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Synthesis of trimethyl (2*S*,3*R*)- and (2*R*,3*R*)-[2-$^2H_1$]-homocitrates and the corresponding dimethyl ester lactones—towards elucidating the stereochemistry of the reaction catalysed by homocitrate synthase and by the Nif-V protein

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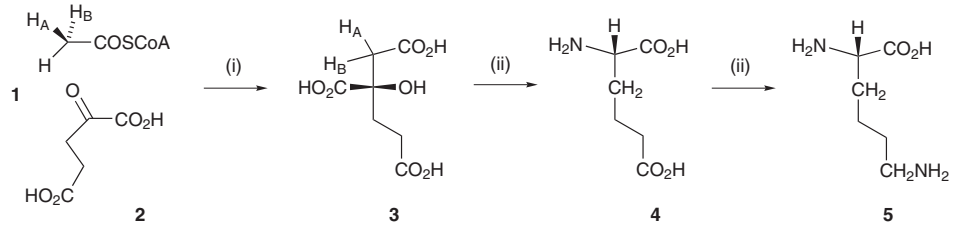
Abstract—Trimethyl (2*S*,3*R*)- and (2*R*,3*R*)-[2-$^2H_1$]-homocitrates, **10b** and **10c** respectively, and dimethyl (2*S*,3*R*)- and (2*R*,3*R*)-[2-$^2H_1$]-homocitric lactones, **11b** and **11c** respectively, have been synthesised from shikimic acid and [2-^{2}H]-shikimic acid by a route which defines the stereochemistry of the two chiral centres in each compound. The NMR spectra of these products will enable the stereochemistry of the biological reaction catalysed by homocitrate synthase and by the protein from the *nifV* gene to be elucidated.

Homocitric acid **3**, a key intermediate in the biosynthetic pathway to the essential amino acid lysine **5** in fungi and euglenids, is synthesised from acetyl CoA **1** and α-ketoglutarate **2** in a reaction catalysed by the enzyme homocitrate synthase (EC 4.1.3.21) as shown in Scheme 1.[1] This pathway involves the intermediate α-aminoadipic acid **4**, required in the biosynthesis of penicillins and cephalosporins and there has been a suggestion that homocitrate synthase limits α-aminoadipic acid formation in penicillin biosynthesis.[2] Homocitric acid is also required in nitrogen fixation, where the reaction in Scheme 1 is catalysed by the protein derived from the *nifV* gene.[3] Patients with the disease propionic acidaemia have been shown to excrete homocitric acid.[4]

The steps in the lysine pathway to α-aminoadipic acid **4** parallel closely those steps in the citrate cycle leading from acetylCoA and oxaloacetate via citrate to glutamic acid. It has been shown that homocitric synthase catalyses attack on the carbonyl group of α-ketoglutarate from the *re*-face,[5] unlike the more common *si*-citrate synthase (EC 4.1.3.7) which catalyses attack on the carbonyl group of oxaloacetate from the *si*-face.[6] The stereochemistry at the acetate methyl group in the reaction



Scheme 1. Reagents and conditions: (i) homocitrate synthase or the protein from the *nifV* gene; (ii) other enzymes in the biosynthetic pathway to lysine in fungi and euglenids.

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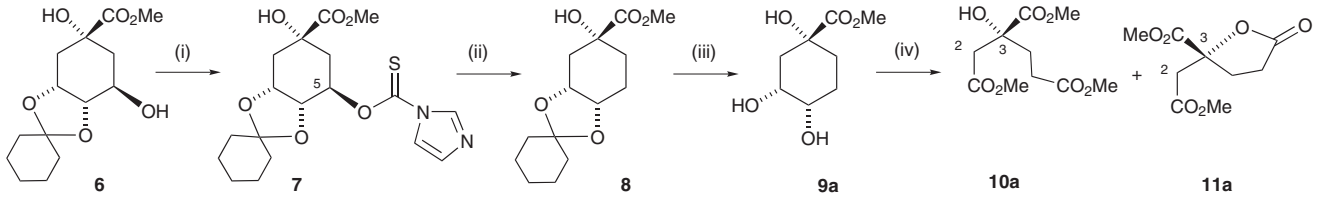


