Effects of PMSG/hCG treatment on female rats exposed to the respective diets in utero.

Treatment	Serum progesterone (ng/ml)	Ova shed	Antral follicles	Corpora lutea
Ethanol	119.2 ± 4.6	46 ± 9	9 ± 1	17 ± 1
Pair fed	74.4 ± 11.5 [b]	38 ± 5	4 ± 1 [b]	10 ± 1 [b]
Control	89.8 ± 12.0 [a]	38 ± 5	6 ± 1	11 ± 1 [a]

[a] $p < 0.05$ vs ethanol; [b] $p < 0.01$ vs ethanol.

Furthermore, the table indicates that histological examination of the ovaries showed a greater number of corpora lutea present in the ovaries from animals exposed to ethanol in utero than in the female rats whose mothers received the pair fed diet ($p < 0.01$) or the control diet ($p < 0.05$) during pregnancy. There were also more antral follicles present in the ovaries in female rats subjected to ethanol in utero compared to animals treated with the pair fed diet or control diet during gestation.

The results indicate that the exogeneously administered gonadotrophins stimulated uterine growth equally in both the offspring from ethanol-exposed mothers and offspring from control mothers and that the ovaries of the offspring from each treatment are capable of responding to the exogenous gonadotrophins. However, administration of PMSG and hCG stimulated ovarian growth greater in fetal ethanol-exposed female rats than in offspring from the pair fed and control groups as evidenced by the enhanced ovarian weights and greater numbers of ova shed. The increase in ovarian weights in the ethanol animals were due to the large numbers of corpora lutea and antral follicles present in the ovary relative to those in ovaries from animals of control groups. The presence of the increased number of corpora lutea observed by histological examination corresponds to the increased number of total ova recovered and higher levels of serum progesterone measured in the ethanol-exposed offspring compared to the same parameters in offspring from control mothers. These data collectively indicate that fetal ethanol exposure may increase the sensitivity of the ovaries to the exogenous gonadotrophins.

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Secretion of the Dufour gland of the ant *Nothomyrmecia macrops* (Hymenoptera: Formicidae)

J. P. J. Billen[a], B. D. Jackson[b] and E. D. Morgan*[b]

[a] *Zoological Institute, University of Leuven, Naamsestraat 59, B-3000 Leuven (Belgium), and* [b] *Department of Chemistry, University of Keele, Staffordshire ST5 5BG (England)*
Received 24 March 1988; accepted 2 May 1988

Summary. The Dufour gland of the primitive Australian ant *Nothomyrmecia macrops* contains microgram quantities in total of linear alkanes and alkenes from C_{13} to C_{30} with heptadecene the major component (66%). In all, 50 substances, including methyl-branched hydrocarbons, aldehydes and acetates, α-farnesene and α-springene in low nanogram amounts, were identified by gas chromatography-mass spectrometry.
Key words. Ant; *Nothomyrmecia;* Dufour gland; hydrocarbons; farnesene; springene.

Nothomyrmecia macrops Clark is considered to be the most primitive living ant and is therefore interesting from many aspects, but it is extremely elusive and was probably also the species most sought by myrmecologists. Several expeditions have been organized to the region of Balladonia in Western Australia, where the first two specimens were collected [1] in 1931. All but the first were unsuccessful in finding this "holy grail to ant specialists" [2], until it was rediscovered in 1977 at Poochera, South Australia by a CSIRO field party [3].

Because of its primitive behaviour and some rather peculiar anatomical features [3], *N. macrops* has been placed in the subfamily of Nothomyrmeciinae, in which it is the only species [4]. Since its rediscovery, it has been the subject of several studies, dealing with its general anatomy and phylogenetic position [3], its behaviour [5], genetics [6], sting morphology [7], and, in connection with the present work, its gland ultrastructure [8]. However, until now, no investigations have been made on the chemical secretions of this species. During a recent field trip to Poochera, foraging workers of *N. macrops* were collected and various tissues prepared for chemical analysis.

