levels of aldehyde oxidase activity. All such flies display the engrailed phenotype. Aldehyde oxidase, therefore, is probably not responsible for the transformation of the posterior compartment to an incomplete anterior compartment. It is probably present in the transformed compartment because at compartment formation during embryogenesis (*1, 11*) in the absence of the en^+ posterior compartmental marking gene, instructions are given for an anterior compartment in which the aldehyde oxidase structural gene is or will be active. During the first instar stage of development the dorsoventral compartmental boundary is established and is apparently unaffected by the engrailed mutation (*12*). The wing margin, which is marked by aldehyde oxidase, carries instructions from the previously established anterior compartments so that bristle patterns in the adult wing will be characteristically anterior along the anterior and posterior wing margin.

Studies of enzymatic compartmentalization should lead to a better understanding of the logic behind gene deployment in pattern formation (*6*).

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Flavonoids and Other Chemical Constituents of Fossil Miocene *Zelkova* (Ulmaceae)

Abstract. *Organic solvent extractions of* Zelkova oregoniana, *a Miocene angiosperm compression fossil, indicate the chemical preservation of kaempferol, dihydrokaempferol, an* n*-alkane chain length range of 10 to 32 carbons, hydroxy acids, steranes, triterpenoids, and methyl pheophorbide a. This appears to be the oldest occurrence of flavonoids in fossil sediments reported.*

Green-colored angiosperm leaves have been reported by Weigelt and Noack (*1*), while Dilcher *et al.* (*2*) have isolated methyl pheophorbide a from middle Eocene sediments. We report here the organic chemical constituents isolated from the Succor Creek Flora from Oregon (*3*), which is Oligocene to Miocene in age (36 to 25 × 10^6 years old). In particular, data are presented from an analysis of *Zelkova oregoniana* compression fossils, which are vivid green and represent 90 percent of the megafossils seen on the fracture planes of fossiliferous sediments. A chemical investigation by means of paper chromatography and combined gas chromatography–mass spectroscopy (GS-MS) allowed for the detailed identification of associated organic constituents (*4*). The isolation of various fatty acids, aromatic compounds, steroids, and chlorophyll derivatives is precedented in the literature from older sediments [Green River Oil Shales of Eocene age, 53 × 10^6 years old (*5*)], as well as from Paleozoic plant debris (*4*). To our knowledge, material from the Succor Creek Flora represents the first case of the preservation of flavonoid compounds in comparably aged rock strata.

A portion of the rock containing fossil leaves of *Z. oregoniana* was pulverized and extracted for 72 hours in absolute methanol. The resultant pale yellow solution was concentrated under vacuum without heat and chromatographed on paper (Whatman, 3 mm) using standard techniques (*6*). Two spots were obtained on paper chromatography, the largest being bright fluorescent yellow, with an R_F value of 0.78 in solvent I and 0.06 in solvent II (*7*). Ultraviolet (UV) spectroscopy (*6*) showed the compound (*8*) to be the flavonol kaempferol. This identification was confirmed by cochromatography with authentic kaempferol (*9*) in additional solvents (*10*).

The second compound, occurring in much smaller amounts, appeared black under UV and UV with NH_3 and had R_F values of 0.85 and 0.56 in solvents I and II, respectively. A comparison of the UV spectra of this compound (*11*) with published data (*6*) show it to be dihydrokaempferol, the flavanonol form of kaempferol. The limited amounts of material precluded further analysis.

Previous work on wood of the extant, related taxon *Zelkova serrata* (*12*) also showed the presence of kaempferol and dihydrokaempferol, but as the 7-*O*-methyl and 6-*C*-glycosyl derivatives keyakinin and keyakinol, respectively. However, analysis of flavonoids in leaves of *Z. serrata, Z. sinica,* and *Z. verschaffeltii* available to us showed only the presence of kaempferol and quercetin 3-glycosides; that is, they lacked 7-*O*-methylation and 6-*C*-glycosylation. These data suggest that there may be considerable flavonoid variation in extant *Z. serrata* itself and, indirectly, that the flavonoids from the

Table 1. Carbon number data from gas chromatography (GC) (Apiezon L column).

