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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[2] M. P. DRAKE, P. F. DAVISON, S. BUMP and F. O. SCHMITT, Biochemistry *5*, 303 (1966).
[3] J. ROSMUS, M. P. DRAKE and Z. DEYL, Biochim. biophys. Acta *140*, 507 (1967).
[4] C. PREVIERO, C. M. PREVIERO and P. JOLLES, Biochim. biophys. Acta *124*, 400 (1966).
[5] A. L. RUBIN, M. P. DRAKE, P. F. DAVISON, D. PFAHL, P. T. SPEAKMAN and F. O. SCHMITT, Biochemistry *4*, 181 (1965).

in the smallest amount of water and dried again. Finally it was homogenized, dissolved in double distilled water and dialyzed against double distilled water twice overnight. Both the residue in the dialyzing bag and the dialyzate were tested for the UV-absorbance. It appeared that the liberated N-formylkynurenine derivative is dialyzable, suggesting the presence of the tryptophyl residue near the C-end of the collagen molecule. The dialyzate containing the N-formylkynurenine derivative was thereafter made 0,5 M according to $NaHCO_3$ and heated at 100 °C for 4 h. A part of this reaction mixture (0.1 ml) was directly mixed with 50 μl of saturated solution of dimethylamino-naphtalene-5-sulphonylchloride in acetone in order to estimate the N-terminal amino acid residue. The second aliquot (0.2 ml) was used for testing the homogeneity of the resulting peptide by means of paper chromatography (solvent system n-butanol-pyridine-acetic acid-water 30:20:6:24). The main part of the sample was degraded stepwise by EDMAN's technique, modified by GRAY and HARTLEY[6]. The N-terminal amino acid was determined after each degradation step, as described by GRAY and HARTLEY[6], but the identification of resulting dimethylaminonaphtalene sulphonyl derivatives of amino acids has been performed by means of thin-layer chromatography as described by DEYL and ROSMUS[7]. The following amino acid sequence was found: trp-leu-lys-arg[8].

No difference in the sequence of the resulting peptide was found if, instead of acid-soluble collagen, the insoluble collagen was used.

The analysis of the peptide mixture released from the native insoluble collagen by means of trypsin resulted in the detection and isolation of a tryptophane containing peptide[9]. The sequence of this peptide was determined in the same was as described above and the following sequence was found: glu[8]-trp-leu-lys.

Therefore one can conclude that one of the C-terminal sequences in the native collagen is: glu-trp-leu-lys-arg, which is supported by the fact that DEYL et al.[10] found one C-terminal arginine among 6 C-terminal amino acids of the native collagen molecule.

Zusammenfassung. Es wurde die Lage des Tryptophanrestes in der α_3 Tropokollagenkette bestimmt: Das Tryptophan befindet sich auf dem 4. Platz vom C-Ende der Kette, in der Sequenz trp-leu-lys-arg[8]. Der Vergleich mit dem tryptischen Hydrolysat führte zur Verbreiterung der Sequenz um eine weitere Aminosäure: glu-trp-leu-lys-arg[8].

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Laboratory for Gerontology, Czechoslovak Academy of Sciences, Prague-Krc, and Central Research Institute of Food Industry, Prague-Smichov (Czechoslovakia), 6 March 1967.

[6] J. GRAY and B. S. HARTLEY, Biochem. J. *89*, 380 (1963).
[7] Z. DEYL and J. ROSMUS, J. Chromat. *20*, 514 (1965).
[8] trp, tryptophane; leu, leucine; lys, lysine; arg, arginine; glu, glutamic acid.
[9] J. ROSMUS, O. MIKES and Z. DEYL, unpublished observations.
[10] Z. DEYL, J. ROSMUS and S. BUMP, Biochim. biophys. Acta *140*, 515 (1967).

Cholesteryl Esters in Flue-Cured Tobacco

The constituents of tobacco in all its various forms have received extensive chemical study[1], and several sterols, sterol esters, and sterol glycosides have been reported in tobacco – the sterols commonly obtained being stigmasterol, β-sitosterol, ergosterol and campesterol (JOHNSTONE et al.[1,2]). Cholesterol, often considered to be an 'animal' sterol, has been found only recently in plants and to our knowledge has been conclusively identified in only 4 species[3] although its presence has been inferred in others[4]. In spite of extensive work on tobacco, cholesterol and its derivatives have never been isolated from this plant[5]. We now report a simple sequence of separation steps (chart) which results in the ready isolation of a sterol ester fraction from the hexane extract of flue-cured tobacco, together with proof of the presence of a significant percentage of cholesteryl esters in this fraction.

Flue-cured tobacco leaves[6] were ground and extracted continuously with warm hexane[7]. The extract was chilled, filtered, and evaporated, and the residue (5%[8]) was dissolved in acetone and chilled. Precipitated solids were removed by filtration and the acetone evaporated. The residue (4.5%) was subjected to a 14 transfer countercurrent distribution using hexane as the stationary phase and acetonitrile as the mobile phase. The residue from evaporation of tube 0 (i.e., the least polar material, 1.3%)

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