A GENERAL APPROACH TO THE SYNTHESIS OF ^{14}C-LABELED PHOTOACTIVE ACRYLIC ACIDS

Taj Mohammad[*] and Harry Morrison

Department of Chemistry, Purdue University
West Lafayette, IN 47907

SUMMARY

Condensation of aromatic carboxaldehydes with [2-^{14}C]-malonic acid under Knoevenagel conditions provides corresponding β-aryl substituted [α-^{14}C]-acrylic acids. The reaction is applicable to both aromatic and heteroaromatic aldehydes under controlled reaction conditions leading to moderate to good yields.

Key words: Acrylic acids, Carbon-14, Synthesis, Knoevenagel condensation, DNA

INTRODUCTION

A variety of aromatic and heteroaromatic acrylic acid derivatives are naturally occurring compounds. The 4(5)-imidazole derivative, 3-(1H-imidazol-4-yl)-2-propenoic acid i.e. (E)-urocanic acid (2a) is of particular interest because of its presence in the epidermis as a result of the deamination of histidine and its photoreactivity with DNA (1). The photoisomer i.e. (Z)-urocanic acid, is also of considerable current interest because of its role as an immunosuppressant (2). 3-(1H-Indol-3-yl)-2-propenoic acid i.e. (E)-indoleacrylic acid (2b) is a metabolic product of tryptophan found in animals and, more extensively, in plants which is also photoreactive with DNA (3). The 2-ethylhexyl ester of the naturally occurring p-methoxyphenyl analog, 3-(4-methoxyphenyl)-2-propenoic acid i.e. (E)-p-methoxycinnamic acid (2c) is a common ingredient of commercial sunscreens used in photoprotective cosmetics. The potential phototoxicity of this ester has been debated (4), but it too is photoreactive with DNA (5). Coumarin is an invariable chromophore of the furocoumarins (commonly known as "psoralens") used in the PUVA (i.e. psoralen + ultraviolet A radiation) phototherapy of psoriasis. The probable mechanism of PUVA therapy involves the formation of interstrand crosslinks in double stranded DNA (6). We were interested in combining the DNA photoreactivity of the acrylic acid functionality with the coumarin chromophore and have prepared the synthetic bifunctional derivative, (E)-6-coumarinylacrylic acid (2d).

Because of our interest in the photochemical interaction of these acrylic acids with DNA, and the need to detect the covalent binding to the nucleic acid at the nano- and subnanomole levels, we needed the ^{14}C-labeled analogs of these compounds, and report herein the general synthetic approach to the derivatives **2a-2d** shown in the Scheme. This approach can be adapted to both aromatic as well as heteroaromatic derivatives with slight modifications in the reaction conditions.
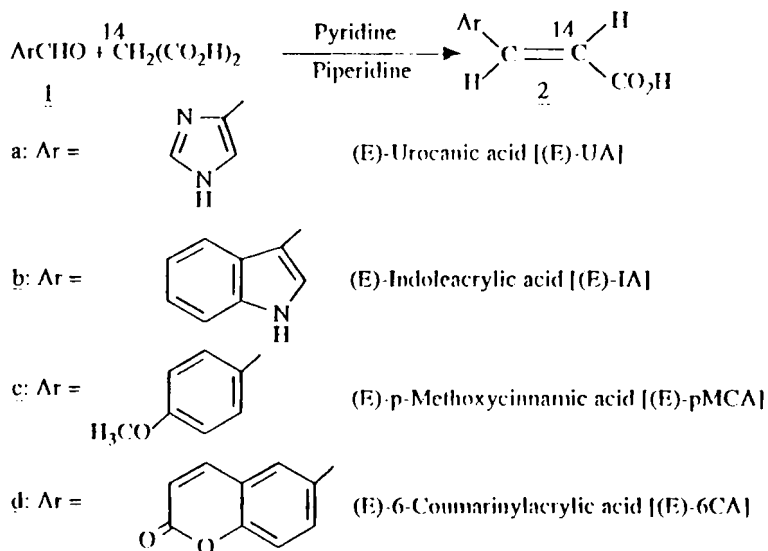
DISCUSSION

