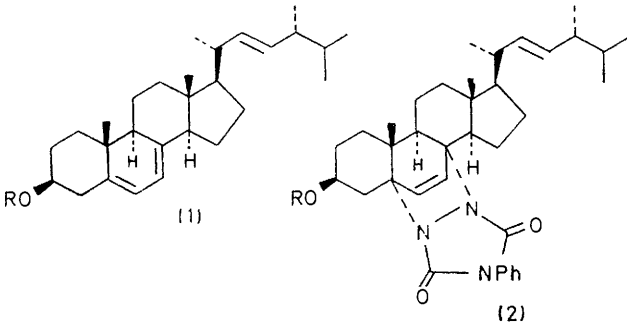
Synthetic Uses of Steroidal Ring B Diene Protection: 22,23-Dihydroergosterol

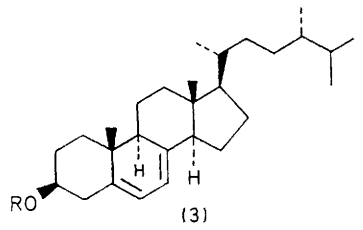
By **Derek H. R. Barton,* A. A. Leslie Gunatilaka, Tsutomu Nakanishi, Henri Patin, David A. Widdowson, and Brian R. Worth,** Department of Chemistry, Imperial College, London SW7 2AY

Protection of the ring B diene system of ergosterol by (i) a two-step addition of the elements of water across the 5,6-double bond, (ii) formation of the 4-phenyl-1,2,4-triazoline-3,5-dione adduct, or (iii) formation of the iron tricarbonyl adduct by treatment with *p*-methoxybenzylideneacetonetricarbonyliron, allowed selective reduction of the 22,23-double bond by catalytic or, as appropriate, ionic hydrogenation. Regeneration of the 5,7-diene system in each case gave a high yield of 22,23-dihydroergosterol.

SYNTHETIC manipulation of ergosterol (1; R = H) other than at the 5,7-diene system requires an efficient method for the protection of this function. The Diels–Alder



(1) (2)

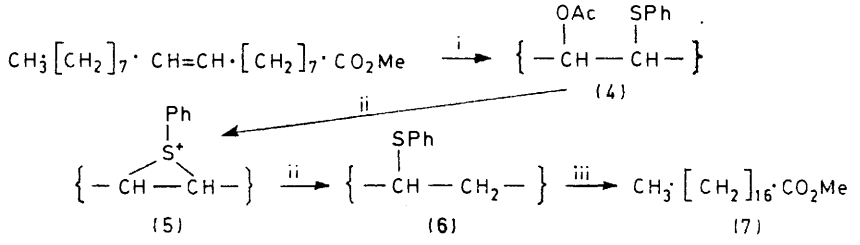


(3)

adduct with maleic anhydride [1] and the 5,8-epidioxide [2] are not useful. In contrast, the effective use of the 4-phenyl-1,2,4-triazoline-3,5-dione [3] adduct (2) for protection of the diene has been established.[4] We report here further exploitation of this approach and the development of two alternative methods which complement the first.

Our synthetic objective was 22,23-dihydroergosterol (3; R = H), which occurs in a number of fungi [5] and which we have recently isolated from a mutant yeast enzymatically blocked at the 22,23-dehydrogenase.[6] 22,23-Dihydroergosterol (of uncertain purity) has been synthesised *via* hydrogenation of the maleic anhydride adduct of ergosterol.[7] The overall process is, however, very inefficient. Our initial approach was to use the azo-adduct (2). Electrophilic attack on this system generally occurs selectively at the 22,23-double bond,[6] but catalytic hydrogenation shows a preference for 6,7-attack and a simple synthesis by this means was thus precluded. Instead, use was made of the selective electrophilic attack on the side chain to introduce reducible groups at C-22 and C-23. Conventional chemical reductions of the difunctionalised side chain however predominantly regenerated the Δ^{22}-system and a variant on ionic hydrogenation [8] (Schemes 1 and 2) was developed for the successful method.

As a simplified model system, methyl oleate was treated with benzenesulphenyl chloride and mercury(II) acetate in dichloromethane at room temperature [9] (Scheme 1). The product was an isomeric mixture of



SCHEME 1 Reagents: i, $Hg(OAc)_2$–PhSCl; ii, $CF_3{\cdot}CO_2H$–$PhCH_2{\cdot}SiHMe_2$; iii, Raney Ni

9,10-phenylthioacetoxy-derivatives of methyl stearate (4) (70%). This was treated with benzyldimethylsilane in trifluoroacetic acid solution [8] to give the mixed methyl 9- and 10-phenylthiostearates (6) (62%). Other work [10]

[1] (*a*) D. N. Jones, P. F. Greenhalgh, and I. Thomas, *Tetrahedron*, 1968, **24**, 297; (*b*) K. D. Bingham, G. D. Meakins, and J. Wicha, *J. Chem. Soc.* (*C*), 1969, 671.

[2] D. H. R. Barton, R. K. Haynes, G. Leclerc, P. D. Magnus, and I. D. Menzies, *J.C.S. Perkin I*, 1975, 2055.

[3] R. C. Cookson, S. S. H. Gilani, and I. D. R. Stevens, *J. Chem. Soc.* (*C*), 1967, 1905.

