TECHNICAL PAPERS

The Dreywood Anthrone Reaction as Affected by Carbohydrate Structure

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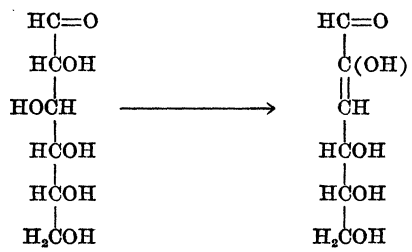
The recent work of Roman Dreywood (*1*) and of Daniel Luzon Morris (*3*) has shown that anthrone in sulfuric acid is a general reagent for carbohydrates, including sugars, polysaccharides, glycosides, carbohydrate ethers, and esters. The writers have confirmed this by tests with methylated glucose, diheterolevulosan, levulose tetraacetate, maltose octaacetate, hydroxymethylfurfural, and the diphenylacethydrazide of hydroxymethylfurfural. However, the phenylosazones of glucose and galactose and the phenylosotriazole of glucose do not give this test, whereas mannose phenylhydrazone gives a positive reaction. These facts must be connected in some way with the structure of these compounds. The osazones and osotriazoles differ from the other compounds by yielding osones with strong acids, while the hydrazones are reconverted to the original sugars. These, in common with the other carbohydrates and carbohydrate derivatives mentioned previously, initially split off water, with the formation of hydroxyaldehydes, with glucose for example:



This was postulated by Hurd and Isenhour (*2*) and confirmed by Wolfrom, Schuetz, and Cavalieri (*5*). By further loss of water and ring formation the hydroxyaldehyde goes over to hydroxymethylfurfural. In an analogous manner pentoses yield furfural, and hexomethyloses yield methylfurfural. The anthrone reaction thus substantiates this mechanism.

Incidentally, the anthrone reagent gives a cherry-red color with ascorbic acid and a negative test with levulinic acid, acetol, and methylglyoxal.

The study of the anthrone reaction further suggested the testing of melanoidins for residual sugar groups, since these substances are high polymers and Dreywood (*1*) obtained a positive reaction with cellulose plastics. The natural melanoidin extracted by Weast and MacKinney (*4*) from sun-dried apricots gave a negative test, but the synthetic melanoidin prepared by heating a solution containing fructose and aspartic acid, and having practically the same elementary composition as the natural product, gave a positive carbohydrate reaction.

In view of the fact that the anthrone reaction seems to require the presence of a furfural structure, the above observations lend support to our belief that melanoidins may be produced in two ways. The first way is through the formation of 3-carbon fragments (acetol, glucic acid, methylglyoxal) which polymerize and then react with amino acid. The reaction is favored by mild temperature conditions such as obtain in the drying of apricots. The second route leading to melanoidin formation requires higher temperatures, and under these conditions hydroxymethylfurfural is formed, which then yields melanoidins by reacting with amino acid.

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Inhibition of Hair Growth by the Percutaneous Application of Certain Adrenal Cortical Preparations

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Accumulating evidence suggests that the adrenal cortex may act as an inhibitor of hair growth. Butcher (*2*) and Ralli and Graef (*5*) found in the adult rat that replacement of hair on depilated areas of skin is accelerated by adrenalectomy. Another report by Butcher (*4*) points to the cortical part of the gland as being responsible for this effect. He also found (*3*) that the skin of the adrenalectomized rat exhibits an increased rate of oxygen consumption paralleling the increased rate of hair growth. Ralli and Graef (*5*) observed that melanin deposition in hair is also stimulated by adrenalectomy under conditions that ordinarily inhibit its production. The same workers (*6*) discovered that injected desoxycorticosterone acetate and, to a lesser degree, adrenal cortical extract prevented the stimulation of hair growth and melanin deposition which otherwise follows adrenalectomy in the black-hooded rat. The following experiment was conducted to determine whether or not various adrenal cortical preparations might exert an inhibiting influence on hair growth when applied directly to the skin.

