Chemistry of Insect Antifeedants from *Azadirachta indica*, Part 14[1]: Absolute Configuration of Azadirachtin

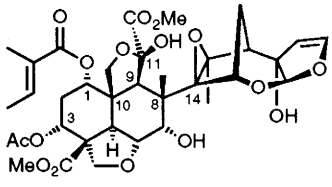
Steven V. Ley,* Helen Lovell and David J. Williams

Department of Chemistry, Imperial College of Science, Technology and Medicine, London SW7 2AY, UK

The absolute configuration of the insect antifeedant azadirachtin is determined by high field NMR application of the Mosher method and confirmed by X-ray crystallographic analysis.

Azadirachtin **1**, isolated from the Indian neem tree, *Azadirachta indica*, has been shown to have powerful biological activity as an insect antifeedant and ecdysis inhibitor.[2] Interest in this and related antifeedants has increased dramatically over recent years as they can be used in pest control schemes without causing environmental damage.[3]

Although the relative stereochemistry of azadirachtin has been assigned by X-ray[4] and NMR[5] techniques,† attempts to elucidate the absolute configuration have so far proved unsuccessful. This has necessitated synthetic studies on both the natural product and simple mimics to facilitate the preparation of both antipodes.[6,7] Kakisawa and coworkers recently reported an extension of standard Mosher methodology using high field FT NMR techniques to allow determination of the absolute configuration of complex chiral molecules.[8,9] This adaptation has been successfully applied to azadirachtin, and confirmation of the result was given by an X-ray crystal structure.



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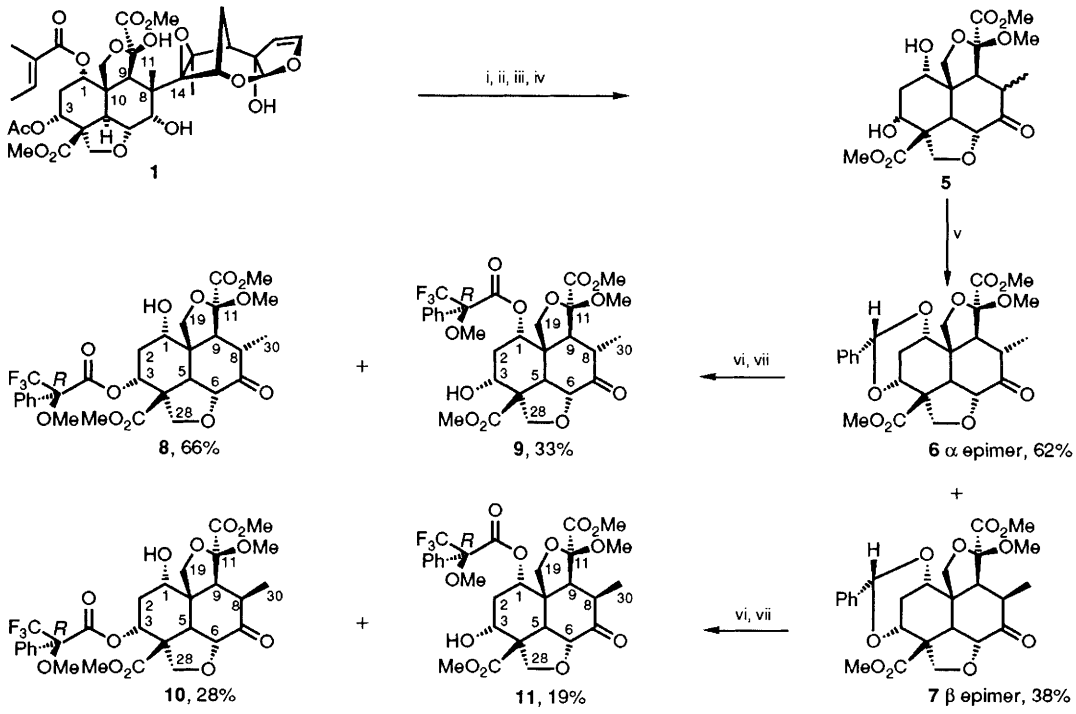
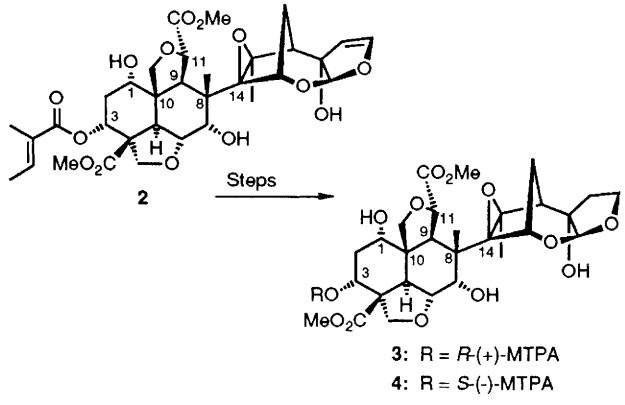
Initial studies were carried out using 3-substituted *R*-(+)- and *S*-(−)-MTPA-esters [MTPA = α-methoxy-α(trifluoromethyl)phenylacetic acid] of 22,23-dihydro-azadirachtol **3**, **4**.

