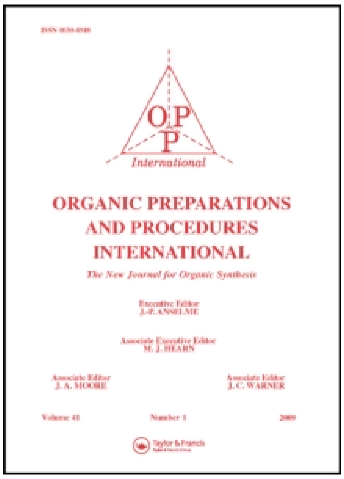

Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

"ONE POT" FISCHER SYNTHESIS OF (2, 3, 3-TRIMETHYL-3-H-INDOL-5-YL)-ACETIC ACID. DERIVATIVES AS INTERMEDIATES FOR FLUORESCENT BIOLABELS

Philip L. Southwick[a]; Jennifer G. Cairns[a]; Lauren A. Ernst[a]; Alan S. Waggoner[a]
[a] Center for Fluorescence Research, Carnegie Mellon University, Pittsburgh, PA

To cite this Article Southwick, Philip L. , Cairns, Jennifer G. , Ernst, Lauren A. and Waggoner, Alan S.(1988) '"ONE POT" FISCHER SYNTHESIS OF (2, 3, 3-TRIMETHYL-3-H-INDOL-5-YL)-ACETIC ACID. DERIVATIVES AS INTERMEDIATES FOR FLUORESCENT BIOLABELS', Organic Preparations and Procedures International, 20: 3, 279 — 284

To link to this Article: DOI: 10.1080/00304948809355822

URL: http://dx.doi.org/10.1080/00304948809355822

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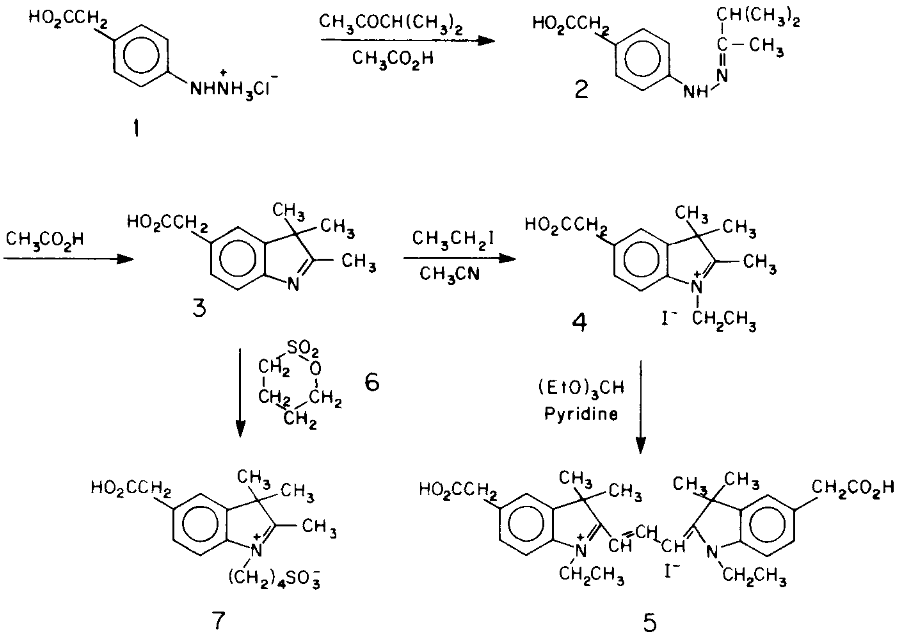
Submitted by (03/10/87) **Philip L. Southwick*, Jennifer G. Cairns, Lauren A. Ernst, and Alan S. Waggoner**

Center for Fluorescence Research
Carnegie Mellon University
4400 Fifth Avenue, Pittsburgh, PA 15213

(2,3,3-Trimethyl-3-H-indol-5-yl)-acetic acid (3) is expected to possess exceptional advantages as the precursor of a family of dyes of cyanine and related types intended for use as covalently attached fluorescent labels for biological research. In contrast, cyanines utilized previously[1] in such research have not carried a functional group to provide for covalent attachment to proteins. We now describe a convenient synthesis of 3 and its conversion to derivatives used to prepare carboxy cyanines.

