

## SYNTHESIS OF DEUTERIUM AND TRITIUM LABELED PSORALENS

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## SUMMARY

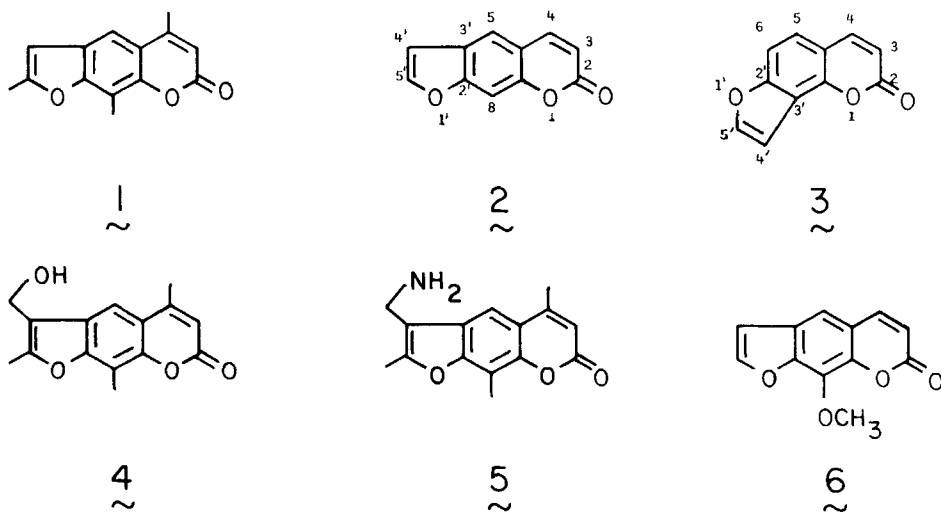
Deuterium and tritium labeled 4,5',8-trimethylpsoralen (1), psoralen (2), and angelicin (3) have been prepared by D<sub>2</sub>O and T<sub>2</sub>O exchange with specific activities of 228-253 mCi/mmol. Tritium labeled 4,5',8-trimethylpsoralen (1) with a specific activity of 17.6 Ci/mmol was prepared by catalytic reduction with tritium gas followed by dehydrogenation. Tritiated 4'-hydroxymethyl-4,5',8-trimethylpsoralen (4) with a specific activity of 12.0 Ci/mmol was obtained by reduction of the corresponding aldehyde with sodium borotritide and elaborated to 4'-aminomethyl-4,5',8-trimethylpsoralen (5) (12.0 Ci/mmol) by conversion of 4 to the chloromethyl derivative followed by the Gabriel synthesis. Tritium labeled 8-methoxypsoralen (6) was prepared by demethylation of unlabeled 6 to the phenol followed by methylation with tritiated methyl iodide.

Key Words: 4,5',8-Trimethylpsoralen, Psoralen, Angelicin, 4'-Hydroxymethyl-4,5',8-trimethylpsoralen, 4'-Aminomethyl-4,5',8-trimethylpsoralen, 8-Methoxypsoralen

## INTRODUCTION

The linear furocoumarin derivatives known as psoralens are used as medicinal agents for the treatment of psoriasis, leukoderma and mycosis fungoides (1-3), as molecular probes for investigating nucleic acid secondary structure (4,5), for studies dealing with bacterial and viral repair mechanisms (6,7), and for the inactivation of DNA and RNA viruses (8). Psoralens have been shown to intercalate within the double stranded helical regions of both DNA and RNA and, upon irradiation with 320-380 nm light, undergo covalent photoaddition to the pyrimidine bases of the nucleic acid. Some of the psoralen-pyrimidine adducts which result can undergo a second photoaddition to a second pyrimidine base, resulting in a crosslinking of the adjacent strands (4,9). The biological effects of the psoralens are currently thought to result from this nucleic acid reactivity.

To investigate the various aspects of the psoralen-nucleic acid interaction, it is often essential to have the psoralen labeled. In this report, procedures for the preparation of deuterated and tritiated 4,5',8-trimethylpsoralen (1), psoralen (2), angelicin (3), 4'-hydroxymethyl-4,5',8-trimethylpsoralen (4), 4'-aminomethyl-4,5',8-trimethylpsoralen (5), and 8-methoxypsoralen (6) are described.



