**From Plant Extracts.** The plant material investigated consisted of beets, potatoes, carrots, and alfalfa.

Beet and potato extracts could be cleaned up according to the procedure used for soils. Carrots and especially alfalfa extracts, after being decolorized with 1 gram of Nuchar, had to be passed through a 4-gram Florisil column, to remove all interfering substances.

When a column of 10 grams of Florisil was used, the interfering substances were removed too, even without having added activated carbon to the extract.

With alfalfa, it seems to be advisable to remove waxes as described by Ordas, Smith, and Meyer (*1*).

**Recoveries.** Between 95 and 98% of known amounts of heptachlor, added to soils in a pentane or acetone solution, were recovered from a Miami silt loam and 85 to 92% from a muck soil. For crops recoveries between 87 and 93% were obtained.

Soils were usually treated on a 10-p.p.m. basis and crops on a 2-p.p.m. basis. The solvent in which the heptachlor was added to the soils or crops was evaporated before final extraction. The percentage figures for recoveries presented above are based on 23 recovery tests using a Miami silt loam, 9 tests with a muck soil, and between 8 and 17 recovery tests conducted with each of the four crops investigated.

### Analytical Setup and Calculations

Each analysis was run in duplicate, using a soil or crop blank for determination of apparent heptachlor. In addition, known amounts of heptachlor were added to heptachlor-free soil or crop samples. The unknowns, after the value for apparent heptachlor had been subtracted, were calculated on the basis of the value obtained for the known amounts. As the effectiveness of the color reagent changes with time, analyze known amounts of heptachlor with each analysis. Results were expressed in parts per million, based on dry weight for soils and fresh weight for plants.

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**FORAGE CONSTITUENTS**

# Detection of Saponins and Sapogenins on Paper Chromatograms by Liebermann-Burchard Reagent


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**Developed and dried paper chromatograms of saponins or sapogenins are drawn through a warm bath of sulfuric acid and acetic anhydride (1 to 1, by volume) and spread on stainless steel. Characteristic colors usually begin to appear immediately and reach optimum intensity in about 15 minutes. Within this time very little background color develops. Colored photographic slides provide accurate records of the chromatograms.**


OCCURRENCE OF triterpenoid saponins in alfalfa, ladino clover, and bur clover has been reported in earlier publications from this laboratory (*5, 9, 10, 11*). In further work with the same plants and other legume forages, need arose for a means to locate saponins and sapogenins on paper chromatograms.

The Liebermann-Burchard reagent (*4, 7*), a mixture of sulfuric acid and acetic anhydride, recommended itself, because it gives strong colors with many steroids and triterpenoids (*1, 3*). However, because of its destructive action, few attempts to use it on paper chromatograms appear to have been made (*2, 6, 8*). Trials of published methods failed to give satisfactory results with legume saponins and sapogenins and after some experimentation the procedure described here was devised.

### Apparatus

35-mm. camera with Kodak Ektachrome F film and Wratten-82A filter.

Horizontal easel with adjustable light and camera supports (Figure 1).

Photoflood lamp bulbs No. RFL2.

Stainless steel sheet, 14-gage, one surface etched to dull finish with aqua regia. Suggested size 30 × 55 cm.

### Procedure

The staining reagent is prepared by pouring acetic anhydride (ACS) into an equal volume of sulfuric acid (specific gravity 1.84). Mixing in the reverse order results in very rapid heating accompanied by violent sputtering and excessive darkening. Promptly after mixing, the reagent is cooled to about 80° C. by swirling the container in a stream of tap water. During mixing and cooling a small quantity of gas is evolved and the liquid becomes straw colored. The reagent is poured into an open container such as a glass evaporating dish, where it is allowed to cool to 70° C., at which temperature dipping may be started. The reagent deteriorates with use and a solution that has cooled below working temperature tends to become excessively dark and viscous if rewarmed. Thus, only quantities sufficient for immediate use should be prepared.

Oven dried chromatographic strips are drawn through the reagent at a rate of about 3 cm. per second and then for full color development they are laid on a sheet of stainless steel which has been warmed to about 40° C. A thermometer is used to submerge the papers as they pass through the bath and to check the temperature of the liquid, which should be between 60° and 70° C.

Stained areas corresponding to the locations of saponins or sapogenins begin to appear as red spots almost immediately. Stain intensification, which is accompanied by color changes to blue or purple with some substances, continues for about 20 minutes. The paper gradually disintegrates and darkens. However, if it is of a clean and firm textured grade such as Whatman's No. 3MM, its appearance changes but little within the time needed for optimum development of stains. Chromatograms of legume forage saponins and sapogenins are photographed 15 to 17 minutes after dipping.

The temperatures and times indicated are satisfactory for Whatman's 3MM paper. Softer papers need temperatures as low as 50° to 60° C. for the reagent, 25° C. for the plate, and periods up to 30 minutes for color development.