Scheme 2. Reagents and conditions: (i) thiocarbonyldiimidazole/CH_2Cl_2/rt, 16 h (89%); (ii) Bu_3SnH–AIBN/toluene/reflux, 3 h (83%); (iii) Amberlite IR-120 (H^+)/MeOH/reflux, 3 h (70%); (iv) (a) $NaIO_4$–silica gel/CH_2Cl_2, (b) H_2O_2/HCO_2H/rt, 6 h, (c) Amberlite-120 (H^+)/MeOH/reflux,16 h (36% **10a** + 15% **11a**).

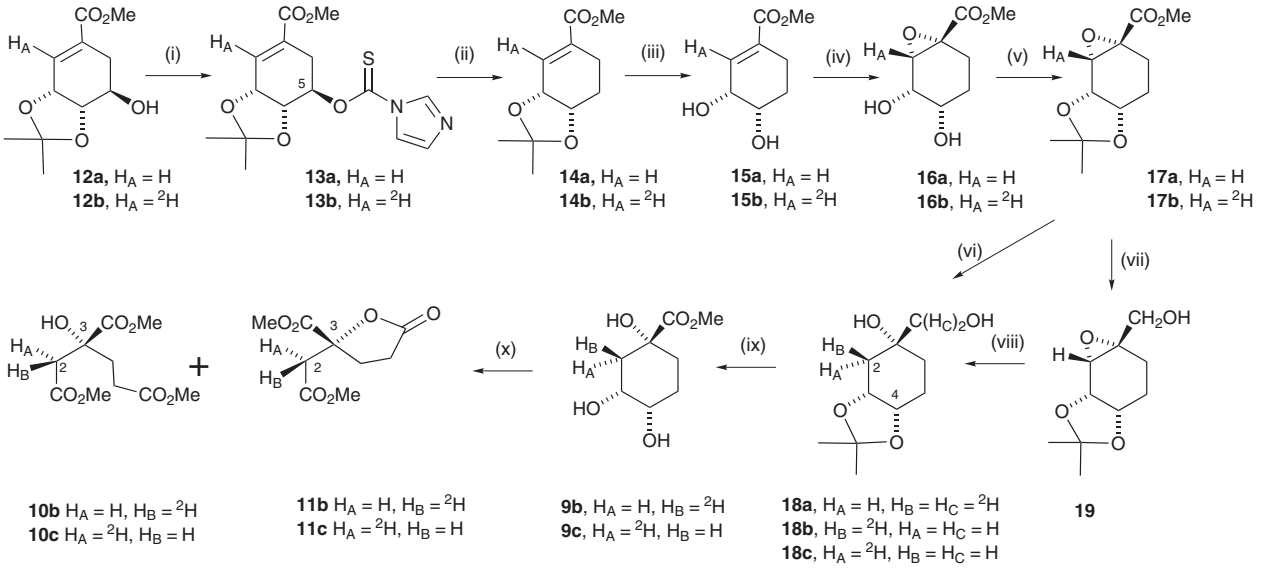
catalysed by citrate synthase has been shown to be inverted, using (*R*)- and (*S*)-[2-$^2H_1$,2-$^3H_1$]-acetylCoA, **1** (H_A = ^{3}H, H_B = ^{2}H) and **1** (H_A = ^{2}H, H_B = ^{3}H), respectively,[7] and it is of interest to investigate this aspect of the stereochemistry in the analogous reactions catalysed by homocitrate synthase and by the protein from the *nifV* gene. Unlike citric acid, homocitric acid **3** is asymmetric and so the two hydrogens, H_A and H_B, arising from acetate in this product are diastereotopic. Assignment of stereochemistry to the chemical shifts arising from these hydrogens will therefore enable the stereochemistry of step (i) in Scheme 1 to be assessed by ^{3}H NMR spectroscopy when (*R*)- and (*S*)-[2-$^2H_1$,2-$^3H_1$]-acetylCoA are used in the enzymatic reaction. We now report a synthesis of trimethyl (2*S*,3*R*)- and (2*R*,3*R*)-[2-$^2H_1$]-homocitrates **10** and dimethyl (2*S*,3*R*)- and (2*R*,3*R*)-[2-$^2H_1$]-homocitric lactones **11**, involving reactions of unambiguous stereochemistry. Since homocitrate from the enzyme reactions can readily be converted to these esters without racemisation,[8] this synthesis constitutes an assay for the stereochemistry of the enzymic reaction.