#### RESULTS AND DISCUSSION

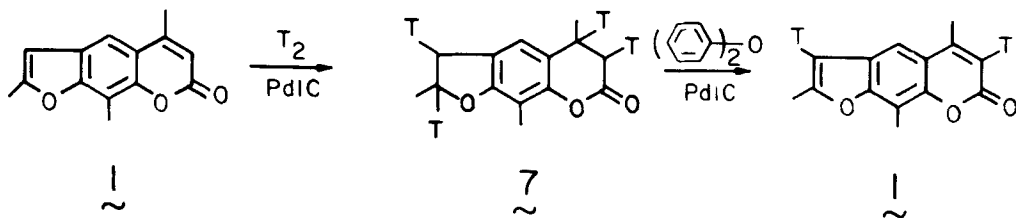
4,5',8-Trimethylpsoralen undergoes acid catalyzed exchange in *p*-dioxane with both  $D_2O$  and  $T_2O$  (10). The deuterium or tritium is incorporated predominately but not exclusively at the 4' position. With  $D_2O$ , the PMR signal for the 4' proton was depressed 30, 54, and 100% following 6, 9, and 24 hours under the reaction conditions. Various other protons were exchanged to a lesser degree as shown by the mass spectrum, which revealed that up to six deuteriums had been incorporated into the structure.

Psoralen and angelicin likewise undergo acid catalyzed deuterium and tritium exchange. The PMR spectrum of deuterated psoralen found the 4' position to be the primary exchange site while the mass spectrum indicated that up to three deuteriums had been incorporated into the molecule. Angelicin was also found

to exchange mainly at the 4' position.

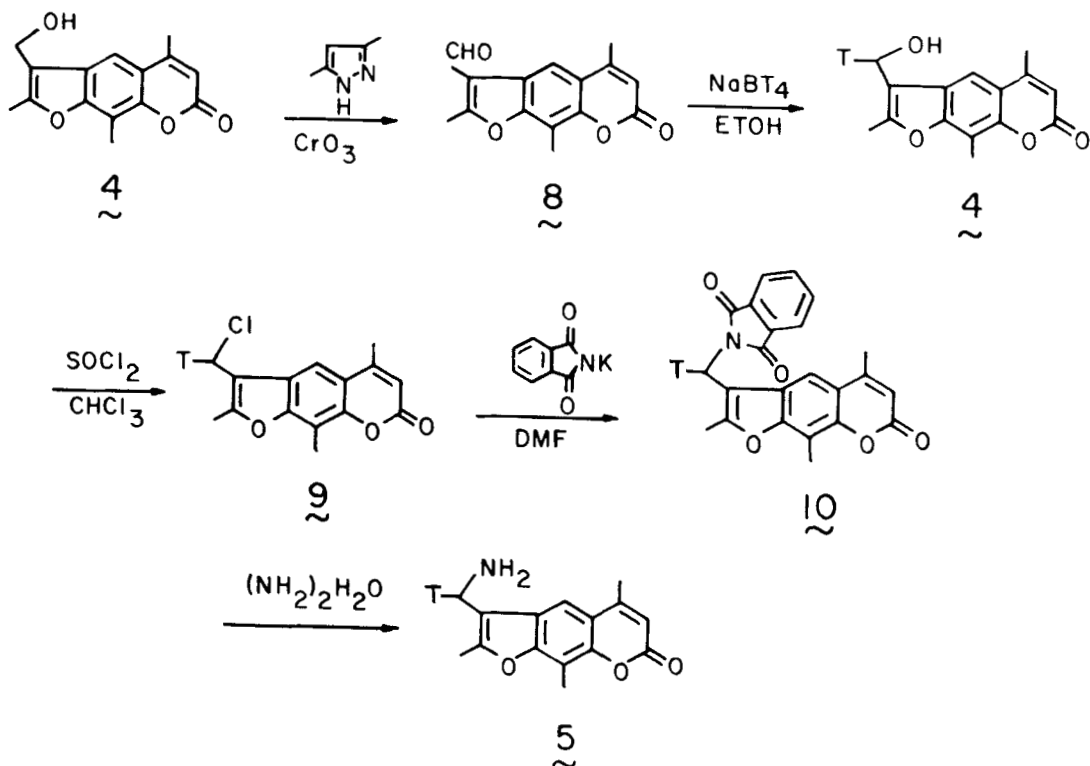
Tritiation by exchange of 4,5',8-trimethylpsoralen, psoralen, and angelicin resulted in a specific activity of 200-250 mCi/mmol for all three compounds. As the specific activity of the  $T_2O$  used was approximately 300 mCi/mmol, the results suggest that the isotope exchange approached equilibrium in all three cases. Degradation of the three compounds was evident during the exchange reactions with psoralen being the most stable to the conditions used.