GC peak number	Abundance (%)	Carbon number	Coinjected standards	Molecular formula
1	0.5	29.58	5β-Cholestane	$C_{27}H_{48}$
2	1.8	29.90	5α-Cholestane	$C_{27}H_{48}$
7	3.7	30.82		$C_{28}H_{50}$
8	1.5	30.94		$C_{30}H_{50}$
9	1.1	31.00		$C_{29}H_{52}$
12	1.3	31.23	Onocerane III	$C_{30}H_{54}$
13	1.5	31.35		$C_{30}H_{54}$
14	6.6	31.42	Lupane	$C_{30}H_{52}$
15	10.0	31.53	Stigmastane	$C_{29}H_{52}$
16	0.7	31.59		$C_{30}H_{54}$
17	11.2	31.98		$C_{30}H_{52}$
21	2.4	32.60		$C_{30}H_{52}$
24	1.3	33.02		$C_{30}H_{52}$
25	2.5	33.52		$C_{31}H_{54}$
26	3.6	33.72	Friedelane	$C_{30}H_{52}$
31	10.0	36.82	β-Carotene	$C_{40}H_{78}$

Table 2. Mass spectral data and deduced structural types. Peak numbers refer to Table 1.

GC peak number	GC-MS scan number	Parent ion	Molecular formula	Structural type
1	4	372	$C_{22}H_{48}$	Sterane (C_{27})
3	5	386	$C_{28}H_{50}$	Tetracyclic triterpane
		416	$C_{30}H_{56}$	Tricyclic triterpane
5	6	460	$C_{33}H_{64}$	
7	8	386	$C_{28}H_{30}$	Sterane (C_{28})
8	9	416	$C_{30}H_{50}$	Tricyclic triterpane
9		400	$C_{29}H_{52}$	Sterane (C_{29})
15	11	400	$C_{29}H_{52}$	Sterane (C_{29})
21	13	412	$C_{30}H_{52}$	Pentacyclic triterpane
24	15	412	$C_{30}H_{52}$	Pentacyclic triterpane
25	16	426	$C_{31}H_{54}$	Pentacyclic triterpane

fossil *Z. oregoniana* may represent original, unaltered flavonoids.

Chloroform extracts yielded a green compound having a visible-light absorption spectrum similar to that of pheophytin a, as well as an R_F value identical to that of methyl pheophorbide a synthesized from fresh chlorophyll a (*13*). Mass spectra of this green compound had a parent ion of 606, with prominent peaks at M − 28 and M − 59. Chloroform extracts were taken from freshly cleaved surfaces and are not thought to represent surface contaminations.

Isolated cycloalkanes identified on an Apiezon L–greased stainless steel column (20 feet by 0.01 inch) are listed in Table 1; retention data and Kovats indices (*14*) were calculated by coinjection of authentic compounds. The percentage abundance of each peak was based on peak area. Where coinjection of a standard produced peak enhancement, the standard was entered (Table 1) and considered along with mass spectral similarity as corroboration of identity. Compounds identified in this way are 5β-cholestane, 5α-cholestane, onocerane III, lupane, stigmasterane, friedelane, and β-carotene, with peaks 1, 2, 12, 14, 15, 26, and 31, respectively. The identification of cycloalkanes was limited by the number of standards available; mass spectral data for some compounds and their deduced structural types are given in Table 2.

