RELATIONSHIP OF MOLECULAR CONFIGURATION TO THE ACTIVITY OF FUROCOUMARINS WHICH INCREASE THE CUTANEOUS RESPONSES FOLLOWING LONG WAVE ULTRAVIOLET RADIATION*

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The furocoumarin group of compounds includes psoralen, 8-methoxypsoralen (methoxsalen) and other related compounds. The leaves and fruits of plants containing naturally occurring furocoumarins have been used for centuries in Egypt (1), India (2), and other oriental countries in the treatment of skin depigmentation. Methoxsalen has more recently been used in the treatment of vitiligo. In addition, Musajo (3), Fitzpatrick *et al.* (4) have reported increased pigmentation of skin in individuals exposed to sunlight in presence of this compound. Musajo has also investigated the relative activity of these compounds when applied topically to human skin. Fowlks *et al.* (5) have recently reported the photosensitizing action of furocoumarin, coumarin and several other substances in bacteria in presence of long wave ultraviolet light (>3200 Å).

The purpose of this investigation was (a) to set up a bioassay method using mammalian albino guinea pig skin to study the relative activity of the compounds, (b) to correlate the erythema-inducing property of various compounds in terms of molecular configuration, (c) to determine the minimum effective concentration of different compounds on guinea pig skin and correlate these data with the results obtained with human skin.

It appears that albino guinea pig skin can be conveniently used for assaying the photodynamically active and the inactive compounds. Using long wave ultraviolet light of 3200 Å and above wavelength, erythema and pigment inducing compounds can be given orally or applied topically and tested for their relative activity. The parent substance, psoralen, exhibits maximum activity. The order of activity of other active compounds appears to be (1) psoralen, (2) 4,5′,8-trimethylpsoralen, (3) 4-methylpsoralen, (4) 5′8-dimethylpsoralen, (5) 8-methoxypsoralen, (6) 5-methoxypsoralen, (7) 4′,5′-dihydroxanthotoxin (topically only), (8) psoralen glucoside, (9) 8-isoamyleneoxypsoralen. Compounds like isobergapten and 4,5′-dimethylisopsoralen showed weak activity. The furan ring and coumarin ring configuration compounds do not show any photoactivation. The compound to be active photodynamically in inducing erythema and pigmentation should have a linearly annulated fused ring structure of furan and coumarin ring as present in psoralen molecule. A non-linear angular structure like isopsoralen molecule does not exhibit any activity. Carbon atoms 5 and 8, valence bonds between carbons 3-4 and 4′-5′, and attachment of furan ring at carbon 6 and 7 are the active centers in the molecule. An intact lactone ring is essential for the compound to be active. Methyl substitutions at carbon atoms 4,5′,8 in psoralen molecule, though, do not increase the relative activity of the basic molecule, but still they retain its photodynamic activity. Methoxy substitution in 8 or 5 position, however, decreases the activity. Hydroxyl, nitro, amino group substitution render the compound inactive. Substituting radicals which increase the electron density of reactive centers retain the activity of the molecule, whereas radicals which decrease the electron density lower the photodynamic activity.

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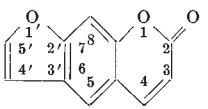
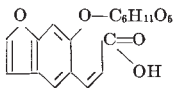
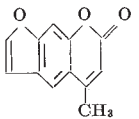
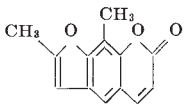
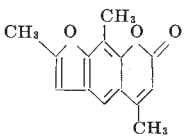
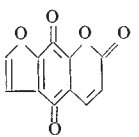
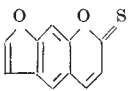
MATERIALS AND METHODS

Using albino guinea pig and human skin, conditions and requirements for a bioassay method were standardized to evaluate the relative activity of various compounds. The degree of erythema produced in presence of a known amount of a substance and the minimum effective concentration which produces erythema following topical application and oral administration were determined. On the back of a clean-shaved, smooth skinned guinea pig, and on the innerside of forearms of medical students, varying concentrations of each compound dissolved in 95% ethanol were applied topically in a number of squares of one inch area marked on the skin with adhesive tape. For oral administration the compound was given in the form of capsules. In topical studies with guinea pig and

