UV-A Photolysis of Khellin: Products and Reaction Mechanism

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Photolysis at 365 nm of khellin in various solvents was studied: the degradation rate strongly depends on solvent but not on polarity, since it is related to the singlet oxygen lifetime in each solvent. Eight products were isolated and characterized, some of which had previously been described as coming from chemical oxidation of khellin. Both facts support an oxic pathway for photolysis involving singlet oxygen and leading to unstable intermediates. These are rapidly decomposed by nucleophiles, suggesting possible interference—if they are also formed *in vivo*—with physiological processes during photochemotherapeutic treatment with khellin.

Introduction

Khellin (4,9-dimethoxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one, Figure 1) is a natural furochromone extracted from *Ammi visnaga* and has sometimes been used in the past as a coronary vasodilator. However, its phototherapeutic effectiveness has also been recognized in the last few years, leading some dermatologists to test it in association with ultraviolet light for the treatment of skin diseases such as psoriasis and vitiligo. These diseases are commonly treated by PUVA (psoralens + UV-A) therapy: the most frequently used drug is 8-methoxypsoralen (8-MOP), a furocoumarin which, however, has severe side effects, such as erythema and oedema, genotoxicity, risk of skin cancer, and cataracts.[1] The various efforts made to overcome these side effects have mainly been devoted to study of the structural analogs of psoralens; of these, khellin is one of the most promising agents against vitiligo. KUVA (khellin + UV-A) therapy can, in fact, restore pigmentation in affected areas of the skin without severe phototoxicity. Conversely, the efficacy of khellin in clearing psoriasis is very low,[2] suggesting that different mechanisms are involved in the two therapeutic actions. While the treatment of psoriasis may mainly rely on the antiproliferative activity of the compounds, it is still an open question how these sensitizers stimulate melanogenesis. Much work has been done to correlate physicochemical properties with pigmentogenic activity, but no conclusive results have been obtained.[3] One of these research lines concerns the photolysis of photosensitizers themselves, both to identify the products—and to study their particular biological relevance—and to understand whether the intermediate by which these products are formed interfere in some way with biological processes.

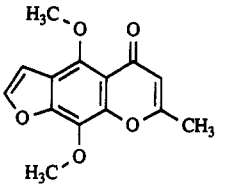

Figure 1. Molecular structure of khellin.

Table I. Pseudo-First-Order Rate Constants ($min^{-1} \times 10^{-3}$) for Khellin Photolysis in Various Solvents

	proteated	deuterated
carbon tetrachloride	>250[a]	
chloroform	12	28
dichloromethane	3	
benzene	7	9
cyclohexane	5	
acetone	5	6
ethanol	≪1[b]	
methanol	≪1[b]	<1[c]
water	≪1[b]	<1[c]

[a] Photolysis was almost complete after 2 min irradiation. [b] No photodegradation after 2 h irradiation. [c] Less than 5% degradation after 2 h irradiation.

Results

Kinetics. Table I shows the pseudo-first-order rate constants of the disappearance of khellin, as measured by HPLC, when its solutions in various solvents are irradiated at 365 nm. These data show that photolysis is strongly affected by the solvent. However, no correlation at all is seen with the polarity of the medium, this being the case of furocoumarins.[4] On the contrary, the decomposition rate is higher in those solvents in which singlet oxygen has a longer lifetime.[5] Thus, some deuterated solvents were used ($CDCl_3$, CD_3COCD_3, CD_3OD, C_6D_6, D_2O) and compared with the corresponding proteated ones. In all cases photolysis proceeded faster.

Photoproducts. Using commercial $CHCl_3$ as solvent, many products formed, of which compounds 1–4, 7, and 8 were isolated. When irradiation was carried out in freshly distilled $CHCl_3$, 4 was absent, while the addition of MeOH afforded 1–3 and 5–8. These products were characterized as shown in Figure 2.

• Abstract published in *Advance ACS Abstracts*, November 1, 1993.

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(5) Monroe, B. M. In *Singlet $^1O_2$*; Frimer, A. A., Ed.; CRC Press: Boca Raton, 1985; Vol. I, p 177.

