females was not indicated by a significant difference in hemoglobin concentration of the blood at the beginning of the test period. Elvehjem[3] has stressed the necessity of exhaustion of body iron stores in preparation of test animals, and the question arises as to what evidence of exhaustion can be accepted. Allowing the hemoglobin level to fall too low results in animals which are sickly and not capable of a normal response to the iron supplement given subsequently.

Whatever is the explanation of this greater hemoglobin regeneration in anemic female rats as compared with males, it is the authors' belief that ignorance of this fact may explain some of the discrepancies of the same magnitude in the findings in various laboratories relative to the availability of iron in foodstuffs.

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A CATALYTIC METHOD FOR THE PREPARATION OF α-PYROABIETIC ACID

THE preparation of the so-called pyroabietic acids by the usual prolonged high temperature treatment of rosin[1, 2] entails very considerable pyrolytic decomposition, with consequent contamination of the resulting product. In connection with recent experiments on dehydrogenation of rosin products (rich in pimaric acids), by way of palladium charcoal,[3] it was noted that an appreciable proportion of a positive rotating acid survived the high temperature (300–325° C.), a region normally well above that at which decarboxylation of rosin acids takes place. The isolated acid did not give a crystalline sodium salt characteristic of α-pimaric acid, nor did it have its optical properties.[4] Its melting point (171–172° C.), rotation ($[\alpha]_d^{20} + 54°$; $[\alpha]_j^{20} + 58°$) and other properties agree well with those of α-pyroabietic acid described by Dupont-Dubourg, Fanica and others. Subsequent experiments with the palladium charcoal catalyst showed that the isomerization can be carried out at much lower temperatures (250° C.) and completed in about two or three hours. The yield at the lower temperature is excellent, the product quite uniform and apparently unaccompanied by the usual intermediate isomers. Acids with the same properties were obtained with this catalytic procedure from α-pimaric acid, l-abietic acid (Schulz), mixed rosin acids and rosins from longleaf and slash pines (*Pinus palustris* and *Pinus caribaea*) and French gum (*Pinus pinaster*). This finding, which would indicate highly selective isomerizing action for the catalyst, is in marked contrast with results obtained by the usual 100-hour heating without a catalyst when applied to rosin acids and rosins from different sources.[2]

Preliminary experiments showed that palladium charcoal catalyzes the isomerization even at 200° C., but not as effectively as at higher temperatures. Platinum charcoal, nickel charcoal and, to a lesser extent, activated charcoal itself also catalyzed the formation of pyroabietic acid.

This laboratory is at present engaged in a systematic study of the application of various catalysts and different types of carriers to the primary rosin acids, as well as the rosin acids or partially isomerized acids. Publication of more comprehensive data is contemplated in the near future.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

PRESERVING THE NATURAL COLOR OF GREEN PLANTS[1]

IMPROVED teaching methods of botanical subjects demand better demonstration materials. Living specimens should be preferred to non-living ones. However, increased difficulties in obtaining living specimens forces the use of more preserved forms. Better methods of preservation are needed to increase attractiveness of dead specimens. Several methods have been published which are more or less useful. Keefe's[2] method is outstanding among these. The writer has experimented with older formulae as well as new combinations for a period of about three years. Out of this work success with one new, general method seems to justify publication.

Formalin-acetic acid-alcohol solutions (5 cc of commercial formalin, 5 cc of glacial acetic acid and 90 cc of 50 per cent. ethyl alcohol; or 10 cc of commercial formalin, 5 cc of glacial acetic acid and 85 cc of 70 per cent. ethyl alcohol) are in general excellent preservatives. They are being used extensively for museum and histological materials. By adding 0.2 gram of copper sulfate to 100 cc of either of these F.A.A. formulae, a preservative results which will bring about an almost normal green color in nearly all

[1] Dupont and Dubourg, *Bull. Inst. Pin.*, 51: 181, 1928.
[2] Fanica, *Bull. Inst. Pin.*, 44: 155, 1933.
[3] Method of Ruzicka and Waldman, *Helv. Chim. Act.*, 16: 842, 1933.
[4] S. Palkin and T. H. Harris, *Jour. Am. Chem. Soc.*, 55: 3683, 1933.

[1] Papers from the Department of Botany, the Ohio State University, No. 383.

