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Improvements in Optical Methods for Measuring Rapid Changes in Membrane Potential

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Summary. In an effort to increase the utility of optical methods for measuring membrane potential in excitable cells, an additional 369 dyes were tested on giant axons from the squid. Several promising dyes with relatively large absorption and fluorescence signals are described. In addition, a simple modification of the apparatus led to a sixfold increase in the size of dye-related birefringence signals. In preparations with a suitable geometry, these signals are as large as absorption signals but photodynamic damage and bleaching are eliminated when wavelengths longer than the absorption band are used.

Dye-related absorption and fluorescence signals have provided a new and sometimes powerful method for monitoring membrane potential in a wide variety of preparations (for reviews *see* Cohen & Salzberg, 1978; Waggoner, 1979). Utilizing giant axons from squid as a screening preparation, we had already tested several hundred dyes looking for useful signals (Cohen et al., 1974; Ross et al., 1977). However, the application of optical techniques to neurons of ordinary dimensions remained limited by several interrelated factors: the small size of the signals, pharmacologic effects, photodynamic damage, and dye bleaching. For example, the signal-to-noise ratios were small in experiments on invertebrate neurons in intact ganglia or on tissue cultured mammalian neurons; only large action potentials (> 50 mV) in cell bodies are easily detected in single trials (Salzberg, et al., 1977; Woolum & Strumwasser, 1978; Ross & Reichert, 1979; Grinvald et al., 1981*a*; Grinvald, Ross & Farber, 1981*b*). Measurements of synaptic potentials or measurement of spikes from smaller membrane areas (e.g., growth cones) usually required signal-averaging techniques. Care was needed to avoid pharmacologic effects in experiments monitoring activity that depended on several synaptic relays (Grinvald et al., 1981). Finally, using higher intensity illumination or longer sweeps increases the relative rate of dye bleaching in tissue cultured neurons to such a degree that each record had to be corrected for this effect (Grinvald et al., 1981*a*). We have continued to synthesize additional dyes and attempted to improve the measuring techniques in order to obviate the above problems; our progress is reported here.

Roman numerals are used to refer to dyes in Table 2 of this paper, Table 2 of Ross et al. (1977), and Tables 3 and 4 of Cohen et al. (1974). Arabic numerals refer to dyes listed in the Appendices of the three papers. Preliminary reports have been published (Cohen et al., 1977; Grinvald et al., 1978; Salzberg, 1978, 1979).

Materials and Methods

Giant axons with diameters of 380 to 660 μm were dissected from the hindmost stellar nerves of the squid, *Loligo pealii*, and cleaned of small fibers. The apparatus used for measurements of absorption, birefringence, and fluorescence is shown schematically in Fig. 1; it was similar in most respects to that described previously (Ross et al., 1977). Light from a quartz-halogen tungsten-filament lamp was passed through heat and interference filters (*filter 1*) and focussed to a 5-mm spot centered on the axon. Barrier filters (*filter 2*) passed the emitted fluorescence and prevented scattered incident light from reaching the photodetector in the fluorescence measurement. For the absorption and birefringence measurements, an image of the axon was formed with a microscope objective and a variable slit was positioned in the image plane parallel to the axis of the axon so that only light passing through the axon reached the detector. For birefringence experiments a calcite Glan-

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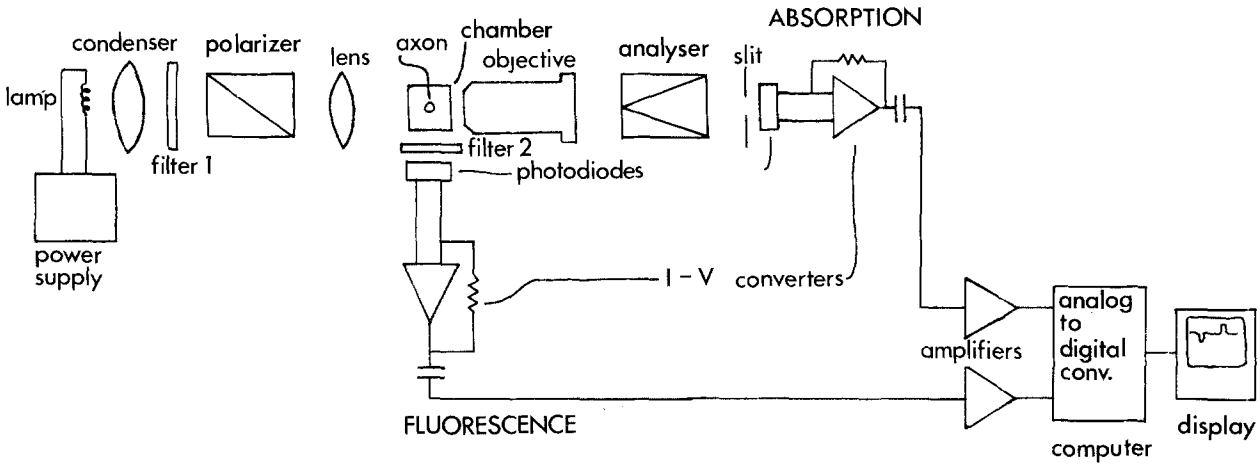


Fig. 1. Schematic diagram of the apparatus used for simultaneous optical and electrode measurements from squid axons. Most of the apparatus was similar to that described previously (Ross et al., 1977); however, the recording system was changed. For most experiments the amplified outputs of both current-to-voltage converters as well as the voltage clamp currents and potentials were fed to the first two channels of two data acquisition cards (600-11-DMA, Adac Corp., Woburn, Mass.) mounted in a PDP 11/34 minicomputer (Digital Equipment Corp., Maynard, Mass.). The clock of one adac card was disabled, and both were driven by the clock from the second card. Both cards were short-cycled to 8 bits, and the two bytes combined into the 16-bit word on a direct-memory-access interface (DR11B, DEC). The two end-of-conversion pulses from the adac cards were passed to an AND gate which then triggered the cycle request of the DR11B. In most experiments, data from each of the four channels was recorded every 24 μsec. For display, digital-to-analog converters were used and the analog signals displayed on a graphics terminal (Tektronix 4012), used as an oscilloscope by means of its analog multiplexor card (Mod. 940C). For some experiments the recording system used a Tracor-Northern TN-1500 digital signal averager

Table 1. Dye sources

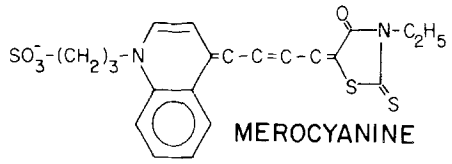
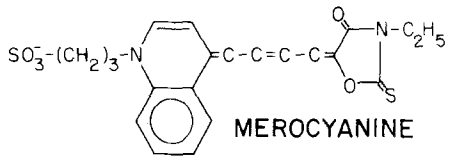
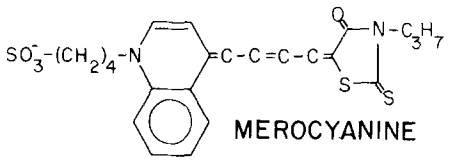
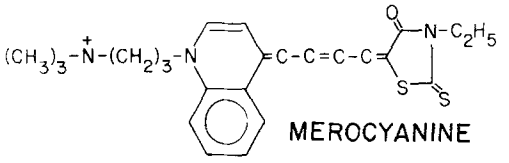
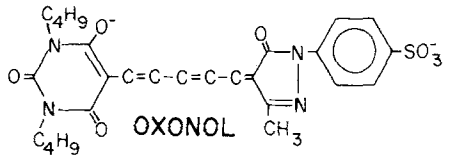
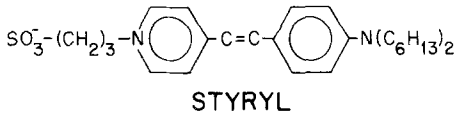
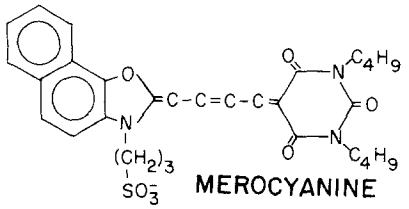
A.	Chroma-Gesellschaft Schmidt & Co.
C.	MC & B Manufacturing Chemists
D.	Allied Chemical Corp.
H.	Synthesized for these experiments
M.	Eastman Kodak Co.
Y.	Dr. B. Chance
AC.	Accurate Chemical and Scientific Co.
AH.	American Hoechst Corp.
EA.	E.R. Atkinson
EK.	Dr. E.M. Kosower
GW.	Dr. G. Weber
KG.	Krongauz and Goldburt
LL.	Dr. L.M. Loew
MP.	Molecular Probes, Inc.
NK.	Nippon Kankoh Shikiso Kenkyusho Co., Ltd.
PS.	Polysciences Inc.
SD.	Searle Diagnostic
WS.	Dr. W. Stewart

Thompson polarizer was inserted in the incident beam and a calcite Ahrens polarizer positioned between the objective and photodetector was used as an analyzer. The polarizer axis was at +45° to the longitudinal axis of the axon, and the analyzer axis was crossed at −45°. These polarizers (Karl Lambrecht Corp., Chicago) were removed from the light path during absorption and fluorescence measurements. Absorption and fluorescence were measured simultaneously; birefringence was measured in separate trials. The field of view of the objective lens restricted the absorption and birefringence measurements to light passing through a 1.5-mm length of axon.

The electrodes and amplifiers used for voltage-clamp experiments were in most instances the same as previously described (Cohen, Keynes & Landowne, 1972; Davila et al., 1974), and compensation for the resistance in series with the membrane was used in some of the experiments. The internal perfusion experiments were carried out using a different arrangement of voltage clamp electrodes and amplifiers, and a horizontal chamber positioned on the stage of a Reichert Zetopan microscope. In these experiments, the internal electrode was of the "piggy-back" type described by Chandler and Meves (1965) consisting of a platinized platinum or platinized platinum-iridium wire 75–100 μm in diameter for passing current and a 75–80 μm pipette filled with 0.57 M KCl for measuring voltage. This pipette also contained an electrically floating 25-μm platinum wire to lower the longitudinal impedance. The currents were guarded. Axons were perfused using a modification (Bezanilla & Armstrong, 1972) of the technique originally devised by Tasaki, Watanabe and Takenaka (1962) but adapted to a chamber designed for optical measurements having top, bottom, and front made of glass. The usual internal perfusion solutions consisted of 350 mM Sucrose, 20 mM HEPES, and either 350 mM KF or 300 mM KGlutamate and 50 mM KF, adjusted to pH 7.3.

In experiments on intact axons several different concentrations of each dye (0.01–1.0 mg/ml) were tried in an effort to find the concentration which gave the largest signal. After incubation with the dye for 10–20 min, the dye solution was usually replaced with seawater that had been bubbled with nitrogen or argon gas. The deoxygenation effectively eliminated any photodynamic damage to the axon. The preparation of the dye solutions has been described previously (Cohen et al., 1974). Of the dyes in Table 2, only dye XXVII required the use of 0.2% Pluronic F127 (a nonionic surfactant, BASF Wyandotte Corp., Wyandotte, Mich.). All dye solutions were prepared just prior to the incubation since standing at room temperature often led to loss of color or to an increase in turbidity. Dyes of the merocyanine-rhodanine group were stored

Table 2. Dyes that have relatively large changes in absorption, birefringence and/or fluorescence when added externally

		Source	Wavelength (nm)	$t_{1/2}$ (sec)	Mode	$\Delta F/F_r$ or $\Delta A/A_r$	S/N
XVII	 MEROCYANINE	H	750	700	abs. biref.	5×10^{-4}	25 15
XXII	 MEROCYANINE	NK2367	720	450	abs. biref.	5×10^{-4}	25 15
XXIII	 MEROCYANINE	H	750	260	abs.	7×10^{-4}	40
XXIV	 MEROCYANINE	H	750	400	abs. biref.	2×10^{-4}	10 6
XXV	 OXONOL	H	640	250	fluor. abs. biref.	10^{-3} 10^{-4}	6 5 3
XXVI	 STYRYL	LL	510	250	fluor. abs.	10^{-3} 5×10^{-4}	5 6
XXVII	 MEROCYANINE	H	570	50	fluor. biref.	10^{-3}	5 5

The source of the dye, the incident wavelength for the largest signal in absorption or fluorescence, the time required for the inward current to be reduced by 50% in oxygenated sea water, the fractional changes and the signal-to-noise ratios are given. Only relatively large signals are indicated. Birefringence measurements were not made for dyes *XXIII* and *XXVI*.

in the dark at 4° C. The incubation of the axon was carried out at temperatures between 20 and 30° C. All the experiments were carried out at room temperature, 20–24 °C.

