Fluorescence Spectra and Quantum Yields: Quinine, Uranine, 9,10-Diphenylanthracene, and 9,10-Bis(phenylethynyl)anthracenes

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Spectra and quantum yields of the title compounds taken with a Turner corrected spectrum spectrofluorometer are carefully tabulated. Our spectra of quinine sulfate and of fluorescein are compared to other corrected spectra by a simple ratio method, which is suggested as generally useful. The effects of chemical substituent changes on the fluorescence spectra and quantum yields of 9,10-bis(phenylethynyl)anthracene are shown.

The spectra and quantum yield of fluorescers are of both scientific interest and of practical value. We have had difficulty finding data which we can use in our own work. The present paper mainly concerns several substituted 9,10-bis(phenylethynyl)anthracenes (BPEA's).

The instrument used for this work was the Turner Model 210 spectrofluorometer (*15*) which automatically corrects the emission spectra and the excitation energy. We were interested in determining the accuracy of these corrections by comparing standard spectra.

A recent publication by Melhuish (*11*) listed 11 spectra taken of quinine sulfate on different instruments. He noted the difficulty of reading values off small published graphs. We have noted the same problem. Only in a 1960 article of Melhuish (*9*) have we found a tabulated spectrum which warranted much detailed comparison with our own spectrum.

We have attempted to make our spectra useful for comparison purposes. They are all taken at low concentration to avoid reabsorption and are tabulated at a fairly close wavelength interval and normalized at the peak.

The choice of giving emission vs. wavelength rather than emission vs. frequency was made deliberately, despite suggestions that only the latter be used (*11, 14*). Our instrument reads in wavelength, and the data have to be transformed mathematically to frequency. In the process, some precision is lost. We think data should be presented in its original form and transformed by the user to fit his needs.

Experimental

Synthesis of substituted 9,10-bis(phenylethynyl)anthracenes. These compounds were prepared from anthradiols by the method of Donner et al. (*5*). BPEA and 1-chloro-BPEA (1-CBPEA) were obtained from Michael Rauhut of American Cyanamid. 2-Chloro-BPEA (2-CBPEA) was described previously (*5*).

Data on the precursor anthradiols and substituted BPEA's prepared by us are shown in Table I. All the compounds gave satisfactory analysis for C, H, and F. The structure of BPEA is shown in Figure 1.

Our abbreviations for the substituted compounds are 2-EBPEA (2-ethyl-BPEA), 1-PBPEA (1-phenyl-BPEA), 1,4-DMBPEA (1,4-dimethyl-BPEA), and 1,4-DPBPEA (1,4-diphenyl-BPEA).

Other materials. Benzene was spectro grade from Matheson Coleman & Bell. Dibutyl phthalate (DBP) was a special photographic grade from Eastman-Kodak and was used after filtering. Tertiary-butanol (*t*-BuOH) was analyzed reagent grade from Baker.

Aqueous solutions were made from distilled water. The NaOH solution was made with Harleco concentrate and was stored in polyethylene bottles. Reagent grade sulfuric acid from Allied Chemical Co. was used in making up quinine sulfate solutions, and these were stored in glass. Quinine sulfate was from Merck and Co. Some was used as purchased and another sample had been recrystallized by Fletcher (*3*).

Uranine (or sodium fluorescein) was purchased from Harleco. We purified additional samples in two ways: uranine was recrystallized from isopropanol and ether; or fluorescein diacetate was prepared, recrystallized several times, hydrolyzed to the sodium salt, and the fluorescein precipitated in its acid form [method of Orndorff and Hemmer (*12*)]. These samples gave results similar to the original. 9,10-Diphenylanthracene (DPA) was purchased from Aldrich Chemical Co. and was used without purification.

Table I. Substituted 9,10-Bis(phenylethynyl)anthracenes and -9,10-Anthradiols

Position, no., and kind of substituents	Anthradiols[a]		Anthracenes	
	Mp, °C	Formula	Mp, °C	Formula
2-Ethyl	166–167[b]	$C_{22}H_{24}O_2$	171–174[c]	$C_{32}H_{22}$
2-*t*-Butyl	170–171[d]	$C_{34}H_{28}O_2$	147–148.5[e]	$C_{34}H_{26}$
1,4-Dimethyl	204–207[b,f,g]	$C_{32}H_{24}O_2$	176–177[b]	$C_{32}H_{22}$
1,4,5,8-Tetramethyl	275–280[b,h]	$C_{34}H_{28}O_2$	[i]	
1,4,6,7-Tetramethyl	199–200[j,k]	$C_{34}H_{28}O_2$	230–231[b]	$C_{34}H_{26}$
1,4,6,7-Tetramethyl (**meso**)	271–273[d]	$C_{34}H_{28}O_2$		
1,5-Diethoxy	191–192[j,l]	$C_{34}H_{28}O_4$	213–215[c]	$C_{34}H_{26}O_2$
1,5-Diethoxy (**meso**)	256–258[m]	$C_{34}H_{20}O_4$		
1-Phenyl	114–117[d]	$C_{36}H_{24}O_2$	192–193[b]	$C_{36}H_{22}$
1,4-Diphenyl	185–190[j]	$C_{42}H_{28}O_2$	237–238[j]	$C_{42}H_{26}$
1-Fluoro	190–192[b]	$C_{32}H_{19}FO_2$	232–233[b]	$C_{30}H_{17}F$

