SYNTHESIS OF TRITIUM AND CARBON-14 LABELED TIMEFURONE

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SUMMARY

Timefurone (*1*), a methyl thioether analogue of khellin (*2*), was labeled with tritium at the C-2 position and with carbon-14 at the C-5 position for conducting *in vivo* biotransformation studies with *1*. Tritium labeled *1* was prepared by lithiating and tritiating a silyllated derivative of khellinone (*3*) at C-2, followed by converting the labeled khellinone into *1*. Carbon-14 labeled *1* was obtained by first dismantling the pyrone ring of khellin, followed by reconstruction of the ring with incorporation of carbon-14 into the carbonyl position.

Key Words: Synthesis, khellin analogue, tritium, deuterium, carbon-14, carbon-13, Fries rearrangement

INTRODUCTION

Cardiovascular disease, specifically atherosclerosis, has been linked to the distribution of cholesterol associated with various plasma lipoproteins (1). Low density lipoproteins (LDL) are considered atherogenic (2), whereas high density lipoproteins (HDL) are believed to be antiatherogenic (3), or protective against atherosclerosis. In hopes of reducing risk of atherosclerosis by favorably altering serum HDL/LDL ratios, considerable efforts have been expended to identify drugs which would evaluate HDL cholesterol levels while lowering LDL cholesterol in the serum (4). The discovery that khellin (*2*) (5), a naturally occuring furochromone isolated from Ammi visnaga L., can reduce LDL and raise HDL cholesterol levels in

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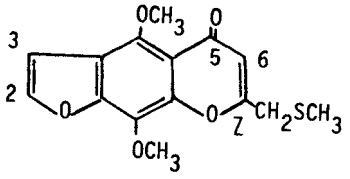
normocholestrolemic male S.E.A. Japanese quail (6) and in man (7) prompted an investigation of the structural features of furochromones, and khellin in particular, responsible for these lipid-altering and antiatherogenic activities. An analogue program associated with this investigation yielded, among other compounds, timefurone (*1*), a methyl thioether analogue of khellin,



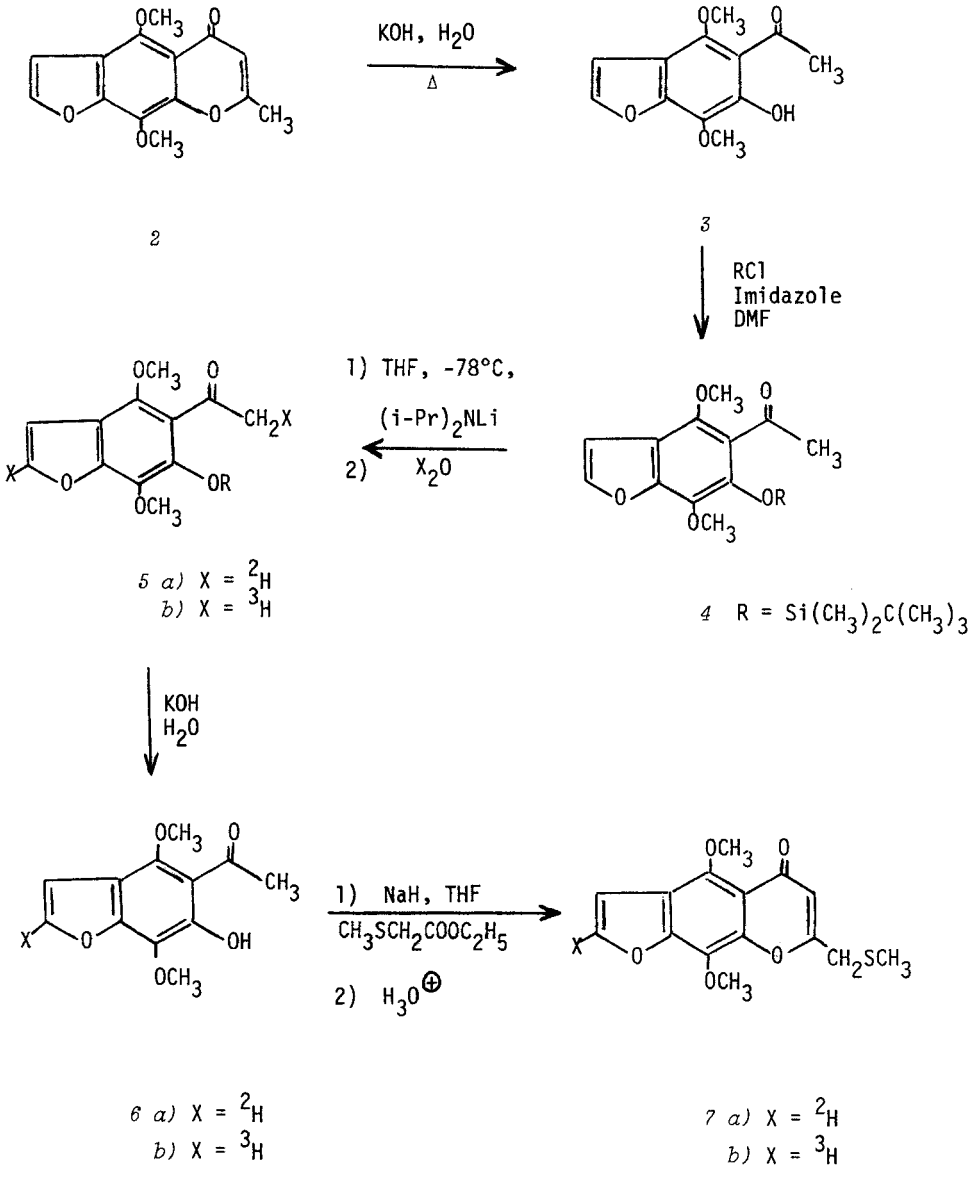
1

which exhibited the desired lipid-altering property (8). This report describes the syntheses (9) of radioactive forms of *1*, labeled with tritium in the furan ring, and with carbon-14 in the pyrone ring, for conducting *in vivo* absorption, distribution, metabolism and excretion studies on this compound.

DISCUSSION AND RESULT

As part of the khellin analogue program, we examined the metallation of selected ethers of khellinone (*3*), the base catalyzed hydrolysis product of khellin, as a potential means of introducing substituents into the furan ring. Treatment of the *t*-butyldimethylsilyl ether of khellinone (*4*) with lithium diisopropylamide afforded a dilithium derivative which on contact with deuterium oxide underwent deuteration at C-2 and the carbonyl methyl carbons, as shown in Scheme 1. When the resulting doubly deuterated silyl ether *5a* was treated with dilute potassium hydroxide solution, the protecting silyl group was removed, and the deuterium at the carbonyl methyl carbon readily underwent proton exchange, but the deuterium at C-2 proved non-exchangeable and remained

Scheme 1. Synthesis of Deuterium and Tritium Labeled *1*.