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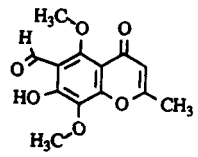
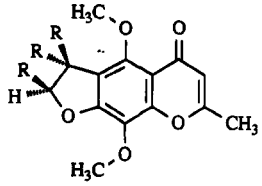
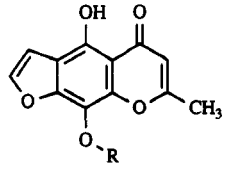




Compound	2	3	4	5	6
R'	H	OH	H	H	H
R''	OH	H	OH	OH	OMe
R'''	OH	OH	OEt	OMe	OH



Compound	7	8
R	Me	H

Figure 2. Molecular structures of isolated compounds 1–8.

Aldehyde 1. On the basis of spectral data, this compound proved to correspond to that obtained from khellin by either oxidation with $NaIO_5$ or ozonolysis.[6]

Diol 2. The NMR spectrum indicates that the benzopyrone moiety is unchanged, while the signals of both protons 2 and 3 are shifted upfield with respect to khellin, although not so much as expected by saturation of the 2,3 double bond by hydrogen or carbon atoms. The values thus suggest saturation of the double bond by two hydroxyls: although they do not give signals in the NMR spectrum, their presence is shown by the large broad peak centered at 3340 cm^{-1} in the IR spectrum. MS also shows peaks belonging to the molecular ion and to fragments due to the loss of one hydroxyl and one molecule of water.

As regards stereochemistry, the very low coupling constant between H(2) and H(3) suggests that they have a *trans* configuration.

Diol 2 was acetylated, giving **2a**. Its NMR spectrum is very similar to that of parent **2**, both furan protons being further deshielded by the acetoxy groups. Two new singlets were also found at 2.14 and 2.16 ppm, each accounting for three protons, which further supported the proposed structure.

Diol 3. The NMR spectrum of **3** differs from that of **2** only in that H(2) and H(3) are coupled to each other with a constant of 5.2 Hz, showing that they are in a *cis* arrangement.

This compound corresponds to that obtained by Hg$(NO_3)_2$ oxidation of khellin.[6] The NMR data show small discrepancies with those reported, but our spectrum lacks hydroxyl protons, thus allowing us to observe the above coupling.

Acetylation gave rise to **3a**, in which only one hydroxy group was acetylated, namely that at C(3), perhaps because of steric hindrance due to the *cis* configuration.

Ethoxy Derivative 4. In compound **4** too, only the furan ring of khellin is involved in the photolysis: as in diols **2** and **3**, its protons resonate upfield, again indicating the proximity of electron-withdrawing groups without mutual coupling. Moreover, an ethoxy group is present. The IR spectrum shows the broad band of a hydroxyl which, however, is not visible in the NMR spectrum.

These data suggest that the 2,3 double bond is saturated by addition of a hydroxy and an ethoxy group. Mass spectroscopy confirmed the structure, showing the molecular peak at m/z 322 and its fragments due to successive loss of hydroxy, ethyl, and ethoxy groups.

Comparison of NMR data with similar compounds[7,8] and acetylation (in **4a** only the signal of H(3) moved downfield) established that the ethoxy group is bound to C(3), the configuration being *trans*.

The addition of an ethoxy group may derive from the presence of EtOH as chloroform stabilizer. Thus, distillation of the solvent led to the lack of compound **4** among the photoproducts, while addition of 10% MeOH to the freshly distilled $CHCl_3$ yielded two methoxy derivatives, **5**, and to a lesser extent, **6**, which were in turn acetylated to **5a** and **6a**: since the two dihydrofuran protons are still in a *trans* configuration—like in both diol **2** and ethoxy derivative **4**—**5** and **6** are regioisomers. Their acetylation allowed us to establish that in **5** the methoxy group is bonded to C(3) and the hydroxyl to C(2), the reverse occurring in **6**.

The same conclusion arose from the comparison of the MIKE spectra of $M^{\cdot+}$ of **5** and **6**. In fact, as shown in Figure 3, the molecular ion of compound **5** presents, among its primary fragment ions, the ionic species at m/z 264, corresponding to the loss of 44 Da. This fragmentation can be explained by the loss of a neutral moiety CH_3OCH through a concerted mechanism involving a first cleavage of the C(2)–C(3) bond and a further cleavage of the C(3)–C(3a) bond. A fragment ion is formed, which could be stabilized by cyclization through formation of a new O–Ph bond involving the OH(2). This 1,3-benzodioxole cannot be formed by the same mechanism for the molecular ion of **6**, and in fact, in its MIKE spectrum this product is not present.

