Low Molecular Weight Phenolic Compounds in Spanish Oak Woods

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Low molecular weight phenolics were analyzed by HPLC in oak heartwood of *Quercus robur* L., *Quercus petraea* Liebl., *Quercus pyrenaica* Wild., and *Quercus faginea* Lam. Data were processed with the STATGRAPHICS program. Gallic, ellagic, vanillic, syringic, and ferulic acids, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, aesculetin, and scopoletin were found. Ellagic and gallic acid derivatives were also recognized according to their hydrolysis products and their UV spectra. *Q. robur* and *Q. petraea* differ from the others by the relative concentration of these compounds.

Keywords: *Phenolic compounds; Quercus robur; Quercus petraea; Quercus pyrenaica; Quercus faginea; wood*

INTRODUCTION

Chemical composition of oak wood is similar to that of other kinds of wood in relation to cellulose, hemicellulose, and lignin contents that represent about 40%, 25%, and 20%, respectively. Several studies were carried out about the extractives from oak wood, specially about phenolic compounds. The phenolic constituents of oak heartwood are not flavonoids (Singleton *et al.*, 1971); they are essentially ellagitannins (Mayer *et al.*, 1967; Markman, 1974; Quinn and Singleton, 1985). Nowadays, the structures of the main ellagitannins have been established, and the presence of some of them like castalagin, vescalagin, grandinin, and roburin A-E in the wood of oak species is well documented (Mayer *et al.*, 1967; Scalbert *et al.*, 1988a,b; Hervé du Penhoat *et al.*, 1991a,b; Viriot *et al.*, 1994; Klumpers *et al.*, 1994; Chatonnet *et al.*, 1994; Masson *et al.*, 1995a,b).

Moreover, in 1953, Black *et al.* identified syringaldehyde, vanillin, coniferaldehyde, and sinapaldehyde as natural constituents of oak wood, and these results were confirmed by Chen (1970) and Seikel *et al.* (1971). Pearl *et al.* (1957) found very different contents of benzoic and cinnamic acids among six oak species analyzed. These findings are similar to those of Guymon and Crowell (1968) and Miller *et al.* (1992), which indicate that species is the most important factor contributing to the content of benzoic and cinnamic acids and aldehydes in oak wood. Site, however, does not exert a significant influence on some of these components, although there is much variation among the samples.

Here we report an analysis of low molecular weight polyphenols by high-performance liquid chromatography in oak heartwood of four species grown in the north of Spain: *Quercus robur*, *Quercus petraea*, *Quercus pyrenaica*, and *Quercus faginea*. The first two species are commonly used for cooperage, especially in France, due to their unique mechanical, physical, and chemical properties, giving highly appreciated sensory characteristics.

EXPERIMENTAL PROCEDURES

Collection of Wood Samples. Transversal sections from the trunks of oak trees of *Q. robur* (seven trees), *Q. petraea* (three trees), *Q. pyrenaica* (five trees), and *Q. faginea* (three trees) grown in Alava province (Spain) were provided by Centro Técnico de la Madera del País Vasco, SA (Euskal Herriko Zuraren Teknica Bazkunea, SA). The heartwood was separated and processed into sawdust using a hammermill. The sawdust ranged in size from 0.75 to 0.28 mm.

Extraction. The sawdust samples (10 g) were extracted with 300 mL of methanol/water (1:1) at room temperature for 24 h. The extracts were filtered on a Büchner funnel, and methanol was removed in a rotary evaporator, at a temperature below 40 °C. The acqueous solution (solution I) was fractionated by liquid−liquid extraction with diethyl ether and ethyl acetate. The organic fractions were dried and redissolved in methanol. The remaining aqueous phase was freeze-dried.

Standards. Reference compounds were purchased from Fluka (gallic acid, aesculetin, and scopoletin), Aldrich (vanillic, sinapic, and ferulic acids, syringaldehyde, and coniferaldehyde), Apin (ellagic acid), Chem Service (syringic acid), Merck (vanillin), and Sigma (catechin).

Global Determinations. In solution I, total polyphenols were determined by the Folin−Ciocalteu assay with gallic acid as a standard (Singleton and Rossi, 1965), condensed tannins by the vanillin method with catechin as a standard (Swain and Hillis, 1959), and total ellagitannins by HPLC quantification of ellagic acid released after acid hydrolysis, taking into account the free ellagic acid content of the solution (Puech *et al.*, 1990) and color as the absorbance at 420 nm.

