GK1.5 treatment (Table 3). Although nearly all thymocytes express L3T4 (*5*), the percentage of L3T4$^+$ thymocytes was not significantly changed in treated mice (*8*).

The mechanism of the therapeutic actions of GK1.5 administration is still unknown. The observed decrease in the numbers of peripheral L3T4$^+$ helper (inducer) or effector T cells may be sufficient to account for the amelioration of the disease. Alternatively, the ability of the antibody to bind L3T4 and block the functional activities of this molecule could also play a role. In vivo administration of monoclonal antibodies to the I-A subregion of the major histocompatibility complex can prevent and reverse EAE (*9*). It is possible that antibody to L3T4 and antibodies to I-A effect EAE because both antibodies block T-cell activation, either at the site of the T-cell receptor for I-A [in the case of treatment with antibody to L3T4 (*5, 10*)] or at the site of I-A on antigen-presenting cells (in the case of treatment with antibody to I-A). In addition, if there is a reduction in the number of L3T4$^+$ cells or if their activation is blocked (or both), there may be a shift in the balance between regulatory helper and suppressor T cells to an alternate stable state (*11*) in which suppression becomes the dominant response to the encephalitogenic components of MSCH.

Regardless of the mechanism of action of GK1.5 treatment, we have shown that in vivo administration of GK1.5 has a dramatic effect on the course of EAE. The successful treatment of murine EAE with GK1.5 suggests that therapy with monoclonal antibodies to the human equivalent of the murine L3T4$^+$ subset might prove effective in the treatment of MS and possibly in other diseases where this T-cell subset plays a central role.

MATTHEW K. WALDOR*
*Departments of Genetics* and *Neurology, Stanford University, Stanford, California 94305*
SUBRAMANIAN SRIRAM
*Department of Neurology, Stanford University*
RICHARD HARDY
LEONORE A. HERZENBERG
LEONARD A. HERZENBERG
*Department of Genetics, Stanford University*
LEWIS LANIER
*Becton Dickinson Monoclonal Antibody Center, Mountain View, California 94043*
MAE LIM
LAWRENCE STEINMAN
*Departments of Neurology* and *Pediatrics, Stanford University*

**References and Notes**

1. E. C. Alvord, M. W. Kies, A. J. Suckling, *Experimental Allergic Encephalomyelitis, A Useful Model for Multiple Sclerosis* (Liss, New York, 1984); P. Y. Paterson, in *Autoimmunity*, N. Talal, Ed. (Academic Press, New York, 1977), pp. 644–692.
2. B. G. Arnason *et al.*, *J. Exp. Med.* **116**, 177 (1962); N. K. Gonatas and J. C. Howard, *Science* **186**, 839 (1974); L. Ortiz-Ortiz and W. O. Weigle, *J. Exp. Med.* **144**, 604 (1976).
3. U. Traugott, E. L. Reinherz, C. S. Raine, *Science* **219**, 308 (1982).
4. C. B. Pettinelli and D. E. McFarlin, *J. Immunol.* **127**, 1420 (1981); Z. Lando and A. Ben-Nun, *Clin. Immunol. Immunopathol.* **30**, 290 (1984); J. Trotter and L. Steinman, *J. Immunol.*, in press.
5. D. P. Dialynas *et al.*, *Immunol. Rev.* **74**, 29 (1983).
6. E. L. Reinherz and S. F. Schlossman, *N. Engl. J. Med.* **303**, 370 (1980); E. L. Reinherz *et al.*, *ibid.*, p. 125.
7. C. Bernard and I. R. MacKay, *J. Neuroimmunol.* **4**, 61 (1983).
8. M. K. Waldor *et al.*, unpublished data.
9. L. Steinman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7111 (1981); S. Sriram and L. Steinman, *J. Exp. Med.* **158**, 1362 (1983).
10. P. Marrack *et al.*, *J. Exp. Med.* **158**, 1077 (1983).
11. L. A. Herzenberg, S. J. Black, L. A. Herzenberg, *Eur. J. Immunol.* **10**, 1 (1980).
12. J. A. Ledbetter and L. A. Herzenberg, *Immunol. Rev.* **47**, 63 (1979).
13. K. Hayakawa, R. R. Hardy, D. R. Parks, L. A. Herzenberg, *J. Exp. Med.* **157**, 202 (1983).
14. D. R. Parks, R. R. Hardy, L. A. Herzenberg, *Immunol. Today* **4**, 145 (1983).
15. Supported by NIH grants NS-571, NS-18235, GM-17367, HD-01287, and CA-04681; a grant from the Multiple Sclerosis Society 1440-A-1; and a grant from the Kroc Foundation.
* To whom correspondence should be addressed at Department of Neurology, C-338, Stanford University, Stanford, Calif. 94305.

