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CHEMICAL ECOLOGY OF THE MEAT ANT, *IRIDOMYRMEX PURPUREUS* SENS. STRICT.

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Abstract—Twelve volatile constituents have now been characterized from the common meat ant, *Iridomyrmex purpureus* sens. strict. 2,5-Dimethyl-3-ethyl-, 2,5-dimethyl-3-propyl- and 2,5-dimethyl-3-butylpyrazine were mandibular gland constituents, and in total represented < 1 ng/gland. The major constituents were confirmed as the known 6-methylhept-5-ene-2-one and the iridodials. The oxygenated compounds: 1,3,3-trimethyl-2,7-dioxabicyclo[2,2,1]heptane, not previously reported from an insect source, together with 2-acetyl-3-methylcyclopentene and 6-methylhept-5-ene-2-ol were minor products. Minor iridoids were 2-(3-methylcyclopentyl)propional, a dihydroneptalactone and an iridolactone, plus actinidine. The oxygenated volatiles and iridoids were isolated both from extracts of whole insects, and bodies of *I. purpureus* workers.

The same chemical pattern was repeated in samples of *I. purpureus* sens. strict., collected over a wide area of south-eastern Australia. The several series of pyrazines, iridoids, carbonyl compounds and associated volatiles, together with the previously described hydrocarbons, serve to distinguish *I. purpureus* sens. strict. chemically.

Key Word Index: Chemical ecology, ants, *Iridomyrmex purpureus*, pyrazines, iridoids and actinidine, 1,3,3-trimethyl-2,7-dioxabicyclo[2,2,1]heptane, 2-acetyl-3-methylcyclopentene, 6-methylhept-5-ene-2-one and 6-methylhept-5-ene-2-ol

INTRODUCTION

As part of a continuing study of the chemical ecology of the ant genus *Iridomyrmex* (Formicidae, Dolichoderinae) we have reported (Brophy *et al.*, 1983) on the hydrocarbon constituents from two widely distributed Australian species, *Iridomyrmex purpureus* (= *detectus*), the common meat ant, and *I. nitidiceps*, a member of the *itinerans* group. There were distinct differences in the hydrocarbon patterns of the two species, which in turn, differed from the pattern noted for the cosmopolitan species, the Argentine ant, *Iridomyrmex humilis* (Cavill and Houghton, 1973).

The prime objective of the present study has been to assess the extent of chemical variation, if any, that may be detected in one clearly recognized species of *Iridomyrmex* taken over a wide geographical range. In Australia, the wide distribution and accessibility of populations of *I. purpureus* has led to a detailed chemical investigation of this species. The work complements the original characterization of 6-methylhept-5-ene-2-one (Cavill and Ford, 1953), and of iridodial (Cavill *et al.*, 1956, 1960), the major exocrine constituents of *I. purpureus* (= *detectus*).

The Australian meat ant studied in the present investigation was described in 1858 under the genus *Formica*, with the species names *detecta* and *purpurea* being used for female reproductives and workers respectively. *Iridomyrmex* was later separated off as a distinct genus (Mayr, 1862), to which *detectus*, for long accepted as a legitimate species name, was assigned. But some confusion related to designation by Emery (1912) of type of genus as "*Iridomyrmex purpureus* (Fred. Smith) = *detectus* (Fred. Smith)", while listing *I. detectus* as a legitimate species of *Iridomyrmex* and *I. purpurea* as a synonym. It was only recently (Greaves, 1971; Greenslade, 1974) that recognition was given to the requirements of the Rules of Zoological Nomenclature that *purpureus* be accepted and *detectus* rejected, as the correct name for the type of *Iridomyrmex*.

In Australia ants of the genus *Iridomyrmex* are dominant arthropods of the soil surface, and meat ants are dominant amongst species of *Iridomyrmex* (Greenslade, 1979). The species *Iridomyrmex purpureus* s. strict., i.e. in the sense of Greaves (1971), is by far the most widespread meat ant, extending over one third of the Australian continent. Thus, of the almost three million square miles of mainland Australia, *I. purpureus* is likely to occur in up to one million square miles of it. Its range extends from east to west of the continent and from its southern extremities at least as far north as the Tropic of Capricorn. But limitations to the distribution of *I. purpureus* are imposed by (a) rainfall: it does not occur below the 14 in. isohyet level except in the presence of rivers and food trees: (b) vegetation: it is absent from dense bush, tropical rainforest, and from open, treeless areas; (c) altitude: it occurs little if anything above 3000 ft; (d) ocean barriers: it is absent from a number of outlying islands, including Tasmania: and (e) soil types: it cannot construct nests in quartz sand soils (Greaves, 1971).