HPLC Analysis. Low molecular weight phenolic compounds extracted with diethyl ether and ethyl acetate were analyzed by HPLC (Conde *et al.*, 1995a). An apparatus equipped with an autosampler and a diode array detector was used with a C_{18} Hypersil ODS (5 μm) column (20 cm × 4 mm i.d.), protected with a precolumn of the same material. The elution conditions were as follows: flow rate, 1 mL/min; temperature, 30 °C; solvent A = H_2O/PO_4H_3 (999:1), solvent B = $MeOH/PO_4H_3$ (999:1); linear gradient from 20% to 100% B, in 40 min; detections at 325 nm (with a bandwidth of 150 nm), 280 nm (bandwidth of 4 nm), and 360 nm (bandwidth of 4 nm). UV spectra (240−400 nm) were also recorded. Injection volume was 20 μL. Chromatographic peaks were identified by comparison of their retention time and UV spectra with those of reference compounds. Quantitative determinations were carried out by the external standard method.

Acid Hydrolysis. Those chromatographic peaks with similar UV spectra as gallic and ellagic acids, but with different retention times, were collected at the outlet of the chromatograph. The methanol was removed in a rotary evaporator, and the acqueous solution was freeze-dried; 1 mL of 12 N HCl was added to the lyophilized, and the mixture was heated at 100 °C for 24 h under vacuum. The hydrolyzed sample was divided in two equal parts. One of them was dissolved in methanol and analyzed by HPLC. The other one

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Table 1. Average (*x*), Standard Deviation (SD), and Coefficient of Variation (cv) Values for Dry Extracts (DE) in Methanol (50%) and Their Fractions Soluble in Diethyl Ether, Ethyl Acetate, and Water (mg/g of wood), Optical Density (OD) at 420 nm, Total Polyphenols (TP) (mg of gallic acid/g of wood), Total Ellagitannins (TE) (mg of ellagic acid/g of wood), and Proanthocyanidins (PRO) (mg of catechin/g of wood) in Spanish Oak Woods[a]

	Q. robur			*Q. petraea*			*Q. pyrenaica*			*Q. faginea*		
	x	SD	cv	*x*	SD	cv	*x*	SD	cv	*x*	SD	cv
DE MeOH (50%)	77.7	12	15	78.7	18	23	82.6	17	21	89.3	13	15
DE Et_2O	4.5	1.9	41	8.6	4.4	76	10.8	1.2	10	30.7	2.8	9
DE AcEt	6.5	1.5	21	7.6	1.1	14	7.7	1.3	17	11.8	3.5	30
DE H_2O	72.5	20	28	62.3	20	33	73.7	19	26	78.3	20	26
OD	0.59	0.13	22	0.41	0.05	13	0.76	0.15	19	0.99	0.28	29
TP	56.6	16.8	30	70.3	25.4	36	78.5	21.4	27	89.2	18.1	20
TE	7.6	2.7	36	8.5	2.5	30	5.1	0.8	16	4.0	1.5	38
PRO												

[a] Number of samples for average: *Q. robur*, 7; *Q. petraea*, 3; *Q. pyrenaica*, 5; *Q. faginea*, 3. Degrees of freedom: between groups = 3; within groups = 14.

