

Table 1. Intensity of colors produced by different methods for assay of oxidative changes in fats.

| Method | Thio-barbituric acid reaction | Kreis-test | Peroxide method of Hartmann and Glavind |
|---|-------------------------------|-------------|---|
| Wave length | 532 m μ | 550 m μ | 520 m μ |
| Substance | Extinction | | |
| Lard, 400 mg | 0.175 | 0.325 | 0.73 |
| Fatty acids from cod liver oil, 1.3 mg* | 0.165 | 0.35 | 1.65 |
| Methyl oleate hydroperoxide**, 2.5 mg | 0.10 | 0.36 | 7.3 |
| Epihydrin aldehyde*** | 0.64 | 0.60 | 0.89 |
| Benzoyl peroxide, mg | 0.00 | 0.00 | 2.5 |

* Highly unsaturated fraction precipitated at -75°C from acetone solution after more saturated acid had been removed at higher temperatures. Stored for 7 years at room temperature.

** Prepared from purified oleic acid by the method of Swift *et al.*⁷. Peroxide value 5 000 milliequivalents per 1 000 grammes.

*** To 330 mg freshly distilled acrolein dissolved in saturated KCl was added 1 ml 30% H_2O_2 . Since all reactions were to be carried out in a non-aqueous medium, the mixture was extracted with 40 ml CHCl_3 , and 2.5 ml of the extracts was used in each trial.

while there is no such parallelism with Hartmann and Glavind's method for the determination of peroxides. Benzoyl peroxide reacts quantitatively with leucodichlorophenolindophenol in the latter reaction but produces no color in the Kreis- or thiobarbituric acid tests. Purified methyl oleate hydroperoxide produces rather weak colors in the thiobarbituric acid as well as the Kreis-test as compared with the colors produced with leucodichlorophenolindophenol. On the other hand, strong colors are produced in the two tests in comparison with Hartmann and Glavind's method by such substances as oxidized fatty acids and lard. Especially intense colors are produced with epihydrin aldehyde which, according to Powick⁸, is the carrier of the Kreis-test.

These observations seem to justify the conclusion that while Hartmann and Glavind's method is a specific reaction for peroxide groups, the thiobarbituric reaction like the Kreis-test is indicative of more complex structures formed in the course of the peroxidation process, and which, in accordance with Powick's explanation, are split off in the form of epihydrin aldehyde by the treatment with strong acids used in both methods.

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Paper Chromatography on Borate-Impregnated Paper

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Paper impregnated with phosphate buffers has been employed with advantage for the separation of organic acids^{1,2}. As emphasized by Levi in a review³ it should also be possible to utilize paper treated in various other ways, and in connection with work on lichen acids capable of forming complexes with boric acid it seemed desirable to investigate the possible use of borate buffers.

A number of phenols, phenolic aldehydes and phenolic acids as well as some sugars were studied. With every substance or group of isomeric substances parallel

chromatograms were run, using paper impregnated with a sodium borate buffer and, for comparison, a sodium phosphate buffer of approximately the same pH, 8.7. The buffers were 0.1 M with respect to boric or phosphoric acid. Spots of phenolic compounds were detected by spraying the chromatograms with a solution of bis-diazotised benzidine³ or in some cases by examination of the strips in ultra-violet light. The sugar chromatograms were developed with the aniline hydrogen phthalate reagent.

On the addition of sodium borate the distribution between water and an organic solvent of substances capable of forming strongly acidic complexes with boric acid (cf. Böeseken⁴) should be displaced in favour of the aqueous phase. Accordingly, such substances would be expected to show decreased R_F values on borate-impregnated paper as compared with the values on unimpregnated or phosphate-impregnated paper.

The experiments (Table 1) proved the decrease in R_F values to be considerable in the case of phenols and phenolic aldehydes containing two adjacent hydroxyl groups and less but still distinct in the case of saligenin. On the other hand, compounds with isolated hydroxyl groups as well as *o*-hydroxyaldehydes moved at approximately the same rate on phosphate- and borate-impregnated paper. Isomeric substances often do not separate well on chromatograms of the conventional type, but as shown in Table 1, borate-impregnated paper may facilitate the separation if one of the isomers is reactive towards boric acid.

Phenolic acids are known to form complexes with boric acid if they contain two adjacent hydroxyl groups or a hydroxyl group in ortho-position to a free carboxyl group. The results obtained (Table 2) are in accordance with these facts, although there are small differences in the R_f values of the unreactive acids also, probably due

Table 1. R_F values for phenols and phenolic aldehydes.