[a] Except where indicated, these diols are probably a mixture of **dl** and **meso**-epimers. [b] Recrystallized from benzene. [c] Recrystallized from cyclohexane. [d] Recrystallized from ethanol. [e] Recrystallized from cyclohexane–hexane. [f] This diol formed a very stable monodioxanate. [g] The monoadduct (phenylacetylene: dimethylanthraquinone, 1:1) melted 179.5–180.5° after recrystallization from cyclohexane. [h] The monoadduct (phenylacetylene: tetramethylanthraquinone, 1:1) melted 249–250° after recrystallization from benzene. The infrared spectrum showed both OH (3420 cm^{-1}) and CO (1645 cm^{-1}). [i] Conversion of the anthradiol to the substituted anthracene in the usual manner gave a material which was easily soluble in benzene or cyclohexane; solutions had an intense orange-red fluorescence. However, both the color and fluorescence rapidly disappeared from solutions exposed to air and light. Analysis of the recovered product suggested a photodioxide. [j] Recrystallized from cyclohexane–benzene (8:2). [k] The monoadduct melted 191–193° after recrystallization from benzene. [l] The monoadduct melted 160–161° after recrystallization from cyclohexane. [m] Recrystallized from toluene.

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Instrumentation and procedures. All the measurements were done with a Turner Model 210 spectrofluorometer, Serial No. D110. Fletcher (*6*) reported earlier fluorescence studies with the same instrument. The precision with which emission peak areas can be repeated is about the same now as when he used the instrument. Thus, a σ of about 3% can be obtained by a reasonable number of repetitions.

This instrument permits one to measure absorbances with the same monochromator and slit as are later used for excitation in fluorescence studies. This is valuable when exciting on a narrow peak where absorbance depends upon slit width.

Stock solutions were prepared with at least 30 mg of sample so that the error in mass concentration is less than 1%. Solutions were all stored in glass or teflon containers in the dark.

Absorbance solutions were prepared by diluting the stock solutions by weight. The dilutions were made to give optical densities at the excitation peak of 0.7, which again means a 1% precision. Absorbance solutions were about 20 μM for the BPEA's and somewhat higher for uranine and quinine sulfate.

Absorbance solutions were diluted carefully by weight to give "fluorescence" solutions with calculated optical densities of less than 0.01 for most cases. The fluorescence solutions were about 0.1–0.2 μM for the BPEA's. These solutions were used to obtain fluorescence emission spectra. Two such solutions were compared to obtain relative quantum yields. Generally, one solution for such comparisons was of quinine sulfate.

Fluorescence solutions in two cuvets, A and B, were run in the order A, B, A as a check on the constancy of the fluorometer with time. Only if the two runs of A were equal within 3% were the spectra used to calculate quantum yields as described in the following section.

The absorbances or optical densities of the absorbance solutions were measured in matched cells. The base lines were set by filling both cells with solvent. The absorbance at the wavelength of excitation was measured with the monochromator scan stopped.

Quenching by oxygen was looked for by a simple two-point test. A cuvette with a teflon stopper was filled with a fluorescence solution in a box filled with forming gas (97% N_2; 3% H_2). This cuvette was removed from the box, and a spectrum taken in the fluorometer. Then the teflon stopper was removed, air was shaken into the solution, and the spectrum run again. The ratio of the two areas is the Stern-Volmer ratio for quenching by oxygen dissolved. Oxygen partial pressure at China Lake can be calculated from the barometric pressure of 700 torr as 147 torr. The stoppers have been shown to be air tight by several tests. There was a slight question as to whether oxygen affected the absorbance rather than the emission. Tests could only be made at higher concentrations where no effect was seen.