2

3

5 a) X = ^{2}H
b) X = ^{3}H

4 R = $Si(CH_3)_2C(CH_3)_3$

6 a) X = ^{2}H
b) X = ^{3}H

7 a) X = ^{2}H
b) X = ^{3}H

in the product. Thus these transformations produced [2-^{2}H]khellinone (*6a*), which was condensed with ethyl methylthioacetate and cyclized to give the title compound labeled with deuterium at the C-2 position in the furan ring (compound *7a*). The presence and positions of the deuterium labels in the product and intermediates were ascertained with NMR spectroscopy. By carrying

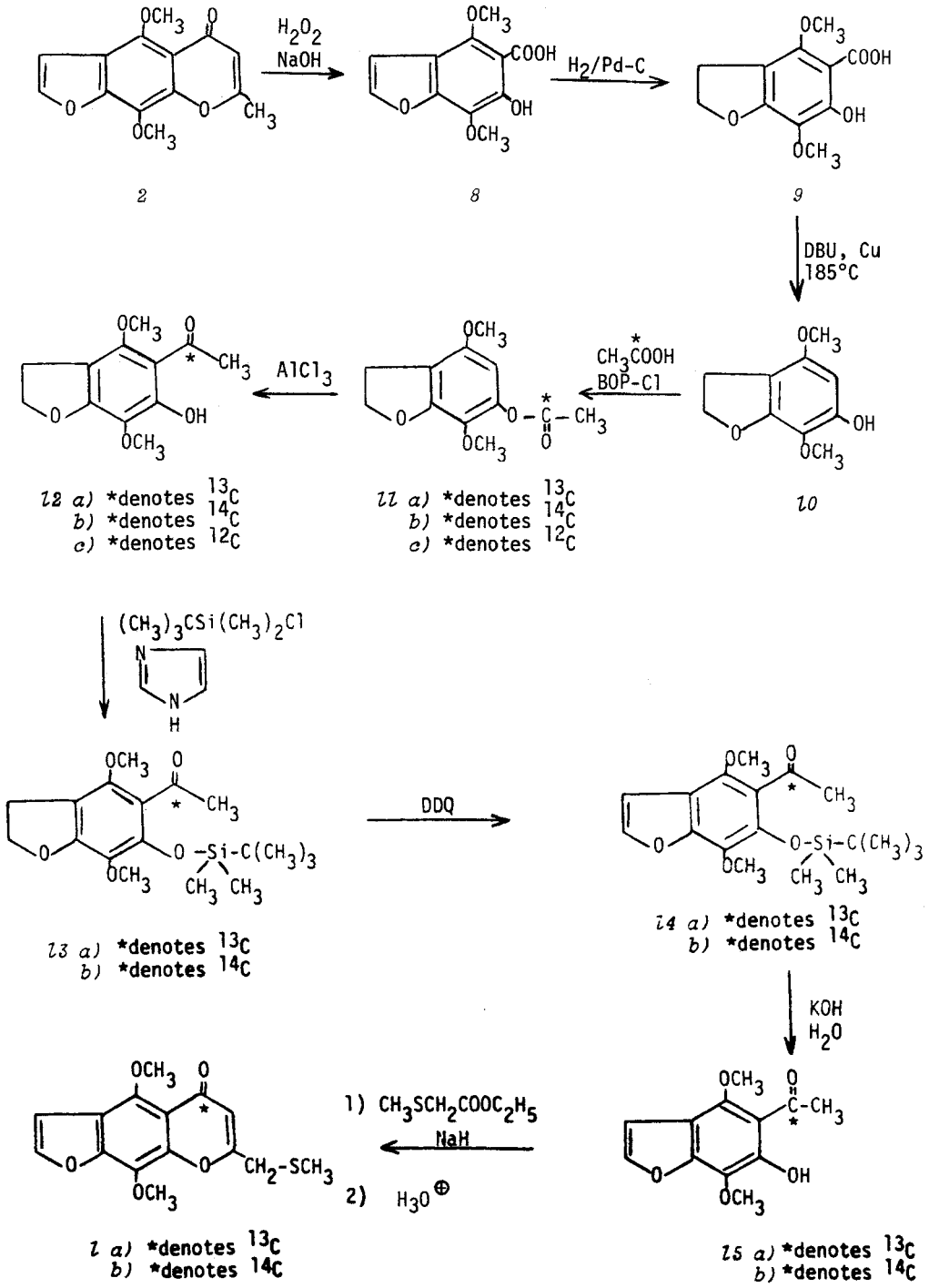
out an analogous series of reactions with substitution of tritiated water for deuterium oxide, we obtained tritium labeled khellinone (*6b*) which was then used to prepare tritium labeled compound *7b*.

We had determined that the label at C-2 was non-exchangeable by subjecting khellinone to both acid and base treatment in the presence of deuterated water. However, in a preliminary metabolism study, in which *7b* was administered both orally and intravenously to rats, significant amounts of tritiated water was excreted in the urine, indicating metabolic instability of the C-2 label. Although this finding had interesting metabolic implications, *7a* must be considered unsuitable for certain metabolism studies. We therefore sought an alternate form of radioactive *1* with which to conduct comprehensive metabolism studies with compound *1*. We turned to labeling with carbon-14.

To select a suitable site in compound *1* for incorporating carbon-14, we evaluated the various carbon positions in the molecule in terms of accessibility and potential vulnerability to separation from the bulk of the ring skeleton. The furan and benzene ring carbons appeared too inaccessible. The C-7 position and the side chain attached thereto would be susceptible to removal from the molecule *via* hydrolysis. This hydrolytic dismantling of the pyrone ring, on the other hand, would provide access to the C-5 and C-6 positions. We chose, as carbon-14 incorporation site, the C-5 position because it appears to be potentially the more metabolically stable of the two. Our strategy was to remove the carbons at C-5, C-6, and C-7 in the pyrone ring, and reconstruct the ring with inclusion of carbon-14 at C-5.

The synthetic route devised for that purpose is shown in Scheme 2. In a single step, khellin was oxidatively degraded to the phenolic acid *8*. Reduction of the furan ring in the presence of palladium-on-charcoal catalyst afforded the dihydrobenzofuran compound *9*, which was decarboxylated to give 2,3-dihydro-4,7-dimethoxy-6-hydroxybenzofuran (*10*). This completed the destruction of the pyrone ring. The phenolic acid *8* could be decarboxylated directly, but the resulting 4,7-dimethoxy-6-hydroxybenzofuran proved highly

Scheme 2. Synthesis of Carbon-13 and Carbon-14 Labeled *1*.



susceptible to air oxidation, and could only be obtained in poor yields with difficult handling. Reduction of the double bond between C-2 and C-3 positions in the furan ring, though necessitating its reintroduction later on in the synthesis, not only removed this problem, but also made possible the crucial Fries rearrangement described later.

The reconstruction of the pyranone ring began with the acylation of the phenol *10* with [1-^{14}C]acetic acid in the presence of N,N-*bis*[2-oxo-3-oxazolidinyl]-phosphorodiamidic chloride (BOP-Cl)(10). Several other carboxylic acid activating agents and coupling agents, including N,N'-carbonyldiimidazole (CDI) and dicyclohexylcarbodiimide (DCC) were tried for effecting the acetylation of *10*, but only BOP-Cl maximized the efficient utilization of acetic acid, an important consideration when labeled acid was to be used. The carbon-14 labeled phenyl acetate *11b* was subjected to Fries rearrangement (10), which produced the substituted acetophenone *12b* labeled with carbon-14 at the carbonyl carbon. The destination of the label in the rearrangement reaction was demonstrated with an analogous reaction sequence in which phenol *10* was acetylated with [1-^{13}C]acetic acid, and the resulting carbon-13 labeled acetate was rearranged under the same conditions to afford the carbon-13 labeled acetophenone *12a* whose label position was confirmed by ^{13}C-NMR spectroscopy. The absence of the double bond between C-2 and C-3 in the benzofuran ring system is essential for the success of the Fries rearrangement. When the reaction was attempted with the analogous fully aromatic compound, 6-acetoxy-4,7-dimethoxybenzofuran, only an intractable tarry mixture was produced, which contained none of the desired khellinone (*3*).