Retrosynthetically, quinic acid might serve as the source of the 3*R* centre of homocitrate if the hydroxyl group at C-5 were to be specifically removed and the remaining *cis*-diol cleaved. We therefore used the method of Shing and Tang[9] to prepare the quinate derivative **6**, in which the *cis*-3,4-diol moiety is protected. Conversion to the 5-thiocarbonylimidazole derivative **7**† in 89% yield followed by reduction using Bu_3SnH and AIBN afforded the protected 5-deoxyquinate **8**† in 83% yield, as shown in Scheme 2. Deprotection using Amberlite IR-120 (H^+) gave the diol **9a**† in 70% yield and this was cleaved using periodate on silica gel followed by oxidation of the intermediate dialdehyde with H_2O_2 and formic acid. Methylation then gave a mixture which was separated by chromatography to afford, as oils, trimethyl (3*R*)-homocitrate, **10a**,† in 36% yield and dimethyl (5*R*)-homocitric lactone, **11a**,† in 15% yield. The ^{1}H NMR spectra of these compounds (Figs. 2a and 3a) showed good separation between the signals due to the diastereotopic hydrogens which would originate from acetate in the enzyme catalysed reaction.

Having obtained the unlabelled target molecules, the next step was to synthesise the two diastereoisomerically labelled derivatives by methods which would allow unambiguous assignment of their stereochemistry. The isopropylidene derivative **12a** was therefore prepared from shikimic acid by the method of Chahoua et al.[10] and this was reduced in good yield to the 5-deoxy derivative **14a**† via **13a**,† as shown in Scheme 3, using the method described above for the preparation of the quinate analogue **8** (Scheme 2). Deprotection was now required so that epoxidation of the double bond would be directed to the same face as the 3- and 4-hydroxyl groups by the well known Sharpless directed epoxidation of allylic and homoallylic alcohols.[11,12] This was achieved in 79% yield using Amberlite IR-120 (H^+) in methanol, and epoxidation of the product **15a**† was carried out using *tert*-butylhydroperoxide and vanadyl acetylacetonate in dichloromethane, giving **16a**† in 57% yield. W-coupling between H-2 and H-4 was observed in the ^{1}H NMR spectrum of **16a**, suggesting that these hydrogens are quasi-equatorial, as shown in Figure 1. This implies that the desired (1*R*,2*S*)-stereochemistry had been induced in the epoxidation reaction. Reprotection of the secondary alcohol groups was now necessary to allow for selective oxidation of the primary alcohol which would result from reduction of the ester when the epoxide was reduced from the re-face to create the stereospecifically labelled centre at C-2. This was achieved using 2,2-dimethoxypropane and camphorsulfonic acid, giving the isopropylidene derivative **17a**.†