The sources of the dyes used in these experiments are given in Table 1. The letters following the dye structure in Table 2 and the letters in parentheses in the Appendix refer to the letter and source in Table 1. Small samples of the dyes that we synthesized for these experiments (H) can be obtained by writing to Dr. A.S. Waggoner, Department of Chemistry, Amherst College, Amherst, Mass. 01002. The dyes labeled NK can be purchased from Nippon Kankoh-Shikiso Kenkyusho Co., Ltd., 2–3 Shimoishii 1 Chome, Okayama-Shi, Okayama, Japan. Synthetic procedures used for making two of the dyes labeled H are given below.

Synthetic Procedures

The dyes were synthesized according to general procedures described by Hamer (1964) and Strumer (1977). All dyes decomposed upon melting. When chromatographed on Eastman Chromagram silica gel thin-layer sheets with a variety of solvent systems, each dye showed a single visible spot. The synthetic procedures for the oxonol, XXV, and the merocyanine, XXIII, are described below as examples.

[1,3-dibutylbarbituric acid-(5)]-[3-methyl-l-*p*-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (XXV). To a stirring solution of 3-methyl-l-*p*-sulfophenyl-5-pyrazolone (1.26 g, 5 mmol) and glutaconaldehyde dianilide hydrochloride (1.42 g, 5 mmol) in 150 ml of acetonitrile at room temp. was added triethylamine (2 ml).

After stirring for 1 hr, 1,3-dibutylbarbituric acid (1.4 g, 5.8 mmol) was added to the reaction flask and stirring continued for another 1 hr at room temp. Removal of the solvent under vacuum and washing the residue with ether (50 ml) gave 3.3 g of a semisolid which was column chromatographed over silica-gel (400 g, 60–200 mesh). Elution with acetone (2000 ml) and with mixtures (250 ml each) of acetone and ethanol (2, 5, 7, and 15%) removes most of the higher R_F value impurities. Next the column was eluted with a mixture (2:1) of acetone and ethanol and the eluent collected in fifty 20-ml fractions. Fractions 27 to 39 were combined (after the TLC and absorption spectrum comparison) and afforded 1.37 g (36%) of the bluish red product, mp 192–5 °C, having absorption maximum at 613.5 nm and emission maximum at 638 nm in ethanol.

[3-propylrhodanine-(5)]-[1-δ-triethylammonium sulfobutyl-1,4-di-hydroquinoline-(4)]-tetramethinemerocyanine (XXIII). A mixture of 5-(γ-acetanilidoallylidene)-3-propylrhodanine (0.52 g, 1.5 mmol) and anhydro-4-methyl-l-δ-sulfobutylquinolinium hydroxide (0.4 g, 1.5 mmol) in a mixture of methanol (50 ml and DMF (50 ml) was warmed to 50 °C and to the resulting solution was added triethylamine (4 ml). The reaction mixture was refluxed for 1 hr, solvent removed under vacuum, and the residue column chromatographed over silica-gel (60 g, 60–200 mesh). Elution with acetone (600 ml) and mixtures (250 ml each) of acetone and ethanol (10, 15, and 20%) removed the higher R_F impurities. Elution with a mixture (800 ml) of acetone and ethanol (30%) gave 190 mg (21%) of the product, mp 250–8 °C (softens at 245 °C). Its absorption spectrum in ethanol showed two broad peaks with maxima at 665 and 720 nm.

Results

Birefringence

Because extrinsic birefringence signals in axons exist at all wavelengths longer than the dichroic absorption band, over much of the near infrared spectrum (750–1000 nm) it is possible to measure a birefringence signal even though no photons are absorbed by the dye molecules (Fig. 5, Ross et al., 1977). Since there is no absorption, there can be no bleaching and no photodynamic damage, and thus a birefringence measurement might present considerable advantages over absorption or fluorescence. In previous measurements (Ross et al., 1977), the incident light was passed through two filters, a Shott RG9 which passes most near infra-red wavelengths and a band-pass interference filter with a width at half-height of 30 nm. Because the birefringence signal occurs at all wavelengths in the near infra-red, it was expected that removal of the interference filter would increase the light level and thereby increase the signal-to-noise ratio by reducing the relative effects of shot and dark noise. The results of a birefringence measurement during a single action potential (Fig. 2) using only the RG9 filter show that this expectation was fulfilled. With polarizer and analyzer (Fig. 1) crossed and at 45° to the longitudinal axis of the axon, the birefringence change in a single trial (Fig. 2, top trace) has a signal-to-noise ratio of 19:1 when the observed signal-to-noise ratio was normalized to a 50-mV voltage step and to a response time constant of the light measuring system of 250 μsec. This signal is similar in size to the absorption signal measured with the same dye; thus dye-related birefringence signals can be relatively large. We used the method of de Senarmont-Friedel (Bennett, 1950) to introduce additional retardation; the signal-to-noise ratio was, however, not substantially improved by the adding of either positive or negative retardation.

To show that the birefringence change that occurred during the action potential (Fig. 2) depended on the change in membrane potential rather than the ionic currents or changes in membrane permeability that also occur during action potentials, birefringence was measured during hyperpolarizing and depolarizing voltage clamp steps (Fig. 3). During the hyperpolarizing step where the current density is very small and there was no change in permeability, there was a birefringence change and it had the time course of the change in potential. Even though the current density and permeability changes during the depolarizing step were large, the birefringence change again had a time course similar to the change in potential. When experiments using four different voltage steps were carried out, the size of the birefringence change was linearly related to the size of the potential change over the range ±100 mV from the resting potential. Thus we conclude that this birefringence signal was in some manner dependent on the change in membrane potential.

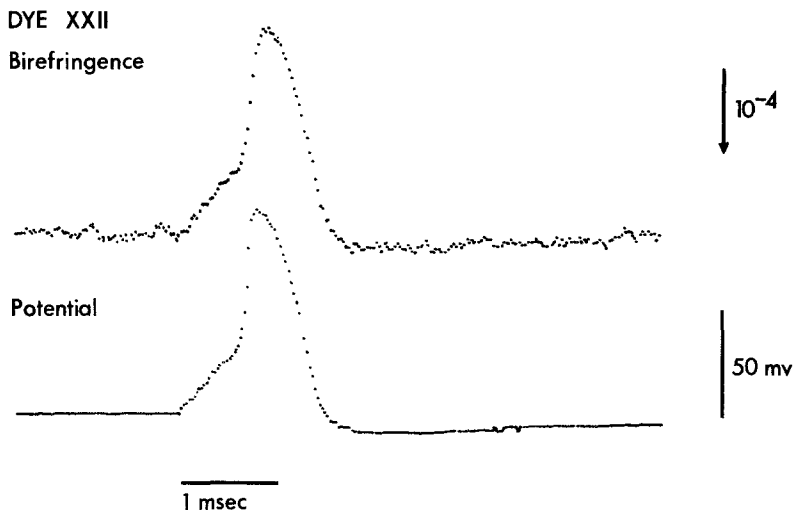


Fig. 2. Birefringence change (upper trace) during a single action potential (lower trace) in a giant axon. The birefringence signal appears slightly slower than the action potential because the high-frequency response time-constant of the light-measuring system was limited to 60 μsec to reduce noise. When measurements were made before the addition of dye, intrinsic birefringence signals (Cohen, Hille & Keynes, 1970) were measured which were about 20 times smaller than this dye related signal. In this and subsequent figures showing birefringence signals, the direction of the vertical arrow to the right of the optical trace indicates the direction of an increase in light intensity; the size of the arrow represents the stated value of the change in intensity in a single sweep, ΔI, divided by the resting intensity, I_r. The axon had been stained for 15 min with a 0.1 mg/ml solution of the merocyanine dye, XXII, in seawater. A Schott, RG9, filter was used in the incident beam for the experiments illustrated in Figs. 2–4. All the records are traced from Tektronix 4631 hard copy recordings of data displayed on the Tektronix 4012 terminal, or from polaroid print enlargements made from the Tracor-Northern signal averager

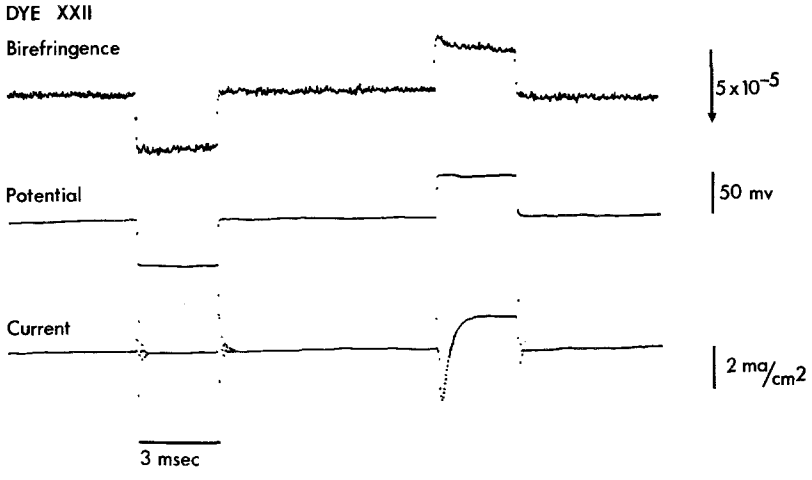


Fig. 3. Birefringence changes (top trace) during 55 mV hyperpolarizing and 45 mV depolarizing voltage clamp steps (middle trace). The current density is shown in the bottom trace. The time course of the birefringence signal was similar to the time course of the potential change. In this and subsequent figures hyperpolarization is represented downward; inward currents are downward. Sixteen trials were averaged. The response time constant of the light measuring system was 20 μsec. The axon had been stained for 20 min with a 0.1 mg/ml solution of the merocyanine dye, XXII, in seawater

In fact, voltage clamp experiments similar to that shown in Fig. 3 were generally used to measure the absorption, birefringence, and fluorescence changes reported in this paper. And, with rare exceptions, the signals during hyperpolarizing and depolarizing steps had similar time courses. Similarly, in most instances where four voltage steps were used, the absorption, fluorescence and birefringence signals were linearly related to potential over the range ± 100 mV from the resting potential. For the absorption signals of the merocyanine dyes, XVII and XXII, larger steps (± 175 mV) were also tested; the signals remained linear. Thus, essentially all of the optical signals described in this paper appeared to depend upon potential in a simple way.

In some applications it may be necessary to use optical methods to monitor potential changes that last much longer than action potentials. For such applications it would be useful to know if the dye signals follow membrane potential for times that are substantially longer than the 3-msec step that was normally used. Absorption measurements with 1000-

msec steps were attempted, but the potential dependent absorption signals were smaller than the intensity changes due to bleaching that occurred over such long times. While the abrupt changes in absorption that occurred at the beginning and end of such steps were approximately equal in size suggesting that the dye signals did follow the longer step, interference from bleaching should be eliminated in a birefringence measurement at infra-red wavelengths. Figure 4 illustrates the results of a birefringence measurement during a 1500-msec hyperpolarizing step using dye XVII. The birefringence signal has a time course that is generally similar to the potential. The differences in the two time courses might result from drift in the optical apparatus, a small component with a slow relaxation time in the birefringence signal, drift in the voltage clamp electrodes, and/or bleaching resulting from light of shorter wave-length that passed through the RG9 filter.