Recent work (Greenslade, 1979; Anon, 1981) has been concentrated on demonstrating that *I. purpureus*

in its original sense (s. lat.), should be regarded as a polytypic species-group (Zeuner, 1943) rather than a single species. Such a species-group is most likely to diversify genetically in response to the isolation effects of deserts, low rainfall areas, lack of vegetation, etc., factors seen to operate in South Australia and the central part of the continent. It is here particularly that divergent forms of the *purpureus* species-group are currently being recognized. But Greaves' (1971) information on the distribution of the species *I. purpureus* s. strict. is comprehensively and thoroughly based on 32 years' collecting around Australia. From his map it would appear that during this time approx. 250–300 collections of *I. purpureus* s. strict. were made and his definitions of distribution remain unchallenged.

MATERIALS AND METHODS

Status and range of insect material

Iridomyrmex purpureus sens. strict. is the subject of the present investigation. It is considered to agree with Greaves' (1971) concept of the species on the basis of a 1965 series of samples collected by one of us (P.L.R.) from 20 meat ant nests occurring within a 60 km radius of the city of Sydney. The series formed part of Greaves' study, and all samples were identified by Dr G. Ettershank (Monash University, Victoria), who examined Greaves' material, as the species now recognized as *Iridomyrmex purpureus*, sens. strict.

Samples for the current study were obtained in the first place from Sydney and its environs over approx. 60 km in an east-west direction [Ir. 49, Ir. 50, Ir. 68, Ir. 123]. Then, radiating out from Sydney, samples were collected over a distance of 890 km south to Melbourne [Ir. 1, Ir. 31, Ir. 40-Ir. 41]; 1020 km west to Mildura [Ir. 72, Ir. 80]; and 570 km north to Armidale [Ir. 95, Ir. 103].

Wherever possible, the samples were taken at intervals of 100–300 km and were almost all located well within an optimal distribution range for *I. purpureus* sens. strict. They were taken in a limited area of eastern Australia clearly removed from restrictions of rainfall, vegetation, soil types, etc. which might act as ecological barriers favouring the long-term establishment and maintenance of divergent populations. But the extensive range of the current samples was nevertheless selected, within present limits of manpower and resources, adequately to demonstrate the chemical and morphological characteristics of *I. purpureus* sens. strict. as a whole.

A morphological check of the above samples showed some variation in overall body size, general level of pigmentation, pigmentation relationships, contours of head, trunk and gastral sclerites, length and strength of major body setae, etc. But the range of morphological variation appeared to be minor. It might be regarded as reaching a normal level for a widespread and abundant species and not as casting any doubt on the identity and status of that species.

Gas chromatography and mass spectrometry

Analytical gas chromatography was carried out on a Shimadzu GC6-AMP gas chromatograph fitted with a flame ionization detector, using helium as carrier gas at approx. 2 ml/min. Three columns were used: (a) SCOT OV17 (100 m × 0.5 mm i.d.); (b) SCOT OV1 (30 m × 0.5 mm i.d.) and (c) SCOT FFAP (105 m × 0.5 mm i.d.). Columns (a) and (b) were programmed from 90° at 5°/min, and column (c) from 90° at 3°/min. For combined gas chromatography–mass spectrometry (GC–MS) the instrument was coupled directly to an AEI MS12 mass spectrometer through an all glass straight-split system. Throughout the GC run mass spectra were recorded every 6 sec at 70 eV ionizing voltage and ion source temperature 175°C. The spectra were acquired and processed by a VG Digispec Display data system.

Isolation of constituents from I. purpureus

(a) Extraction methods correspond to those reported for the isolation of constituents from *I. nitidiceps* (Cavill *et al.*, 1982), *I. purpureus* workers (140 g approx.) collected from one nest at Richmond, N.S.W. were ground in a glass mortar with anhydrous sodium sulphate and the mixture was extracted with methylene chloride (400 ml) for 6 hr, using a Soxhlet apparatus, then further extracted with diethyl ether (400 ml) for 6 hr. The methylene chloride extract, after filtration, was washed with saturated sodium hydrogen carbonate solution (2 × 50 ml), then water (2 × 50 ml). The methylene chloride layer was separated, dried with anhydrous Na_2SO_4, the solvent evaporated, and the resultant neutral oil (4.74 g) was then examined by GC–MS (see Tables 1 and 2). The ethereal extract, worked-up in the same way, yielded additional material (340 mg). The sodium hydrogen carbonate extracts and washings were combined (200 ml approx.) acidified with 2 M HCl, then extracted with ether for 16 hr. The ethereal solution, after drying (anhydrous $MgSO_4$), and evaporation of the solvent, gave an acidic oil (106 mg).

