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**Oxygenated Sterol Derivatives. Their Identification from
the Fungus-Infected Silkworm Carcass, *Bombyx cum*
Botryte and their Effects on Growth and Sterol
Metabolism of the Silkworm, *Bombyx mori****

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Oxygenated sterol derivatives such as 7-oxocholesterol (2), 7 α - and 7 β -hydroxycholesterol (3 and 4) and 25-hydroxycholesterol have been shown to be potent inhibitors of sterol biosynthesis and β -hydroxy- β -methylglutaryl (HMG) coenzyme A (CoA) reductase activity when added to mammalian cultured cells. Following the blockage of sterol synthesis, the concentration of sterol in the cells declines, growth ceases, and eventually the cells die. On the other hand, Cheng *et al.* isolated ergosterol peroxide (1), 7 α -hydroxycholesterol (3) and 7 β -hydroxy-cholesterol, -campesterol, and -sitosterol (4—6) from *Bombyx cum Botryte*, consisting of silkworms (*Bombyx mori*) killed by infection with the microscopic fungus *Botrytis bassiana* Bals. The "animal drug" *B. cum Botryte* (僵蚕) has been used in China for treatment of several diseases including cancer, and some of the above-mentioned 7-hydroxylated compounds and other oxygenated sterols were reported to be cytotoxic to rat hepatoma cells.

We considered that the death of silkworms following fungus infection might be due to the cytotoxic effect of the oxygenated sterols, which may be produced by a cooperative action of the host (silkworm) and invader (fungus). However, since the insect lacks *de novo* sterol synthesis, these compounds should be nontoxic to the insect if HMG CoA reductase is the sole target enzyme involved in the inhibitory effect of oxygenated sterols. In order to examine these possibilities, we first attempted to confirm the presence of oxygenated sterols in *B. cum Botryte*, and then the effects of several oxygenated cholesterol derivatives on the growth and sterol metabolism of the silkworm *B. mori* were investigated.

The sterols 1—6 were identified, *albeit* in very minute amounts (less than 1% of total sterol) from *B. cum Botryte* by gas chromatography-mass spectrometric analysis.

Then the larvae of the silkworm *B. mori* were reared on a diet which contained 0.1% cholesterol or sitosterol in combination with 0.01% oxygenated sterols (1, 2, 4, 15-oxo-5 α -cholest-8(14)-en-3 β -ol, (20S)-20-hydroxycholesterol and 25-hydroxycholesterol).

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Most of the larvae developed to the third instar and survived at least till 20 days after hatching. Their mean body weights were not very different from the control value. It may be concluded that the effect of the oxygenated sterols, which were added to diet at a much higher concentration than was found in the carcass of *B. cum Botryte*, was too small to induce mortality of *B. mori*. Thus, the death of *B. cum Botryte* is probably not caused by the oxygenated sterols.