607. *A New Sterol from a Strain of* Aspergillus niger.

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A new tetraethenoid sterol, isolated from the non-saponifiable matter of a strain of *Aspergillus niger*, has been proved to be 14-dehydroergosterol. It occurs as a minor component in admixture with ergosterol.

The mould *Aspergillus niger* is of industrial importance for the manufacture of citric acid. A by-product of this process is ergosterol, isolated from the mycelium by extraction with alcohol and hydrolysis. Many strains of this mould are known, only a few of which are useful commercially. One such strain, under investigation in the laboratories of Messrs. J. and E. Sturge Ltd., Birmingham, was found to furnish an ergosterol which was *more* lævorotatory than pure ergosterol (see Barton and Cox, *J.*, 1948, 783, 1357). Other strains of *A. niger*, investigated in these laboratories and equally satisfactory for the production of citric acid, have always furnished essentially pure ergosterol. The existence of this anomaly was first brought to our attention by Dr. E. R. S. Winter, Director of Research at Messrs. J. and E. Sturge Ltd., who kindly advised us during the initial stages of the investigation and provided us with an adequate supply of raw material.

The main component of the anomalously rotating sterol was the expected ergosterol, present to an extent of 82% as judged by the absorption spectrum. That the strongly lævorotatory contaminant was possibly a sterol was shown by its digitonin precipitability. It was separated from ergosterol by prolonged recrystallisation of the mixed benzoates—care was needed because even traces of acids, such as the hydrogen chloride present in aged batches of chloroform, were sufficient to destroy the strongly lævorotatory benzoate (see Experimental). In this way a pure benzoate, $C_{35}H_{46}O_2$, m. p. 208—212°, $[\alpha]_D$ −274° (in chloroform), −260° (in carbon tetrachloride), was finally obtained. This showed a characteristic absorption band (see figure) at 319 mμ. ($\varepsilon_{max.}$ 16,000 in ethanol). It was shown to be 14-dehydroergosteryl benzoate (I; R = Bz) by the reactions summarised in the sequel on p. 2730.

On catalytic hydrogenation with a platinum catalyst in acetic acid solution the benzoate afforded, in almost quantitative yield, " α "-ergostenyl hexahydrobenzoate (II; R = C_6H_{11}·CO), the constitution * of which was confirmed (*a*) by hydrolysis to " α "-ergostenol and hexahydrobenzoic acid and (*b*) by its preparation by the similar hydrogenations of " α "-ergostenyl benzoate (II; R = Bz) and of ergosteryl benzoate (III). This established the nature of the carbon skeleton and indicated the presence of at least one double bond at position 7(8), 8(9), and/or 8(14).

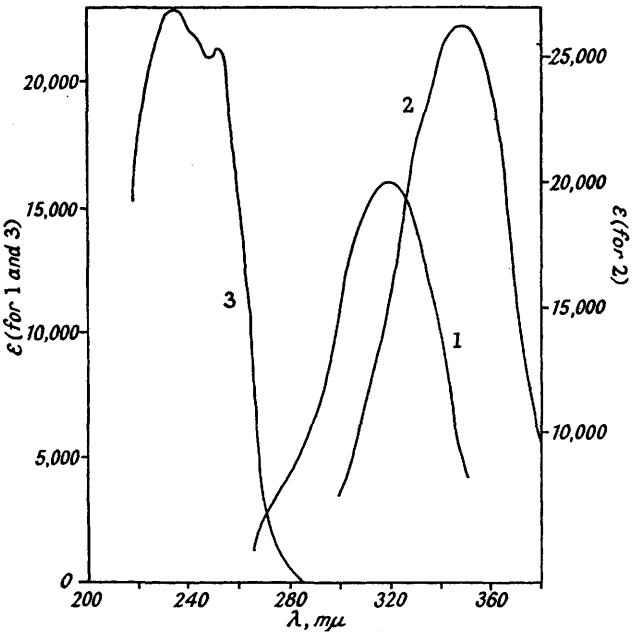
With maleic anhydride in refluxing benzene—conditions under which ergosteryl benzoate itself does not react (Inhoffen, *Annalen*, 1934, **508**, 81)—the new benzoate gave a beautifully crystalline adduct (IV), which showed no butadienyl absorption in the ultra-violet region. The position of attachment of the maleic anhydride residue was proved by catalytic hydro-