† All NMR analysis carried out on Bruker 500 MHz spectrometer.



Scheme 1 *Reagents and conditions*: i, H_2, Pd/C, MeOH, 4 h, 80%; ii, MeI, Ag_2O, reflux, 3 h, 86%; iii, PCC, 4 Å mol. sieves, CH_2Cl_2, 48 h, 45%; iv, NaOMe, MeOH, 0 °C–room temp., 16 h, 79%; v, $PhCH(OMe)_2$, PPTS, PhH, reflux, 2 h, 100%; vi, PPTS, H_2O, MeCN, 16 h, 94%; vii, MTPA–Cl, DMAP, py, CH_2Cl_2, 16 h; PCC = pyridinium chlorochromate, PPTS = pyridinium toluene-*p*-sulfonate, DMAP = 4-dimethylaminopyridine, py = pyridine



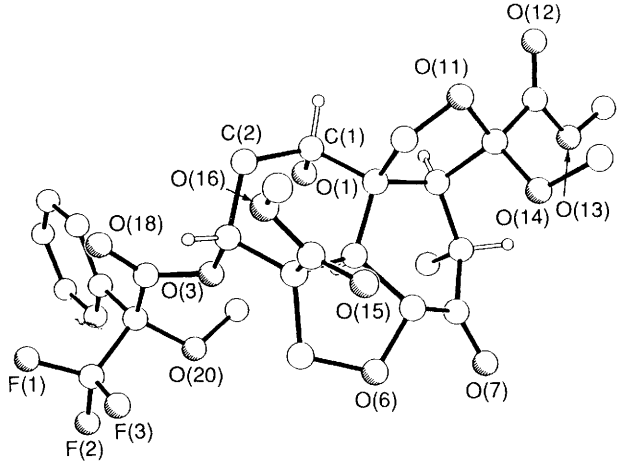


Fig. 1

These were formed in a straightforward manner from the naturally occurring 3-tigloyl azadirachtol **2**. Unfortunately, when subjected to the analytical procedure the results were inconclusive. It was thought that the axial position of the C-3 hydroxy group may have caused steric compression of the MTPA moiety. Attempts to perform a Mitsunobu inversion under both standard and modified[10] conditions failed so a comparison could not be made with an ester at the less hindered equatorial position.

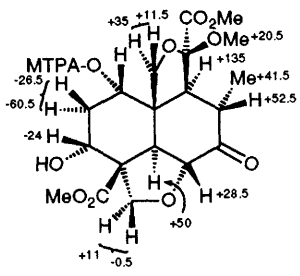
It was decided to repeat the study on the left-hand fragment of azadirachtin alone, as it was reasoned that the MTPA group would be subjected to less steric hindrance. This was readily available from degradation of the natural product[11] and isolated as a mixture of epimers at the C-8 position. Separate *R*-(+)- and *S*-(−)-MTPA esterification of diols **6** yielded a mixture of esters at the 1 and 3 positions. Separation of the C-8 epimers of both 3-*R*-(+)-MTPA and 3-*S*-(−)-MTPA esters (**10a**, **11a** and **10b**, **11b**) was carried out by HPLC. HPLC separation of the corresponding 1-substituted esters was not successful.