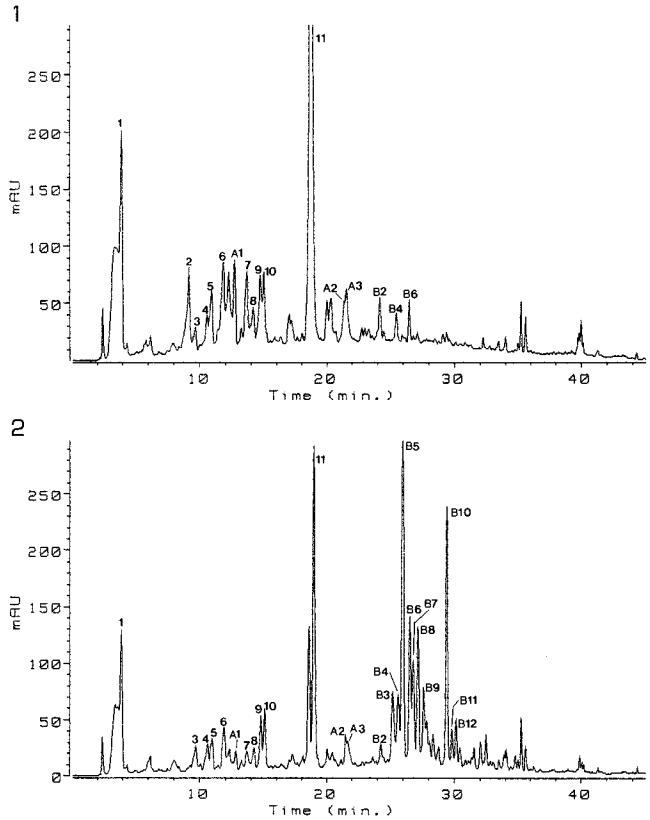

Figure 1. HPLC chromatograms (325 ± 75 nm) of ether extracts from heartwood of *Q. robur* L. (1) and *Q. faginea* Lam. (2): 1 = gallic acid; 2 = aesculetin; 3 = vanillic acid; 4 = syringic acid; 5 = vanillin; 6 = syringaldehyde; 7 = scopoletin; 8 = ferulic acid; 9 = coniferaldehyde; 10 = sinapaldehyde; 11 = ellagic acid; A = **A** compounds; and B = **B** compounds.

was dried and trimethylsilylized in order to analyze the sugars by GC−MS (Conde *et al*., 1995b).

Statistical Analysis. Data were analyzed with the STATGRAPHICS program. Univariate analysis and discriminant canonical multivariate analysis were carried out. In univariate analysis, using a single-variable model, mean, standard deviation, and coefficient of variation values are calculated for each variable in each species. In discriminant canonical multivariate analysis, some mathematical functions, called discriminant functions, are calculated. On projecting the points of the euclidean space R^n and representing them graphically in two dimensions, the geometrical distances among the points were identical with the statistical distances.

RESULTS AND DISCUSSION

Table 1 shows average, standard deviation, and coefficient of variation values for dry extracts in methanol (50%) and their fractions soluble in diethyl ether, ethyl acetate, and water, total phenols, and total ellagitannins contents in the four species considered. The condensed tannins assay by the vanillin method turned out to be negative. It means that the content of these compounds in oak heartwoods is inferior to the method sensibility, according to Scalbert *et al.* (1988b). The presence of this type of tannins has been described in oak bark, where they can be implicated in phytopathologic reactions (Scalbert and Haslam, 1987; Chatonnet, 1993).

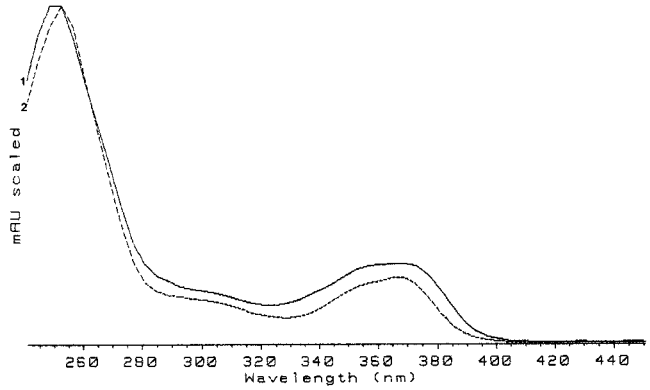

Figure 2. UV spectra of **A-2** (1) and ellagic acid standard (2).

In the four species, most of the polyphenols present in the heartwood are water-soluble, and insoluble in diethyl ether and ethyl acetate, and include probably high molecular weight polyphenols (ellagitannins oligomers and polymers) (Scalbert *et al.*, 1990; Viriot *et al*., 1993).

Heartwoods of *Q. faginea* and *Q. pyrenaica* present higher values than *Q. robur* and *Q. petraea* for all the global determinations, except for total ellagitannins. However, it must be explained that on carrying out the acid hydrolysis for quantification of the ellagic acid released, *Q. robur* and *Q. petraea* yielded only ellagic acid, whereas *Q. pyrenaica* and *Q. faginea* gave similar amounts of ellagic and gallic acids and other components with UV spectra of ellagic acid. It implies that these two latter species have a type of ellagitannins different to those of *Q. robur* and *Q. petraea*.

