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DEFENSIVE AND OTHER SECRETIONS OF THE AUSTRALIAN COCKTAIL ANT, *IRIDOMYRMEX NITIDICEPS*

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Abstract—Iridodial and isovaleric acid were characterized as major components of the total extract, and of the anal gland secretion, of *Iridomyrmex nitidiceps*. Minor constituents identified from the total extract included iridolactones, dihydronepetalactones, actinidine, 2- and 3-pentenoic acid, and fatty acids.

Behaviour experiments demonstrated isovaleric acid to be primarily an alarm substance, developing repellency only at high concentration. Iridodial was deduced to be the basic repellent. Together the two served a defensive function. Their combination as products of one pair of glands showed a high level of social development.

INTRODUCTION

Cyclopentanoid monoterpene derivatives are well known to be employed by insects in a defensive role.[1,2] Of these monoterpenoids, iridomyrmecin (**1**) was first isolated from the anal gland secretion of the cosmopolitan pest, the Argentine ant, *Iridomyrmex humilis* (Dolichoderinae).[3] Elucidation of the structures of iridomyrmecin, together with the *cis, trans*-(**2**) and *trans,cis*-iridodials (**3**), and the dolichodials (e.g. **4**) has followed on their inter-relation with nepetalactone (**5**)—an attractant for members of the Felidae—isolated from the cat-mint plant, *Nepeta cataria*.[4,5]

The latter cyclopentanoid monoterpene derivatives, generally referred to as iridoids, are now known to be characteristic constitutents of the anal gland secretions of many dolichoderine ants. Iridoids have not been reported from representatives of other ant sub-families. They have been isolated from Phasmatidae or stick-insects,[6,7] and from members of several genera of beetles.[8-10]

A recent extensive review[11] lists the iridoids, and associated aliphatic and alicyclic carbonyl compounds, acids and alcohols characterized as anal gland constituents, from 22 species of dolichoderine ants. For example, 6-methylhept-5-ene-2-one[12] was first found in association with *cis, trans*- and *trans, cis*-iridodial in the common Australian meat ant, *Iridomyrmex purpureus* (= *detectus*).[13] Following on the isolation of methylheptenone and iridodial from the meat ant, the same compounds were identified[14] from what was then considered to be the cocktail ant, *Iridomyrmex nitidiceps*, this material being collected in the Bago State Forest, N.S.W.

Subsequent chemical and biological studies have been based on a series of samples of *Iridomyrmex nitidiceps*,† collected on the trunks of various forms of *Eucalyptus* and *Angophora* distributed through the Syndey area, from Kuringai-Chase National Park in the north to the Royal National Park in the south. Of the known material of *I. nitidiceps*, that collected from the Royal National Park has given iridodial and isovaleric acid as the major constituents of the anal gland secretion.[2] The same constituents have been characterized from total extracts of *I. nitidiceps* collected throughout the Sydney area. Methylheptenone was not detected from samples collected intermittently from the same locality in the Royal National Park during the period 1965–1980.

Although the involvement of dolichoderine anal gland secretions with a combined "alarm-defense" behavioural system has been recognized[15-17] experimental data supporting the views held is meagre. Thus from early in its inception the chemical investigation was associated in an inter-disciplinary undertaking with a biological study directed towards assessing experimentally the relative behavioural functions of major anal gland constituents The results of the above studies, now reported in detail, form part of a comparative chemical and biological investigation of exocrine gland secretions of members of the genus *Iridomyrmex*.

Constituents of the cocktail ant, I. nitidiceps

Total extraction of *I. nitidiceps* workers with light petroleum, or methylene chloride was carried out as described for *I. humilis*.[18] The use of methylene chloride as solvent has, in general, resulted in a higher proportion of total extract.[22] The total extract was separated into a neutral and an acidic fraction. Gas chromatographic comparisons of the total extract, the neutral fraction, and the acidic fraction with *cis, trans*- and *trans,cis*-iridodial from *I. purpureus* (= *detectus*), and with synthetic isovaleric acid on column (3) showed that iridodial and isovaleric acid were the major constituents.

