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Research Article

Synthesis of the ^{15}N-labelled insecticide imidacloprid

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Summary

A five-step synthesis of the neonicotinoid insecticide imidacloprid (**1**) labelled with ^{15}N is reported in an overall yield of 10%. The stable isotope ^{15}N was introduced in the pyridine part by reaction of $^{15}NH_3$ with coumalic acid methyl ester (**2**) to ^{15}N-hydroxy nicotinic acid (**3**) followed by further reactions to ^{15}N-2-chloro-5-(chloromethyl) pyridine (**6**) which was coupled with *N*-nitroimidazolidin-2-imine to yield **1**.

Key Words: imidacloprid; stable isotope; ^{15}N

Introduction

Pesticides are used worldwide in order to reduce crop loss caused by insects and fungi. The evolution of more resistant pests means that there is a constant need to develop new and more effective pesticides with different modes of action. In the early 1990s a new group of insecticides, the neonicotinoids, of which imidacloprid[1] is a prominent member, were commercialized.

Degradation and metabolism studies, an essential prerequisite prior to approval by the various agencies, are best performed using a labelled form of the pesticide. Especially the determination of non-extractable residues can only be carried out with a labelled pesticide. This can either be radioactive, for which there are advantages and disadvantages, or as in the present study where a stable isotope (^{15}N) is employed.

Results and discussion

Although the synthesis of [^{2}H]- and [^{3}H]-imidacloprid has been reported[2] no such information is available for the ^{15}N-labelled compound (**1**, ^{15}N-1-[(6-

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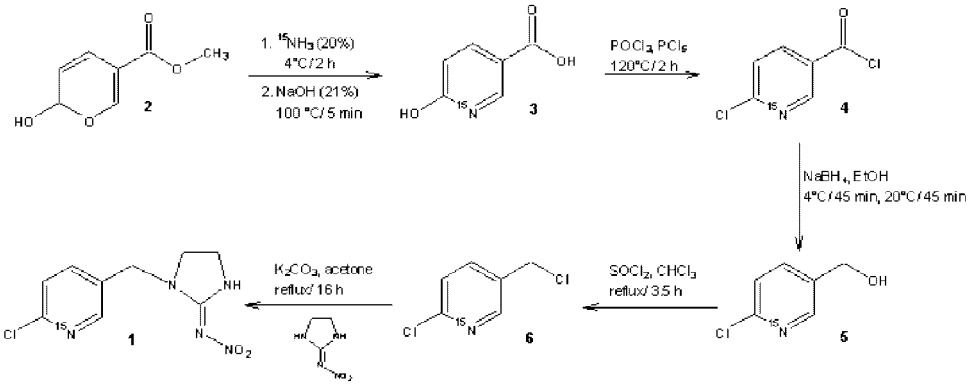

Scheme 1. Synthesis of ^{15}N-labelled imidacloprid

chloropyridin-3-yl)methyl]-*N*-nitroimidazolidin-2-imine). Our strategy was influenced by the fact that the pyridine part of the molecule is retained in the degradation products.[3–5] Several reports of the synthesis of ^{15}N-labelled pyridine have appeared in the literature[6–8] but the subsequent synthetic procedure is not an attractive one. The preferred alternative takes advantage of the fact that in the synthesis of imidacloprid itself the most common final step involves reaction of 2-chloro-5-chloromethyl pyridine and *N*-nitroimidazolidin-2-imine.[9,10] The former is usually synthesized from nicotinic acid[11] but because the ^{15}N-version of the latter is expensive we chose to react $^{15}NH_3$ with coumalic acid methyl ester (**2**) to form the hydroxy nicotinic acid (**3**). This route to the preparation of imidacloprid proposed by Tan *et al.*[12] was partly modified (Scheme 1) in order to improve yields and generate the product in a simpler and faster manner.

The complete synthesis of ^{15}N-labelled imidacloprid is shown in Scheme 1. The product was purified by preparative HPLC but no purification of the intermediates was performed. Successful labelling of all the intermediates and the ^{15}*N*-imidacloprid was confirmed by mass spectrometry and ^{15}N-NMR spectroscopy.

