

# Fluorescence Assay of Hydroxynaphthoquinones

JOHAN G. VAN DAMME \* and ROELAND E. DE NÈVE \*

Received November 17, 1977, from the *Laboratorium voor Galenische en Magistrale Farmacie, Farmaceutisch Instituut, Vrije Universiteit Brussel, Paardenstraat, 67, B-Sint-Genesius-Rode, Brussels, Belgium.* Accepted for publication April 5, 1978. \*Present address: S. A. Unda-Harzé, B 4071 Aywaille, Belgium.

**Abstract** □ Three procedures for fluorescence assay of hydroxynaphthoquinones are reported. The first procedure, based on prior sodium dithionite reduction and determination of the fluorophore in butyl acetate, is applicable to all of the hydroxynaphthoquinones investigated (detection limit:  $\pm 0.020 \mu\text{g}$  of quinone/g of butyl acetate). The second method, specific for 5-hydroxy-1,4-naphthoquinones (juglone series), involves warm reduction with stannous chloride in an acid medium and determination of the fluorophore in chloroform (detection limit:  $\pm 0.020 \mu\text{g}$  of quinone/g of chloroform). The third method, based on the reaction of Guilbault and Kramer, is applicable to 5-hydroxy- and 5,8-dihydroxy-1,4-naphthoquinones having both free quinonoid positions (detection limit:  $\pm 0.020 \mu\text{g}$  of juglone/g of dimethyl sulfoxide and  $0.070 \mu\text{g}$  of *p*-naphthazarin/g of dimethyl sulfoxide). The nature of the fluorophores also was investigated.

**Keyphrases** □ Hydroxynaphthoquinones, various—fluorometric analyses, three different procedures □ Fluorometry—analyses, various hydroxynaphthoquinones, three different procedures

Hydroxynaphthoquinones of considerable interest (1) include the perihydroxy-1,4-naphthoquinones, juglone [occurring in the green parts of *Juglans regia* L. (Juglandaceae)], and plumbagin [in the roots and leaves of Plumbaginaceae and in *Drosera rotundifolia* L. (Droseraceae)], as well as lawsone [in *Lawsonia inermis* L. (Lythraceae)], lapachol (in the heartwood of several genera of the Bignoniaceae, especially the tribe Tecomeae), and phtiocol (isolated from *Mycobacterium tuberculosis*).

Dried plants, crude extracts, tinctures, or the pure quinones are still in use. In Belgium and France, tincture of *Drosera* is reputed to be of value in chronic bronchitis; but, since the drug deteriorates even when carefully stored, it is often inert (2). Henna (powder of dried leaves of *Lawsonia*) has been used in intestinal amoebiasis (3) and is perhaps one of the oldest cosmetics still in use. The fungicidal and bactericidal properties of pure plumbagin and juglone also have found medicinal use (4), and the antitumor properties of lapachol are presently of interest (5).

The chemistry of the hydroxynaphthoquinones has been studied extensively, especially lapachol (6) and juglone (1). However, the usual colorimetric and spectrophotometric methods for quantitative analysis of these compounds suffer from a lack of sensitivity and specificity. Fluorometric methods have not been reported until now and have to be based on chemical conversion because hydroxynaphthoquinones are nonfluorescent.

This paper describes simple fluorometric procedures for the pure compounds, permitting determinations at the submicrogram level. The most general method involves biphasic reduction with sodium dithionite solutions and determination of the fluorophore in butyl acetate. Since the procedure is also applicable to naphthoquinones with alkyl or prenyl (3-methyl-2-butenyl) groups (7), the method is not specific but is highly sensitive. Another procedure is based on warm reduction with stannous chloride followed by determination of the fluorophore

formed in chloroform. The method is specific for 5-hydroxy-1,4-naphthoquinones (juglone and plumbagin) and is sensitive. A third procedure uses the reaction of Guilbault and Kramer (8) (nucleophilic addition of potassium cyanide) and appears to be specific for 5-hydroxy- and 5,8-dihydroxy-1,4-naphthoquinones with both quinonoid positions free.