In order to re-introduce the double bond between C-2 and C-3 in the dihydrokhellinone *12b* it was found that the phenol function must be first masked. This was done with the *tert*- butyldimethylsilyl group. Treatment of the silyl ether *13b* with 2,3-dichloro-5,6-dicyano-1,4-quinone (DDQ) gave the carbon-14 labeled silyl ether of khellinone (*14b*). The protecting silyl group

was removed with dilute base, and the resulting [^{14}C]khellinone (*15b*) was then condensed with ethyl methylthioacetate and cyclized to produce the carbon-14 labeled compound *1b*. A parallel series of reactions was also carried out, starting with [^{13}C]dihydrokhellinone *12a*, to give carbon-13 labeled compound *1a*.

Rat Excretion Study with [^{3}H]Timefurone (*6b*)

a) Formulation and Administration of *6b*

The solution used for intravenous administration was prepared by dissolving 1.1 mg of a sample of *6b*, 80.2 μCi/mg) and 5.9 mg of unlabelled timefurone in a mixture of 0.35 ml ethanol and 0.35 ml Emulphor EL-620, and then diluting with 6.3 ml of warm (35°C) 5% glucose solution. For oral administration 2 ml of this solution was further diluted to 8 ml by the addition of 5% glucose.

Two young adult, male Sprague-Dawley rats were each injected with 0.5 ml intravenously (tail vein), and two male rats were each dosed with 2 ml orally. The actual amount of radioactivity administered to each rat was determined by weighing the syringe containing the drug before and after dosing, and determining the specific activity of the formulations gravimetrically (Table 1).

Table 1

Amount of [^{3}H]Timefurone Administered to Individual Rats

Rat Number	1	2	3	4
Route of Drug Administration	IV	IV	Oral	Oral
Weight of Rat				
At time of dosing	205 g	200 g	184 g	178 g
End of experiment	244 g	236 g	225 g	218 g
Amount of Drug Administered				
Weight	0.516 mg	0.513	0.510	0.516
mg/kg	2.52	2.57	2.77	2.90
μCi	6.62	6.58	6.32	6.41

Table 2

Recovery of Radioactivity Following a Single Dose of [^{3}H]Timefurone Administered Intravenously (Rats 1 and 2) and Orally (Rats 3 and 4)

	Percent of Dose			
Urinary Excretion*	Rat 1	Rat 2	Rat 3	Rat 4
0-7 hr	29.35	39.73	21.06	27.09
7-24 hr	9.55	6.25	19.76	14.82
24-48 hr	2.46	1.78	1.80	5.80
48-72 hr	1.40**	1.11**	0.75**	1.33**
72-96 hr	0.62**	0.75**	0.47**	0.85**
Total	43.38	49.62	43.84	49.89
Fecal Excretion				
0-24 hr	28.40	22.79	26.55	0.97
24-48 hr	2.34	1.36	2.01	20.98
48-72 hr	0.30	0.25	0.26	0.36
72-96 hr	0.16	0.14	0.13	0.14
Total	31.20	24.54	28.95	22.45
Tritiated Water				
Excreted in Urine 0-96 hr	5.99	5.67	4.97	4.58
Remaining in Rat at 96 hr***	6.65	7.80	7.93	11.44
Total Non-Volatile Radioactivity	68.59	68.49	67.82	67.76
Total Recovery of Radioactivity	81.23	81.96	80.72	83.78

*Includes tritiated water.
**Greater than 90% of radioactivity in these samples was in volatile materials, e.g., water.
***Body water assumed to be 70% of body weight and blood water is representative of body water.

b) Sample Collection and Processing

The rats were individually housed in stainless steel metabolism cages designed for the separation and collection of urine and feces. Continuous access to food and water was available throughout the entire experimental period. Urine was collected 7, 24, 48, 72 and 96 hours after dosing. At each collection period the screen floor of the cage and the collection funnel were rinsed with 5-10 ml of water and the washings combined with the urine sample. Duplicate 0.5 ml aliquots of urine were counted in 10 ml of ACS® aqueous counting scintillant (Amersham-Searle). Feces were collected 24, 48, 72 and 96 hours after dosing and homogenized to a uniform consistency using a Polytron. Two aliquots (0.4 to 0.5 g) were weighed directly into small paper combustion cups, dried and combusted in a Packard Tri-Carb Sample Oxidizer, Model 306. Tritiated water from the combusted sample was counted in Monophase 40. Tritiated water content of urine was determined by pooling a 2% aliquot from each collection, lyophilizing and counting duplicate 1 ml aliquots of the trapped water in 10 ml of ACS® solvent. Tritiated water content of blood taken 96 hours after dosing was determined in a similar way.

c) Excretion of Radioactivity

The excretion of radioactivity from individual rats following a single IV or oral dose of [^{3}H]timefurone is summarized in Table 2.

EXPERIMENTAL SECTION

Radioactivity determinations were carried out with a Packard Tri-Carb Model 2425 liquid scintillation spectrometer. The external standard method was used, and counting samples were prepared in Diotol. Thin layer chromatographic (TLC) analyses were done on 2 x 10 cm glass plates precoated with a 250 μm thick layer of silica gel GF (Analtech). Developed zones were visualized under ultraviolet light (254 nm). Radioactive zones were detected with a Packard Model 7220/21 Radiochromatogram Scanner equipped with Model 7222 Thin Layer Chromatograph Scanner. Proton and carbon-13 NMR spectra were

obtained with Varian Model EM-390 and Model CFT-20 spectrometers, respectively. Melting points were determined with a Thomas-Hoover Unimelt apparatus and were uncorrected.

Effect of DCl-D_2O on Khellinone* (*3*)

A mixture of 250 mg of *3*, 1 ml of 20% DCl in D_2O, and 5 ml of CH_3OD was stirred at 50°C for 64 hours. The mixture was diluted with 4 ml of D_2O and concentrated at room temperature under reduced pressure to remove methanol. The remaining aqueous suspension was filtered, and the solids were washed with D_2O, and dried under vacuum to give 247 mg of yellow crystals, which showed a single component identical to the starting material *3* by TLC [1:99 (v/v) methanol:methylene chloride, R_f 0.40; 1:1 (v/v) ethyl acetate:hexane, R_f 0.52]. The proton NMR spectrum of this material showed 81% deuteration at the carbonyl methyl carbon, but no deuteration at C-2 or C-3; ^{1}H-NMR ($CDCl_3$, TMS) δ: 2.67 (s, 0.56H, $-COCH_3$), 4.00 and 4.10 (s, 3H each, $-OCH_3$), 6.83 (d, J=2Hz, 1H, H at C-3), 7.43 (d, J=2Hz, 1H, H at C-2).

