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The Fluorescence of Ellagic Acid* and Its Borax Complex

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The absorption and fluorescence properties of the plant phenol ellagic acid have been studied in alcohol and aqueous solutions. Fluorescence is weak in all kinds of solvents, but is greatly enhanced by addition of borax. The resulting complex emits much more intensely in methanol than in water solution. UV and fluorescence data reveal that the complex formed in methanol is different from the complex formed in aqueous solution. The enormous fluorescence enhancement of ellagic acid by borax offers a simple method for the visualization and quantification of the potent anti-mutagen ellagic acid as well as a fluorimetric method for the determination of boron.

(Keywords: Ellagic acid; Absorption spectra; Fluorescence spectra)

Die Fluoreszenz der Ellagsäure und ihres Borax-Komplexes

Die Absorptions- und Fluoreszenzeigenschaften des Pflanzenphenols Ellagsäure wurden in methanolischer und wäßriger Lösung untersucht. Die Fluoreszenz ist in allen Lösungsmitteln schwach, wird aber durch Zugabe von Borax gewaltig erhöht. Der resultierende Komplex emittiert in methanolischer Lösung viel intensiver als in wäßriger. Die UV- und Fluoreszenzdaten zeigen, daß der in Methanol gebildete Komplex von dem in wäßriger Lösung gebildeten verschieden ist. Der enorme Anstieg in der Fluoreszenzintensität, welcher durch Borax bewirkt wird, kann einerseits zur Visualisierung und Bestimmung von Ellagsäure (einem potenten Antimutagen) dienen, andererseits bietet er eine Möglichkeit für die fluorimetrische Quantifizierung von Bor.

Ellagic acid ("alizarin yellow", "bezoaric acid", "kajidin") is a plant phenol frequently encountered in leaves and barks of *Dicotylidone* species[1,2]. Its structure is depicted in Figs. 1 and 2. The compound has also

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* The IUPAC name for ellagic acid is 2,3,7,8-tetra-hydroxy[1]benzopyrano[5,4,3—*c,d,e*][1]-benzopyran-5,10-dione.

been shown to occur in a variety of various other plants and to be responsible for the faint yellow color of certain flowers[3].

Recently, it has been shown that ellagic acid undergoes an extremely facile reaction with the ultimate carcinogen benzo[a]pyrene-7,8-diol-9,10-epoxide[4]. As a result, it exhibits exceptional activity in the inhibition of the mutagenicity and cytotoxicity of bay-region diol epoxides of polycyclic aromatic hydrocarbons[5].

In continuation of our studies[6] on the intrinsic fluorescence of natural products we have focused our interest on this compound. Its UV spectra in organic solvents have been reported[7—9], whereas the fluorescence properties have not been described so far.

Experimental

Compounds and Solvents

Ellagic acid was purchased from Aldrich Chemie (Steinheim, FRG) and was purified by recrystallization from pyridine and glacial acetic acid. The identity was proved by a correct m.p. and by UV spectra in agreement with published data.

Boric acid (H_3BO_3) and borax ($Na_2B_4O_7$, waterfree) were of analytical reagent grade. Solutions of borax in water or methanol showed weak blue fluorescence. This background had to be subtracted from the technical spectra of the complexes. Methanol was dried over sodium sulfate and distilled.

Spectra

The absorption spectra were run on a Perkin-Elmer Lambda 5 spectrophotometer in 1 × 1 cm quartz cells. Fluorescence spectra were run on an Aminco SPF 500 instrument. Data were handled with a HP 9815 A desk calculator.

Results and Discussion

The absorption spectra of ellagic acid in isopropanol and in water are shown in Fig. 1. They are highly *pH*-dependent in the near neutral *pH* range. The fluorescence spectra in isopropanol and water of different *pH* are shown in Fig. 2. Because of the weak fluorescence of ellagic acid the emission spectra were obtained under 300-nm excitation, rather than by excitation into the longest-wave absorption band, thus minimizing interferences from *Rayleigh* and *Raman* scatter. Evidently, changes in the spectra with *pH* are more significant in fluorescence than in absorption (Tab. 1).

