pores with increasing adsorption. The fact that the entropy of desorption is smaller than that of adsorption (fig. 3) can be explained by the ink bottle theory of adsorption hysteresis suggested by Kraemer and McBain[8], and suggests that the activated carbon used has micropores with narrow entrance and wide interior.

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o-Aminoacetophenone: Identification in a primitive fungus-growing ant *(Mycocepurus goeldii)*

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Summary. *o*-Aminoacetophenone is the major volatile product present in the mandibular gland secretion of the primitive fungus-growing ant *Mycocepurus goeldii*. This novel arthropod natural product is biosynthetically far removed from the aliphatic ketones and alcohols found in those genera of the tribe Attini that represent the main line of evolution. The divergent phylogenetic position of *Mycocepurus*, and possibly of other closely related genera, is emphasized.

Recent investigations[2-6] on the natural products chemistry of attine ants have demonstrated that these hymenopterans are a rich source of 2- and 3-alkanones, the corresponding alcohols, and in some cases, oxygenated monoterpenes. The ethyl ketones, which are the main releasers of alarm behavior in species in the more highly evolved genera[2,5], are generally the major compounds produced in the mandibular glands. An investigation[5] of the mandibular gland products of fungus-growing ants in several genera that reflect the accepted phylogeny of the tribe Attini[7] indicates that the distribution of these exocrine compounds is in accord with the recognized evolution of the genera in this taxon. However, whereas this chemosystematic study utilized species in genera whose established relationships clearly defined them as pivotal taxa in the phylogeny of the tribe[8], it did not include species in the small genera that appear to have diverged from the general attine stem[7]. We now wish to report that the mandibular gland chemistry of *Mycocepurus goeldii*[9], a species in one of these divergent genera, is dominated by *o*-aminoacetophenone, an exocrine compound unique to the tribe Attini or for that matter, to any other arthropod species.

Materials and methods. Colonies of *M. goeldii* were collected near Presidente Prudente, Brazil. Crushed heads of workers possess a strong grape-like fragrance and in southern Brazil these ants are sometimes referred to as the 'formica perfume' (perfume ant). Extracts were prepared either by dissecting mandibular glands or by crushing heads in spectrograde *n*-pentane; volatile compounds were resolved gas-chromatographically on both 1% OV-1, programmed from 100–250 °C at 5 °C/min, and 10% Carbowax 20 M, isothermally at 180 °C. Eluting compounds were collected on graphite and their mass spectra obtained by direct insertion into the ion source of a Bell and Howell 21-490 mass spectrometer.

Behavioral studies were undertaken on either field or laboratory colonies by exposing the ants to mandibular gland extracts, crushed heads, or pure compounds applied to filter paper squares (1 cm^2) or to the tips of wood applicator sticks. The activity of compounds as alarm releasers for another attine species, *Atta texana*, was determined as described previously[2,10].

Results. Four compounds, all present in mandibular gland extracts, were detected by gas chromatography, the major and final eluting one possessing the grape-like odor associated with *M. goeldii*. The mass spectrum of this compound was characterized by a molecular ion and base peak at *m/z* 135, with major fragments being present at m/z 120 (loss of CH_3), *m/z* 92 (further loss of $-COCH_3$), and *m/z* 65 (aromatic ring). The mass spectrum and retention times of this substance were completely congruent with those of authentic *o*-aminoacetophenone.

Three minor constituents, eluting earlier than aminoacetophenone, were not conclusively identified. The mass spectrum of the 1st of these compounds, possessing a strong molecular ion at *m/z* 150 and a base peak at *m/z* 135, was similar to o-methoxyacetophenone, but differed in minor respects. The other 2 compounds possessed molecular ions at *m/z* 218 and m/z 232, and their mass spectra were very similar to homofarnesene and bishomofarnesene[11]. Insufficient quantities of these compounds prevented their complete characterization.

Workers of *M. goeldii* are attracted to a crushed mandibular gland or head and respond similarly to 1 µg of *o*-aminoacetophenone placed on filter paper squares or tips of wood applicators. One the other hand, workers of the highly evolved attine species *Acromyrmex nigra, Atta laevigata* and *A. sexdens* do not appear to react to a crushed head of *M. goeldii*. High concentrations of *o*-aminoacetophenone are repellent to workers of *M. goeldii* whereas workers of the *Acromyrmex* and *Atta* species are slightly attracted to high concentrations of this compound. *o*-Aminoacetophenone was completely inactive as a releaser of alarm behavior for workers of *Atta texana* when compared at all concentrations to their natural alarm pheromone, 4-methyl-3-heptanone[1,10].

