Syntheses and Antibacterial Activities of Tizoxanide, an *N*-(Nitrothiazolyl)salicylamide, and its *O*-Aryl Glucuronide†

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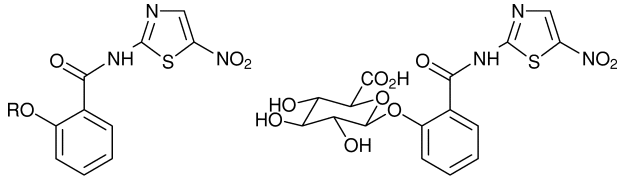
Jean-François Rossignol[a] and Andrew V. Stachulski[b]

[a]*The Romark Institute for Medical Research, 6200 Courtney Campbell Causeway, Suite 870, Tampa, Florida, USA*
[b]*Salford Ultrafine Chemicals and Research Ltd., Synergy House, Guildhall Close, Manchester Science Park, Manchester M15 6SY, UK*

Mild hydrolysis of the broad-spectrum anaerobic antibacterial and antiparasitic agent nitazoxanide **1** affords tizoxanide **2**, which is a major metabolite of **1** retaining most of its activity; further metabolism of **2** leads to the *O*-aryl glucuronide **3**, efficiently synthesised in four steps from benzyl salicylate and showing slight antibacterial activity.

The thiazole derivative nitazoxanide **1**, first described by Rossignol,[1] is a broad-spectrum antibacterial and anti-parasitic agent, particularly efficacious against anaerobic bacteria[2] and as an anthelmintic and antiprotozoal agent.[3,4] The desacetyl metabolite of **1**, tizoxanide **2**, is itself a potent antibacterial and antiparasitic agent.[2] Further metabolism of **2** leads to the glucuronide **3** and sulfate **4** conjugates. In this paper, we describe the conversion of **1** to **2** and a convenient synthesis of **3**, both to test for any remaining bioactivity and as an analytical standard.

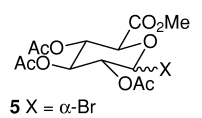


1 R = CH_3CO
2 R = H
4 R = SO_3H

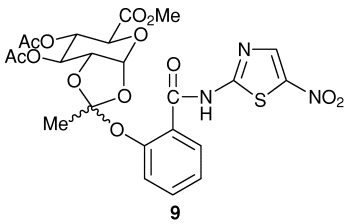
3

Tizoxanide **2** was readily obtained from nitazoxanide **1** in excellent yield by hydrolysis with aqueous HCl at 50 °C as previously given in the patent literature.[5] However, **2** could not be coupled to the bromosugar **5** using either the Koenigs–Knorr or lithium phenolate[6] methods, and acid-catalysed reactions with the tetraacetate **6**[7,8] or imidate **7**[9] were also unavailing, the low organic solubility of **2** being a major problem.

A conjugate was obtained when **2** was reacted with the 1-hydroxysugar **8**[10] under Mitsunobu conditions.[11] The ^{1}H NMR spectrum [in particular δ_H 1.8 (s) and 5.9 (d)] was consistent with the orthoester **9**[11] rather than the desired glucuronide.



5 X = α-Br
6 X = β-OAc
7 X = α-OC(=NH)CCl_3
8 X = α/β-OH



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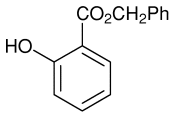

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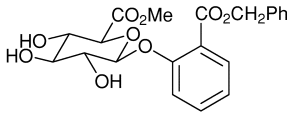

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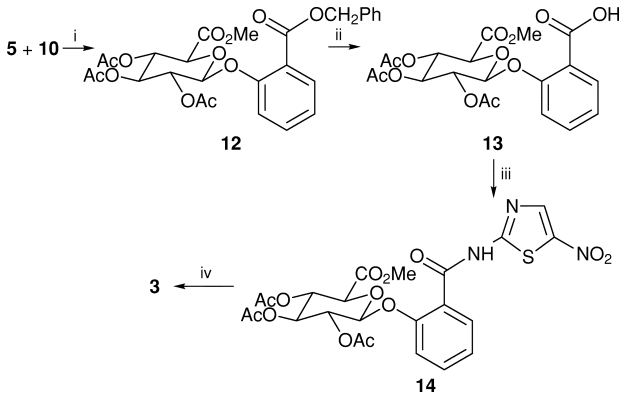