5-Acetyl-6-*t*-butyldimethylsilyloxy-4,7-dimethoxybenzofuran (khellinone *t*-butyldimethylsilyl ether) (*4*)

A solution of 2.362 g of *3* (10 mmols), 2.261 g of *t*-butyldimethylsilyl chloride (15 mmols), and 2.042 g of imidazole (30 mmols) in 20 ml of dry dimethylformamide was stirred at 65°C for 1 hour. The mixture was diluted with 45 ml of water and well stirred at 0°C. The resulting precipitates were filtered, washed with cold water and dried under vacuum to afford 3.48 g (99.3% yield) of *4*, m.p. 98-98.5°C; single component by TLC [1:99 (v/v) methanol:methylene chloride, R_f 0.52; 1:1 (v/v) ethyl acetate:hexane, R_f 0.69]; IR νmax (cm^{-1}): 3150, 3120 (=CH), 1705 (C=O), 1605, 1540 (C=C), 1345,

*The khellinone sample used in this experiment was obtained by hydrolyzing khellin and was recrystallized from methanol, m.p. 101-102°C [lit. m.p. 92-95°C (12), 99-100°C (13)].

1250, 1150, 1075 (C-O-Si-O-C), 850, 825 (Si-O-); UV λmax in EtOH [nm (ε)]: 214 (30,200), 242 (15,450); mass spec. m/z at 350, ten most intense ions m/z (rel. inten.): 295 (6.1), 294 (20), 293 (100), 279 (4.3), 278 (22), 264 (7.9), 263 (42), 248 (6.0), 235 (3.6), 73 (7.0); ^{1}H NMR ($CDCl_3$, TMS)δ: 0.20 (s, 6H, Si-CH_3), 0.95 (s, 9H, $C(CH_3)_3$), 2.50 (s, 3H, $COCH_3$), 3.95 and 4.00 (s, 3H each, OCH_3), 6.80 (d, J=2Hz, 1H, H at C-3), 7.50 (d, J=2Hz, 1H, H at C-2); anal. calc'd. for $C_{18}H_{26}O_5Si$ (mol. wt. 350.48): C, 61.68, H, 7.48; found: C, 61.71, H, 7.42.

5-Acetyl-4,7-dimethoxy-6-hydroxy-[2-^{2}H]benzofuran ([2-^{2}H]khellinone, *6a*)

A solution of 1.113 g of freshly distilled diisopropylamine (11 mmols) in 50 ml of dry tetrahydrofuran* (THF) was cooled under N_2 with a dry ice-acetone bath. Eleven ml of 1M solution of *n*-BuLi (11 mmols) in hexane was added. The mixture was stirred at -78°C under N_2 for 15 minutes and 1.753 g of *4* (5 mmols) was added. Stirring at -78°C under N_2 was continued for 30 minutes and 0.5 ml of deuterium oxide was added dropwise in 10 minutes. The cooling bath was removed, and when the chunks of ice had melted (~10 minutes), 10 ml of 6N HCl was added to the yellow milky suspension. The mixture was concentrated at reduced pressure to remove THF and hexane. The aqueous residue was diluted with 10 ml of water, cooled in an ice bath, and the solids were filtered, washed with water and briefly air dried. The solids (*5*a) were redissolved in 20 ml of THF and 5 ml of 50% KOH in water was added. The two-phased wine-colored mixture was well stirred at room temperature overnight (20 hours). The mixture was diluted with 10 ml of water and concentrated at reduced pressure. The aqueous residue was extracted with ether to remove *t*-butyldimethylsilanol, cooled in an ice bath, and acidified with 10 ml of 6N HCl. The resulting precipitates were filtered, washed with cold water and dried under vacuum to give 1.099 g of bright yellow crystals, *6a* (93% yield), which showed a single component identical to khellinone (*3*) by TLC [1:99 (v/v)

*Freshly distilled over CaH_2.

methanol:methylene chloride, R_f 0.40; 1:1 (v/v) ethyl acetate:hexane, R_f 0.52]. The ^{1}H-NMR of *6a* showed that it was 85% deuterated at C-2 and there was no deuterium at C-3 or the carbonyl methyl carbon, δ($CDCl_3$, TMS): 2.67 (s, 3H, $COCH_3$), 4.00 and 4.10 (s, 3H each, OCH_3), 6.83 (s, 1H, H at C-3), 7.45 (d, J=2Hz, 0.15H, H at C-2), and 13.24 (s, 1H, OH).

5-Acetyl-4,7-dimethoxy-6-hydroxy-[2-^{3}H]benzofuran ([2-^{3}H]khellinone, *6b*)

Tritium labeled *6b* was prepared in the same manner as *6a*, except that the dilithio salt of *4* was decomposed with tritiated water (nominally 1 Ci**, 5 Ci/ml, 11 mmols) instead of deuterium oxide. The product obtained after the same workup as described above was subjected to two more aqueous base-acid treatment cycles to ensure removal of the last traces of tritium which might have remained on the carbonyl methyl carbon. From 1.753 g of *4*, there was obtained 1.116 g of *6b* (95% yield), sp. act. 104.6 μCi/mg (103.2 μCi/mg after the first repeat base-acid treatment cycle), radiochemically homogeneous by TLC with identical R_f as khellinone (*3*) in 1:99 (v/v) methanol:methylene chloride and 1:1 (v/v) ethyl acetate:hexane.

4,9-Dimethoxy-7-[(methylthio)methyl]-5H-[2-^{2}H]furo[3,2-g][1]benzopyran-5-one (*7a*)

A solution of 945 mg of *6a* (4.0 mmols) and 805 mg of ethyl methylthioacetate (6.0 mmols) in 5.5 ml of dry dimethylformamide (DMF) was added dropwise with stirring to a suspension of 500 mg of sodium hydride (57% mineral oil suspension, 12 mmols) in the same solvent. After the initial vigorous evolution of gases had subsided, the mixture was stirred under N_2 at 65°C for 45 minutes, cooled to room temperature, and 2 ml of conc. HCl was carefully added dropwise with stirring. The mixture was again heated at 65°C with stirring under N_2 for 45 minutes, cooled in the bath, and 10 g of ice was added, followed by 20 ml of 1N NaOH. The resulting suspension was stirred with cooling for 10 minutes and filtered.

**Tritiated water was supplied by Amersham Corp., Arlington Heights, IL.

The collected solids were washed with cold water and air dried. The crude product was triturated with 20 ml of ether in several portions, dissolved in 10 ml of methylene chloride, and treated with activated charcoal (Darco G-60), The solution filtered and concentrated at reduced pressure. The residue was recrystallized from 1:3 (v/v) ethyl acetate:hexane to give 870 mg of *7a* (71% yield), cream-colored needles, m.p. 151-152°C; single component identical to authentic *1* [m.p. 151-152°C (8)] by TLC [R_f 0.58 with 1:19 (v/v) methanol:methylene chloride, R_f 0.47 with ethyl acetate]; ^{1}H-NMR showed *7a* was 83% deuterated at C-2, δ ($CDCl_3$, TMS): 2.23 (s, 3H, SCH_3), 3.57 (s, 2H, SCH_2-), 4.07 and 4.20 (s, 3H each, OCH_3), 6.13 (s, 1H, H at C-6), 6.97 (s, 1H, H at C-3), 7.60 (d, J=2Hz, 0.17H, H at C-2).

