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Synthesis of hydantoin analogues of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine with insulinotropic properties

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This article is dedicated to Professor Pierre Potier, in memory of his second anniversary.

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ABSTRACT

The first synthesis of an optically pure (2*R*,3*R*,4*S*)-hydantoin **2**, analogue of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine, was achieved in two steps in un-optimized 35% overall yield from previously reported aldehyde synthon **1**. (2*R*,3*R*,4*S*)-Hydantoin is stable at acidic pH. This solves the major drawback of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine that easily cyclizes into inactive lactone. Furthermore, (2*R*,3*R*,4*S*)-hydantoin stimulates the insulin secretion by 150% at 25 μM compared with 4-hydroxyisoleucine and insulin secretagogue drug repaglinide. In view of its stability and biological activity, (2*R*,3*R*,4*S*)-hydantoin represents a good candidate for type-2 diabetes management and control.

Type-2 diabetes, formerly known as non-insulin dependent diabetes mellitus, has reached epidemic proportions with currently 180 million persons worldwide. This number is projected to reach 300 million by 2030.[1] Besides increasing morbidity and mortality, type-2 diabetes remains the most costly disease undergoing social, familial, and professional impacts.[2] All therapeutic protocols and trials focus on the prevention of short- and long-term complications, mainly due to micro- and macro-vascular disorders. Microvascular injuries lead to retinopathy, nephropathy, and neuropathy in long-term diabetes patients. However, macro-vascular complications provoke coronary heart disease, stroke, and myocardial infraction, and represent the major cause of handicap and death. Type-2 diabetes is a metabolic disorder characterized by at least three major defects, decreased insulin secretion, altered insulin efficiency (tissue resistance), and increased release of hepatic glucose. Each of these disorders involves specific physiological targets justifying the need for large spectrum therapeutic agents.

Besides insulin, several drugs have proven their efficacy to reduce hyperglycemia-induced disorders. Each possesses its own pharmacological mechanism of action and exhibits diverse toxicological risks.[3,4] For instance, sulfonylureas that stimulate insulin secretion through interaction with a specific islet cell receptor, may cause light to severe hypoglycemia. Bisguanide metformin that suppresses glucose biosynthesis and enhances tissue sensitivity to insulin, causes severe lactic acidosis. Thiazolidinediones that enhance tissues sensitivity and repaglinide, a rapid acting insulin secretaguogue, both undergo hepatic injury.[5]

Fenugreek (*Trigonella foenum-graecum*) seeds have been traditionally used in Asia and Africa to decrease hyperglycemia in diabetic patients.[6,7] The non-proteinogenic amino acid, (2*S*,3*R*,4*S*)-4-hydroxyisoleucine, extracted from the seeds of fenugreek, was proven to possess interesting insulinotropic properties. Indeed, (2*S*,3*R*,4*S*)-4-hydroxyisoleucine enhance insulin secretion proportionally to glucose concentration without any hypoglycemic risk.[8]

We have published the first total synthesis of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine.[9] The key steps were the bio-conversion of ethyl-2-methylacetoacetate to ethyl-(2*S*,3*S*)-2-methyl-3-hydroxybutanoate and an asymmetric Strecker synthesis that provides the desired amino acid in a stereoselective manner. The major drawback of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine lies in its high propensity to form a five-membered lactone between the OH group and the carboxylic group under acidic conditions. It has been demonstrated that the lactone of 4-hydroxyisoleucine possesses no insulinotropic activity.[10] In a stomach, it is likely that the pH is low enough to convert most, if not all, of the hydroxyisoleucine into its inactive lactone analogue. In order to solve this problem, we have focused our effort to develop derivatives of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine that may have similar pharmacological profiles of those of the parent compound yet are more stable under acidic conditions. We have synthesized for the first time hydantoin analogues that are stable in acidic conditions and stimulate insulin secretion.

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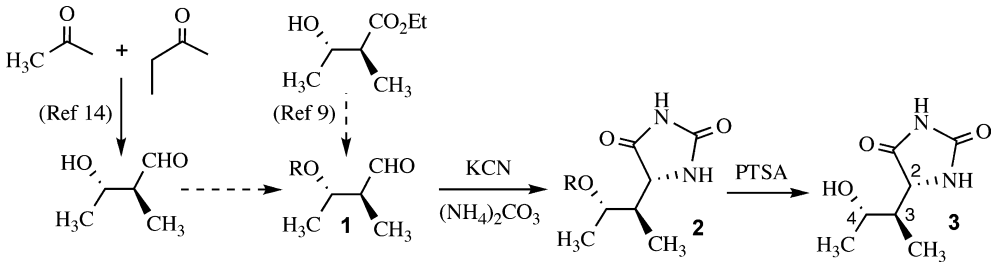

Figure 1. Synthesis of 5-(2-hydroxy-1-methyl-propyl)-imidazolidine-2,4-dione **3** (hydantoin) from aldehyde **1**.

In order to improve our previously reported synthesis of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine, and to avoid the Strecker step, we opted for the hydantoin approach. Despite the synthetic challenge, hydantoin offers various advantages compared to the parent amino acid. The first advantage is their expected stability in acidic conditions that overcome the major drawback in hydroxyisoleucine development. The second advantage is the prodrug character of hydantoins that may generate the parent amino acid through liver hydantoinase hydrolysis.

