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TRAIL PHEROMONE OF Kalotermes flavicollis

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The trail pheromone of the termite Kalotermes flavicollis Fabr. has been isolated with the aid of column, liquid, and gas chromatography. Four of its components have been identified by PMR and chromato-mass spectrometry: nonan-1-ol, decan-1-ol, undecan-1-ol, and dodecan-1-ol, which are present in the natural trail pheromone in a ratio of 1:2:4:4. This has been confirmed by comparison with standard substances by means of gas chromatography and biotesting.

Termites are serious pests of articles, materials, and structures [1]. Their chemical signals have acquired exceptional diversity and regulate practically all aspects of their lives. Chemical signaling is considered as one of the conditions of the appearance of social behavior in termites [2], and it therefore appears desirable to use it in the development of effective methods for the fight against these harmful insects. The most promising in this respect are the trail pheromones. At the present time, the trail pheromones of a number of species of termites have been isolated and identified [3]; however, the trail pheromones of termites living in the USSR are unknown.

The trail pheromone from a population of the termite Kalotermes flavicollis Fabr. living in France has been isolated; it was identified as hex-cis-3-en-ol [4]. However, a number of facts cast doubt on the correctness of this determination, at least in relation to a population of this species of termite living on the territory of the USSR. Hex-cis-3-en-1-ol is a widely distributed substance characteristic of many plants and an attractant for many insects [5]. Furthermore, the hypothesis of the multicomponent nature of the pheromones of insects in general [6] and of termites in particular [3] is generally accepted. In view of the facts presented, we have carried out work on the isolation and identification of the trail pheromone of K. flavicollis.

To isolate and identify the trail pheromone we prepared an extract from 10,000 pseudoergates of the K. flavicollis. After two-stage column chromatography of the extract a single active fraction was obtained which, as GLC analysis showed, consisted of several

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TABLE 1. Results of Investigations of the Active Substances for K. flavicollis

Substance	Efficacy, TU per 1 g, M±m	Characteristic ions, m/z (%)
Substance 1 (nonan-1-ol)	$1{,}09\pm0{,}06\times10^9$	31 (65), 98 (17), 126 (2), 143 (1)
Substance 2 (decan-1-ol)	$0{,}92\pm0{,}04\times10^9$	31 (35), 112 (22), 140 (4), 157 (1)
Substance 3 (undecan-1-ol)	$3{,}17\pm0{,}12\times10^9$	31 (17), 126 (19), 154 (2), 171 (1)
Substance 4 (dodecan-1-ol)	$1{,}42\pm0{,}13\times10^9$	31 (16), 140 (18), 168 (2), 185 (1)
Trimethylsilyl derivative of substance 1	—	201 (100)
Trimethylsilyl derivative of substance 2	—	215 (100)
Trimethylsilyl derivative of substance 3	—	229 (100)
Trimethylsilyl derivative of substance 4	—	243 (100)
Natural trail pheromone	$2{,}32\pm0{,}12\times10^{12}$	—
Mixture of standard substances in a weight ratio of 1:2:4:4	$2{,}45\pm0{,}12\times10^{12}$	—
Hex-cis-3-en-1-ol	$2{,}89\pm0{,}18\times10^6$	—

substances. On a gas chromatography, it was established by the micropreparative selection of fractions corresponding to a single substance on the chromatogram followed by their biotesting that four substances possessed activity (Table 1). GLC analysis on capillary columns with different polarities showed the individuality of the substances isolated and the identical nature of their chromatographic behaviors.

By the preparative gas-chromatographic separation on a column with the medium-polarity phase XE-60 we isolated one of the components of the trail pheromone (substance 4, see Table 1) and recorded its PMR spectrum. Analysis of the spectrum permitted the assumption that it was a normal alcohol with 10-12 carbon atoms. The further identification of the active substances was carried out by chromato-mass spectroscopy, which showed the complex nature of the fragmentation of all the active substances. The mass spectra contained ions with m/z 31 of different intensities, corresponding to the $(CH_2OH)^+$ ion and the $(M - H_2O)^+$ ions. In addition, fragments were present corresponding to the hydrocarbon breakdown of the $(M - H_2O)^+$ ions. To determine the molecular masses of the substances more accurately and to show the presence of OH groups, the active substances were silylated. The mass spectra of the trimethylsilylated derivative of each of the active substances showed the presence of an ion corresponding to $(M - CH_3)^+$, where $M = R{-}O{-}Si(CH_3)_3$, which showed the presence of an OH group in the initial compound and enabled the molecular masses of the active substances to be calculated. Thus, analysis of the PMR spectra and the mass spectra of the active substances enabled the structures of the substances comprising the trail pheromone of the termite K. flavicollis to be determined. They are the normal alcohols nonan-1-ol, decan-1-ol, undecan-1-ol, and dodecan-1-ol present in the natural trail phermone in a weight ratio of 1:2:4:4.

To confirm the correctness of the establishment of the structures we used standard substances. GLC analysis in capillary columns with different polarities showed that the components of the trail pheromone that had been identified coincided completely in retention times with standard substances of the presumed structures. Biotesting also showed that the substances taken as standards had the same level of trail activity as those isolated from the natural trail pheromone (see Table 1).

It must be mentioned that all the components of the trail pheromone of K. flavicollis possessed a higher trail efficacy than hex-cis-3-en-1-ol (see Table 1). Moreover, GLC analysis did not show the presence of this substance in the natural trail pheromone of K. flavicollis. The results that we have obtained indicate substantial differences in the compositions of the trail pheromones of termites from different populations. This statement agrees well with hypotheses on the existence of geographical pheromone races [7, 8] and the possible dependence of the composition of termite trail pheromones on the chemical composition of their food [9, 10].

