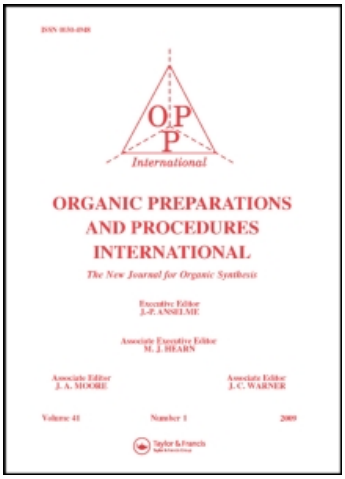

Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

A CONVENIENT SYNTHESIS OF cis-UROCANIC ACID, AN ENDOGENOUS IMMUNOSUPPRESSANT

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To cite this Article Mohammad, Taj and Morrison, Harry(2000) 'A CONVENIENT SYNTHESIS OF cis-UROCANIC ACID, AN ENDOGENOUS IMMUNOSUPPRESSANT', Organic Preparations and Procedures International, 32: 6, 581 — 584
To link to this Article: DOI: 10.1080/00304940009355956
URL: http://dx.doi.org/10.1080/00304940009355956

A CONVENIENT SYNTHESIS OF *cis*-UROCANIC ACID, AN ENDOGENOUS IMMUNOSUPPRESSANT

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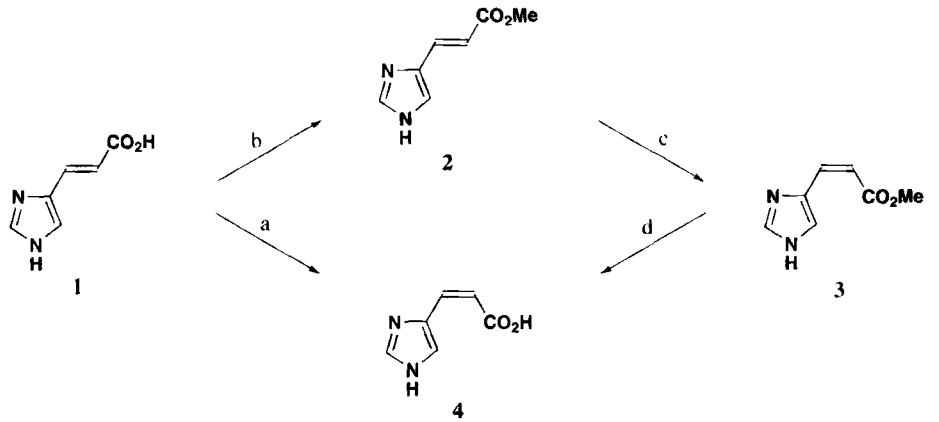
There is continuing interest in the photochemistry and photobiology[1,2] of urocanic acid (2-propenoic acid, 3-[1H-imidazol-4(5)-yl]), primarily due to its presence in the skin and its role as an immunomodulator.[3] The naturally occurring isomer, *trans*-urocanic acid (**1**), is the metabolic product of histidine, and accumulates in the epidermis (*ca.* 30 mg/cm^2). Sunlight converts the *trans* isomer **1** to the *cis* compound **4**, and the latter causes immunosuppression in several animal models.[2,3] For our collaborative studies on the mechanism of *cis*-urocanic acid-induced immunosuppression,[4] we needed sufficient quantities of this compound. Previously, the *cis* isomer **4** has been synthesized in one step by photoisomerization of the *trans* isomer **1**[5,6] (step a in *Scheme*). Owing to the long reaction times, this procedure involving cumbersome workup and purification protocol, is not convenient for the preparation of large quantities of the *cis* isomer. The restricted reaction scale is partly attributed to the poor solubility of *trans*-urocanic acid in a majority of solvents. We report herein a simple three-step method for the synthesis of **4**, which can be adapted for any convenient preparative scale.

Accordingly, the *trans* acid **1** was esterified with anhydrous methanol in the presence of sulfuric acid to obtain the *trans* ester **2** in 95% yield. Photoisomerization of **2** with a low pressure mercury lamp (λ_{irr} = 254 nm) in dichloromethane[7] provided the *cis* ester **3** in 74% yield. The ester **3** has been reported as an oil;[7,8] however, in our hands it was obtained as a low-melting solid (mp 51-53°). The spectral data for the esters **2** and **3** were consistent with the literature values (see Experimental Section). The *cis* ester **3**, upon hydrolysis with aqueous sodium hydroxide followed by chromatographic purification, yielded *cis*-urocanic acid (**4**) in 94% yield.



