Tetrahedron Letters,Vol.30,No.35,pp 4737-4740,1989 0040-4039/89 $3.00 + .00
Printed in Great Britain Pergamon Press plc

THE STRUCTURE OF TWO NEW CLERODANE DITERPENOID POTENT INSECT ANTIFEEDANTS FROM *Scutellaria woronowii (Juz);* JODRELLIN A & B.

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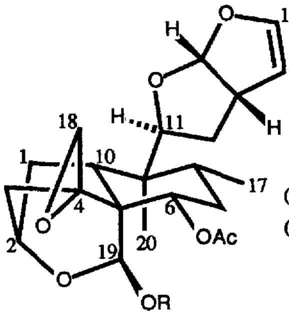
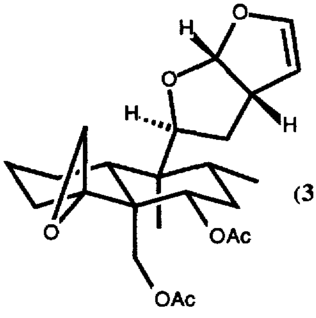
Summary: Two new clerodane diterpenoids isolated from *Scutellaria woronowii* (Juz) were shown to be the most potent clerodane antifeedants yet discovered. Their structures were rigorously determined by ^{1}H, ^{13}C, nuclear Overhauser difference 1-D, ^{1}H COSY 2-D n.m.r. and mass spectroscopy.

As part of our continuing search for enviromentally safe insect pest behaviour modifying chemicals from natural sources, a systematic survey of the labiate genus *Scutellaria*[1] was undertaken for feeding deterrent materials. The northern temperate species *Scutellaria woronowii,* was found to contain two new clerodane type diterpenoids, which we call jodrellin A (**1**) and jodrellin B (**2**): both demonstrate antifeedant properties.[2,3] In particular (**2**) is by far the most potent clerodane antifeedant known to date.[3,4,5]

The ethanolic extract from 10.7g of freeze dried aerial material from *Scutellaria woronowii* was subjected to extensive chromatography to yield the two active diterpenoids (**1**)[6] and (**2**).[7]



(**1**) R=Ac jodrellin A
(**2**) R=CO.^{i}Pr jodrellin B



(3) clerodin

By direct comparison of the n.m.r. spectra of (**1**) and (**2**) with that of an authentic sample of the natural product clerodin[8] (**3**) it was obvious that the compounds contained common functionalities. Both jodrellin A and B contain the dihydrofurofuran unit, the AB spin system corresponding to the C4-C18 epoxide, the C17 equatorial methyl doublet and a C20 axial methyl singlet. The 500 MHz. ^{1}H n.m.r. spectrum of jodrellin A (**1**) indicates the presence of two acetate functional groups while that of jodrellin B (**2**) indicates the substitution of one of the acetate groups by an isobutyrate ester. The two acetate signals of jodrellin A (**1**) were assigned to C6 and C19 by comparison with clerodin. The position of the isobutyrate ester in jodrellin B (**2**) is in agreement with the n.m.r. and in accord with the mass spectrum which indicates a strong fragmentation ion corresponding to the loss of the isobutyrate ester from the C-19 anomeric centre. The AB spin system corresponding to the C19 methylene of clerodin was absent and replaced by a downfield singlet in both molecules [δ 6.74 and δ 6.70 respectively for (**1**) and (**2**)]. From these data it was clear that these molecules must contain a bridging oxygen substituent originating from the C19 position. The 1H 2-D COSY 90 spectra of (**1**) and (**2**) were virtually superimposable (Table 1). The connectivity of the B ring may be deduced from analysis of the coupling constants (Table 1) and the 2-D spectrum. Selective decoupling experiments on jodrellin A (**1**) showed that the proton signal at δ 4.18 was coupled with at least three other protons, none of which appeared as doublets. The H-1eq - H-3eq W coupling in (**1**) and (**2**) was apparent from the COSY spectrum.

