Nitrations of 4',5'-Dihydropsoralens: A Route to Radiopharmaceutical Precursors

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Unlike the nitration of the fully-aromatic furocoumarin, methoxsalen, which yields a single nitration product, the nitration of the 4',5'-dihydro compound generates a condition-variant mixture of three nitro products. Reduction, diazotization, and pyrrolidine-trapping of one of these, the 4',5'-dihydro-5-nitro-8- methoxypsoralen, provides a pyrrolidine triazene precursor for radioiodination of the dihydropsoralen system.

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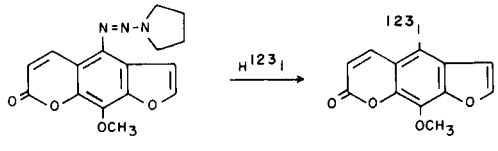
The linear furocoumarins or psoralens have a long and fruitful tradition of use in therapy for pathological skin conditions [1,2]. In fact, their uptake and binding to dermal melanocytes has caused pharmacologists to propose their use as carriers for cytotoxic functionalities to malignant melanomas [3]. Recent syntheses of new and dermally-active psoralens demonstrate the continuing interest in the field [4,5]. Our own interests in radioiodinated pharmaceuticals for imaging metastatic dermal melanoma have made the psoralens attractive candidates for diagnostic purposes [6,7].

For radiohalogen labeling of imaging agents a variation of the Sandmeyer reaction, in which diazoniums are trapped as triazenes by secondary amines, has achieved wide popularity [8-10]. The preparation and decomposition of a triazene has already been applied to the synthesis of I-123 5-iodo-8-methoxypsoralen (see Scheme I), a radioimaging candidate for occult melanomas [11]. Metabolically, these fully aromatic psoralens possessing unsaturation at both 3-4 and 4'-5' sites are more mutagenic because they photobind intracellularly to form less repairable cross-linked DNA adducts [12]. An improved class of melanoma imaging agents might be the labeled analogs of the less mutagenic dihydro-psoralens. A convenient way to prepare such partially-reduced 4',5'-dihydropsoralens has appeared but these have not been functionalized as triazenes for use as radioiodination precursors [13].

The usual approach to preparing these triazeno precursors is the nitration, reduction, nitrous acid treatment, and diazotrapping of the candidate drug [9-11]. In the course of such a study on a partially reduced version of the most clinically active drug, methoxsalen (**1**), we have found that the site of nitration of 4',5'-dihydro-8-methoxypsoralen is extremely dependent upon reaction conditions. Earlier workers reported a mono-nitrated product (mp 192-198°) from 4',5'-dihydro-8-methoxypsoralen (**3**) but were unable to assign a structure [14]. Later researchers claimed the separation by fractional crystallization of two mononitrated isomers (mp 175-176 and 220-222°) from the same nitration of **3** but neither agreed in properties with the earlier report [15]. Access to modern laboratory techniques such as nmr and hplc resolves the issue.

Scheme I



Unlike the nitration of **1** which yields exclusively the 5-nitro-8-methoxypsoralen (**2**) under a variety of conditions [14,16], the nitration of the 4',5'-dihydro compound, **3**, gives a mixture of 3-nitro, 5-nitro, and 3,5-dinitro- compounds throughout a nearly 100° difference in reaction temperatures (Table I/Scheme 2). Furthermore, traditional fractional crystallization to analytical purity proved impossible since eutectic mixtures are formed between the nitrated species. Quantification was possible by either carefully integrated proton-nmr spectra or hplc assay of the nitration mixtures. The methoxyl resonances on the purified nitro psoralens were distinct and authentic mixtures of these substances displayed no interaction-induced shifts. In the 3-nitro compound **4** the methoxyl was observed at 4.07, in the 5-nitro **5** at 4.17, and in the 3,5-dinitro **6** at 4.20 ppm. In the starting material, **3**, the methoxy signal was evident at 4.02 ppm. All comparisons were performed in anhydrous deuterochloroform against TMS with the average of three separate integrations per sample