4,9-Dimethoxy-7-[(methylthio)methyl]-5H-[2-^{3}H]furo[3,2-g][1]benzopyran-5-one (*7b*)

Tritium labeled *7b* was prepared in the same manner as *7a*. From 945 mg of *6b* (4.0 mmols), there was obtained 714 mg of *7b*, m.p. 151-152°C, same as authentic *1*; sp. act. 80.2 μCi/mg; UV λmax in ethanol [nm(ε)]: 208 (21,200), 249 (38,400), 334 (4,600); radiochemically homogenous with identical R_f as authentic *1* by TLC [1:19 (v/v) methanol:methylene chloride, ethyl acetate]. The mother liquor yielded another 150 mg of *7b*, m.p. 150.5-151°C, sp. act. 79.5 μCi/mg (15491-RSH-9E), after chromatographic purification on 60 g of silica gel with 1:39 (v/v) methanol:methylene chloride.

5-Carboxy-4,7-dimethoxy-6-hydroxybenzofuran (*8*)

To a stirred suspension of 10 g of khellin (*2*, 38.5 mmols) in 200 ml of 1.5N NaOH was added 12 ml of 30% H_2O_2. The mixture, which became warm spontaneously was stirred for 2.5 hours, heated on a steam bath to 75°C, and stirred for another hour without heating. The yellow mixture was filtered to remove small amounts of solids. The filtrate was acidified to pH 1 with 25 ml of conc. HCl and cooled in an ice bath. The precipitates were filtered,

washed with cold water, dried, and recrystallized from 60 ml of ethyl acetate to afford 5.88 g of *8* (64% yield), m.p. 153-154°C, single component by TLC [95:5:0.1 (v/v) methylene chloride:methanol:acetic acid, R_f 0.28]; ^{1}H-NMR δ(DMSO-d_6, TMS): 3.90 and 3.97 (s, 3H each, OCH_3), 7.07 (d, J=2Hz, 1H, H at C-3), 7.85 (d, J=2Hz, 1H, H at C-2).

5-Carboxy-4,7-dimethoxy-6-hydroxy-2,3-dihydrobenzofuran (*9*)

A suspension of 650 mg of palladium on charcoal catalyst in a solution of 3.962 g of *8* (16.63 mmols) in 18 ml of DMF and 12 ml of methanol was stirred under H_2 at room temperature and atmospheric pressure until uptake of H_2 ceased (2.5 hours). The mixture was filtered and the collected catalyst was washed with 10 ml 1:1 v/v DMF:methanol. The combined filtrate and washings were concentrated at 45°C and 25 torr to remove methanol. The remaining DMF solution was diluted with 100 ml of water and cooled in an ice bath. The resulting precipitates were filtered, washed with cold water and dried under vacuum to give 3.804 g of *9*, (95.2% yield), m.p. 142-143°C; TLC R_f 0.16 with 95:5:0.1 (v/v) methylene chloride:methanol:acetic acid; UV λmax in ethanol [nm(ε)]; 222 (26,550), 269 (10,850), and 304 (3,400); IR νmax in Nujol (cm^{-1}): 3532 (OH), 2953, 2929, 2856 (C-H), 1683 (C=O), 1619, 1464-1355 (C=C), 1282, 1265, 1237, 1204, 1128, 1098, 1049 (C-O); mass spec. m/z at 240, ten most intense ions m/z (rel. inten.): 222 (100), 207 (54), 240 (41), 165 (21), 193 (17), 223 (14), 221 (12), 164 (10.5), 151 (10.4), 192 (10.0); ^{1}H-NMR δ (DMSO-d_6, TMS): 3.20 (t, J=8Hz, 2H, H at C-3), 3.70 and 3.77 (s, 3H each, OCH_3), 4.63 (t, J=8Hz, 2H, H at C-2); anal. calc'd. for $C_{11}H_{12}O_6$ (mol. wt. 240.21): C, 55.00, H, 5.04, found:C, 54.76, H, 5.02.

4,7-Dimethoxy-6-hydroxy-2,3-dihydrobenzofuran (*10*)

A mixture of 2.162 g of *9* (9.0 mmols), 2 ml of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU, Aldrich) (13.4 mmols), 550 mg of copper powder*, and 250 mg of fine glass powder** was heated under N_2 at 185°C with stirring for 30

*The copper powder was freshly ground with mortar and pestle and screened through an 80 mesh sieve.
**The glass powder was ground from glass wool with mortar and pestle.

minutes. The mixture was cooled slightly and sonicated with 10 ml of water which dissolved organic materials. The mixture was acidified with 2 ml of conc. HCl and well stirred with 25 ml of methylene chloride. The layers were separated and the aqueous layer was extracted with 15 ml of methylene chloride. The combined methylene chloride extracts were washed with brine and dried over $MgSO_4$. Removal of solvent gave a cream-colored solid residue which was chromatographed on 150 g of silica gel with 2.5% v/v methanol in methylene chloride. There was obtained 1.659 g of *9* (94% yield) as white crystals, single component by TLC [R_f 0.67 with 95:5:0.5 (v/v) methylene chloride:methanol:acetic acid; R_f 0.54 with 1:1 (v/v) ethyl acetate:hexane]. An analytical sample was recrystallized from 1:4 (v/v) ethyl acetate:hexane, m.p. 114-115°C [lit. m.p. 112.5-113°C (12), 114°C (13)]; UV λmax in ethanol [nm(ε)]: 206 (41,350), 272 (638); IR νmax in Nujol (cm^{-1}): 3407 (OH), 3071-2859 (C-H), 1618, 1509, 1481, 1461, 1448 (C=C), 1197-1033 (C-O); mass spec. m/z at 196, ten most intense ions m/z (rel. inten.): 181 (100), 196 (91), 163 (25), 153 (21), 197 (11.2), 182 (11.2), 125 (4.8), 69 (3.4), 123 (2.9), 167 (2.8); ^{1}H-NMR δ($CDCl_3$, TMS): 3.03 (t, 2H, J=8Hz, H at C-3), 3.72 and 3.83 (s, 3H each, OCH_3), 4.56 (t, 2H, J=8Hz, H at C-2), 5.71 (S, 1H, OH), 6.00 (s, 1H, H at C-5); anal. calc'd. for $C_{10}H_{12}O_4$ (mol. wt. 196.20): C, 61.21, H, 6.17, found: C, 60.97, H, 6.18.