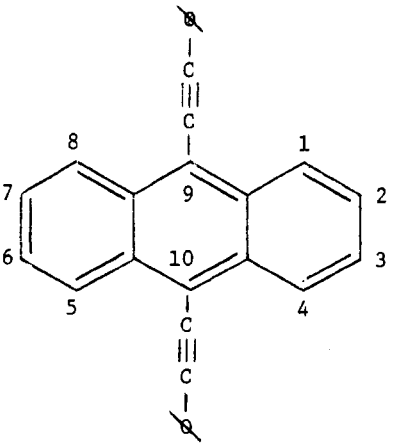

Figure 1. Structure of BPEA

Table II. Spectra of Standards

λ, nm	*A*, rel	λ, nm	*A*, rel	λ, nm	*A*, rel
Quinine sulfate 1.0N H_2SO_4 Absorption (10 nm)		Uranine 0.1N NaOH Absorption (10 nm)		DPA Benzene Absorption (10 nm)	
300	528	420	36	300	0
310	726	440	100	340	323
318.2	823	460	322	350	491
330	800	480	678	357.0	652
340	944	490	985	363.6	605
346.5	1000	491.5	1000	374.6	1000
$\epsilon = 10{,}564\ M^{-1}\,cm^{-1}$		$\epsilon = 89{,}320\ M^{-1}\,cm^{-1}$		$\epsilon = 13{,}390\ M^{-1}\,cm^{-1}$	
350	985	500	757	385	651
360	816	510	243	395	930
370	500	520	43	400	651
380	227	530	0	410	129
400	25			420	11
430	0				

λ, nm	$F_q(\lambda)$, rel	λ, nm	$F_q(\lambda)$, rel	λ, nm	$F_q(\lambda)$, rel
Emission (2.5 nm) Exc, 346.5 nm (10 nm)		Emission (10 nm) Exc, 322 nm (10 nm)		Emission (10 nm) Exc, 385.0 nm (10 nm)	
310	0	470	0	380	0
350	4	480	7	390	39
380	18	490	151	400	423
400	151	495	360	412	993
410	316	500	567	422	914
420	538	505	795	432	1000
430	735	510	950	440	882
440	888	512	1000	450	607
445	935	515	985	460	489
450	965	520	933	470	346
455	990	525	833	480	222
457.2	1000	530	733	490	150
400	998	540	533	500	103
465	979	550	417	550	4
470	951	560	333	600	0
475	916	570	233		
480	871	580	167		
490	733	600	83		
500	616	620	42		
520	408	640	17		
550	171	650	8		
600	19	670	0		
650	3				
700	0				

$\bar{\nu}\ \mu M^{-1}$	$F_q(\bar{\nu})$, rel
Recalcd by Formula 3	
2.5	114
2.4	390
2.3	755
2.2	963
2.151	1000
2.1	969
2.0	728
1.9	461
1.8	211
1.7	67

Quantum Yield Calculations

These were made by use of the following equation:

$$q_x = q_R \frac{A_R \cdot \lambda_R \cdot Q_x \cdot n_x^2}{A_x \cdot \lambda_x \cdot Q_R \cdot n_R^2} \quad (1)$$

Here, subscripts x and R refer to the unknown and reference solutions, q is a quantum yield, A is a calculated optical density, λ is an excitation wavelength, Q is the area under the emission curve which equals $\int F(\lambda)d\lambda$, and n is the index of refraction of the solvent. The wavelength is required in this equation since the Turner fluorometer is corrected for excitation energy (*15*) which must be changed to excitation quanta. The excitation correction on this instrument is good as shown by the lack of change of q for a given solution of quinine sulfate excited at different wavelengths.

The absorbance is calculated from the dilution ratio of the absorbance solution. This assumes that Beer's law holds. Tests of this assumption were made for BPEA at absorbances of 1.5 down to 0.3. We then assumed it held for the other compounds.

Results

Tabulated spectra. The spectra for quinine sulfate, uranine, and DPA are presented in Table II, and those for the BPEA's are given in Table III. The pertinent data for absorbance and emission are listed in the tables. The compound and solvent are listed, and the slit widths are given in parentheses. The absorbance spectra are normalized at the higher peak in the long wavelength band.

The molar absorptivity, ϵ, is given for the higher peak. This is based upon the usual equation:

$$A = \epsilon cd = \log I_0/I \quad (2)$$

Here, A is the absorbance (optical density) read from the Turner in its spectrometer mode of operation. All cells had an optical path, d, of 1 cm.

The emission spectra were obtained in the same solvent as the absorbance. The concentrations were those used for quantum yield runs; 1 μM or lower. Reabsorption and concentration quenching are thus minimized. The emission slit width is given in parentheses. The excitation wavelength and slit width are also given. We think these data are sufficient to allow others to reproduce our conditions and to test the spectra.