We have previously reported the preparation of E-IA (**2b**) with the ^{14}C label at the 2-position of the indole ring (7). This involved the Doebner modification of the Knoevenagel reaction which employed 3-[2-^{14}C]indolecarboxaldehyde (prepared from [2-^{14}C]-indole) and malonic acid, using the former as the limiting reagent. We made several modifications of the literature procedure for the "cold" preparation, the most important being the use of equimolar quantities of 3-indolecarboxaldehyde and malonic acid. With the availability of [2-^{14}C]-malonic acid this modification becomes attractive for the synthesis of a variety of acrylic acids with the label at the α-position in the side chain. The availability of both side chain and ring labeled acrylic acids allows for one to differentiate between total molecule incorporation into *e.g.* DNA and the incorporation of side chain cleaved photoproducts. The advantage of using radiolabeled malonic acid is that the label is incorporated in the last step of the synthesis, as opposed to previous method that used the labeled compound as the starting material. We have recently utilized this modification for the synthesis of (E)-[α-^{14}C]p-methoxycinnamic acid (**2c**)(5) and report herein the extension of this approach to the synthesis of [α-^{14}C]-labeled (E)-UA (**2a**) and (E)-6CA (**2d**).

(E)-[α-^{14}C]Urocanic acid (**2a**) has been previously synthesized by Kraml and Bouthillier (8) in two steps by heating [2-^{14}C]-malonic acid and imidazole-4(5)-carboxaldehyde **1a** in water. The intermediate malonic acid derivative was subsequently decarboxylated by refluxing in pyridine for 14 h. An analogous procedure was reported (9) for the preparation of "cold" **2a** (60% yield) in which pyridine was present at the outset, piperidine was added as a catalyst, and the intermediate diacid was decarboxylated *in situ*. Utilizing this procedure with slight modification, we prepared radiolabeled **2a** and found the reaction to be optimal within 2.5 h; further heating led

to degradation of the UA.

Scheme

$$ ArCHO + \overset{14}{C}H_2(CO_2H)_2 \xrightarrow[\text{Piperidine}]{\text{Pyridine}} \underset{H}{\overset{Ar}{>}} C \overset{14}{=} C \underset{CO_2H}{\overset{H}{<}} $$

1   **2**

a: Ar =   (imidazole structure)   (E)-Urocanic acid [(E)-UA]

b: Ar =   (indole structure)   (E)-Indoleacrylic acid [(E)-IA]

c: Ar =   H₃CO (benzene ring structure)   (E)-p-Methoxycinnamic acid [(E)-pMCA]

d: Ar =   (coumarin structure)   (E)-6-Coumarinylacrylic acid [(E)-6CA]

Thus, condensation of the carboxaldehyde **1a** with [2-^{14}C]-malonic acid under Knoevenagel conditions for 2 h provided E-[α-^{14}C]UA in 38% yield. The crystallized product indicated > 98% chemical and radiochemical purity.

For the synthesis of coumarin analog **2d**, the desired carboxaldehyde **1d** was prepared by formylation of coumarin with chloroform and sodium hydroxide, adopting the modified literature method (10). The only report (11) on the synthesis of "cold" **2d** in 56% yield utilized a Perkin reaction on **1d**. We have synthesized **2d** by the Knoevenagel reaction of **1d** with malonic acid in pyridine and piperidine. The product was obtained in 80% yield and was fully characterized by spectroscopic data. The (E) configuration about the exocyclic double bond in **2d** was established from the large coupling constant value (ca. 16 Hz) as compared to the smaller J value (ca. 10 Hz) for the (Z)-isomers of **2a** and **2b** (12) and other acrylic acids (2b). The radiolabeled analog **2d** was synthesized by the above procedure using [2-^{14}C]-malonic acid, with the crude product isolated in 63% yield and 94% purity. One crystallization from acetic acid provided (E)-6CA in > 98% chemical and radiochemical purity. The identity of **2d** was confirmed

by comparison with "cold" material by TLC, co-TLC and HPLC analyses.

Our initial studies demonstrate that the coumarin analog 2d photolytically binds to calf thymus DNA. The detailed structure-photoreactivity studies of these acrylic acid derivatives are currently in progress and these results will be published elsewhere.