Because the hydrazone 2 deteriorated rapidly during storage, attention was directed to "one pot" versions of the Fischer synthesis. However, the procedures listed by Robinson[2] proceeded slowly and gave poor yields of 3. It was found that, in acetic acid, hydrazone formation is nearly complete within 30 minutes in the presence of acetate ion. Indolization to 3 (UV max 260 nm) is essentially complete after 30 minutes at reflux. The generation of small amounts of a purple-red by-product



often accompanied indolization, generally to a greater extent when sodium acetate had been added than when it had not. Yields of recrystallized product of satisfactory purity were 60% based on the arylhydrazine hydrochloride 1.

Compound 3 is a fluorescent substance; its solutions produce spots with a blue-white fluorescence on thin-layer plates when irradiated at 360 nm. Reaction with ethyl iodide yielded the ethiodide 4, which was converted to the fluorescent carbocyanine 5 by treatment with triethyl orthoformate in refluxing pyridine.[3] Reaction of 3 with 1,4-butanesultone (6) afforded 7, a type of quaternary salt useful as an intermediate for water-soluble cyanine dyes.[4]

EXPERIMENTAL SECTION

Microanalyses are by Atlantic Microlab, Inc., Atlanta, GA. Spectra were determined with the following instruments: ultraviolet and visible spectra--Hewlett Packard 8451 H diode array spectrophotometer; infrared spectra--Perkin Elmer 580 spectrophotometer; NMR spectra--Perkin Elmer R 24 and IBM NR/80 spectrometers; fluorescence spectra (data are uncorrected)--Hitachi-Perkin Elmer MPF-3 fluorescence spectrophotometer.

With NMR spectra abbreviations used are the following: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

<u>(2,3,3-Trimethyl-3-H-indol-5-yl)-acetic Acid (3)</u>. <u>Procedure A</u> (Acetic acid and potassium acetate).- The hydrochloride of p-carboxymethylphenylhydrazine (1)[5] (6.06 g, 30 mmol) and potassium acetate (6.0 g, 60 mmol) were suspended in a mixture of methyl isopropyl ketone (3.7 g, 42 mmol) and glacial acetic acid (30 ml). The mixture was stirred at room temperature for 30 min. A spectrum taken on a sample withdrawn from the mixture at this time showed the strong absorption at 275 nm of the phenylhydrazone (2) and the 232 nm maximum of the hydrazine (1) was absent. The mixture was then stirred and heated at reflux for 1 hr. By that time the maximum at 275 nm had disappeared and been replaced by the 260 nm maximum of 3. Removal of the solvent under reduced pressure on a rotary evaporator left a residue, which solidified when triturated with water (20 ml). It was collected and recrystallized from ethyl acetate to give 3.89 g (60%) of crystals with a yellow-brown tint, mp. 155-165°. An additional crystallization from ethyl acetate gave flat needles with a light tan tint, mp. 168-170°.

^{1}H-NMR ($CDCl_3$): δ 1.25 (s, 6H, 3-methyls), 2.25 (s, 3H, 2-methyl), 3.60 (s, 2H, methylene of 5-carboxymethyl), 7.15-7.56 ppm (m, 3H, benzene ring).

<u>Anal</u>. Calcd. for $C_{13}H_{15}NO_2$: C, 71.86 H, 6.96 N, 6.45

Found: C, 71.69 H, 6.97 N, 6.41

<u>Procedure B</u> (Acetic acid).- A mixture containing 1 (6.06 g, 30 mmol), methyl isopropyl ketone (3.7 g, 42 mmol) and acetic acid (30 ml) was stirred for 30 min. at room temperature; at this point the UV spectrum of the solution showed hydrazone formation to be incomplete. The mixture was then stirred and heated at reflux for 30 additional min., at which time the spectrum coincided with that of 3 (maximum at 260 nm). The product, mp. 163-170°, was isolated as described above in 61% yield (3.95 g).

(2,3,3-Trimethyl-3-H-indol-5-yl)-acetic Acid Ethiodide (4).- Compound 3 (0.5 g, 2.3 mmol) was suspended in a mixture of acetonitrile (5 ml) and ethyl iodide (2 g, 12.8 mmol) and the mixture was refluxed with stirring for 1 hr. More ethyl iodide (2 g) was then added, and heating and stirring were continued for an additional 5 hrs. The mixture was then cooled and diluted with ether (20 ml). The precipitated deliquescent product was collected by gravity (not suction) filtration and washed on the filter with isopropyl alcohol (10 ml), yield 0.42 g (49%) of 4, mp. 210-217° (dec.). Recrystallization, effected by diluting a concentrated methanol solution with isopropyl alcohol to cloudiness, afforded colorless flakes; mp. 217° (dec.) with shrinking from 205°.

