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A Convenient Synthesis of 5′-Iodoresiniferatoxin (I-RTX)

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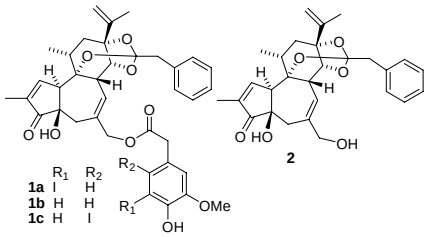
Dedicated to the memory of Professor Ivano Morelli.

Starting from resiniferonol orthophenylacetate (ROPA, **2**) and commercial 5-iodovanillin (**5a**), a convenient synthesis of the ultrapotent vanilloid antagonist 5′-iodoresiniferatoxin (**1a**) was achieved, overcoming the problems involved in the direct iodination of either resiniferatoxin (**1b**) or homovanillic acid (**3a**).

Keywords: Vanilloid antagonists, 5'-iodoresiniferatoxin, resiniferatoxin, TRV1, resiniferonol orthophenylacetate.

The study of ion channels strongly depends on the availability of compounds that can either activate or inhibit their function with high selectivity and potency[1]. While there is no shortage of ligands for sodium-, potassium-, and calcium-channels [1], most channels of the TRP type still await de-orphanization in terms of small molecule activators and/or inhibitors [2]. A remarkable exception is TRPV1, the capsaicin receptor, for which a large number of ligands (vanilloids) are available [3]. Most TRPV1 activators are either natural products or compounds derived from (or inspired by) natural products. Conversely, vanilloid antagonists are mainly synthetic compounds that have emerged from the random screening of chemical libraries. Nevertheless, none of them approaches the potency of 5'-iodoresiniferatoxin (I-RTX, **1a**), a natural product - derived ligand. I-RTX inhibits TRPV1 activation with a one-digit nanomolar $K_{(i)}$, [4]. While impressive, this value probably even underestimates the actual potency of I-RTX, whose intracellular penetration is slow compared to the time frame of most assays for vanilloid activity [5]. I-RTX is not only important as a molecular probe, but also as a potential drug, and has been investigated, with impressive results, in animal models of antitussive [6] and analgesic [7] activity.

I-RTX was serendipitously discovered by Wahl while attempting to prepare a radioactive derivative of the ultrapotent vanilloid agonist resiniferatoxin (RTX, **1b**) [4]. The molecular bases for the reversal of activity induced by aromatic iodination *ortho* to the phenolic hydroxyl are unknown, but a similar observation was made with capsaicinoids for iodination at the carbons *ortho*- and *meta*- to the phenolic hydroxyl [8]. Remarkably, iodination of RTX *meta* to the phenolic hydroxyl generated instead a partial agonist (**1c**) [9].

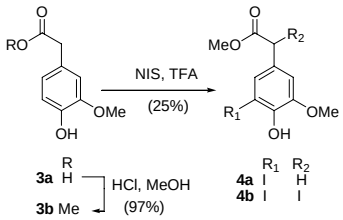


Despite the relevance of I-RTX for pharmacological research, a convenient synthesis of this compound has not yet been reported. The original synthesis by Wahl [4] was improved by a Merck group [10], and is based on the iodination of RTX with the sodium iodide/chloramine T system. Since RTX is labile in these conditions, the reaction requires careful control, and must be quenched at incomplete conversion. [10]. After HPLC purification, I-RTX was eventually

obtained in *ca* 22% yield. Given the low yield of the reaction, its problematic scale-up, and the exorbitant price of RTX [11], this synthesis is unsuitable to produce the amounts of I-RTX needed to profile its bioactivity in *in vivo* experiments.