Iridodial was characterized from the neutral fraction as the *bis*-2, 4-dinitrophenylhydrazone.[13] Gas chromatographic comparisons showed the iridodial to be largely *cis,trans*-(**2**), with a small proportion of the *trans,cis*-isomer/s (**3**). The predominant *cis,trans*-configuration for iridodial was confirmed by oxidation of the neutral fraction with zinc permanganate, as previously described[19] for

†Identified by Dr. R. W. Taylor, CSIRO, Division of Entomology, Canberra.

synthetic iridodial. The major products of oxidation were the epimeric *cis,trans*-neptalinic acids (**6**).

GC-MS analyses of the neutral fraction (Fig. 1), and after chromatography, of the hydrocarbons, terpenoids and other lipid fractions were undertaken. The hydrocarbons comprised 15% of the total extract, and were predominant consitituents in the heads of *I. nitidiceps*. The hydrocarbons will be reported on separately—they are of higher molecular weight and include normal alkanes, alkenes and branched mono-, di and tri-methylalkanes.

Ion monitoring of the broad iridodial peak (1) (Fig. 1) showed that the tail of the peak (2) contained actinidine (**7**), [*m/z* (%): 147 (50), 146 (25), 132 (100), 117 (30)]. Of the remaining peaks (3) and (4) have mass spectra corresponding to those of the epimeric dihydronepetalactones (**8**).[20] Similarly peaks (5) and (6) have mass spectra corresponding to those of the iridolactones, e.g. iridomyrmecin (**1**).[21] The four lactone peaks were not present in sufficient quantity for complete chemical characterization, peak (3) showed ions at *m/z* (%): 168 (10), 153 (55), 113 (100), 95 (25), 81 (80), 67 (55), and peak (5) at *m/z* (%): 168 (8), 153 (5), 109 (45), 95 (100), 81 (90), 67 (90). The ratio of peaks (3) to (5), and of (4) to (6), correspond to the ratio of the *cis,trans*-to the *trans,cis*-iridodials (see above).

The odour of the acidic fraction indicated the presence

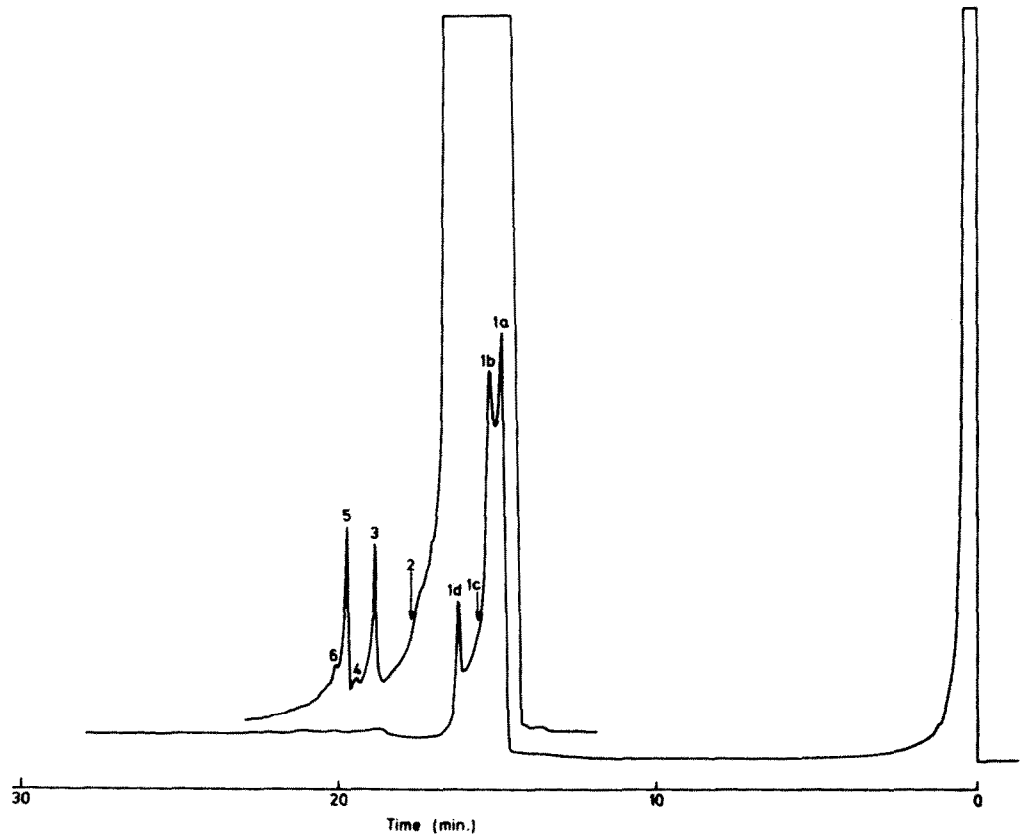
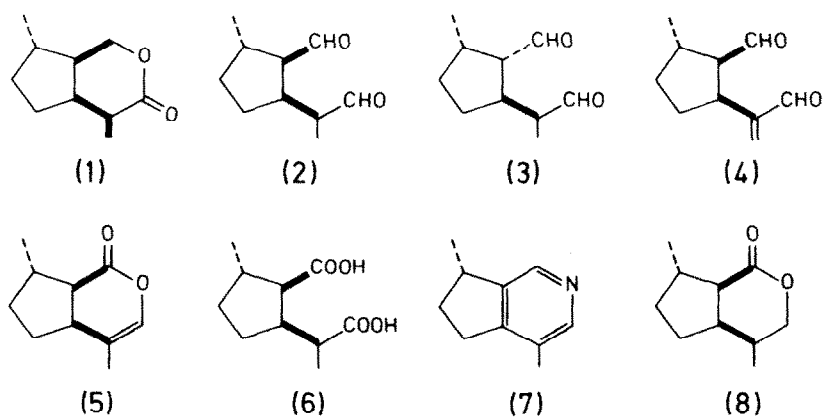