## EXPERIMENTAL

**Instrumentation**—The fluorescence spectrometer, equipped with a xenon arc<sup>1</sup> fed by a dc current supply<sup>2</sup>, had a magnetic stabilizer<sup>3</sup>, two quartz prism monochromators<sup>4</sup> with continuously adjustable slit widths, fluorescence attachment<sup>5</sup>, a potted photomultiplier<sup>6</sup>, and a microammeter<sup>7</sup> coupled to a transmission concentration converter<sup>8</sup> with four-digit display. The coarse gain of the instrument was variable in steps up to 15,000 $\times$ , and the instrumental sensitivity in terms of quinine sulfate units is  $1 \mu\text{g}/\text{ml}$  of  $0.1 N H_2SO_4$ , obtaining full-scale deviation (100 in arbitrary units) by a gain of 3000 $\times$ , an excitation maximum of 355 nm (slit width 10 nm), and an emission maximum of 460 nm (slit width 10 nm). The blank under these circumstances was 0.1 in arbitrary units.

For recording (uncorrected) spectra, the monochromators were equipped with wavelength drives and the transmission concentration converter was coupled to a one-axis recorder<sup>9</sup> with a wavelength marker.

Absorption spectra were obtained on a recording spectrophotometer<sup>10</sup>.

**Materials**—Lawsone<sup>11</sup>, lapachol<sup>12</sup>, and juglone<sup>12</sup> were used without further purification, and *p*-naphthazarin<sup>13</sup> was purified by recrystallization in benzene. Plumbagin was prepared and purified by the method of de Buruaga (9) (mp<sup>14</sup> 75°). Phtiocol also was prepared (10) and purified by preparative TLC<sup>15</sup> (mp 172°).

All reagents were analytical or reagent grade purity. The assay solutions, prepared in double-distilled water, were 15% sodium dithionite, 1% potassium cyanide in phosphate buffer (pH 7.5), and 5% stannous chloride in 4 M HCl.

Chloroform<sup>16</sup> and dimethyl sulfoxide<sup>16</sup> were fluorescent grade, and butyl acetate<sup>16</sup> was analytical grade.

All glassware was treated with chromic acid (24 hr) and, after rinsing with distilled water, oven dried. To avoid adsorption losses, the inner surfaces were deactivated by soaking for 10 min in a 1% solution of a silicizing agent<sup>17</sup>. The glassware was then rinsed thoroughly and oven dried (105°, 30 min).

Quartz cells were treated with fuming nitric acid (11).

**Procedure A**—The appropriate quantity of quinone was dissolved in 5 ml of butyl acetate in a screw-capped tube. Then 5 ml of sodium dithionite reagent was added, and the mixture was shaken for 15 min at 200

<sup>1</sup> Type Osram X B O 450 W mounted in a housing LX 501, C. Zeiss.

<sup>2</sup> Type V X 501 r, C. Zeiss.

<sup>3</sup> Type B V X 200 K, C. Zeiss.

<sup>4</sup> Type M 4 Q III, C. Zeiss.

<sup>5</sup> Type Z F M 4 C, C. Zeiss.

<sup>6</sup> RCA 1 P 28.

<sup>7</sup> P M Q II, C. Zeiss.

<sup>8</sup> TKR, C. Zeiss.

<sup>9</sup> Servogor S Type RE 541, Goerz-Austria.

<sup>10</sup> Perkin-Elmer model 124.

<sup>11</sup> Fluka AG, Switzerland.

<sup>12</sup> Aldrich Chemical Co.

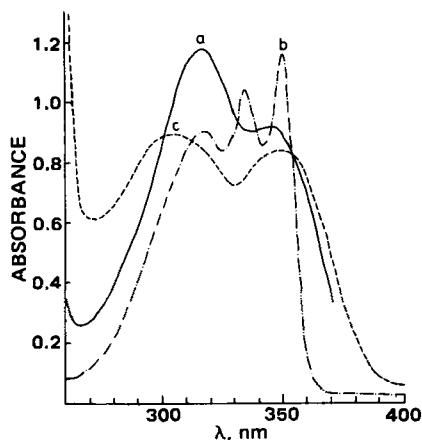
<sup>13</sup> Bayer A. G., West Germany.

<sup>14</sup> Uncorrected on a Tottoli apparatus, Buchi.