For quinine sulfate we have given the spectrum in both wavelength and frequency units. The interrelationship of these units is given by (*11*):

$$\lambda^3 F(\lambda) = \lambda^2 F_q(\lambda) = \lambda F(\tilde{\nu}) = F_q(\tilde{\nu}) \quad (3)$$

Here, $\tilde{\nu} = 1/\lambda$ and the spectral flux $F(\lambda)$ is given in both energy $F(\lambda)$, and quantum $F_q(\lambda)$ units. $F(\lambda) = dF/d\lambda$, i.e., spectral flux is the flux within a wavelength interval. The same holds for $F(\tilde{\nu}) = dF/d\tilde{\nu}$. Flux is in watts through a given surface; quantum flux is in Einstein sec^{-1}. Quantum spectral flux is in Einstein sec^{-1} nm^{-1} or Einstein sec^{-1} $(\mu m^{-1})^{-1}$. All curves are normalized at the peaks.

There is an observation to make about transforming fluorescence curves from wavelength to frequency and the reverse. The position of the peak moves in the transformation except for sharp peaks where $\tilde{\nu}(\text{peak}) = 1/\lambda(\text{peak})$. In a regraphing of data by use of Formula 3, the transformed graph will generally have its peak in a new position. For this reason, we wish Melhuish had presented the original and transformed peak λ and $\tilde{\nu}$ in his table (*11*). As will be seen below, the peak position has a particular importance in comparing spectra.

Graphical spectra. Figure 2 summarizes the qualitative spectral shifts and the quantitative fluorescence yield changes with structure of the BPEA's. All spectra were taken at a 10-nm slit width. The wavelength of excitation was unimportant for the three compounds we tested. That is, we excited in their second band around 310 nm and also in the first band and found no change in their emission spectra nor quantum yields.

The spectra become less structured and more red shifted as the molecular structure is more strained. The substituents in the 2 position show small effect. Substituents in the 1 position strain the ring owing to spatial problems vs. the phenylethynyl group. Substituents in the 1,4 position induce similar but larger strain. In our discussions, Mohan and Rauhut reported similar results with 1,5 and 1,8 substitution. Tetra-substitution in the 1,4,5,8 position by Cl or CH_3 (Table I) shifts the spectra into the red and also makes the molecule unstable. Presumably, the steric strain affects both the singlet energy levels and the reactivity (*7*). The steric effects seem much larger than any electronegativity differences between alkyl and chloro substituents.

Quantum yields. Table IV presents the results of our relative fluorescence quantum yield studies. All values are relative to quinine sulfate in 1.0*N* H_2SO_4 as being 0.55 (*4*). In the case of BPEA in DBP and *t*-BuOH, the actual comparison was with BPEA in benzene.

Concentrations were kept low so that the absorbance at the excitation wavelength was less than 0.01. Twofold variations in absorbance such as from 0.008 to 0.004 showed no effect with quinine vs. BPEA. Solutions were

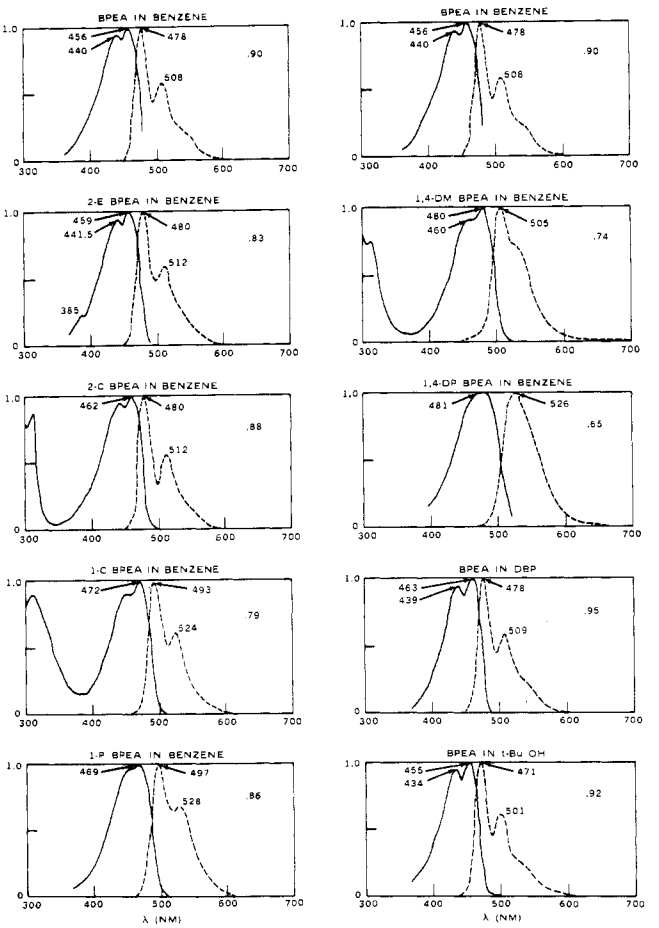

Figure 2. Spectra of BPEA's
These absorbance and emission spectra and quantum yields are meant to give a picture of change with molecular structure and solvent. Numbers are slightly different from those reported in tables which are preferred. Abbreviations are given in text