Fluorescence is greatly enhanced by addition of boric acid or borax. Intensity increases by a factor of 150 and 1 000, respectively. The absorption and emission spectra in methanol in the presence and absence of boric acid and borax are shown in Fig. 3. Both the UV and fluorescence spectra indicate the formation of complexes, which are different for the boric acid and borax case. Moreover, the spectra indicate that addition of borax does not create the ellagic acid anion, but rather a chelate complex with absorption and emission maxima different from those of the anion.

Complex formation between ellagic acid and borax is also evident from the spectra of aqueous solutions (not shown), but the increase in fluorescence intensity after addition of borax is much smaller than that of methanol solutions. According to the emission spectra, complexation takes place at *pH* values above 6, and intensity increases steadily as the *pH* is raised to 11. Most interestingly, the absorption maxima of the borax complex undergo changes with *pH*, whereas the emission maxima practically do not.

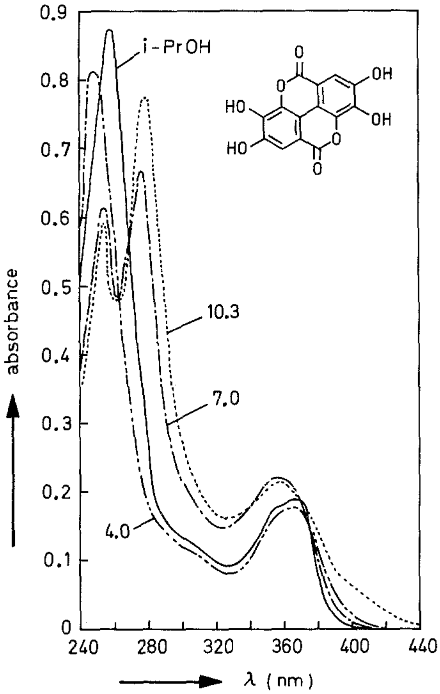

Fig. 1. Absorption spectra of ellagic acid in isopropanol (————) and water of *pH* 4.0 (—··—··), 7.0 (—·—·—), and 10.3 (······); conc. 17.5 μ*M*

The *pH* dependence of the absorption spectra of ellagic acid are governed by two *pK* values of 6.59[10] and 11.00[11]. The changes in fluorescence with *pH* parallel those in absorption and there is no evidence that ellagic acid (as a phenol) undergoes excited state dissociation[12].

We assume that the tremendous increase in fluorescence after addition

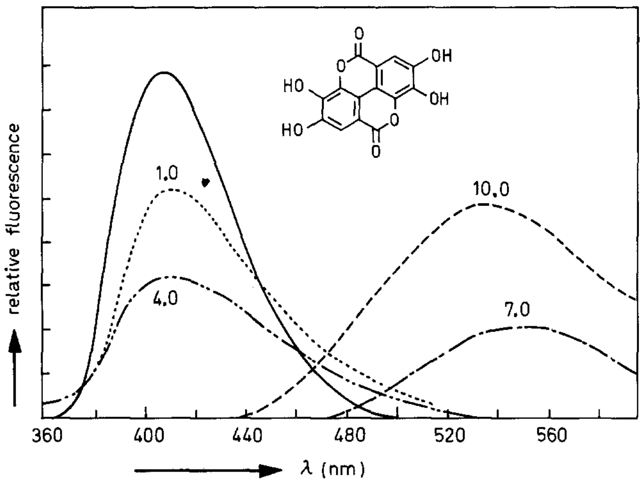

Fig. 2. Fluorescence spectra of ellagic acid in isopropanol (————) and in water of *pH* 1.0 (······), 4.0 (—··—··—), 7.0 (—·—·—), and 10.0 (— — — —) containing 40% isopropanol; excitation wavelength 300 nm, absorbance at 300 nm 0.08

Table 1. *Absorption and fluorescence maxima (in nm) of ellagic acid (46 μM) and its borax complexes in alcohols and water of various pH.* The aqueous solutions contained 40% isopropanol, when no borax was present

