

# THE CHEMISTRY AND PHYSIOLOGICAL ACTION OF KHELLIN AND RELATED PRODUCTS

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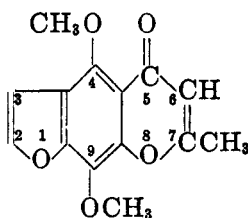
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## CONTENTS

I. Introduction.....	543
II. Natural sources of khellin and related products.....	544
A. <i>Ammi visnaga</i> L.....	544
B. <i>Ammi majus</i> L.....	547
III. Chemistry of khellin.....	548
A. Degradations.....	548
1. Khellin.....	548
2. Visnagin and visnaginone.....	550
3. Khellol glucoside.....	552
4. Khellaktone.....	554
5. Visnagan.....	554
6. Fatty acids.....	554
B. Identification and assay.....	555
C. Syntheses.....	556
1. Khellin.....	556
2. Norkhellin.....	562
3. Visnagin and visnaginone.....	564
4. Isovisnagin and isovisnaginone.....	565
IV. Physiological action.....	568
A. Pharmacological data.....	568
1. Khellin.....	568
2. Khellol glucoside.....	571
3. Visnagan.....	571
B. Comparative assays and correlation of structure with activity.....	572
C. Clinical data.....	574
1. Khellin.....	574
2. Physiological effects of products isolated from <i>Ammi majus</i> L.....	574
V. References.....	575

## I. INTRODUCTION

Khellin (4,9-dimethoxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one or 5,8-dimethoxy-2-methylfuro(4',5',6,7)chromone) is obtained from the fruits and seeds of the plant *Ammi visnaga* L. The fruits contain about 1 per cent of this



Khellin (visammin)

compound. Pure khellin,  $C_{14}H_{12}O_5$ , m.p. 153–155°C., occurs in colorless, odorless needle-shaped crystals, having a bitter taste. It has also been called visammin by some authors (77).

Khellin is very soluble in chloroform and less soluble in cold ether and light petroleum. It is soluble at 25°C. in 130 parts of 95 per cent alcohol, 6750 parts of water, 500 parts of a saturated aqueous solution of theophylline, and 33 parts of a saturated solution of sodium benzoate. It is also soluble in glacial acetic acid and in dilute mineral acids, from which it is recovered unchanged (31).

A number of pharmaceutical laboratories in Egypt and in the United States prepare khellin on a commercial scale.<sup>1</sup> It is generally dispensed in the form of tablets or injectable solutions. Tests for identification and purity and methods of assay have been developed (31) (see Section III, B).

The plant from which khellin is obtained and its use as a home remedy were known to the Egyptians centuries ago.

One of the worst plagues in Egypt is schistosomiasis or bilharzia, which, according to Strong (99), affects some six million out of twelve million Egyptians. It is caused by the trematode *Schistosoma hematobium*, *mansoni*, or *japonicum*. This worm, which lives as so-called Miracidium larvae in molluscs or water-snails, enters the human body in the form of cercaria by way of the drinking water, or penetrates through the skin of bare-legged bathers. It lives in the portal veins and deposits eggs in the mucous membranes of the bladder, causing hematuria, cystitis, etc. The irritation caused by developing worms frequently results in the formation of kidney, gall bladder, or ureteral stones.

The active principles of the Khellah plant (*Ammi visnaga* L.) were known to the Egyptians to be useful in relieving the pain of renal colic and ureteral spasms, and often to facilitate the passing of ureteral stones. Khellah was also known to have a beneficial effect on caries, odontitis, and gingivitis. This knowledge must have spread to France, where one of the first published papers (63) on Khellah appeared, and where it is mentioned with the French name, "Herbe aux curedents" ("tooth-pick" plant).

A decoction and tincture of *Ammi visnaga* L. was included in the *Egyptian Pharmacopoeia* (1934) and recommended as an antispasmodic in renal colic.

Following the elucidation of the chemical structure of khellin (54, 82, 93, 94), additional pharmacological properties were discovered which led to its clinical application as a coronary vasodilator in bronchial asthma and in angina pectoris.

## II. NATURAL SOURCES OF KHELLIN AND RELATED PRODUCTS

### A. AMMI VISNAGA L.

*Ammi visnaga* L. is a perennial herbaceous plant found in the waste lands of the Eastern Mediterranean and particularly in the region of the Nile delta. It is called Chellah, Khilla, or Khella by the Egyptians. A close relative of this plant is *Ammi majus* L., which is often mistaken for *Ammi visnaga* L. but differs,

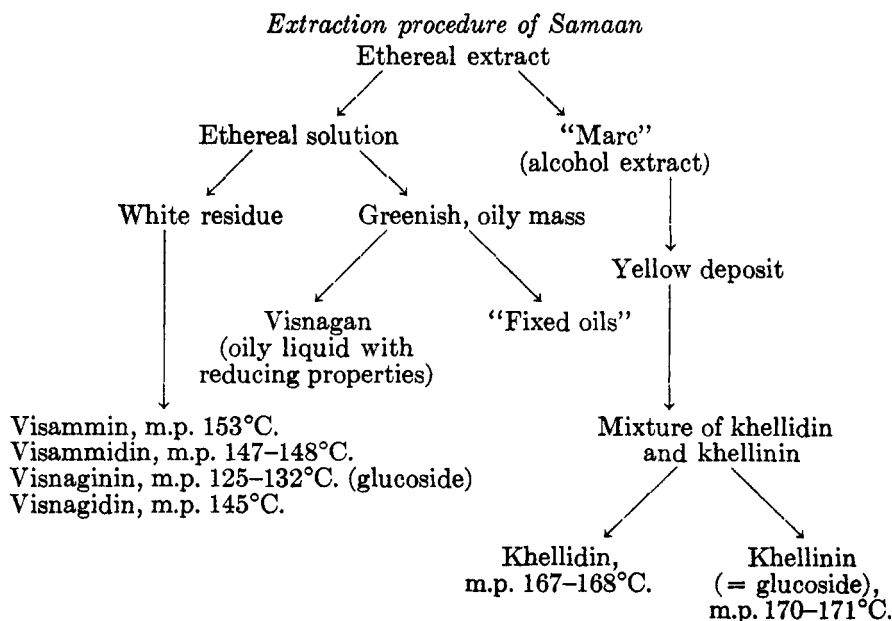
<sup>1</sup> For instance: Ammicardin (pure khellin), Alpha Laboratories, Cairo, Egypt; Ammivin (pure khellin), The National Drug Company, Philadelphia, Pennsylvania; Eskel (a mixture of khellin and visnagan, 75:25), Smith, Kline and French Laboratories, Philadelphia, Pennsylvania.

not only morphologically but also in its active principles, from *Ammi visnaga* L. *Ammi majus* L. was called Ami by Galenus and at the time of Charles the Great, Ameni. In Egypt it is also known by the names "Aatrillal," "Gazar et Shaytam," "Regl el Ghorab," and "Chella Sheytaniya." Both plants belong to the family of Umbelliferae, in which we find many other pharmaceutically well-known plants, such as *Carum carvi* (caraway) and *Conium maculatum* (hemlock). *Ammi visnaga* L. actually looks to a layman's eye very much like *Conium* (hemlock) and is of quite a hardy nature. For example, it was possible by using seeds from Egypt to plant it successfully in the much colder climate of Minnesota (102). Photographs of *Ammi visnaga* L. and its fruit can be found in the publications of Upsher Smith (102) and Fahmy and Abu-Shady (29). The plant grows 1.5 m. high. It is odorless when fresh but develops a pleasing aromatic odor on drying. The fruits are comparable to caraway seeds, being greyish brown, ovoid schizocarp. The fruits of *Ammi visnaga* L. are pear-shaped, while those of *Ammi majus* L. are more cylindrical. Macroscopic and microscopic differentiation of the two plants and their fruits is possible (29).

Alcoholic extracts of powdered fruits of *Ammi majus* L., when examined under filtered ultraviolet light, emit a blue fluorescence which is not given by alcoholic extracts of the powdered fruits of *Ammi visnaga* L. (52). These two plants can also be differentiated by triturating their powders or extracts with solid sodium hydroxide; a pink color is produced only with *Ammi visnaga* L. (73).

In 1879 Moustapha (68, 69) extracted a white crystalline glycoside, as silky needles of bitter taste, from the fruits of *Ammi visnaga* L.; for this substance he proposed the name "kelline."

In 1881 Malosse (63) obtained 2 per cent of a brown oily material, which he called visnagol, three crystalline products which he called  $\alpha$ -,  $\beta$ - and  $\gamma$ -visnagin, and another oil, which after saponification yielded Ammi stearic acid, m.p.

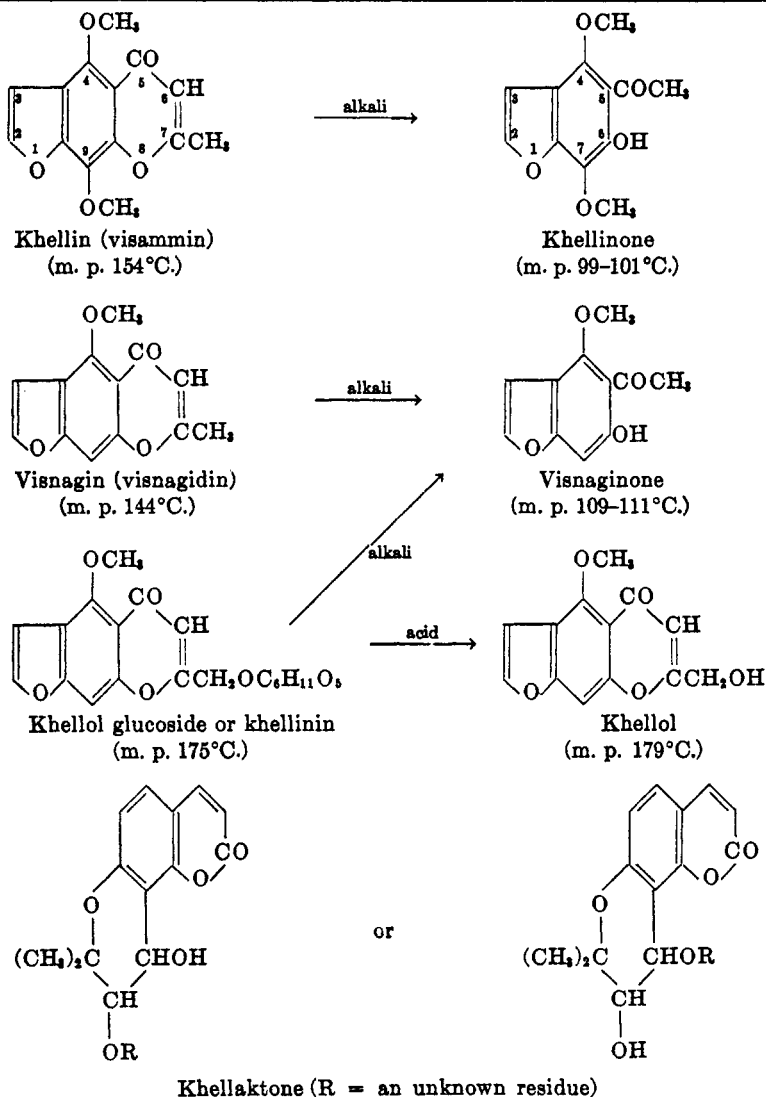


17°C. He also isolated a number of constituents such as albuminoids, glucose, etc.

Attempts by Samaan (77) to isolate the active principles of *Ammi visnaga* L. led only in a few instances to chemically pure substances. No analytical data

TABLE 1

*Products isolated from Ammi visnaga L. and their hydrolysis products (5, 42, 64, 93, 94, 95)*



were given. The nomenclature used by Samaan differs considerably from that used later by Spaeth and Gruber. His khellinin is identical with Spaeth's khellol glucoside (correct melting point 175°C.) and his visammin with khellin (correct melting point 154°C.).

Spaeth and Gruber (93) obtained pure khellin by extracting the fruits of *Ammi visnaga* L. with ether. The ether was evaporated, and petroleum ether was added. Crystals and also a greenish-yellow material were obtained on chilling. The latter was separated from the petroleum ether, treated with boiling water, and the filtrate was concentrated until crystals appeared. All crystalline material was combined and extracted with chloroform; concentration of the chloroform extract gave crude khellin, m.p. 144–145°C. (0.38 per cent yield). After recrystallization from methyl alcohol it had a melting point of 154–155°C.

Visnagin was isolated (94) from a mother liquor of khellin by evaporating it to dryness, dissolving the residue in benzene, and adding petroleum ether to the hot solution until turbidity appeared. Khellin precipitated out on cooling and was filtered off; upon further addition of petroleum ether, a crude fraction of visnagin (m.p. 139–142°C.) was obtained. The pure visnagin had a melting point of 144–145°C.; the yield was 0.045 per cent.

Pure khellol glucoside was obtained (95) by extracting the fruits of *Ammi visnaga* L. first with ether and then with methyl alcohol. The methyl alcohol fraction gave the glucoside, which crystallized with water and melted at 142–144°C.; the anhydrous glucoside melted at 174–176°C.

The crystalline principles that have been isolated from *Ammi visnaga* L. and identified and their hydrolysis products are shown in table 1. In general, the fruits contain about 1 per cent of pure khellin, 0.1 per cent of pure visnagin, and 0.3 per cent of pure khellol glucoside (31, 41).

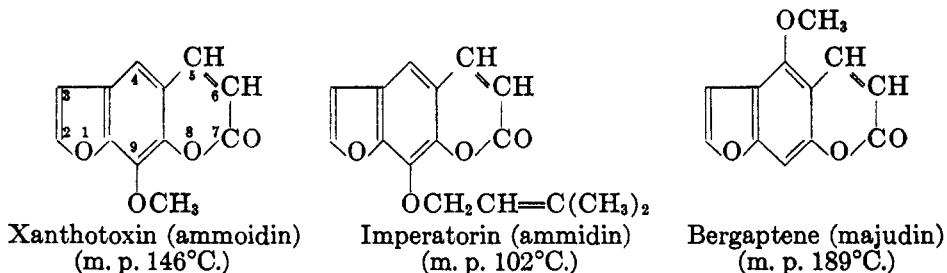
#### B. AMMI MAJUS L.

Schoenberg and Sina (90) identified ammoidin, which had been isolated from *Ammi majus* L. (29, 30, 92a), as xanthotoxin, which had previously been isolated by Thoms (100) and Bose and Mookerjee (18) and synthesized by Spaeth and Pailer (98).

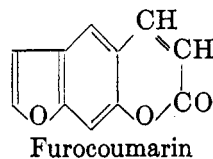
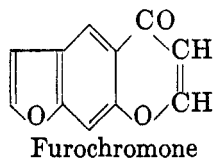
The second crystalline substance obtained by Fahmy and Abu-Shady, ammidin, was identified as the coumarin imperatorin, the structure of which had been elucidated by Spaeth and Holzen (96) as early as 1933.

