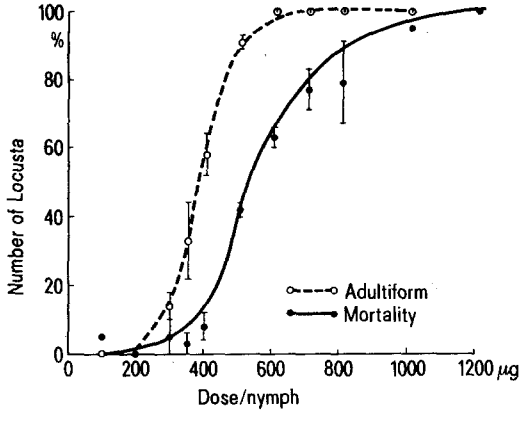
Results and discussion. Of the 6 new compounds (**3–8**) tested for anti-juvenile hormone activity, only the 5,7-dimethoxy-2,2-dimethylchromene **6** gave rise to symptoms characteristic of a disturbance in juvenile hormone function. All compounds were revealed to be more or less toxic but failed to produce precocious metamorphosis when applied in a single dose of up to 1500 µg per nymph.

The effects of analog **6** are illustrated in the figure; brackets represent standard error of the mean (SEM) and where no bracket is shown, SEM was smaller than the symbol used. The mortality curve includes all deaths occurring during the 4th instar and the subsequent molting. Mortality rate among control insects receiving only acetone was less than 5%. The adultiform curve describes the percent of adultiforms among the survivors. The effects of the 5,7-dimethoxy analog were very similar to those of precocene I and II published elsewhere[8]. The lethal dose evaluated graphically (LD_{50} = 520 µg) was comparable to those of precocene I (420 µg) and precocene II (295 µg). However, the effective dose (ED_{50} = 375 µg) was much higher than those of precocene I (85 µg) and precocene II (30 µg) indicating a loss of activity.



Dosage-mortality and dosage-adultiform production curves for *L. migratoria* exposed to 5,7-dimethoxy-2,2-dimethylchromene.

Besides the results illustrated in the figure, we have also noted the following effects:

- The compound greatly delayed the moult, and this delay increases with higher doses.
- The morphology of adultiforms varies from moderate adult characteristics to advanced adult characteristics. The effect has already been reported for precocene II by Pener et al.[4].
- The adultiforms are viable and survive at least 1 month after moult.
- The deaths occurred mostly within 24 h after the application. Only a few died during the moult.

Our results clearly indicate that any change in the chemical structure of precocene results in total or great loss of activity. The double bond is also an essential feature for the activity of precocene: we have prepared the saturated compounds corresponding to precocene I and II and found them inactive. It is also known that precocene II resistant insects species metabolise it via the epoxy-precocene[9,10]. These facts could be a severe limitation for the practical application of precocene analogs as insecticides. Search for compounds with similar biological action is under current investigation.

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Action spectra for bilirubin photodisappearance

D.A. Lightner[1], T.A. Wooldridge, S.L. Rodgers and R.D. Norris

Department of Chemistry, University of Nevada, Reno (Nevada 89557, USA), 15 September 1978

Summary. The excitation wavelength dependence of bilirubin photodestruction, as measured by quantum yields, has been determined in benzene, chloroform-1% ethanol, chloroform-1% hexane, methanol-1% concentrated ammonia, pH 8.5 aqueous buffer and pH 7.4 aqueous buffer with added albumin. The results show that in the visible spectrum the 370–490 nm excitation wavelength region is very effective in the photodestruction, but excitation in the UV-region ($\lambda < 320$ nm) is even more effective.

Although phototherapy has been used increasingly for over 20 years to treat physiologic jaundice in newly born infants[2,3], and the efficacy of the treatment as it pertains to the lamps used has been discussed from time to time[4–8], no action spectra have been reported for bilirubin, except an incomplete study of bilirubin (BR) in plasma[9]. In that work the jagged-looking action spectrum may have been an artifact of not correcting for the emission characteristics of the light source, i.e. the lamp did not have a constant emission (quanta/sec) over the region used. It is also not clear whether higher energy harmonic emission (UV) from the monochromator was filtered out – an important consideration since BR is highly UV-active. Since phototherapy owes its success, in part, to BR photodestruction[2–4,10] we judged it important to bring information to bear on the wavelength response of BR photodisappearance, especially since this crucial facet of BR photochemistry has been one of obvious interest for some time[4–9,11].