(b) A total of 2500 *I. purpureus* workers (32 g approx.) were collected at Cottles Bridge in the greater Melbourne area, Victoria and stored in methylene chloride. The whole ants (1550, 20 g approx.) were extracted with methylene chloride as in (a), and gave a neutral fraction (2.36 g) and an acidic fraction (34 mg).

The heads of the remainder of the ants (950) were removed, then extracted with diethyl ether for 6 hr and the extract was worked-up as in (a). The neutral ethereal extract was dried (anhydr. $MgSO_4$), carefully evaporated to small volume (~2 ml) under N_2 and the product transferred to a vial for GC–MS characterization (see Tables 1 and 2).

A further collection of 1000 *I. purpureus* workers from the same district was undertaken. Heads and bodies were immediately separated and extracted with methylene chloride. Each of the neutral extracts was isolated, as above, and transferred to a vial for GC–MS characterization (see Table 2).

(c) Aliquots of acidic fractions, isolated as described in (a) and (b), were methylated with diazomethane in diethyl ether [prepared from *p*-toluenesulphonyl methyl nitrosamide (Fieser and Fieser, 1967)]. After evaporation of the solvent under N_2, the methyl esters were examined by GC–MS.

(d) Individual samples of *I. purpureus* workers (5–15 g), collected from the localities shown in Table 2, were extracted with methylene chloride as in (a), and/or heads and bodies were extracted separately as in (b). Neutral and acidic fractions were isolated save that the solvent was not removed completely on evaporation. Neutral extracts were concentrated to small volume (1–2 ml), then transferred to a vial prior to GC–MS characterization (see Table 2). Extracts were concentrated further, under N_2, as required. Where the quantity permitted, the neutral concentrate was transferred to a distillation tube and heated to 150°C (air bath); the distillate enriched in the more volatile constituents was then re-examined by GC–MS.

(e) Mandibular glands (265) were dissected from *I. purpureus* workers, collected at Riverstone, N.S.W. The dissected mandibular glands were stockpiled in diethyl ether (0.5 ml) in a round-bottom vial at −40°C. The whole glands were crushed under the storage ether using a glass rod. The extract was decanted into a second vial and the glands were again titurated under ether (0.5 ml). The combined extract, after drying (anhydrous Na_2SO_4), was evaporated at room temperature under N_2, to give an oil (~2 μl) which was examined by GC–MS.

Table 1. Mass spectral data for exocrine constituents (1–12)

Peak (see Fig. 1)	Compound	Mass spectral data *m/z* (%)	Ref.
1	2,5-Dimethyl-3-ethylpyrazine	136 (84), 135 (100), 108 (23), 107 (18), 56 (40), 42 (20)	*
2	2,5-Dimethyl-3-propylpyrazine	150 (15), 149 (10), 135 (25), 122 (100), 121 (12), 42 (15)	*
3	2,5-Dimethyl-3-butylpyrazine	164 (2), 163 (2), 149 (7), 135 (10), 122 (100), 121 (12)	*
4	2-Acetyl-3-methylcyclopentene	124 (17), 109 (28), 81 (74), 79 (25), 53 (19), 43 (100)	†
5	1,3,3-Trimethyl-2,7-dioxabicyclo[2,2,1]heptane	142 (5), 127 (14), 114 (5), 100 (15), 99 (15), 84 (70), 83 (3), 82 (55), 72 (90), 67 (13), 57 (18), 55 (12), 43 (100)	‡
6	6-Methylhept-5-ene-2-one	126 (7), 111 (16), 108 (38), 71 (18), 69 (45), 68 (18), 67 (18), 58 (25), 55 (52), 43 (100)	
7	6-Methylhept-5-ene-2-ol	128 (12), 110 (25), 95 (98), 73 (32), 71 (36), 69 (66), 67 (50), 55 (52), 45 (43), 53 (60), 41 (100)	
8	2-(3-Methylcyclopentyl)propional	140 (1), 125 (6), 111 (18), 83 (27), 82 (45), 81 (20), 72 (35), 71 (30), 69 (100), 67 (48), 58 (95), 55 (85), 43 (15), 41 (60)	§
9	Iridodial	168 (3), 153 (4), 150 (7), 135 (25), 111 (50), 109 (34), 81 (100), 67 (67), 58 (80), 41 (80)	
10	Dihydronepetalactone and/or isodihydronepetalactone	168 (10), 153 (52), 113 (100), 95 (25), 81 (80), 69 (3), 68 (15), 67 (55)	‖
11	Iridomyrmecin and/or isoiridomyrmecin	168 (7), 153 (3), 109 (47), 95 (100), 82 (40), 81 (90), 69 (40), 68 (60), 67 (90), 41 (50)	‖
12	Actinidine	147 (52), 146 (28), 132 (100), 117 (47)	¶

*Heller and Milne (1978, 1980). †Wheeler *et al.* (1975). ‡Klein and Rojahn (1967). §Meinwald *et al.* (1977). ‖Cavill *et al.* (1982). ¶Wheeler *et al.* (1977).