In order to provide high specific activity 4,5',8-trimethylpsoralen, the following reduction-dehydrogenation scheme was utilized (11,12). Unlabeled 1 was reduced with tritium gas over palladium on charcoal to give [3,4,4',5'- $^3H_4$ ]-3,4,4',5'-tetrahydro-4,5',8-trimethylpsoralen (7). Compound 7 was then dehydrogenated by refluxing in diphenylether over the same catalyst, giving [3,4'- $^3H_2$ ]-4,5',8-trimethylpsoralen with a specific activity of 17.6 Ci/mmol. The catalytic reduction-dehydrogenation procedure was not applied to psoralen or angelicin. However, it would be expected to give results similar to those found with 4,5',8-trimethylpsoralen.

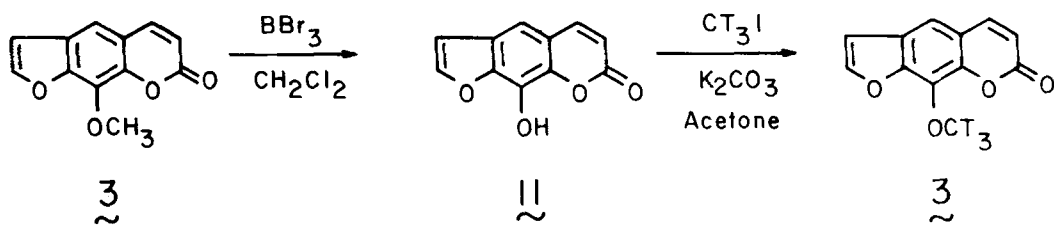


The preparation of tritiated 4'-hydroxymethyl-4,5',8-trimethylpsoralen and 4'-aminomethyl-4,5',8-trimethylpsoralen with specific activities less than 100 mCi/mmol has been reported (10). To provide these compounds with higher specific activities, the following scheme was devised. Unlabeled 4 was oxidized with chromium trioxide-3,5-dimethyl pyrazole complex (13) to give 4'-formyl-4,5',8-trimethylpsoralen (8). Reduction of 8 with sodium borotritide (48 Ci/mmol) provided tritiated 4 with a specific activity of 12 Ci/mmol. Tritiated 4 was then treated with thionyl chloride in chloroform, giving 4'-([ $^3H$ ]-chloromethyl)-4,5',8-trimethylpsoralen (9). Compound 9 was reacted with potassium

phthalimide to give 4'-(phthalimido- $^{3}\text{H}$ -methyl)-4,5',8-trimethylpsoralen (10). Hydrazinolysis of 10 with hydrazine hydrate in ethanol afforded 4'-( $^{3}\text{H}$ -aminomethyl)-4,5',8-trimethylpsoralen with a specific activity of 12.0 Ci/mmol.



With 8-methoxypsoralen, a variety of acid and base catalyzed deuterium exchange reactions were run. However, no incorporation of the isotope was detected. To provide tritiated **6**, a different scheme was utilized which placed the tritium label on the methoxy group at the 8-position. As shown below, unlabeled 8-methoxypsoralen was demethylated to 8-hydroxypsoralen (11) with boron tribromide, followed by O-methylation with tritiated methyl iodide. The specific activity of the 8-methoxypsoralen so prepared was controlled by the activity of the methyl iodide used.



The results of the above experiment are summarized in Table I.