[4] D. H. R. Barton, T. Shioiri, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1971, 1968.

[5] (*a*) N. J. McCorkindale, S. A. Hutchinson, B. A. Pursey, W. T. Scott, and R. Wheeler, *Phytochemistry*, 1969, **8**, 861; (*b*) P. Singh and S. Rangaswami, *Current Sci.*, 1966, **35**, 515; (*c*) A. Santos Ruiz, *Anales real acad. farm.*, 1943, **3**, 201.

[6] D. H. R. Barton, J. E. T. Corrie, D. A. Widdowson, M. Bard, and R. A. Woods, *J.C.S. Perkin I*, 1974, 1326.

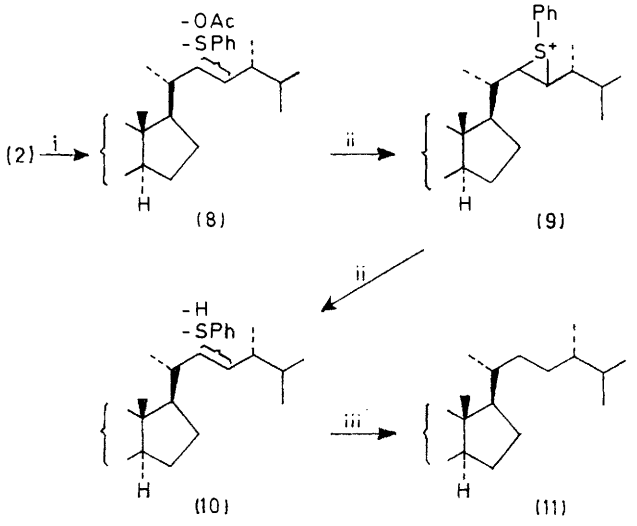
[7] (*a*) A. Windaus and R. Langer, *Annalen*, 1934, **508**, 105; (*b*) H. H. Inhoffen, *ibid.*, p. 81; (*c*) J. Brynjolffssen, D. Hands, J. M. Midgley, and W. B. Whalley, following paper.

[8] D. N. Kursanov, Z. N. Parnes, and N. M. Loim, *Synthesis*, 1974, 633, and references there cited.

[9] T. Mukaiyama, T. Endo, S. Ikengaa, and K. Osonoi, *J. Org. Chem.*, 1968, **33**, 2242.

[10] D. H. R. Barton, J. E. T. Corrie, and D. A. Widdowson, unpublished results.

has shown that this process should occur with apparent retention of configuration by involvement of an episulphonium intermediate [as (5)] which is opened by a hydride ion delivered from the silane. Final desulphurisation with Raney nickel gave methyl stearate.

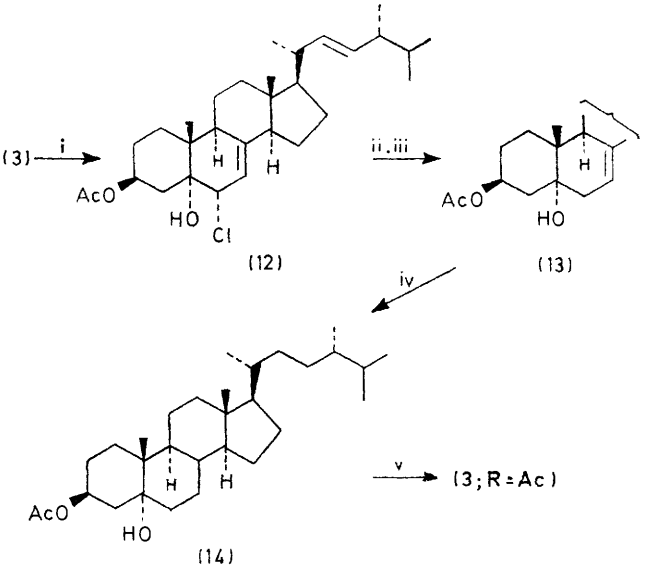


SCHEME 2 Reagents: i, $Hg(OAc)_2$–PhSCl; ii, $CF_3 \cdot CO_2H$–$PhCH_2 \cdot SiHMe_2$; iii, Raney Ni

In the steroid series (Scheme 2) the azo-adduct (2; R = Ac) was treated with a five-fold excess of benzenesulphenyl chloride and mercury(II) acetate in dichloromethane to give the mixed 22,23-acetoxy-phenylthio-adducts (8) in 60% yield. Without separation (analytical t.l.c. indicated 3 components) these were reduced at 0 °C in trifluoroacetic acid with benzyldimethylsilane to give the 22- (and/or 23-)phenylthio-adducts (10) (76%) *via* the presumed episulphonium intermediate (9). The starting ergosterol acetate azo-adduct (2; R = Ac) was also reformed in 16% yield. No conditions were found whereby the formation of this could be completely suppressed. Desulphurisation of the thioether (6) with Raney nickel in refluxing tetrahydrofuran–ethanol gave the azo-adduct of 22,23-dihydroergosterol (11) in 33% yield (based on recovered starting material). This product contained traces of the 22,23-olefin which could be removed by treatment with *m*-chloroperbenzoic acid.[6] The 22,23-epoxide formed was readily separated from the required dihydro-adduct (7) (see Experimental section). Finally, reduction of the adduct with lithium aluminium hydride gave 22,23-dihydroergosterol (85%), m.p. and mixed m.p. 128—130°, $[\alpha]_D$ −121°. This material was also spectroscopically identical with that obtained from the *pol* 5 mutant of *Saccharomyces cerevisiae*.