Materials and methods. Bilirubin IX*α* (BR IX*α*) was obtained from Sigma and purified by dissolving in chloro-

form, washing that solution 3 times with dilute aqueous $NaHCO_3$, evaporation and crystallization from 1:1 chloroform methanol. It contained less than 5% of the III*a* and XIII*a* isomers as determined by high performance liquid chromatography with a Dupont Zorbax-SIL column (25 cm long by 4.6 mm inner diameter) using $CHCl_3$/1% ethanol/1% acetic acid and a DuPont model 848 liquid chromatograph equipped with a model 837 variable wavelength detector set at 450 nm[12]. Action spectra were determined on 1.5×10^{-5} M solutions of BR IX*a* in benzene, carbon tetrachloride, chloroform-1% ethanol, chloroform-1% (v/v) hexane, methanolic ammonia (1%v/v concentrated NH_4OH), pH 8.5 Tris (Sigma) buffer and pH 7.4 phosphate buffer with added albumin. Some selected wavelength studies were done on Gunn rat serum. The BR solutions were irradiated in 10 mm pathlength quartz cuvettes (Pyrocell) with monochromatic light (10 nm bandpass) from a Bausch and Lomb monochromator equipped with a 200 W super pressure Hg source. At excitation wavelengths between 400 and 500 nm a 0.45 cm thick Lucite filter was placed between the cuvette and monochromator to eliminate lower wavelength harmonics. Spectral changes following irradiation were monitored using a Beckman 25 spectrophotometer.

Potassium ferrioxalate actinometry[13] was performed on the lamp at each excitation wavelength used and checked frequently. Similarly, Reinecke's salt actinometry[14] was performed at wavelengths greater than 480 nm. (All manipulations were carried out in a darkroom equipped with a red safelight filter (Kodak No. 1A).) At wavelengths between 350–480 nm a 0.15 M potassium ferrioxalate solution was used and $\Phi_{Fe^{+2}}$values were obtained from a linear least squares plot of the values obtained by Parker and Hatchard[13]. Between 250 and 350 nm a 0.006 M ferrioxalate solution was used and the $\Phi_{Fe^{+2}}$value of 1.24 (Parker and Hatchard) was used. The % of light absorbed by the actinometer solutions (a proplem at wavelengths greater than 440 nm), was, when necessary, estimated from the visible spectrum and a correction was made. The estimate is not very good because of the bandwidth difference between the spectrophotometer (narrow) and the monochromator (10 nm). Light intensities were measured before and after bilirubin irradiation and the average was taken.

Bilirubin solutions were prepared by weighing (microbalance) samples of appropriate size to produce 1.5×10^{-5} M concentration when diluted to 25 or 50 ml with solvent. Chloroform and methanolic ammonia dissolved BR immediately, whereas benzene required up to 1 h to form a clear solution. Solutions in these solvents appear to remain stable for at least 1 week when kept under refrigeration. Buffered aqueous solutions of BR were prepared by dissolving the required amount of BR in a few drops of concentrated