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# Hernandulcin: An Intensely Sweet Compound Discovered by Review of Ancient Literature

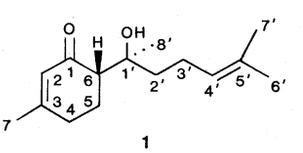
Abstract. *Ancient Mexican botanical literature was systematically searched for new plant sources of intensely sweet substances.* Lippia dulcis *Trev., a sweet plant, emerged as a candidate for fractionation studies, and hernandulcin, a sesquiterpene, was isolated and judged by a human taste panel as more than 1000 times sweeter than sucrose. The structure of the sesquiterpene was determined spectroscopically and confirmed by chemical synthesis. Hernandulcin was nontoxic when administered orally to mice, and it did not induce bacterial mutation.*

Because of such factors as the caloric and cariogenic potential of sucrose, alternative means are being sought to satisfy the human habit of consuming sweets. Synthetic substances such as saccharin, cyclamate, and aspartame have been or are being used in the United States for this purpose, while naturally occurring substances such as phyllodulcin, stevioside and glycyrrhizin are used in Japan (*1, 2*). None of these substances is ideal, however, either because of taste characteristics that are generally perceived as unpleasant, questionable safety, chemical instability, or because of the relatively high cost of production (*1, 3*).

The discovery of practically every intensely sweet, prototype molecule has been fortuitous; there is still insufficient knowledge to design sweet compounds that are structurally unrelated to existing sweeteners (*3*). In addition, large-scale screening of potential sweeteners is impractical because of the lack of convenient bioassay systems (*4*). Thus, as part of a program to develop new sweetening agents and to provide compounds that could aid in understanding the relation between moleclular structure and sweetness, the Mexican ethnobotanical literature was searched with the express purpose of uncovering records of intensely sweet-tasting plants.

While examining a monograph entitled *Natural History of New Spain*, written between 1570 and 1576 by the Spanish physician Francisco Hernández (*5*), our attention was drawn to a remarkably sweet plant known to the Aztec people by the Nahuatl name *Tzonpelic xihuitl*. The literal translation of these words is "sweet herb." The accurate description and illustration of the plant provided by Hernández combined with another mention (*6*), enabled the assignment of *Tzonpelic xihuitl* as *Lippia dulcis* Trev. (Verbenaceae). The constituents of this plant do not appear to have been studied in recent years, but investigators have suggested that the sweet principle is volatile (*7*) and distinguishable from the sweet glycoside glycyrrhizin (*8*).

The principal sweet component of *L. dulcis* (*9*), which was present mainly in the leaves and flowers, was isolated in pure form after solvent partition and chromatographic fractionation. This compound, **1**, which has been named

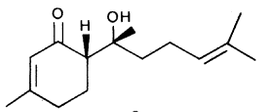



**1**

hernandulcin in honor of Francisco Hernández, was obtained as a colorless oil $[\alpha]_D^{25}$ +109° (c, 0.11 g/100ml in ethanol). Its molecular formula is $C_{15}H_{24}O_2$, based on a high-resolution mass spectral measurement of the molecular ion, 236.18005 amu. Analysis of the $^1$H- and $^{13}$C-NMR

(nuclear magnetic resonance) spectra (Table 1), as well as other spectroscopic measurements (*10*), allowed the tentative identification of hernandulcin as a sesquiterpene of the bisabolane class, with the structure 6-(1,5-dimethyl-1-hydroxyhex-4-enyl)-3-methylcyclohex-2-enone. Two-dimensional NMR experiments (*11, 12*), which measured both the $^1$H-$^1$H and $^1$H-$^{13}$C chemical-shift correlations, supported this identification and permitted the unambiguous assignment of the $^{13}$C-NMR spectrum of hernandulcin (Fig. 1). The data obtained are consistent with those from other bisabolane sesquiterpenes (*13, 14*).