a) H_2O, hυ b) MeOH, H_2SO_4 c) CH_2Cl_2, hυ d) H_2O, NaOH

EXPERIMENTAL SECTION

The melting points (mp) are uncorrected and were determined on a Fisher-Johns apparatus. Thin layer chromatography (TLC) analysis was performed on 0.2 mm thick plates pre-coated with fluorescent silica (60 F_{254}), and the spots were visualized under short wave ultraviolet (UV) light and/or iodine vapors. The spectral data were acquired on the following instruments: Perkin-Elmer 1800 FT spectrophotometer for infrared (FT-IR); Perkin-Elmer Lambda 3B UV/Vis spectrophotometer for UV spectra; Varian Gemini-200 and/or Inova-300 spectrometer for nuclear magnetic resonance (NMR) spectra, which were referenced relative to either residual $CHCl_3$ at δ 7.26 ppm or internal standard DSS at δ 0.00 ppm; Finnegan 4000 spectrometer for electron impact (EIMS) and chemical ionization (CIMS) mass spectra of probe samples at 70 eV (relative intensities of fragment ions are noted in parentheses).

***trans*-Methyl Urocanate (2).**- A stirred suspension of *trans*-urocanic acid (**1**, 6.9 g, 0.05 mol) in 10% (v/v) sulfuric acid in anhydrous methanol (75 mL) was heated at reflux for 21 h. The resultant clear solution was allowed to cool to ambient temperature and diluted with 125 mL of methanol. The solution was made basic, with stirring, using solid anhydrous sodium carbonate. The basic solution was filtered on a Celite pad, and the filtrate was evaporated *in vacuo*. The residual solid was extracted with hot ethyl acetate (5 x 50 mL). Removal of solvent followed by drying *in vacuo* yielded 7.2 g (95%) of ester **2** as white solid which was recrystallized from ethyl acetate, mp 100-101°, *lit.*[8-10] mp 94-96°, 92-94°, 100-101°. TLC in $CHCl_3$:95% EtOH (4:1) showed only one spot of higher mobility than the starting acid **1**. The purity was further confirmed by high-performance liquid chromatography (HPLC) analysis on a C-8 reversed-phase column (250 x 4.6 mm, 10 µm) using 30% methanol in 50 mM phosphate buffer (pH 6.9) at 1.0 mL/min and detection at 280 nm. The ester **2** eluted at 11.3 min in > 99% purity. UV: λ_{max}, nm (ε, $M^{-1}cm^{-1}$), 284 (19, 900) in CH_2Cl_2, 287 (20, 600) in 0.1 M phosphate buffer (pH 6.8); FT-IR (KBr): 3135, 1719, 1649, 1431, 1327, 1299, 1261, 1165 cm^{-1}; 200 MHz ^1H NMR ($CDCl_3$): δ 3.79 (s, 3 H, OCH_3), 6.48 (d, J = 15.8 Hz, 1 H, C_α-H), 7.30 (s, 1 H, $C_{4(5)}$-H), 7.63 (d, J = 15.8 Hz, 1 H, C_β-H), 7.72 (s, 1 H, C_2-H); EIMS: *m/z* (rel. int.) 152 (50, M^+), 121 (100), 120 (29), 93 (73), 92 (1), 67 (2), 66 (24), 59 (1), 65 (6); CIMS: *m/z* (rel. int.) 153 (100, $M+H^+$).

***cis*-Methyl Urocanate (3).**- A stirred clear colorless solution of the *trans* ester **2** (1.1 g, 7.2 mmol) in dichloromethane (350 mL) was irradiated with a low pressure mercury lamp, under continuous argon bubbling, for 2-4 h.[7] The reaction was repeated three more times and the NMR of the combined dark brown viscous oil obtained upon evaporation of solvent indicated the formation of ca. 85% of the *cis* ester **3**. The liquid was distilled *in vacuo*, bp. 106°/0.9 mm Hg, *lit.*[10] bp 85° *in vacuo* (at an unspecified pressure) to give a nearly colorless, viscous oil (3.15 g, 74%). Upon standing, the viscous oil slowly solidified to a white solid, mp 51-53°. TLC analysis in $CHCl_3$:95% EtOH (4:1) showed only one spot of higher mobility than the starting *trans* ester **2**. The HPLC analysis under the above conditions indicated 98% purity for **3** which eluted at 20.5 min. UV: λ_{max}, nm (ε, $M^{-1}cm^{-1}$), 308 (21, 000) in CH_2Cl_2, 295 (14, 000) in 0.1 M phosphate buffer (pH 6.8); FT-IR ($CHCl_3$): 3267, 1697, 1621, 1352, 1216, 1107, 758, 669 cm^{-1}; 200 MHz ^1H NMR ($CDCl_3$): δ 3.80 (s, 3 H, OCH_3), 5.69 (d, J = 12.5 Hz, 1 H, C

$_{\alpha}$-H), 6.86 (d, J = 12.5 Hz, 1 H, C_{β}-H), 7.38 (s, 1 H, $C_{4(5)}$-H), 7.75 (s, 1 H, C_2-H), 12.81 (br s, 1 H, NH); 75 MHz ^{13}C NMR: δ 51.97, 111.36, 127.77, 131.37, 136.29, 137.44, 169.04; EIMS *m/z* (rel. int.): 152 (100, $M^{+\cdot}$), 121 (77), 120 (91), 93 (48), 92 (26), 67 (3), 66 (26), 65 (14), 59 (2); CIMS *m/z* (rel. int.): 153 (100, $M+H^+$).

