5. BLOCH, K. *Phys. Rev.*, 1947, **27**, 574.
6. BUCHANAN, J. M., and HASTINGS, A. BAIRD. *Physiol. Rev.*, 1946, **26**, 120.
7. EVANS, E. A., JR. *A symposium on respiratory enzymes.* Madison: Univ. of Wisconsin Press, 1942. P. 197.
8. GAFFRON, H., FAGER, E. W., and BROWN, A. Reported at Isotope Symposium, Univ. Wisconsin, 1947.
9. HILL, R., and SCARISBRICK, R. *Nature, Lond.*, 1940, **146**, 61.
10. HOLT, A. S., and FRENCH, C. S. *Arch. Biochem.*, 1946, **9**, 25.
11. LORBER, V., LIFSON, N., and WOOD, H. G. *J. biol. Chem.*, 1945, **161**, 411.
12. MCALISTER, E. D., and MYERS, J. *Smithsonian Instn Publ.* (Misc. Coll.), 1940, **99**, No. 6.
13. OCHOA, S., MEHLER, A., and KORNBERG, A. *J. biol. Chem.*, 1947, **167**, 871.
14. RUBEN, S., KAMEN, M. D., and HASSID, W. Z. *J. Amer. chem Soc.*, 1940, **62**, 3443.
15. VENNESLAND, B., CEITHMAL, J., and GOLLUB, M. C. *J. biol. Chem.*, 1947, **171**, 445.
16. WARBURG, O., LÜTTGENS, W. *Naturwiss.*, 1944, **32**, 161, 301.
17. WEINHOUSE, S., and MILLINGTON, R. H. *J. Amer. chem. Soc.*, 1947, **69**, 3089.
18. WOOD, H. G. *Physiol. Rev.*, 1946, **26**, 198.

IN THE LABORATORY

Sodium Chlorite as an Aid in Paleobotanical and Anatomical Study of Plant Tissues

E. S. BARGHOORN

Biological Laboratories and *Botanical Museum, Harvard University*

In the study of plant tissues, both living and fossil, it is frequently desirable to prepare bleached and cleared whole mounts of various plant parts. The identification of leaf and root fragments as well as other plant remains in peats, brown coals, and organic sediments is frequently hindered by the presence of "humic" substances and amorphous brown residues which obscure the structural residues of the original plant tissues. Similarly, in the anatomical study of living plants it is frequently necessary to extract a wide variety of pigments and other colored substances in order to clarify cellular details of floral organs, epidermal structures, or the vascular tissues of various organs or parts prepared as whole mounts. Plant remains which occasionally occur abundantly in archeological sites are often very fragmentary and friable, a condition which renders them difficult to study by the ordinary histological methods of embedding and sectioning. Such plant remains, however, commonly possess distinctive anatomical features of value in identification when the delicate tissues are carefully bleached and cleared.

Partial decolorizing and clearing of plant parts, both living and fossil, is often accomplished by the use of dilute aqueous NaOH or KOH. Fossil plant fragments and delicate tissues of living plants, however, frequently become excessively soft and almost gelatinous after caustic treatment, making it extremely difficult, if not impossible, to prepare satisfactory slides for study and reference. This is particularly true of fossil plant tissues, which not uncommonly dissolve after prolonged exposure to caustic.

In recent studies of the macroscopic plant remains in postglacial peats and tertiary lignites it was found necessary to develop a technique for extraction of the lignin and "humic" substances with a minimum of chemical and physical alteration of cellulosic residues. By such a technique structurally intact tissues as well as microfossils represented by pollen grains, spores, and diatoms might be freed from obscuring colloidal residues.

The method developed for this purpose has proven entirely satisfactory. Subsequent experimentation has shown it to be adaptable for many other purposes in the study of plant tissues where there is need for strong bleaching with little loss of strength or tendency for maceration. The procedure is essentially a modification of the Cross and Bevan method for the extraction of lignin from cellulose. It is based on techniques developed in cellulose research in the paper industry, utilizing sodium chlorite as the oxidizing and bleaching agent (*1–5*).

For the extraction of heterogenous plant fragments from peats and incompletely coalified brown coals the following procedure has been useful:

(1) Swell and partially deflocculate the material in 5–10% aqueous KOH for 6–24 hrs at room temperature. The action, if too drastic, can be curtailed by the use of 50% alcohol instead of water. After treatment, the softened mass may be carefully broken apart to accelerate subsequent treatments. Brown clays rich in organic matter often deflocculate completely after boiling in 10% sodium carbonate.

(2) Wash with water by successively decanting to remove excess caustic and soluble humic substances. If microfossils are to be recovered, a portion of the mixture may be washed and centrifuged after alkali deflocculation and the pollen grains and spores removed for acetolysis or other treatment. At this stage, larger plant fragments such as roots, rhizomes, and small seeds may be removed.

(3) Flood the residues with lactic acid (85%) or dilute mineral acid sufficient to neutralize the remaining alkali. With acidification the color of the plant tissues becomes much lighter, and the solution clarifies somewhat.

At this stage the peat or lignite residues may be examined in a binocular microscope and the discrete fragments removed.