Furthermore, an elimination reaction occurs in both molecular ions: **5** loses methanol, yielding the peak at an energy value corresponding to m/z 276, while **6** gives a fragment ion having m/z 290, showing loss of water.

Phenol 7. In the NMR spectrum of **7** only one methoxy group was found, the second one being substituted by a signal at 13.16 ppm, which may be explained by the presence of a phenol strongly involved in hydrogen bonds. This fact suggests that the loss of the methyl group occurred from position 4 instead of 9, allowing the OH to be linked by C(5)=O. This involvement may also account for both the chromatographic behavior of **7**, which is much less polar than was expected for a phenol, and the absence of OH stretching in IR spectrum. The main CO peak is present at 1662 cm^{-1}, that is, very close to the corresponding

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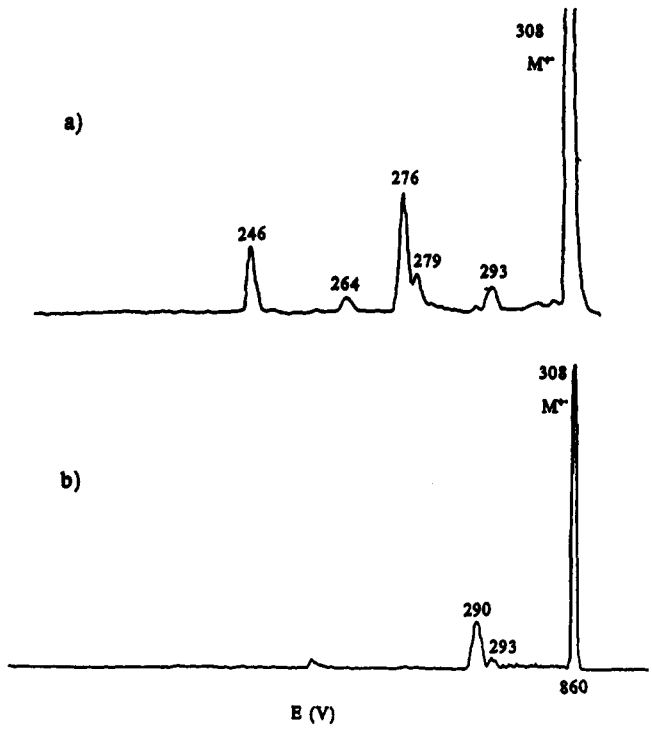

Figure 3. Mass-analyzed ion kinetic energy spectra of the molecular ions of **5** (a) and **6** (b).

absorption in khellin (1655 cm^{-1}); this scarce effect of hydrogen bonding has, however, been already reported.[9]

Furthermore, in compound **7** we could not observe any NOE interaction between methoxy protons and 3(H), which was instead clearly visible in the parent khellin.

Thus, compound **7** corresponds to khellinol, which is present in *Ammi visnaga*, but was also obtained by oxidation of khellin.[6]

Phenol 8. The NMR spectrum of **8** lacks both methoxy signals and indicates the presence of two phenol protons at 8.81 and 13.00 ppm. This compound thus corresponds to khellinquinol (dinorkhellin), obtained by oxidation of khellin with V_2O_5.[6]

The structure of **7** and **8** was also confirmed by submitting khellin to chemical demethylation. When it was refluxed in the presence of pyridinium chloride for very short periods (10–30 s), both products were present, while longer heating (2 min) yielded only **8**.

Discussion

Due to its structural analogy with furocoumarins, khellin was first studied in terms of photoreactivity toward DNA.[2a] Its very low DNA-photobinding—and consequently poor antiproliferative and antipsoriasic activity—was attributed to the hindering effect of the two bulky methoxy groups which prevent intercalation into the double helix. A similar effect had in fact been observed with isopimpinellin (5,8-dimethoxypsoralen) when compared with the mono-substituted analogs 5-MOP and 8-MOP.[3b]

Taking into account that the photoreaction of furocoumarins with DNA mainly involves the furan ring,[1] this explanation appears reasonable. As well as in DNA,[2] the furan-side C_4-cycloadduct between khellin and thymine

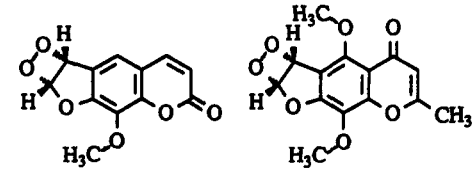

Figure 4. Molecular structure of the presumed dioxetane intermediates of 8-MOP and khellin, the latter accounting for compounds **1–6**.

