Analysis of the Solvent Effect on the Photophysics Properties of 6-Propionyl-2-(dimethylamino)naphthalene (PRODAN)

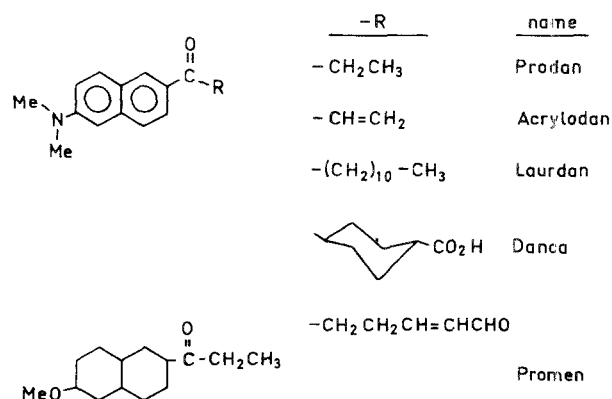Javier Catalan,[1,4] Pilar Perez,[1] Jose Laynez,[2] and F. Garcia Blanco[3]

The absorption and emission spectroscopic properties of 6-propionyl-2-(dimethylamino)naphthalene (PRODAN) have been studied in a large number of protogenic, nonprotogenic, and amphiprotic solvents. The data obtained can be explained by the inclussion of a new term in the Lippert equation which takes into account the acidity of the solvent. This finding indicates that some precaution should be taken when using PRODAN as an indicator of the polarity of protein cavities if the environments involved include acid sites.

INTRODUCTION

The simultaneous presence in a conjugated system of an electron-releasing and an electron-withdrawing group in resonant positions is known to increase the polarity as a result of the electron charge traveling along the system, which leaves the electron donor and acceptor positively and negatively charged, respectively. Such a charge movement is normally more important in, for example, charge transfer (CT) electronic excited states. This movement produces a higher dipolar moment if the two groups occupy relatively distant positions on the molecule. Consequently, electronic jumps from the ground electronic state to an excited electronic state (CT) will be very sensitive to the polarity of the molecule environment and the molecule will make a suitable probe for studying the polarity of the surrounding environment.

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PRODAN, a 6-acyl-2-dimethylaminonaphthalene (Scheme I) introduced by Weber and Farris [1], is a classical example of this type of probe and features a high spectral sensitivity to its environment. On the basis of the effect of temperature on the spectral distribution and fluorescence bandwith of PRODAN–albumin complexes, these authors showed the occurrence of dynamic relaxation of the surrounding protein within 2–4 ns of

-R	name
$-CH_2CH_3$	Prodan
$-CH=CH_2$	Acrylodan
$-(CH_2)_{10}-CH_3$	Laurdan
$\diagup\!\!\diagdown CO_2H$	Danca
$-CH_2CH_2CH=CHCHO$	Promen

Scheme I

the fluorescence lifetime. PRODAN and a chemical variant, DANCA (Scheme I), were used to study the polarity of many protein cavities by spectroscopic techniques [2,3]. The results showed that many protein cavities have a high effective polarity despite the lining of nonpolar amino acid residues [4]. Also, an analogue of PRODAN (R = $-CH_2CH_2CH=CHCHO$; Scheme I) was used to study electrostatic interactions at the binding site of bacteriorhodopsin [5].

PRODAN was used to study the effect of pressure on ligand–protein complexes [6] and on the dielectric constant in phosphatidylcholine lipid bilayers [7,8] and goldfish brain synaptic membranes [8]. More recently Prendergast et al. [9] used one of its chemical modifications, namely, ACRYLODAN (Scheme I), a compound that binds covalently to protein $-SH$ groups with a high specificity and has proved to be very useful to study hydrophobic domains in conformational changes. Finally, Laurdan (Scheme I), another variant of PRODAN, was used to study dipolar relaxation in dioleoyl-L-α-phosphatidylcholine vesicles [10] and dipalmitoyl-L-α-phosphatidylcholine vesicles [11].

Obviously, if full advantage is to be taken of these probes, the factors responsible for the high spectral sensitivity of the chromophore 6-acyl-2-dimethylnaphthalene to the environment must be studied in depth since spectroscopic changes are usually related to polarity changes.

So far, attempts at rationalizing the effect of the solvent on the photophysical behavior of PRODAN have relied on considering the solvent to be a continuous dielectric and hence to be applicable to the Lippert equation [12], which allows calculation of the excited electronic state. By using the Stokes shifts of this chromophore in cyclohexane and water, Weber and Farris [1] estimated dipole variations of 20 D. However, Balter et al. [13] recently reported a μ^* − μ value of 8.0 D, which was obtained by analyzing the Stokes shift measured by Weber and Farris [1] in the solvents benzene, triethylamine, chlorobenzene, chloroform, acetone, dimethylformamide, and acetonitrile.