(4) Decant the excess acid and add an excess of 2–5% aqueous solution of sodium chlorite. If the mixture is sufficiently acid, the prompt evolution of chlorine dioxide takes place. The degree of acidity determines the speed of the reaction. Chlorine dioxide resembles chlorine in its corrosive properties, and the reaction is best carried out in a hood, although a covered (not sealed) container may be used in a well-ventilated room.

The bleaching action may take several hours. If necessary, the acidified chlorite solution should be renewed.

(5) After chlorination the bleached cellulosic and cuticular residues are washed thoroughly in water and mounted in glycerin, glycerin jelly, or other media.

This procedure, which may be employed on any unconsolidated organic sediment, can be variously modified. The primary purpose is to extract discrete plant fragments and to bleach them with a minimum of chemical or mechanical degradation. If the plant fragments can be removed without swelling and deflocculation of the matrix, steps 1 to 3 may be eliminated. Pretreatment is not essential for the bleaching action, although it accelerates it.

The sodium chlorite method was tried after numerous modifications of the Cross and Bevan procedure had been tested. With other methods little success was encountered in the recovery of delicate structural residues such as the epidermal layers of roots, leaf cuticles, and other structures which tend to fragment, "clump," or even completely dissolve during successive washings, centrifuging, and other drastic manipulation. The great advantage of the chlorite procedure is that virtually complete delignification and "dehumification" can be accomplished in one solution and at an easily controlled rate. The speed of the action may be varied with the concentration and acidity of the chlorite solution. Similar results are obtainable by the use of sodium hypochlorite solutions (commercial bleaches). These, however, are unsatisfactory because of more intense oxidative action resulting frequently in the dissolution of delicate cellulosic residues.

Sodium chlorite is a salt of somewhat unstable composition. Under ordinary laboratory conditions it is perfectly safe to handle without special precautions. High temperatures and intimate contact with easily oxidizable substances such as sulfur and rubber, however, can lead to rapid decomposition or explosion, and some care is necessary in using the reagent. In aqueous solution sodium chlorite slowly decomposes, particularly in the presence of light.

Sodium chlorite has an oxidation potential between hypochlorite and hydrogen peroxide, even when used in fairly acid solutions (*3*). Chlorite oxidation is sufficiently slow, therefore, that presumably no oxidative degradation of plant cellulose takes place except after prolonged exposure. This is apparently true of its behavior not only with normal plant tissues but also on the degraded cellulosic residues of fossil plants.

Extensive use of sodium chlorite solutions in the preparation of fossil plant fragments for anatomical study indicates that the resulting residues are essentially the remaining "holocellulose" fractions of the fossil plant tissues. Determination of the cuprammonium fluidity of such residues indicates that the fossil plant "holocellulose" fractions are composed largely of highly degraded cellulose which differs both chemically and physically from unaltered plant cellulose.

Because of its unusual properties of delignifying and dehumifying woody tissues, sodium chlorite has proven useful in the histological study of cell-wall degradation in peats and other fossil plant deposits. A major difficulty in such studies results from the accumulation of obscuring lignin and humic residues, which often take the form of amorphous granules simulating bacterial colonies. Careful delignification and bleaching by chlorite solutions removes the obscuring substances and reveals the unmodified or partially modified cellulosic fractions of the wall. In recent studies of cell-wall degradation in woods from peat deposits it has been possible to demonstrate a selective retention of various lamellae of the cellulosic fractions of the cell wall. These studies have been greatly facilitated by staining thin sections after delignification. Experience has shown that stains such as ruthenium red, safranine, and Hiedenhains hematoxylon are selectively absorbed by chlorinated plant tissues in a manner similar to that in untreated plant tissues, although the intensity of the stain is less.

After prolonged treatment with dilute chlorite there is no detectable reduction in the intense optical activity of cellulose in polarized light.

References

1. JAYME, G. *Cellulosechem.*, 1942, **20**, 43–49.
2. LOVELL, E. L. *Ind. eng. Chem.*, 1945, **37**, 1034–1037.
3. TAYLOR, M. C., WHITE, J. F., and VINCENT, G. P. *Tech. Ass. pulp pap. Ind.*, 1940, **23**, 251–256.
4. TAYLOR, M. C., WHITE, J. F., and CUNNINGHAM, R. *Ind. eng. Chem.*, 1940, **32**, 899.
5. WISE, L. E., *et al. Pap. Trade J.*, 1946, **122**, No. 2, 35–43.

Paper Chromatography Using Capillary Ascent[1]

ROGER J. WILLIAMS and HELEN KIRBY

Biochemical Institute, University of Texas, The Clayton Foundation for Research, and *Department of Chemistry, University of Texas*

Chromatography, which has most often involved the use of a column of finely divided adsorbent (e.g. alumina) through which solutions and solvents are allowed to percolate, has proved an invaluable tool in separating and analyzing constituents of mixtures. Consden, Gordon, and Martin (*1*) have successfully developed the use of strips or sheets of filter paper as the "column" by dipping the upper edge in a reservoir of an organic solvent saturated

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