12 **13** **14**

Scheme 1 *Reagents and conditions*: i, Ag_2O, isoquinoline, 0–20 °C; ii, cyclohexene, Pd–C, Pr^iOH, heat; iii, $EtN{=}C{=}N(CH_2)_3NH^+Me_2Cl^-$, 1-hydroxybenzotriazole, 4-dimethylaminopyridine, 2-amino-5-nitrothiazole, DMF; iv, NaOH, MeOH aq., 0–20 °C then pH 6

Acid-catalysed condensation of benzyl salicylate **10** with **6** or **7** also proved quite ineffective: by contrast a 2,6-dimethylphenol has been successfully glucuronidated[12] using the classical Helferich procedure (**6** + tosic acid). Coupling of the lithium phenolate of **10** with **5** in methanol gave a low yield (24%) of a more polar product which proved to be the partially deprotected ester **11**. Rather than try to progress **11**, whose unprotected OH groups were likely to cause problems, a literature procedure for the glucuronidation of methyl salicylate[13] was very satisfactorily adapted (Scheme 1).

Koenigs–Knorr reaction of **5** with **10** gave the conjugate **12** in 61% yield after chromatography. The chemical shift and coupling constant ($J = ca.$ 8) of the anomeric proton in **12** were consistent with a β-glucuronide. Debenzylation of **12** using catalytic transfer hydrogenation gave acid **13** in 80% yield: the condensation of **13** with 2-amino-5-nitrothiazole was performed using the water-soluble carbodiimide method shown.[14] Chromatography afforded product **14** in excellent purity and 67% yield. The esters were cleaved using aq. NaOH, and after acidification to pH 6 the sodium salt of glucuronide **3** precipitated in 80% yield. By high-performance liquid chromatographic analysis this material appeared identical with the authentic metabolite.

Biological Data

The antibacterial activities of compounds **1**, **2** and **3** were compared. Minimum inhibitory concentrations (MICs) in the range 1–10 μg cm^{-3} were observed for all three compounds against *Helicobacter pylori*, **3** being about tenfold

†This is a **Short Paper** as defined in the Instructions for Authors, Section 5.0 [see *J. Chem. Research (S)*, 1999, Issue 1]; there is therefore no corresponding material in *J. Chem. Research (M)*.

less effective than **1** or **2**. Against *Sarcocystis neurona*, **1** and **2** showed MICs of 2 μg cm^{-3} while the MIC of **3** was 40 μg cm^{-3}. Against strains of the aerobic Gram-positive and Gram-negative bacteria *Staphylococcus aureus, Enterococcus faecalis, Morganella morganii, Escherichia coli* and *Pseudomonas aeruginosa* all three compounds were inactive at up to 512 μg cm^{-3}.

Further biological results, with a discussion of the mode of action of these compounds, will be published separately.

Experimental

For general directions, see an earlier paper from these laboratories.[15] Mass spectra were recorded on a Varian-Saturn GC-ITD instrument in the electron-impact (EI) mode for compound **2**, on a Kratos MS 25 instrument for chemical ionisation (CI) spectra and on a Kratos Concept 1S instrument for the fast atom bombardment (FAB) mode. Antibacterial screening was performed using either an agar dilution technique in a Wilkens Chalgren medium containing 10% blood at an inoculum of 10^9 colony forming units (CFU) cm^{-3}, for the anaerobic bacteria, or in a Mueller Hinton agar medium at an inoculum of 10^6 CFU cm^{-3} in Mueller Hinton broth for the aerobic bacteria.

2-*Hydroxy*-N-(5-*nitrothiazol*-2-*yl*)*benzamide* (*Tizoxanide*) **2**.—A suspension of 2-acetoxy-*N*-(5-nitrothiazol-2-yl)benzamide (nitazoxanide, **1**, 100 g, 0.326 mol) in 37% w/v HCl (500 cm^3) was stirred and heated at 50 °C for 24 h.[5] The resulting slurry was cooled and filtered, then the filtrate was well washed with deionized water until the washings were neutral and dried at 50 °C to give tizoxanide **2** (85 g, 98%), mp 254 °C; ν_{max}/cm^{-1} (Nujol) 1670; δ [220 MHz, $(CD_3)_2SO$] 7.00–7.15 (2 H, m, ArH), 7.60 (1 H, t, ArH), 8.00 (1 H, d, 6-H) and 8.75 (1 H, s, 4′-H); *m*/*z* (Me_3Si derivative, EI) 338 [$MSi(CH_3)_3^+$], 193 (100%, cleavage of thiazole fragment).

