Table 2. ^{13}C-NMR spectrum of amoorastatone (**3**) measured at 22.6 MHz in deuteriochloroform-pyridine d_5 (~20%) recorded in ppm downfield from tetramethylsilane[6]

C-1	70.6	d	C-14	61.0	d
C-2	36.1	t	C-15	218.7	s
C-3	74.5	d	C-16	47.6**	t
C-4	40.4*	s	C-17	40.9	d
C-5	28.4	d	C-19	64.1	t
C-6	27.5	t	C-20	122.1	s
C-7	68.7	d	C-21	143.1	d
C-8	42.2*	s	C-22	110.5	d
C-9	51.3	d	C-23	140.2	d
C-10	44.5*	s	C-28	95.9	d
C-11	211.8	s	*CO*CH$_3$	169.9	s
C-12	50.8**	t	CO*CH$_3$*	23.5	q
C-13	45.7*	s	C*CH$_3$*	21.4	q
				20.1	q
				19.6	q

* and ** These values may be interchanged.

We are also pleased to report that in comparison cell growth (P388) inhibition studies sendanin (**4**)[7] was found quite (ED_{50}= 0.01 µg/ml) active, anthothecol (**7**)[14] significantly (ED_{50}= 1.2 µg/ml) active and both rohitukin (**8**)[15,16] and limonin (**9**)[17] were found to be inactive (ED_{50}> 100 µg/ml). Based on these observations, an intact steroid D-ring bearing a 14,15*β*-epoxide and the 17*α*-3'-furan system seem important structural requirements for inhibiting growth of the P388 lymphocytic leukemia cell line by limonoid-type triterpenes.

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An Argentine ant aggregation factor

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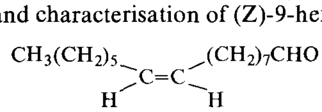
Summary. (Z)-9-Hexadecenal, a general aggregation factor of the Argentine ant, *Iridomyrmex humilis*, has been isolated and chemically characterized. It is implicated as a component of the trail-pheromone complex on evidence that it is a constituent of the ventral gland secretion, and that it both activates and attracts *I. humilis* workers.

Biological and chemical studies directed towards identifying the various aggregation factors produced by the Argentine ant, *Iridomyrmex humilis*, to activate and control trailing and other behavioural functions, have resulted in the isolation and characterisation of (Z)-9-hexadecenal (**1**).



(1)

The hexadecenal was obtained by total extraction of *I. humilis* workers with methylene chloride as previously described[3]. The extract, after removal of acidic constituents, was concentrated and then subjected to column chromatography on silica gel H. A lipid fraction, eluted with light petroleum/ether (90/10), had a characteristic fragrant odour and attracted *I. humilis* workers in multi-choice olfactometer tests. This fraction, predominantly glycerides, contained a number of trace constituents. Further column chromatography, followed by gas chromatography gave the hexadecenal (**1**), of linear retention index (LRI) 1770[4]. This trace constituent represents some 6 ppm of the body weight of the insect.

The mass spectrum of (**1**)[5] was consistent with the compound being a long chain unsaturated aldehyde of molecular formula $C_{16}H_{30}O$. It showed M^+ 238 (3%) and an M-18 ion m/e 220 (7%) indicative of the loss of the elements of water. The remainder of the spectrum was consistent with that of an alkene, with the base peak at m/e 55. Microhydrogenation of (**1**) (5 µg) gave only one product of increased retention time (LRI 1790). The mass spectrum of this product was indistinguishable from that of an authentic specimen of hexadecanal[6]. Micro-ozonolysis of (**1**)[7] (10 µg) yielded heptanal, identified by GC-MS. Compound (**1**) is 9-hexadecenal.

Behavioural tests in a multi-choice olfactometer of fractions/constituents isolated by total extraction of *I. humilis* workers

Test series	Treatment choices				'F' Ratio for treatments
	Blank	Solvent (1)	Solvent (2)	Test sample	
Lipid fraction from column chromatography	+0.2371	−0.1094	+0.1636	+1.0314***	12.284 (p<0.005)
Lipid fraction LRI <1000 from GC	+0.1413	+0.1877	+0.1755	+0.3565	0.240
Lipid fraction LRI 1000–1400 from GC	+0.3903	+0.3547	+0.2374	+0.4475	0.263
Lipid fraction LRI 1400–2000 from GC	+0.0584	−0.1363	+0.1672	+0.6653***	15.010 (p<0.001)
(Z)-9-hexadecenal (natural)	+0.2483	+0.2862	+0.3642	+0.9374***	14.205 (p<0.001)
(Z)-9-hexadecenal (natural)	+0.2571	+0.4616	+0.3432	+1.2172*	11.860 (p<0.005)
(Z)-9-hexadecenal (synthetic)	+0.1660	+0.3167	+0.1317	+0.7687	5.638 (p<0.025)

