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CHEMOTAXONOMY OF THE AUSTRALIAN DOLICHODERINAE: VOLATILE CONSTITUENTS OF *IRIDOMYRMEX DISCORS*

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ABSTRACT.—Fifteen components have been identified in the CH_2Cl_2 extract of *Iridomyrmex discors* by combined gc-ms techniques. Ten of these (6-methylhept-5-en-2-one, actinidine, the two *cis, trans*- and the two *trans, cis*-isomers of iridodial, 1,3,3-trimethyl-2,7-dioxabicyclo[2.2.1]heptane, 2-(3-methylcyclopentyl)propional, 2-acetyl-3-methylcyclopentene, and isodihydronepetalactone) have been noted in other species of *Iridomyrmex*. A further component has been tentatively identified as an isomer of 1,3,3-trimethyl-2,7-dioxabicyclo-[2.2.1]heptane. The remaining four components (1-methycyclopentene, 1-methyl-2-ethylcyclopentane, 2-methylcyclopent-1-ene-carboxaldehyde, and geranylacetone), which were confirmed by co-injection with authentic samples prepared by unambigious syntheses, have not been reported previously from insect sources.

Ants of the genus *Iridomyrmex* (Dolichoderinae) are the dominant arthropods of the soil in all climatic zones of Australia (1). Chemical interest in this genus was sparked in 1950 when the antibiotic and insecticidal activities of iridomyrmecin, a terpenoid isolated from *Iridomyrmex humilis* (Mayr), were reported (2). Since then, a wide variety of compounds, including hydrocarbons, fatty acids, iridoids, other terpenoids, and pyrazines have been reported from *Iridomyrmex conifer* (Florel) (3), *I. humilis* (3–10), *Iridomyrmex myrmecodiae* (Emery) (11), *Iridomyrmex nitidiceps* (5,11,12), *Iridomyrmex nitidus* (Mayr) (3, 11–13), *Idiomyrmex purpureus* (=*detectus*) (3,5,11,14,15), and *Idiomyrmex rufoniger* (Lowne) (11). It is noteworthy that specimens of worker ants of *I. purpureus* collected over a wide area of southeastern Australia possessed similar chemical profiles (15). Terpenoid constituents have also been noted, but not identified, in *Idiomyrmex gracilis* var. *rubriceps* (Forel), *Idiomyrmex gracilis* (Lowne), and *I. rufoniger* (11).

From the point of view of biological activity, several of the compounds noted above have been shown to elicit behavioral responses in ants. For example, 9-hexadecenal, the lipid fraction, and the exocrine gland constituents of *I. humilis* are all involved in the trailing behavior of this species (4,10,16,17), and isovaleric acid and iridodial form the basis of the defensive secretion of *I. nitidiceps* (12).

As part of our continuing interest in the chemotaxonomy of Australian ants, we now report our results from examination of the volatiles from the CH_2Cl_2 extract of two collections of *Idiomyrmex discors* (Forel), one collected from New South Wales and the other from Western Australia.

RESULTS AND DISCUSSION

A representative glc trace of the volatile constituents of the CH_2Cl_2 extract of *I. discors* collected in New South Wales is presented in Figure 1, and the peak identifications are given in Table 1. Assignments were made by comparison of gc-ms data and retention times with those from reference compounds. The two major components are the commonly occurring 6-methyhept-5-en-2-one (peak 6) and the various *cis, trans*- and *trans, cis*-iridodials (three isomers are accounted for by peak 9 and the fourth isomer by