* It is a curious discrepancy in the literature that our " α "-ergostenyl hexahydrobenzoate appears to be identical with a substance described as ' " α "-ergostenyl benzoate ' by Wieland, Rath, and Hesse (*Annalen*, 1941, **548**, 34). The latter was prepared by methods comparable to those employed here for the preparation of the hexahydrobenzoate. We have always found that α-ergostenyl benzoate melts at 116° and has $[\alpha]_D$ ±0° (in chloroform). It is possible that the ' " α "-ergostenyl benzoate ' of Wieland, Rath, and Hesse is a higher-melting form but against this possibility stand (*a*) the methods of preparation and (*b*) the fact that there was no depression in m. p. on admixture of our " α "-ergostenyl hexahydrobenzoate with the ' " α "-ergostenyl benzoate ' of Wieland, Rath, and Hesse. We are greatly indebted to Professor H. Wieland, for the latter observation.

genation to give the monoethenoid dihydroergosteryl B_3 hexahydrobenzoate–maleic anhydride adduct (V). An authentic specimen of (V) was prepared by reaction of ergosteryl B_3 benzoate (VI; R = Bz) (see Barton, *J.*, 1946, 513; Barton and Brooks, *J.*, 1951, 277) with maleic anhydride to give the adduct (VII), followed by catalytic hydrogenation. These experiments indicated the existence of double bonds at positions 7(8) and 14(15) in the adduct precursor.

The presence of an ethylenic linkage at the 22(23)-position in the side chain was proved by ozonolysis of the benzoate–maleic anhydride adduct. This afforded the same 2 : 3-dimethylbutanal, characterised as the lævorotatory semicarbazone, as is obtained in the same way from ergosterol and its derivatives retaining the 22(23)-ethylenic linkage.

It was mentioned above that the new strongly lævorotatory benzoate was characterised by an absorption band at 319 mμ. This was interpreted to imply the presence of three double bonds in conjugation, two being present in one ring (Fieser and Fieser, " Natural Products Related to Phenanthrene," Reinhold Publ. Corp., 3rd Edn., p. 188; the calculated $\lambda_{max.}$ for 14-dehydroergosterol is 323 mμ, in excellent agreement with that found by experiment).