Experimental

Melting points were determined on a digital melting point apparatus model 8100 (Electrothermal, Southend-on-Sea, UK) and were not corrected. UV spectra were recorded with a Cary 1E spectrophotometer (Varian, Darmstadt, Germany). ^{1}H, ^{13}C and ^{15}N NMR spectra were obtained using a Unity Inova 300 spectrometer (Varian, Darmstadt, Germany) at 298 K at 300, 75 and 50 MHz (nominal frequency), respectively. Infrared attenuated total reflectance (IR-ATR) spectra were recorded on a Fourier transform infrared (FT-IR) spectrometer Avatar 320 E.S.P. (Nicolet, Offenbach, Germany).

LC–MS was performed on a VG platform II quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with an electrospray interface (ESI), operating in the positive mode. MS parameters: source temperature: 120°C, capillary 3.5 kV, HV lens 0.5 kV, cone ramp 20–60 V, full-scan mode ($m/z = 80-1200$). LC was carried out with an HP 1100 system (Agilent, Waldbronn, Germany) equipped with a degasser, an autosampler, a quarternary pump and a diode array detector (DAD, 200–400 nm). For separation, a reversed phase column (Eurospher 100-C18, 5 μm, 250 × 3 mm, Knauer, Berlin, Germany) with a guard column (C_{18}, 5 μm, 5 × 3 mm), kept at 25°C and a gradient system with methanol/formate buffer (10 mM, pH 4.0, 2% methanol) was used. Gradient conditions at a flow rate of 0.5 ml/min were: 0/5, 2/25, 7/40, 13/50, 18/50, 20/100, 30/100, 35/5 (minutes/percent of methanol). For preparative HPLC an YMC column (YMC-Pack ODS-A, 10 μm; column 250 × 20 mm, guard column 50 × 20 mm) with the same mobile phase and gradient (flow rate: 20 ml/min) as described above was employed.

^{15}N-6-hydroxy nicotinic acid (**3**)

Ten grams of aqueous $^{15}NH_3$ (obtained from Chemotrade, Leipzig, Germany; 20% aqueous solution, ^{15}N 98 at.%) was added to 4.0 g (25.6 mmol) of ice-cooled coumalic acid methyl ester (**2**, synthesized from malic acid which was subsequently converted to coumalic acid which itself was esterified with absolute methanol)[12] in portions of 1 ml in a 22 ml centrifuge tube as reaction vessel. After stirring for 2 h at 4°C in the sealed tube, the ester was saponified with 12 ml of an aqueous solution of sodium hydroxide (21%) by heating for 5 min at 100°C in the closed reaction vessel. The product was precipitated by acidifying the solution with concentrated hydrochloric acid until a pH of 4.5 was reached. The light orange precipitate was filtered, washed with ice water and dried to yield 2.33 g of crude ^{15}N-6-hydroxy nicotinic acid (yield = 64.5% of theory). Spectroscopic data: ^{1}H-NMR: (DMSO-d_6): δ (ppm) = 3.38 (OH, s), 6.36 (1 H, dd, $^3J = 9.6$ Hz, $^3J = 2.4$ Hz), 7.79 (1 H, dd, $^4J = 2.4$ Hz), 8.00 (1 H, dd, $^2J = 2.6$ Hz), 12.38 (acid OH, s); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 109.8 (s), 120.1 (d, $^2J = 7.5$ Hz), 140.5 (s), 141.2 (d, $^1J = 11.4$ Hz), 163.1 (d, $^1J = 10.5$ Hz), 166.1 (d, $^3J = 2.1$ Hz); ^{15}N-NMR (DMSO-d_6): δ (ppm) = −209.0 (pyridine-^{15}N); UV (methanol): $\lambda_{max1} = 203$ nm, $\lambda_{max2} = 258$ nm, $\lambda_{max3} = 295$ nm; IR (ATR, cm^{-1}): 3115 (w), 3078 (m), 2980 (w), 2595 (w), 2474 (m, br), 1703 (m), 1630 (s), 1595 (s), 1553 (m), 1467 (w), 1412 (m), 1337 (m), 1270 (m), 1257 (m), 1225 (s), 1128 (s), 1117 (s), 996 (m), 906 (m), 842 (m), 776 (s), 722 (m); MS (ESI+): m/z 141 ($[M+H]^+$). Spectroscopic data correspond to those documented in the literature for unlabelled 6-hydroxy nicotinic acid.[13]