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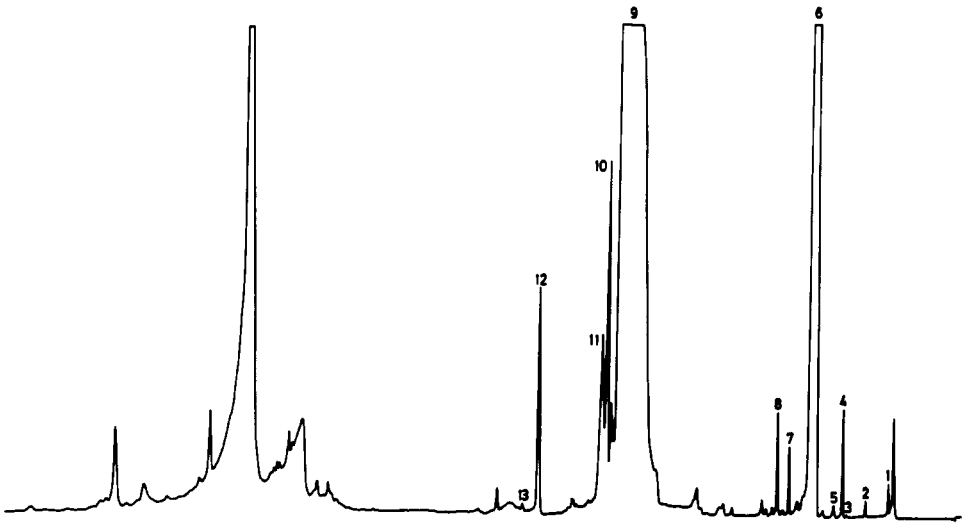


FIGURE 1. Representative gc trace of the CH_2Cl_2 extract of *Iridomyrmex discors* collected from the Botany Bay Breakwater, run on an OV1 column (30 m × 0.5 mm), temperature programmed 100–250°, 4°/min. Unlabelled peaks represent hydrocarbon components. At lower attenuation, peak 9 was seen to consist of 3 components (isomeric iridodials).

peak 11). Five of the minor constituents have also been previously identified in various species of *Iridomyrmex* (Table 1).

Of the remaining components, peak 1 gave a molecular ion *m/z* 82 and a base peak of *m/z* 67. The rather simple fragmentation pattern was suggestive of 1-methylcy-

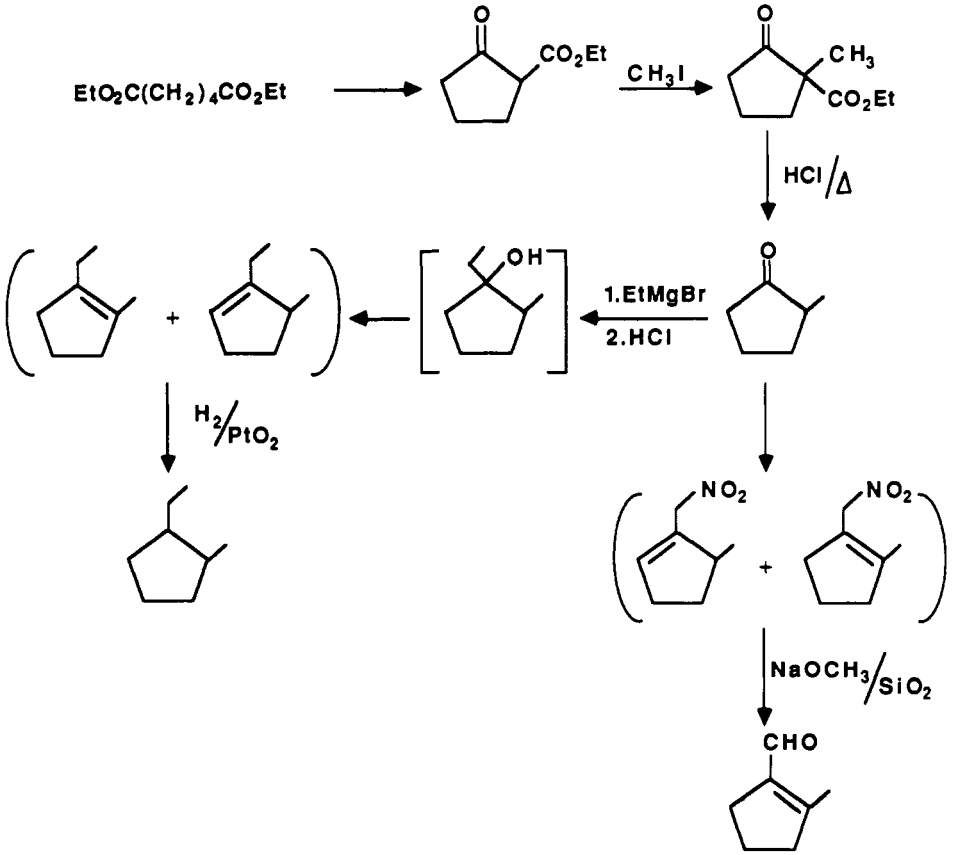
TABLE 1. Constituents Identified in the CH_2Cl_2 Extract of *Iridomyrmex discors*.

