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The Skin-Photosensitizing Furocoumarins

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Skin photosensitization is at present the best known property of some furocoumarins. Several photodermatites, which occur when the skin comes into contact with plants or vegetable products and is later exposed to sunlight, have been known for some time: erythemas of varying degrees, followed by pigmentation, appear after a latent period. This is the case e.g. in dermatitis bullosa and in those caused by Colony water, Ficus and celery.

In the Dermatological Clinic of Bern University, in 1938, KUSKE[1] studied some of these photodermatites; he found that besides plant extracts (*Ficus carica*, *Ruta graveolens*, *Pastinaca sativa*, *Heracleum mantegazianum*, *Angelica officinalis*) two pure furocoumarins, oxy-peucedanin and particularly bergapten, were also photodynamically active. He then considered furocoumarins as the agents of photodermatites.

More recently FAHMI and ABU-SHADY[2], and SCHÖNBERG and SINA[3] isolated three furocoumarins—xanthotoxin, bergapten and imperatorin—from the fruits of *Ammi majus*; these fruits had been used since ancient times by the Egyptians to cure leucodermic spots. Consequently, the Egyptian dermatologist EL MOFTI[4] started a clinical investigation for the purpose of treating vitiligo by an association of xanthoxin and imperatorin. The two compounds were administered orally, or applied locally on the leucodermic spots, which were then exposed to sun or UV-light.

When we started research in this field, we first noticed the very strong photosensitizing effect of psoralen, which is the parent furocoumarin. This fact explains why, in the popular Indian practice, the seeds of *Psoralea corylifolia* were used for the treatment of vitiligo[5].

We later studied the relationships between structure and photodynamic activity. Accordingly, we extracted many natural coumarins from plants; we synthetized other terms, and established some tests for the measure of photodynamic activity. Up to the present, we have done experiments using 59 furocoumarin derivatives, besides a great number of coumarins and related compounds. Although we do not feel that the problem has been exhausted, we have undoubtedly achieved some definite points on the relationships between chemical composition and photodynamic properties in this family of compounds[6–13].

Two tests have been used, one in sunlight, the other in UV-light. The compounds were dissolved in ethanol and applied on 2–4 cm² sized areas on the skin of volunteers.

The sunlight tests were performed by painting 25 μg of compound per cm² and exposing the skin to sunlight for 20 min in the early afternoons of June-July.

For UV-experiments, a Philips HPW 125 lamp, emitting almost exclusively radiations at 3655 Å, has been used throughout. After application of 25 μg per cm² of skin, this was irradiated for 30 min, keeping it at 15 cm from the lamp: this distance was kept constant throughout all the experiments. The data obtained in the two sets of experiments—with sun and UV-light—agrees almost completely. Compounds studied and results obtained in the UV-tests are reported in Table I.

Besides these qualitative tests, we made some quantitative ones, determining for each compound (5 μg per cm² of skin) the minimum time of irradiation which was necessary to produce erythema on human skin.

Tests were repeated many times, and average values are reported in Table II. To the activity of psoralen,

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[1] H. KUSKE, Archiv Dermatol. Syphil. *178*, 112 (1938); Dermatologica *82*, 273 (1940).

[2] J. R. FAHMY and H. ABU-SHADY, Quart. J. Pharm. Pharmacol. *20*, 281 (1947); *21*, 499 (1948).

[3] A. SCHÖNBERG and A. SINA, Nature *161*, 481 (1948); J. Amer. chem. Soc. *72*, 4826 (1950).

[4] A. M. EL MOFTY, J. Roy. Egyptian M.A. *31*, 651 (1948); *35*, 1 (1952); Brit. J. Dermatol. *64*, 431 (1952).

[5] H. S. JOIS, B. L. MANJUNATH, and S. VENKATA RAO, J. Indian chem. Soc. *10*, 41 (1933); Ber. dtsch. chem. Ges. *69*, 964 (1936).

[6] L. MUSAJO, G. RODIGHIERO, and G. CAPORALE, La Chimica e l'Industria *35*, 13 (1953); Bull Soc. Chim. Biol. *36*, 1213 (1954).

[7] L. MUSAJO, Il Farmaco, Ed. Sci. *10*, 539 (1955).

[8] L. MUSAJO, G. RODIGHIERO, G. CAPORALE, and C. ANTONELLO, Il Farmaco, Ed. Sci. *13*, 355 (1958).

[9] G. RODIGHIERO and G. CAPORALE, Il Farmaco, Ed. Sci. *10*, 760 (1955).

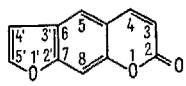
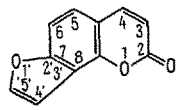
[10] G. RODIGHIERO and C. ANTONELLO, Il Farmaco, Ed. Sci. *10*, 889 (1955).

[11] G. CAPORALE and C. ANTONELLO, Il Farmaco, Ed. Sci. *13*, 363 (1958).

[12] C. ANTONELLO, Gazz. Chim. Ital. *88*, 415 (1958).

[13] G. CAPORALE, Il Farmaco, Ed. Sci. *13*, 784 (1958); Ann. Chim. (Roma) *48*, 650 (1948); *50*, 1135 (1960).