We were now able to introduce a deuterium label stereospecifically at C-2 whilst retaining the *R* stereochemistry at C-1 (which was to become C-3 in homocitric acid) by reacting **17a** with lithium aluminium deuteride. The product **18a**† was obtained in 83% yield. The unlabelled analogue **18d**† of this compound was prepared independently from the quinic acid derived **9a** as shown in Scheme 4 by protection followed by reduction using $LiAlH_4$. Comparison of the spectra of the shikimate-derived **18a**† and the quinate-derived **18d**† confirmed the stereochemistry at the centre C-1 in the former compound. All attempts to oxidise the primary alcohol group in **18a** to an acid failed, although, when we oxidised the unlabelled compound **18d** using oxygen and a platinum catalyst, followed by methylation and deprotection, we were able to obtain the desired methyl 5-deoxyquinate **9a**.† An isotope effect had evidently prevented oxidation of **18a** and indeed when the dideuterio-compound **18e**† was obtained from methyl deoxyquinate, as in Scheme 4, this was also resistant to oxidation. The problem was circumvented by selectively reducing the ester in **17a** without affecting the

†This compound had the required analytical and spectroscopic properties.



Scheme 3. Reagents and conditions: (i) thiocarbonyldiimidazole/CH_2Cl_2/rt, 16 h (95% using **12a**; 99% using **12b**); (ii) Bu_3SnH–AIBN/toluene/reflux, 3 h (72% using **13a**; 80% using **13b**); (iii) Amberlite IR-120 (H^+)/MeOH/reflux, 3 h (79% using **14a**; 80% using **14b**); (iv) ^{t}BuOOH/CH_2Cl_2/V(acac)$_3$, 0 °C/rt, 24 h (57% using **15a**; 59% using **15b**); (v) $(MeO)_2CMe_2$/(±)-10-camphorsulfonic acid/CH_2Cl_2/rt, 3 h (quant, unpurified); (vi) $LiAl^2H_4/Et_2O$/−78 °C, 5 h/0 °C, 1 h (83% **18a** from **17a**); $LiAlH_4/Et_2O$/−78 °C, 5 h/0 °C, 1 h (87% **18c** from **17b**); (vii) $NaBH_4$/THF, 0 °C/rt, 14 h (92% using **17a**) (viii) $LiAl^2H_4/Et_2O$/−78 °C, 5 h/0 °C, 1 h (83% **18b**); (ix) (a) O_2/Pt/$NaHCO_3/H_2O$–MeOH/55 °C, 16 h, (b) Amberlite IR-120 (H^+)/MeOH/reflux, 16 h (69% **9b** using **18b**; 65% **9c** using **18c**); (x) (a) $NaIO_4$–silica gel/CH_2Cl_2/rt 10 min, (b) H_2O_2/HCO_2H/rt, 6 h, (c) Amberlite-120 (H^+)/MeOH/reflux, 16 h (38% **10b** + 16% **11b**; 40% **10c** + 15% **11c**).

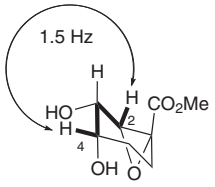
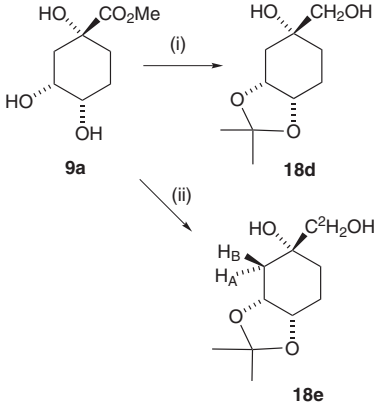


Figure 1. W coupling between H-2 and H-4 in **16a**.



Scheme 4. Reagents and conditions: (i) (a) $(MeO)_2CMe_2$/(±)-10-camphorsulfonic acid/CH_2Cl_2/rt/1 h, (b) $LiAlH_4/Et_2O$ −78 °C, 5 h/0 °C, 1 h (89%); (ii) (a) $(MeO)_2CMe_2$/(±)-10-camphorsulfonic acid/CH_2Cl_2/rt/1 h, (b) $LiAl^2H_4/Et_2O$/−78 °C, 5 h/0 °C, 1 h (89%).

epoxide, using sodium borohydride, as in Scheme 3. The product **19**† was then reduced with lithium aluminium deuteride to afford the desired alcohol **18b**.† This compound was oxidised, methylated and deprotected, as for **18d**, giving the ester **9b**.† Cleavage of the diol and

