plets at 2.3 (2H, H_2-7) and 1.75 (4H; H_2-5 and H_2-6)ppm for the remaining methylene groups. Finally, the alternative structure **6**, equally compatible with above evidence, but unlikely from the biogenetic point of view, seems also less probable on the basis of the following evidence. Osmilation of spiniferin-1 gave a mixture of compounds, from which a diol (**7**) M^+/e 246, ν_{max} 3400 cm^{-1}, λ_{max} 250 nm, whose ^{1}H-NMR-spectrum (table 1) still contained the olefinic signals for H-1 and H-2 could be separated by repeated TLC-chromatography. The upfield shift of the H-1 signal in **7** (table 2; 6.26 ppm in **3** and 5.26 ppm/in **7**) was suggestive for the location of the hydroxyl groups at C-9 and C-10 and the relative intensities of the paramagnetic shifts induced by Eu (fod-d_9)$_3$ on the signals for H-1, H-12 and H-13 in its ^{1}H-NMR-spectrum ($\Delta\delta_{Eu}^{n=0.3}$: 0.3, 0.12, 0.12 for H-1, H-12 and H-13, respectively) can be easily explained by the structure **7**; in the case of the alternative structure **6** addition of Eu(fod-d_9)$_3$ to the corresponding 9,10-diol would be expected to produce in its ^{1}H-NMR different shifts for H-12 and H-13.

Evidence which has allowed us to choose structure **2a** for spiniferin-2 has been obtained by its conversion via the γ-hydroxy-α,β-butenolide **8** (m-Clperbenzoic acid in $CH_3CO_2HCH_3CO_2Na$)[6], m.p. 155–157 °C, M^+/e 244, ν_{max} 3350, 1740 and 1640 cm^{-1} (^{1}H-NMR in table 2) to the α,β-unsaturated-γ-lactone **9** (treatment of **8** with $NaHB_4$), m.p. 143–145 °C, M^+/e 228, ν_{max} 1750 and 1640 cm^{-1}. The protons at C-6 appeared as an AB quartet at 3.4 and 2.6 (J = 14) ppm further split by coupling (J = 9, 4) to a single proton at C-7 resonating at 4.08 ppm. This definitively ruled out the alternative structure **2b** for spiniferin-2. The ^{13}C-NMR-data for spiniferin-2 are collected in table 1; the assignment of the signals of the benzenoid and furan carbons are based on published data[4,7], the assignment of the sp^3 carbons are based on selective decoupling.

The skeletons of **3** and **2a** are so far unique among sesquiterpenoids, and they would seem to arise by C–C cyclization involving lateral Me groups of the poly-isoprene chain (**10** and **11**).

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Neobonellin, a new biologically active pigment from *Bonellia viridis*[1,2]

L. Cariello, M. De Nicola Giudici, L. Zanetti and G. Prota

Stazione Zoologica, and Istituto di Chimica Organica dell'Università, Via Mezzocannone 16, I-80121 Napoli (Italia), 20 March 1978

Summary. A difference of possible physiological significance is reported in the chemical nature of the green integumentary pigment of the echiuroid *Bonellia viridis*, the proboscis of which contains the known bonellin (**1**, R = H) as the major constituent, while the body skin is mainly pigmented by an isoleucine conjugate, neobonellin, formulated tentatively as **2** (R = H) by spectral and chemical data.

The inhibition of growth and the development of masculinity in *Bonellia viridis* (Echiurida) is currently ascribed[3–5] to bonellin, the green tegumentary pigment of the mature female.

Notably, this animal consists of a large body of about 8 cm long with a proboscis reaching a length of more than 1 m. The pigment was first isolated by Lederer[6] some 40 years ago from the proboscides of the worm and was for a long time considered to be closely related to mesopyrrochlorine, until a recent study by Pelter et al.[7] showed that bonellin has most probably the unusual chlorin structure **1** (R = H), unrelated to chlorophyll.

