Quinine Calibration of the Aminco-Bowman Spectrophotofluorometer

Recently, spectrophotofluorometers have been developed that make possible the scanning of excitation and fluorescent spectra in the visible and ultraviolet regions. They can thereby be used for the identification and quantitative assay of organic compounds (*1*). Such instruments present the most sensitive method devised to date for the measurement of 5-hydroxyindole compounds (*2*), which are of interest in psychopharmacology (*3*) and in diagnosis of carcinoid tumor (*4*).

To date, we know of no data that have been reported on scanning spectrophotofluorometric measurements of quinine. It is of interest, therefore, to present such data, for quinine is a compound frequently used as a standard in the calibration of instruments of this type (*5*).

The recently available Aminco-Bowman spectrophotofluorometer (*6*) was used in this study. Calibration of this instrument was made with N.F. quinine alkaloid (*7*) as the reference compound. Using a quinine solution of 0.1 μg/ml in 0.1*N* H_2SO_4, we determined the excitation (activation) and fluorescent spectra by the method described in the instruction manual for the Aminco-Bowman spectrophotofluorometer (*8*). Quinine samples from two other sources gave similar results.

Several peaks were observed in both the activation and fluorescent spectra for all three samples tested. Activation peaks occurred as follows: at 265 mμ, a prominent peak; at 365 mμ, a maximum peak; at 455 mμ, a scatter peak; at 510 mμ and 750 mμ, occasional harmonics of the 265-mμ and 365-mμ peaks, respectively. These were the observed, uncorrected values. Corresponding fluorescence peaks occurred as follows: at 360 mμ, a scatter peak; at 450 mμ, a maximum peak. Thus the activation maximum was at 365 mμ (uncorrected) and the fluorescence maximum was at 450 mμ. These peaks checked to within 5 mμ on repeated tests.

Serious question exists whether 365 mμ is the true activation maximum for quinine. Other investigators using the Aminco-Bowman instrument have reported activation maxima similar to ours within 5 mμ (*9*). Activation maxima obtained on the afore-mentioned quinine samples with the Farrand recording spectrofluorometer model C occurred at 350 mμ (*10*). Absorption maxima that we obtained with the same samples on the Beckman DU spectrophotometer occurred at 348 mμ to 350 mμ. Since activation maxima should theoretically be identical with absorption maxima (*11*), this would prejudice evidence in favor of 350 mμ being the more precise activation maximum. Until more is known about the nature of the chromatic response to the activation process, it is in order to consider a correction factor of −15 (correct to within 5 mμ) for activation spectra obtained on the Aminco-Bowman instrument.

There is no question whether 450 mμ is the true fluorescence maximum for quinine, for this was the value obtained on both the Aminco-Bowman and the Farrand instruments when these were set at their respective activation maxima, namely, 365 mμ and 350 mμ. Although it would be highly desirable to have the activation and fluorescence maxima of a given compound measure to within 1-mμ precision on instruments of different manufacturers to permit absolute standardization, this is an ideal yet to be activated in the development of spectrophotofluorometry. Many instrumental factors that are known to affect fluorescence (*11*) must be standardized before this can be effected. According to Bowen and Wokes (*11*), the best practical solution to the problem at present is "to use a *figure of merit,* the lowest concentration of suitably selected fluorescent solutions whose fluorescence can be measured with an average error of some agreed amount."

Once the activation and fluorescent maxima had been worked out, the following data were obtained: (i) the widest range of quinine concentrations within which these maxima were constant (this was an essential preliminary to the development of a quinine calibration curve); and (ii) the range of quinine concentrations (within the aforementioned range) that gave a linear response as measured by fluorescence intensity. From these observations, a linear calibration curve for quinine could be plotted. By determining one or two values on the quinine calibration curve from day to day, we could thus rapidly check the instrument for accuracy before proceeding to the assay of other compounds.

The results are set forth in detail in Table 1. For this work, all quinine concentrations were made up in 0.1*N* H_2SO_4. Only maximum activation and fluorescence peaks are listed; activation maxima were corrected by subtracting a factor of 15 mμ for the reasons described. Corresponding activation and fluorescence maxima were constant over a quinine range from 0.005 to 10 μg/ml. A reagent blank consisting of 0.1*N* H_2SO_4 exhibited per se only scatter peaks throughout the range of the wavelength disks. Fluorescence intensity was obtained by multiplying the galvanometer needle deflection (transmission scale) by the meter multiplier readings on the Aminco photomultiplier microphotometer. A plot from data in Table 1 of fluorescence intensity (ordinate) versus quinine concentration (abscissa) measured at activation maximum of 350 mμ (corrected) and fluorescence maximum 450 mμ reveals that the region of linearity in the resulting calibration curve extends from a concentration range of 0.005 to 0.1 μg/ml, approximately. This compares favorably with data obtained by Wokes *et al.* (*12*) on the Spekker fluorimeter.