Fig. 1. Glc trace of the neutral fraction from *I. nitidiceps* run on column (5), programmed from 90° at 5°C/min. The insert is an expansion of the region below it. The numbers refer to text.



of lower molecular weight fatty acids. The major constituent, isovaleric acid was characterized as its *p*-bromophenacyl ester. The acidic fraction was examined, after methylation with diazomethane, by GC–MS (Fig. 2 and Table 1). Peak (1) was identified as methyl isovalerate, peak (2) was shown to be methyl pent-3-enoate, and peak (3) to be methyl pent-2-enoate. This peak has the same mass spectrum, but a shorter retention time than an authentic specimen of methyl *trans*-pent-2-enoate, and is assigned the *cis*-configuration. Peak (4) is the methyl ester of an unidentified methylpentenoic acid. Peaks (5) and (7) are methyl benzoate and methyl phenylacetate respectively.

The mass spectrum of peak (6) corresponded to that of a methyl methylcyclopentane carboxylate. It showed a parent ion $M^{+\cdot}$ 140, and substantial ions at *m/z* 125 (M − 15), 109 (M − 31). The base peak at *m/z* 81 is characteristic of the methylcyclopentenyl ion. Peaks (8) and (9) represent the iridodials which have appreciable water solubility. Peaks (10), (11) and (12) are dimethyl nepetalinates, possibly they arise from autoxidation of iridodial[22] whilst the mass spectrum of peak (13) has characteristics suggesting that it could be the methyl ester of an iridoid hydroxyacid, it is not identical with either of the methyl esters derived on hydrolysis and methylation of isoiridomyrmecin or isodihydronepetalactone. Peak (14) is unidentified. The remaining peaks are methyl esters of normal fatty acids: myristic, palmitic, oleic and stearic; peak (16) corresponds to an unsaturated fatty acid.

Of the above compounds, iridodial, actinidine and isovaleric acid have been identified by gas chromatography and mass spectrometry, as constituents of the anal gland secretion. The iridoid lactones were not found in extracts of the anal glands, however, they may have been present in quantities insufficient for detection.