Any study involving the rate of hair growth in the rat demands recognition of the cyclic nature of this growth. Butcher (*1*) found that, up to the age of 90 days at least, there are alternating periods of growth and quiescence. Each period is about 17 days in length. Thus,

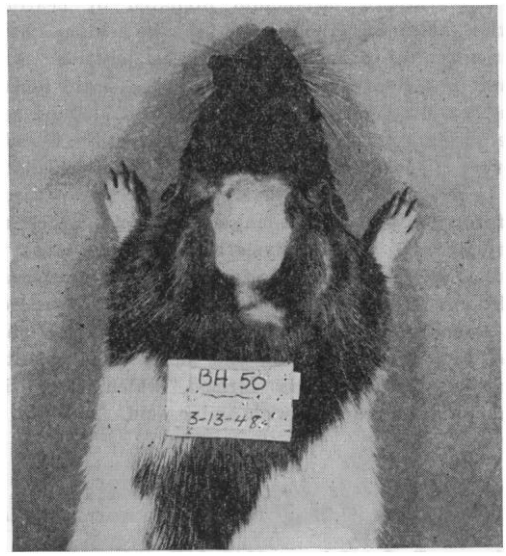

FIG. 1

a hair grows for about 2½ weeks and then stops. After the rest period the follicle resumes activity by starting another hair, while the first will remain for a variable period and then fall away. Secondly, not all of the follicles of the body are producing hair at one time, although all follicles within a limited region are synchronous in their activity. At any one time the pattern of growth falls into one of a number of fairly well-defined configurations. Fig. 1, a photograph of an alcohol-treated control rat, illustrates one of the normal patterns on the neck in which the active areas are bands extending caudad from the ears. This hair had grown during the preceding week. None had grown along the midline or on the extreme lateral aspect of the neck. These patterns are, with minor variations, bilaterally symmetrical. Furthermore, a dark pigment is present in the skin during the time that dark hair is being formed. Such pigmentation is not apparent during the period of follicular quiescence.

The cutaneous area chosen for study was the dorsolateral aspect of the neck. This region was selected because it is one from which the animal is least likely to remove, by licking or scratching, any materials applied thereon. The hair was clipped from this and adjacent areas at weekly intervals. The extent of replacement of hair on the denuded area was recorded by sketches or photographs a week later. By this means the hair growth patterns became evident.

Albino rats of the Wistar strain and black-hooded animals of the Long-Evans strain were used. They were divided into 3 groups and treated daily by applying measured amounts of material onto the skin of the right dorsal side of the neck with a tuberculin syringe without a needle and by rubbing lightly. The 5 animals of Group I received 0.1 ml of adrenal cortical extract[1]; the 13 animals of Group II, 0.1 mg of 11-dehydro-17-hydroxycorticosterone in 0.1 ml of 25% ethyl alcohol[1]; and the 8 rats of Group III, 0.1 ml of 25% alcohol. Not only did the animals of Group III serve as controls, but also the left untreated side of all of the animals served as references for the study of the alteration of growth rate in areas under treatment.

Within 3 weeks after initiation of treatment the replacement of hair was stopped on all animals in the areas treated with 11-dehydro-17-hydroxycorticosterone. This inhibition is illustrated by the interruption of the band of hair caudal to the right ear of the rat pictured in Fig. 2 as contrasted with the continuous band on its left side. The region of inhibited growth coincides with the area to which this purified cortical hormone was applied. Neither hair nor pigment appeared in this area during the 6 weeks of treatment. Inhibition of hair growth occurred also in the animals receiving the aqueous cortical extract, but to a lesser degree. The effect on hair growth in these rats after 6 weeks of treatment corresponded roughly with the results in the animals treated for 2 weeks with 11-dehydro-17-hydroxycorticosterone.

FIG. 2

Histological examination of biopsy samples of skin from the areas treated with this cortical fraction show that a true inhibition of hair growth had occurred. No detectable change in the rate of hair replacement was produced by the use of 25% alcohol on the controls.

