SPECIFICITY AND POSSIBLE MECHANISM OF REACTION BETWEEN 9-ANTHRALDEHYDE AND UNSATURATED ALIPHATIC COMPOUNDS DURING ULTRA-VIOLET IRRADIATION

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In a previous work (5) we described a new luminescent reaction for the detection of unsaturated aliphatic compounds. This reaction consists in the appearance of an intense blue luminescence subsequent to short-term ultraviolet irradiation of a mixture of 9-anthraldehyde alcoholic solution with substances containing unsaturated carbon linkages, included in the aliphatic chain. In the course of rather protracted irradiation, the initial luminescence turns to white-bluish. This new analytical possibility was also applied to tissues in view of its utilisation in the differential cytochemical analysis of lipids. Subject of the present work are the results of the investigations, carried out with the aim to study the specificity of this reaction and its probable mechanism.

The experiments were performed on sample (model) systems with known chemical substances and upon tissue specimens. Freezing microtome sections were employed as tissue objects (specimens), obtained from human liver, with pronounced centrolobular fatty dystrophy and skin of 7-month-old human foetus. The material was fixed in formol-calcium after Baker. The sections were treated for two hours in room temperature with recently prepared saturated at 56° 9-anthraldehyde solution in 70° ethanol, and thereafter washed in 50° ethanol; next they were spread over object glass in alkalized with several drops ammonia distilled water and finally included in 90° glycerin. Irradiation and observation of the preparations was performed under luminescent microscope, type "Zetopan-Reichert", with HBO-200 as light source, excitation filter UG 1/2.5 mm + BG 12/3 mm and protective filter GG 9/1 mm.

Investigations on the reaction specificity

I. Experiments on sample specimens — Irradiation was carried out of the substance 9-anthraldehyde and of its mixtures with chemically pure organic compounds as well. The organic compounds were chosen in view of their possible presence in the model tissue sample or else, in view of the characteristic features of their chemical structure. Experiments were performed with a mixture of 9-anthraldehyde, solved in 70° ethanol, with glycerin, with oleic acid, stearinic acid, squalene, with amino acids (leucine, glycine, serine), with egg albumin (ovalbumin), with hydrazide of 3-hydroxy-2-naphtoic acid and with hydroquinone. It was established that saturated blue luminescence after short term irradiation, subsequently turning to blue-whitish, was produced merely during irradiation of 9-anthral-

dehyde with oleic acid and with squalene. In the latter instance, the luminescence appears much more quickly and is considerably more intensive. Under the identical conditions, the stearinic acid displays whitish yellow-green, induced light emission, whereas the amino acids and the egg albumin — yellow-green. The Schiff base obtained of the 9-anthraldehyde to which the hydrazide of 3-hydroxy-2-naphtoic acid was added produced stable, during longterm irradiation, yellow luminescence. The results of the experiments described show that of all the substances investigated, merely the unsaturated aliphatic compounds react by producing intense blue luminescence during 9-anthraldehyde irradiation. The reaction is stronger in compounds, containing a greater number of unsaturated carbon bonds.

2. Experiments over tissue specimens — At an earlier stage the reactions of Barolier and Suhovski, O_2-Schiff, Schiff-periformic and Schiff-peracetic acids, UV-Schiff were carried out on tissue specimens with the aim to prove the presence of unsaturated carbon linkages. The homophasic lipids produced positive reaction in all instances. The sections liable to 9-anthraldehyde impregnation were previously treated with N/100 Lugol's solution for 30 min or with brominized water for $^1/_2$ to 1 hour. During the irradiation the reaction was negative in both cases — blue luminescence did not appear. The results of these experiments demonstrate that during halogenization of unsaturated carbon bonds the reaction is suppressed. It should be assumed that the development of blue luminescence is associated to the chemical changes taking place in these linkage groups in particular, with the participation of 9-anthraldehyde.

Investigations on the probable mechanism of reaction

1. Experiments are performed aiming to prove whether the reaction is due to the direct participation of unsaturated carbon bonds, or else, it is accomplished at the expence of products of their oxidation, produced during ultra-violet irradiation of the preparations. The negativation of the reaction during halogenization of the double bonds proves their participation in the formation of products with 9-anthaldehyde, displaying intense blue luminescence, but by no means resolves the problem of whether they participate directly or the reaction is realized at the expence of products of their oxidation — aldehydes, carbon acids, peroxides. For the further elucidation of this question, additional experiments were carried out: sections were treated with peracetic acid for two hours (after Lilli) or were subjected to irradiation for from 6 to 18 hours under bactericide ultraviolet lamp; thereupon, part of the sections underwent blocking of the aldehyde groups with hydroxylamine and an attempt was made for peroxide blocking with stanochloride. The control irradiated sections and the sections subjected to blocking as well were impregnated with 9-anthraldehyde and repeatedly irradiated upon observation. In all instances the reaction was delayed and weaker regardless of whether the preparations were blocked or not. The results of the experiments just described are interpreted in the following manner: in the course of treatment of the sections with peracetic acid and during their ultra-violet irradiation, one part, but not all unsaturated bonds are oxidized until aldehydes and peroxides are formed; this fact explains the

weakening of the reaction too. The blocking does not account for alterations whatsoever, a fact indicating that aldehyde or peroxide groups presumably do not participate in the reaction.