<sup>15</sup> On 20  $\times$  20-cm precoated silica gel plates with 2-mm layers (E. Merck, Darmstadt, West Germany). The eluent was heptane-butyl acetate (1:9); the *R<sub>f</sub>* of phtiocol was 0.55.

<sup>16</sup> E. Merck, Darmstadt, West Germany.

<sup>17</sup> Siliclad, Clay-Adams, New York, N. Y.



**Figure 1**—UV spectra in butyl acetate. Key: a, reduced lawsone,  $7.0 \times 10^{-5}$  M; b, reduced juglone,  $6.5 \times 10^{-5}$  M; and c, reduced *p*-naphthazarin,  $6.0 \times 10^{-5}$  M.

movements/min on a horizontal shaker. In a nitrogen atmosphere, about 3 ml of the butyl acetate phase was transferred to a 1-cm path quartz cell.

**Procedure B**—The appropriate quantity of quinone was dissolved in 1 ml of ethanol in a screw-capped tube. Then 5 ml of stannous chloride reagent was added; the mixture was covered with a small funnel and left for 1 hr over a boiling water bath (evaporation of the ethanol). After cooling, 5 ml of chloroform was added, and the mixture was shaken for 5 min at 200 movements/min. Following a brief centrifugation (5 min at  $3000 \times g$ ), about 3 ml of the organic phase was transferred to a 1-cm path quartz cell.

**Procedure C**—The appropriate quantity of quinone was dissolved in 5 ml of dimethyl sulfoxide, 0.5 ml of the potassium cyanide reagent was added, and the mixture was left to stand for 10 min. About 3 ml was transferred to a 1-cm path quartz cell prior to measurement.

**Assay**—For the determination of linearity, two concentration regions were chosen. In each region, seven samples and one reagent blank were tested. The samples were: Procedure A, 0.5–2 and 1–10  $\mu\text{g}$  of quinone/g of butyl acetate; Procedure B, 1–10 and 5–25  $\mu\text{g}$  of quinone; and Procedure C, 0.4–4 and 2–10  $\mu\text{g}$  of quinone/g of dimethyl sulfoxide.

To determine the precision of the method, 12 blanks and 12 1- $\mu\text{g}$  quinone standards were analyzed, starting with the weight operation, and the standard deviation was calculated.

The detection and determination limits as defined by Currie (12) were determined for each procedure.

## RESULTS AND DISCUSSION

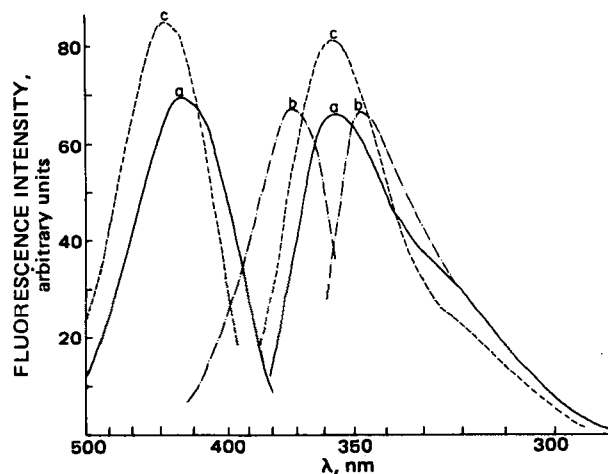
**Nature of the Fluorophores**—*Procedure A*—By applying the biphasic dithionite procedure to alkyl- and phenyl-naphthoquinones, it was shown that the fluorophores formed were the corresponding hydroquinones; furthermore, the conversion of the quinone into the hydroquinone was nearly complete (7).

Although formation of hydroquinones should logically be involved for hydroxynaphthoquinones, the problem is more complex because of interferences due to phenol–keto tautomerism. Indeed, in contrast to the alkylnaphthohydroquinones where the phenolic form predominates in solution and only one form exists in the solid state (13), different tautomeric forms with perihydroxynaphthohydroquinones have been obtained in the solid state. In this case, the term “isomerism” instead of tautomerism must be used (14).

According to Fieser and Gates (10), dithionite reduction of phthiocol yields 3-methyl-1,2,4-trihydroxynaphthalene, and the phenolic tautomer predominates in solution.

