Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron Letters
Tetrahedron Letters 46 (2005) 6457–6460

Remarkable Chichibabin-type cyclotrimerisation of 3-nitrotyrosine, tyrosine and phenylalanine to 3,5-diphenylpyridine derivatives induced by hypochlorous acid

L. Panzella,[a] P. Di Donato,[b] S. Comes,[b] A. Napolitano,[a] A. Palumbo[b] and M. d'Ischia[a,*]

[a]*Department of Organic Chemistry and Biochemistry, Complesso Universitario di Monte S. Angelo via Cinthia, University of Naples Federico II, I 80126 Naples, Italy*
[b]*Zoological Station Anton Dohm, Villa Comunale, I 80121, Naples, Italy*

Received 9 May 2005; accepted 22 July 2005

Abstract—Reaction of 3-nitrotyrosine with HOCl in aqueous phosphate buffer (pH 7.4) leads to a mixture of extractable products, including 3,5-di(4-hydroxy-3-nitrophenyl)pyridine (15% isolated yield) and 3,5-di(4-hydroxy-3-nitrophenyl)-2-(4-hydroxy-3-nitrophenylmethyl)pyridine (3%) arising by a Chichibabin-like pyridine synthesis via *N*-chloroimine intermediates. Under the same conditions, phenylalanine gives 3,5-diphenylpyridine in 9% isolated yield, while tyrosine leads to 3,5-di(4-hydroxyphenyl)pyridine (3%) and 3-(3-chloro-4-hydroxyphenyl)-5-(4-hydroxyphenyl)pyridine (3%).

The reactions of α-amino acids with hypochlorous acid (HOCl)[†] have been extensively investigated over the past decades for their potential synthetic interest[2–4] as well as for their importance in biology and medicine,[5,6] being implicated in the inflammatory response mediated by activated neutrophils through the myeloperoxidase–H_2O_2–Cl^- system.[7] As a rule, the HOCl-mediated oxidation of the amino acidic functionality at neutral pH proceeds with decarboxylation to yield mainly aldehyde and nitrile products.[4–6] In the case of tyrosine, aromatic ring chlorination may occur as well, leading, besides 4-hydroxyphenylacetaldehyde, to 3-chlorotyrosine, 3,5-dichlorotyrosine and 3-chloro-4-hydroxyphenylacetaldehyde.[5,8,9] The accepted mechanism of the deamination/decarboxylation of amino acids by HOCl involves formation of *N*-chloramines, unstable intermediates that spontaneously decompose with loss of NH_3, CO_2 and Cl^- to give aldehyde species.[4,10,11] With excess HOCl, dichloramines may also be formed, which are responsible for nitrile formation.[12]

Recently, we revisited the reaction[13] of HOCl (1.5 molar equiv) with 3-nitrotyrosine (**1**) (0.02–2 mM), a biochemical marker of peroxynitrite, at pH 7.4, and noticed the presence in the ethyl acetate extractable fraction of two main products whose properties did not match with those of any of the known oxidation products of amino acids and that were relatively retained on reverse phase HPLC (t_R 33.4 and 36.2 min) and TLC (R_f 0.23 and 0.33).[‡] The product at R_f 0.23 could be isolated by precipitation from the organic residue dissolved in ethyl acetate, while that at R_f 0.33 was isolated by preparative TLC.

The product at R_f 0.23 displayed in the ESI-/MS spectrum, a pseudomolecular ion peak at m/z 352 and

Keywords: Aminoacid chlorination; *N*-Chloroimines; 3,5-Diphenylpyridines; Cyclotrimerisation.
* Corresponding author. Tel.: +39 081674132; fax: +39 081674393; e-mail: dischia@unina.it
† HOCl has a pK_a of 7.46,[1] therefore it exists as a mixture of the protonated and dissociated form ($^-$OCl, hypochlorite) at pH 7.4; throughout this paper HOCl will be used to designate this mixture unless otherwise stated.

‡ HPLC analyses were run on octadecylsilane coated columns, 250×4.6 mm, 5 μm particle size, at a 1.0 mL/min flow rate; eluent: 2% acetic acid in water (solvent A) and 2% acetic acid in acetonitrile (solvent B), gradient from 10% to 60% B (25 min), from 60% to 90% B (15 min). Analytical and preparative TLC was performed on 0.25 and 0.5 mm silica gel plates, respectively (eluent: cyclohexane–ethyl acetate 60:40 v/v).