Table 1. Effect of concentration on the spectral activation and fluorescence maxima of quinine.

Quinine concentration (μg/ml)	Wavelength (mμ)			Fluorescence intensity
	Activation maximum		Fluorescence maximum	
	Observed	Corrected		
100.0	380	365	450	59.5
10.0	365	350	450	20.7
1.0	365	350	450	2.44
0.10	365	350	450	1.87
0.08	365	350	450	1.59
0.05	365	350	450	1.04
0.03	365	350	450	0.65
0.01	365	350	450	0.23
0.008	365	350	450	0.19
0.005	365	350	450	0.12
0.001	365	350	410	0.06
0.0005	365	350	405	0.04
0.0001	375	360	405	0.05

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Minnesota Cretaceous Pine Pollen

Although plant fossils from Cretaceous sediments in Minnesota are generally poorly preserved and fragmentary, there have been occasional noteworthy finds. Chaney (*1*) has described a pine cone (*Pinus clementsii*) from Springfield, Minn., that is morphologically identical to the cones of *P. resinosa* Ait., the red pine, a species still found in the northeastern part of the state. Contemporaneous deposits from New Ulm, 25 miles east of Springfield, have yielded impressions of pine needles also similar to *P. resinosa.*

This report (*2*) is based on a preliminary analysis of sediments that occur in the vicinity of New Ulm. These deposits are assigned to the early Upper Cretaceous Dakota Series [(sensu lat.) (*3*)] and represent sediments deposited on flood plains, in stream and river channels, and in lakes or catchment basins. Extensive exposures occur sporadically along both banks of the Big Cottonwood River between Springfield and New Ulm. Coarse to fine sands, often cross-bedded and occasionally cemented, comprise the main fabric of the formation in the area, but seams of shaly clays ranging in thickness from a few millimeters to several feet are not uncommon. In some of the clay seams, intact leaf cuticles occur; because of their excellent preservation, it was thought that plant microfossils might also be present.

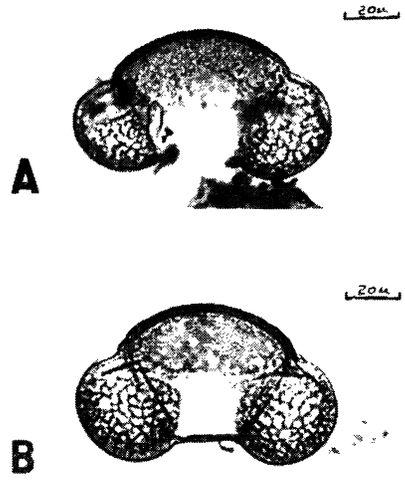

Fig. 1. (*A*) Lateral view of Cretaceous pine pollen. *Pinus resinosipites* sp. nov. (low mid-focus; slide PKP 24L-3; location, 18.9 × 119.2 (ref. 57.7 × 127.6). Vesiculated grain; bladders with coarse, internal reticulations, constricted at base (*sylvestris* type); marginal crest poorly developed; exine granular; size across widest part of body exclusive of wings, 58.4 μ; size including wings, 84.5 μ. The holotype is in the paleobotanical collection at the University of Minnesota (*7*). (*B*) Lateral view of red pine pollen.

Past analysis (*4*) of sediments of this age yielded little information, for only spores of undetermined botanical origin were recovered. My samples, when treated by Traverse's methods (*5*), provided moderately abundant spores and pollen the preservation of which is comparable to that of pollen from early postglacial peat deposits. The comparative analysis of these microfossils is still in its initial stages, but sufficient observations have been made to indicate that abietineous pollen is the most abundant (*6*).

In the accompanying figure, one such grain (Fig. 1*A*) is shown above a grain from red pine (Fig. 1*B*); both grains have been acetolyzed. There is no discernible difference between this grain and the pollen of *P. resinosa*; however, this is not unequivocal evidence that the two grains are specifically identical. Interspecific variation in the pollen of the Abietineae is slight. Descriptively, the pollen of *P. strobus, P. echinata, P. sylvestris,* and Wodehouse's pollen species *P. scopulipites* seem quite similar to the pollen of *P. resinosa.* Differences in body size and marginal crest development are not great enough for reliable use in identification of single grains, but Cain's (*8*) size-frequency data do show that this grain's body size corresponds with the average for *P. resinosa.*

With the addition of this information to the fossil record, together with the cone and foliage previously described by Chaney, it appears likely that one of the Cretaceous pines of Minnesota has a counterpart in the living red pine. It would seem unwise to conclude that red pine has existed as a specific entity since the Cretaceous, for its Tertiary record is unknown, but the morphological evidence is temptingly suggestive. If the relationship indicated by the fossil record was accompanied by an ecological similarity, there must have been heights of land sufficiently elevated to cause a vertical floral zonation, for the remainder of the fossil record of this stage indicates warm temperate conditions.

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