(purified through the methoxy compound) in 500 cc. of water containing 4.3 g. of sodium bicarbonate was treated with 20 cc. of 30% hydrogen peroxide (superoxol). The solution rapidly darkened and, after standing for thirty-four hours, a crop of dull red large leaves had separated, 3.2 g. (43% based on 2-hydroxy-1,4-naphthoquinone utilized). One crystallization from dioxane gave pure isonaphthazarin. Acidification of the aqueous filtrate yielded 2.1 g. of orange precipitate which consisted of unchanged 2-hydroxy-1,4-naphthoquinone plus a small amount of isonaphthazarin. On several occasions, use of less pure 2-hydroxy-1,4-naphthoquinone as starting material led to much lower yields.

2-Hydroxy-3-(β-cyclogeranyl)-1,4-naphthoquinone.—Isonaphthazarin (2.0 g.) was reduced as described by Fieser and Gates[1] and the leuco compound heated in the dark under nitrogen for forty-eight hours at 65–70° with 1.0 g. of β-cyclogeranioi[2] (m. p. 44°), 0.6 g. of anhydrous oxalic acid and 20 cc. of dioxane. The processing of the reaction mixture included the following steps: extraction of the unchanged leucoisonaphthazarin with aqueous hydrosulfite, reduction with concentrated aqueous hydrosulfite, and extraction from ether–petroleum ether with Claisen's alkali. The crude phenolic portion thus obtained was chromatographed after air oxidation on freshly ignited magnesium sulfate. On development with petroleum ether, a weakly adsorbed bright yellow band readily passed into the filtrate. Similar filtrates from systematic readsorptions of the column eluate were combined and on concentration to dryness under reduced pressure afforded 99 mg. of solid residue which after three crystallizations from ether–petroleum ether gave 36 mg. of golden yellow rectangular plates, m. p. 135–135.5°. It is quite soluble in the ordinary organic solvents, fairly soluble in warm petroleum ether, much less soluble cold, and dissolves in dilute alcoholic alkali to give the beautiful scarlet characteristic of alkali salts of 2-hydroxy-1,4-naphthoquinones. It dissolves in concentrated sulfuric acid to give a deep orange-red solution. Two further crystallizations to obtain a sample for analysis did not alter the melting point.

Anal. Calcd. for $C_{20}H_{22}O_3$: C, 77.38; H, 7.15. Found: C, 77.50; H, 7.23.

β-Cyclogeranolapachone (I).—A solution of 11 mg. of 2-hydroxy-3-(β-cyclogeranyl)-1,4-naphthoquinone in ice-cold concentrated sulfuric acid (0.3 cc.) was allowed to stand several minutes, then diluted with ice water. The precipitated dark orange-brown material was taken into ether, washed with water, bicarbonate and brine, and concentrated to dryness. The residue was taken into benzene–hexane and chromatographed on freshly ignited magnesium sulfate. Development with 50% benzene–hexane left a broad salmon-pink band in the middle of the column which was sectioned out and eluted with ether. After evaporation of the ether, the solid residue was crystallized twice from pure acetone to give 3.5 mg. of orange-red prismatic blades, m. p. 232–233.3°. A mixed melting point with β-geranolapachone prepared according to Fieser and Gates[1] showed no depression.

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Riboflavin Estimation in Fruits and Vegetables

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As part of a collaborative project,[1] it was recently necessary to make a series of chemical determinations of thiamin and riboflavin in certain fruits and vegetables, and the Conner–Straub procedure[2] was followed. Unfortunately, at the beginning, Supersorb,[3] the specific adsorbent for riboflavin was unavailable. An empirical method was, therefore, evolved, and we hoped, later, to correlate results into the series by concurrent assays on additional samples on arrival of the adsorbent. This comparison may now be made and, subject to certain provisos, we believe the modification accurately reflects differences in riboflavin content within a series. With respect to absolute values, it is in accord with microbiological assay by means of *Lactobacillus casei*. It has, further, certain advantages: increased light stability, no adsorbent is needed and the riboflavin in the aqueous buffer exhibits approximately twice the fluorescence found in pyridine–acetic solution, with consequent decrease in the percentage reading error.

The Conner–Straub procedure is followed in detail in extraction and preparation of the sample, except that, in the case of fruits, 10 ml. of pectinol (1 g. in 25 ml.) is added per 50-ml. of sample, in addition to the clarase. The whole is then incubated at 45° for two hours. The pectinol is absolutely necessary for prunes, apricots, dates, etc., to produce a satisfactory solution. A 10–20 ml. aliquot is then heated to boiling with 5 ml. of 2% acetic, as in (1), made to volume, 50 ml., with buffer, and a 15-ml. aliquot treated for a minimum of three minutes with 1 ml. of potassium permanganate, and decolorized with 3 ml. of 3% hydrogen peroxide. The solution is then filtered and compared with buffered standards at *p*H 6.0 in a Coleman fluorophotometer. The B_2 filter for the exciting light (Hg arc) cuts out completely above 4900 Å. and for the fluorescent light, below 5100 Å. The cut-out is sharp, and, for the latter filter, the transmission rises from zero at 5100 to over 90% at 5400 Å. Quinine sulfate and thiochrome have no effect on the galvanometer with these filters, at least in the concentrations used. In the majority of plant extracts treated as above, there appear to be no other water-soluble fluorescent compounds in sufficient quantities to interfere, though trouble might be anticipated in those botanical families where anthraquinone glucosides occur. However, since we do not know the behavior of these compounds on Decalso or Supersorb, similar difficulties might arise with either method.