The Dufour gland is a bulbous organ attached to the poison apparatus in all aculeate Hymenoptera. In all ant species that have been studied, it is filled with an oily mixture of organic substances [9]. Its primary purpose is unknown, though in individual cases it has been shown to contain a trail pheromone, a home range or territorial marking pheromone, and to have other pheromonal properties. Hölldobler and Taylor have already shown, in a simple behavioural test, that *N. macrops* workers were alerted by, and attracted to, the secretion of their Dufour glands [5].

In view of the uniqueness of this species and also because the contents of the Dufour gland are a useful systematic and taxonomic indicator, we report our chemical investigation of it here.

Results and discussion. Dufour glands, dissected from the abdomens of workers of *N. macrops* were sealed in glass capillaries and brought by air to Keele, where they were analyzed using our solid sampling method[10] without the intervention of solvent. A typical gas chromatogram of one of these glands is seen in figure 1. More than 99.9% of the material consists of straight chain alkanes, alkenes and alkadienes, with heptadecene the major component, followed by other odd-numbered saturated and unsaturated hydrocarbons. Ten individual glands were analyzed in this way. It can be seen from figure 2 that although the total amount in the gland varied widely from 1730 ng in worker A to 17,780 ng in worker J (fig. 2A), the composition of the mixture did not vary much (fig. 2B). The variation in amount may reflect the physiological condition of the ants (they forage singly) as well as the efficiency of dissection. In most cases the Dufour gland was analyzed with the sting attached. In four cases, ants C, F, H and I, the sting was removed to demonstrate that these substances originate only from the Dufour gland. Two of the glands were analyzed by linked gas chromatography-mass spectrometry and another sample consisting of seven glands sealed in a single capillary was analyzed by GC-MS (fig. 3).

In all cases heptadecene was the major component, followed by other linear alkanes and alkenes (fig. 2B). Some 60 components in all were separated and all but eight of them identified. Among the minor components were 7-methyl and 5-methyl branched alkanes, a sesquiterpene and a diterpene and some acetates. All these substances are indicated on figure 3. Insufficient material was available to locate the positions of the double bonds in the unsaturated compounds, but where fully identified in ants, the heptadecene and tricosene are (Z)-8-heptadecene and (Z)-9-tricosene respectively[9]. The farnesene isomer usually found in ants is (Z,E)-α-farnesene[9]. Here we detected the most stable isomer (E,E)-α-farnesene, but think this may have been formed by isomerization. A high injection port temperature (220 °C) had to be used to ensure volatilization of the whole mixture, and farnesenes isomerize[11] above 140 °C. A very small quantity of α-springene[12] (3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene) was identified by comparison of its mass spectrum with that of a synthetic sample[13] (M^+ absent, m/z (rel. intensity) 191 (5); 161 (1); 147 (4); 134 (12); 119 (15); 107 (27); 93 (46); 81 (46); 69 (100); 55 (33); 41 (95)). While farnesenes are well known in insects and occur frequently in ant Dufour glands, this is the first time its diterpene homologue has been found in insects. A series of homologous alkenes I–VIII (fig. 3) were the only compounds that could not be identified. These are probably branched C_{23} to C_{30} dienes. They are typified by compound III, the most abundant of the group, a C_{25} diene (t_r 40.1 min, M^+ 348 (2); 222 (6); 208 (10); 194 (6); 123 (16); 109 (26); 96 (72); 82 (91); 67 (74); 55 (100); 43 (83); 41 (94)). 9-Pentacosyne and some linear dienes, synthesized for comparison did not have closely similar retention times or spectra. Compounds IV, VI and VIII had very weak spectra but their retention times and their characteristic low mass ions placed them in the same series. Squalene was identified by comparison of its retention time and mass spectrum with an authentic specimen. It has been recognized in analyses of Dufour glands from another species[4] but it is either present in the tissue, or is a contaminant from handling since it was also present in similar amounts in a sample of cuticular tissue taken from an abdominal sternite. The two phthalate esters (t_r 28.4 and 41.3 min) are frequently found in our work. They are not introduced in the laboratory, they may be picked up by the ants in their environment or through contact with plastic materials before killing and dissection.

