An Unusual Ecdysteroid, (20*S*)-Cholesta-7,14-diene-3β,5α,6α,20,25-pentaol (Bombycosterol) from the Ovaries of the Silkworm, *Bombyx mori*

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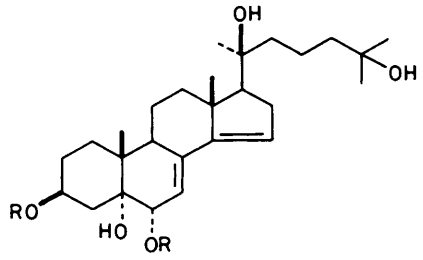
A novel ecdysteroid, for which the name bombycosterol is proposed, has been isolated from the ovaries of the silkworm *Bombyx mori* and its structure was determined as (20*S*)-cholesta-7,14-diene-3β,5α,6α,20,25-pentaol (**1**) by spectroscopic means and ^{1}H n.m.r. comparison with a reference compound (**3**).

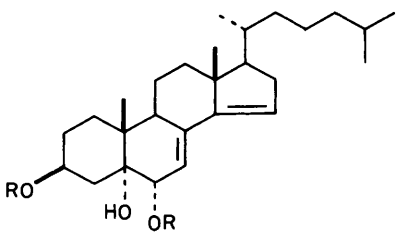
A number of ecdysteroids have been isolated from animal and plant sources.[1] An A/B-*cis*-14α-hydroxy-7-en-6-one structure which is commonly involved in ecdysteroids is known to be important for ecdysone activity.[2] We previously characterized four ecdysteroids, including 2,22-dideoxy-20-hydroxyecdysone,[3] which meet this structural criterion, in the pupal ovaries of the silkworm *Bombyx mori*[4,5] and indicated the presence of an unidentified steroidal substance.[4] We have now characterized this substance as (20*S*)-cholesta-7,14-diene-3β,5α,6α,20,25-pentaol (**1**), for which we propose the name bombycosterol, by spectroscopic means and ^{1}H n.m.r. comparison with compound (**3**).[6] The structure of (**1**), particularly the 5α,6α-glycol moiety, is novel[7] and casts light on the biosynthesis of ecdysteroids; however, a preliminary study showed that bombycosterol does not exhibit moulting hormone activity as bioassayed with *Sarcophaga peregrina*.

The ecdysteroid fraction [hydrolysate of ecdysone conjugate fraction was combined as it also contains (**1**)] was obtained from the ovaries (wet weight 3.5 kg) of *B. mori* as reported previously.[4] Reverse-phase h.p.l.c. (Wakogel ODS-10K, 61.5% aq. methanol) yielded 0.5 mg of bombycosterol as an amorphous solid: $C_{27}H_{44}O_5$ (M^+ 448) by field-desorption mass spectrometry; u.v. (EtOH) 243 nm; Fourier-transform i.r. 3400 (OH) and 1638 cm^{-1} (weak, diene) with no carbonyl absorption near 1660 cm^{-1}; diacetate (**2**) (obtained using Ac_2O, pyridine, room temp.), $C_{31}H_{48}O_7$ (M^+ 532) by electron-impact mass spectrometry (e.i.–m.s.). The following evidence supports the assignment of structure (**1**).

A series of e.i.–m.s. peaks for (**1**) at *m/z* 145 (side chain, $C_8H_{17}O_2$), 127, 109, 59, and 43 supported the presence of a



(**1**) R = H
(**2**) R = Ac



(**3**) R = H
(**4**) R = Ac

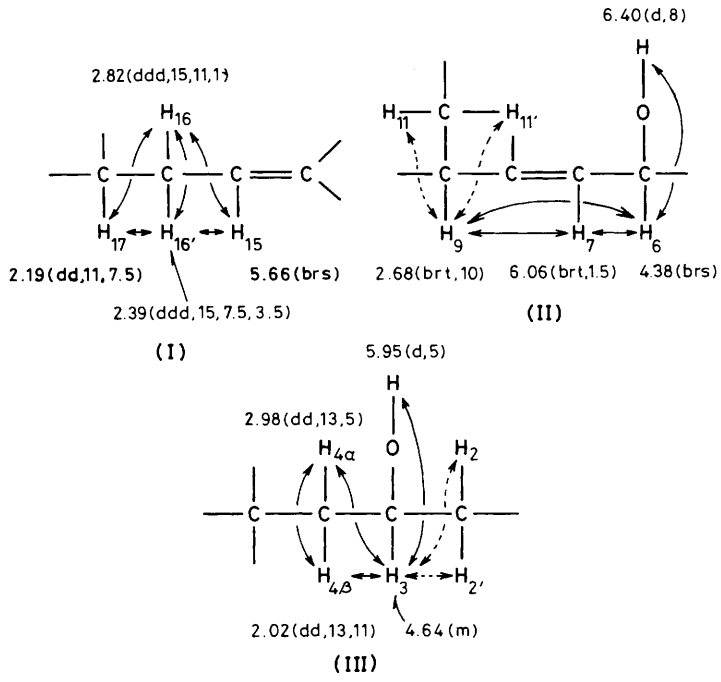
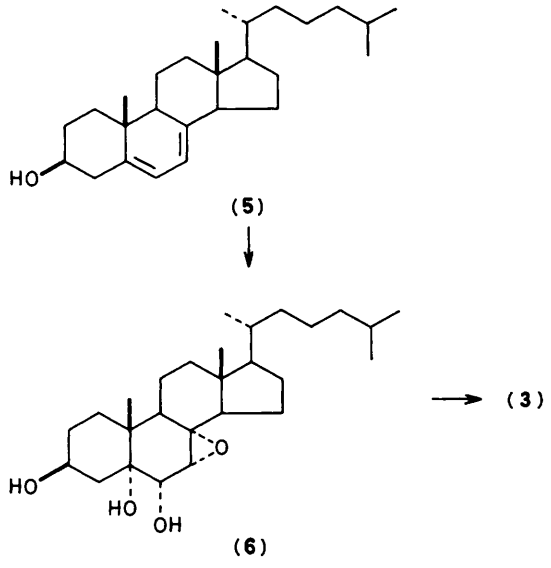