In the course of a study on the growth inhibitory properties of extracts of *B. viridis*, we have found a remarkable difference between the bonellin content of the proboscis and body integument, the latter containing mainly a hitherto-unknown derivative (**2**, R = H), which we named neobonellin. Indeed, when first examined by TLC in various solvents, extracts of the proboscides and body integuments of *B. viridis* showed comparable pigment patterns, since bonellin and neobonellin have very close R_f-values. However, the difference appeared after conversion of the pigments into the corresponding methyl esters which show different chromatographic behaviour.

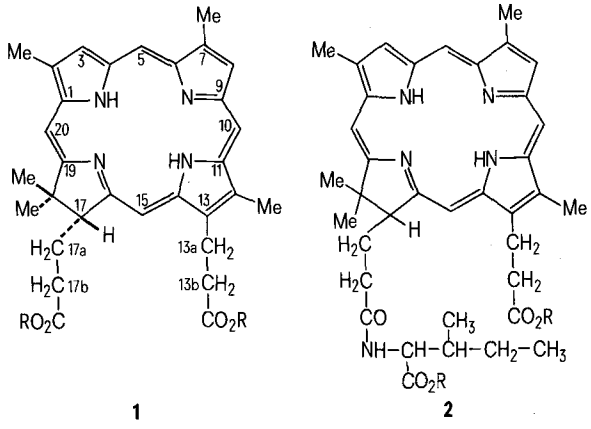
In a typical experiment, the pigments from the proboscides and body skin of *B. viridis* were separately extracted with methanol, transferred into ether, and then esterified by treatment with methanol saturated with HCl (24 h at room temperature). Subsequent fractionation of the crude esters so obtained by preparative TLC on Merck F_{254} silica gel

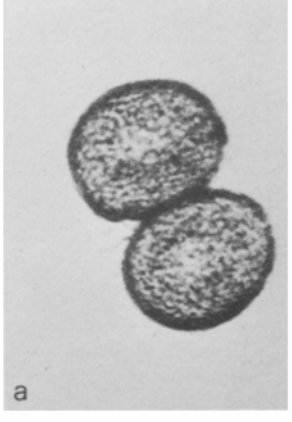
Yields and absorption spectra of bonellin and neobonellin as the methyl esters

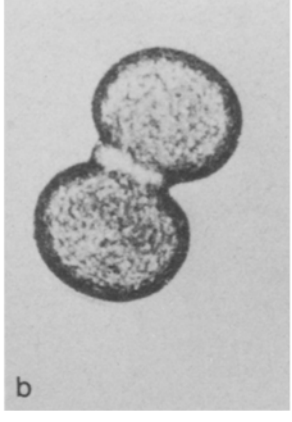
Pigment	Yields (g/100 g wet tissue) Proboscides	Body-tegument	λ_{max} ($CHCl_3$) nm
Bonellin	0.16	0.04	641, 620 (sh), 590, 539, 521, 494, 488 and 394
Neobonellin	0.01	0.18	640, 613, 588, 536 (sh), 521, 491 and 391

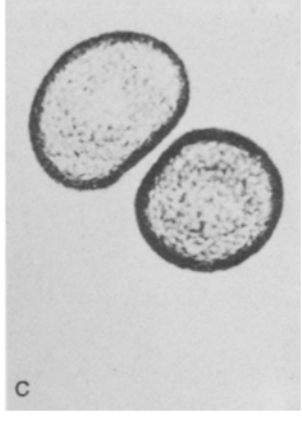
with CCl_4-AcOEt-MeOH (80:20:1, v/v) gave pure bonellin dimethylester (**1**, R = Me), m.p. 54–55 °C (from cyclohexane) and a more polar band containing essentially neobonellin dimethylester (**2**, R = Me) together with a small amount (about 7%) of a valine analogue, as evinced a) by mass spectrometry ($M^{\pm}$ at m/e 667 analyzed 653) and b) by acid hydrolysis leading to a mixture of isoleucine (93%) and valine (7%)[8]. Eventually, neobonellin dimethylester could be isolated in the pure form after separation from the accompanying pigment by careful column chromatography on fine silica gel (Kieselgel G, Merck, 10–40 µm) with CCl_4-AcOEt-MeOH (80:20:1 v/v).