0040-4039/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2005.07.106

exhibited a chromophore characterised by maxima at 260 and 368 nm. The ^{1}H NMR spectrum (DMSO-d_6) showed as salient features: (a) the lack of aliphatic proton signals; (b) the typical spin system of the 4-hydroxy-3-nitrophenyl moiety and (c) a highly deshielded doublet at δ 8.84 ($J = 2.0$ Hz) coupled to a multiplet at δ 8.31 partially overlapped to one of the aromatic proton resonances. Scrutiny of the spectrum run in different solvents revealed that the integrated area of the signal at δ 8.31 was invariably half that of the other resonances. Based on ^{1}H, ^{13}C heteronuclear multiple quantum coherence and heteronuclear multiple bond correlation experiments, the compound was identified as 3,5-di(4-hydroxy-3-nitrophenyl)pyridine (**2**).

The product at R_f 0.33 displayed in the ESI-/MS spectrum a pseudomolecular ion peak at m/z 503. The ^{1}H NMR spectrum was characterised by the presence of spin systems for three different 4-hydroxy-3-nitrophenyl moieties. Moreover, the low field region showed a doublet ($J = 2.4$ Hz) at δ 8.84, coupled to a doublet at δ 7.68. Finally, the aliphatic region of the ^{1}H NMR spectrum was characterised by the presence of a 2H singlet at δ 4.17. On this basis, the product was identified as 3,5-di(4-hydroxy-3-nitrophenyl)-2-(4-hydroxy-3-nitrophenylmethyl)pyridine (**3**).

NMR resonances of compounds **2** and **3** and assignments based on 2D correlation experiments are listed in Table 1.

Isolated yields of **2** and **3** were 15% and 3%, respectively, based on reacted **1**.

Table 1. NMR spectral data for **2** and **3**

	2 (DMSO-d_6)		**3**($CDCl_3$)	
	^{1}H (J, Hz)	^{13}C	^{1}H (J, Hz)	^{13}C
1[a]	—	—	—	—
2	8.84 (d, 2.0)	146.1	—	157.2
3	—	133.9	—	136.0
4	8.31 (m)	131.4	7.68 (d, 2.4)	135.8
5			—	133.0
6			8.84 (d, 2.4)	147.1
1′	—	126.8	—	130.5
2′	8.33 (d, 2.4)	123.7	8.00 (d, 2.0)	125.3
3′	—	138.0	—	135.0
4′	—	153.1	—	155.8
5′	7.19 (d, 8.8)	120.4	7.30 (d, 8.4)	121.2
6′	8.00 (dd, 8.8, 2.4)	133.5	7.48 (d, 8.4, 2.0)	137.9
1″			—	131.5
2″			8.34 (d, 2.4)	123.1
3″			—	134.5
4″			—	156.0
5″			7.28 (d, 8.8)	120.7
6″			7.84 (d, 8.8, 2.4)	135.8
$CH_2\alpha$			4.17 (s)	40.1
1‴			—	132.0
2‴			7.76 (d, 2.4)	124.5
3‴			—	134.0
4‴			—	154.5
5‴			7.04 (d, 8.4)	120.1
6‴			7.39 (dd, 8.4, 2.4)	138.2