^{15}N-(6-chloropyridin-3-yl) methanol (**5**)

^{15}N-6-hydroxy nicotinic acid (2.33 g, 16.6 mmol), phosphorous pentachloride (3.56 g) and phosphorous oxychloride (1.7 ml) were mixed and heated at 75°C in a 25 ml-flask.[12] When no more gas was evolved the temperature was raised to 120°C and held for 2 h. Subsequently, phosphorous oxychloride was removed under reduced pressure resulting in a brown residue of ^{15}N-6-chloronicotinoyl chloride (**4**) which was used in the next step without further purification. **4** was reduced by adding to a solution of 700 mg $NaBH_4$ dissolved in 150 ml absolute ethanol and 1.5 ml sodium hydroxide solution (0.01 N) while stirring for 45 min at 4°C and for 45 min at room temperature.[2] After removal of the solvent the residue was treated with saturated sodium chloride solution, extracted with diethyl ether and the organic phase was dried over anhydrous sodium sulfate. Evaporation of the solvent provided ^{15}N-(6-chloropyridin-3-yl) methanol (0.95 g), yield 39.4% of theory. Melting point: 53°C (lit.: 52–53°C[14] (unlabelled)); Spectroscopic data: ^{1}H-NMR: (DMSO-d_6): δ (ppm) = 4.54 (2 H, d, ^3J = 5.5 Hz), 5.43 (1 H, t), 7.49 (1 H, d, ^3J = 8.5 Hz), 7.80 (1 H, dd, ^4J = 1.7 Hz), 8.36 (1 H, d); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 60.5 (s), 124.5 (d, ^2J = 10.8 Hz), 138.0 (d, ^2J = 9.5 Hz), 138.9 (d, ^3J = 8.4 Hz), 148.8 (s) 149.3 (d, ^1J = 20.4 Hz); ^{15}N-NMR (DMSO-d_6): δ (ppm) = −75.6 (pyridine-^{15}N); UV (methanol): λ_{max1} = 215 nm, λ_{max2} = 268 nm (UV data correspond to literature[15] for the unlabelled compound); IR (ATR, cm^{-1}): 3296 (m, br), 3091 (w), 2891 (w), 2842 (m), 2719 (w), 1587 (m), 1566 (m), 1446 (s), 1381 (m), 1295 (m), 1284 (m), 1233 (w), 1207 (w), 1101 (s), 1063 (s), 1010 (s), 917 (m), 819 (s), 722 (m), 666 (m); MS (ESI+): *m/z* 145 ([M+H]$^+$).

^{15}N-2-chloro-5-(chloromethyl) pyridine (**6**)

6 was obtained by reaction of ^{15}N-(6-chloropyridin-3-yl) methanol with thionyl chloride in chloroform[2] (yield = 88.1% of theory). Melting point: 40°C; Spectroscopic data: ^{1}H-NMR: (DMSO-d_6): δ (ppm) = 4.84 (2 H, s), 7.57 (1 H, d, ^3J = 8.4 Hz), 7.96 (1 H, dd, ^4J = 2.5 Hz), 8.51 (1 H, dd, ^2J = 11.5 Hz); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 42.9 (s), 125.2 (d, ^2J = 10.8 Hz), 134.0 (d, ^2J = 10.8 Hz), 141.0 (d, ^3J = 8.4 Hz), 150.6 (s), 150.7 (d, ^1J = 18.9 Hz); ^{15}N-NMR (DMSO-d_6): δ (ppm) = −74.1 (pyridine-^{15}N); UV (methanol): λ_{max1} = 218 nm, λ_{max2} = 269 nm; IR (ATR, cm^{-1}): 3087 (w), 3052 (w), 3007 (w), 2968 (w), 1585 (m), 1561 (m), 1457 (m), 1446 (s), 1373 (m), 1283 (m), 1233 (m), 1218 (w), 1163 (w), 1137 (m), 1105 (s), 1077 (w), 1019 (m), 941 (m), 908 (w), 838 (m), 820 (s), 736 (s), 700 (s), 655 (m); MS (ESI+): *m/z* 163 ([M+H]$^+$).