***cis*-Urocanic Acid (4)**.- A suspension of the *cis* ester **3** (1.1 g, 7.2 mmol) in 1 N sodium hydroxide (8.4 mL) was stirred for 3 h in a flask covered with aluminum foil. The suspension slowly dissolved resulting in a clear solution. The cooled solution was acidified to pH ~ 4 by dropwise addition of 5 N hydrochloric acid. The solvent was stripped off on the rotary evaporator, and the residual solid was desalinated on a 1.5 cm x 20 cm column of XAD-7 adsorbent (20-60 mesh) (Aldrich Chemical Co.). The column was eluted with 0-15% $CHCl_3$ in MeOH, and the collected fractions (25 mL each) were analyzed by UV at 276 nm. The pooled fractions (# 4-13) containing *cis* acid **4** were evaporated to get a white solid (0.91 g, 94%), mp 176-178°, *lit.*[5,6] mp 175-176°, 178-180°. TLC in n-BuOH:EtOH:H_2O (4:1:5) (top alcohol layer was used after equilibration) showed only one spot of R_f = 0.4. This purity was substantiated from HPLC analysis on the above column using 100% 50 mM phosphate buffer (pH 7.0) with detection at 254 nm. Compound **4** eluted at 7.9 min as a single peak (> 99%). UV (H_2O): λ_{max}, nm (ε, $M^{-1}cm^{-1}$) 261 (13,000); FT-IR (KBr): 3124, 1646, 1570, 1473, 1406, 1325, 1087, 838 cm^{-1}; 200 MHz ^{1}H NMR (D_2O): DSS internal standard at δ 0.00 ppm, 6.11 (d, J = 12.8 Hz, 1 H, C_{α}-H), 6.81 (d, J = 12.8 Hz, 1 H, C_{β}-H), 7.69 (s, 1 H, $C_{4(5)}$-H), 8.67 (s, 1 H, C_2-H); 75 MHz ^{13}C NMR: δ 121.31, 124.19, 124.51, 129.06, 133.49, 172.56; EIMS: *m/z* (rel. int.): 138 (100, $M^{+\cdot}$), 120 (72), 93 (16), 92 (39), 68 (1), 65 (15); CIMS: *m/z* (rel. int.): 139 (100, $M + H^+$).

Acknowledgement.- Partial support from the Gerontology Program at Purdue is gratefully acknowledged.

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[2+2] CYCLOADDITIONS OF BENZOFURAN-3(2H)-ONE ENAMINES WITH DIMETHYL ACETYLENEDICARBOXYLATE

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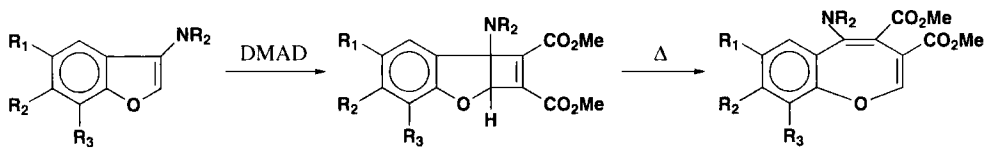
[2+2]Cycloaddition reactions have been used for the ring expansion by two-carbon atoms.[1] The reactions of enamines derived from cyclic ketones with activated acetylenes via cycloadditions involve the formation of fused cyclobutene adducts which may then undergo thermal isomerization to yield two carbons-enlarged rearrangement products.[2] Different cyclic products can be obtained by modifying the structure of the cyclic enamine and of the acetylene. We were interested in the synthesis of enamines derived from substituted benzofuran-3(2H)-ones[3] which might react similarly with electron-deficient acetylenes to yield [2+2]cycloadducts and then isomerize to substituted 1-benzoxepins. Even though, seven-membered heterocycles with 8π electrons have been synthesized, 1-benzoxepins belong to a class of little known heterocycles which have proved difficult to prepare. Herein we describe the [2+2]cycloaddition reaction of enamines of substituted benzofuran-3(2H)-one with dimethyl acetylenedicarboxylate (DMAD) in benzene and methanol.



1 2 3

a) NR_2 = pyrrolidine, R_1 = H, R_2 = OCH_3, R_3 = H
b) NR_2 = pyrrolidine, R_1 = CH_3, R_2 = H, R_3 = H
c) NR_2 = pyrrolidine, R_1 = Cl, R_2 = H, R_3 = Cl
d) NR_2 = morpholine, R_1 = Cl, R_2 = H, R_3 = Cl