Table III. Spectra of BPEA's

BPEA Benzene Absorption (10 nm)

λ, nm	A, rel
360	55
400	344
420	652
440	938
448	907
457	1000
$\epsilon = 32{,}900\ M^{-1}\ cm^{-1}$	
470	808
480	185

Emission (2.5 nm) Exc, 440.0 nm (10 nm)

λ, nm	$F_q(\lambda)$, rel
450	9
460	111
470	703
475	909
478	1000
480	870
490	425
495	370
500	425
508	555
510	537
520	333
530	240
540	203
550	166
570	79
700	0

2-EBPEA Benzene Absorption (10 nm)

λ, nm	A, rel
390	221
400	326
410	467
420	634
430	845
440	937
441.5	944
448.7	910
459	1000
$\epsilon = 32{,}300\ M^{-1}\ cm^{-1}$	
460	994
470	845
480	281
490	28

Emission (10 nm) Exc, 440 nm (10 nm)

λ, nm	$F_q(\lambda)$, rel
450	11
460	99
470	516
480	1000
490	703
500	484
512	604
520	495
530	341
540	264
550	220
560	154
570	104
508	77
590	55
600	44
620	16

2-CBPEA Benzene Absorption (10 nm)

λ, nm	A, rel
300	802
304	769
312	880
320	393
330	86
343	44
360	70
370	96
390	237
400	314
410	458
420	622
430	822
443	943
450	919
462	1000
$\epsilon = 32{,}000\ M^{-1}\ cm^{-1}$	
470	890
480	381
490	61
500	5
520	0

Emission (2.5 nm) Exc, 443.0 nm (10 nm)

λ, nm	$F_q(\lambda)$, rel
450	0
460	61
470	469
480	1000
490	571
500	408
512	571
520	449
530	286
540	225
550	204
560	133
570	92
580	61
650	0

1-CBPEA Benzene Absorption (10 nm)

λ, nm	A, rel
400	208
420	471
430	619
440	818
451	905
458	897
472	1000
$\epsilon = 36{,}500\ M^{-1}\ cm^{-1}$	
480	865
490	354
500	62
510	8
520	0

Emission (2.5 nm) Exc, 451.0 nm (10 nm)

λ, nm	$F_q(\lambda)$, rel
450	0
470	57
480	341
490	976
492.5	1000
500	841
510	523
515	500
520	568
526	614
530	591
540	409
550	295
560	227
570	182
600	68
630	23
650	0

1-PBPEA Benzene Absorption (10 nm)

λ, nm	A, rel
360	76
380	115
400	232
410	359
420	505
430	680
440	864
450	956
460	963
469	1000
$\epsilon = 30{,}900\ M^{-1}\ cm^{-1}$	
470	994
480	812
490	346
500	54
510	8
520	0

Emission (10 nm) Exc, 469 nm (10 nm)

λ, nm	$F_q(\lambda)$, rel
460	0
470	60
480	380
490	94
495	1000
500	880
510	640
517	580
520	620
526	640
530	620
540	480
550	360
560	280
570	220
580	160
600	80
630	20
650	0

1,4-DMBPEA Benzene Absorption (10 nm)

λ, nm	A, rel
300	825
308	735
312	743
320	593
330	300
340	153
350	93
366	60
380	73
390	104
400	153
410	246
420	309
430	498
440	674
450	857
460	911
465	905
482	1000
$\epsilon = 30{,}400\ M^{-1}\ cm^{-1}$	
490	819
495	620
500	377
510	80
520	8
550	0

Emission (2.5 nm) Exc, 460 nm (10 nm)

λ, nm	$F_q(\lambda)$, rel
440	0
480	71
490	393
500	928
505	1000
510	928
520	732
525	696
530	714
535	732
540	714
550	553
560	411
570	321
580	250
590	196
600	161
650	0

1,4-DPBPEA Benzene Absorption (10 nm)

λ, nm	A, rel
400	177
410	241
420	345
430	482
440	647
450	819
460	939
470	984
480	1000
$\epsilon = 31{,}600\ M^{-1}\ cm^{-1}$	
490	931
500	647
510	283
520	75
530	0

Emission (10 nm) Exc, 481 nm (10 nm)

λ, nm	$F_q(\lambda)$, rel
450	0
480	9
490	73
500	310
510	710
520	964
524	1000
530	982
540	818
550	764
560	673
570	527
580	418
590	336
600	282
640	55
700	0

BPEA DBP Absorption (10 nm)

λ, nm	A, rel
370	45
380	104
400	298
420	612
439	940
449	880
460	990
463	1000
$\epsilon = 35{,}312\ M^{-1}\ cm^{-1}$	
470	856
480	194
490	0

Emission (10 nm) Exc, 463.0 nm (10 nm)