Peak No (see Figure 1)	Assignment	Species of ant from which previously reported	Reference
1	1-Methylcyclopentene	—	—
2	1-Methyl-2-ethyl-cyclopentane	—	—
3	2-Acetyl-3-methyl-cyclopentene	*Iridomyrmex purpureus*	15
4	1,3,3-Trimethyl-2,7-dioxabicyclo [2.2.1]-heptane	*I. purpureus*	15
5	2-Methylcyclopent-1-ene carboxaldehyde	—	—
6	6-Methylhept-5-ene-2-one	*Iridomyrmex conifer*	3
		Iridomyrmex nitidiceps	11
		I. purpureus	3,11,15
		Iridomyrmex rufoniger	11
7	An isomer of peak no. 4	—	—
8	2-(3-Methylcyclopentyl)-propional	*I. purpureus*	15
9	Iridodial	*I. conifer*	3
		Iridomyrmex nitidiceps	11,12
		I. purpureus	3,14,15
		I. rufoniger	11
10	Actinidine	*Iridomyrmex humilis*	10
		I. nitidiceps	12
		I. purpureus	15
11	Iridodial	see peak 9	
12	Geranylacetone	—	—
13	Isodihydronepetalactone	*Iridomyrmex nitidus*	12,13
		I. purpureus	15

clopentene, which was subsequently confirmed by direct comparison with authentic material prepared as described below.

Peak 2 also produced a simple mass spectral fragmentation pattern. The molecular ion, *m/z* 112, was consistent with the formula C_8H_{16}, and major fragment ions were noted at *m/z* 83 and 55. The compound was assigned as an isomer of 1-methyl-2-ethylcyclopentane, and synthetic material was prepared for direct comparison as illustrated in Scheme 1. It is noteworthy that even under mild conditions the implied tertiary alcohol was not isolated but underwent ready dehydration to give a mixture of olefins. Subsequent hydrogenation gave the desired isomeric cyclopentanes in a 2:1 ratio, and these had essentially identical mass spectra. The isomer in lower concentration possessed the longer retention time on an OV1 column and also co-chromatographed with the naturally occurring material. On the basis of relative retention times, this compound was assigned the *trans*- stereochemistry.

The approach used in the preparation of the above compound proved useful in the synthesis of reference material for peak 5. This compound was assigned as 2-methylcylopent-1-ene-carboxaldehyde, also on the basis of its mass spectral fragmentation pattern. A molecular ion was noted at *m/z* 110 assignable as $C_7H_{10}O$, and the loss of 29 amu was considered to indicate a -CHO functionality. Synthetic material was prepared via methoxide treatment of the mixture of nitro-olefins (Scheme 1) whence a single product was obtained. Although yields were low, possibly as a result of adsorption of the product to the Si gel during purification procedures, sufficient material was ob-



SCHEME 1. Preparation of 1-methyl-2-ethylcyclopentane and 2-methylcyclopent-1-ene-carboxaldehyde from diethyl adipate.

tained for it to be characterized. Direct gc-ms comparisons of the product aldehyde and the naturally occurring material showed them to be identical.

Peak 7 was assigned as an isomer of the known 1,3,3-trimethyl-2,7-dioxabicyclo-[2.2.1]heptane on the basis of its mass spectrum and relative retention time.

The most abundant of the new minor components (peak 12) had a molecular ion of *m/z* 194 indicative of a molecular formula $C_{13}H_{22}O$. The base peak at *m/z* 43 was typical of a methyl ketone, and these data, together with considerations of retention time, led to an assignment as geranylacetone (6,10-dimethyl-5,9-undecadiene-2-one). The assignment was subsequently confirmed by direct comparisons with synthetic material derived from a retro-aldol reaction of farnesal. Farnesal was prepared from selective oxidation of commercially available farnesol. Glc analysis of the synthetic geranylacetone on an SP 1000 column indicated a single isomer. Comparison of the semicarbazone derivative mp with literature data suggested it to have a *Z* configuration.