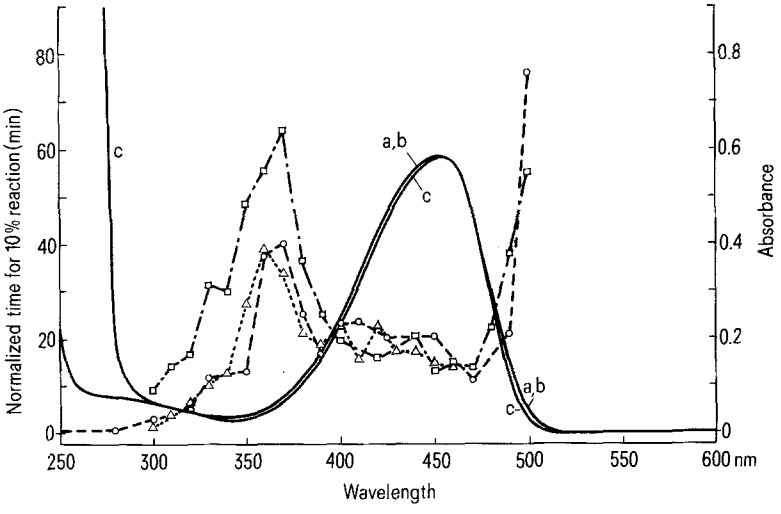

Fig. 1. Action spectra (A, B, C) and absorption spectra (a, b, c) for 1.5×10^{-5} M solutions of bilirubin-IX*a* in: 1. $CHCl_3$-1% ethanol, A [○] (----) and a (——); 2. $CHCl_3$-1% n-hexane, B [△] (....) and b (——); 3. benzene, c [□] (–.–.) and c (——). All reaction times for action spectra are normalized to a constant 4.5×10^{15} quanta/sec lamp output intensity for each excitation wavelength. Times in min.

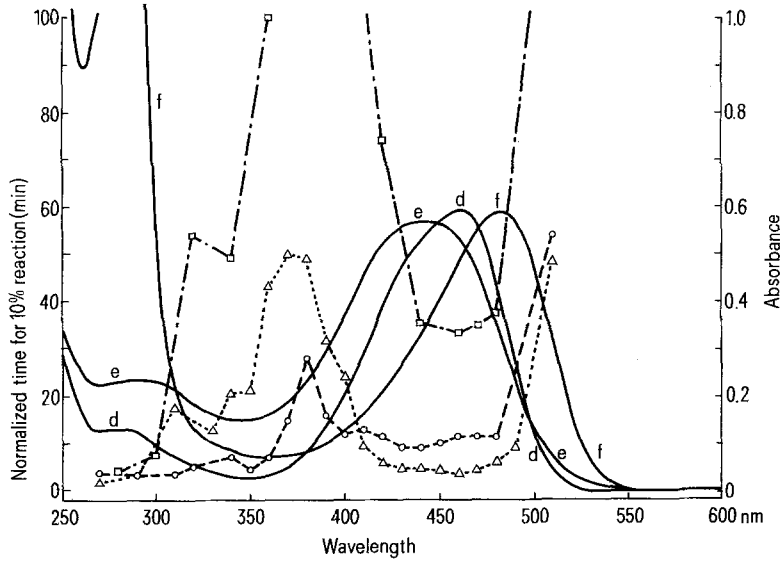

Fig. 2. Action spectra (D, E, F) and absorption spectra (d, e, f) for 1.5×10^{-5} M solutions of bilirubin-IX*a* in: 1. CH_3OH-1% concentrated NH_4OH, D [○] (----) and d (——); 2. pH 8.5 aqueous Tris buffer, E [△] (....) and e (——); 3. pH 7.4 phosphate buffer with *ca* 3×10^{-5} M fract V bovine serum albumin, F [□] (–.–.) and f (——). On curve F, the points not shown are at 370 nm (189 min), 390 nm (127 min) and 500 nm (143 min). All reaction times are normalized to a constant 4.5×10^{15} quanta/sec lamp output intensity for each excitation wavelength. Times in min.

NH_4OH and diluting at once with 0.1 M phosphate or Tris buffer as described earlier[15]. The pH 7.4 buffer contained bovine serum albumin (fract V, Sigma) as described previously[16]. The absorbance of a 2-ml aliquot of the solution was determined versus air and the sample irradiated long enough to produce as close as possible to a 10% reaction, as determined by reduction in intensity of the band at approximately 450 nm. The data were normalized to an arbitrary constant light intensity of 4.5×10^{15} quanta/sec. Benzene, chloroform-1% ethanol, carbon tetrachloride and methanol are Fisher reagent grade, re-distilled. Ethanol-free chloroform stabilized with 1% hexane was prepared by distilling 500 ml of Fisher reagent grade chloroform from P_2O_5 into 5 ml of hexane (Fisher, reagent grade) then passing the solution through 2 1.5 cm inner diameter columns each containing 25 g of Woelm (Eschwege) W200 basic alumina, activity grade Super I. Potassium ferrioxalate was prepared from ferric chloride and potassium oxalate as described[17]. Potassium Reinecke's salt was prepared and used as described[14]. Bovine serum albumin (fract V) was obtained from Sigma.