λ, nm	$F_q(\lambda)$, rel
440	0
450	0
460	70
470	786
478	1000
490	554
496	428
500	446
510	571
520	411
530	286
540	232
550	196
560	125
570	89
580	63
590	36

BPEA *t*-BuOH Absorption (10 nm)

λ, nm	A, rel
370	103
400	404
410	549
420	750
430	912
434	941
440	894
442.5	863
450	978
455	1000
$\epsilon = 34{,}043\ M^{-1}\ cm^{-1}$	
460	956
470	368
480	29
490	0

Emission (10 nm) Exc, 455 nm (10 nm)

λ, nm	$F_q(\lambda)$, rel
430	0
450	52
460	552
468	1000
480	552
485	431
490	474
500	586
510	379
520	259
530	233
540	172
550	121
560	86
580	43
600	26
610	9

Table IV. Quantum Yields in Air

Compound	Solvent	Exc (nm)	Emission peaks (nm)		q^a
Quinine	1.0N H_2SO_4	346.5	457		0.55 ± 0.03
Quinine	0.1N H_2SO_4	346.5	457		0.52 ± 0.04
Quinine	1.0N H_2SO_4	322	457		0.54 ± 0.03
Uranine[b]	0.1N NaOH	492	515		0.92 ± 0.02
Uranine[c]	0.1N NaOH	492	515		0.91
Uranine[c]	0.1N NaOH	465	515		0.94
Uranine[c]	0.1N NaOH	322[d]	515		0.92 ± 0.02
DPA	Benzene	357	432	412	0.67 ± 0.01
BPEA	Benzene	440	477	508	0.84 ± 0.03
2-EBPEA	Benzene	440	480	512	0.88
2-CBPEA	Benzene	443	480	512	0.92 ± 0.09
1-CBPEA	Benzene	451	493	526	0.90
1-PBPEA	Benzene	460	495	525	0.91 ± 0.04
1,4-DMBPEA	Benzene	440	507	~525	0.84 ± 0.01
1,4-DPBPEA	Benzene	479	523		0.80 ± 0.05
BPEA	Benzene	457.5	477	510	0.81 ± 0.05
2-EBPEA	Benzene	310	480	512	0.92
1,4-DPBPEA	Benzene	440	524		0.72
BPEA	DBP	463	479	510	1.00
BPEA	*t*-BuOH	455	468	500	0.88

[a] Ref. *4* suggests 0.55 as the best quantum yield value for quinine sulfate. All other values are referred to quinine sulfate excited at 346.5 nm. The error limit is sigma for a series of runs comparing a quinine sulfate standard sample with different samples of the named compound. [b] Uranine purified by recrystallization from 2-propanol and ether. [c] Fluorescein purified by method of Orndorff and Hemmer (*12*) as recommended by Demas and Crosby (*4*). The pure acid in low concentration was then dissolved in 0.1*N* NaOH to give the sodium salt uranine. [d] In this one case, the quinine sulfate reference sample was also excited at 322 nm rather than at 346.5 nm.

Table V. Air Quenching[a] of Fluorescence

Compound	$q(N_2)/q$(air), measd	$q(N_2)$, calcd
DPA	1.30	0.87
1,4-DPBPEA	1.06 ± 0.02	0.80
BPEA	1.00 ± 0.01	0.84

[a] P(air) = 700 torr; $P(O_2) \simeq 147$ torr.

Table VI. Comparison of Uranine Fluorescence Emission Spectra from Crosby (*3*) and from the Turner 210

λ	Turner / Crosby	λ	Turner / Crosby
490	1.14	520	0.994
495	1.08	525	1.03
500	0.958	530	1.08
505	1.02	540	1.03
510	1.00	550	0.990
512	Peak-Turner	560	0.968
513.3	Peak-Crosby	570	0.932
515	0.992	580	0.923

made and run in one day with uranine and the 1,4-disubstituted BPEA's.

Quinine sulfate showed no changes with excitation at wavelengths of 322 or 346.5 nm. In 0.1*N* H_2SO_4 the yield decreased to 0.52 ± 0.04, which result led us to prefer the 1.0*N* acid for our reference solutions.

The sigma given for BPEA in benzene was obtained for 10 runs over a long period of time. It probably represents the best precision possible with our spectrofluorometer. Single values in Table IV represent only one run.