PRODAN was recently the subject of two theoretical studies [14,15] aimed at identifying the highly polar electronic state responsible for its high spectroscopic sensitivity. The results obtained in this respect showed that, according to X-ray diffraction data, the compound has a planar structure at 50°C; however, no highly polar states were detected. In order to contrast these data with the experimental results, Weber and Farris [1], Novak et al. [14], and Ilich and Prendergast [15] investigated the possibility that the high polarity of PRODAN might result from a twisted intramolecular charge transfer (TICT)

in the excited state arising from the torsion of the $-N(CH_3)_2$ group of PRODAN via a mechanism similar to that proposed for other molecules containing -N-alkyl$_2$ groups [16–18]. The TICT electronic states found by these authors lay at a sufficiently high energy level with respect to the first excited state to be considered photophysically irrelevant. This led them to conclude that a stabilization of the environment was the reason that these states were those of the lowest energies among the possible excited states. Novak et al. [14] estimated this contribution from the solvent by applying the Amos–Burrows theory [19] and concluded that polar solvents place the polar state below the other TICT states, though this was markedly dependent on the Onsager radius used in the calculations. On the other hand, Ilich and Prendergast [15] prompted the need for specific interactions with the solvent in the formation of the highly polar (18.4-D) excited state.

The emitting state of PRODAN has been studied by several authors, who have come to controversial conclusions. Rollington and Drickamer [20] suggest that PRODAN emitted from a CT state in polar solvents but from a locally excited state in nonpolar solvents. However, their lifetime measurements indicated the existence of a single state. Lakowicz and Balter [21,22] and Lakowicz [23] stated that the emission of PRODAN in n-butanol at 218 K was a multistep process, while Heisel et al. [24] reported that the fluorescence of PRODAN in the same solvent over the temperature range from −75 to −24°C was influenced by an interaction between the solute and the solvent capable of inducing a relaxation process of the excited state. Balter et al. [13] pointed out that the first excited state of PRODAN in cyclohexane was a singlet with (n, π^*) connotations, so it could not be compared with the fluorescent state of the compound in polar solvents.

The effect of the solvent on the emission of PRODAN was studied only in broad terms by Weber and Farris [1] in their original work, where they analyzed the changes on emission brought about by 13 solvents.

In order to analyze more deeply the dependency of PRODAN fluorescence on the solvent, we studied 38 solvents including 12 of those used by Weber and Farris [1]; protic, nonprotic, and amphiprotic solvents were used. We also attempted to provide a joint spectral description of the spectral behavior of this chromophore in order to be able to predict more precisely the polarity of the PRODAN environment when used as a biochemical probe.

To help clarify the behavior of PRODAN we have also studied the behavior of PROMEN (6-propionyl-2-methoxynaphthalene) in the same solvents for two mo-

tives. First, the presence of the -OCH_3 substituent group (Scheme I) removes the TICT effect due to the -N-$(CH_3)_2$ group. In this way, we could verify that this effect actually occurs in PRODAN. Second, because the -O-CH_3 group is a poorer electron donor than the -N-$(CH_3)_2$ group, charge transfer will occur to a small extent in this compound, and therefore, it should be less sensitive to the polarity of the solvents. The existence or lack of a relationship in the spectroscopic behavior of the two probes in different solvents would give an understanding of the TICT mechanism in PRODAN. Furthermore, it would allow us to rationalize the effect of the solvents on PRODAN. Finally, we attempted to quantify the chromophore–solvent interactions by using an orientation polarizability term (Δf) and a polarizability term [$f(n_D)$] for the general contributions [12] and the SB (solvent basicity) and SA (solvent acidity) values calculated by thermodynamic analysis [25,26] for the specific contributions of the group of probes, benzene, toluene, pyrrole, and N-methylimidazol for SB [25] and N-methylimidazole and N-methylpyrrole for SA. In this case, we have taken into account the effect of the polarity difference of the two probes for each solvent [26]. The SB and SA terms are similar, in general, to the hydrogen-bond basicities β and acidities α as described by Taft et al. [28].

MATERIALS AND METHODS

PRODAN and PROMEN were from Molecular Probes Inc. (Eugene, Oregon). All solvents used were of the highest available purity and were supplied by Aldrich, Fluka, and Merck. The purity and water contents of the solvents used were checked by gas chromatography.

The absorption spectra were recorded on a 2100 UV-VIS Shimadzu spectrophotometer. The monochromator was calibrated with the 486.0 and 656.1 lines of a deuterium lamp. The corrected fluorescence spectral data were obtained with a phase-modulation spectrofluorometer (SLM 48000). The emission monocromator was calibrated with an Oriel's 6035 Hg (Ar) spectral calibration lamp.

All emission spectra were obtained with λ_{exc} corresponding to the λ_{max} of absorption. When necessary, magic-angle polarization was used for some of the sample measurements. When the solubility of the chromophores was too low, the absorption measurements were carried out with cuvettes of 10-cm path length. All solutions were air equilibrated and measurements were performed at 25°C.