| Substance | R_F values on paper impregnated with | |
|---|--|-----------|
| | borate | phosphate |
| <i>a. n-Butanol-water</i> | | |
| Pyrocatechol | 0.40 | 0.88 |
| Resorcinol | 0.91 | 0.96 |
| Hydroquinone | 0.91 | 0.94 |
| Pyrogallol | 0.16 | 0.78 |
| Phloroglucinol | 0.78 | 0.80 |
| 3-Methoxy-pyrocatechol | 0.45 | 0.92 |
| 4- " " | 0.46 | 0.95 |
| Methoxyhydroquinone | 0.87 | 0.90 |
| 3,4-Dihydroxybenzaldehyde | 0.28 | 0.84 |
| 2,4- " " | 0.92 | 0.91 |
| <i>b. Benzene-water</i> | | |
| Saligenin | 0.07 | 0.31 |
| <i>p</i> -Orsellinaldehyde (Atranol) | 0.48 | 0.52 |

Table 2. R_F values for phenolic acids (*n*-Butanol-water).

| Substance | R_F values on paper impregnated with | |
|---|--|-----------|
| | borate | phosphate |
| <i>o</i> -Hydroxybenzoic acid | 0.35 | 0.60 |
| <i>m</i> - " " | 0.22 | 0.27 |
| <i>p</i> - " " | 0.21 | 0.27 |
| 3,4-Dihydroxybenzoic acid | 0.02 | 0.06 |
| 2,4- " " | 0.17 | 0.38 |
| 3,5- " " | 0.13 | 0.18 |
| 4-Methoxy-2,6-cresotic acid (Evernicic acid) | 0.42 | 0.70 |

Table 3. R_F values for hexoses (*n*-Butanol-ethanol-water 4,1,5).

| Substance | R_F values on paper impregnated with | |
|-----------|--|-----------|
| | borate | phosphate |
| Glucose | 0.04 | 0.09 |
| Fructose | 0.02 | 0.09 |
| Sorbose | 0.02 | 0.08 |

to the sensitivity of the R_f values to small changes in pH.

Experiments with a few hexoses (Table 3) indicate the usefulness of the method also in sugar chemistry. The sugars investigated move more slowly on phosphate-impregnated than on untreated paper, but their sequence is not changed. On borate-impregnated paper, however, a good separation of *e. g.* glucose and sorbose can

be achieved, although these two sugars appear to move at the same rate in the systems ordinarily used.

Generally, the preparation of parallel chromatograms on borate- and phosphate-impregnated paper affords a simple micro-method for the detection of borate reactive groupings. Apart from the analytical use in separating isomers, the method may be of value for the determination of the relative positions of hydroxyl groups in complex natural products. Thus it will sometimes be possible to localize the position of methoxyl- or acyl-groups.

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Hemicellulose Extracted from Wood Holocellulose Swollen in Liquid Ammonia

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The chemistry of the so-called hemicellulose portion of the woods is still far from being clarified^{1,2}. This is partly due to the difficulties encountered in the extraction of the hemicellulose portion without changing it chemically. The preparation and the use of holocellulose as a starting material in the isolation of hemicelluloses was a large step forwards, but still rather strong alkaline solution had to be applied in the isolation of the various fractions.

It is well known³ that liquid ammonia will swell cellulosic fibers, and after eva-

Table 1. Yield of hemicelluloses extracted from ammonia-swollen wood.

| Fraction | Birch <i>Betula verrucosa</i> | Spruce <i>Picea excelsa</i> |
|----------------------|--------------------------------------|------------------------------------|
| Hot water | 14.4 % | 7.1 % |
| 1 % sodium carbonate | 2.3 » | 2.8 » |
| 2 » » hydroxide | 19.1 » | 7.3 » |
| 5 » » » | 7.9 » | 9.9 » |
| Residue | 52.3 » | 69.0 » |
| Total recovery | 96.0 % | 96.1 % |

poration of the ammonia, change the native cellulose structure (Cellulose I) into the hydrate structure (Cellulose II). The swelling of wood in liquid ammonia to render the lignin portion more accessible was first applied by Yan⁴. Later Purves and Neubauer⁵ used this swelling on maple wood. They investigated the carbohydrates in the water soluble portion. A deesterification was noted at the conditions applied, pressure and room temperature. Bishop and Adams⁶ recently described the isolation of hemicellulose fractions from wheat straw holocellulose swollen in liquid ammonia at the boiling temperature of ammonia (— 33° C). Noteworthy, was the pronounced increase in the water soluble portion (an increase from 3 to 20.2 %) from the unswollen to the swollen material. It is believed that the

Table 2. The pentosan and uronic acid content of birch and spruce holocellulose and extracted hemicellulose fractions (expressed in %).

| Fraction | Pentosan | | Uronic acids | |
|----------------------|----------|--------|--------------|--------|
| | birch | spruce | birch | spruce |
| Holocellulose | 28.0 | 7.8 | 6.0 | 5.1 |
| Hot water | 62.7 | 25.3 | 12.8 | 16.6 |
| 1 % sodium carbonate | 50.4 | 47.3 | 17.9 | 20.3 |
| 2 » sodium hydroxide | 40.5 | 40.8 | 4.9 | 8.0 |
| 5 » sodium hydroxide | 52.3 | 13.8 | 7.1 | 7.5 |
| Residue | 6.4 | 1.4 | 0.14 | 0.01 |