Concise Synthesis of Arogenate. A Biosynthetic Precursor of Phenylalanine and Tyrosine

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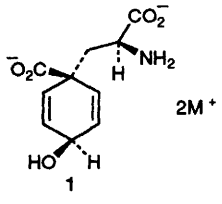
A concise new route to arogenate [L-(8*S*)-β-(1-carboxy-4-hydroxy-2,5-cyclohexadien-1-yl)alanine diammonium salt] **1** in which Michael addition of the anion derived from methyl 1,4-dihydrobenzoate **2** to the dehydroalanine derivative **3** is the key C–C bond-forming step is described.

Arogenate [L-(8*S*)-β-(1-carboxy-4-hydroxy-2,5-cyclohexadien-1-yl)alanine ion(2-)] **1** is a precursor in the biosynthesis of L-phenylalanine and L-tyrosine in some microorganisms and plants.[1] The free acid, arogenic acid, is very unstable and is quantitatively converted into L-phenylalanine.[2] Arogenate is moderately stable at pH 7.5 in the solid state but decomposes in strong base or on heating. In view of the precarious stability of arogenate **1** and its difficult accessibility from natural sources,[2] an efficient synthesis seemed to be an attractive alternative to isolation. A synthesis of arogenate **1** that relied on a Diels–Alder reaction to establish the carbon skeleton has been communicated previously,[3] and synthesis *via* immobilized microbial proteins has also been reported.[4] The synthesis that we now report is concise, affords enantiomerically pure arogenate, is amenable to scaling up, and should allow an entry to arogenate analogues.

Our retrosynthetic analysis suggested that the carbon framework of arogenate could be constructed in one step by the Michael addition of the anion derived from methyl 1,4-dihydrobenzoate **2** to a suitable dehydroalanine derivative **3**. Subsequent oxygenation of the doubly allylic C-4′ position and removal of the protecting groups would then give arogenate **1**. This approach has provided a very efficient route to arogenate (Scheme 1). Additionally it was found to be possible to resolve the initially formed racemic products and good stereoselectivity was achieved for the C-4′ hydroxylation using the methods described below.

Methyl 1,4-dihydrobenzoate **2** was prepared by the Birch reduction of benzoic acid followed by esterification with $MeOH/BF_3{\cdot}Et_2O$.[5] Deprotonation of **2** with LDA in THF at −78 °C followed by quenching with a THF solution of methyl 2-acetamidoacrylate[6] **3** afforded the spirolactam **4** (51% yield, m.p. 98–100 °C).† Hydrolysis of the methyl ester **4** with NaOH–MeOH–H_2O mixture gave the corresponding spirolactam carboxylic acid, which was converted to the acid chloride with thionyl chloride and thence the esters **5** with (+)-menthol (71% overall yield). The resulting mixture of diastereoisomeric esters **5** was separated by fractional crystallization. Thus 1 g of the diastereoisomeric mixture **5** was dissolved in boiling EtOAc (2.3 ml), then hot hexane (3 ml) was added. Allowing the solution to cool resulted in crystallization of 370 mg of the less soluble (3*S*) diastereoisomer **6** [mp 160–162 °C, $[\alpha]_D$ +78.5 (*c* 4.0, $CHCl_3$)].‡ The homogeneity of this crystalline material **6** was easily demonstrated by normal-phase analytical HPLC.

It was found to be necessary to acylate the lactam nitrogen of **6** with the methoxycarbonyl group to avoid problems with the subsequent oxidation and reduction steps.§ This could only be achieved satisfactorily with methyl *p*-nitrophenyl carbonate and DMAP which gave **7** (mp 109–110 °C) in 92% yield, as both methyl chloroformate and dimethyl pyrocarbonate were decomposed very readily under the conditions employed.