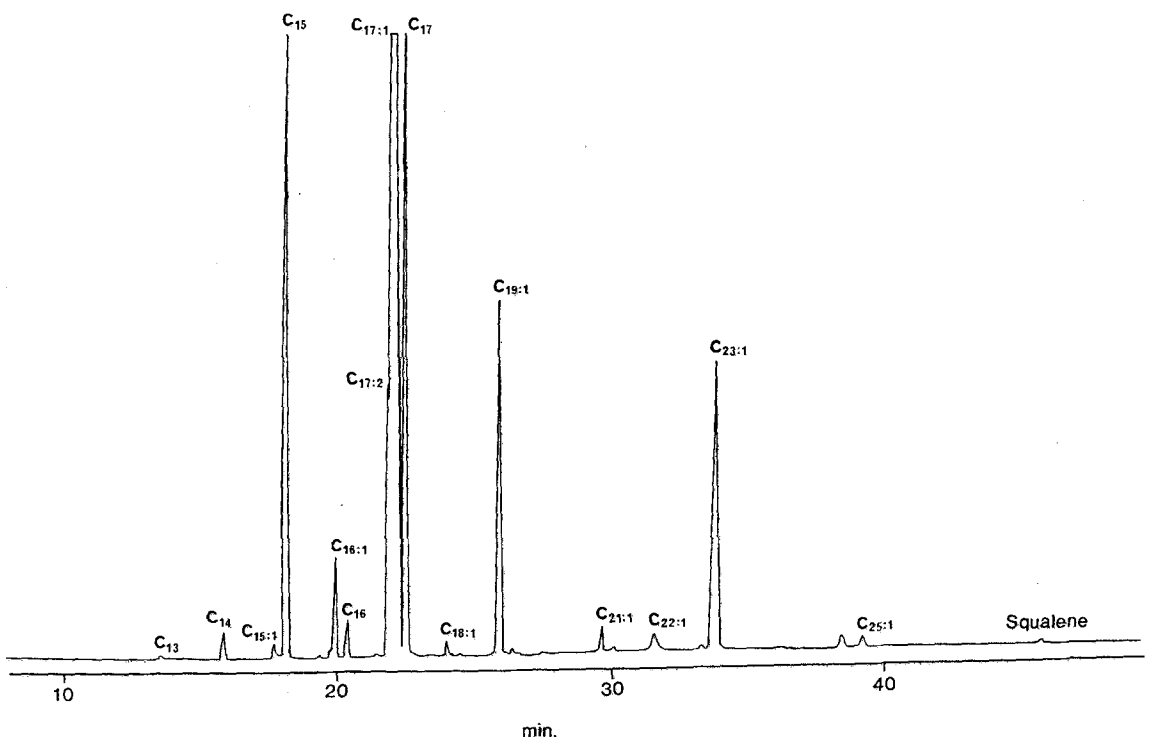

Figure 1. Gas chromatogram of the Dufour gland contents of a single worker of *N. macrops*. C_{17} is heptadecane, $C_{17:1}$ heptadecene, etc. Conditions as described in the experiment section.

