ther investigations on the quantity of cochliophilin A exuded from spinach roots, and the development of a quantitative bioassay, are under way.

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7 Cochliophilin A (5-hydroxy-6,7-methylenedioxyflavone, **1**). Pale yellow needles. HR-EI-MS: 282.0524 ($C_{16}H_{10}O_5$ calcd. 282.0528). EI-MS *m/z* (%): 282 (M^+, 100), 253 (5.4), 224 (16.7), 180 (12.6), 102 (6.4). UV λ_{max}(MeOH) nm: 276, 317. ^{1}H-NMR δ ($CDCl_3$, 500 MHz): 6.10 (2H, s, O-CH_2-O), 6.60 (1H, s, H-8), 6.68 (1H, s, H-3), 7.54 (3H, m, H-3′,4′ and 5′), 7.87 (2H, m, H-2′ and 6′), 12.70 (1H, s, 5-OH). ^{13}C-NMR δ ($CDCl_3$, 125 MHz): 89.5 (C-8), 102.7 (O-CH_2-O), 105.5 (C-3), 107.8 (C-10), 126.2 (C-2′ and 6′), 129.1 (C-3′ and 5′), 130.1 (C-6), 131.2 (C-1′), 131.9 (C-4′), 142.2 (C-5), 153.3 (C-9), 154.1 (C-7), 164.0 (C-2), 183.0 (C-4).
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Nest cell lining of the solitary bee *Hylaeus bisinuatus* (Hymenoptera: Colletidae)

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Abstract. The nest cell lining of *Hylaeus bisinuatus* (Hymenoptera: Colletidae) was shown by high-resolution solid-state [^{13}C]NMR to be composed of lipid polymer and protein. The lipid polymer was shown by reduction and subsequent GC/MS analysis to be comprised of ω-hydroxy fatty acids (C_{20}, C_{22}, C_{24} and C_{26}) and fatty alcohols (C_{16} to C_{30}). The protein portion of the lining had a silk-like amino acid composition.
Key words. Solitary bees; lipid polymer; silk; CP/MAS ^{13}C NMR; Hymenoptera; Colletidae; *Hylaeus bisinuatus.*

The earthen walls of nest cells of most species of ground-nesting bees are endowed with a thin, hydrophobic lining or membrane[1–3]. Exceptions include members of some genera of the andrenid subfamily Panurginae that apparently lack nest cell linings[4] and ground-nesting leaf-cutter bees (Megachilidae) that line their nest cells with cut leaf fragments like their stem-nesting relatives[5]. Lining membranes are produced by adult nesting females prior to provisioning the cell with pollen and nectar. Linings have been variously described as waxy, silken, varnish-like or cellophane-like[1,2,6]. A few taxa, such as *Macropis*[7] and *Centris*[8], collect floral oils or plant resins, respectively, which they utilize in the construction of their cell linings. Chromatographic and spectroscopic analyses of the nest cell linings of most other representative species of ground-nesting bees have positively implicated the lipoidal secretion of the hypertrophied abdominal Dufour's gland as the glandular source of the nest cell lining[9–15]. As no species of the related sphecid wasps have been reported to construct a nest cell lining, secreted nest cell linings of bees may prove to be a critical, derived taxonomic character that unifies the bees (Apoidea) as a single evolutionary lineage.

The cellophane-like nest cell linings of species of the Colletidae, long considered the most primitive family of bees, were until recently thought to be comprised of secretions from thoracic salivary glands or mandibular glands. Microscopic examination of cell linings of the colletid genera *Hylaeus*[16], *Colletes*[11,12], and *Ptiloglossa*[17] has revealed the variable presence of fiber-like

strands embedded in a solid matrix, suggesting that their linings are comprised of two different components. Using their unusual bifid tongue as a brush, females of *Colletes*[18] and *Hylaeus*[19] nesting in glass observation nests have been reported to paint a liquid onto the walls of their nest cells that quickly solidifies or polymerizes. Females of *Colletes* were seen to acquire this liquid from drops secreted at the tips of their abdomens[18]. Chemical analyses demonstrated that their linings are derived from macrocyclic lactones, produced in the Dufour's gland, which subsequently polymerize on the cell walls to form a matrix of polyester sheets[9,11–13,15].