The *n*-alkanes isolated from the fossil material show a bimodal distribution from C_{10} to C_{32} with maxima at C_{27}, C_{29}, and C_{31} (typical for plant waxes) and at *n*-C_{17} (typical for algal sources); from 30 to 42 percent of the *n*-alkanes isolated are referable to the range C_{27} to C_{31}. The isoprenoid alkanes farnesane, pristane, and phytane are present and comprise $\approx$ 0.32 percent (dry weight) of the sediment. Thin-layer chromatography on silica gel [solvent system, hexane, ether, and methanol (40 : 10 : 1 by volume)] of the ether-soluble acids released by methanolic KOH hydrolysis indicates the presence of mono-, di-, and trihydroxy acids ($R_F \approx$ 0.60, 0.30, and 0.16, respectively). Mass spectra of the trimethylsilyl ethers are characterized by mass-to-charge ratio (*m/e*) 73 [$Si(CH_3)_3$], *m/e* 75 [$(CH_3)_2Si^+{=}O$], M − 15, M − 31, and M − 47. Two series of monohydroxy acids (0.21 percent) were detected: one series of *w*-hydroxy acids *n*-C_{24}, *n*-C_{26}, and *n*-C_{28}, and another as yet unidentified series. The relative amounts of fatty acids ($\approx$ 0.28 percent) and hydroxy acids suggest their derivation from plant waxes. Of the higher hydroxylated acids, 10,16-dihydroxyhexadecanoic and 9,10,18-trihydroxyoctadecanoic acids were identified.

These data indicate that the original biochemistry of leaf material has undergone chemical diagenesis. Cycloalkanes isolated from *Zelkova* have not been reported in any present-day biological source and most probably represent the products of steroid or triterpenoid alteration attending fossilization. The isolation of kaempferol, β-carotene, and methyl pheophorbide a indicates that extensive reduction-decarboxylation reactions, associated with most fossiliferous material, did not occur to any considerable extent. Preliminary data from experiments designed to determine the thermal and geochemical stability of flavonoids suggest that flavanonol (for example, dihydrokaempferol) and flavonoid-*O*-glycosides exist within a very restricted *p*H window under simulated geophysical conditions (100°C in bentonite clay). With significant variations in *p*H, thermolysis of these compounds is observed at 80°C, suggesting that the Succor Creek Flora experienced a very mild geothermal gradient. The preservation of chlorophyll derivatives corroborates the presence of relatively low temperatures (70°C) during fossilization (*15*). Unlike the anaerobic, poorly drained Geisel Valley (Eocene), which yields green angiosperm fossils and brown coals (*16*), the Succor Creek Flora is the result of pyroclastic fall deposition (tephra). Streams of lava blocked rivers and created numerous lakes. The surrounding vegetation, as well as accumulated aquatic debris (algal blooms in part), was rapidly buried under ash. Sediments bearing *Zelkova* were selected from upper strata of the Succor Creek locality and showed no evidence of overlying lava flows. The rapid burial of these leaves and the apparent lack of elevated temperatures in their subsequent geologic history may explain the preservation of the consortium of organic compounds reported here. The preservation of vascular and nonvascular plant material may explain the bimodality of *n*-alkane ranges and the presence of steranes associated with algal sources (for example, sigmastene).

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8. Major spectral maxima (nanometers) were: methanol, 365 and 267; $AlCl_3$, 425 and 267; $AlCl_3$/HCl, 423 and 268; sodium methoxide, 440 and decomposes; sodium acetate, 375, 274, and procedure II(6); and H_3BO_3, 367 and 267.
9. Authentic kaempferol is available from K & K Laboratories, Plainview, N.Y.
10. The additional solvents were: solvent III, *n*-butanol, acetic acid, and water (5 : 1 : 1 by volume) and solvent IV, water.
11. Major spectral maxima (nanometers) were: methanol, 330 (shoulder) and 292; $AlCl_3$, 378 and 318; $AlCl_3$/HCl, 375 and 316; sodium methoxide, 327 and 246 (shoulder); sodium acetate, 327, 284 (shoulder), and 254 (shoulder); and H_3BO_3, 336 (shoulder) and 296.
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