Absorptiometric analysis, showing the similarity of the spectra of reduced lawsone and phthiocol, indicates that lawsone also yielded 1,2,4-trihydroxynaphthalene upon dithionite reduction (Fig. 1).

The reaction product with juglone was isolated by performing the procedure with ethyl acetate instead of butyl acetate and evaporating the solvent at room temperature in a nitrogen atmosphere. The melting point ( $170^\circ$ ) and chemical properties (15) of the compound indicate the presence of an  $\alpha$ -hydrojuglone (1,4,5-trihydroxynaphthalene). The absorption spectrum (Fig. 1) of  $\alpha$ -hydrojuglone in butyl acetate is very



**Figure 2**—Uncorrected excitation and emission spectra in butyl acetate. Key: a, reduced lawsone,  $1.4 \times 10^{-4}$  M; b, reduced juglone,  $1.3 \times 10^{-4}$  M; and c, reduced *p*-naphthazarin,  $1.2 \times 10^{-4}$  M.

characteristic with three maxima: 317, 334, and 349 nm. Dithionite-reduced plumbagin yielded a similar spectrum, showing the formation of  $\alpha$ -hydroplumbagin.

The reaction product of *p*-naphthazarin was obtained in a similar manner; physical and chemical properties (16) (e.g., violet fluorescence in ethanol, mp  $140^\circ$ ) suggest the presence of 1,4,5,8-tetrahydroxynaphthalene. As shown by the absorption spectrum (Fig. 1), the phenolic tautomer must also predominate in solution.

In contrast to the biphasic dithionite reduction, other reduction procedures (stannous and titanous chloride and zinc powder plus hydrochloric acid) did not always give the phenolic tautomers. With *p*-naphthazarin, cold stannous chloride reduction did not yield a fluorophore upon extraction in butyl acetate; on the other hand, addition of alcoholic hydrochloric acid to  $\alpha$ -hydronaphthazarin solution in butyl acetate yielded fluorescence quenching. The formation of  $\beta$ -hydronaphthazarin (see Procedure B) is also unlikely, so another tautomer is probably involved.

From fluorescence analysis, it appears that one can distinguish between 2-hydroxynaphthoquinones (lawsone, lapachol, and phthiocol) and 5-hydroxynaphthoquinones (juglone and plumbagin), the spectra being identical within the same group (Fig. 2).

*Procedure B*—According to Thomson (17), warm stannous chloride reduction of juglone gives a mixture of  $\alpha$ - and  $\beta$ -hydrojuglone in a 35:65 ratio, the latter being extractable with chloroform. Zahn and Ochwat (18) showed that the probable structure of  $\beta$ -hydrojuglone is 5-hydroxy-1,4-dioxo-1,2,3,4-tetrahydronaphthalene.

The fluorescent species obtained with juglone by application of Procedure B is thus probably  $\beta$ -hydrojuglone and is, as shown by its excitation and emission spectra, completely different from  $\alpha$ -hydrojuglone (Fig. 3). With plumbagin, a fluorophore also was obtained, which is different from  $\alpha$ -hydroplumbagin. Since the absorption spectrum of the compound in chloroform is similar to the spectrum of  $\beta$ -hydrojuglone (with respect to a bathochromic shift of approximately 40 nm), the fluorescent species is presumably a 1,4-dioxo-1,2,3,4-tetrahydronaphthalene compound ( $\beta$ -hydroplumbagin).

In contrast, warm stannous chloride reduction of *p*-naphthazarin did not yield fluorophores upon extraction either in chloroform or in butyl acetate, which illustrates that the formation of either  $\alpha$ - or  $\beta$ -hydronaphthazarin is not probable.

*Procedure C*—Thiele and Meisenheimer (19) observed that hydrogen cyanide yields a diaddition product with 1,4-benzoquinone: 2,3-dicyano-1,4-dihydroxybenzene. The fluorescent properties of this compound were reported by Guilbault and Kramer (8), who demonstrated that the same compound is obtained upon reaction with potassium cyanide in dimethyl sulfoxide and that the green fluorescence must be assigned to a tautomeric ionic form.