Tab. I. Qualitative tests on human skin irradiated with long-wave ultraviolet light (3655 Å)[6,8]; ++++ maximum of activity; – inactivity

Nr.	Natural furocoumarins		Activity
1	Psoralen		++++
2	Xanthotoxin	(8-methoxy-psoralen)	+++
3	Bergapten	(5-methoxy-psoralen)	+++
4	Angelicin		+
5	Isobergapten	(5-methoxy-angelicin)	+
6	Oxypeucedanin	[5-(β,γ-oxido-isoamyloxy)-psoralen]	sunlight only
7	Xanthotoxol	(8-hydroxy-psoralen)	–
8	Imperatorin	(8-isoamylenoxy-psoralen)	–
9	Bergaptol	(5-hydroxy-psoralen)	–
10	Isopimpinellin	(5,8-dimethoxy-psoralen)	–
11	Ostruthol	[5(β,γ-dihydroxy-isoamyloxy)-psoralen angelic acid monoester]	–

Synthetic furocoumarins

	Related to psoralen	
12	4',5'-dihydro-psoralen	–
13	3,4-dihydro-psoralen	–
14	4'-methyl-psoralen	+++
15	4,4'-dimethyl-psoralen	+++
16	4'-phenyl-4-methyl-psoralen	–
17	Dimer of psoralen	–
18	Thyopsoralen	–
	Related to xanthotoxin	
19	3,4-dihydro-xanthotoxin	–
20	3-methyl-xanthotoxin	+
21	4-methyl-xanthotoxin	+++
22	4'-methyl-xanthotoxin	++
23	4',3-dimethyl-xanthotoxin	+
24	4',4-dimethyl-xanthotoxin	+
25	5',4-dimethyl-xanthotoxin	+++
26	5'-phenyl-4-methyl-xanthotoxin	–
27	5-chloro-xanthotoxin	+
28	5-nitro-xanthotoxin	–
29	5-amino-xanthotoxin	–
30	5-acetylamino-xanthotoxin	–
31	8-benzyloxy-psoralen	+
32	Thyoxanthotoxin	–
	Related to bergapten	
33	4'.5'-dihydro-bergapten	–
34	4-methyl-bergapten	+
35	4-methyl-4',5'-dihydro-bergapten	–
36	Allobergapten	+
37	4',5'-dihydroallobergapten	–
38	4',5',3,4-tetrahydro-allo-bergapten	–
39	4-methyl-allobergapten	+
40	4-methyl-4',5'-dihydro-allobergapten	–
41	5-ethoxy-psoralen	++
42	5-isopropyloxy-psoralen	+
43	5-*n*-propyloxy-psoralen	– (sunlight +)
44	5-*n*-butyloxy-psoralen	– (sunlight +)
45	5-isoamyloxy-psoralen	– (sunlight +)
46	Psoralen-5-oxyacetic acid ethyl ester	–
47	5-benzyloxy-psoralen	–
48	8-nitro-bergapten	–
49	8-amino-bergapten	–
50	8-acetylamino-bergapten	–
51	Bergapten-8-carboxylic acid methyl ester	+
52	4',5'-dihydro-bergapten-8-carboxylic acid methyl ester	–
53	Bergapten-8-carboxylic acid	–
54	4',5'-dihydro-bergapten-8-carboxylic acid	–
55	Dimer of bergapten	–
	Related in angelicin	
56	4-methyl-angelicin	+
57	Dimer of angelicin	–
	Related to isopimpinellin	
58	5,8-dihydroxy-psoralen	–
59	Psoralenequinone	–

which is the highest observed, was given the arbitrary value 100.

PATHAK *et al.*[14–16] have recently examined a series of 36 furocoumarin derivatives, as well a great number of other compounds, including many coumarins. Most of these compounds have been tested on guinea pigs. Amounts up to 1000 μg of each compound were applied on the skin of the back, which was then irradiated for 45 min with an UV-lamp of $\lambda > 3200$ Å at a 12–15 cm distance[16]. Furocoumarins tested in these experiments, and not included in Table I, are listed in Table III.

[14] M. A. PATHAK and T. B. FITZPATRICK, J. Investig. Dermatol. *32*, 255 (1959).

[15] M. A. PATHAK and T. B. FITZPATRICK, J. Investig. Dermatol. *32*, 509 (1959).

[16] M. A. PATHAK, J. H. FELLMAN, and K. D. KAUFMAN, J. Investig. Dermatol. *35*, 165 (1960).

PATHAK and FITZPATRICK[14,15] have also determined the minimum amounts of some furocoumarins causing erythema—at the irradiation time and distance reported—either after application on the skin of guinea pigs or of humans or after ingestion by the guinea pig. Also in these experiments, psoralen proved the most active compound, followed by some of its derivatives, xanthotoxin, bergapten and, with very low activity, isobergapten.

The main relationships between structure and photodynamic activity arising from our researches are the following (see some examples in Table IV).

(1) Photodynamic activity is fundamentally tied to the furocoumarinic ring: psoralen, the parent furocoumarin, has in fact the highest activity, whilst coumarin[17], benzofuran and the dihydroderivatives (4′,5′-dihydropsoralen and 3,4-dihydropsoralen) are inactive. All dihydroderivatives of xanthotoxin, bergapten and of allobergapten are also inactive.

(2) In the furocoumarinic system the linear structure is more active than the angular one: in fact psoralen and bergapten are much more active than angelicin, isobergapten and allobergapten.

(3) The introduction of a –OH group in the molecule of psoralen removes the activity (xanthotoxol, bergaptol); when the –OH group is methylated, the activity is restored (xanthotoxin, bergapten); lengthening of the alkyl chain reduces the activity gradually to zero (5-ethoxy-psoralen, 5-isopropyloxy-psoralen, 5-*n*-propyloxy-psoralen, 5-*n*-butyloxy-psoralen, etc.).

Introduction of two methoxy-groups respectively in the 5 and 8 positions causes the compound to be inactive (isopimpinellin).

(4) Introduction of methyl groups in various positions of the molecules of psoralen, xanthotoxin and bergapten leads in general to a lessening of activity.

(5) Introduction of nitro-, amino-, acetylamino-groups cancels the activity of the parent compound.

