formation of pyridine or 3-hydroxypyridine. It is likely that during the transformation of these substrates compounds are produced that contain carboxyl groups. The failure to detect carboxylated intermediate products may have been due to concentrations below the detection limit even though the method used would allow detection of carboxylated compounds in concentrations as low as 1 µmol/l[24]. The fact that no carboxylated end products (e.g. acetate) accumulated agrees with our radiolabeled experiments where it has been shown that the pyridine-carbon is efficiently transformed to $^{14}CO_2$.

In conclusion, microorganisms obtained from digested sewage sludge were able to oxidize pyridine and 3-hydroxypyridine to carbon dioxide under anaerobic conditions when an electron acceptor like nitrate or sulfate was available.

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Sexual dimorphism in the defensive secretion of a carabid beetle*

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Summary. The defensive secretions of male and female *Oodes americanus* display striking qualitative differences. Altogether 13 carboxylic acids were identified in the secretions of the two sexes. Methacrylic, crotonic, and tiglic acids are produced exclusively by the female; the male lacks these unsaturated components, but produces their saturated analogs. 2-Methylbutyric acid is a major component produced by both sexes. Shared components also include hexanoic, (*E*)-2-hexenoic, benzoic, and (*E*)-2-octenoic acid, of which the latter two had not previously been reported from carabid beetles.
Key words. Carabidae; defensive secretion; sexual dimorphism; carboxylic acids.

The chemistry and morphology of the defensive glands of carabid beetles have been intensely investigated. Over 300 species have been studied[1], and a wide range of chemicals has been characterized, including carboxylic acids, esters, carbonyl compounds, and aromatic components[2–5]. In no species had evidence been presented indicating that the secretion might differ in males and females. We here report the demonstration of sexual dimorphism in the defensive secretion of *Oodes americanus*, a member of the carabid tribe Oodini of the supertribe Callistitae[6], a lineage of relatively recent evolutionary origin within the family Carabidae.

Materials and methods

Beetles. The seven *O. americanus* studied (5 ♂♂, 2 ♀♀) were taken live at lights on January 30, 1990, on the grounds of the Archbold Biological Station, Lake Placid, Florida, U.S.A. Beetles of this species are ordinarily rare, and we were unable to collect them in additional numbers. They were maintained in the laboratory for several weeks on freshly-killed mealworms (larvae of *Tenebrio molitor*) and water. For gland excision they were killed by freezing and dissected under Ringer's solution. Dissected specimens have been deposited in the Cornell University Insect Collection, voucher lot number 1193.

Gas chromatography (GC). This was carried out on a Hewlett-Packard 5890 Series II instrument equipped with a splitless injector, a flame ionization detector, and a HP 3396A integrator. The analyses were performed on either a 30 m × 0.26 mm fused-silica capillary column coated with DB-1 (methyl silicone) (0.25 µm), or a column of similar dimensions coated with DB-Wax (0.25 µm). The samples were introduced by splitless injection. The GC oven temperature was kept at 30 °C for 5 min and programmed to 260 °C at 15 °C/min for the DB-1 column; or at 60 °C for 4 min and programmed to 180 °C at 10 °C/min for the DB-Wax column.

Gas chromatography – FTIR-MS. Infrared and mass spectra were obtained on a Hewlett-Packard 5890 Series II gas chromatograph linked in series to a HP 5965A IR detector and a HP 5970 mass selective detector. Analyses were performed using either a 25 m × 0.32 mm fused-silica column coated with SE-54 (0.25 µm), or a 30 m × 0.26 mm fused-silica column coated with DB-WAX (0.25 µm).

Preparation of pentafluorobenzyl derivatives[7–9]. The defensive glands of individual beetles were excised and placed in cooled (dry ice) vials. To each vial pentafluorobenzyl bromide (5 µl, Aldrich Chemicals, USA) was added followed by triethylamine (10 µl, Aldrich). The vials were kept with occasional shaking at room temperature for 3 h, after which 30 µl H_2O was added and the products were extracted with hexane (20 µl). The hexane layer was removed and analyzed by GC and GC-FTIR-MS. Authentic acids, obtained from commercial sources, were similarly derivatized.