TABLE I

Relative activity of furocoumarins

Furocoumarin	Structure	Relative Activity on Albino Guinea Pig Skin					Relative Activity on Human Skin				Remarks
		Topical	Oral	M. E. C.* for erythemal response			Erythemal response		Pigmentation response at		
				Topical μg./sq. in.	Oral mg./kg.	Degree of erythema	Topical	M. E. C.* μg./sq. in.	M. E. C.*	50 μg./sq. in.	
1. Psoralen		++++	++++	5 μg.	3.72 mg. (0.02 mM)†	++	++++	<10 μg.	++	+++	Blistering 25μg.
2. Psoralen glucoside		+	+	125 μg.	7.32 mg. 14.7 mg. (0.04 mM)†	± +	+	100 μg.	+	−	No blistering
3. 4-Methylpsoralen		+++	+++	10 μg.	8 mg. (0.04 mM)†	++	+++	10 μg.	+	++	Blistering 25μg. and above
4. 5′, 8-Dimethylpsoralen		+++	+++	5 μg.	4.3 mg. (0.02 mM)†	+	+++	10 μg.	+	++	Blistering 25μg. and above
5. 4,5′,8-Trimethylpsoralen		++++	++++	2.5 μg.	4.6 mg. (0.02 mM)†	++	+++	10 μg.	+	++	Blistering 25μg. and above
6. Psoralenquinone		inactive	inactive	inactive up to 2000 μg.	inactive 25 mg.	inactive	inactive	inactive	inactive	inactive	
7. Thiofurocoumarin		inactive	inactive	inactive up to 2000 μg.	inactive 10 mg.	inactive	inactive	inactive	inactive	inactive	

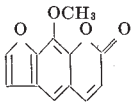
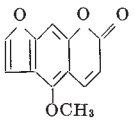
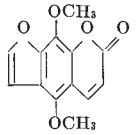
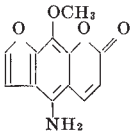
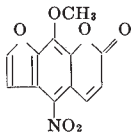
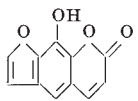
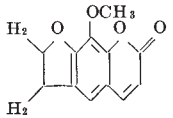
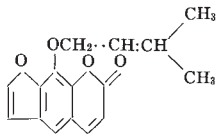
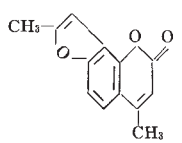
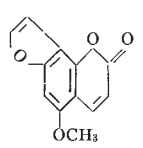
8. 8-Methoxypsoralen (8-MOP) xanthotoxin		+++	+++	15 μg.	4.32 mg. (0.02 mM)†	+	++	10 μg.± 25 μg.+	±	+	No blistering up to 150 μg.
9. 5-Methoxypsoralen (5 MOP) bergapten		++	++	50 μg.	8.6 mg. (0.04 mM)†	++	+	25 μg.± 50 μg.+	±	±	No blistering up to 150 μg.
10. 5,8-Dimethoxypsoralen (Isopimpinellin)		inactive	inactive	inactive. No erythema up to 2000 μg.	inactive 25 mg.	inactive	inactive	inactive	no pigmentation	no pigmentation	
11. 5-Amino, 8-methoxypsoralen (5 NH_2 8-MOP)		inactive	inactive	inactive. No erythema up to 2000 μg.	inactive 32 mg.	inactive	inactive	inactive	no pigmentation	no pigmentation	
12. 5-Nitro, 8-methoxypsoralen (5 NO_2 8-MOP)		inactive	inactive	inactive. No erythema up to 2000 μg.	inactive 25 mg.	inactive	inactive	inactive	no pigmentation	no pigmentation	
13. 8-Hydroxypsoralen (xanthotoxol)		inactive	inactive	inactive. No erythema up to 2000 μg.	inactive 28.5 mg.	inactive	inactive	inactive	no pigmentation	no pigmentation	
14. 4′, 5′-Dihydroxanthotoxin (4′,5′-dihydro 8-methoxypsoralen)		+	inactive	75 μg.	inactive 25 mg.	inactive	+	150 μg.	±	no pigmentation	