Table 1: ^{1}H n.m.r. data of jodrellin A (**1**) and jodrellin B (**2**)

jodrellin A (1)				**jodrellin B (2)**			
δ	Assignment	Multiplicity	J	δ	Assignment	Multiplicity	J
6.74	19	s	-	6.70	19	s	-
6.45	15	t	2.5	6.45	15	t	2.5
6.01	16	d	6.2	6.01	16	d	6.2
4.81	14	t	2.6	4.81	14	t	2.6
4.63	6	dd	11.6, 4.6	4.65	6	dd	11.4, 4.5
4.18	2	m	-	4.17	2	m	-
3.99	11	dd	11.7, 4.6	3.99	11	dd	11.7, 4.6
3.53	13	m	-	3.53	13	m	-
2.99	18b	d	4.4	2.99	18b	d	4.4
				2.58	1'	hep.	7.0
2.52	3_{eq}	dt	14.3, -	2.50	3_{eq}	dt	14.3, 2.6
2.42	18a	d	4.4	2.42	18a	d	4.4
2.35	1_{eq}	ddd	14.6, 7.7, 4.7	2.34	1_{eq}	ddd	14.4, 7.8, 4.9
2.13	OAc	s	-				
2.03	10	dd	11.4, 4.3	2.05	10	dd	11.4, 4.4
1.96	OAc	s	-	1.94	OAc	s	-
1.85	12	td	11.9, 8.4	1.85	12	td	11.9, 8.5
1.76	3_{ax}	dd	14.3, 2.8	1.77	3_{ax}	dd	14.3, 2.6
1.71-1.66	7_{ax}, 12	m	-	1.69	12	dd	12.2, 5.2
1.65-1.61	8	m	-	1.66-1.63	7_{ax}, 8	m	-
1.59	1_{ax}	dd	14.4, 11.5	1.59	1_{ax}	dd	14.3, 11.5
1.40	7_{eq}	ddd	12.8, 4.5, 2.7	1.40	7_{eq}	ddd	12.8, 4.5, 2.7
				1.26	2'Me	d	7.1
				1.22	2'Me	d	7.0
1.17	20Me	s	-	1.17	20Me	s	-
0.89	17Me	d	6.5	0.89	17Me	d	6.4

Nuclear Overhauser diference experiments supported these assignments (Table 2). For example, irradiation of H-6 (δ 4.65) produced enhancements indicating the close mutual proximity of H-8ax and H-10ax. The H-18a and H-18b signals indicate a colinear relationship with the H-6 proton. Irradiation of the H-2 proton produced four n.O.e.'s strongly indicative of the assigned structure. Finally, irradiation of the C20-Me group gave a strong enhancement of H-19 (+16.9%) providing substantial evidence for the assigned structures. The n.O.e.'s confirmed the previous assignments arising from the 1-D and 2-D ^1H n.m.r. spectra.

Table 2: ^1H nuclear Overhauser effect data of jodrellin B (2)

Irradiation	Response
H-6	H-18b (+12.9%), H-18a (-1.6%), H-10 (+5.0%), H-8ax (+4.5%), H-7eq (+4.6%).
H-2	H-3eq (+3.8%), H-1eq (+3.8%), H-3ax (+5.1%), H-1ax (+2.4%).
C20-Me	H-19 (+16.9%), H-11 (+6.1%), H-1eq (+6.3%), H-7ax, H-12 (~2.5%), C17-Me (~1.8%).

The high resolution accurate mass spectra were in accord with the molecular formulae and measured ^{13}C and ^1H n.m.r. spectra of jodrellin A (1) and jodrellin B (2).[9]

The antifeedant potential of the compounds was assessed by presenting them in combination with a phagostimulant, sucrose, on glass fibre discs (Whatman GF/A 2.1 cm diam.) to larvae of *Spodoptera littoralis* (Boisduval). These larvae were 24-36 hours into the final stadium and had been deprived of food for 4 hours before being placed individually into Petri dishes with two glass fibre discs (GFD). Both discs had been treated with 100μl of a 50mM sucrose solution and allowed to dry. One disc acted as the control and the other disc, the treatment disc, was treated additionally with 100μl of a solution containing a test compound. The dried discs were weighed and presented to the larvae. The bioassay terminated after the larvae had eaten approximately 50% of one of the discs, which took between 8-24 hours. The discs were reweighed and the Antifeedant Index [(C-T)/(C+T)]% calculated where C and T are the weight eaten of the control and treated disc, respectively. A potent antifeedant would be represented by a value greater than 75%.

