hexane), $[\alpha]_D^{21}$ −145° (*c* 0·44), $\lambda_{max.}$ (Et_2O) 225 nm (ε 8500), $\nu_{max.}$ (KBr) 1740, 1655, 1245, and 965 cm^{-1}; δ 0·83 (3H, s, 18-H_3), 0·98 (3H, s, 19-H_3), 2·00 (3H, s, 3-OAc), 3·93br (2H), 4·52br (1H, 3-H), 5·26br (2H, 22- and 23-H), 6·50 (2H, AB, *J* 8 Hz, 6- and 7-H), and 6·56 p.p.m. (2H, s); *m/e* 548 (M^+) and 378 [M^+ − (60 + 110)] (Found: C, 74·5; H, 8·7; N, 5·3. $C_{34}H_{48}N_2O_4$ requires C, 74·4; H, 8·8; N, 5·1%).

Hydrogenation of the Adduct (IV) *over* 10% *Palladium–Charcoal*.—A mixture of the adduct (IV) (183 mg) and 10% palladium–charcoal (100 mg) in ethanol (40 ml) was hydrogenated at 50 lb in^{-2} for 18 h. Filtration and evaporation, followed by p.l.c. [chloroform–methanol (95 : 5)] gave the *hexahydro-adduct* (V) (133 mg) as white needles, m.p. 244—245° (from methylene chloride–hexane), $[\alpha]_D^{21}$ +67° (*c* 0·45), $\nu_{max.}$ 1735, 1660, and 1165 cm^{-1}; δ 0·83 (3H, s, 18-H_3), 1·13 (3H, s, 19-H_3), 2·00 (3H, s, 3-OAc), 2·50 (4H, s), 3·40br (2H), and 4·65br p.p.m. (1H, 3-H); *m/e* 554 (M^+) and 380 [M^+ − (60 + 114)] (Found: C, 73·5; H, 9·7; N, 5·2%; M^+, 554·4083. $C_{34}H_{54}N_2O_4$ requires C, 73·6; H, 9·8; N, 5·05%; *M*, 554·406).

The 1,4-*Cycloadduct from Ergosteryl Acetate and Phthalazine*-1,4-*dione* (XII).—This cycloadduct (XII) was similarly prepared from ergosteryl acetate (438 mg) and phthalazine-1,4-dione [4] (148 mg); δ 1·98 (3H, s, 3-OAc), 4·00br (2H), 4·60br (1H, 3-H), 5·30br (2H, 22- and 23-H), 6·56 (2H, AB, *J* 8 Hz, 6- and 7-H), and 8·00 p.p.m. (4H, A_2B_2).

The 1,4-*Cycloadduct from Ergosteryl Acetate and* 4,5-*Dihydropyridazine*-3,6-*dione*.—This was similarly prepared from ergosteryl acetate (175 mg) and 4,5-dihydropyridazine-3,6-dione * (46 mg). The residue obtained after the work-up, purified by p.l.c. [benzene–methanol (90 : 10)], gave pale yellow *crystals*, m.p. 215° (from ether–hexane), $\lambda_{max.}$ (EtOH) 225 (ε 4200) and 255 nm (3600); $\nu_{max.}$ (KBr) 1745, 1716, 1680, 1240, and 975 cm^{-1}; δ 0·83 (3H, s, 18-H_3), 0·98 (3H, s, 19-H_3), 2·00 (3H, s, 3-OAc), 2·40br (4H, $W_{\frac{1}{2}}$ 6 Hz), 3·67 (2H, s), 4·75br (1H, 3-H), 5·27br (2H, 22- and 23-H), and 6·37 p.p.m. (2H, AB, *J* 8 Hz, 6- and 7-H); *m/e* 550 (M^+) and 378 [M^+ − (60 + 112)] (Found: C, 74·2; H, 9·1; N, 5·6. $C_{34}H_{50}N_2O_4$ requires C, 74·15; H, 9·15; N, 5·1%).