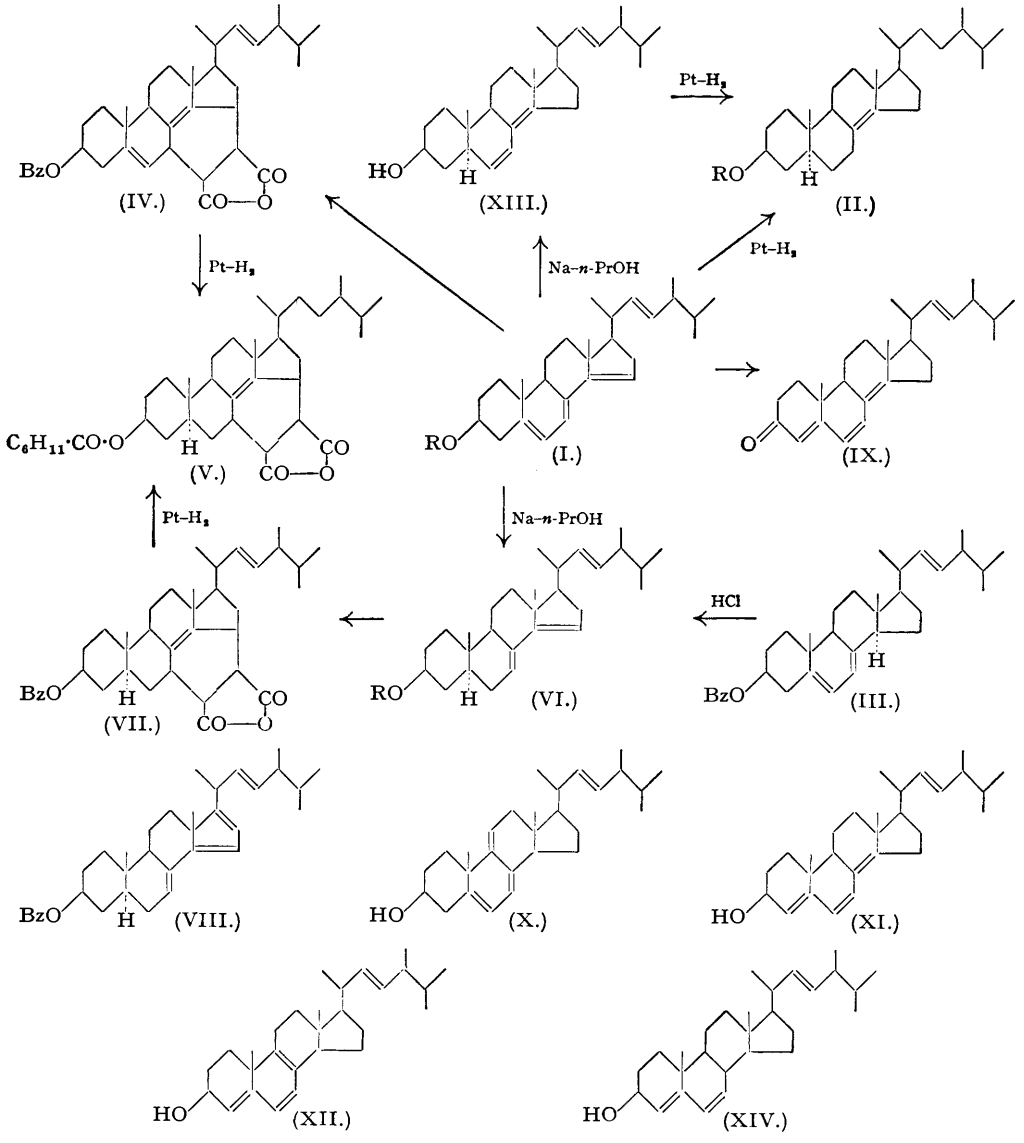


1. 14-*Dehydroergosteryl benzoate.*
2. *Ergosta*-4 : 6 : 8(14) : 22-*tetraen*-3-*one.*
3. *Ergosta*-6 : 8(14) : 22-*trien*-3β-*yl benzoate.*

There are, then, two possible formulæ (I; R = Bz) and (VIII), for the benzoate. A choice between them was made as follows. Although alkaline hydrolysis of the benzoate led to destruction of material by resinification, reductive hydrolysis by lithium aluminium hydride afforded without difficulty the parent sterol (I; R = H), m. p. 198—201° (decomp.), $[\alpha]_D$ −396° (in carbon tetrachloride), characterised as the acetate and by reconversion into the benzoate. Oxidation of the sterol by the Oppenauer method, followed by careful chromatography, gave ergosta-4 : 6 : 8(14) : 22-tetraen-3-one (IX), m. p. 114—115°, $[\alpha]_D$ +590° (in chloroform). The absorption spectrum of this ketone [$\lambda_{max.}$ 348 mμ; $\varepsilon_{max.}$ 26,500 in ethanol (see Fig.)] showed that there were three double bonds in conjugation (in three different rings) with the keto-group (cf. Fieser and Fieser, *op. cit.*, p. 192). In agreement, the corresponding 2 : 4-dinitrophenylhydrazone had $\lambda_{max.}$ 427 mμ, $\varepsilon_{max.}$ 41,000 in chloroform (cf. Braude and Jones, *J.*, 1945, 498). This excludes (VIII) from further consideration, for a conjugated ketone could not have been obtained on Oppenauer oxidation of the corresponding sterol.