During stain intensification the reagent on the paper absorbs moisture from the air. The dilution that occurs in this way retards darkening of the papers by the action of concentrated sulfuric acid and is thus an essential feature of the staining process. Satisfactory re-

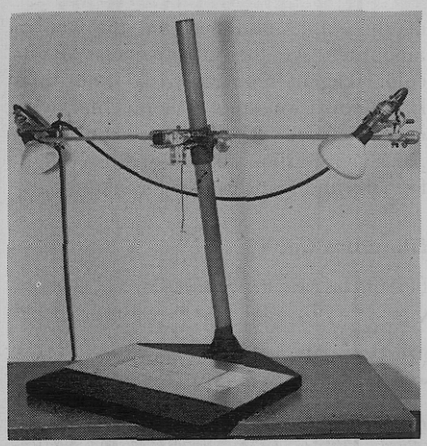

**Figure 1. Horizontal easel, with stainless steel sheet, supports camera and lights**

sults have been obtained on papers exposed during stain development to air having relative humidities in the range 21 to 40% of saturation at about 25° C. In contrast excessive and rapid darkening has occurred when relative humidities of about 15% or lower were encountered. For this reason humidification may be necessary for satisfactory staining in arid localities.

For viewing finished color slides, use a 300-watt projector fitted with a 3-inch lens which permits table top projection at very short lens-to-screen distances with brilliant color reproduction.

The final colors obtained with some known substances are given in Table I. Examples of stained chromatograms of sapogenins and saponins from alfalfa are shown in Figure 2. The smallest

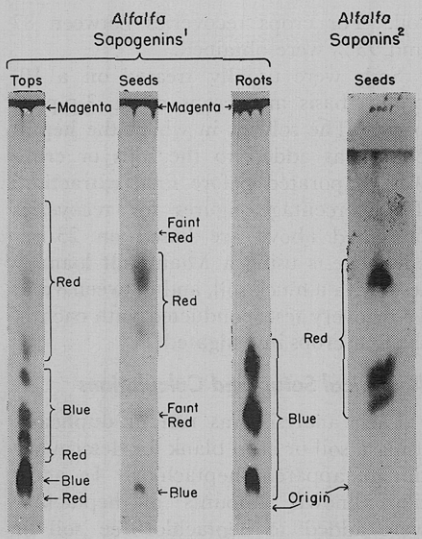


**Figure 2. Ascending paper chromatograms stained with sulfuric acid to acetic anhydride 1 to 1 by volume**

1. Developing solvent. Ethyl acetate–95% ethyl alcohol–0.05N ammonium hydroxide, 68:15:13.5
2. Developing solvent. n-Butyl alcohol–acetic acid–water, 65:10:32 (upper phase)

quantities of single triterpenoids detected were 5 to 10 γ. Somewhat larger quantities produce very intense stains.

**Table I. Triterpenoids and Steroids Stained with Liebermann-Burchard Reagent**

| *Test Substance* | *Final Color* |
|---|---|
| Abietic acid | Red |
| Castanogenin | Blue |
| Cholesterol | Purple |
| Cholic acid | Red |
| Echinocystic acid | Red |
| Erythrodiol | Red |
| Hederagenin | Blue |
| Medicagenic acid | Blue |
| Oleanolic acid | Magenta |
| Sitosterol | Purple |
| Smilagenin | Purple |
| Soyasapogenol B | Red |
| Soyasapogenol C | Magenta |
| Ursolic acid | Red |
| Uvaol | Magenta |

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**MUSHROOM ANALYSIS**

# Chromatographic Identification of the Amino Acids and Carbohydrates in the Cultivated Mushroom *Agaricus Campestris* L. exFries


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In recent years the cultivated mushroom, *Agaricus campestris* L. exFries, has become prominent in the American diet. Correspondingly the mushroom industry has become a more important member of our agricultural community. The increased popularity is due to the high nutritive value and low caloric content of the cultivated mushroom. These properties were recognized and partially measured by use of the microbiological assay and standard chemical methods by a group of workers at the University of Massachusetts around 1940. Their work has been summarized by Esselen and Fellers (*5*). With the advent of chromatographic techniques (*1, 8*), it has become possible to identify the amino acid and carbohydrate spectra of the mushroom. A number of amino acids and carbohydrate constituents previously unrecognized in the cultivated mushroom are reported herein.

Composition studies utilizing microbiological assay, as an analytical tool, were conducted by Esselen and Fellers (*5*) in 1946. These workers identified and measured quantitatively six of the amino acids known to be essential to man. The remaining four essential amino acids were identified by qualitative chemical tests. In 1951, Block, Stearns, Stephens, and McCandless (*2*), using two-dimensional chromatography, found 12 amino acids present in the sporophore of *Agaricus campestris*. These studies represent the present status of knowledge, in this country, of the amino acid content of the cultivated mushroom. In 1953, two-dimensional chromatography employed on a closely related species, *Agaricus hortensis* var. *alba*, identified 16 and possibly 21 amino acids in their alcohol extracts and acid hydrolyzates (*16*).

The carbohydrate moiety of *Agaricus campestris* was investigated by McConnell and Esselen (*11*) in 1947. Employing standard chemical methods they identified and quantitatively measured: mannitol, reducing sugars in the form of dextrose, glycogen, crude hemicellulose, and furfural-yielding substances. The above study, as far as it is known, is the only report in the literature on the carbo-