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# The structure of scytonemin, an ultraviolet sunscreen pigment from the sheaths of cyanobacteria

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*Abstract.* Despite knowledge of the existence of the pigment called scytonemin for over 100 years, its structure has remained unsolved until now. This pigment, the first shown to be an effective, photo-stable ultraviolet shield in prokaryotes, is a novel dimeric molecule (molec. wt. 544) of indolic and phenolic subunits and is known only from the sheaths enclosing the cells of cyanobacteria. It is probable that scytonemin is formed from a condensation of tryptophan- and phenylpropanoid-derived subunits. The linkage between these units is unique among natural products and this novel ring structure is here termed the 'scytoneman skeleton'. Scytonemin absorbs strongly and broadly in the spectral region 325–425 nm (UV-A-violet-blue, with an in vivo maximum at 370 nm). However, there is also major absorption in the UV-C ($\lambda_{max} = 250$ nm) and UV-B (280–320 nm). The pigment has been recently shown to provide significant protection to cyanobacteria against damage by ultraviolet radiation. The pigment occurs in all phylogenetic lines of sheathed cyanobacteria and possibly represents a UV screening strategy far more ancient than that of plant flavonoids and animal melanins. How diverse organisms deal with UV radiation is considered of vital importance to global ecology.
*Key words.* Indole alkaloid; UV-sunscreen pigment; natural products; blue green algae; cyanobacteria.

Scytonemin, a cyanobacterial sheath pigment (fig. 1) with potent ultraviolet absorbing properties has recently been characterized in some detail[1,2] but its structure remained unsolved, although its existence was reported over 100 years ago[3]. It is the only sunscreen pigment from prokaryotes now fully identified[4], and there is compelling evidence that it is an essential component of many microbial ecosystems[1,2]. Herein, we report the structure of this novel yellow-green pigment.

Scytonemin is known only from the extracellular sheath of cyanobacteria (blue-green algae) and primarily when these photosynthetic prokaryotes are exposed to high solar irradiance[1,5,6]. The sheath pigmentation was first noted by Nägeli[4], who later introduced the term

Figure 1. *Scytonema* sp. with scytonemin-containing laminated sheaths; collected originally as a dry crust in a periodically flooded marine sabkha in Bonaire, Netherlands Antilles by R. P. Sheridan. A heterocyst may be seen centrally in the lower filament. Bright field, oil immersion. Filament diameter (includes sheath): 20–22 μm.

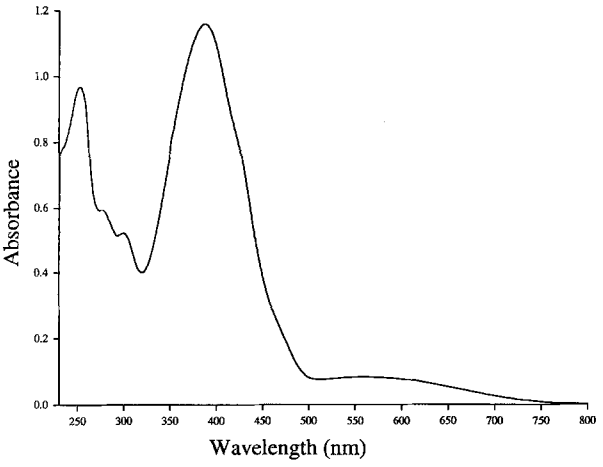


Figure 2. Ultraviolet-visible spectrum of scytonemin (concentration = $2.2 \times 10^{-5}$ M) in tetrahydrofuran ($\lambda_{max} = 252$, 278, 300, 386 nm; a smoothing function was applied to the 500–800 nm region).

'scytonemin' in 1877[7]. Sheathed cyanobacteria or similar ancestral forms occur commonly as microfossils from the Proterozoic ($2.5–0.54 \times 10^9$ y B.P.) in strata of biogenic origin (i.e. stromatolites) and even earlier[8,9,10]. Since UV fluxes (UV-B and -C, the latter <280 nm) were considerably higher then than now[11], it is likely that development of scytonemin for its UV-screening properties was important to the evolution of cyanobacteria. The absorption spectrum of scytonemin in intact sheaths and in various degrees of purity has been recorded by several authors[1,2,12]; the complete spectrum of the purified pigment is here (fig. 2). The pigment absorbs most strongly in the UV-A spectral region (315–400 nm) with an in vivo $\lambda_{max} = 370$ nm[1]. However, there is also significant absorbance in the violet and blue region as well as in the UV-B (280–320 nm) and UV-C (190–280 nm). The pigment has been isolated and identified from more than 30 species of sheathed cyanobacteria from diverse geographic regions, including freshwater, terrestrial, and marine habitats, wherever exposure to strong solar irradiance occurs[1,13].