It is a possible criticism of this proof of structure that rearrangement of the three conjugated ethylenic linkages might occur during the formation of the maleic anhydride adduct (IV), although such is not the case with ordinary dehydroergosterol (X) (Honigmann, *Annalen*,

1934, **508**, 89). If for the moment this point of view is accepted, there would then be three possible formulæ (I; R = H), (XI), and (XII) which would explain the formation of (IX) on Oppenauer oxidation. Of these, (XI) is possibly excluded on spectral grounds as it should show $\lambda_{max.}$ about 279 mμ in ethanol (Fieser and Fieser, *op. cit.*, p. 188) rather than $\lambda_{max.}$ 323 mμ (see above). Decisive chemical evidence for the exclusion of (XI) and (XII) was obtained by reduction of the new sterol by sodium and boiling *n*-propanol. After benzoylation of the



product, the presence of ergosteryl B_3 benzoate (VI; R = Bz) was shown by adduct formation with maleic anhydride. Careful chromatography of the portion of the benzoate which did not react with maleic anhydride afforded one fraction which, from its absorption spectrum, contained about 50% of ergosteryl benzoate. The main product of reduction was, however, a new conjugated-dienyl benzoate, m. p. 129—130°, $[\alpha]_D$ −104° (in chloroform), $\lambda_{max.}$ 252 mμ, $\varepsilon_{max.}$ 23,000 in ethanol (see Fig.), which had the formula $C_{35}H_{48}O_2$ and was, therefore, a triene. It was further characterised by hydrolysis to the parent sterol and by conversion of the latter into the acetate. As justified in detail below, this triene must be ergosta-6 : 8(14) : 22-trien-

3β-ol (XIII). The formation of this compound, and especially of ergosterol B_3 (VI; R = H) by reduction under conditions where double-bond migrations, outside the extent of the original conjugated triene system, are excluded, provides final proof for the correctness of the formula (I; R = H) for the new sterol from *A. niger*. Incidentally the formation of ergosterol B_3 in this way also proves that the configuration about the 22(23)-ethylenic linkage is the same (*trans*) (Turnbull, Whiffen, and Wilson, *Chem. and Ind.*, 1950, 626; R. Norman Jones, *J. Amer. Chem. Soc.*, 1950, **72**, 5322) as in ergosterol.

The formula (XIII) assigned to the new conjugated diene was deduced from the following evidence. The two conjugated double bonds must be in two different rings because of the absorption spectrum. Catalytic hydrogenation of the acetate afforded "α"-ergostenyl acetate (II; R = Ac) in excellent yield. This excludes formula (XIV), which in any case is known (Güntzel, *Ber.*, 1939, **72**, 1317), in favour of (XIII). In confirmation, isomerisation of the benzoate by hydrogen chloride in chloroform gave ergosteryl B_3 benzoate, characterised as the maleic anhydride adduct. The formation of (XIII) by reduction is an excellent example of 1 : 6-addition.

Formula (XIII) was previously assigned, on the basis, mainly, of exclusion evidence (Barton, *J.*, 1946, 512), to ergosterol B_2. The constants that we now record for (XIII) are in moderate agreement only with those reported for ergosterol B_2 (Windaus, Dithmar, Murke, and Suckfüll, *Annalen*, 1931, **488**, 91).

This is the first recorded occurrence af a tetraethenoid steroid in Nature and also the first example of a naturally occurring sterol with olefinic unsaturation at position 14(15).

Experimental.

M. p.s are uncorrected. Unless specified to the contrary, rotations were determined in carbon tetrachloride at room temperature (15—25°) in a 1-dm. tube. For polarimetry all specimens were dried *in vacuo* at 20° below their m. p.s or at 110°, whichever was the lower. Values of $[\alpha]_D$ have been approximated to the nearest degree.

Absorption spectra were determined, unless specified to the contrary, in absolute ethanol by a Unicam S.P. 500 spectrophotometer.

Savory and Moore's standardised alumina was used throughout, unless specifically indicated to the contrary.

Light petroleum refers in all cases to the fraction of b. p. 40—60°.

In the text below, the phrase " in the usual way " refers to dilution with water, extraction with ether, washing successively with aqueous potassium hydroxide, aqueous hydrochloric acid, and water, followed by evaporation of the ethereal solution *in vacuo*. Where necessary, water was removed from the residue by azeotropic distillation with benzene *in vacuo*.

Alkaline hydrolyses were effected by using several equivalents of potassium hydroxide and refluxing the reactants for 30—60 minutes in methanol or dioxan–methanol according to the solubility of the ester.