was in fact produced by irradiation in a frozen state,[10] when steric effects should be less important, that is, in the same condition in which several furan-side adducts of furocoumarins were prepared.[1]

Instead, different behavior is shown by the γ-pyrone ring of khellin with respect to the α-pyrone ring of furocoumarins: when the latter compounds are irradiated with UV-A in solution, they mainly undergo C_4-cyclodimerization at that level.[4] When irradiation is carried out in the presence of suitable substrates (e.g., pyrimidine bases, unsaturated fatty acids) the pyrone ring is still involved. No evidence for such behavior was found with khellin, probably because the 6,7 olefinic double bond is not conjugated with the benzofuran moiety, and therefore should not be involved in the excited states of the molecule.

The present work confirms the above results in that no cyclodimers were detected among the photolysis products of khellin. The main mechanism leading to photolysis must therefore be different.

C_4-cycloadditions appear to proceed through the formation of radical intermediates[11] and should thus be favored by polar solvents, as occurs with furocoumarins.[4] This is not the case with khellin: photolysis may proceed through a photodynamic mechanism, i.e., by the oxidizing action of singlet oxygen produced by khellin itself.

It is known that the lifetime of singlet oxygen depends on the solvent, in that it is quenched by both C–H and, to a greater extent, O–H bonds. Although literature values on singlet oxygen lifetimes in various solvents[5] show an ample range of variation because they were measured with different methods, they do show good correlations with the rate constants of khellin photolysis. In particular, the higher rate in deuterated solvents with respect to proteated ones strongly indicates the involvement of this active form of oxygen in the reaction.

Until the end of 1991, only one of the products characterized had been reported for furocoumarins, that is, the aldehyde derived from the fission of the furan ring (corresponding to **1**).[12] The mechanism was studied in detail with 8-MOP: it was hypothesized that singlet oxygen—generated by energy transfer from the excited state of 8-MOP to molecular oxygen—forms a dioxetane intermediate (Figure 4) which is rapidly hydrolyzed to yield the aldehyde through cleavage of the C–C bond.[12]

More recently, nonphotochemical production of a dioxetane of 8-MOP with $NaOCl/H_2O_2$ in a water–methanol solution (Mizuno *et al.*)[8] and of the corresponding epoxide with dimethyldioxirane in a methanol–acetone solution (Adam and Sauter)[7] succeeded in isolating a new type of

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(10) Abeysekera, B. F.; Abramowski, Z.; Towers, G. H. N. *Photochem. Photobiol.* **1983**, *38*, 311.

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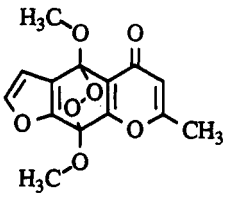

Figure 5. Molecular structure of the presumed endoperoxide intermediate of khellin accounting for compounds 7 and 8.

compound deriving from nucleophilic attack by methanol with cleavage of a C–O bond.

The formation of such compounds as 2–6 may be explained by the same pathway. The intermediate is known to be unstable, and we observed the formation of water adducts 2 and, to a lesser extent, 3. When irradiation was carried out in the presence of EtOH or MeOH, these solvents behaved like water, yielding 4–6.

According to Adam and co-workers,[7] the addition of the nucleophile should occur at C(3), as happens in compounds 3 and 5, yielding mainly *trans* isomers. The higher reactivity of C(3) appears to be further supported by the different fragmentation pattern of the molecular ions of 5 and 6: in both cases an elimination reaction occurs involving C(3) rather than C(2).

The same authors suggest that the regioisomer in which the nucleophile is bonded to C(2) (Mizuno's compound and 6) is due to secondary reactions: hydrolysis of dioxetane to diol 2, followed by nucleophilic substitution at C(2) (now a hemiacetalic carbon). In this hypothesis, however, a *cis* configuration would be expected for the 2-alkoxy derivatives, or at least racemization. Instead, both Mizuno's compound and 6 still have a *trans* configuration: only in diol 3, which is formed to a much lower extent than 2, are the two oxygens *cis*. Moreover, we did not succeed in transforming 2 into 6 by treatment with methanol, even under acid catalysis. Thus, although we cannot exclude the presence of *cis* isomers among the numerous products which could not be characterized because of their low yields, a direct solvolytic reaction on C(2) does seem reasonable.

