# SPECIALIA



## Furanosesquiterpenoids in sponges VI. Further structural studies for spiniferins, sesquiterpenes from *Pleraplysilla spinifera*

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*Summary.* The methylene bridged structure **3** is reassigned to spiniferin-1, unstable sesquiterpene from the sponge *Pleraplysilla spinifera.* Evidence leading us to choose structure **2a** between the 2 alternative formulations **2a** and **2b** earlier proposed for spiniferin-2 are also reported.

On the basis of spectroscopic studies, mainly $^1$H-NMR, 2 alternative structures (**1a–b**) and (**2a–b**) were earlier assigned to spiniferin-1 and -2, unstable furanosesquiterpenes from the sponge *Pleraplysilla spinifera,* respectively[1]. The key-argument which suggested to us the presence of a cyclopropane moiety in spiniferin-1 was the upfield doublet at 0.75 ppm coupled with a doublet at 3.62 ppm; the former was assigned to H-5 and the latter to H-6. Indeed, we noted that the value of the coupling constant (J = 10 Hz) was slightly larger than expected for vicinal coupling constants in substituted cyclopropane derivatives, which for J trans are reported to range from 4.0 to 9.6 Hz[2]. Next, 2 singlets at 6.28 and 6.32 ppm were considered the central bands of an ABq, and assigned to H-9 and H-10. Now the study of this molecule on a freshly prepared sample by $^{13}$C NMR-spectroscopy has clearly revealed that the proposed structures **1a – b** were both incorrect. In this paper we wish to suggest for spiniferin-1 the revised structure **3,** as deduced from spectral data, and confirmed by degradation and chemical transformations. Chemical transformations of spiniferin-2 and related spectroscopic properties, which have now allowed us to choose **2a** between the earlier proposed structures **2a** and **2b,** are also described.

Table 1. $^{13}$C-NMR chemical shifts[a] for spiniferin-1 (**3**), dihydrospiniferin-1 (**4**) and spiniferin-2 (**2a**)

| | 3 | 4 | 2a |
|---|---|---|---|
| C-1 | 130.3 d[e] | 36.4 t[e] | 128.0 d[b] |
| C-2 | 124.9 d[e] | 25.6 t[e] | 126.3 d[b] |
| C-3 | 44.3 t[e] | 41.7 t | 134.4 s[c] |
| C-4 | 39.6 s | 37.5 s | 132.5 s[c] |
| C-5 | 127.4 s[b] | 128.0 s[b] | 138.9 s[d] |
| C-6 | 109.41 d[c] | 108.1 d[c] | 27.7 t[e] |
| C-7 | 118.4 s | 124.0 s | 147.8 s |
| C-8 | 153.2 s | 152.1 s | 117.0 s |
| C-9 | 112.4 d[c] | 111.1 d[c] | 25.9 t[e] |
| C-10 | 131.4 s[b] | 131.2 s[b] | 32.8 t[e] |
| C-11 | 34.1 dd[e] | 34.7 dd | 137.3 s[d] |
| C-12 | 109.4 d | 108.9 d | 112.0 d |
| C-13 | 140.9 d | 138.9 d | 138.9 d |
| C-14 | 28.2 q[e] | 26.8 q | 21.0 q |
| C-15 | 30.7 q[e] | 28.9 q | 15.7 q |

[a] $^{13}$C-NMR-spectra were determined in $CDCl_3$ at 25.20 MHz on a Xl-100 Varian F.T. Spectrometer; [b–d] assignments may be reversed; [e] assignments confirmed by selective decoupling.