Biological evaluation of anal gland constituents

In view of general statements[15–17] in functional terms

Table 1. Acids identified as their methyl esters in *Iridomyrmex nitidiceps*

No	Acid	Ratio	Mass spectral data for methyl esters, m/z (%)	Ref.
1	Isovaleric acid	100	116(3),101(20),85(50),74(100),59(55),57(50)	a
2	Pent-3-enoic acid	4	114(30),99(8),86(10),83(30),82(35),74(60), 55(100)	a
3	Pent-2-enoic acid	35	114(40),99(7),83(100),55(50)	a
4	Methylpentenoic acid	2	128(15),113(3),96(15),83(15),74(15),69(95), 41(100)	a
5	Benzoic acid	2	136(35),105(100),77(55)	a
6	Methylcyclopentene carboxylic acid	4	140(38),125(60),109(35),105(15),81(100),79(40)	
7	Phenylacetic acid	1	150(28),105(8),91(100)	a
8	[Iridodial]		168(3),153(3),150(7),135(14),109(34), 81(100),67(70)	b.
9	[Iridodial]		168(3),153(4),150(8),135(18),109(35), 81(100),67(65)	b
10	Nepetalinic acid	15	228(1),197(16),196(10),168(65),141(27),109(60) 81(100)	c
11	Nepetalinic acid	1	228(0),197(16),196 (7),168(32),141(19), 109(47),81(100)	c
12	Nepetalinic acid	5	228 (0),197(15),196(10),168(60),141(20), 109(60),81(100)	c
13	Unknown acid	9	200(2),169(6),158(45),143(28),126(40),111(100) 85(90),57(85)	
14	Unknown acid	8	158(12),145(10),113(85),87(100),85(90),67(55), 55(60)	
15	Myristic acid	4	242(10),211(5),199(15),87(38),74(100),59(60)	d
16	Unknown acid	6	180(8),172(15),169(70),87(90),74(80),55(100)	
17	Palmitic acid	9	270(5),239(4),227(6),87(60),74(100),55(35)	d
18	Oleic acid	10	296(3),265(10),264(20),222(10),87(48), 74(70),69(75),55(100)	d
19	Stearic acid	7	298(10),267(3),255(8),87(70),74(100),55(65)	d

[a] EPH-NIH Mass Spectral Data Base [Ed: S.R. Heller and G.W.A. Milne], U.S. Government Printing Office, Washington, 1978

[b] Ref. 22

[c] F.E. Regner, E.J. Eisenbraun and G.R. Waller, *Phytochem.*, 6, 1271 (1967)

[d] Registry of Mass Spectral Data [Ed: E. Stenhagen, S. Abrahamsson and F.W. McLafferty] Wiley-Interscience, New York, 1974

that major anal gland constitutents in the doclichoderine ants are considered to be alarm and defence substances, or may combine the two effects, a series of behavioural experiments was directed towards these areas.

Synthetic iridodials were unavailable for experimentation, while natural iridodials were likely to contain additional trace constituents (Fig. 1). *Cis,trans-* and *trans,cis*-iridodial from the meat ant, *I. purpureus* (= *detectus*), which was available in sufficient quantity, tended to retain traces of methylheptenone, whose presence would render suspect any behavioural effect the iridodial might have on *I. nitidiceps*. With these limitations, materials for behaviour studies were restricted to isovaleric acid (synthetic) and whole anal glands obtained by micro-dissection.

Alarm in itself was not readily measureable directly in behavioural terms and could best be quantified by effects resulting from it. Further, it required delimitation from other facets of the complex behaviour patterns of which it normally formed part. The two primary effects resulting from alarm in ants are changes in the *activity rate*, and a striking pattern of *activity interruption*, in which movement is not smooth and continuous, but punctuated by brief, repeated movement arrests.[23] These two reactions were used to define alarm in *I. nitidiceps*. Treatment by both isovaleric acid and whole anal glands produced a high level of increase in activity rate (Table 2), which was recorded as the number of times the test ant crossed beneath a single diameter marked on the glass cover of a cylindrical observation chamber within 10 min. There was no significant difference between the two treatments. Activity interruption, recorded by one observer using a tally counter or event recorder, was counted as the number of times in 10 min the test ant displayed momentary arrests of movement producing the characteristic jerky alarm effect. Here the figure for whole anal glands was significantly less than for isovaleric acid alone, although at a low level.

Taken together, and assuming that the two reactions produce a comparatively complete picture of alarm behaviour as a restricted entity, isovaleric acid must be considered a major alarm constituent of the anal gland secretion. Further experimentation would be needed to reach a decision on whether or not isovaleric is the only alarm substance involved, although this possibility should be recognized. The reduced activity interruption recorded for whole anal glands suggests that there must be a counteraction, or damping down, of alarm by one or more of the anal gland components additional to isovaleric.

Test insects were likely to be repelled by a secretion which was defensive in effect, whether from their own or another species. But the assessment of repellency as a negative reaction introduced a measurement problem, arising from the need to differentiate between passive "failure to attract" and active "repulsion". In the present case an active attractant (honey) was introduced into the experiments as control and the effects of materials thought to be repellent were then weighed up against its activity. At the low, 5-ant treatment level, whole anal glands showed an increase in repellency, in which repellency/attractancy percentages changed from approximately 20:80 to 50:50 with treatment. But isovaleric acid treatment had no effect on the percentage levels established in the controls (Table 3). There was a clear indication from this result that the whole anal glands contained a repellent other than isovaleric acid.