Fluorescence quenching by oxygen. Three compounds

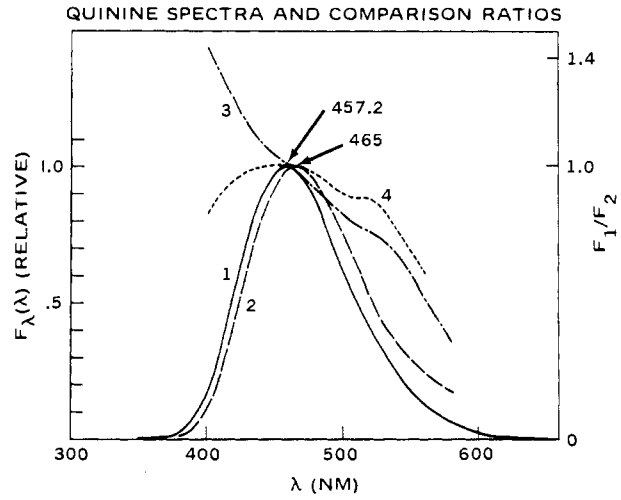

Figure 3. Comparison of quinine sulfate spectra
Curve 1: Quinine spectra from this work
Curve 2: Quinine spectra from Melhuish (*9*)
Curve 3: Ratio of curve 1 to curve 2
Curve 4: Ratio of curve 1 to curve 2 after shifting curve 2 so that its peak is at 457.2 nm

were run in deaerated solutions and with air readmitted (Table V). DPA showed strong quenching with our single value equal to Melhuish's (*10*). BPEA shows no quenching by oxygen. This is rather remarkable for an anthracene derivative as is the fact that it does not react with singlet oxygen molecules. These properties suggest it as a possible fluorescence standard although it does undergo a slow photochemical reaction of some sort. 1,4-DPBPEA did show some quenching by oxygen; although small, this is undoubtedly real.

Solution stability. Quinine sulfate solutions are stable for weeks even if left in a quartz cell in room light. A uranine solution was stable for three days (q = 0.91) but unstable after eight days (q = 0.84). BPEA is stable for a week when kept in the dark; it does react slowly in light, but not to a photoperoxide.

Comparisons with Earlier Data

We will suggest a quantitative technique for comparing fluorescence spectra from different instruments. We have compared our quinine sulfate spectrum using this simple technique with Melhuish's (*9*) tabulated spectrum. The results suggest more effort along this line.

The accuracy of the spectral correction of a fluorometer is of major importance in relative quantum yield measurements. From a tabulation of quantum yields, it is difficult to decide whether the spectral correction, chemical purity, or some other factor causes the differences. If the normalized emission and the peak wavelength are carefully tabulated, one can isolate one factor. Certainly, if two instruments give different spectra for two compounds, the relative quantum yields would be expected to differ. This idea is by no means a new one (*11*), but we have found no quantitative comparisons being made or suggested.

Quinine sulfate spectra. We have made simple quantitative comparison of our spectrum with one of Melhuish's from 1960 (*9*). If both our fluorometers were properly corrected, the ratios of the normalized $F_q(\lambda)$ would be unity everywhere. Figure 3 shows that this is not the case. Curve 3 shows the ratio of our $F_q(\lambda)$ values to his, and the curve obviously wanders far from unity.

Moreover, the peak position influences the shape of the comparison ratio curve. Two spectra otherwise perfectly corrected will give a sloped ratio line if one is shifted slightly in wavelength. Curve 3 looks much like such a

sloped straight line. Therefore, we have calculated curve 4 (also in Figure 3), showing the effect of aligning the spectra at their peaks. It is clear from this that Melhuish's fluorometer and our Turner fluorometer are indeed giving different spectra. Discounting the concentration difference of the quinine sulfate solutions, one or both of the instruments must be badly corrected.

We think that spectra from several instruments should be compared in this fashion. A number of comparison curves nearly flat at unity would give cause for thinking that all the spectra were correct. Our spectra are given in careful tabular form to allow such comparisons. This same idea was expressed by Melhuish in 1960 but does not seem to have been implemented. Perhaps no one else has wanted to throw his spectra open to critical comparisons.

Peak wavelengths. The peak wavelength is important in comparing different spectra. This wavelength varies with mathematical transformations such as sensitivity corrections and wavelength-to-frequency changes. However, there is an interesting difference between sharp and broad peaks in this respect. Sharp peaks such as the mercury lines should fall at the same wavelength, whether the spectrofluorometer is corrected or not. A broad peak like quinine can be shifted several nanometers if the spectrofluorometer sensitivity is changing steeply near the peak. Compounds like BPEA fall intermediate but are probably not shifted on most instruments. A formula might be worked out connecting steepness of sensitivity change, steepness of $F(\lambda)$ vs. λ and $\Delta\lambda$ the shift of the peak.

Of more immediate interest is that the wavelength found for the peak is a measure of the flatness of a corrected instrument or accuracy of a numerical correction. All properly corrected spectra of equal samples should show the same peak wavelength, for this reason, that wavelength is important to report.

