Synthesis of Stereoselectively Labelled Citric Acids transformable into Chiral Acetic Acids

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The ketones (**1b**) and (**1c**) have been transformed into the monodeuteriated citric acids, (**4b**) and (**4c**) respectively, which are suitable starting materials for the synthesis of chiral acetic acids and other labelled compounds; under base catalysis, the axial 2-H in (**1a**) exchanges with deuterium more than 1000 times faster than the axial 2-H in 4-t-butylcyclohexanone.

The use of stereoselectively isotope-labelled compounds is a powerful technique for the elucidation of various stereochemical phenomena. We report here that a synthetic route (Scheme 1) which has previously been used for the synthesis of citric acid and some of its analogues[1] is also suitable for the preparation of stereoselectively monodeuteriated citric acids (**4b**) and (**4c**) and the enantiomer of (**4c**). In addition, we report an unusually fast base-catalysed exchange of the axial 2-H in (**1a**) with deuterium.

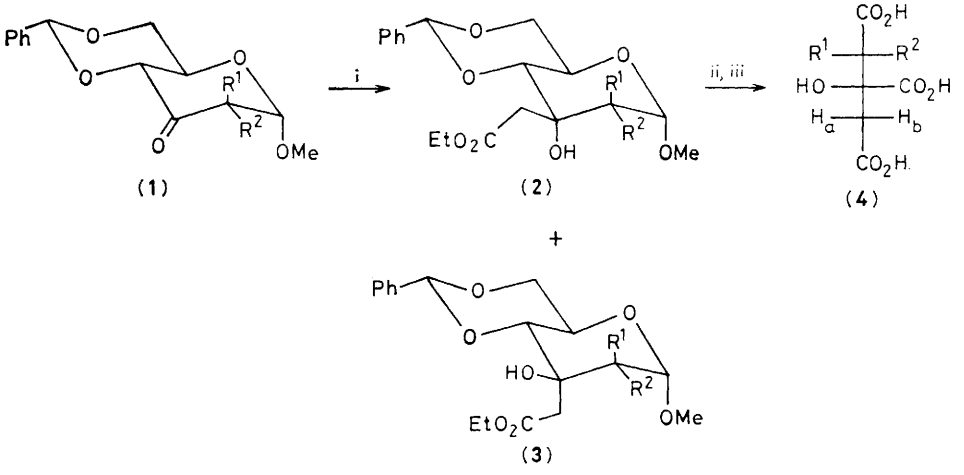
When the stereoselectively monodeuteriated ketone (**1b**)[2] was transformed according to the reaction sequence (**1**) → (**2**) → (**4**) in Scheme 1, we obtained (1*S*,2*S*)-[1-$^2H_1$]-2-hydroxypropane-1,2,3-tricarboxylic acid (**4b**) {$[\alpha]_{578}^{20}$ −0.4°, $[\alpha]_{436}^{20}$ −0.77° (*c* 2.5, H_2O); in saturated aq. $(NH_4)_2MoO_4$[1]: $[\alpha]_{436}^{20}$ −3.11° (*c* 2.5)}. The ^1H n.m.r. spectrum (Figure 1) corroborates the high stereochemical purity of (**4b**) and shows in addition that the δ 3.04 and 2.86 signals of the AB spectrum must be due to H_a and H_b, respectively; the assignment for citric acid (**4a**) is obtained by replacing D with H_a and H with H_b in (**4b**). The introduction of a deuterium atom into citric acid leads to a small (0.018 p.p.m.) upfield shift of the signal from the geminal proton. The trimethyl ester (CH_2N_2) of (**4b**) was obtained in a 20% overall yield from the epoxide precursor of (**1b**) and was contaminated with 4—5% of the nondeuteriated analogue (mass spectra).

Degradation of the minor Reformatsky product (**3b**) obtained from (**1b**) (Scheme 1) gave the enantiomer of (**4c**), contaminated with 3—4% of the nondeuteriated analogue (mass spectra) {found: $[\alpha]_{578}^{20}$ + 1.1°, $[\alpha]_{436}^{20}$ + 1.6° (*c* 2.5, H_2O); in saturated aq. $(NH_4)_2MoO_4$[1]: $[\alpha]_{436}^{20}$ + 29.4° (*c* 2.5)}. A 65% deuteriated sample of this citric acid was synthesised previously by axial deuteriation and subsequent oxidation of 3-dehydroquinic acid.[3] A similar sequence involving dideuteriation–monodedeuteriation gave the enantiomer of (**4b**) which was 70% deuteriated.[3] These samples were not characterised further.

Compound (**1b**) can also be obtained in a less pure form (4.9% [$^2H_0$], 87.3% [$^2H_1$], 7.8% [$^2H_2$]), by treatment (10 min) of (**1a**) (200 mg) with potassium carbonate (61 mg) in D_2O–CH_3OD–tetrahydrofuran (2:2:10 ml) at 10 °C. By analogy with results from other six-membered ring ketones,[4] there was a rapid exchange of the axial 2-H and a slower exchange of the equatorial 2-H (^1H n.m.r. spectroscopy). A rate ratio k_{ax}/k_{eq} of about 35† was found by mass spectral analysis of the mixture of deuteriated trimethyl citrates obtained by degradation of the mixture of deuteriated ketones (**1**). This ratio is smaller than those obtained for ketones which are presumably conformationally less flexible.[4] However, the reactivity of the axial 2-H is unusually high. Ketone (**1a**) (50 mg) or 4-t-butyl-cyclohexanone (30 mg) in dioxane (5 ml) was treated at 11.5 ± 0.5 °C with 1.25 ml of a solution of NaOD in D_2O which showed 11.2 on the pH meter. Aliquots were acidified to *ca.* pH 6 and worked up and the formation of (**1b**) or deuteriated 4-t-butylcyclohexanone was studied by ^1H n.m.r. or mass spectroscopy, respectively. The half-lives found for (**1a**) and 4-t-butylcyclohexanone were of the order of 1 min and 30 h, respectively. A study of the cause of this difference in reactivity is in progress.