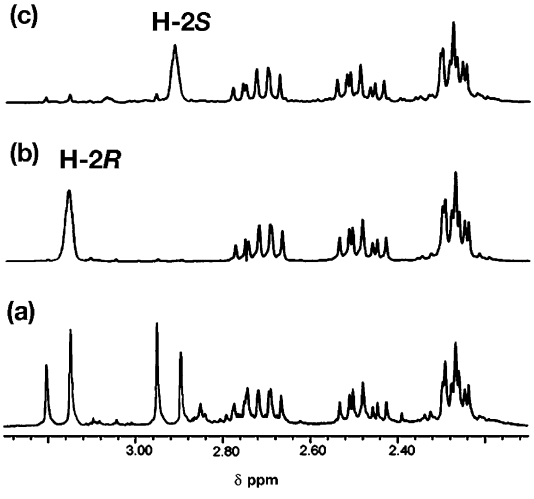


Figure 2. Part of the 300 MHz ^{1}H NMR spectra in C^2HCl_3 of (a) trimethyl (3*R*)-homocitrate **10a**; (b) trimethyl (2*S*,3*R*)-[2-^{2}H$_1$]-homocitrate **10b**; and (c) trimethyl (2*R*,3*R*)-[2-^{2}H$_1$]-homocitrate **10c**.

methylation using methanol and Amberlite IR-120 (H^+) gave a mixture from which trimethyl (2*S*,3*R*)-[2-^{2}H$_1$]-homocitrate **10b**† and dimethyl (2*S*,3*R*)-[2-^{2}H$_1$]-homocitric lactone **11b**† could be separated.

To obtain the diastereoisomeric [2*R*-^{2}H$_1$]-compounds, we prepared the labelled protected shikimate derivative **12b** from D-mannose by modification of a method developed for the preparation of [2-^{2}H$_1$]-shikimate by Floss and co-workers[13] who adapted a synthesis of the unlabelled compound and its derivatives by Fleet et al.[14] This compound was then taken through the steps **12b**† →**13b**†→**14b**†→**15b**†→**16b**†→**17b**†→**18c**†→**9c**†→**10c**† + **11c**†

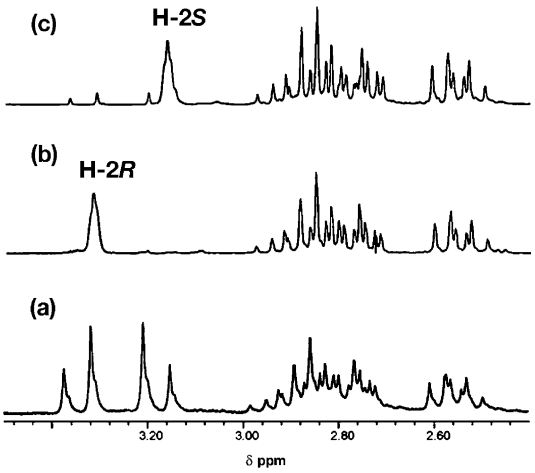


Figure 3. Part of the 300 MHz ^{1}H NMR spectra in $C^{2}HCl_3$ of (a) dimethyl (3*R*)-homocitric lactone **11a**; (b) dimethyl (2*S*,3*R*)-[2-$^{2}H_1$]-homocitric lactone **11b**; and (c) dimethyl (2*R*,3*R*)-[2-$^{2}H_1$]-homocitric lactone **11c**.

as described in Scheme 3. The ^{1}H NMR spectra of the various samples of trimethyl homocitrate, **10a**, **10b** and **10c**, are shown in Figure 2 and those for the samples of dimethyl homocitric lactone, **11a**, **11b** and **11c**, are shown in Figure 3. The chemical shifts of the diastereotopic hydrogens which are biosynthetically derived from acetate have clearly been defined by these experiments and thus the synthesis affords an assay for the stereochemistry of the biosynthetic reaction.

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References and notes

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