1676, 1632, 1553, 1495, 1470, and 1447 cm^{-1} (Ref. 1 (nujol) 1673 cm^{-1}). (Found: C 64.2; H 5.34. Calc. for $C_{12}H_{12}O_4$: C 65.5; H 5.48.)

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Gel Filtration of Lignin Model Compounds

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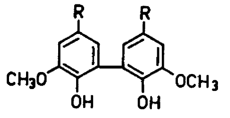
Gel filtration has been found to be useful in lignin degradation studies (see, *e.g.*, Refs. 1 and 2). It was found to be of importance to use a suitable combination of gel material and eluent in experiments with lignin degradation products. In order to get a basis for the selection of a system for the gel filtration, we have subjected a number of lignin model compounds, ranging from 124 to 512 in molecular weight, to gel filtration under different conditions. In the selection of model compounds a maximum of structural variation has been pursued.

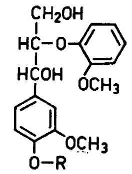
For the system Sephadex (G-series)/water the elution properties of a solute are described by a distribution coefficient, K_d, defined as the volume fraction of the water imbibed in the gel particles, which is available to the solute.[3] In the present communication a similar parameter, K_d', has been used to compare results obtained in different systems. K_d' is defined as $(V_e - V_p)/(V_a - V_p)$, where V_e = the elution volume of the solute; V_p = the elution volume of a polymer such as Björkman lignin or India Ink (V_p corresponds approximately to the void volume of the column); V_a = the elution volume of acetone. In the systems investigated $V_a - V_p$ was found to roughly correspond to the volume of eluent imbibed by the gel particles.

Results obtained on Sephadex G-25 with water as eluent are given in Table 1. Apparently the compounds examined are not eluted in the order of molecular size and furthermore the elution is delayed (all K_d'-values are > 1). These phenomena have been observed previously on gel filtration of aromatic compounds under these conditions and have been attributed to a preferential adsorption to the gel matrix.[5]

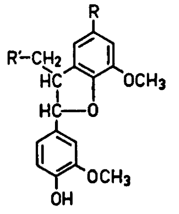
By the use of dioxane-water (1:1) as eluent adsorption effects were greatly diminished or eliminated (Table 1). Irregularities observed, *e.g.* the relatively high



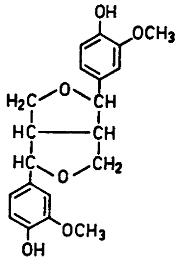
III R=CH₂OH
V R=CH₂-CH=CH₂



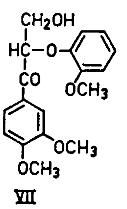
IV R=H
VIII R=CH₃



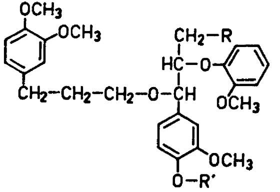
VI R=CH₂CH₂CH₃ R'=H
X R=CH=CHCH₂OH R'=OH



IX



VII



XI R=R'=H
XII R=OH R'=CH₃

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Table 1. K_d'-values of lignin model compounds on gel filtration under different conditions.

Compound[a]	Molecular weight	K_d'-values				
		Sephadex G-25/ water	Sephadex G-25/ dioxane-water (1:1)	Sephadex G-25/ dimethyl sulfoxide	Sephadex LH-20/ dioxane-water (9:1)	Polystyrene gel/ tetrahydro-furan
R–H	124	2.3	0.86	0.88		
R'–H	138	2.1	0.81	0.94		
R–CHO	152	2.8	0.85	0.83		
R'–CHO	166		0.81	0.90		
R–$CH_2CH_2CH_3$ (I)	166	2.6	0.63	0.74	0.68	0.77
R–COOH	168	2.7	0.78	0.74		
R–$COCH_2CH_3$	180	3.0	0.74	0.75		
R–$CH=CHCH_2OH$	180	3.2	0.81	0.73		
R–$COCH(OH)CH_3$	196	2.5	0.82	0.76		
R–CH_2COCH_2OH	196	1.9	0.85	0.75		
R–$[CH(OH)]_2CH_2OH$ (II)	214	1.6	1.01	0.69	1.22	0.67
III	306	3.7	0.74	0.65		
IV	320	2.3	0.65	0.61		
V	326		0.40	0.59		
VI	328		0.40	0.56	0.43	0.65
VII	332	3.1	0.56	0.68		
VIII	334		0.61	0.63		
IX	358		0.54	0.56	0.58	0.61
X	358	7.1	0.57	0.54		
XI[b]	482		0.29	0.48		
XII[b]	512		0.33	0.49		

[a] R = 4-hydroxy-3-methoxyphenyl, R' = 3,4-dimethoxyphenyl.