*Results and discussion.* The $^{13}$C-NMR-spectrum of spiniferin-1 exhibits signals for only 5 $sp^3$ carbons and the remaining signals are due to $sp^2$ carbons (table 1). Spiniferin-1, $C_{15}H_{16}O$, is therefore a tricyclic sesquiterpene and all the available data for this compound can be explained by the methylene bridged formula **3.** Thus, $^1$H-NMR doublets at 0.75 and 3.62 are easily accomodated in this formula and assigned to H-11a and H-11b, respectively, the former being heavily shielded because it lies over the plane of the conjugated $\Delta^{5,7,9}$ triene system[3], and singlets at 6.28 and 6.32 ppm are assigned to H-6 and H-9 or viceversa (table 2). Further, decoupling experiments showed that H-11b is allylically coupled to H-6 and H-9 and also established the existence of a long-range interaction between H-11a and H-1. The $^{13}$C-NMR-data of spiniferin-1, along with those of its 1,2-dihydroderivative (**4**)[1], are collected in table 1. The assignment of the signals of the furan carbons is based on published data[4]. Distinction between resonances due to C-3 and C-11 was facilitated by the multiplicity of the latter in the off-resonance spectrum giving rise to a dd because of the largely magnetically non-equivalence of the 2 attached protons[5]. Selective decoupling confirmed this assignement in **3** and also the assignments for the remaining $sp^3$ protonated carbons. The C-1 and C-2 olefinic resonances were readily identified by the disappearance in the spectrum of the 1,2-dihydrospiniferin-1 (**4**) of the signals at 124.9 and 130.4 ppm and differentiated by selective decoupling. Comparison of the UV-data for spiniferin-1 ($\lambda_{max}$ 240 and 302 nm) with those for 1,2-dihydrospiniferin-1 ($\lambda_{max}$ 230 and 266 nm)[1] clearly indicated further conjugation of the $\Delta^{1,2}$-double bond in **3,** thus supporting the new formulation. Further support was obtained by osmilation of 1,2-dihydrospiniferin-1 (**4**) which gave a tetrahydroxyderivative, $M^+/e$ 282, whose $^1$H-NMR-spectrum contained 2 singlets for 2 CHOH protons (4.52 and 4.34 ppm, 1H each). Chemical confirmation of the new formulation **3** was obtained by ozonolysis of **4** (3% $O_3$ for 5 min at room temperature) which gave a diketone, $C_9H_{14}O_2$, $M^+/e$ 154, $\nu_{max}$ 1720, 1690 $cm^{-1}$, whose $^1$H-NMR-data completely accord with the structure **5**. The spectrum, in fact, contained singlets at 1.1 (6H) for 2 tert-methyl groups, and at 3.4 (2H; $H_2$-2)ppm for an isolated methylene group between 2 carbonyl functions, and multi-