Raw Material.—The sample of " ergosterol " from the new strain of *A. niger*, kindly supplied by Messrs. J. and E. Sturge Ltd. through the courtesy of Dr. E. R. S. Winter, had m. p. 156—160°, $[\alpha]_D$ −150° (*c*, 1·06), compared with $[\alpha]_D$ −135° (*c*, 0·59) for pure ergosterol. The absorption spectrum of the crude sterol showed the triple maxima characteristic of ergosterol, the intensity corresponding to 82 ± 3% of ergosterol. The maximum at 320 mμ indicative of 14-dehydroergosterol (see below) corresponded to a content of 13%. The total sterols accounted for thus amounted to 95 ± 3%.

Isolation of 14-*Dehydroergosterol.*—Preliminary experiments failed to show any marked separation of 14-dehydroergosterol on crystallisation of the sterols or their acetates, or on careful chromatography of the latter. The first fraction of the chromatogram (11 fractions in all) had m. p. 171—173°, $[\alpha]_D$ −92° (*c*, 7·63) in chloroform, and was identified as pure ergosteryl acetate. The 14-dehydroergosteryl acetate was more strongly adsorbed. For example the penultimate fraction had m. p. 166—168° $[\alpha]_D$ −180° (*c*, 5·00) in chloroform (see below) and contained, according to the absorption spectrum, 57% of ergosteryl acetate. Fractional precipitation with digitonin indicated that 14-dehydroergosterol formed an insoluble digitonide similar to that given by ergosterol.

The following procedure was ultimately adopted for the separation of 14-dehydroergosterol (as the benzoate). Crude ergosterol (see above) (125 g.) was dissolved with heating in 1 l. of benzene with addition of 150 ml. of dry " technical pyridine." After the mixture had been cooled to room temperature benzoyl chloride (150 ml.) was added with further cooling. After the whole had been left overnight at room temperature water was added and the precipitated crude benzoate filtered off. The benzene layer of the filtrate was separated and a further quantity of crude benzoate precipitated by the addition of an equal volume of alcohol. The combined benzoate fractions were recrystallised eight times from benzene, to give a product (3·5 g.) melting at approx. 205° (decomp.), with $[\alpha]_D$ between −230° and −250° (*c*, approx. 0·5). Four further recrystallisations gave pure 14-*dehydroergosteryl benzoate* (*ca.* 0·5 g.), m. p. 210—212° (decomp.), $[\alpha]_D$ −260° (*c*, 0·49), −274° (*c*, 3·04) in chloroform, $\lambda_{max.}$ 319 mμ, $\varepsilon_{max.}$ 16,000 (Found : C, 84·2; H, 9·4. $C_{35}H_{46}O_2$ requires C, 84·25; H, 9·3%). The m. p. is not sharp and depends on the rate of heating; the same applies to the simple derivatives.

During early experiments on the fractionation of the mixed benzoates, solvent mixtures containing chloroform were employed. Owing to acidity of the chloroform, the results were erratic and on some occasions no 14-dehydroergosteryl benzoate could be isolated. There was, further, difficulty during polarimetry with chloroform which was some months old. In one case 14-dehydroergosteryl benzoate mutarotated in 19 hours at room temperature from $[\alpha]_D$ −75° (after 1 hour in chloroform) to $[\alpha]_D$ +60°. The dextrorotatory product was shown to be a hydrocarbon but was not investigated further. Since 14-dehydroergosteryl benzoate was stable in carbon tetrachloride this solvent was used subsequently for polarimetry.

Alkaline hydrolysis of 14-dehydroergosteryl benzoate in the usual way caused much resinification. Reductive hydrolysis by lithium aluminium hydride proved satisfactory. Because of its sparing solubility in ether the benzoate was extracted from a Soxhlet thimble into ether containing an excess of lithium aluminium hydride. Addition of water, extraction with dilute sulphuric acid, and evaporation of the dried (Na_2SO_4) ethereal solution to small volume gave fine needles, m. p. 195—200° (decomp.), $[\alpha]_D$ −351° (*c*, 0·31). Two recrystallisations from carbon tetrachloride–methanol afforded pure 14-*dehydroergosterol*, m. p. 198—201° (decomp.), $[\alpha]_D$ −396° (*c*, 0·21; *l* = 2-dm.), $\lambda_{max.}$ 319 mμ, $\varepsilon_{max.}$ 17,500 (Found : C, 85·6; H, 10·7. $C_{28}H_{42}O$ requires C, 85·2; H, 10·75%).