Signal Size

For each dye tested we calculated the signal-to-noise ratio during a single 50-mV depolarizing step using the procedures described in Cohen et al. (1974). The signal-to-noise ratios obtained with several dyes with relatively large signals are given in the last column of Table 2; results with additional dyes are given in the Appendix. Since the signal-to-noise ratio is dependent on the apparatus used in the measurement, we also calculated the fractional change in absorption and fluorescence which is less dependent on the particular experimental arrangement. These values are given for the dyes in Table 2. $\Delta F/F_r$ is the change in fluorescence intensity divided by the resting fluorescence intensity due to the dye, while $\Delta A/A_r$ is the change in transmitted intensity divided by the reduction in resting transmission due to staining by the dye. $\Delta A/A_r$ is equal to $\Delta T/T_{r,\text{before}}$-$T_{r,\text{after}}$). For the dyes in Table 2 with large absorption changes, $\Delta T/T_{r,\text{after}}$ ($\equiv \Delta I/I_r$) was also calculated. The values for XVII, XXII, XXIII, XXIV, XXV, and XXVI were, respectively, 10^{-4}, 10^{-4}, $2\text{–}10^{-4}$, $5\text{–}10^{-5}$, $5\text{–}10^{-5}$, and $5\text{–}10^{-5}$. Thus the values of $\Delta I/I_r$ were between 2 and 10 times smaller than $\Delta A/A_r$. The fractional change in birefringence was not calculated because the change in resting intensity resulting from the addition of the dye was small and varied considerably from axon to axon. For the best dyes the fractional change in light intensity, $\Delta I/I_r$, in birefringence measurements was about 5×10^{-4}, when intact axons were used. In experiments with perfused axons removal of the axoplasm greatly reduced the resting retardation and increased the fractional change (Fig. 4).

The merocyanine dye, XVII, gave the largest signal at the time of our previous report (Ross et al., 1977). (The signal-to-noise ratio reported in Table 2 is smaller than that reported previously; we found that signal size was dependent on the condition of the axon. When the inward current density resulting from a 50-mV depolarizing step decreased from 4 mA/cm^2 to 1 mA/cm^2 the signal size for dyes XVII and XXII increased by 10–30%). The signal-to-noise ratio obtained using the related merocyanine, XXIII, was substantially larger. The peak wavelength for excitation of the oxonol, XXV, is at 640 nm, a wavelength that would allow the use of either a helium-neon or a krypton laser as a light source. Grinvald et al. (1981*b*) used a He-Ne laser as a light-source and an analog of this dye in experiments on tissue cultured neurons. Loew et al. (1979) have presented suggestive evidence that the optical signals from an analog of the styryl, XXVI, were the direct effect of the change in potential gradient on the chromophore (an electrochromic effect). Because of their unusual signals we tried to reinvestigate two dyes which had been studied earlier, J. Red-Brown (69) and Jalapeña Red Chandler (69′) (Ross et al., 1977). Unfortunately, they were found to be degenerate.

While the signal with the positively charged merocyanine, XXIV, was somewhat smaller than that found with the negative analog, XVII, the direction of the signal was the same. The sign of the charge on this pair, as well as four other merocyanines, I, 610, XXIII, and 659 were checked by means of electrophoresis. The electrophoretic results were, in each case, consistent with the given structures. The use of pairs like XVII–XXIV and I-610 might be useful for ruling out charge specific origins for optical signals in situations where electrodes cannot be used to verify that the signals result from membrane potential changes.

Photodynamic Damage and Dye Bleaching

Because photodynamic damage and dye bleaching can cause serious problems in the use of dyes for monitoring membrane potential, these effects were measured for several dyes. The severity of both effects depend on the intensity of the measuring light and the duration of the monitored activity. In experiments where relatively low intensities (tungsten filament sources) and brief durations were used, the difficulties were not serious (Salzberg et al., 1977; Baylor & Chandler, 1978; Hirota, Fujii & Kamino, 1979; Grinvald et al., 1981*a*; Nakajima & Gilai, 1980). However, with higher intensities (laser source focused onto a small area) or longer durations, both photodynamic damage and

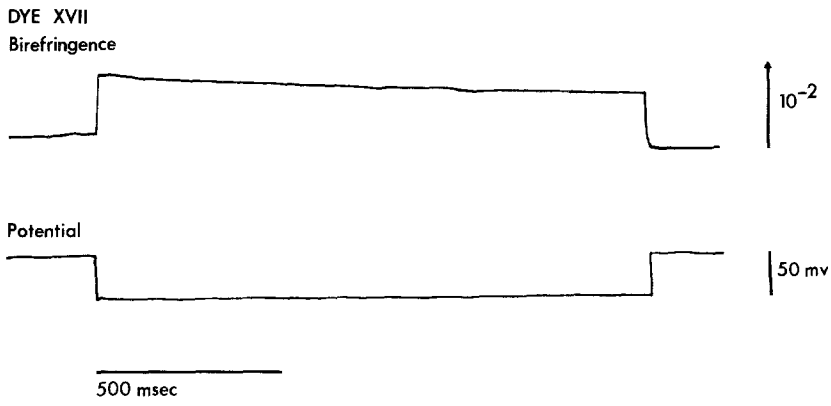


Fig. 4. The birefringence change (upper trace) during a 1500 msec hyperpolarizing step (lower trace). The time course of the birefringence change was similar to the time course of the potential change for this relatively long step. Eight sweeps were averaged. The axon was stained for 10 min with a 0.2 mg/ml solution of the merocyanine dye, XVII, in seawater. The response time constant of the light measuring system was 180 μsec. Similar results were obtained with the merocyanine dye, XXII

bleaching were more obvious (Grinvald et al., 1981*b*; Senseman & Salzberg, 1980).

Photodynamic damage was measured in air-equilibrated seawater with the light intensity that was used to measure the optical signals. The damage was assessed by measuring the interval (kill time) between turning on the light and the time when the peak inward current resulting from repeated 50-mV depolarizations declined by 50%. This time is indicated for the dyes in Table 2 as $t_{1/2}$. Similarly, the bleach time is defined as the interval between turning on the light and the time it took for the optical signal to decline by half. Generally the bleach time was similar to the kill time. The ratio of the bleach time and kill time was calculated for 26 dyes; the median ratio was 1.0 with a range between 0.1 and 5. Dyes with short kill times had bleach times that were longer than the kill time, while dyes with long kill times had bleach times that were shorter. Thus, the five dyes with kill times of less than 50 sec had bleach/kill ratios between 2 and 5 while the 11 dyes with kill times greater than 150 sec had ratios between 0.1 and 1. The kill time for the benzoxazole-merocyanine dye, XXVII, was five times longer than that obtained for the following analogs: I, IX, 137, 610, 615 and 632.

Valenzeno and Pooler (1978) reported that the photodynamic damage with dye I was blocked by incubation with 2, 4, 6-trinitrobenzene sulfonic acid (TNBS). We confirmed their result, finding that 5 mM TNBS slowed the photodynamic damage by a factor of 10. Unfortunately, the signal was also reduced by a factor of about 5. This result suggests that the TNBS may compete with the dye for membrane binding sites.

Spectra

The spectra of the absorption and dichroism signals were remeasured for the merocyanines, XXII and 665, using interference filters with narrower bandwidths (10 nm width at half height). The resulting spectra were more complex than had been realized from earlier experiments using 30 nm filters (Fig. 6, Ross et al., 1977). Figure 5 illustrates the spectra of the changes in absorption for linearly polarized light for an axon stained with dye 665. There are two positive peaks for the perpendicular light and two (or three) negative peaks for parallel light. Similar complexities were observed by Nakajima and Gilai[8] in their experiments with dye XVII on frog muscle. For dye XXII we found even more complex spectra exhibiting four peaks for both planes of polarization. The amount of dichroism found with dye XXII was less than that found with dyes XVII and 665; with XXII the absorption changes were negative for all wavelengths for both planes of polarization. Furthermore, when the absorption and dichroism spectra of the signals with the positive dye XXIV were compared to that found with the negative dye XVII, they were similar but not identical. With the positive dye the corrected signal at 630 nm for light polarized parallel to the axon was larger than the signal at 750 nm, while the converse was true for the negative dye. Thus the spectra of the absorption signals for these merocyanine dyes are complex and they are sensitive to small alterations in dye structure. These complex spectra should be useful in choosing between possible mechanisms for these signals.

Inside Dyes

For some kinds of experiments it may be useful to employ dyes that are added to the inside of cells. One example would be an attempt to monitor potential changes in a dendritic tree that was buried in a neuropil. In this kind of experiment it would be important that the dye be confined to a single neuron to avoid confusion about the origin of the signal.

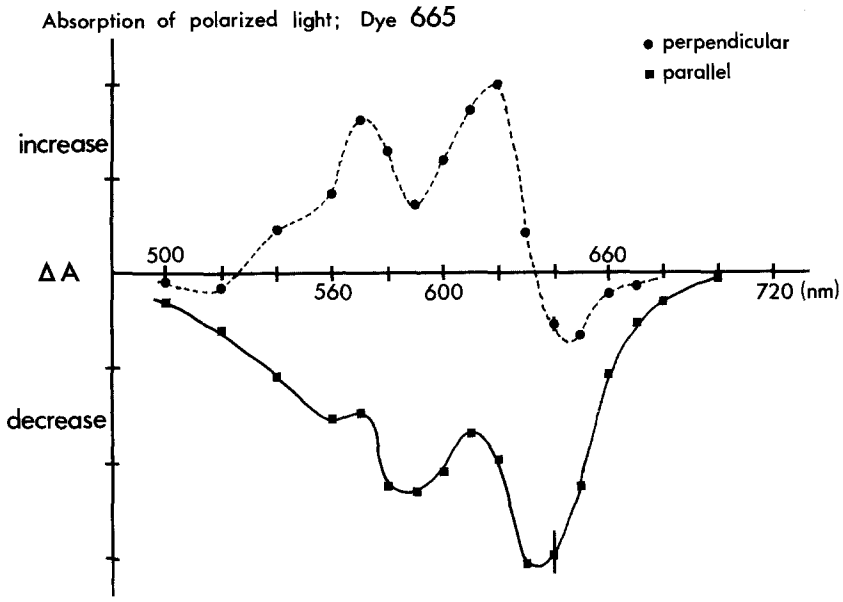


Fig. 5. Absorption changes, using polarized light, as a function of wavelength. The absorption signal with dye 665, the dimethine, 3-hexylrhodanine analogue of dye XVII, is quite dichroic at some wavelengths. The curves are drawn by eye. Repeated measurements at 640 nm were made throughout the experiments, and the decrease with time of these signals (they declined by 47% during 54 measurements) was used to adjust the size of the signals at other wavelengths. The vertical lines through the two 640-nm points indicates the scatter of these values about a linear decay. The axon was stained with a 0.2-mg/ml solution of the dye in seawater plus 0.2% Pluronic F127

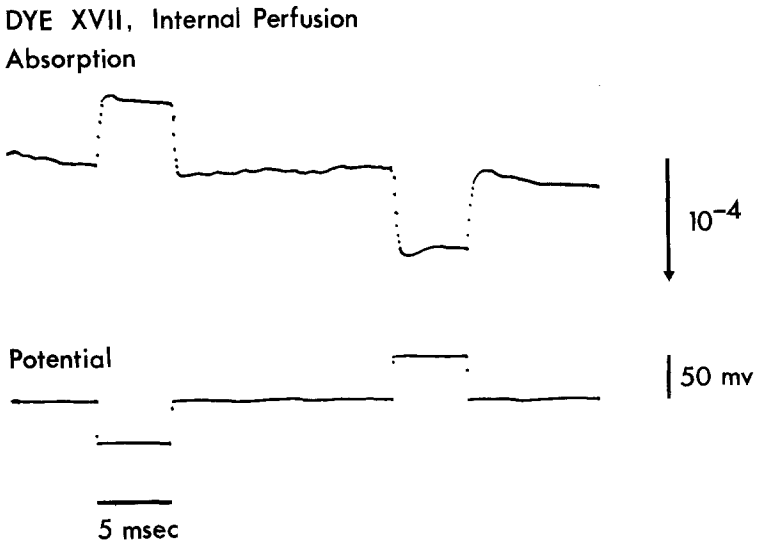


Fig. 6. Absorption changes (upper trace) during voltage-clamp steps (lower trace) from an axon that had been internally perfused for 15 min with a 0.1-mg/ml solution of the merocyanine dye, XVII. Just prior to the experiment, the axon was perfused with a dye-free solution for 1.5 min. The absorption changes of the internally perfused axon were opposite in direction to those obtained with external addition of the same dye (Fig. 3 of Ross et al., 1977). The direction of the vertical arrow to the right of the absorption trace indicates the direction of an increase in light absorption; the size of the arrow represents the stated value of the change in absorption in a single sweep, ΔA ($\equiv \Delta I$), divided by the resting absorption due to the dye. 160 sweeps were averaged. The incident light filter had a peak transmission at 750 nm and a full-width at half-height of 60 nm. The response time constant of the light-measuring system was 180 μsec. The peak inward current density resulting from the depolarizing step was 1 mA/cm^2. The holding potential was −60 mV

Accordingly, some experiments with internal perfusion of dyes were carried out in an attempt to determine which dyes at which concentration are likely to give the largest signals when injected into neurons. Figure 6 illustrates the results of an absorption measurement following internal perfusion with a 0.1 mg/ml solution of dye XVII. Comparison of this result with that obtained when the dye was added externally (Fig. 3 of Ross et al., 1977) shows that the internal signal is opposite in sign but otherwise similar to the external signal, a result consistent with the hypothesis that dye XVII is membrane impermeant.