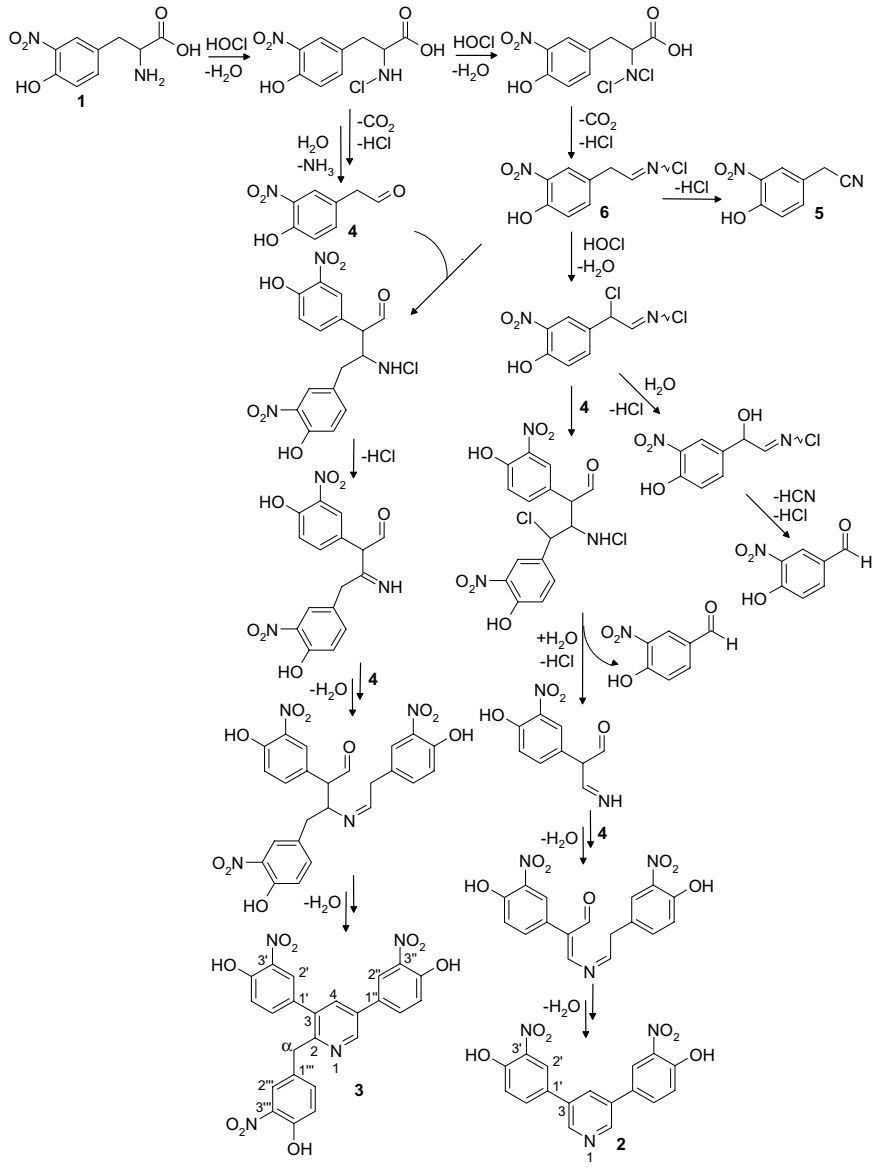
[a] Numbering as shown in structural formulas **2** and **3**.

Other reaction products that were isolated from the ethyl acetate fraction after removal of the solvent at rt were 4-hydroxy-3-nitrophenylacetaldehyde **4** (20% isolated yield), 4-hydroxy-3-nitrophenylacetonitrile **5** (20% isolated yield), the *N*-chloroimines **6** (inseparable mixture of *E*/*Z* isomers, 17% isolated yield)[§] and little 4-hydroxy-3-nitrobenzaldehyde.

Formation of products **2** and **3** is reminiscent of the Chichibabin pyridine synthesis via condensation of carbonyl compounds with ammonia or amines in boiling ethanol or in autoclave under pressure.[14–17] Whereas **3** is evidently the result of a cyclotrimerisation process involving **4** and an imine intermediate, the pyridine **2** would arise by a more complex mechanism in which a 4-hydroxy-3-nitrophenylmethyl moiety is lost. In the classical Chichibabin condensation, phenylacetaldehyde reacts with ammonia to give 3,5-diphenylpyridine with loss of toluene,[16] whereas in the reaction of **1** with HOCl no detectable formation of 4-hydroxy-3-nitrotoluene was observed after the usual work-up, suggesting that a different mechanism was operative involving direct loss of 4-hydroxy-3-nitrobenzaldehyde. Since in separate experiments it was found that 4-hydroxy-3-nitrobenzaldehyde does not arise by oxidation of 4-hydroxy-3-nitrotoluene under the reaction conditions, a series of experiments were performed to gain a deeper mechanistic insight. When the pure *N*-chloroimines **6** were heated in ethyl acetate at 40 °C for 30 min, the nitrile **5** was formed as the main product, along with the pyridines **2** and **3**, the aldehyde **4** and 4-hydroxy-3-nitrobenzaldehyde, whereas at rt the trimer **3** was the prevalent species along with **5**. Addition of the aldehyde **4** to the solution of **6** in ethyl acetate at 40 °C led to an increase in the yield of formation of **2** and **3**. Addition of formaldehyde[16] to the reaction mixture of **1** with HOCl did not affect formation of **2** or **3**, thus ruling out its involvement in the build-up of the pyridine ring. On this basis, it is argued that formation of pyridine derivatives depends on the generation and reactivity of the *N*-chloroimines **6**. These can undergo condensation with two molecules of the aldehyde **4** to give eventually **3** (Scheme 1). Alternatively, further chlorination of **6** by HOCl or by interaction with another molecule of **6** may give the α-chloroderivatives, which after condensation with **4** would eliminate 4-hydroxy-3-nitrobenzaldehyde.