The structure proposed for hernandulcin was confirmed by chemical synthesis resulting from a directed aldol-condensation reaction (*15*). The reaction afforded (±)-hernandulcin and its diastereomeric counterpart, (±)-epihernandulcin, **2**, in a




**2**

ratio of 95 to 5, respectively. On the basis of this, the relative stereochemistries of hernandulcin and epihernandulcin were established, since directed aldol condensations are known to proceed through a chairlike intermediate in which the conformer with the greater number of equatorial substituents is preferred (*16*). Further supporting evidence for the stereochemistry of the products was obtained by the observation of a 28 percent $^1$H-NMR nuclear Overhauser enhancement (nOe) for (±)-epihernandulcin, indicating that the C-8′ methyl protons and the C-6 proton are arranged in a *syn* conformation. No such nOe effects were evident for (±)-hernandulcin. The differences in the $^{13}$C-NMR chemical shifts for C-5, C-6, C-2′, and C-8′ in hernandulcin and epihernandulcin are consistent with expected differences in intramolecular steric interactions of the compounds (*17*).

Table 1. $^1$H- and $^{13}$C-NMR spectral data for hernandulcin, **1**, and epihernandulcin, **2**, recorded with deuterochloroform as the solvent and tetramethylsilane as the internal standard. Abbreviations: δ, chemical shift; M, multiplicity; *J*, coupling constant in hertz; s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; m, multiplet; br, broad.

| Site | $^1$H-NMR* δ, M, *J* | | $^{13}$C-NMR† δ, M | |
|---|---|---|---|---|
| | **1** | **2** | **1** | **2** |
| C-1 | | | 204.0, s | 203.6, s |
| C-2 | 5.88, s (br) | 5.85, s (br) | 127.4, d | 127.4, d |
| C-3 | | | 163.6, s | 163.6, s |
| C-4 | 2.34, m | 2.33, m | 31.2, t | 31.6, t |
| C-5 | 1.69, m; 2.05, m | 1.75, m; 2.06, m | 25.0, t | 22.1, t |
| C-6 | 2.42, dd, 14.1, 4.5 | 2.35, dd, 14.1,4.5 | 52.0, d | 55.4, d |
| C-7 | 1.97, s (br) | 1.92, s (br) | 24.1, q | 24.2, q |
| C-1′ | | | 73.9, s | 74.4, s |
| C-2′ | 1.48, t (br), 8.0 | 1.38, m; 1.55, m | 40.1, t | 36.9, t |
| C-3′ | 2.05, m; 2.15, m | 2.00, m; 2.20, m | 21.5, t | 22.1, t |
| C-4′ | 5.19, t (br), 8.1 | 5.08, t (br) | 124.4, d | 124.7, d |
| C-5′ | | | 131.4, s | 131.5, s |
| C-6′ | 1.68, s (br) | 1.66, s (br) | 25.7, q | 25.8, q |
| C-7′ | 1.63, s (br) | 1.60, s (br) | 17.6, q | 17.7, q |
| C-8′ | 1.18, s | 1.19, s | 23.6, q | 25.5, q |
| –OH | 5.29, s | 5.07, s | | |

*Recorded at 360 MHz. †Recorded at 90.8 MHz.

