THE CHEMISTRY OF THE PSORALENS*

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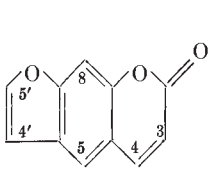
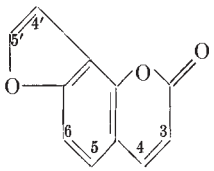
The psoralens belong to a group of compounds which have been considered as derivatives of coumarin, the furocoumarins. There are twelve different ways a furan ring can be condensed with the coumarin molecule and each of the resulting compounds could be the parent for a family of derivatives. Examples of most of these possible furocoumarins have been synthesized; but nature is more conservative so that all of the naturally occurring furocoumarins so far described turn out to be derivatives of psoralen I or angelicin II (1).



I



II

These natural derivatives of psoralen and angelicin have one or more of the following substituents at the 5,8 or 5,6 positions respectively: hydroxy, methoxy, butanyloxy isoamyleneoxy, geranyloxy and substituted isoamyleneoxy derivatives such as epoxy, dihydroxy or esterified hydroxy. In addition derivatives of both psoralen and angelicin are found with isopropyl or substituted isopropyl groups at the 5′ position and some of these derivatives have oxygen functions; keto, hydroxy or methoxy; at the 4′ position. Altogether about two dozen furocoumarins have been isolated from natural sources.

Although the first furocoumarin was isolated over a hundred years ago, when in 1834 Kalbrunner isolated bergapten (5-methoxypsoralen) from bergamot oil, the biological importance of these compounds has not been known until very recent times. The fact that these compounds are found in various plant materials leads naturally to teleological speculation as to what biological function they fulfill. Such speculation was justified since biological activity of the psoralens and angelicins has been demonstrated. They appear to have specific biochemical properties which may contribute to the survival of certain plant species. Specifically these compounds belong to that group of substances which can inhibit certain plant growth without otherwise harming the plant (2, 3, 5).

It is interesting that it was this property which led to the isolation of the only new naturally occuring furocoumarin discovered in the United States. Bennett and Bonner (2) isolated thamnosmin from leaves of the Desert Rue (*Thamnosma montana*) because a crude extract of this plant was the best growth inhibitor found among the extracts of a number of desert plants surveyed for this property, although all the extracts showed seedling growth inhibition. One could speculate as to the role such growth inhibition plays in the economy of those desert plants when survival may depend upon a successful fight for the little available water. The structure of thamnosmin was determined by Crosby (4) to be a derivative of angelicin namely 5-methoxy-6-(2,3-epoxyisopentanlyoxy-) angelicin. Rodighiero (5) has also shown that psoralen, 8-methoxypsoralen and angelicin inhibit seed germination, root growth and seedling growth.

Such findings suggest that further investigation may reveal certain furocoumarins in the role of natural growth regulator of certain plants just like another coumarin derivative, scopoletin, which Goodwin and others (6, 7) have shown regulates cell division in the root of *Avena sativa*. This idea is strengthened if one recalls that the psoralens of *Ammi majus*, 8-methoxypsoralen, 5-methoxypsoralen and 8-isoamylenoxypsoralen (imperatorin) are found concentrated in the pericarp of the fruit (seed). The psoralen and angelicin of *Psoralea corylifolia* are also found in the pericarp of the fruit (28) and thus the germ of the seed is surrounded by a tissue containing a germination inhibitor, which could regulate the time when sprouting will occur by the rate at which it diffuses into the surrounding soil. Chakraborty, DasGupta, and Bose (8) have very recently shown that out

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Presented at the Brook Lodge Invitational Symposium on the Psoralens, sponsored by The Upjohn Company, Kalamazoo, Michigan, March 27-28, 1958.

of seventeen natural coumarin derivatives which they tested, the psoralens, including psoralen and imperatorin, were the most effective antifungal agents tried. And thus the economy of nature would be beautifully demonstrated if the same compound acts as germination inhibitor and also decontaminates the soil by killing pathogenic fungi as it diffuses away.

Musajo (3) has mentioned some hitherto unreported results of Dolcher, Rodighiero and Caporale who described the mutagenic properties of five furocoumarins and found 5-methoxypsoralen and psoralen to be almost as effective as the most effective mutagenic agent known, trypaflavin, when tested on onion root tips. Psoralen at 5.6×10^{-5} M. and 5-methoxypsoralen at 5.0×10^{-5} M. induced 40% of mitosis with chromosome mutations when incubated with the onion roots for 4 hours at 20°C. At higher concentrations there was total inhibition of mitosis. Chromosome aberrations noted were agglutinization of chromosomes and liquification of the chromosome surface (stickiness effect). It is not reported if these experiments were performed in the absence of light which could have caused these effects due to photosensitization in the presence of the furocoumarins (29). The photosensitizing properties of several of the psoralens have been described elsewhere (3, 9).

