

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.

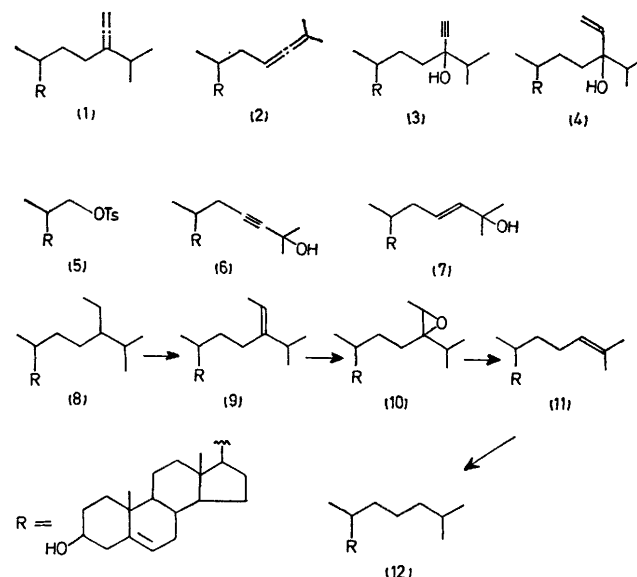
THE terminal stages of sterol biosynthesis in Δ^{24} -sterols such as fucosterol (9) and desmosterol (11),¹ consist of hydrogenation, alkylation and/or dealkylation. We have chosen the allenic sterols (1) and (2) as possible inhibitors of sterol biosynthesis on the basis of previous observations² that 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 acetylide³ in liquid ammonia followed by treatment with acid, gave the ethynyl alcohol (3) (61%). Reduction of (3) with $\text{LiAlH}_4\text{-AlCl}_3$ ⁴ in tetrahydrofuran gave saringosterol (4)³ (42%) and stigmasta-5,24(28),28-trien-3 β -ol (1) (46%), m.p. 105–106°, ν_{max} 1950 and 850 cm^{-1} (allene), δ (CDCl_3) 4.68 (2H, m, 29-H), M^+ 410.3586.

The second allene, cholesta-5,23,24-trien-3 β -ol (2), was prepared from 3-acetoxybisanorcholeonic 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 solution⁵ to yield the propargylic alcohol (6) in 70% yield after cleavage of the THP group. Treatment of (6) with $\text{LiAlH}_4\text{-AlCl}_3$ 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* transforms⁶ 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 compositions⁷ 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) $\rightarrow$ (9) and/or (9) $\rightarrow$ (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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¹ L. J. Mulheirn and P. J. Ramn, *Chem. Soc. Rev.*, 1972, **1**, 259.

² K. Endo, G. M. Helmkamp, and K. Bloch, *J. Biol. Chem.*, 1970, **245**, 4293; M. Morisaki and K. Bloch, *Bioorg. Chem.*, 1971, **1**, 188; *Biochemistry*, 1972, **11**, 309. For an excellent review on related inhibitors, see: R. R. Rando, *Science*, 1974, **185**, 320.

³ N. Ikekawa, K. Tsuda, and N. Morisaki, *Chem. and Ind.*, 1966, 1179.

⁴ W. T. Borden and E. J. Corey, *Tetrahedron Letters*, 1969, 313; L. A. van Dijck, B. J. Lankwerden, J. G. C. M. Vermeer, and A. J. M. Weber, *Rec. Trav. Chim.*, 1971, **90**, 801.

⁵ J. J. Partridge, S. Faber, and M. R. Uskoković, *Helv. Chim. Acta*, 1974, **185**, 320.

⁶ Y. Fujimoto, N. Awata, M. Morisaki, and N. Ikekawa, *Tetrahedron Letters*, 1974, 4335; M. Morisaki, H. Ohtaka, M. Okubayashi, N. Ikekawa, Y. Horie, and S. Nakasone, *J.C.S. Chem. Comm.*, 1972, 1275.

⁷ Experimental procedures were essentially the same as those described in previous papers, see: M. Morisaki, H. Ohtaka, N. Awata, N. Ikekawa, Y. Horie, and S. Nakasone, *Steroids*, 1974, **24**, 165; Y. Fujimoto, M. Morisaki, N. Ikekawa, Y. Horie, and S. Nakasone, *ibid.*, p. 367.