2. Experiments carried out for establishing the nature of some of the products obtained during 9-anthraldehyde irradiation:

a) Investigations on irradiated 9-anthraldehyde — During irradiation of tissue sections in which the formation of 9-anthraldehyde crystals was permitted, a gradual waning was noted of its characteristic canary-colour luminescence. For the elucidation of the latter phenomenon, ultra-violet-irradiation was resorted to of a crystalline anhydrous substance 9-anthraldehyde and of a substance obtained from the crystallization of a 70° ethanol solution. In addition, a solution of 9-anthraldehyde in ethanol was continuously irradiated under direct sun-light. During the irradiation of the 9-anthraldehyde crystalline substance, a gradual decrease was established of its initial bright canary-yellow luminescence, without destroying the crystalline structure. During long-term irradiation (exceeding one hour on the microscope or 48—72 hours on the UV-bactericide lamp) a thin luminescent layer appeared over the surface of the crystals with a clouded (not bright) orange colour. The latter luminescence corresponds in tinge and intensity to the luminescence, characteristic of the 9-anthraquinone. During irradiation of the 9-anthraldehyde crystals, included under covering glass in 90% glycerin, a gradual extinction was observed of luminescence without the appearance of a superficial orange-luminescent layer. A similar phenomenon was observed also during irradiation of 9-anthraldehyde, crystallized from 70° ethanol solution.

During long-term exposure of 9-anthraldehyde ethanol solution to direct sun-light, a gradual bleaching is observed accompanied by white crystalline sedimentation, with whitish fluorescence in Wood filter light. The chemical analysis of the latter product demonstrates that anthracene carbonic acid is concerned.

b) Chromatographic investigations for establishing if a separate product results from the reaction, displaying intense blue luminescence. Chromatography was performed on a previously irradiated with ultra-violet rays mixture of 9-anthraldehyde and squalene over a silicagel-plaster layer with mobile hexaneacetone (100/1) phase. 9-anthraldehyde and anthracene were plotted as witness controls. The examination of the chromatograms in Wood's filter light showed that two intense blue luminescent spots appeared in the irradiated mixture of 9-anthraldehyde and squalene, situated nearest to the starting line; above them a canary-yellow spot was discerned, corresponding to the spot produced by the 9-anthraldehyde, whereas at the highest point of the frontal line a light blue luminescent spot was noted, corresponding to the anthracene insofar disposition and luminescence were concerned (Fig. 1). The experiments performed prove that the reaction was commenced by the formation of several products with intense blue luminescence. These products are with higher molecular weight than the 9-anthraldehyde.

c) Experiments aiming to establish whether during the reaction 9-anthraldehyde join-up products are produced at the site of double linkages. Chromatography was carried out on irradiated mixtures of 9-anthraldehyde with squalene and 9-anthraldehyde with oleic acid. Bright blue fluorescent

stains appeared at a variable distance from the starting line for both products. The latter finding demonstrates that in each individual case, products are formed with variable molecular weight. The latter finding warrants the assumption that the compound exhibiting intense blue luminescence is produced at the expence of the integration of the 9-anthraldehyde at the site of the double linkage, and by no means concerns an isolated chemical transformation of the 9-anthraldehyde.