Results

Altogether 13 carboxylic acids were identified from the defensive secretion of male and female *O. americanus* (fig. 1). The FTIR and MS data obtained for the pentafluorobenzyl derivatives of these acids are summarized in the table. The identifications were confirmed by comparing GC retention times, mass spectra, and infrared spectra of the derivatives with those obtained from derivatized authentic samples.

The relative ratio of components in the secretion of the two sexes (average for 2 individuals of each sex) is given in figure 2. The three additional males examined (but not assessed quantitatively) had the same qualitative composition as the males in figure 2.

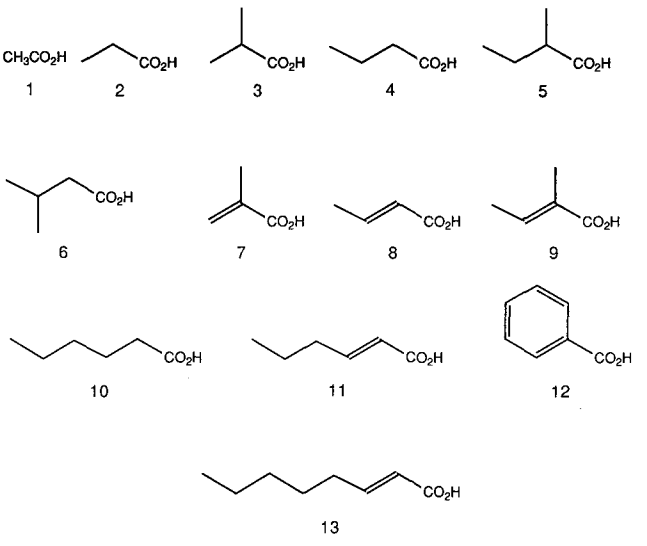

Figure 1. Carboxylic acids in the defensive secretion of *Oodes americanus*.

Chemical composition of defensive gland secretion of male and female *Oodes americanus*: Summary of analytical evidence for structure assignments. * = sample insufficient for a reliable IR spectrum; w = weak; m = medium; s = strong.

	Spectra of pentafluorobenzyl derivatives	
Compound	Mass spectrum [*m/z* (%)]	Infrared [cm^{-1}]
1 Acetic acid	240(M^+,34), 181(100), 161(17), 117(9), 43(42)	2978(w), 1774(m), 1510(s), 1219(s)
2 Propionic acid	254(M^+,16), 181(100), 161(10), 57(19)	*
3 Isobutyric acid	268(M^+,16), 220(2), 181(100) 161(14), 43(63)	2983(w), 1762(m), 1510(s), 1138(s)
4 Butyric acid	268(M^+,12), 181(100), 161(9) 71(14), 43(19)	2975(w), 1766(m), 1510(s), 1162(m)
5 2-Methyl-butyric acid	282(M^+,7), 254(19), 181(100) 161(13), 57(88), 41(49)	2978(w), 1760(m), 1510(s), 1136(s)
6 Isovaleric acid	282(M^+,5), 240(3), 181(100) 161(9), 101(10), 85(9), 57(21)	2971(w), 1764(m), 1510(s), 1137(m)
7 Methacrylic acid	266(M^+,23), 221(33), 181(100) 161(13), 69(23), 41(28)	2983(w), 1748(m), 1510(s), 1146(s)
8 Crotonic acid	266(M^+,16), 221(23), 181(100) 161(28), 117(9), 69(70), 41(28)	*
9 Tiglic acid	280(M^+,15), 265(13), 235(17) 181(100), 161(12), 83(30), 55(46)	2983(w), 1741(m) 1510(s), 1134(s)
10 Hexanoic acid	296(M^+,4), 240(4), 181(100), 161(9), 97(20), 69(23), 43(21)	*
11 (*E*)-2-Hexenoic acid	294(M^+,2), 251(21), 239(13) 181(100), 161(12), 97(16), 55(30)	2971(w), 1750(m), 1510(s), 1162(s)
12 Benzoic acid	302(M^+,77), 257(37), 181(100) 161(14), 105(88), 77(57), 51(28)	3076(w), 1750(m), 1510(s), 1262(s)
13 (*E*)-2-Octenoic acid	322(2), 262(13), 251(8), 181(100), 161(9), 95(15), 55(33)	2941(w), 1750(m), 1510(s), 1153(m)