Fahmy and Abu-Shady (28) amplified the findings of Schoenberg and Sina by identifying majudin, a third crystalline product from *Ammi majus* L., as bergaptenone, which had been isolated in 1912 by Thoms and Baetcke (101) from another plant and also studied in detail by Bhar (17).

The crystalline principles that have been isolated and identified from *Ammi majus* L. are shown below (17, 96, 98, 100, 101):



It is interesting to note that all the known active principles of *Ammi visnaga* L. are furochromones with the exception of khellaktone, while those of *Ammi majus* L. and of *Psoralea corylifolia* L. are furocoumarins.

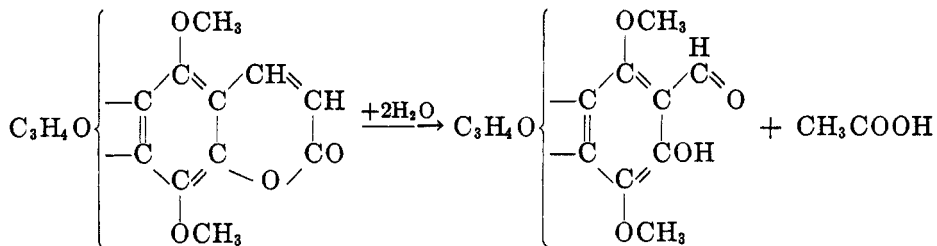


### III. CHEMISTRY OF KHELLIN

#### A. DEGRADATIONS

##### 1. *Khellin*

Fantl and Salem (33) found that khellin, which had the empirical formula  $C_{14}H_{12}O_5$ , gave on hydrolysis with barium hydroxide a compound having the formula  $C_{12}H_{12}O_5$  and melting at  $100^\circ C$ . Both khellin and the split product were observed to have two methoxyl groups and their formulas were resolved as follows:

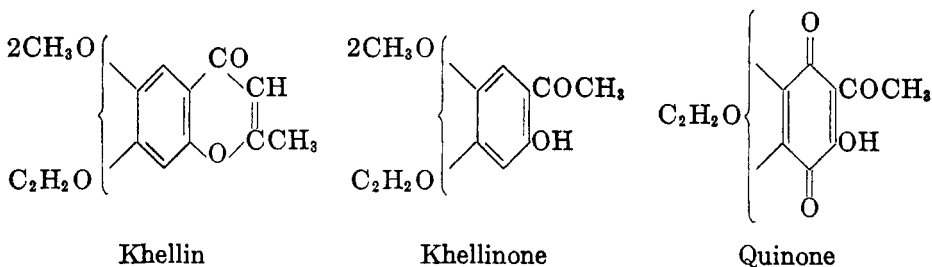


Khellin  
(according to Fantl and Salem)

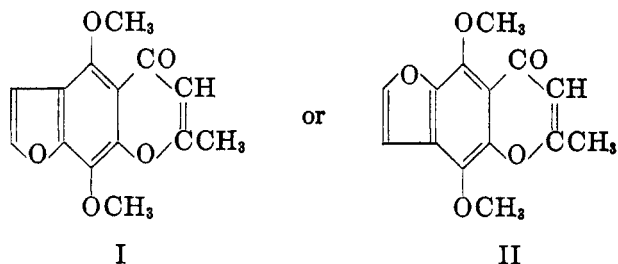
Three years later, in 1933, the complete and correct formula for khellin was published for the first time by Samaan (82), but it was left to Spaeth and Gruber (93) to prove the structure by degradation and partial synthesis. Samaan's publication was based, according to Anrep, Barsoum, Kenawy, and Misrahy (2), on the work by Hassan (49) and Malik (62).

Spaeth and Gruber (93) confirmed the empirical formula of khellin. The second split product was again found to be acetic acid. This meant that two moles of water had been taken up (while in the case of ester hydrolysis only one mole of water would have been taken up). Since khellin could be easily hydrolyzed with 1:1 potassium hydroxide, it was likely to be a chromone. Khel-

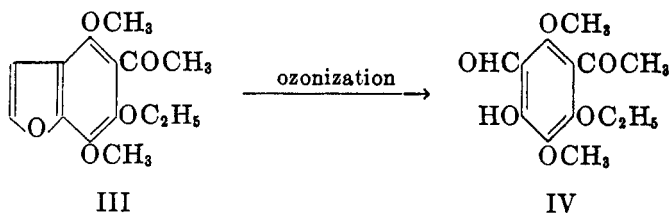
linone on oxidation with nitric acid in ether gave the yellow quinone  $C_{10}H_6O_5$ , thus proving that the two methoxyl groups were in the *p*-position. Therefore khellin had to have the structure shown below:



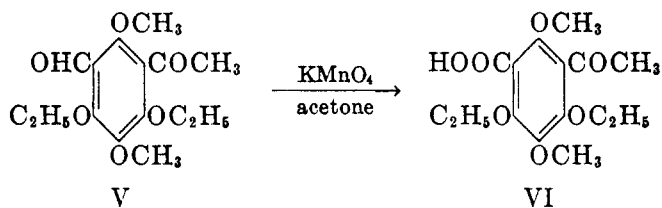
The remaining  $C_2H_2O$  was likely to be a furan ring, which is often found in related natural products. According to a method used in the furocoumarin series, Spaeth and Gruber oxidized with alkaline hydrogen peroxide and obtained the expected 2,3-furandicarboxylic acid. The furan ring could be connected with the rest of the structure in two ways.



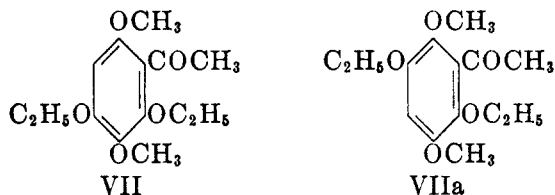
The decision in favor of I was based upon the following facts: Khellinone ethyl ether, tentatively assumed to have structure III (proven later to be correct), gave compound IV on ozonization.



Since the hydroxyaldehyde (IV) could not be oxidized directly, the ethyl ether (V) was prepared and oxidized to VI.

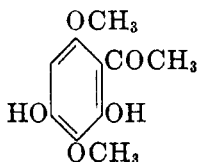


Compound VI, when treated with quinoline and copper, split off carbon dioxide and gave the ketone VII.



If formula II were correct, this would be VIIa.

Wessely and Moser (103), however, had prepared from 2,5-dimethoxyresorcinol the ketone



which, with diethyl sulfate and potassium hydroxide, gave compound VII, as proved by mixed melting points of semicarbazones. This showed conclusively that khellin must have structure I. The conclusion of Spaeth and Gruber was further corroborated by Geissman's synthesis (36) (see page 556), and also by Samaan's spectrophotometric data (89) (see page 555).

Spaeth and Gruber finally accomplished a partial synthesis of khellin, starting from khellinone, which will be discussed later on.

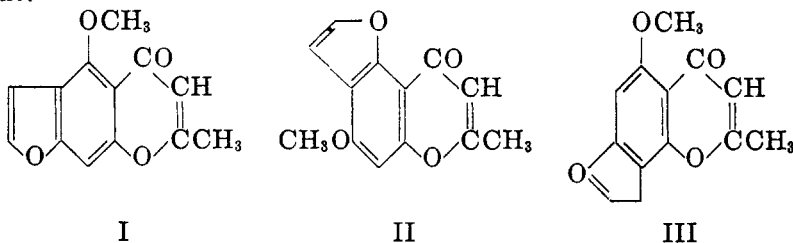
## 2. *Visnagin and visnaginone*

Spaeth and Gruber (94) also elucidated the structure of visnagin. They found the empirical formula to be  $\text{C}_{13}\text{H}_{10}\text{O}_4$  and one methoxyl group to be present. On warming with 1 per cent potassium hydroxide two molecules of water were added, giving acetic acid and visnaginone,  $\text{C}_{11}\text{H}_{10}\text{O}_4$ , m.p. 109–111°C.

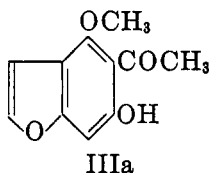
Visnaginone had a phenolic hydroxyl group (giving an ethyl ether of melting point 153–154°C.). The presence of a carbonyl group (ortho to the phenolic hydroxyl group) was proven by ring closure according to Kostanecki; in an analogous manner to the formation of 3-acetylkhellin from khellinone, 3-acetylvisnagin, m.p. 192–193°C., was formed. Visnagin treated with hydrogen peroxide gave furandicarboxylic acid. This proved that *one* oxygen atom was present in a coumarone complex. Visnaginone produced phloroglucinol upon



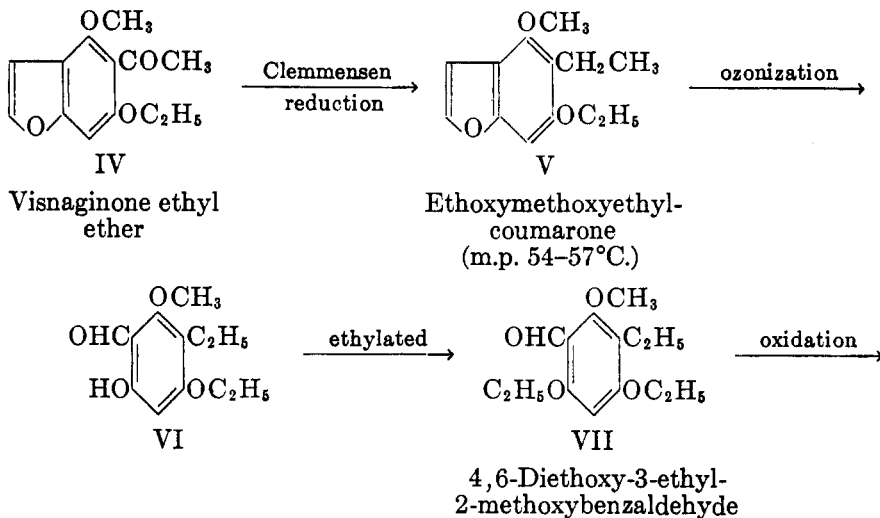
alkaline fusion. Consequently, the decision between formulas I, II, and III was difficult:



In all three cases, visnaginone had to be a hydroxymethoxyacetocoumarone, which (if formula I were correct) should be:

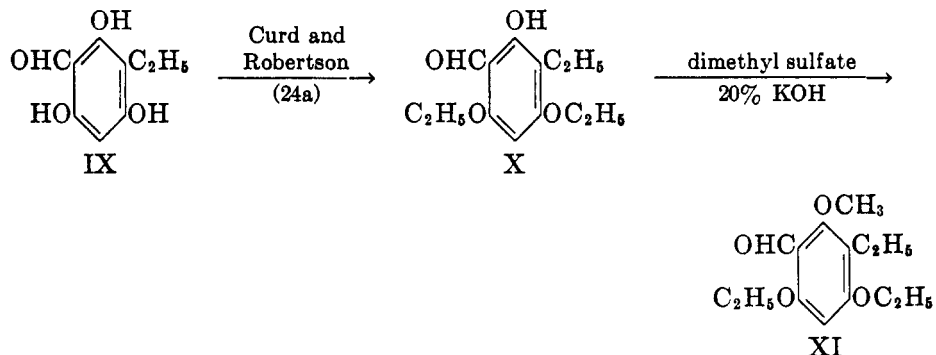


Structure proof in favor of I was accomplished as follows:

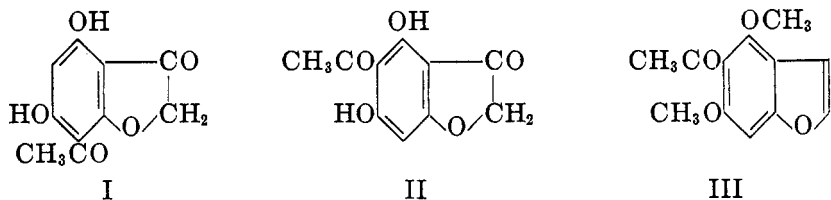


Spaeth and Gruber proved the structure of VII (and thereby of I) by synthesizing both VII and VIII, starting from ethylphloroglucinol (prepared by

Clemmensen reduction from phloroacetophenone), introducing an aldehyde group according to Gattermann (IX), ethylating (X), and methylating (XI). The semicarbazone of XI, obtained by synthesis, was shown by analysis and mixed melting points to be identical with the one of VII, obtained by degradation. In this way the structure of I has been proven.



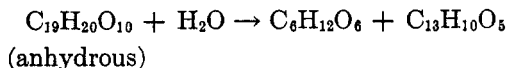
Additional structure proof for visnaginone methyl ether has been supplied by the work of Gruber and Hoyos (46), who were able to show that visnaginone methyl ether (III) could not be formed from isomer I as a starting material, whereas it should be possible to obtain it from isomer II as a starting material.



This latter synthesis, however, was not carried out, owing to insufficient quantities of isomer II.

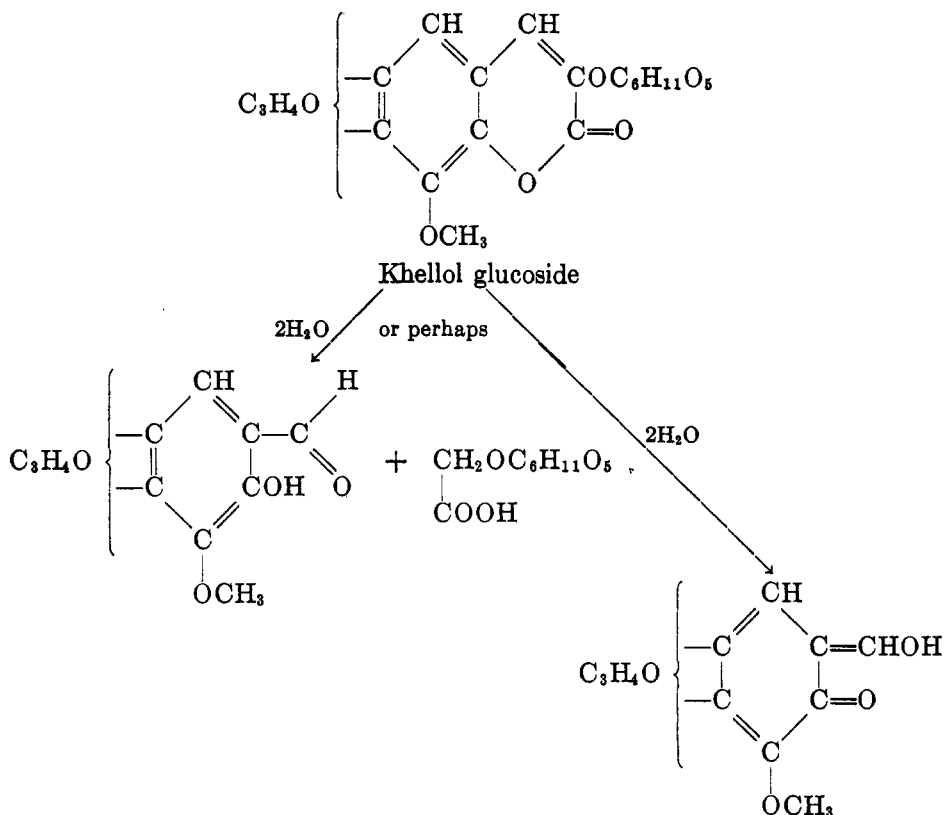
### 3. *Khellol glucoside*

Fantl and Salem (33) attempted to determine the structure of khellol glucoside. They found the sugar to be glucose and the empirical formula to be  $C_{19}H_{24}O_{12}$  (including  $2H_2O$ ). Acid hydrolysis proceeded as follows:



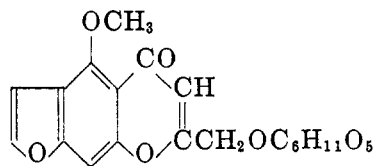
The latter compound was called khellol and was believed to contain a basic oxygen atom, such as is present in chalcones, lactones, and pyrones.