SEPARATION OF FUROCOUMARINS

With new discoveries of the biological activities of the several furocoumarins which have appeared in recent years there has also been a renewed activity concerning the chemistry and physical properties of these compounds. In particular a number of investigators have sought ways of isolating, identifying and quantitatively determining the furocoumarins in general and certain of them in particular.

There is no simple quantitative method for the isolation of the furocoumarins of plant origin. The usual methods which have been employed for the isolation of numerous natural products have been used for the furocoumarins namely: extraction with lipid solvents or alcohol followed by partition between immiscible solvents with or without the use of acids or alkalies, then further purification by repeated recrystallizations or combinations of sublimation and recrystallization. Such methods are not suitably quantitative, nor are they technically satisfactory.

A number of investigators have therefore turned to paper chromatography as a means of separation and identification of furocoumarins. The first effort along these lines was that of Svendsen (14) who reported the separation of pimpinellin, isopimpinellin, bergapten, and isobergapten from *Pimpinella magna* and *P. saxifraga*. He chromatographed on paper the microsublimates from the crude plant extracts using petroleum ether (65–70° C.)–benzene–95% methanol (5:4:2) to develop the chromatograms. At about the same time in 1952 Riedl and Neugebauer (15) also published a chromatographic method for the isolation and identification of coumarins from plant sources. They used a paper that had been presprayed with glycol (ethylene or propylene) and was developed with benzene at 10°C. for their separation. Swain (16) studied a number of solvent systems for the separation of a wide variety of naturally occurring coumarins including three furocoumarins. Rodighiero, Caporale and Ragazzi (17) reported the identification of psoralen and bergapten from *Ruta graveolens* using chromatographic separation on paper developed with methanol-pyridine-water (18:1:1) solution. They determined the Rf. of a number of different furocoumarins in the methanol-pyridine-water solvent and also in methanol-phenol-water (45:4:1). Chakraborty and Bose (18) have made the most extensive study of the paper chromatographic separation of natural coumarins. They studied the separation of 12 representative coumarin derivatives, including furocoumarins, in 21 different solvent systems. Unlike other investigators they also attempted the separation of a mixture of their coumarin derivatives and discovered that most spots contained more than one compound and when a single compound was separated from the mixture its Rf. was usually altered sufficiently so that positive identification was rarely possible. The author has confirmed these observations of difficulties with the chromatographic separation of mixtures and also that the best separations on paper are obtained with solvent mixtures containing 85% or more water. It is our observation that the addition of up to 15% of solvents such as acetic acid or methylethylketone to the water increased the capacity of the system and usually sharpens the separation somewhat. Paper chromatography alone can not be relied upon for the separation and identification of furocoumarins unless a preliminary purification has first been carried out.

The separation of a number of furocoumarins

using paper electrophoresis (19) on a circular disk apparatus has also been reported. Difficulties are encountered with this method in mixtures. Preliminary separations of coumarins from natural sources using aluminum oxide column chromatography has been reported (20–22) but in general pure compounds have not been obtained.

ANALYTICAL REACTIONS

The analytical determination of the furocoumarins remains a problem. One might expect from general considerations that furocoumarins would be quite reactive or at least undergo reactions typical of activated aromatic compounds but in fact they are rather unreactive in the usual sense. No one for example has reported coupling a diazonium compound with a furocoumarin, a reaction which is characteristic of activated aromatic compounds. Likewise efforts to prepare derivatives based upon reactions designed to open the lactone ring, *i.e.*, the phenylhydrazides or hydroxamic acids, are met with failure. Such lack of reactivity has frustrated efforts to find specific reactions which will lead to colored derivatives with somewhat specific absorption spectra. The only reaction known which goes with facility is a reaction with diluted nitric acid. In acetic acid this reaction appears to give a quantitative yield of nitro derivative. But the absorption spectrum of the resulting compound has little to recommend it over the spectrum of the parent compound for analytical purposes.

The absorption spectra of psoralen, 5-methoxypsoralen, 8-methoxypsoralen and 5,8-dimethoxypsoralen are reproduced in Figure I. These spectra were obtained on a Cary, model 14, recording spectrophotometer. One should note that as with coumarins (31) there are three regions in which one or more absorption maxima occur, these are the less than 225 region, the 230–270 region and the 290–330 region. Distinct minima occur between these regions. Hydrogenation in the 3,4 position (30) or opening of the lactone ring greatly reduces the absorption coefficient in the 295–325 region. Psoralen, 5-methoxypsoralen 8-methoxypsoralen and 5,8-dimethoxypsoralen obey Beers' law to the limit of the solubility of the compounds.

