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.

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Paper Chromatography Using Capillary Ascent[1]

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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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with water and allowing the liquid to flow down over the paper. This method has found wide and important applications for both qualitative and quantitative work (*2–10*).

In the present study we have applied the general idea of Consden, *et al.* in a preliminary way to the analysis of amino acid mixtures and have found that allowing the solvents to *ascend* by capillary action, rather than to descend, as has been common in all types of chromatography, has tremendous advantages with respect to consistency of results, simplicity of apparatus, and particularly the ease with which large numbers of analyses can be made. By introducing other modifications we have approximately doubled the sensitivity of the method for amino acids.

The apparatus which we have developed for the purpose of analysis on a relatively large scale is extremely simple, consisting of a series of 6-gal earthenware jars, in the bottoms of which are placed 10″ Pyrex pie plates, each containing about 100 ml of a suitable solvent mixture. The jar is fitted with a cover in the form of a square of plate glass. By grinding the upper edge of the jar to a plane surface and sanding the glass cover at the area of contact, the seal can be made airtight by the use of desiccator grease. Airtightness is not always essential.

In its simplest operation the filter paper (Whatman No. 1) to be used is in the form of sheets 18″ × 11¼″, obtained by cutting the larger sheets as purchased in two, using a power-driven paper cutter. Along one long edge of the paper, about 1″ from the margin, are placed, at intervals of about ¾″, solutions of the samples to be analyzed, and after drying, the sheet is formed into a cylinder about 6″ in diameter. The edges are stapled together with wire staples so that they do not quite touch.

In operation the paper cylinder is allowed to stand upright, with the dried spots comprising the samples near the bottom, in the Pyrex dish containing the solvent mixture. The whole vessel is covered with the plate glass and allowed to stand until the solvent front has nearly reached the top of the paper cylinder. This requires 12–18 hrs, depending on the solvent used. We have not yet been concerned with analyses in which close temperature control was desirable. Obviously, this simple apparatus would lend itself to such control more readily than more complicated arrangements.

For practical purposes various modifications of this setup are valuable. For example, in exploratory work we have found it desirable to carry out adsorptions in 1,000-ml cylinders or in plain glass cylinders of similar dimensions. In this case, the solvent mixture is merely placed in the bottom and a paper cylinder of suitable size is used. Several samples can be run simultaneously in this manner.

In case the output of the apparatus needs to be expanded beyond what is possible using a single paper cylinder in the 6-gal jar, concentric cylinders, e.g. 7″, 6″, and 5″ in diameter, can be used simultaneously. By this means a series of 6 jars fitted in the manner described can be used for the production of about 450 individual one-dimensional chromatograms per day. The parallel production of a large number of chromatograms under substantially identical conditions is particularly valuable in quantitative estimations involving matching of the size and intensity of color of the spots obtained (as in amino acid analyses), because several dilutions of the unknown sample and of the standard solutions are thus made available for direct comparison.

TABLE 1

	Consden, et al. (descending) (various addenda)	*Dent* (descending) (NH_3 added)	*W and K* (descending) (28 hrs, 38 cm)	*W and K* (ascending) (28 hrs, 28 cm)
Glycine	.40–.42	.42	.37	.36
Alanine	.54–.59	.62	.58	.55
Norvaline	.78–.81	..	.78	.75
Valine	.76–.78	.80	.75	.72
Norleucine	.85–.89	..	.80	.83
Leucine	.83–.88	.85	.84	.80
Isoleucine	.81–.86	..	.82	.83
Serine	.33–.36	.35	.33	.30
Threonine	.41–.50	.50	.45	.43
Cystine	.13–.30	.21	.24	.24
Methionine	.76–.83	.82	.74	.74
Proline	.85–.91	.86	..	.88
Aspartic acid	.12–.17	.17	.25	.22
Glutamic acid	.13–.28	.28	.27	.23
Phenylalanine	.86–.90	.86	..	.83
Tyrosine	.59–.64	.63	..	.55
Tryptophane	.76–.86	.77	.74	.71
Histidine	.68–.72	.77	..	.62
Arginine	.59–.89	.87	.48	.54
Lysine	.46–.82	.80	.48	.41

The same apparatus is readily adapted to the production of two-dimensional chromatograms which can conveniently be run two at a time. In this case, a single sample is placed about 1″ from the lower corner of a sheet 11″ square. After drying, two such sheets are formed into a cylinder by clipping them along two edges and allowed to stand with the lower edge immersed in the solvent mixture as before. After the solvent front has risen nearly to the top, the cylinder is removed and dried, unfastened, and reformed into a new cylinder in which the samples are distributed along the bottom. After the regular period, the two-dimensional chromatogram is ready for further processing and evaluation.