Reference compounds

2,5-Dimethyl-3-alkyl-, and 2,6-dimethyl-3-alkyl-pyrazines, in which the respective 3-alkyl substituent was ethyl, propyl or butyl, were synthesized from 2,5-dimethyl- or 2,6-dimethyl-pyrazine (Aldrich Chemical Co.) as described previously (Klein and Spoerri, 1951). Pyrazines were also provided by Pyrazine Specialties (U.S.A.).

Synthetic 6-methylhept-5-ene-2-one and -2-ol, and specimens of natural iridodial, *iso*iridomyrmecin, *iso*dihydronepetalactone and actinidine were available from earlier studies (Cavill *et al.*, 1976, 1982). The acetal, 1,3,3-trimethyl-2,7-dioxabicyclo[2,2,1]heptane, was provided by Dragoco (see Klein and Rojahn, 1967).

RESULTS

A representative gas chromatograph of the volatile constituents isolated by total extraction of *I. purpureus* workers from Richmond, N.S.W. is shown in Fig. 1. Peaks numbered 1–12 correspond to the compounds listed in Table 1. Individual compounds were identified from mass spectral data. Gas chromatographic–mass spectrometric (GC–MS) comparisons were made with reference compounds. With the exception of unidentified trace constituents, the unnumbered peaks in Fig. 1 represent hydrocarbons. These predominantly higher molecular weight alkanes, alkenes, alkadienes and monomethylalkanes from *I. purpureus* have been characterized (Brophy *et al.*, 1983).

Peaks 6 and 9 represent the known major constituents of the anal gland secretion of *I. purpureus*: 6-methylhept-5-ene-2-one and iridodial respectively. Originally these compounds were isolated and characterized as their 2,4-dinitrophenylhydrazones and iridodial was shown to be a mixture of the *cis,trans*-(13) and *trans,cis*-(14) isomers (Cavill and Ford, 1960).

Peaks 1, 2 and 3 have been identified as 2,5-dimethyl-3-ethyl-, 2,5-dimethyl-3-propyl- and 2,5-dimethyl-3-butyl-pyrazine respectively (see Table 1). As mass spectral data do not distinguish between the corresponding 2,5- and 2,6-dimethyl-3-alkyl-derivatives, the natural pyrazines (1), (2) and (3) were subjected to comparative gas chromatography. The linear retention indices for (1), (2) and (3) on column (c), 1400, 1455 and 1530, correspond to those of the respective synthetic 2,5-dimethyl-3-alkylpyrazines.

The mass spectrum of peak 4 (Table 1) showed a parent ion at *m/z* 124, indicating a molecular formula, $C_8H_{12}O$. The base peak at *m/z* 43 suggested a methylketone, whilst the ion at *m/z* 81 (79%) was characteristic of a methyl cyclopentenyl moiety. The spectrum closely corresponds to that reported for 2-acetyl-3-methylcyclopentene, a volatile ketone isolated from two *Azteca* spp. of dolichoderine ants (Wheeler *et al.*, 1975).

The spectrum of peak 5 showed a small parent ion, M^+ 142, corresponding to a molecular formula, $C_8H_{14}O_2$. Whilst the base peak was again indicative of a methyl ketone the short retention time noted for peak 5 suggested an acetal or ether structure. As methylheptenone (6) was a major exocrine constituent, the spectrum of peak (5) was compared with that of the synthetic acetal (5) which, biogenetically, could be formed from (6) *via* the intermediate epoxyketone. Gas chromatographic and mass spectrometric com-