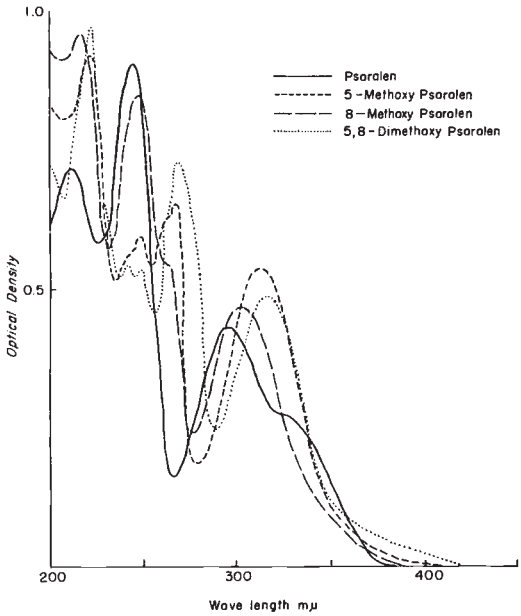


FIG. I. Traces of the individual Cary recording spectrophotometer records made of 4.0 × 10^{-5} M solutions of each of the furocoumarins: psoralen, 5-methoxypsoralen, 8-methoxypsoralen and 5,8-dimethoxypsoralen in 25% ethanol.

CHEMISTRY OF PSORALENS

Excellent reviews have appeared, some in recent years, which summarize the chemistry and natural origin of the coumarins including the furocoumarins (1, 3, 10–13). These reviews have adequately covered the methods of synthesis of furocoumarins and the proofs of structure of most of the naturally occurring compounds. Some degradation reactions and photochemical reactions will be considered here.

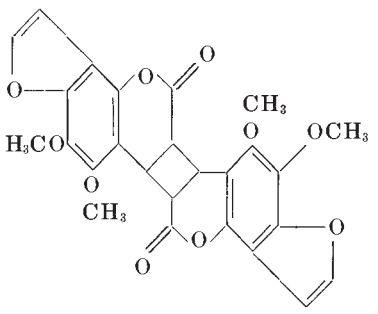
The methods used to degrade psoralens for structure determination have been well described, particularly in papers by Späth and his coworkers (for references see 10–13), therefore, only a brief description of three degradation reactions will be given here. One of the three reactions gives information as to the presence of a condensed furan ring which positively identifies an unknown compound as a furocoumarin if other reactions have identified it as a coumarin. Furan-2,3-dicarboxylic acid is obtained when either psoralen or angelicin derivatives are oxidized with alkaline peroxide. Reaction of an alkoxy furocoumarin, other than a methoxy derivative, with an acetic acid-sulfuric acid mixture results in cleavage of the ether. One may then identify the resulting furocoumarin phenol and the alcohol by other reactions. The third reaction can give information as to whether the compound is a psoralen or angelicin derivative. For this reaction

the lactone ring is opened in strong sodium hydroxide solution and the resulting phenolic compound is methylated. Permanganate oxidation followed by methylation gives derivatives of 4,6-dimethoxyisophthalic acid from psoralen and its derivatives and derivatives of 2,4-dimethoxyisophthalic acid from angelicin and its derivatives. This method fails to distinguish 5-methoxypsoralen from 5-methoxyangelicin and pimpinellin from isopimpinellin.

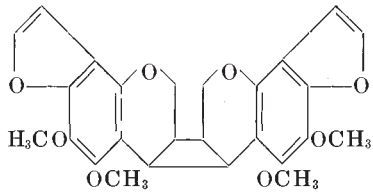
PHOTOCHEMICAL REACTIONS OF PSORALENS

Wessely and Dinjaski (23) exposed 5,6-dimethoxyangelicin, pimpinellin, in a thin layer to diffused sunlight for 2.5 months and obtained a dimer which regenerated the parent compound upon heating and gave furan-2,3-dicarboxylic acid upon alkaline oxidation with hydrogen peroxide. Hydrogenation of the dimer gave a tetrahydro derivative which was split with heat to a single compound that melted at 132–3° and added a mole of hydrogen. Irradiation of 5,6-dimethoxyangelicin in acetic acid solution gave a different dimer that underwent the identical reactions of the first dimer. In a later paper (24) Wessely with Plaichinger investigated the hydrogenation of photodimers of coumarin and herniarin and a synthetic dicoumarin and reaffirmed the earlier conclusion that the photodimers of pimpinellin have a cyclobutane structure either III or IV.

