orange-yellow. Microscopic examination of the crushed galls showed that they consisted largely of teliospores and mycelium with the color confined to the teliospores.

Entire galls weighing 10 to 15 g were diced, weighed and placed in Erlenmeyer flasks. One hundred ml of saturated alcoholic potash was added and the whole refluxed for one-half hour. The gall residue was separated by suction filtration and the residue mixed in a Waring blender for 2 minutes with 50 ml of alcohol. The mixture was again refluxed for 15 minutes and the alcoholic extracts combined.

Complete removal of all the carotenoids present was accomplished with three extractions, using small amounts of petroleum ether (b.p. 30–60°). The pigments were epiphasic against 90 per cent. methanol, indicating the absence of free or esterified xanthophylls.

The combined petroleum extracts were dried over anhydrous Na_2SO_4 and brought to a small volume by distillation *in vacuo*. They were then chromatographed by the Strain[3] technic, using as adsorbent a mixture of MgO and Hyflo Super Cel (1:1). Only two zones separated. The lower zone was subsequently shown by spectrum analysis to be β-carotene. The upper, more strongly adsorbed red-orange zone had absorption maxima in petroleum ether at 4600Å and 4900Å with a minimum at 4800Å. The latter pigment, by its behavior on the adsorbent and its absorption spectrum, appears to be identical with γ-carotene.

The total carotene concentration of the gall was 3.31 mg per 100 g, of which 36 per cent. was β-carotene and 64 per cent. the γ-isomer. By comparison, a similar chromatographic study of the leaves gave 4.03 mg per 100 g of total petroleum-phasic carotenoids distributed as follows: 7 per cent. α, 83 per cent. β and 10 per cent. γ-carotene. Small amounts of xanthophylls were present in the leaves, but these were not investigated.[4]

The remarkably high content of the γ-isomer in the gall is of particular interest. This isomer is quite rare in plants, constituting only 0.1 per cent. of the total carotene prepared from ordinary sources.[5] Small amounts have been found in apricots (*Prunus armeniaca*).[6] Mackinney[7] has reported the marsh dodder (*Cuscuta salina*) to be a relatively rich source. Emerson and Fox[8] found a remarkably high concentration of γ-carotene in the male gametangia of the aquatic Phycomycete *Allomyces*. The latter workers point out the probability that "carotenoids may play important biological roles in sexuality and the process involved in the metabolism of reproduction."

The gall described is the richest source of γ-carotene which has come to the attention of the authors.

SUMMARY

In an investigation of the pigments of the telial galls of the common rust fungus *Gymnosporangium juniperi-virginianae* Lk. β- and γ-carotenes were shown to be the only carotenoids present, with the γ-isomer predominating. The identification of γ-carotene was based on its more characteristic properties, behavior on an adsorbent, and its absorption spectra. Neither free nor esterified xanthophylls were present, and only traces of chlorophyll.

The leaves of the juniper, besides containing chlorophyll, showed the presence of α-, β- and γ-carotene.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

CHROMATOGRAPHIC ANALYSIS IN REVERSE

THE adsorption of substances from solution has generally been accomplished in one of two ways: by shaking the solution with the adsorbent and then filtering or by means of a chromatographic adsorption column. In the latter, the solution is allowed to percolate through a column packed with an adsorbent and the various adsorbable substances in the solution form bands in the adsorbent column, which can later be removed and eluted separately.

Because of the recognized value of chromatographic analysis, a modification of this technique found in this laboratory appears to have interesting possibilities as a research method. This modification consists in reversing the usual Zwett technique. Instead of passing the solution through the adsorbent column and then separating the bands by washing, the solution is placed in a tube and the adsorbent allowed to settle slowly through it, a small portion at a time. The powdered adsorbent falling through the solution sets up eddy currents which mix the solution sufficiently.

[3] H. Strain, *Jour. Biol. Chem.*, 105: 523, 1934; *ibid.*, 111: 85, 1935.

[4] M. Tswett has reported the presence of the xanthophyll, rhodoxanthin, in *Juniperus virginiana. Compt. rend.*, 152: 788, 1911.

[5] R. Kuhn and H. Brockmann, *Naturwiss.*, 21: 44, 1933; *Ber.*, 66: 407, 1933.

[6] H. Brockmann, *Z. physiol. Chem.*, 216: 45, 1933.

[7] G. Mackinney, *Jour. Biol. Chem.*, 112: 421, 1935.

[8] R. Emerson and D. L. Fox, *Proc. Royal Soc. London*, 128: 275, 1940.