Table 2. Exocrine constituents of *I. purpureus* workers

Geographical location of *I. purpureus*	Ref. No. Ir.	Extraction of: whole ants (W) heads (H) bodies (B)	Pyrazines: 2,5-Dimethyl-3-ethyl (1)	Pyrazines: 2,5-Dimethyl-3-propyl (2)	Pyrazines: 2,5-Dimethyl-3-butyl (3)	Methylheptenone and associated volatiles: 2-Acetyl-3-methylcyclopentene (4)*	Methylheptenone and associated volatiles: Bicyclic acetal (5)	Methylheptenone and associated volatiles: 6-Methylhept-5-ene-2-one (6)	Methylheptenone and associated volatiles: 6-Methylhept-5-ene-2-ol (7)	Methylheptenone and associated volatiles: 2-(3-Methylcyclopentyl)propional (8)*	Iridoids: Iridodial (9)	Iridoids: Dihydronepetalactone/s (10)	Iridoids: Iridolactone/s (11)	Iridoids: Actinidine (12)
Armidale	103	H, B	+	+	+	+	+	+	+	+	+	+	+	+
Tamworth	95	H, B	+	+	+	+	+	+	+	+	+	+	+	+
Sydney														
Richmond	68	W, H, B	+	+	+	+	+	+	+	+	+	+	+	+
Riverstone district	49	H, B	+	+	+	+	+	+	+	+	+	+	+	+
	123	Mandibular glands	+	+	+									
Terry Hills	48	H, B	+	+	+	+	+	+	+	+	+	(+)†	(+)	+
Mittagong	45	H, B	+	+	+	+	+	+	+	+	+	+	+	+
Bookham	44	H, B	+	+	+	+	+	+	+	+	+			
Chiltern	43	H, B	+	+	+	+	+	+	+	+	+			+
Kilmore	42	H, B	+	+	+		+	+		+	+	(+)	(+)	+
Melbourne:														
Cottles	40	H, B	+	+	+		+	+	+		+	+	+	+
Bridge district	41	H, B	+	+	+	+	+	+	+	+	+	(+)	(+)	+
Mildura-Dareton	72	H, B	+	+	+	+	+	+	+	+	+	+	+	+‡

*Compounds (4) and (8) identified by MS data only. †(+) Bracket indicates GC identification only. ‡Proportion of actinidine greater than in other samples.

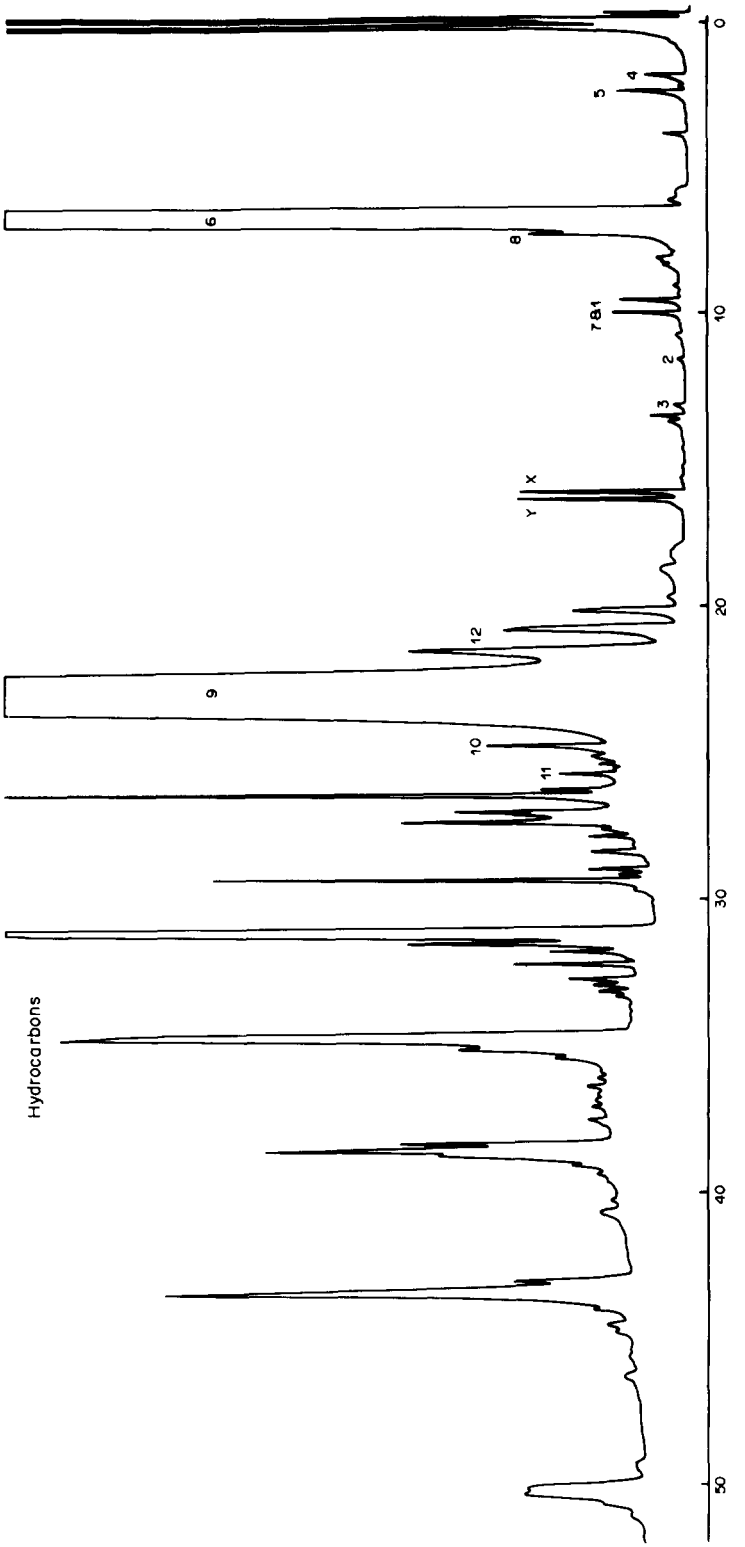


Fig. 1. GC trace of the volatiles from *I. purpureus* run on FFAP (column c) 90°, programmed at 3°C/min to 230°C. The numbers refer to Table 1.