In the present work no evidence was obtained for the presence of pyrazines in this ant, although they have been noted in other species of *Iridomyrmex*. However, this may be a reflection of the collection techniques used. For example, Cavill *et al.* (15) have reported that pyrazines were always found when *I. purpureus* workers were collected onto dry ice and the heads removed and extracted immediately but were not usually detected when the ants were stored in CH_2Cl_2 prior to dissection.

The co-occurrence of the variety of compounds noted in *I. discors* and in *Iridomyrmex* species in general poses interesting questions in relation to their biosynthesis as well as to chemotaxonomy. While this is the first report of geranylacetone from an insect source, it is perhaps noteworthy that farnesal has been reported in trace amounts from the ant *Lasius fuliginosus latreille* (subfamily Formicinae) (18). Geranylacetone bears the same relationship to farnesal as does methylhept-5-en-2-one to citral, and the latter has recently been shown (19) to be derived from the terpenoid pathway. The origins of the various small cyclopentanoids are less apparent, but one possibility is that they may be considered as nor-iridoids. Related compounds, 2-methylcyclopentanone and *cis*-2-methyl-1-acetylcyclopentane as well as 2-acetyl-3-methylcyclopentene, have earlier been noted in other members of the Dolichoderinae, in particular *Azteca* nr. *velox* and *Azteca* nr. *nigriventis*, and such a biosynthetic proposal made (20). These latter compounds were found to elicit alarm behavior in *Azteca*; the biological function(s) of the compounds in the present study is unknown.

With respect to the distribution of this species of ant, several subspecies names which have been applied to *I. discors* indicate it to be a putative, widespread, and polytypic species. It is uncertain at this time whether or not the species name, in fact, covers a complex. It is of interest to note that the gc trace of the CH_2Cl_2 extract from *I. discors* collected from Western Australia showed 6-methylhept-5-en-2-one and the isomers of iridodial as the major constituents with trace amounts of actinidine and geranylacetone.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler hot stage and are uncorrected. Infrared (ir) spectra were recorded on an Hitachi 260-10 spectrophotometer, and ^{1}H-nmr spectra for $CDCl_3$ solutions were recorded on either a Varian 360L (60 MHz) or a Bruker AM 500 spectrometer (500 MHz). Mass spectra (ei) were recorded on an AEI MS 12 mass spectrometer operating at 70 eV and an ion source temperature of 200°. Gc-ms was performed on either an OV1 column (30 m × 0.5 mm) or a SP 1000 column (85 m × 0.5 mm) using a Shimadzu GC6-AMP gas chromatograph coupled to the mass spectrometer through an all glass straight split. Spectra were recorded every 6 seconds and were processed by a V.G. Display Digispec data system.

COLLECTIONS AND EXTRACTIONS OF *I. DISCORS*.—Several collections of ants were made from various colonies on the Botany Bay Breakwater, New South Wales, and from a suburban lawn in North Perth,

Western Australia, either with a hand-operated vacuum pump or with an artist's brush. Voucher specimens from each collection have been lodged in the National Insect Collection, CSIRO Division of Entomology, Canberra.

A number of the Botany Bay ants (0.88 g) were sacrificed and ground with Na_2SO_4 in a mortar and pestle, and the mixture was extracted in a Soxhlet apparatus with CH_2Cl_2. Careful removal of the solvent, in vacuo, afforded a pale yellow oil (199 mg) which was analyzed by gc and gc-ms. Ants from Western Australia were likewise extracted, and the extract examined in the same manner.