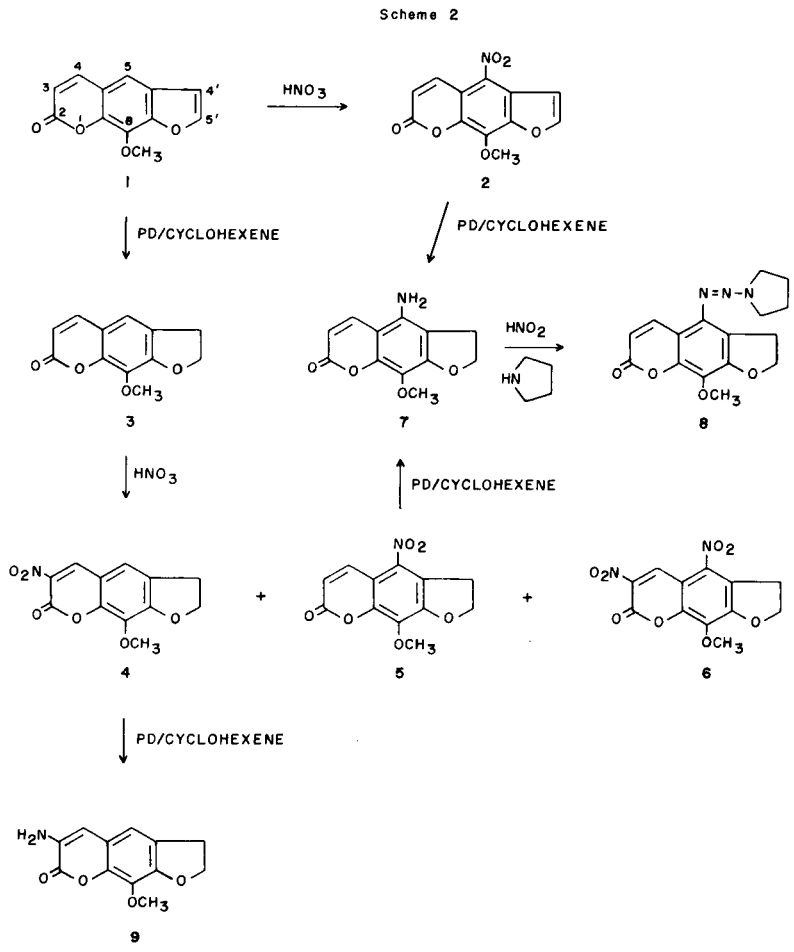
Table I

Nitration Results on 4',5'-Dihydro-8-methoxypsoralen (**3**)

Run	Temperature °C	% **4**	% **5**	% **6**
1 [a]	25	66	18	c
2	40	75	25	c
3	70	64	24	12
4	100	22	18	60
5 [b]	117	c	10	90

[a] Sixteen percent of the recovered solid was unreacted **3**. [b] In runs 1-4 the weights of dried recovered solids were 0.235 ± 0.005 g except for run # 5 in which 0.0579 g was recovered. [c] Peak detected on hplc but at concentrations too small to quantify. Undetectable by ^{1}H-nmr.

Scheme 2



employed to calculate the stated percentages. For hplc detection the elution times were 3.35 minutes for **6**, 5.71 minutes for **4**, and 6.90 minutes for **5**, on a Waters C-18 radial compression module with 60:40 methanol:water at 2 ml/minute as the moving phase.

In the nitration runs the recovery of the dried mixed nitration products represented 70-80% of the product mixture. The chilled aqueous phase, after precipitation of these products was deep yellow in color and contained by tlc 4-6 other components (possibly oxidized byproducts) but only barely detected traces of the nitro compounds. All of these had negligible solubility in water. In run # 5 at the highest reaction temperature, 117°, these water-soluble components predominated and a 19% combined yield of **5** plus **6** was observed. For chemical isolation of the 3,5-dinitropsoralen, **6**, the nitration at 100° proved to be the most convenient synthesis. Even though only 60% of the dried solid was the desired compound by nmr assay, there was considerably less water-soluble byproduct formed and the chemical recovery of isolated **6** was 56%.

The nitration results are in accord with both bromination results and C-13 chemical shift data. Kaufman and Worden reported that bromination of **3** yields a 4',5'-dihydro-3-bromo-8-methoxypsoralen with further bromination yielding a 4',5'-dihydro-3,5-dibromo-8-methoxypsoralen [17].