Although successful, this approach was cumbersome and a more direct synthesis was sought (Scheme 3). In another connection, it had been observed that 5α-hydroxy-Δ^7-steroids were readily dehydrated to 5,7-dienes,[11] free of 4,6- and 6,8(14)-diene analogues.[12] Direct hydration of a 5,7-diene to the 5α-hydroxy-derivative is not possible but it was found[13] that, at −78 °C, chromyl chloride reacted with ergosteryl acetate to give, in 95% yield, the 6α-chloro-5α-hydroxy-derivative (12), m.p. 192—193°. We immediately reduced this compound with lithium aluminium hydride to give, after reacetylation the 5α-hydroxy-derivative (13) (80%), m.p. 231—233°, $[\alpha]_D$ 0°. Hydrogenation of compound (13) over *freshly prepared* Adams catalyst in ethyl acetate led to saturation of the side chain without migration of the 7,8-double bond[14] (see spectral data in Experimental section). The 22,23-dihydro-product (14), m.p. 227—230°, $[\alpha]_D$ +15.2°, was dehydrated with freshly purified thionyl chloride in pyridine at room temperature to give 22,23-dihydroergosterol acetate (80%), m.p. 146—148°, $[\alpha]_D$ −82°.

Although this approach gave an improved overall yield, it was still considered that a more efficient process was desirable. The problem with azo-adduct protection of the 5,7-diene lay in the normal, if sterically constrained, reactivity of the 6,7-double bond generated. If the total π-system of the diene were complexed, as in the tricarbonyliron complexes,[15] then the problem would be avoided. Accordingly, ergosterol benzoate was converted into the tricarbonyliron complex (15) in 70% yield, by treatment with pentacarbonyliron in refluxing di-n-butyl ether (144 °C). When formed in this way, the product always contained 3—5% of unchanged ergosterol benzoate. This could be removed

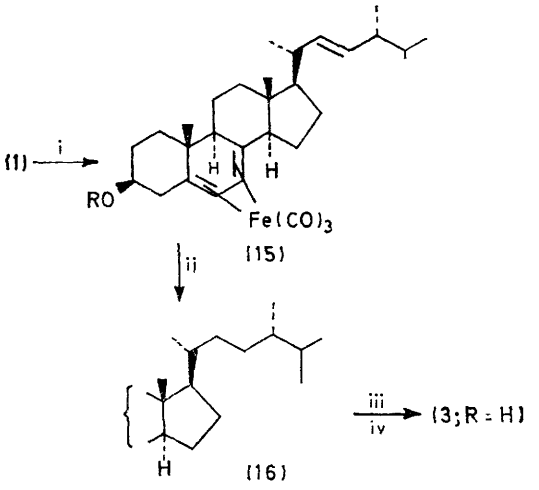


SCHEME 3 Reagents: i, CrO_2Cl_2; ii, $LiAlH_4$; iii, Ac_2O–C_5H_5N; iv, H_2–PtO_2; v, $SOCl_2$–C_5H_5N

by formation of the azo-adduct (2; R = PhCO) with 4-phenyl-1,2,4-triazoline-3,5-dione.[4] In order to circum-

[11] D. H. R. Barton and A. G. M. Barrett, unpublished results.
[12] D. Freeman, A. Acher, and Y. Mazur, *Tetrahedron Letters*, 1975, 261.
[13] D. H. R. Barton and R. K. Haynes, *J.C.S. Perkin I*, 1975, 2065.
[14] D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1948, 1354.
[15] H. Alper and J. T. Edward, *J. Organometallic Chem.*, 1968, **14**, 411.

vent the problem of high reaction temperature andn-complete reaction, use was made of the arylmethylene-acetone-tricarbonyliron complexes which have been



SCHEME 4 Reagents: i, $Fe_2(CO)_9$–$MeOC_6H_4$·CH:CH·COMe; ii, H_2–PtO_2–$PhCH_2$·SiHMe$_2$; iii, $FeCl_3$,$6H_2O$; iv KOH

shown to be efficient tricarbonyliron transfer agents.[16] Benzylideneacetonetricarbonyliron reacted with ergosteryl acetate at 90 °C to give a 69% yield of the complex (15; R = Ac) (see Table). The more electron-rich

Formation of tricarbonyliron complexes of ergosterol derivatives

No.	Reagent [b]	Ergosterol derivative	Conditions (T/°C; t/h)	Yield [a] (%)
1	$Fe(CO)_5$	Benzoate	144; 20	70
2	[$Fe(CO)_3$(bza)]	Acetate	90; 24	69
3	[$Fe(CO)_3$(mba)]	Acetate	90; 16	64
4	[$Fe(CO)_3$(bza)]	Benzoate	90; 20	65
5	[$Fe(CO)_3$(mba)]	Benzoate	90; 16	60
6	[$Fe(CO)_3$(mba)]	Acetate	60; 48	66
7	[$Fe(CO)_3$(mba)]	Benzoate	60; 48	59
8	mba + Fe_2CO_9	Benzoate	55; 120	80

[a] Purified product. [b] bza = benzylideneacetone; mba = methoxybenzylideneacetone.

p-methoxybenzylideneacetonetricarbonyliron reacted at 90 °C during 16 h or at 60 °C during 48 h to give a 60% yield. It was subsequently found that the arylmethyleneacetone could be used catalytically for the reaction between ergosteryl benzoate and nonacarbonyldi-iron. By the use of reagents in excess after 120 h at 55 °C the yield of the purified tricarbonyliron complex of ergosteryl benzoate was raised to 80%. The results of the evaluation of this method are given in the Table.

