evidence for the release of endogenous opioid peptides after physiological stimulation is required to establish their neurotransmitter role at this site.

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References and Notes

1. See review by R. A. North [*Life Sci.* **24**, 1927 (1979)].
2. D. G. Amaral and H. M. Sinnamon, *Prog. Neurobiol. N.Y.* **9**, 147 (1977).
3. S. F. Atweh and M. J. Kuhar, *Brain Res.* **129**, 1 (1977); C. B. Pert, M. J. Kuhar, S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3929 (1977).
4. V. M. Pickel, T. H. Joh, D. J. Reis, S. E. Leeman, R. J. Miller, *Brain Res.* **160**, 387 (1979).
5. R. Simantov *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2167 (1977).
6. M. Sasa, K. Munekiyo, Y. Osumi, S. Takaori, *Eur. J. Pharmacol.* **42**, 53 (1977).
7. S. J. Bird and M. J. Kuhar, *Brain Res.* **122**, 523 (1977); P. J. Guyenet and G. K. Aghajanian, *ibid.* **136**, 178 (1977); J. Korf, B. S. Bunney, G. K. Aghajanian, *Eur. J. Pharmacol.* **25**, 165 (1974); W. S. Young, S. J. Bird, M. J. Kuhar, *Brain Res.* **129**, 366 (1977).
8. Coronal slices (300 μm thick) of guinea pig pons were mounted in a recording chamber [P. A. Schwartzkroin, *Brain Res.* **85**, 423 (1975)] and superfused with Krebs solution (2 ml/min) at 37°C. The Krebs solution was of the following composition (m*M*): NaCl, 126; KCl, 5; NaH_2PO_4, 1.2; $MgSO_4$, 1.3; $CaCl_2$, 2.4; $NaHCO_3$, 26; and glucose, 10; and was gassed with 95 percent O_2 and 5 percent CO_2. To prevent movement of the slice, a titanium electron microscopy grid was placed on top of it and held in position by a platinum loop attached to a micromanipulator. Viewed from above at a magnification of ×10 to ×40, the locus coeruleus could be seen as a dark gray area below the fourth ventricle and medial to the mesencephalic root of the trigeminal nerve. Recording microelectrodes, filled with 4*M* potassium acetate and having tip resistances of 50 to 100 megohms were positioned over this area and manually advanced into the tissue until a cell was impaled; then the position of the recording electrode was noted. At the end of each experiment the tissue was fixed in formaldehyde and stained with cresyl violet. The experiments reported here are those in which subsequent histological examination of the slice confirmed that the recordings had been made from neurons of the locus coeruleus.
9. C. M. Pepper and G. Henderson, unpublished observations.
10. R. A. North, Y. Katayama, J. T. Williams, *Brain Res.* **165**, 67 (1979).
11. G. M. Lees, H. W. Kosterlitz, A. A. Waterfield, *Agonist and Antagonist Actions of Narcotic Analgesic Drugs*, H. W. Kosterlitz, H. O. J. Collier, J. E. Villareal, Eds. (University Park Press, Baltimore, 1972), p. 142; K. Jhamandas, J. Sawynock, M. Sutak, *Nature (London)* **269**, 433 (1977); K. Starke, *Rev. Physiol. Biochem. Pharmacol.* **77**, 1 (1977).
12. W. Zieglgänsberger and H. Bayerl, *Brain Res.* **115**, 111 (1976).
13. B. R. Sastry, *Neuropharmacology* **18**, 367 (1979); E. Carstens, I. Tulloch, W. Zieglgänsberger, M. Zimmerman, *Pfluegers Arch.* **379**, 143 (1979).
14. J. L. Barker, J. H. Neale, T. G. Smith, Jr., R. L. Macdonald, *Science* **199**, 1451 (1978); A. W. Mudge, S. E. Leeman, G. D. Fischbach, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 526 (1979).
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Evidence for Lignin-Like Constituents in Early Silurian (Llandoverian) Plant Fossils

