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The aphid sex pheromone

Glenn W. Dawson, Norman F. Janes, Alan Mudd, John A. Pickett, Alexandra M.Z. Slawin*, Lester J. Wadhams and David J. Williams*

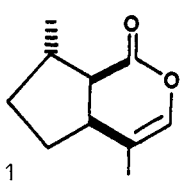
AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts., AL5 2JQ, U.K.

* Chemistry Department, Imperial College of Science and Technology, Prince Consort Road, London SW7 2AY, U.K.

Abstract - Many species of pest aphids reproduce sexually on the winter host. The sex pheromone of the vetch aphid, *Megoura viciae*, has recently been identified as comprising the (4a*S*,7*S*,7a*R*)- isomers of nepetalactone and nepetalactol. The stereochemistry at carbon-1 of the lactol was not defined, but is now shown, by X-ray crystallography on the 3,5-dinitrobenzoate ester, to have the *R* configuration. This lactol is the major product from reduction of the lactone with diisobutylaluminium hydride. Results from the similar reduction of other nepetalactone isomers are presented.

INTRODUCTION

Aphids reproduce asexually on their host plants during the summer, but many species migrate to winter or primary hosts, where sexual reproduction occurs. Pettersson (ref. 1) demonstrated that females of *Schizaphis* spp. attract males by means of a sex pheromone released from the hind tibiae. Marsh (ref. 2) also demonstrated a sex pheromone for the vetch aphid, *Megoura viciae*, and this has now been identified as a synergistic mixture of (4a*S*,7*S*,7a*R*)-nepetalactone (1) and (4a*S*,7*S*,7a*R*)-nepetalactol (2a or b) (ref. 3). However, the stereochemistry at carbon-1 of the lactol (2a or b) was not defined and therefore required further investigation.



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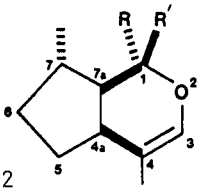


2

2a; R = OH, R' = H
b; R = H, R' = OH.

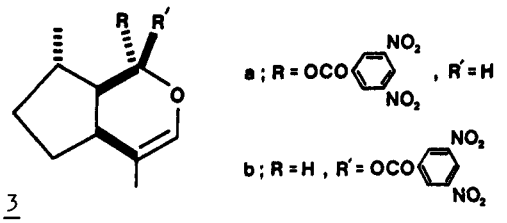
PREPARATION OF THE LACTOL 2

(+)-(4a*S*,7*S*,7a*R*)-Nepetalactone (1), isolated from *Nepeta cataria* (refs. 3 and 4), was treated with diisobutylaluminium hydride (DIBAL) at -78°C for 5 min and, after work-up with methanol, gave the lactol 2 as mostly one isomer: $[\alpha]_D^{20}$ = -40° (c = 0.5, EtOH); ^{1}H-NMR (nuclear magnetic resonance) (400 MHz, JEOL JNM-GX 400, $CDCl_3$), major isomer (93%), 1.09 ppm (d, 3H, J=6), 1.15 (m, 1H), 1.35 (m, 1H), 1.55 (s, 3H), 1.65 (m, 1H), 1.82 - 2.00 (m, 3H), 2.45 (m, 1H), 2.96 (d,1H, $J_{1,OH}$=6Hz,ex D_2O), 4.84 (t, 1H, $J_{1,7a}$ = 6Hz), 6.01 (s, 1H); diagnostic protons, minor isomer (7%), 2.90 (d,1H,$J_{1,OH}$=6Hz,exD_2O), 5.20 (dd,1H,$J_{1,OH}$=6Hz, $J_{1,7a}$=3Hz); ^{13}C-NMR (100 MHz), major isomer, 16.3 (4Me), 20.6 (7Me), 30.8 (C-5), 33.4 (C-6), 35.9 (C-7), 38.7 (C-4a), 50.5 (C-7a), 94.3 (C-1), 113.6 (C-4), 134.1 (C-3); minor isomer, 15.9 (4Me), 22.7 (7Me), 29.3 (C-5), 31.8 (C-7), 33.2 (C-6). 41.2 (C-4a), 50.4 (C-7a), 94.3 (C-1), 113.3 (C-4), 134.9 (C-3), assigned using DEPT, C-H and H-H correlation spectroscopy. GC-MS (gas chromatography coupled mass spectrometry) (fused SiO_2 capillary column, HP1 bonded phase; VG 70-250, 70 eV, 240°C), only one peak obtained, m/z 168 (M^+, 8), 150 (18), 135 (37), 122 (31), 81 (93), 67 (68), 58 (70), 55 (59), 41 (100). This material was identical to that found in the extract of hind tibiae from *M. viciae* (ref. 3) as determined by GC (fused SiO_2 capillary column, HP1 and Carbowax 20M bonded phases). However, because NMR results demonstrated the presence of the other isomer which was not separated on GC, the sample was acetylated with

