On the stereochemical outcome of the reaction between (−)-chorismic acid and diazomethane: absolute proof of stereochemistry of the major pyrazoline by X-ray crystallography of a cyclopropane based derivative

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Reinvestigation of the reaction between (−)-chorismic acid and diazomethane in diethyl ether on a larger scale has shown that the previously reported single pyrazoline based product is accompanied by a stereoisomer that results from the addition of diazomethane to the more hindered α-face of the chorismate 1,2-double bond (α:β addition *ca.* 1:6). Thermolysis of the two cycloadducts at 80 °C afforded a pair of cyclopropane derivatives with stereochemistry that could not be confidently assigned using data from coupling constants alone. NOE data allowed a more confident assignment of the stereochemistry of the two cyclopropanes; the β-cyclopropyl derivative was saponified and hydrolysed to yield a bicyclo[4.1.0]hept-2-ene-1-carboxylic acid derivative that was unequivocally shown to possess (1*S*,4*R*,5*R*,6*R*)- stereochemistry by an X-ray crystallographic study.

The shikimate pathway is a biosynthetic pathway utilized by plants, fungi and micro-organisms for the synthesis of several essential aromatic metabolites including the three commonly occurring aromatic L-α-amino acids (Phe, Tyr, Trp).[1–3] As a result of the pathway being foreign to all higher forms of life including mammalian systems, compounds that inhibit the enzymes which catalyse the varied synthetic transformations *en route* from acyclic C_3 and C_4 precursors to aromatics have been highlighted as important pharmaceutical and agrochemical targets.

As part of a programme to utilize both (−)-shikimic acid **1** and (−)-chorismic acid **2** as precursors to substrate analogues of shikimate pathway enzymes in which we have replaced olefinic functionality with other rigid moieties that mimic a double bond (*e.g.* cyclopropanes and epoxides), we had course to reinvestigate on a much larger scale the reaction between (−)-chorismic acid **2** and diazomethane first described by our group in 1976. We had originally reported[4] that treatment of a solution of **2** with diazomethane at −78 °C on a small scale afforded a single highly crystalline pyrazoline (along with several minor byproducts) whose structure was assigned as **3** on the basis of ^{1}H NMR spectral data recorded at 100 MHz for its acetate **4**; a coupling constant $J_{5,6}$ of 5 Hz in **4** was seen to be indicative of a *cis* relationship between H-5 and H-6.

We now wish to report the indirect observation of a second and minor pyrazoline product **5** in the residues from crystallization of the major isomer **3** (ratio **3**:**5** *ca.* 6:1). Structural elucidation of cyclopropanes **6** and **7** (derived from **3** and **5**, respectively) has shown the original assignment[4] of the stereochemistries of **3** and **4** to be inconclusive based upon coupling constant data alone and we describe herein experiments to rigorously and unambiguously assign the stereochemical details of **3**, **4**, **6** and **7**.

Treatment of **2**‡ with diazomethane at −78 °C afforded a crude product from which a pyrazoline product (previously assigned as **3**) could be crystallized (68% yield), thermolysis of which (C_6H_6, 80 °C) afforded the cyclopropyl derivative (previously assigned as **6**)[4] that showed a similar coupling to **4** between H-5 and H-6 ($J_{5,6}$ 4 Hz). Thermolysis of the residues from crystallization of **3** afforded a crude product which upon chromatography on silica yielded cyclopropyl **6** (4%) together with a previously overlooked diastereoisomeric cyclopropyl derivative **7** (12%) resulting from the addition of diazomethane to the more sterically crowded α-face of **2**.