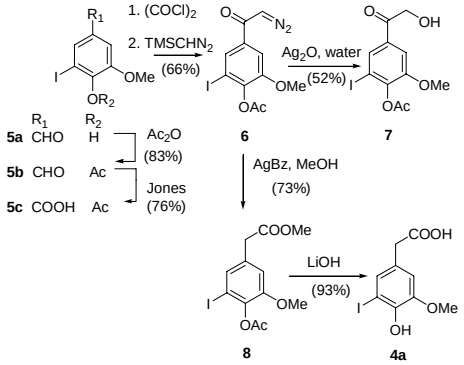
We reasoned that resinifernol orthophenylacetate (ROPA, **2**), the terpenoid core of RTX, would be a more convenient starting point for the synthesis of I-RTX, both in terms of availability of the starting material and purification of the final product. Thus, while RTX is a highly offensive compound, ROPA can be manipulated under normal laboratory conditions, and can be obtained relatively easily from the partially hydrolyzed latex of *Euphorbia resinifera* Berg., a household plant [12]. Conversely, the isolation of RTX from the native latex is difficult and hazardous due to its obnoxious properties and to the occurrence of irritant and tumor-promoting ingenol and deoxyphorbol esters that share the polarity and chromatographic behavior of RTX [12]. Finally, carrying out the iodination at the stage of a simple vanillyl derivative will also solve the problem of the instability of the terpenoid core of ROPA in the iodinating conditions. A similar strategy has been reported for the preparation of 6'-iodoresiniferatoxin (**1c**) [9], but, surprisingly, no attempt has been made so far to extend this strategy to its more important 5'-isomer.

The iodination of homovanilllic acid (**3a**) was first investigated (Scheme 1). This compound and its esters have been reported to be poor substrates for aromatic iodination [9], and also, in our hands, complex mixtures were obtained with a variety of iodinating conditions. However, methyl homovanillate (**3b**) could be iodinated, albeit in poor yield, with the *N*-iodosuccinimide (NIS) - trifluoroacetic acid (TFA) protocol [13]. The compound obtained (**4a**) contained *ca*. 5-10% of an impurity, tentatively identified as the product of α, 5′-bis-iodination (**4b**) on the basis of MS and ^{1}H NMR spectroscopic evidence. Thus, a peak corresponding to the incorporation of two iodine atoms was observed in the MS, while the ^{1}H NMR spectrum showed two additional *meta*-coupled aromatic protons. This by-product could not be removed by either chromatography or crystallization. After hydrolysis and Mitsunobu esterification [14] with ROPA, I-RTX (**1a**) was obtained, still contaminated, however, with the corresponding bis-iodinated impurity. Since preparative HPLC could not afford a completely pure material, this approach, though simple, was abandoned, and an alternative strategy based on the homologation of 5-iodovanillic acid was explored (Scheme 2).



Scheme 1: Iodination of methyl homovanillate (**3b**).

While homovanillic acid (**3a**) is expensive, 5-iodovanillin (**5a**) is cheap and commercially available in high purity [15]. After acetylation and oxidation, an acetylated carboxylic acid precursor for the one-carbon Arndt-Eisert homologation was obtained (**5c**). Reaction with oxalyl chloride and next with trimethylsilyldiazomethane [16] afforded the stable diazoketone **6**. The Wolf rearrangement of **6** in water with silver oxide gave mainly the corresponding acyloin **7**, while the reaction was successful after switching to the methanol-silver benzoate system. [17]. The resulting acetylated methyl ester was next hydrolyzed (LiOH, THF-water), affording 5'-iodohomovanillic acid (**4a**) as a crystalline compound in 43% yield overall from **5a** (Scheme 2).



Scheme 2: Synthesis of 5'-iodohomovanillic acid (**4a**) from commercial 5-iodovanilline (**5a**).

The final Mitsunobu coupling of **4a** and ROPA (**2**) could be carried out with crude ROPA (*ca*. 80%, HPLC) and the DIAD-TPP redox couple. After solvent removal, the residue was dissolved in toluene and cooled to remove the hydrazodicarboxylate-triphenylphosphine oxide crystalline adduct [18] Further purification by gravity column chromatography on neutral alumina affording I-RTX (**1a**) as a colorless foam in a reproducible yield of 52% and a HPLC purity of *ca* 95%.

In conclusion, a convenient synthesis of I-RTX, an ultrapotent vanilloid antagonist, has been reported,

overcoming the problems posed by the iodination of RTX and homovanillic acid and filling an important gap in vanilloid research.