When treatment samples were increased to 14-gland

Table 2. Alarm behaviour in terms of changes produced by 5-gland (i) and 5-gland-equivalent (ii) treatments tested on single ants in closed system. Twenty replicates

Materials Tested	Means for Activity Rate: Control	Treatment	Effect due to Treatment	t^a
(i) Whole anal glands	81.55	125.25	+ 43.7	10.45***
(ii) Isovaleric acid	103.18	145.85	+ 42.67	10.48***
Difference between (i) and (ii) in treatment effect levels			+ 1.02	0.18 N.S.

Materials Tested	Means for Activity Interruption: Control	Treatment	Effect due to Treatment	t^a
(i) Whole anal glands	67.75	104.55	+ 36.8	5.25***
(ii) Isovaleric acid	78.85	141.6	+ 62.75	7.72***
Difference between (i) and (ii) in treatment effect levels			- 25.95	2.42*

a Student's t-value for significance of difference between control and treatment

* Probability that treatment effect is not significant < 5%;

*** Probability that treatment effect is not significant < 0.01%

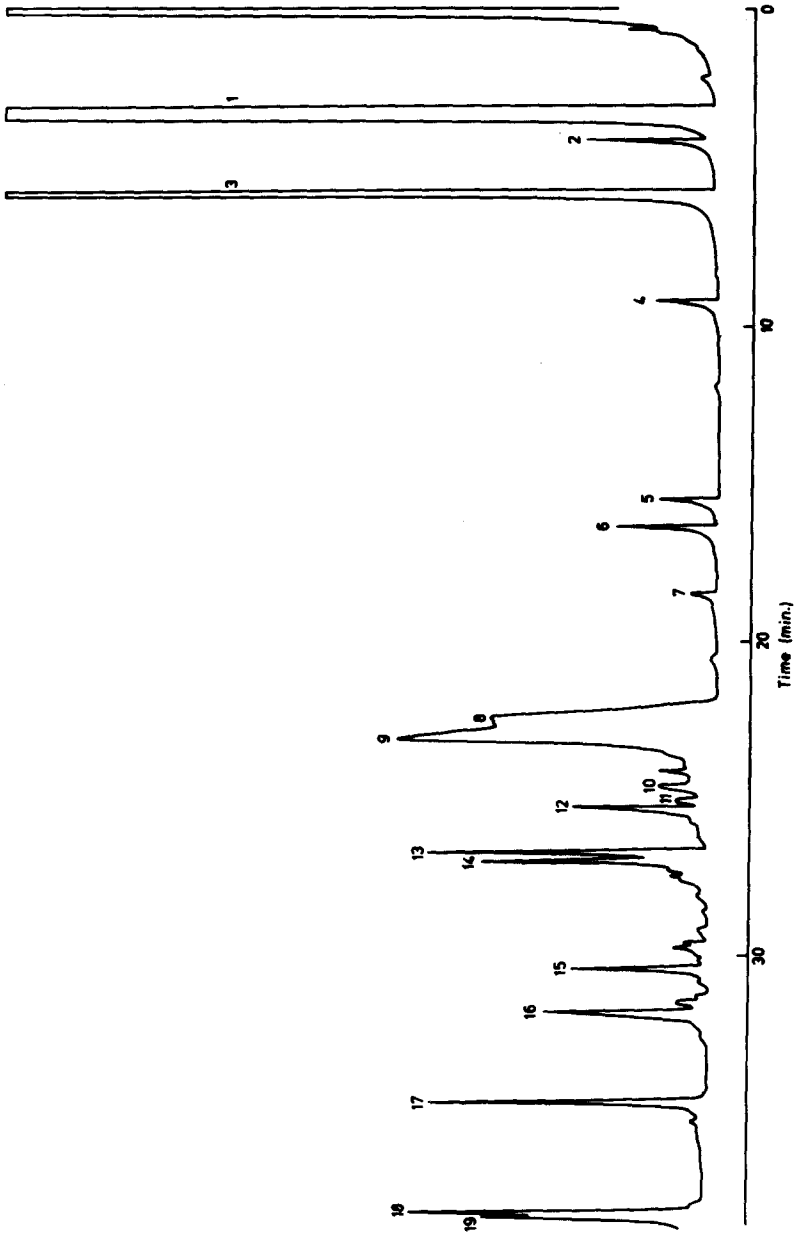


Fig. 2. Glc trace of the acidic fraction after methylation of *I. nitidiceps* run on column (5), programmed from 70° at 5°C/min. The numbers refer to Table 2.

concentrations, whole anal glands again showed a positive repellency increase, but to a somewhat higher level than for the 5-gland tests. Results for isovaleric acid showed a complete reversal from the control repellency/attractancy relationship to figures in the percentage range of 65:35 (Table 3).

