followed closely so as to add fresh droplets as the xylol and chloralphenol evaporate. Finally the material is mounted in pure balsam and can be kept indefinitely.

Counterindication of this technique holds only for hyaline spores or mycelia which are scarcely visible in balsam. But wherever a slight coloration or a sufficiently thick membrane is present, details are sufficiently visible to permit taxonomical or anatomical observations.

There is another drawback to this technique when free spores are present on the slide. These can be displaced or drawn out from under the cover slip when the slide is levigated with the two media. When such is foreseen, one can place the material directly in a mixture of chloralphenol and balsam diluted in xylol in the proportion of two droplets to one. The chloralphenol insures the clarification of the material and the penetration of the balsam. With the cover slip on, the slide is gently heated until the chloralphenol has completely evaporated. Here again, attention must be paid to feeding fresh droplets of Canada balsam as the mounting medium dries up.

This latter procedure does not apply to fresh material but only to herbarium material or one that has been dried beforehand.

This technique has been very useful to the writer to prepare quickly permanent mounts of abundant herbarium material that had to be examined at short notice, while keeping a permanent record of the observations.

Chloralphenol is prepared by mixing by weight two parts of chloral hydrate to one part of phenol crystals. The mixture, liquefied by gently heating it on a flame, can be kept in dropping bottles.

This technique may not be totally unknown, as Langeron seems to infer. In any case, the writer has seen no reference in the botanical or phytopathological literature to its having been used.

Fluorophotometric Determination of Rutin and Other Flavones

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A great deal of interest has developed in the therapeutic use of rutin for the treatment of reduced capillary resistance in certain hemorrhagic diseases (*1*, *3*). Rutin is a flavone glucoside which yields quercetin, glucose, and rhamnose on hydrolysis with dilute acids (*2*). Most flavones react with boric acid to form highly colored derivatives which can be adapted to colorimetric determinations (*5*, *6*). We have applied the boric acid reaction to the determination of rutin and have also improved the sensitivity of the method by measuring the intensity of fluorescence with a photometer, instead of measuring the optical density of the colored solution. We have also found that the fluorometric method can be used to advantage with purified quercetin, and presumably with other flavones which react with boric acid.

A stock solution of recrystallized rutin[1] in dry acetone was diluted to give a series of standards containing known concentrations of rutin. The boric acid reagent was prepared by mixing equal volumes of a saturated solution of boric acid in acetone and a 10 per cent citric acid solution in acetone, as described by Wilson, Weatherby, and Bock (*6*). The reagent was added to the rutin standards in the ratio of four volumes to one of rutin solution. Colorimetric measurements were made with a Klett-Summerson photoelectric colorimeter using a blue filter (No. 42), and the fluorescence measurements were made with a Pfaltz and Bauer Model B fluorophotometer. The mercury vapor light in the fluorophotometer was passed through a set of filters having a peak transmission in the violet band at 430–440 mμ. The degree of fluorescence was measured with a photocell through a yellow filter having a sharp cutoff at 520 mμ. Fluorescence was also observed using ultraviolet light below 370 mμ; but the photometer readings were higher with the violet light source which was finally chosen for this work. The rutin-boric acid solutions exhibited a green fluorescence which, on examination with a spectroscope, appeared to extend from 510 mμ to 620 mμ.

Calibration of the fluorophotometer with rutin standards produced the results shown in Table 1. The degree of fluorescence appears to be directly related to the concentration of rutin and affords a convenient method for its determination. The advantages of fluorimetry over colorimetry are also demonstrated in Table 1, where the readings with different concentrations of rutin are compared. However, the boro-citric reagent itself exhibits some fluorescence which varies with different lots of reagent (*6*). The effects of moisture, concentration of reagents, time of standing, and other variables have been worked out, and the fluorimetric method is now being applied to the determination of rutin in biological materials, using procedures for extraction similar to those already developed for flavones (*4*, *6*).

TABLE 1

COMPARISON OF FLUORIMETRIC AND COLORIMETRIC READINGS WITH RUTIN-BORIC ACID SOLUTIONS

Rutin/ml. sample (μg.)	Colorimeter readings	Fluorimeter readings
10.0	27.0	67.0
8.0	22.0	55.0
6.0	17.0	43.0
4.0	12.0	30.0
2.0	8.0	16.0
1.0	5.0	9.0
0.50	3.5	5.5
0.25	2.0	4.0
0.10	1.0	3.0
0	0	2.0

[1] The rutin used in these experiments was obtained through the courtesy of Dr. M. J. Copley, Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia.

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