[2] Keefe, Anselm Maynard. SCIENCE, 64: 331–332, 1926.

chlorophyll-bearing plant organs. It is not necessary to weigh the copper. A stock of the preservative may be saturated by adding an excess, and the remaining undissolved copper removed, or a lump may be dropped in the preservative along with the specimens and left until proper color fixation occurs. When the latter method is employed the specimens should be shaken after standing a few hours to insure complete distribution of the copper. Successful results also may be obtained even if the copper is not added for six to eight hours after the specimens enter the solution. Some difficulty may be experienced, especially with certain algal cultures in which excess carbonates are present. A bluish-white precipitate (probably copper carbonate) may accumulate if excess copper is added and allowed to remain. Removal of the extra copper after saturation prevents much of this. Ordinarily the usual discoloration occurs soon after specimens enter the preservative, but after three to four days in the F.A.A.-copper sulfate solution usually a green color appears. By watching development of its intensity and removing specimens when the proper color is obtained, excellent specimens may be secured. They are transferred then to a copper-free F.A.A. solution, 70 per cent. alcohol or other preservatives for permanent storage. In a few plants, such as *Berberis* and *Ophioglossum,* some difficulty may be experienced in obtaining sufficient penetration for rapid development of the proper green color. However, if such specimens are boiled in the preservative for fifteen to twenty minutes good results follow. Care must be taken to stop the heating when coloration has developed to the proper point, and to transfer specimens to a copper-free solution. This quick method may be employed wherever heating is not injurious to the plants.

The color reaction may also be hastened by exhausting air from tissues immediately after specimens enter the preservative. This can be done easily by means of the common vacuum pump which is run by water-supply pressure. This is especially usable for fern gametophytes and young sporophytes. In these two cases permanent coloration can be obtained within fifteen to thirty minutes. Thicker tissues should be allowed to stand in the copper solution for a day or so after air exhaustion.

The addition of copper sulfate to Transeau's Algal Preservative also gave similar results as for the above. Dr. E. N. Transeau developed this preservative over twenty years ago. It is an excellent preservative for algae as well as for general preservation. The formula calls for 6 parts water, 3 parts 95 per cent. ethyl alcohol and 1 part commercial formalin. If marine algae are to be preserved sea water is used in making up the solution. After fixation has occurred, 5 to 10 per cent. glycerine may be added to prevent destruction of algal specimens in case of loss of preservative by evaporation.

Several tissues preserved in F.A.A.-copper sulfate solution have been sectioned and stained. Cellular structure is preserved the same as for straight F.A.A., and no apparent difficulty in staining has been encountered. The copper sulfate even enhances differentiation in some cases. This may be due to the copper salt rendering the tissues more acid. The green color can not be held with sufficient intensity to permit use in the Venetian Turpentine Method without further staining. Efforts in this direction have been made, using fern gametophytes and moss protonemata.

The chief advantages of this method and its modifications are: (1) a green color closely approximating that of ordinary chlorophyll is obtained; (2) preservative ingredients are easily secured and inexpensive; (3) the method is rapid; (4) fixation is sufficiently good for many histological problems; (5) color fixation does not interfere with staining; (6) and the preservative gives successful results with numerous representatives of Algae, Bryophytes, Pteridophytes, Gymnosperms and Angiosperms.

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A MODIFIED QUINHYDRONE ELECTRODE FOR TISSUES[1]

THE quinhydrone electrode has enjoyed extensive use in the determination of the pH of physiological systems. Its reliability in solutions containing proteins was investigated extensively by Shau-Kuang Liu[2] in 1927. Recently, Pierce[3] and Pierce and Montgomery[4] developed a micro-modification of the Cullen[5] electrode and used it successfully to determine the pH of the glomerular urine of Necturus and the aqueous humor of rachitic rats. With this type quinhydrone electrode the broken skin of the animal in contact with the saturated potassium chloride solution completes the junction of the two half cells. To avoid this, which gives rise to erratic potentials with the intact skin and practical difficulties with the broken skin, the following modification of the quinhydrone electrode was designed and found serviceable in tumor tissues where sufficient fluid was present to fill the capillary.

With this quinhydrone electrode a series of Hastings[6] and Sendroy's phosphate buffer mixtures was measured at 20° C.

[1] The expense of this work was defrayed in part by a grant from the International Cancer Research Foundation.

[2] Shau-Kuang Liu, *Biochem. Z.*, 185: 243, 1927.

[3] J. A. Pierce, *Jour. Biol. Chem.*, 111: 501, 1935.

[4] J. A. Pierce and H. Montgomery, *Jour. Biol. Chem.*, 110: 763, 1935.

[5] G. E. Cullen, *Jour. Biol. Chem.*, 83: 535, 1928.

[6] A. B. Hastings and J. Sendroy, *Jour. Biol. Chem.*, 61: 695, 1924.