Tab. II. Quantitative test on human skin[6,8]: substance 5 μg per cm²; irradiation with a Philips HPW 125 lamp (3655 Å) at 15 cm from the skin

Compounds	Minimum length of irradiation requested for outcome of erythema (min)	Relative activity
Psoralen	6	100
4′-methyl-psoralen	10	60
Xanthotoxin	16	37,5
4,5′-dimethyl-xanthotoxin	18	33,3
4,4′-dimethyl-psoralen	20	30
4-methyl-xanthotoxin	20	30
Bergapten	22	27,5
5-ethoxy-psoralen	25	24
4′-methyl-xanthotoxin	25	24
5-isopropyloxy-psoralen	35	17,1
4-methyl-bergapten	40	15
5-chloro-xanthotoxin	40	15
Angelicin	50	12
4′,4-dimethyl-xanthotoxin	50	12
Allobergapten	50	12
4-methyl-allobergapten	50	12
Bergapten-8-carboxylic acid methyl ester	50	12
3,4′-dimethyl-xanthotoxin	55	11
3-methyl-xanthotoxin	60	10
Isobergapten	60	10
4-methyl-angelicin	60	10
8-benzyloxy-psoralen	60	10

The research here reported indicates as typically active three naturally occurring furocoumarins, psoralen, xanthotoxin, and bergapten. Their great diffusion (Table V) gives an explanation for many photodermatites of vegetable origin and leads us to wonder whether they act in human nutrition through vegetables in normal use[18].

Table VI shows the results of our investigation concerning foods of vegetable origin (vegetables, fruits, cereals, oils).

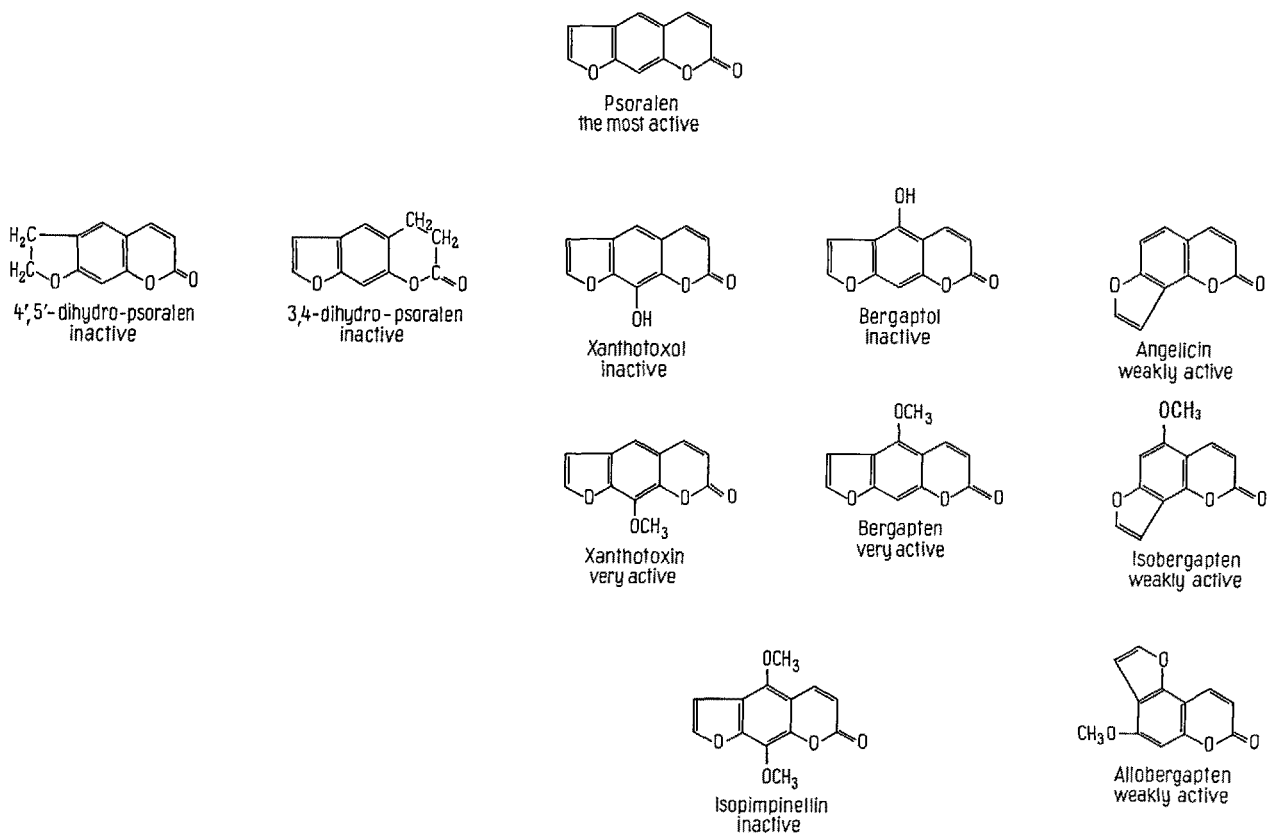
[17] Coumarin derivatives proved inactive throughout our investigation; 53 coumarin derivatives behaved in the same way with PATHAK et al.[15,16]. These authors, however, seem to have noticed a slight or minimum activity with high doses of some 7-allyloxy-coumarins, of hydroquinone monoallyl-ether and of two benzofurane derivatives.
We feel that the extremely low activities observed in these compounds do not overshadow our conclusion attributing the photosensitizing activity essentially to the furocoumarin nucleus.
On the other side, we also noticed that, so far as photosensitizing activity is concerned, there are some relationships between furocoumarins and compounds of different nature as benzpyrene (see below).

[18] L. MUSAJO, G. CAPORALE, and G. RODIGHIERO, Gazz. Chim. Ital. *84*, 870 (1954).

