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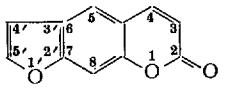
Skin-Photosensitizing Activity of some Methylpsoralens

For years the photosensitizing properties of the furocoumarins (or psoralens) and their mechanism of action have been under study in this institute[1–11]. Psoralen is the parent linear furocoumarin and its highest activity was pointed out in the first experiments, in which the skin photosensitizing properties of 59 natural or synthetic furocoumarin derivatives were tested, to find the relationship between activity and chemical structure. The quantitative, even though rather approximate, test consisted in determining for each compound the minimum irradiation time necessary to produce erythema on human skin in the following standardized conditions: 5 μg of substance/cm^2 of skin irradiated with a Philips HPW 125 lamp (emitting rather exclusively at 3.655 Å) at a 15 cm distance.



psoralen

Psoralen was always considered the most active in this group of photosensitizers, and the relative activity of the other furocoumarins was referred to it, making its activity equal to 100[1].

Successively PATHAK et al.[12,13], working out an analogous screening on many furocoumarin derivatives, also concluded that psoralen is the most active compound. They applied to guinea-pig skin different increasing amounts of the substances and observed the various degrees of erythema after a constant period of irradiation. They reported that some methylderivatives of psoralen have an activity of the same order as psoralen (++++).

We also observed in recent research the very high skin-photosensitizing activity of 2 trimethylpsoralens (i.e. 4,5′,8- and 4,4′,8-) which, tested on human skin, as reported above, appeared even more active than psoralen. Therefore we extended these studies by examining the activity on the skin of several other known and unknown methyl-derivatives of psoralen[14] (see Table).

As in the test previously indicated, we now determined the minimum irradiation time necessary for the appearance of erythema. However, because of the very high activity of the substances, the experiments were performed on guinea-pig skin, making some changes in the test conditions. The guinea-pig skin appeared to have a lower sensitivity than the human skin, e.g. the minimum erythemal dosis of radiation (3.655 Å) for psoralen (5 μg/cm^2) is 33.15×10^{16} hν/cm^2 on human skin and 66.31×10^{16} hν/cm^2 on guinea-pig skin. The modified test was performed as follows: 2.5 μg of substance/cm^2 were placed, using an acetonic solution, on a portion of non-pigmented skin of the abdomen of common guinea-pigs,

Compounds	No. of animals used	Minimum irradiation time necessary for the outcome of erythema (min)	Relative activity (psoralen = 100)
8-Methylpsoralen [a]	37	5	540
5-Methylpsoralen [a]	22	6	450
5′,8-Dimethylpsoralen	20	8	337
4,8-Dimethylpsoralen [a]	10	8	337
4,5′,8-Trimethylpsoralen [b]	9	10	270
4,4′,8-Trimethylpsoralen [a]	9	10	270
4′,8-Dimethylpsoralen	7	12	225
4-Methylpsoralen	10	12	225
Psoralen	31	27	100
Xanthotoxin (8-methoxypsoralen)	15	38	71
4′-Methylpsoralen	12	40	67
Bergapten (5-methoxypsoralen)	13	44	61
4,4′-Dimethylpsoralen	8	45	60
3,4,8-Trimethylpsoralen [a]	19	50	54
3,4-Dimethylpsoralen	6	inactive until 60 min	
3,4,4′8-Tetramethylpsoralen [a]	10	inactive until 60 min	

[a] Compounds not previously known. [b] Recently proposed in USA for the treatment of vitiligo, like xanthotoxin or 8-methoxypsoralen.

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[14] The preparative work of the unknown compounds will be published elsewhere.

previously freed from hair with scissors. The skin was then irradiated with an Osram HWA 500 W lamp (strongly emitting at 3.655 Å, besides in the visible region) at a distance of 45 cm. With radiation doses near to the minimum required, erythema appeared as a rule after 24 h, but sometimes after 48 h. Each substance was assayed on several animals (Table). Psoralen, xanthotoxin and bergapten were also tested for sake of comparison.

The data reported in the Table show that, among the tested substances, 8-methylpsoralen has the highest activity, more than 5 times higher than that of psoralen, and 7 other methylpsoralen are also more active than psoralen.