In reading the peak wavelength, an automatically corrected fluorometer such as the Turner 210 has an advantage over uncorrected instruments requiring numerical correction. In the former, one can scan slowly and locate the corrected peak within the precision of the monochromator calibration and recorder reproducibility. The latter is only as precise as one is willing to space the data points. The uncorrected scan can only locate a conditional peak. The mathematical correction will move this peak toward the lower sensitivity end of the spectrum. In general, the corrected peak can only fall at a wavelength where one has taken a data point either manually or off a graph.

Owing to the above reasons, we think the review of Melhuish (*11*) should have listed the quinine peaks found by the different authors. Our peak is 457.2 nm. Fletcher (*6*) reported 453 nm on the same instrument, but he had not recalibrated the monochromator with the mercury lamp at the time of taking his spectrum. Melhuish (*9*) reported 465 nm (Figure 3). Velapoldi (*16*) shows about 454 nm on a graph from another Turner instrument.

Berlman (*1*) reports the spectrum of BPEA in graphical form as $E_q(\tilde{\nu})$ vs. $\tilde{\nu}$. The peaks are sharp and probably convert directly to nm by inversion. His values are 471 and 508 nm, as compared to 478 and 510 nm on our Turner. This may be due to a solvent difference since he used cyclohexane. Maulding and Roberts (*8*) report a short wavelength peak of 486 nm for BPEA in benzene.

Quantum yield comparisons. Considering all the spectral problems discussed above, the literature values would not be expected to agree closely. For BPEA, values of 0.96 (*8*) and 1.0 (*1*) have been reported as compared to our value of 0.84 of quantum yield. Our measured value for DPA yield in air is 0.67. We also measured the ratio between fluxes from N_2 and air-saturated solutions as 1.30. This gives us a value of 0.87 as compared to Melhuish's (*10*) 0.84 in deaerated benzene.

There have been many values (*4, 13*) for the uranine yield, with 0.90 considered (*4*) as a good compromise within 5%. Thus, our value of 0.92 is reassuring.

Conclusions

We wish that the spectra and quantum yields reported here could be quantitatively evaluated for accuracy. Instead, we can only place them in the record for future comparison with other people's values and for use as the best available data for the newly reported compounds.

The time is surely approaching when two or more workers can compare fluorescence spectra, as in Figure 3, and get flat comparison curves. Our tabulated data seem to be only the second attempt, since Melhuish in 1960, to provide the basic data for such comparison.

The comparison method shown in Figure 3 may not be the best. It appears to be the only one offered so far except for visual comparison of graphs reproduced in single-column figures in journals (*2, 11*).

Note: Uranine Spectra Comparison

Since writing the above, we have received a corrected fluorescence emission spectrum of uranine from Crosby (*3*). His group has built and calibrated a spectrofluorometer so that they can correct the original point by point spectra. Table VI shows a comparison similar to that in Figure 3. From 490 to 580 nm, the two corrections are reasonably close. The peak wavelengths are also close. The areas under the two normalized spectral curves are nearly equal—Crosby's is about 1–2% larger.

Crosby's original data were at random wavelengths where the flux output had been corrected. We plotted these points and drew a careful graphical spectrum. From this, we read off flux at the wavelengths of our own spectrum. Crosby's original corrected curve can be reconstructed quite well with the comparison data of Table VI and our uranine spectrum from Table II.

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Literature Cited

(1) Berlman, I. B., "Handbook of Fluorescence Spectra of Aromatic Molecules," 2nd ed., Academic Press, New York, N.Y., 1971.
(2) Chen, R. F., NBS Special Publ. 378, 183 (1973).
(3) Crosby, G. A., Washington State University, Pullman, Wash., private communication, 1973.
(4) Demas, J. N., Crosby, G. A., *J. Phys. Chem.*, **75** (8), 991 (1971).
(5) Donner, W., Reid, W., Schlegelmilch, W., *Chem. Ber.*, **94**, 1051 (1961).
(6) Fletcher, A. N., *Photochem. Photobiol.*, **9**, 439 (1969).
(7) House, H. O., Koepsell, D. G., Campbell, W. J., *J. Org. Chem.*, **37**, 1003 (1972).
(8) Maulding, D. R., Roberts, B. G., *ibid.*, **34**, 1734 (1969).
(9) Melhuish, W. H., *J. Phys. Chem.*, **64**, 762 (1960).
(10) Melhuish, W. H., *ibid.*, **65**, 229 (1961).
(11) Melhuish, W. H., *J. Res.*, **76A** (6), 547–60 (1972).
(12) Orndorff, R. W., Hemmer, A. J., *J. Amer. Chem. Soc.*, **49**, 1272 (1927).
(13) Parker, C. A., "Photoluminescence of Solutions," p 266, Elsevier, New York, N.Y., 1968.
(14) Parker, C. A., *ibid.*, p 153.
(15) Turner, G. K., *Science*, **146**, 183 (1964).
(16) Velapoldi, R. A., *J. Res.*, **76A** (6), 641 (1972).

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