Abstract. *Chemical evidence is presented with previously reported morphological features for banded-tube cell types in the earliest known plant fossils associated with stream-deposited sediments. Phenolic aldehydes (p-hydroxybenzaldehyde, vanillin) and aromatic compounds from pyrolysis (2-methoxy-4-hydroxybenzaldehyde, methylsyringaldehyde) derived from cellular remains are interpreted as evidence for lignin or lignin-like degradation products. The presence of parallel-aligned banded tubes, with annular to spiral thickenings and occasional end walls, in conjunction with lignin-like constituents fulfill most of the morphological and chemical criteria for cell types that could have functioned as water-conducting cells.*

Parallel-aligned banded tubes with annular to spiral ribbing have been reported in early Silurian (Llandoverian) plant fossils found in stream-deposited sediments (*1*). The parallel alignment, possession of end walls, and ribbing in some of these cellular remains suggest a structural similarity to tracheids and a functional specialization related to water-transport tissues. Owing to the extreme age of this early Silurian material, which predates the earliest known vascular plants, the structural similarities between the banded tubes and tracheids may be misleading. Tracheids must be defined on the basis of their morphology and biochemistry, as well as their function. They are highly specialized cells, nonliving at maturity, more or less elongated, having a variety of potential pit types (*2*), and are chemically characterized by having lignified walls with secondary thickenings. Within the evolutionary context, the tracheid is the single most important anatomical criterion for affirming a vascular plant habit (*3*). Other features such as trilete spores, cuticles, sporopollenin, and stomata provide only inferential evidence for a vascular plant. Lignin, a necessary aspect of the tracheid definition, has been isolated from Paleozoic, Mesozoic, and Cenozoic fossil vascular plants (*4*-*6*). Its presence may be detected by alkaline oxidation with nitrobenzene or pyrolysis, which yields *p*-hydroxybenzaldehyde, vanillin, syringaldehyde, and various aromatic acids (*7*). We have chemically analyzed the Llandoverian plant fossils from the Massanutten Sandstone, Virginia (*1*), for phenolics and possible lignin decomposition products. The chemical data confirm the presence of low concentrations of what we interpret to be lignin-like constituents. The presence of these chemicals in early Silurian plant remains found in stream-deposited sediments suggests that these plants may have had cell types that were both structurally and biochemically similar to those of water-conducting cells.

Fossiliferous siltstone containing the carbonaceous plant remains was collected from the lower Massanutten Sandstone, Virginia, which is considered to be Albion (Llandoverian) in age (*1*). The fossils appear as mostly amorphous coalified remains showing little distinguishable megastructure. They were mechanically removed from freshly parted samples and washed in double-distilled water. Duplicate samples of the associated siltstone and a high-fired clay were tested with the plant samples so as to check for organic contaminants in the extraction or handling procedure. None were detected. The chemical constituents reported here are considered indicative of the plant assemblage's biochemistry. Organic solvent extractions (*4*), pyrolysis (*5*), and oxidation with alkaline nitrobenzene (*6*) of separate subsamples were performed, and the chemical constituents were identified by gas chromatography (GC) and by combined GC-mass spectroscopy (GC-MS) (*4*). Of particular interest is the isolation of significant amounts of vanillin and *p*-hydroxybenzaldehyde, both considered by-products of lignin degradation (Fig. 1A). Pyrolyzates (450°C) from the carbonaceous plant material contained indanone, naphthalenes, and benzofurans; and 600°C pyrograms yielded as their primary aromatic products benzene, alkyl-substituted benzenes, phenols, xylenols, and benzofurans (Fig. 1B). Organic solvent extractions with a mixture of benzene and methanol, 3:1 (by volume), yielded a consortium of constituents similar to those isolated from Devonian vascular or presumed vascular land plants: straight-chain fatty acids (C_{10} to C_{24}, maximum C_{17}); phenols, $C_nH_{(2n-18)}O$ (C_{10} to C_{22}, maximum C_{14}); the aromat-

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ic acids, phenyl, $C_nH_{(2n-8)}O_2$ (C_{10} to C_{14}, maximum C_{11}), and naphthyl, $C_nH_{(2n-14)}O_2$ (C_{11} to C_{16}, maximum C_{16}); and various aromatic dicarboxylic acids, $C_nH_{(2n-10)}O_4$, $C_nH_{(2n-16)}O_4$ (C_{10} to C_{16}, maxima C_{14} and C_{16}).