The C-13 nmr shifts of two different 4',5'-dihydropsoralens, specifically 4',5'-dihydro-5-methoxypsoralen and 4',5'-dihydro-5-(1-hydroxy-1-methylethyl)psoralen, have been reported [18]. In both of these compounds the C-3 carbon appears at higher field than the C-5 carbon. In fully aromatic psoralens, in which electrophilic substitution occurs preferentially at C-5, these assignments are reversed [18]. We have confirmed these published assignments and have shown that in **3** the C-3 carbon appears at 112 ppm and the C-5 carbon at 117 ppm. Reduction of the furan-ring unsaturation clearly favors electrophilic substitution alpha to the coumarinic carbonyl.

Both the 3-nitro, **4**, and the 5-nitro, **5**, reduced smoothly by the palladium/cyclohexene method [13] to **9** and **7** in 77 % and 80% yields respectively. The 5-amino could also be obtained by this exchange hydrogenation of 5-nitro-8-methoxypsoralen in 31% yield. The 5-amino gave the desired triazene, **8**, in 67% conversion through intercep-

tion of the transient diazonium ion with pyrrolidine [19]. Several attempts to trap a diazonium intermediate from **9** were unsuccessful and > 6 products were observed on hplc. Other workers have reported on the hydrolytic lability of coumarins nitrated adjacent to the carbonyl in which the ring opens to yield *o*-hydroxyaldehydes [15,20]. The electron-withdrawing effects of a transient diazonium ion might be expected to show similar behavior. The multiple product mixture from this diazotization was not further investigated.

EXPERIMENTAL

Melting points were determined in capillaries in a Thomas-Hoover apparatus and are reported uncorrected. Infrared spectra were taken on a Perkin Elmer Model 283 spectrometer and ^1H and ^{13}C nmr spectra taken on a JEOL-FX90Q spectrometer in the indicated solvents. Combustion analyses were performed by the Robertson Microanalytical Laboratory, Florham Park, NJ. The hplc separations were performed on a Perkin-Elmer Series 2 Liquid Chromatograph with an LC-75 variable UV-VIS spectrophotometric detector. Methoxsalen **1** was pharmaceutical-grade material supplied by the Elder Pharmaceutical Co., Bryan, Ohio.

4',5'-Dihydro-8-methoxypsoralen (**3**).

This dihydro compound was prepared in 73% yield by the published method [13]. It was recrystallized from 95% ethanol to analytical purity (hplc), mp 163-164°, lit mp 164-165°.

Standard Nitration Conditions.

A suspension/solution of 0.218 g (1.00 mmole) of **3** in 4.5 ml of glacial acetic acid was heated in an oil bath to the indicated temperature 25, 40, 70, 100 and 117° (Table I). A solution of 1.0 ml of concentrated nitric acid in 2.5 ml of glacial acetic acid was added dropwise over *ca.* 10-15 minutes and the reaction mixture maintained at the selected temperature for 1.5 hours. The mixture was cooled to room temperature, diluted with 250 ml of water, and the precipitated solids filtered from the supernatant. These solids were washed with water until the washings were neutral, dried *in vacuo*, and weighed. All weights were 0.235 ± 0.005 g except for the highest temperature run at 117° for which only 0.057 g was recovered. The ^1H-nmr analysis, with quantitative integration of the non-overlapping methoxy singlets, was employed as an analytical method (See Table I). Authentic nitrodihydropsoralens were isolated and characterized as indicated.

4',5'-Dihydro-3-nitro-8-methoxypsoralen (**4**).

A solution of 0.75 g (3.4 mmoles) of **3** in 15 ml of glacial acetic acid maintained at room temperature was treated to the dropwise addition of 3.0 ml of concentrated nitric acid in 7.0 ml of acetic acid. External cooling (ice/water bath) was employed to maintain the temperature at 25° during the addition. The reaction mixture was diluted with 250 ml of water and filtered. The crude solids were recrystallized from methanol (2x) to give 0.41 g, 46% yield of **4** as yellow needles, mp 233-234°; ^1H-nmr (deuteriochloroform): δ 3.36 (dt, 2H, C-5' CH_2, $J_{4',5'}$ = 8 Hz, $J_{5',5}$ = 1.3), 4.07 (s, 3H, CH_3O), 4.85 (t, 2H, C-4' CH_2, $J_{4',5'}$ = 8 Hz), 7.21 (t, 1H, C-5 H, $J_{5',5}$ = 1.3 Hz), and 8.72 (s, 1H, C-4 H).