<u>COMPOUND</u>	<u>STRUCTURE AND LABEL POSITION</u>	<u>TRITIATION METHOD</u>	<u>SPECIFIC ACTIVITY (Ci/mM)</u>
4,5',8-Trimethylpsoralen $\underset{\sim}{1}$	<p>(Major Position)</p>	$\text{T}_2\text{O}$ Exchange	0.253
Psoralen $\underset{\sim}{2}$	<p>(Major Position)</p>	$\text{T}_2\text{O}$ Exchange	0.231
Angelicin $\underset{\sim}{3}$	<p>(Major Position)</p>	$\text{T}_2\text{O}$ Exchange	0.228
4'-Hydroxymethyl- 4,5',8-trimethylpsoralen $\underset{\sim}{4}$		$\text{NaBT}_4$ Reduction	12.0
4'-Aminomethyl- 4,5',8-trimethylpsoralen $\underset{\sim}{5}$		$\text{NaBT}_4$ Reduction	12.0
8-Methoxypsoralen $\underset{\sim}{6}$		Alkylation ( $\text{CT}_3\text{I}$ )	2.5

**TABLE I.** The structure, label position, specific activity and method of tritiation for each of the psoralen compounds.

## EXPERIMENTAL

PMR spectra were obtained at room temperature in  $\text{CDCl}_3$  using tetramethylsilane as an internal standard on a Varian T60 NMR spectrometer, with chemical shifts expressed in PPM. Mass spectra were obtained with an AEI MS-12 Mass Spectrometer; ion potential 70 V, probe temperature 120-170°C. Radioactivity measurements were made in a Beckman LS-230 liquid scintillation counter. Specific activities were determined by counting aliquots of standard solutions of each tritiated psoralen in omnifluor-triton/toluene containing 10% water (v/v). The concentration of the standard solutions was determined by using the extinction coefficients of each psoralen. Radiochemical purity was determined by thin layer chromatography of each purified product in at least three solvent systems ( $\text{CHCl}_3$ ,  $\text{CHCl}_3/\text{CH}_3\text{OH}$  98:2,  $\text{C}_6\text{H}_6/(\text{CH}_3)_2\text{CO}$  1:1) using Eastman No. 13181 silica gel plates with fluorescent indicator. Each plate was divided into equal sections which were placed into scintillation vials and the amount of radioactivity in each section determined. Radiochemical purity is expressed as the percent of the total radioactivity found in the section which contained the tritiated compounds. Tritiated water (0.5-1.0%  $\text{T}_2\text{O}$ ) was provided by Lawrence Berkeley Laboratory and deuterated water (99.8%) purchased from Bio-Rad Labs. 4,5',8-Trimethylpsoralen was synthesized according to Bender et al. (14), 8-methoxypsoralen was purchased from Sigma Chemical Company, while psoralen and angelicin were provided by Professors M. Pathak (Harvard University) and J. Cleaver (University of California, San Francisco), respectively. All solvents, drying agents, etc. were of reagent grade. Tritiated methyl iodide and sodium borotritide were purchased from New England Nuclear.

[4'- $^3\text{H}$ ]-4,5',8-Trimethylpsoralen (1). The preparation of this compound by  $\text{T}_2\text{O}$  exchange has been previously described (10). The following improved purification procedure has subsequently been developed. The crude tritiated product was dissolved in a small volume of chloroform, loaded on a silica gel column (60-200 mesh) of appropriate size and eluted with either methylene chloride or chloroform. The fractions containing the purified material were combined and the

solvent removed, giving the purified product (253 mCi/mmol) which was greater than 99% radiochemically pure.

