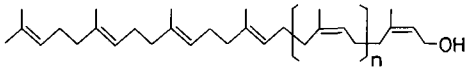
Biotransformation of polyprenols by the larvae of *Pieris rapae crucivora*

T. Suga and T. Shishibori

Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Hiroshima 730 (Japan), 8 February 1979

Summary. The biotransformation of cleomeprenols-1-^{3}H by the larvae of *Pieris rapae crucivora* Boisduval has been investigated. It was found that cleomeprenols fed to the larvae are transformed into long chain fatty acid esters that are excreted in the feces.

During recent years, polyprenols have received much attention because of their biological functions as carriers of sugars in the biosyntheses of bacterial wall polysaccharides[1] and eucaryotic glycoproteins[2,3]. Besides being found in microorganisms and animal tissues, polyprenols have been reported in higher plants[4]. Polyprenols with 9–12 isoprene units were found as a main constituent in the leaves of *Cleome spinosa* L. and the structures of the polyprenols, named cleomeprenols, were established to be (**1**)–(**4**)[5]. It is

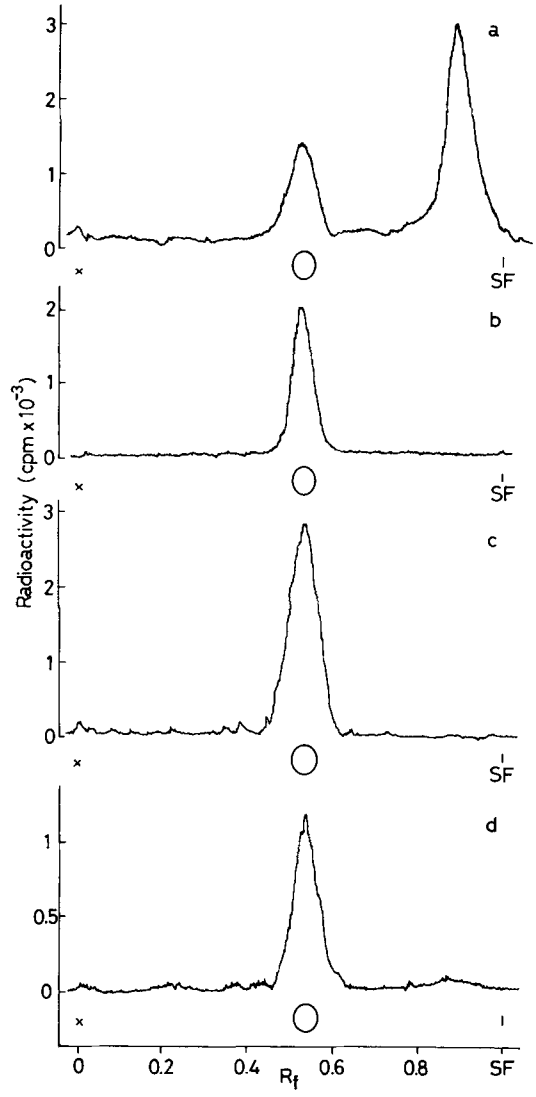


1 : n=4, 2 : n=5, 3 : n=6, 4 : n=7

a well-known fact that the larvae of *Pieris rapae crucivora* Boisduval feed on the leaves of *C. spinosa* of Caparidaceae and plants belonging to the Cruciferae[6]. We have now investigated the in vivo transformation of cleomeprenols in the larvae of *P. rapae crucivora*, and report here the first evidence that cleomeprenols fed to the larvae are transformed into higher fatty acid esters.

To prepare tritium-labelled cleomeprenols, natural cleomeprenols (23 mg) composed of **1** (20%), **2** (41%), **3** (33%) and **4** (6%) were oxidized with active manganese dioxide (180 mg) in hexane for 8 h to give a mixture of the corresponding aldehydes (15 mg). The mixture was then reduced with sodium borohydride-^{3}H (1 mCi, 203 Ci/mole) to yield cleomeprenols-1-^{3}H (5 mg, 163 μCi/mg). The leaves of *C. spinosa* (20 g) were coated with a specimen of the tritiated cleomeprenols (114 μCi) by applying it in ethereal solution, and fed to 15 head of 10-day-old larvae for 2 days until almost all the leaves were eaten.