Alkaline hydrolysis of the glucoside gave a compound having the composition  $C_{11}H_{10}O_4$ . From attempts to resolve the structure of this split product the following structures were assumed:

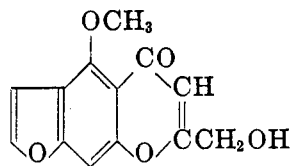


Work on khellol glucoside was continued by Hassan (49) and by West (104), but their theses were not available to the authors of this review.

In 1941 Spaeth and Gruber (95) elucidated the structure of khellol glucoside. Hydrolysis with alkali gave a yellow product, m.p. 109–110°C., which was identical with visnaginone. Contrary to Fantl and Salem, Spaeth and Gruber did not find that the khellol glucoside had lactone character. Instead, they suggested the following formulas, which are the same as those proposed previously by Samaan (82) but without chemical data:



Khellol glucoside



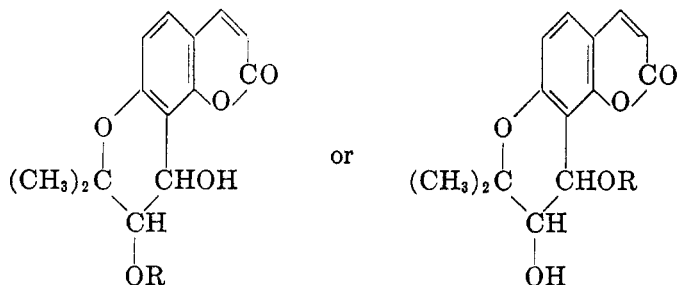
Khellol

They were able to show that the sugar residue was *d*-glucose.

Anrep, Kenawy, Barsoum, and Fahmy (5) repeated Spaeth and Gruber's work on the crystalline principles of *Ammi visnaga* L. and obtained the same results.

4. *Khellaktone*

In 1945 Matzke (64) published a thesis on the structure of khellaktone, which originally had been isolated in 1938 by Gruber from *Ammi visnaga* L. (42). Khellaktone was found to have the following structure:



R = an unknown residue.

5. *Visnagan*

As a by-product of the ether extraction of seeds of *Ammi visnaga* L. (see page 545), Samaan isolated visnagan as a dark oily liquid distilling with decomposition at 165°C. at 20 mm. and obtained in about 2 per cent yield. Floriani (33b) also did a complete extraction and isolated an oily principle which he called visnagol. It is not possible to determine whether or not this oil is identical with the visnagan described by Samaan. Floriani also claims to have isolated an alkaloid (visnine), but no further data are given and no one in later reports made reference to a compound of alkaloid nature. Cavallito and Rockwell (20) have prepared similar material, which upon further purification yielded a colorless, hard glass which did not distill at 10<sup>-6</sup> mm. at 120°C. Analyses indicated a molecular formula of C<sub>20</sub>H<sub>26-28</sub>O<sub>7</sub> for visnagan. It formed no derivatives which would indicate the presence of hydroxyl, carbonyl, or carboxyl groups, contained no methoxyl groups, and was optically active ( $[\alpha]_D^{25} = +30.5^\circ \pm 0.5^\circ$  (16 mg./ml. in dioxane)). The material was sufficiently fluid at 60°C. to allow a refractive index determination:  $n_D^{60} = 1.5345$ . Mild alkaline hydrolysis yielded one acidic group, stronger hydrolysis nearly two. Ultraviolet absorption spectra indicated the possible presence of an aromatic ring conjugated to another unsaturated system. Infrared absorption data suggested the presence of a para-substituted phenyl group, methyl groups, a bonded hydroxyl group, and perhaps a strained ring carbonyl but no carboxyl group. A crystalline impurity also was found in crude visnagan which melted slowly between 133–140°C. and whose empirical formula was tentatively given as C<sub>15</sub>H<sub>12</sub>O<sub>5</sub>.

6. *Fatty acids*

The composition of the fatty acids from the seed fat of *Ammi visnaga* L. has been shown (41) to be 5 per cent palmitic acid, 50 per cent petroselinic acid, 32 per cent oleic acid, and 13 per cent linoleic acid.

## B. IDENTIFICATION AND ASSAY

Fahmy, Badran, and Messeid (31) describe three identification tests that have been developed for use in the *qualitative* determination of khellin:

- (a) When one drop of 0.01 per cent w/v solution in alcohol or in water is added to a piece of solid sodium or potassium hydroxide, a rose-red color is developed within 2 min.; this color is still detectable in a dilution of 1:500,000.
- (b) When a few crystals of khellin are treated with one drop of concentrated sulfuric acid on a white porcelain plate, a deep orange color is developed which on dilution with water turns yellow.
- (c) When a solution of 10 mg. of khellin in 2 ml. of 50 per cent alcohol is poured on a freshly prepared mixture of 0.5 ml. of *N*/2 iodine and 0.5 ml. of 10 *N* potassium hydroxide solution, a yellow color is formed followed by a yellow precipitate, which redissolves gradually on shaking, imparting to the solution a wine-red color.

Rahman (73) developed a *quantitative* (gravimetric) method for the assay of khellin in fruits of *Ammi visnaga* L., but his method, based on weighing the residue of a chloroform extract, gave too high results because the extracted material was not pure. This assay could *not* be decidedly improved by modifying the method of extraction or by purifying the residue (31a).

Fahmy and Badran (31a) suggested a modification which reduced the assaying time to 3 hr. and gave satisfactory results. Their modification consisted mainly in dissolving the residue from the chloroform extract in 10 *N* sulfuric acid and measuring the nonglucosidal chromones by comparing the resulting yellow color with a suitable standard, using a photoelectric colorimeter.

Assays of different varieties of khellin fruits showed that the highest values were given by fruits collected in the lower Nile delta, whereas fruits collected in upper Egypt, Lebanon, or Morocco gave lower results.

Furthermore, a spectrophotometric method was developed for the identification and assay of khellin and khellol glucoside (88). It was found by using the potassium hydroxide color test that the ultraviolet spectrum of khellin had two maxima at 250 and 338  $m\mu$  and that khellol glucoside had two maxima at 246 and 334  $m\mu$ . With this method it was possible to determine concentrations of 3-5 parts per million of khellin and khellol glucoside.

A reddish-violet color reaction also develops when certain simple 2-methyl- $\gamma$ -pyrones are treated with potassium hydroxide, e.g., 2,6-dimethylpyrone, 2,6-dimethylchromone, 2,3-dimethylchromone, visnagin, etc. The presence of a methyl group in position 2 is essential, as no such color is given with chromone, 6-methylchromone, flavone, norkhellin, or 2-phenylnorkhellin (91, 92).

It has also been shown that it is possible to use the ultraviolet absorption spectra of khellin and khellol glucoside as indications of their chemical structure (89). The short-wave band of high intensity given by khellin at 240-250  $m\mu$  was interpreted as indicating conjugated double bonds. The long-wave band of low intensity at 380-340  $m\mu$  indicated a ketone group. The ultraviolet spectrum

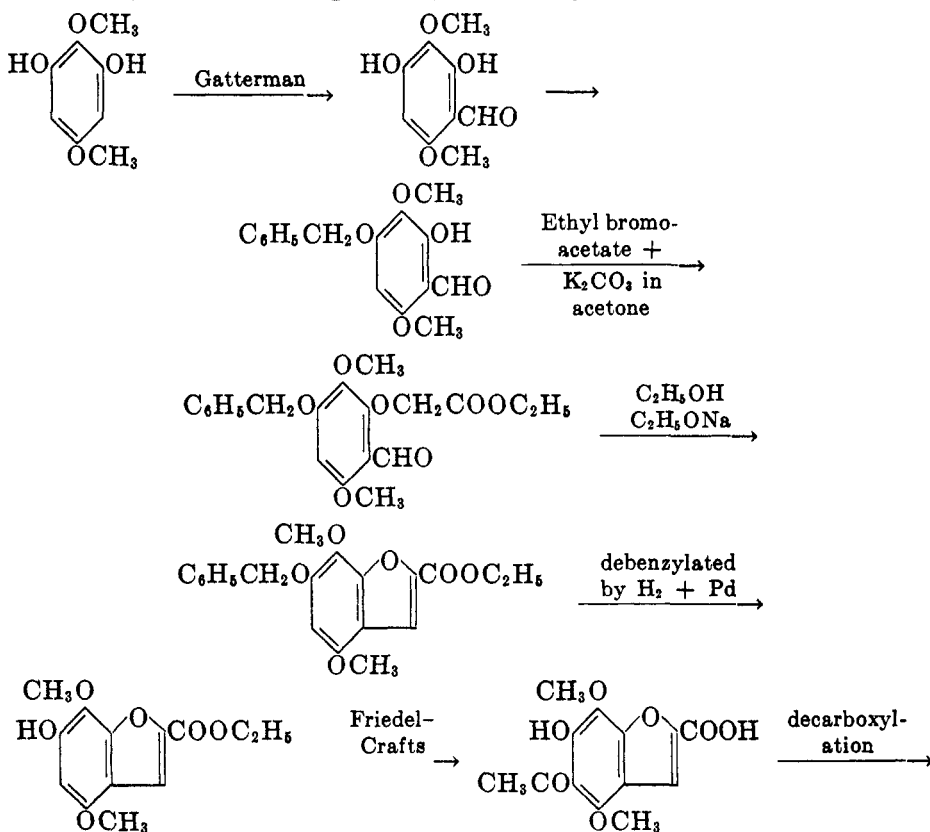
of khellol glucoside gave similar results. The data obtained on methylheptenone and mesityl oxide were compared and resulted in the conclusion that both compounds are  $\alpha,\beta$ -unsaturated ketones, a result which was in accordance with the structural formulas proposed by Spaeth and Gruber.

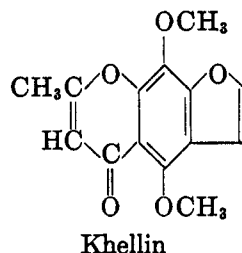
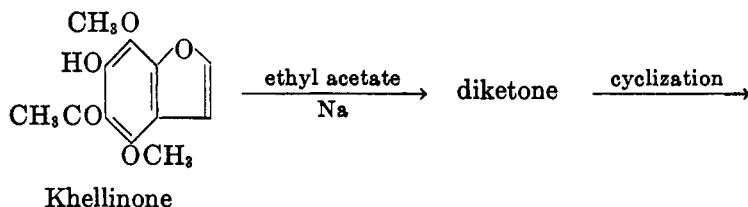
### C. SYNTHESSES

#### 1. *Khellin*

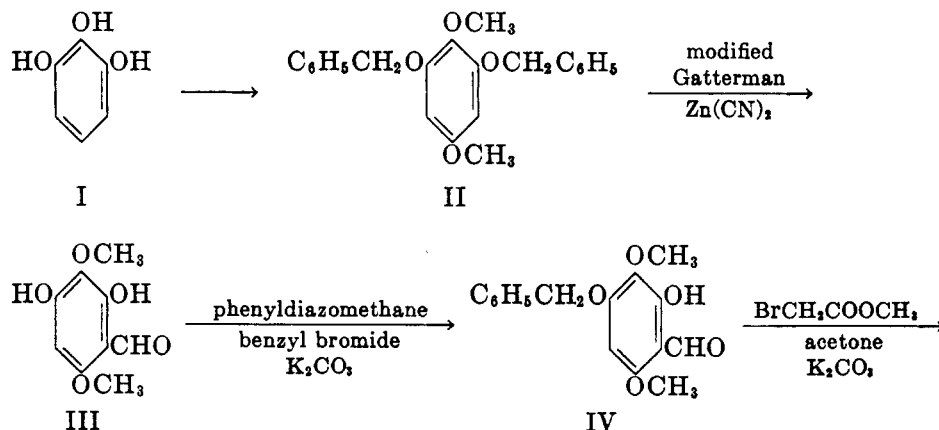
Khellin has been *partially* synthesized by Spaeth and Gruber (93) from khellinone, using the Kostanecki-Robinson method. Since this method sometimes yields mixtures of the desired chromone and the isomeric 4-methylcoumarin, this partial synthesis was repeated by Geissman (36), using a modified Wittig method (105) which gave unequivocal results. On reacting khellinone (as well as khellinone acetate) with sodium hydride in ethyl acetate, and treating the reaction product with cold hydrochloric acid, khellin was obtained. This removed any doubt as to the 2-methylchromone nature of khellin as indicated by Spaeth and Gruber's formula.

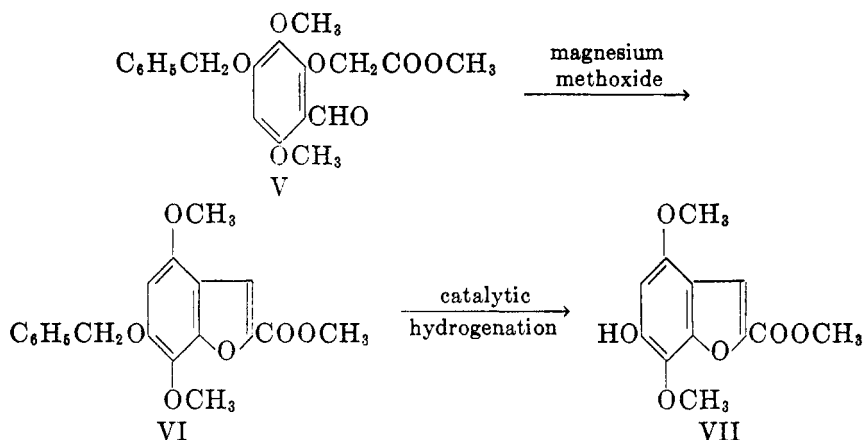
Clarke and Robertson (24) achieved a *total* synthesis of khellin (and related substances) in 1949, starting from 2,5-dimethoxyresorcinol.