Figure 1. The partial structure and coupling networks derived from *J*-resolved two-dimensional ^{1}H n.m.r. spectroscopy (360 MHz, in C_5D_5N): δ values, multiplicity, and *J*/Hz. The couplings shown by dotted lines were not ascertained owing to the cross peaks, but were indicated from the splitting pattern of 3- and 9-H.

20,25-dihydroxy side chain as observed with 2,22-dideoxy-20-hydroxyecdysone.[3] The ^{1}H n.m.r. spectrum of (**1**) (C_5D_5N) showed five singlet methyl resonances (Me-26 and -27 are counted separately) [δ 1.12 (Me-19), 1.42 (Me-18), 1.425 (Me-26, 27), and 1.54 (Me-21)], two CHO*H* (δ 4.38 and 4.64), and two olefinic protons (δ 5.66 and 6.06). The presence of the partial structures (I)–(III) (Figure 1) was deduced by *J*-resolved two-dimensional ^{1}H n.m.r. spectroscopy.† The u.v. (243 nm in ethanol)‡ and i.r. (1635 cm^{-1}) data of (**3**) are in good agreement with those of (**1**). The ^{1}H n.m.r. data of (**1**) and (**3**), and their corresponding 3,6-acetates (**2**) and (**4**), were compared.§ The signals for (**1**) and (**2**) are superimposable on those of (**3**) and (**4**) except for minor differences for 15- and 16-H which are apparently due to the 20-OH group.

For the determination of the stereochemistry at C-6 of the 5α,6ξ-glycol moiety, the chemical shift and coupling patterns of the 4-methylene protons in the ^{1}H n.m.r. spectrum (C_5D_5N) were useful. This method is simpler than that based on $J_{6,7}$ values.[8] The 4α-protons of 6α-isomers such as (**1**), cholest-7-ene-3β,5α,6α-triol, cholesta-7,14-diene-3β,5α,6α-triol (**3**), and cholestane-3β,5α,6α-triol consistently resonate at δ *ca.* 3.0, whereas the 4β-protons of 6β-isomers such as cholest-7-ene-3β,5α,6β-triol and cholestane-3β,5α,6β-triol resonate at δ *ca.* 3.0.[8,9] In addition pyridine-induced deshielding was observed for the Me-19 signal of the 6β-isomers as expected.



Scheme 1

The stereochemistry at C-20 in (**1**) was assumed to be *S* since (20*S*)-2,22-dideoxy-20-hydroxyecdysone is also present together with (**1**) in the same source[3] and this was supported by ^{1}H n.m.r. data (C_5D_5N) for (20*S*)- and (20*R*)-20-hydroxycholesterol; the Me-21 resonance (δ 1.54) of (**1**) is closer to the value (δ 1.51) for the Me-21 resonance of the 20*S*-isomer than that (δ 1.36) of the 20*R*-isomer.

Permanganate oxidation of 7,8-didehydrocholesterol (**5**) afforded 7α,8α-epoxycholestane-3β,5α,6α-triol (**6**) which

† The 2-dimensional *J* spectra were recorded with a Nicolet NT-360 spectrometer; we are indebted to Drs. T. Iwashita (Suntory Institute for Bio-organic Research) and M. Ishiguro (Suntory Institute for Biomedical Research) for the measurements.

‡ The isomeric 7,9(11)-diene was reported in ref. 6(b) to have a similar u.v. absorption maximum but with shoulders ($\lambda_{max.}$ 236, 242, and 250 nm).