EXPERIMENTAL SECTION

Melting points were determined on a Fisher-Jones and/or Mel-Temp melting point apparatus and were not corrected. Pre-coated fluorescent silica gel (60 F_{254}) plates of 0.2 mm thickness were employed for thin layer chromatography (TLC) analysis and the spots were visualized under short wave UV light and/or iodine vapor. Infrared (IR) spectra in KBr pellets were recorded on a Perkin-Elmer 1800 Fourier transform spectrophotometer. Ultraviolet (UV) absorption spectra in buffer ("buffer" was sodium phosphate unless otherwise specified) were measured on a Perkin-Elmer Lambda 3B UV/VIS and/or Gilford modified Beckman DU spectrophotometers. Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Varian Gemini-200 spectrometer operating at 200 and 50 MHz for ^{1}H and ^{13}C, respectively, and the chemical shift values are expressed in δ units relative to internal tetramethylsilane (TMS) referenced at δ 0.00 ppm. Electron impact (EIMS) and chemical ionization (CIMS) mass spectra of probe samples were obtained on a Finnegan 4000 spectrometer at 70 eV; relative intensities are noted in parentheses after each fragment. Radioactive samples of appropriate dilutions or fractions collected from HPLC column were counted by liquid scintillation counting (LSC) on a Packard 300C liquid scintillation spectrometer using ACS® cocktail (Amersham Corp., Arlington Heights, IL). High performance liquid chromatography (HPLC) analyses were effected on a Varian 5000 liquid chromatograph fitted with a Rheodyne 7125 injection port and a 0.2 ml injection loop. A Varian 2050 variable wavelength detector (set at 254 and 290 nm for UA and 6CA, respectively) was used to monitor the column injections which were recorded and processed with a Perkin-Elmer LCI-100 computing integrator. Alltech Econosil C-8 reverse phase stainless steel column (4.6 mm x 250 mm) was used with the following isocratic solvent programs: 100% 0.05 M buffer pH 7.0 at a flow rate of 1.0 ml min^{-1} (method A), 80% 0.05 M buffer pH 7.0 : 20% methanol (v/v) at a flow rate of 1.0 ml min^{-1} (method B).

<u>Time course reaction of imidazole-4(5)-carboxaldehyde (1a) and malonic acid</u>: A clear stirred solution of the carboxaldehyde **1a** (13) (24 mg, 0.25 mmol) and malonic acid (29 mg, 0.28 mmol) in dry pyridine (0.7 ml) containing piperidine (6 μl) was heated at 90 °C under nitrogen atmosphere. Within 10 min, a white solid separated out from the reaction mixture and, after holding the temperature for 15 min, the mixture was refluxed. The progress of the reaction was monitored qualitatively by HPLC. A small volume of the clear solution was dissolved in buffer and 50 μl was analyzed by HPLC using method A. Three major peaks were observed at 3.1 (peak 1), 3.7 (peak 2) (both unidentified) and 4.5 min [(E)-UA] with their relative composition after 2.5 h equal to 6, 21 and 73%, respectively (these normalized percent areas have not been corrected for differences in molar extinction coefficients at the detector wavelength). Analysis after 2.5 h indicated that the amounts of all three products decreased, with > 80-90% of the detectable product having disappeared by 21 h. As was also observed in the thermolysis of (E)-UA (*vide infra*), a solid deposited on the sides of the reaction flask which increased gradually with the reaction time; analysis of this material after 7.5 h gave a relative composition of peaks 1, 2 and (E)-UA as 28, 35 and 37%, respectively.

<u>(E)-[α-^{14}C]Urocanic acid (2a)</u>: A reaction of carboxaldehyde **1a** (48 mg, 0.5 mmol), "cold" malonic acid (58 mg, 0.56 mmol) and [2-^{14}C]-malonic acid (0.25 mCi, specific activity 51.0 mCi/mmol; NEN-Du Pont, Boston, MA) in dry pyridine (1.5 ml) and piperidine (10 μl) was carried out under nitrogen atmosphere as described for the time course condensation. After initial reaction at 90 °C for 15 min, the mixture was refluxed for 2 h. The solvent was removed under reduced pressure and the residual solid was dissolved in water (3.0 ml) and treated with Norit. The solution was filtered through a 0.2 μm hydrophilic nylon membrane and the residue was washed with additional water. The product crystallized upon refrigeration and was collected by filtration and washed with chilled water to afford 26 mg (38%) of **2a**. The specific activity of the product was 0.53 mCi/mmol and the combined HPLC-LSC analysis indicated the material to be > 98% chemically and > 99% radiochemically pure [based on the presence of about 1% (Z)-isomer, t_R 9.6 min]. The identity of the product was established by comparison with an authentic sample of "cold" (E)-UA through TLC, co-TLC and HPLC analyses.