TABLE I—(*Continued*)

Furocoumarin	Structure	Relative Activity on Albino Guinea Pig Skin					Relative Activity on Human Skin				Remarks
		Topical	Oral	M. E. C.* for erythemal response			Erythemal response		Pigmentation response at		
				Topical μg./sq. in.	Oral mg./kg.	Degree of erythema	Topical	M. E. C.* μg./sq. in.	M. E. C.*	50 μg./sq. in.	
15. 8-Isoamyleneoxypsoralen		+	inactive	100 μg.	inactive 25 mg.	inactive	+	200 μg.	±	no pigmentation	
16. 4,5′-Dimethylisopsoralen		±	inactive	500 μg.	inactive 20 mg.	inactive	±	150 μg.	+	no pigmentation	
17. Isobergapten (5-methoxy isopsoralen)		+	inactive	250 μg.	inactive 30 mg.	inactive	±	200 μg.	±	no pigmentation	

* Minimum effective concentration.
† Millimols.

human skin, a lag period of 30 to 45 minutes after applying the compound was observed to give optimum response of erythema. In oral studies with guinea pig, a lag period of 1½ hours after feeding the compound was required before the animals could be subjected to radiation. With guinea pigs, a total time of 45 minutes' exposure was found to give optimum response, whereas on human skin 15 minutes' exposure was observed to be adequate.

Ultraviolet light lamp (G. E. Wood's light model no. 70) emitting light of wavelengths above 3200 Å (which had 52%, 25%, 35% and less than 1% transmission at 3650 Å, 3400 Å, 3800 Å and 3200 Å respectively) was used as a source of radiation. A distance of 14 cm. was kept constant between the lamp and the radiated skin area.

The degree of erythema which developed at the end of 18 and 36 hours after radiation was employed as a measure of relative activity of the compound. Erythema grading was evaluated photometrically (Photoelectric reflection meter model 610, Photovolt Corporation, New York) and compared with the visible intensity which was recorded in terms of conventional + or − signs.

In all these studies, care was taken to avoid exposure of skin directly to fluorescent light or sunlight before and after radiation.

RESULTS

Using albino guinea pig and human skin, the relative activity of various furocoumarins, coumarins and other related compounds has been investigated and a relationship of molecular configuration to the activity of these compounds postulated. In Table I, the relative activity of furocoumarins has been briefly summarized and expressed in graded response of + or − signs for topical as well as oral studies. The relative activity has been expressed after comparing the erythema response as observed by visible intensity grading and reflection meter reading.

It can be seen that out of 17 furocoumarin compounds tested, eleven showed photoactivation. Six compounds, namely psoralen, 4-methylpsoralen, 5′,8-dimethylpsoralen, 4,5′,8-trimethylpsoralen, 8-methoxypsoralen, 5-methoxypsoralen, exhibited more activity than psoralen glucoside, dihydroxanthotoxin, 8-isoamyleneoxypsoralen, dimethylisopsoralen and isobergapten. These latter five compounds are active topically at comparatively high concentration. Compounds like psoralenquinone, 2-thiofurocoumarin, 5,8-dimethoxypsoralen (isopimpinellin), 5-nitroxanthotoxin, 5-aminoxanthotoxin, xanthotoxol are inactive topically, as well as orally. All compounds active topically, however, do not show the same response when given orally. Dihydroxanthotoxin, 8-isoamyleneoxypsoralen, dimethylisopsoralen, isobergapten, do not produce any erythema even when given in large doses. On a molar basis psoralen and methylated psoralen derivatives possess high activity.

Furochromone compounds represented by khellin, chellol glucoside, and visnagin in Table II do not exhibit any photodynamic response. Compounds like furan, benzofuran have been reported to be inactive (3). Furacin and the open chain compound, cinnamic acid, were found to be inactive.

Coumarin compounds totaling 27 did not exhibit any erythema inducing action except thiocoumarin which showed some response at very high concentration. It is evident from Table III that coumarin and its other derivatives do not possess the active configuration for photodynamic action.