Table 3: Bioassay Results Antifeedant Index [(C-T)/(C+T)]% [mean* ±SEM]

Concentration applied (ppm)	**jodrellin A (1)**	**jodrellin B (2)**	**clerodin (3)**
100	92 ± 7.6	100 ± 0.0	74 ± 8.4
25	53 ±13.3	83 ±10.3	24 ±9.8
1	43 ±15.9	54 ±14.4	14 ±19.6

* = 10 replications

The results of the bioassay (Table 3) indicate that the structural placement of the extra oxygen atom of the C2-C19 etheral linkage enhances the antifeedant activity of jodrellins A and B when compared with clerodin. Indeed, jodrellin B (2) is still a very potent antifeedant at 25ppm, and is the most potent clerodane antifeedant so far described.[3,4,5] The conformational constraint present in (1) and (2) relative to other clerodanes suggeste functional importance and may stimulate renewed interest in the preparation of simple analogues showing this type of activity. Furthermore, as our desire to discover more ecologically acceptable pest control methods increases, these new natural compounds, with behaviour modifying activity, may well find future applications.

Acknowledgements: *We thank the S.E.R.C. (J.C.A.) and R.B.G. (M.C.) for financial support, the S.E.R.C. Mass Spectrometry Service Centre, University College of Swansea, for Mass Spectra, and Miss S. Johnson, and the staff of Technical Glass, Kew for cultivation of plant material. This work was carried out under MAFF licence No. PHF 1020/10 issued under the Import and Export (Plant Health Great Britain) Order 1980 and Plant Pests (Great Britain) Order 1980.*

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6. 0.005% of dry mass.
7. 0.023% of dry mass.
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9. Jodrellin A (**1**).ν_{max} (film) 2926, 1729, 1616, 1463, 1373, 1257, 1141, 1090, 1008 and 951 cm^{-1}; EIMS *m/z* 448 (M^+), 389 (M^+-AcO^-), 328 (M^+-2AcOH), 317, 300, 279 (M^+-AcO^--$C_6H_7O_2^+$), 218 (MH^+-$C_6H_7O_2^+$-2AcOH), 172, 111 ($C_6H_70_2^+$), 91, 83, 69 and 55; (Found: (M^+), 448.2106. $C_{24}H_{32}O_8$ requires 448.2114).

   jodrellin B (**2**). ν_{max} (film) 2968, 2933, 1727, 1615, 1465, 1371, 1249, 1195, 1156, 1141, 1082, 1009 and 950 cm^{-1}; ^{13}C δ 175.6(q), 170.0(q), 146.7, 108.1, 102.0, 91.5, 85.5, 66.4, 67.2, 60.4(q), 50.1(CH_2), 45.7, 41.5(q), 41.3, 41.0(q), 36.7(CH_2), 35.6, 34.3, 33.1(CH_2), 32.4(CH_2), 28.5(CH_2), 21.3, 19.0, 18.4, 16.7 and 14.2; EIMS *m/z* 476 (M^+), 417 (M^+-AcO^-), 389 (M^+-$^iPrCO_2^-$), 363, 346, 328 (M^+-$^iPrCO_2H$-AcOH), 292, 272, 218 (MH^+-$C_6H_7O_2^+$-AcOH-$^iPrCO_2H$), 189, 172, 157, 145, 111($C_6H_7O_2^+$), 91, 83, 71 and 55; (Found (M^+), 476.2413. $C_{26}H_{36}O_8$ requires 476.2416); $[\alpha]_D^{20}$=-11.4°, c=0.07g/100ml ($CHCl_3$).

(Received in UK 30 June 1989)