Tetrahedron Letters,Vol.25,No.15,pp 1533-1534,1984 0040-4039/84 $3.00 + .00
Printed in Great Britain

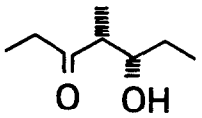
THE CHEMICAL IDENTIFICATION OF THE RICE WEEVIL AND MAIZE WEEVIL AGGREGATION PHEROMONE

Norman R. Schmuff[1]*, Joel K. Phillips[2], Wendell E. Burkholder[2], Henry M. Fales[1], Chi-Wan Chen[3], Peter P. Roller[4], and Michael Ma[5]

[1]Laboratory of Chemistry, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20205
[2]Stored Products and Household Insects Laboratory, ARS, USDA, Department of Entomology, University of Wisconsin, Madison, WI 53706
[3]Laboratory of Theoretical and Physical Biology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205
[4]Laboratory of Experimental Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205
[5]Department of Entomology, University of Maryland, College Park, MD 20742

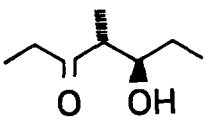
ABSTRACT: (R*,S*)-4-Methyl-5-hydroxy-3-heptanone is identified as the major component of the aggregation pheromone of the rice weevil (Sitophilus oryzae L.) and the maize weevil (S. zeamais Motsch.).

Rice weevil (Sitophilus oryzae L.) infestation of stored cereal grains results in hundreds of millions of dollars of loss annually[1]. Phillips and Burkholder[2] reported the existence of a male rice weevil-produced aggregation pheromone and Walgenbach et al[3] found evidence for a similar compound in the maize weevil (S. zeamais Motsch.). We now report the isolation, purification and identification of the male rice weevil and maize weevil pheromone for which we propose the name "sitophilure" (1a).



R*S*

1a



R*R*

1b

Two hundred vials each containing a single 2-day-old virgin male rice weevil, a single cracked wheat kernel, and a highly absorbent antibacterial assay disc[4] were maintained under standard rearing conditions[2] for two weeks. Disks were then batch extracted with hexane. After concentration, the crude extract was chromatographed on Florisil® (1:1 hexane, ether). The pooled bioactive fractions were then further purified by preparative GC (3% SE-30® on Gas Chrom Q®, temperature programmed at 5°C per min from 50°C to 200°C with the pheromone eluting at 82°C). The collected material was fully active.

Solutions containing 5-15 µg of material in 500 µl of hexane/ether were then analyzed by GC-MS. Material stored at -40°C showed a single major GC peak (accounting for <95% of the integrated area) on several different non-polar stationary phases. When stored at room

temperature for any length of time, this major peak decreased in intensity and gradually disappeared. The EI mass spectrum [129 (0.3), 128 (0.3), 126.1037 (2,$C_8H_{14}O$), 115.0767 (1,$C_6H_{11}O_2$), 97.0658(3,C_6H_9O) 86.0716 (18,C_5H_{10}), 70 (5), 69(3), 57(91), 29(100)] suggested a molecular weight of 144 and a formula of $C_8H_{16}O_2$. This was confirmed by positive (CH_4) and negative (CH_4/N_2O-hydroxide) CI spectra which show ions at m/z 145 $(M+H)^+$ and m/z 143 $(M-H)^-$ respectively. Material isolated from the maize weevil exhibits virtually identical physical and spectral properties. The mass spectrum suggests the linkage $CH_3CH_2COCH(CH_3)CHXY$ (loss of ethyl, McLafferty rearrangement to the ion at m/z 86). Supporting evidence for this fragment was obtained from a 500 MHz ^1H NMR spectrum of a small sample of impure material [δ0.90(d,J=7.0 Hz,-CH_3)] that is coupled to a multiplet at δ1.96 (-C<u>H</u>-CH_3)]. Attachment points of the remaining C_2H_5 and OH (water loss from $M^{+\cdot}$) units were not obvious from the mass spectrum so several α-methyl branched 3-ketoalcohol isomers of formula $C_8H_{16}O_2$ were synthesized by established procedures. Synthesis of a mixture of <u>1a</u> and <u>1b</u>[5a] and separation by preparative GC (10% SP-1000 at 100°C) provided pure <u>1a</u> whose R*S* configuration has been established by ^{13}C NMR[5b]. This racemic diastereomer was found to be identical by GC-MS and ^1H NMR with the natural material from both weevils. This substance seems to have its biogenesis in the polyketide pathway, as do a number of other beetle and weevil pheromones[6], e.g., serricornin and <u>exo</u>-brevicomin, but it is the first of its class to exhibit the expected 1,3 oxygenation pattern.

There are preliminary indications that minor amounts (<5%) of the R*R* isomer <u>1b</u> (separated from <u>1a</u> on 10% SP-1000 at 100°C) are present in both the rice weevil and maize weevil extracts[7]. In addition, trace amounts of both isomers appear to be present in similarly prepared extracts of the granary weevil, <u>S. granarius</u> (L.). There are also detectable amounts of 3-pentanone (from retro-aldol ?) in both rice and maize weevil extracts and bioassay indicates that it has approximately the same bioactivity as compound <u>1a</u>. It is possible that 3-pentanone is an attractive chemical breakdown product of the pheromone; perhaps this also explains the instability of the extract on storage. The question of whether these minor components in the extract are indeed of functional significance or are merely artifacts will be addressed in our full paper. Synthesis of each enantiomer of <u>1a</u> is in progress.

ACKNOWLEDGMENT: This research was supported in part by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by a cooperative agreement between the University of Wisconsin and ARS, USDA. The assistance of James L. Cone in determining mass spectra is gratefully acknowledged.

References

1. D. B. Reddy, <u>J. Econ. Entomol.</u> <u>43</u>, 203 (1950).
2. J. K. Phillips, W. E. Burkholder <u>ibid</u> <u>74</u>, 539 (1981).
3. C. A. Walgenbach, J. K. Phillips, D. L. Faustini, W. E. Burkholder, <u>J. Chem. Ecol.</u> <u>9</u>, 831 (1983).
4. W. E. Burkholder, R. J. Dicke, <u>J. Econ. Entomol.</u> <u>59</u>, 540 (1966).
5. a. A. B. Smith III, P. A. Levenberg, <u>Synthesis</u> 567 (1981).
   b. C. H. Heathcock, M. C. Pirrung, J. E. Sohn, <u>J. Org. Chem.</u> <u>44</u> , 4294 (1979).
6. a. T. Chuman, <u>Yuki Gosei Kagaku Kyokaishi</u> 39, 1183 (1981) (1983).
   b. W. Francke, W. Mackenroth, W. Schröder, A. R. Levinson, <u>Colloq. INRA</u> <u>7</u>, 85 (1982).
7. Synthetic sitophilure <u>1a</u> as a 2:1 mixture with <u>1b</u> exhibits significant activity (comparable to the natural extract) at levels of ∿20 ng as judged from the dual pitfall bioassay[2].

(Received in USA 24 January 1984)