6-Acetoxy-4,7-dimethoxy-2,3-dihydrobenzofuran (*11c*)

A) With BOP-Cl

To a solution of 392 mg of *10* (2.0 mmols), 120 mg of acetic acid (2.0 mmols), and 607 mg of triethylamine (6.0 mmols) in 5 ml of methylene chloride was added 764 mg of BOP-Cl* (3.0 mmols). The mixture was stirred at room temperature for 4 hours, during which time a fluffy precipitate was formed as the BOP-Cl went into solution. The mixture was filtered and the collected solids were washed with 10 ml of methylene chloride. The combined filtrate and washings were concentrated at reduced pressure to give an oil, which on

*BOP-Cl was supplied by Chemical Dynamics Corp., South Plainfield, NJ.

trituration with water afforded a crystalline product. Chromatography of this material on 75 g of silica gel with 1:39 (v/v) methanol:methylene chloride yielded 385 mg of *11c* (82% yield) as white crystals, TLC R_f 0.75 with 1:39 (v/v) methanol:methylene chloride and 0.47 with 1:1 (v/v) ethyl acetate:hexane. An analytical sample was recrystallized from 1:4 (v/v) ethyl acetate:hexane, m.p. 90-91°C [lit. m.p. 87-88.5° (12)]; UV λmax in ethanol [nm(ε)]: 205 (41,900), 281, (1,650); IR νmax in Nujol (cm^{-1}): 2950, 2928, 2855 (C-H), 1761 (C=O), 1628, 1603, 1585 (C=C), 1230, 1209, 1183, 1175, 1131, 1062, 1031 (C-O); mass spec. m/z at 238, ten most intense ions m/z (rel. inten.): 181 (100), 196 (92), 238 (38), 163 (14.1), 153 (11.3), 197 (10.3), 182 (10.2), 239 (4.8), 167 (3.6), 66 (2.7); ^{1}H-NMR δ ($CDCl_3$, TMS): 2.27 (s, 3H, $COCH_3$), 3.10 (t, J=8Hz, 2H, H at C-3), 3.73 and 3.80 (s, 3H each, OCH_3), 4.60 (t, J=8Hz, 2H, H at C-2), and 6.03 (s, 1H, H at C-5); anal. calc'd. for $C_{12}H_{14}O_5$ (mol. wt. 238.23): C, 60.50, H, 5.92, found: C, 60.25, H, 5.86.

B) With DCC

A solution of 387 mg of *10* (2.0 mmols), 896 mg of DCC (4.2 mmols), and 252 mg of acetic acid (4.2 mmols) in 10 ml ethyl acetate was stirred under N_2 at 75°C overnight. TLC analysis of the mixture showed the reaction was incomplete. Three additional portions of 1 mmol each of DCC and acetic acid in 2 ml of ethyl acetate were added at two-hour intervals while the mixture was maintained at 75°C. The mixture was filtered and the filtrate was concentrated. The residue was chromatographed on 75 g of silica gel with 1:49 (v/v) methanol:methylene chloride to give 400 mg of *10* (84% yield based on *10*, 23% yield based on acetic acid) after recrystallization from ethyl acetate:hexane, m.p. 90-91°C, identical by TLC to the sample of *11c* prepared from *10* with BOP-Cl described above.

6-[1-^{13}C]Acetoxy-4,7-dimethoxy-2,3-dihydrobenzofuran (*11a*)

A solution of 2.97 g of *10* (15 mmols) and 3.31 g of DCC (16 mmols) in 10 ml of dry THF was cooled in an ice bath and 1.0 g of [1-^{13}C]acetic acid* (16.4

*[1-^{13}C]Acetic acid of 90% enrichment was supplied by Prochem, B.O.C.Limited, London, U.S. distributor U.S. Services, Summit, NJ.

mmols) in 5 ml of dry THF was added. The mixture was stirred at 0°C for 0.5 hour and stored in a freezer (-15°C) for 16 hours. TLC analysis showed little acetylation had occurred. The mixture was stirred at room temperature for three days and finally heated with stirring at 75°C under N_2 for 18 hours. The mixture was filtered and the solids were washed with 30 ml of methylene chloride. The combined filtrate and washings were concentrated at reduced pressure to give an oil, which was chromatographed twice on 150 g of silica gel with 1:49 (v/v) methanol:methylene chloride to give 1.468 g of *11a* (41% yield based on *9*, 37% yield based on [1-^{13}C]acetic acid), m.p. 90-91°C, and 0.930 g of the unreacted starting phenol *10*, m.p. 114-115°C. Both ^{1}H-NMR and ^{13}C-NMR spectra of *11a* showed ^{13}C-enrichment at the carboxy carbon. In the ^{1}H-NMR spectrum, the signal attributed to the methyl protons adjacent to the carbonyl carbon was a doublet at 2.27 with a ^{13}C-^{1}H coupling constant of 6 Hz, indicating high ^{13}C enrichment in the carbonyl carbon, which was also evident in the ^{13}C-NMR spectrum as a disproportionately strong signal for the carbonyl carbon at 169.37 ppm and a 60 Hz ^{13}C-^{13}C coupling between the carbonyl carbon and the adjacent methyl carbon at 20.66 ppm. Aside from these features just described, these spectra corresponded to those of the phenol acetate *11c* prepared from *10* and acetic acid with BOP-Cl described earlier.

6-[1-^{14}C]Acetoxy-4,7-dimethoxy-2,3-dihydrobenzofuran (*11b*)

To a solution of 2.671 g of triethylamine (26.4 mmols) in 10 ml of methylene chloride was added nominally 438 mg of [1-^{14}C]acetic acid* (200 mCi, 7.3 mmols) in 3 ml of methylene chloride. BOP-Cl (3.374 g, 13.25 mmols and phenol *10* (1.727 g, 8.8 mmols) were added with stirring, followed by 10 ml of methylene chloride. The suspension was stirred under N_2 at room temperature for 3 hours and 2 ml of 1M acetic acid in methylene chloride was added. The mixture was stirred for 4 more hours and 0.7 ml of 1M acetic acid

*[1-^{14}C]Acetic acid of 27.4 mCi/mmol was supplied by Pathfinder Laboratories, Inc., St. Louis, MO.

in methylene chloride, 1.8 ml of 2M triethylamine in methylene chloride, and 446 mg of BOP-Cl (1.75 mmol) were added. This addition pattern of reactants, to reach the final ratio of 8.8 mmols phenol:10 mmols acid:15 mmols BOP-Cl:30 mmols triethylamine, was intended to optimize the utilization of both the phenol and labeled acid with minimal isotopic dilution. After another 4 hours of stirring, the reaction mixture was partitioned with 30 ml of saturated sodium bicarbonate solution. The aqueous layer was extracted with 25 ml of methylene chloride. The combined organic phases were washed with brine and dried over $MgSO_4$. Removal of solvent afforded a largely crystalline residue which was chromatographed on 150 g of silica gel with 1:39 (v/v) methanol:methylene chloride to give 1.785 g of white crystalline *11b* (85% yield). A recrystallized sample of this material (from ethyl acetate-hexane) had a specific activity of 92 μCi/mg (21.9 mCi/mmol) and was radiochemically homogeneous, with identical TLC R_f [0.68 with (v/v) methanol:methylene chloride, 0.45 with 1:2 (v/v) ethyl acetate:hexane] as the authentic sample of *11c* described earlier. The recrystallized *11b* and mother liquor residue were recombined for conversion to the ketone *12b*.