Experimental

Acetyl-5-iodovanillic acid (**5c**)**:** To a solution of 5-iodovanillin acetate (**5b**, 1g, 3.12 mmol, prepared from commercial 5-iodovanillin (**5a**) by reaction with Ac_2O-pyridine) in acetone (10 mL), freshly prepared Jones reagent [19] was added (3 mL). After stirring overnight at room temperature, the reaction was worked up by concentration and filtration over Celite. The filtrate was then extracted with diethylether, and the organic phase was washed with brine. After drying and removal of the solvent, the residue was crystallized from diethylether to afford 800 mg (76%) of **5c** as a white powder.

MP: 199ºC.
Rf: 0.37 (light petroleum -EtOAc 7:3).
IR (KBr): 3072, 1764, 1683, 1572, 1409, 1294, 1193, 1168, 1037 cm^{-1}.
^1H NMR (300 MHz, $CDCl_3$): 2.39 (3H, s, Ac), 3.89 (3H, s, OMe), 7.64 (1H, d, *J* = 1.5 Hz, Ar-H), 8.16 (1H, d, *J* = 1.5 Hz, Ar-H).
^{13}C NMR (75 MHz, $CDCl_3$): 20.9 (CH_3), 56.5 (CH_3), 91.7 (C), 113.8 (CH), 129.0 (CH), 132.8 (C), 145.4 (C), 151.6 (C), 167.5 (C), 170.3 (C).
CI-EIMS: m/z $[M+H]^+$ 321 $[C_{10}H_9IO_5 + H]^+$

α-Diazo-5-iodoacetovanillone acetate (**6**)**:** To a cooled solution of **5c** (700 mg, 2.1 mmol) in dry CH_2Cl_2 (4 mL), oxalyl chloride (0.73 mL, 8.4 mmol, 4 mol. equiv.) and cat. DMF (0.20 mL) were added. After stirring for 1 h at 0°C and 90 min at room temperature, the reaction was worked up by evaporation, and the residue dissolved in THF-acetonitrile (1:1, 10 mL). After cooling to 0°C, $TMSCHN_2$ (2M in diethylether, 1.84 mL, 3.94 mmol, 1. 9 mol. equiv.) was added. The brownish-colored reaction was stirred at 0°C for 30 h, and then quenched by the addition of 0.5 N acetic acid. The reaction was then worked up by the addition of satd $NaHCO_3$, and the organic phase was separated, washed with brine and evaporated. The residue was purified by gravity column chromatography on silica gel (25 g, light petroleum -EtOAc 8:2 as eluant) to afford 471 mg (66%) **6** as a yellowish powder.

MP: 143ºC.
Rf : 0.32 (light petroleum -EtOAc 7:3).
IR (KBr): 3113, 3027, 2412, 2117, 1768, 1566, 1407, 1282, 1196, 1027, 903 cm^{-1}.
^1H NMR (300 MHz, $CDCl_3$): 2.23 (3H, s, Ac), 3.73 (3H, s, OMe), 5.71 (s, 1H), 7.29 (1H, d, *J* = 1.5 Hz, Ar-H), 7.49 (1H, d, *J* = 1.5 Hz, Ar-H).
^{13}C NMR (75 MHz, $CDCl_3$): 20.9 (CH_3), 56.5 (CH_3), 91.7 (C), 110.8 (CH), 128.7 (CH), 136.6 (C), 144.3 (C), 151.9 (C), 167.6 (C), 184.0 (C), 225.1 (C).
CI-EIMS: m/z $[M+H]^+$ 361 $[C_{11}H_9IN_2O_4 + H]^+$

Methyl 5′-iodohomovanillate (**8**)**:** To a refluxing solution of **6** (350 mg, 0.97 mmol) in methanol (4 mL), freshly prepared silver benzoate [17] (140 mg) and triethylamine (2 mL) were added. After refluxing for 1 h, the reaction was worked up by filtration over silica gel and evaporation, and the residue was crystallized from diethylether to afford 260 mg (73%) **8** as an amorphous brownish powder.