Figure 1 shows HPLC chromatograms of ether extracts from *Q. robur* and *Q. faginea* heartwoods, which are the most representative of the differences among species. The acids gallic, vanillic, syringic, ferulic, and ellagic, the aldehydes vanillic, syringic, coniferylic, and sinapic, and the coumarins aesculetin and scopoletin were identified. Moreover, two other types (**A** and **B**) of low molecular weight phenolics have been found. The **A** compounds, whose UV spectra are similar to that of ellagic acid (Figure 2), yielded by acid hydrolysis ellagic

Table 2. Average (x), Standard Deviation (SD), and Coefficient of Variation (cv) Values for Phenolic Compounds in Spanish Oak Woods (μg/g of Wood)[a]

	Q. robur			*Q. petraea*			*Q. pyrenaica*			*Q. faginea*		
compd	x	SD	cv	x	SD	cv	x	SD	cv	x	SD	cv
gallic acid	100	89	89	145	72	49	63	34	53	176	52	29
aesculetin	1.83	1.65	90	2.54	1.77	70	0.83	0.36	44	1.26	0.97	77
vanillic acid	1.98	0.63	32	1.90	0.04	2	1.84	0.63	34	1.72	0.61	36
syringic acid	2.69	1.85	69	2.31	0.32	14	1.59	0.44	27	1.66	0.92	55
vanillin	2.91	1.20	41	2.77	0.55	20	1.91	0.99	52	1.54	0.94	61
syringaldehyde	3.75	1.56	41	3.55	1.03	29	1.79	0.81	45	2.03	0.42	21
scopoletin	2.27	0.91	40	1.07	0.54	40	2.04	0.78	38	1.35	0.69	51
ferulic acid	1.30	0.43	33	1.16	0.78	68	0.51	0.29	58	0.50	0.26	52
coniferylaldehyde	3.77	1.53	40	3.85	0.73	19	3.26	1.45	44	4.26	1.23	29
sinapaldehyde	3.94	1.32	34	4.92	0.53	11	2.48	1.50	60	3.53	1.93	55
ellagic acid	186	36	19	195	18	9	183	41	22	213	45	21

[a] Number of samples for average: *Q. robur*, 7; *Q. petraea*, 3; *Q. pyrenaica*, 5; *Q. faginea*, 3. Degrees of freedom: between groups = 3; within groups = 14.

Table 3. Average (x), Standard Deviation (SD), and Coefficient of Variation (cv) Values for A and B Compounds in Spanish Oak Woods (μg of ellagic (A) or gallic acid (B)/g of wood)[a]

		Q. robur			*Q. petraea*			*Q. pyrenaica*			*Q. faginea*		
compd	t_R	x	SD	cv	x	SD	cv	x	SD	cv	x	SD	cv
A-1	12.7	9.6	6.2	65	8.2	3.0	36	10.1	4.2	41	8.2	5.7	68
A-2	21.3	24.5	9.3	38	49.8	19.9	40	25.1	11.9	47	42.6	29.6	69
A-3	21.6	14.7	12.7	86	1.5	2.6	172	1.8	1.8	100	8.1	10.0	123
B-1	23.5	39.6	30.8	77	48.8	17.6	36	11.2	25.1	223			
B-2	24.1	2.6	3.3	128	0.9	0.8	87						
B-3	25.0	4.6	2.8	60	2.8	2.4	86				5.5	5.6	100
B-4	25.4	3.0	4.3	147	1.2	0.2	15				3.6	5.3	146
B-5	25.8	23.4	15.6	66	16.3	14.1	86	18.4	20.8	113	33.4	29.6	88
B-6	26.6	2.1	3.0	143	0.5	0.8	173	2.2	2.5	112	4.1	4.5	110
B-7	26.7	0.9	0.8	87	0.6	0.6	97	1.7	0.9	55	8.2	5.4	65
B-8	27.1	5.1	6.9	135	16.1	16.5	102	49.3	9	18	45.0	15.5	34
B-9	27.5	2.6	2.8	107	1.3	1.5	119	14.7	6.1	41	6.6	3.0	45
B-10	29.3	30.1	18.5	61	30.1	13.5	44	55.0	4.4	26	68.7	14.4	20
B-11	29.7	1.7	1.1	65	0.7	0.9	125	1.7	1.5	87	1.3	1.2	92
B-12	30.0	1.3	0.9	66	1.6	1.7	104	3.2	1.9	59	3.6	4.0	113
B-13	32.1	0.6	0.6	100	0.7	0.6	87	10.6	8.8	83	2.4	0.9	38