*^{15}N-imidacloprid (***1***, ^{15}N-1-[(6-chloropyridin-3-yl)methyl]-N-nitroimidazolidin-2-imine)*

One gram of *N*-nitroimidazolidin-2-imine (7.7 mmol, synthesized[16] from ethylene diamine and nitro guanidine yield: 41%) and 3.9 g of K_2CO_3 were

suspended in 80 ml of acetone and a solution of ^{15}N-2-chloro-5-(chloromethyl) pyridine (0.94 g in 20 ml of acetone) was added. The mixture was heated under reflux for 16 h after which the solvent was evaporated under reduced pressure. After addition of water the solution was extracted three times with ethyl acetate. The organic layers were combined and the solvent was removed yielding 1.01 g of crude ^{15}N-imidacloprid (68.3% of theory). Purification was performed by preparative HPLC (yield after purification: 44.7%). The overall yield calculated from the initially used amount of coumalic acid methyl ester, was 10.0%. Melting point: 140°C (lit.: 143–144°C[17] (unlabelled imidacloprid)); Spectroscopic data: ^{1}H-NMR: (DMSO-d_6): δ (ppm) = 3.51 (2 H, t, ^{3}J = 9.1 Hz), 3.65 (2 H, t) 4.49 (2 H, s), 7.54 (1 H, d, ^{3}J = 8.2 Hz), 7.81 (1 H, dd, ^{4}J = 2.5 Hz), 8.38 (1 H, dd, ^{2}J = 11.6 Hz), 8.99 (1 H, s); ^{13}C-NMR (DMSO-d_6): δ (ppm) = 42.3 (s), 45.2 (s), 45.8 (s), 125.0 (d, ^{2}J = 9.6 Hz), 132.3 (d, ^{2}J = 10.8 Hz), 140.1 (d, ^{3}J = 7.2 Hz), 150.0 (s), 150.2 (d, ^{1}J = 20.1 Hz), 161.0 (s); ^{15}N-NMR (DMSO-d_6): δ (ppm) = −74.7 (pyridine-^{15}N); UV (methanol): λ_{max1} = 212 nm ($\log \varepsilon$ = 4.06), λ_{max2} = 269 nm ($\log \varepsilon$ = 4.29); IR (ATR, cm^{-1}): 3331 (m, br), 3047 (w), 2981 (w), 2906 (w), 1557 (s), 1479 (m), 1462 (w), 1433 (m), 1387 (m), 1359 (m), 1289 (s), 1273 (s), 1226 (s), 1202 (s), 1139 (m), 1128 (m), 1098 (s), 1048 (m), 932 (m), 839 (m), 816 (m), 816 (m), 737 (m), 707 (m), 664 (m); MS (ESI+): m/z 257 ($[M+H]^+$). NMR data, UV data and IR data agree with the respective data for the unlabelled compound.[2]

Conclusion

^{15}N-labelled imidacloprid, required for degradation and metabolism studies, as well as an internal standard in isotope dilution mass spectrometry, has been synthesized as part of a 5-step procedure. One of the intermediates – ^{15}N-2-chloro-5-chloromethyl pyridine – can be conveniently used for the labelling of other neonicotinoids such as thiacloprid and acetamiprid. Other pharmaceuticals, such as the cardiotonics amrinone and milrinone[18,19] or cosmetic products[20] containing the 2-pyridone structure can also be synthesized by the reported method and the use of substituted 2H-pyran-2-ones.

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