Tab. III. Furocoumarins tested on guinea pig by PATHAK et al.[16]: administered amounts varied till 1000 μg per cm²; 250 W lamp, emitting UV-radiations with $\lambda >$ 3200 Å; distance 12–15 cm; irradiation time 45 min

4-methyl-psoralen	++++	3,4-benzo-5′,8-dimethyl-psoralen	+
5′-methyl-psoralen	++++	3,4-cyclohexeno-5,8-dimethyl-psoralen	+
5′,8-dimethyl-psoralen	++++	Anhydromarmesin	+
4,4′-dimethyl-psoralen	+	Marmesin	+
4,5′,8-trimethyl-psoralen	++++	5′-methyl-angelicin	+
3,4,5′,8-tetramethyl-psoralen	+	3-bromo-4′,5′-dihydro-xanthotoxin	±
8-*n*-propyl-4,5′-dimethyl-psoralen	++	8-amino-4,5′-dimethyl-psoralen	–
3-*n*-butyl-4,5′,8-trimethyl-psoralen	+	4′,5′-dihydro-xanthotoxin	–
8-acetyl-4,5′-dimethyl-psoralen	++	4′,5′-dihydro-4-methyl-psoralen	–
8-bromo-4,5′-dimethyl-psoralen	++	3,5-dibromo-4′,5′-dihydro-8-methoxy-psoralen	–
8-acetamido-4,5′-dimethyl-psoralen	+	5-bromo-8-methoxy-psoralen	–
8-acetyl-4,5′-dimethyl-psoralen semicarbazone	+	4′,5′-dimethyl-angelicin	–

Tab. IV. Relationships between structure of some furocoumarins and their photosensitizing properties on the skin



Tab. V. Diffusion of photosensitizing furocoumarins in nature

Psoralen: *Ficus carica, Psoralea corylifolia, Phebalium argenteum, Coronilla glauca, Zanthoxylum flavum.*

Xanthotoxin: *Fagara xanthoxyloides, Luvunga scandens, Angelica archangelica, Ammi majus, Ruta chalepensis, Ruta montana, Ruta graveolens.*

Bergapten: *Ficus carica, Fagara xanthoxyloides, Skimmia laureola, Citrus bergamia, Citrus acida, Seseli indicum, Ligusticum acutilobum, Heracleum sphondilium, Heracleum giganteum, Heracleum nepalense, Heracleum panaces, Heracleum sibiricum, Heracleum lanatum, Ruta graveolens, Fagara schinifolia, Pastinaca sativa, Angelica archangelica, Ammi majus, Pimpinella magna, Pimpinella saxifraga, Petroselinum sativum, Apium graveolens.*

Isobergapten: *Pimpinella saxifraga, Pimpinella magna, Heracleum sphondilium, Heracleum panaces, Heracleum sibiricum, Heracleum lanatum.*

Angelicin: *Angelica archangelica, Psoralea corylifolia.*

Oxypeucedanin: *Peucedanum officinale, Imperatoria ostruthium.*

Tab. VI. Vegetable products used in human nutrition which have been tested for the presence of photosensitizing furocoumarins

The photosensitizing furocoumarins are present in: parsley, celery and figs.

They are absent in: cabbage, cauliflower, chicory, french beans, crab apples, capsicum, radish, spinach, fennel, artichoke, carrots, green peas, lupines, beans, large beans, onions, garlic, rosemary, sage, olives, pears, apples, bananas, oranges, tangerines, lemons, grape fruit, potatoes, dates, chestnuts, grapes, almonds, walnuts, flour of wheat, of maize, of rice, tea, coffee, pepper, olive oil, raisin pip oil, almond oil, walnut oil, sunflower oil, soybeans oil.

Photodynamic furocoumarins have been found in parsley, celery, and figs. Negative data may not have an absolute significance since season, varieties and environmental influences cannot be excluded. It has, in fact, been demonstrated that the activities of parsley and celery vary with the seasons[19], and also that the amounts of psoralen and bergapten in leaves of *Ficus carica* show great seasonal variations, particularly related to the rain-fall before the harvest[20].

We isolated in pure form bergapten from celery (*Apium graveolens*) and parsley (*Petroselinum sativum*)[18]. Coumarins had not been previously reported to be present in celery; it was known, though, that celery could cause a dermatitis on the hands and arms of people who handle it, (e.g. farm workers)[21–23]; but the cause was not known. Our research indicates that bergapten is the active agent Parsley seeds were known to contain a coumarin which had been neither isolated in pure form, nor identified[24].

[19] G. RODIGHIERO and G. ALLEGRI, Il Farmaco, Ed. Sci. *14*, 727 (1959).
[20] G. RODIGHIERO and C. ANTONELLO, Il Farmaco, Ed. Sci. *14*, 679 (1959).
[21] M. M. LEGRAIN and R. BARTHE, Bull. Soc. Franc. dermatol. syphil. *33*, 662 (1926).
[22] S. A. HENRY, Brit. J. Dermatol. Syphilis *50*, 342 (1938).
[23] J. F. PALUMBO and E. V. LINN, J. Amer. pharm. Ass. *42*, 57 (1953).
[24] P. CASPARIS and E. MANELLA, Pharm. Acta helvetiae *19*, 158 (1944).

We therefore ascertained that photodynamic coumarins enter our diet, although in very small amounts. The significance of this fact was not thoroughly understood hitherto. From quantitative determinations on celery and parsley[19], it was concluded that 1 mg bergapten is the highest amount that can be ingested with food during summer, in 24 h. This quantity is certainly low, but it must not be overlooked, since bergapten is active at few μg (per cm^2 of skin) and it seems to be able to become fixed on the skin itself.

Tab. VII. Photodynamic oxydation of α-terpinen into ascaridol

Compounds 10^{-4} *M* conc.	% Ascaridol after irradiation in oxygen stream: Visible light 200 W wire lamp at 15 cm (10 h)	UV-light Hanau high pressure mercury arc lamp 250 W at 15 cm (4 h, 30 min)	No irradiation (oxygen for 10 h)
Hematoporphyrin	72.6	65.9	9.8
Chlorophyll	39.6	40.7	2.92
Hypericin	34.0	23.9	—
Methylene blue	67.1	22.4	0.98
Bengal rose	52.9	41.7	0
Erythrosin	42.6	42.5	0
Eosin	20.6	18.0	1.46
3,4-benzpyrene	—	29.7	—
Psoralen	5.4	12.8	1.31
Xanthotoxin	5.1	7.9	1.59
Bergapten	1.8	11.8	1.08
Angelicin	3.2	6.5	—
Xanthotoxol	1.6	6.5	—
Control (no photodynamic compound)	1.4	11.6	—

