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ISOLATION AND IDENTIFICATION OF THE MALE SEX PHEROMONE OF THE GRAPE BORER XYLOTRECHUS PYRRHODERUS BATES (COLEOPTERA: CERAMBYCIDAE)

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The sex pheromone of grape borer, Xylotrechus pyrrhoderus Bates, was isolated from body extracts and container washings of male insects, and was determined to be a mixture of (2S,3S)-octanediol (**1**) and (2S)-hydroxy-3-octanone (**2**). Each compound showed special response in electroantennogram experiments. The females were attracted by 80:20 to 95:5 mixtures of **1** and **2**. This is the first time that the male sex pheromone from Cerambycidae species has been isolated and determined.

The grape borer, Xylotrechus pyrrhoderus Bates, is a major pest of grapevines in Japan. The existence of a sex pheromone produced by the male grape borer which attracts females was first observed and reported by one of the authors (K.I.).[1] The male sex pheromone has now been identified as a two-component mixture of (2S,3S)-octanediol (**1**) and (2S)-hydroxy-3-octanone (**2**). We report here the isolation and identification of the sex pheromones from the male grape borer.

Airborne collection, whole body extraction, and container washing methods were carried out to collect the pheromone from the male insects. The airborne collection method using Porapak Q gave less satisfactory results, but the latter two methods gave satisfactory results. Fifty-six males were immersed in hexane at 10 min, and the solvent was decanted. The solvent was evaporated to give a yellow residue (13.5 mg). By the same procedure, the female body extract (1.2 mg) was obtained from 101 females. Twenty insects were placed in a 3-l glass container for 3 days under laboratory conditions. After the insects were removed, the container was washed two times with 100 ml hexane. The hexane washings from more than 20 containers was combined, and the solvent was removed to give 35.8 mg and 44.3 mg of oily residues, respectively, from the males and females.

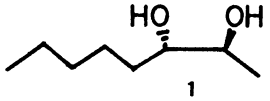
The whole body extracts and container washings of the male insects showed similar gas chromatographic profiles, whereas the female extracts showed a quite different pattern from the male extracts. GC/MS analysis (CP Wax 51 glass WCOT column, 0.24 mm i.d. x 50 m, 150 °C) revealed the presence of two major components in the male extracts, but none of these components were found in the female extracts. The minor components in male and female extracts exhibited mass spectra typical of alkanes which probably originated from the cuticle. The mass spectrum of major component (**1**) exhibited fragment ions at m/z 101(23%), 100(12), 83(79), 55(100), 45(55), 43(29), and 41(43). The mass spectrum of **1** is closely similar to that of published data for 2,3-octanediol.[2]

To identify these components, we collected larger quantities. From 1800 males (20 insects x 90 vessels), a yellow wax-like residue (1.80 g) was obtained by the container

washing method. By means of preparative GLC, each component was isolated as a single peak to give 4.8 mg of **1** and 0.8 mg of **2**.

The IR spectrum of **1**, $[\alpha]_D^{23}$ -19.2° (c 0.48, $CHCl_3$), showed a strong band at 3380 cm^{-1} due to a hydroxyl group and no bands due to carbonyl and ether groups. The ^{13}C NMR spectrum showed that **1** contains 8 carbons: two CH_3's, four CH_2's, and two CH(OH)'s. The ^1H NMR spectrum exhibited the presence of CH_3CH_2- (δ 0.90, 3H, t, J=7 Hz), $CH_3CH(OH)$- (δ 1.20, 3H, d, J=7 Hz and δ 3.59, 1H, dq, J=2, 7 Hz), and -CH(OH)- (δ 3.34, 1H, unresolv) groups. From these spectral data **1** was determined to be 2,3-octanediol, and the ^1H-NMR spectrum of which was identical to that published for threo-2,3-octanediol by Katzenellenbogen et al.[3] In order to establish the absolute configuration of **1**, we synthesized all enantiomers[4] from dimethyl D- or L-tartrate according to the method of exo-brevicomin synthesis by Mori.[5] Starting from dimethyl D-tartrate, (2S,3S)-octanediol ($[\alpha]_D^{21}$ -19.2°, c 1.45, $CHCl_3$) was obtained. [(2R,3R)-Octanediol, $[\alpha]_D^{25}$ +17.5°, c 0.40, $CHCl_3$]. Thus, the major component (**1**) of male sex pheromone was established as (2S,3S)-octanediol.