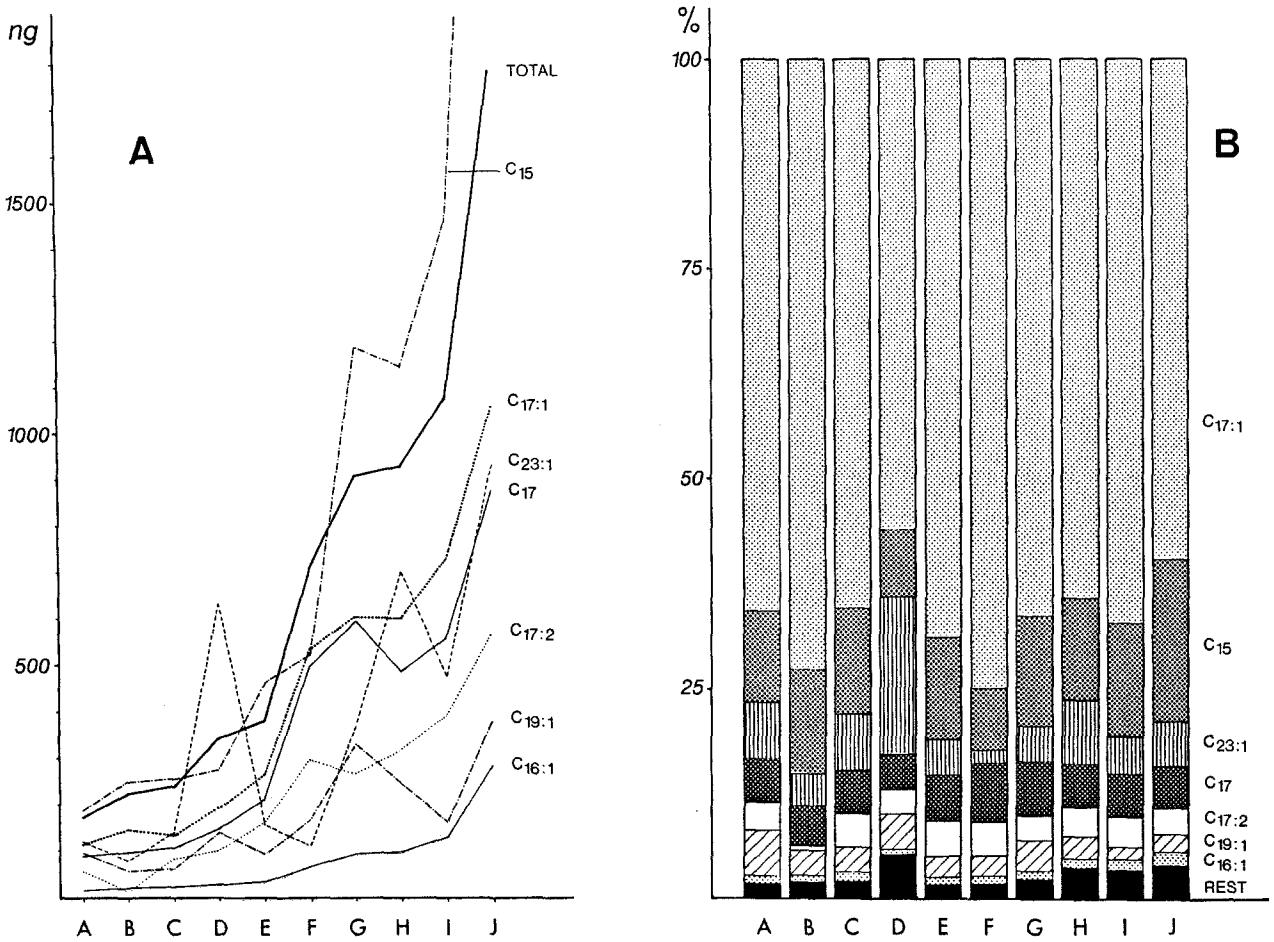

Figure 2. *A* The variation in amount of the different components between individual workers, graded from A, the smallest, to J, the largest gland. The scale is reduced 10-fold for the total amount and $C_{17:1}$. *B* The relative proportions of the major components in each individual gland. In most cases the sting was attached, but in samples C, F, H and I, this was carefully dissected away, to demonstrate that none of the materials analyzed came from the sting.

Mean quantities (ng) per individual of the major substances in the Dufour gland of workers of *Nothomyrmecia macrops* and the percentage composition, determined by gas chromatography on 10 samples.

