Isolation and Synthesis of an α-Malamic Acid Derivative from *Justicia ghiesbreghtiana*

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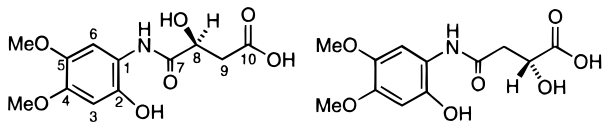
A polar extract of leaves of *Justicia ghiesbreghtiana* yielded *N*-(2-hydroxy-4,5-dimethoxyphenyl)-(*S*)-α-malamic acid, **1**. Incomplete spectral analysis yielded a hypothetical structure, which was then proven by total synthesis. Coupling of the trifluoroacetate of malic anhydride (trifluoroacetoxysuccinic anhydride) with an arylamine provided the key to regiospecific preparation of the α- rather than *β*-malamic acids.

Several specimens of an Acanthaceous plant labeled *Justicia ghiesbreghtiana* Lem., of presumed Mexican origin, are found worldwide in botanical or horticultural gardens, but the connection between these specimens and collection data is obscure. The name *Justicia ghiesbregtiana* was originally published in 1847[1] by Lemaire, who stated that the specimen he saw was "introduced from Mexico by the naturalist M. Ghiesbregt". The collector was, more properly, August Ghiesbreght, and the name has therefore been preserved as *J. ghiesbreghtiana*. It was not mentioned in the most recent comprehensive treatment of *Justicia*, although not all of the some 600 species of *Justicia* are discussed therein.[2] *J. ghiesbreghtiana* is sometimes treated (particularly in the botanical garden literature) as synonymous with the better known Mexican species *J. spicigera* Schlechtend., which has been used as a medicinal herb in Mexico[3] and cultivated as such in Texas.[4] *J. spicigera* is said to be used as a stimulant, to treat scabies and various gastrointestinal disorders such as dysentery, and as a source of blue dye.[2−5] Chemical investigations of *J. spicigera* reported the presence of the flavanoid kaempferitrin,[4] *β*-sitosterol glucoside, cryptoxanthin, alantoin, and a black, resinous dye.[3] We report here isolation of a novel malamic acid derivative from a Cairo, Egypt, botanical garden specimen of *J. ghiesbreghtiana*.

A water-soluble fraction remaining after removal of nonpolar materials and extensive column chromatography yielded a pure (TLC, NMR), gummy compound, $C_{12}H_{15}NO_7$ by HRMS. The ^{13}C and ^1H NMR spectra accounted for the presence of two carbonyls, two methoxy groups on a tetrasubstituted benzene, one methylene, and one methine to which an oxygen was attached. Two 1H singlets at *δ* 6.50 and 6.94 suggested that the benzene ring was 1,2,4,5-substituted. A base peak of *m*/z 154 in the HRMS was due to a $C_7H_8NO_3$ fragment, while a peak of second intensity at *m*/z 169 was established as $C_8H_{11}NO_3$. These formulas suggested that the aromatic portion of the unknown was substituted with two methoxy, one hydroxy, and one −NHR group. The ^{13}C NMR resonances seemed best to fit a 2-hydroxy-4,5-dimethoxyaniline derivative, although a 2,5-dimethoxy-4-hydroxyaniline could not be absolutely ruled out. Cleavage at the N−R bond in the mass spectral fragmentation with concomitant rearrangment of one H from the side chain would yield the *m*/*z* 169 ion. Decoupling of the ^1H NMR spectrum, along with the chemical shifts of the methine and methylene protons, suggested the presence of a −CO−CH(OH)−CH_2−CO− moiety attached at one carbonyl to the aniline NH. The IR spectrum showed a complex series of absorptions in the 1550−1650 cm^{-1} region, suggesting amide, carboxyl, and/or carboxylate functionalities. The data to this point were interpreted as best being consistent with either structure **1** or **2** for the unknown, where the absolute stereochemistry at the side chain OH was that expected if it were derived from L-malic acid. Compounds **1** and **2** are α- and *β*-malamic acids, respectively.