^{1}H-NMR ($CDCl_3$-CF_3CO_2H): δ 1.60 (s+t, 9H, 3-methyls (s) coinciding with methyl (t) of 1-ethyl), 2.85 (s, 3H, 2-methyl), 3.90 (s, 2H, methylene of 5-carboxymethyl), 4.54 (q, 2H, methylene of 1-ethyl), 7.60 ppm (center of multiplet) (m, 3H, benzene ring).

Anal. Calcd. for $C_{15}H_{20}INO_2$: C, 48.27 H, 5.40 N, 3.75

Found: C, 48.38 H, 5.41 N, 3.71

5,5'-Dicarboxymethyl-3,3,3',3'-tetramethyl-1'-diethylindocarbocyanine Iodide (5).- A solution of ethiodide 4 (0.373 g, 1.0 mmol) and triethyl orthoformate (0.44 g, 3.0 mmol) in pyridine (5 ml), was heated at reflux for 1 hr. The mixture turned a deep purple-red color within the first few minutes of heating. The pyridine was removed under reduced pressure using a rotary evaporator. The solid residue was dissolved in warm methanol (4 ml) and 15 ml of 0.067 N hydriodic acid (1.0 mmol) was added with stirring to precipitate 0.265 g (84%) of the product as a dark-purple powder. It was recrystallized by adding 0.1 N aqueous hydriodic acid to a solution of the dye in methanol until the product separated in the form of a granular blue-black crystalline precipitate, mp. 250-253° (dec.).

IR (Nujol): 3400 (broad), 2900 (broad), 1725, 1613, 1555, 1450, 1420, 1370, 1335, 1270, 1245, 1190, 1160, 1140, 1112, 1080, 1070, 1030, 925, 780 cm^{-1}. Visible spectrum (ethanol): λ_{max}(log ε) 530 (4.97), 564 nm (5.13). Fluorescence spectra (ethanol): emission maximum, 580 nm; excitation maxima, 532 (shoulder), 565 nm. When spotted on TLC plates, 5 shows a brilliant red fluorescence under irradiation at 360 nm.[6]

Anal.[7] Calcd. for $C_{31}H_{37}IN_2O_4$: C, 59.24 H, 5.93 N, 4.45 I, 20.19

Found: C, 60.35 H, 6.20 N, 4.52 I, 18.51

5-Carboxymethyl-2,3,3-trimethyl-3-H-indolium-1-(4'-sulfobutyl)-betaine (7).- Compound 3 (1.0 g, 4.61 mmol) was dissolved in hot n-butyronitrile (10 ml) and 1,4-butanesultone 6 (1.0 g, 7.35 mmol) was added. The mixture was stirred and maintained under reflux for 22 hrs (bath at 123°). During this time, the product separated as a crystalline powder. The mixture was cooled, diluted with ether (35 ml), and the product was collected as a light-brown granular powder, mp. 285-288°; yield: 1.0 g (61%). Dilution of a hot methanol solution of 7 with isopropyl alcohol afforded granular crystals with a light purple tint, mp. 287-290°.

^1H-NMR ($CDCl_3$-CF_3CO_2H): δ 1.61 (s, 6H, 3-methyls), 2.00-2.40 (m, 4H, 2'- and 3'-methylenes of butyl), 2.81 (s, 3H, 2-methyl), 3.20-3.45 (m, 2H, 4'-methylene of butyl), 3.90 (s, 2H, methylene of carboxymethyl), 4.30-4.60 (m, 2H, 1'-methylene of butyl), 7.45-7.75 ppm (m, 3H, benzene ring).

Anal. Calcd. for $C_{17}H_{23}NO_5S$: C, 57.77 H, 6.56 N, 3.96

Found: C, 57.59 H, 6.61 N, 3.95

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6. Reversed-phase silica-gel plates (Analtech RPS-F) were used with carboxyl-containing cyanines, with development by methanol-water or acetone-water mixtures.

7. Hamer (ref. 4) had reported that samples of a dye of the same indocarbocyanine series as 5 also showed a low iodine content. Apparently recrystallizations of these cyanine dyes in the presence of water may yield products in which iodide ion is partly replaced by another anion. In the case of 5, loss of iodide ion together with a proton from the carboxyl would produce a betaine. The iodine content of this analyzed sample would correspond to that of a mixture consisting of 5 (91.7%) and 8.3% of an iodine-free form of the dye. If 5 and the betaine were present in that proportion, the calculated values would be C, 60.48; H, 6.04; N, 4.55; I, 18.51, in quite good agreement with the values found.