Previous work in this laboratory has shown that the epimeric C-8 diols **5** are separable by flash chromatography on formation of one corresponding 1,3-benzylidene acetal **6** and **7**.[11] These were formed in quantitative yield and the two diols were obtained after facile deprotection of the separated acetals (Scheme 1). Separate *R*-(+)- and *S*-(−)-MTPA esterification was carried out as before and the 1- and 3-MTPA esters **8** and **9**, **10** and **11** were separated by flash chromatography.

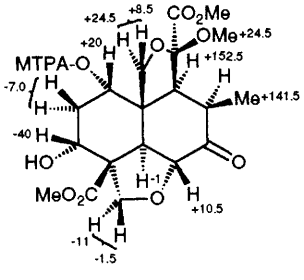
All of the compounds were crystalline, and one **8a** was suitable for X-ray structural analysis.‡ The structure (Fig. 1) gave an independent corroboration of our NMR results.

‡ *Crystal data*: **8a** $C_{30}H_{33}F_3O_{12}$, M = 642.6, monoclinic, space group $P2_1$, a = 8.,018(4), b = 12.888(7), c = 13.921(5) Å, β = 97.33(2)°, U = 1427 Å^3, Z = 2, D_c = 1.50 g cm^{-3}, μ(Cu-Kα) = 11 cm^{-1}. Data were measured on a Siemens P3/PC diffractometer with graphite monochromated Cu-Kα radiation using ω-scans. The structure was solved by direct methods and refined anisotropically to give R = 0.048, R_w = 0.052 for 1889 independant observed reflections [$|F_o| > 3\sigma(|F_o|)$, θ ≤ 116°].

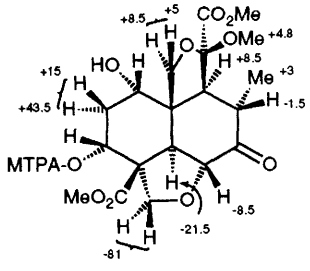
Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.



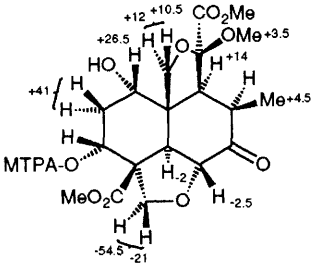
8a: 1*R*-(+)-MTPA-
8b: 1*S*-(-)-MTPA-



9a: 1*R*-(+)-MTPA-
9b: 1*S*-(-)-MTPA-



10a: 3*R*-(+)-MTPA-
10b: 3*S*-(-)-MTPA-



11a: 3*R*-(+)-MTPA-
11b: 3*S*-(-)-MTPA-

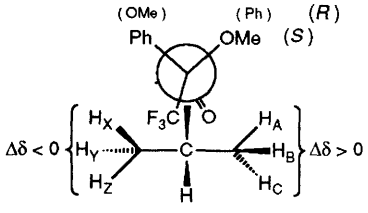


Fig. 2(*a*) $\Delta\delta = \delta S - \delta R$; all values $\Delta\delta$ expressed in Hz; (*b*) $\Delta\delta = \delta(S\text{-MTPA}) - \delta(R\text{-MTPA})$

NMR analysis was successful for all the molecules, as shown in Fig. 2(*a*). Data from both the 1- and 3-MTPA esters predict the same absolute configuration and agree with the conditions laid out in the model.[9] Placing protons with $\Delta\delta > 0$ on the right side of the MTPA plane and those with $\Delta\delta < 0$ on the left side as shown in Fig. 2(*b*) gives a 1*S*-centre from **8** and **9** and a 3*R*-centre from **10** and **11**. Both these are in agreement with the X-ray crystal structure, which also shows that the MTPA moiety lies in the ideal conformation required for the application of this model. Careful examination of the NMR data also shows that absolute values of $\Delta\delta$ decrease as distance from the MTPA moiety increases, as expected.

In conclusion, the four sets of data obtained using the modified Mosher method have allowed determination of the absolute configuration on the left hand fragment of azadirachtin, and the results have been confirmed by X-ray analysis. As the relative stereochemistry of azadirachtin has already been established,[3] we can now confirm that the absolute configuration of azadirachtin is as shown in Fig. 1.

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