Isomer (**4c**) was prepared from (**1c**), which was obtained

† This value is approximate only, the main reason being the unexpected consumption of base during the exchange reaction.



a; $R^1 = R^2 = H$. **b**; $R^1 = D$, $R^2 = H$. **c**; $R^1 = H$, $R^2 = D$.

Scheme 1. Synthesis of the citric acids (**4**) from the ketones (**1**). *Reagents:* i, Zn, $BrCH_2CO_2Et$; ii, HOAc, H_2O, heat; iii, $KMnO_4$, aq. NaOH (ref. 1). The Reformatsky reaction produces (**2**) and its C-3 epimer (**3**) in the ratio 94:6 (ref. 1). The overall yield (**1**) → (**2**) → (**4**) is about 25% (ref. 1).

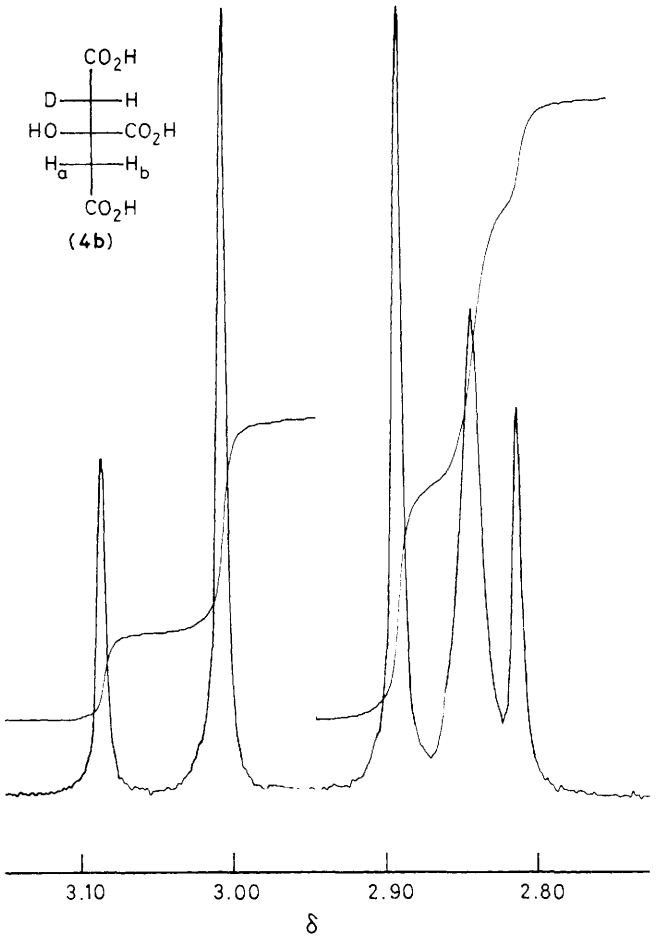
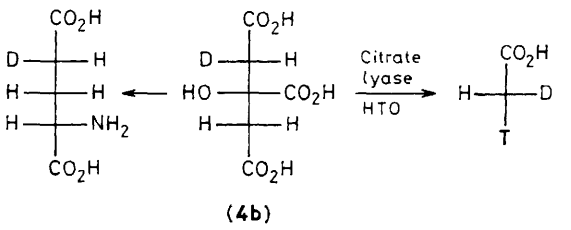


Figure 1. Methylene region of a 200 MHz ^{1}H n.m.r. spectrum of the monodeuteriated citric acid (**4b**) in D_2O.

by dideuteriation[1] of (**1a**) followed by treatment with potassium carbonate as for (**1b**) but for 36 min and in non-deuteriated solvents. As found by mass spectral analysis of the trimethyl ester (CH_2N_2), the sample of (**4c**) had the following composition: 17.9% [$^2H_0$], 81.2% [$^2H_1$], 0.9% [$^2H_2$] {found for this mixture: $[\alpha]_{578}^{20}$ −0.5°, $[\alpha]_{436}^{20}$ −1.0° (*c* 2.6, H_2O); in saturated aq. $(NH_4)_2MoO_4$[1]: $[\alpha]_{436}^{20}$ −20.8°}.

The technique presented here is the first to allow stereoselective introduction of a deuterium atom into the *pro-S* carboxymethyl group of citric acid. The new compounds



Scheme 2. Enzymatic conversions of (**4b**) into (*R*)-[2-$^2H_1$,$^3H_1$]-acetic acid or (4*S*)-[4-$^2H_1$]-L-glutamic acid.

(**4b**) and (**4c**) can be transformed into chiral acetic acids[5] by using the enzyme citrate lyase (commercially available) in tritiated water (Scheme 2). To determine the inversion stereochemistry of this enzymatic reaction, tritiated analogues of (**4b**) and (**4c**) were prepared by means of a three-[6] or four-enzyme[7] synthesis which included the use of the enzymes aconitate isomerase[6] or *re*-citrate synthase[7] which are difficult to obtain.

It should also be possible, by means of enzymatic conversions of (**4b**) or (**4c**), to prepare stereoselectively deuteriated samples of aconitic acid, isocitric acid, α-ketoglutaric acid, and glutamic acid (Scheme 2) (*cf.* ref. 8).

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