Under similar conditions, tyrosine reacted with HOCl to give 3,5-di(4-hydroxyphenyl)pyridine (**7**) and 3-(3-chloro-4-hydroxyphenyl)-5-(4-hydroxyphenyl)pyridine

[§] ^{1}H NMR ($CDCl_3$) δ (ppm) (major/minor 1:0.4): 3.69 (2H, d, $J = 5.6$ Hz), 3.89 (0.8H, d, $J = 4.8$ Hz), 7.14 (1H, d, $J = 8.4$ Hz), 7.16 (0.4H, d, $J = 8.4$ Hz), 7.43 (1H, dd, $J = 8.4$, 2.0 Hz), 7.46 (0.4H, dd, $J = 8.4$, 2.0 Hz), 7.95 (1H, d, $J = 2.0$ Hz), 7.99 (0.4H, d, $J = 2.0$ Hz), 8.06 (0.4H, t, $J = 4.8$ Hz), 8.25 (1H, t, $J = 5.6$ Hz), 10.50 (1.4H, br s); ^{13}C NMR ($CDCl_3$) δ (ppm) major/minor: 40.1/38.3 (CH_2), 120.7/120.9 (CH), 125.1/124.9 (CH), 126.1/126.3 (C), 133.4 (C), 138.2/137.9 (CH), 154.4 (C), 173.8/171.5 (CH); ESI-/MS: m/z 215 ([M+2−H]$^-$, 33), 213 ([M−H]$^-$, 100), 177 ([M−HCl−H]$^-$, 38).

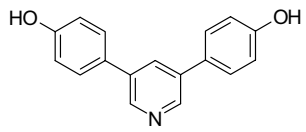


Scheme 1.

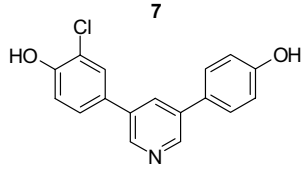
(**8**) isolated in 3% yield each,[¶] in addition to 3-chlorotyrosine, 4-hydroxyphenylacetaldehyde, 3-chloro-4-hydroxyphenylacetaldehyde and 4-hydroxyphenylacetonitrile as the main species, consistent with previous reports.[5,8,9] Extensive chlorination of the reactive phenol ring accounts in part for the lower yields of pyridine products. Phenylalanine also gave a 9% yield of 3,5-diphenylpyridine (**9**),[‖] previously prepared by reaction of phenylacetaldehyde with methyleniminium chloride,[18] while representative aliphatic amino acids, such as serine, leucine and aspartic acid, failed to give pyridine derivatives with HOCl under a variety of conditions. It may be relevant to notice that Chichibabin reactions depend on the degree of enolisation of the aldehyde intermediates,[16] a factor that may be implicated in the lack of pyridine formation from aliphatic amino acids.

[¶] Preparative TLC fractionation (eluent chloroform–methanol 95:5 v/v). **7** (R_f 0.25): ^{1}H NMR ($(CD_3)_2CO$) δ (ppm): 7.01 (4H, d, $J = 8.4$ Hz), 7.66 (4H, d, $J = 8.4$ Hz), 8.10 (1H, t, $J = 2.0$ Hz), 8.73 (2H, d, $J = 2.0$ Hz); ^{13}C NMR (CD_3OD) δ (ppm): 117.1 (4×CH), 129.4 (4×CH), 131.4 (2×C), 131.9 (2×C), 134.0 (CH), 144.6 (2×CH), 159.4 (2×C); ESI+/MS: m/z 264 ($[M+H]^+$, 100). **8** (R_f 0.33): ^{1}H NMR ($(CD_3)_2CO$) δ (ppm): 7.01 (2H, d, $J = 8.8$ Hz), 7.18 (1H, d, $J = 8.4$ Hz), 7.63 (1H, dd, $J = 8.4$, 2.0 Hz), 7.68 (2H, d, $J = 8.8$ Hz), 7.83 (1H, d, $J = 2.0$ Hz), 8.16 (1H, t, $J = 2.0$ Hz), 8.76 (1H, d, $J = 2.0$ Hz), 8.77 (1H, d, $J = 2.0$ Hz); ^{13}C NMR (CD_3OD) δ (ppm): 117.1 (2×CH), 118.2 (CH), 127.8 (CH), 129.4 (2×CH), 129.6 (CH), 130.9 (C), 131.2 (C), 131.4 (C), 131.9 (C), 133.4 (CH), 145.5 (CH), 146.1 (CH), 154.9 (C), 159.4 (C); ESI+/MS: m/z 300 ($[M+2+H]^+$, 33), 298 ($[M+H]^+$, 100).