Results and discussion. Rather than representing our action spectra in the traditional format of plotting % concentration change vs excitation wavelength, we have found it more meaningful to plot the normalized reaction time required to cause an exactly 10% drop in concentration of BR vs excitation wavelength (figures 1 and 2). In this way, all reactions in each solvent and each excitation wavelength are carried to the same point. Since lamps have variable output (quanta/sec) at each wavelength as a function of lamp type and age, we routinely performed actinometry measurements to determine emission intensities at each wevelength. With those data, all reaction times could be normalized to an (arbitrary) lamp output of 4.5×10^{15} quanta/sec at each wavelength. Thus, the data are internally consistent and the profiles of figures 1 and 2 will be invariant from lamp to lamp, using the same normalization factor, since it will appear that the lamp had a continuous output of 4.5×10^{15} quanta/sec over the entire spectral range of the action spectra for each experiment.

Figure 1 shows BR action spectra in the aprotic solvents, chloroform and benzene, and figure 2 shows action spectra for the protic solvents, methanol and water. The use of organic solvents is not necessarily unrealistic. It has been a long held view that the principal photoactive site in vivo is in the skin (lipid tissues); therefore, studies of BR in lipid-like organic solvents need not be viewed as inappropriate modelling. In order to show that the ethanol stabilizer present (1%) in $CHCl_3$ has no effect on the photochemical behavior, an action spectrum was also determined in chloroform with n-hexane stabilizer[18] (figure 1). An entirely similar action spectrum was found for CCl_4 solvent. Several observations can be drawn readily upon examination of the data of figures 1 and 2: 1. expectedly, BR is very reactive in the region of its visible absorption maximum, less reactive on the short wavelength side (λ 370 nm), when the absorbance drops, and considerably less reactive at $\lambda > 500$ where absorbance approaches zero; 2. unexpected excitation at $\lambda < 340$ leads to an extremely efficient photodisappearance (even more than excitation near 450 nm), yet, BR absorbance in the region 340–250 nm is considerably less than that near 450; and 3. photodisappearance is generally slower in protic solvents with added base than in neutral aprotic solvents, but added albumin supplies the well-known protective influence on BR photo-oxidation[2,16,19,20]. A partial action spectrum of Gunn rat serum is in agreement with the aqueous albumin curve in figure 2. The Gunn rat is congenitally jaundiced and has an excess (about 10 mg%) of unconjugated BR in its serum. With Sprague-Dawley rat serum (no jaundice) and added BR, there were no qualitative differences from the data in pH 7.4 aqueous buffered albumin. With Gunn rat serum the irradiation times were (curiously) considerably greater than the above – so great in fact that literally days would have been necessary for a 10% disappearance in some spectral regions.

Observation 1. appears to be physically consistent with the absorption curve profiles. Observation 2. leads to several deductions: a) a different mechanism for BR photodisappearance must be obtainable with irradiation at shorter ($\lambda < 340$ nm) wavelengths and b) this mechanism probably contains a significant radical component, as judged from these and other relevant experiments with added free radical quenchers[21].

The data suggest that the most effective region for phototherapy is 400–470 nm. The region $\lambda < 340$ nm becomes an even more effective region, but the safety of using lamps emitting radiation at those wavelengths is questionable not only because of possible photocarcinogenicity and tissue damage by UV-radiation, but also because of the possibility of inducing free radical reactions with irradiated BR as the initiator. Particularly, for the latter reason, it seems important not to irradiate infants with lamps emitting radiation with wavelength less than 370 nm.

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