Substance	Amount	± SD	Percent	± SD	Rank
C_{14}	32	35	0.3	0.1	14
$C_{15:1}$	30	39	0.3	0.2	12
C_{15}	918	999	12	3.3	2
$C_{16:2}$	13	2.5	0.1	0.03	16
$C_{16:1}$	78	85	1	0.3	7
C_{16}	33	30	0.4	0.1	10
7 MeC_{17}	9	5	0.07	0.01	20
$C_{17:2}$	226	175	3.3	1.1	5
$C_{17:1}$	4360	3170	66	5.6	1
C_{17}	365	272	5	0.8	4
$C_{18:1}$	17	8.4	0.2	0.03	15
$C_{19:1}$	174	112	3	1.1	6
C_{19}	11	6.2	0.1	0.01	18
$C_{21:1}$	19	14	0.3	0.2	13
C_{21}	10	6.7	0.1	0.05	17
$C_{22:1}$	22	17	0.4	0.2	11
$C_{23:1}$	372	303	6	4.7	3
$C_{24:1}$	9	2.5	0.08	0.02	19
III	28	22	0.5	0.5	9
$C_{25:1}$	34	25	0.5	0.3	8
Total	6700	5160			

The composition (table) is not unlike that reported by Cavill and Williams[15] for *Myrmecia gulosa*. Compared to the Dufour gland secretion of formicine and myrmicine ants, many examples of which are now known[9,16], the *N. macrops* contents resemble those of myrmicine ants more closely, but the wide range of minor components, and the presence of acetates, albeit in very small amounts, is characteristic of many formicines. The composition of the gland in *Nothomyrmecia*, however, shows the highest affinity to that reported by Cavill and Williams[15] for *Myrmecia gulosa* where (Z)-8-heptadecene (62%), pentadecane (17%) and heptadecane (4%) were the major substances among a series of hydrocarbons including alkenes and branched hydrocarbons. Comparable percentages indeed were found in *N. macrops* (66, 12 and 5%, respectively; table), in addition to the very similar general chemical profile, especially in the more volatile region. Regarding the value of the Dufour gland composition in chemotaxonomy[17], the apparent similarity between *Nothomyrmecia* and *Myrmecia* may indicate a closer relationship between these two endemic Australian genera than has been thought so far.

Experimental. Workers of *Nothomyrmecia macrops*, identified by R. W. Taylor, were collected in South Australia, 24 and 25 February 1987 and taken immediately to Canberra (CSIRO) and there anaesthetized and killed by placing for 3 min in a Biofreezer at − 50 °C. The glands were removed by dissection under water. They were immediately sealed, either individually or in groups, in soft-glass capillaries (2 mm × 2 cm), and kept cool until brought by air to Keele. Gas chromatography was carried out on a Carlo Erba Fractovap 4160 series instrument with a flame ionization detector and a Shimadzu Chromatopac C-R3A data processor. A fused silica capillary column (25 m × 0.32 mm) coated with OV1 stationary phase of 0.4-μm film thickness was used for the analysis. Helium was used as the carrier gas at a flow rate of 1.0 ml min^{-1}.

The capillary tubes containing the samples were kept in the solid injector[10] in the injection port at 220 °C for 2 min before crushing. The split vent was kept closed during the injection and opened after 1 min. The oven temperature was ini-

