Steroidal Allenes as Inhibitors of Sterol Biosynthesis

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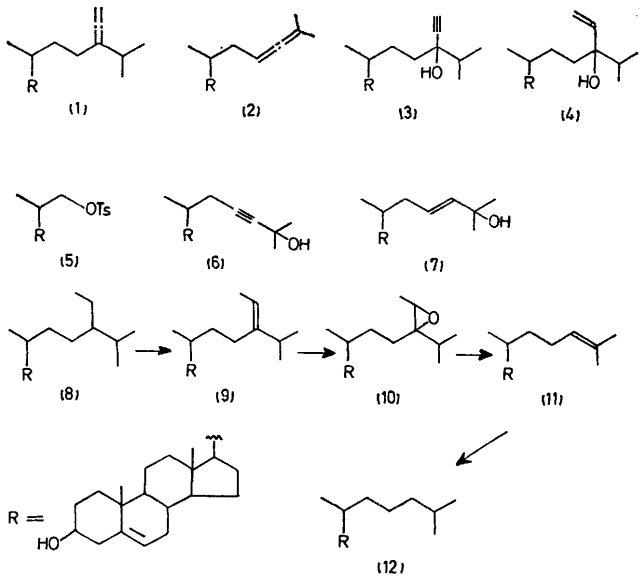
Summary Allenic analogues (**1**) and (**2**) of fucosterol (**9**) and desmosterol (**11**) were synthesized and the former was found to be a specific inhibitor of sterol metabolism in the silkworm.

THE terminal stages of sterol biosynthesis in Δ^{24}-sterols such as fucosterol (**9**) and desmosterol(**11**),[1] consist of hydrogenation, alkylation and/or dealkylation. We have chosen the allenic sterols (**1**) and (**2**) as possible inhibitors of sterol biosynthesis on the basis of previous observations[2] that some allenic compounds with close structural similarity to the olefinic substrates are potent and specific inhibitors of β-hydroxydecanoyl thioester dehydrase, a key enzyme system in unsaturated fatty acid biosynthesis.

Reaction of 24-oxocholesterol tetrahydropyranyl (THP) ether with sodium acetylide[3] in liquid ammonia followed by treatment with acid, gave the ethynyl alcohol (**3**) (61%). Reduction of (**3**) with $LiAlH_4$–$AlCl_3$[4] in tetrahydrofuran gave saringosterol (**4**)[3] (42%) and stigmasta-5,24(28),28-trien-3β-ol (**1**) (46%), m.p. 105—106°, ν_{max} 1950 and 850 cm^{-1} (allene), δ ($CDCl_3$) 4·68 (2H, m, 29-H_2), M^+ 410·3586.

The second allene, cholesta-5,23,24-trien-3β-ol (**2**), was prepared from 3-acetoxybisnorcholenic acid, which was converted in 5 steps to the tosylate (**5**) in 70% overall yield. This was coupled with lithium 2-methyl-3-butyn-2-ol THP ether in dioxan solution[5] to yield the propargylic alcohol (**6**) in 70% yield after cleavage of the THP group. Treatment of (**6**) with $LiAlH_4$–$AlCl_3$[4] provided the allylic alcohol (**7**) (80%), and the allene (**2**) (13%), m.p. 109—111°, ν_{max} 1970 cm^{-1} (allene), δ ($CDCl_3$) 1·66 (6H, d, J 3 Hz, 26,27-Me_2) and 4·85 (1H, m, 23-H), M^+ 382·3229.

The silkworm *Bombyx mori* transforms[6] sitosterol (**8**), *via* (**9**), fucosterol-24,28-oxide (**10**) and (**11**), into cholesterol (**12**) which is the major sterol in the insect and is the precursor of ecdysone. When examples of *B. mori* were reared on (**10**), (**11**) or (**12**), addition of the allene (**1**) to the diet brought about almost no effect on insect growth and development.[7] Gas chromatographic analysis of sterol compositions[7] on the eleventh day after hatching (Table) indicated that sterol metabolism in *B. mori* was also not significantly influenced by the added allene. However, the inhibitory effect of (**1**) emerged when the sterol source was (**8**) or (**9**). Dealkylation of the dietary sterols (**8**) or (**9**) was severely retarded and the silkworms remained in the second instar due to moulting disruption which is probably caused by depletion of (**12**). Thus, these results strongly suggest that the allene (**1**) specifically inhibits the conversion of (**8**) → (**9**) and/or (**9**) → (**10**) in *B. mori*.



When the insects were fed with (**8**), (**11**) or (**12**), in combination with the same amount of the other allene (**2**), growth and development were severely retarded resulting in mortality before reaching the second instar. In this situation the critical sterol appeared to be (**12**). Thus, the allene (**2**) may be acting not *via* inhibition of sterol dealkylation but in another unspecified manner.

TABLE. Sterol compositions (%) in *B. mori*.

Added sterol[a]	Cholesterol (**12**)	Desmosterol (**11**)	Sitosterol (**8**)	Fucosterol (**9**)	Others[b]
Sitosterol (**8**)	25	12	55	0	8
Sitosterol (**8**) + allene (**1**)	11	0	75	0	14
Fucosterol (**9**)	49	16	2	30	3
Fucosterol (**9**) + allene (**1**)	21	3	0	51	25
Epoxide (**10**)	38	51	5	0	6
Epoxide (**10**) + allene (**1**)	44	49	4	0	3
Desmosterol (**11**)	36	57	4	0	3
Desmosterol (**11**) + allene (**1**)	39	54	4	0	3
Cholesterol (**12**)	80	3	6	0	11
Cholesterol (**12**) + allene (**1**)	66	2	5	0	27

[a] Both the nutritional sterols and the allene (**1**) were added at 0·2% of the semi-synthetic diet.[7]
[b] When allene (**1**) was added, a large amount of the remaining allene (**1**) was detected in addition to 'others.'

A comparison of the results observed with the allenes (**1**) and (**2**) seems to suggest a structural dependence for their respective inhibitory activity.

It is interesting to note that *B. mori* reared on the allene (**1**) as the sole sterol source have survived into the second instar for more than 20 days without moulting to the third instar, showing that the allene (**1**) partially satisfied the insect sterol requirement. In contrast, the allene (**2**) was completely inadequate as an essential nutrient, because all insects reared on (**2**) died in the first instar.

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