[3,4'-<sup>3</sup>H<sub>2</sub>]-4,5',8-Trimethylpsoralen (1). 4,5',8-Trimethylpsoralen (33 mg, 0.14 mmol) 10% palladium on charcoal (16.5 mg) and glacial acetic acid (2 mL) were placed in a 25 mL round bottom flask and stirred with tritium gas (150 Ci) until the uptake of tritium had ceased (4 h). The catalyst was removed by centrifugation, followed by evaporation of the supernatant under vacuum. The residual solid was dissolved in ~1 mL methylene chloride, loaded on a 1/2" x 5" silica gel column (60-200 mesh), then eluted with methylene chloride. The fractions containing the product were combined and the solvent removed, giving [3,4,4',5'-<sup>3</sup>H<sub>4</sub>]-3,4,4',5'-tetrahydro-4,5',8-trimethylpsoralen (7, 36 Ci/mmol). Compound 7 was then placed in a 25 mL round bottom with diphenyl ether (5 mL) and 10% palladium on charcoal (30 mg). A nitrogen bubbler was attached and the mixture refluxed for 28 hours. After cooling to room temperature, ethanol (5 mL, 100%) was added and the catalyst removed by centrifugation. The supernatant was partially evaporated, then loaded on a 1/2" x 5" silica gel column (60-200 mesh) and eluted with chloroform. The fractions containing the product were combined, the solvent volume reduced and the chromatography repeated on a 1/2" x 10" column as above. The purified product (4 mg, 12.1% yield from unlabeled 1) so obtained was stored in absolute ethanol to inhibit radiolysis. The specific activity of the material was 17.6 Ci/mmol while the radiochemical purity was determined to be greater than 95%.

[4'-<sup>3</sup>H]-Psoralen (2). Psoralen (285 mg, 1.53 mmol), p-dioxane (10 mL), and fuming sulfuric acid (30% SO<sub>3</sub>, 1.5 mL) were placed in a 50 mL round bottom flask followed by the addition of T<sub>2</sub>O (8 Ci, 0.4 mL). A condenser was attached and the mixture refluxed for 23 h, forming a dark amber solution. After cooling to room temperature, the acidic reaction mixture was neutralized by dropwise addition of NaOH (4 N). The solution was transferred to a 250 mL separatory funnel and the psoralen extracted with chloroform (60 mL), resulting in an emulsion. This was cleared by the addition of sodium chloride (1.0 g). After additional chloroform extractions (3 x 40 mL), the combined extracts were dried (MgSO<sub>4</sub>) and filtered, giving a final volume of 200 mL. TLC of this

extract found psoralen along with two minor low  $R_f$  fluorescent spots. The radiochemical purity of the psoralen at this point was 88%. An aliquot (50 mL) was removed from the 200 mL chloroform extract and the solvent removed by rotary evaporation. The residual yellow-orange solid (37 mg) was dissolved in a small volume of chloroform, loaded on a 1/2" x 5" silica gel column (60-200 mesh), then eluted with chloroform. The fractions containing psoralen only were combined and the solvent removed giving a white solid (14 mg). The analytical TLC of the product found the radiochemical purity to be greater than 98%. The yield of recovered psoralen, based on the 50 mL aliquot, was 19.6%.

4'-<sup>3</sup>H]-Angelicin (3). Angelicin (21 mg, 0.11 mmol), p-dioxane (5.0 mL), and fuming sulfuric acid (30%  $\text{SO}_3$ , 0.7 mL) were placed in a 25 mL round bottom flask, stirred magnetically, and a second portion of fuming sulfuric acid (0.4 mL) added dropwise.  $\text{T}_2\text{O}$  (0.5 mL, ~10 Ci) was added and the resulting yellow solution placed in a heating mantle and heated at reflux for 4.5 h. The resulting amber solution was cooled to room temperature, then neutralized by the careful addition of saturated sodium bicarbonate solution (45 mL). After transfer to a separatory funnel, the product was extracted with chloroform (2 x 8 mL), the extracts dried ( $\text{Na}_2\text{SO}_4$ ), filtered, then evaporated, giving a yellow residue. TLC of this material found mostly product with two faint low  $R_f$  spots. The solid was dissolved in a small volume of chloroform, loaded on a 1/2" x 10" silica gel column (60-200 mesh), then eluted with chloroform. The fractions which contained the angelicin only were combined and the solvent removed giving the purified product (6 mg, 28% recovery). The analytical TLC of this material found the radiochemical purity to be greater than 99%.