As regards 7 and 8, their formation may again be explained by the action of singlet oxygen: if its attack occurs at the central benzene ring instead of at the 2,3 double bond, an endoperoxide may be formed[12] (Figure 5). In this case, two acetalic carbons would originate and may be prone to nucleophilic attack by water, resulting in the loss of one or both methoxy groups.

Conclusions

The photodynamic activity of khellin is known to be rather low,[2a,13] when compared with that of most furocoumarins. Nevertheless, the kinetics of khellin photolysis in various solvents and the structure of the resulting products suggest that singlet oxygen is involved in the process, the mechanism being further supported by literature reports on chemical oxidation. Two different intermediates are probably formed, one being dioxetane at the furan ring and the other an endoperoxide at the benzene ring. The eight characterized photoproducts may all be accounted for by one of these two intermediates.

Several other products are present in the irradiated solutions of khellin, although in smaller amount. Some probably underwent double lysis, since both furan and benzene rings are modified, while the γ-pyrone ring appears to be more stable. Unfortunately, such a high number of products, and the several steps necessary for their purification, prevented any quantitative evaluation.

Further research will be undertaken to establish whether the isolated products are involved in the biological activity of khellin. Indeed, Mizuno *et al.* showed that a photolysis product of 8-MOP—corresponding to our methoxy derivative 4—has, even in traces, a strong inhibitory effect on polymorphonuclear neutrophiles toward anaphylatoxin C5a.[8] Moreover, several pieces of evidence indicate that in preirradiated solutions of furocoumarins some product is present, able to bind to proteins[14] and to lyse red blood cells.[15] Conversely, if the intermediates which have been postulated (dioxetanes, endoperoxides, or epoxides) are easily attacked by water or alcohols, they may also bind to nucleophilic biomolecules, possibly resulting in the severe side effects, e.g., mutagenicity, clearly described by Adam and co-workers.[7]

Experimental Section

Khellin, purchased from Sigma Chem. Co., St. Louis, MO, was used without further purification, although it contained traces of visnagin, its 9-demethoxy analog. Most spectral data are available,[16] but ^{1}H-NOE measurements allowed a better assignment of the chemical shift of the two methoxy groups.

Solvents used were to HPLC grade; deuterated solvent had a deuteration degree ≥99%.

Khellin solutions (0.2 mM), in glass test tubes cooled by water circulation, were irradiated by two Philips HPW 125 lamps, mainly emitting at 365 nm. Light dose on the tube was 55 J s^{-1} m^{-2}.

HPLC analysis was performed with a RP-18 column, 250 × 4 mm, 7 μm particles, eluted with MeOH–H_2O (6:4) or *i*-PrOH–H_2O–HCOOH (50:50:1) for irradiation in benzene, at a flow rate of 1.0 or 0.6 mL/min, respectively, with UV detection at 250 nm. Except for water, naphthalene (2 mM) was used as internal standard, and the percentage of khellin remaining at the various irradiation times was calculated through the ratio of the peak area of khellin over the internal standard.

Photolysis products were obtained from concentrated solutions irradiated for 48 h. Preliminary separation was achieved through column chromatography, followed by TLC on precoated silica gel plates developed with cyclohexane–EtOAc (40:60) (eluent **A**) or $CHCl_3$–MeOH (98:2) (eluent **B**), unless otherwise indicated. The bands were scraped and eluted with $CHCl_3$ or EtOH and filtered, and the solvent was removed under reduced pressure. The purities of the compounds were generally higher than 90%, on the basis of their ^{1}H NMR spectra; only for 6, because of its low yield, purity was lower; however, it was sufficient for structural assignment.

For FT-IR spectra, solutions of the compounds were applied to NaCl disks and left to evaporate.

^{1}H-NMR spectra were obtained at 200 MHz, using TMS as internal standard; coupling constants are given in Hz.

Electron impact (EI) mass spectrometric measurements were performed on a double-focusing, reverse-geometry instrument. EI mass spectra were obtained at 70 eV electrons (200 μA). Samples were introduced by the direct inlet system. Ion source temperature was 180 °C. Metastable transitions were detected by mass-analyzed ion kinetic energy (MIKE) spectroscopy.

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(14) Yoshikawa, K.; Mori, N.; Sakakibara, S.; Mizuno, N.; Song, P.-S. *Photochem. Photobiol.* **1979**, *29*, 1127.