[a] t_R = retention time (min). Number of samples for average: *Q. robur*, 7; *Q. petraea*, 3; *Q. pyrenaica*, 5; *Q. faginea*, 3. Degrees of freedom: between groups = 3; within groups = 14.

acid, but no sugar was obtained. The **A-1** compound is eluted at 12.7 min, so it is more polar than ellagic acid: It could be an ellagic acid derivative with a radical that provides a higher polarity, linked by a C−O bond, because the UV spectrum did not change. This type of structure, such as valoneic acid, is part of the ellagitannins composition of related *Quercus* sp. (Mayer *et al.*, 1976a,b). The chromatographic behavior of this compound was described by Yoshida *et al.* (1992), who analyzed by RP-HPLC the hydrolysis products of eucalbanin B and found gallic acid (t_R = 2.7 min), ellagic acid (t_R = 12.3 min), and valoneic acid dilactone (t_R = 5.5 min). This later compound presents an intermediate retention time between the other two acids, which is in agreement with our results.

The **A-2** and **A-3** compounds are eluted at higher retention time (21.3 and 21.6 min, respectively) than ellagic acid (t_R = 18.7 min), and so, they could be ellagic acid derivatives less polar than this one, such as methyl ethers. In order to probe this statement, we have carried out the ellagic acid methylation with trimethylsilyldiazomethane (Cadahía, 1995) and obtained different methyl derivatives. The monomethyl derivatives have the same retention time and UV spectra as the **A-2** and **A-3** compounds. Although the literature related to these species does not describe the presence of this type of compound, they were identified in other species such as *Eucalyptus* (Hillis *et al.*, 1974; Yazaki and Hillis, 1976).

The **B** compounds, which have a UV spectrum very similar to that of gallic acid (Figure 3) and show higher retention times than ellagic acid, by acid hydrolysis produce mainly gallic acid and a small amount of ellagic acid and glucose. They seem to be gallic acid derivatives but much less polar than this one and even than ellagic acid. However, the galloyl esters reported in literature

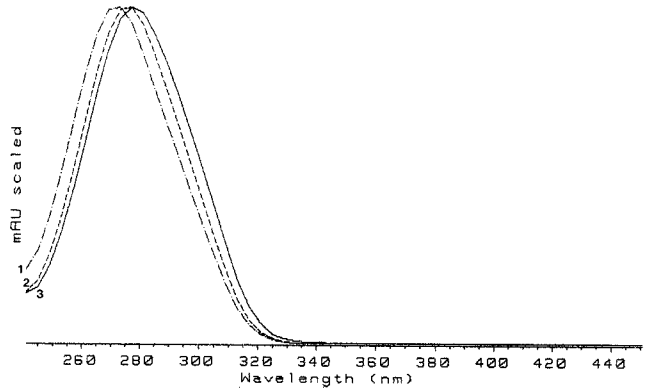

Figure 3. UV spectra of **B-5** (1), **B-10** (2), and gallic acid standard (3).

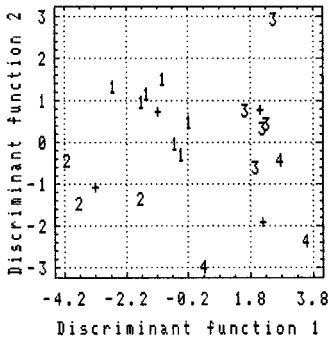

Figure 4. Multivariate analysis for the group of low molecular weight phenolics (gallic, ellagic, vanillic, syringic, and ferulic acids, vanillin, syringaldehyde, coniferaldehyde, sinapaldehyde, aesculetin, and scopoletin). Projections of the points of each species on the two principal canonical axes: 1 = *Q. robur*; 2 = *Q. petraea*; 3 = *Q. pyrenaica*; and 4 = *Q. faginea*.