Acetylation in pyridine–acetic anhydride overnight at room temperature gave 14-*dehydroergosteryl acetate*, crystallising as needles (from benzene–methanol), m. p. 164—167° (decomp.), $[\alpha]_D$ −322° (*c*, 0·11; *l* = 2 dm.), $\lambda_{max.}$ 319 mμ, $\varepsilon_{max.}$ 15,000 (Found : C, 82·2; H, 9·85. $C_{30}H_{44}O_2$ requires C, 82·5; H, 10·15%).

Benzoylation of 14-dehydroergosterol regenerated the benzoate, $[\alpha]_D$ −257° (*c*, 0·11; *l* = 2-dm.), $\lambda_{max.}$ 319 mμ, $\varepsilon_{max.}$ 16,000.

14-*Dehydroergosteryl Benzoate–Maleic Anhydride Adduct.*—In a preliminary experiment the crude acetate was refluxed with maleic anhydride in benzene solution. After hydrolysis and re-acetylation of the neutral material pure ergosteryl acetate, m. p. 172—174°, $[\alpha]_D$ −92° (*c*, 2·09 in chloroform), was readily obtained. The facility of reaction with maleic anhydride thus indicated was confirmed as follows. 14-Dehydroergosteryl benzoate (0·7 g.) was refluxed in benzene (10 ml.) with freshly distilled maleic anhydride (0·25 g.) for 3 hours. On cooling, the crystalline adduct was deposited. Recrystallisation from benzene gave felted needles of the *adduct*, m. p. 235—240° (decomp.), $[\alpha]_D$ −96° (*c*, 1·93; *l* = 4-dm.), no selective absorption in the region above 235 mμ (Found : C, 78·5; H, 8·05. $C_{39}H_{48}O_5$ requires C, 78·5; H, 8·1%).

In perbenzoic acid titration in chloroform at 0—5° two equivalents of per-acid were rapidly consumed. After 3 days the uptake corresponded to 2·4 equivalents.

Ozonolysis. The adduct (1·0 g.), suspended in acetic acid (50 ml.), was treated with ozonised oxygen for 3 hours until all had dissolved. The reaction mixture was diluted with water (50 ml.) and heated for 10 minutes whilst zinc dust was added. The solution was heated to boiling. 20 Ml. of distillate were collected and treated with semicarbazide hydrochloride (1 g.), and sodium hydrogen carbonate was added (to excess). After cooling at 0—5° overnight the semicarbazone was filtered off; recrystallised from benzene–light petroleum, it had m. p. 133—134°, $[\alpha]_D$ −44° (*c*, 1·05 in chloroform). There was no depression in m. p. on admixture with an authentic specimen of (−)-2 : 3-dimethylbutanal semicarbazone, m. p. 133—134°, $[\alpha]_D$ −47° (*c*, 1·03 in ethanol), −39° (*c*, 1·09 in chloroform), prepared in the same way from " α "-dihydroergosteryl acetate.

Hydrogenation of 14-*Dehydroergosteryl Benzoate.*—Pure 14-dehydroergosteryl benzoate (325 mg.) in anhydrous ether (125 ml.) was added to " AnalaR " acetic acid (100 ml.) and pre-reduced platinum catalyst (200 mg.), and the whole hydrogenated overnight. Working up in the usual way gave, after digestion with methanol, " α "-ergostenyl hexahydrobenzoate (320 mg.), m. p. 128—130°. One recrystallisation from chloroform–methanol furnished the pure hexahydrobenzoate (230 mg.), $[\alpha]_D$ +4° (*c*, 1·77 in chloroform), m. p. 134—135° alone or mixed with an authentic specimen (see below).

