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First synthesis of 3-*O*-methyl-scyllo-inosamine, a natural product which favors the Rhizobium–Leguminosae symbiosis

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Abstract—Rhizopine, extracted in small amounts from nodules induced by *Sinorhizobium meliloti* strain L5-30 infection of *alfalfa*, was proved to possess the 3-*O*-methyl-scyllo-inosamine gross structure.

Members of the bacterial genera *Rhizobium*, *Azorhizobium*, and *Sinorhizobium* form symbiotic associations with the plant family Leguminosae allowing the conversion of atmospheric nitrogen into an ammonium salt that the plant can utilize (symbiotic nitrogen fixation).[1] This process is important in agriculture since it favors the growth of Leguminosae in soil with low bioactive nitrogen content.

Both partners benefit from the interaction, which involves the invasion of the root hair by the *Rhizobia*, which enters into the plant, differentiates into bacteroids and leads to nitrogen-fixing nodules.[2]

Rhizopines are opine-like compounds produced only by the bacteroid in the nodule. They are neither detected in other parts of the plant nor in free-living bacteria.[3] They are known to favor the growth of free-living *Rhizobia*, which are not only able to catabolize them but also use them as a source of carbon and nitrogen.[1,3,4] Genes involved in rhizopine catabolism (*moc* genes) and synthesis (*mos* genes) have been recently identified and characterized.[1,4]

One of the rhizopines (rhizopine) has been extracted in small amounts from nodules induced by *Sinorhizobium meliloti* strain L5-30 (*S. meliloti*) infection of alfalfa (3.3 ng/g fresh weight, 21 days after inoculation).[2] It was detected by high voltage electrophoresis on Whatman 3MM paper stained with silver nitrate.[4] Furthermore it has been suggested[4] that it possesses the L-3-*O*-methyl-scyllo-inosamine structure (3-*O*-MSI) **1** but this has not yet been firmly established (Fig. 1).

The structure determination for rhizopine relies on the GC–MS of a poorly purified extract, which was subjected to extensive acetylation (see below).[2] It has a molecular weight of 403, but the molecular ion was not observed[5] and has been characterized by the presence of peaks at m/z 223 (loss of three acetic acid molecules), 181 (most abundant base peak, loss of one ketene group), 136 (loss of another ketene group). These ions along with several others, including that at m/z 167, have been used as a fingerprint to identify rhizopine.[2]

We disclose the first synthesis of *3-O-methyl-scyllo-inosamine* **1** and its fully acetylated derivative whose

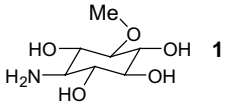

Figure 1. Structure of rhizopine: 3-*O*-methyl-scyllo-inosamine.

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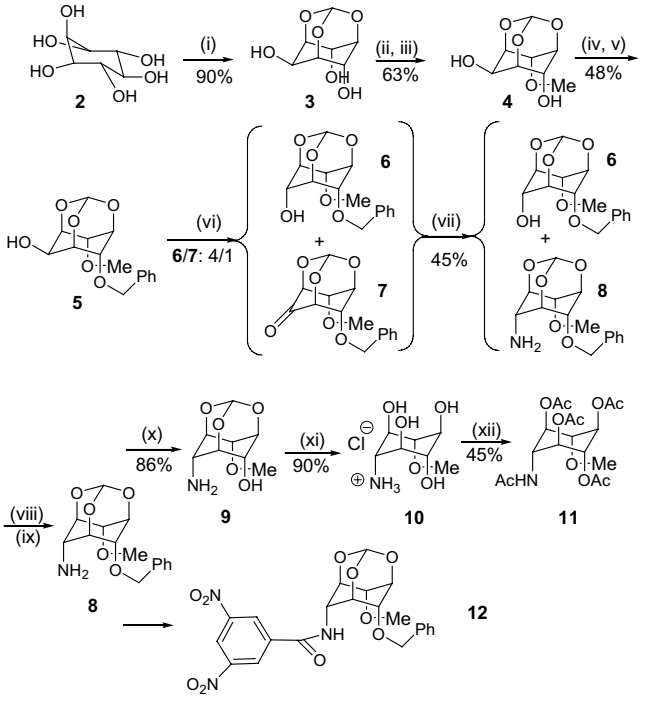
physical properties favorably compare with those of authentic samples of rhizopine isolated from alfalfa.[2]