Hydrogenation of the complex (15) over Adams catalyst, palladium–charcoal, Raney nickel, or chlorotristriphenylphosphinerhodium was in each case very slow or non-existent. The addition of acid catalysts caused concomitant decomposition of the diene complex. The addition of a catalytic amount of benzyldimethylsilane [17] to a hydrogenation over Adams catalyst in ethyl acetate gave smooth reduction of the 22,23-double bond (in 94% yield) together with some saturation of the phenyl ring of the benzoate (indicated by the mass spectrum). The diene was regenerated by oxidative cleavage of the complex (16) with iron(III) chloride hexahydrate.[18] Saponification of the esters (benzoate + cyclohexanecarboxylate) without purification, gave the 22,23-dihydroergosterol (92%), m.p. 129—131°, $[\alpha]_D$ −131°, spectroscopically identical with the previous samples. This material was converted into the 3β-acetate (3; R = Ac) and the benzoate (3; R = PhCO). The latter was converted into the azo-adduct (2; R = PhCO), m.p. 201—203°. These were all identical with the corresponding derivatives of the natural material.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus. Unless otherwise stated, $[\alpha]_D$ values were recorded for solutions in chloroform and i.r. spectra for solutions in carbon tetrachloride or Nujol mulls. N.m.r. spectra were recorded with $CDCl_3$ as solvent and tetramethylsilane as internal standard. U.v. spectra were recorded for solutions in absolute ethanol.

Unless otherwise stated, light petroleum refers to the fraction of b.p. 40—60° and solvent mixtures used are described in ratios of volumes. T.l.c. and p.l.c. were carried out on Merck Kieselgel GF_{254} plates (0.2 and 1 mm layers, respectively).

The Mixture of Methyl 9,10-Acetoxy-phenylthio-stearates (4).—Benzenesulphenyl chloride (1.17 g, 8.34 mmol) in dry dichloromethane (10 ml) was added gradually at room temperature during 30 min [9] to a stirred mixture of methyl oleate (2.25 g, *ca.* 7.57 mmol) and mercury(II) acetate (1.30 g, 4.08 mmol) in dry dichloromethane (10 ml). After 3.5 h, t.l.c. showed that most of the starting material was consumed. Mercury(II) chloride was removed by filtration, the filtrate was evaporated, and the residue was chromatographed over alumina (grade III; 80 g). Elution with light petroleum (*ca.* 250 ml) gave a small amount of starting material. Further elution with light petroleum (1.6 l) and then light petroleum–ether (10 : 1; *ca.* 175 ml) yielded the isomeric methyl 9,10-acetoxy-phenylthio-stearates (4) as an oil (2.47 g, 70%), $\nu_{max.}$ (film) 1 730, 1 582, and 1 236 cm^{-1}, τ 9.12 (3 H, CH_3), 8.75 (*ca.* 26 H, CH_2), 7.98 (3 H, s, Ac), 7.70 (2 H, CH_2·CO_2Me), 6.77 (1 H, m, *H*C·SPh), 6.35 (3 H, s, CO_2Me), 4.95 (1 H, m, *H*C·OAc), and 2.65 (5 H, m, ArH).

Methyl 9- and 10-Phenylthio-stearates (6).—The mixture of methyl 9,10-acetoxy-phenylthio-stearates (4) (49.5 mg) in benzyldimethylsilane (0.13 ml, *ca.* 120 mg) and trifluoroacetic acid (2 ml) was stirred at room temperature for 20 h.[19] T.l.c. [light petroleum–ether (20 : 1)] showed that the desired compound (R_F 0.33) was generated almost exclusively. After the reaction was complete (t.l.c.), the solution was poured into saturated aqueous sodium hydrogen carbonate and extracted with ether. The extract was washed with N-sodium hydroxide and water, dried ($MgSO_4$), and evaporated. P.l.c. [light petroleum (b.p. 60—80°)–ether (20 : 1)] gave the methyl 9- and 10-phenylthiostearates (6) (27 mg, 62%), as an oil, $\nu_{max.}$ 1 730 and 1 170 cm^{-1}, τ 9.13 (3 H, CH_3), 8.75 (*ca.* 28 H, CH_2), 7.72

[16] J. A. S. Howell, B. F. G. Johnson, P. L. Josty, and J. Lewis, *J. Organometallic Chem.*, 1972, **39**, 329.
[17] G. F. Emerson and R. Pettit, *J. Amer. Chem. Soc.*, 1962, **84**, 4591.
[18] G. F. Emerson and R. Pettit, *J. Org. Chem.*, 1964, **29**, 3620.
[19] C. T. West, S. J. Donnelly, D. A. Kooistra, and M. P. Doyle, *J. Org. Chem.*, 1973, **38**, 2675.