1-METHYLCYCLOPENTENE.—Cyclopentanone (10 g, 0.12 mol) in Et_2O (10 ml) was added slowly and with vigorous stirring to a cooled (−10°) ethereal solution of MeMgI (0.13 mol). On completion of the addition the reaction mixture was warmed to room temperature, then stirred for a further 65 h prior to workup in the normal manner. The Et_2O was removed by careful distillation at atmospheric pressure and the product distilled as a colorless liquid (2.1 g, 21%), bp 66–68°, [lit. (21) 76°]; ms *m/z* (%) 82 (26), 81 (16), 67 (100), 41 (19), 39 (16), 30 (56); ir (neat) 3020, 1640 cm^{-1}; ^1H nmr (500 MHz) δ 1.7 (3H, br s), 2.2–2.3 (6H, m), 5.3 (1H, m).

1-METHYL-2-ETHYLCYCLOPENTENE.—*2-Ethoxycarbonylcyclopentanone.*—Diethyl adipate (75.5 g, 0.37 mol) was cyclized with molecular sodium (12.5 g, 0.54 g atom) in dried C_6H_6 (300 ml) as described by Pinkney (22). Workup gave the product as a colorless, sweet-smelling liquid (45.5 g, 78%), bp. 73–76°, 0.7 mm, [lit. (22) 105°, 11 mm].

2-Methyl-2-ethoxycarbonylcyclopentanone.—Alkylation of 2-ethoxycarbonyl-cyclopentanone (17.2 g, 0.11 mol), as described by Barco *et al.* (23), with MeI (14.5 ml) in Me_2CO (270 ml) containing anhydrous K_2CO_3 (55.6 g) gave the desired product, after appropriate workup, as a colorless liquid (16.5 g, 88%), bp 96°, 18 mm [lit. (23) 107°, 15 mm].

2-Methylcyclopentanone.—The β-ketoester (4.9 g, 0.03 mol) was dissolved in HCl (14 ml) and H_2O (3 ml) added until the solution became turbid, using the general method of Shive *et al.* (24). After refluxing for 16 h, workup followed by distillation at atmospheric pressure gave the desired product as a colorless liquid (1.1 g, 39%), bp 140–141° [lit. (25) 139°]; ir (neat) 1715 cm^{-1}; ^1H nmr (60 MHz) δ 1.0–1.1 (3H, d), 2.0–2.2 (7H, m).

1-Methyl-2-ethylcyclopentane.—2-Methylcyclopentanone (2.3 g, 0.02 mol) was added dropwise with stirring to a cooled ethereal solution of EtMgI (0.03 mol). The reaction mixture was warmed to room temperature, then refluxed overnight. Workup followed by distillation of the crude product at atmospheric pressure gave a mixture of the isomeric 1-methyl-2-ethyl- and 2-ethyl-3-methylcyclopentenes (0.51 g, 19%), bp 110–115°. Hydrogenation of this mixture, neat, over Pt (iv) oxide (0.012 g) gave, after removal of the catalyst, the diastereomeric 1-methyl-2-ethylcyclopentanes (400 mg, 79%); ir (neat) 2970–2890, 1460 cm^{-1}; ^1H nmr (60 MHz) δ 1.0 (m), 1.6 (m). Gc examination of this mixture showed two peaks in a ratio of approximately 2:1; ms isomer 1 *m/z* (%) 112 (15), 84 (29), 83 (69), 82 (19), 70 (52), 69 (85), 65 (15), 57 (14), 56 (57), 55 (100), 43 (10), 42 (40), 41 (69), 39 (21); isomer 2 *m/z* (%) 112 (7), 84 (27), 83 (65), 82 (15), 70 (48), 69 (30), 67 (14), 57 (14), 56 (59), 55 (100), 44 (18), 43 (15), 42 (58), 41 (74), 39 (27).

2-METHYLCYCLOPENT-1-ENE-CARBOXALDEHYDE.—*5-Methyl- and 2-methyl-1-(nitromethyl)cyclopentene.*—2-Methylcyclopentanone (2.0 g, 0.02 mol), $MeNO_2$ (6.2 g, 0.1 mol), $(CH_3)_2N(CH_2)_2NH_2$ (2.2 ml, 0.02 mol) and C_6H_6 (50 ml) were refluxed overnight in a flask fitted with a Dean and Stark apparatus according to the method of Tamura *et al.* (26). Workup followed by vacuum distillation gave a pale yellow liquid comprising a 1:7 mixture of 5-methyl and 2-methyl-1-(nitromethyl)cyclopentene (1.1 g, 38%), bp 112°, 23 mm [lit. (26) 120–125°, 13 mm]; ir (neat) 1675, 1560, 1375 cm^{-1}; ^1H nmr (500 MHz) δ 1.02–1.04 (3H, Me for 5-methyl isomer, d, $J = 6.8$ Hz), 1.76 (3H, Me for 2-methyl isomer, s), 1.84–1.90 (2H, m), 2.4–2.5 (2H, m), 4.98 (2H, s), 5.9 (1H, br s).