Tubes of various sizes may be used. Best results have been obtained using tubes of 4 to 12 mm inside diameter and 100 cm long. The tube is mounted vertically. This is important, since otherwise the solid will tend to pile up unevenly on the bottom and will not settle uniformly through the solution. A small funnel is attached with a rubber tube to the top end and the bottom is closed with a cork. Placing a funnel at the top of the tube makes it easier to add the adsorbent to a small tube without spilling. It also allows the powder to be wetted by the solvent and to fall slowly through the narrow neck of the funnel in such a way that it does not enter the tube in lumps. Six or more inches of the tube (depending on the total length) are filled with pure solvent and the solution to be treated is carefully poured on top of it. The tube is filled completely, the liquid extending up into the small funnel to a depth of several centimeters. The powder is added in equal measured amounts of 0.2 to 1 gram. The accuracy of separation which is desired determines the size of the portions, and this in turn depends on the width of the tube, the quantity of substance to be adsorbed and the ease with which it is adsorbed.

In a solution containing three adsorbable substances, A, B and C, whose affinity for the adsorbent decreases in the same order, a portion of adsorbent settling through the solution will adsorb substance A first. The following portions will continue to adsorb A as long as any remains in solution. When A is removed further adsorbent will begin to adsorb B and finally C in succession. If such substances are colored, the column of the settled layers of adsorbent thus built up will be found to be colored by A on the bottom, then by B and finally by C, while the top layer will be entirely colorless if an excess of adsorbent has been added. It is interesting to observe by this method that more strongly adsorbed solutes are eluents for less strongly adsorbed substances. This is, of course, obvious by definition, but is clearly demonstrated in the apparatus described. When several portions of adsorbent have passed through the tube and have adsorbed all substance A from the upper part of the tube, some will still remain in the lower portion. The next portion of adsorbent will adsorb substance B in the upper part of the tube and, as it falls further, will lose the color due to B and assume the color due to A because the B has been eluted by the more strongly adsorbed A remaining in the lower part of the tube. When finally all the A is gone and B is only present in the lower parts of the tube, the process is repeated by C being adsorbed at the top and then being eluted by B as it falls into the lower end of the tube.

The relative adsorptive abilities of two different materials can easily be determined by adding first one and then the other. When colored materials are being adsorbed, the least effective adsorbent will be least colored and will also serve to mark the positions of adjacent portions of the more active adsorbent. This effect can be utilized by using a non-adsorbing white powder with colored substances and a colored non-adsorbent when colorless substances are being treated. The addition of a small amount of such inert material between portions of adsorbent in this way very clearly indicates their position in the column thus built up and is a help in correctly sectioning it at the end of the experiment.

After the last portion of adsorbent has settled, the solvent can be carefully poured off and the tube allowed to drain. Then the cork is removed and the whole core pushed out and sectioned into its components. It is better to add quite a little adsorbent above the last colored layer so that when the solvent is poured off, just a little of this unimportant material will slide down the tube with the solvent while the desired portion of the core is not disturbed.

Investigation of this matter has been an incidental result of other work, the more pressing nature of which has, as yet, precluded further development of its potentialities. However, it is thought that the method has wide possibilities as a research tool in the field of adsorption analysis and may prove to be applicable to cases not amenable to methods previously described. Consequently, this method is brought to the attention of other research workers with the hope that those having the knowledge of the pitfalls and advantages of adsorptive procedures will investigate and develop it further.

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BOOKS RECEIVED

Advances in Internal Medicine. J. MURRAY STEELE, editor-in-chief. Illustrated. Pp. xi + 292. Interscience Publishers, Inc. $4.50.

Fading Trails: The Story of Endangered American Wildlife. Illustrated. Pp. xv + 279. $3.00.

FUSON, REYNOLD C. and HAROLD R. SNYDER. *Organic Chemistry.* Pp. viii + 506. John Wiley and Sons, Inc. $3.50.

GARDNER, VICTOR R. *Basic Horticulture.* Illustrated. Pp. x + 441. Macmillan. $3.75.

Organic Reactions. ROGER ADAMS, editor-in-chief. Pp. vii + 391. John Wiley and Sons, Inc. $4.00.

Organic Syntheses. Edited by L. I. SMITH. Pp. v + 114. Chapman and Hall, Ltd., London: John Wiley and Sons, Inc., New York. $1.75.

THOMPSON, SIR D'ARCY WENTWORTH. *On Growth and Form.* Revised edition. Illustrated. Pp. 1116. Cambridge University Press, England: Macmillan, New York. $12.50.

War Medicine. A Symposium. Edited by WINFIELD SCOTT PUGH. Illustrated. Pp. 565. Philosophical Library, New York. $7.50.