Hylaeus bisinuatus cell lining material was extracted with hexane to remove soluble lipid components and analyzed by cross-polarization/magic-angle spinning carbon-13 nuclear magnetic resonance (CP/MAS[^{13}C]NMR). The spectrum of the cell lining was dominated by a large resonance band at 33.1 ppm due to aliphatic CH_2 (fig. 1). The spectrum is characteristic of a lipid polyester polymer which has long chain, primary fatty alcohols esterified to long chain fatty acids[20,21] with additional resonance bands at 173 ppm for carbonyl and at 72 ppm for CHO carbon. This assignment was confirmed by reduction of portions of the cell lining material with $LiAlH_4$ and with $LiAlD_4$. The monomers that were recovered from the separate reduction reactions were derived and analyzed by GC/MS (fig. 2). The major components were even chain length fatty alcohols (C_{16} to C_{30}) and α, ω-diols (C_{20} to C_{26}). Analysis of the mass spectra of the cell lining material that was reduced with $LiAlD_4$ showed that there was deuterium incorporation at C_1 of both the alcohols and the diols, indicating that the original monomers in the lipid polymer of the cell lining were fatty acids and ω-hydroxyfatty acids, respectively[22,23].

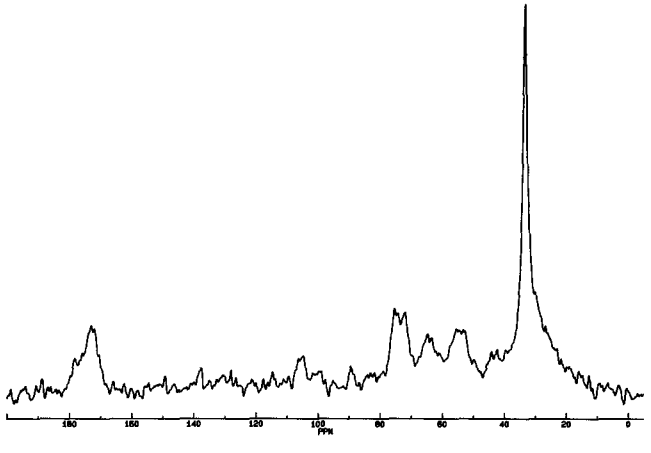

Figure 1. CP/MAS [^{13}C]NMR spectrum of *Hylaeus bisinuatus* nest cell lining. The spectrum was obtained on a Bruker MSL 300 NMR spectrometer operating at 75.47 MHz for ^{13}C and equipped with high-power amplifiers and a medium-bore (89 mm) probe. The sample was packed in a 4 mm Zirconia rotor and spun at 6.0 kHz. Data were collected by taking 4 K scans using a 2.5-μs 90° ^{1}H pulse, a 1-ms contact time, and a 5-s delay time. The spectrum was externally referenced to the benzene ring in hexamethylbenzene at 132.3 ppm with respect to tetramethylsilane.