Tab. VIII. Photodynamic haemolysis

Compounds 10^{-5} *M* conc. in saline buffered at pH 7.4	% Haemolysis with Visible light 500 W wire lamp at 40 cm (60 min)	UV-light (3655 Å) Philips HPW 125 lamp at 20 cm (20 min)
Hematoporphyrin	100	60.8
Chlorophyll	100	65.2
Hypericin	100	41
Phagopyrin	100	29
Methylene blue	67	28.3
Bengal rose	100	50.5
Erythrosin	100	30
3,4-benzpyrene	62.8	85.2
Phenanthrene	40	52.3
Anthracene	19.8	30.9
Psoralen	19.4	20.3
Xanthotoxin	20.0	15.9
Bergapten	24.5	21.1
Angelicin	24.2	23.1
Xanthotoxol	21	18.2
Imperatorin	17	20.6
Control (no photodynamic compound)	20–22	20–22

Photodynamic furocoumarins might, as a consequence, bear some importance in the biochemistry of normal human skin; this hypothesis had been forwarded by us in 1957[25] and was later also made, by TUCKER[26].

We desired to set our conclusions about chemical structure and photodynamic activity in the general pattern of photodynamics and to explain the intimate biological mechanism of the photosensitizing activity of furocoumarins.

Since from the data of the literature it was not possible to make a concrete comparison between the properties of our compounds and those of many other photodynamic compounds widely studied, (haematoporphyrin, methylene blue, fluoresceine, hypericin, phagopyrin, benzpyrene, etc.), we selected the most significant and frequently used tests, and, through them we compared the photodynamic activity of furocoumarins with that of the more important related compounds[26].

(1) *Photooxidation of α-terpinen to ascaridol*[27]. α-terpinen is known to add a molecule of oxygen, being transformed into ascaridol (the latter compound is known also for its antihelminthic properties). The test was performed by irradiating, under a slow but constant stream of oxygen, a series of 1% ethanolic solutions of α-terpinen added with a photodynamic compound in 10^{-4} *M* concentration.

Two sets of experiments were made with all compounds studied, that is by irradiating both with visible and UV-light. The amounts of ascaridol formed after irradiation are reported in Table VII.

(2) *Haemolysis of red cells.* This is one of the best known effects of photodynamic compounds. The following procedure has been used[28]. All compounds have been tested in saline buffered at pH 7.4, at conc. 10^{-5} *M*. 15 ml of each solution were carefully mixed with 0.5 ml of a red cell suspension; this was in turn obtained by centrifuging 5 ml of calf blood; the red cells were then washed three times with saline, and made up to volume ml 100.

The amount of hemolysis was checked spectrophotometrically, by measuring the transmission at 5.400 Å before and after irradiation (Table VIII).

(3) *Photooxidation of blood serum proteins.* This was followed by determining polarographically the concentration of the dissolved oxygen before and after irradiation[29]. Calf blood serum was used, after 1:10

[25] L. MUSAJO, G. RODIGHIERO, and L. SANTAMARIA, Atti Soc. ital. Patol. *5*, 1 (1957).
[26] H. A. TUCKER, J. Investig. Dermatol. *32*, 279 (1959).
[27] G. RODIGHIERO and C. BERGAMASCO, Il Farmaco, Ed. Sci. *13*, 368 (1958).
[28] G. RODIGHIERO and G. CAPORALE, Il Farmaco, Ed. Sci. *13*, 373 (1958).
[29] E. FORNASARI and G. RODIGHIERO, Il Farmaco, Ed. Sci. *13*, 379 (1958).

dilution with Britton-Robinson buffer at pH 7.1; the samples were added at $5.8 \cdot 10^{-4}$ *M* concentration.

The solutions were placed in straight-walled quarze cells, set in place for the polarographic reading. The effect was followed determining the oxygen concentration before irradiation and checking its decrease after 10, 20 and 30 min (Table IX).

(4) *Sensitizing effect through application on guinea-pig skin.* The test was performed both by painting the compounds on the skin, and by injecting them intradermically[25]. A portion of non-pigmented skin of common guinea-pigs was freed from hair with scissors taking care that the skin remained intact. No difference in result was noted as to skin site.

(a) On 4 cm^2 of the naked skin, the compound was placed in an alcoholic or hydroalcoholic solution, which was evaporated by a stream of warm air. Doses of $2.7 \cdot 10^{-7}$ *M* of each compound (equal e. g. to 50 μg of psoralen per cm^2), were used.

(b) 0.2 ml of 0.1% solution in water or propylene glycol were injected intradermically.

The treated zones were then irradiated; each animal was kept under control up to 8 days after irradiation. Each test was repeated several times in order to exclude variations due to individual sensitivity. Results obtained in these tests are summarized in Table X and XI.

From the bulk of the results obtained in the tests mentioned, some regularities emerged, which suggested the following conclusions:

(1) Furocoumarins show photodynamic properties in a way which is different from those of other groups: for instance, haematoporphyrin—and, to a similar extent, hypericin, phagopyrin, bengal rose and erythrosin—photooxidizes terpinen, causes haemolyses, photooxidizes blood serum; while it is not active if painted on the skin, on intradermical injection it causes an immediate, though short, photoreaction. Furocoumarines, on the contrary, do not influence the photooxidation of terpinen, do not cause haemolysis, do not photooxidize, to any appreciable extent, blood serum proteins; but, both on epicutaneous application and on intradermical injection, they provoke dermatitis characterized by latent period, erythema and later pigmentation.