That the newly discovered cyclopropyl **7** was diasteroisomeric with that previously reported **6** was apparent from its ^{1}H NMR spectrum in $CDCl_3$; it was clear from the observation of two singlets at δ 3.78 and 3.68 (each 3 H), a broad singlet centred at δ 3.35 (OH) and the presence of two pairs of signals corresponding to both the 2,3- and enolpyruvyl double bonds (δ 6.28 and 5.67, and δ 5.50 and 4.88, respectively) that addition of diazomethane had occurred to both acidic groups as well as the 1,2-double bond of **2**. Stereochemical assignment of both cyclopropyls **6** and **7** was shown to be ambiguous on the basis of coupling constant data alone since in the newly discovered isomeric cyclopropyl **7** $J_{5,6}$ (3.5 Hz) was found to be similar in magnitude to that in **6** ($J_{5,6}$ 4 Hz).

Absolute stereochemical assignments of cyclopropyls **6** and **7** was achieved by way of NOE enhancements observed upon irradiation of key signals in their high-field ^{1}H NMR spectra (400 MHz). Upon irradiation of H-7β of **6** an enhancement of *ca.* 4% was observed in the signal corresponding to H-4 whilst irradiation of H-7α of **7** resulted in an enhancement in the signal assigned as that of H-5 (*ca.* 2.5%); no enhancements in H-4 upon irradiation of H-7β of **7** and in H-5 upon irradiation of H-7α of **6** were observed in similar experiments. These data are consistent only with the cyclopropyls **6** and **7** being derived from pyrazolines in which addition of CH_2N_2 to **2** has occurred from the β- and α-faces respectively. Thus cyclopropyl **7** is thought to arise from the thermolysis of a previously overlooked pyrazoline **5** present in the mother liquors of the crude reaction mixture after crystallization of **3**.

Saponification of **6** occurred smoothly at room temperature to afford the diacid **9** (contaminated with around 5% of the monoester **8**) which was hydrolysed upon ion-exchange

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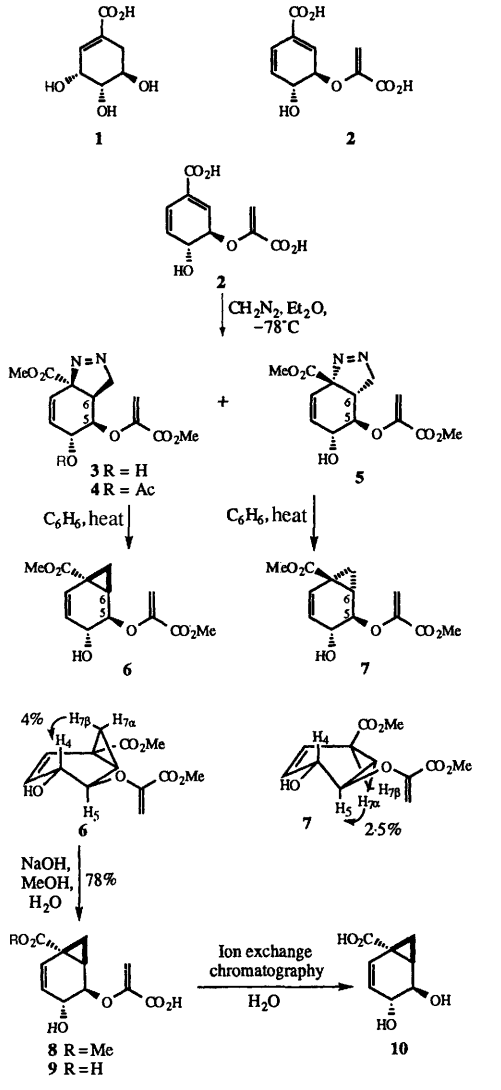
‡ (−)-Chorismic acid **2** was isolated[5] from the accumulation media of a culture of *Klebsiella pneumoniae* 25 306 and crystallized at −18 °C from light petroleum (bp 60–80 °C)–ethyl acetate as pale yellow needles [mp 116–117 °C (decomp.)] that were stored at −18 °C.



chromatography in water (Amberlite IRA-400) to afford the cyclopropyl acid **10** (77%). Crystallization from methanol afforded crystals of sufficient quality to allow for structure determination by X-ray crystallography (Fig. 1)§ acid **10** is thus shown to contain a β-cyclopropyl subunit and to possess (1*S*,4*R*,5*R*,6*R*) stereochemistry.