Anal. Calcd. for $C_{12}H_9NO_6$: C, 54.76; H, 3.45; N, 5.32. Found: C, 54.69; H, 3.56; N, 5.33.

4',5'-Dihydro-5-nitro-8-methoxypsoralen (**5**).

This nitro isomer was isolated by preparative hplc from the recrystallization mother liquors, after removal of the 3-nitro isomer, in the nitration described above. Employing a C-18 Whatman Magnum 20 column with 1:1 water:methanol as the moving phase at a flow rate of 18 ml/minute the desired 5-nitro isomer elutes in 9.90 minutes. Evaporation of the water/methanol and recrystallization from anhydrous methanol gave **5** as yellow microneedles, in *ca* 10% recovery, mp 192-194°; ^1H-nmr (deuteriochloroform): δ 3.65 (t, 2H, C-5' CH_2, $J_{4',5'}$ = 8 Hz), 4.17 (s, 3H, CH_3O), 4.81 (t, 2H, C-4' CH_2, $J_{4',5'}$ = 8 Hz), 6.44 (d, 1H, C-3 H, $J_{3,4}$ = 10 Hz), and 8.35 (d, 1H, C-4 H, $J_{3,4}$ = 10 Hz).

Anal. Calcd. for $C_{12}H_9NO_6$: C, 54.76; H, 3.45; N, 5.32. Found: C, 54.48; H, 3.40; N, 5.39.

4',5'-Dihydro-3,5-dinitro-8-methoxypsoralen (**6**).

The dinitro compound was prepared by dropwise addition over 10 minutes of 3.0 ml of concentrated nitric acid in 7 ml glacial acetic acid to 0.750 g (3.35 mmoles) of **3** in 10 ml of acetic acid maintained at 100° in an oil bath. The solution was heated at 100° for 1.5 hours, diluted with 250 ml of cold water, and filtered. The crude yellow solid was recrystallized from methanol (2x) to give 0.58 g, of yellow felted needles of **6**, mp 172.5-174.5°. Subsequent crystal crops could be obtained from the mother liquors but nmr and hplc invariably showed them to be mixtures of 3-nitro, 5-nitro and 3,5-dinitro compounds; ^1H-nmr (deuteriochloroform): δ 3.76 (t, 2H, C-5' CH_2, $J_{4',5'}$ = 8 Hz), 4.20 (s, 3, CH_3O), 4.91 (t, 2H, C-4' CH_2, $J_{4',5'}$ = 8 Hz), and 9.46 (s, 1H, C-4 H).

Anal. Calcd. for $C_{12}H_8N_2O_8$: C, 46.76; H, 2.62; N, 9.09. Found: C, 47.05; H, 2.80; N, 8.85.

4',5'-Dihydro-5-amino-8-methoxypsoralen (**7**).

This compound could be prepared by the cyclohexene/palladium reduction of **5** in 80% yield (hplc) or by a similar reduction of **2** in 31% isolated yield. Since analytically-pure **5** is only available in low yield by a preparative hplc separation, the direct reduction of **2** is preferable. A suspension of 1.00 g (3.83 mmoles) of **2** [14] 100 ml of 95% ethanol, 2.5 ml cyclohexene, and 2.5 g 5% palladium on carbon was refluxed with vigorous stirring for 20 minutes and filtered hot to remove the catalyst. The catalyst was washed twice with 10 ml portions of hot ethanol and the washings and filtrates combined. Evaporation to dryness *in vacuo* gave a pale yellow solid which was subjected to successive fractional recrystallizations from ethanol to give 0.40 g, 44%, of 5-amino-8-methoxypsoralen (the less soluble component) and 0.29 g, 31%, of **7** (the more soluble component). The 5-amino-8-methoxypsoralen, mp 242-243°, was identical (mp, mixed mp, ir, nmr) with an independently prepared sample [13,14]. Because of the similar melting points the 4',5'-dihydro-5-amino compound **7**, mp 241-243°, was established as a different substance by non-identity with 5-amino-8-methoxypsoralen (depressed mixed mp, different nmr and ir spectra) and by direct analytical support for its structure: ^1H-nmr (DMSO-d_6): δ 3.04 (t, 2H, C-5' CH_2, $J_{4',5'}$ = 9.0 Hz), 3.76 (s, 3H, CH_3O), 4.71 (t, 2H, C-4' CH_2, $J_{4',5'}$ = 9.0 Hz), 5.82 (br s, 2H, NH_2, exchangeable in deuterium oxide), 6.02 (d, 1H, C-3 H, $J_{3,4}$ = 10 Hz) and 9.24 (d, 1H, C-4 H, $J_{3,4}$ = 10 Hz).