(2 H, m, CH_2·CO_2Me), 6.95 (1 H, m, HC·SPh), 6.37 (3 H, s, CO_2Me), and 2.70 (5 H, m, ArH), *m/e* 406 (*M*, 14%), 378, 297, 296, 110 (100%), and 109.

Methyl Stearate (7).—The methyl 9- and 10-phenylthiostearates (6) (80 mg) in 90% ethanol was refluxed with Raney nickel (1.5 g) for 3 h. T.l.c. [light petroleum–ether (20 : 1)] showed that the starting material was converted into one product. The bulk of the nickel was removed by decantation and centrifugation and then washed several times with ethanol. Dimethylglyoxime was added to the combined ethanolic solutions, which were then evaporated. The residue was separated by p.l.c. [light petroleum–ether (20 : 1)] to give methyl stearate (7) as a solid, spectroscopically and chromatographically identical with authentic material.

4-Phenyl-1,2,4-triazoline-3,5-dione Adduct (8) *of the Mixture of 22-Acetoxy-23-phenylthio- and 23-Acetoxy-22-phenylthioergosta-5,7-dien-3β-yl Acetates*.—To a stirred mixture of the 4-phenyl-1,2,4-triazoline-3,5-dione adduct of ergosteryl acetate [4] (III) (860 mg, *ca.* 1.4 mmol) and mercury(II) acetate (2.16 g, *ca.* 6.75 mmol) in dry dichloromethane (3 ml), benzenesulphenyl chloride (1.96 g, *ca.* 13.5 mmol) in dry dichloromethane (4 ml) was added slowly at room temperature during 40 min. The mixture was stirred at room temperature for a further 20 h. T.l.c. showed the presence of the polar isomeric mixture of the desired compounds as three spots overlapping each other, R_F 0.51, 0.45, and 0.38 [benzene–ether (5 : 1)] or two spots overlapping each other, R_F 0.39, 0.33 [ether–light petroleum (3 : 2)] in addition to the small amount of the starting material, R_F 0.62 [benzene–ether (5 : 1)] or 0.61 [ether–light petroleum (3 : 2)]. After the removal of mercury(II) salts, p.l.c. [ether–light petroleum (3 : 2)] gave the mixture of the adducts (8) (650 mg, 60%) as a solid (from aqueous ethanol), $\nu_{max.}$ 1 749, 1 733, 1 702, 1 510, and 1 245 cm^{-1}, τ 7.95br (6 H, s, OAc), 6.77 [1 H, m, 23-(or 22-)H], 4.65 [1 H, m, 22-(or 23-)H], 3.80 and 3.60 (2 H, ABq, *J* 8 Hz, H-6 and -7), and 2.67 (10 H, m, ArH), *m/e* 781 (M^+), 779, 546 (100%), 486, 437, 436, 377, 376, 361, 110, and 109 (Found: C, 70.55; H, 7.45; N, 5.25. $C_{46}H_{59}O_6N_3S$ requires C, 70.65; H, 7.6; N, 5.35%). From the less polar band, starting material (105 mg, 12%; identified by n.m.r.) was obtained.

Reduction of the Acetoxy-phenylthio-adducts (8).—To the mixed isomers (8) (650 mg) in benzyldimethylsilane (5 ml, *ca.* 4.75 g), trifluoroacetic acid (30 ml) was added dropwise with stirring at 0 °C during 1 h. The mixture was stirred at 0 °C for a further 2 h, then poured into saturated aqueous sodium hydrogen carbonate at 0 °C and extracted with ether. The ether layer was washed with sodium hydrogen carbonate solution until neutral and then water and dried ($MgSO_4$). T.l.c. [light petroleum–ether (1 : 1)] showed the presence of two products (R_F 0.5 and 0.42). After evaporation of the ether, the product was chromatographed over aluminium oxide (grade III; 30 g) in order to remove the excess of benzyldimethylsilane. Elution with light petroleum (200 ml) gave unchanged benzyldimethylsilane. Elution with ether gave the product mixture, which was separated by p.l.c. [light petroleum–ether (1 : 1), twice developed]. From the less polar band (R_F 0.69), the regenerated 4-phenyl-1,2,4-triazoline-3,5-dione adduct of ergosteryl acetate (1; R = Ac) (100 mg, 16%) was obtained. From the more polar band, the 4-phenyl-1,2,4-triazoline-3,5-dione adduct of the isomeric mixture (10) of 22- and 23-phenylthioergosta-5,7-dien-3β-yl acetates (10) (480 mg, 76%) was isolated. Recrystallisation from aqueous ethanol gave fine needles, m.p. 130.5—132.5°, $\nu_{max.}$ 1 749, 1 735, 1 705, 1 511, and 1 247 cm^{-1}, τ 8.00 (3 H, s, OAc), 6.78 [1 H, m, 22-(23-)H], 4.55 (1 H, m, H-3), 3.80 and 3.80 (2 H, ABq, *J* 8 Hz, H-6 and -7), and 2.68 (10 H, m, ArH), *m/e* 723 (M^+), 721, 488, 379, 378, 363, 119 (100%), 110, and 109 (Found: C, 3.0; H, 7.85; N, 5.6. Calc. for $C_{44}H_{57}N_3O_4$: C, 3.0; H, 7.95; N, 5.8%).