4'-([<sup>3</sup>H]-Hydroxymethyl)-4,5',8-trimethylpsoralen (4). 4'-Formyl-4,5',8-trimethylpsoralen 8 (20 mg, 0.08 mmol) and ethanol (95%, 10 mL) were placed in a 50 mL round bottom flask followed by the addition of sodium borotritide (390  $\mu\text{g}$ , 10  $\mu\text{mol}$ , 48 Ci/mmol, 500 mCi). The heterogeneous mixture was magnetically stirred while the progress of the reaction was monitored by TLC ( $\text{CH}_2\text{Cl}_2$ ). Although the mixture was stirred 2 h, the reaction was essentially over after



10 min. The ethanol was removed under reduced pressure and the residual solid dissolved in 10 mL methanol which was then removed by rotary evaporation. This was repeated four more times and the resulting solid dissolved in a small volume of chloroform, loaded on a 1/2" x 7" silica gel column (60-200 mesh), then eluted with chloroform. The fractions containing the product were combined, the volume reduced, then rechromatographed on an identical column using methylene chloride as the eluant. The product fractions were combined and the solvent removed giving the purified product (5 mg, 48% yield), which was stored in absolute ethanol to inhibit radiolysis. Analytical TLC found the radiochemical purity of this material to be greater than 99%.

4'-([<sup>3</sup>H]-Aminomethyl)-4,5',8-trimethylpsoralen (5). 4'-(Phthalimido-[<sup>3</sup>H]-methyl)-4,5',8-trimethylpsoralen (10, ~6 mg, ~0.02 mmol), ethanol (95%, 5 mL), and hydrazine hydrate (0.01 mL, 0.18 mmol, 85% solution in water) were placed in a 25 mL round bottom flask with a small magnetic stirrer. The mixture was heated at 50° for several hours until no starting material remained by TLC (CHCl<sub>3</sub>). After this, the ethanol was removed on the rotovap giving a light yellow residue. Chloroform (5 mL) and 1 N sodium hydroxide (5 mL) were added and the mixture transferred to a small separatory funnel. The basic solution was further extracted with chloroform (2 x 5 mL), the combined extracts washed with water (3 x 5 mL), dried (MgSO<sub>4</sub>), filtered and evaporated, giving the amine as the free base. Absolute ethanol (2 mL) was added, then saturated with anhydrous HCl gas. The ethanol was removed on the rotovap and the amine hydrochloride (~2 mg, ~50% yield) placed under vacuum to remove residual HCl. Analytical TLC (benzene/acetone 1:1) found the radiochemical purity of the product to be greater than 99%. The product was stored in absolute ethanol to inhibit radiolysis.

8-([<sup>3</sup>H<sub>3</sub>]-Methoxy) psoralen (6). A modified 10 mL pear bottom flask to which a glass valve had been attached at the top was used as a low pressure bomb for the reaction. 8-Hydroxypsoralen (11) (20.4 mg, 0.10 mmol), anhydrous potassium carbonate (57.5 mg, 0.59 mmol), and acetone (1.0 mL) were added to the reaction flask followed by [<sup>3</sup>H<sub>3</sub>]-methyl iodide (50 mCi, 2-3 Ci/mmol) in acetone (0.5 mL). The valve was shut and the mixture heated at 55°C for 15 h while being magnetic-

ally stirred. After this period, the reaction flask was cooled and then frozen. A vacuum line was attached and the solvent pumped off. Water (5 mL) was added to the dry residue and the product extracted with chloroform (4 x 3 mL) from the basic solution. The combined chloroform extracts were washed with water (1 x 5 mL), then passed through a short column of anhydrous sodium sulfate. Next, the solvent volume was reduced to ~0.5 mL, then loaded on a 1/2" x 4" silica gel column (60-200 mesh) and eluted with chloroform. The first 20 mL off the column contained all the product, which was found to be greater than 98% radiochemically pure with a specific activity of 2.5 Ci/mmol. The yield was approximately 50% based on the amount of [ $^3\text{H}$ ]-methyl iodide used.