(2) Benzpyrene shows a unique behaviour, since it manifests properties of compound of the haematoporphyrin group and properties similar to those of furocoumarins. In fact it oxidizes terpinen, causes haemolysis and, when painted on guinea-pig skin, provokes,

Tab. IX. Photo-oxydation of blood serum proteins

Compounds $5.8 \cdot 10^{-4}$ *M* conc.	% decrease of dissolved oxygen after 30 min irradiation	
	Visible light 100 W wire lamp at 12 cm	UV-light (3655 Å) Philips HPW 125 lamp at 12 cm
Hematoporphyrin	96	56
Chlorophyll	100	71
Hypericin[a]	35	12
Methylene blue	92	23
Bengal rose	80	59
Erythrosin	85	52
Eosin	42	24
Psoralen	12	35
Xanthotoxin	10	10
Bergapten[a]	10	13
Angelicin	12	15
Xanthotoxol	12	11
Control (no photodynamic compound)	10–12	10–12

[a] $^1/_{10}$ of the concentration indicated

Tab. X. Erythema formation after epicutaneous application on guinea pigs and irradiation

Compounds	Visible light Osram HWA 1000 lamp with filter for 3655 Å; at 30 cm (1 h)	UV-light (3655 Å) Philips HPW 125 lamp at 18 cm (1 h)
Hematoporphyrin	—	—
Chlorophyll	—	—
Hypericin	—	—
Methylene blue	—	—
Bengal rose	—	—
Erythrosin	—	—
Eosin	—	—
3,4-benzpyrene	±	+++
Phenanthrene	—	+
Anthracene	—	+
Psoralen	—	+++
Xanthotoxin	—	+++
Bergapten	—	+++
Angelicin	—	+
5-isopropyloxy-psoralen	—	++
Control (no photodynamic compound)	—	—

Tab. XI. Effects of intradermical injection of test compounds in guinea pigs followed by 60 min of irradiation at 25 cm. Visible plus long wave UV-light, obtained with an Osram HWA 1000 lamp

Compounds	Solvent	Effects Prevalently wheal	Prevalently erythema	Latence
Eosin	water	+++		none
Erythrosin	water	+++		none
Bengal rose	water	+++		none
Haematoporphyrin	water	++		none
Fluorescein	water	+		none
Trypaflavine	water	+		none
Psoralen	glycol		+++	about 6 h
Bergapten	glycol		+++	about 6 h
Xanthotoxin	glycol		+++	about 6 h
Angelicin	glycol		+	about 6 h
Imperatorin	glycol		—	
Xanthotoxol	glycol		—	

after a latent period, a distinct and long photoreaction, followed by pigmentation.

As far as the mechanism of the activity of these compounds is concerned, it is generally agreed that the compounds that we recorded as the haematoporphyrin group, act by a photooxidation mechanism; the effects actually disappear when oxygen is excluded, or reducing agents such as ascorbic acid or cystein, are added. Many reaction patterns have been proposed; for instance, by FIALA[30] and, more recently, by SCHENCK *et al.*[31].

SANTAMARIA[25] has studied extensively and by means of various techniques, the effects produced by irradiation on blood serum proteins, in presence of haematoporphyrin. He reached interesting conclusions regarding the changes undergone by the protein macromolecules. More recently, the same author[32] demonstrated evidence of the formation of an oxyradical from irradiated haematoporphyrin, thus giving experimental confirmation of SCHENCK'S pattern.

Photooxidative properties have been demonstrated to be lacking in furocoumarins, or to be present so slightly that they could not justify the great effects provoked on the skin.

With these experiments we have to conclude that furocoumarins are a group of photodynamic compounds having peculiar properties and that their effect on the skin cannot be explained as a photooxidation of proteic substrates.

Further research has been going on in our laboratory (and others):

(a) We were able to demonstrate that light does not transform furocoumarins into biologically active compounds. Our compounds do undergo modifications, namely into dimers, but the dimers proved inactive when applied on the skin, with or without irradiation[33].

(b) No conclusive decrease of sulphydril groups has been noted after irradiation, in presence of furocoumarins, of simple compounds such as cysteine and glutathione.

(c) We confirmed previous research[34,35], showing that furocoumarins do not influence the tyrosine-tyrosinase reaction even under irradiation. Recent work by JUDIES[36] would indicate that xanthotoxin causes the change of dihydroxyphenylalanine (Dopa) into melanine upon UV-irradiation, whilst it is inactive towards phenylalanine and tyrosine.

(d) Photosensitizing activity of furocoumarins has been studied also on bacteria[37,38]: psoralen, xanthotoxin, bergapten, as well as some synthetic derivatives, were found very active, causing death of bacterial cells.

(e) Xanthotoxin has been studied in the USA., mainly by FITZPATRICK *et al.*[39,40], in relationship with the problem of sunburn prevention and of suntan.

(f) Also in the USA. furocoumarins have attracted the attention of many authors[41–43] because of a possible influence upon the provocation of light-cancer. Different conclusions were drawn, but most recent results[44] show that furocoumarins do not seem to be dangerous in this respect.

(g) PATHAK and FELLMAN[45] have recently pointed out interesting relationships between activating and fluorescent wavelengths and photodynamic properties. of a great number of furocoumarins; they noted that active furocoumarins show a maximum of excitation in the region 340–380 mμ and a maximum of fluorescence in the region 420–460 mμ. Inactive furocoumarins have maxima in different spectral regions.

PATHAK et al.[46] found also that UV-irradiation of psoralen and xanthotoxin produces an excitation of molecules to a triplet state and generation of free radicals, which can eventually evoke biological changes in the irradiated system.

All these results, however, are not conclusive for an explanation of the mechanism of action of the furocoumarins.

After additional fruitless research, we found in our laboratory in Padova a reaction which might throw some light on the problem. We noticed that the flavin-mononucleotide (FMN) can undergo a reaction with photodynamic furocoumarins. A preliminary report on the subject has been given in May, 1960[47]; a first communication appeared recently in Nature[49].

Aqueous or hydroalcoholic solutions of FMN added with psoralen, xanthotoxin or bergapten were irradiated with a Philips HPW 125 lamp (3655 Å). The mixtures were then checked by paper chromatography

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[42] A. C. GRIFFIN, J. Investig. Dermat. *32*, 367 (1959).
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[47] At the 'Istituto Veneto di Scienze, Lettere ed Arti' in Venice[48].
[48] L. MUSAJO and G. RODIGHIERO, Atti Ist. Veneto Sci., Lett. Arti *118*, 271 (1960).
[49] L. MUSAJO and G. RODIGHIERO, Nature *190*, 1109 (1961).

using as developing solvent *n*-butanol, acetic acid, water (4:1:5). Spots are present which do not appear after irradiation of FMN alone or of furocoumarins alone.