2-Methylcyclopent-1-ene-carboxaldehyde.—The above nitro-olefin mixture (0.5 g, 0.004 mol) was added dropwise to dry methoxide-activated Si gel (26 g) according to the method of Hogg *et al.* (27). After thorough mixing, the mixture was sealed in a brown bottle and left undisturbed for 120 h at room temperature. The crude product was eluted with Et_2O (300 ml) and then purified by vacuum distillation as a yellow liquid (24 mg, 6%), bp 64°, 118 mm (Kugelrohr). Ms *m/z* (%) 110 (84), 109 (40), 95 (39), 81 (100), 79 (58), 67 (58), 51 (40), 41 (48), 39 (39); ir (neat) 1660 cm^{-1}; ^1H nmr (60 MHz) δ 1.7 (2H, m), 2.05 (3H, s), 2.5 (2H, m), 9.9 (1H, s).

GERANYLACETONE.—*Farnesal.*—Pyridinium chlorochromate (89 g) prepared according to the method of Cheng *et al.* (28), was added to petroleum ether (60–80°, 120 ml) containing farnesol (6.2 g) and the suspension stirred at room temperature for 4.5 h. The mixture was filtered through florasil (149 g). Removal of the solvent followed by vacuum distillation gave farnesal (3.6 g, 58%), bp 140, 0.6 mm; ms

m/z (%) 220 (trace), 161 (11), 136 (14), 107 (28), 93 (57), 91 (15), 81 (26), 79 (25), 71 (31), 69 (100), 67 (25), 55 (35), 43 (39), 41 (82); ^{1}H nmr (500 MHz) δ 1.59–1.60 (12H, d, J = 1.5 Hz), 1.7 (6H, s), 2.0 (6H, s), 2.13–261 (16H, m), 5.07–5.11 (4H, m), 5.87–5.89 (2H, d, J = 7.9 Hz), 9.90–9.92 (1H, d, J = 8.1 Hz) 9.98–10.00 (1H, d, J = 8.1 Hz). The compound was characterized as the semicarbazone when it crystallized as white crystals from EtOH/H_2O, mp 98–100°.

Geranylacetone.—A solution of farnesal (1.0 g) in 95% EtOH (50 ml) and aqueous 0.48 M NaOH (35 ml) was refluxed for 20 min. The mixture was cooled, diluted with H_2O (58 ml), neutralized with 10% H_2SO_4 (12 ml), and exhaustively extracted with CH_2Cl_2. The combined organic extracts were washed with H_2O, decolorized with activated charcoal, and dried. Removal of the solvent followed by vacuum distillation gave geranylacetone as a yellow oil (0.8 g, 96%), bp 135°, 10 mm [lit. (28) 124°, 10 mm]; ms *m/z* (%) 194 (2), 151 (19), 136 (20), 125 (15), 107 (19), 93 (13), 69 (75), 67 (14), 43 (100), 41 (57); ir (neat) 1700 cm^{-1}; ^{1}H nmr (500 MHz) δ 1.59–1.62 (6H, d, J = 1.5 Hz), 1.67 (3H, s), 1.97–2.06 (4H, m), 2.13 (3H, s), 2.25–2.29 (2H, m), 2.43–2.46 (2H, t, J = 7.5 Hz), 5.05–5.09 (2H, t, J = 5.1 Hz). The compound was characterized as the semicarbazone when pale yellow needles were crystallized from EtOH, mp 86–88° [lit. (29) *Z*-isomer 90–91°, *E*-isomer 96–97°].

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