Discussion

Most acids found in *O. americanus* are of frequent occurrence in carabid defensive secretions[2,3]. However, nei-

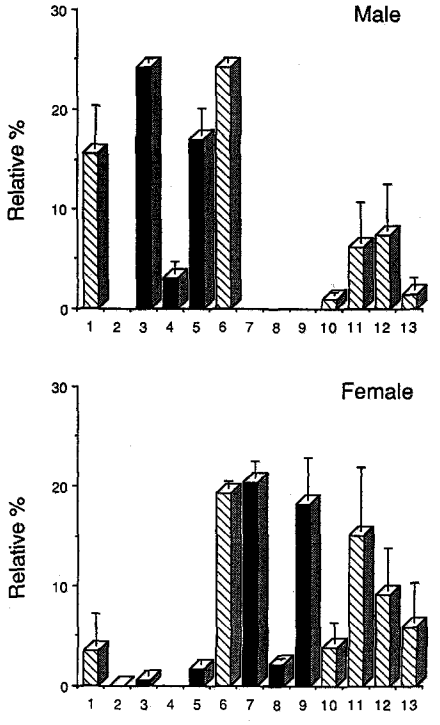

Figure 2. Relative percent of acids in defensive secretion of male and female *Oodes americanus*; N = secretion samples from 2 individuals of each sex. Column height = mean; bar = higher of the two values. Propionic acid was present as trace in the female only. Rationale for column depiction differences in text.

ther benzoic acid nor (*E*)-2-octenoic acid had previously been reported from Carabidae (benzoic acid has, however, been reported from a cicindellid [10]). Crotonic acid is of only restricted distribution in the family [11]. One of two species of *Oodes* previously examined, the Australian *O. modestus*, is reported to produce methacrylic and tiglic acid [12]. The other, the Japanese *O. vicarius*, produces 2-methylbutyric, isovaleric, and senecioic acid [13].

Most remarkable is the finding that the secretion of *O. americanus* is sexually dimorphic. The three unsaturated acids, methacrylic, crotonic, and tiglic acid, were not detected in the male secretion. However, in the female secretion, methacrylic and tiglic acid are two of the major components. Although the biosynthetic pathways of these unsaturated acids have not been fully established, the compounds are believed to be amino acid-derived, as follows [5]:

valine → isobutyric acid → methacrylic acid
isoleucine → 2-methylbutyric acid → tiglic acid
leucine → isovaleric acid → senecioic acid

Benn et al. [14] have shown that valine is converted to methacrylic acid in *Carabus taedatus*. If we assume similar biosynthetic capacities to be in force in *O. americanus*, it seems possible that the female produces methacrylic and tiglic acid by desaturation of isobutyric and 2-methylbutyric acid respectively. Indeed, in the female, the two presumed precursor acids, isobutyric and 2-methylbutyric acid, are present only as minor components, while in the males they are major components. Hence, it appears that males lack the desaturase(s) required for introduction of double bonds into the precursors. It is also noteworthy that while crotonic acid is found only in females, its saturated counterpart, butyric acid, is present in the male.

Isovaleric acid is a major component of both male and female secretion. If we regard this acid to be a potential precursor for senecioic acid, a component found in other carabid secretions, it appears that *O. americanus* lack the desaturase needed to effect this conversion since neither sex was found to produce senecioic acid. The pentafluorobenzyl esters of senecioic and tiglic acids coelute on methylsilicone columns. However, on our polar DB-wax columns the two derivatives were resolved, and their mass and FTIR spectra were distinctly different [15]. The absence of senecioic acid from the *O. americanus* secretion could thus be established with certainty.

Acids such as hexanoic, hexenoic, and octenoic acid are considered not to be amino acid-derived, but to be produced possibly by condensation of acetate units [14]. These particular acids, together with benzoic acid, were found in both sexes of *O. americanus*.

