minima on the time axis and the absolute values of heat flow vary somewhat from experiment to experiment – due to the amount of inoculum and also to the length of the lag phase associated with the adjustment of the organism to the growth medium – the general characteristics of the curves are well reproducible. In each example, the thermograms of two independent experiments with the same organism are shown. In some cases, the calorimetric characterization could be improved for characterizing different strains or mutants of the same organisms. Two examples are shown in Figures 10 and 11.

5. *Mixed cultures*

Only a few calorimetric investigations on the growth of mixed populations have been undertaken. Figure 12 shows the thermogram of a soil sample with different salt additions, and Figure 13, thermograms of microbial growth in cattle manure. In both cases it is impossible to recognize any characteristic profiles of the growing organism. The smoothing of the curves is caused by the superposition of the heat profiles of the growing species, but also by the unknown change in metabolism caused by the mutual interference of the species.

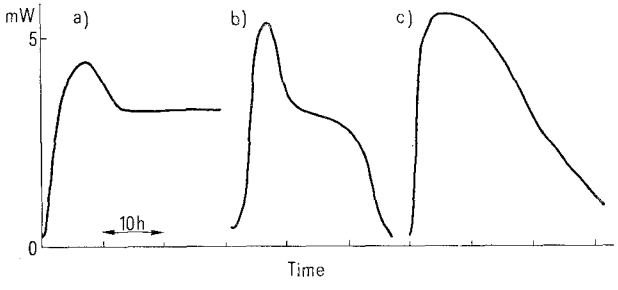

Fig. 13. Heat production from bacterial activity in cattle manure. a) solid manure at 40 °C; b) liquid manure at 45 °C; c) liquid manure at 60 °C.

6. *Conclusion*

It was shown in chapter 4 that, under exactly defined experimental conditions, a sufficient identification of some groups of bacteria is possible. In comparison with the usual microbiological methods, the calorimetric method has the great advantage that results are already at hand after 5 to 10 h. But, as with every other method, the organism under investigation has to be isolated before it can be identified. It is therefore not possible to carry out any thermic spectroscopy in a mixed culture. For clinical application, this question is of less interest, because contamination in blood or urine, for instance, is usually caused by just one species. At present, the growth profiles obtained are most sensitive to differences in experimental conditions, for example oxygen potential. Therefore, the calorimetric method is not yet suitable for practical use in clinical microbiology. However, with technical improvement and development of suitable culturing conditions, the microcalorimetric method may become a useful tool in the routine laboratory, even if it will never supersede any other conventional method[17].

[17] Acknowledgment. The kind permission of the cited authors and journals to reproduce their figures is gratefully acknowledged.

SPECIALIA

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Defensive Substances of Opilionids[1]

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Summary. The defensive secretions of 4 species of opilionids were analyzed. *Leiobunum ventricosum* and *Hadrobunus maculosus* produce 4-methylheptan-3-one, while *L. calcar* produces E-4,6-dimethyl-6-octen-3-one. *L. longipes* produces E-4,6-dimethyl-6-nonen-3-one, a new natural product.

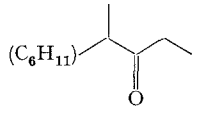
Arachnids of the order Opiliones possess a pair of dischargeable defensive glands[3,4] that produce volatile odorous secretions. Chemical work has so far been done only on 2 species of the suborder Laniatores and 3 species of the suborder Palpatores. The Laniatores produce methylated 1,4-benzoquinones[5–7], while the Palpatores produce acyclic ketones, including 4-methylheptan-3-one (I) and E-4,6-dimethyl-6-octen-3-one (II)[8,9]. We here report on the chemistry of the secretions of 4 additional Palpatores. Two of these, *Leiobunum ventricosum* and *Hadrobunus maculosus*, produce compound I, and a third, *L. calcar*, produces compound II. The fourth, *L. longipes*, produces E-4,6-dimethyl-6-nonen-3-one (III), a previously undescribed natural product.

The animals (12 *L. ventricosum*, 12 *H. maculosus*, 60 *L. calar*, 60 *L. longipes*) were collected in the environs of Ithaca, New York, and on the Huyck Preserve in Rensselaerville, New York. All were 'milked' of their secretion by the techniques previously described[9].

Gas chromatographic examination (2.4 m × 2 mm, 3% OV-225, Chromosorb W, 80–150 °C)[10] of the secretions of *L. ventricosum* and *H. maculosus* revealed the presence of a single volatile component in each, with the same retention time. The mass spectrum of this component was identical to that reported for 4-methylheptan-3-one (I)[9].

The secretion of *L. calcar* revealed 3 volatile components upon gas chromatographic analysis. The mass spectrum and retention time of the major component (ca. 70% of mixture) was identical to that of an authentic sample of E-4,6-dimethyl-6-octen-3-one (II). Confirmation of this identification was obtained by microozonolysis of the mixture, which yielded only one important volatile product, whose mass spectrum was identical to that reported for 4-methylheptan-2,5-dione (IV)[9].