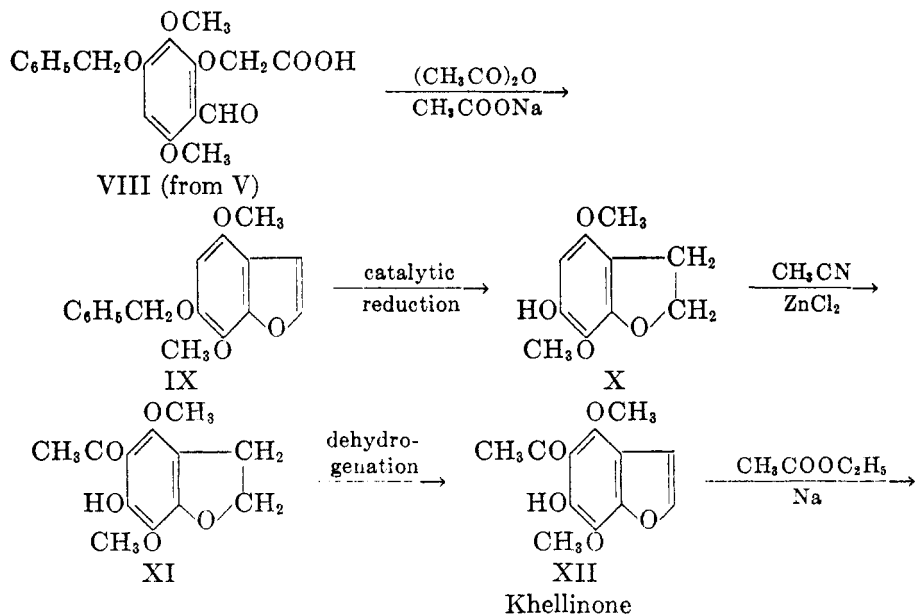


In the same year Baxter, Ramage, and Timson (16, 25) also achieved a *total* synthesis of khellin by first preparing 2,6-dibenzyloxy-1,4-dimethoxybenzene (II) from pyrogallol (I), essentially by the method of Baker, Nodzu, and Robinson (13). 2,4-Dihydroxy-3,6-dimethoxybenzaldehyde (III) was obtained by subjecting II to a modified Gatterman reaction, using zinc cyanide. Treatment of III with phenyldiazomethane or benzyl bromide and potassium carbonate gave 4-benzyloxy-2-hydroxy-3,6-dimethoxybenzaldehyde (IV). This compound was condensed with methyl bromoacetate in acetone in the presence of potassium carbonate to give methyl 5-benzyloxy-2-formyl-3,6-dimethoxyphenoxyacetate (V). Cyclization of this compound with magnesium methoxide gave methyl 6-benzyloxy-4,7-dimethoxycoumarone-2-carboxylate (VI) in better yields than with sodium or potassium methoxide. Catalytic hydrogenolysis of the cyclized ester (VI) gave methyl 6-hydroxy-4,7-dimethoxycoumarone-2-carboxylate (VII). Since it was found impossible to introduce an acetyl group into compound VII, the authors turned their attention back to compound V.

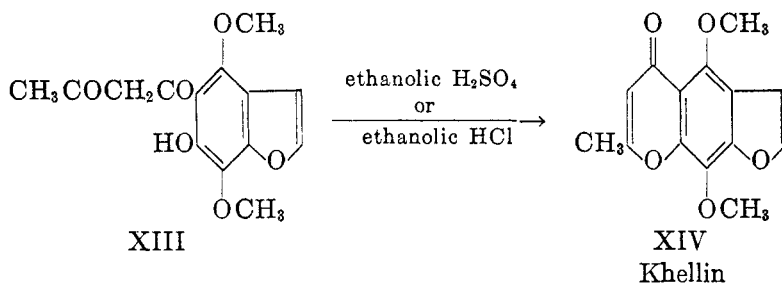




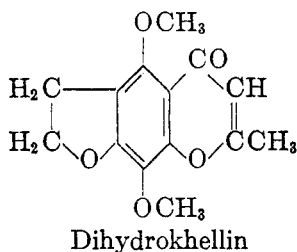
Cyclization and simultaneous decarboxylation of 5-benzyloxy-2-formyl-3,6-dimethoxyphenoxyacetic acid (VIII, from V) with acetic anhydride and sodium acetate gave 6-benzyloxy-4,7-dimethoxycoumarone (IX), which on catalytic reduction at 3 atm. pressure absorbed two molecular proportions of hydrogen to give 6-hydroxy-4,7-dimethoxycoumaran (X). This product reacted with acetonitrile in the presence of zinc chloride by the Hoesch method to give 5-acetyl-6-hydroxy-4,7-dimethoxycoumaran (XI), which was dehydrogenated to khellinone (XII) by sublimation under reduced pressure through a heated column containing palladium-Norit (30 per cent). Condensation with ethyl acetate and sodium gave 5-acetoacetyl-6-hydroxy-4,7-dimethoxycoumarone (XIII), which cyclized readily with ethanolic sulfuric acid or ethanolic hydrogen chloride to give khellin (XIV).



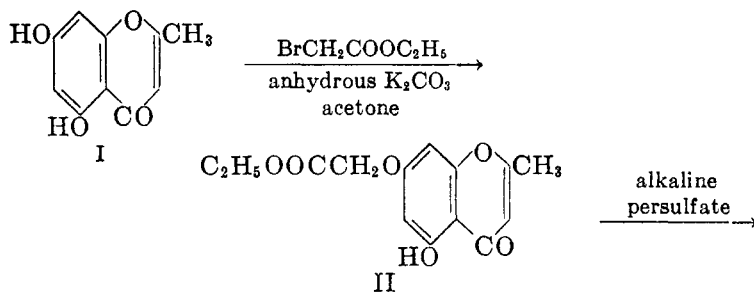


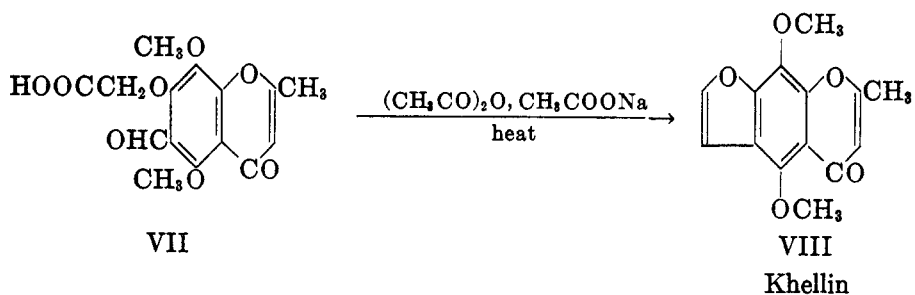
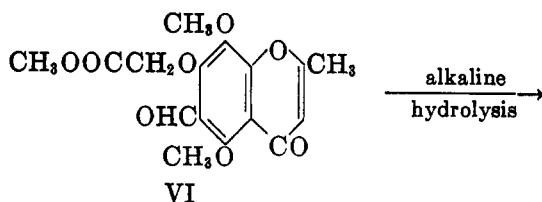
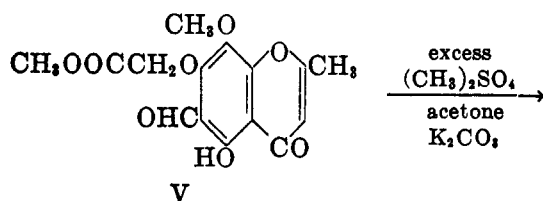
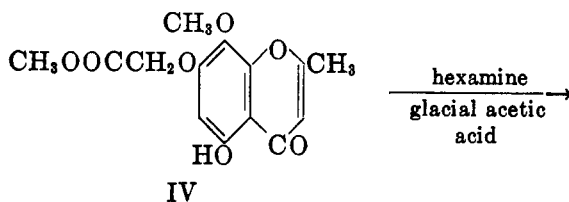
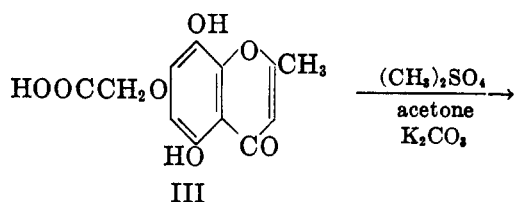


Cyclization of the diketone obtained from the condensation of 5-acetyl-6-hydroxy-4,7-dimethoxycoumaran with ethyl acetate gave dihydrokhellin (16).

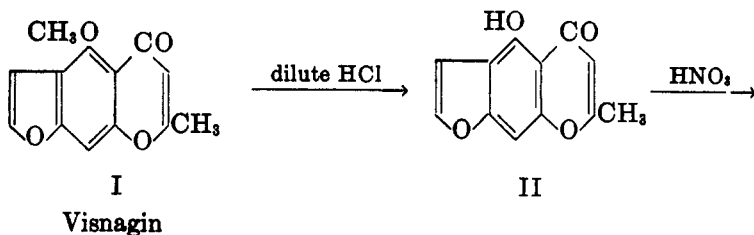


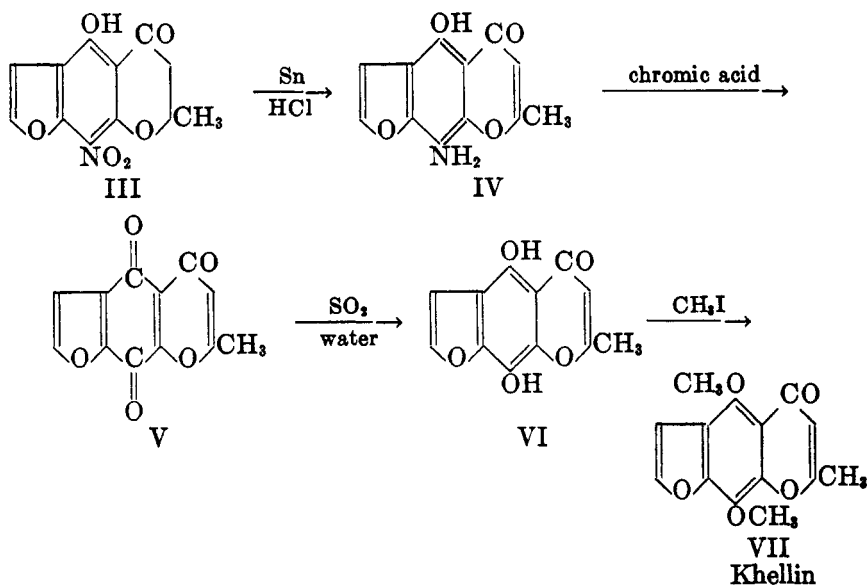
Murti and Seshadri (70a) also published a total synthesis of khellin in 1949. Whereas in the two syntheses described above the pyrone ring was built up on the appropriate coumarone, this synthesis involved building up the furan ring on the chromone system. 5,7-Dihydroxy-2-methylchromone (I) was used as the starting material. (This compound was prepared from phloracetophenone by a modified procedure with improved yields.) Condensation of I with one mole of ethyl bromoacetate, using anhydrous potassium carbonate in acetone, gave the phenoxyacetic ester (II). Treatment of II with alkaline persulfate introduced a hydroxyl group into position 8 and at the same time hydrolyzed the ester group, giving the required carboxylic acid (VII). This compound, when boiled with acetic anhydride and sodium acetate, formed the furan ring with the simultaneous evolution of carbon dioxide, giving khellin (VIII).





Schoenberg and Badran (89a) synthesized khellin from visnagin:

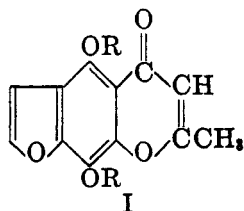




Since visnagin (which has little biological activity) is a by-product in the manufacture of khellin, it was more than a matter of academic interest to find a method whereby visnagin could be converted into khellin.

Murti and Seshadri also prepared the orange-yellow quinone derivative (V) by oxidation of khellin with nitric acid (70a). Further reduction of the quinone was effected with sulfur dioxide in alcoholic solution, giving the demethylated khellin (VI).

Schoenberg and Sina (91) succeeded in demethylating khellin to 5,8-dihydroxy-2-methylfuro(4',5',6,7)chromone (Ia) by heating with magnesium iodide in the absence of a solvent, followed by hydrolysis with dilute sulfuric acid. Khellin (Ib) was regenerated by methylation with diazomethane in the presence of methyl alcohol or by the methyl iodide-potassium carbonate method. Other ethers were also prepared: namely, the diethyl (Ic), the dipropyl (Id), the diallyl (Ie), and the di( $\omega$ -carboethoxymethyl) (Ig) ethers. The synthesis of these compounds was carried out by allowing Ia to react with ethyl iodide, *n*-propyl iodide, allyl iodide, and ethyl bromoacetate, respectively. The di( $\omega$ -carboxymethoxy) derivative (If) was obtained by acid hydrolysis of Ig.