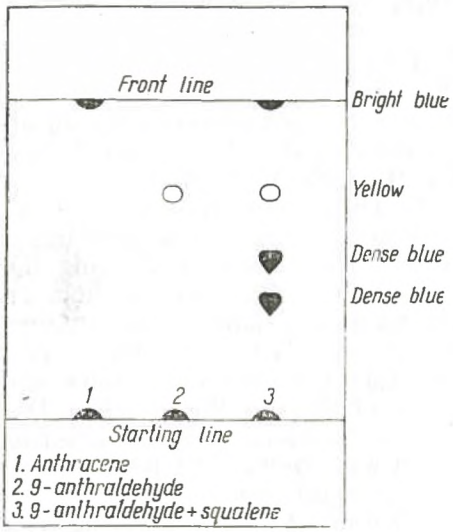
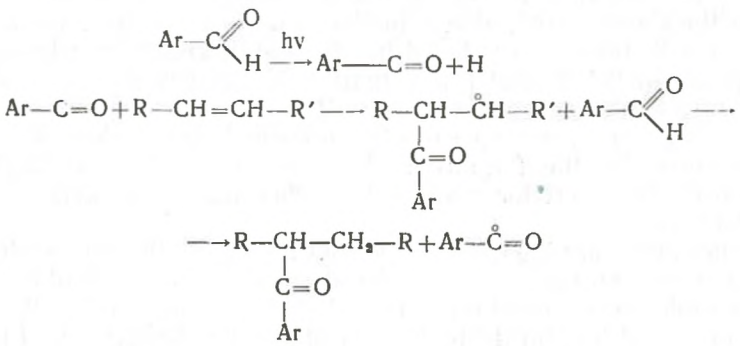


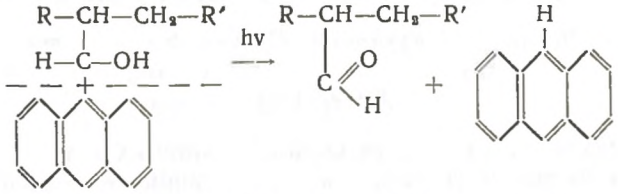
Fig. 1

d) Experiments for establishing the relationship between anthracene, produced during the reaction, and the product exhibiting intense blue luminescence. A mixture of squalene 9-anthraldehyde, after continuous irradiation, was subjected to chromatographic study according to the system already described. A substantial reduction was established of the intense blue glowing spots, localized nearby the starting line, and intensification of the spot corresponding to the anthracene.

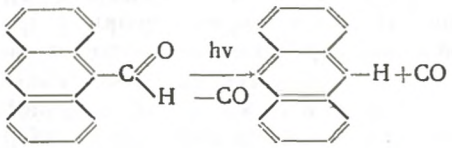
3. Considerations on the possible mechanism of the reaction. The experiments performed in this direction warrant the following assumptions: a) during the reaction products are formed with similar molecular weight and different from the anthraldehyde; b) the chromatographic motility, resp. the molecular weight of these products is determined by the aliphatic component; c) the products obtained during the reaction are produced at the expence of the reactive capacities of the unsaturated carbon linkage (on behalf of the aliphatic component); d) the prolonged ultra-violet irradiation accounts for decomposition of these products with anthracene formation. The considerations listed justify up to a certain extent the assumption of the mechanism of reaction as follows:



In theory, anthracene production might occur also during the formation of compounds of the following type:



This compound, subjected to further irradiation, undergoes decomposition with splitting of anthracene. It is probable that certain anthracene quantities might be also produced directly, as a result of the splitting of a single carbonyl group from the 9-anthraldehyde under the effect of ultra-violet irradiation (4):



In conclusion, it should be pointed out that the experimental material herein presented provides sufficient ground for lending support to the statement that the reaction displayed by 9-anthraldehyde during ultra-violet irradiation in the presence of unsaturated aliphatic compounds, is highly specific. The studies aimed at establishing the properties of the products obtained justify the accepting, of course, with certain degree of probability, the would be mechanism through which the reaction referred to in this paper occurs.

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О СПЕЦИФИЧНОСТИ И ВОЗМОЖНОМ МЕХАНИЗМЕ РЕАКЦИИ 9-АНТРАЛЬДЕГИДА С НАСЫЩЕННЫМИ АЛИФАТНЫМИ СОЕДИНЕНИЯМИ ПРИ УЛЬТРАФИОЛЕТОВОМ ОБЛУЧЕНИИ

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РЕЗЮМЕ

Проведены исследования на модельных системах с известными веществами и на тканевых объектах, с целью установления специфичности и возможного механизма реакции, которую дает 9-антральдегид при ультрафиолетовом облучении в присутствии ненасыщенных алифатных соединений. Эта реакция состоит в появлении интенсивной синей люминесценции, которая при более длительном облучении, переходит в синевато-белую. Устанавливается, что положительная реакция появляется только у алифатных соединений с ненасыщенными углеводными связями. При халогенировании двойных связей, реакция негативируется. Проведены хроматографические исследования, которые показывают, что при реакции образуется группа продуктов, молекулярный вес которых выше чем 9-антральдегида, а в то-же время получается и антрацен. Его количество увеличивается с удлинением срока облучения. Вещества, имеющие интенсивную синюю люминесценцию, имеют разную хроматографическую подвижность при варьировании алифатной составной. Делается попытка объяснить механизм реакции на почве присоединения 9-антральдегида к месту двойной связи, следуемого отщеплением антрацена.