(15) Potapenko, A. Ya. *J. Photochem. Photobiol. B. Biol.* **1991**, *9*, 1.

(16) Hassan, M. A.; Zubair, M. U. In *Analytical Profiles of Drug Substances*; Florey, K., Ed.; Academic Press: London, 1980; Vol. 9, p 371.

7-Hydroxy-5,8-dimethoxy-2-methyl-4-oxo-4*H*-1-benzopyran-6-carbaldehyde (aldehyde 1): CAS 92611-82-0 (all registry numbers supplied by author); TLC R_f = 0.47 (eluent A), 0.64 (B); ^{1}H-NMR ($CDCl_3$) δ (ppm) 2.39 (d, 3H, 2-CH_3, J = 0.8), 3.97 and 4.03 (2s, 3H each, 5-OCH_3 and 8-OCH_3), 6.04 (q, 1H, 3-H, J = 0.8), 10.36 (s, 1H, CHO), 12.2 (br, 1H, PhOH (lit.[6] 11.01 in DMSO-d_6); IR $\bar{\nu}$ (cm^{-1}) 1742 (CHO). MS m/z 264 ($M^{\bullet+}$), 263, 249, 246, 231.

***trans*-2,3-Dihydro-2,3-dihydroxy-4,9-dimethoxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one (diol 2)**: TLC R_f = 0.13 (A), 0.25 (B); ^{1}H-NMR ($CDCl_3$) δ (ppm) 2.35 (d, 3H, 7-Me, J = 0.7), 3.96 and 4.09 (2s, 3H each, 4-OMe and 9-OMe), 5.51 (d, 1H, 3-H, J = 0.5), 6.02 (q, 1H, 6-H, J = 0.7), 6.41 (d, 1H, 2-H, J = 0.5); IR $\bar{\nu}$ (cm^{-1}) 3340 (br, OH); MS m/z 294 ($M^{\bullet+}$), 293, 276, 259.

***cis*-2,3-Dihydro-2,3-dihydroxy-4,9-dimethoxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one (diol 3)**: CAS 102830-25-1; TLC R_f = 0.30 (B), 0.29 (AcOEt/HCOOH (99:1)); ^{1}H-NMR (acetone-d_6) δ (ppm) 2.33 (d, 3H, 7-CH_3, J = 0.7), 3.92 and 3.93 (2s, 3H each, 4-OCH_3 and 9-OCH_3), 5.36 (d, 1H, 3-H, J = 5.2 (lit.[6] 4.96, br, in DMSO-d_6)), 5.77 (d, 1H, 2-H, J = 5.2), 6.13 (q, 1H, 6-H, J = 0.7); IR $\bar{\nu}$ (cm^{-1}) 3395 (br, OH); MS m/z 294 ($M^{\bullet+}$), 293, 276, 259.

***trans*-2,3-Dihydro-2-hydroxy-4,9-dimethoxy-3-ethoxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one (ethoxy derivative 4)**: TLC R_f = 0.21 (A), 0.32 (B); ^{1}H-NMR (acetone-d_6) δ (ppm) 1.21 (t, 3H, $-OCH_2CH_3$, J = 7), 2.35 (d, 3H, 7-Me, J = 0.8), 3.75 (q, 2H, $-OCH_2CH_3$, J = 7), 3.90 and 3.92 (2s, 3H each, 4-OMe and 9-OMe), 4.90 (d, 1H, 3-H, J = 1.3), 5.96 (q, 1H, 6-H, J = 0.8), 6.02 (not resolved d, 1H, 2-H). ^{13}C-NMR (acetone-d_6) δ (ppm) 18 ($-OCH_2CH_3$), 60.5 and 61.5 (2 × –OMe), 63 ($-OCH_2CH_3$); IR $\bar{\nu}$ (cm^{-1}), 3350 (br, OH); MS m/z 322 ($M^{\bullet+}$), 305, 293, 277, 276.

***trans*-2,3-Dihydro-2-hydroxy-3,4,9-trimethoxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one (methoxy derivative 5)**: TLC R_f = 0.21 (B), 0.50 (Et_2O/CH_3CN (88:12)); ^{1}H-NMR (acetone-d_6) δ (ppm) 2.35 (d, 3H, 7-Me, J = 0.8), 3.48 (s, 3H, 3-OMe), 3.89 and 3.92 (2s, 3H each, 4-OMe and 9-OMe), 4.82 (s, 1H, 3-H), 5.96 (q, 1H, 6-H, J = 0.8), 6.12 (br, 1H, 2-H); IR $\bar{\nu}$ (cm^{-1}) 3295 (br, OH); MS m/z 308 ($M^{\bullet+}$), 293, 279, 276, 261.