Oxidation by alkaline nitrobenzene and subsequent GC-MS analyses, and GC-MS pyrograms of the plant fossils from the Massanutten Sandstone yield profiles similar to those of known vascular land plants (*4*-*6*) and suggest the presence of lignin or lignin-like remnants in these early Silurian cell types (Fig. 1A). Quantitative data, indicating very low amounts of these constituents, may be interpreted as evidence for low original concentrations or as the effect of diagenesis. Results of analyses of contemporary lignin differ significantly from those of the Massanutten Sandstone flora, and are interpreted as evidence that the chemical profiles for the fossil remains are not the result of contamination from living plants. Significant in this regard is the almost total lack of syringaldehyde from the fossil material. Lignin appears to undergo decomposition by initial methoxyl content reduction. Geocatalysis resulting in demethoxylation proceeds differentially, preferentially converting syringl groups into vanillyl groups. At elevated temperatures, demethoxylation is followed by alkyl substitution (*4*, *6*). The data may be interpreted as indicative of a geothermal or pressure diagenesis.

Reports of "lignin" or "pseudolignin" in some mosses (*8*) and the structural similarities between banded-tube cell types and the hydroids and conducting cells of some mosses and liverworts raise the possibility that the early Silurian fossils may represent a level of organization parallel with that seen in some nonvascular land plants. When highly selective procedures have been applied to mosses and liverworts they have yielded negative results for the presence of lignin (*9*).

The oxidative degradation procedure of Erikson *et al.* (*10*) when applied to six moss and two liverwort species yielded other types of phenolics in cell walls, but did not show the presence of lignin. Similarly, the degradation by-products of oxidation did not conform to those produced by truly vascular plants. Hébant (*11*) has shown with a broad survey of bryophyte taxa that a positive Wiesner reaction (phloroglucinol hydrochloride) was not present, whereas various "primitive" vascular plant controls gave a positive reaction for lignin. The Wiesner reaction may be weak in plants having high amounts of syringl-propane units; however, this condition is known to occur only in the higher land plants such as angiosperms. We agree therefore with Hébant, who states that "no true convincing evidence for the presence of lignin in bryophytes seems to have emerged" (*12*).

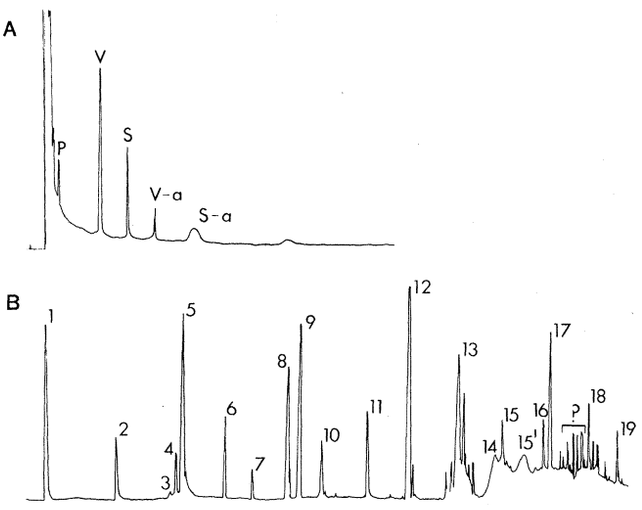

Fig. 1. (A) Gas chromatogram on Apiezon L of trimethylsilyl ethers of *p*-hydroxybenzaldehyde (*P*), vanillin (*V*), syringaldehyde (*S*), vanillic acid (*V-a*), and syringic acid (*S-a*) from Silurian (Llandoverian) plant remains. (B) Partial chromatogram of the 600°C pyrolyzates of Silurian plant remains: peak 1, benzene; peak 2, toluene; peak 3, ethylbenzene; peak 4, *p*-xylene; peak 5, *m*-xylene; peak 6, *o*-xylene; peak 7, styrene; peak 8, ethyltoluene; peak 9, trimethylbenzene; peak 10, benzofuran; peak 11, indene; peak 12, 2-methylbenzofuran; peak 13, phenol; peak 14, cresol; peak 15, dimethylbenzofuran; peak 15', naphthalene; peak 16, unknown; peak 17, naphthalene; peak 18, xylenol; and peak 19, 1-methylnaphthalene.