Rf: 0.45 (light petroleum -EtOAc 7:3).
IR (KBr): 3644, 1767, 1737, 1463, 1415, 1279, 1189, 1042, 1010, 901 cm^{-1}.
^1H NMR (300 MHz, $CDCl_3$): 2.35 (3H, s, Ac), 3.54 (2H), s), 3.69 (3H, OMe), 3.80 (3H, s, OMe), 6.86 (1H, d, *J* = 1.5 Hz, Ar-H), 7.29 (1H, d, *J* = 1.5 Hz, Ar-H).
^{13}C NMR (75 MHz, $CDCl_3$): 20.9 (CH_3), 40.5 (CH_2), 52.4 (CH_3), 56.2 (CH_3), 91.7 (C), 113.8 (CH), 130.9 (CH), 134.2 (C), 141.9 (C), 151.4 (C), 168.1 (C), 171.3 (C).
CI-EIMS: m/z $[M+H]^+$ 365 $[C_{12}H_{13}IO_5 + H]^+$

5′-Iodohomovanillic acid (**4a**)**:** To a solution of **8** (240 mg, 0.66 mmol) in water-THF 2:1 (3 mL), LiOH (194 mg, 4.6 mmol, 7 mol. equiv.) was added. After stirring at room temperature overnight, the reaction was diluted with water, extracted with EtOAc, sequentially washed with 2 N H_2SO_4 and brine, and then evaporated. The residue was purified by crystallization from CH_2Cl_2, affording 190 mg of a white powder.

MP: 178ºC.
Rf: 0.15 (light petroleum -EtOAc 6:4).
IR (KBr): 3412, 1710, 1506, 1273, 1222, 1161, 1025, 884, 824 cm^{-1}.
^1H NMR (300 MHz, $CDCl_3$): 3.54 (2H, s), 3.88 (3H, s, OMe), 6.10 (1H, -OH, s), 6.76 (1H, d, *J* = 1.5 Hz, Ar-H), 7.21 (1H, d, *J* = 1.5 Hz, Ar-H).
^{13}C NMR (75 MHz, acetone-d_6): 44.7 (CH_2), 55.5 (CH_3), 88.9 (C), 114.2 (CH), 124.9 (CH), 129.6 (C), 148.0 (C), 148.1 (C), 172.0 (C).
CI-EIMS: m/z $[M+H]^+$ 309 $[C_9H_9IO_4 + H]^+$

5′-IodoRTX (**1a**)**:** To a cooled (0°C) stirred solution of ROPA (**2**, 220 mg, 0.47 mmol) and 5′-iodohomovanillic acid (**4a**, 145 mg, 0.47 mmol, 1

mol. equiv.) in dry THF (2 mL), triphenylphosphine (TPP, 147 mg, 0.56 mmol, 1.2 mol. equiv.) and diisopropylazodicarboxylate (DIAD, 0.101 mL, 0.56 mmol,. 1.2 mol. equiv.) were added. After stirring at room temperature for 2 h, the reaction was worked up by evaporation, and the residue was dissolved in toluene (*ca*. 5 mL) and cooled to 4°C overnight. After filtration of the copious white precipitate, the filtrate was purified by gravity column chromatography on alumina (25 mL, light petroleum-EtOAc 8:2 as eluant) to afford 184 mg (52%) of **1a**, having physical and spectroscopic (^{1}H NMR) properties identical to those reported in ref. 10.

^{13}C NMR (75 MHz, $CDCl_3$): 10.4 (CH_3), 18.9 (CH_3), 19.9 (CH_3), 33.1 (CH), 35.8 (CH_2), 39.2 (CH), 39.4 (CH_2), 40.5 (CH_2), 41.1 (CH_2), 55.4 (CH_3), 56.4 (CH), 72.0 (C), 73.5 (C), 80.6 (CH), 81.2 (C), 84.5 (C), 110.8 (CH_2), 112.0 (CH), 117.9 (C), 126.7 (CH), 127.3 (C), 127.4 (CH), 128.9 (CH), 131.2 (CH), 131.4 (CH), 134.0 (C), 135.0 (C), 136.7 (C), 145.1 (C), 146.1 (C), 146.5 (C), 158.5 (CH), 171.1 (C), 208.4 (C).

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