During the course of obtaining the spectral data and allowing the unknown to stand in solution, the aromatic proton resonances eventually disappeared and were replaced by singlet resonances at *δ* 5.45 and 5.55, with the remainder of the spectrum not greatly changed. MS of the product showed the loss of two hydrogens from the original formulation and suggested transformation of the unknown into an acyl quinone-imine structure **3**.[6] This transformation led to decomposition, and more detailed spectral studies were not possible.



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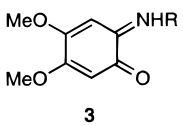

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At this point, several α- and *β*-malamic acids analogous to structures **1** and **2** were synthesized in an attempt to discover unique spectral data for each that would allow assignment of either regiochemistry to the

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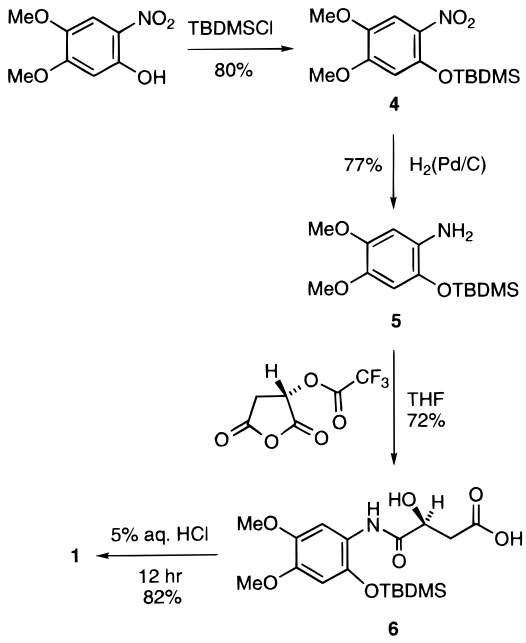
Scheme 1



unknown. The results were not unequivocal and will be reported elsewhere, but they suggested that the α-malamide regiochemistry was the proper one for the unknown. A total synthesis of **1** was therefore undertaken (Scheme 1). The key to the regiospecific synthesis was coupling of the amine **5** with (*S*)-trifluoroacetoxysuccinic anhydride.[7,8] The final product acid had NMR spectra very similar to, but not identical with, those of the originally isolated unknown. The NMR spectral data and optical rotation became essentially identical when the synthetic product was converted to the ammonium salt, thus indicating that the original isolation had resulted in isolation of **1** as a salt. Both the isolated and synthetic **1** were originally obtained as almost colorless or with a faint pinkish or purplish cast, but upon obtaining spectra, or even storage in the refrigerator, they became a darker blue-purple.

Justicia species, particularly some used as medicinal herbs, often contain lignans, but we were unable to isolate any from *J. ghiesbreghtiana*. One medicinal species, *J. gendarussa*, was also reported to be lignan-free, but did contain two 2-aminobenzyl alcohol derivatives,[9] the only other aminobenzene derivative we could find reported from *Justicia*. Structurally, and perhaps biosynthetically, **1** may be related to the benzoxazolinones and 1,4-benzoxazinones common to seedlings of the Gramineae. One such (2,4-dihydroxy-1,4-benzoxazin-3-one) was reported from *Acanthus mollis* of the same family as *Justicia* (Acanthaceae).[10]

Compound **1** was inactive as an antimicrobial when tested against Gram-positive and Gram-negative bacteria and a fungus. A synthetic analogue lacking the C-2 OH group was inactive against *Mycobacterium tuberculosis*. Almost all solutions of extracts and partial isolates in this study were dark wine to purple colored, reflecting the known use of several *Justicia* species for the preparation of dyes.