RESULTS AND DISCUSSION

Absorption

The spectroscopic data obtained for PRODAN and PROMEN are collected in Tables I and II. 1

The first absorption band of PRODAN is extremely broad (7700 cm^{-1}) and, as a rule, shows an ill-defined maximum (Fig. 1). For this reason, we used the first derivative, λ_{max}, or—and more appropriately to our minds—the average energy point that would correspond to 90% of the maximum intensity, λ'_{max}, to locate the maximum. The maximum of the first band of PROMEN,

Table I. PROMEN Absorption

	Solvent	λ^{max} (nm)	λ'^{max} (nm)	$\Delta\nu_{0,9}$ (cm^{-1})
1.	Cyclohexane	301.7	301.9	1529
2.	Carbon tetrachloride	305.0	305.0	1643
3.	Triethylamine	302.8	302.1	1697
4.	Ethyl acetate	303.5	303.0	1707
5	Dioxane	304.4	304.3	1708
6.	Toluene	306.0	305.0	1729
7.	Benzene	306.3	305.5	1712
8.	Acetone	—	—	—
9	2-Butanone	—	—	—
10.	Fluorobenzene	307.2	305.8	1689
11.	Propionitrile	304.8	304.4	1726
12.	Acetonitrile	305.0	304.4	1747
13.	Tetramethylurea	307.5	305.7	1797
14.	Cyclohexanone	—	—	—
15.	Anisole	—	—	—
16.	Hexamethylphosphoramide	307.8	306.7	1678
17.	Chlorobenzene	309.2	307.5	1618
18.	N,N-Dimethylformamide	307.8	306.6	1701
19.	Dichloromethane	308.1	306.7	1700
20.	Water	311.1	310.2	1931
21.	1,2-dichlorobenzene	310.0	308.6	1637
22.	Chloroform	309.6	308.3	1661
23.	Pyridine	—	—	—
24.	Benzonitrile	310.0	309.0	1591
25.	1-Octanol	308.9	308.1	1790
26.	Dimethyl sulfoxide	309.6	308.3	1693
27.	2-Propanol	309.4	307.8	1846
28.	Methanol	308.5	307.4	1734
29.	2,2,2-Trifluoroethanol	309.9	309.3	1765
30.	1-Propanol	308.8	308.2	1726
31.	1-Butanol	309.4	308.2	1788
32.	Ethanol	308.2	308.1	1832
33.	Cyclohexanol	310.4	309.6	1720
34.	Formamide	311.8	311.1	1714
35.	Ethylene glycol	311.2	310.6	1843
36.	Pyrrole	312.3	310.5	2072
37.	2,2,2-Trichloroethanol	316.4	315.8	1683
38.	Nitromethane	—	—	—

Table II. PRODAN Absorption

Solvent	λ_{max} (nm)	$\lambda'_{max\,0.9}$ (nm)	$\Delta\nu_{0.9}$ (cm^{-1})
1. Cyclohexane	343.2	341.6	1336
2. Carbon tetrachloride	344.6	342.1	1904
3. Triethylamine	344.0	342.6	1405
4. Ethyl acetate	347.8	347.8	1627
5 Dioxane	347.8	348.0	1420
6. Toluene	348.7	348.9	1559
7. Benzene	349.6	350.2	1491
8. Acetone	350.8	351.7	1826
9 2-Butanone	352.2	352.7	2431
10. Fluorobenzene	350.5	352.4	1738
11. Propionitrile	351.4	352.6	1928
12. Acetonitrile	352.2	352.9	1972
13. Tetramethylurea	353.6	353.7	1877
14. Cyclohexanone	353.0	354.8	1904
15. Anisole	352.5	355.0	1855
16. Hexamethylphosphoramide	354.2	355.0	2218
17. Chlorobenzene	353.6	355.1	1719
18. N,N-Dimethylformamide	355.2	357.4	2080
19. Dichloromethane	353.9	357.9	2590
20. Water	356.8	358.0	2712
21. 1,2-dichlorobenzene	355.4	359.3	2073
22. Chloroform	354.9	359.5	2187
23. Pyridine	355.9	359.9	2166
24. Benzonitrile	355.8	360.7	2194
25. 1-Octanol	358.0	361.5	2611
26. Dimethyl sulfoxide	358.5	361.8	2378
27. 2-Propanol	360.5	362.0	3120
28. Methanol	361.4	363.2	2576
29. 2,2,2-Trifluoroethanol	373-367	363.3	3740
30. 1-Propanol	360.6	363.6	2536
31. 1-Butanol	362.0	363.7	2520
32. Ethanol	362.4	364.6	1998
33. Cyclohexanol	364.7	365.3	2564
34. Formamide	374.1	369.2	2694
35. Ethylene glycol	375.8	370.8	2634
36. Pyrrole	374.8	371.6	2673
37. 2,2,2-Trichloroethanol	387.2	383.6	1921
38. Nitromethane	—	—	—