Five dyes were tried in perfusion experiments. Dye XVII gave the largest signal (absorption signal-to-noise ratio of 5:1 in a single sweep). Other dyes, with signal-to-noise ratios in parenthesis were: XXII (2:1); Lucifer Yellow CH (n.c.); Nile Blue A (0.4, abs; 0.1, fluor); and chlorotetracycline (n.c.); In contrast with the result obtained with dye XVII, the sign of the signals found after perfusion with Nile Blue A were the same as that found upon external addition.

Discussion

The birefringence signals reported here are relatively large and their use would appear to be especially attractive in situations where photodynamic damage and/or dye bleaching cause difficulty. Using a birefringence measurement will be most straightforward when the shape of the object to be monitored is cylindrical and its longitudinal axis is oriented at 45° to the plane of polarization of the incident light. This situation holds for the present experiments and would also be true for an axon in tissue culture. However, when a spherical structure with a radial optic axis (a cell body) is placed between crossed polarizers, it will appear bright in four quadrants and the sign of the birefringence is opposite in the two pairs of diagonally situated quadrants. Birefringence signals from a single barnacle neuron that are opposite in sign in the diagonal quadrants have been obtained (Boyle & Cohen, 1980). Thus, while birefringence signals may be relatively complex in certain measuring situations, in other instances birefringence might be the signal of choice.

While the signal-to-noise ratio in the birefringence experiments on giant axons was not improved by adding additional retardation, in barnacle experiments, introducing additional retardation was required to detect the signals (Boyle & Cohen, 1980). We presume that this difference results from the difference in resting retardation, 56 nm in the axon (Cohen, Hille & Keynes, 1970) vs. less than 15 nm in the barnacle ganglion (Boyle & Cohen, 1980). When the resting retardation is low, the dark noise in the measuring system reduces the signal-to-noise ratio.

Although the birefringence signal-to-noise ratios reported here were five times larger than those found previously, the size of absorption signals increased by less than a factor of two, and the size of fluorescence signals remained constant. While these modest improvements may indicate that we have reached the upper limit for optical signals with the classes of dyes we have investigated most carefully, Waggoner and Grinvald (1977) suggested on theoretical grounds that it might be possible to increase the absorption signal size by up to a factor of 10, and they thought that it should be possible to find much larger fluorescence signals, especially if specific binding to excitable membranes could be achieved. The fact that fractional change in fluorescence of dye XXV in tissue cultured neurons (5×10^{-2}; Grinvald et al., 1981*b*) and single muscle cells (3×10^{-2}; Baylor et al., 1981) is 20 times larger than in squid axons (Table 2) may in part result from the nonspecific binding to Schwann cells and connective tissue that occurs in the axon preparation. The nonspecific binding adds to the resting fluorescence and reduces the fractional change. Because larger signals would provide considerable benefits, we plan to continue synthesis and screening. Furthermore, inspection of the appendix shows that there are four dyes with absorption signals and 22 dyes with fluorescence signals within a factor of two of the largest. Some of these have significantly different chemical structures. We think that the existence of a large number of relatively good probes should be advantageous when attempting to use dyes in experiments where pharmacologic sensitivity is high.

These dye screening experiments have been carried out on giant axons from a decapod mollusc and there is no *a priori* reason to expect that the results can be directly transferred to other preparations. In terms of relative signal size there is no other preparation where dyes have been systematically tested. However, 23 dyes that worked well in squid experiments were tried in absorption measurements on barnacle supraesophageal ganglia; about half gave detectable signals in single trials (Grinvald et al., 1981*a*) and among the dyes that did give signals, the ranking according to size was not identical in squid and barnacle experiments. In addition, the wavelength dependence of the dye XVII signals are different in different preparations (Ross & Reichardt, 1979; Nakajima & Gilai, 1980). Thus, if optimization of signal size is important, it will be necessary to rescreen a selection of dyes on each new preparation. Since the squid axon is surrounded by a Schwann cell, it may be a better model for neurons surrounded by glia than it is for naked cells.

We are especially grateful to Nippon Kankoh-Shikiso Kenkyusho, Co., Ltd., for synthesizing dyes at our request, and we thank Eastman Research Laboratories, Drs. B. Chance, E.M. Kossower, L.M. Loew, W. Stewart and G. Weber for providing dyes used in this paper. We are indebted to the director and staff of the Marine Biological Laboratory for their assistance. This work was supported by U.S. Public Health Service grants NS 08437 and NS 12253, NSF grant BNS-7705025 and a long-term EMBO Fellowship to A.G.

Appendix

Signal-to-Noise Ratios for Additional Dyes Added Externally

The signal-to-noise ratio of the change in fluorescence, absorption, and birefringence for a single 50-mV voltage-clamp step and the source of the dye according to the list in Table 1 are given in parentheses. The signal-to-noise ratio for absorption is given in italics. The signal-to-noise ratio in birefringence is followed by a B. Dyes from Nippon Kankoh-Shikiso Kenkyusho, Ltd., Okayama, Japan (NK) are designated by the catalogue number in the company's organic chemical list (1969), and supplement (1974). In most instances a maximum of 128 trials was averaged which limited the minimum detectable signal-to-noise ratio to about 0.1. When no change was detected n.c. was recorded. The classification and nomenclature of most of the dyes conform to that of Fry (1977). Within groups, the dyes are listed so that similar structures are near one another. Structural formulae are available from the authors. S. Lesher has modified ADAPT (automatic data analysis using pattern recognition techniques) (Stuper & Jurs, 1976) to run on a PDP11 so that structural formulae can be transmitted via disk files.

Cyanine Dyes

Oxacyanines: 545. bis-[3-methylbenzoxazole-(2)]-β,δ-neopentylene-pentamethinecyanine iodide (2, *1*, H); 546. bis-[7-isopropyl-3-methylbenzoxazole-(2)]-heptamethinecyanine iodide (n.c., *n.c.*, NK1708); 547. anhydro-bis-[3-γ-sulfopropylnaphth(2,1-d)oxazole-(2)]-pentamethinecyanine hydroxide sodium salt (0.7, *0.3*, H); 548. bis-[3-ethylnaphth(2,1-d)oxazole-(2)]-β-ethyltrimethinecyanine bromide (n.c., *n.c.*, NK1520); 549. bis-[3-ethylnaphth(2,1-d)oxazole-(2)]-pentamethinecyanine iodide (n.c., *n.c.*, NK2034); 550. anhydro-[5-phenyl-3-γ-sulfopropylbenzoxazole-(2)]-[1-γ-sulfopropylnaphth(1,2-d)oxazole-(2)]-trimethinecyanine hydroxide (0.2, *n.c.*, NK2073); 551. bis-[3-ethyl-5-phenylbenzoxazole-(2)]-β-ethyltrimethinecyanine chloride (n.c., *n.c.*, H); 552. bis-[3-ethyl-5-phenylbenzoxazole-(2)]-pentamethinecyanine iodide (n.c., *n.c.*, NK2037); 553. bis-[3-ethyl-5-phenylbenzoxazole-(2)]-heptamethinecyanine iodide (n.c., *n.c.*, NK1960); 554. bis-[3-ethyl-5-methoxybenzoxazole-(2)]-β-methyltrimethinecyanine iodide (n.c., *n.c.*, NK1974); 555. bis-[3-ethyl-5-methoxybenzoxazole-(2)]-pentamethinecyanine iodide (1, *n.c.*, NK2035).

Thiacyanines: 556. bis-[3-ethyl-6-methoxybenzothiazole-(2)]-heptamethinecyanine iodide (n.c., *0.1*, NK1557); 557. [5-carboethoxy-3-ethyl-4-methylthiazole-(2)]-[3-ethyl-4-methylthiazole-(2)]-pentamethinecyanine iodide (0.1, *0.1*, NK2357); 558. bis-[5-carboethoxy-3-ethyl-4-methylthiazole-(2)]-pentamethinecyanine iodide (0.3, *0.4*, NK2358); 559. bis-[3,5-dimethyl-2-thiazoline-(2)]-heptamethinecyanine iodide (1, *1.5*, NK746); 560. anhydro-bis-[3-γ-sulfopropyl-5-methylthiazole-(2)]-pentamethinecyanine hydroxide triethylamine salt (n.c., *n.c.*, H); 561. anhydrobis-[3-γ-sulfopropylbenzothiazole-(2)[-β-δ-neopentylene-pentamethinecyanine hydroxide triethylamine salt (2, 1.5, NK2371); 562. bis-[3-methylbenzothiazole-(2)]-β,δ-neopentylene-pentamethinecyanine iodide (0.1, *0.1*, H); 563. anhydro-bis-[1-γ-sulfopropylnaphtho(1,2-d)thiazole-(2)]-heptamethinecyanine hydroxide (n.c., *n.c.*, NK1978); 564. anhydro-[1-ethylnaphtho(1,2-d)thiazole-(2)]-[1-γ-sulfopropylnaphtho(1,2-d)-thiazole-(2)]-trimethinecyanine hydroxide (n.c., *n.c.*, NK2075); 565. bis-[1-methylnaphtho(1,2-d)thiazole-(2)]-β-methyltrimethinecyanine p-toluenesulfonate (n.c., n.c., NK1859); 566. bis-[1-ethylnaphtho(1,2-d)thiazole-(2)]-γ-chloropentamethinecyanine p-toluenesulfonate (0.2, *n.c.*, NK460); 567. bis-[3-β-acetoxyethyl-4-methylthiazole-(2)]-γ-(3-β-acetoxyethyl-4-methylthiazolium iodide-(2)]-pentamethinecyanine iodide (0.2, *0.2*, NK1820); 568. bis-[3-methylbenzothiazole-(2)]-β-p-dimethylaminostyryl-trimethinecyanine iodide (n.c., *n.c.*, NK865); 569. bis-[3-heptylbenzothiazole-(2)]-β-p-dimethylamino-styryltrimethinecyanine iodide (n.c., *n.c.*, NK208); 570. 3′,3′-trimethylenebis-[(3-octylbenzothiazole-(2))-benzothiazole-(2′))-penta-methinecyanine iodide] (n.c., *n.c.*, EK); 571. 5,5′-dimethoxy-3,3′,9-triethyl-2,2′-thiacarbocyanine bromide (0.3, *n.c.*, AC); 572. 3-ethyl-3′-methylthiathiazolinotricarbocyanine iodide (0.9, *0.6*, AC).