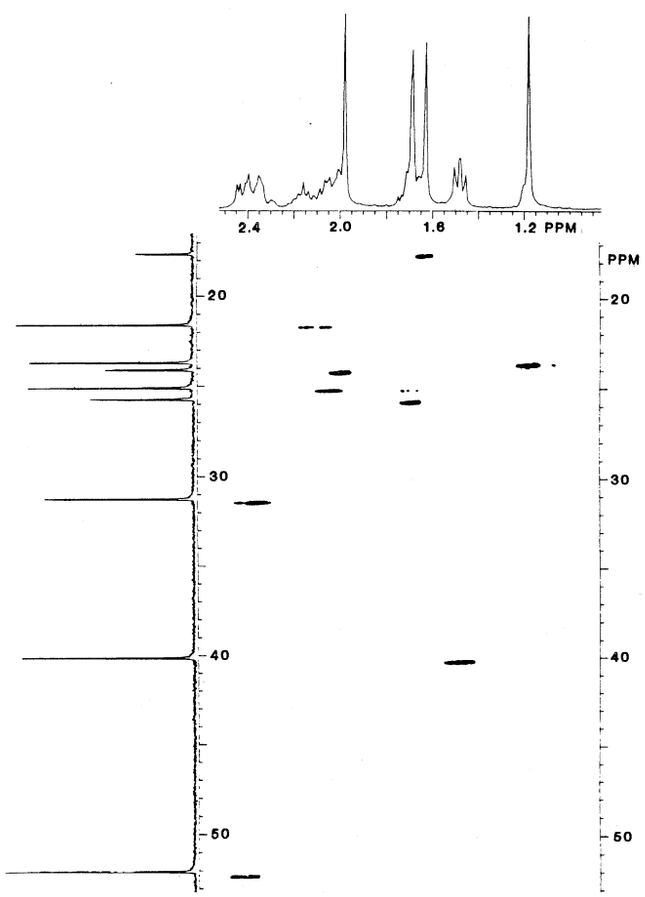



Fig. 1. Two-dimensional $^1$H-$^{13}$C shift-correlated NMR spectrum of hernandulcin (upfield region only), obtained with a Nicolet NIC-360 spectrometer at 90.8 MHz ($^{13}$C) and 360 MHz ($^1$H). The pulse sequence used was that of Bax and Morris (*12*), with $\Delta_1 = 4$ msec and $\Delta_2 = 2.4$ msec. The initial data matrix consisted of 256 blocks of 1024 points each with 200 acquisitions per block. Zero-filling resulted in a final data matrix of 512 blocks of 2048 points. The sine-bell function was used to enhance the resolution in both dimensions, and the spectrum shown is the absolute value. The one-dimensional $^1$H- and $^{13}$C-NMR spectra, respectively, are shown at the top and along the side of the figure.

The mutagenic potential of (±)-hernandulcin was examined by a forward mutation assay utilizing *Salmonella typhimurium* strain TM677, according to published procedures (*18, 19*). No significant mutagenic activity was observed when (±)-hernandulcin was assayed in either the presence or absence of a metabolic activating system derived from the livers of rats treated with Aroclor 1254. When suspended in 1 percent sodium carboxymethyl cellulose and then administered by gastric intubation to male Swiss-Webster mice at single doses up to 2 g per kilogram of body weight, (±)-hernandulcin produced no mortality.

Preliminary studies with a human taste panel were performed to test the sensory properties of naturally occurring (+)-hernandulcin (*20*). A $2.0 \times 10^{-4}M$ aqueous solution of this compound was perceived to be equivalent in sweetness to a 0.25*M* solution of sucrose; thus, on a molar basis, (+)-hernandulcin was judged to be more than three orders of magnitude sweeter than sucrose. Nevertheless, (+)-hernandulcin was considered by the panel to be somewhat less pleasant than sucrose and to exhibit perceptible off- and aftertastes as well as some bitterness.

It is generally accepted that sweet-tasting compounds bind stereoselectively with a receptor located in the taste-cell

membrane (*3*). For hernandulcin, two possible binding sites are the C-1 carbonyl and C-1′ hydroxyl groups because the sweet taste of the compound is lost when these groups are modified (*21*). Since these two functional groups are located about 2.6 Å apart in the preferred conformation, this molecule closely fits the Shallenberger model (*22*) for sweet-tasting compounds. Thus, the structural simplicity of hernandulcin, the availability of a facile synthesis for it, and the intense sweetness it exhibits make it a target molecule for the further investigation of the relation between structure and sweetness.