while broad, is well defined and thus easy to locate. PROMEN also featured consistent λ_{max} and λ'_{max}. The greatest deviation corresponded to the tetramethylurea and was only 1.8 nm. On the other hand, we should note that the width at 0.9 I_{max}, namely, $\Delta\nu_{0.9}$ was virtually constant at about 1700 cm^{-1} in all the solvents studied, while in PRODAN it varied between 1300 cm^{-1} in cyclohexane and 3700 cm^{-1} in trifluoroethanol. The maximum was so flat in some cases that its location was occasionally rather imprecise, even if the first derivative was used. This is the basic reason why the whole discussion on the position of the absorption maxima is referred to the λ'_{max} values.

Figure 2 shows the values of the maxima of the first absorption band of PROMEN and PRODAN. As can be seen, the two are proportional by a factor of 2.4. We should note the occurrence of two outstanding deviations (carbon tetrachloride and water). In principle, an analysis of the absorption spectra appears to suggest that the second absorption band of PRODAN in these solvents might overlap with the first, so the maxima of the latter in these solvents should lie at a higher wavelength. This might account for the deviations observed in Fig. 2. On the other hand, the proportionality also shown in Fig. 2 indicates that the first absorption band of PRODAN is about 2.4 times as sensitive to the effect of the solvent compared to that of PROMEN in the same solvent.

The general effect of the solvent on the absorption is defined by the Lippert equation [12]

$$\bar{\nu}_a = -(\mu^* - \mu)\mu\frac{2}{hca^3}\Delta f -$$
$$(\mu^{*2} - \mu^2)\frac{2}{hca^3}f(n_D) + (\bar{\nu}_a)_0 \quad (1)$$

where μ and μ^* are the dipole moments of the ground and excited state, respectively,

$$\Delta f = \frac{\epsilon - 1}{2\epsilon + 1} - \frac{n_D^2 - 1}{n_D^2 - 1}; \quad f(n_D) = \frac{n_D^2 - 1}{n_D^2 - 1}$$

a is the Onsager cavity radius and $(\bar{\nu}_a)_0$ is the frequency corresponding to the vacuum transition.

The experimental results suggest the occurrence of specific interactions between some solvents and PRODAN, the chemical structure of which includes no acid group liable to change in the electron transition. However, PRODAN does have basic sites (the carbonyl group, the tertiary amine group), as does PROMEN (the methoxy group), that can undergo significant changes with the electron transition. Therefore, electron transitions in these chromophores may be markedly dependent on the acidic nature of the solvents. It thus seems sensible to apply a modified form of Eq. (1), which includes a term representing the SA [26], to the absorption of these chromophores (Table III).

The energy of the first absorption band of PROMEN (kK) conforms to the equation

$$\bar{\nu}_a = -1.43\,\Delta f - 9.18\,f(n_D) - 1.48\,\text{SA}$$
$$+ 34.99$$
$$(n = 30; \quad r = 0.96; \quad \sigma = 0.07) \quad (2)$$

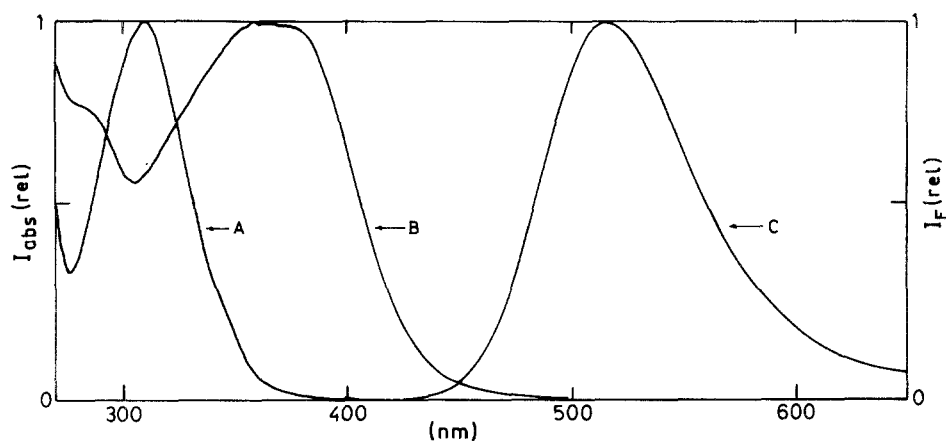and excluding the acidity term SA (Table III), $r = 0.75$.

Fig. 1. Absorption spectra of PROMEN (A) and PRODAN (B) in 2,2,2-trifluorethanol. Corrected emission of PRODAN in the same solvent (C).