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.76; N, 6.06. Found: C, 61.54; H, 4.81; N, 5.74.

4',5'-Dihydro-3-amino-8-methoxypsoralen (**9**).

By reduction of **4** the title compound was prepared in 77% yield, mp 154-155° (from methanol) by using the cyclohexene/palladium method described above: ^1H-nmr (deuteriochloroform): δ 3.17 (t, 2H, C-5' CH_2, $J_{4',5'}$ = 8.5 Hz), 3.30 (br s, 2H, NH_2, exchangeable in deuterium oxide), 3.97 (s, 3H, CH_3O), 4.58 (t, 2H, C-4' CH_2, $J_{4',5'}$ = 8.5 Hz), 6.56 (s, 1H, CH), and 6.70 (s, 1H, CH).

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.76; N, 6.01. Found: C, 61.82; H, 4.69; N, 5.78.

Pyrrolidine Triazene of 4',5'-Dihydro-5-amino-8-methoxypsoralen (**8**).

A suspension of 0.233 g (1.00 mmole) of **7**, 0.076 (1.1 mmoles) of sodium nitrite, and 10 ml of ice water was stirred vigorously while 1.0 ml of trifluoroacetic acid was added dropwise. The addition required about 5 minutes and during this time the temperature was held at 0 to 5° by external cooling. The cooling bath was then removed and the contents of the reaction flask stirred for 5 minutes, added slowly to an ice-cold solution of 0.10 g (1.41 mmoles) of pyrrolidine in 15 ml of 1 *M* potassium hydroxide. The triazene began to precipitate at once but the stirring was

continued at ice-bath temperatures for 10 additional minutes before filtration. A small second crop was obtained by adjusting the *p*H of the filtrate to neutrality. The combined solids were dried *in vacuo* and recrystallized from 95% ethanol, 0.210 g, 67% mp 194-195°; ^{1}H-nmr (deuteriochloroform): δ 2.00 (m, 4H, CH_2CH_2), 2.83 (t, 2H, C-5′ CH_2, $J_{4',5'}$ = 8.0 Hz), 3.70 (m, 2 x 2H, pyrrolidyl N-CH_2), 3.80 (s, 3H, CH_3O), 4.61 (t, 2H, C-4′ CH_2, $J_{4',5'}$ = 8.0 Hz), 6.12 (d, 1H, C-3 H, $J_{3,4}$ = 9.0 Hz), and 8.36 (d, 1H, C-4 H, $J_{3,4}$ = 9.0 Hz).

Anal. Calcd. for $C_{16}H_{17}N_3O_4$: C, 60.94; H, 5.44; N, 13.33. Found: C, 60.87; H, 5.58; N, 13.10.

Pyrrolidyl Triazene of 4′,5′-Dihydro-3-amino-8-methoxypsoralen.

Several attempts to prepare this substance by the procedure described above for **8** resulted in the recovery of approximately 5% of the amine **9**, which precipitated when the presumed diazonium intermediate was added to the basic pyrrolidine. A methylene chloride extract of the neutralized supernatant was analyzed on a 20 cm C-18 hplc reverse phase column with water-methanol as the moving phase. It possessed 6 major components in addition to pyrrolidine. The starting material **9** was barely detectable. Similar results were obtained when the reaction was repeated with a 10-fold excess of pyrrolidine and no aqueous base present.

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