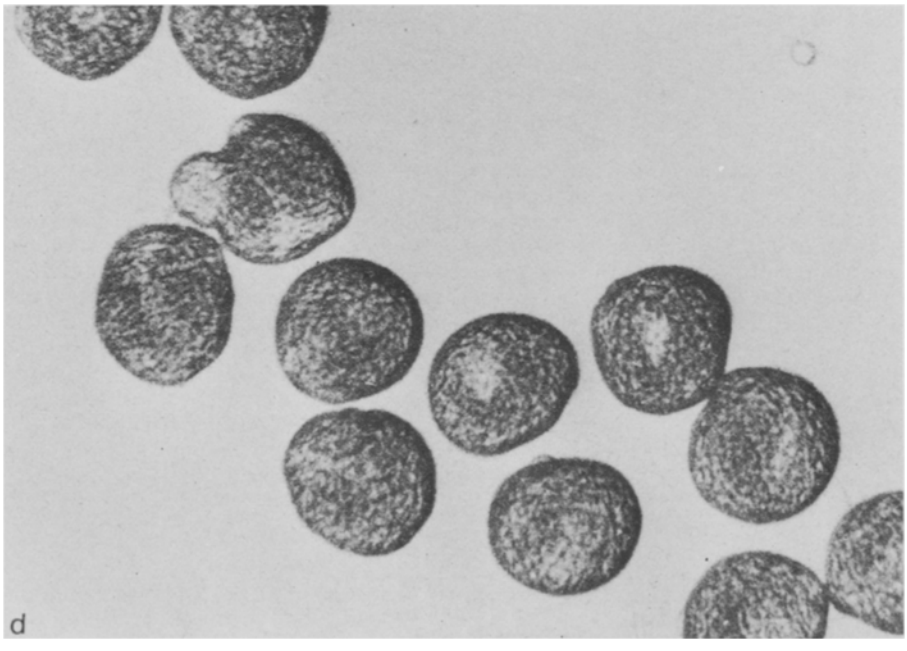
The pigment exhibited the typical bonellin chromophore (table) and had a molecular formula[9] of $C_{39}H_{49}N_5O_5$ including 2 methyl esters groups. The H^1 NMR-spectrum ($CDCl_3$) was largely superimposable by that[7] of bonellin dimethylester (**1**, R = Me) except for a) the presence of additional signals in the sp^3 aliphatic region for total 10 protons including 2 C-Me groups (0.70–0.87 δ) and a low-field methine proton overlapping the multiplets at δ 4.1–4.7 (17 and 13a protons), and b) a D-exchangeable broadened doublet centred at δ 6.35 (**1**H, J = 10 Hz), consistent with the presence of an amide bond. Hydrolysis of neobonellin dimethylester with 6N HCl in a sealed tube at 110 for 12 h gave bonellin (**1**, R = H) and the conjugated amino acid which was isolated in crystalline form, m.p. 285–6 °C with dec. (from MeOH), and identified as L-isoleucine by comparison with an authentic sample. The combined evidence led us to conclude that neobonellin was an isoleucine conjugate of bonellin in which the amino acid residue is linked through an amine bond to the propionic acid side-chain at C-17 rather than at C-13, on account of a more pronounced modification observed in the resonances of the











Fertilized eggs of *Sphaerichinus granularis* (mechanically deprived of the fertilization membrane) at 2-cell stage were allowed to stand in a 10^{-7} M solution of bonellin in sea water containing 0.01% of Tween 80: *a* control; *b* after 15 min treatment; *d* after 5 h treatment (× 820). Neobonellin acts similarly, but at same concentrations it exhibits about half the activity shown by bonellin.

17a and 17b methylene signals with respect to those of 13a and 13b. Such a conjugation reaction is reminiscent of the detoxication processes[10] which most foreign compounds and some natural metabolites, usually unwanted by the organism, undergo in the body to produce more polar and relatively less active products.

Indeed, the occurrence of neobonellin in the body skin more than in the proboscis of female *Bonellia viridis* would suggest a role of this pigment in the storage or scavenger of bonellin, responsible, as reported[3-5], for the inhibition of growth and the development of masculinity of the worm larvae.

While the physiological role of these pigments in *B. viridis* awaits further investigation, we have found that both bonellin and neobonellin have a strong blocking effect on the development of sea urchin embryos.