The PRODAN conforms to

$$\tilde{v}_a = -4.21\,\Delta f - 16.99\,f(n_D) - 3.31\,SA + 32.85$$

$$(n = 34; \quad r = 0.96; \quad \sigma = 0.16) \quad (3)$$

and excluding the acidity term SA (Table III), $r = 0.72$.

Equations (2) and (3) provide an accurate description of the experimental data in terms of features of the solvent and allow us to quantify the different contributions of the solvent to S_0-S_1 transitions in these chromophores and also account for the proportionality observed in Fig. 2 as a result of the fact that the three contributions increase by roughly the same factor (2.9, 2.0, and 2.2) in passing from PROMEN to PRODAN. According to Eq. (3), the data listed in Table III for carbon tetrachloride and water should correspond to higher wavelengths (346 and 361 nm, respectively), so they would not deviate significantly as in Fig. 2.

The behavior of the S_0-S_1 transition in PRODAN and PROMEN is also indicative of the absence of any discriminating action on the methoxy or tertiary amino groups of some solvents (e.g., the typical complexes formed between tertiary amines and halogen-containing solvents).

The higher solvent sensitivity of PRODAN compared to PROMEN can logically be ascribed to the greater releasing capacity of the -NMe$_2$ group compared to the methoxy group and the fact that such a capacity increases in jumping to the Frank–Condon (FC) excited state, which will increase the basicity of the carbonyl group and the dipole moment (μ); this in turn will result in a greater relative increase in the specific and general interactions of the solvent on the S_0-S_1 transition of PRODAN com-

pared to that of PROMEN, consistent with the situation depicted in Fig. 2.

EMISSION

Table IV lists the fluorescence emission data of PRODAN. Note the consistency between λ_{max} and λ'_{max}. Also, the width $\Delta\tilde{v}_{0.9}$ is practically constant (1100 cm^{-1}) and significantly smaller than that of the absorption band. In summary, the uncertainty inherent in this absorption value does not exist in its emission counterpart, which indicates that the emission transition value is more appropriate for characterizing this probe against any solvent.

On the other hand, at room temperature, the fluorescence lifetime of these species is long enough for the FC state formed in the absorption process to undergo the appropriate solvent ordering [1,21,27] and emit from the equilibrium situation. One can thus wonder about a potential proportionality between the absorption and the emission transition energies of PRODAN in the solvents studied. As shown in Fig. 3 there is a trend in this respect, but with marked deviations, particularly in halogen-containing solvents. According to Lippert [12] the general solvent effect can be described by Eq. (4):

$$\tilde{v}_f = -(\mu^* - \mu)\mu^*\frac{2}{hca^3}\Delta f$$

$$- (\mu^{*2} - \mu^2)\frac{2}{hca^3}f(n_D) + (\tilde{v}_f)_0 \quad (4)$$

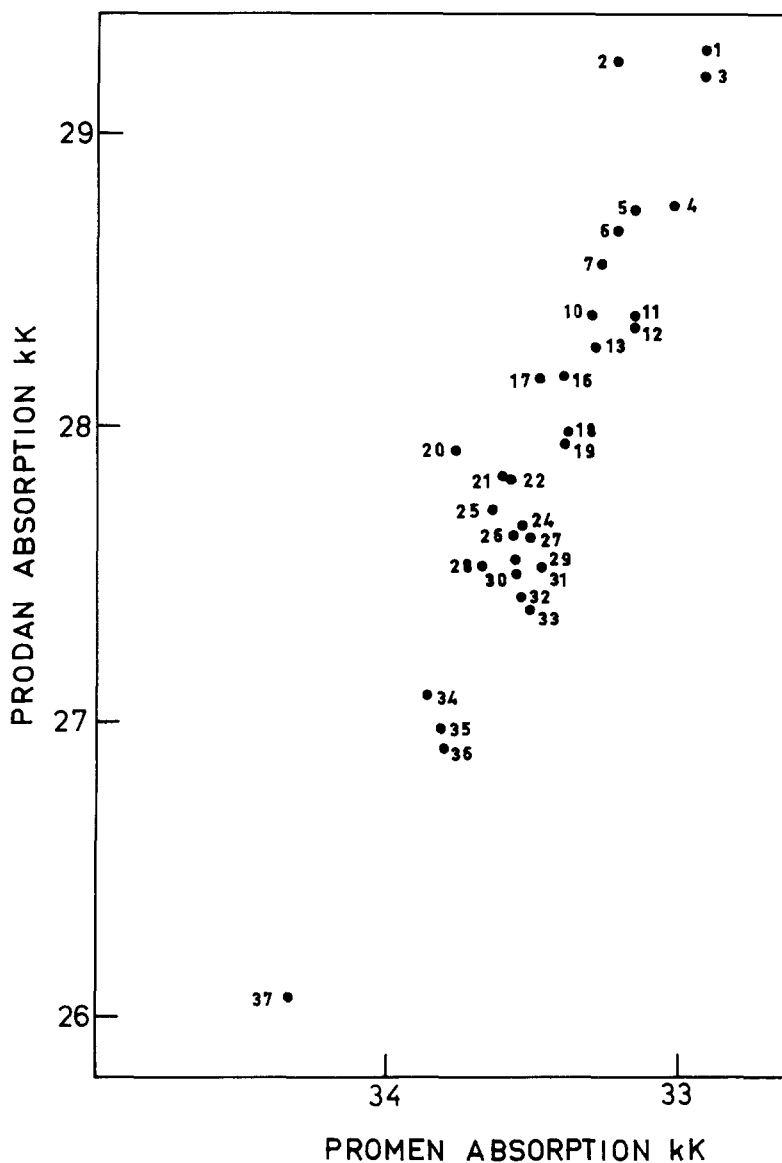However, if the effect of the solvent acidity is also con-