4-Phenyl-1,2,4-triazoline-3,5-dione Adduct (11) *of 22,23-Dihydroergosteryl Acetate*.—Raney nickel (2 g) in tetrahydrofuran (25 ml) and ethanol (12 ml) and the isomeric phenylthio-adducts (10) (220 mg) were refluxed for 1 h (t.l.c. control). T.l.c. [light petroleum–ether (1 : 1)] showed three spots (R_F *ca.* 0.5, 0.42, and 0.34). The nickel was removed by decantation and centrifugation and washed with absolute ethanol several times. The residual nickel contained in the combined solutions was removed by filtration. The solvents were evaporated off and the residue was separated into the three components by p.l.c. [light petroleum–ether (1 : 1), twice developed]. From the second band, the starting material (10) was recovered (90 mg, 40.9%). From the less polar band, the adduct (11) (50 mg, 26.7%) was obtained. Recrystallisation from aqueous methanol and then acetone–methanol gave plates, m.p. 179—181°, $[\alpha]_D^{21}$ −95.2° (*c* 0.69), $\nu_{max.}$ ($CHCl_3$) 1 742, 1 726, and 1 694 cm^{-1}, n.m.r. data as below, *m/e* 615 (M^+), 613, and 380 (100%) (Found: C, 74.2; H, 8.55; N, 6.7%). Though physical data supported the structure of (11), the appearance of an $M^+ - 2$ peak in the mass spectrum suggested the presence of a small amount of a didehydro-compound. The product (11) was therefore purified as follows.

To the crude material (50 mg) from p.l.c., in dry dichloromethane (3 ml), *m*-chloroperbenzoic acid (15 mg) was added at 0 °C, and the solution was kept at 0 °C for 43 h.[6] It was then diluted with cold dichloromethane and filtered through an alumina (grade III; 3 g) column to remove acidic material. P.l.c. [light petroleum–ether (1 : 1), twice developed] of the resulting mixture gave the pure 4-phenyl-1,2,4-triazoline-3,5-dione adduct (11) (28 mg, 56%), m.p. (plates from acetone–methanol) 188—190°, $[\alpha]_D^{18}$ −96.5° (*c* 0.29), τ 7.97 (3 H, s, OAc), 4.52 (1 H, m, H-3), 3.76 and 3.57 (2 H, ABq, *J* 8 Hz, H-6 and -7), and 2.62 (5 H, m, ArH), *m/e* 615 (M^+), 440, 438, 380 (100%), 378, and 365 (Found: C, 74.15; H, 8.85; N, 7.0. $C_{38}H_{53}N_3O_4$ requires C, 74.1; H, 8.7; N, 6.8%).

22,23-Dihydroergosterol (3; R = H).—To the acetate (11) (20 mg) in dry tetrahydrofuran (8 ml), lithium aluminium hydride (62 mg) was added with stirring, and the mixture was refluxed for 18 h under nitrogen.[4] A few drops of water were added to the stirred mixture at 0 °C and the solution was dried ($MgSO_4$) and evaporated. The residue was fractionated by t.l.c. [benzene–ether (1 : 1)] to give 22,23-dihydroergosterol (3; R = H) (11 mg, 85%), m.p. (needles from chloroform–methanol) 128—130°, $[\alpha]_D^{19}$ −121° (*c* 0.1), $\lambda_{max.}$ 262 (ε 8 000), 272 (11 200), 282 (11 800), and 294 nm (6 800) {lit.,[7] m.p. 152—153°, $[\alpha]_D^{19}$ −109°; lit.,[6] m.p. 148—150°, $[\alpha]_D$ −128.7° (*c* 0.41), $\lambda_{max.}$ 262 (ε 8 900), 272 (12 800), 282 (13 600), and 294 nm (7 700)}, *m/e* 398 (M^+) (mass spectrum identical with that of the authentic specimen [6]). The m.p. of the original specimen from yeast was 128—130°; the m.p. we gave earlier [6] is incorrect.

3β-Acetoxy-6α-chloroergosta-7,22-dien-5α-ol (12).—Chromyl chloride (310 mg) in dry methylene chloride (4 ml) was added, over 2—3 min at −78 °C, to a deoxygenated solution of ergosteryl acetate (438 mg) in redistilled toluene (85 ml)

under nitrogen. The mixture was stirred at −78 °C (5 min) and a saturated solution of sodium borohydride in ethanol (1 ml) was added. After 15 min (−78 °C) the mixture was poured into N-hydrochloric acid (150 ml) and benzene (100 ml). The organic layer was separated, washed successively with water (100 ml) and brine solution (3 × 50 ml), dried ($MgSO_4$), and filtered through a plug of anhydrous sodium sulphate. Evaporation of the filtrate gave the crude chlorohydrin (12) (465 mg, 95%). Recrystallisation from ethyl acetate gave material (12) (320 mg, 65%), m.p. 192—193° (lit.,[13] 192—194°), identical with an authentic sample.