Mere knowledge of the absorption properties of scytonemin is not proof of its effectiveness as a UV screen. However, it has been shown that common levels of scytonemin in cyanobacterial sheaths may be sufficient to prevent about 85–90% of incident UV-A radiation from entering the cells[1,2]. In a series of experiments with cultures of *Chlorogloeopsis sp.*, isolated from a terrestrial crust, it was shown that high UV-A radiation inhibited photosynthesis and delayed growth until substantial amounts of scytonemin had been deposited in the sheaths[2]. It is synthesized only by living cells and its synthesis is induced by high irradiance, most effectively by UV-A and -B radiation[1,2]. In addition, resistance to photobleaching of chlorophyll *a* by UV-A was inherent in cells surrounded by a scytonemin-containing sheath but not in identical cells with the sheath removed[2]. The regulation of this pigment appears to reflect the requirements of a sunscreen, because final sheath content varies directly with UV flux. In addition, it is not subject to rapid photodegradation as evidenced by long term persistence in terrestrial crusts or dried cultures[1,2,14].

*Methods and results*

*Stigonema sp.* from Waldo Lake, Oregon, collected in September 1990, *Scytonema sp.* from Curacao, Netherlands Antilles, December 1991, and *Lyngbya sp.* from Huahine, French Polynesia, June 1992, provided scytonemin pigment for chemical studies. These were extracted, essentially as previously described[1], to yield a crusty green solid in all cases. Attempts to obtain crystals of a quality suitable for x-ray diffraction analysis, a possibility suggested by Kylin's work[7], were not successful since only small, disordered needle clusters were obtained from several solvents (5:2 acetone/water; 5:1 THF/EtOH; EtOAc). These crystals are extremely stable (mp > 325 °C) and burn in a fashion reminiscent of coal dust. While reported by Kylin as relatively insoluble in most solvents[7], scytonemin shows a variable degree of solubility in N,N-dimethylformamide (DMF), pyridine or tetrahydrofuran (THF). Simple chemical reduction of scytonemin with a variety of mild reducing agents (e.g. ascorbic acid) yields a bright red pigment with slightly improved solubility properties[1,7]. This reduction product often appears in scytonemin-containing sheaths when the cyanobacteria become buried in anoxic sediments and in microbial mat layers and when the cells are no longer viable[1]. Scytonemin is easily reformed from this reduced pigment by air oxidation on silica gel or $NaIO_4$ oxidation. The redox relationship of these two forms of the sheath pigment is consistent with their incidence in nature[1].

Scytonemin and its reduction product both analyzed for $C_{36}H_{22}N_2O_4$ (obs. $M^+$ 546.1578, −0.2 mamu dev.) by high resolution fast atom bombardment mass spectrometry (HR FAB MS), and hence, appeared to possess 27 degrees of unsaturation. Scytonemin, the oxidized form of the pigment with 28 degrees of unsaturation, apparently undergoes a facile reduction in the mass spectrometer. By $^{13}C$ NMR spectroscopy, scytonemin showed only 16 carbon lines, and thus had several elements of symmetry (table). By $^1H$ and $^{13}C$ NMR, a *para*-substituted phenol was apparent in the pigment which accounted for degeneracy of two carbon and two proton absorption bands. Hence, an empirical carbon and hydrogen molecular formula of $C_{18}H_{10}$ was deduced, indicating that the pigment was a symmetrical dimer of $C_{18}H_{10}NO_2$ units. As all of the carbon atoms in scytonemin are $sp^2$ hybridized as shown by $^{13}C$ NMR spectroscopy, the pigment must contain 8 rings.

Detailed 2D-NMR analysis employed the reduced pigment[15] as it was both more soluble and showed one additional proton resonance ($\delta$ 11.79 in DMF-$d_7$, table). This latter signal was shown by a variety of NMR techniques and comparisons to model compounds[16] to belong to the N*H* proton of a 2,3-disubstituted indole ring. In addition, the carbons corresponding to one of the 2H signals of the phenolic ring (C11, C15) showed long range $^1$H-$^{13}$C coupling to an isolated vinyl proton at $\delta$ 7.42, thus defining a trisubstituted ethylene group attached in the *para* position. The remaining atoms in the reduced pigment, a '$C_2O$' unit, must provide a connection between these partial structures and between the two dimeric halves of the molecule, as well as form one more ring. The ketonic nature of this oxygen atom was shown by the characteristic chemical shift of the carbon component ($\delta$ 196.41 in DMF-$d_7$). While several NMR experiments designed to view long range $^1$H-$^{13}$C couplings (LR HETCOSY B, COLOC) confirmed the above partial structures and positioned the isolated vinyl proton $\beta$ to the carbonyl, they could not unequivocally define the nature of the linkage between the dimeric units. The two structural possibilities for this final ring and connection between units (fig. 3), a cyclopentanoid (*A*) with a single connection between carbon atoms in each half versus a cyclohexanoid (*B*) with the two bridgehead carbon atoms in common to both halves, were indistinguishable by NMR analysis.