The general precautions used in this type of manipulation are similar to those described by other workers, and the procedures used in drying and processing the paper for amino acid determinations are essentially the same. The use of paper cylinders which can stand unsupported is of great convenience in connection with drying and handling. By the use, for example, of phenol which has been purified by vacuum distillation over zinc dust in an all-glass apparatus, we have been able to obviate the undesirable and extraneous color streaks which otherwise develop (*1*). Moreover, using saturated salt solution instead of water to mix with the phenol (or other solvent) prevents "water logging" (*1*) and inhibits the production of a brown front (*1*), presumably due to copper in the filter paper, which in amino acid analyses accompanies

glycine. We have found in the case of amino acid analyses that by using a 0.25% ninhydrin solution in saturated butanol-water, we can obtain about the same intensities of color from 2 γ amounts of the amino acids as can be obtained from 4 γ amounts using the usual 0.1% solution.

ing'' and ''ascending'' chromatograms made under parallel conditions by us have been compared, and the ''ascending'' chromatograms are in general definitely superior.

Table 2 gives R_F values for the various amino acids when different solvents are used as determined by Cons-

TABLE 2*

	Collidine		Benzyl alcohol		1–1 v/v benzyl alcohol butanol		Butyl alcohol		Isobutyric acid	
	Consden, et al.	*W and K*	*Consden, et al.*	*W and K*	*Consden, et al.*	*W and K*	*Consden, et al.* (3% NH_3)	*W and K*	*Consden, et al.*	*W and K*
Glycine	0.25	0.26	0.02	0.02	0.03	0.02	0.05	0.03	0.36	0.34
Alanine	0.32	0.32	0.03	0.05	0.05	0.04	0.09	0.06	0.44	0.42
Norvaline	0.48	0.44	0.12	0.14	0.19	0.15	0.31	0.25	0.71	0.68
Valine	0.45	0.43	0.11	0.14	0.15	0.15	0.22	0.21	0.65	0.63
Norleucine	0.60	0.55	0.27	0.21	0.36	0.26	0.51	0.41	0.79	0.79
Leucine	0.58	0.55	0.21	0.21	0.31	0.25	0.46	0.35	0.78	0.77
Isoleucine	0.54	0.53	0.18	0.20	0.27	0.23	0.40	0.34	0.76	0.74
Serine	0.28	0.30	0.01	0.01	0.02	0.02	0.05	0.04	0.34	0.32
Threonine	0.32	0.32	0.02	0.02	0.04	0.02	0.08	0.05	0.43	0.41
Cystine	0.14	0.11	0.00	0.00	0.00	0.01	0.01	0.01	0.25	0.14
Methionine	0.57	0.53	0.17	0.14	0.21	0.15	0.05	0.22	0.69	0.63
Proline	0.35	0.34	0.12	0.15	0.12	0.11	0.14	0.08	0.57	0.55
Aspartic acid	0.22	0.23	0.00	0.00	0.00	0.01	0.01	0.01	0.31	0.27
Glutamic acid	0.25	0.27	0.00	0.00	0.07	0.00	0.01	0.02	0.38	0.33
Tyrosine	0.64	0.59	0.14	0.07	0.19	0.11	0.14	0.14	0.58	0.47
Tryptophane	0.62	0.59	...	0.15	0.30	0.20	...	0.20	...	0.63
Histidine	0.28	0.30	0.02	0.01	0.03	0.02	0.09	0.02	0.45	0.36*
Arginine	0.16	0.17	0.01	0.01	0.01	0.01	0.05	0.03	0.40	0.38*
Lysine	0.14	0.11	0.00	0.01	0.01	0.01	0.03	0.01	0.27	0.34*

* When chromatographed in an acid solvent, these basic amino acids yield diffuse bands. The values given represent the maxima.

Values obtained by Consden, *et al.* are from *descending* chromatograms. Those under "W and K" are *ascending* values.

Table 1 gives R_F values for various amino acids as determined by Consden, *et al.* and by Dent, using the descending procedure, and by ourselves using both descending and ascending methods. All of these values apply to the use of phenol-water as the solvent. In the work of Consden, *et al.* and Dent, various agents, NH_3, HCN, Cupron, were added to the system to avoid the production of extraneous colors. We have used only purified phenol.

It may be noted that the ascending values do not differ very significantly from the descending values, though they are frequently a little lower. We have found, in the ''ascending'' procedure using the paper cylinder, that the solvent front is likely to be far more even than when a sheet is used employing the descending method. The use of the paper cylinder makes it possible to introduce the entire edge of the paper into the solvent mixture at one time, and the paper extends straight upward, avoiding the bend necessary in the descending method. Furthermore, the paper throughout its length maintains a vertical position without any tendency to wrinkle or roll. These factors are doubtless responsible for a more even solvent front and, in addition, cause the separations to be more clear cut. A considerable number of ''descend-

den, *et al.* (*1*) and in the present study using the ascending procedure.

Our experience in applying this method to the quantitative determination of minute amounts of amino acids is as yet limited, but from the experience of others, as well as our own, it appears that, especially with the simplification and refinement in procedure here described, paper chromatography will be found to be an increasingly important research tool in analyzing for amino acids and numerous other substances.

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