The Partial Synthesis of Ergosta-5,7,22,24(28)-tetraen-3β-ol

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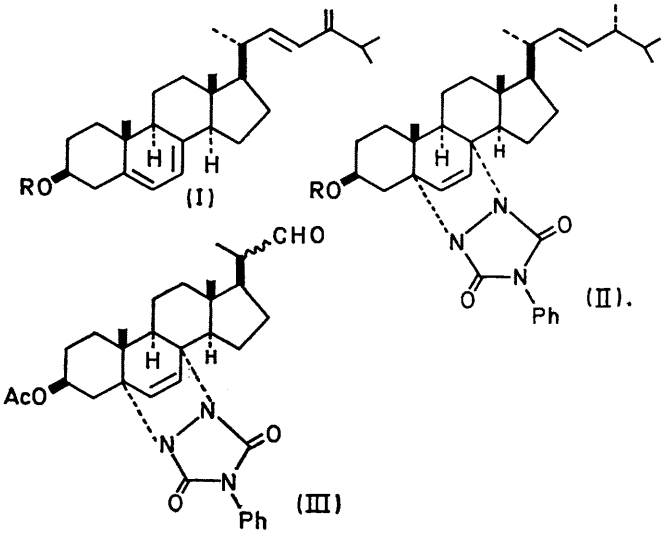
Summary The Diels–Alder adduct between ergosterol acetate and 4-phenyl-1,2,4-triazolin-3,5-dione can be efficiently re-converted into ergosterol by lithium aluminium hydride reduction; using this reaction as a key step, ergosta-5,7,22,24(28)-tetraen-3β-ol has been synthesised in labelled form and shown to be an efficient precursor of ergosterol in yeast.

DURING work on ergosterol biosynthesis[1] we required radioactively labelled ergosta-5,7,22,24(28)-tetraen-3β-ol[2] (I;

R = H). Unlabelled material was synthesised first in the following way. 4-Phenyl-1,2,4-triazolin-3,5-dione,[3] unlike maleic anhydride,[4] gives in high yield a Diels–Alder adduct[5] (II; R = H), with ergosterol. This adduct offers scope for chemical modification of the ergosterol side-chain, provided that the ring-B diene system can be regenerated efficiently. We now report a synthesis of (I; R = H) based upon this approach.



Ergosteryl acetate was treated with the triazoline[3] to give the adduct† (II; R = Ac), (87%), m.p. (from EtOH), 173—175°, $[\alpha]_D^{25}$ − 118°, (*c* 0·98). Similarly prepared in quantitative yield was the benzoate (II; R = Bz), m.p. 191—192°, $[\alpha]_D^{25}$ − 92° (*c* 0·92). The α-orientation of the urazole residue was assigned by analogy with the known chemistry of ergosterol,[6] and by the lack of significant change in the chemical shift of the 19-H_3 protons in the n.m.r.

Reversal of the Diels–Alder reaction was carried out by several methods (Table). Pyrolysis of (II; R = Ac) at 230°/4·5 × 10^{-2} mm. gave a mixture of ergosteryl acetate (36%) and 9(11)-dehydroergosteryl acetate (17%), identified by comparison with an authentic specimen.[7] Of the solvolytic and reductive methods attempted, reduction with lithium aluminium hydride in tetrahydrofuran was the most efficient (Table, No. 1). This process was used in all subsequent experiments. The mechanism of the reversal is the subject of continuing study.

The adduct (I; R = Ac) was ozonised in methylene chloride–methanol at −70° for 37 min. (*ca.* 1·1eq. O_3). Working-up with tris-diethylaminophosphine gave 28% recovered starting material and 34% of hexanoraldehyde

Removal of protecting group from (I;R = Ac)

No.	Reagent	Conditions	Yield of ergosterol (%)
1	$LiAlH_4$	THF, reflux, 18 hr.	99
2	Heat	230—235°, 4·5 × 10^{-2}mm	36[a]
3	Na/EtOH	reflux, 24 hr., under N_2	53
4	$NH_2NH_2 \cdot H_2O$	EtOH, reflux, 14 hr.	30
5	Furan	85°, 16 hr., 100°, 22 hr.	34[a]

[a] The acetate group was retained.

(III), which was shown by n.m.r. spectroscopy to be a 7 : 1 mixture of the 20*S*- and 20*R*-epimers, respectively. Repeated crystallisation from benzene–ether gave pure 20*S*-aldehyde, as an ether solvate, m.p. 175—176°, ν_{max} 1750, 1725, and 1700 cm^{-1}. Alumina chromatography of the aldehyde caused epimerisation to a mixture of aldehydes in the ratio *S* : *R* = 1 : 4 from which the *R*-isomer, m.p. 194—195°, ν_{max} 1750, 1725, and 1703 cm^{-1} could be crystallised. This ready epimerisation was avoided if the ozonolysis was carried out in methylene chloride containing 1% of pyridine at −70° and the pyridine was removed by washing the reaction mixture quickly with dilute hydrochloric acid. The diene side-chain of (I) was constructed by a Wittig reaction of the 20*S*-aldehyde (III) with the phosphorane derived from 2-isopropylallyl bromide (prepared from the corresponding alcohol[8]) and triphenylphosphine. After acetylation of the product and reduction with lithium aluminium hydride ergosta-5,7,22,24(28)-tetraen-3β-ol (approx. 50%) was obtained. It was identified as such, and as its benzoate (I; R = Bz) by full comparison with authentic specimens kindly supplied by Dr. O. N. Breivik (Fleischmann Laboratories, New York). This synthesis confirms the structure assigned to the natural tetraene. This structure rests otherwise mainly on physical data.

The tetraenol (I; R = H) was re-synthesised in radioactive form using [1,3-$^{14}C_2$]-2-allylisopropyl bromide.[9] This was fed to growing cultures of *Saccharomyces cerevisiae* (Strain NRRL Y2250), under aerobic and anaerobic conditions. After 2 days, the cells were harvested and the ergosterol produced was rigorously purified *via* the adduct (II; R = Ac).[10] The incorporations were 26% and 25%, respectively. The reverse process, from labelled ergosterol to tetraenol (I; R = H), was shown by similar experiments not to occur. These results are in agreement with the conclusion of Katsuki and Bloch[11] as to the terminal step in ergosterol biosynthesis.

The simple selective and reversible protection of the ring-B diene system of ergosterol may be of importance in the synthesis of side-chain-substituted analogues of vitamin D.

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† All new compounds had correct analyses, and where not specifically discussed, the expected spectroscopic data.

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