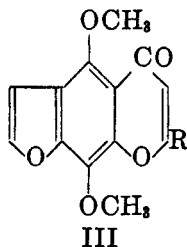
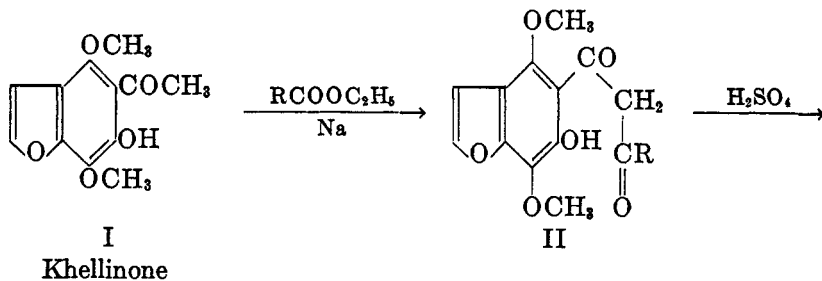


Ia: R = H  
 Ib: R = CH<sub>3</sub>  
 Ic: R = C<sub>2</sub>H<sub>5</sub>  
 Id: R = *n*-C<sub>3</sub>H<sub>7</sub>

Ie: R = C<sub>3</sub>H<sub>5</sub>  
 If: R = CH<sub>2</sub>COOH  
 Ig: R = CH<sub>2</sub>COOC<sub>2</sub>H<sub>5</sub>

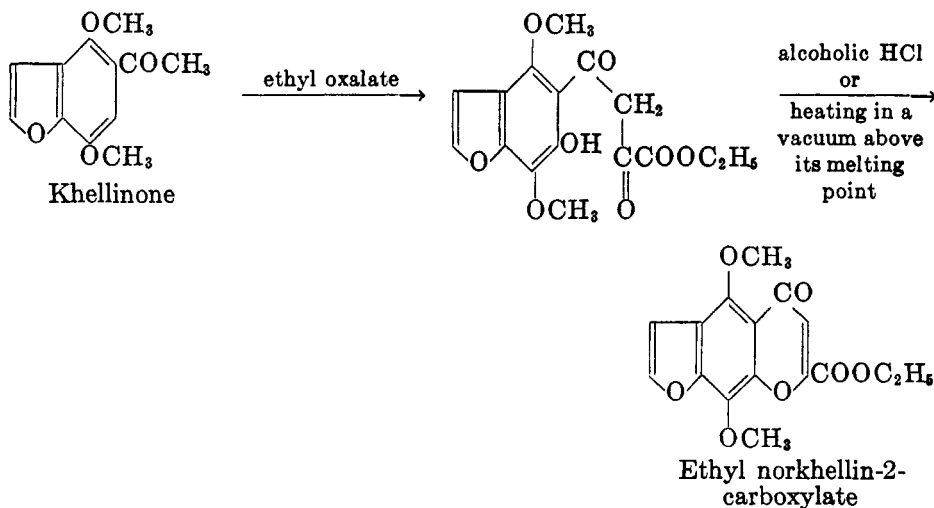
2. *Norkhellin*

Norkhellin (IIIa) and its 2-methyl, 2-ethyl, 2-*n*-propyl, and 2-phenyl derivatives were prepared from khellinone (I) (92), thereby effecting a partial syn-

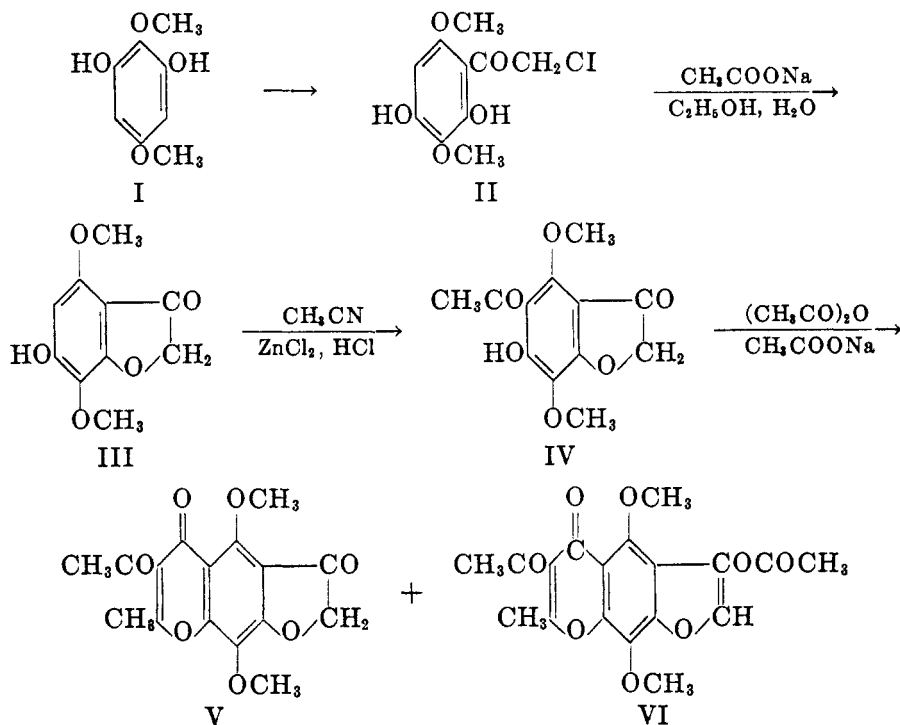


- IIIa: R = H (norkhellin)  
 IIIb: R =  $\text{CH}_3$  (khellin)  
 IIIc: R =  $\text{C}_2\text{H}_5$   
 IIId: R = *n*- $\text{C}_3\text{H}_7$   
 IIIe: R =  $\text{C}_6\text{H}_5$

thesis of khellin (IIIb) (2-methylnorkhellin). Ethyl norkhellin-2-carboxylate was prepared in a similar way from khellinone:



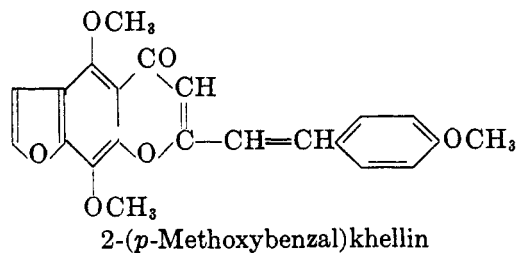
6-Acetyl-3(2*H*)-dihydro-3-ketokhellin has been synthesized from 2,5-dimethoxyresorcinol (I) (34, 35). The latter compound on chloroacetylation gave 2,4-dihydroxy-3,6-dimethoxy- $\omega$ -chloroacetophenone (II). This was cyclized to 6-hydroxy-4,7-dimethoxy-3(2*H*)-benzofuranone (III), which on treatment with acetonitrile according to the method of Hoesch gave 5-acetyl-6-hydroxy-4,7-dimethoxy-3(2*H*)-benzofuranone (IV). On cyclizing, 6-acetyl-3(2*H*)-dihydro-3-ketokhellin (V) and 3-acetoxy-6-acetylkhellin (VI) were formed.



6-Acetyl-3(2*H*)-dihydro-3-ketokhellin

3-Acetoxy-6-acetylkhellin

Khellin when condensed with anisaldehyde gives 2-(*p*-methoxybenzal)khellin (92):

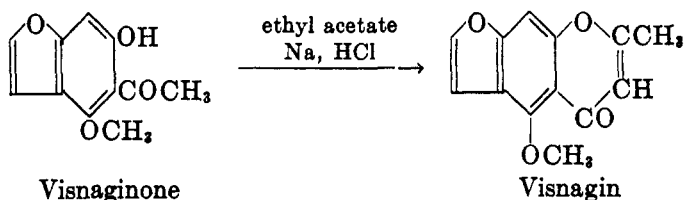


Khellinone and visnaginone, when condensed with aromatic aldehydes such as anisaldehyde, *p*-dimethylaminobenzaldehyde, piperonal, and vanillin, gave the corresponding colored chalcones, which deserve interest for their potential vitamin P activity.

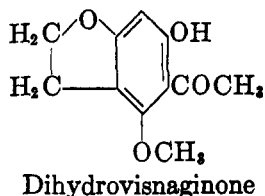
Compounds believed to be oxonium salts of khellin have been prepared by direct treatment of khellin with sulfuric and hydrochloric acids. These are khellin sulfate and khellin hydrochloride, respectively (67).

### 3. *Visnagin and visnaginone*

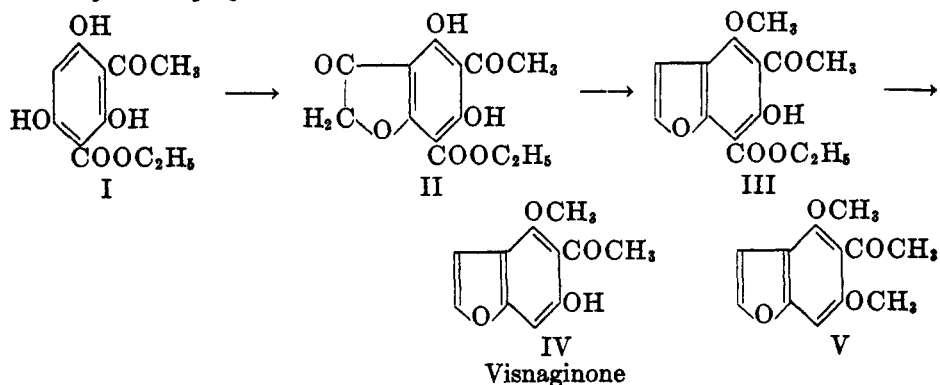
Visnagin has been synthesized after preliminary work with dicarbonyl derivatives of phenol (47, 48) by the deacetylation of 3-acetylvisnagin, obtained from visnaginone (45). Visnagin has also been synthesized by treating visnaginone with ethyl acetate, sodium, and hydrochloric acid (23),



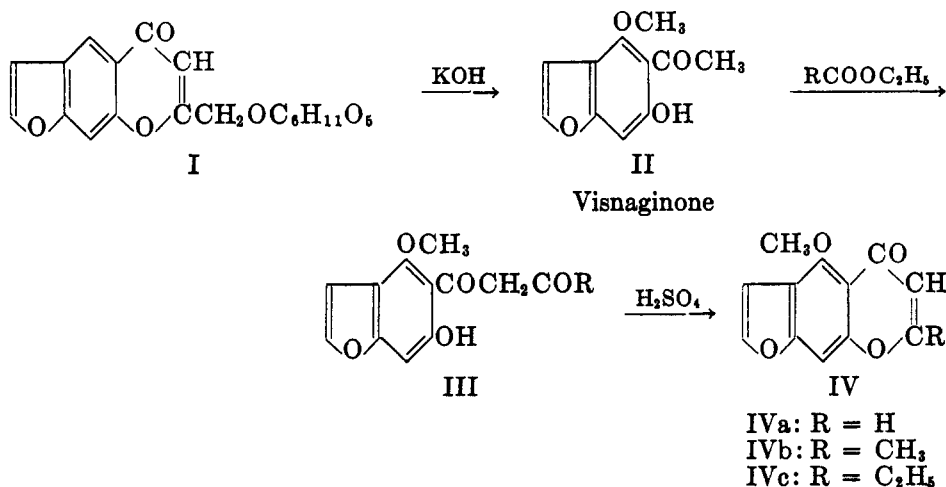
and by a "dehydrogenation" procedure involving reaction of *N*-bromosuccinimide with dihydrovisnaginone (37).



Subsequent to the partial syntheses of visnagin from visnaginone, Gruber and Horváth (43) synthesized visnaginone. As a starting material, phloroacetophenonecarboxylic acid ester (I) was used. Ring closure according to Hoesch gave only one of the expected isomers—namely, the desired one (II)—which, after acetylation, hydrogenation, and methylation, gave the monomethyl ether (III); saponification of III and distillation gave compound IV, m.p. 108–110°C. IV was found to be identical with natural visnaginone, m.p. 109–111°C. In the same way visnaginone methyl ether (V) was prepared and found identical with a methyl ether prepared from natural visnaginone.



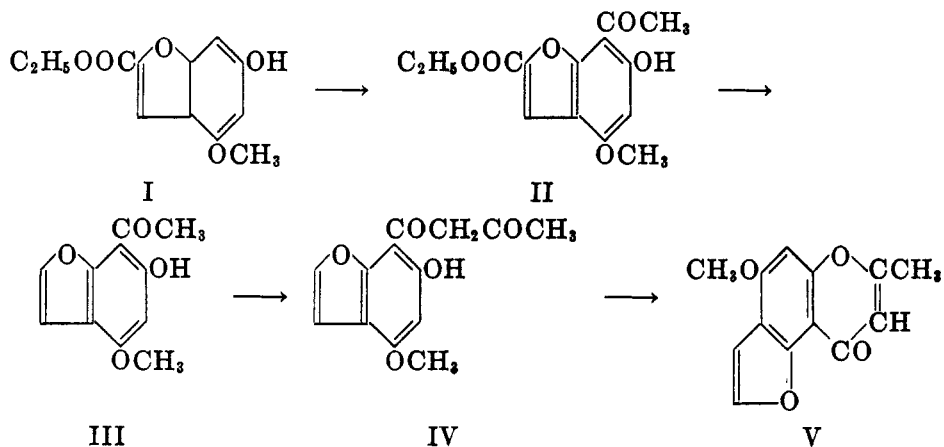
By reactions similar to those used in the preparation of norkhellin, Schoenberg and Sina prepared norvisnagin (IVa) and its 2-substituted derivatives (91).



By a similar route, using *o*-hydroxyacetophenone and ethyl formate, these authors succeeded in synthesizing chromone in good yields, thereby improving older methods (12, 50).

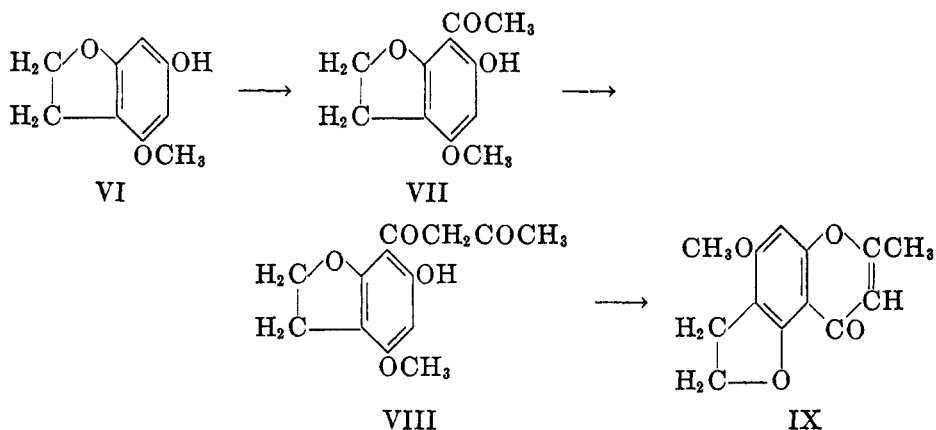
#### 4. Isovisnagin and isovisnaginone

Clarke, Glaser, and Robertson (23) synthesized isovisnagin, m.p. 247°C., by first applying the Hoesch reaction with acetonitrile or the Friedel-Crafts reaction with acetyl chloride to the coumarone I to obtain II. Compound II on hydrolysis and subsequent decarboxylation gave the ketone III, which was shown to be isomeric and not identical with visnaginone and proved to be the

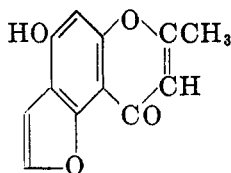


7-acetyl derivative. From the ketone III, isovisnagin (V) was synthesized by cyclization of the diketone (IV) obtained from III according to the procedure employed for visnagin (reacting with ethyl acetate in the presence of sodium).

Similarly, the application of the Friedel-Crafts reaction to compound VI gave only the 7-acetylcoumaran (VII), identical with a specimen prepared by the hydrogenation of isovisnaginone; on condensation with ethyl acetate this ketone (VII) gave the diketone VIII, which on cyclization gave dihydroisovisnagin (IX).