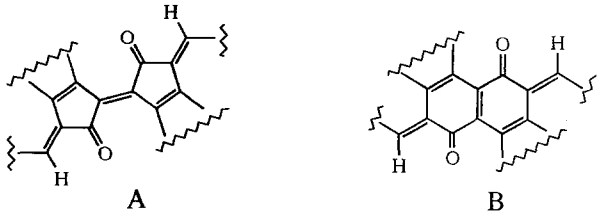


Figure 3. Two conceivable connections between the dimeric halves of scytonemin.

This dilemma was solved by fragmenting the reduced pigment via ozonolysis[17]. A major product (10%, obs. $[M+H]^+$ $C_{18}H_{11}NO_3$, 290.0816, 0.1 mamu dev.) was completely described by a variety of spectroscopic techniques, including 2D NMR methods (table, ref. 17). The data showed that the fragment possessed a cyclopentyl ring containing an $\alpha$-dicarbonyl functionality as well as the indole and phenolic subunits. This product is only conceivable beginning with a dimeric pigment that is joined through an olefinic carbon atom unique to each half of the molecule. The geometry of the trisubstituted olefin was shown by observing nOe between the indole N*H* proton band and C11, 15 (C11′, 15′) proton band. The geometry of the tetrasubstituted olefin in the reduced pigment is predicted as *E* by inspection of molecular models; this was confirmed as the lower energy isomer by MM2 calculation. Oxidation of the reduced pigment involves formal

Table. NMR data for scytonemin, reduced scytonemin, and the ozonolysis fragment derived from reduced scytonemin (chemical shifts in ppm, J in Hz)

| C # | Scytonemin[a,c] $^{13}$C | $^1$H | Reduced Scytonemin[b,c] $^{13}$C | $^1$H | Ozonolysis fragment[d] $^{13}$C | $^1$H |
|---|---|---|---|---|---|---|
| 1 | 129.83 | | 123.38 | | 177.01 | |
| 2 | 194.17 | | 196.41 | | 193.25 | |
| 3 | 118.67 | | 124.69 | | 119.41 | |
| 3a | 174.30 | | 145.76 | | 158.41 | |
| 4a | 163.94 | | 142.21 | | 140.63 | |
| 5 | 122.08 | 7.76 d (7.7) | 113.51 | 7.65 d (8.0) | 114.09 | 7.68 dd (8.0, 0.9) |
| 6 | 135.14 | 7.49 ddd (7.7, 7.6, 1.1) | 124.93 | 7.31 ddd (8.0, 7.2, 1.2) | 126.38 | 7.42 ddd (8.0, 7.3, 1.1) |
| 7 | 126.64 | 7.22 dd (7.6, 7.2) | 121.30 | 7.21 ddd (8.0, 7.2, 1.0) | 123.90 | 7.34 ddd (7.5, 7.3, 0.9) |
| 8 | 129.67 | 7.89 d (7.2) | 125.98 | 7.75 d (8.0) | 120.69 | 7.85 d (7.5) |
| 8a | 125.61 | | 124.37 | | 120.79 | |
| 8b | 158.63 | | 127.73 | | 123.60 | |
| 9 | 139.42 | 8.00 s | 128.22 | 7.42 s | 128.84 | 7.32 s |
| 10 | 126.36 | | 126.94 | | 124.44 | |
| 11, 15 | 136.86 | 9.00 d (8.7) | 131.71 | 7.81 d (8.6) | 131.71 | 7.70 d (8.5) |
| 12, 14 | 117.08 | 7.34 d (8.7) | 116.91 | 7.01 d (8.6) | 116.40 | 6.97 d (8.5) |
| 13 | 163.55 | | 160.22 | | 159.84 | |
| OH | | | | 10.34 bs | | 10.26 bs |
| NH | | | | 11.79 bs | | 12.21 bs |