Discussion. The production of *o*-aminoacetophenone by workers of *M. goeldii* demonstrates that the exocrine chemistry of this fungus-growing ant differs considerably from those in genera representing the main line of attine evolution. Whereas aliphatic compounds such as 3-octanol and 4-methyl-3-heptanone are typical of the mandibular gland products identified in a variety of attine genera[4,5], aromatic natural products are clearly atypical of species in

taxa identified with the phylogeny of the Attini[7]. On the other hand, *Mycocepurus* is one of a series of small genera whose members cultivate specialized fungi that differ considerably from those produced by polymorphic attines such as *Acromyrmex* and *Atta*[7]. If the mandibular gland chemistry of *M. goeldii* is typical of other attine genera that have diverged from the main stem of attine evolution in not emphasizing simple aliphatic ketones and alcohols, then a large potential treasure-trove of natural products remains to be characterized. This could be particularly true for species in the genera *Mycetophylax* and *Mycetarotes*, two divergent taxa that are considered to be closely related to *Mycocepurus*[7].

Although *o*-aminoacetophenone exhibits demonstrable pheromonal activity for workers of *M. goeldii*, it possesses no pronounced activity for workers of attine species in more specialized genera. Species of the latter, such as *Atta texana*, utilize 4-methyl-3-heptanone as an alarm pheromone and in view of the great olfactory acuity they manifest for their own alarm pheromone[1], it is not surprising that they can readily distinguish this minty ethyl ketone from the unrelated grape-like aromatic ketone produced by *M. goeldii*. It is worth noting that another ant pheromone, methyl anthranilate[12], also is characterized by a powerful grape-like odor, and it may not be insignificant that both *o*-aminoacetophenone and methyl anthranilate possess similar shapes. Since insect pheromones have been utilized as paradigms for studying the relationships of molecular shape to odor quality[13], it would appear that these natural products, because they can be evaluated behaviorally, may be particularly useful for studying olfactory theory. Finally, as the degradation of tryptophan could possibly lead to the synthesis of *o*-aminoacetophenone, this species may provide a particularly useful model for a worthwhile biosynthetic study.

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The effect of diphosphonates on periosteal and bone cells in culture

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Summary. Calvaria cells were separated into periosteal and bone cells and cultured in the presence of ethane-1-hydroxy-1,1-diphosphonate (EHDP) or dichloromethanediphosphonate (Cl_2MDP). Both cell types were affected to the same degree with respect to the effect on cell number and lactate production. The action of the diphosphonates seems therefore not to be specific for one of the cell types.

Diphosphonates are compounds which contain a P-C-P bond and are thus related to pyrophosphate, but they are resistant to metabolic destruction. They have a strong affinity for calcium phosphate crystals; they inhibit both formation and dissolution of this mineral in vitro and in vivo, and they prevent ectopic calcification and bone resorption[2,3]. Recently these effects have been used clinically. Thus, EHDP has been found to decrease the development of ectopic ossification after total hip replacement[4] and in paraplegia[5]. Furthermore various diphosphonates have proved useful in the management of Paget's disease, a disease in which bone turnover is increased[6–8], and in tumoral bone disease[9,10].

These effects of diphosphonates in vivo have been mainly attributed to their physicochemical interactions with calcium phosphate crystals. Recently, however, it was found that the diphosphonates also influence cellular metabolism. Thus, in cultured calvaria and rabbit ear cartilage cells both Cl_2MDP and EHDP decrease the production of lactate[11,12]. Furthermore Cl_2MDP increases glycogen content[13], palmitate oxidation[14] and alkaline phosphatase activity in cultured calvaria cells[15], and increases the synthesis of collagen and glycosaminoglycans in cultured cartilage cells[16,17].

Bone cells have been separated into osteoclast- and osteoblast-like cells by Luben et al.[18], and into periosteal and bone cells by Peck et al.[19]. According to the latter authors, the periosteal cells seemed to be the osteoclast-like and the bone cells the osteoblast-like cells.

In previous studies on the effect of diphosphonates on calvaria cells a mixture of these cells has always been used. The question arises whether the action of the diphosphonates varies according to the type of cells; it is also possible that only certain types of cells survive when cultured in the presence of diphosphonates. To answer these questions, the cells were separated according to Peck[19] and the effect of EHDP and Cl_2MDP on cell number and lactate production was studied in both types of cells.

Material and methods. Bovine parathyroid hormone (PTH) (248 units/mg) was obtained from Inolex Corp., Chicago, Ill., USA. It was dissolved in 0.01 M HCl, 10 mM ascorbic acid, 2 mg/ml bovine serum albumin at a concentration of 40 units/ml, divided into aliquots and frozen at −80 °C. Salmon calcitonin was obtained in solution from Sandoz AG, Basel, Switzerland. 100 units were dissolved in 1.0 ml water containing 2 mg acetic acid, 2 mg sodium acetate and 7.5 mg NaCl. The solution was divided into aliquots and frozen at −80 °C until used. EHDP and Cl_2MDP were obtained as the disodium salt from Procter & Gamble Co., Cincinnati, OH, USA.

The cells were cultured as described earlier[11]. Briefly, calvaria of 1-day-old Wistar rats were digested with collagenase and the liberated cells cultured in an atmosphere