Indocyanines: 573. bis-[1,1,3-trimethyl-1H-benz[ε]indole-(2)]-trimethinecyanine iodide (3, *0.2*, NK2610); 574. anhydro-bis-[1,1-dimethyl-3-γ-sulfopropyl-1H-benz[e]indole-(2)]-heptamethinecyanine hydroxide sodium salt (*8*, NK2611); 575. bis-[3,3-dimethyl-1-carboxymethyl-3H-indole-(2)]-heptamethinecyanine chloride (0.6, *0.6*, NK1405); 576. anhydrobis-[1-δ-sulfobutyl-3-spirocyclohexane-3H-indole-(2)]-heptamethinecyanine hydroxide sodium salt (1, *12*, H); 577. 1′,1′-pentamethylenebis-[(1,3,3-trimethyl-3H-indole-(2))-(3′,3′-dimethyl-3′H-indole-(2′))-pentamethinecyanine iodide] (2, *3*, H); 578. 1′,1′-tetramethylenebis-[(1,3,3-trimethyl-3H-indole-2))-(3′,3′-dimethyl-3′H-indole(2′))-pentamethinecyanine iodide] (0.2, *0.3*, NK2477); 579. bis-[3,3-dimethyl-1-γ-sodium sulfopropyl-3H-indole-(2)]-trimethinecyanine iodide (n.c., *n.c.*, NK1751); 580. anhydrobis-[3,3-dimethyl-1-γ-sulfopropyl-3H-indole-(2)]-pentamethinecyanine hydroxide sodium salt (n.c., *n.c.*, NK1840).

Quinolino or Pyrido-cyanines: 581. anhydro-bis-[1-γ-sulfopropylquinoline-(4)]-γ-chloropentamethinecyanine hydroxide (n.c., *n.c.*, NK2061); 582. bis-[4-ethyl-benzo(t)quinoline-(3)]-trimethinecyanine iodide (n.c., *n.c.*, NK1095); 583. 1,1′-diethyl-2,2′-quinotricarbocyanine iodide (n.c., *n.c.*, AC); 584. 1,1′-diethyl-4,4′-quinotricarbocyanine iodide (n.c., *n.c.*, AC); 585. Neocyanine (n.c., *n.c.*, M).

Other cyanines: 586. [3-methylbenzothiazole-(2)]-[1,3,3-trimethyl-3H-indole-(2)]-pentamethinecyanine iodide (2, *4*, NK2378); 587. [1-ethylquinoline-(4)]-[1,3,3-trimethyl-3H-indole-(2)]-pentamethinecyanine iodide (0.8, *0.3*, NK2359); 588. anhydro-[1-γ-sulfopropylquinoline-(4)]-[1,3,3-trimethyl-3H-indole-(2)]-trimethinecyanine hydroxide (0.15, *n.c.*, NK2483); 589. anhydro-[1-γ-sulfopropylquinoline-(4)]-[1,3,3-trimethyl-3H-indole-(2)]-pentamethinecyanine hydroxide (n.c., *n.c.*, NK2356); 590. anhydro-[1-γ-sulfopropylquinoline-(4)]-[1,1,3-trimethyl-1H-benz[e]indole-(2)]-trimethinecyanine hydroxide (4, *5*, NK2484); 591. anhydro-[1-γ-sulfopropylquinoline-(4)]-[1,1,3-trimethyl-1H-benz[e]indole(2)]-pentamethinecyanine hydroxide (3, *3*, NK2360); 592. bis-[1,3-dimethylbenzimidazole-(2)]-trimethinecyanine iodide (n.c., *n.c.*, NK1193); 593. anhydro[1,1-dimethyl-3-γ-sulfopropyl-1H-benz[e]indole-(2)]-[3-γ-sulfopropyl-naphth(2,1-d)oxazole-(2)]-pentamethinecyanine hydroxide (1.5, *5*, NK2374); 594. [1,1-dimethyl-3-ethyl-1H-benz[e]indole-(2)]-(1-ethylnaphth(1,2-d)oxazole-(2)]-pentamethinecyanine iodide (0.1, *0.1*, NK2380); 595. anhydro-[1,1-dimethyl-3-γ-sulfoprophyl-1H-benz[e]indole-(2)]-[3-γ-sulfopropylnaphtho(2,1-d)thiazole-(2)]-pentamethinecyanine hydroxide (1, *1*, NK2373); 596. [1,1-dimethyl-3-ethyl-1H-benz[e]indole-(2)]-[3-ethylnaphth(2,1-d)oxazole-(2)]-pentamethinecyanine iodide (n.c., *n.c.*, NK2379); 597. anhydro-[3-γ-sulfopropylbenzoxazole-(2)]-[1,3,3-trimethyl-3H-indole-(2)]-pentamethinecyanine hydroxide (0.1, *n.c.*, H); 598. 8,9-diazo-3,3′-diethylthiacarbocyanine iodide (0.2, *n.c.*, M); 599. 1,1′-dimethyl-2,2′-diphenyl-3,3′ indolocarbocyanine bromide (n.c., *n.c.*, M); 600. 3′-ethyl-1-methyl-6′-nitro-2-(3-pyridyl)-3-indolothiacarbocyanine *p*-toluenesulfonate (0.05, *n.c.*, M); 601. 5′-chloro-1,3′-dimethyl-2-phenyl-6′-nitro-3-indolothiacarbocyanine p-toluenesulfonate (n.c., *n.c.*, M); 602. 6-chloro-1,3-diallyl-1′,2′-diphenylimidazo[4,5-b]quinoxalino-3′-indolocarbocyanine *p*-toluenesulfonate (n.c., *0.3*, M); 603. 1,1′-dimethyl-2,2′-diphenyl-3,3′-indolocarbo-

cyanine bromide (n.c., *n.c.*, M); 604. 1,1′,3,3′-tetraethylimidazo[4,5-b]quinoxalinocarbocyanine chloride (2, *0.3*, M); 605. 1′,3′-diallyl-1,3-diethyl-(2-imidazo[4,5-b] pyrido[2,3-e]pyrazino)-2-imidazo[4,5-b] quinoxalinocarbocyanine perchlorate (0.2, *n.c.*, M).

Merocyanine Dyes

Benzoxazole merocyanines: 606. [1-butyl-3-γ-dimethylaminopropyl-barbituric acid-(5)]-[3-propylbenzoxazoline-(2)]-tetramethinemerocyanine (0.1, *0.2*, H); 607. [1-butyl-3-γ-sulfopropylbarbituric acid-(5)]-[3-propylbenzoxazoline-(2)]-tetramethinemerocyanine (n.c., *n.c.*, H); 608. [1,3-diethylbarbituric acid-(5)]-[3-γ-sodium sulfopropylnaphth(2,1-d)oxazoline-(2)]-tetramethinemerocyanine (0.8, *0.6*, H, NK2592); 609. [1,3-dihexylbarbituric acid-(5)]-[3-γ-sodium sulfopropylnaphth(2,1-d)oxazoline-(2)]-tetramethinemerocyanine (0.2, *0.1*, H); 610. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[3-(γ-N,N,N-trimethylammoniumpropyl iodide)-benzoxazoline-(2)]-tetramethinemerocyanine (3, *2*, H); 611. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[3-ω-sodium sulfohexylbenzoxazoline-(2)]-tetramethinemerocyanine (10, *2*, H); 612. [1-butyl-3-γ-sodiumsulfopropyl-2-thiobarbituric acid-(5)]-[3-γ-sodium sulfopropylbenzoxazoline-(2)]-tetramethinemerocyanine (n.c., *n.c.*, H); 613. [1,3-dimethyl-2-thiobarbituric acid-(5)]-[3-γ-triethylammonium carboxypropylbenzoxazoline-(2)]-tetramethinemerocyanine (0.1, n.c., H); 614. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[1-γ-sulfopropylnaphth(1,2-d)oxazoline-(2)]-tetramethinemerocyanine (0.1, *0.1*, H); 615. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[3-γ-triethylammonium sulfopropylnaphth(2,1-d)oxazoline-(2)]-tetramethinemerocyanine (6, *1*, H); 616. [1,3-dimethyl-2-thiobarbituric acid-(5)]-[3-γ-dimethylaminopropylbenzoxazoline-(2)]-tetramethinemerocyanine (n.c., *0.3*, H); 617. [3-ethylrhodanine-(5)]-[3-γ-triethylammonium sulfopropylbenzoxazoline-(2)]-dimethinemerocyanine (n.c., *n.c.*, H); 618. [3-ethylrhodanine-(5)]-[5-phenyl-3-γ-triethylammonium sulfopropylbenzoxazoline-(2)]-tetramethinemerocyanine (n.c., *0.1*, NK2364); 619.[3-ethylrhodanine-(5)]-[3-γ-triethylammoniumsulfopropylbenzoxazoline-(2)]-tetramethinemerocyanine (0.3, *0.3*, NK2375); 620. [3-ethyl-2-methylthio-4-oxothiazolonium iodide-(5)]-[3-γ-triethylammonium sulfopropylbenzoxazoline-(2)]-dimethinemerocyanine (0.3, *n.c.*, H); 621. 5-(3′-ethyl-2′-benzoxazolylidene-ethylidene)-3-carboxymethyl rhodanine (n.c., *n.c.*, AC); 622. [3-phenyl-5(4)-isoxazolone-(4)]-[3-γ-sodium sulfopropylnaphth(2,1-d)oxazoline-(2)]-tetramethinemerocyanine (0.3, *0.1*, H, NK2591); 623. 4-[3-γ-sodium-sulfopropyl-2-benzoxazolylidene-2-butenylidene]-3-phenyl-5(4)-isoxazolone (0.1, *0.5*, M, NK2353); 624. [3-methyl-1-phenyl-5-pyrazolone-(4)]-[3-γ-sodium sulfopropylbenzoxazoline-(2)]-tetramethinemerocyanine (n.c., *n.c.*, H); 625. [malononitrile]-[3-γ-sulfopropylbenzoxazoline-(2)]-tetramethinemerocyanine (n.c., *0.1*, NK2355); 626. [1,3-diethyl-2-thiohydantoin-(5)]-[3-γ-sodium sulfopropylnaphth(2,1-d)oxazoline-(2)]-tetramethinemerocyanine (0.1, *0.1*, NK2593).

Benzothiazolo merocyanines: 627. [1,3-dibutylbarbituric acid-(5)]-[3-γ-sodium sulfopropylnaphtho(2,1-d)thiazoline-(2)]-tetramethinemerocyanine (2, *0.3*, H); 628. [1,3-dibutylbarbituric acid-(5)]-[3-δ-triethylammonium sulfobutylnaphtho(2,1-d)thiazoline-(2)]-tetramethinemerocyanine (2, *1*, H); 629. [1,3-dibutylbarbituric acid-(5)]-[1-γ-sulfopropylnaphtho(1,2-d)thiazoline-(2)]-tetramethinemerocyanine (1.5, *0.3*, H); 630. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[6-iodoacetamido-3-methylbenzothiazoline-(2)]-tetramethinemerocyanine (0.1, *n.c.*, H); 631. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[4-methyl-3-γ-sulfopropylthiazoline-(2)]-tetramethinemerocyanine (2, *1.5*, NK2384); 632. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[3-γ-triethylammonium sulfopropylnaphtho(2,1-d)thiazoline-(2)]-tetramethinemerocyanine (4, *7*, NK2370); 633. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[3-γ-sulfopropylnaptho(2,1-d)thiazoline-(2)]-tetramethinemerocyanine (5, *2*, H); 634. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[1-γ-sulfopropylnaphtho(1,2-d)thiazoline-(2)]-tetramethinemerocyanine (n.c., *n.c.*, H); 635. [3-ethyl-2-thioxo-4-oxazolidinone-(5)]-[3-methylthiazolidene-(2)]-dimethinemerocyanine (n.c., *n.c.*, AC); 636. [3-ethyl-4,5-dihydrothiazoline-(2)]-[3-ethylrhodonine-(5)]-dimethinemerocyanine (0.5, *0.3*, NK1268); 637. [3-carboxymethylrhodanine-(5)]-[3-ethylbenzothiazoline-(2)]-dimethinemerocyanine (n.c., *n.c.*, AC); 638. [3-ethylrhodanine-(5)]-[5-methyl-3-γ-triethylammonium sulfopropylthiazoline-(2)]-tetramethinemerocyanine (n.c., *n.c.*, H); 639. [3-β-carboxyethylrhodanine-(5)]-[3-γ-sulfopropylnaphtho(2,1-d)thiazoline(2)]-tetramethinemerocyanine (0.1, *0.2*, NK1943); 640. [3-ethylrhodanine-(5)]-[5-methyl-3-γ-sodium sulfopropylthiazoline-(2)]-tetramethinemerocyanine (n.c., *1*, NK2481); 641. [2,2-dimethyl-1,3-dioxane-4,6-dione-(5)]-[3-ethylbenzothiazoline-(2)]-α-methyl-dimethinemerocyanine (n.c., *0.3*, H); 642. [3-ethylbenzothiazoline-(2)]-[3-methyl-1-p-triethylammonium sulfophenyl-5-pyrazolone-(4)]-tetramethinemerocyanine (3, H).