3β-*Acetoxyergosta*-7,22-*dien*-5α-*ol* (13).—The crude chlorohydrin (12) (980 mg) in dry tetrahydrofuran (100 ml) was added slowly to a stirred suspension of lithium aluminium hydride (120 mg) in dry tetrahydrofuran (50 ml) at room temperature under nitrogen. The mixture was refluxed (3 h) and cooled to 0 °C, and the excess of hydride destroyed with saturated sodium sulphate solution. Filtration through a plug of anhydrous sodium sulphate and evaporation of the filtrate gave a solid, which was treated with acetic anhydride (5 ml) and pyridine (10 ml) at 0 °C (18 h). Removal of the solvents under reduced pressure afforded the acetate (13) (735 mg, 80%), m.p. 231—233° (from acetone) (lit.,[20] 230—232°), $[\alpha]_D^{23}$ ±0° (*c* 2.0) (lit.,[20] ±0°).

3β-*Acetoxyergost*-7-*en*-5α-*ol* (14).—The diene (13) (200 mg) was shaken (4 h) with freshly prepared Adams catalyst (20 mg) in redistilled ethyl acetate (75 ml) under hydrogen (*ca.* 1 atm). The catalyst was removed by filtration, fresh catalyst was added, and the procedure was repeated. Filtration through a plug of anhydrous sodium sulphate and evaporation of the filtrate gave the *acetate* (14) (189 mg, 94%), m.p. 227—230° (from acetone), $[\alpha]_D^{23}$ +15.2° (*c* 2.0), τ 4.95 (2 H, m, 3α- and 7-H), 8.00 (3 H, s, 3β-OAc), 9.07 (3 H, s, 19-H_3), 9.15 (6 H, d, *J* 7 Hz, 26- and 27-H_3), 9.22 (6 H, d, *J* 6.5 Hz, 21- and 28-H_3), and 9.44 (3 H, s, 18-H_3) (Found: C, 78.45; H, 10.85. $C_{30}H_{50}O_3$ requires C, 78.55; H, 11.0%), *m/e* 440 $(M - H_2O)^+$, 438, 380, 379, 336, 341, and 340.

22,23-*Dihydroergosteryl Acetate* (3; R = Ac).—Redistilled thionyl chloride (0.2 ml) in dry pyridine (0.5 ml) was added to compound (14) (100 mg) in dry pyridine (8 ml) at room temperature under nitrogen. After 45 min the solution was diluted to 50 ml with water and extracted with toluene (2 × 25 ml). The combined organic layer was washed with water (2 × 25 ml) and brine (2 × 25 ml), dried ($MgSO_4$), and evaporated. The residue afforded the acetate (3; R = Ac) (77 mg, 80%) as plates (from acetone–methanol), m.p. 140—145°. A purified sample had m.p. 146—148° and other constants in agreement with the literature.[6]

(*Ergosteryl Benzoate*)*tricarbonyliron* (15; R = PhCO).—A mixture of ergosteryl benzoate (2.00 g) and pentacarbonyliron (10 ml) in anhydrous di-n-butyl ether (80 ml) was refluxed (bath temperature 155—165 °C) under a slightly positive pressure of nitrogen for 17—20 h (u.v. control). The mixture was cooled to room temperature and filtered through a short bed of alumina (grade V). The black residue was washed several times with di-n-butyl ether to obtain a yellow solution. Evaporation at *ca.* 20 mmHg and 60 °C afforded a yellow crystalline solid (1.99 g, 78%), the u.v. spectrum of which showed the presence of *ca.* 3—5% of the starting material. It was purified by the following procedure.

To a well stirred solution of the solid (1.99 g) in dry dichloromethane (20 ml) was added dropwise 4-phenyl-1,2,4-triazoline-3,5-dione (10% in anhydrous acetone) at −78 °C, until the mixture became permanently red. Stirring was continued for 3 h at this temperature, alumina (grade V; 5.0 g) was added, the cooling bath was removed, and the solution was allowed to warm to room temperature. It was filtered and the yellow solution thus obtained was evaporated to dryness. The residue was taken up in hexane and the solution filtered through a column of alumina (grade V). The eluates belonging to the distinct yellow band were evaporated to yield a yellow crystalline solid, which when recrystallised from ethyl acetate–methanol afforded bright yellow needles of (*ergosteryl benzoate*)*tricarbonyliron* (15; R = PhCO) (1.81 g, 70%), $\nu_{max.}$ 2 040, 1 965, 1 715, 1 600, 1 585, and 975 cm^{-1}, τ 1.88—2.05 (2 H, m, ArH), 2.40—2.70 (3 H, m, ArH), 4.83 (2 H, m, 22- and 23-H), 4.96 (2 H, ABq, J_{AB} 4 Hz, 6- and 7-H), 5.03br (1 H, m, 3α-H), 7.27—8.83 (CH_2 envelope), and 8.93, 9.03, 9.13, 9.23, and 9.27 (Me), $\lambda_{max.}$ (cyclohexane) 230 nm (log ε 4.42), *m/e* 640 (M^+), 612, 584, 556, 541, 529, 500, and 378 (100%) (Found: C, 71.15; H, 7.45. $C_{38}H_{48}FeO_5$ requires C, 71.25; H, 7.55%).

p-*Methoxybenzylideneacetonetricarbonyliron*.—To a solution of *p*-methoxybenzylideneacetone (29 g) in toluene (100 ml) was added nonacarbonyldi-iron (29 g). After 24 h at room temperature under nitrogen, the yellow solution was warmed at 60 °C until t.l.c. showed complete transformation into the complex (*ca.* 4 h). The solution was cooled and chromatographed on silica gel (elution first with light petroleum and then with mixtures progressively enriched in ether). The red solution was concentrated under vacuum and the *complex* precipitated on cooling as red crystals (8.05 g, 32%), m.p. 110—112° (decomp.) (Found: C, 53.1; H, 3.85. $C_{14}H_{12}FeO_5$ requires C, 53.2; H, 3.8%), *m/e* 316 (M^+) 288, 260, 232, 176, and 161 (100%).