" α "-*Ergostenyl Hexahydrobenzoate* [with J. D. Cox and E. Miller].—Hydrogenation overnight of " α "-ergostenyl benzoate, ergosteryl benzoate, or ergosteryl B_3 benzoate, in acetic acid over a platinum catalyst, in each case afforded " α "-*ergostenyl hexahydrobenzoate* which, crystallised from ethyl acetate–methanol, had m. p. 134—135°, $[\alpha]_D$ +3° (*c*, 1·32 in chloroform) (Found : C, 82·1; H, 11·2. $C_{35}H_{58}O_2$ requires C, 82·3; H, 11·4%).

The constitution assigned to this compound was confirmed by alkaline hydrolysis which furnished " α "-ergostenol and hexahydrobenzoic acid, both giving no depression in m. p. on admixture with synthetic specimens of the same m. p.s.

Ergosteryl B_3 *Benzoate–Maleic Anhydride Adduct* [with J. D. Cox].—Ergosteryl B_3 benzoate (1 g.) in dry benzene (25 ml.) was refluxed with excess of maleic anhydride for 30 minutes. The crystalline *adduct* was rapidly deposited. Recrystallised from benzene, it had m. p. 262° (decomp.), $[\alpha]_D$ −62° (*c*, 0·55 in chloroform) (Found : C, 78·8; H, 8·35. $C_{39}H_{50}O_5$ requires C, 78·2; H, 8·4%).

Hydrogenation. Hydrogenation of this adduc tin acetic acid in the presence of a platinum catalyst and working up in the usual way afforded the dihydroergosteryl B_3 hexahydrobenzoate–maleic anhydride *adduct*, m. p. 217—220° (from chloroform–methanol), $[\alpha]_D$ −32° (*c*, 2·22 in chloroform; *l* = 0·5 dm.) (Found : C, 76·6; H, 9·4. $C_{39}H_{58}O_5$ requires C, 77·2; H, 9·65%).

Hydrogenation of 14-*Dehydroergosteryl Benzoate–Maleic Anhydride Adduct.*—Hydrogenation in acetic acid with a platinum catalyst and working up in the usual way also gave dihydroergosteryl B_3 hexahydrobenzoate–maleic anhydride adduct, m. p. 220—222° (from chloroform–light petroleum), $[\alpha]_D$ −28° (*c*, 1·74 in chloroform; *l* = 0·5 dm.), undepressed in m. p. on admixture with the authentic specimen (see above).

Ergosta-4 : 6 : 8(14) : 22-*tetraen*-3-*one*.—14-Dehydroergosterol (0·65 g.) was dissolved in dry acetone (25 ml.)–benzene (25 ml.) with addition of aluminium *tert.*-butoxide (2·0 g.). The solution was refluxed for 4 hours, then poured into cold dilute sulphuric acid, and the benzene layer separated and washed with water. After removal of the benzene *in vacuo*, the gummy residue was dissolved in light petroleum and chromatographed over alumina. Benzene–ether (9 : 1) eluted crystalline ergosta-4 : 6 : 8(14) : 22-tetraen-3-one which, recrystallised from methanol or light petroleum, had m. p. 114—115°, $[\alpha]_D$ +590° (*c*, 0·21 in chloroform; *l* = 2 dm.), $\lambda_{max.}$ 348 mμ, $\varepsilon_{max.}$ 26,500 (Found : C, 85·1; H, 10·4. $C_{28}H_{40}O$ requires C, 85·65; H, 10·25%).

In ethanolic hydrochloric acid the ketone gave the corresponding 2 : 4-*dinitrophenylhydrazone* as very dark red needles, purified by chromatography and recrystallisation from ethyl acetate; it had m. p. 236—237° decomp., $\lambda_{max.}$ 427 mμ, $\varepsilon_{max.}$ 41,000 in chloroform (Found : N, 10·2. $C_{34}H_{44}O_4N_4$ requires N, 9·95%).