Methyl 1-[2-(*Benzyloxycarbonyl*)*phenyl*]-β-D-*glucopyranuronate* **11**.—The bromosugar **5** (0.60 g, 1.5 mmol) was added in one portion to a solution of benzyl salicylate **10** (0.34 g, 1.5 mmol) and $LiOH{\cdot}H_2O$ (0.063 g, 1.5 mmol) in methanol (1.5 cm^3) which was stirred at 0 °C. After 1 h, the temperature having risen to 20 °C, the solution was diluted with water containing a few drops of acetic acid, then extracted with CH_2Cl_2 (3 × 5 cm^3). Evaporation gave crude product (0.60 g) which was chromatographed to afford the product **11** as a solid (0.15 g, 24%) which on trituration with diethyl ether and recrystallisation (methanol–diethyl ether) had mp 164–166 °C (Found: C, 60.1; H, 5.3. $C_{21}H_{22}O_9$ requires C, 60.3; H, 5.3%); δ [$(CD_3)_2SO$], *inter alia*, 3.68 (3 H, s, CH_3O), 4.12 (1 H, d, 5-H), 5.23 (1 H, d, 1-H), 5.34 (2 H, m, ArCH_2O) and 7.10–7.70 (9 H, m, ArH); *m*/*z* (CI, NH_3) 436 (MNH_4^+, 12%).

Methyl 1-[2-(*Benzyloxycarbonyl*)*phenyl*]-2,3,4-*tri*-*O*-*acetyl*-β-D-*glucopyranuronate* **12**.—Silver(I) oxide (2.12 g, 0.91 mmol) was added in portions to a stirred mixture of bromosugar **5** (3.30 g, 8.31 mmol) and benzyl salicylate **10** (3.78 g, 16.6 mmol) in isoquinoline (4.6 g) at 0 °C, giving a thick slurry. On warming to 20 °C over 1 h no remaining **5** was seen (TLC in 1:1 EtOAc–hexane), so the mixture was diluted with diethyl ether and filtered through Celite, then the filtrate was worked up for a neutral product, followed by evaporation to an orange oil which was washed with hexane (2×), decanting the mother liquors, to remove the bulk of the unreacted **10**. Chromatography afforded the product **12** as a foam (2.75 g, 61%) (Found: *m*/*z*, 562.1933. $C_{27}H_{32}NO_{12}$ requires MNH_4^+, 562.1924); ν_{max} ($CHCl_3$)/cm^{-1} 1750 (vs), 1610, 1590 (sh) and 1490; δ ($CDCl_3$) 2.09 (9 H, s, 3 × CH_3CO), 3.77 (3 H, s, CH_3O), 4.21 (1 H, d, 5-H), 5.20 (1 H, m, 1-H), 5.30–5.40 (3 H, m, 2-H + 3-H + 4-H), 5.37 (2 H, s, PhCH_2O), 7.10–7.25 (2 H, m, ArH) 7.35–7.55 (6 H, m, ArH) and 7.81 (1 H, dd, ArH); *m*/*z* (CI, NH_3) 562 (MNH_4^+, 65%).

Methyl 1-(2-*Carboxyphenyl*)-2,3,4,-*tri*-*O*-*acetyl*-β-D-*glucopyranuronate* **13**.—A solution of ester **12** (2.71 g, 4.98 mmol) in propan-2-ol (75 cm^3) and cyclohexene (5 cm^3) was stirred and heated at gentle reflux for 0.5 h with Pd-C (0.3 g). The catalyst was filtered off, then the filtrate was evaporated to a foam which was dissolved in 4%. aq. $NaHCO_3$ (25 cm^3) and washed with diethyl ether (2×). Cautious acidification of the aq. phase then extraction with Et_2O gave on evaporation the acid **13** as a colourless foam (1.84 g, 80%) (Found: C, 52.4; H, 4.9; *m*/*z*, 472.1465. $C_{20}H_{22}O_{12}$ requires C, 52.85; H, 4.8%; MNH_4^+, 472.1455); ν_{max} (Nujol)/cm^{-1} 3700–2500(br), 1760 (br, s), 1610 (m) and 1495; δ (220 MHz, $CDCl_3$) 2.00–2.10 (9 H, 3 s, 3 × CH_3CO), 3.73 (3 H, s, CH_3O), 4.34 (1 H, d, 5-H), 5.35–5.45 (4 H, m, 1-H to 4-H), 7.28 (2 H, m ArH), 7.62 (1 H, t, ArH) and 8.11 (1 H, d, ArH); *m*/*z* (CI, NH_3) 472 (MNH_4^+, 100%).

