similar decomposition, but evidently not as rapid as that of GBR. The formation of glucose and sulphate and in case of sinalbin also of SCN^- (in accordance with its enzymatic cleavage[5]), as well as the decrease of the pH value of these samples was the same as in the GBR.

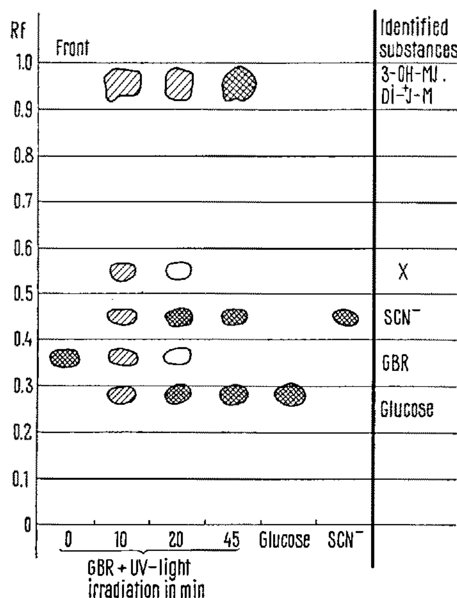

Fig. 2. Identification of products of glucobrassicin decomposition by means of paper chromatography. After applying aliquote parts of the investigated solutions on the paper Whatman 1, the developing was effected in the system butanol-acetic acid-water (4:1:3). Detection: indoles by the EHRLICH[1] and PROCHÁZKA (formaldehyde) reagent[4]; glucose and GBR: $AgNO_3 + NH_4OH$[1]; SCN^-, GBR: $Fe(NO_3)_3 + HNO_3$[1].

However, the isothiocyanates expected from other glucosinolates were not detected even after attempted conversion to thiourea derivatives. This indicates a certain difference between the photolysis and the enzymatic hydrolysis of these glucosides.

The identity of the products of UV-photolysis of the examined glucosinolates, especially of GBR, and of the products of their enzymatic hydrolysis suggests aspects on the function of these substances in plant physiology, previously not considered[6-8].

Zusammenfassung. Durch die Wirkung von UV-Licht auf wässrige Lösungen von Glucobrassicin und Sinalbin entstehen dieselben Zersetzungsprodukte wie bei der Spaltung durch das Enzym Myrosinase (HSO_4^- und SCN^-, Glucose und diesbezügliche Hydroxyverbindungen). Bei anderen Glucosinolaten führte der UV-Einfluss zwar auch zur Abspaltung von HSO_4^- und Glucose, eine gleichzeitig erwartete Bildung von Isothiocyanaten wurde hier jedoch nicht gefunden.

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Pigment Formation from Tyrosine Derivatives by UV-Irradiation in Thin-Layer Chromatography

Thyroxine is known to be sensitive to light, undergoing oxidation with the formation of a visible yellow pigment[1]. Brown pigments were described as appearing from tyrosine and dopa, following oxidation by UV-light[2]. Autooxidation of tyrosine[3-5], dopa[6-7] and dopamine[8] leading to melanin formation has been widely reported. Since all the compounds mentioned are derivatives of tyrosine, the conditions for pigment appearance on thin-layer chromatography plates, particularly from thyroid hormones, have been investigated.

Ascending chromatography was carried out in ethyl acetate:methanol:$2N$ ammonia (100:40:60 V/V)[9] for 1 h on glass plates coated with a 250 μ thick layer of Kieselgel G.

Three plates, applied with 25 μg of 3-monoiodo-L-tyrosine (MIT), 3,5-diiodo-L-tyrosine (DIT), 3,5-diiodo-L-thyronine (T_2), 3,5,3'-triiodo-L-thyronine (T_3), L-thyroxine (T_4), DL-thyronine (T), L-tyrosine (Tyr.) and with a mixture consisting of MIT, DIT, T_2, T_3, T_4, 5 μg of each dissolved in $3N$ ammonia, were chromatographed. One plate was sprayed with a 0.2% ninhydrin solution in acetone and heated at 100 °C for 5 min, the second was UV-irradiated and the third was left uncovered in the laboratory for 5 days. Two h of UV-irradiation or exposure to ordinary light for 2–5 days revealed dark spots at the sites corresponding to all examined compounds, as located in the ninhydrin stained plate. The presence of iodine in the molecule does not seem to be essential, since tyrosine and thyronine also yielded pigmented spots, although lighter than those of the iodinated compounds.

Similarly chromatographed plates, when UV-irradiated through an ordinary glass cover or when kept in the dark for nearly 2 months, did not reveal any spots. However, upon exposure to irradiation (the former one after re-

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moval of the cover) the spots appeared as expected, indicating the UV-light to be the responsible factor for this process.