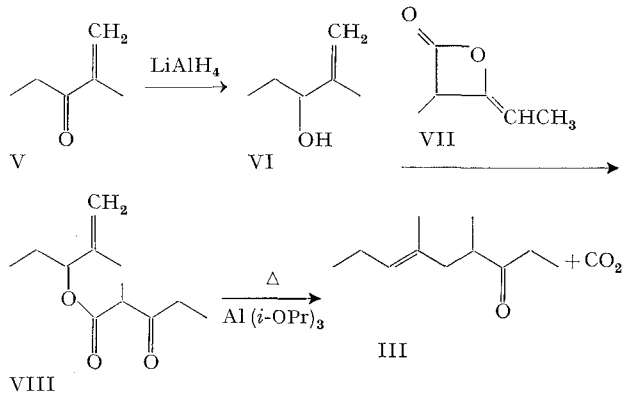
Gas chromatographic analysis of the defensive secretion of *L. longipes* revealed the presence of one major (ca. 90%) and one minor volatile component. The mass spectrum of the major component contained the following prominent peaks at 70 eV: *m/e* 168 (4), 139 (10), 111 (15), 86 (48), 83 (39), 69 (100), 57 (87), 55 (65), 41 (53). The spectrum is consistent with expectations for an unsaturated ketone of molecular formula $C_{11}H_{20}O$. The rearrangement peak at *m/e* = 86, along with peaks at M-29, M-57, and 57, indicate that this component is closely related to 4-methylheptan-3-one (I)[9]. With this assumption, partial structure *a* seems attractive. Spectral data alone, however, do not permit the assignment of an unambiguous structure to the C_6H_{11} residue comprising the distal portion of the molecule.



a

Further information was obtained by microozonolysis. Ether was added to the natural mixture, and ozone was passed through the solution at –78 °C for 5 min. The mixture was purged with nitrogen, allowed to warm to room temperature, and treated with triphenylphosphine. Analysis by gas chromatography/mass spectrometry revealed the presence of one major component with a mass spectrum identical to that reported for 4-methylheptan-2,5-dione (IV)[9]. In a second, similar experiment, using amyl acetate as a solvent, analysis by gas chromatography/mass spectrometry revealed the presence of propionaldehyde. On this basis, the second component can be formulated as 4,6-dimethyl-6-nonen-3-one. E-4,6-dimethyl-6-nonen-3-one (III) was prepared in a manner analogous to the previously described preparation of E-4,6-dimethyl-6-octen-3-one (II)[9]. Reduction of 2-methyl-1-penten-3-one (V)[11] with lithium aluminum hydride afforded 2-methyl-1-penten-3-ol (VI) in 71% yield. The addition of VI to methylketene dimer (VII) gave 2-methyl-1-penten-3-yl 2-methyl-3-oxopentanoate (VIII) in 85% yield. Compound VIII was heated at 205 °C for 15 h in the presence of aluminum isopropoxide while gas evolution was monitored. The reaction was carried to approximately 90% conversion as shown by gas chromatography, and the product, III, was purified by preparative gas chromatography (3.7 m × 1.0 cm, 10% SE-30 on Chromosorb W, 135 °C). The IR-spectrum (neat) of III showed the expected carbonyl absorption at 1710 cm^{-1}.

The 60 MHz NMR-spectrum ($CDCl_3$) showed the following absorptions: δ 5.08 (1, t, J = 7 Hz, HC=C), 2.64 (1, m, $CHCO$), 2,42 (2, q, J = 8 Hz, CH_2CO), 1.9 (4, m, CH_2–C=C), 1.58 (3, s, CH_3–C=C), 1.07 (3, t, J = 8 Hz, CH_3CH_2CO), 1.02 (3, d, J = 7 Hz, CH_3CHCO), 0.91 (3, t, J = 8.5 Hz, CH_3CH_2C=C). E-4,6-Dimethyl-6-nonen-3-one (III) was indistinguishable from the major component of the *L. longipes* secretion on the basis of gas chromatographic/mass spectrometric comparison.



A small portion (~5%) of the product mixture obtained from the Claisen rearrangement[12] of VIII was the *Z* isomer (shorter retention time on OV-225 compared to the major component). That the 2 products were geometrical isomers was shown by their having identical mass spectra. The assignment of the more stable *E* stereochemistry to the major isomer is made by analogy to the known thermodynamic course of the Claisen rearrangement[13].

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[2] Acknowledgments. Partial support by the National Institutes of Health (Grant Nos. AI–12020 and AI–02908 and Fellowship No. 1–F32–CA05139 to T. H. J.) and the National Science Foundation (Grant No. BMS 75–15084) is gratefully acknowledged. We thank Dr. W. A. Shear for identifying the opilionids.
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