EXPERIMENTAL

Mass spectra were taken on a Finnigan 4021 GC-MS chromato-mass spectrometer at 70 eV. Glass column 2 mm × 2 m, stationary phase 5% of XE-60 on Inerton Super 0.125-0.160 mm. PMR spectra were taken on a Bruker CXP-200 instrument in $CDCl_3$.

Column chromatography was conducted on a column of silica gel L 40-100 mesh. The eluent used was the hexane–diethyl ether (0-50%) system. After biotesting, the behaviorally active fractions were rechromatographed on a column of Florisil 100-200 mesh. Elution was carried out with hexane–diethyl ether (0-40%).

The behaviorally active fractions were analyzed on a Chrom-5 gas chromatograph (Czechoslovakia) with a flame-ionization detector. Glass columns 3 mm × 2.5 m, stationary phase 5% XE-60 and 5% of OV-101 on Inerton Super 0.125-0.160 mm. Capillary glass columns 0.2 mm × 50 m with the stationary phases XE-60 and OV-101. The carrier gases were nitrogen and helium. The temperature of the evaporators and of the detectors was 300°C. The temperature of the column varied according to the conditions of GLC analysis.

The isolation and accumulation of the substances were carried out by preparative GLC. The substances were taken in glass capillaries cooled with a mixture of dry ice and acetone or with liquid nitrogen. Fractions corresponding to a single substance on the chromatogram were taken (for this purpose, a 1:100 outlet flow splitter was used) and were biotested.

A 50-μl portion of an active fraction after rechromatography was placed in a conical flask and 1 ml of pyridine, 0.5 ml of hexamethyldisilazane, and 5 μl of trifluoroacetic acid were added and the mixture was left on a shaking machine for 15 min. Then the samples were analyzed on a gas chromatograph and a chromato-mass spectrometer [11].

The normal $C_nH_{2n+1}OH$ alcohols, where n = 6-13, were used as standards for HGLC analysis and biotesting.

We used pseudoergates of the termite K. flavicollis collected on the territory of the Pitsudno-Myusserskii reservation, Abkhaz ASSR. To isolate the trail pheromone an extract of the sternal glands of the termites which produce the trail pheromone, was prepared. The abdominal sternites were dissected out from the termites and after homogenization they were extracted with hexane at 4°C for 24 h. The extracts were filtered and evaporated, and the residue after weighing and biotesting, were used for further study.

Biotesting was carried out by Karlson's method [12]. The level of trail reaction was expressed in arbitrary trail units (TUs) per 1 g of substance. Each biotest was performed in not less than 10 replicates, and the results were calculated with the use of Student's criterion [13].

SUMMARY

The trail pheromone of the termite Kalotermes flavicollis Fabr. has been isolated and identified; it consists of four components – nonan-1-ol, decan-1-ol, undecan-1-ol, and dodecan-1-ol – in a ratio by weight of 1:2:4:4.

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SYNTHESIS OF THE RACEMIC SEX PHEROMONE OF Pseudococcus comstocki

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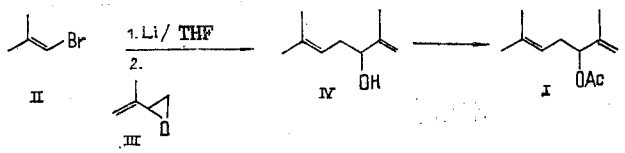
The synthesis of the acetate of 2,6-dimethylhepta-1,5-dien-3-ol – the sex pheromone of the Comstock bug – has been carried out by condensing isobutenyllithium with 3,4-epoxy-2-methylbut-1-ene and acetylating the 2,6-dimethylhepta-1,5-dien-3-ol formed. The overall yield of pheromone was 46%.

(+)-3-Acetoxy-2,6-dimethylhepta-1,5-diene (I) has been identified as the sex pheromone of the Comstock bug Pseudococcus comstocki (Kuwana) [1, 2]. At the present time it has been adopted in the USSR for wide use in the practice of the quarantining and protection of plants [3].

Synthesis of (+)-(I) are known that are based on the oxidation of 2,6-dimethylhepta-2,5-diene [2, 4], the condensation of methacrolein with prenyllithium [5] and of 4-methylpent-4-enal with isopropylmagnesium bromide [6], and an eight-stage transformation of 2,5-dimethylpyridine [7]. In the present paper we discuss the synthesis of the racemic acetate (I) by the condensation of the Li derivative of isobutenyl bromide (II) with 3,4-epoxy-2-methylbut-1-ene (III).

It is known that monohydroxy-1,3-dienes interact with organocuprates and with organomagnesium and organolithium compounds in the majority of cases nonstereoselectively with the formation of products of formal 1,4- and 1,2-addition [8-13]. The latter predominates on the use of vinyllithium reagents in THF [10]. In actual fact, on the interaction of the isobutenyllithium obtained from the bromide with the oxide (III) in THF the required alcohol (IV) was formed regioselectively. Its acetylation led to the desired acetate (I) with an overall yield of 46%, calculated on (II). The physicochemical characteristics (boiling point n_D, and IR and PMR spectra of the alcohol (IV) and acetate (I)) that had been synthesized corresponded to the characteristics published for these compounds [5].



The acetate (I) obtained, which, according to GLC, had a purity of 96% exhibited a high attractant activity in the field with respect to males of the Comstock bug.

EXPERIMENTAL

IR spectra of solutions in CCl_4 were taken on a UR-20 instrument. PMR spectra were measured relative to TMS on a Tesla BS-467A spectrometer (60 Hz) in CCl_4, and GLC was

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