In a paper on photochemical reactions in sunlight Schönberg *et al.* (25) investigated the reaction of phenanthraquinone with a number of ethylene derivatives including 8-methoxypsoralen and some benzofuran derivatives. He postulated the reaction as between the 4′,5′ positions of the furocoumarin and the orthoquinone oxygens of phenanthraquinone to give a derivative of furobenzodioxin V. Schönberg gives no proof of this structure but reasons from analogy. He found that coumarin does not react with phenanthraquinone in sunlight while benzofuran and its derivatives do.



III



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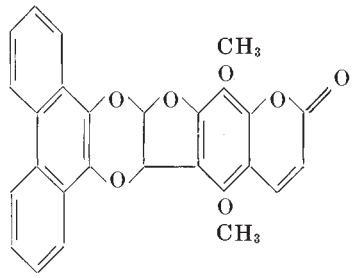

V

In a paper by Lerner *et al.* (26) changes in the absorption spectra of 8-methoxypsoralen were reported as a result of irradiation. The change consisted of a loss of the characteristic peaks and valleys of the spectrum and a generalized absorption getting stronger toward shorter wavelengths. Upon irradiation of psoralen the author noted this same response and chromatography of the resulting solution revealed at least two new fluorescent compounds had been formed. One of these compounds, on the basis of its chromatographic behavior and by analogy with the results obtained with 5,6-dimethoxyangelicin, has been designated a dimer. The other proved to be furocoumaric acid identical with the one reported by Stoll (20). Similar results are obtained upon irradiation of 8-methoxypsoralen and 5-methoxypsoralen. The ultraviolet absorption spectra of the three compounds eluted from the irradiated psoralen chromatograms are given in Figure II. From the differences in the absorption spectrum of the compound designated a dimer as compared with the parent compound it would appear that while the assignment of a cyclobutane derivative for its structure as made by Wessely (24) might be possible in this case it is not probable. Absorption in the 295 mμ to 325

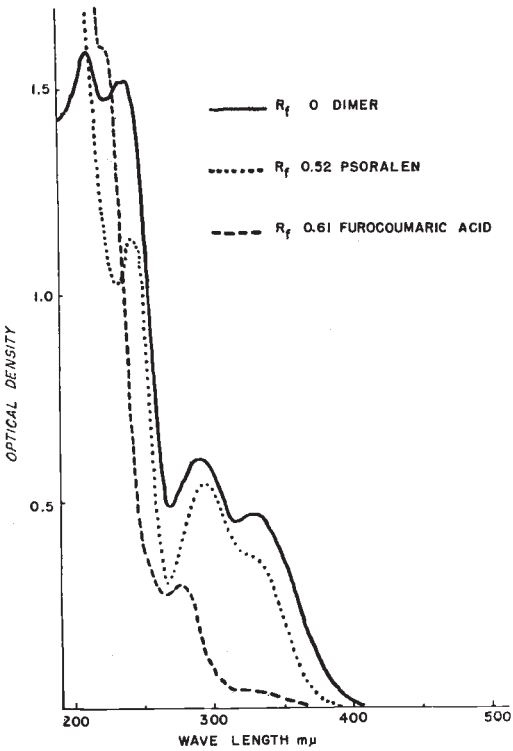

FIG. II. Traces of a Cary recording spectrophotometer record of the spectra made of the eluates of the three fluorescent bands of a paper chromatograph on which an irradiated psoralen solution in 40% ethanol had been streaked. The chromatograph was developed with 15% acetic acid in water, air dried 24 hours and eluted with 95% ethanol. The concentrations are unknown.

mμ region for coumarin and its derivatives appears to be due principally to the lactone ring (30) since if this ring is open or the 3,4-double bond is hydrogenated absorption in this region is lost. The furano ring also makes a contribution in the 295–325 mμ region but it is slight as is shown by the spectrum of furocoumaric acid. The spectrum of the dimer on this basis suggests that the dimerization involves a bond between 3 or 4 atom of one molecule of psoralen and the 4′ or 5′ atom of another molecule with saturation of the double bond of the furano ring. Such photodimers would be consistent with the findings of both Wessely and Schönberg. More work will be done in these laboratories on this problem to ascertain the exact structure of these photodimers.

We would like to thank E. W. Peterson who ran the absorption spectra reported here and the Paul B. Elder Company of Bryan, Ohio for gifts of 8-methoxypsoralen and Sandoz Ltd. of Basle, Switzerland for generous supplies of psoralen. These investigations were supported by a grant from the United States Public Health Service, Grant No. C2837.

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