***trans*-2,3-Dihydro-3-hydroxy-2,4,9-trimethoxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one (methoxy derivative 6)**: TLC R_f = 0.21 (B), 0.41 (Et_2O/CH_3CN (88:12)); ^{1}H-NMR (acetone-d_6) δ (ppm) 2.35 (d, 3H, 7-Me, J = 0.8), 3.56 (s, 3H, 2-OMe), 3.91 and 3.96 (2s, 3H each, 4-OMe and 9-OMe), 5.12 (s, 1H, 3-H), 5.54 (s, 1H, 2-H), 5.96 (q, 1H, 6-H, J = 0.8); IR $\bar{\nu}$ (cm^{-1}) 3358 (br, OH); MS m/z 308 ($M^{\bullet+}$), 293, 279, 259.

4-Hydroxy-9-methoxy-7-methyl-5*H*-furo[3,2-*g*][1]-benzopyran-5-one (phenol 7): CAS 478-42-2; TLC R_f = 0.85 (A), 0.85 (B); ^{1}H-NMR ($CDCl_3$) δ (ppm) 2.46 (d, 3H, 7-Me, J = 0.7), 4.16 (s, 3H, 9-OMe), 6.09 (q, 1H, 6-H, J = 0.7), 7.02 (d, 1H, 3-H, J = 2.3), 7.62 (d, 1H, 2-H, J = 2.3), 13.16 (s, 1H, 4-OH); IR $\bar{\nu}$ (cm^{-1}) 1662 (CO); MS m/z 246 ($M^{\bullet+}$), 245, 231, 230.

4,9-Dihydroxy-7-methyl-5*H*-furo[3,2-*g*][1]benzopyran-5-one (phenol 8): CAS 2159-83-3; TLC R_f = 0.40 (A), 0.41 (B); ^{1}H-NMR (acetone-d_6) δ (ppm) 2.45 (d, 3H, 7-Me, J = 0.6), 6.13 (q, 1H, 6-H, J = 0.6), 7.03 (d, 1H, 3-H, J = 2.3), 7.89 (d, 1H, 2-H, J = 2.3), 8.81 (s, 1H, 9-OH), 13.00 (s, 1H, 4-OH); IR $\bar{\nu}$ (cm^{-1}); 1661 (CO); MS m/z 232 ($M^{\bullet+}$), 231, 230, 204, 192, 191, 190.

When required, acetylation was obtained by mixing about 3 mg of the compound with 5 mg of anhydrous NaOAc and 1 mL of Ac_2O. The mixture was refluxed for 2 h, poured onto ice (≃10 g) and stirred for 1 h. The resulting solution was extracted three times with EtOAc which was then washed with water, treated with Na_2SO_4, and taken to dryness. Acetyl derivatives were purified by TLC and characterized by NMR spectrometry ($CDCl_3$).

These procedures gave **2a–6a** (only relevant resonances are indicated, the others being practically identical to those of the parent compounds).

2a: δ 2.14 and 2.16 (2s, 3H each, 3- and 2-Ac), 6.31 (d, 1H, 3-H, J = 0.8) 6.64 (d, 1H, 2-H, J = 0.8).

3a: δ 2.17 (s, 3H, 3-Ac), 5.82 (d, 1H, 2-H, J = 5.5), 6.29 (d, 1H, 3-H, J = 5.5).

4a: δ 2.13 (s, 3H, Ac), 5.02 (s, 1H, 3-H), 6.69 (s, 1H, 2-H).

5a: δ 2.10 (s, 3H, Ac), 6.30 (s, 1H, 3-H), 6.64 (s, 1H, 2-H).

6a: δ 2.12 (s, 3H, Ac), 5.51 (s, 1H, 2-H), 6.18 (s, 1H, 3-H).

Khellin was demethylated by mixing it with a 5-fold excess of pyridinium chloride and heating at reflux for 0.5 min. The cooled residue was dissolved in 2 N HCl, extracted with EtOAc, and used as chromatographic standard for phenols 7 and 8.

Supplementary Material Available: ^{1}H NMR spectra of compounds **2–6** (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and can be ordered from the ACS; see any current masthead page for ordering information.