Previous reports on the chemistry of carabid secretions, as a rule, fail to specify whether the analyzed samples stemmed from males, females, or both sexes combined. It is impossible to tell, therefore, whether the chemical sexual dimorphism found in *O. americanus* is restricted to this species or prevalent in other carabids as well. In at least one species, *Pasimachus subsulcatus*, the secretion was shown to be qualitatively and quantitatively similar in males and females [16]. Efforts to classify carabids into groups based on defensive gland chemistry [5, 11, 17], should be exercised with caution. Whereas *Oodes* secrete aliphatic acids, taxa in two other member tribes of the Callistitae – Licinini and Callistini – respectively secrete as major compounds formic acid, and m-cresol or quinones [12, 17, 18]. Ambiguity due to such evolutionary lability would be compounded by unrecognized sexual dimorphism of secretion composition.

No definite functional interpretation can be offered for the chemical dimorphism of *O. americanus*. However, it is conceivable that the defensive secretion also plays a pheromonal role. If one assumes that the glands are not hermetically sealed but subject to slow leakage of their contents in vapor form, one could envision such emission, given its dimorphism, to provide a basis for sex attraction and/or recognition in courtship. There is certainly no reason why the tubular efferent ducts of the *O. americanus* glands, which we found to conform closely in gross structure to those of carabid glands generally, might not be prone to leakage. To take on signal value in a pheromonal context, the evaporative output from the glands would need to be no more than minimal. A further possibility is that the secretion is used by the female to repel courting males when she is unreceptive. Such

employ of secretion has been claimed for the carabid *Pterostichus lucublandus*[19].

Little is known about the chemical basis of sexual behavior in carabid beetles. The possibility that the defensive secretion plays a role in the courtship of some species is worth investigating. With respect to *O. americanus*, it would clearly have been desirable to have had a larger number of individuals for analysis, to determine whether factors such as age or reproductive status also affect secretory composition.

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Purealidin A, a new cytotoxic bromotyrosine-derived alkaloid from the Okinawan marine sponge *Psammaplysilla purea*

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Summary. A new bromotyrosine-derived alkaloid with antileukemic activity, purealidin A (**5**), has been isolated from the Okinawan marine sponge *Psammaplysilla purea* and its chemical structure elucidated on the basis of the spectroscopic data.
Key words. Antileukemic activity; purealidin A; bromotyrosine-derived alkaloid; sponge; *Psammaplysilla purea.*

Recently, several bromotyrosine-derived alkaloids have been isolated from marine sponges[1-7], especially from the family Verongidae, and we have also reported isolation of purealin (**1**)[8] and lipopurealins A ~ C (**2** ~ **4**)[9] from the Okinawan marine sponge *Psammaplysilla purea*. Purealin (**1**) was shown to activate myosin K,EDTA-ATPase, while purealin (**1**) and lipopurealins (**2** ~ **4**) exhibited inhibitory activity against Na,K-ATPase. Purealin and its related alkaloids, therefore, have been shown to have properties which are at present unique, which could make them valuable as a biochemical tool for regulating enzyme activity[10-13]. During our studies on bioactive substances from Okinawan marine organisms[14-17], we further investigated the sponge *P. purea* to see whether we could obtain other purealin-related compounds which might show similar unusual biological activity. In this paper we describe the isolation and structure elucidation of a new purealin-related alkaloid, named purealidin A (**5**), which exhibits potent antileukemic activity.

The sponge *P. purea* was collected at Minna Island, Okinawa, by SCUBA and kept frozen until used. The methanol extract was partitioned between ethyl acetate and water and the aqueous layer was subsequently extracted with *n*-butanol. The *n*-butanol-soluble material was subjected to silica gel flash column chromatography; it was eluted with $CHCl_3$/*n*-BuOH/AcOH/H_2O (1.5:6:1:1) followed by a Sephadex LH-20 column with $CHCl_3$/MeOH (1:1). The fraction containing purealin-related compounds (detectable by ninhydrin test on TLC) was further purified by reversed-phase HPLC [ODS, CH_3CN/H_2O (25:75) with 0.1% trifluoroacetic acid] to furnish purealidin A (**5**, 0.0003% yield, wet weight).

Purealidin A (**5**)[18] showed intense M + H ions in the ratio of about 1:2:1 at *m/z* 517, 519, and 521 in the positive FABMS spectrum, which indicated the presence