The minimal time required for the beginning of appearance of pigment from 25 μg of material developed chromatographically was as follows: for MIT, DIT, T_2 and T_3 2–4 min, for T_4 – 10 min, for T – 30 min and 60 min for tyrosine. The shorter time required for the iodinated compounds suggests that the presence of iodine has some influence on the reaction.

With dopa and dopamine (applied in aqueous solution, 25 μg each) already during the chromatography pigments began to appear, which were intensified further by UV-irradiation. The much faster reaction observed with these compounds might be explained by the fact that dopa is a better precursor than tyrosine in both enzymatic and non-enzymatic production of melanin[5,10].

The minimal amounts of substances needed for the formation of a visible pigment after chromatography and 2 h irradiation were estimated. The sensitivity limit for MIT, T_3, T_4, Tyr and T was 2.5 μg while 1 μg was adequate for DIT and T_2.

The chromatographic properties of the UV-irradiated material were investigated by applying a plate with 25 μg of the above substances and immediate exposure to UV-irradiation for 22 h, prior to chromatography. The pigmented spots obtained at the origin did not migrate with the solvents used. However, upon subsequent reirradiation for 2 h, additional spots were obtained at the usual sites, except T_4 which occupied the place corresponding to T_2 or T_3, probably as the consequence of deiodination. The additional spots seem to be caused by small amounts of unaltered substances, present together with the pigment formed during the first irradiation. When various amounts of MIT and T_2 were treated as above, it was observed that the dose required for the development of additional spots was 5 times higher than the sensitivity limit. The formation of compounds with modified chromatographic mobilities from thyroxine and triiodothyronine upon exposure to UV has been explained as a possible polymer formation[11].

In our work, the chemical nature of the brown pigment has not been determined, but data available in the literature suggest that this pigment is a melanin-like polymer. Melanin can be formed not only enzymatically but also by autoxidation with the participation of free radical intermediates[6,7]. Such compounds were described to be formed from dopa[7,8] and also from thyroxine[1,12,13]. The effect of UV in the autoxidative process consists in a conversion of a 'nonautoxidizable compound into an autoxidizable one'[7,14].

Zusammenfassung. Nach Bestrahlung mit UV- und gewöhnlichem Licht entstehen in Dünnschichtchromatogrammen von Tyrosin, Thyronin und ihren Jodderivaten wie auch Dopa und Dopamin dunkle Flecken an den entsprechenden Stellen dieser Substanzen. UV-Licht ist für das Auftreten der Pigmente verantwortlich, welche melaninähnliche Polymere zu sein scheinen.

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Inhibition of Steroid Δ^4 Reductase by Heparin: Studies with Desoxycorticosterone, Progesterone, Androstenedione and Testosterone

Heparin has recently been shown to inhibit C-4 double bond reduction of cortisone, cortisol, corticosterone and aldosterone by rat liver homogenates[1,2]. Reduction of the C-20 carbonyl function and oxidation-reduction at C-11 of cortisone were not modified by heparin.

Hepatic enzyme systems for steroid reduction at the C-4 double bond are thought to interact with several widely scattered sites on the substrate molecule, i.e. the double bond, the 1 and 2 positions of ring A, carbon 11, and the C-17 side chain[3]. This being the case, information as to whether heparin interferes with the attachment of steroid to enzyme protein might be obtained by investigating steroids with various structural differences at C-11 and C-17. In the previous studies[1], heparin inhibited reduction of the C-4 double bond regardless of whether the C-11 position was occupied by a ketone or a hydroxyl group.

This report concerns the effect of heparin on ring A reduction of four C-11 desoxy, Δ^4-3 keto steroids by rat liver. Each of the steroids investigated has different structural characteristics at C-17.

Female Holtzman rats weighing between 200 and 250 g were used in this study. Details of preparing whole liver homogenates and of the incubation and extraction procedures have been published[1]. Steroid ring A reduction was determined by UV-absorption at 240 nm.

The Figure shows the manner in which increasing concentrations of heparin inhibited ring A reduction of androstenedione (4-androsten-3, 17-dione) progesterone, desoxycorticosterone and testosterone. It appears that heparin will inhibit reduction of the C-4 double bond of a variety of C-19 and C-21 steroids with various structural configurations at C-17. Rates of reduction for these 4 compounds were similar in the absence of heparin.

MCGUIRE and TOMKINS[4], using a crude liver preparation, found about the same order of activity for reduction of the C-4 double bond with a wide variety of steroid

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