To sum up experimental results of the biological study of behavioural functions of anal gland constituents of *I. nitidiceps*: (a) isovaleric acid is a major alarm constituent of the anal glands; (b) the alarm effect of isovaleric tends to be somewhat modified in the presence of other anal gland constituents; (c) the anal glands contain a repellent other than isovaleric, which chemical analysis of anal gland constituents suggests is likely to be iridodial; (d) increased production of whole anal gland secretion leads to an increase in the repellency effect; (e) at high concentrations the function of isovaleric acid shows a major changeover from alarm to repellency, possibly at the expense of the alarm effect.

DISCUSSION

Only the earlier studies[24–26] on alarm-defence substances in dolichoderine ants showed a direct experimental approach to problems of function. More recent lists, for example of alarm substances, have tended to be conjectural, recording them as "known and probable", or "presumed".[16,17] This situation, indicating a failure of biological investigations to keep pace with chemical studies, might have arisen from the fact that, while chemical studies have been stimulated by the intrinsic interest of the compounds involved, as well as by their availability in terms of quantitative levels, yet the biological functions of alarm and defence remain difficult to define and delimit.

The present investigation is the first to demonstrate experimentally the function of isovaleric acid as an alarm substance. Nevertheless, the latter possibility had already been put forward on theoretical grounds[17] arising from the recovery of isovaleric from the total extract of a myrmicine ant, *Myrmicaria.*

The biological function of iridodial is more obscure than that of isovaleric. It had been recorded previously from the anal glands of *Tapinoma nigerrimum*[27] and more recently from a number of *Conomyrma* spp.[28] It has been suggested as a possible fixative for included anal gland volatiles, and also as a mechanical deterrent,[29] both credible possibilities. But the present study represents an advance on earlier explanations in that it not only demonstrates the origin of iridodial from the anal glands, but also pinpoints its position as a defensive secretion in the face of evidence of the strong repellency effect of whole anal glands, and the absence of any other major constituent which could function as a repellent.

Iridodials have been reported[11] from many species of dolichoderine ants. In *I. nitidiceps*, the *cis,trans*-isomers (**2**) predominate. Iridolactones (e.g. **1**) and dihydronepetalactones (e.g. **8**) were also found in small proportions in the total extract (Fig. 1). Previously iridodial, predominantly of one configuration, was found[30] in association with the iridolactone of the same configuration as the major iridodial isomer, in total extracts of *Iridomyrmex pruinosus*, and of *Tapinoma sessile.* The biological significance, if any, of isomer variations in the iridodials, confirmed as defensive secretions, is unresolved. Possibly the significance does not lie in the iridodials *per se* but in stereospecific transformation products—iridoid lactols, lactones and/or hydroxyacids—some of which are minor and trace constituents (Figs. 1 and 2).[30]

The occurrence of actinidine (**7**) as a trace constituent of the total extract of macerated ants was viewed with some caution as iridodial may be converted into actinidine by the action of ammonia. The presence of actinidine was confirmed as a constituent of the anal gland secretion of *I. nitidiceps.* Recently it was reported[28] as a major constituent of two species of dolichoderine ants of the genus, *Conomyrma.*

Isovaleric acid was isolated previously[31] as the major component of a mixture of volatile acids from a total extract of the dolichoderine ant, *Liometopum micro-*

Table 3. Defensive behaviour in terms of changes produced in the repellency/attractancy ratio by 5-gland (i), 5-gland-equivalent (ii), 14-gland (iii) and 14-gland-equivalent (iv) treatments on worker ant populations in cage arena. Ten replicates

Materials Tested	Proportional Changes in Repellency/Attractancy		t^a
	Control	Treatment	
(i) Whole anal glands	$19.53\%/_{80.47\%}$	$49.89\%/_{50.11\%}$	9.64^{***}
(ii) Isovaleric acid	$25.33\%/_{74.67\%}$	$27.0\%/_{73.0\%}$	1.23 N.S.
Difference between (i) and (ii) in treatment effect levels			8.77^{***}
(iii) Whole anal glands	$18.06\%/_{81.94\%}$	$58.59\%/_{41.41\%}$	11.63^{***}
(iv) Isovaleric acid	$28.88\%/_{71.12\%}$	$63.46\%/_{36.54\%}$	7.94^{***}
Difference between (iii) and (iv) in treatment effect levels			0.81 N.S.