Fig. 2. Absorption maxima of PRODAN vs absorption maxima of PROMEN in the different solvents studied (see Tables I and II).

sidered (Table III), the data in Table IV can be analyzed to obtain the following:

$$\bar{\nu}_f = -10.38\,\Delta f - 11.35\,f(n_D) - 7.97\,SA + 27.76$$

$$(n = 34; \quad r = 0.96; \quad \sigma = 0.42) \quad (5)$$

and excluding the acidity contribution term SA (Table III), $r = 0.76$; i.e., as expected, there is a substantial increase, both in the acidity and in the polarity term. Equation (5) allows one to quantify the effect of the solvent on the emission of PRODAN and show for the

first time that it is highly sensitive to the solvent acidity. This finding is rather significant, as it may change views on this polarity probe.

Likewise, the lack of outstanding deviations between the polar and the nonpolar solvents in Fig. 3 contradicts the interpretation of the emission of PRODAN via TICT states [3,8,14,15,20].

On the other hand, the appropriate description of cyclohexane, both in absorption, Eq. (3), and in emission, Eq. (6), refutes the assumption of the first singlet in this solvent having (n, π^*) connotations as suggested by Balter et al. [13] and should therefore be excluded

Table III. Polarizability Terms and Solvent Acidity[a]

	Solvent	Δf	$F(n_D)$	SA
1.	Cyclohexane	0.002	0.204	0.0
2.	Carbon tetrachloride	0.0011	0.215	0.08
3.	Triethylamine	0.0048	0.195	0.07
4.	Ethyl acetate	0.200	0.184	0.08
5	Dioxane	0.021	0.202	0.13
6.	Toluene[b]	0.012	0.227	0.10
7.	Benzene[b]	0.003	0.227	0.14
8.	Acetone	0.284	0.180	0.07
9	2-Butanone	0.273	0.187	0.06
10.	Fluorobenzene	0.155	0.218	0.05
11.	Propionitrile	0.288	0.164	0.05
12.	Acetonitrile	0.305	0.175	0.07
13.	Tetramethylurea	0.257	0.211	0.07
14.	Cyclohexanone	0.268	0.192	0.04
15.	Anisole	0.112	0.233	0.10
16.	Hexamethylphosphoramide	0.260	0.215	0.04
17.	Chlorobenzene	0.144	0.233	0.07
18.	N,N-Dimethylformamide	0.275	0.205	0.08
19.	Dichloromethane	0.217	0.203	0.13
20.	Water	—	—	0.30
21.	1,2-dichlorobenzene	0.187	0.242	0.05
22.	Chloroform	0.148	0.210	0.20
23.	Pyridine	0.211	0.230	0.07
24.	Benzonitrile	0.235	0.236	0.05
25.	1-Octanol[b]	0.226	0.205	0.26
26.	Dimethyl sulfoxide	0.264	0.221	0.09
27.	2-Propanol	0.276	0.187	0.22
28.	Methanol	0.308	0.169	0.30
29.	2,2,2-Trifluoroethanol	0.318	0.154	0.56
30.	1-Propanol[b]	0.277	0.187	0.26
31.	1-Butanol	0.263	0.195	0.26
32.	Ethanol	0.289	0.180	0.25
33.	Cyclohexanol	0.235	0.216	0.25
34.	Formamide	0.281	0.212	0.22
35.	Ethylene glycol[b]	0.275	0.205	0.32
36.	Pyrrole	0.183	0.229	0.37
37.	2,2,2-Trichloroethanol	—	—	—
38.	Nitromethane	0.291	0.188	0.15

[a] See Ref. 26.
[b] Data to be published.