The synthetic strategy developed is reminiscent to that of Kirby for a related compound.[6] It uses commercially available myo-inositol **2** as starting material and takes advantage of the conformational rigidity of the corresponding orthoformate **3** in which two hydroxyl groups are axially oriented whereas the remaining one is equatorial [2 mol equiv $HC(OEt)_3$, Amberlyst 15, DMF, 100 °C, 3 h, 90% yield, Scheme 1].[7]

Alkylations, sequentially carried out with sodium hydride in DMF and methyl iodide then with benzyl bromide led to the monomethyl–mono benzyl derivative **5** as well as some dibenzylated compound from which it could be easily separated (Scheme 1). The selective methylation of one of the axially oriented hydroxyl group has been attributed to the easier formation of the corresponding alkoxide due to intramolecular stabilization from the remaining alcohol.[6] The choice of the sequence is crucial since reversing the order of alkylation dramatically lowers the overall yield due to the competing formation of the dibenzyl ether in addition to the desired monobenzyl derivative.

It was decided to take advantage of the better accessibility of reagents from the β-face to introduce the amino group with the required axial orientation. This was effectively achieved in two successive steps involving oxidation of the equatorial hydroxyl group to a ketone followed by its reductive amination.



Scheme 1. Synthesis of racemic 3-*O*-methyl-scyllo-inosamine from myo-inositol. (i) 2 mol equiv $HC(OEt)_3$, Amberlyst 15, DMF, 100 °C, 3 h; (ii) NaH, DMF, 25 °C, 1 h; (iii) MeI, 25 °C, 15 h; (iv) NaH, DMF, 25 °C, 2 h; (v) BnBr, 25 °C, 15 h; (vi) 1.5 equiv NMO, 0.2 equiv TPAP, CH_2Cl_2, molecular sieves; (vii) 10 equiv NH_4OAc, 1.05 equiv $NaBH_3CN$, 20 °C, 72 h; (viii) HCl, ether; (ix) 1 M NaOH; (x) 4 equiv Na, liq. NH_3, *t*-BuOH, THF, −78 to 20 °C, 18 h; (xi) HCl (g), methanol, 20 °C, 3 h; (xii) Ac_2O, AcONa, 20 °C, 1.5 h.

The first step, performed with tetrapropylammonium perruthenate (TPAP) in the presence of *N*-methyl morpholine *N*-oxide (NMO) surprisingly gives, beside the desired ketone **7**, the epimeric alcohol **6** with the hydroxyl group in an axial position (80/20 ratio).[8]

Reaction of the crude mixture with ammonium acetate and sodium cyanoborohydride[6,9] leads to the required amine **8**, which was separated from the accompanying alcohol **6** by taking advantage of the insolubility of its ammonium salt in ether (Scheme 1, step viii). Pure amine **8** was finally obtained in 45% yield after a basic workup.

The presence of the axial alcohol **6** as well as the ketone **7** was unexpected. Its formation was also observed when the oxidation was carried out under Swern conditions.[5] Furthermore we have been unable to oxidize **6** under conditions, which allow the oxidation of its epimer **5** and not even when using PDC or PCC instead.

The structure and stereochemistry of **8** has been ascertained on the basis of X-ray crystallography of its 3,5-dinitrobenzoate **12**.[10]

3-*O*-Methyl-scyllo-inosamine hydrochloride **10** was synthesized from **8** by (i) debenzylation using sodium in liquid ammonia [Scheme 1, step x, 86% yield of **9**][1] and (ii) acid hydrolysis of the tripode [Scheme 1, step xi, 90% yield of **10**].

The electrophoretic mobilities of natural rhizopine **1** extracted from nodules induced by *S. meliloti* strain L5-30 and our sample of racemic-3-*O*-methyl-scyllo-inosamine are similar.

Both samples remain unaffected when incubated with *S. meliloti* Rm1021 (*moc*−) strain missing the gene coding for rhizopine catabolism and are catabolized when incubated with *S. meliloti* L5-30 (*moc*+) strains. It is surprising that our racemic sample was fully catabolized by (*moc*+) type Rhizobium, which would be expected to react with only one of the two antipodes.