[a] Student's *t*-value for significance of difference between control and treatment

*** Probability that treatment effect is not significant < 0.01%

cephalum. The acids, isovaleric, isobutyric and acetic, were found in the approximate ratio 60:20:5. The glandular origin of these acids was not established, but methylheptenone was identified as a constituent of the anal gland secretion. In *I. nitidiceps*, the acidic fraction from total extraction gave the 2- and 3-pentenoic acids, a methylpentenoic acid, and a methylcylopentene carboxylic acid in addition to isovaleric acid. Whilst isovaleric acid has been identified as a major constituent of the anal gland secretion, the glandular origin and function of the additional acids is not yet known.

EXPERIMENTAL

Biological materials and methods. Biological investigations were carried out at a different period in time from chemical studies, and were independent of them. Living populations were collected from the same area as that drawn on for chemical material, but from only one type of host tree—"scribbly gum". Whereas chemical characterization referred to "workers-only" material collected from trials on the tree surface, biological material was obtained directly from nests located within damaged and decayed heart wood in the tree bases. Long-term laboratory colonies of this origin were established which were of mixed caste with plentiful brood. Colonies were held in perspex laboratory cages[32] under conditions of natural daylight, in an air-conditioned laboratory at 23°.

Experiments were carried out at 25°, under "natural daylight" lamps, and were of two types: (a) Alarm, studied in the "closed system" already developed[32] produced information on two behavioural reactions,[23] from tests against single ants of samples of isovaleric acid in freshly distilled ether, and of whole anal glands without solvent. Two observers using tally-counters recorded results in 10-min runs. (b) An repellency/attractancy relationship was recorded in experiments carried out in the arenas of laboratory cages. The base of the arena was lined to the walls with cork, and a circle of plastic 5″ in diameter was attached to its centre by cellotape. The whole was covered by a sheet of filter paper, held down by entomological micro-pins. A treatment area, consisting of 3 concentric circles of 1/2″ dia., 1–1/2″ diameter and 2–1/2″ dia. was marked out on the centre of the filter paper above the plastic. Four to six hundred worker ants were allowed to merge from the nest into the arena and were enclosed there by the trapdoor. The cage was conditioned in the 25° C.T. room for 1 hr, the small innermost treatment area was then coated with crystallized honey to act as an attractant, and the population in the arena was allowed to stabilize to this for a further 30 min. For experiments the zone between the 1/2″ dia. circle and the 1–1/2″ dia. circle was designated the "attractancy zone", that between the 1–1/2″ dia. circle and the 2–1/2″ dia. circle the "repellancy zone", and treatment samples of either isovaleric acid or whole anal glands were applied around the circumference of the 1–1/2″ dia. circle. Controls for isovaleric acid and whole anal glands respectively were of ether alone (allowed to evaporate for 1 min before the commencement of an experiment), or without any solution applied to the treatment circle. Counting runs were of 5 min, and each experimental figure recorded was then the mean of two runs.

Gas chromatography and mass spectrometry. Analytical gas chromatography was carried out using a Perkin–Elmer 800 instrument with FID detector and fitted with stainless steel columns (2 m × 3 mm) packed with: (1) 25% DEGS on Celite (30–80 mesh), (2) 5% DBS on Chromosorb W (80–100 mesh), (3) 10% LAC–IR–296 with 2% H_3PO_4 on Celite (30–80 mesh), (4) 5% Apiezon on Celite (30–80 mesh). Nitrogen was used as carrier gas (60 ml/min).

GC-MS (electron impact) was carried out using a Shimadzu GC6-AMP gas chromatograph connected *via* a straight-split interface to an AEI MS12 mass spectrometer operating at 70 eV, ion source at 175°. A SCOT column (5) coated with OV-17 (30 m × 0.5 mm) was used with helium as carrier gas. Data was recorded and processed by a VG display Digispec 2025 system. Temps are in degrees Celsius.