In summary, we have highlighted the presence of a previously overlooked pyrazoline **5** in the mother liquors that remain from the reaction between (−)-chorismic acid **2** and diazomethane after crystallization of the major product **3** (**3**:**5** *ca.* 6:1). We have shown that pyrazolines **3** and **5** give rise to diastereoisomeric cyclopropyls **6** and **7** upon thermolytic extrusion of nitrogen at 80 °C for which structures cannot be confidently assigned based upon coupling constants alone ($J_{5,6}$ 4 and 3.5 Hz, respectively). Data from NOE experiments has allowed for the structural assignment of **6** and **7** with a much greater degree of confidence and the X-ray crystal structure of a cyclopropyl acid **10** (derived from **6**) has allowed the absolute proof of structure of compounds **3**, **4**, **6** and **7**, indicating that the structures of **3**, **4** and **6** were previously assigned correctly.[4]

§ Atomic coordinates, thermal parameters and bond lengths and angles have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, 1996, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 207/19. Data were collected only at low resolution and the structural parameters therefore have rather high levels of uncertainty.

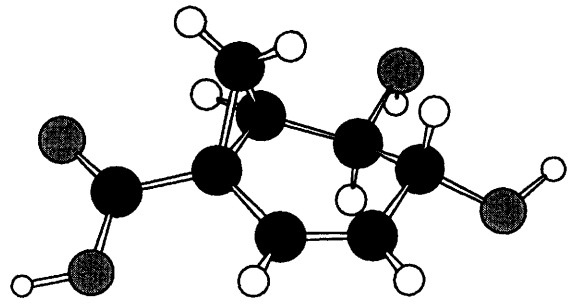


Fig. 1 X-Ray crystal structure of **10**

Experimental

General

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Microanalyses were performed by The University of Sheffield Department of Chemistry Microanalytical Service and by the Microanalysis Department at Zeneca Pharmaceuticals, Alderley Park, Macclesfield, Cheshire. Mass spectra were recorded by chemical ionization (+CI) (ammonia as the ionizing agent) using a Kratos MS-25 mass spectrometer. Nuclear magnetic resonance spectra (^{1}H and ^{13}C) were recorded in the solvents specified using a Bruker AM-250 spectrometer operating at 250.1 and 62.9 MHz, respectively; *J* values are given in Hertz. Flash column chromatography was performed using silica gel 60 (Merck 9385). Ethyl acetate, methanol, light petroleum (bp 40–60 °C) and water were distilled prior to use. Benzene was dried over sodium wire and distilled prior to use.

Methyl (1*R*,4*R*,5*R*,6*S*)-4-hydroxy-5-[(1-methoxycarbonyl)-ethenyloxy]bicyclo[4.1.0]hept-2-ene-1-carboxylate 7

A solution of the residues from treatment of (−)-chorismic acid **2** (350 mg, 1.55 mmol) with an ethereal solution of diazomethane [after crystallization of pyrazoline **3** (331 mg, 68%)][4] in benzene (10 cm^3) was stirred and held at reflux for 3 h, the solvent removed *in vacuo* and the residues purified by column chromatography. Elution with light petroleum (bp 40–60 °C)–ethyl acetate (1:1) afforded *cyclopropanes* **6**[4] and **7** as a colourless oil (67 mg, 4 and 12%, inseparable 1:3 mixture) (Found: C, 58.0; H, 6.15. $C_{13}H_{16}O_6$ requires C, 58.2; H, 6.0%); *m*/*z* 268 (M^+); data for **7**: $\delta_H(CDCl_3)$ 6.28 (1 H, ddd, *J* 10, 2.5 and 1, H-2), 5.67 (1 H, dd, *J* 10 and 3.5, H-3), 5.50 (1 H, d, *J* 3, H-3′), 4.88 (1 H, d, *J* 3, H-3′), 4.51 (1 H, dddd, *J* 6, 3.5, 2.5 and 1, H-4), 3.87 (1 H, dd, *J* 6 and 3.5, H-5), 3.78 (3 H, s, CO_2Me), 3.68 (3 H, s, CO_2Me), 3.50–3.20 (1 H, broad s, OH), 1.99 (1 H, dddt, *J* 10, 6.5 and 3.5, 1, H-6), 1.79 (1 H, dd, *J* 10 and 3.5, H-7β), 1.03 (1 H, dd, *J* 6.5 and 3.5, H-7α).