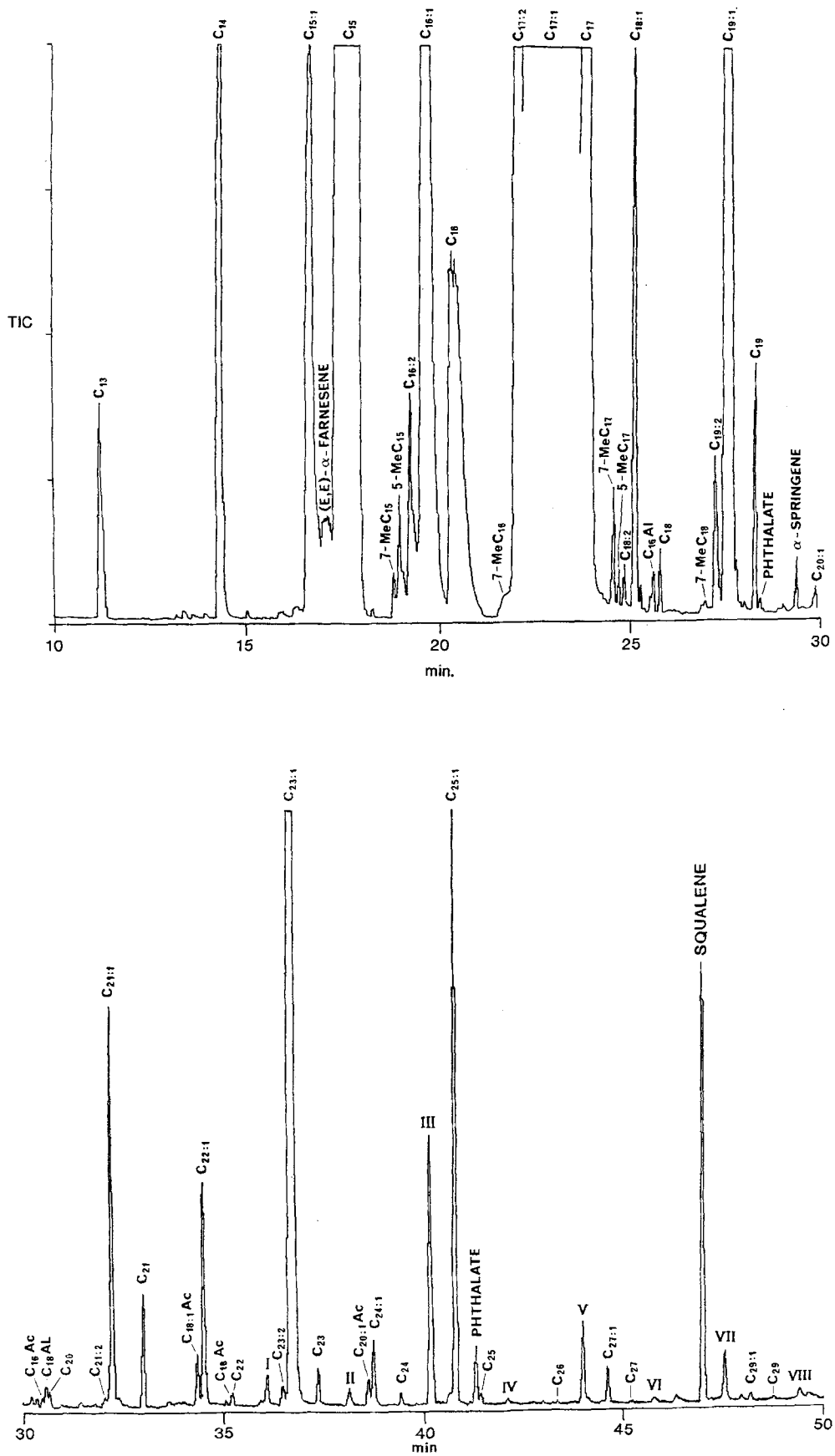

Figure 3. The total ion current from the GC-MS of 7 Dufour glands. C_{17} is heptadecane, $C_{17:1}$ heptadecene, etc. C_{16}AL is n-hexadecanal, $C_{18:1}$Ac is octadecenyl acetate, etc. I–VIII are unknown dienes, see text. The smallest labeled peaks represent less than 1 ng per individual worker.

tially 100 °C and increased at a rate of 6 °C min^{-1} to 270 °C. Ten single Dufour glands (6 with sting attached, 4 without) were analyzed and the absolute quantity of each component was determined by using a solution of pentadecane in hexane as an external standard.