Solvent extraction of whole ants

(a) *I. Nitidicep*: workers (9.0 g) were extracted (Soxhlet) with light petroleum, b.p. 40–60° for 24 hr. The extract, on cooling, was treated with $NaHCO_3$aq. further washed (H_2O), dried (Na_2SO_4), and the solvent removed. The crude extract (0.47 g) gave an almost colourless viscous oil (0.18 g) on distillation under reduced pressure. The bicarbonate extract (and aqueous washings) were acidified (dil HCl) and continuously extracted with ether. The ethereal layer, after drying and distillation, gave a colourless acidic oil (0.07 g).

(b) Seven samples of *I. nitidiceps* workers from different locations in the Sydney area were each extracted with light petroleum, as in (a). The total extracts were subjected to gas chromatography, using column (3). The major peaks corresponded to isovaleric acid and iridodial.

(c) Whole ants (35 g) were ground in a glass mortar with anhyd Na_2SO_4, and the mixture extracted (Soxhlet) with CH_2Cl_2 (6 hr), then with ether (6 hr). Solvent extracts were treated separately with an excess of $NaHCO_3$aq and the two aqueous extracts were then combined, acidified (dil HCl) and worked-up as described in (a), giving an acidic fraction (136 mg). The organic extracts were dried, and solvent was removed using a Büchi evaporator. The extraction with methylene chloride gave a neutral oil (2.16 g), (Fig. 1). The ether extraction gave additional material (0.15 g). The neutral fraction represents 6–7% of body weight, and the acidic fraction 0.4%. Aliquots of the acidic fraction were methylated (diazomethane) for GC-MS characterization as required (Fig. 2). Data is given in Table 1.

Characterization of iridodial and isovaleric acid

The freshly distilled neutral fraction from (a), predominantly iridodial, gave a yellow *bis*-2,4-dinitrophenylhydrazone, m.p. 220–225° on treatment with 2,4-dinitrophenylhydrazine sulphate in EtOH. The m.p. of this derivative was undepressed on admixture with an authentic specimen of iridodial *bis*-2,4-dinitrophenylhydrazone from *I. purpureus* (= *detectus*).[13]

Freshly isolated iridodial (neutral fraction 0.1 g) in water (25 ml) was heated with a soln of $KMnO_4$ (1.5 g) and $ZnSO_4$ (2.0 g) in water (100 ml) for 3 hr. The mixture was worked-up as described.[19] The nepetalinic acids were methylated (diazomethane), the methyl esters being isolated as a colourless oil (45 mg), on distillation under reduced pressure. Gas chromatography [column (2)] showed two peaks at 12′ 40″ and 13′ 50″, whose retention times correspond to those of the epimeric dimethyl *cis,trans*-nepetalinates (**6**).

The acidic oil from (a), on treatment with *p*-bromophenacyl bromide in the usual manner, gave *p*-bromophenacyl isovalerate, m.p. and mixed m.p. 67–68°, with an authentic specimen. [Found: C, 52.4; H, 5.1% $C_{13}H_{15}O_3Br$ requires C, 52.2; H, 5.0%]. The acidic oil was methylated with diazomethane in ether. Gas chromatography of the methylated fraction showed a broad peak of retention time 1′ 47″ on column (1), temp 58°, and of retention time 4′ 0″ on column (4), temp. 52°. The respective retention times are identical with those of methyl isovalerate.

Characterization of anal gland constituents

(a) Anal glands (30) freshly excised from *I. nitidiceps* workers were crushed under light petroleum (1 ml) with a needle. An aliquot was methylated using diazomethane in ether, and the mixture was allowed to stand (10 min). A gas chromatographic comparison using column (1) showed that the major peaks of the methylated extract corresponded to those of the reference compounds—the *cis,trans*- and *trans,cis*-iridodial, from *I. purpureus* (= *detectus*), and methyl isovalerate.

(b) Anal glands (50) were excised, then extracted with ether (0.5 ml). An aliquot was methylated (diazomethane) and the ethereal soln concentrated under N_2 GC–MS characterization showed methyl isovalerate [*m/z* 116 (3), 74 (100)], iridodial [*m/z* 168 (3), 109 (35), 81 (100)], and actinidine [*m/z* 147 (40), 146 (30), 132 (100), 117 (25), subtracted spectrum].

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