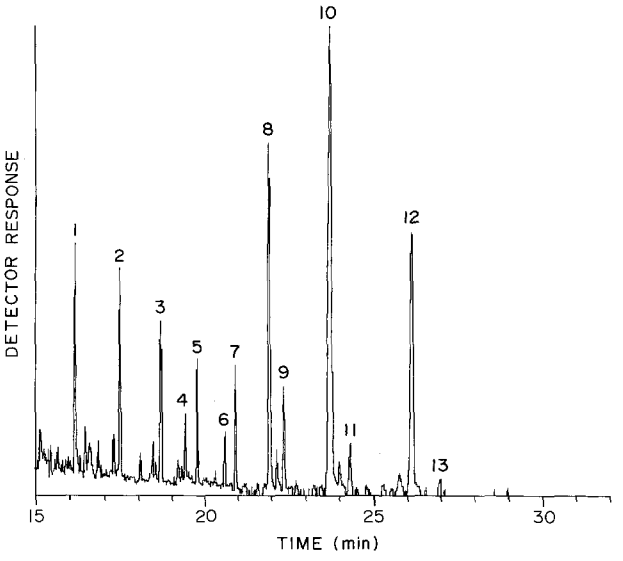

Figure 2. Ion chromatogram of the derivatized reduction product of the nest cell lining of *Hylaeus bisinuatus*. The cell lining was extracted with hexane, reduced with $LiAlD_4$ in tetrahydrofuran, partitioned into chloroform, derivatized with *N, O*-bis(trimethylsily)acetamide, and analyzed by combined gas chromatography/mass spectrometry (Hewlett Packard 5890 A/5970) on a capillary column (25 m cross-linked methyl silicone)[34]. The oven temperature was held at 55 °C for 3 min after injection and then raised to 305 °C at 15°/min. Spectra were taken at intervals of 1.2 s at 70 eV. Primary fatty alcohols identified by their mass spectra are: 1, C_{16}; 2, C_{18}; 3, C_{20}; 5, C_{22}; 7, C_{24}; 9, C_{26}; 11, C_{28}; 13, C_{30}. α, ω-Diols are: 6, C_{20}; 8, C_{22}; 10, C_{24}; 12, C_{26}. Peak 4 is 1, 7, 16-trihydroxyhexadecane (shown by deuterium incorporation[22] to be derived from 10, 16-dihydroxyhexadecanoic acid).

Amino acid composition[a] of protein in the nest cell lining of *Hylaeus bisinuatus*

Amino acid		
Asx	20.9	(22.7)
Glx	24.0	(28.7)
Ser	20.2	(17.3)
Gly	9.3	(5.7)
His	2.4	(3.0)
Arg	0.8	(1.1)
Thr	0.1	(0.1)
Ala	6.6	(4.8)
Pro	1.2	(1.1)
Tyr	0.4	(0.6)
Val	5.3	(5.1)
Met	1.0	(1.2)
Ile	1.5	(1.6)
Leu	4.5	(4.8)
Phe	0.6	(0.8)
Lys	1.1	(1.3)

[a] Number of residues per 100 identified amino acids. Values in parentheses are mol %. Sample was hydrolyzed in 6 N HCl at 110 °C for 24 h and amino acids were analyzed with a Microsystem HPLC[33].

The NMR spectrum of the *H. bisinuatus* cell lining (fig. 1) also indicated the presence of protein with resonance bands in the carbonyl region and at 75, 65, 55 and 43 ppm[24–26]. These assignments were confirmed by elemental analysis (C, 49.03; H, 7.20; N, 8.77) and amino acid analysis (table). Aspartic acid, glutamic acid, serine, and glycine accounted for 74% of the residues of the protein. This composition, with a high proportion of

small amino acid residues, is similar to that of cocoon silk proteins produced by both lepidopteran and hymenopteran larvae[26–28]. The proportion of acidic residues is higher than in most previously examined insect silks, although similar compositions have been reported for the cocoon silk of some hymenopteran species[29] and the sericin portion of *Bombyx mori* cocoon silk[30]. Very few silk proteins produced by adult Hymenoptera have been chemically examined. However, silk proteins utilized in nest construction by *Polistes annularis* and *P. metricus* adults have recently been shown to have amino acid compositions which are similar to that found for the nest cell lining of *H. bisinuatus*[26,31]. The combined data indicate that the nest cell lining of *H. bisinuatus* is a mixture of a lipid polyester and silk protein. The ω-hydroxyfatty acids which form the backbone of the lipid polymer have been found as monomers in the cell linings of other solitary bees, but the average chain length (24.0) is longer for the polymer in the lining of *H. bisinuatus* than those reported for other species where ω-hydroxyoctadecanoic acid is frequently the dominant monomer[9,11]. The composition reported here is similar to that found for the nest cell lining of several species of *Colletes* which contained: a lipid polymer composed of ω-hydroxyfatty acids (C_{18}, C_{20} and C_{22}), small amounts of dihydroxy fatty acids (similar to peak 4, fig. 2), and a protein rich in glutamic acid and alanine[11]. The hexane extract of the *H. bisinuatus* cell lining was analyzed by GC/MS and found to contain primarily *n*-alkanes with the major components being *n*-heptacosane (28% of the extract), *n*-nonacosane (22%), and *n*-pentacosane (20%). These hydrocarbons may be derived from the cuticular lipids of *H. bisinuatus*; the composition and chain length distribution is very similar to the cuticular hydrocarbon pattern reported for the solitary bee, *Nomia bakeri*[32]. Macrocyclic lactones were not found in the GC/MS analysis of the hexane extract of the *H. bisinuatus* cell lining, although these components (which are produced in the Dufour's gland in other solitary bees) serve as the precursors of the lipid polyester of the nest cell lining[9,13].

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