Experimental Section

General Experimental Procedures. NMR spectra were obtained at 300 MHz (^{1}H) and 75 MHz (^{13}C) in $CDCl_3$ unless otherwise indicated. IR spectra were from thin films on NaCl unless otherwise indicated. Organic extraction solvents were dried over Na_2SO_4 and evaporated in vacuo.

Plant Material. Fresh plant material of *Justicia ghiesbreghtiana* Lem. was collected on December 16, 1992, in the Giza zoological garden, Cairo, Egypt. It was identified by Professor Dr. Nabeil El Hadedi, Department of Botany, Cairo University, and a voucher was deposited in the Department of Pharmacognosy herbarium, Al-Azhar University.

Extraction and Isolation. Air-dried and powdered aerial parts (700 g) were extracted with 3:2 hexane−EtOAc and the marc dried and extracted with MeOH. The MeOH was evaporated, the residue was triturated with H_2O, and the aqueous layer was washed with Et_2O and evaporated to dryness. The residue was triturated with MeOH, and the MeOH was then evaporated to a gummy residue (29 g). This was dissolved in H_2O, chromatographed on a C_{18} Si gel column, and eluted with H_2O−MeOH. Fractions 4−8 (5−25% MeOH) showed orange-red spots on TLC with 2,4-DNPH reagent. These were combined and evaporated to leave 2.1 g of residue. This was chromatographed on Si gel with $CHCl_3$−MeOH as eluting solvent. Fractions 21−33 (45−70% MeOH) were combined to yield 0.85 g of residue, which was similarly rechromatographed. Fraction 9 (25% MeOH; 130 mg) gave a single spot on Si gel TLC (R_f 0.47; solvent $CHCl_3$−MeOH−H_2O 61:32:7) as an almost colorless amorphous solid, eventually identified as a salt of **1**: $[\alpha]^{20}_D$ −23° (*c* 1.7, H_2O); IR (KBr) ν_{max} 3445 (br), 1640, 1610, 1535 cm^{-1}; ^{1}H NMR (D_2O) δ 2.56 (dd, 1H, J = 8.0, 15.5 Hz), 2.70 (dd, 1H, J = 4.0, 15 Hz), 3.67 (s, 3H), 3.71 (s, 3H), 4.51 (dd, 1H, J = 4.0, 8.0 Hz), 6.50 (s, 1H), 6.94 (s, 1H); ^{13}C NMR (D_2O) δ 41.9, 56.8, 57.3, 70.1, 102.2, 110.2, 116.0, 142.4, 144.4, 148.2, 175.9, 178.6; negative EIMS (electrospray) *m/z* 284 (100); positive EIMS (electrospray) *m/z* 286 (30), 214 (100); HRFABMS *m/z* 285.0844 (calcd for $C_{12}H_{15}NO_7$ 285.0849); HREIMS *m/z* 169.0739 (calcd for $C_8H_{11}NO_3$ 169.0739) and *m/z* 154.0506 (calcd for $C_7H_9NO_3$ 154.0504).

Isolated **1** became colored (eventually blue-purple) upon standing and/or as spectral data were obtained. It eventually converted completely to a substance (presumably **3**) whose aromatic ^{1}H resonances (δ 6.50 and 6.94) were replaced by two singlets at δ 5.45 and 5.55. This material underwent further decomposition.