§ ^{1}H N.m.r. data: (**2**) ($CDCl_3$) 5.09 (3α-H), 5.24 (6β-H), 5.47 (7-H), 5.68 (15-H), 1.03 (Me-18, 19), 1.23 (Me-21, 26, 27), 2.02 (3-OAc), and 2.14 (6-OAc); (**3**) (C_5D_5N) 5.57 (15-H), 2.49 (16-H), 2.30 (16′-H), 0.64 (Me-18), 0.97 (Me-21), and 0.89 (Me-26, 27); (**4**) ($CDCl_3$) 5.65 (15-H), 0.84 (Me-18), 0.92 (Me-21), and 0.86 (Me-26, 27). The data for (**3**) and (**4**) omit resonances which coincide with those for (**1**) and (**2**), respectively.

could be converted by acid treatment into the 7,14-diene-5α,6α-glycol (**3**) (Scheme 1).[6] This type of reaction could be considered as a biomimetic one involved in the biosynthesis of bombycosterol. Further, the 5α,6α-diol moiety in (**1**) is reminiscent of the 7-en-5α,6α-epoxide which was previously postulated[10] as the intermediate in the bioconversion[11] of cholesterol into the 7-en-6-one compound, although the stereochemical course of the epoxide ring opening is still in question.[12]

Furthermore it is suggested that a Lewis acid-catalysed type rearrangement of a 7-ene (or 7,14-diene) 5α,6α-epoxide could be a possible mechanism for the formation of a 7-en-6-one with the 5β-stereochemistry of ecdysone because the reaction is chemically conceivable, and should afford a compound with the correct 5β-stereochemistry.

Since there remains the possibility that bombycosterol is derived from some unstable compound¶ such as the 5α,6α-epoxide during the course of extraction and isolation procedures, experiments are in hand to see whether it is actually present in ovaries as such.

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References

1 C. Hetru and D. H. S. Horn, 'Progress in Ecdysone Research,' ed. J. A. Hoffmann, Elsevier–North-Holland Biomedical Press, Amsterdam, 1980, p. 13.

2 R. Bergamasco and D. H. S. Horn, ref. 1, p. 299.

3 N. Ikekawa, T. Ikeda, T. Mizuno, E. Ohnishi, and S. Sakurai, *J. Chem. Soc., Chem. Commun.*, 1980, 448.

4 E. Ohnishi, T. Mizuno, N. Ikekawa, and T. Ikeda, *Insect Biochem.*, 1981, **11**, 155, in which bombycosterol is referred to as compound V.

5 (a) E. Ohnishi, T. Mizuno, N. Ikekawa, and N. Awata, *J. Insect Physiol.*, 1977, **23**, 317; (b) E. Ohnishi, T. Mizuno, F. Chatani, N. Ikekawa, and S. Sakurai, *Science*, 1977, **197**, 66.

6 Compound (**3**) and the corresponding 3,6-diacetate (**4**) were prepared by a slight modification of the published method: (a) M. Anastasia, A. Fiecchi, and A. Scala, *Tetrahedron Lett.*, 1979, 3323; (b) M. Anastasia, G. C. Galli, and P. Allevi, *J. Org. Chem.*, 1979, **44**, 4984.

7 To our knowledge there is only one precedent for ecdysteroids having a 7,14-diene system: S. Imai, E. Murata, S. Fujioka, T. Matsuoka, M. Koreeda, and K. Nakanishi, *J. Am. Chem. Soc.*, 1970, **92**, 7510.

8 *E.g.*, S. P. Gunasekera and F. Schmitz, *J. Org. Chem.*, 1983, **48**, 885. This reference includes ^{1}H n.m.r. data for a hydroxylated sterol (9α,11α-epoxycholest-7-ene-3β,5α,6β,19-tetraol 6-acetate), isolated from a sponge, which is in conflict with our observation. Detailed analysis of the original spectra, kindly provided by Professor Schmitz, led to the conclusion that the C-6 stereochemistry of this marine sterol should be revised.

9 Y. Fujimoto and N. Ikekawa, unpublished work.

10 H. H. Rees, T. G. Davies, L. N. Danan, W. J. S. Lookley, and T. W. Goodwin, in ref. 1, p. 125.

11 C. C. Hetru, C. Kappler, J. A. Hoffmann, R. Nearn, L. Bang, and D. H. S. Horn, *Mol. Cell. Endocrinol.*, 1982, **26**, 51.

12 For the stereochemistry of the peracid epoxidation and simultaneous epoxide opening to the diol of a 5,7-diene system, see: (a) M. Fieser, A. Quilico, A. Nickon, W. Rosen, E. J. Tarlton, and L. F. Fieser, *J. Am. Chem. Soc.*, 1953, **75**, 4066; (b) G. H. Alt and D. H. R. Barton, *J. Chem. Soc.*, 1954, 1356.

¶ For instance, we observed that 5α,6α-epoxycholest-7-en-3β-ol acetate decomposes when in contact with SiO_2 over an extended period.