acetic anhydride and 4-(dimethylamino)pyridine (DMAP) in pyridine, to aid chromatographic resolution. Two peaks were obtained from the acetylated product, in the ratio indicated by NMR on the original sample: the major component chromatographed first on both columns. The first peak was enhanced on coinjection with acetylated leg extract from *M. viciae*. Only a trace of the second isomer could be found in the aphid leg extract. Designation of the stereochemistry at carbon-1 could not be made by NMR spectroscopy because it was not possible to define the conformation of the molecule, so the 6 or 3 Hz coupling constants between protons on carbons-1 and 7a for the major and minor isomers might have arisen from either absolute stereochemistry.

X-RAY CRYSTALLOGRAPHY ON DERIVATIVE OF LACTOL 2

The lactol (i.e. mainly 2a or b) (50 mg) was treated with 3,5-dinitrobenzoyl chloride (200 mg) and DMAP (200 mg) in pyridine (5 ml) overnight at room temperature. The solvent was removed and the residue purified by chromatography on a silica plate, eluting with hexane/ether. The product, which contained the two isomers in the original ratio, was then crystallised from acetonitrile at -15°C to give the 3,5-dinitrobenzoate of the major isomer only (i.e. 3a or b), m.p. 145°C (35 mg, 32%): ^{1}H-NMR (400 MHz) 1.14 (d, 3H, J=6), 1.27 (m, 1H), 1.59 (s, 3H), 1.63 (m, 1H), 1.98 (m, 3H), 2.72 (m, 1H), 6.00 (s, 1H), 6.32 (d, 1H, J=3), 9.14 (d, 2H, J=2), 9.25 (t, 1H, J=2); ^{13}C-NMR (100 MHz) 15.9, 19.8, 28.9, 32.8, 35.1, 36.7, 48.8, 93.9, 114.2, 122.6, 129.6, 133.4, 133.9, 148.7, 161.7.



3

An X-ray diffraction study (Fig. 1) on 3 revealed the structure with the *S* stereochemistry at carbon-1, i.e. structure 3a. Based on these findings, the aphid-derived nepetalactol is assigned structure 2a, (-)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol.

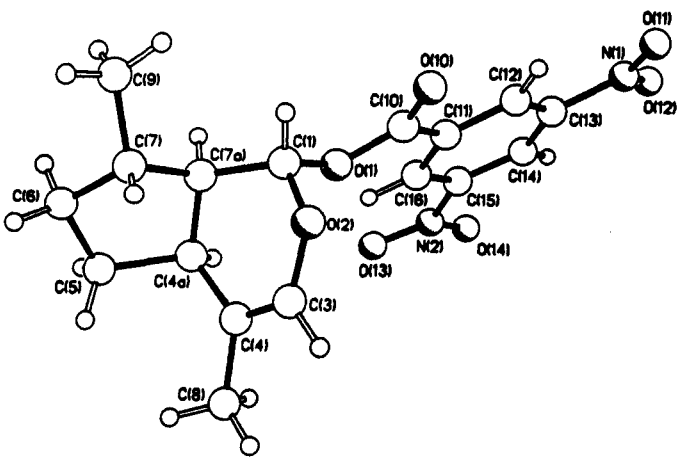
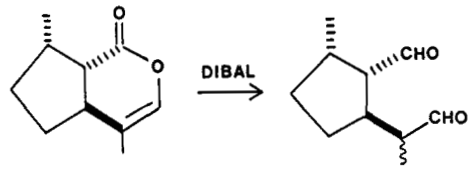


Fig. 1. The molecular structure of the lactol 3,5-dinitrobenzoate ester (3) (Note a).

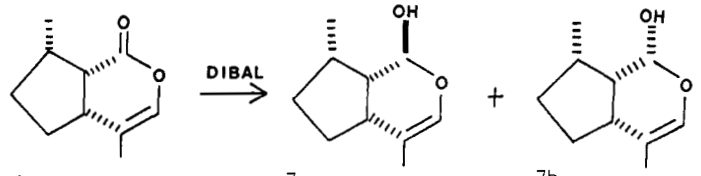
Note a: *Crystal data for* 3: $C_{17}H_{18}N_2O_7$, M = 362.3, monoclinic, a = 9.553(4), b = 5.884(2), c = 16.008(7)Å, β = 95.60(3)°, U = 896Å^3, space group $P2_1$, Z = 2, D_c = *1.34 gcm*$^{-3}$, μ(Cu-K_α) = 9 cm^{-1}. Data were measured on a Nicolet R3m diffractometer with Cu-K_α radiation (graphite monochromator) using w-scans. The structure was solved by direct methods and refined anisotropically to give R = 0.055, R_w = 0.060 for 1238 independent observed reflections [$|F_o| \geq 3\sigma(|F_o|)$, $\theta \leq 58°$]. Atomic coordinates, bond lengths and angles, and thermal parameters, have been deposited at the Cambridge Crystallographic Data Centre.