Reynolds and Van Allen (20) showed that 2,3-dicyano-1,4-naphthalenediol is obtained with 2,3-dichloro-1,4-naphthoquinone and sodium cyanide. 1,4-Naphthoquinone should react much more slowly and presumably yield the same compound.

Performance of the Guilbault–Kramer procedure on 2,3-dichloro-1,4-naphthoquinone yielded a yellow fluorescent solution, and no ap-

**Table I—Quantitative Determinations (Procedure A)**

Parameter	2-Hydroxy-1,4-naphthoquinones			5-Hydroxy-1,4-naphthoquinones		
	Lawsonone	Phthiocol	Lapachol	Juglone	Plumbagin	Naphthazarin
Excitation (slit width <sup>a</sup> )	355 (3.6)	343 (3.6)	345 (6)	350 (9)	350 (6)	360 (6)
Emission (slit width <sup>a</sup> )	429 (22)	432 (23)	430 (24)	370 (14)	375 (14)	440 (24)
Gain	9500×	12,000×	12,000×	12,000×	15,000×	12,000×
Blank <sup>b</sup>	0.5 ± 0.1 <sup>c</sup>	1.5 ± 0.2 <sup>c</sup>	2.5 ± 0.2 <sup>c</sup>	0.5 ± 0.2 <sup>c</sup>	0.2 ± 0.2 <sup>c</sup>	2.2 ± 0.2 <sup>c</sup>
1-μg standard <sup>b</sup>	49.0 ± 0.2 <sup>c</sup>	62.5 ± 0.3 <sup>c</sup>	57.0 ± 0.3 <sup>c</sup>	52.0 ± 0.3 <sup>c</sup>	53.5 ± 0.3 <sup>c</sup>	61.6 ± 0.3 <sup>c</sup>
Detection limit <sup>d</sup>	0.015	0.020	0.022	0.023	0.022	0.020
Lower determination limit <sup>d</sup>	0.045	0.060	0.066	0.071	0.066	0.061

<sup>a</sup> In nanometers. <sup>b</sup> In arbitrary units. <sup>c</sup> Standard deviation. <sup>d</sup> In micrograms of quinone per gram of solvent (butyl acetate).

preciable fluorescence was noted with 1,4-naphthoquinone after a reaction time of 10 min. Application of the same procedure on perihydroxy-naphthoquinones with both quinonoid positions free (juglone and naphthazarin) yielded highly fluorescent species, and no fluorescence upon reaction with plumbagin was detectable. From fluorescence spectra, it appears that only one fluorophore (Fig. 4) was involved with juglone and that several fluorophores were formed with *p*-naphthazarin (Fig. 5). In the last case, the presence of more than one fluorescent component was suspected because the shape of the emission spectrum changed when the wavelength of the exciting light was varied (21).

Since no monoaddition product could be isolated (8, 19, 20) with juglone upon reaction with *p*-benzoquinone, *p*-naphthoquinone, and 2,3-dichloro-1,4-naphthoquinone, formation of a diaddition compound again was presumed.

**Choice of Solvent—Procedure A**—Biphasic reduction with butyl acetate is preferred, although the procedure yields similar results with amyl alcohol and ether. The use of an ester such as butyl acetate is indicated, because the solubility of this solvent for the dithionite reagent is extremely low. On standing, dithionite solutions become turbid through sulfur precipitation.

The aprotic character of the solvent is of interest since interferences due to electrolytic excited-state dissociation, as found with naphthols (22) and naphthalenediols (23), are avoided. Furthermore, absorption, excitation, and emission spectra in butyl acetate are bathochromically shifted compared to the spectra in cyclohexane (24), illustrating hydrogen bonding. Bathochromic shifts may be important, because the energy output of the light source is higher in the near UV region of the spectrum, resulting in a higher instrumental sensitivity.

**Procedure B**—Although  $\beta$ -hydroquinones are fluorescent in solvents such as ether, butyl acetate, and amyl alcohol, they are also fluorescent in chloroform, in contrast to the  $\alpha$ -hydroquinones which produce non-fluorescent chloroform solutions because of an external heavy atom effect (25). Interferences of other quinones due to simultaneous reduction to hydroquinones are avoided, and the selectivity of the method is greatly enhanced. The absence of an external heavy atom effect (spin orbit

coupling) in  $\beta$ -hydroquinones can be explained by intramolecular hydrogen bonding.

