number 1 is a common feature of the 3-carbon aliphatic substrates, with a 2nd primary alcohol on carbon atom number 3, and separated by a secondary alcohol (as in the case of glycerol) or a ketone group (as in the case of dihydroxyacetone). Alternatively, an aldehyde may replace the 2nd secondary alcohol group (as in diglyceraldehyde). Neither an acid nor a methyl group permits substrate activity as seen by the absence of such activity with glyceric acid and propanediol, respectively[9]. Thus, the active component of PPP awaiting identification is likely to be a small molecule of the order of mass probably of the monomer or dimer, but possibly with several sites for accepting ATP, since from the table the presence of a concentration of LC 1259 of 80 μg ml^{-1} produced an apparent concentration of glycerol of 26 μmoles ml^{-1} or approximately 240 μg ml^{-1}.

In view of these findings, the previously-reported lipolytic effects of PPP are being re-examined.

It is possible that the inhibitory action of PPP on (ATP-dependent) glucose uptake by fat cells[5] may be associated also with phosphorylation of PPP.

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2 Present address: Unit for Metabolic Diseases, Synthelabo-LERS, 31, Avenue Paul Vaillant-Couturier, F-92220 Bagneux, France.

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Identification of both fucosterol and isofucosterol in the silkworm, *Bombyx mori*

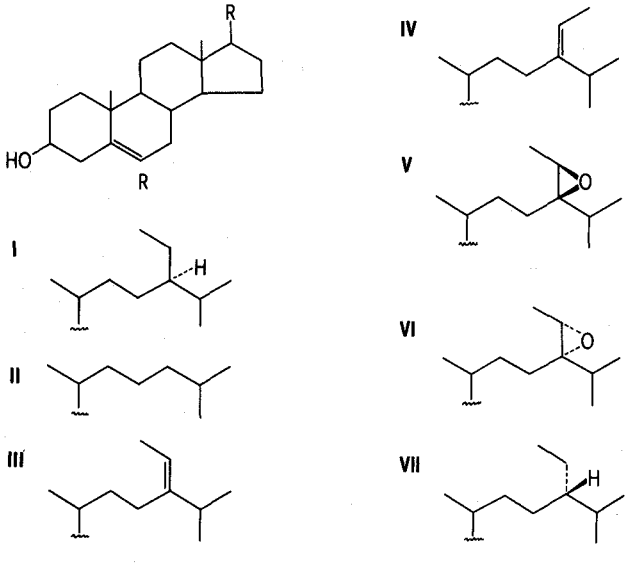
M. Morisaki, B. Ying and N. Ikekawa

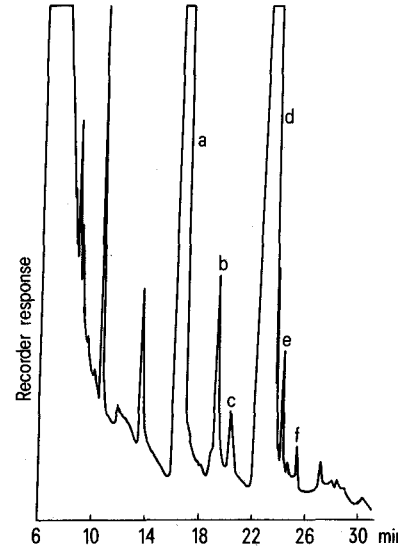
Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 227 (Japan), 8 September 1980

Summary. Fucosterol and isofucosterol were identified in the silkworm, *Bombyx mori.*

Since phytophagous insects have no ability to synthesize sterols de novo, they utilize plant sterols, e.g. sitosterol (I), by converting them into cholesterol (II) for their growth and development. Therefore, the C-24, 28 carbon-carbon bond cleavage reaction of I is an important metabolic process for insects. Although fucosterol (III) seems to be established as an intermediate in the conversion of sitosterol to cholesterol[1-4], a similar role for isofucosterol (IV) has remained obscure[3-5]. Recent observations by Nicotra et al.[6] that *Tenebrio molitor* transforms [^{3}H]-sitosterol into both fucosterol and isofucosterol prompted us to describe our successful identification of fucosterol and isofucosterol in the silkworm, *Bombyx mori.*

The 5th instar larvae (50 species) of *B. mori* reared on mulberry leaves were extracted with chloroform-methanol (1:1). The extract was fractionated with column chromatog-





Sterols in the silkworm, *Bombyx mori* as analyzed with their trimethylsilyl ethers by gas chromatography. An open-tubular glass capillary column (40 m) coated with OV-17 was used at 270 °C. Peaks at retention times of 17.0, 19.4, 20.5, 23.6, 24.4 and 25.5 min were assigned by comparison with the authentic samples, to cholesterol (a), campesterol (b), stigmasterol (c), sitosterol (d), fucosterol (e) and isofucosterol (f), respectively.