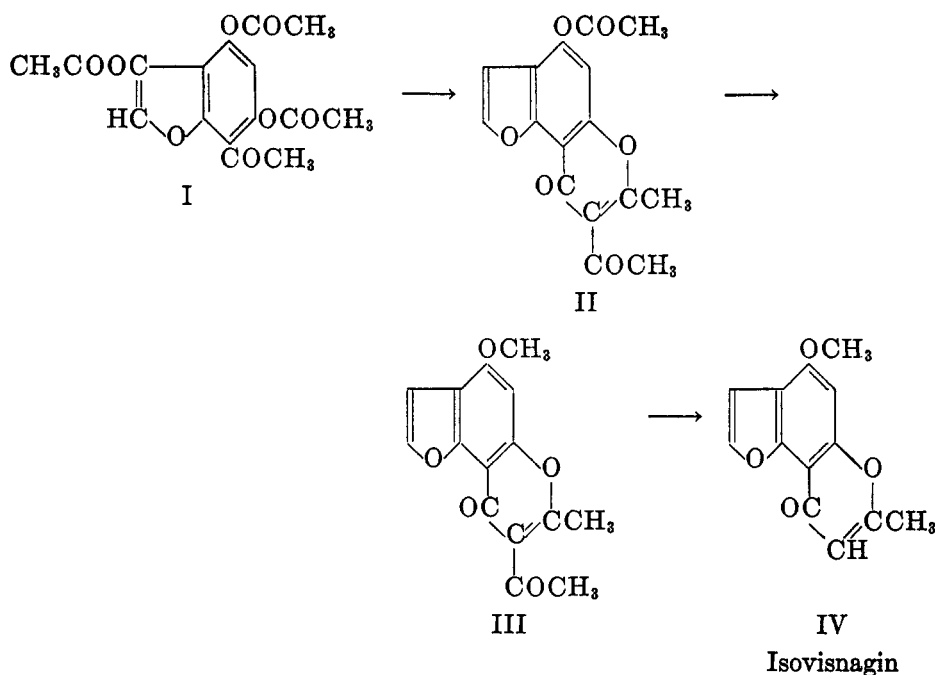
Norisovisnagin was prepared in an unexpected manner, during an attempt to prepare the parent hydroxyfuranochromone from visnagin by demethylation (23). This latter compound on being gently refluxed with concentrated hydriodic acid gave rise to a somewhat resinous product, from which a well-crystallized phenolic compound was isolated. This substance gave isovisnagin on methylation and was therefore the furanochromone, norisovisnagin.



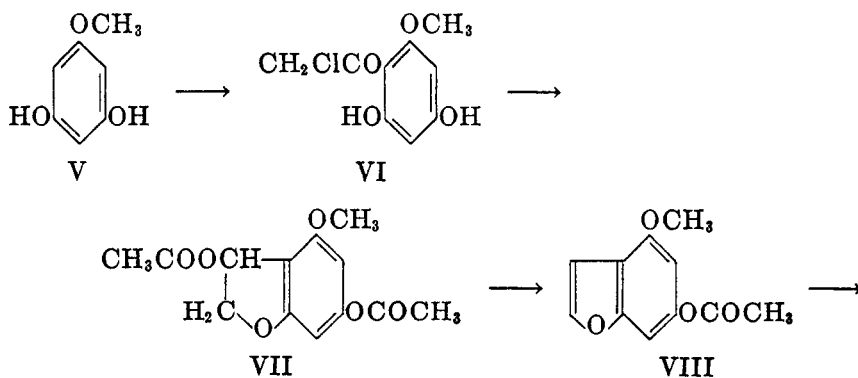
Norisovisnagin

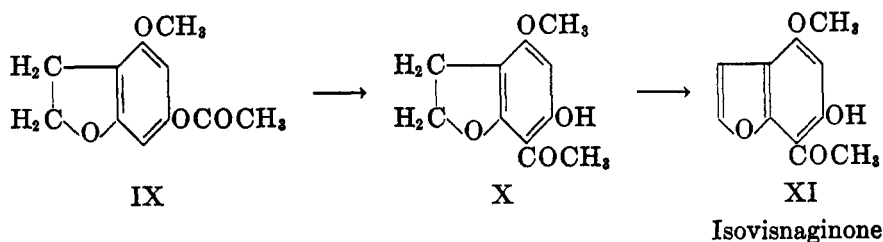
Isovisnagin, m.p. 239–241°C., has also been synthesized by Gruber and Horváth (44) from 3,4,6-triacetoxy-7-acetylcoumarone (I) by catalytic hydrogenation with platinum oxide catalyst and subsequent ring closure according to Kostanecki, obtaining as a main product the chromone II, which after saponification and treatment with diazomethane gave 3-acetylisovisnagin (III). The splitting of the acetyl group was more difficult than in the case of 3-acetylvisnagin and 3-acetylkhellin, owing to the diminished reactivity of the angular position of the ring. Gruber and Horváth succeeded in splitting it off with 0.5 *N* sodium ethoxide and so obtained isovisnagin (IV), m.p. 239–241°C. Further proof of structure was obtained by opening the  $\gamma$ -pyrone ring of 3-acetylisovisnagin, leading to isovisnaginone (XI), m.p. 135–136°C.



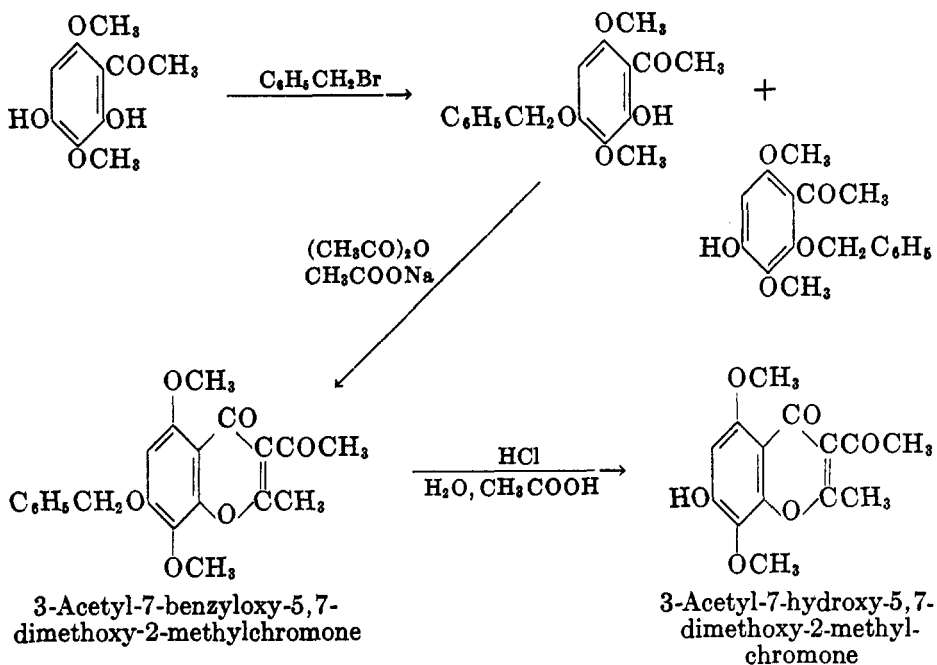


Isovisnaginone was also prepared in a second way, starting with phloroglucinol monomethyl ether (V). This compound when treated with chloroacetonitrile, zinc chloride, and hydrogen chloride gas gave  $\omega$ -chlorophloroacetophenone monomethyl ether (VI), which after ring closure and subsequent acetylation and hydrogenation yielded the coumaran VII. VII lost one molecule of acetic acid on vacuum distillation, giving 6-acetoxy-4-methoxycoumarone (VIII). Further hydrogenation gave the coumaran IX, and application of the Hoesch reaction and saponification gave dihydroisovisnaginone (X). The same product, however, was obtained in much better yields by using the Fries rearrangement. Dehydrogenation with palladium black finally gave isovisnaginone (XI) (44).





3-Acetyl-7-benzyloxy-5,7-dimethoxy-2-methylchromone and 3-acetyl-7-hydroxy-2-methylchromone have been synthesized, starting from 4-acetyl-2,5-dimethoxyresorcinol (34):



#### IV. PHYSIOLOGICAL ACTION

Khellin has long been known to be an effective antispasmodic; it has been used particularly for the relief of spasms of the ureter, bile duct, and gall bladder as well as in bronchial asthma, the anginal syndrome (increasing coronary flow), and against whooping cough. More recently it was found to be a potent coronary vasodilator.

##### A. PHARMACOLOGICAL DATA

###### 1. *Khellin*

The minimum lethal dose of a 1:10 alcohol tincture (prepared from the fruits of *Ammi visnaga* L.) was determined by Samaan (76) to be as follows: 45 ml./kg.

for the toad, intralymphatically (within 24 hr.); 22 ml./kg. for the rabbit, intravenously (within 24 hr.); 36 ml./kg. for the rabbit, subcutaneously (within 24 hr.). Postmortem examination revealed no abnormalities besides dilatation of the visceral vessels. According to Samaan, the drug relaxed all smooth muscles, especially the uterus. Two years later (79) the same author determined median lethal doses for *crystalline* khellin: 90 mg./kg. for the rabbit, hypodermically (within 22 hr.); 105 mg./kg. for the toad, intralymphatically (within 3 hr.).

Toxicity determinations made at the Sloan Kettering Institute<sup>2</sup> gave the following results:

0.5 ml./20 g. mouse in 5 per cent gum acacia in isotonic saline, intraperitoneally, given daily for 7 days at each dosage level

DOSE	MORTALITY
mg./kg.	
512	3/3
256	3/3
128	0/3
64	0/3

Intravenous injection of khellin (4-5 mg./kg.) into dogs produces an immediate and pronounced fall in blood pressure which, however, is only of short duration (59a). Repeated injections have *no* cumulative effect upon the blood pressure. Previous atropinisation does not eliminate these effects, thereby excluding a muscarinic type of activity. Khellin is not a sympatholytic, but acts directly on smooth muscle. It should be noted that the lowering effect on the blood pressure is apparent only in dosages which are 20 times the therapeutic dose for man (59a).

On comparing khellin with papaverine and eupaverine, khellin was found to be the most effective in ureteral spasms. Khellin and papaverine were equally effective in their relaxation of the gall bladder, bile duct, and urinary bladder, and both were found to be more active than eupaverine (83). The effect of khellin was also superior to that of enatin and bromsalizol (86). Further experiments (88) confirmed the observation that khellin has a selective antispasmodic effect upon the ureter, bronchial muscles, gall bladder, and bile duct.

*Quantitative* evaluation of the antispasmodic activity of crystalline khellin was attempted by Anrep, Barsoum, and Kenawy (1), using the perfusion technique on excised lungs of guinea pigs (see table 2).

The effect of crystalline khellin on the anginal syndrome was studied (2) in experiments on heart and lung preparations from dogs. The minimal active concentration was 1:2,000,000. Dilutions of 1:200,000 increased the coronary blood flow to three to four times the initial volume. The activity of khellin was evaluated as being about four times that of aminophylline (4). The action of khellin was inferior to that of amyl nitrite but much more prolonged. Similar

<sup>2</sup> Data kindly supplied by Dr. C. Chester Stock, Sloan Kettering Institute, New York City.

experiments on rabbits and cats gave indefinite and inconclusive results. Contrary to the statements of Samaan (79), higher doses did *not* weaken the heart. In a comparative study of the response of the heart to khellin and khellol glucoside, Samaan, Hossein, and Fahim stated (87) that pure khellin depresses the heart by producing a less complete systole with diminished cardiac output. The drug is active in dogs in doses of 2-4 mg./kg. given intravenously.

Further perfusion experiments have been reported by Killam and Fellows (33a, 59). In 157 experiments in which 1:100,000 khellin was administered within 2 min. to isolated rabbit hearts, perfused with oxygenated Locke's solution (the rate of injection being proportional to the rate of flow of perfusion fluid), the average increase in flow of perfusion fluid was 32 per cent, as compared with a preliminary control period; in 67 experiments with 1:10,000 khellin, the increase was 67 per cent, whereas 1:10,000 visnagin caused an average increase of 57 per cent. In 8 of 11 experiments on anesthetized, heparinized dogs, 2, 3, and 5 mg./kg. khellin administered intravenously produced increases in coronary flow of 20-88, 49-100, and 75-230 per cent, lasting 2-20, 28-60, and 90 (duration

TABLE 2  
*Action of khellin on bronchi and perfused lungs of guinea pigs*

INITIAL FLOW	CONCENTRATION OF KHELLIN	MAXIMUM FLOW	INCREASE
<i>ml./min.</i>	<i>mg./l.</i>	<i>ml./min.</i>	<i>per cent</i>
5.0-5.4	10	42.0-43.0	700
4.4-4.8	10	39.0-39.6	750
2.5-2.6	6	11.6-12.0	350
3.5-3.7	2	5.2- 5.4	47

of experiment) min., respectively. In all experiments, the blood pressure fell moderately and returned to normal in 2-4 min. Bagouri (11) determined the minimum effective concentration which would increase the coronary outflow in the isolated perfused rabbit heart as being approximately 2 mg./l. In this experiment, the systemic blood vessels were found to be considerably less sensitive to khellin than the coronary vessels.

Khellin was found to be a potent coronary vasodilator for men (6). In the dosages used, it did *not* affect the general blood pressure nor did it increase the oxygen requirements of the heart. Its action lasted many hours.

The antispasmodic activity of khellin and analogs was also determined by another biological method (14), using the caecum of the fowl as test object. Detailed results, as obtained with this method (33a, 91, 92), are presented in table 3.

Significant antihistamine activity is exhibited by khellin as well as visnagin (33a). Oral administration of 20-100 mg./kg. khellin or of 50-100 mg./kg. visnagin protected guinea pigs against otherwise lethal histamine aerosols.

A method was worked out to determine the absorption of khellin and its

estimation in blood and tissues (15). Ethanol extraction is followed by extraction with chloroform, and khellin is determined by the colorimetric method of Fahmy, Badran, and Messeid (31) or by the biological method of Barsoum and Gaddum (14). The recovery is 90–95 per cent. Blood plasma was found to contain 10–20 per cent *more* khellin than the red cells, but khellin bound to the cells is *not* inactive and is readily released into the plasma (unlike histamine).

Administration *per os* is advisable if maintenance of high blood levels is desired, whereas *intramuscular* administration is recommended if it is necessary to raise the khellin concentration within a short time. Khellin is *not* excreted in the urine in an unchanged form, and disappears from the blood and tissues very slowly. Repeated administration therefore leads to accumulation in the body (59a). The single administration of 100–200 mg. khellin raises its concentration in the blood above the minimum effective concentration which has been shown to cause coronary vasodilation and relaxation of the bronchi.

Recently, it has been demonstrated by Charlier and Philippot (22, 59a) that khellin diminishes or prevents the otherwise fatal ventricular fibrillation induced in anesthetized dogs by the administration of chloroform–adrenaline.

### 2. *Khellol glucoside*

The pharmacological activity of khellol glucoside (khellinin) was first investigated by Samaan (79, 80), who reported that fatal doses given to toads depressed the respiratory center, with subsequent paralysis. The respiration was arrested long before the heart. Solutions of 1:100,000 increased the contractility of the heart, without changing the rate of the beat. In the intact dog the blood pressure was raised and the respiration depressed. In a subsequent paper, Samaan reported (85) that khellol glucoside had a coronary vasodilating effect, but this was in contradiction to his own previous statements (79) and to experimental results of Anrep, Kenawy, Barsoum, and Fahmy (5) and Bagouri (11). Subsequent experiments of Samaan and coworkers (87), however, confirmed his previous findings: namely, that pure khellol glucoside possesses a persistent and rather selective stimulating action on the heart, producing a more complete systole and diastole with corresponding increase in cardiac output. It is active in doses of 1–1.5 mg./kg. if given intravenously to dogs. It increases the coronary flow and is *not* cumulative. Moderate stimulation of the dog's heart by khellol glucoside has also been reported by Fellows, Killam, and coworkers (33a). The same authors found that khellol glucoside, contrary to khellin and visnagin, afforded *no* protection to guinea pigs against poisoning by histamine aerosol. According to Barsoum, Kenawy, and El Sheehy (15), khellol glucoside is *not* converted into khellin in the digestive tract or in the body tissues.