**Procedure C**—Guilbault and Kramer (8) observed that the maximum rates of reaction and fluorescence were obtained with benzoquinone in a solvent with a high dielectric constant such as dimethyl sulfoxide. This finding illustrates that the fluorescent species in an ionic molecule and solvents favoring the ionic form should be best. The effect of water and pH of the added reagent also was reported (8). With benzoquinone, the total water content should not exceed 9% (v/v) to avoid a deleterious effect on the reaction rate, and the pH should be held at 6.5–7.5 for the reaction to proceed smoothly.

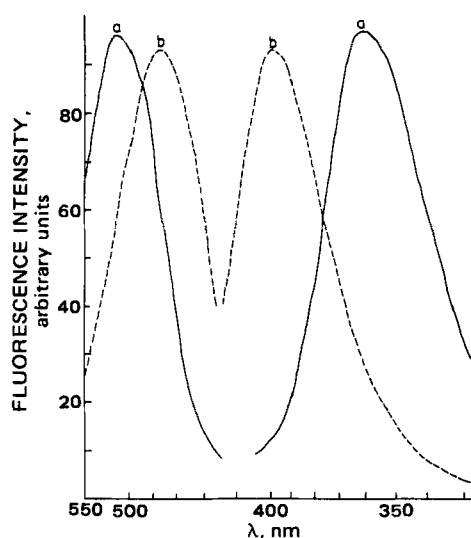
With juglone, similar results were obtained. The maximum fluorescence intensity and reaction rate were obtained in dimethyl sulfoxide with a total water content not exceeding 2% (v/v). The potassium cyanide reagent should also be made up in a pH 7.5 phosphate buffer.

**Quantitative Results—Procedure A**—As indicated in Table I, the precision of the method is high, mainly due to the high photochemical stability of the fluorophores formed and the stoichiometric progress of the reduction. Moreover, working in a nitrogen atmosphere is not required for reliable results.

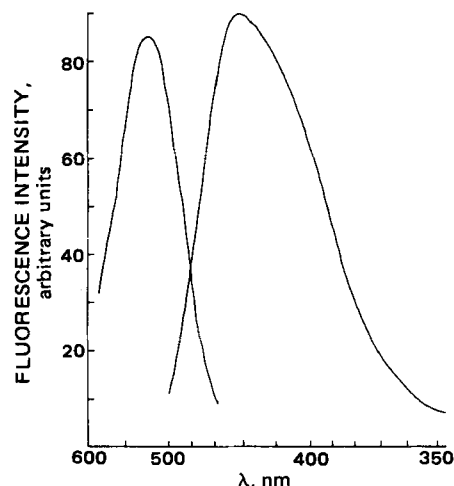
The luminescence blank is low, and the main factors contributing to the blank are the luminescence of the cell, the surroundings, and scattered light. Insertion of properly chosen stray light filters at the exits of excitation and emission monochromators is recommended. The dithionite reagent itself does not contribute to the blank.

From excitation and emission maxima or spectra, it appears that 2-hydroxy- and 5-hydroxy-1,4-naphthoquinones can be determined separately in mixtures. Separate determinations of 5-hydroxy- and 5,8-dihydroxy-1,4-naphthoquinones seem rather difficult to perform. The (lower) determination limit, defined as the concentration for which the relative standard deviation is 10%, is calculated as indicated by Currie (12). The obtained values ( $\pm 0.020 \mu\text{g/g}$  of solvent) show the high sensitivity of the method.

To determine the linearity, the upper determination limit was defined as the concentration for which a 10% deviation of the regression line is obtained, calculated from the first seven points. A value of  $4 \mu\text{g/g}$  of solvent was obtained, showing the deviation from linearity due to concentration quenching.



**Figure 3**—Uncorrected excitation and emission spectra in chloroform. Key: a,  $\beta$ -hydrojuglone,  $3.3 \times 10^{-5}$  M; and b,  $\beta$ -hydroplumbagin,  $3.7 \times 10^{-5}$  M.