REDUCTION OF OTHER NEPETALACTONE ISOMERS

In addition to the nepetalactone 1, there are three other diastereoisomers, and of these, 4 and 6 were obtained from *Nepeta mussinii* (refs. 3 and 4). On reduction with DIBAL, the lactone 4 gave the two iridodial isomers 5: ^{1}H-NMR (400 MHz), aldehydic protons, major isomer (80%), 9.63 (d, 1H, J=3), 9.78 (d, 1H, J=3); minor isomer (20%), 9.57 (d, 1H, J=2), 9.78 (obs); ^{13}C-NMR (100 MHz), major isomer, 12.1, 16.2, 29.5, 34.7, 37.7, 38.8, 50.5, 57.6, 204.3, 204.6; minor isomer, aldehydic carbons, 204.4, 204.5. The formation of the ring-opened products from 4 contrasts with the unopened product from 2, and presumably reflects the greater steric strain in the *trans*-fused cyclopentane/δ-lactol ring systems. Corresponding ring-opened products would also be expected from the fourth nepetalactone diastereoisomer, which also has *trans*-fused rings. The *cis,cis* lactone 6 gave both diastereoisomers of the corresponding nepetalactol 7: ^{1}H-NMR (400 MHz), diagnostic protons, major isomer (70%), 5.02 (t, 1H, $J_{1,7a}$=5), 6.02 (s, 1H); minor isomer (30%), 5.33 (dd, 1H, $J_{1,7a}$=3), 5.99 (s, 1H); ^{13}C-NMR (100 MHz), *major isomer* 16.3 (4Me), 16.6 (7Me), 29.5 (C-5), 32.4 (C-6), 35.8 (C-7), 38.3 (C-4a), 45.3 (C-7a), 92.7 (C-1), 113.7 (C-4), 134.4 (C-3); minor isomer, 15.1 (7Me), 17.1 (4Me), 30.8 (C-5), 32.4 (C-6), 36.6 (C-7), 38.8 (C-4a), 44.3 (C-7a), 92.6 (C-1), 114.5 (C-4), 132.3 (C-3), assignments made as above. The major isomer therefore appears to be 7a.

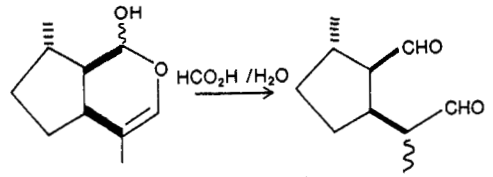


4 5



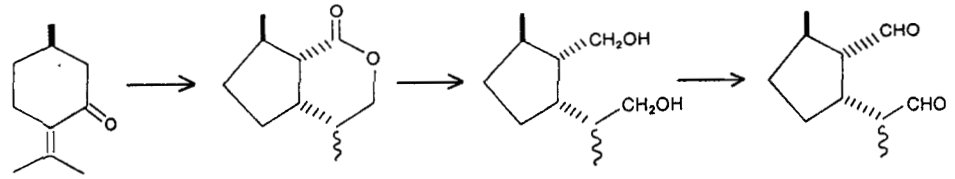
6 7a 7b

The *cis* ring-fused nepetalactols, 2 and 7, were stable in neutral solution, but on treatment with formic acid, the nepetalactol 2 gave the corresponding iridodial isomers 8: ^{1}H-NMR (400 MHz), aldehydic protons, major isomer (60%), 9.66 (d, 1H, J=1), 9.74 (d, 1H, J=2); minor isomer (40%), 9.63 (d, 1H, J=3), 9.73 (d, 1H, J=4). As further proof of the structure of the iridodial isomers 8, they were shown to have the same NMR and GC properties as the iridodial



2 8

isomers 12 (i.e. enantiomeric with 8). These were prepared by Swern oxidation of the diol 11 from reduction of the dihydronepetalactone 10 (available by the method of Wolinsky and Eustace, ref. 5, from *R*-pulegone 9).



9 10 11 12

Thus, the absolute stereochemistry of the lactol component of the sex pheromone of *M. viciae* has been defined. The formation of this diastereoisomer in such a large proportion compared with the alternative isomer must result from a particular stereochemical feature, but the exact rationale remains unexplained. The lactol 2a has now been shown to comprise the sex pheromone of the greenbug or wheat aphid, *Schizaphis graminum* (ref. 6).

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