Table IV. PRODAN Emission

	Solvent	λ_{max} (nm)[a]	$\lambda'_{max\,0.9}$ (nm)	$\Delta\nu_{0.9}$ (cm^{-1})	Stokes Shift (cm^{-1})
1.	Cyclohexane	392.4	394	1392	3904
2.	Carbon tetrachloride	—	—	—	—
3.	Triethylamine	404.0	404.2	1163	4452
4.	Ethyl acetate	429.9	430.1	1076	5503
5	Dioxane	427.5	428.0	1140	5372
6.	Toluene[b]	416.0	416.6	1036	4658
7.	Benzene[b]	417.2	417.9	1053	4627
8.	Acetone	446.7	447.3	1151	6076
9	2-Butanone	442.9	443.3	1144	5798
10.	Fluorobenzene	424.8	423.3	1277	4758
11.	Propionitrile	449.7	449.8	1134	6130
12.	Acetonitrile	455.6	456.0	1153	6407
13.	Tetramethylurea	447.9	447.8	1160	5946
14.	Cyclohexanone	443.3	443.8	1097	5652
15.	Anisole	428.5	429.3	1057	4878
16.	Hexamethylphosphoramide	451.6	451.9	1131	6035
17.	Chlorobenzene	425.1	426.3	1095	4705
18.	N,N-Dimethylformamide	455.0	455.9	1115	6046
19.	Dichloromethane	439.8	440.1	1156	5219
20.	Water	527.5	527.3	1035	—
21.	1,2-Dichlorobenzene	427.8	429.6	1075	4553
22.	Chloroform	434.3	435.9	1037	4878
23.	Pyridine	447.3	446.9	1071	5412
24.	Benzonitrile	448.1	448.5	1073	5427
25.	1-Octanol	476.4	476.8	1122	6689
26.	Dimethyl sulfoxide	464.5	464.6	1116	6117
27.	2-Propanol	479.8	480.5	1073	6817
28.	Methanol	502.7	502.8	1174	7465
29.	2,2,2-Trifluoroethanol	517.6	517.6	1144	8204
30.	1-Propanol	486.8	487.7	1139	6995
31.	1-Butanol	484.4	485.0	1151	6923
32.	Ethanol	493.0	493.7	1169	7173
33.	Cyclohexanol	475.1	475.0	1121	6320
34.	Formamide	501.8	503.1	1077	7209
35.	Ethylene glycol	513.9	513.5	1148	7497
36.	Pyrrole	498.4	498.8	1109	6865
37.	2,2,2-Trichloroethanol	500.8	502.2	1018	6157
38.	Nitromethane	452.9	455.7	1452	—

from the analysis of Stokes shifts. The seemingly anomalous behavior of this solvent may arise from an erroneous assignation of the emission maximum (401 nm) by Weber and Farris [1].

The ratio coefficient of the Δf term of eqs. (4) and (1), and (6) and (3), allows us to calculate $\mu^*/\mu = 2.5$. This conclusion is independent of the Onsager radius.

According to Lippert [12], the Stokes shift of a chromophore in different solvents behaving as continuous dielectric (general solvent effect) depends only on the orientation polarization according to

$$\tilde{\nu}_a - \tilde{\nu}_f = \frac{2}{hca^3}(\mu^* - \mu)^2 \, \Delta f + cte \qquad (6)$$

However, according to experimental evidence, this shift includes the effect of the SA. Taking into account this effect, we calculated the equation

$$\tilde{\nu}_a - \tilde{\nu}_f = 6.59 \, \Delta f + 4.96 \, SA + 3.79$$
$$(kK) \; (n = 34; \quad r = 0.95; \quad \sigma = 0.35) \qquad (7)$$

and excluding the acidity term SA (Table III), $r = 0.77$.