DISCUSSION

The furocoumarin ring configuration represents a fused structure of furan and coumarin rings. The unsubstituted psoralen molecule has been shown to exhibit a photodynamic action in presence of long wave ultraviolet (5). Photodynamic action in mammalian skin in presence of these compounds and ultraviolet light above 3200 Å would be reflected by biological changes of increased erythema and pigmentation. By topical application and oral feeding of different furocoumarin, coumarin, furochromones and other compounds to albino guinea pig and also comparing the response on human skin, the relationship of molecular configuration to the activity of various compounds has been evaluated.

The furan ring structure does not possess any erythema inducing action. The coumarin ring configuration compounds totaling 27 do not show any photoactivation. Orally as well as topically these different derivatives of coumarin do not show any erythemal response. Even the monomethyl, dimethyl and trimethyl derivatives which presumably possess more electron density, do not exhibit photodynamic effect. The psoralen molecule which has a fused ring structure of furan and coumarin shows highest activity. The absorption spectra of this molecule reveals four peaks of 212 mμ, 254 mμ, 294 mμ and 320 mμ.

TABLE II

Relative activity of furochromones and other compounds

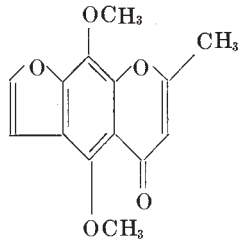
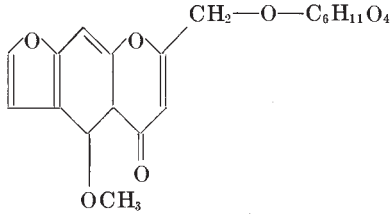
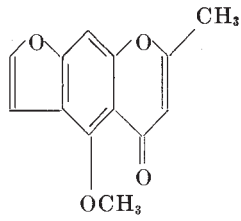
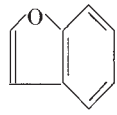
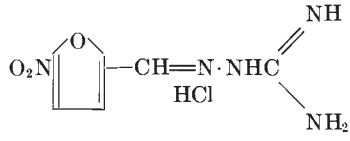
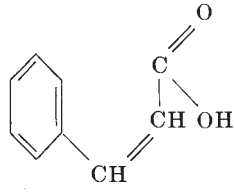
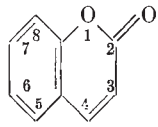
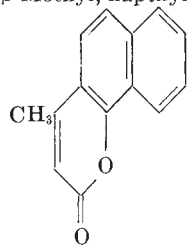
Compound	Structure	Topical erythema response (guinea pig skin)
1. Khellin		Inactive 1000 μg./sq. in.
2. Chellol glucoside (2-glucosoxy methyl-5-methoxy furochromone)		Inactive 1000 μg./sq. in.
3. Visnagin		Inactive 1000 μg./sq. in.
Other Compounds		
1. Furan		Inactive 1000 μg./sq. in.
2. Benzofuran		Inactive 1000 μg./sq. in.
3. Furacin (5-nitro-2-furaldehyde amino guanidine HCl)		Inactive 1000 μg./sq. in.
4. Cinnamic acid		Inactive 1000 μg./sq. in.