parisons with the synthetic compound, 1,3,3-trimethyl-2,7-dioxabicyclo[2,2,1]heptane, confirmed the identity of the natural product (5).

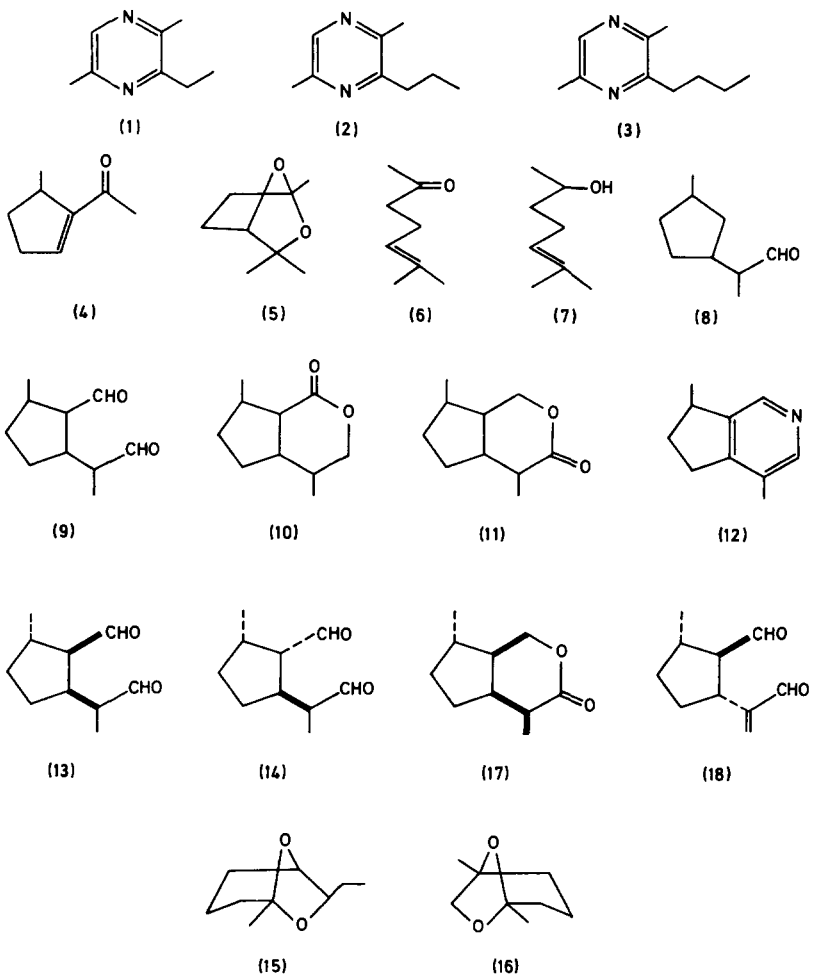
The mass spectrum of peak 7 showed M^+ 128, corresponding to a molecular formula $C_8H_{16}O$, plus a strong M-18 ion, *m/z* 110, indicative of an alcohol. A GC–MS comparison with an authentic specimen of 6-methylhept-5-ene-2-ol confirmed the identity of 7.

Two minor constituents (X) and (Y) (see Fig. 1) have not been identified. The spectrum of compound (X) shows ions at *m/z* 124 (5%), 109 (15%), 97 (15%), 94 (18%), 82 (55%), 81 (35%), 69 (12%), 55 (22%), 54 (25%), 53 (25%) and 43 (100%). The spectrum of compound (Y) shows ions at *m/z* 140 (5%), 125 (5%), 112 (16%), 94 (43%), 82 (15%), 69 (65%), 55 (15%), 43 (100%) and 41 (90%). Proportions of (X) and (Y) were minimal in other samples. The mass spectra of peaks 8, 9, 10 and 11 (Table 1) were indicative of iridoids (see Cavill *et al.*, 1976, 1982). Compound (8) was identified from its mass spectrum as 2-(3-methylcyclopentyl)propional (Meinwald *et al.*, 1977). The dominant peak 9 was the known *cis,trans*- and *trans,cis*-iridodials. Peaks 10 and 11 had mass spectra corresponding to those of a dihydronepetalactone and iridolactone, respectively. *Iso*dihydronepetalactone (type 10) and *iso*iridomyrmecin (type 11) were the major exocrine constituents isolated from *I. nitidus* (Cavill and Clark, 1967). Compounds (8), (10) and (11) were not available in sufficient quantity for stereochemical investigation. Finally, the spectrum of peak 12 was shown to be identical with that of the iridoid alkaloid, actinidine (12).

