# ISOLATION AND IDENTIFICATION OF A TRAIL ATTRACTANT FOR THE TERMITE *Reticulitermes lucifugus* FROM THE PLANT *Zizyphus jujuba*

S. G. Klochkov, V. I. Kozlovskii, and A. N. Pushin

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Two trail attractants for the termite *R. lucifugus* have been isolated from the plant *Zizyphus jujuba*: α- and β-bisabolenes. The threshold concentrations of the actions of these substances amounts to $10^{-9}$ g/liter. β-Bisabolene in the range of concentrations of $1\text{-}10^{-6}$ g/liter is a powerful repellant for termites.

Attractive substances for xylophagous insects can be found in their food [1, 2]. Under natural conditions, dry residues of the plant *Zizyphus jujuba* Mill. (Rhamnaceae) form one of the components of the food of the termite *Reticulitermes lucifugus* Rossi. Behavioral experiments have shown the presence in extracts prepared from this plant of substances causing a trail reaction in the termite *R. lucifugus*. By the methods of CC, GLC, and preparative GLC, in combination with biotesting on termites, two active substances have been isolated from *Z. jujuba* extracts. GLC analysis on capillary columns with different polarities showed the individuality of the substances isolated. Their PMR and mass spectra were then taken. Mass-spectrometric analysis revealed the presence in both substances of the same molecular ions with m/z 204 and a complex nature of the subsequent fragmentation (Table 1). A comparison of the results of GLC analysis and PMR and mass spectroscopy permitted the substances under investigation to be assigned to the sesquiterpene monocyclic hydrocarbons ($C_{15}H_{24}$). Further identification was performed by $^{13}C$ NMR spectroscopy. Analysis of mass and $^{13}C$ NMR spectra (Tables 1 and 2) and their comparison with literature information enabled the fine structures of the active substances to be established. They were both bisabolenes. The first substance (from its position on an analytical chromatogram) was α-bisabolene. The chemical shifts of all 15 carbon atoms in the $^{13}C$ NMR spectrum were identical with those given previously for this substance [3] (the difference in the chemical shifts did not exceed 0.5 ppm; see Table 2). The structure of this substance was also confirmed by its mass spectrum [4] (see Table 1). The mass spectra and chemical shifts of the carbon atoms of the second substance (see Tables 1 and 2) were identical with the corresponding characteristics of β-bisabolene [5].

To study the specific action of the individual substances isolated, we carried out behavioral experiments on *R. lucifugus* termites. The results of biotesting demonstrated considerable differences in the reactions of the termites to these substances. For them, α-bisabolene is an active attractant (threshold concentration $10^{-9}$ g/liter, Table 3), while the reaction of the termites to β-bisabolene strongly depended on the concentration of the substance presented to them. β-Bisabolene possessed attractant properties only at concentrations of $10^{-8}\text{-}10^{-9}$ g/liter and in the remaining range of concentrations this substance was a powerful repellent (see Table 3).

Thus, the presence of active attractants in the food of the termite *R. lucifugus* has been shown. The results obtained confirm the hypothesis expressed previously on the food origin of many signals of the system of chemical communication of insects in general [6] and of termites in particular [7].

## EXPERIMENTAL

Mass spectra were taken on a Finnigan 4021 GC-MS chromato-mass spectrometer at 15 and 70 eV. Quartz capillary column, 0.1 mm × 25 m with SE-30 as the stationary phase. PMR and $^{13}C$ NMR spectra were taken on Bruker CXP-200 and Varian VXR-300 instruments in $CDCl_3$.

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TABLE 1. Results of the Mass-Spectrometric Investigation of the Active Substances

| Substance | Characteristic ions (m/z, %) | |
|---|---|---|
| | 70 eV | 15 eV |
| α-Bisabolene | 69 (11 %), 79 (15 %), 80 (25 %), 93 (100 %), 94 (25 %), 95 (5 %), 105 (7 %), 107 (13 %), 109 (40 %), 119 (52 %), 121 (26 %), 161 (7 %), 189 (5 %), 204 ($M^+$, 4 %) | 204 ($M^+$, 100 %) |
| β-Bisabolene | 69 (100 %), 93 (85 %), 94 (40 %), 95 (13 %), 105 (12 %), 107 (21 %), 109 (30 %), 119 (16 %), 133 (19), 147 (10 %), 161 (45 %), 189 (8 %), 204 ($M^+$, 29 %) | 204 ($M^+$, 100 %) |