The isolation of chemical constituents that may be the degradative by-products of a lignin-like moiety from banded cell types in early Silurian plant fossils suggests that these plants had the capacity both to synthesize a lignin-like constituent and to isolate it in specialized cell types. Such a capacity was a necessary but not sufficient antecedent evolutionary event to the appearance of bona fide vascular land plants. The role of lignin in contemporary plant tracheids appears to be that of a controlling agent for the hydration of cell wall hydrophilic constituents. The parallel-aligned banded-tube cell types associated with lignin chemistry and that of the tracheid suggest the probability of their being evolutionary separate solutions to similar physiological or structural problems. The lack of compressions with a characteristic morphology precludes the identification of a thalloid or axial habit for these fossil plants, and the nature of these remains is such that an assemblage of plants, rather than a single plant type, may be represented. The structural interpretation of the banded tubes therefore remains speculative. There are other reports of banded tubes similar to those isolated from the Massanutten material (*13*); however, we do not conclude that all such cell types possess similar chemistries, nor that they are necessarily functional equivalents.

The presence of chemical and anatomical features which in tandem parallel those seen in the vascular plant habit may be shown for the Massanutten Sandstone plant fossils. Collectively, these features indicate the genomic potential for lignified and structurally reinforced cell types that may have been functionally analogous to hydroids or tracheids.

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References and Notes

1. L. M. Pratt, T. L. Phillips, J. M. Dennison, *Rev. Palaeobot. Palynol.* **25**, 121 (1978).
2. A. Fahn, *Plant Anatomy* (Pergamon, Oxford, 1967); K. Esau, *Anatomy of Seed Plants* (Wiley, New York, ed. 2, 1977).
3. H. P. Banks, *BioScience* **25**, 730 (1975); K. J. Niklas, *Ann. Bot.* **40**, 1239 (1976).
4. K. J. Niklas, *Brittonia* **28**, 113 (1976); _____ and W. G. Chaloner, *Rev. Palaeobot. Palynol.* **21**, 81 (1976); K. J. Niklas and P. G. Gensel, *Brittonia* **30**, 216 (1976).
5. A. C. Sigleo, *Science* **200**, 1054 (1978).
6. R. F. Leo and E. S. Barghoorn, *ibid.* **168**, 582 (1970).
7. True lignins are polymeric natural products arising from an enzyme-initiated dehydrogenation polymerization of *trans*-coniferyl-1-ol, *trans*-sinapyl-2-ol, and *trans-p*-coumaryl-3-ol; K. Freudenberg and A. C. Neish, *Constitution and Biosynthesis of Lignin* (Springer-Verlag, New York, 1968).
8. S. M. Siegel, *The Plant Cell Wall* (Pergamon, New York, 1962); *Am. J. Bot.* **56**, 175 (1969); D. C. Scheirer, *Bryologist* **75**, 305 (1972).
9. M. Erikson and G. E. Miksche, *Phytochemistry* **13**, 2295 (1974); *Acta Chem. Scand. Ser. B* **28**, 109 (1974).
10. M. Erikson, S. Larsson, G. E. Miksche, *Acta Chem. Scand.* **27**, 127 (1973).
11. C. Hébant, *J. Hattori Bot. Lab.* **38**, 565 (1974).
12. _____, *The Conducting Tissues of Bryophytes* (Lubrecht S. Cramer, Monticello, N.Y., 1977).
13. P. K. Strother and A. Traverse, *Palynology* **3**, 1 (1979).
14. Supported by NSF grant DEB 78-22646 (to K.J.N.).

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