The minor component (0.8 mg) isolated as a single peak was revealed by GC/MS to be a mixture of two compounds (ca. 3:2 ratio) which was not separable by GCL, TLC, and HPLC. The IR and NMR spectra of this fraction (**2'**) showed strong bands at 3440 (OH) and at 1700 cm^{-1} (CO) and the presence of CH_3CH_2- (δ 0.90, 3H, t, J=7 Hz), $CH_3CH(OH)$- (δ 1.40, 3Hx3/5, d, J=7 Hz), CH_3CO- (δ 2.21, 3Hx2/5, s), -$COCH_2$- (δ 2.46, 1Hx3/5, d, J=7 Hz and δ 2.49, 1Hx2/5, t, J=8 Hz), -CH(OH)- (δ 4.23, combined 1H, unresolv), and -OH (δ 3.51, 1Hx3/5, d, J=5 Hz and 3.63, 1Hx2/5, d, J=5 Hz) groups. These IR and NMR spectra suggested **2'** to be a mixture of 2-hydroxy-3-octanone (**2**) and 3-hydroxy-2-octanone (**3**) (3:2). GC/MS analysis also suggested the major and earlier eluting compound to be 2-hydroxy-3-octanone (**2**). The mass spectrum of **2** exhibited major fragment ions at m/z 101(10%), 99(51), 83(28), 71(43), 55(49), 45(86), 43(100), and 41(34). The mass spectrum of **3** showed major fragment ions at m/z 101(13%), 99(17), 83(46), 71(19), 55(93), 45(48), 43(100), and 41(48). In order to establish the structure of **2**, we also synthesized (2S)- and (2R)-hydroxy-3-octanones from L-lactic acid and from D-alanine by treatment with amyllithium. GLC and mass spectral data of **2** were identical with those of synthesized **2**. Purification of synthetic specimen by preparative GLC induced the isomerization from **2** to **3** to give the same mixture as **2'**.



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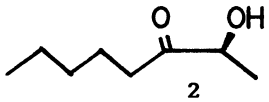

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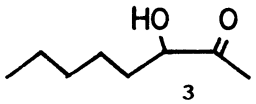

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Each component alone was not active in laboratory bioassays based on sexual response in flight tunnel. In EAG experiments, however, each compound showed special response. Of the various combinations of the two components which were tested, ratios of 80:20 to 95:5 of the two components, (2S,3S)-octanediol (**1**) : (2S)-hydroxy-3-octanone, attracted the most females. However, mixtures of **1** and (2R)-hydroxy-3-octanone did not show any attractancy. In conclusion, the present results suggest that the male sex pheromone is a mixture of (2S,3S)-octanediol and (2S)-hydroxy-3-octanone in ratios of 80:20 to 95:5.

References

1) K. Iwabuchi, Appl. Ent. Zool., 17, 494 (1982).

2) "EPA/NIH Mass Spectral Data Base", ed. by Heller and Milne, Vol. 1, NSRDS-NBS 63.

3) J.A. Katzenellenbogen and S.B. Bowlus, J. Org. Chem., 38, 627 (1973).

4) One of the isomers (2S,3R)-octanediol, $[\alpha]_D^{18}$ + 22.7°, had been synthesized by J. Kawabata, S. Tahara, and J. Mizutani, Agr. Biol. Chem.(Tokyo), 42, 89 (1972).

5) K. Mori, Tetrahedron, 30, 4223 (1974).

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