Methyl 1-[2-N-(5-*Nitrothiazol*-2-*yl*)*carboxamido*]*phenyl*-2,3,4-*tri*-*O*-*acetyl*-β-D-*glucopyranuronate* **14**.—1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.81 g, 4.25 mmol) was added to a stirred suspension of acid **13** (1.75 g, 3.85 mmol), 4-*N*,*N*-dimethylaminopyridine (0.5 g, 4.10 mmol), 1-hydroxybenzotriazole monohydrate (0.65 g, 4.25 mmol) and 2-amino-5-nitrothiazole (0.615 g, 4.24 mmol) in DMF (25 cm^3) at 0 °C. After 2 h at 20 °C, then 16 h at 0 °C the solution was concentrated to near dryness, then extracted with CH_2Cl_2 (2 × 25 cm^3) and worked up for a neutral product. Evaporation gave a brown solid (2.63 g) which was chromatographed on silica. Appropriate fractions were pooled and evaporated to a sticky solid which on trituration with diethyl ether deposited the product **14** as a flaky yellow solid (1.51 g, 67%), mp 262–264 °C (Kofler block, from CH_2Cl_2–methanol–diethyl ether) (Found: C, 47.5; H, 4.15; N, 7.15. $C_{23}H_{23}N_3O_{13}S$ requires C, 47.5; H, 4.0; N, 7.2%); ν_{max} (Nujol)/cm^{-1} 3350 (sharp), 1750, 1665, 1625 (w), 1605 (m), 1530 and 1350; δ [$(CD_3)_2SO$] 1.94, 1.98, 2.05 (9 H, 3 s, 3x CH_3CO), 3.68 (3 H, s, CH_3O), 4.80 (1 H, d, 5-H), 5.05 (2 H, t) and 5.48 (3 H, m, 2-H + 3-H + 4-H), 5.67 (1 H, d, 1-H), 7.20–7.30 (2 H, m, ArH), 7.55–7.70 (2 H, m, ArH) 8.71 (1 H, s, 4″-H) and 13.39 (1 H, br s, NH); *m*/*z* (CI, NH_3) 582 (MH^+, 100%).

1-[2-N-(5-*Nitrothiazol*-2-*yl*)*carboxamido*]*phenyl*-β-D-*glucopyranosiduronic Acid* **3**.—A 2.5 mol dm^{-3} NaOH solution (5 cm^3) was added in one portion to a stirred suspension of the ester **14** (1.45 g, 2.50 mmol) in methanol (17.5 cm^3) at 0 °C. On warming to 20 °C over 1 h, a yellow solution resulted which was acidified to pH 6.9 with acetic acid, followed by evaporation to dryness. The residue was triturated with aq. ethanol, 1:4 (20 cm^3) then the yellow solid was filtered to give the sodium salt of the product **3** (1.03 g, 89%), mp > 200 °C (decomp.) from aq. ethanol (Found: *m*/*z*, 464.0367. $C_{16}H_{15}N_3O_{10}SNa$ requires MH^+, 464.0376); ν_{max} (Nujol)/cm^{-1} 3700–2500 (br), 3540, 3260, 3100 (w), 1645 (sh), 1620, 1600, 1535 and 1350; δ(D_2O) 3.53 (2 H, m) and 3.67 (1 H, t, 2-H + 3-H + 4-H), 3.83 (1 H, d, 5-H), 5.14 (1 H, d, *J* 8, 1-H), 7.16 (1 H, t, ArH), 7.29 (1 H, d, ArH), 7.54 (1 H, dt, ArH), 7.74 (1 H, dd, ArH) and 8.38 (1 H, s, 4″-H); *m*/*z* (FAB +ve ion, glycerol) 442 (MH^+, free acid), 464 (MH^+) and 486 (MNa^+). High-performance liquid chromatographic analysis of the product (C_{18} μ-Bondapak reverse-phase column, aq. acetonitrile eluent) showed an area purity of 99.25%.

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