From the results obtained, it appears evident that by introduction of 1 methyl-group in the psoralen molecule the skin-photosensitizing activity increases if the methyl-group is in the positions 4, 5 or 8; its influence increases in this order.

On the contrary, the methyl-group in position 4′ has a quenching effect on the activity. Very negative is the influence of the methyl-group when it is in the 3 position, as was observed also by PATHAK et al.[12,13]: in fact the tested derivatives which have a methyl-group in this position (3,4-dimethyl-, 3,4,8-trimethyl- and 3,4,4′,8-tetramethylpsoralen) have only a very weak activity or they are inactive.

It is not possible to group together the results now obtained with those obtained previously on the human skin because of the changed experimental conditions in which the present test was performed and the different reactivity of the guinea-pig skin.

The explanation of the different activity of the various substituted psoralens is undoubtedly bound to the problem of the mechanism of action of furocoumarins. The photoreactions between furocoumarins and nucleic acids, recently made known by our laboratory[10,11], which lead to the formation of C_4-cyclo-adducts, in which the 5,6 double bond of pyrimidine bases (thymine, cytosine, uracil) and the 4′,5′ or 3,4 double bonds of furocoumarins are involved, seem to shed a light on this problem[15]. We are now still extensively studying these photoreactions, to obtain more complete information in this field[16].

Riassunto. Alcuni metil-derivati del psoralene hanno una attività fotosensibilizzatrice cutanea notevolmente più forte dello stesso psoralene, considerato finora la più attiva sostanza di questo gruppo. Il derivato, finora trovato con maggiore potere fotosensibilizzante è l'8-metilpsoralene.

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[15] A photo-C_4-cyclo-adduct between psoralen and thymine, which appears identical with that obtained in the photoreaction between the 2 substances, in which the 4′,5′ double bond of psoralen and 5,6 double bond of thymine are involved, was isolated also by hydrolysis of DNA extracted from Ehrlich ascites tumour cells irradiated at 3.655 Å in the presence of psoralen (not yet published).

[16] These studies were aided by Consiglio Nazionale delle Ricerche, Roma.

On the Localization of Tryptophane in the α_3 Chain of Collagen

PIEZ[1] recently reported the non-homogeneity of the peptidic chain of collagen called α_1. He was able to show that this α_1 fraction consists essentially of 2 polypeptidic fragments with molecular weight 9×10^4, one of them being called now α_1, the other α_3. The difference between those 2 fragments is not only in their chromatographic behaviour but in the amino acid composition as well: there is a considerable amount of evidence available for the presence of one tryptophane residue within the α_3 chain. As tryptophane is an aromatic amino acid with reactive character, it is of considerable interest to investigate the localization of this residue in the polypeptide chain: DRAKE et al.[2] and later ROSMUS et al.[3] were able to demonstrate that nearly all the aromatic residues (tyrosines) in the collagen molecule are located in the so-called telopeptides, which could be split off from the native collagen molecule by the action of pepsin, trypsin or pronase. The sequential analysis of the above-mentioned telopeptides from pepsin and pronase digests of native collagen did not show any evidence of the presence of a tryptophyl residue. Nevertheless, this might be due to some losses during the telopeptide fractionation. For this reason we looked for a specific technique of splitting of the peptidic chain in the place of the tryptophane residue.

PREVIERO et al.[4] published a simple technique of the non-enzymatic cleavage of the tryptophyl residue through its conversion into a N-formylkynurenine derivative. We have used this technique for splitting both the acid soluble and insoluble collagens. The preparation of the acid soluble and insoluble collagen was done according to RUBIN et al.[5]. For cleavage of the peptidic chain, samples of the soluble or insoluble collagen were dissolved or suspended in 100% formic acid (1000 mg in 250 ml of formic acid) and about 10 mg of resorcinol were added to the reaction mixture. The mixture was treated with a slow stream of ozone from a laboratory made ozonizer at 12 °C for 16 h. The conversion was followed by measuring the optical density of a 0.5 ml sample in 3 ml of ethanol. When the maximum of the optical density increase was reached, the ozonization was stopped, formic acid was carefully removed in vacuo, the dry residue was suspended

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