2-*tert*-Butyldimethylsilyloxy-3,4-dimethoxynitrobenzene (4). A solution of 3,4-dimethoxy-6-nitrophenol[11] (3.0 g, 15 mmol), *tert*-butyldimethylsilyl chloride (2.41 g, 16.0 mmol) and pyridine (50 mL) was stirred for 24 h. The pyridine was removed in vacuo and the crude oily residue purified by VLC (vacuum layer chromatography) on Si gel (*n*-hexane−EtOAc, EtOAc gradient) to give 3.95 g (84%) of **4** as a yellow solid (purity by GC, 98%): mp 63−65 °C; IR (ν_{max}) 2932, 2859, 1620, 1583, 1514, 1336, 1273, 1222, 1086, 1006, 838 cm^{-1}; ^{1}H NMR δ 0.22 [s, 6H, $Si(CH_3)_2$], 1.02 [s, 9H, $SiC(CH_3)_3$], 3.88 (s, 3H, OCH_3), 3.89 (s, 3 H, OCH_3), 6.41 (s, 1H, CH aromat), 7.48 (s, 1H, CH aromat); ^{13}C NMR δ −4.5 [$Si(CH_3)_2$], 18.2 (*C*), 25.6 (CH_3), 56.2 (OCH_3), 56.3 (OCH_3), 105.3 (*C*-3), 107.7 (*C*-6), 132.8 (*C*-1), 143.0 (*C*-5), 146.1 (*C*-2), 154.2 (*C*-4); EIMS *m/z* 313 [M$^+$] (2), 298

(24), 258 (38), 257 (65), 256 (100), 240 (38), 211 (57), 196 (26), 183 (26), 166 (16), 137 (6), 75 (28); *anal.* C 53.65%, H 7.40%, N 4.47%, calcd for $C_{14}H_{23}NO_5Si$ C 53.91%, H 7.47%, N 4.53%.

2-*tert*-Butyldimethylsilyloxy-4,5-dimethoxyaminobenzene (5). A mixture of **4** (3.5 g, 11 mmol), Pd/C, 10% Pd (0.22 g), and THF (60 mL) was stirred under H_2 for 12 h. After filtration, the solvent was evaporated. VLC of the residue on Si gel (*n*-hexane−EtOAc; EtOAc gradient) gave **5** as an beige oil (2.75 g, 87%): IR (ν_{max}) 3363, 2930, 2857,1621, 1518, 1465, 1230, 1200, 1013, 907, 838, 782 cm^{-1}; ^1H NMR δ 0.27 [s, 6H, $Si(CH_3)_2$], 1.03 [s, 9H, $SiC(CH_3)_3$], 3.84 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 6.47 (s, 1 H, aromat CH), 7.96 (s, 1 H, aromat CH); ^{13}C NMR δ −4.4 [$Si(CH_3)_2$], 18.0 (*C*), 25.5 (CH_3), 56.1 (OCH_3), 56.3 (OCH_3), 103.1 (*C*H), 104.6 (*C*H), 119.5 (*C*NH), 138.4 (*C*OR), 143.4 (*C*OR), 146.4 (*C*OR); positive HRCIMS *m/z* 284.1695 (calcd for $C_{14}H_{26}NO_3Si$, 284.1682); GC−MS *m/z* 283 [M^+] 51, 268(22), 226(98), 210(16), 195-(50), 180(19), 168(14), 73(100).