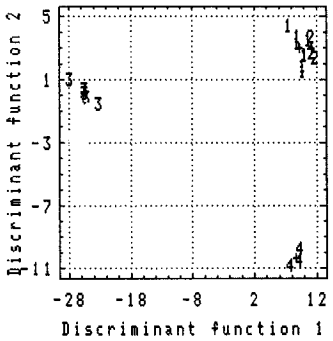

Figure 5. Multivariate analysis for the group of **B** compounds. Projections of the points of each species on the two principal canonical axes: 1 = *Q. robur*; 2 = *Q. petraea*; 3 = *Q. pyrenaica*; and 4 = *Q. faginea*.

are not less polar than ellagic acid (Haddock *et al.*, 1982; Nishimura *et al.*, 1986; Krajci and Gross, 1987; Sheu *et al.*, 1990), and their occurrence in wood of European oak has never been reported (Viriot *et al.*, 1993). The products obtained by us in the hydrolysis could be explained supposing that syringyl groups are implicated in the structure. This type of group yields by hydrolysis gallic acid, according to our own experience. So, the compound would present less polarity than the galloyl esters reported in the literature.

The **A** compounds are present in the four species in the same quantities, and the **B** compounds are more abundant in *Q. pyrenaica* and *Q. faginea*. So, it is possible to distinguish the woods of these species by the proportion of these two kinds of compounds.

The concentrations of low molecular weight phenolics are different according to the species and tree considered (Tables 2 and 3). A comparison of different samples from the same species shows that there is an interindividual variability in relation to the polyphenols contents, as happens also with global determinations (Table 1). This can be explained because the age of wood and the distance from base can also influence the composition of extractable polyphenols (Singleton, 1974; Scalbert *et al.*, 1986; Masson *et al.*, 1995b). The main component is ellagic acid (Table 2), and the second is gallic acid. On the other hand, aldehyde contents are always higher than those of acids, and cinnamic compounds are more abundant than the benzoic ones, with the exception of ferulic acid, which is present in very low concentrations. *Q. robur* and *Q. petraea* show higher amounts of these components, except for gallic and ellagic acids and coniferaldehyde, whose highest concentrations correspond to *Q. faginea*. The concentrations found for these components are lower than those cited by Miller *et al.* (1992), but vanillin and syringaldehyde concentrations are similar to those described by Nabeta *et al.* (1987) (1.2−3.6 μg/g of wood).

The discriminant canonical multivariate analysis of the benzoic and cinnamic acids and aldehydes and coumarins concentrations provides two discriminant functions, which accounted for 95.33% of the total variance, with a canonical correlation of 0.9202 and 0.7762, respectively. The graphical representation of the results according to these discriminant functions allows to differentiate four groups of samples corresponding to one of the four species analyzed (Figure 4). Function 1 contains the main contribution of coniferaldehyde, sinapaldehyde, vanillin, vanillic acid, and aesculetin in this sequence. This function presents the highest discriminant capacity and distinguishes *Q. robur* and *Q. petraea* from the other two species. When the **B** compound concentrations (calculated as gallic acid) are used for discriminant canonical multivariate analysis, the two discriminant functions obtained account for 99.84% of the total variance, with a canonical correlation of 0.9984 for function 1 and 0.9831 for function 2. The graphical representation of the results according to these discriminant functions shows three groups of samples, belonging to *Q. robur* and *Q. petraea* (group 1), *Q. pyrenaica* (group 2), or *Q. faginea* (group 3) (Figure 5). Function 1, made up mainly of **B-1**, **B-3**, **B-4**, **B-9**, **B-11**, and **B-13** in this sequence, distinguishes *Q. pyrenaica* from the other three species, while the function 2, made up of **B-4**, **B-6**, **B-5**, **B-1**, **B-10**, and **B-3**, differentiates *Q. faginea* from the other ones.

These results allow us to conclude that the benzoic and cinnamic acids and aldehydes and coumarins present differences among species similar to those described for different populations of a same oak species (Miller *et al.*, 1992; Marco *et al.*, 1994). However, the **B** compounds, from an enological point of view, could be more suitable, since they differenciate the woods of species habitually used for cooperage from others that could be used for this purpose.

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