Typical long-chain fatty acids—myristic, palmitic, oleic, stearic and linoleic—were characterized from the acidic fraction of *I. purpureus* workers, after methylation. Of these the C18 acids, oleic and stearic were predominant. Fatty acids represent < 1% of the total extract. The acidic fraction from the bodies of *I. purpureus* workers, collected at Riverstone, N.S.W. was examined in further detail. Nepetalinic acids, identified as the dimethyl nepetalinates, and several oxoacids characterized as monomethyl esters were also present. The oxidation products are likely to be formed from iridodial during isolation and work-up (Cavill *et al.*, 1976, 1982). Extractives of the neutral fraction which have some water solubility, for example, the acetal (5), iridodial and the iridoid lactones, were also detected in the acid fraction.

DISCUSSION

The volatile constituents (1) to (12), characterized from *I. purpureus* workers collected at Richmond, N.S.W. were also detected in samples of *I. purpureus* collected from Sydney south to Melbourne, west to Mildura and north to Armidale. The results sum-



marized in Table 2 show no obvious chemical differences in the pattern of the exocrine constituents.* Variation in the proportions of minor and trace constituents are considered to lie within experimental limits.

Of the constituents, the 2,5-dimethyl-3-ethyl-2,5-dimethyl-3-propyl- and 2,5-dimethyl-3-butyl-pyrazines (1,2 and 3 respectively) were isolated from the heads and from total extracts of the meat ant. The pyrazines were shown to be mandibular gland constituents following on the extraction of 265 glands, individually dissected from *I. purpureus* workers (see above). The pyrazines were present in approximately equal amounts, but in total, represented < 1 ng/gland. The pyrazines were also found in an extract of *I. purpureus* pupae. Pyrazines have not been detected from all samples of *I. purpureus* examined. Initially, when pyrazines were characterized from the heads and from a total extract of the Argentine ant, *I. humilis* (Cavill and Houghton, 1974), pyrazines were sought but not detected in the heads of the meat ant, *I. purpureus*. Subsequently, it appeared that failure to detect the highly volatile pyrazines, in trace amounts, might well relate to collection and isolation procedures. When ants were collected and stored in methylene chloride and heads were removed at a later stage, then extracted, pyrazines were not necessarily detected. But when ants were collected into "dry ice" and heads were immediately removed and extracted, pyrazines were found (see Table 2).

Wheeler and Blum (1973) recorded the presence of dimethylalkylpyrazines in the mandibular glands of three species of the primitive ponerine ant genus, *Odontomachus*, in which they were shown to release intense alarm behaviour. A range of dimethylalkylpyrazines have since been isolated as mandibular gland constituents from numerous species of ponerine ants (for a review see Wheeler *et al.*, 1982). In our earlier studies on *I. humilis*, 2,5-dimethyl-3-*iso*pentyl- and the *cis*- and *trans*-isomers of 2,5-dimethyl-3-styryl-pyrazine were isolated from the heads of adult workers. Recently we have confirmed the presence of these pyrazines in the mandibular glands of *I. humilus* pupae. Despite the occurrence of pyrazines in the Argentine ant, we have been unable to record any alarm effect in relation to adult mandibular glands. Instead there is a clear, but transitory stimulus to trailing (Robertson *et al.*, 1980).

The venom glands of representatives of two myrmicine genera, *Atta* (Cross *et al.*, 1979) and *Myrmica* (Evershed *et al.*, 1982) have yielded trace proportions of the 2,5-dimethyl-3-ethylpyrazine (1). A representative of a third myrmicine genus, *Tetramorium caespitum*, has yielded the 2,5-dimethyl-3-ethyl- together with 2,5-dimethyl-pyrazine (Attygalle and Morgan, 1983). These pyrazines have been shown to act as trail pheromones. Pyrazines have also been detected from two formicine ants, a *Calomyrmex* species (Brown and Moore, 1979) and *Notoncus ectatommoides* (Brophy *et al.*, 1982).