### 3. *Visnagan*

According to Samaan (81, 84) visnagan has low toxicity, an accelerating effect upon the respiration of dogs, and a coronary vasodilating effect in dilutions of 1:25,000 to 1:200,000. Cavallito and Rockwell (20), on further purification of

visnagin, isolated a crystalline product with the tentative empirical formula  $C_{15}H_{12}O_5$ . This compound, however, did *not* have any coronary-dilating properties.

B. COMPARATIVE ASSAYS, AND CORRELATION OF STRUCTURE  
WITH ACTIVITY

Anrep and coworkers (5), in criticizing Samaan's papers of 1932 (79, 80) and 1946 (85), decided to determine the antispasmodic activities of khellin, visnagin,<sup>3</sup> khellol glucoside, and their hydrolysis products *quantitatively*. For these assays, both the colorimetric method of Fahmy (31, 32, 73) and the biological method of Barsoum and Gaddum (14) were used. The compounds were dissolved in tyrode solution in 0.5 millimolar concentration. The activities reported below refer to equimolecular amounts. Taking the activity of khellin as 100, the following activity indices were calculated:

COMPOUND	ACTIVITY INDEX	COMPOUND	ACTIVITY INDEX
	<i>per cent</i>		<i>per cent</i>
Khellin.....	100	Khellol.....	25
Visnagin.....	70	Visnaginone.....	20
Khellinone.....	33	Khellol glucoside.....	0

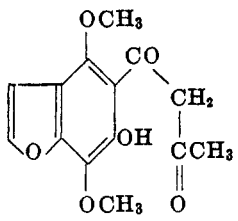
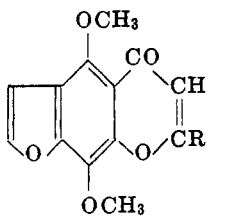
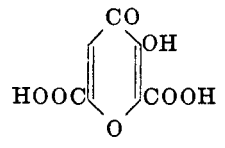
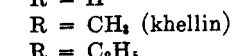
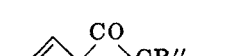
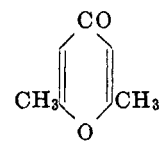
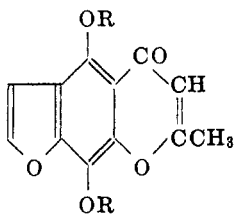
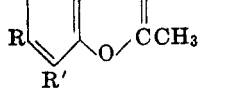
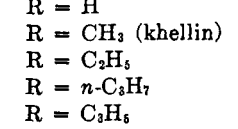
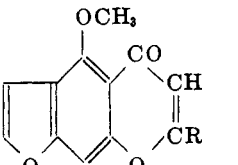
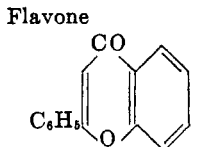
More recently, these and a number of other khellin analogs have been evaluated in a similar manner (91, 92) with the results shown in table 3.

From these data the following conclusions regarding the antispasmodic activity are possible: (1) So far, no khellin analog has been found—at least according to published literature<sup>4</sup>—which has an activity superior to that of khellin. (2) Generalizations which apply to the dimethoxy compounds (khellin type) are equally applicable to the monomethoxy compounds (visnagin type). (3) Loss of the furan ring leads to decisive (70–90 per cent) diminution but not to complete loss of activity. (4) Opening of the  $\gamma$ -pyrone ring results in considerable (75 per cent) but not complete loss of activity. (5) Removal of the 9-methoxyl group results in 30–50 per cent loss of activity. (6) Removal of *both* the 4- and the 9-methoxyl groups reduces the activity by 90 per cent. (7) Simultaneous replacement of both the 4- and the 9-methoxyl groups by hydroxyl reduces the activity by 90 per cent, replacement by ethoxyl only by 25 per cent, whereas higher alkoxy groups reduce it by 80–90 per cent. (8) Replacement of the 7-methyl group by hydroxymethyl reduces the activity more than 50 per cent. (9) Replacement of the 7-methyl group by the glucose radical renders the compound practically inactive. (10) Replacement of the 7-methyl group by hydrogen

<sup>3</sup> According to the authors visnagin occurs in *Ammi visnaga* L. in such small amounts that commercial extraction from the plant would hardly be worthwhile.

<sup>4</sup> However, Hudson of the British Schering Research Institute lays claim (25) to the discovery of khellin analogs with enhanced activity.

TABLE 3  
Activity index of khellin and analogs

COMPOUND	ACTIVITY INDEX	COMPOUND	ACTIVITY INDEX
	5	Meconic acid	<10
			
		2,6-Dimethylpyrone	3
			
<p>R = H</p> <p>R = CH<sub>3</sub> (khellin)</p> <p>R = C<sub>2</sub>H<sub>5</sub></p>	55		
	100	<p>R = H</p> <p>R = CH<sub>3</sub> (khellin)</p> <p>R = C<sub>2</sub>H<sub>5</sub></p> <p>R = n-C<sub>3</sub>H<sub>7</sub></p> <p>R = C<sub>3</sub>H<sub>5</sub></p>	10
<p>R = CH<sub>3</sub>, R' = R'' = H</p> <p>R = CH<sub>3</sub>, R' = H, R'' = CH<sub>3</sub></p> <p>R = OCH<sub>3</sub>, R' = R'' = H</p> <p>R = R' = OCH<sub>3</sub>, R'' = H</p> <p>R = OC<sub>2</sub>H<sub>5</sub>, R' = R'' = H</p> <p>R = OC<sub>3</sub>H<sub>7</sub>-n, R' = R'' = H</p> <p>R = OC<sub>4</sub>H<sub>9</sub>-n, R' = R'' = H</p> <p>R = OC<sub>2</sub>H<sub>5</sub>, R' = R'' = H</p> <p>R = OH, R' = R'' = H</p>	75		100
	22		75
	15		20
	10		27
	20		30
	27		30
	30		30
	30		<10
	<10		
Flavone	15	<p>R = H (norvisnagin)</p> <p>R = CH<sub>3</sub> (visnagin)</p>	30
		<p>R = C<sub>2</sub>H<sub>5</sub></p>	50
			(previously found, 70% (4))
			40

results in 45 per cent and replacement by ethyl in 25 per cent loss of activity.

## C. CLINICAL DATA

1. *Khellin*

Extracts of *Ammi visnaga* L. have been used for centuries as a home remedy to relieve spasms of the ureter, kidney, bladder, and gall bladder (53, 54, 78, 82, 99).

Following the more recent discovery of the coronary-vasodilating effect of khellin, a number of clinical papers have been published dealing with beneficial or promising results in the treatment of bronchial asthma and angina pectoris (3, 4, 7, 8, 39, 40, 55, 59b, 71, 75).

Administration of 100 mg. intramuscularly or 50–100 mg. *per os* one to three times daily was found to be effective in the treatment of 150 cases of angina pectoris (3). Treatment usually started with 100 mg. administered intramuscularly one to three times daily for 2 weeks and was then continued with 1 tablet (50 mg.) three times daily, given after meals. According to Ayad (8) the attacks disappeared completely in 61 per cent of the cases and showed marked improvement in 22 per cent, whereas 17 per cent showed no response. Similar results were reported by Lian and Charlier (59a), whereas Scott and coworkers (92b) obtained only questionable improvements with frequent side effects.

Anrep *et al.* (4) noticed only few side effects and *no* influence on bleeding or blood coagulation time.

Another recent clinical paper by Rosenman *et al.* (74) describes in detail the treatment of 14 patients with angina pectoris, 11 of whom showed good response; also the treatment of 21 patients with acute bronchial asthma, 9 of whom experienced significant and sometimes dramatic relief after a single intramuscular injection of 200 mg. of khellin. Similar observations were reported by Major (60) on 12 patients whose asthma was refractory to treatment with epinephrine and/or aminophylline. Armbrust and Levine (7) and Osher and Katz (71) obtained comparable results. Beneficial effects were obtained in 9 out of 14 patients suffering from coronary insufficiency (16a) as controlled by electrocardiographic tests.

In a series of 45 patients suffering from bronchial asthma 41 obtained complete and prolonged relief after administration of a single dose of 200–300 mg. of khellin given intramuscularly (3). Relief was complete 5–15 min. after the injection and lasted about 24 hr. The action of khellin was found to be as prompt as that of epinephrine or ephedrine, but longer lasting and less toxic; it was also safer than aminophylline. These findings were substantiated in a subsequent study on 138 cases of bronchial asthma (56).

Cases of "angina of effort," according to Greiner and Gold (39, 40), have *not* improved under the administration of khellin, but contrary observations were reported by Dewar and Grimson (26). Khellin has also been used successfully in the treatment of whooping cough (57).

2. *Physiological effects of products isolated from Ammi majus L.*

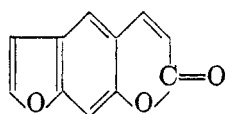
Well known for ages, the extract of *Ammi majus* L. has been used in Egypt as a home remedy against leucoderma. Whereas the crude extract is very toxic to cold- and warm-blooded animals, *pure* ammoidin (xanthotoxin) is nontoxic in therapeutically effective doses. Pure ammoidin has been used successfully in



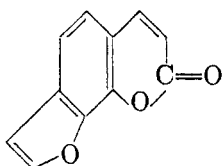
the treatment of leucoderma by oral administration of 0.05 g. three times daily, or when applied topically in the form of a 1 per cent liniment to leucodermic patches (28, 72). According to Mozem (70), repigmentation occurs without necessarily being accompanied by vesiculation.

In discussing the mechanism of activity, the author left the question open as to whether the effect was due to stimulation of dopase or its coenzyme in the functionally depressed melanoblasts, to removal or neutralization of the cause of depression of the melanoblasts, or to the stimulation of other mechanisms of repigmentation and photosensitization as yet unknown.

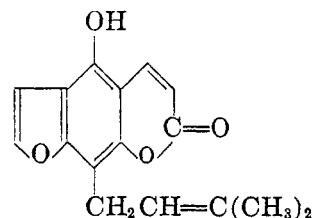
In this connection it is interesting to note that Panja (72) and others before him have used successfully extracts from *Psoralea corylifolia* L. (oil of bouchi), a leguminose, for the treatment of leucoderma. From such extracts psoralen and related furocoumarins have been isolated (38). Their chemical structure has been elucidated (21, 97), and their synthesis accomplished (51). The relationship to the products isolated from *Ammi majus* L. becomes evident upon comparison of their structures (see xanthotoxin, imperatorin, and bergapten, page 547).



Psoralen  
(m.p. 162°C. (51);  
171°C. (97))



Isopsoralen  
(m.p. 142°C.)



Psoralidin  
(m.p. 315° C.)

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#### V. REFERENCES<sup>5</sup>

- (1) ANREP, G. V., BARSOUM, G. S., AND KENAWY, M. R.: *J. Pharm. Pharmacol.* **1**, 164 (1949).
- (2) ANREP, G. V., BARSOUM, G. S., KENAWY, M. R., AND MISRAHY, G.: *Brit. Heart J.* **8**, 171 (1946).
- (3) ANREP, G. V., BARSOUM, G. S., KENAWY, M. R., AND MISRAHY, G.: *Lancet* **252**, 557 (1947).
- (4) ANREP, G. V., KENAWY, M. R., AND BARSOUM, G. S.: *Am. Heart J.* **37**, 531 (1949).
- (5) ANREP, G. V., KENAWY, M. R., BARSOUM, G. S., AND FAHMY, I. R.: *Gaz. Fac. Med. Cairo* **14**, 1 (1947).
- (6) ANREP, G. V., AND MISRAHY, G.: *Gaz. Fac. Med. Cairo* **13**, 33 (1945) (original not available).
- (7) ARMBRUST, C. A., AND LEVINE, S. A.: *Am. J. Med. Sci.* **220**, 127 (1950).

<sup>5</sup> An attempt has been made to cover the literature on khellin as completely as possible up to September 1950. Several publications, particularly from Egyptian sources, are included, even though they were not available to the authors of this review and consequently are not discussed.