It appears from these studies that at least one of the 11-oxysteroid hormones of the adrenal cortex (11-dehydro-17-hydroxycorticosterone) is able to exert a direct depressing effect on the growth of hair in the rat. It is this group of compounds that is known to exert a glu-

[1] The aqueous cortical extract and the 11-dehydro-17-hydroxycorticosterone were supplied by J. J. Pfiffner, of Parke, Davis & Company.

coneogenic action in general metabolism. Apparently, this action is accomplished, in this case, by some local mechanism in the tissue to which it is applied. It does not depend on the intermediary action of any recognized endocrine gland and, in the doses employed, affects no hair follicles not directly treated. The less significant growth-inhibiting potency of the aqueous cortical extract agrees with biological assay of the material, showing it to contain "gluconeogenic" steroids in low concentration.

Detailed histological and histochemical analysis of the treated skins and other studies of the action of adrenal cortical hormones on growth will be reported elsewhere.

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Effect of 2,4-Dichlorophenoxyacetic Acid on Root Development in Bean Cotyledons

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One of the well-known effects of plant-growth regulators on plants is the forced production of roots in various excised as well as intact organs of the shoot. In addition to being an excellent selective herbicide, the synthetic plant-growth regulator, 2,4-D, is also a good root-inducing compound.

In the course of an experiment in which the persistence of 2,4-D in soils is being studied, it was observed that the cotyledons of garden beans (*Phaseolus vulgaris* L. var. Lualualei)[1] planted in certain soils treated with 2,4-D (10 lbs/acre of 95% acid dusted on soil surface) developed roots. As far as is known to the writer, this is the first reported instance of this phenomenon. Although the root primordia are capable of developing in any part of the cotyledon, they seemed to develop more frequently in the basal portion. The first effect of the 2,4-D in the soil on the bean was the dying and rotting of the young, growing, embryonic axis, followed by the rotting of the cotyledons. After the embryonic axis died and if the cotyledons survived the effects of the 2,4-D, the latter remained turgid and developed root primordia. These rooted cotyledons, however, failed to develop any shoots when they were transferred to normal untreated soil. In a few cases, roots were observed on exposed cotyledons which were still intact on the stems of bean seedlings growing in 2,4-D-treated soil.

In order to prove that the roots observed above did not develop from axillary buds in the embryo after the primary root had died as a result of the presence of 2,4-D in the soil, the following experiment was conducted. Garden beans of the variety Bountiful were soaked in tap water at room temperature for 3 hrs. After the soaking period, the testa was removed from each swollen bean, and the cotyledons were separated. The germ end of each cotyledon (approximately one-fourth of the length of the cotyledon) was then severed to remove the embryonic axis entirely. The excised cotyledons were planted in soil previously treated with 2,4-D (5 lbs/acre of 95% acid dusted on soil surface). Similar cotyledons were planted in untreated soil. Twelve days after planting, the cotyledons were dug up and examined. It was observed that about half the number of the cotyledons in the 2,4-D–treated soil had developed roots, whereas none in the untreated soils showed any signs of root development (Fig. 1). These roots could not have developed from the axillary buds in the embryo.

FIG. 1. Cotyledonary roots induced by 2,4-D: above, in 2,4-D-treated soil; below, in untreated soil.

An attempt was made to induce root development in bean cotyledons on filter paper in Petri dishes containing 2,4-D solutions varying in concentrations from 1 to 32 ppm. Three temperatures were employed for each concentration: room temperature (74.9°–86.0° F), 84.9°–93.5° F, and alternation between these temperatures (8 hrs at the higher and 16 hrs at the lower temperature range daily). The germ ends of Bountiful beans were excised before being placed in Petri dishes containing the 2,4-D solutions (15 cc). Two days later, the testa from each bean was removed, and the cotyledons were separated and allowed to remain in the Petri dishes. After two weeks it was observed that all concentrations of 2,4-D used induced rooting of the cotyledons. At room temperature and at alternating temperatures the cotyledons rooted most readily at 2–8 ppm of 2,4-D. At 84.9°–93.5° F they rooted most readily at 1–4 ppm. At the highest temperature range, the cotyledons rooted slightly more readily than at room temperature or at alternating temperatures, and the latter two temperatures were about equal in their root-inducing capacity. In this experiment, one cotyledon (6% of total) in the distilled water control at room temperature produced roots. In no other case did an untreated cotyledon produce roots, whether cultured in Petri dish or in soil.

[1] Supplied by Truck Crops Department, Hawaii Agricultural Experiment Station.