Merocyanine rhodanine dyes: 643. [3-methylrhodanine-(5)]-[1-δ-triethylammonium sulfobutyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*13*, H); 644. [3-ethylrhodenine-(5)]-[1-δ-triethylammoniumsulfobutyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*25*, 20B, H, NK2377); 645. [3-ethylrhodanine-(5)]-[1-δ-sodium sulfobutyl-1,4-dihydroquinoline-(4)] -α,γ-neopentylene-tetramethinemerocyanine (*11*, NK2482); 646. [3-ethylrhodanine-(5)]-[1-ω-triethylammonium sulfopentyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *10*, H); 648. [3-ethylrhodanine-(5)]-[1-ω-triethylammoniumsulfohexyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*9*, H); 649. [3-ethylrhodanine(5)]-[1-p-triethylammonium sulfobenzyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*17*, 15B, NK2472); 650. [3-butylrhodanine-(5)]-[1-δ-triethylammonium sulfobutyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*20*, H); 651. [3-phenylrhodanine-(5)]-[1-γ-sodium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*5*, H); 652. [3-allylrhodanine-(5)]-[1-δ-triethylammonium sulfobutyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*15*, H); 653. [3-α-furylrhodanine-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*8*, H); 654. [3-p-ethoxyphenylrhodanine-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *0.1*, H); 655. [3-aminorhodanine-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *0.3*, H); 656. [3-β-triethylammonium sulfoethylrhodanine-(5)]-[1-γ-sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *0.9*, NK2389); 657. [3-β-sodium carboxyethylrhodanine-(5)]-[1-γ-sodium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *n.c.*, H); 658. [3-β-triethylammonium sulfoethylrhodanine-(5)]-[1-methyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *1*, NK2385); 659. [3-ethylrhodanine-(5)]-[1-propyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*0.3*, H); 660. [3-ethylrhodanine-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydrobenzo(h)quinoline-(4)]-tetramethinemerocyanine (*16*, 30B, NK2607); 661. [3-phenylrhodanine-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydrobenzo (h)quinoline-(4)]-tetramethinemerocyanine (n.c., *7*, 9B, NK2609); 662. [3-ethylrhodanine-(5)]-[4-γ-sodium sulfopropyl-1,4-dihydrobenzo (t) quinoline-(1)]-tetramethinemerocyanine (0.3, *8*, NK2597); 663. [3-ethylrhodanine-(5)]-[1-δ-triethylammonium sulfobutyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (0.1, *12*, H); 664. [3-hexylrhodanine-(5)]-[1-δ-sodium sulfobutyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (n.c., *14*, H); 665. [3-hexylrhodanine-(5)]-[1-γ-sodium sulfopropyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (*13*, H); 666. [3-butylrhodanine-(5)]-[1-δ-sodium sulfobutyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (*12*, NK2479); 667. [3-phenylrhodanine-(5)]-[1-γ-sodium sulfopropyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (n.c., *12*, H); 668. [3-dodecylrhodanine-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-dimethinemero-

cyanine (n.c., *0.2*, H); 669. [3-benzylrhodanine-(5)]-[1-γ-sodium sulfopropyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (n.c., *0.8*, H); 670. [3-hexylrhodanine-(5)]-[1-β-sodium sulfoethyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (n.c., *5*, H); 671. [3-hexylrhodanine-(5)]-[1-(γ-N,N,N-trimethylammoniumpropyl iodide)-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (n.c., *2*, H); 672. [3-sodium carboxymethylrhodanine-(5)]-[1-γ-sodium sulfopropyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (n.c., *n.c.*, H); 673. [3-ethylrhodanine-(5)]-[1-(γ-N,N,N-trimethylammoniumpropyl iodide)-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (n.c., *2*, H); 674. [3-ethyl-4,5-dihydrothiazoline-(2)]-[rhodanine-(5)]-dimethinemerocyanine (n.c., *n.c.*, NK1773). 675. [3-amino-2-methylthio-4-oxothiazolonium iodide-(5)]-[1-propyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (0.1, *0.5*, H); 676. [3-ethyl-2,4-thiazolidinedione-(5)]-[1-γ-pyridiniumsulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *3*, NK2596); 677. [3-propyl-2-thioxo-4-oxazolidinone-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*7*, H); 678. [3-butyl-2-thioxo-4-oxazolidinone-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *1*, H); 679. [3-ethyl-2-thioxo-4-oxazolidinone-(5)]-[1-δ-sodium sulfobutyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*15*, H); 680. [3-ethyl-2-thioxo-4-oxazolidinone-(5)]-[1-ω-triethylammonium sulfopentyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (*10*, H); 681. [3-ethyl-2-thioxo-4-oxazolidinone-(5)]-[1-p-triethylammonium sulfobenzyl-1,4-dihydroquinoline-(4)-tetramethinemerocyanine (*8*, 12B, NK2373); 682. [3-ethyl-2-thioxo-4-oxazolidinone-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydrobenzo(h)quinoline-(4)]-tetramethinemerocyanine (*4*, NK2608); 683. [3-ethyl-2-thioxo-4-oxazolidinone-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (*4*, H); 684. [3-isopropyl-2-thioxo-4-oxazolidinone-(5)]-[1-γ-sodium sulfopropyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (*6*, H); 685. [3-butyl-2-thioxo-4-oxazolidinone-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (*8*, H); 686. [3-phenyl-2-thioxo-4-oxazolidinone-(5)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (*5*, H); 687. [3-phenylrhodanine-(5)]-[1-(γ-N,N,N-trimethylammoniumpropyl iodide)-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (n.c., *0.3*, H).

Other merocyanines: 688. [3-ethylrhodanine-(5)]-[1-ethyl-3-γ-triethylammonium sulfopropylbenzimidazoline-(2)]-tetramethinemerocyanine (0.05, *0.1*, H); 689. 5-acetanilidoallylidene-3-ethylrhodanine (n.c., *n.c.*, NK1227); 690. [3-ethylrhodanine-(5)]-[6-methyl-2-γ-sulfopropyl-2,3-dihydropyridazine-(3)]-tetramethinemerocyanine (n.c., *n.c.*, H); 691. [3-carboxymethylrhodanine-(5)]-[1-ethyl-1,2-dihydroquinoline-(2)]-dimethinemerocyanine (n.c., *n.c.*, AC); 692. [3-phenyl-5(4)-isoxazolone-(4)]-[1-methyl-1,4-dihydropyridine-(4)]-hexamethinemerocyanine (1, *1*, AC); 693. [1,3-dibutylbarbituric acid-(5)]-[1-γ-sodium sulfopropyl-1,4-dihydropyridine-(4)]-tetramethinemerocyanine (n.c., *0.1*, H); 694. [1,3-dibutylbarbituric acid-(5)]-[1-γ-sodium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (0.5, *1.5*, H); 695. [3,3-dimethyl-1-γ-sulfopropylindoline-(2)]-[3-phenyl-5(4)-isoxazolone-(4)]-tetramethinemerocyanine (n.c., *1*, NK2366). 696. [1-methyl-1,4-dihydropyridine-(4)]-[3-phenyl-5(4)-isoxazolone-(4)]-dimethinemerocyanine (n.c., *n.c.*, NK1980); 697. [3-phenyl-5(4)-isoxazolone-(4)]-[1-γ-sodium sulfopropyl-1,4-dihydropyridine-(4)]-tetramethinemerocyanine (n.c., *n.c.*, H); 698. [3-phenyl-5(4)-isoxazolone-(4)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *n.c.*, NK2474); 699. 4-anilinomethylene-3-phenyl-5(4)-isoxazolone (n.c., *n.c.*, NK1225); 700. [3-methyl-1-phenyl-5-pyrazolone-(4)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *0.3*, H); 701. 1-ethyl-3-methyl-5-(1′-methylpyrolinylidene-2′-ethylidene)-imidazolinethione-(2)-one-(4) (n.c., *n.c.*, AC); 702. [1,3-indanedione-(2)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *n.c.*, NK2475); 703. [1-ethylinodolin-2-one-(3)]-[1-γ-triethylammonium sulfopropyl-1,4-dihydroquinoline-(4)]-tetramethinemerocyanine (n.c., *2*, NK2476).

Rhodacyanines: 704. anhydro-[5-(3,3-dimethyl-1-γ-sulfopropyl-3H-indolin-2-ylidene-ethylidene)-3-ethylthiazolid-4-one-(2)]-[1,3,3-trimethyl-3H-indole-(2)]-methinecyanine hydroxide (0.2, *n.c.*, NK2383); 705. [3-ethyl-5(1,3,3,trimethyl-3H-indolin-2-ylidene-ethylidene)thiazolic-4-one-(2)]-[1,3,3-trimethyl-3H-indole-(2)]-methinecyanine iodide (1.5, *0.2*, NK2382); 706. [3-ethyl-5-(1,1,3-trimethyl-1H-benz[e]indolin-2-ylidene-ethylidene)thiazolid-4-one-(2)]-[1,1,3-trimethyl-1H-benz[e]indole-(2)]-methinecyanine iodide (3, *0.3*, NK2491); 707. 2-(1,3-dibutyl-2-thiobarbituric acid-5-ylidene)-3-ethyl-5-(3-γ-triethylammonium sulfopropylbenzoxazolin-2-ylidene-ethylidene)thiazolid-4-one (0.2, *0.5*, H); 708. 3-ethyl-2-(3-ethylrhodanine-2-ylidene)-5-(3-γ-triethylammonium sulfopropylbenzoxazolin-2-ylidene-ethylidene)thiazolid-4-one (0.2, *n.c.*, H); 709. anhydro-[3-carboxymethyl-5-(3-ethylnaphtho(2,1-d)thiazolin-2-ylidene-ethylidene)thiazolid-4-one-(2)]-[5-carboxy-3-ethylbenzothiazole-(2)]-methinecyanine hydroxide (0.2, *n.c.*, NK2059); 710. 3-amino-2-(3-ethylrhodanine-2-ylidene)-5-(1-propyl-1,4-dihydroquinolin-2-ylidene-ethylidene)thiazolid-4-one (n.c., *n.c.*, H).