Furthermore, not only are the IR spectra of both the synthetic product and the natural rhizopine superimposable (Fig. 2) but in addition the GC mass spectra of the fully acetylated 3-*O*-methyl-scyllo-inosamine **11** (Fig. 3) exhibits strong similarities with that of an acetylated authentic sample recently published.[2]

The data described above strongly support the structural identification of 3-*O*-methyl-scyllo-inosamine **1** as rhizopine. We are planning the synthesis of scalemic-3-*O*-methyl-scyllo-inosamine to understand its biological activity and to determine the absolute stereochemistry of rhizopine.

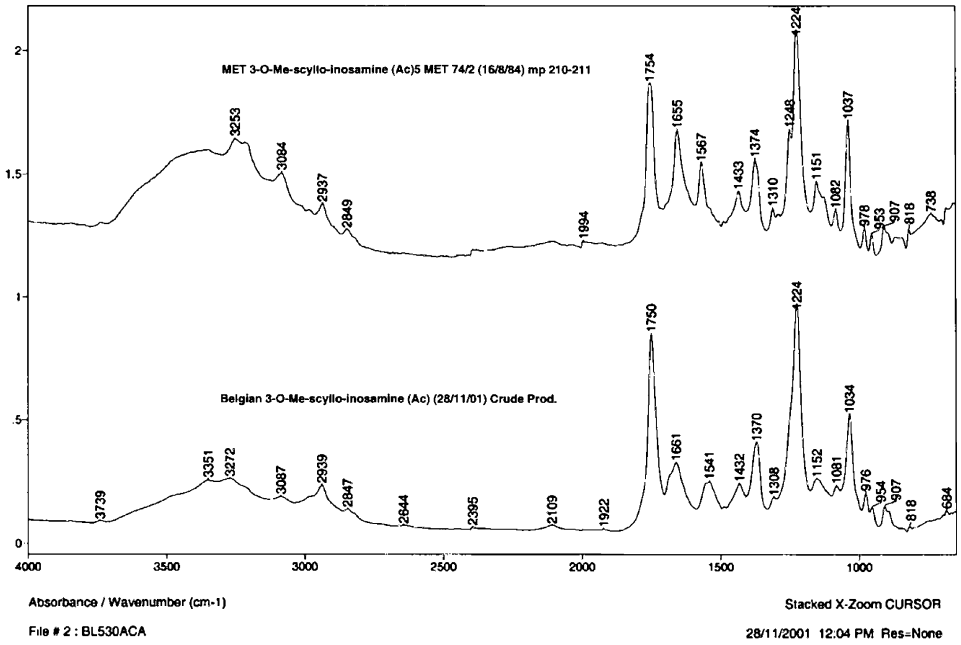


Figure 2. IR spectra of *rac*-3-*O*-methyl-scyllo-inosamine and rhizopine.

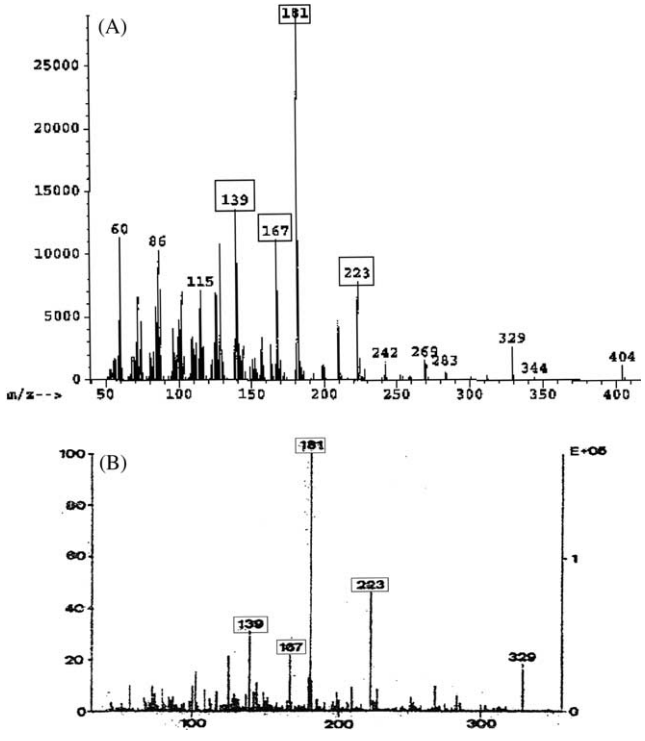


Figure 3. Mass spectra of natural rhizopine (A) and of our sample of racemic 3-*O*-methyl-scyllo-inosamine (B).

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10. The X-ray structure of **12** will be published elsewhere.