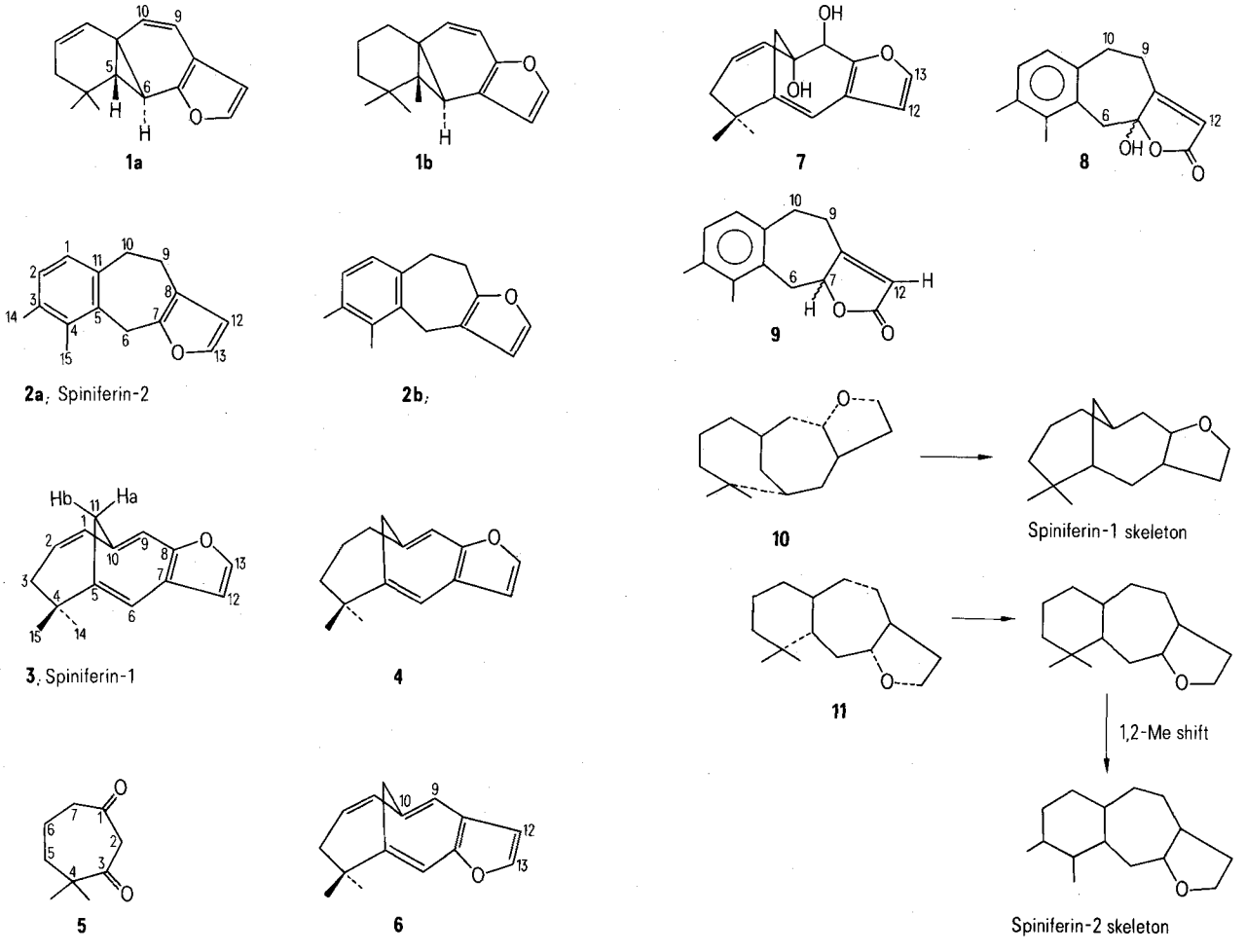


Table 2. $^1$H-NMR-data for spiniferin-1 (**3**), spiniferin-2 (**2a**) and derivatives[a]

| Position | 3 | 4 | 7 | 2a | 8 | 9 |
|---|---|---|---|---|---|---|
| 1 | 6.26 dd (J = 10,3) | 2.28 m | 5.26 bd (J = 10) | 6.82 s | 6.88 d (J = 8) | 6.82 d (J = 8) |
| 2 | 5.34 m | 1.5–1.7 bm | 5.8 m | 6.82 s | 6.70 d (J = 8) | 6.62 d (J = 8) |
| 3 | 2.88 dt, 2.02 dd (J = 16,3) (J = 16,8) | 1.5–1.7 bm | 2.65 bm[b], 1.9 m | – | – | – |
| 6 | 6.28 s or 6.32 s | 6.17 s | 5.84 s | 4.02 s | 3.6 d, 2.8 d (J = 12) | 3.4 dd, 2.6 dd (J = 14,4) (J = 14,9) |
| 7 | – | – | – | – | – | 4.08 ddd (J = 9, 4, 2) |
| 9 | 6.32 s or 6.28 s | 6.17 s | 4.42 s | 2.61 complex | 2.2–2.5 complex | 2.4–2.2 complex |
| 10 | – | – | – | 2.92 complex | 2.2–2.5 complex | 2.2–2.4 complex |
| 11 | 3.62 d, 0.75 d (J = 10) | 3.04 d, 1.08 d (J = 10) | 2.65 bm[b] | – | – | – |
| 12 | 6.57 d (J = 2) | 6.45 d (J = 2) | 6.18 d (J = 2) | 5.96 d (J = 2) | 5.30 bs[c] | 5.30 q (J = 2) |
| 13 | 7.34 d (J = 2) | 7.25 d (J = 2) | 7.36 d (J = 2) | 7.03 d (J = 2) | – | – |
| 14 | 0.80 s | 1.00 s | 0.98 s | 2.25 s or 2.21 s | 2.08 s or 2.16 s | 2.04 or 1.92 |
| 15 | 1.40 s | 1.25 s | 0.98 s | 2.21 s or 2.25 s | 2.16 s or 2.08 s | 1.92 or 2.04 |

[a]Spectra were determined at 100 MHz in $CDCl_3$ except those of **8** and **9** which were run in $C_6D_6$; [b]H-3eq and $H_2$-11 overlap; [c]this signal sharpened on irradiation at $\delta$ 2.2 (H-9); while irradiation at both $\delta$ 3.6 and 2.8 ($H_2$-6) did not cause modification of the signal.

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, $\nu_{max}$ 3400 $cm^{-1}$, $\lambda_{max}$ 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 $\gamma$-hydroxy-$\alpha,\beta$-butenolide **8** (m-Clperbenzoic acid in $CH_3CO_2HCH_3CO_2Na$)[6], m.p. 155–157 °C, $M^+$/e 244, $\nu_{max}$ 3350, 1740 and 1640 $cm^{-1}$ ($^1$H-NMR in table 2) to the $\alpha,\beta$-unsaturated-$\gamma$-lactone **9** (treatment of **8** with $NaHB_4$), m.p. 143–145 °C, $M^+$/e 228, $\nu_{max}$ 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]


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*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 | $\lambda_{max}$ ($CHCl_3$) 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 |