**Figure 4**—Uncorrected excitation and emission spectra of the fluorophores of  $1.2 \times 10^{-4}$  M juglone (Procedure C).

**Table II—Quantitative Determinations (Procedure B)**

Parameter	Juglone	Plumbagin
Excitation (slit width <sup>a</sup> )	363 (13)	397 (18)
Emission (slit width <sup>a</sup> )	510 (38)	474 (30)
Gain	1500×	750×
Blank <sup>b</sup>	2.9 ± 0.1 <sup>c</sup>	2.0 ± 0.1 <sup>c</sup>
1- $\mu$ g standard <sup>b</sup>	31.3 ± 0.1 <sup>c</sup>	27.5 ± 0.1 <sup>c</sup>
Detection limit <sup>d</sup>	0.016	0.018
Lower determination limit <sup>d</sup>	0.050	0.054

<sup>a</sup> In nanometers. <sup>b</sup> In arbitrary units. <sup>c</sup> Standard deviation. <sup>d</sup> In micrograms of quinone per gram of solvent (chloroform).

Correlation coefficients were calculated from the first seven points (the first concentration region) and were not less than 0.998.

**Procedure B**—Quantitative results are summarized in Table II. The fluorophores formed do not suffer from either photodecomposition or reoxidation by air. Furthermore, as indicated by the precision of the method, the isomerization ratio of  $\alpha$ - to  $\beta$ -hydrojuglones is independent of the concentration involved.

The luminescence blank is sufficiently low. In contrast to Procedure A, the reagents contribute to the blank, as shown by insertion of a spectrograde labeled chloroform blank.

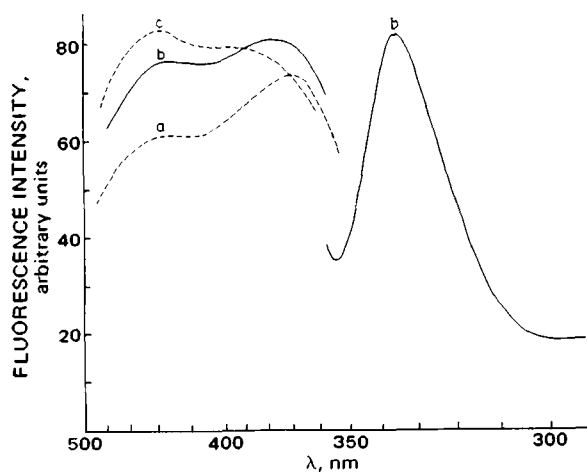
From excitation and emission maxima, it appears that juglone and plumbagin can be determined separately in mixtures. The lower and upper determination limits in both cases were 0.020 and 4  $\mu$ g/g of chloroform, respectively, which indicates a sufficiently large linearity.

Correlation coefficients calculated for the first seven points (first concentration region) were not less than 0.999.

The method is highly specific for 5-hydroxy-1,4-naphthoquinones; other quinones do not interfere when chloroform is the solvent.

**Procedure C**—Although more fluorophores are involved with *p*-naphthazarin, reliable quantitative results were obtained of a sufficiently high precision at a 375-nm emission maximum (Table III). The detection and lower determination limits of *p*-naphthazarin were three times higher than the juglone limits, and the upper determination limits were 5 and 7  $\mu$ g/g of dimethyl sulfoxide with juglone and naphthazarin, respectively.

Fluorescent grade labeled dimethyl sulfoxide is recommended as the solvent since analytical grade solvents yield high blanks, probably due to impurities.



**Figure 5**—Uncorrected excitation and emission spectra of the fluorophores of  $1.2 \times 10^{-4}$  M *p*-naphthazarin. Key: a, emission spectrum (excitation = 330 nm); b, emission spectrum (excitation = 335 nm); and c, emission spectrum (excitation = 340 nm).