Reduction of 14-*Dehydroergosterol with Sodium and* n-*Propanol*.—14-Dehydroergosteryl benzoate (2 g.) was added to boiling *n*-propanol (100 ml.) in which several atomic proportions of sodium had been dissolved. When hydrolysis was complete (0·5 hour) more sodium was added until the *n*-propanol was saturated (4 hours). Dilution with water, extraction with ether, and working up in the usual way gave a mixture which was then benzoylated and combined with the benzoates from the reduction of a further 2·0 g. of 14-dehydroergosteryl benzoate. The benzoate mixture, crystallised once from chloroform–methanol, had $[\alpha]_D$ −92° (*c*, 0·58 in chloroform), $\lambda_{max.}$ 250, 280, and 293 mμ, $\varepsilon_{max.}$ 9000, 1500, and 480 respectively (as well as benzoate absorption). The last two maxima were clearly due to about 10% of ergosteryl benzoate. There was no absorption at 320 mμ and the reduction was therefore substantially quantitative. The mixed benzoates were refluxed in benzene (5 ml.) with maleic anhydride (0·4 g.) for 3 hours. Concentration of the benzene solution *in vacuo* and extraction of unreacted maleic anhydride with water afforded, in small yield, ergosteryl B_3 benzoate–maleic anhydride adduct, m. p. 262° (decomp.) alone or mixed with an authentic specimen (see above), $[\alpha]_D$ −54° (*c*, 0·50 in chloroform).

The residual benzoates were carefully chromatographed in light petroleum over alumina, the progress of the fractionation being followed by both rotation and absorption spectrum. Benzene–light petroleum (1 : 3) eluted material not of relevant interest. Benzene–light petroleum (1 : 1) then eluted material which was collected in five fractions. The last of these contained (absorption spectrum) about 50% of ergosteryl benzoate. Material from the other four, in which the conjugated diene ($\lambda_{max.}$ 252 mμ) had been concentrated, was combined and recrystallised from chloroform–methanol, to give *ergosta*-6 : 8(14) : 22-*trien*-3β-*yl benzoate*, m. p. 129—130°, $[\alpha]_D$ −104° (*c*, 2·13 in chloroform), $\lambda_{max.}$ 234 and 252 mμ, $\varepsilon_{max.}$ 23,000 and 21,500 respectively (Found : C, 83·9; H, 10·05. $C_{35}H_{48}O_2$ requires C, 83·95; H, 9·9%).

Alkaline hydrolysis afforded *ergosta*-6 : 8(14) : 22-*trien*-3β-*ol*, m. p. 113—114° (from methanol), $[\alpha]_D$ −115° (*c*, 2·10 in chloroform), $\lambda_{max.}$ 253 mμ, $\varepsilon_{max.}$ 17,000 (Found : C, 78·3; H, 10·45. $C_{28}H_{44}O,2CH_3{\cdot}OH$ requires C, 78·2; H, 11·4%).

Acetylation with pyridine–acetic anhydride overnight gave the corresponding *acetate*, m. p. 113—114° (from methanol), $[\alpha]_D$ −107° (*c*, 1·98 in chloroform) $\lambda_{max.}$, 253 mμ, $\varepsilon_{max.}$ 17,000 (Found : C, 82·3; H, 10·85. $C_{30}H_{46}O_2$ requires C, 82·15; H, 10·55%).

Treatment of ergosta-6 : 8(14) : 22-trien-3β-yl benzoate (200 mg.) in chloroform with dry hydrogen chloride at 0° for 10 minutes (cf. Barton and Brooks, *J.*, 1951, 277) and working up in the usual way gave crude ergosteryl B_3 benzoate. This was refluxed in benzene (3 ml.) for 3 hours with maleic anhydride (40 mg.). After cooling, water was added and the mixture left overnight. The crystals deposited were recrystallised twice from benzene, to give ergosteryl B_3 benzoate–maleic anhydride adduct, m. p. 260—261° alone or mixed with an authentic specimen (see above), $[\alpha]_D$ −55° (*c*, 0·51 in chloroform).

Hydrogenation of ergosta-6 : 8(14) : 22-trien-3β-yl acetate (100 mg.) in acetic acid overnight over a platinum catalyst and working up in the usual way furnished " α "-ergostenyl acetate, $[\alpha]_D$ ±0° (*c*, 1·74 in chloroform), m. p. 109—110° (from chloroform–methanol) alone or mixed with an authentic specimen of the same m. p. and rotation (Barton and Cox, *J.*, 1948, 783).

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