CESAR M. COMPADRE
JOHN M. PEZZUTO
A. DOUGLAS KINGHORN*
*Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, Health Sciences Center, University of Illinois at Chicago, Chicago 60612*

SAVITRI K. KAMATH
*Department of Nutrition and Medical Dietetics, College of Associated Health Professions, University of Illinois at Chicago*

### References and Notes

1. E. Newbrun, *Cariology* (Williams & Wilkins, Baltimore, ed. 2, 1983), pp. 122–160.
2. G. A. Crosby and R. E. Wingard, Jr., in *Developments in Sweeteners*, C. A. M. Hough, K. J. Parker, A. J. Vlitos, Eds. (Applied Science, London, 1979), vol. 1, pp. 135–164.
3. G. A. Crosby, G. E. DuBois, R. E. Wingard, Jr., in *Drug Design*, E. J. Ariëns, Ed. (Academic Press, New York, 1979), vol. 8, pp. 215–310.
4. G. T. Shearman, M. P. Cafaro, H. Lal, J. L. Howard, *Drug Dev. Res.* **1**, 145 (1981).
5. This monograph has been published at intervals in Latin and Spanish. The last edition that appeared is F. Hernández, *Obras Completas Tomo II: Historia Natural de Nueva España* (Universidad Nacional de México, Mexico City, 1959).
6. M. Martinez, *Las Plantas Medicinales de México* (Ediciones Botas, Mexico City, ed. 5, 1969).
7. M. Rio de la Loza, *Farmacía, Méx.* **1**, 3 (1892).
8. K. Brietwieser, *Pharm. Ind.* **10**, 76 (1943).
9. *L. dulcis* was collected at Tlayacapan in central Mexico, and a voucher specimen (*Compadre 105*) is deposited in the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, Ill.
10. Hernandulcin showed infrared absorptions at 3479 $cm^{-1}$ (0.002 to 0.005*M* in carbon tetrachloride) and 1644 $cm^{-1}$ (film) for an intramolecularly bonded hydroxyl group and an α,β-unsaturated keto group, respectively. The latter signal was confirmed by an observed maximum in the ultraviolet spectrum in ethanol at 236 nm (ε, 7775). Prominent fragment ions in the high-resolution mass spectrum of hernandulcin were observed at *m/z* 110 ($C_7H_{10}O$), *m/z* 95 ($C_6H_7O$), and *m/z* 82 ($C_5H_6O$), which suggested that it is a monocyclic sesquiterpene.
11. R. Benn and H. Günther, *Angew. Chem. Int. Ed. Engl.* **22**, 350 (1983).
12. A. Bax and G. A. Morris, *J. Magn. Reson.* **42**, 501 (1981).
13. M. Becker and P. Weyerstahl, *Helv. Chim. Acta* **62**, 2724 (1979).
14. F. Bohlmann, C. Zdero, S. Shöneweiss, *Chem. Ber.* **109**, 3366 (1976).
15. The reaction was performed by adding 6-methyl-5-hepten-2-one (8.5 mmol) to a cold (−15°C) mixture of 3-methyl-2-cyclohexen-1-one (8.5 mmol) and lithium diisopropylamide (8.5 mmol) in anhydrous tetrahydrofuran (15 ml). After the reaction mixture was stirred for 5 minutes, it was worked up in the usual manner; purification by column chromatography afforded (±)-hernandulcin (4.1 mmol) and (±)-epihernandulcin (0.2 mmol). This type of reaction has been reviewed [T. Mukaiyama, *Org. React.* **28**, 203 (1982)]. Whereas (±)-hernandulcin is sweet, (±)-epihernandulcin exhibits no sweet taste.
16. D. A. Evans, J. V. Nelson, T. R. Taber, in *Topics in Stereochemistry*, N. L. Allinger and E. L. Eliel, Eds. (Interscience, New York, 1982), vol. 13, pp. 1–115.
17. C. H. Heathcock, M. C. Pirrung, J. E. Sohn, *J. Org. Chem.* **44**, 4294 (1979).
18. J. M. Pezzuto *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1427 (1980).
19. J. M. Pezzuto *et al.*, *Chem.-Biol. Int.* **43**, 323 (1983).
20. Sensory studies were performed by a panel of 17 previously trained volunteers according to guidelines [H. R. Moskowitz, R. R. Kluter, J. Westerling, H. L. Jacobs, *Science* **184**, 583 (1974)]. Sucrose was dissolved in distilled water in the following concentrations, 0.031*M*, 0.06210*M*, 0.125*M*, 0.25*M*, 0.50*M*, 1.0*M*, and 2.0*M*; and (+)-hernandulcin was prepared as $1.06 \times 10^{-5}M$, $2.11 \times 10^{-5}M$, $4.24 \times 10^{-5}M$, $8.47 \times 10^{-5}M$, $16.9 \times 10^{-5}M$, $33.9 \times 10^{-5}M$, and $67.8 \times 10^{-5}M$ aqueous solutions. The solutions were presented at random (in duplicate) to panel participants, and the participants expectorated and rinsed their mouths with distilled water between tastes. Using an open-ended magnitude-ratio scale, sucrose and hernandulcin were rated for sweetness intensity and sweetness pleasantness. The off-taste and aftertaste intensities and the pleasantness and bitterness intensities were estimated in a similar manner. The standard panel response for the sweetness pleasantness of sucrose reached a maximum for a 0.25*M* solution.
21. Derivatives of (±)-hernandulcin in which the C-1 carbonyl group was reduced to an alcohol or the C-1′ hydroxyl group was acetylated were not sweet.
22. R. S. Shallenberger, T. E. Acree, C. Y. Lee, *Nature (London)* **221**, 555 (1969).
23. We thank R. C. Gesteland, C. E. Costello, D. D. Soejarto, A. N. Abdel-Sayed, and R. L. Lopez de Compadre for their technical assistance. Supported by the National Institute of Dental Research (contract N01-DE-02425), an NCI Research Career Development Award for 1984 to 1989 (J.M.P.), and the National Autonomous University of Mexico (C.M.C.).
* To whom correspondence should be addressed.