(1) With the Department of Home Economics.

(2) Conner and Straub, *Ind. Eng. Chem., Anal. Ed.*, **13**, 385 (1941).

(3) Supersorb, Florisil or Floridin is a Fuller's Earth; Decalso, a synthetic zeolite, obtainable through supply houses. Clarase (Takamine Laboratories, N. Y.) and Pectinol (Röhm and Haas, Philadelphia) are commercial enzyme preparations.

TABLE I

RIBOFLAVIN ESTIMATES IN FRUITS AND VEGETABLES[a] IN MICROGRAMS PER GRAM

Material	Aqueous buffer, no adsorption	20 hours later	Pyridine–acetic adsorbed	20 hours later	Microbiological[b]	Thiamin, μg./g.
Asparagus, fresh	1.15	1.00	1.13	0.45		2.19
Asparagus, blanched	1.25		1.18			2.60
Broccoli, fresh	...		0.79			0.77
Broccoli, dehydrated	6.28		4.30			3.71
Broccoli, dehydrated	8.66		7.16			3.97
Broccoli, dehydrated	13.0		13.4		12.6	7.25
Peas, fresh	0.86	0.65	0.67	.22	1.5	1.16
Peas, fresh	.80		.72			0.91
Peas, cooked	.83	0.63	.78	.33		1.00
Peas, dehydrated	4.01		5.73		5.5	4.38
Peas, dehydrated	3.36		4.78			4.16
Spinach, dehydrated	23.4		18.6		23.7	10.2
Rice bran, concd.	7.74	6.85	5.44	2.71		141.0
Apricots, dried, sulfured	1.94	1.94	1.63	0.51		0.21
Prunes, dried	1.59	1.64	1.27	.20		1.24
Dates, Deglet Noors	1.14		0.30			0.53
Dates, Deglet Noors	0.73	0.77	0.40	.05		0.52
Grass, dehydrated	8.23	7.80	11.5	4.78		5.28

[a] It should be understood that the estimates on the materials assayed are valid only for these samples, purchased for the most part on the local market, without consideration of variety, maturity or possible abnormal local conditions caused by climate, Jan.–March, 1942. [b] We thank Miss M. B. Smith of the Department of Home Economics for these values.

Riboflavin estimates are given, Table I, in column 2 by the above procedure, and in column 4 after passage over Decalso and Supersorb, where the Conner–Straub procedure for both B_1 and B_2 is followed. The same samples measured twenty hours later, columns 3 and 5, after standing in the laboratory away from direct lighting, clearly show the higher light stability of our extract. We are greatly indebted to Professor Agnes Fay Morgan for permission to include, in column 6, certain microbiological assays on the same samples with *Lactobacillus casei*. They indicate very satisfactory agreement with the chemical method over the range 1 to 20 μg. per gram of sample. We include thiamin values in column 7. The rice bran concentrate, a trade product, is of interest because the thiamin value is approximately 10% higher than the minimum stated on the label, the riboflavin roughly 8 or 35% lower, depending upon whether we take the value of column 2 or 4. The thiamin content of the sulfured apricot is low, as might have been predicted.

With two reproducible exceptions, grass and peas *after* dehydration, our simpler procedure yields consistently higher results. In the case of the dehydrated peas there is definitely interference, possibly from a compound which forms a discrete yellow zone on the Supersorb, not found in the fresh peas, removed with the riboflavin by the eluting solvent. The values listed in Table I for a given vegetable are from different samples, variously treated. In general, at levels of 20 μg. per g., the results are reproducible within 5–10%, and 5–20% at 1.0 μg. per g.

The effect of diffuse light on the standards is shown in Table II. The concentration used at zero time is approximately 0.1 μg. per ml.

Our simpler procedure is certainly worth consideration where the adsorbent is unavailable, because comparative variations are reflected with accuracy in the figures. It is necessary to be more cautious in considering absolute values. Discussing specifically the vitamin A potency of foods, Booth[4] suggests that physiological responses should be expressed in International Units, and that only chemical results should be based on the gram and the liter. We may also note that it does not follow *a priori* that either of the above procedures has been absolutely (as distinct from comparatively) calibrated against *Lactobacillus* or any other bio-assay. In other words, the true chemical concentration may not represent the real biological potency. This may be illustrated in the case of carrots and spinach, where the former may have 2 to 4 times the carotene content, but where there is still doubt concerning the relative biological potencies, in terms of vitamin A bio-assay.

TABLE II

EFFECT OF LIGHT ON PERCENTAGE RETENTION OF RIBOFLAVIN IN STANDARDS

Time in hours	0	3	6	24
Pyridine–acetic	100	81.1	64.9	32.4
Buffer, *p*H 6.0	100	91.5	88.1	76.3

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(4) Booth, *Food Manuf.*, **17**, 60 (1942).