On the other hand, we should note that, according to Lippert (12), the Stokes shift does not depend on $f(n_D)$.
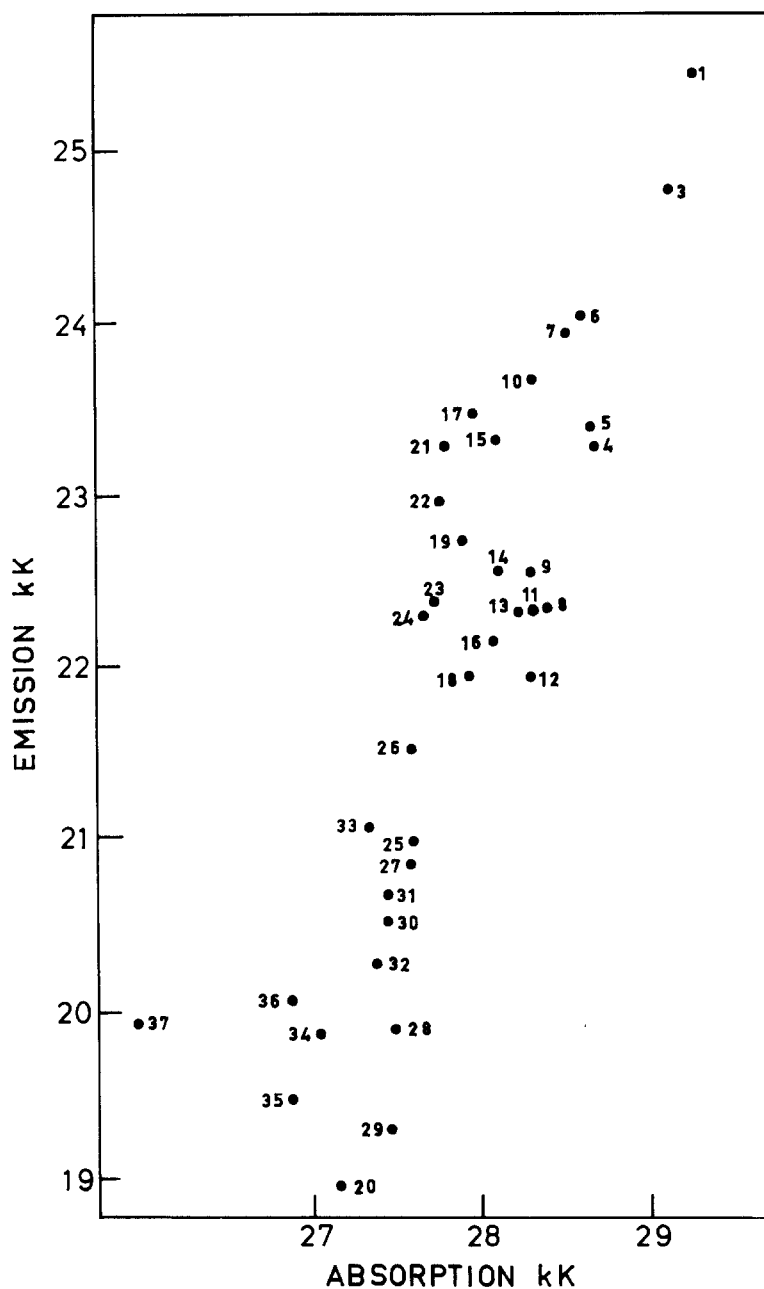
Fig. 3. Emission maxima vs absorption maxima of PRODAN in the different solvents studied (see Tables II and IV).

From the above equations, it follows that

$$\frac{2}{hca^3}(\mu^* - \mu)^2 = (6.59)\ 10^3 \quad (\text{cm}^{-1}) \quad (8)$$

If the Onsager radius is assumed to be 4.2 Å (the value used by Weber and Farris [1] and by Balter et al. [13]), the difference between the dipole moment of the excited state and that of the ground state is 7.0 D.

Weber and Farris [1] reported an independent method for the estimation of the $\mu^* - \mu$ variation from the fluorescence shifts of PRODAN measured in the same solvent at two temperatures yielding the fluorescence from the FC formed by absorption and that from the equilibrium state. These authors showed these situations to occur for PRODAN in propylene glycol at -50 and 20°C and obtained a shift of 2226 cm^{-1}, which, together with

the Onsager radius (4.2 Å), allowed them to estimate $\mu^* - u = 7.79$ D, i.e., consistent with our results (7.0D).

The dipole moment of the excited state is 2.5 times larger than that of the ground state. On the basis of this value and using the same Onsager radius as Weber and Farris [1] and Balter et al. [13] (4.2 Å), we obtained $\mu = 4.7$ D and $\mu^* = 11.7$ D, which are similar to the values of 10.9 recently estimated by Balter et al. [13]. The μ^* moment value determined is also consistent with that calculated theoretically by Novak et al. [14] for the first (π, π^*) singlet of the planar form of PRODAN (12.59 D). In summary, there seems to be no need to resort to electronic states with distorted geometries (e.g., TICT type and related) or to change the nature of the excited state on changing the polar or nonpolar nature of the solvent to rationalize the effect of the solvent on the chromophore PRODAN.

Also, the conclusions drawn by Weber and Farris [1] on the photophysical features of PRODAN based on the ratio, $\mu^*/\mu = 8$ should be reconsidered in view of the new value arrived at in this work, 2.5.

In conclusion, the electron absorption and emission transitions of PRODAN are highly sensitive to the solvent acidity. Therefore, the conclusions arrived at so far in relation to the polarity of protein cavities obtained using this probe should be reconsidered if the environments involved include acid sites. Thus, the extremely significant conclusion [2,3] on the high polarity of the myoglobin heme pocket would also be consistent with the occurrence of N-H sites, a fraction of which would interact with PRODAN by solvating it. The occurrence of acid sites would also account for the heterogeneity observed and the spectral shifts attributed to polar environments. Thus, with pyrrole type N-H sites, the interaction would further stabilize the fluorescence to an extent similar to that reported by MacGregor and Weber [2].

Finally, we should note the inequivocal sensitivity of PRODAN to its environment; this makes it a rather suitable probe for polar and acidic media. No doubt, the best parameter for these characterizations is the fluorescence emission, which covers a wide spectral range (394 nm in cyclohexane and 527 nm in water), as shown in Table IV.

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