[a]Spectra recorded in pyridine-$d_5$. $^1$H NMR at 300.13 MHz, $^{13}$C NMR at 75.46 MHz. $^1$H NMR spectrum referenced to TMS at 0.00 ppm; $^{13}$C NMR referenced to pyridine centerline at 149.90 ppm. The centerline of the 7.22 ppm pattern in the $^1$H NMR spectrum is obscured by the furthest upfield pyridine signal. Assignments based on $^1$H-$^1$H COSY, XHCORR, and HMBC spectra.
[b]Spectra recorded in DMF-$d_7$. $^1$H NMR at 300.13 MHz, $^{13}$C NMR at 75.46 MHz. $^1$H NMR spectrum referenced to upfield methyl of DMF at 2.74 ppm; $^{13}$C NMR referenced to DMF centerline at 162.70 ppm. Assignments based on $^1$H-$^1$H COSY, XHCORR, LRHETCOSYB, and COLOC spectra.
[c]Due to the dimeric nature of these compounds, indicated shifts represent atoms in both halves of the molecule (see text and fig. 4).
[d]Spectra recorded in DMSO-$d_6$. $^1$H NMR at 400.13 MHz, $^{13}$C NMR at 100.61 MHz. $^1$H NMR spectrum referenced to TMS at 0.00 ppm; $^{13}$C NMR referenced to DMSO centerline at 39.50 ppm. Assignments based on XHCORR and HMBC spectra.

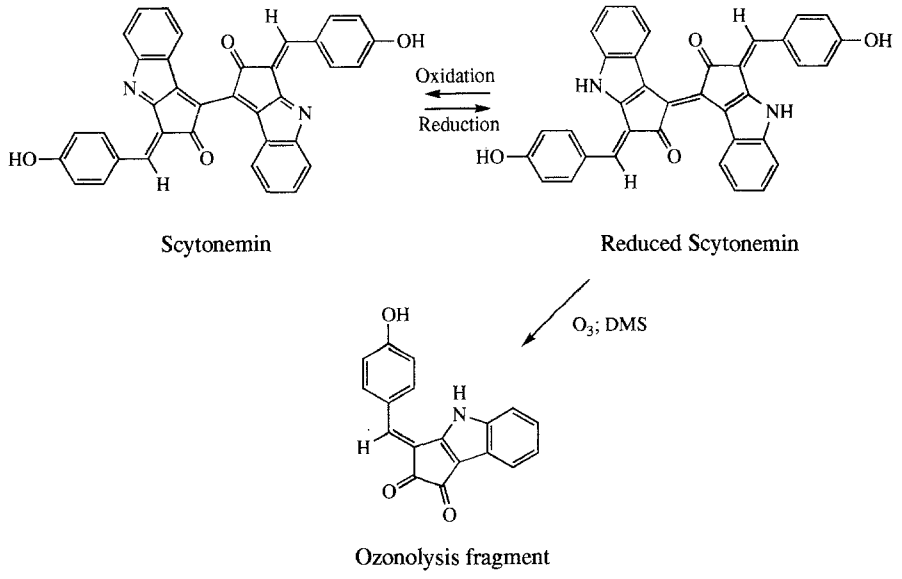


Figure 4. Scytonemin, its reduction product (see text and references 1, 7), and monomer formed by ozonolysis[17].

loss of $H_2$ with consequent reorganization of olefinic bonds to reform scytonemin containing imine-type nitrogens in both halves of the molecule[18] (figs 4 and 5). The imine-type functionality in scytonemin is supported by a $^{13}C$ NMR shift of $\delta$174.30 for C3a and C3a' (pyr-$d_5$).

Scytonemin likely derives from condensation of tryptophan- and phenylpropanoid-derived subunits; however, the linkages between these units in this pigment are unique among natural products. Hence, these molecules possess a new ring system in nature for which we propose the trivial name 'the scytoneman skeleton' (fig. 6). The elucidation of the chemical structure and biological function of scytonemin adds a microbial analog to the short list of compounds known to have an ultraviolet sunscreen action in living organisms (e.g. animal melanins[19,20] and plant flavonoids[21,22]).

Figure 5. 3D-representation of the oxidized form of scytonemin (energy minimized using CHEM 3D Plus: grey = carbon, white = hydrogen, black = nitrogen, stippled = oxygen).

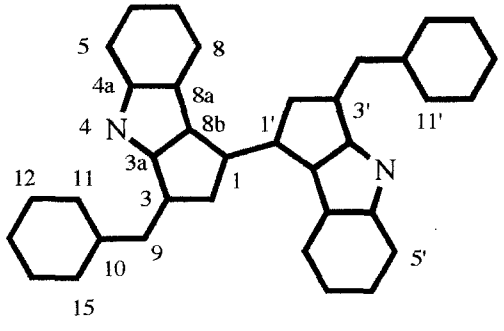

Figure 6. The 'scytoneman skeleton' is a new carbon skeletal type in nature composed of indolic and phenolic subunits.

In addition to the important adaptive role of scytonemin to the cyanobacteria which produce it, this pigment must certainly play an important role in microbial communities exposed to high solar radiation. The high concentration of scytonemin in many cyanobacterial sheaths will unquestionably provide significant protection to other microorganisms living within and beneath the upper layer of sheathed cyanobacteria. Because of these attributes and the possible role scytonemin may have played in the early evolution of photosynthetic organisms, new insights are certain to result from knowledge of its molecular structure.

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