Actually two types of spots have been located: (1) spots with yellow colours and yellow fluorescences, at low Rf (0.15–0.22); (2) spots with violet or blue fluorescences at high Rf (0.85–0.90)[50].

The intensities of these spots increase to a certain extent with the lengthening of irradiation time; temperature has some influence on the presence of the spots. We are at present investigating these photoreactions. Some of the compounds, corresponding to the spots mentioned, have been isolated. The study of all compounds formed is in progress[53].

Data hitherto obtained, indicate that the compounds giving chromatographic spots of type (1) are complex flavin derivatives, those giving chromatographic spots of type (2) correspond to products of transformation of the furocoumarins.

In case of the photoreaction between FMN and bergapten, in aqueous ethanolic solution, so far three new compounds have been isolated in small quantity: substance (a), $C_{14}H_{14}O_6$, melting point 153°, with violet fluorescence; substance (b), $C_{12}H_{10}O_6$, melting point 178°, with violet fluorescence; substance (c), $C_{12}H_8O_5$, melting point 208°, with grey-blue fluorescence. Compound (a) is an ethyl ester giving, by saponification, an acid identical to compound (b). All these compounds appear to have coumarinic structure, showing that the furanic ring of the bergapten undergoes modifications.

We believe that a full knowledge of this photoreaction, noticed *in vitro*, will give some insight into the mechanism of the sensitizing effect of furocoumarins. This suspicion comes from the following data:

(a) We noticed an evident or even complete protection when guinea pigs, treated with bergapten or psoralen and then irradiated, are administered with high doses of FMN (subcutaneous injection in aqueous solution). The protection was shown by a delay in the outbreak of a lesser degree—or even an absence—of erythema in respect of a group of animals irradiated under the same conditions, but without FMN. We are uncertain of the significance of these experiences on guinea pigs; the amount of FMN which is necessary to give a protection is actually very high: 50 mg per animal had, in fact, to be injected soon after irradiation and then 80 mg divided in 4 doses at 12 h intervals. The dosage was far from LD_{50}, but some toxic effects were noticed.

(b) In a chromatographic study of irradiated solutions of FMN with several furocoumarins or coumarin derivatives, spots indicating new compounds are present only when photodynamically active furocoumarins are tested (see Table XII).

Having observed the agreement between biological response and photochemical reactivity *in vitro* of the compounds mentioned, we feel we are justified in considering the possibility of a relationship between these two facts.

Tab. XII. Chromatographic studies on the photoreaction between FMN and some furocoumarin derivatives

Furocoumarin derivatives with skin photosensitizing properties in sun and UV-light	Spots of new compounds Rf 0.15–0.22	Rf 0.85–0.90
Psoralen	+	+
4′-methyl-psoralen	+	+
4′,4-dimethyl-psoralen	+	+
Xanthotoxin	+	—
3-methyl-xanthotoxin	+	—
3′,4-dimethyl-xanthotoxin	+	—
4′-methyl-xanthotoxin	+	+
4′,4-dimethyl-xanthotoxin	+	—
5′,4-dimethyl-xanthotoxin	+	—
5-chloro-xanthotoxin	+	—
8-benzyloxy-psoralen	+	—
Bergapten	+	+
4-methyl-bergapten	+	+
5-isopropyloxy-psoralen	+	+
Isobergapten	+	+
Allobergapten	—	+
4-methyl-allobergapten	+	—
Angelicin	—	+
Bergapten-8-carboxylic acid methyl ester	+	—
Active only in sunlight		
5-*n*-propyloxy-psoralen	—	+
5-*n*-butyloxy-psoralen	—	+
5-isoamyloxy-psoralen	—	+

No new spots have been noted with the following *photodynamically inactive* derivatives: xanthotoxol, bergaptol, imperatorin, isopimpinellin, 5,8-dihydroxy-psoralen, psoralenquinone, 4-methyl-4′-phenyl-psoralen, 4-methyl-5′-phenyl-xanthotoxin, 3,4-dihydropsoralen, 4,5′-dihydro-psoralen, 4′,5′-dihydro-bergapten, psoralen-5-oxyacetic acid ethyl ester, ostruthol, 5-nitro-xanthotoxin, 5-acetamino-xanthotoxin, 8-nitro-bergapten, dimer of psoralen, thyo-psoralen, thyo-xanthotoxin, umbelliferon, erniarin, citropten, seselin.

Riassunto. Gli autori riassumono un complesso di ricerche da essi condotte sulle proprietà fotosensibilizza trici cutanee, nell'uomo e negli animali, delle furocumarine.

Sono stati stabiliti i rapporti fra costituzione chimica ed attività, sperimentando con sostanze naturali e con numerosi prodotti di sintesi appositamente preparati.

[50] We give here for comparison the Rf-values reported in the literature for the following flavin derivatives, conditions being equal: FMN 0.10; riboflavin 0.31; lumiflavin 0.40; lumichrome 0.70; 6,7-dimethylflavin-9-acetic acid 0.30; its methyl ester 0.70; its ethyl ester 0.76[51,53].

[51] E. LEDERER, *Chromatographie en chimie organique et biologique*, II, 678 (1960).

[52] C. FUKAMACHI and Y. SAKURAI, J. Vitaminology *1*, 219 (1955).

[53] FMN and FAD (flavin-adenin-dinucleotide) behave differently towards furocoumarins. FAD does not seem to react appreciably; in fact, the irradiation of solutions of the latter containing equimolecular quantities of psoralen, xanthotoxin or bergapten did not give significant variations in the activity of FAD as coenzyme for *D*-aminoacid-oxidase, when determined by WARBURG and CHRISTIAN's method[54].

[54] O. WARBURG and W. CHRISTIAN, Biochem. J. *296*, 294 (1938).

Fra le sostanze naturali, la più attiva è la furocumarina fondamentale, psoralene, seguita da due suoi metossiderivati, xantotoxina e bergaptene. Anche alcuni metilpsoraleni di sintesi sono molto attivi.