raphy on silica gel and the sterol mixture (285 mg) was obtained from the eluate with benzene-ethyl acetate (9:1). This was analyzed as trimethylsilyl (TMS) ethers by gas chromatography with an open-tubular glass capillary column coated with OV-17 (40 m) at 270 °C. As indicated in the figure, fucosterol (1.6%) and isofucosterol (0.8%) were detected, together with cholesterol (41.5%), campesterol (3.3%), stigmasterol (1.2%) and sitosterol (51.6%). To confirm this, the sterol mixture was treated with pyridine-acetic anhydride and the resulting acetate was subjected to TLC on a silver nitrate impregnated silica gel G plate. Development with hexane-benzene (4:1) 5 times separated fucosterol acetate ($R_f = 0.35$) from isofucosterol acetate ($R_f = 0.30$). Each fraction was saponified, derivatized to the TMS ethers and then analyzed with a gas chromatograph-mass spectrometer using 3% OV-17 on Chromosorb W HP (1.5 m) at 248 °C. Selected ion monitoring on m/z 484 (M^+), 469 ($M-CH_3$) and 386 (M-McLafferty fragment ion) and 129 gave prominent peaks at the retention time of 12.2 min (fucosterol TMS ether) or 12.9 min (isofucosterol TMS ether). These results clearly showed the presence of both fucosterol and isofucosterol in *Bombyx mori*. Separate experiments indicated that mulberry leaves, on which the insects were reared, contain no appreciable amount of these sterols.

We have previously identified both (24R, 28R)- and (24S, 28S)-fucosterol epoxide (V) and (VI) in *B. mori* as intermediates of fucosterol dealkylation[7,8]. These results, together with the findings that sitosterol (I) and clionasterol (VII) indifferently satisfied the sterol requirement of *B. mori*[7], appear to suggest a rather loose stereospecificity in sitosterol dealkylation. However, species differences in the stereochemical course seem also to be evident from the recent observations that isofucosterol but no fucosterol was identified in the honey bee, *Apis mellifera* fed synthetic diets[9], and *Tenebrio molitor* larvae converted (24R, 28S)-isofucosterol epoxide, but not its (24S, 28R)-diastereoisomer, into cholesterol[10].

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Chitin synthesis inhibiting insect growth regulators do not inhibit chitin synthase

R.T. Mayer, A.C. Chen[1] and J.R. DeLoach

Veterinary Toxicology and Entomology Research Laboratory, Agricultural Research, Science and Education Administration, US Department of Agriculture, College Station (Texas 77841, USA), 22 August 1980

Summary. Tunicamycin, an antibiotic, and 5 insect growth regulators (diflubenzuron, Bay Sir 6874, Bay Sir 8514, CGA-19255 and Lilly 494) do not inhibit chitin synthase obtained from 4-day-old *Stomoxys calcitrans* pupae.

Since the introduction of insect growth regulators that putatively inhibit chitin production a number of articles have appeared that have dealt with their mode of action. Because diflubenzuron[2] (Dimilin, TH-6040; N-[[(4-chlorophenyl)amino]carbonyl]-2,6-difluorobenzamide) was one of the first chitin synthesis inhibitors (CSI) reported[3-5] it has received the most attention. Although there is agreement that diflubenzuron disrupts the formation of insect cuticle there is disagreement as to the molecular basis for this.

Ishaaya and Casida[6] observed an increase in chitinase activity in tissues obtained from house fly larvae reared on diets containing diflubenzuron. This observation led them to suggest that the reduced amounts of chitin in diflubenzuron-treated insects was the result of enhanced chitinase activity. However Deul et al.[5] repeated the experiments using *Pieris brassicae* (L.) larvae and found no effect on chitinase activity, but chitin deposition was affected.

Other investigators have suggested that diflubenzuron acts directly on the chitin synthase (EC 2.4.1.16; UDP-2-acetyl-amino-2-deoxy-D-glucose chitin 4-β-acetamidodeoxyglucosyltransferase)[5,7-9] which is the terminal enzyme in the synthesis of chitin. These suggestions are based on in vivo and in vitro organ culture work that showed a buildup of UDP-N-acetyl-D-glucosamine (UDP-GlcNAc) in tissues treated with certain benzoylphenylurea CSI. Because UDP-GlcNAc is the obligatory substrate for chitin synthase it was logical to assume that chitin synthase was inhibited by diflubenzuron and UDP-GlcNAc accumulated as a result. Although this is a logical explantation for the mode of action of diflubenzuron and other CSI, Van Eck[9] and Mayer et al.[10] suggested that caution should be exercised in interpreting results from in vivo and in vitro organ culture experiments. Such experiments, at best, can only give indirect evidence for a mode of action.

Meola and Mayer[11] showed that in diflubenzuron treated stable fly (*Stomoxys calcitrans*, L.) pupae chitin synthesis did not occur because adult epidermis was not produced; diflubenzuron appeared to inhibit the proliferation of imaginal epidermal cells. This observation indicated that a different mode of action was operating, but it did not eliminate the possibility that there could be a direct inhibitory effect of diflubenzuron on chitin synthase.

Recent work performed by Cohen and Casida[12] and Mayer et al.[13] provided evidence that diflubenzuron did not inhibit chitin synthase. Both groups were able to isolate the chitin synthase in cell-free preparations and demonstrate that diflubenzuron had no effect on the enzyme.

The purpose of the present communication is to extend the work of Mayer et al.[13] by testing other CSI.

Materials and methods. Chemicals[2]. Radiolabeled UDP-[glucosamine-6-^{3}H]GlcNAc (6.6 Ci/mmole) and Bray's scintillation cocktail were purchased from New England Nuclear, Boston, MA and UDP-GlcNAc purchased from Sigma Chemical Co., St. Louis, MO. Tunicamycin was