[b] Synthesis.[4]

K_d'-value of the hydroxyl rich compound II, can be explained by partition chromatography superimposed on the "molecular sieving" action of the gel rather than adsorption effects. When ethanol-water (1:1), acetone-water (1:1), and 2-methoxyethanol-water (65:35) were used as eluents the retardation of compound II was more pronounced. A few experiments on Sephadex LH-20 with dioxane-water (9:1) as eluent indicate that partition chromatography was operating in this system as well (Table 1).

Using the system Sephadex G-25/dimethyl sulfoxide all the compounds investigated were eluted essentially according to molecular size and marked side-effects were not observed (Table 1).

Some experiments on polystyrene gel with tetrahydrofuran as an eluent have also been made (Table 1). In this system the monomeric compound II is eluted closer to the dimeric compounds VI and IX than to the monomeric compound I. In this connection it is of interest to note that compound II is eluted prior to other compounds investigated in the system Sephadex G-25/water and also that compound II has a relatively low K_d'-value in the system Sephadex G-25/dimethyl sulfoxide.

Formamide and dimethylformamide have been used in gel filtration studies of lignin and lignin degradation products.[6–8] It was demonstrated that lignin model compounds were eluted according to their molecular size on Sephadex G-50 with formamide as eluting agent.[6] Dimethylformamide has been used for the separation of low molecular weight lignin degradation products on Sephadex LH-20.[8] In the present study a few experiments with lignin model compounds in the system Sephadex LH-20/dimethylformamide were performed. The results (I, $K_d'=0.72$; II, $K_d'=0.54$; IX, $K_d'=0.46$) indicate that the gel filtration properties of this system in certain respects resemble those of the systems polystyrene gel/tetrahydrofuran and Sephadex G-25/dimethyl sulfoxide.

Experimental. A Waters Gel Permeation Chromatograph Model 200 (column set B1: 3×10^3 Å, 5×10^2 Å, 10^2 Å, and 60 Å) was used for gel filtration on polystyrene gel with tetrahydrofuran as an eluent. For the experiments with Sephadex gels the following columns and solvents were used:

Sephadex G-25 (fine)/water. Amount of gel, 27 g; bed volume, 133 ml; column length, 35 cm. *Sephadex G-25 (fine)/dioxane-water (1:1).* Amount of gel, 47 g; bed volume, 199 ml; column length, 57 cm. *Sephadex G-25 (fine)/dimethyl sulfoxide.* Amount of gel, 32 g; bed volume, 198 ml; column length, 56 cm. *Sephadex LH-20/dioxane-water (9:1).* Amount of gel, 8 g; bed volume, 32 ml; column length, 18 cm. *Sephadex LH-20/dimethylformamide.* Amount of gel, 8 g; bed volume, 36 ml; column length, 20 cm.

Gel filtration. The samples (1 – 3 mg) were dissolved in 1 – 2 ml of the eluent and applied to the columns. The UV absorbance at 280 nm of the effluent from the columns was continuously recorded by means of a Beckman DB spectrophotometer equipped with a 1 mm flow cell and a recorder. In most experiments the flow rate was 5 – 10 ml/h.

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Fungal Carotenoids

VII.* Synthesis of β,γ- and γ,γ-Carotene with Terminal Methylene Groups

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Recently Arpin *et al.*[1] reported the isolation and identification of β,γ-carotene (*10*) from the discomycete *Caloschypha fulgens*. The new IUPAC nomenclature rules for carotenoids [2] are used here. The γ-terminus features an exocyclic methylene group common to terpenes, but previously unreported in carotenes. We now report the synthesis of β,γ-carotene (*10*) according to the route outlined in Scheme 1 and confirm the assigned structure. Symmetrical γ,γ-carotene (*8*) was also synthesized according to the same basic route.

Synthetic β,γ-carotene (*10*), purified by column chromatography on deactivated alumina and silica gel plates and crystallized from benzene-methanol solution, melted at 174°C, undepressed on admixture with a natural specimen.

The synthetic and natural pigments could not be separated chromatographically.

Synthetic β,γ-carotene (*10*) had λ_{max} 421, 444 [E(1 %, 1 cm) = 2600] and 472 [E(1 %, 1 cm) = 2370] nm in petroleum ether and 425, 447.5, [E(1 %, 1 cm) = 2520] and 475 [E(1 %, 1 cm) = 2300] nm in acetone. The IR spectrum (KBr) resembled those of common carotenes except for a strong absorption at 889 cm^{-1} ascribed to $R_1R_2C=CH_2$. The NMR spectrum (60 Mc) in $CDCl_3$ solution exhibited singlets at τ 9.17 (3H) and 9.10 (3H) due to *gem.* dimethyl on the γ-ring, broad singlets at τ 5.31 (1H) and 5.46 (1H) attributed to a $R_1R_2C=CH_2$ grouping with magnetically non-equivalent protons and a doublet at τ 7.57 (1H, $J=8$ cps) ascribed to the methne proton at C-6′. Signals at τ 8.98 (singlet, 6H, *gem.* dimethyl) and τ 8.30 (singlet, 3H, end-of-chain methyl) were caused by the β-end group. Four in-chain methyl groups gave a singlet at τ 8.03

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