Gli autori hanno anche stabilito, esaminando una cinquantina di alimenti di origine vegetale, che le furocumarine fotosensibilizzatrici possono entrare nella nostra dieta, ponendo il problema di una loro possibile funzione biologica a livello cutaneo.

Inoltre, per affrontare lo studio del meccanismo di azione di queste furocumarine, essi le hanno confrontate con altre note sostanze fotodinamiche, rispetto a quattro test scelti fra i più significativi: fotoossidazione dell'α-terpinene ad ascaridolo; emolisi dei globuli rossi; fotoossidazione delle proteine del siero di sangue; effetto ottenuto per applicazione epicutanea od iniezione intradermica nella cavia e successiva irradiazione.

Le furocumarine fotosensibilizzatrici hanno dimostrato di possedere proprietà particolari, diverse da quelle delle altre sostanze fotodinamiche, cioè il loro meccanismo d'azione, a differenza di queste ultime, non può essere riportato ad una fotoossidazione di substrati proteici.

Nonostante queste e successive ricerche degli autori e di altri ricercatori, il meccanismo d'azione delle furocumarine è rimasto oscuro.

Solo in questi ultimi tempi gli autori sono riusciti a mettere in evidenza alcuni fatti che possono servire a chiarire la complessa questione. Essi hanno trovato che il flavin-mononucleotide (FMN) e le furocumarine fotosensibilizzatrici per irradiazione ultravioletta danno luogo alla formazione di nuove sostanze, talune delle quali sono derivati flavinici complessi ed altre sono composti cumarinici derivanti da trasformazioni che intervengono nella parte furanica delle furocumarine.

Ci sono indicazioni per ritenere che questa fotoreazione possa servire per interpretare l'attività sulla cute. Solo le furocumarine attive infatti fotoreagiscono con l'FMN, quelle inattive non danno alcuna reazione; inoltre con l'FMN si può ottenere una protezione nelle cavie trattate con furocumarine e poi irradiate.

Brèves communications – Kurze Mitteilungen – Brevi comunicazioni – Brief Reports

Oxygen Heterocycles[1]. Maackiain, a New Naturally Occurring Chromanocoumaran[2]

Previous papers[1,3] have been concerned with the isolation and elucidation of the structure of sophorol, a new isoflavanone from *Maackia amurensis* Rupr. et Maxim. var. Buergeri (Maxim.) C. K. Schneid. In a continued investigation of the heartwood constituents of the same plant material, the present author has isolated a further new oxygen heterocyclic compound, named maackiain, the presence of which has been forecast from biogenetical considerations[1].

Maackiain, $[\alpha]_D^{20} -251.7°$ ($CHCl_3$), *R* D[4] in methanol (C, 0.074), 26°; $[\alpha]_{700} -175.8°$, $[\alpha]_{589} -227.0°$, $[\alpha]_{400} -559.0°$, $[\alpha]_{360} -684.0°$, formed colourless leaflets (m.p. 178.5° ~ 179.0°) containing a half equivalent molecule of water from aqueous methanol. Analytical data of the crystals are in excellent agreement with the formula $C_{16}H_{12}O_5$ $^1/_2H_2O$. (Found: C, 65.68; H, 4.69; H_2O, 3.7, 3.6%. $C_{16}H_{12}O_5$ $^1/_2H_2O$ requires: C, 65.53; H, 4.46; $^1/_2H_2O$, 3.6%. Found: (In a sample, m.p. 180.0° ~ 181.0°, dried at 100° ~ 110° *in vacuo*) C, 67.67, 67.33; H, 4.41, 4.58. $C_{16}H_{12}O_5$ requires C, 67.60; H, 4.26).

Maackiain has a phenolic hydroxyl group (IR OH 3472 cm^{-1} in Nujol) and gave a mono-O-methyl ether, m.p. 168 ~ 169°. (Found: C, 67.92; H, 4.74; OCH_3, 10.35%. $C_{17}H_{14}O_5$ requires C, 68.45; H, 4.37; OCH_3, 10.40%.) The remaining four oxygen atoms were indifferent to reagents under standard conditions. The IR-spectrum of maackiain showed characteristic absorption bands[5] at 1035 cm^{-1} and 929 cm^{-1}, assigned to a methylenedioxy group. The methylenedioxy protons were confirmed by the 56.4 mc/sec N.M.R. spectrum[6] of O-methylmaackiain, which shows sharp singlet lines of the type Ar–O–CH_2–O–Ar and OCH_3 at higher fields of 77 cps and 196 cps each from the signal of solvent chloroform. Pterocarpin[7] as a reference compound showed the corresponding singlet peaks at 76 and 196 cps respectively.

On the basis of the above evidence, the formulation of maackiain can be expanded into the formula $C_{15}H_9$, –O–CH_2–O–, –O–, –O–, –OH.

Biogenetic considerations and the above results suggested the chromanocoumaran skeleton, having a 2,4-di-

[1] Paper IV. Paper III in this series see H. Suginome, Tetrahedron Letters No. 19, 16 (1960).

[2] Read before the 14th Annual Meeting of the Chemical Society of Japan, Tokyo (April 1961).

[3] H. Suginome, J. org. Chem. *24*, 1655 (1959).

[4] The R.D.-curve was kindly determined by Dr. M. Maruyama through the courtesy of Professor S. Fujise of Tohoku University.

[5] L. H. Briggs, L. D. Colebrook, H. M. Fales, and W. C. Wildman, Anal. Chem. *29*, 904 (1957).

[6] Spectra were determined in chloroform solution. The band positions were read by the side-band method.

[7] A. McGookin, A. Robertson, and W. B. Whalley, J. chem. Soc. *1940*, 787; *1954*, 2794. – F. E. King, C. B. Cotterill, D. H. Godson, L. Jurd, and T. J. King, J. chem. Soc. *1953*, 3693. – A. Akisanya, C. W. L. Bevan, and J. Hirst, J. chem. Soc. *1959*, 2679.