5-Acetyl-4,7-dimethoxy-6-hydroxy-2,3-dihydrobenzofuran (*12c*)

Aluminum chloride (1.60 g, 12 mmols) was dissolved in 6 ml of nitrobenzene with warming and the solution was cooled in an ice bath. To the cold solution was added 1.43 g of *11c* and the mixture was stirred at 0°C. The resulting yellow-brown solution was kept in a refrigerator (10°C) for 23 hours. The reaction mixture was quenched with 12 g of ice and stirred with 5 ml of 6N HCl. The mixture was extracted with 50 ml of ethyl acetate in three portions. The combined extracts were washed with water and brine and dried over $MgSO_4$. The solution was concentrated and the residue was chromatographed twice on 150 g of silica gel, first with 1:49 (v/v) methanol:methylene chloride, then with 1:1 (v/v) ethyl acetate:hexane to afford 913 mg of *12c* (64% yield) as pale yellow crystals and 249 mg of phenol *10*, which accounted

for 17% of the starting material *11*. An analytical sample of *12c* recrystallized from ethyl acetate-hexane melted at 105-106°C [lit. m.p. 102-103 (12,14), 105°C (13)]; TLC R_f 0.75 [1:39 (v/v) methanol:methylene chloride] and 0.60 [1:1 (v/v) ethyl acetate:hexane]; UV λmax in ethanol [nm(ε)]: 204 (16,200), 274 (15,600); λsh [nm(ε)]: 315 (3,400); IR νmax in Nujol (cm^{-1}): 2950, 2924, 2855 (C-H), 1727 (C=O), 1615, 1584 (C=C), 1428, 1418, 1370 (C-O); mass spec. m/z at 238, ten most intense ions m/z (rel. inten.): 223 (100), 238 (83), 208 (21), 205 (17), 195 (13), 224 (12.4), 239 (12.2), 193 (12.0), 192 (4.6), 165 (4.5) ^{1}H-NMR δ ($CDCl_3$, TMS): 2.63 (s, 3H, $COCH_3$), 3.30 (t, J=8Hz, 2H, H at C-3), 3.86 and 3.90 (s, 3H each, OCH_3), 4.65 (t, J=8Hz, 2H, H at C-2), 13.95 (s, 1H, OH); ^{13}C-NMR ppm ($CDCl_3$, TMS): 28.13 (C-3), 32.04 (carbonyl CH_3), 59.33 and 60.59 (OCH_3), 73.05 (C-2), 108.18 and 108.75 (C-5 and C-3a), 110.62 (C-7), 128.39 (C-6), 158.84 and 159.36 (C-4 and C-7a), 203.38 (C=O); anal. calc'd. for $C_{12}H_{14}O_5$ (mol. wt. 238.23): C, 60.50, H, 5.92, found: C, 60.39, H. 5.88.

5-[1-^{13}C]Acetyl-4,7-dimethoxy-6-hydroxy-2,3-dihydrobenzofuran (*12a*)

The carbon-13 labeled *12a* was prepared by Fries rearrangement of *11a* in the presence of aluminum chloride with the procedure described above, except that the reaction was carried out at room temperature. This change resulted in a lower yield, and the crude product was more tarry and required more extensive purification by chromatography. From 1.436 g of *11a*, there was obtained 685 mg of *12a*, which was identical to *12a* by TLC. A sample of recrystallized from ethyl acetate-hexane melted at 105-106°C [lit. m.p. 104-105°C (8)], and its ^{1}H and ^{13}C-NMR spectra clearly showed carbon-13 enrichment at the carbonyl carbon. The ^{1}H-NMR spectrum showed a doublet with a coupling constant of 6 Hz at 2.63 attributed to coupling between the carbonyl ^{13}C and carbonyl methyl protons. The ^{13}C-NMR spectrum showed an intense signal at 203.38 ppm attributed to the carbonyl carbon.

5-[1-^{14}C]Acetyl-4,7-dimethoxy-6-hydroxy-2,3-dihydrobenzofuran (*12b*)

The procedure for preparing *12c* from *11c* described earlier was followed, with the reaction being carried out at 0-10°C for 22 hours. From 1.570 g of *11b* (7.3 mmols), there was obtained 1.204 g of *12b* (77% yield), m.p. 105-106°C; sp. act. 89.1 μCi/mg (21.2 mCi/mmol); radiochemically homogenous by TLC with same R_f as *12c*.

5-Acetyl-6-*t*-butyldimethylsilyloxy-4,7-dimethoxy-2,3-dihydrobenzofuran (*13c*)

A mixture of 2.382 g of *12c* (10 mmols), 2.260 g of *t*-butyldimethylchlorosilane (15 mmols) and 2.042 g of imidazole was stirred with 20 ml of dry DMF until all solids had dissolved. The solution was stirred under N_2 at 75°C for 1.5 hour, cooled in an ice bath and diluted with 50 ml of ice water. The mixture was stirred well at 0°C for 0.5 hour and filtered. The collected crystals were washed with cold water and dried under vacuum. There was obtained 3.422 g of *13c* (97% yield), white crystals melting at 80.5-81.5°C; TLC R_f 0.60 with 1:99 (v/v) methanol:methylene chloride, R_f 0.67 with 1:1 (v/v) ethyl acetate:hexane; UV λmax in ethanol [nm(ε)]: 205 (31,350), 273 (3,750); λsh [nm(ε)]: 225 (13,900); IR νmax in Nujol (cm-1): 2954, 2927, 2857 (C-H), 1699 (C=O), 1611, 1591, 1425-1471 (C=C), 1053-1037, 1251, 1256, 1283, 1349 (C-O), 785-851 (+Si-O-C); mass spec. m/z at 352, ten most intense ions m/z (rel. inten.): 295 (100), 265 (31), 296 (24), 280 (10.8), 73 (10.5), 297 (7.3), 75 (6.4), 266 (5.8), 337 (4.7), 140 (3.5); ^{1}H-NMR δ($CDCl_3$, TMS): 0.17 [s, 9H, $C(CH_3)_3$], 0.95 (s, 6H, $Si(CH_3)_2$], 2.45 (s, 3H, $COCH_3$), 3.23 (t, J=8Hz, 2H, H at C-3), 3.76 and 3.79 (s, 3H each, OCH_3), 4.59 (t, J=8Hz, 2H, H at C-2); anal. calc'd. for $C_{18}H_{28}O_5Si$ (mol. wt. 352.49): C, 61.33, H. 8.01, found: C, 61.05, H. 7.97.

5-[1-^{13}C]Acetyl-6- -butyldimethylsilyloxy-4,7-dimethoxy-2,3-dihydrobenzofuran (*13a*)

The carbon-13 labeled *13a* was prepared with the same procedure as described for *13c*. From a mixture of 1.132 g of *12c* and 60 mg of *12a* (1.192 g

total, 5.0 mmols), there was obtained 1.719 g of *13a* (98% yield) with 5% carbon-13 enrichment, m.p. 80.5-81.5°C, identical to *13c* by TLC, R_f 0.60 with 1:99 (v/v) methanol:methylene chloride, R_f 0.67 with 1:1 (v/v) ethyl acetate:hexane.

5-[1-^{14}C]Acetyl-6-*t*-butyldimethylsilyloxy-4,7-dimethoxy-2,3-dihydrobenzofuran (*13b*)

Similarly, 1.050 g of *12b* (4.4 mmols) was stirred under N_2 at 80°C for 4 hours with 1.356 g of *t*-butyldimethylchlorosilane (9 mmols) and 1.225 g of imidazole (18 mmols) in 9 ml of dry DMF to produce 1.527 g of *13b* (98%yield). This material melted at 80.5-81.5°C and was radiochemically homogeneous with identical TLC R_f as *13c* [0.60 with 1:99 (v/v) methanol:methylene chloride, 0.67 with 1:1 (v/v) ethyl acetate:hexane].