Thermolysis of (E)-urocanic acid (2a): A clear colorless solution of (E)-UA (30 mg, Sigma Chemical Co.) in dry pyridine (0.7 ml) containing piperidine (6 μl) was refluxed with stirring under nitrogen atmosphere for 21 h. A 25 μl aliquot was dissolved in 1.0 ml buffer and 50 μl was analyzed by HPLC using method A, before and after heating. More than 76% of the urocanic acid had decomposed.

Coumarin-6-carboxaldehye (1d): The carboxaldehyde **1d** was prepared by a modification of the literature procedure (10). A stirred yellow solution of coumarin (16.0 g, 0.11 mol) in 17 N sodium hydroxide (40 ml) at 80 °C was treated with chloroform (20 ml) by dropwise addition over 3 h. The resultant deep red reaction mixture was heated at 100 °C for 8 h. The excess chloroform was rotoevaporated and the residual mixture was cooled in ice. The solid was removed by filtration and the dark red filtrate was acidified with conc. HCl. The precipitated pale yellow solid was extracted with chloroform (5 x 60 ml) and the combined organic extracts washed with water and dried (Na_2SO_4). The solvent was stripped off on a rotavapor and the residual yellow solid was extracted with a 100 ml mixture of warm ethanol:ether (4:1). The insoluble solid **1a** (2.05 g, 11%) melted at 190-192 °C. One crystallization from ethyl acetate raised the mp to 194-195 °C [lit.(10) mp 187-189 °C]. IR: 1724 (lactone C-O), 1690 (carboxaldehyde C-O), 1604, 1375, 1340, 1263, 1156, 962 cm^{-1}; UV (95% EtOH): λ_{max} (ϵ, M^{-1} cm^{-1}) 254 (31,940), 316 nm (5,030); ^{1}H NMR (CDCl$_3$): δ 6.54 (d, J=9.5 Hz, 1H, 3-H), 7.49 (d, J=8.5 Hz, 1H, 8-H), 7.81 (d, J=9.5 Hz, 1H, 4-H), 8.06 (m, 2H, 5-H, 7-H), 10.05 (s, 1H, CHO); EIMS: m/z (rel. int.) 174 (100, M$^{+\cdot}$), 173 (74), 145 (77), 117 (26), 89 (48), 63 (85), 51 (14); CIMS: 175 (100, M+H$^+$).

(E)-[α-^{14}C]β-Coumarin-6-ylacrylic acid (2d): A stirred mixture of [2-^{14}C]-malonic acid (0.25 mCi, specific activity 2.5 mCi/mmol; Sigma Chemical Co., St. Louis, MO) and "cold" malonic acid (40 mg) and the carboxaldehyde **1d** (70 mg, 0.4 mmol) in dry pyridine (0.8 ml) containing piperidine (10 μl) was heated at 100 °C under a nitrogen atmosphere for 41 h. After being allowed to cool to ambient temperature, the resultant reaction mixture was diluted with water (3.0 ml) and the cold solution was acidified with 5 N HCl. After refrigeration an off-white solid was collected by filtration and washed with cold water to afford **2d** (54 mg, 63%, specific activity 0.6 mCi/mmol). The combined HPLC-LSC analysis with method B (t$_R$ 12.3 min) indicated 94% radiochemical purity

(including 0.6% (Z)-isomer, t_R 8.8 min). A product of > 98% chemical and radiochemical

purity was obtained after crystallization from glacial acetic acid.