Solvent	absorption maximum	(M^{-1} cm^{-1})	fluorescence maximum
methanol	364	10.990	412
isopropanol	367	10.880	408
water, *pH* 1.0	366	—	408
water, *pH* 5.0	362	—	420
water, *pH* 7.0	356	12.150	540
water, *pH* 10.0	356	15.210	540
methanol plus borax	380	11.100	463
methanol/boric acid	369	10.160	480
water, *pH* 9.5/borax	376	—	510
water, *pH* 7.0/borax	371	—	510
water, *pH* 3.0/borax	365	—	410

of borax is due to the formation of a chelate. Borax is known to complex *ortho*-dihydroxy groups, a fact that has been utilized to recognize the presence of these groups in various natural products by virtue of significant spectra shifts in UV spectra[13].

A plot of fluorescence intensities of an ellagic acid solution in

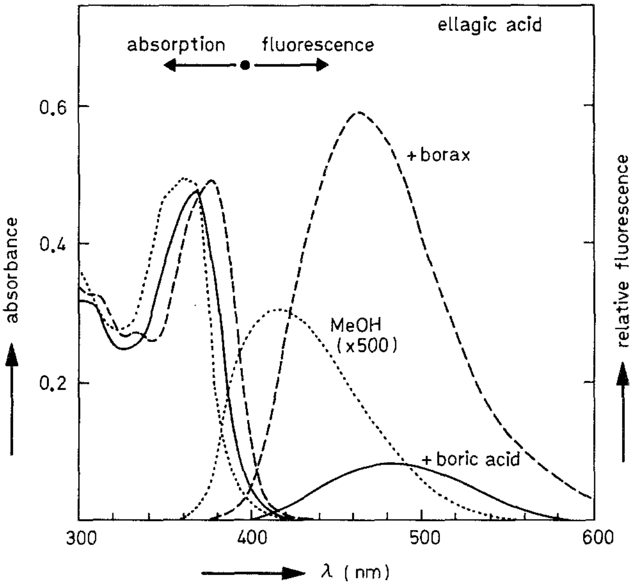

Fig. 3. Absorption and fluorescence spectra of ellagic acid (ca. 20 μM) in methanol (······), methanol containing 10 mM boric acid (————), and methanol containing 10 mM borax (— — — —). The emission spectrum of ellagic acid in methanol is multiplied by a factor of 500

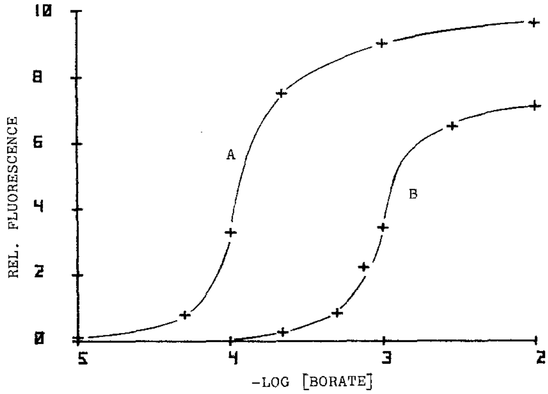

Fig. 4. Plot of relative fluorescence intensity of ellagic acid (40 μM) in methanol (curve A) and ammoniacal methanol (curve B) vs. -log of borax concentration

methanol vs. borate concentration is shown in Fig. 4. Assuming a 1 : 1 stoichiometry between borax and ellagic acid, a binding constant of 5 556 M^{-1} can be calculated.

Although the emission maxima remain the same, a different curve shape (Fig. 4) and binding constant (952 M^{-1}) is obtained when ammoniacal methanol (1 ml conc. ammonia to 99 ml methanol) is used as a solvent. The difference can be explained by assuming complexation of ellagic acid (in pure methanol) and ellagic acid monoanion (in alkaline methanol), respectively.

The dramatic increase in the fluorescence of ellagic acid in methanol after addition of borax may be utilized for two purposes: Firstly, it may serve to visualize and determine ellagic acid in cells and tissue during studies on its mutagenicity-inhibiting action. Secondly, it offers a possibility for the fluorimetric determination of boron in neutral organic solvent. Most of the methods published so far employ strong sulfuric acid as a solvent[12].

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