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## Disk-to-Disk Transfer as the Rate-Limiting Step for Energy Flow in Phycobilisomes

Abstract. *A broadly tunable picosecond laser source and an ultrafast streak camera were used to measure temporally and spectrally resolved emission from intact phycobilisomes and from individual phycobiliproteins as a function of excitation wavelength. Both wild-type and mutant phycobilisomes of the unicellular cyanobacterium* Synechocystis *6701 were examined, as well as two biliproteins, R-phycoerythrin (240 kilodaltons, 34 bilins) and allophycocyanin (100 kilodaltons, 6 bilins). Measurements of intact phycobilisomes with known structural differences showed that the addition of an average of 1.6 phycoerythrin disks in the phycobilisome rod increased the overall energy transfer time by 30 ± 5 picoseconds. In the isolated phycobiliproteins the onset of emission was as prompt as that of a solution of rhodamine B laser dye and was independent of excitation wavelength. This imposes an upper limit of 8 picoseconds (instrument-limited) on the transfer time from "sensitizing" to "fluorescing" chromophores in these biliproteins. These results indicate that disk-to-disk transfer is the slowest energy transfer process in phycobilisomes and, in combination with previous structural analyses, show that with respect to energy transfer the lattice of approximately 625 light-harvesting chromophores in the* Synechocystis *6701 wild-type phycobilisome functions as a linear five-point array.*

Conversion of light energy to chemical potential in biological photosynthetic systems is accomplished in macromolecular complexes composed of polypeptides and pigment molecules. A general feature of these complexes is the presence of numerous chromophores ("antenna pigments"), which absorb light and transfer the excitation quantum to a special chlorophyll or bacteriochlorophyll molecule in the "reaction center" for subsequent conversion to electron flow (*1*). Such complexes often contain several hundred antenna chromophores per reaction center. Since the overall quantum efficiency for the transfer of energy from the antenna to the reaction center is typically ~90 percent (*1*), energy transfer to the reaction center must compete successfully with pathways of energy dissipation in the antenna, such as fluorescence and nonradiative decay. Because most light-harvesting complexes are integral membrane components, information on the details of their molecular architecture is limited (*2*). Consequently, the structural basis for the highly efficient transfer of energy in these systems is not understood. However, an opportunity to correlate the structure and function of one light-harvesting system is provided by the phycobilisome, an antenna complex that efficiently funnels light energy collected over a broad spectral region and a large spatial area to the reaction center of photosystem II in cyanobacteria and red algae (*3*).

Phycobilisomes are peripheral membrane complexes that can be readily isolated without perturbing their structural and functional properties (*3*). Phycobilisome morphology depends on organismal origin. These particles have molecular weights from $7 \times 10^6$ to $15 \times 10^6$, contain between 300 and 800 tetrapyrrole (bilin) chromophores, and absorb light