ting the ratio of absorbance of fraction 45 (relatively pure protein) to that of various non-protein fractions at a range of wavelengths (Fig. 2) it is evident that, in the region 220 to 225 mμ, absorbance due to protein reaches a maximum relative to absorbance due to contaminants. Absorbance in this region should therefore be reasonably specific for protein and also be very sensitive.

TOMBS *et al.*[1], in recommending spectrophotometric determination of serum protein at 210 mμ as an extremely sensitive procedure, pointed out that absorbance in this region is largely due to the peptide bond. Consequently, proteins from different sources have similar absorption coefficients at this wavelength. Instrumental limitations to analysis at these wavelengths have been discussed by SAIDEL *et al.*[6] and TOMBS *et al.*[1] The use of this procedure places certain restrictions on the choice of buffers. The strong absorbance of succinate, phthalate and barbiturate makes estimations at 220 mμ difficult in their presence. Sodium hydroxide, acetate, glycine and Tris can be used at a concentration of 0.01 *M* and sodium chloride, cacodylate, borate, phosphate and ammonium sulphate are satisfactory up to and above 0.1 *M*. The absorbance of protein at 220 mμ accords with Beer's Law up to 2.0 and is virtually independent of pH between values of 3 and 11.

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Fractionation of indole compounds on Sephadex G-10*

GELOTTE[1] and PORATH[2] observed that aromatic amino acids and other aromatic compounds are adsorbed to Sephadex and consequently pass through the column slower than non-aromatic compounds of comparable size. Sephadex should adsorb the clavine alkaloids and other indole-containing compounds, since these compounds are aromatic. It was found that the indole-containing fermentation products of *Claviceps purpurea* PRL 1980 are separated from carbohydrates and salts in the fermentation medium and are themselves separated into fractions on a Sephadex G-10 column.

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Methods

Seven-day old submerged cultures of *C. purpurea*[3] were adjusted to pH 6.0 and homogenized. Ethyl alcohol was added to 67 % and precipitated material was removed by filtration. The solution was left in the refrigerator overnight. The crystalline material, mainly mannitol, was removed and the alcohol was evaporated with a rotary evaporator. The aqueous solution was partially clarified by extraction with an equal volume of petroleum ether, and then lyophilized. The solid material was dissolved in a small volume of water and adjusted to pH 6.0. In some cases the less polar alkaloids and acidic indole compounds were removed with acidic and basic diethyl ether. The residue was then dissolved in water and adjusted to pH 6.0.

The resulting extract was placed on a Sephadex G-10 column which had been equilibrated with 0.02 *M* ammonium acetate buffer pH 6.0. The same buffer was used as eluant. The color with Van Urk's reagent (indole group) and ninhydrin (α-amino acids, alkaloids) and the absorbance at 280 mμ (aromatic compounds) were followed. The bulk of the material comes through in the first fractions. The later fractions were dissolved in ethyl alcohol and analyzed by thin-layer chromatography (TLC) on Silica Gel G. Indole-containing compounds were detected with Ehrlich's reagent. The clavine alkaloids were separated with ethyl acetate–acetone–dimethylformamide (5:5:1)[4], and lysergic acid, tryptophan, and the acidic indole compounds were separated with methyl acetate–isopropanol–25 % conc. NH_3 (45:35:20)[5]. To improve separation of some of the spots other solvents and two-dimensional TLC were employed. The compounds were identified primarily from the R_F values. Indoleacetic acid and 5-hydroxyindoleacetic acid were shown to be acidic by extractions into ether at pH 2–3.

Results and discussion

The order of elution of the fermentation products is indoleacetic acid, tryptophan, chanoclavine, 5-hydroxyindoleacetic acid, (molliclavine (?), elymoclavine, festuclavine, penniclavine), unknowns b and c, and (agroclavine, lysergic acid, unknowns, a, d, e). As expected, the more polar compounds, which are more soluble in the aqueous mobile phase, tend to pass through the column first. However, 5-hydroxyindoleacetic acid comes off much later than indoleacetic acid. Evidently the increased adsorption to Sephadex as a result of the hydroxyl group is more important than the increased solubility in water of 5-hydroxyindoleacetic acid. The same argument probably applies to lysergic acid, which is more soluble in water than the clavine alkaloids, but which may be strongly attracted to Sephadex through its carboxylate group.

Five compounds were observed which migrate near lysergic acid on TLC in several solvents (Fig. 1). These unknown compounds were concentrated in the last fraction off the Sephadex column of a sample which had been extracted with acidic and basic diethyl ether. Compound b was eluted from TLC plates. The compound migrates in an electric field similarly to lysergic acid, it is non-fluorescent, has absorption maxima at 280 and 291 mμ with a shoulder at 271 mμ, does not react with ninhydrin, and upon reduction with hydrogen–platinum dioxide is converted to a very non-polar product. One of these compounds is probably 6-methyl-$\Delta^{8,9}$-ergolene-8-carboxylic acid.[6] The other compounds are apparently new alkaloids which contain the ergoline ring structure.

Fractionation on Sephadex permits the simultaneous separation of acidic,

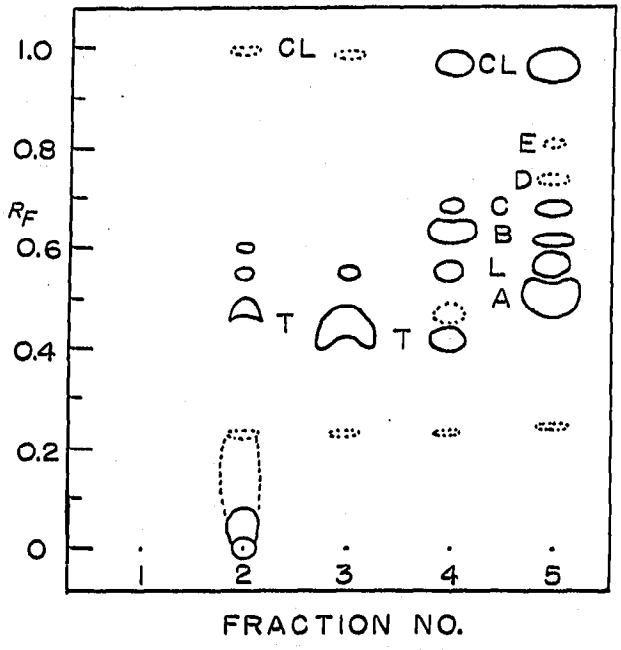

Fig. 1. Thin-layer chromatogram of fractions obtained from an aqueous extract of a *Claviceps purpurea* culture on Sephadex G-10. Five grams of lyophilized sample were extracted with ether (acid and base). The pH of the aqueous residue was adjusted to pH 6.0. Sample volume was 10 ml. The column was 1 in. × 8 in. Each tube off the column contained 12 ml. The fractions were: No. 1, tubes 7–10; No. 2, 11–16; No. 3, 17–23; No. 4, 24–38; No. 5, 39–64. After lyophilization, fractions No. 2–5 were redissolved in 0.1 ml 80% ethanol. The volume per TLC spot was 0.01 ml. TLC solvent was methyl acetate—isopropanol—25% conc. NH_3 (45:35:20). Blue to blue-grey spots with Ehrlich's reagent are shown. T = tryptophan, L = lysergic acid, CL = clavine alkaloids, A, B, C, D, E = unknown alkaloids.

basic, and neutral indole compounds from non-aromatic bulky contaminants. The identification of compounds which overlap on TLC is facilitated, since compounds with the same R_F values often appear in different Sephadex fractions. The method is particularly useful for the separation of more polar derivatives which are not readily extracted with organic solvents.

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