5-[1-^{13}C]Acetyl-6-*t*-butyldimethylsilyloxy-4,7-dimethoxybenzofuran (*14a*)

A dark yellowish-green solution of 1.410 g of *13a* (4 mmols) and 1.135 g of DDQ (5 mmols) in 10 ml of dioxane was gently refluxed with stirring under N_2 for 2 hours. Within minutes after reflux began, crystalline plates appeared in the mixture as the solution turned dark reddish brown. The mixture was cooled and filtered and the solids were washed with 20 ml of methylene chloride. The combined filtrate and washings were concentrated at 45°C and 25 torr. The residual viscous dark red oil was chromatographed on 75 g of silica gel twice with 1:99 (v/v) methanol:methylene chloride to give 797 mg of *14a* (57% yield), which was identical by TLC to the khellinone silyl ether *4* prepared from khellinone (*3*) described earlier [R_f 0.64 with 1:99 (v/v) methanol:methylene chloride, 0.59 with 1:2 (v/v) ethyl acetate:hexane].

5-[1-^{14}C]Acetyl-6-*t*-butyldimethylsilyl-4,7-dimethoxybenzofuran (*14b*)

Similarly, the carbon-14 labeled dihydrobenzofuran *13b* (1.500 g, 4.26 mmols) was dehydrogenated with 1.205 g of DDQ (5.32 mmols) in 10 ml of

refluxing dioxane. The crude product was first chromatographed on 150 g of silica gel with 1:2 (v/v) ethyl acetate:hexane, then on 80 g of silica gel with 1:99 (v/v) methanol:methylene chloride to yield 861 mg of *14b* (58%). This material was radiochemically homogeneous and identical to *4* by TLC [Rf 0.64 with 1:99 (v/v) methanol:methylene chloride, 0.61 with 1:2 (v/v) ethyl acetate:hexane].

5-[1-^{13}C]Acetyl-4,7-dimethoxybenzofuran ([^{13}C]khellinone, *15a*)

A mixture of 795 mg of *14a* (2.26 mmols) and 10 ml of methanol was warmed on a steam bath until all solids were in solution. Addition of 2 ml of 50% KOH in water turned the solution golden yellow. The solution was stirred at room temperature for 45 minutes and concentrated at 32°C and 25 torr. The resulting wet paste was mixed with 20 ml of water and acidified with 5 ml of 6N HCl. The mixture was filtered and the solids were washed with water to give a mixture of bright yellow khellinone and colorless *t*-butyldimethylsilanol. The latter material was volatile and readily removed by drawing air through the filter funnel. The remaining solids were dried under vacuum to afford 531 mg of *15a* (99% yield), m.p. 101.5-102.5°C, identical to khellinone (*3*) by TLC [R_f 0.56 with 1:99 (v/v) methanol:methylene chloride, 0.40 with 1:2 (v/v) ethyl acetate:hexane]; ^{13}C-NMR ppm ($CDCl_3$, TMS): 33.13 (keto methyl C), 60.45 and 60.90 (OCH_3), 105.87 (C-3), 110.51 and 110.81 (C-5 and C-3a), 128.79 (C-7), 143.85 (C-2), 151.75, 152.44 and 153.53 (C-6, C-4 and C-7a), 205.29 (C=O, ^{13}C-enriched).

5-[1-^{14}C]Acetyl-4,7-dimethoxybenzofuran ([^{14}C]khellinone, *15b*)

Similarly, 860 mg of *14b* (2.45 mmols) was desilylated to afford 572 mg of *15b* (99% yield), m.p. 101.5-102.5°C; sp. act. 90.4 μCi/mg (21.3 mCi/mmol); radiochemically homogeneous by TLC with identical R_f as khellinone (*3*) [R_f 0.56 with 1:99 (v/v) methanol:methylene chloride, 0.41 with 1:2 (v/v) ethyl acetate:hexane].

4,9-Dimethoxy-7-[(methylthio)methyl]-5H-[5-^{13}C]furo[3,2-g][1]benzopyran-5-one (*1a*)

The procedure for preparing deuterium labeled *7a* from [^{2}H]khellinone (*6a*) was followed. Trituration of the crude product from 472 mg of *15a* (2.0 mmols) with ether and decolorization with activated charcoal were ommitted. Instead, the crude product was chromatographed on 80 g of silica gel with 1:39 (v/v) methanol:methylene chloride. The purified material was recrystallized from 1:4 (v/v) ethyl acetate:hexane to afford 428 mg of *1a* (70% yield), m.p. 150.5-152°C, identical by TLC to an authentic sample of *1* [R_f 0.57 with 1:19 (v/v) methanol:methylene chloride, 0.56 with ethyl acetate, 0.25 with 1:39 (v/v) methanol:methylene chloride]; ^{13}C-NMR ppm ($CDCl_3$, TMS): 15.98 (SCH_3), 36.03 (S-CH_2), 61.64 and 62.35 (OCH_3), 105.21 (C-3), 110.40 (C-6), 113.94 (C-4a), 119.60 (C-3a), 130.09 (C-9), 145.66 (C-2), 146.28 and 146.69 (C-8 and C-4a), 147.47 (C-9a), 163.08 (C-7), 178.03 (C-5, ^{13}C-enriched).

4,9-Dimethoxy-7-[(methylthio)methyl]-5H-[5-^{14}C]furo[3,2-g][1]benzopyran-5-one (*1b*)

Carbon-14 labeled *1b* was prepared from *15b* in the same manner as deuterium labeled *7a* from [^{2}H]khellinone (*6a*). The crude product obtained from 555 mg of *15b* (50.2 mCi, 2.35 mmols) was chromatographed on 80 g of silica gel (packed in methylene chloride) with 150 ml of 1:39 (v/v) methanol:methylene chloride followed by 500 ml of 1:19 (v/v) methanol:methylene chloride. The product was further chromatographed on 80 g of silica gel with 1:1 (v/v) ethyl acetate:methylene chloride to give 484 mg of pure product which was recrystallized from 1:1 (v/v) ethyl acetate:hexane to afford 442 mg of *1b*, m.p. 151-152°C (same as an authentic sample of *1*); sp. act. 70.7 μCi/mg (21.6 mCi/mmol); UV λmax in ethanol [nm(ε)]: 211 (19,750), 216 (19,700), 248 (37,900), 334 (4,600); λsh [nm(ε)]: 284 (5,750); radiochemically homogeneous by TLC with same R_f as authentic *1* [R_f 0.49 with

1:19 (v/v) methanol:methylene chloride, 0.56 with ethyl acetate]. The residue from the mother liquor (40 mg) was co-crystallized with 160 mg of *1* to give a second crop of 175 mg of *1b*, sp. act. 14.4 μCi/mg, m.p. 151-152°C, also shown to be radiochemically pure by TLC. The total radiochemical yield was 33.8 mCi, 67.3% from *15b*.

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