Oxonol Dyes

Pyrazolone-barbituric acid dyes: 711. [1,3-dipentylbarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (4, *18*, 12B, H); 712. [1,3-diphenylbarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (0.1, *2*, H); 713. [1,3-diisopropylbarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrozolone-(4)]-pentamethineoxonol ditriethylamine salt (2, *1*, H); 714. [1-butylbarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (0.15, *2*, H); 715. [1, 3-dihexylbarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (6, *20*, H); 716. [1,3-dibutylbarbituric acid-(5)]-[3-methyl-1-γ-sulfopropyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (0.3, *1*, H); 717. [1,3-dibutylbarbituric acid-(5)]-[3-carboxypropyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (3, *2*, H); 718. [1,3-dipropylbarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamenthineoxonol ditriethylamine salt (1.5, *0.8*, H); 719. [1,3-dibutylbarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-α, γ-neopentylene-pentamethineoxonol ditriethylamine salt (1, *2*, H); 720. [1,3-dibutylbarbituric acid-(5)]-[3-carboxy-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol tritriethylamine salt (n.c., *n.c.*, H); 721. [1-butyl-3-γ-dimethylaminopropylbarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (1.5, *1.5*, H); 722. [1,3-dibutylbarbituric acid-(5)]-[1-p-carboxylphenyl-3-methyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (2, *5*, H); 723. [1-butyl-3-γ-sulfopropylbarbituric acid-(5)]-[3-methyl-1-phenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (0.3, *0.3*, H); 724. [1-butyl-3-γ-sulfopropylbarbituric acid-(5)]-[3-methyl-1-phenyl-5-pyrazolone-(4)]-α',γ-neopentylene-pentamethineoxonol ditriethylamine salt (n.c., *n.c.*, H); 725. [1,3-dibutylbarbituric acid-(5)]-[3-methyl-1-phenyl-5-pyrazolone-(4)]-α',γ-neopentylene-pentamethineoxonol triethylamine salt (0.4, *3*, H); 726. [1,3-dibutylbarbituric acid-(5)]-[3-methyl-1-phenyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 727. [1,3-dibutylbarbituric acid-(5)]-[3-carbopropoxy-1-phenyl-5-pyrazolone-(4)]-pentamethineoxonol triethylamine salt (3, *0.5*, H); 728. [3-carboethoxy-1-phenyl-5-pyrazolone-(4)]-[1,3-dibutylbarbituric acid-(5)]-pentamethineoxonol triethylamine salt (4, H); 729. [1-benzyl-3-carbopropoxy-5-pyrazolone-(4)]-[1,3-dibutylbarbituric acid-(5)]-pentamethineoxonol

triethylamine salt (0.2, *n.c.*, H); 730. [1,3-diethyl-2-thiobarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol (4, *13*, H); 731. [1,3-dipropyl-2-thiobarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (1.5, *6*, H); 732. [1,3-dibutyl-2-thiobarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (2, *20*, H); 733. [1,3-dipentyl-2-thiobarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (7, *7*, H); 734. [1,3-dihexyl-2-thiobarbituricacid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (0.1, *0.3*, H); 735. [1,3-diphenyl-2-thiobarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (0.15, *1.5*, H); 736. [1,3-dioctyl-2-thiobarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (n.c., n.c., H); 737. [1-butyl-3-octyl-2-thiobarbituric acid-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (n.c., *n.c.*, H); 738. [1-*p*-carboxyphenyl-3-methyl-5-pyrazolone-(4)]-[1,3-diethyl-2-thiobarbituric acid-(5)]-pentamethineoxonol (3, *4*, H).

Barbituric acid oxonols: 739. bis-[1,3-diisopropylbarbituric acid-(5)]-trimethineoxonol (0.3, n.c., H); 740. bis-[1,3-diphenylbarbituric acid-(5)]-trimethineoxonol (n.c., *n.c.*, H); 741. bis-[1,3-dibutylbarbituric acid-(5)]-α,γ-neopentylene-pentamethineoxonol (3, *3*, H); 742. bis-[1,3-diethylbarbituric acid-(5)]-α,γ-neopentylene-pentamethineoxonol (0.15, *n.c.*, M); 743. bis-[1,3-diphenylbarbituric acid-(5)]-pentamethineoxonol (n.c., *n.c.*, H); 744. bis-[1-butylbarbituric acid-(5)]-pentamethineoxonol (1, *0.5*, H); 745. [1,3-dicarboethoxybarbituric acid-(5)]-[3-methyl-1-*p*-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 746. [1-butyl-3-γ-sulfopropylbarbituric acid-(5)]-[1,3-dibutylbarbituric acid-(5)]-α',γ-neopentylene-pentamethineoxonol ditriethylamine salt (0.8, *2*, H); 747. 3′,3′-trimethylenebis-[(1,3-dibutylbarbituric acid-(5))-(1′-butylbarbituric acid-(5′))-pentamethineoxonol] (0.1, *1*, H); 748. 3′,3′-hexamethylenebis-[(1,3-dibutylbarbituric acid-(5))-(1′-butylbarbituric acid-(5′))-pentamethineoxonol] (0.4, *n.c.*, H); 749. bis-[1-butyl-3-γ-sulfopropylbarbituric acid-(5)]-pentamethineoxonol (0.3, *0.2*, H); 750. [1-butyl-3-γ-dimethylaminopropylbarbituric acid-(5)]-[1,3-dibutylbarbituric acid-(5)]-pentamethineoxonol (n.c., *n.c.*, H); 751. bis-[1-butyl-3-γ-dimethylaminopropylbarbituric acid-(5)]-pentamethineoxonol (n.c., *n.c.*, H); 752. [1,3-dibutylbarbituric acid-(5)]-[1-butyl-3-γ-sulfopropylbarbituric acid-(5)]-pentamethine oxonol (0.1, *0.3*, H); 753. [1,3-diethyl-2-thiobarbituric acid-(5)]-[1,3-di-β-methoxyethylbarbituric acid-(5)]-pentamethineoxonol (2, *0.5*, M); 754. bis-[1,3-diphenyl-2-thiobarbituric acid-(5)]-pentamethineoxonol (n.c., *n.c.*, H); 755. [1,3-diphenyl-2-thiobarbituric acid-(5)]-[2-thiobarbituric acid-(5)]-trimethineoxonol (n.c., *n.c.*, H).

Pyrazolone dyes: 756. bis-[3-methyl-1-phenyl-5-pyrazolone-(4)]-methineoxonol (n.c., *n.c.*, H); 757. bis-[1-β-hydroxyethyl-3-methyl-5-pyrazolone-(4)]-trimethineoxonol (n.c., *n.c.*, H); 758. bis-[3-carbopropoxy-1-phenyl-5-pyrazolone-(4)]-trimethineoxonol (0.4, *2*, H); 759. bis-[3-carboethoxy-1-phenyl-5-pyrazolone-(4)]-trimethineoxonol (1, *2*, H) 760. bis-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-trimethineoxonol (n.c., *n.c.*, NK1447); 761. bis-[1,3-dimethyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 762. bis-[1-p-methoxyphenyl-3-methyl-5-pyrazolone-(4)]-pentamethineoxonol (0.1, *1*, H); 763. bis-[3-methyl-1-o-methylphenyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 764. bis-[1-β-hydroxyethyl-3-methyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 765. bis-[3-methyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 766. bis-[3-methyl-1-α-naphthyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 767. bis-[3-methyl-1-phenyl-5-pyrazolone-(4)]-pentamethineoxonol (*1*, H); 768. bis[3-carboethoxy-1-β-hydroxethyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 769. bis-[3-carboethoxy-1-phenyl-5-pyrazolone-(4)]-pentamethineoxonol (0.4, *0.5*, H); 770. bis-[1-benzyl-3-carboethoxy-5-pyrazolone-(4)]-pentamethineoxonol (0.2, *0.6*, H); 771. bis-[1-benzyl-3-carbopropoxy-5-pyrazolone-(4)]-pentamethineoxonol (0.2, *1*, H); 772. bis[3-ethoxy-1-phenyl-5-pyrazolone-(4)]-pentamethineoxonol (0.1, *0.5*, H); 773. bis[3-pentyl-1-phenyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 774. bis-[1-phenyl-3-propyl-5-pyrazolone-(4)]-pentamethineoxonol (0.1, *1*, H); 775. bis-[3-carboxy-1-phenyl-5-pyrazolone-(4)]-pentamethineoxonol (0.4, *0.4*, H); 776. bis-[1-benzyl-3-p-fluorosulfonylbenzamido-5-pyrazolone-(4)]-pentamethineoxonol triethylamine salt (n.c., *n.c.*, H); 777. bis-[3-p-nitroanilino-1-(2,4,6-trichlorophenyl)-5-pyrazolone-(4)]-pentamethineoxonol triethylamine salt (n.c., *n.c.*, H); 778. bis-[3-amino-1-phenyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 779. bis-[3-phenyl-1-thiocarbamoyl-5-pyrazolone-(4)]-pentamethineoxonol (0.3, *0.8*, H); 780. bis-[3-carboxy-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol (n.c., *n.c.*, H); 781. bis-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol (0.2, *0.5*, H); 782. bis-[1-benzyl-3-carbopropoxy-5-pyrazolone-(4)]-γ-phenyl-pentamethineoxonol (n.c., *n.c.*, H).

Other oxonols: 783. bis-[3-propyl-5(4)-isoxazolone-(4)]-pentamethineoxonol (0.6, *3*, Y); 784. bis-[3-ethylrhodanine-(5)]-methineoxonol triethylamine salt (1, *0.3*, H); 785. bis-[3-propylrhodanine-(5)]-methineoxonol triethylamine salt (1, *1*, H); 786. bis-[3-butylrhodanine-(5)]-methineoxonol triethylamine salt (0.1, *0.2*, H); 787. bis-[3-hexylrhodanine-(5)]-methineoxonol triethylamine salt (0.03, *0.1*, H); 788. bis-[3-ethylrhodanine-(5)]-trimethineoxonol triethylamine salt (0.4, *0.1*, H); 789. bis-[3-propylrhodanine-(5)]-trimethineoxonol triethylamine salt (0.1, *n.c.*, H); 790. bis-[3-p-ethoxyphenylrhodanine-(5)]-trimethineoxonol (n.c., *0.8*, H); 791. bis-[3-α-furylrhodanine-(5)]-trimethmeoxonol (0.4, *n.c.*, H); 792. bis[3-β-carboxyethylrhodanine-(5)]-trimethineoxonol (n.c., *n.c.*, H); 793. [1,3-dibutylbarbituric acid-(5)]-[3-phenyl-5(4)-isoxazolone-(4)]-pentamethineoxonol (0.3, *0.4*, H); 794. [1,3-diethylbarbituric acid-(5)]-[3-phenyl-5(4)-isoxazolone-(4)]-α',γ-neopentylene-pentamethineoxonol (3, *n.c.*, M); 795. [3-ethylrhodanine-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-methineoxonol ditriethylamine salt (n.c., *0.2*, H); 796. [3-hexylrhodanine-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-methineoxonol ditriethylamine salt (0.3, *3*, H); 797. [3-ethylrhodanine-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-trimethineoxonol ditriethylamine salt (1, *8*, H); 798. [3-butylrhodanine-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-trimethineoxonol ditriethylamine salt (0.5, *3*, H); 799. [3-hexylrhodanine-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-trimethineoxonol ditriethylamine salt (0.2, *5*, H); 800. [3-ethyl-2-thioxo-4-oxazolidinone-(5)-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (1.5, *9*, H); 801. [3-ethyl-2,4-oxazolidinedione-(5)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (n.c., *n.c.*, H); 802. [3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-[3-phenyl-5(4)-isoxazolone(4)]-pentamethineoxonol ditriethylamine salt (0.1, *4*, H); 803. [3-aminorhodanine-(5)]-[1,3-indanedione-(2)]-pentamethineoxonol-(0.5, *0.2*, H); 804. [1,3-indanedione-(2)]-[3-methyl-1-p-sulfophenyl-5-pyrazolone-(4)]-pentamethineoxonol ditriethylamine salt (n.c., *n.c.*, H).

Styryl Dyes

Quinoline dyes: 805. 9-p-dimethylaminostyrylacridine (0.03, *n.c.*, NK2016); 806. 4-p-dimethylaminostyrylquinoline (0.3, *0.1*, NK1837); 807. 4-p-diethylaminostyryl-1-ethylquinolinium iodide (2, *0.4*, H); 808. anhydro-4-p-dimethylaminostyryl-1-γ-sulfopropylquinolinium hydroxide (n.c., *n.c.*, H); 809. anhydro-4-p-dimethylaminostyryl-1-ω-sulfohexylquinolinium hydroxide (0.1, *n.c.*, H); 810. anhydro-4-p-diethylaminostyryl-1-γ-sulfopropylquinolinium hydroxide (2, *n.c.*, H); 811. 4-(4′-p-dimethylaminophenylbuta-

1′:3′-dienyl)quinoline (0.5, *1*, NK1905); 812. 4-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-1-methyquinolinium iodide (0.2, *0.2*, H); 813. anhydro-4-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-1-γ-sulfopropylquinolinium hydroxide (2, *1*, NK2354); 814. anhydro-4-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-1-δ-sulfobutylquinolinium hydroxide (1.5, H).