The 2-acetyl-3-methylcyclopentene (4) was first isolated, together with 2-methylcyclopentanone and *cis*-1-acetyl-2-methylcyclopentane, from the anal glands of several *Azteca* spp. of the *Dolichoderinae* (Wheeler *et al.*, 1975). In *I. purpureus* this nonterpenoid (4) was found in association with the terpenoids, 6-methylhept-5-ene-2-one (6), 6-methylhept-5-ene-2-ol (7), and 1,3,3-trimethyl-2,7-dioxabicyclo[2,2,1]heptane (5). The methylheptenone (6) is present in substantially greater amount than the associated compounds (4), (5) and (7). Of the minor volatiles the bicyclic acetal (5) has not been reported previously from an insect source. Biogenetically, the acetal is readily derived from methylheptenone and hence is also considered to be an anal gland constituent. Structurally, it is closely related to exobrevicomin (15) and frontanalin (16). The latter bicyclic acetals are aggregation pheromones of the western pine beetle, *Dendroctonus brevicomis* (Brand *et al.*, 1979, and refs therein).

The remaining compounds (8–12) are known cyclopentanoid monoterpene derivatives and of these, the iridodials dominate the group (Fig. 1). The iridoids (9–12) and the associated carbonyl compounds and alcohols, (4), (6) and (7) are well recognized as anal gland constituents of the *Dolichoderinae* (review by Blum and Hermann, 1978), and as such, are considered to be involved in the "alarm–defence" mechanisms of dolichoderine ants (Cavill *et al.*, 1982, and refs therein). Whether individual components, for example, the bicyclic acetal (5) now characterized from *I. purpureus*, have other functions is yet to be determined.

The iridoid alkaloid, actinidine (12), was identified as a major anal gland constituent in two *Conomyrma* spp. (Wheeler *et al.*, 1977). It was a minor constituent in *Iridomyrmex humilis* and *I. nitidiceps* (Cavill *et al.*, 1980, 1982). It is also a minor constituent in *I. purpureus*, but was found in relatively greater proportion in one sample collected near Mildura (Ir. 72, see Table 2).

The current study has yielded a chemical pattern of volatile constituents, major and minor, which was consistent throughout many samples of the meat ant species *Iridomyrmex purpureus* sens. strict. The meat ant samples on which the study was based could only be collected at intervals along radii extending out from Sydney in a zone of eastern Australia, although the distribution of *purpureus* was clearly continental. But the repetition of the chemical pattern throughout all the samples studied has led to the view that the range and complexity of the pattern was adequate to define *purpureus* chemically at species level. Moreover, the initial record of a substantially greater proportion of actinidine in one sample (Ir. 72), collected from the edge of the arid zone in which speciation was likely to have been active, suggested that the pattern might well prove significant in helping to assess the status of intra- and inter-specific forms. These studies are in progress.

The patterns of the exocrine constituents differ markedly for the three species of *Iridomyrmex*—*purpureus*, *nitidiceps* and *humilis*—that have now been examined in more detail. 6-Methylhept-5-ene-2-one (6), the biogenetically related alcohol (7) and acetal (5), together with 2-acetyl-3-methylcyclopentene (4) comprise a series of volatiles characteristic of *I. purpureus*—they were not found in *nitidiceps* or

*Preliminary examination of a sample collected near Perth, Western Australia also showed the same pattern.

humilis. *Iso*valeric acid was the comparable volatile component of the anal gland secretion of *I. nitidiceps*. Additionally, pent-2-enoic, pent-3-enoic and two trace C_6 acids were isolated from a total extract of *nitidiceps* workers (Cavill *et al.*, 1982). A comparable group of oxygenated volatiles were not detected in *I. humilis*. Rather, the proportion of lower molecular weight alkenes and monomethylalkanes, in the range C_{15}–C_{20}, is greater for the Argentine ant than for *I. purpureus* or *nitidiceps* (see Brophy *et al.*, 1983).

Iridoids remain the dominant exocrine products (see Fig. 1). The *cis,trans*- (14) and *trans,cis*- (15) iridodials, together with the minor lactones (10) and (11) plus actinidine (12), were found in both *purpureus* and *nitidiceps*. Additionally, *purpureus* gave the nor-iridoid, 2-(3-methylcyclopentyl)propional (8). Iridomyrmecin (17) has been shown to be the major iridoid from *I. humilis* (Pavan, 1952), whilst *trans,trans*-dolichodial (18) and actinidine have since been characterized (Cavill *et al.*, 1976). Finally, the pyrazine pattern of *I. purpureus* is distinct from that of *humilis*. Pyrazines were sought, but not found in *nitidiceps*.

The overall pattern of the volatile constituents—pyrazines, carbonyl compounds, alcohols and/or acids, iridoids and actinidine—together with the hydrocarbons, readily enables chemical differentiation between the three species of *Iridomyrmex*—*purpureus*, *nitidiceps* and *humilis*.

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