TABLE III

Relative activity of coumarin derivatives

Compound	Activity (Erythemal response)	
	Topical effect 1000 μg./sq in.	Oral effect
1. Coumarin 	Inactive	No response 30 mg./kg.
2. 7-Hydroxycoumarin (umbelliferone)	Inactive	No response 30 mg./kg.
3. 4-Hydroxycoumarin	Inactive	
4. 7-Hydroxy, 4-methylcoumarin (4-methylumbelliferone)	Inactive	
5. 3-Methylcoumarin	Inactive	
6. 4-Methylcoumarin	Inactive	No response 25 mg./kg.
7. 6-Methylcoumarin	Inactive	
8. 7-Methylcoumarin	Inactive	
9. 5,7-Dimethylcoumarin	Inactive	
10. 6,7-Dimethylcoumarin	Inactive	
11. 4,5,7-Trimethylcoumarin	Inactive	No response 33 mg./kg.
12. 3-Ethylcoumarin	Inactive	No response 26.1 mg./kg.
13. 3,4-Dihydro, 5-methylcoumarin	Inactive	
14. 3,4-Dihydro, 6-methylcoumarin	Inactive	No response 28.6 mg./kg.
15. 3,4-Dihydro, 7-methylcoumarin	Inactive	No response 28.6 mg./kg.
16. 3,4-Dihydro, 6,7-dimethylcoumarin	Inactive	
17. 5,7-Dihydroxy, 4-methylcoumarin	Inactive	
18. 8-Isopropyl, 5-methylcoumarin	Inactive	No response 25.3 mg./kg.
19. 4-Methyl, 7-ethoxycoumarin	Inactive	
20. 2-Thiocoumarin	+	No response 25 mg./kg.
21. 3-Chlorocoumarin	Inactive	No response 20 mg./kg.
22. 4 α Methylbenzocoumarin	Inactive	
23. 4 β Methylbenzocoumarin	Inactive	
24. Aesculine (6,7-dihydroxy, 6-glucoside)	Inactive	
25. 7-Acetoxy, 4-methylcoumarin 7-(—O—C(=O)—CH_3)	Inactive	
26. 7-Allyloxycoumarin 7-(—O—CH_2—CH_2=CH_2)	Inactive	
27. β Methyl, napthylcoumarin 	Inactive	

Though the action spectra and absorption spectra for photodynamic activity of this molecule have not been completely determined, characteristic absorption of this compound in long wave ultraviolet region can be said to be one of the factors which makes this molecule photodynamically active. Long wave ultraviolet does not cause increased erythema and pigmentation, but

in presence of this molecule, these biological changes are observed.

There are active sites in the psoralen molecule which are favorable for photon induced activation. The active regions in psoralen molecule are (a) valence bonds between carbon 3 and 4, (b) carbon atoms 5 and 8, (c) intact lactone ring, (d) furan and coumarin rings fusion at carbon atoms 6 and 7 to give linearly annulated structure of furocoumarin molecule, (e) unsaturated linkage between carbon 4′ and 5′.

Furochromone molecule as studied in three of its derivatives, has an altered 3-4 valence bond. These compounds are inactive. Fowlks (5) has shown that open lactone ring compound furocoumaric acid without any substitution at other carbon atoms, is an inactive molecule.

Methyl radicals which are known to increase the electron density and carcinogenic activity in carcinogenic compounds, however, do not appear to increase the erythema inducing property of psoralen on human skin. However, trimethylpsoralen exhibits highest response in guinea pig skin as compared to other active compounds. Methyl substitution does not affect the active centers in psoralen molecule by virtue of its electron donating property.

Substitution with a methoxy group at carbon 5 or 8 results in decreased activity. 5,8-Dimethoxypsoralen is completely inactive. Substitution with amino or nitro group at carbon 5 or hydroxyl group at carbon 8 reveals the same effect of inactivation. It appears that substitution with methoxy, nitro, amino or hydroxy group results in decreased electron density at carbon atoms 5 and 8 which therefore render the compound inactive.

4′5′-dihydro, 8-methoxypsoralen possesses very low activity when applied topically and when given orally does not show any photoreactivation. Methyl substitution at carbon 5′ does not alter the activity. The unsaturated linkage is probably needed at these carbon atoms.

The linkage of furan ring with coumarin ring at carbon 6 and 7 is essential. Furan ring attached to carbon 7 and 8 as shown in isobergapten and dimethylisopsoralen structure inactivates these compounds.

It is thus evident that the linearly annulated unsubstituted structure of furocoumarin molecule possesses the maximum activity. Non-linear structure loses its activity.

SUMMARY

Compounds of furocoumarin group which induce erythema and pigmentation of skin were tested on albino guinea pig and human skin for their relative activity along with other structurally related coumarin, furochromone and furan ring configuration compounds. A bioassay method for screening active and inactive compounds has been developed.

Of 17 furocoumarin compounds tested, psoralen was found to be most active. A relationship of molecular configuration to the activity of various compounds is discussed. A linearly annulated, unsubstituted compound having fused furan and coumarin rings, as present in psoralen molecule exhibits highest activity.

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