***N*-(2-*tert*-Butyldimethylsilyloxy-4,5-dimethoxyphenyl)-(*S*)-α-malamic acid (6).** Trifluoroacetic anhydride (TFAA) (2.02 g, 9.62 mmol) was added dropwise at 0 °C and stirring to (*S*)-malic acid (0.43 g, 3.2 mmol). Stirring was continued for 1 h at 0 °C when excess TFAA was distilled off under reduced pressure. The obtained anhydride solid residue was dried for 45 min (oil pump vacuum), dissolved at 0 °C in THF (15 mL), and a solution of **5** (1.80 g, 6.35 mmol) in THF (15 mL) was added dropwise under stirring. The reaction mixture was allowed to warm to room temperature and was stirred for another 1 h. The solvent was removed in vacuo, and the crude residue was purified by VLC on Si gel (*n*-hexane−EtOAc, gradient EtOAc) to yield **6** (0.91 g, 72%): UV (MeCN) λ_{max} (log ϵ) 304 (6960), 254 (8914); IR (ν_{max}) 3370, 2933, 2859, 1719, 1659, 1534, 1203, 928, 840 cm^{-1}; ^1H NMR δ 0.24 [s, 6H, $Si(CH_3)_2$], 1.00 [s, 9H, $SiC(CH_3)_3$], 2.77 (dd, 1H, J = 9.0, 17.4 Hz, H-9), 3.13 (dd, 1H, J = 3.2, 17.4 Hz, H-9), 3.81 (s, 3H, OCH_3), 3.83 (s, 3H, H-8), 4.66 (dd, 1H, J = 3.0, 9.0 Hz, CH), 6.44 (s, 1H, H-3), 8.00 (s, 1H, H-6), 9.15 (s, 1H, NH); ^{13}C NMR δ −4.3 [$Si(CH_3)_2$], 18.0 (*C*), 25.6 (CH_3), 38.3 (CH_2), 56.1 (OCH_3), 56.3 (OCH_3), 68.6 (*C*H), 103.6 (*C*H), 104.8 (*C*H), 121.1 (*C*NH), 138.4 (*C*OR), 143.1 (*C*OR), 145.4 (*C*OR), 169.7 (C═O), 176.4 (C═O); positive HRCIMS *m/z* 400.1806 (calcd for $C_{18}H_{30}NO_7Si$ 400.1792).

***N*-(2-Hydroxy-4,5-dimethoxyphenyl)-(*S*)-α-malamic acid (1).** A mixture of **6** (0.60 g, 1.5 mmol) in THF (10 mL) and 5% HCl (25 mL) was stirred for 12 h. The THF was removed in vacuo, the aqueous solution was extracted with EtOAc (3 × 20 mL). After removal of the combined and dried EtOAc extracts, the residue was purified by VLC as above to give pink crystals. These were washed with CH_2Cl_2 to yield **1** (0.35 g, 82%): (mp 175−176 °C); $[\alpha]_D^{25}$ −56° (*c* 0.98, MeOH); UV (MeCN) $\lambda_{max.}$ (log ϵ) 302 (7009), 252 (7249); IR (ν_{max}) 3360, 2971, 2939, 1720, 1657, 1525, 1454, 1204, 1001, 858 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.47 (dd, 1H, J = 8.4, 15.9 Hz, H-9), 2.76 (dd, 1H, J = 3.9, 15.9 Hz, H-9), 3.65 (s, 3H, OCH_3), 3.68 (s, 3H, OCH_3), 4.39 (dd, 1H, J = 3.9, 8.4 Hz, H-8), 6.55 (s, 1H, H-3), 7.85 (s, 1H, H-6), 9.19 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 39.6 (CH_2), 55.7 (OCH_3), 56.3 (OCH_3), 68.7 (*C*H), 101.1 (*C*H), 105.7 (*C*H), 118.5 (*C*NH), 140.4 (*C*OR), 141.1 (*C*OR), 145.2 (*C*OR), 170.8 (C═O), 172.2 (C═O); positive HRCIMS *m/z* 286.0928 (calcd for $C_{12}H_{16}NO_7$ 286.0926); positive CI *m/z* 285 [M^+] (33), 267 (25), 196 (18), 180 (13), 169 (100), 168 (44), 154 (81), 140 (21), 126 (19), 109 (17), 71 (19), 69 (25); *anal.* C 50.20%, H 5.55%, N 4.61%, calcd for $C_{12}H_{15}NO_7$, C 50.53%, H 5.30%, N 4.91%.

The acid **1** (31 mg, 0.11 mmol) was dissolved in 1 mL of concentrated aqueous NH_3, evaporated, and dried to yield 33 mg of the ammonium salt as a pale reddish solid whose color gradually changed to a deeper, more purplish color: mp 169−171° dec; $[\alpha]^{25}_D$ −26° (*c*, 0.43, MeOH). Its ^1H and ^{13}C NMR spectra were essentially identical to those of the material originally isolated from the plant.

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