Other Styryl Dyes

815. 4-p-dihexylaminostyryl-1-methylpyridinium iodide (n.c., *n.c.*, LL); 816. 4-p-dipentylaminostyryl-1-methylpyridinium iodide (1.5, *1*, LL); 817. anhydro-4-(4′-p-dihexylaminophenylbuta-1′:3′-dienyl)-1-γ-sulfopropylpyridinium hydroxide (1, *0.4*, LL); 818. 2-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-6-maleimidyl-3-methylbenzothiazolium p-toluenesulfonate (0.3, *n.c.*, H); 819. 2-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-6-iodoacetamido-3-methylbenzothiazolium iodide (n.c., *n.c.*, H); 820. 2-(4′-p-dimethylaminophenylbuta-1′-3′-dienyl)-3-ethylbenzothiazolium iodide (0.4, *0.2*, NK375); 821. 2-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-3-propylbenzothiazolium iodide (1, *1*, H); 822. 3-butyl-2-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-4-methylthiazolium iodide (0.2, *0.2*, NK365); 823. 3-carboxymethyl-2-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-6-iodoacetamido-benzothiazolium iodide (n.c., *n.c.*, H); 824. 3-β-carboxyethyl-2-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-benzothiazolium iodide (n.c., *n.c.*, H); 825. anhydro-3-p-dimethylaminostyryl-6-methyl-2-γ-sulfopropylpyradizinium hydroxide (n.c., *n.c.*, NK2490); 826. 3-(4′-p-dimethylaminophenylbuta-1′:3′-dienyl)-2-ethyl-6-methylpyradizinium iodide (n.c., *n.c.*, NK2352); 827. 2-p-dimethylaminostyryl-1,3-diethylbenzimidazolium iodide (n.c., *n.c.*, NK1189); 828. 2-p-diethylaminostyryl-3,3-dimethyl-3H-indole (n.c., *n.c.*, NK2020).

Other Structural Types

Aminovinyl: 829. 3,5-dimethyl-2-(β-p-carboethoxyanilinovinyl)oxazolium iodide (n.c., *n.c.*, NK936); 830. 2-(β-carboethoxyanilinovinyl)-3,4,5-trimethyloxazolium iodide (n.c., *n.c.*, NK996); 831. 2-(β-acetanilidovinyl)-3-ethylbenzoxazolium iodide (n.c., *n.c.*, NK252); 832. 3,4-dimethyl-2(β-N-methylanilinovinyl)oxazolium iodide (n.c., *n.c.*, NK2013); 833. 3,4-dimethyl-2-(β-p-methoxyacetanilidovinyl) thiazolium iodide (n.c., *n.c.*, NK252); 834. 2-(β-p-carboethoxyanilinovinyl)-1-ethyl-6-methylpuridinium iodide (n.c., *n.c.*, NK618); 835. 2-(β-anilinovinyl)-6-dimethylamino-1-ethylquinolinium iodide (n.c., *n.c.*, NK574); 836. 2,6-di(β-2-pyrimidylaminovinyl)-1-ethylpyridinium iodide (n.c., *n.c.*, NK1116); 837. 2-(4′-acetanilidobuta-1′:3′-dienyl)-3-ethylbenzoxazolium iodide (0.1, *n.c.*, NK1185); 838. 2-(4′-acetanilidobuta-1′:3′-dienyl)-3-ethylbenzothiazolium iodide (3, *2*, NK351); 839. anhydro-2(6′-p-carboxyanilinohexa-1′:3′:5′-trienyl)-3-ethylbenzothiazolium hydroxide (0.2, *0.5*, NK415); 840. 2-(6′-acetanilidohexa-1′:3′:5′-trienyl)-1-ethylquinolinium iodide (n.c., *n.c.*, NK483); 841. 2-(6′-acetanilidohexa-1′:3′:5′-trienyl)-1,3,3,-trimethyl-3H-indolinium iodide (0.2, *0.2*, NK484); 842. 4-(5′-anilino-2′,4′-pentadienylidene)-3-methyl-1-p-triethylammonium sulfophenyl-5-pyrazolone (n.c., *0.3*., H).

Benzals: 843. 1,3-diethyl-5-p-dimethylaminocinnamylidenebarbituric acid (4, NK2362); 844. 1,3-dibutyl-5-p-dimethylaminocinnamylidenebarbituric acid (0.05, *0.2*, H); 845. 1,3-dibutyl-5-p-dimethylaminocinnamylidene-2-thiobarbituric acid (0.1, 0.1, H, NK2363); 846. 5-p-dimethylaminocinnamylidene-1,3-dimethylbarbituric acid (0.2, *n.c.*, H).

Hemicyanines: 847. 2-p-dimethylaminoanilinomethylbenzothiazole ethiodide (n.c., *n.c.*, NK1851); 848. 3-ethyl-2-(6′-piperidinohexa-1′:3′:5′-trienyl)benzothiazolium iodide (0.4, *1*, NK439); 849. anhydro-2-(6′-piperidinohexa-1′:3′:5′-trienyl)-3-γ-sulfopropylbenzoxazolium hydroxide (n.c., *n.c.*, NK2376); 850. 3-ethyl-2-(6′-piperidinohexa-1′:3′:5′-trienyl)benzoxazolium iodide (0.4, *n.c.*, NK2381); 851. anhydro-3,3-dimethyl-2-(6′-dimethylaminohexa-1′:3′:5′-trienyl)-1-γ-sulfopropyl-3H-indolinium hydroxide (0.3, *0.3*, NK2361); 852. 2-(4′-dimethylaminobuta-1′:3′-dienyl)-3-ethylbenzothiazolium iodide (0.3, *0.6*, NK673).

Triphenylmethane: 853. Coomassie violet R-150 (n.c., *n.c.*, M); 854. Methyl blue (0.2, *n.c.*, SD); 855. Alcian brilliant blue FFR (0.1, *0.4*, SD); 856. Acilon Fast Green BBF (n.c., *0.6*, SD); 857. Methyl-green (0.4, *n.c.*, A);

Pyronin: 858. lissamine rhodamine B sulfonyl chloride (0.05, *n.c.*, MP); 859. Sulforhodamine 101 (n.c., *n.c.*, M); 860. 5-aminoeosin (n.c., *n.c.*, MP); 861. eosin-5-maleimide (n.c., *n.c.*, MP); 862. eosin-5-isothiocyanate (0.5, *1*, MP); 863. eosin-5-iodoacetamide (0.03, *0.1*, MP); 864. 5-(3,5-dichlorotriazinyl)aminoeosin (n.c., *n.c.*, MP); 865. Rose bengal (0.4, *0.6*, D).

Other types: 866. Trypan red (n.c., *0.1*, SD); 867. Benzopurpurine 4B (n.c., *0.2*, SD); 868. Benzo brilliant red 8BS (n.c., *n.c.*, SD); 869. Rhodanile blue (1.5, SD); 870. Lacmoid (n.c., *n.c.*, C); 871. 9-amino acridine (0.02, *n.c.*); 872. Supranol fast cyanine B (n.c., *n.c.*, SD); 873. 1-pyrenemethyl iodoacetate (n.c., *n.c.*, MP); 874. pyrene-1-sulfonyl chloride (n.c., *n.c.*, MP); 875. 3-(1-pyrene)propenoic acid (0.1, *n.c.*, MP); 876. 2-(1-pyrenesulfonamido)ethyltrimethylammonium iodide (n.c., *n.c.*, MP); 877. 4,4′-di-p-methylanilino-8,8′-disulfo-1,1′-binaphthyl (0.4, *n.c.*, GW); 878. 4,4′-dianilino-8,8′-disulfo-1,1′-binaphthyl (0.2, *n.c.*, GW); 879. 1-anilinonaphthalene-2-sulfonic acid (n.c., *n.c.*, MP); 880. 2-(4′-maleimidylanilino)naphthalene-6-sulfonic acid (n.c., *n.c.*, MP); 881. 8-[5-(6,7-dihydro-2,4-diphenyl-5H-1-benzopyran8-yl)-2,4-pentadienylidene]-5,6,7,8-tetrahydro-2,4-diphenyl-1-benzopyrylium perchlorate (n.c., *n.c.*, H); 882. 2-[3-(4,6-diphenyl-2H-pyran-2-ylidene)-2-propylidene]-4,6-diphenylpyrylium perchlorate (n.c., *n.c.*, H); 883. 8-[3-(6,7-dihydro-2,4-diphenyl-5H-1-benzopyran-8-yl)-2-propylidene]-5,6,7,8-tetrahydro-2,4-diphenyl-1-benzopyrylium perchlorate (n.c., *n.c.*, H); 884. 7-[3-(5,6-dihydro-2,4-diphenylcyclopenta[β]pyran-7-yl)-2-propylidene]-6,7-dihydro-5H-cyclopenta[β]pyrylium perchlorate (n.c., *0.6*, H); 885. 8-[(6,7-dihydro-2,4-diphenyl-5H-1-benzopyran-8-yl)methylidene]-5,6,7,8-tetrahydro-2,4-diphenyl-1-benzopyrylium perchlorate (n.c., *n.c.*, H); 886. Cibacron blue F3GA (n.c., *n.c.*, PS); 887. Cibacron Brilliant Blue (n.c., *n.c.*, PS); 888. Anthralan blue G conc. (n.c., *2*, AH); 889. Remazol brilliant blue R (n.c., *1.5*, AH); 890. Anthralan Blue B (n.c., *1*, AH); 891. Remazol Brilliant Blue B (0.2, *0.3*, AH); 892. [3,3-dimethyl-1-γ-sulfopropyl-3H-indole-(2)]-[cyclohexa-1,3-dien-4-one-(6)]-dimethinemerocyanine (n.c., *0.1*, H); 893. bis-[1-ethylquinoline-(2)]-azamethinecyanine iodide (n.c., *n.c.*, M); 894. 3-ethyl-2-(6′-oxohexa-2′,4′-dienylidene)benzothiazoline (0.3, *0.5*, NK349); 895. [cyclohexa-1,3-dien-4-one-(6)]-[1-ethyl-1,4-dihydroquinoline-(4)]-dimethinemerocyanine (n.c., *n.c.*, NK1937); 896. Adriamycin (n.c., *n.c.*, EA); 897. Lucifer yellow CH (n.c., *n.c.*, WS); 898. Riboflavin (n.c., *n.c.*, M); 899. 0-Cresolphthalein (n.c., *n.c.*, M); 900. Brilliant yellow (n.c., *n.c.*, M); 901. 1′-(β-hydroxyethyl)-6-nitro-1′,3′,3′-trimethylspiro-2H-1-benzopyran-2,2′-indoline (n.c., *n.c.*, KG); 902. 6-decanoyl-2 naphthol (n.c., *n.c.*, GW); 903. 6-phenacetyl-2-dimethylamino naphthalene (n.c., *n.c.*, GW); 904. 6-decanoyl-2 methylaminopropyl-methyl naphthylamine (n.c., *n.c.*, GW); 905. 1[[2-(5-dimethylaminonaphthalene-1-sulfonyl) aminoethyl]-amino]-3-(1-naphoxy)-2-propanol, HCl (0.1, *n.c.*, MP); 906. 4-(n-iodoacetoxyethyl-N-methyl)-7-nitrobenz-2-oxa-1,3-diazole (n.c., *n.c.*, MP); 907. monobromobimane (0.2, *0.02*, EK); 908. 2,4-bis-(p-dimethylaminostyryl)-1-ethylpyridinium iodide (1, *2*, AC).

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