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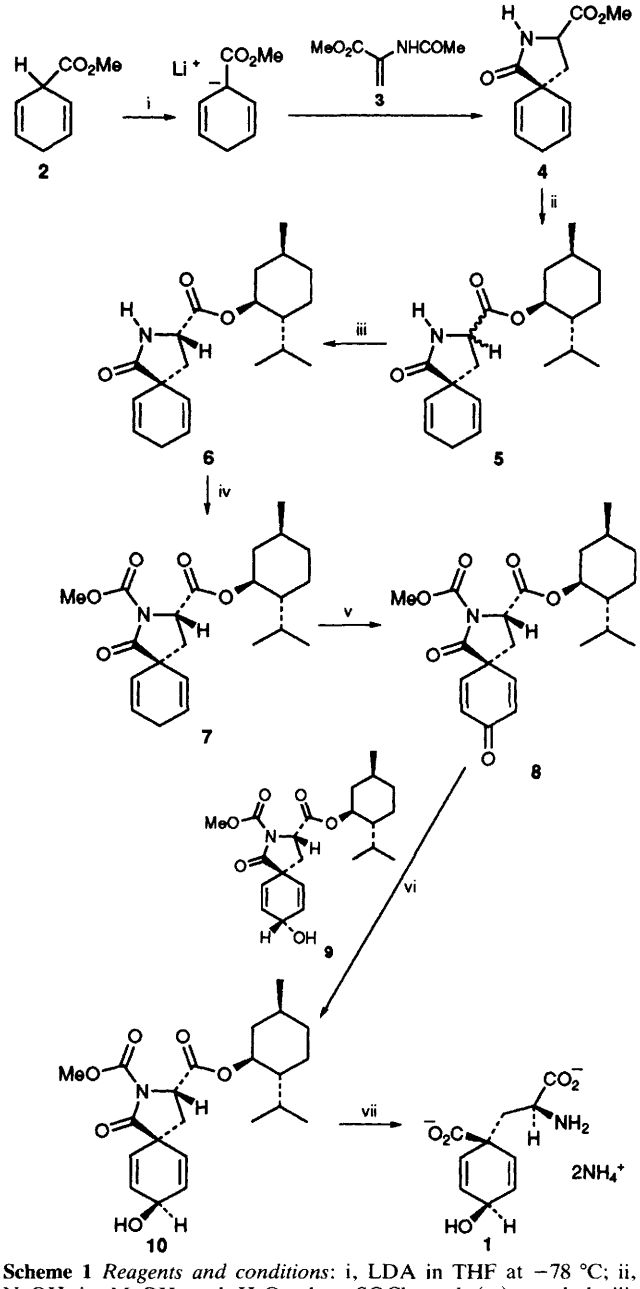
Oxidation of the doubly allylic C-4′ position of **7** was achieved with CrO_3/3,5-dimethylpyrazole (3,5-DMP) complex[7] in CH_2Cl_2 at −20 °C giving the cyclohexadienone **8** (56%, mp 163–164 °C). Reduction of **8** with diisobutylaluminium hydride (DIBAL) in CH_2Cl_2 at −78 °C afforded the epimeric alcohols **9** and **10** (protected arogenate) in the ratio of 1:4 in quantitative yield. The mixture was separated by chromatography to give **9** (16%) and **10** (62%).¶



Scheme 1 *Reagents and conditions*: i, LDA in THF at −78 °C; ii, NaOH in MeOH and H_2O, then $SOCl_2$ and (+)-menthol; iii, fractional crystallization; iv, methyl *p*-nitrophenyl carbonate and DMAP; v, CrO_3/3,5-DMP in CH_2Cl_2 at −20 °C; vi, DIBAL in CH_2Cl_2 at −78 °C; vii, NaOH in MeOH and H_2O at 70 °C for 36 h

Deprotection of **10** was achieved by hydrolysis with NaOH–MeOH–H_2O at 70 °C for 36 h. After extraction to remove the liberated (+)-menthol, arogenate **1** was purified by ion-exchange chromatography on Sephadex A-25 using ammonium hydrogencarbonate buffer as eluent. Fractions were analysed by TLC on cellulose using the solvent system: 70% propan-1-ol, 29% H_2O and 1% pyridine for development and ninhydrin was used to reveal the amino acids. The R_F values were: phenylalanine 0.60, tyrosine 0.55, arogenate 0.40. Fractions containing arogenate were basified with ammonia and freeze-dried giving diammonium arogenate **1** as a white powder (66%).‖

We expect that the enzymes prephenate dehydrogenase, prephenate aminotransferase, arogenate dehydrogenase and arogenate dehydratase, which are not found in mammals, may be inhibited by analogues of arogenate, which would then constitute a new safe class of antibiotics and herbicides. By simple modifications to the general methodology presented here, we envisage that such analogues may be made available.

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Footnotes

† Interestingly, no trace of the *N*-Acetyl derivative of **4** was observed.

‡ The fact that **8** has the (3*S*) stereochemistry was proven by the conversion of a derivative of **8** into L-tyrosine (+)-menthyl ester which was identical to an authentic sample. Full details will be published elsewhere.

§ Acylation was necessary prior to oxidation otherwise decomposition occurred. A carbamate was needed otherwise DIBAL reduction at the imide carbonyls predominated over reduction of the ketone.

¶ The configuration at C-4′ of **9** and **10** was deduced after **10** was converted into arogenate **1**. The NMR data for compounds **9** and **10** were similar to that reported by Danishefsky[3] for analogous compounds with the same stereochemistry at C-4′.

‖ Arogenate **1** was always found to be contaminated with some amount (about 5%) of both L-tyrosine and L-phenylalanine due to spontaneous decomposition. The mechanisms by which these products are produced will be discussed in a full paper.

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