

**Marginalin, a Substance from the Pygidial Glands of *Dytiscus Marginalis* (Coleoptera): Molecular Associations with Polyamines *in vitro***

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Z. Naturforsch. **45b**, 1455–1456 (1990);  
received May 7, 1990

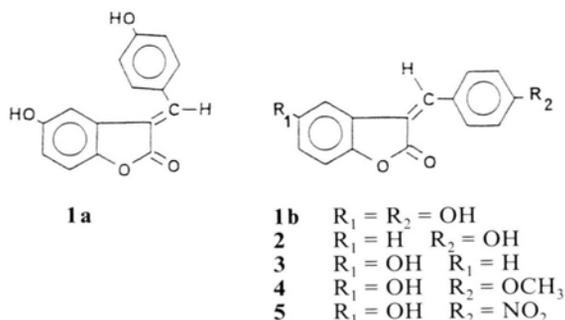
Marginalin, *Dytiscus marginalis*, Coleoptera,  
Defensive Secretions, Molecular Associations

Marginalin, a product previously isolated from the pygidial glands of the water-beetle *Dytiscus marginalis* (Coleoptera), strongly reacts *in vitro* to form complexes with polyamines of biological interest. With spermin and spermidin, this interaction is specific giving 1:1 complexes which were isolated and studied (physico-chemical data). The results so far observed are discussed in relationship with the known biological properties of spermin and spermidin and the significance of marginalin in the defensive secretion of the beetle.

Z-isomer **1b** produced by opening of the lactone and relactonization.

The technique of the chromatographic barrier [5] was applied to marginalin in order to determine molecular interactions with substances of biological interest. Series of substances are deposited on a SiO<sub>2</sub> TLC at a short distance of the starting point where is the tested product (here marginalin). During the migration, three cases are possible at the limit: the tested product goes through the barrier by keeping the same *R<sub>f</sub>* as a standard in lateral position, or it is slowed in its migration, or it is definitely captured within the deposited reactant. A wide series of substances were tested with marginalin (**1a** or **1b**) such as for example, glycin, tryptophane, tryptamine, aspartic acid, urea, calf thymus ADN, albumin, *etc.* Strong interactions were noticed with spermin and spermidin and at a less extent, with cadaverin and putrescin (hexane-ethyl acetate 1:1 on Schleicher-Schüll fluorescent films, UV observation at 254 nm). The two phenolic groups of marginalin are implicated in the molecular association, as exemplified by the negative results observed with the monohydroxy analogues **2** or **3**, the methoxy derivative **4** [6] and nitromarginalin **5** [7]. From molecular models, it appears that interactions between putrescin and cadaverin are possible with the E-isomer of marginalin **1a** while the distance which separates the two hydroxy groups is too big in the Z-isomer **1b**. The natural marginalin **1a** thus appears to be more adapted that the isomer **1b** for interactions with polyamines, as far as the stability of the final complex is concerned.

Marginalin **1a** is a substance which was isolated [1][2] from the defensive glands of the water-beetle *Dytiscus marginalis* (Coleoptera). These pygidial glands of the *Dytiscidae* play a role in the protection of such aquatic insects against mosses, algae, bacteria and other parasites which could grow on their body surface, more particularly on the wing covers. Benzoic acid, *p*-hydroxybenzaldehyde, methyl *p*-hydroxybenzoate, were previously found [1][2] in these secretions, embedded in a protective hydrophobic paste made of high molecular weight glycoproteins [3], a rather sophisticated system. Marginalin as a lactone is sensitive to moisture and its biological significance as such, is of course bound to this hydrophobic film renewed very often by the beetle, a *sine qua non* condition for existence. However, as far as we know, no biological properties were reported for marginalin, the only known natural benzylidene isocoumaranone, thus suggesting the present experiments. The stereospecific synthesis of the Z-isomer **1b** of marginalin [4] allowed by comparison of the physicochemical data with the reported values to determine the E-stereochemistry **1a** for the natural product. This E-isomer **1a** could also be obtained from a different synthesis [1] as from isomerization of the



Spermin:  $\text{H}_2\text{N}-(\text{CH}_2)_3-\text{NH}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$

Spermidin:  $\text{H}_2\text{N}-(\text{CH}_2)_4-\text{NH}-(\text{CH}_2)_3-\text{NH}_2$

The study of the molecular associations formed between marginalin and spermin or spermidin was greatly facilitated by the fact that the correspond-

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen  
0932-0776/90/1000-1455/\$ 01.00/0



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ing complexes precipitate from the methylene chloride solution. By proceeding from a known weight of marginalin with an excess of the polyamines, it is possible to determine the 1:1 proportion as the reaction is quantitative (total disappearance of **1a** or **1b** from the  $\text{CH}_2\text{Cl}_2$  solution). The UV spectra of the isolated complexes (DMSO) showed that the chromophores of marginalin were unchanged but that a considerable increase of the intensity of absorption at 518 nm had occurred (from  $1 \times 10^3$  to  $2.2 \times 10^4$ ). This hyperchrome effect was noticed by the change of colour from yellow for marginalin itself to orange for the two complexes. The  $^1\text{H}$  NMR spectra showed a total modification by reference to marginalin, all signals being now shifted in complicated multiplets between 6.62 and 6.85 ppm while the original  $\text{A}_2\text{B}_2$  and ABX systems are no longer apparent. The end amino groups of spermin or spermidin are known to protonate quite easily even at physiological pH, leading to conformationally mobile cations so that they can associate with the negative charges of marginalin to give stable additions compounds which precipitate. The resulting complexes may have a pseudo-cyclic structure. They were found to decompose immediately in presence of water to give back marginalin and the polyamine.

The biological significance of the polyamines and in particular of spermin and spermidin [8] begins to be understood. These substances serve as growth promoters for most cells as for many bacteria. They also play a determinant role in cellular

differentiation and proliferation by their interaction with nucleic acids. As component of the secretion of the pygidial glands of the aquatic insect *Dytiscus marginalis*, marginalin can fix spermin and spermidin thus providing protection against unicellular algae [9], mosses, bacteria and other microorganisms. The hydrophobic coating in which marginalin is embedded is of course necessary for a full activity.

In hydrophobic conditions, marginalin is a pigment which may fix solidly on a variety of supports (*in vitro* on a cotton plug). When mixed in an organic solution with a grease or a silicon oil and deposited by evaporation on such a support, marginalin will stay for days even in presence of water. The protective role of the hydrophobic glycoprotein *in vivo* is thus certain. When in contact with microorganisms such as algae, marginalin may fix itself as a dye on the proteins of the cell, or dissolve in the lipids and interfere by its powerful yellow colour on the role that carotenoids play in photosynthesis. The transposition of these results to corresponding *in vivo* systems is a problem out of our competence, so that the real study of the raison d'être of marginalin in the pygidial glands of *Dytiscus marginalis* will wait for more specialized experiments.

#### Acknowledgements

Thanks are due to Mrs. C. Fontaine and C. Pasquier for the  $^1\text{H}$  NMR determinations (Bruker 200 MHz) which were carried out at Gif sur Yvette.

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