A large-scale "cold" synthesis of **2d** was carried out several times with varying

reaction times and temperatures. The optimum reaction conditions were found to be 100

°C for > 24 h. The crude product obtained in > 80% yield (> 94% pure by HPLC) was

crystallized from hot glacial acetic acid as white leaflets, mp 317-318 °C [lit.(11)

mp 306 °C]; R_f 0.61 (CHCl₃:95% EtOH :: 9:1), 0.85 (nBuOH:95% EtOH:H₂O ::: 4:1:5); IR:

1731 (lactone C—O), 1686 (acid C—O), 1618, 1437, 1383, 1317, 1171, 824 cm⁻¹; UV (0.1

M buffer pH 7.1): λ_{max} (ϵ, M⁻¹ cm⁻¹) 272 (39,400), 333 nm (4,600); ¹H NMR (DMSO-d₆): δ

6.56 (d, J=9.6 Hz, 1H, 3-H), 6.59 (d, J=16.1 Hz, 1H, α-H), 7.45 (d, J=8.7 Hz, 1H, 8-H),

7.65 (d, J=16.1 Hz, 1H, β-H), 7.97 (dd, J=8.7, 2.0 Hz, 1H, 7-H), 8.05 (d, J=9.6 Hz, 1H,

4-H), 8.08 (d, J=2.0 Hz, 1H, 5-H), 12.45(br s, 1H,O-H); ¹³C NMR (DMSO-d₆): δ 117.09 (o),

117.19 (o), 119.24 (e) 119.99 (o), 128.55 (o), 130.94 (e), 131.74 (o), 142.61 (o),

144.23 (o), 154.73 (e), 159.97 (e), 167.76 (e) (the attached proton test (APT)

spectrum was used to assign signals as 2° and 4° or 1° and 3°, using (e) and (o)

notation, respectively. EIMS: m/z (rel int.) 216 (100, M⁺·), 215 (9), 199 (10), 188

(22), 187 (21), 171 (24), 143 (11), 142 (13), 131 (11), 115 (38), 89 (20), 63 (27), 51

(18); CIMS: 217 (100, M+H⁺); high-resolution mass spectrum: calcd for C₁₂H₈O₄ 216.0423,

found 216.0423.

REFERENCES

1. (a) Farrow S. J., Mohammad T., Baird W. and Morrison H.-Chem.-Biol. Interact. **75**:
   105 (1990); (b) Farrow S. J., Jones C. R. Severance D. L., Deibel R.M., Baird
   W. M. and Morrison, H. A.-J. Org. Chem. **55**: 275 (1990).

2. (a) Rasanen L., Jansen C. T., Hyoty H., Reunala T. and Morrison H.-Photodermatol.
   6: 287 (1989) and references cited therein; (b) Norval M., Simpson T. J.,
   Bardshiri E. and Howie S. E. M.-Photochem. Photobiol. **49**: 633 (1989).

3. Farrow S. J., Mohammad T., Baird W. and Morrison H.-Photochem. Photobiol. 51, 263 (1990).

4. Forbes P. D., Davies R. E., Sambuco C. P. and Urbach F.-J. Toxicol.-Cut. & Ocular. Toxicol. 8: 209 (1989) and references cited therein.

5. Mohammad T., Baird W. M. and Morrison H.- Bioorg. Chem. 19: 88 (1991).

6. Cadet J. and Vigny P.-In "Bioorganic Photochemistry: Photochemistry of the Nucleic Acids", Morrison H. (Ed.), John Wiley & Sons, New York, NY, 1990, Vol.I, pp 210-224.

7. Morrison, H., Mohammad T. and Severns B.-J. Labelled Compd. Radiopharm. 27 565 (1989).

8. Kraml M. and Bouthillier L. P.-Can. J. Biochem. Physiol. 33: 590 (1955).

9. Horii Z., Sakurai K. and Tomio K.-J. Pharm. Soc. Japan 74: 408 (1954).

10. Sen R. and Chakravarty D.- J. Amer. Chem Soc. 50: 2428 (1928).

11. Jainamma K. M. and Sethna S.-J. Indian Chem. Soc. 50: 606 (1973).

12. Mohammad T. and Morrison H.-unpublished results.

13. Battersby A. R., Nicoletti M., Staunton J. and Vleggaar R.-J. Chem. Soc. Perkin Trans I: 43 (1980).