4'-Formyl-4,5',8-trimethylpsoralen (8). 3,5-Dimethylpyrazole (410 mg, 4.3 mmol) was added to a suspension of chromium trioxide (425 mg, 4.3 mmol) in methylene chloride (12.5 mL) and the mixture stirred at room temperature under argon for 15 min. To the resulting dark red solution, 4'-hydroxymethyl-4,5',8-trimethylpsoralen (400 mg, 1.6 mmol) was added in one portion and the reaction mixture then stirred at room temperature for 2 h. TLC ( $\text{CHCl}_3$ ) after this period found no starting material remained. The solvent was removed on the rotovap and the residue dissolved in a small volume of chloroform, loaded on a 1/2" x 8" silica gel column (60-200 mesh) and eluted with methylene chloride. The fractions containing product were combined and the solvent removed giving the aldehyde (290 mg, 73% yield). Further purification was accomplished by recrystallization from 95% ethanol giving white crystals, NMR ( $\text{CDCl}_3$ )  $\delta$  2.4-2.6 (6H, d), 2.8 (3H, s), 6.3 (1H, s), 8.2 (1H, s), 10.2 (1H, s). Analysis: Calculated for  $\text{C}_{15}\text{H}_{12}\text{O}_4$ : C, 70.3; H, 4.7. Found: C, 70.1; H, 4.9.

4'-([ $^3\text{H}$ ]-Chloromethyl)-4,5',8-trimethylpsoralen (9). A 2% solution (v/v) of thionyl chloride in chloroform (2 mL) was added to [ $^3\text{H}$ -4'- $\alpha$ ]-4'-hydroxymethyl-4,5',8-trimethylpsoralen (4, 5 mg, 0.02 mmol, 12.0 Ci/mmol) and the solution magnetically stirred until no starting material was detected by TLC ( $\text{CHCl}_3$ ). After this period (~30 min), the solvent was evaporated at room temperature, giving the product which was one spot on TLC ( $\text{CHCl}_3$ ). The white solid was placed under high vacuum at room temperature for 1 h to remove

any residual thionyl chloride, then used directly for the preparation of 10. The yield was essentially quantitative.

4'-(Phthalimido-[<sup>3</sup>H]-methyl)-4,5',8-trimethylpsoralen (10). 4'-([<sup>3</sup>H]-Chloromethyl)-4,5',8-trimethylpsoralen (9, ~5 mg, ~0.02 mmol), freshly prepared potassium phthalimide (13 mg, 0.07 mmol, 2 h, acetone reflux, 6 h vacuum drying at 100°C) and DMF (2 mL, dried over 4 Å molecular sieves) were placed in a small round bottom flask and stirred overnight at room temperature after which no starting material remained by TLC (CH<sub>2</sub>Cl<sub>2</sub>). The DMF was removed under reduced pressure and the yellow residue suspended in 1-2 mL chloroform, which was then removed by rotary evaporation. The resulting solid was placed under high vacuum for 10 min then suspended in ~1 mL chloroform, loaded on a 1/2" x 8" silica gel column (60-200 mesh) and eluted with chloroform. The fractions containing the product were pooled and the solvent evaporated. The resulting white solid was placed under vacuum 30 min, then used directly for the preparation of 5. The yield was approximately 80%.

8-Hydroxypsoralen (11). 8-Methoxypsoralen (10 g, 46 mmol) was dissolved in methylene chloride (200 mL, distilled from P<sub>2</sub>O<sub>5</sub>) in a three-neck 500 mL round bottom flask with attached reflux condenser and dry nitrogen line. Boron tribromide (10 mL, 100 mmol) was added to the stirred reaction mixture in one portion. Precipitation of a yellowish solid ensued within 15-20 min from the orange reaction mixture. After 6.5 h, a small amount of cold water (5 mL) was cautiously added, causing an exotherm which subsided after a few minutes. Additional water (500 mL) was added and the heterogeneous mixture stirred overnight followed by collection of the crude product by suction filtration. After sucking dry on the filter, the cream colored solid was recrystallized from acetonitrile (200 mL), giving very fine slightly yellow crystals (5.48 g, 58% yield); m.p. 247-249°C, NMR (CD<sub>3</sub>COCD<sub>3</sub>) δ 6.2-6.4 (1H, d, C3-H), 7.0-7.1 (1H, d, C4'-HO), 7.4 (1H, s, C5-H), 7.9-8.2 (2H, m, C4-H, C5'-H). Mass spectrum m/e (relative abundance) 202, M<sup>+</sup> (100).

Analysis: Calculated for C<sub>11</sub>H<sub>6</sub>O<sub>4</sub>: C, 65.4; H, 3.0. Found: C, 65.1; H, 3.1.

## ACKNOWLEDGMENTS

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