The larval body and the feces were extracted separately with a mixture of methanol and chloroform (2:1, v/v). The extracts contained 1.6% and 21.7% of the radioactivity of the given substrate, respectively. Thin-layer radiochromatographic analysis indicated that the extract of the larval body comprises polar compounds which remained unidentified. On the other hand, a combination of thin-layer radiochromatography and liquid scintillation measurement showed the extract of feces to comprise 2 radioactive components with 40% (2.18×10^7 dpm) and 60% (3.32×10^7 dpm) of the total radioactivity at R_f 0.53 and 0.89 on TLC, respectively, as shown in figure a. Co–TLC showed that the less mobile component was unchanged cleomeprenols. The more mobile component was confirmed to be composed of esterified cleomeprenols, as is shown by what follows. The component was purified by TLC (silica gel G; hexane-ethyl acetate, 4:1, v/v), followed by continuous development-TLC (hexane-ether, 19:1) using the radioactivity as an indication. The purified component, on reduction with lithium aluminum hydride, gave radioactive cleomeprenols which were identified with the natural cleomeprenols by co-chromatography on the TLC (silica gel G; hexane-ethyl acetate, 4:1) (figure c) and the reversed phase TLC (Kieselgur G impregnated with 5% liquid paraffin in hexane; acetone-water, 9:1)[7]. On the other hand, saponification of the purified component with 5% methanolic potassium hydroxide afforded radioactive cleomeprenols (figure d) and fatty acids. Identification of fatty acids and determination of their compositions were performed by GLC analysis of the methyl esters (prepared with CH_2N_2) on a 15% DEGS-Chromosolve W column at 160 °C, and the acids were found to be lauric (5.6%), myristic (7.7), palmitic (16.7), palmitoleic (2.7), stearic (7.4), oleic (12.8), linoleic (12.7) and linolenic acids (8.8) and others (25.6). The results constitute evidence that the more mobile component con-



Thin-layer radiochromatograms of *a* the extract of feces of the larvae which ate the leaves coated with a specimen of cleomeprenols-1-^{3}H, *b* cleomeprenols-1-^{3}H fed to the larvae, *c* a $LiAlH_4$-reduction product of cleomeprenol esters isolated from the feces and *d* unsaponifiable material obtained from cleomeprenol esters of feces. TLC was performed on a silica gel G plate with hexane-ethyl acetate (4:1, v/v). Spots indicate reference cleomeprenols.

sists of cleomeprenols esterified with these fatty acids. Comparison of the fatty acid composition of cleomeprenol esters of the feces with that of the esters present in the leaves of *C. spinosa* showed that the concentrations of stearic and oleic acids in the former were about 3 times those of the acids in the latter, while linolenic acid was one-third. Other fatty acids in both the esters were similar in composition.

In pig liver, more than half of the dolichols are present as the fatty acid esters[8]. Formation of the dolichol esters is observed during the incubation of dolichols with rat liver microsomes[9]. However, no accumulation of cleomeprenol esters in the larval body of *P. rapae crucivora* was observed. Thus, it was found that the larvae transform the cleomeprenols of *C. spinosa* to long chain fatty acid esters to excrete them in this form in the feces.

1 Y. Higashi, J.L. Strominger and C.C. Sweeley, Proc. natl Acad. Sci. USA *57*, 1878 (1967).
2 J.F. Caccam, J.J. Jackson and E.H. Eylar, Biochem. biophys. Res. Commun. *35*, 505 (1969).
3 R.M. Baar and F.W. Hemming, Biochem. J. *126*, 1193 (1972).
4 F.W. Hemming, in: Natural Substances Formed Biologically from Mevalonic Acid, p. 105. Ed. T.W. Goodwin. Academic Press, London 1970.
5 T. Suga, T. Shishibori, S. Kosera, T. Tanaka and M. Itoh, Chem. Lett. *1975*, 771.
6 T. Shirōzu, in: Butterflies of the Japan in Colour, p. 159. Hokuryu-kan, Tokyo 1965.
7 P.J. Dunphy, J.D. Keer, J.F. Pennock and K.J. Whittle, Chem. Ind. *1966*, 1549.
8 P.H. Butterworth and F.W. Hemming, Archs Biochem. Biophys. *128*, 503 (1968).
9 R.W. Keen and M.E. Kruczek, Biochemistry *15*, 1586 (1976).