TABLE 2. $^{13}C$ NMR Chemical Shifts of α- and β-Bisabolenes (δ, ppm, $CDCl_3$)

| Carbon atom | α-Bisabolene | β-Bisabolene | Carbon atom | α-Bisabolene | β-Bisabolene |
|---|---|---|---|---|---|
| C-1 | 133,5 | 133,5 | C-9 | 18,5 | 106,9 |
| C-2 | 121,5 | 120,6 | C-10 | 123,3 | 124,1 |
| C-3 | 29,7 | 30,7 | C-11 | 26,9 | 26,8 |
| C-4 | 35,1 | 39,7 | C-12 | 123,3 | 124,1 |
| C-5 | 27,9 | 28,2 | C-13 | 131,0 | 130,4 |
| C-6 | 30,8 | 31,4 | C-14 | 25,8 | 25,7 |
| C-7 | 23 6 | 23,5 | C-15 | 17,7 | 17,7 |
| C-8 | 139,0 | 154,0 | | | |

TABLE 3. Activities of α- and β-Bisabolenes for the Termite R. lucifugus

| Concentration of the substances, g/liter | Type of activity | |
|---|---|---|
| | α-bisabolene | β-bisabolene |
| $1-10^{-5}$ | Trail attractant | Repellent |
| $10^{-6}$ | " | " |
| $10^{-7}$ | " | No activity |
| $10^{-8}$ | " | Trail attractant |
| $10^{-9}$ | " | " |
| $10^{-10}$ | No activity | No activity |
| Trail efficiency, TU per 1 mg | $2.14 \pm 0.02 \cdot 10^9$ for concentrations of $1\text{-}10^{-9}$ g/liter | $1.45 \pm 0.1 \cdot 10^9$ for concentrations of $10^{-8}\text{-}10^{-9}$ g/liter |

Column Chromatography. Chromatography was performed on a column containing Florisil, 40-80 mesh. The hexane–diethyl ether (0-50%) system was used as eluent. After biotesting, the active fractions were rechromatographed on a column of Florisil, 100-200 mesh. Elution was carried out with hexane–diethyl ether (0-40%).

Gas-Liquid Chromatography. The behaviorally active fractions were analyzed on a Chrom-5 gas chromatograph (Czechoslovakia) with a flame-ionization detector. Glass column, 3 mm × 2.5 m; stationary phases 5% of XE-60 and 5% of SE-30 on Inerton Super, 0.125-0.16 mm. Glass capillary columns, 0.2 mm × 50 mm with the stationary phases XE-60 and SE-30. The carrier gases were nitrogen and helium. The temperature of the injectors and of the detectors was 250°C. The column temperature was varied according to the conditions of GLC analysis.

Preparative Isolation of the Active Substances. The isolation and accumulation of the substances was carried out by preparative GLC on the Chrom-5 gas chromatograph. Glass column 3 mm × 2.5 m; stationary phase 15% of SE-30 on Inerton Super 0.125-0.16 mm. Fractions corresponding to a single substance on the chromatogram were collected. For this we used an outlet flow split of 1:100. One part was directed to the FID and the other to a cluster of detectors modified for preparative purposes [8]. The substance was collected in 5 × 300 mm glass capillaries (internal diameter 1 mm) cooled with a mixture of dry ice and acetone or with liquid nitrogen.

Preparation of the Extract. The plant material was collected in the foothills of the Western Kopet-Dagh, Turkmen SSR. The air-dry leaves and stems of the plant Z. jujuba were homogenized and extracted with petroleum ether (40-70° fraction) at 4°C for 48 h. The extracts were filtered, evaporated, and weighed and, after biotesting, were used in the investigation.

Biotesting. The R. lucifugus termites were collected on the territory of the Syunt-Khasardagskii reserve (Krasnyi Kopet-Dagh, Turkmen SSR). Biotesting was carried out by Karlson's method [9]. The level of the trail reaction of the termites was expressed in nominal trail units – TUs – per 1 mg of substance. Each biotest was repeated not less than 10 times and the results were calculated with the use of Student's criterion [10].

## SUMMARY

Two trail attractants for the termite R. lucifugus have been isolated from the plant Z. jujuba: α- and β-bisabolenes.

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