Ozonolysis of the Cycloadduct (IV).—The cycloadduct (IV) (5·14 g), in dry methylene chloride (200 ml) containing 1% pyridine,[12] was ozonized (flow rate 0·007 mol h^{-1}) at −70° for 2 h, set aside, and allowed to warm to room temperature overnight. The mixture was treated with hexamethylphosphorous triamide (10 ml), left for 0·5 h, then washed rapidly once with ice-cold 1·0N-hydrochloric acid (100 ml) and twice with cold water, and dried. Evaporation left a yellow gum which was filtered through a silica-gel column, with benzene as eluant. Evaporation and crystallisation gave the *hexanor-aldehyde* (IX) (3·15 g, 70%) as dark yellow crystals, m.p. 165—166° (from ether), $[\alpha]_D^{21}$ −144° (*c* 0·36), $\nu_{max.}$ (KBr) 1730, 1645, and 1250 cm^{-1}; δ ($CDCl_3$) 0·85 (3H, s, 18-H_3), 1·03 (3H, s, 19-H_3), 1·23 (3H, d, *J* 7 Hz), 2·00 (3H, s, 3-OAc), 3·67br (2H), 4·75br (1H, 3-H), 6·50 (2H, AB, *J* 8 Hz, 6- and 7-H), 6·60 (2H, s), and 10·16 p.p.m. (1H, d, *J* 3 Hz, CHO); *m/e* 480 (M^+) and 310 [M^+ − (60 + 110)] (Found: C, 69·8; H, 7·8; N, 5·9. $C_{28}H_{36}N_2O_5$ requires C, 69·95; H, 7·55; N, 5·85%).

The Pyridazine-3,6-*dione Adduct* (X) *of* 3β-*Acetoxycholesta*-5,7,22-*triene*.—Isopentyltriphenylphosphonium bromide [7] (2·5 g) in diethyl ether (50 ml) was treated with a solution of n-butyl-lithium in hexane (2 ml, 3·32 mmol), under prepurified nitrogen at room temperature. After 2 h at room temperature the orange-red ylide solution was added to a solution of the hexanor-aldehyde (IX) (1·01 g, 2·10 mmol) in tetrahydrofuran (50 ml) (freshly distilled from lithium aluminium hydride) during 0·5 h. The betaine separated immediately as a yellow precipitate. After 1 h at room temperature, the solvent was evaporated off by passing dry nitrogen over the mixture. Tetrahydrofuran (80 ml) was introduced with a syringe and the solution was refluxed for 1 h. The solvent was removed and the residue dissolved in ether and washed with water. The ethereal solution was dried and evaporated and separated by p.l.c. [ether–methylene chloride (95 : 5)]. The *adduct* (X) gave yellow crystals (250 mg), m.p. 172—173° (from ether), $\nu_{max.}$ (KBr) 1730, 1645, 1245, and 955 cm^{-1}; δ ($CDCl_3$) 0·85 (3H, s, 18-H_3), 0·93 (3H, s, 19-H_3), 1·97 (3H, s, 3-OAc), 3·83br (2H), 4·67br (1H, 3-H), 5·00br (2H, 22- and 23-H), 6·13 (2H, AB, *J* 8 Hz, 6- and 7-H), and 6·30 p.p.m. (2H, s); *m/e* 534 (M^+) (Found: C, 74·0; H, 8·3; N, 5·7. $C_{33}H_{46}N_2O_4$ requires C, 74·1; H, 8·65; N, 5·25%).

Retro-1,4-*cycloaddition of the Adduct* (IV).—A mixture of the adduct (IV) (28·5 mg) and potassium hydroxide (1 g)

* Prepared by reduction of pyridazine-3,6-diol with aluminium amalgam.

[11] J. P. Connolly, S. F. O'Muircheartaigh, and J. B. Thomson, *J. Chem. Soc.* (*C*), 1970, 508.

[12] G. Slopm, jun., and J. L. Johnson, *J. Amer. Chem. Soc.*, 1953, **80**, 915.

in 80% ethanol was refluxed under nitrogen. The reaction was followed to completion by u.v. spectroscopy (appearance of 282 nm absorption). After 4 h the mixture was diluted with water and extracted with chloroform. After the usual work-up, crystalline ergosterol (19 mg, 90%) was obtained (from ether).

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