Tests on fertilized eggs of *Sphaerichinus granularis*, with or without fertilization membrane, provided evidence that aqueous solution of these pigments in their natural forms (R=H)[11] readily inhibit cleavage at any stage of development, and cause a complete cellular lysis within a short time. These effects are qualitatively similar to those described by previous authors[4,5] for crude or partially purified pigment extracts of female *Bonellia viridis*. Interestingly enough, while in dilute solution up to 10^{-6} M bonellin and neobonellin are toxic for the embrionic cells, at lower concentration they produce a different effect resulting in the separation of blastomeres in the first stages of cellular division. Subsequently, the apparently undamaged blastomeres lose the ability for cleavage, although they continue to survive for several hours (figure).

The observed inability of separated embryonic cells to enter in mitosis is of interest in view of the possibility that bonellins can also be active on proliferating cells, such as tumoral tissues. Experiments in this direction are now in progress in our laboratory.

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8 Using similar procedures Pelter et al. (unpublished results) have found that specimens of *Bonellia viridis* collected from the Bay below the Fort San Lucian Marine Station of Malta exhibit a different and more complex pattern of amino acid conjugates of bonellin including the valine (63%), the isoleucine (23%), the leucine (5.9%), and the alloisoleucine (4%) derivatives, as well as some others present in very small amounts. These remarkable differences in the composition of bonellin conjugates found in Maltese and Neapolitan specimens of *Bonellia viridis* are reproducible and may be attributed to environmental factors or to a species difference.
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11 Notably, both bonellin and neobonellin in the form of the methyl esters show no activity on the development of sea urchin embryos.

Activators of serum lipoprotein lipase in alloxan diabetic rats

S.S. Mukerjee and S.K. Mukherjee

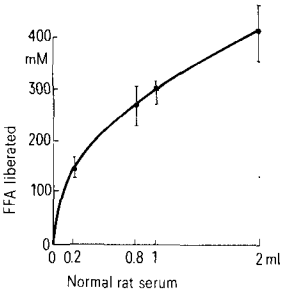
Division of Toxicology and Experimental Medicine, Central Drug Research Institute, Lucknow-226001 (India), 14 March 1978

Summary. The amount of free fatty acid (FFA) liberated with intralipid in post-heparin guinea-pig serum, when serum from alloxan diabetic rat is incubated, is higher than that liberated when serum from healthy rats is used. The above effect is probably due to larger quantities of lipoprotein lipase present in the diabetic serum.

Endogenously produced lipids and lipids available in plasma from diet, when transported, provide a balanced caloric supply of energy to various organs and tissues. The normal metabolism of the lipoproteins responsible for this transport is dependent on the proper functioning of a group of lipolytic enzymes. Lipoprotein lipase (LPL) is primarily responsible for hydrolysis of the triglycerides present in chylomicra and very low density lipoproteins (VLDL) before their uptake as fatty acids by extrahepatic tissues. Heparin-released LPL represent the only enzyme system involved in the catabolism of triglyceride fatty acid (TGFA) in peripheral tissues[1].

Schnatz and Williams[2] studied the effect of acute insulin withdrawal on the LPL activity of epididymal fat from alloxan diabetic rats. They observed a rapid decline in LPL activity causing an inverse correlation with blood glucose, plasma triglycerides and fatty acids. They attributed the decline in LPL activity to the elevated circulating triglycerides of the uncontrolled diabetes.

It has been shown that heparin released LPL activity is dependent on the presence of a protein cofactor, which is a component of natural lipoprotein substrates[3]. Subsequently it has been observed that this cofactor protein increased the catalytic rate of LPL but was not a prerequisite for its activity. Its optimum pH is similar to the pH optimum of lipase (8.0-8.5) rather than the pH which is obtained in physiological states. Its reaction depends upon lipid-water interface. Its ability to induce an increase in the LPL activity is due to the approximation of a positively charged amino-acid residue to the lipase active site[4]. It is shown that preparations of human or rat post-heparin LPL were



The assay system consisted of 10% intralipid, tris-buffer (1.35 M; pH 8.4) 15% bovine albumin, ammonia solution (0.025 M), post-heparin guinea-pig plasma and varying amounts of rat serum.