Triglyceride metabolism in *Ephestia cautella* pupae exposed to carbon dioxide[1]

A. Friedlander and S. Navarro[2]

Agricultural Research Organization, Institute for Technology and Storage of Agricultural Products, Division of Stored Products, P.O. Box 6, Bet Dagan (Israel), 15 February 1979

Summary. The triglyceride content of *Ephestia cautella* pupae exposed to increased carbon dioxide atmospheres at low relative humidity was not markedly affected. There was a significant increase in weight loss of pupae exposed to low relative humidity. Results indicate that for *E. cautella*, metabolic water formation by fat utilization can hardly regulate water exigencies for the pupae and cannot fully compensate for water losses in high carbon dioxide atmospheres.

Recently the effect of atmospheres containing low oxygen or high carbon dioxide concentrations was studied in an attempt to apply the controlled-atmosphere method for controlling insects in large bulks of dry grain[3]. To clarify the effect of carbon dioxide on stored product pests, we investigated the basic biochemistry of its influence on *Ephestia cautella* (Walker) pupae[4]. It is well known that stored-product insects can survive at very low humidities. Therefore, the means of conserving water in these insects is an important aspect of their structure and physiology.

Although carbon dioxide plays an important role in keeping insect spiracles open, thus exposing the organism to desiccation[5], it is now generally accepted that the effect of carbon dioxide is complex and not restricted to this physiological effect only[6].

As a process of dehydrogenation, the oxidation of organic substrates takes place with the consequent formation of metabolic water[7]. The present investigation was carried out to clarify the effect of carbon dioxide on triglyceride metabolism, and to ascertain whether metabolic water, formed in fat metabolism, may be a regulating phenomenon that replaces water lost through increased desiccation rate induced by carbon dioxide under conditions of low relative humidity.

E. cautella pupae were reared according to Navarro and Gonen[8]. They were examined after exposure to various gas compositions and relative humidities at 26 °C as described by Navarro and Donahaye[9]. Weight loss was determined by weighing each sample before and after treatment, and was calculated as percent of the starting weight. Triglycerides were extracted by the method of Bligh and Dyer[10], which is essentially as follows: The sample (0.5 g, about 60 insects) were homogenized in the extraction fluid (chloroform-methanol 1:2 v/v, 5 cm^3). The homogenate was filtered and the filtrate treated as described in the original paper[10]. After removal of the aqueous phase, an aliquot of the chloroform phase was diluted 100-fold and used for the determination of triglycerides by the method of Wahlfeld[11], with the following modifications: The aliquot was evaporated in a small test tube and heated subsequently to 100 °C

Effects of different atmospheric compositions at 2 relative humidities on triglyceride levels and weight loss of *E. cautella* pupae (in relation to time)

Atmospheric composition (%)			Relative humidity	Exposure time	Triglycerides	Weight loss
O_2	N_2	CO_2	(%)	(h)	(% ± SE)	(% ± SE)
20	80	0	100	24	12.04 ± 0.630	0.83 ± 0.177
20	80	0	100	48	13.00 ± 0.772	1.62 ± 0.524
20	80	0	100	144	9.87 ± 0.445	1.62 ± 0.630
20	80	0	20	48	13.45 ± 0.697	4.46 ± 0.793
20	80	0	20	144	9.46 ± 0.766	12.17 ± 0.650
20	0	80	100	24	15.64 ± 1.020	3.02 ± 0.281
20	0	80	100	48	12.91 ± 1.186	5.06 ± 0.294
20	0	80	100	144	10.06 ± 0.835	7.53 ± 1.513
20	0	80	20	48	12.60 ± 0.514	25.91 ± 1.414
20	0	80	20	144	9.16 ± 1.496	58.77 ± 1.173