**Table III—Quantitative Determinations (Procedure C)**

Parameter	Juglone	Naphthazarin
Excitation (slit width <sup>a</sup> )	444 (24)	337 (12)
Emission (slit width <sup>a</sup> )	525 (45)	375 (14)
Gain	1500×	9000×
Blank <sup>b</sup>	0.5 ± 0.1 <sup>c</sup>	1.4 ± 0.1 <sup>c</sup>
1- $\mu$ g standard <sup>b</sup>	21.0 ± 0.1 <sup>c</sup>	12 ± 0.2 <sup>c</sup>
Detection limit <sup>d</sup>	0.023	0.070
Lower determination limit <sup>d</sup>	0.070	0.210

<sup>a</sup> In nanometers. <sup>b</sup> In arbitrary units. <sup>c</sup> Standard deviation. <sup>d</sup> In micrograms of quinone per gram of solvent (dimethyl sulfoxide).

*p*-Benzoquinone and 2,3-dichloro-1,4-naphthoquinone also yield a positive reaction.

## REFERENCES

- (1) R. H. Thomson, "Naturally Occurring Quinones," 2nd ed., Academic, New York, N.Y., 1971.
- (2) R. Paris and H. Moyses, "Matière Médicale," vol. 2, Masson, Paris, France, 1967, pp. 225–229.
- (3) M. E. Hanke and S. M. Talaat, *Trans. R. Soc. Trop. Med. Hyg.*, **55**, 56 (1961).
- (4) P. Lebeau and M. Janot, "Traité de Pharmacie Chimique," vol. 2, Masson, Paris, France, 1955, pp. 1160–1164.
- (5) K. V. Rao, T. C. McBride, and J. J. Oleson, *Cancer Res.*, **28**, 1952 (1968).
- (6) "The Constitution and Properties of Lapachol, Lomatiol and Other Hydroxynaphthoquinone Derivatives," L. F. Fieser, Ed., 1937.
- (7) J. G. Van Damme, Ph.D. thesis, Vrije Universiteit Brussel, Brussels, Belgium, 1977.
- (8) G. G. Guilbault and D. N. Kramer, *Anal. Chem.*, **37**, 1395 (1965).
- (9) S. de Buruaga, *An. Soc. Fis. Quim.*, **31**, 134 (1933).
- (10) L. F. Fieser and J. W. Gates, *J. Am. Chem. Soc.*, **63**, 2948 (1941).
- (11) J. R. Edisbury, "Practical Hints on Absorption Spectrometry," Hilger and Watts, London, England, 1966, pp. 127–132.
- (12) L. A. Currie, *Anal. Chem.*, **40**, 586 (1968).
- (13) G. W. Wheland, "Resonance in Organic Chemistry," Wiley, New York, N.Y., 1955, pp. 401–405.
- (14) B. Pullmann and A. Pullmann, "Les Théories Électroniques de la Chimie Organique," Masson, Paris, France, 1952, pp. 247, 248.
- (15) "Elsevier's Encyclopaedia of Organic Chemistry," Carboisocyclic Condensed Compounds, No. 3, vol. 12B, F. Radt, Ed., Elsevier, New York, N.Y., 1950, p. 2155.
- (16) *Ibid.*, pp. 2179–2181.
- (17) R. H. Thomson, *J. Chem. Soc.*, **1950**, 1737.
- (18) K. Zahn and P. Ochwat, *Ann. Chem.*, **462**, 72 (1928).
- (19) J. Thiele and L. Meisenheimer, *Chem. Ber.*, **33**, 675 (1900).
- (20) G. Reynolds and J. Van Allen, *J. Org. Chem.*, **29**, 3591 (1964).
- (21) C. A. Parker, "Photoluminescence of Solutions," Elsevier, New York, N.Y., 1968, p. 19.
- (22) A. Weller, *Z. Phys. Chem.*, **17**, 224 (1958).
- (23) L. D. Derkacheva, *Opt. Spektrosk.*, **9**, 110 (1958) (English).
- (24) I. Berlman, "Handbook of Fluorescence Spectra of Aromatic Molecules," Academic, New York, N.Y., 1965, pp. 112–115.
- (25) C. A. Parker, "Photoluminescence of Solutions," Elsevier, New York, N.Y., 1968, pp. 373–386.

## ACKNOWLEDGMENTS

Abstracted from a thesis submitted by J. G. Van Damme to the Vrije Universiteit Brussel in partial fulfillment of the Doctor of Philosophy degree requirements.

Supported in part by the Nationaal Fonds voor Geneeskundig Onderzoek.