Two single Dufour glands and a sample of seven glands were analyzed by GC-MS, on a Hewlett Packard 5890 Gas Chromatograph and 5970B Mass Selective Detector with HP59970C ChemStation. A fused silica capillary column (12 m × 0.2 mm) coated with HP-1 (cross-linked methylsilicone gum ≅ OV – 1) of 0.33-µm film thickness was used. The carrier gas was helium at 10 psi column head pressure (≅ 1 ml min^{-1} flow rate). The samples were introduced by the solid injection method [10] described above. The oven temperature was initially 60 °C and increased at a rate of 4 °C min^{-1} to 250 °C. The mass selective detector was set to monitor m/z 35–350 in the scan mode (≅ 1.5 scan s^{-1}) under 'Autotune' conditions using 70-eV ionization.

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* Please address all correspondence to E.D.M., Dept of Chemistry, University of Keele, Staffordshire ST5 5BG, England.

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Marine eicosanoids: Occurrence of 8-(R)-HETE in the starfish *Patiria miniata*

M. V. D'Auria, L. Minale, R. Riccio and E. Uriarte [1]

Dipartimento di Chimica delle Sostanze Naturali, Università di Napoli, Via L. Rodinò, 22, I-80138 Napoli (Italy)
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Summary. The first isolation of 8-(R)-hydroxy-5Z, 9E, 11Z, 14Z-eicosatetraenoic acid [8-(R)-HETE] from a marine source, the pacific starfish *Patiria miniata,* is reported. 8-(R)-HETE occurs together with 8-(R)-hydroxy-5Z, 9E, 11Z, 14Z, 17Z-eicosapentaenoic acid.
Key words. Starfish; 8-(R)-HETE; arachidonic acid metabolism; marine prostanoids biosynthesis.

Marine prostanoids, first discovered in a gorgonian [2], have since been isolated from octocorals [3–5] and from red algae [6]. A feature of the octocoral-derived clavulones [3], claviridenones [4] and punaglandins [5] and the algal metabolite, hybridalactone [6] is C-12 oxygenation. This led Corey [7] to suggest a distinctive biosynthetic pathway for these marine eicosanoids. More recently, biosynthetic experiments with two different species of coral, *Clavularia viridis* and *Pseudoplexaura porosa,* have shown the intermediacy of 8-(R)-HPETE [i.e., 8-(R)-hydroperoxy-5,11,14(Z), 9 (E)-eicosatetraenoic acid] in the conversion of arachidonic acid to pre-clavulone [8, 9], from which clavulones and possibly other prostanoids as well can arise by a series of hydroxylations, esterifications etc. These results led Corey and Matsuda [10] to propose a new biosynthetic pathway for marine prostanoids through the 2-oxidopentadienyl cation, totally different from the biosynthetic path to mammalian prostanoids. Bundy et al. [11] discovered an arachidonic acid C-8 lipoxygenase in the gorgonian coral *Pseudoplexaura porosa,* capable of converting exogenous arachidonic acid into 8-(R)-HPETE, and this gives further support to the importance of the 8-oxygenation pathway in the biosynthesis of marine prostanoids. The corresponding alcohol, 8-HETE, has been previously obtained by a variety of procedures: enzymatically [12], after in vivo stimulation by a tumor promoter [13], chemically via regio-random oxidation [14] and via total chemical synthesis [15], but has not yet been identified in marine organism extracts.

In this paper we report the isolation of 8-(R)-HETE (**1**) along with its 17,18-didehydro derivative (**2**) from the starfish *Patiria miniata.* The recent discovery that 8-(R)-HETE triggers oocyte maturation in starfish [16] provides still another reason for interest in this result.

The compounds **1** and **2** were purified from the aqueous extracts of the animals (3.5 kg fresh wt), collected from the Gulf of California, by the following successive chromatographic steps: recovery of the polar material on a column of Amberlite XAD-2, chromatography of methanol eluates on a column of Sephadex LH-60 (eluent: methanol-water, 2:1) to separate the steroidal glycosides from the eicosanoids, which were eluted as the last moving compounds, chromatography on silica gel (eluent: chloroform-methanol, 98:2) and finally HPLC (C_{18} µ-Bondapak, 75% aq. methanol) to obtain 8-(R)-HETE (0.5 mg) and **2** (1 mg).