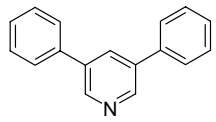
[‖] Preparative TLC fractionation (eluent: cyclohexane–ethyl acetate 80:20 v/v). **9** (R_f 0.25): ^{1}H NMR ($(CD_3)_2CO$) δ (ppm): 7.48 (2H, t, $J = 7.2$ Hz), 7.56 (4H, t, $J = 7.2$ Hz), 7.84 (4H, d, $J = 7.2$ Hz), 8.28 (1H, m), 8.89 (2H, m); ^{13}C NMR ($(CD_3)_2CO$) δ (ppm): 128.1 (4×CH), 129.3 (2×CH), 130.0 (4×CH), 134.4 (CH), 137.9 (2×C), 139.2 (2×C), 146.0 (2×CH); ESI+/MS: m/z 232 ($[M+H]^+$, 100).



7



8



9

To our knowledge, this is the first example of one-pot formation of 3,5-diarylpyridine derivatives by mild oxidation of an α-amino acid with HOCl. Although the yield of **2** is low, the reaction is nonetheless of preparative value because of the simple procedure and the ease of isolation of the product in pure form (>98% NMR analysis) without chromatographic steps. Pyridine **2** bears two nitro groups that may be conveniently modified thus offering a valuable option for the preparation of structural variants of potential synthetic and pharmacological interest, for example, as inhibitory agents of interleukin-6.[19]

Acknowledgements

This study was carried out in the frame of the MIUR projects ('Sostanze naturali ed analoghi sintetici con attività antitumorale', PRIN 2003). Thanks are also due to the Centro Interdipartimentale di Metodologie Chimico Fisiche of Naples University for NMR and mass spectrometric facilities and to Mrs. Silvana Corsani for technical assistance.

References and notes

1. Morris, J. C. *J. Phys. Chem.* **1996**, *70*, 3798–3805.
2. Larionov, O. V.; Kozhushkov, S. I.; de Meijere, A. *Synthesis* **2003**, *12*, 1916–1919.
3. Brands, K. M. J.; Wiedbrauk, K.; Williams, J. M.; Dolling, U.-H.; Reider, P. J. *Tetrahedron Lett.* **1998**, *39*, 9583–9586.
4. van Tamelen, E. E.; Haarstad, V. B.; Orvis, R. L. *Tetrahedron* **1967**, *24*, 687–704.
5. Hazen, S. L.; d'Avignon, A.; Anderson, M. M.; Fong, F. H.; Heinecke, J. W. *J. Biol. Chem.* **1998**, *273*, 4997–5005.
6. Hawkins, C. L.; Pattison, D. I.; Davies, M. J. *Amino Acids* **2003**, *25*, 259–274.
7. Weiss, S. J.; Klein, P.; Slivka, A.; Wei, M. *J. Clin. Invest.* **1992**, *70*, 1341–1349.
8. Fu, S.; Wang, H.; Davies, M.; Dean, R. *J. Biol. Chem.* **2000**, *275*, 10851–10858.
9. Pereira, W. E.; Hoyano, M.; Summons, R. E.; Bacon, V. A.; Duffield, A. M. *Biochim. Biophys. Acta* **1973**, *313*, 170–180.
10. Armesto, X. L.; Canle, L. M.; Santaballa, J. *Tetrahedron* **1993**, *49*, 275–284.
11. Armesto, X. L.; Canle, L. M.; Gamper, A. M.; Losada, M.; Santaballa, J. A. *Tetrahedron* **1994**, *50*, 10509–10520.
12. Roberts, J. T.; Rittberg, B. R.; Kovacic, P. *J. Org. Chem.* **1981**, *46*, 4111–4115.
13. Whiteman, M.; Halliwell, B. *Biochem. Biophys. Res. Commun.* **1999**, *258*, 168–172.
14. Chichibabin, A. E. *J. Russ. Phys. Chem. Soc.* **1906**, *37*, 1229.
15. Chichibabin, A. E. *J. Prakt. Chem.* **1924**, *107*, 122–128.
16. Eliel, E. L.; McBride, R. T.; Kaufmann, S. *J. Org. Chem.* **1953**, *75*, 4291–4296.
17. Farley, C. P.; Eliel, E. L. *J. Org. Chem.* **1956**, *78*, 3477–3484.
18. Winter, A.; Risch, N. *Synthesis* **2003**, *17*, 2667–2670.
19. Tagat, J. R.; McCombie, S. W.; Barton, B. E.; Jackson, J.; Shortall, J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2143–2146.