* Reaction significantly greater than to 'blank'; ***reaction significantly greater than to all other treatments. Each test series was based on 4 choices and 4 replicated experiments. Mean differences, for each choice within an experiment, between numbers of worker ants aggregating on controls and on treatments, were subjected to analysis of variance, using log(X+1) transformation of data. Comparison of treatment means was based on their ranges (G.W. Snedecor and W.G. Cochran, Statistical Methods. 6th ed. Iowa State University Press, 1967).

The cis- and trans-9-hexadecenals were prepared for comparison with (**1**), by oxidation of the corresponding alcohols[8] with chromic oxide-pyridine[9]. Gas chromatographic (3 columns) and mass spectral data did not differentiate the natural product from the synthetic (Z)- and (E)-isomers. The Raman spectrum[10] of the natural product (30 µg, purified as above) showed a band at 1656 cm^{-1}, characteristic of a cis-disubstituted ethylene. This assignment was confirmed by comparison with the spectra of the synthetic isomers, the (Z)-isomer giving a band at 1656 cm^{-1}, and the (E)-isomer at 1670 cm^{-1}.

Biological studies, which were related throughout to the chemical investigations, first examined a number of exocrine glands which might be involved in trailing control[11]. In experiments comparing the effects of samples of dissected glands on field trails, the ventral (i.e. Pavan's) gland of *I. humilis* was indicated as a major source of stimulus to trailing, in conformity with earlier work[12]. (Z)-9-Hexadecenal was implicated as a trailing pheromone constituent when its presence in a sample of 370 dissected ventral glands of *I. humilis,* held in solvent at -70 °C during preparation, was confirmed by comparative GC-MS[13].

Further assessment of the behavioural effects of chemically-separated fractions and constituents, isolated chromatographically from total extract (see above), and of dissected ventral and other glands, was made by a recently-developed multi-choice olfactometer technique[14]. The method was quantitative, based on populations of laboratory-conditioned *I. humilis* workers. Experiments were carried out under controlled physical conditions, each producing a direct comparison of the effect of a test sample with the effects of solvents and a blank (table).

The test series listed in the table showed significant activity in the gross lipid fraction. After further separation by gas chromatography[4], activity was concentrated in the fraction of LRI 1400–2000. However, fractions LRI < 1000 and 1000–1400 respectively, showed no activity. Finally, activity was demonstrated for the natural (Z)-9-hexadecenal, a constituent of the fraction, LRI 1400–2000. The synthetic (Z)-9-hexadecenal also shows a high level of activity, but not statistically at the same level as the natural product.

Possible explanations for the reduced level of attractancy to the synthetic compound may involve the concentration level selected for the present test series, or the absence from the synthetic material of some trace constituent present in the natural product, whose behavioural effect materially augmented the reactivity of the test population. Certainly, the latter suggestion is supported by the current concept of behaviour patterns commonly controlled by multi-component pheromone blends rather than single constituents at least in the Coleoptera and Lepidoptera[15], and for the primitive ant, *Myrmecia*[16]. The possible existence of additional pheromone constituent/s as yet undetected is further emphasized by slight differences which exist in the behavioural reaction pattern recorded[14] to natural ventral glands as compared with either natural or synthetic (Z)-9-hexadecenal. The differences, which may be in either degree or kind, will require further experimentation for interpretation.

The characterisation of (Z)-9-hexadecenal as a probable ant trail-pheromone constituent relates it to bombykol, (E)-10,(Z)-12-hexadecadienol, from the silkworm moth, *Bombyx mori*[17] - the first insect sex-attractant pheromone to be characterized - to other long chain unsaturated alcohols and acetates[18], and more recently to aldehydes[19], all isolated from the Lepidoptera. Now the isolation of bombykal, (E)-10,(Z)-12-hexadecadienal, as a second sex attractant pheromone from *Bombyx mori,* again points to multicomponent systems being involved as chemical messengers[20]. Further chemical and biological studies on aggregation factors of *I. humilis* are proceeding.

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