(1*S*,4*R*,5*R*,6*R*)-4,5-Dihydroxybicyclo[4.1.0]hept-2-ene-1-carboxylic acid 10

A solution of diester **6**[4] (317 mg, 1.18 mmol) in methanol (10 cm^3) and water (1 cm^3) was treated with sodium hydroxide (100 mg, 2.5 mmol) and the resulting solution was stirred at room temperature for 16 h and then neutralized by the addition of Amberlite IR-120 (H) ion-exchange resin (*ca.* 200 mg). After stirring for 15 min the resin was removed by filtration and the solution applied to a column of Amberlite IRA-400 (OAc). Elution with 25% aq. acetic acid and removal of the solvent *in vacuo* afforded the *title compound* **10** (154 mg, 77%) as colourless prisms (from MeOH), mp 186–8 °C (decomp.) (Found: C, 56.35; H, 6.1. $C_8H_{10}O_4$ requires C, 56.45; H, 5.9%); *m*/*z* 188 ($M + NH_4^+$); $\delta_H(CD_3OD)$ 6.36 (1 H, dd, *J* 10.5 and 2.5, H-2), 5.37 (1 H, dd, *J* 10.5 and 2, H-3), 3.80 (1 H, ddd, *J* 8, 2.5 and 2, H-4), 3.71 (1 H, dd, *J* 8 and 4.5, H-5), 2.12 (1 H, ddd, *J* 9, 7.5 and 4.5, H-6), 1.56 (1 H, dd, *J* 9 and 4, H-7β), 1.07 (1 H, dd, *J* 7.5 and 4, H-7α); $\delta_C(CD_3OD)$ 176.2 (C=O), 127.8 and

127.8 (C-2 and C-3), 71.8 and 71.1 (C-4 and C-5), 27.7 (C-1), 27.5 (C-6), 23.4 (C-7).

Crystal data for $C_8H_{10}O_4$ 10. M = 170.16; crystallizes from methanol as colourless, elongated blocks; crystal dimensions 0.60 × 0.20 × 0.20 mm. Orthorhombic, a = 6.517(3), b = 7.448(7), c = 16.013(13) Å, U = 777.3(10) Å^3, D_c = 1.454 g cm^{-3}, Z = 4; space group $P2_12_12_1$ (D_2^4, No. 19); Mo-Kα radiation (λ = 0.71069 Å), ν(Mo-Kα) = 1.10 cm^{-1}, F(000) = 359.96.

Three dimensional, room temperature X-ray data were collected in the range $3.5 < 2\theta < 40°$ on a Nicolet R3 diffractometer by the omega scan method. The 399 independent reflections (of 474 measured) for which $|F|/\sigma(|F|) > 3.0$ were corrected for Lorentz and polarization effects, but not for absorption. The structure was solved by multiple solution direct methods and refined by block cascade least-squares. Hydrogen atoms were included in calculated positions, or along identified hydrogen bonds, with isotropic thermal parameters related to those of the supporting atom, and refined in riding mode. Refinement converged at a final R 0.0422 (R_w = 0.0396, 109 parameters, final mean and maximum delta/sigma 0.001 and 0.003 respectively), with allowance for the thermal anisotropy of all non-hydrogen atoms. A final difference electron density synthesis showed minimum and maximum values of −0.20 and +0.19 e Å^{-3}. Complex scattering factors were taken from the program package SHELXTL as implemented on the Data General DG30 computer. A weighting scheme $w^{-1} = [\sigma^2(F) + 0.00029(F)^2]$ was used in the latter stages of refinement.

Acknowledgements

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