

be observed that the corresponding ninhydrin values are below zero. This might be caused by the formation of complexes between the substances eluted and the THAM buffer, and such a binding of THAM by some components of snake venom has already been suggested as an explanation of a phenomenon encountered in ion exchange chromatography of venom^{4,5}. By gel-filtration of the chromatographic fractions in question we have indeed shown that they are partly identical with the fourth zone in Fig. 1.

Gel-filtration of the rattle-snake venom (Fig. 2) was performed on the same dextran-gel column and under the same experimental conditions as described above. This venom does not contain any cholinesterase but has a much higher concentration of L-amino acid oxidase and a considerably stronger UV-absorption than the cobra venom. As in the former case lecithinase A was the only enzyme retarded to any appreciable degree. The ninhydrin and UV-extinction patterns, however, were quite different from those obtained with the ringhals venom. About 85 % of the material applied appeared in the first two peaks, and the large ninhydrin zone corresponding to the tubes 35—50 in Fig. 1 was missing. The most retarded zone had a very flat and elongated shape, and the quotient E_{250}/E_{350} was lower than unity in all fractions. As the ringhals and the rattle-snake belong to different families (*Elapidae* and *Crotalidae*, respectively), it would be of great interest to know to what extent these differences are typical. In the general mapping of the components of snake venoms gel-filtration may prove to be a valuable tool.

We have also compared gel-filtration experiments carried out at different pH values and with buffers of varying concentrations. The only deviation found was a slightly more pronounced tailing of the lecithinase A at low ionic strengths. This might be interpreted as a weak adsorption tendency, but at present no definite opinion can be formed on this question.

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Quantitative Determination of Carotene by Paper Chromatography

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It has been shown previously¹ that carotenes can be quantitatively separated from the other chloroplast pigments by chromatography on a filter paper with a kieselguhr filler. On the basis of this observation a method for the quantitative determination of carotene in dried grass and seaweed meal has been worked out.

Procedure. Approximately 1 g of the finely ground sample is moistened* with 2 ml of water in a 100 ml Erlenmeyer flask. After standing for 15 min (to allow swelling), acetone (25 ml) is added and the sample left over night under nitrogen in the dark. The solution is then decanted through a sintered glass funnel, and the residue is extracted with another 25 ml of acetone. The extraction is repeated until the filtrate remains colourless. Then the combined extracts are concentrated *in vacuo* to a small volume, transferred quantitatively to a volumetric flask (10 or 25 ml according to the content of carotene) and made up to the mark with acetone.

An aliquot of the extract (0.2—0.8 ml) is applied onto the centre of a circular filter paper; (Schleicher & Schüll, No. 287, Kieselguhr-filter, 18 cm diameter) the solvent being continuously evaporated by a stream of nitrogen. When the spot is dry, the paper is placed between similar halves of 15 cm petri dishes, and the chromatogram is developed with pure petroleum ether (b.p. 60—80°C) according to the technique of Rutter². As soon as the carotenes, which migrate close to the solvent front, are separated from the other pigments,

* Essential for quantitative extraction of carotene from the seaweed meal.

Table 1. Carotene content of dried grass and seaweed meal, mg/kg.

| Material | Method | |
|--------------|----------------------|-----------------------|
| | Paper chromatography | Column chromatography |
| Dried grass | 274, 277, 284, 284 | 250 |
| » | 248, 249, 252 | 240 |
| Seaweed meal | 101, 103 | 95 |

the development is interrupted, and the carotene zone is cut out immediately. The strip is then packed tightly in a glass tube (of approximately 8 mm inner diameter) which has a capillary end. The elution is carried out with 5 ml of acetone applied in several small portions and the eluate is made up to precisely 5 ml. The optical density is read in a spectrophotometer at 456 $m\mu$ and the carotene content calculated from the extinction coefficient of β -carotene ($E_{1\text{ cm}}^{1\%} = 2490$ in acetone). No interference with the absorption at 456 $m\mu$ was obtained with the eluates from a blank chromatogram.

The method described above has certain advantages over the conventional method, mainly because it avoids time consuming transference of the carotenes from acetone to petroleum ether. It is simple, requires very little equipment, and may, with a few alterations, be used for microdetermination of carotenes. From Table 1 it is seen that the accuracy of the method was satisfactory. The results obtained with the above procedure were comparable with, although somewhat higher than, those obtained by the conventional analysis³. This is believed to be caused by the higher over all recoveries obtained with the new method.

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The Structure of the Benzene Diazonium Ion

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The structure of benzene diazonium chloride ($C_6H_5N_2Cl$) has been examined by X-ray methods. As the general features of the diazonium group is settled after one Fourier projection owing to the high crystal symmetry, a preliminary report of the first part of the structure determination is given.

Benzene diazonium chloride single crystals were grown from an ethanol solution by slow precipitation with ether at 0°C. The space group is $C222_1$ and the unit cell dimensions are $a = 15.16 \text{ \AA}$, $b = 4.95 \text{ \AA}$, $c = 9.05 \text{ \AA}$ as determined from oscillation and Weissenberg photographs taken at -90°C. With four molecules in the unit cell (calculated density 1.374) this space group demands the molecules to be in special positions, i.e. the molecules have twofold axes of symmetry parallel to the x or y axis.

Integrated Weissenberg photographs were taken at -90°C with rotation about the b axis and the intensities measured with a microphotometer. Approximate x and z parameters were determined from a Patterson projection and refined on the basis of successive Fourier syntheses. Finally, a few cycles of least squares refinements brought the reliability factor R down to 6.5%. Absorption and secondary extinction corrections were not applied, and the thermal vibrations were regarded as isotropic.

A part of the Fourier projection (plane group pmg ; $c' = c$, $a' = a/2$) along the b axis is shown in Fig. 1. The chloride ion is situated at the origin and the twofold axis of the diazonium ion has the coordinates $(x, 1/4)$. The interatomic distances within the diazonium ion to be found in true lengths in this projection are as follows: $N_1-N_2 = 1.11 \text{ \AA}$, $N_1-C_1 = 1.42 \text{ \AA}$ and $C_1-C_4 = 2.70 \text{ \AA}$. Supposing the line through C_2-C_3 to be parallel to the x axis