Hydantoin **2** was prepared from aldehyde **1** through the classical Bucherer–Bergs reaction.[11–13] Aldehyde **1** represents the key intermediate and could be prepared according to our previously reported approach[9] or by the method of Cordova[14] (Fig. 1). The hydantoin **2** is obtained in 70% un-optimized yield and with 90% diastereomeric excess. The latter was measured by NMR signal integration of the proton at position 2. The hydantoins reported in this Letter are members of a new family of compounds that have never been reported before.

Compound **2** was deprotected to give the hydantoin **3** in un-optimized 50% yield and with 90% isomeric excess (NMR-peak integration).[15] The two isomers of **3** were converted separately to their corresponding five-membered lactones and were compared with the lactones obtained from (2*S*,3*R*,4*S*) and (2*R*,3*R*,4*S*)-4-hydroxyisoleucine, respectively. Lactonization was conducted in acidic conditions to exclude any epimerization risk. Analysis of thus obtained lactones by NMR shows unambiguously that the minor hydantoin bears the natural (2*S*,3*R*,4*S*) stereochemistry (Fig. 2). The major one has the inverse stereochemistry at position 2 being (2*R*,3*R*,4*S*)-hydantoin.

Major and minor isomers of hydantoin **3** were hydrolyzed,[16] to give the expected (2*R*,3*R*,4*S*) and (2*S*,3*R*,4*S*)-4-hydroxyisoleucine.[17] Hydantoin approach is so far the shortest and efficient way to (2*R*,3*R*,4*S*)-4-hydroxyisoleucine.

Stability to pH. The stability of 4-hydroxyisoleucine and its insulinotropic activity is compromised by the proximity between the carboxyl and the hydroxy groups that favors the formation of the corresponding lactone. In the fenugreek seeds, a small proportion of 4-hydroxyisoleucine exists as a lactone (5–10%) that accounts for the aromatic properties of the curry spice (15–30% of fenugreek seeds powder). Pure 4-hydroxyisoleucine has no particular odor. Prior to biological evaluations, we studied the benefit of the hydantoin form in terms of acidic stability. For the pH stability of hydantoin versus 4-hydroxyisoleucine, we measured the integration of NMR signals of the five-membered lactones. The NMR tubes were filled with 0.5 ml of D_2O containing 10 mg of compound. HCl was added to the final concentration of 0.5 M and the tubes were kept at 37 °C during the experiment. NMR spectra were recorded for every 1 h and compared.

As shown in Figure 3,[18] Hydantoin is largely more stable than 4-hydroxyisoleucine. Thus, after 4 h of incubation (histograms and NMR details), 29% of 4-hydroxyisoleucine is converted to lactone (Fig. 3a) compared to 5.7% for the hydantoin (Fig. 3b). This is easily understandable since the hydantoin must undergo hydrolysis before cyclization to lactone.

Biological activity. Stimulation of insulin secretion was evaluated on rat pancreatic Langerhans islets.[19] (2*R*,3*R*,4*S*) and (2*S*,3*R*,4*S*)-hydantoins were compared to 4-hydroxyisoleucine and repaglinide, as positive controls. In each experiment, the insulin stimulation must be compared to the basal insulin level (untreated islets). Basal secretion is 45 pg/islet/h for 4-hydroxyisoleucine experiment and 100 pg/islet/h for repaglinide and hydantoin. In our conditions, repaglinide, as well as other compounds, failed to induce insulin secretion at concentrations below 25 μM. This is not in agreement with literature.[20] Figure 4 confirms that 4-hydroxyisoleucine enhances the insulin secretion by the islets, compared to untreated rats (100 pg/islet/h at 75 μM instead of 45 pg/islet/h). This result obtained in vitro by using buffered neutral solution needs to be balanced in case of oral 4-hydroxyisoleucine administration. Stimulation of insulin secretion by 4-hydroxyisoleucine is not dose dependent and remains constant

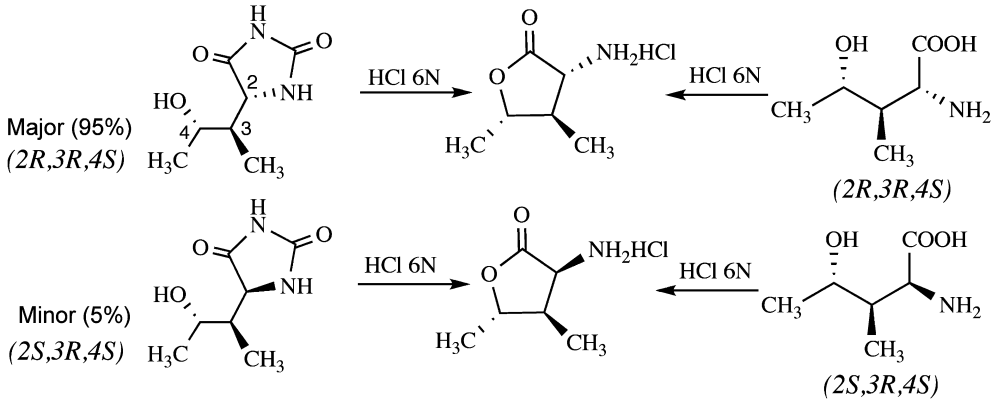

Figure 2. Determination of the absolute configuration of hydantoins.