- (8) AYAD, H.: *Lancet* **254**, 305 (1948).
- (9) AZIZ, S.: Thesis, Faculty of Medicine, Cairo, 1947-48 (original not available).
- (10) BAGOURI, M. M.: Thesis, Cairo, 1947 (original not available).
- (11) BAGOURI, M. M.: *J. Pharm. Pharmacol.* **1**, 177 (1949).
- (12) BAKER, W.: *J. Chem. Soc.* **127**, 2349 (1925).
- (13) BAKER, W., NODZU, R., AND ROBINSON, R.: *J. Chem. Soc.* **1929**, 74.
- (14) BARSOUM, G. S., AND GADDUM, J. H.: *J. Physiol.* **85**, 1 (1935).
- (15) BARSOUM, G. S., KENAWY, M. R., AND EL SHEEHY, A.: *J. Roy. Egypt. Med. Assoc.* **30**, 312 (1947).
- (16) BAXTER, R. A., RAMAGE, G. R., AND TIMSON, J. A.: *J. Chem. Soc.* **1949**, S30.
- (16a) BEST, M. M., AND COE, W. S.: *Circulation* **2**, 344 (1950).
- (17) BHAR, C. H.: *Science and Culture* **12**, 504 (1947); *Chem. Abstracts* **41**, 5879 (1947).
- (18) BOSE, P. K., AND MOOKERJEE, A.: *J. Indian Chem. Soc.* **21**, 181 (1944).
- (19) BUTLER, W. J.: *Bull. Am. Soc. Hosp. Pharm.* **4**, 242 (1947); a review.
- (20) CAVALLITO, C. J., AND ROCKWELL, H. E.: *J. Org. Chem.* **15**, 820 (1950).
- (21) CHAKRAVARTI, K. K., BOSE, A. K., AND SIDDIQUI, S.: *J. Sci. Ind. Research (India)* **7B**, 24 (1948); *Chem. Abstracts* **42**, 7492c (1948).
- (22) CHARLIER, R., AND PHILIPPOT, E.: *Arch. intern. pharmacodynamie* **81**, 404 (1950).
- (23) CLARKE, J. R., GLASER, G., AND ROBERTSON, A.: *J. Chem. Soc.* **1948**, 2260.
- (24) CLARKE, J. R., AND ROBERTSON, A.: *J. Chem. Soc.* **1949**, 302.
- (24a) CURD, F. H., AND ROBERTSON, A.: *J. Chem. Soc.* **1933**, 442.
- (25) DAVIES, J. S. H.: *Lancet* **254**, 887 (1948).
- (26) DEWAR, H. A., AND GRIMSON, T. A.: *Brit. Heart J.* **12**, 54 (1950).
- (27) DOBIE, E.: *Pharm. J.* **133**, 645 (1934); a review.
- (28) FAHMY, I. R., AND ABU-SHADY, H.: *Quart. J. Pharm. Pharmacol.* **21**, 499 (1948).
- (29) FAHMY, I. R., AND ABU-SHADY, H.: *Quart. J. Pharm. Pharmacol.* **20**, 281 (1947).
- (30) FAHMY, I. R., ABU-SHADY, H., SCHOENBERG, A., AND SINA, A.: *Nature* **160**, 468 (1947).
- (31) FAHMY, I. R., BADRAN, N., AND MESSEID, M. F.: *J. Pharm. Pharmacol.* **1**, 529, 535 (1949).
- (31a) FAHMY, I. R., AND BADRAN, N.: *J. Pharm. Pharmacol.* **2**, 561 (1950).
- (32) FAHMY, I. R., AND EL KEIY, M.: *Rept. Pharmac. Soc. Cairo* **3**, 36 (1931) (original not available).
- (33) FANTL, P., AND SALEM, S. I.: *Biochem. Z.* **226**, 166 (1930).
- (33a) FELLOWS, E. J., KILLAM, K. F., TONER, J. J., DAILEY, R. A., AND MACKO, E.: *Federation Proc.* **9**, 271 (1950).
- (33b) FLORIANI, L.: *Rev. farm. (Buenos Aires)* **2**, 14 (1929).
- (34) GARDNER, T. S., AND WENIS, E.: Personal communication, 1950.
- (35) GARDNER, T. S., WENIS, E., AND LEE, J.: *J. Org. Chem.* **15**, 841 (1950).
- (36) GEISSMAN, T. A.: *J. Am. Chem. Soc.* **71**, 1498 (1949).
- (37) GEISSMAN, T. A., HALSALL, T. G., AND HINREINER, E.: *J. Am. Chem. Soc.* **72**, 4326 (1950).
- (38) GHOSH, J. C.: *Pharm. J.* **121**, 54 (1928).
- (39) GREINER, T., AND GOLD, H.: *J. Pharmacol. Exptl. Therap.* **98**, 10 (1950).
- (40) GREINER, T., GOLD, H., *et al.*: *Am. J. Med.* **9**, 143 (1950).
- (41) GRINDLEY, D. N.: *J. Sci. Food Agr. (England)* **1**, 53 (1950).
- (42) GRUBER, W.: Thesis, University of Vienna, 1938.
- (43) GRUBER, W., AND HORVÁTH, K.: *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse, Abt. IIb*, **158**, 874 (1949); *Monatsh.* **81**, 819 (1950).
- (44) GRUBER, W., AND HORVÁTH, K.: *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse, Abt. IIb*, **158**, 563 (1949); *Monatsh.* **80**, 571 (1949).
- (45) GRUBER, W., AND HOYOS, F. E.: *Monatsh.* **78**, 417 (1948); *Chem. Abstracts* **42**, 7290 (1948).
- (46) GRUBER, W., AND HOYOS, F. E.: *Sitzber. Akad. Wiss. Wien, Math.-naturw. Klasse, Abt. IIb*, **158**, 303 (1949); *Monatsh.* **80**, 306 (1949).
- (47) GRUBER, W., AND TRAUB, F.: *Monatsh.* **77**, 414 (1947).

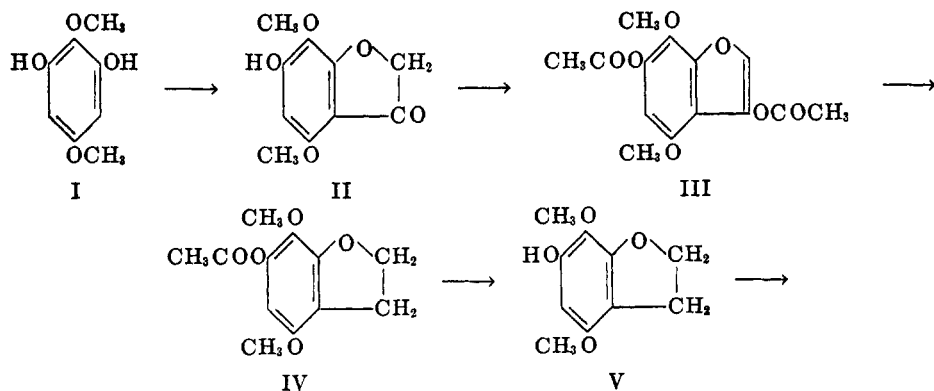
- (48) GRUBER, W., AND TRAUB, F.: *Monatsh.* **78**, 417 (1948).
- (49) HASSAN, M. K.: Thesis, Cairo, 1932 (original not available).
- (50) HEYWANG, R., AND KOSTANECKI, S.: *Ber.* **35**, 2887 (1902).
- (51) HORNING, E. C., AND REISNER, D. B.: *J. Am. Chem. Soc.* **72**, 1514 (1950).
- (52) HUSSEIN: Thesis Pharmac. Cairo, 1941 (original not available).
- (53) IBRAHIM, ALI BEY: *J. Roy. Egypt. Med. Assoc.* **12**, 71 (1929).
- (54) IBRAHIM, ALI PASCHA: *Brit. J. Urol.* **1**, 396 (1929).
- (55) KENAWY, M. R., AND BARSOUM, G. S.: *Gaz. Fac. Med. Cairo* **13**, 39 (1945) (original not available).
- (56) KENAWY, M. R., BARSOUM, G. S., AND BAGOURI, M. M.: *Eye, Ear, Nose Throat Monthly* **29**, 79 (1950).
- (57) KHALIL, A., AND SAFWAT, A.: *Am. J. Diseases Children* **79**, 42 (1950).
- (58) Khellin (Editorial): *J. Am. Med. Assoc.* **137**, 758 (1948).
- (59) KILLAM, K. F., AND FELLOWS, E. J.: *Federation Proc.* **9**, 291 (1950).
- (59a) LIAN, C., AND CHARLIER, R.: *Acta Cardiol.* **5**, 373 (1950).
- (59b) LIPMAN, B. S., AND MASSIE, E.: *J. Lab. Clin. Med.* **36**, 956 (1950).
- (60) MAJOR, R. H.: *J. Kansas Med. Soc.* **1**, 114 (1949).
- (61) MALEK, ABD EL.: Thesis, Cairo, 1938 (original not available).
- (62) MALIK, W. S. ABD-EL: Thesis, Cairo, 1932 (original not available).
- (63) MALOSSE, TH.: Thesis, Montpellier, 1881 (original not available); see also *Am. J. Pharm.* **53**, 639 (1881) and *Union méd. Paris* **41**, 569 (1886).
- (64) MATZKE, O.: Thesis, University of Vienna, 1945.
- (65) MOHAMMED, HASSAN KH.: Thesis, Cairo, 1940-41 (original not available).
- (66) MOLFINO, J. F.: *Chacra (Buenos Aires)* **16**(190), 78 (1946); a review.
- (67) MOUBASHER, R., AND BARAKAT, M. Z.: *J. Am. Chem. Soc.* **72**, 2307 (1950).
- (68) MOUSTAPHA, I.: *Compt. rend.* **89**, 442 (1879).
- (69) MOUSTAPHA, I.: *Union méd. Paris* **41**, 569 (1886); also *Gaz. hebdom. sci. méd. Montpellier* **30** (1886) (original not available).
- (70) MOZEM, ABDEL EL MOFTI: *J. Roy. Egypt. Med. Assoc.* **31**, 651 (1948).
- (70a) MURTI, V. V. S., AND SESHADRI, T. R.: *Proc. Indian Acad. Sci.* **30A**, 107 (1949).
- (71) OSHER, H. L., AND KATZ, K. H.: *Boston Med. Quart.* **1**, 11 (1950).
- (72) PANJA, G.: *Indian J. Venereal Diseases* **13**, 56 (1947).
- (73) RAHMAN, ABD EL: Thesis, Faculty of Medicine, Fouad I University, Cairo (1943) (original not available); also *Rept. Pharmac. Soc. Cairo* **17**, 5 (1944) (original not available).
- (74) ROSENMAN, R. H., FISHMAN, A. P., KAPLAN, S. R., LEVIN, H. G., AND KATZ, L. N.: *J. Am. Med. Assoc.* **143**, 160 (1950).
- (75) SALAMA, S.: *Gaz. Fac. Med. Cairo* **13**, 10 (1946) (original not available).
- (76) SAMAAN, K.: *Quart. J. Pharm. Pharmacol.* **3**, 25 (1930).
- (77) SAMAAN, K.: *Quart. J. Pharm. Pharmacol.* **4**, 14 (1931).
- (78) SAMAAN, K.: *Brit. J. Urol.* **3**, 294 (1931).
- (79) SAMAAN, K.: *Quart. J. Pharm. Pharmacol.* **5**, 6 (1932).
- (80) SAMAAN, K.: *Quart. J. Pharm. Pharmacol.* **5**, 183 (1932).
- (81) SAMAAN, K.: *Quart. J. Pharm. Pharmacol.* **6**, 13 (1933).
- (82) SAMAAN, K.: *Brit. J. Urol.* **5**, 213 (1933).
- (83) SAMAAN, K.: *Quart. J. Pharm. Pharmacol.* **9**, 23 (1936).
- (84) SAMAAN, K.: *Quart. J. Pharm. Pharmacol.* **18**, 82 (1945).
- (85) SAMAAN, K.: *Quart. J. Pharm. Pharmacol.* **19**, 135 (1946).
- (86) SAMAAN, K., AND EL ASREEGY, M. I.: *Brit. J. Urol.* **7**, 116 (1935).
- (87) SAMAAN, K., HOSSEIN, A. M., AND FAHIM, I.: *J. Pharm. Pharmacol.* **1**, 538 (1949).
- (88) SAMAAN, K., HOSSEIN, A. H., AND EL RIDI, M. S.: *Quart. J. Pharm. Pharmacol.* **20**, 502 (1947).
- (89) SAMAAN, K., HOSSEIN, A. H., AND EL RIDI, M. S.: *Quart. J. Pharm. Pharmacol.* **20**, 504 (1947).

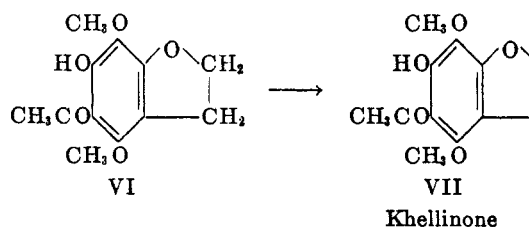
- (89a) SCHOENBERG, A., AND BADRAN, N.: Personal communication; to be published in *J. Am. Chem. Soc.*
- (90) SCHOENBERG, A., AND SINA, A.: *Nature* **161**, 481 (1948).
- (91) SCHOENBERG, A., AND SINA, A.: *J. Am. Chem. Soc.* **72**, 3396 (1950).
- (92) SCHOENBERG, A., AND SINA, A.: *J. Am. Chem. Soc.* **72**, 1611 (1950).
- (92a) SCHOENBERG, A., AND SINA, A.: *J. Am. Chem. Soc.* **72**, 4826 (1950).
- (92b) SCOTT, R. C., *et al.*: *Circulation* **3**, 80 (1951).
- (93) SPAETH, E., AND GRUBER, W.: *Ber.* **71**, 106 (1938).
- (94) SPAETH, E., AND GRUBER, W.: *Ber.* **74**, 1492 (1941).
- (95) SPAETH, E., AND GRUBER, W.: *Ber.* **74**, 1549 (1941).
- (96) SPAETH, E., AND HOLZEN, H.: *Ber.* **66**, 1137 (1933).
- (97) SPAETH, E., OKAHARA, K., AND KUFFNER, F.: *Ber.* **70**, 73 (1937).
- (98) SPAETH, E., AND PAILER, M.: *Ber.* **69**, 767 (1936).
- (99) STRONG, R. P.: In *Nelson's Loose-Leaf Medicine* **2**, 467 (1947).
- (100) THOMS, H.: *Ber.* **44**, 3325 (1911).
- (101) THOMS, H., AND BAETCKE, E.: *Ber.* **45**, 3705 (1912).
- (102) UPSHER SMITH, F. A.: *J. Am. Pharm. Assoc.* **22**, 184 (1933); a review.
- (103) WESSELY, F., AND MOSER, G. H.: *Monatsh.* **56**, 97 (1930).
- (104) WEST, R. W.: Thesis, Cairo, 1938 (original not available).
- (105) WITTIG, G., BANGERT, F., AND RICHTER, H. E.: *Ann.* **446**, 155 (1925).

## ADDENDUM

The following articles have appeared since this review was prepared:

- (a) DAVIES, J. S. H., AND DEEGAN, T.: *J. Chem. Soc.* **1950**, 3202. The synthesis of a visnagin isomer, 8-methoxy-2-methylfurano(3', 2', 6, 7)chromone and its derivatives.
- (b) DAVIES, J. S. H., MCCREA, P. A., NORRIS, W. L., AND RAMAGE, G. R.: *J. Chem. Soc.* **1950**, 3206. The synthesis of 2-methyl(3', 2', 6, 7)chromone and derivatives.
- (c) DAVIES, J. S. H., AND NORRIS, W. L.: *J. Chem. Soc.* **1950**, 3195. The synthesis of visnagin and related compounds.
- (d) GEISSMAN, T. A., AND HALSALL, T. G.: *J. Am. Chem. Soc.* **73**, 1280 (1951). A total synthesis of khellin was effected partly to determine whether it might be prepared synthetically more advantageously than it could be isolated from the natural source. Attempts were made to obtain khellinone in better yields. Like Baxter *et al.* (16) and Clarke and Robertson (24), they proceeded to khellinone by way of 2,5-dimethoxyresorcinol. The steps in their synthesis are presented as follows:





Considerable attention was directed toward the problems involved in the preparation of the 2,5-dimethoxyresorcinol (I) from pyrogallol. Numerous experiments were also performed in the dehydrogenation of dihydrokhellinone; one method involved the use of *N*-bromosuccinimide. The conclusion of the authors is that the production of khellin by synthesis is not practicable. The overall yield of VI from I is of the order of 10 per cent; and a number of additional steps are involved in the preparation of I from pyrogallol and the conversion of VI into khellinone (VII).

- (e) GEISSMAN, T. A., AND HINREINER, E.: *J. Am. Chem. Soc.* **73**, 782 (1951). The synthesis of visnaginone from phloroglucinol.
- (f) SLAUGHTER, D., AND HAZEL, R.: *J. Pharmacol. Exptl. Therap.* **101**, 33 (1951). Toxicity studies of visammin (khellin) and Mefurone (a brand of khellinin, khellol glucoside). Both compounds had low toxicity to rats, mice, dogs, and rabbits.