Complex Formation with Arylmethyleneacetonetricarbonyliron.—(i) The following procedure was used for runs 2—7 in the Table. Ergosteryl acetate (0.44 g, 1 mmol) and the arylmethyleneacetone complex (4 mmol) in toluene (10 ml) were warmed at 60 or 90 °C under nitrogen during 16—48 h. The cooled solution was diluted with ether, filtered, and evaporated. The residue was fractionated by p.l.c. [light petroleum–ether (9 : 1)] to give a yellow oil. Crystallisation from methanol gave the complex (15; R = Ac), m.p. 94—95°.

(ii) (with Dr. A. Fernandez Mateos) Ergosteryl benzoate (4.5 g, 9 mmol), *p*-methoxybenzylideneacetone (5.4 g, 17 mmol), and nonacarbonyldi-iron (10.9 g, 30 mmol) in toluene (40 ml) were warmed to 55 °C under nitrogen for 5 days. T.l.c. (4% light petroleum–ethyl acetate, 4 times developed) showed complete reaction. The solution was filtered and evaporated *in vacuo* to give an oily residue. Trituration with methanol and recrystallisation from ethyl acetate gave the complex (15; R = COPh) (4.3 g, 80%), m.p. 163—165°, $[\alpha]_D$ −67.9° (*c* 1.20).

Catalytic Hydrogenation of (*Ergosteryl benzoate*)*tricarbonyliron*.—(Ergosteryl benzoate)tricarbonyliron (640 mg) in AnalaR ethyl acetate (35 ml) was hydrogenated at atmospheric pressure and room temperature for 14 days over Adams platinum oxide (100 mg) and benzyldimethylsilane (10 drops). The resulting black solution was filtered through Celite and the Celite was washed with ether. The combined solutions were evaporated under reduced pressure to yield a yellow solid which was taken up in ethyl acetate.

[20] G. F. Laws, *J. Chem. Soc.*, 1953, 4185.

Trituration with methanol afforded a mixture of 22,23-dihydroergosterol benzoate and cyclohexanecarboxylate tricarbonyliron complexes (16; R = COPh or COC_6H_{11}, respectively) as bright yellow plates (602 mg, 94%), $\nu_{max.}$ 2 040, 1 965, 1 715, 1 600, and 1 585 cm^{-1}.

To a stirred solution of the above mixture (325 mg) in tetrahydrofuran (20 ml), absolute ethanol (15 ml), and water (2 ml), was added powdered iron(III) chloride hexahydrate (2.5 g). After 2 h at room temperature the mixture was refluxed over a steam-bath for ½ h and cooled to room temperature, and a solution of potassium hydroxide (4.5 g) in absolute ethanol (25 ml) was added. The mixture was refluxed for a further ½ h, cooled, diluted with brine, and extracted into ether. The organic layer was washed with brine until neutral, dried ($MgSO_4$), and evaporated to yield a pale yellow crystalline solid which when recrystallised from chloroform–methanol afforded needles of 22,23-dihydroergosterol (3; R = H) (184 mg, 92%), m.p. 129—131°, $[\alpha]_D^{25}$ −131° (*c* 0.75).

A portion of the above sterol was acetylated with acetic anhydride–pyridine to obtain the acetate (3; R = Ac). The *benzoate* was analogously obtained as plates, m.p. 151—152° (from acetone–methanol), $[\alpha]_D^{25}$ −59.3° (*c* 0.44), τ 1.90—2.05 (2 H, m, ArH), 2.40—2.70 (3 H, m, ArH), 4.50 (2 H, ABq, *J* 6 Hz, 6- and 7-H), 5.00 (1 H, m, 3α-H), 7.43 (2 H, m, 4-H), 7.60—9.00 (CH_2 envelope), and 9.00, 9.07, 9.13, 9.17, 9.23, and 9.37 (Me) (Found: C, 83.7; H, 9.85. $C_{35}H_{50}O_2$ requires C, 83.6 and H, 10.0%).

4-Phenyl-1,2,4-triazoline-3,5-dione Adduct (11) *of* 22,23-*Dihydroergosteryl Benzoate*.—The benzoate (1; R = PhCO) was treated with 4-phenyl-1,2,4-triazoline-3,5-dione (7) as before [4] to obtain the adduct as needles, m.p. 201—202° (from light petroleum–benzene), mixed m.p.[6] 201—203°.

We thank the Nuffield Foundation and the S.R.C. for support. We acknowledge a gift of ergosterol from Roussel-Uclaf, the help of Dr. J. E. T. Corrie in the early stages of this work, and that of Dr. A. Fernandez Mateos for the experiment indicated.

[5/1730 *Received*, 9*th September*, 1975]